Frogs about town: Aspects of the ecology and conservation of frogs in urban habitats of South Africa

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In loving memory of my grandmother, Kitty Lombaard (1934/07/09 – 2012/05/18), who has made an invaluable difference in all aspects of my life.

"Act as if what you do makes a difference.
It does." ~ William James

CONSERVATION

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Thesis summary

Globally urbanisation impacts on 88% of amphibian species and is recognised as a major cause for the observed amphibian declines. This is as result of habitat fragmentation, alteration in habitat morphology and degradation of habitat quality. The interference of anthropogenic noise on anuran communication and the impacts thereof on their breeding success has become a major research focus in recent conservation studies. . However, within the African continent very little research has been conducted on the effects of urbanisation on anuran habitat and the acoustic environment, which is the main focus of this study. The thesis is structured as follows:

CHAPTER ONE provides an introduction to the field of urban ecology and relates it to amphibian conservation. The chapter reviews the far reaching and diverse effects of urbanisation on frog populations reported in literature across the world and also supply a broad introduction to the succeeding chapters. It also briefly summarises evidence from literature on the positive contributions brought about by the developed world. Following the vast negative impacts of urbanisation, the importance of amphibians is briefly discussed to motivate their conservation in urban environments, before concluding with a motivation for the need for urban ecological research on amphibians in South Africa.

CHAPTER TWO addresses the distribution of amphibian communities across an urban-rural gradient in the city of Potchefstroom and assesses the habitat determinants explaining distribution at both local (pond) and landscape scales. Four surveys conducted spanned the breeding seasons of all species occurring in this region and included three different sampling techniques to detect fish and anuran larvae species. Seven micro-habitat and seven landscape variables were included to evaluate determinants of habitat use among local species and species richness. Using Bayesian modelling, aquatic vegetation, predatory fish and pond size was found to be major determinants shaping species richness on a local scale, whereas surface area of urban central business district had only a slightly negative correlation with species richness on a landscape scale. This is a pioneer study for documenting effects of urbanisation on amphibian communities along an urban-rural gradient in Africa.

CHAPTER THREE evaluates the extent of the influence of aircraft acoustic noise on the calling behaviour of the critically endangered Pickersgill's Reed Frog, *Hyperolius pickersgilli*. Literature documenting the effects of airplane noise on anuran calling activity is very limited and this study aimed not only to contribute to existing knowledge, but also to provide the first study of its kind within South Africa. Effects on five call properties of *H. pickersgilli* were determined

using passive and directional recording equipment at two sites, reflecting presence and absence of aircraft flybys. Results showed an increase in calling rate of *H. pickersgilli* during aircraft flybys. *Hyperolius pickersgilli* was found to call throughout the night until just before sunrise. The calling behaviour, frequency structure and call sound pressure level of *H. pickersgilli* suggest that this species is prone to be effected by continuous anthropogenic noise. However, the lack of flights between midnight and sunrise provides a period of no disturbance for the frogs. Future studies on the effects of change in calling behaviour should be supported by playback studies at quiet sites and connected to breeding success to determine if these effects are detrimental to the survival of this critically endangered species.

CHAPTER FOUR focussed on the Western Leopard Toad, *Amietophrynus pantherinus* and was divided into two major parts. One component focussed on the migration of this species across roads and aimed to firstly quantify the number of individuals migrating over a 500 m stretch of road using a drift fence system operated by public volunteers. The drift fence proved very successful, with no roadkill observed during the time it was in place. This study also stressed that large numbers of toads (average of 20.47% of 2 384 toads over six breeding seasons) are still being killed on the urban and suburban roads. Road patrol statistics collected by volunteers are biased in the sense that it is prone to human error, but when a drift fence is constructed, bias is excluded and space for human error limited. The study also provided road sensitivity areas analysed using geographic information systems to create digital buffer zones of 250 m, 500 m and 1 000 m around selected breeding sites.

Secondly the study aimed to evaluate the use of data collected by these citizens occupying a volunteering role in the toad's conservation. The second part of this study was directed towards the acoustic analysis of the call of *A. pantherinus*. The two main objectives of this component were to 1) evaluate the extent of variation of the call properties in order to 2) assess whether the ambient anthropogenic noise have an effect on these properties. Seven call properties for advertisement calls and four for release calls were analysed. Call properties were found to vary significantly between populations ($P<0.05$). Although sound pressure level was found to have an effect on variation by using canonical redundancy analysis, variation can also be explained by the geographical isolation of the populations.

CHAPTER FIVE provided novel data on the extensive repertoire of *Amietia quecketti* in terms of its unique calling behaviour. Directional recordings were used to examine the extent of the variation in the two-part call (click-note followed by a whine-note). The whine-note was re-described and four different notes were designated, including the tonal-note, creak-note,

pulsatile- / rip-note, and whine-note. Furthermore, the newly assigned whine-note was divided into nine phases that differed in frequency structure. Also, evidence is provided that *A. quecketti* males call at high frequencies. The success of *A. quecketti* in urban environments as observed in Chapter 2 is described in terms of this species' extensive repertoire and unusual frequency structure.

CHAPTER SIX provides insight into the effects of atmospheric conditions on the calling behaviour of *Amietia quecketti*, giving the proximate impact urbanisation has on weather conditions as well as the potential impact human activities can have on climate change on the long term. Calling activity was monitored over a nine-week period together with data from a mobile weather station which logged atmospheric variables every five minutes. *Amietia quecketti* was found to call most intensely between 00h00 and 03h00 in the morning and was most active in May, June and August. Humidity, temperature and wind velocity were found to have significant effects ($P<0.05$) on the calling activity of *A. quecketti*.

CHAPTER SEVEN is concerned with the attitudes of people towards frogs in South Africa. The first part of this study assessed the attitudes of people towards frogs in Potchefstroom. Surveys were distributed via the internet as well as manually to reach people with no internet access as well. Attitudes of people of Potchefstroom were mostly positive with more than half of the sampled population of 295 respondents indicating a strong liking in frogs. This study provides evidence that the presence of myths and knowledge can highly affects people's attitudes towards frogs. The second part of this study focussed on the motivations of volunteers saving Western Leopard Toads from roadkill in Cape Town, South Africa. Volunteers were motivated by a strong value-driven approach to saving toads.

CHAPTER EIGHT provides a general discussion and outline on the contributions this study presented and also the new areas where more research is needed within the extent of the field of urban ecology from a South African perspective.

Keywords: acoustic analysis, acoustic degradation, anuran communities, anuran distribution, bioacoustics, habitat alteration, habitat use, urban ecology, urban-rural gradient

Opsomming

Verstedeliking het ‘n impak op 88% van amfibieërspesies wêreldwyd en is ‘n primêre oorsaak van populasie-afnames as gevolg van habitatfragmentering, habitatwysiging asook algemene degradasie in habitatkwaliteit. Die bestudering van die effek van lawaai op paddakommunikasie, en die impak daarvan op hul reproduktiewe sukses, is van kardinale belang. Byna geen navorsing oor die effekte van verstedeliking op paddas is al op die Afrika vasteland uitgevoer nie. Hierdie studie fokus dan op die effek van die wysiging in die akoestiese omgewing op die roepgedrag van paddas. Hierdie proefskrif is as volg gestruktureer:

HOOFSTUK EEN verskaf ‘n inleiding tot die veld van stedelike ekologie en koppel dit aan die toepasbaarheid daarvan op amfibieërbewaring. Die hoofstuk hersien verder die verreikende en diverse effekte van verstedeliking op paddapopulasies soos gerapporteer in literatuur. Dit dien ook as ‘n algemene inleiding tot die daaropvolgende hoofstukke. Positiewe bydraes deur verstedeliking word uitgelig, maar die oorwegend negatiewe effek word uitgelig. Die hoofstuk word afgesluit met ‘n rasionaal vir ‘n behoefte aan stedelike ekologie navorsing oor amfibieë in Suid-Afrika.

HOOFSTUK TWEE handel oor die verspreiding van amfibieërgemeenskappe oor die stedelike-landelike gebied van Potchefstroom en evalueer faktore wat die verspreiding van paddas op mikro en makro-skaal beïnvloed. Vier opnames seisoenale opnames wat gestrek het oor die broeiseisoene van alle spesies wat in hierdie area voorkom is onderneem. Drie verskillende opname-tegnieke is gebruik om vis- en paddavisspesies te versamel. Deur van Bayesiaanse modellering gebruik te maal, is gevind dat akwatiese plantegroei, predatoriese vis en damgrootte noemenswaardige bepalend is om spesierykheid op ‘n plaaslike skaal te verklaar. Die tipe oppervlak van die stedelike sentrale besigheidsdistrik het slegs ‘n geringe negatiewe korrelasie getoon het met spesierykheid. Hierdie studie kan gesien word as ‘n pionierstudie vir die dokumentering van die effekte van verstedeliking op amfibieërgemeenskappe as ‘n geheel oor ‘n stedelike-landelike gradiënt in Afrika.

HOOFSTUK DRIE evalueer die invloed van lugvaartgeraas op die roepgedrag van die krities bedreigde Pickersgill se Rietpadda, *Hyperolius pickersgilli*. Literatuur wat die effekte van vliegtuiggeraas op paddas se roepgedrag dokumenteer, is skaars. Hierdie is dan ‘n eerste vir Suid-Afrika. Effekte op vyf roepeienskappe van *H. pickersgilli* is bepaal deur die gebruik van passiewe klankopname- en gerigte mikrofone by twee lokaliteite wat die aanwesigheid en afwesigheid van lugvaartaktiwiteit reflekteer. Resultate het ‘n toename in die roepsnelheid van

H. pickersgilli aangetoon met toenemende lugvaartaktiwiteit. Daar is bevind dat *Hyperolius pickersgilli* regdeur die nag tot net voor sonsopkoms roep. Die roepgedrag, frekwensie struktuur en die roep SPL van *H. pickersgilli* stel voor dat hierdie spesie kwesbaar is om deur deurlopende mensgegenereerde geraas negatief geaffekteer te word. Alhoewel toekomstige studies moet fokus op die effekte van verandering in roepgedrag, moet dit ondersteun word deur studies wat vlieggeraas terugspeel by stil lokaliteite, daarby moet die verband met broeisuksesse aangetoon word om te bepaal of hierdie effekte afbrekend is tot die oorlewing van hierdie kritiese bedreigde spesie.

HOOFSTUK VIER het gefokus op die Westelike luiperdskurwepadda, *Amietophrynus pantherinus,* en is ingedeel in twee hoofelemente. Een komponent fokus op die migrasie van die spesie oor paaie, en poog om die aantal individue te kwantifiseer wat oor 'n 500 m padafstand, toegerus met 'n keerheiningsisteem met putvalle, migreer, Plaaslike publieke vrywilligers het telling gehou van die getalle skurwepaddas wat in die pitvalle beland en het die paddas dan veilig oor die pad geneem an aan die anderkant vrygelaat. Die keerheining was suksesvol met geen padsterftes ("roadkill") wat waargeneem is gedurende die duur van die opstelling. Hierdie studie het ook beklemtoon dat groot hoeveelhede paddas (gemiddeld van 20.47% van die 2 384 paddas wat oor ses broeiseisoene getel is) steeds aangeteken word as padsterftes. Padpatrollie statistiek wat deur vrywilligers ingesamel is, is subjektief in die sin dat dit blootgestel is aan menslike foute, maar wanneer 'n keerheining gebou word, word hierdie bevooroordeling uitgeskakel menslike foute word beperk. Die studie verskaf ook inligting oor sensitiewe paaie wat geanaliseer is deur die gebruik van geografiese inligtingsisteme om sodoende 'n digitale bufferzone van 250m, 500m en 1 000m rondom die geselekteerde broei-areas te skep. Tweedens het die studie gepoog om die gebruik van data deur hierdie vrywilligers in die *A. pantherinus* se bewaring speel, te evalueer.

Die tweede komponent van hierdie studie was gerig op die akoestiese analise van die roep van *A. pantherinus*. Die twee hoofdoelwitte van hierdie komponent was 1) om die variasie van die roepeienskappe te evalueer om sodoende 2) te assesseer of die omliggende antropogeniese geraas 'n effek het op hierdie eienskappe. Sewe roepeienskappe vir adverteringsroepe en vier vir vrylating roepe is geanaliseer. Daar is bevind dat roepeienskappe noemenswaardig tussen populasies varieër ($P<0.05$). Alhoewel gevind is dat klankdrukvlakke 'n effek op variasie het deur die gebruik van "canonical redundancy analysis", kan variasie deur die geografiese isolasie van die populasies verklaar word.

HOOFSTUK VYF verskaf nuwe data oor die uitgebreide "repertoire" van *Amietia quecketti*. Gerigte klankopnames is gebruik om die mate van variasie in die tweeledige roep (klik gevolg deur ‘n kwaak) te bepaal. Die kwaaknoot is heromskryf en gevolglik is vier verskillende note geïdentifiseer. Dit sluit in die tonusnoot, knarsnoot, pulserende- skeurnoot, en kwaaknoot. Daarbenewens is die nuuttoegekende kwaaknoot verder verdeel in nege fases wat verskillende frekwensiestrukture het. Verder het die studie ook bewyse gelewer van *A. quecketti* mannetjies se hoë akkoord roepfrekwensies. Die sukses van *A. quecketti* in stedelike omgewings, soos in Hoofstuk 2 waargeneem, word omskryf in terme van hierdie spesie se uitgebreide repertoire en komplekse frekwensiesamestelling.

HOOFSTUK SES verskaf insig rakende die effekte van atmosferiese toestande op die roepgedrag van *Amietia quecketti*. Gegewe die onmiddellike impak van verstedeliking op weersomstandighede sowel as die potensiële impak wat menslike aktiwiteite in die langtermyn op klimaatsverandering het. Roepaktiwiteit is oor ‘n periode van negе weke gemoniteer, tesame met data van ‘n mobiele weerstasie wat atmosferiese veranderlikes elke vyf minute aangeteken het. Daar is bevind dat *A. quecketti* meesal tussen 00h00 en 03h00 in die oggend roep en dat die aktiefste roepaktiwiteit waargeneem is in die periode Mei, Junie en Augustus. Humiditeit, temperatuur en windsterkte het die mees noemenswaardige effek ($P<0.05$) op die roepaktiwiteit van *A. quecketti* gehad.

HOOFSTUK SEWE handel oor die houding en persepsies van mense teenoor paddas. Die eerste deel van hierdie studie het die houdings van mense teenoor paddas in Potchefstroom geassesseer. Vraelyste is via die internet, sowel as per hand versprei om ook mense sonder internettoegang te bereik. Houdings van mense van Potchefstroom was meestal positief met meer as die helfte van die populasiemonster van 295 respondente wat aandui dat hulle baie van paddas hou. Hierdie studie verskaf bewyse dat die teenwoordigheid van mites en kennis ‘n groot impak het op mense se houdings teenoor paddas. Die tweede deel van hierdie studie het gefokus op die motivering van vrywilligers wat die Westelike luiperdskurwepaddas in Kaapstad se paaie red, Suid-Afrika, beskerm. Vrywilligers word gemotiveer deur ‘n sterk waardegedrewe benadering tot die red van paddas.

HOOFSTUK AGT verskaf ‘n algemene bespreking en ‘n uiteensetting van die bydraes wat hierdie studie bied, asook die nuwe areas waar meer navorsing benodig word in die uitgebreide veld van stedelike ekologie vanuit ‘n Suid-Afrikaanse perspektief.

I, Donnavan Kruger, declare that this thesis is my own, unaided work, except where otherwise acknowledged. It has not been submitted for any degree or examination in any other university.

David Johannes Donnavan Kruger

30 April 2014

Abbreviations used in text

°C	degrees Celsius
dB	decibel
df	degrees of freedom
CCA	canonical correspondence analysis
DF	dominant frequency
DIC	deviance information criterion
Hz	hertz
kHz	kilohertz
km	kilometres
kPa	kilopascal
m	meter/meters
MWF	mean weighted frequency (also called emphasised frequency)
P	capital and italised; refers to significance, i.e. the *P*-value (i.e. $P < 0.05$)
Pa	Pascal
PCA	principal component analysis
RDA	redundancy analysis
RH	relative humidity
SPL	sound pressure level
t	metric tonnes
VIF	variable inflation factor

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CHAPTER ONE

General introduction

"The enemy is chronic disturbance, cumulative and irreversible, that moves the world systematically down the curve of biotic impoverishment, place by place, until the effects fuse and ends this phase in the evolution of the biosphere. The cure is the loud and relentless pursuit of the restoration and preservation of the physical, chemical, and biotic integrity of Earth—all of Earth—as the special preserve of this civilization. That step requires the elevation of global conservation to a level competitive with other political and economic interests."

– George Woodwell (ecologist) 2010

1.1 Introduction to urban ecology

Humans are part of nature and not apart from nature. The paradigm that human beings are connected to ecosystems as any other biotic components are, and that biotic and abiotic processes in cities can only be fully explained in terms of their ecological background to nature, dates back a century (Geddes 1915) and possibly more (*sensu* Alberti 2008). The idea of interdependence between cities and natural resources manifested in multiple disciplines over the past century including sociology (Park *et al.* 1925; Duncan 1960), geography (Berry 1964; Williams 1973; Zimmerer 1994), ecology (Odum 1953; Sukopp 1990; McDonnell *et al.* 1993), anthropology (Rapoport 1977), history (Cronon 1991), and urban design and planning (McHarg 1969; Spirn 1984; Lynch 1961), to name a few. This is reflected in a substantial increase in academic books published on aspects of urban ecology over the past decade (Table 1.1).

Table 1.1 Selected academic books published on aspects of urban ecology over the past decade (2005 – 2013).

Year	Authors	Title
2005	Konijnendijk *et al.*	Urban forests and trees: A reference book
2005	Kowarik and Körner	Wild urban woodlands: New perspectives for urban forestry
2006	Bucur	Urban forest acoustics
2008	Alberti	Advances in urban ecology: integrating humans and ecological processes in urban ecosystems
2008	Marzluff *et al.*	Urban ecology: An international perspective on the interaction between humans and nature
2008	Mitchell *et al.*	Urban Herpetology
2008	Carreiro *et al.*	Ecology, planning, and management of urban forests international perspectives
2009	McDonnell *et al.*	Ecology of cities and towns: A comparative approach
2011	Endlicher *et al.*	Perspectives in urban ecology: Studies of ecosystems and interactions between humans and nature in the metropolis of Berlin
2011	Huggenberger and Epting	Urban Geology: Process-oriented concepts for adaptive and Integrated Resource Management
2011	Loeb	Old growth urban forests
2013	Boone and Fragkias	Urbanisation and sustainability: Linking urban ecology, environmental justice and global environmental change
2013	Elmqvist *et al.*	Urbanisation, Biodiversity and Ecosystem Services: Challenges and opportunities, a global assessment
2013	Ewing and Clemente	Measuring urban design: Metrics for livable places
2013	Nilsson *et al.*	Peri-urban futures: Scenarios and models for land use change in Europe
2013	Pickett *et al.*	Resilience in ecology and urban design: Linking theory and practice for sustainable cities
2013	Vescovi	Designing the urban renaissance: Sustainable and competitive place making in England

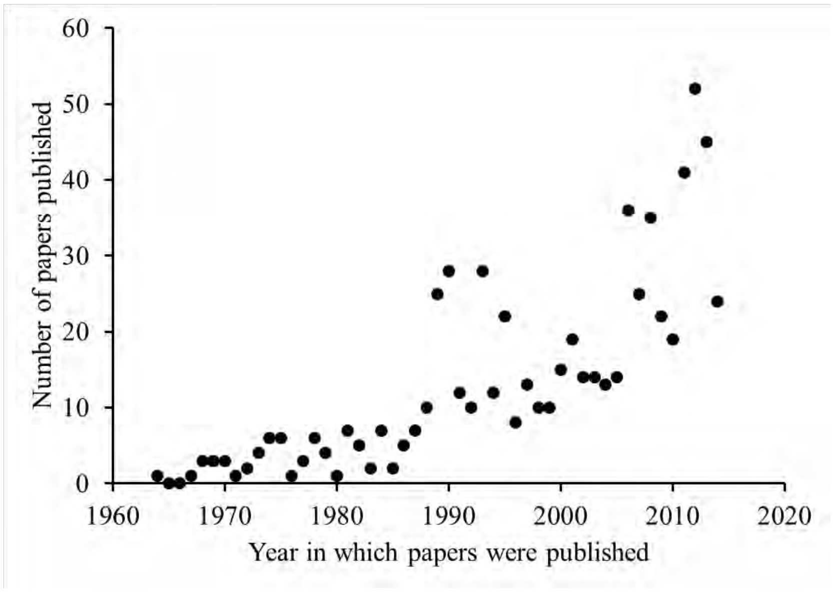


Figure 1.1 Number of papers published per year from 1964 to 2014 obtained from a basic keyword search in Web-of-Science for papers that contain the words “urban” and “ecology”.

The preceding paragraph, Figure 1.1 and Table 1.1 demonstrate the variety of disciplines that are intertwined with the study of urban processes and systems in which the term “urban ecology” is used today (Alberti 2008). Therefore, it is important to place this thesis’ approach into perspective. Due to the inter-disciplinary nature of the field of urban ecology, McDonnell *et al.* (2009) narrowed the definition of urban ecology by first defining the elemental words “urban” and “ecology” and thereafter centralising the definition around natural sciences. They narrowed down the definition by describing the field of urban ecology in terms of an applied natural science by first defining the elemental definitions, but keeping its other definitions in mind. Consequently, urban ecology is defined as the study of the interactions between the physical, biotic, and abiotic environment within the urban setting. Furthermore, McDonnell *et al.* (2009) distinguish between two complementary approaches within urban ecology, namely

1)

“*Research into ecology* **in** *cities refers to studies on the physical environment, soils, fauna and flora, and differences between urban and other environments”*

(McDonnell *et al.* 2009).

2)

"Research into the ecology **of** *cities… uses the ecosystem framework and studies the urban area as an interactive system including both human and ecological components."*

(McDonnell *et al.* 2009)

A multidisciplinary approach should therefore be followed when aiming to explain the interactions between biodiversity, the abiotic environment and anthropogenic systems mentioned in these definitions. Throughout this thesis these approaches will both apply when referred to aspects of urban ecology.

McDonnell and Pickett (1990) recognised the gap in the field of ecology and proposed a framework to guide the integration of ecological studies along urban-rural gradients. Taylor *et al.* (2011) have studied urban biodiversity from an Australian context and stated that ecologists in general ignored biodiversity of cities until the later decades of the 20th century. However, research into urban biodiversity has been escalating in recent years, featuring books (Table 1.1), journals and increasing memberships in urban ecosystem ecology in ecological societies (Mayer 2010). The rapidly increasing urban landscape presents an alarming challenge specifically for wildlife (Lowry *et al.* 2013). Urban wildlife often exhibit behaviours that differ from those of their rural equivalents, from changes in food and den preferences, to adjustments in the structure of their signals, where other species are intolerant of habitat change and show no plasticity to change (Lowry *et al.* 2013). Ecological research in urban ecosystems concerning wildlife can focus on a variety of factors, including behavioural (Donaldson *et al.* 2007; Parris *et al.* 2009; Evans *et al.* 2010; Kitchen *et al.* 2010), physiological (Partecke *et al.* 2005; Partecke *et al.* 2006b), and genetic (Partecke *et al.* 2006b; Noël *et al.* 2007) responses to increased urbanisation. Although research conducted in the field of urban ecology is rapidly increasing, it remains a small fraction (<2%) of ecological research as a whole (Mayer 2010; Magle *et al.* 2012).

1.2 Urban ecology of anurans

Magle *et al.* (2012) performed a thorough assessment of urban wildlife research published between 1971 and 2010 and found that rates of publication are increasing but remain relatively low. They highlighted that most research was published in conservation, landscape ecology, and wildlife biology journals and were nearly all from North America, Europe, and Australia. Furthermore, Magle *et al.* (2012) demonstrate that urban wildlife research is predominantly conducted on birds and mammals, and that the most critical gaps for urban wildlife research is in rapidly urbanising areas in South America, Africa, and Asia, and on understudied taxa, which include amphibians, reptiles, fish and arthropods.

Amphibians are among the least studied taxonomic groups in urban and suburban areas (Pickett *et al.* 2001; McDonnell & Hahs 2008; Magle *et al.* 2012). Despite the widespread declines documented for many amphibian species (Stuart *et al.* 2004), being the most threatened vertebrate group on Earth (IUCN 2013) and their significance for ecosystem function (Carey & Wahl 2010; Davenport & Chalcraft 2012). Hardly any research on how anurans cope in urban habitats have been undertaken in any of the emerging countries such as South Africa. Hamer and McDonnell (2008) developed a conceptual framework on Amphibian Population Dynamics in urban areas using existing global and interdisciplinary knowledge (Figure 1.2). They expanded on each of the aspects of this framework in their study and identified areas in need of research and future research opportunities in order to develop conservation strategies for amphibians in urban and suburban areas. In their paper they also summarise the key areas that affect and interact with amphibians and discussed observed and possible failures and successes of amphibians in urban environments. In conclusion, Hamer and McDonnell (2008) stated that critical issues that need to be addressed included: 1) the need for more research on amphibians in urban habitats of tropical and sub-tropical environments, 2) standardisation of the definition of urban, and 3) standardisation of an appropriate landscape scale for the study of amphibians. Aspects of recent research on the urban ecology of amphibians included genetics (Noël *et al.* 2007; Measey & Tolley 2011), local and landscape habitat use (Hamer & Parris 2011; Hamer *et al.* 2012), bioacoustics (Bee & Swanson 2007; Parris *et al.* 2009), movement patterns (Hamer *et al.* 2008; Hamer & McDonnell 2008), and climate change (Ospina *et al.* 2013).

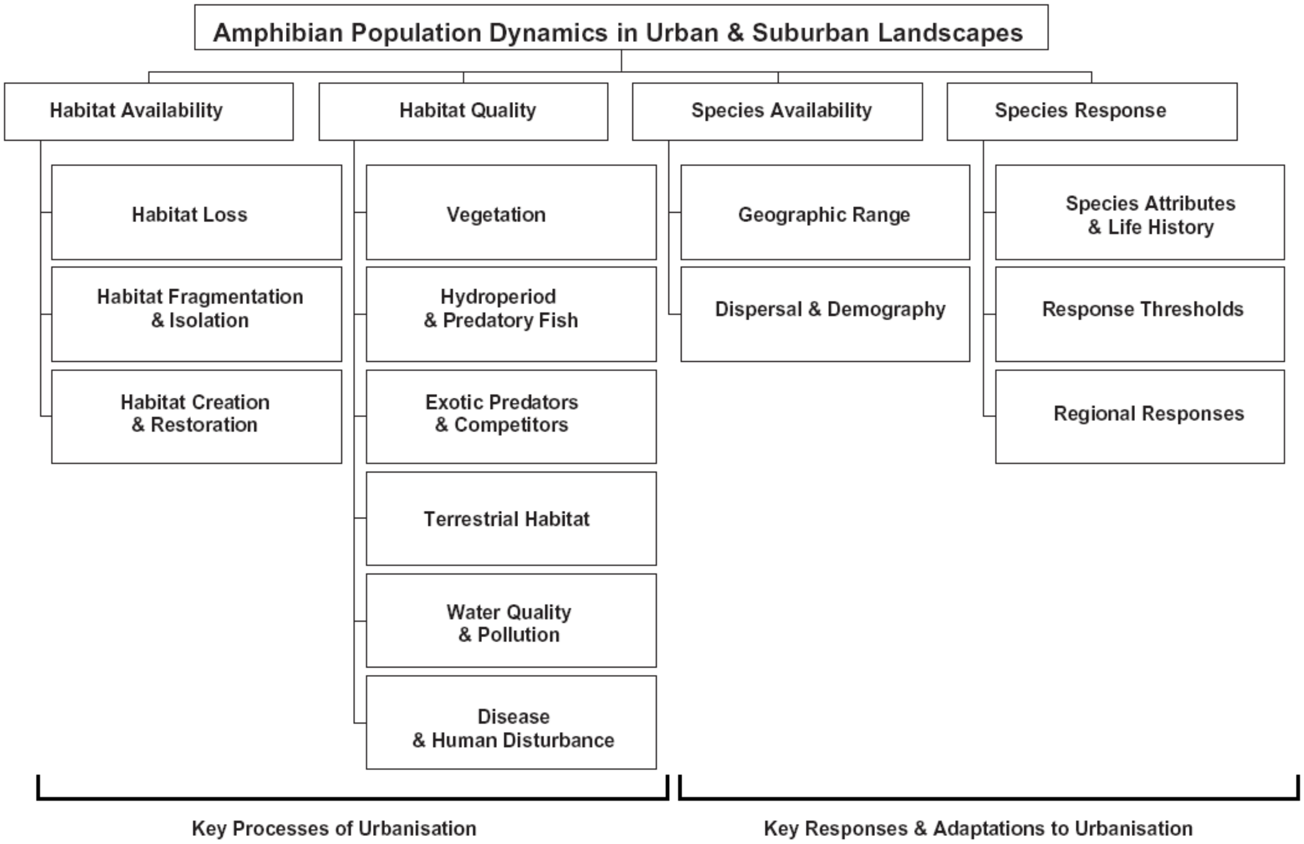


Figure 1.2 A conceptual framework presented by Hamer and McDonnell (2008) for assessing extant knowledge of amphibian ecology and conservation in urban and suburban landscapes.

1.3 Effects of urbanisation on anurans

1.3.1 Habitat degradation, fragmentation and destruction

Degradation and subsequent loss of natural habitat is possibly the single best studied example of a long lasting and significant effect of urbanisation on amphibians (Cushman 2006; Hamer & McDonnell 2008). Habitat loss, fragmentation and degradation (McKinney 2002, 2006) have a cumulative impact on 88% of threatened amphibians (Baillie *et al.* 2004). Furthermore, nine out of ten threatened species are experiencing population declines because of habitat loss (Dodd & Smith 2003). Human transformation of the landscape will not only continue, but likely expand and intensify, and therefore urbanisation is regarded among the greatest present and future threats to amphibian populations (Stuart *et al.* 2004; Beebee & Griffiths 2005; Cushman 2006; Hamer & McDonnell 2008).

Aside from the obvious direct effects, e.g. loss of breeding habitat, alteration and loss of habitat indirectly threatens amphibians in a variety of ways, including: 1) the relatively low dispersal capabilities (vagility) of amphibians are further decreased (Sinsch 1990; Gibbs 1998; deMaynadier & Hunter 2000; Bowne & Bowers 2004); 2) high road mortalities lower population growth rates (Fahrig *et al.* 1995; Carr & Fahrig 2001; Carr *et al.* 2002); 3) narrow habitat preferences in most species is intensified by the effects of habitat loss, degradation, and edge effects (Findlay & Houlahan 1997; Semlitsch 2000; Houlahan & Findlay 2003); and 4) increased vulnerability to pathogens, invasive species, climate change, increased ultraviolet-B exposure and environmental pollution (Pounds *et al.* 1999; Broomhall *et al.* 2000; Kiesecker *et al.* 2001; Blaustein *et al.* 2000; Hecnar 1995; Bridges & Semlitsch 2000; Davidson *et al.* 2001; Stuart *et al.* 2004). Habitat loss and fragmentation contribute directly to most of these threats (Carr & Fahrig 2001; Bowne & Bowers 2004; Houlahan & Findlay 2003; Cushman 2006). These threats are briefly summarised in the following sections.

1.3.2 Chemical pollution

Due to their amphibious lifestyle and semi-permeable skin, their skin can easily absorb chemicals they come into contact with in both their aquatic and terrestrial environments (Bridges 2000; Blaustein & Johnson 2003). Blaustein & Wake (1995) stated that amphibians are perhaps the most sensitive group of vertebrates to aquatic and atmospheric pollution. Therefore, amphibians can be deemed as highly useful early warning systems of environmental change (Bridges 2000; Blaustein & Johnson 2003; Simon *et al.* 2011) (see section 1.4.5 on amphibians as bio-indicators). Amphibians generally have a low tolerance for most pesticides and chemical pollution, which can cause mortalities (Hecnar 1995; Bridges & Semlitsch 2000; Davidson *et al.* 2001; Blaustein & Johnson 2003) and deformities (Gardiner & Hoppe 1999; Burkhart *et al.* 1998; Carr *et al.* 2003).

1.3.3 Light pollution

Due to our own inability to see in the dark, humans have developed and positioned widespread outdoor and indoor electrical lighting to such an extent that the outline of the continents is now visible from outer space (Elvidge *et al.* 2007; Figure 1.3). Literature distinguishes between "astronomical light pollution", which obscures the view of the night sky, and "ecological light pollution", which alters natural light regimes in terrestrial and aquatic ecosystems (Longcore & Rich 2004).

Light pollution is the introduction of artificial lighting into areas where it changes the illumination or spectral composition of natural lighting (Rich & Longcore 2006). Anurans may be particularly susceptible to adverse effects from light pollution (Buchanan 2006). For anurans, a rapid increase in lighting causes a reduction in visual capability from which the recovery time may be minutes to hours (Buchanan 1993) and after adjusting to a light, frogs may be attracted to it (Jaeger & Hailman 1973), where they most likely capture insects attracted to the lights (Baker 1990). However, this might be disadvantageous, i.e. frogs might feed on insects around lamp post, but end up being more prone to roadkill or predators. Species that have evolved under very low illuminations have adaptations that may restrict their capabilities to compensate for increases in illumination intensity or that may allow them to take advantage of slight increases in environmental illumination (Buchanan 2006). Even though the effect of light intensity (i.e. natural diurnal lux measurement) constitutes an important factor in anuran activity (Jaeger *et al.* 1976; Pancharatna & Patil 1997; Hatano *et al.* 2002; Almeida-Gomes *et al.* 2007), the effect of light pollution on frogs specifically has not been well studied (Buchanan 1993; Buchanan 2006).

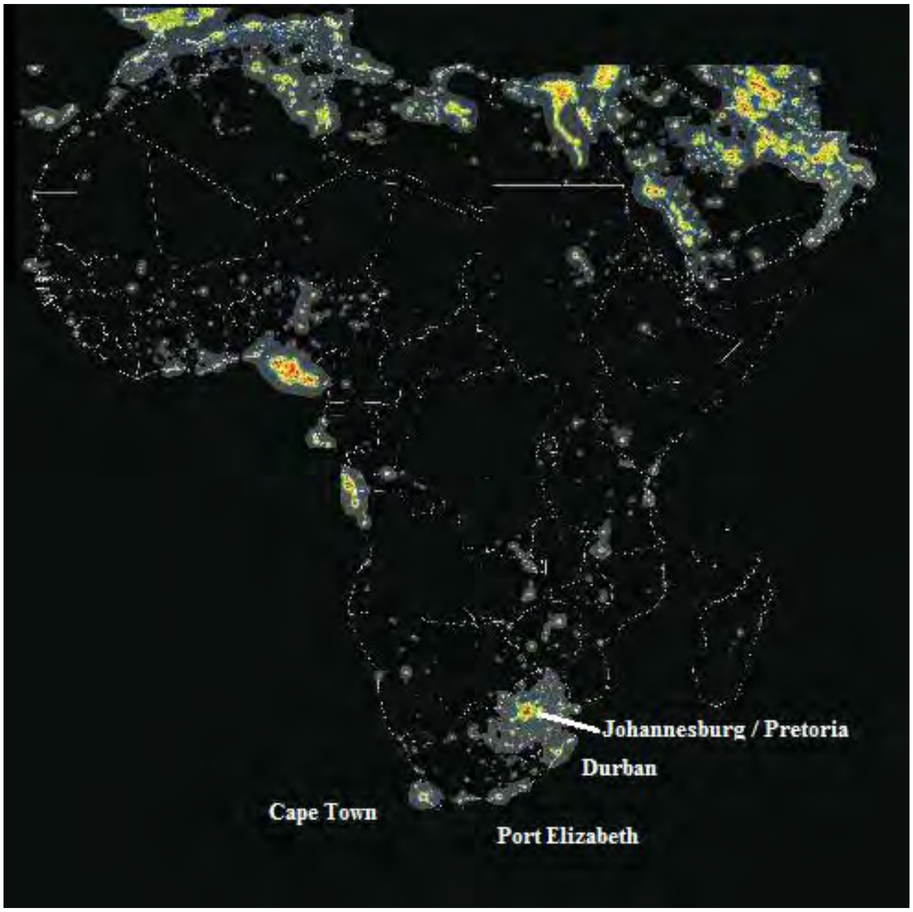


Figure 1.3 Light pollution shown on the African continent. Within South Africa, Johannesburg, Cape Town, Durban and Port Elizabeth emit the most light into the night sky. Source: Cinzano *et al.* (2001); City labels inserted.

1.3.4 Acoustic habitat degradation and effects of anthropogenic noise

Urbanisation brings about not only degradation to the physical habitat (i.e. sufficient habitat that meets breeding, foraging and hibernating requirements), but also alters the acoustic environment by masking the signals of vocally active animals in marine (Clark *et al.* 2009) and terrestrial habitats (Bee & Swanson 2007). Anurans have a diversity of call types to communicate signals to both males and females, to conspecifics as well as interspecific. This repertoire includes advertisement calls (principle signals given by males to attract mates), male courtship calls (altering of vocal behaviour when females are nearby), female courtship- or reciprocation calls (in response to male courtship calls), aggression calls (given by males to defend their calling sites against conspecifics), release calls (anurans usually give release calls when clasped by other males, unreceptive females or have finished oviposition give similar calls), and distress calls (also called alarm- and defensive calls; describe vocalisations given by frogs being attacked by predators, usually relatively loud screams and often delivered with the mouth open) (Wells 2007).

As studies have long-established, the effectiveness of anuran communication and ultimately breeding success is not only subject to the detection of signals against background noise but also discrimination of differences among signals by the receiver (Wiley & Richards 1978; Gerhardt 1982; Penna *et al.* 2005; Lengagne 2008; Wilkens *et al.* 2012). The spread of urbanisation can have extensive detrimental effects on anuran communication by interfering with their acoustic signalling for mates (Sun & Narins 2005; Parris *et al.* 2009; Cunnington & Fahrig 2010). It has already been shown that species richness in some anuran communities is negatively correlated with anthropogenic noise and road density (Fahrig *et al.* 1995; Findlay & Houlahan 1997; Fahrig & Rytwinski 2009). The detrimental effects that anthropogenic noise have on the communications systems in a variety of taxa are of growing concern (Barber *et al.* 2009a, 2009b, Landon *et al.* 2003; Dowling *et al.* 2012; Popper & Hastings; Melcón *et al.* 2012). It was only during the past decade that some studies started focussing on the effects of anthropogenic noise on amphibians (Parris *et al.* 2009; Love & Bee 2010; Boeckle *et al.* 2009; Cunnington & Fahrig 2010; Hoskin & Goosem 2010; Kaiser & Hammers 2009).

Studies that focus on the effects of human-generated noise on anurans include, impaired sender and receiver detection thresholds and spatial orientation, breeding success, masking effects of noise, vagility of different species, distribution changes, vocalisation plasticity in temporal and spectral properties and sexual selection (Herrera-Montes & Aide 2011; Cunnington & Fahrig 2010; Fahrig & Rytwinski 2009; Hoskin & Goosem 2010; Parris *et al.* 2009; Kaiser *et al.* 2010; Carr & Fahrig 2001; Eigenbrod *et al.* 2009; Wiley 1994; Wollerman & Wiley 2002; Brumm & Slabbekoorn 2005; Langemann & Klump 2005, Bee & Swanson 2007). These effects, mostly conducted on road traffic noise, have emerged as a field of interest and concern and have been well documented over the past two decades and almost all report negative effects and apprehension.

1.3.5 Invasive species introductions

One of the most harmful indirect effects of urbanisation is the intentional or unintentional introduction of alien and invasive species to ecosystems. This is mainly as a result of humanity's urge to control the environment and our unprecedented ability to disperse inter-continentally (Kiesecker 2003; Molnar *et al.* 2008; Keller *et al.* 2011). On a global scale, invasive species cover just about all existing taxa, including aquatic (Bruton & Van As 1986; Figuerola & Green 2002) and terrestrial vegetation (Foxcroft *et al.* 2004; Pyšek *et al.* 2009), fungi (Liebold *et al.* 1995), molluscs (Bij de Vaate & Jazdzewski 2002; Van der Velde & Platvoet 2007), insects (Roques *et al.* 2009), fish (Kats & Ferrer 2003; Gozlan *et al.* 2010), amphibians (Kark *et al.* 2009), reptiles (Kark *et al.* 2009), birds (Hulme *et al.* 2008; Klark *et al.* 2009), and mammals (Hulme *et al.* 2008; Genovesi *et al.* 2009) to cite only a few examples of the exhaustive list of biota that impact on ecosystems they were introduced to. The situation for South Africa does not look much different, with over 3000 publications that date back more than 150 years on South African alien invasive flora and fauna (Musil & MacDonald 2007).

In most parts of the world invasive species have enormous impacts on freshwater ecosystems (Lowe *et al.* 2000; Kiesecker 2003; Musil & MacDonald 2007; Keller *et al.* 2011). The introduction of over 160 predatory fish species to 120 countries worldwide is a major global threat to amphibian biodiversity (Barber & Babbitt 2003; Kiesecker 2003). Bearing in mind that most amphibians depend on freshwater systems to complete their life cycles, invasive species is acknowledged as a key universal problem causing amphibian declines (Gascon *et al.* 2007). Larvae of many anurans are susceptible to exotic and invasive fish predation (Knapp & Matthews 2000; Gillespie 2001; Kats & Ferrer 2003). Invasive fish species in South Africa that are known to feed on anuran eggs or larvae include Bluegill sunfish (*Lepomis macrochirus*) and Smallmouth bass (*Micropterus dolomieu*)

(Skelton 2001). Although invasive Mosquitofish species, especially *Gambusia affinis* and *Gambusia holbrooki* are known to predate on amphibian eggs and larvae across the world (Morgan & Buttemer 1996; Komak & Crossland 2000; Smith *et al.* 2013), no studies were found to report predation on South African anuran species. Studies on the impact of invasive fish as threat for threatened amphibian species in South Africa are long overdue (Minter *et al.* 2004; Measey 2011).

Several examples exist where frogs are the invasive species. Detrimental effects of introductions of alien frogs to their non-natural environments have been clearly demonstrated by the invasive Cane Toad (*Rhinella marina*) in Australia, (Lever 2001). *Rhinella marina* was originally introduced into Puerto Rico in the 1920's to be used as a means of biological control for the sugar cane (*Saccharum* spp.) pest, white-grub (*Phyllophagum* spp.), and its success led to further introduction to Australia, Florida, Papua New Guinea, the Philippines and many small islands in this region (Van Volkenberg 1935; Lever 2001). Thereafter *R. marina* became notorious as a major threat to native biodiversity due to competition between native species for food and breeding sites (Lampo & De Leo 1998; Lever 2001; Kiesecker 2003) and because it is highly toxic to anything that tries to eat it (Hayes *et al.* 2009; Shine 2010). A recent study has shown that choruses of the invasive Cuban Tree Frog (*Osteopilus septentrionalis*) in Everglades National Park affected the acoustic behavior of native treefrogs (Tennessen *et al.* 2013). More examples of invasive amphibian threats are summarised in Pilliod *et al.* (2012).

In South Africa, range extensions and accidental introductions of three endemic anuran species are of particular concern, namely *Hyperolius marmoratus*, *Amietophrynus gutturalis* and *Xenopus laevis* in Cape Town (De Villiers 2006; Tolley *et al.* 2008). However, it is not yet known if these species have an effect on the threatened species which territories they invade (Tarrant 2012). Infectious diseases and pathogens are often accompanied by alien species introductions (e.g. Weldon & Fisher 2011). As many as 37% of South African frogs are under threat as a result of invasive species introductions. This figure is much higher than the global average of 15.7% (Measey 2011). Alien vegetation (e.g. the water hyacinth (*Eichhornia crassipes*); Minter *et al.* 2004) is especially problematic to South African frogs. Forty out of 43 species are impacted by alien plant species, due to spreading alien vegetation and afforestation (Measey 2011).

1.3.6 Climate change

Increased levels of atmospheric carbon dioxide produced by the use of fossil fuels (e.g. vehicle fuel and generation of electricity using coal), globally contribute to climate change and correlates strongly with increased urbanisation and population growth (Grimmond 2007; Solomon *et al.* 2007; Huong & Pathirana 2013). The connection between amphibian disease-related declines and climate change has recently been debated amongst scientists (Pounds *et al.* 2006; Alford *et al.* 2007) and it is generally established that changes in climate can have multiple effects on amphibians due to their reliance on seasonal precipitation for breeding (Corn 2005; Minter 2011). Midgley *et al.* (2001) noted in a preliminary review of climate change in South Africa that higher temperatures are expected over the whole of South Africa. Summer rainfall will decrease by between 5% in northern regions and 25% in the south. Within the next 50–100 years the biomes, as we currently know them, may be reduced to 35–55% of their present extent. Furthermore, Minter *et al.* (2004) listed a number of threatened amphibian species that climate change will impact on. Minter (2011) stressed the necessity for increased research and long-term monitoring of South African frog populations. Observations of biological and ecological changes and their contribution to climatic change that is separated from other factors are in its infancy in South Africa and Africa as a whole (Midgley *et al.* 2001).

1.3.7 Constructive contributions of urbanised landscapes

Except for the numerous detrimental and cascading effects of urbanisation, there are some constructive contributions as a side-effect of urbanisation. Tyler and Watson (1998) demonstrated that out-houses for sanitation facilities for staff in the populated farming communities of Victoria in Australia, were used by frogs for hibernation refuges. Furthermore, the construction of roads also creates trenches on either side of the road, and these become new seasonal pools in which open habitat specialist frog species such as the Spring peeper (*Pseudacris crucifer*) can breed (Tyler *et al.* 2007). Amphibians with wide habitat tolerances might be more capable of persevering within urban landscapes since they are able to exploit artificial habitats such as garden ponds, ornamental lakes and dams, retention ponds and drains (Hamer *et al.* 2012). For instance, the Common Frog (*Rana temporaria*) in Britain continues to exist in urban and suburban areas more so than in rural areas, which is largely a result of the large quantity of garden ponds (Carrier & Beebee 2003). Together with the help of habitat modification that benefits a few species, the numbers of frogs sacrificed for physiological, research and teaching purposes has also diminished over the years, however, numbers lost as a result of habitat destruction and large-scale environmental degradation overpower these mitigation measures (Tyler *et al.* 2007). Therefore, the overall effect of urbanisation on amphibians remains negative and detrimental.

1.4 Importance of amphibians

On Tuesday 11 September 2012, Sam Masters from *The Independent* newspaper published an article about the recently compiled list of the hundred top bird species that are at risk of extinction. One of the comments she quoted from the interview with the Zoological Society of London's director of conservation, Professor Jonathan Baillie, said:

> *"The donor community and conservation movement are leaning increasingly towards a 'what can nature do for us' approach, where species and wild habitats are valued and prioritised according to the services they provide for people."*

From this statement it is clear that human perceptions and views of amphibians can influence their conservation to a great extent, by for example, willingness to fund conservation actions (Ceríaco 2012). In an economically driven world and in light of humanity's selfish nature, underscored by the comment above, nature is weighed up against monetary and self-centred beneficial value. Up to this point the detrimental and far-reaching effects of urbanisation on the world's amphibians were briefly summarised. However, to emphasise the importance of anuran amphibians to man, I provide a concise discussion on key areas where these invaluable animals have played, and will continue to play, their part in the success, progress and sustenance of humanity.

1.4.1 Pivotal position in the food web – trophic importance

Amphibians play a vital role in the ecosystem where they occupy an intermediate trophic position (Rubbo *et al.* 2006; Davenport & Chalcraft 2012). They are substantial predators, particularly of invertebrates, and simultaneously they fall prey to many predators including fish, other frogs, snakes, birds and mammals (Duellman & Trueb 1986). The herbivorous tadpoles consume significant amounts of algae and vegetable detritus while also serving as food for aquatic predators, both invertebrate and vertebrate (McDiarmid & Altig 1999). Amphibians comprise a large proportion of the vertebrate biomass in forests and wetlands (Burton & Likens 1975; Gibbons *et al.* 2006) and can therefore cumulatively consume large amounts of arthropods at a time. For example, Abdulali (1985) is one of very few studies who estimated daily insect biomass intake in a frog species. He found that an adult Indian bullfrog (*Hoplobatrachus tigerinus*) devours approximately 10% of its own weight in insects every day. During illegal exports of Indian frog legs, 9 000 t of *H. tigerinus* were harvested annually, thereby 900 t of insects, including mosquitoes and agricultural pests, survived daily and must be controlled via chemical insecticides (Abdulali 1985; also see Mohneke 2011), this in itself leads to compromised environmental health (Bridges 2000). However, it should be recognised that their diet consists of a variety of beneficial insects as well, and frogs cannot be used as an independent intervention for disease vector control (Raghavendra *et al*. 2008).

1.4.2 Educational importance

The use of amphibians in education is prominent in history and everyday life (e.g. 'Reptiles and amphibians in the classroom zoo' in Netting 1948). For example, using frogs as models for teaching basic anatomy to students has been a standard practice for more than 150 years (Tyler *et al.* 2007). In experiments in physiology and pharmacology, neurotransmitters and inhibitors such as acetylcholine and atropine were added to muscles and nerve bundles to demonstrate basic physiological concepts in vertebrates. Furthermore, because of the place amphibians hold in the evolutionary transition from primary aquatic vertebrates to terrestrial tertrapods, they also hold an evolutionary important place in education (Hickman *et al.* 2012). Furthermore, frogs and reptiles are used to reconnect children to the natural world to address modern day problems such as obesity and nature alienation as well as to evoke an interest and understanding about conservation issues (Louve 2008; Grant *et al.* 2011).

1.4.3 Research and medical importance

Frogs have formerly presented an important learning platform for man and will continue to do so in future. In a review of the roles that frogs played in the lives of humanity, Tyler *et al.* (2007) noted that about 10% of the Nobel prizes in physiology and medicine were awarded to investigations using frogs (see http://nobelprize.org/). Amphibians have been models for research in developmental biology (Gurdon & Hopwood 2000), eye research (Tsonis 2008), bioacoustics (Gerhardt & Huber 2002; Ryan 2001; Narins *et al.* 2006; Leary 2009), pharmacology (Bertaccini *et al.* 1968; Spande *et al.* 1992; Nogawa *et al.* 2001), neurobiology (Narins *et al.* 2006), endocrinology (Leary 2009), and bioindicators of ecosystem health (Waddle 2006; Swell & Griffiths 2009; Simon *et al.* 2011, see Section 1.4.5). In the past, using anurans for pregnancy testing was the standard practice for 20 years from the 1930's (Shapiro & Zwarenstein 1934; Hansen 1960). Throughout the globe a human pregnancy test was developed that involved injecting a small quantity of urine of a patient into a male or female anuran, usually *Rhinella arenarum, R. marina*, *Xenopus laevis* or *Rana* species (Galli-Mainini 1948; Bettinger & O'Loughlin 1950; McDonald & Taft 1953; Gurdon & Hopwood 2000). Pregnancy could be confirmed by the release of spermatozoa into the frog's bladder and cloaca in the case of a male, or the release of eggs into the container in the case of a female, within three to four hours of administering the urine (Galli-Mainini 1948; Gurdon & Hopwood 2000).

Skin secretions (peptides and alkaloids) from glandular anuran skin have been extensively researched for antibiotic compounds, hallucinogens, anti-tumour agents, analgesics, anti-inflammatory compounds, natural adhesives, repellents for mosquitoes and other predators and parasites, and spermicidal compounds (see Tyler *et al.* 2007 for a review). Furthermore, there are also indications that one of the alkaloids, caerulein, can provide relief for sufferers of chronic schizophrenia (Maroji *et al.* 1982; Watanabe *et al.* 1984; Sleigh 2012).

1.4.4 Contact with nature promotes health and well-being

Literature suggest that when humans come into contact with nature they are provided with beneficial physiological effects, such as recovery from mental fatigue, enhanced ability to cope with and recover from stress, restored concentration and improved productivity, and stimulates positive thinking (Table 1.2). No studies were found to use frogs with regards to these matters. However, frogs can be an especially useful model and are currently used to reconnect children with nature in urban areas (Louve 2008)

Table 1.2 A summary of evidence supporting the assertion that contact with nature promotes health and well-being. Table adapted from Maller *et al.* (2006).

What the research demonstrates with certainty	
Statement	Key references
There are some known beneficial physiological effects that occur when humans encounter, observe or otherwise positively interact with animals, plants, landscapes or wilderness	Friedmann *et al.* 1983a; Friedmann *et al.* 1983b; Parsons 1991; Ulrich, *et al.* 1991b; Rohde & Kendle 1994; Beck & Katcher 1996; Frumkin 2001
Natural environments foster recovery from mental fatigue and are restorative	Furnass 1979; Kaplan & Kaplan 1989; Kaplan & Kaplan 1990; Hartig *et al.* 1991; Kaplan 1995
There are established methods of nature-based therapy (including wilderness, horticultural and animal-assisted therapy among others) that have success healing patients who previously had not responded to treatment	Levinson 1969; Katcher & Beck 1983; Beck *et al.* 1986; Lewis 1996; Crisp & O'Donnell 1998; Russell *et al.* 1999; Fawcett & Gullone 2001; Pryor 2003
When given a choice people prefer natural environments (particularly those with water features, large old trees, intact vegetation or minimal human influence) to urban ones, regardless of nationality or culture	Parsons 1991; Newell 1997; Herzog *et al.* 2000
The majority of places that people consider favourite or restorative are natural places, and being in these places is recuperative	Kaplan & Kaplan 1989; Rohde & Kendle 1994; Korpela & Hartig 1996; Herzog *et al.* 1997; Newell 1997; Herzog *et al.* 2000
People have a more positive outlook on life and higher life satisfaction when in proximity to nature (particularly in urban areas)	Kaplan & Kaplan 1989; Kaplan 1992a; Lewis 1996; Leather *et al.* 1998; Kuo 2001; Kuo & Sullivan 2001
Exposure to natural environments enhances the ability to cope with and recover from stress, cope with subsequent stress and recover from illness and injury	Ulrich 1984; Parsons 1991; Ulrich *et al.* 1991b
Observing nature can restore concentration and improve productivity	Tennessen & Cimprich 1995; Leather *et al.* 1998; Taylor *et al.* 2001
Having nature in close proximity, or just knowing it exists, is important to people regardless of whether they are regular 'users' of it	Kaplan & Kaplan 1989; Cordell *et al.* 1998

1.4.5 Amphibians as models for bioindicators

Due to the permeability of amphibians' hairless, shell-less and scale-less skins and their naked jelly-encapsulated eggs (Duellman & Trueb 1986), they are especially sensitive to changes in the chemical composition of air and water (Blaustein *et al.* 1994). Amphibians have also recently been considered by many biologists to be exceptional biological indicators (or bioindicators) of environmental health (Blaustein 1994; Blaustein & Wake 1995). The global decline in amphibian populations and the increase of deformities can be seen as a warning of severe environmental deprivation (Blaustein & Johnson 2003). For example, Horn & Dunson (1995) investigate the effects of heavy metals, pH and water hardness on larval amphibian stages of the Jefferson Salamander (*Ambyostoma jeffersonianum*) and the Wood Frog (*Rana sylvatica*). They found that larval survival was significantly decreased with the presence of Aluminium and Copper.

1.5 The need for urban ecological research on amphibians in South Africa

The term "urban ecology" is seldom used in South African scientific literature (Cilliers & Siebert 2012). Although there is a good body of literature on urban floral communities (Cilliers *et al.* 1998; Cilliers & Bredenkamp 2000; Dingaan *et al.* 2001; Grobler *et al.* 2002; Cilliers & Siebert 2011, 2012), a few studies on birds (Smith 2004; Pauw & Louw 2012), biodiversity as a whole (Holmes *et al.* 2012), nuisance baboon troops (Hoffman & O'Riain 2012), feral cats (Tennent & Downs 2008), and some studies conducted on social aspects (Cilliers *et al.* 2012; Graham & Ernstson 2012) very few have touched on the predicaments faced by amphibians in urbanised regions of South Africa (Minter *et al.* 2004; Measey 2011; Mokhatla *et al.* 2012). South Africa hosts 122 species, representing 12.1% of the total global families with seven new species described or allocated in the past three years (Frost 2014). Despite this wide anuran diversity in South Africa, there has been surprisingly little literature found on urban ecological aspects of frogs.

Urbanisation is one of the most important causes of global amphibian declines (Baillie *et al.* 2004; Hamer & McDonnell 2008). South Africa's total human population growth between 1996 and 2001 was 10.4% and demonstrated further growth to 15.5% from 2001 to 2011, with the urban population increasing with >10% in the last decade (Statistics South Africa). Furthermore, among the most threatened groups are freshwater-dependent animals, which include amphibians, (Masters *et al.* 2000). Amphibians face many threats within southern Africa, but the threat most frequently attributed to declining populations is habitat destruction due to wetland drainage, afforestation, crop farming, invasive alien vegetation and urbanisation (Harrison *et al.* 2001; Minter *et al.* 2004; Measey 2011).

Within urban ecology, there are two fast developing fields in the acoustic arena, namely "urban bioacoustics" and "soundscape ecology", neither of which has been studied from a South African perspective. Indeed, there is a large gap in the field of amphibian bioacoustics as a whole in South Africa, with most previous studies from a research group at the University of Witwatersrand headed by Professor Neville Passmore and Dr Phil Bishop between the 1970's and 1990's.

1.6 Thesis scope and objectives

This thesis is essentially concerned with the response of anurans to an increasingly urban South Africa in terms of habitat on a local and landscape scale (Chapter 2), the increasingly noisy acoustic environment (Chapter 3–5), the extent of road carnage (Chapter 4), urban climate (Chapter 6), and also includes a social approach to amphibian conservation (Chapter 7). Although the thesis covers a wide variety of topics on anuran urban ecology some degree of overlap in some aspects was inevitable, but limited.

The city of Potchefstroom was the chosen study area to evaluate anuran community distribution across an urban-rural gradient and the response of species richness to urbanisation using habitat variables on both local (pond) and landscape scales. This study is aimed to provide a starting point to further gradient analysis on other towns in South Africa to inspect the habitat use and severity of the effects of urbanisation along this gradient. To study the effects of anthropogenic noise on the acoustic behaviour and call properties of anurans, the critically endangered Pickersgill's Reed Frog (*Hyperolius pickersgilli*, occurring at patchy landscapes in coastal Kwa-Zulu Natal; IUCN 2013) and the endangered Western Leopard Toad (*Amietophrynus pantherinus*, occurring in urban habitats of the Western Cape) were used as model species. Calling behaviour of the widely distributed Common River Frog (*Amietia quecketti*) was also analysed in relation to climatic variables within an urban green space.

Finally, the attitudes of people towards frogs were explored to evaluate to what extent it may influence the tolerance of frogs in urban habitats. These themes were selected to cover a broad spectrum on the aspects of the ecology and conservation of frogs in urban habitats of South Africa.

CHAPTER TWO

An assessment of frog communities along an urban-rural gradient in Potchefstroom, South Africa

Human nature is potentially aggressive and destructive and potentially orderly and constructive.

– Margaret Mead, American anthropologist

2.1 Introduction

Habitat change is among the biggest threats to amphibian populations worldwide (Czech *et al.* 2000; Marzluff 2001; Stuart *et al.* 2004; Beebee & Griffiths 2005; Cushman 2006; Hamer & McDonnell 2008; Measey & Tolley 2011; *Mokhatla et al. 2012*). No less than 88% of threatened amphibians are impacted by urbanisation through habitat loss, fragmentation and degradation (Baillie *et al.* 2004). The life history stages of amphibians necessitates a multi-spatial approach when defining habitat, for as the name amphibian suggests (*amphi* = two/both + *bios* = life/living), these animals require both a suitable aquatic habitat to breed and metamorphose from free-swimming larval stages, as well as a proper terrestrial habitat to mature, forage and hibernate (Pope *et al.* 2000; Semlitsch & Bodie 2003; Cushman 2006; Pillsbury & Miller 2008; Lemckert *et al.* 2012). Therefore, urbanisation can affect amphibians at a local micro-habitat level by changing water quality and surface hydrology (Pechmann *et al.* 1989; Vershinin & Tereshin 1999; Babbitt & Tanner 2000) as well as at larger landscape scales such as habitat alteration, fragmentation, creating movement barriers and loss of metapopulation connectivity (Hager 1998; Vos & Chardon 1998; Houlahan & Findlay 2003; Semlitsch & Bodie 2003; Rothermel 2004; Trenham & Shaffer 2005; Hamer *et al.* 2008; Heard *et al.* 2013). Although habitat alteration, degradation and fragmentation have a more direct effect on anurans, urbanisation can also affect anurans indirectly via changes in acoustic environments (see Chapters 3–5), changes in urban atmospheric conditions (see Chapter 6) as well via the attitudes of people towards frogs that can affect the persistence and success of anuran populations (see Chapter 7). In this chapter I will only focus on the effects of urbanisation pertaining to habitat change.

On a landscape scale, vast amounts of surface area is altered by replacing natural habitat with impervious surfaces associated with business districts, industrial, residential and agricultural landscapes, leaving only fragments of terrestrial and aquatic habitats that are transformed and altered for recreation purposes. The built environment creates multiple barriers via roads, houses and walls or fences, leaving anurans with challenging and in most cases impossible migration route that weaken ecological connectivity between wetlands and dispersal habitat (Vos & Chardon 1998). Although anurans are able to cross and climb over or through some of these perceived barriers, this might differ between species. Studies have frequently shown a negative correlation between species richness and urban density (Findlay & Houlahan 1997; Pillsbury & Miller 2008; Hamer & Parris 2010). Urban landscapes can be defined by almost decomposable, nested spatial hierarchies, in which hierarchical levels correspond to structural and functional units functioning at distinct spatial and temporal scales (Reynolds & Wu 1999; Wu & David 2002; Alberti 2008). The different spatial and hierarchical units of the landscape vary with socioeconomic and biophysical processes, i.e. from households and buildings to habitat patches or remnant ecosystems (Alberti 2008). Therefore, it is necessary to understand the underlying community dynamics of anurans within this complex network of an altered environment.

Anurans can have a primarily terrestrial or aquatic existence, but the vast majority need both terrestrial and aquatic habitats to complete their life-cycles (Duellman & Trueb 1986; McDiarmid & Altig 1999; Wells 2007). Therefore, on the long-term, micro-habitats are more important for amphibians than for any other vertebrates (Lemckert *et al.* 2012). As ectotherms and having a permeable skin, amphibians naturally have physiological constraints, namely to maintain body temperature and stay hydrated while foraging (Duellman & Trueb 1986; Laurila 1998; McDiarmid & Altig 1999; Wells 2007). Therefore, choosing a fitting aquatic habitat for breeding, protecting their naked gelatinous eggs from predators and finding a suitable terrestrial habitat to hibernate is of utmost importance (Lemckert *et al.* 2012; Searcy *et al.* 2013). Furthermore, apart from skin toxins and avoiding predators behaviourally, anurans in general have few physical attributes to protect them from predators and therefore rely on habitat for concealment and camouflage.

Urbanisation affects the microhabitat in several ways, including the introduction of invasive plant and animal species (Pilliod *et al.* 2012), altering water quality, i.e. increased levels of heavy metals (Paul & Meyer 2001; Alberti 2008) and changing hydrology (Wellborn *et al.* 1996). Terrestrial and aquatic habitat for calling, hiding during daytime, hibernating, foraging, migrating, dispersing and breeding are also decreased (Lemckert *et al.* 2012). Urbanisation has a profound effect on predation in water bodies through the introduction of invasive fish species (Pilliod *et al.* 2012). Predation by fish on tadpoles and amphibian eggs decreases amphibian species chance of survival and impact on species richness and occurrence in urban areas (Ficetola & De Bernardi 2004; Pearl *et al.* 2005; Hamer & Parris 2011; Hamer & Parris 2013). Along with hydroperiod, it has the potential to produce alterations in the structure of larval amphibian communities (Wellborn *et al.* 1996; Hamer & Parris 2013). Even low densities of invasive fish species may have detrimental effects on native amphibians (Lydeard & Belk 1993; Pilliod *et al.* 2012). Globally, several species have been shown to have adverse effects on amphibian populations. These include poecilids (mosquitofish), salmonids (trout and bass) and cyprinids (carps and domesticated cyprinids such as gold fish, *Carassius auratus*) (Formas 1995; Gillespie & Hero 1999; Monello & Wright 2001), and Odontubutid (Rotan) (Pilliod *et al.* 2012). Two mosquitofish species (*Gambusia affinis* and *G. holbrooki*), that are native to southern and eastern United States, for example now have global distributions due to their former popularity as biological control agents for mosquito larvae (Courtenay & Meffe 1989). They are small and effective aquatic predators and have been considered a major driver for native fish and amphibian declines in Australia, New Zealand and the Western United States (Bence 1988; Courtenay & Meffe 1989; Lydeard & Belk 1993; Mahony 1999; Komak & Crossland 2000; Hamer *et al.* 2002; Hamer & Parris 2013).

To sustain biodiversity within urban landscapes, features that support local amphibian populations must be identified (Scheffers & Paszkowski 2013). Hazell *et al.* (2004) demonstrated that both natural and constructed ponds in agricultural landscapes have a part to play in conservation of anuran diversity and providing habitat for frogs, given that heterogeneity in waterbody characteristics across the landscape is preserved. However, amphibian health may be compromised by the use of pesticides and other chemicals in agricultural landscapes (Mann *et al*. 2009). Studies have shown that landscape determinants that play a role promoting urban anuran diversity include larger areas of green open space for metapopulation connectivity and provision of terrestrial foraging habitat, (Hazell *et al.* 2004; Hamer & Parris 2010; Scheffers & Paszkowski 2013) and constructed ponds and stormwater wetlands (Hazell *et al.* 2004; Scheffers & Paszkowski 2013). Except for urban density, threats on a landscape scale include invasive plant species, land transformation and road density (Eigenbrod *et al.* 2009; *Mokhatla et al. 2012*).

The perseverance of anuran populations in urban landscapes have been shown to be subjective to a dynamic set of variables at different spatial scales, from individual breeding sites to larger landscapes (Pellet *et al.* 2004a, b; Drinnan 2005; Rubbo & Kiesecker 2005; Pillsbury & Miller 2008; Hamer & Parris 2010). Very few studies report how anuran communities as a whole respond to urbanisation at both local (micro-habitat) and landscape scales (Pillsbury & Miller 2008; Hamer & Parris 2010). This study is the first to assess effects of urbanisation on anuran communities as a whole on a multi-spatial scale in the African continent (but see Measey & Tolley 2011; Mokhatla *et al. 2012*).

The aim of this study was to assess anuran community assemblages across an urban-rural landscape in Potchefstroom and determine supportive information for the amphibian distribution by looking at landscape variables and micro-habitat determinants. A gradient analysis contributes to the understanding of the ecology of cities, which is important for comparative urban ecological research (Du Toit & Cilliers 2011).

2.2 Materials and Methods

2.2.1 Study area

Potchefstroom is an academic city located in the North-West Province, South Africa, governed by the Tlokwe City Council Municipality. The town is one of the oldest in South Africa and has a rich history with the first European people settling in 1838 (Badenhorst *et al.* 1939; Jenkins 1971). Although Potchefstroom is one of the oldest cities in the country, it has not developed into a large city such as Cape Town and Johannesburg. It is a multi-cultural town of 2 573 km^2, populated by more than 162 700 residents (71.6% African, 20.7% Caucasian, 6.8% Coloured and 0.9% Indian). The Tlokwe City Council Municipality reported a 2.4% population increase in a decade spanning from 2001 to 2011. This is a 1.8% increase on the previous decade and 0.8% higher than that of the North-West Province as a whole for the same period (Tlokwe City Council, Census 2011). This underscores the potential rapid future growth rate for this developing town. Potchefstroom is also home to five tertiary institutions and 30 schools, as well as numerous research bureaus and training centres. In addition, Potchefstroom is an important industrial, service and agricultural growth point of the North-West Province. Industries include steelworks, agriculture and chemical processing.

Potchefstroom is close to the Vredefort Dome world heritage site, the oldest and largest reported astrobleme (an eroded remnant of a large crater made by the impact of a meteorite) globally (McCarthy & Rubridge 2005; Norman & Whitfield 2006). The geological alterations that formed from the impact 190 million years ago brought about a relatively rich faunal and floral diversity within the Grassland and Savanna biomes (Mucina & Rutherford 2006; Figure 2.1). Within the Tlokwe City Council Municipality there occurs three threatened terrestrial ecosystems, namely Rand Highveld Grassland, Vaal-Vet Sandy Grassland and Vredefort Dome Granite Grassland, covering more than 13% of the district area, and holds 401 wetlands covering 3 664 ha (SANBI 2009).

2.2.2 Larval amphibian and fish survey

Site selection

A pilot survey on 104 wetlands (i.e. water bodies in a broader sense) was conducted between 1 February and 14 April 2012 in order to evaluate physical properties of sites to be selected for primary surveys. Taking time- and man-power constraints into consideration, 68 wetlands were randomly selected using the RANDOM function in Microsoft Office Excel 2010 and divided into nine wetland-type categories namely: roadside pool (n = 6), formal ornamental pond (n = 12), garden pond (n = 12), permanent river (n = 6), former quarry (n = 4), big dam (n = 6), farm dam (n = 6), vlei (n = 7), and large peri-urban pond (n = 9). The availability of 68 wetlands for sampling was further reduced to 61 sites by drought conditions experienced at the time of the formal survey (see Appendix A for site descriptions).

As defined by MacKenzie *et al.* (2006), season refers to the sampling period in which the target species is either present or absent from the sites, i.e. sites are closed to changes in occupancy or changes occur at random. Four aquatic surveys at the 68 selected wetland sites were conducted during autumn, spring, and summer: 1) 8 May - 28 May 2012, 2) 10 September - 30 September 2012; 3) 14 November - 4 December 2012, and 4) 19 January - 8 February 2013. The study was designed to include the breeding and larval development periods (adults not included) of all 11 species occurring in the region (Du Preez & Carruthers 2009). This approach was followed because it showed that the species is both present and breeding. During the four surveys it was assumed that the wetlands were closed to local colonisation and or extinction by frogs. The sequence in which wetlands were visited was randomised.

Detection methods

Three techniques were used simultaneously for aquatic sampling of amphibian larvae and fish (see Appendix B for list of wetland effort quantification):

- Electro-fishing

  A backpack electro-fisher (SAMUS725M) for electro-narcosis was used to collect fish and tadpoles. An anode carried in one hand and a cathode trailing behind electrifies water within an approximate radius of 2 m, depending on water conductivity. Any fish within the sensitivity radius go into a state of narcosis (stunned). Tadpoles were less affected, but swam from their hiding spots out to the open water. Stunned fish and startled tadpoles were then collected by means of a landing net (2.5 mm mesh and square frame, 300 x 300 mm) fitted with a rubberised handle and placed into buckets (separate buckets for tadpoles and fish). Electro-fishing was the first technique applied to each pond. To standardise the effort, the total amount of time spent electro-fishing was relative to the surface area of a wetland (two minutes minimum, plus one additional minute per doubling of surface area >25 m^2, maximum of 22 minutes).

- Bottle trapping

  Plastic funnel traps were made from 2-L soft drink bottles (Richter 1995; Lauck 2004) and perforated to allow water ventilation. To standardise the effort, the total amount of bottle traps set out was relative to the surface area of a wetland (two traps minimum, plus one additional trap per doubling of surface area >25 m^2, maximum of 12 traps). Bottle traps were set after electro-fishing and left in wetlands overnight. Sampling protocol for bottle traps followed Hamer & Parris (2010).

- Dip-netting

  Dip-netting was performed using a square framed dip net (300 x 300 mm, 2.5 mm mesh). Dip-netting followed trap retrieval in the mornings. To standardise effort, the total amount of time spent on dip-netting was the same as for electro-fishing. Due to the irregular distribution of larval amphibians in wetlands (Shaffer *et al.* 1994), traps and dip-net sweeps were scattered proportionally and randomly among micro-habitat types. Traps were placed into ponds between 20:00 and 00:30 hours (GMT +2), and retrieved the following morning. Traps were set for one night at each pond per survey due to time limitations within a sampling season. Sampling protocol for dip-netting followed Hamer & Parris (2010). A binomial key was used to identify amphibian larvae species (Du Preez & Carruthers 2009) and fish species (Skelton 2001).

2.2.3 Habitat variables

Micro-habitat

A set of seven variables were used to measure micro-habitat for each wetland throughout each survey, namely: wetland surface area (measured at the high-water mark using a meter-wheel for smaller ponds and ArcGIS 10.0 for large dams); bank slope (water depth measured 1 m from the water edge; conductivity (µS/cm^3); pH; vegetation index (total percentage of the pond covered by aquatic vegetation = emergent + submerged + floating vegetation); shade index; and presence of predatory fish. Emergent and submerged vegetation were defined as vegetation extending above and below the water surface respectively. If 50% of the pond was covered by emergent vegetation, 70% by submerged vegetation and 30% by floating vegetation, index values of 0.5, 0.7 and 0.3 were given respectively and the mean were then calculated from these values. Shade index was calculated by using a digital clinometer to measure the angle between the surveyor at eye-level and the highest peak at the horizon, facing north, east, and west, taking an average value of the three readings, whether the highest structure be a tree, building or hill. The surveyor always measured the shade index at the same location at each site, which was in the middle of the survey edge area. The mean of the continuous measurement for all variables was taken to be used in canonical analysis (see below). As demonstrated by Paul & Meyer (2001), there is usually an increase in conductivity in urban waterways, likely caused by contaminants washed in from roads and other impervious surfaces via surface runoff. Pond water conductivity and pH were measured in 500-mL water samples collected ≈1 m from the edge of the water at a depth of 50–100 mm using a handheld electronic multi-meter (YSI 556 MPS). I calculated the mean number of predatory fish at each pond using the numbers caught with all detection methods. All survey detection methods and variable measurement techniques were performed by the candidate to eliminate surveyor bias. Predatory fish were defined as those species caught in the study area that is known to prey on frog eggs or tadpoles (Pilliod *et al.* 2012; K. McHugh pers. comm.). Although several cyprinid species were detected, they were not included as predatory fish because of the uncertainty and inconsistency of data on the effects of these on amphibian populations in literature (Pilliod *et al.* 2012).

Landscape variables

In addition to micro-habitat variables, a set of landscape variables were categorised and calculated as measures of urbanisation using ArcGIS 10.0 package. Earlier studies on the effects of urbanisation on amphibians have used the proportion of the landscape surrounding wetlands covered by urban land as a metric of urbanisation (Houlahan & Findlay 2003; Gagné & Fahrig 2007; Hamer & Parris 2010). Buffers of 250 m were constructed around each wetland (at the location of sampling) and landscape features were categorised and digitised within each of the 68 buffers. Land surface area was divided into 12 categories as a personal interpretation from aerial imagery (SPOT 2010; 1:5 000 map resolution), namely agricultural lands, natural veld, impervious surfaces (parking areas and paved surfaces), industrial area, railway, roads, rural settlements, residential areas, urban central business district (CBD), urban recreational grass or fields, residential area, water surface area, and planted woody patches (including residential gardens, where point data for trees were used, but not surface area). The "calculate area" tool was used to obtain the surface area covered by the categories for each wetland's buffer. Buffer zones of 250 m were used because of the relatively small size of Potchefstroom. Using larger buffer zones would possibly have resulted in homogenising landscape variables. Furthermore, distances from each site to the CBD and average distances to other ponds were calculated using "distance to point" and "average distance between points" tools respectively in ArcMap (Esri 2011).

Geographical analysis

An Inverse Distance Weighting (IDW) interpolation analysis was conducted using number of species per pond (32 classes were chosen, thus eight increments of four). Since the number of species did not exceed four in any given wetland and the vast number of tadpoles collected at a time, especially for *Amietophrynus spp.*, species richness would bias towards these species. Therefore the Menhinick's index for species richness and the Shannon index for species diversity would have given an incorrect perspective of the number of species occurring at each wetland. Therefore, only the number of species was used in the interpolation and species richness in this chapter refers to the number of species and not the Menhinick's or Shannon index. For species relative abundance, the number of individuals recorded at each pond was divided into three classes (low, medium and high) using natural breaks (Jenks') for each species within all wetlands. All geographical analyses were conducted with the ArcGIS 10.0 package.

2.2.4 Ordination and statistical analysis

Ordination analysis

For ordination analysis, a biplot diagram was created of the canonical correspondence analysis (CCA) for the number of amphibian larvae according to seven micro-habitat and seven landscape explanatory variables that were recorded for 45 wetlands (7 wetlands were excluded from the analysis due to drought; a further 16 wetlands were excluded due to the absence of frogs from the ponds). CCA is a direct gradient ordination analysis that produces ordination axes confined to linear groupings of environmental variables (ter Braak 2000). Arrows at the end of each ordination axis point in the direction of the environmental gradient as identified by the explanatory variables. The longer the arrows in each ordination axis, the stronger correlated explanatory variables are with the ordination axes than those with short arrows. In order to comply with a general rule of thumb to have one variable for every ten samples (Harrell 2001; Wintle *et al.* 2004) some landscapes were merged to represent broader categories in order to obtain seven useable variables: altitude, green open space (GreenSpace, m^2, represented agricultural lands and urban recreational grass or fields), roads (m^2), urban CDB (UrbCBD, m^2, represented CBD and industrial area), residential area (UrbRes, m^2, represented high-income residential area as well as rural residential settlements; no gardens were included), distance to CBD (CBD_Dist, m), average distance between ponds (PondDist, m). Surfaces categorised as railway, impervious surfaces and woody patches were left out of the analysis as there was too little surface area to represent a gradient. Species data were presented by the total number of individuals of each species over the four survey periods for *Amietia quecketti* (*Aq*), *Amietophrynus gutturalis* (*Ag*), *A. rangeri* (*Ar*), *Cacosternum boettgeri* (*Cb*), *Kassina senegalensis* (*Ks*), *Strongylopus fasciatus* (*Sf*), and *Xenopus laevis* (*Xl*). Species richness (Sp_Rich) was also included to make an inference on general species diversity from the landscape variables (Hamer & Parris 2011). All ordination analyses were performed using CANOCO 4.5. Transformations included a log10 transformation for pond area, embankment slope, conductivity, pond shading, aquatic vegetation, altitude, distance between ponds and CBD, average distance to other ponds and urban residential area. A log10(x+1) transformation was calculated for roads area, green open space area, and urban CBD area.

Bayesian analysis

The effects of six micro-habitat variables and four landscape variables on species richness (i.e. number of larval frog species detected at a pond) were assessed using Bayesian inference. The six micro-habitat variables and four landscape variables were incorporated into 10 Poisson regression models in a variety of combinations, and included: pond area, pH, slope of embankment, conductivity, aquatic vegetation, predatory fish, area of roads, area of green open space, area of urban CBD, and average distance between ponds. We used uninformative priors for model intercept terms (a ~ dnorm[0, 1.0 x 10^{-6}]) and the regression coefficients (beta[j] ~ dnorm[0, 1.0 x 10^{-6}], where j is an explanatory variable) in OpenBUGS (Lunn *et al.*, 2000, Spiegelhalter *et al.*, 2007). . Explanatory variables were examined for collinearity using Spearman rank correlations and consequently highly correlated variables ($|\rho| \geq 0.4$) were not included into the same model (Appendix C). One null model ('no effect') was also included (constant only), resulting in a total of 11 models

OpenBUGS was used to produce 100 000 samples from the posterior distribution of 11 models after discarding an initial "burn-in" of 10 000 samples. Three Monte Carlo Markov chains were run for each model with a suitable number of iterations so that convergence was reached for all variables on the basis of the Brooks-Gelman-Rubin statistic (i.e., $R<1.05$). We obtained 95% Bayesian credible intervals from the 2.5th and 97.5th percentiles of the distribution. Relative fit of the models against model complexity were evaluated using the Deviance Information Criterion (DIC; Spiegelhalter *et al.*, 2002). The best fit of the models were considered to be those with a $\Delta DIC \leq 2$ ($\Delta DIC = DIC – DIC_{min}$), although any model with $\Delta DIC<10$ was also considered as being potentially relevant (Spiegelhalter *et al.*, 2007, Anderson, 2008).

Estimating effect sizes in ecology has the advantage of facilitating comparison of credible intervals with values that are ecologically meaningful, rather than focusing on statistical significance (McCarthy 2007). We therefore assessed the relative importance of the ten explanatory variables by calculating the multiplicative effect (with 95% credible interval) of each variable on species richness across the range of the variable. In Poisson regression, the multiplicative effect is calculated as the exponent of the standardized coefficient:

$E_i = \exp(b_i \text{ x } range_i)$

where E_i is the multiplicative effect of variable i, b_i is the regression coefficient of variable i, and range i is the range of values for variable i. A multiplicative effect size of 1 corresponds to no change in species richness, and so an explanatory variable with E_i substantially different than 1 is likely to have a biologically important effect on species richness. Multiplicative effect sizes > 1 indicate a positive effect of the explanatory variable on species richness; effect sizes < 1 indicate negative effects.

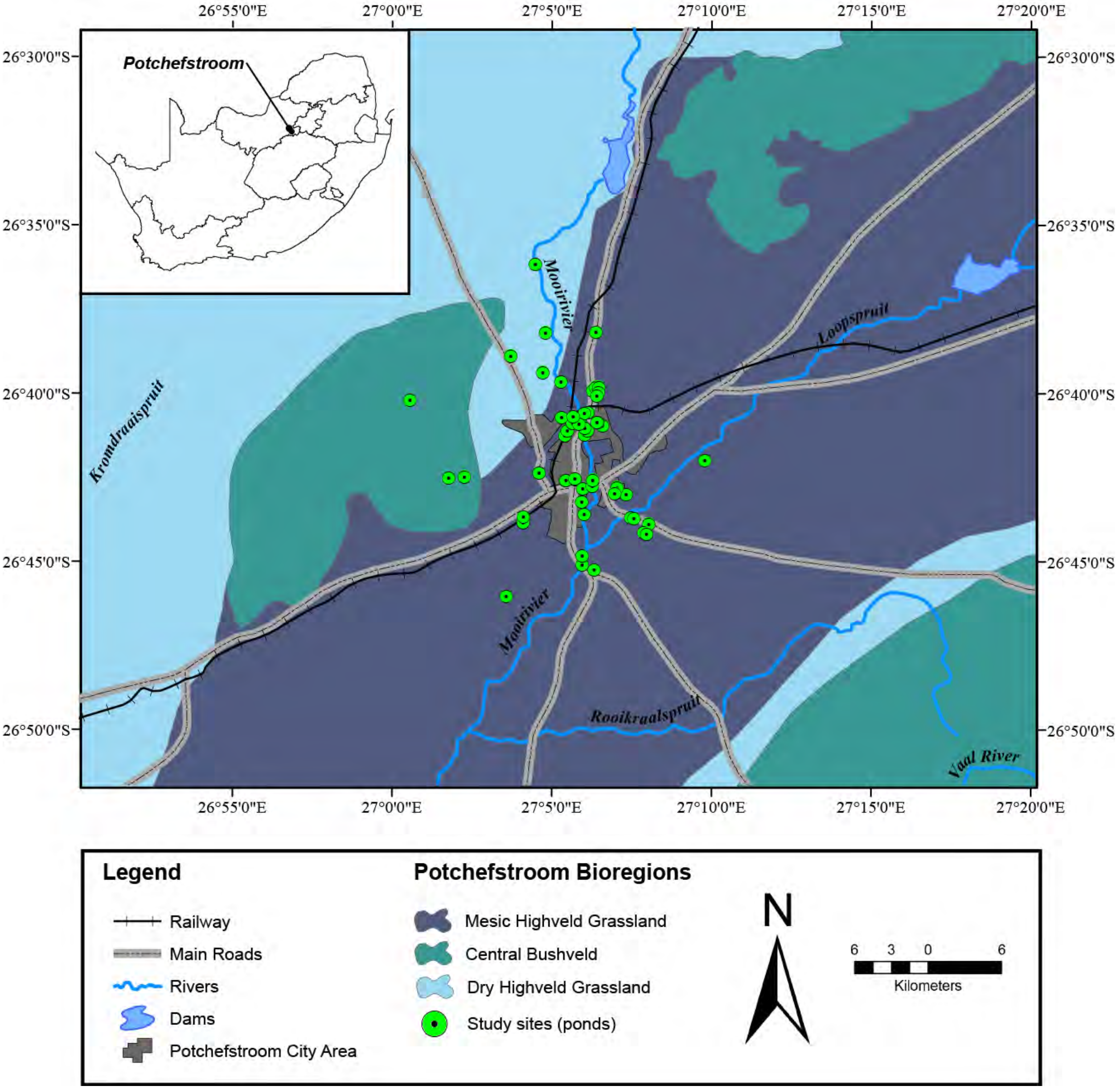


Figure 2.1 Map showing the vegetation bioregions surrounding Potchefstroom. Shapefiles obtained from SANBI; vegetation map and legend after Mucina & Rutherford (2006).

Bayesian statistics are net popular among ecologists (McCarthy 2007). However, good reasons exist to make Bayesian analysis a preferred data analysis method, including: Bayesian methods 1) include previous data to strengthen estimation of the presence or absence probability of a specific species occurring at a given site; 2) uses likelihood margins instead of *P*-values to provide a measure of probability; 3) differs from frequentist methods by asking what the probability is of the hypothesis being true given the data, instead of what the probability of observing the data is given that the various hypothesis is true (McCarthy 2007).

2.3 Results

2.3.1 Species distribution

Anuran larvae of eight species were detected during the field surveys (Table 2.1). *Amietia quecketti* was the most frequently detected species, occurring in 39.2% of the 61 wetlands (seven sites were excluded because of drought conditions), whereas the Bubbling Kassina (*Kassina senegalensis*) was detected in only one pond. The remaining six species were detected in 6.6–26.2% of the sites. The mean number of species per wetland was 1.2 (± 1.07 SD), ranging from 0–4. Forty five (73.8%) wetlands were occupied by at least one species. Species' distribution that overlapped the study area but were not detected during the surveys included *Amietophrynus poweri*, *Pyxicephalus adspersus*, *Schismaderma carens*, and *Tomopterna cryptotis*. Although both ephemeral and permanent wetlands were included in the study, drought caused all seven ephemeral ponds to have no water during the four survey seasons and therefore surveys were not conducted at these dry sites. Predatory fish (Sharptoothed Catfish, Mosquitofish, Largemouth Bass, Banded Tilapia) were detected in 64% of the wetlands (in 2, 27, 12, 26 wetlands respectively), with Mosquitofish and Tilapia occurring most frequent (Table 2.1).

Table 2.1 Larval frog species and predatory fish detected during the study and the number of wetlands (out of 61) where they occurred.

Family / *Species*	Common name	No. of ponds
Anurans		
Pyxicephalidae		
Amietia quecketti	Common River Frog	24
Cacosternum boettgeri	Common Caco	5
Strongylopus fasciatus	Striped Stream Frog	4
Bufonidae		
Amietophrynus gutturalis	Guttural Toad	16
Amietophrynus rangeri	Raucous Toad	12
Hyperolidae		
Kassina senegalensis	Bubbling Kassina	1
Pipidae		
Xenopus laevis	Common Platanna	16
Predatory fish		
Clariidae		
Clarias gariepinus	Sharptoothed Catfish	2
Poeciliidae		
Gambusia affinis	Mosquitofish	27
Centrarchidae		
Micropterus salmoides	Largemouth Bass	12
Cichlidae		
Tilapia sparrmanii	Banded Tilapia	26

Ponds were representative of a gradient between urban to rural landscapes, with a varying degree of CBD, residential (gardens included), green open space and water surface areas as demonstrated in Figure 2.2. Species richness was mostly focussed on the urban fringe and further away from the Potchefstroom CBD (Figure 2.3). Ponds occurring in or near the Potchefstroom CBD didn't host any species, whereas wetlands occurring on the urban fringe had higher species richness and were dominated by *A. quecketti*, *Amietophrynus gutturalis*, *A. rangeri* and *X. laevis* (Figure 2.4). However, due to the dry seasons experienced during the summer of 2012 and 2013, the interpolation of species richness may reflect an incomplete and distorted idea of the true species richness of the area (Figure 2.3) as well as the distribution of *C. boettgeri* and *K. senegalensis* requiring ephemeral ponds (Figure 2.4C, D). *Amietophrynus gutturalis*, *A. rangeri* and *X. laevis* featured in more ponds inside the urbanised border than outside (Figure 2.4A, B, F), whereas *S. fasciatus* was present in one of the wetlands in the urbanised border and the remaining three populations fell outside of it (Figure 2.4E). *Cacosternum boettgeri* and *K. senegalensis* were only found outside of the urbanised area (Figure 2.4C, D), and *A. quecketti* occurred in an almost equal number of sites outside and inside of the urbanised border (Figure 2.4G).

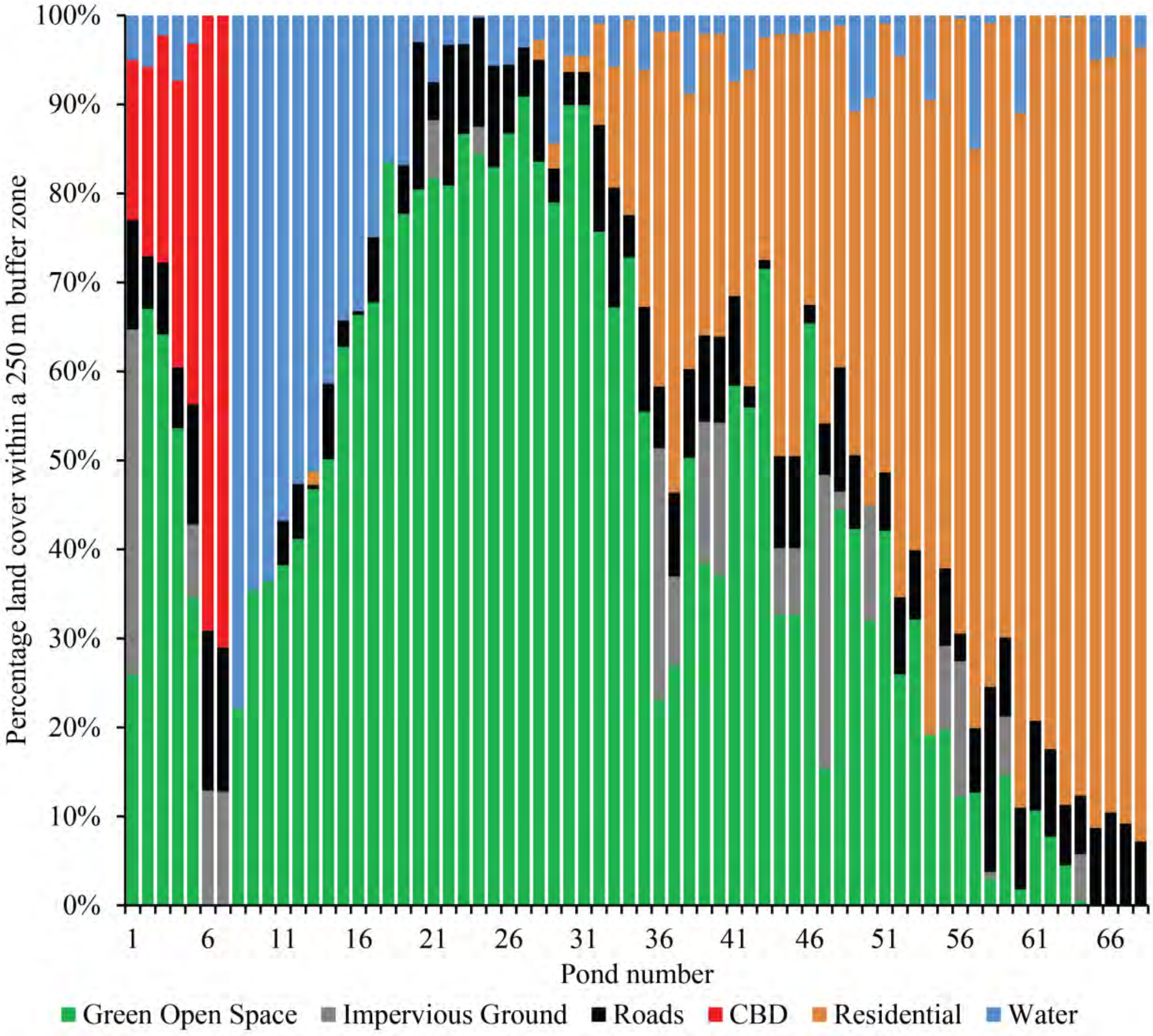


Figure 2.2 A column chart showing the collective contribution of different types of surface area (%) within the 250 m radius buffer zones of each of the 68 wetlands to illustrate the indirect urban-rural gradient in which sites were distributed. CBD = central business district. The figure does not depict a gradient in a given direction, but rather shows the percentage of each land cover for the area 250 m around each surveyed pond.

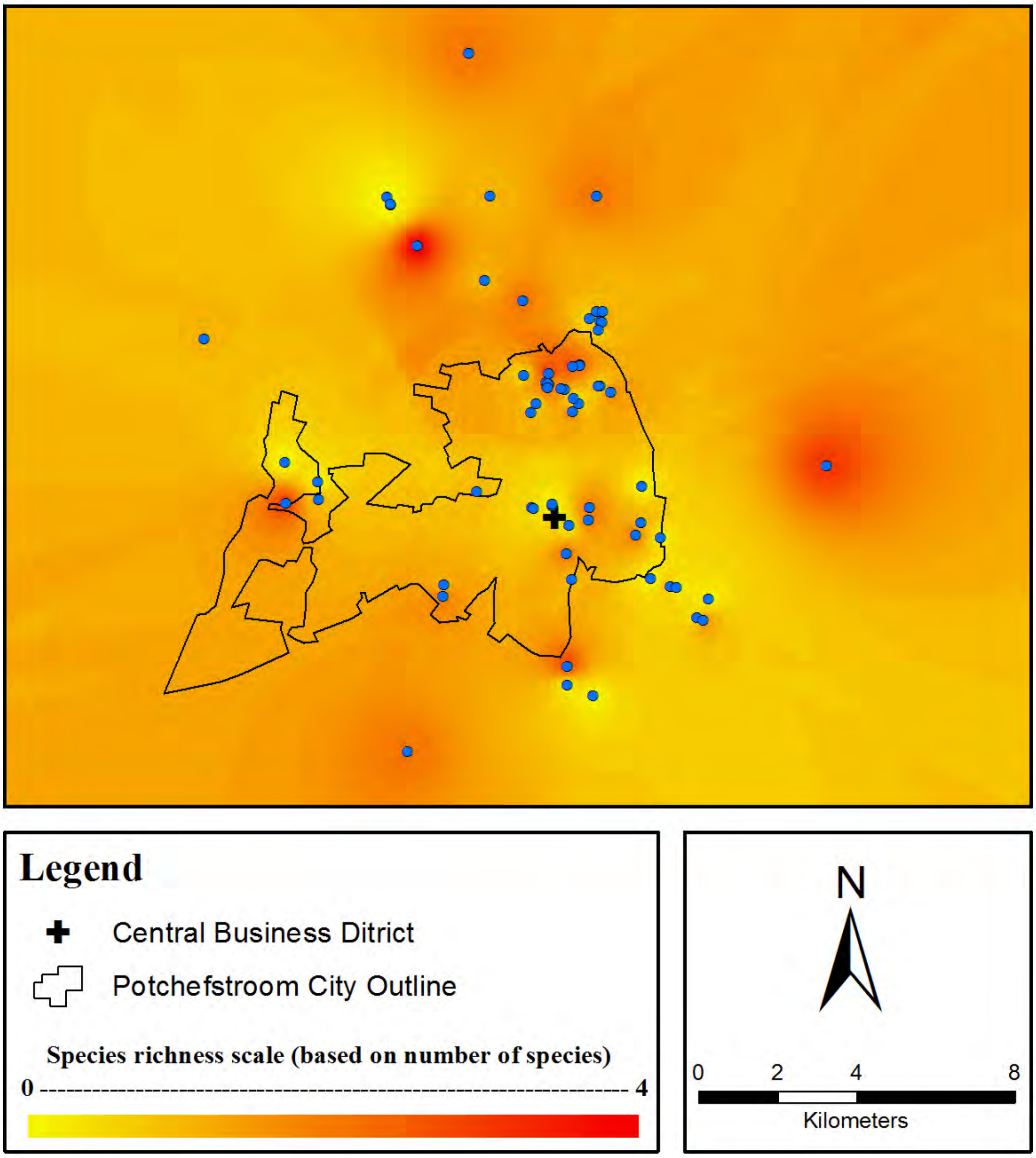
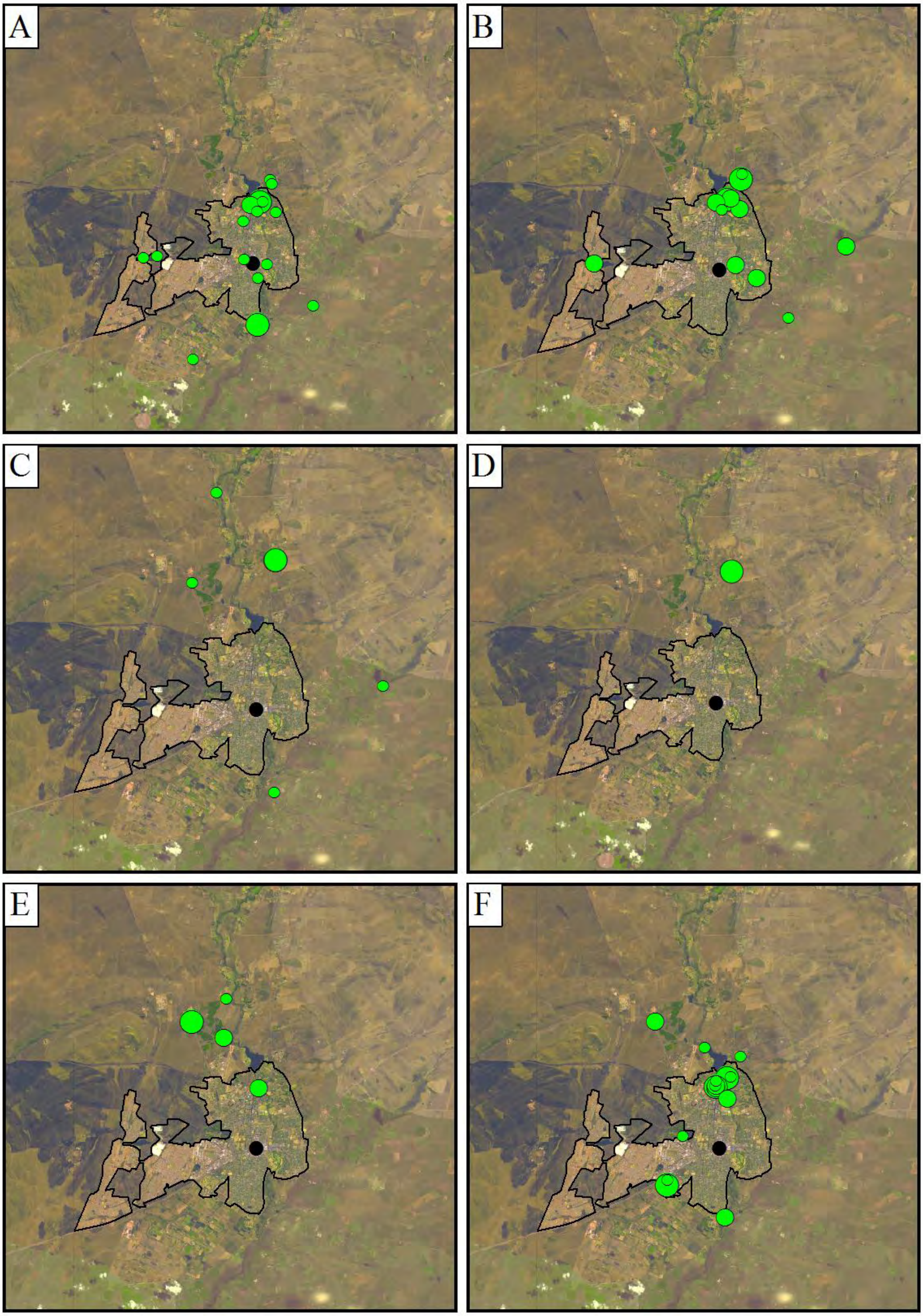


Figure 2.3 An Inverse Distance Weighting (IDW) interpolation analysis of the number of species that occurred at each of the 68 surveyed ponds. City outline were manually drawn from SPOT 2010 (1:5 000 map resolution) to provide a guideline of the city's location. Blue circles depict the surveyed ponds.


A
B
C
D
E
F

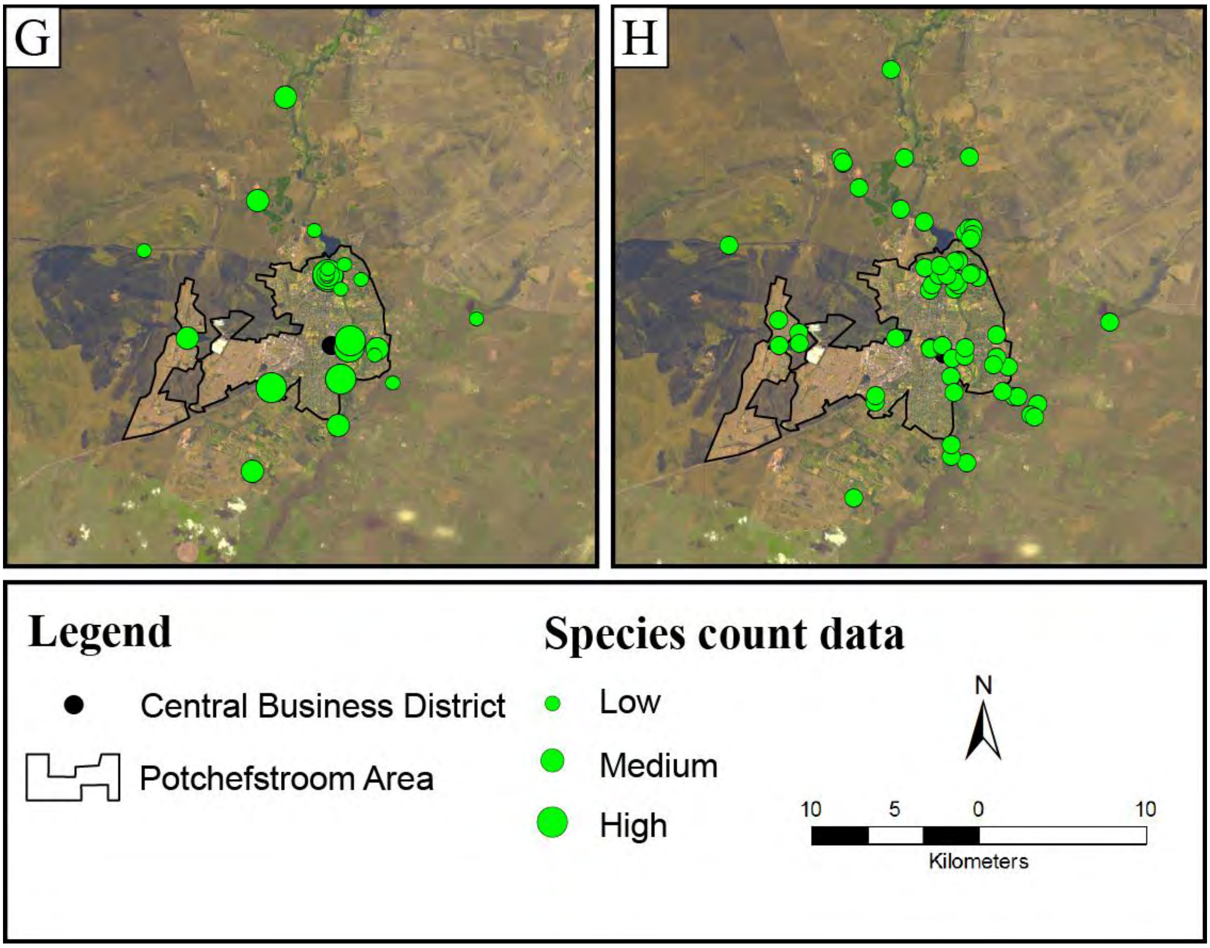


Figure 2.4 Distribution maps (continued from previous page) of each of the seven anuran species detected over a four-season survey. Green circles are representative of the relative count data of each pond for *Amietophrynus gutturalis* (A), *A. rangeri* (B), *Cacosternum boettgeri* (C), *Kassina senegalensis* (D), *Strongylopus fasciatus* (E), *Xenopus laevis* (F) and *Amietia quecketti* (G). Green circles in map H show the locations of each of the surveyed ponds in order to compare the presence of the species distribution on maps A-G. Potchefstroom area excludes agricultural landscape. Black patches on the aerial image show areas of field that were burned.

2.3.2 Landscape habitat determinants

The first CCA axis for landscape variables (Figure 2.5; axis 1) explain 22.9% of the variability in the species data and 58.0% of the variability in the species-environment relationship (eigenvalue = 0.721; *F* = 10.972; *P* = 0.0020). Axis 1 described a landscape gradient from low altitudes, less roads and green open space, larger residential surface areas with closer average distances between wetlands that are located closer to the CBD (negative values), to higher altitudes with higher surface areas of roads and green open space with wetlands that are spaced further away from one another and also located further away from the CBD (positive values; Figure 2.5; Table 2.2). Only two species were associated with negative axis 1 scores (*Amietophrynus gutturalis* and *A. rangeri*). *Amietia quecketti* was weakly associated with the positive axis 1 scores, whereas *Xenopus laevis*, *Strongylopus fasciatus*, *Cacosternum boettgeri* and *Kassina senegalensis* had a greater affinity with ponds located at higher altitudes further away from the CBD that had a lot of green open space. Species richness was associated with higher surface areas of roads and green open space as well as ponds that have a higher average distance between them.

The second CCA axis (Figure 2.5; axis 2) explained 9.8% and 24.9% of the variability in the species data and species–environment relationships, respectively (eigenvalue = 0.721; *F* = 10.972; *P* = 0.0020). Axis 2 described a gradient from higher altitudes and residential surface areas, which are located further away from the CBD but low surface areas of green open space (negative scores), to low residential surface areas as well as high CBD, roads and green open space surface areas located at lower altitudes closer to the CBD (positive scores). *Xenopus laevis* was associated with negative axis 2 scores, whereas *S. fasciatus, K. senegalensis* and *C. boettgeri* were slightly associated with positive axis 2 scores. *Amietia quecketti* and *A. rangeri* had a high affinity for high CBD surface areas associated with green open space. Species richness was also associated with positive values of axis 2.

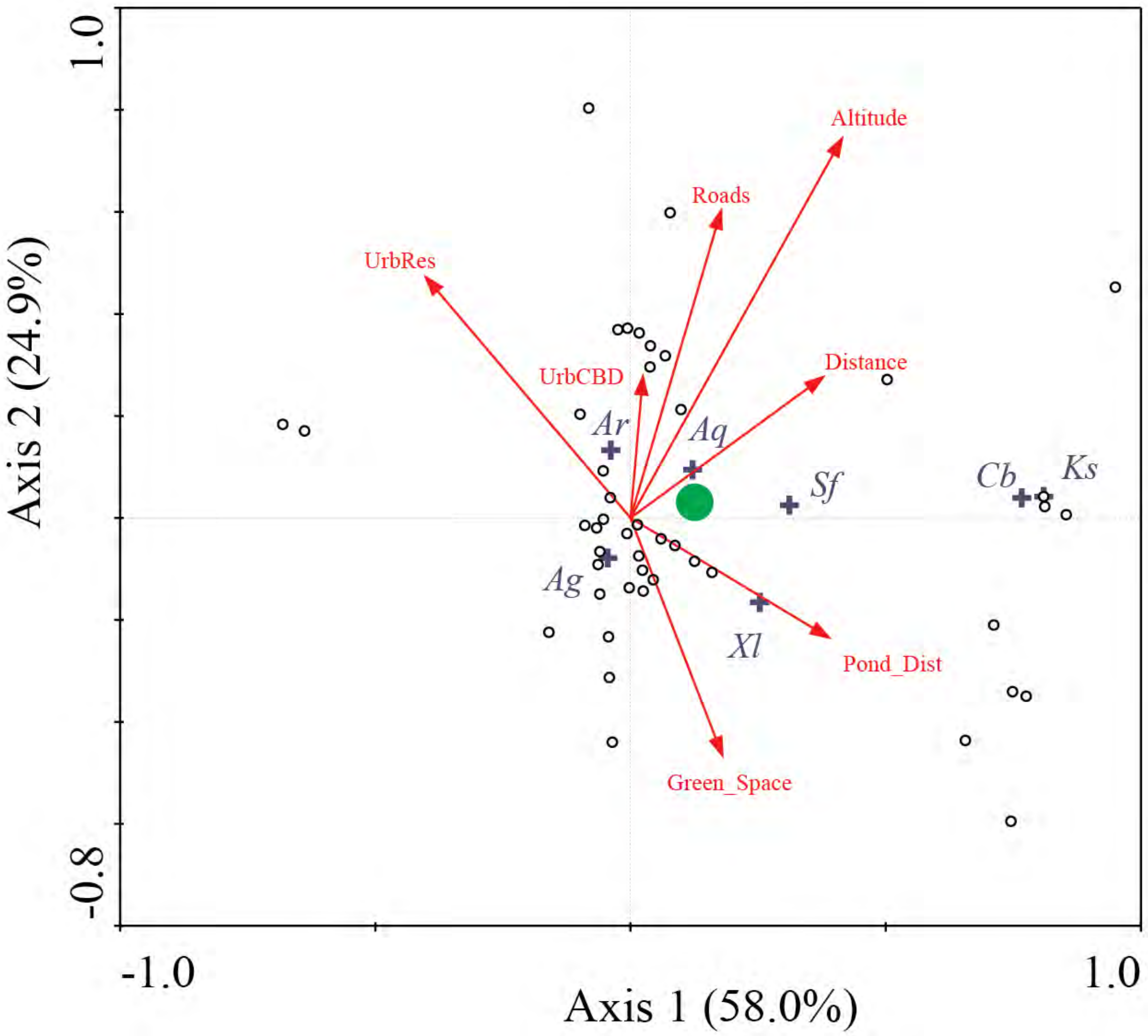


Figure 2.5 Ordination diagram (biplot) of the canonical correspondence analysis (CCA, *P*<0.05) for the number of amphibian larvae according to seven landscape explanatory variables recorded at 45 wetlands in Potchefstroom, South Africa, 2012–2013. Species are presented as blue crosses; sample sites are presented as open circles; number of species occurring at a wetland is presented as a solid green circle.

2.3.3 Micro-habitat determinants

The first CCA axis for micro-habitat variables (Figure 2.6; axis 1) was also significant and explained 20.3% of the variability in the species data and 66.6% of the variability in the species-environment relationship (eigenvalue = 0.640; F = 9.418; P = 0.0020). Axis 1 described a gradient from shaded, small and well-vegetated ponds with steep embankment slopes, high conductivity and lower pH levels hosting large number of predatory fish (negative values), to sunny habitats with large pond areas and gentle slopes having less vegetation and a low number of predatory fish, low conductivity and higher pH levels (positive values; Figure 2.6; Table 2.2). *Cacosternum boettgeri*, *K. senegalensis*, and *S. fasciatus* were associated with the positive scores of axis 1, whereas *A. quecketti* and *X. laevis* were slightly associated with negative scores. *Amietophrynus gutturalis* and *A. rangeri* were both grouped in the middle of the CCA-plot where axis 1 and 2 crossed, possibly indicating that they exhibit generalised habitat preferences, and species richness was not associated with either positive or negative values in axis 1. The second CCA axis (Figure 2.6; axis 2) explained 6.3% and 20.7% of the variability in the species data and species–environment relationships, respectively (eigenvalue = 0.640; F = 9.418; P = 0.0020). *Cacosternum boettgeri* and *K. senegalensis* were associated with ponds with limited vegetation, steep bank slopes and larger pond areas with lower conductivity and pH levels (Figure 2.6), whereas *A. quecketti*, *X. laevis* and *S. fasciatus* were more associated with these conditions.

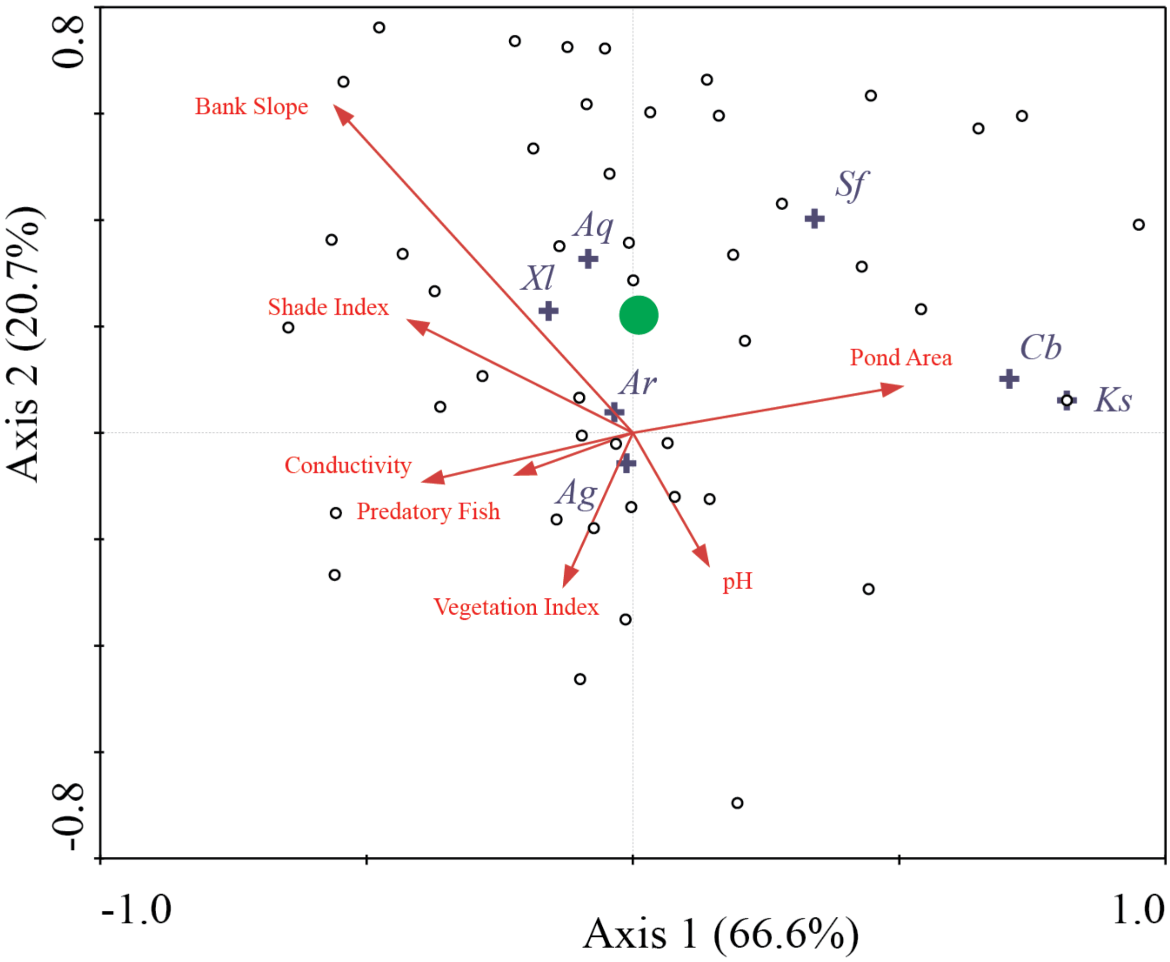


Figure 2.6 Ordination diagram (biplot) of the canonical correspondence analysis (CCA; $P<0.05$) for the number of amphibian larvae according to seven micro-habitat explanatory variables recorded at 45 wetlands in Potchefstroom, South Africa, 2012–2013. Species are presented as blue crosses; sample sites are presented as open circles; number of species occurring at a wetland is presented as a solid green circle.

Table 2.2 Variables and their ordination correlations used to quantify habitat on a landscape and local scale for 45 wetlands in Potchefstroom, South Africa.

Variable	Mean (weighted)	SD	Range	Weighted species correlation	
				Axis 1	Axis 2
Landscape variables					
Altitude (m a.s.l.)	1 348.3	14.6	1 324–1 420	0.3642	0.5933
Green open space (m^2)	20 640.4	15 118.2	0–106 518	0.1582	-0.3709
Roads area (m^2)	2 504.4	2 374.4	0–13 107	0.1559	0.4801
Central business district area (m^2)	270.9	1 873.0	0–50 511	0.0213	0.2206
Residential area (m^2)	22 863.4	21 455.2	0–62 851	-0.3523	0.3763
Distance to central business district (m)	3 982.9	1 250.1	297–11 974	0.3314	0.2202
Average distance to nearest other wetland (m)	4 753.3	1 265.4	3 723–10 353	0.3413	-0.187
Micro-habitat variables					
Wetland area (km^2)	22 540.2	109 960.7	3.6–1 180 021.9	0.4135	0.0479
Slope of wetland bank (m)	330.8	171.1	30–990	-0.459	0.3375
Conductivity ($\mu S/cm^3$)	669.3	379.6	42.0–2 340.3	-0.322	-0.0503
pH	8.4	0.6	7.2–9.7	0.1159	-0.1361
Vegetation index	0.7	0.4	0–1.7	-0.1062	-0.1575
Shade index	59.4	51.3	12–188	-0.3452	0.1162
Number of predatory fish	90.9	120.1	0–427	-0.1798	-0.0431

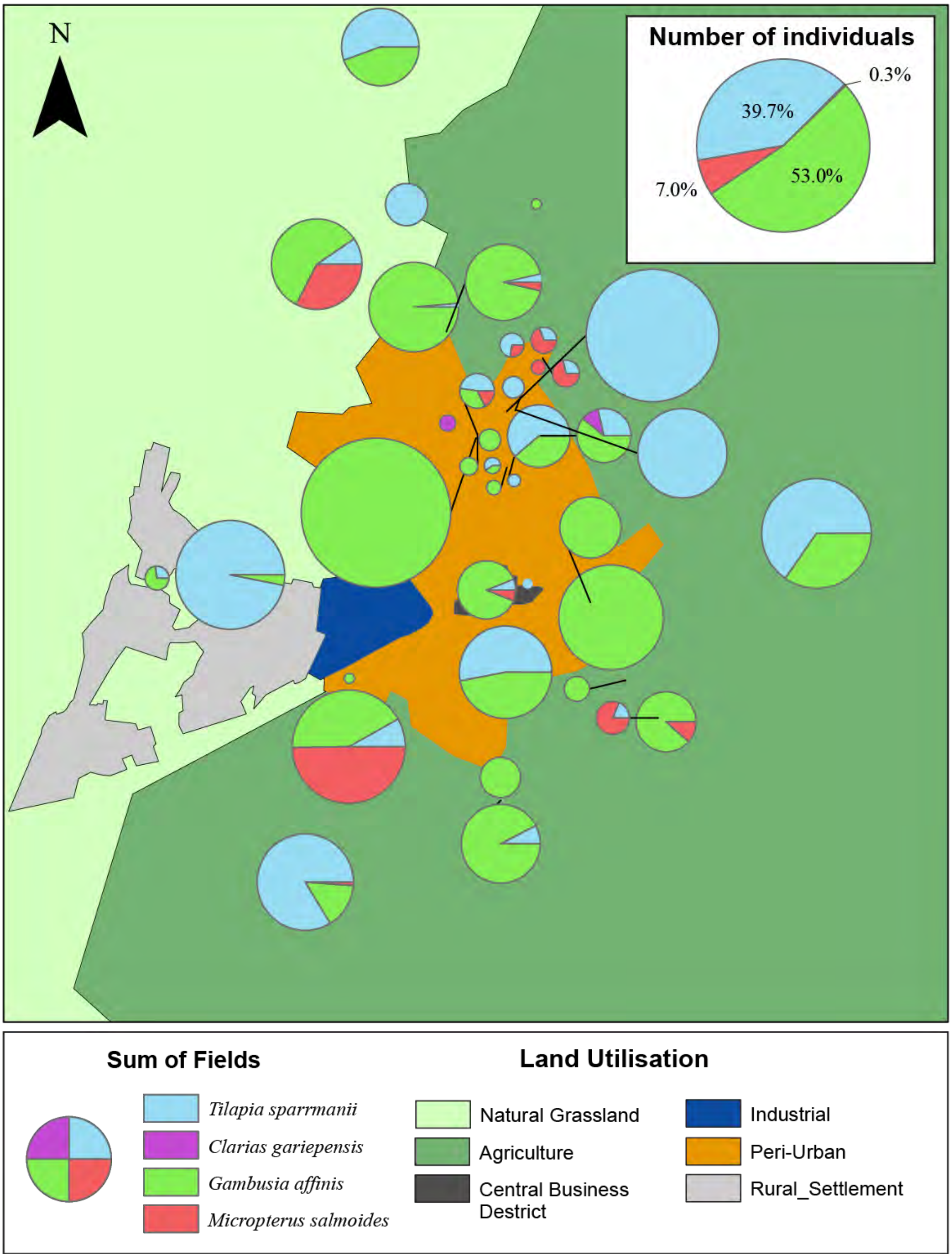


Figure 2.7 Occurrence of four predatory fish species. *Gambusia affinis* and *M. salmoides* are alien invasive species against a background of land-use is the greater Potchefstroom District.

Table 2.3 Distribution of anuran species among 68 wetlands divided into nine pond types.

Pond type	Total no. of ponds	Ponds occupied	Percentage occupied (%)	Max. no. of species
Roadside pool	6	2	33	1
Formal ornamental pond	12	5	42	2
Garden Pond	12	12	100	3
Permanent river	6	3	50	2
Former quarry	4	0	0	0
Big dam	6	6	100	3
Farm dam	6	4	67	4
Vlei	7	6	86	3
Large peri-urban pond	9	7	78	4

Note: Roadside pools and former quarries reflect poor/no occupancy because of drought conditions.

Predatory fish (*Tilapia sparrmanii*, *Clarias gariepinus*, *Gambusia affinis* and *Micropterus salmoides*) were detected in 63.9% (39) of the ponds, with *G. affinis* and *T. sparrmanii* being the dominant species (Figure 2.7). *Gambusia affinis* and *M. salmoides* are invasive species and occurred widespread over the study area. The number of predatory fish species occurring at ponds ranged from 0–3 ($\bar{X}$ = 1.0; SD = 1.03) and were associated with well-vegetated ponds (Figure 2.6). Other predatory fish species detected in ponds included cyprinids such as European Carp (*Cyprinus carpio*), Grass Carp (*Ctenopharyngodon idella*), Barbs (*Barbus spp.*), goldfish (*Carassius auratus*) and koi (*Cyprinus carpio*, domesticated cyprinids).

2.3.4 Bayesian analysis for habitat variables

Three models (models 4, 9 and 10) that predicted species richness of the frog breeding assemblages were identified based on DIC values (ΔDIC<2) as having better relative fit than the remaining eight models (Table 2.4). Model 4 included local habitat variables vegetation, predatory fish and pond area (see Table 2.5 for regression coefficients). Vegetation was the only variable in model 9 (together with the constant). In terms of supported models, model 10 included a landscape variable (surface area of urban CBD) and each model included vegetation. In terms of the remaining eight models, there were lowest support for the models in which landscape variables dominated (model 6; ΔDIC=7.4) and for the null model there was also low support (constant only; model 1; ΔDIC=5.1). No models were essentially unsupported (ΔDIC>10).

Local habitat variables showed stronger evidence in the multiplicative effect sizes in the models in which they were included (Figure 2.8A-F) than the comparatively weaker evidence for landscape variables (Figure 2.8G-I; also see Table 2.4). Multiplicative effects of landscape variables showed only weak predictions, with negative effects of surface area covered by roads and urban CBD (multiplicative average effect sizes of 0.84 and 0.46, respectively). This translates to the prediction that, holding all other variables constant, the pond surrounded by the highest proportion of urban CBD within 250m radius (i.e. 5.1ha) would have 42–51% of the species detected at the pond with no surrounding urban CBD and there was an average multiplicative effect size of 0.46 across the four models that included this variable (Figure 2.8I). There was also very weak evidence of a positive effect of green open space on frog species richness, with average multiplicative effect sizes of 1.26 and 1.01 for models 6 and 8, respectively (Figure 2.8H).

At the local scale, there was strong evidence of a positive effect of aquatic vegetation on species richness, with an average multiplicative effect size of 2.7 for models 2–5 and 8–10, with 95% credible intervals encompassing 1 in only models 4 and 8 (Figure 2.8D). This translates to the prediction that, when all other variables are held constant, the pond containing the highest proportion of aquatic vegetation would have 2.4–3.1 times the number of species detected at the pond with no vegetation.

Table 2.4 Deviance information criterion (DIC) values for the 11 Poisson regression models of species richness at a pond. Best fit models are presented in bold with ΔDIC<2.

Model	Variables	DIC	ΔDIC†
1	constant	175.2	5.1
2	constant, area, slope, conductivity, vegetation, fish	172.6	2.5
3	constant, slope, conductivity, vegetation, shade, fish	173.8	3.7
4	**constant, area, vegetation, fish**	**170.6**	**0.5**
5	constant, area, pH, slope, conductivity, vegetation, fish	174.5	4.4
6	constant, roads, green, urbCBD	177.5	7.4
7	constant, ponddist	176.4	6.3
8	constant, area, vegetation, fish, roads, green, urbCBD	175.2	5.1
9	**constant, vegetation**	**170.6**	**0.5**
10	**constant, vegetation, urbCBD**	**170.1**	**0**
11	constant, urbCBD	174.6	4.5

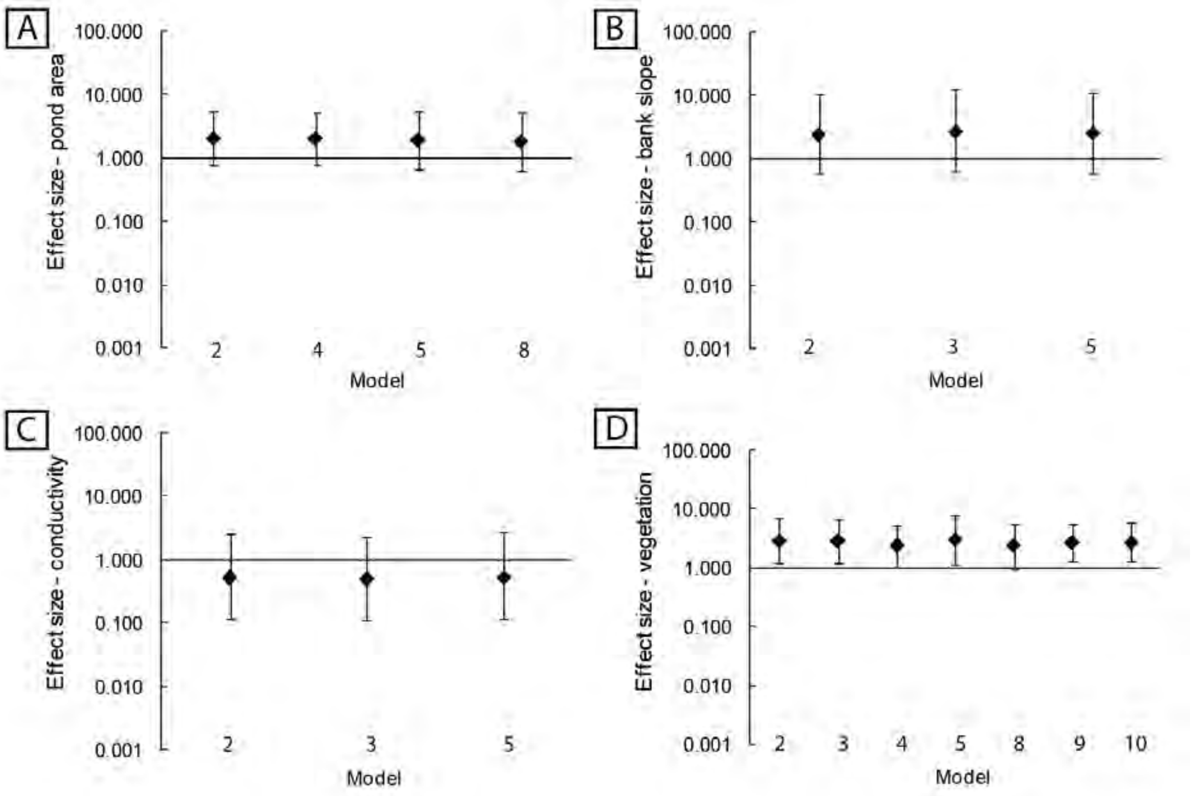
Note: area, log10(area); slope, log10(slope); conductivity, log10(conductivity); shade, log10(shade); fish, mean [*Gambusia affinis* + *Clarias gariepinus* + *Micropterus salmoides* + *Tilapia sparrmanii*]; roads, log10(roads + 1); green, (green + 1); urbCBD, log10(urbCBD + 1); ponddist, log10(ponddist).

†Difference in DIC value for a particular model when compared to the top-ranked model (model 10).

Table 2.5 Coefficients of the explanatory variables included in the three best Poisson regression models (models 4, 9 and 10).

Variable	Mean	SD	2.5%	97.5%
Model 4				
Constant	0.15	0.12	-0.09565	0.3857
Area	0.13	0.09	-0.04071	0.305
Vegetation	0.50	0.24	0.0246	0.9625
Fish	0.14	0.09	-0.04617	0.3069
Model 9				
Constant	0.19	0.12	-0.0439	0.4175
Vegetation	0.58	0.22	0.1397	1.005
Model 10				
Constant	0.16	0.12	-0.09444	0.3901
Vegetation	0.59	0.23	0.14	1.043
urbCBD	-0.18	0.12	-0.4318	0.01919

Notes: See Table 2.4 for a description of variables. SD = standard deviation.



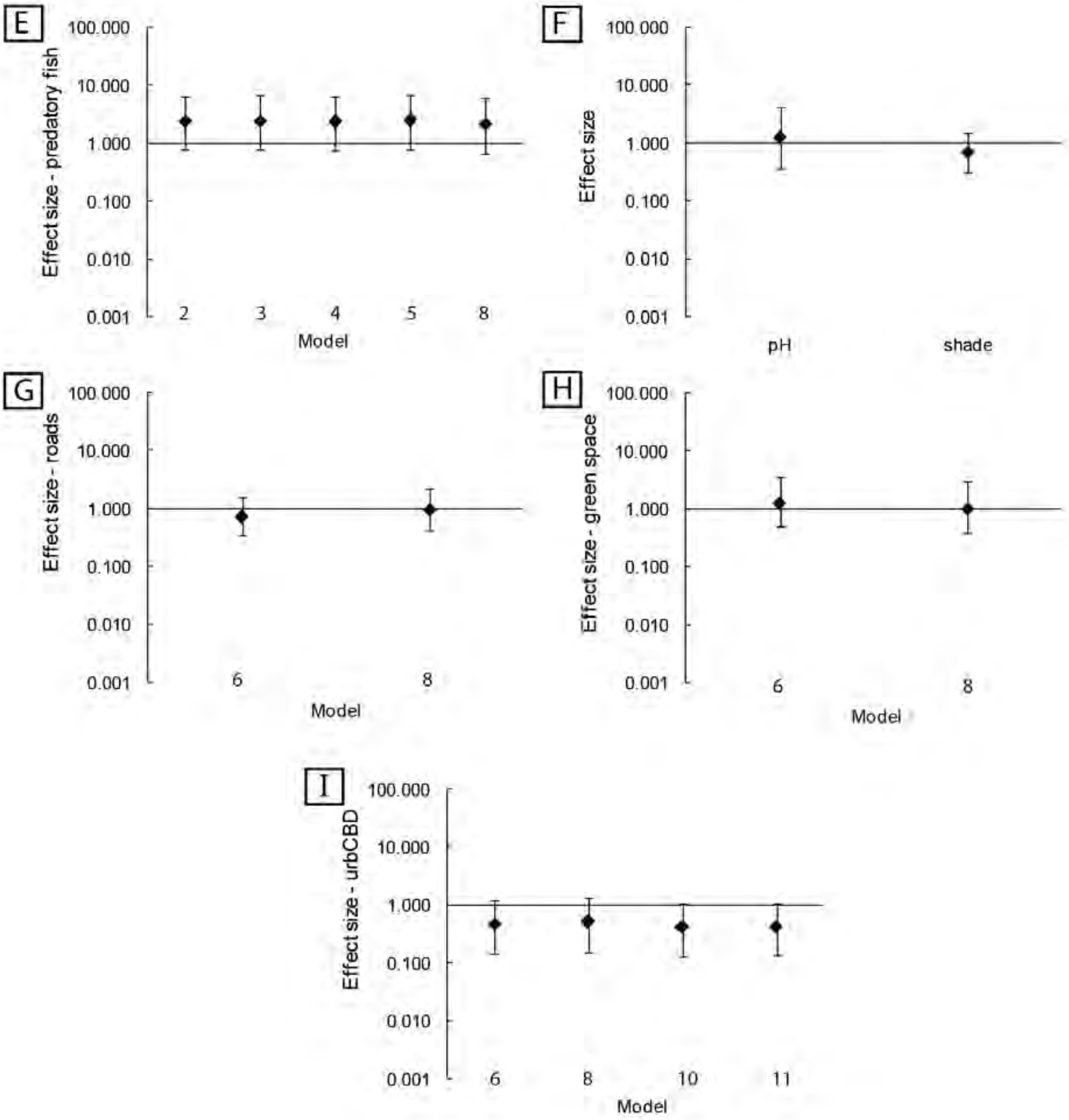


Figure 2.8 The multiplicative effect of ten explanatory variables (mean and 95% credible interval) on larval frog species richness predicted by models with ΔDIC<10 for ponds in Potchefstroom, South Africa (continued from previous page). Multiplicative effects for pond area were predicted by model 2, 4, 5 and 8 (A); effects for bank slope and conductivity were predicted by models 2, 3 and 5 (B, C); effects for vegetation were predicted by models 2–5 and 8–10 (D); effects for the presence of predatory fish were predicted by models 2–5 and 8 (E); effects for pH and shade were predicted by models 5 and 3 respectively (F); effects for roads and green open space surface area were predicted by models 6 and 8 (G, H); effects for urban CBD surface area were predicted by models 6, 8, 10 and 11 (I). Multiplicative effect sizes > 1 indicate a positive effect of the explanatory variable on species richness; effect sizes < 1 indicate negative effects.

2.4 Discussion

2.4.1 Species-specific responses to habitat variables

Results generated many species-specific responses to urbanisation and agree with other studies in that it stresses the importance of terrestrial habitat and aquatic vegetation for amphibians that are situated further away from CBD (see Semlitsch 1998; Smith & Green 2005; Pillsbury & Miller 2008; Hamer & Parris 2010; Scheffers & Paszkowski 2013). *Amietophrynus spp*. were spaced close to where axes meet within the CCA plot of landscape variables (Figure 2.6), meaning they were habitat generalists and proved to fare well in the urbanised landscape and had no specific affinity for any of the micro-habitat variables. *Amietia quecketti* was especially abundant and were detected in 39.2% of the wetlands, the highest for all species. This may be explained by the exclusion of competition for habitat availability due to winter-breeding behaviour (Bonnet *et al.* 1998; Voituron & Lengagne 2008). It was also grouped with the *Amietophrynus spp.* in terms of micro-habitat, but showed a high affinity for urban landscapes (especially buffer zones containing residential areas) as well as green open space and roads. Due to specific habitat requirements (gradual bank slope, well-vegetated large ponds) and association with ephemeral wetlands, *Cacosternum boettgeri* and *Kassina senegalensis* were not found near developed areas, and therefore more sensitive to urbanisation. None of these species occurred within the urbanised landscape. *Strongylopus fasciatus* was associated with large, well-vegetated ponds and could be found in residential areas at wetlands that met their local pond requirements. *Xenopus laevis* was associated with larger and deeper (i.e. steeper embankment slope) ponds showing an affinity to urbanised landscapes, good wetland connectivity and presence of predatory fish, which is typical of primarily aquatic breeders.

Amietia quecketti occurred at more ponds than any other species (39.2% of the 61 wetlands) within a variety of wetland types. This might prove *A. quecketti* a practical species to serve as a bioindicator species for assessing habitat degradation or to assess habitat connectivity in urban landscapes because of its abundance, wide distribution, extended breeding season and broad habitat utilisation (Blaustein 1994; Blaustein & Wake 1995), or serve as a species to evaluate urban wetlands (Ehrenfeld 2000).

2.4.2 Species richness response to local habitat variables

In modelling habitat variables using Bayesian methods, results demonstrated a strong correlation between species richness and aquatic vegetation, which were included in all supported models. Species diversity is known to escalate with an increase in vegetation diversity and result in habitat heterogeneity, as shown in a number of studies (Hazell *et al.* 2001; Jansen & Healey 2003; Hazell *et al.* 2004; Lemckert *et al.* 2006; Lane *et al.* 2007). Aquatic vegetation provides locations for oviposition for frogs (Egan & Paton 2004) and consequently also for predator avoidance (Tarr & Babbitt 2002).

Our results show that higher species richness and an increase in aquatic vegetation were accompanied by an increase in predatory fish. Studies have demonstrated that the presence of vegetation would create considerable habitat heterogeneity and therefore shelter for tadpoles (Hecnar & M'Closkey 1997; Hazell *et al.* 2001; Lane *et al.* 2007). Evidence for the refuge hypothesis has been provided by Tarr & Babbitt (2002). The most abundant and widely distributed predatory fish species in this study were mosquitofish (*Gambusia affinis*) and the Banded Tilapia (*Tilapia sparrmanii*) (44.3% and 42.6% of the 61 wetlands, respectively). Literature show that the presence of predatory fish can affect amphibian community assemblages negatively (Hamer & Parris 2011; Hamer & Parris 2013).

In this study, conductivity and pH showed only weak effects on species richness. Conductivity is known to reduce larval survival and development (Griffis-Kyle & Ritchie 2007; Snodgrass *et al.* 2008) and negatively impacts anuran species richness and community composition (Hamer & Parris 2011). Urban stormwater run-off may cause increased conductivity levels (Dow & Zampella 2000; Paul & Meyer 2001), but may also be influenced by other factors. For example, one of the study sites (Wasgoedspruit, vlei) is downstream of a gypsum mine that explains abnormally high conductivity levels. No anuran larvae or fish species were detected in this wetland.

2.4.3 Species richness response to landscape habitat variables

The best model of species richness (model 10) included vegetation (positive effect) and surface area of urban CBD (negative effect). Urban CBD surface area indicated a moderately strong multiplicative effect size of 0.46. On larger spatial scales than this study, decreases of higher magnitude in amphibian species richness have been observed in wetlands surrounded by high human population densities and urban land (Rubbo & Kiesecker 2005; Pillsbury & Miller 2008; Hamer & Parris 2011). Decreases observed in these studies were attributed to landscape fragmentation by means of roads, buildings, and houses that obstruct many frog species to disperse among corresponding habitats and disrupts metapopulation dynamics (Gibbs 1998; Parris 2006). However, the remaining landscape variables (namely, roads surface area, green open space surface area and average distance to ponds) only indicated weak effects, and inferences on these statistics should be made with great care.

Although different gradient classification methods were used, the very weak support demonstrated by the model including purely landscape variables (ΔDIC=7.4) contradicts previous studies on anuran assemblages across urban-rural gradients (Pillsbury & Miller 2008; Hamer & Parris 2011; Scheffers & Paszkowski 2013). The unsupported models do not imply that landscape variables do not play a role in habitat determinants; rather I propose that it is due to the small size of Potchefstroom as a city, lacking the road density of larger cities (e.g. Melbourne, Australia; Hamer & Parris 2011). Ponds surrounded by large areas of green open spaces may have greater habitat connectivity with surrounding ponds and wetlands, thereby assisting in dispersal and may also provide habitat to forage and hibernate (Hamer & Parris 2011). The Mooi River bisects Potchefstroom, providing a green open area strip on both sides of the river. Green open space extending along rivers provides dispersal corridors for both fauna and flora (Good 1998; Säumel & Kowarik 2010).

Overall, species richness was associated with well-vegetated wetlands, low urban CBD surface area and conductivity, large pond areas and steeper bank slopes. Habitat generalist species bias the distribution towards the urban fringe of the town and species showing habitat specificity occurred in areas of low habitat alteration due to urbanisation. This study underscores the importance of habitat heterogeneity within wetlands and shows that the effects of urbanisation in smaller developing towns shape the amphibian community structure at early stages of infrastructure development. Although Potchefstroom demonstrated that a number of habitat generalist anuran species are still persisting close to the CBD, literature show that ponds that have been isolated by urban infrastructure cannot be easily recolonised by individuals following local extinction (Semlitsch 2000).

In conclusion, this study gives emphasis to the value of a multi-spatial approach to amphibian conservation in urban and urbanising areas. Although the drought condition biased the ponds towards permanent wetlands and consequently inferences on hydroperiod were a limitation, this study highlights the species specific responses to urbanisation as observed within an urban and urbanising landscape. Although restricted to the Potchefstroom area, the response of the community structure of anurans to an urbanisation gradient was reported for the first time from a South African perspective and link well with studies reported on the effects of land use on anuran functional groups and species composition in the eastern coast of southern Africa (Russell & Downs 2012; Trimble & Van Aarde 2014). With the correct resource management and urban design of the fast development of Potchefstroom, the river may provide a key area for supplying habitat connectivity, not only for anurans, but other riverine dependent fauna and flora as well (Good 1998; Säumel & Kowarik 2010; Hamer & Parris 2011).

CHAPTER FOUR

Toads and roads: Migration and call structure of the Endangered city dwelling Western Leopard Toad (*Amietophrynus pantherinus*)

Slowly, hopping

sometimes stopping

to blink and look around

the leopard toads

approach the roads

which resonate with sound

- Freda Jones, South Africa (excerpt from SAVE THE FROGS! poetry contest)

4.1 Introduction

4.1.1 Anuran migrations

Anuran amphibians migrate between important habitat resources that are spatially separated, e.g., sites for reproduction, nutrition and hibernation (Sinsch 1990; Pilliod *et al.* 2002). Movement between these spatially separated resources are considered migration, whereas movement within a foraging site for example, is not (Sinsch 1990). The migration ranges for anurans are relatively small when compared to other animals, with most frogs and toads migrating not more than a 1000 m from their natal ponds (Duellman & Trueb 1986; Sinsch 1990). Migration distances range from 150 m for *Rhinella marina* (Zug & Zug 1979), to 237 m for Hyla regilla (Jameson 1956), up to 3 km for the *Bufo bufo* (Heusser 1969). Females of *Pyxicephalus adspersus* are known to travel up to a mean of 446.8 m before digging down their hibernation burrows in South Africa (Yetman & Ferguson 2011). However, many anurans, like eleutherodactyline frogs (frogs of the genus *Eleutherodactylus*), do not migrate to breeding ponds and reproduction occurs within their home range, an area considered in which an individual carries out its daily activities (Duellman & Trueb 1986). Home ranges sizes vary among species, with larger species having larger home ranges, but require at least a suitable shelter and foraging component and in some cases a suitable calling site for males (Duellman & Trueb 1986).

Whether migrating for short or long distances, anurans are bound to face certain challenges along the way. Firstly, due to their ectothermic physiology and their proneness to dehydration due to their skin's permeability to water, frogs are inherently constrained by temperature and ambient moisture levels in terms of migration distances and hours of the day suitable for migration (Duellman & Trueb 1986; Sinsch 1990). Secondly, except for physiological limitations, predator avoidance also poses a formidable challenge to migrating anurans. Travelling under low light conditions, presumably to spot predators (Tuttle & Ryan 1982; Nunes 1988), or complete darkness to avoid detection (Vignoli & Luiselli 2013) are both behavioural adaptations to mitigate this problem. Thirdly, geographical barriers such as mountains, deserts, rivers and oceans are major obstructions for anuran dispersal and are well known ancient isolating mechanisms that drive species genetic diversion and diversification (e.g. Van de Vliet *et al.* 2014). However, with the urbanisation revolution, frogs are faced with a whole new set of human-produced barriers such as roads, walls, fences and constructed waterways for stormwater management and agricultural purposes (Peterson *et al.* 2012).

Millions of vertebrate animals are killed each day by vehicular traffic (Forman & Alexander 1998; Trombulak & Frissell 2000; Coffin 2007). A rapidly growing literature in the field of road ecology suggests vehicle/wildlife collisions are a major source of vertebrate mortality (Glista *et al.* 2009; Van der Grift *et al.* 2003; Jaeger & Fahrig 2004; Aresco 2005; Fahrig & Rytwinski 2009; Van der Ree *et al.* 2009; Benítez-López *et al.* 2010; Borda-de-Agua *et al.* 2010; Kociolek *et al.* 2011; Van der Ree 2011). This field of research is in its infancy in South Africa, but is starting to gain momentum (EWT 2013). In addition to high mortality rates, roadways can also affect wildlife by impeding movement patterns, and ultimately causing isolation and decline of populations (Spellerberg 1998; Forman *et al.* 2003; Eigenbrod *et al.* 2008; Canessa & Parris 2013). There is ample evidence that shows that amphibians are especially susceptible to the effects of roads because they are slow-moving animals that frequently pause to sit and observe and they access multiple habitats seasonally to complete their life cycles (Hels & Buchwald 2001; Steen *et al.* 2006; Glista *et al.* 2007; Roe & Georges 2007; Eigenbrod *et al.* 2009; Baskaran & Boominathan 2010). Road mortalities as a result of roads crossing amphibian migration routes can have a direct effect on two levels, namely a decrease in population sizes and a limitation of movement between corresponding resources and conspecific populations (Carr *et al.* 2000), together with the indirect effects on calling and breeding behaviour (Fahrig *et al.* 1995; Findlay & Houlahan 1997; Fahrig & Rytwinski 2009, 2011; Parris *et al.* 2009; Barber *et al.* 2010).

The distance of roads to breeding sites is one of the major factors influencing mortality as a result of a difference in anurans' vagility i.e. movement distance and frequency (Langton 1989; Carr & Fahrig 2001). The more frequently individuals move, and over longer distances, the higher the potential impact of the road (Carr & Fahrig 2001). Puky (2005) summarised the factors determining amphibian road kill (Table 4.1). The "road-effect zone" is the extent of major ecological effects from the edge of a road (Figure 4.1; Langton 1989; Forman & Alexander 1998) and has been quantified for anurans by Eigenbrod *et al.* (2009).

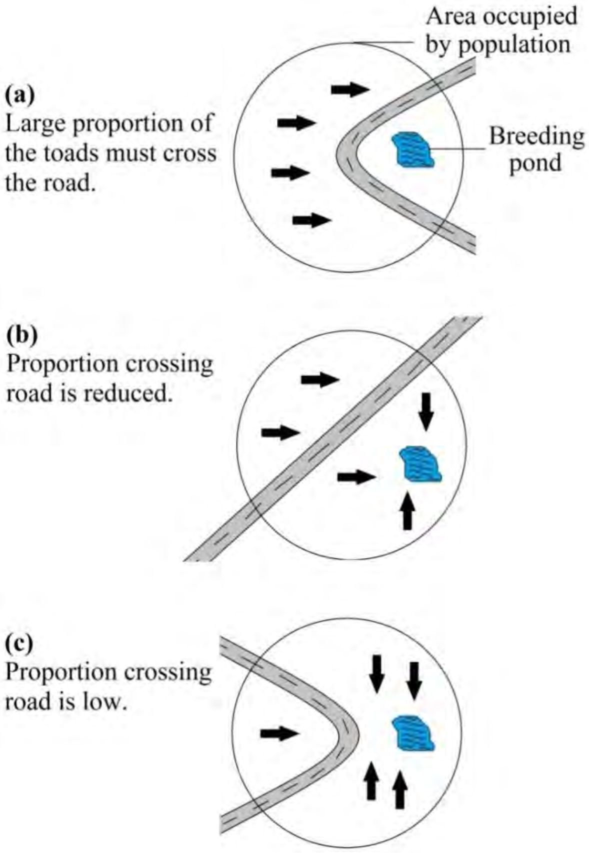


Figure 4.1 A representation of the possible influence of roads on populations of amphibians determined by the relative position of the road to the breeding pond; influence in decreasing order from a-c. Image adapted from Langton (1989).

In one study, road effect zones were calculated to be between 250 - 1000 m for four anuran species and well beyond a 1000 m for two other anuran species (Eigenbrod *et al.* 2009). Roads located next to wetlands may cause unsustainable rates of mortality, especially in the case of toads and other large species (Langton 1989; Eigenbrod *et al.* 2009). Large proportions of populations can be exterminated during migration to and dispersal from the breeding site over time on a road with high traffic density, and this may lead to local extinction (Minter *et al.* 2004; Van der Grift *et al.* 2013). Incidentally, several human fatalities have also occurred as a result of drivers swerving and losing control of their vehicles in an attempt to miss migrating animals (Langton 1989).

Table 4.1 Factors determining amphibian road kill (Puky 2005).

Amphibian specific factors	Amphibian movement types Length and direction of movement Velocity Temporal movement pattern Behavioural changes on roads
Main habitat characteristics	Vegetation Available aquatic habitats
Transportation characteristics	Road density Traffic intensity Vehicle speed Position and structure of roads Awareness of drivers
Weather conditions	Precipitation Temperature Wind Annual changes of weather conditions

Moral and welfare issues are increasingly dictating stronger demands for measures to protect individual animals, as well as motorists and their passengers. As such, socio-ecological relationships are becoming more recognised as a key component of conservation planning (Langton 1989; Toerpe 2013; Wilson *et al.* 2013). People can relate to animals and other components of natural ecosystems and the general public increasingly shows a high level of interest in amphibians (Langton 1989; Kriger 2012; Ruiz-Mallén & Corbera 2013; this study). With this increasing interest in the environment, attitudes of the general public are becoming "green" on roads, i.e. the public have a greater consideration for animals. However, active avoidance of amphibians on roads i.e. swerving can be hazardous both to the driver and other road users and is therefore seldom a feasible option. Interventions such as tunnel systems for example, will save human as well as amphibian lives (Dodd *et al.* 2004; Woltz *et al.* 2008; Glista *et al.* 2009). The argument may also relate to "amphibian patrols", where volunteers are at risk when removing amphibians from the roads, as is the case with the Western Leopard Toad (*Amietophrynus pantherinus*) (Fischer 2013).

Due to the adverse effects of roads on animals across the globe, biologists and engineers have struggled with methods to assist animal movement across transportation passages and came up with several possible solutions (Langton 1989; Forman *et al.* 2003; Dodd *et al.* 2004; Taylor & Goldingay 2010). A popular and widely used mitigation measure is to channel animal movement towards a tunnel or culvert that provides means of access over or under a road, usually with a type of drift-fence (Langton 1989; Forman *et al.* 2003; Dodd *et al.* 2004; Taylor & Goldingay 2010). However, the use of culverts, or any other method, within South Africa has rarely been implemented. A culvert with a drift fence has been constructed at an anuran breeding site next to the N4 highway at Onderstepoort, Pretoria (Vincent Carruthers pers. comm.). However, no peer-review literature was published on this matter.

Another method for mitigating anuran mortality on roads is to involve public participation. Volunteer groups are actively saving amphibians and reptiles off roads around the globe, including Europe (Toadsonroads 2014; Froglife 2014), America (The Schuylkill Center for Environmental Education 2014) and South Africa (Anderson 2014). Although this approach holds a number of advantages like cost-effectiveness and data acquisition for monitoring, the success of the technique is mainly determined by man-power and the determination of champions driving these initiatives.

4.1.2 Vocalisation, explosive breeding and sexual selection in toads

Anurans are one of the most vocally active vertebrates in the animal kingdom, communicating spatial, physiological, aggression, and reproductive information to conspecific males and females (Duellman & Trueb 1986; Ryan 2001; Narins *et al.* 2006; Wells 2007). The advertisement call is the most commonly known and heard vocalisation and is used to advertise readiness to mate and also the level of fitness of an individual male (Wells 2007). In some species females select males based on different call properties that convey information on fitness of the specific male (Gerhardt & Huber 2002). However, in other species it is a matter of “first come first served” where males rush for anything that looks like a female (Cherry 1992; Channing *et al.* 1994; Bergen *et al.* 1997). This behaviour is usually found in explosive breeding toads (Wells 2007).

A large breeding aggregation of amphibians that lasts for short periods at a time is defined as explosive breeding behaviour (Wells 2007). Explosive breeding behaviour is especially common among toads (Table 4.2), but is known to occur in other non-bufonids, for example

Pyxicephalus adspersus (see Channing *et al.* 1994). In some explosive breeders males shift from calling to active searching behaviour when populations become denser (Wells 1977b; Wagner & Sullivan 1992; Wells 2007). More time is spent on active searching and displacing other males from females than is spent on calling (Davies & Halliday 1979). Searching is confined to small areas and results in a form of spatial organisation within the aggregation at high densities (e.g. *Bufo boreas*: Black & Brunson 1971; *B. regularis*, now *Amietophrynus gutturalis*: Tandy & Keith 1972). Conversely, at low densities some males will desert active searching behaviour altogether and adjust to stationary calling. In this way direct competition between males for females is lowered and the possibility for female choice increases (Wells 1977b; Höglund & Robertson 1988). Therefore, acoustic interference might have limited effects once a female has reached the breeding site.

Another behavioural trait explosive-breeding anurans display is similar to that of a lek (aggregation of males for the purposes of courtship and display), where males often temporarily defend small territories around themselves (Channing *et al.* 1994). Generally a mating lek offers ample chance for females to choose the male to their liking. However, within anuran aggregations males leave little choice for females, as males will either intercept females approaching another male or initial mates are displaced by larger males, known as scramble competition (Klöckner 1982; Radwan & Schneider 1988; Cherry 1992; Channing *et al.* 1994; Bergen *et al.* 1997).

Table 4.2 Species of the family Bufonidae that exhibit some form of explosive breeding behaviour.

Species	**Reference**
Bufo woodhousii	Sullivan 1986b
Amietophrynus pantherinus	Cherry & Francillon-Vieillot 1992 This study
Bufo americanus	Sullivan 1992
Incilius valliceps	Wagner & Sullivan 1992
Anaxyrus cognatus	Brown & Pierce 1994
Anaxyrus boreas	Marco 1998
Bufo bufo	Hettyey *et al.* 2012
Melanophryniscus aff. *Montevidensis*	Cairo *et al.* 2013

Direct male competition can become strong in large breeding aggregations of species that demonstrate explosive breeding behaviour, especially for smaller males (Krupa 1989; Leary *et al.* 2005). These studies show that smaller males will gather around large males that have more attractive calls, without the smaller males calling. When a female approaches one of these males, the smaller silent male will intercept the female and engage in amplexus. This is called satellite behaviour and is also density dependant like the alteration between searching and calling discussed above. Larger males are not necessarily older (i.e. they have a slower growth rate), but more likely to assume satellite behaviour earlier in the evening than larger males that have a faster growth rate, although, satellite behaviour is more common in prolonged breeders (Wells 2007). Several authors proposed different criteria that females may use in explosive-breeding aggregations (Davies & Halliday 1977; Gatz 1981; Lee & Crump 1981; Cherry & Francillon-Vieillot 1992; Greene & Funk 2009; Hettyey *et al.* 2012). Even so, there is little proof to substantiate these criteria (Halliday & Tejedo 1995; Sullivan *et al.* 1995; Liao & Lu 2011). Any criteria used by females for mate selection is largely unknown (Wells 2007). Mate choice would have to be based on phenotypic or behavioural characteristics of males. Castellano & Giacoma (1998) reported a case where females of *Bufo viridis* choose males with specific call traits. However, in explosive breeders, active female choice is improbable because females are exposed to coercion and male-male competition (Wells 1977, 2007; Hedengren 1987). Greene & Funk (2009) found that mating success was not related to body size but to larger nuptial pads and thicker, more muscular forearms. Although other males tried to dislodge amplecting males, they did not succeed. These findings suggest that morphology might be influenced by sexual selection in species displaying scramble competition, although not via female choice (Liao & Lu 2011). However, Cairo *et al.* (2013) states that the operational sex ratio is the key that regulates the intensity of sexual selection and females of explosive breeders potentially may have chances to select their mates.

The Passenger Pigeon (*Ectopistes migratorius*) was also an explosive breeder species that travelled *en masse* between sites and as such was at risk of large numbers of individuals being killed in a short space of time, with the result that it went extinct within 50 years as a result of hunting and habitat loss (Ehrlich *et al.* 1988; Greenberg 2014). Problems associated with traffic for animals exhibiting explosive breeding behaviour, particularly species that travel *en masse* such as *E. migratorius* and *A. pantherinus* is that a large number of individuals are killed in a short space of time (Langton 1989; Koons *et al.* 2013). The proximate effect of mortality on population size will be underestimated and extinction might occur more rapidly due to high mortality rates, so that loss of the very last individuals of the last population of a species possibly will not be evidently related to habitat loss (Simberloff 1994). *Ectopistes migratorius* illustrates an essential principle of conservation biology: it is not always necessary to kill the last pair of a species to force it to extinction (Ehrlich *et al.* 1988).

Findings of a study conducted by Sullivan (1983a) suggest that females might choose a mate on the basis of frequency of calls (i.e. call rate) in *Bufo cognatus*, where Sullivan (1992) provided evidence of *Bufo americanus* females choosing males for their calling effort (calling rate times call duration), but found that females did not differentiate between high and low frequencies. Later studies performed on the same population showed inconsistencies between call characteristics of males (Howard & Palmer 1995; Howard & Young 1998). Overall, call frequencies were negatively correlated with male body size and females choose larger males calling at lower pitches, although females did not show uniformity in mate body size choices. While Howard & Young (1998) did not test for these variables, calling rate and call duration were highly variable, both between and within males, but were not related to body size. Thus, for some bufonid species at least, female mate choice exists to some degree, but are jeopardised by either the interception of males actively searching, satellite males or scramble competition.

4.1.3 Geographic variation of advertisement calls

Literature report many examples of geographic variation in advertisement calls of anurans (Ryan & Wilczynsji 1991; Bernal *et al.* 2005; Smith & Hunter 2005; Forti *et al.* 2012; Kaefer & Lima 2012). The genetic foundation for the advertisement call is shown by distinct calls produced by interspecific hybrids (Duellman & Trueb 1994). Forester (1973) shows intermediate call properties between natural hybrids of *Scaphiopus bombifrons* and *S. hammondii* and the parental species. Natural hybrid toads of *Bufo americanus* and *Bufo woodhousii fowleri* have distinct differences in pulse rates compared to parental toads (Zweifel 1968). Bernal *et al.* (2005) found evidence for genetic and bioacoustic differentiation between populations living on opposite slopes of the Columbian Andes in the dendrobatid *Colostethus palmatus*. Thus, dispersal capacity of anurans will greatly influence variation in call properties among populations (Jang *et al.* 2011). Although gene flow is a major driver for variation in acoustic properties, other variables such as temperature, male size, sexual selection and possibly noise pollution explain the remainder of the variation (see Chapters 3 and 6).

Anuran vocalisations evidently attract conspecifics to breeding sites (Duellman & Trueb 1994; Swanson *et al.* 2007; Wells 2007). Playbacks of a chorus recording from *Bufo terrestris* evoked positive responses of receptive males and females at distances of up to 40 m (Bogert

1960). Male Chorus Frogs, *Pseudacris triseriata*, orient themselves to conspecific recorded and natural choruses at distances between 50 - 75 m (Landreth & Ferguson 1966). This is known as the active distance (distance over which an acoustic signal can be detected).

The noise created by passing traffic can extend more than 4 km from a road, depending on the volume and speed of traffic and the current weather conditions (Department of Transport Welsh Office 1988). Studies have shown that traffic noise reduces the active distance of frogs and that some species respond by altering call properties (Parris *et al.* 2009), changing calling behaviour (Sun & Narins 2005). A study conducted by Bee & Swanson (2007) shows that traffic noise reduces the ability of frogs to orientate them toward the call source.

4.1.4 The Western Leopard Toad - a case study

The Western Leopard Toad (*Amietophrynus pantherinus*) is a large, charismatic toad reaching a maximum length of about 140 mm (Du Preez & Carruthers 2009). It is easily distinguished by its striking, symmetrical, dark red-brown markings edged in black and yellow (Figure 4.2). The species is distributed in Cape Peninsula and Cape Flats along the south-western coastline of the Western Cape, South Africa (Figure 4.3). Populations in rural habitats of Overstrand are also known. *Amietophrynus pantherinus* is known from selected sites on the Cape Flats and Cape Peninsula. It is threatened throughout most of its range by urban development and habitat degradation (De Villiers 2004; IUCN 2013). Research on the natural history of the Western Leopard Toad (*Amietophrynus pantherinus*) has been conducted mainly by Michael Cherry (but see Measey & Tolley 2011) in the late 80's and early 90's (Cherry 1989; Cherry 1992; Cherry & Francillon-Vieillot 1992; Cherry & Grant 1994).

Every year between July and September (weather dependent) hundreds of *A. pantherinus* cross the busy roads of the Cape Peninsula to reach their breeding destinations. Individual populations breed over a period of approximately 10 days and this occurs over the entire area for around 30 days (occasionally longer). Importantly, however, this short period falls into different winter months (John Measey pers. comm.). While breeding generally takes place in larger, more secure wetlands, urban development poses obvious threats around these wetlands by causing habitat fragmentation, restricting the foraging area and movement of toads as well as high road mortalities. In the urban environment, toads face challenges in crossing roads and barriers (for example walls, embankments, canals) while foraging and migrating to and from breeding sites.

Urban expansion and increased road traffic causes the death of hundreds of toads each year during the breeding season (De Villiers 2004). This may result in reduced population size and restricted or completely interrupt gene flow between populations. Measey (2011) ranks the priority of *A. pantherinus* as 4/10 for conservation research of threatened South African amphibian species, where 1 = highest priority.

Figure 4.2 The Western Leopard Toad (*Amietophrynus pantherinus*).

Amietophrynus pantherinus is well-known for being an explosive breeder (Cherry 1992; Cherry & Francillon-Vieillot 1992; Minter *et al.* 2004), but can also be considered a capital breeder, a term that has not been used for this species yet. Capital breeders typically breed earlier than most species (usually at the end of winter), in order to minimize the risk of predation, reducing interspecific competition and allowing juveniles ample time to develop (Bonnet *et al.* 1998; Voituron & Lengagne 2008). Since the Western Cape is subjected to a winter rainfall regime, it also provides opportunities for anurans to employ the adaptive behaviour of capital breeders. During the breeding season it is likely that capital breeders are exposed to a sudden return of cold conditions, interfering with migration and mating activities and will have to rely on their energetic reserves to ensure survival (Voituron & Lengagne 2008).

Taxonomic note

In the past *Amietophrynus pantherinus* was considered an allopatric population of *A. pardalis*, separated by a distance of more than 300 km from the closest *A. pantherinus* population in the furthest Eastern locality (De Villiers 2004). Poynton & Lambiris (1998) raised *A. pantherinus* to the status of full species on the basis of colouration, markings and morphological differences between the two populations. In addition, Eick *et al.* (2001) agreed with the separation of the species based on genetic divergence between *A. pantherinus* from the Western Cape and *A. pardalis* from the Eastern Cape. However, Cunningham and Cherry (2000) found just 0.5% divergence among the populations and thought that this was inadequate to justify full species status for the Western Cape population. Passmore (1977) described the advertisement call of *A. pardalis* (in the Eastern Cape), but a sufficient comparison between the calls of *A. pardalis* and *A. pantherinus* has not yet been published (Poynton & Lambiris 1998). Therefore, I here provide a brief comparison of the calls of both species as part of an additional objective of this study.

Citizen science

Amietophrynus pantherinus has enjoyed a growing interest from the citizens of Cape Town since a "toads on roads" volunteer movement was launched in 2007. Each district has a coordinator that collects data on the quantity of toads dead or alive for each evening that they patrol. Volunteer groups are overseen by eight coordinators which cover many suburbs in the Cape Peninsula.

Traffic on roads is ranked as the number one threat requiring mitigation in the biodiversity management plan for *A. pantherinus* (Rebelo *et al.* submitted). Although the species is tolerant of habitat alteration, it is being negatively impacted by increased urbanisation and agricultural expansion in its entire range. Road kill, urban development, alien vegetation and introduced alien fish are all thought to be important factors affecting its population numbers (Measey 2011). A recent introduction and rapid expansion of the Guttural Toad, *Amietophrynus gutturalis* into the Western Cape poses the additional threats of competition and possible hybridisation (IUCN 2013). Public participation and awareness has contributed to reducing toad mortality, but no quantitative studies have been published to date.

4.1.5 Objectives of study

This study was divided into two major parts concerning the urban dwelling Western Leopard Toad, *Amietophrynus pantherinus*. One component focussed on the migration of this species across roads and aimed to 1) quantify the number of individuals migrating over a 500 m stretch of road using a drift fence system operated by public volunteers, and 2) to evaluate the use of data collected by these citizens occupying a volunteering role in the toad's conservation. The second part of this study was directed towards the acoustic analysis of the call of *A. pantherinus*. The two main objectives of this component were to 1) evaluate the extent of variation of the call properties in order to 2) assess whether ambient anthropogenic noise has an effect on these properties.

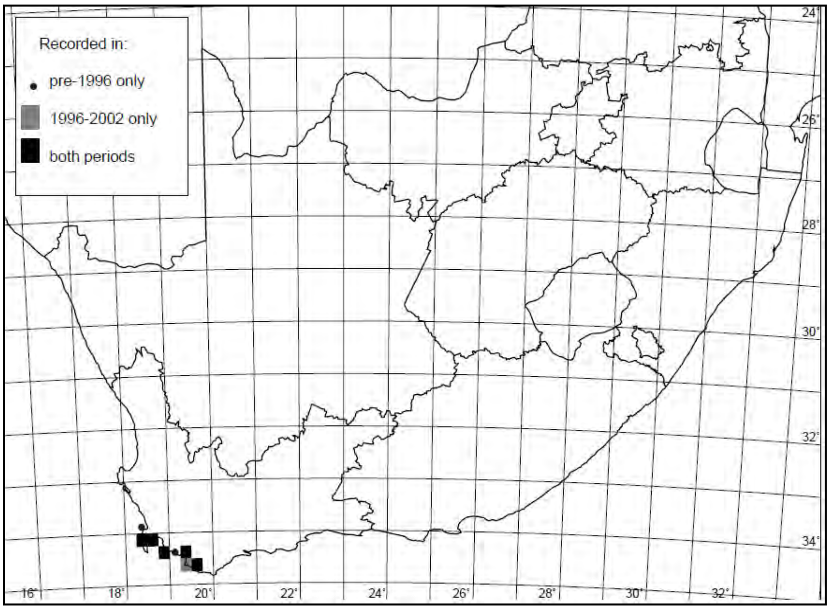


Figure 4.3 Distribution map of *Amietophrynus pantherinus* (source Minter *et al.* 2004).

4.2 Materials and methods

4.2.1 Collection of migration count data

4.2.1.1 Study area

Toad NUTS (Noordhoek Unpaid Toad Savers) is a volunteer group that rescues *A. pantherinus* from becoming road kill in Noordhoek. Toad NUTS' focus area is mainly in Noordhoek (Western Cape, South Africa, Figure 4.4A) and some surrounding suburbs, including Sun Valley, Fish Hoek, Clovelly and Kommetjie. Noordhoek Main Road is a two-lane motorway bisecting the entire suburb and is situated next to Lake Michelle, where the toads breed each year. It was only in 2010 that toad mortalities were noticed by this volunteer group (ToadNUTS) at the southern end of Noordhoek Main Road. Two of the breeding sites are located close to the road (range: 22.7–1057.8 m perpendicular to Noordhoek Main Rd; Figure 4.4C), where the toads migrate to and from the urban gardens across the road to the main lake and surrounding ponds (Figure 4.4B and C). In 2013, drift fencing was erected for the first time along 500 m on both sides of Noordhoek Main Road South, running from S 34.115749°, E 18.385599° to S 34.112209°, E 18.381627° (Figure 4.4C, Figure 4.5).

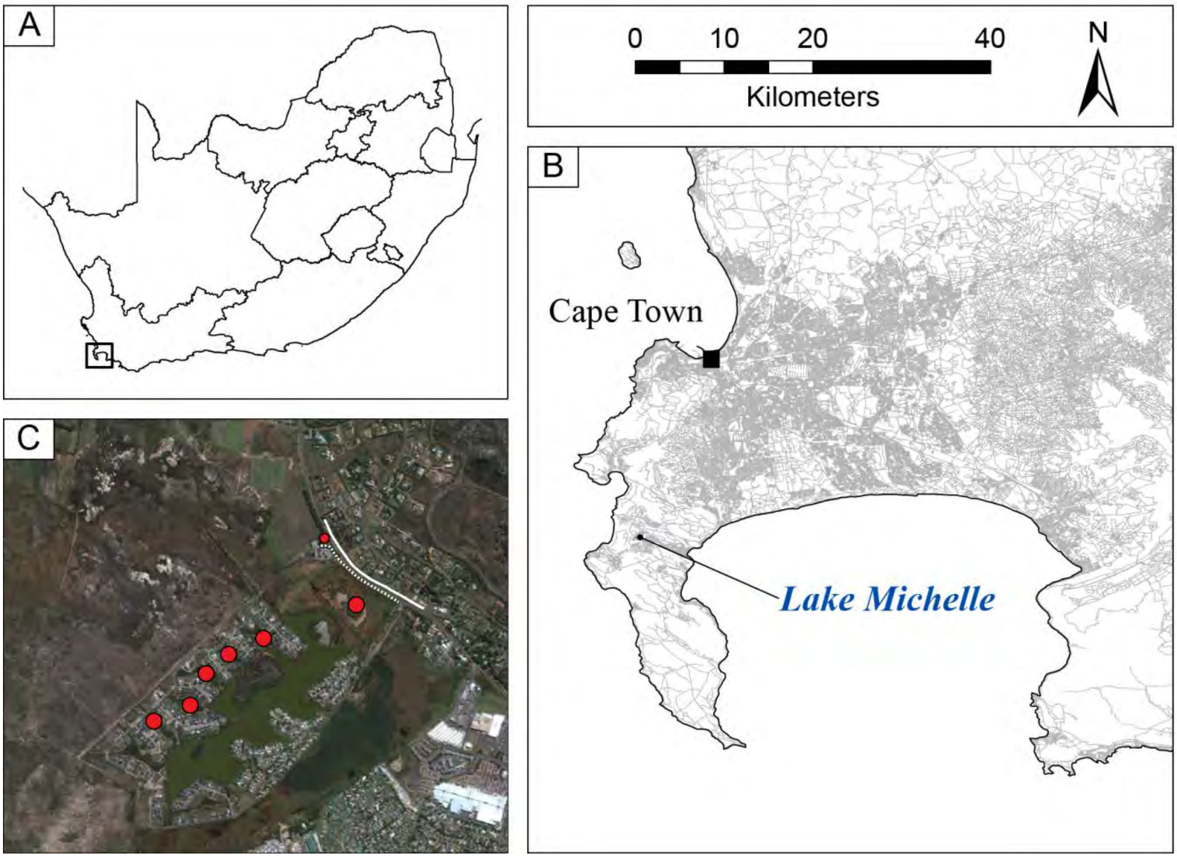


Figure 4.4 Map of the study area, showing (A) Cape Town within South Africa; Lake Michelle located in Cape Peninsula. The Cape Town central business district is indicated by the black square. (B) The road network is shown in grey; and (C) an aerial image of Lake Michelle showing the western (dotted white line) and eastern (solid white line) drift fences. Solid red circles indicate breeding sites.

4.2.1.2 Drift fence specifications

Temporary drift fences were erected to the east-side of Noordhoek Main Road using 500 m x 1 m of emergency shade cloth. The cloth was fixed in place with cable ties attached to wooden stakes knocked into the ground at about a 60° angle. Approximately 700 mm of shade cloth was fixed above ground and 300 mm of cloth was buried into a trench running the length of the fence. The cable ties and buried section of cloth ensured that the drift fence was securely fixed and impervious to wind. The drift fence on the west-side of Noordhoek Main Road was positioned within the residential estate of Lake Michelle. Yellow and orange shade cloth of 400 m x 700 mm was used. This colour was chosen in order to blend in with the natural surroundings. Plastic plant pots (height 280 mm; diameter 300 mm, from here on referred to as "buckets") with six drainage holes were used as pitfall traps, and positioned at approximately 40 m intervals and buried level with the ground with approximately 100 mm drainage below. The location of the buckets was indicated by means of reflective tape. Lids were provided next to each bucket in case of flooding during heavy rains to prevent animals from drowning. The drift fence was erected on 4 and 5 August 2013 which was a week after the start of the breeding season for *A. pantherinus* and dissembled on 2 October 2013, having remained in place for 59 nights.

4.2.1.3 Patrolling the drift fence

Walking patrols of the fence and inspecting of pitfall buckets were conducted at least once every 24 hours either at night (20:30, 21:30 and 24:00) or early in the morning (between 05:00 and 06:00). A total of 105 drift fence patrols were carried out by 24 patrollers over 59 nights for 2013. A complete patrol on both sides of the road (about 1000 m) usually took patrollers about 30 to 45 minutes depending on the quantity of animals in the buckets. During peak periods of toad movement, patrols were carried out up to four times over a 24 hour period. Records were kept of which fence (east or west) the toads were found at, and if they were found inside or outside of the buckets. In addition, all other species found were noted.

4.2.1.4 Road patrols

This study includes data obtained from volunteer road patrols between 2008 and 2012. All road patrols in Noordhoek were coordinated by the Toad NUTS. Meticulous records were kept of details from each patrol over this period, although it is recognised that records from 2008 and 2009 lack complete information. I also recognise that data might be subject to a certain level of bias and therefore attempt to bridge this by supplying as much detail as possible. All patrollers were trained to: remove the toad from the road and placing it next to the road; taking account of the direction in which the toad was facing; record the general location of the toad (i.e. name of road); keep a record of all living and all dead toads and to also move dead toads off the road to avoid double counting; and determine the gender of the toad. The main objective for road patrols was to ensure patrollers were out on the busy roads over the period from darkness (18:30) until the traffic quieted down, or until the toads had stopped moving. On any night when toads were moving, patrols may have continued until midnight. If no toads were observed and if there was little likelihood that they would emerge later in the evening, the patrols may have ended by 20:00.

Figure 4.5 Eastern drift fence along Noordhoek Main Road South, used to protect *Amietophrynus pantherinus* migrating towards Lake Michelle from road kill.

4.2.1.5 Geographical analysis

Traffic flow rates were supplied by Cape Town government (Development, Information and GIS Department) for main roads intersecting *A. pantherinus* migration routes. Average traffic flow rates were calculated for both directions between 19:00 and 00:00 in the evening when toads were most likely to migrate. Traffic flow was divided into three orders of magnitude namely, low (100–299 cars/hour), moderate (300–799 cars/hour) and high (800–2000 cars/hour) using the effect of traffic intensity as demonstrated by Hels & Buchwald (2001) as a guide. Breeding site coordinates were provided by volunteer coordinators, Jeanne Tarrant and John Measey. Buffer zones of 250 m, 500 m and 1000 m were digitally constructed around each breeding site and assigned sensitivity zones as high, moderate and low sensitivity areas respectively. These buffers were chosen on account of the dispersal distances of the American Toad (*Bufo americanus*; Forester *et al.* 2006). All geographic analyses were carried out in ArcGis 10.0.

4.2.2 Variation in call properties

4.2.2.1 Study area

Study sites included three populations, two occurring at higher noise levels, Die Oog and Raapkraal Road, and one reference site, Strawberry Lane, with low noise levels (Table 4.3; Figure 4.6). Since expensive equipment had to be left unattended and the investigator had to make directional recordings of calls that continued past midnight, these sites were selected for safety reasons and ease of access.

Table 4.3 Population growth rate from 2001 to 2011 populations for Cape Town residents. The three sites from which the recordings were made are also included in their respective wards, i.e. governmental voting area divisions (www.capetown.gov.za; also see Figure 4.6).

Urbanisation variable	2001	2011	Change 2001 to 2011		Ward description	Site monitored
			Number	% change		
Ward: 62						
Population	18 046	24 832	6 786	37.60%	Includes the areas of Bishopscourt, Constantia, Fernwood, Plumstead, Porter Estate, Trovato, Upper Newlands and Wynberg.	Strawberry Lane
Households	7 105	9 000	1 895	26.70%		
Average Household Size	2.54	2.76				
Ward: 64						
Population	20 732	24 489	3 757	18.10%	Includes the areas of Clovelly, Fish Hoek, Kalk Bay, Lakeside, Marina da Gama, Muizenberg and St. James.	Raapkraal Rd
Households	8 460	9 707	1 247	14.70%		
Average Household Size	2.45	2.52				
Ward: 69						
Population	20 931	37 172	16 241	77.60%	Includes the areas of Capri, Fish Hoek, Kommetjie, Noordhoek, Ocean View, Sunnydale and Sunvalley.	Noordhoek, drift fence location
Households	6 928	13 085	6 157	88.90%		
Average Household Size	3.02	2.84				
Ward: 71						
Population	28 605	27 943	-662	-2.30%	Includes the areas of Bergvliet, Heathfield, Kirstenhof, Lakeside, Retreat, Steenberg and Tokai.	Die Oog
Households	7 422	9 281	1 859	25.00%		
Average Household Size	3.85	3.01				

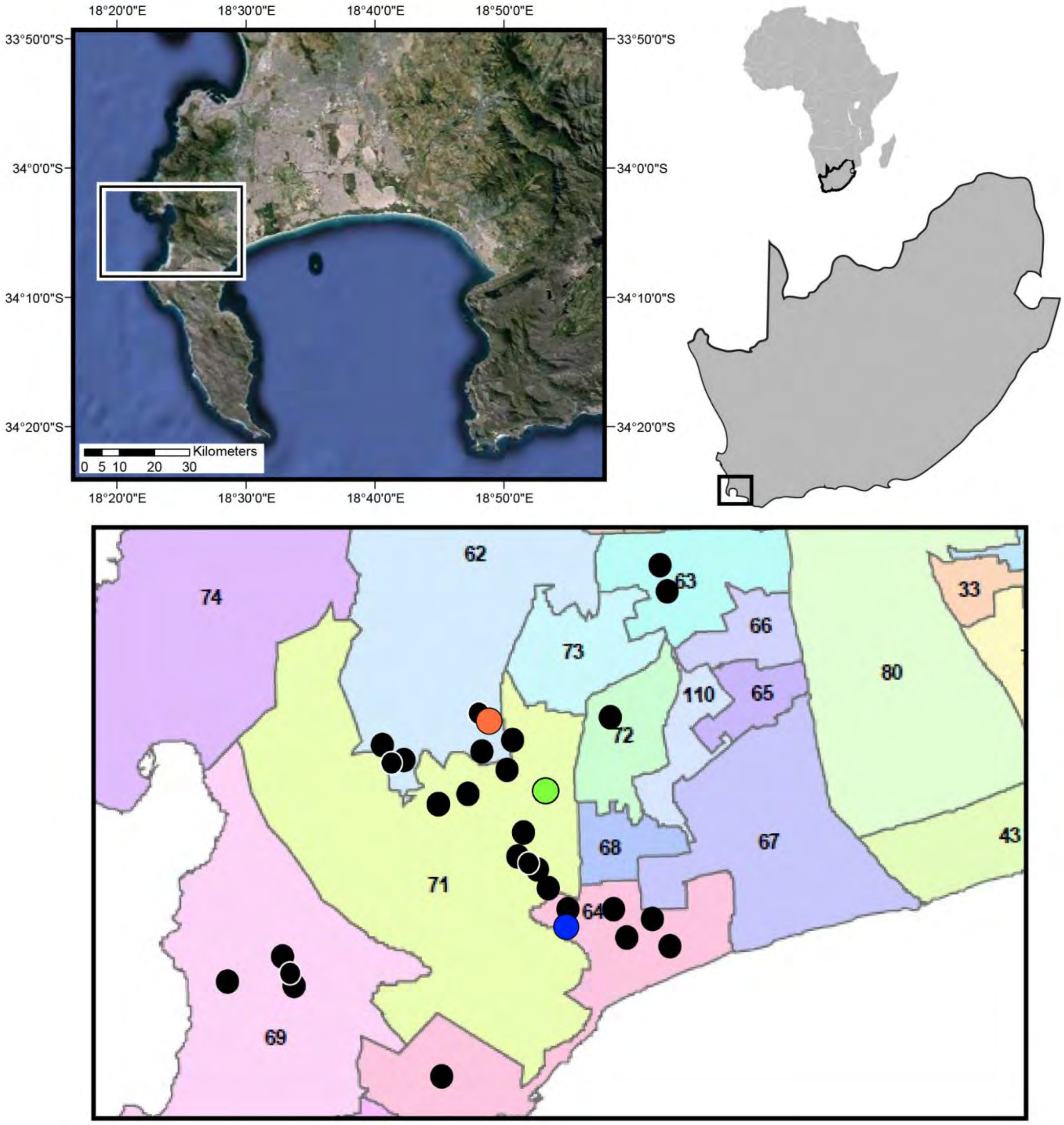


Figure 4.6 A map showing the study area. Raapkraal Road (blue), Die Oog (green), Strawberry Lane (orange) and other known breeding sites (black) are included as circles in the area of the Cape Peninsula. The demarcated area is also divided into the subsequent districts according to the census 2011 voting districts. Ward map excerpt from www.capetown.gov.za.

4.2.2.2 Chorus monitoring

Passive acoustic recordings were used to monitor chorus behaviour. Automated acoustic recorders, SongMeter SM2 and SM2+ (Wildlife Acoustics) were deployed at three localities with differing noise levels as mentioned in section 4.2.1. Chorus duration and inter-chorus intervals were measured in seconds using the Song Scope® software package (Wildlife Acoustics). SongMeters were set to record 10 minutes of the hour over a period of 72 hours for each site. All soundscapes (e.g. spectrographic representations of chorus recordings over an extended amount of time) were compiled from the aforementioned software package. The SongMeter SM2 and SM2+ also recorded ambient and water temperature every five minutes throughout recording time.

4.2.2.3 Advertisement and release call recording and description

Advertisement and release (or distress) calls of *A. pantherinus* were recorded in the field between 28 and 31 August 2012. At all times the operator attempted not to disturb the chorus during the recording procedure in order to obtain feasible and consistent recordings of entire bouts.

Directional recordings of the advertisement and release calls of *A. pantherinus* were collected using a Nagra ARES-ML digital recorder equipped with a dedicated Sennheiser ME66 directional microphone for acoustic analyses (44.1 kHz, 16 bit). Advertisement and release calls were recorded from a total of 30 males from three sites, Raapkraal Road, Die Oog and Strawberry Lane (Table 4.4), except for one individual which did not produce a release call. Release calls were recorded by catching a male after the recording of an advertisement call was made, holding the male firmly with two fingers (thumb and index finger) behind the forearms of the male, simulating amplexus by another male. The snout-vent lengths (SVL) of all frogs were measured with Vernier callipers, and weight was measured with a Pessola scale (1000 g limit). Ambient and water temperature were taken with each measurement. SoundRuler (version 0.9.6.0, 2007-03-10) software was used to compile spectrograms, oscillograms and power spectra for the descriptions of advertisement and release calls. A Hanning FFT window was used with a 256 sample length (0.9 overlap between FFTs) with minimum frequency set to zero and maximum to 10 kHz.

Ten successive call-notes for each of the 30 advertisement- and 29 release call recordings were analysed for males (15, 12 and 7 males respectively for Raapkraal Road, Die Oog and

Strawberry Lane). Because pulses at the start of the call have a slower rate than the last part (see results), in order to compare pulse rates with *A. pardalis* an average pulse rate is calculated by using call duration and number of pulses. Seven call properties were measured for advertisement calls: call duration (s), ACD; inter-call interval (s), AICI; pulse number, APN; dominant frequency (Hz), ADF; mean weighted frequency (Hz), AMF; duration of first five pulses (s), AFP and the duration of last five pulses (s), ALP. Four call properties for release calls were measured: call duration (s), RCD; inter-call interval (s), RICI; pulse number, RPN and mean weighted frequency (Hz), RMF. A comparison between dominant frequency (DF: obtained from BatSound© version 4.1.4 by Pettersson Electronics and Acoustics AB) and mean weighted frequency (MWF: obtained from Song Scope® version 4.1.3A) were made to evaluate the viability of the two frequency measurements. Call duration is temperature-corrected (Platz & Forrester 1988).

Table 4.4 Profiles of the three study areas with descriptive statistics of sound level (SPL) measurements for each site. Raapkraal Rd was measured on two occasions to investigate the difference in SPLs at two different times during the night. nd = no data.

Locality		Strawberry Lane	Raapkraal Rd 1	Raapkraal Rd 2	Die Oog
Date		8/28/2013	8/27/2013	8/27/2013	8/27/2013
Temperature		11.2 °C	12.1°C	12.0 °C	13.4 - 13.8°C
Starting time		21:30	20:45	22:30	18:35
Coordinates		34.041464S 18.439357E	34.078024S 18.454550E	34.078024S 18.454550E	34.048399S 18.447176E
Distance to closest Main Road (m)		396	181	181	179
Distance to closest calling male within chorus (m)		nd	10	5	1.5
SPL distance from ground (m)		1.5	1.5	1.5	1.5
Ambient SPL (dB)	Median	59.6	61.8	61.9	67.85
	Maximum	73.2	70.1	66.7	78.4
	Minimum	50.2	57.7	57.3	65

Sound pressure levels (SPLs) were measured (dB intervals, C-weighting) for five males using a calibrated Brüel and Kjaer (calibration number: 2013-0285) precision sound-level meter (Model 2235) and microphone (B & K 4176). Ambient noise levels were measured in one minute intervals over 10 minutes from 17:00 18:00 on 27 and 28 August 2013 for all sites (Table 4.4). Peak SPLs of 15 consecutive advertisement calls of five males were measured 0.5 m from tip of snout, at a 180° angle, 28 August 2013. Ambient temperatures varied from 10.9-12.5°C.

4.2.2.4 Call analysis and statistical tests

Coefficients of Variation

Coefficient of variation (CV) is useful for meaningful comparisons between two or more magnitudes of variation, even if they are different means or different scales of measurement (Lewontin 1966; Hallgrimsson & Hall 2005). Coefficient of variation was calculated for each individual call property to examine within and between-male variation in call structure as well as variation between populations, using:

$$CV = \frac{SD}{\bar{X}} * 100$$

where SD is the standard deviation and $\bar{X}$ the mean; the result is then multiplied by 100 to obtain a percentage value.

Significance of the CV between-populations was tested using Levene's *F* test (Levene 1960) for coefficient of variation to test for homogeneity of variances. The test performs a one-way ANOVA on the ratio of the absolute deviations associated with each observation from its respective group (or population) mean divided by the group mean:

$$ANOVA\left\{\frac{(|X_{ij} - \bar{X}_j|)}{\bar{X}_j}\right\}$$

where X_{ij} is the value of a certain call property of a single male and $\bar{X}_j$ is the mean of the population of the same call property.

Within-male CVs was used to characterise the variability of each call properties as static or dynamic. Within-male CVs ≤5% were classified as static, ≥12% as dynamic, and 5%<CV<12%

as intermediate (Gerhardt 1991; Smith & Hunter 2005). Between-male and between-population CVs was also calculated. All statistical analyses were performed in SPSS 21.0 (SPSS 2013).

Canonical ordination analysis

To determine variation in advertisement call assembly between populations and overcome problems associated with collinearity between call properties, a redundancy analysis (RDA) was conducted to derive orthogonal components. Canonical ordination was used to compile RDA graphs in Canoco for Windows (version 4.56). In order to find the combinations of explanatory variables that best explains the variation between individual calls and populations, averages of each of the 30 males' calls were used.

4.3 Results

4.3.1 Migration counts

The drift fences were not yet installed during the first week of toad migration in July 2013 and patrollers relied on road patrols only. Seventy toads were found on the 500 m stretch of Noordhoek Main Road, of which 19 (27.1%) were run over by traffic. After the installation of the drift fence, 61 more toads were found migrating to the breeding sites and 27 (44.3%) returning to the gardens (Figure 4.7). No road-kill was observed over the 59 day period during which the fences were in place.

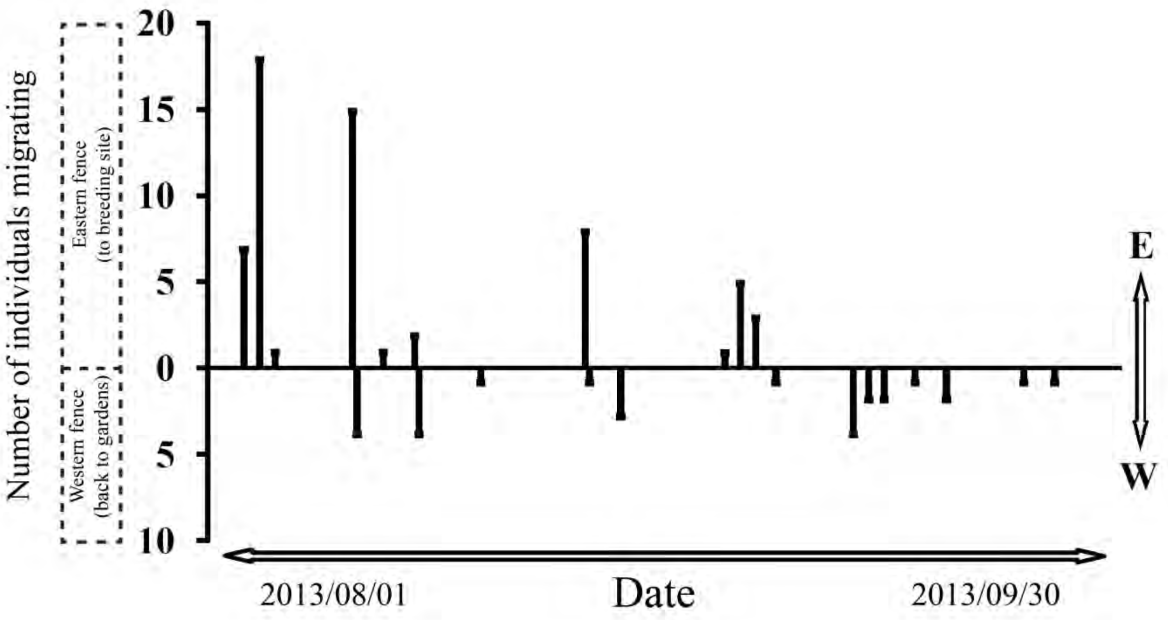


Figure 4.7 Number of *Amietophrynus pantherinus* found along each of the drift fences, showing the migration activity either to the breeding sites (East fence) or back towards the urban gardens where they aestivate (West fence).

Aside from *A. pantherinus*, other wildlife recorded in the pitfall buckets included Sand frogs (*Tomopterna delalandi*), Clicking Stream frogs (*Strongylopus grayii*), a Sand Toad (*Vandijkophrynus gariepensis*), Common Platannas (*Xenopus laevis*), Common Slug-eater snakes (*Duberia lutrix*), Cape River crabs (*Potamonautes perlatus*), Cape skinks (*Trachylepis capensis*), a Marsh terrapin (*Pelomedusa subrufa*), Tiger moth caterpillars (Family Arctiidae), and stink bugs (Family Pentatomoidea).

Over a period of six years, Toad NUTS volunteer group have found 2 384 toads on the roads of Noordhoek (including drift fence data for 2013) of which 20.47% were killed by traffic

(Table 4.5). Although there were seven less patrollers in 2013 compared to 2012, there was a 55.18% increase in number of toads found and 5.9% decrease in toad mortality.

Based on geographical analyses and road kill data for road sensitivity zones (i.e. high roadkill probability) I calculated 21 096 m within low sensitivity areas, 29 451 m within medium sensitivity areas and 47 550 m within high sensitivity areas of the respective main roads (Figure 4.8; Table 4.6). Only main roads with available traffic flow data were analysed for each sensitivity rating.

Table 4.5 A summary of toad and patroller data collected between 2008 and 2013 since the commencement of volunteer road patrols in Noordhoek, Western Cape. NR = Data not recorded at the time.

Year	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Breeding season dates	NR	NR	22/07–21/08	28/07–12/09	26/07–09/09	18/07–15/09
No. of individuals on the road	228	173	405	371	473	734
Percentage dead	27.2%	19.1%	19.8%	19.7%	23.5%	17.6%
Male : Female ratio	NR	NR	NR	1.3 : 1	0.6 : 1	2.2 : 1
No of patrollers	NR	NR	36	48	47	40
No of hours patrolled	NR	NR	125.50	183.25	225.33	255.75
Average Hours per patroller	NR	NR	3.5	3.8	4.8	6.4
No of road patrols	NR	NR	92	124	146	190
No of days Patrolled	NR	NR	31	47	46	60

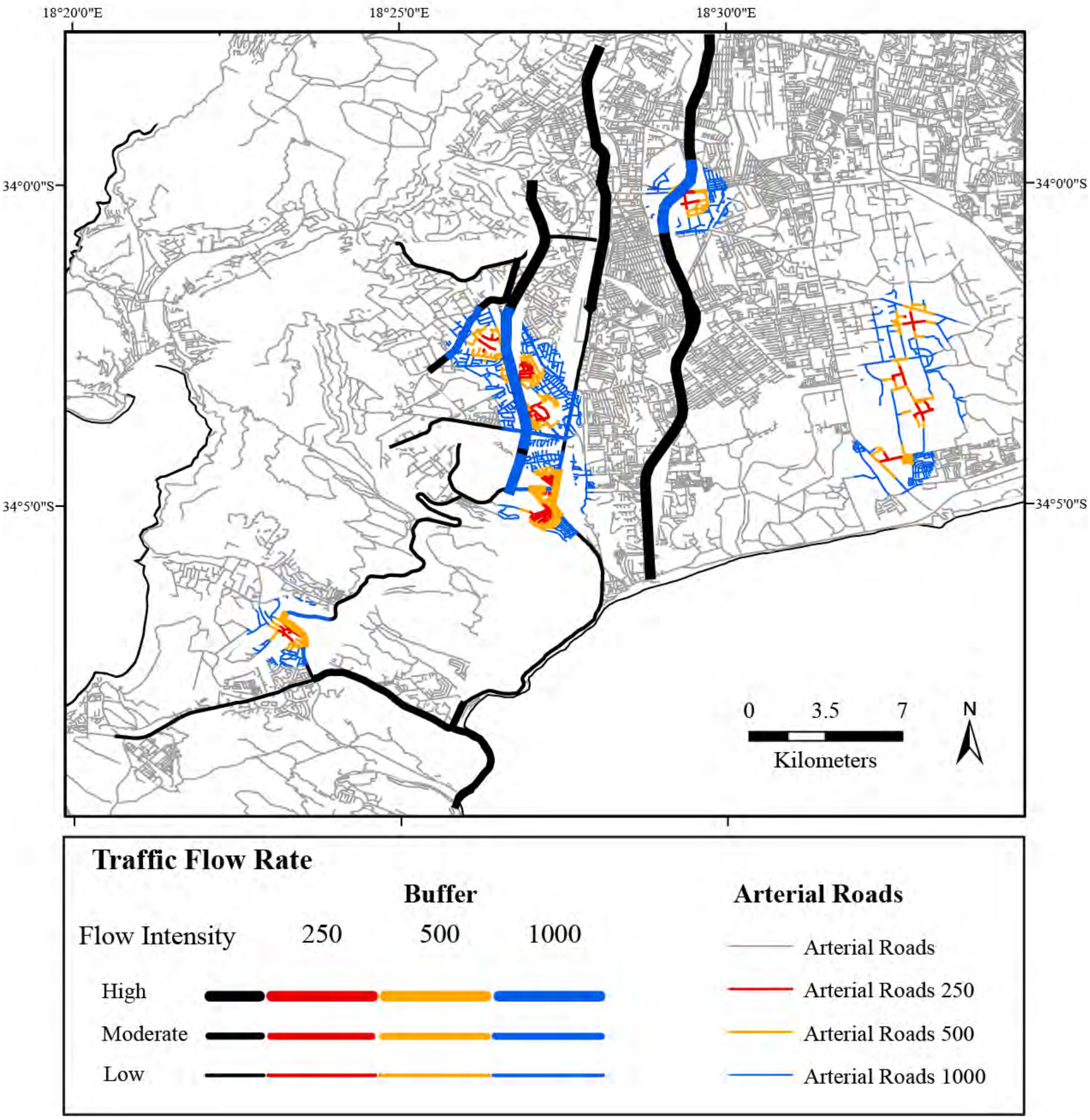


Figure 4.8 A map showing road sensitivity zones within 250, 500 and 1000 m buffer zones around breeding sites of *Amietophrynus pantherinus*. Sensitivity is graded as blue, orange and red for the three zones indicating low, moderate and high sensitivity respectively. Traffic flow rates for main roads (black) are graded with three line weightings, with heaviest lines indicating the highest traffic flow intensity.

Table 4.6 Roads lengths (meters) occurring within buffer zones of 250, 500 and 1000 meters of 14 *Amietophrynus pantherinus* breeding sites.

Main Roads			Arterial Roads		
Buffer250	*Buffer500*	*Buffer1000*	*Buffer250*	*Buffer500*	*Buffer1000*
21 096	29 451	47 550	130 977	233 100	431 243

4.3.2 Call analysis

4.3.2.1 Variation of mean weighted and dominant frequency values

Both the mean weighted frequency (MWF) and dominant frequency (DF) show a strong negative correlation with snout-vent length, but the trendline for DF fits the data better (Figure 4.9; $R^2 = 0.35$; *P*>0.05) as a result of lower variation shown in Figure 4.10 and Table 4.7.

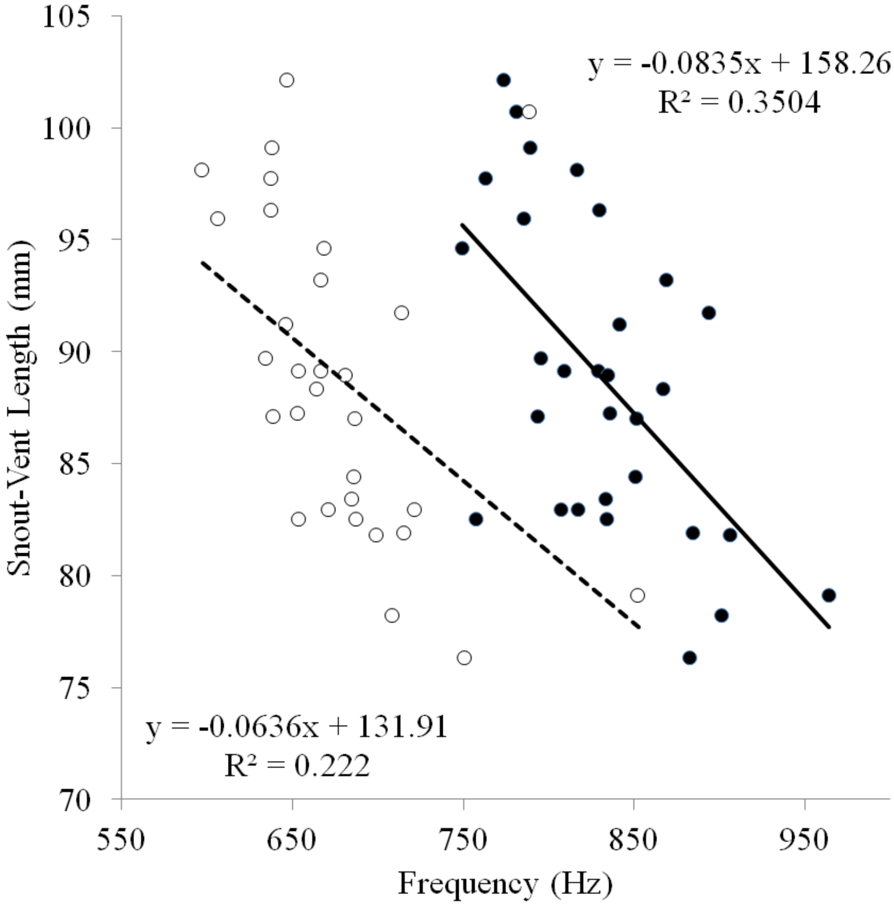


Figure 4.9 A linear regression plot of snout-vent length and two frequency measurements: mean weighted frequency (open circles, stippled trendline), and dominant frequency (solid circles, solid trendline). Each regression's trendline equation and coefficient of determination (R^2) is given.

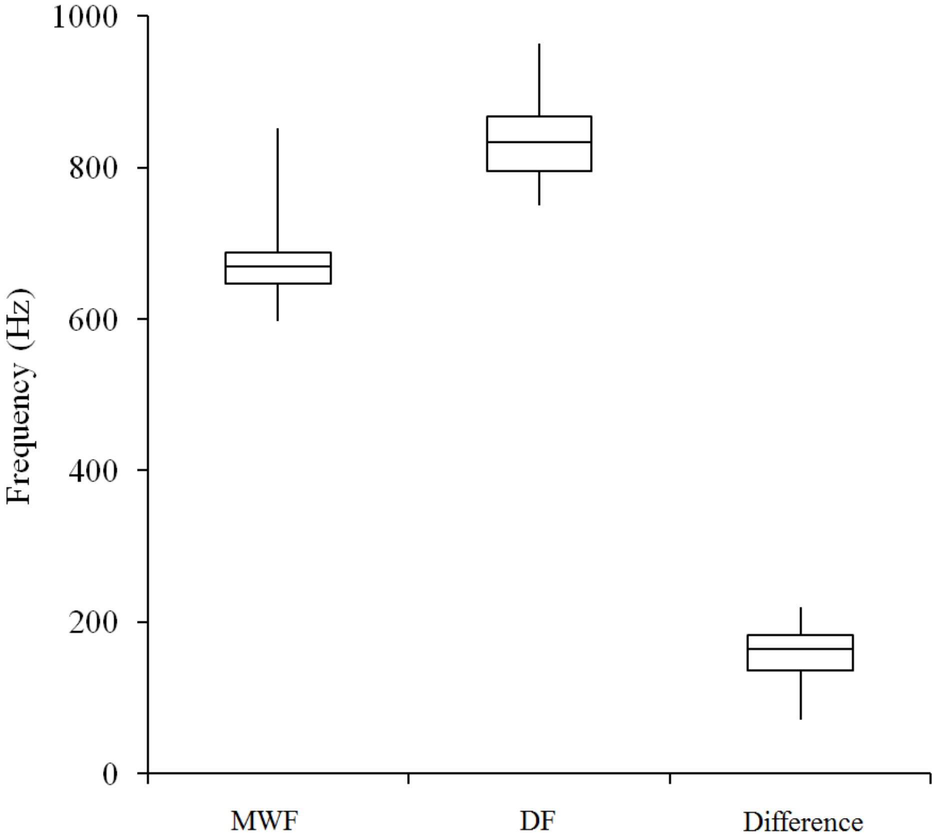


Figure 4.10 Box and whisker plots of variation in values of mean weighted frequency (MWF), dominant frequency (DF) and the difference in these values (Difference). Box plot indicate the median, 1st and 3rd quartile and whiskers indicate maximum and minimum values.

4.3.2.2 Advertisement and release calls

Amietophrynus pantherinus produces a pulsed advertisement call with a mean pulse train of 31.4 pulses per call (range 20 – 44) at a mean dominant frequency of 678.3 Hz (range 577–864 Hz) that lasts an average of 1198 ms (range 1006 –1461 ms). Descriptive statistics of call properties were summarised from all recorded subjects (n=30) in their three respective populations in Tables 4.7 and 4.8.

Table 4.7 Descriptive statistics for each advertisement and release call property at each study population of *A. pantherinus*, as well as for weight and snout-vent length for all males and temperatures at which recordings were made.

	Raapkraal Rd n = 15		Die Oog n = 12		Strawberry Lane n = 3	
	Mean	SD	Mean	SD	Mean	SD
Advertisement call						
Call duration (ms)	1 226	157	1 118	109	1 283	132
Inter-call interval (ms)	1 292	625	1 144	413	1 444	518
Pulse number (per call)	30.8	4.4	31.9	3.3	32.1	3.5
Dominant frequency (Hz)	697.0	54.3	656.5	46.0	668.5	30.4
Mean weighted frequency (Hz)	849.5	59.4	828.2	43.0	797.8	53.8
First five pulse duration (ms)	233	30	197	18	206	15
Last five pulse duration (ms)	128	23	109	21	163	25
Release call						
Call duration (ms)	0.157	0.074	0.129	0.031	0.143	0.013
Inter-call interval (ms)	0.349	0.113	0.360	0.123	0.281	0.032
Pulse number	6.1	1.8	6.5	1.5	5.7	1.5
Mean weighted frequency (Hz)	884.5	89.8	835.0	128.2	800.9	80.2
Male descriptives and temperature						
Weight (g)	80.0	18.3	95.8	11.2	93.3	12.7
SVL (mm)	86.0	7.2	91.2	5.2	92.7	6.3
Water temperature (°C)	14.9	0.6	15.1	1.2	14.8	0.0
Ambient temperature (°C)	12.8	1.0	14.8	1.0	12.2	1.3

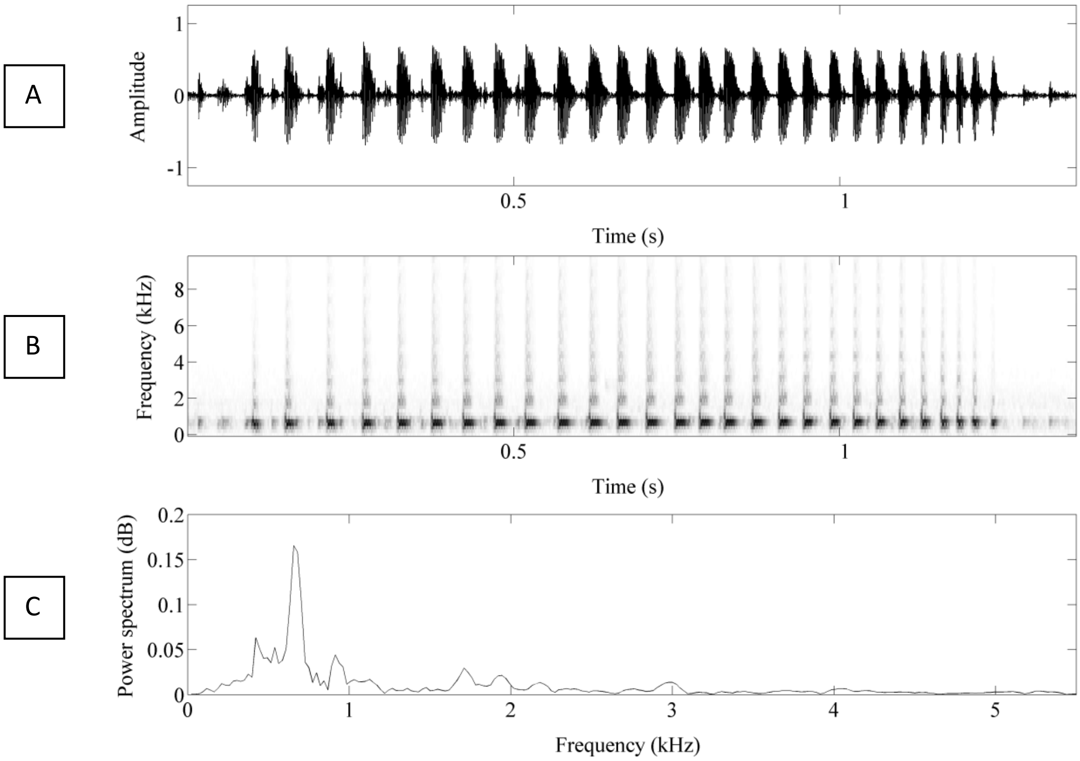


Figure 4.11 Advertisement call of *Amietophrynus pantherinus*. A, oscillogram of a sample call; B, spectrogram of the call presented in A; C, Power spectrum of the call presented in A.

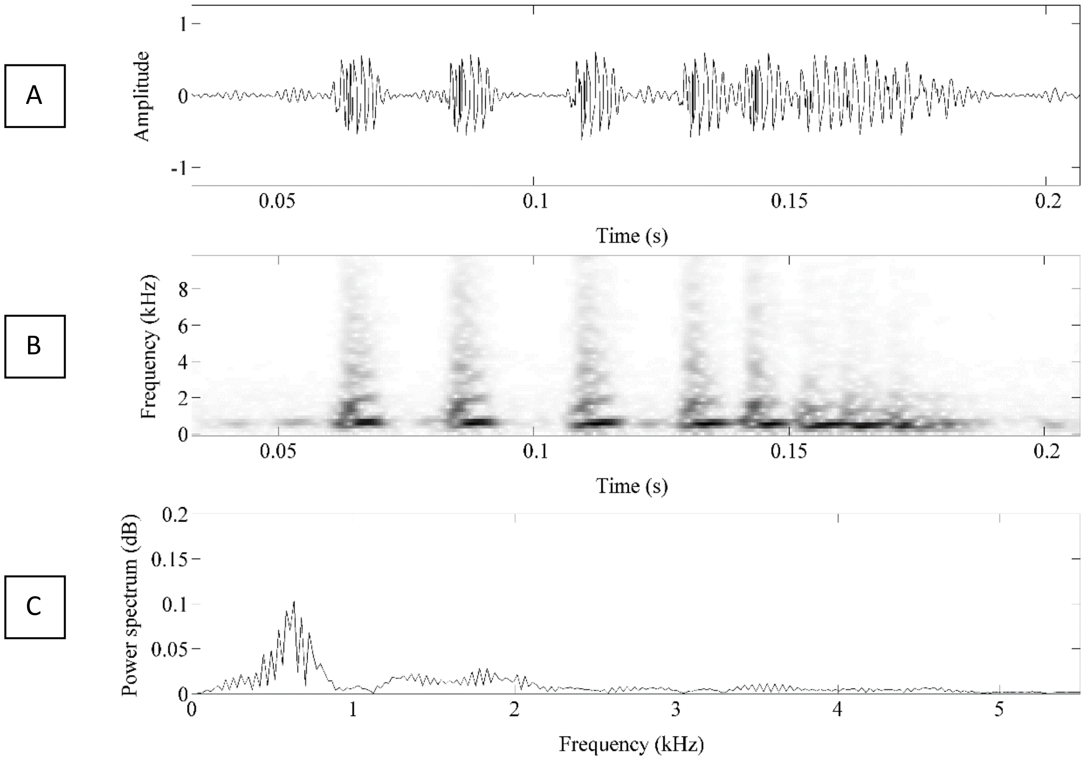


Figure 4.12 Release call of *Amietophrynus pantherinus*. A, oscillogram of a sample call; B, spectrogram of the call presented in A; C, Power spectrum of the call presented in A.

Table 4.8 A summary of sound pressure levels (dB) of *Amietophrynus pantherinus* advertisement calls of five males. Peak SPLs of 15 consecutive calls were measured 0.5 m directly in front of the tip of snout. Ambient temperatures varied from 10.9-12.5°C.

Subject	SPL (dB)		SVL (mm)
	Median	Range	
1	110.3	110.1-110.4	79.1
2	110.9	110.8-111.0	82.5
3	113.3	113.2-113.4	97.5
4	111.2	110.0-111.3	83.6
5	108.8	108.7-108.9	79.1

4.3.2.3 Advertisement call and male release call variation and correlation

Variation within and between individuals

Inter-call interval of advertisement calls and call duration, inter-call interval and pulse number of release calls were found to be dynamic properties (CV≥12%); spectral properties were static (CV≥5%) and the remaining properties were not included in dynamic or static ranges and subsequently classified as intermediate (5%<CV<12%; Table 4.9).

Table 4.9 Mean based coefficient of variations of advertisement and release call properties within- and between-males of *Amietophrynus pantherinus* of three populations. Classification of static, intermediate and dynamic call properties is based on within-male averages. Minimum and maximum values indicated below averages.

	Within-male				Between-male	
	Raapkraal Rd (n=15)	Die Oog (n=12)	Strawberry Lane (n=3)	Average (n=30)	All individuals	Classification
Advertisement call						
Call duration	8.1 4.6–12.1	6.3 3.4–10.8	9.3 4.2–12.2	7.5 3.4–12.2	12.6	Intermediate
Inter-call interval	33.8 12.4–60.5	28.7 12.2–69.5	33.8 30.4–39.3	31.8 12.2–69.5	43.9	Dynamic
Pulse number	8.5 4.1–14.0	6.6 3.4–14.6	9.5 5.5–13.8	7.8 3.4–14.6	12.6	Intermediate
Dominant frequency	1.8 1.0–3.4	2.1 0.9–4.1	3.2 1.2–4.3	2.0 0.9–4.3	7.8	Static
Mean weighted frequency	2.2 0.5–4.6	2.9 0.5–7.6	3.3 1.5–4.2	2.6 0.5–7.6	6.6	Static
First five pulse duration	8.3 4.0–23.2	5.7 3.0–8.2	7.0 5.8–7.7	7.1 3.0–23.2	13.9	Intermediate
Last five pulse duration	9.6 1.5–17.1	13.9 7.5–32.9	10.5 7.9–11.9	11.4 1.5–32.9	22.1	Intermediate
Release call						
Call duration	26.1 4.1–118.0	16.5 3.8–47.1	7.8 6.1–9.5	20.3 3.8–118.0	39.5	Dynamic
Inter-call interval	20.3 9.1–43.7	15.2 9.6–28.2	10.3 7.2–12.2	17.2 7.2–43.7	32.9	Dynamic
Pulse number	16.1 3.9–43.7	14.8 6.2–38.4	13.5 7.7–17.8	15.3 3.9–43.7	26.7	Dynamic
Mean weighted frequency	4.2 2.2–11.5	6.3 2.3–10.6	6.7 4.5–8.5	5.3 2.2–11.5	12.8	Static

Variation between populations

Levene's test showed a significant statistical difference ($P < 0.05$) among populations for call duration, pulse number, mean weighted frequency and duration of first five pulses for advertisement calls, as well as inter-call interval and mean weighted frequency of release calls (Table 4.10). The first RDA axis (axis 1) reveals the four environmental variables (weight, SVL, water and ambient temperature) significantly account for 14.4% of the variability in the call property data and 74.0% of the variability in the species-environment relationship, with axis 1 being by far the most important gradient (Figure 4.13; eigenvalue = 0.144, F-ratio = 4.206, P = 0.0120). The RDA triplot shows that axis 1 is dominated by a gradient of negatively correlated variables, which include ambient temperature, water temperature and weight, all which are negatively correlated with the mean weighted frequency, call duration and inter-call interval of the advertisement call, as well as the mean weighted frequency and call duration of the release call (Figure 4.13). However, there is a strong positive correlation between weight and release call pulse number as well as with advertisement call pulse number and SVL. No environmental variables were redundant as shown by the variance inflation factor (VIF), $1 > \text{VIF} < 2.4$. The three populations seem to show some separation of sample distribution, but also with considerable overlap (colour-coded dashed lines). Overlapping is excluded when average SPLs of the background noise from each site is included as one of the environmental variables within the RDA analysis (Figure 4.12; axis 1 eigenvalue = 0.163, F-ratio = 4.876, P = 0.01; all canonical axes: trace = 0.327, F-ratio = 3.035, P = 0.002).

Table 4.10 Levene's test of homogeneity of variances showing call properties that significantly differs between populations. Significant values are in bold. df = degrees of freedom.

	Levene Statistic	df1	df2	Significance
Advertisement call				
Call duration	6.770	2	264	**.001**
Inter-call interval	.372	2	255	.690
Pulse number	4.476	2	264	**.012**
Dominant frequency	1.577	2	264	.209
Mean weighted frequency	10.996	2	264	**.000**
First five pulse duration	15.795	2	264	**.000**
Last five pulse duration	1.802	2	264	.167
Release call				
Call duration	1.866	2	271	.157
Inter-call interval	6.680	2	261	**.001**
Pulse number	2.182	2	271	.115
Mean weighted frequency	8.851	2	271	**.000**

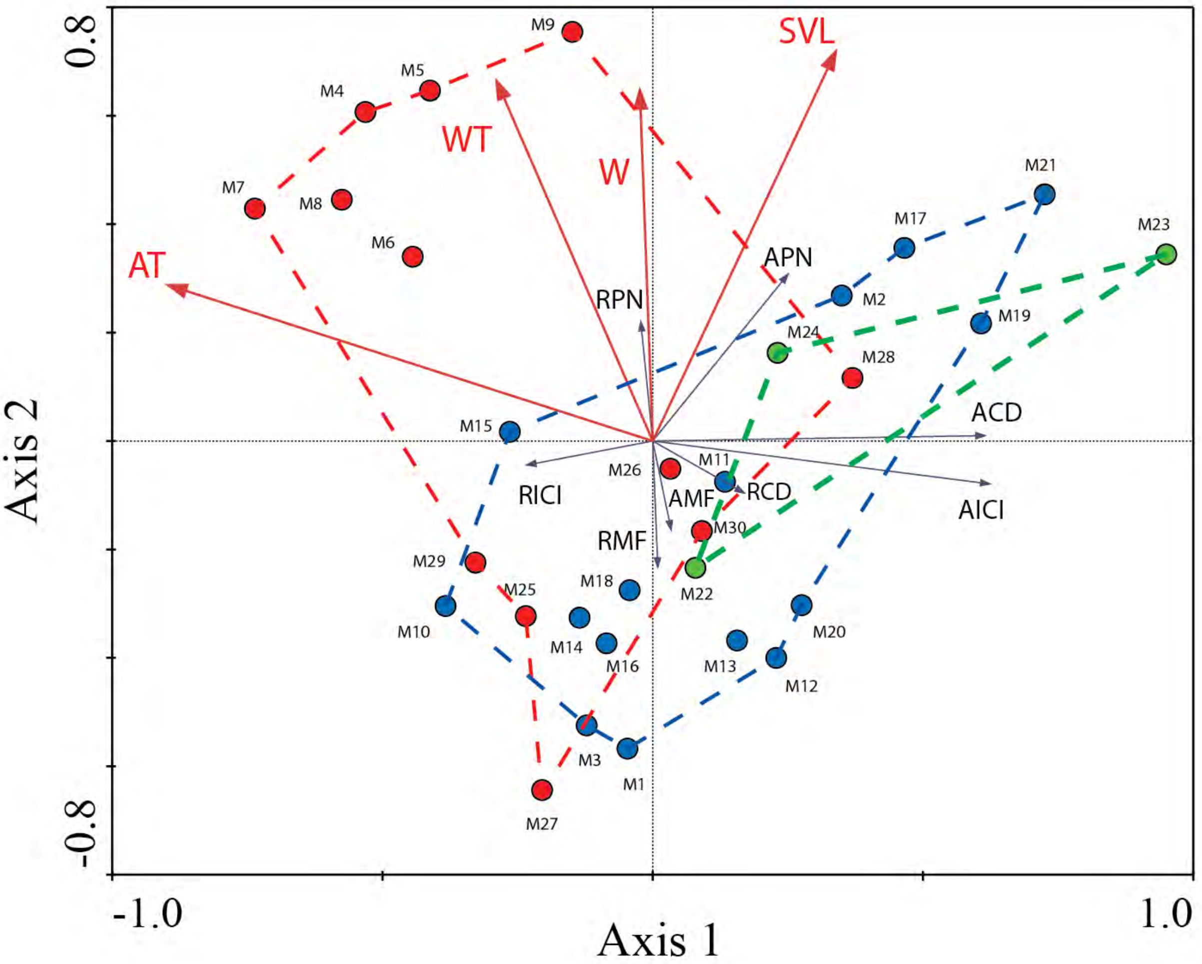


Figure 4.13 Ordination diagram (triplot) of the redundancy analysis (RDA) for the difference in call properties among populations according to four explanatory variables showing 30 *Amietophrynus pantherinus* male call samples (M1–30) at three populations, Raapkraal Rd (blue), Die Oog (red) and Strawberry Lane (green) Cape Town, South Africa. Weight (W), snout-vent length (SVL), water temperature (WT) and ambient temperature (AT) were used as explanatory environmental variables. The following species variables were used for advertisement calls: call duration (ACD), inter-call interval (AICI), pulse number (APN), mean weighted frequency (AMF), first five pulse duration (AFP), and last five pulse duration (ALP); and for release calls: call duration (RCD), inter-call interval (RICI), pulse number (RPN), and mean weighted frequency (RMF).

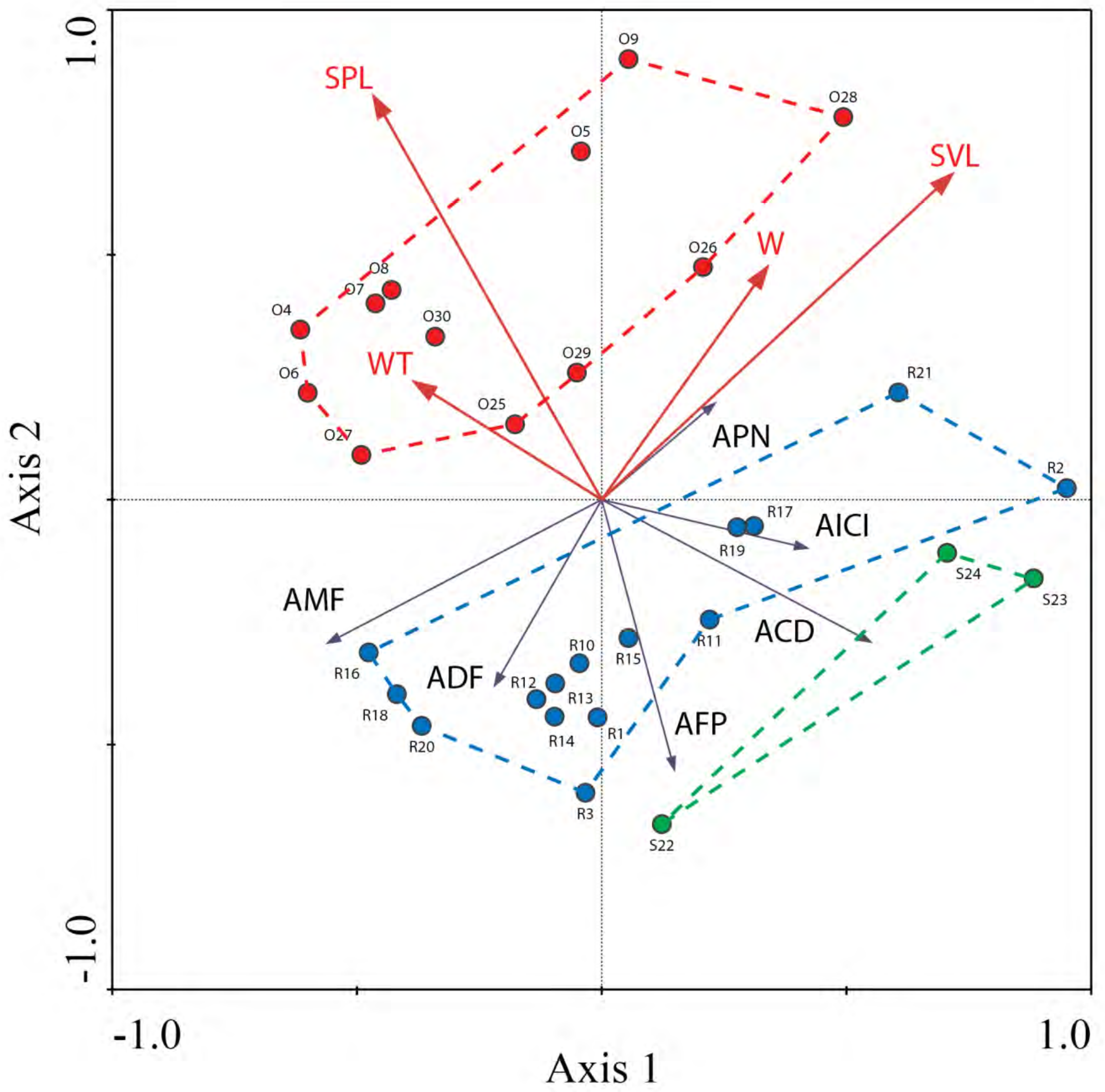


Figure 4.14 Ordination diagram (triplot) of the redundancy analysis (RDA) for the difference in advertisement call properties among populations according to four explanatory variables showing 30 *Amietophrynus pantherinus* male call samples (R = Raapkraal Rd; O = Die Oog; S = Strawberry Lane; 1–30) at three populations, Raapkraal Rd (blue), Die Oog (red) and Strawberry Lane (green) Cape Town, South Africa. Weight (W), snout-vent length (SVL), water temperature (WT) and ambient average sound pressure level of each locality (SPL) were used as explanatory environmental variables. The following species variables were used for advertisement calls: call duration (ACD), inter-call interval (AICI), pulse number (APN), dominant frequency (ADF), mean weighted frequency (AMF), first five pulse duration (AFP), and last five pulse duration (ALP).

Pulse number for both advertisement and release calls are strongly correlated (positively) with male SVL, weight and water temperature, though less so with water temperature (Figure 4.13 and 4.16). As expected, temperature-uncorrected call duration for advertisement and release calls were negatively correlated with ambient temperature. Surprisingly and although weak, mean weighted frequency for both calls was negatively correlated with both weight and SVL.

4.3.2.4 Chorus structure

Bout and inter-bout duration of choruses varied considerably with uninterrupted calling for almost 10 minutes (598 seconds) and some choruses could be as short as 34 seconds (Table 4.11). Choruses have a SPL range of 63–92.9 dB measured at a distance of five meters (Table 4.12). Figure 4.15 depicts the commencement of a chorus started by one male and followed haphazardly by more males, although when looking at the oscillogram, unison bout calling could be discerned.

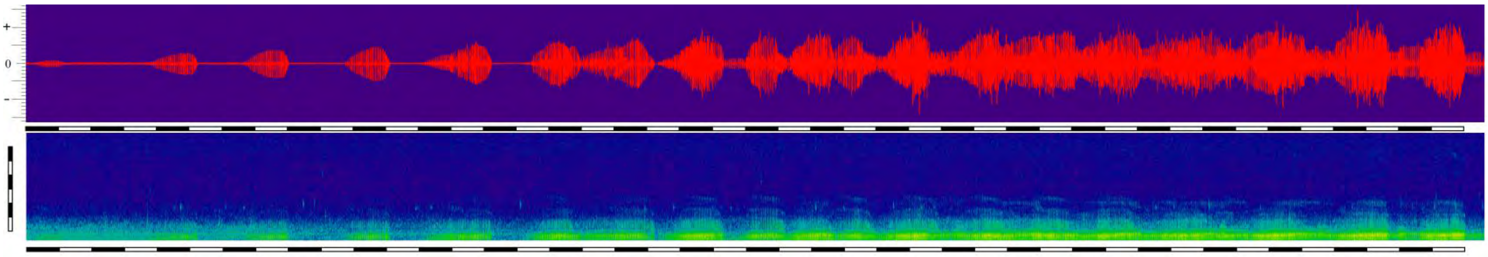


Figure 4.15 A spectrogram sample and associated oscillogram from a recording of a chorus of *Amietophrynus pantherinus* showing unison bout calling. Horizontal scale = 1 second; Vertical scale starts at 1 000 Hz and represents the same amount.

Table 4.11 Bout and inter-bout duration of choruses in four localities that were obtained from Song Meters.

	Raapkraal Road		Die Oog		Strawberry Lane	
	Bout duration (s)	Inter-bout duration (s)	Bout duration (s)	Inter-bout duration (s)	Bout duration (s)	Inter-bout duration (s)
Median	199.0	118.0	243.0	174.0	134.8	227.6
Range	34–589	18–325	95–598	37–933	58–306	119–341

Table 4.12 Sound pressure levels (dB) of choruses measured at Raapkraal Rd and Die Oog at a distance of approximately 5 meters. Raapkraal was measured twice. nd = no data.

Locality		Strawberry Lane	Raapkraal Rd 1	Raapkraal Rd 2	Die Oog
Chorus SPL (dB)	Median	nd	65.3	73.4	91.5
	Range	nd	63.9–66.1	72.4–78.4	85.1–92.9

4.4 Discussion

4.4.1 Effect of road traffic on *A. pantherinus*

Owing to its explosive breeding behaviour, *A. pantherinus* is easier to monitor than anurans with longer breeding seasons. I found that use of the drift fence system on both sides of the road successfully prevented 100% of the toads from crossing the road along that stretch. While the drift fencing was undoubtedly successful in preventing the toads accessing the road, these data do not account for the individuals that may have changed their migration behaviour (Arntzen *et al.* 1995; Baker 1999; Ortmann *et al.* 2006).

Methods to reduce road mortality of amphibians have been used since the early 1970s in European countries, mainly Hungary, Austria, Germany, Switzerland and the Netherlands (Schmidt & Zumbach 2008). Surprisingly, only 27 (24.1%) of the total recorded 112 *A. pantherinus* individuals were found on the western fence, returning to their aestivating destinations among urban gardens. Studies show that the capture efficiency of drift fences decreased over time for a number of amphibians and reptiles (Baker 1999; Crosswhite *et al.* 1999; Ortmann *et al.* 2006). More research on the migration behaviour of *A. pantherinus* is needed to determine where the toads migrate to after the breeding season, if they return to the same breeding locality each year and if obstructions along their way influence their migration behaviour (Ortmann *et al.* 2006). Furthermore, research on the minimum viable population is critical to the long-term persistence of populations (Simberloff 1994; Hanski *et al.* 1996; Rai 2003). Estimating population size of breeding adults is the first step in generating a population viability analysis (Rai 2003), which is needed for *A. pantherinus*. The technique draws conclusions from trends in population growth to predict increases, stability or decline to extinction caused by mutation accumulation (Higgins & Lynch 2001; Bulman *et al.* 2007). Higgins & Lynch (2001) shows that metapopulation structure, habitat loss or fragmentation, and environmental stochasticity (the variation in population growth rate that takes place because expected rates of survival and reproduction differ from one season to the next) can be expected to largely accelerate the build-up of mildly harmful mutations. This lowers the genetic effective size to such an amount that surprisingly large metapopulations may face risk of extinction. Even though in the case of *A. pantherinus* volunteers are helping to mitigate the effects of road mortality (coordinated by the South African National Biodiversity Institute), there is still a 17.6–27.2% mortality rate recorded annually in sections where only road patrols are conducted (Table 4.5; also see Zuiderwijk 1989; Bonardi *et al.* 2011).

4.4.2 Citizen science

The general public have increasingly formed part of the scientific arena over the last few years for a number of reasons, from community education to new opportunities for data collection (Maisonneuve *et al.* 2010; Kawrykow *et al.* 2012; Abolafya *et al.* 2013; Freitag & Pfeffer 2013; Wilson *et al.* 2013). However, standards of competency, ethics, assessment, subjectivity and oversight are major challenges that need to be addressed. This study not only demonstrates the possibility of how citizens can play an invaluable role in saving a species from local extinction, but also shows practical evidence of methods that can be used to overcome the above mentioned challenges in conservation and monitoring biology.

Studies on road mortality before and after installation of wildlife structures are sparse (Glista *et al.* 2009) and are therefore impractical to evaluate their efficiency. However, if a community willingly takes part in a citizen science project, where they can rescue an endangered species while enjoying the activity at the same time, one has a winning recipe that adds a new spin to science (see Chapter 7 on attitudes towards frogs). Citizen science has recently become popular among different disciplines and has been published in high impact journals such as *PLOS ONE* (see Maisonneuvea *et al.* 2010; Kawrykow *et al.* 2012; Surasinghe & Courter 2012; Jones *et al.* 2012; Tippins & Jensen 2012; Abolafya *et al.* 2013; Devisch & Veestraeten 2013; Freitag & Pfeffer 2013; Havens & Henderson 2013; Rosner 2013; Scripa & Moorefield-Lang 2013; Toerpe 2013).

4.4.3 The possibility of installing under-road passes

While drift fencing is much cheaper, tunnel systems are permanent and have been widely implemented across the globe from the 1980’s to reduce road death of a variety of animal species, with ACO Wildlife Company Limited being the leading supplying company of these systems (Langton 1989; Yanes *et al.* 1995; Dodd *et al.* 2004; Mata *et al.* 2007; Woltz *et al.* 2008; Glista *et al.* 2009). Thirty-two studies investigated the effectiveness of installing culverts or tunnels as road crossings for amphibians (Smith & Sutherland 2014). However, only a handful of studies have investigated the population-level effects of wildlife crossing structures (Van der Ree *et al.* 2011; Van der Grift *et al.* 2013). Maintenance of tunnel and fence systems has also been erratic, causing vegetation to completely take over and affect the use of the tunnels. One of the biggest problems with the tunnel systems is that the breeding timeframe is usually determined by sporadic events throughout the breeding season, which may be several months for some species.

Amietophrynus pantherinus is an explosive breeder and has a distinct winter-early spring breeding season of only a few weeks, which would enable maintenance teams to clear culverts before the breeding season commences. Tunnels or culverts have the added advantage in that they avoid handling of animals or crowding of animals in pitfall traps. Crowding of metamorphs in a confined space might provide further problems for cannibalistic species such as *Pyxicephalus adspersus*.

Installing culverts is not a viable solution in all cases. In some residential areas toads might have to cross several residential roads before getting to their intended breeding site. In these cases volunteers are very effective in removing the toads from the roads and directing them towards the breeding site. Nevertheless, for busy roads like Noordhoek Main Road and Silvermine Road (another main road bisecting Noordhoek) where the roads are elevated and the barriers will hardly be seen from the road, motivation for installing these structures is well supported. Traffic density is high which leads to high toad mortality and this in turn also jeopardises volunteer safety.

With the data provided for this study, public participation and awareness for *A. pantherinus* has increased steadily in suburbs in the Cape Peninsula over the last five years. Although the exact numbers of individuals that faced inevitable death cannot be calculated, assisting1860 toads across roads and recording 20.5% road mortality provides some perspective on the fate that these *A. pantherinus* populations would have faced without public intervention. No quantitative study has yet been conducted on juveniles that migrate *en masse* in the summer months. However, some studies proposed that fluctuation in terrestrial juvenile survival has the largest effect of all life history stages on amphibian population growth rate (Som *et al.* 2000; Vonesh & De la Cruz 2000; Biek *et al.* 2002). Subtle alterations in terrestrial juvenile survival could cause population declines. Biek *et al.* (2002) showed that a 15% reduction in terrestrial survival could reduce *Bufo boreas* population growth rate by 19% whereas a 46% reduction in embryo survival is necessary to lead to a comparable decline in population growth rate. Subtle changes are unlikely to be detected using count data that are not adjusted for the probability of detection (Schmidt 2003). Estimates of population parameters based on capture-recapture analyses are sufficiently sensitive (e.g. Fujiwara & Caswell 2001) such that I can predict population declines and conservation action can hopefully reverse the negative trends (Schmidt 2003). Remote sensing techniques such as PIT-tagging (Arntzen *et al.* 2004) and motion camera monitoring (Pugnucco *et al.* 2011) are often easier and more effective within amphibian culverts since tunnels will guide frogs to a common entrance and exit points.

Before construction of permanent systems, decisions should be guided taking the following into account: long term objectives of installing a crossing system in the landscape, habitat type/fauna/soils, relationship with and shared function amongst all planned bridges/purpose built eco-passages, existing or planned drainage/water management options, locations in respect of major crossing opportunities (mainly bridges), road size, number of lanes etc., traffic volume (both existing and predicted), ground conditions and water level fluctuation including extremes, monitoring capacity and maintenance capacity. Roads in 250 m, 500 m and 1000 m proximities were identified in the results of this study. These roads can serve as important areas for volunteers to focus man-power and for guidance of potential future installations of toad tunnels along roads with the highest impact.

4.4.4 Dominant frequency and mean weighted frequency

This is the first known study to report the use of mean weighted frequency (MWF) to describe call spectral properties. Most literature uses dominant frequency (DF) for standardisation when analysing animal calls (Parris *et al.* 2009; Bastos *et al.* 2011). However, Wildlife Acoustics makes use of a calculation to determine the MWF in their sound recognition and analysis software, Song Scope. Song Scope calculates the MWF as follows: given a time series of Fast Fourier Transforms (FFTs), the frequency center of each bin is taken, multiplied by the absolute power level of energy in that bin, and divided by the total of power levels. Thus, Song Scope takes all sounds in the selected frequency into account when calculation the MWF. Therefore, the use of MWF in call descriptions and analysis has its limitations.

Male frogs typically call in large choruses and it is sometimes unavoidable to include distant calls from conspecifics or background noise from other sources. If there is no clear recording, Song Scope will detect nearby males or other noise and this will influence the measurement to some extent. However, MWF does give a more comprehensive idea on the distribution of frequencies expressing the highest amplitude.

4.4.5 Advertisement and release calls description and variation

Amietophrynus pantherinus produces a pulse train of approximately 31 pulses that end at a higher pace than they start. Our multi-level study of within- and among-male and inter-population variation in the advertisement and release call structure resulted in several important and interesting outcomes. I discovered contrasting patterns of variation inter-population in some call properties. Call properties were also categorised as static, intermediate and dynamic as described by Gerhardt & Huber (2002) and Smith & Hunter (2005).

Temperature can influence pulse rate and call duration, although in this comparison of the populations, the difference in temperature was maximum (5.9 °C) and call duration and body size were temperature-corrected (Platz & Forester 1988). Regardless, temperature-induced effects on the acoustical properties of calls are most likely comparatively small in relationship to taxonomical or ecological influences (Hoskin *et al.* 2009; Gingras *et al.* 2013). Furthermore, DF has not often been shown to be affected by temperature (Gerhardt & Huber 2002).

Passmore (1976) was the first to analyse and describe the call properties of *A. pardalis* (Table 4.12), a species thought to be synonymous to *A. pantherinus* until 1998 when Poynton & Lambiris (1998) elevated them to two full species (see section 4.1.4). Passmore (1976) subsequently gave a brief review on the phonology used to describe the advertisement call of *A. pantherinus*. The first advertisement call for *A. pardalis* was depicted by Ranger (in Hewitt, 1935) as “a loud snore - a drawn out sound”. Poynton (1964) notes that the “snore” *of A. pardalis* sounds similar to the call of *A. gutturalis*. The call has also been expressed as a “ghurr ghurr” sound by Hewitt (1937) and Wager (1965) observed that they produce short, soft grunts audible only a few feet away. As Passmore (1976) noted, Wager might have referred to the release call. Passmore (1976) also noted that the main characteristics of the call of *A. pardalis* are the long call duration and slow pulse rate (Table 4.14). Because of the close relatedness to *A. pantherinus* and a lack of incorporating call properties to supplement the motivation for the separation of the species (Poynton & Lambiris 1998), it is important to focus on the differences of their calls.

Table 4.13 Call properties of the advertisement call of *Amietophrynus pardalis* from Passmore (1976) in comparison with *A. pantherinus* in this study. DF = dominant frequency.

		Species	
		A. pardalis (n=11)	*A. pantherinus* (n=30)
DF	Range (Hz)	750–1300	597.2–852.5
Call duration	Mean (ms)	1410	1198
	Range (ms)	1060–1610	1006–1461
Call pulse rate	Mean (call/s)	21.71	26.1
	Range (call/s)	18.0–25.2	23.6–26.6
Pulse number	Mean	30	31.3
	Range	24–35	23.7–38.8
Ambient temperature C		15	10.5–16.4

Advertisement calls have been found to be useful in separating closely related species (Porter 1964; Forti *et al.* 2012; Gingras *et al.* 2013; Minter *et al.* 2004) and is a particularly valuable taxonomic aid when dealing with closely related species groups such as *A. pantherinus* and *A. pardalis* where distinction by morphological means alone is difficult. As a consequence of the recognition of full species status of *A. pantherinus*, it has also now been classified as Endangered (Poynton & Lambiris 1998; Harrison *et al.* 2001; De Villiers 2004). Comparing the call property data generated by Passmore (1976) on *A. pardalis* to this study, the advertisement call of *A. pantherinus* is produced at a lower pitch (approximately 300Hz difference), has a shorter “snore” (as described by most authors for *A. pardalis*; Hewitt 1937; Poynton 1964; Wager 1965; Passmore 1976) with a faster pulse rate, but approximately the same number of pulses per call (Table 4.13). The data is comparable without temperature correction necessary for duration, due to the similar ambient temperature in which both the studies were conducted. Data presented herein thus support *A. pantherinus* as valid species.

One central outcome is that a number of call properties vary greatly within individuals (dynamic properties) where as others vary little (static properties; Gerhardt 1991). Based on coefficients of variation (CV), dominant frequency of both advertisement and release calls showed less variability within and among males ($CV \leq 3.2\%$ and $CV \leq 7.8\%$ respectively) compared with temporal properties ($6.3\% < CVs < 118.0\%$) within males. A study on 91 populations including 41 species representing four families revealed that CV for pulse rate, call length and call rate typically exceed 20% (Gerhardt 1991). Therefore, variation in these dynamic properties is too common to be assigned to a noise-related source.

Castellano *et al.* (2002) argued that call properties under morphological or physiological restrictions will be likely to be more static while properties under energetic constraints or social selection pressure are often more variable. However, some highly static properties have not proven to be under morphological limitations and so control factors other than body size appear to influence these properties (Gerhardt & Huber 2002; Smith & Hunter 2005).

Both females and males use the spectral and temporal properties of calls to assess conspecifics during breeding, which may drive sexual selection in some species (Wells 1977; Sullivan *et al.* 1995; Giacoma & Castellano 2001; Ovaska & Rand 2001; Gerhardt & Huber 2002; Wells 2007). Females are known to choose mates on the basis of their call properties such as frequency, pulse rate, amplitude, call rate, or call duration (Sullivan 1983; Ovaska & Rand 2001; Gerhardt & Huber 2002). Parris *et al.* (2009) showed that *Litoria ewingii* produce calls with a higher pitch in the presence of traffic noise as opposed to the absence thereof. *Physalaemus pustulosus* females select larger males on the basis of their fundamental frequency (Ryan 2001). Therefore, if females choose mates on the basis of a lower pitch that signifies overall fitness (i.e. older, larger males that call at lower frequencies *post-facto* demonstrates ability to survive), traffic noise might affect males calling at higher frequencies adversely. However, some studies show a negative correlation for body size (i.e. SVL and weight) to call frequency (Bastos *et al.* 2011; present study). Consequently, larger males will have an advantage over low-frequency traffic noise.

This study found significant variation in dynamic call properties of *A. pantherinus* among males and between geographically separated populations (Table 4.10). Although an explosive breeder, the high male:female operational sex ratios indicates that female mate choice could very possibly exist in this species (Cherry 1989; Cherry & Francillon-Vieillot 1992; Cairo *et al.* 2013; present study). Cherry & Francillon-Vieillot (1992) found no evidence for correlations between male mating success and snout-vent length, age or weight, nor of female preference to low frequency calls for *A. pantherinus* (Cherry 1989). In this study I demonstrated that larger males produce higher frequency calls, suggesting that it is possible for smaller males to advertise their size information in their advertisement calls. Females will only breed when they have reached a critical size and males will breed as soon as they have reached reproductive maturity (Cherry & Francillon-Vieillot 1992). They also stated that there was no sexual selection for male body size in *A. pantherinus* and that the correlations that are shown between body weight and size may indicate redundancy. However, this remains speculative.

It is known that toads occasionally return to breed where they originally metamorphosed (Bogert 1947; Jameson 1957; reviewed in Sinsch 1990; Breden 1987; Reading *et al.* 1991; Waldman & McKinnon 1993), even when the pond was altered and areas paved over (Heusser 1969). Thus, anurans that return to their natural ponds must employ methods via morphological characteristics or behavioural tendencies to avoid interbreeding with closely related individuals (Waldman 2001). One such method is via acoustic signalling (Waldman 1987). Cherry (1989), however, noted that individuals of *A. pantherinus* did not return to the same pond in three consecutive years. Whether this was just a case of coincidence and the individuals fell prey to predators or got run over by vehicles, or whether it was a behavioural trait, was undetermined.

The relatively low number of males recorded (three) at the Strawberry Lane locality may not permit robust statistical inferences from this site. However, Levene's test for equality of variance requires a minimum of three observations. Also, all the males had 10 calls analysed, which contributes to the significance of the observations (Levene 1960; Lewontin 1966; Down 1976; Hallgrimsson & Hall 2005; Gastwirth *et al.* 2010).

4.4.6 Chorus behaviour

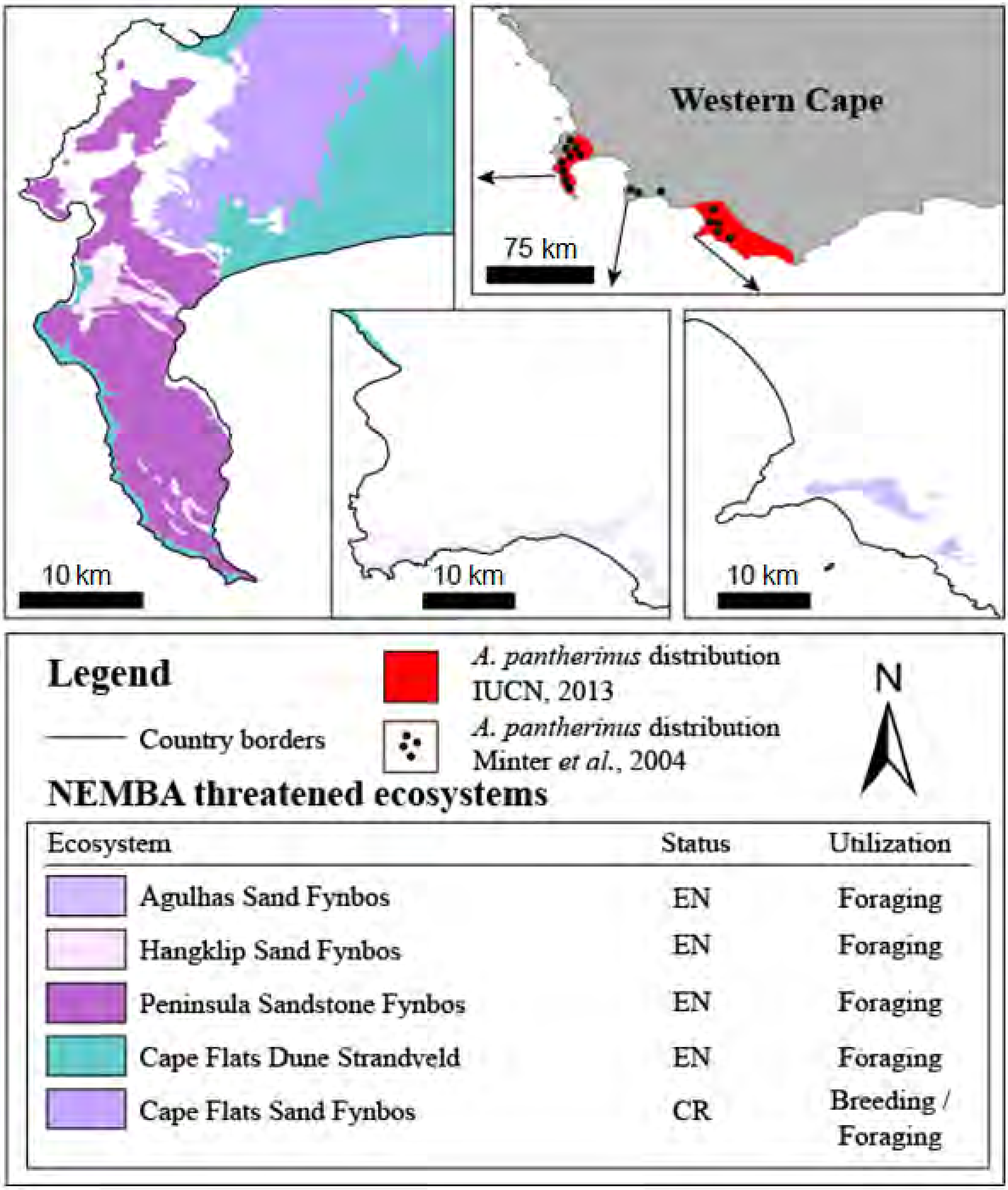
Amietophrynus pantherinus exhibits unison bout calling. Calling in a chorus confuses predators by preventing them from focussing on one individual (Wells 2007). Males start calling when other males call, simply because they have to compete for the attention of females (Brooke *et al.* 2000; Schwartz 2001). Unison bout calling also has its disadvantages in that high levels of background noise and acoustic interference is generated (Wells 2007).

4.4.7 Ecological advantages for conserving the species

The concept of a holistic approach to biodiversity conservation has been welcomed by many authors (Weber & Schmid 1995; Auyang 1999; Bekoff 2000; Maass 2008; Ibisch *et al.* 2010). Holism in systems biology (opposed to reductionism) states that natural systems (physical, biological, chemical, social, economic, mental, linguistic, etc.) and properties of the whole can't be explained by the properties of its parts (Mazzocchi 2012). Therefore, in a holistic view, in order to conserve species, the basic needs for the entire ecosystem must be met. Not all species enjoy the same level of conservation action due to unattractiveness and a history of misconceived folklore or perceptions (Czech *et al.* 1998; Ceríaco 2012). Accordingly, many species have acted as umbrella species to help conserve other sympatric threatened species and habitats. Because of the attractiveness, size and curious migration behaviour, *A. pantherinus* has elicited a major public engagement for conserving the species. This can help conserve sympatric species that are red listed and National Environmental Management: Biodiversity Act threatened ecosystems (Figure 4.14; Table 4.14). The charismatic appearance of *A. pantherinus* and its threatened status may permit inclusion on the CITES list to protect the species against possible illicit trade (Rebelo *et al.* submitted). However, Rebelo *et al.* (submitted) also states that because *A. pantherinus* breeds easily and the large number of toadlets that migrate *en masse* would result in easy population introduction in large numbers of hand-reared specimens to protected localities, thus conflicting its scarcity value. Even so, *A. pantherinus* has an advantage in sharing habitat with other threatened species. As an umbrella species it can support protection of six species (1 mammal and 5 amphibians) within the City of Cape Town (Table 4.14). In addition, it will serve to highlight the conservation of NEMBA threatened ecosystems (Table 4.14; Figure 4.16).

Table 4.14 A list of threatened species that overlap in distribution with *A. pantherinus* to indicate its viability as an umbrella species. VU = Vulnerable; NT = Near Threatened; EN = Endangered; CR = Critically Endangered.

Taxon	Common Name	Status	Population trend
Mammalia			
Amblysomus corriae	Fynbos Golden Mole	NT	Unknown
Amphibia			
Breviceps gibbosus	Cape Rain Frog	NT	Unknown
Cacosternum capense	Cape Caco	NT	Unknown
Capensibufo rosei	Rose's Mountain Toadlet	VU	Unknown
Microbatrachella capensis	Micro Frog	CR	Decreasing
Xenopus gilli	Cape Platanna	EN	Unknown



Ecosystem	Status	Utilization
Agulhas Sand Fynbos	EN	Foraging
Hangklip Sand Fynbos	EN	Foraging
Peninsula Sandstone Fynbos	EN	Foraging
Cape Flats Dune Strandveld	EN	Foraging
Cape Flats Sand Fynbos	CR	Breeding / Foraging

Figure 4.16 Map of NEMBA (2009) listed threatened ecosystems where *A. pantherinus* distribution overlaps. Map created with ArcGIS using vegetation data from South African National Biodiversity Institute. EN = Endangered; CR = Critically endangered. The extent of population occurrences are derived from Minter *et al.* (2004) (solid circles), but do not indicate actual populations; and a shapefile (distribution demarcated in red) obtained from IUCN (2013). Populations between Hangklip and Hermanus remain unconfirmed (Measey 2011).

CHAPTER FIVE

Hitting the high notes–the extraordinary vocal repertoire of the Common River Frog (*Amietia queckettii*)

The most important thing in communication is hearing what isn't said.

- Peter Drucker

5.1 Introduction

5.1.1 Call repertoire of frogs

With the exception of a few frog species most anuran vocalisations are stereotyped and have a relatively straightforward acoustic structure (Wells 1988; Narins *et al.* 2000; Feng *et al*. 2002). For the majority of species variation within a species usually comprise only of slight modifications of the signature advertisement call, for example consisting of a single note, a series of identical repeated notes, or a long trill (Hauser 1996; Bradbury & Vehrencamp 1998; Ryan 2001; Fitch *et al.* 2002; Walkowiak 2006; Wells & Schwartz 2006). As scientists studied anuran acoustics, modifications to the original call terminology described by Bogert (1960) were inevitable. For example, Bogert (1960) coined the term "mating call" to describe the primary signals produced by males during the breeding season. However, these calls are now generally known as the advertisement calls (Littlejohn 1977; Wells 1977; Ryan 2001; Wells 2007). Calls can be classified either from a behavioural perspective namely advertisement calls, courtship calls, aggression calls (including close-range and long-distance territorial calls), distress calls, warning calls and release calls (Walkowiak 2006), or according to their physical properties such as trills, chuckle calls, rapping, creak calls, pulsating or tone-like calls (Narins *et al.* 2000; Wells & Schwartz 2006).

Apart from the primary function of attracting females to a breeding site, anuran advertisement calls can serve a variety of functions, including species identity, sexual receptivity, position, size and in some cases, the individual identity of males in a chorus (Gerhardt & Huber 2002; Wells & Schwartz 2006). In some species where males call over extended breeding periods, advertisement calls can promote hormone production and reproduction condition in females (Lea *et al.* 2001). In addition, advertisement calls provide information about the relative position and spatial proximity to other males and facilitate spacing between calling individuals by means of call intensity (Brenowitz *et al.* 1984; Wilczynski & Brenowitz 1988; Brenowitz 1989; Gerhardt *et al.* 1989; Wells & Schwartz 2006).

5.1.2 High frequency harmonics

Anuran call frequencies rarely exceed 6,000 Hz (Ryan 2001; Wells 2007; Du Preez & Carruthers 2009). Among the anurans, only two species, *Odorrana tormota* and *Huia cavitympanum*, are known to produce calls in the ultrasonic (US) range (Feng *et al.* 2006; Arch *et al.* 2008, 2009). These species are also morphologically adapted to hear ultrasound, having deeply sunken tympana and ear canals as a resonator (Feng *et al.* 2006), which have been proposed to play a critical role for ultrasonic hearing. There are also sexual differences in the audiograms of males and females of *O. tormota* and other sympatric species within this genus (Liu *et al.* 2014), suggesting that communication in the ultrasonic range is exclusively used by males.

5.1.3 Study species

Despite the widespread distribution, abundance in numbers, and extended breeding season of *Amietia quecketti*, there is surprisingly limited information on the natural history and vocal behaviour of this species. *Amietia quecketti* calls most actively in autumn and early spring (Chapter 6). Such breeding behaviour can be compared to those of capital breeders, which breed earlier than sympatric species to avoid resource competition (see Chapter 4). This species has a wide range of habitat preferences, including pools, ponds, dams, lakes, and the banks of slow-flowing streams and rivers. They also show no preference to whether wetlands are man-made formal ornamental ponds or natural (Du Preez & Carruthers 2009; Chapter 2).

Amietia quecketti inhabits the forest, grassland, savanna and agricultural areas that are associated with permanent water (Harper *et al.* 2010; Chapter 2). The species endures habitat disturbance and is regularly associated with human residency. Some habitat descriptions by Measey *et al.* (2009) and Harper *et al.* (2010) were described for *A. angolensis* that is part of the recently split *A. angolensis*-complex (see *Taxonomic note*).

Males tend to have preferred calling sites underneath overhanging structures such as vegetation, rocks or man-made constructions and usually will stay within four meters from this spot (Viviers 2013). Although this suggests strong territorial behaviour, males will select several such territories over the course of the breeding season. Males also exhibit aggressive behaviour, for example, attacking a cell phone speaker during playback of conspecific calls (pers. obs.). The present study focussed on the extraordinary vocal behaviour of this common urban garden frog and argues that its unique vocal properties contribute to its success of survival in an increasingly noisy urban environment with high levels of traffic and other anthropogenic noise.

5.1.4 Calling and social behaviour of *Amietia quecketti*

Other than call descriptions in frog field guides, the call of *A. quecketti* has received little attention. Passmore & Carruthers (1979) described the biphasic advertisement call as two different calls that are "commonly heard". The click-note is defined as a "sharp rattle" preceding a "short croak". Passmore & Carruthers (1979) also noted that these two calls are often sequential, although not always. Wager (1986) phonetically termed click-notes "*kik- kik- kik*" or "*cluck-cluck-cluck*" that are produced at slow intervals and the whine-notes he described as a "melodiously and mournfully, drawn-out "*keraak-owwwwww*". Du Preez (1996) stated that two different calls are produced–an extended "*kūâârk*" or "*kūâââ*" and a rapid series of "*kik*" or "*klack*" sounds. Channing (2001) described the biphasic call more comprehensively, noting the two-part call consisting of 6–7 frequency modulated clicks, followed by a short croak. The average duration of the two-part call was 0.35 s and 0.54 s for the click-note and croak respectively. Channing additionally described that the click-note dominant frequency rose from 2.7–2.9 kHz and that the dominant frequency for the croak was 0.8 kHz. Du Preez & Carruthers (2009) described the call as a rapid series of six or seven short "*kik-kik-kik-kik*" clicks, followed by a short "*keroip*" croak.

There is very little known about the calling and social behaviour of *A. quecketti*. Wager (1986) and Du Preez & Carruthers (2009) noted that males are known to call throughout the day and often at night, throughout the year, including mid-winter. Wager (1986) also stated that males are often found beneath a protruding rock near the edge of the water, whereas Du Preez & Carruthers (2009) added that males are also found calling from both partially concealed and exposed sites. Wager (1986) reported that he came across an amplecting pair around 10:00 am on a sunny day, pestered by three continuously calling males. Furthermore, Wager (1986) observed that "thousands of eggs" are laid at a time in shallow water, often in an eddy of a swiftly-flowing stream, while Channing (2001) noted that this species is found in most permanent water bodies within its range. These anecdotal observations made by Wager (1986) underlines the little-known, rich social behaviour of this species.

5.1.5 Taxonomic note

The southern African populations of the *Amietia angolensis* and *A. fuscigula* clades have recently undergone taxonomic revision (Channing & Baptista 2013). The revision was based on genetic analysis and advertisement call comparisons. The species *A. angolensis* was split into two geographically separate groups, namely *A. angolensis* and *A. quecketti*. Previously, *A. dracomontana* (Channing 1978) was identified on a longer, slower call than "*A. angolensis*" (now *A. quecketti*), and also minor differences in body proportions. According to Channing & Baptista (2013), *A. dracomontana* has an advertisement call closely related to the call of *A. quecketti* and showed little genetic difference and consequently they assigned it as a junior synonym of *A. quecketti*. Channing & Baptista (2013) used “emphasised frequency” (among seven other temporal call characteristics) to determine the spectral differences in species advertisement calls, assuming it describes the zone on a spectrogram where most energy is concentrated, which is similar to mean weighted frequency (see Chapter 4). They described the biphasic call of *Amietia* spp. as “an initial long series of clicks forming a pulse train, and a second short phase made up of rapid pulses”. Channing & Baptista (2013) only measured the emphasised frequency of the first phase (click-note). They found first phase advertisement call differences between *A. angolensis* and *A. quecketti* to differ with a mean of 182.2 Hz ($\bar{X}$ = 1465.2, range 962–1894, N = 13; $\bar{X}$ = 1647.4, range 780–2153, N = 5 respectively).

5.1.6 Study objective

Amietia quecketti demonstrated to be a habitat generalist and to successfully breed in and around cities (Chapter 2). The main objective of this study was to evaluate the frequency composition of the advertisement call of *A. quecketti* in order to provide an additional argument for the success of this species across a wide habitat range observed in a noisy, acoustically altered environment. In addition to frequency characteristics, it is argued that the success of *A. quecketti* in urban environments is partially attributable to its extensive repertoire and unusual call properties. This study also provides evidence that suggest that harmonics of some calls extend into the ultrasonic range (≥20kHz).

5.2 Materials and Methods

5.2.1 Advertisement call recording and description

Many authors of various field guides used different terms to describe the call of *A. quecketti* as summarised in section 5.1.4. For consistency, I will use the term “click-note” to describe the first, rapid clicking phase (“kik-kik-kik”) of the two-part advertisement call. For the second croak-like call note I will use the term “whine-note”. A third call not previously recognised in literature, is the aggression call, which the term “iambic-note” after Narins *et al.* (2000) will be used.

An automated recorder (Song Meter SM2, Wildlife Acoustics) was set up in the North-West University Botanical Gardens, Potchefstroom, South Africa (Chapter 6). The Song Meter was set to record at a 16-bit sample rate (16 kHz), which allowed recording a frequency band of only 8 kHz. Calls recorded in a continuous, passive manner enabled us to capture call types that could easily have been missed in comparison to actively recording (i.e. in person) a few minutes per individual. In addition to the Song Meter recordings, three individuals were recorded with a Nagra ARES-ML digital recorder equipped with a Sennheiser ME66 directional microphone for acoustic analyses (44.1 kHz, 16 bit). Each male recorded was captured directly after the recording, weighed with a Pesola scale (60 g limit) and measured using a Vernier calliper. Frogs were also individually marked by injecting a “pit-tag” micro-transponder subcutaneously on the dorsum to avoid duplicate recordings of males. Ambient and water temperature were taken with each measurement.

Five consecutive calls for each of three males were analysed using two spectral properties (dominant frequency, DF; mean weighted frequency or emphasised frequency, MWF) and five temporal properties (call duration; pulse number; inter-pulse interval; inter-click-whine interval; inter-call interval). See Table 5.1 for a full description of all properties measured. Mean weighted frequencies (or emphasised frequency) are compared to one another on account of the recent taxonomic review (Channing & Baptista 2013) to evaluate the viability of using MWF for call description purposes. Mean weighted frequency was calculated using Song Scope software (version 4.1.3A, Wildlife Acoustics). Dominant frequency (DF) was obtained from power spectrums compiled in BatSound Sound Analysis (version 4.1.4, Pettersson Electronics and Acoustics). The ASCII values of the power spectrum of each call were copied to Microsoft Excel (2010) in order to calculate the dominant frequency at the highest amplitude instance. All temporal properties were analysed using Song Scope (W). Spectrograms and associated oscillograms were compiled using Bat Sound, Song Scope and Adobe Soundbooth (CS5). Sound pressure levels (SPLs) were recorded (dB intervals, C-weighting) using a calibrated Brüel and Kjaer (calibration number: 2013-0285) precision sound-level meter (Model 2235) and microphone (B & K 4176). Distance from microphone to the tip of recorded males' snout was kept constant at closely 300 mm, measured with a wire extended from the device.

Table 5.1 Description of the call properties measured for the biphasic advertisement call and aggression call of *Amietia quecketti.*

Call property measured (unit)	Description
Biphasic advertisement call	
Call type	Call type was assigned on the basis of whether the male produced a click-note (1), whine-note (2) or a combination of the two (3).
Call note duration (ms)	Duration from the onset of the first pulse to the offset of the last pulse in a single call. Measured for both click- and whine notes.
Pulse number	The amount of pulses in a single call note.
Inter-pulse interval (ms)	Measured between the second and third click-pulse.
Mean Weighted Frequency (Hz)	Also described as "emphasised frequency". Mean frequency at which the call is at greatest intensity. Measured for both click- and whine notes.
Dominant frequency (Hz)	Frequency at which amplitude is the highest. Measured for both click- and whine notes.
SPL (dB)	Sound pressure level is the force of sound on a surface area perpendicular to the direction of the sound at a given distance; measured in decibels, 300 mm from the snout of the subject. Measured for both click- and whine notes.
Inter-click-whine period (ms)	When both notes in the biphasic call are present; period between the two call note types.
Inter-call Interval (ms)	The interval between two call notes. Whether only click-notes, whine-notes or a combination of the two call notes were produced.
Aggression call	
Call duration (ms)	Duration from the onset of the first pulse to the offset of the last pulse in a single call.
Pulse number	All pulses counted, including narrowband FM pulse and tone-like pulse.
Narrowband FM pulse duration (ms)	Duration of FM narrowband pulse.
Narrowband FM pulse position	Position relative to pulse number, i.e. occurring at nth pulse.
Tone-like pulse duration (ms)	Duration of tone-like narrowband pulse.

Tone-like pulse position	Position relative to pulse number, i.e. occurring at n^{th} pulse.
Dominant frequency (Hz)	Whole note; same above.
Mean weighted frequency (Hz)	Whole note; same as above.

5.2.2 Ultrasonic recording

To assess the extent of the frequency spectrum of the advertisement call of *A. queckettii*, one male was recorded with an ultrasonic detector (Pettersson D1000X, Pettersson Electronics and Acoustics) with a frequency range of 5–235 kHz. Ambient temperature at the time of recording (2013-09-10, 23:49) measured 11.7 °C and water temperature 16.0 °C.

5.2.3 Coefficients of Variation

Coefficient of variation (CV) allows for meaningful comparisons between two or more magnitudes of variation, even if they are different means or different scales of measurement (Lewontin 1966; Hallgrimsson & Hall 2005). Coefficient of variation was calculated for each individual call property to examine within and between-male variation in call properties using:

$$CV = \frac{SD}{\bar{X}} * 100$$

where SD is the standard deviation and $\bar{X}$ the mean, the result is then multiplied by 100 to obtain a percentage value.

The within-male CVs were used to characterize the variability of each call property as static or dynamic. Within-male CVs ≤5% were classified as static, ≥12% as dynamic, and 5%<CV<12% as intermediate (Gerhardt 1991; Smith & Hunter 2005). I also calculated between-male CVs. All statistical analyses were performed in Microsoft Excel (Microsoft Office 2010).

5.3 Results

5.3.1 Call description

Amietia quecketti has a two-part call consisting of a click-note of 4–6 pulses ($\bar{X}$ = 5.1), followed by an intricately structured whine-note (Figure 5.1). The two spectral properties MWF and DF varied considerably between both parts of the call. Mean weighted frequency of click-notes varied from 1482.8–2568.4 kHz ($\bar{X}$ = 2206.4, SD = 276.26, n=3) and DF from 703.0–2109.0 kHz ($\bar{X}$ = 1753.9, SD = 325.03, n=3). For whine-notes MWF ranged from 1034.7–1352.0 kHz ($\bar{X}$ = 1236.4, SD =102.42) and DF from 703.0–1500.0 kHz ($\bar{X}$ = 1054.7, SD = 325.03) (see Table 5.2 for descriptive statistics of all analysed call properties). The two parts are typically sequential (60% of the time), but either call can also be separately produced (Figure 5.2). Call SPLs averaged 82.9 dB and 74.3 dB for click-notes and whine-notes respectively.

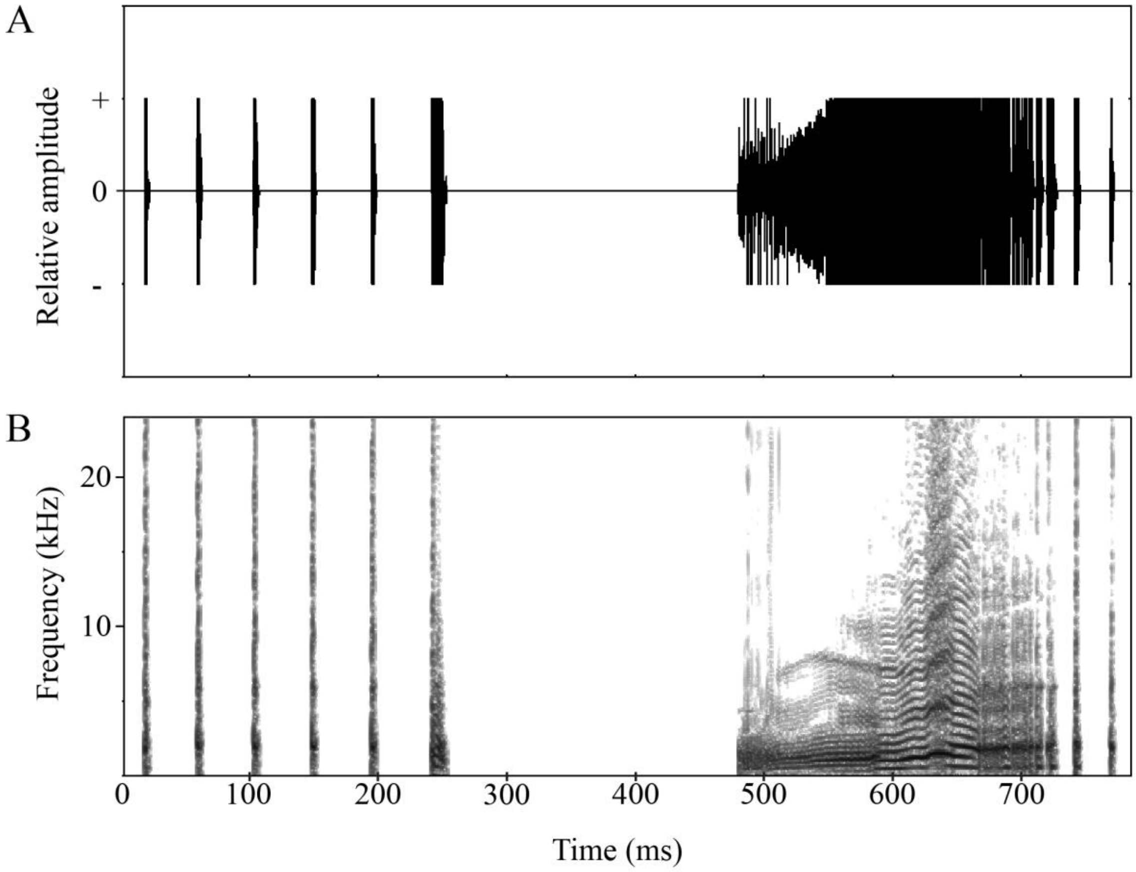


Figure 5.1 An oscillogram (A) and associated spectrogram (B) of an example of the two-part call of *Amietia quecketti*.

Table 5.2 Variation of the biphasic advertisement call properties of *Amietia quecketti*, n=3.

Note type	Call property	SD	Mean min.–max.
Click-note	Call duration (ms)	59.60	347.6 253.0–444.0
	Pulse number	0.67	5.1 4.0–7.0
	Inter-pulse interval (ms)	3.28	70.3 65.0–76.0
	Mean Weighted Frequency (Hz)	276.26	2206.4 1482.8–2568.4
	Dominant frequency (Hz)	375.74	1753.9 703.0–2109.0
	SPL (dB)	2.27	82.9 78.7–87.3
	Inter-click-whine interval (ms)	92.67	305.7 127.0–463.0
Whine-note	Call duration (ms)	222.66	753.8 499.0–1147.0
	Mean Weighted Frequency (Hz)	102.42	1236.4 1034.7–1352.0
	Dominant frequency (Hz)	325.03	1054.7 703.0–1500.0
	SPL (dB)	2.25	74.3 70.6–77.6
	Inter-call Interval (s)	31.45	17.0 2.6–123.7

Table 5.3 Mean-based coefficient of variation of the biphasic advertisement call properties within- and between-males of *Amietia quecketti*. Classification of static, intermediate and dynamic call properties is based on within-male averages, n=3.

Note type	Call property	Coefficient of variation (%)		Classification
		Within-male	Between-male	
Click-note	Call duration (ms)	16.3 10.2–20.9	17.1	Dynamic
	Pulse number	12.8 9.3–16.1	13.2	Dynamic
	Inter-pulse interval (ms)	4.2 2.5–6.9	4.7	Static
	Mean Weighted Frequency (Hz)	12.8 5.2–25.4	12.5	Dynamic
	Dominant frequency (Hz)	24.6 4.3–62.9	21.4	Dynamic
	SPL (dB)	2.4 2.4–2.4	2.7	Static

	Inter-click-whine interval (ms)	20.3 1.4–30.7	30.3	Dynamic
Whine-note	Call duration (ms)	6.6 1.1–13.7	29.5	Intermediate
	Mean Weighted Frequency (Hz)	6.2 4.1–8.2	8.3	Intermediate
	Dominant frequency (Hz)	20.0 4.6–33.6	30.8	Dynamic
	SPL (dB)	2.0 0.9–3.0	3.0	Static
	Inter-call Interval (s)	75.5 32.4–152.4	184.8	Dynamic

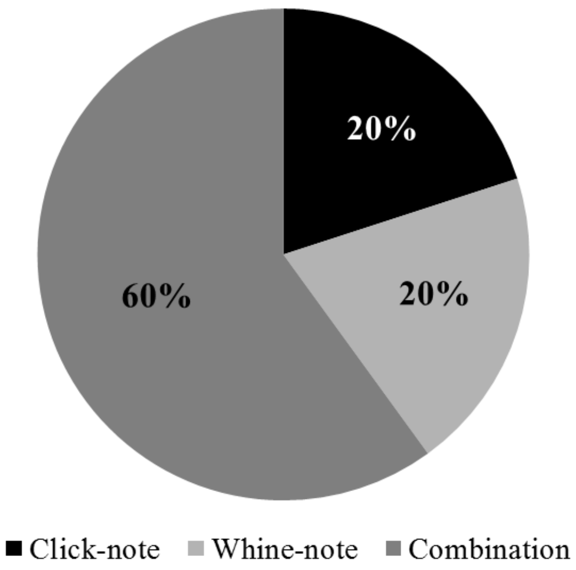


Figure 5.2 Relative incidence of the occurrence of each phase of the biphasic advertisement call, n=3.

Some temporal properties showed more within-male variation than spectral properties (Table 5.3). Call note duration for instance varied from 253.0–444.0 ms for click-notes, having 4–7 pulses per note with a relatively consistent inter-pulse interval that ranged from 65.0–76.0 ms. Concurrently, call duration of whine-notes ranged from 499.0–1147.0 ms. Inter-click-whine interval ranged from 127.0–463.0 ms ($\bar{X}$ = 305.7, SD = 92.67) and call rate fluctuated between one call every 2.6–123.7 s ($\bar{X}$ = 17.0, SD = 31.45).

Surprisingly, the only call properties that were classified as static were inter-pulse-interval and SPLs of click- and whine notes. Both MWF and DF were dynamic, and even more unexpectedly, DF showed a higher CV than MWF (Table 5.3), which contradicts other studies (Gerhardt & Huber, 2002).

5.3.2 Call repertoire

The calls of *A. quecketti* included an unusually broad range of sounds with extreme variation in spectral structure, beyond the two previously recognised call parts. Calls were defined to be click-notes (Figure 5.3), tonal (Figure 5.4), whine-notes (Figures 5.4–5.9), creak-notes (Figure 5.10A), iambic-tonal-combination notes (Figure 5.10B), pulsatile- / rip-notes (Figure 5.10 C) and aggression iambic-notes (Figure 5.11a-j).

5.3.2.1 Advertisement click-note

Click-notes are produced in pulse groups of 4–7 (usually 5 or 6) broadband pulses. Short pulses in general tend to be broadband (Peter Narins pers. comm.). All pulses are roughly similar in frequency characteristics, except for the last pulse having a longer duration that is produced at random (only one example of such an occurrence; Figure 5.3).

5.3.2.2 Advertisement whine-note

Whine-notes were highly variable, as demonstrated by the recorded signals from two males (Figures 5.4, 5.5, 5.8–5.10). No single whine note was found to be exactly the same as any other note. Whine-notes that were also described as croaks, a drawn-out "*keraak-owwwwww*" and "*keroip*" croak by other authors, exhibited a wide range of frequency characteristics and were subsequently divided into unique notes:

- *Tonal-note*: whine-notes with a narrowband tonal frequency. Many subharmonics can be distinguished, each a doubling of the fundamental frequency of the call (Figures 5.4a, c). Variation within the frequency structure includes shallow, rapid frequency modulation (FM) nearing the end of the tone (Figure 5.4d), as well as additions of a range of iambic pulses (Figures 5.4b, d-f; see section on aggression iambic notes below). Dominant frequencies (same as fundamental frequency in this case) of tonal whine-notes ranged from 591–676 Hz ($\bar{X}$ = 631.7, SD = 31.59, N = 9) and call duration varied between 387–776 ms ($\bar{X}$ = 658.1, SD = 117.74, N = 9).

- *Creak-notes*: Notes had a very high pulse-rate of 130–170 pulses/second. Dominant frequency was similar to that of the rip-note and measured 1420 Hz. Only one note was recorded (Figure 5.7A).

- *Pulsatile- / rip-note*: Notes start off with a high pulse-rate (90 pulses/second) up to 477 ms into the call, finishing the remainder of the call with a slower pulse-rate (40 pulses/second). Dominant frequency measured 1410 Hz. Only one note was recorded (Figure 5.7C). A variation of this call is seen in Figure 5.6j, where pulses on the ends are slower than those in the centre.

- *Whine-notes*: This call type dominated recordings. Extensive variation was demonstrated within whine-notes and therefore an attempt has been made to subsequently divide whine notes into phases, each displaying unique spectral characteristics. Although not unequivocally defined, an example of one selected whine-note could be divided into nine phases (Figure 5.5):
    - Phase 1 - Defined by chaos (non-periodic, irregular vibrations) occupying up to 2.5 kHz of the frequency band, amplitude modulated (AM) (DF = 1078 Hz).
    - Phase 2 - Demarcated by numerous distinct, shallow, rapid FM bands (DF = 1219 Hz).
    - Phase 3 - Defined by linear, tonal, harmonic-rich frequency bands (DF = 1125 Hz).

- Phase 4 - Shows a linear upward frequency shift from the linear frequency bands in phase 3, some evidence of chaos is visible (DF = 1219 Hz).
- Phase 5 - Following the upward frequency shift, this phase consists of another linear, tonal, harmonic-rich frequency set of bands (DF = 1313 Hz)
- Phase 6 - Consists of a rapid transition of tonal frequencies to broadband deterministic chaos, with traces of subharmonics visible (DF = 1547 Hz).
- Phase 7 - From the previous phase, phase seven exhibits a dominant frequency jump of approximately 500 Hz. Contrary to phase four, this phase shows a linear upward frequency, some evidence of chaos is also visible in the second half of this phase (DF = 2063 Hz).
- Phase 8 - Consists of deterministic chaos, with very brief (<10 ms) harmonics becoming visible at random (DF = 1875 Hz).
- Phase 9 - Consists of click-like or iambic-note-like pulses and are produced in differing numbers (DF = 2334 Hz).

The nine described phases are chronologically numbered for ease of the descriptions. However, these phases can occur haphazardly in an inexhaustive number of orders within whine-notes (Figure 5.6a-p). Whine phases 1, 2 (partly), 8 and 9 display amplitude modulation (AM) and phases 2 (partly) and 3–7 exhibit frequency modulation (FM). Figures 5.6a, b, e, i, g, l, n, o and p show a rapid transition of rapid upward and downward FM so that it represents a dome shape or chevron shape in the start of the call, usually after phase 1. Highest amplitude contributions come from phases 4–7 (1200<DF<2100), with the tonal, harmonic-rich phase 5 being the largest contributor to the DF (Figures 5.8, 5.9). Maximum amplitude is reached approximately ¾ into the whine-note (Figure 5.9).

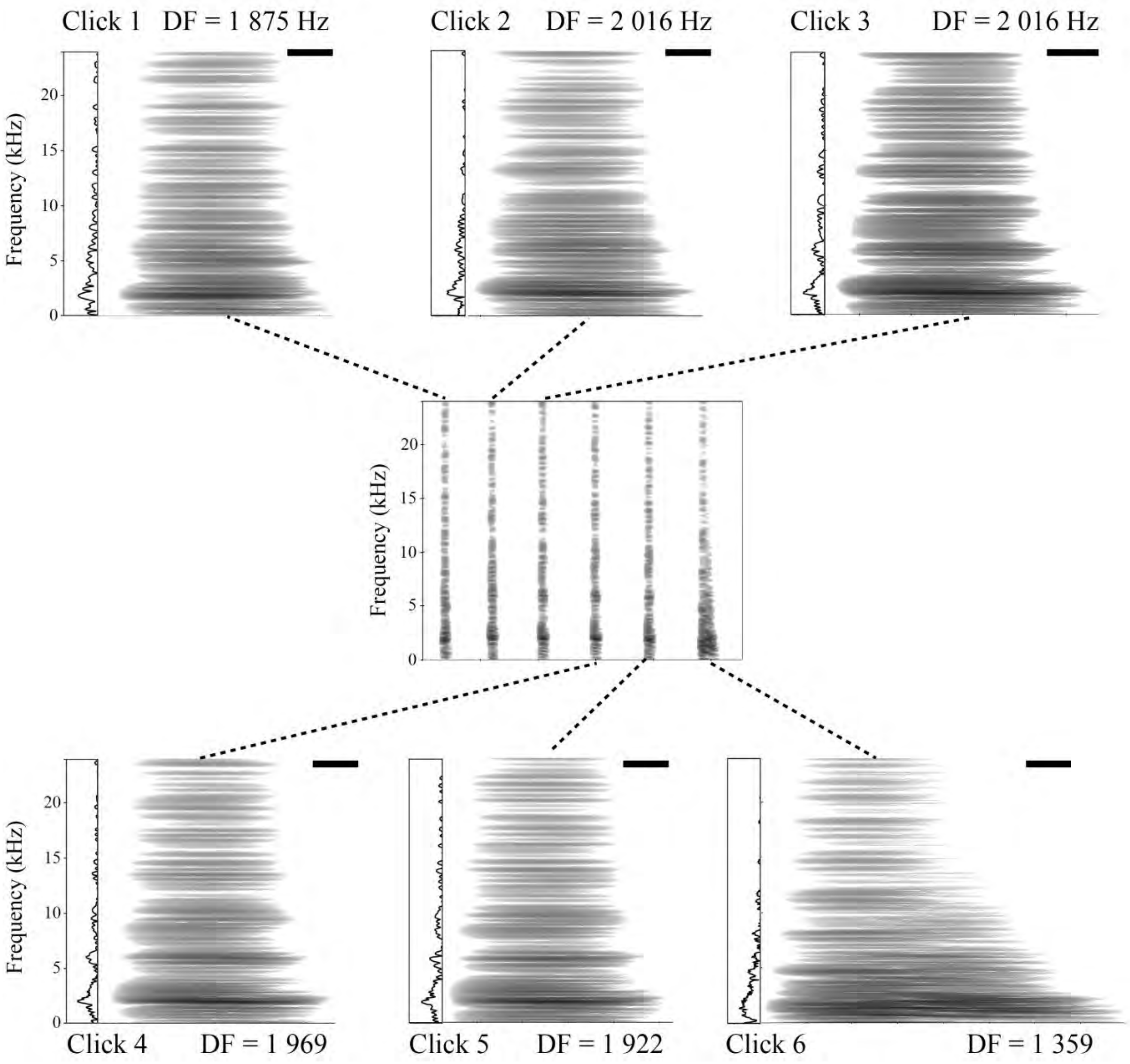


Figure 5.3 A spectrographic presentation of an example of a six-pulse click-note of *Amietia quecketti*. Power spectra for each pulse (left of spectrogram) were cut off at a minimum of -50 dB relative amplitude. Scale bars = 5 ms.

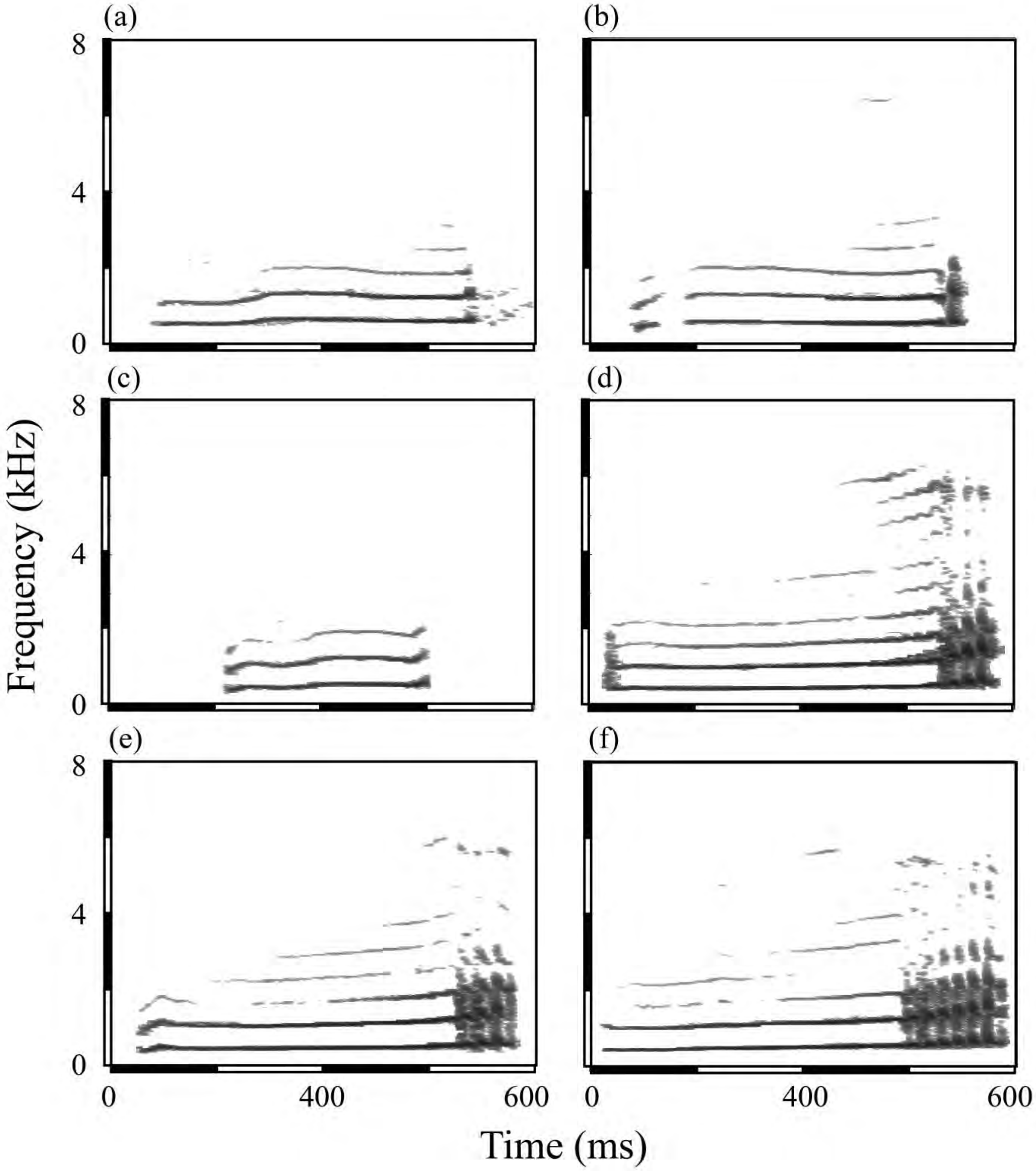


Figure 5.4 A sequence of examples of nine tonal whine-notes produced by one male of *Amietia quecketti* within the extent of two hours (01:00 - 03:00 am) to show the tone-like call. The click- notes vary in number and are a precursor to the tone-like note that follows. This tone-like note is one of the phases in the whine-note, and is sometimes produced alone, such as in this case. Ambient temperature ranged from 16.9–17.5 °C. Calls recorded with Song Meter SM2 passive recorder (16 bit sample rate). Noise removed. Threshold set to 18 (Bat Sound), therefore not all subharmonics are shown.

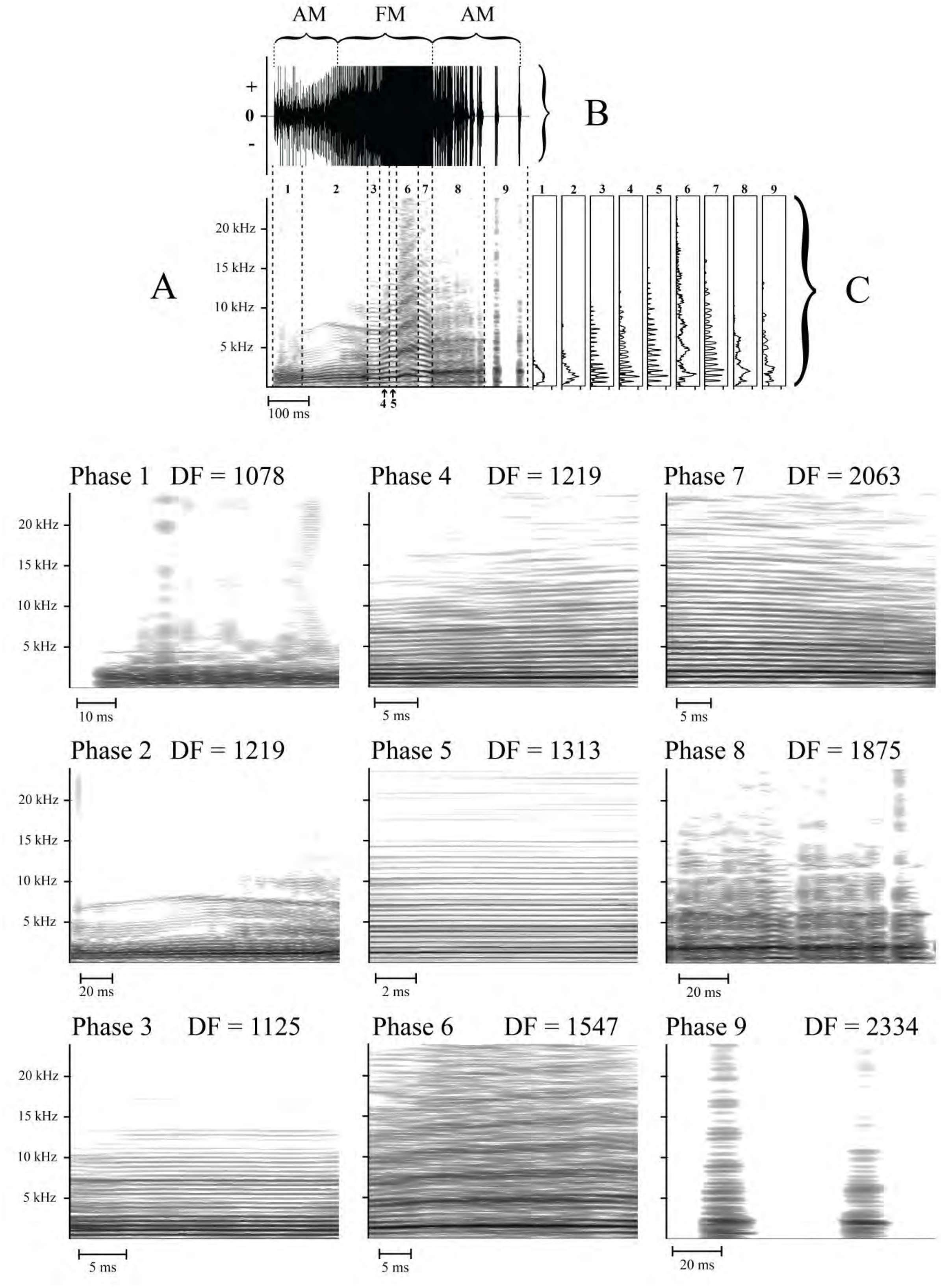


Figure 5.5 A spectrographic presentation (A) and associated oscillogram (B) of an example of the complex composition of a call of *Amietia quecketti* to show up to nine phases into which a whine-call note can be broken down. Dashed lines represent the approximate borders of each phase and an instantaneous amplitude spectrum is given for each phase (C). Phases 1–9 have been magnified to provide increased temporal resolution. Each phase's dominant frequency (DF) is also provided. AM = amplitude modulation; FM = frequency modulation.

5.3.2.3 Nonlinear events within whine-notes

Phases demonstrate rapid change in frequency structure, varying from broadband deterministic chaos to moderate-band and narrowband tonal frequencies. Harmonics are independent and show frequency onset and offset (i.e. harmonics appear and disappear on and from spectrogram). This is known as nonlinear phenomena and whine-notes demonstrate four separate nonlinear events that occur in a single whine-note (Figure 5.10). These nonlinear events included biphonation, subharmonics, deterministic chaos and frequency jumps within a single note (Figure 5.10A-D).

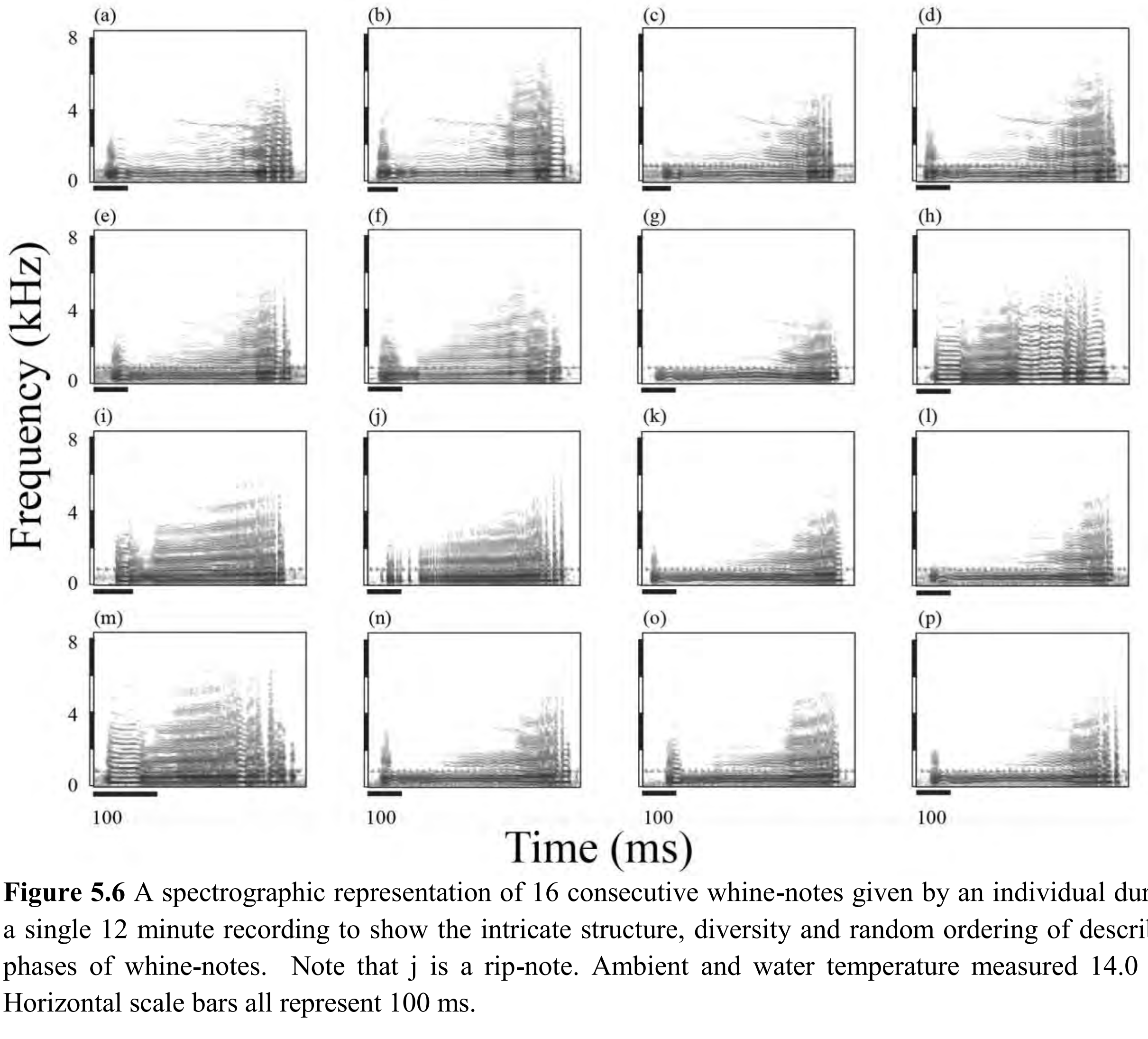


Figure 5.6 A spectrographic representation of 16 consecutive whine-notes given by an individual during a single 12 minute recording to show the intricate structure, diversity and random ordering of described phases of whine-notes. Note that j is a rip-note. Ambient and water temperature measured 14.0 °C. Horizontal scale bars all represent 100 ms.

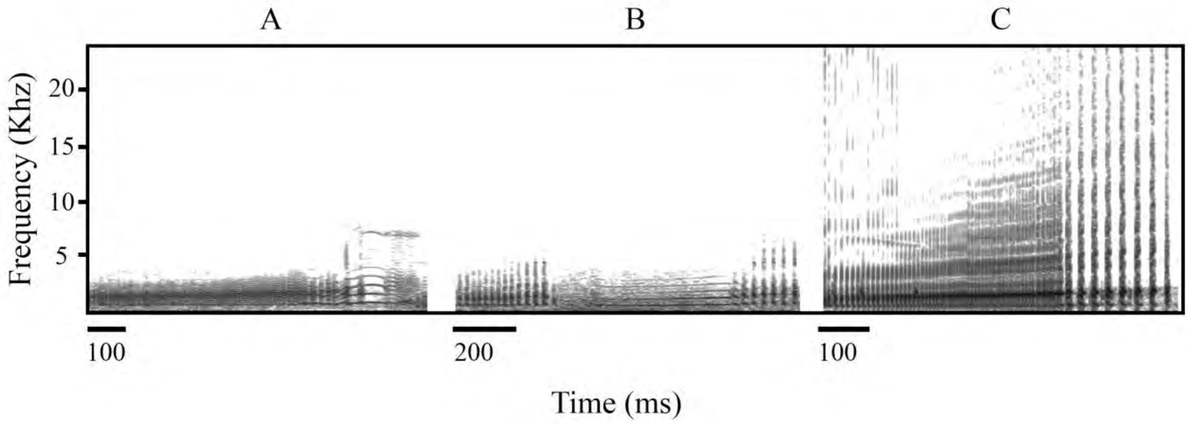


Figure 5.7 Examples of three more variations of whine-notes subsequently referred to as a creak-note ending in dome-shaped harmonics (A), iambic-tonal-combination notes (B) and pulsatile- / rip-note (C). Tonal part of the iambic-tonal-note is partially obscured by background noise.

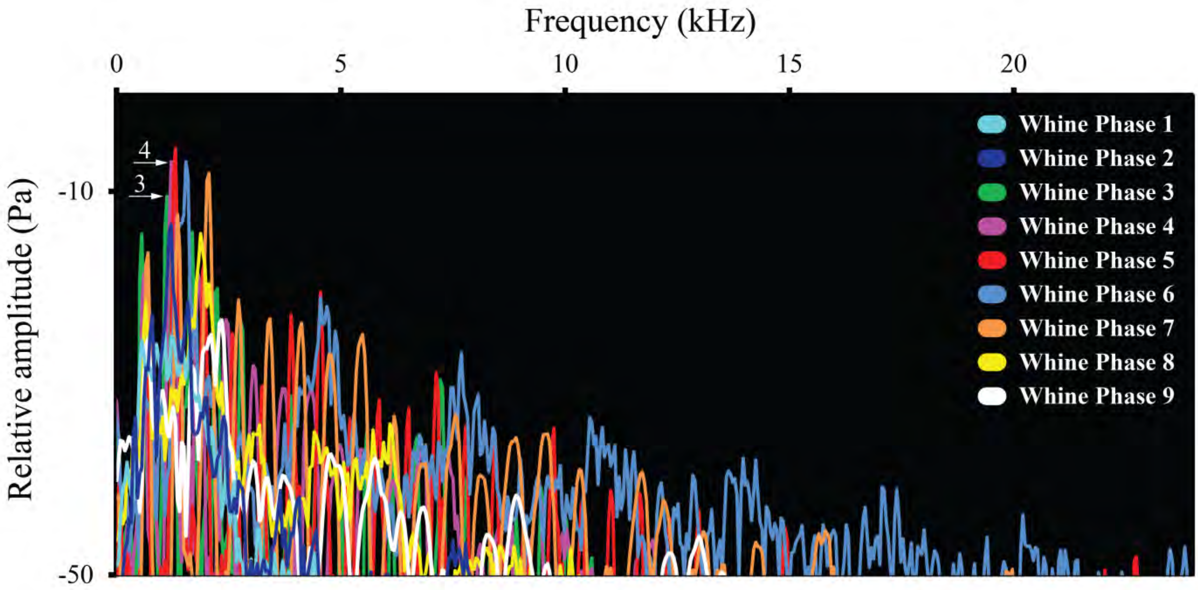


Figure 5.8 Frequency power contribution of each of the nine phases of the whine-note example in Figure 5.5. Relative amplitude was extracted from -50 Pa from an original of -120–0 Pa. Arrows indicate obstructed phases.

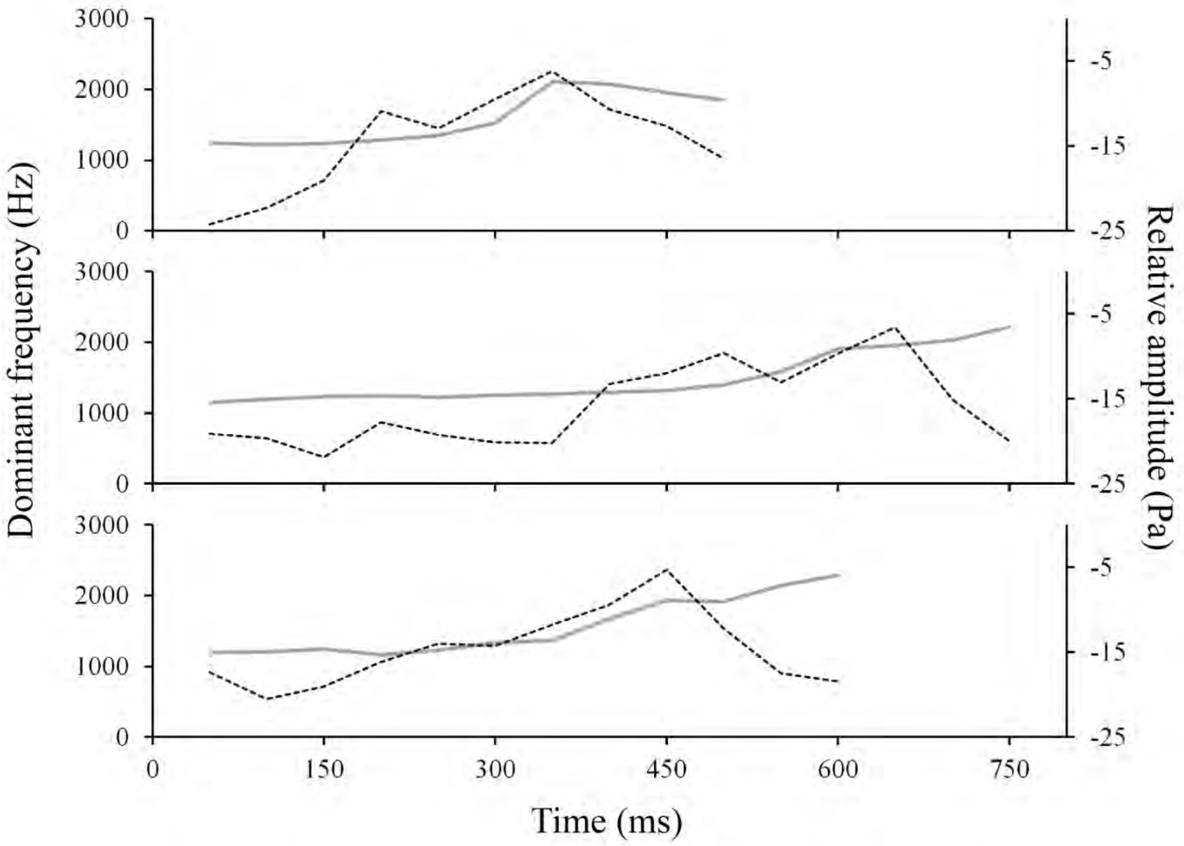


Figure 5.9 Whine-notes of three consecutive calls from one individual showing how the dominant frequency and relative amplitude changes in 50 ms intervals. Solid grey line represents dominant frequency; dashed line represents relative amplitude.

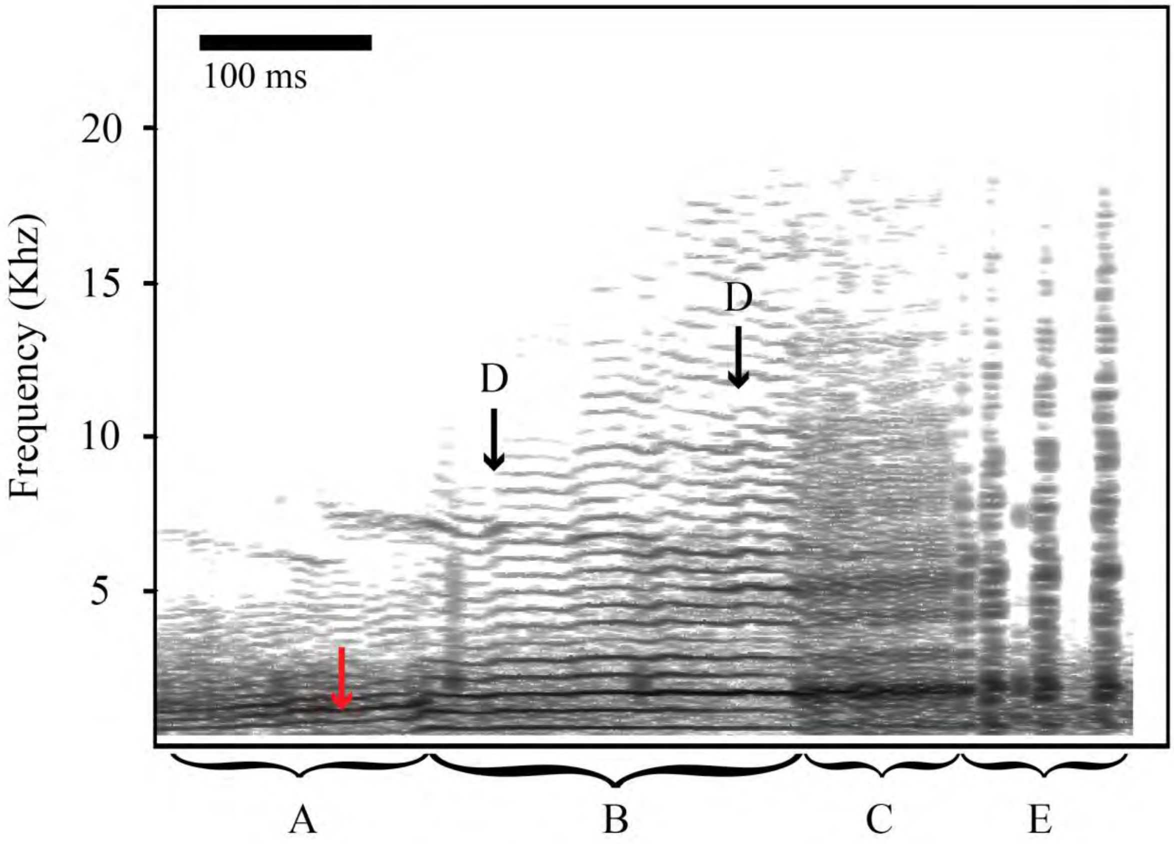


Figure 5.10 An example of a whine-note to demonstrate four nonlinear events, namely biphonation (A), subharmonics (B), deterministic chaos (C) and frequency jumps (D) within a single note. Red arrow indicates the independent harmonic which is the defining feature of biphonation. Black arrows indicate the vertical area where narrow frequency jumps occur. Three iambic-note-like pulses are indicated at (E).

5.3.2.4 Aggression iambic-note

The aggression iambic-note is similar to the click-note in that it is pulsed, but has a lower mean and range of DFs and MWFs (DF: $\bar{X}$ = 626.6 Hz, 531–1281, MWF: $\bar{X}$ = 872.4, 769.8–1073.4; Table 5.4; compare with Table 5.2). Pulses ranged from 5–13 ($\bar{X}$ = 10.9, N = 10) with a call duration of 290–709 ($\bar{X}$ = 476.6). Two insertion types integrated into the iambic aggression note and differences in frequency structure were noted. One insertion type occurred in 40% of calls and could be distinguished by a portion of a shallow, rapid, narrowband FM pulse, were positioned next to pulses 4–7 ($\bar{X}$ = 4), were 106–427 ($\bar{X}$ = 227.8) in length, and similar to the frequency structure of phase 2 of the whine-note (Figure 5.11a-c, g). The next addition type was a short tone-like pulse that only occurred in one of the calls and was positioned at the end (Figure 5.11a).

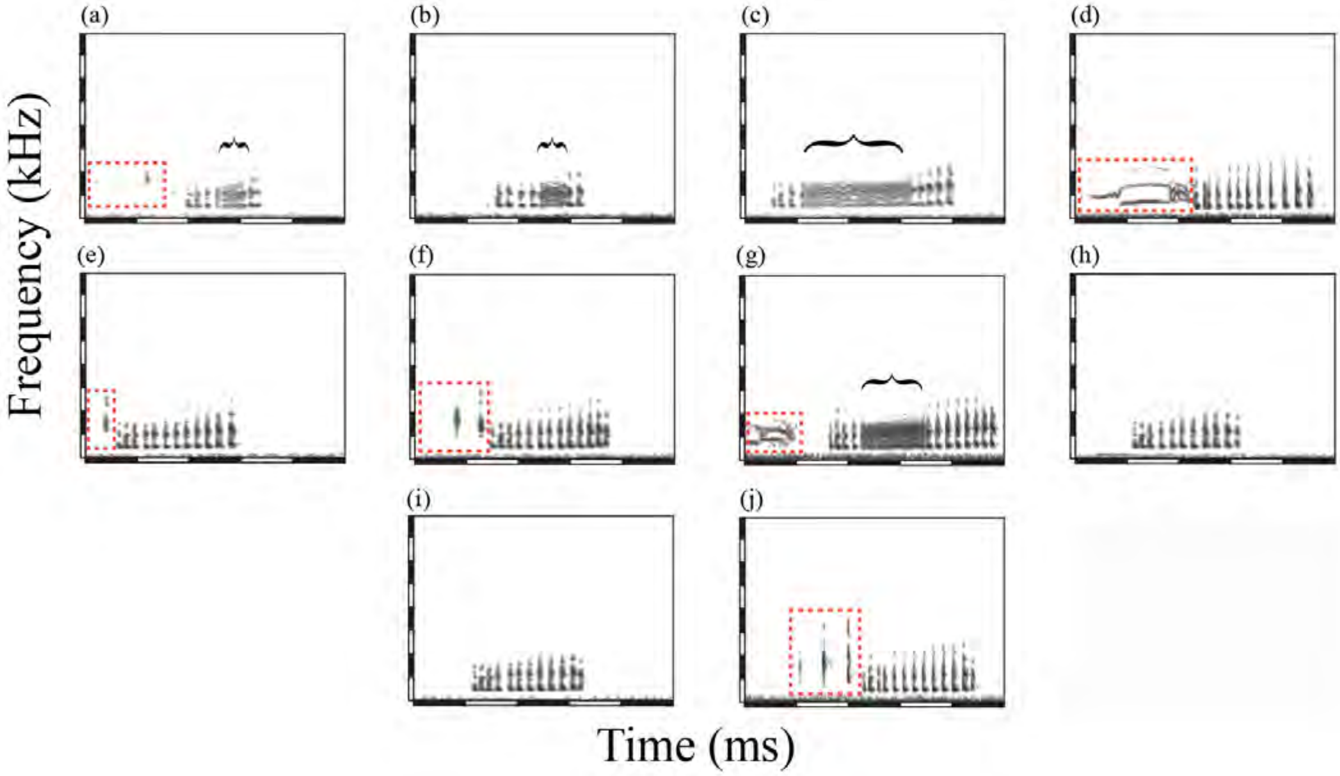


Figure 5.11 Examples of ten iambic aggression notes (a-j) produced by one *Amietia quecketti* male following playbacks of a conspecific. Vertical scales represent 1 kHz starting at 0 kHz; Horizontal scales represent 200 ms intervals; braces represent nonlinear, chaotic or rapid FM events included in some aggression notes; red dashed line rectangles represent portions of playback calls. Call notes with no conspecific call precursor fell outside of the time-frame.

Table 5.4 Aggression call analysis recorded of one male (N = 10).

Call property	Mean	Range
Call duration (ms)	476.6	290–709
Pulse number	10.9	5–13
Narrowband FM pulse duration (ms) (N = 4)	227.8	106–427
Narrowband FM pulse position	4	4–6
Tone-like pulse duration (ms) (N = 1)	51	N/A
Tone-like pulse position	5	N/A
Dominant frequency (Hz)	626.6	531–1281
Mean weighted frequency (Hz)	872.4	769.8–1073.4

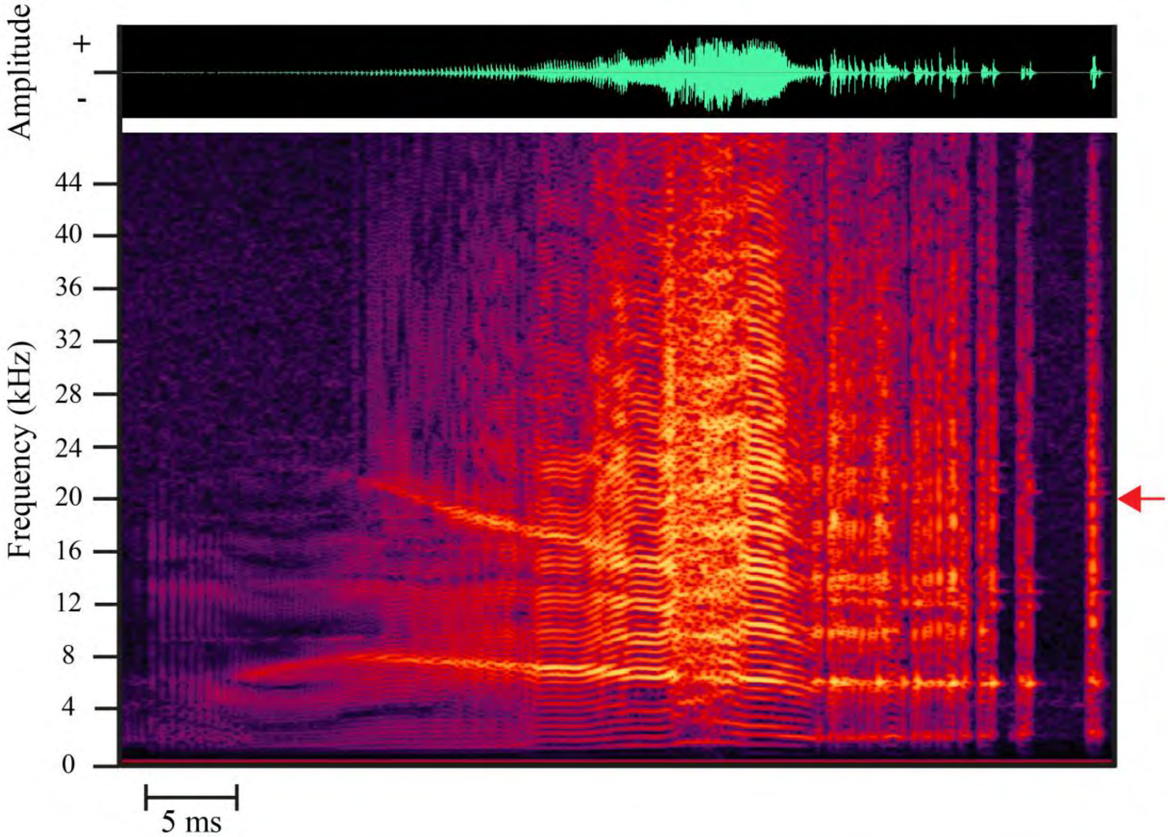


Figure 5.12 A spectrogram and associated oscillogram compiled in Adobe Soundbooth CS5 to show the harmonic rich whine-note containing harmonics well into the ultrasonic spectrum. The red arrow represents the highest frequency (20 kHz) audible to the human ear. Because of the frequency sensitivity range of the Pettersson US detector, the 0–5 kHz frequency band is irrelevant.

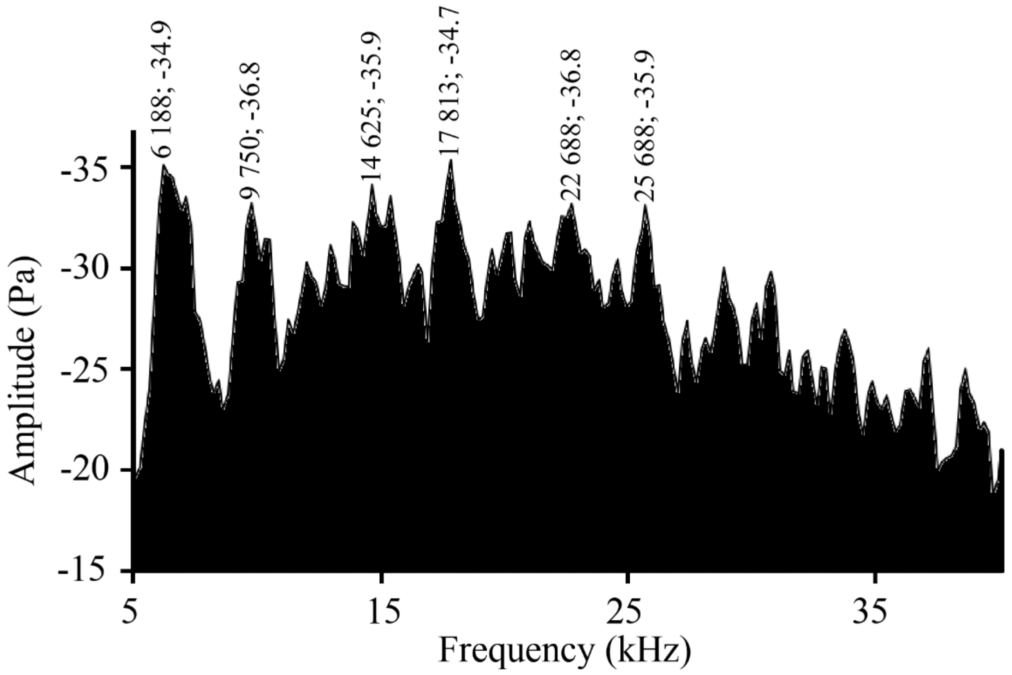


Figure 5.13 The power spectrum from the whine-note presented in Figure 5.12 was obtained from BatSound. Peak frequencies and amplitude values are labelled. Frequency band 0–5 kHz falls outside of the US recorder's range. Hanning FFT window was used at 1024 sample rate.

5.3.3 High-frequency harmonics

Following recognition of harmonic-rich whine-notes, further investigation on the frequency spectrum was conducted. Although click-notes also showed frequencies extending towards the US range, they were not included because short pulses tend to be broadband (Peter Narins pers. comm.). The spectrogram revealed harmonics well into the US range (Figure 5.12). The same call recorded in Figure 5.5 was simultaneously recorded with an US recorder and showed a similar frequency structure. Two distinct emphasised areas are visible around 6.5 kHz and 17.6 kHz. The power spectrum of the whine-note shows harmonics at high frequencies, even frequencies extending into the ultrasonic range (25.523 kHz), that have similar relative amplitude levels as the harmonics in the audible range (6.492 kHz; Figure 5.13).

5.4 Discussion

5.4.1 Call repertoire of *A. quecketti*

Two calls for *A. quecketti* are described (advertisement and aggression call) and for the first time an aggression note emitted by a species of the *Amietia* genus is reported. Advertisement calls consisted of an extraordinary vocal repertoire with four note-types that are described (tonal-notes, creak-notes, rip-notes, and whine-notes). The calls range widely in spectral characteristics from a narrowband tonal to broadband chaotic phases, to even broader band, pulsatile advertisement click-notes as well as pulsatile iambic aggression notes consisting of up to 13 pulses. Temporal variation within note-types could be produced in different rates, from 16 pulses/second in advertisement click-notes, to 90 pulses/second in rip-notes, to extremes of 170 pulses/second in creak-notes.

Other frog species' reported to have comparable complexity to the repertoire of *A. quecketti* include *Boophis madagascariensis* (see Narins *et al.* 2000), *Odorrana tormota* (see Feng *et al.* 2002) and *Physalaemus spiniger* (see Costa & Toledo 2013). A typical response of many of the species studied is that aggression call components are added to the normal advertisement call (Wells & Schwartz 1984; Grafe 1995), but in other species the advertisement call itself contains an aggressive component (Narins & Capranica 1978). Aggression calls are often graded, i.e. their dominant frequency, duration and intensity can be varied during male-male interactions (Lopez *et al.* 1988; Grafe 1995). Males of *Philautus leucorhinus* are likely to equal the length of aggression calls to that of the opponent (Arak 1983). An explanation given for call complexity in some species is that calls are used for strength calculation in male-male interactions (Wagner 1992), but in other frogs it has been proposed that female choice can lead to cumulative call complexity (see reviews in Gerhardt 1994; Castellano & Giacoma 1998; Wilczynski *et al.* 2001). Variation in repertoires from other anuran species have been described by many authors, including Narins *et al.* (2000), Toledo & Haddad (2005), Pettitt *et al.* (2012), Costa & Toledo (2013). It was suggested that variation of sporadic calls can represent advertisement calls produced sub-optimally, referring to the vocal apparatus being cold and therefore needing to be "warmed up" (Jameson 1954; Haddad 1987; Christensen-Dalsgaard *et al.* 2002; Toledo & Haddad 2005).

Classification of call properties of *A. quecketti* into either static, intermediate or dynamic on the basis of their CVs (Gerhardt 1991; Smith & Hunter 2005) demonstrated that DF and MWF are dynamic properties. Most studies show that spectral properties are usually static, i.e. does not have a CV larger than 5% (Gerhardt 1991; Gerhardt & Huber 2002; Smith & Hunter 2005; Bee *et al.* 2013). However, this study's finding is inconsistent with numerous studies of frogs showing that spectral properties tend to be static features of frog calls (Gerhardt 1991) and display little variation within individuals (e.g., Bee & Gerhardt 2001; Bee *et al.* 2001, 2010; Castellano *et al.* 2002; Friedl & Klump 2002; Bee 2004; Gasser *et al.* 2009; Schrode *et al*. 2012; Bee *et al.* 2013). Castellano *et al.* (2002) reasoned that call properties mostly affected by morphological or physiological status tend to be more static while properties under energetic limitation or social influence are frequently more fluctuating. Most frogs have restricted ability (e.g., compared with songbirds and mammals) to modify the spectral properties of their calls and the biophysics of sound production limits the frequencies males can produce (Gerhardt & Huber 2002).

The results of this study demonstrate that each of the whine-phases can contribute to the MWF, or emphasized frequency as used in Channing & Baptista (2013). Surprisingly, the dominant frequency is shown to be highly variable in both the click- and whine-notes, more so than MWF. Therefore, this study on the call of *A. quecketti* supports the use of MWF for this species (see Chapter 4, not supporting MWF as measurement). The large repertoire of this species has been overlooked and brings about new challenges for systematists to describe calls for this recently revised genus.

5.4.2 Nonlinear phenomena in the whine-note

The connection between acoustic structure and function advocates that nonlinear acoustic phenomena should commonly act to stimulate arousal and antipredator reactions in multiple species, although their probable usefulness is not restricted to antipredator behaviour (Rendall & Owren 2010). Sound production systems could, regularly or in particular situations, produce a set of acoustic features that are collectively referred to as nonlinear acoustic phenomena (Wilden *et al.* 1998; Fitch *et al.* 2002; Slaughter *et al.* 2013). These nonlinear acoustic phenomena may include rapid frequency jumps, subharmonics, biphonation, and deterministic chaos, which look like noise on a spectrogram (Fitch *et al.* 2002). These nonlinear events are tied to different functions. Sounds with rapid frequency jumps can function to capture attention (McConnell 1991) and may provoke aggressive responses in chimpanzees and birds (Hope 1980; Slocombe & Zuberbüler 2005). It has been suggested that the occurrence of nonlinear phenomena (i.e. biphonation) may sub-serve individual recognition and estimation of size (Fitch *et al.* 2002; Volodina *et al.* 2006). In some birds, calls that that are produced in flocks are characterized by upward frequency shifts and chaos, whereas alarm calls are demarcated by chevron-shaped frequency modulations (Hope 1980). Slaughter *et al.* (2013) noted that animals hearing these sounds may have specific responses to them. For example, tonal-harmonic calls may induce calm behavior, while abrupt signal onsets may increase arousal in mammals and birds (Morton 1977; Götz & Janik 2011). A range of nonlinear acoustic phenomena have also been shown to commonly cause fear or arousal in receivers (Blumstein & Récapet 2009; Blesdoe & Blumstein 2014). *Amietia quecketti* is one of few frog species that exhibit a variety of these nonlinear phenomena (Narins *et al.* 2000; Feng *et al.* 2002; Costa & Toledo 2013). Responses for frogs with complex repertoires have rarely been tested and were limited to call types (Narins *et al.* 2000; Christensen-Dalsgaard *et al.* 2002). Therefore, many questions are raised to experimentally test the responses of nonlinear events on frog vocal and social behaviour. The existence of calling site preference (Viviers 2013), aggressive behaviour during conspecific call playbacks (pers. obs.), aggression calls and the existence of an extensive repertoire may suggest strong female sexual selection in this species, although choice discrimination experiments and quantitative male and female courtship behaviour must be conducted to confirm this.

5.4.3 Significance of vocal behaviour and spectral structure in communicating in noisy environments

Amietia quecketti commonly occur in a wide range of habitats, including streams and rivers of varying flow intensities where there is constant acoustic interference from the flow of water (Wager 1986; Channing 2001; Du Preez & Carruthers 2009; Chapter 2). This species is also closely associated with human habitation (Minter *et al.* 2004; Chapter 2). Despite an increasingly noisy urban environment, *A. quecketti* seems to do well in cities. Recent studies demonstrate that frogs emit diverse broadband signals as an evolutionary adaption in noisy environments (Feng *et al.* 2006; Shen *et al.* 2011a, b; Liu *et al.* 2014).

Unlike other South African frogs, males of *A. quecketti* call throughout most of the year, although spawn has only been observed in autumn (May) and early spring (September) when this species breeds (Chapter 2 and 6). Although not experimentally demonstrated, in prolonged breeding species where males and females share habitat, male advertisement calls can stimulate hormone production in females and maintain reproductive condition in females (Lea *et al.* 2001; Wells & Schwartz 2006). This might also hold true for *A. quecketti* because of their prolonged calling behaviour.

The call repertoire of *A. quecketti* displays a variety of spectral properties that are suggested in literature to aid vocal animals to communicate in different habitats, including noisy environments. Another way to maximize contrast with wide-spectrum noise of running water would be to have calls with energy concentrated into a narrow frequency band. Some frogs i.e. *Hyperolius marmoratus*, *Odorrana tormota*) and birds that call near torrents have narrowly tuned calls (Greer & Wells 1980; Wells 1980; Passmore 1981; Dubois 1977a, b; Dubois & Martens 1984; Haddad & Giaretta 1999; Feng *et al*. 2002), but whether this behavior represents a specific adaptation to that environment has not been tested (Wells 2007). Zimmerman (1983) found that more frog species used tonal sound as did forest birds and frequency modulated sound in forest than in open habitat where broadband calls were most prevalent. However, higher-frequency sounds attenuate more rapidly with distance (Morton 1975; Lawrence & Simmons 1982; Surlykke 1988; Römer & Lewald 1992) and are more directional than low frequencies (Kinsler & Frey 1962). In addition, the short wavelengths of high-frequency sounds are susceptible to reflection and scattering by relatively small objects, such as twigs, leaves and blades of grass (Marler 1955; Sales & Pye 1974). However, additional environmental and behavioural complexities can place further, and in some cases competing, selective constraints on signal frequency (Arch & Narins 2008).

In order to assess whether males and females of *A. quecketti* respond to the high-frequency harmonics, a pilot study should first examine whether phonotaxis experiments can be used to quantify responses (Backwell & Passmore 1990; Backwell & Passmore 1991; Jennions *et al.* 1995; Bee 2007; Shen *et al.* 2008; Bee 2010). Furthermore, if the ultrasounds have a consistent influence on conspecific behaviour, then the vocalization may be considered exapted (i.e. a secondary, additional attribute that developed with the adaptation to a specific selection pressure) for communication. In these cases, the evolution of the US communication system may have been guided by conspecifics' favourable use of inadvertently providing sensory information (Arch & Narins 2008).

One of the most likely reasons for overlooking the frequency in previously recorded calls of *A. quecketti* is probably the distance from which calls were recorded, because attenuation is positively correlated to frequency (Morton 1975; Lawrence & Simmons 1982; Surlykke 1988; Römer & Lewald 1992). Another possible reason is the vacant field of expertise in anuran bioacoustics in South Africa.

CHAPTER SIX

Calling activity of the Common River Frog (*Amietia quecketti*) in an urban green space setting

"...in the afternoon, a haze gathered in the air; a veil, of thinnest gauze, seemed to be drawn over the heavens; a halo surrounded the sun; the tree-frog sang louder than ever..."

– Herbert, H.W. and Picton, T. (1882)

6.1 Introduction

Residential and urban fractal landscapes contain recreational areas with manicured lawns and woody vegetation such as parks and gardens, which are known as green open spaces or green areas (Alberti 2008; Pillsbury & Miller 2008; Hamer & Parris 2010). Green open spaces are vital elements of all cities and municipalities and symbolise a crucial part of the basic infrastructure of urban areas (Georgi & Dimitriou 2010). Apart from aesthetics, they can deliver important secondary benefits for people from associated residential areas (De Vries *et al.* 2003; Cohen *et al.* 2012; Van Renterghem *et al.* 2012) as well as for biodiversity and wildlife (Hamer & Parris 2010; Kong *et al.* 2010; Muller *et al.* 2010). Urban green open spaces also have important implications for the local climate regarding the effects of urban heat islands (Ellefsen 1991; Oke 2004; Alberti 2008; Jacobson & Ten Hoeve 2011; Cohen *et al.* 2012; Henninger 2012; Santamouris 2013; Figure 6.1). Cohen *et al.* (2012) demonstrated that urban parks with a dense tree canopy reduce temperatures by up to 3.8°C in summer and 2.0°C in winter in Tel Aviv, Israel.

Being ectothermic, ambient temperature regulates a vast amount of physiological processes in anurans, including metabolic rate, feeding and digestion, development and growth, gametogenesis, locomotion and hearing, and also anuran calling activity (summarised in Wells 2007). Several previous studies have demonstrated that temperature and/or rainfall are crucial factors for anuran reproductive activity (Blair 1960; Heusser & Ott 1968; Heinzmann 1970), but few have included more micrometeorological variables (Oseen & Wassersug 2002; Lemckert *et al.* 2013). Furthermore, many studies have focussed on anurans occurring in temperate zones that primarily breed in warmer months (Obert 1975; Howard 1980; Gilbert *et al.* 1994).

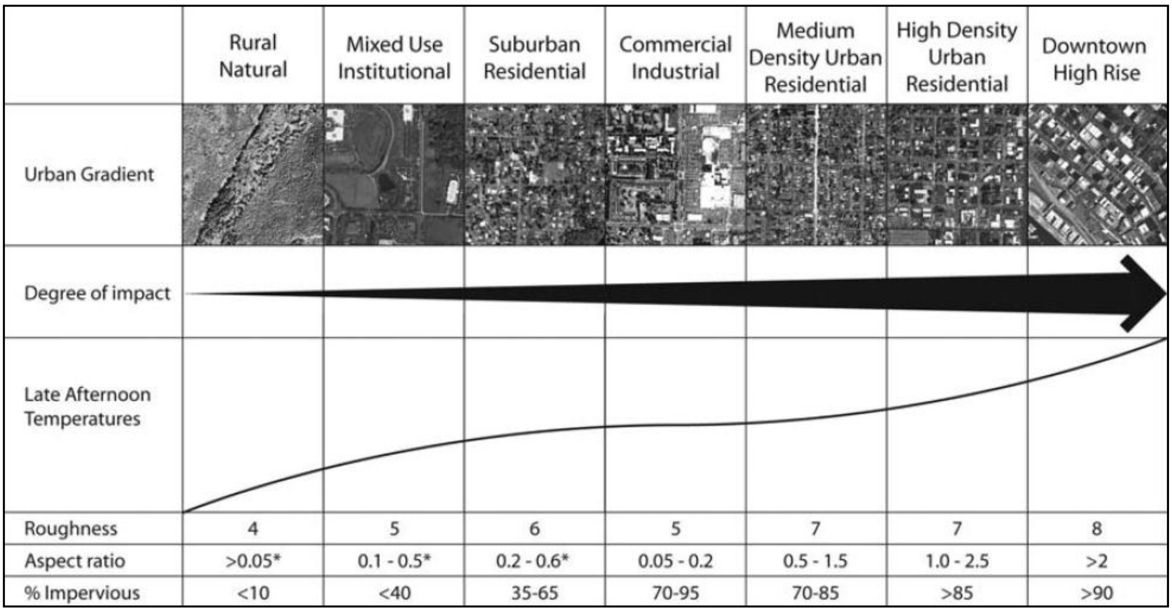
Little experimental evidence exists for relationships of anuran calling activity and environmental atmospheric variables in South African species (Henzi *et al.* 1995). Information on the effects of atmospheric variables on calling activity is important when using surveys to detect frog species for management and research (Lemckert *et al.* 2013) and for monitoring changes in breeding activity as a result of climate change (Ospina *et al.* 2013). Furthermore, with the increased use of passive acoustic surveys (Sueur *et al.* 2008; Depraetere *et al.* 2012), it becomes more essential to identify temporal and atmospheric conditions under which frog species will be most vocally active to allow maximum detectability and minimise resource utilisation.

Most studies assessing the effects of atmospheric condition on calling activity focus on temperature (Jackson 1952; Einem & Ober 1956; Storm 1960; Almeida-Gomes *et al.* 2007), precipitation (Balinsky 1969; Telford & Dyson 1990; Krupa 1994; Lemckert 2001), or both (Storm 1960; Humphries 1979; Okuno 1985; Radwan & Schneider 1988; Ospina *et al.* 2013) and have consistently showed mostly positive relationships with the number of males calling, or the intensity of their calling activity. In some anuran species, humidity also shows a positive relationship with calling activity (Almeida-Gomes *et al.* 2007; Hauselberger & Alford 2005). These variables are most strongly linked to calling, apparently since anurans desiccate when environmental conditions are dry and, being ectothermic, are able to call most effectively when conditions are warm (Lemckert *et al.* 2013). However, species will respond to these variables differently based on the breeding environments and seasons in which breeding occurs (Saenz *et al.* 2006).

Drivers of anuran reproductive activity may include more than one environmental variable. Steen *et al.* (2013) found that eight of their 11 target species were influenced by month of survey and four of those were influenced by at least one other atmospheric condition such as temperature or humidity. Precipitation was comparatively inconsequential in influencing calling activity for all of the species studied by Steen *et al.* (2013). It is well-known that features of both acoustic signal production (Blair 1958; Zweifel 1968; Navas 1996) and reception (Moffat & Capranica 1976; Stiebler & Narins 1990; Van Dijk *et al.* 1990) in ectotherms are temperature dependent (Narins 2001). Other single abiotic environmental factors, such as a drop in barometric pressure (FitzGerald & Bider 1974; Obert 1976; Bauch & Grosse 1989), light intensity (Heinzmann 1970; Schneider 1977), humidity (Bellis 1962), wind (Henzi *et al.* 1995), and moon phases (FitzGerald & Bider 1974) also affect the onset and offset of calling (i.e. when calling starts and ends) and/or locomotive activity in certain anuran species. Ultimately, there is no single set of atmospheric variables to explain calling activity in all anuran species (Oseen & Wassersug 2002; Lemckert *et al.* 2013).

There is ample literature found on the effect periodicity of the moon on the activity of birds and mammals (e.g. Kotler 1984; Nelson 1989; Penteriani *et al.* 2011). However, amphibians were found to be less explored regarding lunar phase (Tuttle & Ryan 1982; Baugh & Ryan 2010; Vignoli & Luiselli 2013). A few studies have shown frogs to exhibit lunar "phobia", reducing their calling activity on bright moonlit nights, possibly to avoid predation (FitzGerald & Bider 1974; Tuttle & Ryan 1982; Nunes 1988; Grafe 1997; Wells 2007). Effects of other abiotic environmental variables covered in some studies included photoperiod (e.g. Jaeger *et al.* 1976; Whittier & Crews 1987), light intensity (Hatano *et al.* 2002; Almeida-Gomes *et al.* 2007) and anthropogenic noise (Sun & Narins 2005; Chapter 3). The relative importance of the above mentioned environmental factors on calling activity of males may differ among species (Brooke *et al.* 2000). For example, Sinsch (1987) found that European Toads orient themselves towards breeding sites almost as well under an overcast sky compared to a clear sky. Furthermore, Vignoli & Luiselli (2013) found that *Hyla intermedia* and *Rana dalmatina* avoided full moon and deposited eggs in new moon phases or conditions where moonlight arriving on the ground was low due to an overcast sky. They also interpreted this as anti-predatory behaviour, but did not include any other environmental variables.

Due to differences in the immediate micrometeorological conditions of urban environments in green open space where the majority of the surface area is covered with vegetation, calling activity might differ in these areas. This study's objective was to investigate the influence of meteorological conditions in an urban green space on the calling activity of *Amietia quecketti*. Although ambient noise might affect calling behaviour (Krause 2004; Sun & Narins 2005; Parris *et al.* 2009; Chapter 3), this variable was not included as part of the objective. Determining the atmospheric and temporal variables influencing anuran calling activity is important for management and research when using acoustic surveys to detect species (Lemckert *et al.* 2013).



	Rural Natural	Mixed Use Institutional	Suburban Residential	Commercial Industrial	Medium Density Urban Residential	High Density Urban Residential	Downtown High Rise
Urban Gradient							
Degree of impact							
Late Afternoon Temperatures							
Roughness	4	5	6	5	7	7	8
Aspect ratio	>0.05*	0.1 - 0.5*	0.2 - 0.6*	0.05 - 0.2	0.5 - 1.5	1.0 - 2.5	>2
% Impervious	<10	<40	35-65	70-95	70-85	>85	>90

Figure 6.1 Correlation between the urban gradient and urban heat island based on Urban Terrain Zones (Ellefsen 1991) and Urban Climate Zones (Oke 2004). Late Afternoon temperatures are synthesised from Oke (1973). The temperature increases along the urban gradient. Roughness refers to the effective terrain roughness according to the Davenport classification (Davenport *et al.* 2000). The roughness Aspect ratio = ZH/W is average height of the main roughness elements (buildings and trees) divided by their average spacing. In the city centre this is the street canyon height/width. The aspect ratio is related to flow regimes (Oke 1987) and thermal controls other impervious areas (shading and screening) (Oke 1982). Percent impervious reflect the proportion of ground plane that is covered with buildings, roads and other impervious areas. The amount of pervious ground cover affects soil moisture and evaporation (source Alberti 2008).

6.2 Materials and Methods

6.2.1 Study area and focal species

This study was conducted at a constructed permanent pond in the North-West University (NWU) Botanical Gardens in Potchefstroom, South Africa (Figure 6.2). The pond is moderately sized (>900 m^2), ≈2 m deep, and part a series of seven other constructed ponds within the gardens (S26.681883, E27.095511; 1 346 m a.s.l.). A railway track and a tarred road flank two opposite borders of the botanical garden, which is further surrounded by mulit-story buildings (incl. student residence) and sports fields (Figure 6.2). Potchefstroom falls within a summer rainfall regime with average minimum and maximum temperatures of 18–28°C for summer and 1–16°C for winter (http://www.worldweatheronline.com). Minimum temperatures in winter can drop well below zero (<-5°C) and maximum temperatures >30°C are common in summer.

The Common River Frog (*Amietia quecketti*) has a wide distribution within South Africa (Du Preez & Carruthers 2009; Channing & Baptista 2013) and is a frequent urban garden inhabitant throughout its range (Chapter 2). This species calls for much of the year but is most active in winter months and early spring (Du Preez & Carruthers 2009; Viviers 2013). *Amietia quecketti* has a previously overlooked unique vocal repertoire consisting of click-notes, whine-notes, rip-notes, iambic-like notes, none-like notes and creak notes (Chapter 5). The success of this species can be attributed to its adaptability to a wide habitat range (Chapter 2) and possibly also its unique vocal behaviour (Chapter 5).

6.2.2 Calling intensity as a measure of calling activity

Call surveys provide a practical measure of species abundance and calling intensity that can be compared over geographic and temporal ranges (Hemesath 1998; Mossman *et al.* 1998; Pillsbury & Miller 2008). *Amietia quecketti* has an extensive repertoire as demonstrated in Chapter 5, consisting of a two-part call, a click-note and a whine-note, with many variations of whine-notes, including other note-types. Click-notes occur 80% of the time (60% combination of the notes, 20% click-notes only; Chapter 5). Furthermore, click-notes are produced in 4–7 short and simple pulses per note, lacking the complexity and variability of whine-notes and the rest of the repertoire and are therefore easily identified by call recognition software. Consequently, click-notes were a good measure for calling activity in this species. Along with the weather station, a passive recorder (Song Meter SM, Wildlife Acoustics) was set to record 15 hours continuously from 17h00–08h00 the following day next to a pond containing a known breeding population of *A. quecketti* in the NWU Botanical Gardens (Figure 6.2; Chapter 2). The recordings were made on the nights of full moon and new moon (approximately every two weeks) starting from 2013/02/25 – 2013/09/19 (atmospheric variables were measured only from the date of installation, 2013/05/24). The recorder was fitted with two multi-directional microphones (Wildlife Acoustics) directed to the two furthest embankments of the pond.

Click-notes were detected and counted using the call recognition component of Song Scope software (version 4.1.3A, Wildlife Acoustics). Four recognisers were compiled using training data (click-pulses from within the recordings). Each recogniser was tested by running it through a set of recordings and the recogniser with the highest detection count was chosen to detect click-pulses in all recordings. False negatives (click-pulses missed) and false positives (sounds wrongly detected as click-pulses) were determined for the chosen recogniser. For quantification of calling activity, number of click-notes produced per hour on the half-hour (i.e. 17h30–18h30) was used to centre calling activity on each hour.

6.2.3 Measuring atmospheric variables

To document meteorological variables, a weather station was placed (2013/05/24) on an open lawn approximately 10 metres from the pond where automated recordings were conducted (Figure 6.2). The weather station consisted of a ± 9 meter high tower with sensors installed for temperature (Model 41003), relative humidity (RH), precipitation (Model 61002), wind speed and wind direction (Model 05103), (Sensors brand: R.M. Young). All data were logged with a Campbell Sci Logger (CR10X). Data for moon percentage illumination were obtained from Time and Date AS (http://timeanddate.com/s/2kvd). Water temperature was measured at one hour intervals using the Song Meter external probe. Probe was lowered to 20 mm into the water. Hourly barometric pressure and a ten year continuum (2001–2011) measurement for wind speed, barometric pressure and temperature changes in Potchefstroom were obtained from the South African Weather Services (approximately 2 km from study site; Plot 460, Vyfhoek; 26.7S, 27.1E; 1350 m a.s.l.) in order to make inferences on the general diel variations of these variables. Absolute values for change in barometric pressure ($|\Delta \text{kPa}|$) were calculated and also included as an abiotic variable.

6.2.4 Ordination and statistical analysis

For ordination analysis, a biplot diagram was created of the principle component analysis (PCA), for the dependent variable, number of click-pulses produced (for a measure of calling intensity), and six explanatory variables namely: ambient temperature, water temperature, relative humidity, wind velocity, barometric pressure and percentage of moon illuminated. Arrows at the end of each ordination axis point in the direction of the environmental gradient as identified by the explanatory variables. The longer the arrows in each ordination axis, more strongly correlated variables are with the ordination axes than those with short arrows. No rainfall was observed within the time frame of the study and was therefore excluded from the analysis. Data in analysis was centred and standardised. Ordination analysis was conducted in CANOCO (version 4.5). Call intensity (click-pulses per hour) was log(x+1) transformed.

To support the ordination analysis a multiple linear regression was conducted on the data to test for correlation significance ($P<0.05$) of the dependent (call intensity) and independent (atmospheric conditions) variables using the "enter" input method to generate one model. A correlation matrix was run for the eight atmospheric variables to determine collinearity. Variables with $0.400<r<0.600$ were regarded as moderately collinear, while $r>0.600$ were regarded as highly collinear (after Lemckert 2013). Significance was tested at $P<0.05$. All calculations were conducted in SPSS (version 22, IBM Corporation 2013). Box plots were created for biweekly and hourly calling intensity. Box plots followed the conventional format of inter-quartile ranges represented by the "box" and minima and maxima represented by whiskers. Median is represented by the division mark within the "box". Outliers are values greater than 1.5 interquartile ranges away from the 25th or 75th percentiles.

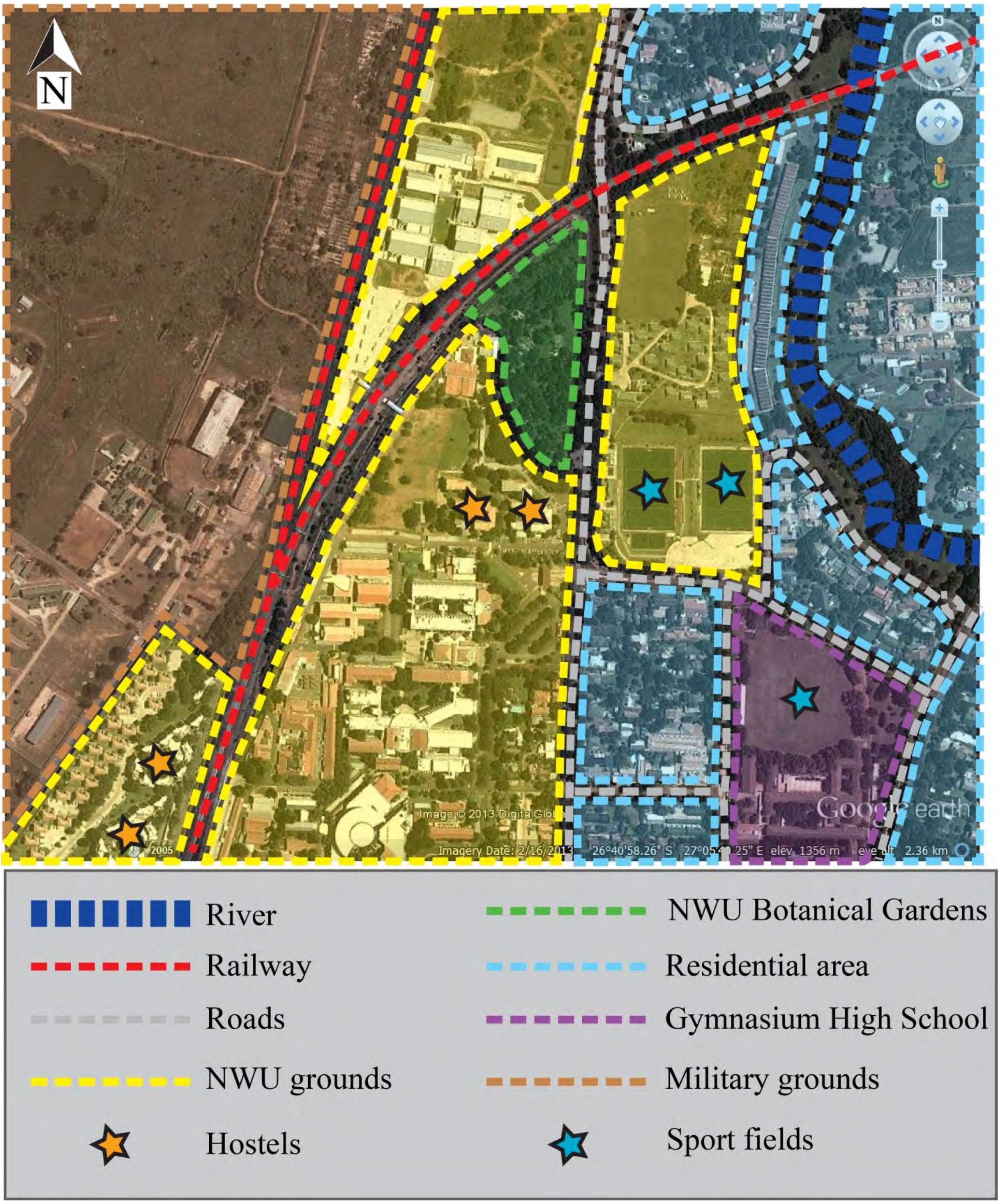


Figure 6.2 Modified Google Earth map of the North-West University (NWU) Botanical Gardens in Potchefstroom, North-West Province, South Africa. Anthropogenic activity surrounding the gardens are colour coded. White arrow indicates position of weather station.

6.3 Results

Diel variations in wind velocity, barometric pressure and ambient temperatures in Potchefstroom show that, generally, when temperature starts to decrease from its highest value at approximately 15h00, barometric pressure increases from its lowest value from around 16h00, followed by a rapid decrease in wind velocity from 16h30–18h30 (Figure 6.3). Wind velocity fluctuates from 18h30–00h00, but decreases in variation and velocity from 00h30–06h00 before wind picks up again with a rise in temperature. Males of *A. quecketti* started calling in May and reached two peak intensities, one between late June and early July, decreasing activity within two weeks thereafter, and reached another peak between August and early September, after which calling decreased again (Figure 6.4A). Calling usually commenced from around 19h00 and reached peak intensity between 23h00 and 02h00 in the evenings (Figure 6.4B). Two lower peaks were also seen between 20h00 and 21h00 and again at around 04h00 in the early morning (Figure 6.4B; median marks in box plots). Detection accuracy ranged from 54.2–91.5% ($\bar{X}$=82.2%) of the clicks that were detected, correcting for false positives and false negatives (Table 6.1). Sounds detected as click-pulses in the early evening between 17h00–19h00 and early morning between 07h00–08h00 were mostly finches and general traffic noise (i.e. false positives).

The PCA ordination of the first axis (axis 1) explained 40.1% (eigenvalue = 0.401) of the variation in all variables. Axis 1 represented a strong gradient from high humidity and calling intensity and low ambient and water temperatures (negative values), to low humidity, high ambient temperature and water temperature and low calling intensity (positive values). Axis 2 explained 18.7% of the variance (eigenvalue = 0.187) and represented a gradient from high barometric pressure, low wind velocity and full moon phases (negative values), to low barometric pressure, higher wind velocity and new moon phases (positive values; Figure 6.5). Calling intensity was slightly associated with positive values in axis 2.

Click-notes per hour for the population ranged from 0–6309 ($\bar{X}$=884.5; SD=1332.4). Atmospheric variables that the regression model included were wind velocity, RH and ambient air temperature. There was a slight breeze throughout most of the recording period, with wind velocity ranging from 0.1–4.4 m/s ($\bar{X}$=0.920; SD=0.835). Relative Humidity ranged from 6–94% ($\bar{X}$=53.1; SD=23.0) and ambient temperature ranged from -0.2–27.2°C ($\bar{X}$=9.8; SD=6.6). Four other variables were excluded by the model (see Table 6.3 for descriptive statistics of these variables).

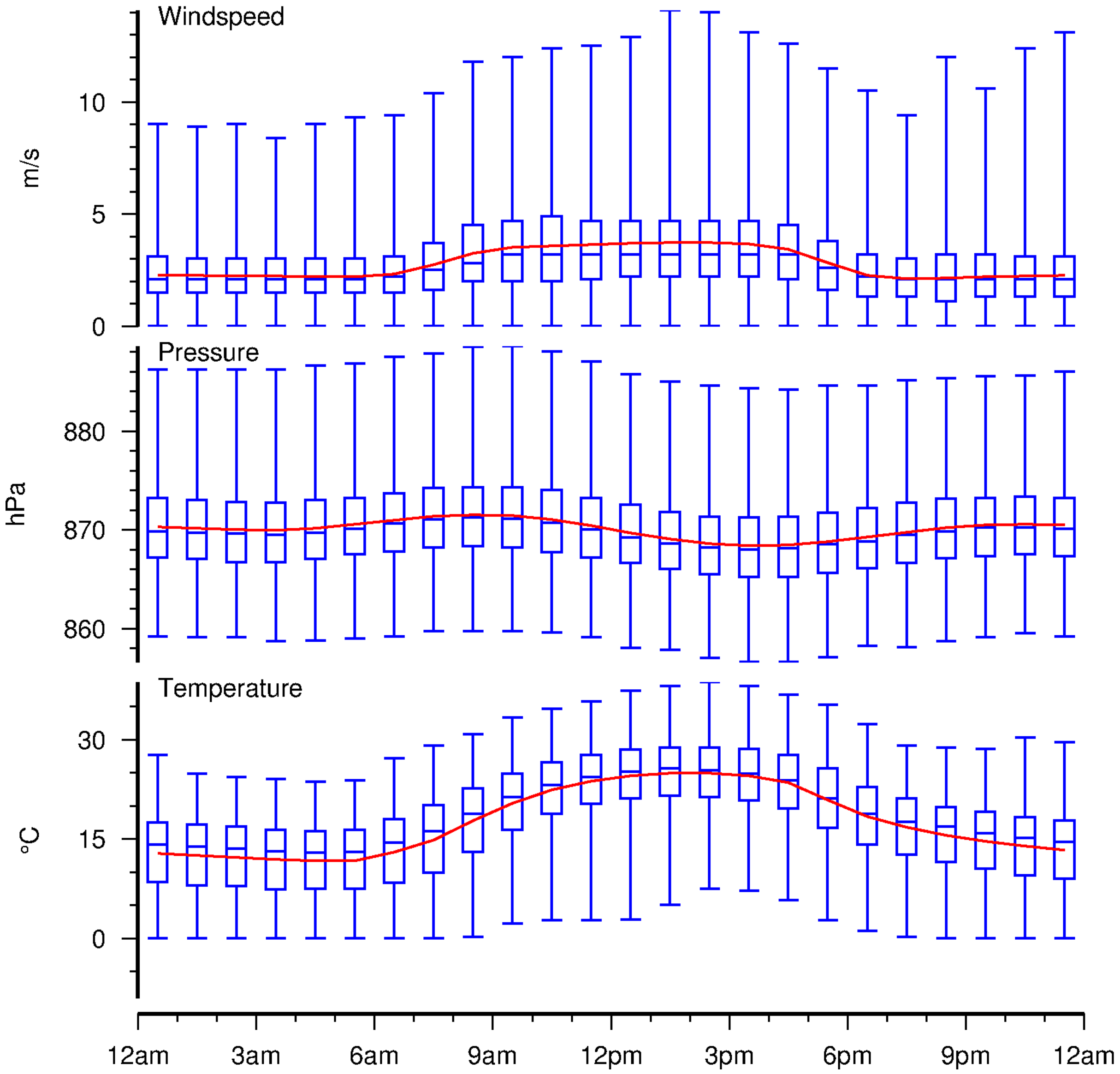
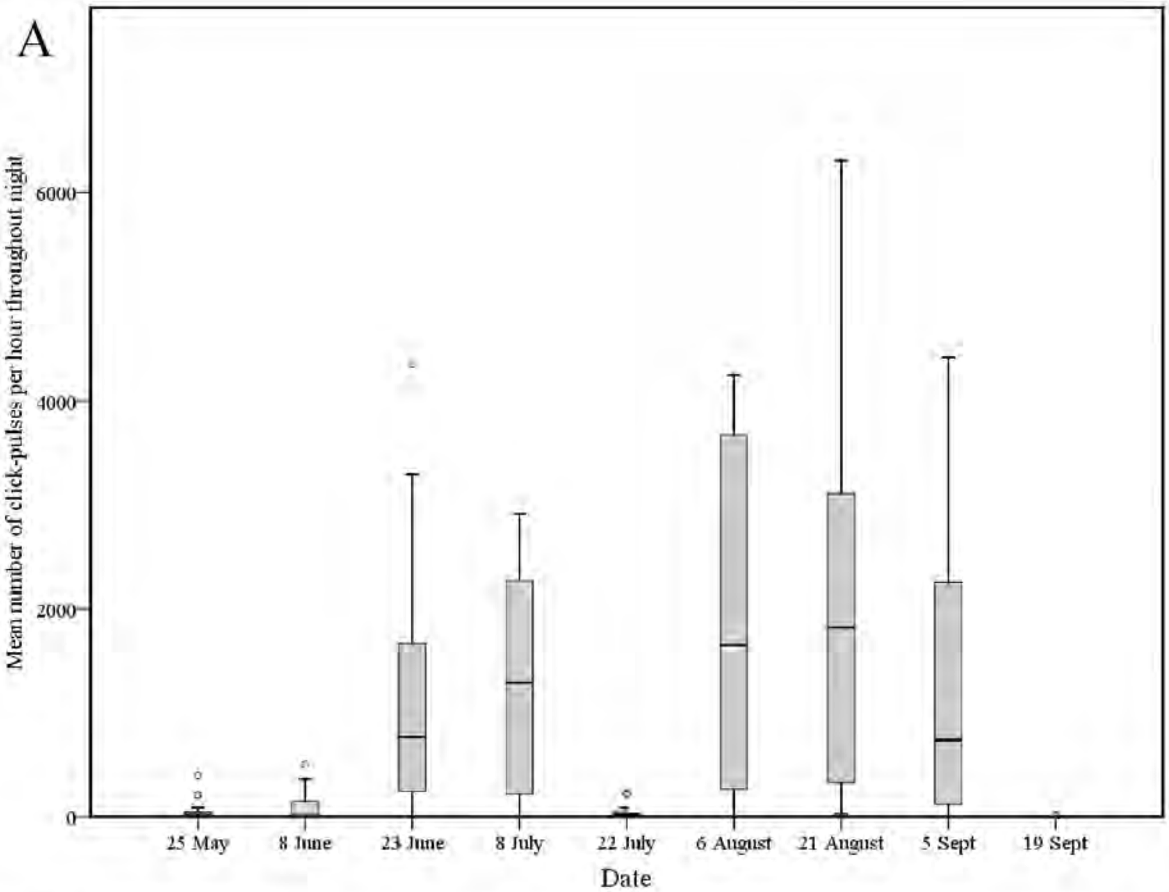


Figure 6.3 Box plots showing diel variations in wind speed, barometric pressure and ambient temperature per hour over a continuum of ten years (2001–2011) in Potchefstroom to show interdependence between these variables. Red lines show a curved regression. Data obtained from the South African Weather Station.



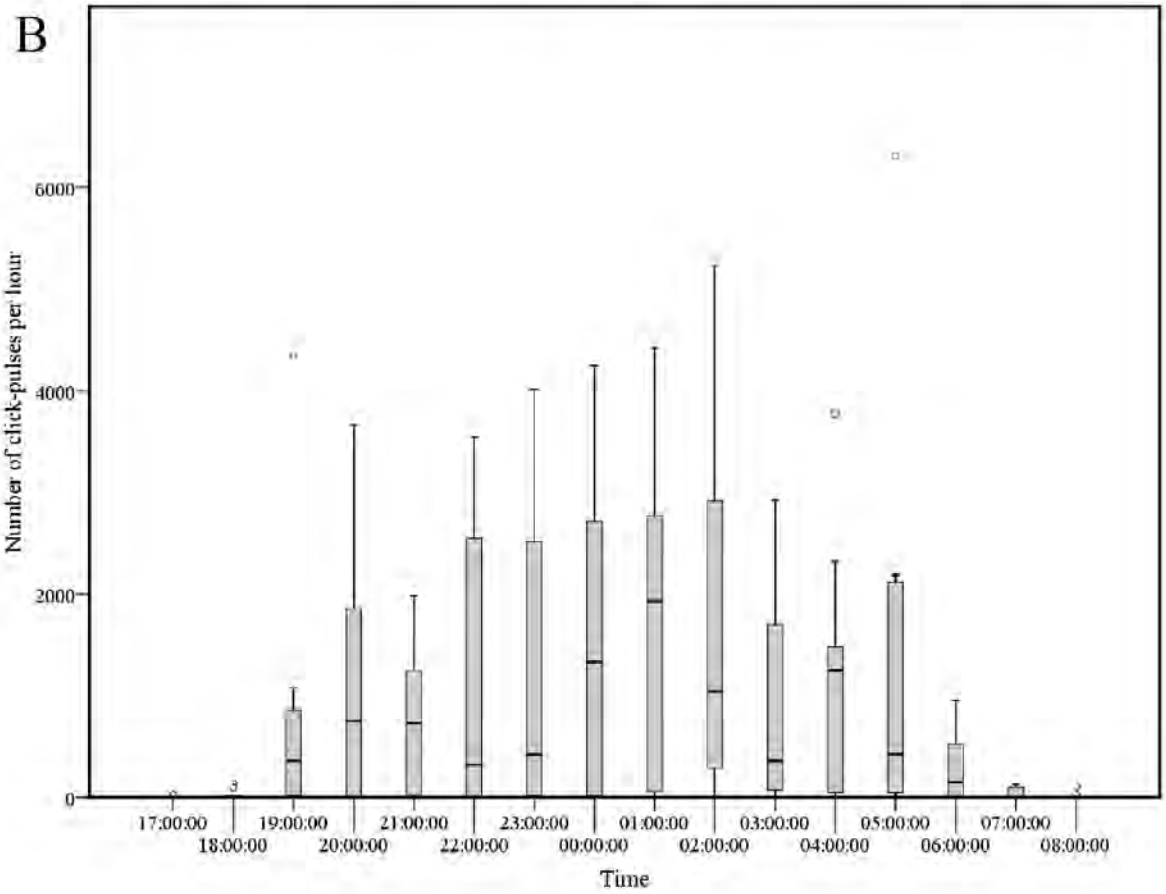


Figure 6.4 Calling intensity expressed as number of click-pulses per hour as a function of date (A; surveys were recorded biweekly on the occurrence of new moon and full moon) and time (B; measured continuously over a 15-hour period). Open circles represents outliers.

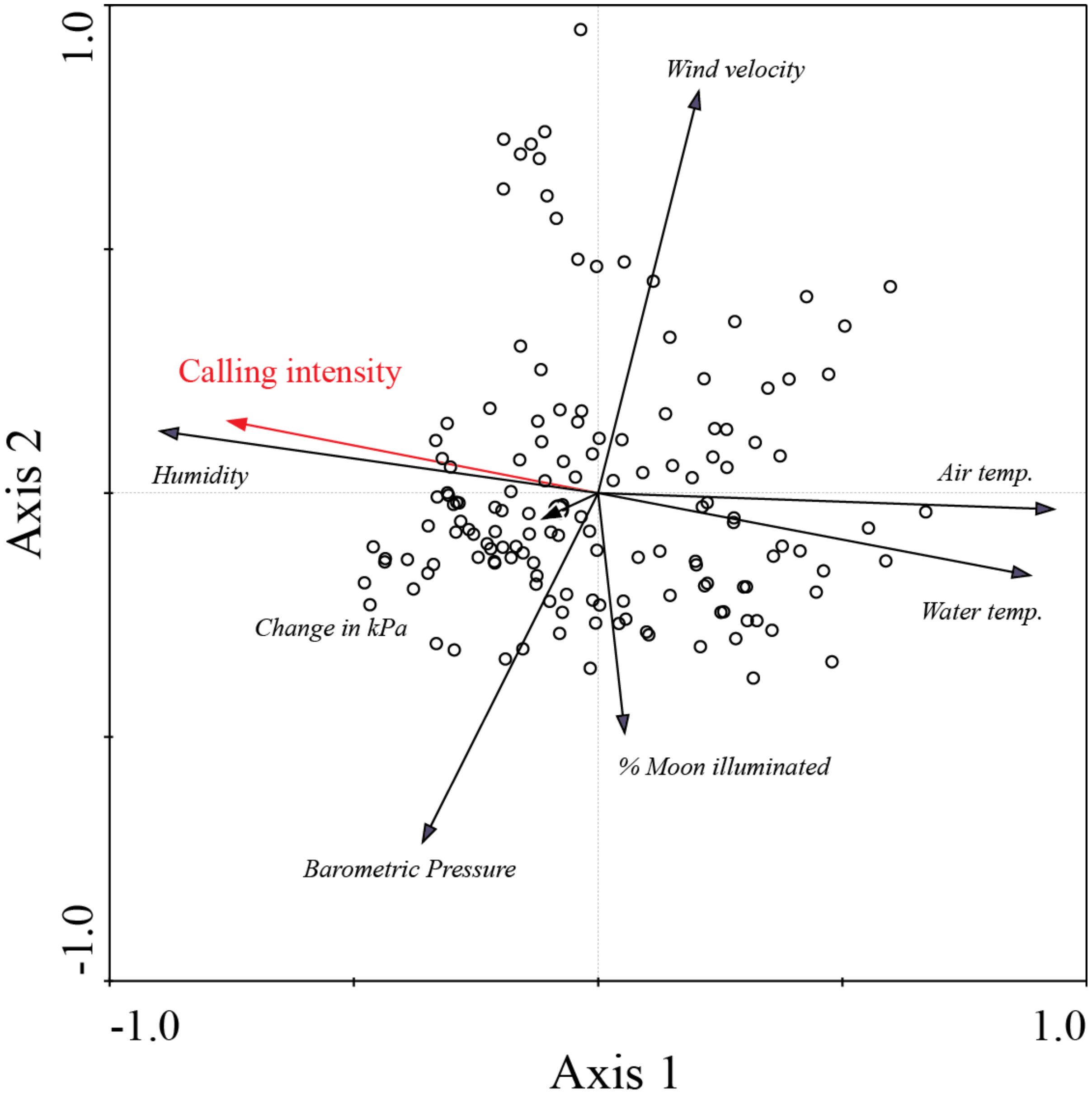


Figure 6.5 A biplot showing a principle component analysis (PCA) analysing seven micrometeoroligcal variables (wind velocity, m.s^{-1}; air and water temperature, °C; percentage of moon illuminated, %; barometric pressure, kPa; change in barometric pressure, ΔkPa; and relative humidity, %) and calling intensity.

Table 6.1 Descriptive statistics for detection accuracy measured for recording nights (n=15).

	Mean	Range	Standard deviation
% False Positives	4.7	0–40.9	10.9
% False Negatives	27.8	9.4–86.6	21.6
Total clicks detected	7609.1	17–31337	10756.3
Predicted click-count	8498.2	21–34390	11722.5
% Detected from predicted	**82.8**	**54.2–91.5**	**10.3**

Table 6.2 Descriptive statistics of the nine atmospheric or meteorological conditions measured. SD = standard deviation.

Atmospheric variable	Mean	Range	SD
Number of click-notes per hour	884.5	0–6309	1332.4
Wind Velocity (m/s)	0.920	0.1–4.4	0.835
Relative Humidity (RH%)	53.1	6–94	23
Barometric pressure (kPa)	871.3	864.1–875.6	2.6
Barometric pressure change (|ΔkPa|)	0.283	0–1.4	0.276
Ambient temperature (°C)	9.8	-0.2–27.2	6.6
Water temperature (°C)	12.7	8.5–17.6	2.1
Percentage of moon illuminated (%)	55.5	0.1–100	49.6

Table 6.3 Coefficients* calculated from a linear regression to compile a fixed model (enter-method) with the independent variables with the highest significance of influence on calling intensity.

Model (Significance, *P*<0.001)		Unstandardised Coefficients		Standardised Coefficients	t	Significance ***P*≤0.05**
		B	Std. Error	Beta		
1	(Constant)	-51150.77	44300.38		-1.155	.250
	Hour (1–16)	-49.74	30.01	-.173	-1.657	.100
	Wind Velocity (m/s)	**700.89**	**170.04**	**.439**	**4.122**	**.000**
	Relative Humidity (RH%)	**-17.35**	**8.77**	**-.300**	**-1.979**	**.050**
	Barometric pressure (kPa)	60.08	50.99	.117	1.178	.241
	Barometric pressure change (|ΔkPa|)	634.79	375.96	.131	1.688	.094
	Ambient temperature (°C)	**-161.68**	**37.63**	**-.804**	**-4.296**	**.000**
	Water temperature (°C)	149.77	110.61	.237	1.354	.178
	Percentage of moon illuminated (%)	-2.10	2.15	-.078	-.977	.331

Note: Significant variables in the model are in bold.

**Dependent Variable: Number of click-notes per hour, not log(x+1) transformed.*

The linear regression model produced three atmospheric variables that had a significant influence on calling activity (Table 6.3). As the night progressed, temperatures fell considerably and calling activity increased. Therefore ambient temperature was negatively correlated with calling intensity and is included as a significant variable. Due to the negative correlation of humidity to temperature, humidity was positively correlated with calling intensity. Wind velocity was also shown to have a significant contribution ($P = 0.015$) to calling intensity and displayed a positive correlation. In a Pearson correlation between the variables, RH was not significantly correlated (*P*=0.052; one-tailed), with calling intensity, (Appendix D). Variables that showed significant correlations in the Pearson one-tailed correlation matrix, but not included in the model, were Change in barometric pressure (|ΔkPa|) (*P*=.047; r=0.140), Water temperature (*P*=0.035; r=-0.151) and Percentage of moon illuminated (*P*=0.017; r=-0.177).

6.4 Discussion

The results of this study show that calling activity of *A. quecketti* is influenced by air temperature, RH and wind velocity. Results were based on more than 127 000 click-pulse detected observations, extending over 144 hours of nine 15-hour biweekly recordings. Passive acoustic monitoring and automated species identification algorithms made this approach possible, which, together with a nearby mobile weather station, permitted us to assess the effects of seven meteorological conditions on calling activity of *A. quecketti* in detail. The temporal scale of this study can aid in future passive acoustic monitoring to detect population changes as a result of climate change (Stewart 1995).

6.4.1 Monthly and diel variation in calling activity

Two peaks in calling activity were observed over the nine-week acoustic monitoring period. One period of more intense calling activity was between 2013/06/23 and 2013/07/08 and another between 2013/08/06 and 2013/09/05. There are a number of advantages to being an early and/or late winter breeder, including minimal acoustic interference with males of other species (Littlejohn 1977). Due to the winter-breeding habits of *A. quecketti*, there were no other frog species calling at the same space and time as this study and they are subsequently classified as calling in a monospecific chorus. Diel variation in calling activity in *A. quecketti* had one peak between 00h00 and 02h00 and two smaller peaks at around 20h00 and again at 04h00. Ospina *et al.* (2013) found a similar hourly variation in four *Eleutherodactylus* spp. with a peak in calling activity around 01h00, and another peak at around 05h00. During the dawn chorus (at 05h00), *Eleutherodactylus* spp. males move from calling to retreat sites (Woolbright 1985; Ospina *et al.* 2013), while producing aggressive calls to defend retreat sites (Stewart & Rand 1991, 1992). Although the behaviour of *A. queckettii* was not monitored in the present study, territoriality in this species is known to occur (Viviers 2013; Chapter 5). Furthermore, being most active when people, and therefore traffic, are least active is an added advantage when communicating in an urban environment. Calling in the early hours of the morning between 00:00 and 03:00 eliminates most of the acoustic noise interference.

6.4.2 Calling activity and species responses to weather variables

Calling intensity of males of *A. queckettii* was inversely correlated with temperatures and slight increases in wind velocity. Although it is not uncommon for frogs to be active at low temperatures (Navas 1997, 2006), the literature shows that the majority of anurans will wait for more favourable temperatures for breeding (Kam & Chen 2000; Chen *et al.* 2001; Oseen & Wassersug 2002; Hsu *et al.* 2006; Lemckert *et al.* 2013; Ospina *et al.* 2013), including winter-breeding species (Kirlin *et al.* 2006). However, males of *A. queckettii* preferred calling at low temperatures in early and late winter (calling intensity peaking at around 01h00 and again at 04h00). A possible explanation for this unusual behaviour is that sound travels further in certain atmospheric conditions, especially at night when temperature gradients are high, causing temperature inversions (Larsson & Israelsson 1991; Ovenden *et al.* 2009). Furthermore, temperature inversions occur at a thin layer where a normal decrease in temperature with height shifts to temperature increasing with height (Schreiber & Beckenbauer 2013). Temperature inversion causes upward directed sound waves to be refracted downwards because sound speed is proportional to the square root of the absolute temperature (Schreiber & Beckenbauer 2013). Temperature inversion is often observed during clear nights when the ground cools off by thermal radiation. Temperature, and therefore sound speed as well, escalate with height, and the sound waves are refracted toward the ground, which is comparable with the sound propagation on downwind conditions (Schreiber & Beckenbauer 2013). The distance of the temperature inversion can vary between 17 m at night in the summer and as low as seven meters in winter (Schreiber & Beckenbauer 2013). The lower the boundary of the temperature inversion is, the more sound (i.e. including sound with lower intensities) is redirected towards the ground. For example, traffic noise can be louder than expected over a distance of 300 m in some atmospheric conditions, because sound can rebound from a boundary layer that originates from wind shear or temperature layers at a height of 30–50 m. (a wind shear is where wind speed increases considerably with height, particularly when there is a relatively sharp boundary between low and higher wind speeds; Ovenden *et al.* 2009).

Except for low temperatures, calling activity in *A. quecketti* is also positively correlated to RH (P=0.05), with mean RH ranging from 58–74% between 00:00 06:00 in the morning. Together with low temperatures, the temporal offset of calls coincides with atmospheric conditions that minimises sound attenuation through atmospheric absorption (Larsson & Israelsson 1991; L'Espérance *et al.* 1995; Harris 1966; Schreiber & Beckenbauer 2013). *Amietia quecketti* has a complex frequency structure, with a dominant frequency ranging from 703–2109 Hz for click-notes and 703–1500 for whine-notes (Chapter 5). Although higher-frequency sounds attenuate more rapidly with distance (Morton 1975; Lawrence & Simmons 1982; Surlykke 1988; Römer & Lewald 1992), attenuation is very low for sound propagated in low temperatures (0–5°C) together with high RH (50–80%), which explains why sound travels further at night (Harris 1996). Furthermore, low wind velocities have been shown to carry sound further than it would travel in its absence (Larsson & Israelsson 1991; L'Espérance *et al.* 1995). A decrease in chorus attendance of both male and female individuals of *Hyperolius marmoratus* during windy conditions is thought to prevent or allow recovery from desiccation (Henzi *et al.* 1995). However, males of *A. quecketti* call mostly while in water or close to the water's edge (Viviers 2013), thereby overcoming the problem of desiccation, or having water close-by to rehydrate if necessary.

In a Pearson correlation matrix, calling intensity of *A. quecketti* is negatively correlated to percentage of moon illuminated (P=0.017; r=-0.177), although not included by the regression model. Several other species show a response to lunar activity, for example orientation when the moon is visible (for summary of species see Wells 2007), are less active (Fitzgerald & Bider 1974; Backwell & Passmore 1990) or decrease calling activity on moonlit nights (Wells 2007). General lunar phobia behaviour is mostly attributed to predation avoidance (Grafe 1997; Wells 2007). Conversely, *Smilisca sila* is more likely to call in the open on moonlit nights than on dark nights, presumably to better detect bats that prey on the species (Tuttle & Ryan 1982; Nunes 1988). Diego-Rasilla & Luengo (2002) demonstrated that *Triturus marmoratus* orientated towards celestial bodies even when magnets were used to disrupt the ambient magnetic field. In the current study, *A. quecketti* displays the tendency to be more acoustically active during nights when moon illumination is low, i.e. new moon. This might may also be attributable to anti-predatory behaviour due to its tendency to sometimes call on floating vegetation in the open or on banks with little vegetation cover (Viviers 2013). It is also possible that it is because of a slight negatively correlation of percentage illumination to wind speed, although no collinearity was found between these variables (Appendix D).

6.4.3 Future studies

Due to the wide distribution of *A. quecketti*, there might be differences in temporal offset in calling activity, and hence breeding activity, across a latitudinal and altitudinal gradient (Terhivuo 1988). This study can be used as a baseline to identify differences in calling activity across higher or lower latitudes to determine effects of climate change. Furthermore, because of the wide habitat distribution across an urban-rural landscape (Chapter 2), calling activity across this gradient can be quantified to determine if micro-climates within cities affect breeding offset within narrow dispersion ranges.

CHAPTER SEVEN

Attitudes of people towards frogs – implications for conservation

"Most amphibia are abhorrent because of their cold body, pale colour, cartilaginous skeleton, filthy skin, fierce aspect, calculating eye, offensive smell, harsh voice, squalid habitation, and terrible venom; and so their Creator has not exerted His powers to create them".

– Carolus Linnaeus 1758

7.1 Introduction

Attitudes toward animals are shaped by our ideas, values, folklore, myths, perceptions, knowledge and the nature of existing relationships between animals and people (Thornhill 1993; Davey *et al.* 1998; Arrindell 2000; Drews 2002; Knight 2008; Prokop & Tunnicliff 2010). Whether endangered or not, not all species hold sufficiently enough beauty to be appreciated and adored by humans. Existence of mistaken ideas and negativistic attitudes causes a subjective approach to the conservation of unpopular species (Gibbons 1988; Knight 2008; Ceríaco 2012). Frogs and reptiles, or herpetofauna, are among the most maligned groups of vertebrates by humanity (Capitão 2009; Ceríaco 2012).

Besides the diverse superstition that exists in a variety of sociodemographic groups (Capitão 2009; Minter 2005; Ceríaco 2012) frogs were considered to have very little impact on everyday human life mainly because they are harmless to humans. As a result of drastic knowledge increase of their importance in all ecosystems (Buffico & Úbeda 2006; Lopez *et al.* 2009) and contributions to medicine (Gomes *et al.* 2007; Samgina *et al.* 2012), in combination with the alarming declines in frog populations worldwide over the past three decades (Stuart *et al.* 2004; Mendelson *et al.* 2006; Allentoft & O'Brien 2010), it is of utmost importance for the wellbeing of amphibians and of humans that society learns to appreciate the value of these slandered and poorly understood animals.

The relationship between frogs, research, and human society may seem vague for many people. Apart from remembering (not necessarily warm-heartedly) the frog dissection in biology class, most people would find it difficult to describe even a single contribution frogs have made to civilisation. However, in the past, frogs have played important roles in scientific accomplishments, for example pregnancy testing and models in pharmacological research (Elkan 1938; Simmaco *et al.* 1996). Though not well-known to the general public, current research suggests they will continue to do so (Arch *et al.* 2009; Samgina *et al.* 2012; Ferguson *et al.* 2013; Hartigan *et al.* 2013).

Literature shows that negative attitudes towards amphibians are a global trend (Tomažič 2011; Capitão 2009; Knight 2008; Czech & Krausman 2001; Minter 2005). In a study conducted by Minter (2005) as part of her masters' mini-dissertation, she found that apart from the negative attitudes held by South African Grade 7 learners towards frogs, they also do not appreciate the value of frogs in the environment and are unaware of their conservation needs. Minter (2005) also included five proposed lessons in her dissertation in order to promote knowledge about, and positive attitudes towards frogs and conservation. Gordon (2009) built on this study and investigated prior knowledge that South African Grade 7 learners and their educators have about amphibians in order to develop a resource pack for future improvement of teaching science. Gordon found that both educators and learners showed severe limitations in their knowledge base of scientific concepts about amphibians, partly due to language difficulties.

The consequences of the superstitions, folklore, dislike of frogs or even batrachophobia (fear of amphibians) among people extends further than a few annual frog deaths (Capitão 2009; Ceríaco 2012). As an indirect consequence, negativistic attitudes toward frogs (and other disliked animals) results in a lack of attention in the fields of conservation and funding (Gibbons 1988; Czech *et al.* 1998; Knight 2008; Ceríaco 2012). Thus, attitudes of people towards an animal group are critical to the long-term conservation needs in several respects.

Some countries seem to realise the importance of the role in public participation in conservation action. Frogs and their enthusiastic and entertaining supporters have captivated Australians. Frog and tadpole study groups, herpetological societies, landcare groups, recovery teams, zoos, science teachers and conservation groups participate in habitat rehabilitation and survey/monitoring activities of threatened as well as non-threatened amphibians (Ehmann 1999). Unfortunately, a negative consequence of such activities that result in aesthetic appeal of the animals, compassion, affection and a desire to please and stimulate children is the global increase of the possession of wild animals as pets (Drews 2002; Pagani *et al.* 2007; Prokop & Tunnicliff 2010). These positive feelings and a mistaken empathy for animals boomerang by condemning these pets to an unfamiliar environment and insufficient care (Drews 2002).

South African frogs have become popular in the pet trade as well (see Appendix E: Pet Trade). Some southern African species available at international pet stores include the Giant Bullfrog (*Pyxicephalus adspersus*), African Bullfrog (*Pyxicephalus edulis*), Bubbling Kassina (*Kassina senegalensis*), Red-legged Kassina (*Kassina maculata*), Banded Rubber Frog (*Phrynomantis bifasciatus*), Mottled Shovel-nosed Frog (*Hemisus marmoratus*), and Clawed Frog (*Xenopus laevis*) which are amongst 56 species from a variety of countries (Appendix E, Pet trade: Frogs for sale). One South African frog that has become particularly famous in recent months is the Desert Rain Frog (*Breviceps macrops*). Dean Boshoff uploaded a video on YouTube of this frog making a defensive scream-like call. This video received nearly 10 million views (by March 2014) and even made the international news with broadcasts on CNN and other prominent TV stations (see Appendix E: Desert Rain Frog).

Scores of species at risk because they are of no use

LONDON: The spoon-billed sandpiper, three-toed sloth and a long-beaked echidna named after Sir David Attenborough are among the 100 most endangered species in the world, according to a new study.

The list of at-risk species has been published as conservationists warn that rare mammals, plants and fungi are being sacrificed as their habitats are appropriated for human use.

pairs of the birds left in the wild and their number drops by a quarter annually.

There are also just 500 pygmy three-toed sloths left on the uninhabited Isla Escudo de Veraguas, 16km off the coast of Panama. They are half the size of sloths found on the mainland and are the smallest and slowest sloths.

But their numbers are declining with fishermen and lobster divers "opportunistically"

Figure 7.1 Newspaper extract from *The Independent*, Tuesday 11 September 2012.

With reference to South African frogs, Branch & Harrison (2004) noted that amphibians feature well in folklore and scientific research, but perform badly in comparison to the devotion mammals enjoy. They also state that in the existing conservation paradigm that highlights sustainable utilisation, frogs are largely neglected because they are considered to have limited value in ecotourism or in the upliftment of disadvantaged communities (Branch & Harrison 2004; Figure 7.1).

Volunteering can be defined as pro-social behaviour, done of one's own free will and without monetary reward, to benefit another person, group or cause (Noble 1991; Clary *et al.* 1998; Cordingley 2000; Penner 2004; Measham & Barnett 2008; Finkelstien 2009). Volunteering is central to democracy (Verba *et al.* 1995) and plays a fundamental part in political, social and economic systems (Warburton & Oppenheimer 2000; Measham & Barnett 2008). However, it was only recently that the importance and value of volunteerism has been evaluated and became a growing area of research (Warburton & Oppenheimer 2000; Clary & Snyder 2002; Newman *et al.* 2003; Bekkers 2007; Dolcinar & Randle 2007). Research suggests a positive relationship between levels of voluntary activity, physical health and life satisfaction (Whiteley 2004). A key area of growing interest is the role that environmental volunteers play in natural resource management (Ryan *et al.* 2001; Byron & Curtis 2002; Hunsberger *et al.* 2005; Measham & Barnett 2008). However, some researchers have raised questions about the reliability of data quality and methodological comparability regarding studies that, for the most part, rely on volunteers for labour and data acquisition (Fore *et al.* 2001; Newman *et al.* 2003; Measey 2011). Despite volunteers having been perceived as amateurs in the past, they have contributed to ecological understanding for centuries through collecting botanical specimens and participating in monitoring programs such as the bird banding/ringing programs or the National Audobon Society Christmas Count (Fore *et al.* 2001; Gouveia *et al.* 2004; Measham & Barnett 2008) and are continuing to do so (Abolafya *et al.* 2013; Chapter 4; this study). This collaboration of the general public with professional scientists is aptly called citizen science. Citizen science is well developed in other countries as seen from the examples above, but limited in South Africa. The largest citizen science project may be through an online portal in collaboration with the South African National Biodiversity Institute where citizens can capture images of plants, invertebrates, amphibians, reptiles, birds, fungi, fish, mammals and diseases and share identifications, comments and knowledge.

For these reasons, it is essential to understand why people volunteer and what motivates them in order to enhance the recruiting process and also to better provide the basic needs for volunteers to keep them motivated. There has been considerable research conducted on the motivations for volunteerism (Clary *et al.* 1998; Clary & Snyder 2002; Dolcinar & Randle 2007). Most of the research explains the motivation of volunteers by compiling a volunteer functional index (VFI) by using a set of questions which requires volunteers to rate their agreement to each statement. One particular VFI used by a number of authors is that proposed by Clary *et al.* (1998) (see Clary & Snyder 2002; Penner 2002; Stukas *et al.* 2002). The authors use a functionalist approach to volunteering, examining the functional motives individuals have for choosing to volunteer. The scale is divided into 6 separate functional motives (or factors), including: 1) Protective motives - a way of protecting the ego from the difficulties of life; 2) Values - a way to express ones altruistic and humanitarian values; 3) Career - a way to improve career prospects; 4) Social - a way to develop and strengthen social ties; 5) Understanding - a way to gain knowledge, skills, and abilities; and 6) Enhancement - a way to help the ego grow and develop.

This study had two main objectives, namely to 1) explore the attitudes of Potchefstroom residents towards frogs in general, which will enable us to provide insight into the extent that people like or dislike frogs, and 2) to assess the motivation and perceptions of volunteers for saving Western Leopard Toads (*Amietophrynus pantherinus*) from getting run over by traffic in the city of Cape Town.

7.2 Materials and methods

7.2.1 Study areas

Potchefstroom, North-West Province

A questionnaire survey distributed to 295 people in the district of Potchefstroom, North-West Province, South Africa, was used to evaluate the attitudes of citizens towards frogs. Potchefstroom is a multi-cultural town of 2 573 km^2, accommodating 162 762 residents (Tlokwe City Council, Census 2011). Potchefstroom can be considered an academic city, since it hosts one of three North-West University campuses (Figure 7.2).

Cape Town, Western Cape

Hundreds of *A. pantherinus* adults migrate from their foraging habitat in fynbos heathland, farmland, suburban gardens, and urban open areas to breeding sites in large wetlands, vleis, dams, and sluggish water in lowland fynbos heathland, as well as in altered habitats with permanent water bodies (IUCN 2013). These animals are then caught from the roads in the city of Cape Town (Western Cape Province, South Africa) by volunteers, saving them from possibly getting run over by traffic (see Chapter 4). Volunteers cover over 20 suburbs where the toads migrate to and from breeding ponds between late July and early September and again when the metamorphosed toadlets emerge in their thousands between October and January. Volunteer groups are overseen by eight coordinators which cover the suburbs of Muizenberg, Frogmore Estate, Kirstenhof, Lakeside, Marina Da Gama, Tokai, Blue Route, Constantia, Nova Constantia, Steenberg, Sweet Valley, Westlake, Zwaanswyk, Grassy Park, Lotus River, Phillipi, Zeekoeivlei, Bergvliet, Die Oog, Meadowridge, Plumstead, Retreat, Observatory, Ottery, Pinelands, Hout Bay, Capri, Clovelly, Fish Hoek, Glencairn, Kommetjie, Sun Valley and Noordhoek.

7.2.2 Participants and procedure

Potchefstroom

Two survey methods were deployed to ensure the inclusion of people residing in rural communities as well as suburban communities. Citizens were approached and asked whether they would be willing to participate in the survey. The questionnaire was facilitated by both Sakele Magodla (employed at the department of Zoology at the North-West University) and the researcher. Sakele helped in verbally translating the questionnaire to Sotho-, Tswana- or Zulu-speaking residents that had difficulty understanding English (Figure 7.2). We visited Ikageng and Promosa suburbs, the local taxi ranks as well as the Potchefstroom Industria and the Mooiriver Mall to include citizens that do not have access to internet. Although this sample of respondents is not representative of Potchefstroom, the aim of this study was only to investigate the general attitudes.

Studies have shown that web-based surveys are more reliable than telephonic interviews (Braunsberger *et al.* 2007). Citizens of Potchefstroom were also given the option to access the questionnaire online via a link (https://docs.google.com/forms/d/1xEoXNtYnPKKkqQ-CYK6fgtAyJH9sCXNICWtv-x0oQXc/viewform for English and https://docs.google.com/forms/d/1YxGWKnfP9-jXzFmunDmlfngKPD6Xy3gQ3O_XkObRSiM/viewform for Afrikaans). The questionnaire was made available via the North-West University intranet, where citizens were asked to share this with friends, family and colleagues residing in Potchefstroom. However, this has made the results slightly biased towards university staff members. See Appendix F for questionnaire distributed to Potchefstroom citizens.

Cape Town

Cape Town volunteer coordinators were asked to distribute an electronic questionnaire. Volunteers were asked to access the questionnaire via the following link: https://docs.google.com/forms/d/122k0LzfBn0bb81Kn6ISLGltFVPx7M_Gy6tLsX-iPpLw/viewform (see Appendix G given to Western Leopard Toad volunteers). Both the electronic questionnaires were compiled using Google Docs that provides live and immediate access to the feedback. Feedback was downloaded in a Microsoft Excel file format.

Approach towards attitude study

A functionalist approach was applied to question the attitudes of people towards frogs. A set of 15 questions were compiled to rate four attitude dimensions of people towards frogs (Table 7.1). Interested attitude dimensions included:

- Liking - five questions were used to determine to what extent people liked or disliked frogs.
- Inquisitive - one question (Would you like to learn more about frogs?) were used to establish if people were keen on knowing more about frogs.
- Knowledge - five questions were used to determine the level of general knowledge people have regarding frogs. Question 5 (Table 7.1) was shared by the themes "Liking" and "Knowledge", as both of these variables can influence the response (Drews 2002).
- Myths / Beliefs – five questions were used to score the level of a variety of myths and beliefs held by people. High scores indicate few myths or beliefs.

Analysis

Each question was scored either -1, 0 or +1, which corresponded to whether people agreed (+1), disagreed (-1) or had no opinion (0) to the question or statement. Responses to the five attitude dimensions were transformed into percentages. Certain questions were negatively scored to adjust the scores in order for it to reflect either an agreement or disagreement of the four attitude dimensions. Participants' scores were calculated to range from 0 - 100% in agreement of the attitude dimension.

Structural equation modeling

Structural equation modelling (SEM) is a multivariate analysis technique for testing and estimating causal relations using a combination of statistical data and qualitative causal assumptions (Ziegler *et al.* 2011). The use of SEMs is common in studies in several fields including conservation biology (Iriondo & Albert 2003), environmental psychology (Corral-Verdugo *et al.* 2008; Ceríaco 2012), and human-wildlife dimensions (Vaske *et al.* 2001). Applications of SEM include causal modeling (path analysis), confirmatory factor analysis, second order factor analysis, regression models, covariance structure models and correlation structure models. A path analysis representing a causal model for the attitudes of Potchefstroom residents towards frogs was staged. The model was compiled using AMOS Version 22 (SPSS 2013) in order to explore the regression of the four attitude dimensions.

Age, gender, race, and education were also added into causal models as demographic covariates (independent variables). Inquisitiveness, knowledge, myths or beliefs and the demographic indicators were specified as predictors for liking frogs or not. Covariances between the endogenous factors (variables within the model) and the rest of the independent factors were estimated.

Figure 7.2 Assistant surveyor Sakele Magodla interviewing residents of the Ikageng township.

Table 7.1 Set of 15 questions and statements within the questionnaire that was used to identify five functions regarding frogs.

	Questions	**Yes / No / Undecided**
1	Do you like frogs?	
2	Are you scared of frogs?*	
3	Will you pick up and handle a frog?	
4	Would you like to learn more about frogs?	
5	It would be best if all frogs were killed.*	
6	Would you consider having a frog picture as the wallpaper or screen saver on your cell-phone/computer?	
7	Do you know that frogs belong to the most threatened group of animals on Earth?	
8	Conservation of wetlands and freshwater habitat may help to reduce loss of frog species and numbers?	
9	It is important to conserve South Africa's frogs	
10	Certain frogs are important to medical research	
11	Touching a frog/toad gives you 'warts' on your hand*	
12	It is safe to collect water from rivers or dams where frogs occur*	
13	Frogs cause lightning*	
14	You should sprinkle salt on a frog's skin*	
15	Frogs spread disease to humans*	

Functions	**Score totals**
Liking, 1 - 3, 5 & 6	-5 to 5
Inquisitive, 4	-1 to 1
Knowledge 5, 7 - 10	-5 to 5
Myths / Beliefs 11 - 15	-5 to 5

*Questions negatively scored.

7.3 Results

7.3.1 Attitudes of Potchefstroom residents toward frogs

Sociodemography

The demography of people interviewed was skewed towards caucasian (White) females, and residents having a higher level of education, but age was normally distributed within male and female participants (Figure 7.3). Female participants covered 57% (169) of the total survey sample and males the remaining 43% (126). Africans (Blacks) included 33% (97) of the total survey, caucasians 66% (196) and coloureds the remaining ≈1% (2). Sixty-three per cent (106) of females and 55% (70) of males had a tertiary qualification.

Attitude dimensions

Overall the 295 surveyed residents of Potchefstroom scored high within each of the four attitude dimensions (Figure 7.4). Generally the sample showed a liking in frogs, with an average score above 50% and the third quartile (Q3) equal to 80%, suggesting a high liking. However, the first quartile suggest a slight dislike in frogs (Q1 = 40%). Inquisitiveness was only tested with one question ("Would you like to learn more about frogs?"; Table 7.1) and revealed a general high interest in learning more about frogs, scoring a mean of 64% (Figure 7.4, also see Figure 7.5). Results from the knowledge dimension scores were also high ($\bar{X}$ = 77%) in accordance with the high Myths / Beliefs-score ($\bar{X}$ = 77%) which suggest that only few people from the sample were either not sure of the myth or believed in some of them (Figure 7.4). When looking at the distribution of the four attitude dimensions it shows that female participants had a greater dislike of frogs opposed to males, i.e. higher proportion of females scored 20% and 40% in the "Liking attitude" dimension (Figure 7.5). Also, a greater extent of dislike of frogs in African participants in comparison to caucasian participants was observed. All participants exhibited more or less the same level of curiosity, i.e. the desire to learn more about frogs. Most participants showed a good level of general knowledge about frogs, except for africans where some uncertainty was observed in a portion of the participants scoring 40%. All participants showed a strong disbelief in myths, except for a fraction of the african participants who showed some belief in certain myths (Figure 7.5).

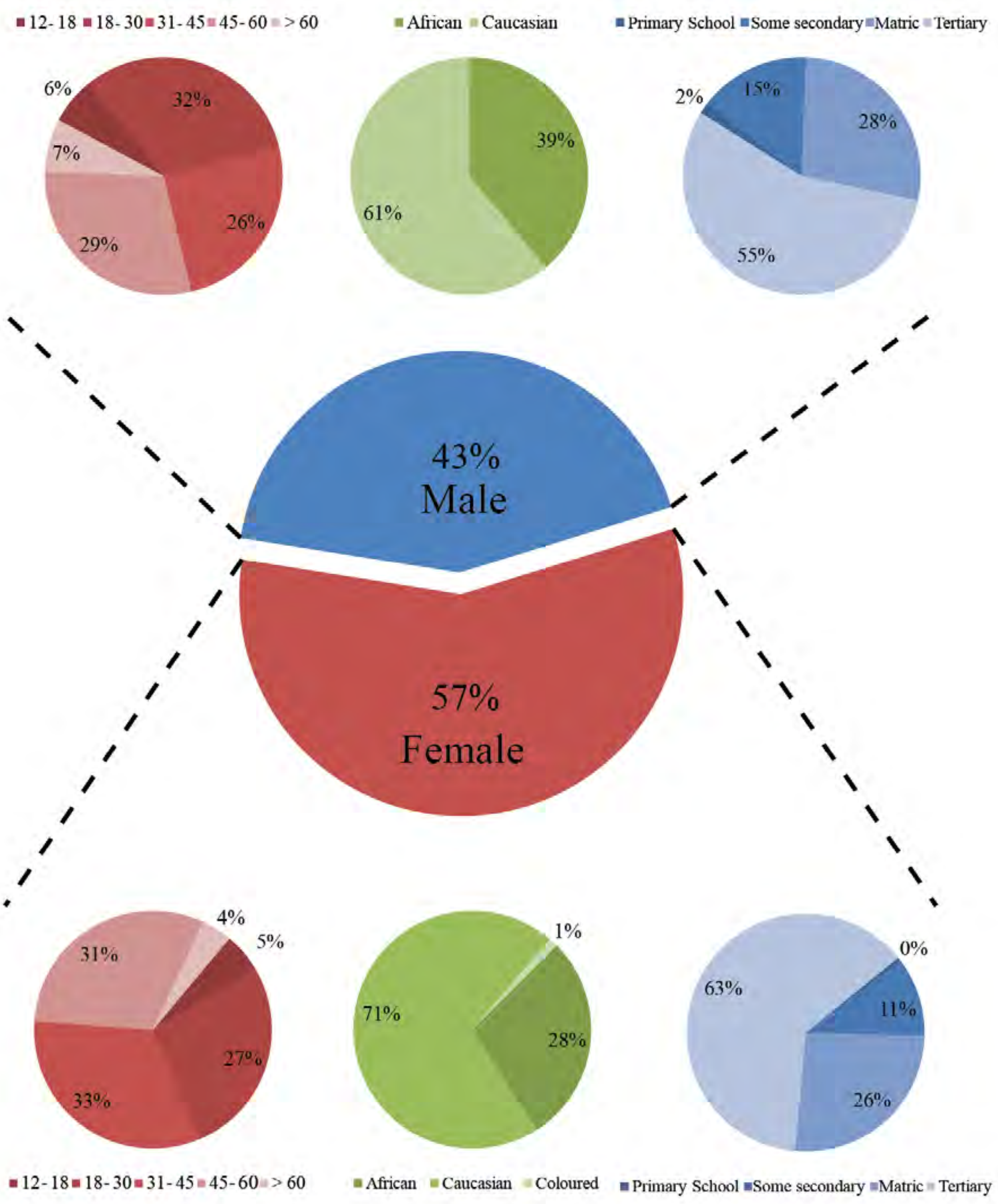


Figure 7.3 A combination of pie charts showing the distribution of the demography between gender (centre chart), age, race and level of education (small charts, left to right respectively) of 295 Potchefstroom residents.

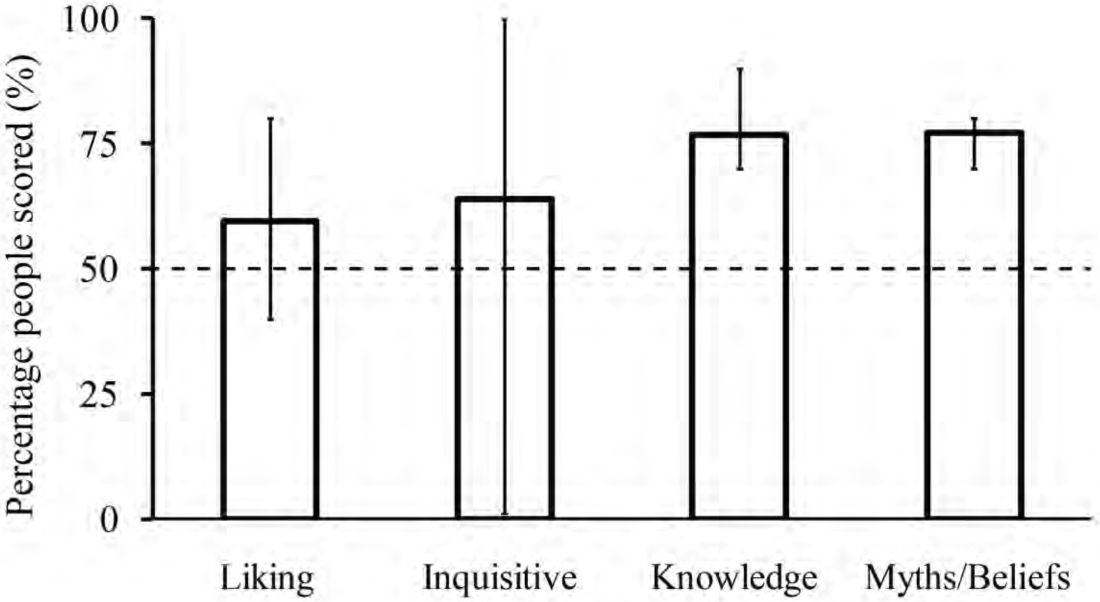


Figure 7.4 Levels of four tested attitude dimensions toward frogs among 295 Potchefstroom residents, showing means and inter-quartile ranges (whiskers). Values above 50% show a general positive inclination toward the attitude, whereas values below 50% reflect a general disapproval (after Drews 2002).

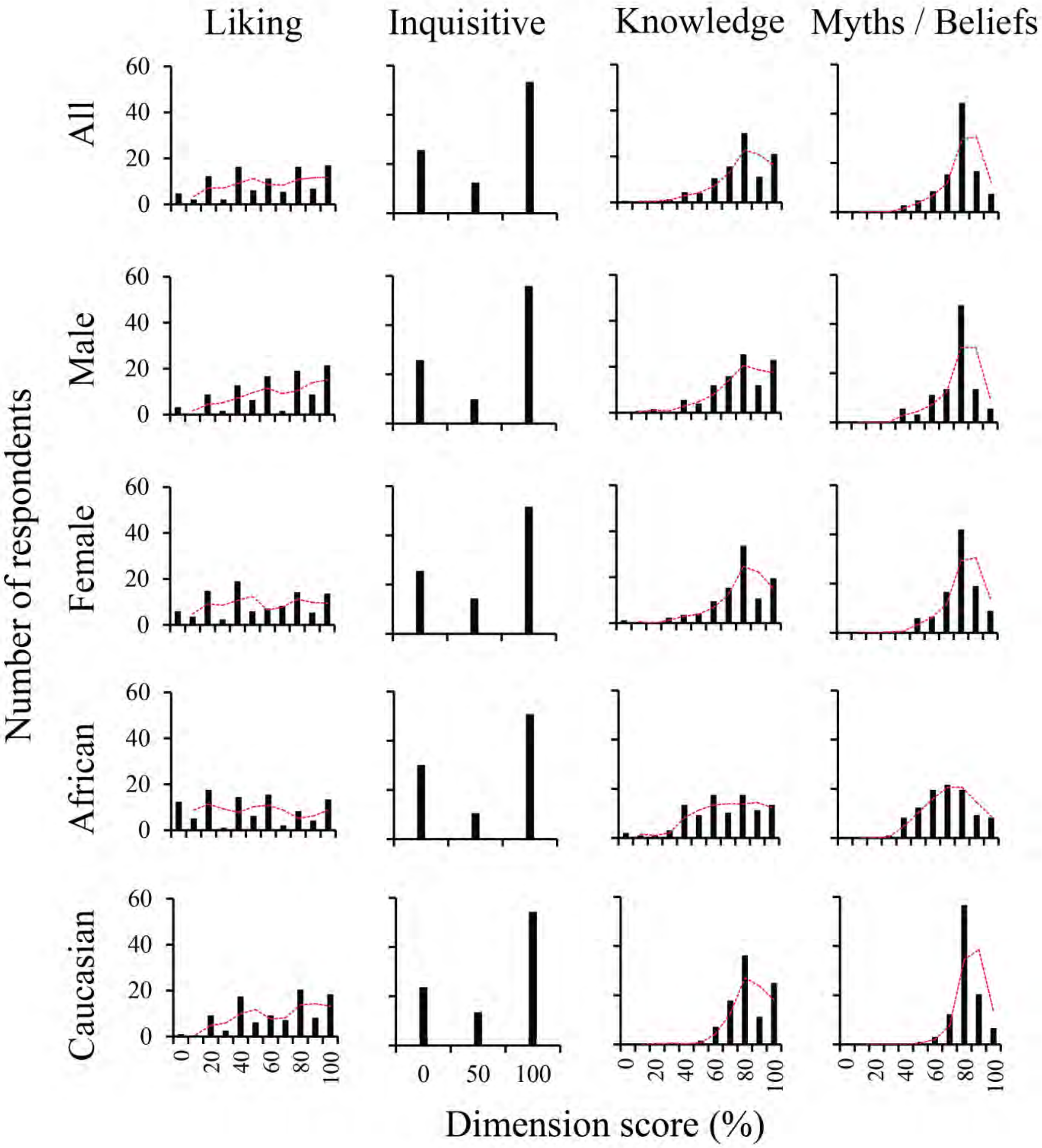


Figure 7.5 Column charts of four tested attitude dimensions toward frogs among 295 Potchefstroom residents, showing the tendencies of the distribution of agreement and disagreement in responses between the entire sample, males, females, africans and caucasians. Values above 50% show a positive inclination toward the attitude, whereas values below 50% reflect disapproval and 50% indicates neutral. The centre of each polygon represents zero, the further away the edge is from the centre, the higher is the proportion of individuals at the respective score. Higher Myth / Belief scores indicate a low belief in myths. Moving average trendline depicted in red dashed line.

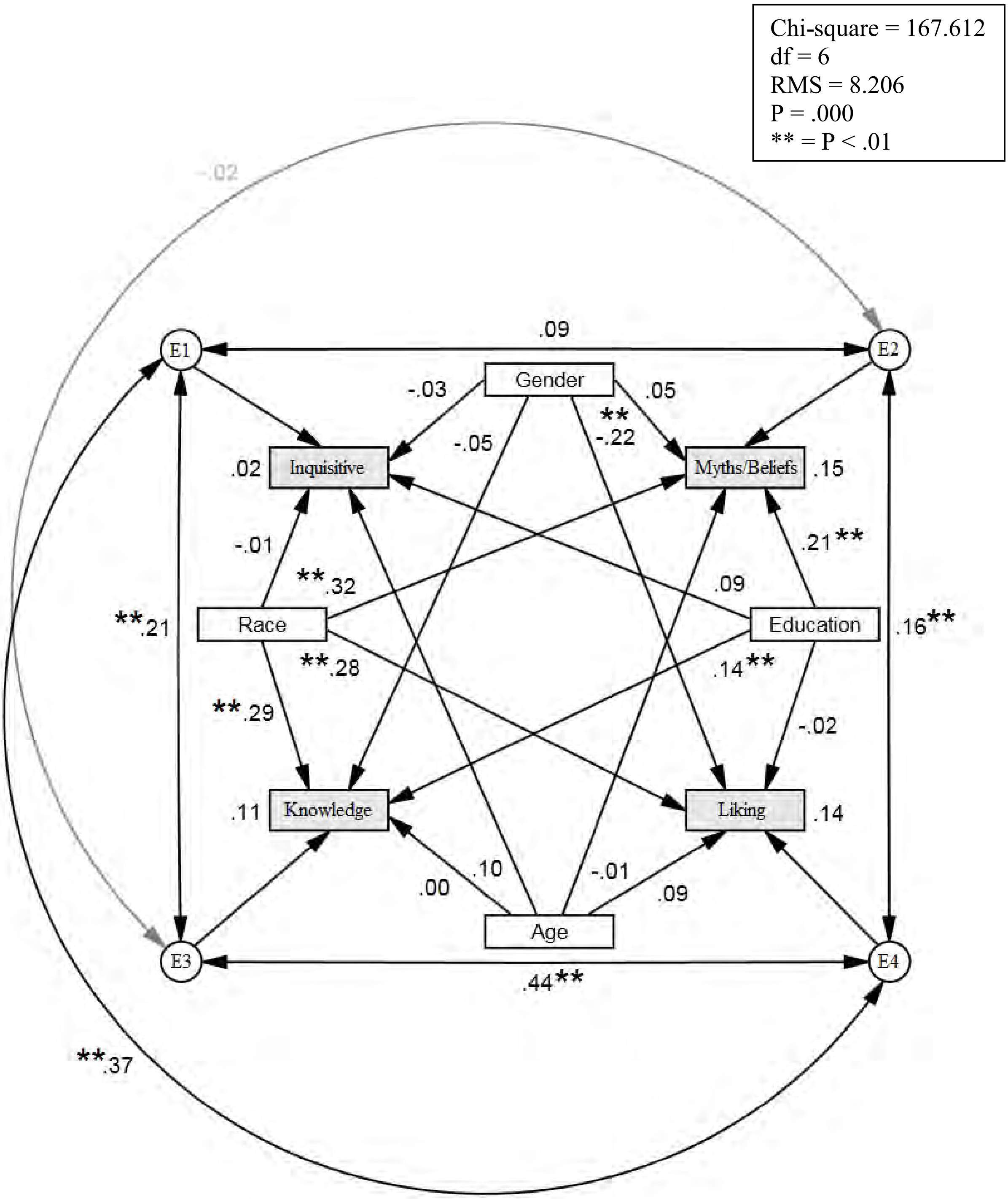


Figure 7.6 Path analysis representing a causal link between the four attitude dimensions and sociodemographic variables that best explained the relationship among them. Path coefficients are indicated for each path (link) between variables. E1-4 = error terms of unobserved independent variables; df = degrees of freedom; RMS = root mean square. Negative correlations with gender indicate favouritism towards females.

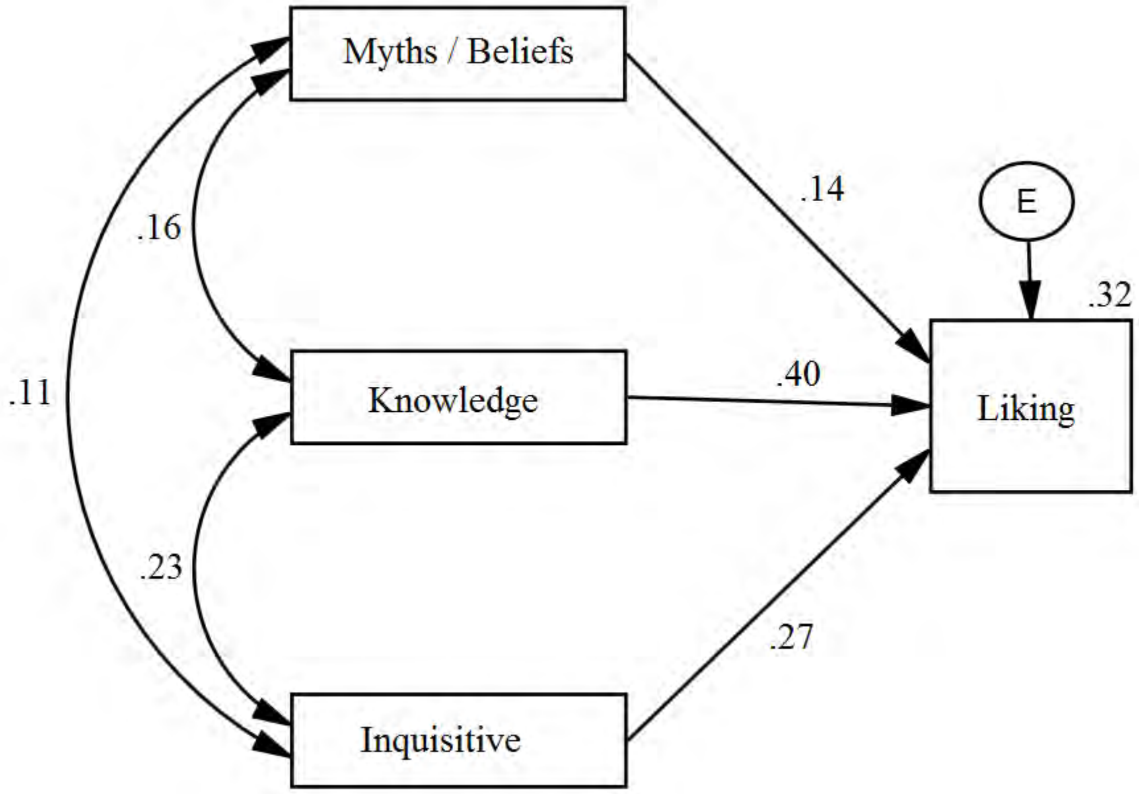


Figure 7.7 Path analysis representing the causal model that best explained the relationship among the measured variables Inquisitive, Knowledge and Myths / Beliefs (treated as independent variables) and Liking (treated as dependent variable) to show the contribution for each of the independent variables ($R^2 = 0.32$). Correlation coefficients are indicated for each path (link) between covariates. Standardised regression weights are indicated for each path between dependent variable and independent variables. E represents the error term (68%) which accounts for exogenous variables (unobserved effects) possible explained by the sociodemographic variables in Figure 7.6. All coefficients are significant ($P < 0.05$), except for Inquisitive – Linking where $P = 0.054$.

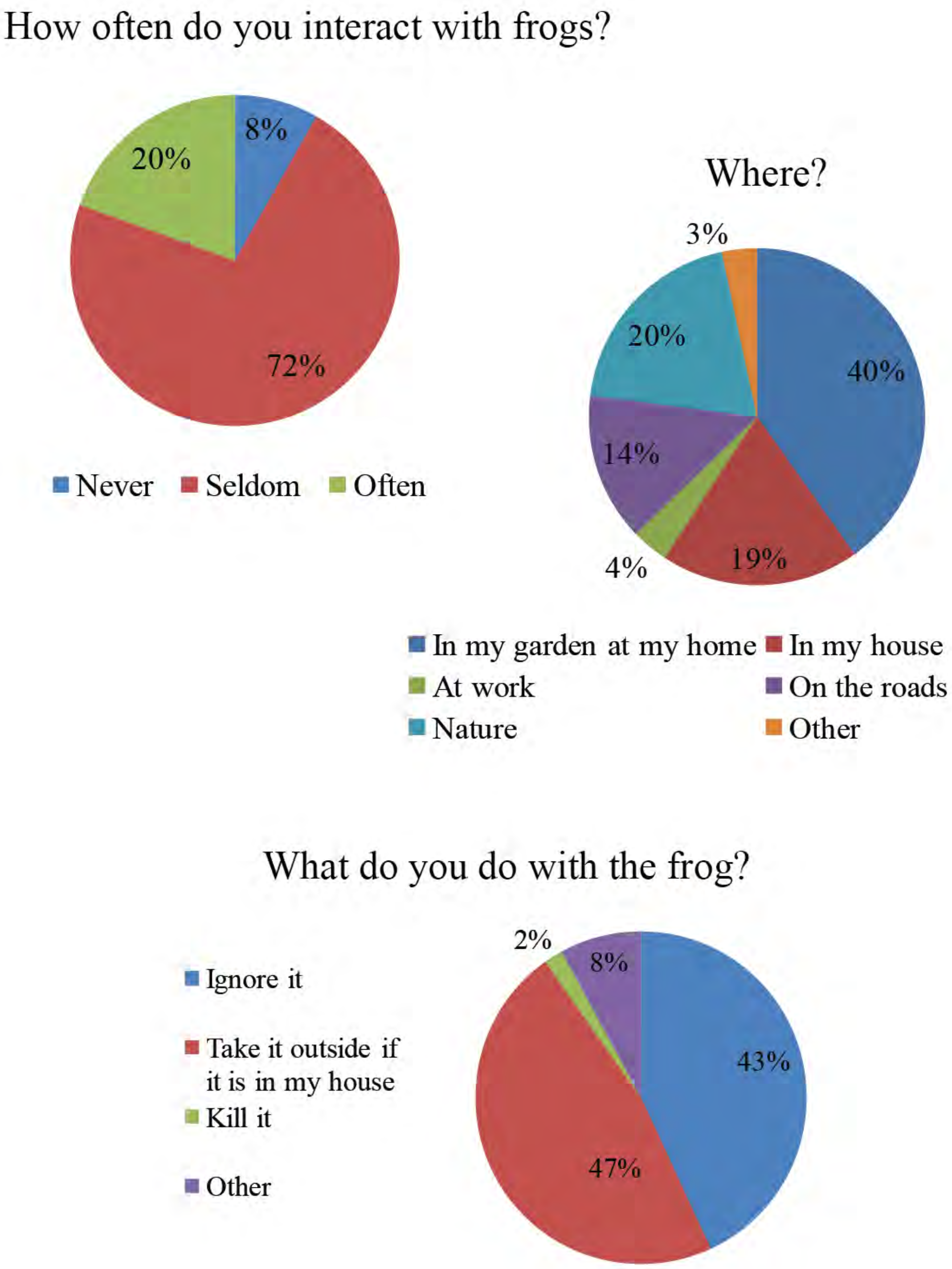


Figure 7.8 Pie charts from the total resident responses of Potchefstroom pertaining to their interaction with frogs. See text for elaboration of "Other".

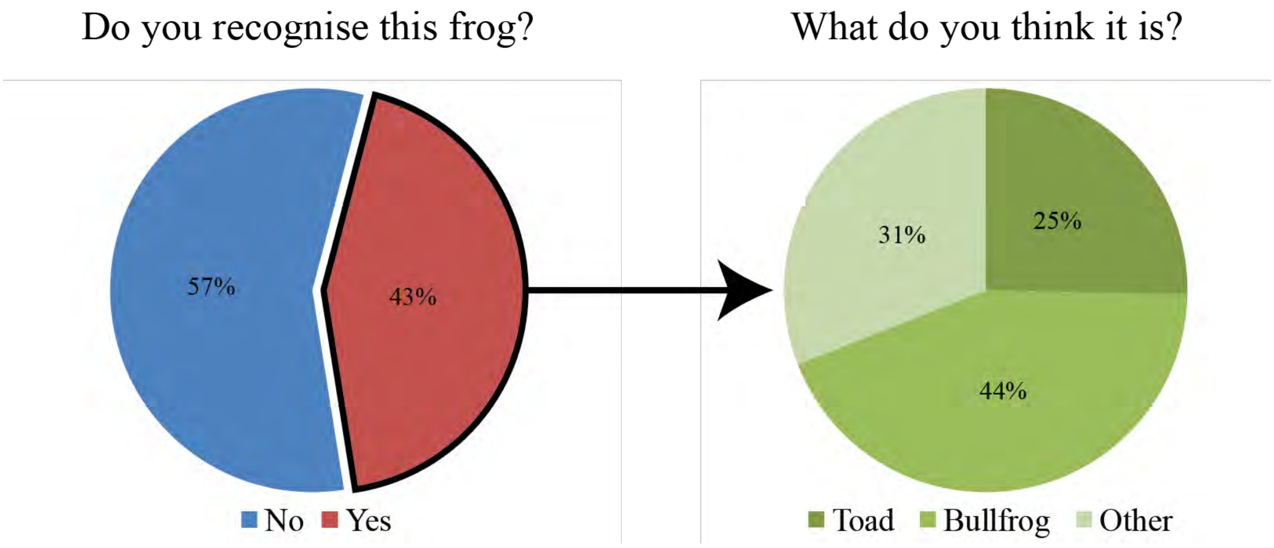


Figure 7.9 Pie charts showing the responses of Potchefstroom residents to the questions "Do you recognise this frog?" and "What do you think it is?" after showing a photo of a Guttural Toad (*Amietophrynus gutturalis*).

Attitude dimensions path analysis

The sociodemographic characteristic education was significantly linked to myths and knowledge; race was significantly linked to myths, knowledge and liking; and gender was significantly linked to myths ($P<0.05$). Unobserved independent variables were significantly correlated, E1‹–›E3 and E4; and E4‹–›E3 and E2 (Figure 7.6). The variation that is explained by unobserved independent variables in this model is most likely elucidated by the variables in the causal model for "Liking" (Figure 7.7) and, conversely, the proportion of variation not explained in the "Liking"-model will likely be described by the model for sociodemographic variables (Figure 7.6).

All correlation coefficients in the path analysis for the degree in which knowledge about frogs, inquisitiveness towards frogs and myths about frogs were correlated to the level of liking frogs were significant ($P<0.05$), except for the correlation between knowledge and liking ($P=0.054$). Knowledge had the highest correlation of 0.40, followed by inquisitiveness (0.27) and lastly myths (0.14). Liking had a relatively high R^2 of 0.32, meaning that 68% of the variation in liking frogs were explained by unobserved variables (Figure 7.7).

Frog interaction and recognition

Only 8% of all participants never interact with frogs, 72% have seldom interaction and 20% often interact with them (Figure 7.8). The most common locations for these interactions (92%) are in the garden at home (40%), in nature (20%), in the house (19%), and on the roads (14%). Other less common locations include: at work, by the river, at the zoo, at the museum, at recreation areas, on the farm, on hiking trips, at friends' house, at the swimming pool and at the fish pond (remaining 7%). When participants (92%) do find frogs, they will take it outside the house if it is inside (47%), ignore it (43%), or kill it (2%). Other actions include: calling someone to catch and remove it, photographing it, pouring water on it to prevent it from dehydrating, avoiding it, taking it someplace to avoid getting it hurt, rescuing it from the pets, scaring people with it or talking to it (8%).

After providing a picture of the common Guttural Toad (*Amietophrynus gutturalis*), participants were asked if they recognised the frog. Fifty-seven percent (167) of the participants did not recognise the toad (5% could not open the image on the internet due to technical problems) and the remaining 43% (128) did recognise the toad (Figure 7.20). One in every four people (25%) identified it as a common toad and 44% (31) branded it as a bullfrog. Other names included native Sotho, Xhosa and Tswana names, including: Ixoxo, Letlametlu (bull / land frog); Lenkogwane, Segodikae, Itshele (Xitsonga), Rain frog, Leopard frog, garden frog and house frog (31%).

7.3.2 Motivations of Cape Town volunteers to save the endangered *A. pantherinus*

The vast majority (92%, 34) of the volunteers were older than 30 years of age and also strongly skewed towards female citizens, which comprised 81% (Figure 7.21). More than a third (35%) of the volunteers has been saving toads for more than four seasons and there is an increase of 16% in the number of volunteers since 2009 (Figure 7.22). Almost all of the volunteers (92%) will continue participating in this voluntary conservation action and the remaining 8% (three individuals) will either continue for one more year or have finished in 2013 due to relocation.

Most of the volunteers (60%) meet co-volunteers at predetermined destinations. Other social groups include family (11%), friends (4%), other volunteers (3%) and partners (2%). The remaining 20% of the volunteers are not surveying roads for toads, but monitoring breeding populations at ponds (Figure 7.23).

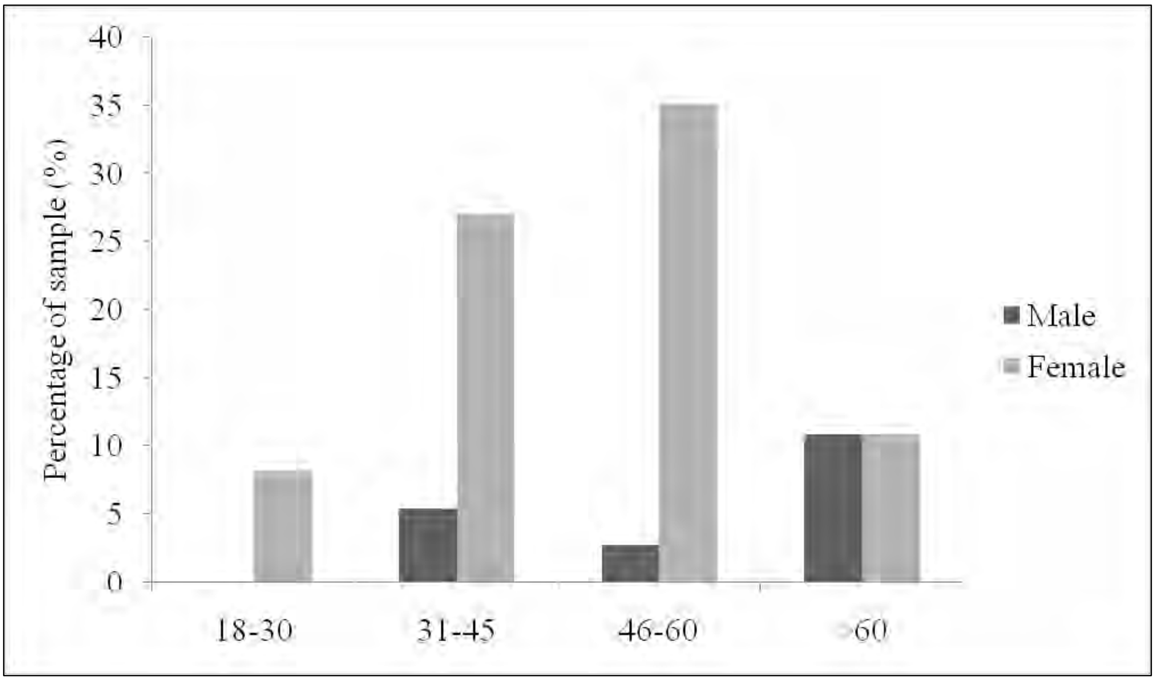


Figure 7.10 Age and gender distribution among 37 volunteers saving the endangered Western Leopard Toad (*Amietophrynus pantherinus*) from the roads during toad-migration in Cape Town.

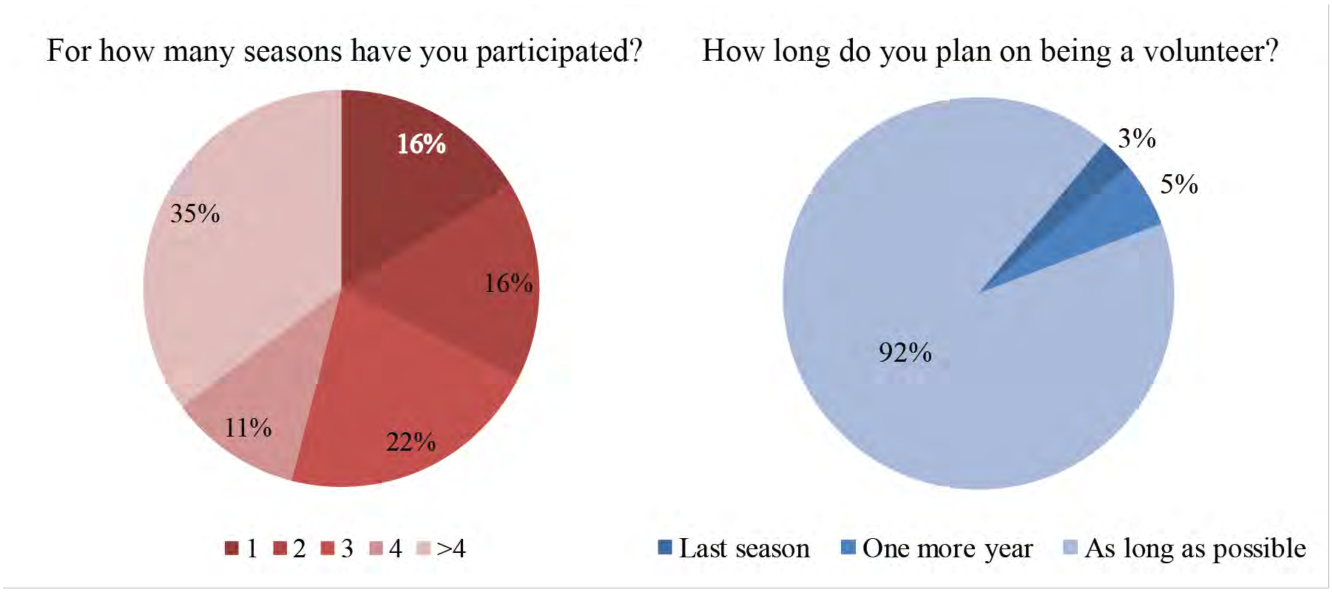


Figure 7.11 Pie charts showing the percentages of the total of 37 volunteers' responses to two questions pertaining to the active period and planned active period of volunteering.

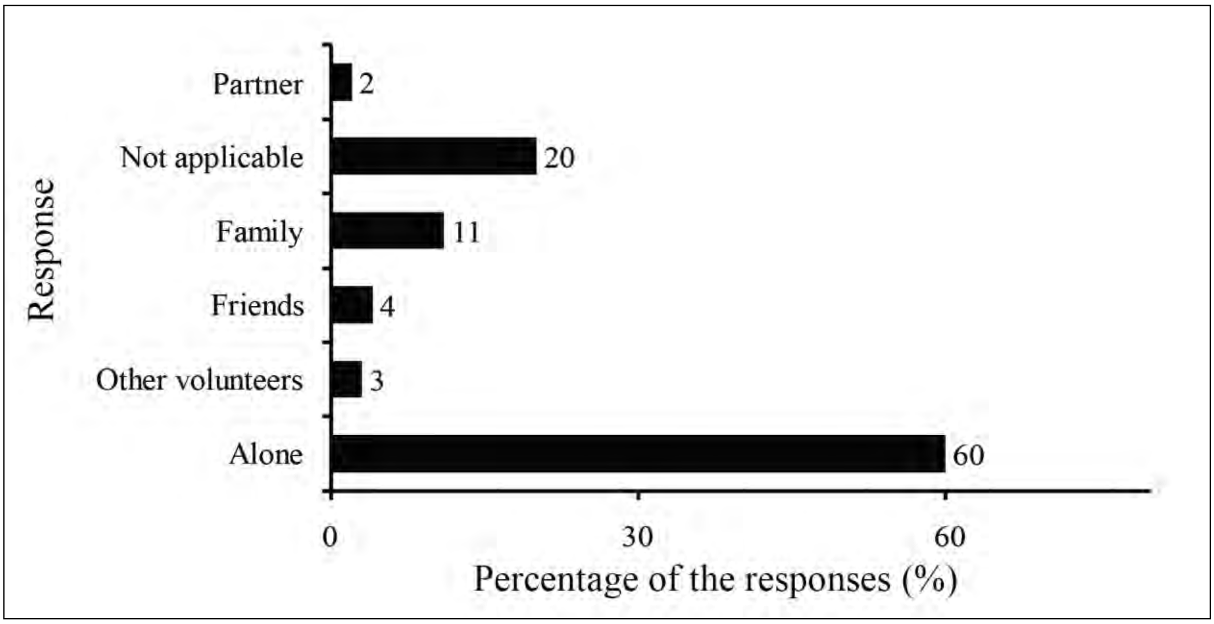


Figure 7.12 Representation of the ratio between volunteers who work alone and those that are accompanied by one or more people. "Not applicable" refers to volunteers monitoring breeding populations but do not partake in toad-saving. "Alone" refers to all volunteers that meet co-volunteers at a predetermined destination.

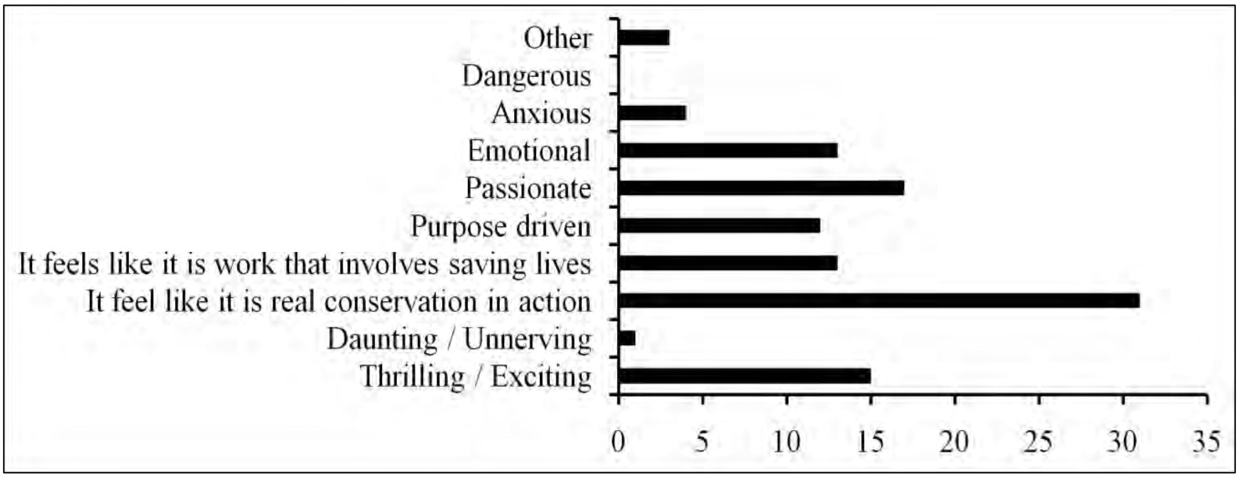


Figure 7.13 The number of responses to various feelings experienced by volunteers during road patrol. Participants could have selected more than one feeling. See text for a description of “Other”.

Feelings mostly experienced by volunteers when saving toads from getting run over ranged from passionate, emotional, purpose driven, thrilling or exciting, to feelings that it is work that involves saving lives (Figure 7.24). The feeling that had the highest frequency was that volunteering felt like it is conservation in action. No volunteer experienced a feeling of danger. Descriptions of the feelings that was not provided in the multi-choice but added by the volunteers were:

- If I don't go out, animals will die
- Every dead one gives me a bit of eco-depression and anger
- It's good to connect with people with positive, constructive values who do something practical to show it

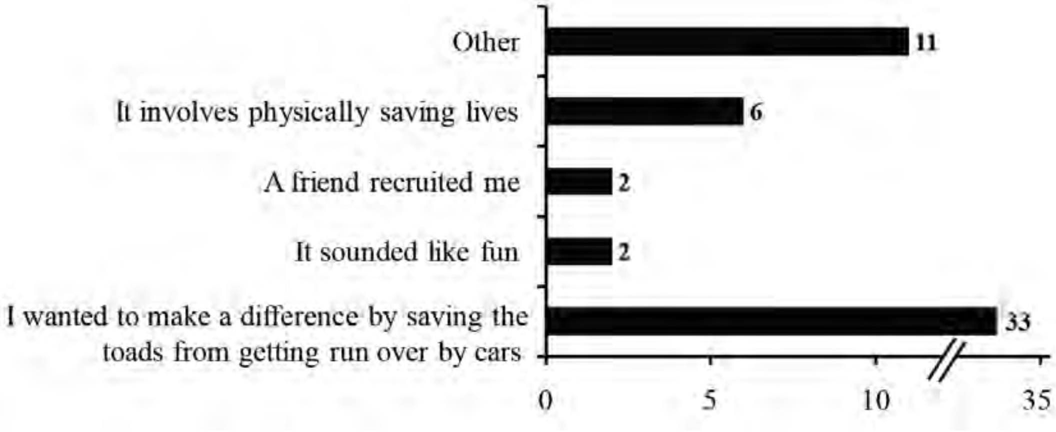


Figure 7.14 Responses of volunteers when asked why they decided to become a volunteer. See text for description of “Other”. Participants were able to choose more than one option.

When asked why they decided to participate in volunteering, most volunteers (33) felt they wanted to make a difference by saving the toads from getting run over by cars (Figure 7.25). As shown in Table 7.2, this reason and other motivations supplied by the volunteers are very specific but can however be divided into the six VFI functions as described by Clary *et al.* (1998).

Table 7.2 Volunteer responses divided into the six volunteer functions described by Clary *et al.* (1998).

	Protective motives	Values	Career	Social	Understanding	Enhancement
I want to create a community that cares		X		X		X
We have several toads living in our garden but it's sad to see the numbers have diminished over the years. Finding dead or injured toads (or frogs) in the road is upsetting.	X	X				
To save a species that would otherwise have gone extinct on 10 years		X				
I want to gain experience in the conservation field			X		X	X
As a custodian of this planet it is my privilege.		X				X
Wanted to contribute to conserving our wonderful environment		X				
We all have to do something	X	X				X
Nature conservation list			X			
To preserve an endangered species		X				
Practical hours for studies			X		X	X
I have Western Leopard Toads in my garden - I want to ensure they and their offspring survive and enjoy urban wildlife adventure.	X	X				
I wanted to make a difference by saving the toads from getting run over by cars	X					
It sounded like fun				X		X
A friend recruited me				X		
It involves physically saving lives		X			X	

7.4 Discussion

7.4.1 Attitudes of Potchefstroom residents toward frogs

The method of data acquisition resulted in a skewed demography (Figure 7.3), thus this study is not representative of the community of Potchefstroom, but nevertheless provides a good idea of the attitudes of people towards frogs. This may be due to Potchefstroom being an academic town (Jenkins 1971) and that people living in rural areas might not have had access to email and internet. Four attitude dimensions revealed an overall positive attitude of residents towards frogs, even though 44% of the respondents did not like frogs and 29% were scared of frogs. Drews (2002) observed five attitude dimensions of Costa Rican adults towards animals:

- sentimental attitude (an expression of feelings of affection toward animals)
- materialistic attitude (which regards animals as resources and praises acts of control over them)
- inquisitive attitude (interest in learning about the biology of animals and their habitats)
- ethical attitude (indicate concern for the ethical treatment of animals and nature)
- schematic attitude (role of aesthetic appearance in the preferences for certain animals and acknowledges feelings of aversion, dislike or fear of some animals)

This study shared the inquisitive attitude of Drews (2002) and found that most Potchefstroom residents (56%) that participated in the survey had a high level of interest in learning more about frogs. This underlines the curious nature of humankind and perhaps also indicates a subliminal fascination with frogs. The schematic attitude dimension used by Drews (2002) is incorporated into the Liking-attitude of this study and shows that people have a widespread perception in the degree to which they like or dislike frogs. Although on average residents took a liking in frogs, there is still more than a third of the people (37%) showing a dislike in frogs.

There are still some uncertainties about frogs among residents. Results demonstrated that 11.5% of the total surveyed residents scored ≤50% in the Knowledge attitude dimension and 7.8% scored ≤50% in the Myths / Beliefs dimension, showing a noteworthy level of ignorance among residents. Ceríaco (2012) demonstrated by means of a model that the presence of folklore and negative values clearly predicts persecution and anti-conservation attitudes towards amphibians and reptiles. In accordance with the findings of Ceríaco (2012), the existence of folklore varied socio-demographically, but negative attitudes concerning frogs are widespread in the Potchefstroom population (Figure 7.5, first column). The attitude profile of Potchefstroom residents is probably incomplete, given the small series of questions used in this study and differences between the studies of Kellert (1993), Drews (2002) and Ceríaco (2012) in the assignment of questions to particular attitudes using factor analysis. Nonetheless, knowledge was positively and highly correlated with the degree to which people like frogs ($P < 0.05$; Figure 7.7). This study's results stress the importance we need to place in education and awareness of the importance of frogs and their need for conservation effort. This can be achieved by holding educational outreaches and inviting schools to attend a "frog day (see Minter, 2005).

Before trying to swing the attitudes of citizens more positively toward animals, the challenge is to raise the awareness about frogs' needs and thereby generate the ethical concern for their well-being (Drews 2002). Children have the capacity for positive perceptions about frogs that is gained by insight and knowledge, changing their perspective of frogs from a negativistic to a positive attitude (Minter 2005; Tomažič 2011). Ceríaco (2012) demonstrated via structural equation models that certain ideas and values can directly influence human attitudes towards herpetofauna and that they can be a severe conservation issue. If benefits to humankind can be proven from frog conservation, there is a better chance of persuading politicians and the general public to carry out actions to conserve populations and species (Tyler *et al.* 2007).

The recipe for successful implementation of creating positive attitudes in people has been refined over the years. Tomažič (2011) concluded that education should place greater importance on permitting students to experience a variety of different animal species directly with the purpose of cultivating a positive transformation in their feelings and attitudes toward them. Prior knowledge about animals was shown to have a significant influence in the perception of children towards wild animals (Randler *et al.* 2005). Randler *et al.* (2012) experimentally confirmed the suggestion that attitudes toward animals are positively influenced by physical contact with them. These findings can be used to influence children's perceptions about frogs effectively. Furthermore, education about biodiversity in schools must focus on a small number of species, start in primary schools, take place outdoors, and be linked with classroom teaching (Randler *et al.* 2005).

In South Africa, a group of scientists have already started to actively promote education, awareness and capacity building as part of a framework of prime areas needing prioritisation for threatened species in the first stage of a three-stage strategy (Measey 2011). Aims included building capacity in professionals practicing conservation and taxonomy of amphibians in South Africa, as well as to advance awareness and appreciation among the general public with regard to the intrinsic importance of biodiversity, in which amphibians play a central component (Tolley *et al.* 2011). National frog awareness day was organised in South Africa for the first time in 2013 and repeated in 2014, to build on the public awareness strategy proposed by Tolley *et al.* (2011) (Jeanne Tarrant pers. comm.). Although South Africa is lagging behind countries like Australia in creating awareness about amphibians (Ehmann 1999), we are well underway with increasingly more organisations and people getting involved.

7.4.2 Motivations of Cape Town volunteers to save the endangered *A. pantherinus*

In our small sample of 37 volunteers that participated in saving toads off roads and contributing to monitoring of the breeding sites, I found that more females are willing to volunteer than are males (81% females to 19% males). This might be due to preferences between females and males in their choice of the type of project or organisation (Wymer 2011). Wymer (2011) found that females have a preference in volunteering for organisations dedicated to helping the needy and distressed. Females prefer working with children or teenagers and in addition favour organisations in which the leadership style is categorised by participatory decision making. Furthermore, females choose volunteer positions in which they can build relational ties with others and have a stronger keenness to volunteer for groups that focus on community building and seek co-volunteers' ideas and input (Wymer 2011). The latter might be the main reasons for the high female sample in the survey. Nevertheless, females are globally the majority in volunteer groups (Cox 2011; BLS 2013).

One of the major reasons for volunteering is to improve things / surroundings / help people (Clary *et al.* 1998; Measham & Barnett 2008; Cox 2011; this study). Literature shows that older citizens are more likely to join volunteer organisations than younger people (Lie *et al.* 2009; Cox 2011). For older people volunteering is an expression of citizenship and have two components namely, volunteering as leisure and work, and volunteering as care and civic consciousness (Lie *et al.* 2009).

The increased reputation of environmental volunteering is derived, in part, from the benefits for the volunteers themselves (Clary *et al.* 1998; Table 7.2). Usually, volunteering assists people to believe they can make a positive difference in the world and this feeling can help people feel good about themselves (Mirowsky & Ross 1989; Clary *et al.* 1998; Clary &

Snyder 1999). Environmental volunteering has been related to improved social networks, and an enhanced sense of place (Gooch 2005).

Volunteerism also plays a fundamental role in promoting environmental sustainability (UNV 2014). Government agencies have been decreasing their own participation in natural resource management and placing the responsibility of environmental monitoring on volunteerism to save costs (Hunsberger *et al.* 2005; Savan *et al.* 2003; Measham & Barnett 2008). Governments should acknowledge the value of public participation and proactively cultivate an enabling environment with the suitable resources and support for volunteering infrastructure. Careful planning is necessary on the part of governments and development agencies to make sure that local programmes that involve volunteers in environmental activities are acknowledged, encouraged and sustained (UNV 2014).

The results of the study underline the importance of knowledge and need for public awareness about frogs in enhancing conservation of frogs in South Africa. Even though the study sample included a large proportion of educated individuals, folklore and myths about frogs persisted. The outcomes also suggest that when people have a broader knowledge and interest in frogs, they tend to have fewer misconceptions (myths or beliefs; Figure 7.6). Therefore, human curiosity can be exploited to enhance the conservation paradigms for frogs. This work supports the study of how certain ideas and values can directly influence human attitudes towards frogs and how they can be a serious conservation issue (Ceríaco 2012).

The volunteers saving *A. pantherinus* from road kill in Cape Town is an excellent and maybe the most pronounced example for conservation volunteering of the general public within South Africa. The enormous quantity of toads that are helped to cross roads cannot be overlooked (Chapter 4). Volunteer groups should be recognised, morally boosted and overall encouraged by NGO's in conservation, scientific institutions and the government in order to sustain and advance this growing and important practice.

CHAPTER EIGHT

General Conclusion

It seems to me that if you wait until the frogs and toads have croaked their last to take some action, you've missed the point.

– Kermit the Frog

This thesis examined aspects of the effects of urbanisation on selected frog populations in a South African context. These included the effects of urban development on population dynamics; habitat determinants on local and landscape scales; the effects of anthropogenic noise and roads; as well as the concealed effects of the residential social structure on the perceptions of frogs.

Urbanisation has a two-way effect on anuran community composition in that it may cause local extinctions through altering, degrading and fragmenting habitats, and conversely contribute the expansion of frog populations by creating a variety of suitable habitats in urban areas for some species (Chapter 2). Urbanisation may also affect anurans indirectly via changes in acoustic environments (Chapters 3, 4 and 5), changes in atmospheric conditions (Chapter 6), and because of the attitudes of people (e.g. negative persistence of anurans) (Chapter 7). Here I draw on the conclusions, contributions and questions generated from the results obtained:

8.1 Frog communities along an urban-rural gradient

Although restricted to Potchefstroom, the response of the community structure of anurans to an indirect urbanisation gradient was reported for the first time in South Africa. Species habitat affinity differed at both landscape and micro-habitat scales. Pond breeding species that are habitat generalists were well represented in urban environments (*Amietia quecketti*, *Amietophrynus gutturalis*, *Amietophrynus rangeri*, and *Xenopus laevis*), whereas other species that showed high habitat specificity did not occur within developed environments (*Cacosternum boettgeri*, *Kassina senegalensis* and *Strongylopus fasciatus*). However, local drought conditions during the study period caused the lack of detection of certain species at sites where they were known to occur. Although four surveys were conducted to include the spectrum of breeding seasons of all species in the range of the study area, two species were not detected, namely the Giant Bullfrog (*Pyxicephalus adspersus*) and the Red Toad (*Schismaderma carens*). Firstly, to include more ephemeral ponds, follow-up surveys should be conducted during a summer with adequate precipitation, and secondly, survey methods should be adapted to include species missed in previous surveys, for example by including adult presence and absence via passive acoustic surveys.

Results obtained confirmed finding of other studies (e.g. Shochat *et al.* 2006) that diversity is highest at the urban fringe, not merely due to low-density development in such locations, but because resource inputs are generally high in managed green spaces and might provide a higher diversity of habitat types (Shochat *et al.* 2006). Urbanising regions show a species richness relationship where diversity concentrates along edges of urban gradients (Blair 1996; Allen & O'Connor 2000; Pei-Fen Lee *et al.* 2004). This relationship can be expected to vary between cities with different biophysical surroundings and development outlines (Shochat *et al.* 2006). Urban ecosystems may diverge from nonhuman-dominated systems in factors that control food web dynamics. Trophic dynamics in urban ecosystems are influenced by intricate human social processes and feedback methods (Shochat 2004; Faeth *et al.* 2005). Therefore, species absence and presence might not only be driven by habitat type, but also prey availability. The diversity in prey should therefore also be taken into consideration for future surveys. Furthermore, one species in particular, *A. quecketti*, was observed to inhabit more urban ponds than any other species. This might be due to its generalised habitat preferences and the abundance of permanent wetlands in urban environments in the study area. *Amietia quecketti* might be a practical species to serve as a bioindicator species for assessing habitat degradation or to assess habitat connectivity in urban landscapes because of its abundance, wide distribution, extended breeding season and broad habitat utilisation (Blaustein 1994; Blaustein & Wake 1995). However, more studies on species with similar behavior than *A. quecketti* will be needed to develop a type of scoring system such as the South African Scoring System for river quality (SASS) (Dickens & Graham 2002). *Amietia quecketti* presence was found to be negatively correlated with pH ($P < 0.05$), thus they occurred more frequently at lower pH levels. Horn & Dunson (1995) found that larval survival of the Jefferson Salamander (*Ambyostoma jeffersonianum*) and the Wood Frog (*Rana sylvatica*) were higher at low pH levels. However, experiments to determine the tolerance of *A. quecketti* larvae to different levels of heavy metals, pH and water hardness are needed.

This study demonstrated that models including purely landscape variables (e.g. road surface area within a 250 m radius) were not supported. This contradicts previous studies on anuran assemblages across urban-rural gradient (Pillsbury & Miller 2008; Hamer & Parris 2011; Scheffers & Paszkowski 2013). The unsupported models do not imply that landscape variables do not play a role in habitat determinants; rather I propose that it is due to the small size of Potchefstroom as a city, lacking the road density of larger cities, thus emphasising the importance of considering scale when addressing the challenges of urban ecology conservation (e.g. Melbourne, Australia; Hamer & Parris 2011). However, the results of vegetation, a local habitat variable, being well supported in the models (ΔDIC<2.0) confirm the findings of studies in other countries (Pillsbury & Miller 2008; Hamer & Parris 2011; Scheffers & Paszkowski 2013).

To facilitate perseverance of amphibian diversity in urban and suburban landscapes in South Africa, it is necessary to 1) circumvent and mitigate further habitat loss and degradation of habitat quality, terrestrial as well as aquatic habitat; 2) ensure/improve the availability, as well as accessibility, of habitat for anuran species to maintain viable metapopulations and regional communities, and/or individuals for reintroduction into re-established or newly-created habitats; and 3) expand on strategies to reconnect the landscape to permit dispersal of anurans between suitable habitats (Hamer & McDonnell 2008). Furthermore, the effect of invasive species on frog populations are not well studied in South Africa and this is known to have important implications in shaping anuran communities (Semlitsch & Bodie 1998; Porej & Hetherington 2005).

To recall the definitions of the two approaches to urban ecology mentioned in Chapter 1 (i.e. ecology *in* cities and ecology *of* cities, p. 3), the research conducted on the effects of frog species distribution and habitat determinants driving their distribution belongs to the approach where ecology *in* a city was investigated (McDonnell *et al.* 2009). Habitats on a local and landscape scale were used to investigate how tendencies of frogs differed in which habitat they might occupy, be it in a rural or urbanised environment. Chapter 6 also followed this approach by investigating the effect of atmospheric variables on the calling behaviour of *A. quecketti*. In the remaining chapters the "ecology *of* cities" approach were used, where both human and ecological components were included to study the effects of urbanisation on certain species and amphibian conservation. These components included the acoustic arena (effects of noise on vocal behaviour of frogs), city infrastructure (effects of roads on migration in *A. pantherinus*), and a social component (attitudes of people towards frogs and how race, gender, age and education effects their attitudes). To answer the questions asked within these studies, a multidisciplinary approach needed to be followed. Studies were designed with the help of an environmental sociologist, weather scientist, ecologists and bioacousticians.

8.2 The effect of airplane noise on the calling behaviour of Pickersgill's Reed Frog (*Hyperolius pickersgilli*)

Although our results show that *H. pickersgilli* males change their calling behaviour in response to high intensity airplane flyby noise, it was also observed that males of this species tend to call much longer than any other local species, calling for at least 12 hours and halting only before sunrise (Figure 3.10), which temporally separates their calling behaviour from sympatric species. Even though airplane activity can continue well into the night at some airports, concomitant with chorusing frogs, flights at King Shaka international airport stop around 21h00 for a period of at least five hours. *Hyperolius pickersgilli* males therefore have up to five hours to attract a mate without airplane flyby noise interference. Future studies that connect anthropogenic noise to reproductive success are urgently needed for *H. pickersgilli* (and other southern African anurans) to develop reasonable action plans (Brumm 2010). Although this study focussed on *H. pickersgilli*, sympatric species at the study area may be adversely affected by airplane noise and studies are therefore needed for a frog species community approach to include all species and investigate how their calls are influenced. A more comprehensive monitoring study, which must include the influence of pollutants and pesticides, must be conducted to monitor the population dynamics over time. This will help identify the severity of the compromised soundscape.

8.3 Migration and call structure of the Western Leopard Toad (*Amietophrynus pantherinus*)

Some call properties of *A. pantherinus* have been shown to be highly dynamic which are typically subject to sexual selection and the influence of anthropogenic noise (Cherry 1992; Gerhardt & Huber 2002; Parris *et al.* 2009). However, the variability of call properties across the geographical range of *A. pantherinus* needs to be studied more extensively. Therefore, no threats are apparent in terms of acoustic interference.

Road traffic is one of the major threats to *A. pantherinus* and will cause significant population declines and local extinctions if not mitigated (Minter *et al.* 2004). Results of this study contributed to the identification of the most sensitive areas where large quantities of toads might be crossing, and also gives an indication of the roads with high traffic flows in relation to breeding sites. This can be used to focus volunteer resources and mitigate the effects of roadkill more effectively in the Cape Peninsula. Furthermore, roads represent 14% of the localities where people interact with frogs (Chapter 7). Therefore, roads might present an advantageous opportunity to both educate people about the importance of frogs, create awareness, as well as saving amphibians from roadkill.

Amietophrynus pantherinus is listed as Endangered (IUCN 2013) and is legally protected by the Nature Conservation Ordinance 19 of 1974, but is not listed by NEMBA or CITES. Therefore, public participation is critical to the future existence and prosperity of this species. The biodiversity management plan for *A. pantherinus* (Rebelo *et al.* submitted) states that exclusion of the species on the CITES list is motivated by the fact that the large numbers of toadlets travel *en masse* and can be propagated and hand reared adult populations can be reintroduced. However, the question remains whether if necessary, we will be able to successfully introduce enough adults to sustain long-term populations, or will it be another Passenger Pigeon-case?

Writing a draft proposal for permission and funding for wildlife road culverts will be the next step in identifying effective mitigation procedures. Wildlife culverts will generate efficient ways to monitor population sizes and density, giving insight to the natural history, population dynamics and conservation of *A. pantherinus* needed to elevate its status as an Endangered species. Volunteers must be supported and motivated for their efforts to save *A. pantherinus*. They are playing, and will play, a critical role in the conservation of this species.

8.4 The extraordinary vocal repertoire of the Common River Frog (*Amietia quecketti*)

Amietia quecketti exhibits an extraordinarily extensive calling repertoire. Two calls for *A. quecketti* are described (advertisement and aggression call) and for the first time an aggression note emitted by a species of the *Amietia* genus is reported. There is considerable variation within three main call types namely, advertisement click-note, advertisement whine-note and aggression rip-note. Playback experiment responses for frogs with complex repertoires, especially with nonlinear acoustic phenomena, have rarely been tested and were limited to call types (Narins *et al.* 2000; Christensen-Dalsgaard *et al.* 2002). Therefore, a plethora of questions are opened to experimentally test the responses of nonlinear events on frog vocal and social behaviour.

Further in-depth phonotaxis experimental studies on the repertoire of *A. quecketti* by conducting play-back experiments with different call types, will not only provide a better understanding in how these frogs communicate, but will also provide valuable insights to the evolution of animal communication systems, since this species produces a wide variety of calls, each with extensive variation. This study has not only produced novel results on the behaviour of anuran bioacoustics, it has also generated an inexhaustive list of questions that can be explored by empirical studies. For example, does the extensive variation in the whine-notes provide information to either male or female, and if so, what are the components of the call that convey this information? Furthermore, results suggest that call composition of *A. quecketti* might contribute to its success that is observed in urban environments (Chapter 2). However, empirical studies are needed to test this hypothesis.

8.5 Calling activity of the Common River Frog (*Amietia quecketti*) in urban green space conditions

In studying the diel calling activity and the effects of atmospheric variables on the intensity of calling, results demonstrated that *A. quecketti* was most active in the hours when acoustic interference from anthropogenic noises are the least likely to occur, thereby adding another possible explanation for the apparent success of this species in urban environments. However, the absence of a control site, i.e. calling activity from a natural habitat, is a limitation to this study. Therefore it is unknown if the calling behaviour (i.e. calling when acoustic interference is lowest) is an adaptation to urban noise, or if it is due to the natural behaviour that makes this species adept to the urban environment. Few studies have followed a more inclusive approach to the effects of atmospheric variables on calling activity (Henzi *et al.* 1995; Oseen & Wassersug 2002; Kirlin *et al.* 2006; Lemckert *et al.* 2013). Furthermore, most of the species studied were summer breeding anurans that demonstrated a positive relationship to precipitation and temperature, with few studies including winter-breeding anurans (Kirlin *et al.* 2006; Lemckert *et al.* 2013). *Amietia quecketti* called more under atmospheric conditions where temperature was low, wind velocity was slightly elevated and relative humidity increased. This study was one of only two studies on the effects of micrometeorological conditions on frog calling activity in South Africa (Henzi *et al.* 1995) and demonstrates the extensive gap in this field of research. Furthermore, given the issue of climate change and its effects on amphibians (Minter *et al.* 2004; Minter 2011; Ospina *et al.* 2013), as well as the effects of urbanisation on the climate of the surrounding environment (Jacobson & Ten Hoeve 2011; Cohen *et al.* 2012), studies incorporating the effects of abiotic environmental factors are urgently needed for South Africa.

8.6 Attitudes of people towards frogs

The social aspect of the urban ecological study in this thesis was focused on the attitudes of the greater Potchefstroom residents towards frogs and the motivations for volunteering to save toads from roadkill in Cape Town. This was the first study on the attitudes of people towards frogs conducted from a South African perspective and will fit into a larger study being conducted country-wide (see Minter 2005 for study on perception of seventh-graders). With 56% of the people surveyed having a strong liking in frogs, there is still a high percentage of the sample that showed evidence of a dislike in anurans. Results showed that knowledge about frogs and the presence or absence of myths or beliefs contributed mostly to the degree to which people liked frogs. The results largely confirm the findings of other studies on the attitudes of people towards frogs (Minter 2005; Tomažič 2011; Ceríaco 2012). However, this study was not representative of the Potchefstroom population, and future studies will have to focus on including more citizens that do not have access to internet.

Volunteerism plays an important role in promoting environmental sustainability (UNV 2014). Volunteers saving *A. pantherinus* from road kill in Cape Town are an excellent, and maybe the most pronounced example of conservation volunteering of the general public in South Africa. The enormous quantity of toads that are annually assisted to cross roads cannot be overlooked. Volunteer groups should be recognised, financially supported, morally boosted and encouraged by NGO’s in conservation, scientific institutions and the government in order to sustain and advance this growing and important practice. Motivations of volunteers show a strong value- and self-enhancement driven origin.

In conclusion, results represented in this thesis symbolise only a drop in the ocean for the multitude of research that is needed for urban ecological studies on amphibians from a South African perspective in order to provide insights in the mitigation of the effects of the urban sprawl. Effects of light pollution (Buchanan 1993; Buchanan 2006) for example has not been studied and needs attention, not only from a South African perspective, but globally.

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CHAPTER NINE

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Appendix A: Locations and site descriptions of 68 ponds surveyed in Potchefstroom, South Africa

Pond Code	Locality description	Coordinates	Altitude	Pond Area	Hydroperiod	Pond Type	Pond Utilization
A1	Parys Road Roadside pool 1	S26 43.955 E27 08.098	1331	1912.8	Ephemeral	Roadside pool	Agriculture
A2	Parys Road Roadside pool 2	S26 43.774 E 27 07.530	1328	434.0	Ephemeral	Roadside pond	None
A3	Parys Road Roadside pool 3	S26 43.789 E27 07.630	1329	518.2	Ephemeral	Roadside pond	None
A5	Roadside pool between Viljoenskroon-Prison roads	S26 45.317 E27 06.388	1328	174.8	Permanent	Roadside pond	Recreational
A6	Potchefstroom HTS roadside pool	S26 42.363 E27 07.111	1354	538.1	Permanent	Roadside pond	Recreational
A7	Promosa roadside pool	S26 42.027 E27 01.813	1414	260.0	Permanent	Roadside pond	Recreational
B11	98 Lombaard Street, Mooivet, Pond 2	S26 42.664 E27 05.485	1346	36.0	Permanent	Formal ornamental pond	Agriculture
B13	98 Lombaard Street, Mooivet, Pond 4	S26 42.672 E27 05.503	1346	13.0	Permanent	Formal ornamental pond	None
B15	100 Strydom Street, Raised Garden Pond 2	S26 43.082 E27 07.386	1347	41.3	Permanent	Formal ornamental pond	Agriculture

B16	Botanical Garden Raised Pond	S26 40.924 E27 05.726	1356	3.6	Permanent	Formal ornamental pond	Agriculture
B17	Tina Scholtz Academy, Garden Pond 1 (front)	S26 42.639 E27 05.787	1353	52.0	Permanent	Formal ornamental pond	Aesthetics
B18	Tina Scholtz Academy, Garden Pond 2 (back/reception)	S26 42.610 E27 05.784	1343	33.6	Permanent	Formal ornamental pond	None
B2	Banquet Hall Pond	S26 42.910 E27 06.031	1337	13546.1	Permanent	Formal ornamental pond	None
B5	Botanical Gardens Pond 7	S26 40.899 E27 05.694	1348	10.2	Permanent	Formal ornamental pond	Aesthetics
B6	Calfyn, NWU Campus	S26 41.325 E27 05.468	1355	782.0	Permanent	Formal ornamental pond	Historic_quarry
B7	Electronic Services pond, NWU Campus	S26 41.192 E27 05.544	1355	62.0	Permanent	Formal ornamental pond	Historic_quarry
B8	Tuscany Ridge Garden Pond, near Dam 5, Carletonville Road	S26 40.014 E27 06.448	1370	8.7	Permanent	Formal ornamental pond	Agriculture
B9	Tuscany Ridge Front Pond	S26 39.996 E27 06.337	1370	96.0	Permanent	Formal ornamental pond	Recreational
C1	Oudrift Riverside Lodge Pond 1	S26 40.642 E27 06.189	1350	456.3	Permanent	Garden Pond	None
C10	Botanical Gardens Pond 2	S26 40.952 E27 05.697	1346	108.8	Permanent	Garden Pond	Aesthetics

C12	Botanical Gardens Pond 4	S26 40.925 E27 05.722	1346	919.6	Permanent	Garden Pond	Aesthetics
C13	Botanical Gardens Pond 5	S26 40.901 E27 05.711	1340	344.5	Permanent	Garden Pond	Aesthetics
C16	Leonie & Hennie Stander Pond 2	S26 40.992 E27 05.969	1344	32.4	Permanent	Garden pond	Aesthetics
C19	9 Roselt Street, Baillie Park 1 (back)	S26 42.873 E27 07.102	1348	6.0	Permanent	Garden Pond	None
C2	Oudrift Riverside Lodge Pond 2	S26 40.655 E27 06.186	1350	903.8	Permanent	Garden Pond	None
C4	Oudrift Riverside Lodge Pond 4	S26 40.668 E27 06.078	1350	518.9	Permanent	Garden Pond	None
C6	17 Strydom Street, Baillie Park	S26 43.044 E27 07.024	1346	55.1	Permanent	Garden Pond	None
C7	Olivier Street 3 Garden pond, Mieder Park	S26 43.313 E27 05.993	1338	33.6	Permanent	Garden Pond	Aesthetics
C8	5 Schubart Street garden pond	S26 41.309 E27 06.083	1344	58.3	Permanent	Garden Pond	Aesthetics
C9	Botanical Gardens Pond 1	S26 40.971 E27 05.709	1336	2487.7	Permanent	Garden Pond	Aesthetics
D1	River, Residential area	S26 41.195 E27 06.172	1343	346.7	Permanent	Permanent river	Recreational

D2	Urban River, Mooi River Mall 1	S26 42.837 E27 06.326	1333	142.4	Permanent	Permanent river	Aesthetics
D3	Rural River, Govan Mbeki	S26 45.162 E27 06.013	1325	1087.5	Permanent	Permanent river	River
D4	Rural River, Oudedorp Farm	S26 36.247 E27 04.538	1366	3888.9	Permanent	Permanent river	Agriculture
D5	Suburban river, Van Graan Street	S26 41.116 E27 06.094	1343	546.7	Permanent	Permanent river	Recreational
D6	Urban River, Mooi River Mall 2	S26 42.655 E27 06.338	1335	202.2	Permanent	Permanent river	Aesthetics
E1	Daly quarry 1	S26 38.281 E27 03.327	1396	5517.8	Ephemeral	Former quarry	Recreational
E2	Daly quarry 2	S26 38.397 E27 03.377	1395	14722.5	Ephemeral	Former quarry	Aesthetics
E3	Daly quarry 3	S26 38.377 E27 03.377	1395	14722.5	Ephemeral	Former quarry	Aesthetics
E4	Promosa former quarry	S26 42.300 E27 02.307	1394	765.2	Permanent	Former quarry	Recreational
F1	Potchefstroom dam	S26 40.103 E27 05.781	1351	18376.0	Permanent	Big dam	None
F2	Daly Farm Dam 1	S26 38.262 E27 04.909	1360	674565.4	Permanent	Big dam	Aesthetics

F3	Daly Farm Dam 2	S26 39.510 E27 04.739	1357	269421.6	Permanent	Big dam	Aesthetics
F4	Poortjies Dam, Primosa	S26 42.638 E27 02.154	1383	1180021.9	Permanent	Big dam	River
F5	Modderdam	S26 41.916 E27 09.717	1338	412 078.6 9	Permanent	Big dam	Agriculture
F6	Gerrit Thomas Dam	S26 46.095 E27 03.668	1334	68 993.17	Permanent	Big dam	Run_off
G2	Daly farm pond 1	S26 38.963 E27 03.769	1378	5326.9	Permanent	Farm dam	Aesthetics
G3	Eleazer dam	S26 40.290 E27 00.612	1420	32729.8	Permanent	Farm dam	Aesthetics
G4	Carletonville Auction farm Dam	S26 38.264 E27 06.439	1378	72025.9	Permanent	Farm dam	Aesthetics
G5	Parys Road Dam 1	S26 44.208 E27 07.931	1331	5062.3	Permanent	Farm dam	Aesthetics
G6	Parys Road Dam 2	S26 44.252 E27 08.025	1334	12505.6	Permanent	Farm dam	River
G7	Hendrik se Gat, Military Base	S26 40.799 E27 05.363	1368	45645.6	Permanent	Farm dam	River
H1	Parys road vlei	S26 43.661 E27 07.249	1328	110814.7	Ephemeral	Vlei	Aesthetics

H2	Oudebrug Road Vlei	S26 41.035 E27 06.646	1349	51011.4	Permanent	Vlei	Recreational
H3	Vlei next to river, Groen Wilgers Retiring home	S26 40.984 E27 05.907	1346	1141.4	Permanent	Vlei	None
H4	Wasgoedspruit, S. faciatus site	S26 42.440 E27 04.658	1351	24854.1	Permanent	Vlei	Run_off
H6	Corner of Chris Hani St and Govan Mbeki Dr Vlei	S26 43.680 E27 06.071	1330	878.3	Permanent	Vlei	Recreational
H7	Promosa vlei ditch	S26 42.595 E27 01.828	1389	3490.6	Permanent	Vlei	Recreational
H8	Govan Mbeki Vlei	S26 44.897 E27 06.010	1324	4 539.41	Permanent	Vlei	River
I1	Agriculture Golf club Pond 1	S26 43.912 E27 04.163	1358	5118.7	Permanent	Large peri-urban pond	Aesthetics
I2	Agriculture Golf club Pond 2	S26 43.752 E27 04.171	1359	102970.2	Permanent	Large peri-urban pond	Aesthetics
I3	Oudebrug Street Dam 1, Johan Rens Dam	S26 40.942 E27 06.497	1351	2241.3	Permanent	Large peri-urban pond	Aesthetics
I4	Oudebrug Street Dam 2 , next to Johan Rens	S26 40.948 E27 06.471	1350	4261.0	Permanent	Large peri-urban pond	Aesthetics
I5	Engineering Faculty Dam, NWU Campus	S26 40.768 E27 05.727	1353	3863.2	Permanent	Large peri-urban pond	River

I6	Tuscany Ridge Dam 1, Carletonville Road	S26 39.895 E27 06.432	1372	43393.3	Permanent	Large peri-urban pond	Historic_quarry
I7	Tuscany Ridge Dam 2, Carletonville Road	S26 39.896 E27 06.532	1374	19453.4	Permanent	Large peri-urban pond	Historic_quarry
I8	Tuscany Ridge Dam 4, Carletonville Road	S26 40.048 E27 06.519	1369	8995.0	Permanent	Large peri-urban pond	Recreational
I9	Tuscany Ridge Dam 6, Carletonville Road	S26 40.152 E27 06.466	1364	1871.8	Permanent	Large peri-urban pond	Agriculture

Appendix B: Survey effort quantification

	Locality number	Locality description	Electro-fishing (minutes)	Dip-netting (minutes)	Bottle traps
1	B15	100 Strydom Street, Raised Garden Pond 2	3	3	2
2	C6	17 Strydom Street, Baillie Park	3	3	3
3	C8	5 Schubart Street garden pond	3	3	3
4	C19	9 Roselt Street, Baillie Park 1 (back)	2	2	2
5	B11	98 Lombaard Street, Mooivet, Pond 2	3	3	2
6	B13	98 Lombaard Street, Mooivet, Pond 4	2	2	2
7	I1	Agriculture Golf club Pond 1	22	22	12
8	I2	Agriculture Golf club Pond 2	22	22	12
9	B2	Banquet Hall Pond	22	22	12
10	B16	Botanical Garden Raised Pond	2	2	2
11	C9	Botanical Gardens Pond 1	22	22	12
12	C10	Botanical Gardens Pond 2	4	4	3
13	C12	Botanical Gardens Pond 4	20	20	11
14	C13	Botanical Gardens Pond 5	9	9	5
15	B5	Botanical Gardens Pond 7	2	2	2
16	G4	Carletonville Auction farm Dam	22	22	12
17	H6	Corner of Chris Hani St and Govan Mbeki Dr Vlei	22	22	12
18	F2	Daly Farm Dam 1	22	22	12
19	F3	Daly Farm Dam 2	22	22	12
20	G2	Daly farm pond 1	22	22	12
21	E1	Daly quarry 1	22	22	12
22	E2	Daly quarry 2	22	22	12
23	E3	Daly quarry 3	22	22	12
24	G3	Eleazer dam	22	22	12
25	B7	Electronic Services pond, NWU Campus	3	3	3
26	I5	Engineering Faculty Dam, NWU Campus	22	22	12
27	H8	Govan Mbeki Vlei	6	6	4
28	G7	Hendrik se Gat, Military Base	22	22	12
29	B6	Kalfyn, NWU Campus	17	17	10
30	C16	Leonie & Hennie Stander Pond 2	3	3	2
31	F6	Lieb dam	22	22	12
32	F5	Modderdam	22	22	12
33	C7	Olivier Street 3 Garden pond, Mieder Park	3	3	2
34	H2	Oudebrug Road Vlei	22	22	12
35	I3	Oudebrug Street Dam 1, Johan Rens Dam	22	22	12
36	I4	Oudebrug Street Dam 2 , next to Johan Rens	22	22	12
37	C1	Oudrift Riverside Lodge Pond 1	11	11	7

38	C2	Oudrift Riverside Lodge Pond 2	20	20	11
39	C4	Oudrift Riverside Lodge Pond 4	12	12	7
40	G5	Parys Road Dam 1	22	22	12
41	G5	Parys Road Dam 2	22	22	12
42	A1	Parys Road Roadside pool 1	22	22	12
43	A2	Parys Road Roadside pool 2	6	6	4
44	A3	Parys Road Roadside pool 3	6	6	4
45	H1	Parys road vlei	22	22	12
46	F4	Poortjies Dam, Primosa	22	22	12
47	F1	Potchefstroom dam	22	22	12
48	A6	Potchefstroom HTS roadside pool	8	8	5
49	E4	Promosa former quarry	22	22	12
50	A7	Promosa roadside pool	6	6	4
51	H7	Promosa vlei ditch	22	22	12
52	D1	River, Residential area	10	10	6
53	A5	Roadside pool between Viljoenskroon-Prison roads	6	6	4
54	D3	Rural River, Govan Mbeki	6	6	4
55	D4	Rural River, Oudedorp Farm	5	5	3
56	D5	Suburban river, Van Graan Street	5	5	3
57	B17	Tina Scholtz Academy, Garden Pond 1 (front)	3	3	3
58	B18	Tina Scholtz Academy, Garden Pond 2 (back/reception)	3	3	2
59	I6	Tuscany Ridge Dam 1, Carletonville Road	22	22	12
60	I7	Tuscany Ridge Dam 2, Carletonville Road	22	22	12
61	I8	Tuscany Ridge Dam 4, Carletonville Road	22	22	12
62	I9	Tuscany Ridge Dam 6, Carletonville Road	22	22	12
63	B9	Tuscany Ridge Front Pond	4	4	3
64	B8	Tuscany Ridge Garden Pond, near Dam 5	2	2	2
65	D2	Urban River, Mooi River Mall 1	4	4	3
66	D6	Urban River, Mooi River Mall 2	5	5	4
67	H3	Vlei next to river, Groen Wilgers Retiring home	22	22	12
68	H4	Wasgoedspruit	22	22	12

Appendix C: Pearson correlations between local and landscape variables to test for collinearity.

		AREA	PH	SLOPE	COND	VEG	SHADE	PREDFISH	ALT	ROADS	GREEN	URBCBD	URBRES	DISTCBD	PONDDIST
AREA	Pearson Correlation	1	.319*	-.096	-.149	.120	-.710**	-.069	.297*	-.307*	.336**	-.182	-.411**	.506**	.515**
	Sig. (2-tailed)		.012	.461	.253	.359	.000	.596	.020	.016	.008	.161	.001	.000	.000
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
PH	Pearson Correlation	.319*	1	-.137	-.238	-.331**	-.483**	-.218	.112	.165	-.264*	.006	-.058	-.011	.158
	Sig. (2-tailed)	.012		.294	.064	.009	.000	.091	.391	.204	.040	.964	.659	.933	.224
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
SLOPE	Pearson Correlation	-.096	-.137	1	.037	-.268*	.299*	-.051	.070	.029	.074	-.302*	.307*	-.070	-.323*
	Sig. (2-tailed)	.461	.294		.777	.036	.019	.694	.590	.823	.569	.018	.016	.594	.011
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
COND	Pearson Correlation	-.149	-.238	.037	1	.158	.129	.087	-.386**	-.120	-.080	.090	.177	-.201	-.312*
	Sig. (2-tailed)	.253	.064	.777		.224	.321	.507	.002	.356	.537	.492	.172	.119	.014
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
VEG	Pearson Correlation	.120	-.331**	-.268*	.158	1	-.164	.261*	-.010	-.263*	.132	-.070	-.028	.285*	.210
	Sig. (2-tailed)	.359	.009	.036	.224		.207	.042	.941	.041	.312	.590	.829	.026	.103
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
SHADE	Pearson Correlation	-.710**	-.483**	.299*	.129	-.164	1	.168	-.223	.061	-.213	.111	.402**	-.519**	-.671**
	Sig. (2-tailed)	.000	.000	.019	.321	.207		.195	.085	.641	.099	.394	.001	.000	.000
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
PREDFISH	Pearson Correlation	-.069	-.218	-.051	.087	.261*	.168	1	-.091	-.003	.121	-.109	.020	.070	.126
	Sig. (2-tailed)	.596	.091	.694	.507	.042	.195		.485	.980	.353	.403	.876	.594	.334
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61

ALT	Pearson Correlation	.297*	.112	.070	-.386**	-.010	-.223	-.091	1	.097	-.106	-.283*	.020	.405**	.226
	Sig. (2-tailed)	.020	.391	.590	.002	.941	.085	.485		.456	.418	.027	.880	.001	.080
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
ROADS	Pearson Correlation	-.307*	.165	.029	-.120	-.263*	.061	-.003	.097	1	-.187	.189	.189	-.248	-.221
	Sig. (2-tailed)	.016	.204	.823	.356	.041	.641	.980	.456		.150	.146	.144	.054	.087
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
GREEN	Pearson Correlation	.336**	-.264*	.074	-.080	.132	-.213	.121	-.106	-.187	1	-.208	-.222	.349**	.273*
	Sig. (2-tailed)	.008	.040	.569	.537	.312	.099	.353	.418	.150		.108	.085	.006	.034
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
URBCBD	Pearson Correlation	-.182	.006	-.302*	.090	-.070	.111	-.109	-.283*	.189	-.208	1	-.471**	-.608**	-.091
	Sig. (2-tailed)	.161	.964	.018	.492	.590	.394	.403	.027	.146	.108		.000	.000	.484
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
URBRES	Pearson Correlation	-.411**	-.058	.307*	.177	-.028	.402**	.020	.020	.189	-.222	-.471**	1	-.012	-.593**
	Sig. (2-tailed)	.001	.659	.016	.172	.829	.001	.876	.880	.144	.085	.000		.928	.000
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
DISTCBD	Pearson Correlation	.506**	-.011	-.070	-.201	.285*	-.519**	.070	.405**	-.248	.349**	-.608**	-.012	1	.499**
	Sig. (2-tailed)	.000	.933	.594	.119	.026	.000	.594	.001	.054	.006	.000	.928		.000
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61
PONDDIST	Pearson Correlation	.515**	.158	-.323*	-.312*	.210	-.671**	.126	.226	-.221	.273*	-.091	-.593**	.499**	1
	Sig. (2-tailed)	.000	.224	.011	.014	.103	.000	.334	.080	.087	.034	.484	.000	.000	
	N	61	61	61	61	61	61	61	61	61	61	61	61	61	61

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix D: Correlation matrix of calling intensity and atmospheric variables. Values highlighted in yellow represent significance (*P*<0.05).

		Clicks per hour	Hour (1–16)	Wind velocity (m/s)	RH (%)	Barometric pressure (kPa)	Change in pressure (|ΔkPa|)	Air temperature (°C)	Water temperature (°C)	Percentage of moon illuminated
Pearson Correlation	Number of click-notes per hour	1.000								
	Hour	.007	1.000							
	Wind Velocity (m/s)	.180	.000	1.000						
	Relative Humidity (RH%)	.052	.618	.015	1.000					
	Barometric pressure (kPa)	-.088	.067	-.477	.324	1.000				
	Barometric pressure change (|ΔkPa|)	.140	.041	-.095	.012	-.075	1.000			
	Ambient temperature (°C)	-.216	-.586	.253	-.738	-.214	-.115	1.000		
	Water temperature (°C)	-.151	-.503	.020	-.784	-.173	-.071	.853	1.000	
	Percentage of moon illuminated (%)	-.177	.000	-.165	-.067	.117	.071	.102	.054	1.000
Sig. (1-tailed)	Number of click-notes per hour									
	Hour	.468								
	Wind Velocity (m/s)	.015	.499							
	Relative Humidity (RH%)	.268	.000	.431						
	Barometric pressure (kPa)	.146	.213	.000	.000					
	Barometric pressure change (|ΔkPa|)	.047	.312	.130	.446	.185				
	Ambient temperature (°C)	.005	.000	.001	.000	.005	.086			
	Water temperature (°C)	.035	.000	.407	.000	.019	.199	.000		
	Percentage of moon illuminated (%)	.017	.500	.024	.211	.082	.199	.111	.260	

Appendix E: Websites on information about frogs

This appendix serves as an example of the information about frogs that is obtainable via the internet. Websites are alphabetically arranged by topics about different information aspects about frogs. Selected video sites and forums are included.

Desert Rain Frog

Yahoo News: http://uk.news.yahoo.com/world-s-cutest-frog-desert-rain-frog-dean-boschoff-weird-sound-181250460.html#tSDKFIc

Youtube video posted by Dean Boshoff: http://www.youtube.com/watch?v=cBkWhkAZ9ds

Frog friendly gardening

http://www.theguardian.com/environment/2008/jan/09/wildlife.conservation

http://frogs.org.au/frogwatch/friendly.html

http://frogwatch.museum.wa.gov.au/Backyard+Frogs/Building+Frog-Friendly+Gardens/default.aspx

http://www.abc.net.au/gardening/stories/s2246709.htm

http://www.backyards4wildlife.com.au/uploads/BFW%20FS%20-%20Frog%20Friendly.pdf

http://www.telegraph.co.uk/gardening/gardeningadvice/4933439/Frog-friendly-gardens-Helen-Yemms-Thorny-Problems.html

http://www.qldfrogs.asn.au/01_cms/details.asp?ID=15

General importance of frogs (as bioindicators, in the ecosystem, medical research)

http://www.savethefrogs.com

http://www.greenosai.org/environment/diversity/58-significance-of-frogs-in-our-environment.html

http://cgee.hamline.edu/frogs/science/

http://science.howstuffworks.com/zoology/reptiles-amphibians/frog5.htm

http://www.ask.com/question/why-are-frogs-important

http://frogsaregreen.org/tag/frogs-as-bioindicators/

http://ces.iisc.ernet.in/biodiversity/amphibians/ecological.htm

http://frogmatters.wordpress.com/category/medical-research/

http://articles.timesofindia.indiatimes.com/2012-04-28/guwahati/31451748_1_amphibians-frog-s-day-new-species http://www.irec.org.au/farmer_f/pdf_177/Native%20frogs%20born%20in%20rice%20bays%20consume%20rice%20pests.pdf

http://srel.uga.edu/ecoviews/ecoview060820.htm

Myths about frogs

http://allaboutfrogs.org/weird/general/myths.html

http://en.wikipedia.org/wiki/Frogs_in_popular_culture

http://www.exploratorium.edu/frogs/folklore/

https://www.burkemuseum.org/herpetology/frog_myths

Pet trade

Frogs as Pets - Contributing to spread of chytrid and amphibian extinction crisis

http://exoticpets.about.com/od/frogsandtoads/qt/pettradechytrid.htm

Frogs for sale

http://www.exotic-pets.co.uk/frogs-for-sale.html

Is the Namaqua Rain Frog in the Pet Trade?

http://www.frogforum.net/frogs/21247-namaqua-rain-frog-pet-trade.html

The pet trade: Frogs and toads

http://www.youtube.com/watch?v=7D8-nRfXsRY

http://www.savethefrogs.com/frogblog/uncategorized/save-the-frogs-is-ending-the-trade-in-wild-caught-frogs/

http://news.mongabay.com/2010/0908-goyenechea_neme_frogs.html

Appendix F: Questionnaire distributed to Potchefstroom residents

Attitudes to Frogs – Potchefstroom

Please return to Donnavan Kruger:

Donnavan.Kruger@nwu.ac.za

A. GENERAL

Please place a cross (X) in the appropriate box

1. **Age**

1 < 12	2 12–18	3 18–30
4 31–45	5 46–60	6 > 60

2. **Gender**

1 Male	2 Female

3. **Race**

1 African	2 Caucasian	3 Coloured	4 Indian	5 Other

4. **Level of education**

1 None	2 Primary	3 Some secondary	4 Completed secondary	5 Tertiary

B. Please Indicate the degree to which you agree or disagree with EACH statement by placing a cross (X) in the box to the right of each statement or question.

	Yes	No	Undecided
5. Do you like frogs?			
6. Are you scared of frogs?			
7. Will you pick up and handle a frog?			
8. Would you like to learn more about frogs?			
9. It would be best if all frogs were killed			
10. I would like to have a frog picture as the wallpaper or screen saver on my cell-phone.			
11. Do you know that frogs belong to the most threatened group of animals on Earth?			
12. Conservation of wetlands and freshwater habitat may help to reduce loss of frog species and numbers			

	Yes	No	Undecided
13. It is important to conserve South Africa’s frogs			
14. Certain frogs are important to medical research			
15. Touching a frog/toad gives you ‘warts’ on your hand			
16. It is safe to collect water from rivers or dams where frogs occur			
17. Frogs cause lightning			
18. If you catch a frog, you should sprinkle salt on its skin			
19. Frogs spread disease			

Please place an (X) in the box for your answer

20. How often do you interact with frogs?

1 Often	2 Seldom	3 Never

21. Where? More than one answer is applicable

1 In my garden at my home	2 In my house	3 At work	4 On the roads	5 Other

22. What do you do when you encounter a frog?

1 I ignore it	2 I take it outside (if it is in my house)	3 I kill it	4 Other

23. Do you recognize this frog? Y/N………

24. Do you know what it is? ....

25. If you could access more information about frogs, how would you like to obtain it?

1 E-mail	2 Pamphlet	3 Presentation	4 Other

Specify:

………………………………………………………………………..................

....

....

Please return form to Donnavan Kruger:

Donnavan.Kruger@nwu.ac.za

Appendix G: Questionnaire distributed to volunteers

Attitudes of Western Leopard Toad Volunteers

Please return to Donnavan Kruger:
Donnavan.Kruger@nwu.ac.za

A. GENERAL

Please place a cross (X) in the appropriate box

1. **Age**

1 < 12	2 12–18	3 18–30
4 31–45	5 46–60	6 > 60

2. **Gender**

1 Male	2 Female

3. **Race**

1 African	2 Caucasian	3 Coloured	4 Indian	5 Other

4. **Level of education**

1 None	2 Primary	3 Some secondary	4 Completed secondary	5 Tertiary

When did you start participating in the Western Leopard Toad volunteer activities (choose one)?

☐ 2013

☐ 2012

☐ 2011

☐ 2010

☐ More than four years ago.

For how many seasons have you participated as volunteer (choose one)?

☐ 1

☐ 2

☐ 3

☐ 4

☐ >4

Why did you decide to become a volunteer (you may select multiple options)?

☐ I wanted to make a difference by saving the toads from getting run over by cars

☐ It sounded like fun

☐ A friend recruited me

☐ It involves physically saving lives

☐ Other____

When I go out to save the Western Leopard Toads from getting run over by cars, I generally go out... (choose one)

- ☐ Alone
- ☐ Alone and meet the other volunteers on site
- ☐ With friends
- ☐ With family
- ☐ With colleagues
- ☐ Other____.

Mark the feelings that would best describe your experience as a volunteer (you may select multiple options).

- ☐ Thrilling / Exciting
- ☐ Daunting / Unnerving
- ☐ Feel like it is real conservation in action
- ☐ Feel like it is work that involved saving lives
- ☐ Sense of gratification / satisfaction / fulfillment
- ☐ Passionate
- ☐ Emotional
- ☐ Anxious
- ☐ Dangerous
- ☐ Other____.

In one brief sentence, what would you consider the best aspect of the Western Leopard Toad volunteer program?

In one brief sentence, what would you consider the worst aspect of the Western Leopard Toad volunteer program?

In one brief sentence, what would you change about the Western Leopard Toad volunteer program?

How long do you plan on being a volunteer for the Western Leopard Toads (choose one)?

- ☐ This was my last season
- ☐ 1 year
- ☐ 2 years
- ☐ >2 years
- ☐ As long as it is possible