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An assessment of the current distribution, biodiversity and health of the frogs of the Kruger National Park in relation to physical and chemical factors

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EXECUTIVE SUMMARY

The first reports indicating that global frog populations were declining, were not only received with disbelief but lead to questions being asked as to what caused these changes and what should be done to curtail the trends. Scientists were in agreement that there was no easy solution and when the potential issues related to the declines in populations were analysed it was clear that the problem is complex and multi-faceted and that no simple solution exists. The impacts and issues range from habitat loss and modification to global warming and chemical pollution. As is the case with most animals the loss of habitat can be ascribed to activities such as agriculture, abstraction and human development which include mining and industries. Added to that is the human appetite for frog's legs that have resulted in severe losses of frog populations across the world. In addition frogs and toads, and in particular rare and threatened species, are being targeted for the fast growing pet trade and these specimens, that are illegally collected, are translocated across the globe. On an ecological level, these introduced species and predators pose a threat and in many instances, infectious diseases are carried with the translocated animals. In South Africa limited research has been done to investigate threats to amphibian populations and the majority of the reported problems are anecdotal.

In South Africa only one of the three orders of the class Amphibia, namely Anura, is present. The Anura, which includes frogs and toads, display a spectacular diversity of body form while their calls are a unique trait used for identification. Of the 117 species known in South Africa more than 20% is regarded as threatened, while approximately 7% is regarded "Data deficient" which implies that limited data is available and it is therefore difficult to determine their conservation status.

As a conservation organisation the Kruger National Park (KNP) realised that the decline in amphibian populations would extend into the park and requested that the current distribution of frogs in the park be investigated. In addition, it was requested that the potential impact of acid rain on frog health and the impact of mega herbivore grazing on the habitat be determined. Based on these questions the following objectives for the project were formulated:

- Identify and quantify physical factors, such as habitat alteration, that may influence amphibian distribution.
- Identify and quantify chemical factors, such as metals and pH, of the rainwater and water in the pans and rivers that may influence amphibian distribution.

- Determine the current distribution pattern of amphibians in the different wetland systems (pans) and the major river basins of the KNP.
- Evaluate current biodiversity and distribution against historical distribution.
- Determine the effects of chemical pollution on the biology of amphibians through histopathology, bio-accumulation and bio-marker (geno-toxicity) studies.

For the study, 45 sites, representative of the ecoregions of the KNP, were identified. The sites were selected to cover as much vegetation, geological and habitat types as possible. All 45 sites were summer survey sites and 14 winter survey sites were selected. Sampling was done twice a year at all summer sites (4 surveys in total). As part of the frog diversity survey, frogs and tadpoles were physically collected and night surveys conducted. During the night surveys calls were recorded and frogs crossing roads were collected.

Where water was present, a water sample was collected and *in situ* water parameters measured. Sediment samples to use for sediment classification and chemical analyses were collected from all the sites. During the winter surveys the basal and additional cover were assessed and scored in four transects, each 50 m long and 10 m wide. The woody vegetation of these transects were identified, counted and heights estimated. The vegetation survey intended to get an understanding of the possible habitat available for frogs during the non-breeding season while the water samples were analysed for metals and nutrients in an attempt to identify drivers that could be linked to frog diversity.

Adult and sub-adult frogs and tadpoles were identified *in situ* and or representative samples were preserved for species confirmation. Preserved specimens were submitted to the Southern African Institute for Aquatic Biodiversity (SAIAB) and to the Skukuza Museum as part of the Kruger National Park's collection. Tadpoles of *Chiromantis xerampelina*, *Pyxicephalus edulis*, *Amietophrynus maculatus* and *Hildebrandtia ornata* were collected to be used in acute and chronic acid tolerance bio-assays to determine the LC50 pH values. Adult specimens of the same species were sacrificed and after blood smears were collected, the specimens were preserved in 10% neutrally buffered formalin for the histology analysis.

Fresh foam nests of *C. xerampelina*, where evidence of eggs laid the previous evening existed, were collected, taken to a field laboratory and sprayed with a range of pH adjusted water to simulate acid rain. Tadpoles were collected after hatching and preserved in 10% neutral buffered formalin to determine possible impacts of acid on development.

The diversity in study sites is reflected by the variation in surface area ranging from 0.01 ha (S15 – N'watimbuti) to 3.4 ha (S43 – Maroela). The vegetation at the sites was as diverse with some of the pans that were depressions with no vegetation in or around the fringes of

the water body (S36 – Crocodile Bridge) to pans with dense basal cover surrounding it and extensive "in-pan" vegetation (S22 – Rietpan). The organic material content in the majority of the pans were high, indicating that they act as nutrient sinks in the landscape. Pan depths varied from as little as 0.15 m (S32 – Shulungwane) to 1.275 m (S39 – Nkaya Pan). It was observed that high frog diversity existed at pans with basal cover in excess of 60% and "in-pan" vegetation classified as "mosaic" or "entire". Statistical analyses showed that the best relationship was observed between selected frog species and additional cover in the area surrounding the pan perimeter. The above emphasises the importance of cover for breeding during the breeding periods. In addition, pans with a high percentage of logs and stumps, ant hills and tree clumps had the highest frog diversity recorded during this study.

The extent and impacts of pollution on South African frogs have not been addressed, even though pollution and pesticides pose serious threats. Wetland habitats are biologically diverse and productive areas that support numerous species of both aquatic and terrestrial organisms and are critical habitats. Frogs appear to be particularly vulnerable to population losses. Most frogs within the Kruger National Park are terrestrial, with an aquatic larval stage associated with a seasonally inundated wetland system. The majority of frogs use these ephemeral wetland habitats for breeding, and many are found in or near bodies of water outside the breeding season including *Xenopus laevis, Xenopus muelleri*, *Amietia angolensis* and *Ptychadena anchietae*.

The potential use of amphibians as biological indicators is still in its infancy in Southern Africa. More intensive long-term amphibian population studies as well as intensive autecological studies need to be conducted. One of the most difficult problems in conservation biology is the lack of baseline data against which to measure population changes. The result has been that much of the information concerning amphibian declines is anecdotal; this is especially pertinent for South Africa. There is an extensive lack of accurate knowledge on the specific habitat requirements (migratory, foraging and breeding) and species tolerance to environmental degradation.

Regular monitoring of amphibian populations is the best way of determining population trends within species. This may be the only way of assessing conservation actions for many amphibians. Some amphibians undergo large natural fluctuations in their population numbers and so long-term datasets are required in order to determine the direction of trends over time. Monitoring data can be used to assess the effects of conservation and other land management practices.

Historic surveys in the Kruger National Park resulted in 33 frog species being recorded. During the current survey 30 frog species were recorded. A combination of historic and recent field surveys result in 34 frog species being recorded from the Kruger National Park. The results of this survey indicate that long term monitoring projects specifically focused on amphibians are required within the Kruger National Park pan systems; which specific emphasis on water quality and micro-habitat selection of the adults and tadpoles within selected pans. Long-term experiments using caged tadpoles in selected sites in specific drainage systems must still be conducted. A comparative toxicity data-base for South African amphibians needs to be compiled as a means of assessing the potential toxicity risks.

The current distribution of Striped Stream Frog (*Strongylopus fasciatus*), Raucous Toad (*Amietophrynus rangeri*), Natal Sand Frog (*Tomopterna natalensis*) and Shovel-Footed Squeaker (*Arthroleptis stenodactylus*) within the Kruger National Park needs to be addressed. The limited records of Sharp-nosed Grass Frog (*Ptychadena oxyrhynchus*) warrants further surveys in suitable habitat. More detailed studies of specific habitat requirements, breeding biology, duration of the larval stage and development are recommended for several frog species within the Kruger National Park. The tadpoles of *Strongylopus fasciatus*, *Ptychadena mossambica, Tomopterna marmorata* and *Tomopterna krugerensis* are presently un-described as well as aspects of the tadpole ecology such as the larval duration of *Chiromantis xerampelina, Hildebrandtia ornata, Hyperolius tuberilinguis, Kassina maculata, Leptopelis mossambica*. Extremely limited information of the non-breeding ecology of the majority of frog species is known. One can refer to the African Bullfrog (*Pyxicephalus edulis*), Southern Ornata Frog (*Hildebrandtia ornata*) and Golden-Leaf-folding Frog (*Afrixalus aureus*) as examples in this regard.

From the results of the water quality analysis it was observed that the geology of the study area and the chemical properties of the water have no major influence on the metal concentrations in the water. Weathering of rock led to high concentrations of Na and SO_4 associated with the Ecca shales in the area. Study sites with higher Na and SO_4 levels had increased concentrations of metals, but none were at levels of concern. When analysing the nutrients (PCA ordination bi-plot), a number of the wetlands displayed alkaline dystrophic characteristics, whilst a larger number had saline eutrophic characteristics. Gibbs diagrams were used in two plots, the first based on cations with the Ca and Na and the second using anions (Cl and HCO_3^-). The plots indicated that majority of the wetlands studied were rock dominance, with a smaller group evaporation dominance pans. In addition to this, the Maucha diagrams used indicates that most of the study sites are SO_4 dominated, with Cl and Na contributing in the ion patterns.

When analysing the results, it is reasonable to assume that most of the salts present in the pans are from geological origin (Karoo Super Group). The high SO_4 concentrations are normally associated with the Vryheid Formation (not present in the KNP) which is known for the rich coal ore formations. It is therefore reasonable to assume that the acid rain can contribute to the elevated values of sulphate.

In the acute acid tolerance bio-assay, the tadpoles from *P. edulis* were the most sensitive for pH changes and having a LC50 at pH 4.55. This can be attributed to the fact that they spend a long time in the water and may be more sensitive to sudden changes in the environment. The tadpoles of *C. xerampelina* had a LC50 at pH 4.07 and this can be linked to the fact that they are mainly arboreal, spending a very short period in the water once the eggs have dropped from the nests. *A. maculatus* and *H. ornata* had LC 50 pH values of 3.75 and 3.75 respectively. During the chronic acid tolerance bio-assays, *P. edulis* had the higher mortality rates with values not lower than 36.7% compared to the rest with values as low as 20%.

The blood smears revealed no blood parasites in *C. xerampelina*, whilst parasites were recorded in the other three species. In the analysis, *P. edulis* had a 40% infection rate, *A. maculatus* a 60% infection rate and *H. ornata* had an 80% infection rate with a prevalence of 3-10 blood parasites per 100 blood cells.

The histopathology analysis shows some histological alterations in the liver and kidney of *Tomopterna cryptosis* and *Breviceps adspersus*. The lung, heart, gonad, stomach, ventral skin and dorsal skin samples showed no alterations. The alterations in the liver and kidney were not present in all specimens investigated. The alterations are early toxicopathic, non-neoplastic lesions which includes non-focal hepatocellular and nuclear pleomorphism, hepatocelluar vacuolation and focal necrosis. Some vascular congestion, hepatocellular atrophy and the presence of melanomacrophage centres were observed in the liver tissue. The alterations in the tissue can be a result of unfavourable stressors possibly related to changes in the water quality (SO_4) and some increases in metals.

As final conclusions the following is listed:

- During the study, the water analysis indicated very little issues regarding possible pollutants that are currently affecting the environment. The only constituent of possible concern is the sulphate levels (SO_4 – refer to Chapter 4) were elevated levels were observed in the southern section of the Kruger National Park. This can be related to possible acid rain associated with the industrial complex and coal power stations in Mpumalanga (Mphepya et al. 2006).

- The study indicated that frogs depend on good vegetation cover and additional habitat structure (i.e. dead wood, rocks and termite mounds) where they possibly over winter. This is one of the aspects suggested for further investigation.

- The current distribution pattern of species was similar to the historic record, but a few new distribution records are added to the KNP database. The species not recorded are of no real concern, as the sampling times may have led to them not being found.

- The analysis of the acid rain experiments are continuing. This will be published as soon as the data interpretation is completed.

- The initial histo-pathological study gave good results which indicated that the frogs can be effectively used as bioindicators. A larger sample size will be needed to develop the frog health index.

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1   INTRODUCTION

1.1   General introduction

Amphibian declines have been an on-going global phenomenon, first reported as such in 1990 and gathering in number with increasing quantities of studies and interpretations (e.g. Blaustein and Wake 1990; Houlahan et al. 2000). These reports have highlighted the complexity of the global extent of amphibian decline together with numerous factors in both pristine and disturbed habitats. "Enigmatic declines", a term coined to refer to declines in pristine habitats (Stuart et al. 2004), became the focus of many studies which postulated a range of factors including increased ultraviolet radiation, climate change, infectious disease and their synergistic effects. However, the primary cause of global biodiversity loss (including amphibians) is through habitat change (Stuart et al. 2008) which ultimately stems from increasing human population and per capita consumption. Through the 1990s there was little in the way of a class-level assessment of how widespread the phenomenon of global amphibian decline was, and of the potential factors that could be involved in amphibian declines at a global level. It is in this context that the Global Amphibian Assessment (GAA; now Amphibians on the IUCN Red List), a joint initiative led by IUCN, Conservation International and NatureServe, was completed in 2004, comprising the first ever comprehensive conservation assessment of all of the world's amphibian species known at that time (5 743 species; Stuart et al. 2008). The GAA's main objectives were to   1) determine the scale of the amphibian extinction\ crisis, 2) identify key geographical areas and habitats for amphibian conservation, 3) identify the main threats and propose priority conservation actions to address these threats, and 4) establish an amphibian-focused expert network (Angulo et al. 2011).

Early in 1994 two South African herpetologists, Phil Bishop and Les Minter pondered the global fate of amphibians and wondered what they could achieve in South Africa. The legacy of a strong tradition in herpetological research in the region had culminated in frog distribution maps that were by then 30 years old (Poynton 1964). Red List assessments for the region were also out of date (Branch 1988). Their brainchild which became known as the South African Frog Atlas Project (SAFAP), was nurtured to fruition by the Animal Demography Unit at the University of Cape Town, and resulted in a collaboration of almost the entire regional herpetological community and culminated in the publication of the first comprehensive Atlas and Red Data book for the frogs of South Africa, Lesotho and Swaziland (Minter et al. 2004). This publication and the updated Red List it contained, coincided with the global GAA initiative and allowed this most recent regional assessment to provide one of the most comprehensive data sets into the GAA (Angulo et al. 2011).

The data for South African amphibians compiled in 2004 and 2010 can best be compared to understand current trends that are present. In 2004, there were 117 (assessed) amphibian species in South Africa. Of these, twenty species were considered to be in a threatened category, with four as Critical (CR), eight as Endangered (EN) and eight as Vulnerable (VU). In addition five species were listed as Near Threatened (NT), eighty-four as being of Least Concern (LC) and eight as Data Deficient (DD). In terms of proportions and in comparison to global figures in 2004, 17% of South African amphibians were considered to be threatened, whereas 32% of global amphibians were considered to be in a threatened category. Nearly 7% of South African species were listed as Data Deficient, as opposed to the global 23%, which indicated that the amphibian fauna in South Africa is comparatively well-studied when compared to the global figures. This pattern is repeated again in the 2010 assessment, as the proportion of globally threatened species was at 30% and the proportion of Data Deficient species had increased globally to 25%, largely due to new species descriptions (Angulo et al. 2011).

When South African amphibian data is compared only to the global anuran data, a similar pattern is observed than when compared to the entire amphibian data set. From the information it is evident that 29% of all frogs are assessed as in a threat category (CR – 7%, EN – 12% and VU – 10%), whereas 6% are assessed as NT, 39% as LC and 26% as DD. Figures for the entire global dataset thus closely follow those of the anuran subset, in no small part because anurans comprise 88% of assessed amphibians. In relation to the global proportions then, South African amphibians appear to be faring relatively better, with proportionately less species in a threatened category or in the Data Deficient category. This may be due, to some extent, to the potential causes of enigmatic declines having a greater effect at the global level, e.g. chytridiomycosis and climate change (Angulo et al. 2011).

There is some congruence between the patterns of major threats affecting global amphibians and those affecting South African amphibians. It should be stressed that in the vast majority of cases (both global and in South Africa) most threats are as perceived by Red List assessors. Very few examples of scientific studies have actually quantified the relative impact of each threat, although this should be done (Angulo et al. 2011).

Habitat loss or modification as a result of agriculture and other forms of human activity remains the most important single threat to the survival of amphibian populations, because of the scale of these changes and their relative permanence. Species that have limited distributions are at greatest risk. Global warming has a serious effect on climate, leading to altered rainfall patterns that may adversely affect amphibian reproductive cycles. The depletion of stratospheric ozone causes an increase in UV-B radiation that greatly reduces

hatching success of embryos and the survival of the tadpoles of certain frog species (Kiesecker et al. 2001; Blaustein et al. 2003). Chemical pollution has been shown to have devastating effects on amphibian populations, including developmental and behavioural abnormalities (Bridges and Semlitsch 2000; Sparling et al. 2001). Pollutants include not only residues released by industry, but fertilizers, herbicides, fungicides and pesticides. Several of these pollutants disrupt the endocrine and reproductive systems of amphibians (Colborn and Clement 1992; Stebbins and Cohen 1995). Human appetite for frogs' legs has an enormous impact. It was estimated that in 1993, no less than 4700 tons of frogs' legs were exported into Europe from Indonesia alone (Patel 1993). This represents between 94 and 235 million individual frogs that were slaughtered in the process (Veith et al. 2000). The pet trade has driven various amphibian species to the brink of extinction. The rarer a species, the higher the asking price for specimens resulting in a more severe threat to those few that remains in the wild. Introduced predators can have a serious impact on amphibian populations. Such predators may compete with native species for natural resources, carry and transmit disease, hybridize with native species, and prey on amphibians (Kiesecker and Blaustein 1998; Lawler et al. 1999; Kats and Ferrer 2003).

Another threat is infectious disease and like most other animals, amphibians fall prey to viruses, fungi and parasitic infections transmitted by protozoans and various helminths. In recent years it has become increasingly clear that infectious disease is one of several causes of global amphibian declines (Carey 2000; Williams et al. 2002; Collins and Storfer 2003; Daszak et al. 2003).

Emerging infectious diseases are defined as diseases that are newly recognized, have recently appeared in a population, or are rapidly increasing in incidence, virulence or geographical range (Daszak et al. 2000). Three of the most commonly implicated diseases of amphibians associated with recent population declines are chytridiomycosis, saprolegniosis and Ranavirus infections (Cunningham et al. 1996; Berger et al. 1998; Kiesecker et al. 2001). In England, Ranavirus was implicated in 3400 outbreaks over a five-year period that led to the death of thousands of frogs (Langton 2002).

Chytridiomycosis is caused by a single-celled fungus, *Batrachochytrium dendrobatidis* and has the strongest association with amphibian population declines (Longcore et al. 1999; Daszak et al. 2003). This fungus was identified as the most likely cause of recent amphibian population declines and extinctions in Australia, Central America, the United States of America and Spain (Daszak et al. 1999). However, in the same areas, the fungus co-exists with non-declining frog species (Speare and Berger 2000). It is not known which inherent factors make some amphibians more susceptible to infectious disease than others. The

emergence of an infectious disease such as chytridiomycosis is usually linked to several factors and may be triggered by environmental change. A classic example is that of *Bufo boreas*, a species in which high mortality of its embryos was attributed to a variety of factors, including the EI Nino/Southern Oscillation, water depth, ultraviolet-B radiation and infection by a fungus, *Saprolegnia ferax* (Kiesecker et al. 2001). Climate-induced reduction in water depth caused an increase in the exposure of the embryos to UV-B radiation, thus increasing the vulnerability of this toad to infection. Blaustein et al. (2003) ascribed outbreaks of chytridiomycosis in Costa Rica to changes in rainfall and temperature (Lips 1998: Pounds et al. 1999) and the concentration of contaminants by cloud build-up (Pounds and Crump 1994), while an outbreak of chytridiomycosis in populations of *Alytes obstetricans* in Spain was associated with increased pH of the water (Bosch et al. 2001).

Amphibian declines in South Africa

Evidence for a countrywide decline in frog populations in South Africa is lacking (Channing and Van Dijk 1995). Amphibian declines in southern Africa have been observed, but only at the local population level and are usually confined to areas directly impacted upon by relevant threats. Among many threats faced by amphibians in southern Africa, the most frequently implicated is habitat destruction resulting from wetland drainage, afforestation, crop-farming, invasive alien vegetation and urbanization (Harrison et al. 2001). In 1998, the mass mortality of a species assumed to be *Amietia vertebralis* was reported by hikers at an upland site in the Drakensberg escarpment (O'Grady 1998). The cause of death was undetermined, but at the time the river had been reduced by drought to a series of isolated stagnant pools (Weldon and du Preez 2004).

In an overview of the major threats affecting Red List species (2000), habitat loss was the most pervasive and over-riding threat to mammals, birds, and plants (groups for which the database was considered to be most comprehensive). Three primary causes of habitat loss were identified: agricultural activities (including afforestation), extraction activities (mining, timber logging, fisheries, etc.) and activities associated with human development (e.g. housing, industry and transport) (Hilton-Taylor 2000). More difficult to assess, but perhaps equally damaging, are the consequences of habitat degradation and fragmentation on population recruitment and ecosystem functioning. Few threats to amphibians in the atlas region have been formally documented, and identification of threats is largely anecdotal (Branch and Harrison 2004). Although the rates are often listed as distinct and separate factors, it is important to appreciate that when concurrent many threats act synergistically, each intensifying the damaging effect of the other. The most important threats, namely habitat loss, fragmentation and degradation, are dynamically interrelated and result from the

other listed threats. Although illegal trade in southern African reptiles has existed for some time, similar exploitation of amphibians for the market in exotic pets is unknown. It is unlikely to be extensive and it was not listed as a known threat for species evaluated here.

Habitat loss, fragmentation and degradation

Habitat loss, in all its many forms, was cited as the most pervasive threat facing amphibians. Consequent habitat fragmentation threatens many species. Habitat loss most commonly results from agricultural development (including sylviculture), but localized urban development and associated infrastructure threatens some species, particularly those with restricted ranges (e.g. *Microbatrachella capensis*, *Xenopus gilli*, and *Amietophrynus* (*Bufo*) *pantherinus* on the Cape Flats and *Afrixalus knysnae* on the south coast; *Hyperolius pickersgilli* in coastal KwaZulu-Natal; and *Pyxicephalus adspersus* in Gauteng (Branch and Harrison 2004). A few species appear to benefit from artificial habitats such as dams, ditches and irrigated parks and gardens (e.g. *Xenopus laevis*, *Amietophrynus gutturalis*, *Hyperolius marmoratus*, *Strongylopus grayii*, *Amietia* (*Afrana*) *fuscigula*, and *A. angolensis*). Some threatened species appear able to persist in radically altered habitats, although we lack information on how their population densities may have been affected by the changes (e.g. *Cacosternum capense* and *Breviceps gibbosus*). Habitat loss and fragmentation can lead to genetic depletion in isolated populations (Garner and Pearman 2001) and this may be reflected in lowered larval fitness (Hitchings and Beebee 1997; Rowe et al. 1999). Loss of amphibian diversity is related to habitat size in forest patches (Vallan 2000) and isolated wetlands (Semlitsch 2000).

Dams, overgrazing, and siltation

Habitat loss is often consequence of agricultural development, including the construction of dams, overgrazing, and trampling by domestic stock. Dams, while providing additional habitat for some species, tend to reduce or completely eliminate downstream flow of streams and rivers, reducing suitability for riparian species. This is an especially important factor for *Heleophryne* spp. that requires perennial streams with good-quality, clear water for successful breeding. Although grazing and trampling do not cause major direct habitat loss, they do lead to increased run-off, erosion and consequent siltation in water catchments. These may directly affect tadpole survival and the habitats of adults of aquatic species (e.g. *Heleophryne* spp., *Xenopus* spp*., Amietia* spp., etc.). The impacts may be particularly severe where suitable breeding sites for amphibians are limited. This is of concern in the semi-arid habitats of Namaqualand where herds of goats threaten isolated freshwater springs (Branch and Braack 1995). Domestic stock levels have increased and herds overgraze vegetation around natural water sources leading to siltation and increased evaporation. Current designs of troughs at artificial water points are rarely suitable as frog breeding sites.

Afforestation and alien invasive plants

Not only is habitat lost directly to exotic plantations and alien vegetation, but indirect effects on hydrodynamics and fire frequency in these areas are often significant. The encroachment of invasive alien vegetation reduces groundwater levels and increases the risk of wildfires. When wildfires occur, the increased burden of woody material causes fires to be especially hot and damaging. Management of mosaics, including those of natural grasslands and indigenous forests and plantations requires an integrated approach that determines not only the viability of plant communities and plantations, but also assesses the requirements of the diverse vertebrate fauna (Castley 1997). Afforestation in South Africa has been noted to have a marked impact on biodiversity and some plant and animal species have become extinct or threatened as a result of afforestation (Allan et al. 1991; Armstrong et al. 1998). Lawes et al. (1999) developed environmental criteria and indicators for sustainable plantation management in South Africa.

Pollution

A reduction in water quality may arise from direct or indirect contamination. This may include direct chemical contamination, or secondary run-off of petroleum and rubber compounds from roads, agricultural pesticides and herbicides, acid precipitation from atmospheric pollution and eutrophication from fertilizer run-off. Owing to the widespread use of pesticides, increasing numbers of non-target species are exposed to chemical contamination. Amphibian populations are particularly susceptible to such contamination and the causal relationships between pesticide usage and amphibian declines are of increasing concern (Boone et al. 2001; Sparling et al. 2001).

The extent and impacts of pollution on South African frogs have not been adequately addressed. Langton (2002) noted high levels of cadmium and copper in frog carcasses in the United Kingdom and the latter (as copper pthalocyanide) was linked to the use of slug pellets in suburban gardens. Channing (1998) discussed the toxicity of many pesticides to tadpoles, but gave no examples of deaths of South African frogs in the wild resulting from their use. The use of Dieldrin and DDT in the control of Tsetse Fly in the Kariba Wilderness area (Zimbabwe) has been implicated in the local decline of amphibians (Taylor 2002).The effects of acid precipitation on montane amphibians has been studied in the United States (Corn et al. 1989), but its impact on amphibians in the eastern highveld of South Africa (an area of intensive coal-based power generation) has not been investigated (Branch and Harrison 2004).

Herbicides and endocrine-disrupting contaminants have also been shown to cause amphibian abnormalities and mortalities (Pickford and Morris 1999; Saka 1999; Bettaso et

al. 2002). Frog mortalities and deformities can arise secondarily via synergistic links between trematode infections and pesticide exposure (Kiesecker 2002). Pollution, probably by heavy metal contaminants, was implicated in developmental deformities in juvenile *Pyxicephalus adspersus* at Bullfrog Pan (P.J. Bishop pers. obs.) as well as three-legged froglets (no left forearm) of Red Toads (*Schismaderma carens*) emerging at Monte Casino.

Fire

In many ecosystems, especially in the Grassland and Fynbos biomes, fire is a natural phenomenon, but fire in forest habitats is naturally infrequent. However, change in water-flow dynamics following road construction or afforestation may lower the water table locally, drying vegetation to unnatural levels and making it more susceptible to fire. Fire was indicated as a current or future threat for nine frog species, all in the south-western Western Cape Province. This region has a Mediterranean climate and experiences hot, dry summers; thus fires caused by humans are an annual threat (Branch and Harrison 2004). Midgley et al. (2001) predicted an increase in the frequency and severity of fires in the region with global warming.

Road mortalities

Many amphibians are killed or injured while crossing roads. This may occur during normal movements within their home range or during breeding migrations. Awareness of this problem is not new (Stoner 1925; Dickerson 1939) and mortalities on roads, particularly in pristine areas, may impact significantly on long-lived, wide-ranging species such as tortoises (Nicholson 1978) and *Pyxicephalus adspersus*. Similarly, explosive breeders that migrate *en masse* to well-defined and long-established breeding sites are vulnerable to vehicles when crossing roads, although such impact varies with the agility of the species (Carr and Fahrig 2001). Roads situated next to wetlands may cause unsustainable rates of mortality, particularly in the case of toads and other large species. Populations can be decimated during migration to and dispersal from the breeding sites and this may lead to local extinction. Branch (1990) noted high road mortalities during mass breeding migrations of *Amietophrynus (Bufo) pardalis* in Port Elizabeth. Similar impacts were recently documented for *Amietophrynus (Bufo) pantherinus* on the Cape Peninsula (De Villiers 2003; Le May 2003). Road mortalities also threaten burrowing species, including *Pyxicephalus adspersus* and several rain frogs, for example, *Breviceps gibbosus* and *B. sylvestris* when they emerge to feed and breed. Several hundred adult Giant Bullfrog males have been observed in Randjiesfontein as well as Heidelberg areas when killed on the roads. Various solutions have been proposed for some amphibians threatened by excessive road mortalities, including specially constructed tunnels and temporary restrictions (7-10 days) on traffic movements at night along roads adjacent to breeding ponds. Large under-road culverts for

storm water control may also serve as safe transit corridors in areas of high impact. However, road underpasses only work in association with costly barriers that prevent access onto roads by frogs and are only feasible in certain situations. Non-migratory fences have been erected along the Bakwena N4 highway near Onderstepoort in Gauteng Province as well as the N1 outside Polokwane in Limpopo Province to prevent Giant Bullfrogs (*Pyxicephalus adspersus*) moving onto the roads. Moreover, experience at the Suikerbosrand Nature Reserve in Gauteng suggests that measures to reduce speeds on roads such as posting reduced speed limits to minimize inadvertent vehicle collisions with wildlife are impractical to enforce. Precautionary signs have been erected around the major Giant Bullfrog breeding sites in Gauteng with little success. The emphasis needs to be on the position and design of new roads rather than post hoc mitigation measures (Branch and Harrison 2004).

Chytridiomycosis and other diseases

The chytrid fungus has been identified in a number of South African frogs, particularly *Xenopus laevis* (Speare 2000; Weldon 2002). As part of a global initiative aimed at studying the distribution of chytrid fungus world-wide, a retrospective survey was conducted on archived museum specimens in South Africa. Of the nine species surveyed, seven (representing three families) showed histological evidence of chytridiomycosis, including *Ametia umbraculata*. Although this does not prove that the chytrid fungus was responsible for the *A. umbraculata* deaths reported in 1998, it seems a plausible explanation as chytridiomycosis epidemics often occur during periods of drought and affect high-altitude species (Berger et al. 1998; Pounds et al. 1999; Berger et al. 2000). An investigation following a mass die-off of *Amietia (Afrana) fuscigula* on the west coast of South Africa revealed that all of these frogs were infected by *B. dendrobatidis* (Du Preez unpubl. data).

As this parasite exhibits low host-specificity and is therefore likely to infect any amphibian species (Speare et al. 2001), the number of infected species and geographical extent of the disease in South Africa are probably underestimated at present. Available data indicate that chytridiomycosis has been present in South Africa longer than in any other country in which it occurs and it is therefore possible that the fungus originated in Africa (Weldon et al. unpublished data). Its prevalence in *Xenopus laevis*, for example, appears to be stable over time and it produces little or no apparent clinical symptoms in this species. The scientific trade in *X. laevis* provides a feasible means whereby the parasite could have spread from South Africa to other parts of the globe (Weldon et al. unpublished data). Comparisons of DNA from South African strains with strains from Australia and the USA are under way to determine the origin of *B. dendrobatidis*. Recently, the Office International des Epizooties

(OIE) accepted amphibian chytridiomycosis as a disease requiring quarantine restrictions on amphibians moved between countries. South Africa is a member state of the OIE and a major supplier of *X. laevis* for scientific research facilities in many countries. There is, therefore, an urgent need to investigate and quantify the risks involved in exporting these frogs, both to protect the trade and to prevent the spread of disease to native frog populations elsewhere in the world (Weldon and du Preez 2004).

Climate change

In a preliminary assessment of climate change in South Africa, Midgley et al. (2001) noted that higher temperatures are predicted over the whole of South Africa. Summer rainfall will decrease by between 5% in northern regions and 25% in the south. Within the next 50-100 years, the biomes as we currently know them (including the Fynbos, Succulent Karoo, Grassland and Forest biomes) may be reduced to 35-55% of their present extent. The northern arm of the Fynbos Biome may disappear altogether and fires may become more frequent and extensive in fynbos and could disrupt many of the close and essential relationships between indigenous plants and animals. The impact of predicted future climate change in South Africa will be most devastating on the semi-arid Succulent Karoo and adjacent karroid regions. Although these areas have a relatively depauperate amphibian fauna, the Succulent Karoo is a minor centre of endemism.

Moreover, assessment of the response of many species is hindered by a basic lack of information on their biology and distribution patterns. Although there are reports citing increased pollution in river catchments as a contributing factor to amphibian declines, the impact of these activities on the long-term health of amphibian populations is unclear, in part because of a lack of long-term research on amphibian populations inhabiting specific areas such as the Kruger National Park (KNP).

Conservation efforts to protect the planet's vertebrate diversity have been disproportionate for the various groups and have tended to favour mammals and birds. The so-called 'lower vertebrates' such as fish, amphibians and reptiles; generally have a lower public appeal and are typically neglected in conservation programmes, yet these groups are of fundamental importance at an ecosystem level. In terms of species richness, amphibians outnumber mammals with more than 4700 living species currently recognised and with an expected total exceeding 5000 (Glaw and Köhler 1998). Ironically, at a time when taxonomists are unravelling and describing this richness at an unprecedented rate, alarming reports of amphibian population declines and species extinctions are being recorded around the world. Amphibians are proportionally the most threatened group of vertebrates (Branch 1988).

Amphibians are an important component of South Africa's exceptional biodiversity (Siegfried 1989) and are such worthy of both research and conservation effort. Amphibian populations are declining throughout the world (Kiesecker et al. 2001; Blaustein and Kiesecker 2002). It has become clear that declines cannot be attributed to any single causative factor and those complex mechanisms involving abiotic and biotic interactions are responsible for this phenomenon (Blaustein and Kiesecker 2002). These declines have been attributed to a combination of factors, including climate change, chemical pollution, habitat loss and disease (Blaustein et al. 2003).

The majority of frogs use wetlands for breeding, and many are found in or near bodies of water outside the breeding season. In South Africa 88 of the 105 (84%) described species use wetland habitats. In South Africa, 19 frog species are permanent residents of wetlands or surrounding areas, 60 use wetlands for breeding and feeding during the rainy season, and 17 don't use wetlands (Channing and Van Dijk 1995). Destruction of essential aquatic breeding habitats negatively impacts on certain frog species and may act synergistically with factors such as habitat deterioration and fragmentation resulting in population declines. Wetland and riverine habitats are biologically diverse and productive areas that support numerous species of both aquatic and terrestrial organisms and are critical habitats. Frogs appear to be particularly vulnerable to population losses. Most South African frogs are terrestrial, with an aquatic larval stage associated with a freshwater system.

1.2   Aim and Objectives

The main aim of this project is to determine the current status of the amphibian biodiversity in the Kruger National Park wetlands. Most of the pans are rain fed and therefore critical habitat and require special attention.

The study objectives are to:

- Identify and quantify physical factors such as habitat alteration that may influence amphibian distribution.
- Identify and quantify chemical factors such as metals and pH of the rainwater and water in the pans and rivers that may influence amphibian distribution.
- Determine the current distribution pattern of amphibians in the different wetland systems (pans) and the major rivers basins of the KNP.
- Evaluate current biodiversity and distribution against historical distribution.
- Determine the effects of chemical pollution on the biology of amphibians through histopathology, bioaccumulation and bio-marker (geno-toxicity) studies.

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2   STUDY SITES

2.1   Introduction – site selection

Historically, frog surveys in the Kruger National Park (KNP) were done on a limited scale. Historic records are reflected in Addendum 2.1 (Kruger National Park records) and Addendum 2.2 (South African Frog Atlas Project (SAFAP) records). The records for the KNP reflect collections by rangers and scientific staff and the area or site description of where the specimens were collected. The sampling for the South African Frog Atlas Project was conducted between 1996 and continued into 2003, a period of more than seven years (the atlas period). In addition to data collected during the atlas period, data that predated 1996 were accessed from several institutions and from the field records and databases of individuals.

For the study, the project team met with Dr Andrew Deacon (previously of Scientific Services, SANParks) and Dr Harold Braack (Consultant) to discuss the possible sites that can be used during the study. A preliminary survey was conducted in February 2009 and a reconnaissance trip was carried out. The survey started in the north of the Kruger National Park and all sections were visited. Section Rangers were approached and the possibility of sites discussed. One aspect that was used for the criteria in the selection was accessibility to the pan. In most cases this was achieved, but a few exceptions were made to ensure that the widest possible distribution of pans was achieved. During initial discussions it was decided that 40 pans (summer survey sites) and 10 rivers (winter survey sites) will be used. After two visits to the park, the final number of sites selected was 45 summer sites (mostly pans) and 14 winter sites (mostly rivers and streams).

Although the initial idea was to use pans for the summer survey and rivers for the winter surveys some exceptions were made. Apart from ensuring a good spread of sites throughout the park, this was done to accommodate additional historic frog "hotspots" or areas of high species diversity. These were added after discussions with members from the reference committee. The exceptions were Xipudza, Bangu, Nshawu and Nkaya Pan (Satara) which were used during the summer and winter surveys as study sites. Although Xipudza is a spring fed stream, it hosts both summer and winter breeders and it was considered an important site in the northwest of the park. Bangu was mentioned as an important site in the central area near the eastern border of the park. A low weir was present at the site and the pools below the weir (fed by seepage from the weir) again hosted summer and winter species. The large floodplain wetland associated with the Nshawu area is regarded as an important area and its sheer size have different habitats that a variety of frog species can

use throughout the year. This site was selected to determine if both groups of breeders can use it. A similar approach was applied in Nkaya Pan to the south of Satara.

Sites were selected across a diversity of geological and vegetation types across the entire park (Figure 2.1). The selected sites were overlaid onto the ecoregion, geology and vegetation maps used by the Kruger National Park (Figure 2.2-2.4). Another criteria used during the selection of sites was to ensure that sites represented different types of wetlands (grass pans, pans with vegetation on the fringes and pans with no vegetation in the fringes of the pans). The surface area of the pans vary and this was another criteria used, as it was important to see if the small water bodies are used and what diversity can be associated with it (Addendum 3.1). The eight pans selected in the Tshokwane area were used for the more detailed studies (Chapter 5 and 6) and again the geological regions were used during the selection. One of the sites is an old borrow pit and although it is not a classical pan, it was used to determine which species will utilise this type of habitat, as there are a large number of these artificial wetlands distributed throughout the park. It will be valuable to study such a site and how species diversity can change if it is rehabilitated.

The coordinates for each site was recorded (summer and winter sites). In addition the circumference and depth profile were determined and this information was used to calculate the surface area and volume of each pan (summer sites only). At each of the summer survey sites, four 10 m wide transects were laid out along the northern, eastern, southern and western axes. These transects were 50 m long and were used to determine the available cover and habitat for frogs (Chapter 3).
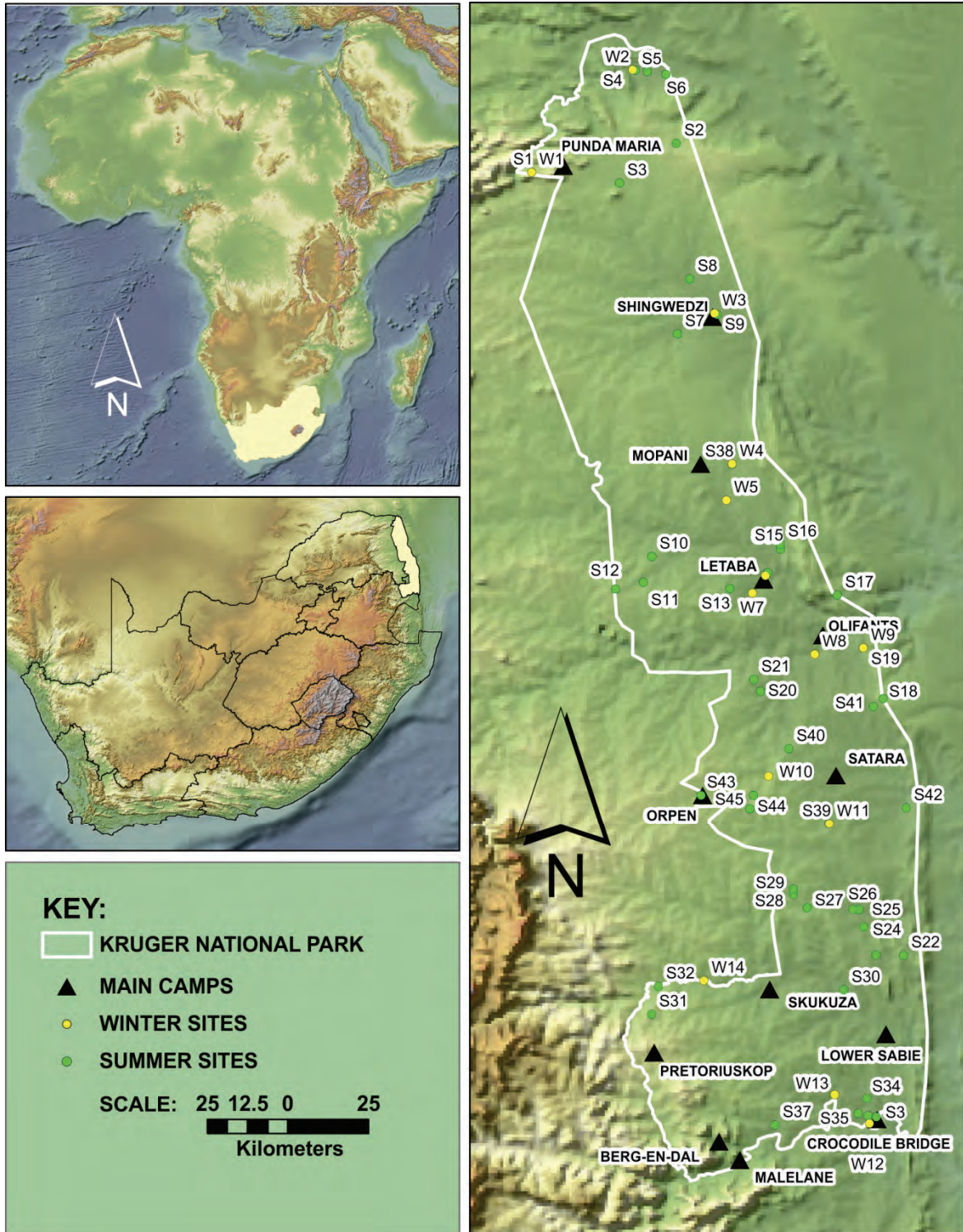
Figure 2.1: Sites selected for the project. (S1-S45 are the summer survey sites and W1-W14 are the winter survey sites).
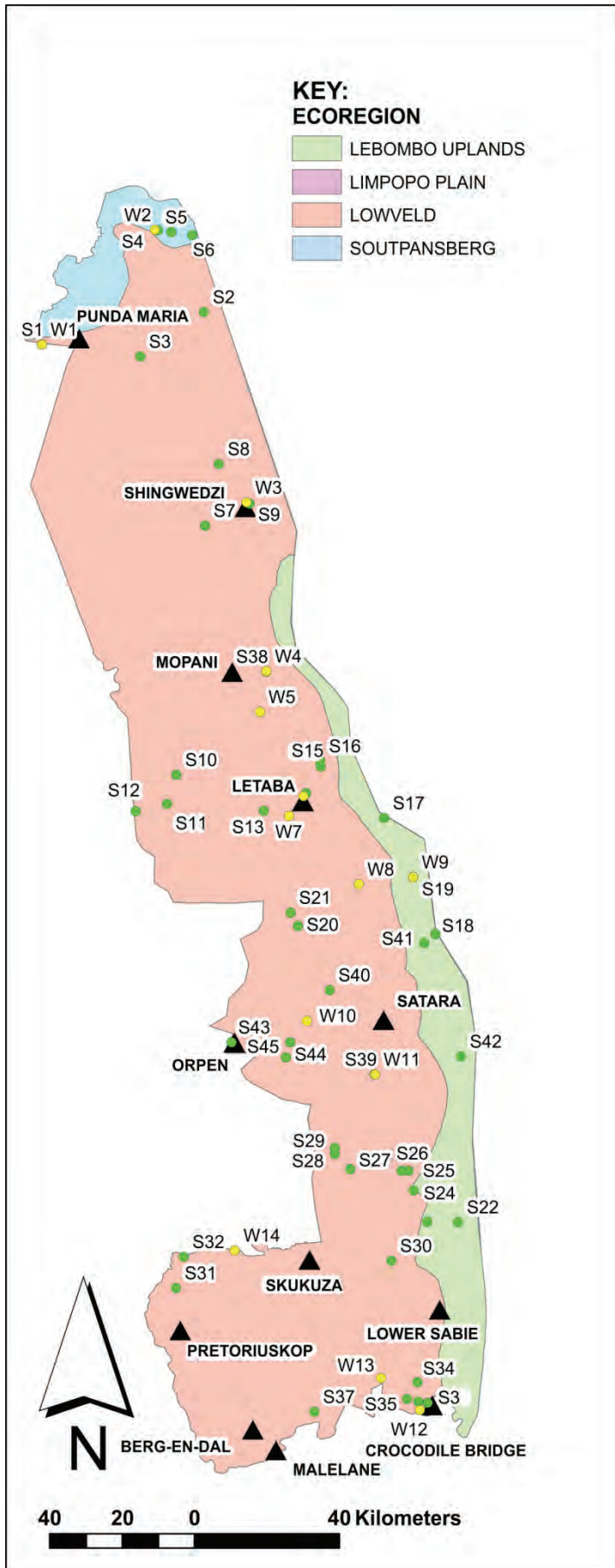
Figure 2.2: Survey sites overlaid on the Ecoregion map (KNP division) of the Kruger National Park.
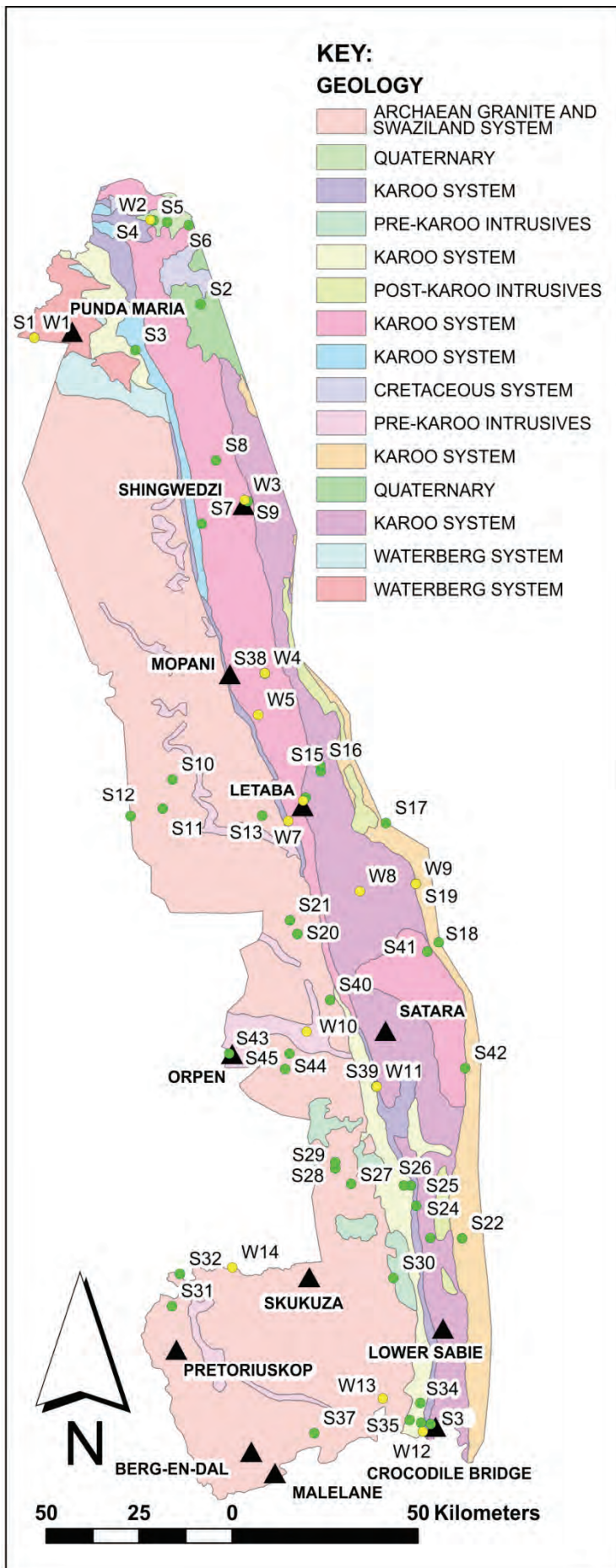
Figure 2.3: Sites overlaid on the geology map of the Kruger National Park.
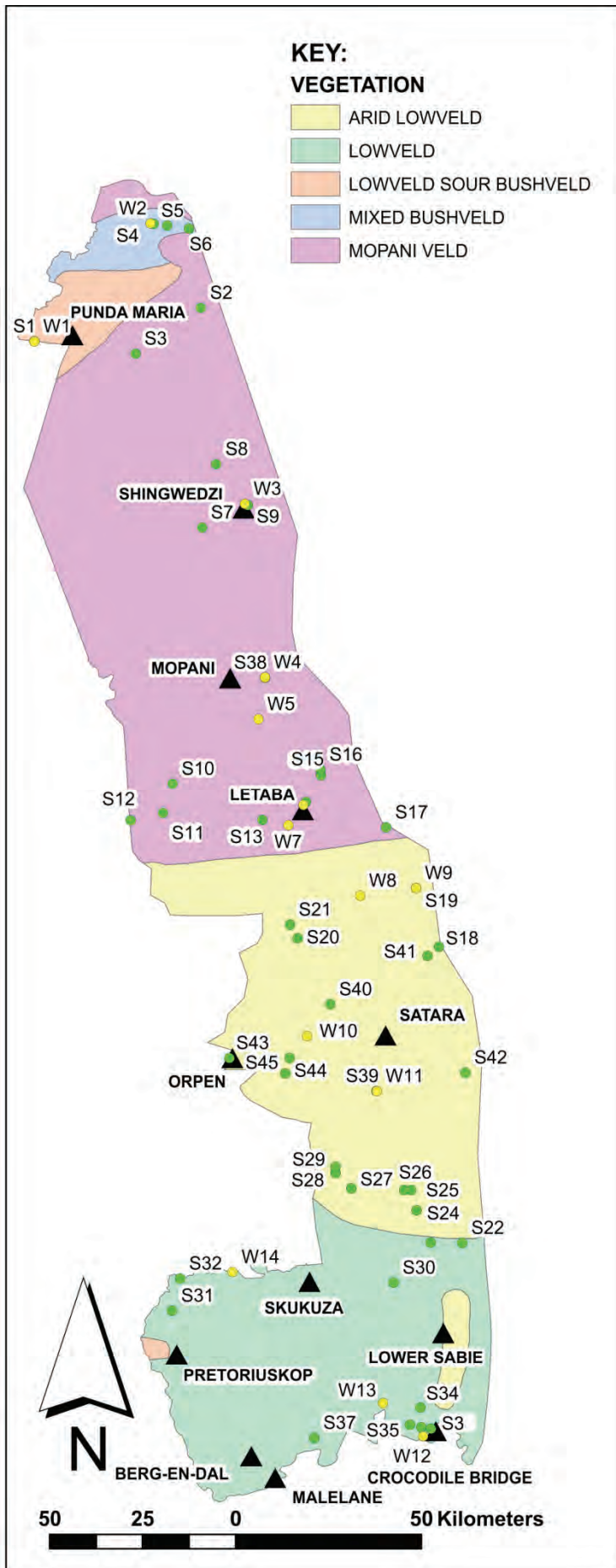
Figure 2.4: Sites overlaid on the vegetation map of the Kruger National Park.

2.2   Summer survey sites (Addendum 2.3)

A short description of each site is listed in Addendum 2.4. In addition, a general photograph will be used to illustrate the site, but additional photographs are included as Addendum 2.5. The photograph used in the chapter will be referred to as (a) with two or three additional photographs of the site. These will include a general view of the site and some of the biotopes present. The last photographs represent the four transects that were used during the plant and basal cover surveys.

The ecoregion, geology and vegetation maps (Figure 2.2-2.4) are the maps used by the Kruger National Park and it was used to correlate the information from this project to specific management areas and strategies.

2.3   Winter survey sites (Addendum 2.6)

A short description of each site is listed in Addendum 2.7. In addition, a general photograph is used to illustrate the site, but more photographs are included as Addendum 2.5. The first photograph in the folder is the one used in this chapter as a general illustration of the site. The others represent some of the habitat present and the last pictures will show the stream/river banks.

The reference to the ecoregion, geology and vegetation (Addendum 2.6) are the classification used by the Kruger National Park (Figure 2.2-2.4).

3   THE PHYSICAL STRUCTURE AND RELATED VEGETATION OF THE PANS

3.1   Introduction

The term "'pan" is a generic term used in South Africa to describe a wetland type that consists of a shallow depression, or basin, and that is usually a closed, or endorheic, system. Commonly the shape of a pan is ovoid but can also be "clam", "kidney" or "pork chop"-shaped (Goudie and Wells 1995) when two or more pans have spread and combined (Allan et al. 1995). The size of pans vary greatly from a few meters to a few hundred meters in diameter (Johnson and Rogers 2003) and the depth is usually less than three meters even when fully inundated (Cowan and Van Riet 1998).

Precipitation is the principal source of water for isolated systems (Brooks 2005) and water can enter a pan either directly from the atmosphere or via surface run-off. Although not as visible the linkage to groundwater and subsurface lateral drainage is as important sources (Shaw and Bryant 2011). In floodplain pans, *juxta* positioned to water courses, the filling occurs as overland lateral spill when the watercourse overtops its banks (Denny 1993). The water loss of pans can be mainly attributed to direct evaporation and leaching although vegetation transpiration also contributes (Brooks 2005). Allan et al. (1995) emphasized that the existence of pans depend entirely on the water regime with factors such as rainfall intensity, evaporation rate and groundwater level that influence the duration of inundation. Accordingly the residence time of water, or "hydroperiod" of pans will vary from permanent to temporary and even to dry in any given year depending on the climate and the ability of a system to retain water. With regard to water retention the physical structure, or shape of a pan, as well as the permeability of the underlying sediments, play a crucial role (Semeniuk and Semeniuk 1995). Although some permanent pans exist many of the pans in South Africa have inundation periods which can be described as ephemeral and in general pans are principally regarded as ephemeral and sporadic (Meintjies et al. 1994). The latter situation applies in particular to the dry western regions of the country, where some pans are dry for years between flooding events, while the eastern wetter regions have pans that are more permanent with some sufficiently large and permanent enough to be classified as lakes (Cowan and Van Riet 1998).

Pans can become very turbid after rainfall events and saline through time (Masing et al. 1990). Although it is often stated that pans are restricted to lowlands or plains (Masing et al 1990), they are not uncommon on ridge tops or crests of ridges.

3.2 South African pans

3.2.1 Distribution of pans in South Africa

As far as distribution is concerned, pans are a common feature of the western, drier parts of South Africa where the highest concentrations are in the Kalahari region of the Northern Cape and in the western Free State (Breen et al. 1993). Shaw (1988) states that the highest concentration of endorheic pans are found in areas with a mean annual rainfall of less than 500 mm and an average net evaporation of 1000 mm per annum. Despite their relation to low rainfall regions pans also occur in the eastern regions of the South Africa below and above the escarpment (Ashton and Schoeman 1983; Rogers et al. 1989; Goudie and Wells 1995).

3.2.2 Pan terminology

Globally a vast array of terms is used to describe aquatic systems which in essence are depressions in the landscape, and which are akin to the pans of South Africa. These similar systems are located in a variety of climates and have fundamental structural and functional characteristics corresponding to their South African counterparts. These include vernal pools (Zedler 1987), playas (Briere 2000; Parker et al. 2010), prairie wetlands (Bolen et al. 1989), prairie potholes (Keddy 2000), ephemeral wetlands (Johnson and Rogers 2003), barlkarras (Semeniuk and Semeniuk 1995), billabongs (Bunn and Boon 1993), sabkhas (Yechieli and Wood 2002), hemi-arid lake basins (Currey 1994), storage floodplains (Noble and Hemens 1978) and dambos (Denny 1993).

Depressions in a landscape can be the result of various activities which *inter alia* include tectonic activity, volcanic activity, meteorite impact, karstic solution, thermokarstic subsidence, glacial excavation and aeolian (wind action) erosion (Shaw and Thomas 1989 ; Goudie and Wells 1995).

In the low rainfall regions of South Africa, several conditions can contribute to pan formation and Goudie and Wells (1995) stated that the following conditions are necessary in order for depressions to form:

- The areas must be arid,
- The area should not be one where fluvial processes are fully integrated and
- The area should be one where wind driven accumulation does not result in the infill of any irregularities in the land surface.

It should however be noted that aridity need not in every case act as a predisposing condition for pan development as is indicated by the numerous pans in the Lake Chrissie

district of South Africa which characterized by high rainfall (Ashton and Schoeman 1983). In southern Africa factors that have been have been known to lead to the development of pans include salt weathering, wind deflation and animal activity such as grazing, wallowing and trampling (Goudie 1991). Allan et al. (1995) emphasized the role of wind action which contributes to the development of low mounds, also known as lunette dunes, beyond the shorelines on the leeward side of pans.

Soil permeability is an important factor determining the hydroperiod of pans. The influx of inorganic material in the form of sand, silt and clay into depressions results in the accumulation of sediment on the floor of the depression. The nature or composition of the deposit will determine the permeability and consequently the hydroperiod. Although the deposit composition varies between pans it is common that all pans will have a relatively impervious substrate that permits the collection of surface water for a period. Often this layer may be cemented by calcium or magnesium carbonates creating a floor or "hardpan" which decreases permeability. In addition the hardpan can act as a barrier to plant roots and can be the reason why vegetation may be absent or sparse at some pans (MacVicar 1991). The soil horizons, or layers, made up of an assortment of grain sizes, mineral substances and organic materials, differ from pan to pan and Jenny (in Venter et al. 2003) stated that no two soil horizons are identical because each is a unique product of the local parent material as well as aspects such the climate and slope. In addition the soil horizon will differ laterally away from the centre, an aspect caused by the very nature of the pan's physical structure.

When a pan receives water it will infiltrate into the soil, mainly due to gravity, filling the air voids between constituent particles. This will continue until the saturation rate exceeds the infiltration rate leading to a cessation of vertical flow of water into the sediments (Cox and McFarlane 1995) and thereby causing water accumulating to occur above ground (Denny 1993). The surplus water remains in the depression until it infiltrates or it is removed through evaporation or uptake by plants or animals.

3.2.3   Chemical characteristics of pans in South Africa

Concentric drainage due to pan basin shape (Figure 3.1) leads to material and minerals from the surrounding landscape accruing in the pans and concentrating in the centre of the pan (Parris 1984). Because of the organic content of the sediments pans are often referred to as "nutrient sinks" as they accumulate nutrients in the long term (Howard-Williams 1984). The velocity and volume of run-off entering the pan basin determines the quantity and characteristics of suspended matter (Whitford 2002). After rainfall events pans can become turbid due to sediment load. When the sediments settle the chemical constituents are

trapped sediments and bacteria, fungi and protozoa can break them down to simpler molecules which other organisms can utilise or are otherwise lost to the atmosphere (Malan and Day 2005). As a result Bachelor et al. (2002) refer to pans as "nutrient rich systems that cycle and transform chemical constituents". It is important to note that the capacity of a system to store nutrients is finite (Howard-Williams 1984) and therefore the fact that a pan operating as a nutrient sink, transformer or source needs to take the inputs and outputs to the system, as well as the ability of the system to assimilate and process any quantity of chemical constituents entering the pan under various conditions over time into account.

It should be considered that pans can also accumulate pollutants. Metals commonly precipitate and bond to sediments under aerobic conditions. During periods of inundation soils tend to become anoxic or anaerobic and under these conditions metals can be released into the water column and can become available to organisms which can lead to deleterious effects (Malan and Day 2005).

Classic biogeochemical processes associated with wetlands include nitrification and de-nitrification, nitrogen fixation, nitrogen mineraliszation, nitrogen volatilization, phosphorous precipitation, phosphorous adsorption and absorption, ferrolysis, gleying, sulphur reduction, fermentation of organic carbon and methanogenesis (Mitsch and Gosselink 1986). In arid landscapes pans are critical biogeochemical cycling stations where seasonal and temporary pans undergo fluctuating conditions often switching from inundated to desiccated stages. The alternating dry and wet phases drive the biogeochemical processes taking place in the water column and the substrate. In this context pans can be regarded as biogeochemical "hot spots" (McClain et al. 2003).
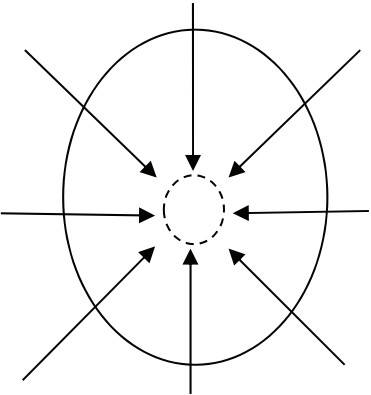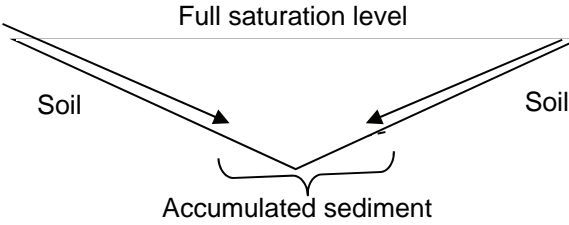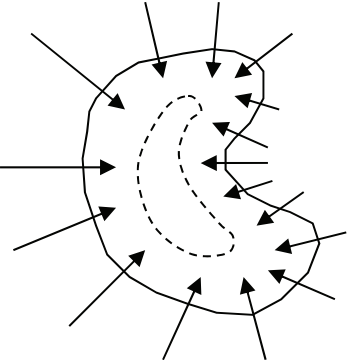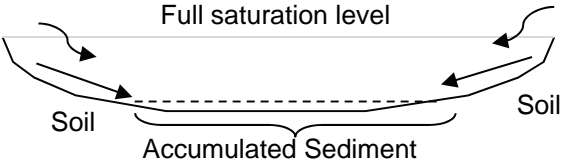
Aerial view	Cross-sectional view
1a) Concentric drainage into ovoid shaped pan wetland.	1b) Concentric drainage into ovoid pan wetland with conic shaped basin.
2a) Concentric drainage into kidney shaped pan wetland.	2b) Concentric drainage into pan wetland with a parabolic shaped basin.

Figure 3.1: Conceptual aerial and cross-section views of concentric drainage in ovoid shaped (1a) and kidney shaped (2a) pans with parabolic-shaped (1b) and cone-shaped (2b) basins. Arrows indicate the direction of flow and the dashed lines indicate the concentrated zone of sediment accumulation (Parris 1984).

The majority of information available on the physico-chemical conditions of pans in South Africa stem from research conducted in the drier central regions such as the Orange Free State (Meintjies 1996) and the Northern Cape (Meintjies et al. 1994). However studies have also been conducted on the water chemistry of pans in the south Western Cape (Silberbauer

and King 1991) and Western Cape (Coetzer 1981) and to a lesser extent on the pans of Gauteng (Ashton and Schoeman 1983; Rogers et al. 1989) and Mpumalanga (Batchelor et al. 2002). Others studies provide more a general synthesis on pans countrywide (Seaman et al. 1991; Day 1993) but it is important to note that the focus is on water quality in pans while little is known about the chemistry of the sediment.

The water chemistry of pans is highly variable and can change considerably over short and long periods of time, with a pan changing freshwater to saline conditions, making these ecosystems unpredictable (Meintjies et al. 1994). The characteristic temporal and spatial variability and diversity of pans are regarded as important requisites that determine biodiversity (Rogers et al. 1989). The major cation composition of salt pans studied by Seaman et al. (1991) were found to be dominated by sodium, while the concentration of other cations such as magnesium, calcium and phosphorous varied. Conversely the most common anion was chloride followed by sulphate and carbonate or bicarbonate respectively. This was confirmed by Day (1993) who showed that pans with saline water are dominated by sodium chloride, while freshwater pans are dominated by sodium or a combination of sodium and calcium with carbonate and sulphate prevailing as the dominant anions. Knowledge of the water and sediment chemistry is important as it not only determines the species of organisms that could potentially be present but has implications for the utilisation and management of these wetlands (DWAF 1999).

3.2.4 Vegetation characteristics of South African pans

According to Parris (1984) vegetation establishment in and around pans is a function of the physical gradients of the basin and the chemical gradients in the soil. Venter and Gertenbach (1986) stated that the distribution of vegetation is determined by a combination of soil properties and the genetic ability of the plants to overcome the biogeochemical and water availability obstacles. Based on the latter the hydrological gradients play a crucial role, as vegetation will vary depending on the hydroperiod, with species increasingly tolerant of flooding established closer to the centre of the wetland (Brooks 2005). The fluctuation of the water level in less permanent pans maintains the plant diversity with different species, each with their specific levels of tolerance, present in dry and wet phases (Rogers et al. 1989). The influence of nutrient enrichment cannot be excluded in the context of vegetation establishment as it influences both species composition and productivity (Howard-Williams 1984) and the seasonal influx of nutrients can therefore, have a major influence on vegetation (Coetzer 1987).

Vegetation studies mirror those on water chemistry with the majority of research done on the drier central regions of South Africa (Geldenhuys 1982) followed by work in Mpumalanga (Allan 1987) and the Highveld regions (Rogers et al. 1989; Allan et al. 1995) while other research projects included pans country-wide (Cowan and Van Riet 1998). In these studies various approaches, using a range of parameters, were employed to classify vegetation. These parameters included aspects such as *inter alia* the nature of hydrophytic vegetation, presence of vegetation, spatial distribution of vegetation, relative abundance of vegetation, degree of inundation, vegetation physiognomy, species composition of vegetation and salinity. It can be accepted that some parameters may be more applicable in specific instances than in others, since specific climatic conditions have a suite of factors that apply.

Although the abovementioned studies resulted in different classification systems it was found that pans could be divided into two main groups, namely those that were bare and those that to some extent were vegetated. This vegetation mainly included short grasses, reeds and sedges. There was also consensus that pans have lower plant diversity which is ascribed to the high degree of variability of pans and specifically to relatively short hydroperiods and the variable water chemistry when compared to other wetland types. However Allan et al. (1995) recorded a total of 97 different species in the Highveld region, with pans in the east and the west of the area showing the highest diversity with 81 and 58 species respectively. This high diversity could be associated with the lower degree of variability of the highveld when compared to the arid central and south western areas of the Orange Free State where pans are inclined to be drier and more bare (Geldenhuys 1982).

Semeniuk et al. (1990) proposed a generic vegetation classification system for basin wetlands that potentially could be applied apply to vegetated pans in South Africa. This classification system is based on a number of parameters namely a) the areal extent and pattern of distribution of vegetation cover, b) the internal organisation of that vegetation, c) the predominant vegetation structure or the range of structural types in zones and d) the floristic details (Semeniuk et al. 1990). Based on vegetation cover and internal organisation total nine categories were identified by Semeniuk et al. (1990) (Figure 3.2). It should however be noted that the vegetation of pans has the propensity to change distinctly from one season to the next (Rogers et al. 1989) which leaves this classification system open to question particularly in relation to its long-term application to pans.
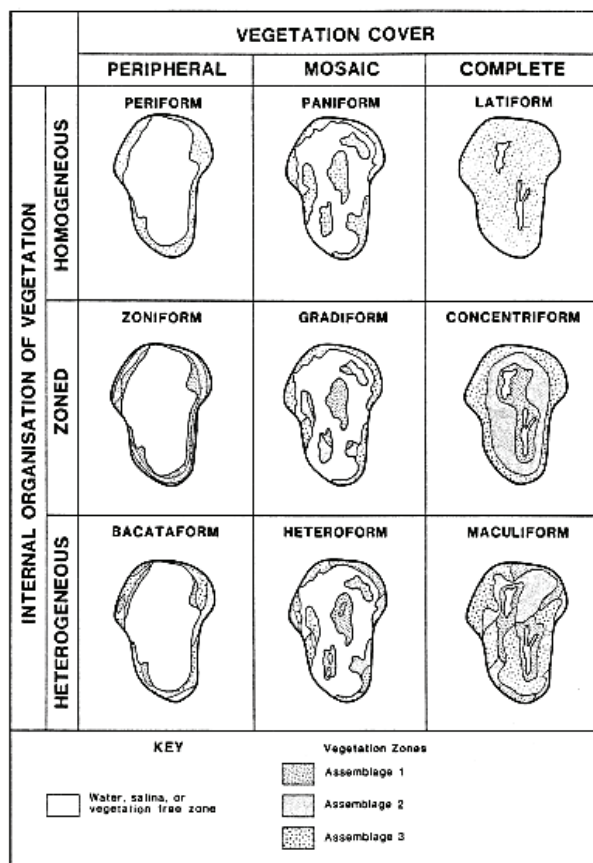
Figure 3.2: The nine categories of vegetation organisation and cover within basin wetlands as proposed by Semeniuk et al. (1990).

Table 3.1: Direct and indirect physical impacts threatening wetlands (Malan and Day 2005).

Direct Impacts
- Drainage for agriculture, forestry, mosquito control.   - Poorly managed grazing, mowing and burning.   - Flooding due to construction of dams.   - Dredging for navigation, flood control.   - Filling for solid waste disposal, road construction, residential, commercial and industrial development.   - Erosion.   - Groundwater abstraction.   - Discharges of pollutants from point-sources, diffuse sources (agriculture, informal settlements, urban areas), air pollution.   - Mining of wetland soils for peat, coal, gravel, phosphate, salts and other materials.
Indirect Impacts
- Sediment diversion by dams, channels roads and other structures.   - Hydrological alterations by canals, roads and other structures.   - Subsidence due to extraction of groundwater, oil, minerals.

3.3 Potential threats to pans

The threats to wetlands as listed by Malan and Day (2005) shown in Table 3.1 are applicable to pans. With an excessive inflow of nutrients, in particularly nitrogen and phosphates, pans are prone to eutrophication while drainage from mines and salinisation pose further threats (Malan and Day 2005). Since pans tend to accumulate sediments they are potentially vulnerable to sediment pollution (Cowan 1995). Because pans can act as a sink for clay-sized particles, with their relatively high adsorption ability, more pollutants can potentially enter the system (Coetzee 1995). Sediment pollution has serious implications for biota as contaminated sediments can serve as a source and route of entry into food webs where organisms absorb or consume toxic substances.

The impact of climate change has recently attracted added attention and Abrahams (2008) stated that climate change is expected to cause a significant change in the hydrology of lakes, reservoirs and other wetlands. This has been investigated by applying hydrological modelling techniques in conjunction to various predicted climate change scenarios (Pyke 2005). Evidence supporting these theories is increasingly coming to the fore with Hulmans et al. (2008) showing that ephemeral aquatic ecosystems are hydrologically sensitive to precipitation patterns, thereby enhancing the potential sensitivity of these ecosystems to future climate change. This is a concerning aspect regarding pans in the Kruger National Park since the climate is projected to become significantly warmer with an estimated two to six degrees centigrade (°C) and an uncertainty associated with future rainfall trends which could be up to 20% higher or lower (Venter et al. 2003).

3.4 The ecological importance of pans

Pans constitute distinct habitats or subsystems of the broader ecosystem and form an important and integral part of the greater landscape. As such, pans can enhance the overall ecosystem by increasing the spatial heterogeneity of the environment and by providing key habitats for species (Parris 1984). The management and conservation of pans is therefore, both necessary and vital

The importance of wetlands as habitat is often stressed with Cowan (1995) pointing out that due to the highly variable nature of inundation of South African wetlands, wetlands of no apparent importance may become essential habitats at certain times. This is especially applicable to pans which when inundated provide a valuable water resource for wildlife in regions where water scarcity prevails. Breen et al. (1993) indicated that the outstanding value of pans lies in providing areas for wildlife feeding and breeding where the seasonal provision of roosting areas for vast flocks of water birds should be noted. Whigham (1999)

argued that pans may be more valuable than other types of wetlands due to the important landscape and biodiversity functions they perform, such as the provision of habitat for migrating birds, improvement of water quality and maintenance of high biodiversity. Whitford (2002) points out that in arid landscapes ephemeral waters are essential for the long-term survival of amphibians.

Notwithstanding the acknowledgement of the importance of pans one of the main problems remains the lack of knowledge regarding their physical characteristics and its relation to frog biodiversity. The aim of this component of the project was therefore to determine the physical characteristics of selected pans in the Kruger National Park and relate this to frog biodiversity.

3.5   Methodology

To determine their physical characteristics the pans that formed part of the study were surveyed once during the dry season. Where possible the vegetation was assessed in seasons when active growth occurred in order to enable the identification of species.

3.5.1   Physical dimensions of the pans

The outer perimeter of the pan is regarded as point of the pan basin profile where evidence of the highest water level was evident. The maximum depth and maximum surface area occurs when shallow depression wetlands hold water up to the overflow depth.

Pan circumference

A Garmin 500 GPS was used to record waypoints along the outer most perimeter, or perimeter of each pan at intervals of approximately two meters in order to delineate the outer most boundaries. In larger pans with the intervals were extended to 5 meters. These waypoints were then imported into Garmin MapSource (version 6.13.6) and the distance tool was used to calculate the circumference and surface area of the pan.

Pan depth and basin profile

It is important to note that this was not a detailed bathymetric survey of the pan basin profile but rather a rapid survey to assess the basic pan basin profile. Transects across the length and width of each pan were identified and the number of transects used were determined by the size of the pan with more transects selected in the larger pans and vice versa. In general, two or three transects were selected. A theodolite (Figure 3.3) was used to measure the depths along each transect. The theodolite was set up at the perimeter of the pan at the start of each transect and the height of the front end of the theodolite (H_1) measured. Heights were then measured and recorded, at five meter intervals along the

transect. During calculations H_1 was deducted from each value to determine the actual depth at each point. For each pan a profile was drawn and the results graphically presented.

A dimensionless profile coefficient (*p*) is used to represent the average basin shape or profile of a pan. When the basin shape is an inverted cone with a straight slope profile from the margin to the deepest point, (*p*) is regarded as 1. A value less than 1.0 indicates a basin with a generally convex profile while a value greater than 1.0 indicates a generally concave to parabolic basin profile (Hayashi and van der Kamp 2000; Brooks and Hayashi 2002).

The drawn profile of each pan was studied and a *p* value awarded by applying the methodology of Hayashi and van der Kamp (2000) as is shown in Figure 3.4. Because it was observed that the surveyed pans were shallow it was decided to end the scale at a maximum value of five.

Figure 3.3: The theodolite and measurement staff used during measurements of pan depths in the Kruger National Park.
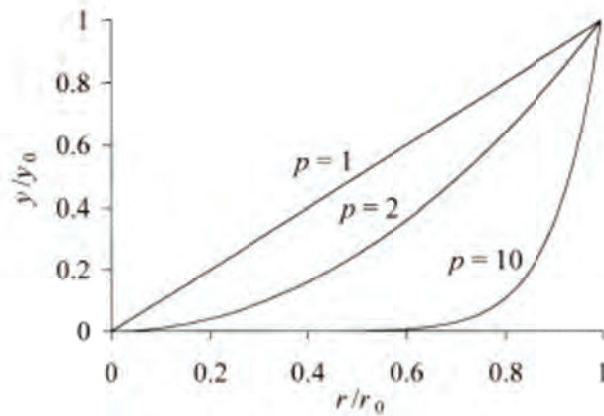
Figure 3.4: Graph illustrating an averaged basin profile for symmetric basins where *y/y0 = (r/r0)* to the power of *p* with a dimensionless constant of 1, 2 and 10 respectively (Hayashi and van der Kamp 2000).

Pan size and volume

According to Hayashi and van der Kamp (2000) simple power functions can be used to approximate area-depth and volume-depth relations for shallow depression wetlands. However, the equations dealing with volume used did not explicitly contain maximum pool depth, which is presumed to be an important parameter for pool hydroperiod and Brooks and Hayashi (2002) then adapted the formula which was then used in this study:

$$V_{max} = \frac{A_{max} \times d_{max}}{1 + 2/p}$$

Where:

V_{max} = the volume of water

d_{max} = maximum depth

A_{max} = the area of water surface and

p = the pan profile coefficient.

3.5.2   Sediment characteristics

Two sediment samples were collected at each pan in polypropylene zip lock bags. Each sample consisted of a number of smaller subsamples taken at random points in the pan basin. The one sample was used for chemical analyses of which the results are reported separately in this document. The second was used to determine the sediment characteristics as indicated below.

In the laboratory the sample was removed from the bag and thoroughly mixed in a plastic dish. A subsample was then removed and placed in pre-weighed glass beakers. The combined weight of the sample and the glass beaker was determined after which it was placed in an oven at 60° C for 72 hours to dry. The dried samples were then weighed and the difference between the two samples were recorded and expressed as a percentage and represents the moisture content.

To determine the organic content in the sediment three sub-samples were taken from the dried samples and placed in pre-weighed crucibles and the total weight determined. The crucibles were then incinerated in a furnace at 600° C for six hours after which the weight of the samples was determined. The weight difference was then expressed as percentage of the dried mass and the average for each pan calculated. The organic content of each sample was then classified according the characteristics listed in Table 3.2 (USEPA, 1991).

Table 3.2: Classification categories of organic content, based on the percentage organic content, in sediment samples (USEPA 1991).

Calculated percentage	Category
< 0.05%	Very low
0.05-1%	Low
1-2%	Moderately low
2-4%	Medium
> 4%	High

In order to establish the grain size composition of the sediment, a sub sample of the dried samples of each pan was sieved through an Endecott sieve system with mesh sizes ranging from 53µm to 4000µm. Each fraction collected by the individual mesh sizes was weighed and expressed as a percentage of the dried mass and categorised according to Table 3.3.

Table 3.3: Classification categories of grain size categories for sediment samples (Cyrus et al. 2000).

Grain size	Category
> 4000 µm	Gravel
4000-2000 µm	Very coarse sand
2000-500 µm	Coarse sand
500-212 µm	Medium sand
212-53 µm	Very fine sand
< 53 µm	Mud

3.5.3   Vegetation assessment

The extent of basal cover, as well as woody species diversity and population structure, was assessed along four transects oriented north, south, east and west of each pan. Each

transect was 50 m long and started at the pan perimeter. The length of transect was marked by rolling out a tape measure and the width extended five metres to each side resulting in a transect 50 m long and 10 m wide. The extent of basal cover was assessed and rated according to the characteristics listed in Table 3.4 at each five meter interval starting at the perimeter (0 meters). At each interval the woody species in the preceding five meters were identified using the keys provided by van Wyk and van Wyk (1997) and Schmidt et al. (2007), counted and their heights estimated. During the assessment additional cover, such as termite mounds or dry woody material, at each interval were identified. For statistical analyses the average basal cover ratings for each interval was calculated. In addition the type and cause of disturbances were recorded and the vegetation cover in the pan basin assessed and classified into the three main categories shown in Figure 3.2.

Table 3.4: Rating of the extent of basal cover.

Estimated basal cover percentage	Rating
0%	0
0-20%	1
20-40%	2
40-60%	3
60-80%	4
80-100%	5

3.5.4   Relating physical pan structure and vegetation to frog diversity

The environmental variables' role in explaining a frog species' presence or absence were analysed with generalised linear models in R (R Development Core Team, 2011). The GLM was done with alogit and the binomial family of distributions. Vegetation cover in the pan was analysed as a factor variable. Model selection was done with the command 'step' and evaluated with the command "Anova" in the "car" package. Although difficult to assess, model performance was assessed by calculating R^2 values.

3.6   Results

3.6.1   Pan profiles

The profiles of each pan drawn using the depths measured with the theodolite along these transects (Addendum 3.1) are shown with an example of a resulting profile shown in Figure 3.5.
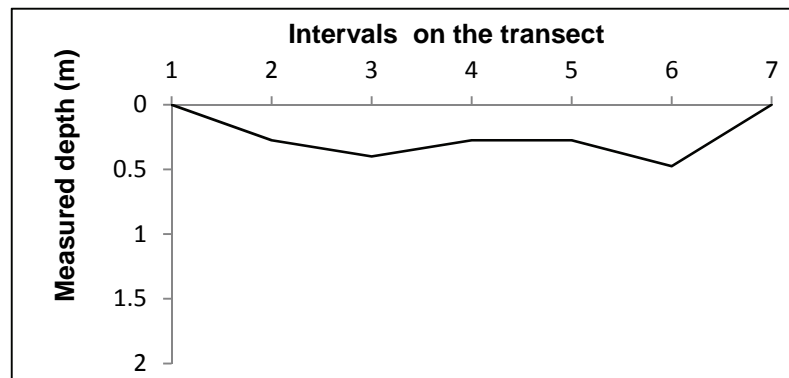
Figure 3.5: The profile, based in the measured depths along, transect 1.of Zombe Pan (S16) in the Kruger National Park.

3.6.2 Pan dimensions

Table 3.5 shows the measured circumferences and calculated surface areas and volumes as well as the awarded p value of the pans. Table 3.5 shows that the average of surface area of the pans was of 3775 m^2 but that it ranged from the smallest, N'watimbuti (S15), at 109 m^2 to the largest, Maroela (S43), with a surface area in excess of 3 ha. The average depth of the pans was 0.6728 m with Nkaya Pan (S39) pan was the deepest, at 1.275 m, while the shallowest pan was Shulungwane (S32) at 0.15 m.   The volumes of the pans varied with Maroela Pan the largest at an excess of 11,000 m^3 and Shulungwane the smallest at a mere 11 m^3. The dimensions of pans S1, S19 and S38 are not shown as they are classified as rivers rather than pans.

3.6.3 Sediment characteristics

Organic content

Table 3.6 shows that with the exception of Shipukuyila and Airstrip Pans (S13 and S14) the organic content of the sediments were classified as high. It is however the organic contents of pans S21, S31, S33 and S34, where the organic contents exceeds 20%, that should be noted.

Particle size

The composition of the sediment of the pans (Table 3.7) varied considerably with 67 % of the pans dominated by very fine sand or mud where the latter condition dominated in more than 50%. Particles larger than 2000 µm, which constitutes gravel, dominated in only three pans but the situation in pan S18 (Pumbe) where more than 70% of the sediment consist of gravel is of particular interest.

Table 3.5: The physical dimensions and characteristics of the pans surveyed in the Kruger National Park.

Site number/name	Circumference (m)	Surface area (m²)	Maximum depth (m)	Calculated capacity (m³)	Awarded p value*
S2 Malonga	221	1412	0.5	353.00	2
S3 Bullfrog	95	626	0.225	46.95	1
S4 Hapi West	221	3012	0.6	1084.32	3
S5 Hapi East	295	3597	0.55	1187.01	3
S6 Hlanganani	1000	11594	0.95	6608.58	3
S7 Shingwedzi West	161	1027	0.35	119.82	1
S8 Shingwedzi North	156	1712	0.3	171.20	1
S9 Shingwedzi Camp	253	2747	0.9	1483.38	3
S10 Oorgenoeg	143	1246	0.525	392.49	3
S11 Ngwenyeni	71	282	0.815	153.22	4
S12 Lochaka	84	243	0.75	109.35	3
S13 Shipukuyila	262	4841	0.775	2251.07	3
S14 Air Strip	133	998	0.775	464.07	3
S15 N'watimbuti	58	109	0.575	31.34	2
S16 Zombe	154	717	0.525	225.86	3
S17 Ramiti 2	146	1208	0.35	253.68	3
S18 Pumbe	320	7657	0.35	1339.98	2
S20 Mujatu	282	5142	1.075	3685.10	4
S21 William's Pan	63	285	0.5	85.50	3
S22 Rietpan	453	12216	1.05	7696.08	3
S23 Sabie gravel pit	242	3331	0.7	1554.47	4
S24 Seribye	341	7412	0.82	3646.70	3
S25 Lannea	388	6627	1.1	4373.82	3
S26 Munywini	209	1426	0.65	617.93	4
S27 Hildebrandtia	159	567	0.4	136.08	3
S28 Lugmag	173	1216	0.8	583.68	3
S29 Nhlanguleni	213	1228	0.5	368.40	3
S30 Onder Sabie	93	555	1	333.00	3
S31 Famehlo	179	1161	0.85	657.90	4
S32 Shulungwane	77	220	0.15	11.00	1
S33 Giraffe	112	826	0.75	371.70	3
S34 Randspruit	166	825	0.3	165.00	4
S35 Soswanini	163	1283	0.65	500.37	3
S36 Crocodile Bridge	148	1033	1.25	860.83	4
S37 Gardenia	203	1511	1	1007.33	4
S39 Nkaya	223	3687	1.275	3133.95	4
S40 Tshokweni	427	13257	0.65	5170.23	3
S41 Nkaya (Pumbe)	349	5743	0.75	2153.63	2
S42 Sweni	116	516	0.175	30.10	1
S43 Maroela	1000	34706	0.55	11452.98	3
S44 Majekejekeni	259	4497	0.825	2473.35	4
S45 Phelwana	400	6259	0.675	2112.41	2

Table 3.6: The organic content of the sediments collected from pans in the Kruger National Park.

Pan number and name	Mass of dried sample (g)	Mass of organic content (g)	% organic content	Classification of the organic content
S1 Xipudza	38.4332	1.9465	5	High
S2 Malonga	26.6941	1.696	6	High
S3 Bullfrog	19.4153	0.989	5	High
S4 Hapi West	34.1206	3.6093	11	High
S5 Hapi East	30.9874	3.3014	11	High
S6 Hlanganani	18.7325	2.4313	13	High
S7 Shingwedzi West	19.7254	1.7384	9	High
S8 Shingwedzi North	29.5868	4.0078	14	High
S9 Shingwedzi Camp	30.0754	2.2059	7	High
S10 Oorgenoeg	32.9776	2.7252	8	High
S11 Ngwenyeni	34.5463	3.3961	10	High
S12 Lochaka	22.7646	1.3426	6	High
S13 Shipukuyila	44.0737	0.8719	2	Medium
S14 Air strip	24.2899	0.9902	4	Medium
S15 N'watimbuti	30.4716	1.6179	5	High
S16 Zombe	33.2098	3.3979	10	High
S17 Ramiti 2	32.0657	3.625	11	High
S18 Pumbe	19.9984	3.6185	18	High
S19 Bangu	48.9728	3.0712	6	High
S20 Majuta	30.7279	1.6758	5	High
S21 William's Pan	20.7377	4.4074	21	High
S30 Onder Sabie	30.3393	3.1608	10	High
S31 Famehlo	18.0844	4.332	24	High
S32 Shulungwane	14.3969	1.9109	13	High
S33 Giraffe	13.4931	2.816	21	High
S34 Randspruit	5.0117	1.2881	26	High
S35 Soswanini	13.3137	1.755	13	High
S36 Crocodile Bridge	22.7783	3.3611	15	High
S37 Gardenia	22.9447	1.653	7	High
S38 Nshawu	30.8082	5.3298	17	High
S39 Nkaya	29.3644	3.0766	10	High
S40 Tshokweni	26.9603	1.6349	6	High
S41 Nkaya Pumbe	23.351	2.7108	12	High
S42 Sweni	21.3164	2.6865	13	High
S43 Maroela	22.4808	2.6362	12	High
S44 Majekejekeni	29.9203	1.893	6	High
S45 Phelwana	28.2677	3.6196	13	High

Table 3.7: The classification of the sediment based on particle size of the pans surveyed.

Site number and name	Classification of particles				
	Gravel	Coarse sand	Medium sand	Very fine sand	Mud
S2 Malonga	5.2	28.8	14.8	41.4	9.8
S3 Bullfrog	3.9	16.8	20.4	46.2	12.7
S4 Hapi West	0.5	20.9	23.4	35.4	19.8
S5 Hapi East	4.3	14.6	7.4	37.3	36.4
S6 Hlanganani	2.0	24.2	8.4	31.0	34.5
S7 Shingwedzi West	0.8	12.3	11.1	46.8	28.9
S8 Shingwedzi North	2.2	15.3	16.2	39.2	27.1
S9 Shingwedzi Camp	3.7	9.8	10.0	32.8	43.8
S10 Oorgenoeg	6.9	29.5	12.3	23.9	27.5
S11 Ngwenyeni	5.6	20.4	17.1	49.3	7.6
S12 Lochaka	30.6	23.8	12.9	22.7	10.0
S13 Shipukuyila	7.3	19.9	17.1	6.4	49.2
S14 Air strip	31.0	3.6	5.6	26.2	33.6
S15 N'watimbuti	30.9	8.1	6.1	18.0	37.0
S16 Zombe	16.5	13.3	8.0	16.6	45.6
S17 Ramiti 2	2.6	29.8	10.0	22.9	34.7
S18 Pumbe	75.4	6.6	2.0	3.1	12.9
S20 Majuta	24.3	15.3	7.5	26.7	26.2
S21 William's Pan	18.2	22.9	8.1	9.5	41.4
S22 Rietpan	29.1	35.3	15.1	12.5	7.9
S23 Sabie gravel pit	17.0	42.9	18.8	17.0	4.3
S24 Seribye	14.4	30.5	16.6	32.7	5.7
S25 Lannea	14.9	12.0	17.6	50.1	5.4
S26 Munywini	2.9	9.9	17.8	59.7	9.7
S27 Hildebrandtia	3.3	46.5	31.5	15.9	2.9
S28 Lugmag	6.9	52.7	21.3	15.6	3.4
S29 Nhlanguleni	6.4	54.9	20.3	13.5	5.0
S30 Onder Sabie	4.7	26.3	10.7	16.5	41.7
S31 Famehlo	1.6	21.5	20.8	22.9	33.3
S32 Shulungwane	3.5	28.4	12.8	14.8	40.5
S33 Giraffe	0.3	8.8	5.1	26.1	59.8
S34 Randspruit	5.5	26.2	11.0	14.9	42.5
S35 Soswanini	4.0	27.7	10.9	16.3	41.0
S36 Crocodile Bridge	6.7	24.4	14.0	11.2	43.6
S37 Gardenia	2.9	15.0	11.0	6.0	65.2
S39 Nkaya	29.7	14.1	10.2	27.9	18.2
S40 Tshokweni	24.4	27.9	9.8	26.0	11.9
S41 Nkaya Pumbe	12.5	13.7	10.1	10.0	53.6
S42 Sweni	2.6	14.8	16.1	11.9	54.5
S43 Maroela	0.9	25.8	8.2	14.7	50.4
S44 Majekejekeni	9.9	28.1	13.3	10.7	38.1
S45 Phelwana	7.5	22.7	12.9	9.8	47.0

3.6.4   Vegetation

"In-pan" vegetation

Table 3.8 shows that pans where the "in-pan" vegetation cover could be classified the "mosaic" organisation dominated, followed by pans with a "peripheral" organisation with 44% and 40% respectively. Only seven pans, or approximately 16 %, displayed a "complete" vegetation cover organisation

Vegetation related to pans

The complete results of the basal cover assessment, the woody plant distribution and population structure are shown in Addendum 3.2. The average of the ratings of all four transects at each interval was then calculated and this is graphically represented as shown in the example in Figure 3.6. In addition the presence of identified woody species in each transect interval is also shown in Addendum 3.2. These two aspects, namely the extent of basal cover and the presence of woody species, are recorded for each interval in order to represent the availability of cover in 5 m wide concentric areas around the pan. This cover in the concentric areas, or available frog habitat, was used to statistically determine the relationship with frog biodiversity.
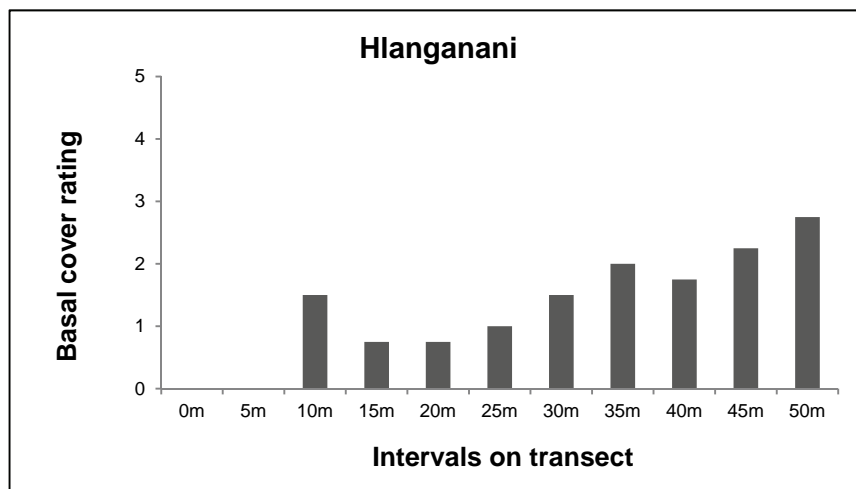
Figure 3.6: The average extent of basal cover at five meter intervals of these transects at Hlanganani Pan (S6) in the Kruger National Park.

Table 3.8: The classification of the "in-pan" vegetation cover organisation as observed in the Kruger National Park.

Site number and name	Peripheral	Mosaic	Complete
S2 Malonga		✓	
S3 Bullfrog		✓	
S4 Hapi West	✓		
S5 Hapi East	✓		
S6 Hlanganani	✓		
S7 Shingwedzi West		✓	
S8 Shingwedzi North			✓
S9 Shingwedzi Camp		✓	
S10 Oorgenoeg		✓	
S11 Ngwenyeni	✓		
S12 Lochaka	✓		
S13 Shipukuyila			✓
S14 Air strip	✓		
S15 N'watimbuti		✓	
S16 Zombe	✓		
S17 Ramiti 2			✓
S18 Pumbe		✓	
S20 Majuta		✓	
S21 William's Pan	✓		
S22 Rietpan			✓
S23 Sabie gravel pit	✓		
S24 Seribye		✓	
S25 Lannea		✓	
S26 Munywini	✓		
S27 Hildebrandtia	✓		
S28 Lugmag	✓		
S29 Nhlanguleni		✓	
S30 Onder Sabie	✓		
S31 Famehlo	✓		
S32 Shulungwane		✓	
S33 Giraffe		✓	
S34 Randspruit	✓		
S35 Soswanini	✓		
S36 Crocodile Bridge	✓		
S37 Gardenia		✓	
S39 Nkaya	✓		
S40 Tshokweni			✓
S41 Nkaya Pumbe		✓	
S42 Sweni		✓	
S43 Maroela			✓
S44 Majekejekeni		✓	
S45 Phelwana		✓	

During the project a total number of woody species, both trees and shrubs were identified. The list of these plants is attached in Addendum 3.3. The population structure of the woody species is also shown in Addendum 3.2 both as physical counts of the specimens observed and in graphic format.

3.6.5 The relationship between physical pan structure, vegetation and frog diversity

A preliminary investigation of the relationship between the abovementioned aspects showed that in general the lowest frog diversity, with scores of five species and lower occurred in pans with a peripheral or mosaic in-pan vegetation organisation with less than 60% basal cover in the first ten meter concentric area around the pan (Table 3.9). The sediments of these pans were in general dominated by fine material. The exceptions on this were Bangu (S19), which is a river with a well-established riparian zone and gravel and coarse sand dominating the sediment as well Ngwenyeni Pan (S11) where the basal cover exceeded 60% in the first ten meters. Where higher diversity is concerned the trend observed was not as clear. It was observed that the pans with highest diversity, of nine and higher mostly had a mosaic or complete "in-pan" vegetation organisation and in general the basal cover in the first ten meter concentric area around the pan exceeded 60% (Table 3.10). Although pan size did not seem to correlate with diversity note should be taken that in the group with lowest diversity a number of pans with area smaller than 600 m^2 were observed while in the high scoring group no pan was smaller than 600 m^2.

Statistical analyses of the relationship between selected frog species and environmental variables showed that the best relationship were obtained between maximum depth and additional cover in the case of *Phrynobatrachus mababiensis, Ptychadena anchietae* and *Hildebrandtia ornata.*

Table 3.9: Summary of the relationship between frog diversity and physical aspects at pans with a low diversity.

						Pan numbers						
	S4	S8	S10	S11	S12	S15	S19	S30	S31	S32	S41	S42
In pan vegetation cover	Peripheral	Complete	Mosaic	Peripheral	Peripheral	Mosaic	Mosaic	Peripheral	Peripheral	Mosaic	Mosaic	Mosaic
Ave. basal cover 1st 5 m	1	2	1	4	2	3	4	3	1	3	2	2
Ave. basal cover 1st 10 m	1	2	2	4	2	3	3	2	2	3	3	2
Gravel and coarse sand	-	-	36	-	54	-	60	-	-	-	-	-
Very fine sand and mud	55	66	-	57	-	55	-	58	56	55	64	66
Total surface area (m²)	3000	1700	1246	282	243	109	River	555	1160	220	5700	516
Frog diversity	5	3	5	5	5	4	4	2	5	4	5	5

Table 3.10: Summary of the relationship between frog diversity and physical aspects at pans with a high diversity.

				Pan numbers					
	S1	S2	S3	S5	S13	S18	S34	S35	S40
In pan vegetation cover	Mosaic	Mosaic	Mosaic	Peripheral	Complete	Mosaic	Peripheral	Peripheral	Complete
Ave. basal cover 1st 5 m	5	3	4	1	2	4	1	4	2
Ave. basal cover 1st 10 m	5	3	4	1	2	3	2	4	2
Gravel and coarse sand	46	-	-	-	-	82	-	-	52
Very fine sand and mud		51	59	74	56	-	57	57	-
Total surface area (m²)	River	1412	626	3500	4800	7657	825	1283	13300
Frog diversity	9	9	10	9	9	9	10	10	10

Table 3.11: Generalised linear model (binomial error distribution, logitlink function with quasi-likelihood estimation in the case of over dispersion) outcomes for the relationships of environmental variables on species presence of frog species in the 36 pans in the Kruger National Park. (PMAB : *Phrynomantis bifasciatus*, KSEN: *Kassina senegalensis*, HMAR: *Hyperolius marmoratus*, XMUE: *Xenopus muelleri*, PANC: *Ptychadena anchietae*, HORN: *Hildebrandtia ornate*, PMAB: *Phrynobatrachus mababiensis*).

Species	Peripheral	Mosaic	Complete	Basal cover (first 10 m)	Basal cover (first 25 m)	Other cover	Gravel	Coarse sand	Medium sand	Mud	Max depth	R²
KSEN	-	-	-	-	-	-	-	-	-	-	-	-0.39
PMAB	-	-	-	-	-	2.253	-	-	-	-	-5.361*	0.095
PBIF	-	1.685*	-	-	-	-	-	-	-	-	-	-0.15
HMAR	-	-	-	-0.904·	-	-	-0.072·	-0.132*	-	-	-	0.015
XMUE	-	-	-	-	1.844*	-	-	-	-	0.057·	-	0.07
PANC	-	-	-	-	-	-2.027*	0.056	-	-	-	-	-0.05
HORN	-	2.312	-	-	-	-	-	-	-	-	3.873*	-0.2

Estimates of coefficients and their significance, *, <0.05,

3.7   Discussion

The organic content of all the pans that formed part of the study were high which reflects their role as nutrient sinks within the environment as indicated by Bachelor et al. (2002). In addition it should be noted that all the pans showed evidence of a high amount of animal activity as many were not only used for drinking but as wallows that were frequented by elephants, rhinoceros, buffaloes and warthogs in particular. This emphasises the important role that these wetlands play in water stressed areas as was shown by Cowan (1995). These animals do not only contribute to the organic load of the pans but also assist with basin formation and pan shape and this latter aspect can be recorded as ways in which pans are formed in the study area. On the negative side the high frequency of use depletes the basal cover in particular at the edge of the pan and this can decrease the habitat availability. The ecological importance of pans, and in particular with regard to frog habitat, are also illustrated by the fact that in a relative shallow pan with a maximum depth of only 22 cm and with a small surface, such as Bull frog Pan (S3) ten frog species were recorded. In the same vein it should be noted that at the smallest pan, N'watimbuti (S15) with a surface area109 m^2 and an estimated volume of 31 m^3 provided habitat for four species of frogs.

The fact that in general the pans were small, with small surface area and volumes, emphasises their ephemeral characteristic as described by Whigham (1999). As these aspects directly relate to their short hydroperiod the available habitat in the adjoining areas is of extreme importance for the survival of the frogs during dry periods. It should be noted that in pans where basal cover exceeding 60 % in the first ten meters from the pan the frog diversity is in general the highest.

The composition of the sediments varied considerably between pans but in general it can be accepted that these sediments reflect the geology of the catchment of each pan in particular. Although the pan sediment was in general dominated by the finer material it important to note that all the pans had amounts of coarse material that could be utilised as cover by the tadpoles and sub-adults. With the exception of the Pumbe Pan, which is situated on the crest of a mountain, all the other pans are in low lying areas and valleys.

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4   WATER QUALITY AND SEDIMENT

4.1   Introduction

The water quality of inland depression water bodies such as pans is strongly influenced by both the mineralogy of the surrounding rocks and by fractionation due to mineral precipitation (Rosen, 1994). Lithology and in particular porosity and water-rock interactions is an important factor influencing the chemical composition of pans. Because pans are hydrologically closed basins, the salts will be determined by solute input and evaporative concentration. Therefore the chemical composition is the product of a series of interactions such as silicate hydrolysis, carbon dioxide uptake from the atmosphere, uptake of sulphate from oxidized sulphite, precipitation of alkaline compounds from solution and precipitation of solutes from rainwater (Rosen, 1994). According to Eugster and Jones (1979) these different interactions result in the water quality of water bodies that are influenced by evaporation to be dominated by three to four salt species.

A key aspect in freshwater ecology is the trophic state of freshwater ecosystems. When considering the trophic state of the pans it is important to include the nutrient concentrations. The concentrations of nitrates (NO_3^-), nitrites (NO_2^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) ions are important as these are the major nutrients that are involved in eutrophication of aquatic resources. According to Hutchinson et al. (1932) pans can be divided into two types of trophic states that are variations of eutrophic and dystrophic lakes. These variations are known as alkaline dystrophic types and saline eutrophic. Alkaline dystrophic waters are shallow and are formed by wind erosion, are often dark in colour (grey or brown) with very little light penetration, the pH is usually above 8, they are rich in suspended organic material from allochthonous origin and support very little organisms. Saline eutrophic waters are also shallow and occupy basins close to estuaries or those that have been formed by wind erosion. The water is turbid, with little light penetration and a pH above 7. The water is rich in organic material from both allochthonous and autochthonous origin. These lakes often support a variety of plants and animals in large numbers.

4.2   Materials and methods

Water and sediment samples

Water samples were collected in acid-washed polyethylene bottles from each of the study sites (Chapter two). The polyethylene bottles were rinsed with water from the pan before a sample was taken and after collection, these samples were stored at –4ºC until further analysis. Along with the collection of the samples, *in situ* measurement of the following variables were taken using a WTW 340i Multi meter: oxygen saturation (%), dissolved

oxygen concentration (mg/l), temperature (^{o}C), pH, conductivity (µS/cm) and Total Dissolved Solids (mg/l). The frozen samples were transported to the laboratory where they were allowed to defrost. A Merck Spectroquant Pharo 100 Spectrophotometer and relevant test kits were used to measure various chemical variables. These variables were: tot alkalinity, ammonium, chloride, nitrates, nitrites, sulphates, ortho-phosphates and turbidity. In addition water samples were analysed for macro elements (Ca, K, Mg, Na) and trace metals (Ag, Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn) using an ICP-OES and ICP-MS methods respectively. Prior to analysis 50 ml of water was filtered through 0.45 µm cellulose acetate filter using a Millipore (Millipore Corp., Bedford, MA) system.

Statistical Analysis

In order to assess the spatial and temporal variation in the water quality of the selected pans univariate and multivariate statistical analyses were carried out. Canoco version 4.5 was used to complete ordination of the sampling sites. The Principle Component Analysis (PCA) is based on a linear response model relating species and environmental variables (Ter Braak and Šmilauer, 2002; Van den Brink *et al*., 2003). Results of the ordination are a *map* of the samples being analysed on a 2 dimensional bases, where the placements of the samples reflect the (dis)similarities between the samples; in this case the sampling sites.

4.3   Results

General temporal characteristics

For the interpretation of the water quality of the pan wetlands in the Kruger National Park, the water quality data presented in Addendum 4.1 were subjected to different graphical and multivariate statistical analyses. Data on two sets of samples are presented. The wetland pans were sampled during the summer of 2010 and selected pans were sampled for a second time during the summer of 2011 (indicated by an "a" and "b" respectively). During the winter period the riverine habitats of frogs were sampled and these are indicated by W and the site number. There were two separate trends present with the winter, riverine samples displaying a positive relationship between water pH and TDS (Figure 4.1). This relationship was not present in the pan water samples with some pans displaying both high TDS concentrations and low pH values (Figure 4.1). This phenomenon is known to occur in temporary water bodies and similar finding were reported in pans of the Mpumalanga lakes district (Russell 2008; Ferreira 2010). According to Zhu and Anderson (2002) this can be attributed to degassing of bicarbonate when TDS starts increasing in response to lower volumes during evaporation to form CO_2, water and CO_3^-. Addendum 4.2 will have the figures in colour.
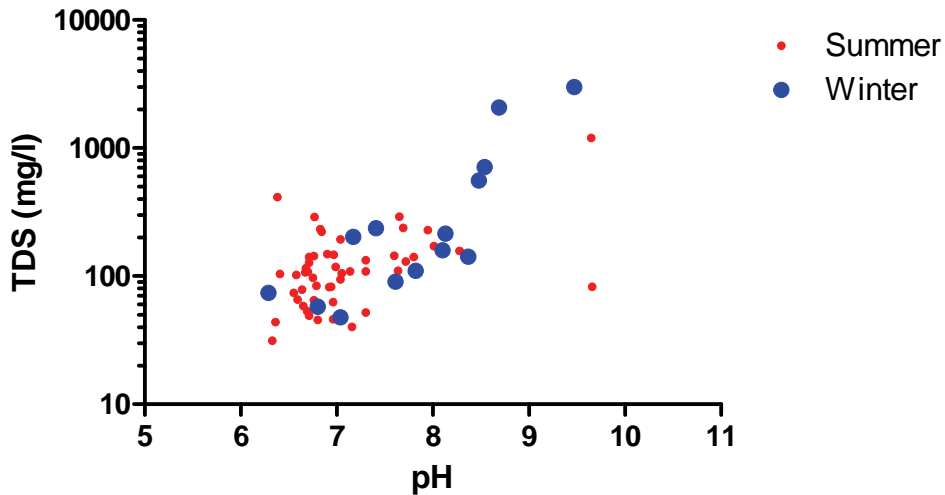
Figure 4.1: The relationship between pH and TDS (mg/l) in river water (winter) and pan water (summer).

These temporal relationships are also indicated in the PCA bi-plot of all the sites and water quality parameters (Figure 4.2). The ordination explains 62% of the variation in the data with the riverine sites (W1-W14) from the majority of the pans sites on the second axis due to higher pH and TDS / conductivity values.

General spatial characteristics

Graphical representations are often used to display differences or similarities in water quality between sites (Hem 1985). The graphical methods used during this study are PCA ordinations, Gibbs diagrams and Maucha diagrams. Based on the PCA ordination, three distinct spatial water quality patterns could be distinguished based on the physico-chemical data collected during this study (Figure 4.2). The first grouping was discussed in the previous section and consists of all the riverine sites and 7 pan sites. The water quality at these sites is dominated by high TDS concentrations, alkaline pH's and higher dissolved oxygen levels. The second grouping consists of pans situated in the central and southern regions of the park. The water quality of these pans is characterized by higher nutrient values and SO_4 concentrations. The third grouping displays higher dissolved metal concentrations, with the exception of the two common earth metals, Al and Fe.
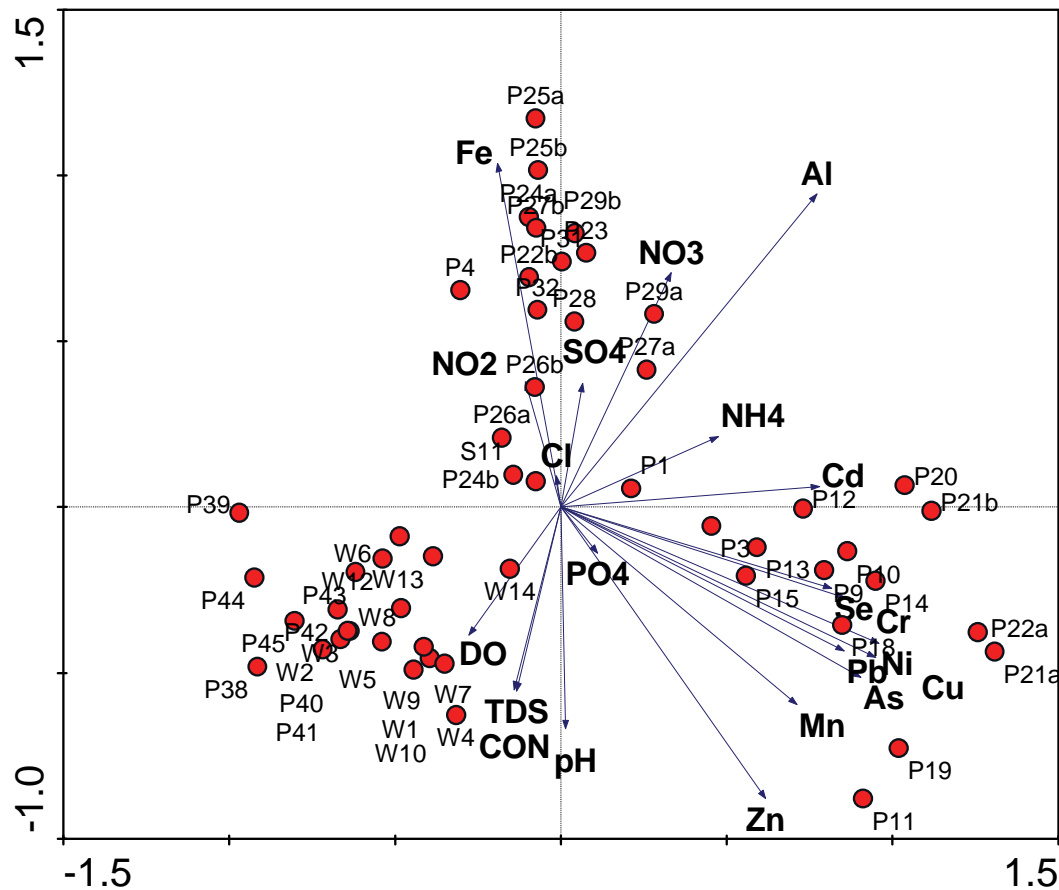
Figure 4.2: Principal component analysis bi-plot of the spatial and temporal water quality characteristics of 45 pan and 14 riverine sites in the Kruger National Park. The two axes explain 62% of the variation in the data (38% on PC1 and 24% on PC2).

4.4   Discussion

The geology of the study area and the chemical properties of the water have a major influence on the metal concentrations of the water (Russell 2008). The weathering of the shales of the Ecca Group can cause high concentrations of Na and SO_4. The salts that are formed with these two ions are very effective in the breakdown of rocks (Russell 2008). The pans with higher Na and SO_4 levels (Table 4.1) then also display higher dissolved metal concentrations (lower right hand quadrant in Figure 4.2).

The use of the nutrient status of pans as a classification system for pans has been proposed by Ferreira (2010). A PCA ordination bi-plot was constructed using only the nutrient, pH, conductivity and suspended matter concentrations (Figure 4.3). Although sampling was not specifically conducted to distinguish between alkaline dystrophic and saline eutrophic systems a fair measure of these characteristics could be observed. The pans in the upper two quadrants of the bi-plot could be regarded as displaying alkaline dystrophic

characteristics, whilst the bottom two quadrants represent pans with varying degrees of saline eutrophic characteristics.
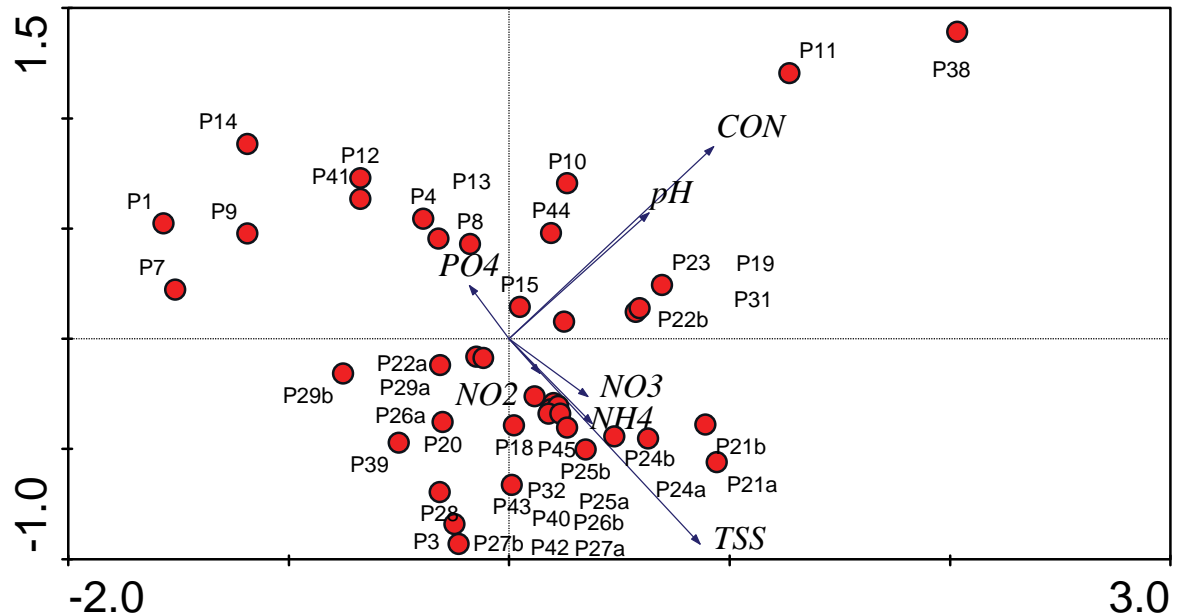
Figure 4.3: Principal component analysis bi-plot of the spatial and temporal nutrient, conductivity and suspended matter levels in 45 pan sites in the Kruger National Park. The two axes explain 78% of the variation in the data (48% on PC1 and 30% on PC2).

Gibbs diagrams are often used to distinguish between the three major mechanisms that control surface water chemistry, i.e. atmospheric precipitation, rock dominance and evaporation-crystallisation processes (Gibbs 1970). This is done using two diagrams. The first is based on cations with Ca and Na representing fresh and saline water bodies respectively. The second uses the major anions in saline (Cl) and fresh (HCO_3^-) as end-members. The water samples from the 45 pans that were studied are predominantly found within the central rock-dominated region and edge towards the upper right hand evaporation dominated region (Figure 4.4). This stands to reason as evaporation is the main mechanism for water loss from these systems.
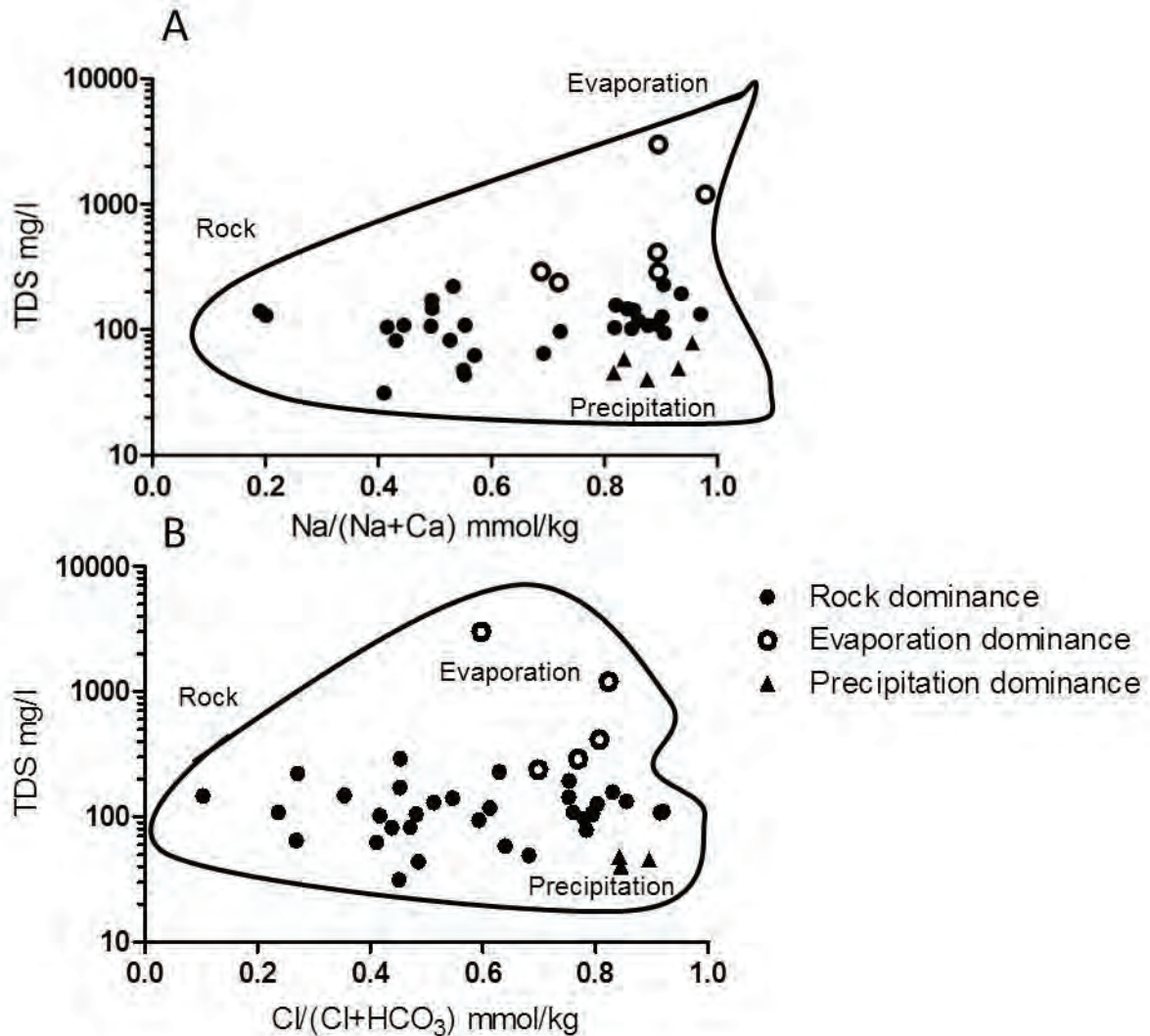
Figure 4.4: Gibbs diagrams of (A) TDS vs. Na/Na+Ca and (B) TDS vs. Cl/CL+ HCO3- showing the rock, evaporation and precipitation dominated water samples from the 45 pans studied.

The predominant source of dissolved salts or the majority of the pans appear to be the rocks and soils of their respective catchments. It is possible that those pans that show precipitation dominance are fed by springs from perched aquifers as they clearly have less rock influence on their salt composition.

The relative contribution of the different anions and cations to the totals dissolved salt concentrations in the pan water samples are displayed using Maucha diagrams. A Maucha diagram presents the ion concentrations in milli-equivalents per litre that allows for the balance of cations and anions to be viewed at a glance. Silberbauer and King (2001) successfully used Maucha diagrams to show spatial trends in water quality of wetlands in the south-western Cape, while Ferreira (2010) applied these diagrams to indicate the potential

influence of acid mine drainage on pans in Mpumalanga. In this study, Maucha diagrams were constructed for all 45 pans and 8 distinct ionic patterns were noted (Table 4.1). These progress from Group 1 that shows a natural freshwater state with calcium carbonate predominating (only one pan) to a state where the ionic composition is almost entirely dominated by SO_4 (Group 8 with 9 pans). In between there are varying changes of other cations, most notably Na. It is evident that the majority of pans are dominated by anions in the form of Cl (Groups 2 and 3) and SO_4 (Groups 4 to 8).

Based on the results from the Gibbs diagrams, it reasonable to assume that most of the salts are from geological sources (Seaman et al. 1991). Since the geology of the Kruger National Park is dominated by Karoo Super Group immature mineralogical composition is expected to be rich in feldspar, lithofragments and carbonate elements (Johnson et al. 2006). These rocks tend to weather easily and will be rich in cations such as Na, Ca, Mg and K. The lower Ca and Mg levels is attributed to loss through precipitation of calcite and its Mg-rich forms, while the higher Na and K are due to their involvement in fewer reactions (White et al. 2001). The Maucha diagrams indicate that SO_4 are an important contributing anion to the water chemistry in the majority of the pans that were studied. These findings are surprising since natural freshwaters usually contain low SO_4 concentrations with the only geological source being shales from formations such as the Vryheid Formation that is characteristic of rich coal ores (Pinetown et al. 2007). It is therefore likely that the high input of SO_4 into the pans could be the result of precipitation of acid rain. However Singer et al. (1999) do suggest that other processes that precipitate SO_4 rich complexes such as thenardite, bloedite, etc.

Table 4.1: Grouping of pans using Maucha diagrams with the particular Maucha pattern above the group. The key to the different components of the diagram is presented below the table.

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
P8	P11	P19	P4, P21b, P22a, P38	P1, P22b, P23, P24a, P24b, P25a, P25b, P26a, P26b, P27a, P2b, P28, P39, P41	P21a, P31	P3, P10, P14, P20, P40, P42, P44, P45	P9, P12, P13, P15, P18, P29a, P29b, P32, P43
High alkalinity, equal concentrations of Cl, SO_4, Ca, Mg	High Cl and Na concentrations	Predominantly Cl and Na with a high alkalinity	Na and Cl still high but SO_4 now dominant anion	Anions Cl and SO_4 dominant ions	Cl decreasing with increasing SO_4 dominance, high K	SO_4 dominated with increased alkalinity	SO_4 dominance, high Na

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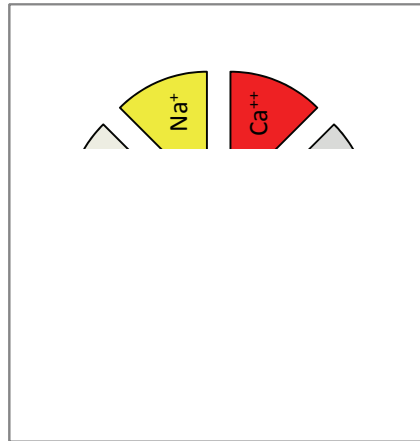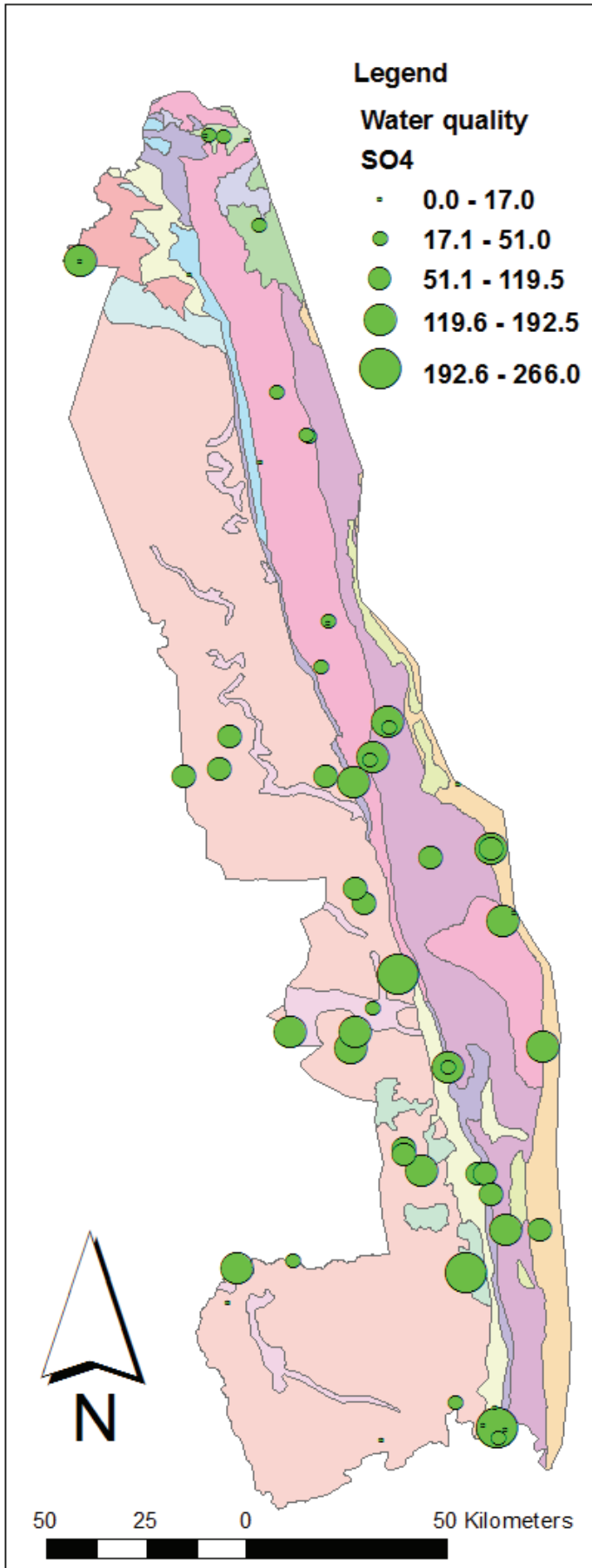
Figure 4.5: The presence of SO_4 at each site represented by the bubble – bubble size indicative of concentration in mg/l.

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5   FROG DIVERSITY AND CURRENT DISTRIBUTION IN THE KRUGER NATIONAL PARK

5.1   Introduction

The Kruger National Park is dominated by vast stretches of open woodland inter-dispersed by a varying grass layer. There are many environmental factors which influence the distribution and numbers of frogs in the Kruger National Park (KNP). These factors include the geographic position of the Kruger National Park, its basic geology and soils, vegetation and habitats, rainfall in both seasonality and abundance, the availability, distribution and duration of surface water, other climatic forces, fire and the impact of large herbivores.

The geological history of the Kruger National Park has in general terms resulted in the western half of the Park being constituted mainly by sandy granitic soil, while the eastern half consists of more clayey basaltic soils. In the southern half the granites and basalts are separated by a narrow strip of sedimentary soils (the Karoo sediments). The granitic areas are undulating in nature, while the basalts are flatter and plains-like. The western granites tend to support broad-leaved woodland and sour grasses on the crest of hills due to the very sandy nature of the soils there, and *Acacias* in the valleys where the clays and leached nutrients have accumulated over years. The eastern basaltic soils tend to support a good grass cover (particularly in wetter years) (Chittenden et al. 2008).

Rainfall is of course the main driver of the ecology of frogs as it influences both habitat quality and food availability. In the Kruger National Park the rainfall shows two distinct patterns or cycles. The first is the usual annual cycle in which the large majority of rain (85%) falls in the summer months between October and March. The first heavy summer rains are the stimulus for the annual breeding activities of the majority of frog species within the park. The second cycle is one driven by the effects of the El Nino Southern Oscillation (ENSO-commonly referred to simply as El Nino). El Nino is a global phenomenon which is driven by ocean-atmosphere interactions which alters the distribution of rainfall, causing floods in certain areas and droughts in others. In South Africa this usually manifests typically as a cycle of approximately 20 years in duration with 10 years of above average rainfall followed by 10 years of below average, though the durations are variable. Dry years may also be experienced in a wet cycle and wet years in a dry cycle. Typically, the wet years (wet cycles) are characterised by flowing rivers, full dams and pans, and an abundant grass biomass. Dry cycles have a depleted grass biomass through the grazing actions of herbivores, fires and termites (Chittenden et al. 2008).

In a study by (Kennedy et al. 2003) which was replicated in many parts of the KNP it was found that none of the 18 key grass species were lost following the 'severest drought in living memory'. The percentage abundance of these grasses declined during the drought to 87.5% of the pre-drought value. The relative abundance of species such as *Digitaria eriantha* declined while that of *Urochloa mosambicensis* increased, with the latter also spreading to new sites after the drought had past. Many of the major rivers cease to flow, and pools, pans and dams dry up. This has a detrimental impact on the breeding success of many species who deposit their eggs in the shallow ponds or pans which desiccate prior to the metamorphosis of their tadpoles. Desiccation of breeding sites is probably the greatest cause of mortality of frogs within the Kruger National Park.

The strong seasonality of rainfall in the KNP allows for plant material produced in the wet season to dry and be burned during the dry season. Fire has undoubtedly been an important factor in the savanna since the ascension of the grass layer to dominance. Fires are a natural phenomenon and are mainly a result of early summer thunderstorms. Average shrub and tree mortality of 1.3% has been quoted for 43 fires across a broad range of savanna areas in the Kruger National Park (Shea et al. 1996). Fires during the early winter or dry period can have a detrimental impact on the vegetation and on aestivating frogs under logs or stumps. Three candidate approaches to fire management have been put forward in the KNP; a lightning fire (letting nature take its course) approach; a patch mosaic burning approach which aims to establish a mosaic of vegetation structure types; and an approach based on the assessment of grass biomass and the species composition of the grass sword. A patch-mosaic system of burning is based on the premise that fire pattern is a surrogate for diversity, producing a range of patches in the landscape with unique patch characteristics and fire histories (Parr and Brockett 1999).

A total of 16 Vegetation units and 35 different Landscape Types have been observed within the Kruger National Park (Mucina and Rutherford 2006). This classification is too fine in regards to the frog distributions within the park. From an amphibian reproductive perspective the most fundamental of the habitats is the aquatic habitats. These include the major rivers, seasonal pools and pans, seasonally inundated grassland and the artificially created dams and waterholes. The more permanent rivers drain the bushveld hinterland and tend to support resident species within the riparian zones such as the Common River Frog (*Amietia angolensis*), Brown-backed Tree Frog (*Leptopelis mossambicus*) as well as Shovel-footed Squeaker (*Arthroleptis stenodactylus*). Several other species use seasonal pools, backwaters in major rivers on a temporary basis for breeding events such as the Flat-backed

Toad (*Amietophrynus maculatus*), Plain Grass Frog (*Ptychadena anchietae*) and Russet-backed Sand Frog (*Tomopterna marmorata*).

The savanna of the KNP provide valuable habitat for several large herbivores. The dependence on animals on plants has many wide-ranging effects on the plants and vegetation in the savanna. Elephants have the potential to literally shape the African savanna. Elephants clearly have an impact on the woody plant population. This is clearly evident where there are a few scattered trees outside an elephant-proof enclosure in the KNP and a more spread woody structure inside the enclosure without elephant (Trollope et al. 1998). The destruction of trees around the pans could potentially have a negative impact on arboreal species such as the Brown-Backed Tree Frog (*Leptopelis mossambicus*) and Southern Foam Nest Frog (*Chiromantis xerampelina*). A current study; as part of a broader research programme being run by SANparks Scientific Services looking at the impact of elephants on biodiversity; is being conducted by the Organisation for Tropical Studies (OTS) within the Kruger National Park (KNP). The focus of this particular study is looking at different frog communities in vegetation of different heights along the Sabie River. The idea behind this study is to see if areas with shorter vegetation and more open areas have different frog species composition compared to those sections of the river which have tall trees and a closed canopy.

There have been numerous studies on the nature of the piosphere effect where animals trample and utilise the vegetation in the vicinity of water points. There is an almost complete lack of woody plants in the vicinity of artificial watering points on Satara basaltic soils with a zone of high utilization of woody vegetation extending beyond this area (Brits et al. 2002). Borehole closure led to an increase in the relative abundance of decreased grass species, suggesting that the piosphere effects on the herbaceous composition may be reversible (Gaylard et al. 2003).

The majority of frog species within the Kruger National park are explosive breeders completing their short-duration reproductive cycles within ephemeral wetland habitats such as seasonal pans, seasonal pools and inundated grassland. The character of the pans differs substantially in different parts of the park. In the west they are shallow and typically circular or oval in shape with muddy banks exposed by the fluctuating water-level. In the wetter east the pans are surrounded by dense stands of emergent reeds and sedges, inundated grassland and copses of trees. Competition for suitable call sites results in different species occupying different zones of the pan. Some species such as the Plain Grass Frog (*Ptychadena anchietae*), Mozambique Grass Frog (*Ptychadena mossambica*) and Mottled Shovel-nose Frog (*Hemisus marmoratus*) call from exposed mud-banks whilst

others species such as the Banded Rubber Frog (*Phrynomantis bifasciatus*), African Bullfrog (*Pyxicephalus edulis*), Snoring Puddle Frog (*Phrynobatrachus natalensis)*, Bubbling Kassina (*Kassina senegalensis*), Golden Leaf-folding Frog (*Afrixalus aureus*) and Southern Ornate Frog (*Hildebrandtia ornata*) call from concealed positions within the seasonally inundated hygrophilous sedge and grass dominated littoral zones of the pan.

Other species like the Red Toad (*Schismaderma carens*) calls whilst floating in open water or amongst reeds. The Red-legged Kassina (Kassina maculata) calls from the middle of well vegetated pans. The partially submerged males use the floating and emerging vegetation to call from and readily duck underwater if approached. Several pans as well as rivers have dense reed beds which are favoured by the Painted Reed Frogs (*Hyperolius marmoratus*) and Tinker Reed Frog (*Hyperolius tuberilinguis*). The Water lily Frog (*Hyperolius pusillus*) calls from floating water lily pads (*Nymphae spp.*) and deposits it eggs between overlapping lily pads. Trees are used for calling sites for the arboreal frog species such as the Southern Foam Nest Frog (*Chiromantis xerampelina*) and Brown-backed Tree Frog (*Leptopelis mossambicus*). The Southern Foam Nest Frog (*Chiromantis xerampelina*) constructs foam nests on natural objects overhanging the water such as the branches of trees and shrubs, grass tussocks and rocks as well as artificial structures such as dam walls and bridges. Brown-backed Tree Frog (*Leptopelis mossambicus*) calls from exposed positions within trees and shrubs and deposits eggs in a shallow burrow under leaf litter next to the shallow pans, pools or streams (L.R. Minter pers. obs.).

The Lowveld region of South Africa lies at the convergence of three of southern Africa's climatic influences. These are mesic influences from the south, a more humid and tropical influence from the north, and an arid influence from the west. The spatial pattern in species richness is another important dimension when considering the biogeography of southern Africa. Poynton (1964) found that the amphibian fauna of South Africa appeared to be polarized into a tropical fauna and a south-western Cape fauna, with a large, but essentially heterogeneous assemblage of forms occupying the intermediate or transitional area. He determined that the 18°C July isotherm can be used to delimit the tropical faunal region, and the limits of the main portion of the Cape flora can be used to delimit the Cape faunal region.

Of the 34 frog species recorded in the Kruger National Park, 17 indicator species represent the tropical fauna of the **Eastern Sub-region** (in descending order of importance) *Kassina senegalensis, Amietia angolensis, Amietophrynus gutturalis, Tomopterna cryptosis, Cacosternum boettgeri, Xenopus laevis, Phrynobatrachus natalensis, Breviceps adspersus, Amietophrynus rangeri, Tomopterna natalensis, Amietophrynus garmani, Hyperolius*

marmoratus, Schismaderma carens, Strongylopus fasciatus, Ptychadena anchietae and *Chiromantis xerampelina.*

Fourteen species are indicator species of the **Bushveld District** namely *Phrynomantis bifasciatus, Ptychadena anchietae, Chiromantis xerampelina, Poytonophrynus fenoulheti, Ptychadena mossambica, Pyxicephalus edulis, Amietophrynus maculatus, Amietophrynus garmani, Breviceps adspersus, Hemisus marmoratus, Schismaderma carens, Phrynobatrachus natalensis, Tomopterna marmorata* and *Tomopterna cryptosis.* Indicator species of the **Maputoland Assemblage** included *Hyperolius tuberilinguis, Hyperolius pusillus, Arthroleptis stenodactylus, Kassina maculata* and *Phrynobatrachus mababiensis.*

5.2   Methodology

Questions concerning amphibian biodiversity basically fall into two categories:

(1) Those related to habitats, sites, or areas and

(2) Those concerned with species assemblages.

The primary goal of habitat or area-based questions is to inventory the species that occur in habitats or areas at a specific site. Species-based studies focus on one or more populations over an extended period of time. Several techniques are available for generating species lists or information on species richness for a site. For the most part, field techniques are methods of general collecting, as historically practiced by herpetologists. Typically, they involve searching for and collecting amphibians in all possible microhabitats both during the day and at night. Below a few general collecting techniques are discussed. These techniques described by Heyer et al. (1994) have been adapted for the monitoring of frog species in the Kruger National Park. The Kruger National Park survey entailed monitoring of 45 selected pans and localities from February 2009 until the end of December 2011. Four survey methods were used during this amphibian survey to obtain the species inventory (Table 5.1). These included visual encounter surveys (VES) of the terrestrial and aquatic habitats, nocturnal and diurnal road surveys for live and road-killed animals especially after rainfall events, anuran call surveys at selected breeding habitats (nocturnal and diurnal) and dip-netting for tadpoles in the 45 summer and 14 winter sites. This included nocturnal and diurnal surveys conducted after a sufficient rainfall period.

Nocturnal surveys were conducted of selected habitats to determine the number of adult male frogs calling. Daytime surveys were conducted within the 45 pans searching for evidence of any previous breeding activities (eggs, tadpoles) as well as emerging metamorphs. A SASS macro-invertebrate net was used for the dip-netting of tadpoles within

the larger pan systems and rivers and a smaller aquarium net in the smaller pools or shallow inundated grasslands. Voucher specimens of the tadpoles were collected and preserved in ethanol. Tadpole specimens and metamorphs were sent to Professor Louis du Preez and Donnavan Kruger at North-West University for positive identification.

5.2.1 Surveys at breeding sites

Many amphibians are most conspicuous only at breeding ponds during appropriate weather conditions. This is especially pertinent to the majority of frog species occurring within the Kruger National Park. Therefore, surveys conducted at breeding sites are especially effective. Sampling at the breeding site involves counting the frogs are some predetermined fashion. Generally, adults are counted along visual or aural transects. Data from surveys at breeding sites can be used to estimate species richness at one or several sites. Across-site comparisons are useful for identifying areas most suitable for development or preservation, studying the effects of pollution from point sources and determining the presence of predators. The techniques can also be used to monitor changes in population levels of species, to detect changes in species assemblages through time, or to carry out detailed autecological studies.

The current survey entailed the monitoring of 45 summer and 14 winter sites situated throughout the Kruger National Pak between February 2009 and December 2011. Surveys were conducted during the early summer months (November-December) as well as during the later summer months (February-March) in order to cover the majority of frog species reproductive events. Surveys were conducted during the autumn months (May) in order to determine the presence of autumnal or winter breeding frog species such as *Amietia angolensis* and *Strongylopus fasciatus*. The winter surveys were restricted mainly to the river systems as there was little or no water within the pans.

5.2.2 Target organisms and habitat

The techniques described below can be adapted for the study of any amphibian that breeds in communal aggregations in temporary or permanent ponds, vleis, streams or rivers. Breeding site surveys can focus on adults or larvae. Adults are usually more conspicuous and easier to sample and identify than larvae. However, larvae are typically present at the breeding site for longer periods than adults. Sampling both adults and larvae is the best approach and the one adopted for the current Kruger National Park amphibian diversity survey. Monitoring adults at a breeding site is easiest when breeding is concentrated in a narrow, well-defined period, but it can also be implemented when the breeding period is extended. In arid areas such as the Kruger National Park most breeding is rain-dependent,

and developmental times of larvae are often short; surveys must be undertaken during suitable weather conditions, whenever they occur (Wells 1977). Due to administrative problems regarding accommodation and availability of game guides within the Kruger National Park survey periods had to be booked well-in advance; and could not be randomly undertaken after suitable rainfall events. This restricted the surveys to predetermined dates rather than after heavy rainfall periods such as during January and February 2012.

For short, infrequent surveys, larval sampling yields more complete species lists than adult surveys do. This is especially pertinent to the current survey as the majority of sites were only visited for a short duration (single evening) during nocturnal surveys. However, if there is any doubt as to larval identification the larvae should be reared through metamorphosis as in the case of *Tomopterna* and *Ptychadena*. Larval densities can be strongly influenced by local factors (e.g. climate and co-occurring predators) and can vary greatly over short periods (Woodward and Mitchell 1991). Larval densities are not good predictors of adult population size.

Breeding site studies are most thorough in small, shallow bodies of water (<50 cm deep) that are free of emergent vegetation and that can be surveyed by observers in a relatively short period such as numerous seasonal wetland habitats within the Kruger National Park. The majority of pans within the Kruger National Park are open bodies of water although certain pans are dominated by hygrophilous grasses and sedges, floating vegetation as well as dense patches of rank emergent vegetation.

5.2.3   Quantitative sampling of tadpoles

Most tadpoles occur in aquatic habitats including lentic waters (streams and rivers) and lotic waters (ponds and dams). Tadpoles are often found in large concentrations at breeding sites over longer periods. As a result, sampling tadpoles rather than the adults may be a more efficient method for inventorying species at a site, even though eggs and larvae of many species are poorly known. In addition the collection of tadpole voucher specimens is easier and has less impact on the population than collecting adults.

There are various methods for sampling and identification of amphibian larvae (tadpoles) from water bodies. These methods include seining, dip-netting, trapping and enclosure sampling. These techniques provide a fast, relatively thorough, qualitative or quantitative sample with minimal personnel, material and time. In addition, the techniques generally do not harm the animals so can be used to monitor rare or endangered species. The two primary goals of these procedures are to assess the species richness of larvae in a body of water and determine larval population size.

Amphibian larvae occur in three basic habitat types: small bodies of water, ponds/dams and streams/rivers. Several points must be considered when sampling tadpoles. First, most tadpoles are medium to strong swimmers that can out swim a slow-moving net. Second, tadpoles commonly escape by hiding in the bottom substrate, making it essential to keep a seine or dip-net on the bottom when sampling. Third, vegetation and/or irregular bottom surfaces such as the deep clay deposits within the majority of pans within the Kruger National Park make tadpole sampling difficult. Seine and frigid-frame samplers prove ineffective in bodies of water with abundant vegetation. Fourth, many tadpoles are microhabitat specialists, so all the biotopes should be sampled appropriately.

Equipment varied and at one extreme, a small aquarium net (about 10 cm wide) with a bendable frame is useful in capturing tadpoles from small, shallow pools. A slightly larger SASS macro-invertebrate net serves well for the general collecting in both rivers and ponds. Wire-mesh sieves (kitchen strainers) with a handle work well in densely vegetated areas or rocky areas. Net size and mesh size determine passage rates through the water; some experience will be needed to find the net optimal for the body of water and tadpoles to be sampled. Fine mesh nets capture all larvae but are easily clogged with filamentous algae and debris. They are cumbersome and move through the water relatively slowly and tear easily. Large mesh nets are easier to use but may miss small individuals. The use of several mesh size nets is recommended to ensure all sizes of tadpoles are caught. There are no definitive rules about the number of sweeps needed to sample a habitat adequately. It is not uncommon to cover almost all of the surface area in small ponds or pools whereas only a fraction of larger bodies of water are sampled, such as shorelines of larger dams and pans. Approximately five to ten sweeps are adequate to cover the smaller pans and seasonal pools and up to twenty to fifty sweeps can be made in larger pans. The timing of collection varies on how much clay, vegetation and detritus must be removed from the net and how many larvae need to be identified. A reasonable procedure is to survey each habitat for an equal period or with an equal number of sweeps. Making more sweeps in the larger habitats increases the chance of encountering rare species. Increasing the number of sweeps also increases the chance of capturing highly habitat-specific species.

Tadpoles are fragile often with delicate tails, which are easily damaged in nets. Trauma can be minimised by keeping tadpoles, cool, un-crowded and in the net for as little time as possible. Tadpoles should not be picked up with your hands from the net but collected in a glass beaker and placed into a larger bucket. Consideration should also be taken for the water body sampled. Major disturbances, such as massive sediment up-welling by sweeping a net in small ponds may result in the death of all associated animals.

5.2.4   Collection of voucher specimens

Voucher specimens of tadpoles were collected from all the 45 summer and 14 winter sites surveyed. A maximum of 5 tadpoles per species were collected and preserved in 50% ethanol. Tadpole species were sent to Professor Louis du Preez at the University of the North-West for positive identification and archived in their museum.

5.2.5   Data treatment and interpretation

The following information was recorded at the 45 pans:

- Physico-chemical parameters such as pH, TDS, conductivity, oxygen (%; mg/l) and surface-water and deep-water temperatures (see methodology for water quality aspect)
- Presence or absence of calling sites (bushes, trees, stumps, rocks, floating, fringing or emergent vegetation).
- Reproductive activity of adult frogs (Calling, eggs, tadpoles, etc.)
- Developmental stages of any larvae (tadpoles, metamorphs)

The data from breeding-site surveys can be used to produce a list of frog species encountered within the Kruger National Park (Table 5.1). Species lists can be compared across sites; although one should be cautious to attribute too much to species absences if only a few breeding sites are examined during a single season survey or surveys conducted during low breeding activity. Breeding-site surveys can be used to estimate effective population size and operational sex ratio (OSR), two parameters that are important for conservation work (Falconer 1989). For these purposes, surveys must be made over an extended period because breeding populations vary widely from night to night at a single pond (Ryan 1985). Due to the large size of the Kruger National Park and severe time constraints for surveys and the accessibility of certain sites nocturnal surveys or audio transects were not taken at all of the pans. The majority of nocturnal surveys were limited to a single night survey base from the vehicles as well as time constraints. The results obtained from short-term sampling are highly dependent on collecting and the environmental variables. Some of these variables include weather (both prior and during sampling), collector's experience, and level of sampling effort in each habitat, diversity of collecting techniques used, and phenology of the amphibian species. This is especially important when results from similar habitats are compared. Any effects of these variables must be recognised and controlled. Time constrained searches must standardise collecting effort within the selected habitat types (Hyer et al. 1994).

5.2.6   Nocturnal surveys and digital tape recordings

In the vast majority of frog species, males in reproductive condition use distinctive species-specific calls to advertise their position to potential mates and rivals (Wells 1977). The vehicle based nocturnal surveys and making of digital audio recording exploits the species-specific behaviour of frogs. All calling male frogs around a selected pan or pool are counted; in the case of this project all frog aggregates calling around a selected pan or pools within a riverine site. The effectiveness of this method varies according to the detection distance of each species' advertisement call. Certain species such as *Phrynomantis bifasciatus Ptychadena oxyrhynchus, Pyxicephalus edulis* have calls which can be heard over large distances and others are more cryptic such as *Chiromantis xerampelina*, *Poytonophrynus fenoulheti*, *Hemisus marmoratus, Afrixalus aureus* can only be clearly heard over shorter distances. These counts are then used to estimate or determine:

- Relative abundance of calling males
- Relative abundance of all adults
- Species composition
- Breeding habitat or microhabitat use
- Breeding phenology of species.

Counting calling male frogs or aggregations of calling males (choruses) along strip transects can be the most effective way to:

- Inventory species composition
- Provide a first approximation of relative abundance of breeding frogs
- Determine breeding habitat use
- Map distributions of most frog species throughout a large area.

Several factors may affect the accuracy of assessments of abundance and habitat occupancy based on vehicle based digital audio recording. For example, if counts of a species are not made during its peak-breeding period, when the maximum number of males calling, differences between surveys may simply reflect differences in stage of breeding cycle. This is especially pertinent for certain frog species such as *Pyxicephalus edulis*, which may not emerge for several years to breed due to unfavourable environmental parameters such as insufficient rainfall. Observers must be fully knowledgeable of the species-specific frog calls, must have full hearing ability and be experienced in this sampling technique. Observers should ideally make audio recordings of all the species present in the choruses for historic records as well as confirmation of species identification. Audio recordings using a portable digital Olympus LS-10 tape recorder were made at sites visited during vehicle

based nocturnal surveys. The recordings were made for approximately 2 minutes or if specific target species were calling.

Limitations

The digital audio recording method has several limitations including:

- Number of calling males cannot be determined aurally for chorusing species or in situations of high call overlap such as *Cacosternum boettgeri*, *Afrixalus aureus, Hyperolius pusillus, Hyperolius marmoratus, Kassina maculata, Hemisus marmoratus* and *Tomopterna cryptosis*.

- The calling period of certain frog species differ. Species such as *Phrynobatrachus natalensis* only call during the early evening and late evening and early morning and *Ptychadena oxyrhynchus* between midnight (24h00) and 04h00 and *Hildebrandtia ornata* after 22h30.

- Nocturnal surveys in the Kruger National Park were restricted to vehicles and the major roads as well as restricted time constraints of surveys (22h00-23h00); due to increased security risks resulting from increased rhino poaching activities in the park. The majority of late evening/ early morning callers such as *Ptychadena oxyrhynchus* would have been missed during these brief nocturnal surveys.

- Audio sampling should not be restricted to nocturnal surveys as certain frog species call throughout the day including *Pyxicephalus edulis, Hildebrandtia ornata, Ptychadena anchietae, Breviceps adspersus, Cacosternum boettgeri, Chiromantis xerampelina, Amietophrynus maculatus* and *Phrynobatrachus mababiensis.*

- Explosively breeding frog species are acoustically evident for extreme short periods and are probably not sampled adequately. Certain male and female African Bullfrogs (*Pyxicephalus edulis*), Sharp-nosed Grass Frog (*Ptychadena oxyrhynchus*), Southern Ornate Frog (*Hildebrandtia ornata*) are only present for a few hours at the breeding sites during their short-duration reproductive events.

- Absolute population size cannot be estimated, because male and female survivorships may not be related and operational sex ratios (males to females) may differ greatly.

5.3   Results

Historic surveys in the Kruger National Park resulted in 33 frog species being recorded (Pienaar et al. 1976). During the current survey 30 frog species were recorded. A combination of historic and recent field surveys result in 34 frog species being recorded from the Kruger National Park.

Table 5.1: Frog species recorded in the Kruger National Park.

Common Name	Scientific Name	Historic Records Pienaar et al. (1976)	Current Survey 2009-2012
Golden Leaf-folding Frog	*Afrixalus aureus*	√	√
Common River Frog	*Amietia angolensis*	√	√
Shovel-Footed Squeaker	*Arthroleptis stenodactylus*	√	X
Eastern Olive Toad	*Amietophrynus garmani*	√	√
Guttural Toad	*Amietophrynus gutturalis*	√	√
Flat-Backed Toad	*Amietophrynus maculatus*	√	√
Raucous Toad	*Amietophrynus rangeri*	√	X
Bushveld Rain Frog	*Breviceps adspersus*	√	√
Boettger's Caco	*Cacosternum boettgeri*	√	√
Southern Foam Nest Frog	*Chiromantis xerampelina*	√	√
Mottled Shovel-nosed Frog	*Hemisus marmoratus*	√	√
Southern Ornate Frog	*Hildebrandtia ornata*	√	√
Painted Reed Frog	*Hyperolius marmoratus taeniatus*	√	√
Water Lily Reed Frog	*Hyperolius pusillus*	√	√
Tinker Reed Frog	*Hyperolius tuberilinguis*	X	√
Red-Legged Kassina	*Kassina maculata*	√	√
Bubbling Kassina	*Kassina senegalensis*	√	√
Brown-backed Tree Frog	*Leptopelis mossambicus*	√	√
Dwarf Puddle Frog	*Phrynobatrachus mababiensis*	√	√
Snoring Puddle Frog	*Phrynobatrachus natalensis*	√	√
Banded Rubber Frog	*Phrynomantis bifasciatus*	√	√
Northern Pygmy Toad	*Poytonophrynus fenoulheti*	√	√

Plain Grass Frog	*Ptychadena anchietae*	✓	✓
Broad-banded Grass Frog	*Ptychadena mossambica*	✓	✓
Sharp-nosed Grass Frog	*Ptychadena oxyrhynchus*	✓	✓
African Bullfrog	*Pyxicephalus edulis*	✓	✓
Red Toad	*Schismaderma carens*	✓	✓
Striped Stream Frog	*Strongylopus fasciatus*	✓	X
Tremelo Sand Frog	*Tomopterna cryptosis*	✓	✓
Knocking Sand Frog	*Tomopterna krugerensis*	✓	✓
Russet-backed Sand Frog	*Tomopterna marmorata*	✓	✓
Natal Sand Frog	*Tomopterna natalensis*	✓	X
Common Platanna	*Xenopus laevis*	✓	✓
Muller's or Tropical Platanna	*Xenopus muelleri*	✓	✓

5.4   Discussion

5.4.3   Aspects of the ecology of the frog species recorded in the Kruger National Park

The following section gives a basic habitat description as well as general discussion of the breeding biology and ecology of the 34 frog species recorded in the Kruger National Park. The purpose is to highlight possible research projects and give a clearer indication on which species require more intensive research; especially pertaining to aspects of the ecology and general breeding biology of these species. These include the African Bullfrog (*Pyxicephalus edulis*), Southern Ornata Frog (*Hildebrandtia ornata*). The tadpoles of *Strongylopus fasciatus*, *Ptychadena mossambica, Tomopterna marmorata* and *Tomopterna krugerensis* are presently un-described as well as aspects of the tadpole ecology such as the larval duration of *Chiromantis xerampelina, Hildebrandtia ornata, Kassina maculata* are unknown. A distribution map of each species collected during this study is included.

Golden Leaf-folding Frog (*Afrixalus aureus*)

Afrixalus aureus is a savanna species which inhabits Coastal Bushveld-Grassland, a mosaic of vegetation types found from sea level to an altitude of 300 m along the coast of northern Kwazulu-Natal, as well as various other bushveld vegetation types, such as Mixed Lowveld Bushveld, at altitudes of 200-300 m, east of the eastern escarpment (Jacobsen 1989). Breeding takes place in seasonal or semi-permanent water bodies containing emergent vegetation such as *Polygonum pulchrum*, *Ludwigia stolonifera* and *Cyperus papyrus* (Backwell et al. 1991). At most sites the water is fairly shallow, rarely exceeding 50 cm in depth. During the breeding season (November-February) adults can be found close to breeding sites at the margins of pans and grassy pools. The frogs may sit on emergent vegetation, usually in leaf axils or on broad leaved shrubs, fully exposed to the sun, in a head-down position. At dusk, males take up position a few centimetres above the water, where they begin to call, continuing until about 04h00. High-density choruses develop; these contain a high proportion (50%) of satellite males and are characterised by a high level of aggression (Backwell et al. 1991). Amplexus is axillary, and clutches of about 50 creamy white eggs are enveloped in vertically folded leaves (often of *Polygonum pulchrum* or *Ludwigia stolonifera*) or blades of grass, 4-15 cm above the surface of the water (Jacobsen 1989; Backwell et al. 1991; Channing 2001). Nothing is known of the non-breeding behaviour of this species.
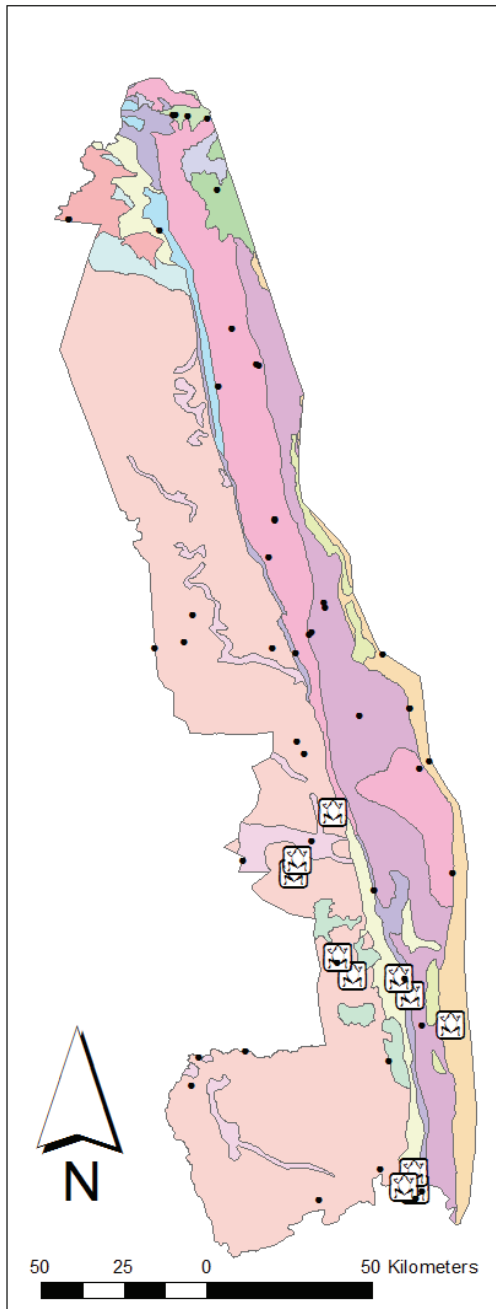
Figure 5.1: Distribution of *Afrixalus aureus* during this study.

Common River Frog (*Amietia angolensis*)

Amietia angolensis inhabits the Grassland and Savanna biomes, and forest fringe. Annual rainfall in these areas is 500-900 mm. The species tolerates some habitat disturbance and is frequently associated with human habitation, taking up residence in ditches and ponds, often where reeds and water lilies are present. Breeding takes place in shallow water along the edges of pools, dams, streams and slow-flowing rivers. These frogs breed in both standing water in flat areas and running water traversing slopes of more than 14 degrees (Channing
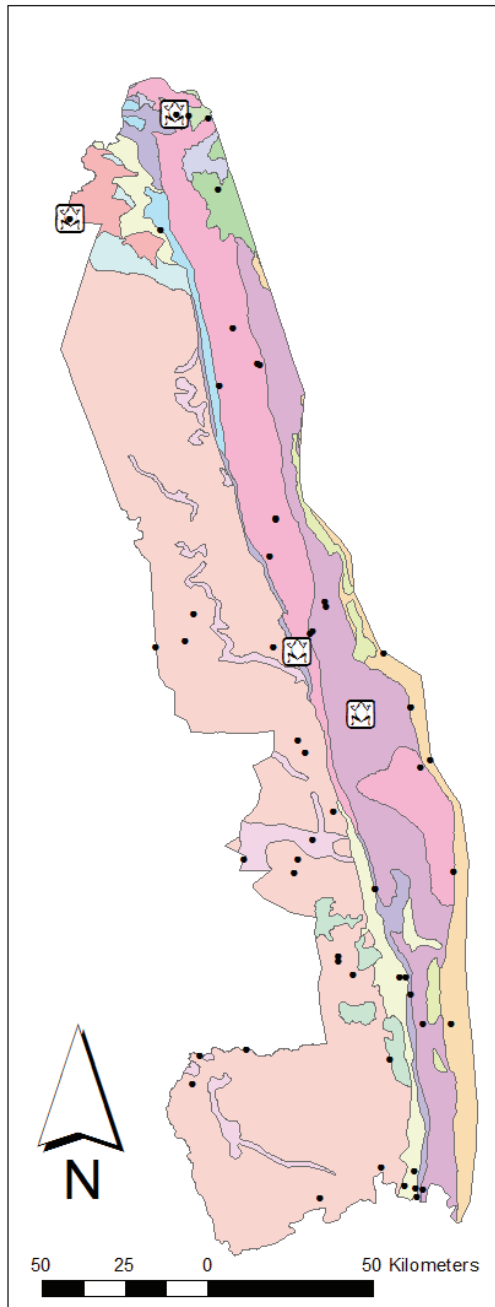
1979). The same habitat is used throughout the year. The adults spend the day floating amongst vegetation or basking on rocks above the water. Larger individuals may be found on banks or in vegetation above the water leaping to the safety of the nearest pool when disturbed. This species has long hind legs and a fair amount of webbing between the toes, and is well adapted to jumping and swimming. *Amietia angolensis* is active throughout the year and breeding has been recorded in all months of the year (Channing 1979). In the Kruger National Park it was collected during the late summer months (March-April) as well as autumn months (May). Males typically call from floating vegetation or from shallow water at the edge. Clutches of 400-500 eggs are laid in shallow, standing water. Tadpoles may grow to 80 mm in length (Wager 1986). Being a common species that is active all year round, these frogs consume large numbers of flying and crawling insects. In turn, they constitute an important prey item for otters, large birds and snakes.

Figure 5.2: Distribution of *Amietia angolensis* during this study.

Shovel-Footed Squeaker (*Arthroleptis stenodactylus*)

The species occurs in wooded areas with abundant leaf litter and sandy soils from sea level up to 2000 m. Along the coast it inhabits Dune Forest and forest patches in Coastal Bushveld/Grassland, while in the Kruger National Park it was found in the decaying leaf litter of riverine woodland (H.H. Braack pers. obs.). They are cryptically coloured matching the leaf litter and usually difficult to locate, even when calling in dense choruses. *Arthroleptis stenodactylus* is a terrestrial breeder and breeding takes place within the decaying leaf litter.

Breeding occurs from December-February (Stewart 1967). Males call from concealed positions under leaf litter. The call is a high-pitched metallic *peep-peep* or *wip-wip-wip* (Carruthers et al. 2009) which sounds like an insect. The eggs are deposited in hollows or shallow burrows in leaf litter beneath bushes or around the roots of trees. Clutch size is 33-80 eggs which develop directly into small froglets (Barbour and Loveridge 1928; Loveridge 1953a).

Eastern Olive Toad (*Amietophrynus garmani*)

The species inhabits various bushveld vegetation types in the Savanna Biome and seems to prefer well-wooded low-lying areas with high daytime temperatures. During the day, individuals may be found under fallen logs, rocks and mats of vegetation, or beneath any object that provides shelter around houses. In northern Kruger National Park, specimens have been found in abandoned termitaria (H.H. Braack pers. obs.) Breeding usually occurs in small, shallow temporary water bodies such as vleis (valley bottom wetlands) and pans (seasonally inundated depressions), but occasionally the quite backwaters of rivers and pools along small, slow-flowing streams are used (Lambiris 1989a). They have also been recorded breeding in artificially created habitats such as farm dams and ornamental ponds around homesteads. *Amietophrynus garmani* adapts to suburban gardens but less readily than Guttural Toads which can be considered urban exploiters. Breeding is initiated by the first heavy rainfalls during spring and summer, continuing into January and February. Choruses of males were observed calling along the Sabie River during March 2011. Males call from exposed mud banks or semi-concealed amongst the fringing grasses around the margins of the pans often forming a small chorus. The call is a loud, braying *kwaa- kwaa,* often antiphonaly (Carruthers et al. 2009). Males exhibit site fidelity, returning to the same site even when removed and released a considerable distance away (Pienaar et al. 1976). Amplexus is axillary, and displacement of amplexing males is frequent, with "knots" of several males and a single female forming at timed (H. Braack pers. obs.). Eggs are laid in double strands containing up to 12 000-20 000 eggs. The eggs are eaten by the Serrated Hinged Terrapin (*Pelusios sinuatus*), Muller's Platanna (*Xenopus muelleri*), and by their own tadpoles, while the adult frogs are taken by young crocodiles (Channing 2001).
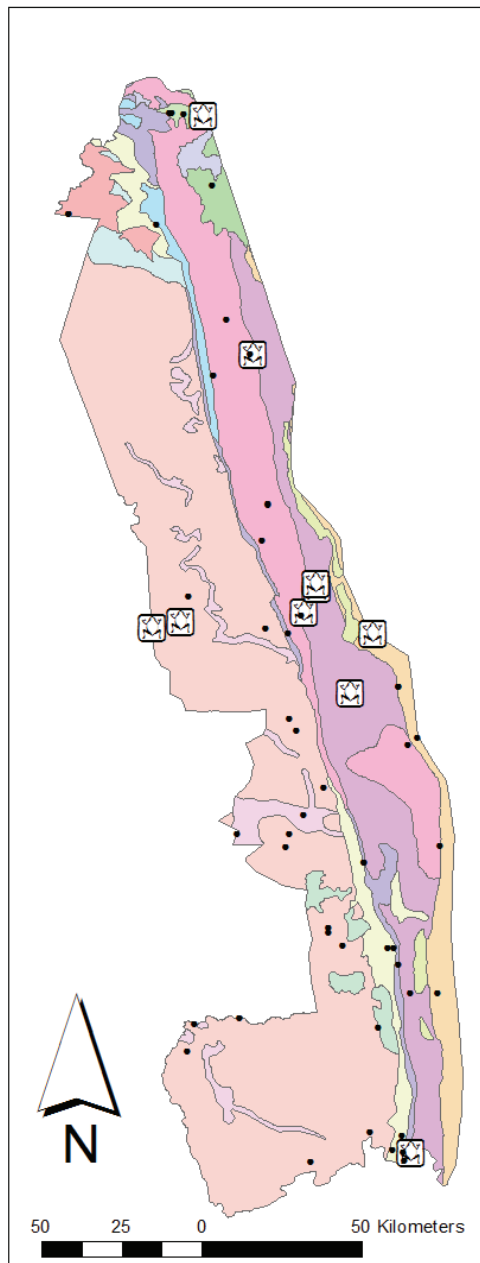
Tadpoles are ovoid and plump and measure up to 36 mm. The tailfin is shallow and curved ending bluntly with the deepest point in the middle of the tail, fin tapering gradually to end in rounded tip. Tadpoles assume a lighter or darker colour to match the substrate. Eggs hatch and tadpoles are free swimming after 24hours and metamorphosis complete after 64 to 91 days (Carruthers 2009).

Figure 5.3 Distribution of *Amietophrynus garmani* during this study.

Guttural Toad (*Amietophrynus gutturalis*)

A. gutturalis inhabits a various vegetation types in the Savanna, Grassland and Thicket biomes at altitudes ranging from sea-level to about 1800 m. In the east, the species is common amongst forest clearings and forest/grassland ecotones, while in the west it has a linear distribution along the wooded banks of the Gariep River (du Preez et al. 2004). Guttural toads are common within suburban gardens and farmlands (Carruthers 2009). Guttural Toads are explosive opportunistic breeders and breeding activities are initiated in small permanent water bodies as soon as temperatures rise in spring, often before the first

rains arrive. In Uvongo, in southern Kwazulu-Natal, breeding has been recorded as early as the end of July and early August with a peak in late September to November, reviving after every rain shower until mid-February. Males call from open positions as well as concealed positions around the margins of the water body or even from floating vegetation within the water body. Calling males exhibit site fidelity, with some individuals returning to the same site year after year. Breeding takes place in open, shallow pools in rivers, farm dams, garden ponds, canals, ditches and borrow pits. During the day, the frogs take shelter under logs, rocks or other objects, in drain pipes and gutters, in burrows, or in holes they excavate in soft ground (du Preez 2004). As many as 25 000 eggs between 1.4-1.5 mm diameter, are laid in two gelatinous strings, each 5 mm thick (Wager 1986). Strings of eggs are often twined around aquatic vegetation. As soon as the toadlets have developed front legs, they leave the water to hide amongst the vegetation until their tales are absorbed, and the start feeding on small insects. The maximum longevity, recorded in captivity, is seven years (Channing 2001).

The tadpoles are small with a plump ovoid body and a rounded snout and measure up to 25 mm. The tailfin is moderately curved ending in a blunt point. The deepest point is in the middle of the tail. The body and tail shaft black with iridescent spots and the fins transparent. Tadpoles are free-swimming after 48-72 hours from being deposited and metamorphosis completed after 35-42 days (Carruthers et al. 2009).
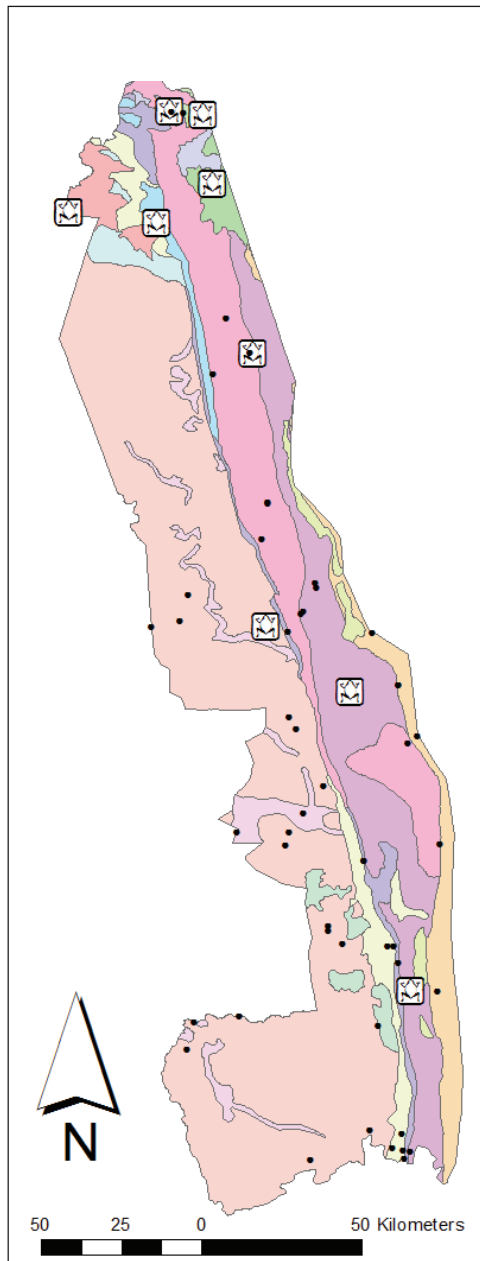
Figure 5.4: Distribution of *Amietophrynus gutturalis* during this study.

Flat-Backed Toad (*Amietophrynus maculatus*)

The species inhabits various vegetation types within the Savanna and Grassland biomes, on the Zululand coastal plains, in the Swaziland Lowveld and Middleveld and in the lowveld and bushveld of north-eastern South Africa (Boycott 2004a). It is usually associated with riverine. Males call from exposed positions on sandbanks or concealed amongst fringing vegetation, rocks along major fast-flowing rivers (Jacobsen 1989). Calling activities occur at night as well as during the day, especially after heavy rainfall. The calls are antiphonal, with individuals calling at a slighter different pitch so that alternate calls are readily distinguishable (Pienaar et al. 1976). Females approach males and make contact with the males prior to amplexus; amplexus is axillary (Channing 2001). Pienaar et al. (1976) and Wager (1986) recorded eggs 1.2 mm in diameter in a single jelly tube 3 mm thick. The egg strings are deposited at the bottom of the pool or wound around stones or twigs with approximately 2000-8000 eggs. Tadpoles reach metamorphosis within six weeks in captivity and as little as two weeks (14 days) in the wild. Tadpoles are preyed upon by Mozambique Tilapia, *Oreochromis mossambicus* (H.H. Braack pers. obs.). Tadpoles were eaten by *Hildebrandtia ornata* tadpoles in experimental containers.
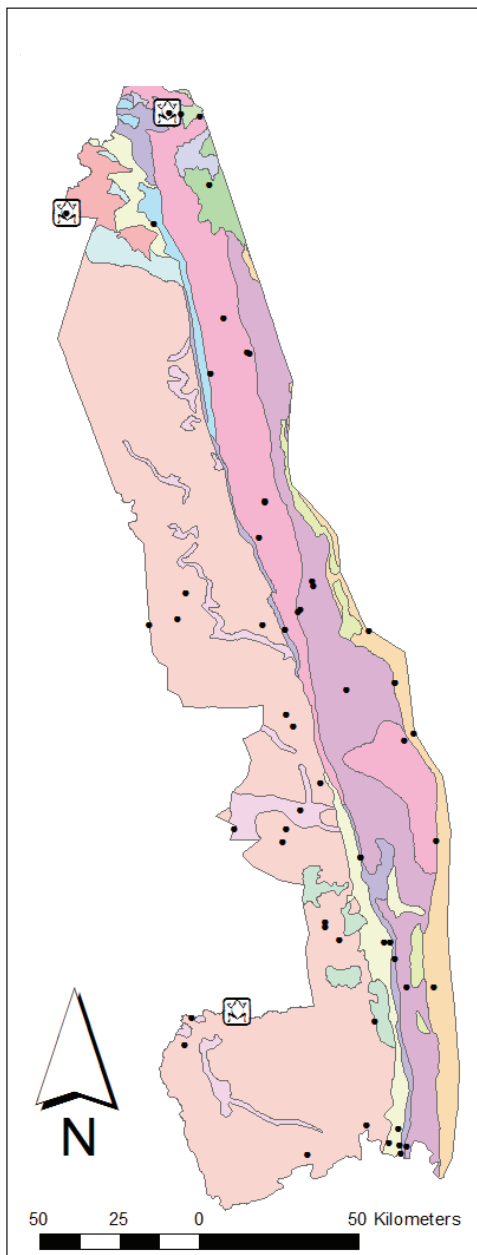
The tadpoles are small with a plump ovoid body and measure up to 25 mm. The tailfin is moderately curved, and ends in a blunt tip. The colour of the body and tail shaft are black with iridescent spots and the fins hyaline with sparse or no pigmentation. Tadpoles are free-swimming after 12-24 hours in captivity and metamorphosis complete after 14 days (Carruthers et al. 2009).

Figure 5.5: Distribution of *Amietophrynus maculatus* during this study.

Raucous Toad (*Amietophrynus rangeri*)

The range of *Amietophrynus rangeri* appears to be contracting in the north and east of South Africa, in Limpopo, Mpumalanga, Gauteng and coastal Kwazulu-Natal provinces. This apparent range contraction seems to compliment the range expansion of *Amietophrynus gutturalis* and it is possible these are linked, that is *Amietophrynus gutturalis* displaces *Amietophrynus rangeri* and/or habitat modification affects these species differently. *Amietophrynus gutturalis* hybridizes with *Amietophrynus rangeri* at sites scattered throughout eastern South Africa. These are not closely related species and it seems that the hybridisation rarely progresses beyond the first generation (Channing 2001). Several authors have suggested that hybridization between the species is a historically recent phenomenon and that the creation of artificial breeding sites such as farm dams, has broken down the natural separation based on breeding habitats (Channing 2001). The apparent complementary range shifts in the two species may also partly reflect the different responses of a tropical savanna species and a mesic temperate species to subtle climatic changes; rather than competitive exclusion (Cunningham 2004a).

In the Kruger Park the species is not abundant and historic records occur mainly along the western higher lying regions. The species has been recorded along the Crocodile, Sabie and Luvuvhu rivers (Pienaar et al. 1976). No records of this species were recorded during the current survey period between 2010 and 2012.

Amietophrynus rangeri inhabits mesic temperate areas of South Africa, Lesotho and Swaziland, a distribution that encompasses much of the Fynbos and Grassland biomes. It also occurs peripherally in the succulent Karoo, Nama Karoo, Thicket, Forest and Savanna biomes. The species is absent from the sub-alpine grasslands of Lesotho, upper montane areas of the Western Cape, the Cape Peninsula, Saldanha Peninsula and Swartland in the southwest, and from the Lowveld and drier parts of Limpopo, Mpumalanga and Kwazulu-Natal provinces and Swaziland (Cunningham 2004b). These toads are frequently encountered at breeding sites around farm dams, large ponds and slow-flowing streams. Non-breeding individuals roam widely and may be encountered crossing trails and roads throughout the year, especially on humid nights. The number of individuals encountered greatly increases around the breeding season as individuals migrate to breeding sites. Males usually call from exposed sites on floating vegetation, in shallow water near banks, or among reed beds. Males show some site fidelity within seasons, but breeding shifts of up to 5km have been recorded across two breeding seasons, and in a three-year ecological study of this long-lived species, few marked individuals were recaptured in subsequent years (Cherry

1993). As in most toads, eggs are laid in spiralling strings and often become entangled in aquatic vegetation (Cunningham 2004b).

The tadpoles are small with a plump ovoid body and measure up to 25 mm. The tailfin is distinctly convex but shallow, end bluntly with the deepest point just anterior to the middle of the tail. The colour of the body is dark, sometimes with golden stipples; dark pigment over tail muscles and characteristically confined to upper two-thirds along full length of tail leaving a bright white band below. The fins are hyaline and they are free-swimming after 24 hours and metamorphosis complete after 64-91 days (Carruthers et al. 2009).

Bushveld Rain Frog (*Breviceps adspersus*)

Breviceps adspersus inhabits semi-arid habitats with sandy to sandy-loam soils. Its distribution closely matches the Savanna Biome, particularly the bushveld vegetation types that are characterised by a "grassy ground layer and a distinct upper layer of woody plants" (Low and Rebelo 1996). The species can survive in suburban environments such as Polokwane. Males were frequently observed calling after rain showers within Letaba, Shingwedzi, Olifants, Lower Sabie, Skukuza, Crocodile Bridge Rest Camps in the Kruger National Park during the day and night.

Breviceps adspersus is a terrestrial breeder with the tadpoles completing metamorphosis within the egg mass. *Breviceps adspersus* is a cryptic species spending most of their time underground. The dry season is spent 15-30 cm below the surface, often in situations where soil moisture is conserved, for example, next to or under rocks, logs, stumps or tree roots (Jacobsen 1989).

In spring or early summer, following heavy rain, males emerge from the soil and establish call sites 5-200 cm from their winter retreats. The call site usually consists of a well-concealed shallow depression at the base of a grass tuft or small herbaceous plant. In overcast, damp conditions calling males may continue unchecked for several days and nights. Males are prompted to call by the calls of their immediate neighbours, and results in bouts of calling which spread through the population in waves. When hot, dry weather returns, or when disturbed, males retreat to their underground burrows.

Males were observed to use the same call site for up to five consecutive nights (Minter 1995, 1998). Amplexus is facilitated by a sticky skin secretion which ensures the male remains attached to the female during nest construction. A mass of about 45 eggs, covered by a smaller mass of fluid-filled jelly capsules lacking yolk, is deposited in a chamber about 30 cm below the ground surface. The eggs measure 5 mm within a 10 mm egg capsule. The

female remains nearby until the froglets are ready to leave the nest (approximately six weeks). The reason for her presence has not been established (Minter 1995; Minter 1998).
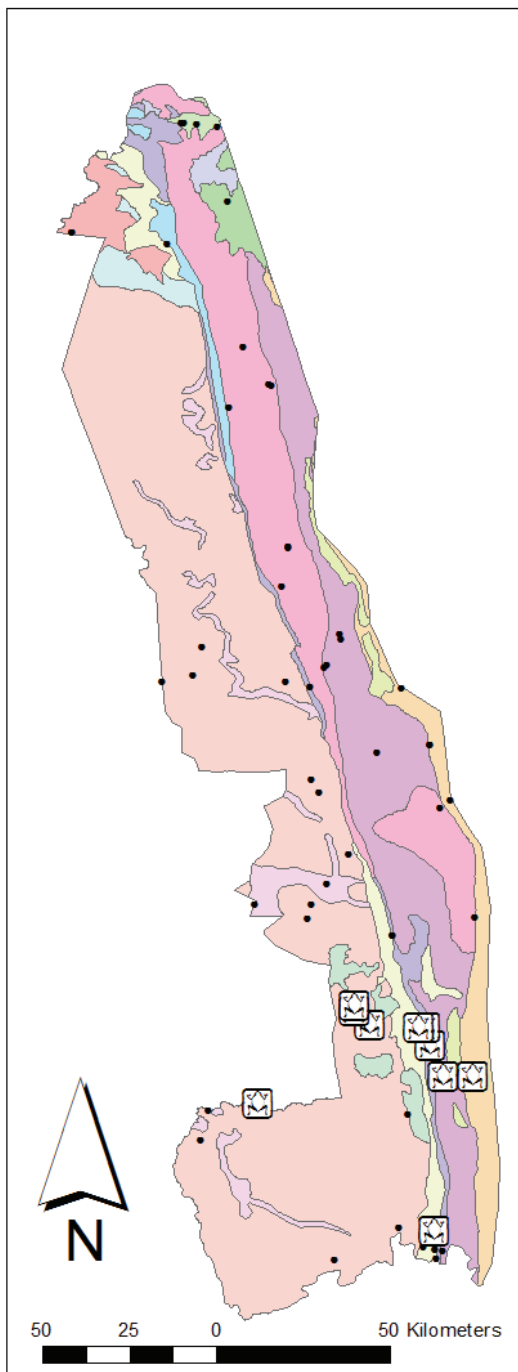
Figure 5.6: Distribution of *Breviceps adspersus* during this study.

Boettger's Caco (*Cacosternum boettgeri*)

Cacosternum boettgeri occurs in the savanna regions of Namibia, eastern Botswana, southern Zambia and the Zimbabwe plateau. It is one of the most abundant and widespread species in Southern Africa, occurring in most suitable habitats throughout its range at both high and low elevations. The range of *Cacosternum boettgeri* may have increased in the last century due to human activity, particularly where bush and reeds have been cleared and

grass has been introduced along with domestic livestock (Van Dijk 1971b). *Cacosternum boettgeri* are common especially in the southern and central portions of the Kruger National Park. Large choruses were observed between Lower-Sabie and Tshokwane in November 2010 after heavy rainfall. *Cacosternum boettgeri* inhabits a wide variety of vegetation types in the Nama Karoo, Succulent Karoo, Savanna, Grassland, Fynbos and Thicket biomes, but is usually absent from the forest, although it is sometimes found in forest clearings. Within these biomes it favours open areas with short vegetation and is especially abundant in grassy areas (Scott 2004). This species can tolerate drier habitats than the Bronze Caco (*Cacosternum nanum*), but is also occurs in high rainfall areas.

Cacosternum boettgeri breeds in almost any small, temporary water body, such as seasonal pools in inundated grasslands, culverts, road side pools and other rain-filled depressions. *Cacosternum boettgeri* was observed calling and breeding within single elephant spoors; situated within the temporary and seasonally inundated grass dominated margins around Seribye Pan, Lannea Pan and Riet Pan in the Tshokwane area during the 2010/2011 wet season.

During the dry season *Cacosternum boettgeri* aestivates in mud-banks, mud cracks and burrows of other animals, disused termitaria and under stones. Two *Cacosternum boettgeri* adults were observed in December 2011 taking refuge within mud-cracks situated within the desiccated Lannea Pan basin. Lannea Pan is situated within the Tshokwane area of the southern Kruger National Park. This species seems to have an extended breeding season. During the rainy season males usually start calling in the late afternoon and call incessantly after dark and will continue until around midnight (large choruses are common). The species has been recorded calling throughout the day within the Kruger National Park; especially after previous rainfall events. Males usually call from concealed positions under vegetation or other cover, at water level, but have also been observed calling from totally exposed positions. Although *Cacosternum boettgeri* were recorded calling around the larger pans in the Kruger National Park they were more abundant around the smaller seasonal pools situated within inundated grassland.

Clutches of approximately 200 eggs are attached to vegetation below the surface of water (Channing 2001). Eggs are 0.9 mm in diameter and are encircles by a jelly capsule. The tadpoles usually hatch within 48 hours and metamorphosis is complete within approximately 18 days (Pienaar et al. 1976).
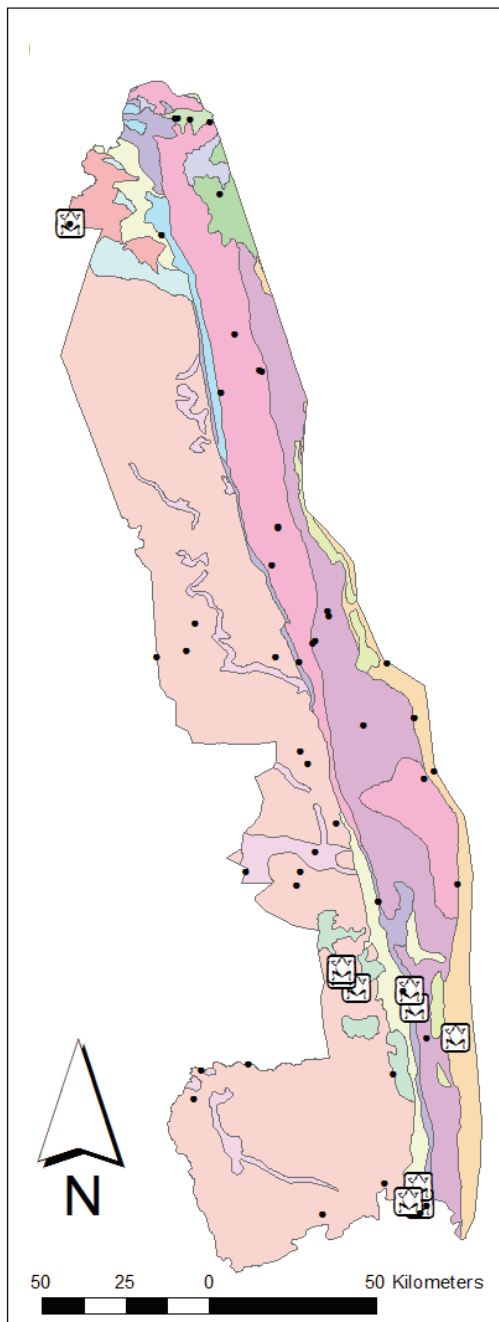
The tadpoles are small with an ovoid, slightly flattened body and a rounded snout and measure up to 25 mm. The tailfin reaches deepest point in middle of tail; tapers to fine rounded tip. The colour of the body is moderately pigmented and varies in colour from cream to brown, can be greenish but is rarely grey. Fin opaque with fine stippling, sometimes with colour reflections. They are free-swimming after 48 hours and metamorphosis complete after 18-21 days (Carruthers et al. 2009).

Figure 5.7: Distribution of *Cacosternum boettgeri* during this study.

Southern Foam Nest Frog (*Chiromantis xerampelina*)

The species inhabits a variety of bushveld vegetation types in the Savanna Biome. Breeding usually takes place in temporary pans and vleis, but also occurs in more permanent water bodies such as dams and quarries. In the absence of trees and shrubs, nests may be attached to the sides of large rocks or man-made structures overhanging water, including bridges, culverts and bird hides.

In summer, these frogs are often seen perched on the branches of trees overhanging or near water, and their white, crusty nests, c.20 cm in diameter, are conspicuous around dams, pans and vleis and along river and stream courses.

In the winter months, they seek shelter under the bark of trees, in rock cracks and on the branches of shady evergreen trees far from the nearest water. They also move into buildings where they take up residence for weeks, or even months, on rafters, walls and window-sills, or behind bookcases and picture frames. In the Kruger National Park they are often found in the rest camps especially in the cold winter months.

Chiromantis xerampelina is adapted in several ways to survive in an arid environment. It possesses a rough, dry skin, and conserves water by means of rectal water re-absorption and by excreting nitrogenous waste in the form of uric acid (Coe 1974). Inactive individuals may be found perched in exposed positions on branches of trees and shrubs where their colour becomes chalky white or pale grey to reflect light and heat. Several specimens were observed sunning on a dead branch during the midday heat in December (39°C); several kilometres from the nearest water body within the Kruger National Park.

The males gather at suitable nesting sites at night where they produce soft, discordant croaks and squeaks. They do not appear to be territorial, and two or more frogs close together, or even on top of each other, will call irregularly and independently. The female leaves the water and climbs up to the nesting site where amplexus with one of the males takes place. Nest construction begins when the female releases an oviducal secretion from her cloaca and churns it into white foam with her hind legs. Peripheral males take up positions on either side of the amplexing pair and attempt to position their cloacae adjacent to that of the female during bouts of oviposition. Thus the female's eggs are fertilized by more than one male; a breeding strategy known as polyandry (Jennions et al. 1992). Neither the amplexing male nor the peripheral males participate in the construction of the foam nest. The nest may take up to seven hours to complete and nest construction is split into 2-4 sessions. Between sessions the female leaves the nest site and returns to the water to rehydrate. At this time the amplexing male may dismount and on returning to the nest the female may amplex with a different male (Jennions et al. 1992). Communal nests, involving two or more females and numerous males are commonly formed. One such nest contained 50 males and 20 females (Passmore and Carruthers 1995). The female usually returns the following night and adds a second layer of foam (not eggs) to the top of the nest. Males seldom attempt amplexus on second night; if they do, they soon release the female and leave (M.D. Jennions pers. obs.). Jennions et al. (1992) recorded a mean clutch size of 1100 eggs for single-female nests. Once the eggs hatch within the nest, the tadpoles rely on

bubbles in the foam for oxygen (Seymour and Loveridge 1994). After 4-6 days, the wriggling tadpoles begin to move downward within the nest, sometimes in a wet squirming mass of several tadpoles or in ones and twos, until they reach the bottom (Wager 1986). It is thought that these movements and the accumulation of tadpoles at the bottom of nest soften the crust, thereby enabling the tadpoles drop into the water below where they complete their development. Egg development within a foam nest may to avoid or reduce predation in the early stages of development. However, in some cases, the water below the nest recedes, and the tadpoles drop onto the ground and perish (Boycott and Theron 2004). The tadpoles are medium sized with an oval body and measure up to 55 mm. The tailfin is deepest midway tapering to an acute point with the upper fin almost twice as deep as lower fin. The colour of the body is uniformly brown to orange-brown and the fins are semi-transparent with specks of grey and orange and whitish below. Tadpoles are free-swimming after 4-6 days and the larval duration is currently unknown. The tadpoles developed within 90 days in the laboratory during exposure experiments.
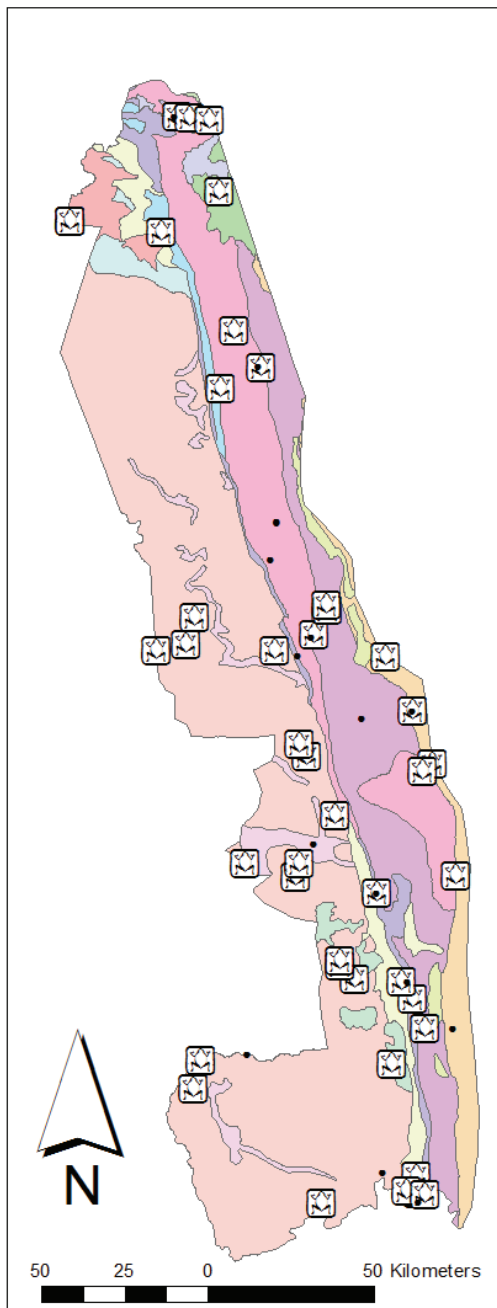
Figure 5.8: Distribution of *Chiromantis xerampelina* during this study.

Mottled Shovel-nosed Frog (*Hemisus marmoratus*)

The species is widely distributed throughout all the regions of the park. They are usually found in the open grassland areas adjacent to seasonally inundated pans and grasslands where the soil is moist and aerated (Pienaar et al. 1976). The species was recorded

throughout the park and males were observed calling from the edges of the pans as well as within seasonally inundated grassland and roadside pools.

This species thrives in semi-arid environment and is well-adapted to breeding in shallow, temporary water bodies. In the atlas region it inhabits a variety of veld vegetation types in the Savanna Biome. Breeding habitat includes pans, waterholes artificial impoundments, as well as the isolated pools form in riverbeds as water levels drop. The substrate usually consists of fine mud or clay, but burrows have been observed in coarser sandy sediments too.

These burrowing frogs spend the dry season in a torpid state, underground. They begin to call as soon as the first spring rains have soaked the ground, sometimes even before standing water has accumulated at the breeding site. At this time they construct extensive, shallow tunnels, in low muddy areas that are likely to be filled with subsequent rains, or close to the edges of pools that have already formed. The tunnels form conspicuous low ridges on the surface, often intersect, and sometimes terminate in larger, rounded chambers. Males usually leave the tunnels and call from the ground surface, but in the absence of ground cover they may call from within the tunnels or chambers which are situated within the seasonal or temporary wet zones of the pans.

Choruses at Hans Merensky Nature Reserve typically comprise groups of 5-8 males that participate in discrete bouts of calling, alternating with periods of silence (L.R. Minter pers. obs.). One male usually initiates calling, followed by the other members of the group. Calling males may be as little as 30 cm apart. Amplexus is inguinal. Once in amplexus, the female selects a suitable oviposition site and disappears beneath the surface, male in tow, to excavate a nest. Nests may be constructed in low-lying areas that are subsequently flooded after rain (Kaminsky et al. 1999), or in more elevated positions away from the water's edge. For example, nests have been found 15 cm to 8 m from the water's edge in South Africa (L.R. Minter pers. obs.; Jacobsen 1989). A chamber is constructed in which a spherical mass of 88-242 eggs are laid (Rödell 2000). Sterile jelly capsules are laid on top of the fertile eggs and the entire mass is bound together by a fibrous substance that prevents the egg mass from being flattened by the female who sits on top of the mass until the eggs hatch. After hatching, the developing tadpoles cling to the body of their mother, who actively defends them against intruders (Rödell et al. 1995).

In dry weather, development is arrested and the tadpoles can remain in the chambers for as long as two months in anticipation of the next rains (Kaminsky et al. 1999). If rising water floods the nest, the tadpoles leave it (at any stage of development) and enter the water to feed. If the nest is not flooded, the female provides an escape route from the nest to the

water by constructing a surface slide down which the tadpoles wriggle to the water (Kaminsky et al. 1999). If this is not possible, the female may carry her tadpoles to the water while they adhere to her body. Since the tadpoles of *H. marmoratus* often begin their development before the breeding site contains water, they have an advantage over tadpoles of species that lay their eggs only after heavy rain in that, once the site is flooded. *Hemisus* tadpoles take less time to reach metamorphosis and are exposed to predators for a shorter period of time. The early development of the tadpoles may be an adaptive advantage in the seasonal Kruger National Park pans which are shared with potentially carnivorous tadpole species such as *Hildebrandtia ornata*. These adaptations are advantageous in environments where rainfall is unpredictably highly variable, such as the Kruger National Park, were pans may desiccate within a short period of time if there are inadequate or subsequent additional rainfall events.

The tadpoles are medium sized with a plump ovoid body and measure up to 55 mm. The tailfin slopes up the body with the deepest point in the middle of the tail. The colour of the body is mottled yellow-brown to grey and abdomen is white. Tadpoles collected from vegetated pans where brown-green in colouration whilst those collected from muddy pans were grey in colour. Tadpoles emerge from jelly capsules in approximately 8 days.
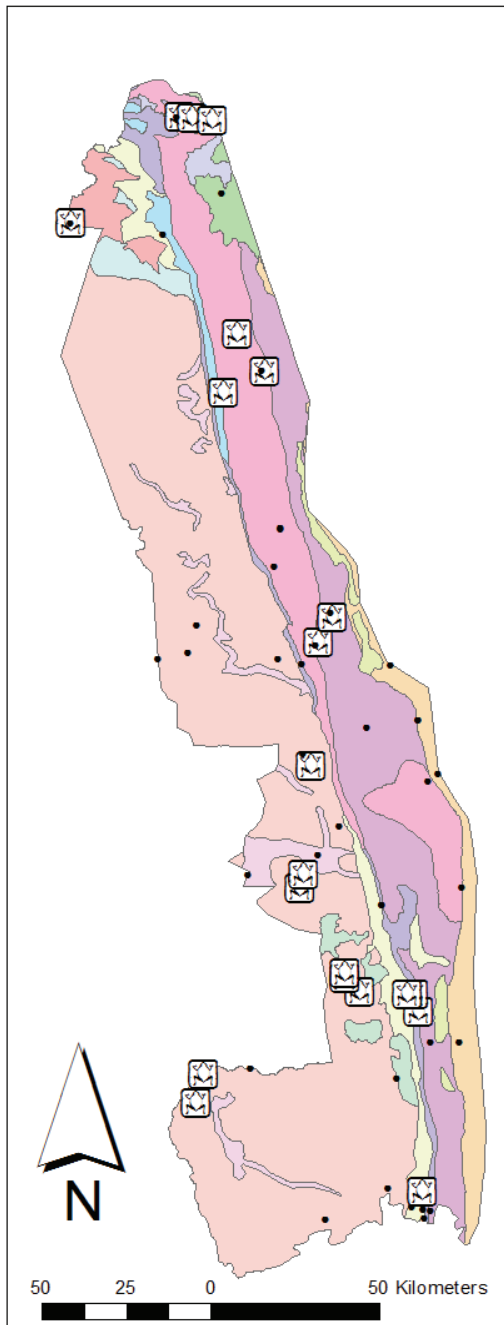
Figure 5.9: Distribution of *Hemisus marmoratus* during this study.

The female remains behind with the eggs until they hatch and the tadpoles migrate out of the brood chamber when it floods or the mother may carry the eggs to the water or even construct a channel from the tadpoles towards the water. In unfavourable weather such as in dry periods the egg development may be arrested for up to two months (Du Preez and Carruthers 2009).

Southern Ornate Frog (*Hildebrandtia ornata*)

The species is widely distributed throughout the park especially in the open bushveld savanna (Pienaar et al. 1976). During the current survey tadpoles and emerging juveniles were recorded throughout all the regions of the park. Adults were recorded calling in low densities at Lannea Pan and Hildebrandtia Pan in the Tshokwane area.

In South Africa, the species inhabits a variety of bushveld vegetation types in the Savanna Biome, particularly areas with deep, sandy soils. It breeds in shallow temporary pans in dry, open woodland, often with emergent grass, and has also been recorded calling from pools on top of granite inselbergs (Jacobsen 1989; Channing 2001). Due to the fossorial habit of the species, the frogs are rarely seen above ground, except during the rainy sea son when adults are sometimes seen crossing roads in wet weather, or feeding on alate termites when they emerge en mass.

In Kruger National Park, adults have been found under rocks and logs during the dry season, within 50 m of their breeding site (H.H. Braack pers. obs.). Adults and emerging froglets were collected from several pans in the Kruger National Park. Two distinct colour morphs were observed emerging from the pans. A more common golden-brown backed morph with a single green stripe stretching from mouth to the anus and a less common green-backed morph. At Hildebrandtia Pan in the Tshokwane area the majority of emerging froglets were the green-backed morph emerging from the dense hydrophilic grasses and sedges on the margins of the pan. Both the colour morphs are extremely cryptic amongst the grasses and sedges and the froglets are extremely agile.

Breeding usually occurs in early summer but may take place in mid- to late summer if rains are delayed (Amiet 1974; Rödell 2000). *H. ornata* is an explosive breeder. Strong choruses develop immediately after heavy rain but die away within a relatively short period (Theron and Minter 2004). At Hans Merensky Reserve, males started to call at dusk as they approached the breeding site and took up positions c.50 cm from the water's edge. Amplexus is axillary and may take place early in the evening and two amplexing pairs were observed at 19:00. Several newly laid batches of eggs, found in a grassy pan on the reserve, each consisted of a single layer of eggs forming one large, floating mass. Two batches of

eggs laid in captivity numbered 838 and 1171 respectively. The mean diameter of the eggs was 1.96 mm (5.12 mm including jelly capsule, n = 20). Embryos emerged from the capsules after 36 hours (L.R. Minter pers. obs.). The tadpoles are medium-large sized with a heavy built ovoid body and measure up to 95 mm. The tailfin starts behind the body and shows a slightly convex border which tapers to an acute whip-like point. The colour varies according to the substrate from a dark brown above and pale below with a metallic sheen when the tadpoles reach maturity. The Y-shaped bands become visible on the throat. The larval duration of the tadpoles is currently unknown. Tadpoles raised in captivity metamorphosed after approximately 30-35 days.

Hildebrandtia ornata tadpoles are voracious predators feeding on the tadpoles of other species. Cannibalism has been reported, although this behaviour may be a laboratory artefact (Lambiris 1989a; Rödel 2000). The tadpoles are also reported to scavenge (Channing 2001). *H. ornata* tadpoles were observed preying on tadpole within the collection containers. Several of the larger *Kassina maculata* tadpoles were severely injured and the entire tail eaten up to the head within two hours. *Hildebrandtia ornata* tadpoles readily fed on *C. xerampelina, P. edulis, L. mossambicus* and *H. marmoratus* tadpoles in experimental containers. They were cannibalistic and preyed on the smaller *H. ornata* tadpoles and scavenged on any injured or dead tadpoles. This tadpole was the dominant species; especially in the smaller muddy pools. The light colouration of the tadpoles may be used for camouflage within the muddy clay pools. These tadpoles are capable of a relatively painful bite when handled. More intensive surveys are required on aspects of the ecology and general biology of this species (Minter and Theron 2004).
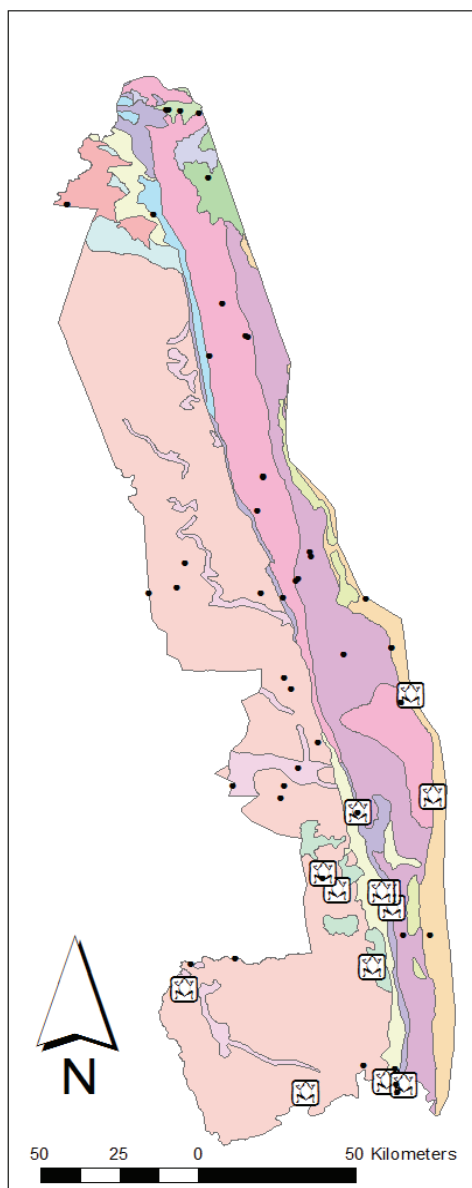
Figure 5.10: Distribution of *Hildebrandtia ornata* during this study.

Painted Reed Frog (*Hyperolius marmoratus*)

This species forms part of a large complex of geographical variants (subspecies), distributed across most of sub Saharan Africa, which are distinguished from one another by differences in dorsal colour patterns. However, adjacent populations with different colour patterns often integrate to a greater or lesser extent, while within populations themselves; there is variation in the colour and markings of juveniles and adult males. These factors, combined with a scarcity of behavioural and other data, have resulted in a confused and unresolved taxonomy (Poynton 1964, Pienaar et al. 1976; Poynton and Broadley 1997; Passmore and Carruthers 1995; Channing 2001).

On-going investigation of the ecology, advertisement call structure and genetics of populations from different parts of Africa has already revealed the presence of cryptic species, and is gradually unravelling the systematics and stabilising the taxonomy of this complex (Channing 2001).

Channing (2001) listed 13 subspecies of *H. marmoratus* distributed through sub-Saharan Africa, of which three occur in the South African region. *H. m. taeniatus* is distributed from Limpopo province, Mpumalanga and Swaziland, southward to about St Lucia, where it intergrades with *H. m. marmoratus* (Lambiris 1989). The latter occurs from St Lucia southward to west of Port Edward, while *H. m. verrucosus* is recorded from the south coast of KwaZulu-Natal, southward along the Eastern Cape coast to Tsitsikamma in the Western Cape (Lambiris 1989; Passmore and Carruthers 1995). There is currently no evidence to suggest that cryptic species are represented within these subspecies of *H. marmoratus*.

In recent years, several populations of *H. marmoratus* have been recorded further west of Tsitsikamma, through Swellendam, to the Cape Peninsula. While these new records may reflect a natural range extension, it is more likely that they have resulted from accidental or deliberate human assisted translocations. Also, some of the westerly records in Limpopo, Gauteng and Mpumalanga provinces may be the result of translocations. For example, specimens of *H. m. taeniatus* have been encountered in consignments of bananas arriving in Cape Town from KwaZulu-Natal or Mpumalanga (A. de Villiers pers. obs.) and have been found in Pretoria on nursery plants originating from KwaZulu-Natal. This species has a loud, distinctive call and forms large choruses that are active for an extended period during the breeding season (Bishop 2004).

Hyperolius marmoratus inhabits a variety of vegetation types within the Savanna. Grassland and Forest biomes, and occurs marginally in the Fynbos Biome. It is a wide-spread and abundant species along the coast and at low altitudes east of the Great Escarpment in

Limpopo Province, Mpumalanga and Swaziland. However, large breeding populations are also recorded at higher elevations, e.g. 1300 m at Ixopo in the KwaZulu-Natal Midlands, and 1400 m at Haenertsburg in Limpopo Province. Jacobsen (1989) recorded this species in montane grassland at 1600 m. *Hyperolius marmoratus* utilizes a wide variety of breeding sites, ranging from temporary ponds, pans and vleis, to permanent bodies of water such as dams, marshes, reed beds, sluggish rivers and streams (Pienaar et al. 1976; Poynton and Broadley 1987; Lambiris 1989, Channing 2001). The species was common and widespread throughout the Kruger National Park (Pienaar et al. 1976) and was confirmed during the current survey of the park.

The adults aestivate during the dry season, and have been found sheltering some distance from their breeding sites in vegetation or under logs and stones. During this time they often take up residence inside houses where they conceal themselves behind cupboards, pelmets, pictures and in toilet cisterns. Although males will call after rain at any time of the year, breeding normally takes place October-February. Males have been observed calling in late March and early April in the Kruger National Park. At low altitudes male calling behaviour is inhibited by temperatures <16°C, while at higher altitudes breeding has been observed at temperatures <10°C (P. Bishop pers. obs.). Before dawn, breeding adults usually move into the canopy of surrounding trees or bask in the sun on emergent vegetation at the edge of the breeding site (P. Bishop pers. obs.).

In the Kruger National Park adult males have been observed resting approximately 80-500 m from the breeding site, especially on the dark-green leaves of Buffalo Thorns (*Ziziphus mucronata*). At dusk they descend to the water body where males take up specific call sites (to which they return on consecutive nights) and call consistently from dusk to just after midnight. Males occasionally call from their resting places in tree canopies in the late afternoon. Where present, tall emergent plants such as reeds and sedges (e.g. *Eleocharis limosa*, *Cyperus papyrus, Cyperus textilis* and *Typha capensis*) are favoured as call sites, but males will also call from trees, grasses, bushes, floating vegetation such as Blue Water Lily *Nymphaea nouchali* or muddy soils at the water's edge.

In the Kruger National Park males are recorded calling and breeding within the seasonal pan systems but they are found in larger densities within the dense reed beds, situated within the backwaters or seasonal pools within the macro-channel banks of the major river systems such as the Crocodile, Sabie, Letaba, Olifants, Shingwedzi, Luvuvhu and Limpopo rivers. Males call from elevated positions within the Lowveld Reed (*Phragmites mauritianus*) beds as well as emerging vegetation such *Papyrus textilis* and *Typha capensis*.

On average, males call for only a few nights in a row, returning to the breeding chorus after a period of about 10 days (Dyson et al. 1992). Gravid females enter the pond shortly after dusk and usually select a mate within a few hours. After several hours in axillary amplexus, the eggs are laid in water. Females have been observed to lay more than one clutch of eggs per season with a month-long interval between layings (P.J. Bishop pers. obs.). From 150 to 650 eggs are laid in flattened clumps of approximately 20, on the surface of submerged leaves, stalks or stones, or amongst the roots of aquatic plants (Pienaar et al. 1976; Channing 2001).

The tadpoles are medium sized with a horseshoe-shape and measure up to 44 mm. The tailfin tapers to a sharp tip. The colour of the body is brown with pale underside and speckled fins with the last 25% of tail often jet-black. Tadpoles hatch within five days and metamorphosis takes 6-8 weeks (Pienaar et al. 1976). Males preach sexual maturity at approximately one year (A. Turner pers. obs.).
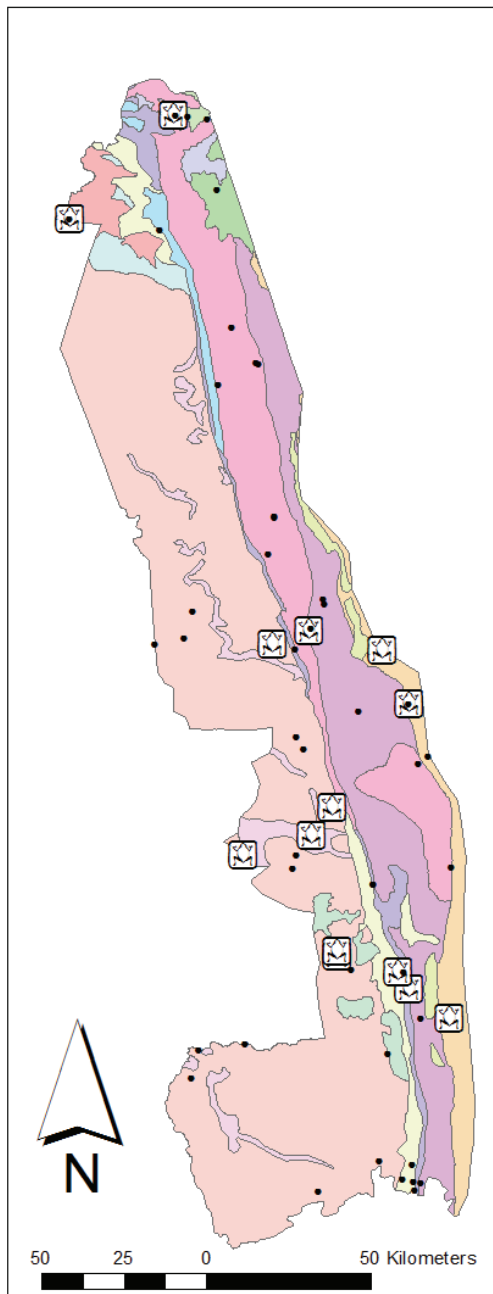
Figure 5.11: Distribution of *Hyperolius marmoratus* during this study.

Water Lily Reed Frog (*Hyperolius pusillus*)

The species usually occurs in low-lying coastal areas but further inland in the northern parts, it is found at higher altitudes, as in Malawi and north-western Botswana (Channing 2001). The species have a widespread distribution throughout the Kruger National Park (Pienaar et al. 1976). *Hyperolius pusillus* inhabits open savanna and grassland, breeding in shallow pans, ponds, vleis and dams with water lilies *Nymphaea spp.*, or at least some form of floating vegetation such as *Ludwigia stolonifera*. Tadpoles and adults were observed throughout all regions of the park in suitable habitat consisting of pans with floating vegetation; especially water lilies (*Nymphaea nouchali*) and *Ludwigia stolonifera.*

In the southern African region it occurs in a variety of bushveld vegetation types, from Coastal Bushveld- Grassland along the coast of the Eastern Cape and KwaZulu-Natal, to Mixed Lowveld Bushveld in the low-lying areas of Limpopo Province, east of the Great Escarpment. Breeding populations were also found in ponds in plantations of pine and *Eucalyptus*, south of Piet Retief (Alexander 2004)

Very little is known about the non-breeding behaviour of *H. pusillus*. In the Kruger National Park breeding takes place from mid-October to the end of March. Males form dense choruses on floating vegetation and leave the breeding site only in the early hours of the morning to ascend into surrounding trees (Passmore and Carruthers 1995). On occasions, males have even been found calling from the tree canopy at the start of the chorus in the early evening, apparently stimulated to call from these atypical sites by the chorus at the breeding site. Choruses are characterised by high levels of aggressive behaviour between males, especially in the early evening when calling is at its peak. At such times, a minimum distance of 25 cm is maintained between calling males by means of the advertisement call, an encounter call, and physical contact between calling males (Cleminson 1991). During territorial disputes, males often butt one another with their vocal sacs (Passmore and Carruthers 1995)

About 500 light green eggs are laid in groups of 20-120, in a single layer between the overlapping margins of lily leaves, with the jelly surrounding the eggs acting as a glue to keep the leaves together (Wager 1965). A clutch of approximately 80 eggs were observed glued between the leaves of *Nymphaea nouchali* at Nhlanguleni Pan in the Tshokwane areas of the southern Kruger National Park. While this specialized oviposition site reduces predation, it may also be a limiting factor at sites where population densities are high (Telford 1982).
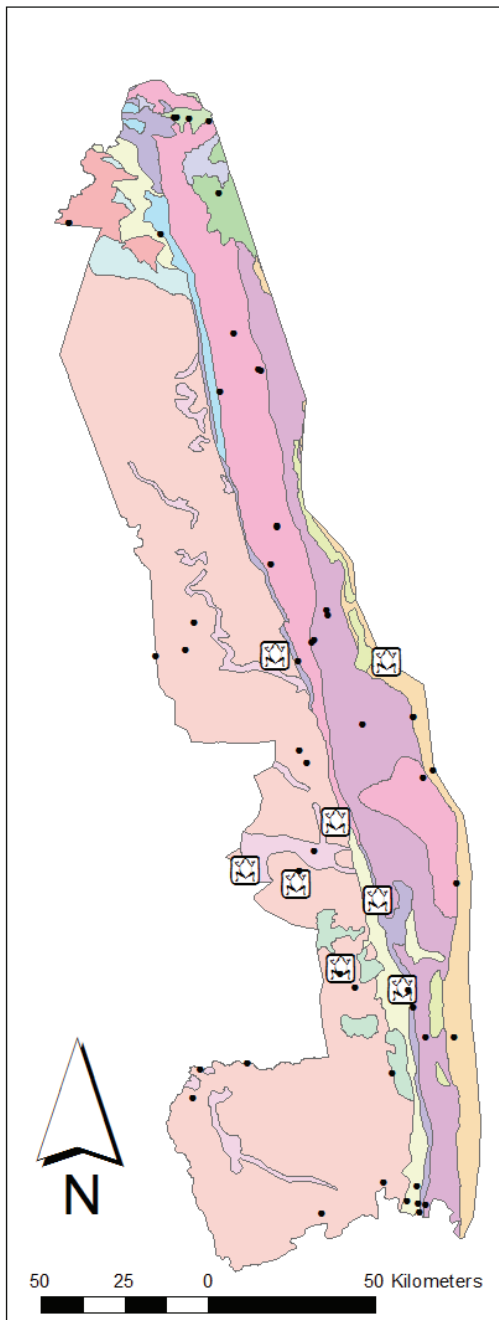
The tadpoles are small-medium sized with an ovoid body and measure up to 35 mm. The tailfin is shallow and tapers to a sharp tip. The colour of the body is greenish-brown, white below and become more green at they develop. Tail fin is slightly mottled with silvery blotches and the last 30% of tail is often jet-black. Metamorphosis is completed after five to six weeks (Du Preez and Carruthers 2009).

Hyperolius pusillus adults feed on ants and flying termites, and are preyed upon by the Yellowbilled Egret *Egretta intermedia* (Channing 2001), Common Brown Water Snake (*Lycodonomorpghus rufulus*), Red-lipped Herald (*Crotaphopeltis hotamboeia*) as well as Mozambique Tilapia *Oreochromis mossambicus.* The call sites favoured by this species make it particularly vulnerable to predation by pisauridae spiders.

Figure 5.12: Distribution of *Hyperolius pusillus* during this study.

Tinker Reed Frog (*Hyperolius tuberilinguis*)

This species was first recorded from the Kruger National park in 1989 at Mabyendzawo stream In the Lower Sabie section and in weir on the Sabie River at Lower Sabie and again in 1990 from the weir on the Sabie River (Kruger Park Database). Approximately 5 adult males were observed calling from dense Lowveld Reed (*Phragmites mauritianus*) beds

around a seasonal pool along the dammed up section of the Sabie River above the weir. The small aggregate of males were observed calling from approximately 19h00 on the 23rd of November 2010.

This species inhabits a variety of bushveld vegetation types in the Savanna Biome, particularly Coastal Bushveld-Grassland. It breeds in reed beds on the periphery of swamps or rivers (Alexander 1990), or dense vegetation surrounding inundated pans, and is often the most numerous frog in multi-species choruses. In the dry season, adults aestivate in secluded places, often entering houses. In summer, they breed over a prolonged period, forming dense choruses (Pallet and Passmore 1988).

In Durban, calling starts at the beginning of September and continues to the middle of April (Alexander 1987). Males call from perches in dense stands of emergent vegetation c.50 cm above the water, and are difficult to locate. Spacing of calling males is maintained by vocal aggression and physical combat (Pallet and Passmore 1988). Males leave breeding choruses in the early hours of the morning and ascend into trees surrounding the breeding site, returning to the breeding site the following evening (Passmore and Carruthers 1995). Telford (1982) noted that males exhibit high call-site fidelity, returning to the same site on successive nights unless disturbed by predators or displaced by competitors.
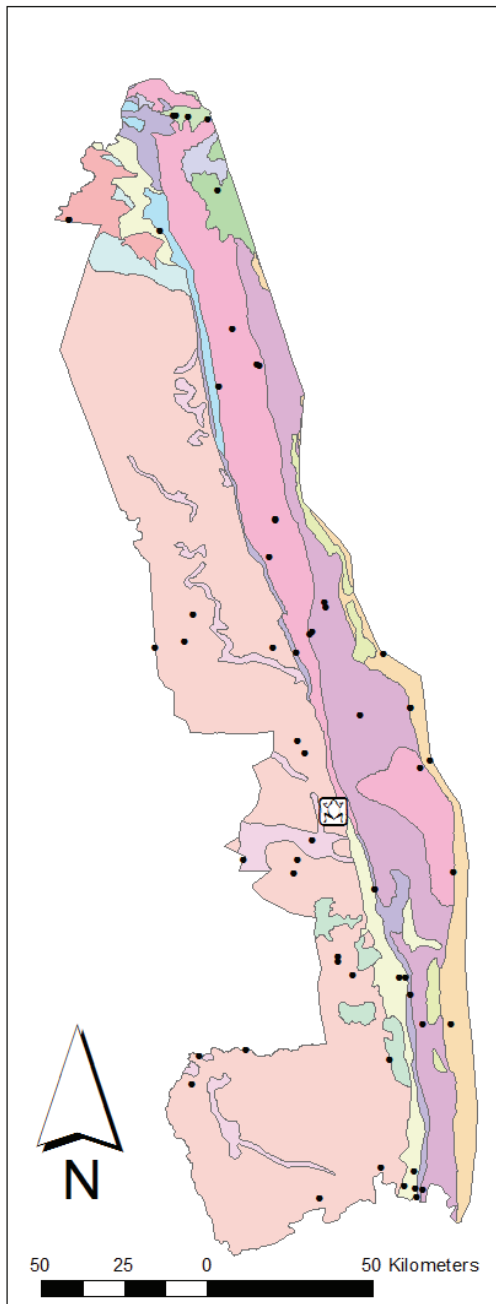
Figure 5.13: Distribution of *Hyperolius tuberilinguis* during this study.

Even though males defend call sites from conspecific males, the call site is not used as the oviposition site (Telford 1982). The eggs are enclosed in a gelatinous cake, which is attached to reed or grass stems above the waterline (Poynton 1964) and the tadpoles drop into the water as they hatch. The white eggs and their jelly capsules are 1.5 mm and 4 mm in diameter, respectively (Wager 1965).

The tadpoles are small-medium sized with an ovoid body and anteriorly dorso-ventrally flattened and measure up to 46 mm. The tailfin is deep and curved with the deepest part in the middle of the tail. The colour of the body is mottled-brown and speckled dorsally with golden pigment cells called iridiophores and the tail shaft dark with the middle third very dark. The larval duration is currently unknown (Channing 2001.)

Red-Legged Kassina (*Kassina maculata*)

Kassina maculata is essentially a lowland species, although there is an unusual record of specimens from the Vumba Mountains (Zimbabwe) at an altitude of 1400 m (Poynton 1964). The species inhabits a wide variety of bushveld vegetation types, predominantly Mixed and Sweet Lowveld Bushveld and Coastal Bushveld-Grassland, in the Savanna Biome. The breeding habitat consists of well vegetated pans, vleis, marshes and dams. Outside of the breeding season, during the dry winter months, *K. maculata* has been found in cavities excavated in the damp mud at the bottom of empty pans (Pienaar et al. 1976), or under moist debris (Loveridge 1953a). The breeding season usually begins in early November and continues until the end of February, depending on weather conditions (Bishop 1994). Males were observed calling in March in the Kruger National Park.

During the day, the frogs are rarely seen, remaining well hidden in clumps of dense vegetation (particularly *Eleocharis limosa*, *Cyperus immensus* and C*. papyrus*) and *Nymphaea nouchali* and occasionally under logs or in the axils of banana leaves. Choruses develop in the late afternoon or early evening, before dusk. Males move closer to the water where they call from the water, well concealed under overhanging vegetation. Later they may be found calling from elevated positions, up to 50 cm above the water, in clumps of emergent vegetation (Bishop 1994). The males appear to have two distinct calling periods, one in the early evening (16:30-20:00) and another in the early morning, peaking between 02:00 and 03:00 (Bishop 1994). The eggs are laid singly or in lines of four or five, attached to submerged vegetation. The tadpoles are large and fish-like, similar to, but larger than those of *K. senegalensis*. They are up to 130 mm long with deep keel-like tails that arise from the top of the head. *Kassina maculata* is a voracious predator of invertebrates and also eats small frogs, such as *Hyperolius marmoratus* and *Afrixalus aureus* (Wager 1965; Channing

2001). In the laboratory tanks *K. maculata* tadpoles preyed on the tadpoles of *Hyperolius pusillus*, *Phrynomantis bifasciatus* and *Afrixalus aureus* (C.L. Cook unpubl. data).

Kassina maculata produces a characteristic odour when handled and is quite unpalatable, due to the production of defensive amines and peptides, such as caerulein and kassinin, by glands in the skin. In spite of these defences, there are records of predation on this species by the Yellowbilled Egret (*Egretta intermedia*) and the Vine Snake, *Thelotornis capensis* (Channing 200I).

The tadpoles are large sized with a deep ovoid body and measure up to 130 mm. The upper fin is high which starts at the back of the head. The colour of the body is mottled-brown with the fin usually darkly mottled but may be bright red. Development is currently unknown but may take two to three months (Du Preez and Carruthers 2009) up to 8-10 months (Channing 2001). Tadpoles raised in captivity developed within 72-90 days (C.L. Cook unpubl. data). The tadpole can consume large amounts of plant matter chewing the stems and leaves. They were only found in well-vegetated pans.
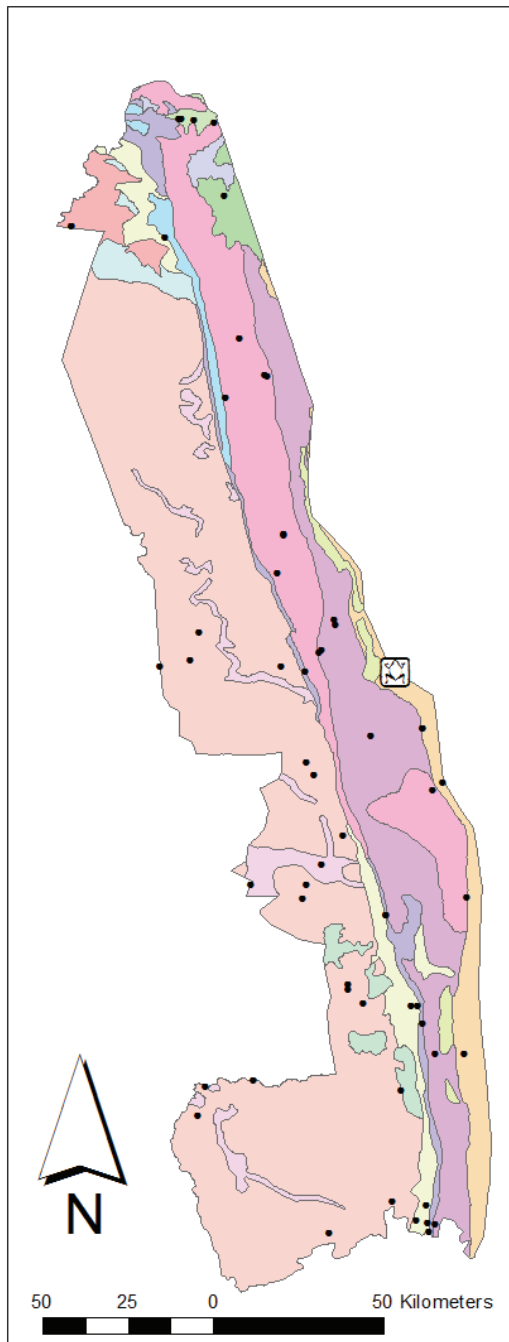
Figure 5.14: Distribution of *Kassina maculata* during this study.

Bubbling Kassina (*Kassina senegalensis*)

Kassina senegalensis is one of the most widely distributed frog species, occurring throughout almost all of sub-Saharan Africa. It is found in suitable habitats at low and high altitudes, from Senegal in West Africa, eastward to Somalia and southward to South Africa.

In the Kruger National Park it is one of the most common frog species and was recorded throughout the park (Pienaar et al. 1976) as well as during the current project. *Kassina senegalensis* has a distinctive call, an explosive, short "boip", reveals the presence of the species from great distances and clearly distinguishes it from all other species (Weldon and du Preez 2004).

Kassina senegalensis inhabits a wide variety of vegetation types in the Savanna and Grassland biomes (Poynton 1964, Passmore and Carruthers 1995). Breeding habitat comprises both temporary and permanent water bodies, including well-vegetated shallow pans, vleis and marshes, as well as deeper dams (Rödel 2000).

During the dry season, *K. senegalensis* aestivates under logs and rocks and inside termitaria and burrows of the Giant Girdled Lizard, *Smaug* (*Cordylus*) *giganteus.* Individuals were found as far as 1200 m from an ephemeral pan, at the end of the breeding season when the frogs moved away from the water to seek shelter for the winter (Kok et al. 1997). Breeding takes place from spring to late summer. During the day, adults hide under vegetation or rocks, or in burrows, emerging in the late afternoon to make their way to the breeding site.

Figure 5.15: Distribution of *Kassina senegalensis* during this study.

They begin to call around dusk (18h00) while some distance from the water (c.20-50 m), before taking up positions near the water's edge, well concealed beneath the grassland vegetation as well as small shrubs. In wet weather, calling takes place during the day as well as at night. The vocal repertoire includes an advertisement call as well as a longer, pulsed, territorial call (Fleischack and Small 1978).

Amplexus is usually initiated out of the water. Between 100 and 500 eggs are laid singly in shallow water: they are adhesive and may stick to submerged vegetation, rocks or other objects, or sink to the bottom. One amplexed pair dispersed their eggs over a distance of 3 m, depositing 1-15 eggs at a number of sites c.30 cm apart (Fleischack and Small 1978). The eggs are 1.1-1.8 mm in diameter in 3-mm jelly capsules, and they hatch within 5-6 days. The tadpoles are medium-large sized with a deep ovoid body and measure up to 80 mm. The tailfin is deep and keel-like and arises from eye level to reach the deepest point about third way along the length and tapers to an acute tip. The tadpole undergoes a slow metamorphosis and may take up to two or three months. During midday they usually feed close to the surface in the mid-water and are voracious feeders consuming large amounts of plant material (Du Preez and Carruthers 2009). *Kassina senegalensis* preys on a variety of arthropods, including termites, caterpillars, ants, flies and spiders (Weldon and du Preez 2004).

Brown-backed Tree Frog (*Leptopelis mossambicus*)

Leptopelis mossambicus is distributed from southern Malawi through south-eastern Zimbabwe to central and southern Mozambique (Poynton and Broadley 1985a; Lambiris 1989a). In Kruger National Park it is widely distributed occurring throughout all the regions of the park (Pienaar et al. 1976) and current survey.

This species inhabits a variety of bushveld vegetation types in the Savanna Biome, as well as Sand Forest and mangrove swamps. It seems to prefer moist, wooded, low-lying areas where it lays its eggs under leaf litter next to shallow pans, pools and streams. *Leptopelis mossambicus* retreats underground during the day and during the dry winter months. Wager (1965) noted that captive individuals spent the dry season (5-6 months) buried in the soil at a depth of 25 cm below the surface. Breeding begins after the first heavy summer rains in November, and continues through January. Males call from elevated positions on grass, reeds, sedges, shrubs and trees, usually no more than 1.5 m above the ground. These call sites are normally near open water, but may be several hundred metres distant. The males are territorial and produce aggressive calls when other males are in close proximity. If the intruder does not move away, protracted fighting may occur (Passmore and Carruthers 1995).

The eggs are laid in a shallow burrow under leaf litter near the water's edge (L.R. Minter pers. obs.). The tadpoles complete part of their development in the nest, and remain in a state of arrested development for several weeks until the next heavy downpour. When the capsules are moistened by rain, the tadpoles immediately break out and wriggle en masse to the water, where they complete their development (L.R. Minter pers. obs.).

The tadpoles are small-medium sized with an ovoid body and measure up to 35 mm. The tailfin is shallow and tapers to a sharp tip. The colour of the body is greenish-brown, white below and become more green at they develop. Tail fin is slightly mottled with silvery blotches and the last 30% of tail is often jet-black. Metamorphosis is completed after five to six weeks (Du Preez and Carruthers 2009).

The tadpoles are medium sized with a streamlined ovoid body and dorso-ventrally flattened and measure up to 60 mm. The tailfin is very long and muscular and terminates in a fine point. The colour of the body is a uniform dark brown to grey above with the tail muscles and fins densely pigmented to mottled. Larval duration is currently unknown (Du Preez and Carruthers 2009). Emerging juveniles are light green in colouration and only change to the brown colouration after 4-6 months.
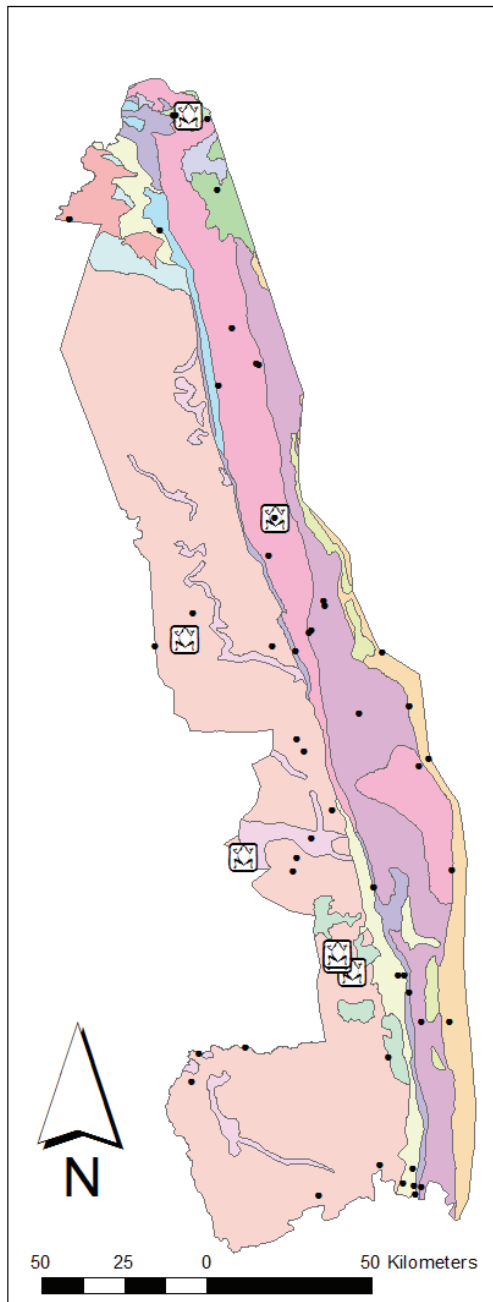
Figure 5.16: Distribution of *Leptopelis mossambicus* during this study.

Dwarf Puddle Frog (*Phrynobatrachus mababiensis*)

Several small *Phrynobatrachus* species were described from central, eastern and southern Africa, but the taxonomy of this group is still unsettled (Poynton and Broadley 1985b; Lambiris 1989). *Phrynobatrachus mababiensis* occurs from the Sahel of East Africa to the Eastern Cape Province, South Africa west to Namibia and southern Angola (Frost 2000).

The colouration and markings of this species vary considerably, but its small size and characteristic insect like call make identification relatively easy. The call may at times be confused with that of *Hemisus marmoratus*, which has a similar pulse rate and emphasized frequency. However, the latter lacks the sporadic clicks that are interspersed in the trill of *P. mababiensis* (Channing 2004).
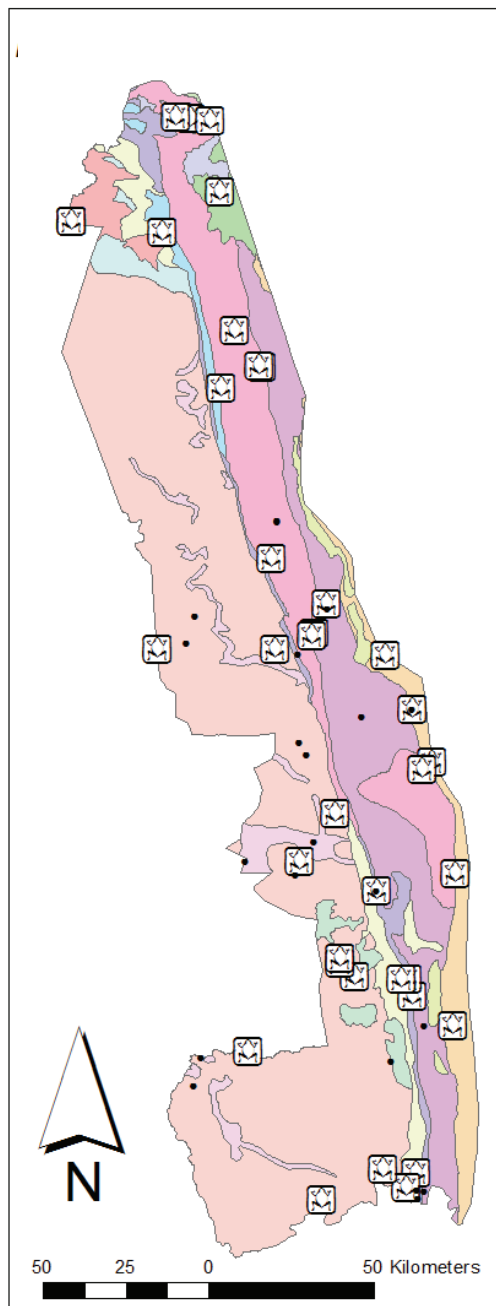
Phrynobatrachus mababiensis inhabits open to wooded savanna and less frequently, grassland, where summer rainfall is 500-1000 mm p.a. It breeds in shallow stagnant water amongst emergent vegetation on the edges of grassy pans, vleis, marshes, small dams and ponds, and in the backwaters of slow-flowing streams. The species is also found in disturbed habitats near villages and other developments (Channing 2004). The species is common with a widespread distribution throughout all the regions of the Kruger National Park (Pienaar et al. 1976). This common species was observed calling during the day as well as night around the majority of pans within the Kruger National Park. The small tadpoles may have been missed in certain of the larger pan systems.

Figure 5.17: Distribution of *Phrynobatrachus mababiensis* during this study.

Dry periods are spent in aestivation. During the dry winter at Xipudza in Kruger National Park adult *P. mababiensis* were found sheltering under stones (H.H. Braack pers. obs.). This species has an extended breeding season that begins after the first spring rains. Males call from the water's edge, well concealed by vegetation. Choruses are usually strongest at dusk, diminishing after nightfall. In overcast or rainy weather, calling continues throughout

the day and night. Aggression between males occurs frequently (Wager I965). Eggs are laid in a single flat layer 0.5 cm in diameter that floats on the water.

The tadpoles are small sized with an ovoid body and blunt snout and measure up to 18 mm. The tailfin is moderately deep and reaches deepest point around the middle of the tail and tapers to a fine point. The colour of the abdomen is dark with silver iridophores and transparent gill and throat regions. Metamorphosis is completed after about five weeks, at which stage the juvenile froglets are only 6 mm long (Wager 1965; Pienaar et al. 1976). This frog was recorded breeding within a single water-filled elephant track as well as in inundated lawns from a leaking tap within the Skukuza research camp.

Snoring Puddle Frog (*Phrynobatrachus natalensis*)

Phrynobatrachus natalensis is widely distributed in the savanna of sub-Saharan Africa, from Senegal in the west to Somalia in the east and southward through East Africa. To the south, it ranges as far as north-eastern Namibia, northern Botswana, and Eastern Cape Province of South Africa. The variation in clutch size, tadpole morphology, size of the adult frog and period of-activity, suggests that this taxon may comprise more than one species (Rödel 2000). This species has a characteristic call (Channing 2004). The species is common and widespread distribution throughout all regions of the Kruger National Park (Pienaar et al. 1976), including the current survey.

Phrynobatrachus natalensis inhabits a variety of vegetation types in the Savanna and Grassland biomes where summer rainfall is >500 mm, although some populations along the western edge of the species range are found in drier areas. The polymorphic colour pattern may be a means of protection against predators, and specific patterns have been correlated with particular habitats (Stewart 1967). Breeding takes place in shallow to fairly deep water in temporary pans and pools, vleis, dams and even small, slow-flowing streams. Wager (1986) recorded the species breeding in brackish pools near the high-water mark at the coast. Breeding sites usually have vegetation or other types of cover along their banks. *Phrynobatrachus natalensis* is tolerant of human disturbance and is often found near human habitation (Channing 2004). The species seems to be declining from wetland habitats within the urban areas of Gauteng Province.

In Kruger National Park *P. natalensis* has been found sheltering under rocks near breeding sites during the dry season (H.H. Braack pers. obs.). Adult specimens were collected from under loosely embedded rocks along the dry river banks of the Letaba, Sand and Olifants River during the current survey. Breeding begins in spring after the first rains and continues to late summer.

Males usually call from concealed sites and may be heard throughout the day and night in wet weather or sunny days following rainfall. Aggressive encounters between males are commonplace (Wager 1965). Mating pairs swim while depositing the small eggs in a single-layered plate that floats at the surface. Reported clutch sizes from West Africa are 200-1652 eggs (Rödel 2000).

The tadpoles are small-medium sized with a rounded body and measure up to 35 mm. The tailfin is of moderate depth reaching the deepest point in the middle of the tail and tapering to a fine point. The colour of the body is brown above and white below. Tadpoles reared by Wager (1965) hatched within 3-4 days and took 4-5 weeks to reach metamorphosis, but other authors report considerable variation in the rate of development (Rödel 2000).

Figure 5.18: Distribution of *Phrynobatrachus natalensis* during this study.

Banded Rubber Frog (*Phrynomantis bifasciatus*)

Banded Rubber Frog (*Phrynomantis bifasciatus*) is a widespread species is distributed from the Democratic Republic of Congo, eastern Ethiopia and Somalia, south through East Africa to north-eastern South Africa. Its range extends westward through northern Botswana and northern Namibia to southern Angola. In South Africa it is recorded from Northern KwaZulu-

Natal (north of 29°S), Swaziland, western Mpumalanga, Limpopo province, northern Gauteng and the central and northern parts of North West Province (north of 27°S and east of 24°E). Historical records from Durban and Kimberley may have been based on accidentally translocated individuals (du Preez 2004). The frog is widely distributed in the Kruger National Park especially in the lower-lying areas (Pienaar et al. 1976). This species was recorded throughout all the regions of the park during the current survey. Large choruses of males were heard calling around the seasonal wetland habitats around the Tshokwane area.

Phrynomantis bifasciatus inhabits a variety of bushveld vegetation types in the Savanna Biome, at altitudes of 50-1450 m. It appears to be adapted to living in hot, semi-arid environments. Breeding takes place in temporary pans and pools, flooded grassland and small, shallow dams (Wager 1965; Jacobsen 1989).

This frog seldom jumps, but walks or runs. When disturbed, it inflates and arches its body, tucking its head in and raising its rump to accentuate the aposematic colours and markings. These frogs may be handled without ill effects, but if unduly alarmed or hurt, they produce copious skin secretions with an unpleasant odour. The secretions are toxic, irritant and lethal to other frogs confined in the same container. They are cardio-toxic, affecting the potassium channels in the membranes of human heart cells, and cause cell death within a short rime (Van der Walt et al. 1992). In humans, prolonged skin contact, or assimilation of the toxin via cuts or scratches on the hands, can cause extremely painful swelling and other symptoms such as nausea, headache, respiratory distress and increased pulse rate. The extreme pain in scratches on fingers was experienced whilst handling a distressed frog during the current survey. The pain and swelling persisted for several hours within my hands.

During the dry season, P*. bifasciatus* takes shelter under rocks or logs, in holes excavated by other animals, in termitaria, in holes in trees or under loose bark, in the axils of banana leaves and in drain pipes (Pienaar et al. 1976; Wager 1986; Lambiris 1989a). It often shelters with other frogs, lizards, scorpions and whip scorpions (Jacobsen 1989). Although this species is not a true climber the expanded digits enable it to climb rock surfaces and tree trunks with ease (du Preez 2004). *Phrynomantis bifasciatus* breeds during spring and summer, after sufficient rain has fallen to produce shallow pools and pans. Males usually call from concealed positions under vegetation or rocks, in holes in trees, the ventilation shafts of termitaria, or from the hoof prints of cattle, but also from more exposed sites. Males begin to call when they are some distance from the water's edge, but as the intensity of the chorus increases they move closer to the water, calling from exposed sites at the water's edge or from emergent or flooded vegetation (L.R. Minter pers. obs.).

At Hildebrandtia Pan in the Tshokwane area of the southern Kruger National Park several males were observed calling from the rank hygrophilous grasses and sedges within the seasonally inundated zone of the pan. Males were also observed calling approximately 100 m from the pan, at the base of large rocks and stumps. The rocks and stumps were possibly chosen for amplifying the males' vocalizations. These frogs are opportunistic in that they will breed in the smallest bodies of water. For example, tadpoles have been seen in the water-filled prints of animals such as elephants (Channing 2001).

No *P. bifasciatus* tadpoles were observed in elephant prints around the pans within the Kruger National Park. Species recorded calling or breeding (eggs and tadpoles) within elephant spoor were *Cacosternum boettgeri*, *Pyxicephalus edulis*, *Hildebrandtia ornata*, *Phrynobatrachus mababiensis*. Recently deposited clutches of eggs as well as tadpoles of *Pyxicephalus edulis* and *Hildebrandtia ornata* in elephants spoor situated within clayey soils in inundated grassland. The spoors were completely desiccated within 96 hours resulting in the death of all tadpoles.
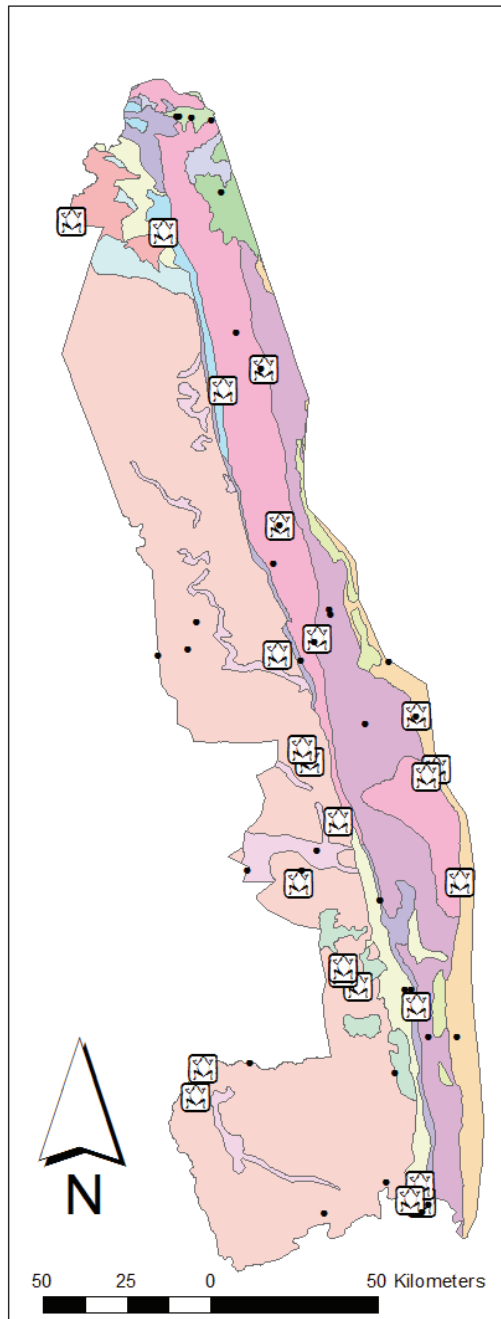
Figure 5.19: Distribution of *Phrynomantis bifasciatus* during this study.

The eggs are light brown at one pole, 1.3-1.5 mm in diameter, and are surrounded by a jetty capsule that expands from 4 to 7 mm in diameter (Stewart 1967). Clutches of 300-1500 eggs

are laid in a mass of jelly, c.75 mm. that is attached to vegetation or sinks to the bottom of the pool. Tadpoles hatch after four days (Power 1927). The tadpoles are gregarious. They resemble *Xenopus* tadpoles, but lack tentacles and have deeper, pigmented fins (black or red). They are filter-feeders, maintaining their position in the water column by means of a rapidly undulating tail tip. Tadpoles usually reach metamorphosis after about a month, depending on the availability of food (Wager 1986), but may take 90 days in captivity (Power 1927). The adults feed mainly on ants, but also consume other Hymenoptera, termites, grasshoppers and spiders (Jacobsen 1982). The Hammerkop (*Scopus umbretta*) is reported to prey on this species (Channing 2001).

Northern Pygmy Toad *(Poytonophrynus fenoulheti*)

Poytonophrynus fenoulheti occurs north of South Africa in Zimbabwe and adjacent parts of eastern Botswana, southern Zambia and Namibia's Caprivi Strip, as well as the higher-lying parts of southern Mozambique (Channing 2001). *Poytonophrynus fenoulheti* occurs at altitudes ranging from sea level to about 1700 m. In the Kruger National Park they occur mainly within the sandstone hills north and west of Punda Maria, the Sandveld around Machayi Pan, the Lebombo around the Klipkoppies rangers' post, Nwanedzi and Pumbe Pan (Pienaar et al. 1976).Tadpoles records of this species were collected from an old gravel borrow-pit within the Tshokwane area during the current survey. Lack of records of this species may be due to the selection of major pan systems for the survey and limited surveys within suitable breeding habitats for *P. fenoulheti.*

Poytonophrynus fenoulheti inhabits a variety of bushveld vegetation types in the Savanna Biome and is occasionally found in adjacent grassland. Its distribution lies within the summer-rainfall region. Although occasionally found in sandy areas, these frogs usually occupy rocky outcrops, taking refuge between rocks or on soil under stones. In these situations they occur singly or in small groups of 5-6 (or as many as nine) individuals, often together with scorpions and lizards (Jacobsen 1989). In Zimbabwe, they have also been found sheltering under shallow, loose, matted layers of sand and roots overlying rocks (Lambiris 1989b). Breeding usually takes place in temporary pools such as those on flat rocky outcrops or shallow rain ponds sometimes in barren areas. Breeding occurs between October and February in the Kruger National Park, but only after heavy rain (H.H. Braack pers. obs.). During the breeding season, males have bright yellow throats and call from exposed positions near the edges of rain pools or while partly submerged near the edge (Lambiris 1989a; Passmore and Carruthers 1995).

Jacobsen (1989) noted that several frogs appeared on the day after an afternoon rain shower, and some of them where observed in amplexus after being placed in bottles. He observed that strings of eggs were abundant at the edge of a rain-filled depression and hatched after 24 hours. The species breeds from late November to late January which time the males congregate in shallow pools. An axillary clasp is used during amplexus. Females produce strings of 2000 eggs that are entwined among stones and vegetation. According to Channing (2001), strings of eggs are 200 mm long and one clutch consisted of only 245 eggs.
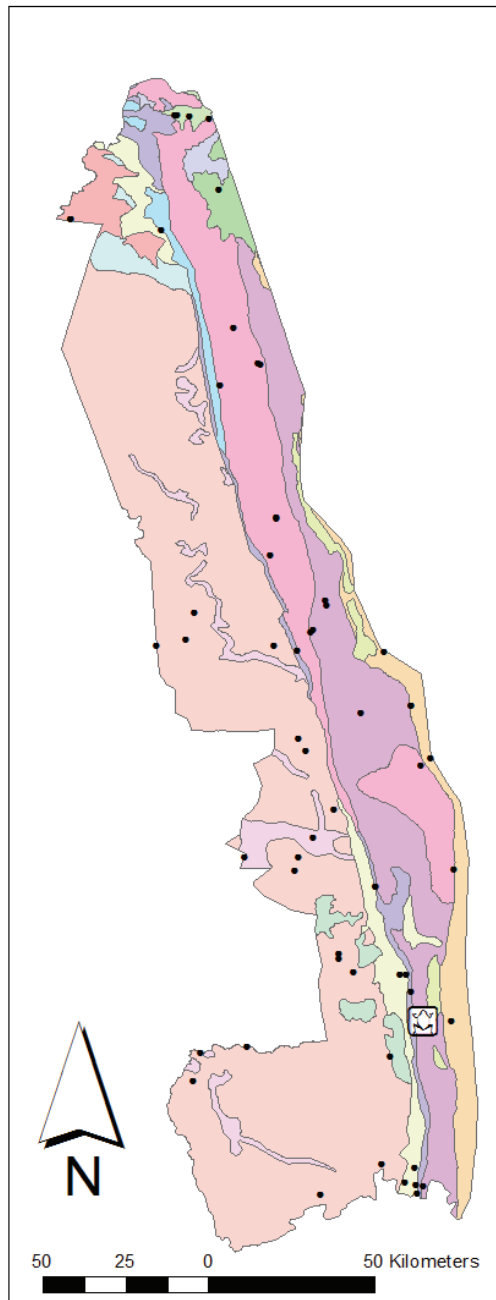
Only one distribution record of this specie was made during the current survey at Sabie Gravel Pit. Tadpoles were collected from the shallow grass dominated shoreline of the open water gravel pit. The tadpoles are small sized with an elongated ovoid body and measure up to 23 mm. The tailfin margins convexly curved and tapers to a sharply rounded tip. The colour of the body and tail shaft is black with scattered golden spots. Tadpoles feed on algae on the bottom and sides of the pools and take c.19 days to complete their development and undergo metamorphosis. Adults feed on soft-bodied arthropods taken largely from sand-free rock surfaces. Frogs kept in sandy termitaria often die after ingesting sand particles which apparently cause internal injury (Lambiris 1989a). Predators of this species in Kruger National Park include the Snouted Night Adder *Causus defilippii* and Herald Snake *Crotaphopeltis hotamboeia* (Pienaar et al.1976).

Figure 5.20: Distribution of *Poytonophrynus fenoulheti* during this study.

Plain Grass Frog (*Ptychadena anchietae*)

Ptychadena anchietae occurs in savanna habitat in all sub- Saharan countries from Angola to Ethiopia in the north, southward to eastern Namibia (Caprivi), eastern Botswana, Zimbabwe and Mozambique (Poynton and Broadley 1985b). This common species has an extended breeding period and is easily detected by its call (Minter and Passmore 2004a).

Ptychadena anchietae is a widespread inhabitant of the savanna biome in the north-eastern parts of South Africa, between 20 and 1 450 m a.s.l. It occurs in relatively moist, coastal bushveld vegetation types with a minimum annual rainfall in excess of 600 mm, as well as in more arid habitats such as Mixed Bushveld, which experiences a minimum annual rainfall of 350 mm (Low and Rebelo 1996). Individuals are often found sheltering amongst grass and plant debris on the edges of their breeding sites, which include temporary pans, shallow pools in riverbeds, borrow pits, waterholes, as well as more permanent vleis and dams (Stewart 1967; Jacobsen 1989).

Ptychadena anchietae is a common a widespread species occurring throughout the Kruger National Park; especially within the open grassland habitats and seasonally inundated pans, inundated grasslands, roadside pools, backwaters and pools within rivers and streams. They are especially abundant in relatively open grassland adjacent to seasonal pans and pools and streams (Pienaar et al. 1976).

These frogs are active on the surface throughout the year in areas where standing water is present or the soil is damp. They are able to survive lengthy periods of hot dry weather by taking shelter to avoid desiccation. In late winter to early spring, adults begin to congregate around permanent water bodies. At this time vocalization is restricted to infrequent, quiet, low trills which differ from the advertisement call (L.R. Minter pers. obs.). These infrequent vocalizations were observed during the April 2010 survey along the rivers and watercourses. Breeding choruses develop after the first spring rains, through to late summer or autumn (October-February at Hans Merensky Nature Reserve). The species was observed calling in May in the Kruger National Park. In wet weather, sporadic calling may be heard during the day, but normally commences in the early evening, peaking between 21h00 and 02h00 (Passmore 1978). Males call from bare or sparsely vegetated areas of the shoreline, usually within 20 cm of the water's edge. The average distance between calling males is about 80 cm; a distance of less than 20 cm usually elicits a territorial call that results in one of the males moving away from the other (Passmore 1978). Eggs are laid in shallow water in floating clumps of up to 300, which adhere to vegetation or sink if disturbed.

The tadpoles are medium sized with a plump ovoid body and measure up to 45 mm. The tailfin reaches the highest point in the middle of tail with a distinct hump and tapering to a fine tip. The colour of the body is grey to brown above and pale below. Larger tadpoles may have a pale triangle on the snout as in the adult frogs. The tadpoles break out of the jelly capsule after 24 hours and develop rapidly (Stewart 1967), undergoing metamorphosis and leaving the water three to four weeks later (Du Preez and Carruthers 2009).

Figure 5.21: Distribution of *Ptychadena anchietae* during this study.

Broad-banded Grass Frog (*Ptychadena mossambica*)

Ptychadena mossambica occurs in open savanna from Kenya and Uganda southward through East Africa to Namibia Caprivi), Botswana, Zimbabwe and Mozambique (Poynton and Broadley 1985b; Channing 2001). This is a highly variable taxon which requires further taxonomic investigation (Poynton and Broadley 1985b; Channing 2001). *Ptychadena*

mossambica has a loud and distinctive call and breeds over an extended period. The frog is widely distributed throughout all the regions of the park in suitable habitat. It is more cryptic during the day compared to *P. anchietae* (Pienaar et al. 1976) as well as this survey.

This savanna species inhabits several bushveld vegetation types in the north-eastern parts of South Africa at altitudes of 200-1200 m. (Jacobsen 1989). Annual rainfall in these habitats is 350->1000 mm. *P. mossambica* and *P. anchietae* are both savanna species and often occupy the same breeding sites. During summer, adults conceal themselves in grass tussocks near vleis, seepage areas, pans and dams Jacobsen 1989), floodplains of rivers and inundated grassland (Passmore and Carruthers 1995). When disturbed they take one long leap into grass, crawl under it and remain concealed (Stewart 1967). Comparatively little is known about the life history of *P. mossambica*. During dry winter months the frogs seek refuge in deep cracks in the dry mud of pans and dams (Pienaar et al. 1976), emerging to breed after the first spring rains. They were observed around the drying pans in May 2010 and December 2011. In flooded or inundated grassland or shallow, grassy pans, males call from completely concealed positions within grass tussocks, usually some distance from the shoreline. At breeding sites, where clumps of emergent vegetation are absent, calling takes place from dense vegetation at the water's edge (Passmore 1978). In more arid areas such as Hans Merensky Nature Reserve, breeding begins before vegetation has developed around the seasonal pans and dams, and *P. mossambica* calls from completely exposed positions, alongside *P. anchietae* (L.R. Minter pers. obs.).

In the southern Kruger Park males were often observed calling from exposed positions around the pans. *Ptychadena mossambica* began calling two days prior to *P. anchietae* during the 2011/2012 wet season. Calling peaks between 20h00 and midnight (Passmore 1978). Pienaar et al. (1976) recorded a batch of 315 eggs that were laid in shallow water and developed rapidly. They were grey-brown on one side, yellow-white on the other and sank to the bottom when laid. A clutch of approximately 250 eggs were laid on the surface of a small shallow pool. The eggs were floating on the surface and sank when disturbed. Diet has not been recorded, but is probably similar that *of P. anchietae* (Minter and Passmore 2004a). The tadpole of this species and the larval development is currently unknown.
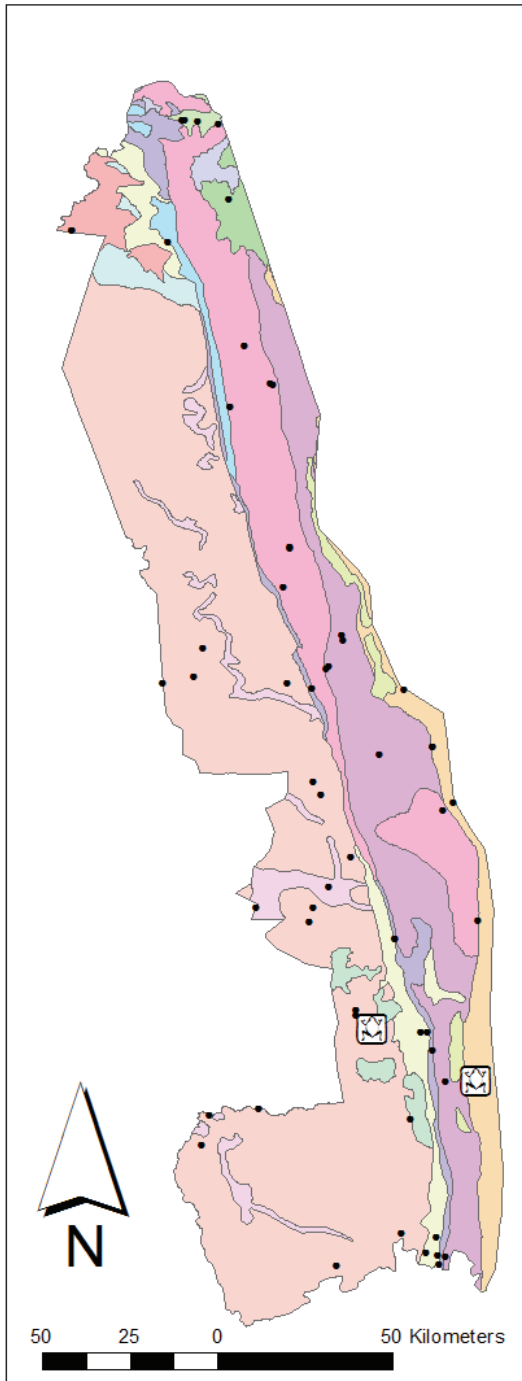
Figure 5.22: Distribution of *Ptychadena mossambica* during this study.

Sharp-nosed Grass Frog (*Ptychadena oxyrhynchus*)

This widespread species occupies savanna and woodland from Senegal, through West Africa and southward to Angola, eastern Namibia (Caprivi), northern Botswana, Zimbabwe and Mozambique (Poynton 1964; Poynton and Broadley 1985b; Channing 2001). This is a

robust species with a distinctive call that can be heard from a considerable distance. It is not as common as *P. anchietae* or *P. mossambica* (Minter and Passmore 2004b).

In the Kruger it was historically recorded in the higher lying western parts of the southern region of the Park around Pretoriuskop (Pienaar et al. 1976). The species was recorded at only two localities during the current survey. Adult males were observed calling during the day from a small seasonally inundated pool adjacent to Ramiti (2) pan in the eastern boundary of the park and an adult was collected under a large rock adjacent to the Sabie River in the south-western sections of the park.

Ptychadena oxyrhynchus inhabits relatively moist, open savanna and woodland, and is less specific in its choice of breeding site than the other *Ptychadena species*, using vleis, inundated grassland and sedge pans, as well as temporary pools, such as roadside puddles and pools on rocky outcrops (Stewart 1967; Passmore 1978; Poynton and Broadley 1985b). It occurs in most of the bushveld vegetation types in the north-eastern parts of the atlas region, from the coast to 850 m a.s.1., which receive 450 -> 1000 mm of rain per annum (Jacobsen 1989; Low and Rebelo 1996). When foraging it may enter indigenous forests and plantations of pine and eucalypts (Poynton and Broadley 1985b; Passmore 1978).

Ptychadena oxyrhynchus presumably survives dry conditions in the same way as other *Ptychadena* species, for example, by retreating into deep mud-cracks, although no specific details are recorded in the literature. In summer these frogs forage a considerable distance from their breeding sites (e.g. 600 m; Passmore 1978), and when disturbed, make use of their exceptional leaping ability to escape. Breeding takes place October-January in KwaZulu-Natal. Males call from the periphery of breeding sites, and most activity takes place within 48 hours of rain. Sporadic calling may occur during the day and early evening, but choruses reach their peak intensity between midnight and 04h00 (Passmore 1978). Eggs are laid in shallow water. The female raises her cloaca out of the water as the eggs are extruded and the male releases his sperm directly onto them by using his feet to form a funnel between his cloaca and that of the female. In this way, fertilization may be achieved before the eggs reach the water (Passmore 1978). Newly laid eggs float, but the slightest disturbance causes them to sink. A recorded batch totalled 3 476 eggs (Channing 2001).

The tadpoles are medium sized with a flattened ovoid body and measure up to 54 mm. The tailfin is fairly shallow with clear mottling and tapers gently to a rounded tip. The colour of the body is grey-brown, throat with little or no pigmentation and abdomen is immaculate.

The tadpoles hatch within two days and metamorphosis is completed in eight weeks (Pienaar et al. 1976). Food items include a variety of terrestrial arthropods- mainly Orthoptera and Arachnida (Passmore 1978).

The occurrence of *P. oxyrhynchus* in South African region is marginal in terms of its global distribution. Jacobsen (1989) noted that it is threatened by extensive habitat destruction and recommended that surveys be undertaken particularly in conservation areas. Future surveys should focus on this species within the Kruger National Park.

Figure 5.23: Distribution of *Ptychadena oxyrhynchus* during this study.

African Bullfrog (*Pyxicephalus edulis*)

Formerly synonymized with *P. adspersus* (Poynton 1964) and later treated as a subspecies of *P. adspersus* (Parry 1982; Poynton and Broadley 1985b; Lambiris 1989a), this taxon was again recognised as a full species by Channing et al. (1994a) on the basis of clear differences in advertisement call and breeding behaviour. *Pyxicephalus edulis* occurs in Mozambique (Channing et al. 1994) and extends into Kenya (Channing 2001). The species

Figure 5.24: Distribution of *Pyxicephalus edulis* during this study.

is probably widespread in Central and East Africa and its presence along the north-eastern border of South Africa indicates that *P. edulis* is also likely to occur in Botswana and Zimbabwe. It is widely distributed in suitable habitat throughout the Kruger National Park. The species was recorded from all the regions of the park with high concentrations around the northern pans around Pafuri. Adult males were observed calling in low densities (<20 males) as well as breeding at Lannea Pan as well as a seasonal pan to the north of Hildebrandtia in the Tshokwane area.

This species inhabits several bushveld vegetation types in the north-eastern parts of the Savanna Biome, from sea level to an altitude of about 1500 m (Jacobsen 1989). Flat, low-lying areas in open grassy woodland that become flooded after heavy rain or contain shallow seasonal pans, constitute prime breeding habitat and support large breeding populations (e.g. in Kruger National Park, Naboomspruit, Soekmekaar and Giyani districts.

Smaller breeding aggregations form in artificial impoundments such as roadside furrows, borrow pits and waterholes, ponds and dams (Jacobsen 1989). Channing et al. (1994) found this species breeding in rice paddies in Mozambique. This species was recorded breeding

within all regions of the Kruger National park including the larger pan systems as well as smaller seasonal pools, inundated grassland and even roadside pools.

Pyxicephalus edulis spends up to 10 months of the year in a dormant state beneath the soil surface (Mitchell 1946). The production of a cocoon to prevent desiccation has not been observed, but Stewart (1967) noted that when hibernating during the dry season, eyes are closed and depressed to the level of the head. Juveniles kept in captivity displayed similar behaviour with several skin layers covering the eyes. Breeding takes place at night (cf. *P. adspersus*) after heavy rain. No aggressive behaviour was observed in a breeding population near Beira (Channing et al. 1994) but in Kruger National park, males calling at distances of 0.5-1.5 m from each other in shallow, flooded grassland were seen charging one another (L.R. Minter pers. obs.). No aggressive behaviour was observed within the low density male aggregates of the species in pans around the Tshokwane areas. However, this species does not appear to display the same level of aggressive behaviour as *P. adspersus*. Males call from the water, with only the head and vocal sac projecting above the surface. Guarding of tadpole swarms and channel construction by males were observed in a breeding population of *P. edulis* near Jock of the Bushveld Rest Camp in the Kruger National Park (H. Braack pers. obs.). No male parental care or channel construction was observed during the current survey. No major breeding aggregations were observed within the Tshokwane area and only low density (<50 adults) breeding events were observed. The aggressive interactions between male bullfrogs are most probably density dependent and require larger breeding aggregations to be initiated.

The tadpoles are medium sized with a rounded plump body and measure up to 46 mm. The tailfin is deep and concave and reaches the deepest point in the middle of the tail and terminates in blunt tip. The colour of the body is black and fin is grey often stippling with iridophores. The skin covering the abdomen is semi-transparent revealing characteristic very long intestine. The eggs develop rapidly and tadpoles are free-swimming after 36 hours. The tadpoles complete metamorphosis in 30 days (Cook et al. 2004).

Food items include a variety of invertebrates and small vertebrates, including frogs. Several bird species, Nile Monitors, *Varanus niloticus,* and humans are known to prey on this species (Channing 2001). Bird species observed preying on migrating froglets around the Pafuri area included Marabou Stork (*Leptoptilos crumeniferus*), Saddle-billed Stork (*Ephippiorynchus senegalensis*), Yellow-billed Stork (*Mycteria ibis*) Hammerkop (*Scopus umbretta*), Fork-tailed Drongo (*Dicurus adsimilis*), Lilac-Breasted Roller (*Coracias caudatus*), European Roller (*Coracias garrulus*), Yellow-billed Kite (*Milvus parasitus*), Wahlberg's Eagle (*Hieraaetus wahlbergi*), Lesser-spotted Eagle (*Aquila pomarina*), Tawny Eagle (*Aquila*

rapax), Brown Snake Eagle (*Circaetus cinereus*), Black-headed Heron (*Ardea melanocephala*), Grey Heron (*Ardea cinerea*), Southern Ground Hornbill (*Bucorvus leadbeateri*), Southern Yellow-billed Hornbill (*Tockus lecomelas*), Black-Headed Oriole (*Oriolus oriolus*) and Woodland Kingfisher (*Halycon senegalensis*).

The effect of human predation outside protected areas should be evaluated as it appears to be relatively common in certain parts of Limpopo. More detailed studies of habitat requirements, breeding biology, duration of the larval stage and development are recommended for this species (Cook and Minter 2004).

Red Toad (*Schismaderma carens*)

The Red Toad occurs from south-eastern Democratic Republic of Congo and Tanzania, southward to Botswana, Zimbabwe and Mozambique. This species is widely distributed throughout the Kruger National Park but never as common as Olive Toads (*A. garmani*) (Pienaar et al. 1976). They don't seem to share their breeding habitat (syntopic) with other toad species and monopolize their chosen breeding habitats (Pienaar et al. 1976). Very few specimens of *S. carens* where recorded during the current survey. Tadpoles were collected from three pans in the Tshokwane area. No adults calling or metamorphs were observed around the pans or on the roads during several nocturnal surveys along the Lower Sabie-Skukuza and Tshokwane-Skukuza roads. This species inhabits a wide variety of vegetation types, primarily in the Savanna biome, but is also found in Grassland vegetation types, such as Rocky Highveld Grassland in Gauteng (Poynton and Broadley 1988; Lambiris 1989a). It breeds in deep, muddy pools or dams in these habitats (Theron and Minter 2004).

When not breeding*, S. carens* has been found in caves, mine adits, burrows and under stones, logs and piles of dead vegetation. It often enters houses, taking shelter in cupboards, plant pots, drawers and other unexpected places (Poynton and Broadley 1988; Jacobsen 1989; Lambiris 1989a). Individuals have even been found 2 m from the ground in trees. This toad seems to emerge earlier in spring and remains active later in autumn than most other summer-breeding species (Jacobsen 1989). Breeding occurs in summer, usually at the peak of the rainy season. Calling has been recorded October-January in the Suikerbosrand Nature Reserve in Gauteng (Carruthers and Carruthers 1979).The low, booming call is produced while floating in water with limbs outstretched. Calling usually occurs at night but also on overcast, humid days.

A large breeding aggregation at Hans Merensky Nature Reserve called throughout the night, laying their eggs in the early hours of the morning. By dawn, amplexing pairs were still present at the site but no egg-laying was observed (L.R. Minter pers. obs.). Eggs are laid in

double strings (cf. Stewart 1967) entwined around submerged vegetation. Estimates of egg numbers vary from about 2500 to 20 000 (Rose 1962; Stewart 1967; Passmore and Carruthers 1995; Channing 2001).

The tadpoles are small-medium sized with an elongated ovoid body and measure up to 35 mm. A peculiar horseshoe shape skin flap extends from eyes to middle of the body. The tailfin is fairly deep and end bluntly. The colour of the body is uniformly black with pigmentation over abdomen sparse to dense. Tadpoles exhibit shoaling behaviour, forming dense clusters, 10-15 cm in diameter, that slowly move through the water, possibly aiding feeding by stirring up the substrate and creating a suspension of food particles (Wager 1965; Pienaar et al. 1976; Passmore and Carruthers 1995). The churning ball of tadpoles may potentially have a respiratory function and increase oxygen levels or a thermoregulatory function by decreasing water temperatures around the tadpoles especially in the warm seasonal pans. This shoaling behaviour does not appear to deter predators as there are many records of fish, terrapins, birds and aquatic insects and their nymphs or larvae feeding avidly on these swarms (Pienaar et al. 1976; Channing 2001.). An interesting feature of the tadpole is the horseshoe-shaped fold of skin that extends backwards from behind the eyes to the middle of the body. This structure has a respiratory function, demonstrated by the fact that it is larger in tadpoles that are reared in polluted water with low oxygen content. Under these conditions the tadpoles swim close to the surface (Channing 2001). Tadpoles take between 37 to 52 days to complete metamorphosis (Du Preez and Carruthers 2009).
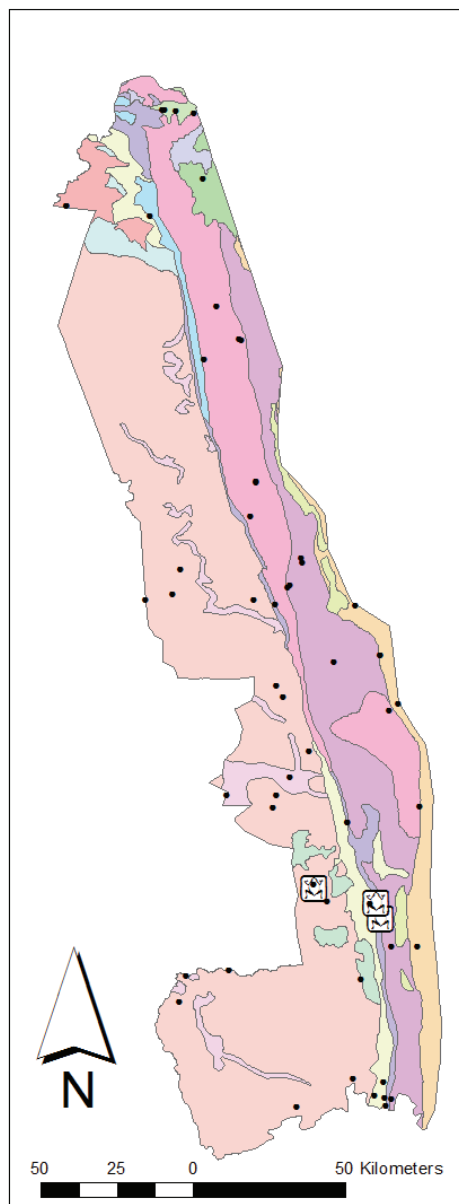
Figure 5.25: Distribution of *Schismaderma carens* during this study.

Striped Stream Frog (*Strongylopus fasciatus*)

Strongylopus fasciatus is found in highland areas of Zimbabwe, Zambia and Mozambique (Channing 2001). The absence of recent records from summer-rainfall regions, such as Limpopo Province, may be due to inadequate surveying during the species' winter breeding period (Boycott 2004b). In the Kruger National Park there is a single historic record (1959) of this species from Folly dam in the Pretoriuskop section (Pienaar et al. 1976). This species was not recorded during the current survey. It may still occur in the Pretoriuskop area; as nocturnal surveys in the area were restricted to a single survey during May 2010. Warm weather still occurred and many of the summer breeding species were still active and calling. More intensive surveys conducted during the correct climatic conditions are required around the Pretoriuskop area in order to ascertain its current status of *S. fasciatus* within the Kruger National Park.

Strongylopus fasciatus inhabits a variety of vegetation types in the Forest, Fynbos, Thicket, Grassland and Savanna biomes. it occurs in well-watered areas with annual rainfall >500 mm, and it is rarely found far from permanent water (Greig et at. 1979).It ranges mainly through the summer-rainfall region, but extends into the winter- rainfall region in the southwest. In montane grassland, these frogs seem to prefer grassy areas and reed beds along streams and rivers and around natural vleis. They are also found in well- vegetated man-made dams and ponds and along irrigation canals. They can tolerate disturbance and have been found in urban parks and gardens, and at dams surrounded by alien vegetation, in commercial forestry plantations. This species is declining from the urban areas of Gauteng (C.L. Cook pers. obs.)

Breeding takes place mainly in winter, and seems to be associated with a drop in temperature. The first calls are usually heard in mid- to late February, but there are records of calling as early as January (R.C. Boycott pers. obs.). In Swaziland, peak calling occurs in March, April and May, and calling activity ceases in November (R.C. Boycott pers. obs.). Outside the winter months, sporadic calling may be triggered by a cold front moving through the subcontinent. In the KwaZulu-Natal midlands, strong choruses have been heard in midsummer (M. Burger pers. obs.) At some breeding sites only a few calling males may be present, while at others, large choruses may form with calling males separated by only a few centimetres. Males call from the water's edge or from elevated positions in reeds and grass. The eggs are laid singly in shallow water on the edges of grassy pools, streams and man-made dams. They soon gather debris and become difficult to see. Although clutch size has not been recorded for *S. fasciatus*, a clutch of 64 eggs was recorded for *S. fuelleborni* in Malawi (Stewart 1967) and another of 44 eggs for the closely related *S. bonaespei* of the

Western Cape Province (Cunningham and Henderson 2000). This suggests that *S. fasciatus* does not lay large clutches of eggs.

The tadpoles are medium to large sized with a rounded plump body and measure up to 70 mm. The tailfin is long and the fin is moderately deep. The colour of the body is dark brown above and the fins yellow-brown with darker spots and the underside is silvery. Tadpole development is extended and metamorphosis is completed in about five months (Wager 1986; Du Preez and Carruthers 2009).

Tremolo Sand Frog (*Tomopterna cryptosis*)

Historical records indicate a wide distribution in the savanna of sub-Saharan Africa from Senegal in the west to Somalia in the east and southward through East Africa to South Africa. *Tomopterna cryptosis* appears to be distributed from Angola through Zimbabwe, Malawi and southward through Namibia, Botswana, Zimbabwe and Mozambique.

There do not seem to be any morphological features that permit one to distinguish between *T. cryptosis* and the cryptic, tetraploid species *T. tandyi*. Their calls differ only slightly in pitch (Channing and Bogart 1996), and only a few people who collected atlas data were able to identify these species in the field. Also, relatively few tape recordings of calls of these species were submitted to the SAFAP. Nevertheless, M. Burger and H.H. Braack collected sufficient reliable records of *T. tandyi*, mainly in the southern parts of its range, to warrant the production of a separate distribution map for that species (Channing 2004a).

This species inhabits various vegetation types in the Savanna and Grassland biomes. Males call from the edge of the pans and smaller roadside pools within the Tshokwane area. Males call antiphonally and form dense choruses which suddenly stop and are silent for a while before resuming calling (Pienaar et al. 1976) as well as observed during the nocturnal surveys around Tshokwane area (2010-2011). Breeding takes place in shallow, standing water at the edges of dams, pans, and even small bodies of water such as roadside puddles (Channing 2004a).

Individuals burrow into sandy soils or dry river beds during the dry season and, in the breeding season, may retreat into termite mounds during the day. Breeding begins after the first spring rains and choruses may be heard throughout the rainy season after showers. Males call from exposed positions near the water's edge, but are well concealed by their cryptic colouration. About 2000-3000 eggs are laid singly in shallow water.

The tadpoles are small-medium sized with a deep plump body and measure up to 37 mm. The tailfin is shallow and usually not as deep or same depth as body reaching highest point about a third way down from the body and tapering to a fine point. The colour of the body is brown to dark brown and the underside is silvery white. Larval development takes about five weeks (Channing 2004a).

Figure 5.26: Distribution of *Tomopterna cryptosis* during this study.

Knocking Sand Frog (*Tomopterna krugerensis*)

T. krugerensis is distributed in a broad band across southern Africa, from north-eastern South Africa and southern Mozambique, through southern Zimbabwe and Botswana to Namibia and southern Angola. Its distribution in the atlas region is rather patchy, especially in Limpopo and North West provinces. It is almost indistinguishable, morphologically, from *T. cryptosis* and, *T. tandyi*, but has an easily recognizable call. The southernmost record is from Hluhluwe in KwaZulu-Natal. *T. krugerensis* was discovered in the Sandveld regions

around Machayi and Mathlakuza Pan in the north-eastern regions of the Kruger National Park (Passmore and Carruthers 1975, Pienaar et al. 1976). During the current survey a single record of a metamorph was collected from Bangu Pan in 2011.

Tomopterna krugerensis inhabits the Savanna Biome at altitudes ranging from sea level to 1500 m, in areas with annual rainfall of 500 >1000 mm. It seems to prefer sandy soils and breeds in temporary water bodies such as large and small pans, vleis, and floodplains. Breeding begins after the first rains and continues into mid-summer. Males call from the open or from partially concealed positions at the water's edge. Approximately 5000 eggs are laid singly in shallow water (Passmore and Carruthers 1975). The tadpoles and details of development are unknown.

Figure 5.27: Distribution of *Tomopterna krugerensis* during this study.

Russet-backed Sand Frog (*Tomopterna marmorata*)

T. marmorata ranges from Botswana eastward through Zambia, Malawi, Zimbabwe to southern Mozambique. In South Africa the species is fairly widespread in Limpopo Province and eastern Mpumalanga at altitudes <1000 m. *T. marmorata* inhabits a range of bushveld vegetation types in the Savanna Biome. It seems to prefer sandy soil and occurs in areas

where annual rainfall is 500-1000 mm. It breeds in slow flowing rivers and streams as well as isolated pools, pans or dams with sandy substrates.

Tomopterna marmorata adults have been found buried in sand banks during the dry season. They emerge after the first rains and begin breeding as soon as bodies of standing or running water have formed. Males call from open areas on sandbanks, near the edge of the water. Amplexus is axillary, and the eggs are laid in shallow water (L.R. Minter pers. obs.). The eggs are laid singly, and a clutch size of 150 eggs has been recorded (Channing 2001). No further life history details are known.

Males were observed calling along the Sabie River as well as several pans and smaller seasonal pools in the Tshokwane area of the southern Kruger National Park. Several emerging metamorphs were collected dispersing approximately 50-100 m from the edge of Nhlanguleni Pan in the Tshokwane area during December 2010. The froglets are cryptically coloured and match the adjacent sand soils. The tadpole and larval duration are currently unknown.

Figure 5.28: Distribution of *Tomopterna marmorata* during this study.

Natal Sand Frog (*Tomopterna natalensis*)

T. natalensis is recorded from Botswana, Zimbabwe and southern Mozambique and its distribution extends into eastern half of South Africa where it occurs from sea level to the high inland plateau at 2000 m. It is largely absent from the upper slopes of the Drakensberg. It is a common species in Limpopo, Mpumalanga, Gauteng and Kwazulu-Natal provinces' as well as in the eastern parts of North West and Eastern Cape provinces and throughout Swaziland. The species is uncommon in the Free State and Lesotho. In the Kruger National Park they are more commonly found at altitudes above 500 m.a.s.l. around the granite hills around Pretoriuskop (Pienaar 1976). No records of the species were made during the current survey. The lack of specimens must not be an indication of absence or decline of the species, but rather insufficient sampling due to inadequate rainfall events in the area and the majority of pans being completely dry during the last sampling survey (December 2011).

Tomopterna natalensis is found in a variety of vegetation types in the Grassland and Savanna biomes. These areas receive annual rainfall of 300->1000 mm. Breeding takes place in streams, rivers or other places where water flows slowly, but also in standing water (Channing 2004b). Breeding begins after the first rains, continuing into midsummer. Males call from exposed positions near the water's edge on bare sand, mud or rock.

The eggs are usually laid singly or in small groups, in running water. The tadpoles are small-medium sized with a deep plump slightly flattened body and measure up to 36 mm. The tailfin is not as deep as body reaching highest point about a third way down from the body and tapering to a fine point. The colour of the body is light brown to dark grey or black, stippled with gold above and the fins are transparent. Development is rapid and is completed within 2-3 weeks. The only recorded predator is the Brown House Snake (*Lamprophis fuliginosus*) (Channing 2004b). The tadpoles are sluggish and are usually located at the bottom of muddy pools.

Common Platanna (*Xenopus laevis*)

This species is widely distributed in sub-Saharan Africa. *X. laevis* is a common and wide-spread species, occurring from sea level to nearly 3000 m in Lesotho. In the west, it is apparently absent in areas of extreme aridity, including much of the Kalahari and Bushmanland in Northern Cape Province, although this may be due to inadequate sampling. Its distribution extends eastward as far as the Great Escarpment, where it comes into contact with *X. muelleri* in the low-lying parts of Limpopo and Mpumalanga provinces. *X. laevis* is a highly invasive species, as is evidenced by the feral populations that have become established in many parts of the world. Its present distribution in the atlas region may not represent its natural range, as this frog is commonly used as live bait by fishermen

which may have been inadvertently translocated to areas which it was previously absent. The proliferation of farm dams and reservoirs over a few hundred years is another factor which may have enabled this species to expand its range.

Xenopus laevis was not a common species in the Kruger National Park and it was only recorded from natural pools, pans and dams in the Pretoriuskop area at altitudes above 500 m.a.s.l. (Pienaar et al. 1976). *Xenopus laevis* was recorded throughout all the regions of the park during the current survey. An adult as well as tadpoles were collected at Xipudza in the Punda Maria area within the north-eastern regions of the park. This is the furthest north that the species has ever been recorded in the Kruger National Park. This is a species which is expanding its range throughout the Kruger National Park. More intensive surveys need to be conducted for the possible presence of hybrids; especially within the pans of the park where both X*. laevis* and *X. muelleri* distribution is sympatric and breeding habitat synoptic, such as Lannea Pan, Soswanini as well as the perennial stream and pools at Xipudza.

This species inhabits all of the biomes in South Africa. Prior to the advent of modern agriculture it probably occurred in low densities in natural water bodies, such as streams, rivers and their pools. Now days, however, the species is also found in a variety of man-made water bodies such as farm dams, ponds, sewage purification works and fish farms. Eutrophic waters seem to produce the highest densities. Breeding and non-breeding habitats appear to be the same, although there are no records of breeding in flowing water. During the current survey a stressed adult specimen collected at Xipudza in the Punda Maria area released a gluey white excretion from its back which killed all other frogs and tadpoles in the bucket.

After heavy rains*, X. laevis* sometimes leave water bodies *en masse*, and single individuals are also encountered on the surface in damp weather. These appearances may be associated with movement to and from breeding sites. Several adult Platannas (*X. laevis* and *X. muelleri*) where observed crossing the Lower-Sabie/Skukuza road on wet evenings. Breeding begins at the onset of the rains, thus at different times in the summer and winter rainfall areas. There is a prolonged breeding period throughout the rainy season, and both females and males are able to reproduce more than once per season. Field studies have suggested that males call around the edges of territories, although this may be density dependent. Spawning takes place during the night when couples in inguinal amplexus, swim around the pond depositing single eggs on any hard substrate (McCoid 1985).

The tadpoles are medium-large sized with a flattened body with a pair of long sensory tentacles resembling a small catfish. The tentacles are as long as body width. The tail is long

and high and translucent and terminates in an acute tip. The colour is translucent with lightly scattering of dark stipples on body and tail. Larvae hatch within two to three days and, after finishing the yolk supply, begin to feed on algae suspended in the water column. Tadpoles display coordinated schooling behaviour, and maintain their position in the water column by means of a characteristic undulating motion of the tail (Wassersug 1996). Time to metamorphosis varies with temperature and the abundance of food. In optimal conditions, metamorphosis is possible within two months (Tinsley et al. 1996). Adults may move from water bodies after breeding, reducing the incidence of cannibalism (Measey et al. 1998).

Adults are generalist predators and scavengers, and can hold food items in their toothed mouths while breaking it apart with their claws using an overhead kick (Avila and Frye 1978). These behaviours can be detected by other adults in the vicinity and sometimes lead to a feeding frenzy (Frye and Avila 1979). Most food items for post-metamorphic *X. laevis* are benthic macro-invertebrates, such as chironomid larvae. However, a wide variety of food sources are used from all microhabitats in water bodies including carrion and terrestrial food items (Measey 1998a, b).
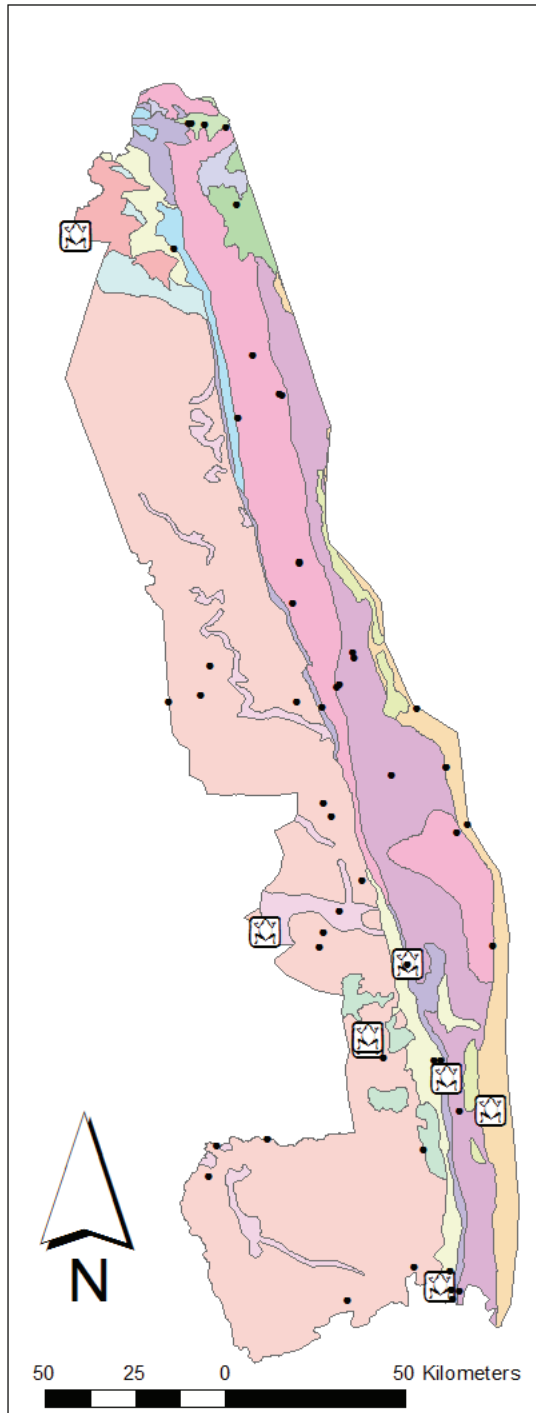
Figure 5.29: Distribution of *Xenopus laevis* during this study.

Even the largest animals take very small prey items, such as zooplankton and ostracods. Toward the peak of the dry season, *X. laevis* will either move from drying water bodies or burrow into the wet mud to aestivate. Longevity is unknown for native animals, but in feral populations and in captivity, individuals are known to have lived for more than 15 years (Measey et al. 1998).

Xenopus laevis plays an important role in the ecology of southern African wetlands because it is widespread and abundant, and it is a voracious predator as well as an important prey item for several mammalian, avian and reptilian predators. A Freshwater Crab (*Potamnautes warreni*) was observed eating an adult along the upper reached of the Ga-Selati, Lekgalameetse area of Limpopo Province (C.L. Cook and L.R. Minter pers. obs.). Adults have been observed preying of Giant Bullfrog (*Pyxicephalus adspersu*s) eggs as well as tadpoles at Glen Austin Pan in Midrand, Gauteng Province (C.L. Cook pers. obs).

Muller's Platanna (*Xenopus muelleri*)

The distribution of *X. muelleri* in sub-Saharan Africa is divided into two distinct areas containing animals that are morphologically similar but probably represent allopatric sibling species (Kobel et al. 1996) Within South Africa, this species is confined to the low-lying areas in northern and eastern Limpopo Province, eastern Mpumalanga and Swaziland, and north-eastern KwaZulu-Natal, which form the western and southern limits of the Mozambique plain. Although Fischer et al. (2000) recorded mixed populations and hybridization between *X. muelleri* and *laevis* in Mpumalanga, the two species are largely allopatric. The ranges of *X. muelleri* and *laevis* are separated by the 18°C mean July isotherm, with *muelleri* part of the tropical faunal assemblage north and east of this climatic boundary, and *laevis* part of a non-tropical assemblage distributed to the south and west of the isotherm (Poynton 1964; Poynton and Broadley 1991). It is possible that the distribution of the species reflects differences in temperature tolerance: *X. laevis* appears to be able to tolerate a wider range of environmental temperatures than *X. muelleri*, which is more tolerant of high temperatures (Tinsley et al. 1996).

Xenopus muelleri is widely distributed throughout the Kruger National Park occurring in all the major River systems including the Limpopo, Luvuvhu, Shingwedzi, Letaba, Olifants, Sabie, Sand and Crocodile Rivers. It is also common within the seasonal wetland habitats such as the larger and smaller pans and seasonal pools within non-perennial watercourses. *Xenopus muelleri* inhabits all types of water bodies, including lowland rivers, lagoons, dams and pans (Poynton and Broadley 1985a), mainly in the Grassland and Savanna biomes. It is seldom found in pristine forest habitats, but readily moves into deforested areas (Tinsley et al. 1996). *Xenopus muelleri* and *X. laevis* do not appear to differ with regard to water-quality

preferences or requirements. The apparent difference in temperature tolerance does not seem to apply in southern Namibia, where *X. laevis* occurs at temperatures at least as high as those from which it is apparently excluded in the east (Tinsley et al. 1996). A possible explanation is that *X. laevis* uses cool refugia within high temperature water bodies. This has been observed in extralimital populations of *X. laevis* (G.J. Measy pers. obs.). Absence of such refugia from some sites would explain the observations of Lambiris (1989a) and Poynton in Broadley (1985a) that the two species are rarely found at the same site. Both species were syntopic* at three different pans within the Kruger National Park namely Seribye Pan (Tshokwane area), Soswanini and Xipudza. No hybrids were observed or collected.
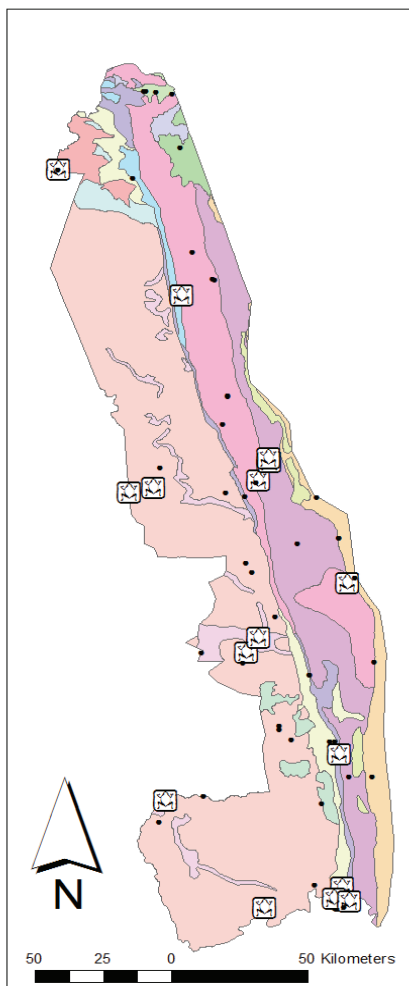
The tadpoles are medium-large sized measuring up to 70 mm with a more rounded flattened body with a pair of long sensory tentacles resembling a small catfish. The tentacles are significantly longer then *X. laevis*. The tail is long and high and translucent and terminates in an acute tip. The body colour is translucent to heavily/ pigmented with the only area covering viscera being semi-transparent. Little is known specifically about the life history of *X. muelleri*, although much can be inferred from the characteristics of the rest of the genus. Like other *Xenopus* they are known to move en masse even under dry conditions (Tinsley et al. 1996). Loveridge (1953) found them aestivating in the mud of a dried pond. Prey items include beetles, beetle larvae and frogs, while predators include Hammerkop (*Scopus umbretta*) and Black-headed Heron (*Ardea melanocephala*) (C.L. Cook pers. obs.).

Figure 5.30: Distribution of *Xenopus muelleri* during this study.

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* Sharing the same habitat within the same geographical range.

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6 THE INFLUENCE OF DECREASED pH LEVELS (ACID PRECIPITATION) ON THE BIOLOGY AND MORPHOLOGY OF SELECTED FROG SPECIES FROM THE KRUGER NATIONAL PARK

6.1 Introduction

The complex life-cycles of frogs, consisting of aquatic larvae (tadpoles) and terrestrial adults, expose them to both aquatic and terrestrial hazards (Duellman and Trueb 1986; Hartwell et al. 1998; Blaustein et al. 2003; Whiles et al. 2006) and they are very sensitive to changes in temperature, precipitation and ultraviolet-radiation (Blaustein et al. 1994). Many frog species are highly specialised to conditions in a particular locality. They are also very important ecologically, being involved in the role of energy flow, as well as nutrient flow in many ecosystems (Whiles et al. 2006). Frogs are the primary vertebrate predators of invertebrates in many freshwater and moist terrestrial environments (Stebbins and Cohen 1995). According to the above mentioned authors amphibians act as a major "conveyor belt" in many habitats, which ultimately provides for transfer of invertebrate energy sources to predatory animals higher up in the food chain, including animal species such as reptiles (especially snakes), and even some birds and mammals that cannot directly access the invertebrate food source. Tadpoles also control algal growth and other aquatic plant growth, and then transfers this stored plant energy to tadpole-eating invertebrates, fish and other vertebrates when preyed upon (Stebbins and Cohen 1995).

As far as we can remember, human activities have been detrimental to the natural biota in one way or another. According to several articles (Blaustein et al. 1994; Stebbins and Cohen 1995; Blaustein et al. 2003; Collins and Storfer 2003; Daszak et al. 1999; Whiles et al. 2006) more and more of the natural habitat and environment has been eliminated or modified as the human population dramatically increases. It is often on such a large scale that many species are in danger of extinction (Whiles et al. 2006). Although amphibians are seldom directly impacted, they are often affected indirectly and with disastrous effects. There have been numerous reports to date regarding global declines in frog populations (Blaustein et al. 1994; Blaustein et al. 2003; Collins and Storfer 2003; Daszak et al. 1999; Whiles et al. 2006; Whitfield et al. 2007), which only show signs of increasing. According to Whitfield et al. (2007) more than a third of amphibian species are globally threatened, and over 120 species have likely suffered global extinction since 1980.

These global declines cannot be explained by pinpointing a specific cause (Collins and Storfer, 2003). It is suspected that several causes are interacting. Major suspected causes include habitat destruction; pollution; introduction of exotic amphibian species and collection of amphibians for commercial purposes such as food, teaching or as aquarium animals

(Duellman and Trueb, 1986). Other possible causes include global change (increased ultraviolet radiation and global warming) and emerging infectious diseases (Blaustein et al. 1994; Blaustein et al. 2003; Collins and Storfer 2003; Daszak et al. 1999; Whiles et al. 2006).

According to Whiles et al. (2006) most research to date has focused on documenting declines of amphibians and identifying causes, but we know little about the ecological consequences of these losses. Hartwell et al. (1998) states that only a few studies have been designed specifically to examine the responses of amphibians to environmental perturbations in aquatic ecosystems. Selvi et al. (2003) states that amphibian tadpoles and eggs have before been used as bio-indicators for contamination monitoring, because of their impervious shells and their highly permeable skins.

Because of the above mentioned attributes of frogs, as well as their water-permeable skins (Stebbins and Cohen 1995), frogs are considered to be excellent "bioindicators" of general environmental health and may serve as an early warning system to environmental degradation (Blaustein et al. 1994; Collins and Storfer 2003; Hartwell et al. 1998). According to Blaustein et al. (1994), Relyea (2004, 2005), Whiles et al. (2006) and Whitfield et al. (2007) there is a basic lack of long-term data on amphibian populations, and the effects of stressors on them, which severely limits our understanding of the distribution of amphibian declines, and therefore the ultimate causes of these declines.

The problem of declining amphibian populations has been drawing special attention since the early 1990s for various reasons, with the most alarming being that frog populations from protected, natural areas were also showing signs of decline (Daszak et al. 1999; Collins and Storfer 2003). As we have seen, amphibians form an important part of South Africa's exceptional biodiversity and it is thus crucial to study, manage and conserve our natural populations. As one of South Africa's premier conservation areas, The Kruger National Park (KNP) and conservation authorities have had growing concerns regarding the possible negative influences of acid precipitation (decreasing pH-levels) on the ecology and biology of their natural amphibian populations. The Southern Foam Nest Frog, *Chiromantis xerampelina*, one of the selected test species, is the only species from the genus *Chiromantis* occurring in South Africa as well as in the KNP.

Some studies have been done in some form or other regarding the effects of decreasing acidic conditions on frog populations. These include studies on acid precipitation (Corn et al. 1989), acid tolerance in amphibians (Pierce 1985) and even some regarding localized adaptation of populations which have been under acid stress for extended periods of time

(Räsänen et al. 2003). Most of these studies are however focussed on frog species from America, Sweden and other countries. Very little or no studies have thus far been conducted with South African species.

Parasites may reflect the immunological status of their hosts, since poor host body condition and lowered immunity may predispose to infection. During periods of stress and lowered immunological condition, the parasite response may be manifest either as an increase, or decrease, in prevalence and parasitaemia (Beldomenico and Begon 2009). Haematozoans are a group of apicomplexan blood parasites that infect the blood of amongst other, frogs. These parasites are considered non-pathogenic; possibly only mild cases of anaemia occur in their presence and reflect a well-adapted host-parasite relationship (Jacobson 2007). Thus the general wide vertebrate host and geographical range of these parasites advocate its possible use as an 'effect' bio-indicator demonstrating a correlation between prevalence, parasitaemia and host body condition. Studies on these parasites thus have the potential to act as bio-indicator of anthropogenic impacts on the hosts or can act as stress indicators. However, currently no information exists on the presence or prevalence of blood parasites in South African's indigenous frog population. When taking into account the large number of these parasites known from frogs world-wide, it is clear that the lack of knowledge on this group of parasites in South Africa is due to the absence of research on this group and not the non-existence of these parasites in the blood of frogs in this region.

This section of the report documents the possible effects of decreasing pH-levels (because of acid precipitation) on frog biology and ecology; and some of the chemical factors that may influence these amphibians through biomarker, bioaccumulation and histological studies.

The following objectives were set for this section of the project:
- To conduct acid tolerance bioassays (LC_{50} – type tests) with eggs as well as tadpoles of 4 frog species occurring in the KNP, using survival and development as endpoints.
- To conduct chronic exposure bioassays with a series of relevant pH-values for the entire embryonic period and as much of the larval (tadpole) period as possible.
- To collect blood smears of all frog species collected within the park, to study and research any blood parasites which might be present.
- To test the effect of decreasing pH-levels on the structure and effectiveness of the foam nests of *C. xerampelina*, as well as the embryonic development whilst eggs are still inside these nests, through exposing foam nests to simulated rain with varying pH-values.

6.2   Methodology

6.2.1   Study and sample area

Figures 6.1 shows the different areas within the Kruger National Park where samples were collected. As can also be seen, sampling was mainly focused in areas comprising the Southern region of the park.
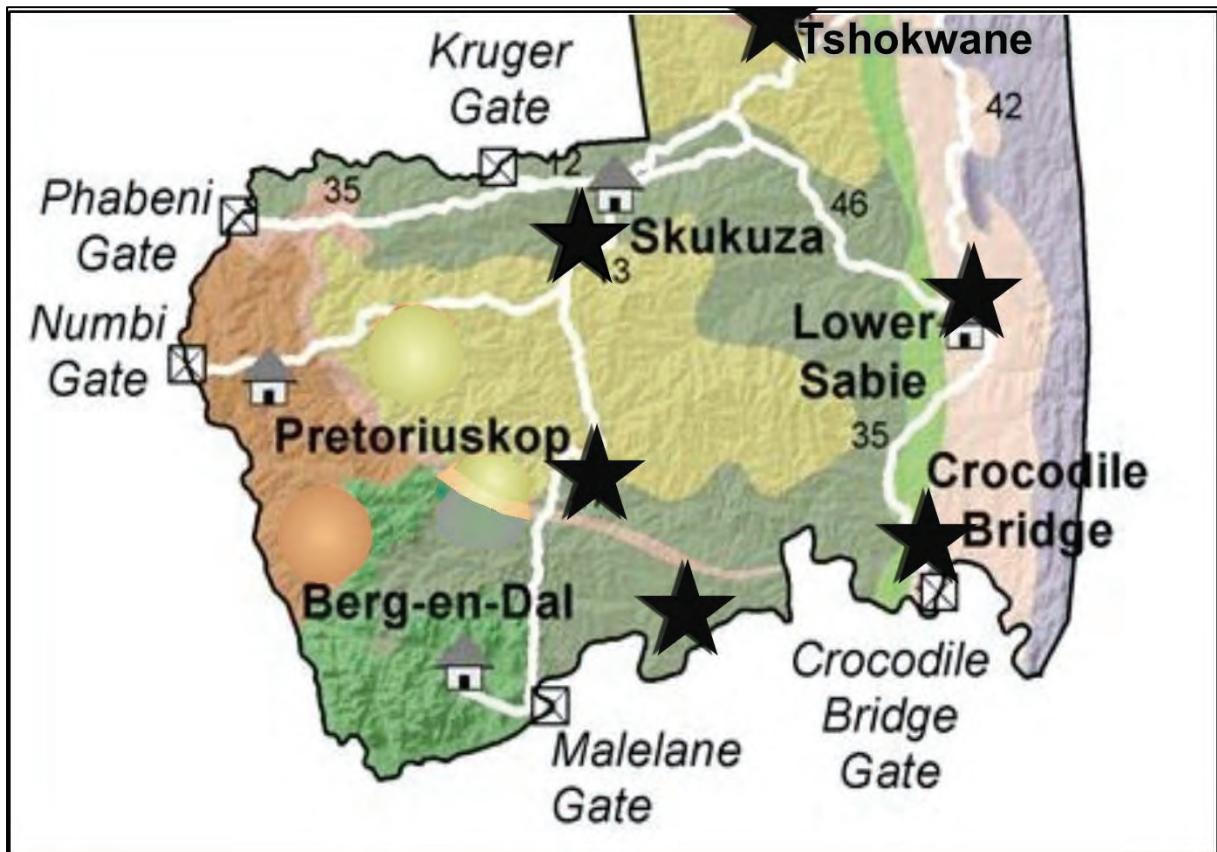
Figure 6.1: A map of the southern region of Kruger National Park, showing the areas in which sampling occurred.

6.2.2   Field techniques

Acute acid tolerance bioassays

This bioassay focuses on the short-term effects that declining pH-levels have on tadpoles over a period of 96 hours. It is similar to a Lethal Concentration 50% (LC_{50}) test, which is normally used to determine the lethal dose of a certain pollutant, toxin or pathogen, or even radiation, that is required to sacrifice half the members of a tested population after a specific test duration, normally 96 hours. Here we have substituted the concentrations with declining pH-values, in order to ascertain at which pH-value 50% of exposed individuals will perish. The tadpoles of *Chiromantis xerampelina* (Southern Foam Nest Frog), *Pyxicephalus edulis* (African Bullfrog), *Amietophrynus maculatus* (Flat-backed Toad) and *Hildebrandtia ornata* (Ornate Frog) were collected from the various areas shown in Figure 6.1. For each of these species, individuals from the same clutch were placed in 10L clear, plastic containers. Ten different pH-values, including a positive control (1 mg/L Cadmium chloride solution), a negative control consisting of pan water (pH=±7), de-chlorinated tap water (pH=±6.85), a pH=6 solution, pH=5.5 solution, pH=5 solution, pH=4.5 solution, pH=4 solution, pH=3.5 solution and a pH=3 solution were used during this bioassay. Only 7 for *P. edulis* (positive control (1 mg/L Cadmium chloride solution), a negative control consisting of pan water (pH=±7), de-chlorinated tap water (pH=±6.85), a pH=6 solution, pH=5.5 solution, pH=5 solution and pH=4 solution). 20-25 tadpoles of each frog species were placed into each container holding one of the 10 different pH-solutions (Figure 6.2A, B, C, D). As mentioned, the tadpoles were then kept here for a period of 96 hours. Every 24 hours the pH was checked (Figure 6.2G) and modified to stay as constant as possible. All tadpole mortalities that occurred during the 96 hours were noted every 24 hours and dead individuals removed from the containers. This setup was triplicated for 3 of the frog species collected (Figure 6.2E, F), but only duplicated for *H. ornata* due to a restricting amount of sampled tadpoles. The same procedure was also performed on the eggs of these 4 frog species, in order to determine at which pH-value 50% of the exposed eggs would develop further into tadpoles.
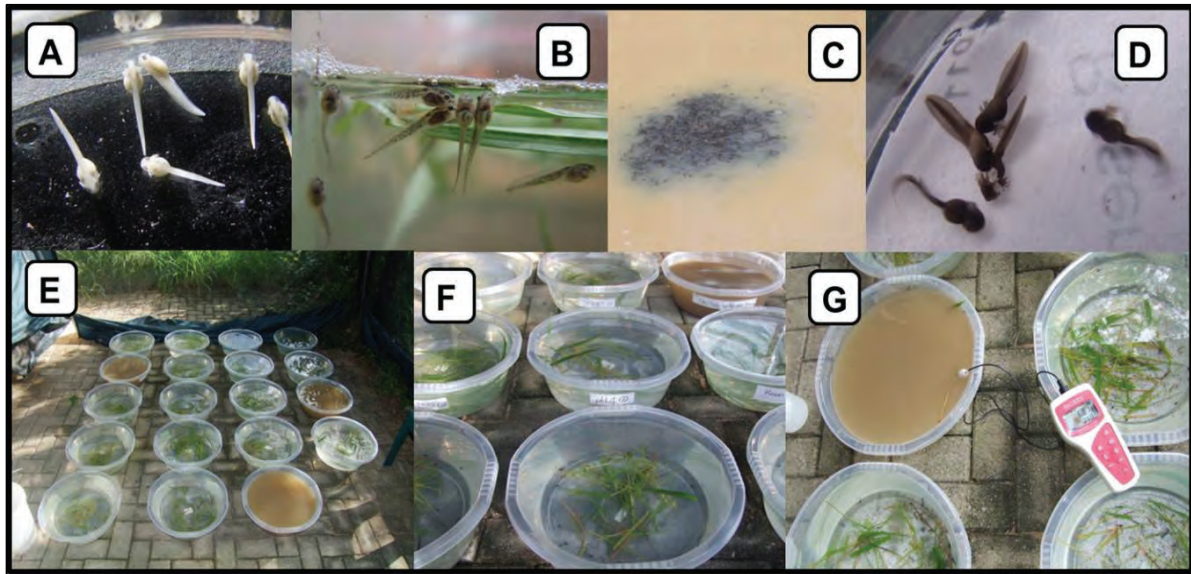
Figure 6.2: A) *C. xerampelina* tadpoles when just dropped from the foam nest. B) *C. xerampelina* tadpoles a few days old. C) School of *P. edulis* tadpoles in shallow water from which samples were collected. D) Newly hatched *P. edulis* tadpoles with external gills still visible. E) Photograph showing the 10L plastic containers containing water with different pH-values. The 7 pH-solutions were triplicated and placed randomly in the area shown here. F) Showing how the containers were marked in order to keep track of the different pH-solutions. G) pH-reading taken every 24 hours.

Chronic acid tolerance bioassays

Once the "LC50"-pH values were calculated for each frog species, according to results from the acute exposures, pH-values were selected to be used during the chronic (long-term) exposures. For this bioassay, 30L plastic containers were used (Figure 6.3A, B). For *C. xerampelina*, *A. maculatus* and *H. ornata*, 7 solutions with differing pH-values were used (solutions included pan water, de-chlorinated tap water, pH=6; 5.5; 5; 4.5 and 4), and for *P. edulis*, only 6 solutions were used (pan water, de-chlorinated tap water, pH=6; 5.5; 5 and 4.8). Each container contained 30 tadpoles, except for *H. ornata*, which only contained 10 tadpoles in each of the species' containers, due to restriction of sampled individuals. Tadpoles were measured before placed into containers. After a period of 3 weeks tadpoles were weighed and measured again, and also at the conclusion of each exposure. During the duration of the exposures temperature was measured and pH-levels were kept as constant as possible (Figure 6.3C). Tadpoles were fed with small amounts of grinded fish food and small pieces of lettuce leaves. The setup was again triplicated for 3 of the frog species mentioned and only duplicated for *H. ornata*.
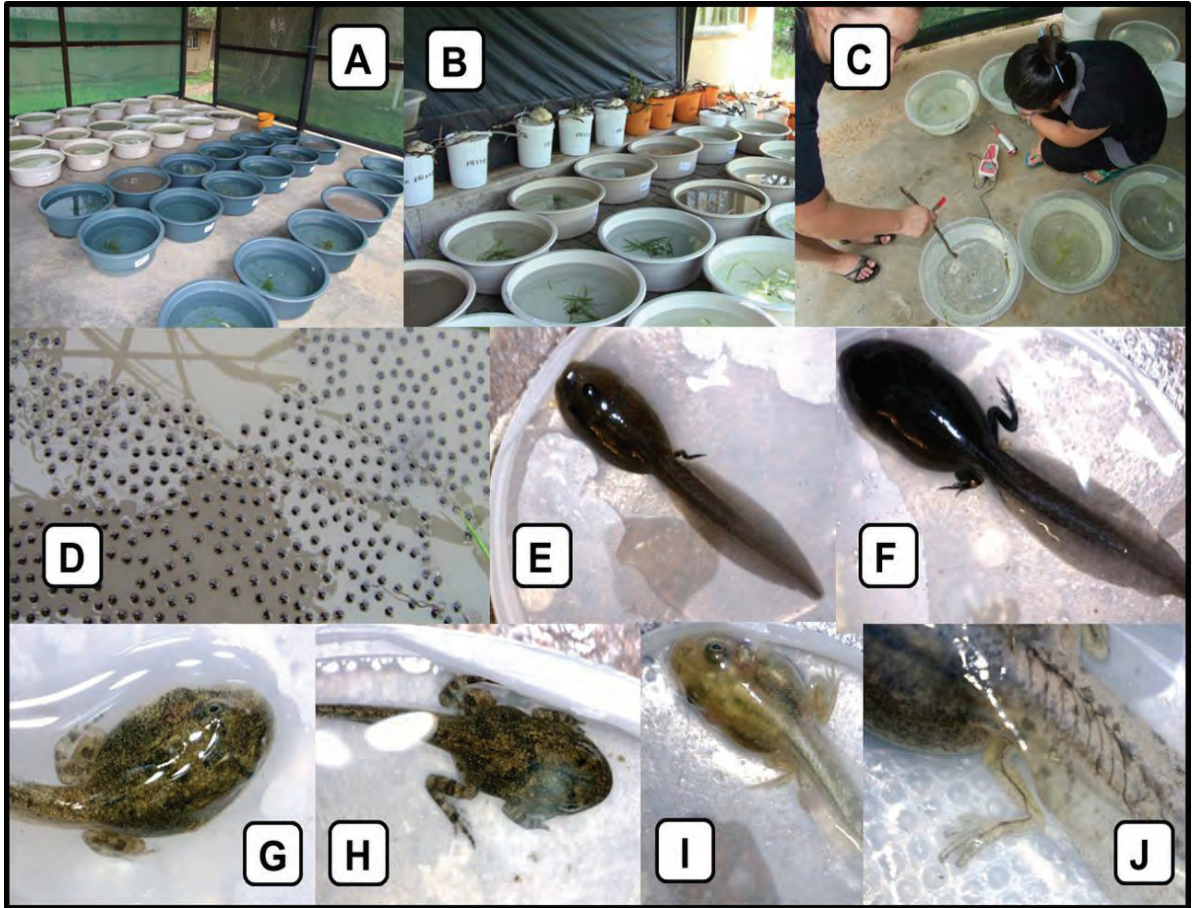
Figure 6.3: A, B) 30L containers used for chronic exposures. Each container marked and placed randomly in area shown in photograph. C) pH adjusting every 24 hours. D) Frog eggs floating on water surface. E, F) Photographs showing development of hind legs in *P. edulis* tadpoles. G, H) *A. maculatus* tadpoles with hind legs. I) *H. ornata* tadpole with hind legs. J) Hind leg of *C. xerampelina* tadpole.

Blood smears

Blood smears were made of all frog species (Figure 6.4) collected according to standard methods; and fixed by placing slides in absolute methanol for ± 10 minutes.
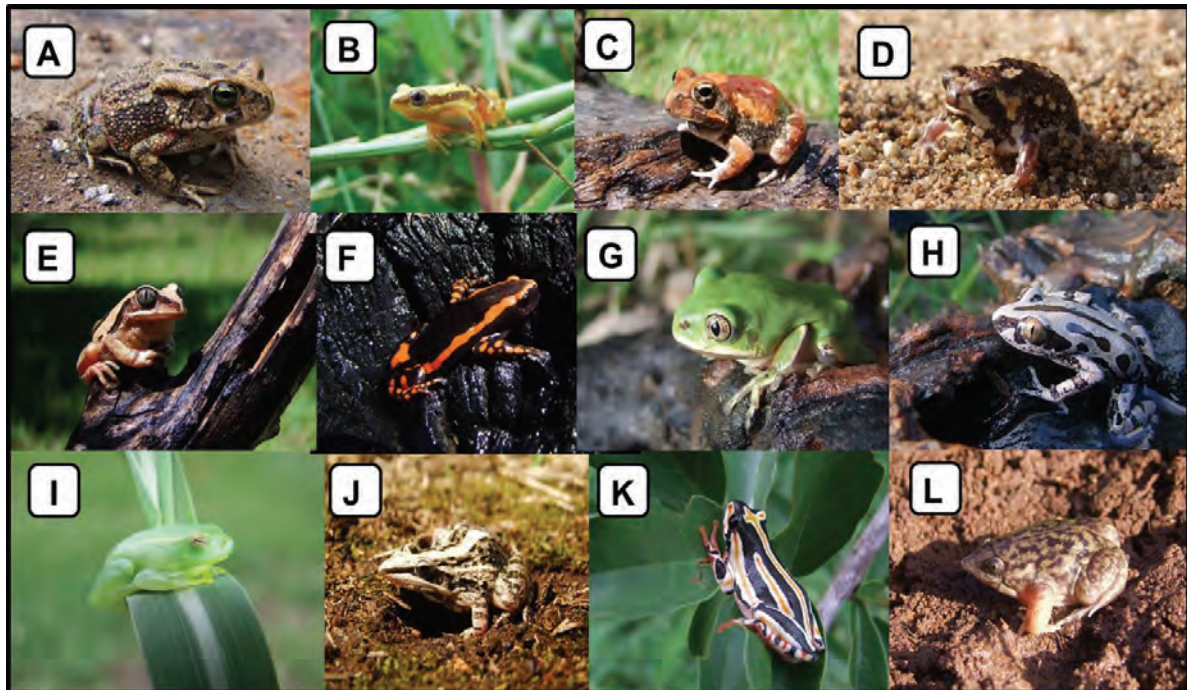
Figure 6.4: Photographs of some of the frog species that were collected for blood smears. A) *Amietophrynus garmani* (Eastern Olive Toad). B) *Afrixalus aureus* (Golden Leaf-folding Frog). C) *Tomopterna marmorata* (Russet-backed Sand Frog). D) *Breviceps adspersus* (Bushveld Rain Frog). E) *Leptopelis mossambicus* (Brown-backed Tree Frog). F) *Phrynomantis bifasciatus* (Banded Rubber Frog). G) Juvenile Brown-backed Tree Frog. H) *Kassina senegalensis* (Bubbling Kassina). I) *Hyperolius pusillus* (Water Lily Frog). J) *Ptychadena mossambica* (Broad-banded Grass Frog). K) *Hyperolius marmoratus* (Painted Reed Frog). L) *Hemisus marmoratus* (Mottled Shovel-nosed Frog).

Foam nest spraying

Foam nests were collected (Figure 6.5A, B, C), placed and then secured on top of 10L buckets (Figure 6.5D, E). Each nest was sprayed with a different pH-value to simulate rain (Figure 6.5F). The solutions used included a control of de-chlorinated tap water, and solutions with pH-values of 6; 5.5; 5; 4.5; 4 and 3.5 (Figure 6.5G). The pH-solutions were all obtained using de-chlorinated tap water mixed with varying amounts of Sulphuric acid. The spraying process was repeated every 24 hours over a period of 5 days, i.e. 5 sprays (rain events) for each of the pH-values and control. Tadpoles were collected after dropping out of foam nests (Figure 6.5H, I) and placed in 15 mL or 50 mL Falcon tubes, depending on

amount of tadpoles, and fixed with 10% NBF (Neutral buffered formalin), and transported to the lab for later analysis.
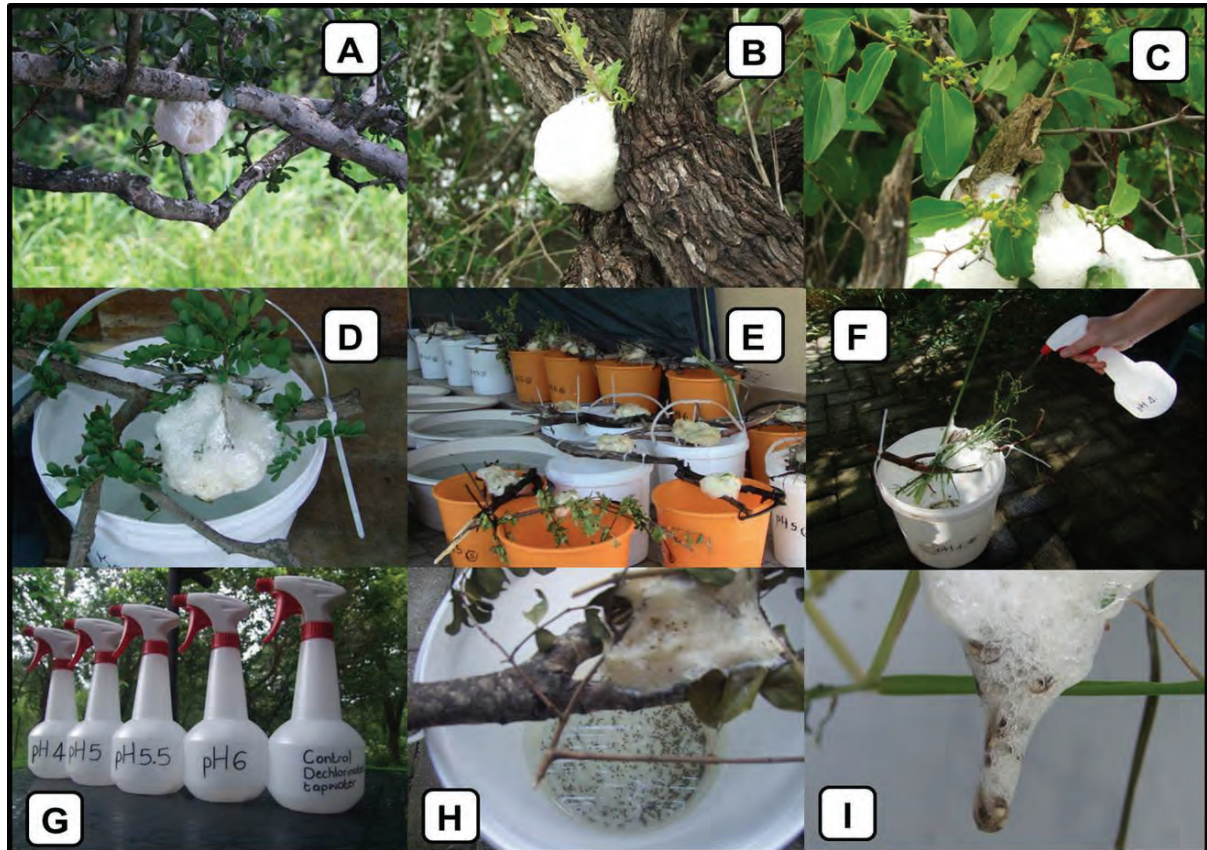
Figure 6.5: A, B) Foam nest collected for spraying experiment. C) *C. xerampelina* adult still on foam nest. D, E) Photograph showing foam nests placed on buckets and secured for spraying. F) Spraying of foam nest to simulate rain. G) Photograph showing spray bottles marked with the different pH-solutions that were used for spraying. H, I) Here we can see how the tadpoles drop through the underside of their foam nests as the nests get wet because of spraying or when they are developed enough to leave the safety of the nest, and get collected at the bottom of the buckets.

6.2.3 Laboratory techniques

Acute acid tolerance bioassays

Tadpole mortalities and survival data noted every 24 hours, for the total duration of 96 hours, was tabulated and the "LC_{50}" pH-value for each of the 4 frog species was calculated by using either the Trimmed Spearman-Karber Method (Version 1.5) or the EPA Probit Analysis Program (Version 1.5).

Chronic acid tolerance bioassays

Exposures were continued until tadpoles of each species began to develop hind legs (Figure 6.5E-J). After this occurred; the daily pH-monitoring was stopped and the tadpoles left to grow to adulthood. The adult frogs will then be removed and checked for any abnormalities, before being stored in formalin for future use.

Blood smears

Blood slides were stained with GIEMSA according to standard methods and studied with a Nikon Eclipse 80i light microscope. Any blood parasites present were noted. Parasite photos and measurements were done with the aid of NIS-Element D program. Any new parasite species will be further studied and ultimately described.

Foam nest spraying

Number and type of abnormalities present in tadpoles were identified with the aid of a light microscope.

6.3   Results

Acute acid tolerance bioassay

The "LC_{50}"- pH values for each of the 4 test species were calculated to be:

- *C. xerampelina* = **pH 4.07** with 95% confidence levels of 3.996 to 4.149
- *P. edulis*   = **pH 4.55** with 95% confidence levels of 4.4 to 4.7
- *A. maculatus*   = **pH 3.75** with 95% confidence levels of 3.742 to 3.762
- *H. ornata*   = **pH 3.74**

The tadpoles of all 4 frog species died within 24 hours of being placed in pH 3.5 or lower. From these results we can see that *P. edulis* is the most sensitive of the 4 frog species, needing a pH of 4.55 for 50% of exposed individuals to die. Whereas the remaining 3 species all need much lower pH conditions before 50% of exposed individuals will die. *A. maculatus* and *H. ornata* will have 50% mortalities at pH levels as low as 3.7. The current average pH of existing pans in the Kruger National Park was measured to be ± pH6.8. During the time these tests were carried out, the pH of rain water was measured to be pH5.42.

The "LC_{50}" pH-values calculated for these 4 frog species were lower than the pH-levels of pH6 and higher, that were measured in various pans throughout the KNP. The pH-levels measured (pH5 and above) at certain times of the year for the rainfall in southern and central KNP are only slightly higher than the pH-tolerances (pH4; 4.5 and pH3.7) of these four species.

Chronic acid tolerance bioassays

All 4 frog species still had mortalities during their long-term exposures to certain pH-concentrations (Table 6.1). *P. edulis* had overall higher mortality percentages, with percentages no lower than 36.7%. The other 3 species all had lower percentages of 20% or less. *P. edulis* had the highest mortality percentage in 4 of the mutual pH-exposure concentrations (pan and tap water, pH6 and pH5) and overall more *P. edulis* tadpoles died during the chronic exposure test, showing again that *P. edulis* is the more sensitive frog species. Also, the pH-concentrations that were decided upon for this species' chronic exposures did not go lower than pH 4.8, whereas the other 3 species all had a pH 4.5 and pH 4 exposure solution. In contrast, *A. maculatus* tadpoles had the overall lowest mortality percentages, the highest being 17.8%, in all of the exposure pH-concentrations, showing this species as the hardier of all 4 of the frog species. There were high mortalities of tadpoles in pH 5.5 for *C. xerampelina* and *H. ornata*, and 100% mortality for *P. edulis* in pan water.

Table 6.1: Tadpole mortality percentages of all four frog species during respective chronic exposure experiments. The lowest pH exposure concentration for *P. edulis* was only 4.8. This was due to the sensitivity of this species. All of the exposed tadpoles would have died in lower pH concentrations, thus defeating the purpose of long term exposure to low pH-conditions of this chronic exposure assay.

SPECIES	EXPOSURE SERIES							
	Pan	Tap water	pH 6	pH 5.5	pH 5	pH 4.8	pH 4.5	pH 4
C. xerampelina	15.6%	52.2%	27.8%	52.2%	20%	-	24.4%	95.6%
H. ornata	55%	40%	10%	90.0%	45%	-	40%	45%
A. maculatus	17.8%	4.4%	5.6%	6.7%	0%	-	2.2%	3.3%
P. edulis	100%	76.7%	43.3%	36.7%	47.8%	51.1%	-	-

Figures 6.6, 6.7, 6.8 and 6.9 all indicate overall tadpole growth that occurred in all 4 frog species during their respective chronic exposure assays. All figures also show a general decrease in tadpole growth as well as size in the lower pH-concentrations such as pH4.5 and pH4. Tadpoles seemed to fair overall better in higher pH-concentrations.

Figure 6.6 shows the mean tadpole lengths for *C. xerampelina* tadpoles that were taken at the start of the exposure, then after 27 days and again at the end of the exposure experiment. A group of 30 random tadpoles were measured for the mean of the unexposed group at the start of the chronic assay. The (*) indicates a significant difference in mean length from the unexposed group of tadpoles. Alphabet letters indicate significant differences

within an exposure period and common numerals indicate significant differences between different exposure periods. Significant difference is when p<0.05.

Mean lengths of all pH-concentrations, except pH4 at 39 days, used for the exposure differed significantly from the mean length of the unexposed group. Within the 27 day time group, mean length of pan water tadpoles differed significantly from that of water, pH4.5 and pH4. Mean length of pH5 differed significantly from that of pH4.5 and pH4. Within the 39 day time group, the mean length of tadpoles in pan water differed significantly from that of water, pH6 and pH4.5. Between the 2 time groups there were significant differences in mean length of pan water, water, pH5.5 and pH4.5.
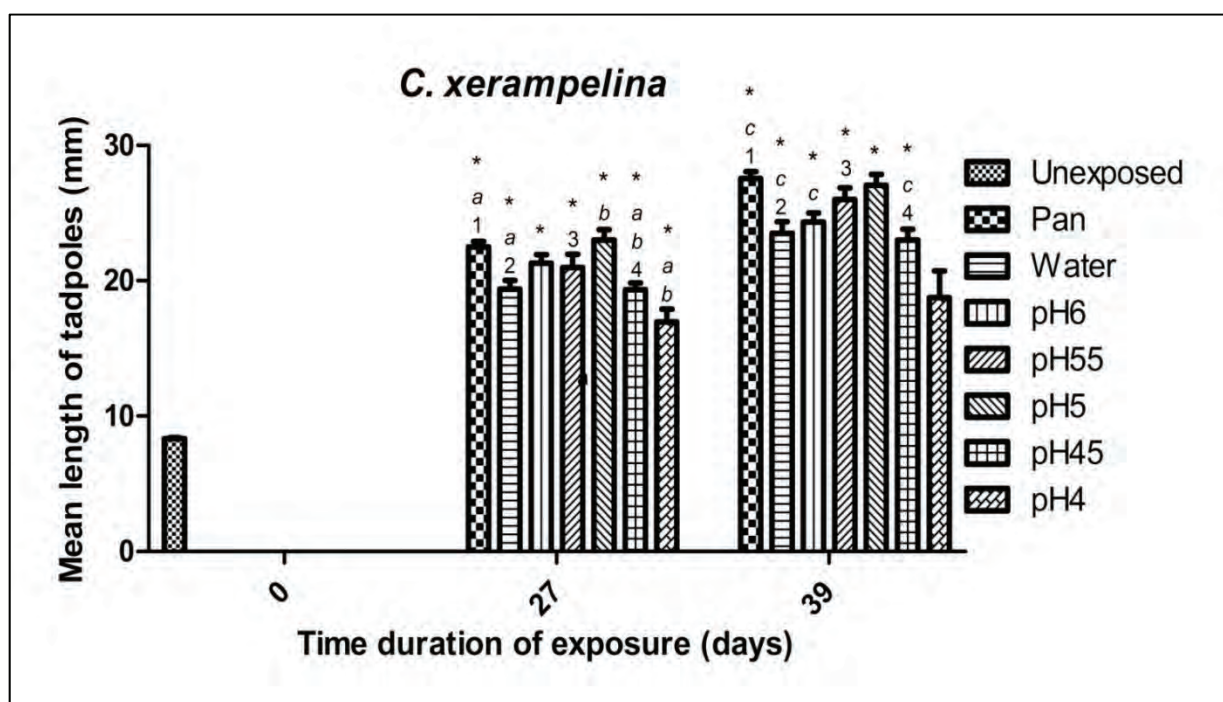
Figure 6.6: Mean tadpole lengths for *C. xerampelina*, from the start of the exposure experiment (time 0), and then for two varying time periods (27 and 39 days) within the duration of the whole experiment.

The mean length of all of the pH-concentrations differed significantly from that of the unexposed group (Figure 6.7). All tadpoles from pan water died during this chronic assay. Pan water therefore does not appear on Figure 6.7. Only pH6 showed a significant difference in length between the time groups.
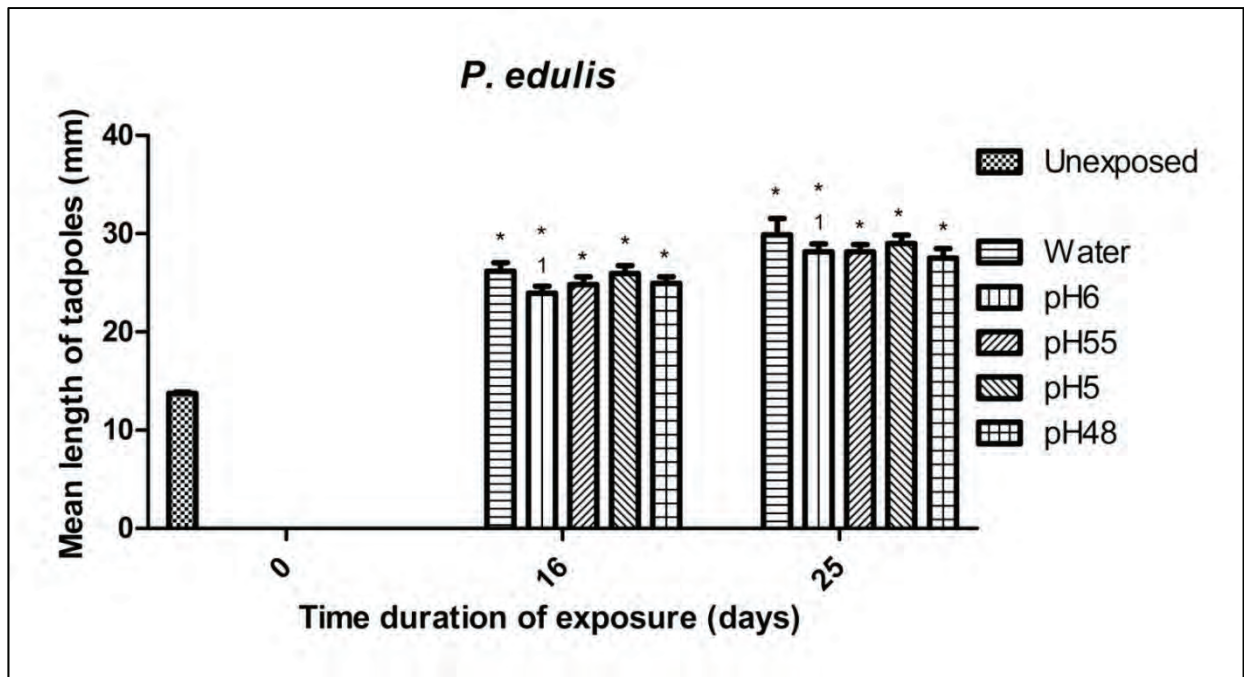
Figure 6.7: Mean tadpole lengths for *P. edulis*, from the start of the exposure experiment (time 0), and then for two varying time periods (16 and 25 days) within the duration of the whole experiment.

All pH-concentrations showed a significant difference from the mean length of the unexposed group (Figure 6.8). Within the 17 day time group, the mean length of tadpoles from the pan water, water, pH6 and pH4 differed significantly from that in pH5.5, pH5 and pH4.5. Within the 27 day time group pH5.5 shows a significant difference from mean lengths in water, pH6 and pH4. Between the 2 time groups the mean lengths of pan water, pH6, pH5.5, pH5, pH4.5 and pH4 all differed significantly.
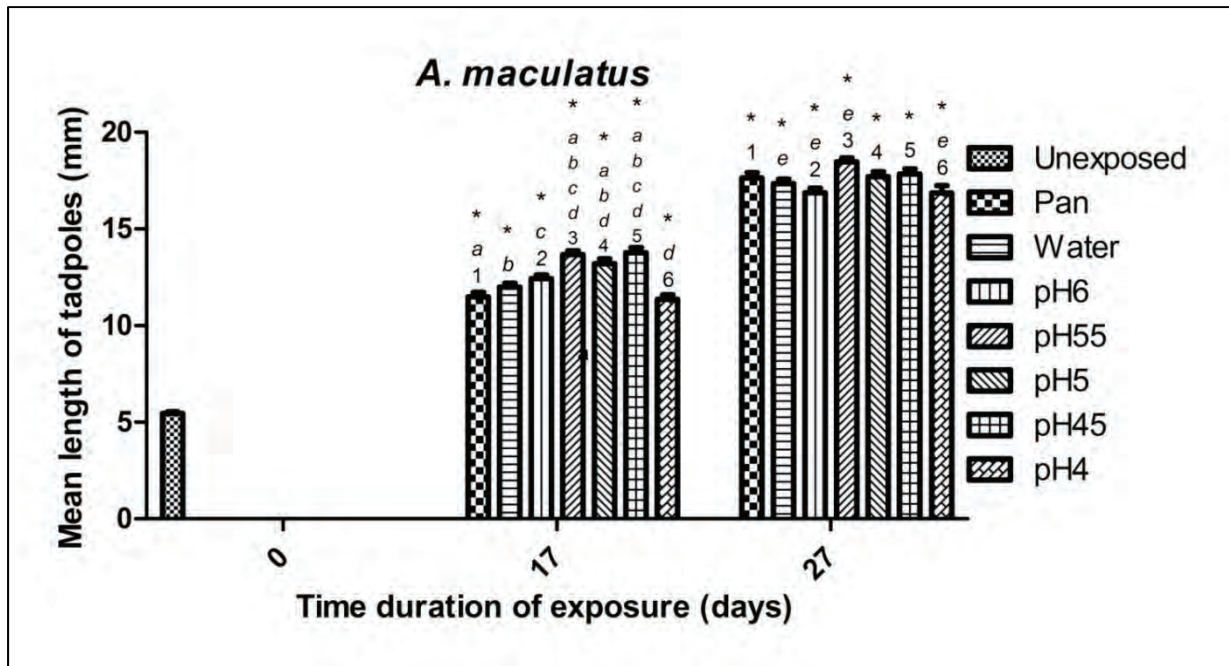
Figure 6.8: Mean tadpole lengths for *A. maculatus*, from the start of the exposure experiment (time 0), and then for two varying time periods (17 and 27 days) within the duration of the whole experiment.

Due to the restriction in sample size of *H. ornata* tadpoles that were collected, all of the individuals from each pH-concentration were measured at the start of this chronic assay to serve as the unexposed group, instead of the 30 random tadpoles that were used for the previous 3 frog species.

Figure 6.9 shows there were no significant differences between pH-concentrations within the unexposed time group and concentrations within the 18 day time group. Within the 36 day time group pH5.5 differed significantly from pan water, water, pH6, pH5, pH4.5 and pH4. Pan water, pH5 and pH4.5 from the 18 day and 36 day time group all differed significantly from the same concentrations in the unexposed group, but there were no significant differences in these pH-concentrations between the 2 time groups. Mean lengths of water and pH6 showed a significant difference from mean lengths of water and pH6 from the unexposed group. There were also significant differences in these pH-concentrations between the 2 time groups. The mean length of pH5.5 showed a significant difference between the start of the chronic assay and at 36 days. There were no significant differences in pH4 from the different time groups. An exponential increase in tadpole length can be seen in the pH5.5 exposure-concentration for both of the time groups.
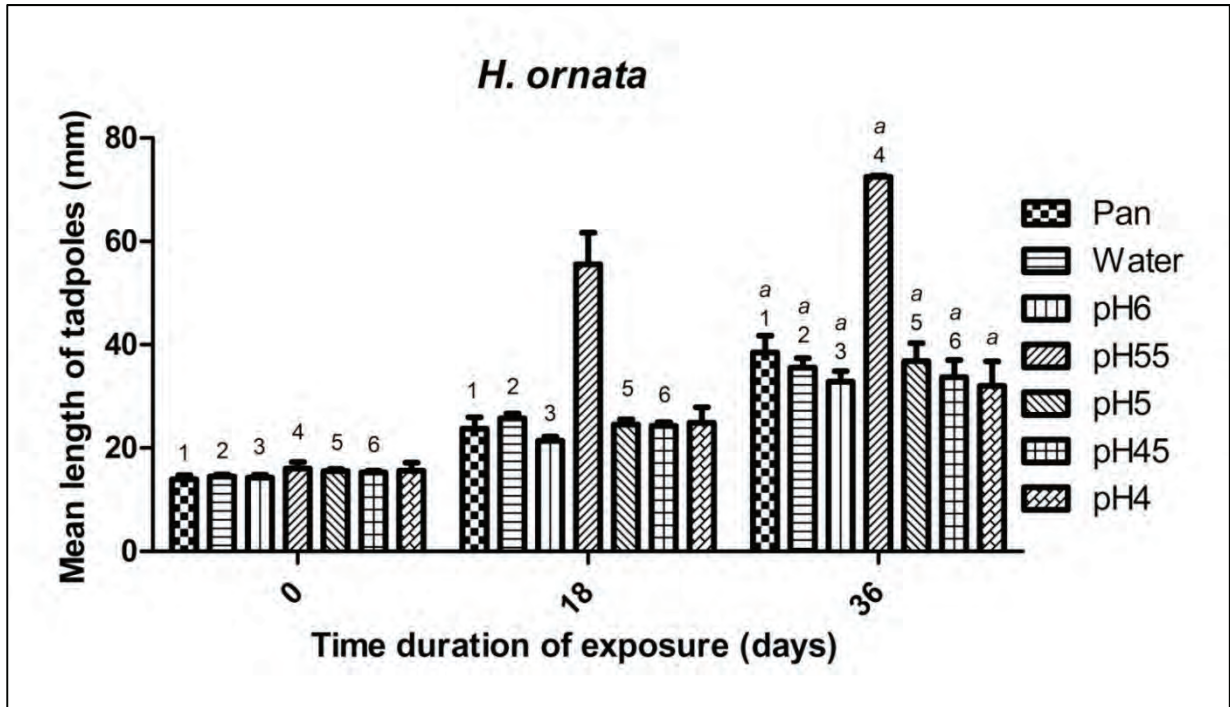
Figure 6.9: Mean tadpole lengths for *H. ornata*, from the start of the exposure experiment (time 0), and then for two varying time periods (18 and 36 days) within the duration of the whole experiment.

Figure 6.10 shows the tadpole weights for each of the 4 frog species at the end of their respective chronic exposure assays. Again we see a general decrease in tadpole weight for the lower pH-concentrations. A significant difference is indicated with a (*) when $p < 0.05$.

Figure 6.10A shows the weights for *C. xerampelina* tadpoles. Pan water weight differed significantly from tap water, pH6 and pH4.5. Tap water weight differed significantly from pH5 and pH5 differed significantly from pH4.5. There were no significant differences in weights for the *P. edulis* tadpoles (Figure 6.10B). Figure 6.10C shows the weights for *A. maculatus* tadpoles. There was a significant difference between weights for pan water and pH6, and pH6 also differed significantly from pH5.5. For *H. ornata* tadpole weights (Figure 6.10D), pH5.5 differed significantly from pan water, tap water, pH6, pH5, pH4.5 and pH4.
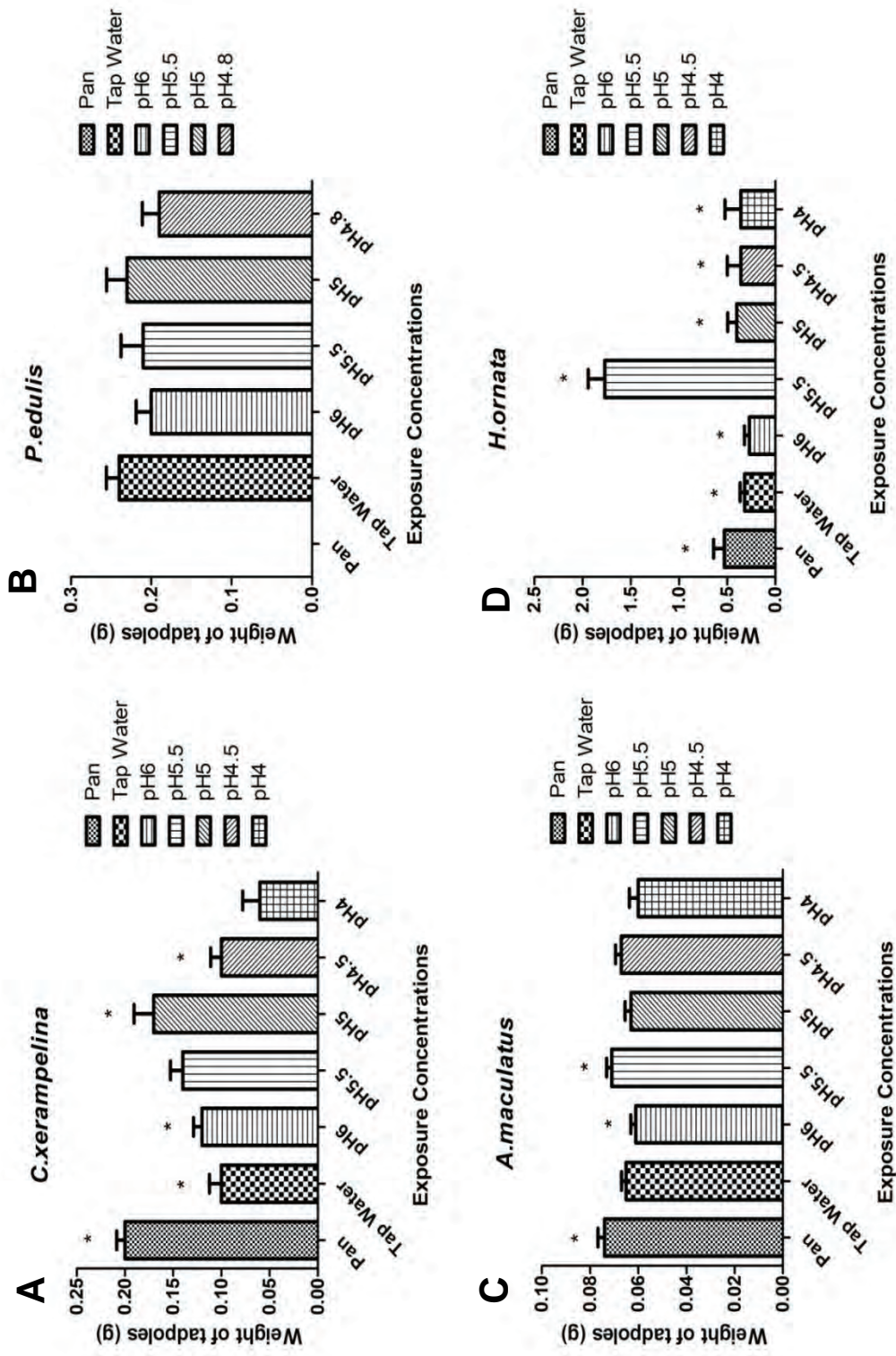
Figure 6.10: Graphs showing mean tadpole weights for all 4 frog species, at the end of their exposures. A) Mean tadpole weights for *C. xerampelina*. B) Mean tadpole weights for *P. edulis*. C) Mean tadpole weights for *A. maculatus*. D) Mean tadpole weights for *H. ornata*.

Blood smears

As indicated in Table 6.2, *C. xerampelina* did not have any parasites present in their blood, whereas *H. ornata*, *P. edulis* and *A. maculatus* did have parasites in their blood. There was a 40%, 60% and 80% parasite infection rate in slides that were screened and found positive for parasite presence, from *P. edulis*, *A. maculatus* and *H. ornata* respectively, with *A. maculatus* showing the highest parasite prevalence of 3-10 parasites per 100 blood cells.

Table 6.2: The frog species found to be positive for the presence of blood parasites when blood slides were screened, as well as their respective parasite infection rates and parasite prevalence. The locality of the frogs from which the blood slides were made is also indicated.

Frog species	*C. xerampelina*	*P. edulis*	*A. maculatus*	*H. ornata*
Frog species that had blood parasites present	NO	YES	YES	YES
Parasite infection rate (amount of slides infected out of slides screened)	0 out of 5	2 out of 5	3 out of 5	4 out of 5
Parasite Prevalence (average number of parasites per 100; 1000 or 10 000 red blood cells)	0	1/1000	1/10 000	1/100
	0	1/100	3/100	1/10 000
	0	0	10/100	1/1000
	0	0	0	1/10 000
	0	0	0	0
Locality of screened slides	Hildebrandtia, Tshokwane, Gardenia	Tshokwane	Skukuza	Tshokwane, Seribye

In Table 6.3 it is shown that 20 parasites were measured of those that were found in *P. edulis* blood slides and 47 parasites were measured from those that were found in *A. maculatus* slides. The average body length and width of parasites found in *P. edulis* was 5.33 µm and 2.56 µm respectively. For *A. maculatus* the average body length and width of the parasites were 13.55 µm and 5.82 µm. Nucleus body length and width was also measured for the parasites found in *A. maculatus*, with an average length and width of 4.7 µm and 4.25 µm. The minimum parasite length and width for *P. edulis* was 4.14 µm and 1.99 µm; and for *A. maculatus* a length of 10.57 µm and width of 4.82 µm. The maximum parasite length and width for *P. edulis* was 5.77 µm and 3.01 µm, and for *A. maculatus*, 15.08 µm and 8.21 µm. Parasites found in *A. maculatus* were found to be a different species from that found in *P. edulis*, and were also much larger in size.

Table 6.3: Body lengths and body widths of parasites found in blood slides of *P. edulis* and *A. maculatus*, also indicating average, minimum and maximum lengths and widths, as well as standard deviation.

P. edulis		*A. maculatus*			
Parasite body length (µm)	Parasite body width (µm)	Parasite body length (µm)	Parasite body width (µm)	Nucleus body length (µm)	Nucleus body width (µm)
5.15	3.01	13.21	5.31	6.94	4.45
5.77	2.39	10.75	6.32	5.64	4.81
5.18	2.53	13.4	5.16	7.11	3.15
5.3	2.53	15.08	8.21	4.29	3.6
5.55	2.84	13.23	5.24	4.73	3.51
5.67	2.96	13.59	5.22	5.35	3.55
5.37	2.67	14.52	4.88	5.78	4.39
4.98	2.33	14.27	5.05	6.2	4.1
5.68	2.47	13.61	5.88	4.27	3.7
4.84	2.3	13.58	5.29	5.03	3.85
5.57	2.43	14.22	6.08	6.03	4.31
5.22	2.1	12.79	5.45	4.92	4.2
5.57	2.92	13.18	5.54	4.08	3.91
5.66	3	12.77	4.82	4.55	3.73
5.52	2.58	13.54	5.44	3.97	4.29
5.21	2.69	13.06	5.52	4.11	3.69
5.22	2.3	13.27	5.49	5.96	4.19
5.48	2.59	14.21	5.83	4.88	4.88
5.46	2.55	14.25	5.63	4.25	4.35
4.14	1.99	13.75	5.53	5.49	3
		12.67	6.85	4.11	3.84
		14.07	5.38	4.41	3.92
		13.64	6.76	3.65	5.07
		13.13	6.91	4.01	4.49
		14.93	5.83	4.73	5.12
		13.07	7.3	4.19	4.73
		13.14	5.57	4.8	4.76
		13.69	4.9	4.81	4.49
		13.14	5.35	5.05	3.83
		13.3	7.35	4.31	4.6
		14.28	5.36	4.29	4.28
		13.92	4.97	5.13	4.44
		14.04	7.31	4.35	4.56
		14.55	6.38	4.47	5.71

		Parasite body length (µm)	Parasite body width (µm)	Nucleus body length (µm)	Nucleus body width (µm)
		13.54	7.38	4.91	4.79
		13.56	5.37	3.63	4.68
		13.7	5.61	4.95	3.65
		12.16	5.33	3.9	4.14
		14.16	5.06	3.9	4.89
		14.16	5.46	4.84	4.75
		12.76	6.36	3.59	4.92
		13.44	6.08	4.16	4.44
		12.52	6.27	3.47	4.9
		13.86	5.71	4.16	3.43
		13.11	5.34	4.86	3.45
		14.18	5.77	4.15	4
		13.71	5.92	4.56	4.09
	Parasite body length (µm)	**Parasite body width (µm)**	**Parasite body length (µm)**	**Parasite body width (µm)**	**Nucleus body length (µm)**	**Nucleus body width (µm)**
Average	5.33	2.56	13.55	5.82	4.7	4.25
Minimum	4.14	1.99	10.57	4.82	3.47	3
Maximum	5.77	3.01	15.08	8.21	7.11	5.71
Standard deviation	0.37	0.29	0.75	0.78	0.82	0.57

Figure 6.11 shows the parasites that were found in the blood slides of *A. maculatus* and *H. ornata*. We can also different parasitic stages such as intraerythrocytic and extraerythrocytic. Some red blood cells have double infections as shown in Figure 6.11J.
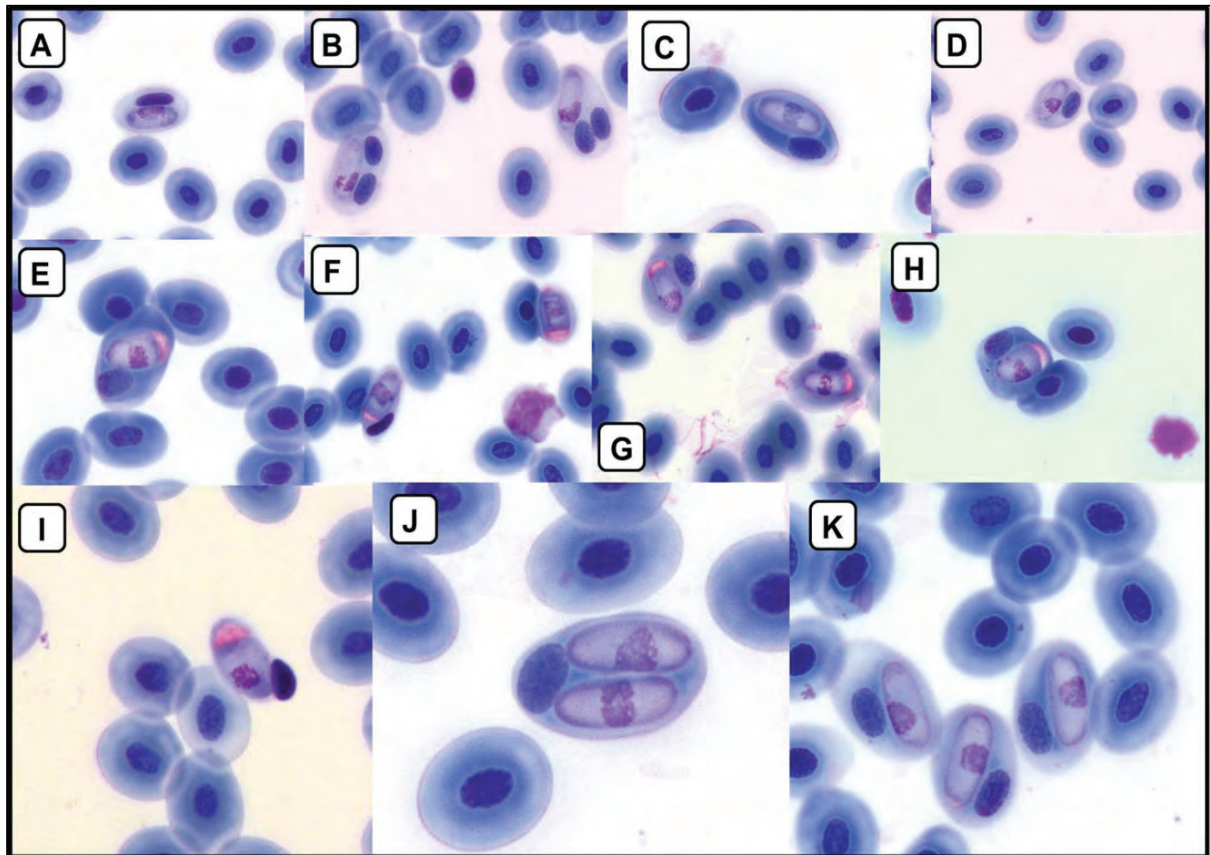
Figure 6.11: Photomicrographs of Giemsa-stained blood smears of *A. maculatus* and *H. ornata*. A-D) Intraerythrocytic parasite stage. E-H) Intraerythrocytic stage with pink-stained cap at one end. I) Extraerythrocytic stage with pink-stained cap. J) Double-infected erythrocyte. K) Photograph showing high parasite prevalence (A-I = 600X; J, K = 1000X magnification).

Figure 6.12 shows the unknown intraerythrocytic apicomplexan parasite that was found in the blood slides of *P. edulis*. As we can see the parasite is a different species from that found in *A. maculatus* and *H. ornata*.
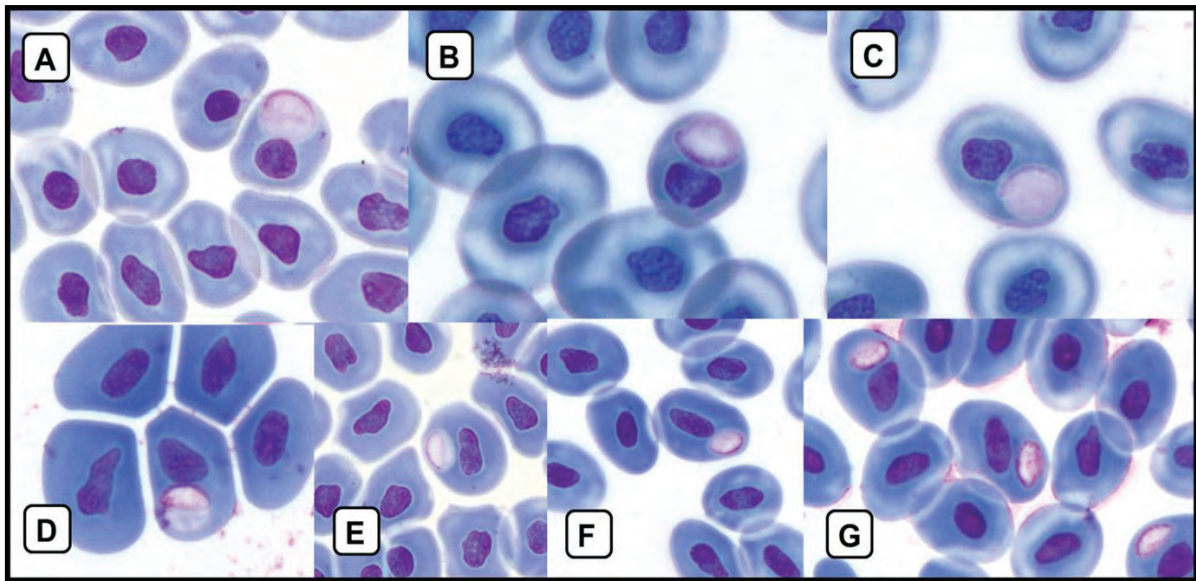
Figure 6.12: A-G) Photomicrographs of an unknown intraerythrocytic apicomplexan parasite found in *P. edulis*. (A-D = 1000X; E-G = 600X magnification).

6.4 Discussion

Acute acid tolerance bioassay

According to literature (Selvi et al. 2003; Sanzo and Hecnar 2006), there have been LC_{50} tests done on frog species before to determine concentrations of certain pollutants at which 50% of exposed individuals would die (ex. Cadmium chloride metal salt ($CdCl_2$ _H_2O) and road de-icing salt such as sodium chloride (NaCl)), but not with the intention of determining the pH value at which the frogs will have a 50% mortality in the exposed individuals. So it is thus very difficult to compare the "LC_{50}" pH-values of different frog species.

According to Sanzo and Hecnar (2006), with short-term exposures to a chemical substance such as sodium chloride (NaCl), tadpoles were shown to experience reduced activity as well as weight, and also displayed physical abnormalities. We thus suspect that the same will happen when tadpoles are exposed to different pH-concentrations. Also the lower the pH-concentration, the worse the possible effect will be on the exposed tadpoles. Räsänen et al. (2003) state that survival of tadpoles as well as hatchling size was reduced with exposure to decreasing pH-concentrations. As we saw from the results all of the frog species' tadpoles died in pH3.5 or lower. Tadpole mortality also increased as the pH-concentrations decreased. So we can safely say that lower pH-concentrations will have negative effects on tadpoles.

According to Du Preez and Carruthers (2009) Bullfrog species spend a lot of time in the water while calling and then later while protecting young tadpoles. This might be a possible

reason for *P. edulis* being more sensitive than the other 3 species. Tadpoles can take up to a month or more to complete their life-cycles, while *A. maculatus* tadpoles finish within 2 weeks. Very little time is spent terrestrially and then only to hibernate until their breeding season starts. *A. maculatus* is a riverine frog species, used to river conditions, which might account for it being hardier. In contrast with *P. edulis* tadpoles, *A. maculatus* tadpoles receive very little to no parental care and are heavily preyed upon by various aquatic predators. The tadpoles must thus be hardier in order to survive. *C. xerampelina* is an arboreal species that spends almost no time in water, and is therefore not used to or tolerant towards the daily changes occurring in water bodies. Eggs are secreted into foam nests that are positioned above the water, where they develop for 4 to 6 days before dropping into the water (Du Preez and Carruthers, 2009). During this time very little predation of eggs occur. This may account for its "LC$_{50}$" pH-value being nearer to that of *P. edulis*. No specific reasons can be pinpointed to explain the differing sensitivities of the 4 frog species. A lot of factor could be contributing to their varying tolerance levels towards changing pH-concentrations.

A lot of acidic fluid is needed to alter the pH of water. The current pH of 5.42 of rain water will most probably not lower the pH-levels of natural water bodies, occurring within the park, sufficiently to have any current negative effects on frog species using these water bodies. The sediment of these water bodies also acts as an excellent buffer in these water bodies, meaning that the slightly acidic rain water will probably have no change on the pH of these water bodies at all. At least we now know the pH-conditions in which these frog species will be detrimentally affected, and can hopefully put in place some preventative measures.

Chronic acid tolerance bioassays

Aquatic eggs and larvae of frogs are particularly susceptible to toxic substances such as pesticides, insecticides, etc., as well as acid precipitation, which may not occur in concentrations high enough to kill adults or even embryos, however several of these toxic substances do affect the development of embryos and larvae to the point of causing a high percentage of abnormalities or a decrease in the rate of development resulting in prolonged larval periods or dwarfed young (Deullman and Trueb, 1986). Amphibian eggs and larvae are especially sensitive to heavy metals and drainage from mines that can have calamitous effects on some populations of amphibians. According to the results of Räsänen et al. (2003), increased acidity generally reduces the survival of embryos and tadpoles. Tadpole size is also reduced with increased acidic conditions (lower pH conditions). Their results also suggest that their test species populations that were breeding in acidic waters had a higher acid tolerance and may have evolved rapidly in response to their environmental acidification

under strong selection. Chronic exposure to sodium chloride (NaCl) revealed significantly lower survivorship in frog tadpoles, a decreased time to metamorphosis, reduced weight and activity, and increased physical abnormalities with an increase in the salt concentration (Sanzo and Hecnar, 2006). Personal observations of tadpoles in the long-term exposure experiment also support this. Tadpoles were much smaller in the lower pH-concentrations; were lighter in their colouring and also tended to develop more axial deformities, where their tails grew crooked.

Because decreased pH-concentrations negatively affect tadpoles as well as embryos, as mentioned above, there are still tadpole mortalities, even though the pH-concentrations chosen for this assay were higher than their "LC_{50}"-pH values. As we already know from our acute acid tolerance assays, *P. edulis* is the most sensitive of the 4 frog species, and thus has the overall higher mortality percentage as well as for several of the other exposure concentrations. In contrast we know that *A. maculatus* is more tolerant and thus has the overall lowest mortality percentages. The pan water that was used was possibly too dirty for the sensitive *P. edulis* tadpoles, accounting for the 100% mortality in this exposure solution. According to Du Preez and Carruthers (2009) tadpole development and metamorphosis of *A. maculatus* tadpoles are generally complete after 2 weeks. For *P. edulis* tadpoles complete metamorphosis in about 30 days. Nothing is mentioned for *C. xerampelina* and *H. ornata*. So from figure 9 we can see that *A. maculatus* tadpole metamorphosis was delayed quite a bit. Tadpole only started to develop hind legs after 27 days (almost 4 weeks) and took several more weeks to finish metamorphosis. The same for *P. edulis* tadpoles, they only started developing hind legs after 25 day and took several more weeks to finish metamorphosis. So we see that decreased pH-concentrations can also delay tadpole metamorphosis.

The general decrease in tadpole lengths as well as weights as seen in Figures 6.6, 6.7, 6.8, 6.9 and 6.10 can be explained by the negative effects that decreased pH-concentrations have on tadpoles as is mentioned above.

The mean *C. xerampelina* tadpole length for pH4 (Figure 6.6) does not differ significantly from the unexposed group's mean length, because it could not be properly compared. All of the tadpoles, except 4, died during the chronic assay and was thus not sufficient to make an accurate comparison. Because this pH-concentration is the closest to the "LC_{50}"-pH value of *C. xerampelina*, it had the most mortalities. *C. xerampelina* tadpoles in pan water differed significantly from those in the lower pH-concentrations, at both of the time intervals, because of the higher pH of pan water and the possibly more optimal conditions, because the pan water was collected from the natural water bodies occurring within the Kruger park (the natural habitats of the frog species). The significant differences for pan water, water, pH5

and pH4.5 between the 2 time-intervals are because exponential tadpole growth occurred within these exposure concentrations as the assay continued.

All of the pH-concentrations used for *P. edulis* (Figure 6.7) showed tadpole growth, and thus all differed significantly from the unexposed group that was measured at the start of the chronic assay. Tadpoles from pH6 showed significant growth from the 16 day time interval to the 25 day time-interval, where they were at first generally smaller than those in pH5.5, but then grew to be of the same size as tadpoles in pH5.5. All of the other pH-concentrations possibly reached their optimal growth rates at the 16 day interval and continued at the same rate until the end of the assay. Thus there were no significant differences in their mean lengths from one time-interval to the next.

Again all of the pH-concentrations (Figure 6.8) were significantly different from the unexposed group, because tadpoles grew as the assay continued. Tadpole growth for the 17 day time-interval was more in pH5.5, pH5 and pH4.5 and less in pan water, tap water, pH6 and pH4, thus there was a significant difference. Because *A. maculatus* is a riverine species pH5.5, pH5 and pH4.5 might be more optimal conditions than the other pH-concentrations and solutions that were used for the assay. The conditions in pan water are more suited to species that prefer static water instead of slow or fast moving water like *A. maculatus*. For the 27 day time-interval pH5.5 showed the highest tadpole growth and thus differed significantly from water, pH6 and pH4, which had the lowest tadpole growth. Again this might be because of the more suited conditions of pH5.5. The significant differences between the time-intervals were because tadpoles in pan water grew from being second smallest in size, to being fourth smallest. Tadpoles in pH6 were fourth smallest at first and then turned out to be the smallest tadpoles at the end of the assay. So their growth rate was very low or actually decreased. Growth rate for tadpoles in pH5.5, pH5 and pH4.5 were exponential, except for tadpoles in pH4.5 which went from being the largest to being second largest. Tadpoles in pH4 were the smallest but showed an increase in growth rate and caught up to tadpoles from the other pH-concentrations at the end of the assay.

Tadpoles from pH5.5 (Figure 6.9) differed significantly from tadpoles in the other pH-concentrations because they grew much larger. This is because of the fact that the *H. ornata* tadpoles are very predatory toward each other, especially in captivity (Du Preez and Carruthers, 2009). As soon as one or more of the tadpoles were slightly bigger than the others; they started to eat the smaller tadpoles. In this instance there was only one remaining tadpole of *H. ornata* left in the pH5.5 solution. The tadpole growth is thus also much higher because of the decreased tadpole-density in this particular container compared to the rest of the containers. According to Crump (1990) cannibalism or intraspecific

predation is now recognized as normal behaviour, rather than aberrant, and it has been documented in more than 1300 animal species. According to this article, cannibalism frequently occurs under conditions of overcrowding and food limitation. Du Preez and Carruthers (2009) also states that *H. ornata* tadpoles show cannibalistic tendencies in captivity. It is suggested that cannibalistic tadpoles have faster developmental or growth rates, are larger in size and have increased survivorship (Crump 1990). Tadpoles in pan water and pH5 had the most growth from the start of the assay to the end. Tadpoles in water, pH6 and pH4.5 went from being amongst the larger sized tadpoles to being among the smallest. Tadpoles from pH4 did not show much growth over the course of the assay because of the low pH-concentration that was affecting both the growth and growth rate of the tadpoles. Conditions in the other pH-concentrations were more suitable.

Blood smears

According to Du Preez and Carruthers (2009) the Flat-backed Toad appears to favour shallow, static or slow-moving water in rivers, weirs and dams which occur in a variety of vegetation types in lowveld grasslands and savannas. Ornate Frogs are a burrowing species, found in a variety of savanna types of vegetation. According to the authors they avoid dense woodland areas and rocky terrain. They also hibernate in deep sandy soils, only surfacing during the breeding season. Shallow temporary pans and marshy areas in open savanna woodlands in eastern and southern Africa are the preferred habitats of the African Bullfrog (Du Preez and Carruthers 2009). They also occur in rice paddies in Mozambique. Adults are also fossorial, like the Ornate Frog, remaining buried for most of the year, except during the breeding season. The Southern Foam Nest Frog can be found in a variety of bushveld vegetation types around seasonal or permanent bodies of open water.

Looking at the different types of habitat as well as behaviour in these 4 frog species, we can see that the Southern Foam Nest Frogs are almost never found in water, preferring to sit in vegetation around water bodies, whereas the Flat-backed Toad is almost always in water. Ornate Frogs and the African Bullfrogs both spend some time out of the year burrowed in the soil, but emerge during their breeding seasons, where the Bullfrogs occur in wetter areas. So we can safely say that Flat-backed Toads, Ornate frogs and African Bullfrogs spend more time in the water than the Southern Foam Nest Frog. Blood parasites in frogs are most likely transmitted by leeches (Barta and Desser 1989), so we then hypothesize that the frog species which spend more time in the water, *H. ornata*, *P. edulis* and *A. maculatus*, will have a higher chance of becoming infected with blood parasites, whereas the species that don't come into contact with water that often, *C. xerampelina*, will be less likely to be infected. We

also hypothesize that if parasites are found in the blood, then they will likely be new species, since very little work has been done on the blood parasites occurring in frogs.

When looking at Table 6.2, it is clear that *C. xerampelina* did not contain any blood parasites, whereas *P. edulis, H. ornata* and *A. maculatus* were indeed infected. So the hypothesis holds true thus far. We also see that *A. maculatus* had the highest prevalence of the 3 infected frog species, also correlating with the fact that they spend the most time in the water and thus in potential contact with vectors.

The blood parasites that were found in these frogs are most likely new species, seeing as very little research has been done thus far on the parasites of frogs of southern Africa. These parasites will be identified and further described in detail at a later stage. Figure 6.2 shows the unknown intraerythrocytic apicomplexan parasite found in the blood of *P. edulis*. This parasite species is different from that found in *A. maculatus* and *H. ornata*, possibly because of the different habitats and environmental conditions of these 3 frog species.
The measurements in Table 6.3 will later be used in aid of identifying or describing the new blood parasite species that are found.

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7 FROGS AS BIOINDICATORS OF ENVIRONMENTAL QUALITY IN THE KRUGER NATIONAL PARK – A PRELIMINARY STUDY USING HISTOLOGY AS ENDPOINT

7.1 Introduction

The exposure of feral populations of animals to environmental contaminants is a global problem (Jenner et al. 1990). Various biological measures have been incorporated into bioassessment programs to evaluate the quality of surface water resources (Adams 2002). To rely on chemical criteria alone without information on biological responses, may be inaccurate and could not portray the biological and ecological condition of aquatic systems.

Histopathological biomarkers or cellular changes in tissues and organs, represent an integration of cumulative effects by physiological and biochemical stressors (Hinton and Lauren 1990) and therefore can be linked to exposure (Van Dyk et al. 2012; Myers and Fournie 2002). The histopathological biomarker approach, linking lower to upper levels of biological organisation, is most useful in multidisciplinary studies. Many lesions are well validated experimentally by exposure to specific stressors. The effects of environmental pollution on liver have previously been done on *Rana esculenta* (Fenoglioa et al. 2005). Amphibians are good bioindicators of environmental pollution due to their susceptibility to chemicals during their freshwater cycles (Fenoglioa et al. 2005). The effects of environmental pollution, together with changes in human activity and climate, have contributed to the reduction in the amphibian population over recent decades (Venturino et al. 2003). However, toxicological research on amphibians has been rather scarce compared with that on other vertebrates.

This project mainly focused to use histology as a tool to assess the effect of environmental pollution on two frog species *Tomopterna cryptosis* and *Breviceps adspersus* in the Kruger National Park. Histology in environmental impact assessment studies aim to detect cellular changes at an early stage before the organ, organism or fish population is affected (Hinton and Lauren 1990). Environmental pollutants such as metals and endocrine disrupting chemicals have a negative on aquatic species and cause cellular changes in organs such as liver, kidney and gonads (Marchand 2006)

The aim of the study was to investigate the health status of two frog species *Tomopterna cryptosis* and *Breviceps adspersus* in the Kruger National Park, using histology as endpoint. Frogs are more susceptible to human impact as breeding occurs in water bodies that are either ephemeral or fluctuate significantly in water level (Pyke 2002).

The species studied were selected on the basis of their diverse breeding strategies. *Breviceps adspersus* is a fossorial group, digging into well drained or loamy soils where they spend most of the time. After good rains, they will emerge and after amplexus, the pair will construct a chamber where the female will deposit her eggs (Du Preez and Carruthers 2009). In contrast, *Tomopterna cryptosis* will leave its burrow (Du Preez and Carruthers 2009) after good rains to lay its eggs in the shallow seasonal pans and even in shallow wallows (Channing 2004).

7.2   Material and methods

7.2.1   Sample collection and processing

Ten specimens were collected of the two frog species *Tomopterna cryptosis* and *Breviceps adspersus* respectively. *Tomopterna cryptosis* was collected using scoop nets to collect the frogs from the pans, whilst *Breviceps adspersus* was collected by hand after and during a rain event when they crossed the road between Skukuza rest camp and the Sand River. The first pan is located near Skukuza and the other near Tshokwane. The frogs were sacrificed on site by severing the spinal cord. Before submersion in the fixative, a ventral incision was made in the abdominal surface of each specimen to expose the visceral organs for optimal fixation. The specimens were fixed whole in 10% neutrally buffered formalin, washed in tap water and dehydrated to 70% ethanol. The width and length of each frog was noted. In the laboratory, each frog was removed from the 70% ethanol solution and dissected, during which a liver, lung, heart, gonad, kidney, stomach as well as a ventral and dorsal skin sample was collected for light microscopy analysis. These samples were stored in 70% ethanol and sent to and independent laboratory, Amanzi Biosecurity, for further processing and preparation for light microscopy analysis using standard techniques for Haematoxylin and Eosin (H&E) staining. The prepared slides were analysed at the University of Johannesburg. This was done by two assessors for increased objectivity and accuracy. Digital images were processed using IM50 Image Manager Software (Pixel IT (Pty) Ltd).

7.3   Results

7.3.1   Specimen data and macroscopic observations

The specimen data for the selected frogs analysed is presented in Table 7.1. The frogs of both species were of similar size. All *T. cryptosis* were identified as female and the *B. adspersus* had a sex ratio of 70% female to 30% male. A necropsy could not be done on site, however, a macroscopic examination was done post-fixation to identify any structural abnormalities. Even though the liver tissue changed colour as a result of the fixation process, the liver of one *T. cryptosis* specimen and two *B. adspersus* specimen showed a

clear difference in liver colour compared to the rest of the sample group. These livers exhibited a light grey colour compared to the dark brown colour of the other specimens. No other macroscopic abnormalities were identified.

Table 7.1: Specimen data for *T. cryptosis* and *B. adspersus* collected from two pans near Skukuza and Tshokwane respectively.

Species	Sample size		Body length	Body width	Sex
	Skukuza	Tshokwane	(mean – mm)	(mean – mm)	
T. cryptosis	3	7	42.7	34.6	100%F
B. adspersus	2	8	39.9	32.7	70%F:30%M

7.3.2   Histopathology

The light microscopy analysis showed that the general tissue architecture of the different target organs of both species were intact and seem to be in a functional state. However, specific histological alterations were identified in the liver and kidney samples of both species, from both sampling sites. However, it should be noted that the difference in sample size of the two sites, compromises the comparability of a site comparison in terms of the histological alterations identified. The percentage prevalence of the histological alterations identified in the selected target organs is presented in Table 7.2.

Table 7.2: Percentage prevalence of histological alterations identified in *T. cryptosis* and *B. adspersus* collected from Skukuza and Tshokwane respectively.

Organ/ alterations	*T. cryptosis*		*B. adspersus*	
	% prevalence		% prevalence	
	Skukuza (n = 3)	Tshokwane (n = 7)	Skukuza (n = 2)	Tshokwane (n = 8)
Liver				
Nuclear pleomorphism	0	43	100	63
Hepatocellular vacuolation	0	43	100	75
Focal necrosis	33	43	50	63
Dilation of blood sinusoids	33	14	0	0
Vascular congestion	66	71	50	0
Fibrillar inclusions	0	0	100	0
Hepatocellular atrophy	33	29	0	0
Melano-macrophage centres	33	57	0	0
Kidney				
Nuclear pleomorphism	0	0	50	0
Glomerulus capillary dilation	0	0	0	13
Inflammatory response	0	0	0	13
Melano-macrophage centre	0	14	0	0
Inter-tubular blood	33	0	0	13
Hydropic change	33	14	0	0
Tubular epithelial vacuolation	0	0	0	0

Tomopterna cryptosis

Most of the liver samples showed the characteristic hepatic cord organisation and the general hepatic architecture was visible in all liver samples of *T. cryptosis.* The cells appear spherical in shape and exhibit a granular cytoplasm. The histological alterations that were only identified in frogs from Tshokwane included nuclear pleomorphism and hepatocellular vacuolation. The nuclear content of the different hepatocytes appeared granular and the nuclei varied greatly in size and shape. The hepatocellular vacuolation was mostly focal in nature although diffuse vacuolation was also identified in a number of specimens. The vacuolated cells in affected specimens was either visible as micro or macro vesicles.

Some of the specimens from both sites also showed focal areas of hepatocyte degeneration and necrosis. These areas were mainly identified in peri-vascular tissue regions and were characterized by pale eosinophilic hepatocytes and nuclei that did not take up Haematoxylin. In other areas, necrotic cells were characterized by dark eosinophilic cytoplasm and pyknotic nuclei.

Dilation of blood sinusoids were only identified in one specimen from Tshokwane and Skukuza respectively, while vascular congestion was identified in a number of frogs from both sampling sites. The presence of melano-macrophage centres and issue regions showing hepatocyte atrophy was visible in specimens from both sampling sites.

Only a few histological alterations were identified in the kidneys of *T. cryptosis.* These included hydropic change of the tubular epithelial cells of two specimens, an increased presence of inter-tubular blood in one specimen from Skukuza and the presence of melano-macrophage centres in one specimen from Tshokwane.

All ten specimens collected were identified as female. The ovary samples showed that 50% of the frogs were mature and 50% immature with regard to the developing stages of oogenesis identified. No histological alterations were identified in any of the ovary, stomach, lung, heart or skin samples analysed. Parasitic infections within the skin samples were identified in four frogs.

Breviceps adspersus

As with *T. cryptosis,* the liver samples showed the characteristic hepatic cord organisation and the general hepatic architecture was visible in all liver samples of *B. adspersus.* Histological alterations identified included, firstly, nuclear pleomorphism. The nuclear content of the different hepatocytes appeared granular and the nuclei varied in size and shape. Hepatocellular vacuolation was also visible in a number of specimens. The tissue regions

affected was mostly focal in nature although diffuse vacuolation was also identified in a number of specimens. Fibrillar inclusions were observed in specimens from Skukuza only. Some of the specimens from both sites also showed focal areas of liver degeneration and necrosis. These areas were mainly in peri-vascular tissue regions and were characterized by pale eosinophilic hepatocytes and nuclei that did not take up Haematoxylin. In other areas, necrotic cells were characterized by dark eosinophilic cytoplasm and pyknotic nuclei. Vascular congestion was identified in one frog from Skukuza.

A few histological alterations were also identified in the kidneys of *B. adspersus.* Nuclear pleomorphism was observed in one of the specimens from Skukuza. A mild inflammatory response, characterized by the infiltration of mono-nuclear leukocytes was identified in one specimen from Tshokwane. Dilation of the glomeruli capillaries was also observed in one specimen from the latter site.

Of the ten specimens collected, three were identified as male and seven were identified as female. The light microscopy analysis confirmed that the testes of the male specimens were all mature in terms of the stages of spermatogenesis observed during the light microscopy analysis. Six of the females were noted as being mature and one female were in the developing stages of oogenesis. No histological alterations were identified in any of the testes or ovary samples collected.

No histological alterations were also identified in any of the stomach, lung, heart or skin samples analysed. The colour plates (figures in this chapter is in Addendum 7.1).
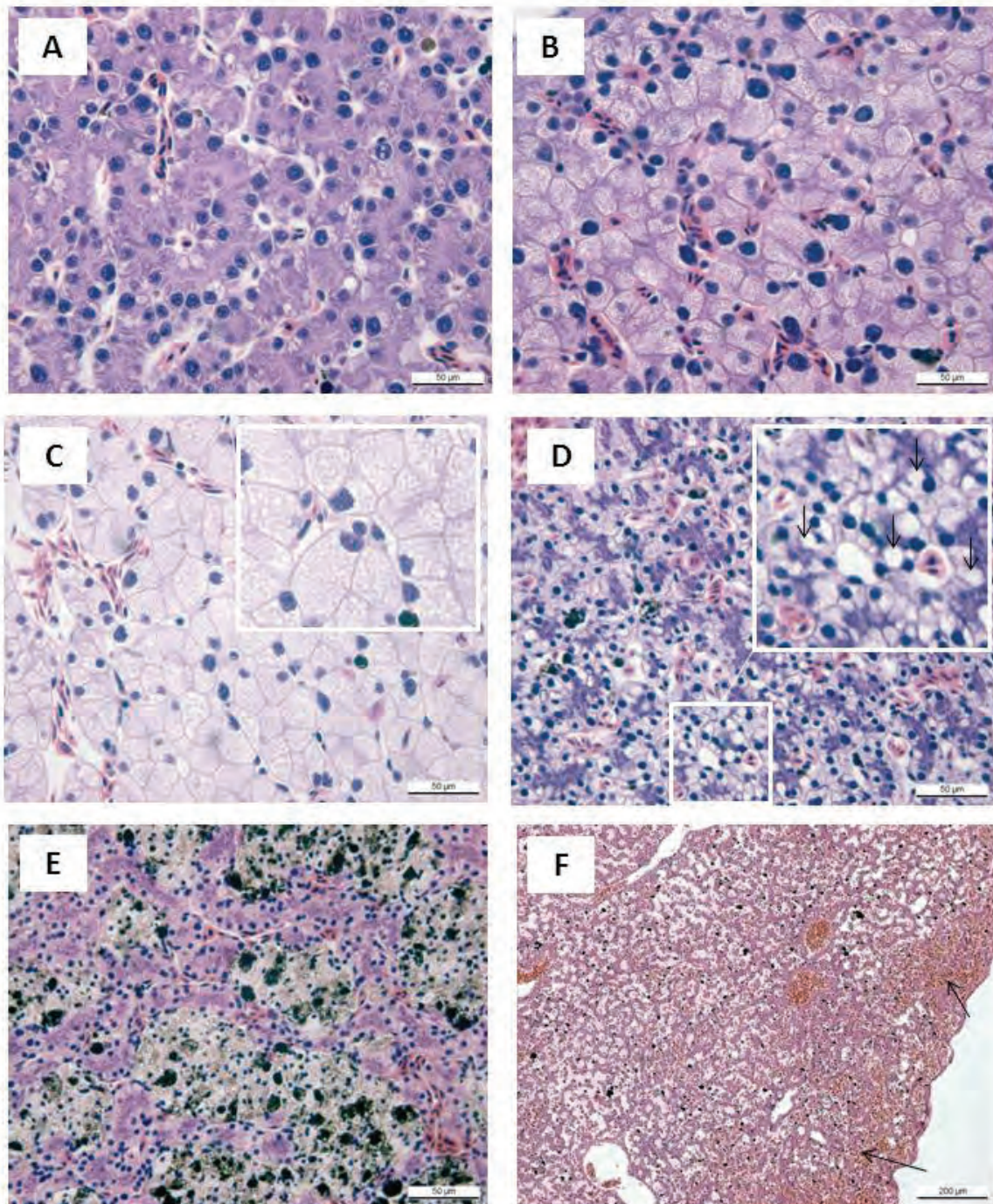
Figure 7.1: Light micrographs (H&E) showing the selected histological alterations identified in the livers of *T. cryptosis* and/or *B. adspersus*: A: Micrograph (x40) showing the normal cord-like structure of the hepatocytes. B: Micrograph (x40) showing granular cytoplasm of hepatocytes. C: Hepatocellular vacuolation characteristic of microvesicular steatosis visible within hepatocytes (x40). D: Hepatocellular vacuolation characteristic of macrovesicular steatosis visible within hepatocytes (x40). Melano-macrophage centres between hepatocytes (x40). F: Low magnification (x10) showing increased blood visible in blood sinusoids.
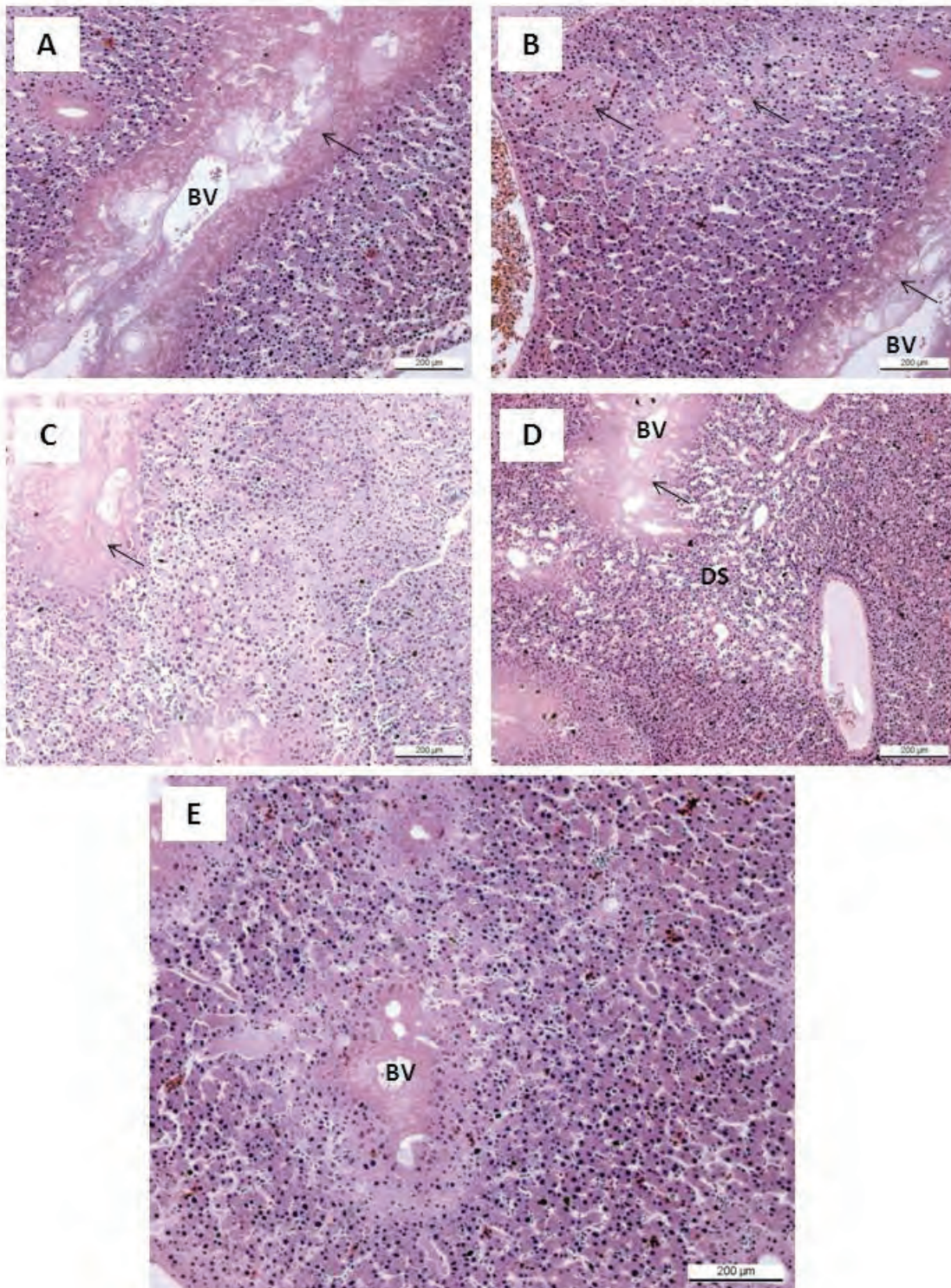
Figure 7.2: Light micrographs (H&E) showing focal areas of liver degeneration and necrosis identified in *T. cryptosis* and *B. adspersus*: A: An area of degenerative hepatocytes (arrow) visible around a blood vessel (BV) (x10). B: Focal areas of necrosis (arrow) visible in close proximity of blood vessels (x10). C: Focal area of degenerative hepatocytes (arrow). Note the pale eosinophilic staining (x10). D: Dilation of blood sinusoids (DS) visible around degenerative hepatocytes (x10). Necrotic hepatocytes (eosinophilic area) surrounding a blood vessel (x10).
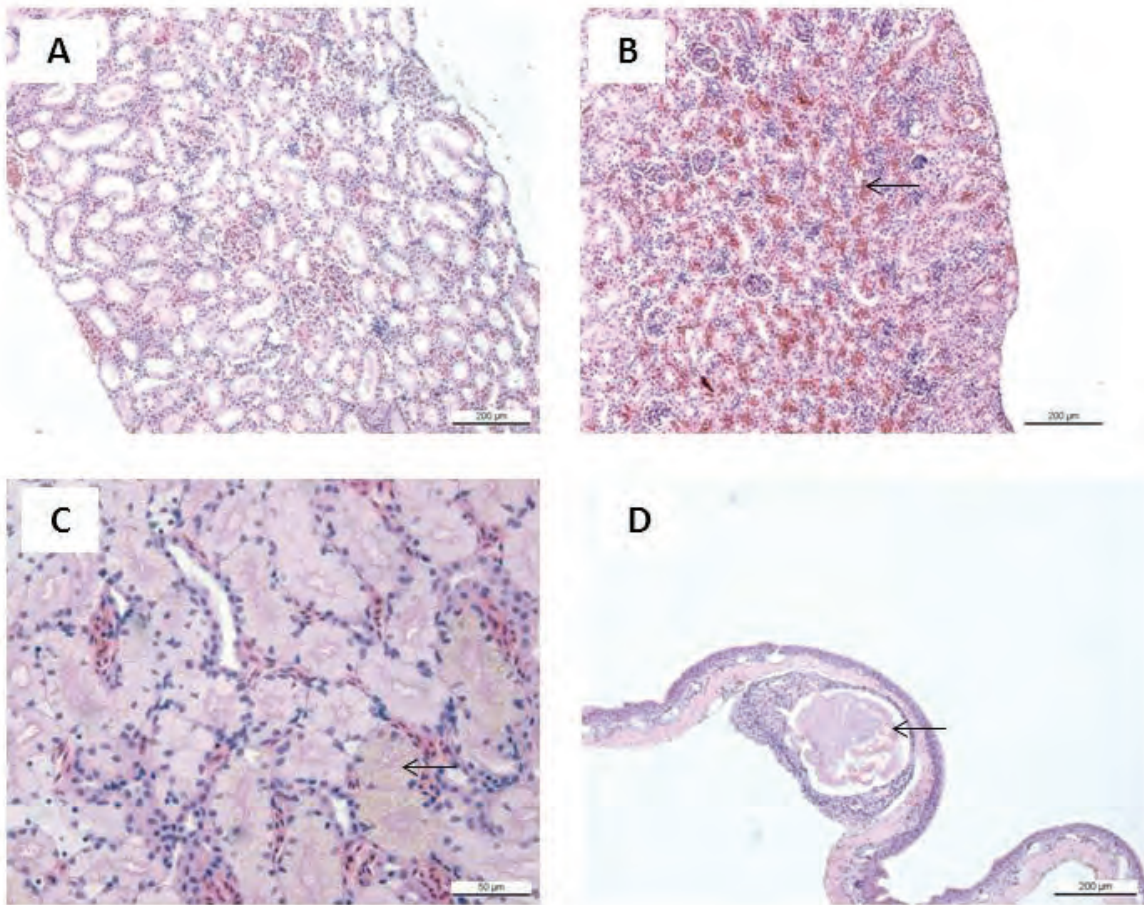
Figure 7.3: Light micrographs (H&E) showing selected histological alterations in the kidney and a parasitic infection identified in the skin samples of *T. cryptosis* and/or *B. adspersus* respectively: A: Micrograph (x10) showing normal kidney structure. B: Increase in inter-tubular blood cells visible (arrow) (x10). C: Hydropic change of tubular epithelial cells visible. Note the increase in size of the epithelial cells and the glassy appearance of the cytoplasm. Also note the intracellular deposits (arrow) stained yellow-brown with H&E. D: A parasite visible within granulomatous tissue in the skin of a *T. cryptosis* specimen.

7.4   Discussion

Examination of the histological sections of the stomach, lung, heart, skin and gonads (testes and ovaries) of the two frog species *T. cryptosis* and *B. adspersus* in the Kruger National Park showed no histological alterations. Histological alterations were noted in the liver and kidney of both species, but not all specimens. It should also be noted that this was a preliminary study with a small sample size in Skukuza, e.g. *T. cryptosis* (n=3) and *B. adspersus* (n=2). As this was a preliminary study to investigate the possibility to use frogs as bioindicators of the environment, a larger sample size is necessary for future studies.

The presence of environmental pollutants was not linked to the histopathological finding of this study. The aim of this study was to identify and describe the cellular changes that were observed and not to link these changes to any specific stressor. Many biological and physical stressors, in addition to chemical stressors, can impact aquatic organisms and aquatic-dependent wildlife. These stressors may be natural, such as daily or seasonal temperature fluctuations, or anthropogenic such as contaminants, thermal effluents, or physical habitat modifications. The effects of these stressors have also not been included in this study. Animal responses to mixtures of chemicals are often different from those by individual chemicals (de Souza-Bueno et al. 2000). It is not possible to measure the impact by chemical stressors individually or the mixtures, without biological and physical stressor impacts. Biomarkers that are sensitive and specific only to the source of exposure are rare (Sorensen et al. 2003).

Early toxicopathic, non-neoplastic lesions identified included non-focal hepatocellular and nuclear pleomorphism, hepatocellular vacuolation and focal necrosis. Fibrillar inclusions were only present in the *B. adspersus* specimens in Skukuza (n=2). Selected liver histological alterations are identified such as vascular congestion, hepatocellular atrophy and the presence of melanomacrophage centres. These changes could reflect the presence of unfavourable stressors. Histological changes were only observed in the liver and kidney.
According to Hinton and Laurén (1990), fish liver microscopic structure is an integrator of physiological and biochemical function which, when altered, may produce biomarkers of prior exposure to toxicants. The liver has a key role in xenobiotic metabolism and excretion, digestion and storage, and the production of yolk protein. Thus, alterations in structure are expected under certain toxic conditions (Hinton et al.1992).

Groman (1982) stated that the kidney, specifically the trunk (posterior) kidney, is one of the more important excretory organs of teleost fish. One would assume that the renal tissues would be at major toxicological risk since they receive large volumes of blood flow from both

the renal portal venous system and the renal arteries. In addition, urine produced collectively or individually through glomerular filtration, tubular reabsorption, or tubular secretion, serves as a major route of excretion for metabolites of various xenobiotics to which fish have been exposed to (Hinton et al. 1992).

For future toxicological studies it should be noted that besides pollution levels, other factors that should be considered when evaluating histopathological results of frogs should include the impact of seasonal variation, the age of the frogs and the sample size of species collected.

For representative and accurate results, the largest possible samples size is recommended. Most fish health studies also incorporate bioaccumulation and other biomarker response tests in conjunction with histopathology (van Dyk et al. 2009; Marchand et al. 2012). The inclusion of other biomarkers could provide valuable information in studies where frogs are used as bioindicators of environmental quality. It should further be noted that only a portion of the organ is sampled for histopathological analysis. It could therefore occur that the histopathological findings could be an underestimation of the actual condition of the organ that is being assessed.

We concluded that frog histology of the liver and kidney could be is a sensitive biomarker to show the effect of environmental pollution.

7.5 Recommendations

In order to use frog histology as a bioindicator for environmental quality to compare different species and different sampling sites, an adequate sample size of at least 10, preferably 20 specimens are required. However, the histological examination of individual specimens may reveal abnormalities and histological changes in the organs examined. It is also necessary to do a necropsy, including a macroscopic examination (externally and internally), body measurements should be taken and blood samples should also be obtained for further biochemical analysis. These should all form part of a standard histology-based frog health assessment protocol for future toxicological studies.

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8   RECOMMENDATIONS and CONCLUSIONS

Amphibian monitoring

This is a summary of the most important components the research team feels should be investigated in future. The most important aspect is the lack of detailed information on amphibian biology and habitat needs.

Amphibians are an important component of South Africa's exceptional biodiversity (Siegfried 1989) and are such worthy of both research and conservation effort. Amphibian populations are declining throughout the world (Wyman 1990; Wake 1991; Kiesecker et al. 2001; Blaustein and Kiesecker 2002). It has become clear that declines cannot be attributed to any single causative factor and those complex mechanisms involving abiotic and biotic interactions are responsible for this phenomenon (Blaustein and Kiesecker 2002). These declines have been attributed to a combination of factors, including climate change, chemical pollution, habitat loss and disease (Blaustein et al. 2003). Evidence for a countrywide decline in frog populations in South Africa is lacking (Channing and Van Dijk 1995). Amphibian declines in southern Africa have been observed, but only at the local population level, and are usually confined to areas directly impacted upon by relevant threats. Among many threats faced by amphibians in southern Africa, the most frequently implicated is habitat destruction resulting from wetland drainage, afforestation, crop farming, invasive alien vegetation and urbanisation (Harrison et al. 2001).

One of the most difficult problems in conservation biology is the lack of baseline data against which to measure population changes. The result has been that much of the information concerning amphibian declines is anecdotal and this is especially pertinent for South Africa. In some instances, especially in the case of disappearances, the anecdotal information is useful, but all ecologists are aware that populations undergo fluctuations in size under ordinary circumstances. Accordingly, a decline over a period of two or three years might be more than offset by a single year of successful recruitment. Those concerned with biodiversity, in general, have long recognised the need to establish long term studies, using standardised methods for natural populations. There is a growing interest in species and groups that might serve as indicators of the state of health of the environment. Many different taxa could be useful indicators, and amphibians have received considerable attention because of a combination of biological attributes:

- Permeable skin that acts in respiration and osmoregulation
- Biphasic life cycle with aquatic and terrestrial phases
- Feeding shifts in many species from herbivorous diets as larvae to carnivorous diets as adults

- Exposed developmental biology offering ease of investigation
- Amphibians are abundant and functionally important in most freshwater and terrestrial habitats in tropical, subtropical and temperate regions.

Regular monitoring of amphibian populations is the best way of determining population trends within species. This may be the only way of assessing conservation actions for many amphibians. Some amphibians undergo large natural fluctuations in their population numbers and so long-term datasets are required in order to determine the direction of trends over time. Monitoring data can be used to assess the effects of conservation and other land management practices. Where possible, all monitoring techniques should be quantitative (i.e. estimating abundance with confidence intervals so that comparisons can be made). Monitoring sites and methods need to be chosen with care so that they can be realistically continued over long periods with consistent methods. Climate change is known to have had strong effects on amphibian distributions in the past, and rapid future changes in climate can therefore be expected to affect both distributions and densities of South African frogs (Measey et al. 2011).

Monitoring can play a vital role in determining whether effects of climate change are reflected in amphibian populations. Models can be used to decide where best to target species or communities of frogs for monitoring of climate change. Monitoring should be enacted across a range of habitats and altitudes to account for local spatial environmental effects. Monitoring entire amphibian communities rather than only threatened species will also enable estimates of the generality of these effects (Measey et al. 2011). When considering an amphibian community assemblage monitoring site, there are many issues worth considering and building into the monitoring design. Some important criteria in selecting a monitoring site include (Measey et al. 2011):
- Importance of using diverse sampling techniques, temporal and seasonal sampling.
- Reduction of sampling error by consistency of recorders—worth training multiple recorders who go out in (at least) pairs to keep consistency. For long-term monitoring it is important to plan for replacement of personnel as they move jobs or retire.
- Importance of flexibility in sampling as diverse range of life-history stages as possible and identification and measurements thereof.
- Detectability may vary with weather, moon brightness, precipitation and prey availability.
- Locating monitoring sites near national weather stations is strongly encouraged wherever possible or installing a weather monitoring station alongside the monitoring site.

- Accessibility is of key importance as sites must be visited during inclement weather when vehicular access is likely to be at its poorest.
- Communication of monitoring results to the wider research community and the general public (Measey et al. 2011).

Long term monitoring projects specifically focused on amphibians are required within the Kruger National Park pan systems which specific emphasis on water quality and micro-habitat selection of the adults and tadpoles within selected pans. Representative pans should be selected from the 45 pans which have been currently surveyed. The data from this survey provides current water quality and species lists which could be supplemented from further surveys. The current distribution of Striped Stream Frog (*Strongylopus fasciatus*), Raucous Toad (*Amietophrynus rangeri*), Natal Sand Frog (*Tomopterna natalensis*) and Shovel-Footed Squeaker (*Arthroleptis stenodactylus*) within the Kruger National Park needs to be addressed. The lack of any recent records of these species during the current survey needs further investigation. The limited records of Sharp-nosed Grass Frog (*Ptychadena oxyrhynchus*) warrants further surveys in suitable habitat.

The low numbers of Eastern Olive Toads (*Amietophrynus garmani*) and Guttural Toads (*Amietophrynus gutturalis*) may be attributed to the increased nocturnal traffic on the roads. Several road fatalities of Am*ietophrynus garmani* and *Amietophrynus gutturalis* were observed during the study. Many frog species disperse along the tarred roads or move onto the roads after heavy rainfall events as well as the emergence of winged-termite alates. Road mortalities of Tremelo Sand Frog (*Tomopterna cryptosis*), Southern Foam Nest Frog (*Chiromantis xerampelina*), Bushveld Rain Frog (*Breviceps adspersus*) and Plain Grass Frog (*Ptychadena anchietae*) were observed during nocturnal surveys.

More detailed studies of specific habitat requirements, breeding biology, duration of the larval stage and development are recommended for several frog species within the Kruger National Park. The tadpoles of *Strongylopus fasciatus*, *Ptychadena mossambica*, *Tomopterna marmorata* and *Tomopterna krugerensis* are presently un-described as well as aspects of the tadpole ecology such as the larval duration of *Chiromantis xerampelina, Hildebrandtia ornata, Hyperolius tuberilinguis, Kassina maculata, Leptopelis mossambica* are unknown. The extremely limited information of the non-breeding ecology of many frog species especially African Bullfrog (*Pyxicephalus edulis*), Southern Ornata Frog (*Hildebrandtia ornata*) and Golden-Leaf-folding Frog (*Afrixalus aureus*) is examples.

Habitat and dispersal movements

The core terrestrial habitat requirements around the breeding habitats of the majority of frog species is unknown as well as the dispersal areas required for juveniles migrating away from the water bodies where they have bred and developed. This has a direct impact on the conservation of the amphibians, as managers can't implement effective strategies to conserve the frogs. As an example, a question was raised during the project – "What is the impact of the closure of artificial water points on the frogs? This was specifically in relation to the possible increased utilisation of the natural water bodies by mega herbivores in particular, but game in general. It was observed that the frogs utilise the natural vegetation and other structures (logs and termite mounds) for refuge as well as calling areas during the breeding season. Increased trampling and grazing can lower the natural vegetation around the pans, which can have a negative impact on the frogs (breeding and calling).

The more sensitive species (in relation to habitat preference) can be negatively impacted if the vegetation cover is reduced and this can in the long term have an impact on species distribution in the Kruger National Park. It is therefore suggested that more detailed studies must be conducted to monitor frog movements during the breeding season in an attempt to determine distances travelled and the habitat utilised during the non-breeding periods. The study should focus on selected areas where the frog diversity is high, as this will allow researchers to gather the most information in small geographical areas. It is suggested that a site with higher impacts is included to determine how this will impact frog breeding dynamics.

In addition, it is suggested that long term monitoring and sampling must be implemented as this will further indicate if the impacts of increased trampling and grazing are having a negative impact on the amphibian populations and community structures in the selected areas.

Water quality and pollution

The long term monitoring must incorporate the possible impacts of pollution (acid rain and other) on the frogs, eggs and tadpoles. The possible changes in the water quality must be monitored to determine if egg development is compromised. In addition, the tadpole development and periods of metamorphoses must be monitored. This can be linked to changes in population structures and possible deformities related to pollution during the development phases of the tadpoles.

During this project, some work was done to determine the possible utilisation of histological analysis to determine possible pollution impacts on the physiology of the amphibians. This

component must be further developed and a good database with baseline information on the frog histology must be compiled. In addition, possible impacts and changes in the tissue types must be recorded in an effort to compile a frog histology index.

During the project, some work was done on the parasites of the amphibians in the Kruger National Park. Other researchers are working on the parasites of amphibians and this can be linked to existing work. The blood parasites need more research, as was seen during this project. Although the final analysis is not completed (when this report is going to press) there are indications that new species of blood parasites were collected. The final analysis will be published once the taxonomical studies are completed.

Conservation

The present study and recommended future research contribute to the conservation of amphibians in the Kruger National Park. It is important to extrapolate the information to conservation outside the park, as these areas are under more threat than the conservation areas. The proposed studies can be carried out in protected areas, as this will give a more realistic view of habitat requirements and needs. However, studies outside conservation areas will give indications of changes in physiological processes, impacts on populations and community structures and the impacts of pollution on the amphibians. This will help us to develop an understanding of the role of the amphibians in the landscape and indicators of changes in biodiversity.

Conclusions

The project has achieved the objectives set in 1.2 and some additional components were added during the project.

- During the study, the water analysis indicated very little issues regarding possible pollutants that are currently affecting the environment. The only constituent of possible concern is the sulphate levels (SO_4 – refer to Chapter 4) were elevated levels were observed in the southern section of the Kruger National Park. This can be related to possible acid rain associated with the industrial complex and coal power stations in Mpumalanga (Mphepya et al. 2006).
- The study indicated that frogs depend on good vegetation cover and additional habitat structure (i.e. dead wood, rocks and termite mounds) where they possibly over winter. This is one of the aspects suggested for further investigation.
- The current distribution pattern of species was similar to the historic record, but a few new distribution records are added to the KNP database. The species not recorded are of no real concern, as the sampling times may have led to them not being found.

- The analysis of the acid rain experiments are continuing. This will be published as soon as the data interpretation is completed.
- The initial histo-pathological study gave good results which indicated that the frogs can be effectively used as bioindicators. A larger sample size will be needed to develop the frog health index.

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Addendum 4.1: Physico-chemical water quality variables measured at 45 pan sites and 14 riverine sites in the Kruger National Park.

Water quality variable	P1	P2	P3	P4	P5	P6
pH	6.38	6.96	6.36	7.3	6.69	7.6
Temperature (°C)	22.9	27.6	30.5	24.6	25.9	26.1
Specific conductivity (µS/cm)	82.6	92.2	86.9	263	106.7	291
Total dissolved solids (mg/l)	413	46.2	43.7	133	53.3	144
Percentage oxygen saturation (%)	90	83	96	80	65.3	89
Dissolved oxygen (mg/l)	4.2	4.75	5.5	4.6	3.6	5.01
Total suspended solids (mg/l)	2	0	42	8	0	0
Nitrate (mg/l NO_3 as N)	0.7		0.7	0.65		
Nitrite (mg/l NO_2 as N)	0.01		0.045	0.028		
Ammonium (mg/l NH_4 as N)	0.06		0.055	0.575		
Ortho-phosphate (mg/l PO_4 as P)	0.01		0.17	0.79		
Sulphate (mg/l)	32		43	35.5		
Chloride (mg/l)	18		10.5	19.5		
Total alkalinity ($CaCO_3$ mg/l)	7.41		19.12	5.71		
Al (µg/l)	100.00		100.00	213.00		
As (µg/l))	11.30		13.40	1.00		
Ca (mg/l)	1.02		3.94	1.00		
Cd (µg/l)	21.70		3.00	0.60		
Cr (µg/l)	2.00		11.40	5.00		
Cu (µg/l)	4.70		9.40	0.20		
Fe (µg/l)	35.00		50.00	326.00		
K (mg/l)	0.47		3.87	2.54		
Mg (mg/l)	1.19		2.26	0.78		
Mn (µg/l)	3.50		15.10	1.80		
Na (mg/l)	4.90		2.78	18.63		
Ni (µg/l)	5.00		15.40	0.50		
Pb (µg/l)	9.40		15.30	2.90		
Se (µg/l)	35.00		7.70	13.80		
Zn (µg/l)	3.10		13.80	0.80		

	P7	P8	P9	P10	P11	P12
pH	6.33	6.9	6.65	7.65	9.65	6.99
Temperature (°C)	25.9	26.9	27.1	27.4	29.3	27.2
Specific conductivity (µS/cm)	66.1	296	114.9	580	2411	237
Total dissolved solids (mg/l)	31.3	148	58.3	291	1200	118
Percentage oxygen saturation (%)	33	21.6	14	8.2	43.1	56.2
Dissolved oxygen (mg/l)	1.9	1.28	0.72	0.42	12.94	3.7
Total suspended solids (mg/l)	4	14	4	14	22	4
Nitrate (mg/l NO_3 as N)	0.05	0.065	0.35	0.3	0.43	1.8
Nitrite (mg/l NO_2 as N)	0.011	0.0085	0.008	0.008	0.005	0.005
Ammonium (mg/l NH_4 as N)	0.1	0.1	0.055	0.435	0.4	0.17
Ortho-phosphate (mg/l PO_4 as P)	0.37	1.44	0.765	0.095	0.02	0.45
Sulphate (mg/l)	27	26	114	63.5	79	96.5
Chloride (mg/l)	9.5	15.5	11	18	160	10
Total alkalinity ($CaCO_3$ mg/l)	19.87	48.52	10.67	37.34	59.19	10.90
Al (µg/l)	32.00	100.00	100.00	153.00	100.00	207.00
As (µg/l))			21.60	23.30	16.80	16.10
Ca (mg/l)	4.19	8.55	1.76	5.82	2.61	2.36
Cd (µg/l)			13.30	2.30	3.00	4.80
Cr (µg/l)			35.50	26.60	36.70	2.70
Cu (µg/l)			24.30	34.10	28.10	29.80
Fe (µg/l)	72.00	12.00	42.00	194.00	0.10	166.00
K (mg/l)	3.85	9.46	3.07	20.22	1.95	7.23
Mg (mg/l)	2.29	6.62	1.53	5.56	12.84	1.22
Mn (µg/l)			28.30	33.00	29.50	29.00
Na (mg/l)	1.66	4.80	5.10	7.34	69.02	8.30
Ni (µg/l)			3.70	33.70	57.90	26.40
Pb (µg/l)			25.70	24.60	25.70	25.90
Se (µg/l)			37.30	37.70	36.60	19.00
Zn (µg/l)			31.10	14.70	28.40	25.10

Addendum 4.1: Continued

	P13	P14	P15	P16	P17	P18
pH	7.72	6.92	7.8	7.3	6.55	9.66
Temperature (°C)	24.1	25.9	25.1	27.5	22.2	23.9
Specific conductivity (µS/cm)	259	164.2	282	107.7	148.4	164.9
Total dissolved solids (mg/l)	130	82.1	141	52	74.1	82.6
Percentage oxygen saturation (%)	31	30	40	40	22.2	142
Dissolved oxygen (mg/l)	3.5	3.1	2.8	2.28	1.31	8.31
Total suspended solids (mg/l)	8	2	18			32
Nitrate (mg/l NO_3 as N)	1.65	0.4	1.35			1.1
Nitrite (mg/l NO_2 as N)	0.005	0.007	0.005			0.005
Ammonium (mg/l NH_4 as N)	0.465	0.825	0.06			0.3
Ortho-phosphate (mg/l PO_4 as P)	0.085	0.305	0.12			0.64
Sulphate (mg/l)	146.5	42	166			184
Chloride (mg/l)	23.5	13.5	14.5			11.5
Total alkalinity ($CaCO_3$ mg/l)	38.34	29.76	20.73			22.19
Al (µg/l)	100.00	100.00	100.00			100.00
As (µg/l))	7.70	31.60	9.00			25.20
Ca (mg/l)	7.28	5.64	5.10			4.73
Cd (µg/l)	9.70	26.20	2.40			4.40
Cr (µg/l)	39.60	24.50	41.50			28.70
Cu (µg/l)	10.30	35.60	26.10			21.60
Fe (µg/l)	24.00	154.00	18.00			25.00
K (mg/l)	19.31	14.10	2.06			7.33
Mg (mg/l)	4.92	3.82	1.95			2.53
Mn (µg/l)	4.10	39.30	18.00			34.60
Na (mg/l)	1.05	2.45	0.69			3.02
Ni (µg/l)	25.10	35.50	5.10			28.90
Pb (µg/l)	17.90	37.70	8.00			32.40
Se (µg/l)	23.60	13.50	29.60			26.20
Zn (µg/l)	12.50	36.80	11.60			25.80

Addendum 4.1: Continued

	P19	P20	P21a	P21b	P22a	P22b
pH	7.95	6.96	8.01	7.04	7.04	8.28
Temperature (°C)	21.7	31.2	27.9	30.3	27.4	31.4
Specific conductivity (µS/cm)	459	125	344	388	188.8	315
Total dissolved solids (mg/l)	229	62.5	171	193	94.1	157
Percentage oxygen saturation (%)	80	76	4	34	99.7	97.7
Dissolved oxygen (mg/l)	4.01	8.71	0.16	2.44	6.3	6.77
Total suspended solids (mg/l)	28	22	72	60	16	18
Nitrate (mg/l NO_3 as N)	2.45	1.5	2	1.9	2.45	3.5
Nitrite (mg/l NO_2 as N)	0.005	0.005	0.005	0.005	0.2325	0.016
Ammonium (mg/l NH_4 as N)	0.155	0.03	3	2.06	0.42	1.07
Ortho-phosphate (mg/l PO_4 as P)	0.47	0.255	0.36	0.36	0.08	0.35
Sulphate (mg/l)	119.5	73.5	72	77	145	139
Chloride (mg/l)	134.5	10	17	18	25	52
Total alkalinity ($CaCO_3$ mg/l)	136.47	24.65	35.29	10.15	29.51	18.33
Al (µg/l)	100.00	391.00	100.00	258.00	75.00	353.00
As (µg/l))	35.00	26.10	56.60	35.20	50.40	0.90
Ca (mg/l)	15.10	5.62	6.81	2.15	5.76	3.75
Cd (µg/l)	14.60	6.30	24.60	25.70	19.00	3.70
Cr (µg/l)	42.20	10.00	45.20	17.60	126.80	2.00
Cu (µg/l)	35.30	33.90	61.00	27.80	49.50	4.10
Fe (µg/l)	0.10	265.00	72.00	309.00	207.00	305.00
K (mg/l)	9.44	5.24	34.24	13.24	18.19	7.15
Mg (mg/l)	24.08	2.59	4.45	1.17	3.68	2.19
Mn (µg/l)	17.70	34.60	65.80	50.20	67.50	1.00
Na (mg/l)	82.03	4.27	3.82	17.90	31.72	9.75
Ni (µg/l)	33.20	34.20	60.80	49.30	47.30	1.90
Pb (µg/l)	38.20	41.80	89.30	34.40	24.00	12.20
Se (µg/l)	40.50	49.40	47.90	132.50	303.60	2.50
Zn (µg/l)	24.50	31.50	47.80	9.30	49.10	1.60

Addendum 4.1: Continued

	P23	P24a	P24b	P25a	P25b	P26a
pH	6.77	6.76	6.71	6.7	6.41	6.75
Temperature (°C)	23.5	29.6	38	30.8	34.8	29.8
Specific conductivity (μS/cm)	583	286	253	219	209	195.5
Total dissolved solids (mg/l)	289	143	127	108	104	96.8
Percentage oxygen saturation (%)	45.2	14.1	20	38.1	20.2	49.7
Dissolved oxygen (mg/l)	3.5	1.01	1.49	3.2	1.71	3.44
Total suspended solids (mg/l)	24	52	54	20	32	20
Nitrate (mg/l NO_3 as N)	4.2	1.45	0.7	11.2	6.85	1
Nitrite (mg/l NO_2 as N)	0.0155	0.13	0.021	0.5	0.15	0.011
Ammonium (mg/l NH_4 as N)	0.13	1.71	0.39	0.22	0.445	0.27
Ortho-phosphate (mg/l PO_4 as P)	0.02	0.02	0.05	0.23	0.05	0.12
Sulphate (mg/l)	85	102.5	107	94	129	141
Chloride (mg/l)	21.5	52	75	78	54	65
Total alkalinity ($CaCO_3$ mg/l)	11.11	29.43	31.67	12.48	24.10	31.53
Al (μg/l)	595.00	387.00	39.00	963.00	674.00	37.00
As (μg/l))	2.00	1.50	5.30	2.70	4.40	0.70
Ca (mg/l)	2.19	5.35	4.86	2.30	4.71	5.93
Cd (μg/l)	1.80	2.00	4.50	5.20	2.80	4.50
Cr (μg/l)	3.40	2.80	1.40	1.80	2.90	9.30
Cu (μg/l)	2.50	1.30	5.20	0.90	0.90	2.90
Fe (μg/l)	1082.00	1127.00	66.00	2114.00	722.00	91.00
K (mg/l)	11.95	17.74	22.61	12.84	7.67	22.79
Mg (mg/l)	1.38	3.92	4.76	1.65	3.01	4.07
Mn (μg/l)	5.40	1.70	5.80	1.40	1.00	3.50
Na (mg/l)	10.70	17.57	25.60	9.38	12.09	8.79
Ni (μg/l)	7.10	2.30	5.10	2.20	0.60	3.10
Pb (μg/l)	4.70	3.50	11.80	1.20	3.10	2.40
Se (μg/l)	5.80	10.10	2.90	5.40	10.10	16.30
Zn (μg/l)	1.40	1.50	3.40	0.80	0.50	2.40

Addendum 4.1: Continued

	P26b	P27a	P27b	P28	P29a	P29b
pH	6.67	7.64	7.16	6.8	6.64	6.71
Temperature (°C)	21.8	33.9	38.4	36.3	28.9	27.9
Specific conductivity (µS/cm)	214	221	80.5	91.3	156.2	98.4
Total dissolved solids (mg/l)	107	110	40.1	45.6	78.4	49.2
Percentage oxygen saturation (%)	4.1	75.6	114.2	44	14.8	17
Dissolved oxygen (mg/l)	0.27	6.46	7.48	2.72	0.9	1.4
Total suspended solids (mg/l)	38	32	46	30	16	14
Nitrate (mg/l NO_3 as N)	0.5	1.8	0.65	1.15	1.95	0.08
Nitrite (mg/l NO_2 as N)	0.005	0.0095	0.005	0.0195	0.038	0.112
Ammonium (mg/l NH_4 as N)	0.185	0.245	0.29	0.625	0.08	0.15
Ortho-phosphate (mg/l PO_4 as P)	0.07	0.1	0.085	0.34	0.975	0.365
Sulphate (mg/l)	135.5	94.5	74	103.5	254	182
Chloride (mg/l)	60	51.5	56	49	10	8
Total alkalinity ($CaCO_3$ mg/l)	26.76	7.75	17.68	9.91	4.75	6.42
Al (µg/l)	100.00	249.00	458.00	150.00	345.00	472.00
As (µg/l))	2.40	2.10	2.60	2.60	4.20	1.50
Ca (mg/l)	6.76	1.81	3.78	2.08	0.98	1.43
Cd (µg/l)	3.60	10.50	4.80	8.70	2.50	3.60
Cr (µg/l)	1.50	8.70	2.40	14.70	22.00	6.60
Cu (µg/l)	2.70	3.70	3.00	3.60	1.70	2.70
Fe (µg/l)	52.00	207.00	489.00	197.00	295.00	512.00
K (mg/l)	7.11	4.24	5.18	5.19	2.01	1.98
Mg (mg/l)	2.41	0.79	2.01	1.15	0.56	0.70
Mn (µg/l)	2.20	6.50	1.10	2.90	1.60	3.70
Na (mg/l)	3.76	8.67	15.19	5.29	11.91	11.03
Ni (µg/l)	2.60	4.80	1.00	2.40	22.20	3.70
Pb (µg/l)	4.90	13.90	3.90	4.70	7.10	1.40
Se (µg/l)	19.00	13.30	4.80	8.60	5.00	5.90
Zn (µg/l)	1.50	4.30	2.20	0.70	5.40	1.90

Addendum 4.1: Continued

	P30	P31	P32	P33	P34	P35
pH	6.68	7.69	6.58	6.59	6.71	6.79
Temperature (°C)	24	36.4	35.9	30.9	31.6	37.2
Specific conductivity (µS/cm)	229	478	206	131.2	283	168.2
Total dissolved solids (mg/l)	115	238	102	65.5	141	83.7
Percentage oxygen saturation (%)	8.6	54	8.6	25.4	58.4	42.3
Dissolved oxygen (mg/l)	0.8	3.4	0.64	1.78	4.33	2.58
Total suspended solids (mg/l)		32	26			
Nitrate (mg/l NO_3 as N)		1	5.2			
Nitrite (mg/l NO_2 as N)		0.024	0.008			
Ammonium (mg/l NH_4 as N)		0.21	0.17			
Ortho-phosphate (mg/l PO_4 as P)		0.055	0.65			
Sulphate (mg/l)		161.5	266			
Chloride (mg/l)		72.5	23.5			
Total alkalinity ($CaCO_3$ mg/l)		53.80	56.44			
Al (µg/l)		487.00	100.00			
As (µg/l))		6.80	0.70			
Ca (mg/l)		5.34	8.31			
Cd (µg/l)		4.20	4.80			
Cr (µg/l)		17.50	3.80			
Cu (µg/l)		1.30	23.80			
Fe (µg/l)		340.00	407.00			
K (mg/l)		76.45	15.47			
Mg (mg/l)		9.87	8.70			
Mn (µg/l)		0.50	2.40			
Na (mg/l)		7.80	26.52			
Ni (µg/l)		3.90	3.70			
Pb (µg/l)		0.40	2.00			
Se (µg/l)		10.30	4.10			
Zn (µg/l)		0.50	0.90			

Addendum 4.1: Continued

	P36	P37	P38	P39	P40	P41
pH	6.94	6.83	9.47	7.04	7.14	7.3
Temperature (°C)	23.1	25.3	22.1	19.3	24.8	28.6
Specific conductivity (µS/cm)	165.1	465	6050	94.7	218	219
Total dissolved solids (mg/l)	82.6	233	3000	47.6	109	109
Percentage oxygen saturation (%)	35.8	16.5	116.7	24.3	37.4	95
Dissolved oxygen (mg/l)	3.01	1.45	9.49	2.05	3.03	7.48
Total suspended solids (mg/l)			38	24	34	6
Nitrate (mg/l NO_3 as N)			0.8	0.3	1.8	0.2
Nitrite (mg/l NO_2 as N)			0.1	0.005	0.005	0.005
Ammonium (mg/l NH_4 as N)			0.035	0.035	0.095	0.04
Ortho-phosphate (mg/l PO_4 as P)			0.445	0.085	0.08	0.06
Sulphate (mg/l)			179	222	192.5	143.5
Chloride (mg/l)			71	80	10	80
Total alkalinity ($CaCO_3$ mg/l)			82.23	25.84	55.27	43.41
Al (µg/l)			0.10	0.10	0.10	0.10
As (µg/l))			3.30	0.50	3.10	1.30
Ca (mg/l)			15.21	4.93	11.16	9.35
Cd (µg/l)			1.60	2.20	1.60	1.60
Cr (µg/l)			6.30	4.80	4.70	6.30
Cu (µg/l)			1.80	0.30	1.60	1.50
Fe (µg/l)			104.00	120.00	47.00	66.00
K (mg/l)			6.16	24.09	6.44	5.97
Mg (mg/l)			10.78	3.30	6.68	4.89
Mn (µg/l)			2.70	0.60	2.60	2.70
Na (mg/l)			74.48	3.45	5.12	6.63
Ni (µg/l)			0.30	0.40	1.60	3.50
Pb (µg/l)			2.40	2.50	6.00	11.80
Se (µg/l)			3.60	4.60	15.80	4.80
Zn (µg/l)			0.60	0.90	2.10	3.20

Addendum 4.1: Continued

	P42	P43	P44	P45	W1	W2
pH	7.05	6.76	6.84	6.97	6.29	6.8
Temperature (°C)	23.9	29.8	28.9	29.6	19.9	23.1
Specific conductivity (µS/cm)	210	131.6	444	212	147.4	115.3
Total dissolved solids (mg/l)	105	64.7	222	146.8	74.1	57.6
Percentage oxygen saturation (%)	21	103.3	56	47.3	66	78.1
Dissolved oxygen (mg/l)	1.66	7.85	4.03	3.58	5.84	6.65
Total suspended solids (mg/l)	36	44	16	40		
Nitrate (mg/l NO_3 as N)	0.2	0.7	0.6	1.1	0.45	0.11
Nitrite (mg/l NO_2 as N)	0.005	0.007	0.005	0.006	0.006	0.039
Ammonium (mg/l NH_4 as N)	0.105	0.17	0.175	0.28	0.032	0.023
Ortho-phosphate (mg/l PO_4 as P)	0.14	0.01	0.05	0.07	0.437	0.364
Sulphate (mg/l)	172.5	174.5	132	141.5	12.5	22
Chloride (mg/l)	32.5	8	7	2.5	8.7	1.2
Total alkalinity ($CaCO_3$ mg/l)	60.06	37.40	32.31	37.21		
Al (µg/l)	0.10	0.10	0.10	0.10	1.74	0.38
As (µg/l))	4.30	3.70	0.90	1.30	3.43	3.44
Ca (mg/l)	10.96	6.41	6.96	7.22		
Cd (µg/l)	3.60	3.40	4.60	2.50	1.53	1.49
Cr (µg/l)	0.70	3.50	2.50	1.00	3.69	2.25
Cu (µg/l)	3.80	3.00	1.00	1.30	2.95	1.33
Fe (µg/l)	86.00	390.00	100.00	39.00	61.85	39.73
K (mg/l)	8.63	4.97	7.10	2.74		
Mg (mg/l)	7.96	5.22	3.64	4.67		
Mn (µg/l)	2.10	1.00	0.50	1.70	24.00	2.55
Na (mg/l)	4.46	8.25	4.53	21.83		
Ni (µg/l)	1.10	3.20	1.10	1.90	3.31	1.66
Pb (µg/l)	6.70	4.80	1.20	7.90	3.05	2.95
Se (µg/l)	11.40	18.30	11.20	5.40	5.29	3.29
Zn (µg/l)	2.40	4.50	0.90	1.40	11.14	9.46

Addendum 4.1: Continued

	W3	W4	W5	W6	W7	W8
pH	7.17	9.47	8.54	8.37	8.69	8.13
Temperature (°C)	26.4	22.1	21.3	24.1	23.8	20.7
Specific conductivity (µS/cm)	404	6050	1425	284	4160	437
Total dissolved solids (mg/l)	202	3000	709	142	2070	214
Percentage oxygen saturation (%)	68.3	116.7	92.1	73.7	81.7	144.5
Dissolved oxygen (mg/l)	5.83	9.49	7.84	5.54	6.49	12.47
Total suspended solids (mg/l)						
Nitrate (mg/l NO_3 as N)	0.54	0.86	0.2	0.97	0.62	0.2
Nitrite (mg/l NO_2 as N)	0.082	0.095	0.09	0.058	0.076	0.056
Ammonium (mg/l NH_4 as N)	0.033	0.419	0.147	0.176	0.111	0.016
Ortho-phosphate (mg/l PO_4 as P)	0.34	0.961	0.294	0.453	0.27	0.156
Sulphate (mg/l)	23	21	47	186	88	84
Chloride (mg/l)	23.4	300	117	18	90	84
Total alkalinity ($CaCO_3$ mg/l)						
Al (µg/l)	1.56	3.59	3.11	7.77	5.70	4.19
As (µg/l))	2.87	2.99	7.94	3.04	2.51	9.89
Ca (mg/l)						
Cd (µg/l)	1.51	1.65	1.45	1.41	1.46	1.34
Cr (µg/l)	2.28	2.91	2.37	2.20	3.57	4.52
Cu (µg/l)	1.73	11.63	2.13	2.05	3.19	2.00
Fe (µg/l)	72.71	85.16	81.60	97.67	109.80	79.23
K (mg/l)						
Mg (mg/l)						
Mn (µg/l)	2.79	2.85	2.33	2.72	7.96	2.40
Na (mg/l)						
Ni (µg/l)	2.35	9.44	3.15	2.20	6.11	2.40
Pb (µg/l)	3.23	2.84	2.88	3.14	2.69	2.65
Se (µg/l)	2.86	16.57	4.66	2.87	9.83	2.94
Zn (µg/l)	11.92	21.13	9.80	10.73	19.31	13.39

Addendum 4.1: Continued

	W9	W10	S11	W12	W13	W14
pH	8.48	7.41	7.04	8.1	7.82	7.61
Temperature (°C)	24.2	23.1	19.3	21.5	22.9	24.9
Specific conductivity (µS/cm)	1118	471	94.7	316	220	181.7
Total dissolved solids (mg/l)	557	236	47.6	159	110	90.5
Percentage oxygen saturation (%)	122.2	37	24.3	112.8	86.5	24.4
Dissolved oxygen (mg/l)	11.22	3.23	2.05	9.99	6.75	2.1
Total suspended solids (mg/l)						
Nitrate (mg/l NO_3 as N)	0.26	0.31	0.2	0.2	0.2	0.23
Nitrite (mg/l NO_2 as N)	0.076	0.054	0.006	0.088	0.122	0.085
Ammonium (mg/l NH_4 as N)	0.55	0.114	0.16	0.03	0.03	0.11
Ortho-phosphate (mg/l PO_4 as P)	0.758	0.248	0.451	0.114	0.218	0.218
Sulphate (mg/l)	36	32	25	51	43	17
Chloride (mg/l)	15	20.6	13.1	7.3	10.4	14.3
Total alkalinity ($CaCO_3$ mg/l)						
Al (µg/l)	3.18	2.60	28.45	6.94	8.87	15.76
As (µg/l))	2.53	3.08	2.14	2.06	5.68	1.79
Ca (mg/l)						
Cd (µg/l)	1.33	1.27	1.58	1.24	1.22	1.24
Cr (µg/l)	2.18	2.33	2.50	2.33	2.25	3.09
Cu (µg/l)	3.30	1.53	8.07	1.82	3.32	2.16
Fe (µg/l)	86.93	96.39	197.70	120.00	132.80	184.10
K (mg/l)						
Mg (mg/l)						
Mn (µg/l)	31.34	28.69	4.31	4.07	3.59	493.50
Na (mg/l)						
Ni (µg/l)	3.09	3.65	8.43	2.04	3.39	3.53
Pb (µg/l)	2.64	9.30	2.73	2.73	3.40	
Se (µg/l)	4.09	2.58	2.16	2.10	2.03	1.90
Zn (µg/l)	9.13	9.22	21.03	13.71	17.51	20.57

Legend

Water quality
Cl

- 0.0 - 11.5
- 11.6 - 38.0
- 38.1 - 90.0
- 90.1 - 160.0
- 160.1 - 300.0

N

50   25   0   50 Kilometers

Legend

Water quality

Cond1

- ·   1.0 - 181.7
- ○   181.8 - 344.0
- ○   344.1 - 1118.0
- ○   1118.1 - 2411.0
- ○   2411.1 - 6050.0

N

50   25   0   50 Kilometers

Legend

Water quality

DO__1

- 1.0 - 25.4
- 25.5 - 47.3
- 47.4 - 68.3
- 68.4 - 103.3
- 103.4 - 144.5

N

50   25   0   50 Kilometers

Legend

Water quality
Na

- ·   0.0 - 1.7
- ○   1.8 - 6.6
- ○   6.7 - 12.0
- ○   12.1 - 42.7
- ○   42.8 - 82.0

N

50   25   0   50 Kilometers

Legend

Water quality
SO4

·   0.0 - 17.0

○   17.1 - 51.0

○   51.1 - 119.5

○   119.6 - 192.5

○   192.6 - 266.0

50   25   0   50 Kilometers

N

Legend

Water quality

TDS1

- 1.0 - 94.1
- 94.2 - 202.0
- 202.1 - 557.0
- 557.1 - 1200.0
- 1200.1 - 3000.0

50   25   0   50 Kilometers

N

Legend

Water quality
Temp1

- 1.0
- 1.1 - 24.2
- 24.3 - 27.6
- 27.7 - 31.6
- 31.7 - 38.4

N

| 50 | 25 | 0 | | 50 Kilometers |