Breeding biology and habitat utilization of the Long-toed Tree Frog (*Leptopelis xenodactylus*)

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ABSTRACT AND KEY TERMS

Leptopelis xenodactylus Poynton, 1963 is a little-known member of the *Leptopelis* genus. It was discovered in 1963 and, since then, minimal work has been carried out on the species. It is listed as Endangered and its distribution restricted to a small area in the Kwa-Zulu-Natal mountain grassland. By means of extensive field work over three seasons, using various techniques which included passive acoustic monitoring, visible elastomer implants, species distribution modelling and ground-truthing to answer specific research questions, this study was able to shed light on many aspects of the secretive frog's biology, ecology and life history. Insight was gained on the distances travelled relating to the frog's movement within and around the wetlands. Two call types were identified and described, along with two variations of these calls and their possible uses. The distribution of calling throughout the recorded period was examined to define the most prolific calling periods on both a monthly and a nightly basis. Observations were made on the mode of amplexus used by this species as well as nest placement, egg dimensions and development, clutch size and dates of laying. Development of tadpoles was also monitored. 19 new localities were identified from the first iteration of the species distribution model and ground-truthing, with plans to re-run the model using the new localities, updated environmental parameters and more updated regularization methods. Recommendations are made for conservation measures and management strategies that could be implemented to better protect and conserve the species as well as the wetlands they inhabit. Finally, suggestions are given of areas where future work on this species would be most beneficial.

Key terms: *Leptopelis xenodactylus*, passive acoustic monitoring, visible implant elastomer, species distribution modelling, reproductive biology, high elevation grasslands

OPSOMMING EN SLEUTELTERME

Leptopelis xenodactylus Poynton, 1963 is een van die meer onbekende spesies binne die genus. Dit is in 1963 ontdek en sedertdien het die spesie min aandag geniet. Die verspreiding is beperk tot ‘n klein area in die KwaZulu-Natal berg grasvelde en is gelys as ‘n bedreigde spesie. Deur gebruik te maak van tegnieke soos passiewe akoestiese monitering, merk van individue deur elastomeer-inplanterings, spesieverspreidingsmodellering met daaropvolgende terrein-verifieëring, kon hierdie studie lig werp op aspekte van biologie, ekologie en lewensgeskiedenis. Insig is verkry oor die migrasie en habitatsbenutting van die spesie in vleilande binne hul verspreiding te bestudeer. Twee tipes roepe is geïdentifiseer en beskryf, tesame met twee variasies van hierdie roepe en hul moontlike gebruike. Die profiel van hierdie roepe is ondersoek vir die duur van die aktiewe seisoen met die oog daarop om seisoenale sowel as sirkadiese patrone te identifiseer. Waarnemings is ook gemaak oor die tipe ampleksus wat deur hierdie spesie gebruik word, asook die morfologie van eiers en die plasing van die eiers binne die omgewing. Eiers is gemeet, getel en die ontwikkeling noukeurig bestudeer en gedokumenteer. Ontwikkeling van paddavissies is ook gemoniteer. Gebaseer op die modellering van verspreidingsmodelle is 19 nuwe lokaliteite vir die spesie bevestig. Gebaseer hierop gaan Ezemvelu opvolgmodellering doen, wat ‘n nog beter begrip van die verspreiding van die spesie sal verseker. Aanbevelings word gemaak vir bewaringsmaatreëls en bestuurstrategieë wat geïmplementeer kan word om die spesies sowel as die vleilande wat hulle bewoon beter te beskerm en te bewaar. Ten slotte word voorstelle gemaak met betrekking tot aspekte van die biologie en lokaliteite waar toekomstige werk op hierdie spesie moet fokus.

Sleutelterme: *Leptopelis xenodactylus*, passiewe akoestiese monitering, elastomeer-inplantering, spesieverspreidingsmodellering, voortplantingsbiologie, hoogliggende grasvelde

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

1.1.1 Frogs and humans

“Though probably few people regard frogs and toads as subjects of any special interest and possibly many look upon them as slimy abominations… to those who take the trouble to study the forms and habits of these humble and harmless creatures, perched on the evolutionary ladder between fish and reptiles, a subject of very considerable interest is unfolded” (Rose 1962).

Some 350 million years ago, amphibians were the first vertebrate group to colonize terrestrial habitats and, in so doing, have outlived the dinosaurs. They are an important group of vertebrates for many and varied reasons. Through their ability to adapt to and overcome change, they have been able to survive, in significant numbers and great diversity, around the world today. They hold many keys to understanding not only how they adapt and function, but how other vertebrates have also evolved to thrive and exploit new realms. This is due to their ability to survive and reproduce in unstable environments and their ability to adapt from one habitat to a significantly different one (Schoch 2014). Whilst not the largest Class of vertebrates, species within the Amphibia have an impressive array of physical and behavioural adaptations and forms which inhabit aquatic and terrestrial niches, undergo metamorphosis and display an impressive variety of modes of locomotion, means of communication, parental care and navigational strategies. Compared to other clades, amphibians also provide essential “missing links” in vertebrate evolution (Iyer and Briggman 2021). Due to combinations of many unique morphological structures, physiological mechanisms and behavioural responses, frogs have adapted to life in nearly all terrestrial habitats, ranging from arctic tundra to some of the driest deserts and from below sea level to elevations of 5 000 m.a.s.l. (Duellman and Trueb 1994).

As of May 2022, 8,455 species of amphibians are globally recognized (Frost 2022). This number has increased by more than 2,000 species in the last 15 years, making amphibians one of the “fastest growing” vertebrate taxa (Dodd 2010). It is not that amphibians are speciating at a particularly rapid pace, but rather that we observe a renewed interest in amphibians, resulting in the description of many, previously unknown, species. Added to this is improved access to and ease of data collection in remote areas, along with an increased activity in scientific communities within megadiverse countries (Stuart et al. 2008). In the 1990s, firm evidence based on long-term monitoring data (Pounds and Crump 1994; Pounds et al. 2003), indicated that many amphibian populations were in decline. Although these initial studies were conducted in highland areas of America and Australia, the same trend was later observed in other ecological realms.

The Class of amphibians is split into three Orders – Urodela (salamanders), Anura (frogs) and Gymnophiona (caecilians) - with frogs comprising 7,468 of the 8,455 recognized amphibian species (Frost 2021). While lacking any salamanders or caecilians, southern Africa enjoys a spectacular diversity of frogs, with over 177 species, representing 13 families (du Preez and Carruthers 2021).

1.1.2 Frogs in the African context

Despite the benefits that frogs provide to people, they have an unfortunate reputation in many, if not most, cultures. They appear extensively throughout the world in literature, art, myths, legends and traditional medicine, but, almost exclusively, as symbols of evil, revulsion, disease, or punishment (Topsell 1608; Ady 1661; Miller 1979; Swenson 2020). In Africa they range from having been considered deities in Egypt, to being considered the devil incarnate in many other parts of the continent. Simultaneously, they are considered the harbingers of rain and good luck, along with being a culinary delicacy (du Preez and Carruthers 2017).

Lack of education and information has a major part to play in the way that African societies view amphibians. This is exacerbated by the reality that, until recently, there was very little literature available in local African languages. Measures are currently being put into place to change this, with the publication of the first bilingual amphibian field guide in 2017 (Phaka et al. 2017) and a bilingual primary school reader focusing on wetlands, with an emphasis on amphibians, in 2021 (Kyle 2021). These can, hopefully, lead to more similar works in other African languages in the future, slowly shifting the generally negative mindset of frogs in Africa, towards a more enlightened and positive one, as people begin to understand them and the benefits they provide to society, agriculture and ecosystems.

There are at least 788 species of frogs on the African continent (Channing and Rödel 2019), with more being discovered and described every year. This is partly due to the sheer size of Africa and a lack of taxonomists in the field of herpetology, but also, to the incredible diversity of habitats and massive physical features which have led to speciation and evolutionary change.

1.1.3 Importance of habitat for amphibians

With amphibians recognized as the most threatened vertebrate Class (Catenazzi 2015) the situation is dire for many amphibian species around the globe. A recent study suggests that the current percentage of amphibian species threatened with extinction is likely 47% worldwide - elevated from the previous estimate of 39% (Borgelt et al. 2022). Habitat destruction and transformation remain among the greatest threats to amphibian populations world-wide and there is a critical need to investigate the mechanisms which underlie patterns of distribution and abundance (Cushman 2006).

In a joint initiative, between the years of 2001-2004, the first Global Amphibian Assessment (GAA) was conducted. This was an attempt to comprehensively assess the known amphibian species world-wide and to accurately classify them on the IUCN Red List, as well as to review the causes of declines and to identify threats (ASG 2021). The GAA identified familiar threats such as habitat loss, chemical contamination and over-harvesting, but also recognized, with alarm, the novel threats of emerging infectious diseases and climate change (Stuart et al. 2008). Collins and Storfer (2003) responded to the global amphibian crisis by identifying six major causes of threats to amphibians. These were: habitat change, over exploitation, alien invasive species, global climate change, pesticides and environmental toxins and emerging infectious diseases with disease and habitat loss/transformation/degradation being recognized as the most serious threats to amphibians around the world (AmphibiaWeb 2021).

Anthropogenic disturbances are now unprecedented in intensity and spatial extent, affecting approximately 75% of Earth's land surface (Cuaron 2000). Research into habitat suitability for amphibians provides valuable information that assists with management and conservation of amphibian species (Dodd 2010). Suitable environmental conditions are particularly important for amphibians, especially at breeding sites, with most species needing to locate specific habitats for survival as well as breeding (du Preez and Carruthers 2017). Whether these sites are in streams, wetlands, grasslands, ponds, dams or rivers, they are directly affected by both the natural and anthropomorphically modified chemical and physical characteristics which, in turn, affect the amphibians' survival, growth, maturation and physical development. In addition to these direct impacts, they will also interact with other factors including predators, prey, parasites and competitors, all of which have impacts on populations (Dodd 2010).

The ideal habitat for an amphibian species includes a complex combination of climatic and geographic variables that interact to create a suitable environment for the species. Due to the passage of moisture through their skins, water is perhaps the most important variable for frogs, deeming all aspects that impact water, whether climatological or environmental, of critical importance (Dodd 2010). Weather parameters that influence frogs include temperature, precipitation, humidity and evaporation, while other aspects would include elevation, physical and chemical variables, vegetation type and soil type (Dodd 2010).

Several factors dictate where frogs may occur, with habitat requirements usually being species specific. Some families and species are more tolerant of wider ranges in environmental variables while others have very “narrow” requirements. Some frogs are more adaptable to a changing environment in the long term, allowing them to inhabit a wider variety of localities than those species that are not able to adapt to change or tolerate a range of environmental variables. Due to combinations of many unique morphological structures, physiological mechanisms and behavioural responses, frogs have adapted to life in nearly all terrestrial habitats, ranging from arctic tundra to some of the driest deserts and from below sea level to elevations of 5,000 m.a.s.l. (Duellman and Trueb 1994).

Frog skin is a complex organ which, in many ways, defines and dictates the environmental parameters frogs can tolerate and those in which they thrive. Their skin is important, not only with regard to temperature regulation, water relations and gaseous exchange, but is also relevant to current evaluations of amphibian declines in the context of disease susceptibility and transmission, impacts of xenobiotics and sensitivity of responsiveness to climate change (Dodd 2010). Being ectothermic, frogs are very sensitive to changes in abiotic conditions and this not only impacts where they can occur, but also what times of the day and season they are active (Dodd 2010). Their skin is semi-permeable, which allows carbon dioxide to be expelled and oxygen to be absorbed through it and also allows for moisture regulation through osmosis (du Preez and Carruthers 2017). Amphibians can avoid excessive water loss by selecting microhabitats that provide sufficient moisture to balance water loss with water uptake from the environment (Wells 2007).

The physiological interactions of amphibians with their abiotic environment is a complex, dynamic system of related processes (Duellman and Trueb 1994). While many species have adapted in various ways to become less dependent on water, most amphibian species still have a close relationship with water for survival as well as reproduction (du Preez and Carruthers 2017). Amphibians handle their water balance through morphological and physiological adaptations as well as behavioural modifications (Wells 2007). Adaptations include behavioural avoidance of harsh environmental conditions and physiological adaptations that increase desiccation tolerance, increase thermal tolerance and decrease cutaneous evaporative water loss (Hillman et al. 2009). Behavioural avoidance behaviors include diurnal retreat, seasonal aestivation, fossoriality and scansoriality (Hillman et al. 2009).

The diel and seasonal activities of amphibians are regulated by environmental conditions, principally moisture and temperature (Duellman and Trueb 1994). Due to their complex life cycles and their movement and behavioural patterns within a season, frogs often require a wide variety of habitats for their various life stages and activities. This necessitates the protection of not only the breeding sites but also the sites where they hunt or over-winter (Baldwin et al. 2006). In many species these habitat-use patterns are seasonal, with areas being entirely unused for a large percentage of the year and yet being critical for the species as, for example, breeding or over-wintering sites (Dodd 2010). Requirements differ among species, but shelter and food are mandatory components (Duellman and Trueb 1994). In most species, successful reproduction usually relies on aquatic habitats for the eggs and larvae, while the juveniles and adults rely more on the terrestrial landscape. This terrestrial landscape is also particularly important in the non-breeding season for thermoregulation, hydroregulation, dispersal, migration and overwintering (Blomquist and Hunter 2009). Newly metamorphosed frogs disperse from the breeding site, sometimes travelling large distances, in their search for suitable habitat. Some species are very specific in the conditions that they can inhabit which can lead to large concentrations of young frogs in limited areas of ideal conditions, which may, in turn, lead to high mortality rates (Patrick et al. 2008). Amphibian populations respond not only to landscape composition, but also to landscape configuration (Dodd 2010).

Due to their bi-phasic life cycle, most amphibians have both aquatic and terrestrial habitat requirements. This aquatic habitat includes lentic (ponds or wetlands) and lotic (streams or rivers) environments. The spatial and temporal variability and systemic connections between the aquatic habitat and the surrounding terrestrial landscape, are key features that structure amphibian communities (Dodd 2010). Physical variables that affect the aquatic environment for frogs include morphometry (habitat's shape and structure), hydroperiod (timing and duration of the habitat holding water), hydrologic connectivity (the extent to which aquatic environments are linked) and flow intensity (velocity and variability) (Dodd 2010).

Chemical variables within the aquatic environment can also significantly impact amphibian growth, survival and behaviour. These pollutants can be toxic or can act as endocrine disrupters and impact the life processes, for example, sexual development (Dodd 2010). Vegetation is a major component of terrestrial environments and influences many factors including thermal gradients, relative humidity, moisture retention, wind flow and habitat suitability due to the provision of shelter or refugia. Aquatic vegetation affects the productivity and the structural and chemical suitability of a habitat (Dodd 2010). Many amphibians spend a significant proportion of their lives underground and, as such, edaphic features are also important aspects of their environment - soil pH, moisture, temperature and texture all play important parts in habitat suitability (Dodd 2010).

1.3.1 Land use impacts on amphibians

A combination of interacting mechanisms result in a suite of attributes that adapt a species for existence under a set of environmental variables (Duellman and Trueb 1994). Human-induced transformation of landscapes alters the structure and function of ecosystems (Vitousek et al. 1997). This in turn has its impacts on the biodiversity of these ecosystems, with frogs in particular - due to their sensitivity - being affected. In recent years more attention has been paid to these impacts and changes but, what is becoming clearer, is that broad studies often allow impacts on important individual species to slip through the cracks without being noticed (Thompson et al. 2015). To fully appreciate and document the changes that occur, an overall understanding of ecosystems and community breakdowns is essential when research of this nature is conducted. For example, the presence of healthy populations of a species of frog does not necessarily mean that the ecosystem is healthy, as unnaturally skewed amphibian communities can be a cause for serious concern. Continued habitat loss and expansion of agricultural and urban land uses in the coming years will likely continue to shift species composition towards communities dominated by generalist and disturbance-associated species (Thompson et al. 2015). Great abundances and diversity of species in altered habitats does not necessarily indicate that those habitats are capable of supporting stable communities into the future. Long-term studies that evaluate population dynamics and trajectories should be conducted in altered habitats. Populations in altered habitats can often be sinks that are actually in decline (Thompson et al. 2015).

1.3.2 Climate change impacts on amphibians and their habitats

The decline of amphibian populations world-wide requires increased physiological studies relating to threats such as climate change (Dodd 2010). Climate is of particular significance due to the impact it can have on reproduction, invasive disease outbreaks and their spread across landscapes, transmission processes and population declines (Dodd 2010). Climate, however, only “loads the dice” - it does not dictate when and where declines will occur and whether or not they will continue. It exerts its influence within the constraints of history, geography, natural history and potential interacting agents (Pounds 2001). Given the complexity of dynamic ecological systems, it is argued that invoking single stressors, or risk factors, to explain amphibian population declines may, in general, be too simplistic (Blaustein and Kiesecker 2002). Differences in susceptibility to stressors depend on numerous variables including life stage, species, population, geography, weather parameters, water chemistry, history of experiencing particular stressors and numerous other factors (Blaustein and Kiesecker 2002).

Trying to predict the impact that climate change will have on a species of frog within an area is challenging as the impacts will often be indirect, unclear, and progressive. In several case studies, for example, the depth of water in which eggs and tadpoles develop has decreased as a result of climate change. This, in turn, has changed the amount of UV-B to which they are exposed, thus increasing their vulnerability to disease and increasing their mortality rates (Kiesecker et al. 2001). This highlights how difficult it is to predict the impacts of climate change and what factors need to be considered when it comes to different species - especially those that are endangered or at high risk.

1.3.4 Disease

The chytrid fungus, which was discovered in 1998 in Australia and Central America during mass amphibian die offs, has resulted in emerging infectious diseases becoming prominent in the world of amphibians in recent years. These were the first cases of a chytridiomycete fungus attacking vertebrates (Berger et al. 1998). Since then, the disease causing organism was described as *Batrachochytrium dendrobatidis* (Bd) - the amphibian chytrid fungus (Longcore et al. 1999).

From the offset Bd has been recognised as an unprecedented threat to amphibian biodiversity. It has infected over 500 species of frog in 54 countries, on all the continents where amphibians occur. Some areas in Central America have already lost over 40% of their amphibian biodiversity which, in turn, has effects and ramifications on entire ecosystems (Fisher et al. 2012). When first discovered, it was thought to be one of the most challenging threats to herpetological conservation in particular and wildlife conservation in general, that the world had yet faced (Cunningham 1998). Fifteen years later, it was still considered one of the greatest threats faced by any vertebrate group (Fisher et al. 2012). Bd is easily spread around the globe by human activity and it is able to persist in the environment on non-susceptible species, giving it the greatest potential to date to cause dramatic amphibian extinctions (Bishop et al. 2012). Bd has shown how catastrophic a single threat can be.

1.2 *Leptopelis* Günther, 1859

1.2.1 Description

African tree frogs belong to the family Arthroleptidae, which includes squeakers (*Arthroleptis* Smith, 1849) and tree frogs (*Leptopelis)* (Frost 2022). *Leptopelis* were first described by Albert Günther in 1858 in his "Catalogue of the Batrachia Salientia in the collection of the British Museum". This classification was based on differences in their vertebral structure and the shape of the tongue. Three specimens were in the collection that he was describing, one from Gabon, one from Ashantee and one from "Africa". The description of the genus included vomerine teeth, smooth skin on the back, discs large, toes webbed and fingers lightly webbed, tympanium distinct, moderate eustachian tubes and a heart-shaped tongue - slightly nicked behind, with the males having an external sub-gular sac; and they are from Africa. These first *Leptopelis* all turned out to be *L. aubreyi* (Günther 1858).

Interestingly, in 1882, Boulenger reconsidered the situation and placed *Leptopelis* as a synonym of *Hylambates* (on what Poynton believed to be inadequate grounds) and it was only in 1924 that Noble revived the former genus again (Poynton 1964).

Directly translated from Greek, "*Leptopelis*" means narrow pelvis and refers to their narrow "waist" (du Preez and Carruthers 2017). According to the most recent literature, there are 54 species of *Leptopelis* in Africa (Frost 2022), occurring throughout the continent south of the Sahara to the Transkei (South Africa), with the exceptions of Namibia, Botswana and the driest areas of South Africa (Poynton and Broadley 1985).

In appearance the genus is easily identifiable and unmistakable, although they display an impressive diversity among the species. They range from having well developed digital discs to these being absent; webbed fingers to not webbed fingers and toes webbed strongly in some, while very weakly in others (Poynton and Broadley 1985). They range from green, smooth skinned, arboreal species with webbing and discs, to brown, warty-skinned fossorial species with hind feet adapted to digging. They are robust, medium to large frogs, with long slender limbs and strong toes used for climbing and digging (Schiøtz 1999).Their toes can have extremely adhesive terminal discs, facilitating their agility manoeuvring through leaves, branches and grass stems (Passmore and Carruthers 1995). The inner metatarsal tubercles are prominent and often flange-like and digital intercalary cartilage is present (Poynton 1964). Vomerine teeth are present, as well as a well-developed gular sac without a gular flap (Schiøtz 1999); the vocal sac is internal (Lambiris 1988), while the tympanum is conspicuous (Passmore and Carruthers 1995). They have vertical pupils (Poynton and Broadley 1985) in large protruding, forward facing eyes (Passmore and Carruthers 1995) which facilitate their nocturnal behaviour. The omosternum is not forked (Lambiris 1988) and is said to be entire to notched posteriorly (Poynton and Broadley 1985) and the metasternum is a narrow to broad cartilaginous plate (Poynton 1964).

1.2.2 Distribution

Throughout their distribution, limited to Sub-Saharan Africa (AmphibiaWeb 2021), they are mostly arboreal with a savanna-living trend showing in several species within the genus and tendencies to become fossorial (Poynton and Broadley 1985). Those that are fossorial have adapted - reducing their digital discs and developing flattened metatarsal tubercles - which they use for digging backwards into the substrate. Cocoon forming in *Leptopelis* is a recognised adaptation employed to overwinter or to survive drought (Vitt et al. 2001). Some are essentially ground-dwelling species, well adapted to burrowing (Minter et al. 2004).

1.2.3 Sexual dimorphism

Males are smaller than females and produce characteristic and often bi-syllabic calls. Physical combat is not uncommon in large choruses of competing males - usually during or after rainfall events (Passmore and Carruthers 1995). The presence/absence of pectoral glands in males is a seasonal feature in certain species of this genus (Schiøtz 1999). The gular region of males is dark in colour.

1.2.4 Reproduction

The eggs of *Leptopelis* species, that have been studied thus far, have been reported to be large, yolky (Poynton and Broadley 1985) and lightly pigmented (Passmore and Carruthers 1995), with oviposition either at ground level (Poynton and Broadley 1985), or underground, in close proximity to water or where rainfall puddles will develop (Schiøtz 1975). The tadpoles, though slow developers, are described by Linden (1971) as powerful and elongated when they emerge, breaking the egg casing by thrashing their tails, while Passmore and Curruthers (1995) described them as being sluggish with long tails. They are able to make their own way to the water after hatching, even navigating substantial obstacles (Schiøtz 1999). Poynton notes that they reached the water by wriggling and jumping, but only once the yolk has been fully absorbed (Poynton

1964). Their navigation towards the closest water body is influenced by relative humidity and topographical gradients (Oldham 1977). Wager (1986), however, theorised that they used their sense of smell. The tadpoles are benthonic and belong to the generalist pond-type (Passmore and Carruthers 1995). A long, pointed, rather shallow tail, the continuity of the super-angular and infra-angular oral papillae without an angular notch intervening and the two rows of oral papillae across the mentum are all characteristics of *Leptopelis* tadpoles across the genus (Van Dijk 1966). The juveniles are an overall green colour, turning browner as they mature (Poynton and Broadley 1985). There are indications that at least one species has direct development, without an aquatic phase (Schiøtz 1999).

1.2.5 Vocalisation

The vocalisations of this genus are usually either a short, sharp “clack” or a longer, buzzing call and several species use a combination of both. In some forest species the “clack” noise is composed of numerous harmonics close together, giving it an odd acoustic quality. It is hypothesized that their “clack” is an advertisement call to attract females while the buzzing is more of a territorial call (Schiøtz 1999). Wager describes the buzzing part of the call as the frogs being wound up (Wager 1986).

1.2.6 Identification

The different species of *Leptopelis* are notoriously difficult to distinguish due to similar morphology (Poynton and Broadley 1985) and, in many cases, overlapping ranges. Their differential characteristics within the genus are described as being exceptionally difficult to discern (Poynton and Broadley 1985). Accurate identification of species requires investigation of a combination of aspects including appearance, vocalization, behaviour and preferred habitat. Many species within this genus have two colour forms, green and brown, with or without patterns. In many cases, as mentioned above, the juveniles are green and turn brown as they mature, often developing a darker pattern on their backs (Schiøtz 1999; Poynton and Broadley 1985). Lambiris describes them as a troublesome genus, saying, further, that “morphological characters and colouration tend to be unreliable in differentiating between the various taxa” and going on to say that “extensive field studies are urgently needed” (Lambiris 1988).

1.2.7 In South Africa

South Africa falls within the range of three *Leptopelis* species - *Leptopelis natalensis* (Smith, 1849), *Leptopelis mossambicus* (Poynton, 1985) and *Leptopelis xenodactylus* (Poynton, 1963) and as Passmore and Curruthers (1995) noted, the colloquial name of “tree frog” is more suited to some species than others (Figure 1.1). *Leptopelis natalensis and L. mossambicus* are typical arboreal species while the *L. xenodactylus* is a grassland and wetland fringe, partly fossorial species and the subject of this study (du Preez and Carruthers 2017).

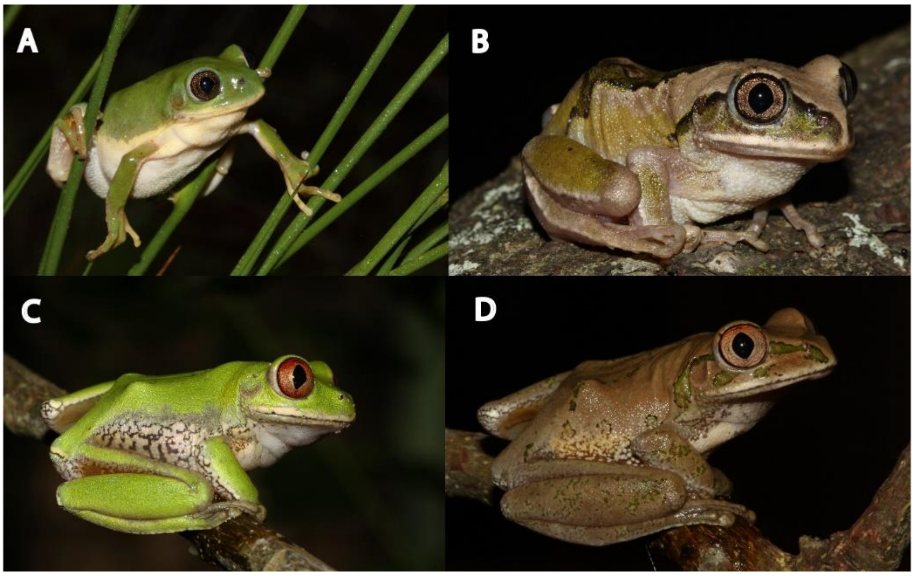

Figure 1.1 Different *Leptopelis* that occur in South Africa (A - *L. xenodactylus*, B - *L. mossambicus,* C - *L. natalensis* green form, D – *L. natalensis brown form*).

1.3 Leptopelis xenodactylus

1.3.1 Introduction

The first *L. xenodactylus* was collected in March 1961 by L. Franklin at the Natal Parks Board trout hatchery in Underberg, Kwa-Zulu Natal (Figure 1.2). It was deposited at the Natal Museum where J.C. Poynton recognized it as something new and the species was described from this single specimen (holotype no: 1147), which was a gravid female (Poynton 1963). The species has remained elusive since then with little information being gathered or documented. Van Dijk (1966) theorized that, “It is quite probable that *L. xenodactylus* will be found to be a synonym of *L. bocagei* or a subspecies of this species; *Leptopelis bocagei haasi* Mertens shows variation from the typical form of about the same order as *L. xenodactylus*”. This is in direct conflict with Poynton (1963) where he acknowledged that it was undesirable to describe a species from a single specimen but “the present specimen is so distinctive that there is no possibility of its belonging to any previously known species”. Van Dijk went on to describe it as a doubtful species but this was found to be wrong and, ironically, he later described the second specimen that was found in 1978 at Franklin in East Griqualand (Van Dijk 1978). The specific name “*xenodactylus*” is translated as “strange toes” and refers to their elongated digits (Channing 2001).

Figure 1.2 *Leptopelis xenodactylus* holotype, collected in 1961, housed at the Natal Museum, Pietermaritzburg.

1.3.2 Distribution

The species is endemic to South Africa, mainly inhabiting high elevation grasslands in the province of KwaZulu Natal, but is thought to possibly extend marginally into the Eastern Cape Province (du Preez and Carruthers 2021). It was theorized by Bates and Haacke (2003) that they may also occur in Lesotho but this has not been confirmed. The second Red Data Book listed their distribution as being limited to the upper plateau slopes of western Natal (Branch 1988). Grimsdell and Raw placed them in the “Mist belt” and “Highland” bioclimatic region according to the regions laid down by Phillips (1973; Grimsdell and Raw 1984). These grasslands include moist upland grasslands, short mist-belt grasslands and north-eastern mountain grasslands which all occur between 1,000 m and 1,830 m above sea level (du Preez and Carruthers 2017). In his account, Poynton (1964) described their range as being most likely southern Natal at least, above 1,330 m and in open country. Armstrong predicted that *L. xenodactylus* would occur in higher rainfall areas in the central and western grasslands (Armstrong 2001). In a paper that examined past predicted distributions, it was modeled that six thousand years ago *L. xenodactylus*’ distribution was smaller than present day, while at 21 thousand years ago, they had a larger distribution than it now is (Schreiner et al. 2013).

1.3.3 Habitat

Carruthers hypothesized that *L. xenodactylus* left its arboreal past and developed adaptations to live in the tree-barren marshes of the Drakensberg foothills. This habitat is frequently covered in snow in winter and yet inundated with floodwater in summer, which creates a suitable breeding habitat – largely free from competition (Carruthers and du Preez 2001). The species is limited to temperate marshlands on the south-eastern escarpment where it occurs on, or beneath, the surface of the substrate (Passmore and Carruthers 1995). Measey noted that it does not occur on the steep slopes of the escarpment (Tolley et al. 2011). Lambiris describes it as being a “wetland ground-dweller” (Lambiris 1988). According to Branch it used small areas of seasonally inundated, open grassland within forested areas (Branch 1988). Channing notes that the species inhabits marshy areas that have high grass polls (Channing 2001) which Minter et al. (2004) described as “hammock bog” in grasslands and presumed to be their typical breeding habitat. Poynton, in his original description, concluded that the morphological adaptations made “perfectly good ecological sense” for living in the grasslands and marshes (Poynton 1963). One of the larger known populations inhabits a wetland that consists of eroded channels with an abundance of small, grassy islands (Minter et al. 2004).

1.3.4 Diagnostic characteristics

Poynton’s original description of the species states “internarial distance less than interorbital distance. Tibiotarsal articulation reaching tympanum. Distance from nostril to tympanum approximately 1/3 length of foot, length of hand equal to intertympanic distance. Tips of fingers and toes flattened, but not broader than the proximal parts of the digits. Length of inner metatarsal tubercle equal to length of inner toe. Webbing (except margin) only present at base of fingers - not reaching middle subarticular tubercle of fourth toe on either side; reaching distal tubercle on outer toe. Dorsal surface a completely uniform green. Ventral surface immaculate” (Poynton 1963).

Described as being cryptic and not commonly encountered (Tolley et al. 2011), long-toed tree frogs are fairly large, robust frogs, reaching a maximum size of 60 mm in length. They have long toes with diminutive terminal discs on both fore- and hind limbs (Passmore and Carruthers 1995) and the inner metatarsal tubercle on the hind limb is of moderate size and flanged for digging. They have a blunt snout and large, brown eyes that protrude from the skull. The tympanum is prominent but less than half the diameter of the eye (Channing 2001). The inter-toe webbing is more extensive than that of *L. mossambicus*, but less than that of *L. natalensis,* with the fingers and toes both being noticeably longer than the other two species (Passmore and Carruthers 1995). Most sources agree on a uniform granular green dorsum and a granular, creamy ventrum (Poynton 1963; Passmore and Carruthers 1979; Lambiris 1988; Branch 1988; Passmore and Carruthers 1995) while du Preez and Curruthers (2017) add the occasional presence of bright yellow spots on the dorsum and a yellowish wash on the ventrum. Van Dijk described the second specimen found as being bright green dorsally, off-white ventrally and characterized by smallish discs at the end of the digits, the long toes and each hind foot having a digging spur (Van Dijk 1978). The Red Data Book lists their “uniform green colouration with absence of any dark marks on the sides of the face” as well as their “long slender digits and very reduced webbing” as distinguishing features (Branch 1988). An early record from 1978, where they were simply called

“Green *Leptopelis*”, describes the webbing on the fingers as being “rudimentary, not reaching the middle subarticular tubercle of the fourth toe” (McLachlan 1978).

Lambiris, after examining eight specimens, concluded that they are uniform grass-green above and creamy white beneath, with no facial mask. They have small, weakly developed, digital discs while the digits themselves are elongated and the toes are barely webbed. He estimated the inner metatarsal tubercle to be about as long as the proximal phalange of the first toe and stated that it was weakly flanged (Lambiris 1988).

In the first Red Data Book, published in 1978, *L. xenodactylus* was described as rare and known only from the one specimen and it was concluded that further work was needed to define both distribution and status (McLachlan 1978). The 1988 Red Data Book describes them as being elusive and probably uncommon and goes on to mention that it is a terrestrial species that spends the daylight hours in burrows or concealed under vegetation. It also theorizes that their breeding may be similar to *L. bocagi* (Günther, 1865), breeding in shallow puddles in inundated grassy areas (Branch 1988).

1.3.5 Breeding behaviour

Eggs are laid in soft mud on the edge of wetlands and the tadpoles wriggle into the water once hatched (Channing et al. 2012). The first specimen collected was a gravid female found in March, which, in Poynton’s first account (1963), suggested autumnal or winter breeding; while a year later, in his second account, the same information was used to suggest that they aestivate (Poynton 1964).

The tadpoles (Figure1.3) are found at high elevation in shallow, muddy water between grass polls in bogs and seeps. The earlier descriptions of the tadpole described them as attaining a maximum length of 40.2 mm, with nostrils closer to the eye than the snout and golden iridophores that extend the length of the tail (Channing et al. 2012). A more recent description states that they reach 50 mm in length, with the long, muscular tail taking up more than two thirds of the total length. The nostrils are closer to the snout than the eye. They are uniformly dark brown to black with silver pigments above and the underside is semi-transparent with scattered pigment. The gut is visible through the ventral skin. The mouth is anteroventral with one row of papillae at the mouth corners and two rows below the mouth. The jaw sheaths are moderate and slightly curved (du Preez and Carruthers 2017).

Figure 1.3 Tadpole of *Leptopelis xenodactylus* (Photo: L.H. du Preez).

“Further investigation into distribution, population size, breeding biology and threats are required…” (Tarrant 2012). “Priorities for conservation work include determination of dispersal, requirements in terms of terrestrial habitat and distribution together with categorizing potential threats. The relationship between this and other species without toe-discs needs to be studied. This species would make a good candidate for monitoring” (IUCN 2017). “Little is known about the life of this rare frog…” (Carruthers and du Preez 2001).

1.3.6 IUCN listing

53 species of *Leptopelis* are listed on the IUCN’s Red List. Of these, 29 are listed as Least Concern, 3 are Near Threatened, 5 are Vulnerable and 7 are listed as Endangered, while a further 9 are Data Deficient (IUCN 2021).

According to the IUCN Red List, *Leptopelis xenodactylus* was listed as Rare in 1994, Vulnerable in 1996 and, finally, Endangered since 2004. Justification for this listing included limited area of occupancy (AOO), the severely fragmented nature of its sub-populations and the continuing decline in the quantity and quality of its habitat, number of sub-populations and number of mature individuals (IUCN ASG 2017). Further monitoring and research were suggested in order to better document movement and dispersal, along with identifying threats and the impacts that they would have on the species (Tolley et al. 2011).

According to the IUCN listing it is historically known from 18 localities, with an extent of occurrence (EOO) of around 11,000 km^2 and an AOO of 42 km^2. The AOO was estimated using the known breeding sites, 14 in number, five of which are in close proximity to each other, while the other nine are relatively isolated (more than 40 km apart) from the other sites. According to Tolley et al. (2011) the EOO is estimated at 10,567 km^2 with an AOO of 50 km^2 (0.5% of the EOO), based on 11 known localities.

There is a continuing decline in AOO due to wetland degradation and loss through agriculture, afforestation, dam construction and urbanization. Population fragmentation is considered a serious problem, with over 50% of the individuals being found in isolated patches with the distance between them thought to be too great for dispersal within one generation (IUCN ASG 2017).

It was suspected at the time of this listing that there were further unknown breeding sites that were yet to be discovered (Armstrong 2001). There was, however, concern that the populations in the Weza section may have become locally extinct, due, in part, to afforestation (J. Harvey pers com August 2016).

In the 2004 Red Data Book for Frogs of South Africa, Lesotho and Swaziland, habitat loss, habitat fragmentation, alien plants and afforestation were recognized as present and potential threats (Minter et al. 2004). By 2017, when the listing was made, the recognized threats for *L. xenodactylus* were afforestation, inappropriate burning regimes, cattle trampling, overgrazing and associated eutrophication of breeding sites, spread of alien plants that lower the water table resulting in the drying out of breeding sites, dam construction and urbanization (IUCN 2017).

The South African National Biodiversity Institute's recommended conservation action for this species was to determine its dispersal and distribution, along with categorizing threats. They also suggested that it was a good candidate species for monitoring and that the relationship between *L. xenodactylus* and other members of the *Leptopelis* genus without toe discs, be investigated (Tolley et al. 2011).

1.3.7 Conservation

In the 1988 Red Data Book, attention was drawn to afforestation, felling timber, veld burning and destruction of grassy wetland habitats as threats to this species (Branch 1988). It further theorized that *L. xenodactylus* appear to have specialized habitat requirements and is unlikely to be able to adapt at the rate at which many current environmental changes take place. Branch (1988) suggested that the known localities be demarcated and "be kept strictly free of any activity that may influence drainage, water table levels, vegetation changes, and insect population structure", further stressing the impact and damage that veld burning could have on their limited habitats.

In the most recent Red Data Book of Amphibians, Burger (2004) makes the point that, while the pre-recorded threats remained a concern, an additional concern was the lack of information available about this species' general biology. This, in turn, made it difficult to assess the conservation requirements and limiting factors. Burger (2004) recommended that further work be carried out to demarcate the actual distribution and describe the life history of these frogs and that known populations be protected from further habitat degradation and loss.

Armstrong (2001) conducted a study where he calculated that 19.72% of the known distribution of the species was within protected areas (uKhahlamba-Drakensberg Park) and, as such, was adequately protected. Tarrant (2012) describes how many of the known localities were within forestry areas which made them prime candidates for Stewardship programs.

Tolley et al. (2011) recommend that priority work for the conservation of this species would be to study the dispersal of the juveniles, as well as produce an accurate assessment of the distribution and list of the threats facing the species. Further stakeholder agreement and discussion of management would also be beneficial in areas where it occurs that are not within formally protected areas.

Due to its status as Endangered, some conservation measures have already been put in place by several organizations to monitor and protect this species. Fortunately, a significant number of the known localities that do not fall within the boundaries of established provincial protected areas, are located on district conservancies and well managed privately owned land. Ezemvelo KZN Wildlife has established a monitoring program for the species, along with efforts by the Endangered Wildlife Trust.

1.4 Problem Statement

Leptopelis xenodactylus is an Endangered species that is listed as such due to its restricted distribution, fragmented subpopulations and the decline in the quality of much of its habitat. Because of its extreme crypsis, and its limited active period, little is known about this species. The IUCN suggested that further work be carried out on this species to better inform the situation and how to protect it. There is a baseline with very little good science having been carried out on *L. xenodactylus* and much of what can be found in the literature is informed guesses based on what is known about other *Leptopelis* species. The problem is that it is very difficult to protect or evaluate a species about which so little is known.

1.5 Aims and objectives

The overall aim of this study was to investigate important aspects of the life history and ecology of *L. xenodactylus*, paying particular attention to aspects relevant to its conservation. The following questions were addressed:

1. What types of wetlands are inhabited by *L. xenodactylus*?
2. What is the probable extent of occurrence and area of occupancy of the species?
3. What environmental variables are important in initiating calling by males?
4. During which months do adult *L. xenodactylus* call? In which months should field surveys for the species be conducted?
5. What are the ideal weather conditions for conducting surveillance for the species?
6. What is call repertoire of *L. xenodactylus*?
7. Where are the females?
8. When and where do *L. xenodactylus* lay their eggs and how long do they take to hatch?
9. How much do they move within their wetland on a seasonal basis?
10. How can wetlands be managed to better protect this species?

CHAPTER 2

STUDY AREA, MATERIALS AND METHODS

2.1 Study Site

The known distribution for *Leptopelis xenodactylus* is limited to KZN, however, within this province it has a fairly extensive range, extending from Greytown in the north-west of its distribution, to near Richmond in the south and close to Himeville in the east (Figure 2.1). Historically, it is known from 18 localities, which were included in the study area and highlighted as areas of focus and interest. The study area was, however, expanded to include many possible wetlands within a certain elevational range, where it was thought the frog might occur. As such, the broader study area consisted of most of the Natal Midlands, with frequent trips to as many potential areas as was possible within the parameters of suitable conditions, available fieldwork assistants and the frog's limited annual active periods.

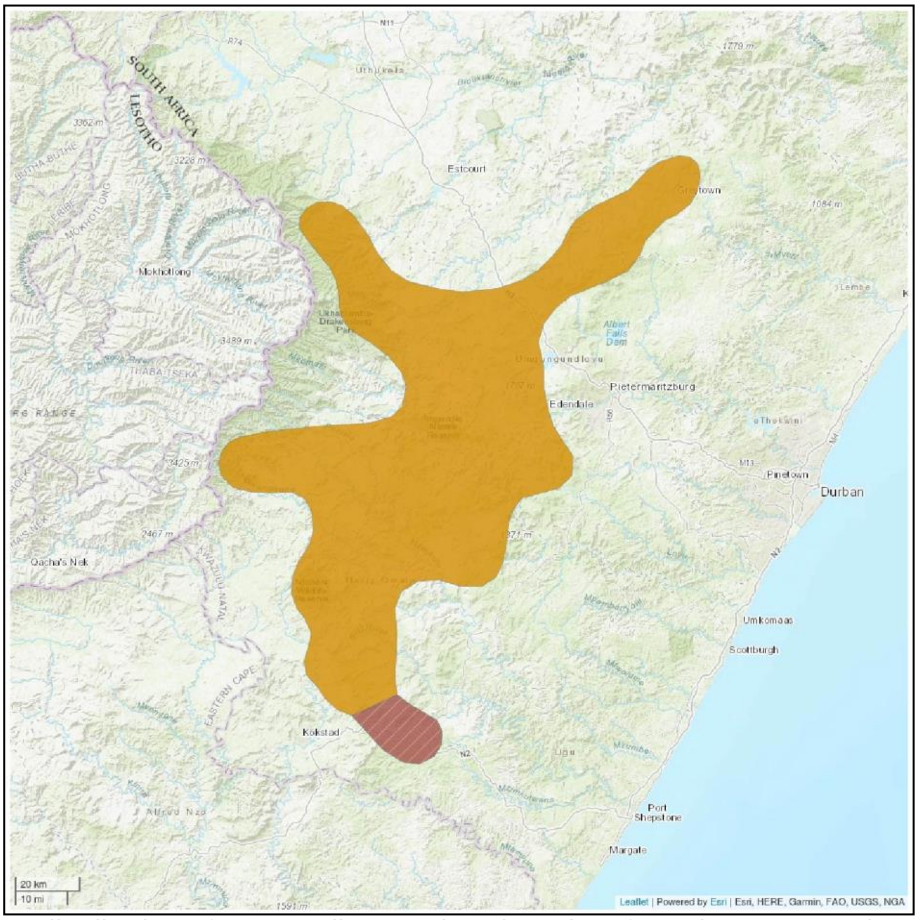

Figure 2.1 Known distribution of *Leptopelis xenodactylus* taken from the IUCN Red Data List (IUCN 2016), orange being the extant area while the red is possibly extinct.

The more intensive aspects of the project were conducted at Fort Nottingham Nature Reserve where a healthy and "protected" population of *L. xenodactylus* was known to occur from the commencement of the project. The area is a relatively safe one where fieldwork could be conducted without undue security concerns.

Fort Nottingham Nature Reserve (Figure 2.2) is managed by Ezemvelo KZN Wildlife, the provincial Nature Conservation Body and was proclaimed in 1909 as a Nature

Reserve to preserve a sample of the high-elevation, montane forest found within the region (Government Gazette No. 1522). The original area of 132 ha. includes a substantial section of indigenous forest, the *Leucosidea sericea* "belt" (which occurs on the periphery of the forest) and several small wetlands and grassland typical of the area. The reserve is surrounded by a diverse array of agricultural land uses which range from grazing for cattle, sheep and horses, to maize and other crops. To further protect the grasslands and wetlands, a further 1,227 ha. adjacent to the reserve, was proclaimed as a private "Stewardship Area" in 2015. The lowest point within the proclaimed area and close to the study area, is 1,470 m above sea level and the highest is 1,750 m above sea level. A desktop survey reveals that it is within the distribution of 34 species of amphibian (du Preez and Carruthers 2021). This area also falls within the Maputaland-Pondoland-Albany Biodiversity Hotspot.

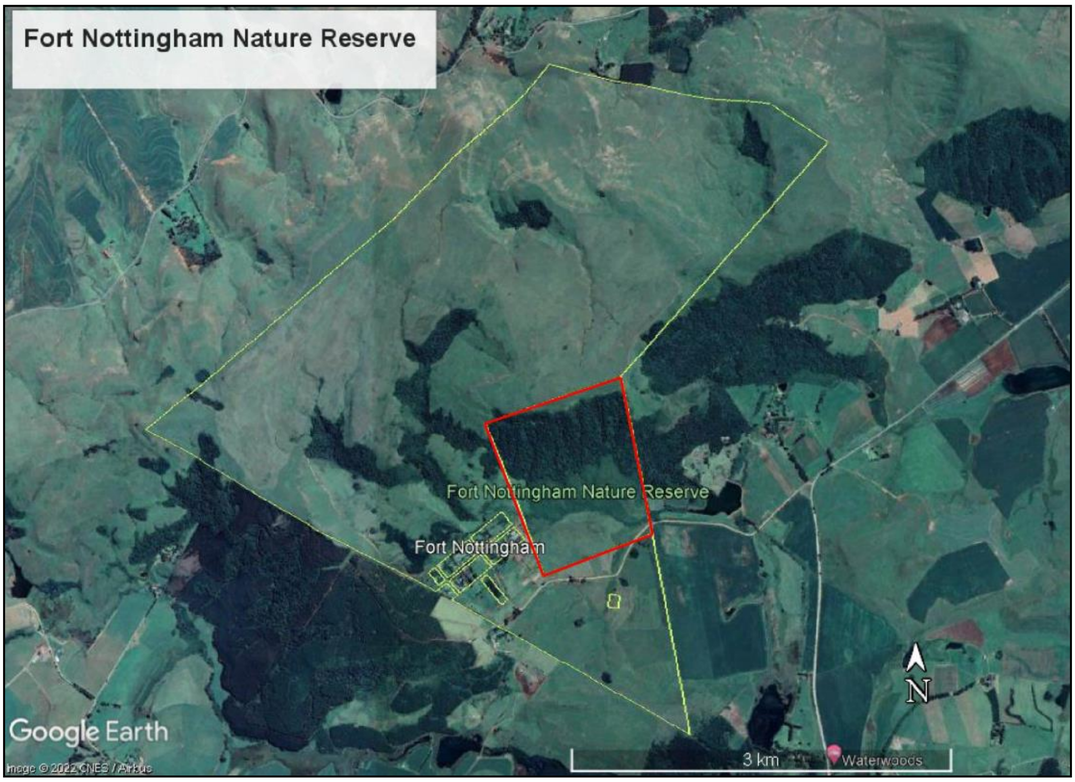

Figure 2.2 Google Earth® image showing the original demarcations of the Fort Nottingham Nature Reserve (red outlined area) as well as the extension made in 2015 (green outlined area); coordinates for the park entrance gate: Lat -29.4201, Lon 29.9118.

According to Mucina and Rutherford (2006) the Fort Nottingham Nature Reserve falls within the Mooi River Highland Grassland (Gs 8) vegetation unit. This vegetation type is characterized by a rolling and partially broken landscape, covered mainly by short bunch grasses, predominantly *Heteropogon contortus*, *Themeda triandra* and *Tristachya leucothrix.* The soil is generally shallow and poorly drained, derived from sedimentary rock, mostly of the Adelaide subgroup (Beaufort Group) of the Karoo Sequence. Some deep, well-drained apedal soils of intrusive igneous rocks also occur.

The region has a mean annual precipitation of 785 mm which falls mostly in summer, with frequent mist and infrequent snow. The mean annual temperature is 14 °C with frequently occurring light frosts for up to six months of the year (Mucina and Rutherford 2006). The vegetation includes many graminoids, herbs, herbaceous climbers and low shrubs.

The vegetation type is considered “Vulnerable” as its area has been reduced by nearly half and is considered poorly protected. The grassland biomes have the greatest annual habitat loss as well as the smallest area of habitat remaining and often relatively little area under formal protection (Jewitt 2018). Threats include transformation for cultivation (maize, beef and dairy farming), as well as commercial plantations (*Pinus* spp. and *Eucalyptus* spp.). Invasion and encroachment by alien woody species, such as American bramble (*Rubus cuneifolius*) and black wattle (*Acacia mearnsii*), are also a concern (Mucina and Rutherford 2006).

The area experiences radical differences between summer and winter weather conditions. As nearly all the rain falls in the warmer months the veld is green and lush in summer while, in winter, most of the vegetation becomes dormant and moribund (Figure 2.3). The Reserve usually experiences fires on an annual or semi-annual basis, both as controlled burns and as uncontrolled fires. The extent and severity of the fires vary with each season.

Figure 2.3 Google Earth® images showing seasonal changes in vegetation at Fort Nottingham.

According to the SANBI Classification system for wetlands and other aquatic ecosystems of South Africa, the study site at the Fort Nottingham Nature Reserve wetland is classified as follows. The spatial framework and regional setting selected is the Department of Water Affairs (DWA) Ecoregions, where it falls within the Eastern Escarpment Mountain, ecoregion 15 (Kleynhans and Louw 2008).The landscape unit is a valley floor. The hydrogeomorphic unit for the wetland at Fort Nottingham is complicated. On the upstream side of the road, it is an unchanneled, valley-bottom wetland, while on the downstream side of the road and culvert it is a channeled, valley-bottom wetland. It is also seasonally inundated and saturated. The substratum type is mineral soil, with a mixture of loamy and organic soils, throughout the wetland. It is a vegetated wetland of the herbaceous form, populated by geophytes, grasses, herbs/forbs, sedges, rushes and palmiets. The vegetation status of the wetland is indigenous. This classification is done at a medium confidence level (Ollis et al. 2013).

2.2 Ezemvelo KZN Wildlife Permit

Application was made and a permit was granted by Ezemvelo KZN wildlife for all the requirements of this project - including collection and mark and recapture. Ezemvelo permits are annual and, as such, new ones were required for each year of the project. For 2019-2021 work was carried out using stand-alone permits - numbers OP 1953/2019, OP 984/2020, OP 2422/2021 and in 2022 permit number OP 2731/2022 was used which is part of a larger project and grants the same permission.

2.3 NWU Ethics permission

The North-West University animal ethics committee, NWU-AnimCareREC, granted ethical clearance for the Project in 2019 and by fulfilling all the annual requirements, clearance is granted until June 2023 - Ethics Number NWU-00058-19-A5.

2.4 Study duration and fieldwork

The project was initiated in 2019 with September 2019 being the start of the first field work season. Methods were revised and updated several times during the study, resulting in only the 2020 season being used for the acoustic work. The majority of the field work was concluded in 2021, with only a small amount extending into September 2022.

Owing to the nocturnal nature of these frogs, most of the fieldwork was carried out in the early evening but extending well into dark. Being in a wetland environment, necessary precautions had to be taken to avoid potential situational dangers, for example, dangerous snakes or accidents in the treacherous terrain. Spring and summer in the Midlands are also prone to severe thunder and lightning storms that might not be predicted by the weather applications and that can move in rapidly. Working in wetlands surrounded by grasslands, such storms can pose a serious threat and need to be treated with respect.

Suitable clothing and foot-ware is required to protect the researcher from mostly exotic vegetation which includes American bramble and Scottish thistle along with discarded bits of metal and old strands of wire and fences are also frequently encountered in these areas. At the beginning of the project gumboots were predominantly used, but it was noted that they had a substantial impact on the wetland. They were also cumbersome and made it very challenging to navigate the thick mud. “Croc” type field shoes and Reef boots were found to be more effective and easier to maneuver and had considerably less impact on the wetland, whilst still affording protection. Strong lighting with long battery life was also essential for locating the frogs and conducting the measurements and other procedures required.

Security in remote, areas after dark, with valuable equipment was another concern. Fieldtrips were always conducted with more than one person and areas with human activity close by were usually avoided or driven past. Where possible, farming neighbourhood watches and security groups were informed of research activity in their area and, when accessing private property, landowner permission was obtained, and the landowner informed of each visit. In the field the group stuck together as much as possible and visual contact was constantly maintained with all members of the group.

Frogs were located using mainly three methods - active searching in the wetland and peripheries, searching for eyeshine in the surrounding taller vegetation and, most effectively, following auditory cues from calling males. Due to the females not calling, the first two methods were used with several females being found by their characteristically large eyeshines in the surrounding bramble bushes and the remaining females were found by active searching around and through the wetland. Many frogs were located using the active searching method whilst in pursuit of a calling male, as this was a laborious and time-consuming process.

Calling individuals are often extremely difficult to locate and require a protracted period of time to triangulate to within a close enough area to warrant closer inspection. Generally, this is carried out in the dark as, early in the season, the light tends to disturb them. Once fairly confident of being within about 30 cm of a calling male, torches are turned on and a search of all vegetation and depressions in the substrate is conducted in minute detail, usually revealing the male. This is not always successful, however, as it was found that they can call from underground burrows. They are unpredictable by nature and can, at times, call from high up in the vegetation and even from exposed perches, rendering them much easier to locate. Throughout the season they often responded to auditory prompts such as mimicry, or a pre-recorded call.

Once located, the frogs were handled only when necessary and as little as possible. The frogs are easy to gently restrain as they are ponderous and clumsy, showing no great desire to escape. They usually perch wherever they are placed for the duration of the process. Caution was always taken to ensure that they were handled with wet hands and restrained as little as possible. They showed no lasting negative response to the handling and usually resumed calling within minutes of being released.

As the wetlands are sensitive environments, care was taken to minimize the impact of all research activities conducted therein. Where possible, existing paths were used to circumnavigate the wetland until reaching the closest access point to a calling frog. To reduce impact to a minimum, older footprints were re-used to enter and exit the muddier sections. The frogs were observed to have a close relationship with the hummocks that are present in these wetlands and, as a result, movement was restricted to the deeper, wetter sections between the hummocks, rather than standing on the hummocks themselves in an attempt to stay dry or to navigate more rapidly. These small changes in fieldworker activity appeared to markedly reduce the impact that was visible on a wetland after work had been carried out.

2.5 Statistical analysis

Statistics were conducted using R Commander (Fox 2005) on the R platform (R Core Team 2013). A probability level of 0.05 was used throughout the statistical analyses, with a null hypothesis being rejected if the probability that it was true was less than 0.05.

CHAPTER 3

CLIMATE AT FORT NOTTINGHAM

3.1 Introduction

The prevailing weather conditions – climate – ultimately control the abiotic environment of an ecosystem. It determines the temperature and availability of water; it influences the natural vegetation and soil development and, consequently, the type of biological community that occurs there. Due to the direct influence that it has on moisture and temperature, climate determines rates of photosynthesis, decomposition and nutrient cycling. Climate, directly or indirectly, relates to the major functional and structural aspects of an ecosystem (Smith 1990). Climate is perhaps the most important factor that determines the geographical distribution of vegetation types as well as species. It also has great bearing on the flows of energy and material through ecosystems, as well as the properties of those ecosystems (Malcolm and Pitelka 2000).

Whether or not an organism can survive at a location is the result of a delicate balance between and complex interplay of biotic and abiotic factors. Ecology is the study of the relationship between living things and their biotic and physical environment (Villee 1977). With this in mind, it is clearly essential that any ecological study of an organism in an ecosystem takes the climate of that area into account.

3.2 Material and methods

The weather data for the duration of this project was recorded using a Davis Vantage Pro® 2 weather station with a MCS137 Logger, supplied and supported by iLeaf. It was located at GPS location -29.416295, 29.937303 on the Glenfern Estate Dairy Farm, within 3 kms of the Fort Nottingham study site (Figure 3.1) (See Chapter 2). The elevation is 1,472 m.a.s.l. and is situated on the edge of the road in flat topography.

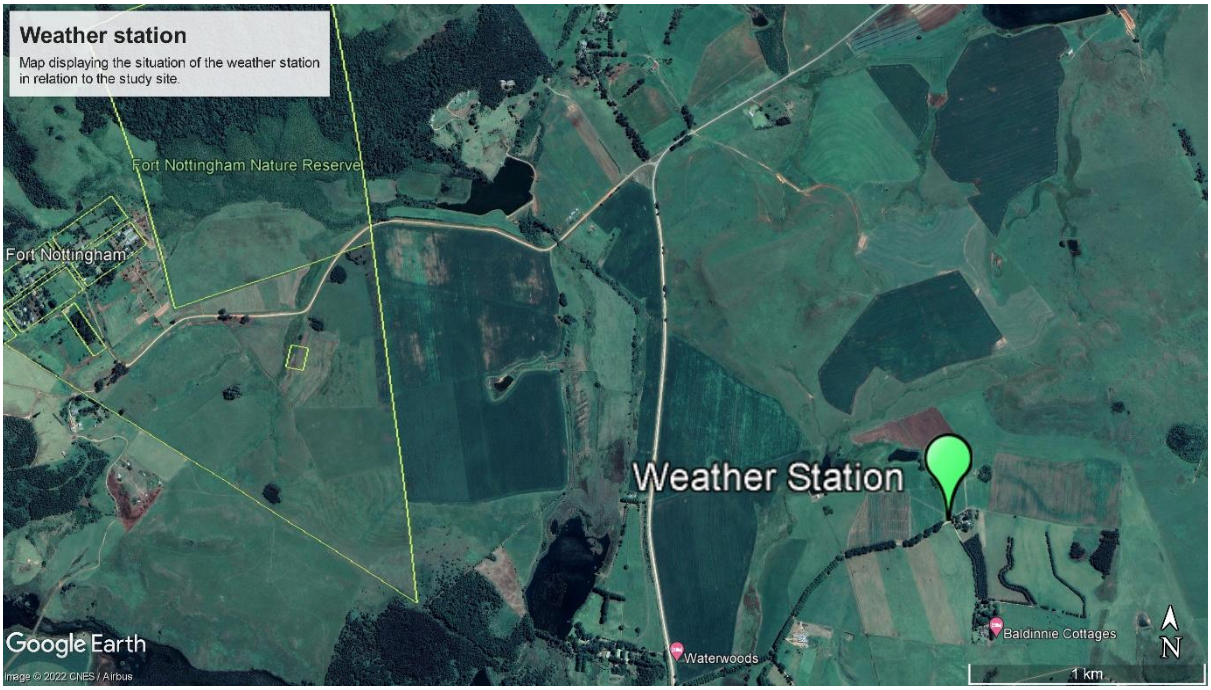

Figure 3.1 Google Earth® image showing the location of the study site in relation to the weather station (green pin) at Fort Nottingham for the 2020 fieldwork season.

Weather data were analysed for the duration of the active fieldwork period for the study site at Fort Nottingham. These weather data were accessed from the above-mentioned weather station and extracted on a monthly basis for the overall averages and annual data.

Monthly data were downloaded in an excel spreadsheet for the months from January 1st 2019 to December 31st 2021. Data retrieved were average temperature dry, daily maximum temperature, daily minimum temperature, average relative humidity, maximum relative humidity, minimum relative humidity and rainfall. Temperatures were recorded in degrees centigrade, humidity as a percentage and rainfall in millimetres.

3.3 Results

3.3.1 Rainfall

The mean annual rainfall for the three-year period (Jan 2019 – Dec 2021) was 905 mm per year with a minimum of 858 mm and a maximum of 949 mm, with most of it falling in summer (Figure 3.2). The maximum monthly precipitation during this period was in December 2021 when 250 mm was recorded.

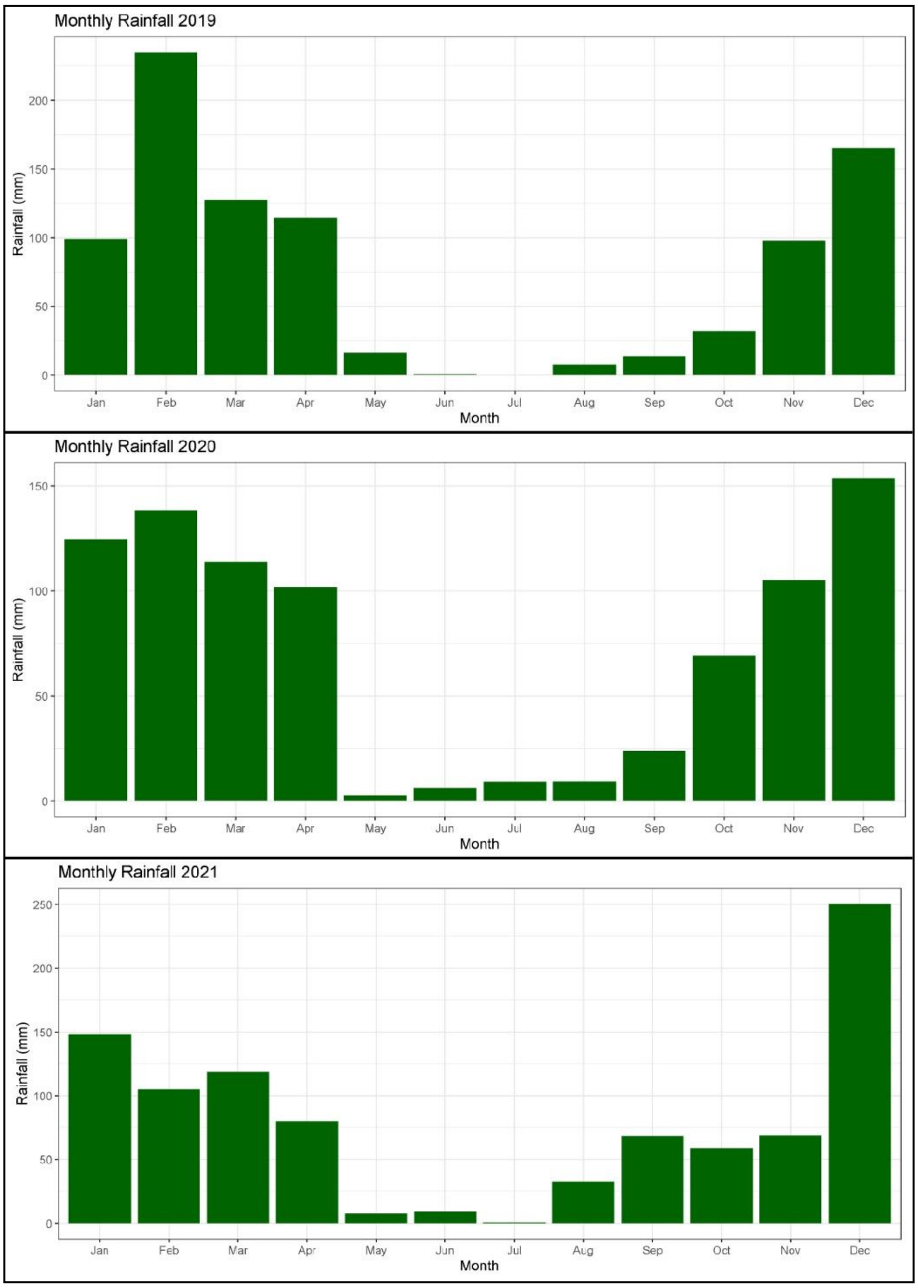

Figure 3.2 Monthly rainfall for 2019-2021 at Fort Nottingham Nature Reserve.

3.3.2 Temperature

Maximum temperatures for the area are relatively high, with daytime temperatures above 23 °C in each month, while minimums fall into the negatives in winter (Figure 3.3). The average temperatures reflect this trend with a dip during June and July, where the mean minimum temperatures were below zero. Warmer temperatures were experienced over the summer months. The maximum temperature during the study was 34.9 °C in October and the minimum was -2.3 °C in July.

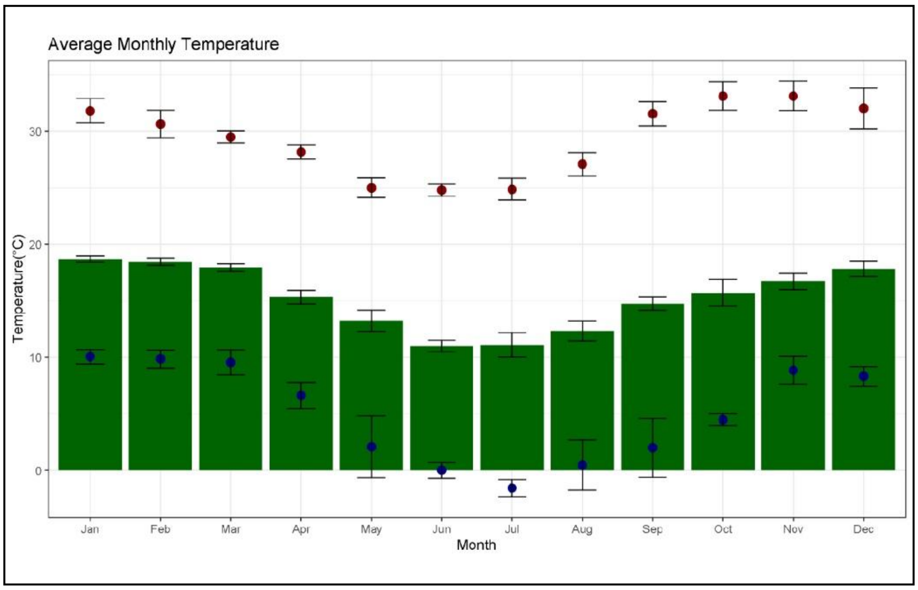

Figure 3.3 Mean monthly temperature (± 1 S.D.) (green) and mean maximum (red) and mean minimum (blue) temperatures per month for the 2019-2021 period at Fort Nottingham Nature Reserve.

3.3.3 Humidity

Mean monthly maximum relative humidity showed very little fluctuation over the three-year period, with days of percentage relative humidity in the high 90's each month of the study period (Figure 3.4). Minimum relative humidity shows a seasonal variation, as might be expected, with lower percentages for the winter months when rainfall is considerably less. The means for the months during the three-year period show a fairly typical pattern of high humidity during summer, dropping to a lower percentage during winter, with a marked dip in July. The lowest relative humidity recorded during the project was 8% in September 2019.

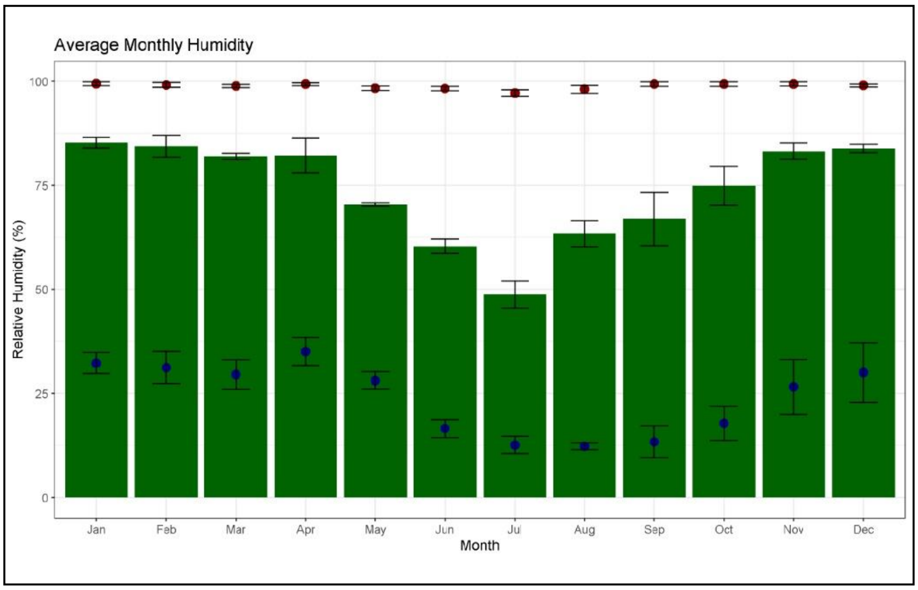

Figure 3.4 Mean monthly (± 1 SD) (green) and mean maximum (red) and minimum (blue) relative humidity as % for the months of the 2019-2021 period at Fort Nottingham Nature Reserve.

3.3.4 Wind

Maximum and minimum daily wind speeds were extracted as well as predominant direction for each day of the months. Over the three years, October always had the highest wind speeds while May had the lowest. Southerly direction was by far the most frequent wind direction, with 225 occurrences of straight southerly wind, 220 of south-south-east and 161 of south-easterly.

3.4 Study Period

Looking at the study period more closely, a total of 540 mm of rain fell during the 9 months. 20% of this fell during September in, mostly, small amounts. Of the 35 rainfall events recorded, only five were greater than 5 mm with the largest single event being 16.5 mm. October accounted for 30% of the rain, also mostly in small amounts, with 9 of the total 49 rainfall events being larger than 5 mm and the largest being 27.4 mm. Finally, 50% fell in December with considerably larger daily rainfall volumes, where 20 of the 71 rainfall events were greater than 5 mm with a maximum amount of 25.4 mm. This shows a steady increase in rainfall during the summer season’s progression.

Considering the mean values over the three years of the project (Table 3.1), it can be seen that there is a steady increase in temperature as the season progresses from winter to summer, with the mean daily temperature increasing by exactly one degree per month, while the maximum and minimum temperatures fluctuated slightly around the one-degree increase mark. Relative humidity, however, increases quite considerably as the season progresses with an overall average increase of 16.3% from September to November.

Table 3.1 Average daily temperature, average maximum and minimum temperatures and average daily relative humidity for the combined three Septembers, Octobers and Novembers of 2019, 2020 and 2021 at Fort Nottingham.

Month	Avg Temp	Max Temp	Min Temp	Rhum%
Sept	14.7	21.8	9.3	66.9
Oct	15.7	22.4	10.4	74.9
Nov	16.7	23	12.2	83.2

CHAPTER 4

GENERAL DESCRIPTION, LIFE HISTORY AND MARK-RECAPTURE

4.1 Introduction

The benefits and problems associated with marking adult anurans have been discussed and debated for many years and by many individuals. Two distinct types of information can be gathered from mark and recapture, depending on how the study is designed, planned and carried out. There is basic information gathered from the recovery of marked animals, but also information gathered from comparing numbers of marked and unmarked animals captured at each sampling occasion (Pollock 1980). This information can be used to estimate abundance, survival, recruitment and population growth (Lettink and Armstrong 2003), as well as growth rates and longevity (Pollock 1980). From these estimates it is sometimes possible to create a population model which can be used to evaluate the relative impacts of different threats, predict how the population will respond to different management strategies and assess the viability of the population over time (Lettink and Armstrong 2003).

Two types of populations have been described - open and closed. Open populations are mostly found in the wild where population numbers are open to additions (hatches or immigration) and permanent deletions (deaths or emigration). Closed populations are found in a controlled laboratory environment where theoretically neither additions nor deletions occur (Pollock 1980).

While marking is becoming more and more commonplace in research, the methods are being constantly refined and ever more carefully scrutinized for methodological ethical issues. A list of criteria were drawn up for marking techniques (Ferner 2007). The criteria are as follows.

Marking should:

- not affect the survivorship or behaviour of the organism
- allow the animal to be as free from stress of pain as possible
- identify the animal as a particular individual, if desired
- last indefinitely or at least as long as the study requires
- be easily read or observable
- be adaptable to organisms of different sizes
- be easy to use in both laboratory and field
- use easily obtainable materials at minimal cost.

Through the years several methods for marking have been developed and implemented for amphibians, including jaw tags, toe-clipping, PIT tags, branding and implants (Donnelly et al. 1994). After looking into a wide variety of possible marking methods used in anurans elsewhere and being cognizant of the species' Endangered status, fairly small size and possible delicacy, Visible Implant Elastomers (VIE) were obtained and tested in the field. At first it was unclear as to whether VIE would be successful or not with *L. xenodactylus* due to it being highly successful in some anurans (Sapsford et al. 2015), while being unsuccessful in others (Brannelly et al. 2013).

Visible Elastomer Implant (VIE) is a method designed by Northwest Marine Technology^{TM} for marking fish. It is a two-part substance, being one part liquid elastomer material and the second part a curing agent. Once the two substances are mixed they harden within a few hours if left at room temperature, but it can be stored in the refrigerator as a liquid, for several weeks. (Nauwelaerts et al. 2000).

For the purposes of this project, the study was dealing with the open population of *L. xenodactylus* found at Fort Nottingham in the Natal Midlands (see chapter 2). The objective of this aspect of the study was to collect movement information within the population and, depending on how the project progressed, possibly add growth, survival and population estimation work.

With the necessity of the frogs being captured for the marking process, it was decided to make the most of the opportunity and gather as much data from each individual as possible. These included measurements as well as behavioural observations.

4.2 Material and methods

4.2.1 Morphometric data

Once located, frogs were gently removed from the vegetation or burrow using moist hands dipped in the closest available water body and then gently restrained. Measurements were taken using electronic digital calipers with a resolution of 0.1 mm and an accuracy of ± 0.2 mm. All measurements were taken with the frogs appropriately restrained or perching on the handler’s hand or equipment. The details recorded were head width, snout-vent-length (SVL) and tibia length. For head width, the calipers were placed at the widest point between the tympanum and the eye on both sides of the frog’s head. For SVL the frog was placed flat in the hand and gentle pressure applied to the “kink” in the back to ensure it wasn’t hunched. The calipers were then placed on the tip of the frog’s snout and at the end of its spinal column. For tibia length the frog was held upside down and the calipers were placed on either end of the frog’s right-side tibia. The frogs were placed unrestrained on a digital pocket scale in order to record body mass (Figure 4.1).

Figure 4.1 Specimen of *Leptopelis xenodactylus* being weighed in the field.

The location of each individual was recorded as accurately as possible using the GPS of the “Frogs of Southern Africa” digital application. The label that was given to each frog on its field data sheet was assigned to the same individual on the list entry, also providing the time of capture and allowing for other notes to be taken. These data were then downloaded as a Microsoft Excel® spreadsheet and added to other field information that was entered on the main spreadsheet following each field trip.

4.2.2 Seasonal movement

During the three seasons of the study, field trips were made in the months of September, October and November, at a frequency listed in Table 4.2, to the field site at Fort Nottingham. During these trips, frogs were located in order to gather morphometric data and for the mark and recapture work. The GPS locality and date of each of these frog captures were noted and entered into a database.

Table 4.2 Frequency of field trips to Fort Nottingham during the study.

Month	Year	Frequency
October	2019	3
November	2019	2
September	2020	2
October	2020	2
November	2020	4
September	2021	1
October	2021	1

The GPS points were entered into “Google Earth®” and were divided into years and months. From these points, using the “Google Earth®” polygon function, it was possible to roughly define the areas within which the frogs were active on the field trip during each month of the three seasons.

The field trips were planned according to the weather, with moist, wind-free evenings being preferred. Due to safety constraints, it was essential to have more than one researcher in the field on the nocturnal field trips, which was a further limiting factor. Suitable evenings for fieldwork, when appropriate people were available, had to be split between the main study site at Fort Nottingham and exploratory trips to other areas where the frogs were thought to occur. Priority was given to conducting at least one trip per month to the study site each year, and more where possible.

4.2.3 Visible Elastomer Implant (VIE)

As *L. xenodactylus* has an Endangered status, it was decided that methods such as toe-clipping and branding should be avoided due to the invasive and damaging nature of the techniques, as well as the permanent and obvious impact on the individual frogs. For VIE marking, six fluorescent colours were available which fluoresce under UV torchlight: red, pink, orange, yellow, green and blue.

The two parts, one-part colour and one part curing agent, were mixed prior to each field trip. Each colour was mixed and stored in separate 31-gauge hypodermic syringes in a refrigerator until the field trip, to prevent the material from hardening. The VIE was transported in a cool box to stop it from hardening on the journey to the study area.

The objective was to mark specimens for individual recognition upon recapture. Six apparently suitable locations for marking were identified on each frog, one on each front limb and two on each hind limb as illustrated in Figure 4.2. It was suggested that the mark be placed in a muscular section of the frog, which is why the limbs were chosen. These sites also reduce the potential for internal damage during marking. In the initial season it was decided to use two marks per frog, either two different colours or two different positions or a combination of both.

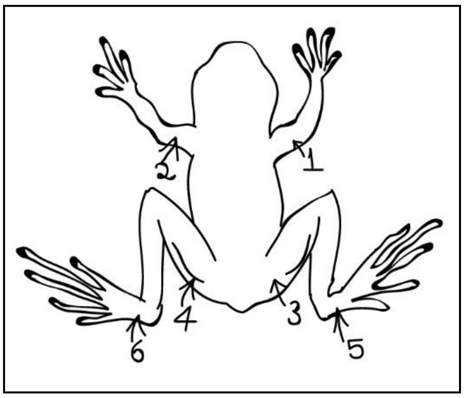

Figure 4.2 Sketch of frog showing the possible positions for the VIE marks for the purposes of the *Leptopelis xenodactylus* mark and recapture project at Fort Nottingham.

Once a frog had been located and captured, standard measurements were taken and sex was ascertained where possible by behaviour (i.e., calling or not) or size (females being considerably larger than males). The frog was then secured firmly to restrict movement and a predetermined combination of colours were injected subcutaneously into the frog. This was done by sliding the needle in under the skin with the bevel upwards. A small amount of the elastomer was then injected (Figures 4.3, 4.4 and 4.5). In most cases it was possible to see the injected gel with the naked eye due to the process being performed on the semi-translucent skin on the underside of the limbs. This made it easier to ascertain when the correct amount of gel had been inserted (<1 mm). The frog was then dipped in water and held in captivity for a few minutes to ensure it was in good health before being released again in the same location. On one occasion, following capture and marking, a frog was heard calling again within 3 minutes of being released.

Figure 4.3 *Leptopelis xenodactylus* about to be marked.

Figure 4.4 *Leptopelis xenodactylus* in the process of being marked.

Figure 4.5 Injecting the elastomer into the *Leptopelis xenodactylus*'s muscular thigh.

While it is sometimes possible to see the marks with the naked eye (Figure 4.6), the fluorescent dyes were selected to ensure easier detection using an ultra-violet light (Figure 4.7). From the commencement of the marking, each captured frog was checked for a VIE mark before the standard measurements were taken. A problem became apparent towards the end of the first marking season when frogs were recaptured with unrecognisable markings, and it became clear that the elastomer had migrated within the frog’s body.

Figure 4.6 Pink VIE mark (indicated by the arrow) visible to the naked eye.

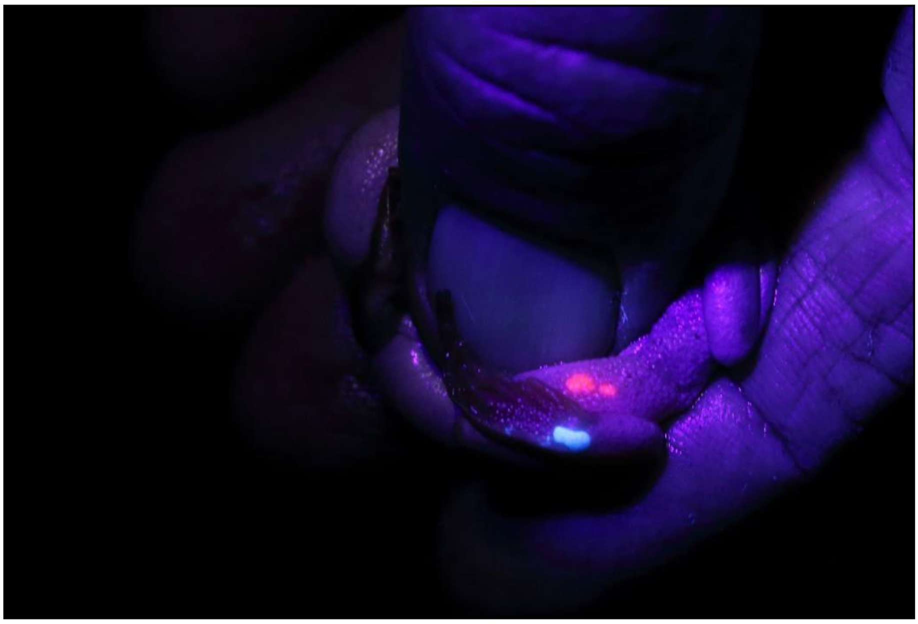

Figure 4.7 VIE mark under the UV light.

For the first season it was decided that a sample size of 30 would be an adequate number of frogs from the Fort Nottingham study site, with as much diversity amongst the sample as possible in terms of sex and size, as even metamorph long toed tree frogs are large enough for this marking method to be effectively used.

From literature it became clear that VIE could be an unreliable method with the possibility of the implants either migrating or being ejected by the frog in some cases, while in other species or individuals it worked consistently and effectively. Following further research, consultation and discussions with researchers who had used the technique successfully, a new method was decided upon for the following season. Instead of relying on the location within the frog, each frog would be marked with a unique three-colour combination and all marks would be inserted into positions 3 and 4 (Fig. 4.2) as these were the largest muscular area and thus the most suitable. This new technique reduced the number of possible combinations but achieved the goal of successful individual frog marking and recognition.

From the end of the 2019 season, when the first frog was marked, each frog that was caught was thoroughly checked with a UV torch to ascertain whether it had a mark or not. While in most cases the marks had migrated from the recorded insertion points, they were usually still visible when a broad UV beam was applied to the ventral and dorsal surfaces of the frog. If the frog was marked, then the mark code was added to that individual's field notes. An example of this code would be O3RB4 which would mean that there was an orange mark on the frog's right thigh and red and blue marks on its left thigh.

4.2.4 Analysis

Unfortunately, several of the colours appeared very similar in the field so it was sometimes difficult to distinguish between a red and an orange mark in a frog. Due to this, along with the movement of the marks, some of the recaptures were difficult to identify. It was done according to four levels of confidence, where the first level of confidence meant there was confirmation that this was a specific marked frog, for example, when there was a naturally occurring unique marking identifying the frog. The second level of confidence was when there were three indications that it was a specific frog, for example, either the three colours matched or two colours and location on the frog. The third confidence level indicated a 2-colour match, for example when only two of the marks were visible and the third had either migrated out of sight or been ejected. Finally, the fourth confidence level was where only 1 of the colours on the recaptured frog was visible and the geographic location and size of the frog fitted for an individual that had been marked with that colour, but there was still a high degree of uncertainty as it could also have been part of another combination.

Once the individuals were identified as conclusively as possible, the records of that individual frog were retrieved from the database and compiled together in a spreadsheet of recaptures. The GPS coordinates of each encounter with that frog were plotted on "Google Earth®" and the distances between the two localities measured using the measuring function in Google Earth®. The dates were calculated on a calendar to ascertain days of liberty between encounters. This resulted in a spreadsheet that listed each marked frog, the number of times it was recaptured, the distance between each recapture and the days of liberty between each recapture.

For the distance calculations, each recapture was considered as a separate record, whether it was a first or second recapture for that particular frog. The recapture events were split into those that occurred within one season, so not more than 90 days at apart, and those that were more than 320 days apart.

4.3 Results

4.3.1 Appearance

Leptopelis xenodactylus look like a typical African tree frog, with their large, protruding eyes, long limbs and "stepped" back. They show relatively little diversity in colour combination, being a generally uniform olive to bright green on their back, bordered by yellow which blends into cream on their bellies. However, the degree of brightness and the shades of the green and yellow differed between individual frogs, as did the extent of the yellow. (Figure 4.8)

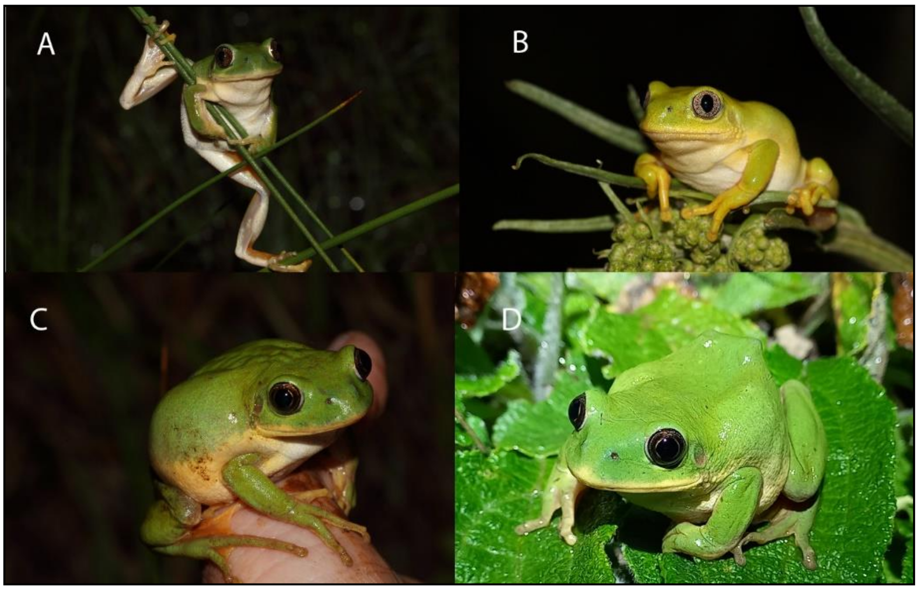

Figure 4.8 Differing brightness and shades of colours in *Leptopelis xenodactylus*: A - whiter belly with little yellow; B - much yellower frog; C - more distinct yellow band between green and cream on the belly; D - vividly bright green frog with little yellow.

Several frogs also displayed small, irregular yellow spots within their green areas, (Figure 4.9); these do not appear to be the result of scarring or injuries as several obviously recovered injuries were found on frogs and these were all a dull brown. James Harvey provided a photograph of one specimen which displayed unusual reddish bars and spots over its dorsal surface. Of the 168 specimens captured, only seven had yellow spots.

Figure 4.9 Irregular colouration on specimens of *Leptopelis xenodactylus*, A - yellow spot by tympanum, B - yellow spot above eye, C - yellow spot on thigh, D - reddish marks on back (Photo: James Harvey).

Young frogs are typical of the genus in appearance, being a uniform dull green dorsally and creamy white ventrally (Figure 4.10). They appear to only develop the yellow colouration in their second year.

Figure 4.10 Typical young *Leptopelis xenodactylus*, assumed to have hatched during the previous season.

4.3.2 Behaviour

Several individuals "feigned death" upon capture, curling their backs and tucking their hind limbs and feet under their body, covering their delicate eyes with their front feet. This posture makes their surface area as small as possible, and they lie motionless for several minutes (Figure 4.11).

Figure 4.11 *Leptopelis xenodactylus* feigning death.

When distressed by capture, the frogs often produced a strong, unpleasant scent. Some frogs smelt a lot more strongly than others, but the strength of odour did not seem related to the amount of handling, as all were exposed to similar amounts of handling. They were also found to excrete a sticky substance from their dorsal surface, possibly from where the scent is produced. This substance is difficult to remove from hands and is extremely bitter and distasteful when in contact with tastebuds. On one occasion, when a fresh, relatively large amount was wiped into the mouth accidentally, a rapid reaction resulted with the throat swelling rapidly and significantly. This was treated by immediate rinsing with water and wiping with a cloth and passed after an hour or so. The secretion did not sting or burn when in contact with cuts or scratches.

The frogs also inconsistently utilized ventriloquy when calling. When used, ventriloquy dramatically increased the difficulty of locating the frogs when an observer was within about a metre of a frog. On most occasions it was relatively easy to locate the frogs, as they were found in the exact locality from which the sound seemed to be coming. On some nights, however, it would take upwards of 10 minutes to locate a frog from within a 1 m area due to the noise being "blurry" and the calls several minutes apart. Triangulation with more than one person looking for the same frog did not always help, as it was sometimes almost impossible to pinpoint exactly from where the call was emanating. Intriguingly, not all frogs on the same night would be using ventriloquy, with some simply calling openly and others apparently using ventriloquy; there was a notable increase in ventriloquy on clear nights when visibility was good.

4.3.3 Morphometric measurements

Measurements were taken on most specimens captured: these were body mass, head width and snout-vent length for the entire sample set and tibial length was added for the specimens caught in 2021 and 2022 (Table 4.2). Two tadpoles were kept in captivity and the metamorphs were measured just prior to release; these both weighed 0.4 g and had head widths of 6.8 mm and 5.8 mm and SVLs of 15.3 mm and 13.9 mm, respectively. The smallest wild caught frog from the project weighed 1 g with a 4 mm head width and a 16 mm SVL. The largest male was 8.2 g with a 17.5 mm head width and 42.7 mm SVL. The largest female was 13.6 g with a head width of 18.5 mm, a SVL of 54 mm and a tibial length of 22 mm.

Body mass, head widths, snout-vent lengths and tibial lengths of the males and females in the sample were approximately normally distributed (Appendix: Figures S4.1, S4.2, S4.3 and S4.4). The difference in mean mass between the males and the females was significant (Welch Two Sample *t*-test: *t* = 5.754, df = 8.679, p-value = 0.001. The difference in mean head width between the males and females was significant (Welch Two Sample t-test: *t* = 5.2257, df = 9.5926, p-value = 0.001). The difference in mean snout-vent length was significant (Welch Two Sample t-test: t = 6.9415, df = 9.2744, p-value = 0.001). And finally, the difference between the mean tibial lengths was significant (Welch Two Sample t-test t = 8.0192, df = 10.447, p-value = 0.001).

Table 4.2 Morphometric measurements of male and female *Leptopelis xenodactylus* captured at Fort Nottingham during the project.

	Mass (g)	Head width (mm)	SVL (mm)	Tibia length (mm)
		Males		
Average	4.52	13.17	35.19	15.88
SD	1.68	2.48	5.35	2.60
Max	8.20	21.50	46.00	23.00
Min	1.00	4.00	16.00	12.50
N = 146				**N = 45**
		Females		
Average	8.50	16.80	45.80	20.90
SD	2.05	2.09	4.56	1.06
Max	13.60	20.00	54.00	22.10
Min	6.60	14.00	41.00	19.80
N = 9				**N = 5**

From the mass of the frogs, it was possible to roughly estimate their ages. Having measured metamorphs at 0.4 g, it was assumed that the many frogs that were captured between 2 g and 4 g were within their first active calling season, most likely having hatched the previous season. It was assumed that frogs less than 2 g were younger than a year and frogs weighing more than 4 g were at least 2 years old. If these assumptions are correct, of the 145 frogs measured, 57 were between 1 g and 4 g while 75 were between 4 g and 7 g and only 13 were greater than 7 g (Figure 4.13).

Figure 4.13 Violin plot showing the distribution of the mass of the *Leptopelis xenodactylus* captured during the project at Fort Nottingham.

The majority of the captured frogs were located by listening for their calls and then homing in on them. Taking into consideration that calling by males is used for reproduction, it was assumed that all of these frogs were sexually mature. There was an audible difference between the calls of the larger frogs and those of the smaller frogs to the extent that, towards the end of the field work, it was possible to target the larger frogs based on their calls (see chapter 6), but there was not enough evidence for firm conclusions.

4.3.4 Burrows

Extensive fieldwork suggests that there are three types of burrows that the frogs utilize. They are made in the top or sides of the hummocks that make up the hummock wetlands that the frogs appear to favour (Figure 4.14).

Figure 4.14 Typical location of an *Leptopelis xenodactylus* burrow on the top of a hummock of grass in a wetland.

The first type consists of substantial, underground burrows into which the frogs can retreat entirely. While many frogs were heard calling from these underground burrows during the project, only one was found where the burrow itself could be examined and described. This was on 12 October 2020 at Fort Nottingham. A frog was calling from the mouth of a burrow approximately 5 cm above the water's surface in the side of a tussock. It vanished when approached and inspection revealed a horseshoe shaped tunnel, ± 2 cm in diameter, with the middle section being highest and the two "legs" closer to the water table. The entrance to the burrow was at the end of one leg. Each leg was approximately 7 cm long and the frog had managed to turn around in the burrow and was in a chamber at the furthest end of the burrow. There was also a small chamber at the bend in the burrow at the highest point. The frog was small, most likely hatched during the previous season.

Secondly, shallow burrows (Figure 4.15 and 4.16), large enough for the entire frog to be below the surface of the soil but usually not much deeper than the length of their bodies (<3cm deep). Males often called from the mouth of these burrows with the lower half of their bodies inside, allowing for a quick retreat if danger threatens.

Figure 4.15 Example of a *Leptopelis xenodactylus* in a shallow burrow where only the head is visible, showing the gular sac that is still stretched from calling.

Figure 4.16 *Leptopelis xenodactylus* hunkered down in a shallow burrow with only the top of his head and his vocal sac visible directly in front of the bottom human finger.

Finally, there were shallow scrapes that the males make and call from early in the season. These scrapes are crafted to fit the individual frog, making it difficult to extricate once it hunkers down. The frog fills the depression completely with all surfaces level with the ground. In many situations the frogs did not even break the surface of the mud and simply reverse into the vegetation, pushing it aside with their hind feet, until it covers them. These simple burrows are not temporary daily shelters as the frogs often remain in them for several days. Generally, frogs that are found in underground burrows are considerably darker and more olive green in colouration, than those above ground.

4.3.5 Seasonal movement

The locality data from the field work at Fort Nottingham clearly displayed two phenomena. Firstly, there is a change in the areas of activity and occupancy with the progression of the seasons. Secondly, the frogs utilized different areas of the wetland in different years.

4.3.5.1 Monthly

From the Google Earth® images (Figure 4.17, 4.18 and 4.19) displaying the areas of utilization for the different years of the project, it is possible to see an overall increase in the area that the frogs utilise as the season progresses. Even though there are differing numbers of records, hours of effort and field trips for each month, there is still firm evidence suggesting a progressive, seasonal increased area of activity and movement within the wetland.

Figure 4.17 Google Earth® image showing the area utilised by *Leptopelis xenodactylus* at Fort Nottingham during the 2019 season.

Figure 4.18 Google Earth® image showing the area utilised *by Leptopelis xenodactylus* at Fort Nottingham in the 2020 season.

Figure 4.19 Google Earth® image showing the area utilised by *Leptopelis xenodactylus* at Fort Nottingham in the 2021 season.

4.3.5.2 Annually

The second phenomenon that the data suggests is different focal areas of use within the wetland in the different years (Figure 4.20). In 2019 the frogs were focused in the area near the road and in three small polygons further south in the wetland. For the next two years they were focused on more southern areas on the eastern side of the wetland. In all three years they were more consistently found at the south end of the wetland, although not in October 2020 and September 2021. This was the least transformed section of the wetland.

Figure 4.20 Composite Google Earth® image demarcating the area within which all the *Leptopelis xenodactylus* were found for the years of 2019, 2020 and 2021 at Fort Nottingham.

4.3.6 Visible Implant Elastomer

Most frogs used in this process were caught and marked on the side of the wetland upstream of the road, due to that apparently being the most heavily populated side during the 2019-2020 seasons when the initial marking process was conducted. During this time, a severe veld fire burned through the wetland resulting in an apparent marked improvement in the overall health of the wetland once it had regrown and there was a considerable increase in the number of frogs calling from the side of the wetland downstream of the road in the 2021 season.

A total of 45 frogs were marked over the two years of fieldwork. 30 were marked in the 2019 season using either one or two colours and location of the mark on the frog and a further 15 in 2020 using three colours.

Of the 45 frogs marked and measured, eight were less than 4 g, so were therefore assumed to be in their first season, 27 individuals were in the 4–7 g range, where the majority of the sample fell, nine were larger than 7 g and one individual was not weighed. The average mass of the marked sample was 5.5 g ± 1.6 g.

4.3.6.1 Recaptures

Of the 59 frogs captured during the 2020 fieldwork season, 12 were recaptures, with 10 being individually identifiable. Of the 40 frogs caught in the 2021 season, three were recaptures, two of which were unidentifiable (Table 4.3). In total, 11 recognizable individuals were recaptured during the project.

Table 4.3 Total number of frogs captured during the 2020 and 2021 season at Fort Nottingham, with the number of recaptures and percentages.

Season	Total	Recaptures	%
2020	59	12	20.34
2021	40	3	7.5

Of the 21 recaptures were the frogs were recognizable, four recaptures were identified to a first level of confidence. Ten individuals were identified to a second level of confidence, while four were identified to a third level and three to a fourth level.

Six individuals were recaptured once, while three were recaptured twice and three were recaptured three times (Table 4.4). The longest period of freedom was 421 days, with four of the individuals spanning two seasons. (Appendix: Table S4.1 for the full data set.)

Table 4.4 Displacement (in meters) of marked *Leptopelis xenodactylus* at Fort Nottingham.

n	Average rate (m / day)	Shortest realized (m; days)	Longest realized (m; days)	Average realized (m)
21	2.76	0; 2	192; 356	36.81

4.3.6.2 Movement

During the two seasons, 21 recaptures were recorded where individuals could be recognised, and movement data gathered. Four of these recaptures spanned two seasons, with the length of time between captures exceeding 320 days. The one and two season recaptures were evaluated separately, designated as those that were within one season (Table 4.5) and those that were over two seasons (Table 4.6).

Table 4.5 Recaptures of marked *Leptopelis xenodactylus* at Fort Nottingham that spanned one season.

N=17	Distance moved (m)	Days between recaptures
Avg	25.40	18.82
SD	25.96	21.32
Min	0.00	2.00
Max	89.34	81.00

Table 4.6 Recaptures of marked *Leptopelis xenodactylus* at Fort Nottingham that spanned two seasons.

N=4	Distance moved (m)	Days between recaptures
Avg	87.15	339.25
SD	75.60	10.71
Min	13.32	327.00
Max	198.85	356.00

To test for a significant difference between the mean displacement of the captured frogs from location of first capture over the single season compared to that over the double season, a nonparametric test was used because the one-season data were not normally distributed (Appendix: Figure S4.5). The Wilcoxon rank sum test with continuity correction did not detect a significant difference between the means ($W = 15$, p-value = 0.09743). However, there was heterogeneity of variance which could not be overcome through standardisation and so the test result may be unreliable.

4.4 Discussion

4.4.1 Appearance

Leptopelis xenodactylus is perhaps most similar in appearance to young *L. mossambicus*, with dark eyes, uniform green ventrum and pale dorsum. The only *Leptopelis* whose distribution they may possibly overlap is *L. natalensis* and they can easily be distinguished by eye colour, being golden red in *L. natalensis* and golden brown in *L. xenodactylus*, as well as by *L. xenodactylus* having more elongated toes. Their vocalisations are similar to the human ear and can easily be confused, however, it is unlikely that the two species would occur in the same location due to different habitat preferences, with *L. natalensis* being associated with wooded areas as well as wetlands.

4.4.2 Defensive behaviour

The observed "curling" behaviour has also been documented in *Semnodactylus wealii* (du Preez and Carruthers 2021), another grassland specialist, as well as *Hylambates maculata* (Liedtke and Muller 2012). It is assumed that this posture makes the frog resemble a leaf or piece of vegetation, possibly allowing them to be passed over by predators as they are not in a shape that a predator would recognize as a living amphibian prey item. This defense mechanism would be particularly effective if the frog was dropped by the predator, as the frogs usually land with their green backs facing upwards and limbs tucked in, making them extremely difficult to re-locate in thick vegetation. Once curled up, individuals generally maintain this posture for the duration of the handling.

The dermal secretion of this species is distasteful and foul smelling. It appears to be a defence mechanism that affects sensitive oral and olfactory tissue, thus probably discouraging predation. When the substance came into contact with cuts and scratches, however, it did not cause any irritation. This secretion would be worth exploring in further research.

Another possible defence mechanism is their "ventriloquy". Similar behaviour was observed in *Leptopelis bocagii*, a semi-fossorial tree-frog in Zimbabwe. The observed increase in this behaviour on clear nights fits in with it being a defence mechanism. On misty nights, the frogs are afforded some shelter by the elements, while on clear nights it is considerably easier for predators to locate them visually after having tracked them down by their call. It would, thus, be beneficial for the species to employ a defence mechanism such as this on clear nights when predators have the advantage. One possible explanation for how it is accomplished is that the frog can project its call, with the sound then reflecting off the surrounding substrate and "sounding" as though it is coming from another location. Further research into this area could prove fascinating.

4.4.2 Burrows and their purpose

The different burrow types seemingly have different purposes and, while not conclusive, a few possibilities seem probable. The first type, described as a substantial, underground burrow where the frogs can disappear entirely from sight, is most likely similar to and possibly even the same as their over-wintering burrows and these are likely where they reside during the day. While only one of these burrows was found, many calling frogs could not be located despite intense searching and the use of these burrows could explain this. Many holes and burrows were found in the hummocks, some are clearly utilized by crabs and potentially crickets and other invertebrates, but others are likely burrows made by frogs. It would be interesting to use an endoscope to investigate these burrows in future studies.

The second type of burrow, the shallower one where the frog is able to retreat subsurface but not entirely disappear from view, is mostly likely used to provide shelter from the elements as well as protection from predators. In the nest that was found, this was the type of burrow that was utilized, with the frogs being partially submerged initially but, by the time all the eggs which filled the cavity entirely were laid, their own bodies had been expelled from the cavity leaving them perched on top of their clutch of eggs (see chapter 6).

The third variety of burrow, little more than a scrape in the mud, possibly provided shelter from the wind and a degree of cover and camouflage and thus protection for the calling frog. It would also have thermoregulatory significance, especially with the ground being wet.

All the burrows were located on hummocks, with no instances of *L. xenodactylus* being found calling either “surface” or “subsurface” on the peripheries of the wetland that appeared to offer similar conditions. Those *L. xenodactylus* that were mobile or elevated when calling showed slightly more flexibility and would occasionally be found in vegetation that was rooted in the peripheral substrate, but most were found on vegetation that originated from these hummocks.

6.4.3 Habitat utilization

The annual changes in area of intense use within the wetlands could be explained by several different factors including rainfall, condition of the wetland, cattle trampling intensity and presence or absence of fires. It is clear, however, that in different years, different parts of the wetland may be inhabited by the frogs, emphasizing the importance of conserving and protecting the entire wetland. During the study, between the 2019 and 2020 season, the wetland underwent a significant change when the culvert under the road in the north was upgraded, allowing for much freer flow of water within that area. This could explain the movement of frogs out of, what was originally, a more densely populated area next to the road, further up into the middle, southern reaches of the wetland.

Other factors that might affect a frog’s location within the wetland are disturbance and competition. About half of the Fort Nottingham wetland is within a farm paddock that is frequently used by cattle and horses, but the impact of their hooves on the soft substrate of the wetland, while obvious, did not appear to affect the abundance of frogs within that part of the wetland.

The southern side of the wetland, where most of the fieldwork was conducted and the majority of the frog population was consistently located, was bisected lengthwise by a livestock fence, with seasonal cattle use on the eastern side and permanent horse use on the western side, for the duration of this study. It is unknown whether historically the entire wetland was suitable hummock wetland but, from the commencement of this study, the western side has been transformed with only a few patches still having hummocks and containing *L. xenodactylus*. The rest was predominantly tall, moribund water grasses that *L. xenodactylus* frogs were seen to move through, but in which the males spent little time calling.

The increase in movement as the summer season progresses is discussed in section 4.3.5.1. where male *L. xenodactylus* probably start calling after the first rains when conditions start to become more suitable for them. This initial calling activity is predominantly given from the surface of the ground or subsurface and may be at, or near, their overwintering site. They do not appear to move around much initially but, as the season progresses, they start to become more mobile as reflected by the data presented. They probably continue to be active for the remainder of the season, but do not call, which makes them extremely difficult to locate. This hampers the continuation of movement research.

The mean displacement of the frogs over one season not being significantly different from the mean displacement over two seasons indicates that the frogs are generally sedentary as adults and that significant movement is not undergone on an annual basis. The sample size was very small, however, and there was heterogeneity of variance, which means that firm conclusions cannot be drawn from the data.

Appendix: Supplementary data

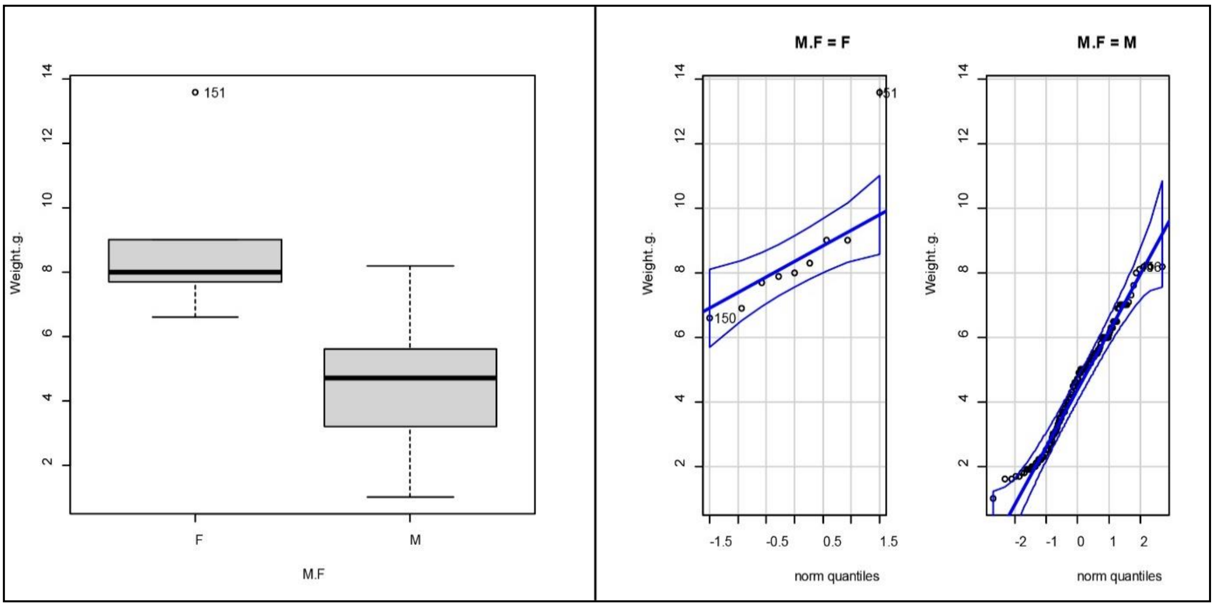

Figure S4.1 Boxplots (left) and QQ plots (right) indicating near-normality of the distribution of sample mass (g) of female *Leptopelis xenodactylus* (F) and male *Leptopelis xenodactylus* (M) respectively. A non-parametric Wilcoxon rank sum test with continuity correction gave a similar result to the two-sample *t*-test: W = 1261.5, p < 0.001.

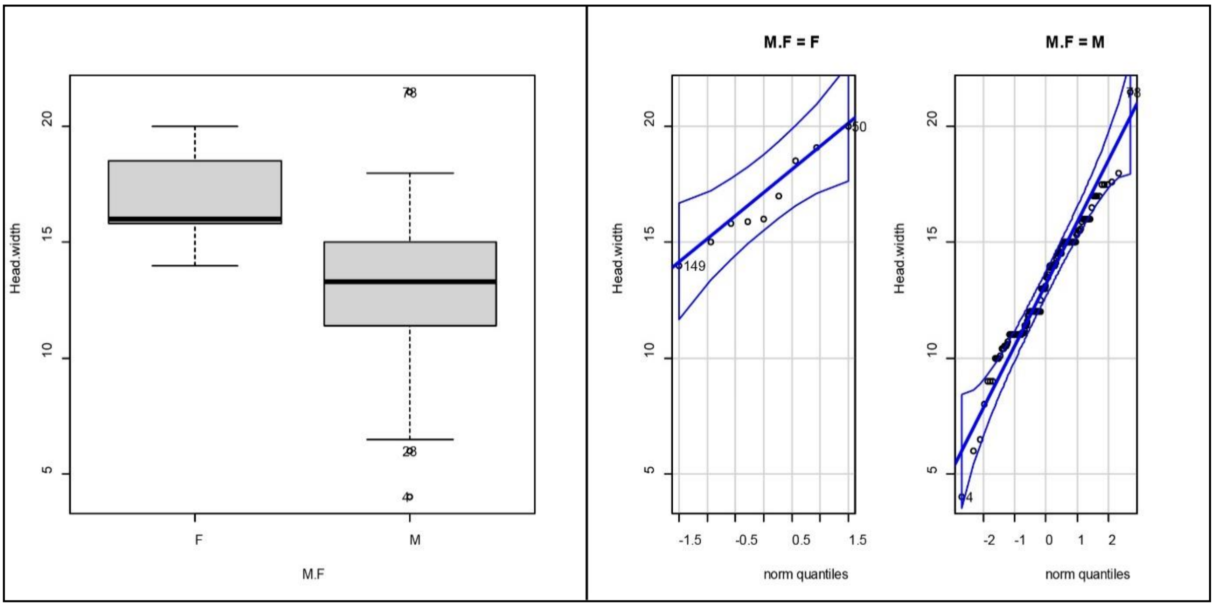

Figure S4.2 Boxplots (left) and QQ plots (right) indicating the normality of the distribution of sample head widths (mm) of female *Leptopelis xenodactylus* (F) and male *Leptopelis xenodactylus* (M), respectively. As they were normally distributed, no non-parametric tests were conducted.

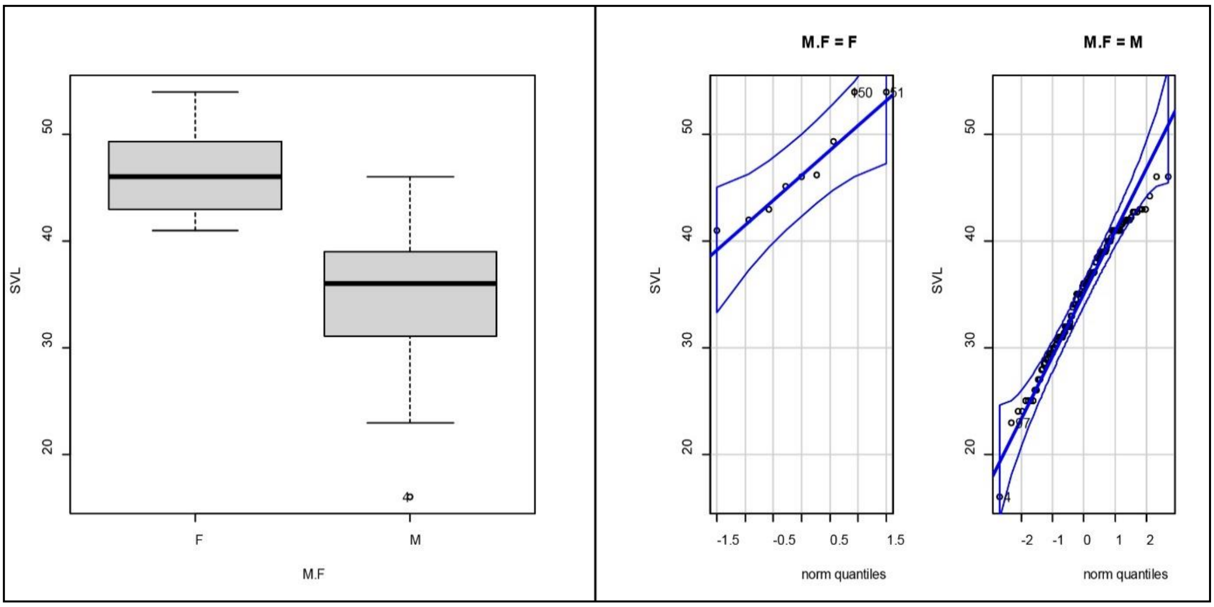

Figure S4.3 Boxplots (left) and QQ plots (right) indicating near-normality of the distribution of snout-vent lengths of female *Leptopelis xenodactylus* (F) and male *Leptopelis xenodactylus* (M) respectively. A non-parametric Wilcoxon rank sum test with continuity correction gave a similar result to the two-sample *t*-test: W = 1273, p-value = 0.000002442

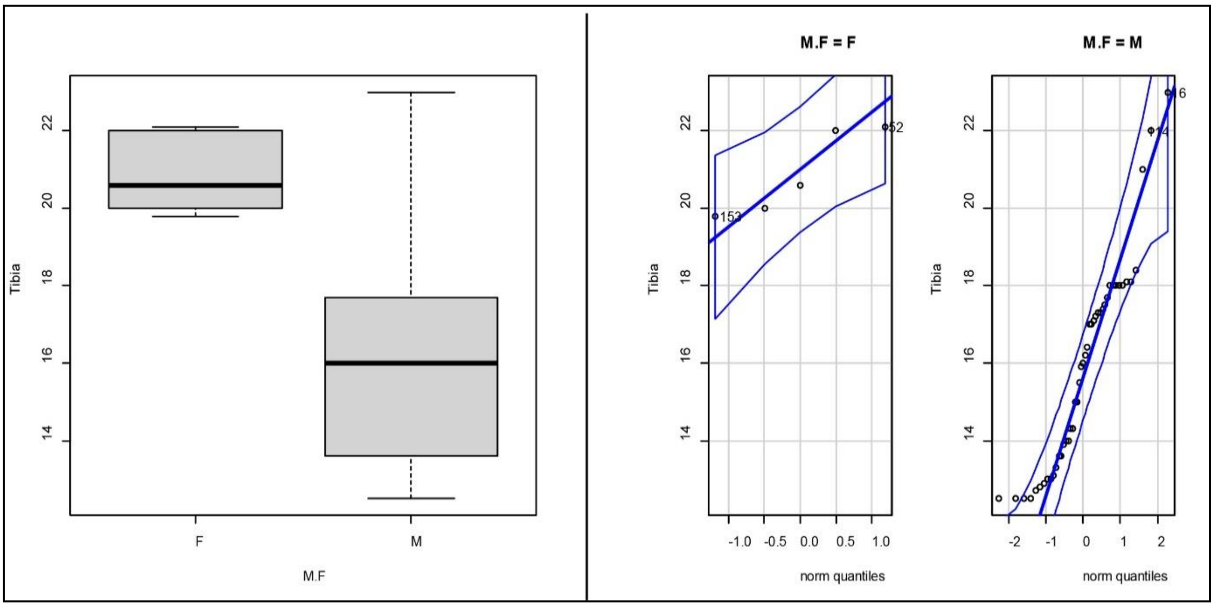

Figure S4.4 Boxplots (left) and QQ plots (right) indicating near-normality of the distribution of the tibia lengths of female *Leptopelis xenodactylus* (F) and male *Leptopelis xenodactylus* (M), respectively. A non-parametric Wilcoxon rank sum test with continuity correction gave a similar result to the two-sample *t*-test: W = 213.5, p-value = 0.001142.

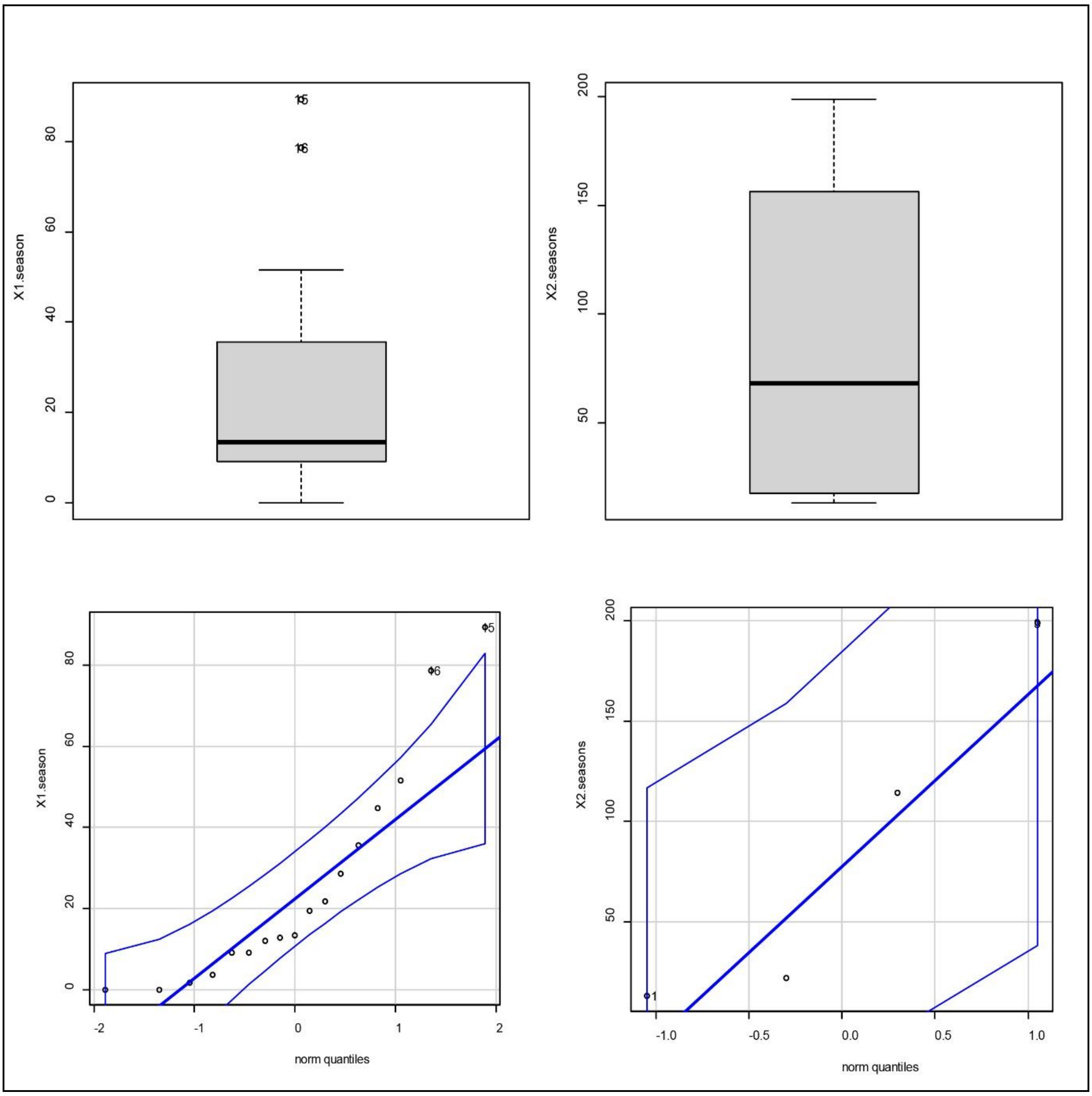

Figure S4.5 Boxplots and QQ plots indicating that the data are not normally distributed for the distances moved by the frogs in one season (left), but the two-season data are normally distributed (right).

Table S4.1 Dates, distances and days between recaptures of the marked and recaptured *Leptopelis xenodactylus* at the Fort Nottingham study site during the 2019-2021 seasons.

Frog No	**Date tagged**	**1st Distance (m)**	**Date 1st recapture**	**Days since tagging**	**2nd distance (m)**	**Date 2nd recapture**	**Days between recaptures**	**3rd distance (m)**	**Date 3rd recapture**	**Days between recaptures**	**Total days**
FN2	20 10 2019	4	23 10 2019	3	45	03 11 2019	11	0	05 11 2019	2	**16**
FN26	05 11 2019	22	04 12 2019	29	192	04 11 2020	334	9	21 11 2020	18	**381**
FN40	15 09 2020	0	27 09 2020	12	2	29 09 2020	2	13	02 10 2020	3	**17**
FN6	20 10 2019	19	05 11 2019	16	114	27 09 2020	327				**344**
FN10	24 10 2019	13	29 09 2020	340	51	21 11 2020	81				**421**
FN42	04 11 2020	29	15 11 2020	11	9	21 11 2020	6				**17**
FN13	24 10 2019	13	05 11 2019	12							**12**
FN20	03 11 2019	12	05 11 2019	2							**2**
FN34	29 09 2020	89	02 10 2020	3							**11**
FN36	29 09 2020	79	21 11 2020	53							**53**
FN39	29 09 2020	36	15 11 2020	47							**47**
FN41	29 09 2020	22	20 09 2021	356							**356**

CHAPTER 5

CALL PROFILE AND SEASONAL ACTIVITY

5.1. Introduction

5.1.1. The frog call

Frog choruses are part of the natural soundscape, to the extent that they are the inspiration for countless poems, songs, children's books and other creations which originated at night, alongside a frog pond. Sound production in animals is primarily a method of advertising the presence of one individual to others of the same species for various reasons - including advertising territories, attracting mates and expressing distress (Duellman and Trueb 1994; Kvsn et al. 2020). To some extent vocalisation dominates the reproductive behaviour of many species of frogs and toads (Gerhardt 1994). Historically, frogs and toads were the first creatures to colonize land and develop vocalisation (Villee 1977; Bass et al. 2008); which is energetically costly to produce (Gerhardt 1994). With their reliance on vocalisation for reproduction, the detection of species-specific calls provides a relatively efficient mechanism for studying and evaluating the status of anuran populations at a site and even on a wider scale (Dodd 2010).

The success of breeding events is impacted by environmental conditions, for example moisture, temperature and food availability, which all have impacts on the embryos, larvae and metamorphosed young, making it essential that conditions are suitable (Pough et al. 2016). Reproduction is hormonally mediated in amphibians; however, external events are proximate stimuli and a complicated combination of hormonal and neural mechanisms are required for a successful breeding event (Pough et al. 2016). Weather conditions act as cues for the release of certain reproductive hormones, signaling the breeding season for certain species. Temperature and photoperiod are particularly significant due to the predictability of their change throughout the year, making them important in the timing of reproductive cycles, especially for frogs in temperate zones (Wells 2007). Many temperate species are highly seasonal (Pough et al. 2016).

For frogs, a breeding site is a resource that often has to be shared with several other species synchronously. As such, differences in calling sites and temporal calling patterns, are essential for appropriate mate-recognition. This temporal utilization of a site ensures that mate recognition is possible, with different species utilizing different periods of the day and night for calling (Schoener 1974; Prado et al. 2005). The calling sites a species chooses can vary according to type of microhabitat, type of vegetation, or height above ground (Wells 2007), with various environmental factors (elevation, vegetation and substrate) having impacts on the sound production (Gerhardt 1994). The type of location that a male occupies will usually vary between species, but will be fairly consist within species (Passmore and Carruthers 1995).

The physical environment distorts calls, making it difficult to predict what signal an animal actually receives. This distortion could result from the attenuation of a signal due to spherical spreading; atmospheric absorption, scattering and boundary interference; the breakdown of signal structure caused by reverberations, or irregular amplitude fluctuations resulting from atmospheric turbulence and the background noise causing the masking of signals (Oseen and Wassersug 2002; Wells 2007). Habitats can have different characteristics that would affect calls, depending on the presence or absence of open water, temperature gradients, type and density of vegetation, humidity and atmospheric turbulence (Wiley and Richards 1982). These features can affect the production, transmission, refraction and reflection of anuran vocalisations (Köhler et al. 2017). Temperature strongly affects anuran calling due to the high energy expenditure that is associated with calling and the link between environmental temperature and metabolic reaction rates in ectothermic animals (Köhler et al. 2017). Temperature appears to have an impact on the temporal parameters of anuran calling more than on the spectral parameters, for example the number and duration of notes (Giacoma et al. 1997). Calling activity of anuran assemblages is related to overall seasonal changes as well as more localised weather factors such as temperature and rainfall (Canavero et al. 2008).

Each vocalizing anuran species has a unique call with many species having complex advertisement calls. The effectiveness of these is dependent on multiple cues, to the extent that females are known to respond differently to different aspects of the males' calls (Duellman and Trueb 1994; Martof and Thompson 1964). Variance in temporal and spectral frequencies, along with chorus distribution, play a role in mate selection (Calsbeek et al. 2022). Body size is well documented as being correlated to the dominant frequency of a frog's call in both intraspecific and interspecific comparisons (Gerhardt 1991; Penna and Veloso 1990) while sexual selection and other forces also have an impact on the frequency of the call (Gerhardt 1994).

Frogs emit a variety of vocalisations for several different reasons (Köhler et al. 2017). These vocalisations can be broadly classified as reproductive, aggressive or defensive (Iyer and Briggman 2021). Reproductive calls are the vocalisations most commonly associated with amphibians and have the highest value and are of greatest interest when it comes to taxonomy (Köhler et al. 2017). Calls can be classified into: (a) advertisement calls - which are emitted by males and serve to attract conspecific females, as well as to announce occupied territory to other males; (b) reciprocation calls - given by receptive females in response to advertisement calls; (c) release calls - given by males or unreceptive females in response to undesired amplexus, and; (d) distress calls - which are loud vocalisations produced by males and females in response to disturbance. Advertisement calls can be further split into (e) courtship calls - produced by males to attract conspecific females; (f) territorial calls - produced by a resident male in response to an advertisement call by another male, and; (g) encounter calls - produced by males during close-range hostile interactions (Duellman and Trueb 1994).

5.1.2. Capturing frog calls

Animals that communicate by the use of sound inadvertently broadcast information about their behaviour, population and distribution (Browning et al. 2017; Wilkins et al. 2013). Recording devices can provide an accurate and valuable record of the species present at a particular locality on a particular night (Passmore and Carruthers 1995) and make it possible to analyse calls quantitively (Rand 2001). Recording devices also enable the detection, recognition, localization and tracking of acoustically active animals (Blumstein et al. 2011). There is great value in capturing the soundscape of a location, using an omnidirectional microphone and allowing it to record for an extended period of time, capturing the available information for the period recorded. Resources are now readily available, with an entry level smart phone being capable of capturing adequate quality recordings, for the majority of purposes one might require. There are also advanced freeware applications to manipulate and analyse this data, making it the “easiest” time in history to work with and on, vocalisations.

The use of passive acoustic monitoring (PAM) on terrestrial ecosystems began in the early 1990s and has increased dramatically since then (Sugai et al. 2019). PAM involves the placing of recording devices, which are programmed to record at set periods of time for the duration of the time they are deployed in the field. The recordings are then analysed and interpreted at a later date (Perez-Granados and Traba 2021). Passive acoustic monitoring can potentially provide efficient, taxonomically broad, non-invasive ways of monitoring populations and communities along with their responses to environmental change (Gibb et al. 2019).

Passive acoustic monitoring, if conducted correctly, provides reliable data on species composition and richness more rapidly and reliably than other survey techniques that rely on human ability (Parker 1991). It offers an effective and efficient method to assess biodiversity as well as capturing long-term changes in relation to seasonal variation, habitat modification, climate change and human activity (Riede 1993; Blumstein et al. 2011).

Acoustic signals are often detectable over greater distances than visual signals, as well as through vegetation and around sight obstacles such as topographic features (Heinicke et al. 2015). They are generally less affected by inclement weather and poor light conditions. Most importantly, acoustic recording can be carried out passively, with little to no obvious impact or effect on the subject in question or it’s environment (Thomas and Marques 2012). Other benefits of PAM include its ability to record constantly for extensive periods of time, which increases the likelihood of capturing information relating to rarer or less vocal species (Klingbeil and Willig 2015). PAM has allowed for advances in species surveys that include broader spatial and temporal sampling, reduced observer bias and long term storage of field recordings, allowing data to be processed at a later date as well as checked for inconsistencies (Blumstein et al. 2011). Another advantage is that human presence is not required after setup of the recording equipment, meaning there is no resulting attraction or deterrent at the site (Klingbeil and Willig 2015).

Negative aspects of PAM include the inability to study non-acoustic species and the challenges associated with distinguishing individual species automatically (Gibb et al. 2019). Other challenges include the sheer quantity of data collected that needs to be processed and analysed, the complications caused by interference from background noise and other species vocalising at the site (Kvsn et al. 2020). There are also the challenges of hardware malfunction and the lack of a visual component making identification of some vocalisations challenging (Klingbeil and Willig 2015). The recording is also subject to the effects of environmental factors, as well as the proximity of the calling subject (Köhler et al. 2017).

Acoustic recordings provide data enabling scientists to collect information in three main areas - biodiversity, density estimation and species identification - each of which have a variety of applications (Kvsn et al. 2020).

5.1.3 Seasonal activity in Anurans

Being ectothermic, anurans are very sensitive to changes in abiotic conditions and this not only impacts where they can occur, but also at what times of the day and season they are active (Dodd 2010). The diel and seasonal activities of amphibians are regulated by environmental conditions, principally moisture and temperature (Duellman and Trueb 1994). It has been suggested, however, that in some cases amphibian activity is more closely linked to the overall progression of seasons and the environmental parameters associated with the changes, rather than with the specific parameters as was initially suspected (Canavero et al. 2008). Cyclical endogenous drivers or variables that change seasonally, such as the photoperiod, are also likely to be important influencers (Graham et al. 2013). This suggests a general anuran activity pattern that is sinusoidal, following the seasonal changes as conditions change from least to most suitable for the frogs (Canavero et al. 2008).

Rainfall may elicit and facilitate the movement of frogs from terrestrial refugia to breeding areas but many species remain located within the wetlands themselves, rendering large scale movement unnecessary (Graham et al. 2013). That said, however, weather superimposes patterns of local activity onto more general seasonal activity patterns (Karns 1986). Variables that are known to impact amphibians include mean annual temperature, mean temperature of the coldest and warmest months, seasonal variation in temperature, annual precipitation, minimum and maximum monthly precipitation, summer rainfall, annual and seasonal evapotranspiration, elevation, topographic relief, vegetation index and moisture index (Qian et al. 2007).

Climatological parameters, such as rainfall and temperature, dictate anuran reproductive behaviour and impact their seasonal activity (Schalk and Saenz 2016). The reproductive mode is described as a combination of traits that include ovum and clutch characteristics, oviposition site, rate and duration of development, size and stage of hatching and type of parental care if there is any (Salthe and Duellman 1973). *Leptopelis xenodactylus* is classified as having non-aquatic eggs, where the tadpoles move to the water from the oviposition site (Wells 2007).

5.1.4 Existing documentation of calling

According to the literature, little was previously known about the calling behaviour of *L. xenodactylus*. The males were said to often call from well-concealed locations at the base of, or within, grass tussocks and from burrows in the mud itself. The call was described as one or two deep, brief croaks, separated by long intervals and sometimes preceded by soft buzzing (Passmore and Carruthers 1979). Channing describes the call as being a short croak, 0.1 s long, with the dominant frequency between 3.0 and 4.0 KHz (Channing 2001). Minter et al. (2004) describe the call as being one or two short, pulsed croaks, uttered at long intervals, with each croak being 0.1 s long with a dominant frequency of about 1,000 Hz, occasionally preceded by soft buzzing. They went on to state that calling was recorded in December and January and was carried out from both well-hidden positions at the base of grass tussocks and the tops of grass-covered islands (Minter et al. 2004). Channing (2008) hypothesizes that they may also vocalize from beneath ground level.

Harvey (2005), in a study spanning the months from November 2004 to March 2005, documented the frogs calling in the months of November, January, February and March, with no calls heard in December, despite an equal number of visits in each month. The estimated number of frogs calling was extremely low during the early months of the year, with the maximum in January being eight individuals on one occasion, five individuals on another occasion, two individuals on three occasions and one frog calling on five occasions.

The Ezemvelo KZN Wildlife Database contains 15 records of frogs found from the months of January to March; only five of these were calling, with the other records being frogs found either mobile during the day (2) or mobile at night in several cases, assumed to be hunting. Of the five that were calling, one was in response to a recorded call, and another was described to only call briefly (3 calls) and was then silent. The other calling records lack information on the situation (EKZNW 2022).

On one occasion Harvey (pers. comm.) estimated five frogs were calling sporadically between 12h00 and 13h00 during drizzly conditions. Another account from Harvey (pers. comm.) was of ± 10 individuals calling actively from a very small wetland at 10h00 in hot, sunny conditions. These frogs were not heard again, even at night, in the following six site visits.

5.2 Material and methods

5.2.1 Call Profile and activity of *L. xenodactylus* based on PAM

The calls of *L. xenodactylus* were recorded at Fort Nottingham in the Natal Midlands. It is a high-elevation wetland (1,470 m.a.s.l.), containing the hummock grasslands favoured by *L. xenodactylus* and where a healthy population was known to occur. Passive acoustic monitoring (PAM) using a Wildlife Acoustics SM4 Song Meter® with on-board, omni-directional microphones was conducted at this site. Songmeters® had been used in a pilot study in 2018 and had been placed at the study site in 2019 with the intention of having two full season’s worth of data by the end of the intended 2020 season. However, in the 2019 season, solar power was used to run the device, as it had been successfully used previously, but it presented challenges for this project, with batteries failing, ant nests affecting the wiring and solar panels not working effectively through expanded metal cages. This, unfortunately, resulted in large gaps in the data where the device had not recorded due to lack of power. This problem was solved by using D-cell batteries in the devices. Having upgraded to the SM4’s, which were much more power efficient, it was cost effective and more reliable to use batteries for the entire period - they only needed changing once. These adaptations finally resulted in a continual recording for the 2020 season. Due to the incomplete data sets from the logistical challenges, it was decided that only the complete data set from 2020 would be used.

5.2.1.1 Song Meter® setup

The SM4 Song Meter® was placed at the Fort Nottingham study site (-29.423187, 29.915301) on 12 August 2020 (Figure 5.1 and 5.2). Theft of Song Meters® was a problem, with a song meter being stolen during the pilot study in 2018 near Underberg. To prevent further theft, expanded metal cages were constructed to house the devices; these were in turn bolted to large, heavy concrete blocks.

The recording device was mounted on a wooden board which was extended between the opposite sides of a metal cage that was designed to house the Song Meter® for protection. The metal cages also circumvented the problem of damage to cables and devices through tampering by humans and wild animals, as had happened in 2018 when a baboon chewed through a cable between the recorder and a mounted solar panel.

Figure 5.1 A – Song Meter® in situ at the Fort Nottingham site; B- Song Meter® open inside the cage, displaying the attachment board and microphone position.

Figure 5.2 Googe Earth® image displaying the location of the Song Meter® within the wetland.

Early in September 2020, an uncontrolled wildfire burnt through the study site, including the area where the device was located. Despite having cleared the grass in the immediate vicinity of the Song Meter®, it was severely burnt (Figure 5.3) and it was assumed that the data would be lost. A replacement SM4 was installed but, although the device's outer housing had been destroyed, the data were intact. The device had even carried on recording after being burnt, resulting in no loss of data.

Figure 5.3 A – Song Meter® cage and surrounding area; B – Close up of burnt Song Meter®.

The Song Meter® was set approximately 50 cm above ground level, and the mounting board was shaped so as not to impede the direct "line of sight" between the device and the wetland from which the frogs were calling. The device was placed on firm ground approximately 5 m from the edge of the wetland, in ± 50 cm high, grassland vegetation (see chapter 3).

The wetland was selected for the passive acoustic monitoring because it was known to contain an active population of *L. xenodactylus* from the preliminary study in 2018. It was also a practical option that fitted within the logistical constraints of the project, given its relative proximity to the main access road and ease of access to the wetland. The siting of the Song Meter® within the wetland was a result of the fortuitous location of a road, near what was known from the preliminary study to be one of the areas within the wetland where *L. xenodactylus* were more actively calling. Due to the weight of the cement block it was difficult to carry any great distance from the road. The presence of a hill obstructing it from sight from the district road, made this location ideal, as it was out of public view and thus probably less likely to be stolen or interfered with.

The Song Meter® was programmed to record the first 10 minutes out of every hour from 16h00 until 08h00 the following day and did so consistently from the starting date until it was retrieved on the 15th of January 2021. In total, 284 hours of recordings and a dataset of 125.3 GB of data were recorded. The recordings were made digitally and in stereo at a sampling rate of 24 000 Hz and a bit rate of 512 kbps. The data were stored in *.wav* format on SD memory cards fitted internally into the device. The data were downloaded periodically throughout the season and stored externally in a home office. The Song Meters® can be set to automatically embed a time and date stamp into the filenames of each recording and they were catalogued according to this scheme.

5.2.1.2 Processing of data

The recordings resulting from this PAM were analysed using Raven Pro® 1.6 (64-bit version, Cornell Laboratory of Ornithology, Bioacoustics Research Program 2019) to establish the temporal, spectral and circadian patterns of the calling of *L. xenodactylus* at this location. Raven Pro® is a program specially designed for bioacoustics research, providing functions specifically suited to analyze animal vocalisations. It provides both an audio and visual representation of the sound file, in the form of a spectrogram (Figure 5.4), allowing the user to select, measure and manipulate specific sections within the recording to suit their individual purposes. A function of Raven Pro® is a selection table which allows the user to select each aspect of interest within the spectrogram of a *.wav* file and save it as an individual file. Various columns can then be added to this selection table and the specific parameters of the selected area will then be listed in these columns.

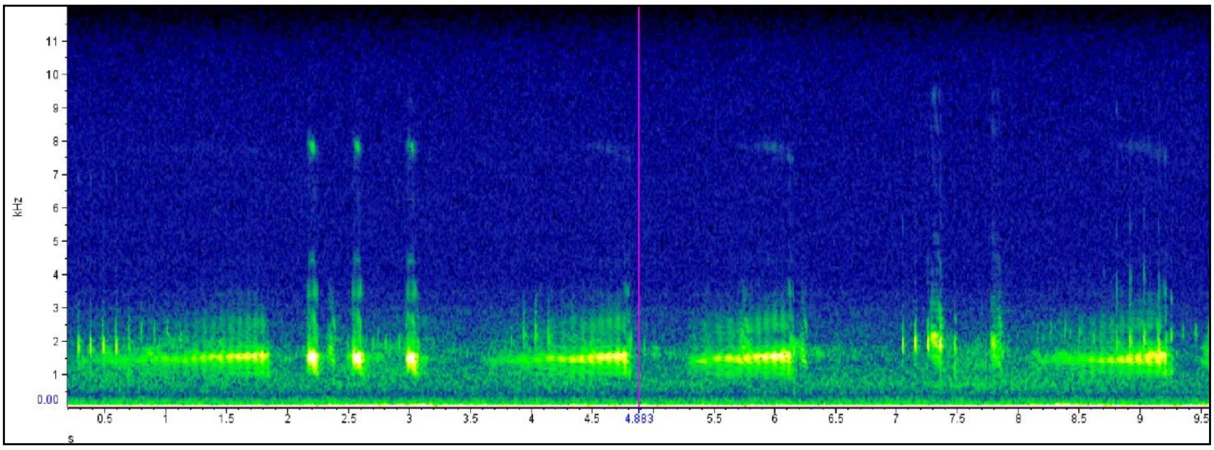

Figure 5.4 Spectrogram of a period of calling of *Leptopelis xenodactylus* exported from Raven Pro®.

The *.wav* files that were downloaded from the Song Meters® were individually opened and a delta time measurement was manually selected from the spectrogram window and committed onto the selection table for each call or call group that occurred on the spectrogram. This resulted in a table with the duration in seconds of each call or calling group within the recording. The measurements were made subjectively by scrutinizing each sound recording in its entirety and manually selecting the calls visible on the spectrogram. In periods of the recording where several individuals were calling concurrently, the entire visible period of vocalisation was selected as one period of calling. These measurements were then linked to the hourly recording time over the five-month period. This was used to estimate calling duration for the hours and days of the season that were recorded.

According to terminology and definitions used by Köhler et al (2017), the call-centered approach was used for this analysis. In this approach, the main, coherent sound unit was defined as a call and was separated by distinct periods of silence. Subunits of this call that were separated by shorter periods of silence were referred to as notes. Within these calls and notes were sound bursts that were known as pulses (Köhler et al. 2017).

To select suitable calls for measurements, Raven Pro®'s spectrogram view allowed for the location and selection of clear calls within the recordings that were of a suitable quality and not cluttered by noise and distortions. Each call was individually selected and saved to the selection table and the delta time measurement was used to measure the duration of these selections. Having used this intermediary tool to select appropriate calls for analysis, they were then opened using the R coding platform (R-Core Team 2013). *Seewave* (Sueur et al. 2008) and *tuneR* (Ligges et al. 2018), with all their dependencies, were the two R packages used for the measurement analysis and graphic representations of the calls. *Seewave* was used for analyzing the call patterns and acoustic properties. These packages were able to process the digitally recorded data to produce the necessary spectrograms, oscilliograms, measurements and statistics of the signals, allowing for the measurements, models and statistics, enabling conclusions to be drawn. Measurements made were call duration (s), number of notes per call, note duration (s), and internote interval (s). Seewave was also used to assess spectral properties of the call by applying a Fast Fourier Transform (FFT) to determine the fundamental and dominant frequencies.

5.2.2 Circadian Frog activity

A measurement of hourly and daily calling activity was extracted from the data by summing the seconds of calling that were selected in the selection table in Raven Pro®. These were then correlated to the hours and days of the recorded period to show the distribution of calling activity. For much of the hourly analysis it was more appropriate to use the hourly percentage of the daily summed calls to establish trends, while for the analysis of daily activity the call sums were used. The hourly percentages were obtained by plotting which hours through the recording period accounted for what percentages of the total amount of calling. Tests were conducted to check for significant monotonic correlations between call sum and the following variables: percentage of moon visible from earth, maximum temperature, minimum temperature and maximum relative humidity.

5.3. Results

5.3.1 Call site and call behaviour

Observations of calling *L. xenodactylus* indicate that they call predominantly from the hummocks, when present, in wetlands. Calling observations were not made in wetlands where hummocks were not present, so it is unclear what they do in the absence of hummocks, but it seems likely that these are degraded wetlands, where the hummocks have been destroyed.

The frogs showed a seasonal shift in locality as the season progressed (see chapter 6), often calling from underground burrows in the hummocks early in the season and moving to the surface and then elevated positions, later in the season. Once the season was well advanced, by mid-October, their affiliation to the hummocks grew weaker as they start to call more and more from elevated positions in the vegetation. They favoured broad leaf vegetation for calling sites, including alien species of *Senecio* as well as various *Juncus* species.

Whilst most calling occurred after dark, there were instances when *L. xenodactylus* were heard calling during the day. Most of these occurrences were under overcast conditions, however, there were exceptions even to this. During the study, frogs were heard actively calling at 08h00 on a hot, clear day in September 2021.

It was observed on several occasions that, during violent weather conditions such as heavy rain and strong wind, the frogs appeared to lose their inhibitions and call more openly. They also became more obvious and active, climbing high onto the vegetation and being considerably more mobile.

5.3.2 Call and Note Measurements

Leptopelis xenodactylus has a complex call and, using the call-centered description, the distinct vocalisations are described as calls with the signals making up these vocalisations being called notes. No inferences were made as to the classification of the calls, as with PAM no observations can be made of the calling individuals. From the calls recorded during the study, the *L. xenodactylus* call repertoire appears to consist of two different advertisement call types which are referred to as A and B.

A-type calls consist of either single, double or triple notes with some occasions where four or more notes occur. The notes are short, individually distinguishable signals. B-type calls are a longer signal that consists of one long, pulsed note. The two calls appear to be given in random combinations and numbers, however, there were no occasions when B calls were made without A calls, while A calls are commonly made without B calls.

5.3.2.1 A-type

The A-type note is a short, sharp, intense signal that begins and ends abruptly and is best described as a “quack”. It is a pulsed vocalisation with a consistent amplitude throughout the signal. A-type calls typically consist of either single, double or triple note calls, with the internote pause between the first and second note being noticeably shorter than the internote pause between the second and third notes (Figure 5.5). Spectral parameters indicated the mean fundamental frequency of single note calls to be 1,594 ± 554 Hz, double note calls to be 1,508 ± 54 Hz, and the triple note calls to be 1,556 ± 151 Hz (n = 5 of each call measured). The dominant frequency of the single note calls was 1,676 ± 226 Hz, the double note calls was 1,514 ± 155 Hz, and the triple note calls was 1,479 ± 98 Hz (n = 5 of each call measured). The bandwidth was between 744 – 1,929 Hz (n = 20).

The mean note length for A calls was 0.085 s ± 0.01 s (n = 125). The first internote pause on the multi-note calls was 0.32 s ± 0.04 s (n = 41), while the second, longer internote pause was 0.392 s ± 0.041 s (n = 20). (Table 5.1)

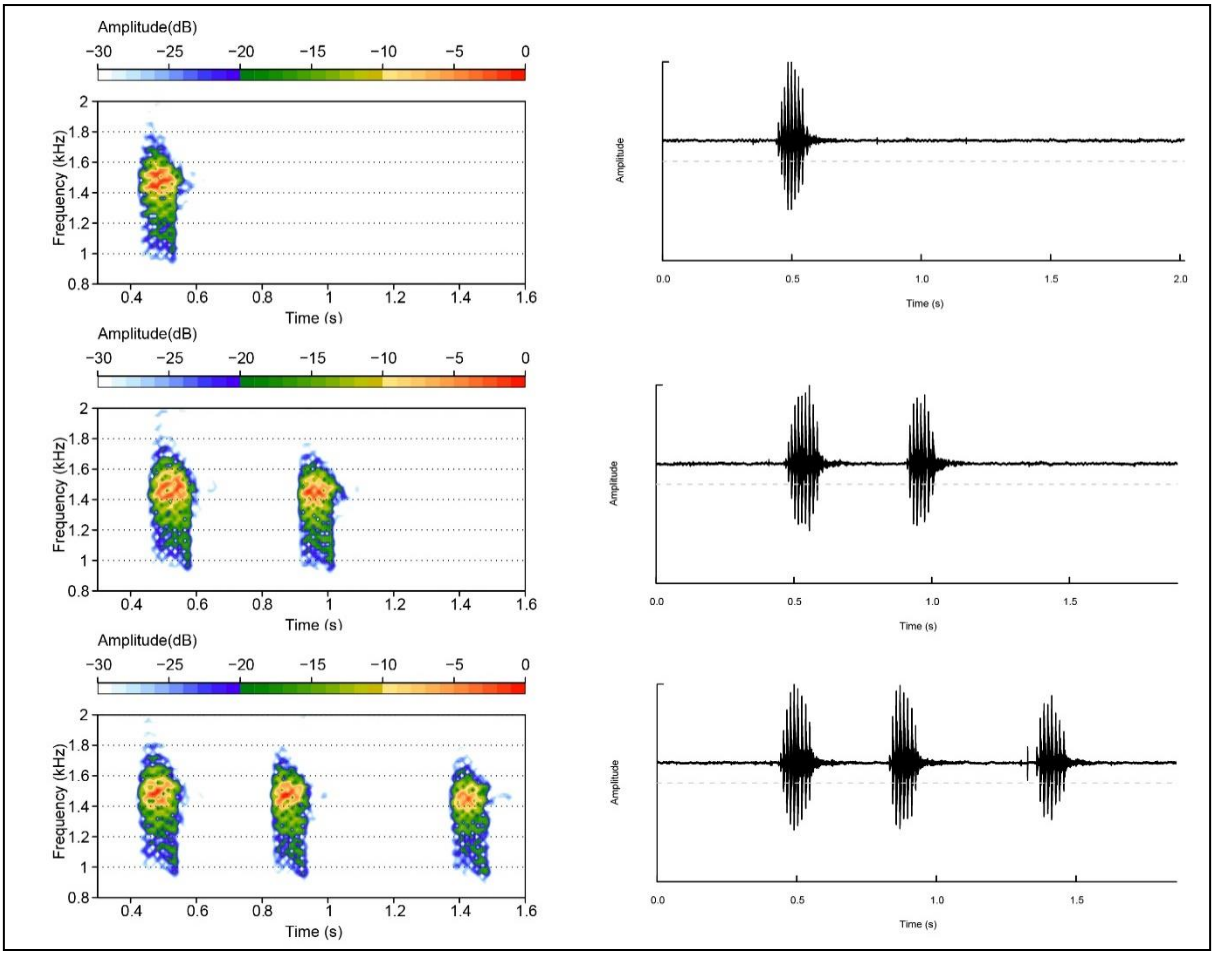

Figure 5.5 The spectrogram and oscillogram of A-type *Leptopelis xenodactylus* calls with one, two and three notes.

Table 5.1 Measurements of the A-type *Leptopelis xenodactylus* calls.

A-type Calls					
	1 Note		**2 Note**		**3 Note**
Call parameter	**mean ± stdev** (min - max)		**mean ± stdev** (min - max)		**mean ± stdev** (min - max)
Mean Fundamental Frequency (Hz)	**1.6 ± 0.53** (0.09 – 2.4)		**1.5 ± 0.16** (1.14 – 1.85)		**1.5 ± 0.15** (1.41 – 1.85)
Mean Dominant Frequency (Hz)	**1.7 ± 0.2** (1.41 – 2.1)		**1.5 ± 0.05** (1.36 – 1.59)		**1.48 ± 0.10** (1.33 – 1.85)
Bandwidth	**744 - 1929**				
Mean Call Duration (s)	**0.07 ± 0.01** (0.05 – 0.08)		**0.52 ± 0.03** (0.44 – 0.57)		**0.95 ± 0.4** (0.89 – 1.01)
Mean Note Duration (s)	**0.09 ± 0.01** (0.05 – 0.11)				
Mean Internote Interval Duration (s)	None	1st Pause	**0.32 ± 0.04** (0.27 – 0.4)	2nd Pause	**0.39 ± .04** (0.32 – 0.5)

The data for the first and second pauses were not normally distributed as indicated by QQ plots and boxplots (See Appendix: Figure S5.1). The second pause is significantly longer than the first pause as indicated by a Wilcoxon rank sum test with continuity correction (W = 93.5, p-value = 0.0000005526). The Bartlett test of homogeneity of variances indicated no significant difference in the variances (Bartlett's K-squared = 0.025315, df = 1, p-value = 0.8736).

5.3.2.2 B-type

The B-type call is a drawn-out signal, with an amplitude that increases gradually, culminating at the highest amplitude and ending abruptly, resembling a buzz. These calls are pulsed calls that are usually given closely following each other in no particular pattern (Figure 5.6).

The mean note length of the B-type call (n = 33) is 0.826 s ± 0.321 s. Spectral parameters indicated the mean fundamental frequency to be 1.867 ± 122 Hz (n = 6 calls measured), the dominant frequency to be 3,323 ± 124 Hz (N = 6 calls measured) and the bandwidth to be between 849 – 2.363 Hz (n = 20) (Table 5.2).

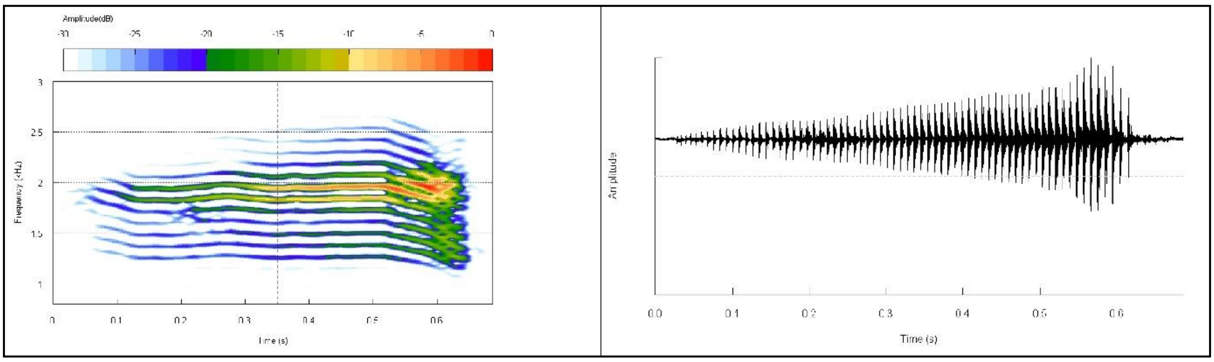

Figure 5.6 A spectrogram and oscillogram of *Leptopelis xenodactylus* B-type calls.

Table 5.2 Measurements of the *Leptopelis xenodactylus* B-type calls.

B-type Calls	
Call parameter	Mean ± stdev (min – max)
Mean Fundamental Frequency (Hz)	1.87 ± 0.12 (1.41 – 2.00)
Mean Dominant Frequency (Hz)	3.32 ± 0.12 (2.99 – 3.50)
Bandwidth	849 – 2,363
Mean Call Duration (s)	0.83 ± 0.32 (0.48 – 1.74)

5.3.2.3 Variations

Two other variations of the calls have been observed in the data. However, at this stage it is unclear if these are different calls or simply variations of the A and B calls. The first variation is similar in structure to an A call, being also a brief signal that appeared as either single or double note calls. It is pulsed and also resembled a "quack" sound, although slightly longer than the A notes and is of increasing intensity, building gradually and ceasing abruptly.

The second variation is similar in structure and duration to the B calls. It is a uniform, "whiney" signal that also resembles a buzz, which begins and ends abruptly. It is pulsed, increasing in amplitude, with a drawn-out period of maximum amplitude, diminishing more gradually than the similar B-type calls.

5.3.2.4 Calling behaviour

On the 21st of September 2021, on a field trip to the study site at Fort Nottingham, an interaction between two males was observed. A large, obviously gravid female had been placed in close proximity to a large, calling male who had continued to call, producing A-type calls. However, a second male who had been calling from ± 1 m away moved towards the male and female. The first male started to make B calls and then advanced towards the intruding male who then fled. This was a fortuitous observation as calling behaviour was not the objective of the field trip; however, two short videos were taken using a smartphone, capturing the interaction. In the first video the second male is already fairly close to the first male who is calling energetically. In the second video the first male is advancing towards the second male, still producing B calls while the second male rapidly moves away. This was the only such calling observation that was made. Figure 5.7 is a spectrogram of the sound file that was extracted from the video, with the B-type calls obvious.

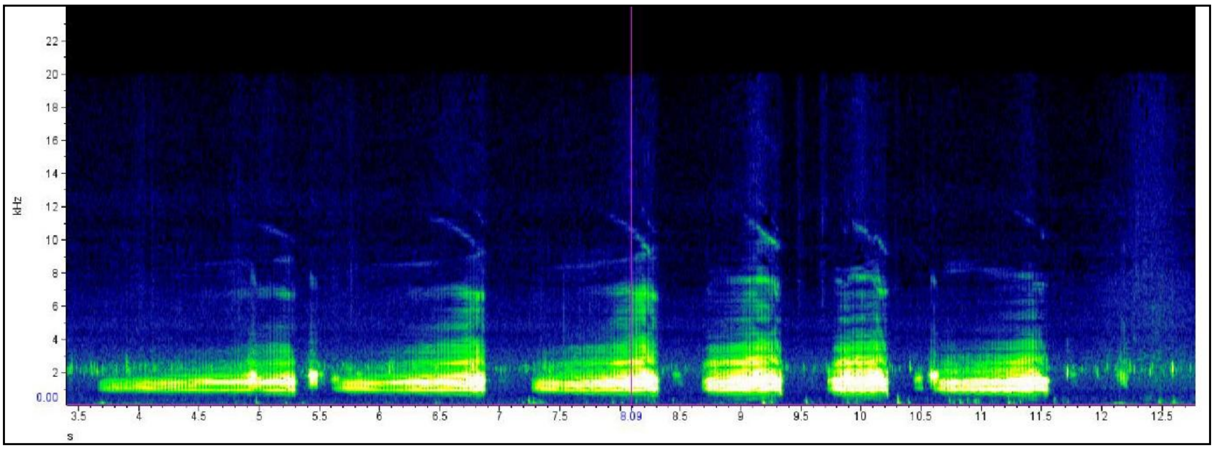

Figure 5.7 Raven Pro® Spectrogram of the calls produced during the video of two male *Leptopelis xenodactylus* interacting.

5.3.3 Calling activity

5.3.3.1 Recorded calling activity per month

Calling activity in the 2020 season started on the first of September with a pulse of high levels of calling followed by intermittent peaks throughout September until the highest peak in the beginning of October (Figure 5.8). This was followed by a consistently active calling period through most of October and then a steady tailing off period into mid-November. During November there was diminishing calling until the final recorded call on the 25th. In Figure 5.8, the x-axis shows the dates of the recordings while the y-axis shows the summed number of seconds that the frogs were calling out of the 11 recorded sessions every day.

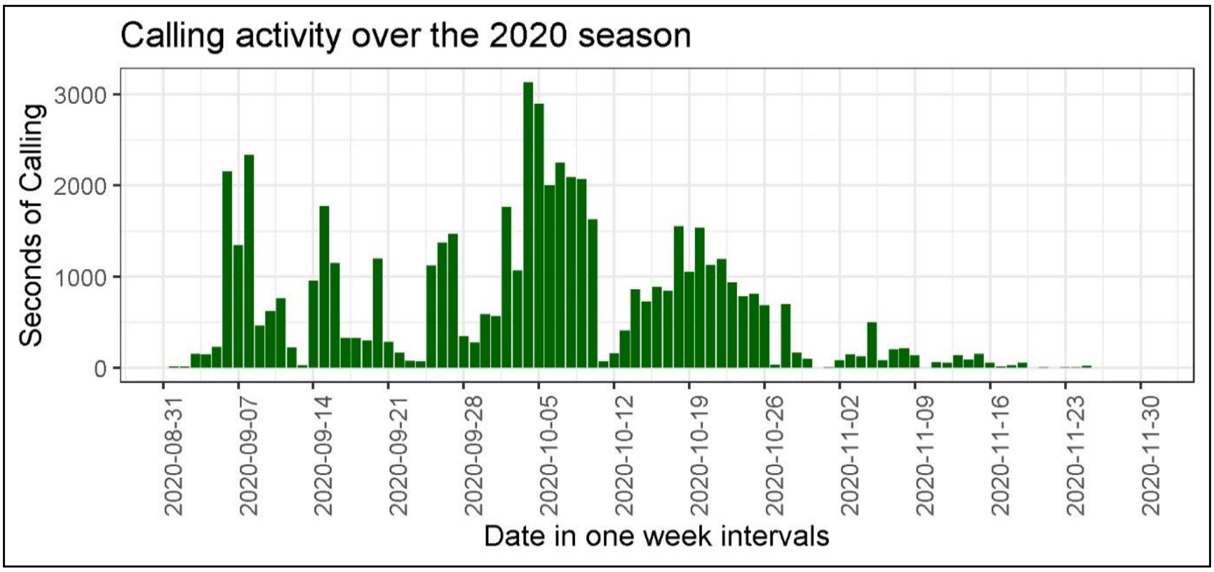

Figure 5.8 Overview of the daily amount of seasonal calling of *Leptopelis xenodactylus* from September 1st to November 30th, 2020, at Fort Nottingham.

5.3.3.2 Recorded calling activity per night

Figure 5.9 shows the distribution of the nightly calling for each month. The calling-time percentage is low early in the evening but increases towards a peak between 22h00 and 01h00 the following morning, after which it tails off. There is a general trend that, as the months progress, the frogs start calling later and later in the evening and the height of activity advances deeper into the evening. Although the time of sunset only advanced by 33 minutes over the three months, the photoperiod increased by 2.21 hours between 1st September and 30th November.

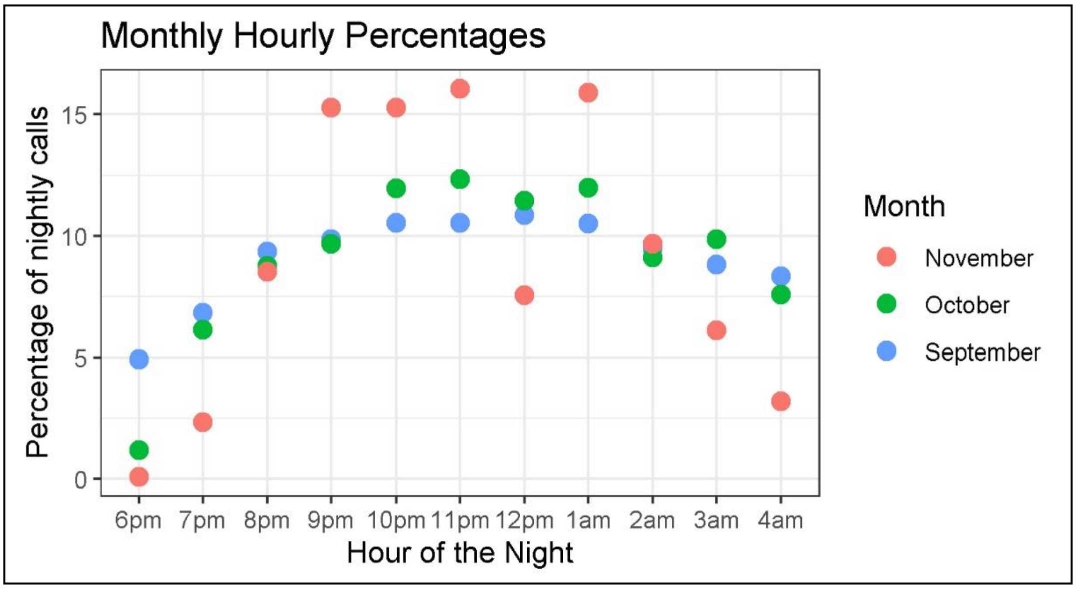

Figure 5.9 The distribution of the call emphasis of *Leptopelis xenodactylus* throughout the night over the three months of the 2020 season at Fort Nottingham. As recorded during the first ten minutes of each hour.

For the preceding figures, the percentages of calls were used to reveal patterns. To see the actual distribution of the total time of calling, the total number of seconds of calling per month were summed in Figure 5.10. Most of the calling takes place in October, with the bulk of the remaining calling happening in September and only a minimal amount of calling taking place in November. There are significant differences between the daily mean call sum of the three months (Kruskal-Wallis rank sum test: Kruskal-Wallis chi-squared = 43.987, df = 2, p-value = 2.808e-10). The mean call sum is significantly greater in October than in September and November, the mean call sum in September is significantly greater than November (Pairwise comparisons using Wilcoxon rank sum test with continuity correction, P value adjustment method: BH. September vs. October p = 0.047; September vs. November p < 0.001; October vs. November p < 0.001.

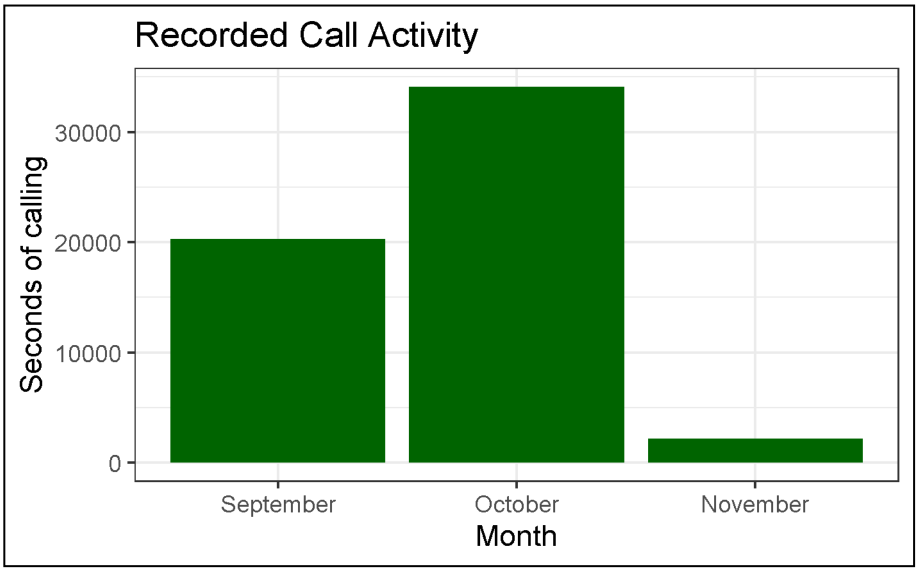

Figure 5.10 Distribution of "seconds of calling" of *Leptopelis xenodactylus* over the months of September, October and November 2020 at Fort Nottingham.

In Figure 5.11, the calls for the most active two weeks of the season (4th – 18th of October 2020) were summed and the percentages per hour were plotted. There is a fairly evenly spread distribution of calling for the hours from 20h00 to 01h00, showing relatively minor increases; 02h00 to 04h00 and 18h00 to 19h00 account for considerably less of the calling percentage.

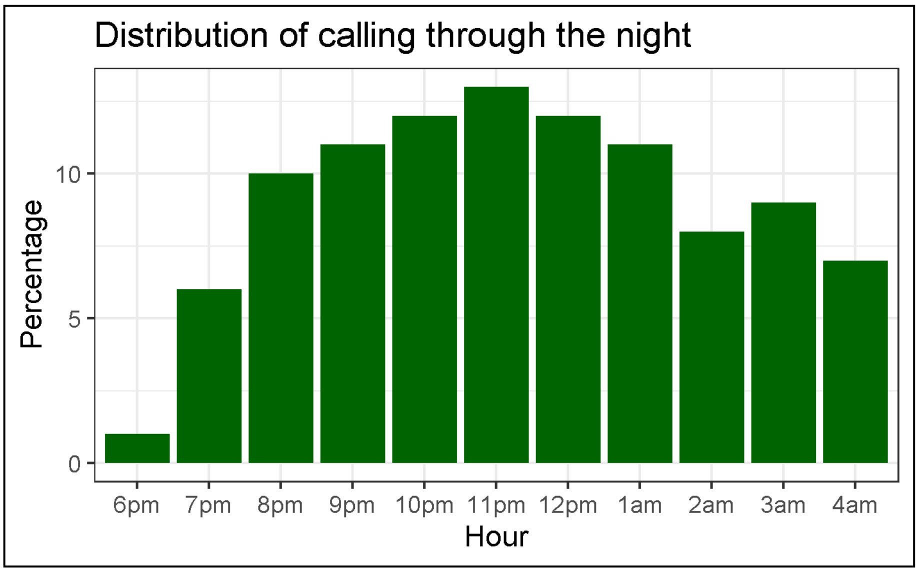

Figure 5.11 Overall hourly distribution of calling percentages for *Leptopelis xenodact*ylus for the hours of the night from 18h00 to 04h00 for the period of 4th to 18th of October 2020 at Fort Nottingham.

5.3.4 Environmental triggers for calling behaviour

Figure 5.12 reveals the clear inhibitory effect that rain has on the calling activity of *L. xenodactylus*, with most calling occurring when there was no rainfall recorded. There is a significant monotonic negative correlation between amount of rainfall (mm) and amount of calling (Spearman's rank correlation rho: S = 155, p-value = 0.024; sample estimate of rho = -0.236). The correlation is, however, not very strong.

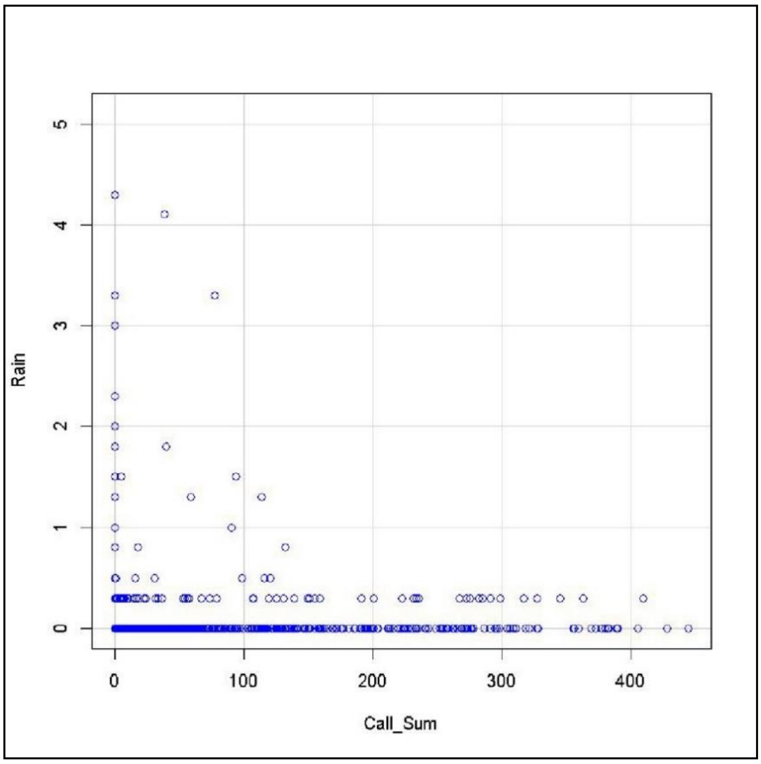

Figure 5.12 Scatterplot of *Leptopelis xenodactylus* call sum in relation to rainfall at Fort Nottingham.

From Figure 5.13 it is apparent that most of the calling happens between 7 °C and 20 °C. Call sum, however, does not have a significant (monotonic) correlation with average daily temperature (Spearman's rank correlation rho: data: S = 125, p-value = 0.966, rho = 0.004).

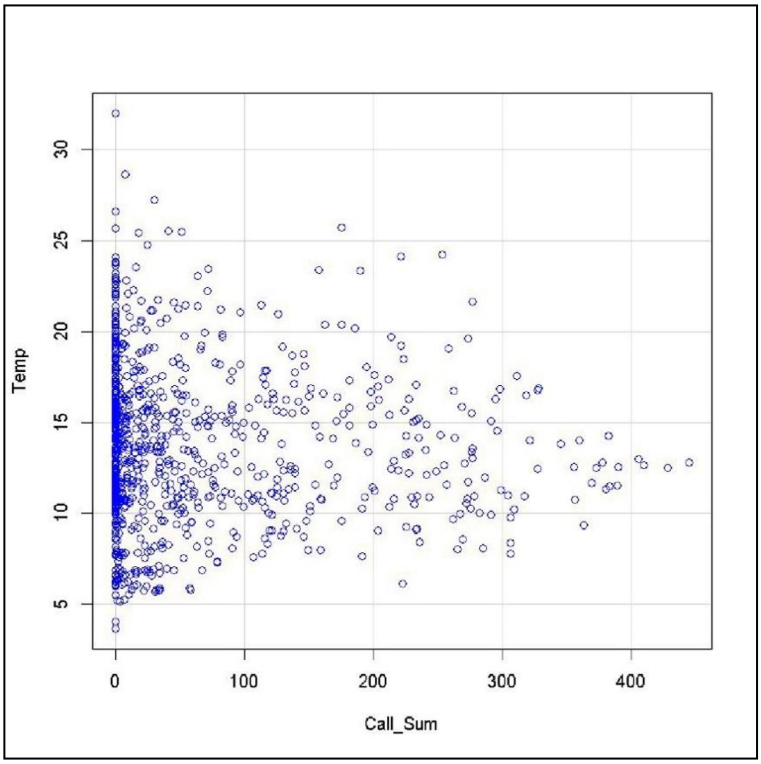

Figure 5.13 Scatterplot showing the *Leptopelis xenodactylus* call sum in relation to average daily dry temperature at Fort Nottingham.

While much of the calling occurred at high percentages of humidity (Figure 5.14), call sum had no significant monotonic correlation with average daily humidity (Spearman's rank correlation rho: S = 149, p-value = 0.080, rho = -0.184).

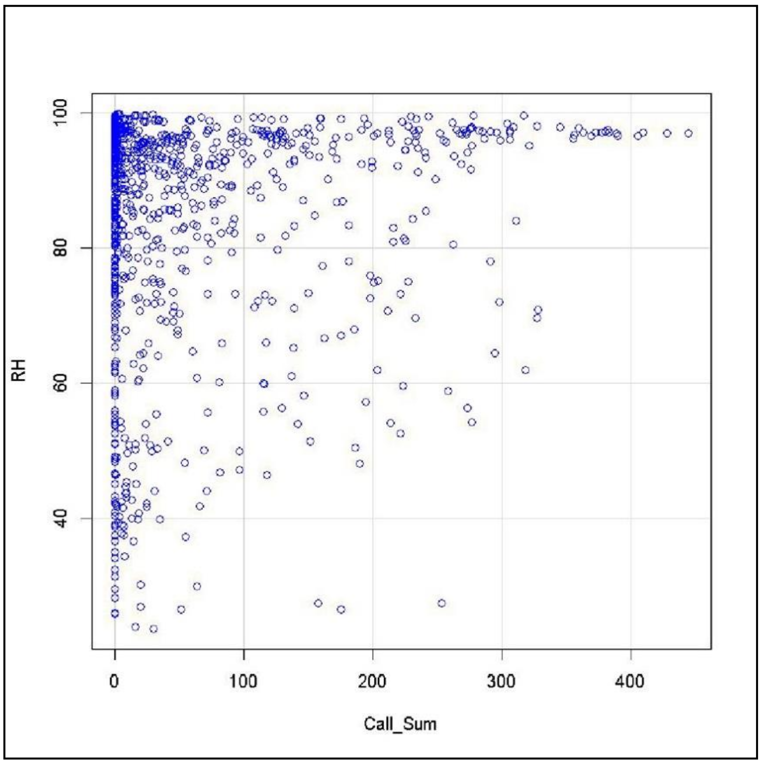

Figure 5.14 Scatterplot showing *Leptopelis xenodactylus* call sum in relation to relative humidity at Fort Nottingham.

From Figure 5.15 it is clear that increasing wind speed had an inhibitory effect on the calling of *L. xenodactylus*, even though call sum had no significant (monotonic) correlation to wind speed (Spearman's rank correlation rho: S = 133, p-value = 0.597, rho =-0.056).

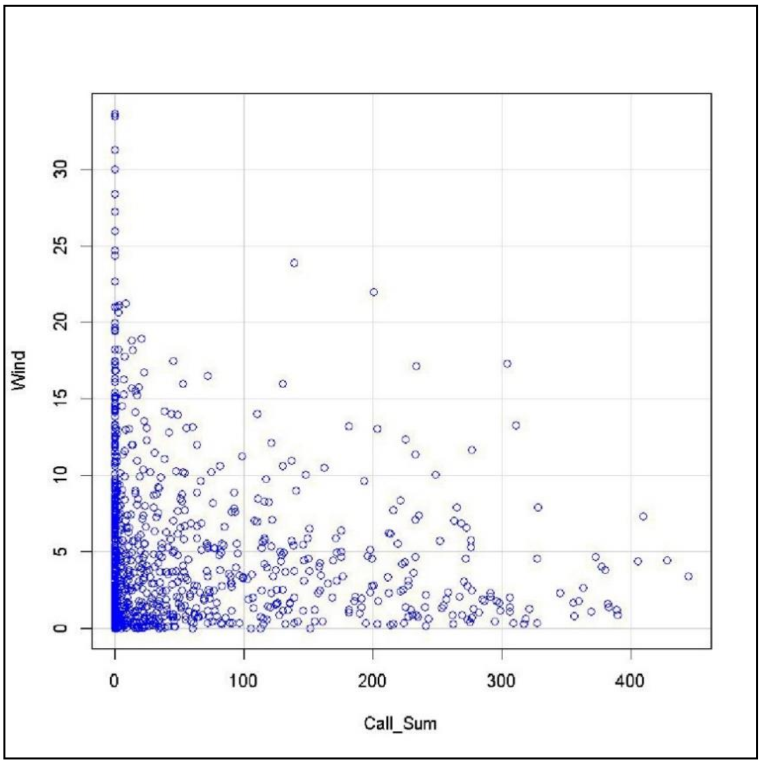

Figure 5.15 Scatterplot showing *Leptopelis xenodactylus* call sum in relation to Windspeed at Fort Nottingham.

Figure 5.16 shows a clear negative relation between the photoperiod and the amount of calling, with it decreasing significantly as the photoperiod rose above 48 000 seconds per day. Call sum shows a significant negative (monotonic) correlation to photoperiod (Spearman's rank correlation rho: S = 196, p-value < 0.001, rho = -0.561).

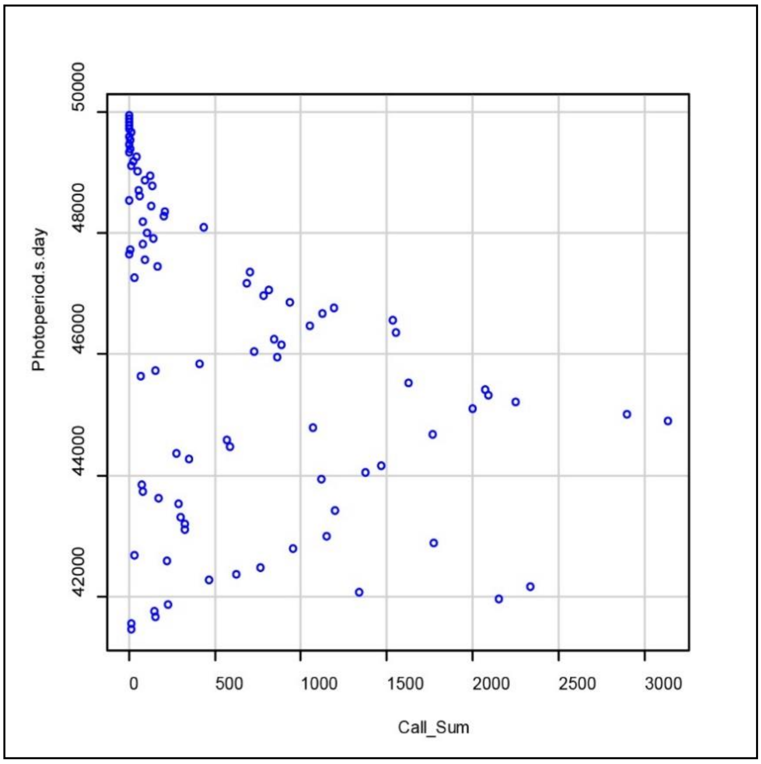

Figure 5.16 Scatterplot showing *Leptopelis xenodactylus* call sum in relation to photoperiod at Fort Nottingham.

From the tests, no significant correlations were found between call sum and percentage of moon visible from earth, maximum temperature, minimum temperature and maximum relative humidity. Call sum was, however, found to have a significant monotonic but not linear correlation to minimum relative humidity (Spearman's rank correlation rho: S = 152, p-value = 0.049, rho = -0.207; Linear regression: Call sum = -0.819* minimum relative humidity + 664.984, residual standard error: 745.9 on 89 degrees of freedom, adjusted R-squared: -0.010, F-statistic: 0.068 on 1 and 89 DF, p-value: 0.796).

5.5 Discussion

5.5.1 Call types and pattern

This study suggests that there are at least two different vocalizations made by these frogs. A-type calls are likely the main advertisement calls, as these are the first calls that start in the evening and are always part of the chorus. All calling frogs present appear to make these calls. B-type calls appear to be aggression or territorial calls based on the sound clip extracted from the video. This hypothesis is supported by Schiøtz (1999) who theorized that the typical short vocalisation of tree frogs is an advertisement call while the buzzing is a more territorial call.

There appears to be very little pattern to the calls that are used or the order of calls in a "chorus". As mentioned, A-type calls are always present in a chorus, while B-type calls are sometimes absent on a night or present only in small numbers. However, when B-type calls are being made, they can be plentiful and usually precede the A-type calls and are frequently repeated several times in quick succession, seldom being made individually.

5.5.2 Call variations

The two variations that were observed in the dataset could be explained in several ways. They may be artifacts in the recordings, simply A and B calls that have been recorded onto the files in an unusual way for some reason. They could also be the result of frogs that are calling at a distance from the recording device, or from a ditch or hollow, or from behind vegetation, obstructing or modifying the call in some way before it reaches the recording device. Another possibility is that the variation could be the result of calls made from underground burrows, with the calls being modified as they pass through the substrate causing the different appearance. Finally, it could be calls made by frogs that are smaller or not calling "properly", either still "warming up" or with injuries or deformities, resulting in their vocalisations not sounding like "standard" A or B calls.

These variations could also be different call types. There are several possibilities for what these calls may be used for, ranging from female release calls to distress calls to male aggression calls. Further work will have to be conducted including targeted recording of individual behaviour to distinguish what the various calls are used for and if, in fact, these are different call types or just anomalies of the A and B calls.

5.5.3 Calling activity

It remains unclear what actually causes frogs to start and stop calling but Figure 5.12 illustrates that rain inhibits calling. It is likely that call triggers will be environmental and climatic factors, however, more work needs to be carried out to reveal which the parameters are and what the relationship is. While calling decreases markedly as the season advances, it is apparent from the Ezemvelo KZN Wildlife Biodiversity Database records, (EKZNW 2022) as well as the forestry report (Harvey 2005) and the observations from this project, that the frogs are still active in the months from November to March. The calling, however, though not ceasing entirely, became erratic and infrequent from mid-November and lacked the "enthusiastic" calling choruses that occurred earlier in the season until they cease calling and become dormant for winter.

Observations showed that the levels of calling activity start slowly in the early evening and usually increase as the evening progressed. This is perhaps due to the frog's nocturnal activity pattern. While the occasional call can be heard in daylight, they generally start to become active during twilight's progression into full darkness. This would also explain why the peak calling period becomes progressively later as the weeks pass and the photoperiod of each day grows longer, meaning that full darkness only comes later in the evening. Their behaviour during violent weather might be explained by predators being less active in the inclement weather and the frogs more visible and less cautious, with less risk of being predated upon.

5.5.4 When to plan fieldtrips

October appears to be the best month to plan field trips for *L. xenodactylus*, at least at Fort Nottingham, as their calling is significantly more consistent and of longer duration than in the other two months of their annual calling period. Rainfall has a significant negative impact on calling activity and should be taken into account when planning trips. While temperature, relative humidity and wind do not have a significant correlation with calling activity, the data shows increased levels of calling at temperatures between 10 °C and 20 °C, high relative humidity and low wind speeds. Photoperiod also has a significant negative correlation to call sum, with calling being markedly reduced beyond 48,000 s of daylight per day (13.33 hours of light per day, 3rd of November).

Appendix: Supplementary data

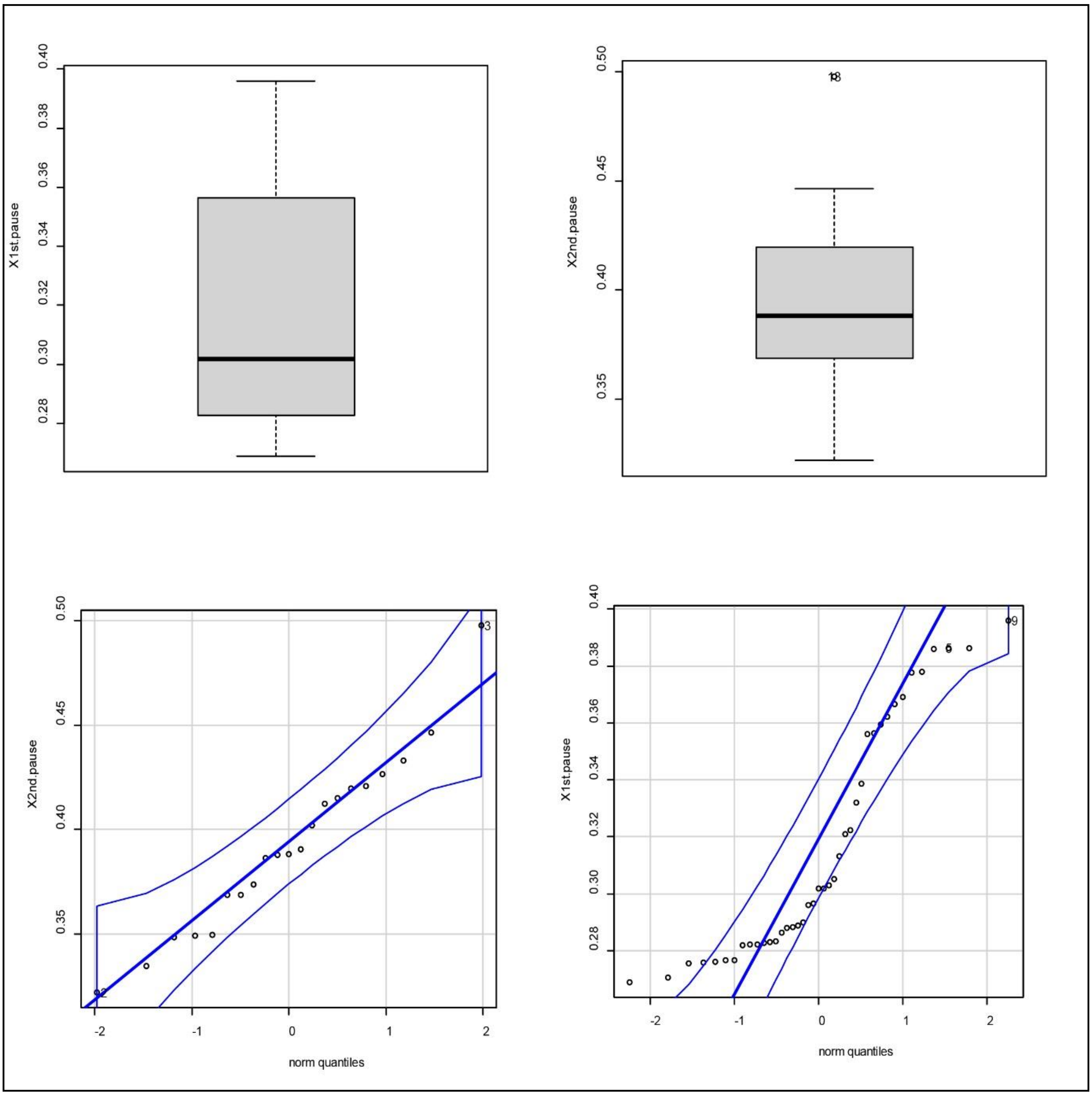

Figure S5.1. Boxplots (top) and QQ plots (bottom) showing that the first pause length for the *Leptopelis xenodactylus* A-type calls is not normally distributed.

CHAPTER 6

REPRODUCTIVE BIOLOGY

6.1 Introduction.

Owing to the long-toed tree frog’s relatively recent discovery, restricted geographical distribution and extremely secretive nature, very little was known about its life history and reproductive behaviour. Much of what was documented in literature was informed guesses based on other species of *Leptopelis* which have been studied and are better known, although the reproductive behaviour of the genus, in general, is little known. Although sometimes prolific in specific sites and relatively common, observations on the breeding behaviour of species in the genus *Leptopelis*, other than their calling, are rare.

The holotype *Leptopelis xenodactylus* was a gravid female found in Underberg in March 1961 (Poynton, 1963). From this, a conclusion was drawn that it is an autumnal to winter breeding species. However, a year later Poynton further hypothesized that this was evidence for aestivation (Poynton, 1964). The original specimen was 52.2 mm SVL with a head width of 19.8 mm and the eggs inside it were said to be “ripe” (Poynton, 1963).

Knowing more about the breeding habits is important for informing how to manage wetlands to better protect and conserve this currently Endangered species. Knowing when and where these frogs lay their eggs would allow for more informed livestock management and burning regimes to ensure that the wetlands are less disturbed during the most sensitive period of the frogs’ development. Information gleaned about this species could also possibly help shed light on other fossorial members of the genus.

6.2 Material and Methods

Most of observations described in this chapter were made at the main study site at Fort Nottingham (See chapter 2) during the course of the four fieldwork seasons spanned within the project’s duration. Observations were documented in a field notebook and photographs were taken in the field. Added to this was much, often incidental, information acquired through observing and working with the species throughout its range on the many field trips and nights spent both locating the frogs and observing the species’ behaviour.

Personal conversations with James Harvey were also helpful for confirming trends that had been observed, as he has a good knowledge of the genus and species from his ongoing work in high elevation wetlands. His field notes also contained dates of observations and behaviour which added depth to the observations and records gathered during this project.

During the final field trip of this study, on the 12th of October 2022, a pair of frogs in amplexus was discovered on the top of a small hummock in the wetland at Fort Nottingham. The frogs, still in amplexus, plus the hummock were very carefully removed from the study site and kept off-site to allow continued observation and monitoring of the development of the eggs. The eggs were kept in the 25 l bucket they had been placed in and humidity was maintained by adding rainwater every week. The bucket was kept at room temperature in an inside office with the lid kept firmly in place except for daily airing when photographs were taken.

Two tadpoles were also captured at a site close to the main Fort Nottingham site and kept off-site to observe development. The tadpoles were kept in a glass aquarium containing rainwater and fed on commercial fish-food flakes. The water was changed frequently, when necessary and the tank was maintained at room temperature. Natural substrate and vegetation was provided and changed frequently. A measuring tape was fixed to the back of the long narrow tank specifically designed for tadpole photography. Photographs were taken at staggered intervals of the tadpoles aligned with the measuring tape to get rough measurements of the tadpoles as well as to document their growth and development.

6.3 Results

6.3.1 Position of calling

Over the three-year study period, the calling positions of 132 male *L. xenodactylus* were recorded as either subterranean, at ground level or above ground in vegetation (elevated). A significant change in calling position from September through October to November was observed (Table 6.1; Pearson's Chi-squared test: χ^2 = 15.695, df = 4, p-value = 0.003457; Fisher's Exact Test: p-value = 0.002416). The χ^2 components (Table 6.1) indicated that proportionately more males than expected were calling from underground in September and proportionately fewer males than expected were calling from underground in November. Also, the relatively large χ^2 component for the elevated position in September indicated that proportionally fewer males were calling from an elevated position that month.

Table 6.1 Calling positions of male *Leptopelis xenodactylus* recorded over the three-year study. The number of calling males, the (Percentages) of the total number, the [Expected counts] and the {χ2 components} are presented.

Month	Subterranean	Ground Level	Elevated	Total
Sept	12 (9.1) [6.55] {4.55}	12 (9.1) [8.36] {1.58}	24 (18.2) [33.09] {2.5}	48 (36.4)
Oct	5 (3.8) [5.3] {0.02}	4 (3.0) [6.80] {1.15}	30 (22.7) [26.89] {0.36}	39 (29.5)
Nov	1 (0.8) [6.14] {4.3}	7 (5.3) [7.84] {0.09}	37 (28.0) [31.02] {1.15}	45 (34.1)
Total	18 (13.6)	23 (17.4)	91 (68.9)	132 (100)

6.3.2 Breeding

Leptopelis xenodactylus breeding adults do not appear to fiercely guard their home ranges and seem to be generally tolerant of conspecifics in close proximity, even if both are calling. The only apparent aggression that was observed was on the evening of the 20th of September 2021, at the

Fort Nottingham study site. Three "pairs" of frogs were found interacting, 25 frogs were located, and many others were heard. These frogs were "grappling" together, not in amplexus, and were of a similar size suggesting that it was male combat. As soon as a light was shone on them, they broke apart and showed no further interaction. Many small frogs were present, of a size that was assumed to be offspring from the previous season. A large gravid female was found, surrounded by males. She appeared to be "ripe" and ready to lay. She was placed near a large male who had been calling (Figure 6.2). Another large male approached her, and the first male "buzzed" several times advancing towards the intruder, who then fled. The male and female, however, showed no further interaction.

Figure 6.2 Large female *Leptopelis xenodactylus* on the left and smaller male on the right.

The 23rd of October 2021 was another extremely active night for the frogs at Fort Nottingham. Upwards of 30 frogs were located, 21 of which were under 2.5 g in mass. All were active and mobile in the grass, and it was hypothesized that they were trying to intercept females. They were all calling and so were assumed to be sexually mature. There were many more frogs calling, but mostly from the surface or underground. Two females were located hunting in an elevated position on nearby American bramble bushes and one was extremely thin. She was thought to have just laid her eggs. Another slightly smaller female, visibly full of eggs, was located later the same night within the wetland amongst the males (Figure 6.3). From this it was assumed that they were actively laying during that evening. From photographs taken of this gravid female it was possible to estimate the minimum number of eggs, (by counting their shapes protruding through the skin), to be 30. A visibly gravid female was also captured on the 4th of December 2019, as was another on the 12th of October 2022, suggesting a prolonged laying period.

Figure 6.3 Large female *Leptopelis xenodactylus*, with eggs visible in her belly.

All of the distinguishable females that were located during the study were found relatively late in the season, with the earliest being on the 20th of September. This was the only one found in September, four were found in October, three in November and one in January.

Harvey (pers coms) reported that on 16th September 2009, 6 adult frogs were caught in a wetland and kept in a bucket overnight to process and release the following night. When the bucket was opened the following day, a pair were found in amplexus, although no eggs had been laid. It is clear, from Figure 6.4, that they use "axillary amplexus" and are reproductively active from fairly early in the "season".

Figure 6.4 *Leptopelis xenodactylus* in amplexus after being kept in a bucket overnight. (Photo: J. Harvey)

On the 12th of October 2022, a pair of *L. xenodactylus* were found in amplexus at the Fort Nottingham site. It was a calm, clear evening upon arrival at the site around 18h30, but, by 19h26 when the pair were located, a storm had blown in with vivid lightning, strongly gusting wind and intermittent, light, yet driving, rain. The temperature ranged from ± 10 °C to a maximum of 14 °C for the evening. The frogs were located on the top of a hummock which protruded above the surrounding substrate by ± 15 cm and was ± 45 cm across. There was little standing water surrounding the hummock, however, the substrate was saturated mud, with vegetated hummocks protruding all around. The hummock itself was densely vegetated with several different types of grasses, forbs and sedges. The frogs had excavated a depression at the base of the vegetation into which their bodies neatly fitted. Both their heads protruded above the ground (Figure 6.5). The frogs ceased activity whilst being observed, hunkering down into the hollow and closing their eyes. The hummock was carefully removed and made to fit snugly into the base of a 25 litre bucket with the frogs, undisturbed and still in their burrow, and the lid was closed. They remained in place for the 70 km drive back to base and, by 22h30, appeared unchanged. They were left undisturbed until the next morning at 07h00 when they were further investigated. They were still in amplexus, but they had filled the entire cavity with eggs to the extent that their bodies were raised out of the hole, and they were perched on top of the egg clutch. By 08h30 they had separated with the male moving to the edge of the bucket and attempting to escape. The female, however, was still sitting over her eggs (Figure 6.6). By 10h30 the female had left this position and was situated above them in the grass and by 11h45 had moved from the eggs and was on the periphery of the bucket. By 12h30 she had settled at the base of a sedge in close proximity to the eggs, but this was possibly coincidental due to the limited options available in the confines of the bucket. The frogs were removed from the bucket and measurements and photographs were taken before they were released back to where they had come from the following evening. The frog's measurements are recorded in Table 6.1.

Figure 6.5 Amplectic pair of *Leptopelis xenodactylus* in situ on top of a hummock in the wetland.

Figure 6.6 Female *Leptopelis xenodactylus* sitting over her eggs.

On the same evening on which the amplectic pair were found, another female was located on the edge of the wetland with visible eggs in her belly – it was thought that she might be heading towards some calling males within the wetland. She was captured and placed in a container with several males who had been calling, however, no interaction was observed. She, however, maintained a constant state of inflation, with the skin visibly distended by air, for the duration of her capture and handling, including the taking of measurements (Table 6.1) and photographs (Figure 6.8).

Table 6.1 Morphometrics of amplectic pair of *Leptopelis xenodactylus*.

	SVL (mm)	Head width (mm)	Tibia (mm)	Mass (g)
Male	37.8	14.5	17.6	4.4
Female	46.2	17	20.6	6.9

Once they had separated and were no longer in amplexus it was observed that the female of the amplectic pair had the remains of a substance on her sides, where the male's forelegs had been seen clasping her (Figure 6.7). This can also be seen in Figure 6.4, suggesting that the frogs may use an adhesive substance to assist with maintaining amplexus during navigation to a suitable nest site and the digging of the nest. The digging appears to be done mostly by the larger female's hind legs as the male's legs are elevated on her back. The substance had the same appearance as the sticky, foul tasting and smelling, substance that the species sometimes excretes when handled.

Figure 6.7 Female *Leptopelis xenodactylus* showing the remnant adhesive substance from the male's grasp during amplexus.

Figure 6.8 An inflated female *Leptopelis xenodactylus* with eggs on right, compared with non-inflated female on left who had just laid eggs.

6.3.3 Eggs

The eggs are fairly large, round spheres of a creamy white appearance, typical of the large yolk sac found in the genus (Poynton and Broadley 1985), but lacking any further pigmentation (Figure 6.9). They were found to be adhesive, sticking to each other and the surrounding substrate. Due to the fact that they were laid in a dense clutch, it was impossible to count them individually and accurately without disturbing them. However, 99 eggs were visible from a photograph taken of the cluster. The eggs were measured using a digital measuring device and, from a sample of 10 eggs that could be measured, had a diameter of 3.6 ± 0.13 mm.

Figure 6.9 The clutch of *Leptopelis xenodactylus* eggs once the female had left them.

After the female had left her eggs, the vegetation was carefully cleared from around and above the eggs with sterilized forceps to allow for better observation. By the 26th of October, the eggs had changed considerably, with the beginnings of small tadpoles, still attached to their yolk sacs, visible inside the eggs (Figure 6.10). The eggs now had a diameter of 4.21 ± 0.12 mm (n=10).

Figure 6.10 Egg clutch *of Leptopelis xenodactylus* at 14 days old, with the covering vegetation trimmed.

By the 9th of November, when the eggs were 4 weeks old, the tadpoles, along with their yolk sacs, were visible within the eggs (Figure 6.11). They twitched violently in response to stimulation by a torch or the vibration of the bucket lid being opened. Several infertile eggs were visible in the photographs, but the majority contained developing tadpoles.

Figure 6.11 *Leptopelis xenodactylus* egg clutch at 28 days old.

6.3.4 Tadpoles

In a survey report, James Harvey mentions finding seven *L. xenodactylus* tadpoles in mid-December 2006 (Harvey 2007). The pictures show the tadpoles to be in stage 36 of the Gosner table (Gosner 1960). Channing (2008) describes collecting three tadpoles near Franklin Vlei on the 15th of January 2007 that were between grass polls in a few mm depth of water over soft mud. The larger two were stage 38 and the smaller, stage 37 of the Gosner table (Channing 2008). On the 6th March 2015, Louis Du Preez (pers coms) captured and photographed tadpoles between Gosner age 40 and 41 at Franklin Vlei.

On the 26th of December 2019, tadpoles were found at a site on the top of the hill near the Fort Nottingham study site (-29.39620, 29.90250). Two tadpoles were located in a small open patch of water in the wetland (Figure 6.12); the puddle was ± 5 cm deep and the water was clear when the tadpoles were first spotted.

Figure 6.12 Situation of the puddles in which the tadpoles *of Leptopelis xenodactylus* were located.

The tadpoles were kept in captivity (Figure 6.13). On the 2nd of January they were approximately 38 mm and 50 mm in length, respectively, with small hindlegs present in the larger, estimated at a Gosner stage 35 and the smaller at Gosner stage 25. They were fed on commercial fish flakes and grew rapidly. By the 11th of January, they were 53 mm and 40 mm in length, respectively. By the 17th of January they were 55 mm and 48 mm, respectively and the larger had well developed hindlegs and was changing colour from near black to green, while the smaller one was developing larger hindlegs. By the 21st of January, the larger individual had developed forelegs, turned brighter green and had shortened to 40 mm. On the 22nd it had shortened further to 29 mm and was, by then, bright green. By the 24th it had absorbed all but the fleshy nub of its tail and measured 21 mm SVL and weighed 0.4 g. By the 25th of Jan, the second tadpole measured 54 mm and the hindlegs had begun to lengthen and fill out. By the 4th of February, both were released where they had been found, one as a fully formed froglet and the other as a metamorph (Figure 6.14).

Figure 6.13 *Leptopelis xenodactylus* tadpoles that were kept in captivity.

Figure 6.14 Composite picture of metamorph of *Leptopelis xenodactylus* prior to release, gills and tail still present on the smaller. Human finger was 14 mm across at widest.

James Harvey (pers coms) raised a tadpole through to maturity and kept the young frog in captivity for over a year. He mentions that the frog began calling at a year of age, although the call was at an audibly higher pitch than the usual calls heard in the field. This confirms a point that was identified during the project fieldwork, where the smaller frogs (assumed to be the previous season's metamorphs) were identifiable by the pitch of their call. This allows researchers to select for the larger specimens when doing the fieldwork. The young frog kept in captivity ate fruit-flies as a metamorph and progressed onto crickets as it grew. Van Dijk mentions those he kept in captivity eating moths (Van Dijk 1978).

6.3.5 Sexual dimorphism

While it is usually difficult to differentiate between males and females of this species in the field (Figure 6.15), several features have been observed that can assist with this. Firstly, as the calling is performed by males only, any calling frog must be male. Secondly - size. Only the females achieve the largest sizes, with the upper SVL limit of males appearing to be around 43 mm while the females can be considerably larger, with the largest formally recorded being 54 mm. The females also seem to have a noticeably sharper nose while the males' noses are more rounded.

Figure 6.15 Four *Leptopelis xenodactylus* for comparison, two females on right, two males on left.

6.4 Discussion

6.4.1 *L. xenodactylus* in comparison to other *Leptopelis*

At the commencement of this study very little was known about the breeding behaviour of *L. xenodactylus*. Minter et al. (2004) suggested they might be similar to *L. bocagi*, breeding in shallow puddles in inundated grassland areas, while Channing et al. (2012) stated that "the eggs are laid in soft mud at the edge of swamps, and the tadpoles wriggle to the water." Carruthers and du Preez (2001) report that *Leptopelis* lay their egg clutches in burrows in the mud or humus in close proximity to water and the tadpoles wriggle into the water upon hatching.

Wager (1986), speaking of *L. natalensis,* relates how they descend to water bodies in forest valleys, in early summer after rain. The eggs are deposited out of water, from around 30 cm to 1 m away from the water's edge on mud or under leaf litter in a "densely sheltered spot". He describes the eggs as large, roughly 7 mm in diameter and pure yellow, with around 200 being laid in a single layer (Wager 1986). *Leptopelis natalensis* is known to breed in riverine bush and swampland along the edge of the north-eastern coastline of South Africa (Passmore and Carruthers 1995), in permanent ponds and densely forested swamps (Channing et al. 2012). *Leptopelis mossambicus* breeds in shallow, temporary pans (Channing et al. 2012), in wooded savanna (Passmore and Carruthers 1995). *Leptopelis natalensis* and *L. bocagi* are both known to construct shallow burrows in mud or leaf litter in which to deposit their eggs - generally in close proximity to water bodies (du Preez and Carruthers 2017). *Leptopelis natalensis* is reported to go into amplexus in the trees and descend to the ground, together, to excavate the shallow nest (du Preez and Carruthers 2017). Les Minter (pers. Comm.) reported observing an amplectic pair of *L. mossambicus* overnight. They burrowed into the soft leaf-litter and soil until completely out of sight. In the morning he carefully excavated the burrow and found the egg clutch which contained 317 eggs.

Wager (1986) went on to mention that the tadpoles of *L. natalensis* can remain unhatched, within the eggs, long after they are mature enough to hatch, awaiting suitably damp conditions to emerge and wriggle to the water where they continue their development. Interestingly, when a rainfall event occurred, several batches of eggs, laid at different times, all hatched simultaneously as the conditions appeared to be suitable (Wager 1986).

6.4.2 Placement of Eggs

At the beginning of the project it was presumed, based on what was known from other *Leptopelis,* that *L. xenodactylus* would lay their eggs in covered burrows on the periphery of the wetland, under grass-litter and substrate. This does not appear to be the case, however, based on the new evidence found. While the hummocks were already thought to be important to the frogs, it was assumed that this was only for calling purposes, but it appears that they may also support and facilitate an important part of the breeding behaviour. The amplectic pair were situated right on the top of the hummock, in an area that was presumably safe from most flooding events. Interestingly, the eggs were completely exposed on the surface of the burrow which would potentially subject them to higher predation - although the clutch was surrounded by relatively tall and dense vegetation. Perhaps, however, this threat is mitigated by the protection afforded them by the “island” effect of the hummock emerging from the surrounding thick mud. In this area the surrounds were heavily trampled by cattle, but they avoided the hummocks when passing through the area. This exposure to the elements may also afford a degree of protection from fungal and other infection, where a closed burrow might create conditions more conducive to pathogens that can cause morbidity and mortality.

6.4.4 General behaviour and movement

All the females found during the project, with the exception of the amplecting individual, were found on the edges of the wetland, either foraging or heading in the direction of the calling males. It is possible that the females emerge with the first substantial rains and head out to the periphery of the wetland to forage and build up energy, while their eggs develop. During this period, with the wetlands perhaps not yet suitable for nesting and egg-laying, the emerging males establish their calling sites and form choruses. In October, when conditions are suitable for successful breeding, the females seem to descend into the wetlands and select a mate from the calling males. The female who was captured and maintained a state of inflation could possibly have been doing this in order to protect and cushion her eggs from impacts and possible harm.

While it is impossible to estimate population size from the data collected in this study, many observations, over extended periods at various locations, allow for informed estimations of populations. For most reasonably sized and suitable sites, the estimated resident populations are in the hundreds as opposed to tens or thousands. On most nights in the field, most of the frogs found were calling, however, there are an unknown proportion of individuals that will not be calling, which complicates the estimations. They are clearly not as abundant as some *Hyperolius* spp., for example. If a site is visited frequently enough, there will be nights when many of the frogs in the population may be active, with the majority of these being smaller frogs, as mentioned previously. This population skew, towards a greater abundance of smaller individuals is normal but may also reflect migration of some younger frogs to the wetland from one or more other wetlands in the vicinity.

It was apparent from fieldwork that early in the season the frogs are often underground or at ground level, while later on in October and November they are generally more mobile and higher up in the vegetation. From this it is assumed that early in the season they are calling from close to their overwintering area, in the most “suited to burrowing” sections of the wetland, while later in the season, as they become more mobile and active, they start to disperse to the other parts of the wetland. They also become considerably easier to locate later in the season, frequently calling from high up in vegetation and from exposed perches. Throughout the season they respond to auditory prompts, but it is unclear whether the males are responding from aggression or are simply being stimulated to form a chorus.

It is also apparent that the frogs often “clump” in close proximity to each other, displaying this behaviour throughout the year. Even later in the season, when they are more dispersed, they are still located within fairly limited areas of the wetland and in close proximity to each other. This could be due to aggregation of calling individuals (Calsbeek et al. 2022), or could suggest that there is a well-defined set of parameters that create a suitable microhabitat for them. These parameters may include the type of vegetation and depth of water and could also contribute to the seasonal movement of the frogs. As the season advances, with the increase in rainfall and the growth of vegetation, more areas become suitable for them, thus enabling the frogs to disperse to other areas of the wetland. From data collected at the Fort Nottingham site, as well as at other sites, the frogs do not appear to utilise the entire wetland, avoiding sections that to the human eye appear similar to parts that they do favour, suggesting there are subtle, but essential, parameters that dictate which areas are and are not, suitable for them.

Another possible explanation for the seasonal increase in mobility and elevation in perch height, is temperature. Early in the season, when the ambient temperature is coolest, the frogs would be considerably sheltered from cooling conditions calling from their underground burrows or surface calling sites, including being sheltered from the wind. On the 27th of September 2020, a total of seven frogs were found, five of which were in shallow, surface burrows. The air temperature was 6 °C while the water and mud temperature was 11 °C.

From the sample data it has been shown that early in the season (September) proportionately more males than expected are calling from underground positions, whereas late in the season (November) proportionately more males than expected are calling from elevated positions. This has implications for fieldwork as it shows that the frogs tend to call from higher up in the vegetation and are therefore easier to locate later in the season.

6.4.5 Conclusion

Through the observations made during this study, a lot more is now known about the reproductive biology and life cycle of this species. The males start calling in the wetlands from the beginning of September, most likely setting up territories, while the females are not to be found in the wetlands and may be on the peripheries or still underground. The females are apparent in the wetlands from the end of September onwards and egg laying events are likely to occur in October and November. The large, yolky eggs are laid in clutches in shallow burrows on the top of the hummocks and take approximately one month to hatch. The tadpoles are assumed to wait for a suitable rainfall event and, when conditions are suitable, break the egg casings and make their way to the water. Tadpoles are found in low numbers in shallow water and appear to develop within the season.

CHAPTER 7

SPECIES DISTRIBUTION MODEL AND GROUND-TRUTHING

7.1 Introduction

The ecological niche that a species occupies is described by Grinnell (1917) as “the conjunction of ecological conditions within which a species is able to maintain populations without immigration.” The correlative summary of a species’ environmental associations and the relationship between those associations and their geographic distribution, is what is dealt with in this chapter (Peterson and Soberón 2012). Species distribution Modelling (SDM) and ecological niche Modelling (ENM), perform statistical tests in an artificial-intelligence-based approach that is designed to predict the geographic distribution of a species (Peterson 2001). Modelling is a mathematical exercise that begins with the geographic space, the model is then fitted in environmental space and finally the outputs are usually visualized in geographic space again (Peterson and Soberón 2012). Ecological niche Modelling and species distribution models consider the biotic interactions of creatures, with their environmental conditions and mobility, in order to better estimate their distributions (Simoes et al. 2020). It can also be used to predict the suitability of an area or a habitat, for the species in question, by examining the existing occurrence records in relation to a set of environmental variables (Peterson et al. 2011). These tools can be extremely useful in a number of fields including quantitative ecological studies, evolutionary biology, conservation planning, resource management and public health.

While potentially a powerful tool, modelling is prone to error, mostly due to incorrect applications. Anderson (2012) lists five pitfalls for this research: firstly — incorrect taxonomic identification; secondly — lack of or inadequate databasing and georeferences; thirdly — the effect of sampling bias across geography; fourthly — violation of assumptions related to selection of the study region fifthly — problems regarding model evaluation to identify optimal model complexity. He goes on to clarify that, if done correctly, this tool can produce high-quality models with valuable applications (Anderson 2012).

There is a growing awareness and concern about the accuracy and usefulness of environmental and ecological niche models (Soberon and Peterson 2004; Simoes et al. 2020). However, if they are considered as an estimation, even when known to not be fully accurate, and are used as such, they can prove useful for certain applications (Warren 2012). Data generated using ENM and SDM maximizes the usefulness of existing locality data and creates a useful evaluation. For sound decisions to be made, ENM and SDM predictions should be followed by well-designed fieldwork and ground truthing to support and confirm outcomes predicted by the models (e.g. (Tarrant and Armstrong 2013). This is especially so when restricted range species are involved (Costa et al. 2009).

The predicted impacts caused by climate change have been calculated according to two separate climate change models (GFDL2.1 and HadCM2) for KwaZulu-Natal, South Africa (Jewitt et al. 2015). These two models show the extremes of the predicted changes, with HadCM2 predicting an average 2.1 °C mean annual temperature increase in KZN, coupled with a mean annual precipitation decrease of 90 mm, while GFDL2.1 predicts a 1.5 °C mean annual temperature increase in KZN with a slight increase in mean annual precipitation of 29 mm (Jewitt et al. 2015).

At the start of the Project there were 22 known localities for *L. xenodactylus* with accurate geographical co-ordinates. Ten of these were from pre-2004 when their IUCN listing was changed to Endangered and 12 in the years between 2004 and the start of this project in 2019 (Figure 7.1).

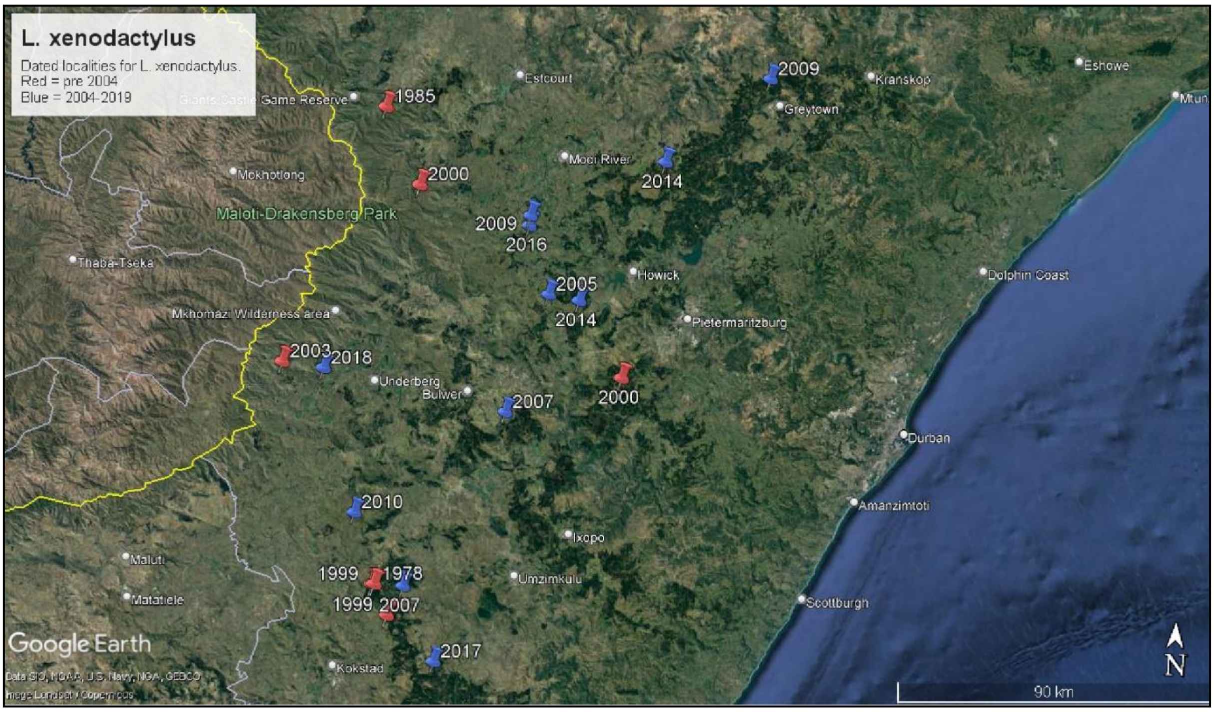

Figure 7.1 Google Earth® image showing known localities for *Leptopelis xenodactylus* in 2019 at the start of the project, red pins indicate localities pre 2004 and blue pins indicate localities added between 2004 and 2019.

7.2 Material and Methods

7.2.1 MaxEnt®

Forty-seven occurrence records for *L. xenodactylus* with geographical co-ordinates accurate to within 250 m of the actual location (WGS84 datum), were extracted from the Ezemvelo KZN Wildlife Biodiversity Database in August 2019. The data was thinned, leaving only one record per wetland, resulting in a dataset of 38 records. Literature was then consulted to assess which environmental predictors would be most likely to influence the distribution of the species (Armstrong 2001; Minter et al. 2004; Elith et al. 2011; IUCN 2017; du Preez and Carruthers 2017).

The variables used were vegetation type (categorical), landform (categorical), elevation (continuous), average summer mean daily maximum temperatures (continuous), average summer mean monthly rainfall (continuous), average summer relative humidity (continuous), soil plant available water (continuous) and average summer potential evaporation (continuous). Summer was defined as September to March. The coverages were developed using the decimal degree Cape (1880) datum by Shulze (Shulze 2007), at a scale of 1' x 1', and were then re-projected onto the WGS84 datum, Transverse Mercator lo31 central meridian and finally resampled to a 20 m x 20 m (400 m^2) grid based on Ezemvelo KZN Wildlife's 2008 version 1 landcover coverage (Ezemvelo KZN Wildlife (EKZNW) 2009; EKZNW 2011). The scale at which the climate variables were developed was larger than the scale at which the wetland coverage and land covers were developed, so the environmental coverages were resampled to a scale that was equal to the climate variables. The resampling was performed to allow for the inclusion of finer resolution data in the form of the wetland and landcover coverages.

MaxEnt® version 3.3.3k was used to develop a species distribution model for *L. xenodactylus*. Three cross-validating replicates were run with the maximum number of iterations being set at 1,000 to allow for and ensure, algorithm convergence, while default settings were used for all the other relevant parameters.

The model's performance was evaluated using a “jack-knife” test and the area under the curve statistics of the receiver operating characteristic plots (Phillips et al. 2006). MaxEnt® provides a regularisation method allowing ecologically relevant, but correlated variables, to be included in the modelling process (Elith et al. 2011). The default MaxEnt® settings for the regularisation parameters were used in the original model because it was a working document that was used in the field for finding new localities. The default feature classes were used due to the number of data points being less than 15 per fold, using a total of 38 points and, as mentioned by Phillips and Dudik (2008), for a dataset of less than 15 points one can only use linear and quadratic features.

The final probability distribution map for *L. xenodactylus* was constructed by overlaying the output probability map with the land transformation and wetlands coverages in the TerrSet® Version 19.0.4 Idrisi Geographical Information System (Eastman 1999) .

7.2.2 Ground-truthing

For the ground-truthing, the predicted distribution was mapped at two probability ranges: 0.5 to 0.74, and ≥ 0.75. Areas of predicted suitable habitat with a probability of occurrence of *L. xenodactylus* of around 0.5 could be considered “typical” habitat (Elith et al. 2011). For each ground-truthing trip, a route was planned to follow the roads that led as close to the wetlands of interest as possible and, once arrived at a wetland in the evening, observers would stop and listen for ± 10 minutes to hear if any *L. xenodactylus* were calling. If they were heard in the wetland, efforts were made to walk in, where legal, and find them, to get a more accurate GPS point and an estimation of frog population density. Where they were not heard, the observers would move on to either another promising looking locality on the same wetland or the next potential location on the route. Routes were selected to cover as many parts of the predicted area of occupancy as possible over the three seasons of the project.

7.2.3 Climate change models

To begin to establish the vulnerability of *L. xenodactylus* to climate change, the *.kml* files of the two models (HadCM2 and GFDL2.1) described in Jewett et al. (2015) were opened in Google Earth® and then overlayed with a *.kml* file of the known localities of *L. xenodactylus* that are accurate to within 250 m (including the new localities recorded during this study). From this it was possible to manually scrutinise each location and ascertain which and how many existing localities fall into each of the four quadrats of the vulnerability framework (Jewitt et al. 2015).

7.3 Results

7.3.1 Localities and Ground-truthing results

Since the commencement of the project in 2019, 21 new localities have been identified (Figure 7.2). This brings the total number of known localities to 43, an increase of 95.5%.

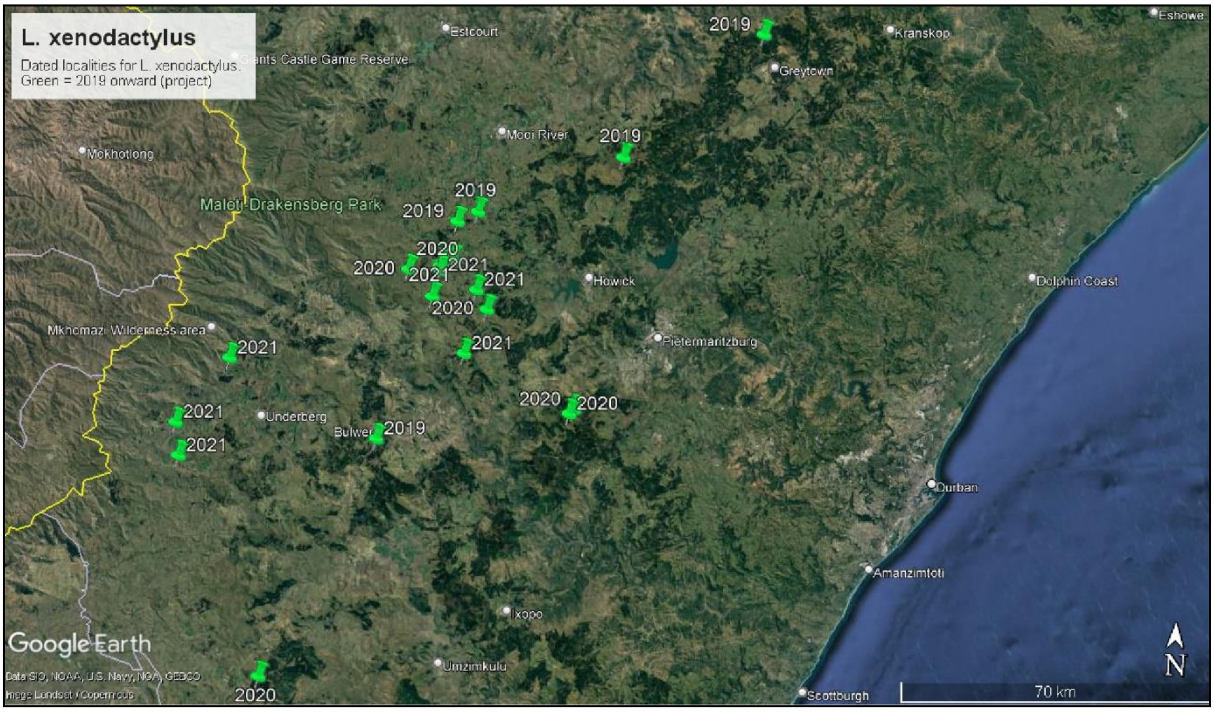

Figure 7.2 Google Earth® image of new localities for *Leptopelis xenodactylus* since the beginning of the project in 2019.

During the fieldwork, using the model for guidance on where to focus attention and effort, 19 new localities for *L. xenodactylus* were discovered as a direct result of ground- truthing the model - one was part of preliminary research before the project and the final locality was provided by a private individual.

Over the three years, 60 different wetland systems that were Modelled to have a ≥ 75% probability of *L. xenodactylus* being present, were visited. *Leptopelis xenodactylus* was confirmed to be present in 19. By nature, these frogs are secretive and could have been present but not vocalising at many of these sites on the night on which they were visited. Only presence data can thus accurately be gathered by this method. The naïve positive predictive power of this model is 0.32 (Fielding and Bell 1997). The mean habitat suitability of the wetlands that were visited was 75.95% ± 20.2% and the mean probability of the wetlands where the frogs were found was 67.26% ± 22%.

7.3.2 Model parameter results

The environmental variables that were included in the distribution model and the frequency of occurrence at the localities with records of *L. xenodactylus,* are presented in Table 7.1. These are the values for each parameter at the co-ordinate locations of the 38 records included in the distribution model, giving an indication of the environment in which *L. xenodactylus* most often occurs.

The predominant vegetation type in which most frogs were located was Temperate Alluvial Vegetation, followed by Mooi River Highland Grassland and Drakensberg Foothill Moist Grassland. The landform type that the frogs favour is U-shaped Valleys with most locations being found in these. All of the localities were situated above 1,000 m.a.s.l., with the bulk of the records falling between 1,300 m.a.s.l. and 1,900 m.a.s.l. and only two records above this. This suggests the presence of both a lower and upper elevation limit. While they are suited to the higher elevational grasslands at the foothills of the Drakensberg, it seems unlikely that they occur much above this elevation range. The results for temperature, rainfall, relative humidity, evaporation and plant available water, all describe a temperate frog species. Their distribution falls entirely within a summer rainfall area.

Table 7.1 Variables used with their frequency of occurrence for *Leptopelis xenodactylus* in the preliminary iteration of the model.

Variable	Frequency	Variable	Frequency
Vegetation type		**Temperature (°C)**	
Temperate Alluvial Vegetation	15	22	3
Mooi River Highland Grassland	7	23	28
Drakensberg Foothills Moist Grassland	6	24	3
Midlands Mistbelt Grassland	5	25	3
Drakensberg Wetlands	3	26	1
uKhahlamba Basalt Grassland	2	**Rainfall (mm)**	
Landform type		90-110	5
U-shaped Valley	23	111-130	27
Upper slopes, mesas	7	131-150	6
Canyons, deeply incised streams	4	**Relative Humidity (%)**	
Mountain tops, high ridges	3	71-73	3
Plains	1	74-76	17
Elevation (m.a.s.l.)		77-79	18
1000-1300	3	**Plant Available Water (mm)**	
1301-1600	17	30-50	1
1601-1900	16	51-70	8
1901-2000	2	71-90	17
Potential Evaporation (mm)		91-110	9
130-140	6	120-140	8
141-150	12		
151-160	19		
161-170	1		

7.3.3 Habitat preference

From the fieldwork and ground-truthing of the model, several common characteristics of the wetlands the frogs were using became apparent. These included the absence of large areas of open water, the presence of more clay-based soil and strongly vegetated wetlands with emergent vegetation. The frogs appeared to prefer broad leafed plants, including invasive alien species, for “roosting”. They also appeared to prefer areas without rapidly moving water and, in wetlands where there was a stream or flowing water, they avoid the flowing areas and were found around the peripheries where the water was almost stagnant. The overriding factor that seemed critical for the presence of these frogs was the vegetated soil hummocks. These appear to be important for calling as well as burrowing and “nesting”.

During the course of the project, all of the frogs found were located either in or in close proximity to a wetland. In March 2016, however, two large, presumably female, individuals were located after dark, high up in the grass heads near the summit of a hill in a grassland approximately 350 metres away from the wetland at the Fort Nottingham study site.

7.3.4 Vulnerability to climate change

The results of the climate change vulnerability analysis shows that according to the GFDL2.1 model, 78% of the localities fall with the “Robust” category, while the remaining 22% are classified as “Vulnerable”. According to the HadMC2 model, 19.5% are in “Robust” areas, 58.5% are in “Susceptible” areas and 22% are in “Vulnerable” areas (Table 7.2).

Table 7.2 Localities of *Leptopelis xenodactylus* (n = 41) in relation to the vulnerability models (Jewitt et al. 2015).

Climate Models: HadCM2 (H), GFDL2.1 (G)				
	Robust	**Susceptible**	**Constrained**	**Vulnerable**
No. Localities	H: 8 G: 32	H: 24 G: 0	-	H: 9 G: 9
Percentage	H: 19.5 G: 78.0	H: 58.5 G: 0	-	H: 22.0 G: 22.0

7.4 Discussion

7.4.1 The model

The initial species distribution model for *L. xenodactylus* was created as a tool to assist with identifying potential localities where the species had yet to be recorded within the area predicted to be their distribution. Due to the initial extreme difficulty surrounding locating these frogs, severe seasonal time constraints, large areas and distances plus limited resources, it was important to narrow the search area as much as possible. This model was not assumed to be definitive or precise. However, it still proved to be a useful tool to identify previously undocumented populations of *L. xenodactylus*. The model was effective for showing the general area in which the frogs were likely to occur, identifying apparently suitable wetlands and allowing for routes to be decided on that would link the highest number of potential sites for an evening’s trip (Figure 7.3). Modelling is by nature a challenging and complex activity, and it may well be that the distribution of this enigmatic species will never be perfectly predicted by any model. This model did, however, provide guidance as to where efforts and resources could best be spent in trying to locate populations of the frog. Using information from the new localities that were located through the initial SDM it is suggested that a second more refined model could be created to more precisely predict the distribution of this species.

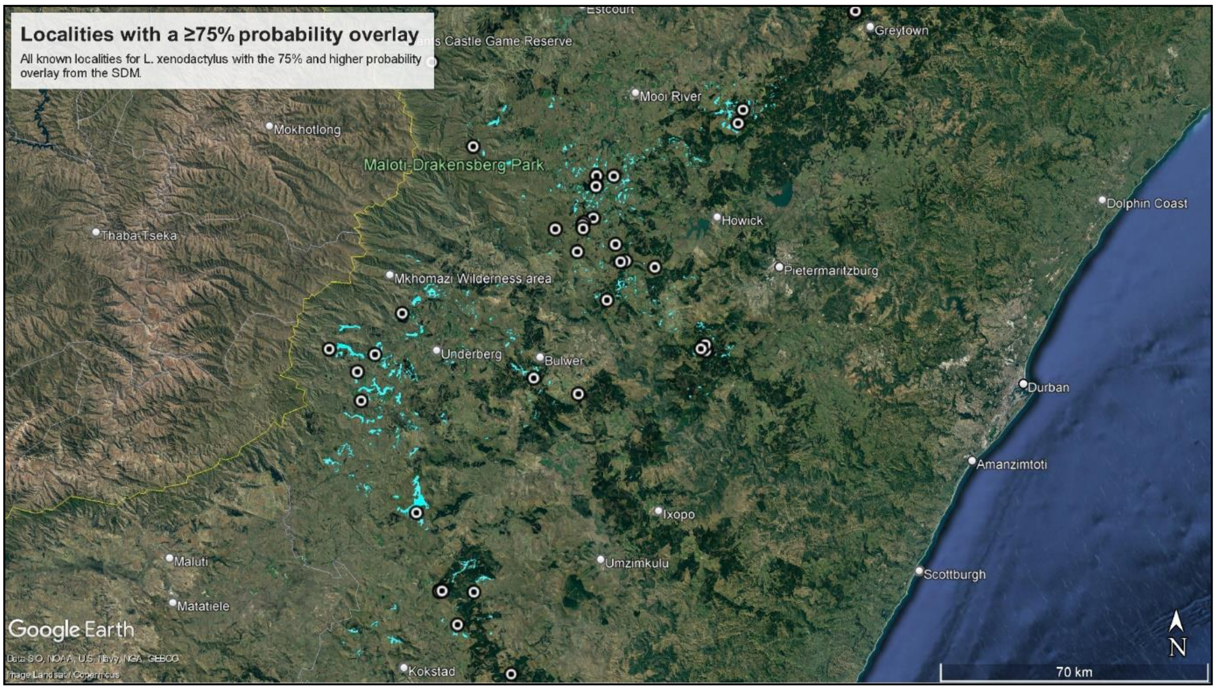

Figure 7.3 Google Earth® image of the known *Leptopelis xenodactylus* localities with the 75% and higher probability overlay.

7.4.2 Ground-truthing

Driving from location to location and listening for ± 10 minutes at each site, while effective and producing positive results, was not found to be a fully comprehensive survey technique for this species. All that can be achieved through this procedure is confirmation of its presence records when the frogs are heard at a locality. If no calls are heard it does not confirm absence as the frogs may be present but silent. The calling frogs are usually situated in small, localised areas within the wetlands which are generally on privately owned land. In order for the frog to be audible an observer is thus limited to sampling parts of the wetland in close proximity to the road and this is often an area that is not suitable for the frogs due to the transformation caused by the construction and presence of the roads.

This ground-truthing technique of listening in the vicinity of wetlands, is clearly strictly confined to the active calling period of the frogs. For this study, the fieldwork was carried out during the peak calling season, which was estimated to be between September and November (Chapter 5). Very little time was spent actively searching for these frogs in the vicinity of known localities during any other months. The frogs, which were found in the grasslands in 2016, were found in January by active searching, but before the searchers knew anything about their behaviour. However, despite a large amount of time and number of visits spent in and near suitable wetlands for other purposes and looking for other species, no further individuals were “stumbled upon” by accident. This indicates that, while they clearly are mobile and active during these months, they are likely more dispersed than during the active calling months. The two individuals found in the grassland had possibly emerged from the wetlands to hunt insects on the flowering heads of the grass, in preparation for aestivation shortly thereafter. These frogs were not calling and, from their large size, were assumed to be females.

7.4.3 Preferred habitat

The records show that the majority of historical frog localities were in wetland vegetation types, with the remainder in grasslands. This is explained by their nature, where resident frogs will be located in the wetlands where they will likely maintain territories and breed, while those that are transient - either youngsters dispersing or adults hunting or dispersing - will be moving through grasslands in search of other wetlands in which to settle. They were likely either found in transit or in small wetlands within grasslands that did not feature as wetlands in the vegetation type coverage.

The hummocks that are located in these wetlands are created by worm castings over extended periods of time. These hummocks are mainly concentrated in valley bottoms and floodplain backswamps of the Drakensberg foothills, where the weathering of dolerite, basalt and clay-rich sedimentary rocks has resulted in 2-5 m thick accumulations of clay-rich sediment (Grenfell et al. 2009). These environments are characterized by shallow, seasonal inundation as well as more prolonged saturation and host seasonal wetland plant species, for example *Carex acutiformis.* The clumps that these sedges form then become the focal point for worm castings, due to the shelter and foraging potential for the earthworms (*Proandricus richerti*), as well as providing the surface litter that is essential for the worms’ survival (Grenfell et al. 2018). From the data available it is unclear if these frogs are present in wetlands that do not, and never have, contained hummocks, or if hammocks are a critical requirement for this species to reproduce. Further research into this aspect might prove very useful for predicting the presence of this species at sites.

7.4.4 Anthropomorphic impacts

Apparent transformation of a wetland was found to not necessarily indicate that *L. xenodactylus* would be absent. In several instances *L. xenodactylus* were present in markedly transformed wetlands. One such example was a remnant wetland identified by the model as potential frog habitat, which was now closely surrounded by a mature *Eucalyptus* plantation. It is uncertain if these were remnant individuals which had managed to persist, or if they represented a persistent population. The fact that they were calling suggests that they were in breeding condition and thus possibly reproducing.

An aspect likely to prove important in the future is the analysis of the land use of the areas surrounding the known population sites and the distance to the closest human habitations and disturbance (de Baan et al. 2013). From this it might well be possible to predict which populations are more likely to decline from either anthropomorphic or climate-related change. If it can be anticipated which ones are most vulnerable to decline and local extinction, targeted protection or mitigation measures could be put in place to prevent this outcome (Dawson et al. 2011).

Close proximity to transformed habitats could impact these populations in a number of ways including through pollution and habitat transformation or disturbance by agricultural activities (either spraying of crops or livestock trampling), tree-farming activities, fires and the introduction of invasive plant species (Sutherland et al. 2019). Ways of mitigating anthropogenic disturbances include careful removal of small dams and unsuitable culverts, blocking of old drains in wetlands, creating buffers around wetland breeding sites, protection of feeding and overwintering sites and maintaining connectivity between suitable wetlands.

Presently, one of the greatest threats facing biodiversity, including amphibians, is climate change. Through establishing the habitat preference of this Endangered species, it allows for a more informed monitoring program to be designed and implemented, which will assist with early detection of threats and changes that are taking place. Presently it is unclear what direct impacts climate change will have on the species, or the specific type of wetland they inhabit in the near future, however, it is predicted that grasslands will decline, posing a risk to their unique biodiversity (Jewitt et al. 2015). Within each domain, micro-refugia will exist, dependent on prevailing wind direction, local topography and cold-air drainage and these, if protected, could provide safe havens for species to persist and potentially expand from (Ashcroft 2010).

According to the vulnerability framework (Mawdsley et al. 2009; Jewitt et al. 2015), the conservation strategies suggested for areas that fall into the “Robust” category are improved representation and replication within protected area networks; ensuring the management effectiveness of those protected areas; development of dynamic conservation plans and the revision of laws, policies and regulations. Those that are “Susceptible” require an increase in the extent of the area protected and that the new protected areas are situated in a manner that maximises climate resilience, that the ecosystem functioning is managed well, and that land-cover change impacts are incorporated into land-management plans. Finally, those areas that fall into the “Vulnerable” quadrat need to be managed to reduce pressures from threats other than climate change and the *L. xenodactylus* populations monitored to assess whether frogs need to be translocated elsewhere or brought into captive-breeding programs. According to the more conservative model (GFDL2.1) only 22% of the localities fall into the “Vulnerable” while the rest are all “Robust” which is encouraging for the future of the species. In the worst-case scenario, according to the HadCM2 model, the majority of the localities fall into the “Susceptible” category, with 22 registered as “Vulnerable” and 19.5 as “Robust”. This provides an indication of the amount of the frog’s habitat that will be affected by climate change as well as giving guidance on which of these locations should be better protected. The vulnerability framework and maps can be used to determine where to set up new protected areas and from where and to where the *L. xenodactylus* should be translocated should conservation translocations be required to prevent a decline in the overall *L. xenodactylus* population.

Leptopelis xenodactylus does, however, have clear and important elevational and temperature constraints, and climate change has already severely impacted some frogs due to these constraints (Li et al. 2013; Cordier et al. 2020). While in some areas there may be suitable wetlands at higher elevations, in others there may not be. Also, dispersal routes between lower elevation and higher elevation wetlands may become transformed through human activities. These factors may become a serious challenge to the conservation of *L. xenodactylus* in the near future. Climate change also often severely alters rainfall patterns, and as this species requires temporary or permanent hummock wetlands many currently suitable habitats may simply dry up while others may become flooded, allowing predators and competitors access. This aspect needs careful monitoring and may require interventions in the future. Better knowledge of the requirements of this species will provide an improved baseline for researchers to monitor and evaluate as time passes and the environment changes, allowing for a more informed understanding of the impacts of environmental changes on the frogs and the wetlands.

CHAPTER 8

CONCLUSION

8.1 Challenges of studying a rare species and the importance of species-specific studies in the light of global amphibian declines

Conducting field based research on rare species is challenging, especially when it has cryptic behaviour and physical appearance and demographic attributes that increase the difficulty of their detection and observation (McDonald 2004). Throughout this study it has been repeatedly reported that *L. xenodactylus* has a very restricted geographical distribution and individuals are secretive and difficult to locate. Its secretive nature is the single phenomenon that affected every aspect of its life history and, therefore, this study. The single amplectant pair that was observed was in a hole under vegetation making it very likely that many other amplectant pairs were simply overlooked. The tadpoles were elusive and difficult to locate, being very sparsely distributed and cryptically coloured. Catching adequate numbers of frogs to mark was challenging and strongly biased towards males due to the fact that their calling made them easier to locate. The frogs used different areas of the wetlands in different years, or parts of a season, making remote recording of their calling behaviour difficult.

The short duration of the calling season, unpredictability of behaviour, time and distance constraints for site visits and the unpredictable nature of any behavioural study, culminated in an extremely challenging study. Problems arise when conservation actions are put in place to conserve at-risk species in the absence of robust, empirical data (Martin et al. 2022). However, improving available knowledge and data about a species is imperative to their conservation, despite the inherent challenges (Martin et al. 2022), validating the investment of time and effort that goes into species-specific studies.

Being characterized by their low abundance and sometimes restricted geographic distribution, rare species are particularly vulnerable to environmental changes as well as human impacts (Foden et al. 2019) and, as such, anthropomorphic impacts on habitats have particularly serious consequences for such species. Knowledge of what conditions allow for a species to persist is often lacking, even though it is a requirement for recognizing threats and assessing recovery potential (Raphael and Molina 2007). Anthropogenic activities such as fencing, draining, damming, ploughing, herbicide application and pesticide use close to wetland, along with livestock management, will potentially dictate which areas are transformed, to what extent there is disturbance and which areas may remain suitable for the frogs. Even positive actions such as alien vegetation management could have negative impacts on the frogs if potentially harmful chemicals are used or changes are made to the structure of the wetland in the efforts to eradicate alien vegetation. Within protected areas, actions such as culvert modification can have serious impacts on the population due to the changes caused in the wetland vegetation and drainage. It is apparent that *L. xenodactylus* have specific requirements in relation to water depth, vegetation type, soil type and wetland structure but, what remains unclear is the level of disturbance and transformation they can tolerate, under what conditions they can temporarily survive and when they can thrive to the extent that the population can support the production and dispersal of youngsters to repopulate other areas.

An important consideration relating to this study and any rare species study, is that data on rare species will be typically limited due to their low prevalence, often compounded by the species' elusive nature, the poor accessibility of their habitat or the limited understanding of their ecological requirements (Hamilton et al. 2015). With females not calling, all aspects of the survey were heavily "male-biased" due to the heavy reliance on calling to locate frogs (Emerson and Boyd 1999). All females found during this study were located fortuitously. In the final two seasons a higher proportion and number of females were located due to lessons learned and knowledge gathered by the team in order to better know where to look and what to look for. However, this still resulted in only 10 females being found compared to 146 males. Due to the males being easier to locate, most of the measurements, movement work and observations were carried out on them. Their "secretive" nature and behavior made describing their calls and calling vitally important as it forms an essential component of any fieldwork conducted on this species. Knowing what the call repertoire consists of and sounds like, as well as when, during the year and during the night, they are most likely to call, affords researchers an advantage in future work. Evidence and information that is gathered via smaller, individual studies, using standardized methods, can offer the best proxy for larger scale research projects or efforts that are infeasible or impractical (Johnson 2002; Nichols et al. 2019).

Another important factor is that during this study it was found that every locality and population differed in size, conditions, numbers and seasonal variability. Because of this, simple numbers of localities with *L. xenodactylus* still present cannot be used as an indication of the overall size or health of the species' population. Whilst it has been established that the frogs are present at these sites, the health and sustainability of these populations remain unknown. Whilst frogs were found calling actively at sites that appeared to be markedly transformed, it is not known whether they were able to successfully breed in these sites or how long they could persist. If it is possible for them to breed, it's unclear what the success rates of these breeding events and survival rates of the tadpoles would be. To establish the health and size of a population, more time and effort would need to be dedicated to each locality.

8.2 Reproductive strategies of *Leptopelis* compared to other frogs

Anurans have extremely diverse reproductive modes, ranging from eggs laid in terrestrial nests where direct development occurs, to those where large numbers of eggs are laid in stagnant water where they develop into tadpoles which feed on the aquatic vegetation (Wells 2007). The breeding biology of *Leptopelis* in general is little known and for some *Leptopelis* spp. accounts are based largely on the life history studies of other, better known, members of the genus (Schiøtz 1999). The records, observations and insights that have been gathered about this species during this study will help shed further light on other similar genus members. The fact that the amplectant pair was found on the last fieldtrip of the last season was in many ways fortuitous, but it was also the stage of the study at which most had been learned about the frog's behaviour. As such, whilst the weather was unpredictable, the knowledge gained from previous seasons guided the timing of this field trip and the area of the wetland in which to concentrate effort.

The variation in the temporal patterns of anuran breeding appears to be related mainly to abiotic factors. For example, rainfall distribution and the presence of bodies of water, and also biotic factors, such as competition, predation and foraging opportunities (Lucas et al. 1996; McCauley et al. 2000). With both the calling and nesting of *L. xenodactylus* occurring on the hummocks, presumably for the protection that they provide, it appears that the link between the frogs' successful breeding events and the hummocks is likely a strong one. The water surrounding these hummocks potentially also plays an important role in the survival rate of the tadpoles. The shallow depth and seasonal fluctuations will reduce the number of predators of the tadpoles in this habitat, as well as reduce the amount of competition with other anurans in the wetland. It remains unclear how long the tadpoles take to develop but, from the instances that were observed during the study and the likelihood of the wetlands drying up during winter, it is unlikely that they would overwinter as tadpoles. It is thought that they probably develop into froglets by the end of the summer season.

8.3 Potential threats to *L. xenodactylus*

Rare species are affected by new and emerging threats that may not be well known, chronic threats that are challenging to mitigate and the interaction of multiple threats that potentially have dire consequences (Brook et al. 2008). As such, a species-specific study focusing on an "Endangered" species should aim to elucidate population level as well as species level threats (Cushman 2006).

While alien species are always a concern for sensitive species (Chornesky and Randall 2003), no evidence was found of alien vertebrates or invertebrates having any impact on *L. xenodactylus*. This is likely due to the frog's habitat, habits and cryptic colour and the general lack of invasive animal species in this area. Alien plants, however, are a potential threat, with the wetlands being frequently surrounded and even invaded, by exotic species. American Bramble (*Rubus cuneifolius*) and tall marsh senecio (*Scenecio inornatus*) are abundant in many of the wetlands in which the frogs were found and, while initially suspected to have a negative impact on the frogs, instead were found to be a favoured hunting and perching place. It was thought that this could be due to the large numbers of insects that were attracted to the flowers of these plants, as well as their broad-leafed foliage. On several trips, calling male frogs were located sitting on the broad leaves of marsh senecio in areas otherwise dominated by indigenous flora. Similarly, female frogs were observed on American bramble at night, presumably hunting for insects that were also observed high on the bramble plants. From field observations it appeared that certain alien plants did not directly negatively impact the frogs but the overall impact that alien vegetation has on the physical structure and water table of the wetland may well have strongly negative impacts (Mack et al. 2000). The impact that *Eucalyptus* plantations, for example, have on the water levels, in and around the wetlands, must pose a severe threat for a species that relies on the shallow, seasonal margins of the wetland.

A fairly recent threat that these frogs are facing is the increasing numbers and spread of bushpigs (*Pomatoceros porcus*) into areas that previously were not inhabited by them. With their highly developed sense of smell bushpigs are probably able to detect frogs and dig them out of the mud in which they bury. This study found much evidence in many of the wetlands of recent bushpig "digging and rooting" activity in the areas that the *L. xenodactylus* would inhabit (Figure 8.1). Bush pigs are spreading into areas where they previously did not occur due to the increased availability of suitable habitat and adequate food. With the increase in commercial agriculture in these areas providing more abundant food and extra cover, they are now developing a much wider distribution in greater densities, which allows them to encroach on and impact, the frog's habitat.

Figure 8.1 Example of damage done by bushpigs in the Fort Nottingham wetland.

Human activities also result in "un-naturally" large numbers and greater densities of animals (Tuomainen and Candolin 2011). In this case, in addition to the bushpigs, chacma baboons (*Papio ursinus)* may be a severe threat to biodiversity in many areas. With humans having removed the baboons' natural predators, as well as providing extra food for them in the form of commercial crops, the number of troops and the number of individuals within a troop, have increased markedly. They have expanded their ranges and placed a lot more pressure on the natural food resources, which include frogs, but have also increased disturbance on their wetland habitats. Many of the rocks surrounding the wetlands studied had been turned over multiple times recently by baboons. Baboons were often seen in the vicinity of wetlands in loose groups of over 50 animals moving methodically over grasslands and they have been reported digging 35 centimetres down to find food (Smithers 1983). Baboons are thus a threat to not only the frogs, but also many other species and their habitat (Moseby et al. 2018).

Agricultural threats for *L. xenodactylus* include the effluents and pollutants resulting from chemical spraying, land preparation and other activities associated with commercial farming. The physical changes, for example, reduction of water supply due to the proximity of alien tree plantations, flooding of wetlands for damming, draining of wetlands for crops or grazing for livestock, as well as the focusing of large numbers of stock into limited areas for brief periods of time that leads to severe trampling and habitat degradation, can all affect this species. While no injured or dead frogs were found in this study, the transformation that high impact grazing of wetlands causes must have a degree of impact on them, their behaviour, feeding and breeding success.

The high elevational range of this species probably currently acts in their favour, placing the wetlands they inhabit generally upstream of most agricultural infrastructure and lands that would be exposed to chemical effluents. This natural, but fortuitous situation has likely been an important factor in the persistence of this species in many of the areas in which it still occurs. However, grazing for high densities of beef cattle and large-scale afforestation is carried out at higher elevations and can result in habitat loss due to land transformation which is one of the major threats to this species, indicating that this is not consistently true.

While many human management actions appear to have an impact on the frogs, several surprising aspects of it appeared not to. One of these was the use of wetlands as watering points for large numbers of stock. This is perhaps due to the natural, historic presence of large herds of ungulates roaming these areas and the frogs being adapted to survive their infrequent visits.

Fires and burning could have a major negative impact on the frogs and their environment. However, in the case of the wildfire experienced at Fort Nottingham, it seemed to possibly “improve” the condition of the wetland and didn’t seem to have a negative effect on the frogs, as they were heard calling on the night after the fire. Their ability to burrow and retreat to underground refugia in the damp substrate probably protects them from the heat of quick burning fires. Hotter, slower burning fires may pose a greater threat as they retain a greater intensity of heat in the area for longer, potentially evaporating the liquid in the substrate, thus removing some of the protective effect this would have given the frogs. Fire, however, is an important management tool for managers of protected areas and farms alike and the impact of fire on these frogs and the wetlands they inhabit needs further investigation to discover when best to burn these sensitive areas. From first principles, it would seem best to burn when the *L. xenodactylus* are still underground, before they emerge, and the breeding season commences.

8.4 Conservation management suggestions

Effective learning can improve conservation management of species through critical and constructive examination of past performance, current problems and the context of these problems (Clark 2002) and then practically applying these lessons. As a result of the observations made and conclusions drawn during this study, several suggestions for management for the conservation of this Endangered species have become apparent.

- The best and most important management for a wetland, from a *L. xendocatylus* perspective, would be to leave it as unmodified as possible, which would mean an active role of preventing any change, as opposed to implementing new management. This would include preventing development upstream of the wetland to stop effluents and pollutants running into the catchment of the water system, preventing afforestation in close proximity of the wetland, or in the catchment, which would affect the water table. Fencing around the wetland would also assist to prevent stock from grazing and trampling the sites continuously or in high densities. Stock fences would allow for naturally occurring ungulates, such as eland (*Taurotragus oryx*), to move through as they are often able to jump over the fences, thus still maintaining the natural movement of animals through areas. Due to their preference for greater elevation, the wetlands that the frogs inhabit are usually high up in the systems, which allows for agricultural and other utilisation of the water supply further down the water course, allowing for management strategies and options where utilisation of water is concentrated in areas below the localities of the frogs.
- In areas that cannot be left unmodified, the control of alien plants should be administered cautiously, with as little chemical use as possible. Fencing could be carried out in such a way as to leave certain parts of the wetland inaccessible to stock. It would be essential that this partitioning of a specific wetland be carried out in an informed manner so that the areas that are fenced off and protected contain suitable habitat for these frogs, for example, areas that contain hummocks.
- Controlled and uncontrolled burns are another aspect that should be managed carefully. As the species are early spring emergers, earlier winter burns would be better, allowing for the vegetation to have grown again by the time they emerge. Burning only a percentage of the wetland at a time would also have less impact, allowing areas to be left unmodified for the frogs. Occasional, low fuel fires appear to be beneficial for the frogs, with the regrowth and health of the wetlands being improved by periodic burning, but timing and frequency are important factors. Frequency of burning would impact the amount of moribund vegetation that accumulates, thus affecting the type and intensity of fire that would occur when a

wildfire reaches the wetland. Slow burning fires that burn for a long time in one area and get into the denser vegetation, could severely impact many frog species, thus it may be better to have more frequent, but less damaging, fires.

- Due to the long-toed tree frog being an Endangered species on the IUCN Red List (IUCN 2021) it is essential that they be highlighted as a species of concern when Environmental Impact Assessments (EIAs) are performed in areas that fall within their distribution, or even within suitable habitats outside the given distribution. Establishing and describing this species' habitat preference is important for this, as it allows for a more accurate estimation of the presence or absence of this, often extremely difficult to detect, species. In the past if the survey or assessment was carried out during non-ideal conditions or time of the year it was difficult to confirm if the frogs were present or not. Having built a distribution model and established their elevational range, landform type and vegetation type, informed EIA practitioners can ascertain whether or not they need to consider this species in their assessments. SANBI has also created a screening tool, enabling practitioners to carry out a desktop survey at a glance, indicating which listed species could occur at a given site. A revised distribution model for *L. xenodactylus* should be included in the EIA screening tool and also in the Spatial Development Frameworks of the municipalities within the distribution range of the species.
- An important aspect relating to *L. xenodactylus'* persistence involves the education of people about this species and the frog's presence in wetlands and on privately owned properties. Being a large, "charismatic" frog species with an easily identifiable call, they can be effective ambassadors for these and other wetlands. With the recent international emphasis on amphibians and how many threats they are facing (Catenazzi 2015), there is an excellent chance that a high proportion of these people, both private landowners and rural populations, might be willing to change some of their practices and improve conditions for this species. Firstly, however, they need to be made aware of their presence and their requirements and informed about aspects such as fire management, pollution, afforestation and general wetland management. Information and documents should be drawn up and made available to all age groups and income brackets, with emphasis on conservancies, farmer's organisations, local schools and NGOs, to disseminate this information along with more general wise environmental practices.

8.5 Achievements of the project

As a result of this study, many gaps in the knowledge of the life history and behaviour of *L. xenodactylus* have been addressed. The mark and recapture aspect of the study offered insights into their seasonal movement within the wetland as well as highlighting that different areas are utilized in different seasons. Different burrow types and the utilization of these burrows were described, as well as various behaviours that were observed. Their calling repertoire was described, with suggestions of the purposes of these calls, as well as the possibility of two further call types. The distribution of their calling over the active calling period and through the night, was also documented, along with the correlation between calling and certain weather parameters. Important reproductive observations were made, including when during the season, they lay their eggs, where in the wetland they lay their eggs, clutch size, egg measurements and sexual dimorphism of the adults. Through the ground-truthing of the model, a further 19 areas were added to the known localities for *L. xenodactylus*, along with describing their preferred habitat based on the environmental parameters at these localities. Finally, the vulnerability of these localities to climate change was assessed using both a conservative and a more severe climate change model with the percentage of their known localities that fell into the various categories being calculated and suggested actions listed.

8.6 Future studies

While this study has shed light on a lot of details of the life history and behaviour of *L. xenodactyus*, it has raised new questions and also left some questions unanswered but revealed areas that would benefit from further research. Some areas that were highlighted through this study included the following:

- A - The exact relationship between the frogs and the hummocks requires further investigation, as this could be extremely useful in habitat evaluation.
- B - Their activity and calling in response to weather remains unclear and could have bearing on knowing when to conduct fieldwork on the species for the best results.
- C - Their ventriloquy or ability to mask their calling location would not only be interesting for this species, but most likely also have bearing on other species of *Leptopelis* as well as possibly other genera.
- D - Through telemetry and further mark and recapture work it would be possible to better document their movements and growth and further insights into abundance. This could also shed light on their movement between wetlands which would inform decisions relating to corridors and rehabilitation work.

Finally, based on the results of this study, it is suggested that the Endangered status of this species be reconsidered. A species is considered Endangered according to a set of criteria laid down by the IUCN, including population size reduction of ≥70% over the last 10 years; extent of occurrence to be less than 5,000 km^2 and severely fragmented; known to exist from no more than five locations with continuing decline; an area of occupancy estimated to be less than 500 km^2

which is also severely fragmented and declining; population estimated to number less than 2,500 mature individuals and continuing to decline or population estimated to be less than 250 mature individuals; quantitative analysis shows a 20% probability of extinction in the wild within 20 years or five generations (IUCN 2001). While *L. xenodactylus* remain a species in need of protection and conservation, the findings of this study show that the area of occupancy is considerably larger than initially assumed and the number of localities is higher than five with many being within close enough proximity of each other for dispersal. It is also highly likely that the population exceeds 2,500 mature individuals and, from the evidence gathered, it seems unlikely that the species will go extinct in the wild within the next 20 years. As such, very important future work would be reassessing their status and possibly relisting them as Vulnerable.

CHAPTER 9

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