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Edwards, Wayne Mark (2019) ***Conservation of the golden mantella in Madagascar: Integrating in situ and ex situ research.*** **Doctor of Philosophy (PhD) thesis, University of Kent,.**

Conservation of the golden mantella in Madagascar: Integrating in situ and ex situ research

Wayne M Edwards

Thesis submitted for the degree of Doctor of Philosophy from the Durrell Institute of Conservation and Ecology, University of Kent.

Date: September 9th, 2019

Word count: 39,638

Abstract

The greatest threats to biodiversity in Madagascar are habitat destruction, fragmentation and climate change. Complementary in situ and ex situ research can aid conservation because many aspects of natural history that can usefully inform conservation measures are difficult to study in the field. The golden mantella is an excellent model as it is unique in that it is a charismatic, high profile Critically Endangered amphibian, but is abundant in captivity and highly suitable for ex situ research. In situ research in a new protected area of Madagascar found surface temperature, litter coverage and the number of tree roots were the most important predictor variables associated with quadrats occupied by golden mantellas. Microclimatic measurements made in the field informed the design of the replicated climatic-controlled enclosures (Froggotrons) for golden mantellas at Paignton Zoo.

Froggotrons revealed golden mantellas had a bimodal activity pattern during daylight hours even under different temperature regimes. At lower temperatures (16 °C – 19 °C) mantellas were overall less active than those at higher temperatures (20 °C – 25 °C), but the phasing and bimodal nature of the activity rhythm was the same under both temperature regimes. Most activity occurred when humidity levels exceeded 85%. Golden mantellas were most active, spent most time in the open and less time on leaves at 21.5 °C. Where temperature deviated either way from 21.5 °C there was an associated decrease in activity and an increased tendency to hide in leaves. Results also show that even under optimum temperature and humidity regimes less than 50% of the frogs were active in open areas at any one time. Ex situ results have been used to assist with the design and timing of field population assessments and shed light on issues concerning imperfect detection when applying models to assess abundance. Species distribution modelling results suggest a potential south-eastwardly shift away from current distribution range and a decrease in suitable habitat from 2110 km^2 under current climate to between 112 km^2-138 km^2 by the year 2085. Golden mantella research is a new development in the area of collaborative, complementary conservation. Integrating in situ and ex situ research may help mitigate the multi-faceted and synergistic threats to biodiversity in Madagascar.

Author contributions

This thesis was written by Wayne M Edwards (WME) under the supervision of Richard A Griffiths (RAG) and Tanya Humle (TH). Part of the work was carried out in collaboration with Michael J Bungard (MJB), Eddie F Rakotondrasoa (EFR), Julie H Razafimanahaka (JHR), Pierre Razafindraibe (PR), Raphali R Andriantsimanarilafy (RRA) and Joseph C Randrianantoandro (JCR). Paignton Zoo carpenter Don Neilson (DN) led the build of the Froggotrons and helped with their design.

Chapter 1: WME wrote the chapter. Review, comments and editorial amendment were suggested by RAG.

Chapter 2: WME was lead author for the chapter and carried out preliminary data collection, analysis and wrote the chapter. RAG, JHF, EFR, PR, RRA, JCR conceived and designed research methods. Following pilot fieldwork by WME, JHF, EFR, PR, RRA, JCR carried out data collection. RAG,TH, MJB, JHF, EFR, PR, RRA, JCR reviewed the manuscript suggesting edits and updates. RAG, MJB, JHF, EFR, PR, RRA, JCR offered critical contributions to the chapter. Chapter 2 was published by the *Herpetological Journal.*

Chapter 3: WME, RAG and MJB conceived the idea of the Froggotrons. WME, DN, MJB, designed and built the Froggotrons. WME wrote the chapter, designed and carried out data collection and analysis. RAG and TH suggested comments and editorial amendments.

Chapter 4: WME conceived the idea and methodology, collected and carried out data analysis and wrote the chapter. Comments, suggestions and feedback were provided by RAG and TH.

Chapter 5: WME wrote the chapter. WME , MJB and RAG conceived the idea, JHF, EFR, PR, RRA, JCR carried out the original data collection. MJB and WME carried out species distribution modelling. RAG and TH provided feedback, review and editorial amendments.

Chapter 6: WME wrote the chapter. Review, comments and editorial amendment were provided by RAG.

Acknowledgements

First and foremost I would like to thank my supervisor Professor Richard Griffiths. Over the years I have come to know the Professor as an incredibly sharp but kind and thoughtful man. I have found his guidance inspiring with questions both brilliant and challenging in equal measure. Thank you, Richard, it has been a pleasure and a privilege to have you as my mentor. Thanks also to my other supervisor Tanya Humle, who was always willing to offer a kind word, advice or review my manuscripts/chapters. I am also especially grateful to Mike Bungard, former Curator of Lower Invertebrates and Vertebrates at Paignton Zoo, UK. Without Mike's help the PhD would never have gotten off the ground, thanks for listening and for being a great friend. Thanks to all at ATASS for providing the first year of funding, it meant we could actually get the PhD underway; DICE and Stiftung Artenschutz for providing funding for equipment and travel to Madagascar; Amy Plowman, Director of Living Collections at Paignton Zoo for the support; Lee Brady (DICE) and Gerardo Garcia (Chester Zoo) for the inspiration and all at Madagasikara Voakajy including Julie Razafimanahaka, Christian Randrianantoandro, Eddie Rakotondrasoa, Pierre Razafindraibe, Raphali Andriantsimanarilafy, and Devin Edmonds and staff at Association Mitsinjo in Madagascar. Thank you all for providing guidance and good company during both my visits to Madagascar and in correspondence since.

Thanks also to my in-laws Ted and Ruth Parkhouse for their ongoing interest in my project and for always being there when we need you. My greatest thanks though are reserved for my wife and best friend Emma, her calm and loving support has been unwavering and critical, it gave me the confidence to get past my fears and believe in my abilities. I must also mention our two young sons Myles and Louis, who have always shown great interest in my studies. Myles (10) with ever more complicated science questions, and Louis (7) who asks almost weekly if I've finished yet as he knows my eventual graduation in Canterbury probably also means a visit to the Harry Potter Museum in London. Thank you for your support boys, and for thinking your dad is cool for doing animal stuff, it means the world to me.

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Chapter 1

Introduction

1.1 The extinction problem and biodiversity conservation

Globally, the human population now stands at over 7 billion and is set to rise to approximately 9.7 billion by 2050 (Lal, 2016). Each day 200,000 more people are added to the planet, and will need space to live, grow food and reproduce (Mills, 2007). Certainly, it is the unprecedented and relentless growth in the human population that has caused, and continues to underwrite, all other major problems associated with the mass extinction of species worldwide (Mittal and Mittal, 2013). Populations, communities and ecosystems are under extreme pressure from land use change, habitat loss, over-harvesting, direct exploitation, invasive species, environmental contaminants and emerging infectious diseases (Beebee and Griffiths, 2005; Araujo et al., 2006; Hamer and McDonnell, 2008; Thuiller et al., 2008; Smol, 2012). Climate change compounds and exacerbates the situation posing significant and serious threats to biodiversity both now and for the foreseeable future (Pounds et al., 1999; Root et al., 2003; Pamesan et al., 2005; Bartelt et al., 2010).

A recent report by the United Nations (2019) estimates that approximately 1 million species are threatened with extinction. Amphibians continue to be among the most threatened of all land vertebrates (Beebee and Griffiths, 2005; Norris, 2007; Bishop et al., 2012; Biega et al., 2017) with 40% assessed as being at risk (IUCN, 2019). Several authors have cited important ecological, biological, economical, medicinal, aesthetic or ethical reasons for preventing further harm or extinctions (Tudge, 1992; Ranvestel et al., 2004; Whiles et al., 2006; Altig et al., 2007; Verburg et al., 2007; Całkosiński et al., 2009; Hocking and Babbitt, 2014). The role of conservation organisations is to increase awareness, education and prevent the decrease in population abundance, community composition or extinction of wildlife (Tudge, 1992). Ex situ, this role is increasingly being taken on by zoos (Biega et al., 2017), with protection of wild places and species, research and education at the top of many mission statements (Barongi et al., 2015). With approximately 700 million visits to zoos per year (Barongi et al., 2015) the potential to raise revenue and promote important conservation issues with the wider public is considerable (Griffiths, 2017). Additionally, research conducted in zoos can provide a unique opportunity to closely observe animal behaviour, which may be difficult to do in the wild (Barongi et al., 2015). In situ conservation programmes may therefore benefit from working closely with zoos. Equally, zoos benefit from the relationship by fulfilling many of their conservation objectives and gaining further insights into the species they hold (Barongi et al., 2015).

Grant et al. (2019) suggest that the success of conservation actions is driven both by research and by the level of communication and collaboration between researchers and conservation managers. Indeed, there is growing support for the use of integrated complementary in situ - ex situ conservation initiatives (IUCN SSC, 2014; Barongi et al., 2015; Trayler-Holzer et al., 2018). The IUCN SSC (2014) have revised their guidelines on ex situ research and now provide a checklist to ensure it has relevance to the conservation needs of the species. The loss of species and habitats is ongoing and serious (IUCN, 2019; Plumptre et al., 2019), and bridging the gap between in situ and ex situ research to better inform conservation management decisions is crucial (IUCN SSC, 2014). It was from this perspective that my golden mantella research project was conceived and undertaken.

1.2 Climate Change, Range Shifts and Species Distribution Models (SDMs)

Climate change is known to be a key player in driving species range shifts worldwide increasing the risk of further extinctions (Heikkenen et al., 2006; Braunisch et al., 2013). Therefore, comprehensive and reliable information regarding the potential for range shift is important for conservation planning (Liu et al., 2013). Species Distribution Models (SDMs) are commonly used to this end and generally operate by exploring relationships between a species' current distribution and its associated environment and then making extrapolations to predict possible range shifts given a warmer or cooler climate (Barbosa et al., 2013; Bateman et al., 2013; Cao et al., 2013; Meynard et al., 2013; Rodriguez-Rey et al., 2013). However SDMs designed to predict species ranges regularly do so without factoring in biotic interactions such as interspecific and intraspecific competition, species dispersal ability and barriers, predation, pathogens, parasites and mutualisms (Guisan and Thuiller, 2005; Vicente et al., 2011; Capinha et al., 2013; Bateman et al., 2013; Higgins et al., 2012). Dormann (2007) provides further insight into the caveats of SDMs; for example, causal drivers are rarely quantifiable and have non-linear synergistic effects, spatial autocorrelation, and limiting factors may also change throughout a species range and differ with environmental change.

According to Kearney and Porter (2009), most distribution modelling follows the correlative method i.e. models treat an organism as a point on a map, statistically linking spatial data to distribution records. Kearney and Porter (2009) suggest that process-based models i.e. those that include information on species and environment interactions, are likely to provide more accurate distribution predictions. There is also growing consensus that although SDMs are useful for identifying common trends among a variety of predictions they are limited when used in isolation and provide more reliable results if used in conjunction with other predictive dynamic process-based models (Anderson et al., 2009: Braunisch et al., 2013; Cao et al., 2013; Rodriguez-Rey et al., 2013). Process-based models have positive and negative aspects e.g. they are more robust and provide more detail but can be less flexible, more biased and data-hungry than correlative models (Kearney and Porter, 2009; Dormann et al., 2012; Gritti et al., 2013; Higgins et al., 2012).

The fundamental point here is that SDMs of rare or cryptic species can be vague or imprecise because they are often built on limited data. Indeed, Marcer et al. (2013) acknowledge a need for using other models in conjunction with rare species SDMs to improve their accuracy but suggest they can still provide a valid and comprehensive insight into species distribution by capturing much of its realised niche. Dormann et al. (2012) recommend a combined workflow by using correlative models to help generate hypotheses on underlying processes which would then, along with ecological theory and experimental data, be used to inform process-based models. Gritti et al. (2013) and Iverson and McKenzie (2013) agree and advocate the use of hybrid SDMs rather than using correlative or processed-based models in isolation which can lead to major differences in the resulting forecasts. Heikkinen et al. (2006) are in favour of using SDMs as an approximation or ‘first filter’ twinned with a thorough understanding of the shortfalls, and Vicente et al. (2011) conclude that more informative projections of species distributions are possible if a combined modelling approach is followed where both regional and local predictors are used instead of the more usual binomial presence versus absence outputs. It is clear that sound ecological theory and more detailed information regarding important aspects of a species niche should be used to improve the accuracy of SDMs (Guisan and Thuiller, 2005; Kearney and Porter., 2009; Huey et al., 2012). It is also important to recognise that natural systems are not closed and therefore it is not possible to account for all driving forces of species distributions, no matter how powerful the model (Heikkinen et al., 2006).

1.3 Climate Change and Protected areas (PAs)

Climate change is having an impact on the distribution of many species as they alter or shift ranges to avoid warming or increased precipitation, and there is great variation in responses between different taxa which may include either reduction, expansion or complete shift in range (Monzon et al., 2011). There are, though, some general global patterns emerging. For example, distributional changes are either towards higher latitudes (estimated to be 6.1 km northward per decade) or elevations (6.1 m upward per decade) in montane habitats (Parmesan and Yohe, 2003; Monzon et al., 2011; Thomas et al., 2012; Anderson et al., 2013). In the distant past, as climate change occurred, species would have had longer to evolve and adapt to changing conditions: this is no longer the case (Monzon et al., 2011).

Protected areas are the keystone of in situ conservation and act as refuges for species and ecosystem processes, they are also a useful tool in the search for ways to mitigate the effects of climate change on species and habitats. However, protected areas and reserves are of course sedentary usually with boundaries agreed and drawn up using political rather than current or future ecological requirements of the species living within them (Monzon et al., 2011; Thomas et al., 2012). Whilst protected areas may go some way to shielding species from habitat destruction, poaching and other anthropogenic pressures, they cannot offset many of the detrimental effects of climate change (Monson et al., 2011). It may be that some species will need to disperse beyond the boundaries of protected areas (Thomas et al., 2012). Monzon et al. (2011) recommend a renewed focus on adaptive strategies such as expanding and connecting future or existing PAs in order to aid the dispersal of vulnerable species or assisted migration, as well as measures that strengthen mitigation through research, community participation or sustainability.

Existing and newly designated PAs will continue to be important if they are able to be colonised by species shifting into new regions. Conservation strategies should retain existing PAs to provide areas for colonisation with substantial effort put into deciding where new PAs are developed. New PAs should be placed in areas where they are able to facilitate and accommodate the leading edge of species shifting range (Monzon et al., 2012). But funding for protected areas is finite and as such should be allocated to those high priority areas where species dispersal and survival is most likely (Buchanan et al., 2011). The current position of many governments worldwide is to increase protected areas from approximately 10% to 17%

of the earth's land surface by 2020, making the identification of suitable areas a priority (Buchanan et al., 2011; Pettorelli, 2012; Thomas et al., 2012).

In summary, species that are unable to adapt to rapid climate change within their current habitats will either have to disperse or risk extirpation or extinction (Foden, 2013). If they are to disperse, they will need uninterrupted habitat as a corridor to new areas (Matisziw and Murray, 2009) and those areas will need to be situated where they are able to provide adequate protection or buffering from increased temperatures. Existing PAs may provide new habitat for some species or a leading edge for other species to facilitate a move into different areas (Thomas et al., 2012). In order to decide which areas optimise a species ability to survive, more studies need to be carried out into species-specific habitat preference and population requirements incorporating information on demographic dynamics such as population size, isolation, density dependent competition, and limits to inter-population movement (Anderson et al., 2013).

1.4 Madagascar

Madagascar is situated in the Indian Ocean around 250 miles off the coast of Mozambique, East Africa. The main sources of national income are agriculture, fisheries and livestock production (Worldbank, 2014). The country is classified as developing and low income where 75% of its 22 million people exist on less than $1.00 per day (Worldbank, 2014). Given the level of poverty across the country it is perhaps not surprising that threats to rainforest habitat remain high with the collection of plants and animals for medicinal or pet trade, logging, hunting and forest clearance for agriculture/industry taking their toll (Harper et al., 2007; Golden et al., 2012; Andriantsiferana et al., 2013). Around 90% of the population relies on an estimated 18 million m^3 of wood for their annual energy needs with approximately half used to make charcoal (Minten et al., 2012). Forest cover decreased by 40% from the 1950's up until the year 2000 with total forest land cover down from 27% to approximately 15% (Harper et al., 2007). A further 0.53% was lost between the years 2000 and 2005 (Eckert et al., 2011). Current estimates for primary forest cover stand at less than 10% (De Wilde et al., 2012). Recent political turmoil has also meant that rates of illegal logging have increased in some areas (Allnutt et al., 2013).

However, Madagascar remains one of the world's foremost biodiversity hotspots (Myers et al., 2000; Raxworthy et al., 2008), and demonstrates one of the highest degrees of amphibian endemism in the world, with at least 244 described species, and if predictions are correct, as many as 465 species in total (Vieites et al., 2009). More than 90% of endemic species on the island are dependent on forest and woodland habitat (Harper et al., 2007). Clearly, the need for biodiversity conservation in Madagascar remains high and should be classed as a priority (Myers et al., 2000, Raxworthy et al., 2008). Climate change is also a threat to species and habitats in the region, warming trends in Madagascar are equal to or above the global average which appears to be driving species upslope 19-51 m per decade (Raxworthy et al., 2008). This is a particular problem for montane endemics which are restricted to narrow elevations close to summits of most of the major massifs in Madagascar (Jenkins, 1987; Andreone et al., 2005; Raxworthy et al., 2008).

1.5 The Malagasy Massif

Mountain ranges hold much of the world's biodiversity, they are also the areas most likely to feel the negative effects of climate change (La Sorte and Jetz, 2010; Sheldon et al., 2011). Despite tropical montane regions exhibiting typically high levels of local endemism, the vulnerability of most tropical montane assemblages to climate change effects has not been well documented (Ricketts et al., 2005; Rull and Vegus-Vilarrubia, 2006). This vulnerability needs to be addressed as the majority of extinctions driven by climate change are likely to occur in tropical areas, which include both high species richness and narrow endemism, particularly in tropical montane systems (Root et al., 2003; Rull and Vegus-Vilarrubia, 2007; Raxworthy et al., 2008; La Sorte and Jetz, 2010; Monzon et al., 2011; Anderson et al., 2013). As montane species are often specialists, it can be assumed that species from such biographical zones may encounter a greater number of range-limiting climatic conditions (Hannah et al., 2002; Sheldon et al., 2011).

A study conducted by Raxworthy et al. (2008) in Malagasy montane habitat revealed overall mean shifts in elevational midpoint of 19-51 m upslope for 30 species of reptile and amphibian with subsequent preliminary reviews of other massifs in the area indicating comparable trends. A number of studies have obtained similar results across a range of different countries and taxa including; plants (Rull and Vegus-Vilarrubia, 2006; Cross and Harte, 2007), mammals (Beever et al., 2010), birds (Sekercioglu et al., 2007; Anderson et al., 2013) and insects (Wilson et al., 2005; Chen et al., 2011).

The main problem with upslope displacement is that mountains tend to be smaller at the top than they are at the bottom which leaves upward range shifting species with less space than they had before (Monzon et al., 2011). Species that do move upwards then have the added stress of increased competition for resources, less suitable and more isolated patches of habitat and different temperature or precipitation regimes which may act independently or together causing further stress (Thomas et al., 2006; Trivedi., 2008; McCain and Colwell, 2011; Monzon et al., 2011; Sheldon et al., 2011).

1.6 Amphibians

Of all the vertebrates amphibians are the group most likely to feel the greatest bio-thermal impact of changing temperatures due to their unique physiology, reproductive processes and highly permeable skins (Williams et al., 2008; Hoffmann et al., 2013). Physiological function and performance levels in ectotherms are highly dependent upon temperature (Deutsch et al., 2008; Amarasekare and Salvage 2012). According to Putnam and Bennet (1981) the rate of biochemical reactions doubles with each 10ºC increase in temperature. Deutsch et al. (2008) describe a temperature performance curve for ectotherms based on Q10 (the rate of change of biological and chemical reactions after a temperature change of 10ºC) i.e. thermal performance rises gradually from critical thermal minimum until an optimum temperature is reached. Temperatures higher than optimum will again decrease performance levels until a critical thermal maximum is reached (Huey and Kingsolver, 1993; Deutsch et al., 2008; Amarasekare and Salvage 2012). Lower thermal limits are more labile as they tend to track or correlate to ambient temperatures, whereas upper thermal limits to heat are not correlated to ambient temperature (Araujo et al., 2013). This means that tropical ectotherms are most at risk from an increase in ambient temperature because they already live close to their optimal temperatures with little distance between upper and lower temperature safety margins (Deutsch et al., 2008; Amarasekare and Savage, 2012; Moritz et al., 2012; Scheffers et al., 2013). Their ability to evolve or adapt to higher temperature is also highly restricted due to the rigidity of their upper thermal boundaries and limited dispersal capabilities (Somero, 2010; Araujo et al., 2013).

According to Kearney et al. (2009) and Chevin et al. (2010), the main thermal challenge for amphibians living in tropical forests is to stay cool, and many species will use behavioural or physical means in order to buffer or regulate the effects of warming. Behavioural adaptations include; avoiding warmer areas, moving into water or shade, burrowing, climbing, behavioural posturing, or by using an altered daily or seasonal timing of activity (Stevenson, 1985; Kearney et al., 2009; Huey et al., 2012). But the control of body temperature by behavioural means will depend on the thermal heterogeneity of the environment as well as the availability of water

(Kearney et al., 2009; Huey et al., 2012). The implication is, if climate change increases the temperature regime in a tropical forest to the point where negative effects on a species cannot be mitigated by the use of behavioural means, the species will have to disperse to more suitable areas or die out. Rare endemics found in only a few localities on mountains with low dispersal ability and limited contact with other populations are the most at risk from extinction and will remain of primary concern (Ricketts et al., 2005; Chen et al., 2011). Of the endangered Madagascan montane amphibian species the golden mantella (*Mantella aurantiaca*) is one of the most threatened and in need of urgent and extensive conservation action.

1.7 *Mantella aurantiaca*

Mantella aurantiaca is a small, terrestrial, diurnal frog with aposematic colouration, endemic to the Eastern rainforests of Madagascar (Andreone et al., 2008; Tessa et al., 2009; Randrianavelona et al., 2010). *M. aurantiaca* are generally found in primary or secondary rainforest (Andreone et al., 2005) at altitudes of approximately 873-1,054m above sea-level (Randrianavelona et al., 2010). Behra et al. (1995) and Rabemananjara et al. (2008) obtained abundance data for this species ranging between 500 and 3,000 individuals per ha and 836 and 1,371 per ha respectively. Their extent of occurrence is centred on Moramanga and estimated to cover approximately 112 km^2 with an area of occupancy at just 10 km^2 (Randrianavelona et al., 2010). Clusters of breeding ponds are found in two main areas (north – Ambatovy; south - Mangabe) together with Torotorofotsy and Analabe forests.

Ex situ populations are held in-country at Parc Botanique et Zoologique de Tsimbazaza in Antananarivo and the Parc Exotique de Madagascar, Mandraka. There is also an assurance colony of around 400 individuals consisting of F1 (Wild founder animals) and F2 (offspring of founders) generations held at the Mitsinjo research facility in Andasibe (Edmonds et al., 2015). Worldwide, zoos have been keeping and breeding golden mantellas since the 1960's, and they are now held by numerous ex situ institutions, private collectors and the pet trade (Edmonds et al., 2015).

M. aurantiaca is classified by the IUCN (2014) as Critically Endangered (CR) B2ab (iii, v) due to having an area of occupancy at less than 10 km^2, fragmented distributions and recent declines in populations and habitat (Andreone et al., 2008; Randrianavelona et al., 2010; IUCN, 2014). *Mantella aurantiaca* are listed on CITES Appendix II (IUCN, 2014) and have been a protected species since 2006, collection for export from natural habitat is allowed with a permit and quota given by Ministry of the Environment and Forest (Randrianavelona et al., 2010). Threats to forests inhabited by this species include logging or clearance for agriculture, illegal collection of individuals, and gold mining. The latter generally impacts pond hydrology, or turbidity, through excavations in close proximity to the water bodies. After initial reports of isolated incidences involving infection by *Batrachochytrium dendrobatidis* (Bd) in individuals exported commercially e.g. single cases for *Heterixalus alboguttatus, Heterixalus betsileo*, and *Scaphiophyryne spinosa* (Kolby, 2014), a more recent study has revealed that Bd is now prevalent in the wild (although not yet recorded in golden mantella populations) in Madagascar and poses a significant threat (Bletz et al., 2015).

M. aurantiaca is under distinct pressure from anthropogenic activities (Woodhead et al., 2007; Andreone et al., 2008; IUCN, 2012; Rabearivony et al., 2010; Randrianavelona et al., 2010) which therefore makes it a prime candidate for in situ and ex situ conservation efforts (Randrianavelona et al., 2010). To date, captive based studies have concentrated on larval morphology (Jovanovic et al., 2009), skin alkaloids (Daly et al., 1997; Andriantsiferana et al., 2009) and bacterial communities (Passos et al., 2018), evolution of colour patterns (Schaefer et al., 2002; Chiari et al., 2004), taxonomy (Odierna et al., 2001), mitochondrial diversity (Vences et al., 2004), tonic immobility (Passos et al., 2017a), calling and fitness (Passos et al., 2017b) and classification (Glaw and Vences, 2006). So far, no papers on species habitat preferences or reaction to projected climate change *in*- or ex situ have ever been produced. The proposed study is therefore designed to fill some of the gaps in our current understanding of the ecology and future needs of this iconic Malagasy anuran.

In situ conservation aims and targets for 2011 – 2015 are set out in the Golden Mantella Species Strategy Document Produced by Madagasikara Voakajy (Randrianavelona et al., 2010). The document advises on a number of key areas such as education, protection, restoration of terrestrial and aquatic habitat and encouraging conservation practices by promoting non-extractive benefits to local communities. The plan also advocates the continued promotion of scientific research into species biology/ecology and engagement with international partner organisations in information sharing.

1.8 Global Action for Amphibians

In 2001 the Global Amphibian Assessment (GAA) was launched in order to document the conservation status of amphibians worldwide (Zippel and Mendelson, 2008; Stuart, 2012). By 2004 the GAA had reported that one third of all described amphibian species were categorised as threatened, with habitat loss and *Batrachochytrium dendrobatidis* (Bd) infection high on the list of drivers behind species declines and extinctions (Stuart, 2012). In 2005 a global initiative identified 595 areas around the world that required conservation to avoid high species extinction, over half of the sites were chosen due to the presence of highly endangered endemic amphibian species (Stuart, 2012). During the Global Amphibian Summit of 2005 the Global Amphibian Conservation Action Plan (ACAP) was formed, designed specifically to address the declines in amphibian species worldwide by providing a framework for prioritising future policies, research, resource use and funding (Zippel and Mendelson, 2008; Stuart, 2012). In 2009 an IUCN summit prioritised specific, and perhaps more achievable, sections of the original ACAP and the Amphibian Survival Alliance (ASA) was formed and charged with implementing ACAP policy (Stuart, 2012). At present there are several other international organisations involved in conservation programs that specifically target amphibians (Gascon et al., 2007).

Working at a finer scale, in-country organisations (e.g. www.MadagasikaraVoakajy.org, and www.AssociationMitsinjo.org) are increasingly important not only for in situ wildlife research but as a way of informing and connecting local people and organisations to international institutions. In-country conservation organisations often have more local knowledge of current political, social and industrial problems impacting on habitats.

Initially menageries, then ‘arks’ breeding animals for reintroduction, zoos are now increasing their contribution to in situ conservation with a much wider ex situ role focusing on reintroduction, research and education (Foster, 1999; Bowkett, 2009). Bowkett (2009) states that in order to optimise conservation success, zoos should balance ex situ management of threatened species with in situ conservation programs. The World Zoo and Aquarium Conservation Strategy developed by Barongi et al. (2015) for The World Zoo and Aquarium Association (WAZA), also advocates and places great emphasis on the one plan/collaborative approach (Trayler-Holtzer et al., 2018). As does the IUCN (2014) who now provide a 5-step checklist for decision makers using the one integrated strategic plan method.

According to Barbosa (2009), zoos could contribute to climate change research in no less than ten subject areas, including detailed studies of responses of individuals to specific variables and investigating those traits/species that respond to climate change. The advantage of studying animals in zoos is that we can simulate a number of different climatic conditions in a controlled environment and then collect and analyse data, a task that would be more expensive, labour intensive and probably almost impossible with some studies in situ. Information gained from such studies could then be used to make predictions regarding the most likely effect of climate change on a population in situ and inform subsequent management options (Barbosa, 2009; Minteer and Collins, 2013).

That said, there are problems commonly associated with ex situ conservation programs and studying species long term. For example, the longer a population spends in captivity the more likely that inbreeding/inbreeding depression will occur (Lacy, 2012). A lack of available space needed to house even the minimum number of individuals required to avoid inbreeding in zoos means inevitably that many ex situ populations can suffer from inbreeding depression (Robert, 2009; Lacy, 2012). Species that have been in captivity for generations may also have changed behaviourally or genetically in order to adapt to their surroundings (Conway, 2011) i.e. survival skills may be lacking such as the ability to recognise threats from predators or finding food (Conde et al., 2011).

Zoos collectively hold 1 in 7 threatened species of various population sizes with approximately 2.6 million species held among 800 organisations including 25% of bird species, 20% of mammal species and 12% reptile species (Conde et al., 2011). However, only 3% of threatened amphibian species are held in zoos; not a good representation given over 30% of amphibians are categorised as threatened in the wild (Browne et al., 2011; Conde et al., 2011). Amphibians are generally neglected in ex situ conservation programs (Balmford et al., 1996; Griffiths and Pavajeau, 2008), although conversely, amphibian life history traits are extremely compatible to re-introduction and captive breeding programs i.e. hard wired physiology and behaviour, high fecundity, small body size and low maintenance (Bloxam and Tonge, 1995; Griffiths and Pavajeau, 2008; Browne et al., 2011).

1.9 Integrating in situ and ex situ research

In situ and ex situ research can and should be carried out in a complementary way (Barongi et al., 2015; Trayler-Holltzer et al., 2018) i.e. there are aspects of behaviour (e.g. microhabitat selection) that are extremely difficult to rigorously assess in situ (Barongi et al., 2015). This is where ex situ research can have a role. In turn, knowledge regarding environmental and habitat variables, ecology and biology in situ can be used to greatly improve the design, methods and execution of ex situ research. The underlying principle of collaborative and complementary research was fundamental to the approach I used for the golden mantella project.

Zippel and Mendelson's (2008) 'Call to Action' paper advocated using a more holistic approach to conservation practices where in situ and ex situ methods are complementary. Although research in both fields has come some way over the last few years, significant gaps in our knowledge remain with pollution, disease, and the design or siting of bio-reserves and impacts of climate change on population declines prioritised as a need for further investigation (Zippel and Mendelson, 2008). Lacy (2012) argues that captive and wild populations should not be considered as mutually exclusive management domains but that the persistence of one relies heavily on the other with the exchange of genes from the wild and research by way of captive studies benefitting wild populations. As an example, research by Schoville et al. (2011) noted severe population decline in the endangered yellow-legged frog (*Rana mucosa*) in California. One population was used to establish a breeding colony ex situ and was subsequently used to obtain information on the genetic variation and possible connectivity to other populations. Their results demonstrated that each of the nine small populations found in three isolated mountain ranges had unique evolutionary lineages and as such should be managed separately.

Captive bred animals have also been successfully used to supplement populations of a species that have become extirpated or extinct in the wild, such as ploughshare tortoises (*Geochelone yniphora*) or the Puerto Rican crested toad (*Peltophryne lemur*) (Pedrono and Sarovy, 2000; Beauclerc et al., 2010). Translocations and re-introductions have also been shown to be viable in a number of other scenarios and taxa including, but not restricted to, California condors (*Gymnogyps californianus*) (Burnett et al., 2013), whooping cranes (*Grus americana*) (Smith et al., 2011), black-footed ferrets (*Mustela nigripes*) (Biggins et al., 2011), Arabian oryx (*Oryx leucoryx*) (El Alqamy,et al., 2012),American bison (*Bison bison*) (Pyne et al., 2010), and Wyoming toads (*Bufo baxteri*) (Dreitz, 2006).

Although re-introductions and translocations can play an important role in the conservation of wild populations each case will need intensive investigation before a release can occur. Science-based studies should first be undertaken to reveal if the proposed habitat is suitable, populations are viable and predicted impacts on existing populations, or whether the problems responsible for the original declines are still a threat (Caughley, 1994; Griffiths and Pavajeau, 2008). Re-introduction should never be driven purely on the basis of the availability of the target species in ex situ breeding programs. The International Union for Conservation of Nature and Natural Resources [IUCN] also provide clarity and concise guidelines if reintroduction or translocation are to be carried out (IUCN, 1998; IUCN, 2013).

While zoos can and do contribute to in situ and ex situ conservation more work and space is needed. Cohesive complementary long term in and ex situ studies that further scientific knowledge, promote greater awareness and conservation actions are needed if we are to stem the decline of species in the short term, and make them sustainable for the long term.

1.10 Problem statement.

- *Mantella aurantiaca* is a specialist tropical montane species, Critically Endangered and threatened with extinction in the wild. Apart from substantial habitat destruction and low levels of occupancy, the species is also extremely vulnerable to climate change. Without a deeper understanding of its specific habitat needs we are currently unable to determine the optimum habitat to save, create or translocate it to in order to allow its persistence in the wild. Without studies such as this future actions will remain best guess, a scenario which is unacceptable.

- Fine-scale climate models still do not tell us how a species will respond to climate change (Raxworthy et al., 2008). Species bioclimatic envelope models have a number of limitations such as the inability to model dynamic interactions, effects of competition, dispersal or other factors relating to amphibian biology/ecology (Hannah et al., 2002).

- There have been a number of studies conducted into physiological and behavioural responses to the environment in amphibians (Wygoda, 1989; De Andrade and Abe, 1997; Bartelt and Peterson, 2005; Whitfield et al., 2007; Tracy et al., 2008) although few have attempted to incorporate the data into a practical species specific model in order to predict future habitat conservation needs.

- There is a clear need for the integration of basic amphibian ecology into modelling, especially more physiological data explaining movement preferences before we are able to predict with any amount of confidence the implications of various landscape configurations and compositions (Semlitsch, 2008; Dodd, 2010).

- The implication is that for those species with limited or restricted ranges, climate change will become a significant threat to survival as these species will exhibit a degree of sensitivity to changing thermal arrays (Lawler et al., 2010). This becomes even more apparent when regions with restricted micro-climates are considered.

1.11 Scope of study

There were three core components to this project, 1) Collection of data on the microhabitat use of golden mantellas in Madagascar, which was used to inform the design of the ex situ experiments; 2) The Froggotrons were used to do manipulations of microhabitat variables that would be impossible in situ, and 3) Climatic data were then used to construct SDMs and predict changes in the distributions under different scenarios.

In order to provide greater predictive power in climate modelling for vulnerable habitats and species, it is important to understand that integrative and sensitivity analysis on the ecology of individual species is an essential supplement to existing models (Hannah et al., 2002). A greater understanding of the target species ecology and its response to changing habitat is desperately needed and undertaking this research in captivity will help illuminate many pressing questions.

There are three main ways used to tackle scientific problems; correlational observational studies, experimental studies or modelling. Correlational studies involve observing what occurs naturally without any interference or manipulation by the observer (Field et al., 2014). Experimental studies are conducted by manipulating one variable to see its effect on another (Field et al., 2014), whereas modelling is used to predict the possible outcomes of, for example, our future conservation actions. This study uses correlational and modelling methods to try to address the problems faced by golden mantellas in Madagascar. Correlational methods were used to explore the relationship between golden mantella presence and environmental variables. However, this study also goes a step further and proposes an additional ex situ experimental approach using the new Froggotron system developed at Paignton Zoo (Chapters

3 & 4). Experimental approaches to solve ecological problems have been used in the past on several species and systems including natterjack toads (*Bufo calamita* Laurenti) (Griffiths et al., 1993), food webs (Wilbur, 1995) and plants (Mishra et al., 2012). Lawton et al. (1993) developed the Ecotron system, composing of 16 large climate chambers where they manipulated environmental variables including moisture, light and temperature and monitored whole community response to climate change. The Froggotron and Ecotron systems are similar in the way they operate, they also both provide a way of monitoring complex systems ex situ, where results can then be used to guide future fieldwork or conservation actions in situ. The Froggotron system differs because we use cameras and recording units connected to each unit to closely monitor single species behaviour over prolonged periods.

1.14 Research objectives and outline of analytical chapters

Chapter 2: What are the predictors for *M.aurantiaca* microhabitat selection in situ?

We surveyed forested habitat adjacent to mantella breeding ponds. Environmental and habitat data were sent back to the UK for analysis where results were used to inform temperature and humidity settings in ex situ climate chambers (Froggotrons). It means we were able to set conditions in the Froggotrons to mimic environmental conditions on the forest floor in Madagascar and also gain an insight into habitat preferences in situ.

Chapter 3: Diel activity budgets of *M.aurantiaca* ex situ. How active are the frogs at different times of day and night, and does the activity pattern change with temperature?

Froggotrons were fitted with cameras capable of filming the frogs continuously over several days. We were interested in identifying the phasing of the activity rhythm and if the intensity of activity changed with a change in temperature. Results could be used to inform husbandry procedures i.e. optimal temperature and humidity levels. Results were also useful to in situ teams to decide best times and/or conditions to survey for frogs.

Chapter 4: Ex situ habitat preferences and response of *M.aurantiaca* to climatic variables. How does behaviour and micro-habitat use change with different environmental parameters?

The study concentrated on how micro-habitat use changed with different environmental parameters, the average proportion of frogs visible at any time and how important leaves are as a component of forest floor micro-habitat. This information is important to in situ research teams if population counts are being conducted under conditions of incomplete and imperfect detection. Ex situ husbandry benefits by the study revealing how long the frogs would spend being active at different temperatures.

Chapter 5: Species Distribution Modelling. How does population distribution change and are there any areas that are less affected by climate change than others?

Our aim was to provide an up-to-date species distribution model for the golden mantella using Maxent and GIS. We used two predicted climate change scenarios for 2085 and compared distributions in each to current distribution. Results are useful to in situ conservation managers in providing new areas classed as climatically suitable. It may mean areas or habitat corridors not previously considered for protection may now be investigated further.

Collectively the analytical chapters will fill gaps in our knowledge on golden mantella, behaviour, micro-habitat use and potential distribution under different temperature regimes, something we know little about. Results can then be used to better inform ex situ husbandry guidelines or help locate further appropriate areas and micro-habitat in situ for assisted colonisation if it is needed.

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Chapter 2

Microhabitat preference of the critically endangered golden mantella frog in Madagascar

[In press: Herpetological Journal]

Wayne M Edwards[1,2], Richard A Griffiths[1], Michael J Bungard[3], Eddie F Rakotondrasoa[4], Julie H Razafimanahaka[4], Pierre Razafindraibe[4] Raphali R Andriantsimanarilafy[4] and Joseph C Randrianantoandro[4]

[1] Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, Marlowe Building, University of Kent, Canterbury, CT2 7NR, UK

[2] Department of Field Conservation and Research, Paignton Zoo Environmental Park, Totnes Road, Paignton, Devon, TQ4 7EU, UK

[3]Department of Environment and Geography, University of York, Heslington, York, YO10 5NG, UK

[4] Madagasikara Voakajy, B.P. 5181, Antananarivo (101), Madagascar

Key words: mantella, Madagascar, amphibian, montane, rainforest, protected area

Corresponding author:

Wayne M Edwards

Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, Marlowe Building, University of Kent, Canterbury, CT2 7NR, UK

Email: wayneedwards11@hotmail.co.uk

2.1 Abstract

The golden mantella (*Mantella aurantiaca*) is a Critically Endangered (CR) frog, endemic to the Eastern rainforests of Madagascar. Although the species is very popular in the pet trade and widely bred in captivity, its specific habitat requirements in the wild are poorly understood. Ten forested sites in the Moramanga district of Madagascar were surveyed for microhabitat, environmental variables and the presence or absence of golden mantellas in quadrats positioned along transects in the vicinity of breeding sites. Mixed models were used to determine which variables best explained microhabitat use by golden mantellas. Sites where golden mantellas were found tended to have surface temperatures of 20-23 C, UVI units of about 2.9, about 30% canopy cover and 30% herbaceous cover. Within sites, golden mantellas preferred microhabitats that had 70% leaf litter coverage and relatively low numbers of tree roots. This information can be used to improve the identification and management of habitats in the wild as well as to refine captive husbandry needs.

2.2 Introduction

Conservation of critically endangered species requires information at different spatial scales. Species Distribution Models (SDMs) can combine climatic and landscape variables from regional or national sources to provide large-scale pictures of habitat preferences and predicted distribution range (Guisan and Thuiller, 2005). However, within the predicted range a species is likely to be patchily and unevenly distributed with occurrence within a habitat patch dependent on microhabitat and its associated microclimate. Microhabitat variables cannot usually be extracted from remote sensing or landcover maps and need to be measured directly on the ground (Stanton et al., 2012). This can be problematical for small, microhabitat specialist species that are difficult to observe. However, understanding microhabitat preferences is crucial to both providing appropriate habitat management in the field and for informing captive management conditions in ex situ programmes (Semlitsch et al., 2009; Piludu et al., 2015; Tapley et al., 2015).

The golden mantella (*Mantella aurantiaca*) is a small, montane, diurnal, frog endemic to the Eastern rainforests of Madagascar (Glaw and Vences, 2007). Its extent of occurrence is 699 km^2 and centred in the Moramanga district (Piludu et al., 2015). The known area of occupancy for this species is low at less than 10 km^2 (Vences and Raxworthy, 2008) with two main population clusters, one to the north of Moramanga at Ambatovy, Torotorofotsy forest and

Analabe forest (Piludu et al., 2015). South of Moramanga clusters of breeding ponds are also found within fragments of Mangabe forest (Piludu et al., 2015). Due to a low area of occupancy, fragmented distribution and a decline in both numbers and suitable forest habitat, this species is categorised as Critically Endangered (CR) B2ab (iii, v) and listed on CITES Appendix II (Vences and Raxworthy, 2008). Current threats to the golden mantella and their rainforest habitat include logging, illegal collection for the pet trade, the destruction of breeding ponds due to mining activity, forest clearance to make way for subsistence agriculture and climate change (Andreone et al., 2008; Vences and Raxworthy, 2008; Piludu et al., 2015). The golden mantella therefore continues to be a prime candidate for in situ and ex situ conservation initiatives, but further research on habitat needs could help fill some knowledge gaps (Randrianavelona et al., 2010).

Most of the forest fragments inhabited by golden mantellas are deemed to have a protected status (Piludu et al., 2015). In reality, the actual practical protection afforded to these areas is low, and forest clearance, mining and the illegal collection of golden mantellas continues regardless. According to Piludu et al. (2015) there are now more threatened golden mantella populations in forests with protected status than there are in forests without protected status. There is clearly a need to identify and prioritise new sites for future conservation actions such as assisted colonisation (Piludu et al., 2015; Andreone et al., 2016). However, without an in-depth knowledge of specific environmental/habitat requirements for the species, finding, creating, restoring or protecting optimum habitat is difficult. This study was therefore designed to determine the environmental and microhabitat variables that influence the presence of golden mantellas in the wild. The results will help to identify areas where this species is most likely to persist and thrive.

2.3 Methods

2.3.1 Data Collection

Ten sites within the protected area of Mangabe-Ranomena-Sahasarotra, Moramanga District, eastern Madagascar, each containing or bordering known golden mantella breeding ponds, were targeted for surveys (Fig.1). Nine of these sites were surveyed between 28 November 2014 – 12 December 2014, and the tenth earlier on in the year in March 2014. These periods correspond to the main breeding activity periods for this species.

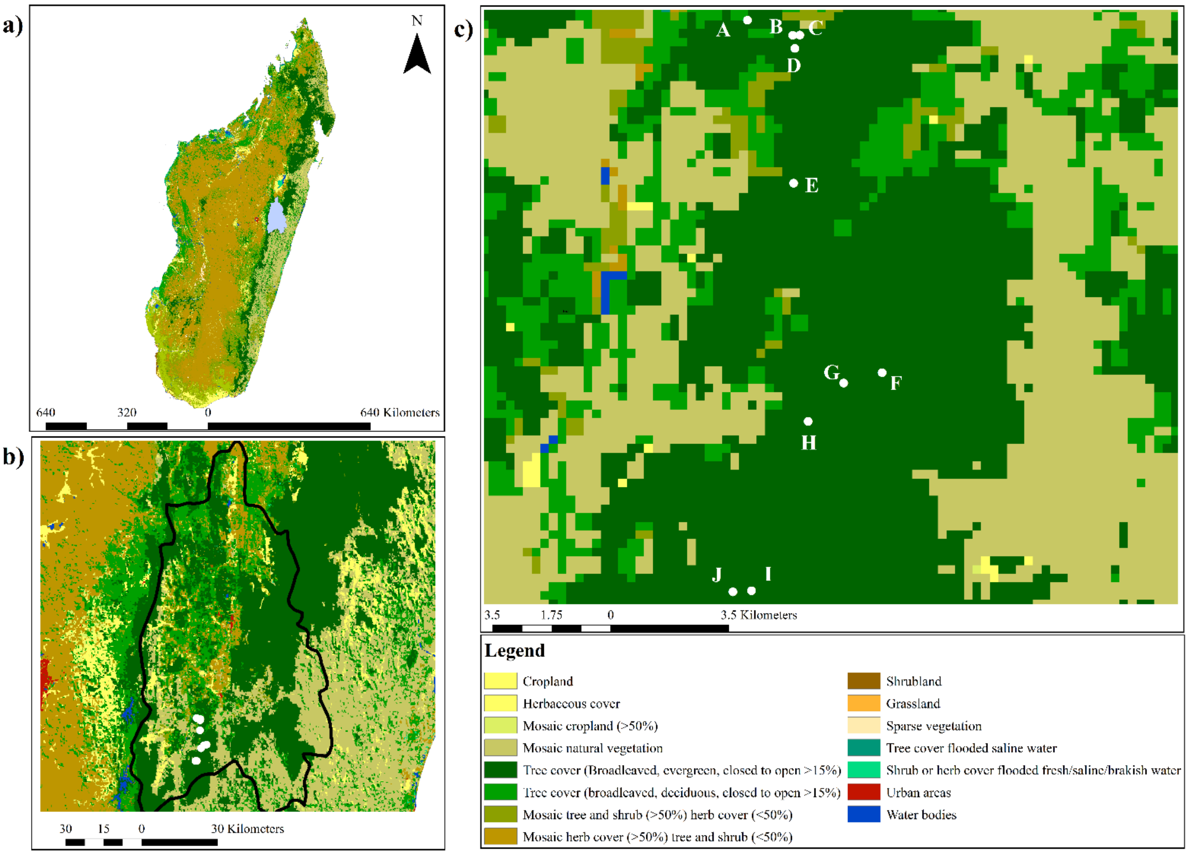

Figure 1: The distribution of ten forested sites surveyed in Madagascar. **(a)**: Madagascar with a highlighted area (grey) denoting the political district of the surveys. **(b)**: Highlights the position of forest surveys within the political district. **(c)**: Shows the distribution and distance between forest sites surveyed (Black dots), sites are labelled (**A**) Antanimbaritsara, (**B**) Ambinanin`I Lemafy, (**C**) Bekomy 2, (**D**) Bejofo, (**E**) Andriamarohangotra 2, (**F**) Andravinala, (**G**) Andavaioka 4, (**H**) Antoko, (**I**) Sasarotra 17, (**J**) Sasarotra 25. Background map data are derived from globcover (European Space Agency) and is at 300m resolution (i.e. each square is 300mx300m in map c).

All surveys took place between 0700-1400 hrs each day, one visit per site. The surveys were centered on breeding pools located in shallow valleys. A series of transects were established on the slope running down to each pool. The first transect was positioned at the valley bottom and ran parallel to the pool. Subsequent transects were positioned at 30 m intervals up the slope, each following the contour at that position, with the last transect positioned along the crest of the slope (Fig.2).

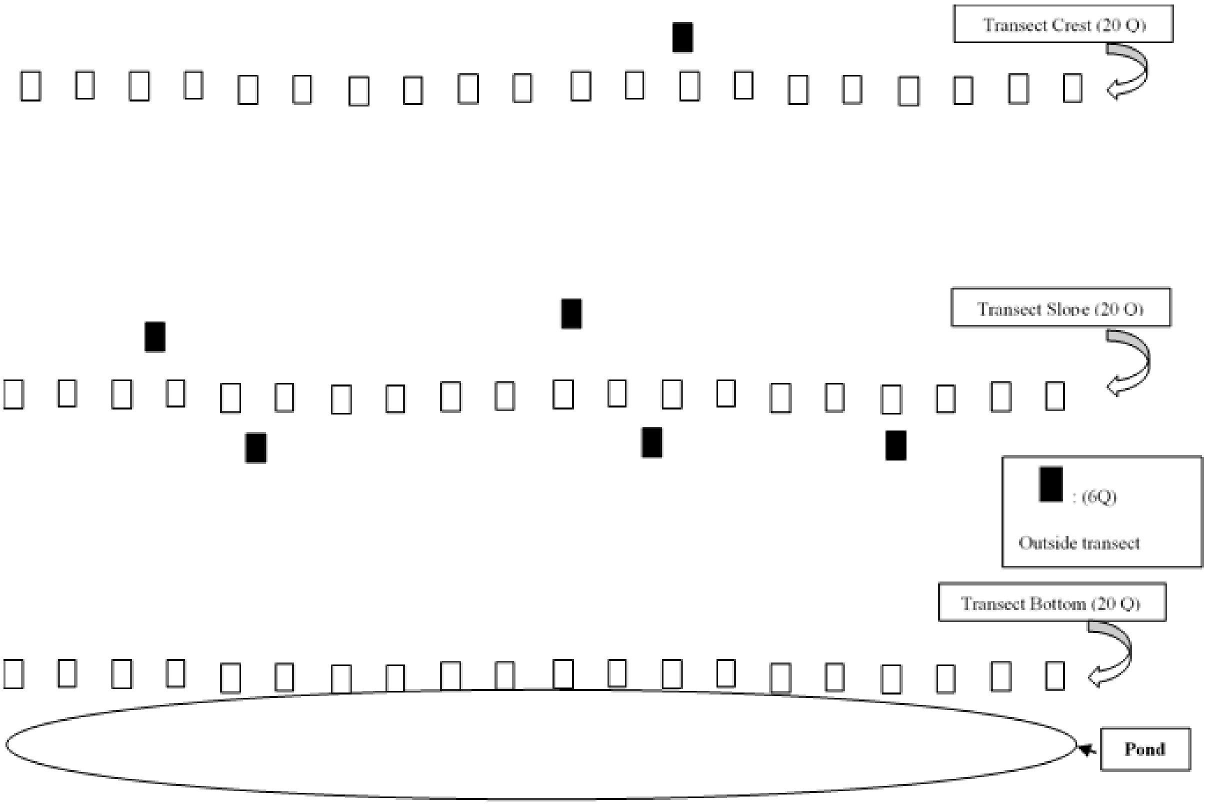

Figure 2: Diagrammatic representation of transect lines of twenty 1 x 1 m quadrats (White boxes) spaced at 30 m intervals running parallel to the breeding pond (White oval). Black boxes indicate where a golden mantella was seen outside of the transect/quadrat line and all environmental and microhabitat data within 1 m^2 of the individual were recorded. (Courtesy of Rakotondrasoa et al., 2015; unpublished report).

The number of transects and the number of associated quadrats surveyed depended on the length, width and topography of the slope accessible to the survey team, i.e. two sites contained five transects, seven sites had three transects and one site had two transects. Where the top of a slope was bordered by a pathway the crest transect was placed 3 m down slope from the pathway, two further transects were then surveyed, one either side of the path. This meant that the two sites with crest paths had five transects in total. Along each transect 1 m x 1 m quadrats were established at 4 m intervals and transects contained between 10–20 quadrats, sites with more transects therefore having more associated quadrats. A two-person research team moved along the transect line stopping, surveying and recording environmental variables (Table 1) and the number of golden mantellas counted in each quadrat.

Table 1**:** Variables, type and method of measurement used to collect data.

Variable	Method of collection
Surface temperature (°C)	Rolson^{TM} Infrared thermometer
Ultra-Violet B (UVI units)	Solarmeter 6.5^{TM} Ultra-Violet Index meter
Canopy cover (%)	Estimate
Herbaceous cover (%)	Estimate
Moss cover (%)	Estimate
Litter Cover (%)	Estimate
Litter depth (cm)	Tape measure
N^{o} dead trees	Count
N^{o} large trees (diameter < 1 m)	Count
N^{o} small trees (~ 1.5 m height)	Count
N^{o} trees cut	Count
N^{o} trees damaged by cyclone	Count
Canopy height (m)	Estimate
Number of tree roots	Count

Transect lines at the valley bottom were surveyed first, followed by next nearest transect as the slope was ascended. Golden mantellas observed outside the transects were also recorded and microhabitat variables measured within 1 m^2 of these locations.

2.3.2 Statistical analysis

Statistical analyses were carried out using the statistical software R (R Core Team, 2017). The quadrats from the ten forests were classified into presence or absence of golden mantella categories and then initially tested for significant differences in microhabitat variables using the Wilcoxon Rank Sum Test. A Generalized Linear Mixed Model (GLMM) was then developed using the number of quadrats occupied and unoccupied to determine which independent variables (Table 1) were most likely to influence the microhabitat preference of golden mantellas (Table 2).

We then followed Zuur et al. (2009) by removing the independent variable with the highest p value and re-running the GLMM. This procedure was repeated until only significant ($p \leq 0.05$) independent variables were left. Sites was held as a random factor in the models and we assumed a binomial error distribution with a logit link function.

2.4 Results

Our analyses showed that for all ten sites combined, two microhabitat variables differed between quadrats with and without mantellas: litter cover and number of tree roots (Wilcoxon tests all $P<0.001$). The GLMM also identified litter cover, number of tree roots and surface temperature as important predictors of golden mantellas (Table 2).

Table 2: Generalised Linear Mixed Model results showing potentially important predictor variables associated with golden mantellas (As canopy cover is alphabetically first in the list of variables it is labelled by R software as the Intercept and then used as a reference point). We provide the z-value ($z = (x - \bar{x})/ s$) and corresponding *p*-value for testing the null hypothesis that the slope and intercept is equal to 0 (Zuur et al., 2009).

Variable	Estimate	Std Error	z value	p (>|z|)
Intercept	-0.682	0.858	-0.795	0.426
Surface temperature	-0.085	0.037	-2.262	0.023
Litter cover	0.011	0.003	3.035	0.002
Litter depth	0.038	0.020	1.851	0.064
Tree roots	0.173	0.050	3.455	0.000

Within the sites, golden mantellas tended to occupy quadrats with at least 70% leaf litter coverage and low (mean = 1.73) numbers of tree roots rather than quadrats with no or very low numbers of tree roots (Table 2). However, across the sites, the number of golden mantellas declined in areas with very dense tree roots (Fig 2).

Although not important at the microhabitat selection level, at the time of the surveys the sites where golden mantellas were found tended to have surface temperatures of 20-23 ºC, UVI units of about 2.9, and about 30% canopy cover and 30% herbaceous cover (Table 3; Figs 2-3).

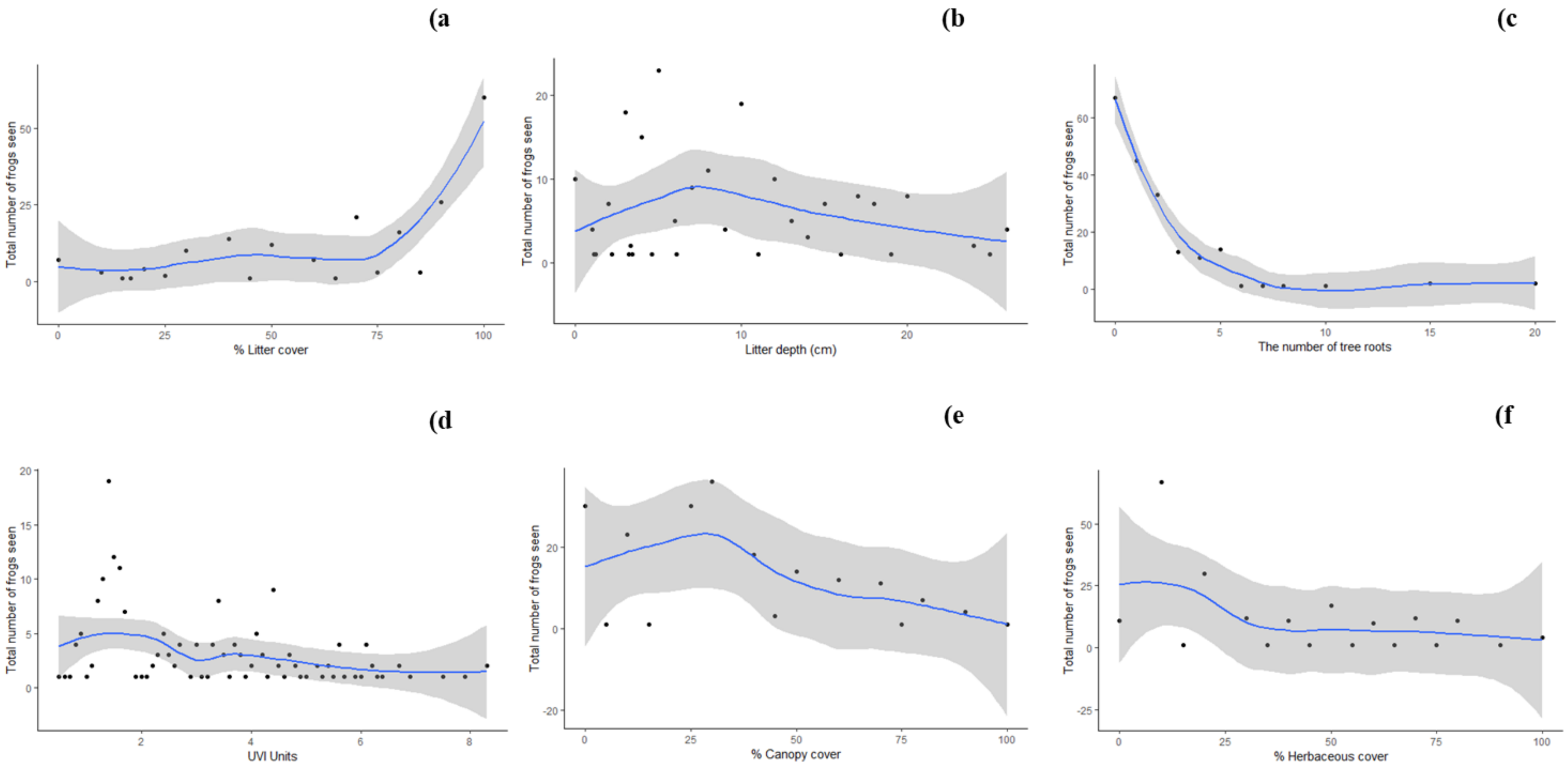

Figure 2: The total number of frogs observed combined for all ten sites versus (a) percentage litter cover, (b) litter depth in cm, (c) the number of tree roots, (d) UVB intensity (UVI units), (e) percentage canopy cover and (f) herbaceous cover. Each of the data points (black dots) represent the specific number of frogs recorded at each associated level of independent variable and are fitted with a LOESS smoother (blue line) to most closely model the relationship between independent variables and the total number of frogs seen. The shaded area represents a 95% confidence interval.

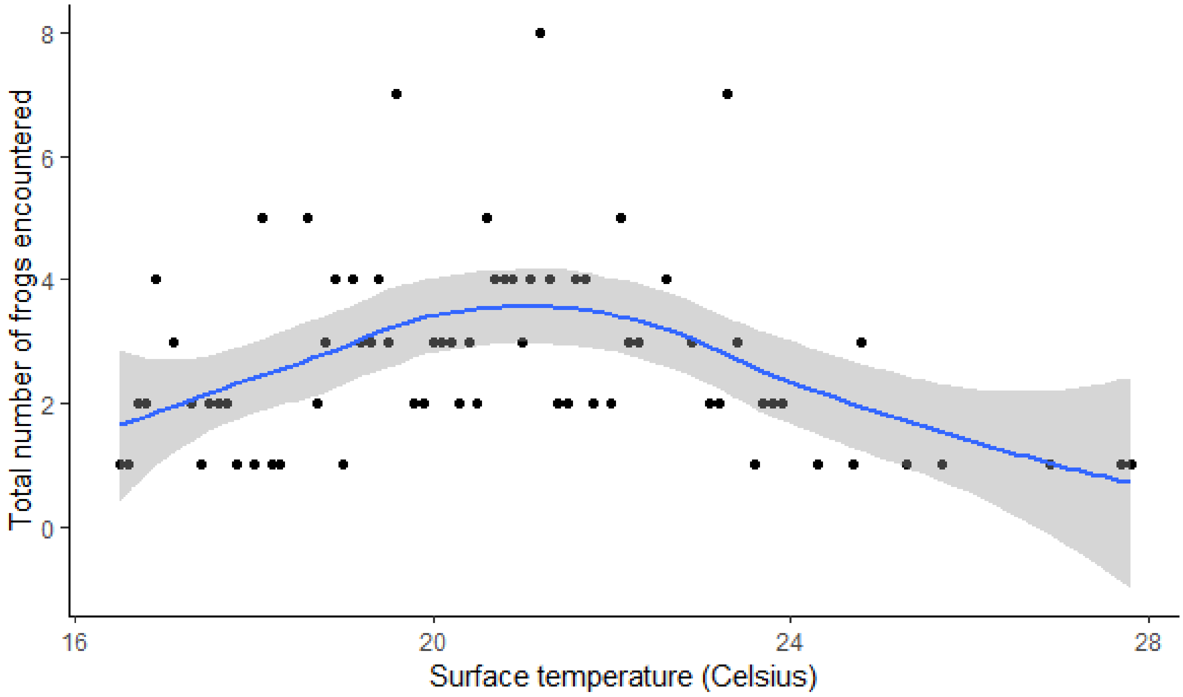

Figure 3: The total number of golden mantellas encountered combined for all ten sites and associated surface temperatures. Each of the data points (black dots) represent the specific number of frogs recorded at each temperature and are fitted with a LOESS smoother (blue line) to most closely model the relationship between surface temperature and the total number of frogs seen. The shaded area represents a 95% confidence interval.

Table 3: Percentage of quadrats surveyed with or without golden mantellas at each of the ten forested sites. The range and mean of the predictor variables associated with mantella presence are also shown (% Litter cover, Litter depth, Number of tree roots, Surface temperature, UVI units, % Canopy cover and % Herbaceous cover). The percentage of quadrats not containing mantellas with associated ranges and means for predictor variables are also shown for each site. The bottom two rows show the differences between predictor variable means for quadrats with or without golden mantellas at all ten sites combined.

Site	Golden mantella	% of Quadrats	% Litter cover		Litter depth (cm)		Number of tree roots		Surface temp (^{o}C)		UVB (units)		% Canopy cover		% Herb cover	
			Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Sassarotra 25	with	51	0 -100	65	0 - 24	10.4	0 - 8	1.6	18.1 - 27.7	22.3	3.0 – 8.3	5.0	0 - 100	30	0 - 100	32
	without	49	0 - 90	39	0 - 17	4.4	0 - 3	0.5	19.2 – 29.8	23.2	3.6 – 8.8	6.0	0 - 70	22	0 - 100	50
Sassarotra 17	with	47	30 - 100	74	2 - 26	13.0	0 - 20	4.7	19.5 – 27.8	22.4	1.2 – 3.6	1.6	0 - 60	29	0 - 80	42
	without	53	30 - 100	67	2 - 16	8.5	0 - 10	2.2	18.7 - 27.9	22.1	1.2 – 4.9	2.3	0 - 80	25	10 - 90	42
Antanimbaritsara	with	36	0 - 100	75	0 - 18	6.0	0 - 3	1.1	16.9 – 22.9	20.0	0.9 – 1.7	1.4	0 - 90	36	0 - 100	25
	without	64	0 - 100	72	0 - 12	5.0	0 - 4	1.2	15.6 – 24.3	19.2	0.3 – 1.6	1.2	0 - 90	33	0 - 100	29
Andriamarohangotra	with	31	0 - 100	39	0 - 5	2.2	0 - 2	0.5	18.6 – 20.3	19.3	1.5 – 6.1	4.3	0 - 60	15	0 - 80	32
	without	69	0 - 90	51	0 - 10	3.2	0 - 4	0.4	19.2 – 20.4	19.7	1.6 – 6.4	3.7	0 - 70	21	0 - 100	37
Andravinala	with	27	40 - 100	79	10 - 25	16.5	0 - 5	1.4	18.6 – 21.1	20.1	2.7 – 5.6	3.9	0 - 60	27	0 - 50	14
	without	73	10 - 100	76	5 - 30	14.0	0 - 4	0.8	17.7 – 21.6	19.8	2.1 – 5.7	3.1	0 - 80	25	0 - 80	18
Andavaioka 4	with	19	20 - 100	73	2 - 20	7.4	0 - 8	2.5	18.1 – 22.1	20.1	2.0 – 3.0	2.4	0 - 90	49	0 - 80	29
	without	81	10 - 100	65	1 - 30	9.1	0 - 7	1.9	15.4 – 25.8	20.2	1.6 – 3.2	2.3	0 - 100	27	0 - 100	30
Ambinanin’I Lemafy	with	18	40 - 100	92	3 - 18	6.7	0 - 5	1.7	16.5 – 26.9	19.0	0.5 – 4.5	1.4	0 - 80	24	10 - 80	33
	without	82	0 - 100	61	0 - 18	6.9	0 - 5	1.1	15.1 – 43.2	24.3	0.3 – 7.8	1.9	0 - 80	18	0 - 90	40
Bejofo	with	14	0 - 85	58	1 - 7	3.2	0 - 1	0.1	16.8 – 22.9	19.1	3.3 – 7.5	4.6	0 - 80	38	10 - 75	39
	without	86	0 - 95	52	0 - 12	3.9	0 - 4	0.2	14.3 – 27.7	19.1	0.8 – 9.6	3.9	0 - 90	39	0 - 90	32
Bekomy	with	11	40 - 80	81	2 - 5	3.9	0 - 6	1.7	17.3 – 22.2	19.2	0.8 – 1.3	1.1	40 - 80	57	10 - 10	10
	without	89	10 - 100	73	0 - 22	6.1	0 - 4	1.2	17.0 – 36.7	21.4	0.9 – 5.8	1.7	0 - 80	40	0 - 90	16
Antoko	with	9	90 - 100	98	5 - 12	9.0	0 - 5	2.0	19.8 – 22.6	21.2	2.4 – 3.7	3.2	0 - 90	17	10 - 80	38
	without	91	10 - 100	84	1 - 18	5.5	0 - 15	1.6	18.2 – 40.4	22.0	1.3 – 6.3	2.8	0 - 100	40	10 - 90	33
Mean of Sites	with	26		73.4		7.8		1.73		20.2		2.9		32.2		29.4
	without	74		64.0		6.7		0.9		21.1		2.9		29.0		32.7

2.5 Discussion

Although the relative number of occupied quadrats varied among sites, this may have been a result of environmental conditions on those survey days being particularly propitious for mantella activity, rather than reflecting real difference in abundances between sites. Nevertheless, our results show that at quadrat or transect level, the number of frogs encountered increases as percentage litter cover increases. Golden mantellas are a tropical forest floor species and are dependent on leaf litter to provide cover, create territories, forage, breed, and more easily regulate hydration state and body temperature. Like all frogs, golden mantellas can mitigate for the effects of evaporative water loss via the skin in drier or warmer conditions by morphological and/or behavioural means (Duellman and Trueb, 1994). Adult frogs take up water via absorption across the skin surfaces when in close contact with moist soils and substrates (Duellman and Trueb, 1994). Granular skin on the ventral surface then facilitates increased capillary action drawing water up from moist soils and provides increased skin surface areas for absorption. However, morphological adaptations such as cutaneous sculpturing or increased permeability and vacuolisation will only be advantageous in moist microhabitat (Hillyard et al., 1998). Therefore, the frogs must move between, or remain in, microhabitats where they are able to reduce the evaporation gradient of water from the body to the surrounding environment and rehydrate at a rate that offsets the amount of water lost. Blomquist and Hunter (2010) obtained similar results for wood frogs (*Rana sylvatica*), which were more likely to inhabit areas with greater humidity, substrate moisture, canopy cover, leaf litter depth and coverage. Seymour (1972) and Walvoord (2003) found that green toads (*Bufo debilis*) and cricket frogs (*Acris crepitans)* were more likely to select moist habitat when exposed to higher temperatures. Several other amphibian studies have obtained similar results and demonstrated that core temperatures, evaporative water loss and subsequent habitat selection were all highly influenced by ambient temperature and humidity (Tracy, 1975; Tracy, 1976; Pough et al., 1983; Semlitsch et al., 2009; Kohler et al., 2011; Tracy et al., 2013). It is now widely regarded that anuran activity is more limited by the effects of dehydration than by temperature, and as such hydroregulation is more important than thermoregulation (Seymour, 1972; Preest & Pough 1987; Tracy et al., 1993; Preest & Pough, 2003; Tracy et al., 2013).

Our results suggest that golden mantellas prefer sites with about 30% canopy cover, and there is a tendency for fewer frogs to be observed in areas with dense canopy cover and tree roots.

Golden mantellas are known to frequent sun-exposed areas within forest (Glaw and Vences, 2007) and the time of day or weather patterns may have an influence on mantella activity in these areas. Sunlight interception and irradiance at ground level depends to a certain extent on the height and positioning of the canopy (Dodd, 2010). The amount of cloud cover and orientation of the sun to the canopy gap can also be important in determining UVB and temperature levels at the forest floor (Pringle et al., 2003). Higher levels of UVB and herbaceous cover may be indicative of higher levels of disturbance or more extensive gaps in the canopy. Larger gaps in the canopy allow more solar radiation to penetrate further towards the forest floor which in turn increases soil and surface temperatures, lowers humidity, reduces leaf litter and food sources, these effects are amplified as canopy gap size increases (Carlson and Groot, 1997, Semlitsch et al., 2009).

It is plausible that as litter depth and the number of tree roots in a given quadrat increase, frog detectability becomes compromised. Greater coverage of herbaceous plants may also impede the ability of researchers to observe the frogs. According to an unpublished report by Rakotondrasoa et al. (2015), direct counts of golden mantella can be biased and challenging. An example is given where a count was carried out and around 400 mantellas were observed, yet further surveys were carried out and 2000 individuals were later captured in the same area. Indeed, it is generally acknowledged that at the population level count data for amphibians may be unreliable given imperfect detection, and where possible should be underpinned by capture mark recapture techniques, good quality habitat data and expert opinion (Schmidt, 2003; Sewell et al., 2010; Griffiths et al., 2015; Barata et al., 2017).

The rainy season begins in November in Madagascar, and this corresponds to the start of the breeding season for golden mantellas. The Bejofo site was surveyed in March, towards the end of the breeding season when frogs may have migrated back up the hill away from ephemeral breeding ponds. Indeed, all golden mantellas encountered in Bejofo were recorded in the hill-top quadrats. The other nine sites were surveyed in November and as such we would expect to observe more frogs in the valley bottom transects near to the breeding ponds. However, this was not the case, as more frogs were observed in the higher transects on the slope or crest of the hill. It may be that the frogs are migrating down to the breeding ponds and laying eggs in leaf litter, then migrating back up to warmer surface temperatures on the slope and crest. Lower average temperatures recorded in valley bottom transects may also mean fewer frogs are active outside of leaves and observed. The timing of the surveys was dictated by logistics and weather, but either way, there was no evidence that the difference in the timing of surveys between sites made any difference to observations of microhabitat use.

2.6 Conclusion

We recommend maintaining the integrity of current golden mantella forest habitat, increasing connectivity between breeding ponds and keeping disturbance of these areas to a minimum by increasing the levels of protection. Piludu et al. (2015) recommend an increase in effort or a new approach to safeguard breeding ponds, involving sampling and surveillance for detection of emerging pathogens, such as the chytrid fungus *Batrachochytridium dendrabatidis* (e.g. Bletz et al., 2015). The monitoring of local climate and the study of predicted climate change effects and further development of species distribution and population viability models to determine future relevant sites should continue (Piludu et al., 2015). Like Rakotondrasoa et al. (2015), we recommend continuing the search for new ponds and the continued monitoring of existing ponds, as well as continuation of research and estimations of population sizes using capture-mark-recapture techniques. Understanding the relationship between rare species and subsequent avoidance by animals of certain microhabitats within their range is vital if we are to plan future management strategies in important forest habitat (Semlitsch et al., 2009; Irwin et al., 2010; Pike et al., 2010). Information on such factors as day-time surface temperatures, canopy cover and litter cover can be used to inform the identification, creation and restoration of suitable habitats in the wild, as well as the requirements of the species in captivity.

Acknowledgements

We would especially like to thank Dr Gerardo Garcia and Chester Zoo for their ongoing support of the golden mantella conservation project. We are also extremely thankful to The Durrell Institute of Conservation and Ecology (DICE), University of Kent and Stiftung Artenschutz (the German Zoos Organisation) for providing funding for travel and equipment expenses associated with initial forest habitat surveys and research in Madagascar. Thanks also to anonymous referees and Tatyana Humle at DICE for manuscript review; Amy Plowman at the Whitley Wildlife Conservation Trust for her continued support; Katy Upton at the lower Vertebrates and Invertebrates Department at Paignton Zoo in Devon; Ben Baker of Chester Zoo for assisting with preliminary fieldwork; Devin Edmonds and the team at Mitsinjo, and all field and office staff at Madagasikara Voakajy in Madagascar.

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Chapter 3

Daily activity profiles of the golden mantella (*Mantella aurantiaca*) under different temperature regimes

Wayne M. Edwards[1], Richard A. Griffiths[1] and Michael J. Bungard[2]

[1] Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation,

Marlowe Building, University of Kent, Canterbury, CT2 7NR, UK

[2] Department of Environment and Geography, University of York, Heslington, York, YO10 5NG, UK

3.1 Abstract

The critically endangered golden mantella (*Mantella aurantiaca*) is an iconic, montane, endemic frog found in the Moramanga district, Madagascar. Ecological and behavioural data for this highly threatened species are sparse, and much of the future mitigation and habitat protection work will need to be based upon scientific evidence provided by both in situ and ex situ studies focused on habitat preferences and requirements. Rare species with cryptic lifestyles are almost impossible to study in the wild, especially if continuous behavioural data over prolonged periods are required. This study therefore utilized environmental information gathered in the field to design a system where these can be measured in captivity. Using climatically controlled chambers (the “Froggotrons”), we analysed the 24-hour activity budget of the golden mantella and how different temperatures impact on their daily activity profile. Golden mantellas showed a bimodal pattern of activity during the day with much less activity during the night. Frogs kept at warmer temperatures (20-25ºC) were more active than those kept under cooler conditions (16-19 ºC). However, the bimodal pattern was retained under the different temperatures, so there was no temperature-induced phase shift. Most activity was observed when humidity levels were above 85%. These findings can inform ongoing field surveys through determining the optimum times of day to either capture or count golden mantellas for further conservation actions.

Keywords: Madagascar; zoo research; activity budgets; habitat preference

3.2 Introduction

Circadian rhythms are driven by an internal biological clock providing species with a way of anticipating, adapting and optimising their behaviour to suit conditions brought about by daily fluctuations in light levels and associated temperatures (Jones et al., 2011; Pita et al., 2011). Such behavioural rhythms have been studied in a wide range of taxa including birds (Ollason and Slater, 1973: Pablos et al., 1995; Singh et al., 2015), mammals (Stephan and Zucker, 1972; Pickard et al., 1995; McMahon et al., 2014), reptiles (Gourley, 1972; Firth and Belan, 1998; Tawa et al., 2014), invertebrates (Campbell, 1976; Shimizu et al., 1997; Jones et al., 2011), fishes (Mueller, 1973; Sanchez-Vazquez et al., 1998; Tolozo-Villalobos et al., 2015), plants (Hoshizaki and Hamner, 1964; Paulsen and Bogorad, 1988; Hartzell et al., 2015) and amphibians (Demian and Taylor, 1977; Griffiths, 1985; Hasegawa and Cahill, 1998: De Carvalho et al., 2014). However, most behavioural rhythm studies have been conducted with mammalian or invertebrate species and are less well understood in amphibians (De Carvalho et al., 2014).

Evolutionary and adaptive reasons for the emergence of behavioural rhythms are wide ranging but are thought to stem from the interaction of several main processes including; predator - prey dynamics, avoidance of competition or for thermoregulatory benefit (Andrews et al., 2009; Donati et al., 2009). Physiological control of the diel cycle is via the hormone melatonin, which acts as a signal to facilitate the onset of an internal clock (Chiba et al., 2005; Trivedi and Kumar, 2014). Melatonin and circadian rhythms may be driven by the internal biological clock, but the intensity of activity in amphibians is also influenced by a number of other factors including temperature and humidity (Griffiths, 1983). There are two measurable aspects of activity rhythm e.g. phase – where the activity occurs in relation to the imposed cycle, and amplitude-how much activity there is during the activity phase. In ectotherms, changing the temperature can affect phase and/or amplitude. Mammals and birds have circadian rhythms that are synchronized by the light-dark cycle and are independent of temperature (Aschoff, 1981). In ectotherms this is not the case; the light-dark cycle may be the main synchronizer of the activity cycle, but the amplitude of activity may depend on temperature. Amphibians are intricately linked to their external environment due to their ectothermic physiology, permeable skins, reproductive cycles and life history traits (Williams et al., 2008; Hoffmann et al., 2012). This means they are driven to seek microhabitats where they are more likely to be able to conserve water and thermoregulate.

The critically endangered golden mantella (*Mantella aurantiaca*) is an iconic, montane, endemic frog found in the Moramanga district, Madagascar. This species is under threat from the effects of continued climate change, habitat destruction, collection for the pet trade and invasive species (Piludu et al., 2015). In-country conservation organisations are working with local communities and others involved in the removal of forests to mitigate the results of forest clearance. However, specific microhabitat preference data for this species are sparse and much of the future mitigation and habitat protection work should be based upon scientific evidence provided by both in situ and ex situ studies focussed on specific habitat requirements - something we know little about. Rare species with cryptic lifestyles are very difficult to study in the wild, especially so if we require continuous behavioural data over prolonged periods. This study therefore utilizes environmental information gathered in the field to design a system where behavioural data can be replicated and continuously measured in captivity.

Historically, activity budgets have been studied ex situ by researchers manipulating or simulating environmental conditions in laboratories and then using direct visual observations (Valdimarsson et al., 1997; Pepin et al., 2006; Dishman et al., 2009; Tan et al., 2013; Mohapatra et al., 2014; Watts et al., 2014), video recording (Murphy et al., 2011; Howerton and Mench 2014) or a combination of both techniques (Weller and Bennett, 2001; Polcak and Gvozdik, 2014). Activity can be studied by using a correlative approach with environmental conditions e.g. collection of data on microhabitat and environmental variables and relating this information to a measure of activity of the target species. However, we are unaware of any other in situ or ex situ study that has concentrated solely on habitat preferences and activity budgets of *M. aurantiaca* under different temperature regimes. Based on in situ and ex situ observations, we predict that *M. aurantiaca* would be most active during daylight hours, but that those activity levels would be related to temperature.

3.3 Methods

3.3.1 Design of enclosures

Research was conducted from 12th – 21st May 2015 at the amphibian biosecure facility at Paignton Zoo Environmental Park. Eight replicated enclosures (termed “Froggotrons”) were constructed on site using compressed plastic fibre boards, each measuring 1 m x 0.78 m x 1.2 m with a Perspex viewing/access window at either end. A 150 mm deep trough at the front of each tank was filled with water; small pebbles were placed at each end to allow the frog’s safe access and exit. Enclosure lids were covered with a fine mesh to allow light in and prevent escape by frogs or the invertebrates used as live food. Each Froggotron was fitted with a misting system operated via a timer set to spray for two minutes twice daily (08:30 hrs and 16:30 hrs). Tank floors were covered in coconut matting and split into a 2 x 2 matrix comprising four equal sections using thin string, the fifth section made up by the water trough area. Leaves were placed on the floor of each tank in piles covering an area equal to approximately 50% of the total floor area (Figure 1). Each tank was fitted with a small camera (420 TVL colour camera with infra-red night vision capability) connected to a digital video recorder set to record 24 hours per day.

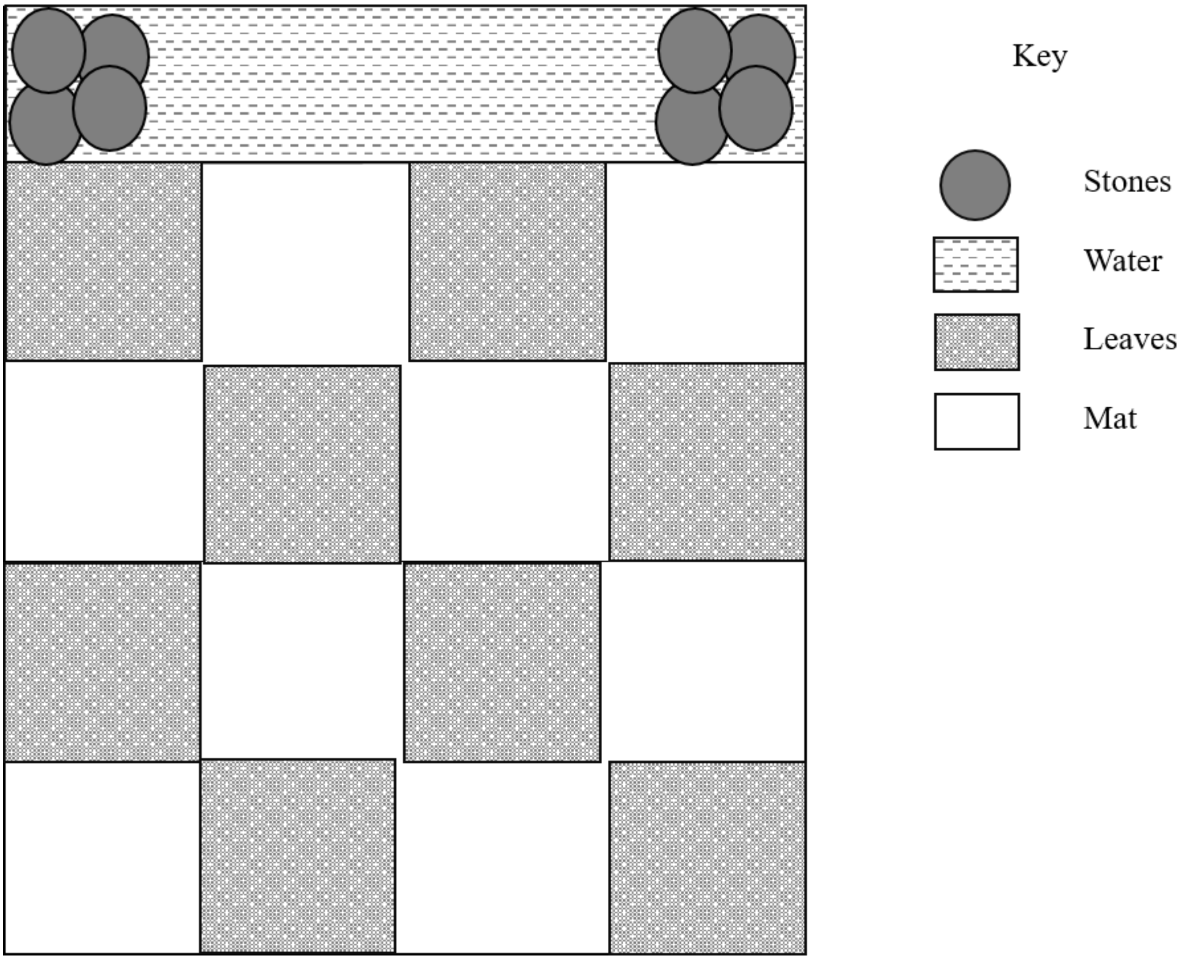


Figure 1: Design of the Froggotron. Leaves were set out to cover approximately 50% of the floor area as indicated by the shaded squares. The clear squares represent coconut matting areas on the tank floor without leaves.

3.3.2 Temperature and light regimes

Two rooms within the amphibian biosecure centre were used to house 4 tanks each; one room was kept at 16-19°C (Cooler Room) and the other at 20-25°C (Warmer Room). Temperatures in both rooms were maintained by air conditioning systems, the presence of lighting and other heating systems meant that the warmer room invariably increased in temperature during the day. Temperature in the cooler room dropped slightly during the afternoon. Our intention was to replicate in-situ wet season light levels for the duration of the study. Therefore, light levels increased in stages in the mornings via a timer starting with small (300 mm) UV-B T5's fluorescent tubes providing first light at 06:15 hrs, followed by larger fluorescent ceiling room lights activated around 08:30 hrs by keepers and finally full daylight bulbs (150 w metal halide, Eye Colour PAR36 ™) directly over each tank timer activated at 09:00 hrs. Full spectrum daylight bulbs were set to turn off at 16:00 hrs followed by ceiling room lights at 17:00 hrs, with the small fluorescent tube lights out and full darkness at 18:15 hrs. In 2014 light and temperature measurements were made at forest floor level at golden mantella sites in Madagascar. Full day time light levels ranged between 200 ~ 400 lux (light meter CEM DT-1300™) and temperatures were between 21 – 23 °C. Camouflage netting was fitted to the lids of each tank to simulate canopy cover, taking light levels down to those recorded in the forests. Frogs were fed every other day between 11:00am – 2:00pm with either fruit flies (*Drosophila melanogaster*) or hatchling crickets (*Gryllus bimaculatus*).

3.3.3 Behavioural monitoring

Eighty golden mantellas were split into two groups of 40, each group (now called Group 1 and Group 2) were again separated into sub-groups of 10 frogs (4 males, 6 females), and each of the sub-groups were placed in identical Froggotrons. Group 1 was allocated to the warmer room; Group 2 were allocated to the cooler room. Our priority was to ascertain the diel activity patterns of the frogs by recording behaviour continuously for 24 hours each day over a period of 10 consecutive days. We reviewed the recorded material via instantaneous scan sampling at 30 min intervals noting frog numbers, behaviour, area of the enclosure and the type of substrate used (leaves or coconut mat). We determined a frog to be active if it had emerged from hiding within leaves (Gunderson and Leal, 2016). Each enclosure was allocated a temperature and humidity data logger (EL-USB-2™) set to record every 30 minutes, and timing was synchronised with the video recording system. Research was carried out with approval from

The Wild Planet Trust's Animal Welfare and Ethics Committee and in compliance with "Guidelines for the use of animals in research," published in Animal Behavior, Vol 99, 2015.

3.3.4 Data analysis

Non-parametric tests were used as the number of mantellas active were shown to deviate significantly ($p \leq 0.05$) from a normal distribution. After the initial twenty-four-hour activity budget was analysed, data were then separated to represent day (06:30am – 18:00 hrs pm) and night (18:30pm – 06:00 hrs am) hours. As activity was minimal during the night, activity, temperature and humidity relationships during daylight were only analysed further. Daytime data recorded for activity, temperature and humidity across all four tanks in each room were averaged, warm and cool room means were then compared using a Wilcoxon Signed Rank Test. Further, the total number of frogs observed were combined across all 8 tanks at each 30-minute time interval point between 06:30am – 18:00 hrs over ten days and plotted against temperature and humidity levels (Fig. 3). Statistical analyses were carried out using Excel^{TM} and the R program ^{TM} (R Core Team, 2016). Data analysis followed the protocol developed by Zuur et al. (2009), a step by step guide for choosing and using General Additive Modelling (GAM) techniques. Therefore, we initially applied a simple linear model which was then modified to include variance structure e.g. room was added as a random variable (See: Appendix 1 for full GAM methodology). We then further developed a maximal model fitted with Maximum Likelihood (ML) and non-significant terms were removed stepwise (Zuur et al., 2009). We compared the fit of models using Akaike's Information Criterion (AIC), and then refitted and validated the final model with Restricted Maximum Likelihood (REML) (Zuur et al., 2009). Residuals from the final model were found to display heterogeneity (a non-random pattern) which meant there was a strong chance of there being a relationship between x and y variables (Zuur et al., 2009). GAM was therefore deemed appropriate because it allows for non-linear relationships between the response variable and multiple explanatory variables to be modelled (Zuur et al., 2009).

3.4 Results

Under both warm and cool conditions golden mantellas showed a bimodal pattern of activity during the day with little activity during night hours (Figure 2). The first peak in activity occurred around or approximately one hour after the larger 150w metal halide lamps were activated.

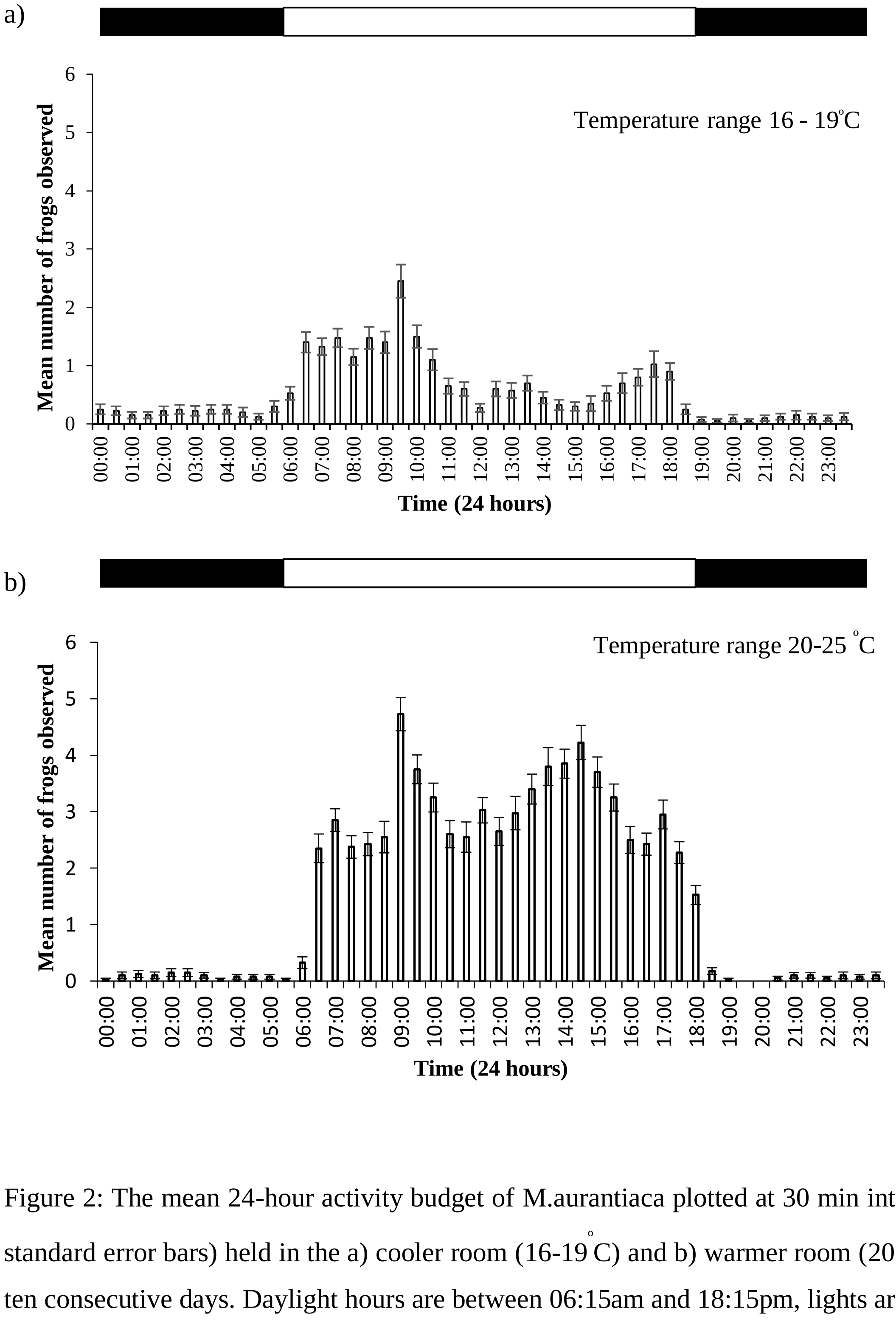


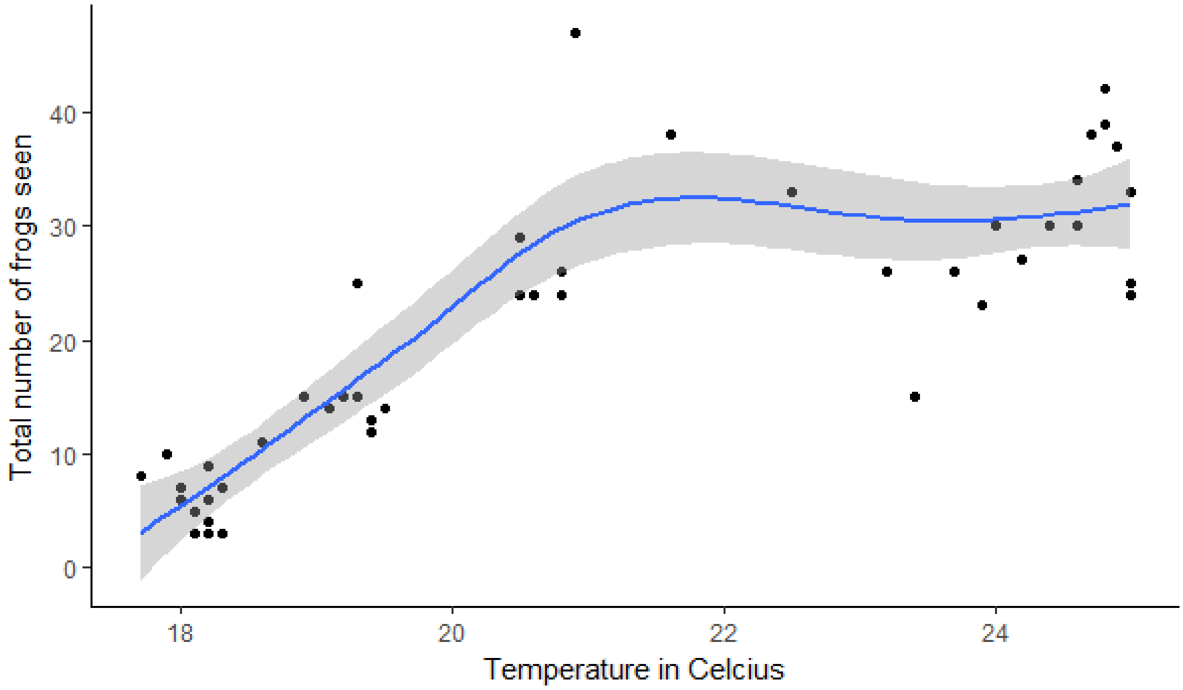
Figure 2: The mean 24-hour activity budget of M.aurantiaca plotted at 30 min intervals (with standard error bars) held in the a) cooler room (16-19 °C) and b) warmer room (20-25 °C) over ten consecutive days. Daylight hours are between 06:15am and 18:15pm, lights are turned out fully and the tanks are in darkness at all times before and after this period and are represented by light-dark bars above each plot.

Frog activity was significantly higher in the warmer room (Median= 2.90) than in the cooler room (Median = 0.75), T = 0, p ≤ 0.01, humidity was not significantly different in the warmer room (Median = 88.69) than in the cooler room (Median = 90.70), T = 99.5, p = 0.25. Activity levels peaked in the morning between 06.15 and 10.00 hrs in the cooler and warmer rooms, with a second peak in activity in the warmer room between 13.00 and 15.00 hrs. The total number of frogs active between the hours of 06.30 and 18.00 hrs increased with an increase in temperature with most activity occurring between temperatures 21˚C – 22˚C (Figure 3a). Activity was also at its greatest when humidity levels were around 85% (Figure 3b). However, even under warm conditions and during the activity peaks, less than half of the frogs were usually active in the open areas of the enclosures.

GAM results

We fitted the temperature data and humidity data with LOESS smoothers which strongly suggested both relationships were non-linear (Figure 3). From here we developed GAMs with smoothing terms on temperature, which was significant (df = 7.346, F = 33.81, p < 0.001), and humidity which was also shown to be significant (df = 3.945, F = 8.86, p < 0.001).

a)



b)

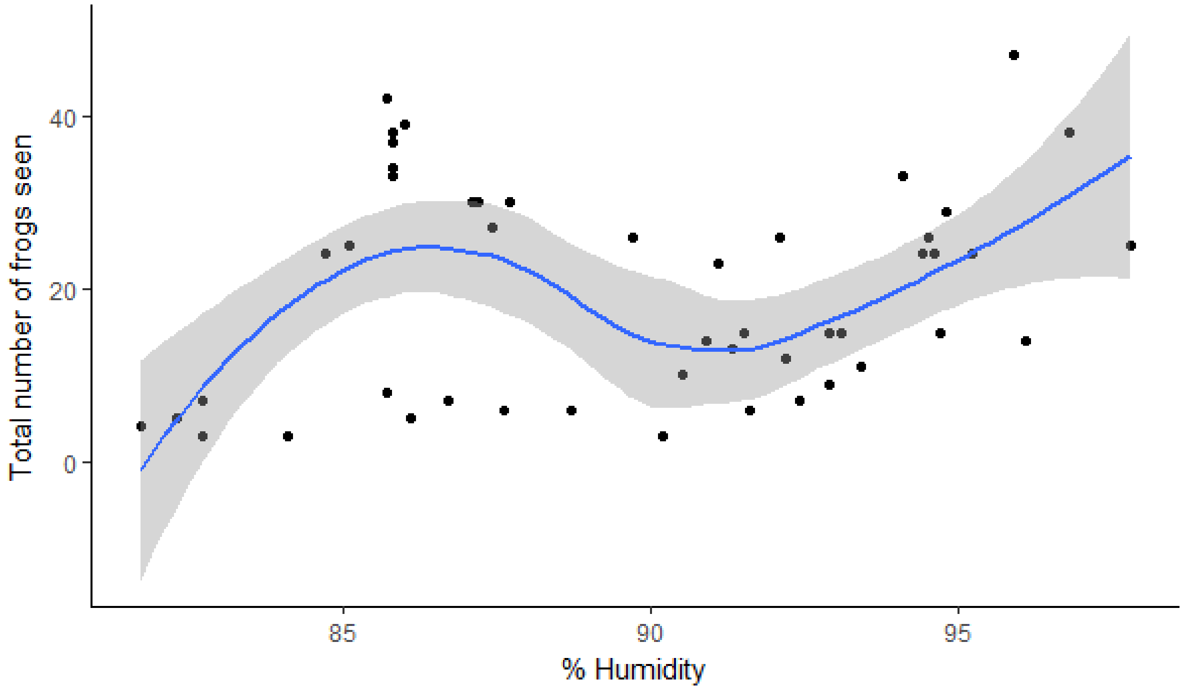


Figure 3: The total number of frogs seen combined across all tanks over ten consecutive days in relation to a) changing temperatures (cooler enclosures 16-19°C; warmer enclosures 20-25 °C) and b) % humidity over the same time period. Data points (black dots) are fitted with a LOESS smoother (blue line) to most closely model the relationship between temperature and the total number of frogs seen. The shaded area represents a 95% confidence interval.

3.5 Discussion

Continuous monitoring showed that golden mantellas were largely diurnal, which is entirely consistent with observations from the field (Glaw and Vences, 2007). The bimodal pattern of activity recorded under both warm and cool conditions suggest a behavioural rhythm that has evolved to avoid the warmest and driest part of the day in the forest.

There was a difference in activity levels at different temperatures, with frogs in the warmer enclosures (20-25 °C) being more active in the open areas outside the cover of the leaves than those in the cooler enclosures (16-19°C). Several studies involving ectotherms have shown that both the amplitude and phasing of activity can shift with extreme changes in temperature (Heckrotte, 1975; Griffiths, 1983; Ellis et al., 2009). However, temperatures experienced during this study were never high enough to induce such behaviour in the frogs. Therefore, there was no shift in activity phasing by the golden mantellas, and only a slight damping of the dusk peak in the cooler tanks.

The difference in intensity of activity between temperatures may be explained by general amphibian physiology and subsequent responses to temperature and humidity levels. Amphibian metabolic rates increase exponentially with an increase in temperature until their body temperature reaches its thermal optimum, above this point metabolic rate then falls until it reaches a critical thermal maximum (Duellman and Trueb, 1994). Although critical thermal minima and maxima for the golden mantella are unknown, the temperatures used here were based on those at which activity has been observed in the field so are probably well within their thermal limits.

Most activity was seen at temperatures of approximately 21 °C to 22 °C in the warm room, which may be indicative of the thermal optimum for this species and corresponding to surface body temperatures observed in the field. Temperatures cooler than 18°C result in lower activity levels even if humidity is above 80%; on the contrary, activity levels drop as temperature decreases. A recent study by Rija et al. (2014) on Kihansi spray toads (*Nectophrynoides asperginis*) obtained similar results after they compared activity levels at different times of day, temperatures and relative humidity. Further, a study by Kohler et al. (2011) concentrated on activity levels and optimal body temperatures for common frogs (*Rana temporaria*) and found that jump lengths peaked at an optimal temperature and shortened with a decrease in temperature. Several other studies focussing on amphibians have also recorded temperature-dependent activity levels and behaviour (Putnam and Bennet, 1981; Samajova and Gvozdik, 2009; Sanabria et al., 2013).

Our results suggest the optimum time to encounter golden mantellas is between first light and approximately 10.00 hrs with another peak in activity under warmer temperatures (20 °C – 25°C) between 13.00 and 15.00 hrs. This information will be useful to in situ conservation managers for determining the best time of day to survey frog numbers or catch individuals in order to translocate them to other areas. However, the results also showed that even under optimum conditions, usually less than half of the frogs present in the enclosures are active at any one time outside the leaf litter refuges. Low levels of detectability in the field remains a challenge for cryptic species such as amphibians (Schmidt, 2003; Sewell et al., 2010; Barata et al., 2017). Ex situ studies in which the actual number of frogs present in an enclosure is known may therefore inform the design and analyses of such field surveys. For example, the study showed that there was never more than 50% of the frogs visible at any one time i.e. results could be used to estimate the ratio of frogs detected to the number of frogs undetected under different temperature and humidity scenarios in the field.

Our findings are also useful in informing best working practices and guidelines for keeping golden mantellas in captivity. Future research should focus on habitat preferences under climate change scenarios. Then, once the climatic envelope and habitat requirements of the species are known, further surveys and habitat assessments should be carried out to identify areas for habitat creation and management for the species, as well as the potential for assisted colonisation.

Acknowledgements

Authors would like to thank; Paignton Zoo Environmental Park for providing the room and frogs in their bio-secure amphibian centre. ATASS for funding the initial set-up and first year stipend costs. The Darwin Initiative and DICE at the University of Kent for funding return trips and in-country costs to Madagascar to collect environmental data later used to set up the Froggotrons. The German Zoos Organisation (Stiftung Artenschutz) for funding equipment used in Madagascar for field surveys. The staff at Association Mitsinjo and Madagasikara Voakajy in Madagascar for their help and expertise in the field. Paignton Zoo carpenter Don Neilson for help with design and build of the Froggotrons. Tanya Humle at the University of Kent for manuscript review. Thanks also to all at the Lower Vertebrate and Invertebrate Department at Paignton Zoo for overseeing the day to day welfare and upkeep of the frogs.

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Chapter 4

The effects of temperature and humidity on microhabitat use by golden mantella (*Mantella aurantiaca*) frogs at Paignton Zoo

Wayne M Edwards and Richard A Griffiths

[1] Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation,
Marlowe Building, University of Kent, Canterbury, CT2 7NR, UK

4.1 Abstract

Climate change impacts biological systems worldwide, none more so than in montane tropical forest and on rare amphibian species living in these habitats. The critically endangered golden mantella frog from Madagascar is facing an uncertain future as temperature rise changes its relationship with the surrounding habitat. Our study was carried out in replicated environmentally controlled enclosures (Froggotrons) based at Paignton Zoo Environmental Park in Devon, UK. Results show that at 21.5˚C more golden mantellas were active, spend the most time active and were most likely to move away from a leaf litter substrate. At 20-23 ˚C, individual mantellas spend periods of approximately 7.5 minutes continuously active in open areas before retreating to a hiding place. Temperatures either side of this optimum saw a decrease in the length of activity bouts, and an increase in time spent on leaves e.g. activity bout length decreased by around 30% to about 5 minutes at temperatures 3-5 ˚C lower or higher than 20-23 ˚C. Continued temperature rises associated with climate change may drive golden mantella populations to shift distribution to higher cooler altitudes. The problem is that this may not be possible in areas where the frogs are confined to small isolated forest patches that are already towards the highest altitude available.

Keywords: Zoo research, Madagascar, amphibian conservation, climate change

4.2 Introduction

Climate change continues to threaten and degrade complex biological systems worldwide and can profoundly change the relationship between a species and its environment (Blaustein et al., 2010). Understanding the implications of rising temperatures on species activity patterns and microhabitat use is needed if we are to mitigate negative effects and plan future conservation measures (Gunderson and Leal, 2015). The amount of time an animal is active impacts upon the time it can apportion to different behaviours such as interspecific competition, hunting, feeding, and reproduction (Gunderson and Leal, 2015). A reduction in time spent reproducing or foraging will lead to a decrease in offspring, energy acquisition, growth rate and fitness (Angilletta, 2001). Thus, when environmental change impacts negatively on species activity patterns, the vulnerability to extinction increases (Sinervo et al., 2011; Logan et al., 2015). Several studies have recorded reduced fitness due to temperature rises affecting activity budgets across a range of ectothermic taxa and species including invertebrates (Corbet et al., 1993; Buckley and Kingsolver, 2012; Kingsolver et al., 2013), reptiles (Grant and Dunham, 1988; Sinervo et al., 2011; Logan et al., 2015) and amphibians (Carouso et al., 2014; Frishkoff et al., 2015). For example, a recent study by Logan et al. (2015) revealed activity in the tropical lizard *Anolis lemurinus* is severely restricted as temperature rises past their thermal optimum, which reduced time available for feeding or reproductive behaviours. Sinervo et al. (2010) suggest that 39% of local lizard populations and 20% of lizard species may be in danger of extinction by 2080 due to a reduction in activity levels associated with climate change. Carouso et al. (2014) documented substantial and rapid changes in body size for plethodontid salamanders due to climate change and acknowledged that where individuals have reduced activity levels and capacity to forage, restrictions on growth occur.

Climate change may be particularly damaging to tropical ectothermic species (Logan et al., 2015). This is partly because many tropical species have become thermally specialised by adaptation to narrow environmental temperature margins (Kingsolver et al., 2013; Logan et al., 2015). Montane tropical forest species face even more of a challenge given that many are considered specialists and highly restricted to specific altitudes (Hannah et al., 2002; Raxworthy et al., 2008). Amphibians are among the most threatened of all montane species by rising temperatures due to their ectothermic physiology, permeable skins, and in many cases, poor dispersal ability (Parmesan, 2006; Raxworthy et al., 2008).

The golden mantella (*Mantella aurantiaca*) is a critically endangered frog from the central eastern rainforests of Madagascar (Vences and Raxworthy, 2008). It lives on forested slopes at altitudes ranging from around 900 – 1000 m above sea level and is restricted to the Moramanga region where the area of occupancy for this species is low at approximately 10 km^2 (Vences and Raxworthy, 2008). Threats to golden mantella habitat include logging and/or slash and burn to make way for subsistence agriculture; artisanal or industrial mining destroying breeding ponds; collection for the pet trade and climate change (Piludu et al., 2015). Temperatures are predicted to rise in Madagascar to between 1.1-2.6 °C by 2050, rainfall across the country is also predicted to increase, apart from along the south-east coast which will become drier in austral summer months (Hannah et el., 2008; Tadross et al., 2008). The implications are that forest floor microhabitats used by the frogs will change. Equally, if temperatures rise beyond the optimum preferred by golden mantellas this may also impact behaviour and, ultimately, fitness i.e. as time spent inactive in refuges increases, time spent on reproduction and hunting decreases. Increasing the protection levels of current areas of habitat will not protect species from climate change. However, the first step towards mitigating the negative effects of climate change on golden mantellas may be to look at exactly how it is they use their habitat, which type of substrate or refuges they prefer and if a rise in temperatures affects the length of activity bouts.

Golden mantellas are ground-dwelling frogs associated with leaf litter (Glaw and Vences, 2007), and like most frogs, need to seek moist shelter in order to cool down and/or re-hydrate (Duellman and Trueb, 1994). As leaf litter is an important component of golden mantella microhabitats (chapter 2), we hypothesised that a sustained change in temperature away from thermal optima would affect the length of activity bouts and time spent hiding in leaves. This type of research is very difficult to carry out in-situ, as following a small frog continuously through forest habitat would be almost impossible. Also, in-situ it would be difficult to estimate the proportion of the population that was unobservable due to non-detection, but this can be done ex-situ in the Froggotrons using known numbers of frogs.

Research was carried out in two parts: in Experiment 1, our intention was to determine how important leaves were as a microhabitat under controlled environmental conditions. In Experiment 2, we explored how temperature influences the length of activity bouts and the time spent hiding in leaves.

4.3 Methods

In Experiment 1 the term "on leaves" was used to refer to any frog, hiding or visible, recorded as being in the area of the tank containing leaf litter. In Experiment 2 the term "in the open" was used for any frog timed as active (visible) in any area of the tank with or without leaves having emerged from hiding.

In Experiment 1, the leaf substrate pattern was designed specifically to test how important leaf cover was to golden mantellas i.e. frogs were given a direct 50/50 choice between staying in leaves or not (Fig 1a). To prevent directional bias tank 1 had all leaves covering the right half of the floor area, tank 2 the left half, tank 3 the back half and tank 4 the front half of the floor area, floor layout was repeated in both warm and cool rooms. In Experiment 2, leaf substrate was set so that areas with and without leaves were interspersed, this was used to better represent the patchy distribution of leaf litter on the forest floor (Fig 1b).

Research was conducted from 10th – 19th Oct 2014 (Experiment 1) and 19th – 23rd Nov 2016 (Experiment 2) at the Amphibian Biosecure Facility at Paignton Zoo Environmental Park in eight custom made climatically controlled environmental units (Froggotrons), each measuring 1m x 780 mm x 1.2 m. A 100 mm wide by 150 mm deep trough at the front of each tank was filled with water. Tanks were covered with a fine mesh to allow light in and prevent escape by frogs, or invertebrates used as live food. Each Froggotron was fitted with a misting system operated via a timer set to spray for two minutes twice daily (0830 hrs and 1630 hrs). Tank floors were covered in coconut matting. In Experiment 1, tank floors were split into two equal sections using thin string, red oak (*Quercus rubra*) leaves were placed on the floor of each tank covering 50% of the total floor area (Figure 1a). In Experiment 2 leaves were placed on the floor of each tank in piles covering an area equal to approximately 50% of the total floor area and set in a checkerboard effect (Figure 1b). Each tank was fitted with a small camera (420 TVL colour camera with infra-red night vision capability) connected to a digital video recorder set to record 24 hours per day.

4.3.1 Behavioural monitoring

Eighty golden mantellas were split into two groups of 40, each group (now called Group 1 and Group 2) were again separated into sub-groups of 10 frogs (4 males, 6 females), and each of the sub-groups were placed in identical Froggotrons. Group 1 was allocated to the Warmer Room (20-25°C); Group 2 was allocated to the Cooler Room (16-19°C).

Our priority in Experiment 1 was to observe substrate preference patterns of the frogs by recording behaviour continuously for each daylight period (06:30-18:00) for 10 consecutive days. We reviewed the recorded material via instantaneous scan sampling at 30 min intervals noting frog numbers in or out of leaves. A frog was categorised as on leaves if at least half of the frog's body was in contact with leaves.

In Experiment 2 our priority was to ascertain the length of activity bouts when frogs were active anywhere in a tank and not hiding under leaves. This was done by recording behaviour continuously for 12 hours for each daylight period over 5 consecutive days. We reviewed the recordings in hourly blocks for each tank in both rooms. As each hour was viewed, the footage would be paused at the first sighting of a frog breaking cover, and the frog would be monitored until it returned to cover. Frogs were categorised as free from leaf cover as soon as they had their full body out in the open and were categorised as back in cover as soon as any part of the head was back under leaves. The difference in time points between free from leaf cover and back in cover gave the total amount of time each frog spent active and not hiding in leaves.

Each tank was allocated a temperature and humidity data logger (EL-USB-2^{TM}) set to record at 30-minute intervals, and timing was synchronised with the video recording system. Research was carried out with approval from The Wild Planet Trust's Animal Welfare and Ethics Committee and in compliance with "Guidelines for the use of animals in research," published in Animal Behavior, Vol 99, 2015.

4.3.2 Data analysis

Statistical analyses were carried out using the R program ^{TM} (R Core team, 2018). Where the number of frogs on leaves, time spent in the open, temperature or humidity data were shown to deviate significantly ($p \leq 0.05$) from a normal distribution non-parametric tests were used. The numbers of frogs on leaves (Experiment 1) were averaged for each room and then compared using a Wilcoxon matched pairs test. Generalised Linear Models were developed to determine which independent variables influenced a) preference for leaves or non-leaves, and b) the time golden mantellas spent in the open.

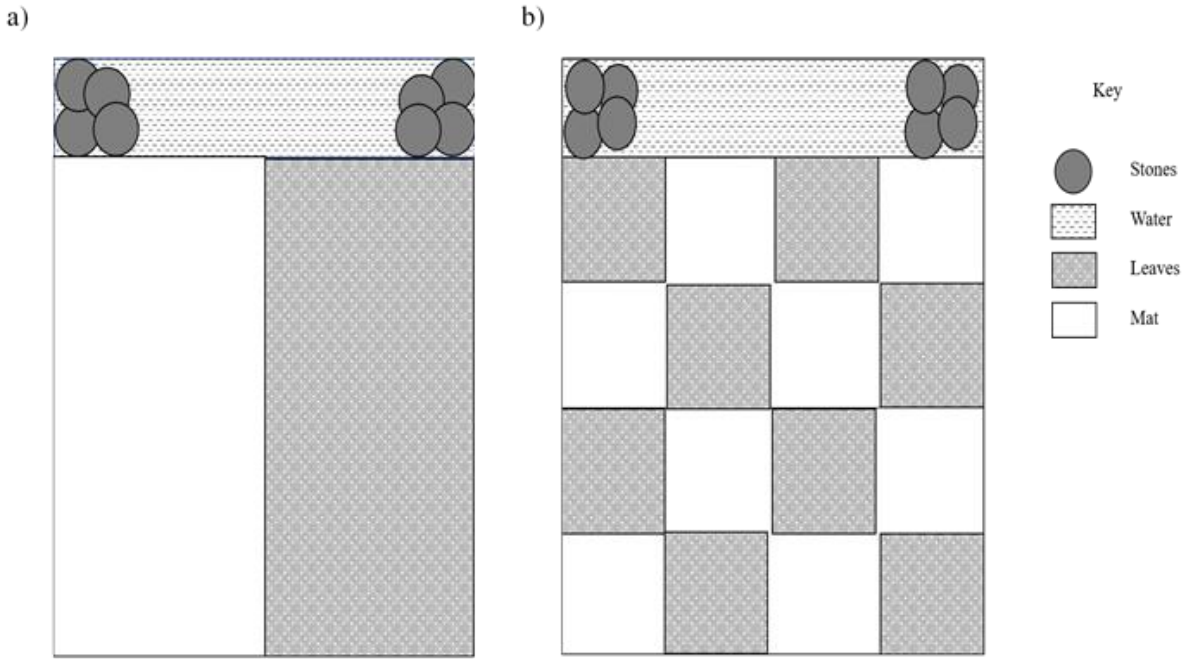


Figure 1(a) Represents the floor area set up within each Froggotron in Experiment 1, leaves were set out to cover approximately 50% of the floor area. (b) Represents the floor area set up within each Froggotron in Experiment 2, leaves were again set to cover approximately 50% of the floor area, this time using a checkerboard configuration. The clear squares represent areas of the tank floor without leaves.

4.3.3 Room set up and light level regime

Temperatures in both rooms were maintained by air conditioning systems, the presence of lighting and other heating systems meant that the Warmer Room invariably increased in temperature during the day. Our intention was to replicate in-situ wet season light levels for the duration of the study. Therefore, light levels increased in stages in the mornings via a timer starting with small (300 mm) UV-B T5's fluorescent tubes providing first light at 0615 hrs, followed by larger fluorescent ceiling room lights activated around 0830hrs by keepers and finally full daylight bulbs (150w metal halide, Eye Colour PAR36 ^{TM}) directly over each tank timer activated at 0900 hrs. Full spectrum daylight bulbs were set to turn off at 1600 hrs followed by ceiling room lights at 1700 hrs, with the small fluorescent tube lights out and full darkness at 1815 hrs. Camouflage netting was fitted to the lids of each tank taking light levels down to those recorded (light meter CEM DT-1300^{TM}) in the forests surrounding golden mantella breeding ponds in Madagascar (See: Chapter 2). Full day time light levels ranged between 200 ~ 400 lux. Frogs were fed every other day between 1100-1400 hrs with either fruit flies (*Drosophila melanogaster*) or hatchling crickets (*Gryllus bimaculatus*) dusted with Nutrobal^{TM} nutritional supplement.

4.4 Results

Experiment 1: On leaves or not on leaves

The number of frogs that preferred to stay in the half of the enclosure with leaves was significantly higher in the cooler room over ten days T = 0, $p \leq 0.01$. (Fig. 1). Temperature and humidity levels are highly significant in determining whether golden mantellas venture into areas without leaves (Table 1). The number of frogs observed on leaves falls as temperature increases to 21°C and as humidity levels rise; the number of frogs seen on leaves then rises again as temperatures increase (Fig. 2). Almost all the frogs in the cooler room spent the majority of their time on leaves.

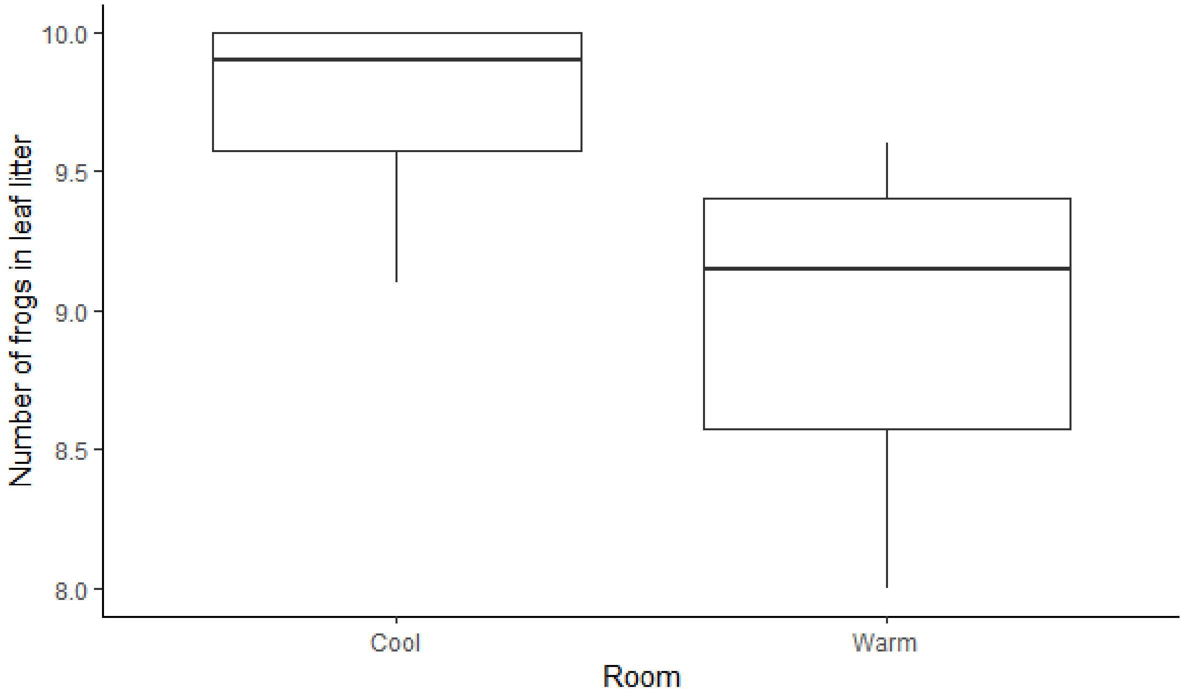


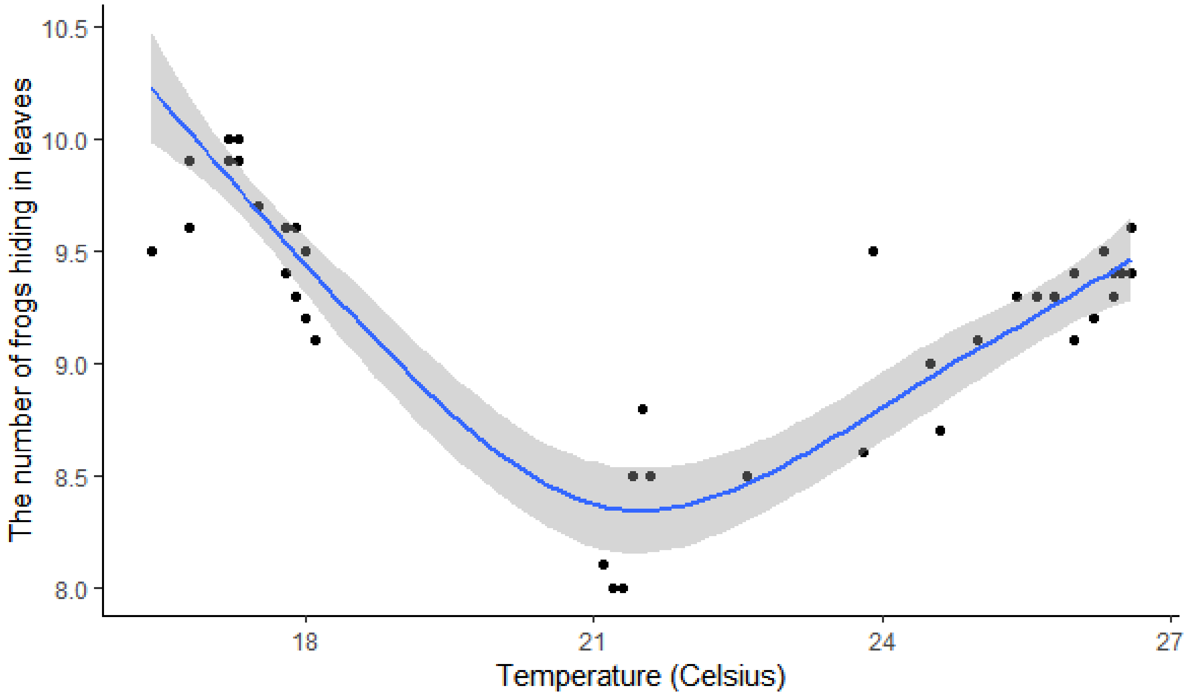
Figure 2: Medians, interquartile ranges and total ranges for the number of frogs observed on leaves in the cooler (16-19°C) versus warmer (20-25°C) rooms.

Table 1: GLM results showing temperature and humidity are highly significant in predicting golden mantella presence in areas of the tanks with leaves and that responses to temperature depend on humidity.

	Estimate	Std. Error	t value	Pr(>|z|)
Temperature	0.619922	0.280421	2.211	0.032
Humidity	0.068392	0.061266	1.116	0.270
Interaction	-0.007189	0.003098	-2.321	0.025

Null deviance: 14.6925 on 47 degrees of freedom
Residual deviance: 4.0543 on 44 degrees of freedom

a)



b)

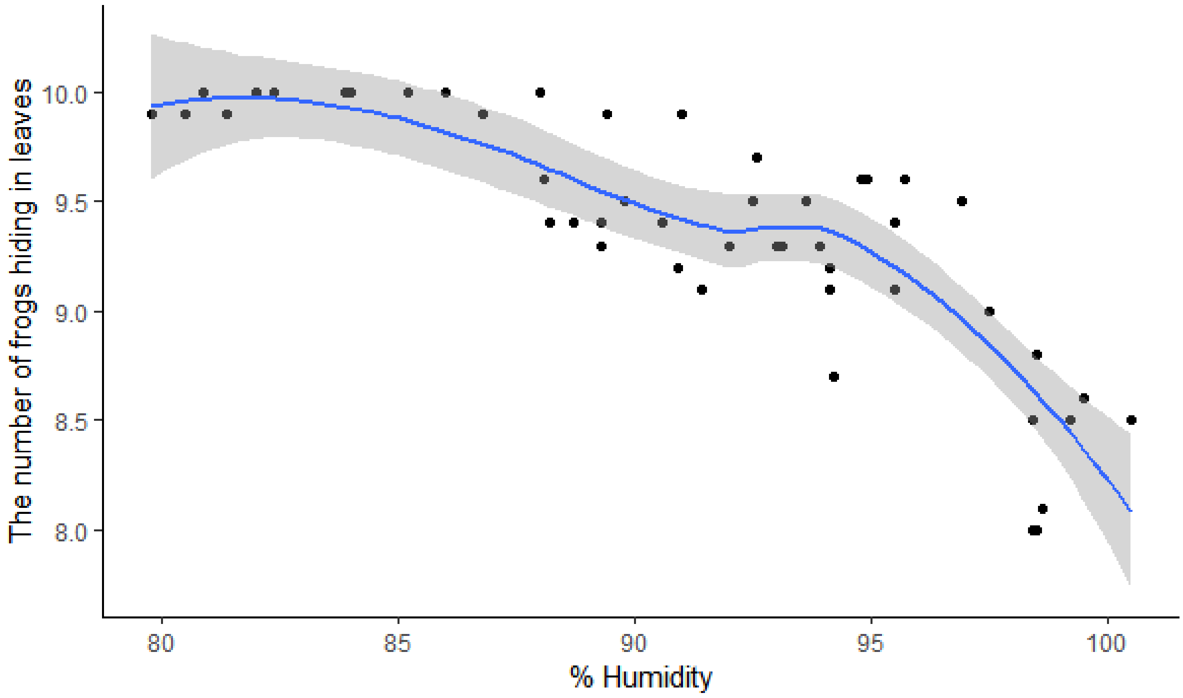


Figure 3: Mean number of frogs seen on leaves averaged across 8 Froggotrons (warm and cool rooms combined) over ten consecutive days relative to a) changing temperature and b) % humidity over the same time period. Data points (black dots) are fitted with a loess smoother (blue line) to most closely model the relationship between temperature and the total number of frogs seen. The shaded area represents a 95% confidence interval.

Experiment 2: **The amount of time spent in the open and not hiding under leaves**

Temperature and humidity levels are highly significant in influencing the amount of time golden mantellas spend active (Table 2**).** The frogs spent longer in the open at the higher temperatures (Fig. 3). Humidity peaks at around 21˚C (Fig. 4), the amount of time spent in the open also peaks at 21˚C (Fig. 5). A combination of temperature at 21˚C and humidity levels over 90% therefore results in frogs spending more time in the open areas, and less time hiding and being inactive (Fig. 5). Golden mantellas spent significantly more time in the open and not hiding under leaves in the warmer room ($W = 1$, $p \leq 0.01$). The length of activity bouts is approximately 7.5 minutes in the warm room, and around 30% shorter in the cooler room (Fig 3).

Table:2: GLM results showing temperature and humidity are highly significant in predicting the length of activity bouts (i.e. number of seconds golden mantella are active outside leaf litter) and that responses to temperature depend on humidity.

	Estimate	Std. Error	Z value	Pr(>|z|)
Temperature	-1.225863	0.151427	-8.095	< 0.001
Humidity	-0.216967	0.033520	-6.473	< 0.001
Interaction	0.013868	0.001646	8.424	< 0.001

Null deviance: 1327.39 on 23 degrees of freedom
Residual deviance: 402.43 on 20 degrees of freedom

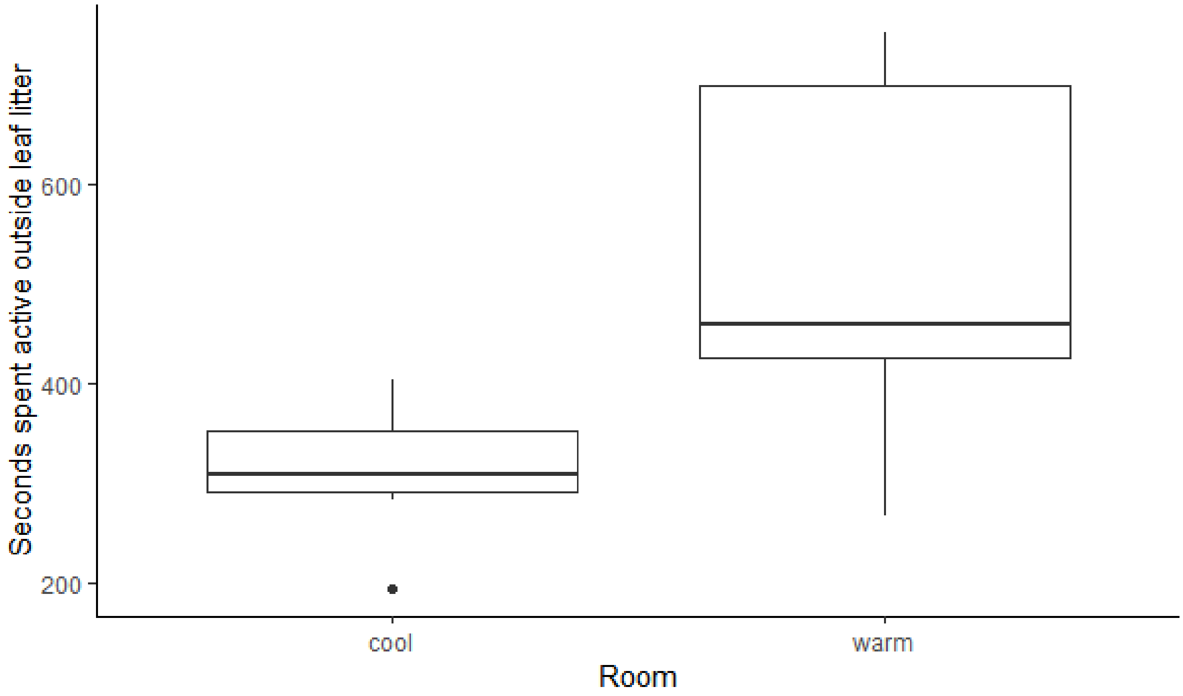


Figure 4: Medians, interquartile ranges and total ranges for the length of activity bouts i.e. number of seconds spent active during a continuous activity bout in the open and not hiding in leaf litter in the cool (16-19°C) or warm (20-25°C) rooms.

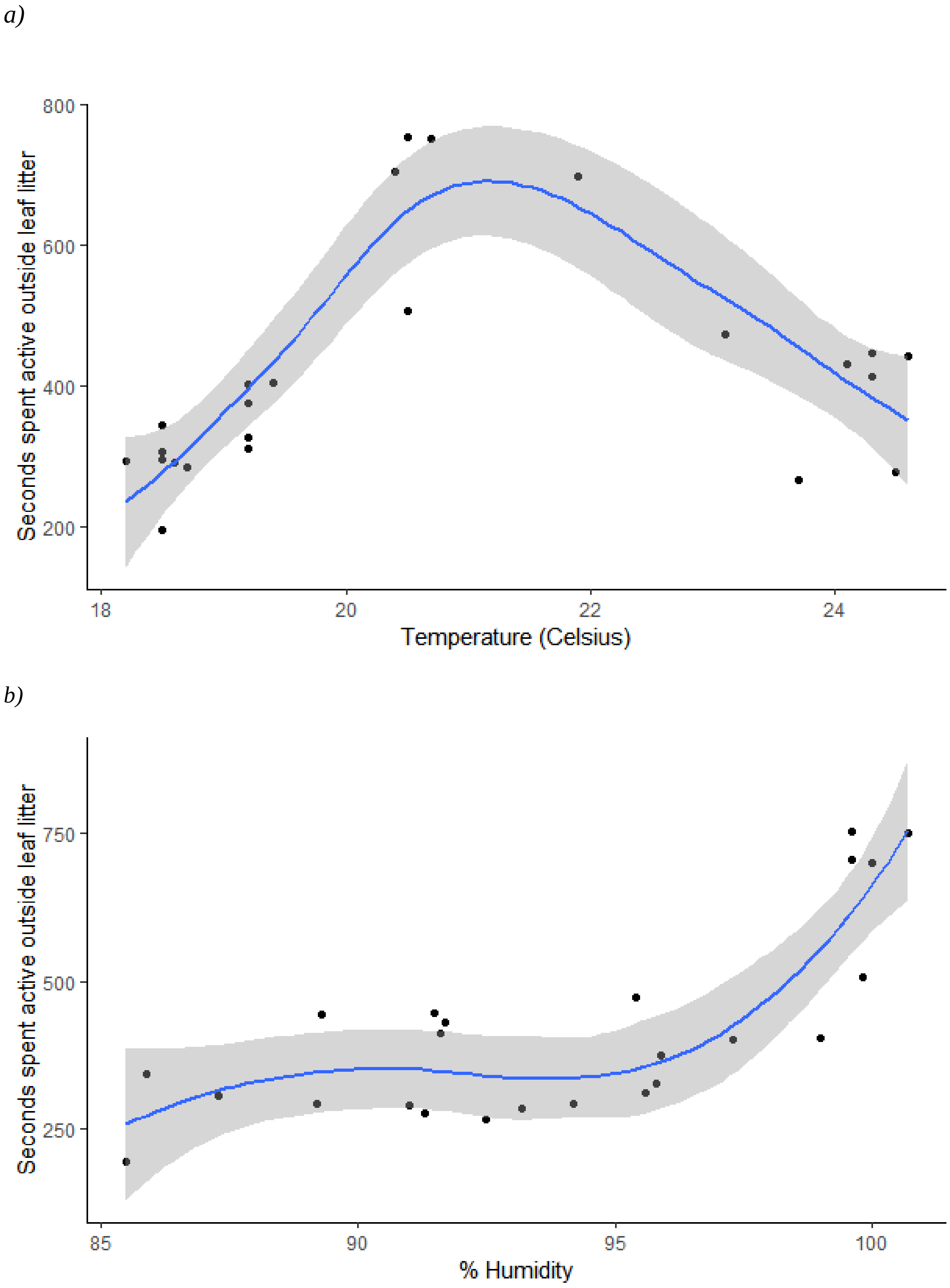


Figure 5: (a) the number of seconds spent in the open (see clarification of terms in methods) peaks as temperature increases to around 21 – 21.5 ˚C and b) the number of seconds spent in the open versus humidity levels for both the cooler room and warmer room data combined.

4.5 Discussion

Golden mantellas spent longer on or in leaves in the cooler *(*16-19°C) room tanks. In the warmer (20-25°C) room more golden mantellas were active and frogs undertook longer continuous activity bouts. Individuals were also more likely to venture away from leaves as temperature increased from 17°C and continued until a peak 21.5 °C was reached. There was a decrease in activity, length of activity bout and the number of frogs in the open again as temperature increased. Humidity levels also peaked at temperatures of approximately 21-22°C. Humidity levels rose and fell with temperature, closely followed by a similar pattern of rise and fall in both the numbers of frogs observed on leaves, and the amount of time spent hiding in them. Activity in amphibians is intricately linked to body temperature and hydration state and is dependent upon ambient temperature and humidity (Duellman and Trueb, 1994). Golden mantellas have highly permeable skins that lose water quickly to the environment via evaporation which in turn affects their hydration state (Duellman and Trueb, 1994). Several physiological systems are negatively affected by a decrease in hydration state including metabolic rate, muscle condition, digestion and development (Bartelt 2010). Frogs can mitigate for the effects of higher temperatures by using physiological or behavioural means i.e. they may move into areas with more favourable conditions and offset evaporative water loss by the intake of water via the skin. Alternatively, lower temperatures will slow metabolic rates, in this instance the frogs will retreat under leaves and conserve body heat by decreasing their activity.

Golden mantellas are a diurnal montane forest floor dwelling species (Vences and Raxworthy, 2008). They prefer to stay upon or within substrates that allow them to offset the detrimental effects of higher or lower than optimum temperatures or humidity levels. However, when temperatures are near to optimum and humidity levels are high the frogs will spend more time in the open and venture away from their preferred substrate.

Madagascar is predicted to see an increase in temperature of 1.1 – 2.6 °C by 2050 (Hannah et al., 2008). This has implications for all species but especially so for montane based amphibians who are often specialist, poor at dispersal and restricted to forest fragments (Araujo et al., 2013; Somero, 2010). A study by Raxworthy et al. (2008) found that several reptile and amphibian species shifted their range upslope in response to a rise in temperature. Golden mantella populations are already distributed close to the summits of the hills they inhabit and so are limited in their ability to shift range upslope in response to an increase in temperature.

Our results show that as temperature and humidity levels moved away from those preferred the frogs were less active i.e. spent more time hidden in leaves and were less likely to venture away from leaf litter. Climate change predictions for golden mantella forest habitat are at the lower end of the scale. Indeed, a subtle rise may in the long-term affect population growth due to a restriction on their activity and ability to utilize microhabitats, in turn impacting upon the length of time spent hunting or interacting with con-specifics.

Golden mantella numbers are falling, their habitat is under threat from logging, mining and encroachment by agricultural practices (Vences and Raxworthy, 2008). Climate change is driving temperature and humidity change at micro-habitat level (Raxworthy et al., 2008). As temperature increases activity will decline, less activity means less time devoted to breeding or feeding. Numbers will continue to fall unless ways can be found to offset the threats faced by the golden mantellas.

We recommend future conservation strategies for climate change mitigation should include species distribution modelling, which may prove useful in identifying climate stable areas within or close to current golden mantella population distributions. In this way it may be possible to prepare or reinstate wildlife corridors or protected areas for either translocation or reintroduction of new populations if current mantella breeding sites become unviable.

Acknowledgements

We would like to especially thank Mike Bungard and his team at the Lower Vertebrates and Invertebrates Department at Paignton Zoo in Devon, UK, for providing the frogs, room and advice during this research. Thanks also to Don Neilson, Paignton Zoo's master carpenter for taking time out from a busy work schedule to build the Froggotrons; Amy Plowman and the team at Field Conservation and Research, Paignton Zoo for their support.

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Chapter 5

Predicted impact of climate change on the distribution of the Critically Endangered golden mantella (*Mantella aurantiaca*) in Madagascar

Wayne M. Edwards[1, 2], **Michael J. Bungard**[3] **and Richard A. Griffiths**[1]

[1] Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, Marlowe Building, University of Kent, Canterbury, CT2 7NR, UK

[2] Department of Field Conservation and Research, Paignton Zoo Environmental Park, Totnes Road, Paignton, Devon, TQ4 7EU, UK

[3] Department of Environment and Geography, Wentworth Way, University of York, York, YO10 5NG, UK

5.1 Abstract

The impact of climate change on Malagasy amphibians remains poorly understood. Equally, deforestation, fragmentation and lack of connectivity between forest patches may leave vulnerable species isolated in habitat that no longer suits their environmental or biological requirements. We assess the predicted impact of climate change by 2085 on the potential distribution of a Critically Endangered frog species, the golden mantella (*Mantella aurantiaca*), that is confined to a small area of the central rainforest of Madagascar. We identify potential population distributions and climatically stable areas. Results suggest a potential south-eastwardly shift away from the current range and a decrease in suitable habitat from 2110 km^2 under current climate to between 112 km^2 – 138 km^2 by the year 2085 – less than 7% of currently available suitable habitat. Results also indicate that the amount of golden mantella habitat falling within protected areas decreases by 86% over the same period. We recommend research to ascertain future viability and the feasibility of expanding protection to newly identified potential sites. This information can then be considered in future conservation actions such as habitat restoration, translocations, re-introductions or the siting of further wildlife corridors or protected areas.

5.2 Introduction

Madagascar is one of the world's mega-biodiversity hotspots, with extremely high levels of endemism across the island (Myers et al., 2000; Vieilledent et al., 2013). Amphibians follow the trend with 325 known frog species, 99% of which are endemic (IUCN, 2018), and potentially many more yet to be described (Glaw and Vences, 2007). Most species are located within the Eastern rainforest belt (Glaw and Vences, 2007). However, forests across Madagascar are being depleted at an alarming rate, i.e. from 1953 to 2014 forested land cover decreased from 27% to 15 % (Brown et al., 2015; Vieilledent et al., 2017). Forest fragments that remain are also decreasing in size with mean distance to forest edge dropping from 1.5 km to 300 m respectively (Brown et al., 2015; Vieilledent et al., 2017). Fragmentation of already depleted forest areas may impede the movement of species with low vagility between habitat patches, increase access for loggers or hunters, expose deep forest species to forest edge effects, increase competition for limited resources, or result in habitat patches too small to sustain viable populations (Cushman, 2006; Echeverria et al., 2006; Vieilledent et al., 2017).

Predictions for climate change across Madagascar suggest a rise in temperature of 1.1 °C –2.6 °C by 2050 (Tadross et al., 2008). Temperatures vary along a gradient from north to south, with the lowest rises predicted in the northern and coastal areas, and highest rises in the southern spiny forest region (Hannah et al., 2008). Rainfall is predicted to increase across the island except along the south-east coast where it will become drier in winter months (Hannah et al., 2008). According to Seidl et al. (2017), climate change has the potential to affect forests in complex ways i.e. an increase in temperature and lower rainfall may lead to higher instances of tree die-off, forest fires, fuel build up, or insect abundance. Under hotter and wetter conditions, soil erosion, runoff and sedimentation become more likely (Seidl et al., 2017). Deforestation and climate change may therefore act synergistically driving species to shift their range to areas with more favourable conditions (Raxworthy et al., 2008). Historically, large tracts of contiguous forest may have made dispersal to higher, cooler or more climatically stable areas easier. However, with many montane forested areas in Madagascar now highly fragmented, dispersal for some species is difficult, if not impossible (Brown et al., 2015).

Golden mantellas (*Mantella aurantiaca*) are Critically Endangered montane forest dwelling frogs from the Central Eastern Rainforest areas of Mangabe and Analamay in Madagascar (Piludu et al., 2015). They are found at altitudes of between 900 m and 1000 m asl and the area of suitable habitat occupied by this species is low at around 10 km^2. A recent survey by Piludu et al. (2015) found 139 breeding sites, many of which were in areas under threat from agricultural expansion, artisanal mining or collection for the pet trade, with the majority in areas already classed as protected.

Climate change may exacerbate problems faced by golden mantellas as they are already found at altitudes close to the summits of the slopes they inhabit, leaving no real opportunity for dispersal to higher, cooler altitudes. It is clear there are few in situ conservation management options remaining i.e. the frogs either adapt to climate change where they are, or alternative viable habitat is located in case it is needed. To this end Species Distribution Modelling (SDM) can play an important part in identifying suitable areas for the possible translocation or reintroduction of golden mantella populations. SDM is the process of exploring the relationships between species distribution and associated environmental and habitat variables, and then predicting spatial relationships (Márcia-Barbosa et al., 2013 Bateman et al., 2013; Cao et al., 2013; Meynard et al., 2013; Rodriguez-Rey et al., 2013). We follow several other authors (Blank and Blaustein,2013; Chunco et al., 2013; Groff et al., 2014; Sharifi et al.,2017) in using SDM to identify and prioritise optimum habitat requirements, where potential anthropogenic disturbance and climate change impacts are at their lowest. Results may then be used to guide future management decisions regarding the placement of protected areas and the reintroduction or translocation of golden mantellas to favourable sites if needed.

5.3 Methods

5.3.1 Data collection and study area

The aim of modelling was to explore potential suitable habitat to inform broader conservation decisions, the extent of the study was an area around Moramanga Province, Madagascar. Records of golden mantella sightings were collected by Madagasikara Voakajy research teams from ten sites within the protected areas of Mangabe, each containing or bordering known golden mantella breeding ponds. Nine of these sites were surveyed between 28 November 2014 – 12 December 2014, and the tenth earlier on in the year in March 2014. These periods correspond to the main breeding activity periods for this species. All surveys took place between 0700-1400 hrs each day, one visit per forest. The surveys were centered on breeding pools located in shallow valleys (See: chapter 2 survey methods).

5.3.2 Species distribution modelling

A total of 198 golden mantellas were recorded across the ten surveyed sites in Moramanga. In order to meet the assumptions of Maxent with environmental data and reduce spatial bias, we needed to reduce golden mantella presence data to one observation (one frog) per 250 m grid square (See: Elith et al., 2011). In doing so we reduced presence data to 98 *Mantella aurantiaca* locations at a 250 m spatial grain.

We used remotely sensed data (Table 1) for climate and habitat variables to model current and future distributions for golden mantellas. Remotely sensed data have greatly improved over recent years and now provide good, useable information to answer ecological questions (Pfeifer et al., 2011). High resolution, baseline current climatic data were from Worldclim (Hijmans et al., 2005) and future climate projections (Representative Concentrations Pathways (RCP) 4.5 and 8.5) were sourced from AFRICLIM (Platts et al., 2015). RCP are greenhouse gas concentration projection scenarios adopted by the Intergovernmental Panel on Climate Change so that climate change studies and modelling might use a set of standardised measures (Van Vuuren et al., 2011). RCP 4.5 assumes CO_2 concentrations will continue to rise to approximately 650 parts per million (ppm) by 2100 and stabilise thereafter (Van Vuuren et al., 2011). RCP 8.5 assumes rising CO_2 concentrations to approximately 1370 ppm by 2100 (Van Vuuren et al., 2011).

Table 1: Data downloaded/used in analysis and associated codes from Worldclim. Bio4, Bio10 and Bio16 are measurements.

Code	Description
Bio4	Temperature seasonality (°C x 10) Standard deviation over monthly values
Bio10	Mean temp warmest quarter (°C x 10) Any consecutive 3-month period
Bio16	Rainfall wettest quarter (mm) Any consecutive 3-month period
Wd	Maximum water deficit (mm) Consecutive months that experience rainfall < monthly PET (Potential Evapotranspiration, Hargreaves method), over which the shortfall in rain is accumulated. Also defined by Stephenson (1998) as the amount of water by which potential evapotranspiration (PET) exceeds actual evapotranspiration (AET).
Canopy_hght	Canopy height (m)

En_veg_ind	Enhanced vegetation index (reflects variation in canopy structure and architecture (Vieilledent et al., 2017). Mean annual Enhanced Vegetation Index is from 16-day 250 m MODIS MOD13Q1 data from the years 2007 – 2017 (Didan, 2015).
Lc250m	Landcover classes cropland, forest etc categorical variable (percentage per 250m grid square). Data is from the European Space Agency (globcover project).
Top wet	Topographic wetness, the presence of water based on upstream contribution, slope and soil content. It is a measure of the potential for water to flow into the grid cell and of how likely it is to remain there.

Potential distributions were modelled using Maxent (v. 3.3.3k), a standard SDM technique using presence-only data (Hernández et al., 2006; Pearson et al., 2007). Climate data were at 1 km resolution and habitat/vegetation data were at 250 m resolution, but for Maxent to work, both sets of data must be at the same scale. All 1 km data were therefore interpolated to 250 m portions, ensuring that values in each grid cell were maintained, e.g. if the 1 km grid square had a temperature of 20°C , then all of the 250 m grid squares that make up that 1 km grid square would also be at 20°C. Habitat variables were included as static variables (a variable that may change with climate change, but we are unable to predict the amount of change due to compounding factors such as anthropogenic disturbance) within the distribution models for future scenarios. We used static variables as it is difficult to model dynamic variable change (e.g. vegetation growth) along with projected climate change. Although we understand vegetation will alter with climate, preliminary runs of the model suffered from excluding vegetation variables altogether, we therefore chose to keep these static variables (See: Stanton et al., 2012).

Maxent makes several assumptions which affect the performance of the model (Phillips et al., 2006) and constrain final spatial patterns of species distribution. We therefore used a regularization multiplier (See: Appendix 2), described by Merow et al. (2013) as placing a Bayesian priori distribution on model parameters (i.e. using current knowledge and reasonable expectation to predict potential distribution). The regularization multiplier effectively constrains or relaxes the fit around the data balancing the need for both accuracy of predictions and generality (Elith et al., 2011). Prior to running final models, we adjusted the regularization multiplier and selected the most appropriate model from Akaike Information Criteria (AIC) (Warren et al., 2010; Warren & Seifert, 2011). In addition, the final models were cross-validated ten times, and to determine drivers of distribution, we jack-knifed environmental data (Phillips et al., 2006)(See: Appendix 2). All other settings were set to default. We used Albers Africa Equal-area projection to equalise grid cell size (Elith et al., 2011) to ~0.250 m^2 and an appropriately scaled kernel density bias file was used to restrict the placement of pseudo-absences (See: Fourcade et al., 2014). Maxent is a presence-only modelling system based upon definite species sightings, which means it does not utilize any known absence information. Instead it fills the gaps using pseudo-absences (estimated absences). Pseudo-absences are estimated by taking known presence data for large numbers of similar species (kernel density file) and then determining the probability of finding a given species across different areas and habitat. This research used a kernel density file constructed from amphibian sightings across Madagascar. We used maximum test sensitivity plus specificity logistic threshold (Liu et al., 2005) for all distributions to identify areas of highly suitable habitat (Appendix 2). The Habitat Suitability Index (Fig.1) (how suitable an area is for a species based upon the variables entered into the model) was calculated using Maxent (Appendix 2). To describe the current golden mantella area of occurrence we developed a Minimum Convex Polygon (MCP) based on the raw data for *M. aurantiaca* occurrences and then added a 10 km buffer (Smith and Green, 2005, suggest maximum dispersal distances for most amphibians would not exceed far beyond 10km), to create an over-estimate of current area. We then used a threshold value to create areas of high likelihood of occupancy within the buffered MCP and the area of potential climate space was assessed (km^2) (Appendix 2).

For each climate scenario we used a metric from Bungard et al. (2018, unpublished) to measure the level of imperilment based on the index of net change (Nc) in area: Nc is calculated for golden mantellas, as the sum of the change for each future scenario; future increase in area (T_{fi}) (km^2) minus future decrease in area (T_{fd}) over the area under current climate conditions (T_c).

Equation 1.

$$Nc = \sum \frac{\left(T_{fi} - T_{fd}\right)}{T_c}$$

Finally, we assessed how well the current system of protected area networks surrounding golden mantella area of occupancy accounts for golden mantella distribution in both current and future climate scenarios. To do this, we calculated for each scenario, the simple metric of area of suitable habitat within the protected area network/total area of suitable habitat using ARCGIS pro^{TM}.

5.4 Results

Our model demonstrated a good fit with the data (AUC = 0.994, SD = 0.001) and showed that two main drivers influence *M. aurantiaca* distributions under current climatic conditions; landcover (contributed 32% to the final model) and the length and severity of the dry season (water deficit; model contribution: 31%) (Fig. 1). Golden mantellas are found mainly in broadleaved evergreen forest (rainforest) and only have a narrow tolerance of extended dry conditions. The potential distribution of golden mantellas under current climate conditions extends outside the current MCP (Fig. 1b) with potentially highly suitable habitat occurring in a narrow south-west to north-east band divided into two distinct areas. These areas embrace the two known population centres for golden mantellas, Mangabe in the south and Torotorofotsy/Analamay in the north. From our models, local protected areas currently offer protection to 24% of potentially suitable habitat for golden mantellas. As climate changes, so does the distribution of golden mantellas, with the area of suitable habitat decreasing from 2,110 km^2 (current climate) to 121 km^2 and 138 km^2 (RCP 4.5 and 8.5 respectively; Fig.1) and protected area decreases by 86% for both climate scenarios. Slightly larger areas of suitable habitat predicted under the higher RCP 8.5 scenario would seem counter-intuitive, however it may be that more variation in topography or changes in range and availability of water at higher altitudes increases available area. Further, we observed a range shift under scenarios RCP 4.5 and RCP 8.5 to the south-east of the current distribution by 10-15 km^2 (Fig 2). Within the projected habitat distribution range under RCP 4.5 and 8.5, there are several areas that are predicted to be climatically stable (Fig 3). By climatically stable we mean consistently provides areas of suitable habitat across climate scenarios. Assuming landcover remains the same then the areas highlighted here also provide suitable habitat in terms of water deficit i.e. the range of water deficit stays within the boundaries needed by golden mantellas.

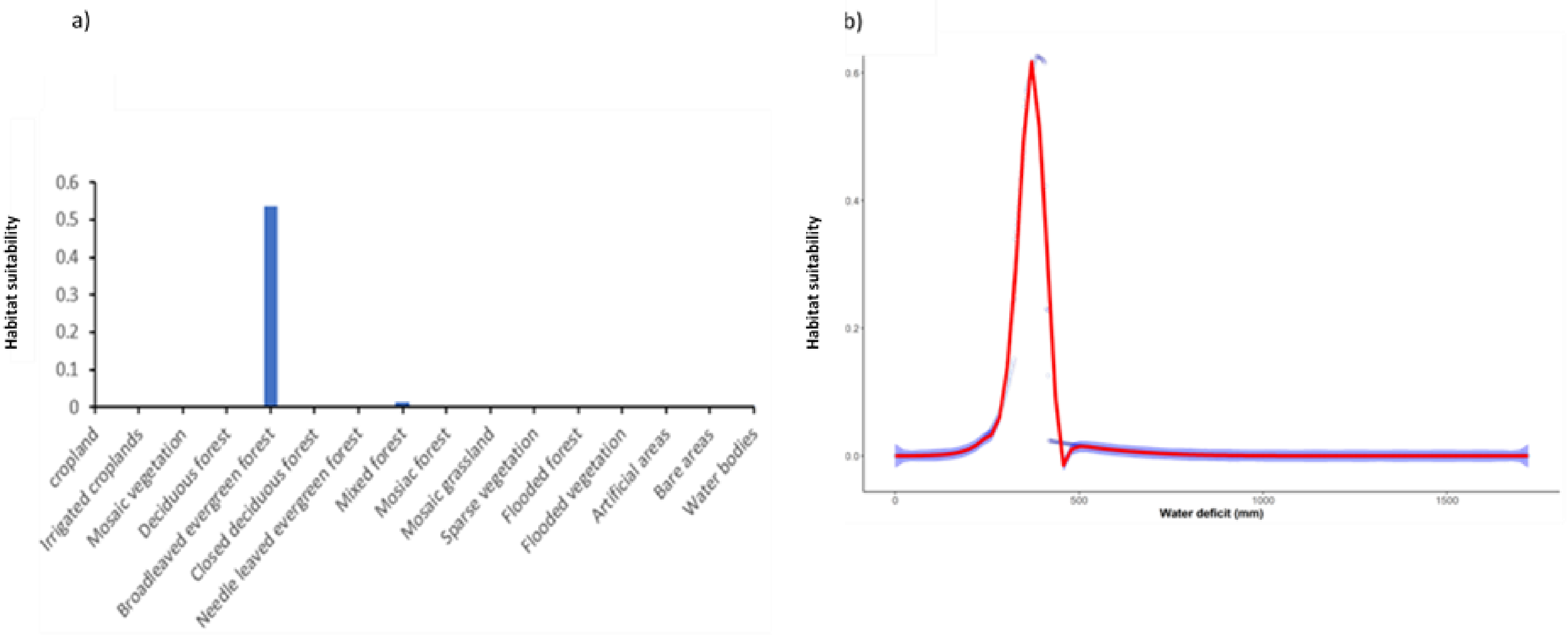

Figure 1: (a) the presence of broadleaved evergreen rainforest and (b) the length and severity of the dry season are the main drivers for the distribution of golden mantellas. Habitat suitability is given as between 0 (unsuitable) and 1 (highly suitable) and is based on variables initially entered in to MaxEnt (Table 1). Water deficit (Wd) is the amount of water by which potential evapotranspiration exceeds actual evapotranspiration (derived from remote sensed satellite data) and is indicative of the severity of the dry season. The red line is the response curve (fit of the data), the blue line is the standard deviation. Our model suggests habitat suitability is high where water deficit remains low at around 400 mm i.e. associated with a short dry season.

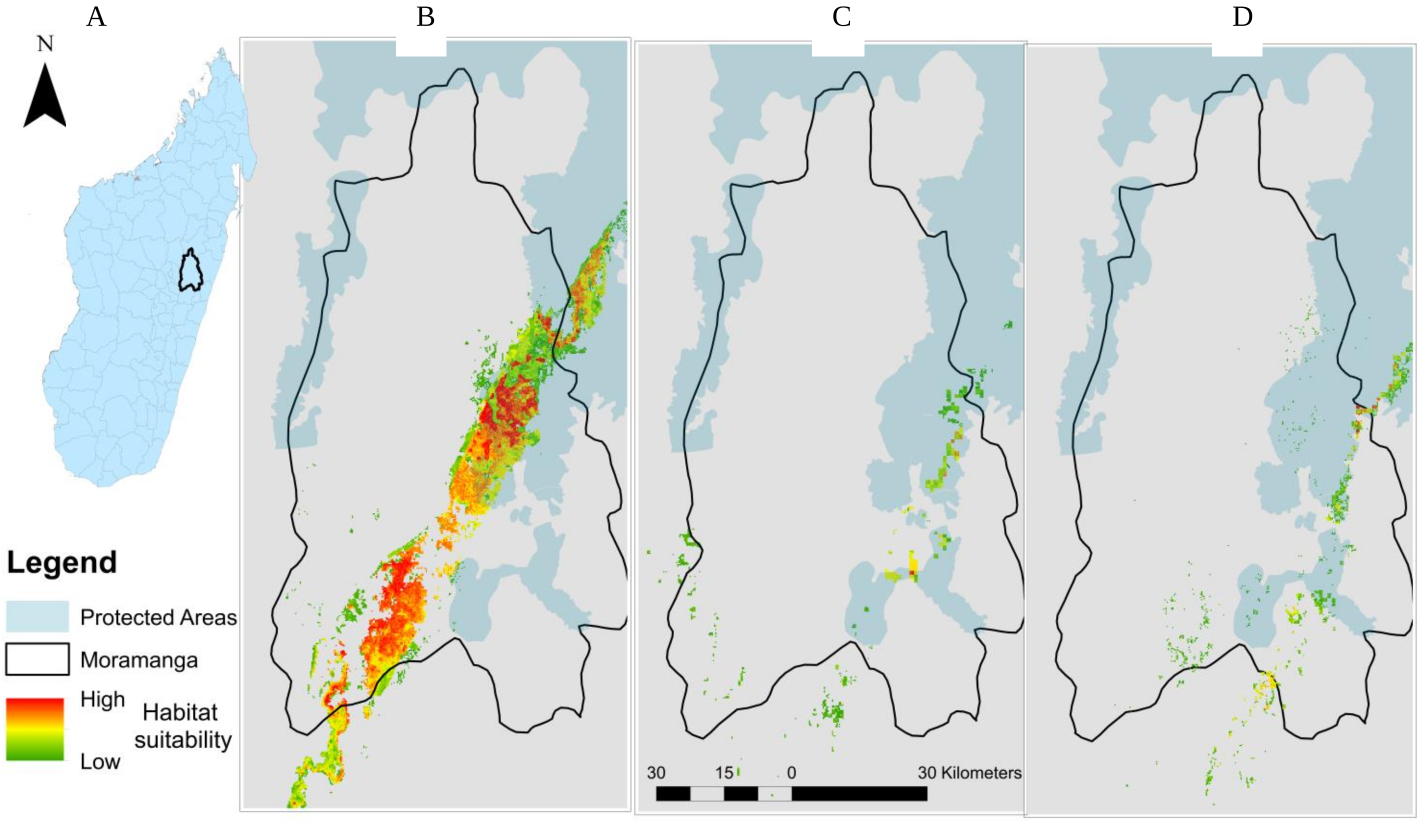

Figure 2. Species Distribution Modelling for the golden mantella showing A) political divisions with Moramanga highlighted with a black border. B) potential distribution under current climate. Potential distributions under C) RCP 4.5, 2085 and D) RCP 8.5, 2085, decrease in size and shift in a south-easterly direction.

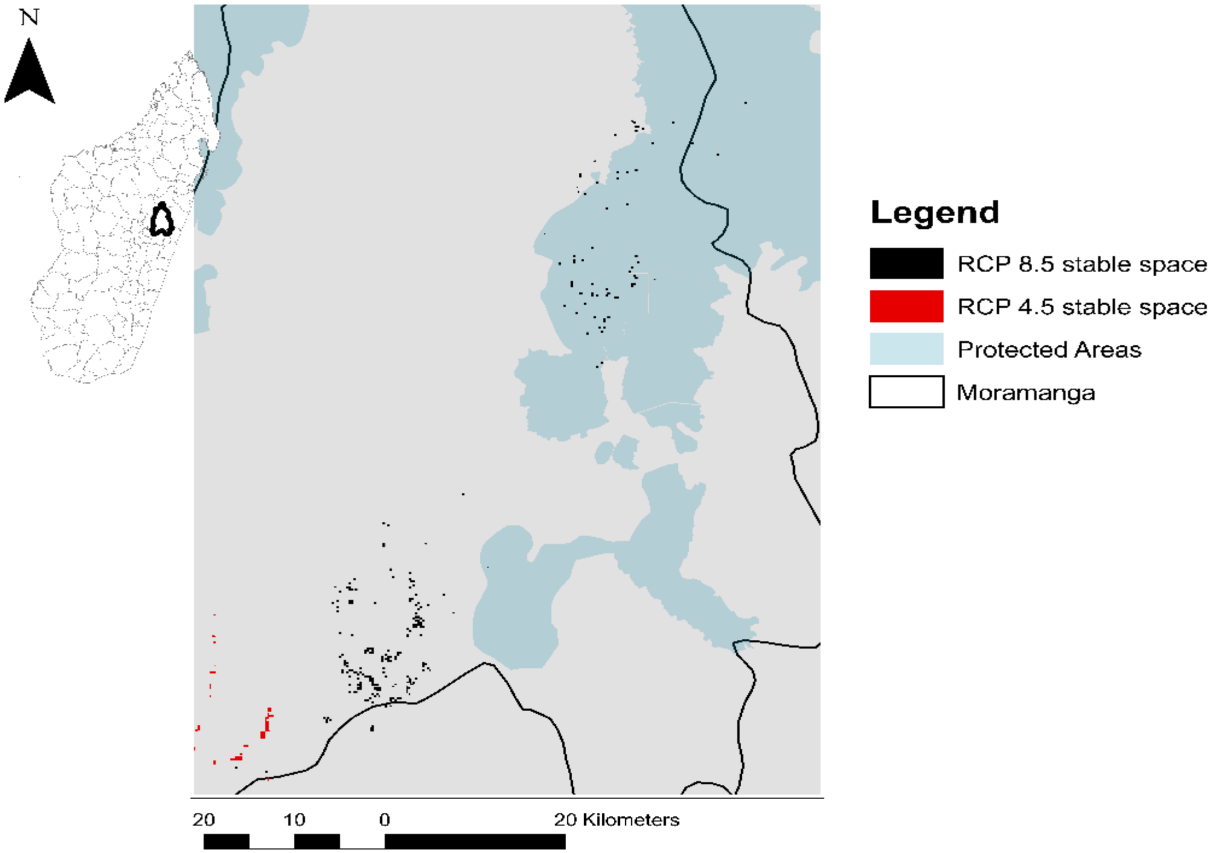

Figure 3. There are several climate stable spaces predicted within the range of projected distributions for RCP 4.5 and RCP 8.5

5.5 Discussion

We investigated whether projected climate change scenarios would influence current golden mantella population distributions in rainforest habitat in Madagascar. Our results suggest golden mantella population distribution is driven by the type of available habitat and the amount of water retained within those habitats. Our models predict that as the length and severity of the dry season increases, the availability of suitable habitat for golden mantellas decreases from 2110 km^2 currently to 121 km^2 under RCP 4.5, and to 138 km^2 under RCP 8.5 by 2085. Consequently, less than 7% of currently available habitat is likely to remain suitable under these scenarios. We also reveal that local protected areas currently offer protection to 24% of potentially suitable habitat for golden mantellas. Models predict that the distribution of viable habitat will shift 10 – 15 km away from its current location with the majority (86%) falling outside of protected areas.

Increased temperatures and reduced rainfall will change forest habitat by restricting the availability of moisture to vegetation, soil and substrate (Bartelt et al., 2010). As microhabitat becomes warmer and drier the opportunity for thermoregulation and hydroregulation become more challenging. Frogs lose water quickly from the skin by evaporation, and to mitigate this loss they need to find moist habitat in which to take up water at least as quickly as it is being lost (Duellman and Trueb, 1994). Several studies have found that montane amphibians may shift range upslope to cooler areas when exposed to prolonged ambient temperature rises (Raxworthy et al., 2008). However, this is not an option for golden mantellas as they already live at, or close to, the crests of the slopes they inhabit. Further, although golden mantellas are known to migrate a few hundred metres between the crest and breeding ponds (Piludu et al., 2015), rather less is known regarding their long-range dispersal ability. Current mantella forest habitat is also highly fragmented and usually bordered by agricultural land or deforested areas. Consequently, land use other than forest may well prevent range expansion or shift to track preferred environmental variables. Indeed, Harrison et al. (2006) state that where a species is in decline they may not automatically shift or expand their current range to track preferred climatic variables. Willis et al. (2015) advise that if climate suitability changes markedly within a species current distribution and there is no suitable climate/habitat within realistic colonisation range, then translocation to suitable areas should be considered.

SDM results identify several locations considered climatically stable and relatively close (within the Moramanga area) to current golden mantella distributions (Fig. 3). However, it is currently unknown if the locations identified contain forest specifically suited for golden mantellas. Ideally, we would hope to survey those new sites and other areas in between current and potential distributions to ascertain if there is a realistic opportunity to place wildlife corridors, which may facilitate golden mantella range shift.

There is already a programme of survey and research which seeks new areas in which to create, restore or protect breeding ponds and habitat (Piludu et al., 2015); however, in light of our current findings, it may be prudent to consider searching further afield for new sites. Our results suggest these new sites should be sought a further 10-15 km south-east from current golden mantella distributions.

The complexity of biological interactions between species, environment and anthropogenic influence over time means there are constraints on the accuracy of any prediction we may make (Harrison et al., 2006). However, climate change is already impacting heavily on species and ecosystems (Hannah et al., 2008; Raxworthy et al., 2008; Tadross et al., 2008), and as such conservation actions should be planned and carried out without delay using the knowledge and techniques we do have, rather than wait until more advanced methods become available (Rowland et al., 2011).

We therefore recommend carrying out surveys to test whether newly highlighted areas identified as climatically stable or within projected distribution under climate change are in fact suitable for the potential translocation or introduction of golden mantellas in terms of microhabitat and water bodies for breeding. Further research should be conducted into the feasibility of placing wildlife corridors between current and potential golden mantella distribution to facilitate range shift to safer areas. Expanding protection and status to potential climate stable areas and projected population distribution ranges should also be a priority.

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Chapter 6

General Discussion

6.1 Summary of in situ and ex situ approaches used

Prior to this project, very little evidence-based ecological research on golden mantellas existed. Several authors had described classification (Zimmerman et al., 1996), known distribution (Glaw and Vences, 2007), skin alkaloids, toxicology and colour (Garraffo et al., 1993; Daly et al., 1997; Chiari et al., 2004), feeding ecology (Woodhead et al., 2007); age structure (Jovanovic and Vences, 2010); chromosome morphology (Pintak et al., 1998) and ex situ husbandry guidelines (Staniszewski, 1998; Walker et al., 2013). However, work on habitat preferences of this Critically Endangered species in the wild was lacking. Similarly, no research had addressed the question of how climate change might affect golden mantella activity and habitat preference, or as a following consequence, future population distribution.

My research has gone some way in addressing these knowledge gaps. To reveal microhabitat preferences, I assessed and analysed a large data set that had been collected in situ across 10 golden mantella breeding sites in Madagascar. Ex situ, I addressed key questions regarding activity budgets including timing and intensity. I examined how activity patterns of golden mantellas compared under different temperatures in order to better understand how thermal challenges through climate change might affect golden mantella behaviour. I followed this by investigating leaf litter as an important component of microhabitat, and how the use of leaf litter as a refuge related to temperature and humidity. I developed a species distribution model for the golden mantella based on the current distribution, and then modelled future distribution under predicted climate change scenarios in Madagascar. I have conducted trial habitat surveys in situ, and throughout the project lifetime worked closely with colleagues involved in conducting surveys or carrying out conservation management actions in Madagascar. I have also made my research results available via the media and through scientific journals. In this way I hoped to not only provide clarity and information on the plight of a critically endangered species, but also to bridge the gap between in situ and ex situ research. Below I discuss my key findings, results and the implications for the golden mantella.

6.2 Key findings

In Chapter 2, I explored in situ mantella habitat, gathered data on environmental and habitat variables and was then able to determine which microhabitat components were important and most likely to determine golden mantella microhabitat use. Results showed the amount of leaf litter coverage and depth; number of tree roots and surface temperature levels were the most important predictor variables associated with quadrats occupied by the frogs. Results revealed optima of over 70% litter coverage, surface temperatures of 20 – 23 °C (range 17 – 29 °C) and comparatively low numbers of tree roots. Information gained in the field was used to inform temperature and moisture settings in zoo-based climate chambers (Froggotrons). By using Froggotrons, I was able to more closely monitor golden mantella behaviour than is possible in the field. This revealed golden mantellas had a bimodal activity pattern during daylight hours even under different temperature regimes. At lower temperatures (16 °C – 19 °C) mantellas were overall less active than those at higher temperatures (20 °C – 25 °C), but the phasing and bimodal nature of the activity rhythm was the same under both temperature regimes. Results also showed that most activity occurred when humidity levels exceeded 85%. Golden mantellas were most active, spent most time in the open and less time on leaves at 21.5 °C. Where temperature deviated either way from 21.5 °C there was an associated decrease in activity and an increased tendency to hide in leaves. Results also show that even under optimum temperature and humidity regimes less than 50% of the frogs were active in open areas at any one time. Species distribution modelling revealed a large decrease in viable habitat by 2080 and a shift in population distribution away from forest currently classed as being in protected areas.

6.3 Implications

Species distribution modelling for the golden mantella predicted climate change has the potential to make current habitat less favourable, or indeed non-viable, as a result of increased temperature and drier conditions. A study by Bartelt et al. (2010) on the western toad (*Anaxyrus boreas*) obtained similar results, i.e. if the toad's forest habitat became drier, balancing their hydrological and thermological needs would become more difficult. There is also evidence to suggest montane amphibians, along with other species and taxa, shift their distributions upslope to avoid rising ambient temperatures (Raxworthy et al., 2008; Feeley et al., 2011; Freeman and Freeman, 2014; Cheng et al., 2019). The problem golden mantellas have is that they already live on the upper slopes in relatively small and isolated patches of remnant forest (Piludu et al., 2015), leaving no opportunity for the frogs to disperse to higher cooler altitudes.

My research highlighted the most important components of microhabitat for the frogs. However, given this will only be useful in the future if any habitat surveyed is in an area considered to be the least affected by climate change, research also highlighted areas predicted to stay climate stable.

6.4 Why the research was needed

Andreone et al. (2005) proposed an urgent need to increase research and gather data on biology, distribution and adaptability of Malagasy species. Raxworthy et al. (2008) advocated carrying out surveys in the Madagascan montane environment on endemics; describing species current elevation distributions; assessing species extinction vulnerabilities to upslope displacement due to climate change; archiving distribution data for future monitoring and where possible, the collection of detailed habitat and microhabitat data as this would enhance our understanding of the mechanism of distribution shifts.

Andreone et al. (2008) produced the Sahongasy Action Plan for Madagascar which prioritised a number of conservation strategies including; predicted shifts in species distribution due to climate change; identification of amphibian refugia for adaptation to climate change; surveys to collect data on priority species and improvement of public awareness. The Sahongasy Action Plan for 2016 – 2020 (Andreone et al., 2016) reviewed the former 2008 plan and went on to develop a revised set of priorities and actions including; the identification of high elevation areas for future surveys; species adaptability to climate change; the identification of priority areas not yet in protected areas; habitat assessments; conduct in situ research in support of ex situ work and the identification of priority sites for assisted colonisation.

My golden mantella research has addressed issues linked to each of the priorities recommended above and will feed into future conservation strategies for Malagasy amphibians. This research bridges the gap between in situ and ex situ conservation by using data collected in the field to inform appropriate temperature and humidity levels in Froggotrons for ex situ research. In turn, research results using Froggotrons has been used to inform in situ survey procedures.

6.5 Limitations of the research

There were of course some unavoidable limits imposed on this project e.g. behavioural research was conducted ex situ in Froggotrons and at potentially higher densities than found in situ. However, research needed to be conducted at realistic and practical levels i.e. each frog may well require more space to closely resemble in situ life than we could ever provide within the confines of a zoo. Further, the golden mantellas used in this project were mainly bred in Paignton Zoo, but several also came from The University of Kent, Bristol Zoo and Heathrow Airport (unknown if wild or captive bred, they were confiscated on landing). None, as far as is known, had been part of a breeding programme to preserve genetic diversity. Therefore, we do not know how their genetic diversity compares to those in the wild or how this might have influenced behaviour. For example, Passos et al. (2018) found that captive golden mantella skin had a lower diversity and relative abundance of associated bacterial species than their wild counterparts. Kraaijeveld et al. (2006) found that anti-predator behaviour in Mallorcan midwife toads (*Alytes muletensis*) tended to dampen after 9 – 12 generations in captivity. Nevertheless, amphibian physiology means they may only maintain hydrated and within optimal body temperature range by responding to ambient temperature and humidity levels in biological patterns e.g. by seeking moist refuge to cool down if conditions become too dry (Duellman and Trueb, 1994). This suggests that the way golden mantellas responded to temperature and humidity regimes provided in the Froggotrons, would be a fair indication of how they might respond under similar conditions in the wild.

6.6 In situ / ex situ research and wider implications

My golden mantella research is complimentary to, and part of, a growing conservation research collective consisting of several teams, each having their own field of expertise e.g. tonic immobility, calling frequency and the composition of bacterial skin communities by Luiza Passos and Gerado Garcia at Chester Zoo (Passos et al., 2017; Passos et al., 2018); evidence based ex situ husbandry including diet, skin micro-biota and UV-B radiation by Richard Preziosi, Christopher Michaels and Racheal Antwis at the University of Manchester (Antwis and Brown, 2009; Antwis et al., 2014; Michaels et al., 2014; Michaels et al., 2015); reproduction and ex situ husbandry by Devin Edmonds at Mitsinjo in Andasibe Madagascar (Edmonds et al., 2015) and the detection and mitigation of *Batrachochytrium dendrabatidis* by Molly Bletz in Madagascar (Bletz et al., 2015). Zoo ex situ conservation is usually carried out for reintroduction, research and education (Harding et al., 2016) or as a way of raising much needed funding for protection and mitigation measures in-country (BIAZA, 2019). For example, Paignton Zoo in the UK has several on-going, long term projects committed to species conservation in situ including, for example, black crested macaque *(Macaca nigra),* Aders duiker *(Cephalophus adersi)* and hazel dormouse (*Muscardinus avellanarius)* (Paignton Zoo, 2019). Indeed, organisations represented by BIAZA (British and Irish Association of Zoos and Aquariums) collectively raise over £20 million each year for this type of conservation project (BIAZA, 2019).

Golden mantella research is a new development in terms of how a combination of in situ and ex situ research can aid conservation. Firstly, the golden mantella is unique in that it is a charismatic, high profile amphibian that is Critically Endangered, but is abundant in captivity (Andreone et al., 2005; Vences and Raxworthy, 2008). This species is also highly suitable for ex-situ research i.e. small, active by day, captive husbandry is well-understood and it breeds well in captivity, which means we can obtain sufficient numbers to ensure rigorous replication in research. Additionally, in situ and ex situ research and surveys were carried out in a complimentary and collaborative way i.e. research teams surveyed habitat and then shared data so that environmental variables could be set to the correct levels in the Froggotrons. In situ habitat data was also used in both the analysis of habitat preferences and for species distribution modelling. Results were then passed back to in situ research teams recommending survey times, potential for detection and new climate stable habitat areas.

Golden mantella research has provided much needed evidence highlighting habitat preferences and the potential negative impacts of climate change. Although this research was species-specific it does feed in to a general and familiar picture when viewed from a worldwide perspective. We know that Montane amphibian species are particularly susceptible to rising temperatures (Hannah et al., 2002; Parmesan, 2006; Raxworthy et al., 2008) and that impacts can be complex and wide-ranging affecting all aspects of life history from species biology/ecology (Jara et al., 2019) to distribution (Kafash et al., 2018). For example, Kissel et al. (2018) found that climate change reduced adult survival and facilitated a decrease in population growth rate in the montane dwelling cascades frog (*Rana cascadae*). Kafash et al. (2018) used species distribution modelling to reveal a potential and substantial loss in suitable habitat for two species of alpine newt (*Triturus ivanbureschi* and *Triturus anatolicus*) due to predicted climate change. The potential for climate change to act synergistically with other threats such as predation pressure or in the dispersal of pathogens is also well known (Campos-

Cerqueira et al., 2017; Polo-Cavia et al., 2017). The negative effects of climate change are not exclusive to montane amphibians, there is evidence to suggest a wide range of species and taxa are also affected including birds (Northrup et al., 2019), mammals (Soultan et al., 2019), reptiles (Logan et al., 2015), fishes (Talloni-Alvarez et al., 2019), invertebrates (Kingsolver et al., 2013) and plants (Mariani et al. 2019). The advantages of the golden mantella research on the wider scale is that the project provides yet more evidence, if it were needed, that anthropogenic induced climate change coupled with habitat destruction/fragmentation has the real potential to drive species to extinction. What the golden mantella research also set out to do was provide evidence to inform the specific needs of the species and relate that to current or future habitat. In doing so we are better placed to deal with immediate threats such as habitat loss and provide greater protection to the most appropriate areas. In producing species distribution models we may also be able to help mitigate for future threats such as climate change by highlighting areas classed as climatically stable.

6.7 Future research and recommendations

Several authors directly involved with golden mantella conservation have made recommendations for future conservation measures (Andreone et al., 2006; Gardner et al., 2014; Bletz et al., 2015; Edmonds et al., 2015; Piludu et al., 2015; Harding et al., 2016). However, perhaps a new and detailed order of hierarchal importance prioritising the measures is needed for clarity.

Habitat destruction is the most pressing problem facing the species. A three-year study by Piludu et al. (2015) has shown that most (over 50%) golden mantella forest habitat is still under threat from either agricultural expansion, mining or frog collection. Therefore, increasing the protection of existing breeding ponds and surrounding habitat should be made the first priority. However, realistic and effective protection of species and habitat can really only be achieved by engaging with local communities (Ormsby and Kaplin, 2005). Indeed, the 2011 – 2015 Species Conservation Strategy (SCS) stated that there was a lack of awareness by local people as to the conservation status, legal status, biology and ecology of golden mantellas (Randrianavelona et al., 2010). The report goes on to suggest that this is due to a lack of easily accessible results and that research results are usually published in overly technical or foreign languages (Randrianavelona et al., 2010). This is important, as Ormsby and Kaplin (2005) found that an increase in knowledge of conservation issues was positively correlated with favourable attitudes towards conservation management strategies. The SCS report points out that there had also been minimal benefit to local communities faced with extremely challenging environments and economic circumstances. According to Fiallo and Jacobson (1995), poverty in rural areas intensifies the need for natural resources found in forests in protected areas. Unfortunately, golden mantella breeding ponds are located in forests that are targeted for the expansion of farming and gold mining activity (Piludu et al., 2015).

Research by Gardner et al. (2015) demonstrated the highly dynamic and complex nature of livelihood change within communities living near protected habitat. Gardner et al. (2015) go on to advocate both developing and monitoring flexible management strategies to rapidly deal with livelihood changes in communities surrounding protected areas as they occur. Understanding local community perceptions and interactions with protected areas are therefore critical components of any future management strategies (Fiallo and Jacobson., 1995; Ormsby and Kaplin., 2005; Yang et al., 2015). Therefore, along with Gardner et al. (2015), I recommend exploratory meetings with farmers operating on the edges of protected areas in order to ascertain if existing farmland could be improved or used in a more productive and sustainable way. This kind of intervention has been shown to work on several occasions in Madagascar (Andrianaiavo et al., 2002; Barrett et al., 2004; Tsujimoto et al., 2009) and may help decrease the need for farmers to expand into forested areas containing golden mantella habitat.

The second priority should be to monitor local climate and climate change effects on current habitat (Piludu et al., 2015). During this project I have already identified, by way of SDM, several areas that could be described as climatically stable within the current area of occurrence of the golden mantellas. I would therefore also recommend that surveys be conducted to ascertain future viability and the feasibility of expanding protection to new areas highlighted in chapter five. This could also require habitat restoration in these areas to improve microhabitats and breeding areas. Such information can then inform potential future conservation actions such as sustainable collection of individuals (Robinson et al., 2018), translocations, re-introductions or the siting of further wildlife corridors or protected areas.

Without protecting golden mantella and their current habitat their numbers will continue to decrease. However, ongoing climate change is a threat that cannot be mitigated for within most of the areas currently occupied by the species. Therefore, a two-pronged approach to golden mantella conservation needs to be applied. On one hand we must protect the species within its current location by keeping habitat intact. On the other hand, we must also assess the future viability of current mantella forest and where connections can be made to potentially climate stable areas under predicted climate change.

6.8 Conclusion

Madagascar is rich in biodiversity and endemic species, but is economically and infrastructure poor, a combination which means conservation intervention on the island is critical but both complex and arduous. Nevertheless, with such a vast array of biodiversity and species endemism at stake, it is a place worth making substantial efforts to protect. The human population of Madagascar increased from 16 million to 24 million during the period 2000 – 2015 and is predicted to continue to around 40 million by 2045 (Vieilledent et al., 2013; Vieilledent et al., 2017). This continued and rapid population growth is positively correlated with increased and progressive rates of deforestation on the island (Vieilledent et al., 2013). Climate change may well aggravate and intensify an already critical situation if predictions for rises in temperature and rainfall are proven to reflect reality (Hannah et al., 2008; Raxworthy et al., 2008; Tadross et al., 2008; Jones et al., 2019).

The first step in attempting to mitigate for the effects of climate change on Malagasy amphibians is to provide evidence on species' responses to rising temperatures and associated hydrological regime change. This is no small problem and will continue to require a concerted and joint effort between local and international, in situ and ex situ organisations in the future. However, the golden mantella conservation initiative has a real chance of being successful, in most part due to the hard work of local people and in-country conservation organisations. The way in which we ex situ organisations can help is by providing infrastructure and resources for research, advice, financial assistance and focussing public opinion internationally. In this way we may, as a collective force, be successful in preventing the extinction of this iconic, charismatic and beautiful Madagascan frog.

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7.1 Appendix 1

Supplementary results to chapter 3

Modelling was carried out by using a protocol developed by Zuur et al. (2009) pages 90-91 and 129 -142 used in conjunction with R. Zuur et al. (2009) suggest the following steps. **Step 1**; plot standardised versus fitted residuals to check if the assumption of homogeneity is valid. A variation in residual pattern is indicative of heterogeneity and so the advised **step 2** is to fit a general least squares model for comparison. **Step 3;** add variance structure i.e. incorporate room as a random factor. **Step 4**; compare models using AIC. **Step 5;** add Maximum Likelihood (ML) estimation to the model. **Step 6**; an ANOVA is used on the ML model and non-significant terms are dropped. **Step 7**; The ML model before and after the non-significant term is dropped are compared. **Step 8**; The final model (the lowest AIC scoring ML model from step 7) is re-fitted with Restricted Maximum Likelihood Estimation (REML). The overall process proposed by Zuur et al. (2009) is a way of validating whether mixed modelling is appropriate for specific data analysis, and if so, which modelling method is best. **Step 9**; add LOESS smoothers to show that data follow a non-linear distribution. **Step 10**; General Additive Modelling with smoothing terms on **a)** temperature and **b)** humidity.

Below I give the R script I used and the results generated at each stage of the process.

Step 1: I initially applied a simple linear model (slm):

```
library (nlme)

library (lme4)

slm <- lm(Activity~Temp+Humidity+Time, data = dframe1)#

plot(slm, select = c(1))
```

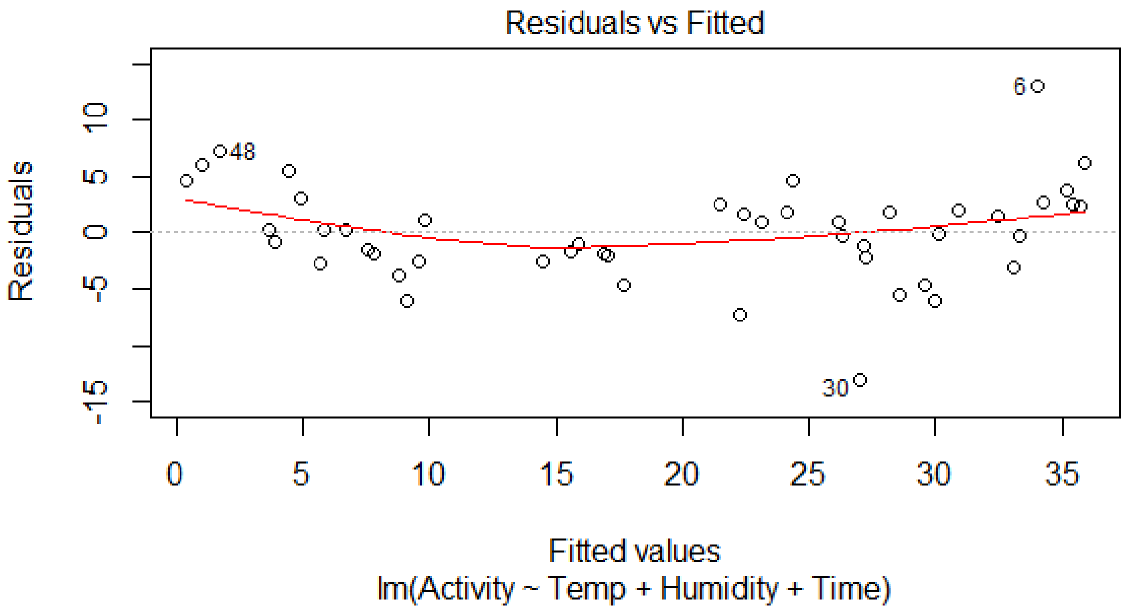


Figure 1: A simple linear regression model where Activity is based upon temperature, humidity and time. Residuals versus fitted values. The residual spread increases for larger fitted values which indicates heterogeneity (Zuur et al., 2009).

Step 2: For model comparison fit the model using a GLS (General least squares)

```
form <- formula(Activity~Temp+Humidity+Time)

m.gls<- gls(form, data=dframe1)

summary(m.gls)
```

```
     AIC         BIC          logLik
 224.0912    253.5493      -85.0456
```

Step 3: Add variance structure i.e. room is added as the random component and models are compared.

```
model1 <- lme(form, random = ~1|froom, method = "REML", data = dframe1)

summary (model1)
```

```
      AIC        BIC       logLik
  223.1484    253.6976    -83.57419
```

Step 4: Compare models m.gls and model1

```
anova (m.gls, model1)
```

```
            Model    df      AIC         BIC         logLik      Test     L.Ratio     p-value
m.gls         1      27    224.0912    253.5494    -85.04560
model1        2      28    223.1484    253.6976    -83.57419   1 vs 2   2.942826     0.0863
```

lme model (model1) improved on the lm (m.gls) , L = 2.940, df = 1, p = < .0864

AIC dropped from 224.0893 to 223.1496 which justified using the mixed model approach.

Step 5: Add ML to model

```
m1.full <- lme(form, random = ~1|froom, method = "ML", data = dframe1)

summary (m1.full)
```

```
Linear mixed-effects model fit by maximum likelihood
 Data: dframe1
      AIC         BIC          logLik
  328.2315     380.6251     -136.1157
```

Step 6: ANOVA is used on the model with ML (m1.full)

```
anova (m1.full)
```

```
            numDF   denDF    F-value     p-value
(Intercept)   1       21    18.244346    0.0003
Temp          1       21     6.439342    0.0192
Humidity      1       21    10.819006    0.0035
Time         23       21     1.049393    0.4581
```

Time is not significant (p = 0.459) and so is removed using shorthand code for dropping a variable e.g:

```
m1.a<- update(m1.full, .~.-Time)
```

Step 7: The old model with Time (m1.full) is compared to the new model without Time (m1.a)

```
anova(m1.full,m1.a)
```

```
         Model  df   AIC       BIC       logLik     Test    L.Ratio    p-value
m1.full    1    28   328.2315  380.6251  -136.1157
m1.a       2     5   316.1407  325.4967  -153.0703  1 vs 2  33.90916   0.0665
```

Removing Time improved the model i.e. AIC decreases from 328.2315 to 316.1407

Step 8: Final model refitted with REML

```
form <- formula(Activity~Temp+Humidity)#creating shorthand use form

model1 <- lme(form,random = ~1|froom, method = "REML", data = dframe1)

anova(model1)
```

```
            numDF    denDF     F-value      p-value
(Intercept)   1        44     18.810236     0.0001
Temp          1        44      6.444054     0.0147
Humidity      1        44     10.612774     0.0022
```

```
summary (model1)
```

```
     AIC              BIC              logLik
  313.3241         322.3574         -151.6621
```

Justified in using a mixed effect model Re-fitted with REML as AIC decreased from 316.1407 to 313.3241

Step 9: We fitted data with LOESS smoothers to better show the pattern in data points, it shows there is a non-linear relationship.

```
library(ggplot2)

 plot1<-ggplot(dframe1,aes(Temperature.in.Celcius,Total.number.of.frogs.seen,))

 plot1+geom_point()+theme_classic()+labs(x ="Temperature in Celcius",y = "Total number of frogs seen")+stat_smooth(method = loess,level= 0.95,aes(fill=room))


plot2<-ggplot(dframe1,aes(Humidity,Total.number.of.frogs.seen,))

plot2+geom_point()+theme_classic()+labs(x ="% Humidity",y = "Total number of frogs seen")+ xlim= c(0,10,20,30,40)+ stat_smooth(method = loess,level= 0.95,aes(fill=room))
```

According to Zuur et al. (2009) Gamm is designed specifically for modelling non-linear so we applied the following in R.

Step 10: General Additive Modelling with smoothing terms on temperature and humidity.

a) Smoothing term on Temperature GAM and output

```
library (mgcv)

n.gam <- gam(Activity~ s(Temp)+Humidity,

          random = list(froom=~1),data = dframe1 )

summary(n.gam)

Family: gaussian
```

```
Link function: identity

Formula:
Activity ~ s(Temp) + Humidity

Parametric coefficients:
                Estimate     Std. Error      t value        Pr(>|t|)
(Intercept)     -24.9982      24.8499        -1.006          0.3207
Humidity          0.4985        0.2768         1.801          0.0796

Approximate significance of smooth terms:
            edf       Ref.df         F           p-value
s(Temp)    7.346      8.305         33.81        <2e-16 ***


R-sq.(adj) =   0.86   Deviance explained = 88.4%
GCV = 26.358  Scale est. = 21.226    n = 48
```

```
plot(n.gam)
```

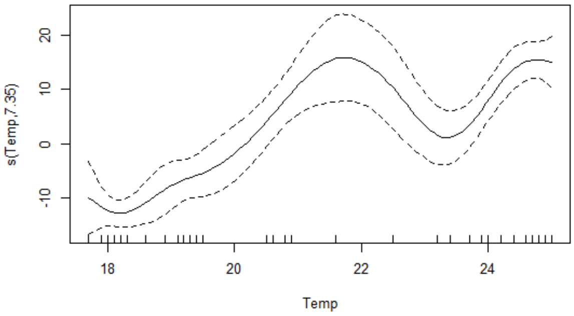


Figure 2: Estimated smoother for the additive mixed model. The solid line is the estimated smoother and the dotted lines are 95% point wise confidence bands. The x axis shows temperature in Celsius; y axis shows the contribution of the smoother to the fitted values, the smoother is centred around 0.

```
anova(n.gam)

Family: gaussian
```

```
Link function: identity

Formula:
Activity ~ s(Temp) + Humidity

Parametric Terms:
              df     F       p-value
Humidity  1     3.242     0.0796

Approximate significance of smooth terms:
              edf     Ref.df      F        p-value
s(Temp)   7.346   8.305      33.81      <2e-16
```

b) Smoothing term on Humidity GAM and output

```
n.gam <- gam(Activity~ s(Humidity)+Temp,
           random = list(froom=~1),data = dframe1 )
summary(n.gam)
Family: gaussian
Link function: identity

Formula:
Activity ~ s(Humidity) + Temp

Parametric coefficients:
                 Estimate   Std. Error     t value      Pr(>|t|)
(Intercept)    -58.8652     6.6357        -8.871       3.49e-11 ***
Temp               3.7635     0.3158       11.919      4.53e-15 ***

Approximate significance of smooth terms:
                   edf      Ref.df    F       p-value
s(Humidity) 3.945    4.885     8.86     5.59e-06 ***
```

```
R-sq.(adj) =  0.825   Deviance explained = 84.3%
GCV = 30.215  Scale est. = 26.473    n = 48
```

```
plot(n.gam)
```

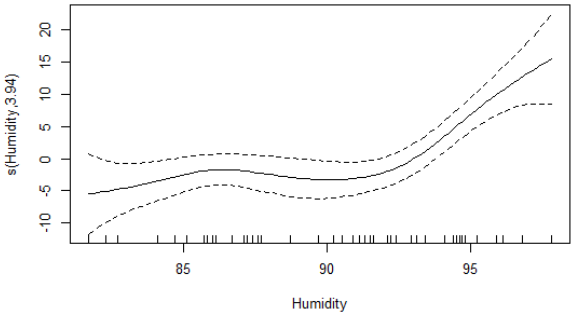


Figure 3: Estimated smoother for the additive mixed model. The solid line is the estimated smoother and the dotted lines are 95% point wise confidence bands. The x axis shows temperature in Celsius; y axis shows the contribution of the smoother to the fitted values, the smoother is centered around 0.

```
anova(n.gam)
Family: gaussian
Link function: identity

Formula:
Activity ~ s(Humidity) + Temp

Parametric Terms:
          df       F        p-value
Temp      1      142.1       4.53e-15

Approximate significance of smooth terms:
               edf      Ref.df        F        p-value
s(Humidity)   3.945     4.885        8.86      5.59e-06
```

7.2 Appendix 2

Supplementary results to chapter 5

Appendix 2 outlines the steps taken and outcomes during Maxent modelling i.e. **Step 1**: We used a regularization multiplier, Prior to running final models, we adjusted the regularization multiplier and selected the most appropriate model from Akaike Information Criteria (AIC) (Table 1). **Step 2**: To determine drivers of distribution, we jack-knifed environmental data. **Step 3**: maximum test sensitivity plus specificity logistic threshold for all distributions to identify areas of highly suitable habitat. **Step 4**: Habitat suitability and validation of variable contribution. The Habitat Suitability Index e.g. how suitable an area is for a species based upon the variables entered into the model was calculated. **Step 5**: To describe the current golden mantella area of occurrence we developed a Minimum Convex Polygon (MCP) based on the raw data for *M. aurantiaca* occurrences and then added a 10 km buffer to create an over-estimate of current area.

Step 1: **Regularization multiplier**

We ran 3 models each time changing the regularization multiplier, i.e. we varied the amount of noise (error) in the model. Because data were collected by Madagasikara Voakajy and are verified from surveys, we would expect that the default value in maxent for the reg multi regularization multiplier (rm =1) would be appropriate, but to be sure, we ran three models (Rm=1, Rm=2, Rm=3). if the AIC score had become more favourable for rm = 3, i.e. AIC had decreased, we would have run more models until the lowest AIC was reached.

Table.1: We ran 3 models, each time changing the regularization multiplier, this produced an AIC value for each model. The model with the lowest AIC value is Rm = 1.

Regularization multiplier (Rm)	Log Likelihood	AIC	BIC
=1	-6152.711308	12435.42262	12698.65529
=2	-6227.322903	12514.64581	1263613781
=3	-6254.776772	12547.55354	12624.49848

Step 2: Jack-knife tests

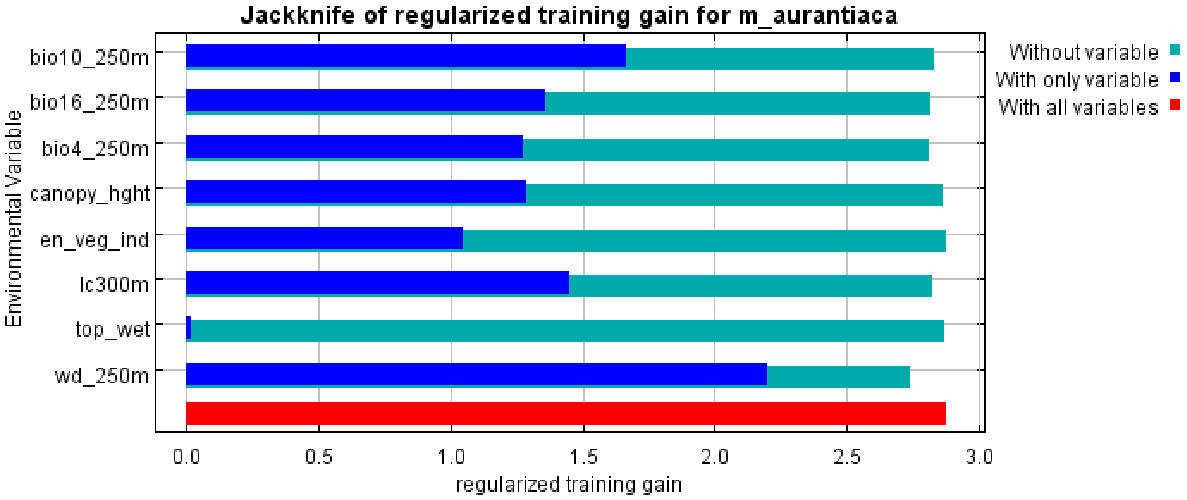


Figure 1: Results of the jack-knife test of variable importance. The environmental variable with highest gain when used in isolation is wd_250m, which therefore appears to have the most useful information by itself. The environmental variable that decreases the gain the most when it is omitted is wd_250m, which therefore appears to have the most information that isn't present in the other variables. Values shown are averages over replicate runs.

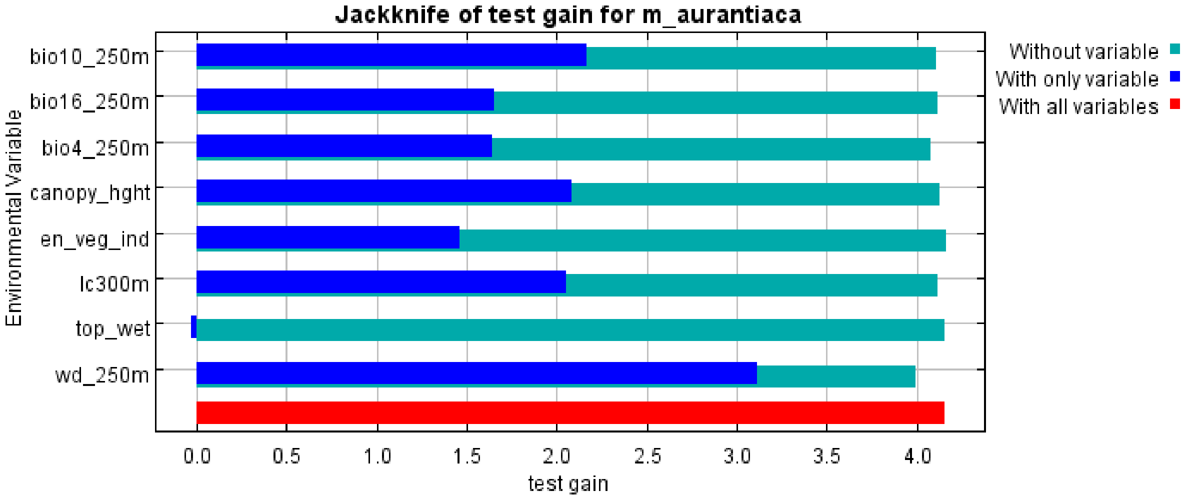


Figure 2: Shows the jack-knife test using test gain instead of training gain. Note that conclusions about which variables are most important can change when using test data.

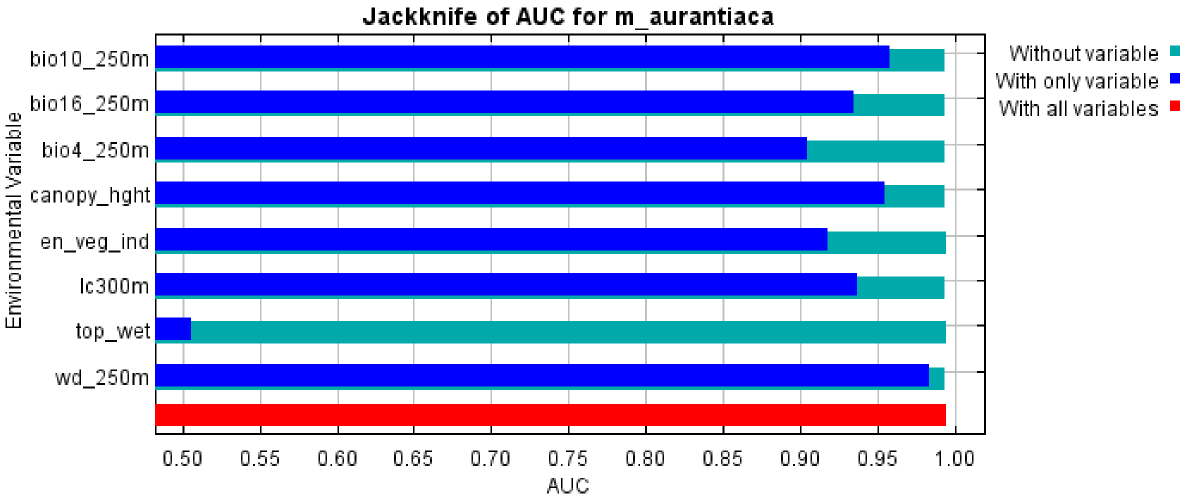


Figure 3: Shows the jack-knife test using AUC on test data.

In the analysis of variable contributions, wd is very clearly the most important variable in the model, if it is removed, the model suffers, if it is the only variable, the model is still good. Together, lc (Landcover) and wd (Water Deficit) are the most important variables influencing the model (Table 2), and of the two, WD is perhaps the most important (Figs. 1, 2 and 3).

Table 2: Gives estimates of relative contributions of the environmental variables to the Maxent model (Variable code descriptions can be found in table 1 chapter 5). To determine the first estimate, in each iteration of the training algorithm, the increase in regularized gain is added to the contribution of the corresponding variable or subtracted from it (if the change to the absolute value of lambda is negative). For the second estimate, for each environmental variable in turn, the values of that variable on training presence and background data are randomly permuted. The model is re-evaluated on the permuted data, and the resulting drop in training AUC is shown in the table, normalized to percentages. Values shown are averages over replicate runs.

Variable	% Contribution	Permutation importance
Lc 300m	32.1	3.1
Wd_250	31.2	3.1
bio16_250m	8.6	16.6

bio10_250m	24.3	2.7
bio4_250m	2.2	73.7
canopy_hght	1.5	0.6
top_wet	0.1	0.1
en_veg_ind	0	0

Step 3: Maximum Test Sensitivity plus Specificity Logistic Threshold (MTSST)

We used Maximum Test Sensitivity plus Specificity Logistic Threshold (MTSST) for all distributions to identify areas of highly suitable habitat (Fig.4). MTSST is the most balanced choice according Liu et al. (2005) and minimises error between specificity and sensitivity (false positives and false negatives). The MTSST gives a threshold value which is specific to the model being run only – there is no ‘universal’ value. To get the value, open the maxent results CSV file and select the average value given at the bottom of each specific column. The value changes for each climate scenario, i.e. current climate = 0.2724, rcp45 = 0.2711, rcp85 = 0.2574. Each MTSST value is used to omit completely unsuitable habitat from the species average distribution map (See: Fig. 5).

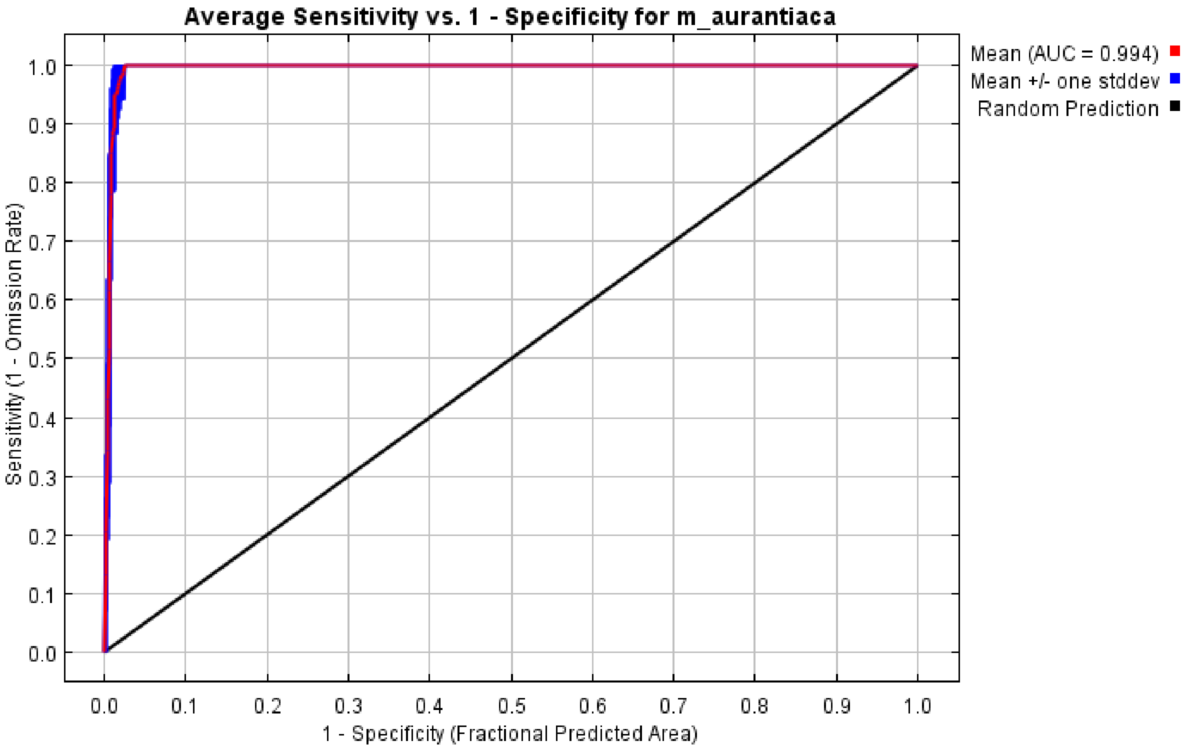
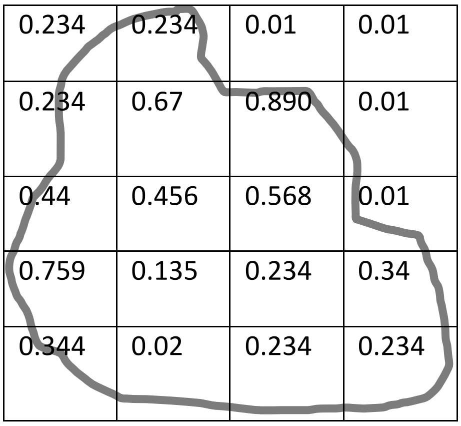


Figure 4: Receiver operating characteristic (ROC) curve averaged over the replicate runs. Note that the specificity is defined using predicted area, rather than true commission. The average test AUC for the replicate runs is 0.994, and the standard deviation is 0.002.

Step 4: Habitat Suitability

We then used a threshold value to create areas of high likelihood of occupancy within the buffered MCP and the area of potential climate space was assessed (km^2). Maxent produces the Habitat Suitability Index as a logistic output (probability of presence). So 1 = highly suitable habitat, 0 = unsuitable habitat. This is where MTSST cuts all values below the threshold value out of the map. An example is given in fig.5.



0.234	0.234	0.01	0.01
0.234	0.67	0.890	0.01
0.44	0.456	0.568	0.01
0.759	0.135	0.234	0.34
0.344	0.02	0.234	0.234

Figure 5: An example of how a Habitat Suitability Index is used to predict species distribution. The grey line is a hypothetical map outline, each grid square has a suitability value produced by maxent. It could be that the maxent html file indicates that water deficit contributes 88% to the final model driving observed distribution i.e. how a species responds to water deficit across its range. The values on the map are then transferred onto a graph for the main driving variable (See: fig 1, Chapter 5).

Step 5: Minimum Convex Polygon (MCP)

To describe the current golden mantella area of occurrence we developed a Minimum Convex Polygon (MCP) based on the raw data for *M. aurantiaca* occurrences and then added a 10 km buffer to create an over-estimate of current area (Fig.6).

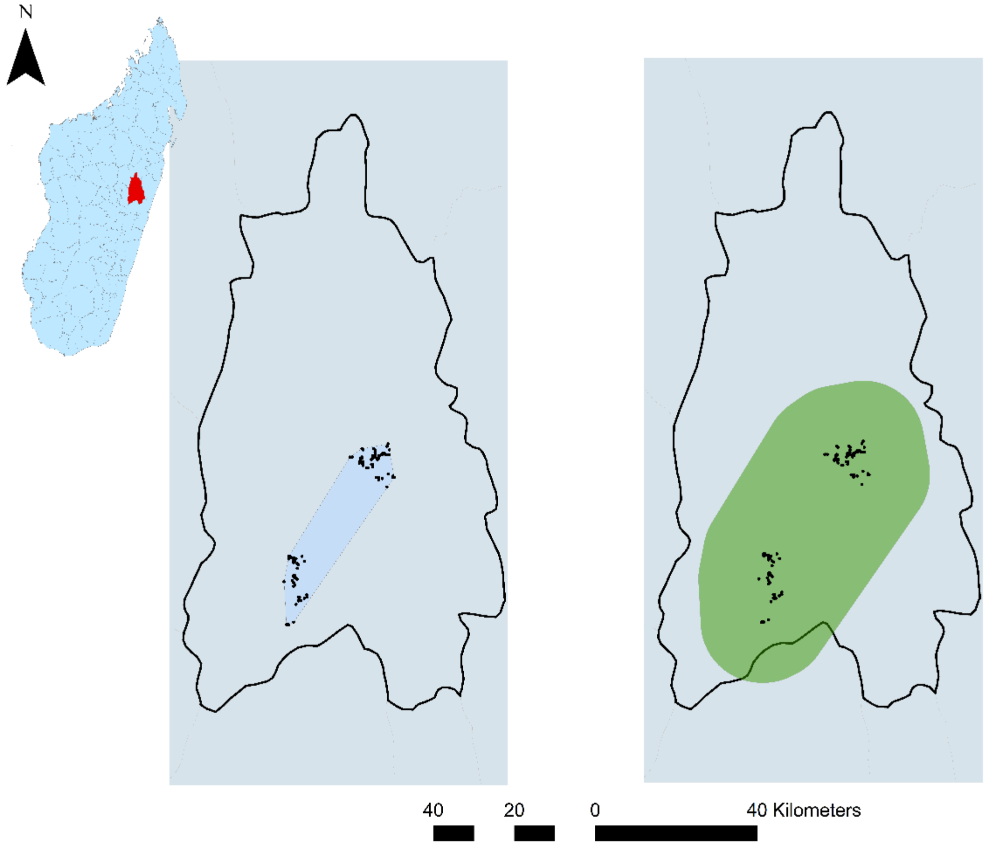


Figure 6: Shows the geographic position of the district (red) and both larger maps are zoomed in. The left-hand large map shows the data (black dots) with the MCP as light blue. The right-hand large map shows the buffered MCP (green) and black dots show original data.