Ecology and calling behaviour of the anurans of northern Zululand, South Africa

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DECLARATION

I, Willem Wentzel Pretorius, declare that this dissertation is my own unaided work, except when otherwise acknowledged in the text. This dissertation is submitted for the degree of Masters of Science in Environmental Sciences to the North-West University, Potchefstroom Campus. It has not been submitted for any degree or examination at any other university.

Willem Wentzel Pretorius

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PREFACE

This project would not have been completed without the guidance, support and help of a number of people. I would like to dedicate this project to all the participants who stood by me in the process of completing this dissertation:

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ABSTRACT

Amphibian population declines and extinctions have become a global problem. In order to address and track the extent of these declines, key information is needed. Therefore, a dire need exists for non-invasive, rapid, labour-efficient and objective monitoring methods. The northern parts of KwaZulu-Natal have a rich anuran diversity comprising more than 50 frog species. This study aims to evaluate the anuran diversity of north-eastern KwaZulu-Natal using automated recorders (Ssong Meters) strategically placed at selected localities across this area. Secondly, the effect of meteorological variables on the calling activity of the anuran community within the Umlalazi Nature Reserve was determined. Finally, active acoustic recordings aided in resolving confusion about a group within the Hyperoliid genus, *Afrixalus*. Six Song Meter recorders were used passively to record the calling activity of vocally active male anurans within north-eastern KwaZulu-Natal. A total of 29 species were recorded in this area. Furthermore, this data indicated peak breeding activity, calling times and species abundance. Additionally, insight into meteorological conditions and their effect on anuran calling behaviour were examined. These studies contribute to addressing a lacuna in the field of South African anuran behavioural studies and the effects of environmental change on these animals. In a case study of the leaf-folding frogs (*Afrixalus* Laurent, 1944), questions raised the concern that due to the practically similar morphology potential among thes species, misidentifications could cause confusion in the field. These matters were clarified using morphological and acoustic analysis. Results obtained from the present study provide important data that could be used to improve the field of anuran behavioural studies, contributing to understanding, guiding and assisting South African anuran conservation.

Keywords: *amphibian declines; acoustic monitoring; automated recorders; north-eastern KwaZulu-Natal; protected areas; breeding activity; meteorological conditions; Afrixalus; anuran conservation*

OPSOMMING

Die Wêreldwye afname in amfibieërpopoulasies dui op die verskynsel van afnames en uitsterwings van amfibieër spesies regoor die wêreld. Ten einde hierdie raaiselagtige wêreldwye dalings te verstaan is dit van kardinale belang om hulle omvang en intensiteit te bepaal. ŉ Groot behoefte bestaan om vinnige en objektiewe moniteringsmetodes te gebruik wat nie 'n nadelige uitwerking op die omgewing sal hê nie. KwaZulu-Natal het ŉ hoë padda-verskeidenheid, veral in die noorde van hierdie provinsie, waar daar meer as 50 paddaspesies voorkom. In die verlede is daar min studies gedoen om die biodiversiteit van hierdie dele te evalueer. Die laaste uitgebreide studie oor die padda-diversiteit van noord KwaZulu-Natal is meer as 30 jaar gelede uitgevoer. Die huidige studie het ten doel om die padda-diversiteit van noord-oos KwaZulu-Natal te evalueer deur gebruik te maak van outomatiese klankopnemers (Song Meters) wat strategies by damme, poele en vleigebiede uitgeplaas is. Tweedens is die effek van meteorologiese veranderlikes op die padda-gemeenskap binne die Umlalazi-natuurreservaat bepaal. Laastens het aktiewe akoestiese opnames gehelp om die verwarring binne die riet- en blaarvoupadda familie (Hyperoliidae) aan te spreek. Ses digitale klankopnemers is gebruik om die roep-aktiwiteit van verskeie paddas in die noorde van KwaZulu-Natal passief op te neem. In totaal het ons 29 spesies in die noorde van KwaZulu-Natal gevind. Hierdie data dui verder op piek-broeitydperke, roeptye en die intensiteite van spesies. Daarbenewens is ŉ insig oor die meteorologiese toestande en die invloed daarvan op paddagedrag ondersoek. Dit dra by tot die aanspreek van die gaping in die veld van Suid-Afrikaanse gedragstudies op paddas en die uitwerking van omgewingsveranderings daarop. In ŉ gevallestudie van Blaarvoupaddas (*Afrixalus* Laurent, 1944), waarvan die roepe en morfologie na aan mekaar is, het dit duidelik geword dat hierdie paddas in die verlede dikwels verkeerd geidentifiseer is. Resultate wat uit die huidige studie verkry word, bied belangrike data wat gebruik kan word om die veld van padda-gedragswetenskappe te verbeter. Dit dra ook verder by om verdere aspekte wat ondersoek moet word uit te lig.

Sleutelwoorde: *amfibieë-afname; akoestiese monitering; outomatiese klankopnames; noord-oos KwaZulu-Natal; beskermde gebiede; broeiseisoen-aktiwiteit; omgewingstoestande; Afrixalus; paddabewaring*

ABBREVIATIONS USED IN TEXT

°C	degrees Celsius
AACRG	African Amphibian Conservation Research Group
ACI	amphibian calling index
ANOVA	analysis of variance
CD	call duration
cm	centimetre
CP	call period
CRR	call repetition rate
dB	decibel
DF	dominant frequency
DF3r/l	third right/left toe disk width
DNA	deoxyribonucleic acid
EAD	distance of the anterior corners of the eyes
EDr/l	eye to snout right/left side
EPD	distance between posterior corners of eyes
F	refers to the ratio of two variances
F3L/HdL	third finger length to hand length ratio
F3Lr/l	third right/left toe length
FFT	Fast Fourier Transform
FLr/l	right/left foot length
FOL/HdL	forearm length to hand length ratio
FOLr/l	right/left forearm length
GB	gigabyte
Ha	hectare
HdLr/l	right/left hand length

HL	head length
HL/SVL	head length to snout-vent length ratio
hPa	hectopascal
HSD	honestly significant difference
HW	head width
HW/SVL	head width to snout-vent length ratio
Hz	hertz
ICI	inter-call interval
IND	inter-nostril distance
IND/HW	inter-nostril distance to head width ratio
INI	inter-note interval
IOD	interorbital distance
IUCN	International Union for Conservation of Nature
kHz	kilohertz
km	kilometres
km/h	kilometres per hour
KZN	KwaZulu-Natal
LHU/HdL	humerus length to hand length
LHUr/l	right/left humerus length
m	meter/metres
m.a.s.l.	metres above sea level
mm	millimetre
MP	M. Pickersgill collection, Leeds, England
ms	millisecond
n.p.s^{-1}	notes per second
ND	note duration

NLr/l	nostril-lip distance right/left side
NM	Natal Museum, Pietermaritzburg, South Africa
No. notes	number of notes
NODr/l	nostril ocular distance on the right/left side
NRR	note repetition rate
NWU	North-West University
P	refers to significance, i.e. the *P*-value (i.e. $P < 0.05$)
p.p.s^{-1}	pulses per second
PAM	passive acoustic monitoring
PCA	principal component analysis
PFLr/l	palpebral fissure length of right/left eye
PPN	pulses per note
PRR	pulse repetition rate
RAMSAR	Ramsar Convention on Wetlands of International Importance
RH	relative humidity
SANBI	South African National Biodiversity Institute
SAWS	South African Weather Service
SD card	secure digital memory card
sec. / s	seconds
SM	Song Meters
spp.	species (plural)
SVL	snout to vent length
T1(2/3/4/5)Lr/l	first(second/third/fourth/fifth) right/left toe length
T1(2/3/4/5)Wr/l	first (second/third/fourth/fifth) right/left toe disk width
T4L/FL	fourth toe length to foot length
T4W/FL	fourth toe disk width to foot length ratio

THL/SVL	thigh length to snout-vent length ratio
THLr/l	thigh length right/left
TL/SVL	tibia length to snout-vent length ratio
TLr/l	tibia length right/left
UNESCO	United Nations Educational, Scientific and Cultural Organization
V	volt
WAV	waveform audio file format
$\bar{X}$ ± SD	mean followed by standard deviation

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CHAPTER 1: GENERAL INTRODUCTION

The thunder would roar, and the lightning would strike,
As I hid under blankets on those long sultry nights.
The rain would come down, like all cats and dogs,
Turning our backyard into Finnegan's bog.
Then it would let up, and out came the frogs.

There were frogs on the lawn, and toads in the streets,
Singing away with one hell of a beat.
They sang in falsetto, there were tenors too,
Out in the muck and the mud and the goo.
They sang like longshoremen, all filled up with brew.

They sang through the night, they sang until dawn,
Putting me at ease with their beautiful songs.
In the morning I'd wake and they were still going strong.
Then they would start to burrow, back where they belonged.
For the next thunderstorm, my heart always longed.

Singing Frogs After A Rain
Juan Olivarez

1.1 Diversity and distribution

Amphibia is a diverse vertebrate class, divided into three orders, namely Anura (frogs), Caudata (salamanders and newts) and Gymnophiona (caecilians). The order Anura is the largest of these, with 56 families and 7 003 species (Frost, 2018). Anurans are the only order of amphibians found in Southern Africa (Du Preez & Carruthers, 2017; Frost, 2018).

Amphibians are found in almost all the terrestrial and freshwater habitats globally, except for some marine and arctic environments (Roelants *et al*., 2007). Anurans have diverse feeding and breeding patterns (Du Preez & Carruthers, 2017). This exposes them to a wide variety of ecological niches where they play an intermediately pivotal role in ecosystems (Davenport & Chalcraft, 2012). Anurans are known as predators (predating on other smaller frogs, invertebrates and other small fauna) and as prey for mammals, fish, reptiles, birds and other frogs (Wells, 2007). Adults feed mostly on small invertebrates such as insects (Semlitsch & Brodie, 2003), whereas tadpoles (mostly aquatic) primarily feed on algae. Anurans have moist skin, allowing cutaneous respiration to take place, which enables them to live in different types of environments (Wake & Vredenburg, 2008). Because of this, they are sensitive to certain changes in their environment, such as changes in air temperatures and water fluctuations (Wake & Vredenburg, 2008).

1.2 Global amphibian declines

Amphibians are declining at an alarming rate and are therefore regarded as the most threatened vertebrate class worldwide (Bishop *et al.*, 2012; IUCN, 2018). Like so many animal taxa today, amphibians are faced with challenges related to anthropogenic influences such as climate change as well as other complex ecological factors, including diseases and parasitism. Most of these factors usually work synergistically with one another and no single factor can therefore be isolated from the others (Lips *et al*., 2008; Ospina *et al*., 2013). Blaustein *et al.* (2010) state that these causes of decline may vary between species and their different regions. Monitoring is needed to improve the understanding of these issues (Aide *et al*., 2013). It can be used as a tool to ensure effective conservation management of natural systems. According to the IUCN (2018), almost 40% of known amphibian species are threatened to some extent (see Figure 1.1). As a consequence, focus on amphibian conservation has increased.

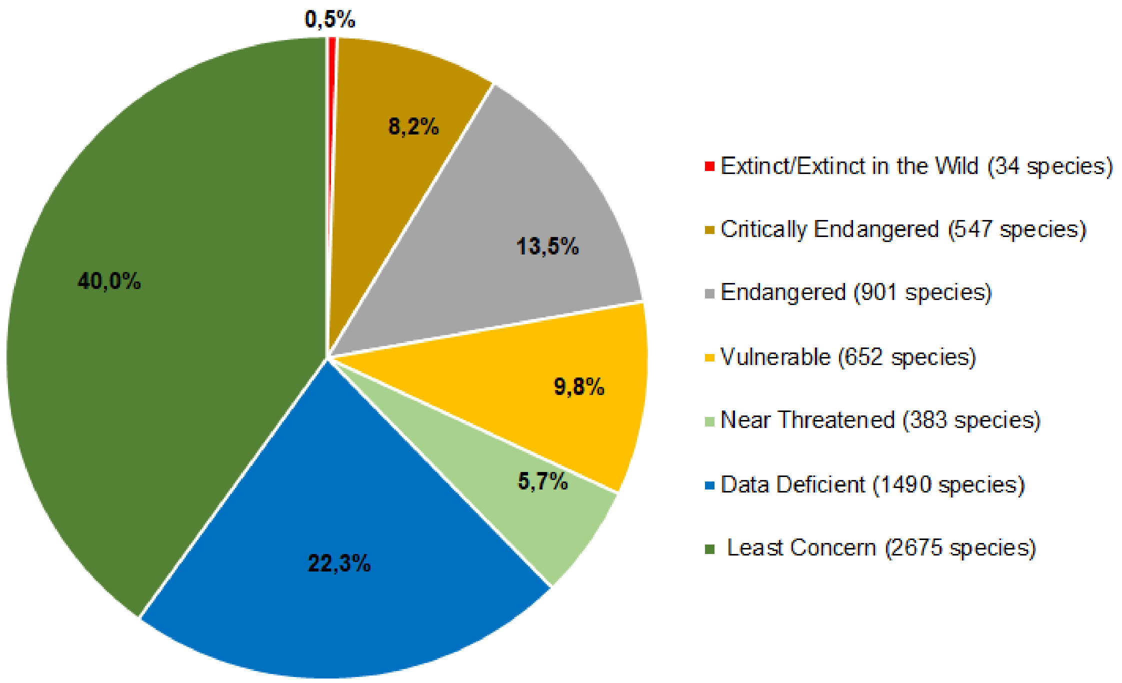

Figure 1.1: Summary of the IUCN Red List categories for amphibians (adapted from Bishop *et al.*, 2012). Data retrieved from the IUCN data list, last updated on 5 July 2018

During the last three decades attention has shifted towards understanding amphibian population declines. In the 1990s, firm evidence based on long-term monitoring data (see McDonald 1990; Pounds & Crump, 1994; Pounds *et al*., 1997)showed that amphibian populations were declining,. These studies showed a drastic decline in amphibian population numbers, especially in the highland areas of America and Australia. This is still the case today, where declines are noted in Australia, South and Central America and some of the high-altitude regions of West Africa (see Blaustein & Dobson, 2006; Lips *et al.*, 2008; Hirschfeld *et al.*, 2016 Carvalho *et al.*, 2017). However, according to Measey *et al.* (2011), Africa as a whole has not experienced severe amphibian biodiversity loss when compared to the other continents.

1.3 Vocalisation

Among vertebrates the anurans is one of the most common groups that use vocalisation for breeding and communication (Köhler *et al.*, 2017). Furthermore, anurans have the ability to emit a diverse set of call types within different contexts. Anurans produce vocalisations by using their respiratory system (Köhler *et al.*, 2017). Positive pressure from the muscles of the buccal cavity causes air to move into the lungs through the nostrils (Wells, 2007; Du Preez & Carruthers, 2017). When anurans call, muscle contractions force air from the lungs via the larynx into the buccal cavity, which causes vocal cord vibrations, producing the sound. These sounds are then transmitted through the buccal cavity into the vocal sacs, which will inflate during calls. This causes the sound to be radiated (Köhler *et al.*, 2017). There are various types of vocal sacs, from the most typical single sub-gular sac to paired- or bilobate sub-gular sacs as well as paired lateral sacs (Köhler *et al.*, 2017). According to Starnberger *et al.* (2014), the colour and form may also play a role as a visual signalling tool. With small frogs that call in air, thin vocal sacs are common, while larger species call within water, where these sacs are mostly thick, with a swollen appearance (Wells, 2007). However, not all anuran species have vocal sacs (Köhler *et al.*, 2017).

Bogert (1960) divides anuran calls into six categories based upon the context of occurrence. This categorisation is still in use. Minor modifications have been made to these categories over the years (Köhler *et al.*, 2017), though. The six categories include 1) mating calls, 2) territorial calls, 3) male release calls, 4) female release calls, 5) distress calls, and 6) warning calls. In a recent study, Toledo *et al.* (2015) stated that these anuran vocalisations can be subdivided into three overarching categories: reproductive, aggressive and defensive calls, including various subcategories.

Calls are species specific and may be used to identify frogs to species level. Whereas calls of some species within a specific genus may vary dramatically, other species only show a slight variation in call structures (Köhler *et al.*, 2017). Call characteristics that distinguish frog species from one another are, for example, pulse rate, dominant frequency, call duration and amplitude (Köhler *et al.*, 2017). Within conspecific anurans, there can be a variety of unique call types, leading to extensive repertoires within species, each acting as different behavioural cues (Bee *et al.*, 2013; Costa & Toledo, 2013).

Calls serve as a means of communication for males and females (Köhler *et al.*, 2017). Males of most species produce more vocal signals compared with conspecific females (Köhler *et al.*, 2017). The primary function of anuran vocalisation of male anurans is to attract gravid conspecific females to mate with. Females usually show preferences for males with a characteristic call within the same species, thereby aiding in pre-mating sexual selection (Bee *et al.*, 2013). Anuran vocalisation not only aids in finding a suitable mate, but also has significance in terms of male-male interactions over territories and females (Köhler *et al.*, 2017). Males use these types of vocalisations to gain information about their rivals (for instance, regarding size and physicality), identify neighbours and their distance from each other (Bee *et al.*, 2013). Some calls may even indicate the reproductive status of individuals (Toledo *et al.*, 2015). Vocalisations are also used to convey information about the size, fitness and quality of genes (Gerhardt, 1991).

Males use reproductive calls to indicate their identity and the most common call of these is the advertisement call (Toledo *et al.*, 2015). Advertisement calls are commonly used as diagnosable characters in taxonomy because this call is emitted more regularly than others and can easily be recorded and analysed (Köhler *et al.*, 2017). Reproductive calls include advertisement (signals emitted by males to attract conspecific females), male courtship (emitted by males who alter their vocal behaviour when conspecific females are near), female courtship or reciprocation (female response to male courtship call), amplectant (emitted by males during courtship), release (males and females emit these calls if they are touched or clasped by another male), and rain calls (emitted during rains or high humidity and may be paired with male excitation prior, during or outside their reproductive season) (Bogert, 1960, Wells, 2007; Toledo *et al.*, 2015).

Aggression calls assist males to defend their calling site against conspecific males (Wells, 1977; Wells, 2007). Most common among these calls is the territorial call (Toledo *et al.*, 2015). Territorial calls are usually emitted if a conspecific rival male is in close proximity and is used to defend a territory, such as breeding and feeding sites. This call also acts as an interspacing cue between males in reproductive choruses (Wells, 2007; Toledo *et al.*, 2015). Aggression calls include territorial encounters. Anurans defend their territory. If an intruder is in close proximity or has entered the signallers' territory, this call is usually more aggressive than the territorial calls. Aggression calls are also made during fighting – these calls are emitted during physical combat between males (Toledo *et al.*, 2015).

Defensive calls are produced when a frog is being attacked or threatened by a potential predator (Wells, 2007). Distress calls is one of the most common call types and are used widely in the bioacoustics of animals (Toledo *et al.*, 2015). Males, females, juveniles and even tadpoles use distress calls when they are seized by predators (Toledo & Haddad, 2005). These types of defensive calls include distress calls, for instance when trying to escape from enemies; alarm calls, which may be emitted for a variety of reasonssuch as when an individual is surprised and escapes or when predators are encountered and the individual alerts other anurans in the area aboutits presence; as well as warning calls – emitted as a signal to warn predators that they will face danger from the frog, for instance that they are toxic or may possess defensive strategies that can harm the predator. These three call types are all grouped under defensive calls (Wells, 2007; Toledo & Haddad, 2005; Toledo *et al*., 2015).

1.4 Amphibian monitoring methods

Channing (1999) states that little interest is shown when it comes to amphibians and their behaviour, especially in Africa. He further notes that there has been a tremendous increase in interest in frogs by the scientific community, worldwide. There is an increasing number of techniques to investigate and describe amphibian biodiversity. However, some of these techniques are considered invasive and there is a need for effective, non-invasive monitoring techniques to survey and describe amphibian species, particularly those that are not well known or newly discovered. Acevedo *et al.* (2009) state that biodiversity surveys will be needed to manage and conserve species in a sustainable and less invasive way. There have recently been major developments in ways to assess amphibian biodiversity more accurately (Depraetere *et al*., 2012), especially in the field of bioacoustics (i.e. Passive Acoustic Monitoring) (Brandes *et al.*, 2006; Villanueva-Rivera, 2007).

According to Toledo *et al.* (2015), anuran call recognition has a long historythat can be dated back to a few hundred years BC. Nonetheless, Bogert (1960) was one of the first to recognise the importance of anuran vocalisation and the need to classify their calls. Among the first scientists to publish sound spectrograms were Schiotz (1967) and Inger (1968).

As far as the actual measurements are concerned, automated recorders can be a useful tool to gather quantitative and qualitative acoustic data (Acevedo *et al*., 2009). Passive acoustic monitoring (PAM) is considered the most effective, cost-efficient long-term monitoring method for detecting biodiversity acoustically (Gannon, 2008). Large areas can be surveyed over an extensive period, leading to large quantities of acoustic data, which can be recorded continuously throughout the year. This helps scientists detect the activity, abundance and behaviour of their study species (Gannon, 2008). The use of passive acoustics is not limited to determining species richness or abundance; it can also be used to provide anuran diversity estimates through manual identification and to determine the structure of local frog communities (Diwakar & Balakrishnan, 2007; Villanueva-Rivera, 2007; Depraetere *et al*., 2012).

In several studies, acoustics have been utilised to track the movement and identify and determine the behaviour of birds (see Frommolt *et al.*, 2008; Volodin *et al.*, 2015; Frommolt, 2017), bats (Vaughan *et al.*, 1997; Lemen *et al.*, 2015; Ossa *et al.*, 2017), frogs (Brandes *et al.*, 2006; Measey *et al.*, 2017; Lee *et al.*, 2017), insects (Diwakar *et al.*, 2007; Schmidt & Balakrishnan, 2015; Symes *et al.*, 2016) and aquatic animals (Johnson *et al.*, 2004; Debich *et al.*, 2015; Frasier *et al.*, 2016).

Through the use of acoustic methods such as PAM, large amounts of high-quality data can be collected over long periods with minimal interference (Acevedo & Villanueva-Rivera, 2006; Diwakar *et al*., 2007; Depraetere *et al*., 2012). However, one of the major drawbacks of these automated data collecting systems is precisely that large amounts of data are collected, which leads to difficulties when it comes to analysis and management (Villanueva-Rivera & Pijanowski, 2012). Furthermore, the fact that ecological events that rely on physical observations may be missed, for instance die-out events or certain behavioural characteristics such as migration.

1.5 Importance of monitoring methods

As discussed previously, anurans mostly make use of vocalisation for communication and reproductive purposes, where advertisement calls are used for mate recognition (Köhler *et al.*, 2017). These calls are mostly species-specific. Therefore they can be used as a tool to distinguish between species (Wells, 2007). According to Passmore (1981), these types of calls can be characterised by the natural environments that species inhabit. It follows that acoustic communication of anurans has evolved as a result of certain adaptations and sexual selection (Wells & Schwartz, 2007). Nevertheless, this is not the only method used to distinguish between species. In recent years, attention has shifted greatly towards molecular analysis for systematic purposes; however, there is still phylogenetic data missing for some genera (Tarrant *et al*., 2008; Tolley *et al*., 2010; Channing *et al*., 2013). For more robust results, the present study suggests that acoustic data and molecular analysis (e.g. DNA sequencing) should be combined, thereby aiding in describing more species, in particular cryptic species (Frost *et al*., 2006; Vences & Wake, 2007; Minter *et al*., 2017), leading to rigorous analysis of relationships between genera (Channing, 1999), while resolving relevant taxonomic uncertainties (Bee *et al*., 2013).

Acoustic data can also aid in conservation efforts, as it is sensitive to environmental changes. Monitoring the bioacoustics of anurans can also help determine the conservation status of some species, since itis able to indicate the relationship between natural and anthropogenic sounds (Bee *et al*., 2013). These results can further show the correlation between such relationships and environmental conditions.

1.6 Anuran diversity of South Africa

South Africa is a biologically rich country with a diverse landscape, consisting of nine major terrestrial biomes. These include Fynbos-, Succulent Karoo-, Grassland-, Savannah-, Desert-, Nama-Karoo-, Albany Thicket-, Indian Ocean Coastal Belt and the Forests Biomes (Mucina & Rutherford, 2006), ranging from the wet tropical regions to desert. However, the country is still regarded as semi-arid with a relatively low number of permanent wetlands consisting of localised stagnant water (Minter *et al.*, 2004).

According to Du Preez and Carruthers (2017), Southern Africa has approximately 171 known frog species consisting of 33 genera and 13 families. Stuart (2008) avers that South Africa is the fourth-ranked country when it comes to the number of threatened amphibian species in the Afrotropical region. Furthermore, Drinkrow and Cherry (1995) highlight that the KwaZulu-Natal coast, north of Durban, is a biodiversity hotspot, based on the number of fauna and flora found in this area. Second only to the Cape Floral Kingdom, the KwaZulu-Natal coast is the most floristically diverse region within South Africa, with vegetation types ranging from forest and grasslands to coastal bushveld and savannah (Mucina & Rutherford, 2006).

The province of KwaZulu-Natal stretches from Port Edward in the south, northwards to the border of Mozambique. KwaZulu-Natal is South Africa’s third-smallest province, covering only 8% of the country’s total area.Simultaneously, 22% of the South African population lives in this province, making it one of the most populated regions in the country (Driver *et al.*, 2015). In terms of its climate, the region has humid tropical conditions ideal for its native fauna and flora, especially along the northern coast. The north coast is a summer rainfall area with an average of 1 000 mm per year and average diurnal temperatures of 28°C in the summer and 23°C in the winter (Driver *et al.*, 2015). KwaZulu-Natal further boasts with the highest species richness of frogs and endemism in South Africa (Minter *et al*., 2004; Measey *et al*., 2011; Driver *et* *al.*, 2015). Currently, 41.5% of the total frog diversity of Southern Africa occurs in KwaZulu-Natal (Du Preez & Carruthers, 2017). Minter *et al.* (2004) states that the southern parts of KwaZulu-Natal contain more endemic species than the northern and western parts. In their turn, the northern parts of KwaZulu-Natal form part of the Maputaland-Pondoland-Albany hotspot area. This area is subject to drastic habitat transformation (Jewitt, 2012; Russell & Downs, 2012; Tarrant & Armstrong, 2013).

The world's tenth largest and one of South Africa's busiest harbours is located in Durban, KwaZulu-Natal. To the north is the city of Richards Bay, where there are major aluminium mining activities and exotic tree plantations (Driver *et al.*, 2015). With the unique weather conditions and landscape in KwaZulu-Natal, this region is popular and ideal for plantations such as sugar cane, bananas and other sub-tropical fruits. It is also a popular tourist destination. All of this leads to a rapid development rate, leading to development on the levels of agriculture,industry and urbanization. In KwaZulu-Natal, there is a growing trend in afforestation, which, in turn, leads to land transformation and habitat destruction. Due to this, some animal species and plants have become threatened or even extinct (Armstrong *et al.*, 1998; Minter *et al.*, 2004). According to a study done by Armstrong *et al.* (1998), more than one-third of KwaZulu-Natal has been transformed. This study was published two decades ago, and since then, there has been a tremendous increase in human population, resulting in more urban developmentsthat carry considerable implications for conservation. In a later study, Armstrong (2001) raised concerns that surveys need to be conducted in the KwaZulu-Natal region to determine whether endemic frogs still occured in their natural environments. This would lead to more effective biodiversity conservation efforts. Of great concern is that terrestrial areas and wetlands along the coast are being classified as critically endangered (Driver *et al.* 2015). These considerations demonstrate that a biodiversity study is much needed in these areas to aid and encourage conservation efforts.

1.7 Study aims and objectives

This study has three main aims: firstly, to determine the anuran diversity in north-eastern KwaZulu-Natal using automated recorders. The data analysed from the recordings will be used to identify the different species present, as well as to calculate their relative abundance. The second aim is to determine the correlation between meteorological variables and anuran breeding behaviour at the Umlalazi Nature reserve. The final aim is to clarify the distribution of two *Afrixalus* species, using call structure and morphological characters. The aims can be arranged as the following objectives:

1. **Strategically placing six automated acoustic recorders (Song Meters) at six localities along West-East and North-South transects along northern KwaZulu-Natal and collecting acoustic data.**

   A lack of recent anuran diversity estimates in north-eastern KwaZulu-Natal has been identified. As a result, improved knowledge is needed of anuran species within this area. This was done by strategically placing six different acoustic recorders within north-eastern KwaZulu-Natal, where the Song Meters recorded the anuran calling activity throughout the year. Species were identified and abundances were determined and correlated with calling activity to determine peak calling times during the year. This information contributes to the understanding and identifying of north-eastern KwaZulu-Natal's natural biodiversity.

2. **Compile atmospheric data and correlate it with collected acoustic data to determine the effect of environmental factors on the calling behaviour of frogs in the Umlalazi Nature Reserve.**

Studies have shown that environmental conditions effect the calling behaviour of anurans. This information can be used to determine the phenology between environmental factors that can act as cues to trigger breeding behaviour such as calling.

3. Clarifying taxonomic confusion within *Afrixalus stuhlmanni* complex.

In this study, two species of the genus *Afrixalus* were found to be closely related. Acoustic and morphological studies were conducted on both species to determine the morphological and acoustic variance between them. The resultant findings will contribute to the systematics and understanding of the *Afrixalus stuhlmanni* complex.

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CHAPTER 2: THE UTILISATION OF PASSIVE ACOUSTIC MONITORING AS A TOOL TO DETERMINE ANURAN DIVERSITY IN NORTH-EASTERN KWAZULU-NATAL, SOUTH AFRICA

The cry of frogs is one of the most wearying, croaking sounds possible, and we have only to place ourselves near some dirty pool in the spring, to convince ourselves of their deep, guttural voices; but bad as this is, it is music compared to the long shrieks, shrill whistlings, snorings, and bellowings of those in other parts of the world.

Mrs R. Lee
Anecdotes of the Habits and Instincts of Birds,
Reptiles and Fishes
(1855)

2.1 Abstract

Various animal groups have developed the ability to produce species-specific vocal signals, each with unique acoustic patterns. Among these groups amphibians is one of the most well-known. The study of vocal signals allows scientists to monitor animals based on their acoustic behaviour. The last known survey done on the anuran diversity and geographical distribution of north-eastern KwaZulu-Natal was conducted by Minter *et al.* (2004). In an attempt to document the anuran diversity in north-eastern KwaZulu-Natal, South Africa, the present study was conducted by making use of passive acoustic monitoring (PAM) via automated recorders. Six localities were identified where recorders were deployed. There were 10 minutes of recording within each hour from 18:00 to 07:00. A total of 54% (29/54) of the expected species were recorded. Anuran activity peaked in the early morning hours. Two species, namely *Leptopelis mossambicus* and *Ptychadena anchietae*, were recorded at all sites. The highest frog diversity was found in St. Lucia with 16 species. Due to El Niño, rainfall patterns were not typical. All of the chosen localities for this study are located in protected areas, which highlights the importance of such reserves. When it comes to choosing automated recorders, PAM proved to be a practical method for medium- to long-term non-invasive biodiversity estimates at specific sites.

Keywords: *anurans; acoustic monitoring; automated recorders; north-eastern KwaZulu-Natal; protected areas; biodiversity estimates*

2.2 Introduction

Biodiversity can be defined as all life on earth in different ecosystems that coexist and and interact within a framework of ecological processes (Hill, 2005; Wake & Vredenburg, 2008). Biodiversity losses has turned into a global phenomenon, with the current estimated extinction rate exceeding those recorded in the last decade (Wilson, 1992; Blaustein *et al*., 2010). This is a major issue of concern, as biodiversity is of critical importance, not only to nature, but also to humankind– in relation to aesthetic, cultural, and economic values (Loreau *et al.*, 2001; Duffy *et al.*, 2017). Biodiversity can be widely used in both economic and scientific fields due to its related values (Hill, 2005; Duffy *et al.*, 2017) such as those mentioned. However, for scientists, the relevance of biodiversity lies in the fact that it can guide understanding of community structure, environmental processes and ecosystem functions (Pullin, 2002). Moreover, biodiversity enjoys direct uses related to food production, tourism and recreation, all of which have economic value, thereby contributing towards global economics (Edwards & Abivardi, 1998; Duffy *et al.*, 2017). Important indirect uses include ecosystem services through ecological processes such as natural pest control as well as flood and erosion protection (Edwards & Abivardi, 1998), underlining the critical nature of conservation, especially in areas with high biodiversity. However, South Africa with its rich biodiversity is under threat given that conservation efforts are of a low priority at a national level (Turpie, 2003). According to Turpie (2003), the main reason for this situation is the fact that the value of biodiversity is either underestimated or completely unknown, which leads to vast quantities of biodiversity loss. When it comes to amphibian diversity the picture appears even bleaker. More than a third of amphibians are in danger of extinction: globally, amphibians is the most threatened vertebrate class (IUCN, 2018).

The process of assessing anuran diversity is time-consuming, requiring a range of sampling methods, which are restrictive due to high cost, large areas to survey and lack of interest. This is, however, starting to change in the field of science. In 2004, the first frog atlas for South Africa was published, showing frog population and distribution patterns across the country (Minter *et al.*, 2004). Since then, several species' names, distribution and conservation statuses have changed due to new data, new discoveries and the use of novel techniques based on morphological -, molecular (DNA barcoding) - and acoustic methodsthat include passive acoustic monitoring (PAM). Passive monitoring equipment now enables scientists to gather behavioural information on communities in remote areas. It contributes to the understanding of critical ecological factors underlying distribution and the effects of seasonal changes and environmental extremes such as droughts, floods and temperature variations on breeding events. One of these novel techniques involves the monitoring of diversity of vocal species by employing PAM.

Riede (1993) has found that the use of sound recordings of acoustic animals enables estimations of biodiversity. These are of great value to science, especially in threatened tropical areas. Riede's statement was made over 25 years ago, and since then technology and biomonitoring methods have seen rapid transformation in terms of new software in addition to improved equipment and technology. Although the use of these methods was widely applied to birds and mammals in the past, this type of monitoring has been progressing into a wider range of fields. Some examples include insects such as Cicadidae and Orthoptera (see Diwakar & Balakrishnan, 2007; Schmidt & Balakrishnan, 2015; Riede, 2017), various avian orders including Coraciiformes (Potamitis *et al.*, 2014; Menon *et al.*, 2015), Accipitriformes (Seress & Liker, 2015; Klingbeil & Willig, 2016) and other avian species (see Gasc *et al*., 2013; Taylor *et al*., 2016; Frommolt, 2017), bats (Chiroptera), terrestrial mammals such as rodents (see O'Farrell *et al*., 2008; Ancillotto *et al.*, 2016), primates (see Boinski & Mitchell, 1997; Heinicke *et al*., 2015; Kalan *et al*., 2015) (see Fenton *et al.*, 2002; Puechmaille *et al*., 2014; Ossa *et al.*, 2017), carnivores (see Banea *et al*., 2012; Stein *et al*., 2013; Comazzi *et al*., 2016) and, last but not least, amphibians such as anurans (see Brandes *et al*., 2008; Obrist *et al.*, 2010; Dey *et al.*, 2015; Measy *et al*., 2017).

Some animal taxa have the ability to produce species-specific vocal signals with unique acoustic patterns. These organisms (including insects, reptiles, birds, amphibians and mammals) use vocalisation to attract mates, communicate with one other and navigate and avoid predation (Le Galliard *et al*., 2012). By using these acoustic signals species can be identified in an area through the use of automated digital recording systems. Acoustic recordings offer one of the best methods to conduct non-invasive population surveys (Le Galliard *et al.*, 2012). It potentially enables scientists to determine and locate calling species within populations in a cost-effective and efficient manner covering a wide area (Le Galliard *et al.*, 2012). Monitoring through the use of passive acoustic ways also enables scientists to collect crucial data (qualitative and quantitative) continuously over extended time periods. The monitoring is not dependent on weather circumstances (Fujioka, 2014), human interactions or landscape change. With automated recorders, species can be detected that may otherwise be difficult to find due to dense vegetation, scarceness, species preference to be active in specific weather conditions and habitat preference. For instance, some male rain frogs (such as. *Breviceps adspersus*) tend to call from shallow depressions or concealed places. *Hemisus guttatus* calls from underground, whereas tree frogs (for instance *Leptopelis mossambicus*) and Southern foam nest frogs (*Chiromantis xerampelina*) call from trees or even dense thorn bushes or shrubs (Du Preez & Carruthers, 2017).

PAM can be very beneficial, especially if some of the detected sites are located in inaccessible areas or areas that tend to vary in terms of the composition (for instance, in the case of dense vegetation) or seasonal changes. The method is ideal especially for nocturnal taxa such as frogs and bats. Le Galliard *et al.* (2012) believe that acoustic technology may prove to be a valuable and versatile tool for ecological studies. This method can also be used to observe and study a species' breeding behaviour and activity. Sueur *et al.* (2008) further aver that the biological quality of an environment based on its soundscapes could be used to determine the impact of human activity on the biodiversity of that area. Pijanowski *et al.* (2011) found that the acoustic diversity of an area, combined with the patterns of the temporal soundscape acoustics, can aid in determining multiple land use types. This has recently been of increasing interest among behavioural scientists and tends to produce improved results compared to other monitoring methods (see Acevedo & Villanueva-Rivera, 2006; Celis-Murillo *et al*., 2009; Dumyahn & Pijanowski, 2011; Stein & Hayssen, 2013; Taylor *et al*., 2016).

Only a few extant studies have attempted to determine the total anuran biodiversity of KwaZulu-Natal, including "A Review of the Amphibians of Natal" (Lambiris, 1989) and "The Atlas and Red Data Book of the Frogs of South African, Lesotho and Swaziland" (Minter et al., 2004). Over the extent of the immediate past, though, this area has undergone considerable anthropogenic transformation. Vast numbers of people settled in the area posing challenges to the natural environment, including human population increases, agricultural impacts, habitat transformation and climate change. According to Jewitt (2012), the rate of land transformation in this province is greater than that of all other cases of land transformation in the country. Indeed, anuran diversity in north-eastern KwaZulu-Natal is under threat due to land invasion not only as a consequence of infrastructure development (Jewitt, 2012), forestry (Armstrong *et al.*, 1998; Minter *et al.*, 2004; Measey *et al.*, 2011) and agriculture (Mittermeier *et al*., 2005), but also factors such as the introduction of invasive species of trees and other plants (Minter *et al*., 2004; Measey *et al.*, 2011)as well as pollution (Angulo *et al*., 2011). These developments pose great concern for the anuran community in this area. For example, the critically endangered Pickersgill reed frog (*Hyperolius pickersgilli*) and the vulnerable spotted shovel-nosed frog (*Hemisus guttatus*) occur in the study area. It is therefore of critical importance to assess the biodiversity of this region to compile effective and appropriate conservation plans and strategies (Turpie, 2003) with regard to these and other species. Long-term monitoring is needed to assess and detect changes in populations of anurans within these parts of South Africa.

The overall aim of this chapter was to establish the importance of conducting a biodiversity study of anurans in north-eastern KwaZulu-Natal by means of acoustic detection using a passive acoustic monitoring approach, in order to identify species within these regions and document their breeding behaviour. It demonstrated the need for the use of automated recorders to record calling males at different localities. It argued that the recordings would be used to determine the anuran diversity for species in these areas.

2.3 Materials and methods

2.3.1 Site selection

The broad study area covered most of north-eastern KwaZulu-Natal, South Africa (see Figure 2.1). Within this area, six different sites were identified within which the recorders (Song Meters) used for passive acoustic monitoring were placed (Table 2.1). Individual sites were chosen based upon the presence of habitats suitable for a variety of species and away from intense anthropogenic influences.

Table 2.1: The six identified localities where recorders were placed

	Locality	Coordinates	
1.	Ndumo Game Reserve	-26.88146 S	32.25062 E
2.	Tembe Elephant Park	-26.94714 S	32.52434 E
3.	Kosi Bay	-26.95712 S	32.82754 E
4.	Bonamanzi Game reserve	-28.07421 S	32.31355 E
5.	St Lucia	-28.38617 S	32.41184 E
6.	Umlalazi Nature Reserve	-28.95540 S	31.76607 E

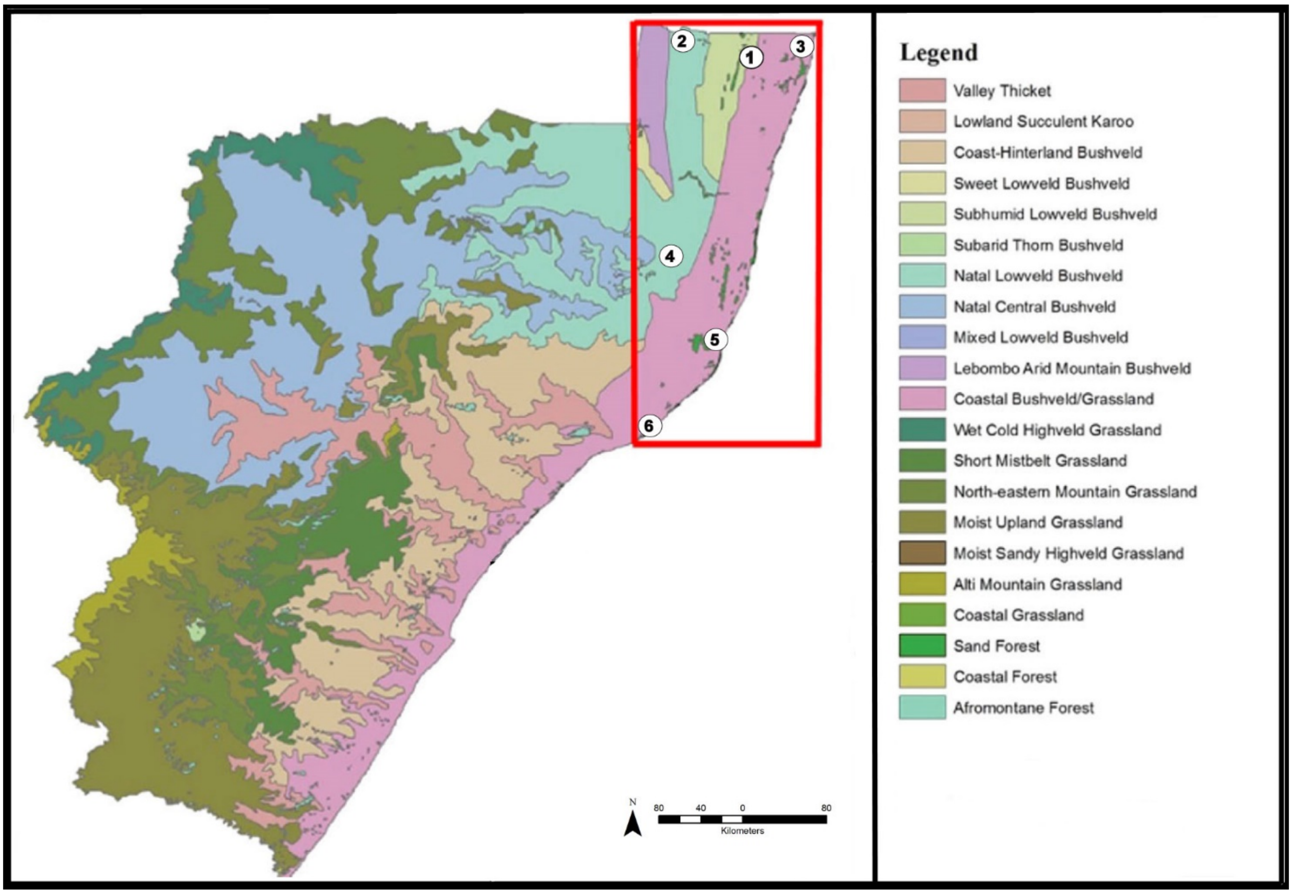

Figure 2.1: Vegetation map and legend of KwaZulu-Natal indicating the study area (red demarcation). The six different sampling sites are shown within the red demarcation. **1:** Ndumo Game Reserve; **2:** Tembe Elephant Park; **3:** Kosi Bay; **4:** Bonamanzi Game reserve; **5:** St Lucia; & **6:** Umlalazi Nature Reserve (adapted from Mucina & Rutherford, 2006)

2.3.2 Site description

All of the identified localities for the study are found within the Maputaland-Pondoland-Albany hotspot area. This area stretches from the Eastern Cape of South Africa (including the Albany centre of plant endemism), through KwaZulu-Natal into the southern parts of Mozambique and Mpumalanga (Figure 2.1). It encompasses six biomes, ranging from ancient sand dunes to the northern low-lying plains with sub-tropical to tropical climates, along the coastal areas (Di Minin *et al.*, 2013). It is internationally recognised as an area with high species richness and endemism levels (Di Minin *et al.*, 2013). A detailed description of each site follows.

Ndumo Game Reserve

The Ndumo Game Reserve is a recognised RAMSAR site situated in the western parts of Zululand. It forms part of the Maputaland bioregion (Haddad *et al*., 2006). This reserve contains 10 000 ha of Bushveld-Savannah, which contributes to the rich species diversity in this area (Haddad *et al*., 2006). The selected site was classified as a temporary pan with characteristic endorheic microhabitats. An SM2 was erected next to the pan (Figure 2.1a).

Tembe Elephant Park

Tembe Elephant Park is situated in the western parts of Zululand (forming part of Maputaland) east from Ndumo Game Reserve (Gaugris *et al*., 2014; Jordaan *et al*., 2016). Tembe Elephant Park covers 30 013 ha of sandy plains, swamps, grass- and woodlands (Matthews *et al.*, 2001; Gaugris *et al.*, 2014; Jordaan *et al.*, 2016). The selected site was classified as a vlei area characterised by palustrine microhabitats. An SM2 was erected close to a bird hide for easy accessibility. Although one SD card got corrupted or faulty, with the result that data could not be retrieved from 20 May to 2 July 2016, it was assumed that no significant data was lost. The SM2 was installed on 17 November 2015 and removed on 5 May 2017.

Kosi Bay Nature Reserve

The Kosi Bay Nature Reserve covers approximately 11 000 ha. It is located east from Tembe Elephant Park, adjacent to the Indian Ocean. Kosi Bay forms the northern part of the iSimangaliso Wetland Park, a UNESCO World Heritage Sites (Warner *et al*., 2016). The selected site was identified as a vlei with characteristic palustrine microhabitats. In Kosi Bay an SM3 was installed from 19 November 2015 to 24 May 2017. Due to unknown technicalities of the SD card, data was lost from 25 August to 9 September 2016 (Figure 2.1b).

Bonamazi Game Reserve

Bonamazi is a 4 000 ha private game reserve and falls within the Hluhluwe region in the heart of Zululand. Declared as a Natural Heritage Site since 1995, habitats range from grassland and savannah to dense thickets (Madsen *et al.*, 2017). The selected site was classified as a temporary pan with characteristic endorheic microhabitats. An SM3 was installed from 24 November 2015 to 24 December 2016. This site was identified as a potential breeding site for *P. edulis*. Due to a battery or solar panel fault, data was lost between 18 August and 24 November 2016. As observed during field trips, this site remained mainly dry throughout the study period (Figure 2.1c).

St. Lucia

St. Lucia is located south of Kosi Bay and viewed as the hub of the iSimangaliso Wetland Park (Warner *et al*., 2016). It is recognised as a RAMSAR wetland and a UNESCO World Heritage Site (Hart *et al*., 2014). Cyrus *et al.* (2009) state that the St. Lucia estuarine system constitutes 80% of the total estuarine area of KwaZulu-Natal, making this the largest and most important estuarine system in Africa (Cyrus *et al*., 2009; Hart *et al.*, 2014). The selected site was identified as a vlei with characteristic palustrine microhabitats. In St. Lucia, an SM3 was installed from 20 November 2015 to 14 November 2016 (Figure 2.1d).

Umlalazi Nature Reserve

Umlalazi Nature Reserve is situated on the north coast of Zululand near Mtunzini (Nevill & Nevill 1995; Traynor, 2008). The 1 028 ha nature reserve forms part of the northern section of the Siyaya Coastal Park (Mabaso, 2002). Diverse vegetation types include grasslands, subtropical dune thicket and mangrove and riverine forests (Zungu, 2017). The site was classified as an ephemeral wetland with both terrestrial and palustrine microhabitats. Within the Umlalazi Nature Reserve an SM3 was installed from 21 November 2015 to 14 November 2016. This site was identified as a breeding habitat for the endangered Pickersgill frog (*H. pickersgilli*) (Figure 2.1e).

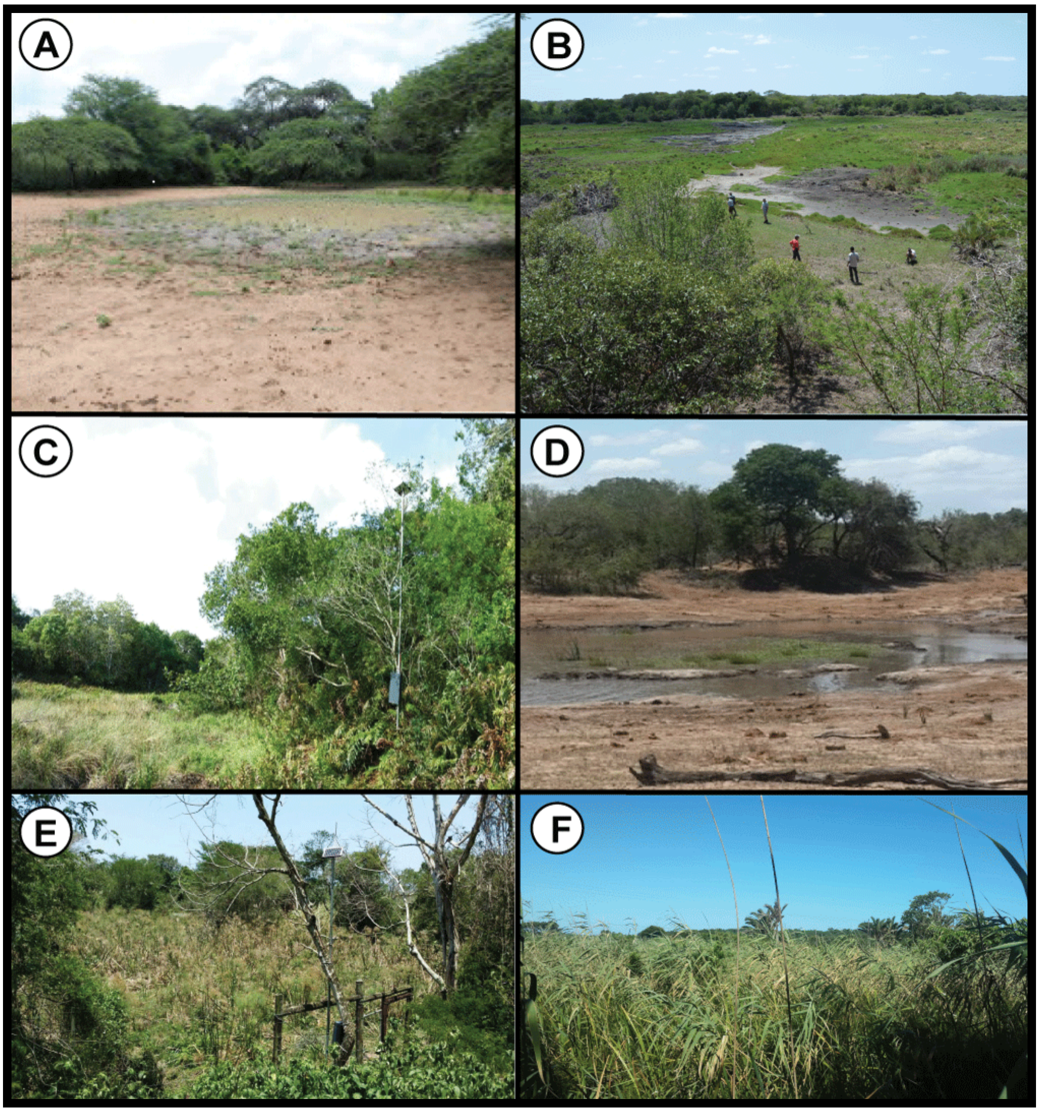

Figure 2.2: The six different sites chosen for the study; **A:** Ndumo Game Reserve, **B:** Tembe Elephant Park, **C:** Kosi Bay Nature Reserve, **D:** Bonamazi Game Reserve, **E:** St. Lucia and, **F**: Umlalazi Nature Reserve

2.3.3 Passive acoustic monitoring equipment

Two second generation Song Meter recorders (model SM2; Wildlife Acoustics Inc., Concord, MA; max capacity of 4 x 32 GB SD cards each) and four third generation Song Meter recorders (SM3; max capacity 4 x 64 GB SD cards each) were used in this study. Each recorder (protected inside a steel case) was equipped with a solar panel connected to a 12V rechargeable lead-acid battery to allow for long-term powering (Figure 2.3).

Since anurans breed at different times of the year, the study was conducted over a period of more than a year to ensure that all possible species could be detected. The Song Meters were left at the sites (see Table 2.1; Figure 2.1) from November 2015 to December 2016, with the exception of two sites (Ndumo and Kosi Bay) where, for logistical reasons, the Song Meters were left until May 2017. Second generation Song Meters (SM2) were installed at Tembe Elephant Park and Ndumo Game Reserve. At the remainder of the four sites, third generation Song Meters (SM3) were installed. The Song Meters were programmed to record 10 minutes of each hour between the hours of 18:00 to 07:00. However, in the case of three of the recorders (Ndumo Game Reserve, Tembe Elephant Park and Bonamanzi Game Reserve) the recording time was extended to 11:00, due to the probability of detecting African bullfrogs (*Pyxicephalus edulis*). Audio data were collected in stereo, 32-bit WAV format at a sampling rate of 16 000 Hz with no dB gain set.

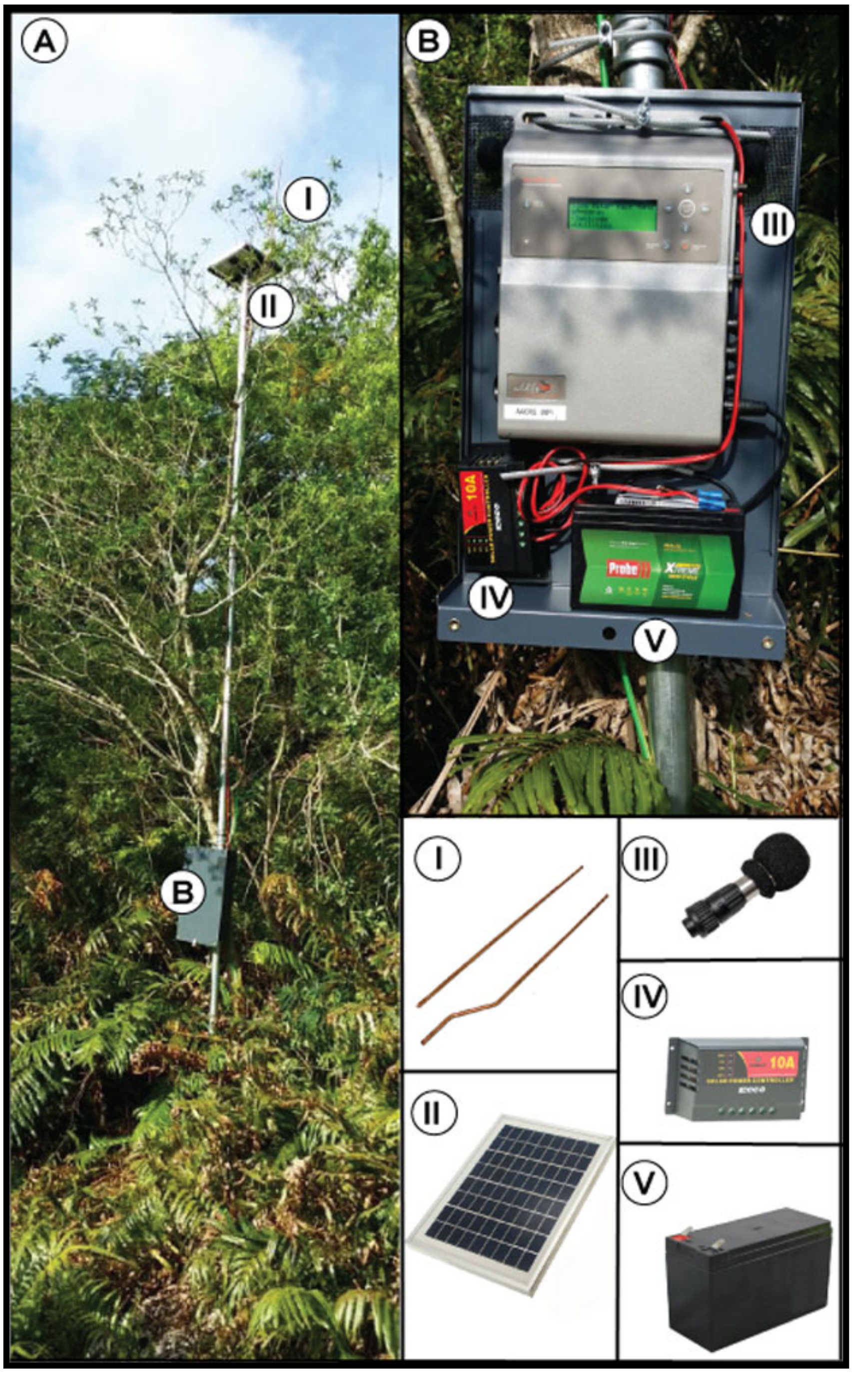

Figure 2.2: Song Meter (SM3) equipment. **A:** Song Meter attached to a tree, connected to a solar panel. **B:** Song Meter housed in protective housing. **I:** Copper lightning conductor rods earthed to the ground. **II:** 25 x 35 cm solar panel. **III:** SM3 microphone. **IV:** 10A solar charger. **V:** 12V Lead-acid battery

2.3.4 Acoustic analysis

In total, the six song meters at the selected sites covered 2 326 nights resulting in a total of 371 580 active recorded minutes. The acoustic data was retrieved during fieldwork trips within the study period, and the data was analysed using Song Scope analysis software (Song Scope™ Wildlife Acoustics Inc., Concord, Massachusetts, USA). Song Scope visualises the data through spectrograms, allowing large audio files to be scanned efficiently. Employing this method to analyse the recorded data allows for detection of species that could be misidentified or missed by the human ear. Visual spectrograms were generated and enhanced by setting the Fast Fourier Transform (FFT) size to 256 with a ½ FFT overlap and a Hamming-window. Frequency bandwidth was narrowed to a minimum frequency set at 300 Hz and a frequency range of 740 Hz with an amplitude gain of 15 to 35 dB (depending on the extent of noise in the specific recording). Recordings were filtered with a 1-second bandpass filter in order to remove background noise outside the set ranges. The display contrast was set between 0 and 25 in order to further enhance the visible dynamic range of the spectrograms. This was done for all the spectrograms in Song Scope ver. 4.1.5 (Song Scope™ Wildlife Acoustics Inc., Concord, Massachusetts, USA). The visualisation of the spectrograms was compared to spectrograms described by Du Preez and Carruthers (2017). Calling males then received an amphibian calling index (ACI) score out of five based on abundance and their intensities (see Table 2.2) (Netherlands, 2014). The intensity for each species was based on the number of calls that where produced within the 10 minutes of recording. If individual males called partially during the recording it was classified as infrequent in contrast to individuals who called repeatedly throughout the recording.

Table 2.2: Amphibian Calling Index scores based on vocal activity of males

Score	Abundance	Intensity
0	No calling males	None
1	One or two males	Infrequently
2	One or two males	Repeatedly
3	Three to four males	Infrequently
4	Three to four males	Repeatedly
5	More than four males or difficult to count males	Repeatedly

2.4 Results

2.4.1 Anuran diversity in north-eastern KwaZulu-Natal

The study identified a total of ten families, fourteen genera and twenty-nine species at the six localities by means of recordings (Table 2.3 and Figures 2.4).

Table 2.3: Expected (based on previous studies) and recorded anuran species at the identified localities listed alphabetically

	Species	Species	Recorded
1	Arthroleptidae	*Arthroleptis stenodactylus*	X
2		*Arthroleptis wahlbergii*	X
3		*Leptopelis mossambicus*	X
4		*Leptopelis natalensis*	X
5	Brevicipitidae	*Breviceps adspersus*	X
6		*Breviceps carruthersi*	
7		*Breviceps mossambicus*	X
8		*Breviceps passmorei*	
9		*Breviceps sopranus*	X
10	Bufonidae	*Poyntonophrynus fenoulheti*	
11		*Schismaderma carens*	
12		*Sclerophrys garmani*	X
13		*Sclerophrys gutturalis*	X
14		*Sclerophrys pusilla*	
15		*Sclerophrys capensis*	
16	Hemisotidae	*Hemisus guttatus*	X
17		*Hemisus marmoratus*	X
18	Hyperoliidae	*Afrixalus aureus*	X
19		*Afrixalus delicatus*	X
20		*Afrixalus fornasini*	X
21		*Afrixalus spinifrons*	
22		*Hyperolius argus*	
23		*Hyperolius marmoratus*	X
24		*Hyperolius pickersgilli*	X
25		*Hyperolius poweri*	
26		*Hyperolius pusillus*	X
27		*Hyperolius semidiscus*	

Table 2.3 continued: Expected (based on previous studies) and recorded anuran species at the identified localities listed alphabetically

28		*Hyperolius tuberilinguis*	**X**
29		*Kassina senegalensis*	**X**
30		*Phlyctimantis maculatus*	
31	Microhylidae	*Phrynomantis bifasciatus*	**X**
32	Phrynobatrachidae	*Phrynobatrachus acridoides*	
33		*Phrynobatrachus mababiensis*	**X**
34		*Phrynobatrachus natalensis*	**X**
35	Pipidae	*Xenopus laevis*	
36		*Xenopus muelleri*	
37	Ptychadenidae	*Hildebrandtia ornata*	
38		*Ptychadena anchietae*	**X**
39		*Ptychadena mossambica*	**X**
40		*Ptychadena nilotica*	**X**
41		*Ptychadena oxyrhynchus*	
42		*Ptychadena porosissima*	
43		*Ptychadena taenioscelis*	
44	Pyxicephalidae	*Amietia delalandii*	
45		*Cacosternum boettgeri*	**X**
46		*Cacosternum nanum*	**X**
47		*Pyxicephalus edulis*	
48		*Strongylopus fasciatus*	
49		*Strongylopus grayii*	
50		*Tomopterna cryptotis*	
51		*Tomopterna krugerensis*	**X**
52		*Tomopterna natalensis*	
53		*Tomopterna tandyi*	
54	Rhacophoridae	*Chiromantis xerampelina*	**X**

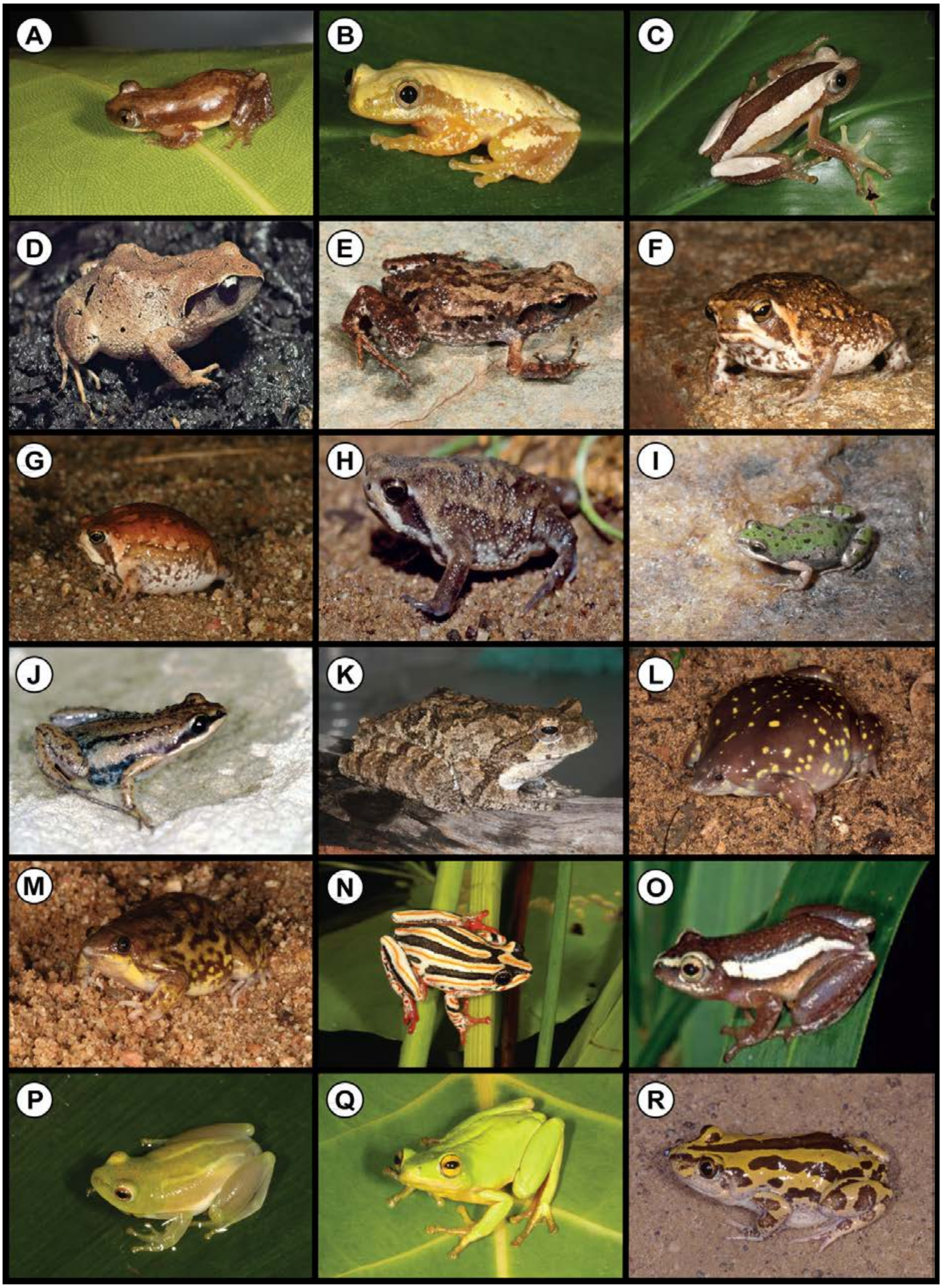

Figure 2.3: Species recorded. **A:** *Afrixalus aureus*, **B:** *Afrixalus delicatus*, **C:** *Afrixalus fornasini*, **D:** *Arthroleptis stenodactylus*, **E:** *Arthroleptis wahlbergii*, **F:** *Breviceps adspersus*, **G:** *Breviceps mossambicus*, **H:** *Breviceps sopranus*, **I:** *Cacosternum boettgeri*, **J:** *Cacosternum nanum*, **K:** *Chiromantis xerampelina*, **L:** *Hemisus guttatus*, **M:** *Hemisus marmoratus*, **N:** *Hyperolius marmoratus*, **O:** *Hyperolius pickersgilli*, **P:** *Hyperolius pusillus*, **Q:** *Hyperolius tuberilinguis* & **R:** *Kassina senegalensis* (© Ed Netherlands)

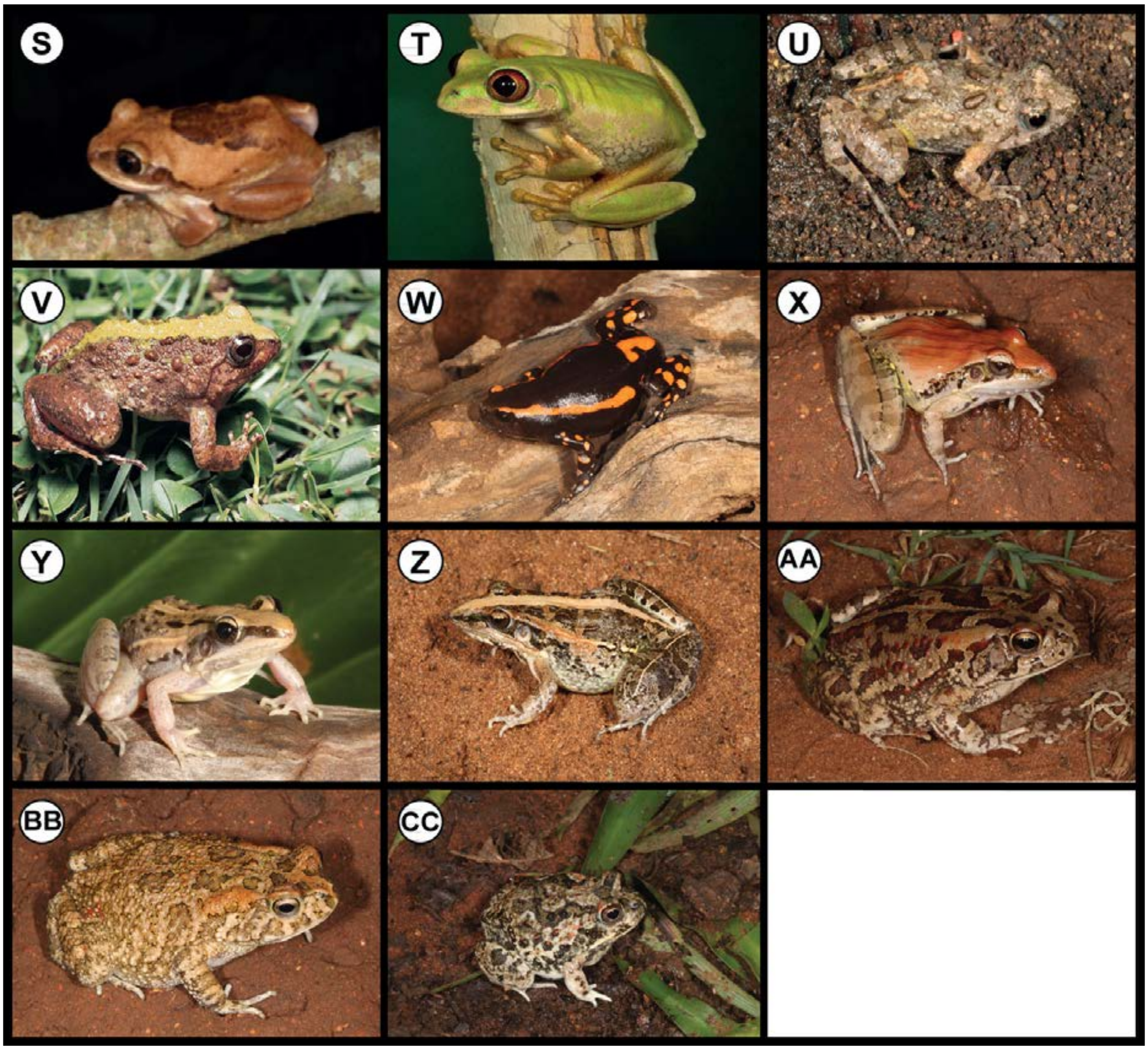

Figure 2.4 continued: S: *Leptopelis mossambicus,* **T:** *Leptopelis natalensis,* **U:** *Phrynobatrachus mababiensis,* **V:** *Phrynobatrachus natalensis,* **W:** *Phrynomantis bifasciatus,* **X:** *Ptychadena anchietae,* **Y:** *Ptychadena nilotica,* **Z:** *Ptychadena mossambica,* **AA:** *Sclerophrys garmani,* **BB:** *Sclerophrys gutturalis* & **CC:** *Tomopterna krugerensis* (© Ed Netherlands)

2.4.2 Frog diversity at sites

The calling activity and intensity were recorded at six different localities within north-eastern KwaZulu-Natal. Table 2.5-2.10 displays anuran species identified by means of acoustic recorders at each of the six localities.

In the Ndumo Game Reserve 12 species (10 genera and seven families) were recorded. The Ndumo SM2 was already erected as part of previous monitoring survey (see Netherlands, 2014). The Song Meter was installed on 17 November 2015 and removed on 19 May 2017. A total of 14 species were recorded at this site. *Ptychadena anchietae* was the dominant calling species, scoring 5/5 for five months (October to December 2016, February 2017 and March 2017). *Kassina senegalensis, P. mababiensis, P. anchietae* and *C. xerampelina* were the first species to call on 5 December 2015. *P. mababiensis* was mostly active on that night, scoring 5/5 at 03:00 in the morning. During November 2016, 83% (10/12) of species called one evening. These species were *L. mossambicus, S. gutturalis, Hy. marmoratus, A. aureus, K. senegalensis, P. bifasciatus, P. mababiensis, P. anchietae, P. mossambica* and *C. xerampelina* (Table 2.5).

Table 2.4: Anuran species recorded using a SM2 recorder at the Ndumo Game Reserve

	Species (n=12)	Family
Ndumo Game Reserve	*Leptopelis mossambicus*	Arthroleptidae
	Sclerophrys gutturalis	Bufonidae
	Hemisus marmoratus	Hemisotidae
	Hyperolius pusillus	Hyperoliidae
	Hyperolius marmoratus	Hyperoliidae
	Afrixalus aureus	Hyperoliidae
	Kassina senegalensis	Hyperoliidae
	Phrynomantis bifasciatus	Microhylidae
	Phrynobatrachus mababiensis	Phrynobatrachidae
	Ptychadena anchietae	Ptychadenidae
	Ptychadena mossambica	Ptychadenidae
	Chiromantis xerampelina	Rhacophoridae

In Tembe Elephant Park 14 species (11 genera and seven families) were recorded using an SM2. *Arthroleptis stenodactylus* was observed as the most abundant calling species at this site over eight months spanning November 2015 to December 2016. *Arthroleptis stenodactylus* and *L. mossambicus* were the first to call on 3 December 2015. December 2015 yielded the most activity with 64% (9/14) species calling, including *A. stenodactylus*, *L. mossambicus*, *S. gutturalis*, *S. garmani*, *He. marmoratus*, *A. fornasini*, *H. tuberilinguis*, *K. senegalensis*, and *P. anchietae* (Table 2.6).

Table 2.5: Anuran species recorded using a SM2 recorder at the Tembe Elephant Park

	Species (n=14)	Family
Tembe Elephant Park	*Arthroleptis stenodactylus*	Arthroleptidae
	Leptopelis mossambicus	Arthroleptidae
	Sclerophrys gutturalis	Bufonidae
	Sclerophrys garmani	Bufonidae
	Hemisus marmoratus	Hemisotidae
	Afrixalus fornasini	Hyperoliidae
	Hyperolius tuberilinguis	Hyperoliidae
	Kassina senegalensis	Hyperoliidae

Table 2.5 continued: Anuran species recorded using a SM2 recorder at the Tembe Elephant Park

	Phrynobatrachus mababiensis	Phrynobatrachidae
	Ptychadena anchietae	Ptychadenidae
	Ptychadena mossambica	Ptychadenidae
	Ptychadena nilotica	Ptychadenidae
	Cacosternum nanum	Pyxicephalidae
	Tomopterna krugerensis	Pyxicephalidae

In the Kosi Bay Nature reserve 13 species (10 genera and eight families) were recorded. *Leptopelis mossambicus* was the dominant calling species of the 13 recorded species at this site over nine months. The greatest abundance of calling occurred in December 2015, January 2016 and January 2017, scoring 4/5. *Hyperolius tuberilinguis* was the first species to call on 19 November 2016, scoring 2/5. During December 2015 a total of 8 out of the 13 species called, including *A. stenodactylus, A. wahlbergii, L. mossambicus, S. gutturalis*, *Hy. marmoratus*, *H. tuberilinguis*, *P. bifasciatus,* and *C. nanum* (Table 2.7).

Table 2.6: Anuran species recorded using a SM3 recorder at Kosi Bay Nature Reserve

	Species (n=13)	**Family**
Kosi Bay Nature Reserve	*Arthroleptis stenodactylus*	Arthroleptidae
	Arthroleptis wahlbergii	Arthroleptidae
	Leptopelis mossambicus	Arthroleptidae
	Breviceps adspersus	Brevicipitidae
	Sclerophrys gutturalis	Bufonidae
	Afrixalus delicatus	Hyperoliidae
	Hyperolius marmoratus	Hyperoliidae
	Hyperolius tuberilinguis	Hyperoliidae
	Phrynomantis bifasciatus	Microhylidae
	Phrynobatrachus natalensis	Phrynobatrachidae
	Ptychadena anchietae	Ptychadenidae
	Cacosternum boettgeri	Pyxicephalidae
	Cacosternum nanum	Pyxicephalidae

In the Bonamanzi Game Reserve nine species (six genera and five families) were recorded. This site had the lowest number of species recorded of all the sites (9 species). *Arthroleptis wahlbergii* was the dominant calling species at this site with a score of 3/5. During December 2015, 89% (8/9) species called, including *A. wahlbergii, L. mossambicus, He. marmoratus, Hy. marmoratus, P. anchietae, P. mossambica, P. nilotica* and *C. nanum* (Table 2.8).

Table 2.7: Anuran species recorded using a SM3 recorder at the Bonamanzi Game Reserve

	Species (n=9)	**Family**
Bonamanzi Game Reserve	*Arthroleptis stenodactylus*	Arthroleptidae
	Arthroleptis wahlbergii	Arthroleptidae
	Leptopelis mossambicus	Arthroleptidae
	Hemisus marmoratus	Hemisotidae
	Hyperolius marmoratus	Hyperoliidae
	Ptychadena anchietae	Ptychadenidae
	Ptychadena mossambica	Ptychadenidae
	Ptychadena nilotica	Ptychadenidae
	Cacosternum nanum	Pyxicephalidae

In St Lucia a total of 16 species (nine genera and eight families) were recorded. *Leptopelis mossambicus* was the dominant calling species at this site, calling for a period of 10 months (except May to July 2016). *Leptopelis mossambicus*, *Hy. marmoratus*, *C. xerampelina* and *S. gutturalis* were the first to call on 20 November 2015 at this site. During December 2015, 69% (11/16) species called, including *A. wahlbergii, L. mossambicus, L. natalensis, B. adspersus, B. mossambicus, B. sopranus, S. gutturalis, H. guttatus, H. tuberilinguis, P. anchietae* and *C. xerampelina* (Table 2.9).

Table 2.8: Anuran species recorded using a SM3 recorder at St. Lucia

	Species (n=16)	**Family**
St. Lucia	*Arthroleptis stenodactylus*	Arthroleptidae
	Arthroleptis wahlbergii	Arthroleptidae
	Leptopelis mossambicus	Arthroleptidae
	Leptopelis natalensis	Arthroleptidae
	Breviceps adspersus	Brevicipitidae
	Breviceps mossambicus	Brevicipitidae
	Breviceps sopranus	Brevicipitidae
	Sclerophrys gutturalis	Bufonidae
	Hemisus marmoratus	Hemisotidae

Table 2.8 continued: Anuran species recorded using a SM3 recorder at St. Lucia

	Hemisus guttatus	**Hemisotidae**
	Hyperolius marmoratus	Hyperoliidae
	Hyperolius tuberilinguis	Hyperoliidae
	Ptychadena anchietae	Ptychadenidae
	Cacosternum boettgeri	Pyxicephalidae
	Cacosternum nanum	Pyxicephalidae
	Chiromantis xerampelina	Rhacophoridae

In the Umlalazi Nature Reserve, 14 species (eight genera and six families) were recorded. *Arthroleptis wahlbergii* was the dominant calling species at this site, calling during every month, with the highest intensity during December 2015 (5/5). *Arthroleptis wahlbergii, L. natalensis, H. guttatus* and *H. marmoratus* were the first species to call on 21 November 2015. December 2015 saw the most activity with 79% (11/14) speciescalling, including *A. wahlbergii, L. mossambicus, L. natalensis, B. sopranus, S. gutturalis, H. guttatus, He. marmoratus, A. delicatus, Hy. marmoratus, H. tuberilinguis* and *P. anchietae* (Table 2.10).

Table 2.9: Anuran species recorded using a SM3 recorder at the Umlalazi Nature Reserve

	Species (n=14)	**Family**
Umlalazi Nature Reserve	*Arthroleptis wahlbergii*	Arthroleptidae
	Leptopelis mossambicus	Arthroleptidae
	Leptopelis natalensis	Arthroleptidae
	Breviceps sopranus	Brevicipitidae
	Sclerophrys gutturalis	Bufonidae
	Hemisus guttatus	Hemisotidae
	Hemisus marmoratus	Hemisotidae
	Afrixalus delicatus	Hyperoliidae
	Afrixalus fornasini	Hyperoliidae
	Hyperolius marmoratus	Hyperoliidae
	Hyperolius pickersgilli	Hyperoliidae
	Hyperolius tuberilinguis	Hyperoliidae
	Ptychadena anchietae	Ptychadenidae
	Ptychadena mossambica	Ptychadenidae

2.4.3 Monthly anuran breeding activity

Displayed in Figure 2.5 is the sum of the total intensities recorded throughout the study period at each site for each species. The horizontal axis represents the months during which the calling activity occurred, while the vertical axis represents the sum of each species' calling intensity. The depth axis represents, in colour, each of the species recorded during the study.

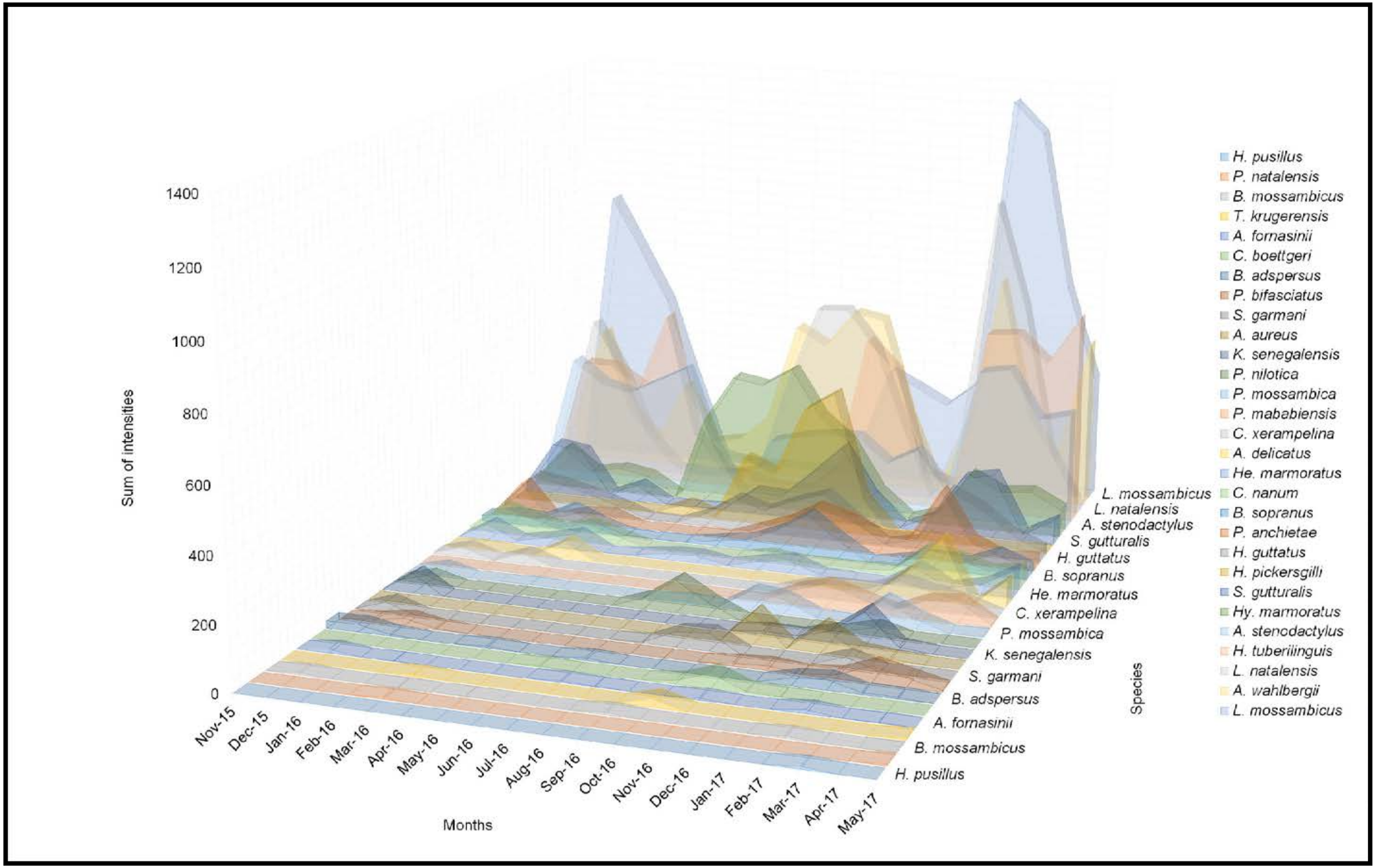

Figure 2.4: Summary for each species 'calling intensities across all study sites for the duration of the study

2.4.4 Hourly anuran activity

Figure 2.6 displays the average calling intensities recorded for each species from 18:00 (factor 1) to 07:00 (factor 14) using the SM2 and SM3 recorders. The horizontal axis represents the factor measured from one to fourteen (1 to 14), where each number represents a specific time in escalating order. The vertical axis shows the average calling intensity (ACI score) of each species (Figure 2.6 and 2.7). Table 2.4 shows the peak calling activity of all recorded males. This indicates the ideal breeding times for the species recorded.

Table 2.10: Hourly and monthly peak calling activity for each species

	Species	Peak time	Peak month
1	*Afrixalus aureus*	19:00-23:00	Oct-16 to Apr-17
2	*Afrixalus delicatus*	19:00-23:00	Nov-15 to Apr-16; Dec-16 to Apr-17
3	*Afrixalus fornasini*	20:00-00:00	Nov-15 to Jan-16; Jun-16 to Aug-16; Oct-16 to Mar-17
4	*Arthroleptis stenodactylus*	19:00-03:00	Nov-15 to Jun-16, Jun-16 to Apr-17
5	*Arthroleptis wahlbergii*	18:00; 05:00-07:00	Whole year
6	*Breviceps adspersus*	19:00-03:00	Nov-15 to Aug-16; Dec-16 to Apr-17
7	*Breviceps mossambicus*	23:00; 04:00-07:00	Nov-15 to Jan-16; Aug-16 to Oct-16; Jan-17 to Mar-17
8	*Breviceps sopranus*	02:00-07:00	Nov-15 to Apr-16; Jul-16 to Apr-17
9	*Cacosternum boettgeri*	19:00-22:00; 01:00-03:00	Sept-16 to Feb-17
10	*Cacosternum nanum*	20:00-04:00	Nov-15 to May-16; Aug-16 to Apr-17
11	*Chiromantis xerampelina*	19:00-03:00	Nov-15 to Mar-16; Oct-16 to Apr-17
12	*Hemisus guttatus*	19:00-02:00	Whole year
13	*Hemisus marmoratus*	23:00-07:00	Whole year
14	*Hyperolius marmoratus*	19:00-01:00	Whole year
15	*Hyperolius pickersgilli*	19:00-04:00	Apr-16 to Jan-17
16	*Hyperolius pusillus*	19:00-21:00	Feb-16 to Apr-17
17	*Hyperolius tuberilinguis*	19:00-04:00	Nov-15 to Jun-16; Aug-16 to Apr-17
18	*Kassina senegalensis*	19:00-23:00	Nov-15 to Jan-16; Oct-16 to Apr-17
19	*Leptopelis mossambicus*	19:00-02:00	Nov-15 to May-16; Sept-16 to Apr-17
20	*Leptopelis natalensis*	19:00-03:00	Nov-15 to Mar-16; May-16 to Apr-17
21	*Phrynobatrachus mababiensis*	20:00-03:00	Oct-16 to Apr-17
22	*Phrynobatrachus natalensis*	02:00-04:00	Feb-16 to Apr-16
23	*Phrynomantis bifasciatus*	20:00-02:00	Nov-15 to Mar-16; Oct-16 to Apr-17

Table 2.4 continued: Hourly and monthly peak calling activity for each species

24	*Ptychadena anchietae*	20:00-03:00	Nov-15 to Apr-16; Jul-16 to Apr-17
25	*Ptychadena nilotica*	23:00-05:00	Nov-15 to Jan-16; Jun-16 to Oct-16; Jan-17 to Mar-17
26	*Ptychadena mossambica*	03:00-05:00	Nov-15 to Jan-16; Jul-16 to Apr-17
27	*Sclerophrys garmani*	21:00-04:00	Nov-15 to Feb-16; Jul-16 to Nov-16; Jan-17 to Mar-17
28	*Sclerophrys gutturalis*	19:00-01:00	Nov-15 to Apr-16; Jul-16 to Apr-17
29	*Tomopterna krugerensis*	20:00-23:00; 01:00-04:00	Feb-16 to Apr-16; Sept-16 to Nov-16

The majority (17/29) of the recorded anurans reached their calling peaks before 00:00. These species include: *A. stenodactylus*, *A. wahlbergii*, *L. mossambicus*, *L. natalensis* (Arthroleptidae); *S. garmani*, *S. gutturalis* (Bufonidae); *H. guttatus*, *He marmoratus, A. aureus*, *A. delicatus*, *A. fornasini*, *Hy marmoratus*, *H. pickersgilli*, *H. pusillus*, *H. tuberilinguis*, *K. senegalensis* (Hyperoliidae); *P. bifasciatus* (Microhylidae); *P. mababiensis* (Phrynobatrachidae); and *C. xerampelina* (Rhacophoridae).

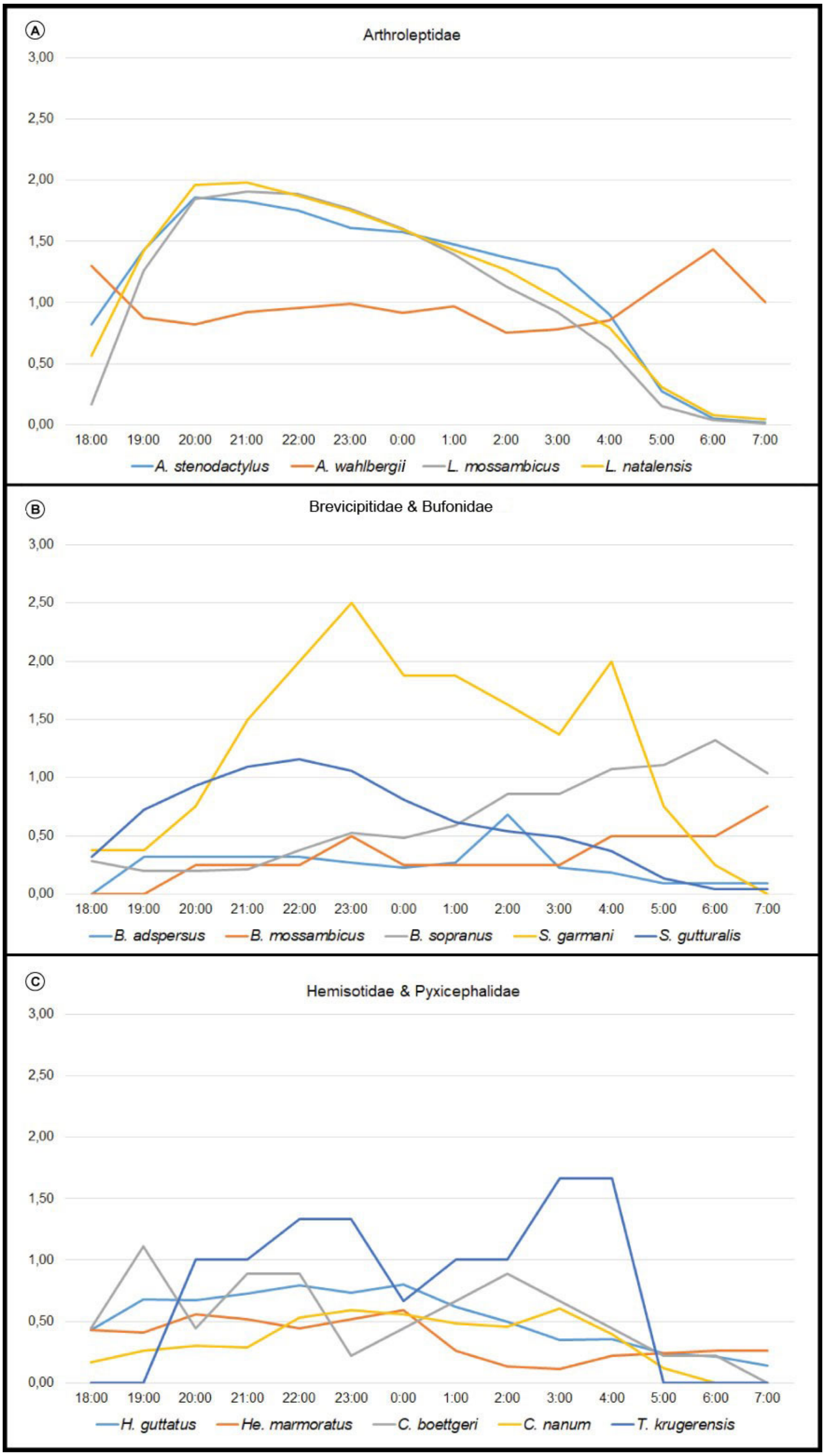

Figure 2.5: Average hourly calling activity of anuran males. **A:** Arthroleptidae, **B:** Brevicipitidae and Bufonidae, **C:** Hemisotidae & Pyxicephalidae

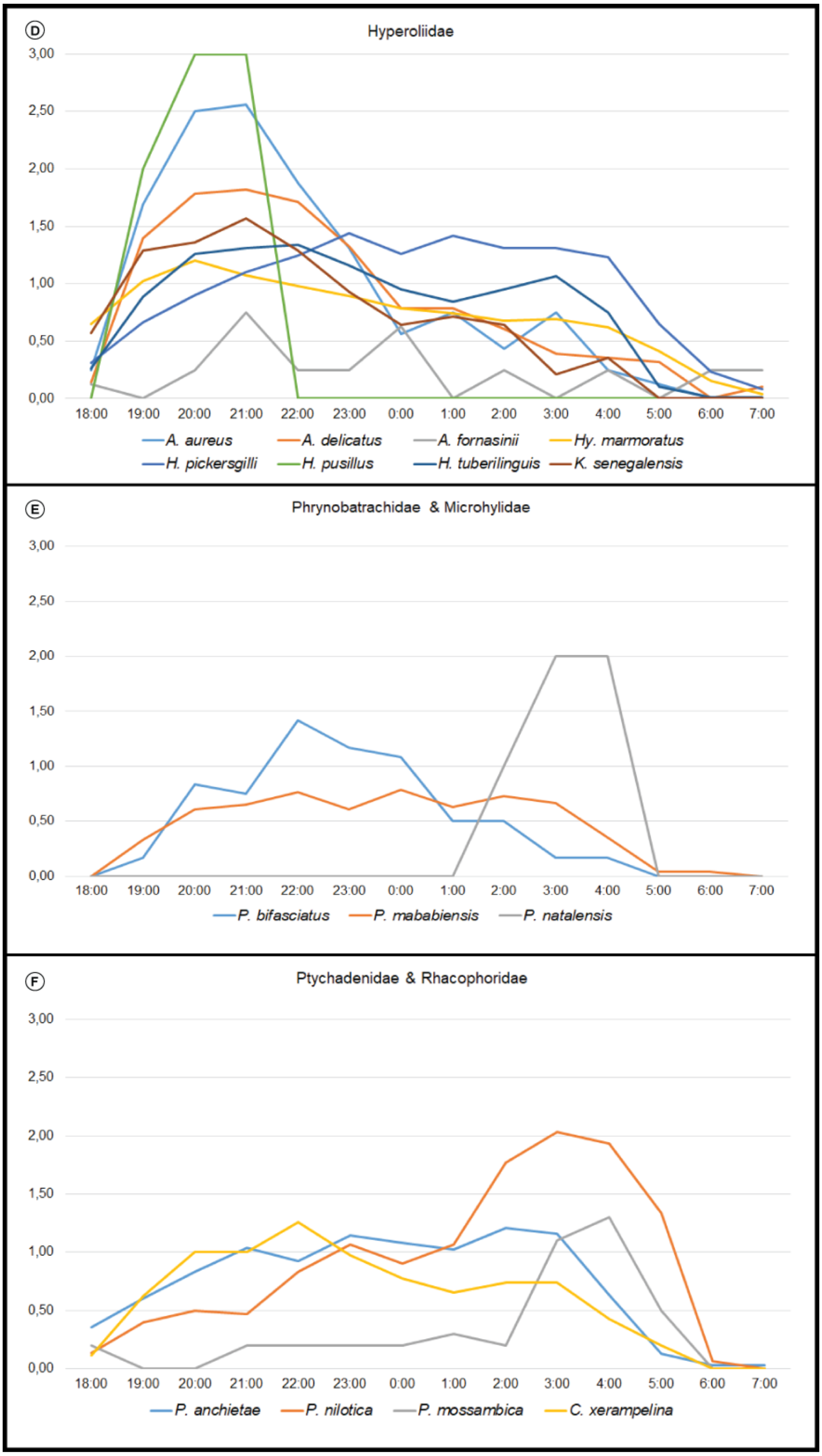

Figure 2.6: Average hourly calling activity of anuran males; **D:** Hyperoliidae, **E:** Phrynobatrachidae & Microhylidae, **F:** Ptychadenidae and Rhacophoridae

2.5 Discussion

Passive acoustic monitoring techniques were used to record calling males. These automated recorders were found to be useful tools to monitor species diversity throughout the study area. A total of 54% of the possible anuran species in north-eastern KwaZulu-Natal were recorded during the study period. Song Scope Analysis software was found to be effective when reviewing the Song Meters' recordings. The presence and abundance of each calling species could be determined and scaled according to the ACI index.

Anuran call surveys are based on the vocalisations of individuals. Information on population structure such as non-calling females, juveniles, tadpoles and eggs cannot be estimated, which restricts data on population abundance and reproduction success (Dorcas *et al.*, 2009). Only males will produce advertisement calls to attract conspecific females (Köhler *et al.*, 2017). This method can therefore only be used to detect changes in the occupancy of vocally active species at a site.

Mis-identification can also be a factor, for instance confusing *Hemisus marmoratus* and mole crickets (Family: Gryllotalpidae). Imperfect detections can also occur due to multiple species calling simultaneously. As a result, not all species present will be detected. Louder, high-pitched calls mask softer calls (Droege & Eagle, 2005). This was the case when *P. anchietae* and *P. mossambica* called. When these two species called simultaneously with high occupancy, it usually masked the other softer calls (i.e. *C. xerampelina)*. These loud calls can also inhibit the calling behaviour of other species (Dorcas *et al.*, 2009). Calls of some lower pitched species (such as *S. gutturalis* and *S. garmani*) will travel longer distances. This could have given a false indication as to which species occupied the specific locality. However, this did not influence the results of the present study, among other reasons since, as Ospina *et al*. (2013) state, species will tend to call during periods with low noise interference. Signals will also travel longer distances during these periods (Waxler, 2004).

Although all Song Meters were located in protected areas away from anthropogenic noise, low anthropogenic noise was detected in the present study. According to Dorcas *et al*. (2009), this noise may influence the detection and behaviour of anurans. However, these sounds were soft or far away and were not considered to have any influence on detection or behaviour. Nonetheless, in urban areas, these interferences may force certain species to adapt their calls or behaviour to compensate for the disturbances (Crouch & Paton, 2002; Kruger & Du Preez, 2016). Technical restrictions were also noted to be a problem. Due to technical faults and capacity restrictions with the SD cards, data was lost, which influenced species detection.

Ospina *et al.* (2013) found that the four *Eleutherodactylus* species would call from 01:00 to 05:00, when noise interference was low. However, during the current study, it was noted that 59% of the species preferred to call before midnight. This suggests that these species will call during warmer night temperatures rather than low noise interference (i.e. temperature is a more important limiting factor than noise interference

During 2014 to mid-2016, South African weather was influenced by a severe drought caused by an El Niño Southern Oscillation event (Archer *et al.*, 2017). El Niño occurs during years when below-normal rainfall and above normal temperatures are experienced (Archer *et al*., 2017). As a result, South Africa experienced two consecutive poor summer rainfall seasons. This led to the worst drought conditions South Africa has faced since 1904 (Archer *et al.*, 2017). During this period, most provinces were declared emergency drought areas (NAC, 2016). These effects were the worst in KwaZulu-Natal, especially in the northern parts (Nel, 2009). Interestingly, most (76%) of the recorded species showed high calling activity during the winter and start of spring months (July-September 2016), calling early in the evenings before midnight. The calling activity was also high during the end of 2016 when rainfall was back to normal. This could be because, during these months, high rainfall was noted, resulting in temporary waterbodies forming and creating ideal breeding sites for the anurans.

A study done by Aide *et al.* (2013) shows that a peak in vocal activity follows the rainy seasons. During this study, the same conclusion can be made. Increased vocal activity during the current study correlated with the precipitation patterns. Interestingly, species seemed to adapt to the precipitation changes. The anurans will most likely call during favourable weather conditions. Rainfall also plays an intermediate role in anuran activity, as some species may be prone to start calling after heavy rains, e.g. *B. adspersus*. On the other hand, some species will decrease calling activity during heavy rains, e.g. *Hy. marmoratus*, or even call infrequently, e.g. *P. anchietae*. Some species have relatively short breeding seasons, e.g. *P. natalensis*, only showing a short period of calling activity.

Cayuela *et al.* (2016) stated that the unfavourable conditions, including drought, lead to a decrease in anuran activity. This can be seen in the current study with the low (49%) species detection. A study done by Netherlands (2014) showed a contradictory picture. This study was done prior to the drought and a higher species richness was recorded at Ndumo Game Reserve (19 species) compared to the current study done in the same area (12 species).

Species with high calling intensities (5/5) over short time intervals are regarded as explosive breeders. Thirty-one percent (9/29) of recorded species received such scores including; *A. stenodactylus*, *A. wahlbergii*, *L. natalensis*, *A. aureus*, *A. delicatus*, *H. tuberilinguis*, *P. anchietae*, *P. mossambica* and *S. garmani*. These nine recorded species occur in either terrestrial, endorheic or palustrine habitats (Du Preez & Carruthers, 2017). These types of habitat only hold water for short periods of time; therefore, mostly without water during long dry seasons. These species can be seen as opportunistic and will flourish once conditions tend to be favourable and waterbodies are formed. Males will have to compete for female access during this time (Bee *et al*., 2013). This will lead to prolonged male activity at these types of habitats.

The highest frog diversity was found in St. Lucia, with 16 species recorded. Two species *L. mossambicus* and *P. anchietae* were recorded at all six of the sites. A total of seven species were found only at one site; these were *B. adspersus, B. mossambicus, S. garmani, A. aureus, H. pickersgilli, H. pusillus* and *T. krugerensis*. The vulnerable species, *H. guttatus,* was recorded at two sites (Umlalazi Nature Reserve and St. Lucia); whereas, the endangered *H. pickersgilli* was only found in the Umlalazi Nature Reserve.

Hyperolius pickersgilli (Raw, 1982) is endemic to only a small part of the coast of northern KwaZulu-Natal. *H. pickersgilli* belongs to the Hyperoliidae family, and has a distinct and unique soft, insect-like chirp with a dominant frequency ranging from 2 890-3 573 Hz call structure (Kruger & du Preez, 2016). It can easily be mistaken for other *Hyperolius* species based on its appearance. According to Bishop (2004a), this species is only found in altitudes below 300 meters above sea level and prefers dense reed-beds in coastal bushveld-grassveld (Mucina & Rutherford, 2006). Both sites where *H. pickersgilli* was found and the type locality, together with the seven additional sites listed in the original description (Raw, 1982) have been destroyed due to urbanisation, according to Tarrant and Armstrong (2013). Agricultural development and urban infrastructure development results in habitat loss, which, in turn, leads to the loss of species diversity, especially within species with restricted ranges, such as *H. pickersgilli* in areas of coastal northern KwaZulu-Natal.

In the current study, eight genera and 30 species were not recorded. Three species (*B. carruthersi*, *B. passmorei*, *B. verrucosus*) from the Brevicipitidae family were not recorded. *Breviceps carruthersi* occurs in the Maputaland Coastal Belt, bushveld areas (Southern Lebombo and Western Maputaland Clay). Males call during and after moderate to heavy rain. These species should have been recorded more south within the study area (Bonamanzi Game Reserve). *B. passmorei* can be found in sandy loam to clay loam bushveld soils (Minter *et al*., 2017). Males usually call after heavy rain. This species should be recorded in Tembe Elephant Reserve and Ndumo Game Reserve. *B. verrucosus* can be found in moist grasslands and coastal forests in southern KwaZulu-Natal (Minter *et al*., 2017). The absence of these three species in the recordings could be attributed to the fact that they have short breeding seasons and also due to a lack of rainfall during the study at these sites.

No recorder was placed near riverine systems due to inaccessibility and security issues. As a result, several species may not have been recorded, including *Amietia delalandii, Strongylopus fasciatus*, *Strongylopus grayii, Tomopterna natalensis* and *Cacosternum striatum* from the Pyxicephalidae family, and *Sclerophrys pusilla,* and *Sclerophrys capensis* from the Bufonidae family.

No species from the Pipidae (*Xenopus laevis, Xenopus muelleri*) were recorded due to their aquatic lifestyle and the fact that their calls are difficult to monitor. *Pyxicephalus edulis* inhabits bushveld areas in the north-eastern parts of the savannah biome in KwaZulu-Natal (Minter *et al*., 2004). Breeding only takes place after heavy rain in flooded open grassy woodlands. Due to the drought and not enough rain for *Pyxicephalus edulis* to breed, no individuals were recorded. *Tomopterna cryptotis* occurs in savannah and grassland areas. Breeding takes place next to shallow, stagnant waterbodies. During dry seasons, individuals will retreat by digging into sandy soils or river beds (Minter *et al*., 2004). *Tomopterna tandyi* inhabits loose, sandy soils within grassland and savannah biomes. Males will call from newly formed pools of flooded areas (Minter *et al*., 2004). It is possible that due to site selection, these species could have been overlooked.

Schismaderma carens can be found in the savannah and grassland biomes, breeding in deep pools or dams (Minter *et al*., 2004). None of the selected sites had deep pools or dams, and this could be the reason for not finding this species. *Poyntonophrynus fenoulheti* can be found in rocky outcrops within bushveld vegetation (Du Preez & Carruthers, 2017). None of the sites had rocky outcrops resulting in the species not being detected. *Hildebrandtia ornata* breeds in shallow, temporary, grassy woodland ponds (Minter *et al*., 2004). This species should have been recorded at most of the locations. However, according to Minter *et al*. (2004), most of the *H. ornata* species found in north-eastern KwaZulu-Natal were found before 1996 and are never abundant at one locality. Netherlands (2014) found *H. ornata* in the Ndumo Game Reserve using active sampling, but not passive acoustic sampling. This is an interesting finding, as the present study also did not record this species. *Ptychadena oxyrhynchus* can be found in most open savannah and woodland areas. This species breeds in temporary pools or sedge pans, calling from the water or water's edge. Due to the majority of the sites not retaining water, this species may have been inactive during the study. *Ptychadena porosissima* can be found in sub-tropical coastal environments, breeding in vleis, sedge pans and inundated grassland areas. Calling will be reduced during dry periods due to the absence of permanent waterbodies (Passmore, 1978). *Ptychadena taenioscelis* is found in woodlands and open grasslands along the coastal plains of northern KwaZulu-Natal. However, Minter *et al.* (2004) reported that the last record of this species was prior to 1996.

Hyperolius argus inhabits coastal grasslands and bushveld, breeding in palustrine habitats (Bishop, 2004b). Males usually call in large choruses from floating or emerged vegetation (Du Preez & Carruthers, 2017). Because of their specialised breeding habitats, this species could have been overlooked due to a lack of permanent waterbodies at the chosen sites. This may also explain the absence of *Hyperolius poweri, Hyperolius semidiscus* and *Phlyctimantis maculatus* species from the same family (Hyperoliidae). The Natal leaf-folding frog (*A. spinifrons*) was also not found during the study. This species would be found to the south of the study area (Umlalazi Nature Reserve and St. Lucia), inhabiting dense sedge beds and inundated grassy areas with still waters.

As mentioned above, numerous factors contributed to not recording all the possible species. These factors combined with technical difficulties, weather conditions or possible personal errors should be kept in mind for future studies. In spite of these problems, this method proved to be an effective sampling tool for conducting anuran diversity monitoring studies.

The purpose of this chapter was to document the breeding and calling activity through the use of automated recorders as a passive long-term approach. A total of 54% (29/54) of expected species were recorded during this study based on call data. However, this may have been a result of to the lack of rain during the study, with most of the sites experiencing a severe drought, with the result that anuran breeding activity was reduced, and many species likely not being detected. The rainfall patterns recorded during the study were not typical of this area. Future studies should include population occupancy and environmental data. This will help scientists to determine whether environmental variations play a role in anuran breeding activities. Studies should also be done to compare data gathered from protected areas with non-protected areas based on anuran breeding activities.

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CHAPTER 3: ENVIRONMENTAL ASPECTS RELATED TO FROG CALLING BEHAVIOUR IN THE UMLALAZI NATURE RESERVE, KWAZULU-NATAL

At the edge of night the sun pulls down
it's soothing shade and peepers creeping
from leafy covers Tune up to sing.

Who will start this evening's song with fluted notes
that serenade the nights?

Someone begins, the same song his ancestors sang,
and the forest fills with an urgent chorus.

It must be high honor, if you're a frog,
to sing the evening's solo
so others will get it right.

David L Harrison
Songs of the tree frogs
Wild Country
(1999)

3.1 Abstract

The validity of bioacoustic approaches for documenting environmental change has been proven increasingly over recent years. Climate change causes modified meteorological variables, resulting in seasonal changes. These meteorological changes do not only act as cues for anuran breeding activity, but also affect the physical properties of sound propagation. Anuran adaptations to facilitate meteorological change play a fundamental role in monitoring surveys, research and management strategies aimed at these animals. Despite the potential importance of its role, research to determine the relationship between these meteorological variables and the calling activity of anurans has been limited to only a few South African species. In the present study, the effects of meteorological conditions on the calling activity of anurans in a northern section of the Umlalazi Nature Reserve, KwaZulu-Natal, were documented. During the study, 14 vocally-active species were recorded. Results may have been affected by severe droughts experienced in South Africa due to the El Niño Southern Oscillation during the survey period. A conclusion was reached that most of the species found in the Umlalazi Nature Reserve react to changes in the relative humidity and rainfall patterns.

Keywords: *bioacoustics; climate change; meteorological variables; anuran monitoring; Umlalazi Nature Reserve; El Niño; breeding behaviour*

3.2 Introduction

The popularity of bioacoustics in the detection and monitoring of environmental change has increased in recent years (see Oseen & Wassersug, 2002; Minter, 2011; Yetman & Ferguson, 2011; Lemckert *et al*., 2013). These studies found interesting correlations between the physical natural environment and the species composition of ecological communities. Saenz *et al.* (2006) note that both biotic and abiotic factors influence anuran community composition, and found that abiotic factors have a stronger influence on anuran breeding activity. Anuran calling behaviour certainly can be correlated with different meteorological and environmental variables (Steelman & Dorcas, 2010; Lemckert *et al*., 2013; Ospina *et al.*, 2013). These variables do not only act as cues for breeding seasons (Moreira *et al*., 2007), but also have effects on the physical properties of individual vocalisation (Wiley & Richards, 1978). Environmental variables can also act synergistically as behavioural cues and certain anurans may act differently in response to different combinations of environmental conditions each year, depending on their geographical range (Oseen & Wassersug, 2002).

The most important meteorological influences are temperature and rainfall (Oseen & Wassersug, 2002), and these have attracted increasing interest in terms of anuran behavioural studies (Telford & Dyson, 1990; Steelman & Dorcas, 2010; Lemckert *et al*., 2013; Ospina *et al*., 2013). According to Wells *et al*. (1996), anuran breeding activities, show a linear correlation with increases in temperature. However, Navas (1996) found that anurans that inhabit high tropical elevations are more vocally active at lower temperatures than those at lower elevations. Conversely, temperate zone anurans will inhibit breeding or migratory activity at lower temperatures, and may cease such behaviour altogether (Oseen & Wassersug, 2002). Moreover, anurans that experience high levels of dehydration have been found to reduce calling activity at high temperatures (Pough *et al*., 1983; Ospina *et al*., 2013). According to Oseen and Wassersug (2002), other atmospheric conditions will become more significant when the temperature is above the required threshold of the species. Such conditions include a decrease in barometric air pressure, precipitation patterns, relative humidity and the wind velocity.

Reduction in barometric pressure precedes weather changes, including rain or snow (Oseen & Wassersug, 2002). Due to the semi-permeable nature of anurans' skin, they are prone to dehydrate easily: anurans need moisture. Rainfall therefore influences anuran behaviour, since it acts as a cue for breeding, effecting the calling behaviour of anurans (Blankenhorn, 1972; Henzi *et al*., 1995; Zina & Haddad, 2005). In some species, rain is also seen as an important cue for migration (Oseen & Wassersug, 2002). Heavy rain furthermore produces temporary pools needed for anuran spawning and larvae development (Telford & Dyson, 1990).

Humidity is seen as another important factor that limits anuran activities (Cree, 1989).When humidity is high the rate of evaporation is low, thereby lowering the risk of dehydration.When it is low, anurans that are at risk of dehydration tend to inhibit their calling activity (Pough *et al*., 1983). High relative humidity also aids in call transmission, as sound will travel easier through the humid air (Oseen & Wassersug, 2002). Moreover, wind has an adverse effect on the calling behaviour of anurans (Larom *et al*., 1997; Waxler, 2004). Increasing wind speeds increase evaporation rate, which negatively affect anurans (Henzi *et al*., 1995). From a vocal point of view, wind tends to interfere, distort and affect the directionality of the sound and calling area, requiring greater efforts from anurans to call effectively (Larom *et al.*, 1997; Steelman & Dorcas, 2010).

Climate change modifies daily meteorological variables (such as temperature increases, rainfall fluctuations, and so on), leading to unusual seasonal changes (Krause & Farina, 2016) which, in their turn, give rise to long-term environmental changes, causing irreversible habitat transformation, rapid species extinction, spread of diseases and community assemblage changes (Blaustein *et al*., 2010; Krause & Farina, 2016). As a result, studies that examine the effects of climate change on animals, especially on anurans, have increased in recent years (Minter *et al.,* 2004; Corn, 2005; Pounds *et al.,* 2006; Alford *et al.*, 2007; Blaustein *et al.*, 2010; Minter, 2011). It has been predicted that South Africa will experience higher temperatures and a decrease in summer rainfall of up to 5% in the northern and 25% in the southern parts of the country and that, as a as a consequence of this and over time, the biomes of these regions will be reduced by 35% to 55% of their present extent (Kruger, 2014). Another prediction made by Turpie (2003) states that in 50 years the country's biomes will have shrunk, with half of the current vegetation replaced by more arid vegetation types. This may have multiple effects on anurans as they rely on seasonal precipitation for breeding (Corn, 2005; Minter, 2011). For instance, the dynamics of species could be changed across landscapes, leading to shifts in the ranges of entire assemblages (Blaustein *et al*., 2010). In addition, climate change could cause changes in breeding behaviour, resulting in a shift in the onset of breeding (Gibbs & Breisch, 2001).

Information on these effects on anuran activity plays a fundamental role in anuran monitoring surveys, research and management (Steelman & Dorcas 2010; Lemckert, 2013). Consideration of this information could help improve the accuracy of calling surveys to ensure effective predictive modelling, most importantly for threatened taxa (Weir & Mossman, 2005; Steelman & Dorcas, 2010, Yetman & Ferguson, 2011). This would increase the probability of detecting vocally-active species based on temporal or seasonal activity (Steelman & Dorcas, 2010).

One infers that the long-term effect that atmospheric conditions such as temperature and humidity have on anurans, therefore amounts to an important aspect of anuran behavioural studies. Despite the importance of such studies, few studies have been conducted to determine the relationship between these environmental variables and the calling activity of anuran males of southern African species (see Henzi *et al*., 1995; Yetman & Ferguson, 2011). In the present study the effects of environmental variation on the calling activity of anurans in a northern KwaZulu-Natal reserve was investigated to determine the ideal environmental conditions that may act as cues to commence breeding activity.

3.3 Materials and methods

3.3.1 Site selection

This study was conducted over a period of one year from November 2015 to November 2016 at an ephemeral wetland in the northern section of the Umlalazi Nature Reserve, KwaZulu-Natal (-28.95540 S; 31.76607 E) (Figure 3.1). The Umlalazi Nature Reserve is situated one kilometre northeast of the town of Mtunzini in northern KwaZulu-Natal at an altitude of less than 100 masl. The 1 028 ha reserve was proclaimed in 1948, and forms part of the Maputaland-Pondoland-Albany Hotspot area. It falls within Indian Ocean Coastal Belt Biome, a biome described in Nevill & Nevill, 1995, Mucina & Rutherford, 2006 and Traynor, 2008. The Umlalazi River forms the northern border of the reserve and flows eastwards to the Indian Ocean (Nevill & Nevill, 1995).

This subtropical region has an annual rainfall of approximately 1 000 mm. Summers are hot and humid, usually exceeding a relative humidity of 80%. Winters are mild with a relative humidity between 50% and 60% (Nevill & Nevill, 1995; Zungu *et al*., 2018). Prevailing winds alternate over the course of the year between a north-easterly and south-westerly direction (Todd, 1994).

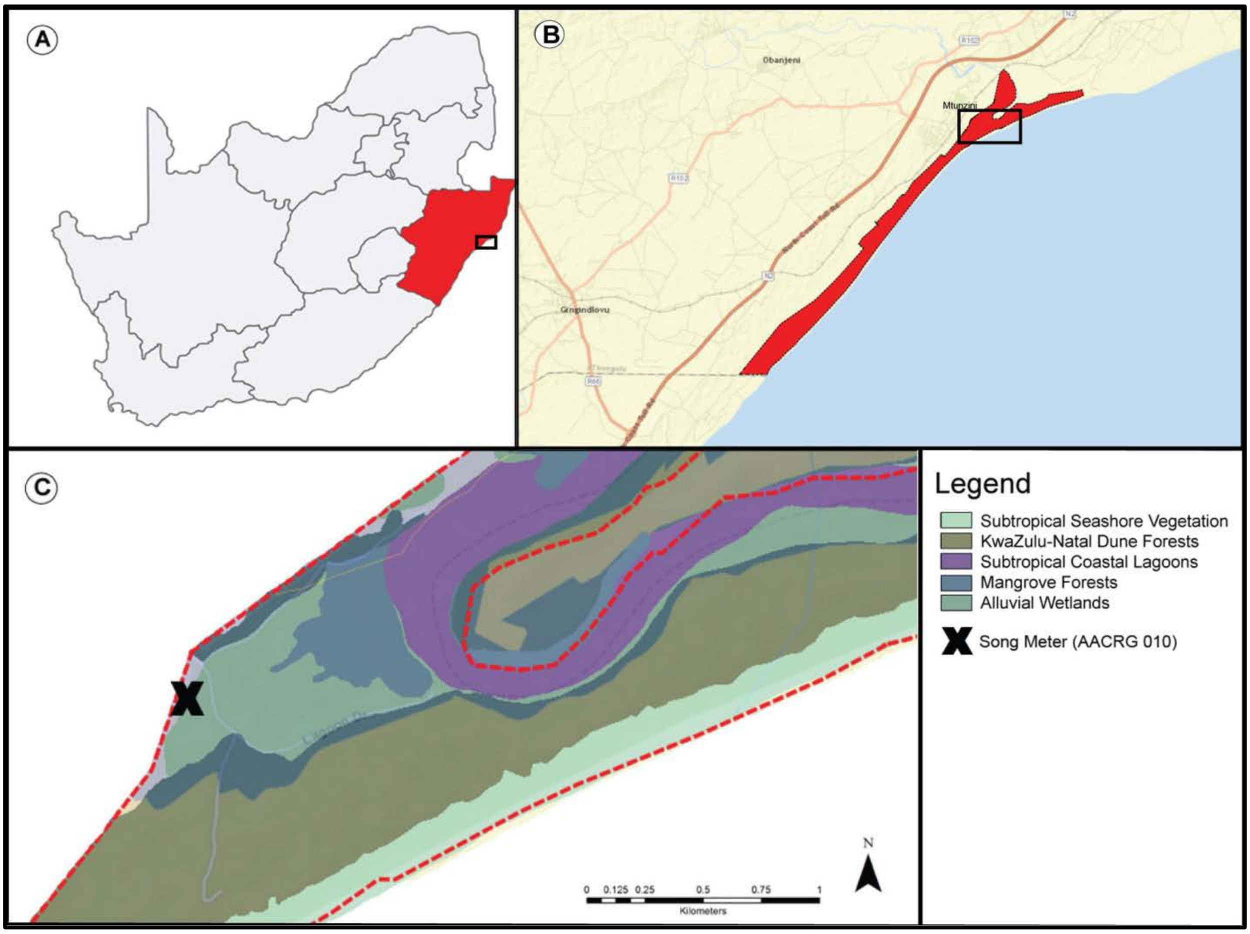

Figure 3.1: Map of the study location (red demarcation). **A:** Map of South Africa, indicating KwaZulu-Natal. **B:** Map of the Umlalazi Nature Reserve. **C:** Map showing the location of the Song Meter within the ephemeral wetland area (adapted from Mucina & Rutherford, 2006)

The microhabitat of the location (-28.95540 S; 31.76607 E) can be described as an alluvial wetland forming a palustrine and terrestrial system. Common reeds (*Phragmites australis*) dominate the wetland and a dune forest surrounds it. This locality was selected since it was close to a wetland, easily accessible and in a secure location. Another reason for its selection was to maximise species diversity by covering both palustrine and terrestrial systems. This location was also identified as a site where *Hyperolius pickersgilli* occurs (Minter *et al*., 2004; Tarrant & Armstrong, 2013).

3.3.2 Meteorological data

Climatic data from November 2015 to November 2016 was obtained from the South African Weather Service's weather station in Mtunzini (Climate Number: 0304357 6: -28.9470 S; 31.7070 E), approximately 6 km north-west of the study location (SAWS, 2017). These data comprised mean temperature (ºC), precipitation (mm), relative humidity (%), barometric pressure (hPa) and wind speed (km/h). Moon illumination data was obtained as a percentage calculated at lunar noon for each night from Time and Date AS (see Thorsen, 2017).

3.3.3 Passive acoustic monitoring equipment

An automated third-generation Song Meter (SM3) was set to record 10 minutes per hour between the hours of 18:00 and 07:00 at the edge of the ephemeral wetland. The recorder was fitted with a solar panel connected to a 12V rechargeable lead-acid battery to ensure long-term powering. Audio data was collected in stereo, 32-bit WAV format at a sampling rate of 16 000 Hz with no dB gain set.

3.3.4 Acoustic analysis

Song Scope analysis software (version 4.1.5: Song Scope™ Wildlife Acoustics Inc., Concord, Massachusetts, USA) was used to score calling intensities, making use of the amphibian calling index (ACI). A digital low-pass frequency filter of 7.4 kHz and a high-pass filter of 300 Hz with a 1-second background filter were set. The Fast Fourier Transform (FFT) size was 256 with a ½ FFT overlap and a Hamming-window.

3.3.5 Statistical analysis

A biplot diagram was created reflecting principal component analysis (PCA) of 7 environmental variables and the calling intensities of 14 species. PCA was used as a method to summarise, in a low-dimensional space, the variance in a multivariate scatter of points of species and environmental data. In doing so, this analysis provided an overview of linear relationships between these variables. Arrows at the end of each ordination axis point in the direction of the species gradient as identified by the explanatory variables. The longer the arrows in each ordination axis, the stronger the explanatory variables are correlated with the ordination axes, in contrast to those with short arrows. Thus the arrow length indicates the degree of correlation between calling intensity and the variable. The ordination analysis was conducted in CANOCO (version 4.5). Descriptive statistics were calculated using IBM SPSS 25 (IBM Corp., 2017).

3.4 Results

3.4.1 Anuran diversity

During the study period (21 November 2015 to 14 November 2016), 14 anuran species comprising 6 families and 8 genera were recorded at the ephemeral wetland in Umlalazi Nature Reserve using an SM3 recorder (Table 3.1).

Table 3.1: Recorded anuran species and family using a Song Meter at the Umlalazi Nature Reserve

	Family	Species
1	Arthroleptidae	*Arthroleptis wahlbergii*
2		*Leptopelis mossambicus*
3		*Leptopelis natalensis*
4	Brevicipitidae	*Breviceps sopranus*
5	Bufonidae	*Sclerophrys gutturalis*
6	Hemisotidae	*Hemisus guttatus*
7		*Hemisus marmoratus*
8	Hyperoliidae	*Afrixalus delicatus*
9		*Afrixalus fornasini*
10		*Hyperolius marmoratus*
11		*Hyperolius pickersgilli*
12		*Hyperolius tuberilinguis*
13	Ptychadenidae	*Ptychadena anchietae*
14		*Ptychadena mossambica*

3.4.2 Monthly anuran breeding activity

Figure 3.2 illustrates the mean calling activity of all the recorded anuran species from November 2015 to November 2016 at Umlalazi Nature Reserve. At the start of the study period, as has been mentioned, below average rainfall was recorded in KwaZulu-Natal, especially in the northern parts. It is clear that the anurans were affected by the precipitation and temperature patterns.

Arthroleptis wahlbergii called most frequently, calling during each month. Species with low intensities included *P. mossambica*, *B. sopranus* and *A. fornasini*.

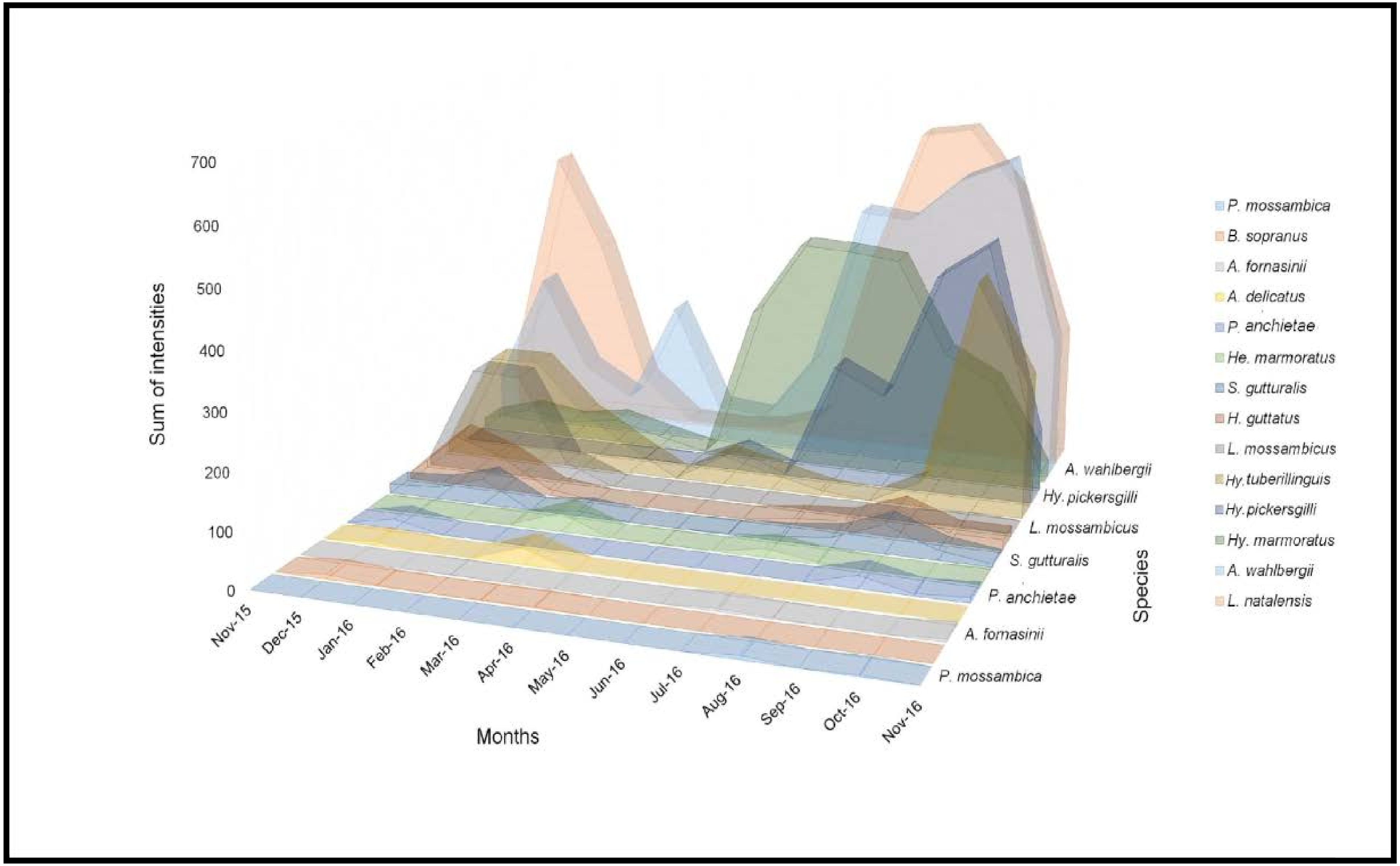

Figure 3.2: The annual calling intensities of anuran species during the study, at an ephemeral wetland within the Umlalazi Nature Reserve, KwaZulu-Natal

3.4.3 Environmental effects

Table 3.2 reflects the preferred mean environmental conditions for each recorded species. The environmental conditions were correlated with the calling intensities of each night. *Breviceps sopranus*, *He. marmoratus*, *A. delicatus*, *A. fornasini*, *P. anchietae* and *P. mossambica* scored below three (3), indicating that no more than 3 individuals were calling, while their calling was also infrequent.

Table 3.2: Mean ($\bar{X}$) and standard deviation (± SD) of prefered environmental conditions measured during active calling for the species detected in the survey

	Temperature (°C)	Humidity (%)	Rain (mm)	Wind (km/h)	Pressure (hPa)	Moon (%)
Arthroleptis wahlbergii	20,4 ± **2,84**	88,2 ± **7,19**	1,42 ± **2,03**	32,76 ± **20,61**	1015,2 ± **5,38**	53,03% ± **0,38**
Leptopelis mossambicus	23,33 ± **1,78**	89,48 ± **8,04**	2,17 ± **3.00**	20,53 ± **12,61**	1010,81 ± **5,14**	26,74% ± **0,29**
Leptopelis natalensis	21,5 ± **2,69**	83,5 ± **10,35**	1,52 ± **2,97**	30,2 ± **19,43**	1013,2 ± **5,26**	42,22% ± **0,36**
****Breviceps sopranus***	25,5 ± **3,44**	75,8 ± **15,46**	0,00 ± **0**	26 ± **6,83**	1009,7 ± **5,20**	60,50% ± **0,21**
Sclerophrys gutturalis	21,59 ± **2,24**	83,78 ± **9,60**	2.80 ± **3,39**	30 ± **14,47**	1014,92 ± **2,34**	67,44% ± **0,40**
Hemisus guttatus	23,20 ± **2,63**	89,54 ± **5,08**	0,84 ± **0,43**	35,00 ± **9,20**	1013,05 ± **5,38**	30,17% ± **0,34**
****Hemisus marmoratus***	18,51 ± **3,94**	93,00 ± **1,41**	0,10 ± **0,14**	22,50 ± **31,82**	1022,30 ± **9,19**	73,05% ± **0,38**
****Afrixalus delicatus***	23,24 ± **2,22**	83,50 ± **9,37**	0,96 ± **0,50**	18,23 ± **16,54**	1010,46 ± **4,24**	71,44% ± **0,32**
****Afrixalus fornasini***	19,07 ± **4,74**	81,60 ± **16,32**	0,70 ± **0,14**	32,75 ± **20,31**	1012,45 ± **9,96**	75,06% ± **0,19**
Hyperolius marmoratus	17,08 ± **4,25**	84,03 ± **11,50**	2.12 ± **3.52**	16,09 ± **12,60**	1017,08 ± **5,85**	41,51% ± **0,34**
Hyperolius pickersgilli	18,28 ± **3,85**	89,85 ± **5,91**	0,60 ± **0,58**	14,58 ± **14,38**	1012,67 ± **4,62**	35,50% ± **0,29**
Hyperolius tuberilinguis	22,16 ± **2,38**	86,04 ± **7,46**	1,11 ± **1.37**	26,68 ± **14,68**	1011,72 ± **4,83**	49,57% ± **0,35**
****Ptychadena anchietae***	20,40 ± **3,21**	84,10 ± **5,47**	1,12 ± **0,77**	27,46 ± **16,01**	1011,63 ± **5,16**	40,36% ± **0,26**
****Ptychadena mossambica***	15,57 ± **4,07**	90,25 ± **1,50**	5,00 ± **0,85**	21,75 ± **2,63**	1008,95 ± **2,03**	64,07% ± **0,30**
**Species that received a score of less than 3.*						

3.4.3.1 Temperature levels

Based on average miminum temperatures, the coldest months were June 2016 (15.77ºC) and July 2016 (14.84ºC). The hottest months were December 2015 to March 2016 with a monthly average of 24.67ºC. Most (64%) of the recorded species preferred to call above 20ºC ambient temperatures (Table 3.2). Species calling below 20ºC included *He. marmoratus*, *A. fornasini*, *Hy. marmoratus*, *H. pickersgilli* and *P. mossambica*.

3.4.3.2 Precipitation

As mentioned, below-normal rainfall was noted due to the El Niño Southern Oscillation that South Africa experienced. The monthly precipitation ranged from the highest in July 2016 (135.2 mm) to the lowest in August 2016 (12.26 mm) (Table 3.3 & Figure 3.3). A total of 11 (79%) of the recorded species called during December 2015, while rainfall over this period amounted to 72.16 mm (see Figure 3.3), excluding *A. fornasini*, *H. pickersgilli* and *P. mossambica*. *Ptychadena mossambica* prefers high precipitation (5.00 mm), calling only during August 2016 and October 2016. *Breviceps sopranus* called during nights with no rainfall (Table 3.3).

3.4.3.3 Relative humidity

For unknown reasons no humidity data were obtained on 29 February 2016 and from 5 October to the end of the study period of 14 November 2016. All the species, except *B. sopranus*, prefer to call at high relative humidity levels (above 80%). Only two months, November 2015 and February 2016, had an average relative humidity below 80% during the observation period (Table 3.2).

3.4.3.4 Wind speed

No wind speed data was obtained for 26 to 27 February 2016 and 5 to 18 October 2016. Wind speed was taken into account, but not wind direction. Greatest wind speed (33.59 km/h) were recorded in February 2016 and least speed (17.11 km/h) during April 2016. Most (79%) of the recorded species preferred to call at wind speeds above 20 km/h. Only *A. delicatus*, *Hy. marmoratus* and *H. pickersgilli* preferred to call in less windy nights (Table 3.2).

3.4.3.5 Barometric pressure

No air pressure data was obtained from 26 to 28 February 2016. Most (89%) of the recorded species, except *B. sopranus* and *P. mossambica*, preferred an air pressure above 1 010 hPa (Table 3.2). Consider in this regard that the elevation of an area is indirectly proportional to barometric pressure. This could explain the high barometric pressures noted during the study since the altitude of the Umlalazi Nature Reserve ranges from 0 to 30 m.a.s.l.

3.4.3.6 Moon illumination

Moon illumination was measured as a percentage, while the percentage (%) of cloud cover was not part of the calculation. Half of the recorded species preferred to call when moon illumination was above 50% (Table 3.2).

Table 3.3: Average monthly measurements of five environmental conditions, excluding percentage moon illumination for each month during the study

Month	Temperature (°C)	Humidity (%)	Rain (mm)	Wind (km/h)	Pressure (hPa)
November 2015	21,62	78,21	69.99	31,63	1012,56
December 2015	24,82	83,45	72.16	30,15	1010,71
January 2016	24,97	81,46	70,86	23,51	1012,22
February 2016	24,82	78,88	75,6	33,59	1010,33
March 2016	24,08	84,73	79,79	24,31	1010,79
April 2016	22,13	84,54	73,42	23,59	1014,49
May 2016	18,35	86,74	69,36	17,11	1019,10
June 2016	15,77	83,57	30,28	19,06	1018,78
July 2016	14,84	85,71	135,2	20,33	1019,24
August 2016	16,64	81,43	12,29	22,54	1017,94
September 2016	19,67	85,85	123,64	24,06	1015,03
October 2016	20,42	88,88	97,59	21,77	1013,29
November 2016	22,22	n/a	99,4	31,61	1012,43

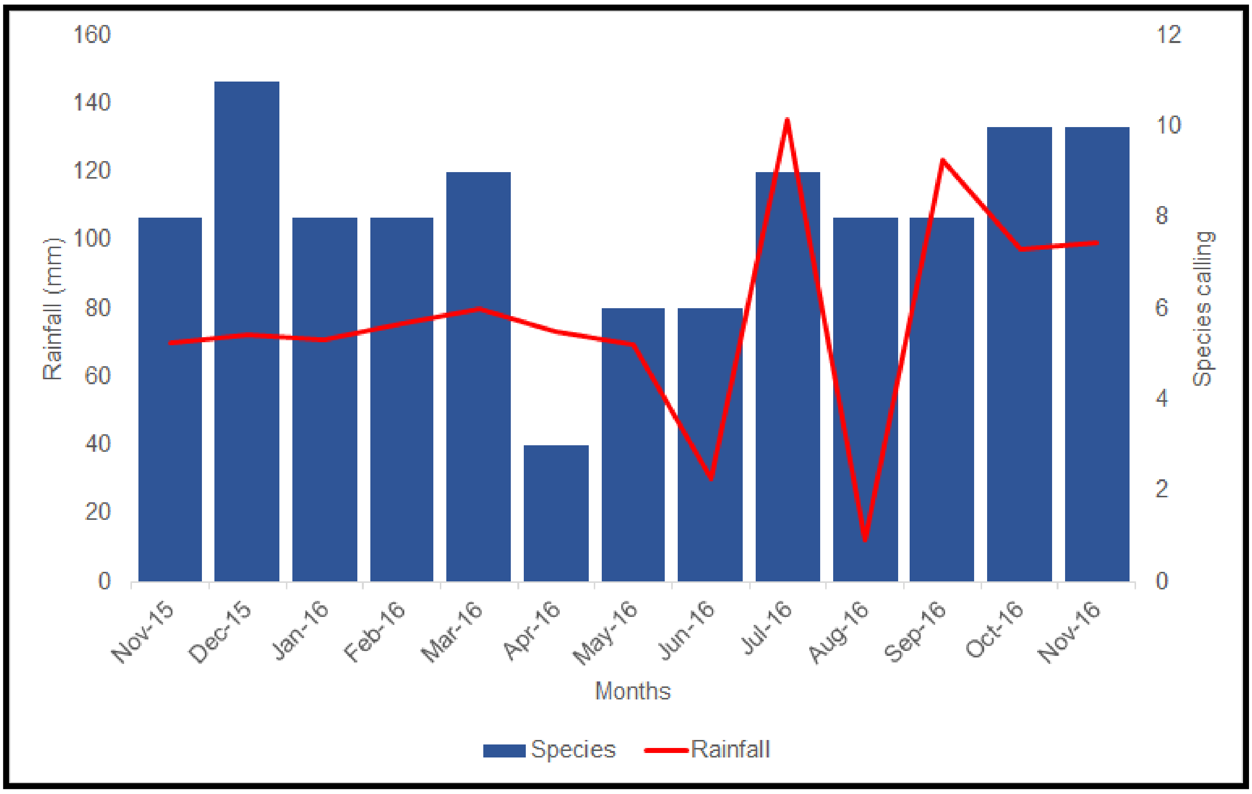

Figure 3.3: Summary of the total rainfall and number of species calling during the study period

The PCA ordination of the horizontal axis (Axis 1) showed a 12.8% explanation (eigenvalue = 0.128) of the variation in all the variables (Figure 3.4). Axis 1 shows a strong gradient between the relative humidity (negative values), rain and barometric pressure (positive values), and a negative correlation with time and moon illumination (negative values). The vertical axis (Axis 2) reflects a 9.5% (eigenvalue =0.095) variance. This axis represents a correlation between temperature and wind speed (negative values), and a negative correlation to rain and barometric pressure (positive values).

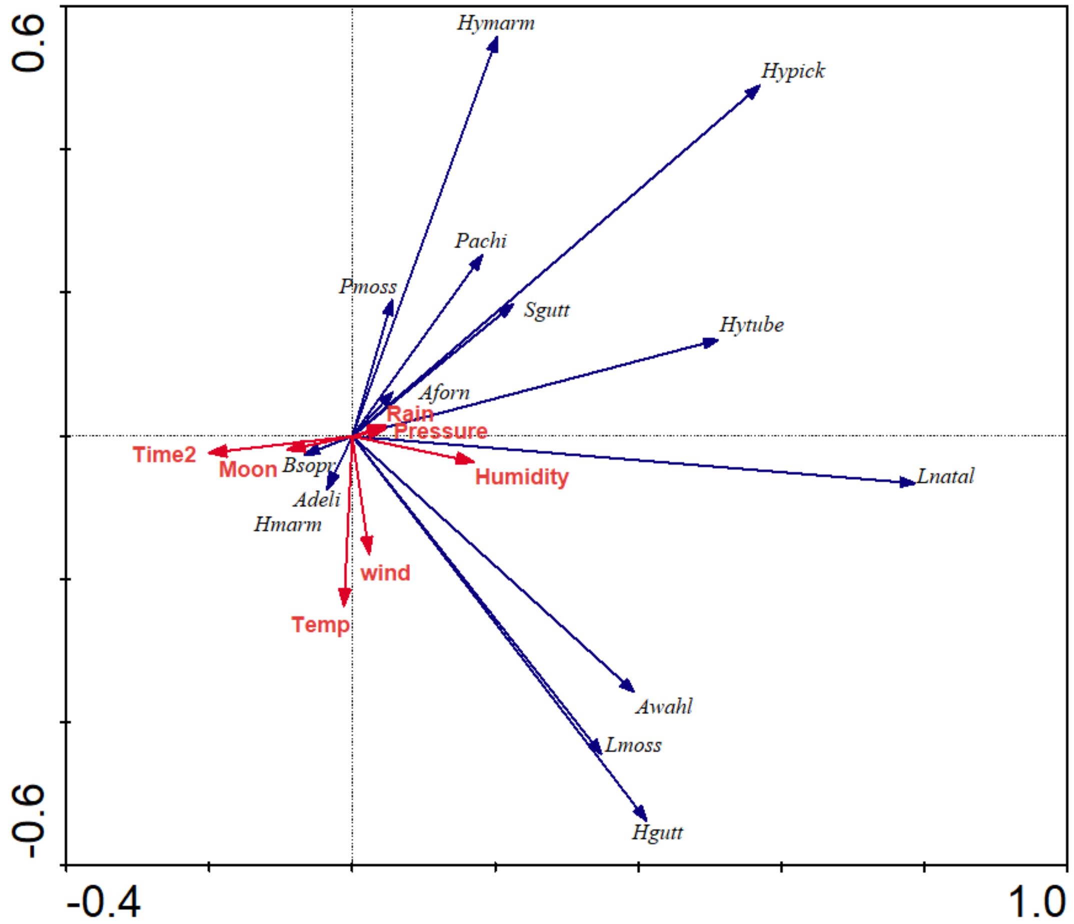

Figure 3.4: A biplot showing a principal component analysis (PCA) of seven environmental variables (wind speed, km/h; ambient temperature, °C; percentage of moon illumination, %; barometric pressure, hPa; relative humidity, %, rainfall, mm and time, hh:mm) and calling intensity of 14 species (*Arthroleptis wahlbergii*, **Awahl**; *Leptopelis mossambicus*, **Lmoss**; *Leptopelis natalensis*, **Lnatal**; *Breviceps sopranus*, **Bsopr**; *Sclerophrys gutturalis*, **Sgutt**; *Hemisus guttatus*, **Hgutt**; *Hemisus marmoratus*, **Hmarm**; *Afrixalus delicatus*, **Adeli**; *Afrixalus fornasini*, **Aforn**; *Hyperolius marmoratus*, **Hymarm**; *Hyperolius pickersgilli*, **Hypick**; *Hyperolius tuberilinguis*, **Hytube**; *Ptychadena anchietae*, **Panchi**; and *Ptychadena mossambica*, **Pmoss**)

Most (11/14) species were shown to have a correlation with relative humidity, rainfall and barometric pressure. Species with positive values include *P. mossambica, P. anchietae, Hy. marmoratus, H. pickersgilli, H. tuberilinguis, S. gutturalis* and *A. fornasini*. Species with negative values include *L. mossambicus, L. natalensis, A. wahlbergii* and *H. guttatus* (see Axis 1). A negative correlation in terms of rainfall and barometric pressure was noted for *B. sopranus*, *He. marmoratus* and *A. delicatus* (Figure 3.4).

3.5 Discussion

As mentioned, KwaZulu-Natal is known for its mesic atmospheric conditions and sub-tropical environment. This creates ideal microhabitats for anurans. Due to the ectothermic nature of anurans, they mostly depend on moisture and temperature for behavioural cues (Du Preez & Carruthers, 2017). Ideal conditions act as cues for certain events including breeding, migration and foraging (Oseen & Wassersug 2002).

The Umlalazi Nature Reserve, where the study was conducted, is situated in the northern parts of KwaZulu-Natal. The Song Meter recorded a total of 14 species within the alluvial wetland on the floodplains of the Umlalazi River. The alluvial wetland is dominated by specialised terrestrial and hydrophtic vegetation such as common reeds (*Phragmites australis*), sedges (*Juncus kraussii),* buffalo grass (*Stenotaphrum secundatum*) and a variety of trees (see Zungu *et al.*, 2018). This type of vegetation is typically found in palustrine systems, which form the breeding ground for most of the South African anurans (Du Preez & Carruthers, 2017). This may explain the relatively high number of species calling during the observations of the present study (14 species). The recorded species were terrestrial (that is, species in Arthroleptidae, Brevicipitidae, Bufonidae and Hemisotidae), arboreal (Hyperoliidae) and semi-aquatic anurans (Ptychadenidae).

Anuran diversity was estimated by the number of calling males present and chorus intensity, using recordings made on an SM3 recorder. These estimates were used to evaluate the effects of variations in temperature, rainfall, relative humidity, air pressure, wind speed and moon illumination. The relationship was between the calling activity of species and the six variables to determine the ideal calling environment for specific species. The relationships found were species-specific and differed among the species. The results show that the calling activity for Umlalazi species differs in their annual calling behaviour. This could be caused by a variety of factors; however, only six environmental factors are considered in the current study, the results of which highlight the importance of changing environmental conditions and the effects these have on the breeding behaviour of anuran species.

3.5.1 Temperature

It is well known that the relative external temperature of the environment affects anuran behaviour. Mainly, the microhabitat is defined by and linked to atmospheric conditions, soil type and vegetation (Van Dijk, 1971; Gayou, 1984). Du Preez and Carruthers (2017) note that South African frogs are more active when temperate temperatures between 20ºC and 30ºC prevail. In the present study, a total of 64% of the species called at temperatures above 20ºC. These results contradict Du Preez’s and Carruthers’statement, because the remaining 46% of the species were recorded at temperatures lower than 20ºC. Nevertheless, the species calling below 20ºC had very high standard deviations (> 3.8), which renders the data inconclusive. Multiple studies on anuran behaviour and temperature do in fact correlate positively with their calling behaviour.

Wells *et al*. (1996) found that the Spring Peeper (*Pseudacris crucifer*) increases its calling behaviour as the temperature rises. Furthermore, Ospina *et al*. (2013) studied the effect of temperature on the calling intensities of four *Eleutherodactylus* species from Puerto Rico. The results showed that the common coqui (*Eleutherodactylus coqui*) and the whistling coqui (*E. cochranae*) both responded positively to increased temperatures, whereas the smaller grass coqui (*E. brittoni*) and the critically endangered plains coqui (*E. juanariveroi*) responded negatively. Recuction in the risk of dehydration explains these behaviours. Smaller species are prone to lose more water when temperatures are high (Van Berkum *et al*., 1982; Tracy *et al*., 2010; Ospina *et al*., 2013). The present study found that 60% of the Hyperoliidae species favoured lower temperatures (below 20ºC) for calling. This could be due to the size of some of these species. The recorded male Hyperoliidae species consist of small to medium-sized species (less than 33 mm), including *A. fornasini*, *Hy. marmoratus* and *H. pickersgilli* (Frost, 2018). This indicates that the size of the species appears to be an important determinant when it comes to the effect of temperature on calling intensity.

However, the activity would only correlate with the temperature within the threshold of the given species (Reading, 1998). At high temperatures (> 30ºC), no species called. Again, anurans most likely reduce calling activity at these high temperatures to lessen the risk of desiccation (Pough *et al*., 1983; Ospina *et al*., 2013). Once the threshold has been crossed, the species develops specific adaptions to ease the effects of high or low temperatures, as demonstrated by a Fleischack and Small (1978), where it was found that the bubbling kasina (*Kassina senegalensis*) would reduce its calling activity during dry periods if air temperatures exceeded 40ºC. Some terrestrial anurans within the family Arthroleptidae and Brevicipitidae will make use of microhabitats within the soil (holes, tunnels or natural crevices), either made by themselves or made by other animals, to reduce water loss that result from high temperatures (Loveridge, 1976). Similarly, *Arthroleptis* species make use of burrows formed by other animals in order to cool down (Loveridge, 1976). *Leptopelis* species have strong metatarsal tubercles, making them effective burrowers (Laurent, 1964). Species of *Leptopelis* are known to burrow during the day and return to trees at night. This could be to avoid high daily temperatures. Furthermore, genera that dig their own burrows include *Breviceps*, *Pyxicephalus* and *Hemisus*, and so forth. Some of these burrowing species (for instance, *Pyxicephalus* and some *Leptopelis* species), form a keratinised cocoon on the stratum corneum (outer layer of the skin) by means of multiple moulting, and this helps to prevent water loss (Loveridge, 1976).

In another kind of temperature adaptation, the arboreal southern foam nest frog (*Chiromantis xerampelina*) uses pigmentation while basking in the direct sunlight, turning their skin chalky white once temperatures exceed 30ºC (Kaul & Shoemaker, 1989).In the process it secretes mucus as it moves. Kaul and Shoemaker (1989) found this process to be similar to mammalian sweating. These anurans will sit in a resting position after the mucus has covered the body and dried. The dry mucus acts as a thin polish-like cutaneous layer hindering water loss (Geise & Linsenmair, 1986). Some reed frogs (such as *Hyperolius marmoratus* and *H. nasutus*) possess a similar mechanism in order to cool down (Withers *et al.*, 1981; Passmore & Malherbe, 1985).

3.5.2 Precipitation

As has been indicated, for the duration of the present study, rainfall was lower than average. This was due to the El Niño Southern Oscillation phenomenon that caused severe droughts all over South Africa, especially in KwaZulu-Natal. During July 2016, however, above normal rainfall was recorded. This caused a spike in anuran activity. This could be explained by the fact that low rainfall was recorded during the preceding months. The rainfall in July 2016 possibly resulted in the formation of several temporary water pools, allowing anurans to breed. It is presumed that these pools kept water for the remainder of the study period.

Anurans will use ponds created by heavy rains as breeding sites (Oseen & Wassersug, 2002). Moist conditions probably reduce the risk of desiccation and aid in migration and calling (Lemckert *et al*., 2013). Henzi *et al*. (1995) made similar findings with regard to *Hy. marmoratus* males, showing high chorus attendance after rainfall but a decrease during rainfall itself (Henzi *et al*., 1995). Similar behaviour was observed by Minter (1995) during the breeding season of the Bushveld rain frog (*Breviceps adspersus*) *after* rain but not *during* rainfall.

The frog species at Umlalazi showed similar responses to rain (see Table 3.2). When it rained, low chorus intensities occurred. However, some individuals did call during low rainfall periods, on average after showers of less than 3 mm. In a similar vein, Marsh (2000) found that the effect of rainfall differed between wet and dry years when it came to the activity of Tungara frogs (*Engystomops pustulosus*). These frogs were more active during the rainy nights of dry years in contrast to wetter years when activity decreased. The current study appears to confirm these findings. For instance, PCA analysis showed that calling of *B. sopranus*, *A. delalandi* and *He. marmoratus* correlated negatively with rainfall itself. This suggests that these species reduce their calling activity during rain. Another factor to bear in mind is that rainfall causes noise interference on the recordings, and that calls were masked by the sound of leaves and raindrops falling on the recorder.

Rain is an important variable for the migration patterns of some anurans (especially for semi- or aquatic anurans). Nonetheless, once individuals reach the breeding site, rain becomes less important (Oseen & Wassersug, 2002). This could possibly explain why anurans continued calling after the July rains in the case of the present study.

3.5.3 Humidity

Relative humidity is the percentage of water vapour in the air relative to the saturation level, and is affected by the ambient temperature (Arundel *et al*., 1986). Higher temperatures will cause more water evaporation and condensation, resulting in increasing relative humidity. However, Arundel *et al*. (1986) note that relative humidity has a direct effect on the temperature. With high relative humidity levels, the rate of water evaporation decreases (Arundel *et al*., 1986). In an early study, Adolf (1932) found that anurans have indirectly proportional behaviour with regard to evaporate water loss rates and relative humidity. Anurans will, therefore, most likely benefit from higher relative humidity levels due to their semi-permeable skin.In the present study, high humidity levels wereobserved in terms of calling behaviours. All the anurans preferred to call at a humidity level above 80%, except for *B. sopranus*, which only called during December 2015. However, the prefered relative humidity was close to 80% (75, 8%) for this species. A positive correlation between humidity and calling intensities of most anurans was noted during the study. This was expected, as many anurans rely on moist environments (Oseen & Wassersug, 2002).

Although aurans probably favour high humidity in order to reduce the risk of desiccation, another aspect that may explain their preference may be that sound travels further because low transition interference occurs in moist environments (Harris, 1966; Oseen & Wassersug, 2002). The sound attenuation is higher in dry air, due to greater air density. In dry conditions, anurans often retreat to moist shelters (Wells, 2007), as mentioned. Terrestrial anurans (families Arthroleptidae and Bufonidae) will find shelter under leaf litter and bark, and so forth (Toft, 1980; Lynch & Myers, 1983). In more rocky environments, humidity tends to be higher. Channing (1976) found that the southern pygmy toad, *Poyntonophrynus vertebralis,* and the marbled rubber frog, *Phrynomantis annectens*, prefer to live under granite rocks in southern Africa. These species use the humid environment of rock crevices as shelter (Wells, 2007). Species such as *Arthroleptis wahlbergii* and *Sclerophrys gutturalis* will most probably exhibit this behaviour when the humidity is below 80% or during the day when the humidity is low.

Other terrestrial anurans (family: Brevicipitidae, Bufonidae and Hemisotidae) construct burrows for shelter as they are more humid (Denton & Beebee, 1993). According to Wells (2007), this adaptation is probably most common among anurans inhabiting dry areas. These burrowing species prefer soils with higher water retention capacities, especially during dry seasons (Mayhew, 1965; Heatwole *et al*., 1971). Toads (such as *Sclerophrys gutturalis*) typically dig a shallow burrow to escape warm or dry conditions (Wells, 2007). The

North American Tree Frog, *Hyla gratiosa*, shows similar adaptions in dry conditions (Neill, 1952). Interestingly, *Agalychnis dacnicolor* and *Hyla gratiosa* seek shelter in rodent burrows, due to the low humidity within these environments (Lee, 1968; Wiewandt, 1971). *Cacosternum boettgeri* and *Semnodactylus wealii* have also been found in burrows of the Giant Girdled Lizard, *Smaug giganteus,* (Branch & Patterson, 1975).

Arboreal frogs make postural adjustments to reduce the surface-volume ratio (Wells, 2007). These frogs are found in the open when conditions are dry, sitting motionless with legs folded and pressed tightly under the ventral surface body (Wells, 2007). This type of behaviour is seen in the family Hyperoliidae (Withers *et al*., 1982; Geise & Linsenmair, 1986) and Rhacophoridae (Kaul & Shoemaker, 1989). *Eleutherodactylus coqui* assumes different postural positions based on the relative humidity of its habitat (Wells, 2007). The individuals become less alert at such times (Wells, 2007). Arboreal frogs use toe pads to stick to wet or moist surfaces (Hanna & Barnes, 1991). These anurans will most likely benefit from humid environments when migrating and breeding. Species of *Hyperolius* and *Afrixalus* will typically use such mechanisms, as they usually climb and call from elevated positions on undergrowth (Laurent, 1964; Du Preez & Carruthers, 2017).

During breeding seasons, anurans rely on high humidity levels for oviposition and larval development (Van Dijk, 1971). *Hemisus* species tend to prefer humid habitats for extra-aquatic oviposition and development (Van Dijk, 1971). Even *E. coqui* males will select high-humid environments as oviposition sites to ensure effective egg development (Townsend, 1989).

3.5.4 Barometric pressure

There is limited literature showing the effect of barometric pressure on anuran behaviour. It is well known that a decrease in barometric pressure occurs before rain (Oseen & Wassersug, 2002).The present study confirms this observation: rainfall and barometric pressure showed a correlation with one another (Figure 3.4). However, Von Seckendorff Hof and Hillyard (1993) state that changes in barometric pressure do not only indicate rainfall. Lowered barometric pressure is frequently associated with storm fronts (Von Seckendorff Hof & Hillyard, 1993); it would presumably also be correlated with other environmental factors such as temperature and humidity. Due to the elevation of the Umlalazi Nature Reserve (0 - 30 m.a.s.l.), high barometric pressure was expected. This was indeed the case: the barometric pressure was above 1 010 hPa throughout the year. Most (86%) of the species preferred to call air pressure was high. In contrast to this general trend, however, Henzi *et al.* (1995) found that the chorus activity of *Hy. marmoratus* increases when barometric pressure is low, prior to the rain.

When there is less rain, barometric pressure could possibly act as a cue for anurans in the breeding season (Oseen & Wassersug, 2002). Oseen and Wasserburg (2002) found this to be the case: *Lithobates clamitans* and *Lithobates catesbeianus* called during low barometric pressure periods late in their breeding season.The present study could however not confirm a similar finding, since barometric pressure was high.

3.5.5 Wind

Anurans will most likely decrease their activity on windy evenings to limit the risk of desiccation. This is a well-known phenomenon in anurans (Bellis, 1962; Boutilier *et al*., 1992; Henzi *et al.*, 1995; Oseen & Wassersug, 2002; Steelman & Dorcas, 2010). The present study found that most species showed a negative correlation with wind velocity. Henzi *et al*. (1995) found that increasing winds led to decreasing chorus attendance with the *Hy. marmoratus*. Similar results were found with the Umlalazi species. If the species did call during windy evenings, preference was shown for low wind velocities (< 35 km/h). It has furthermore been established that *Pseudacris feriarum*, *Lithobates sphenocephala* and *Lithobates catesbeianus* show similar reactions to low wind velocities, increasing calling behaviour during lower wind speeds (Oseen & Wassersug 2002; Steelman & Dorcas, 2010).

Wind moreover influences the physical acoustic properties of calls made by anurans (Waxler, 2004). Sound attenuation is caused by increasing wind speeds leading to sound distortion (Steelman & Dorcas, 2010). Male anurans will therefore avoid high wind speeds when calling. When low wind prevails or on still evenings, sounds tend to travel faster (Taherzadeh *et al.*, 1998). This could also be a way in which frogs avoid high noise interference levels in their environment (Köhler *et al.*, 2017).

3.5.6 Moon illumination

The lunar synodic cycle is known as the period from full moon to the next full moon. The average length of a lunar synodic cycle is 29.5 days (Grant *et al*., 2009). It is well known that lunar cycles influence regular environmental changes (Bradley *et al.*, 1962; Alonso, 1993; Muñoz & la Fuente, 2001; Morton-Pradhan *et al*., 2005; Huba & Drob, 2017). Due to this phenomenon, animals' behaviour changes due to lunar cycles (Fischer *et al*., 2001, Grant *et al*., 2009). Several studies show the effects of lunar cycles and illumination on the activity of anurans (see Fitzgerald & Bider, 1974; Both *et al*., 2008; Grant *et al*., 2009; Yetman & Ferguson, 2011; Grant *et al.*, 2012). The lunar response is species-specific and could vary due to other factors acting synergistically along with lunar cycles (Grant *et al*., 2009).

In the PCA analysis of the present study, calling intensities of most of the recorded species (79%) were negatively correlated with the percentage of moon illumination. However, half of the species seemed to call when illumination was less than 50%. Interestingly, 5/7 of these species were arboreal including *L. mossambicus*, *L. natalensis*, *H. marmoratus*, *H. pickersgilli* and *H. tuberilinguis*. *Hyperolius marmoratus*, *H. pickersgilli* and *H. tuberilinguis* males call from elevated positions in dense vegetation (Minter *et al*., 2004). Furthermore, a study carried out by Backwell and Passmore (1990) found that the calling activity of *Hyperolius marmoratus* was less predictable during moonlit nights. A reason for this may be that they became distracted or frightened by the movement of other anurans or vegetation. Another possible reason could be predation avoidance (Wells, 2007). Visually-orientated diurnal predators (snakes, birds, bats, and so forth) are at a disadvantage during low lunar illumination such as new moon phases (Oseen & Wassersug, 2002), while it could be advantageous for olfactory-oriented predators (foxes, rodents, and so forth) as anurans will not be able to see them (Grant *et al*., 2009). Tuttle and Ryan (1982) found that the neotropical hylid *Smilisca sila* calls during moonlit evenings from open areas to detect predatory bats.

It should also be kept in mind that the sky is not clear every evening, while additional meteorological variables such as rain and cloud cover could mask lunar illumination This highlights the important recognition that various factors could influence anuran activity. These should be included in data collection and analyses. However, the cloud cover percentage (%) was not measured in the present study, illustrating the need to have a weather station available at each site.

3.5.7 Climate change

Changes in seasonal meteorological variables caused by anthropogenic influences, including climate change (that is, changes in temperature and precipitation patterns), have adverse effects on anuran communities (Krause & Farina 2016), causing changes in behaviour such as a shift in breeding events (Corn, 2005). Being forced to breed earlier than usual causes strain for already sensitive species. In the Umlalazi Nature Reserve, for example, this may pose a problem to the already endangered *H. pickersgilli* population. Over prolonged dry periods, individuals suffer from physiological strain, resulting in decreasing fitness levels (Klaus & Lougheed, 2013). Furthermore, climate change causes an increase of extreme natural weather events (such as drought, floods, hurricanes, and so forth), resulting in increased mortalities (Krause & Farina 2016).

Although the effects of climate change on ecological processes are well documented, little emphasis has been placed on how to mitigate or manage such changes on a small topographical scale (Krause & Farina, 2016). Consider furthermore that the effects of climate change on anuran communities are more complex. Bioacoustics should be used in conjunction with other sensing methods (such as manual field surveys) and environmental systems to conduct climate change studies. These considerations underline the importance of such studies anew, especially in South Africa, with a view to ensuring the future of anuran populations.

3.5.8 Conclusion

This study illustrates the importance of environmental changes and the effects these have on anuran breeding behaviour. The recorded data, combined with future studies that would document the effects of weather fluctuations on calling activity of anurans, may be used to predict how climate change might impact the anuran population and their calling behaviour in the Umlalazi Nature Reserve.The results showed that environmental variables are important in relation to the calling activity of anurans in the reserve. However, it remains important to recall that during the present study, as mentioned, South Africa experienced severe droughts due to an El Niño event.

This study has found that calling behaviour of most of the terrestrial breeders (*Leptopelis mossambicus*, *L. natalensis*, *Arthroleptis wahlbergii*; *Hemisus guttatus* and *Breviceps sopranus*) is indirectly proportional to precipitation. It is important to note that these meteorological changes vary from year to year. Anurans also show species-specific adaptations to mitigate the risk of some of these changes such as indirectly proportional adaption of terrestrial anurans to rainfall patterns. As indicated, some of the results seem to contrast with those of other studies. Therefore, further studies should be conducted to estimate the effects of environmental changes over a longer period that would cover both wet and dry years.

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CHAPTER 4: THE LEAF-FOLDING FROGS (*AFRIXALUS* LAURENT, 1944): A CASE STUDY

The sound, which the scientific books describe as "croaking,"
floats far and wide, and produces a beautiful, mysterious effect on
a still evening when the last heavy-footed labourer has trudged
home to his tea, leaving the world to darkness and to me.

WH Hudson
The Book of a Naturalist
(1919)

4.1 Abstract

The leaf-folding frogs (*Afrixalus* Laurent, 1944) forms part of the Hyperoliidae. The genus *Afrixalus* is restricted to sub-Saharan Africa and comprises 33 species. Due to morphological similarities, the taxonomic status of some *Afrixalus* species has led to confusion. In the present study, the differences between *Afrixalus aureus* and *A. delicatus* were examined using morphological and acoustic signal comparison, while the identity of collected specimens was confirmed through DNA barcoding. Results indicate that *A. aureus* is an inland species while *A. delicates*, is coastally distributed. Morphologically the species are fairly similar. However, male *A. aureus* tend to be larger than male *A. delicatus*. Bioacoustic analysis confirms that the call of male *A. aureus* demonstrates a lower frequency and short, pulsed "trilling" whereas *A. delicatus* shows a higher frequency and a biphasic structure. This illustrates the importance of using acoustic parameters as an aid in taxonomic studies of these cryptic species.

Keywords: *Afrixalus; systematic; morphological; acoustic; biphasic call; taxonomic; cryptic species*

4.2 Introduction

The leaf-folding frog genus, *Afrixalus* Laurent, 1944, comprises a diverse and interesting group. The group has challenged taxonomists over the years. Pickersgill (1996) divided the genus into species complexes such as the *brachycnemis* complex (*A. brachycnemis* and *A. delicatus*), the *sylvaticus* complex (*A. sylvaticus, A. aureus* and *A. crotalus*), the *spinifrons* complex (*A. spinifrons* and *A. knysnae*), and the *septentrionalis* complex (*A. morerei* and *A. septentrionalis*). In a later study, Pickersgill (2005) revised the *brachycnemis* and *sylvaticus* complexes, synonymising them with the *stuhlmanni* complex (Frost, 2018).

The genus belongs to the Hyperoliidae, which currently includes 33 recognised species (Frost, 2018). It is restricted to sub-Saharan Africa. The species occupy a variety of habitats, from moist grasslands to tropical rain forests (Pickersgill, 2005). Its distribution ranges from the southern parts of South Africa in the Western Cape (*A. knysnae*) to northern Africa in southern Ethiopia, where *Afrixalus clarkei* and *Afrixalus enseticola* occur as well as western Africa in southern Mali, where *Afrixalus vittiger* and *Afrixalus weidholzi* are found (Frost, 2018). Drewes (1984) considers *Afrixalus* to be a sister taxon of the genus *Hyperolius*. However, vertical pupils easily distinguish members of *Afrixalus* from the latter group (Pickersgill, 2007). All males call, generally above water (Pickersgill, 2005). They produce unique calls for specific purposes. The most conspicuous and widely recognised is the advertisement call. This call is species-specific, making it a valuable taxonomic character (Passmore, 1981; Channing, 1999).

Indeed, anuran vocalisation is widely used for taxonomic purposes, offering a reliable and effective tool for species identification (see Dayrat, 2005; Padial *et al.,* 2009; and Köhler *et al*., 2017). Köhler *et al*. (2017) note that, if bioacoustic data can be integrated with morphological studies, an increase in knowledge of anuran evolution would arise. These advances in taxonomic assessment techniques have led to numerous discoveries of new and morphologically cryptic species (see Vences & Köhler, 2008; Streicher *et al.*, 2012, Forlani *et al.*, 2016; Bosch *et al*., 2017; Minter *et al.,* 2017).

According to Pickersgill (2007), the genus *Afrixalus* is poorly studied in some geographical areas of Africa due to political and civil rivalry during the early 1990s. However, since then, the use of modern technology has improved radically, making it possible to compare such historical data to newfound data. During the present study, two species, namely *A. aureus* and *A. delicatus*, provided an opportunity to study taxonomic differences using morphological and acoustic signal comparisons.

4.3 Materials and methods

4.3.1 Ethics and permits

None of the *Afrixalus* species in KwaZulu-Natal are listed as threatened (IUCN-Red List, 2018) and a collecting permit OP 4092/2016 was obtained from Ezemvelo KZN Wildlife. Ethics clearance, labelled NWU-00006-14-A3, was obtained from the AnimCare ethics committee of the North-West University.

4.3.2 Sampling

Specimens were collected at five different localities within the broader north-eastern KwaZulu-Natal area (Figure 4.3). Collecting was conducted in the summer seasons (November-February) from 2015 to 2017. One to ten specimens were collected per site for morphological assessment, tissue samples and voucher specimens. A total of 23 specimens of both *Afrixalus aureus* (n=16) and *A. delicatus* (n=6) were collected. The captured specimens were weighed using a Sartorius digital balance or a sensitive Pesola scale (max. mass: 100g) and measured with a Vernier calliper before euthanasia. Specimens were photographed in life prior to euthanasia using a Canon EOS20D SLR camera fitted with a Canon twin-flash macro Speedlight. Voucher specimens were euthanased following approved SOP for euthanasia of amphibians (SOP NW-00492-16-A5). Thigh muscle and liver samples were taken subsequent to euthanasia and preserved in 70% molecular grade EtOH or RNA latter for genetic analysis. Euthanased specimens were fixed in 10% neutral buffered formalin (NBF) in a natural position to clearly display all morphological features. Following fixation of at least two days, specimens were rinsed overnight in running tap water and transferred to jars containing 70% EtOH. For prolonged preservation, field numbers were assigned to each specimen (see Appendix C), after which specimens were deposited in the SAIAB Amphibian collection based at the North-West University, Potchefstroom Campus, South Africa. All dates were entered into the SAIAB Specify database (see Appendix C).

4.3.3 Morphometric assessment

4.3.3.1 Specimens

Afrixalus aureus (n=11) and *A. delicatus* (n=6) specimens were examined for morphological assessment. Morphological traits were measured using a Nikon AZ100 (Nikon, Amsterdam) microscope fitted with NIS Elements software. Measurements were taken in accordance with Minter *et al.* (2017), but additional measurements were introduced (Appendix D). Morphometric measurements included snout to vent length (**SVL**), head width (**HW**), head length (**HL**), distance between anterior corners of the eyes (**EAD**), distance between posterior corners of the eyes (**EPD**), eye to snout right side (**EDr**) defined as the distance measured between the central tip of the head to the anterior corner of the right eye, eye to snout left side (**EDl**) defined as the distance measured between the central tip of the head to the anterior corner of the left eye, inter orbital distance (**IOD**), palpebral fissure length of right eye (**PFLr**), palpebral fissure length of left eye (**PFLl**), inter-nostril distance (**IND**) defined as the distance measured between the two nostrils, nostril ocular distance on the right side (**NODr**) defined as the distance between the right nostril to the anterior corner of the right eye, nostril ocular distance on the left side (**NODl**) defined as the distance between the left nostril to the anterior corner of the left eye, nostril-lip distance right side (**NLr**) defined as the distance between the right nostril to the lip on the right, nostril-lip distance left side (**NLl**) defined as the greatest distance between the left nostril to the lip on the left, right foot length (**FLr**) defined as the shortest distance from the fourth right toe to the start of the right tibia, left foot length (**FLl**) defined as the distance from the fourth left toe to the start of the left tibia, first right toe length (**T1Lr**), first right toe disk width (**T1Wr**), first left toe length (**T1Ll**), first left toe disk width (**T1Wl**), second right toe length (**T2Lr**), second right toe disk width (**T2Wr**), second left toe length (**T2Ll**), second left toe disk width (**T2Wl**), third right toe length (**T3Lr**), third right toe disk width (**T3Wr**), third left toe length (**T3Ll**), third left toe disk width (**T3Wl**), fourth right toe length (**T4Lr**), fourth right toe disk width (**T4Wr**), fourth left toe length (**T4Ll**), fourth left toe disk width (**T4Wl**), fifth right toe length (**T5Lr**), fifth right toe disk width (**T5Wr**), fifth left toe length (**T5Ll**), fifth left toe disk width (**T5Wl**), tibia length right (**TLr**) defined as the distance between the right knee to the heel, tibia length left (**TLl**) defined as the distance between the left knee to the heel, thigh length right (**THLr**) defined as the distance between the vent to the right knee, thigh length left (**THLl**) defined as the distance between the vent to the left knee, right hand length (**HdLr**) defined as the distance from the proximal end of outer metacarpal tubercle on the right hand to the tip of the third right finger, left hand length (**HdLl**) defined as the distance from the proximal end of outer metacarpal tubercle on the left hand to the tip of the third left finger, third right toe length (**F3Lr**), third left toe length (**F3Ll**), third right toe disk width (**DF3r**), third left toe disk width (**DF3**), right forearm length (**FOLr**) defined as the distance between the proximal end of outer metacarpal tubercle on the right hand to the corner of the right elbow, left forearm length (**FOLl**) defined as the distance between the proximal end of outer metacarpal tubercle on the left hand to the corner of the left elbow, right humerus length (**LHUr**) defined as the distance between the corner of the right elbow to the body, left humerus length (**LHUl**) defined as the distance between the corner of the left elbow to the body. Measurements were taken on both sides of mature male specimens; however, only the right side was used for comparison. Colouration was determined using life photos.

Four regions were identified to determine asperity distribution: the gular disc area, head region, right tibia and dorsal- and ventral area. The shape of the gular disc was added as a characteristic in some instances. Asperity composition and gular disc shape were compared using Nikon AZ100 micrographs (Nikon, Amsterdam).

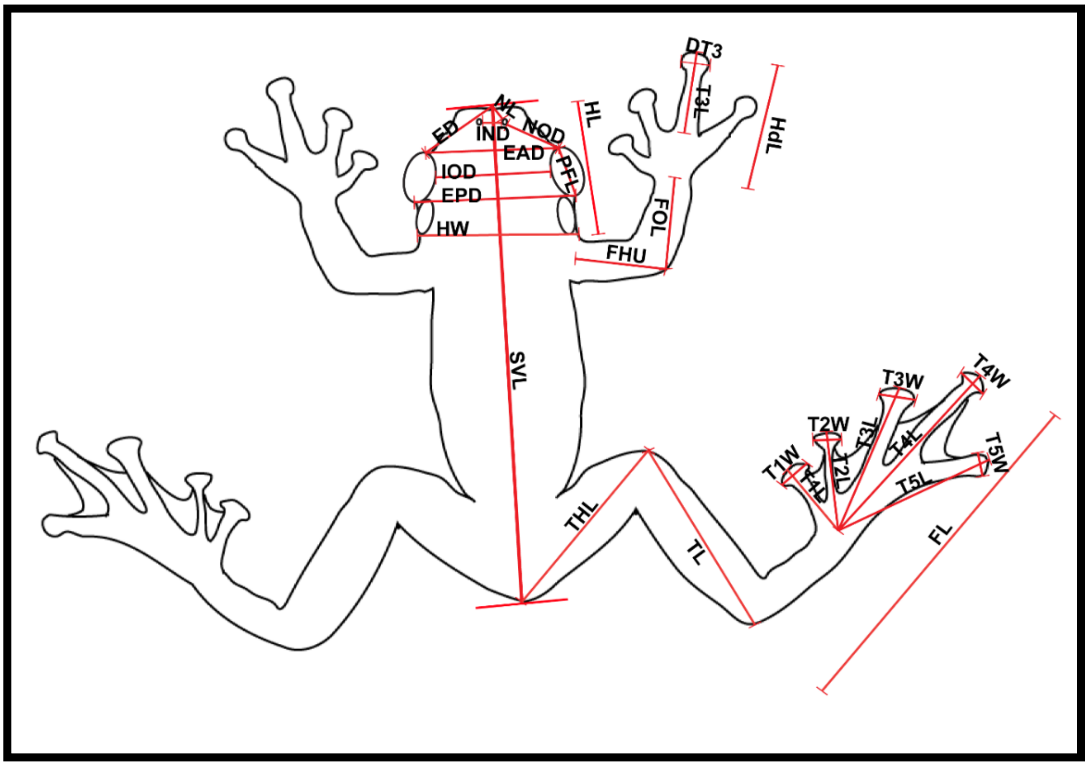

Figure 4.1: Measurements used for morphological analysis

4.3.3.2 Statistical morphological analyses

Standard Excel statistics were executed for measurements (range, mean and standard deviation). Ratios were analysed as percentages of different characteristics for comparative analysis of the right side measurements, including head width to snout-vent length (**HW/SVL**), head length to snout-vent length (**HL/SVL**), inter-nostril distance to head width (**IND/HW**), fourth toe length to foot length (**T4L/FL**), fourth toe disk width to foot length (**T4W/FL**), thigh length to snout-vent length (**THL/SVL**), tibia length to snout-vent length (**TL/SVL**), third finger length to hand length (**F3L/HdL**), humerus length to hand length (**LHU/HdL**) and forearm length to hand length (**FOL/HdL**).

4.3.4 Bioacoustic analysis

4.3.4.1 Field recordings

Vocally active males were observed and recorded on evenings in the field at air temperatures ranging from 20.3 to 26.8°C. A total of 35 (*A. aureus*, n= 23 and *A. delicatus*, n= 12) males were recorded (Table 4.1). Recordings were made using a NAGRA ARES-ML recorder (Software Version 3.24, 2009) fitted with a Sennheiser ME80 external microphone, at a pulse-code modulation (PCM) of 24-bits per sample and a sampling rate of 44.1 kHz, saved as .WAV format files. Prior to the recording, the ambient temperature was measured using an ExTech Instruments waterproof digital thermometer (accurate to ±1°C). After successful recordings, specimens were captured and retained as voucher specimens where possible.

Table 4.1: List of specimens and localities of specimens recorded during the study

Species	Location	GPS coordinates		Date	# of males recorded
Afrixalus delicatus	Wally Farm, Monzi	-28.44891 S	32.28836 E	2016/02/10	7
	Tolla se gat, Sodwana	-27.48863 S	32.66410 E	2016/11/15	2
	Tolla se gat, Sodwana	-27.48863 S	32.66410 E	2017/11/19	3
Afrixalus aureus	Bonamanzi Pond	-28.07421 S	32.31355 E	2015/11/24	2
	Bridge pond, Sihlenga	-27.07540 S	32.46961 E	2016/11/18	2
	Fence pond, Ndumo	-26.89760 S	32.21578 E	2016/11/24	2
	Old SM site, Ndumo	-26.86541 S	32.16652 E	2016/11/25	1
	Wetland, Ndumo	-26.90172 S	32.23740 E	2016/11/26	5
	Fence pond, Ndumo	-26.89760 S	32.21578 E	2016/12/01	3
	Wetland, Ndumo	-26.90172 S	32.23740 E	2016/12/03	4
	Fence pond, Ndumo	-26.89760 S	32.21578 E	2016/12/05	3
	Fence pond, Ndumo	-26.89760 S	32.21578 E	2016/12/10	1

4.3.4.2 Sound analysis

Acoustic signals were analysed manually using Raven Pro Version 1.5.0 software (Cornel Laboratories, USA). Spectrograms were produced for each recording by using the default 1.3 power window with a Hanning window function and FFT size of 512. A maximum of ten advertisement calls per male were analysed. The dominant frequency (Hz) of each call was measured in Audacity version 2.1.2 (http://audacityteam.org/) frequency analysis option with a Hanning window, size 512. *Afrixalus delicatus* has a biphasic call comprising a "zip" and "trill" component (Backwell, 1988). These components were analysed separately. Call descriptions are based on note-centred terminology *sensu* Köhler *et al.* (2017). The oscillograms and spectrograms shown in Figures 4.4-4.7 were generated using BatSound ver. 4.1.4 (Pettersson Elektronik AB), a Hanning window size of 512 and 50% overlap with a bandwidth of between 2 000 Hz to 7 000 Hz.

The present study defines a call as a distinct acoustic entity separated from other calls by distinct silences. Call parameters measured, as reflected in Figure 4.2A, include call duration (**CD**) defined as the start of a call to the end of the same call measured in seconds, inter-call interval (**ICI**) defined as the interval between the end of a call to the start of a consecutive call measured in seconds, call period (**CP**) defined as the start of a call to the start of the next consecutive call measured in seconds and the call repetition rate (**CRR**) defined as the number of calls per minute (min^{-1}). CRR is calculated as the number of calls per group minus one, divided by the duration of the call group, multiplied by 60. Calls consist of notes, that is, subunits within a call separated by silent intervals. Note parameters, as reflected in Figure 4.2B, include the number of notes within a specific call (**No. notes**), note duration (**ND**) defined as the start of one note to the end of the same note measured in seconds, inter-note interval (**INI**) defined as the interval between the end of one note to the beginning of the following consecutive note measured in seconds, and note repetition rate (**NRR**) defined as the number of notes within a call divided by the call duration, measured as number of notes per second (n.p.s^{-1}). Within notes, pulses can be identified. Pulses are discrete quantities of energy. When they are separated distinctly from one another, the call is said to be "pulsed" whereas, if poorly separated from one another, the call is said to be "pulsatile". Pulse parameters, as reflected in Figure 4.2C, include the number of pulses per note (**PPN**) and pulse repetition rate (**PRR**) defined as the number of pulses within a note measured as pulses per second (p.p.s^{-1}). PRR is calculated as the number of pulses within a specific note divided by the duration of that note. The numerical parameters are given as range (minimum-maximum), mean and standard deviation values.

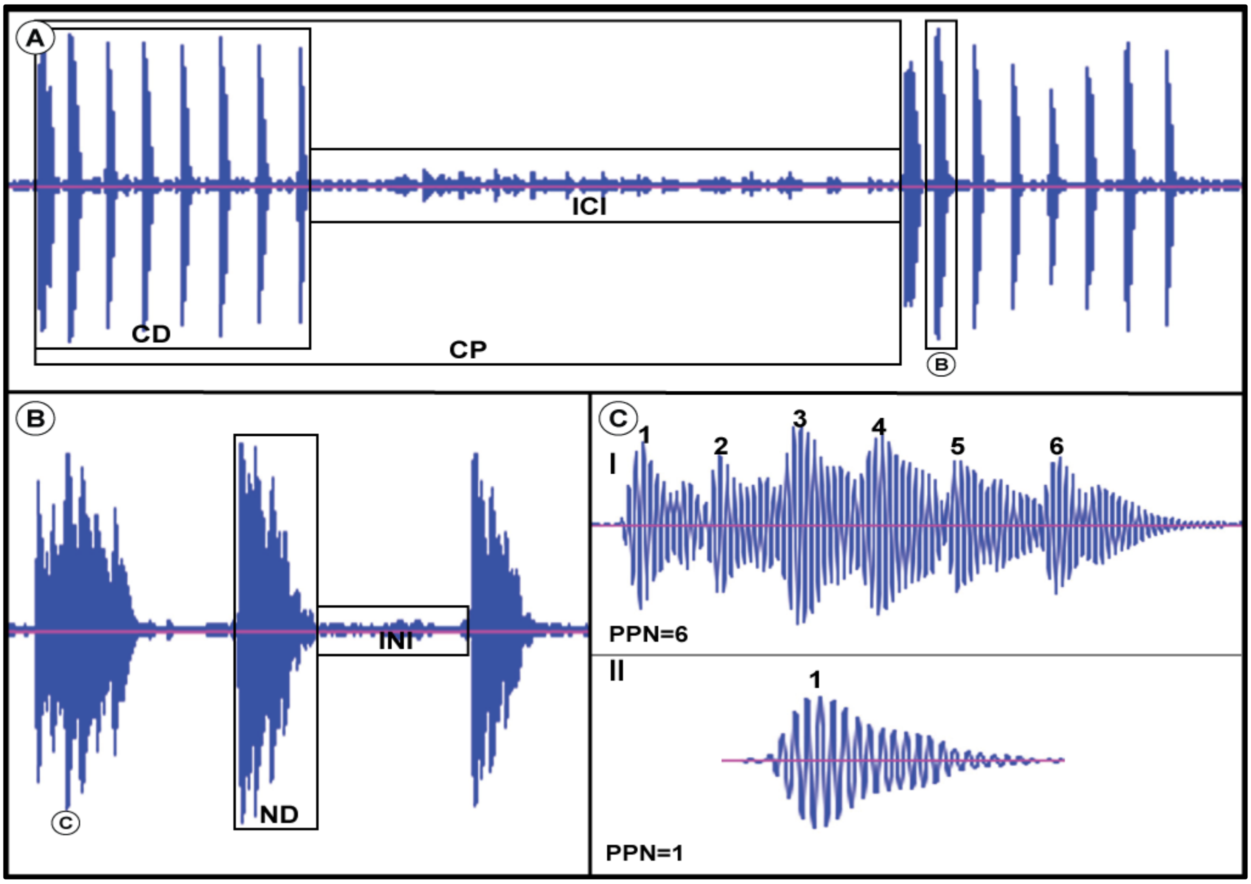

Figure 4.2: Acoustic parameters used for acoustic analyses shown in oscillograms compiled in Raven Pro Version 1.5.0. **A:** Call parameters of *A. aureus*. **B:** Note parameters of *A. aureus*, **C:** Pulse parameters of **I:** *A. aureus* and **II:** *A. delicatus*.

4.3.4.3 Statistical analysis

IBM SPSS 25 (IBM Corp., 2017) was used to perform statistical analysis to determine any significant difference between call parameters and morphometric comparisons between the two species. One-way ANOVA with a Tukey HSD post-hoc test was performed to obtain call parameter comparisons between the two species.

4.4 Results

4.4.1 Distribution

DNA barcoding, which confirmed the distribution formed part of a separate master's dissertation completed by a co-student, Jani Reeder (Amphibians of northern KwaZulu-Natal: A phylogenetic study, NWU). This study confirmed the molecular identity of both species, indicating that *A. aureus* occur inland and *A. delicatus* along the coast.

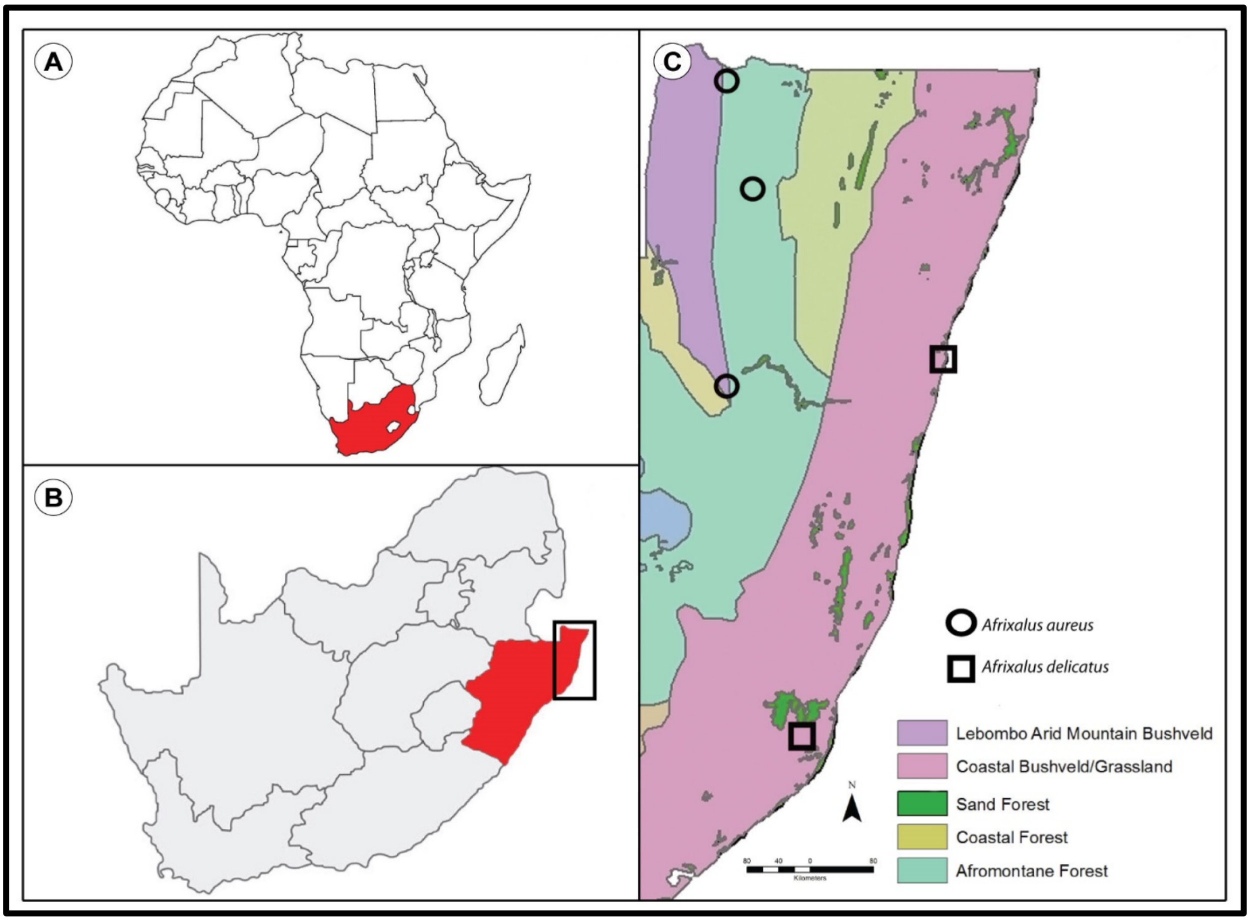

Figure 4.3: Map of northern KwaZulu-Natal showing vegetation types and the distribution of *A. aureus* and *A. delicatus* (adapted from Mucina & Rutherford, 2006)

4.4.2 Morphological differentiation

4.4.2.1 Size

Afrixalus aureus is significantly larger than *A. delicatus.* A one-way ANOVA was conducted to compare differences between morphological measurements of the two *Afrixalus* species. Significant differences of $p < .05$ were noted for the following measurements in these two species: SVL, HW, HL, EPD, IOD, NOD, FL, T4L, T4W, T5L, THL, TL, HdL, F3L and LHU, as reflected in Table 4.2. The remaining measurements showed no significant difference ($p > .05$).

Table 4.2: Mean morphological measurement data for *A. aureus* and *A. delicatus*

	***A. aureus* (n=11)** $\bar{X}$ (min-max) ± **SD**	***A. delicatus* (n=6)** $\bar{X}$ (min-max) ± **SD**
SVL	22,01 (19,62-23,29) ± **1,11**	18,85 (17,28-20,03) ± **0.99**
HW	6,52 (6,05-7,25) ± **0.38**	5,85 (5,22-6,18) ± **0.33**
IOD	2,24 (1,93-2,51) ± **0.18**	2,01 (1,89-2,18) ± **0.11**
IND	1,37 (1,08-1,50) ± **0.11**	1,41 (1,25-1,52) ± **0.10**
NOD	1,76 (1,59-2,04) ± **0.15**	1,50 (1,39-1,73) ± **0.13**
FL	13,45 (12,30-14,44) ± **0.63**	11,72 (11,12-12,45) ± **0.50**
T4L	6,75 (5,69-7,36) ± **0.53**	5,53 (4,26-6,42) ± **0.81**
DT4	0,77 (0,46-1,10) ± **0.19**	0,60 (0,44-0,72) ± **0.10**
THL	8,55 (6,73-9,85) ± **0.83**	6,92 (6,14-7,56) ± **0.49**
TL	8,52 (8,02-9,22) ± **0.43**	6,63 (4,64-7,44) ± **1.03**
HL	5,32 (4,77-5,91) ± **0.38**	4,45 (3,79-4,91) ± **0.40**
F3L	4,30 (3,90-5,00) ± **0.29**	3,41 (3,14-4,38) ± **0.48**
LHU	3,69 (3,24-4,28) ± **0.32**	3,10 (2,76-3,61) ± **0.31**
FOL	3,56 (2,89-4,04) ± **0.33**	3,14 (2,85-3,61) ± **0.26**

4.4.2.2 Statistical analysis of proportions

According to the one-way ANOVA test done on the ratios as percentage, only **IND/HW ($F_{(1,15)} = 20.26$, $p = 0.0004$** and **F3L/HdL ($F_{(1,15)} = 9.84$, $p = 0.0068$** showed significant differences (see Table 4.3). No other ratios showed significant differences ($p > .05$).

Table 4.3: Mean morphological proportions of *A. aureus* and *A. delicatus*

	***A. aureus* (n=11)** $\bar{X}$ (min-max) ± **SD**	***A. delicatus* (n=6)** $\bar{X}$ (min-max) ± **SD**
TL/SVL	39% (36-41%) ± **0,02**	35% (23-40%) ± **0,06**
HW/TL	77% (71-84%) ± **0,04**	90% (81-127%) ± **0,18**
IND/HW	21% (18-25%) ± **0,02**	24% (22-46%) ± **0,01**
F3L/HdL	80% (66-87%) ± **0,05**	64% (49-80%) ± **0,10**

4.4.2.3 Asperity compostion

High variability was noted among individuals within each species. If asperities were present, they were uniformly distributed in both species. Overall, both species showed similar colouration and asperity distribution.

Clearly defined dorsal and lateral bands were observed from the live pictures of these species. *Afrixalus delicatus* has light brown to golden yellow dorsal colouration with dark brown lateral bands concealed by light speckles, weakly defined dorsal markings mostly situated in the lumbar region and oblique bands on the exposed area of the tibia.

Afrixalus aureus has a golden yellow dorsal colouration and lateral bands varying between light and dark brown with light speckles. As in the case of *A. delicatus*, weakly defined dorsal markings are situated mainly in the lumbar region and oblique bands were noted on the exposed area of the tibia.

Dense dorsal and tibia asperities were observed in both species. Ventrally, no asperities were observed for either species. Uniform asperities were also noted on the head and gular region of both species (Gular region: *A. aureus*: 54% and *A. delicatus*: 83%). Asperities were also noted to be less dense on the jaw (*A. aureus*: 73% and *A. delicatus*: 83%). The gular shape of all the specimens was identified as a reuleaux triangular. Table 4.4 summarizes these findings.

Table 4.4: Selected comparative morphological characteristics of *A. aureus* and *A. delicatus*

	***A. aureus* (n=11)**	***A. delicatus* (n=6)**
Gular shape		
	Reuleaux triangle	Reuleaux triangle
Asperities		
Dorsal	Present (100%)	Present (100%)
Ventral	Absent	Absent
Head	Present (100%)	Present (100%)
Gular	Present (54%)	Present (83%)
Lips	Present (73%)	Present (83%)
Tibia	Present (100%)	Present (100%)

4.4.3 Acoustic differentiation

Call structure differed considerably between *A. aureus* and *A. delicatus* (see Figure 4.4). For *A. delicatus,* two call types were noted. The first call was defined as the "zip" call (type A), while the second was defined as a "trill" call (type B; see Backwell 1988). Based on the post-hoc Tukey HSD test on CD, ICI, CP, number of notes, ND, INI and PPN, a significant difference was found between the "trill" call of *A. aureus* and *A. delicatus* ($p < .05$), whereas ND, INI, PPN and pulse rate show a significant difference between the *A. aureus* "trill" call and *A. delicatus* "zip" call ($p < .05$). A significant difference between the two *A. delicatus* calls includes CD, number of notes, ND, INI, NRR and pulse rate ($p < .05$).

In the *A. delicatus* "zip" call, the following parameters were excluded: inter-call interval, call repetition rate and call period. The reason for this is that the "zip" call is produced less frequently and unpredictably. The number of harmonics were not measured, as this parameter is mostly dependent on recording quality. Harmonics were not considered in this study, because the amount of background noise varied between the different recording sites.

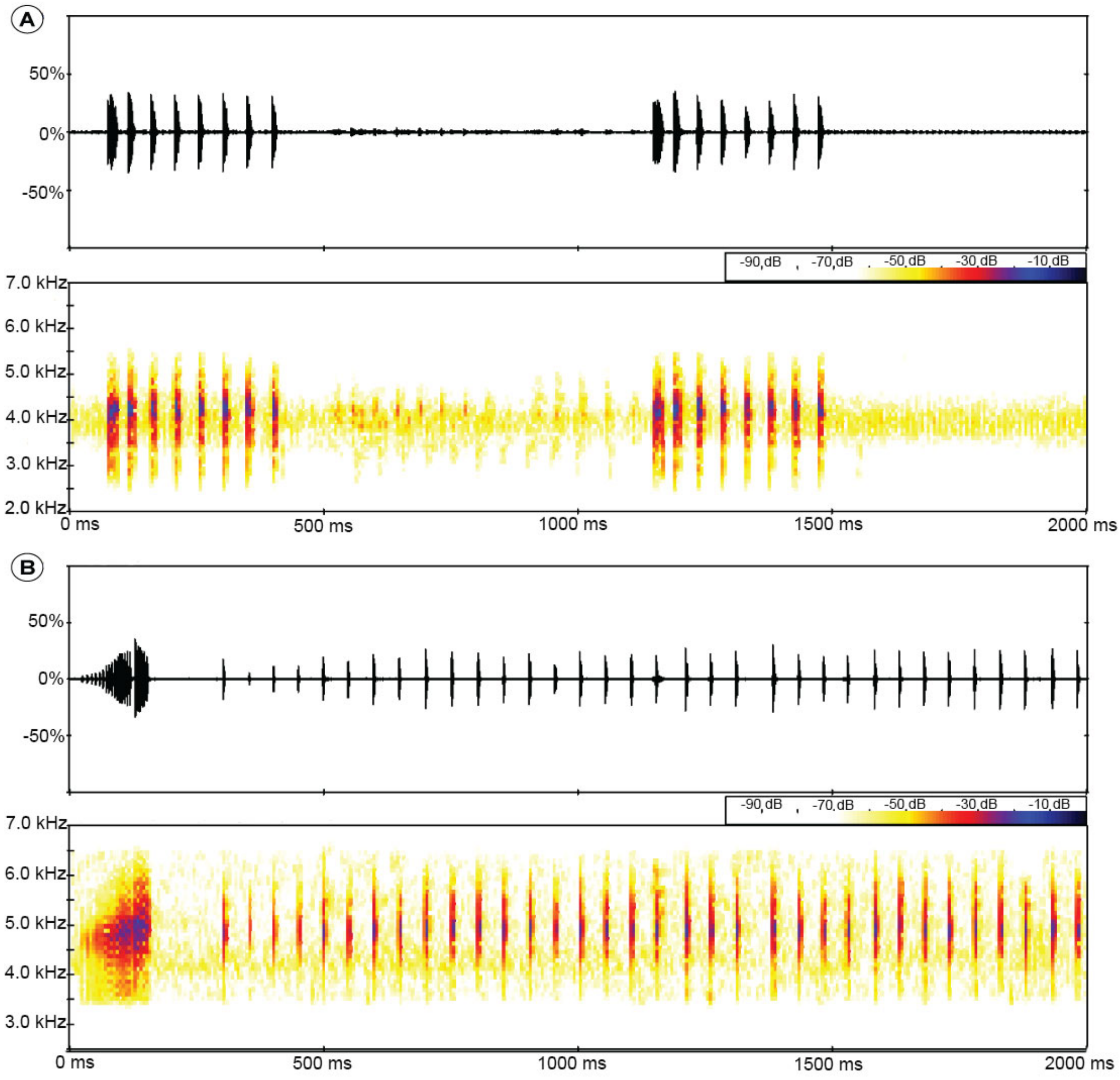

Figure 4.4: Comparative spectrograms and oscillograms of **A:** *A. aureus* & **B:** *A. delicatus* calls. Compiled in BatSound version 4.1.4 at Hanning window function and 512 band resolution with a 50% overlap

Afrixalus aureus advertisement calls consist of multiple pulsed notes within a simple short abbreviated "trill" sound separated by shorter inter-call silences (call duration: 0.412 sec. ± 0.092; inter-call interval: 3.294 sec. ± 1.627; 18.17 notes per second ± 1.812; 3.996 pulses per note ± 0.444) In contrast to the longer *A. delicatus* extended "trill" call, which comprises simple multiple pulsatile notes separated by long inter-call silences (Call duration: 2.826 sec. ± 1.392; inter-call interval: 6.083 sec. ± 3.542; 21.439 notes per second ± 0.977; 1.38 pulses per note ± 0.191), the *A. delicatus* "zip" call consists of short "zip"-like bursts of multiple, single pulsed notes with amplitude modulated pulses (call duration: 0.121 sec. ± 0.067; 215.81 notes per second ± 58.916; 1.125 pulses per note ± 0.143). *Afrixalus aureus* has a higher call rate (17.294 calls per min^{-1} ± 6.783) compared to *A. delicatus'* "trill" call (8.093 calls per min^{-1} ± 5.692).

Table 4.5: Call parameter measurements of *A. aureus* and *A. delicatus*

	***A. aureus* (n=23)**	***A. delicatus***	
		"zip" (n=7)	**"trill" (n=10)**
	Temperature (ºC)		
Mean ($\bar{x}$)	24,4	23,6	24,2
Range	20,3-26,8	21,5-26,3	23,3-26,3
Standard deviation	2,195	1,986	1,449
	Call duration (sec.)		
Mean ($\bar{x}$)	0,412	0,121	2,826
Range	0,07-0,655	0,032-0,373	0,299-6,219
Standard deviation	0,092	0,067	1,392
	Inter-call interval (sec.)		
Mean ($\bar{x}$)	3,294	n/a	6,083
Range	0,629-28,603	n/a	0,304-42,967
Standard deviation	1,627	n/a	3,542
	Call repetition rate (calls p/min-1)		
Mean ($\bar{x}$)	17,294	n/a	8,093
Range	7,62-29,98	n/a	3,13-22,41
Standard deviation	6,783	n/a	5,692
	Call period (sec.)		
Mean ($\bar{x}$)	3,629	n/a	8,756
Range	0,31-28,801	n/a	1,247-45,095
Standard deviation	1,547	n/a	4,178

Table 4.5 continued: Call parameter measurements of *A. aureus* and *A. delicatus*

	Notes per call		
Mean ($\bar{x}$)	7,346	23,337	61,811
Range	2-12	8-43	7-149
Standard deviation	1,407	8,649	32,206
	Note duration (sec.)		
Mean ($\bar{x}$)	0,016	0,003	0,007
Range	0,001-0,12	0,001-0,006	0,001-0,031
Standard deviation	0,002	0,001	0,003
	Inter-note interval (sec.)		
Mean ($\bar{x}$)	0,046	0,003	0,041
Range	0,015-0,52	0,001-0,019	0,001-0,019
Standard deviation	0,005	0,001	0,004
	Note repetition rate (notes p/sec.)		
Mean ($\bar{x}$)	18,17	215,81	21,439
Range	13,0111-28,571	96,849-341,205	12,696-25,837
Standard deviation	1,812	58,916	0,977
	Number of pulses per note		
Mean ($\bar{x}$)	3,996	1,125	1,38
Range	1-10	1-2	1-3
Standard deviation	0,444	0,143	0,191
	Pulse repetition rate (pulses p/sec.)		
Mean ($\bar{x}$)	258,003	461,898	260,748
Range	100-2307,692	166,667-2000	32,258-1000
Standard deviation	26,177	65,928	109,063
	Dominant frequency (Hz)		
Mean ($\bar{x}$)	4346,000	4934,000	5069,000
Range	3234-4981	4031-5906	4835-5434
Standard deviation	176,359	275,517	116,403

The study found that the inner structure of a single *A. aureus* call consists of perfectly distinguishable pulsed notes (see Figure 4.5). Pulses within notes could be distinguished as bursts of energy. In the case of *A. delicatus,* pulsatile notes occured with few distinguishable pulses (see Figures 4.6-4.7).

The dominant frequency for *A. aureus* varied between 3 234 and 4 981 Hz (4346 Hz ± 176.359). For the "zip" call of *A. delicatus,* the dominant frequency ranged from 4 031 to 5 906 Hz (4934 Hz ± 275.517) and the "trill" from 4 835 to 5 434 Hz (5069 ± 116.403). The dominant frequencies for both *A. delicatus* call types are closely related ($p = 0.30$). There is, however, a significant difference between *A. aureus* and *A. delicatus* when it comes to dominant frequency **($F_{(2,37)} = 660554$, $p = 0.000$).**

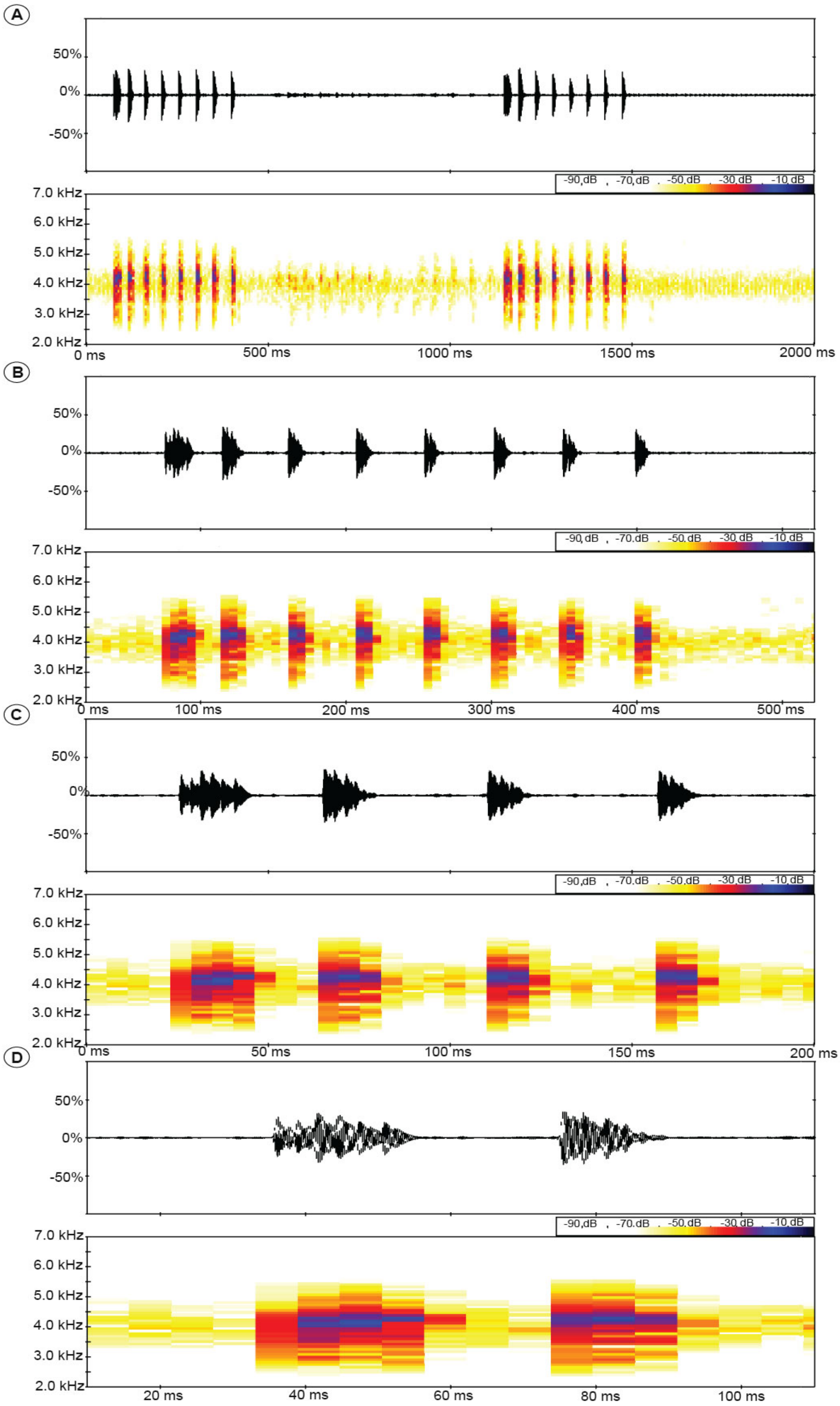

Figure 4.5: Oscillo- and spectrogram visualisations of *A. aureus* notes. Compiled in BatSound version 4.1.4 at Hanning window function and 512 band resolution with a 50% overlap. **A:** 2000 ms. **B:** 500 ms. **C**: 200 ms. & **D:** 100 ms

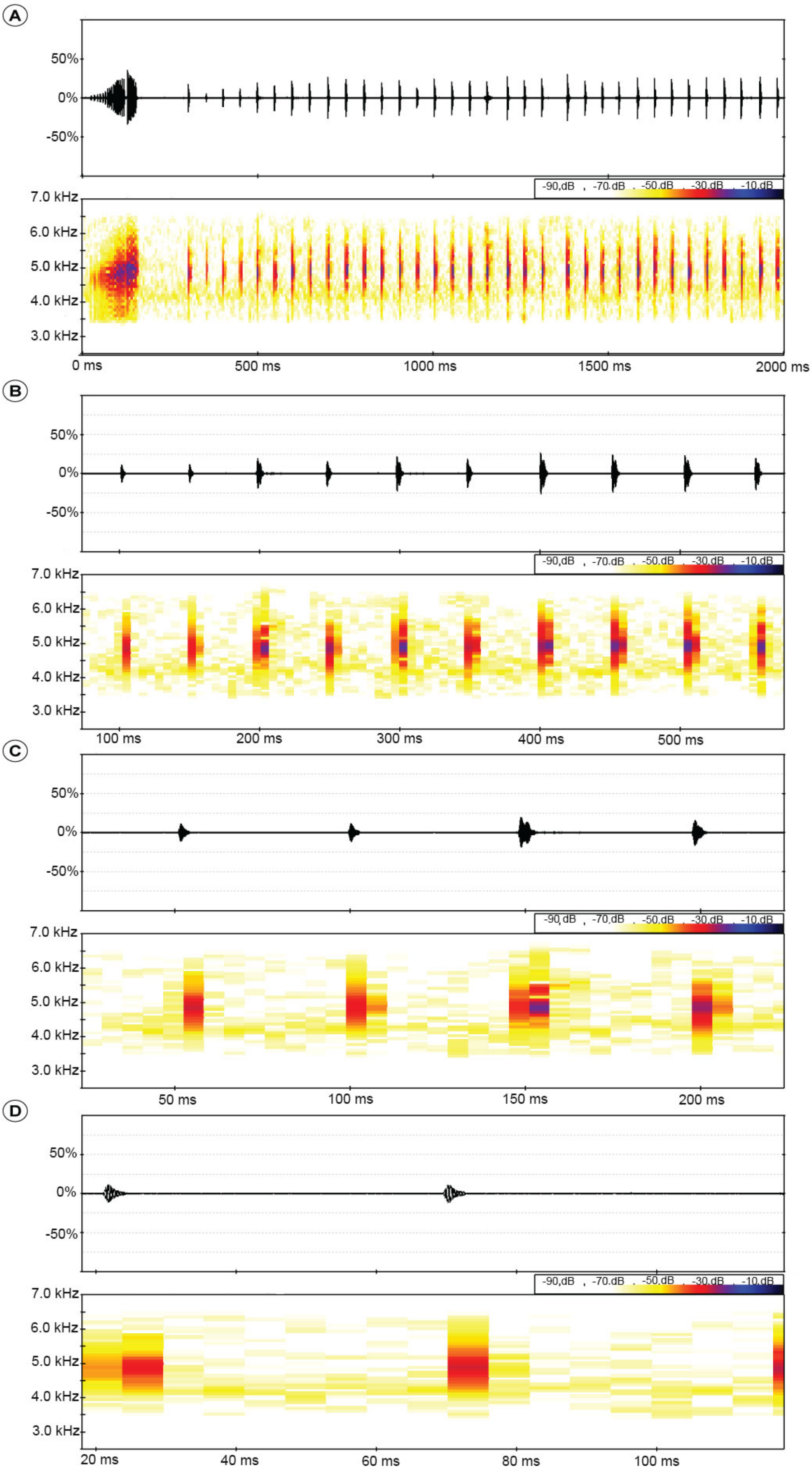

Figure 4.6: Oscillo- and spectrogram visualisations of *A. delicatus* "trill" notes. Compiled in BatSound version 4.1.4 at Hanning window function and 512 band resolution with a 50% overlap. **A:** 2000 ms. **B:** 500 ms. **C:** 200 ms. & **D:** 100 ms

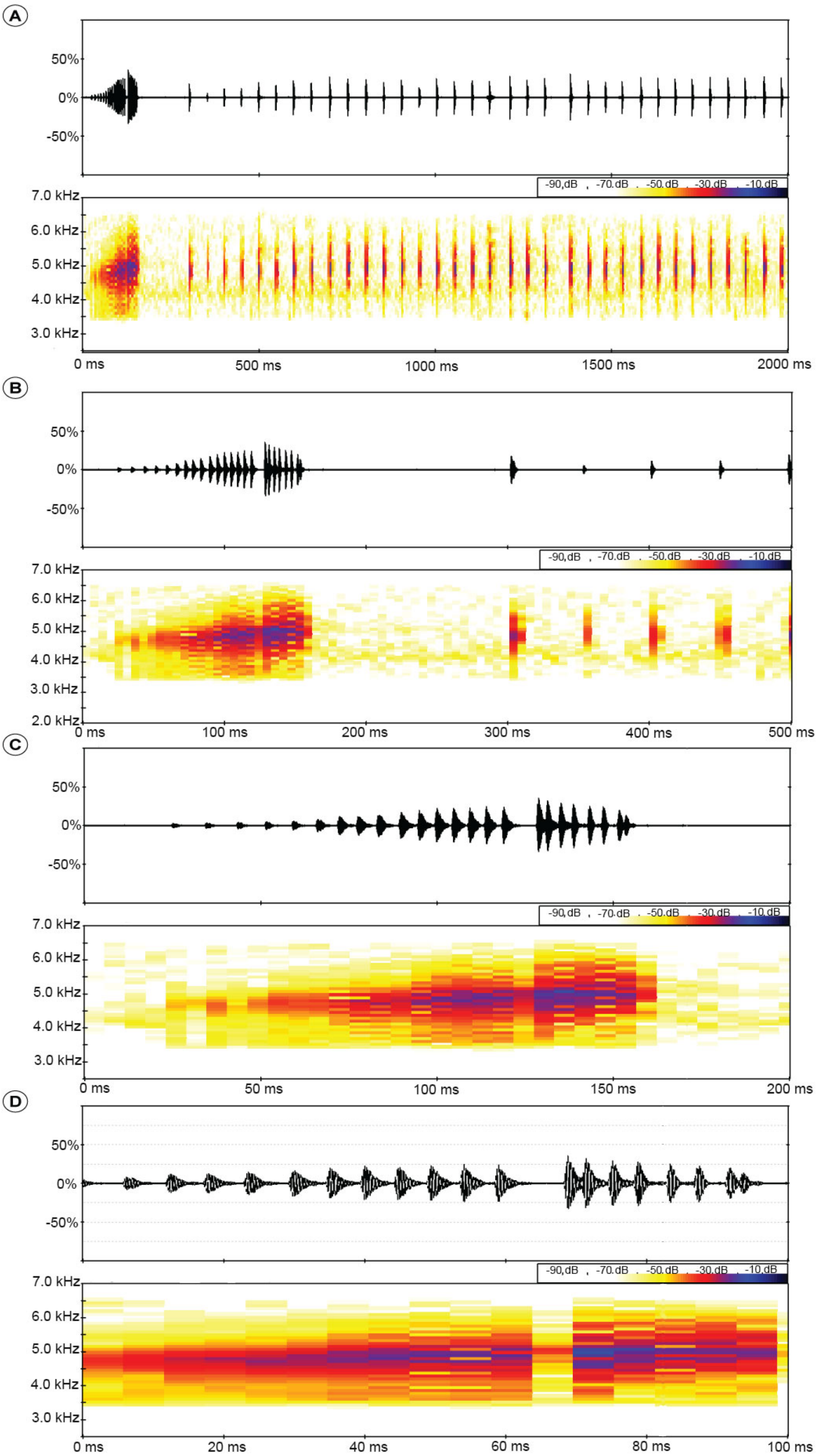

Figure 4.7: Oscillo- and spectrogram visualisations of *A. delicatus* "zip" notes. Compiled in BatSound version 4.1.4 at Hanning window function and 512 band resolution with a 50% overlap. **A:** 2000 ms. **B:** 500 ms. **C**: 200 ms. & **D:** 100 ms

4.5 Discussion

4.5.1 Morphology

Pickergill (1984) avers that colour and morphological variation could aid in distinguishing between similar species and sub-species. In a 1996 paper, Pickersgill used this to distinguish between three taxa (*A. spinifrons spinifrons, A. spinifrons intermedius* and *A. knysnae*) within the *A. spinifrons* complex. Consider in this respect that several morphological traits tend to be the same across the different species. Some may show minor significant differences due to their size. Generally, these species do not exceed a snout-urostyle length of more than 20 mm (Pickersgill, 2005). For this reason, the present study employed multiple measurements were included to determine differences.

Morphological assessments in this study led to the conclusion that *A. aureus* tends to be significantly larger (22 mm **± 1.11**) than *A. delicatus* (18.85 mm ± **0.99**). Due to the limited literature available on the difference between these two species, Pickersgill (2005) and Pickersgill (2007b) were used as guidelines. However, these two studies only yielded a number of valuable morphological comparable characters between the two species. The current study supports these findings, with the exception of the head width to tibia ratio for *A. delicatus*, since the HW/TW ratio within one of the specimens (AACRG: 2945) was greater than expected. However, if this specimen was excluded, similar results would have been observed.

According to Pickersgill (1984; 1996; 2005), skin asperity is a prominent diagnostic character in the genus *Afrixalus*. However, the present study's results in terms of asperity observations did not conform to Pickersgill (2007b). Dorsal asperities were present in both *A. aureus* and *A. delicatus*. Pickersgill (2005) found that asperities were uniform over all upper surfaces, scattered over the gular disk, jaws and confined or prominent on the chest for male *A. delicatus* specimens. However, no asperities were found on the chest region for specimens of *A. delicatus*. In *A. aureus*, asperities were scattered over the dorsum with fine asperities on the gular area and no ventral asperities according to Pickersgill (2005). The same paper describes colour variations of *A. aureus* as yellow-gold dorsally, and the present study was able to confirm this finding.However, whereas Pickersgill (2005) states that tibia bands are characteristic of *A. aureus*, the present study made a different finding. Both species showed tibial bands. Again in the same paper, Pickersgill averst that the southern African *A. delicatus* specimens show weaker dorsal markings and lack lumbar patches (Pickersgill, 2005), whereas the present study found noted lumbar patches were on *A. delicatus* specimens. Pickersgill (2005) describes gular shape (2005) as "squarish" or triangular in both species, and the present study confirms this. However, it is suggested that a more accurate term–reuleaux triangular–should be used to describe this shape.

Previous studies conducted by Pickersgill, (2007b) showed *A. aureus* had a TL/SVL of 37-46% (41%, n=43), HW/TL of 63-70% (66%, n=16) and SVL for males 17-24 mm (21 mm, n=45). The current study showed the TL/SVL is 36-41% (39%, n=11), HW/TL is 71-84% (77%, n=11) while SVL for males stood between 19.6-23.3 mm (22 mm, n=11). In Picersgill (2007b) TL/SVL of 37-56% (43.5%, n=344), HW/TL of 57-83% (69%, n=186) and SVL for males 15-22 (19 mm, n=299) was established in the case of *A. delicatus*. The present study found the TL/SVL to be 23-40% (35%, n=6), HW/TL is 81-127% (90%, n=6) and SVL for males to be between 17.3 and 20 mm (18.9 mm, n=6). Table 4.6 illustrates these results.

Table 4.6: Comparisons between Pickersgill (2007b) and the current study, described by the mean ($\bar{X}$) and minimum to maximum ranges

	Pickersgill (2007b)	Current study
	A. aureus	
SVL (mm)	21 mm (17-24 mm, n=45)	22,01 mm (19,62-23,29 mm, n=11)
TL/SVL	41% (37-46%, n=43)	39% (36-41%, n=11)
HW/TL	66% (63-70%, n=16)	77% (71-84%, n=11)
	A. delicatus	
SVL (mm)	19 mm (15-22 mm, n=299)	18,85 mm (17,28-20,03 mm, n=6)
TL/SVL	43.5% (37-56%, n=344)	35% (23-40%, n=6)
HW/TL	69% (57-83%, n=186)	90% (81-127%, n=6)

The present study found individual *A. delicatus* in sympatry with *A. fornasini*. Pickersgill (2005) notes that both *A. aureus* and *A. delicatus* were recorded in the Ndumo Game Reserve. However, during the present study, no evidence was recorded of *A. delicatus* within this area. Initially, some frogs were identified as *A delicatus*, but molecular studies revealed that all specimens collected at Ndumo Reserve were in fact *A. aureus.* Misidentification may have occurred in the past due to the close morphological relation between the species. A specimen found by Pickergill (2005) in Ndumo was identified as an *A. aureus* juvenile, and the author stated that this might be an incorrect identification. Given the results of the present study, it appears that this could have been correct, while Ndumo specimens identified as *A. delicatus* may have been incorrect. *Afrixalus delicatus* inhabit both tropical and sub-tropical bush and grasslands (Pickersgill, 2005). The present study found *A. delicatus* specimens within these areas. However, our data suggest that *A. delicatus* is confined to the coastal areas whereas, as anticipated, *Afrixalus aureus* occurred in grasslands and forest areas.

4.5.2 Bioacoustics

During sampling, all the recorded males vocalised from dusk until late in the night (between 19:00 to 01:00). Males usually called from elevated positions on vegetation close to the water (Du Preez & Carruthers, 2017). The oscillo- and spectrogram visualisations (see Figure 4.4) show that *A. aureus* and *A. delicatus* advertisement calls differ significantly. *Afrixalus delicatus* shows a two-part (biphasic) call structure. This could suggest that it serves two purposes. Narins and Capranica (1978) were the first to report the two-part diphasic call of the Puerto Rican leptodactylid, *Eleutherodactylus coqui*. They found that the first "co" note functioned as male-male communication. The second "qui" note was used for attracting conspecific females (Littlejohn & Harrison, 1985). In the case under examination here, the first "zip"-like component acted as the male-male communication signal (see also Backwell, 1988). Similar biphasic advertisement call properties were reported by Passmore and Carruthers (1979) for *Amietia delalandii* (then *Rana angolensis*). In fact, at one stage the call was identified as two different calls due to their sequential inconsistency. The present study observed a similar pattern in the case of *A. delicatus*.

Males give the "zip" call early during calling nights. This acts as an indicator as to where each male is located (Backwell, 1988). Males can use this call to determine the optimal position for calling. This call is significantly shorter than the second "trill" part of the call (see Figure 4.7). The overall call structure is more complex. *A. delicatus* employs the second part of its call, identified as a "trill", for male-female communication. Males use more energy for this call as it is longer, albeit also simpler. In certain contexts the "zip" call is less common than the "trill" call, for instance when no females are present (Backwell, 1988). Rand and Ryan (1981) found that females of the Central American leptodactylid, *Physalaemus pustulosus*, prefer complex calls above single note calls. However, the present study found the opposite to be the case. *Afrixalus aureus* has a shorter buzz-like "trill". They have shorter call duration, but emit more calls within a specific time period, accompanied by shorter inter-call intervals. Presumably, *A. aureus* spends its energy on multiple calls rather than one long prolonged call, in contrast to *A. delicatus*.

More recent literature such as Backwell (1988) and Pickersgill (2005) uses pulses to describe notes in a way that the present study emulates. The term "pulse", used by Backwell (1988) and Pickersgill (2005), is therefore equivalent to the term "note" as employed in the present study. One is led to surmise that these studies were based on a call-centred approach in describing call parameters. The call-centred approach does not identify major sub-units as a call, but rather as a call series, as the silent intervals are much shorter than the units themselves. Note-centred approaches define one coherent sound entity as a call and allocates subunits within as notes (Köhler *et al.*, 2017). However, Backwell (1988) and Pickersgill (2005) exclude the term "notes", ignoring the fact that notes form distinguishable subunits within a call (Köhler *et al.*, 2017). The present study therefore presumes that the term "pulses" means "notes". A reason for this development could be that past studies conducted on members of *Afrixalus* were made in the absence of present technological analytical software. Within a study by Backwell (1988), for example, the two call types of *A. delicatus* were defined as two different notes with distinct pulses. However, the present study condiders these to be two separate calls, since two calls occurring after each other showed inconsistency as a result of more refined observing apparatuses.

Afrixalus aureus utters a greater number of pulsed notes than *A. delicatus*, which utters pulsatile notes. The current study is able to endorse Pickersgill (2005), who established that *A. aureus* has a significantly lower dominant frequency when compared to *A. delicatus*. The explanation appears to be that *A. aureus* is larger than *A. delicatus*. As is known, call frequency is negatively correlated with body size (Wells, 2007; Hoskin *et al.*, 2009). Another potential explanation for this difference resides in distribution patterns. Vargas-Salinas and Amezquita, (2013) have demonstrated that abiotic background noise (wind, streams and sea waves) leads to behavioural shifts, and *Afrixalus delicatus* was collected closer to the coast. More energy will be used by senders in high noise areas affecting frequency channels (Vargas-Salinas & Amezquita, 2013).

Pickersgill (2005) has shown that *A. aureus* has a CD of 0.2-0.83 s and 4 -14 pulses per call (mean 8), a pulse rate of10-29 pulses/s, a pulse duration of 21-30 ms and a dominant frequency occurring between 2.8-5.0 kHz. The present study has found a CD of 0.412 s (0.07-0.655 s), showing 2-12 ($\bar{x} = 7.346$) notes per call, a note rate of 13.0111-28.571 (18.17), note duration of between 10-120 ms ($\bar{x} = 16$ ms) and a dominant frequency of 3.2-5 kHz (4.3 kHz).

Again, Pickersgill (2005) found that *Afrixalus delicatus* had a CD of 0.6-21.7 s with 10-684 pulses per call, a pulse rate of 14-35 pulses/s ($\bar{x} = 27$), a pulse duration of 12-23 ms and a dominant frequency occurring between 3.35-5.35 kHz , while the present study found a CD of 2.8 s (0.299-6.219 s), notes per call ranging from 7 to 149 ($\bar{x} = 61.811$), a note rate of 12.696-25.837 (21.439), a note duration between 0.1-31 ms ($\bar{x} =$ 0.7 ms) and dominant frequency between 4.8-5.4 kHz (5.1 kHz).

Table 4.7: Acoustic comparisons between Pickersgill (2005) and the present study described in terms of the mean ($\bar{X}$) and minimum to maximum ranges

	Pickersgill (2005)	Current study
	A. aureus	
CD (sec.)	0.2-0.83	0.412 (0.07-0.655, n=23)
Note per call	8 (4-14)	7 (2-12, n=23)
Note rate (per sec.)	10-29	18.171 (3.0111-28.571, n=23)
Note duration (ms.)	21-30	16 (10-120, n=23)
Dominant frequency (kHz)	2.8-5.0	4.3 (3.2-5, n=23)
	A. delicatus	
CD (sec.)	0.6-21.7	2.8 (0.299-6.219, n=12)
Note per call	10-684	61.811 (7-149, n=12)
Note rate (per sec.)	27 (14-35)	21.439 (12.696-25.837, n=12)
Note duration (ms)	12-23	0.7 (0.1-31, n=12)
Dominant frequency (kHz)	3.35-5.35	5.1 (4.8-5.4, n=12)

This study examined the difference between two *Afrixalus* species. Vocally, the species can be separated on the basis of their call structure in terms of "trill" calls, including call duration, inter-call interval, number of notes, note duration and the number of pulses per note, even though minimal morphological differentiation characterizes the species. This finding potentially demonstrates the value of combining acoustic properties and molecular DNA barcoding. Acoustic parameters combined with molecular findings can therefore aid in future taxonomic studies, as has been mentioned. It has indeed been demonstrated over the past years that genetic data combined with morphological and acoustic data aid in identifying cryptic species (see Streicher *et al.*, 2012 Rakotoarison *et al.*, 2015, Forlani *et al.*, 2016, Bosch *et al.*, 2017, Minter *et al.*, 2017). Further studies should investigate sympatric populations and possible hybridisation between *A. aureus* and *A. delicatus.* To conclude, this study illustrated the value of combining acoustic data with morphological data in frog taxonomy.

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CHAPTER 5: GENERAL DISCUSSION AND CONCLUSION

The moonless sky dotted with bright stars on a cool and damp Winter's night
The breeding frogs in the roadside drain singing at the sound of passing footsteps they grow quiet
I shine my torch but I cannot see them they hide from the glare of the light
The secret of survival for these tiny amphibians is to remain concealed and out of sight

Of nocturnal or diurnal predators their elusiveness is their only means of defence
In wet weather in their breeding season they sing in the drain by the roadside fence
Their songs to me are so familiar in ponds, dams and drains they are rife
Their tiny young their genes will carry and insure the continuance of life.

Yet I seem to know little about them and of Nature even less I seem to know
And at the magical powers of the great Nature Goddess the wonder in me only grow
Her colours they change with the Seasons in her vastness I feel like one grain of sand
All life on this Planet comes from her that live in Ocean and on Land.

The night is dark and the frogs are singing I hear them in the roadside drain
In their breeding season in late Winter they sing in the drizzling rain
Very small and quite shy amphibians and hard to see and hard to find
Their elusiveness is to their advantage it insures the survival of their kind.

Francis Duggan
On Hearing Frogs At Night

This dissertation examined the value of using auditory recording systems (Song Meters) to survey anuran communities within north-eastern KwaZulu-Natal. Chapter 2 asked how these methods could be used to monitor anuran diversity in north-eastern KwaZulu-Natal. Chapter 3 examined how meteorological variables act as cues for the start of the breeding season. Chapter 4 presented a case study on how acoustic recordings of species, prior to and also within the present study, aid in resolving taxonomic confusion in a group of Hyperoliids.

5.1 The anuran community of north-eastern KwaZulu-Natal

Although previous surveys were conducted in this area (Lambiris, 1989; Drinkrow & Cherry, 1995; Minter *et al*., 2004; Russell & Downs, 2012; Netherlands, 2014), they did not employ automated recorders over a variety of locations and an extended period, making the present study unique for this region. The study found, as mentioned, that species compsition differed at each of the six localities, while two species were found at the majority of the sites, namely *Leptopelis mossambicus* and *Ptychadena anchietae*. It is important to note that extreme drought conditions resulted in a relative lack of detecting species. Not all possible species were detected at the sites where they are known to occur. The study duration covered all potential breeding seasons within one year, due to the permanence and durability of the Song Meters.

A total of 29 out of the 54 known species within this area were detected. It would be beneficial to conduct a follow-up survey during normal or La Nina periods and then compare the results obtained with those of the current study. Data could also be improved if surveys were to include habitats not studied by the present survey. These habitats include forests where undetected terrestrial species will be noted and riverine systems that would include aquatic and semi-aquatic anurans. Surveys could further be enhanced by adding active sampling methods such as drift fences, visual encounters and dip netting. As mentioned, the present survey detected only 54% of the species recorded in the past. Part of the reason for this is that locations that were selected as ephemeral ponds ran dry and remained so for the duration of the survey, resulting in non-detection of a number of water-dependent species.

Nevertheless, as demonstrated in Chapter 2, employment of automated recorders proved to be a practical, non-invasive method for medium- to long-term estimates of anuran diversity despite the drought. Acoustic surveys moreover gave insight into the temporal activity patterns of the detected species: the present study endorses that this method can be used to inform active sampling surveys.

5.2 The role of the environment

Chapter 3 shows that atmospheric conditions that acted as cues for the commencement of vocal activity among anurans of the Umlalazi Nature Reserve are in fact species-specific. This species-specific adaptation helps anurans breed during ideal conditions, because it reduces risk such as desiccation, sound distortion, and so forth (see also Oseen & Wassersug, 2002). As mentioned, the present study recorded below normal rainfall and above normal temperatures due to an El Niño event, which posed the problem of detecting a considerable number of species, not least since ambient temperature and rainfall are the most important environmental factors that impact upon breeding, activity and distribution (Oseen & Wassersug, 2002).

Most of the species were active at low levels of anthropogenic noise interference and called during the evenings. Furthermore, the calling activity of most of the recorded species increased after heavy rainfall, regardless of the season. Many studies over the past few years have shown the effects of environmental change on the behaviour of anurans (Henzi *et al*., 1995; Oseen & Wassersug, 2002; Minter, 2011; Yetman & Ferguson, 2011; Lemckert *et al*., 2013). However, the present study is the first of its kind in the north-estern parts of KwaZulu-Natal that focuses on multiple species and different meteorological variables. Henzi *et al*. (1995) did conduct a similar study, but its focus was restricted to *Hyperolius marmoratus* males over a shorter period of time, namely 28 nights.

The present study contributes to addressing the gap in this field of South African anuran behavioural studies and the effects of environmental change. The latter is of major concern, given that climate change will affect future anuran populations (Minter, 2011; Ospina *et al*., 2013). The study therefore reaches the important conclusion that further studies are necessary to investigate abiotic changes and the effect these will have on South African anurans. The present study will also improve the detectability of anurans in future surveys, not least because it points to the ideal season, time and atmospheric conditions for detecting species of interest in the Umlalazi Nature Reserve.

5.3 Bioacoustics and taxonomy

The study employed morphological and acoustic analyses to document the relationship between and variation of two *Afrixalus* species within the *Afrixalus stuhlmanni* complex. *Afrixalus aureus* and *A. delicatus* are primarily distributed within the northern parts of KwaZulu-Natal. Previous studies demonstrated the close relationship between *Afrixalus aureus* and *Afrixalus delicatus*. Their close morphological similarity has led to misidentification in the past and incorrect labelling of museum specimens. The present study corrected this situation, carefully identifying similarities and differences between the species by means of acoustic and morphological comparison.

An interesting find, as demonstrated in Chapter 4, is that *A. aureus* and *A. delicatus* do not occur sympatrically. *Afrixalus delicatus* is distributed along the coastal belt (at Sodwana and Monzi), while *A. aureus* is an inland species. Additional acoustic and morphological data aided in showing the differences between the two species. Nonetheless, most of the findings largely endorse valuable studies conducted by Pickersgill (1996; 2005; 2007a; 2007b) and Backwell (1988).

Consider once more that the two species show a high degree of morphological similarities. However, *A. aureus* males tend to be larger than *A. delicatus* males. When it comes to call structure, however, the differences are considerable. The larger *A. aureus* males have a lower, singular short pulsed "trill" call. Interestingly, *A. delicatus* has a biphasic call structure. It uses the "trill" call for mate recognition. This prolonged call consists of high pitched, pulsatile notes. Bioacoustic analysis of the two species further showed significant differences in the call parameters of each species.

These results indicate the importance of more extensive studies on these species. Future studies should include different individuals across geographical ranges. These studies could conclude whether populations of the two species found in north-eastern KwaZulu-Natal are the same as those found in northern Africa. In conclusion, this study highlights the need to clarify some taxonomic confusions between the *Afrixalus* complexes.

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ANNEXURES

Appendix A: Permits for the study.

ORIGINAL

EZEMVELO KZN WILDLIFE
Conservation, Partnerships & Ecotourism

ORDINARY PERMIT

Fee:	R 50.00	Permit No:	OP 4092/2016
Receipt No:	4147/2016	Contact:	Miss S.M. Hughes

This permit is issued in pursuance of the provisions of the Nature Conservation Ordinance No 15 of 1974 , Chapter 7 and the Regulations framed thereunder.

The permit is issued to:

ID Number: 8905155123088

Mr Edward Charles Netherlands
North-West University
Zoology
Private Bag X6001
Potchefstroom
2530

Residential Address
North-West University
Zoology
Room G16, Building EG
Potchefstroom Campus
Potchefstroom
2531

In the capacity of Researcher

To Collect, Extract blood samples, Release and Export the following species of Amphibians Invertebrates and Reptiles

ALL SPECIES OF FROGS

10 (Ten) per species per protected area or other locality throughout KwaZulu-Natal EXCLUDING KZN Wildlife protected areas but including the following protected areas: Tembe Elephant Park, Vernon Crookes Nature Reserve, Ndumo Game Reserve, Royal Natal National Park, Hluhluwe-iMfolozi Park and iSimangaliso Wetland Park.

REPTILES

10 (Ten) per species per protected area or other locality throughout KwaZulu-Natal EXCLUDING KZN Wildlife protected areas but including the following protected areas: Tembe Elephant Park, Vernon Crookes Nature Reserve, Ndumo Game Reserve, Royal Natal National Park, Hluhluwe-iMfolozi Park and iSimangaliso Wetland Park.

Please read the Terms and Conditions under which this Permit is issued

ISSUED at PIETERMARITZBURG, KwaZulu-Natal, on 08 November 2016

for CHIEF EXECUTIVE

Permit Holder

EZEMVELO KZN WILDLIFE PERMITS OFFICE
PO Box 13053, Cascades, 3202, Pietermaritzburg, KwaZulu-Natal.
Tel +27 33 845 1320 / 1324. Fax: +27 33 845 1747. Fax to Email: 086 529 3320
Email: permits@kznwildlife.com. Website: www.kznwildlife.com

OP 4092/2016 ***Page 1 of 4***

ORIGINAL

Conservation, Partnerships & Ecotourism

INVERTEBRATE

10 (Ten) per species per protected area or other locality throughout KwaZulu-Natal EXCLUDING KZN Wildlife protected areas but including the following protected areas: Tembe Elephant Park, Vernon Crookes Nature Reserve, Ndumo Game Reserve, Royal Natal National Park, Hluhluwe-iMfolozi Park and iSimangaliso Wetland Park.

TERMS AND CONDITIONS UNDER WHICH THIS PERMIT IS ISSUED

1. It is valid only:

   (i) from : 08 November 2016
   to : 07 November 2017

   (ii) in the original

   (iii) if all **4** pages are signed by the permit holder named above

   (iv) to the permit holder named above and the following Nominees :

   Prof. L.H du Preez
   Dr D Kruger
   Dr C. Cook
   Dr J Van As
   Dr C Weldon
   J. Reeder
   Mr W. Pretorius
   Mr F Phaka

2. By signing the permit or licence the holder accepts, and agrees to comply with the conditions under which it is issued.

3. Permit to be returned to E KZN Wildlife, P O Box 13053, Cascades, 3202, upon expiry for renewal or cancellation.

4. Permit shall be carried by holder, or the specified nominees, at all times during use.

5. Outside of E KZN Wildlife areas, use of this permit is subject to landowner's or controlling

Please read the Terms and Conditions under which this Permit is issued

ISSUED at PIETERMARITZBURG, KwaZulu-Natal, on 08 November 2016

for CHIEF EXECUTIVE | Permit Holder

EZEMVELO KZN WILDLIFE PERMITS OFFICE
PO Box 13053, Cascades, 3202, Pietermaritzburg, KwaZulu-Natal.
Tel +27 33 845 1320 / 1324. Fax: +27 33 845 1747. Fax to Email: 086 529 3320
Email: permits@kznwildlife.com. Website: www.kznwildlife.com

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International Air Transport Association live animals regulations and any other Act relevant to the transport, keeping, handling, transport, care and/or welfare of the said species.

16. No collecting is permitted within the road reserve which is a strip 30 (thirty) metres either side of a public road, no matter how small or remote the road may be.

17. No collecting is permitted in the wilderness areas within the Protected Area. For confirmation of boundaries of the wilderness area contact the Officer in Charge.

18. No specimens collected or captured or exported under this permit may be sold.

19. If possible, dead by-catch is to be distributed to the relevant experts for those groups in South Africa under the same conditions of this permit. Please enclose a copy of this permit and the locality and field data associated with the by-catch with the by-catch specimens.

20. This permit/licence/certificate is issued subject to compliance with all other relevant legislation and does not preclude the permit holder from adherence thereto.

Please read the Terms and Conditions under which this Permit is issued

ISSUED at PIETERMARITZBURG, KwaZulu-Natal, on 08 November 2016

for CHIEF EXECUTIVE

Permit Holder

EZEMVELO KZN WILDLIFE

Conservation, Partnerships & Ecotourism

ORIGINAL

authority's written permission.

6. Prior to collecting in areas under the control of the E KZN Wildlife the holders shall contact the Officer-in-Charge of the area at least 48 (Forty-eight) hours before commencing, and shall comply with any conditions which the Officer may impose at his discretion. The officer may refuse collection or capture at his or her discretion.

7. At least one representative specimen (preferably at least one male and one female) of each species collected from each locality must be lodged with a recognised South African museum/herbarium. Holotype specimens, and half the number of paratype specimens, of any new species MUST BE DEPOSITED with a recognised South African museum/herbarium, and may only leave South Africa on a loan basis. These specimens are to be deposited in the SA museums within a year of publishing the description of the new species. The holder shall provide the Chief Executive Officer, KZNNCS with the name of the museum at which the specimens have been lodged, and the accession number of each specimen. This condition relates to unavoidable by-catch of non-target organisms as well.

8. A copy or copies of any publication arising from the authority herein contained will be made available to E KZN Wildlife.

9. Should renewal of this permit be desired, a minimum of one month's notice is required.

10. (i) Reserving accommodation within E KZNWildlife areas is entirely the responsibility of the permit holder. Booking is obtainable at the Central Booking Office, Telephone 033 8451000.(ii) Any assistance required from Board staff will be subject to other demands on the Officer's time and must be arranged in advance with him/her.

11. This permit is valid only if an export/import permit has been issued by the export/import province/country if the legislation applicable in such province/country prescribes such permit.

12. Valid for one consignment only. .

13. Holders shall provide the Chief Executive, with a named list of every specimen collected (including the class, order, family, gender and age), the geographical co -ordinates (to seconds accuracy) of each collection locality and dates of collection, as laid out in the following table. A Global Positioning System with the WGS84 Datum should be used wherever possible to determine the geographical co-ordinates of the collection sites; please state the method used.

14. SPECIMEN (museum Accession) - COLLECTION DATE (ddmmyy) - SPECIES - LOCALITY - LATITUDE (Seconds Accuracy) - LONGITUDE (Seconds Accuracy). Holders are requested to provide additional information, such as the habitat in which each specimen was collected and abundance or relative abundance data (providing standardised sampling methods are used) with the list.

15. The transportation of any live specimen by air shall be done in accordance with the

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Appendix B: Summary of north-eastern KwaZulu-Natal anuran activity.

	Art sten	Art wah	L moss	L nata	Br adsp	Br moss	Br sop	Scl gar	Scl gutt	Hem gutt	Hem mar	Af aur	Af deli	Af for	Hyp mar	Hyp pick	Hyp pus	Hyp tube	Kas sen	Phr biff	Phry mab	Phry nata	Pty anc	Pty mas	Pty moss	Caco boet	Caco nan	Tom krug	Chiro xera
St lucia																													
Nov-15	0	0	25	0	23	0	18	0	6	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Dec-15	0	34	135	3	24	7	24	0	141	16	0	0	0	0	0	0	0	38	0	0	0	0	2	0	0	0	0	0	14
Jan-16	6	0	144	4	0	0	26	0	102	30	0	0	0	0	0	0	0	31	0	0	0	0	0	0	0	0	0	0	0
Feb-16	0	0	237	37	0	0	72	0	6	56	0	0	0	0	0	0	0	74	0	0	0	0	30	0	0	0	0	0	33
Mar-16	0	11	151	8	0	2	32	0	47	37	16	0	0	0	40	0	0	294	0	0	0	0	25	0	0	0	2	0	0
Apr-16	37	0	18	2	0	0	0	0	0	4	0	0	0	0	4	0	0	171	0	0	0	0	0	0	0	0	23	0	0
May-16	0	23	0	0	0	1	0	0	1	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jun-16	0	30	0	0	0	0	2	0	2	40	20	0	0	0	17	0	0	2	0	0	0	0	0	0	0	4	0	0	0
Jul-16	2	61	0	0	8	0	17	0	67	91	7	0	0	0	5	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Aug-16	4	15	1	16	0	0	31	0	46	54	0	0	0	0	37	0	0	3	0	0	0	0	39	0	0	0	1	0	0
Sep-16	23	58	17	8	0	8	73	0	105	65	26	0	0	0	10	0	0	45	0	0	0	0	53	0	0	0	12	0	0
Oct-16	23	0	236	0	0	0	135	0	217	127	10	0	0	0	53	0	0	133	0	0	0	0	70	0	0	11	6	0	0
Nov-16	0	0	138	0	0	0	61	0	88	42	0	0	0	0	54	0	0	76	0	0	0	0	29	0	0	45	0	0	0
Umlalazi																													
Nov-15	0	83	16	120	0	0	0	0	17	12	0	0	0	0	18	2	0	17	0	0	0	0	0	0	0	0	0	0	0
Dec-15	0	285	192	512	0	0	8	0	21	93	2	0	6	0	44	0	0	193	0	0	0	0	18	0	0	0	0	0	0
Jan-16	0	118	188	342	0	0	2	0	47	62	0	0	0	0	30	0	0	194	0	0	0	0	0	0	0	0	0	0	0
Feb-16	0	66	35	81	0	0	0	0	0	12	3	0	0	1	43	0	0	105	0	0	0	0	0	0	0	0	0	0	0
Mar-16	0	252	0	6	0	0	1	0	10	0	34	0	30	0	20	2	0	50	0	0	0	0	0	0	0	0	0	0	0
Apr-16	0	65	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
May-16	0	56	0	10	0	0	0	0	0	0	1	0	0	0	281	37	0	52	0	0	0	0	0	0	0	0	0	0	0
Jun-16	0	174	0	41	0	0	0	0	2	0	0	0	0	0	418	4	0	30	0	0	0	0	0	0	0	0	0	0	0
Jul-16	0	470	0	365	0	0	0	0	8	12	16	0	0	5	413	216	0	9	0	0	0	0	0	0	0	0	0	0	0
Aug-16	0	461	0	607	0	0	0	0	18	19	4	0	0	2	403	171	0	0	0	0	0	0	0	0	7	0	0	0	0
Sep-16	0	542	0	622	0	0	0	0	52	51	0	0	0	0	237	398	0	45	0	0	0	0	23	0	0	0	0	0	0
Oct-16	0	581	4	509	0	0	0	0	18	19	0	0	0	0	189	462	0	417	0	0	0	0	4	0	3	0	0	0	0
Nov-16	0	249	0	242	0	0	0	0	6	19	3	0	0	6	33	115	0	245	0	0	0	0	10	0	0	0	0	0	0
Ndumo																													
Nov-15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dec-15	0	0	10	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	36	0	0	0	40	0	19	0	0	0	18
Jan-16	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Feb-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mar-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Apr-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
May-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jun-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jul-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aug-16	0	0	0	0	0	0	0	0	4	0	0	0	0	0	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sep-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oct-16	0	0	0	0	0	0	0	0	0	2	31	0	0	0	3	0	0	0	0	0	0	0	43	0	37	0	0	0	0
Nov-16	0	0	6	0	0	0	0	0	2	8	2	99	0	0	0	0	0	0	11	8	47	0	64	0	39	0	0	0	32
Dec-16	0	0	0	0	0	0	0	0	4	3	0	2	0	0	0	0	0	0	8	14	52	0	58	0	3	0	0	0	43
Jan-17	0	0	0	0	0	0	0	0	4	0	0	81	0	0	0	0	0	0	34	3	45	0	57	0	0	0	0	0	37
Feb-17	0	0	0	0	0	0	0	0	0	0	0	19	0	0	0	0	0	0	7	0	16	0	56	0	35	0	0	0	28
Mar-17	0	0	0	0	0	0	0	0	0	0	0	8	0	0	15	0	8	0	11	19	58	0	68	0	33	0	0	0	55
Apr-17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	68	0	0	0	0	0	0	0	0
May-17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0
Tembe																													
Nov-15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dec-15	268	0	356	0	0	0	0	32	2	0	5	0	0	12	0	0	0	129	27	0	0	0	18	0	0	0	0	0	0
Jan-16	314	0	292	0	0	0	0	0	0	0	6	0	0	0	0	0	0	66	0	0	0	0	0	0	0	0	8	0	0
Feb-16	304	0	167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	91	0	0	0	0	0	0	0	0	26	0	0
Mar-16	370	0	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	159	0	0	0	0	10	0	3	0	41	6	0
Apr-16	389	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
May-16	157	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jun-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jul-16	196	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	0	0	0	0	0
Aug-16	221	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	103	0	0	0	0	0
Sep-16	218	0	68	0	0	0	0	47	2	0	0	0	0	0	0	0	0	5	0	0	0	0	0	45	0	0	7	0	0
Oct-16	219	0	137	0	0	0	0	55	20	0	0	0	0	0	0	0	0	2	0	0	0	0	16	0	21	0	2	26	0
Nov-16	160	0	184	0	0	0	0	0	2	0	0	0	0	0	0	0	0	130	0	0	0	0	0	0	9	0	7	0	0
Dec-16	214	0	234	0	0	0	0	0	0	0	0	0	0	0	0	0	0	113	0	0	23	0	0	0	0	0	6	0	0
Kosibay																													
Nov-15	46	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	6	0	0
Dec-15	153	12	167	0	0	0	0	0	7	0	0	0	0	0	24	0	0	31	0	8	0	0	0	0	0	0	14	0	0
Jan-16	28	0	115	0	6	0	0	0	7	0	0	0	0	0	16	0	0	98	0	24	0	0	0	0	0	0	13	0	0
Feb-16	8	10	130	0	0	0	0	0	11	0	0	0	0	0	6	0	0	53	0	5	0	0	0	0	0	0	5	0	0
Mar-16	0	28	87	0	0	0	0	0	0	0	0	0	23	0	0	0	0	76	0	0	0	5	0	0	0	0	13	0	0
Apr-16	0	29	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
May-16	0	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jun-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jul-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aug-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sep-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oct-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nov-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dec-16	0	0	40	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	6	0	0	0
Jan-17	39	0	290	0	0	0	0	0	4	0	0	0	0	0	0	0	0	26	0	0	0	0	0	0	0	2	6	0	0
Feb-17	32	0	207	0	8	0	0	0	0	0	0	0	70	0	0	0	0	7	0	4	0	0	1	0	0	0	0	0	0
Mar-17	16	0	122	0	0	0	0	0	0	0	0	0	194	0	0	0	0	2	0	0	0	0	4	0	0	0	4	0	0
Apr-17	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
May-17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bonamanzi																													
Nov-15	0	62	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Dec-15	0	129	62	0	0	0	0	0	0	0	41	0	0	0	65	0	0	0	0	0	0	0	33	26	6	0	72	0	0
Jan-16	0	49	0	0	0	0	0	0	0	0	3	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	37	0	0
Feb-16	12	0	0	0	0	0	0	0	0	0	25	0	0	0	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mar-16	4	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Apr-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
May-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jun-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jul-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aug-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix C: Acoustical and sequence data of *A. aureus* and *A. delicatus*.

ID after Barcoding	AACRG #	Field number	Call Record	Locality	Sequences
Afrixalus aureus	AACRG2972	AL160206O1	n/a	Roadside creche, Mbodla	COI and 16S
Afrixalus aureus	AACRG2510	AE131117A1	n/a	Ndumo site 4	COI and 16S
Afrixalus aureus	AACRG1815	AL120217O1	n/a	Ndumo site 1	COI and 16S
Afrixalus aureus	AACRG3214	AE161118H3	FROG0127	Bridge pond, Sihlenga	16S
Afrixalus aureus	AACRG3240	AE161126G1	FROG0188	Ndumu wetland	16S
Afrixalus aureus	AACRG3242	AE161201A1	FROG0197	Ndumo Fence Pan	16S
Afrixalus aureus	AACRG3243	AE161201A2	FROG0198	Ndumo Fence Pan	16S
Afrixalus aureus	AACRG3255	AW161203A1	FROG0206	Ndumo Wetland	16S
Afrixalus aureus	AACRG3256	AW161203A2	FROG0207	Ndumo Wetland	16S
Afrixalus aureus	AACRG3257	AW161203A3	FROG0209	Ndumo Wetland	16S
Afrixalus aureus	AACRG3258	AW161203A4	FROG0211	Ndumo Wetland	16S
Afrixalus aureus	AACRG3259	AW161205A1	FROG0216	Ndumo Fence Pan	16S
Afrixalus aureus	AACRG3260	AW161205A2	FROG0217	Ndumo Fence Pan	16S
Afrixalus aureus	AACRG3261	AW161205A3	FROG0219	Ndumo Fence Pan	16S
Afrixalus aureus	AACRG3262	AW161205A4	n/a	Ndumo Fence Pan	16S
Afrixalus aureus	AACRG3263	AW161210A1	FROG0223	Ndumo Fence Pan	16S
Afrixalus delicatus	AACRG2945	AL160205J1	n/a	Kosi Bay Lodge Pan	COI and 16S
Afrixalus delicatus	AACRG3022	AL160210B1	n/a	Monzi irrigation pond	COI and 16S
Afrixalus delicatus	AACRG3049	AL160210O6	DON0224	Walley Pond, Monzi	COI and 16S
Afrixalus delicatus	AACRG3050	AL160210O7	DON0222	Walley Pond, Monzi	COI and 16S
Afrixalus delicatus	AACRG3182	AE161115I1	DON0306	Tolla se gat, Sodwana	COI and 16S
Afrixalus delicatus	AACRG3184	AE161115I3	DON0307	Tolla se gat, Sodwana	16S

Appendix D: Measurements and morphological data of *A. aureus* and *A. delicatus*.

	Afrixalus delicatus						*Afrixalus aureus*										
AACRG #	2945	3022	3049	3050	3182	3184	2972	2510	1815	3214	3240	3243	3256	3258	3260	3261	3263
SVL	20028,44	19358,47	17277,31	18116,55	19221,02	19107	23287,3	21754,07	22812,21	23115,72	23059,15	22206,1	21443,83	21862,13	22221,22	20780,31	19615,07
HW	5904,30	5976,42	5222,88	5964,55	6176,70	5833,05	6498,19	6358,41	6498,9	6350,68	7252,51	7116,77	6437,65	6159,52	6744,71	6274,54	6048,23
HL	3915,13	4638,73	4616,35	4838,31	5401,91	4426,62	5572,16	5048,84	5356	5835,19	5916,05	5486,54	5299,98	5464,41	5651,25	4592,51	5122,33
EAD	3608,04	3671,32	3174,80	3471,50	3641,23	3279,84	4624,33	3991,77	4112,8	3547,96	3615,92	3602,85	3839,26	3450,29	3873,7	3374,45	3769,82
EPD	5728,96	5686,67	5140,12	5491,56	5664,19	5441,34	5919,62	5847,24	5748,65	6078,83	5574,03	5746,06	5946,89	5797,28	5800,88	5504,78	5634,11
IOD	1928,71	2175,67	1889,60	2090,19	1991,19	1962,92	2513,89	2307,6	2304,05	2170,08	2068,07	2454,2	2398,37	2222,59	2198,64	1929,45	2078,01
ES (R)	1968,66	2585,8	2525,40	2409,50	2694,21	2491,36	2754,95	2588,4	2814	2687,39	2260,84	2561,08	2414,15	2552,62	2535,48	2022,27	2294,02
ES (L)	2602,67	2610,49	2643,38	2746,28	2378,79	2255,61	2981,97	2483,24	2530,58	2375,55	2350,26	2697,15	2489,62	2304,41	2234,3	2119,47	2530,39
PFL (ED) (R)	1854,93	1863,37	1775,79	1924,16	1840,82	1909,66	1884,57	18646,48	2242,06	2083,23	2081,11	1791,37	2002,23	2148,13	1874,11	1672,74	1783,38
PFL (ED) (L)	1619,56	1870,82	1871,63	1719,29	1607,59	1776,30	1727,49	1860,74	2253,54	2254,56	1876,31	1638,32	1856,58	2090,33	2116,7	1789,9	1729,7
IND	1515,17	1406,16	1245,65	1519,99	1342,98	1416,9	1400,74	1312,68	1500,61	1376,28	1351,63	1348,33	1434,64	1082,81	13631,9	1358,6	1501,64
NOD (R)	1545,82	1729,94	1403,79	1387,21	1483,96	1426,52	1821,3	1638,2	1950,62	1862,93	1732,09	1593,54	1684,82	1674,84	2039,42	1593,38	1727,56
NOD (L)	1533,67	1646,31	1556,56	1265,35	1335,91	1541,16	1747,22	2059,36	1785,18	1775,19	1624,63	1774,91	1779,96	1936,33	1873,64	1914,42	1821,04
NL (R)	1236,45	1342,8	1272,63	1231,32	1050,66	1203,35	1331,35	1351,75	1337,76	1311,49	1400,9	1050,04	1314,28	1290,55	959,84	1200,88	1017,7
NL (L)	1332,09	1265,74	1126,88	1165,75	1121,91	1185,22	1216,55	1381,41	1263,98	1448,41	1446,85	1104,26	1237,7	1348,13	991,89	1170,59	1229,42
FL (R)	12007,78	12450,69	11221,99	11841,63	11121,23	11657,67	14442,65	13059,17	13388,99	13196,39	14251,28	13440,41	13577,86	14153,32	13257,05	12903,72	12298,78
FL (L)	11961,72	12501,51	10149,35	11777,83	11207,53	11979,60	14495,33	12790,09	13272,71	12878,5	13717,54	13714,34	13585,24	13150,87	12128,59	12303,59	12122,53
T1L (R)	2537,74	2036,63	1973,16	1541,21	1893,84	1894,06	2036,83	2732,19	1901,01	2234,66	2264,99	2645,54	2370,25	2160,58	2595,09	2351,33	1665,31
T1L (L)	2476,49	2129,19	1953,30	1909,74	1909,04	2030,79	2716,6	2253,31	2331,84	2002,17	1767,53	2031,44	2139,99	1923,74	2586,25	2097,45	2087,18
DT1 (R)	1847,96	548,51	548,17	571,49	340,09	508,28	798,24	542,69	509,22	833,77	630,29	707,16	745,32	590,61	543,27	587,59	412,37
DT1 (L)	565,7	658,19	542,76	453,21	518,60	547,56	764,76	508,33	478,41	530,33	632,46	583,62	723,46	730,44	487,32	546,92	474,98
T2L (R)	2918,18	2711,27	2575,82	2448,65	2438,96	2891,55	3030,56	3120,6	2867,45	3255,63	3088,32	3535,53	2698,69	2576,98	3199,11	2902,65	2678,66
T2L (L)	3659,58	2876,82	2522,11	2477,62	2092,77	2761,35	3240,52	3134,83	3108,24	2871,38	2390,58	2848,53	2865,76	2846,21	3290,12	2700,39	2447,99
DT2 (R)	498,13	581,62	612,50	600,74	550,96	495,78	764,81	578,08	788,43	530,62	732,44	687,86	607,46	630,76	687,61	567,47	511,31
DT2 (L)	659,23	675,38	575,81	504,44	474,51	564,42	746,24	518,85	852,28	600,44	715,12	655,64	628,48	479,65	745,89	613,69	619,89
T3L (R)	4637,62	4605,32	3737,22	4199,62	3582,74	3997,45	5276,33	4376,46	4623,91	5205,98	5196,29	5406,33	4863,18	4722,37	4733,22	4900,71	4459,66
T3L (L)	5300,68	4465,87	3730,31	3986,13	3905,28	4072,51	5300,1	4693,95	5392,62	4353,73	4617,06	4963,52	4826,16	4476,05	5238,69	4775,28	4244,25
DT3 (R)	632,74	561,96	626,67	579,88	6445,59	472,32	662,45	531,36	893,88	528,36	909,2	812,55	712,1	721,29	574,44	558,56	536,78
DT3 (L)	687,57	703,65	738,19	395,71	612,66	550,5	866,29	671,68	942,07	769,22	846,41	669,19	737,52	702,82	734,9	636,08	524,54
T4L (R)	6407,12	6417,09	5192,04	5387,57	4256,65	5529,39	7362,47	6383,9	6761,15	7098,52	7340,14	7284,45	5694,9	6524,8	7058,63	6474,28	6256,3
T4L (L)	6756,4	6130,88	4531,39	5071,81	5244,13	5594,87	7571,12	6282,12	7305,51	6188,97	6695,26	7158,11	7190,67	6904,93	6751,78	6684,81	6002,53
DT4 (R)	662,9	546,99	717,31	441,96	632,10	601,09	972,47	513,28	789,7	845,49	1098,44	922,06	678,94	806,78	682,52	461,17	706,92
DT4 (L)	664,52	733,54	288,07	447,83	609,88	493,88	969,37	637,33	923,81	915,04	894,69	547,29	793,18	891,12	860,88	609,38	586,83
T5L (R)	5165,76	5195,59	4158,71	3753,40	3081,28	4251,63	5568,02	5416,88	5376,31	5608,54	6326,85	5744,66	5694,9	5042,58	5183,36	4939,71	5138,31
T5L (L)	5650,51	4538,52	4164,43	4094,56	3633,90	4347,19	5971,17	5492,78	6010,71	4852,06	4914,38	5523,38	5354,49	5528,91	5227,75	5156,18	4757,7
DT5 (R)	792,2	755,52	660,12	410,44	639,39	471,69	944,76	699,96	1013,09	810,35	991,04	618,23	781,27	928,84	731,67	514,48	670,01
DT5 (L)	634,66	860,33	790,94	520,24	569,15	532,45	915,33	815,8	1075,14	944,96	864,14	867,16	849,85	908,6	781,4	547,78	600,84
THL (R)	7159,56	7561,16	6972,18	7063,28	6617,42	6141,24	8821,58	8741,49	8796,5	7693,81	8087,05	8489,27	9390,02	9850,67	6734,11	8937,05	8551,86
THL (L)	6924,07	7764	6655,53	6924,15	6503,53	6042,76	8644,89	9378,68	8995,41	7706,69	9106,04	9334,42	9757,98	8274,99	7967,71	6775,17	8107,12
TL (R)	4643,61	6922,44	6445,23	7252,86	7435,60	7064,48	8930,13	8141,85	8439,59	8513,32	9216,03	8943,08	8787,04	8713,66	8033,58	8028,32	8020,12
TL (L)	5891,34	7449,17	6569,39	7198,11	75979,99	9635,2	9555,1	8303,51	8171,14	8579,61	8817,84	8995,44	8769,33	8611,41	7705,54	7904,5	7971,03
HL (R)	3788,95	4906,55	4268,23	4417,19	4754,98	4562,17	5370,84	5611,96	5907,66	5670,05	5584,59	5077,64	5541,16	4772,5	4819,62	5192,77	4943,93
HL (L)	3938,27	4714,39	4228,46	4448,34	4626,43	4622,08	5451,44	5305,87	5833,88	5297,27	5637,94	5326,65	5337,5	5266,37	4832	5281,48	4961,53
F3L (R)	3171,88	3142,19	4380,10	3343,32	3236,29	3213,36	4126,69	4497,04	4998,08	4430,86	4309,6	4238,89	4312,07	4153,13	3899,25	4215,08	4040,81
F3L (L)	2807,9	3129,26	3312,89	3247,49	3526,40	3175,5	4364,47	4513,32	4836,46	4832,28	4464,66	4585,25	3867,44	4450,96	3787,86	4152,89	4211,31
DF3 (R)	867,78	925,53	708,34	702,85	628,36	606,34	895	838,58	1071,75	1007,05	959,27	933,53	702,06	827,32	483,65	852,39	771,76
DF3 (L)	714,53	934,77	669,03	649,26	837,42	513,48	944,77	972,87	1171,02	872,23	922,75	1035,12	743,29	850,01	717,6	788,35	633,03
LHU (R)	3613,25	3299,82	2757,02	2894,46	3046,99	3012,97	4007,32	3312,8	4278,68	3506,19	3898,06	3491,48	3696,95	3548,62	3237,5	3598,48	3966,64
LHU (L)	3779,41	3311,62	3277,62	3139,11	3082,50	2860,46	4154,35	3552,8	3697,93	3942,19	3437,83	3565,39	3692,47	3780,87	3235,23	3385,72	3340,92
FOL (R)	3606,22	3239,83	2846,38	2955,85	3112,19	3087,57	4039,07	3607,82	3363,07	3531,94	3920,68	2894,17	3836,46	3828,39	3319,67	3389,57	3437,62
FOL (L)	3309,5	3058,47	2747,30	3579,76	3349,39	3321,84	4079,64	4023,16	3350,16	3322,35	3522,62	3132,96	3767,53	3254,78	3218,8	3310,04	3574,3

Appendix E: 13th Conference of the Herpetological Association of Africa. Bonamanzi, KwaZulu-Natal, 23-27 January 2017.

twice annually from four rivers: Vemvhane, Tugela, Bilanjil and Ribbon Falls. Presence/absence of *Bd* was determined through cytological screening of tadpole mouthparts. We found no statistical significant difference between the sites, but infection was more consistent between years at sites situated along popular tourist hiking trails. Interestingly, infection prevalence, although higher in summer, did not differ significantly between seasons. High altitude coincides with moderate temperatures resulting in a repressed fluctuation on the pathogen's prevalence between warmer and colder months. Rainfall, however was negatively correlated with infection prevalence. Growth rate ratios of tadpoles indicated that tadpole size and not developmental stage is one of the main drivers of infection. Persistently low to moderate infection prevalence and low pathogen virulence implies that *Bd* acts as an endemic infection in *A. hymenopus*.

An automated approach to amphibian diversity surveys: A case study for northern Zululand

WENTZEL W. PRETORIUS[1]*, DONNAVAN J.D. KRUGER[1] & LOUIS H. DU PREEZ[1,2]

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Global declines of amphibians refer to the phenomenon of the population declines and extinctions of amphibian species around the world. One of the challenges of these enigmatic declines is to assess and track the scope of global decline. Thus there is a dire need for non-invasive, rapid, and objective monitoring techniques. Kwa-Zulu Natal (KZN) has a rich anuran diversity, boasting over 50 frog species in the northern parts of the province. Historically, there have been few studies conducted to assess the biodiversity of KZN, where the last extensive study on frog diversity was performed more than 30 years ago in the northern parts of KZN. This study aims to evaluate 1) the anuran diversity in northern KZN by using automated recorders set at eight different localities, 2) the effects of atmospheric conditions on the frog communities and 3) the amount of spectral and temporal overlap of calls from sympatric frog communities to test the acoustic adaptation hypothesis. The recorders (SongMeter, Wildlife Acoustics) are equipped with an ambient temperature sensor together with a solar panel and rechargeable Lead-Acid batteries to allow long term powering of the recorders. Data will be analysed in the laboratories using Song Scope analysis software and Kaleidoscope Pro software. The data analysed from the recordings can be used to identify the different species present and their abundance at the different localities in northern KZN. These data will then be compared with historical data from previous studies done in the same area. Furthermore, the calling activity will also be combined with temporal environmental data to determine the seasonal patterns together with their mating activity. Recordings of the species will also be described in detail to determine the vocal repertoire and call characteristics of each species. Partitioning of species calling at the same time can be determined together with their characteristics and how they react to one another.

Appendix F: Joint Biodiversity Information Management Forum (BIMF) and Foundational Biodiversity Information Programme (FBIP) 2017: Salt Rock Hotel and Beach Resort, Durban (Postgraduate Forum, Presentation).

composition differs between the existing savanna and the encroached system; determining which environmental variables underlie the differences between ant assemblages and specificity of species in existing systems and also identifying the indicator ant species which are associated with existing savanna and encroached system. A total of six paired sites were chosen for sampling representing open and closed habitat. Sampling was conducted in January 2017 (wet season) and it will be done in September 2017 (dry season). Each site was replicated four times and there are ten traps per sampling grid.

A phylogeny-based comparative study of the phytochemical and pharmacological characteristics of *Croton* species occurring in KwaZulu-Natal

Tanya Mathe

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The genus *Croton* (Euphorbiaceae) has a long history of medicinal use in different parts of the world. In KwaZulu-Natal (KZN, South Africa) five *Croton* species are known to have medicinal value. Two commonly used species *Croton gratissimus* and *Croton sylvaticus* share a common Zulu name 'umahlabekufeni', which may potentially leads to misidentification of the bark used in medicinal trade. Standard barcodes for all the five *Croton* species occurring in KZN will be developed as a tool for the identification of plant parts. These will also be used in phylogenetic analysis to determine if the medicinal properties are clade related. An analysis and comparison of the phytochemical composition of different *Croton* species and different plant parts will be conducted, in order to determine whether the use of the bark can be substituted for the use of leaves. The latter would result in less destructive harvesting. Pharmacological activity will be determined through antimicrobial and antifungal activity tests. Geo-referenced BRAHMS occurrence dataset of KZN *Croton* will be compiled; this can be used to determine their distribution in KZN.

An automated approach to amphibian diversity surveys: A case study for northern Zululand

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Global declines of amphibians refer to the phenomenon of the population declines and even extinctions of amphibian species around the world. One of the challenges of this enigmatic decline is the need for continuous monitoring. Thus a dire need for non-invasive, rapid, and objective monitoring techniques are necessary. Kwa-Zulu Natal (KZN) has rich anuran diversity, boasting over 50 frog species in the northern parts of the province. Historically, there have only been few studies to determine the frog biodiversity of KZN, with the last extensive study conducted more than 30 years ago. This study aims to determine the diversity and effects of atmospheric conditions on the frog communities in northern KZN, through the use of automated recorders set at eight different localities. The data analysed from the recordings are used to identify the different species present and calculate their abundance. Furthermore, the calling activity is combined with temporal (environmental) data to determine the seasonal patterns and mating activity. This monitoring data will provide valuable information on the distribution, diversity and ecological behaviour of frog communities in this area, ultimately assisting in their conservation.

A Taxonomic revision of the *Brevipalpus phoenicis* and *Brevipalpus obavatus* species complexes (Acari: Tenuipalpidae) in South Africa

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[1]*Biosystematics Division, ARC - Plant Protection Research,* [2]*School of Biological Sciences/Zoology, North-West University*

The overall aim of this study is to review the taxonomy and systematics of the flat mites (Acari: Tenuipalpidae) *Brevipalpus phoenicis* and *Brevipalpus obavatus* species complexes to clarify systematic inconsistencies previously reported for South Africa, and explore the relative importance of morphological and genetic traits for the systematics these mites. These flat mites represent some of the most economically important plant feeding

Appendix G: Poster presentation at the annual Joint Biodiversity Information Management Forum (BIMF) and Foundational Biodiversity Information Programme (FBIP) 2018: Cape St. Francis Resort, Eastern Cape.

DETERMINING THE ANURAN DIVERSITY OF ZULULAND USING PASSIVE ACOUSTIC SAMPLING.

Pretorius, W.W., Netherlands, E.C., Kruger, D.J.D. & Du Preez, L.H.

Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa.

Background

Most anuran species have the ability to produce species-specific vocal signals with unique acoustic patterns. These signals enables scientists to monitor anuran communities based on their acoustic behaviour. Anuran diversity within the northern parts of KwaZulu-Natal is out-dated. With the last comprehensive study done more than 30 years ago. Technology has improved significantly since then, leading to new ways to monitor diversity, e.g. Passive Acoustic Monitoring (PAM). PAM enables effective, cost efficient long term monitoring, covering large areas over extensive time periods. In this study the efficiency of PAM was tested and used to conduct anuran diversity surveys in northern KwaZulu-Natal based on their calling activity.

Materials & Methods

The study area covered northern KwaZulu-Natal, South Africa. Six different sites were identified within this area for installation of the recorders (Song Meters) used for Passive acoustic monitoring.

The Song Meters were programmed to record 10 minutes each hour from 18h00 to 07h00. The recorders were left at the sites for the period from November 2015 to December 2016, with the exception of two sites (left until May 2017).

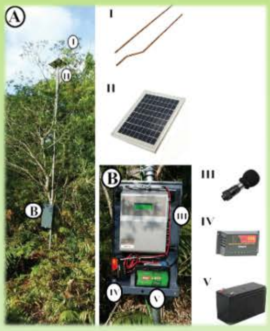

Figure 1: Song Meter (SM3) setup. A: Song Meter attached to a tree connected to a solar panel. B: Song meter housed in protective housing. I: Copper lightning conductor rods earthed to the ground. II: 25x35cm solar panel. III: SM3 microphone. IV: 10A solar charger. V: 12V Lead-acid battery.

Data was analysed using Wildlife Acoustics Inc. Song Scope analysis software. Calling males received an amphibian calling index (ACI) score out of five based on abundance and their intensities.

Results and Discussion

A total of 2326 nights were recorded resulting in 371 580 active recorded minutes. During the study a total of ten (10) families, fourteen (14) genera and twenty-nine (29) species were identified between the six different localities (see Figure 3). Only 48% (29 of 60) of the species that inhabit these areas were recorded. The absence of these species could be attributed to unfavourable weather conditions.

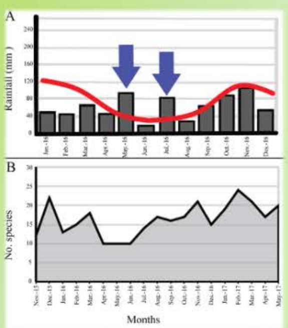

Figure 2: (A) Showing the average monthly rainfall for Zululand in 2016, adapted from a SASRI report, 2016. (B) the sum of each species calling during the study period of November 2015 to May 2017.

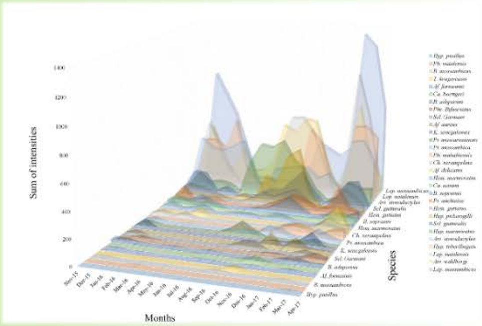

Figure 3: Summary for each species' calling intensities across all study sites for the duration of the study.

Due to the warm phase of the El Niño Southern Oscillation at the end of 2015 and the start of 2016, KZN faced a dry and below average rainfall season during this study. May and July 2016 had above average rainfall with normal rain conditions for the remainder of 2016. Interestingly during the second quarter of 2016 higher than expected calling activity was recorded (10 species). This was possibly due to the abnormal rainfall during May. As the rainfall returned to normal the calling activity of anurans increased (see Figure 2 & 3).

Due to their ectothermic nature, air temperature influences calling activity. Although, not part of this study, during colder months (April – July) of the year, activity decreased. Rainfall also plays an intermediate role in anuran activity, as some species may start calling after heavy rains e.g. *Breviceps* spp. On the other hand, some species will decrease calling activity during heavy rains e.g. *Hyperolius marmoratus*, or even call infrequently e.g. *Ptychadena anchietae*.

Conclusion

The consideration of animal welfare should be essential especially in terms of long term health issues, thus effective non-invasive techniques of monitoring is needed. Through the use of acoustic methods, such as PAM, human habitat invasion can be mitigated and large amounts of high-quality data can be collected over an extensive period with minimal anthropogenic interference. Thus making automated recorders, such as Song Meters, excellent tools to monitor occupancy changes in anuran populations. Furthermore, the calling activity combined with temporal (environmental) data can determine the seasonal patterns and mating activity of observed frog communities. This acoustic data can aid in conservation efforts as it is correlated with environmental change.

African Amphibian Conservation Research Group, North-West University, Potchefstroom

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Appendix H: AARDVARK: Newsletter of the Zoological Society of Southern Africa. December 2017 / January 2018 Edition.

Once this chapter of my life is done, who knows what is next. I dream of learning more about the new and exciting genetic techniques and technologies available. I intend spending more time on research of rare and endangered animals, learning more about diseases that plague our lives directly and indirectly, as well as to have the incredible opportunity to work on epigenetics. My advice to anyone starting up in science; do not be confined to the norm and feel that you have to choose a single discipline. Do what you enjoy doing and pave paths never paved before. Diversity is key to the survival of a species and so it is to a young scientist.

© D. Kleynhans

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Sean is one the 2016 ZSSA Student Award winners.

See page 15 for details

"When Nature calls"
An automated approach to amphibian diversity surveys.

What happened to the days where you could just sit back and relax, enjoying nature and listening to the beautiful natural sounds that surround us? Those noises that echoed through the vast savanna fields or dense forests. A cricket under the bush, a lonely owl trying to leave its mark, or even the local choir of frogs croaking at the nearby pub, where all the animals usually meet for drinks. Someday this privilege will be gone forever and the humbling sounds of nature will be replaced by the overwhelming rumbling noises of the cities that have to cope with the growing population demands of its' inhabitants.

With this growing need, some sacrifices needs to be made, and once again the kind old lady we all know as Mother Nature will have to take the blow. The natural environment will start to lose its touch, as we see the fauna and flora falling on its knees and slowly rendering itself to the mighty wrath of pollution and greed caused by humans. Our smaller friends living on the ground that once entertained us with their songs, hopping around eating their share of insects, will be one of the first to perish.

Frogs belong to the Class Amphibia, viewed as one of the most threatened animal classes in the world. Amphibians are found in most of the terrestrial and freshwater habitats globally, except for some marine and arctic environments. These little guys are vitally important as they can be utilised as environmental indicators, due to their sensitivity to environmental change which in turn then can be used to determine the health of a specific environment. One of the challenges of this enigmatic global frog decline is the need for continuous monitoring. There is a dire need for non-invasive, rapid, and objective monitoring techniques. That is where we come in.

One of the sampling sites included in the study. © W. Pretorius

Southern Africa has more than 170 known frog species. Kwa-Zulu Natal (KZN) represents one of the provinces with the richest anuran diversities in the country, boasting over 50 frog species in the northern parts. Historically, there have only been a few studies done to determine the frog biodiversity of KZN, with the last extensive study conducted more than 30 years ago.

Trekking through the field with the long-term acoustic monitoring equipment and associated solar panel used to power the recording device. © W. Pretorius

The study was aimed at the determination of the biodiversity and the effects of atmospheric conditions on frog communities in Zululand (Northern KZN) through the use of automated recorders set at six different localities. The data analysed from the recordings were used to identify the different species present and calculate their abundance during the end of 2015 to 2016. We observed during the study period, through call identification a total of 46 different species at the various localities. Some interesting findings included: the Endangered Pickersgill reed frog (*Hyperolius pickersgilli*) and the Vulnerable Spotted shovel-nosed frog (*Hemisus guttatus*). During the study we also found two new species of Rain frogs: Phinda rain frog (*Breviceps carruthersi*) and Ndumo rain frog (*B. passmorei*).

The Wildlife Acoustics SongMeter equipment used to automatically record frog calls. Wentzel and colleague setting up one of the recording stations. © W. Pretorius

Furthermore, the calling observations were combined with environmental data in order to determine the seasonal patterns and mating activities. This monitoring data will provide valuable information on the distribution, diversity and ecological behaviour of frog communities in this area, ultimately assisting in conservation actions.

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Frogs, a man's best friend (a tale with a difference)

Once upon a time in a sleepy Zululand village, dogs and frogs had an argument about who was the rightful owner of the title of 'man's best friend'. The dogs based their claim to the title on the loyalty, love and protection they give to people. Frogs then said life in Zululand would be very difficult if they were to all disappear. All the dogs started howling in laughter when they heard this claim from the frogs.

The Natal tree frog* (Leptopelis natalensis) *which only occurs in KwaZulu Natal. © F. Phaka

The dogs were laughing because they did not understand how frogs could make people's lives better if they are only seen when it rains. They also did not