

https://dspace.org/

Amphibian habitat requirements in Highveld Pans: implications for conservation

Thomas, Ryan

2015-05-25
http://hdl.handle.net/10539/18594

Downloaded from a DSpace repository. https://dspace.org/

Amphibian Habitat Requirements in Highveld Pans: Implications for Conservation

Ryan Thomas

0709150P

School of Animal, Plant & Environmental Sciences



Declaration

I, the undersigned, hereby declare that this dissertation is my own, unaided work. Information contained herein which is derived from other published or unpublished works is acknowledged in the text and via a reference list. This dissertation is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg on this the 25th day of May, 2015. It has not been previously submitted for any degree or examination at any other institution of higher learning.



Ryan L. Thomas

Acknowledgements

I would like to firstly thank Airports Company South Africa, Piet and Frikkie for kindly granting me permission to work on their privately-owned land for the full duration of the project. I am also appreciative of the assistance of Dr Craig Whittington-Jones who provided suggestions and historical maps of the study area during the proposal phase of the project. The Ekurhuleni Metropolitan Municipality is also acknowledged for their willingness to assist with providing GIS data.

The project would definitely not have succeeded without the invaluable assistance of those who gave of their time and energy in the field. Thanks go to: Kwaku Acheampong, Connor Beaumont, Liam Inglis, Vidosava Jakovljevic, Shivan Parusnath, Sumeshni Pillay, Kristy Robertson, James Taylor, Shae Thomas and Amy Wardhaugh. Special thanks to Marc Humphries, Ashadee Miller and Sumeshni Pillay for assisting with the laborious task of installing traps. Rose Sephton-Poultney kindly proofread a version of this document.

Thank you to James Taylor for being supportive of me even when I was grumpy, especially toward the concluding stages of writing up this dissertation. I hope to have many more years with you by my side. To the rest of my family, especially my mother, thank you for being supportive of me with my academic endeavours.

Lastly and most importantly, I would like to thank my supervisors Prof. Graham Alexander and Prof. Deanne Drake for their support and guidance over the last few years. A student could not ask for better mentors. They have shown themselves to be more than just academic advisors but have provided advice and support with other aspects of my life as well, particularly when the going got tough.

Permits for scientific research were provided by the Gauteng Department of Environmental Affairs (O27258; O05234) and the Gauteng Department for Agriculture and Rural Development (CPF6/0166; CPB1/0133; CPF6/000152; CPB1/000001). Animal Ethics clearance was provided by the Wits Animal Ethics Committee (2012/44/2A).

Table of Contents

Acknowledgements 3
Abstract 6
Chapter 1 - Introduction and Literature Review 7
1.1. General background 7
1.2. Implications of land transformation 7
1.3. Terrestrial habitat utilisation and frog dispersal 9
1.4. Physical pan characteristics and buffer zones 10
1.5. Water quality 12
Frogs and water quality 12
Influence of water quality indicators and known thresholds on amphibians 14
1.6. Legislation governing wetland conservation in South Africa 17
1.7. Project aims 19
Chapter 2 - An Amphibian-centric Evaluation of Water Quality of Highveld Pans 20
2.1. Abstract 20
2.2. Introduction 20
Urban and agricultural pans in Gauteng 21
Water quality 21
Terrestrial habitat and habitat quality 22
2.3. Methods and materials 23
2.4. Results 25
Site description 25
Chemistry 31
2.5. Discussion 40
Water quality 40
Terrestrial habitat availability and pan metrics 43
Chapter 3 - Frog Biodiversity and Dispersal at Wetland Pans in Urban and Agricultural Gauteng, South Africa 47
3.1. Abstract 47
3.2. Introduction 47
Urban vs agricultural zones – richness & abundance 48
Frog dispersal 49
3.3. Methods and materials 50
3.4. Results 52
Counts and dispersal 52

Intra-seasonal variability 56
Pan metrics and water chemistry 57
3.5. Discussion 62
Chapter 4 - Conserving the Frogs of Gauteng's Highveld Pans 68
Recommendations 70
References 73

Abstract

Land transformation and associated habitat loss has been identified as one of the biggest global factors affecting decreases in frog biodiversity. Gauteng Province is South Africa's economic hub and much of the Highveld grassland, characteristic of the region, has been transformed for urban and agricultural purposes. Large, isolated depressions underlain by impervious soils – known as pans – are typical wetland systems of the Highveld region which form habitat for many frog species. I undertook a coarse assessment of amphibian habitat in eleven pans (six representing urban and five representing agricultural areas) by measuring water quality at one point in time (electro-conductivity, temperature, pH, and concentration of sulphates, ortho-phosphates, ammonia, nitrates + nitrites and metals (Na, Mg, K, Ca)), and pan metrics, such as distance to tarred road, area of available terrestrial habitat and pan area. A frog survey using the pitfall-trap method of capture was also conducted at each of the sample sites for the purposes of evaluating frog biodiversity and spatial habitat utilisation. Evidence of acid mine drainage contamination, extensive dumping of household and building waste, nutrient enrichment and close proximity to roads with heavy traffic were found at urban sites. Agricultural sites were located significantly further away from tarred roads compared to urban sites but some were affected by deposition of eroded material from nearby cropland. A Pearson's Correlation found a strong correlation between NO_2 + NO_3-N concentration and *Amietophrynus gutturalis* abundance across sites. Correlation matrices detected a strong, positive correlation between available terrestrial habitat adjacent to pans and pan proximity to tarred road with abundance of *Cacosternum boettgeri*, *Pyxicephalus adspersus* and *Tomopterna cryptotis*. At least six of the eight recorded frog species were captured at 80 m from the pan shoreline. Based on potential sensitivity of some species to available terrestrial habitat area, I recommend that buffer zones around pans should be between 100 and 500 m to ensure species persistence.

Chapter 1 - Introduction and Literature Review

1.1. General background

Amphibians are currently one of the most threatened classes of vertebrates globally (IUCN 2014), with populations decreasing faster than populations of mammals or birds (Stuart *et al*. 2004). Declines have occurred worldwide for the past three decades (Swanack *et al*. 2009). There are some 7357 known extant species of amphibian worldwide (Frost 2015) and of these, 1959 are globally threatened where 518 are listed as Critically Endangered (IUCN 2015). Of the recorded 116 species of frog in South Africa, 19 species (16.4 %) are threatened and eight are near threatened (IUCN 2015). Forty-three of the 116 species occurring in South Africa are endemic and 16 of these are in a threatened category (IUCN 2015). Although scientists have identified a number of causes for amphibian decline, multiple causes are likely responsible (Storfer 2003). These include: (1) Exposure to ultraviolet radiation with enhanced effects by exposure to contaminants such as pesticides, herbicides and fertilizers (Blaustein *et al*. 2003). (2) Changes in temperature and moisture due to global climate change (Carey & Alexander 2003). (3) Infectious diseases such as chytridiomycosis which has been connected to epizootic mass mortality, species declines, local population extinctions and species extinctions (Daszak *et al*. 2003). (4) Anthropogenic habitat change, which is considered to be the primary cause of global biodiversity loss (Measey 2011). Frogs are thus affected by numerous human impacts on the environment, making conservation efforts a challenge.

1.2. Implications of land transformation

Urbanisation is a major contributor to habitat change, resulting in habitat loss, fragmentation and isolation of subpopulations. Habitat degradation threatens more than one-third of known amphibian species globally (Hamer & McDonnell 2008). Isolation of subpopulations and habitat loss in urban South Africa is especially noticeable when remaining vestiges of wetland environments are viewed in satellite imagery. In some cases development has taken place right up to the shoreline of wetlands, drastically reducing terrestrial habitat surrounding pans or wetlands. In cases where terrestrial habitat

remains, it occurs only as small patches of land adjacent to the wetland. Habitat fragmentation and the accumulated effects of low population numbers can lead to the generation of areas of empty habitat and increased rates of extirpation, especially if genetic isolation occurs (Nunney & Campbell 1993; Thomas *et al*. 1998; Templeton *et al*. 1990). The ability of amphibians to disperse can be severely hindered in developed areas (Hamer & McDonnell 2008). This can be an especially important factor for frogs that breed in temporary water bodies where juvenile dispersal is an important life-history movement that links aquatic habitat to upland habitat (DeMaynadier & Hunter 1999).

Agricultural landscape modification has been included with urbanisation as a major driver of biodiversity loss and constitutes landscape change that affects water bodies and the amphibians that utilise them (Curado *et al*. 2011). Conversely however, agricultural landscapes have also been found to provide adequate alternative breeding habitat for some amphibians (Knutson *et al*. 2004) and may not restrict amphibian movement to the same extent as urban landscapes. In Gauteng Province, undisturbed natural, terrestrial habitat around pans/wetlands in agricultural areas has become limited and fragmented.

Human population growth shows no sign of abating (United Nations 2004, Statistics South Africa 2011). A report by the United Nations estimates that the worldwide human population is projected to grow to 8.9 billion by 2050 (United Nations 2004). As the human population grows and urban areas expand, additional strategies will need to be implemented to help conserve the fauna and flora that currently inhabit areas that in the future may become part of the urban matrix.

As a rapidly developing country, South Africa is no exception with regard to the growing human population, urban sprawl and accompanying environmental issues. The economic hub of South Africa is located in the province of Gauteng which contributes 10% toward the GDP of the African continent and 33% nationally. Gauteng is a major metropolitan area where urban areas cover approximately 17% of the province - and residential areas nearly 9% (Gauteng Government, 2001). Gauteng is also the most densely populated of South Africa's nine provinces (675.1 ppl/km^2) compared with the next highest populated province, Kwa-Zulu Natal at 108.8 ppl/km^2 (Statistics South Africa 2011).

According to the 2011 national census, Gauteng household (a house and its occupants regarded as a unit) density was 215.0/km^2. The Gauteng population measured 12 272 263 people (2011), making the province the most populous in South Africa (Statistics South Africa 2011). This represents a 30.7% growth from 9 388 854 in 2001. Some households are located in environmentally sensitive areas close to wetlands which makes these areas vulnerable to degradation and pollution (Gauteng Government 2001).

1.3. Terrestrial habitat utilisation and frog dispersal

The quantity of terrestrial habitat a frog requires may not only influence its ability to disperse to other populations but also its ability to forage and aestivate. Amphibians are thought to be found only in and immediately adjacent to water because we associate them with where we observe the most visible part of their life cycle. Many amphibians however, have a biphasic life-history pattern, breeding in water but living the greater part of their lives in terrestrial habitats (Dodd & Cade 1998). Numerous amphibians which breed in static bodies of water are fossorial and as a result are not regularly observed in terrestrial habitats (Semlitsch & Bodie 2003).

Once mature, an individual frog may spend its terrestrial existence in close proximity to water or may disperse up to nearly a kilometre away (Gamble *et al*. 2007). For example, the female Giant Bullfrog (*Pyxicephalus adspersus*) which inhabits arid to subtropical grassland (Yetman & Ferguson 2011) can disperse up to 1 km from the water bodies where breeding took place in order to aestivate (du Preez & Carruthers 2009). Yetman & Ferguson (2011) found that both male and female Giant Bullfrogs moved a maximum overnight distance of 350 m when returning to their burrows after spawning. Individuals were also found foraging from 10-20 m from their burrows (Yetman & Ferguson 2011) which indicates the importance of terrestrial habitat for foraging. Semlitsch & Bodie (2003) demonstrated the extent of amphibian terrestrial habitat use in the USA – that showed core terrestrial habitat (mean minimum and maximum distances from aquatic habitat which includes foraging, overwintering and refuge areas) of amphibians can range from 159 to 290 m from the edge of aquatic habitat. Additionally, a study by Rittenhouse & Semlitsch (2007) estimated that frogs can

disperse up to 700 m from breeding sites. Bulger *et al.* (2003) report that the California pond breeding anuran, *Rana aurora draytonii* can occur up to 500 m away from water bodies. Frogs can thus move considerable distances from breeding habitat, however this is likely to be species and habitat specific.

Juvenile frogs can be highly vagile, dispersing in large numbers from where they metamorphose. A study of Columbia Spotted Frogs found that juveniles can disperse more than 5 km from breeding sites within the year following metamorphosis (Funk *et al*. 2005). Thus the terrestrial component of the habitat is of critical importance and must be considered when managing wetland ecosystems that support frogs. Unfortunately, not many studies have investigated spatial habitat use of frogs in South Africa, especially the species which are found in Gauteng Province.

Storfer (2003) suggests that research should occur at a landscape scale and conservation efforts should focus on suitable habitat and dispersal capabilities of species. Because there have been few studies investigating amphibian terrestrial habitat use in the southern hemisphere, there is a need for additional information on the subject for amphibians living in hot environments on the African continent (Yetman & Ferguson 2011). This project assists in addressing that need.

1.4. Physical pan characteristics and buffer zones

There is some variation in how ecological buffer zones are described in literature, but all definitions essentially suggest the same thing; that buffer zones are areas that serve as conserved habitat where animals and plants can thrive in spite of the anthropogenic impacts of adjacent land (biology-online.org 2009). Terrestrial buffer zones for conservation of amphibians provide some level of protection from the effects of urban sprawl (such as pollutants and habitat destruction) and also provide an area for aestivation and foraging. This terrestrial component of aquatic habitat is very important in the management of aquatic resources (Semlitsch & Jensen 2001) and constitutes important core habitat for the survival of amphibians. The size and isolation of the water body, as well as the presence of buildings and roads, can also affect the number of frog and other floral and faunal species found in an area (Houlahan *et al*. 2006, Parris 2006). Urban density (Pillsbury & Miller 2008) and agricultural practices have also been implicated in decreased amphibian abundance and diversity.

The protection of terrestrial buffer zones is touted as a means to ameliorate human impact on receiving waters such as wetlands (Muscutt *et al*. 1993). Buffer zone width is important in buffering against dissolved pollutants (Phillips 1989). A 10 m buffer strip can reduce the phosphorus load of between 65% and 95% and nitrate load between 30% and 50% in surface waters (Vought *et al*. 1995). Vegetated buffer zones also trap sediment and sediment-bound nutrients (Lee *et al*. 2000). Although buffer zone requirements differ depending on the characteristics of wetlands, a minimum of a 15 – 30 m (where minimum buffer widths for conservation of fauna and flora should be closer to 30 m) buffer zone was found to protect wetlands under most conditions in a review which investigated buffer zone effectiveness on sediment and nutrient removal as well as its influence on species diversity (Castelle *et al*. 1994). Castelle *et al.* (1994) also pointed out that appropriate buffer width can be species and locality dependent, a factor which should be considered when buffer zones are delineated.

Buffers zones have been promoted in a number of countries worldwide, particularly developed ones, including the United Kingdom, United States and New Zealand (Muscutt *et al*. 1993). Existing buffer zones mentioned in literature however, vary globally with regards to their size, specific purpose and the legislation that governs their implementation. Many buffer zone requirements may be substantially influenced by political acceptability rather than on scientific backing (Castelle *et al*. 1994). To illustrate, in some states of the United States of America, additional policies are implemented to supplement federal ones thereby increasing buffer zone width over and above what is considered the minimum by federal law (Burke & Gibbons 1995).

Roads have major ecological implications (Coffin 2007) and influence hydrological processes, increase pollutants and noise and improve human access in an area known as the "road-effect zone" (Forman & Alexander 1998, Forman 2000). This zone can extend beyond the edge of the road from 50 – 800 m (Reijnen *et al.* 1995). Fahrig *et al.* (1995) found that the immediate vicinity of roads can also have a major impact on frog survival when they recorded 1856 dead and 591 live frogs along a 506 km stretch of road. Traffic within 1.5 km of ponds had a significant negative impact on *Lithobates pipiens* abundance in the U.S.A. while a less vagile species, *Lithobates clamitans* appeared to be unaffected (Carr & Fahrig 2001).

1.5. Water quality

Frogs and Water Quality

Surface waters are usually collecting points or reservoirs in transformed environments for various non-nutrient substances widely regarded as pollutants, such as heavy metals, pesticides and pharmaceuticals. These can also contain high concentrations of nutrients (e.g. nitrogen and phosphorus compounds), that can greatly influence water quality (Smith & Schindler 2009). The thresholds at which water quality starts to impact negatively on human health may differ considerably from those which have detrimental effects on environmental and amphibian health (DWAF 1996).

Amphibians are good bio-indicators of environmental health and quality (Dunson *et al*. 1992) because of their sensitivity to environmental changes. All life stages of frogs (Boyer & Grue 1995), particularly older amphibian larvae (Howe *et al*. 1998), are susceptible to water quality because they have a semi-permeable skin (which performs respiratory functions) that must be kept moist at all times in order to maintain adequate levels of respiration (Springer & Holley 2013). Wetlands are an essential breeding habitat and are a source of refuge for many species of amphibians. Water quality thus has an effect on species richness and abundance by negatively impacting survival and development through factors such as acidification (Freda 1986, Leuven *et al*. 1986, Beattie & Tyler-Jones 1992, Boyer & Grue 1995), acid mine drainage and leeching of minerals into wetlands (Porter & Hakanson 1976, Haywood *et al*. 2003).

Acid rain in freshwater systems is a result of sulphuric and nitric acid aerosols resulting from fossil-fuel burning, metal smelting and other industrial processes dissolved in rainwater (Haines 1981). Severe acidification is also caused by acid mine drainage (AMD) formed by the oxidation of sulphide minerals when exposed to the atmosphere (Freedman 1995), and is a major problem in Gauteng (GDARD 2011). Water contaminated by AMD typically has high levels of sulphates (> 3000 mg/l) and a low pH (2 < 4), (Naicker *et al*. 2003, Gagliano & Bigham 2006). Calcium hydroxide (slaked lime) is sometimes added to AMD-polluted surface waters to neutralize the acid and raise the pH, and so reduces the concentration of metal pollutants (Johnson & Hallberg 2005), but this

treatment also elevates salinity (Jovanovic *et al*. 1998). Liming of water bodies takes place extensively on the Witwatersrand, South Africa and is a proposed mitigation method in other provinces to reduce the impacts of AMD (McCarthy 2011, Maree *et al.* 2013). The negative effects of AMD on the survival and development of one frog species (*Xenopus laevis*) has been demonstrated (Haywood *et al*. 2003) and likely extends to other frog species.

Eutrophication affects water quality worldwide (Chislock *et al*. 2013) and is a growing problem in sub-Saharan Africa (Nyenje *et al*. 2010). Nutrient enrichment from sources of nitrogen and phosphorus are among the top culprits for eutrophication of freshwater systems (Carpenter *et al*. 1998). Non-natural sources include, run-off from agricultural and urban land using artificial fertilizers, human waste discharge from settlements (where little to no water treatment may occur (GDARD 2011)), detergents, discharge from industry and sewage treatment works (de Jonge *et al*. 2002; Khan & Ansari 2005) as well as deposition of nitrates from industrial air-emissions (Granat 2001). The negative effects of eutrophication include, increased biomass of phytoplankton, increased biomass and species composition change of macrophyte vegetation, algal blooms, increased incidence of fish death, species diversity reductions, decreased water transparency, oxygen depletion, changes to the taste and odour of drinking water and decreased aesthetic value of the water body (Smith & Schindler 2009). Multiple factors affect eutrophication which in turn affects ecosystems in a variety of ways. This may make eutrophication-related problems in wetlands difficult to manage.

Eutrophication influences frog populations directly through elevated ammonia concentration (> 0.73 mg/l) and elevated pH (> 8), (Boyer & Grue 1995). Eutrophication can also affect frogs indirectly (Belden 2006) by increasing parasite load via an increase in abundance and longevity of primary and intermediate hosts such as snails (Lafferty 1997, Johnson & Chase 2004, Johnson *et al*. 2007). Eutrophication also negatively affects tadpoles making them more susceptible to parasitic infection (Peltzer *et al*. 2008). The effects of eutrophication may thus, cause shifts in the richness and abundance of frog communities in complex ways (Johnson & Chase 2004).

Influence of water quality indicators and known thresholds on amphibians

Research regarding concentration thresholds of ions in water and water quality indicators required for the survival of amphibians is generally lacking. The combination of water quality and habitat issues in areas incorporated in this study however, are likely to have profound negative impacts on frogs. I tested the following water quality indicators as part of a coarse water quality assessment in my project where at least some global research has been done to ascertain their direct effects on amphibians.

pH – The pH in aquatic ecosystems is affected by the presence of metals such as aluminium, sulphates, nitrates and temperature. Guidelines for general recommended pH for most drainage basins of the world suggest that it not drop below 6.0 (Carr & Neary 2008) and be within the range of 6.0-7.5 in order to present no threat to most aquatic organisms (Sparling 2009). Southern African, *Pyxicephalus adspersus* have successfully bred in small pans with a mean pH range of 6.6-8.6 (Thomas *et al*. 2014). A pH which lies outside the range of 6.5-8.5 is likely to be detrimental for some amphibians which remain in water for long periods (ILAR 1974).

Alkalinity – refers to the ability of water to "resist" changes in pH when acid-causing substances are introduced (Gupta 2011). Such buffering has clear benefits for amphibians especially in environments that have a higher susceptibility to acidify. Glooschenko *et al*. (1992) found a positive relationship between frog abundance and alkalinity. However, Brodman *et al*. (2003) found a negative relationship which may indicate that alkalinity varies with its benefit to amphibians or that effects are species-specific. Water bodies with low (< 100 mg/l) alkalinity ($CaCO_3$) may acidify more easily and affect amphibian health negatively (Gupta 2011). Ideal alkalinity levels for amphibians are between 150 and 250 mg/l (ILAR 1974). Alkalinity appears to affect frogs differently. This may be because high alkalinity may, in some instances, buffer against pH change at a level still too acidic for some species.

Water Hardness – Water hardness is affected by the presence of dissolved minerals such as calcium (Ca) and magnesium (Mg), (Lentini 2013). The effects of water hardness on amphibian

health have not been well documented and high water hardness may actually mitigate, at least partially, metal toxicity to aquatic life (Weiner 2013). One study by Godfrey & Sanders (2004) suggests that there may be benefits of increased water hardness for embryo development in frogs, to a point. Of the same study, it was reported of South African ponds that *Xenopus laevis* seems to survive and develop better in ponds of moderate to hard water (> 130 mg/ l $CaCO_3$) (Weiner 2013).

Electrical conductivity (EC) – The ability for water to conduct electricity is sensitive to variations in dissolved solid content (Chapman 1996). Electrical conductivity is often used as a measure for water quality because it is easy to measure and serves as a proxy for other water quality indicators that are more difficult to measure (Babbitt *et al*. 2009). Anthropogenic increases in electrical conductivity are largely attributed to agriculture, urbanisation and industry (Carr & Neary 2008; DWAF 1996). Water conductivity that is 150-500 µS/cm is considered adequate for aquatic organisms (Sparling 2009). Research has shown that elevated conductivity can negatively impact consumption of prey in larvae of an amphibian species (*Ambystoma jeffersonium*), (Chambers 2011) as well as correlate negatively with amphibian species richness (Azous 1991). Conductivity is unlikely to affect amphibians directly but may rather relate to turbidity and nutrient concentrations (Schmutzer *et al*. 2008).

Ammonia (NH_3) – is a naturally-occurring substance excreted by many aquatic organisms as a by-product of protein metabolism (Lentini 2013). The compound is also present in the environment in the form of artificial fertilizers used in agriculture (Warren *et al*. 2008). The presence of ammonia is temperature and pH dependant (DWAF 1996) as its prevalence increases in systems at higher pH and temperature while in systems at lower temperature and pH, it disassociates into less toxic ammonium and hydroxide ions (Lentini 2013).

In unpolluted waters ammonia concentration is usually less than 0.1 mg/l NH_3-N with concentrations being typically less than 0.2 mg/l in most surface waters (Chapman 1996). Concentrations higher than 2-3 mg/l of N may indicate organic pollution (Chapman 1996). Frog husbandry guidelines indicate that levels of NH_3 over 0.01 mg/l require immediate attention for

amphibians in captivity (Lentini 2013). In a study by Jofre & Karasov (2009), individuals of three different frog species were exposed to un-ionized ammonia (NH_3). For one species (*Lithobates clamitans*), survival declined, deformities increased and development of embryos was slowed when exposed to NH_3 levels of 0.6 mg/l. For another (*Lithobates pipiens*), these effects were evident at 1.5 mg/l. The third species (*Anaxyrus americanus*) seemed unaffected but was only exposed to a maximum of 0.9 mg/l of NH_3. Ammonia concentrations above 0.2 mg/l are detrimental to fish and are considered to be detrimental to amphibians (ILAR 1974, Odum & Zippel 2008).

Nitrates (NO_3), nitrites (NO_2) and phosphate (usually as PO_4) – are the nutrients most commonly linked to the eutrophication of water bodies and can cause a reduction in water quality, increased turbidity, decreased oxygen levels, loss of habitat and loss of biodiversity (Rabalais 2002). Although nutrients are an essential and natural part of ecosystems, human activity unnaturally increases the concentrations to pollutant levels. In most cases, a nitrogen to phosphorus ratio greater than 25-40:1 could be expected from unimpacted sites whereas, sites impacted by pollutants would typically have N:P ratios of less than 10:1 (DWAF 1996, Bowd *et al.* 2006). Sources of nitrogen and phosphorus include agricultural fertilizers, urban fertilizers, manure application, vehicle emissions, industry, detergents, human waste (sewage) and landfills (Perlman & Milder 2005).

Nitrite- and nitrate-N concentrations are considered toxic to amphibians in captivity when they exceed 0.1 mg/l and 10 mg/l respectively (Lentini 2013). Another source suggests that NO_2 + NO_3-N should not exceed 0.3 mg/l as N (ILAR 1974). In a review of several laboratory studies investigating the toxicity of NO_3 on amphibians, Rouse *et al*. (1999) indicated lethal and sub-lethal ranges of between 2.5 mg/l and 100 mg/l. Odum and Zippel (2008) report that NO_2 and NO_3 in water should not exceed the acceptable concentrations for amphibians of 1.0 mg/l and 50 mg/l respectively. In South Africa, nitrogen concentrations below 0.5 mg/l are considered low enough to limit eutrophication and prevent algal blooms (DWAF 1996). Phosphorus (P) is regarded as the limiting nutrient in freshwater aquatic ecosystems (Rabalais 2002) and is usually found in trace amounts in natural systems (ReVelle & ReVelle 1992). Little information exists regarding P toxicity in frogs (DWAF 1996).

Turbidity – Turbidity reduces light penetration in water and affects plant growth and the quality of habitat at the bottom of water bodies (Boyd 2000). Additionally, when organic matter associated with sediment in turbid waters decomposes, it consumes available oxygen thereby depleting oxygen supply around aquatic organisms (Goudie 2005). Turbidity has been linked to anthropogenic sedimentation and pollution that can affect frog fitness and behaviour (Babbitt *et al*. 2009). Turbidity measures are increasingly recognised as an indication of water quality (Anderson 2005). Some studies have hinted that turbidity may be implicated in impacting negatively on amphibian populations although values for when this negative effect may occur are unclear.

Schmutzer *et al*. (2008) found smaller ranid populations were found in turbid water disturbed by cattle (mean turbidity – 85.82 FTU) versus undisturbed (mean – 23.40 FTU) in the U.S.A, while Knutson *et al*. (2004) found that turbidity, collectively with other water quality indicators, led to lower reproductive success in amphibians in Minnesota. The units NTU and FTU are comparable (Wilde & Gibs 1998). Species richness was also negatively correlated with turbidity (as well as conductivity, hardness, magnesium and chloride) in a study by Hecnar & M'Closkey (1996). Conversely, it has also been suggested that water with very low turbidity may be detrimental to aquatic organisms because of increased bird predation (Boyd 2000). Turbidity thus appears to have both potentially positive and negative effects on amphibians, the magnitude of which can only be ascertained through continued study of the effects of turbidity on frog species.

1.6. Legislation governing wetland conservation in South Africa

Currently legislation which directly addresses wetland conservation in South Africa is limited however, one set of national legislation does address buffer zone requirements around wetlands. The extent of legal protection that addresses issues affecting wetlands is fragmented into various acts that address specific environmental issues such as pollution prevention, pollution-related emergency procedures, lawful water use and stream flow. There are two levels at which South Africa provides for wetlands: (1) internationally agreed management of Ramsar sites, in the form of The Convention on Wetlands of International Importance especially as Waterfowl Habitat (Ramsar 1971) and (2)

nationally recognised acts. These include the National Environmental Management Act 107 of 1998 (NEMA), The National Water Act 36 of 1998 and the National Environment Management Act: Protected Areas Act 57 of 2003.

The Ramsar Convention stipulates that as a signatory, South Africa must ensure that its planning is formulated and implemented in such a way that it promotes the conservation of natural and artificial wetlands. It does not provide official guidelines as to how best to preserve wetlands and does not provide South Africa with any regulations regarding the terrestrial components of a wetland habitat and their potential importance in amphibian conservation. Of the 20 wetlands South Africa has listed as of international importance, only one is located in Gauteng Province (Blesbokspruit, 26º17'S, 28º30'E) and it is listed as a site where changes in ecological character have occurred, are occurring or are likely to occur. This however, does not mean that Gauteng has only one wetland ecosystem worthy of protection (DWAF, 2005).

The National Environmental Management Act provides the principles and legislative guidance regarding decision making on matters that affect the environment. However, it provides limited information on buffer zones. The act does recognise that sensitive, vulnerable, highly dynamic or stressed ecosystems, such as wetlands require specific attention especially when subject to significant human resource management usage and development pressure (Government of South Africa 1998). Section 28 of the NEMA also legislates that landowners take reasonable measures to prevent degradation to the environment. The National Water Act provides guidance regarding development surrounding wetland areas and specifies that the maximum extent of permissible development along a watercourse provided that development does not occur within 500 m of a wetland boundary. The Act is good at focussing on what constitutes acceptable water use and sustainable management. The Act also attempts to address problems that could affect aquatic ecosystems by addressing issues around pollution, irrigation buffer zones (50 m above the 1 in 100 year floodline or at least 100 m from a water course) damming and emergency situations. The Protected Areas Act does not protect areas that fall outside stipulated protected areas and so an amendment would be needed to include all aquatic ecosystems and adjacent terrestrial habitat in order to provide the best possible protection from land

transformation. Limited legislative protection is thus afforded to amphibian populations living in and adjacent to wetlands in South Africa.

The Department of Water and Sanitation (DWS) – previously, Department of Water Affairs and Forestry (DWAF) – addresses the subject of wetland delineation in a document meant to assist in determining whether an area is a wetland or riparian area and for ascertaining its boundaries. The closest a reader will come to a figure supplied by the Department is the mention of a normally used 20 m buffer between a wetland or riparian zone and the proposed land use zone, in a forest environment (DWAF 2005). This figure, however, does not appear in legal documentation in the form of an Act or subsection thereof. The figures presented in the National Water Act of 1998 would be considered in this case however.

1.7. Project aims

This project primarily aimed to quantify the terrestrial spatial use of frogs in Gauteng Province around wetland pans common to the area. Data were collected with the intention of providing information to assist the Department of Environmental Affairs and other environmental management entities in managing frog populations in areas transformed by urbanisation and agriculture through provision of adequate, scientifically substantiated, buffer zone recommendations. Estimates of dispersal distance from water bodies with respect to species richness and abundance were also made. The project additionally aimed to indirectly provide an indication of the relative size of amphibian populations at the study sites and the impact of urban development on frog populations now situated in urban environments compared to less impacted agricultural environments.

Secondarily, possible factors influencing population size and species richness were assessed by recording the proximity of individuals or populations to human development (e.g. tarred roads) and by making a coarse assessment of pan water chemistry. Suggestions for the future delineation of buffer zones along with an estimate of the impact of anthropomorphic-related landscape alteration are made in the final chapter.

Chapter 2 - An Amphibian-centric Evaluation of Water Quality of Highveld Pans

2.1. Abstract

Urbanisation and agriculture often have detrimental effects on wetlands and aquatic ecosystems. Amphibians can be particularly susceptible to anthropogenically transformed environments, making them good bio-indicators of environmental quality. As urbanisation and agriculture grow across the Highveld in Gauteng Province, wetlands that may appear to be suitable for frog habitation could in actuality, be unsuitable. I assessed the state of 11 wetland pans in urban and agricultural areas with a specific focus on evaluating whether these water bodies were suitable for frogs given current knowledge on environmental water and terrestrial habitat quality guidelines. A coarse water quality assessment was conducted along with an evaluation of physical pan characteristics. Variance of urban alkalinity, NO_2 + NO_3-N, Mg and K concentrations differed significantly from relatively comparable values between agricultural sites. Tarred roads were located significantly further away from agricultural sites compared with those at urban sites. Although wetland pans in both urban and agricultural areas showed signs of decreasing habitat quality, agricultural areas generally provided better habitat for frogs because of comparably better water quality and larger areas of undisturbed terrestrial habitat.

2.2. Introduction

Gauteng Province is South Africa's economic hub and land use is predominantly urban and agricultural. Development and land transformation over the last several decades has impacted pans in the province. This impact is especially evident in urban areas when comparing maps from the 1950s to the present (pers. obs.). Environmental health has likely been altered as a result, impacting the suitability of pans as suitable frog habitat. Environmental conditions which impact on habitat suitability for frogs include those that affect water quality and terrestrial habitat availability (Boyer & Grue 1995, Hazell *et al.* 2001).

Urban and agricultural pans in Gauteng

Carpenter *et al*. (1998) indicated that eutrophication (caused by nutrient enrichment) is a problem in aquatic ecosystems in both urban and agricultural areas. It is likely that impacts on water quality vary depending on pan location (urban or agricultural) and adjacent human-related activities. Expansive urban areas in Gauteng are now well integrated with both extant and historical mining operations which have potential to affect water sources (and by extension frog populations) in the province. Water quality in urban areas of Gauteng is also likely to be affected by dense informal settlements that can increase nutrient loads entering water bodies via bad sanitation and other pollutants (Pillay & Terry 1991, Carden *et al*. 2007). Bodies of water in urban areas of Gauteng are also subject to potential contamination from road water run-off (Forman & Alexander 1998, Marsalek *et al.* 1999), light industrial processes, increased air pollution from industry (Morris 1991, Granat 2001) and burning of fossil fuels (e.g. from motor vehicles and aircraft).

Many agricultural areas in Gauteng are under private ownership and thus do not suffer from many of the problems originating from informal settlements. They also tend to be located away from heavy industrial processes. Traffic volumes in agricultural areas tend to be lower by comparison with urban areas and are confined to main roads with little to no traffic on secondary routes. Agricultural land uses may however, be a greater source of nutrient enrichment than urban land uses (Coulter *et al*. 2004) and can also result in high soil erosion and degradation (GDARD 2011).

Water quality

Water quality affects both larval and adult frogs. Acidification (using pH as an indicator) is a well-researched water quality indicator which affects frogs. Increased acidification of water systems is implicated in acid mine drainage (AMD) contamination that results from increased sulphate concentrations. Water salinity may also increase due to AMD treatment processes. The negative effects of AMD have been demonstrated in at least one frog species in South Africa, *Xenopus laevis* (Haywood *et al*. 2003). Eutrophication also affects aquatic habitats through elevated ammonia concentrations (Boyer & Grue 1995) and by creating a favourable environment for the proliferation of

parasites by increasing abundance and longevity of their primary host, the snail (Lafferty 1997, Johnson & Chase 2004, Johnson *et al*. 2007).

Water quality indicators such as alkalinity (Azous 1991, Chambers 2011), water hardness (Weiner 2013, Godfrey & Sanders 2004), electrical conductivity (Azous 1991, Sparling 2009), ammonia (NH_3) concentrations (Jofre & Karasov 2009, Lentini 2013), nitrate and nitrite (NO_2+NO_3-N) concentrations (Rouse *et al.* 1999), and turbidity (Schmutzer *et al*. 2008, Babbitt *et al.* 2009) have all been studied for their potential effect on frog species diversity and abundance. Effects of water quality indicators on frogs vary considerably and appear to be species and location specific.

Terrestrial habitat and habitat quality

Research worldwide has shown that amphibians utilise the terrestrial habitat adjacent to water bodies for burrowing, foraging, aestivation and dispersal and that it forms important core habitat for amphibian survival (Bulger *et al.* 2003, Semlitsch & Bodie 2003, Funk *et al*. 2005, Rittenhouse & Semlitsch 2007, Yetman & Ferguson 2011). The size of the water body situated adjacent to terrestrial habitat and the presence of buildings and roads can also affect frog numbers (Houlahan *et al.* 2006). Sufficient terrestrial buffer zones can help to mitigate against the impact of agricultural and urban activity while also providing adequate terrestrial habitat for use by amphibians and other organisms (Phillips 1989, Castelle *et al*. 1994, Vought *et al*. 1995).

The basic aim of this study was to ascertain whether Gauteng's Highveld pans are suitable habitat for frogs, and if so, whether there are relationships between physical pan characteristics and habitat quality. Sites in urban and agricultural areas were compared. I expected there to be more suitable habitat (aquatic & terrestrial) present in agricultural areas as a result of less intense human activity in comparison to pans present in urban landscapes as well as the expectation that more untransformed land is present around wetland pans in agricultural areas compared to heavily exploited landscapes in urbanised areas. However, due to fertilizer load and soil erosion, evidence of eutrophication was expected to be highest in agricultural landscapes.

2.3. Methods and materials

Sites were selected based on their proximity to transformed land, similarity in size and shape, and public accessibility. Google Earth satellite imagery and Spot 5 Satellite imagery (rendered in ArcGIS) were utilised when estimating pan and site size parameters, accessibility and locality information. Pan edges (shorelines) were mapped using satellite imagery and these demarcations were then verified by ground-truthing for accuracy and to ascertain obvious changes in vegetation type. The GPS locations of pan edges were recorded *in-situ* with a handheld GPS (Garmin eTrex series).

All pans were located on the Highveld in Gauteng Province, South Africa. They were all relatively close to each other geographically (within 25 km) and were thus exposed to similar weather patterns and climatic conditions. Eleven pans were included in the study with six located in urban areas with high levels of transformation. The remaining five pans were located in agricultural areas where transformation appeared to be lower (one pan – located at an agricultural site – was not included for water analysis because it no longer contained water at the time of sampling. It was still part of the evaluation of pan characteristics).

The urban sites ranged from being located in light industrial zones (where warehouses and processing plants were in close proximity), to high density residential zones, to being located within secured areas near National Key Points such as OR Tambo International Airport and Denel SOC Ltd. Agricultural sites were located on private property where mainly plant crops were cultivated. These crops consisted of predominantly maize, potatoes and lettuce. Historically, many more pans were present in Gauteng, but many have been subject to land reclamation processes.

The extent of terrestrial habitat around pans at all sites was mapped out in the same way as shorelines, indicating where potential habitat ended and transformed land began. The extent of terrestrial habitat was also defined by the straight-line distance from the shoreline to the furthest point of terrestrial habitat. I included only habitat which was considered accessible to amphibians that were using the wetland to breed. Transformed land was regarded as being any land that was sufficiently disturbed so as to create a barrier which hindered ease of movement (burrowing or roaming) and

dispersal of frogs, for example: tarred roads, paving, buildings, walls and interlinked fencing. Dirt roads located in agricultural areas, which were derelict or showed signs of disuse, were regarded as minor impact and thus excluded from the definition of transformed land.

Water samples were collected in January 2014. On one day during each sampling season (2012/2013 and 2013/2014), the water temperature, pH, electro-conductivity and a measure of total dissolved solids were recorded at each pan. Measurements were collected using a handheld, portable meter (Consort C5010). In each case, the probe was placed at least one meter from the edge of the water body and between 10 and 15 cm below the surface of the water for one minute. Care was taken to avoid disturbing sediment. No cases arose where the 15 cm depth encountered pan substrate. Recordings could not be made at three sites (all in the agricultural category) in the first season of data (2012/2013) collection because of early drying (lack of rain and inaccessibility due to the blocking of roads during the harvest season). Records were taken from all but one site in the second season (2013/2014) because this pan had dried out.

Water samples were collected in the second season for chemical analyses. A dip sampler was used (a clean container mounted on the end of a 2 m pole) in order to avoid the collection of disturbed sediment in the wading area from research activity. Water was filtered immediately through a vacuum filter (pore size = 6µm) whereafter filtered samples were placed in cooler-boxes kept below 15 °C. Samples were stored in one litre, acid-washed, PE bottles as well as separate 250 ml nitric acid-treated, PVC bottles for each site. These samples were sent for analysis the following day to the Inorganic Laboratory at the Council for Scientific and Industrial Research (CSIR) in Pretoria, South Africa.

The following tests were performed by the CSIR using the methods in parentheses.

1. Alkalinity (Potentiometric titration)
2. Ammonia nitrogen (Automated Flow Injection)
3. Calcium, magnesium, potassium and silicon (Inductively-coupled plasma atomic emission spectrometry)

4. Nitrates and nitrites (Automated Flow Injection)
5. Orthophosphate (Automated Flow Injection)
6. Sulphates (Automated Flow Injection)
7. Total Kjeldahl Nitrogen (Automated Flow Injection)
8. Turbidity (Nephelometric tests)

Total permanent water hardness was calculated from magnesium and calcium ion values according to the equation for water hardness (Weiner 2000):

$$\text{Total Hardness (as CaCO3)} = 2.497(\text{Ca}^{2+},\ \text{mg/l}) + 4.118(\text{Mg}^{2+},\ \text{mg/l}).$$

Statistical analyses

Descriptive statistical analyses (means, range, variance, standard deviation, etc.) were predominantly run on water quality measures since sample size was small. A student's t-test was performed on data to test for significant differences between land-use categories. A Pearson's correlation was also performed on water quality data and pan metrics data to investigate whether a relationship between water quality and physical pan characteristics existed. Because measurements appeared to fluctuate more between sites in urban areas compared to agricultural areas, a Levene's Test for homogeneity was performed to detect for significant differences in variability between site categories.

2.4. Results

Site description

All pans were impacted by a variety of human activity and land transformation types. Urban pans (Fig. 1) were bordered by industry (U1, U3), suburban residences (U1-U6), informal and dense residential settlements (U2, U6), OR Tambo International Airport (U4) and differing areas of vacant land (U1-U6). According to a map of Benoni (Benoni 2628AB, Union of South Africa, 1960) at least five of the six, now permanent urban pans (U1-U5), were classified as non-perennial in 1953. All urban sites are bordered by tarred road on at least one side, which in some cases (U1, U3, U5, U6)

carry high traffic volumes. Evidence of dumping of household and construction waste was seen at all sites except U4. Dumping and litter was most notable at sites U1-U3 and U6, affecting the quality of terrestrial habitat around the pans. Satellite imagery shows that vacant land around all urban sample sites and reed beds within pans experience seasonal fires (likely arson events).

Some factors which may affect habitat quality were site specific and are described below.

- Pan U2 was located less than 200 m from a water treatment plant and the large area of open land north of the pan had electricity pylons running across it from a nearby substation. The vacant land was also frequently used as a thoroughfare and human faeces were encountered on occasion throughout the trapping area. One major water inlet to the pan ran through the nearby informal settlement.
- Pan U3 was historically the site of a derelict horse-racing course which was also used as a motor-racing course until 2005. The car storage yard and an old grandstand that were present to the south of the pan were demolished in early 2014. A large metal-recycling plant lies to the south across the R29 from the pan. The area around the pan was also used regularly as a thoroughfare.
- Pan U4 has been extensively altered over the last six decades. The site is fenced off and access is strictly controlled because it lies adjacent to two National Key Points. The site suffers from noise pollution from nearby airport activity and runoff from the airport property empties into it.
- Pan U5 is located just 760 m north-west of a dam in a conserved area which was affected by a major jet fuel spillage (about 1.2 million litres) in 2006. An estimated 200 000 litres was absorbed into the surrounding soil of the dam and another 56 000 litres into the ground water (http://www.acsa.co.za/home.asp?pid=89). A sewage line runs through Pan U5.
- In addition to being used as a thoroughfare, the land adjacent to Pan U6 is also used for grazing cattle.

Pan: U1
(Benoni South - 26°12’21.66” S, 28°18’50.26” E)



Pan: U2
(Apex - 26°12’52.19” S, 28°18’47.69” E)



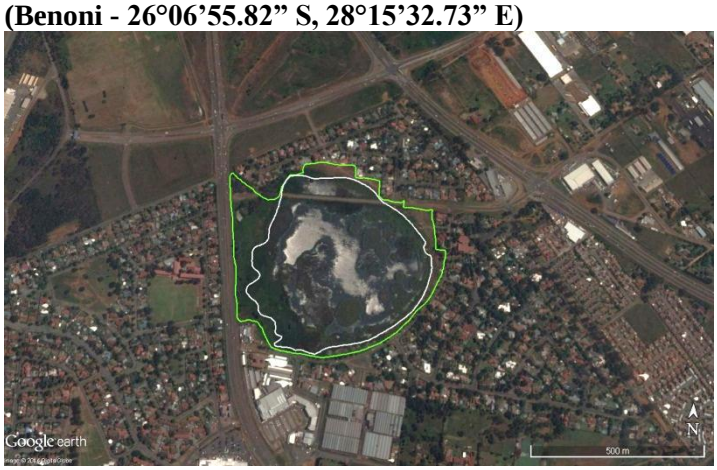
Pan: U3
(Benoni - 26°11’59.61” S, 28°17’12.14” E)



Pan: U4
(Parkhaven - 26°09’18.19” S, 28°15’54.88” E)



Pan: U5
(Benoni - 26°06’55.82” S, 28°15’32.73” E)



Pan: U6
(Esselen Park - 26°01’38.60” S, 28°14’27.42” E)

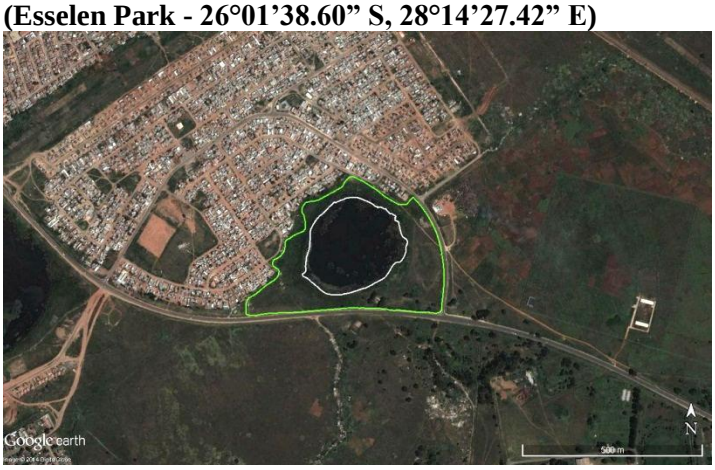


Figure 1: Satellite view of urban sites. The pan boundary (inner, white line) and extent of untransformed land accessible in a straight line from the pan shoreline (outer, green line) is indicated.

Agricultural pans (Fig. 2) were predominantly bordered by cropland which included maize (A1-A5) and vacant land (A1-A5). Potatoes and lettuce were also cultivated adjacent to A2 and A3. Two pans (A2, A5) easily flood adjacent terrestrial habitat after heavy rainfall, Pan A5 especially so because of the very low gradient of the landscape (> 100 m from the pan shoreline). No residences are located near to the sites except for low density farm residences near A2 and A3. Two sites are affected by seasonal mowing of some of the surrounding grassland (A1, A2).

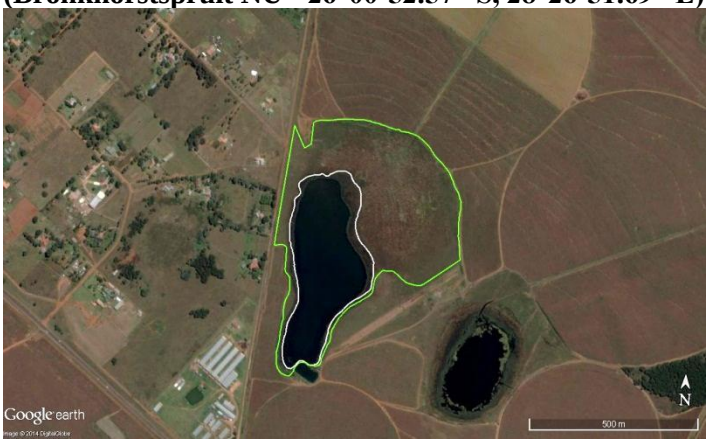
Some factors which may affect habitat quality were site-specific and are described below.

- Pan A1 lies adjacent to a large, vacant plot of land which has an outpost station belonging to the Air Traffic and Navigation Services Limited (ATNS). The pan partially extends into this crudely fenced off property. A busy railway-line is located to the south of the pan.
- The land adjacent to Pan A3 is used to store large quantities of organic fertiliser (compost-manure).
- Some of the land surrounding Pan A4 is currently fallow and was used for crop cultivation. The site is affected by deposition of large amounts of eroded material from surrounding farmland. It is also used as a grazing area for seven head of cattle.

Pan: A1
(Kempton Park NU - 26°01’05.12” S, 28°23’00.46” E)



Pan: A2
(Bronkhorstspruit NU - 26°00’52.57” S, 28°26’51.69” E)



Pan: A3
(Bronkhorstspruit NU - 26°01’23.47” S, 28°27’21.18” E)



Pan: A4
(Bronkhorstspruit NU - 26°02’44.41” S, 28°24’04.47” E)



Pan: A5
(Kempton Park NU - 26°02’44.41” S, 28°24’04.47” E)

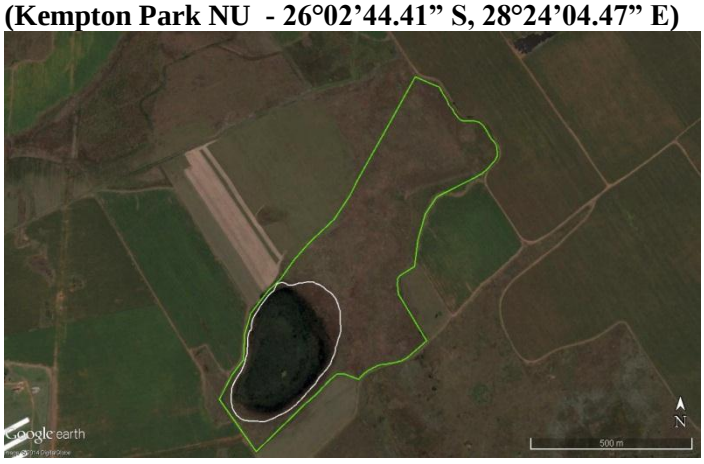


Figure 2: Satellite view of Agricultural sites. The pan boundary (inner, white line) and extent of untransformed land accessible in a straight line from the pan shoreline (outer, green line) is indicated.

A part-summary of results from frog surveys conducted at each of the sites in this project are reproduced below (Table 1) from another chapter (see Chapter 3). Presence data is based on pitfall trap records and anecdotal data from observations made while processing sites. Greater detail on species diversity and abundance can be found in Chapter 3.

Table 1: Select species presence at all sampled sites. Species listed (ordered alphabetically) are a mix of trapped individuals and those observed in the field. This bird list is a limited version compared with the complete observation list where very common bird species (e.g. sparrows, weavers and coots) are excluded from this list.

Common Name	**Species Name**	**U1**	**U2**	**U3**	**U4**	**U5**	**U6**	**A1**	**A2**	**A3**	**A4**	**A5**
Birds												
Yellow-billed Duck	*Anas undulata*							X	X	X	X	X
Squacco Heron	*Ardeola ralloides*					X						
Marsh Owl	*Asio capensis*		X					X			X	X
White-faced Duck	*Dendrocygna viduata*					X						
Amur Falcon	*Falco amurensis*							X				X
Helmeted Guineafowl	*Numida meleagris*				X							
Reed Cormorant	*Phalacrocorax africanus*							X	X	X	X	
Greater Flamingo	*Phoenicopterus ruber*		X			X		X			X	
Glossy Ibis	*Plegadis falcinellus*							X				
African Purple Swamphen	*Porphyrio madagascariensis*						X					
Sacred Ibis	*Threskiornis aethiopicus*						X					
African Grass Owl	*Tyto capensis*											X
Amphibians												
Common River Frog	*Amietia quecketti*								X		X	
Guttural Toad	*Amietophrynus gutturalis*	X	X	X	X	X	X	X	X	X	X	X
Boettger's Caco	*Cacosternum boettgeri*			X	X	X	X	X	X	X	X	X
Bubbling Kassina	*Kassina senegalensis*				X		X	X	X			
Giant Bullfrog	*Pyxicephalus adspersus*							X	X			

Striped Stream Frog	*Strongylopus fasciatus*		X									
Tremolo Sand Frog	*Tomopterna cryptotis*							X	X			
Common Platanna	*Xenopus laevis*				X	X		X	X			
Reptiles												
Bibron's Blind Snake	*Afrotyphlops bibronii*							X				
Rinkhals	*Hemachatus haemachatus*				X					X		
Aurora House-snake	*Lamprophis aurora*											X
Brown Water Snake	*Lycodonomorphus rufulus*							X				
Cape Gecko	*Pachydactylus capensis*							X				
Rhombic Skaapsteker	*Psammophylax rhombeatus*							X				
Cape Skink	*Trachylepis capensis*											X
Mammals												
Lesser red musk shrews	*Crocidura hirta*				X	X						
Blesbok	*Damaliscus pygargus phillipsi*							X				
Duiker	*Sylvicapra grimmia*											X
Species Count		**U1**	**U2**	**U3**	**U4**	**U5**	**U6**	**A1**	**A2**	**A3**	**A4**	**A5**
		1	4	2	7	7	5	17	9	5	7	9

Chemistry

On average, alkalinity was highest in urban areas (170.83 mg/l) with four of six urban sites measuring higher than 100 mg/l $CaCO_3$. Two sites in urban areas had alkalinity higher than 300 mg/l of $CaCO_3$. Alkalinity in agricultural pans (65.25 mg/l) never exceeded 100 mg/l $CaCO_3$ (Table 2). The variance of alkalinity readings was significantly greater ($p < 0.05$; $df = 8$) between urban sites compared with agricultural sites (see Fig. 3 & Table 3). A range of 258 mg/l of $CaCO_3$ was found between urban sites whereas the range of alkalinity between agricultural sites was only 32 mg/l

$CaCO_3$. A student's t-test however, did not detect a significant difference between urban and agricultural data.

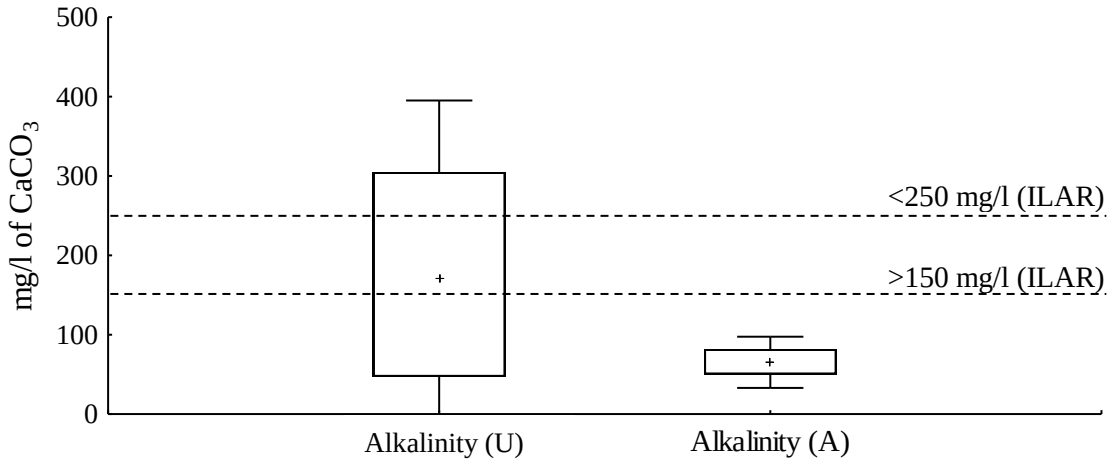


Figure 3: Alkalinity between sites within urban (U) and agricultural (A) areas. Recommended range of alkalinity considered ideal for general frog health are indicated by the dotted line (ILAR 1974).

Most ammonia nitrogen (NH_3-N) measures were below 0.1 mg/l at all sites. Only two sites, one urban and one agricultural had readings higher than this. The highest at 3.2 mg/l was an urban site (U3) while the next highest reading, 1.5 mg/l, was at an agricultural site (A2) (Table 2). The values at both of these sites exceed the NH_3-N concentrations known to have negative effects on amphibian health. There was no significant difference in the concentrations of NH_3-N overall between urban and agricultural pans.

Table 2: Water quality indicators as found per sites along with urban and agricultural means. Analyses were conducted using the lowest number indicated when concentrations or readings were indicated as being below a given figure (e.g. sulphates in agricultural pans were taken as 5.0 mg/l for the purposes of analyses)

	Unit of Measurement	U1	U2	U3	U4	U5	U6	Mean Urban	A1	A2	A3	A4	Mean Agri
Alkalinity	mg/l as $CaCO_3$	305	177	127	47	68	301	170.83	50	53	76	82	65.25
Ammonia nitrogen	mg/l as N	< 0.1	< 0.1	3.2	< 0.1	< 0.1	< 0.1	0.62	< 0.1	1.5	< 0.1	< 0.1	0.45
Calcium	mg/l Ca	129	57	28	7.8	17	22	43.47	3.9	6.7	11	10	7.90
Magnesium	mg/l Mg	157	122	12	7.6	5.8	14	53.07	3.5	3.2	6	5.7	4.60
Nitrate + Nitrite	mg/l N	0.29	0.56	0.46	3.4	0.51	2	1.20	0.46	0.64	0.49	0.31	0.48
ortho Phosphate	mg/l PO_4	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	<0.2	0.20	< 0.2	< 0.2	< 0.2	< 0.2	0.20
Potassium	mg/l K	9.1	57	5.9	1.6	2	35	18.43	3.1	8	9.1	12	8.05
Silicon	mg/l Si	11	3.2	4.3	4.4	0.67	1.2	4.13	0.97	1.2	1.2	1.5	1.22
Sodium	mg/l Na	66	310	21	5.6	13	129	90.77	4.5	5	13	13	8.88
Sulphate	mg/l SO_4	110	1456	40	5.2	5.5	< 5.0	269.53	< 5.0	< 5.0	< 5.0	< 5.0	0.50
TKN	mg/l	0.95	1	4.1	1.7	1.3	3.9	2.16	1.7	3.1	2.4	1.2	2.10
Turbidity	NTU	16	3.3	6.8	6.7	16	3.3	8.68	6.8	6.7	4.4	11	7.23
Total Permanent Water Hardness	mg/l of $CaCO_3$	968.64	644.73	119.33	50.77	66.33	112.59	327.06	24.15	29.91	52.18	48.44	38.67

Table 3: Results of Levene's Test for homogeneity of variance in data sets. Significance ($p < 0.05$) indicates a significant difference of the variance between datasets from urban and agricultural areas. The final column indicates the category (U – urban; A – agricultural) with the significantly greater variance.

Measure	Levene F	df	p-value	Favour
Pan Size	0.51	8	0.496	-
Land Size	12.08	8	**0.008**	A
pH	0.24	7	0.881	-
Temperature	0.37	7	0.562	-
Electro-conductivity	2.64	7	0.148	-
Alkalinity	7.98	8	**0.022**	U
Ammonia nitrogen	0.55	8	0.479	-
Calcium	4.89	8	0.058	-
Magnesium	19.39	8	**0.002**	U
Nitrate + Nitrite	8.84	8	**0.018**	U
ortho Phosphate	-	-	-	-
Potassium	8.81	8	**0.018**	U
Silicon	3.06	8	0.118	-
Sodium	5.31	8	0.050	-
Sulphate	3.95	8	0.082	-
TKN	3.41	8	0.102	-
Turbidity	4.50	8	0.067	-

Metal concentrations varied more between urban sites than agricultural sites (Fig. 4). Sodium concentrations varied the most between sites in urban areas followed by Mg, Ca and K. Urban sites accounted for the highest concentration of metals as well as highest metal concentrations on average overall compared to agricultural sites. Potassium and Mg variability were significantly greater between sites in urban areas compared to between sites in agricultural areas (compare Table 3).

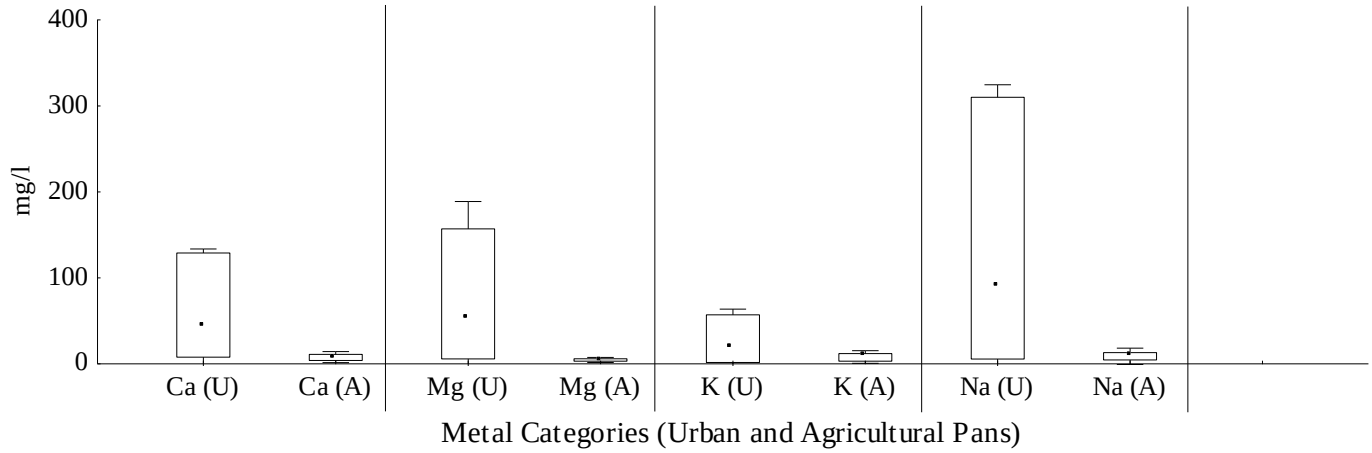


Figure 4: Metal concentrations between pans within urban (U) and agricultural (A) areas

Electro-conductivity measures were most variable between urban sites while showing little variability between agricultural sites (Fig. 5). Between-site variability within urban and agricultural categories were not significant different (Table 3). Electro-conductivity was not significantly higher in urban areas when compared to agricultural areas ($t = 2.2$, $df = 11$, $p = 0.06$). Electro-conductivity was highest at sites U1 and U2 and lowest at one access-controlled urban site (U4) as well as the agricultural sites (Table 4). Electro-conductivity was positively correlated with measures of K ($r = 0.90$, $p < 0.05$), Na ($r = 0.97$, $p < 0.05$) and SO_4 ($r = 0.91$, $p < 0.05$)

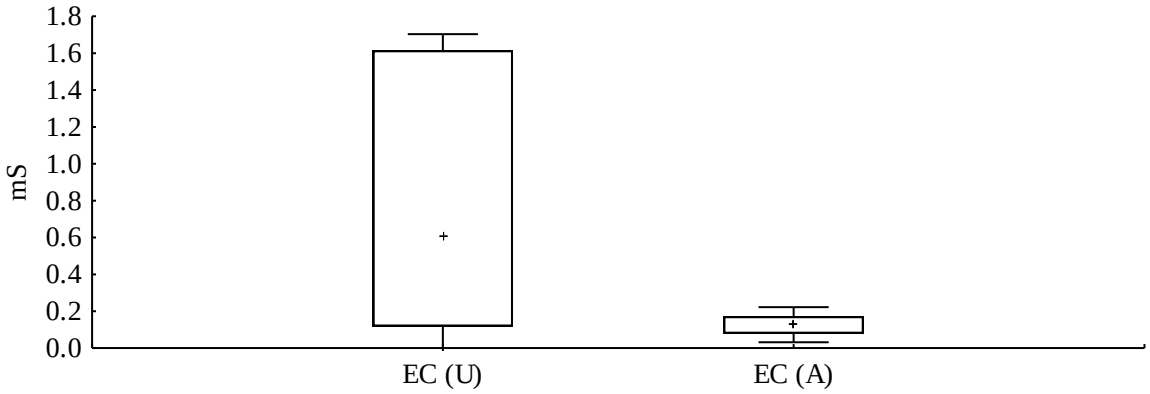


Figure 5: Electro-conductivity between pans within urban (U) and agricultural (A) zones

Table 4: Additional water chemistry values as averages over two seasons of sampling.

Pan ID	pH	Temperature (°C)	Electro-conductivity (µS/cm)
U1	7.3	20.5	740
U2	7.2	23.2	1616
U3	7.7	23.9	383
U4	6.8	20.5	117
U5	6.9	25.1	198
U6	8.7	28.0	613
A1	6.2	30.8	78
A2	7.3	28.0	130
A3	7.1	27.0	173

Nitrite + nitrate concentrations were highest, on average, at urban sites (1.2 mg/l N) compared to agricultural sites, which averaged 0.48 mg/l N. There was significantly higher variation ($p < 0.05$; $df = 8$) in $NO_2 + NO_3$-N concentrations between sites in urban areas (3.11 mg/l N) compared with those

of agricultural areas (0.33 mg/l N), (Fig. 6 & Table 3). No significant difference was found between urban sites and agricultural sites ($p = 0.21$; $df = 5$). All $NO_2 + NO_3$-N concentrations apart from one measured at an urban site (U1), were above the concentration recommended by ILAR (1974), (0.3 mg/l) to prevent health complications in amphibians.

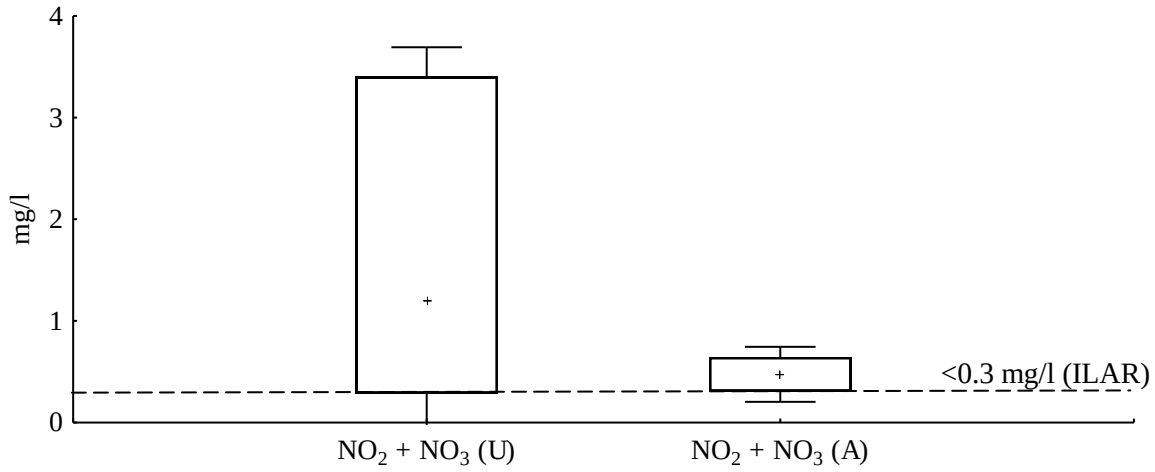


Figure 6: Concentration range of nitrates and nitrites between pans within urban (U) and agricultural (A) categories. Recommended maximum $NO_2 + NO_3$-N concentration before it was considered to be toxic to amphibians (ILAR 1974) is indicated by the dotted line.

Turbidity varied more between urban pans than agricultural ones (Fig. 7). Urban pans also had the most turbid and least turbid waters.

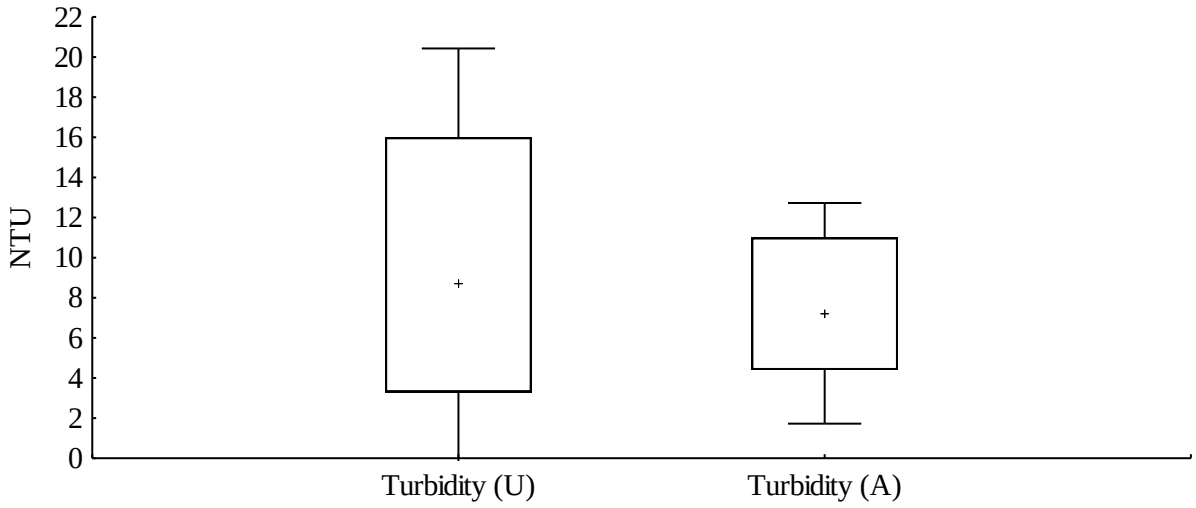


Figure 7: Range of turbidity between pans within urban (U) and agricultural (A) categories.

The mean surface area of pans in urban areas and agricultural areas were comparable (Fig. 8). The area of open terrestrial habitat surrounding pans however, was five times greater on average around agricultural pans compared to pans in urban areas (although not significantly greater ($p = 0.32$, $df = 4$, $t = 2.13$)).

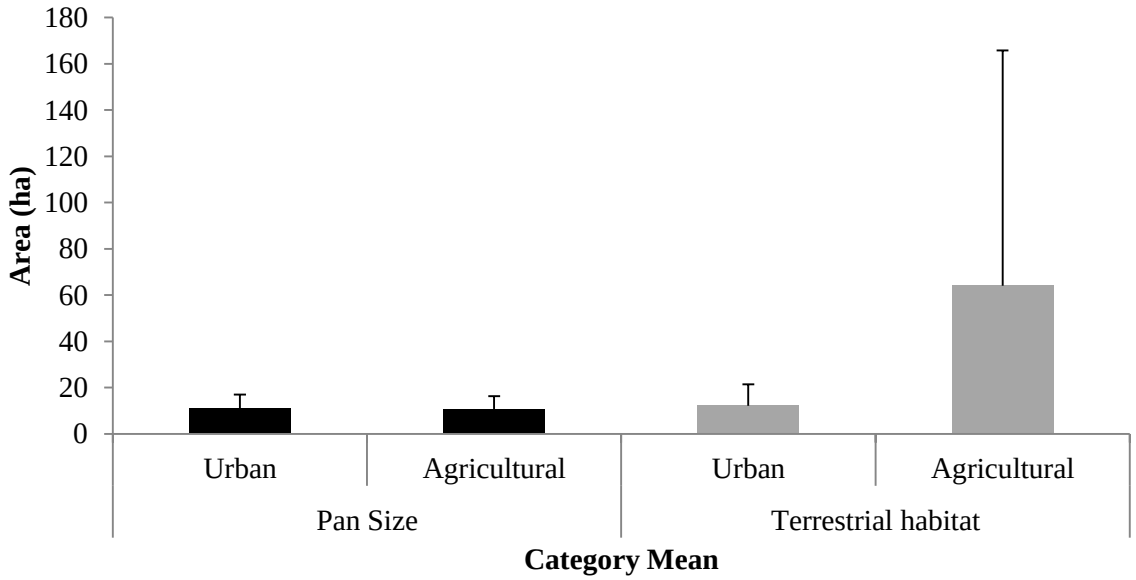


Figure 8: Mean area of pans and surrounding terrestrial habitat in urban (U) and agricultural (A) areas

The distance from the edge of the pans to the edge of transformed land in urban areas (mean = 62.4 m) was significantly different ($t = -3.39$, $df = 130$, $p < 0.005$) from that of agricultural areas (mean = 182.8 m) (Fig. 9). This distance measure was also significantly more variable between agricultural sites compared with urban sites (Levene $F = 19.09$, $df = 130$, $p < 0.00005$), (Table 3).

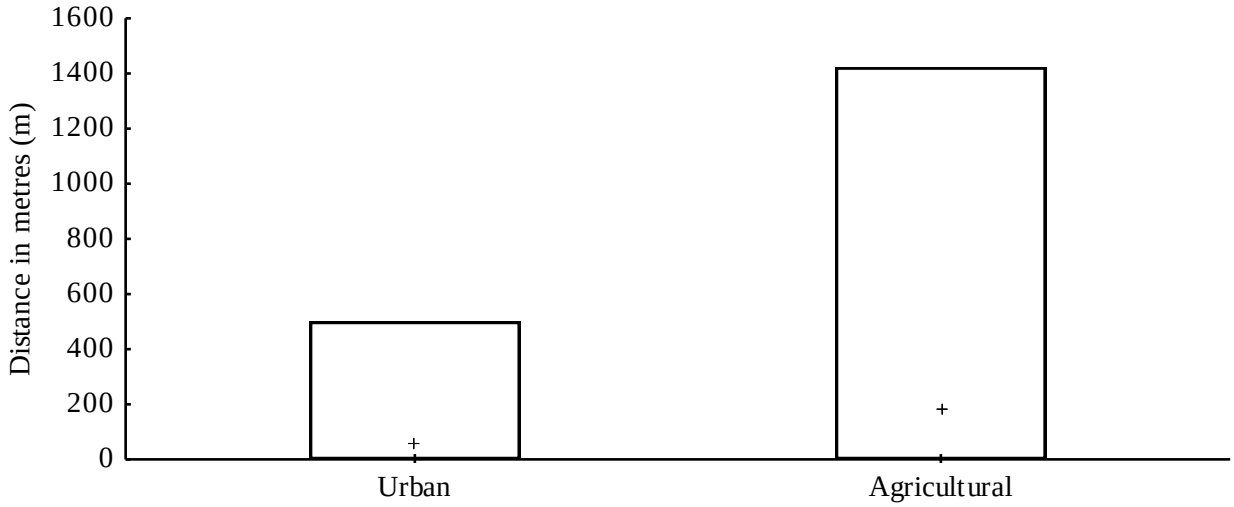


Figure 9: Range of average distance between pan edge and transformed land in urban and agricultural areas.

The shortest mean straight-line distance (5 m) to transformed land from the pan shoreline was recorded at an urban site while the longest (534 m) was recorded at an agricultural site (Table 5). The smallest habitat patches were recorded at urban sites while the largest was recorded at an agricultural site (Table 5).

Table 5: Site dimensions relating to pan size and surround terrestrial land area

Pan ID	Pan Surface Area (m^2)	Mean distance shoreline to nearest transformed land (m)	Area of untransformed land (m^2)
U1	96 200	5	24 600
U2	99 250	61	133 350
U3	129 600	81	129 950
U4	55 950	108	297 400
U5	218 550	39	80 200
U6	54 850	68	84 100
A1	138 700	534	2 511 150
A2	84 050	109	202 550
A3	39 750	73	89 450
A4	72 000	136	221 250
A5	106 650	101	310 000

Tarred roads were located significantly further away from the edge of pans in agricultural areas when compared with urban sites ($t = 2.78$, $df = 4$, $p < 0.05$; Fig. 10). More dirt roads (un-tarred) were present in agricultural areas than at urban sites, but had low vehicle densities and sometimes suitable for 4x4 vehicles only. Buildings were more densely grouped in urban areas when compared to more isolated grouping of structures in agricultural areas. The mean distance from the edge of a pan to the

closest building was much greater in agricultural areas (278 m) compared to urban areas (18 m), although the difference was not significantly different (t-stat = 2.78, df = 4, p = 0.068; Fig. 10).

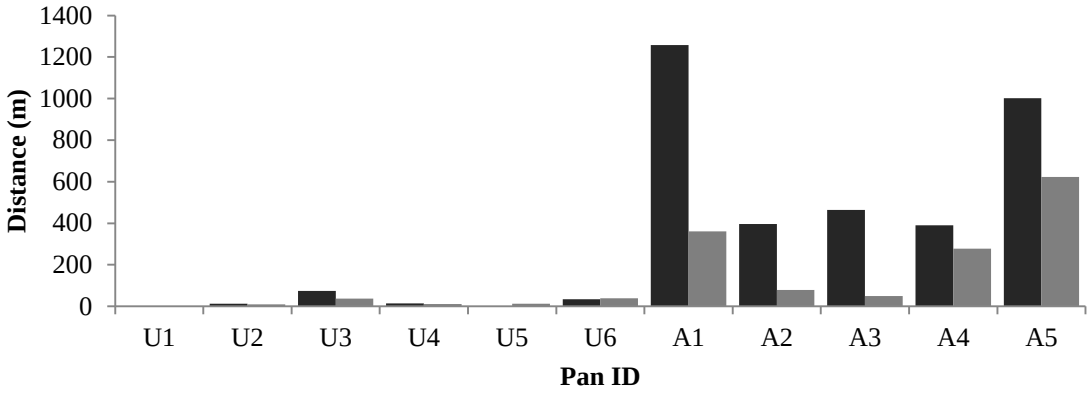


Figure 10: Graphical representation of the shortest possible distance from the edge of a pan to tarred road (black) and buildings (grey) at urban and agricultural sites.

2.5. Discussion

Water quality

The demand for water for urban development is high in Gauteng Province which impacts water resources and as a result the ecosystem health of water sources have been severely affected (GDARD 2011). Four of five (GDARD, 2011) water quality indicators used by the Gauteng government (alkalinity, pH, electro-conductivity and turbidity) were used in this study to evaluate suitable water quality for the optimum survival of amphibians.

Alkalinity varied significantly between sites in urban areas compared to between sites in agricultural areas (Table 3). All but one pan had alkalinity values outside optimum for amphibians, according to ILAR guidelines (ILAR 1974). One pan (U2) had $CaCO_3$ concentrations which fell within the range recommended for keeping amphibians in captivity (ILAR 1974) while only one other (U3) had concentrations suitable to buffer against sudden negative changes in pH (Table 2). It is difficult to ascertain what impact alkalinity measures may have on amphibians in a non-captive context because of a lack of available information and possible varying effects on different species (Glooschenko *et al*. 1992, Brodman *et al*. 2003). The ability for pans in agricultural areas to buffer against pH change is reduced compared with most urban sites (Fig. 3; Table 2). Alkalinity is highly variable between urban pans however (Fig. 3), so caution must be taken when generalising as to the probability that an urban pan would provide better buffering capacity compared to an agricultural pan. Because of the low variability in alkalinity values amongst pans in agricultural areas, similar alkalinity could be expected at most agricultural pans situated in the region (Fig. 3).

At least three of the pans (U1, U2, U3) are located within the vicinity of either historical or extant gold mining and also contained the highest concentration of sulphates of all the study sites. Many of the disused mine shafts on the East Rand of Gauteng are now regarded as being potential sources of Acid Mine Drainage (AMD), (GDARD, 2011). Evidence that AMD related processes affect water quality at some urban sites in Gauteng is clear. One urban site (U2) has sulphate (SO_4) values (1456 mg/l) which are indicative of considerable AMD contamination (Table 2). It is likely that the presence

of large quantities of AMD would have negative consequences for wildlife inhabiting these affected waters as is the case with other similar events in Gauteng (see DWAF 2010 for examples). Sites U1 and U3 may also be affected by AMD, but to a lesser degree (Table 2). Although government data for groundwater shows that most of the province has good quality groundwater, it is acknowledged that AMD threatens this (GDARD 2011). Since groundwater can affect surface waters (Page 1981), AMD's negative effect on ecology may affect more wetland pans in the province as AMD spreads in groundwater. Conversely, high sulphate concentrations were not detected at any of the agricultural sites which show that the agricultural sites are not currently affected by AMD contamination (Table 2).

Nitrate + nitrite concentrations were more variable between urban pans compared with agricultural pans which were relatively consistent (Fig. 6, Table 3). This may again be indicative of the diverse number of impacts experienced by pans in urban areas. It was expected that agricultural areas would have the highest concentrations of nitrate and nitrite compared to pans of urban areas due to the application of fertilizers on croplands. Urban sites however, had higher NO_2 + NO_3–N concentrations overall with a mean of 1.20 mg/l of N versus the agricultural mean of 0.48 mg/l of N (Table 2). This may indicate the presence of sewage contamination at some sites especially when urea-N has had sufficient time to convert to nitrate. Phosphorus (ortho-phosphates (PO_4)) appears to be the limiting nutrient at all sites which is expected in most freshwater systems (Barsanti & Gualtieri 2006) since PO_4 concentrations were all below the detection limit of 0.2 mg/l (Table 2). Although NO_2 + NO_3–N concentrations were higher and more varied across urban sites, concentrations at agricultural sites are not considered suitable for amphibians either.

Mineral concentrations (Na, Mg, K, Ca) in pans were also highly variable between urban sites compared to between agricultural sites (Fig. 4). This led to high variation in total permanent water hardness between pans in urban areas. Water at urban sites was generally hard where two sites were exceptionally hard (where >200 mg/l of $CaCO_3$ indicates very hard water) (Weiner 2013). Conversely, water at all agricultural sites was relatively soft which correlates with the ephemeral properties of these pans and with rainfall being their primary water source (Weiner 2013). Two urban sites (U4,

U5) had comparably soft water to agricultural sites (Table 2) which may indicate that their predominant water source differs in some way to water sources feeding other urban pans. Electro-conductivity of water is used globally as a proxy for water quality and indicates salt saturation in water (Kadlec & Wallace 2009). Electro-conductivity was highly variable between urban sites and correlated positively with Mg, K and Na concentrations (Fig. 5). Pans in urban areas are exposed to greater sources of minerals from increased runoff, and in mining areas, increased liberation of waste minerals from ore, likely reasons for increased electro-conductivity and mineral concentrations at urban sites compared to agricultural sites (Fig. 5, Table 4). Fallout from air pollution (industry, vehicle exhaust) in urban areas may also be contributing to increased mineral content at urban pans.

Higher water hardness can be caused by groundwater which passes through mineral deposits (Weiner 2013) and with the purposeful liming of water bodies to increase alkalinity or water hardness (Boyd & Tucker 1998). The two urban pans (U1 & U2) with the highest water hardness also have the highest sulphate concentrations (Table 2). It appears that liming (addition of $CaCO_3$ to control acidity) of water affected by mining practices may be taking place at or near pans U1 and U2 (compare pH, EC and SO_4 concentrations: Table 2, Table 4). It is unlikely that the higher water hardness of these pans would translate to improved amphibian larval development because the likely reason for the water hardness (AMD amelioration) would make the pan unsuited to most aquatic life.

Turbidity was most variable in urban pans (3.3 NTU – 16 NTU; Fig. 7 and Table 2). Most pans had water which would be considered visibly cloudy (>5 NTU) (AWWA 2003). Turbidity in aquatic ecosystems varies naturally depending on local conditions. Turbidity in the study pans was not notably high or low, and is unlikely to be detrimental to the environment and to frog populations on the dates observed. The negative correlation found between turbidity and amphibian species richness by Hecnar & M'Closkey (1996) was with a turbidity range of 0-185 JTU (Jackson Turbidity Units) and a mean of 9 JTU. This means that all turbidity readings in this study fall at the lower end of this range (Table 2) where amphibian richness in their study was relatively higher. NTU are roughly equivalent to JTU (Wilde 2014). Pans with low turbidity may still provide adequate refugia for amphibians when they contain vast quantities of aquatic vegetation which may offer refuge to

developing frog larvae (for example: U6). While a wider range as well as greater variability of turbidity between pans was experienced in urban areas (Fig. 7), agricultural pan turbidity did not differ significantly from urban pan turbidity.

Terrestrial habitat availability and pan metrics

On average, more terrestrial habitat existed at agricultural sites compared to urban sites (Fig 8, Fig 9). Land utilisation by humans appears to be maximised in urban areas since transformation of land occurs closer to the shoreline for urban pans (compare pans: Fig. 1, Fig. 2, Fig. 10) thus providing little evidence that sufficient buffer zones were delineated historically. Likewise, little evidence of buffer zone delineation was found at agricultural sites. Most crops, however, were sown at a distance from pans, possibly to avoid the risk of flooding. This provides a default buffer area and was especially evident when the landscape had a noticeably shallow gradient.

Thus it appears that pans in agricultural areas were better suited to meeting amphibian terrestrial habitat requirements compared to pans impacted by urbanisation (Fig. 8; Table 5). It should be noted however, that some species of frog move great distances to forage and aestivate (e.g. *Pyxicephalus adspersus* and *Schismaderma carens*) (du Preez & Carruthers 2009). For example, the average amount of available terrestrial habitat in Gauteng's agricultural areas would still fail to meet the requirements of many female *Pyxicephalus adspersus* (endemic to southern Africa) which can disperse up to nearly 1 km away from water bodies in order to aestivate for the dry season. However, the extent of only one agricultural pan's terrestrial habitat (A1) met the mean distance found (446.8 m) for female *P. adspersus* dispersal as defined by Yetman and Ferguson (2011). The dispersal behaviour of other species in Gauteng is largely unknown and limited research has been conducted into how low terrestrial habitat availability would impact population size.

All six urban sites had tarred roads immediately adjacent to the pan site where four of these were busy, main roads with fast moving traffic and/or heavy congestion at peak times. Glista *et al*. (2007) found that traffic is a major source of amphibian mortality since amphibians tend to disperse *en masse* from breeding grounds and so frogs dispersing from these water bodies would be at high risk of road

fatality (Bouchard *et al*. 2009). Conversely, pans located in agricultural areas were significantly ($p < 0.05$, $df = 4$, $t = 2.78$) further from busy roads compared with urban pans (Fig. 10) where the shortest distance (pan edge to tarred road) measured for any agricultural site was more than five times that of the shortest distance measured for any urban site. No agricultural sites were located adjacent to tarred roads. Whilst being located adjacent to dirt roads, risk of road fatality in agricultural areas while dispersing is unlikely since these roads rarely had vehicular traffic.

Property boundary walls can hinder frog dispersal (de Villiers 2004). Urban areas in Gauteng are characterised by walled and fenced-off properties. While fences pose less of a problem for dispersal, walls create an impassable obstacle for many species and result in increased habitat fragmentation. Frogs managing to traverse a busy, urban road would still need to find a way to circumnavigate large townhouse complexes, walled industrial complexes and walled residential properties in suburban areas in order to get to another major water body (Fig. 1). At all six urban sites, frogs would be presented with one or a combination of fences, walls, building development and trenches. Agricultural sites were less affected by walls and fences (Fig. 2). At most, dispersing frogs would encounter farm fencing which would pose little to no hindrance to dispersal since in most instances large gaps between the ground and wiring make it easy to pass. Although no significant difference was found between urban and agricultural shoreline to building distances (Fig. 10), it is noteworthy that farm buildings are likely to pose less of an obstruction to dispersing animals as they are usually standalone structures, distant from other buildings and neighbouring farms (pers. obs.).

Pollution and litter around wetland pans was present in noticeably greater quantities in urban environments which may affect amphibian habitat health. Open spaces, are regularly used for illegal dumping. All urban sites, apart from one (U4), were subject to extensive dumping of refuse and building material. Buried rubble and refuse were also encountered when digging holes for pitfall traps, possibly indicating that these areas have been used over long periods as dumping sites. Agricultural areas, in contrast, did not appear to be as affected by dumping. Some agricultural sites were affected by the deposition of large amounts of soil from cropland during heavy rains which may negatively affect the suitability of terrestrial habitat by burying foraging area and refugia as well as

making the pan basin shallower over time, resulting in reduction of water retaining capability and shortening hydroperiod (Tsai *et al*. 2007).

Rubbo & Kiesecker (2005) found that wetlands in urban areas tended to become more permanent. This is also true of the urban pans exampled here which may have been deepened during the process of urbanisation. Most agricultural pans examined were are ephemeral and appeared to be subject to natural hydrological patterns of seasonal drying and wetting. Because ephemeral pans may provide better habitat for frog breeding (Gibbs *et al*. 2007), Gauteng's agricultural pans likely provide habitat more conducive to the persistence of frog populations. Certain species of frog may also have mating behaviour that is suited only to areas with shallow water such as in *P. adspersus* (du Preez & Carruthers 2009). It is likely that the more natural pans are, the more suitable they will be for the persistence of frog populations.

Water quality indicators which may indicate pan suitability for amphibians are highly variable between sites in urban areas of Gauteng. They are more consistent and less variable by comparison, in agricultural areas (with the exception of available terrestrial habitat). Greater variability in anthropogenic pressures may explain why urban pan water chemistry varies considerably from one pan to the next. Some impacts on urban pans surveyed indicate that mining, nutrient enrichment and extensive urbanisation are all affecting habitat quality. Urban pans, in general, do not appear to provide optimum environmental conditions for frogs (and likely other taxonomic groups) especially for those species that forage and aestivate away from water bodies. Some pans however, may be more suitable than others. Pan U4, which had limited access, had relatively more undisturbed habitat, less litter and water quality parameters which were not unlike those found in agricultural areas. The benefit of fencing (not walling) of surrounding habitat requires further investigation.

Wetland pans in agricultural areas of the Highveld provide better habitat for frogs than urban pans reflected in both higher frog abundance and higher biodiversity (Table 1). This is attributable to a combination of characteristics of agricultural pans including more surrounding undisturbed terrestrial habitat, greater distance to roads and vehicular traffic, relatively natural pan hydrology, and low SO_4

concentrations suggesting no AMD pollution which affects urban pans. Greater diversity of bird fauna (Table 1) was observed in agricultural sites which may translate to increased frog predation by birds but the greater diversity of observed fauna in agricultural areas strongly suggests better overall ecosystem health. The water chemistry of pans in urban areas was much more variable than pans in agricultural areas, suggesting more and more variable pollution inputs. Urbanisation has had a marked impact on ecology and general pan health probably because they have been altered too far beyond their natural state and are located in areas high in pollution and disturbance which affects potential frog terrestrial habitat.

Chapter 3 - Frog Biodiversity and Dispersal at Wetland Pans in Urban and Agricultural Gauteng, South Africa

3.1. Abstract

In order to conserve amphibians effectively in anthropogenically transformed environments, new integrative strategies need to be employed where frog biodiversity is conserved through the preservation of natural land surrounding water bodies. Little is known about the impact that land transformation has on amphibian biodiversity of isolated Highveld pans. Research was undertaken to investigate the relationship between various habitat characteristics (e.g. terrestrial habitat availability and basic water chemistry) and frog biodiversity. I also estimated the extent of terrestrial habitat use by frogs as a function of distance from pan shorelines. Eight of an expected 16 species for the region were detected using the pitfall trap method of sampling. Three of these eight species were found to have population densities that were strongly correlated with the area of available terrestrial habitat. Another three species (two are listed as locally invasive) were found to persist at similar abundances in urban/sub-urban areas and agricultural areas. A positive correlation was found between a water chemistry variable (NO_2 + NO_3-N) and abundance of *Amietophrynus gutturalis*. Pitfall trap captures clearly show that six of eight species utilise terrestrial habitat to at least 80 m from water bodies. Environmental impact assessments and government legislation should take cognisance of the importance of terrestrial habitat for amphibian conservation when legislating for the protection of sufficient terrestrial habitat around wetlands in order to conserve amphibian. The findings and recommended buffer zones presented for the preservation of the studied frog species in this study are in agreement with prescribed buffer zones of the National Water Act of 1998. In all the studied wetland pans however, land alteration within the prescribed buffer has already/is currently taking place.

3.2. Introduction

Urban and agricultural practices threaten amphibians worldwide (Rouse *et al.* 1999, Branch & Harrison 2004, Gibbs *et al.* 2005, Riley *et al.* 2005, Rubbo & Kiesecker 2005, Gallant *et al.* 2007). Since Gauteng Province is South Africa's economic hub, its natural environment has been substantially affected by urban and agricultural land transformation (GDARD 2011). In an attempt to conserve remaining natural areas, legislation that highlights the importance of careful management in areas experiencing development pressure has been drafted (Government of South Africa 1998) along with a prescription of appropriate buffer zones which are needed to protect wetlands (DWAF 2005). The regulations and guidelines of the National Water Act of 1998 attempt to provide guidelines for delineation of buffer zones by requiring that land alteration occur no less than 500 m from the wetland boundary and be subject to specific dimensions. Applications for the alteration of such land are required by the Department of Water and Sanitation. The effective enforcement of this legislation however, is unclear. Environmental impact assessors should still take frog terrestrial use into account when providing a report or processing an application for the development of land near a wetland.

Information about frog terrestrial habitat use, which may influence legislative outcomes preserving frog populations and frog biodiversity in Gauteng, is lacking. Since land transformation is likely to continue to affect natural areas, a solution that involves some level of "co-existence" is needed to facilitate species persistence as well as cater for the economic pressures which dictate the expansion of urban and agricultural landscapes. Knowledge of the extent to which different species of frog move from the edge of a wetland is needed in order to provide estimates as to the size of buffer zones. Since larger patches and a better quality of natural habitat surround pans in agricultural areas (Chapter 1), comparing species richness and abundance between urban and agricultural areas on the Highveld would provide an indication as to the impact urbanisation has had on frog biodiversity in Gauteng. The data will also be useful in indicating the general health and species composition of frog communities in the province.

Urban vs agricultural zones – richness & abundance

Amphibians are affected by water quality (Boyer & Grue 1995, Howe *et al*. 1998), proximity to roads (Fahrig *et al.* 1995), urbanisation and habitat loss and fragmentation (Cohn 1994, Bender *et al.*, 1998, Hamer & McDonnell 2008), and agricultural pollutants (Mann & Bidwell 2001, Mann *et al.* 2009). Because agricultural and urban landscapes are so different from each other, different combinations of the above factors pose a threat to amphibian species in each land use. Some species may tolerate and even thrive in the face of habitat alteration, while others may be extirpated from the landscape (Collins & Crump 2009). Thus, a greater species richness and abundance would be expected in agricultural zones where habitats are less transformed (absence of dumping, fewer roads compared with urban areas and absence of human thoroughfares) despite the use of agricultural fertilisers, absent from urban areas.

Frog dispersal

Published South African frog distributions indicate that 29 of 118 South African frog species (Measey 2010) can be expected to occur in Gauteng Province (Minter *et al.* 2004; du Preez & Carruthers 2009). Of these, 21 are listed as being found regularly in the grassland biome (the predominant biome of the region (Rutherford *et al.* 2010)), and of those, 14 are listed as inhabiting suburbia and agricultural areas (du Preez & Carruthers 2009). Sixteen of the 21 grassland-inhabiting species can be found in the wetland types which were the focus of this study. Three species native to Gauteng (*Amietophrynus gutturalis*, *Hyperolius marmoratus*, *Xenopus laevis*) are recognised as potentially invasive outside of their natural range (DEA 2004, DEA 2014) and this may indicate they are better able to adapt to a wider range of environmental conditions.

Dispersal for the purposes of finding sites for aestivation and foraging has been documented in one southern African species, the Giant Bullfrog (*Pyxicephalus adspersus*). Some evidence suggests that *P. adspersus* forage up to 20 m from their long-term burrows which can be situated up to 900 m from breeding sites for females and up to 330 m for males (Yetman & Ferguson, 2011). As is the case with many pond-breeding anurans, *P. adspersus* is generally philopatric to breeding sites (Yetman &

Ferguson 2011), which adds to the importance of terrestrial buffers around wetland pans. The same study suggests that a 500-1000 m wide buffer zone would be needed to protect most adults aestivating around breeding sites (Yetman & Ferguson 2011). Other pan-breeding South African anurans may need similar conditions in order to persist, making a study which investigates spatial habitat use important.

I investigated amphibian terrestrial habitat use at Highveld pans in Gauteng with the intention of measuring anuran community species composition and abundance with increasing distance from pans. I also took measures of pan metrics and water quality (see Chapter 2) to ascertain whether any correlation could be found with amphibian species abundance. The resulting data were also used to make suggestions about the amphibian specific delineation of wetland buffer zones.

3.3. Methods and materials

Frog surveys were conducted at all sites where water quality and pan characteristics analyses were performed (Chapter 1). Species richness and abundance data were collected by installing pitfall traps in an area adjacent to the edge of each pan. Each pitfall trap consisted of a 9-litre bucket with holes (Ø 5 mm) in the bottom to facilitate drainage after rain. A square, wooden cover mounted on steel stakes provided shelter over the trap and was also used to close the trap when not in use. To provide a damp refuge for captives within the trap, water-soaked sponges were placed on a 3 cm thick layer of soil at the base of the bucket.

Trap layout followed the same design at all 11 sites. The general design followed placement of traps in the order of doubles (i.e. 10 m, 20 m, 40 m and 80 m) from the pan edge. The number of traps at consecutive distances from the pan edge increased by two traps at each increased distance (e.g. there were two traps at 10 m and four traps at 20 m). The arrangement of traps was such that, each subsequent line of traps was offset from those in front of them. This was done in order to maximise trapping rates and ensure one set of traps did not interfere with the potential capture of frogs at another (Fig. 11). Trap placement in the landscape was determined with the use of Google Earth™ (Google Inc.) and ArcGIS (ESRI). Individual trap locations were then plotted out in the field using a

handheld GPS (Garmin eTrex Venture HC). A total of 148 traps were installed at a total of 11 sites in the Ekurhuleni Municipal District of Gauteng Province, South Africa. Six of these sites were located in urban and suburban areas while five sites were located in agricultural areas.

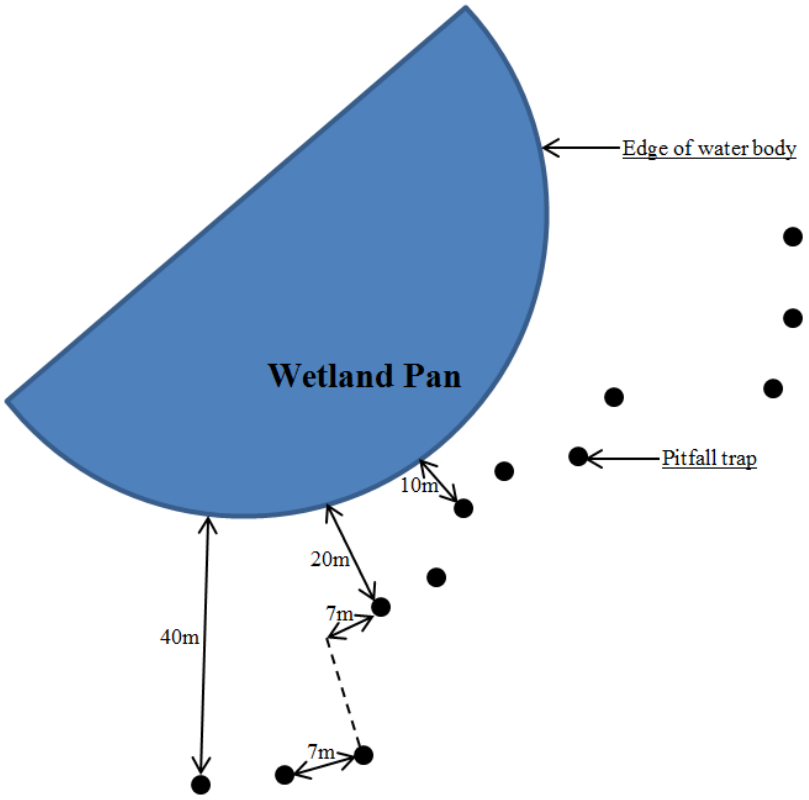


Figure 11: Pitfall trap layout relative to the wetland pan and other traps

Because the area of terrestrial habitat around pans varied between sites in both agricultural and urban areas, the maximum number of traps installed was limited at some of the sites (Table 6). Fieldwork was conducted from the end of November 2012 to the end of March 2013 (Season 1) and the middle of December 2013 to the end of March 2014 (Season 2). Sites were visited every second day (totalling 7790 individual trap checks over both seasons). Frog counts were corrected to reduce bias (arising from differing numbers of installed traps at different sites) by dividing the frog count for each distance category by the number of traps installed in that distance category (e.g. a frog count of 8 at 20 m would result in 2 frogs per trap). Further bias may be introduced when considering that not all sites had the same number of sample days (due to weather and security access). Frog counts were also corrected to accommodate for this bias. All frog counts are documented in their corrected form as

I/T/D (individuals per trap per day). Captured frogs were released immediately after being recorded at each site. To avoid recapture, individuals were released at the shoreline on the opposite side of the wetland pan from where pitfalls were installed.

Table 6: Number of installed traps per site with the associated maximum distance (in metres) traps were installed at from the pan shoreline, in parentheses.

Urban pan ID	Maximum number of installed traps	Agricultural pan ID	Maximum number of installed traps
U1	2 (10)	A1	20 (80)
U2	20 (80)	A2	20 (80)
U3	6 (20)	A3	12 (40)
U4	20 (80)	A4	12 (40)
U5	4 (20)	A5	20 (80)
U6	12 (40)		

The Shannon-Wiener Diversity Index was used to evaluate differences in biodiversity between agricultural and urban areas. A student's t-test was used for most analyses when testing for significance between variables in different site categories. A Spearman's Rank Order Correlation was performed on the data to identify auto-correlated variables. Those showing autocorrelation were removed from the statistical sample set after which, a Pearson's Correlation was run to test for correlation between frog abundance and pan metrics and water chemistry.

3.4. Results

Counts and dispersal

Eight species of amphibian were recorded during the sampling period (Table 7). Greater biodiversity was recorded in agricultural areas (Shannon Index: 1.6) compared with urban areas (Shannon Index: 1.0). Of the eight species recorded, five were recorded in urban areas and seven in agricultural areas. One species was unique to urban areas (*S. fasciatus* – one specimen) while three were unique to agricultural areas (*A. quecketti* – one specimen, *P. adspersus* and *T. cryptotis*) (Table 7).

A single individual of both *A. quecketti* and *S. fasciatus* was recorded during the course of the research project (resulting in a very small value once corrected for traps and sampling days) (Fig. 12). *Amietia quecketti* was recorded at an agricultural site (A2) at 10 metres from the pan shoreline and *S. fasciatus*, 40 metres from the pan shoreline at an urban site (U2) (Fig. 12; Fig. 13). A second *A. quecketti* (not part of the sample set) was observed in a puddle on the outskirts of a cornfield adjacent to Pan A4. Pan U2 contained water which had been contaminated with Acid Mine Drainage (Chapter 1) and was considered as a site unlikely to support frog life.

Table 7: Recorded presence of species across all sites (urban and agricultural site classes are indicated in parentheses)

	(U1)	**(U2)**	**(U3)**	**(U4)**	**(U5)**	**(U6)**	**(A1)**	**(A2)**	**(A3)**	**(A4)**	**(A5)**
A. gutturalis	X	X	X	X	X	X	X	X	X	X	X
C. boettgeri			X	X	X	X	X	X	X	X	X
X. laevis				X	X		X	X			X
K. senegalensis				X		X	X	X			
P. adspersus							X	X			
T. cryptotis							X	X			
A. quecketti								X			
S. fasciatus		X									

Amietophrynus gutturalis

Individuals were recorded at all sites (Table 7). A higher frog count was recorded on average in urban areas compared to agricultural areas (Fig. 12). The number of *A. gutturalis* did not differ significantly between urban and agricultural sites ($t = 0.71$, $df = 9$, $p = 0.49$) however, frog counts differed significantly within the urban category (between urban sites), ($t = 2.82$, $df = 5$, $p < 0.05$). The species was present at pans that had less than 25 000 m^2 area of available terrestrial habitat located in light industrial zones through to pans with more than 2 500 000 m^2 of available terrestrial habitat located in agricultural areas adjacent to corn crops. Sites inhabited by *A. gutturalis*, ranged from having a mean straight-line distance (from pan shoreline to transformed ground) of 5 m – 500 m.

Frogs were trapped at every trapping distance (i.e. 10 m, 20 m, 40 m & 80 m) from the pan edge (Fig. 13). Frog counts were similar at all distances from the pan edge except for the 20 m urban

category where nearly double the number of frogs was found compared with any other distance category. Frog counts however, were only significantly different between the 40 m and 80 m urban categories ($t = 23.86$, $df = 1$, $p < 0.05$). Juvenile captures formed a greater proportion of the sample size than did adults (16:1 respectively).

Cacosternum boettgeri

Individuals were found at all study sites, except for two urban sites which are located nearby to each other (Table 7). The number of *C. boettgeri* did not differ significantly between urban and agricultural sites ($t = -1.50$, $df = 9$, $p = 0.19$) nor did they differ between sites within the urban ($t = 1.91$, $df = 5$, $p = 0.11$) and agricultural ($t = 1.41$, $df = 4$, $p = 0.23$) categories. Despite this, the mean number of *C. boettgeri* in agricultural (0.278 I/T/D) areas is noticeably higher (more than 21 times) than in urban (0.013 I/T/D) areas (Fig. 12). The species was recorded at pans that had surrounding terrestrial habitat that ranged from 80 000 m^2 to more than 2 500 000 m^2. Mean straight-line distances (from the pan shoreline to nearest transformed ground) of these pans ranged from 34 m to 500 m.

Cacosternum boettgeri was found in traps from all distance categories (10 m, 20 m, 40 m and 80 m) in both urban and agricultural areas (Fig. 13). There were no significant differences between capture rates and distance captured from the pan shoreline either within or between urban and agricultural categories. There was however, an increase in the number of captures moving away from the pan until 40 m in the agricultural category and 20 m in the urban category, before dropping (Fig. 13).

Xenopus laevis

Xenopus laevis was found in fewer than half of the sites (Table 7). Individuals from this species were found in two of six urban sites and three of the five agricultural sites. Individuals (I/T/D) recorded in agricultural areas (0.013) amounted to nearly double that of urban areas (0.007) (Fig. 12) but there was no significant difference in capture rates between those of urban and agricultural categories ($t = -0.66$, $df = 9$, $p = 0.53$). No significant difference was found between sites within urban ($t = 1.28$, $df = 5$, $p = 0.26$) and agricultural ($t = 2.21$, $df = 4$, $p = 0.09$) categories. The species was

recorded at pans which had surrounding terrestrial habitat ranging from 80 000 m^2 to over 2 500 000 m^2. Mean straight-line distances (from the pan shoreline to nearest transformed ground) of these pans ranged from 33.74 m to 507.56 m.

Xenopus laevis was recorded in traps from all trap categories (10 m, 20 m, 40 m and 80 m) in agricultural areas and from two in urban areas (10 m and 20 m), (Fig. 13). There were no significant differences between capture rates and distance captured from the pan shoreline either within or between urban and agricultural categories. Adults formed a higher proportion of captures at sites; however, four juveniles were trapped while dispersing from site U5 on one occasion. *Xenopus laevis* tadpoles were also observed at Pan U1.

Kassina senegalensis

Individuals were recorded at four sites (two urban and two agricultural). The number of *K. senegalensis* recorded was similar in urban and agricultural areas ($t = 0.20$, $df = 9$, $p = 0.85$) (Fig. 12) and there was no difference between sites within urban ($t = 1.03$, $df = 5$, $p = 0.35$) or agricultural ($t = 1.03$, $df = 4$, $p = 0.36$) categories. The species was recorded at pans with surrounding terrestrial habitat area ranging from 84 000 m^2 to 2 500 000 m^2. Mean straight-line distances (from the pan shoreline to nearest transformed ground) of these pans ranged from 69 m to 508 m. Individuals of *K. senegalensis* were recorded in traps from three distance categories at urban sites (10 m, 20 m, 40 m) and all distance categories (10 m, 20 m, 40 m & 80 m) in agricultural sites (Fig. 13). There was no significant difference between capture rates and distance captured from the pan shoreline either within or between urban and agricultural categories.

Pyxicephalus adspersus

Individuals were only recorded from agricultural sites (two sites out of five) (Fig. 12). Most captures were juveniles (newly metamorphosed ranging in size from 20-55 mm) dispersing from the pan at U1. A single individual (approximately 80 mm) was found at another agricultural site (U2). Two male *P. adspersus* (not included in the sample set) were observed in a dirt road puddle approximately 3 km from pan U5 (the closest pan to the sighting). Hundreds (estimate) of *P.*

adspersus tadpoles (approx. 20 mm SVL) were found swimming in the flooded grasslands of pan A5. A student's t-test found no significant difference between the overall capture rate of *P. adspersus* in urban and agricultural categories ($t = -1.11$, $df = 9$, $p = 0.29$) or between sites within the agricultural category ($t = 1.01$, $df = 4$, $p = 0.37$). The species was recorded at pans with surrounding terrestrial habitat area ranging from 202 000 m^2 to 2 500 000 m^2. Mean straight-line distances (from the pan shoreline to nearest transformed ground) of these pans ranged from 96 m to 508 m.

Individuals of *P. adspersus* were recorded in traps from all distance categories (10 m, 20 m, 40 m & 80 m). More than 40% (0.25 I/T/D) of all individuals recorded at site A1 were in pitfalls 80 m from the shoreline (Fig. 13). Farmers also reported seeing numerous juvenile *P. adspersus* around houses in the nearby residential area of Cilvale (~ 400 m from the pan edge). There were no significant differences between capture rates and distance captured from the pan shoreline either within or between urban and agricultural categories.

Tomopterna cryptotis

Individuals were recorded exclusively in agricultural areas (two sites out of five) (Fig. 12). Most individuals were recorded at Site A1, while just one individual was recorded in a pitfall trap at Site A2. No significant difference was detected between the number of frogs between urban and agricultural sites ($t = -1.23$, $df = 6$, $p = 0.25$) or within the agricultural site category ($t = 1.11$, $df = 4$, $p = 0.33$). The species was recorded at pans with surrounding terrestrial habitat area ranging from 202 000 m^2 to 2 500 000 m^2. Mean straight-line distances (from the pan shoreline to nearest transformed ground) of these pans ranged from 96 m to 508 m. Individuals of *T. cryptotis* were recorded in traps from three distance categories (20 m, 30 m & 40 m), most being recorded at 20 m from the pan shoreline (Fig. 13). There was a significant difference in the average capture rates between pitfalls at 10 m and 80 m in the agricultural category ($t = -2.63$, $df = 6$, $p < 0.05$). No other significant difference was detected between capture rates of other distance categories.

Intra-seasonal variability

There was an apparent difference (not significant overall) between the number of frogs recorded in Season 1 and Season 2 which was due to large numbers of captures in Season 2, especially at one pan (A1). Frog counts per trap per day amounted to 0.04 I/T/D overall in Season 1 (181 individuals) and 0.11 I/T/D in Season 2 (631 individuals) despite trapping methods remaining the same. Most notable were the differences in capture rates of *P. adspersus* and *C. boettgeri* at agricultural sites between seasons of data collection (Fig. 14). Recorded numbers of *P. adspersus* increased by 174 times in Season 2 compared with Season 1 and those of *C. boettgeri*, by 4 times their number in Season 2 compared to in Season 1. *Kassina senegalensis* and *T. cryptotis* were also recorded more often in agricultural areas in Season 2 compared with Season 1 (Fig. 14), with increased capture rates of 11 and 7 times their Season 1 counts respectively. Approximately half the numbers of *A. gutturalis* and *X. laevis* were recorded in agricultural areas in Season 2 (Fig. 14). Thus, when ignoring species for which only one individual was recorded, four of six species recorded in agricultural areas showed increased capture rates while two showed a reduced prevalence in traps in Season 2. With the exception of *X. laevis*, all species (four when excluding those species for which only one individual was recorded) in urban areas had reduced capture rates (-1.5 to -2.4 times) in Season 2 compared with Season 1 (Fig. 14).

Pan metrics and water chemistry

A strong, significant and positive correlation ($r = 0.81$, $p < 0.05$) was found between *A. gutturalis* counts and $NO_2 + NO_3$-N concentrations (Table 8). Except for one instance, statistical tests were not able to detect any significant relationships between frog biodiversity and water chemistry between sites. Significant correlations between frog trap counts and two physical pan characteristics were detected for three species (Table 8).

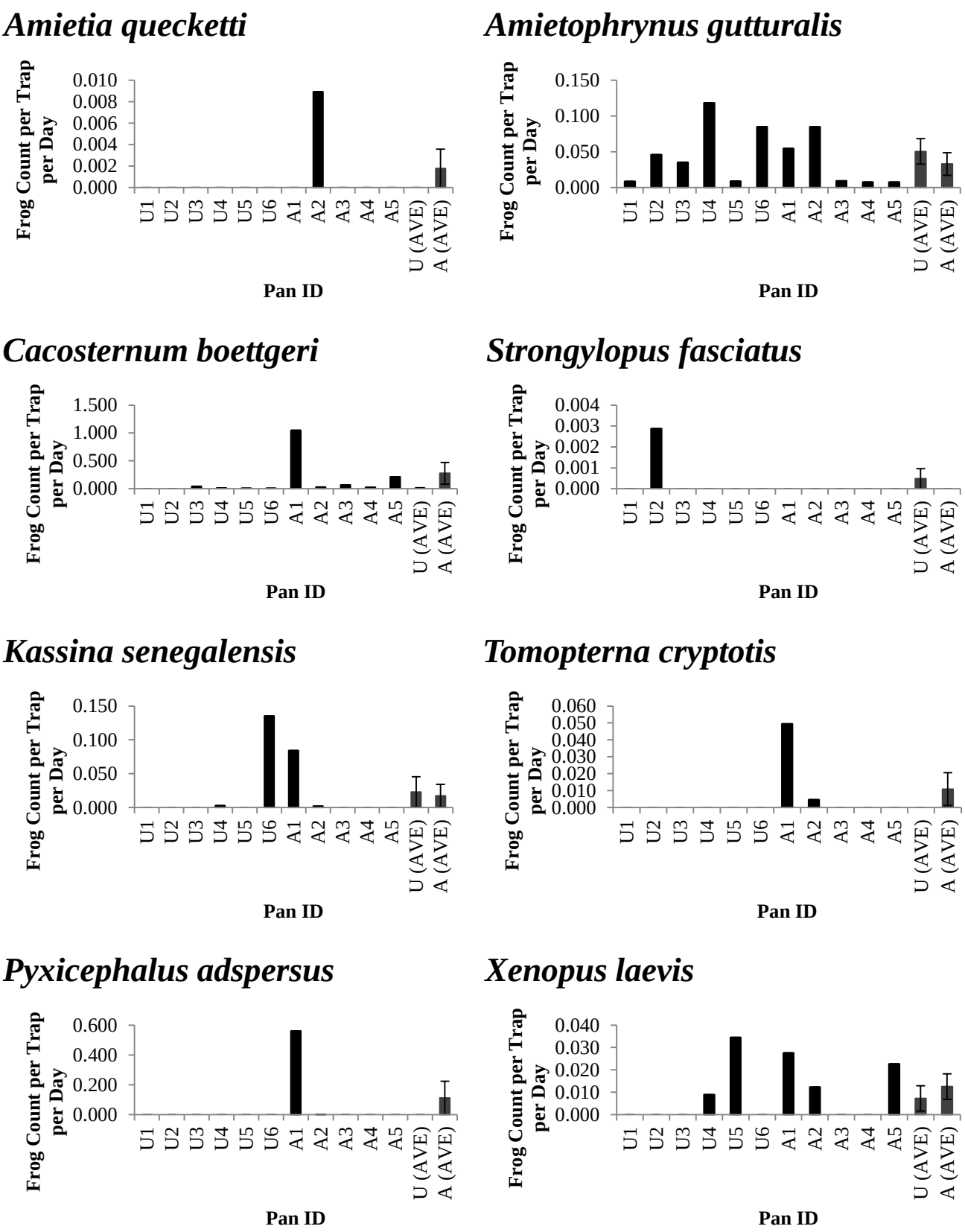


Figure 12: Frog counts (corrected for traps and days sampling) for each recorded species at all sites. Mean number of frogs recorded in urban and agricultural sites is indicated. Standard error is indicated.

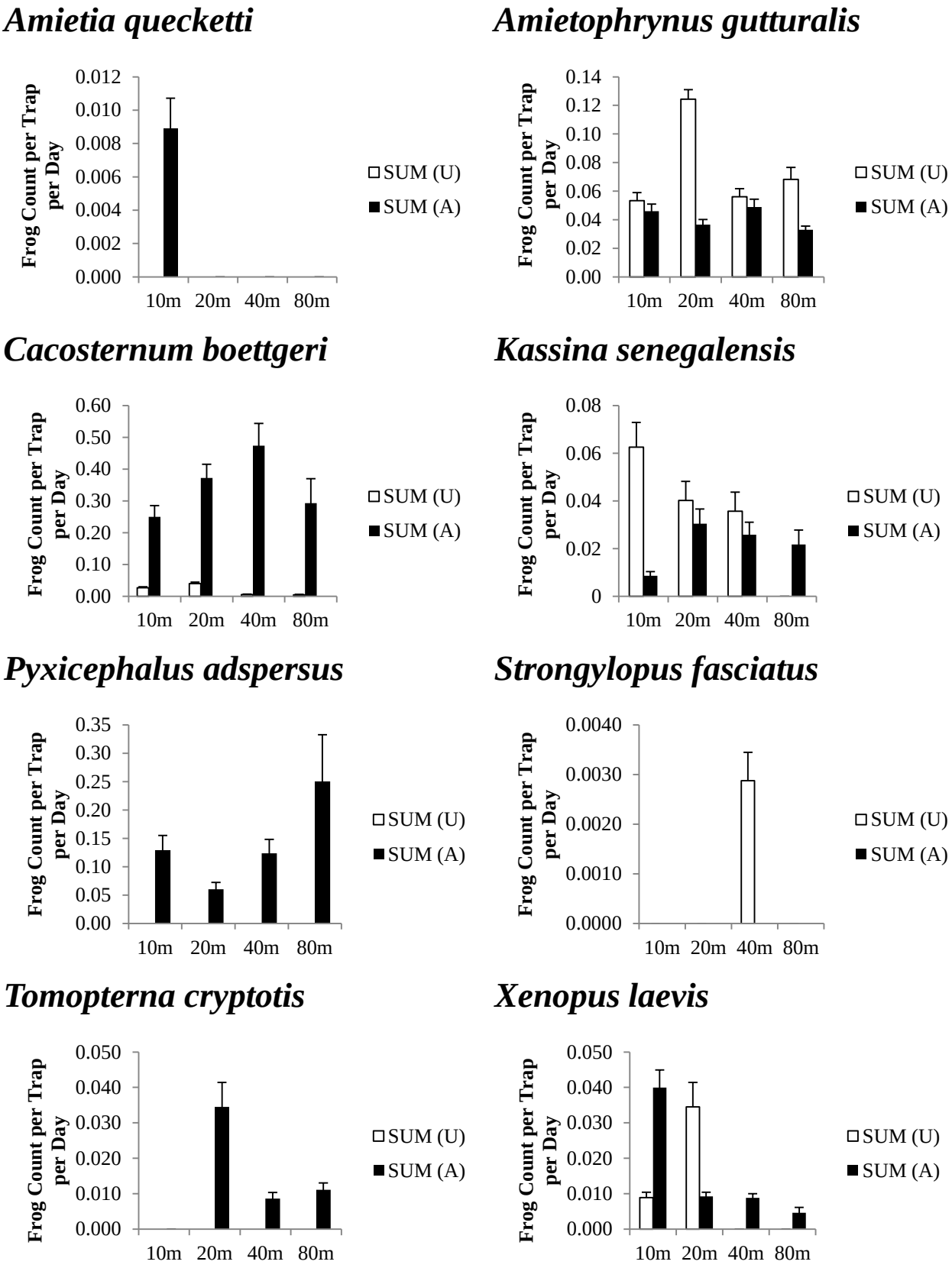


Figure 13: Frog counts (per trap per day) at increasing distance from the pan shoreline for urban (U) and agricultural (A) sites. Standard error is indicated.

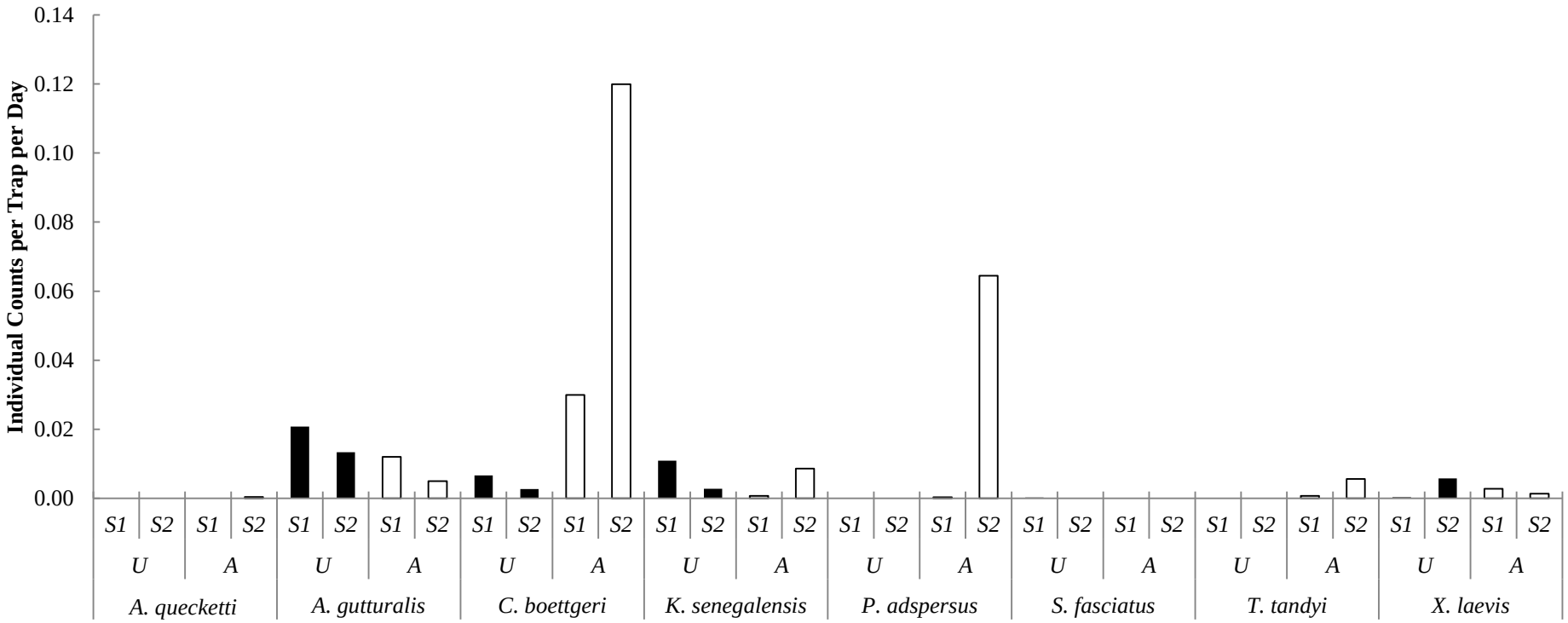


Figure 14: Intra-seasonal variability of average frog counts per species (I/T/D) in urban and agricultural categories.

Table 8: Pearson's Correlations between abundance (I/T/D) and, pan metrics and chemistry. Significant ($p < 0.05$) correlations are indicated in bold type. Strongly auto-correlated variables have been removed from the sample set. Two species do not appear in the table because only one individual from each was captured.

	Frog Abundance (I/T/D) and Pearson's R score					
	A. gutturalis	*C. boettgeri*	*K. senegalensis*	*P. adspersus*	*T. cryptotis*	*X. laevis*
Untransformed Area	0.146	**0.992**	0.436	**0.995**	**0.992**	0.524
Shortest Distance to Tarred Roads	-0.026	**0.901**	0.307	**0.861**	**0.897**	0.386
Silicon	-0.142	-0.245	-0.302	-0.223	-0.242	-0.393
Alkalinity	-0.097	-0.307	0.380	-0.277	-0.302	-0.511
Turbidity	-0.539	-0.119	-0.379	-0.097	-0.107	0.379
Ammonia	0.061	-0.127	-0.222	-0.152	-0.124	-0.170
Sulphates	-0.026	-0.150	-0.186	-0.125	-0.137	-0.251
Nitrates & Nitrites	**0.811**	-0.171	0.270	-0.159	-0.168	-0.064
Total Kjedahl Nitrogen	0.360	-0.099	0.401	-0.128	-0.103	-0.239

3.5. Discussion

Frog abundance appears to be highly variable in Gauteng's Highveld pans, particularly on a temporal scale. Capture rates varied considerably between seasons for some species (*C. boettgeri, P. adspersus*; Fig. 14), although differences were not significantly different probably because of small sample sizes. Seasonal differences may have been due to difference in rainfall patterns over the study or because of the trapping method. Biodiversity of frogs in agricultural areas was higher compared with urban sites, albeit only slightly. The number of species present in agricultural areas, where land transformation appears to occur to a lesser degree when compared to urbanised environments, was 13% (44% = 7 species) higher (based on an expected 16 species) compared with the number found in urbanised areas (31% = 5 species) (Table 7).

One species (*P. adspersus*) was, until recently, considered Regionally Near Threatened by the International Union for Conservation of Nature (IUCN) Red List, but because of its presumed large population size, it was upgraded to the status of Least Concern (IUCN 2014). Species which require large tracts of terrestrial habitat, such as *P. adspersus* (Yetman & Ferguson 2011), are not likely to persist in urban areas where obstructions to dispersal and spatial habitat use (such as roads, paving and buildings) are regularly encountered nearby (Fig. 10). Large scale urbanisation without adequate preservation of terrestrial habitat is thus likely to negatively affect populations of *P. adspersus*.

All *P. adspersus* recorded in the study were juveniles, either newly metamorphosed or approximately a year old. Individuals appear to be highly vagile in comparison to other frogs indicated by most individuals having been captured at 80 m from pan shoreline (Fig. 13) and further supported by frequent reports from farmers encountering them at farmhouses located further away (> 100 m) from breeding sites. Because tarred roads were located significantly further away (t-stat = 2.78, df = 4, $p < 0.05$) from water bodies in agricultural areas compared with urban areas (Fig. 10), individuals at these sites would be able to disperse over greater distances before being at higher risk of traffic fatality. Sand frogs (*Tomopterna*) were also only detected at agricultural sites, coincidentally, matching sites where *P. adspersus* was recorded (Fig. 12; Table 7). Both *Pyxicephalus* and

Tomopterna are known to bury themselves in sandy soil along temporary water bodies (Channing 2004[a]), a feature which was absent from urban sites. This might explain their absence from those areas.

Considering that *P. adspersus* juveniles are known to be highly vagile and adults utilise extensive foraging areas (> 20 m from burrows, which in themselves can be located up to 1 km away from breeding sites; Yetman & Ferguson 2011) and *T. cryptotis*, regularly utilise areas surrounding temporary pans within which to burrow (Channing 2004[b]) and that *C. boettgeri* is found extensively in grasslands (Scott 2004), it is not unexpected that abundance of these species is strongly correlated with increased available terrestrial habitat (Table 8). The population abundance of these species also had a strong, significant correlation with pan distance from tarred roads (Table 8) perhaps suggesting that presence of roads has a detrimental effect on population size and dispersal of certain species. This relationship may also simply be because increased road density is a function of urban density (Zhang *et al.* 2002) that has been implicated in decreased biodiversity (Forman & Deblinger 2000, Hels & Buchwald 2001).

Some species in the study appear to be able to persist in urban areas that were characterised by increased pollution, impermeable surfaces and human related activities close to water bodies as well as agricultural areas (Table 7). Given the age of many of the urban districts in which the study took place (many dating from the historical Witwatersrand Gold Rush in the late 19th century), it can be expected that species which continue to persist in these areas have sufficient resources available to them that, at minimum, provide means for survival and reproductive success. I hypothesise that buffer zones and restricted areas are likely to have reduced benefit for "urban persisting" species compared with those which have population abundances that are strongly correlated with available terrestrial habitat and were only found in areas with more unaffected landscape and higher amounts of penetrable terrestrial surface area. *Xenopus laevis* and particularly *A. gutturalis* appear to persist even when pans in urban areas are negatively impacted by transformation. Given that these two species have become invasive outside of their natural distribution, this is not surprising (DEA 2014). They adapt well to human transformation (Godfrey & Sanders 2004; Measey & Davies 2011) and may even

present a problem to other anurans (Measey 2004) which may be a factor influencing the maximum number of species detected in this study.

Amietophrynus gutturalis was the only species where captures were correlated positively with a measure of water chemistry (Table 8). The possibility that this was due to collinearity where agricultural pans (which had higher frog abundance) would usually have higher nitrogen concentrations but better habitat, was ruled out because urban sites on average, had the higher NO_2 + NO_3-N concentrations (Fig. 6) but comparatively more degraded habitat. Nitrates and nitrites may instead have a detrimental effect on other aquatic organisms (Marco *et al.* 1999; Camargo *et al.* 2005) allowing for the proliferation of *A. gutturalis* through reduced competition for habitat. Research investigating the effect of methemoglobinemia (disorder caused by excess nitrite and nitrate exposure that is characterised by haemoglobin that contains ferric [Fe^{3+}] instead of ferrous [Fe^{2+}] iron reducing the ability for red blood cells to release oxygen to tissues) in *A. gutturalis* and other frogs living in NO_2 + NO_3-N enriched waters may yield interesting results with implications for species competition and conservation (Mylniczenko 2009). Alternatively, higher NO_2 + NO_3-N concentrations may create an environment facilitating increased algal biomass (Rabalais 2002) which could in turn positively benefit herbivorous *A. gutturalis* tadpoles. There may also be an unknown factor positively correlated with NO_2 + NO_3-N in pan water which instead affects population size of *A. gutturalis*.

This study had several strengths as well as areas which could benefit from improvement. Sites were sampled across a range of urban and agricultural pans which differed with respect to land use of the adjacent areas (residential, light industrial, agricultural and minimally impacted open areas). Sampling was performed over two seasons to detect the variability of measures in frog abundance. The study was thus able to provide an indication of how frog populations differ between pans which were impacted in different ways across the land use spectrum. The short temporal resolution of the study highlighted the high variability of frog abundance measures over breeding seasons. Increasing the number of breeding seasons sampled would help in assessing whether frog abundance is correlated with specific environmental variables such as water chemistry, precipitation and pan hydroperiod.

Drift fences and increasing pitfall trap density is likely to vastly improve capture rates and provide a better indication of population size as well as improve the chance of capturing individuals from a wider range of species. Drift fences were originally proposed to increase efficacy of capturing animals, but was abandoned because of difficulties with placement and threat of regular theft. It is possible that the absence of drift fences had a significant effect on the number of captures in this study possibly leading to an underestimation of the effect of habitat on abundance. The measure of abundance in the study was indirect and relied on frog activity. Variation in rainfall may have impacted frog activity (an apparent change in abundance) but could have also influenced frog breeding (an actual change in abundance). Recording species that can move above ground (via thick vegetation) was also a challenge. For example, a large number of visual and auditory observations were made of *C. boettgeri* at pans A4 and A5 but were not included as part of the pitfall trap count. This conundrum could be accommodated for in future projects through the use of frog call surveys. Crime in many areas, particularly densely populated urban areas consisting of informal settlements, would be a hindrance to this method however, as was the case for this project.

Frog abundance appeared to be highly variable from one season to the next, particularly so for those species for which abundance was positively correlated with available terrestrial habitat. Environmental impact assessments (EIAs) which survey at a point in time could lead to serious misinformation and would be inappropriate for reporting on frog abundance and species diversity. For example, neither *P. adspersus* nor *T. cryptotis* were detected in the first breeding season (2012/2013) of this study at Pan A1. In the second season (2013/2014) however, the I/T/D rose to 1.12 (187 individuals) and 0.099 (14 individuals) respectively. To adequately cater for sizeable changes of frog presence in the landscape, EIAs would ideally need to be conducted for several weeks (it is possible that the survey period could be shortened when using frog call surveys as an alternative to pitfall traps) during the wet season and for at least two consecutive breeding seasons. Failure to account for the stochastic nature of frog population abundance could result in considerable over- or underestimates of frog biodiversity.

Some frog species appear to be comparably more sensitive than others to the condition of the terrestrial habitat. These species are unlikely to occur in urban areas. Wetland pans in areas currently under urban development would need to have larger, appropriate areas of terrestrial habitat for the purposes of conservation than that which is currently available around sample pans in urban areas. Consideration would have to be made for dispersal corridors, required to maintain gene pool viability. Although three pans in agricultural areas had comparable species richness and abundance to most urban sites, cropland would allow for easier dispersal from water bodies compared with those water bodies suffocated by urban development.

Individuals from all species (those species with only one recorded capture being excluded) were recorded at 80 m trapping distance from the pan shoreline indicating that these species make use of terrestrial habitat to at least that distance from shore when it is available. This is not unexpected and compared with global studies, 80 m is a short distance. This study shows that some species (*A. gutturalis*, *C. boettgeri*, *K. senegalensis*, *X. laevis*) have been able to persist in urban areas where less than 80 m of linear habitat (mean from pan shoreline to closest transformed land) is available. Some populations may be residual from predevelopment times in some areas. Captures indicating use of terrestrial habitat to the 80 m point indicates that frogs likely rely on terrestrial habitat beyond this point. Trapping distance therefore should not be used to delineate buffer zones but rather the extent of the mean linear distance from the pan shoreline to transformed land.

Conservation of some species may not be possible without considering the implications of the interconnectedness of ephemeral water bodies within the landscape (dispersal corridors) and terrestrial habitat on population size. This study shows that some species may be more reliant on ephemeral water bodies (such as those in agricultural areas) and availability of sufficient terrestrial habitat in order to persist (*P. adspersus*, *T. cryptotis* and *C. boettgeri*). The available terrestrial habitat where these species were found was between 100 and 500 m possibly indicating that buffer zones would have to be at least 100 m to have any conservation merit. This possibly also shows that roads have a detrimental effect on species such as *P. adspersus* which burrow and disperse far from water bodies and supports current literature on the negative impact roads have for some species populations.

Effective frog conservation is a complex conundrum to solve, where a single, blanket-solution paradigm is insufficient. The seasonally stochastic nature of frog abundance, extent of available terrestrial habitat, and distance to roads from water bodies must be taken into consideration while also conserving the ephemerality of pans. Water chemistry may play a part in frog abundance on the Highveld for some species, but further investigation into species-specific effects are needed in order to make a more decisive conclusion. For some species, the anthropogenic changes to the Highveld's grasslands may have already reduced population numbers to a point where they are no longer detected.

Chapter 4 - Conserving the Frogs of Gauteng's Highveld Pans

During the course of this study it was evident that the pans of Gauteng's Highveld have been impacted considerably by human activity, both historically and currently and in both urban and agricultural zones. Urban pans are the most severely altered, being affected by acid mine drainage, nutrient enrichment, dumping of household and construction wastes, loss of peripheral terrestrial habitat to development, close proximity to tarred roads with heavy traffic and loss of the ephemeral quality of pans. In a separate category, agricultural pans are affected by nutrient enrichment, incidents where large quantities of eroded material are deposited onto the wetland area from cropland, loss of peripheral habitat to agriculture and the seasonal mowing of grassland peripheral to water bodies. Sites in both urban and agricultural areas have thus been affected by human development and land transformation.

Despite the effects that human activity has had on altering the landscape, some of Gauteng's frogs (and several species from other taxonomic groups) have managed to persist in and around pans. The most obvious of these is *Amietophrynus gutturalis*, present in comparable abundance in urban and agricultural zones (Fig. 14). The versatility of this species as well as its ability to adapt to human-centric environments (du Preez *et al.* 2004) with differing land uses has likely allowed it to take advantage of an altered environment where other, less adaptable species, cannot. I propose then, that conservation efforts should focus on those species which are more affected by anthropogenic processes on the environment. By aiming to conserve more sensitive species through habitat preservation, those which are more adaptable will by default also be protected.

It is difficult to identify which single factor is most important in its effect on frog biodiversity. This information affects where conservation efforts should focus in order to be effective. A course water quality assessment and evaluation of pan metric data show that variables can differ tremendously from one pan to the next (Fig. 3 – Fig. 7). With a small sample size, this makes it difficult to ascertain whether any one variable has a significant effect on frog population persistence. It is likely however, that multiple variables play a part in affecting abundance and species richness

(Gardner 2001, Williams & Hero 2001, Trenham *et al.* 2003, Koprivnikar *et al.* 2006). Nitrates + nitrites ($NO_2 + NO_3$-N) were the only variable of the coarse water quality assessment which correlated strongly with abundance data (of *A. gutturalis*). Further research may confirm whether there is a link between abundance of *A. gutturalis*, and other species, with $NO_2 + NO_3$-N concentrations of water bodies. If $NO_2 + NO_3$-N concentrations are found to correlate with *A. gutturalis* abundance in future studies, it does not necessarily mean that $NO_2 + NO_3$-N is the cause but that factors affecting $NO_2 + NO_3$-N concentrations are affecting the ecosystem in complex ways. Conducting water quality surveys after the first rains may allow for detection of the maximum concentration of nutrients and ions after being flushed from catchments (Ribarova 2008, Oeurng *et al.* 2010). This potentially significant change in water chemistry is likely to affect frog breeding success for a limited time only, since many species breed for the duration of the rainy season (du Preez & Carruthers 2009), allowing time for water chemistry to become more favourable.

Properties of two physical characteristics of pans (distance from tarred road and terrestrial habitat availability) significantly correlated with the abundance of three species (*C. boettgeri*, *P. adspersus*, *T. cryptotis*). This study highlights that there may be a strong relationship between the persistence of some Highveld species and the availability of essential terrestrial habitat. It is not surprising that *P. adspersus* abundance is strongly correlated with terrestrial habitat availability since the species' extensive spatial land utilisation has been previously documented (Yetman & Ferguson 2011). Global amphibian studies have highlighted the importance of terrestrial habitat for amphibians, numerously indicating that amphibians can move hundreds of metres from water bodies (Bulger *et al.* 2003, Semlitsch & Bodie 2003, Rittenhouse & Semlitsch 2007). Six of eight species were recorded at the maximum trapping distance (80 m) from the edge of pans (only one individual was caught for the remaining two species). I propose that terrestrial habitat availability is one of the most important factors to consider for frog conservation on the Highveld and that a suitable minimum requirement be legislated for appropriate handling of environmental impact assessments around wetlands.

Another important finding is how variable measures of frog biodiversity can be. This is important for conservation as it impacts on the effectiveness and value of spot surveys for environmental impact

assessments. Frog capture rates differed greatly between sample seasons where a boom was detected for at least two species (*C. boettgeri* & *P. adspersus*). The variability of frog biodiversity at Highveld pans could be impacted by a plethora of factors. One noticeable observed (although unmeasured) difference between seasons however, was the late arrival of seasonal rainfall (late December) in the first sample season compared with the second season (mid-November). This may have impacted the filling of ephemeral pans in agricultural areas and may have affected breeding in some species (Telford & Dyson 1990, Marsh 2000, Tryjanowski 2003). Individual pans were also unique and variables that can impact of frog biodiversity (e.g. NO_2 + NO_3-N, habitat patch size) sometimes, differed considerably between them.

Recommendations

Urban pans are heavily impacted by human activity; however, some urban pans may have potential for rehabilitation. Those pans which are located further away from industry and roads, and with larger terrestrial habitat patches would be better candidates for rehabilitation. I suggest that an appropriate approach would be to mitigate negative impact on current systems and to take measures to avoid further degeneration of urban pan ecosystems. Fencing-in sensitive areas, such as pans, may further assist with conservation and prevention of damage to ecosystems while still allowing for dispersal. Pan U4's *A. gutturalis* abundance was the highest of any site and had the highest (four) species presence of any urban site. Restricting access would prevent human thoroughfare with associated paths and dumping of household and construction waste. At least one pan (not part of this study) in the Benoni municipal area of Rynfield has already been fenced for the main purpose of conserving *P. adspersus* populations. In some instances however, very little adjacent terrestrial habitat exists (e.g. Pan A1) because development occurs to the shoreline of pans. In these cases, efforts to mitigate impacts on frog populations are likely better focused elsewhere.

Terrestrial habitat requirements differ considerably between species. However, catering to the needs of those species which appear to rely heavily on terrestrial habitat for survival will not only ensure that the needs of those which can more readily adapt to disturbance are also met but that the

surrounding terrestrial habitat will also buffer against negative impacts on the pan's water quality. Most species in this study were captured at 80 m from the pan edge. The number of captures did not differ significantly between consecutive trapping distances (10, 20, 40, 80 m) for any species which show that individuals are moving through terrestrial habitat similarly up to at least 80 m when it is available. It is difficult to ascertain at which point species such as *C. boettgeri*, *P. adspersus* and *T. cryptotis* would benefit from greater areas of terrestrial habitat unencumbered by tarred roads and development. More research would be needed in areas where individuals could be recorded at distances greater than the maximum of this study (80 m). These species were however, present in abundance at one pan in particular (A1) where at least 500 m of habitat was available from the pan shoreline. I would estimate that terrestrial habitat (in the form of buffer zones) would need to be between 100 m (the minimum distance from pan shoreline to disturbed ground at sites where all three species - *C. boettgeri*, *P. adspersus* and *T. cryptotis* - were detected) and 500 m (the maximum distance from pan shoreline to disturbed ground at sites where all three species - *C. boettgeri*, *P. adspersus* and *T. cryptotis* - were detected).

There is potential to expand this study and improve on methods for the detection of frogs (e.g. using frog call surveys). Finding sites in natural areas of the Highveld (e.g. nature reserves) may add another dimension to the study by highlighting the possible impact that land transformation (both agricultural and urban) has had on ecosystems. Although this study shows that agricultural areas support greater frog biodiversity compared to urban areas, natural areas are likely to support even more. Additionally, to truly evaluate the spatial habitat use of different species, a study on a grander scale is required (with study sites which have larger habitat patches) to get a more valuable population density curve with increasing distance from water bodies.

This study has thus indicated that urban pans suffer from a considerable amount of human impact, and reduced frog biodiversity, and that agricultural pans are in a more natural state and hold greater current potential for the conservation of Highveld frog populations. Proximity to roads and available area of terrestrial habitat may be crucial for the adequate persistence of some species while others have managed to persist in heavily impacted environments. Frog populations appear to be highly

variable, which impacts how environmental impact assessments should be designed. Frogs also use terrestrial habitat up to at least 80 m from the pan shoreline, the limited trapping distance of this study, but shows that current legislated parameters for buffer zones are insufficient to cater for the spatial habitat use of frogs of the Highveld.

References

AWWA (American Water Works Association). 2003. *Principles and Practices of Water Supply Operations – Water Quality (Third edition)*. Library of Congress Cataloging-in-Publication Data. U.S.A.

Anderson, C.W. 2005. In *Geological Survey Techniques of Water-Resources Investigations, Book 9, Chap. A6, sec. 6.7.* Wilde, F.D. (*Ed*). USGS, U.S.A.

Azous, A.L. 1991. An Analysis of Urbanization Effects on Wetland Biological Communities, M.S. Thesis. University of Washington, Department of Civil Engineering, Environmental Engineering and Science Program, Seattle, U.S.A.

Babbitt, K.J., J.S. Veysey & G.W. Tanner. 2009. In *Amphibian Ecology and Conservation: A Handbook of Techniques*. Dodd, C.K. Jr. (*Ed*). Oxford University Press, Oxford, U.K.

Barsanti, L. & P. Guatieri. 2006. *Algae: Anatomy, Biochemistry and Biotechnology*. CRC Press. Florida, U.S.A.

Beattie, R.C. & R. Tyler-Jones. 1992. The Effects of Low pH and Aluminium on Breeding Success in the Frog *Rana temporaria*. J Herpetol. 26: 353-360

Belden, L.K. 2006. Impact of Eutrophication on Wood Frog, *Rana sylvatica*, Tadpoles Infected with *Echinostoma trivolvis* cercariae. Can J Zool. 84: 1315-1321

Bender, D.J., T.A. Contreras & L. Fahrig. 1998. Habitat Loss and Population Decline: A Meta-Analysis of the Patch Size Effect. Ecology. 79: 517-533

Biology-Online.org. 2009. *Ecological Buffer Zone*. [Accessed: 2014-12-11] http://www.biology-online.org/dictionary/Ecological_buffer_zone

Blaustein, A.R., J.M. Romansic, J.M. Kiesecker & A.C. Hatch. 2003. Ultraviolet Radiation, Toxic Chemicals and Amphibian Population Declines. Divers Distrib. 9:123-140

Bouchard, J., A.T. Ford, F.E. Eigenbrod & L. Fahrig. 2009. Behavioral Responses of Northern Leopard Frogs (*Rana pipiens*) to Roads and Traffic: Implications for Population Persistence. Ecol Soc. 14: 23.

Bowd, R., D.C. Kotze, C.D. Morris & N.W. Quinn. 2006. Testing the Applicability of the SASS5 Scoring Procedure for Assessing Wetland Health: a Case Study in the KwaZulu-Natal Midlands, South Africa.

Boyd, C.E. 2000. *Water Quality – An Introdcution*. Kluwer Academic Publishers, Norwell, MA, U.S.A.

Boyd, C.E. & C.S. Tucker. 1998. *Pond Aquaculture Water Quality Management*. Kluwer Academic Publishers, Norwell, MA, U.S.A.

Boyer, R. & C.E. Grue. 1995. The Need for Water Quality Criteria for Frogs. Environ Health Persp. 103: 352-356.

Branch, W. & J.A. Harrison. 2004. Conservation Status and Threats. Pp. 13-29 in Minter, L.R., M. Burger, J.A. Harrison, H.H. Braack, P.J. Bishop & D. Kloepfer, eds. *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland*. SI/MAB Series #9. Smithsonian Institution, Washington, DC.

Brodman, R., J. Ogger., T. Bogard., A.J. Long., R.A. Pulver., K. Mancuso & D. Falk. 2003. Multivariate Analyses of the Influences of Water Chemistry and Habitat Parameters on the Abundances of Pond-Breeding Amphibians. J Freshwater Ecol. 18: 425-436

Bulger, J.B., N.J. Scott, Jr. & R.B. Seymour. 2003. Terrestrial Activity and Conservation of Adult California Red-Legged Frogs *Rana aurora draytonii* in Coastal Forests and Grasslands. Biol Conserv. 110: 85-95

Burke, V.J. & J.W. Gibbons. 1995. Terrestrial Buffer Zones and Wetland Conservation: A Case Study of Freshwater Turtles in a Carolina Bay. Conserv Biol. 9: 1365-1369

Camargo, J.A., A. Alonso, A. Salamanca. 2005. Nitrate Toxicity to Aquatic Animals: A Review with New Data for Freshwater Invertebrates. Chemosphere. 58: 1255-1267

Carden, K., N. Armitage., K. Winter., O. Sichone & U. Rivett. 2007. *Understanding the Use and Disposal of Greywater in the Non-Sewered Areas in South Africa - Report to the Water Research Commission*. University of Cape Town, South Africa.

Carey, C. & M.A. Alexander. 2003. Climate Change and Amphibian Declines: Is There a Link? Divers Distrib. 9:111-121

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley & V.H. Smith. 1998. Nonpoint Pollution of Surface Waters with Phosphorus and Nitrogen. Ecol Appl. 8: 559-568

Carr, L.W. & L. Fahrig. 2001. Effect of Road Traffic on Two Amphibian Species of Differing Vagility. Conserv Biol. 15: 1071-1078

Carr, G.M. & J.P. Neary. 2008. *Water Quality for Ecosystem and Human Health (2nd Ed)*. United Nations Environment Programme Global Environment Monitoring System/Water Programme. Canada.

Castelle, A.J., A.W. Johnson & C. Conolly. 1994. Wetland and Stream Buffer Size Requirements – A Review. J Environ Qual. 23: 878-882

Chambers, D.L. 2011. Increased Conductivity Affects Corticosterone Levels and Prey Consumption in Larval Amphibians. J Herpetol. 45: 219-223

Channing, A. 2004[a]. Genus *Tomopterna* Duméril and Bibron, 1841 (Family Ranidae). Pp. 319-320 in Minter, L.R., M. Burger, J.A. Harrison, H.H. Braack, P.J. Bishop & D. Kloepfer, eds. *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland*. SI/MAB Series #9. Smithsonian Institution, Washington, DC.

Channing, A. 2004[b]. *Tomopterna cryptotis* Boulenger, 1907. Pp. 321-322 in Minter, L.R., M. Burger, J.A. Harrison, H.H. Braack, P.J. Bishop & D. Kloepfer, eds. *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland*. SI/MAB Series #9. Smithsonian Institution, Washington, DC.

Chapman, D. 1996. *Water Quality Assessments – A Guide to Use of Biota, Sediments and Water in Environmental Monitoring – Second Edition*. UNESCO. E&FN Spon. Cambridge, U.K.

Chislock, M.F., E. Doster, R.A. Zitomer & A.E. Wilson. 2013. Eutrophication: Causes, Consequences, and Controls in Aquatic Ecosystems. Nature Education Knowledge. 4: 10

Coffin, A.W. 2007. From Roadkill to Road Ecology: A review of the Ecological Effects of Roads. J Transp Geogr. 15: 396-406

Cohn, J.P. 1994. Salamanders Slip-Sliding Away or Too Surreptitious to Count. Bioscience. 44: 219-223

Collins, J.P. & M.L. Crump. 2009. *Extinction in Our Times – Global Amphibian Decline.* Pp. 82-87 Oxford University Press. New York, U.S.A.

Coulter, C.B., R.K. Kolka & J.A. Thompson. 2004. Water Quality in Agricultural, Urban, and Mixed Land Use Watersheds. J Am Water Resour As. 40: 1593-1601

Correll, D.L. 1998. The Role of Phosphorus in the Eutrophication of Receiving Waters: A Review. J Environ Qual. 27: 261-266

Curado, N., T. Hartel & J.W. Arnitzen. 2011. Amphibian Pond Loss as a Function of Landscape Change – a Case Study Over Three Decades in an Agricultural Area of Northern France. Biol Conserv. 144: 1610-1618

Daszak, P., A. A. Cunningham & A. D. Hyatt. 2003. Infectious Disease and Amphibian Population Declines. Divers Distrib. 9:141-150

deMaynadier, P.G. & M.L. Hunter, Jr. 1999. Forest Canopy Closure and Juvenile Emigration by Pool-Breeding Amphibians in Maine. J Wildlife Manage. 63: 441-450

DEA (Department of Environmental Affairs). 2004. *National Environmental Management: Biodiversity Act, 2004; No. 10 of 2004*. 467: 26436. Government of South Africa. Pretoria, Republic of South Africa.

DEA (Department of Environmental Affairs). 2014. *Government Notice - National Environmental Management: Biodiversity Act (10/2004): Alien and Invasive Species List, 2014.* Government of South Africa. Pretoria, Republic of South Africa.

de Jonge, V.N., M. Elliott & E. Orive. 2002. Causes, Historical Development, Effects and Future Challenges of a Common Environmental Problem: Eutrophication. Hydrobiologia. 475/476: 1-19

DWAF (Department of Water Affairs and Forestry). 1996. *South African Water Quality Guidelines.* Volume 7: Aquatic Ecosystems

DWAF (Department of Water Affairs and Forestry). 2005. *A Practical Field Procedure for Identification and Delineation of Wetlands and Riparian Areas, Edition 1*. Department of Water Affairs and Forestry. Pretoria, Republic of South Africa.

Department of Water Affairs of the Republic of South Africa. 2010. *Mine Water Management in the Witwatersrand Gold Fields with Special Emphasis on Acid Mine Drainage – Report to the Inter-Ministerial Committee on Acid Mine Drainage*. Expert Team of the Inter-Ministerial Committee.

de Villiers, A.L. 2004. *Bufo pantherinus* (A. Smith, 1828). Pp. 71-74 in Minter, L.R., M. Burger, J.A. Harrison, H.H. Braack, P.J. Bishop & D. Kloepfer, eds. *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland*. SI/MAB Series #9. Smithsonian Institution, Washington, DC.

Dodd. Jr., C.K. & B.S. Cade. 1998. Movement Patterns and the Conservation of Amphibians Breeding in Small, Temporary Wetlands. Conserv Biol. 12: 331-339

Dunson, W.A., R.L. Wyman & E.S. Corbett. 1992. A Syposium on Amphibian Declines and Habitat Acidification. J Herpetol. 26: 349-352

Du Preez, L.H., C. Weldon, M. Cunningham & A. Turner. 2004. *Bufo gutturalis* Power, 1927. Pp. 67-69 in Minter, L.R., M. Burger, J.A. Harrison, H.H. Braack, P.J. Bishop & D. Kloepfer, eds. *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland*. SI/MAB Series #9. Smithsonian Institution, Washington, DC.

Du Preez, L. & V. Carruthers. 2009. *A Complete Guide to the Frogs of Southern Africa*. Struik Nature. Cape Town, South Africa.

Fahrig, L., J.H. Pedlar, S.E. Pope, P.D. Taylor & J.F. Wegner. 1995. Effect of Road Traffic on Amphibian Density. Biol Conserv. 73: 177-182

Forman, R.T.T. 2000. Estimate of the Area Affected Ecologically by the Road System in the United States. Conserv Biol. 14: 31-35

Forman, R.T.T. & L.E. Alexander. 1998. Roads and Their Major Ecological Effects. Annu Rev Ecol. 29: 207-231

Forman, R.T.T. & R.D. Deblinger. 2000. The Ecological Road-Effect Zone of a Massachusetts (U.S.A.) Suburban Highway. Conserv Biol. 14: 36-46

Freda, J. 1986. The Influence of Acidic Pond Water on Amphibians: A Review. Water Air Soil Poll. 30: 439-450

Freedman, B. 1995. *Environmental Ecology (2nd Ed) – The Ecological Effects of Pollution, Disturbance, and Other Stresses.* Academic Press, Inc. London, U.K.

Frost, D.R. 2015. Amphibian Species of the World: an Online Reference. Version 6.0. Electronic Database accessible at http://research.amnh.org/vz/herpetology/amphibia/index.php. American Museum of Natural History, New York, U.S.A. Accessed [03 May 2015].

Funk, W.C., A.E. Green., P.S. Corn & F.W. Allendorf. 2005. High dispersal in a frog species suggests that it is vulnerable to habitat fragmentation. Biol Lett. 1: 13-16

Gagliano, W.B. & J.M. Bigham. 2006. Acid Mine Drainage. Pp. 1-4 *In* R. Lal (Ed.) *Encyclopedia of Soil Science – Second Edition (Vol 1)*. CRC Press, Taylor & Francis, NW, U.S.A.

Gamble, L.R., K. McGarigal & B.W. Compton. 2007. Fidelity and Dispersal in the Pond-Breeding Amphibian, *Ambystoma Opacum*: Implications for Spatio-Temporal Population Dynamics and Conservation. Biol Conserv. 139: 247-257

Gallant, A.L., R.W. Klaver, G.S. Casper & M.J. Lannoo. 2007. Global Rates of Habitat Loss and Implications for Amphibian Conservation. 4: 967-979

Gardner, T. 2001. Declining Amphibian Populations: A Global Phenomenon in Conservation Biology. Animal Biodiversity and Conservation. 24.2: 25-44

Gauteng Government. 2001. *Statistics South Africa Census 2001 – Key Results*. Statistics South Africa, Pretoria, Republic of South Africa

GDARD (Gauteng Department of Agriculture and Rural Development). 2011. *Gauteng State of the Environment Report 2011*. Gauteng Provincial Government. Gauteng Province, South Africa.

Gibbs, J.P., A.R. Breisch, P.K. Ducey, G. Johnson, J.L. Behler & R.C. Bothner. 2007. *The Amphibians and Reptiles of New York State – Identification, Natural History, and Conservation*. Oxford University Press. New York, U.S.A.

Gibbs, J.P., K.K. Whiteleather & F.W. Schueler. 2005. Changes in Frog and Toad Populations over 30 Years in New York State. Ecol Appl. 15: 1148-1157

Glista, D.J., T.L. DeVault & J.A. DeWoody. 2007. Vertebrate Road Mortality Predominantly Impacts Amphibians. Herpetological Conservation and Biology. 3: 77-87.

Glooschenko, V., W.F. Weller, P.G.R. Smith, R. Alvo & J.H.G. Archbold. 1992. Amphibian Distribution with respect to Pond Water Chemistry near Sudbury, Ontario. Can J Fish Aquat Sci. 49: 114-121

Godfrey, E.W. & G.E. Sanders. 2004. Effect of Water Hardness on Oocyte Quality and Embryo Development in the African Clawed Frog (*Xenopus laevis*). Can J Comparat Med. 54: 170-175

Goudie, A.S. 2005. *The Human Impact on the Natural Environment: Past, Present and Future*. Wiley-Blackwell, U.S.A.

Government of South Africa. 1998. *National Environmental Management Act, 1998*. Government of the Republic of South Africa. Pretoria, Republic of South Africa.

Granat, L. 2001. Deposition of Nitrate and Ammonium from the Atmosphere to the Baltic Sea. Pp. 133-148 In Wulff, F.V., L.A. Rahm & P. Larsson, eds. *A Systems Analysis of the Baltic Sea*. Springer, Berlin. Germany

Gupta, S.K. 2011. *Modern Hydrology and Sustainable Water Development*. Wiley-Blackwell. West Sussex, U.K.

Haines, T.A. 1981. Acidic Precipitation and Its Consequences for Aquatic Ecosystems: A Review. T Am Fish Soc. 110: 669-707

Hamer, A.J. & M.J. McDonnell. 2008. Amphibian Ecology and Conservation in the Urbanising World: A Review. Biol Conserv. 141: 2432-2449

Haywood, L.K., G.J. Alexander, M.J. Byrne & E. Cukrowska. 2003. *Xenopus laevis* Embryos and Tadpoles as Models for Testing for Pollution by Zinc, Copper, Lead and Cadmium. Afr Zool. 36: 163-174

Hazell, D., R. Cunningham, D. Lindenmeyer, B. Mackay & W. Osborne. 2001. Use of Farm Dams as Frog Habitat in an Australian Agricultural Landscape: Factors Affecting Species Richness and Distribution. Biol Conserv. 102: 155-169

Hecnar, S.J. & R.T. M'Closkey. 1996. Amphibian Species Richness and Distribution in Relation to Pond Water Chemistry in South-Western Ontario, Canada. Freshwater Biol. 36: 7-15

Hels, T. & E. Buchwald. 2001. The Effect of Road Kills on Amphibian Populations. Pp. 25-42 In Irwin CL, P. Garrett & KP. McDermott, eds. *Proceedings of the 2001 International Conference on Ecology and Transportation*. Center for Transportation and the Environment, North Carolina State University, Raleigh, NC.

Houlahan, J.E., P.A. Keddy, K. Makkay & C.S. Findlay. 2006. The Effects of Adjacent Land Use on Wetland Species Richness and Community Composition. Wetlands. 26: 79-96

Howe, G.E., R. Gillis & R.C. Mowbray. 1998. Effect of Chemical Synergy and Larval Stage on the Toxicity of Atrazine and Alachlor to Amphibian Larvae. Environ Toxicol Chem. 17: 519-525.

(ILAR) Institute of Laboratory Animal Resources National Research Council. 1974. *Amphibians – Guidelines for the Breeding, Care, and Management of Laboratory Animals*. National Academy of Sciences, Washington, D.C., U.S.A

IUCN. 2014. *Table 3a: Status Category Summary by Major Taxonomic Group (animals)*. The IUCN Red List of Threatened Species. Version 2014.3. www.iucnredlist.org. Downloaded [03 May 2015].

IUCN. 2015. The IUCN Red List of Threatened Species. Version 2014.3. www.iucnredlist.org. Downloaded [05 February 2015].

IUCN SSC Amphibian Specialist Group 2013. *Pyxicephalus adspersus*. The IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>. Downloaded [02 December 2014].

Jaeger, J.A.G., J. Bowman, J. Brennan, L. Fahrig, D. Bert, J. Bouchard, N. Charbonneau, K. Frank., B. Gruber & K. von Toschanowitz. 2005. Predicting When Animal Populations Are at Risk from Roads: An Interactive Model of Road Avoidance Behavior Ecol Model. 185: 329-348

Jofre, M.B. & W.H. Karasov. 2009. Direct Effect of Ammonia on Three Species of North American Anuran Amphibians. Environ Toxicol Chem. 18: 1806-1812.

Johnson, D.B. & K.B. Hallberg. 2005. Acid Mine Drainage Remediation Options: A review. Sci Total Environ. 338: 3-14

Johnson, P.T.J & J.M. Chase. 2004. Parasites in the Food Web: Linking Amphibian Malformations and Aquatic Eutrophication. Ecol Lett. 7: 521-526

Johnson, P.T.J., J.M. Chase, K.L. Dosch, R.B. Hartson, J.A. Gross, D.J. Larson, D.R. Sutherland & S.R. Carpenter. 2007. Aquatic Eutrophication Promotes Pathogenic Infection in Amphibians. PNAS. 104: 15781-15786

Jovanovic, N.Z., R.O. Barnard, N.F.G. Rethman & J.G. Annandale. 1998. Crops Can Be Irrigated with Lime-Treated Acid Mine Drainage. Water SA. 24: 113-122

Kadlec, R.H. & S. Wallace. 2009. *Treatment Wetlands – Second Edition*. CRC Press, Taylor & Francis. Florida, U.S.A.

Khan, F.A. & A.A. Ansari. 2005. Eutrophication: An Ecological Vision. The Botanical Review. 71:449-482

Knutson, M.G., W.B. Richardson, D.M. Reineke, B.R. Gray, J.R. Parmelee & S.E. Weick. 2004. Agricultural Ponds Support Amphibian Populations. Ecol Appl. 14: 669-684

Koprivnikar, J., R.L. Baker & M.R. Forbes. 2006. Envrionmental Factors Influencing Trematode Prevalence in Grey Tree Frog (*Hyla versicolor*) Tadpoles in Southern Ontario. J Parasitol. 92: 997-1001

Lafferty, K.D. 1997. Environment Parasitology: What Can Parasites Tell Us about Human Impacts on the Environment? Parasitol Today. 13: 251-255

Lee, K.H., T.M. Idenhart, R.C. Schultz & S.K. Mickelson. 2000. Multispecies Riparian Buffers Trap Sediment and Nutrients during Rainfall Simulations. J Environ Qual. 29: 1200-1205

Lentini, A.M. 2013. In *Zookeeping – An Introduction to the Science and Technology*. Irwin, M.D., J.B. Stoner & A.M. Cobaugh (*Eds*). University of Chicago Press, Chicago, U.S.A.

Leuven, R.S.E.W., C. den Hartog., M.M.C. Christiaans & W.H.C. Heijligers. 1986. Effects of Water Acidification on the Distribution Pattern and Reproductive Success of Amphibians. Experientia. 42: 495-503

Mann, R.M. & J.R. Bidwell. 2001. The Acute Toxicity of Agricultural Surfactants to the Tadpoles of Four Australian and Two Exotic Frogs. Environ Pollut. 114: 195-205.

Mann, R.M., R.V. Hyne., C.B. Choung & S.P. Wilson. 2009. Amphibians and Agricultural Chemicals: Review of the Risks in a Complex Environment. Environ Pollut. 157: 2903:2927

Marco, A., C. Quilchano & A.R. Blaustein. 1999. Sensitivity to Nitrate and Nitrite in Pond-Breeding Amphibians from the Pacific Northwest, USA. Environ Toxicol Chem. 18: 2836-2839

Maree, J.P., M. Mujuru, V. Bologo, N. Daniels & D. Mpholoane. 2013. Neutralisation Treatment of AMD at Affordable Cost. Water SA. 39: 245-250

Marsh, D.M. 2000. Variable Responses to Rainfall by Breeding Tungara Frogs. Copeia. 4: 1104-1108

Marsalek, J., Q. Rochfort, B. Brownlee, T. Mayer & M. Servos. 1999. An Exploratory Study of Urban Runoff Toxicity. Water Sci Technol. 39: 33-39

McCarthy, T.S. 2011, The Impact of Acid Mine Drainage in South Africa. S Afr J Sci. 107(5/6): 1-7

Measey, G.J. 2004. *Xenopus laevis* (Daudin, 1802). Pp. 264-266 in Minter, L.R., M. Burger, J.A. Harrison, H.H. Braack, P.J. Bishop & D. Kloepfer, eds. *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland*. SI/MAB Series #9. Smithsonian Institution, Washington, DC.

Measey, G.J. (ed.) 2011. *Ensuring a Future for South Africa's Frogs : a Strategy for Conservation Research.* SANBI Biodiversity Series 19. South African National Biodiversity Institute, Pretoria, Republic of South Africa.

Measey, G.J. & S.J. Davies. 2011. Struggling Against Domestic Exotics at the Southern End of Africa. Pp. 28-30 in Collins, J.P., C. Gascon., R.D. Moore & J.P. Lewis, eds. *Froglog – July 2011 Vol.97*. Arlington, U.S.A.

Morris, J.T. 1991. Effects of Nitrogen Loading on Wetland Ecosystems with Particular Reference to Atmospheric Deposition. Annu Rev Ecol Syst. 22: 257-279

Muscutt, A.D., G.L. Harris., S.W. Bailey & D.B. Davies. 1993. Buffer Zones to Improve Water Quality: a Review of Their Potential Use in UK Agriculture. Agr Ecosyst Environ. 45: 59-77

Mylnivzenko, N. 2009. Amphibians – Diagnostic Testing. Pp. 91-93 in Mitchell, M.A. & T.N. Tully, Jr., eds. *Manual of Exotic Pet Practice*. Saunders-Elsevier, Missouri, U.S.A.

Naicker, K., E. Cukrowska & T.S. McCarthy. 2003. Acid Mine Drainage Arising from Gold Mining Activity in Johannesburg, South Africa and Environs. Environ Pollut. 122: 29-40

Nunney, L. & K.A. Campbell. 1993. Assessing Minimum Viable Population Size: Demography Meets Population Genetics. TREE. 8: 234-239

Nyenje, P.M., J.W. Foppen, S. Uhlenbrook, R. Kulabako & A . Muwanga. 2010. Eutrophication and Nutrient Release in Urban Areas of Sub-Saharan Africa – A Review. Sci Total Environ. 408: 447-455

Odum, R.A. & K.C. Zippel. 2008. Amphibian Water Quality: Approaches to an Essential Environmental Parameter. Int Zoo Yb. 42: 40-52

Oeurng, C, S. Sauvage & J. Sánchez-Pérez. 2010. Temporal Variability of Nitrate Transport Through Hydrological Response During Flood Events within a Large Agricultural Catchment in South-West France. Sci Total Environ. 409: 140-149

Page, G.W. 1981. Comparison of Groundwater and Surface Water for Patterns and Levels of Contamination by Toxic Substances. Environ Sci Technol. 15: 1475-1481

Parris, K.M. 2006. Urban Amphibian Assemblages as Metacommunities. J Anim Ecol. 75: 757-764

Peltzer, P.M., R.C. Lajmanovich, J.C. Sánchez-Hernandez, M.C. Cabagna, A.M. Attademo & A. Bassó. 2008. Effects of Agricultural Pond Eutrophication on Survival and Health Status of *Scinax nasicus* Tadpoles. Ecotox Environ Safe. 70: 185-197

Perlman, D.L. & J. Milder. 2005. *Practical Ecology for Planners, Developers, and Citizens*. Island Press, Washington, D.C., U.S.A.

Phillips, J.D. 1989. An Evaluation of the Factors Determining the Effectiveness of Water Quality Buffer Zones. J Hydrol. 107: 133-145

Pillay, M. & S. Terry. 1991. Informal Settlements – Impact on Water Quality. 17th WEDC Conference – Infrastructure, Environment, Water and People, Nairobi, Kenya. Pp. 25-28

Pillsbury, F.C. & J.R. Miller. 2008. Habitat and Landscape Characteristics Underlying Anuran Community Structure along an Urban-Rural Gradient. Ecol Appl. 18: 1107-1118

Porter, K.R. & D.E. Hakanson. 1976. Toxicity of Mine Drainage to Embryonic and Larval Boreal Toads (Bufonidae: *Bufo boreas*) . Copeia. 1976: 327-331

Prugh, L.A., K.E. Hodges, A.R.E. Sinclair & J.S. Brashares. 2008. Effect of Habitat Area and Isolation on Fragmented Animal Populations. P Natl Acad Sci. 105: 20770-20775.

Rabalais, N. 2002. Nitrogen in Aquatic Ecosystems. Ambio. 31: 102-112

Ramsar. 1971. *The Convention on Wetlands of Internation Importance Especially as Waterfowl Habitat*. UNESCO, United Nations, U.S.A.

Reijnen, R., R. Foppen., C.T. Braak & J. Thissen. 1995. The Effects of Car Traffic on Breeding Populations in Woodland. III. Reduction of Density in Relation to the Proximity of Main Roads. J Appl Ecol. 32: 187-202

ReVelle, P. & C. ReVelle. 1992. *The Global Environment: Securing a Sustainable Future*. Jones and Bartlett Publishers, Inc. London, England.

Ribarova, I., P. Ninov & D. Cooper. 2008. Modeling Nutrient Pollution During a First Flood Event Using HSPF Software: Iskar River Case Study, Bulgaria. Ecol Model. 211: 241-246

Riley, S.P.D., G.T. Busteed., L.B. Kats., T.L. Vandergon., L.F.S. Lee., R.G. Dagit., J.L. Kerby., R.N. Fisher & R.M. Sauvajot. 2005. Effects of Urbanization on the Distribution and Abundance of Amphibians and Invasive Species in Southern California Streams. Conserv Biol. 19: 1894-1907

Rittenhouse, T.A.G. & R.D. Semlitsch. 2007. Distribution of Amphibians in Terrestrial Habitat Surrounding Wetlands. Wetlands. 27: 153-161

Rouse, J.D., C.A. Bishop & J. Struger. 1999. Nitrogen Pollution: An Assessment of Its Threat to Amphibian Survival. Environ Health Persp. 107: 799-803

Rubbo, M.J. & J.M. Kiesecker. 2005. Amphibian Breeding Distribution in an Urbanized Landscape. Conserv Biol. 19: 504-511

Rutherford, M.C., L. Mucina & L.W. Powerie. 2010. Biomes and Bioregions of Southern Africa. Pp. 30-51 in Mucina, L & M.C. Rutherford, eds. *The Vegetation of South Africa, Lesotho and Swaziland*. Strelizia 19. South African National Biodiversity Insititute, Pretoria, South Africa.

Schmutzer, A.C., M.J.Gray, E.C. Burton & D.L. Miller. 2008. Impacts of Cattle on Amphibians Larvae and the Aquatic Environment. Freshwater Biol. 53: 2613-2625

Scott, E. 2004. *Cacosternum boettgeri* (Boulenger, 1882). Pp. 222-224 in Minter, L.R., M. Burger, J.A. Harrison, H.H. Braack, P.J. Bishop & D. Kloepfer, eds. *Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland*. SI/MAB Series #9. Smithsonian Institution, Washington, DC.

Semlitsch, R.D. & J.R. Bodie. 2003. Biological Criteria for Buffer Zones around Wetlands and Riparian Habitats for Amphibians and Reptiles. Conserv Biol. 17: 1219-1228

Semlitsch, R.D. & J.B. Jensen. 2001. Core Habitat, Not Buffer Zone. National Wetlands Newsletter. 23: 5-11

Smith, V.H. & D.W. Schindler. 2009. Eutrophication Science: Where Do We Go From Here? TREE. 24: 201-207

Sparling, D.W. 2009. *Amphibian Ecology and Conservation – A Handbook of Techniques*. Dodd, C.K. Jr (*Ed.*) Oxford University Press Inc. New York, U.S.A.

Springer, J.T. & D. Holley. 2013. *An Introduction to Zoology*. Jones & Bartlett Learning. Burlington, U.S.A.

Statistics South Africa. 2011. *Census 2011 – P0301.4*. Statistics South Africa. Pretoria, Republic of South Africa.

Storfer A. 2003. Amphibian Declines: Future Directions. Divers Distrib. 9: 151-163

Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman & R.W. Walker. 2004. Status and Trends of Amphibian Declines and Extinctions Worldwide. Sciencexpress. 10.1126/science.1103538: 1-4

Swanack, T.M., W.E. Grant, M.R.J. Forstner. 2009. Projecting Population Trends of Endangered Amphibian Species in the Face of Uncertainty: A Pattern-Oriented Approach. Ecol Model. 220: 148-159

Telford, S.R. & M.L. Dyson. 1990. The Effect of Rainfall on Interclutch Interval in Painted Reed Frogs (*Hyperolius marmoratus*). Copeia. 3: 644-648

Templeton, A.R., K. Shaw, E. Routman & S.K. Davis. 1990. The Genetic Consequences of Habitat Fragmentation. Ann Mo Bot Gard. 77: 13-27

Thomas, C.D., J.K. Hill & O.T. Lewis. 1998. Evolutionary Consequences of Habitat Fragmentation in a Localized Butterfly. J Anim Ecol. 67: 495-497

Thomas, R.L., L. Owen-Smith, D.C. Drake, G.J. Alexander. 2014. Restoring Breeding Habitat for Giant Bullfrogs (*Pyxicephalus adspersus*) in South Africa. Afr J Herpetol. 63:1, 13-24

Trenham, P.C., W.D. Koenig, M.J. Mossman, S.L. Stark & L.A. Jagger. 2003. Regional Dynamics of Wetland-Breeding Frogs and Toads: Turnover and Synchrony. Ecol Appl. 13: 1522-1532

Tryjanowski, P., M. Rybacki & T. Sparks. 2003. Changes in the First Spawning Dates of Common Frogs and Common Toads in Western Poland in 1978-2002. Ann Zool Fennici. 40: 459-464

Tsai, J., L.S. Venne, S.T. McMurry & L.M. Smith. 2007. Influences of Land Use and Wetland Characteristics on Water Loss Rates and Hydroperiods of Playas in the Southern High Plains, U.S.A. Wetlands. 27: 683-692

United Nations. 2004. *Economic & Social Affairs - World Population to 2300*. Department of Economic and Social Affairs – Population Division. United Nations, New York, U.S.A.

Vought, L.B., G. Pinay, A. Fugslang & C. Ruffinoni. 1995. Structure and Function of Buffer Strips from a Water Quality Perspective in Agricultural Landscapes. Landscape Urban Plan. 31: 323-331

Warren, J., C. Lawson & K. Belcher. 2008. *The Agri-Environment*. Cambridge University Press, London, U.K.

Weiner, E.R. 2013. *Applications of Aquatic Environmental Chemistry – A Practical Guide*. CRC Press LLC, Florida, U.S.A.

Wilde, F.D. 2014. *Field measurements: U.S. Geological Survey Techniques of Water-Resources Investigations*. Book 9, chap. A6. Sections 6.0–6.8. http://pubs.water.usgs.gov/twri9A6/. Accessed [03 May 2015]

Wilde, F.D. & J. Gibs. 1998. *U.S. Geological Survey TWRI Book 9*. Pp. 1-30. USGS Office of Water Quality, USA.

Williams, S.E. & J. Hero. 2001. Multiple Determinants of Australian Tropical Frog Biodiversity. Biol Conserv. 98: 1-10

Yetman, C.A. & J.W.H. Ferguson. 2011. Conservation Implications of Spatial Habitat Use by Adult Giant Bullfrogs (*Pyxicephalus adspersus*). J Herpetol. 45: 56-62

Zhang ,Q., J. Wang, X. Peng, P. Gong & P. Shi. 2002. Urban Built-Up land Change Detection with Road Density and Spectral Information from Multi-Temporal Landsat TM Data. Int J Remote Sens. 23: 3057-3078