Amphibian diversity and breeding behaviour in the Okavango Delta

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PREFACE AND ACKNOWLEDGEMENTS

This project was made possible by the fact that I was under full time employment in the Okavango Delta, working for the safari operator Okavango Wilderness Safaris. This offered me the opportunity not only to be based out of my study region and conduct my research on a part time basis, but also allowed much time to understand the ecosystem and constantly observe changes in my surrounding environment. During the course of the project, I was first based in Xigera (from Feb 2007 to Feb 2009) and then at Mombo (from Mar 2009 to Jul 2010). Therefore, in addition to the official field trips that were arranged, additional, ad hoc observations were possible outside of these official trips; an opportunity which proved invaluable, especially to Chapter 3. The nature of employment was such that I worked seven days a week for three months at a time, never leaving the safari lodge area, followed by a one month break outside of the Okavango Delta. Although being stationed in the study region for the duration of the study allowed for a thorough investigation of all the aims, full time employment did have certain drawbacks that will be pointed out where necessary in each chapter, the most prominent of which was the difficulty in standardisation of sampling sites and times.

I would like to thank my supervisors, Prof. Louis du Preez and Dr. Ché Weldon, for their constant support, guidance and enthusiasm throughout the course of this project, and for their review and constructive criticism during the creation of this document. Their knowledge and expertise in the field of amphibians is remarkable and my goals would not have been accomplished without their assistance.

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Hyperolius parallelus from the Okavango Delta

ABBREVIATIONS USED IN THE TEXT

Bd	*Batrachochytrium dendrobatidis*
cm	centimetre(s)
g	gram(s)
l	litre(s)
m	metre(s)
mg	milligram(s)
ml	millilitre(s)
mm	millimetre(s)
QDGC	quarter degree grid cell

EXPLANATION OF NON-ENGLISH TERMS

Vlei	Part of a watercourse which spreads out over a flat, depressed valley characterised by specialised water-based vegetation

ABSTRACT

Amphibians are of great ecological importance and a loss of species will have widespread and dire consequences. Recent population declines and extinctions have resulted in amphibians being labelled the most threatened vertebrate class on a global scale. The unique Okavango ecosystem is well known and documented, yet the amphibians of this region are poorly known. This project aimed at assessing diversity in the Okavango Delta by testing isolation as a possible driver for community composition; determining the effect of hydrology on breeding behaviour; and assessing the status and prevalence of the pathogen *Batrachochytrium dendrobatidis* (Bd) responsible for the widespread epidemic chytridiomycosis implicated in amphibian decline.

Using various monitoring techniques, observations of species occurrence were made at three locations representing different degrees of isolation over a 20 month period. Breeding indicators were observed and frogs were screened for amphibian chytrid fungus.

A total of 29 species were recorded, and results indicated that there were no significant differences in community composition between the sampled localities. Species presence, however, was significantly correlated with habitat type. Thus, the availability of suitable habitat appears to be driving amphibian diversity patterns, rather than geographic isolation; and increased habitat diversity near the Delta periphery explains increased amphibian diversity in these areas.

Results from breeding indicators suggested that reproduction in continuous breeders was correlated with the annual flood as well as rainfall, whilst that of explosive breeders was correlated with rainfall alone. It is thus proposed that opportunistic breeding behaviour for some amphibian species is driven by the hydrology of the ecosystem; and this may be explained by increased biological production associated with the flood pulse. Outcomes highlight the unique nature of the Okavango Delta system, and emphasises the need for its preservation.

A total of 249 swab samples were collected and screened for amphibian chytrid fungus. The geographical distribution of collection samples were evenly spread throughout the localities, and were obtained from at least 25 amphibian species. Analyses proved negative for Bd for the 79.92% swabs analysed thus far and it is concluded that Bd seems absent in the study region, a result which has massive conservation implications for the region.

Despite the fact that the Okavango Delta has benefitted from conservation and tourism efforts in the past, the system and its biodiversity remains threatened and effective conservation management strategies must be devised and implemented to ensure its preservation.

Key words: Amphibians; Diversity; Breeding; Chytrid; Okavango Delta.

Xigera Lagoon, the Okavango Delta

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CHAPTER ONE:
GENERAL INTRODUCTION

1.1 The Okavango Delta: a flood-pulsed ecosystem

As the world"s largest inland delta, the Okavango Delta in the north-western portion of Botswana is an oasis in a predominantly desert land. It is the largest wetland in Southern Africa, and at the Ramsar Convention in 1997, the Okavango Delta was declared "a wetland of international importance." Using images over the last three decades, the approximate size of the Delta, which varies remarkably over historical time and time of year, has been estimated at 14 000km^2, with 9 000km^2 classified as true wetland. However, using historical records and including permanently dry areas associated with the Delta, the total area is increased to 28 000km^2 (Ramberg *et al.*, 2006). The Okavango Delta is the result of its location in the centre and deepest point of the Kalahari Basin - a massive sand sheet extending around 3 000km from the Northern Cape in South Africa to the Democratic Republic of Congo - and it may be the relic of an ancient drainage system that originated during the early days of Gondwana"s breakup (McCarthy & Ellery, 1998; Mendelsohn *et al.*, 2010). Water, therefore, is supplied to the region not only by local, annual rainfall, but also by the Okavango River: one of only two perennial rivers in the country. Today, it is among the most pristine wetlands in the world for two reasons. Firstly, it has never been densely populated or inhabited by humans, most probably due to the presence of insect-borne diseases such as malaria and sleeping sickness which have hindered the settlement of humans and their cattle; and secondly, the hydrological regime of the Okavango has never been impacted by any water development projects along the Okavango River, its catchment or the Delta itself (McCarthy & Ellery, 1998).

The Okavango Delta is, strictly speaking, a type of alluvial fan, not a „delta" that by definition discharges into a standing body of water. Alluvial fans of this type are termed „Josimean fans" (adapted from low sinuosity, meandering), owing its name to unique characteristics such as an ultralow gradient, meandering channels and being highly vegetated (Stanistreet & McCarthy, 1993). The Okavango Delta can be divided into the northern, linear Panhandle and the southern, delta-shaped alluvial fan (Figure 1). In the Panhandle, the Okavango River is meandering and dynamic, providing the Delta with a constant supply of water. It is bordered by permanent swamp, with a gradient along this section of approximately 1:5500. In the southern section, the Okavango River divides into four main channels on the alluvial fan: the Selinda flows towards the north-east; the Nqoga channel to the east; the Jao channel to the south-east;

and the Thaoge to the south (McCarthy & Ellery, 1998; Mendelsohn *et al.*, 2010). The gradient of this southern section is approximately 1:3400 (McCarthy & Ellery, 1998).

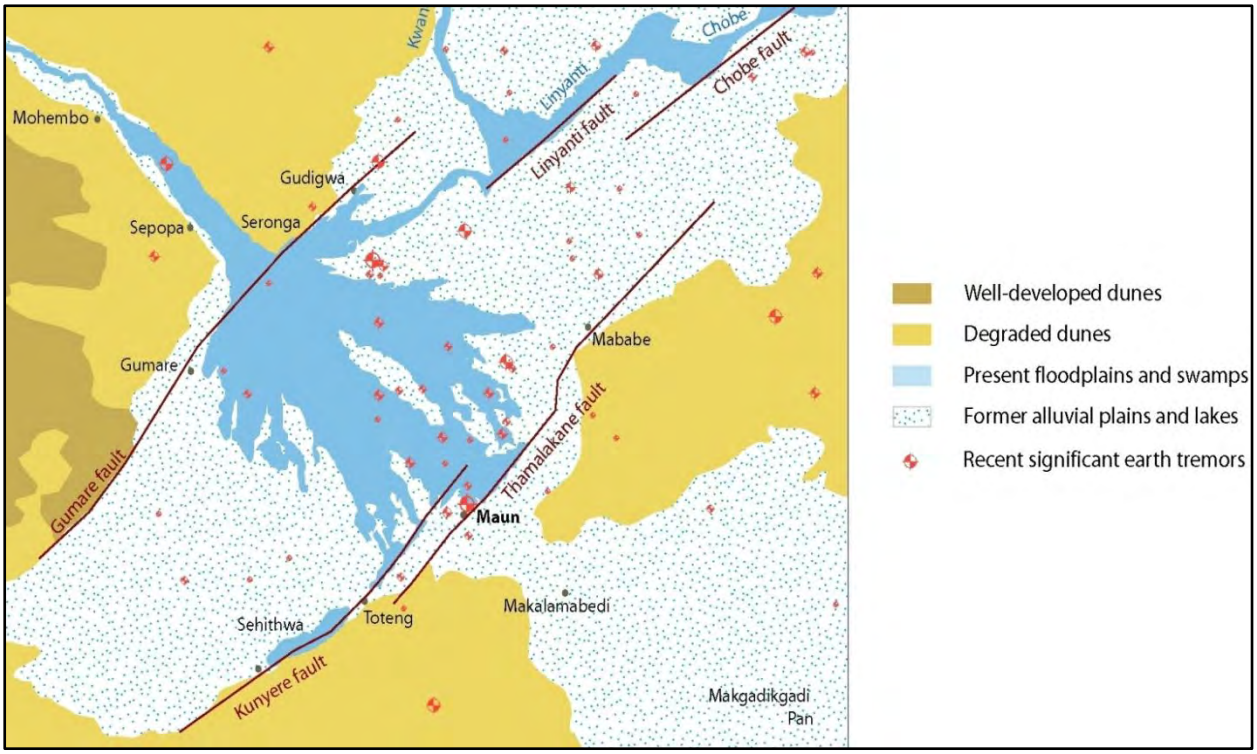

Figure 1: Underlying geological controls responsible for the dynamic Okavango Delta system (Mendelsohn *et al.,* 2010).

Channel margins are defined by peat levees and stabilised by vegetation so that water is able to leak through them, resulting in a situation where the base flow in the river supports a large quantity of water outside the actual channels. This characteristic is what sustains the permanent swamp that is found in the upper section of the fan and along the Okavango River. The lower sections of the fan are seasonally flooded. In this region, water transforms into slow-moving, sheet flooding. Although flood waters cover much of the Delta, it is generally less than one metre in depth, and any higher ground become islands in the mass of water; these islands become more numerous towards the periphery of the seasonal swamp (McCarthy & Ellery, 1998).

In order to understand the Okavango Delta and its dynamic nature, it is necessary to understand the underlying geological controls which are ultimately responsible for the formation of the system and in control of the water movement. The Okavango depression forms part of the much larger East African Rift System. It has resulted from weaknesses in the underlying metamorphic rocks which are represented on the earth"s surface by two major faults running in a north-easterly, south-westerly direction: the smaller Gumare fault in the north and the much larger Thamalakane fault in the south, which is observed as a 12m scarp defining the

Thamalakane River (Figure 1). Relatively speaking, minor subsistence has occurred as a consequence of these faults, demarcating the Okavango Delta, and reaching a depth of around 200-300m at the southern edge of the Delta (Figure 2). A pair of conjugate faults developed at right angles to the abovementioned east-west faults, and these contain the Panhandle and Chief"s Island. Smaller blocks seem to have moved independently of the surrounding subsistence, some even exhibiting uplift and resulting in larger islands. Chief"s Island, in the centre of the Delta, is the resultant feature of such an event. The Okavango depression has gradually been filling with Aeolian (wind-blown sand), fluvial (river sediment) and lacustrine (lake sediments) deposits, resulting in the broad, conical alluvial fan that is the Okavango Delta (McCarthy & Ellery, 1998).

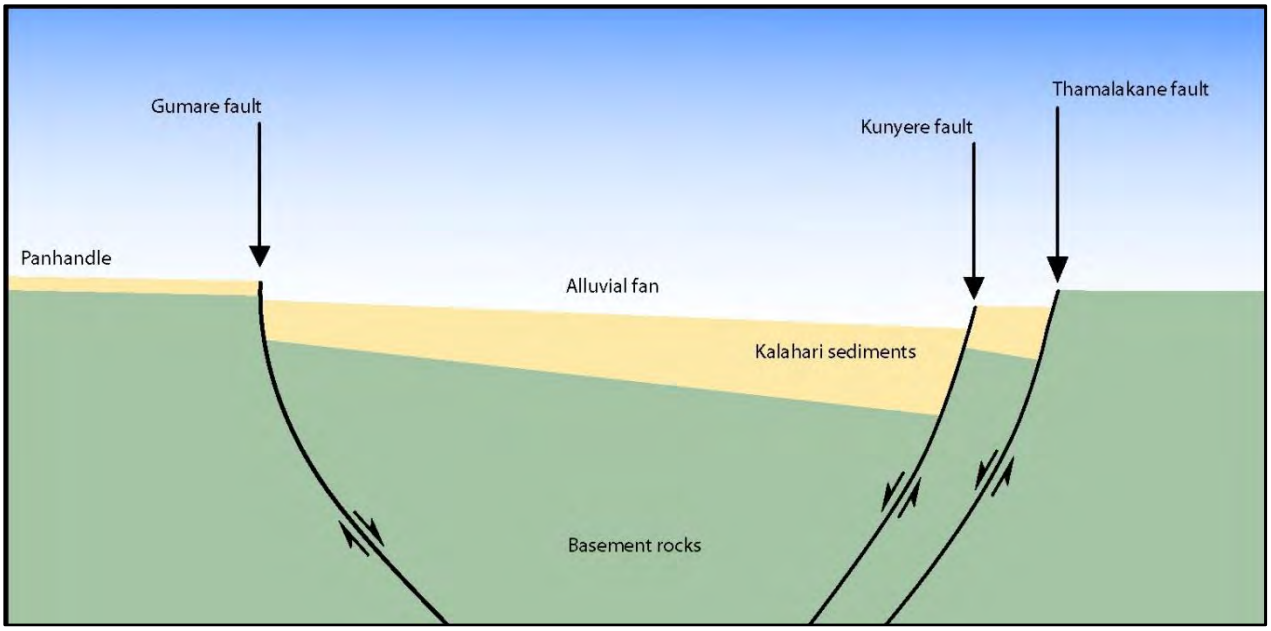

Figure 2: Minor subsistence resulting from fault lines demarcates the Okavango Delta (Mendelsohn *et al.,* 2010).

The Okavango River catchment area is located in the highlands of Central and Eastern Angola, a high rainfall region that receives approximately 1000mm of rain each year from January through to March, the region"s rainy season. This rain water is captured by two major rivers in Angola, the Cubango and the Cuito, and discharge into the Okavango River in Namibia. Around April each year, depending on the time that the bulk of the rainfall was received, the rainwater from Angola reaches Mohembo, the border-post into Botswana at the top of the Panhandle. The result is a flood peak, approximately two meters higher than average water levels, as the seasonal flood wave passes through the area on its way southwards. This rise in water levels diminish as the wave progresses south since the floodwater is no longer confined by channel scarps; instead, it leaks through the vegetated levees and into the surrounding permanent and seasonal swamps through permeable channel margins. In the southern section of the fan,

changes in water levels are again high, around the two meter mark, due to the damming of water against the lower fault lines. Peaks in this section are generally recorded in July and August of the same year (McCarthy & Ellery, 1998).

The advance of flood water is exceptionally slow: it takes approximately four months to cover a mere distance of 250km from the Panhandle to Maun. It follows then, that although the Okavango Delta"s annual rainfall (amounting to approximately 500mm) falls in the summer months between November and March, the Delta contains its largest, yearly volumes of water in the winter months, between April and October. In fact, the Delta is said to display two annual flood pulses: the *minor flood* as a result of localised rainfall in December/January, and the *major flood* as a result of rainwater captured in Angola during the winter months (McCarthy & Ellery, 1998; Mendelsohn *et al.*, 2010). There are several reasons for the retarded water flow, and can be summarised as follows:

- An extremely low, north to south gradient along the length of the Delta;
- heavy, dense vegetation throughout the Delta system;
- an undulating topography dictates that deeper depressions must be filled and saturated before water can continue southwards on its course;
- a large quantity of water is required to renew groundwater levels.

(McCarthy & Ellery, 1998.)

It is estimated that of the massive inflow of water from the Okavango River, combined with the localised rainfall in the region, a yearly average of approximately 9 200 and 6 000 million m^3 respectively, a meagre one and a half percent leaves the Delta as outflow through the Boteti River, and less than two percent leaves as ground water. It is therefore estimated that at least 96% of all water entering the Delta is lost to the atmosphere via evaporation and transpiration (McCarthy & Ellery, 1998).

1.2 Study area

The Okavango Delta is home to an estimated 150 000 islands all varying in shape and size (Gumbricht *et al.,* 2004). Islands are continually being formed and altered by the many biological, physical and chemical processes that characterise the Okavango system, and there is generally a logical explanation behind the position, topography, chemistry and biological diversity of islands. In other words, their location and formation is not a random process. The nuclei of primary islands are formed by three major processes, namely: termite bioengineering; channel death and inversion; and point and scrollbar islands on the concave side of meandering channels, the details of which are beyond the scope of this study (McCarthy 1992; McCarthy & Ellery, 1998; Gumbricht *et al.,* 2004). Once these have been formed, they may extend vertically

or laterally due to chemical precipitation or dust and sediment accumulation, evolving into an irregular and continually changing shape. Topographical studies carried out by Gumbricht *et al.* (2001) reached the conclusion that tectonic activity did not significantly affect island formation, growth or distribution. Based on this information, it can be assumed that Chief‟s Island - like many other large, dry woodland islands - has not been affected by flooding or the influence of a channel for a significantly long period of time (Gumbricht *et al*., 2004).

In February 2001, the Harry Oppenheimer Okavango Research Centre (HOORC) produced a vegetation/habitat map for the entire Ngamiland district which included the Okavango Delta. Although a total of 46 habitats were classified based on dominant plant species and life form characters, Ramberg *et al.* (2006) conclude that, broadly speaking, the vegetation of the Okavango Delta has a "mosaic-like vegetation pattern" that range from permanently flooded swamps, to seasonally and sporadically flooded areas, to dry lands that are never inundated. The result of several vegetation studies have led to the conclusion that hydrology is the major determinant of plant communities in the Okavango Delta, especially the frequency and duration of inundation and the depth of flood water (Ramberg *et al.,* 2006). Therefore, for the purpose of this study, and as classified by Gumbricht *et al.* (2004), the Okavango Delta can be simplified into four broad physiographic regions: (1) the permanent swamp; (2) the seasonal swamp; (3) the occasional swamp (or occasional floodplain as it will be referred to in this study); and (4) large islands or dryland woodlands. A fifth physiographic region that could be included is the Panhandle, but this region does not feature in the present study (Gumbricht *et al*., 2004). Though there is much published work on the various physiographic regions, habitat diversity and plant communities (Paterson, 1976; Smith, 1976; Ellery & Ellery, 1997; Myers *et al.,* 2004; Ramberg *et al.,* 2006), Mendelsohn *et al.* (2010) provides accurate, concise and recent descriptions of each (Figure 3):

1) **Permanent Swamps:** This region is characterised by water levels that never drop below the ground‟s surface, and plant species that are all truly aquatic and evergreen. The giant sedge, papyrus (*Cyperus papyrus),* is the most characteristic species of this region, at times constituting 90% of all plant biomass (Mendelsohn *et al.,* 2010), and is often rooted in peat to constitute channel levees (Ellery & Ellery, 1997). The other three species of reeds and grass that characterise channel margins and immediate back-swamps are *Phragmites australis, P. mauritianus* and *Miscanthus junceus.* The variety of plant species increase in the shallower, open waters away from the channels and back-swamps, and includes species of bulrush (*Typha capensis)*, sedges (*Pycreus* and *Cyperus* species) and water lilies (*Nymphaea* and *Nymphoides* species) to name but a few (Mendelsohn *et al.,* 2010).
2) **Seasonal Swamps:** Distinctive of this region is that inundation and flood water depth is consistent enough to allow the persistence of all aquatic life forms. While reeds and

grass characterise permanent swamps and occasional floodplains respectively, sedges dominate in seasonal swamps (Mendelsohn *et al.,* 2010). The sedge-land that emerges commonly includes species such as *Cyperus articulatus, Oryza longistaminata* and *Schoenoplectus corymbosus* (Ellery & Ellery, 1997). The above ground organs of plant species in this region tend to die back when flood waters recede, and new vegetative shoots and leaves sprout when floodwaters arrive again the following year, initiating a wave of production (Mendelsohn *et al.,* 2010).

3) **Occasional Floodplains:** Although this region is often difficult to distinguish from seasonal swamps, there are distinguishing features that separate the two. Firstly, although alluvial sedimentation is the major contributor to their soils, occasional floodplains are seldom inundated by floodwater. Secondly, many of the plant species present occur exclusively in this region; this is very different from the major overlap of plant species in permanent and seasonal swamps. Thirdly, local, annual rainfall is the driver for plant production, not regular floodwater and inundation (Mendelsohn *et al.,* 2010). Finally, they are dominated by grasses that cannot survive prolonged inundation. Common plant species include *Panicum repens* or *Sorghastrum friesii* (Myers *et al.*, 2004).
4) **Islands and Dryland Woodlands:** Island vegetation is versatile, and show distinct vegetation zones as a result of the wide range of soil chemistry between the periphery and centres of islands, as well as the occurrence of adjacent aquatic and terrestrial plant communities. Dominating tree species in the riverine woodland around fresh-water island peripheries include sycamore figs (*Ficus sycomorus*), wild date palms (*Phoenix reclinata),* mangosteens (*Garcinia livingstonei)*, large fever-berries (*Croton megalobotrys)* and sausage trees (*Kigelia africana*); while various grass and broad-leaved herb species cover the remaining surface area (Mendelsohn *et al.,* 2010). Further towards the interior, wild sage (*Pechuel-loeschea leubnitziae)* and real fan palms (*Hyphaene petersiana)* thrive in the moderately saline soils. Finally, only spike grass (*Sporobolus spicatus)* can survive in the high salt areas before vegetation becomes absent all together (Myers *et al.,* 2004).

   Surrounding the Delta, dryland woodlands can be divided into three types, two of which occur in the present study area: acacia woodlands, associated with deep alluvial sands, to the south, west and on Chief"s Island; and mopane woodlands embedded in clayey, alluvial sediments to the east. In these woodlands, there are often large, homogenous stands of the relevant dominating tree or shrub species, with intermittent patches of mixed woodland communities where soil type allows for such diversity (Mendelsohn *et al.,* 2010).

   A final habitat type that is not necessarily a physiographic region, but will be classified as such for the purpose of this study, is **Rainwater Pans**. They are associated with

islands and dryland woodlands, and are highly important for amphibians. Although their surface-area cover in the Delta is noticeably small, they are known nutrient and biological production hotspots. Due to the temporary nature of water availability, many plant species are shared with the occasional floodplain region. Pan occurring plant species include lesser duckweed (*Lemna aequinoctialis),* rhodes grass *(Chloris gayana)* and carrot-seed grass *Tragus berteronianus (*Mendelsohn *et al.,* 2010).

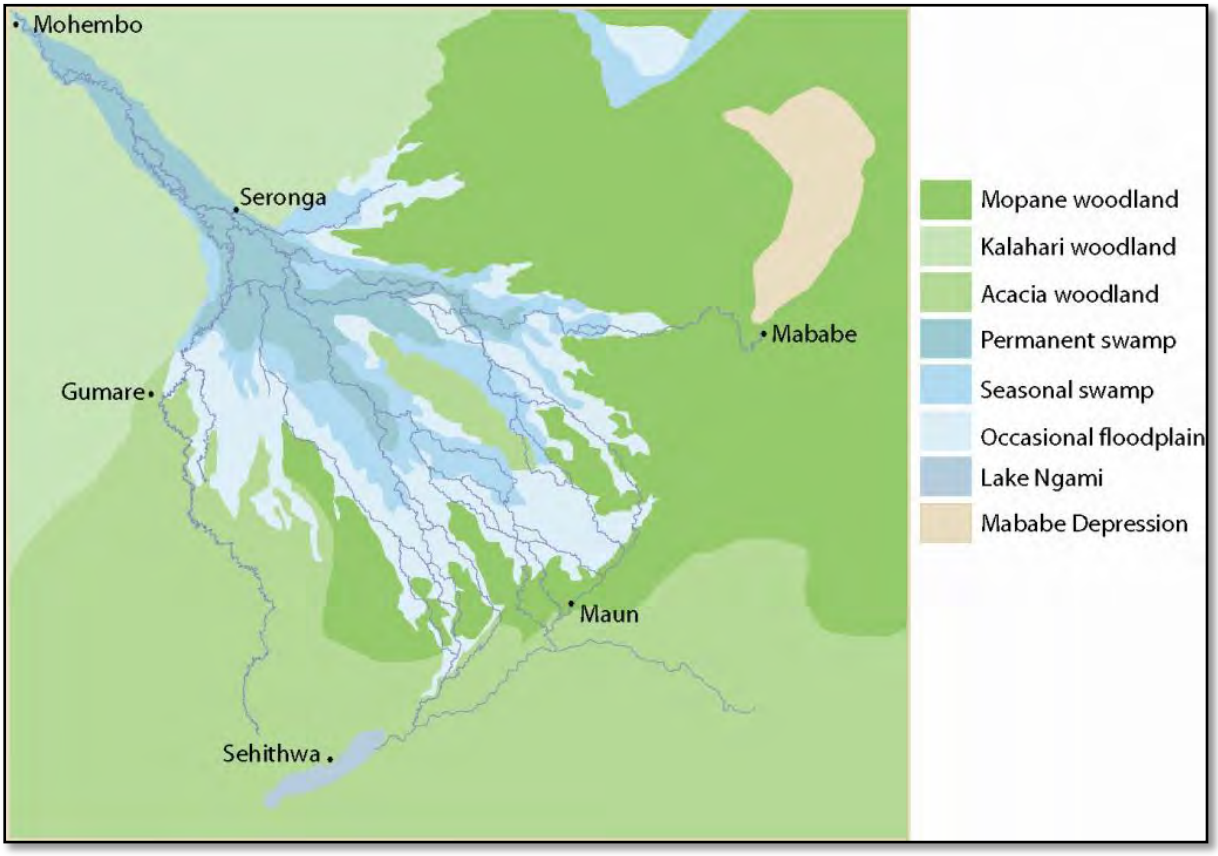

Figure 3: Broad physiographic regions that exist in the Okavango Delta and its surrounds (Mendelsohn *et al.,* 2010).

As an overriding trend of plant diversity in the Delta, habitats gradually increase in both diversity and fragmentation from the permanent swamps of the Panhandle, to the occasional floodplains and increasing dryland along the periphery of the Delta. In fact, approximately 60% of all plant species occurring in the Delta are associated with dryland and island physiographic regions, contrasting drastically with the few species of reeds and papyrus that make up the majority of permanent swamp vegetation. Prominent species such as knob-thorn acacias (*Acacia nigrescens*), lead-woods (*Combretum imberbe*) and jackal-berries (*Diospyros mespiliformis*) require a certain degree of soil and air moisture, yet can only survive where groundwater is sufficiently shallow. Therefore, they are limited in distribution to specific areas with the right moisture conditions in or close to the Delta. In summary, many plant species are restricted to habitats that are never or rarely inundated (Mendelsohn *et al.,* 2010).

The study areas defined for this project were aimed to explore the effect of isolation on the amphibian communities of the Okavango Delta. Isolation in the context of this study is two fold: the first relates to physical isolation by being permanently surrounded by water all year round, in other words, a true island defined by the Oxford English Dictionary (1978) as: “a piece of land completely surrounded by water… or a place insulated at high water or during floods.” The second relates to the exclusion of certain habitat types and plant species (explained above) as a consequence of that area being located within the physiographic regions of permanent and seasonal swamps.

Three study localities (as referred to from here onwards) were identified. They span a range of locations between the coordinates 18.75° to 19.50° latitude and 22.50° to 23.00° longitude, all within the Okavango Delta general coordinates 18.50° to 20.00° latitude and 22.00° to 24.00° longitude. In order to maintain simplicity and consistency with the distribution maps and Gazetteer in Auerbach (1987) and Poynton & Broadley (1985), the present study uses *quarter degree grid cells (QDGC)* as its mapping reference scale (*note: the locality references in both sources uses QDGC with the final division of degree squares into 1, 2, 3, 4; however, the norm in South Africa for this final division is labelled A, B, C, D*. *Therefore, Auerbach’s locality records have been converted to the South African norm, e.g. 1922A1 is converted to 1922AA).* Localities include (1) a set of primary islands formed through channel death or termite activity; (2) the largest island in the Delta, Chief‟s Island, formed through tectonic uplift; and (3) a location on the north-eastern Delta periphery. They range from being completely isolated (surrounded by permanent swamp) to partially or permanently accessible from the mainland. The following study localities were established (Figure 4):

- **Xigera Concession:** A representation of primary islands, this locality consists of several, relatively small islands formed through various biotic and abiotic activities that are periodically inundated to varying degrees by the annual floodwaters. It is situated in the permanently flooded area of the Delta (towards the centre of the alluvial fan, along the western boundary of Moremi Game Reserve) and habitats vary from swamps, permanent channels, floodplains and islands dominated by palms and hardwood riverine woodland (www.wilderness-safaris.com, accessed 07 Sep 09). Due to its location in the permanent swamp physiographic region, this study locality represents one of greatest isolation, at present with no connection to main land whether the flood is at its peak or low. It also contains the least diversity of habitats, lacking any true dry woodlands or rainwater pans (and associated vegetation) as described above; the result of regular inundation and high groundwater levels.

  All sample sites in this locality fall within the coordinates 19.25° to 19.50° latitude and 22.50° to 23.00° longitude (alternatively, QDGC 1922BC and 1922BD).

- **Mombo Concession**: Also located within the Moremi Game Reserve, the Mombo Concession is a cluster of islands located at the north-western tip of Chief‟s Island. Its location just downriver of where the Okavango River splits into its three primary channels, and in a region of deep alluvial sand deposits, means it is arguably the most fertile area in the Okavango Delta. It has a wide variety of habitat types including permanent channels, marshes, swamps, and floodplains, dry (Acacia) and riverine woodland. Approximately 50 years ago (towards the end of the 1970s) the Mombo island group was actually separated from Chief‟s Island due to periods of high flood, but presently they are connected and only about 15% of Mombo is permanently flooded (Myers *et al.*, 2004). In terms of the above described physiographic regions, this locality also supports all the associated habitat types (comparable to the Kwedi Concession) and it is located towards the centre of the Okavango Delta. To the south-east, it is bordered by seasonal swamp and occasional floodplain, and thus it is partially connected to the main land when floodwaters are low (Myers *et al.,* 2004).

  All sample sites in this locality fall within the coordinates 19.00° to 19.50° latitude and 22.75° to 23.00° longitude (alternatively, QDGC 1922BB and 1922BD).
- **Kwedi Concession**: Located on the north-eastern fringe of the Okavango Delta, the Kwedi Concession provides all the habitat types associated with the above physiographic regions. Its location on the periphery of the Delta renders it permanently connected to, and accessible from, the mainland to the north-east. It varies in habitat from permanent swamp, channels and islands; to floodplains and grasslands that are seasonally flooded; to dryland woodlands (dominated by Mopane Woodlands) with rain filled pans containing a mixture of species such as Kalahari apple leaf (*Lonchocarpus nelsii)*, mopane and *Combretum* species. Owing to its wide variety of habitats, it is believed to support the majority of animals occurring in the Okavango Delta (www.wilderness-safaris.com, accessed 07 Sep 09).

  All sample sites in this locality fall within the coordinates 18.75° to 19.25° latitude and 22.75° to 23.00° longitude (alternatively, QDGC 1822DD and 1922BB).

Although the different locality coordinates may fall within the same QDGC, and therefore seem to overlap (e.g. Kwedi and Mombo both have sites in 1922BB), it is important to note that in reality, the actual localities do not overlap, which is obvious when viewed at a larger scale (as in Figure 2). In other words, sample sites of one locality may occur in the upper portion of a specific grid cell, while sample sites for the adjacent location fall in the lower portion of the same grid cell.

Figure 4: Satellite image of the Okavango Delta displaying the three study localities **(**www.google.com/earth/index.html, accessed 22 Sep 10).

Based on the above knowledge of habitat availability in the various localities, and that of habitats conducive to amphibian presence, several sampling sites were randomly chosen to represent all the habitat types present in that study locality. Several preliminary surveys of each locality were needed to identify promising sites that were also logistically feasible to access. Favourable amphibian habitats were identified based on descriptions provided by du Preez & Carruthers (2009): relevant definitions and descriptions below, as well as the most fitting physiographic region (based on vegetation growth forms) as they occur in the Okavango Delta:

- **Pan (Figure 5)**: Mostly semi-permanent water body with widely fluctuating water levels. Surrounding vegetation varies from overhanging trees, reed beds, inundated grass or open mud; corresponds to rainwater pans.

Figure 5: Photograph of a pan sampling site.

- **Floodplain (Figure 6)**: A flat or depressed area lining a river or channel course. It is periodically inundated when water overflows the banks of the water course, and it may retain floodwater for variable periods of time. It is characterised by grasses and not specialised aquatic vegetation; corresponds to occasional floodplains.

Figure 6: Photograph of a floodplain sampling site.

- **Perennial stream (Figure 7)**: A flow of water maintained within a natural channel and through all seasons of the year; corresponds to permanent swamps.

Figure 7: Photograph of a perennial stream sampling site.

- **Vlei (Figure 8)**: A marshy wetland that is characterised by specialised water-based vegetation including sedges, reeds and inundated grass. It is a part of a watercourse which spreads out over a flat, depressed valley. These areas may dry up completely or partially during the dry season; corresponds to seasonal swamps.

Figure 8: Photograph of a vlei sampling site.

- **Forest Floor (Figure 9)**: The area of ground found beneath woodlands with a closed canopy. Large amounts of leaf litter and humus are characteristic; corresponds to dry woodland or islands.

Figure 9: Photograph of a forest floor sampling site.

Due to the remoteness of all study locations, accessibility and safety were limiting logistical factors that had to be taken into consideration when sampling sites were chosen. Sampling sites per locality are representative of all physiographic regions (habitat types) present for that locality. However, due to these limitations, an equal number of sampling sites representing each physiographic region could not be guaranteed. A total of nine or ten formal sampling sites were chosen for each study locality, and, in addition, Xigera and Mombo also acquired additional impromptu sample sites where a new amphibian species for that locality was observed by chance. A summary of the relative proportions of physiographic regions represented by the chosen sampling sites is given in Table 1 for each locality.

Table 1: Proportion (%) physiographic region of the total sampled area for each locality.

Physiographic Region	Study Locality		
	Xigera	Mombo	Kwedi
Permanent Swamp	22.2	10.0	11.1
Seasonal Swamp	44.4	20.0	22.2
Occasional Floodplain	22.2	20.0	22.2
Dryland Woodland/Island	11.1	10.0	11.1
Rainwater Pan	0.0	40.0	33.3

CHAPTER TWO:
AMPHIBIAN SPECIES DIVERSITY IN THE OKAVANGO DELTA: THE EFFECT OF ISOLATION

2.1 INTRODUCTION

2.1.1 Amphibian diversity and conservation: a closer look at the Okavango Delta

The global decline in amphibian populations is one of the most worrisome conservation issues of recent times, and when viewed in the greater context of the environment in which they reside it is not difficult to see why. The ecological importance of amphibians in both terrestrial and aquatic ecosystems suggest that a loss of members from this class will have complicated, widespread and severe consequences. Subsequent to the realisation that not nearly enough data was available on the extent and severity of amphibian declines, the IUCN (International Union for the Conservation of Nature) began to gather data through an initiative called *The World Conservation Union Global Amphibian Assessment* (Stuart *et al.,* 2004).

In light of major concerns about amphibian populations and their wellbeing, and the noteworthy emphasis on conservation and the environment in recent times, this project was launched in 2008, the year proclaimed as the "Year of the Frog" by the World Aquarium and Zoo Association (WAZA), an initiative supported by the IUCN Species Survival Commission and endorsed by Amphibian Ark. It comes in response to the plea for a better understanding of amphibians on all levels and in all regions. For the most of Africa, basic species lists and inventories are often non-existent, and the life histories of many species are wholly or partly unknown. Prior to any assessment of amphibian status and declines, and any decisions on the necessity and creation of a conservation action plan, the first step is to compile species' inventories for the region (Channing, 2001). It was thus timely to undertake this study, to ensure that the unique amphibian diversity of the Okavango Delta ecosystem is better understood, and to facilitate management in such a way so that future generations will also be able to enjoy and appreciate this system with its unique biodiversity.

Worldwide, more than 6000 amphibian species are known with new discoveries and additions expanding this list on an annual basis. In Southern Africa, south of the Zambezi, Okavango and Cunene rivers a total of 13 families, 33 genera and 157 species are currently known. This impressive biodiversity may be attributed to the wide variety of topography, climates and

habitats that occur in the region; and amphibians have managed to invade and establish viable populations in numerous of the microhabitats that result from this diversity (du Preez & Carruthers, 2009).

In a detailed analysis of the distributional patterns of amphibians in Southern African, Poynton (1964) identifies three major faunal groups existing in the region: *tropical fauna* in the north-east, *cape fauna* in the south west and a *transitional complex* where the subtraction margins of the other two groups overlap over an extremely wide area. He further divides the endemic, transitional forms (species) into several zoogeographical units based on their location in warmer, tropical or cooler, temperate areas; as well as their location in the central, western or eastern portions of the region. The area of this study, the Okavango Delta, falls within the transitional complex, in the largest zoogeographical unit called the *central tropical transitional fauna* (Map 2 and Map 3 in Poynton, 1964). This unit is termed as such because it borders the tropical centre and is located in the richest portion of the tropical subtraction margin. Of the sixty forms that are endemic to the transitional complex, this unit is characterised by the following eleven forms: *Xenopus petersii*, *Amietophrynus gutturalis*, *Breviceps poweri*, *Phrynomantis affinis*, *Tomopterna tuberculosa*, *Hylarana darlingi*, *Ptychadena uzungwensis*, *Ptychadena subpunctata, Leptopelis bocagii, Hyperolius rhodesianus* and *Hyperolius parallelus* (Poynton, 1964; Frost, 2009). It should be noted, however, that these divisions cannot be cartographically precise and they are by no means an attempt at rigid classification. The complexity of biological and environmental factors, as well as the wide ecological tolerance of amphibians, makes such an attempt almost impossible (Poynton, 1964).

In an article by Ramberg *et al. (*2006) the total amphibian fauna for the Okavango Delta was quoted at 33 species. However, when the source of this information is traced to its origin in the feasibility study carried out by the CSIR (Council for Scientific and Industrial Research) and Water Transfer Consultants, this figure actually refers to the total number of species *expected* to occur in the region, including the Okavango River (in northern Namibia), the Okavango Panhandle and the Okavango Delta (Murray, 1997). This figure is therefore not a reliable benchmark for actual, recorded observations and collections. The most accurate, published work (in respect of this study), specifically on the amphibians of Botswana, was published by Auerbach (1987) where he attempted to consolidate all available information and museum specimens at that time. His work is especially useful in that he includes in each species description a QDGC distribution map of recorded localities, as well as quoting actual collection points that correspond to quarter degree squares in the Gazetteer section. Auerbach (1987) records a total of 38 amphibian species for the entire Botswana, with 28 of these recorded in the Okavango Delta, between the coordinates 18.50° to 20.00° latitude and 22.00° to 24.00° longitude. In 1990, an assessment of the zoogeography of Botswana"s herpetofauna was

published, but this too used the records in Auerbach (1987) as the source of amphibian information (Simbotwe & Guillette, 1990). Poynton (1964: Map 1) indicates that there is no well-collected locality in or around the Okavango Delta; and furthermore, in preparation for the recent publication *'A complete guide to the frogs of Southern Africa'* (du Preez & Carruthers, 2009), the authors realised that knowledge of the amphibian fauna in the Okavango Delta is severely lacking. Hence a thorough, current investigation of the amphibians of the Okavango Delta is necessary.

For the present study, a desktop study of four credible and up to date sources (mostly on the amphibians of Southern Africa as a whole, not dedicated to Botswana alone) revealed that a possible 51 species may be present in the region (Channing, 2001; Carruthers, 2001; du Preez & Carruthers, 2009 & www.amphibiaweb.org, accessed 31 Oct 09). This figure is based on the visual assessment of distribution maps provided by the sources; however, it should be noted that these distribution maps are not sensitive at the level of latitude and longitude coordinates, nor do they document point localities of records. In addition, this figure is not exclusive to the Okavango Delta alone, but also includes records of species identified in the surrounding areas including the Caprivi Strip, north-eastern Botswana, western Zimbabwe, south-eastern Angola and north-eastern Namibia.

Although there is a possibility that these species occur in the Delta, a more reliable expected species list was compiled using only the detailed Auerbach (1987) publication and the most recently published amphibian guide by du Preez & Carruthers (2009). Annexure I is a table displaying possible species occurring in the Okavango Delta based on previously recorded localities from the above mentioned sources, as well as the resultant expected species list. In summary, records from du Preez & Carruthers (2009) indicate that 33 species may be present in the Okavango Delta, while Auerbach (1987) indicates 28 species, with only three of these previously recorded in the present study area. Therefore, the expected species list for the present study totals 33 species.

According to the IUCN Red Data List, all 33 of the expected Okavango Delta species are categorised as *least concern* (www.iucnredlist.org/amphibians, accessed 31 Oct 10). However, although none of the amphibian species fall into any threatened categories, the Okavango Delta wetland, and thus its amphibians, is under constant threat as discussed further in Chapter 5. The designation of the Okavango Delta as an official Ramsar Site has assisted in its protection for the moment, but more information and knowledge on the system and its biodiversity must be gained to ensure its future conservation. The acquisition of baseline data on all floral and faunal communities is crucial if effective management plans are to be implemented and this pristine wetland is to be maintained (Ramberg *et al.*, 2006). The recent publication by Mendelsohn *et*

al. (2010) was initiated by the IUCN with a major aim to encourage available information and knowledge utilisation, in order to effectively monitor the Delta"s biodiversity composition and abundance; it was hoped the book would combine baseline monitoring information and experience. The authors realised in the early days of the project, however, that not enough baseline data was available to achieve this goal: "it was found that more work was needed to develop appropriate monitoring measures before a synthesis could be attempted," (Mendelsohn *et al.*, 2010).

2.1.2 The effect of isolation on amphibian diversity

A community can be defined as: "an assemblage of species populations that occur together in space and time" (Begon *et al.,* 2006); and the focus of community ecology is to determine natural patterns in species groupings and the factors that affect them. In short, communities are mostly moulded by restrictions to dispersal, environmental limitations and internal dynamics (Begon *et al.,* 2006). The manner in which communities are assembled and organised has interested ecologists for many a decade, and changes in species composition and abundance along some physical gradient (e.g. moisture, temperature, latitude etc.) can provide great insight (Morin, 2003). There are five conventional attributes that are generally analysed when communities are studied: growth form and structure, diversity, dominance, relative abundance and trophic structure (Krebs, 1994).

Information gained from community analyses is usually complex and confusing, but there are various descriptive techniques that can be used to uncover trends in species number, identity and relative abundances (Morin, 2003). The simplest analysis of community composition (but nonetheless a fundamental one) is *species richness*, defined as "the number of species present in a defined geographical unit" (Begon *et al.,* 2006). Although the notion of counting species may seem straightforward, the process is often complicated by the fact that only a subsample of the total population can realistically be counted. Therefore, sampling should preferably continue until a plateau in species number is reached; or alternatively, only samples similar in size in terms of sampling time, area or number of individuals should be compared. A better analysis of community composition, however, not only considers species number, but also incorporates the relative abundance of each species; in other words, how common or rare a particular species is in that community. This gives an estimate of *community diversity.* It combines richness and equitability (or evenness), and this can be quantified using various diversity indices. Prior to the establishment of any effective conservation management plans or establishing priority conservation tasks, knowledge regarding the spatial distribution of species richness must be available (Begon *et al.,* 2006).

Community analyses on islands and the concept of *island biogeography* has been of interest since the early days of Darwin (1859), especially because the factors that control biological diversity are so greatly pronounced in these isolated areas (Morin, 2003). Three major aspects render island biogeography interesting. Firstly, the comparisons of how and why island communities differ from those that are established in the *source-area*, and the specific adaptations of island biota that have allowed them to transverse barriers and become established on islands. Secondly, exploration of the processes that underlies and controls the balance between immigration, extinction and island carrying capacity. And thirdly, the evolutionary changes that biota must undergo in order to become an integrated and functioning ecosystem, whether that involves adaptation to new, island lifestyles or group diversification to penetrate new and available ecological niches (Cox & Moore, 1995).

There are two ecological patterns which are consistent and well recognised in community ecology studies, especially in island biogeography. The first is the inverse relationship between species richness and island remoteness: as the degree of remoteness increases, so species richness decreases. Remoteness, in this case, is defined as the degree of isolation due to physical barriers that hinder dispersal. Therefore, it can be predicted that more remote islands will only contain a fraction of potential colonisers from the source area, since some species have limited dispersal ability. The second pattern is the decrease in species richness as spatial heterogeneity decreases; and the related trend that spatial heterogeneity decreases with a decrease in area size. In other words, the more habitat types an environment contains (more heterogeneous), the higher the species richness due to factors such as increased microhabitats and microclimates (Begon *et al.,* 2006).

There are numerous factors that influence species richness on islands, including area, topography, habitat diversity, remoteness, source-area species richness, immigration, extinction and more; and thus there is a need for a universal theory that can explain what is observed on islands (Cox & Moore, 1995). Such a theory was previously produced by MacArthur and Wilson (1967) who proposed that species richness on islands was more complicated than the straightforward, well known *area size – spatial heterogeneity* relationship; and thus they formulated the *equilibrium theory of island biogeography.* In short, it maintains that island isolation and size play a direct role in immigration and extinction rates respectively. The former is affected by colonisation success, while the latter is influenced by the fact that small islands can only support small populations, rendering them more vulnerable to extinction (Begon *et al.,* 2006). Although relevant, the details of this theory and its application are beyond the scope of the present study, and numerous past studies have been dedicated to exploring its predictions (Nilsson *et al.,* 1988; Kohn & Walsh, 1994; Ricklefs & Lovette, 1999; Herzog & Kessler, 2006).

Barring South Africa with its unique Cape Floral Kingdom, the combined faunal and floral species richness of the Okavango Delta is comparable to that of other biomes in Southern Africa – calculated at 329 species, for six biological groups, per one square kilometre (Ramberg *et al.,* 2006). As previously discussed, habitat density (number of habitats per area) progressively increases from the Panhandle to the drier Delta periphery where fluctuation in water levels are most pronounced. It follows then, that that these areas too will have the greatest biodiversity, especially for those species that require two or more habitats for their development and inhabitation (Ramberg *et al.,* 2006). This assumption holds true for bird, mammal and reptile groups, and Mendelsohn *et al.* (2010) show large increases in species numbers from perennial and seasonal swamp to dryland. There are several major factors determining overall animal biodiversity. Firstly, the great diversity of wetland and dryland habitats, often occurring in very close proximity to one another, allows for niche specialisation and the use of multiple habitats to satisfy one animal"s daily needs. Secondly, the constantly changing landscape resulting from the flood pulse, means that resources such as shelter, food supply and breeding sites are never constant; therefore, opportunistic strategies to locate and exploit productive hot spots, and the ability to move to these, is crucial. Thirdly, the Delta is rich in nutrients and this allows it to support a great abundance of animals (this will be discussed in more detail in Chapter 3). Lastly, the constant supply of fresh water towards the middle of the alluvial fan, and its extended availability to fringe areas, allows most animals to be present all year round; and since the Delta is an oasis in an otherwise desert country, this feature is exceptional (Mendelsohn *et al.,* 2010).

2.1.3 Hypothesis and objectives

Amphibian species diversity is determined by degree of isolation: It is predicted that amphibian species diversity will progressively decrease with increasing degree of isolation.

The key goal of this assessment is to answer the important and often complicated question raised by island biogeography: is lower species richness the result of isolation*,* or is it merely the consequence of a lack of spatial heterogeneity on smaller islands that can support fewer habitats (Begon *et al.,* 2006)? In order to test this hypothesis and add information to areas where current knowledge is lacking, the following objectives were formulated:

- Determine overall amphibian species richness for the Okavango Delta, and compare community composition of each study locality using measures such as species richness and species diversity.

- Compare differences in locality species richness and investigate reasons for the presence or absence of species through quantitative and qualitative analyses of habitat isolation and availability.

2.2 METHODOLOGY

2.2.1 Assessment of locality isolation

In order to assess differences in amphibian richness and diversity between the three localities and investigate the effect of isolation, it was first necessary to ensure each locality did indeed represent different degrees of isolation. Flood frequency data was obtained from the Harry Oppenheimer Okavango Research Institute (HOORC); this data shows the average extent of high and low flood levels. Using ArcGIS 9.2 software, the flood frequency data was overlaid onto the present study area to show each locality in relation to changing flood water levels. The demarcation of each study locality is provided by the coordinates given in Chapter 1, and the reader is reminded that the choice of demarcation was to ensure consistency with the distribution maps of Auerbach (1987). [Note that all figures labelled as *Maps* in the present study were created using ArcGIS 9.2 software.]

2.2.2 Sampling effort

The present study was initiated in 2008 and ended in 2010. After several preliminary visits to the region to assess the area, determine feasibility and finalise study objectives, the first samples were collected in November 2008 and the final samples in June 2010. This section of the study, therefore, lasted 20 months in total. Within this time, three official field trips were conducted: a six day trip to Xigera and Kwedi in Jan/Feb 2009; a six day trip to Xigera and Kwedi in July 2009; and a nine day trip to all three localities in Nov/Dec 2009. There was a lack of sampling initially in Mombo as this locality was only considered and added approximately six months into the study. However, this initial deficit in sampling effort was mostly rectified by the permanent basis of the researcher in that locality for the remaining 14 months.

Despite the skewed sampling efforts (times) implied by the field trips, when all official and ad hoc sampling times were combined for each locality, the final sampling time per locality was relatively even. In total, 49 observer-hours were spent actively searching for specimens. Drift fence-pitfall trap collection (explained below) obscured sampling times dramatically since each trapping event lasted several hours compared to a considerably shorter time for other collection

techniques. However, their contribution to both species richness and abundance, in terms of specimens actually caught, proved minimal. Therefore, for simplicity and accuracy, it was decided that each trapping event would contribute one observer hour to the total sampling effort. In each locality, four trapping events occurred and thus contributed four hours to each one"s total sampling time. In combination for all collection techniques, sampling efforts were as follows: 18.67 hours in Xigera, 14.83 hours in Mombo and 15.50 hours in Kwedi.

2.2.3 Collection of amphibian species in the Okavango Delta

The methodology for this project was guided by the standardised sampling methods of previous, similar studies with comparable objectives (Rödel & Ernst, 2004; Veith *et al.* 2004). Visual and acoustic encounter surveys (*VES* and *AES* respectively) were conducted; these involved the systematic movement of observers through an area or sampling site searching for specimens for a predetermined period of time. In addition, collection by *drift fence-pitfall traps*; *dipnet surveys* and opportunistic *road encounter surveys* provided supplementary data and allowed for the collection of inconspicuous terrestrial and aquatic species (Heyer *et al.* 1994; Rödel & Ernst, 2004; Veith *et al.* 2004).

Specimen collection was undertaken at each of the predetermined sampling sites in each locality. The goal of each sampling session was to collect as many species and specimens as possible; up to a maximum of 20 specimens per species per sampling site, as this provided a sufficiently large sample size for the disease survey of *Batrachochytrium dendrobatidis* (see chapter 4). Several collection techniques were implemented during each sampling session, and as many techniques as possible were used at each site. In general, upon arrival at a sampling site, an AES was first conducted prior to any disturbance of the area by the movement of observers; this was followed by a VES and then a dipnet survey, either at the end of that sampling session or the following day. Drift fence-pitfall traps were randomly erected where habitat was suitable. In combination, these techniques maximised sampling effort: they allowed for the collection of amphibians in all available habitat types and targeted both larval and post-metamorphic life stages.

Preceding each sampling session, all necessary and important information was documented including: start time; sampling site; brief habitat description (especially noting any changes from previous visits); collection technique; current and preceding weather conditions such as temperature, rainfall and wind; and finally any additional information or observations that may have been of importance. At the end of every sampling session, the following information was recorded: end time, all species observed and identified; and number of specimens caught per species with corresponding field numbers.

Here follows a description of the collection techniques employed:

- Acoustic encounter survey (AES)

The fact that amphibians are more often heard than seen, and that each advertisement call is unique to that species, makes this an exceptionally valuable sampling technique (Carruthers, 2001). Each survey consisted of a ten minute listening session, during which time all calls that were heard were documented. Recording of calls were made at all sites using an Olympus digital voice recorder with external microphone.

Calls from the actual sampling site and those heard in its surroundings were recorded. A rank system was used to estimate chorus size, and thus abundance, at each site:

1 = 1 – 2 specimens observed calling

2 = 3 – 5 specimens calling

3 = 6 – 10 specimens calling

4 = 11 – 20 specimens calling

5 = over 21 specimens calling.

- Visual encounter survey (VES)

Searches were conducted from dusk till midnight, and were most productive when carried out in the warm, rainy season when amphibians were most active. Each survey lasted between 30 and 40 minutes, depending on habitat heterogeneity and sampling success, by two to three observers. Predetermined plot sizes were not determined, but every possible niche within the sampling site was systematically investigated; search effort was at the most intense level (including habitat modification) as specified by Heyer *et al.* (1994).

Each observer that assisted in collection was equipped with a torch, plastic bags, field notebook and pencil, permanent marker and disposable gloves. Observers wore gloves during the search to prevent DNA contamination between specimens (important for Bd collection, discussed in Chapter 4), and changed them between specimen capture; or alternatively, thoroughly washed and dried their hands between handling specimens. A thorough habitat search was conducted by walking through each sampling site and carefully searching for specimens on the ground, at the water"s edge, among vegetation and under logs. Calling amphibians were often used to direct searches and calls were traced to their origin.

Once observed, the animal was quickly grabbed at close range by hand. Each specimen was placed in a clean, individual plastic bag, filled with air and a little water from the site, and set aside for storage during the remainder of the sampling session. This was done to prevent recapture of the same individual and to prevent contact between specimens and DNA contamination. Each bagged specimen was labelled with a field number. Searches

were terminated prematurely when observers did not observe (visually or acoustically) any additional specimens or species for at least ten minutes.

The final step was the process of identification and swabbing for Bd in the field, or alternatively at a temporary field lab if specimen identity in the field could not be confirmed, or if the specimen was used in the voucher collection. Using a fresh pair of gloves, a specimen was removed from its bag and while gripped firmly, was identified and swabbed for Bd (also explained in Chapter 4). Once all the necessary data was recorded, the specimen was released on site. Any bagged specimens that had to be analysed or processed in the field lab were placed in an empty cooler box or large storage container and taken back to the field laboratory for analyses and processing. Any specimens not kept as voucher specimens were returned to the original site of collection and released the following day.

- Dipnet survey

This survey procedure required a fine-meshed dipnet with a relatively long handle and solid frame. The aim of this technique was to capture tadpoles and any permanently aquatic amphibian species, i.e. *Xenopus.* Dipnetting was carried out in shallow water and mostly among vegetation where amphibians were most likely to occur. Three different techniques were used to collect specimens. Firstly, wading through shallow water, the net was swept along the floor of the water body, mainly in search of *Xenopus* specimens that often reside on the floor, and for bottom feeding tadpoles. Secondly, standing in one position, the net was kept level with the water surface and alternatively pulled backwards and then pushed forwards several times before checking the net for specimens; this movement created a „current" that trapped specimens. The third method involved several scooping actions in a relatively small area before checking the net for specimens and then moving on to a different area in the site.

As with visual surveys, any juvenile or adult specimens captured were bagged, labelled, identified and processed. Tadpoles, however, were immediately euthanized and fixed by placing them directly in a vial with buffered formalin (described below). It was crucial to keep tadpoles collected at different sites separate; and occasionally some tadpoles were stored in 96% ethanol to preserve DNA for future genetic studies. Once again, vials with specimens had to be labelled with field numbers and recorded in the field data notes.

- Straight line, drift fence-pitfall traps

This technique typically consists of barriers in the form of 6 m long straight-line fences, 30 cm in height and constructed from PVC or shade cloth; with pitfall traps placed at the ends, made from 4 l tins, their bases covered with a little moist soil to prevent desiccation of any specimens caught. Terrestrial animals travelling on the ground surface were directed into

the traps when they encountered the drift fence and began to move along it. Traps were checked early every morning; any specimens caught were removed, identified and processed as per the VES procedure above.

- Road Encounter Survey

Although this is not an official sampling technique of Heyer *et al.* (1994), any specimens that were casually encountered on the road when driving between sampling sites or randomly throughout the study period were captured and identified. Any specimens that had not been previously recorded by other sampling techniques were stored and processed at the field lab as a voucher specimen, since these encounters positively contributed species richness data. The GPS location of where specimen collection occurred was recorded. Those species that had been recorded at official sampling sites elsewhere were immediately released.

2.2.4 Voucher collection and preparation of voucher specimens

A goal of this project was to compile a voucher specimen collection for each of the three localities. To avoid loss and damage to specimens and data, it was crucial to process stored specimens from the various collection techniques as soon as possible once back at the field lab. For the purpose of this research project, only two specimens per species per locality were fixed as vouchers. The procedure for voucher preparation as follows:

1. A specimen number was allocated to the captured specimen: printed specimen tags were used (e.g. AACRG0920, AACRG0921) and followed one another numerically.
2. The following information for each specimen was recorded: field number; specimen number used for labelling the specimen and tissue sample; Bd swab number (for this project MLR001, MLR002 etc. were used), confirmed species name; photograph number and any additional information or observations.
3. Where possible, voucher specimens were photographed, ideally prior to euthanisation.
4. All amphibians were euthanized by immersing them in a MS222 solution (tricaine methane sulphonate): approximately 800mg/l water. After a period of between 5 to 15 minutes the animal was euthanized; this was confirmed by pinching the thigh muscle with forceps and ensuring there was no reflex reaction. The euthanized specimen was then removed from the solution and placed on a clean, dissecting tray; on a piece of paper towel that was discarded after processing. The euthanizing procedures were in accordance with the recommended techniques in Heyer *et al.* (1994) and du Preez & Van Wyk (2006).
5. A tissue sample of every voucher specimen was collected for DNA referencing. Using a pair of sterile sharp-nosed scissors and forceps, a tissue square approximately 2mm x 2mm was removed from the animal"s thigh muscle and immediately transferred to a small, labelled vial

filled with 96% ethanol and tightly sealed. All equipment was sterilised with 70% ethanol followed by flaming.

6. Voucher specimens were labelled using the allocated, printed specimen tag and white linen thread; the thread was tied just above the hind legs with the tag positioned along the ventral side of the animal.
7. The specimen was then transferred to a fixing tray that consisted of a plastic container, the base of which was lined with beeswax. Using pins, the animal was fixed in a normal body posture, following guidelines suggested by Heyer *et al.* (1994) and du Preez & Van Wyk (2006):
    - The limbs were positioned close to the body and flexed in a natural way, as if the animal were squatting;
    - phalanges were straightened and parted so that webbing could be observed;
    - key features were easily accessible for measurement and analysis, even after preservation;
    - the animal was fixed in a way that would allow for easy, effective storage and long-term maintenance.
8. 10% Neutrally buffered formalin (NBF) was used as the fixative: a solution of 900 ml distilled water, 100 ml formalin (100%), buffered with 3.5 g NaH_2PO_4 and 6.5 g Na_2HPO_4 (du Preez & Van Wyk, 2006). The solution was poured into the fixing tray to a level where the animal was either fully submerged, or at least one third of the tray was filled with solution and the exposed portions of the specimen was covered with paper towel soaked in NBF. The animal was left in the fixing tray for a period of at least four days. Formalin was only handled in well ventilated rooms and whilst using gloves.
10. Finally, fixed voucher specimens were transferred to wide-rimmed glass jars filled with NBF for long term storage.

2.2.5 Assessment of amphibian community composition per locality

Species richness is a simple yet highly effective analysis of community composition. For each locality, this richness was calculated by compiling a species inventory of observed species and summing them together; results from all collection methods (adults, tadpoles or calls) were used in this analysis. Species unique to a locality were further analysed in terms of life history traits and habitat or breeding preferences to expose any possible reasons for their presence in one locality alone, and their simultaneous absence in the other two.

When the species inventories had been compiled, it was noticed that several species from the expected species list were surprisingly and conspicuously absent, especially in the Kwedi locality. Records in Auerbach (1987) of these absent species were investigated; and those that

had been previously recorded by him in any QDGC adjacent to a particular locality were noted. The position of these previous records were then checked against Google Earth images (www.google.com/earth/index.html, accessed 22 Sep 10) and Figure 10 to determine whether there were any visible, physical barriers that may have prevented the dispersal of those species from the previously recorded locality in Auerbach, into the adjacent locality of the present study.

A more accurate measure of community composition is species diversity as it accounts for both richness and evenness; this can be quantified using various diversity indices. A diversity index is a statistical method that incorporates richness, evenness and total number of individuals, and attempts to analyse variation within a community (Krebs, 1994; Türkmen & Kazanci, 2010). Such indices have been devised as nonparametric measures of diversity that do not make any assumptions about species-abundance curves (Krebs, 1999). Several different diversity indices were investigated and it was found the most useful results were obtained from the Shannon-Wiener function and the Margalef index.

A favoured technique when assessing communities, the Shannon-Wiener function is based on information theory and aims to correctly predict the species of the next individual if another collection was made. This index of diversity (*H'*) increases with an increase in species number or a more even distribution among species (Krebs, 1994; Krebs, 1999). Its values range from 0.0 to 5.0, with results usually between 1.5 and 3.5 and only seldom above 4.5 (Türkmen & Kazanci, 2010). It is given by the following formula:

$H' = -\Sigma [(ni / N)*(\ln ni / N)]$

Where H' = Shannon diversity index

ni = Number of individuals belonging to i species

N = Total number of individuals

(Türkmen & Kazanci, 2010.)

A measure of evenness based on the above index was also tested. In 1966, Pielou derived an evenness index from the Shannon-Wiener function, ranging from 0.0 to 1.0, with 0.0 indicating no evenness and 1.0 indicating complete even distribution. Pielou"s evenness index is given by:

$J' = H' / H'_{max}$

Where J' = Pielou evenness index

H' = Shannon diversity index

H'_{max} = lnS

S = Total number of species

(Türkmen & Kazanci, 2010.)

The Shannon-Wiener function is strictly speaking only applicable when the total species richness of a large community is known, and when random samples are collected. Often, in community analyses, this is not possible (Krebs, 1999). Therefore, an alternative index was also employed.

Margalef"s diversity index (*d)* is based on an evaluation of the total number of species encountered, against the total number of individuals encountered, (www.neiu.edu/~jkasmer/Biol380/Labs/sp-diversity.htm, accessed 28 Oct 10). It is dependant on variation in the number of species; is useful for site comparisons; and can have any value i.e. there is no limit to the value (Türkmen & Kazanci, 2010). It is given by the following formula:

$d = (S-1) / \ln N$

Where d = Margalef diversity index

S = Total number of species

N = Total number of individuals

Due to the nature of collection techniques and the wide variety of data collected, actual abundance values were not used for these analyses of diversity. Instead, encounter data was used: every time a particular species was encountered during sampling sessions, whether as a calling adult, visually observed specimen or tadpole, it was recorded as one encounter. This was feasible since acoustic and visual surveys were not conducted simultaneously, and thus it was assumed that calling adults observed during an AES were not necessarily the same as observed individuals during the subsequent VES. Moreover, since calling generally occurs in choruses of several individuals, it was decided that one encounter for calls and one encounter for visual observations was not an overestimation of abundance.

2.2.6 Statistical evaluation of differences in community composition

Additional statistical analyses were performed to test whether the differences in locality species diversity were significant. Using the presence/absence data of each species, tests were based on the number of sampling sites in each locality where the particular species was observed. Three different nonparametric analyses were done to test the relationship between species presence and its occurrence within each locality: Kruskal-Wallis test; chi squared test of independence and effect sizes. In the first two analyses, a significance value $p<0.1$ indicated a statistically significant relationship between the two variables. For a more detailed description refer to www.statisticssolution.com/ methods-chapter/statistical-tests/, accessed 28 Oct 10. The latter is discussed in slightly more detail below since it is independent of sample size and thus a better test for the present study where the number of encounters of a single species ranges

from 1 to 26. Those species that displayed significant values for any of the tests were identified and extracted from the overall results for further study.

Effect sizes are calculations that investigate whether a relationship between two variables is *practically significant*: "practical significance can be understood as a large enough difference to have an effect in practice" (Ellis & Steyn, 2003). It is independent of sample size; represented by the phi (ø) coefficient; and calculated by the formula $w=\sqrt{(X^2/n)}$ where X^2 is the chi-square statistic and n is the sample size. The larger the effect size value, the more significant it is: $w \geq 0.3$ indicates medium effect while $w \geq 0.5$ indicates large effect; w is considered practically significant if $w \geq 0.5$.

All statistical analyses were performed using SPSS®[1] and STATISTICA[2] statistical packages.

2.2.7 Investigation of species occurrence related to habitat type

In order to investigate any possible correlation between species occurrence and habitat type, presence/absence data of each species was again used, but this time tests were based on those habitat types in each locality where the species was observed. All sampling sites of similar habitat type were combined in each locality to determine in which habitats species occurred, and then tests were done on the number of localities where species presence was recorded for a particular habitat type. Statistical tests done were the chi squared test of independence and effect sizes, using the programs described previously. Again, those species that displayed significant values for any of the tests were identified and extracted from the overall results for further study.

When communities are analysed across varying environmental conditions, changes in species composition of each community are often apparent and predictable; and individual samples can readily be ordered along one or more imaginary axes. Statistical ordination methods have been developed to highlight *„gradients of species compositional change.'* The resultant graphical representations of these changes in composition, along with independent ecological knowledge of species concerned, allow for an *„ecological interpretation of the gradients,'* and thus each axis can be informally interpreted as a gradient. Also, there is the principal of *„proximity implies similarity*" which suggests that the closer two points are to one another on the ordination, the

[1] SPSS Inc. (2009) SPSS® 17.0 for Windows, Release 17.0.0, Copyright© by SPSS Inc., Chicago, Illinois. www.spss.com.

[2] StatSoft, Inc. (2009). STATISTICA (data analysis software system), version 9.0. www.statsoft.com

more similar they are. An indirect gradient analysis, such as detrended correspondence analysis (DCA), is a type of ordination technique that can be used on multivariate data (Lepš & Šmilauer, 2003).

An encounter matrix (based on the number of times a species was encountered in any survey type) was compiled; sampling sites of similar habitat types were grouped and analysed using the statistical modelling package CANOCO.

2.2.8 Investigation of habitat types available at each locality

Finally, the habitat types present in each of the three localities were analysed. Vegetation data was obtained from the Okavango Delta Information System (ODIS) at HOORC, in the form of a vegetation map that identified a total of 37 different vegetation assemblages covering the entire Delta, listing the dominant plant species present in each assemblage. Using this and the information provided by Ellery & Ellery (1997), Myers *et al*. (2004) and Mendelsohn *et al.* (2010), these vegetation assemblages were simplified into the physiographic regions relevant to the present study, based on those plant species common to each region. It should be noted that there is a possibility of marginal error in this reclassification, but on the whole it is sufficiently accurate for the purpose of the present study.

Using ArcGIS 9.2 software, this vegetation data was overlaid onto each study locality to provide a physiographic map (based on classification in Chapter 1) for each locality.

2.3 RESULTS

2.3.1 Degree of locality isolation

Amphibian collection occurred in three study localities; each of which represented a different degree of isolation. Figure 10 displays localities superimposed on high and low flood data to indicate their degree of isolation. Although each locality spans over two QDGCs and seems to cover a large area of the Delta, in each case the actual sampling sites are positioned on either side, and in close proximity of, where the relevant QDGCs join. Basically towards the centre of each demarcated area, and not spread throughout the entire locality area as implied by the map.

Figure 10 shows that Xigera falls within the permanent swamp physiographic region of the Delta since it is situated within the low flood zone, which implies that when flood waters are at their lowest Xigera is still surrounded by water. From this, it was concluded that Xigera is permanently isolated from any surrounding main land. The sampling sites for Mombo are located on the north-westerly tip of Chief‟s island, visible on the map as the largest island in the centre of the Delta. In this case, the locality is only partially isolated as there is a visible land bridge connecting Chief‟s Island to the main land to the south east. Finally, Figure 10 shows that the Kwedi sampling sites occur on the fringe of the Delta, along its north-easterly border. Therefore, it represents zero degree of isolation as it is permanently connected to, and accessible from, the main land.

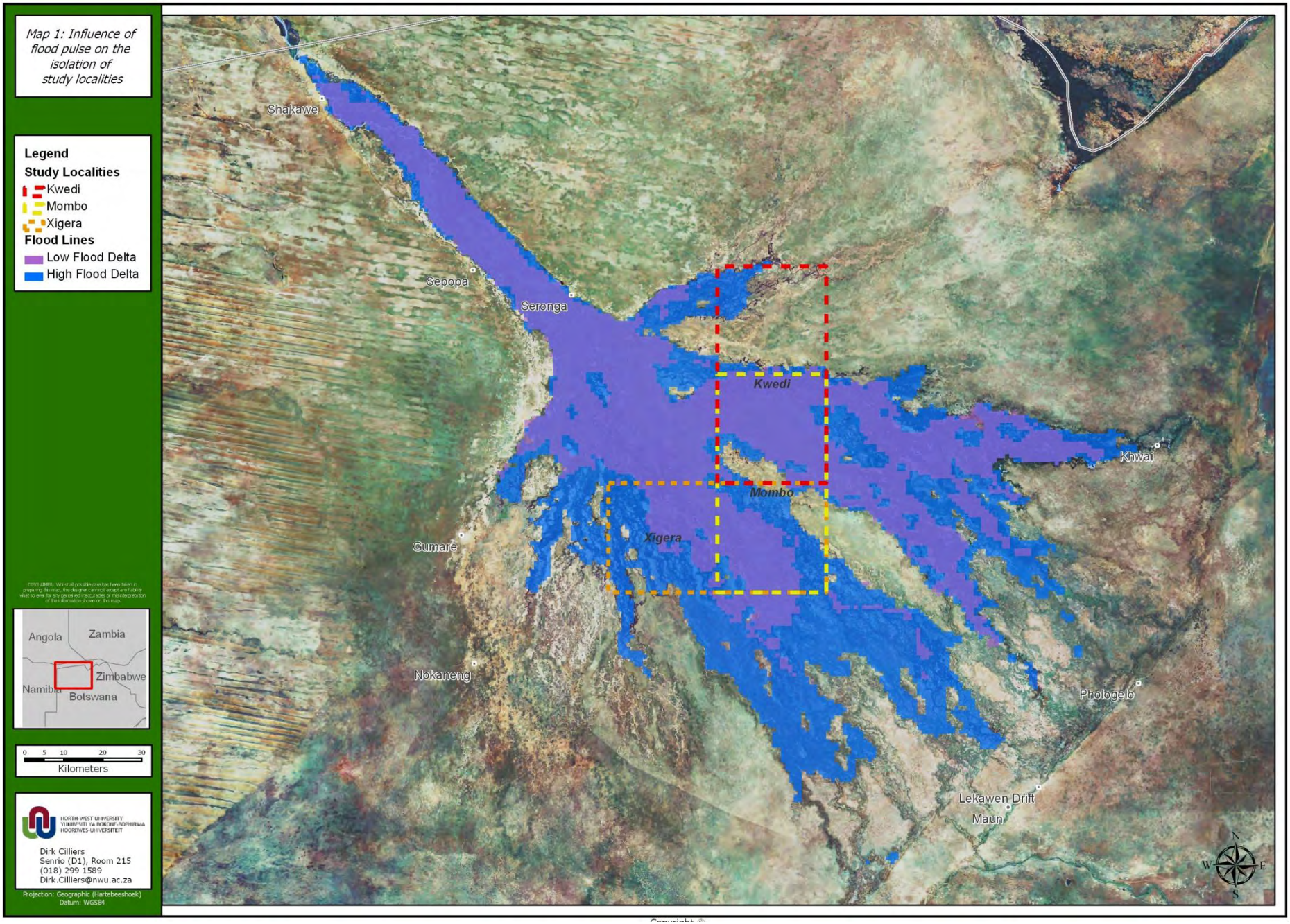

Figure 10: Degree of isolation of each locality where amphibian collection occurred, showing the extent of peak and low flood.

2.3.2 Species richness for the Okavango Delta: actual versus expected

The desktop study resulted in an expected amphibian species list for the Okavango Delta of 33 (Annex. I), but the actual species list based on records from the present study totals at 29. Table 2 below provides this species inventory for the Okavango Delta, together with photographs of specimens collected during this study, where available, since no photos were obtained from external sources in order to ensure accurate morphological representation of species found in the Delta, not elsewhere in their range. Also provided are the number of voucher specimens collected during this study.

Table 2: Amphibian species inventory for the Okavango Delta.

Species	**Number of Voucher Specimens**			**Specimen Photograph**
	Xigera	**Mombo**	**Kwedi**	
Breviceps adspersus adspersus	0	0	0	No voucher specimen collected; identification by vocalisation only.
Amietophrynus gutturalis	2	0	2	
Amietophrynus lemairii	0	2	0	

Amietophrynus maculatus	2	0	0	
Amietophrynus poweri (*garmani* in Auerbach, 1987)	1	2	0	
Chiromantis xerampelina	0	2	2	

Hemisus guineensis	2	0	1	
Hemisus marmoratus	0	2	2	
Hyperolius benguellensis	0	0	2	

Hyperolius nasutus	3	2	0	
Hyperolius parallelus	2	1	2	
Kassina senegalensis	0	2	0	
Phrynobatrachus mababiensis	1	2	1	No voucher photograph available, specimen euthanized at sampling site.
Phrynobatrachus natalensis	1	0	0	No voucher photograph available, specimen euthanized at sampling site.
Phrynobatrachus parvulus	0	1	1	No voucher photograph available, specimen euthanized at sampling site.

Phrynomantis bifasciatus	0	2	0	
Ptychadena anchietae	0	2	2	No voucher specimen photograph available.
Ptychadena guibei	3	0	0	
Ptychadena mascareniensis	4	3	2	
Ptychadena mossambica	0	0	0	Specimen escaped before photograph could be taken.

Ptychadena oxyrhynchus	0	0	2	
Ptychadena subpunctata	3	2	2	
Ptychadena taenioscelis	0	0	3	

Pyxicephalus adspersus	0	1	0	
Pyxicephalus edulis	0	1	0	
Tomopterna cryptotis/tandyi	6	2	0	
Tomopterna tandyi	Combined with above species specimens as they are morphologically indistinguishable.			Same as above; *T. cryptotis* & *T. tandyi* are morphologically indistinguishable in the field. Confirmation of both species in study region was provided by vocalisation.
Xenopus petersii	1	2	1	No voucher specimen photograph available.

Xenopus muelleri	2	0	2	

A total of 49 hours were spent sampling during the course of this study. The increase in species richness over this time is displayed in Figure 11.

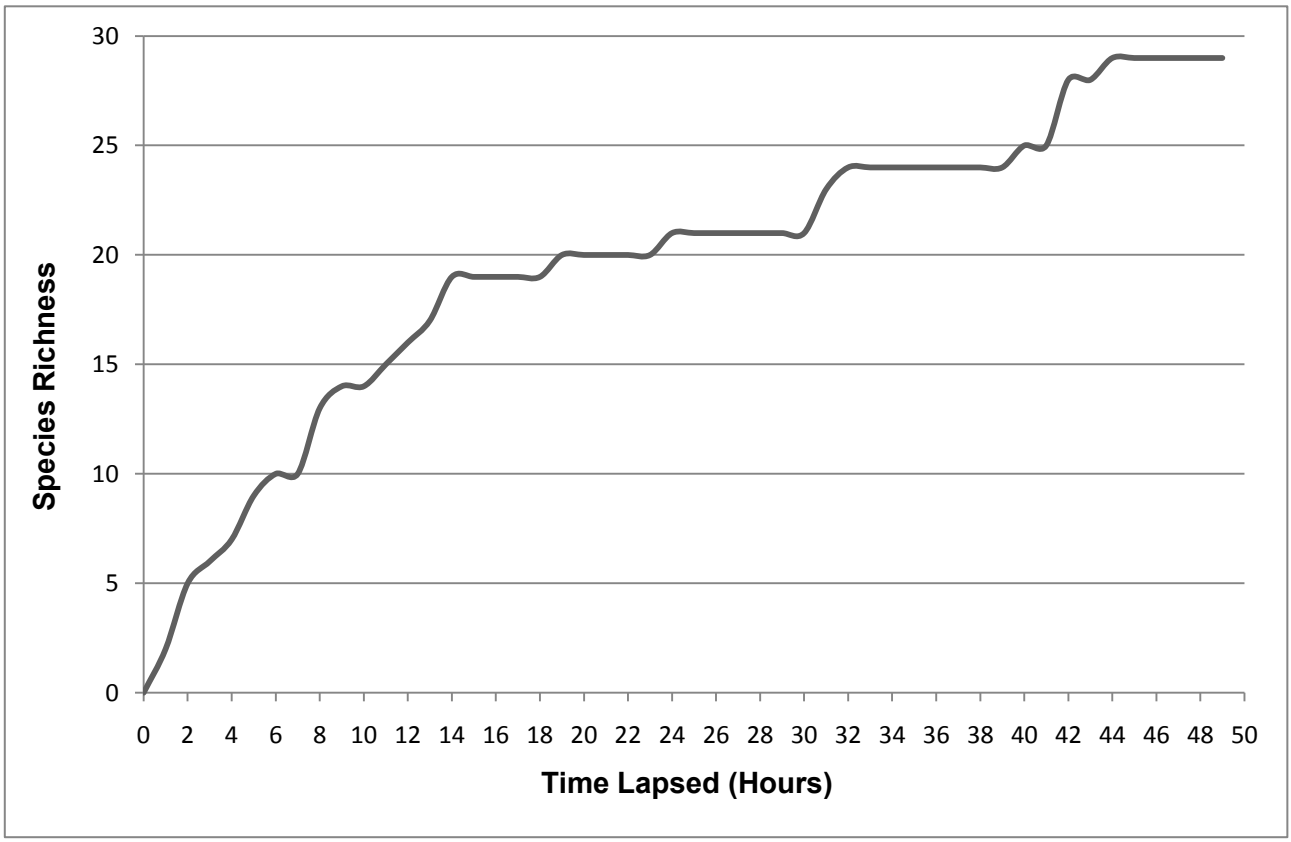

Figure 11: Species richness over sampled time in the Okavango Delta.

The sampling saturation curve displays an initial sharp rise in species richness over the first 14 hours of sampling, with 19 species observed during this time. Species richness then appeared to reach a plateau, barring two periods of additional increase as discussed below. At 44 hours, species richness reached its maximum value of 29, and the additional 5 hours of sampling after

this time did not yield any additional species, indicating that sufficient time was spent to compile representative species inventories.

There were two periods of rapid increase in species richness subsequent to the plateau being reached. The first occurred between 30 and 32 hours, where there was an increase in species number from 21 to 24. This was attributable to the first intense VES and AES sampling sessions in Kwedi during the rainy season; previous visits to this locality were mostly dedicated to area assessment, sampling site selection in summer, and tadpole collection in winter. The second occurred between 40 and 44 hours, where species richness increased from 25 to 29. In this case, the increase was the result of the first sampling opportunity in Mombo during the rainy season. A concerted effort was made during the 2009/2010 rainy season to search for any species that were, at the time, still missing from the expected species list, and thus sampling efforts were concentrated in the preferred habitats of those species.

2.3.3 Species richness per locality

A species inventory was compiled for each locality in order to compare their differences and similarities in species richness. The results are summarised in the diagram below.

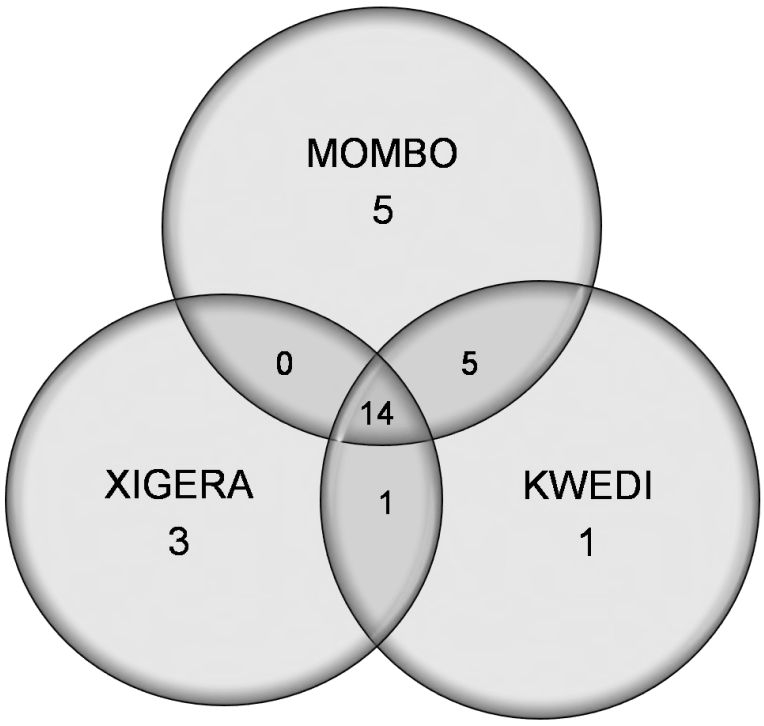

Figure 12: Number of species observed in each locality, also showing the number of unique and commonly shared species among localities.

Xigera had the lowest species richness with 18 species recorded, followed by Kwedi with 21 and finally Mombo with the highest richness of 24. The number of species shared by all three localities was 14. Mombo and Kwedi shared 5 common species, while only 1 species was common to both Xigera and Kwedi, and none were shared by Xigera and Mombo. Finally, Mombo exhibited the highest endemism with 5 species unique to that locality, followed by Xigera with 3 unique species and finally Kwedi with only 1.

Species unique to each locality as follows:

Mombo: *Amietophrynus lemairii, Phrynomantis bifasciatus, Pyxicephalus adspersus, Pyxicephalus edulis* and *Tomopterna tandyi.*

Xigera: *Amietophrynus maculatus, Breviceps adspersus adspersus* and *Phrynobatrachus natalensis.*

Kwedi: *Ptychadena oxyrhynchus.*

Absent species from each locality were compared against previous locality records, and then Google Earth images (Figure 13) and Figure 10 to investigate whether their absence was likely due to a physical barrier to dispersal.

Figure 13: Google Earth satellite images that display any possible, physical barriers to dispersal between present study localities, and previously recorded localities from Auerbach (1987) to the east of the Kwedi QDGC.

The images show that there are physical barriers in the form of large stretches of permanent water and swamp between Kwedi and Mombo, and similarly between Mombo and Xigera. Amphibians are mostly a terrestrial faunal group (Poynton, 1964); therefore, such water barriers may limit the dispersal of species from one terrestrial surface to the next. It thus follows that although a particular species is present in one locality QDGC, observed either in the present

study or documented in Auerbach's records, it does not imply that it has necessarily dispersed to the adjacent QDGC. The possibility of species absence from one locality as a result of barriers must thus be assumed.

The only case where species absence could not be explained by any visible barrier was in Kwedi. 5 Out of the 7 absent species from this locality, which had previously been recorded in an adjacent QDGC in Auerbach (1987), had been observed in 1923AA. This grid cell borders the Kwedi QDGC 1922BB to the east and, as can be seen from Figure 13 and Figure 10, the main land continues without interruption from one cell to the next; in other words, there are no apparent physical barriers separating these two QDGCs. It was therefore assumed that these 5 absent species should occur in the Kwedi locality. This assumption is further supported by the fact that sampling in Kwedi was restricted to official field trips, and there was no opportunity for additional ad hoc observations.

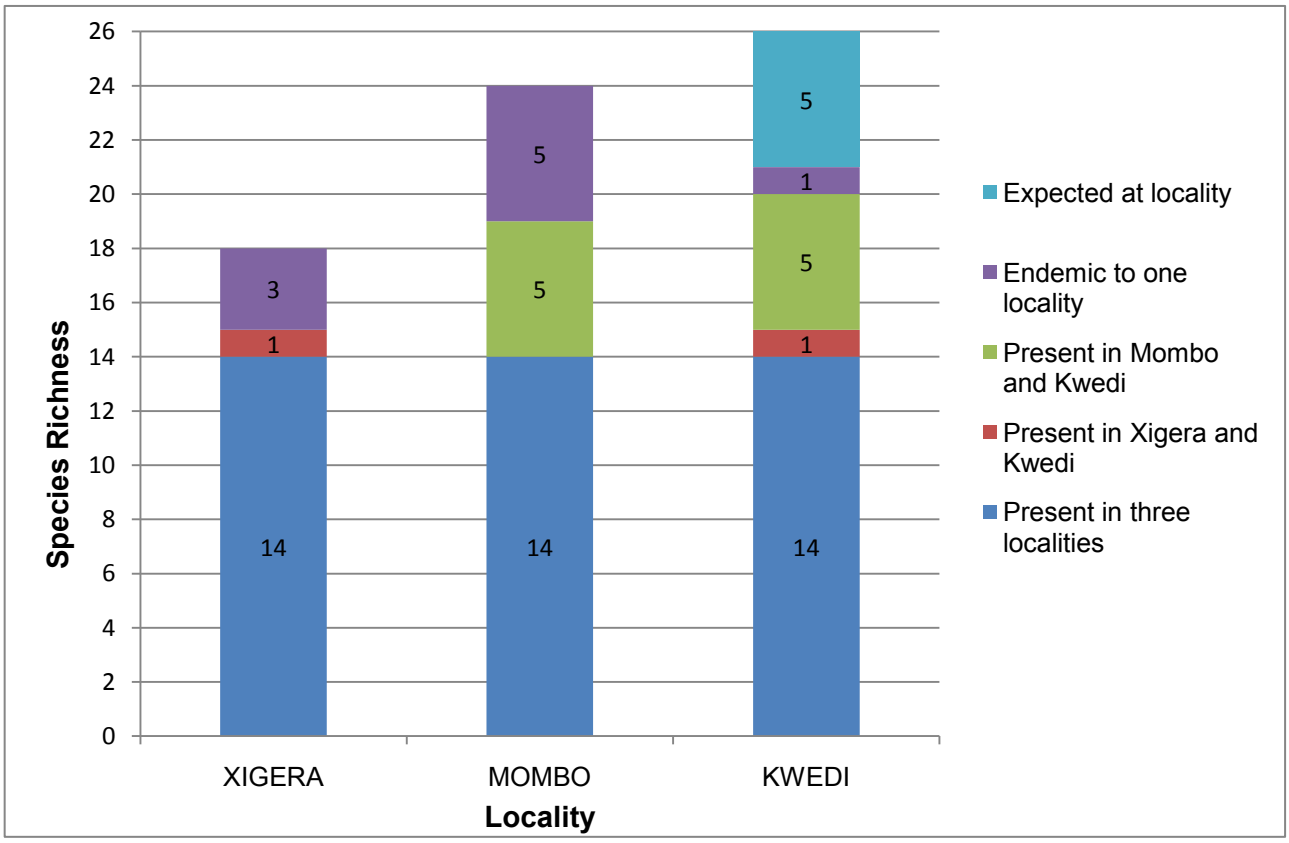

Figure 14: Species richness per locality, showing common, unique and expected species.

An additional 5 species are expected to occur at Kwedi which, when added, make it the most specious site (Figure 14). These species are: *A. maculatus; A. lemairii; P. natalensis; P. bifasciatus* and *P. edulis.* The species richness of Kwedi is thus increased from 21 to 26, giving it the highest richness. Although this analysis is important for the discussion, it will not be included in further analysis of community composition in the present study.

2.3.4 Species diversity and evenness among localities

The remaining descriptive measures of community composition were analysed, namely evenness and diversity. Pielou"s evenness index for all three localities fell within the range of 0.90 and 0.95. Apart from indicating that each location"s community is more or less even (since the maximum value can be 1.0), not much more could be gained from this analysis since all three communities are comparable in equitability.

The results of the Shannon-Wiener and Margalef calculations for species diversity are displayed in Figure 15.

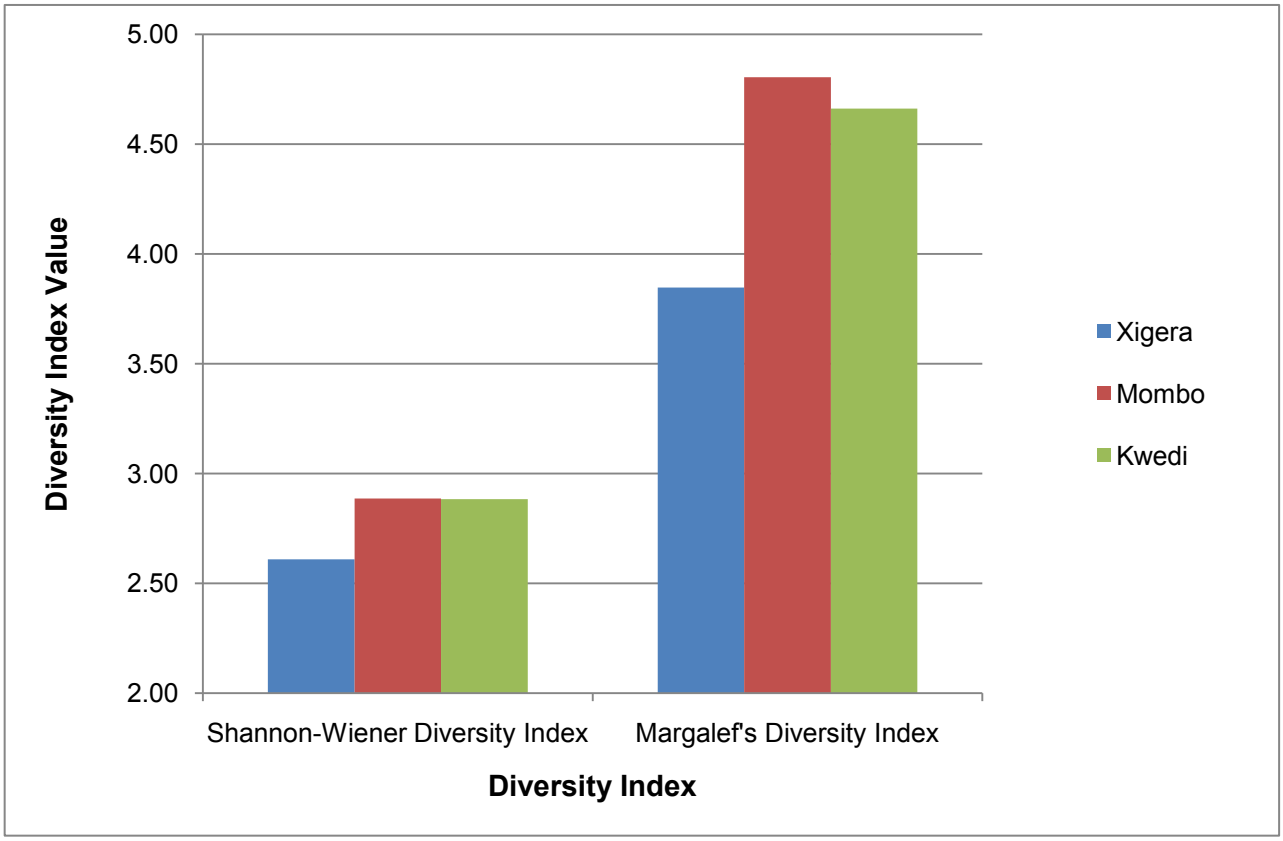

Figure 15: Shannon-Wiener and Margalef diversity index values for each locality.

According to both indexes, Xigera has the lowest species diversity while Mombo has the highest, only slightly higher than that of Kwedi. Since the variation displayed by Margalef"s index is dependant on species richness, and less so on the number of individuals (or encounters in this case), it provides a clearer indication of species diversity at each locality. Using Margalef"s values, there is a sizeable difference between Xigera (3.85) and Mombo (4.80), while the diversity between Mombo and Kwedi are remarkably similar, 4.80 and 4.66 respectively.

A summary of the different measures used to describe the amphibian community at each locality is given in Table 3.

Table 3: Numerical summary of diversity measures used to describe community compositions at each locality.

	Xigera	Mombo	Kwedi
Species Richness	18	24	21
Shannon-Wiener Diversity Index	2.61	2.89	2.88
Pielou's Evenness Index	0.90	0.91	0.95
Margalef's Species Richness Index	3.85	4.80	4.66

2.3.5 Community composition

The results for differences in species presence between localities are summarised in Table 4.

Table 4: Observed species that displayed statistically significant differences in their presence between localities (highlighted in red) for any of the tests performed, and their percentage occurrence in each locality.

Species	Effect Size Value *(ø)*	Chi Squared Test *(p)*	Kruskal-Wallis Test *(p)*	Encounter Occurrence (%)		
				Xigera	Mombo	Kwedi
Amietophrynus lemairii	**0.555**	**0.099**	0.116	0.0	100.0	0.0
Amietophrynus maculatus	**0.853**	**0.004**	**0.007**	100.0	0.0	0.0
Amietophrynus poweri	**0.681**	**0.031**	**0.014**	25.0	62.5	12.5
Breviceps adspersus adspersus	**0.555**	**0.099**	0.116	100.0	0.0	0.0
Hemisus marmoratus	**0.564**	**0.092**	0.161	0.0	25.0	75.0
Phrynobatrachus mababiensis	**0.577**	**0.082**	**0.053**	16.7	66.7	16.7
Ptychadena mossambica	**0.564**	**0.092**	**0.077**	0.0	75.0	25.0
Ptychadena oxyrhynchus	**0.555**	**0.099**	0.117	0.0	0.0	100.0

The above table indicates that only 8 out of the 29 species observed demonstrate any statistically significant differences in their occurrence between localities; and 4 of these are also species previously recorded as unique to one locality. For the present study, effect size was the

best indicator of such differences (*ø≥0.5*); however, the chi squared tests provided similar results and correspond in their values with *p<0.1*. If the effect sizes are looked at more closely, it is evident that they are only slightly greater than 0.5, barring *A. maculatus* and *A. poweri*, indicating that the majority of these species‟ occurrence in a specific locality is only fractionally above the significance level. The results for the Kruskal-Wallis tests are also shown: although not all species exhibit a statistically significant *p* value, those with insignificant values are only slightly above 0.1, the highest being 0.161 for *Hemisus marmoratus*.

The outcome of these results is explained by the percentage encounter occurrence shown in this table. Significant values occurred when at least 62% of total encounters of a particular species were restricted to one locality. In fact, 6 out of the 8 species show that 75% or more encounters were limited to a single locality.

Based on these results, it is not surprising then that the Kruskal-Wallis test for all species combined (multiple comparisons of *p* values) resulted in a *p* value of 0.2094, and thus it can be concluded that there is no statistically significant difference between the species diversity of the three localities. Despite a clear difference in diversity based on the species richness analyses and species diversity indices, this difference has no statistically significant foundation.

It should be noted, however, that this insignificant result cannot be regarded as conclusive evidence. It is known that the value of *p* becomes smaller (indicating significance) as the amount of data collected increases. Since a collection is merely a sub-sample of the total population, and if data is collected through convenience sampling rather than through true random sampling, the values of *p* can be erroneous and cause inaccurate deductions and inferences (Ellis & Steyn, 2003). Although effect sizes are independent of sample size, caution should still be exercised since exceptionally small data sets can lead to incorrect conclusions.

2.3.6 Relation between species occurrence and habitat type

The results for differences in species presence between habitat types are summarised in Table 5.

Table 5: Observed species that displayed statistically significant differences in their presence between habitats (highlighted in red) for the tests performed, and their percentage occurrence in each habitat type.

Species	Effect Size Value (ø)	Chi Square Test (p)	Encounter Occurrence (%)				
			Permanent Swamp	Seasonal Swamp	Floodplain	Rainwater Pan	Dryland
Amietophrynus gutturalis	**0.564**	0.311	27.3	27.3	18.2	9.1	18.2
Amietophrynus lemairii	0.480	0.484	0.0	0.0	50.0	50.0	0.0
Breviceps adspersus adspersus	0.480	0.484	0.0	50.0	50.0	0.0	0.0
Chiromantis xerampelina	0.480	0.484	0.0	0.0	0.0	50.0	50.0
Hemisus marmoratus	**0.564**	0.311	0.0	25.0	25.0	50.0	0.0
Hyperolius benguellensis	**0.784**	**0.056**	100.0	0.0	0.0	0.0	0.0
Hyperolius nasutus	**0.793**	**0.051**	33.3	50.0	16.7	0.0	0.0
Hyperolius parallelus	**0.802**	**0.047**	37.5	37.5	12.5	0.0	12.5
Kassina senegalensis	**0.739**	**0.085**	0.0	50.0	0.0	50.0	0.0
Phrynobatrachus mababiensis	**0.667**	0.155	16.7	50.0	16.7	16.7	0.0
Phrynobatrachus natalensis	**0.535**	0.369	100.0	0.0	0.0	0.0	0.0
Phrynobatrachus parvulus	**0.784**	**0.056**	0.0	100.0	0.0	0.0	0.0
Phrynomantis bifasciatus	**0.535**	0.369	0.0	0.0	0.0	100.0	0.0
Ptychadena guibei	**0.739**	**0.085**	50.0	50.0	0.0	0.0	0.0
Ptychadena mascareniensis	**0.894**	**0.017**	30.0	30.0	30.0	10.0	0.0
Ptychadena mossambica	**0.564**	0.311	0.0	50.0	25.0	25.0	0.0

Ptychadena oxyrhynchus	0.480	0.484	0.0	50.0	0.0	50.0	0.0
Ptychadena subpunctata	**0.793**	**0.051**	33.3	33.3	22.2	11.1	0.0
Ptychadena taenioscelis	**0.894**	**0.017**	0.0	40.0	60.0	0.0	0.0
Pyxicephalus adspersus	**0.535**	0.369	0.0	0.0	0.0	100.0	0.0
Pyxicephalus edulis	**0.535**	0.369	0.0	0.0	0.0	100.0	0.0
Tomopterna spp. (cryptotis/tandyi)	**0.564**	0.311	0.0	0.0	25.0	50.0	25.0
Tomopterna tandyi	**0.535**	0.369	0.0	0.0	0.0	100.0	0.0
Xenopus muelleri	**0.739**	**0.085**	0.0	50.0	0.0	50.0	0.0
Xenopus petersii	**0.739**	**0.085**	50.0	0.0	50.0	0.0	0.0

Table 5 contains 25 species extracted from the overall results. It indicates that 21 out of the 29 observed species displayed practically significant results when their occurrence was tested against habitat type; and a further 4 out of the remaining 8 species displayed medium large to large effect sizes, with ø = 0.480, only marginally below the significance level of 0.5. As in the previous analysis, the effect size value is explained by the percentage occurrence of a species in the respective habitat types. In all but 4 species, where at least 54% of encounters occurred in two habitat types, 50% or more of encounters occurred in one habitat type. Therefore, it can be safely assumed that in totality, species diversity appears to be correlated to habitat type, and that this correlation is practically significant.

The *p* values for the associated chi squared tests were not as consistent as those of Table 4, with 14 out of the 25 species having a value *p>0.1*, indicating an insignificant result. However, as previously explained, *p* is known to decrease with increasing sample size. In the present analysis the same data set, that had previously been split into three categories for the statistical evaluation of locality, was now split into five categories to represent each habitat type. The data in this analysis is therefore progressively sparser, and this may explain the inconsistent *p* values relevant to *ø.*

The CANOCO modelling program was used to graphically display the relationship between species occurrence and sampling sites. The outcome is displayed in Figure 16.

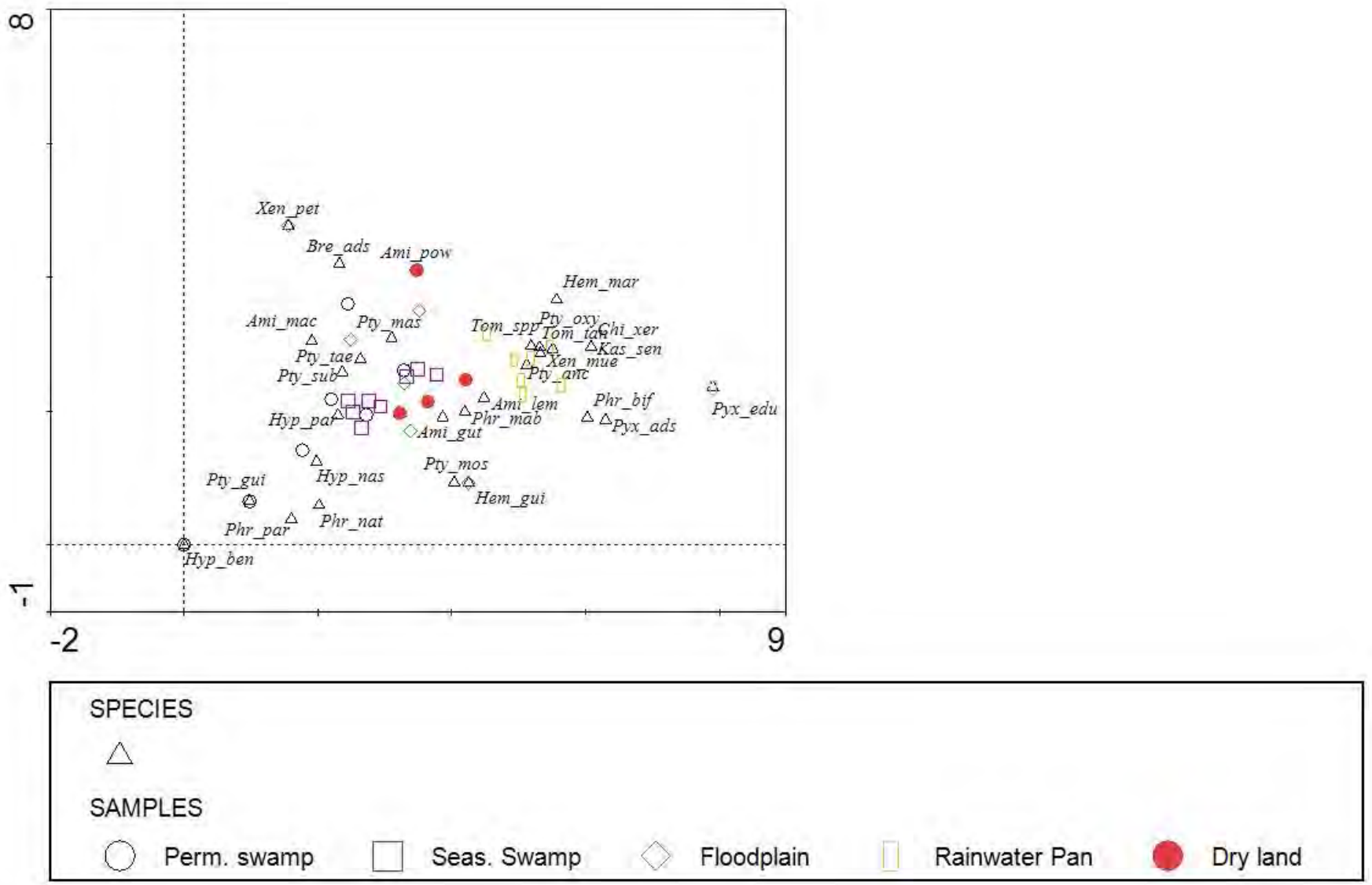

Figure 16: Ordination diagram from DCA of species occurrence and habitat type.

Analysis of the diagram allows the horizontal axis to be informally interpreted as a gradient of water availability: water availability decreases from left to right. This is based, firstly, on the habitat samples where permanent swamp samples are grouped to the left and rainwater pan samples on the right. There is an overlap of seasonal swamp and floodplain samples between these two extremes. The surprising cluster of dry land samples in the middle of the ordination is the result of drift fence-pitfall traps, which was the technique used to sample these habitats. These traps were sometimes opportunistically placed close to water bodies in an attempt to collect rare species migrating towards or away from the water, and thus occasionally trapped species more readily associated with water habitats. Therefore, these samples appear towards the middle of the water availability gradient, rather than clustered on the far right as was expected.

Secondly, the interpretation is due to independent knowledge of species‟ ecological requirements. Amphibians are dependent on water to varying degrees, and this information can be readily obtained from field guides such as du Preez & Carruthers (2009). Based on this knowledge, the water availability gradient became more pronounced. Water dependent species such as *H. benguellensis, P. natalensis, X. petersii* and *P. guibei* occur on the left, and species only dependent on water for breeding purposes on the right, such as *P. edulis, P. adspersus, C. xerampelina* and *P. bifasciatus.* There were some outliers whose position on the ordination was surprising, but these could be readily explained by insufficient sampling of the species e.g. *P. parvulus* and *B. a. adspersus*.

An additional observation of the above diagram results from the proximity of certain species to habitat samples. The clustering of habitat types also resulted in clustering of species, since those species dependent on water were close to those sampling sites with permanent water on the ordination, and visa versa. Proximity indicates that those species occurring closer to a habitat sample are more often present and/or more abundant in that habitat compared with species further away. Take note that several species, such as *H. benguellensis, P. guibei* and *P. edulis,* were actually superimposed on the habitat sample, indicating a high correlation between the species and habitat type.

No interpretation could be made from the y axis. This is understandable since water availability, and the resultant vegetation that accompanies that environmental variable was the basis for sampling site selection and no additional environmental variables were considered.

2.3.7 Physiographic regions and the availability of amphibian habitat types

The results of vegetation data overlaid onto each locality are displayed in Maps 2a, b and c. Each map indicates those physiographic regions that are present in each study locality, and this in turn determines what types of amphibian habitats are available in that locality.

The sampling sites of Xigera (Figure 17A) seem to be dominated by three physiographic regions: permanent swamp, seasonal swamp and occasional floodplain. There appear to be patches of mopane woodland, but experience in the locality confirms dryland woodland on islands with intermittent mopane exists rather than stands dominated by mopane. Percentage cover over the entire locality given by the coordinates as stated in Chapter 1, and demarcated on the map by the solid black border, gives the following values: 60.21% seasonal floodplain, 14.95% permanent swamp and 10.42% occasional floodplain. These values would, however, increase if only the area immediately surrounding the sampling sites was taken into account. Nonetheless, the map points towards a predominantly water covered landscape. It follows then, that amphibian habitat in Xigera is mostly associated with water and lacks dryland and rainwater pan habitats. This is confirmed by Table 1, which indicates sampled area contains 0% rainwater pan habitat and only 11.1% dryland/island habitat.

The sampling sites in Mombo (Figure 17B) are concentrated on the north-westerly peninsula of Chief"s island. The size of the island and lack of flooding beyond its perimeter has allowed for dryland woodland and especially acacia woodland to become established. According to the map, there is mostly an even spread of sampling sites between all five physiographic regions, and Table 1 confirms this: 50% of sampling sites fall in rainwater pan and dryland habitats while the remaining 50% occurs in swamp and floodplain habitats. It can thus be concluded that all amphibian habitats are well represented in this locality.

The sampling sites of the Kwedi locality (Figure 17C) are situated on the north-easterly fringe of the Okavango Delta. This positioning is confirmed by the map and the calculated 56.12% of demarcated area covered by dryland/island woodland of some description to the north, and the remaining 43.88% covered by the water dominated regions of swamp and floodplain to the south. Sampling sites fall predominantly within the margin zone between terrestrial and aquatic habitat; and as with Mombo, the presence of all five physiographic regions in this locality made all five amphibian habitats available for sampling. Since this locality represents a possible source area from which amphibians may have dispersed, it was crucial to sample in all available amphibian habitats. This was accomplished as indicated by the values in Table 1, which show 44.4% of sampling sites occurred in terrestrial habitats while the remaining 55.6% occurred in aquatic habitats.

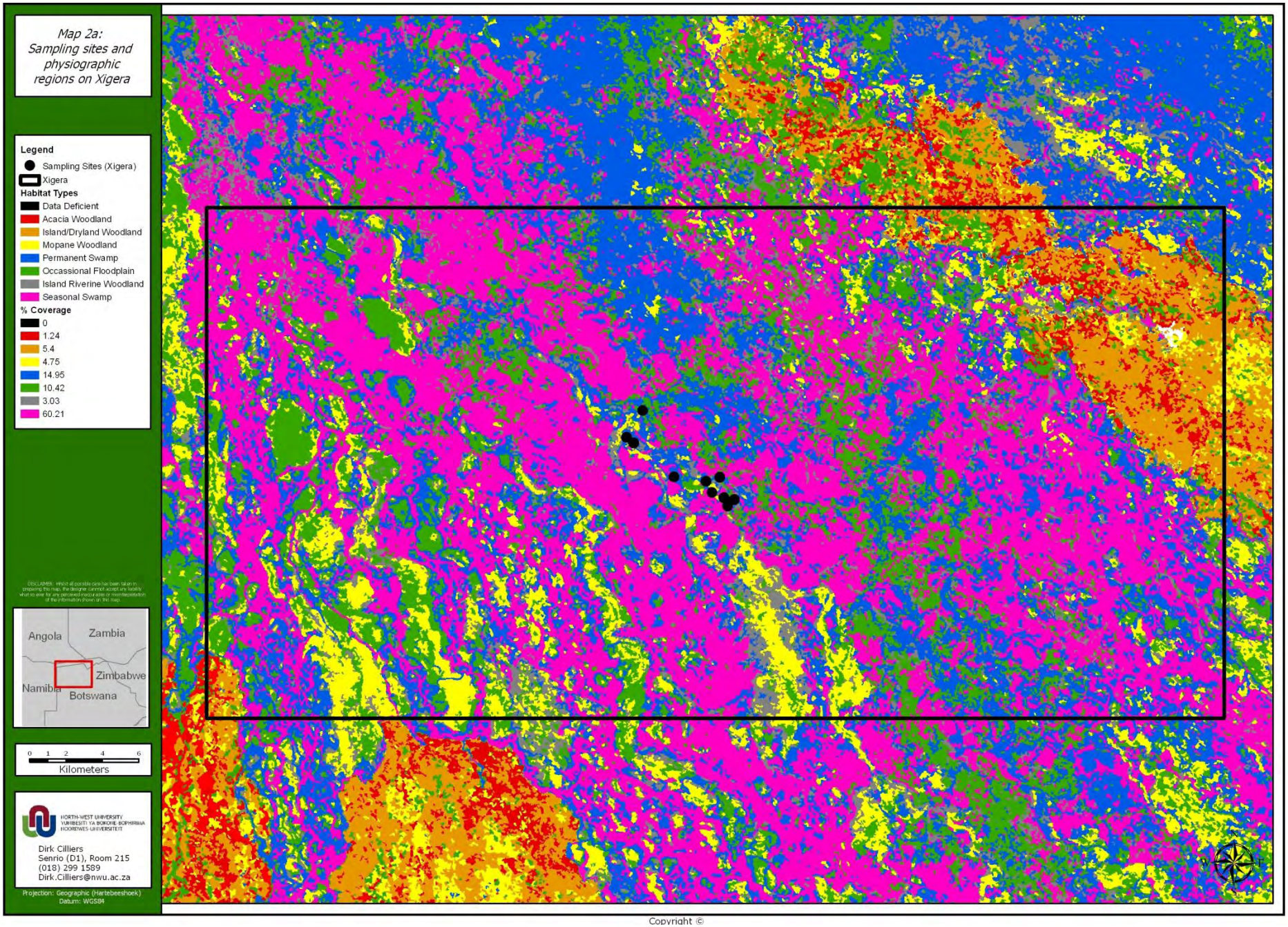

Figure 17A: Sampling sites and physiographic regions in Xigera.

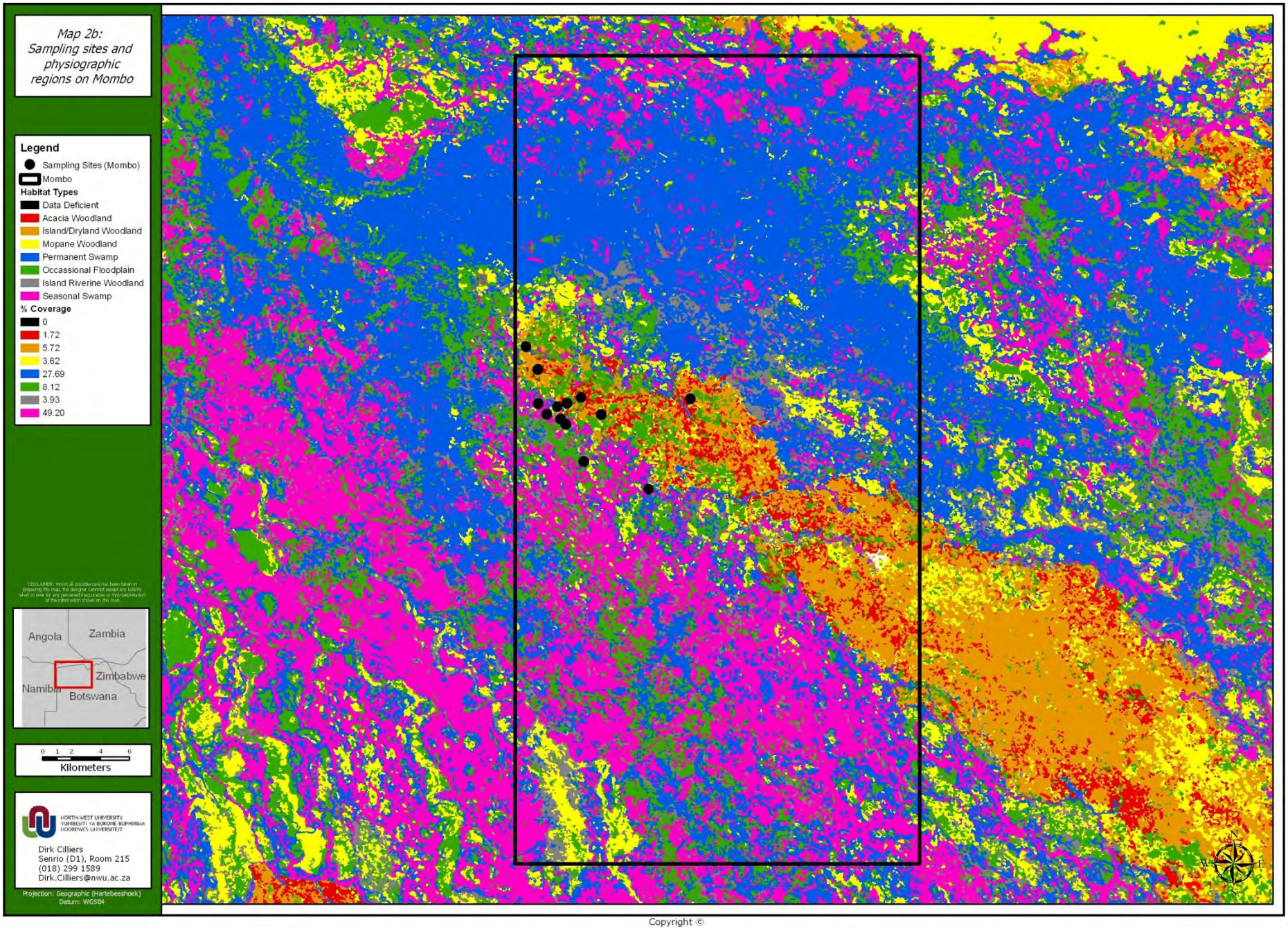

Figure 17B: Sampling sites and physiographic regions in Mombo.

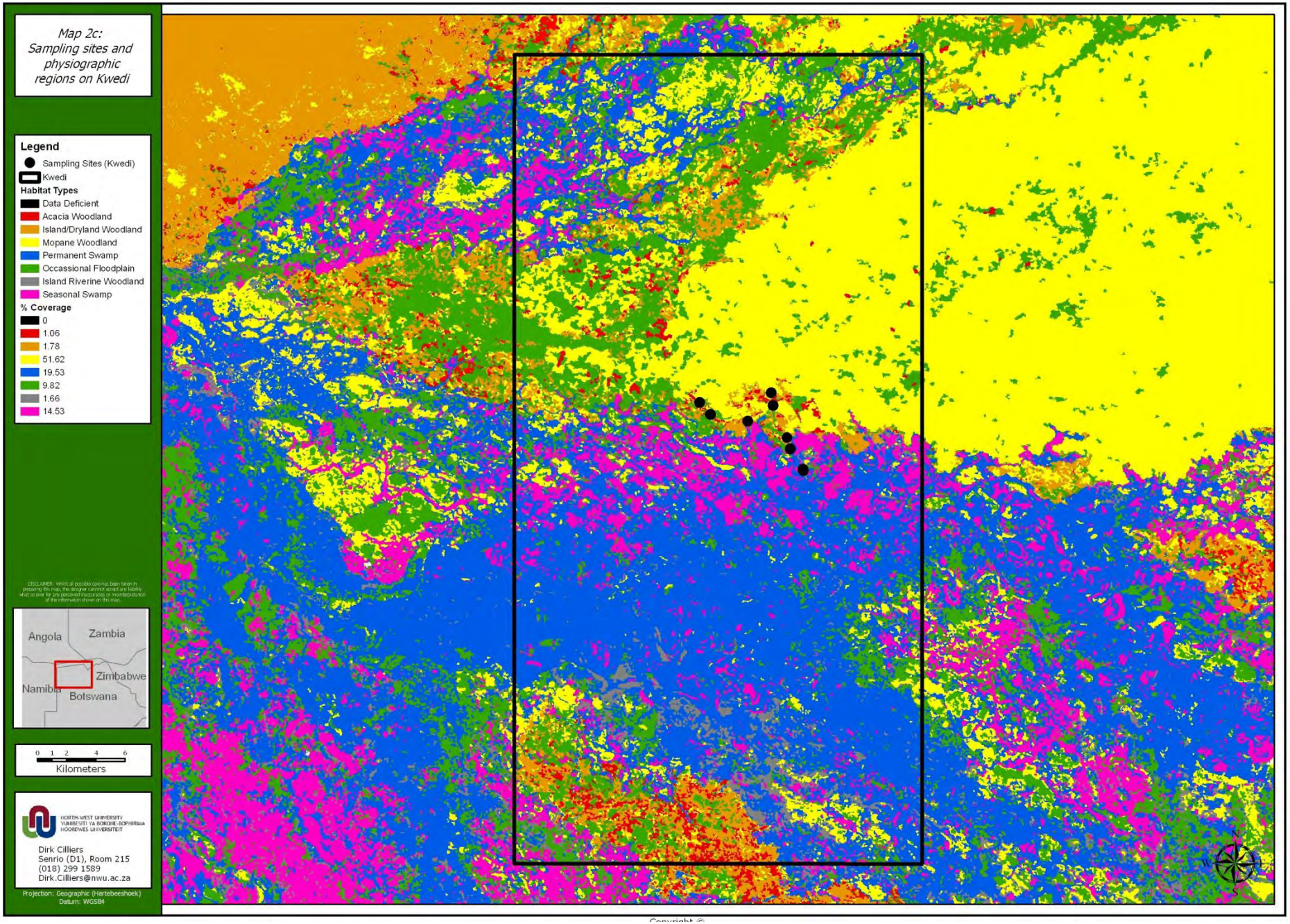

Figure 17C: Sampling sites and physiographic regions in Kwedi.

2.4 DISCUSSION

2.4.1 Amphibian species richness in the Okavango Delta

One of the objectives of this section of the study was to investigate the species richness of amphibians in the Okavango Delta, and compare the results to that of previous sources and the expected species list compiled. The current study recorded 29 species present in the Okavango Delta, collected at three localities, and it is concluded that this figure is an accurate representation of species richness in the region for several reasons. Firstly, Auerbach (1987) only recorded 28 species, and since his is the most detailed work on amphibians in Botswana, and provides distribution maps" clearly indicating previous collection localities within the Okavango Delta"s specified coordinates, it is an extremely reliable reference to use for comparison. Secondly, collection of specimens did reach a plateau, which according to Begon *et al.* (2006) indicates that the subsample collected by the study sufficiently represents the community from which the samples were obtained. The two periods of species richness increase subsequent to reaching the plateau was practically explained, and thus does not diminish the sampling success suggested by the plateau. Finally, when the four absent species (outstanding from the expected species list as well as Auerbach"s records) are investigated more closely using the work by Poynton & Broadley (1985a; 1985b; 1988; and maps in 1991), it was found that these species have not been regularly recorded from the study region:

- *Xenopus laevis:* this species is absent from most of Botswana; and specimens have only been recorded from the extreme south, in 2525BA and 2425DB (Poynton & Broadley, 1985a).
- *Poyntonophrynus kavangensis:* this species has been recorded from only one locality in Botswana, on the eastern side of the Delta in 1923BA, which is at least two QDGCs away from the nearest study locality (Kwedi/Mombo). It is otherwise considerably more common in north-western Zimbabwe, and apparently in the Caprivi Strip and southern Angola, although no point localities for these latter two countries are specified in the source (Poynton & Broadley, 1988).
- *Hildebrandtia ornata:* Although this species distribution is stated as “eastern Transvaal north to Kenya,” it is said to exclude Botswana and no point localities are recorded for this country (Poynton & Broadley, 1985b).
- *Tomopterna krugerensis:* Although this species has been collected in seven locations throughout Botswana, none of the point localities occur within the specified Delta coordinates, and the closest locality recordings to the present study area are 2023AC and 2022BD (Poynton & Broadley, 1985b).

Therefore, although the above investigation is not conclusive evidence that these four species are wholly absent from the Okavango Delta and the present study region, it does indicate that they are extremely rare, if present at all, and thus their absence should not be attributed to insufficient sampling.

2.4.2 Isolation as a driver for amphibian community composition

Species richness is a highly informative descriptor of community composition. Species inventories created for each locality indicated that species richness increased from Xigera to Kwedi to Mombo. However, when the Kwedi inventory was supplemented with those five species that are expected for that locality, based on the lack of visible barriers between previous and current sampling sites, it exhibited the highest species richness of 26 species, followed closely by Mombo with 24, and lastly Xigera with only 18. Furthermore, these five expected species include *Pyxicephalus edulis* and *Phrynomantis bifasciatus* that have characteristically short breeding seasons, and therefore could have been easily overlooked if sampling occurred outside their breeding cycle. It is, therefore, reasonable to assume this decreasing trend in species richness, from least isolated to most isolated localities, is indeed a true reflection of the natural situation; and it will be the basis for the discussion which follows on species richness.

This trend corresponds to established knowledge of island biogeography theory, where the mainland acts as the source pool from which species disperse to other islands; and as the degree of physical isolation of islands and its distance to mainland increases, so its species richness will decrease: largely the result of limitations in species‟ dispersal ability (Begon *et al.,* 2006). Limits to a species‟ distribution are the result of *barriers*. Regardless of its form, for example topographic or climatic, it represents a zone that inhibits a particular species‟ survival; and thus that species never, or seldom, is able to cross the barrier to other favourable yet uncolonised areas. In actual fact, such barriers are better described by the species‟ own physiology, since it is this aspect of the species that ultimately prevents dispersal: “In its distribution a species is therefore the prisoner of its own evolutionary history,” (Cox & Moore, 1995). The classification of amphibians as a predominantly terrestrial group, based on their geographical distribution (Poynton, 1964), implies that in the Okavango Delta, such barriers to dispersal are in the form of large expanses of water, often relatively fast moving, with only small islands or emergent vegetation as stepping stones between favourable areas of inhabitation.

In the present study, Kwedi represents a possible source area; or it can be considered highly similar to the actual source area anywhere on the mainland surrounding the Delta, since there is no indication in the expected species list that species composition differs in a particular direction. Therefore, it is expected that Kwedi has the highest species richness; and that

Mombo will exhibit higher species richness than Xigera, since it is closer to the Kwedi source area and thus there is a greater chance of dispersal success from the source pool on the mainland. This expectation is met by the results of the present study.

There are, however, several flaws to the above argument. Firstly, dispersal to Xigera could have occurred from the mainland to the west of Xigera, not Kwedi. According to Figure 10, Xigera actually appears closer to the mainland towards the west than Mombo does to the mainland towards the east. If the mainland to the east and the west of the Delta are comparable in species composition, it follows then that Xigera should support similar or higher species numbers than Mombo based on the dispersal hypothesis, since it is closer to a possible source pool. Secondly, the presence of the species *Breviceps adspersus adspersus* on Xigera implies it is highly unlikely that the decreased species richness on that island is the result of dispersal limitations. *Breviceps* as a genus has attained complete independence of standing water, and it is able to survive and reproduce using soil moisture alone to satisfy all its moisture requirements. Eggs are laid in underground burrows or chambers and there is no free-swimming larval stage: juveniles (metamorphs) emerge directly from the egg mass (Poynton, 1964). *B. a. adspersus* is known to survive in dry habitats including deserts, and it has been stated that the species is unable to swim, and avoids drowning by inflating the body and floating to the nearest terrestrial surface (www.wildanimalsonline.com/amphibians/rainfrog.php, accessed 01 Nov 10). Therefore, it is safe to assume the specimen observed calling in Xigera did not transverse any major water barrier, unless by a highly random event, but rather arrived there prior to the isolation of Xigera. Lastly, the size of Chief"s island; the fact that it has not been affected by flooding for a significantly long period of time (Gumbricht *et al*., 2004); and the land bridge connecting it to the mainland to the south, arguably renders it comparable to the mainland and Kwedi. This begs the question: why does Mombo have a lower species richness compared to Kwedi? For all these reasons, it does not seem reasonable to attribute lower species diversity on Xigera and Mombo to island isolation.

The lack of evidence supporting degree of isolation as the driver for species richness is further amplified by the results of the species diversity indices and the statistical analyses on species diversity. As with species richness, the diversity indices show that Mombo has the highest species diversity, closely followed by Kwedi and finally Xigera. The Margalef index for Mombo and Kwedi differ by a meagre value of 0.14, indicating high similarity in their species diversity; while they differ substantially from the species diversity of Xigera. Although there are distinct differences in both the measures of richness and diversity between the three localities, especially Xigera, the statistical analyses of species diversity at each locality proved that none of these measures are significant. Therefore, the differences in community composition between the three localities is not large or significant enough to make any further inferences,

and thus it is concluded that isolation does not seem to be the driver for differences in amphibian species diversity in the Okavango Delta. However, it is important to note that other aspects related to degree of isolation such as island area, species turnover and the concept of ecological time were not tested by this study, but should not be excluded as possible causes for the differences observed (Duellman & Trueb, 1994; Begon, 2006).

An explanation for the lack of island effect in the sense of isolation is offered by the dynamic nature of the Delta system. The broad physiographic regions shown in Figure 3, as well as the extent of flooding shown in Figure 10, are only a reflection of water flow in the Okavango Delta over the past few decades; it has not always been this way (Mendelsohn *et al.,* 2010). Within the framework of geological controls discussed in Chapter 1, which constitute the primary forces influencing the Okavango Delta system, there is also a biological aspect that has influenced, and continues to influence, the shape, dynamics and functioning of the system. Thus, the Delta is a product of both biological and geological processes (McCarthy, 1992). Changes in the distribution of water flow and flooding are numerous, but those to the Thaoge channel provide an exceptional and striking example. According to historical records, the Thaoge was the main channel during the 1800s, capturing most of the water that entered the Delta and annually flooding its western half. In approximately 1883 or 1884 this channel failed, and flood water was redirected towards east. Around 1970, there was yet another channel failure, this time in the eastern channel Mboroga; and currently the Xudum and Xene to the south are experiencing higher flow levels that have resulted in the flooding of Lake Ngami for the first time in many decades (Mendelsohn *et al.,* 2010). Examples such as these reflect the constantly changing shape and dynamics of the Delta, and indicate that current water levels in a particular area probably have differed in the past, and will differ again in the future.

Channel failure is often the result of the biological processes causing channel blockages. As the winding channels progress further south, they experience gradual but increased loss of water resulting in a reduced ability to carry bed-load; and consequently, increased deposition and vertical build-up of the channel floor. Papyrus is an astounding pioneer organism, renowned for rapid growth and remarkable ability to form a mat of rhizomes (or roots), so that it is able to colonise and encroach in these areas of newly deposited channel floor, finally leading to papyrus blockages and channel failures. Water is forced to follow alternative routes and quite often these are in the form of hippo trails that become enlarged and eventually form a new channel (McCarthy, 1992).

The dynamic nature of this system, with channels opening and closing and water distribution continually changing, could explain the lack of isolation effect on species diversity between the three localities of this study, since they may not have been isolated for a significantly long

period of time. It is possible, based on the above, that Xigera may have, relatively recently, been accessible from the mainland or other large islands, and this could explain presence of species such as *B. a. adspersus.* Likewise, Chief‟s island may not always have been connected to the mainland to the south, and in recent history may have been completely isolated. In other words, since the degree of isolation of both Xigera and Mombo is not fixed and this study is not focussed on the historical landscape of the Okavango Delta, inferences based on island biogeography are not applicable, and do not seem significant, at the present time.

2.4.3 Habitat availability as a driver for species richness

An alternative approach to isolation in the present study, where the isolation is not physical, results from the concept of *limiting factors*. Such a factor refers to any feature of the environment that hinders or inhibits the presence, growth or reproduction of a species (Cox & Moore, 1995). In the present study, such a limiting factor may be habitat availability, where certain habitat types are excluded in a specific locality due to its position within the permanent swamp physiographic region, as explained in Chapter 1. Therefore, it could be argued that habitat availability might be the driver for amphibian diversity in the Okavango Delta. The position of Xigera within the low flood zone means that it is, at present, not only permanently surrounded by water, but also, that its ground water levels under the islands are at, or just below, surface level throughout the year. The result is that when the annual flood arrives, the landscape of Xigera changes dramatically and shallow water covers most of previously exposed terrestrial ground. Areas that appear as rainwater pans in the summer - when flood waters are low - become inundated vleis and floodplains in the winter. Examples of changes in landscape are shown in Figure 18.

The result of this regular and prolonged inundation is that certain characteristic habitat types, specifically dryland woodland and their associated true pans with accompanying plant species, are unable to establish in Xigera and similar islands.

Independent knowledge of amphibian species ecological requirements and the assessment of the three species inventories highlighted the possibility that species absent from Xigera may be the result of a lack of true pan habitats. Indeed, the absence of at least seven of the eleven species absent from Xigera could be explained by a lack of suitable habitat. These species include: *C. xerampelina, H. marmoratus, P. bifasciatus, P. mossambica, P. oxyrhynchus, P. adspersus* and *P. edulis.* In du Preez & Carruthers (2009) all of these species are said, in some

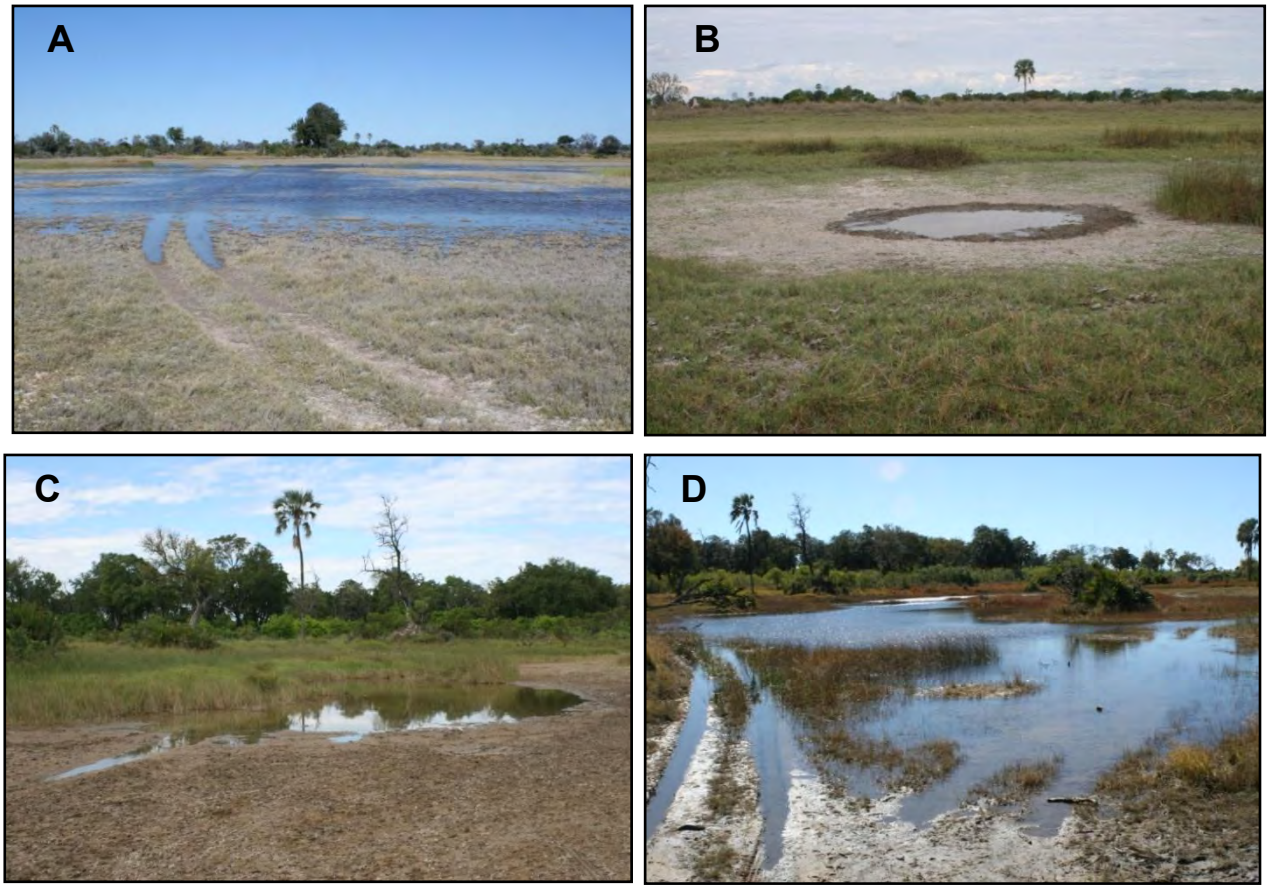

Figure 18: A, Xigera sampling site 1 in November 09; B, Xigera sampling site 1 in July 09; C, Xigera sampling site 2 in November 09; D, Xigera sampling site 2 in July 09.

form or another, to require savanna or bushveld habitats, often mentioning their need for temporary, rain filled depressions for breeding. In addition, both the statistical analyses of species occurrence in various habitat types, as well as the graphical representation created by CANOCO support this argument. The statistical analysis of effect sizes indicates that the occurrence of a species is significantly related to habitat type for at least 72% of observed species; while the CANOCO representation indicates clusters of species influenced by what appears to be a water availability gradient. The above argument can therefore explain why Xigera exhibited the lowest values of species diversity and richness.

Similarity in community composition of Kwedi and Mombo can be explained in the same, but opposite, way. Just as a lack of habitat appears to decrease species richness and diversity in Xigera, the presence of all the habitat types in both Kwedi and Mombo may support similar amphibian communities in the two localities. Therefore, although the aim of this section of the project was not to determine how the various habitat types differ in their species and community composition, and the collected data and subsequent results do not allow for inferences to be made regarding the relative species richness and diversity measures of each habitat type,

results from the present study do indicate that species richness and diversity seem correlated to habitat availability.

This conclusion is not a novel one, and the effect of habitat diversity alone versus island effect *per se* (especially related to island area) has been the focus of many studies in the past (Nilsson *et al.,* 1988; Kohn & Walsh, 1994; Ricklefs & Lovette, 1999; Herzog & Kessler, 2006). Ultimately, the question that must be answered is: "Does richness increase with area at a rate *greater* than could be accounted for by increases in habitat diversity alone?" (Begon *et al.,* 2006.) A relevant, recent study by Ricklefs & Lovette (1999) focussed on exactly this question using four different faunal groups, one being reptiles and amphibians (herps), present on 19 islands in the Lesser Antilles. Their results indicated that species richness for herps was strongly correlated with habitat diversity alone, while that of bats was sensitive to island area alone, and both variables contributed almost equally in birds and butterflies. They conclude that the biology of each taxon likely determines their response to these variables, with dispersal ability and local ecological conditions playing a major role in their presence and survival. In addition, they also speculate that since herpetofauna species diversity is highly correlated with habitat diversity, it is probably also related to the availability of suitable habitat; and furthermore, that island colonisation is possibly direct from similar habitats on the mainland or other islands. Therefore, species composition is determined by habitat filters at the colonisation phase (Ricklefs & Lovette, 1999).

2.4.4 Investigation of available habitat types per locality

As a final investigation of habitat availability and type as a driver for amphibian diversity, vegetation maps were used to verify the presence or absence of habitat types in each locality. This was done to ensure that sampling sites were an accurate representation of each locality as a whole, and not biased towards certain amphibian habitat types. Indeed, the maps generated by the vegetation data did support this habitat hypothesis: Xigera clearly supports fewer habitat types than the other two localities, and these absent habitats are the very ones required by many of the absent amphibian species for colonisation and survival.

In their publication, Ramberg *et al.* (2006) speculate that an increased number of habitats lead to an increased *edge effect*; and that this, in turn, results in higher faunal and floral biodiversity, especially for those species that require two or more habitats for their survival and reproduction. Since the highest diversity of habitats occurs on the Delta periphery where the effects of annual inundation are most varied and pronounced, it follows that these areas will support the highest biodiversity. In the case of amphibians, the results of this study support this premise, with Kwedi on the periphery supporting the highest amphibian diversity, and Mombo comparable since the

characteristics and habitat types of Chief"s Island render it equivalent to the mainland surrounding the Delta. In addition, the fact that the majority of amphibians occurring in the region require both terrestrial and aquatic habitats for their life histories and to complete their various life stages means that the interface between terrestrial mainland or large islands, and aquatic Delta is of utmost importance for the biodiversity of this class.

Such knowledge on biodiversity hotspots and the importance of peripheral areas provides crucial insight into long term preservation of the Delta, and should have a major effect on conservation efforts and direction. This, not only in terms of conserving those areas that display the highest biodiversity, but also with the view that they are potential source areas from where species can disperse to favourable areas within the interior of the Delta; this applies to amphibians at least. From this study and for amphibians, it seems conservation efforts will be best concentrated on preserving various habitat types, as well as interface zones between aquatic and terrestrial regions, as different habitats support different species. This conservation is, for the time being, most important in the areas surrounding the Delta since these areas are often still inhabited and easily accessible by people, and are thus vulnerable to environmental degradation that will inevitably results in a loss of biodiversity.

Several opportunities for further research became apparent during the present study, they are listed below:

- Determine how amphibian diversity is related to the various habitat types in order to highlight which habitats, if any, are more important or sensitive, and whether conservation efforts should be specifically directed at these.
- Since amphibians are important biological indicators that signify the health of an ecosystem, long term monitoring programs of amphibian abundance and diversity should be established in order to detect any early warnings that may indicate potential threats that could cause irreversible damage to the Okavango Delta as a whole.
- A genetic and taxonomic study of the species classed as *Pyxicephalus adspersus* in this project, as several morphological features of specimens collected suggest this may actually be a new species. Since there are “no confirmed endemic species in the Okavango Delta,” (Ramberg *et al.,* 2006), such a discovery may contribute greatly to the importance, and thus protection, of the Delta ecosystem.

CHAPTER THREE:
HYDROLOGY AS DRIVER FOR AMPHIBIAN BREEDING IN THE OKAVANGO DELTA

3.1 INTRODUCTION

3.1.1 Reproductive patterns of amphibians in Southern Africa

The key to survival of any species is its ability to reproduce. Globally, there is a generalised reproductive pattern among anurans that involves short, annual mating episodes; followed by spawning during which time eggs are laid and left unattended; and finally the hatching and development of aquatic larvae. However, this generalised reproductive pattern does not apply to all species. Individual species show great variation and diversity in the reproductive strategies they employ. A reproductive strategy can be described by the combined features of physiology, morphology and behaviour that ensure maximum reproductive output for a defined set of environmental conditions. Reproductive strategy is as critical to a species‟ survival as its morphological and physiological adaptations, and thus it is a product of natural selection aimed at providing optimal fitness levels (Duellman & Trueb, 1994).

Within the Anuran, there appears to be two broad patterns of reproduction dependant on the climate of the region: reproduction in tropical and subtropical species is seemingly all year round (or *continuous*) and highly correlated with rainfall; while the reproductive activity of temperate species seems to be seasonal and short-lived, driven by both rainfall and temperature (Duellman & Trueb, 1994). As discussed previously, the Okavango Delta falls within the transitional complex, in the largest zoogeographical unit called the *central tropical transitional fauna* (Maps 2 and 3 in Poynton, 1964). It contains species from the tropical West African fauna to the north as well as species from the temperate fauna to the south (Auerbach, 1987). It follows then, that reproductive patterns of amphibians in the Okavango Delta would be consistent firstly, with that of the seasonably dry tropics where reproduction is closely linked to the rainy season; and secondly, with the subtropical/temperate regions where reproduction commences with the arrival of seasonal rainfall and, in addition, seems strictly controlled by temperature as well. It should be noted, however, that Hoogmoed & Gorzula (1979) observed typically seasonal breeders reproducing during the dry season when there was an erratic occurrence of rain outside of the normal, wet season. This implies that known seasonal breeders are physiologically capable of continuous breeding, but are limited by local climatic

conditions, such as the presence of rain. It also indicates the possible opportunistic nature of amphibians with regards to breeding habits.

In Southern Africa, it is well established that amphibian reproductive activity generally begins with the first seasonal rains for those species occurring in the summer-rainfall regions; while those occurring in the winter-rainfall region of the Western Cape generally reproduce towards the end of the winter when there is ample water and abundant breeding sites. The actual mating season of a particular species, however, is more complex and further influenced by factors such as temperature and length of day (du Preez & Carruthers, 2009). There are two categories of breeders, depending on the optimal tactic employed by males to exploit the typically limited number of females, whose energy investment into egg production restricts them to only a few reproductive opportunities each season (Passmore & Carruthers, 1995). The first is referred to as *explosive breeders*, and is characterised by the large-scale emergence of males and females, coinciding with heavy rain. They normally congregate around temporary, rainwater pans where breeding and spawning occur in a very short space of time, often accompanied by serious competition and fighting amongst males (du Preez & Carruthers, 2009). Vocalisation by males is normally minimal or lacking since responding females will likely be intercepted and seized by a rival male (due to their great abundance at the time), and energy is better invested by moving around the breeding site, actively looking for potential female mates; a strategy which does occur but is not especially common in Southern Africa. The second category is known as *prolonged/continuous breeders* where reproductive activity may range from a few weeks to several months. Since female arrival at a breeding site is sporadic and relatively random, an active search for females would be mostly futile. Therefore, males rather invest their energy in the establishment and defence of stationary call sites, resulting in large aggregations of calling males. In this case, there is a high turnover of individual males within the chorus, since vocalisation is energetically expensive and males regularly need to break to replenish energy reserves (Passmore & Carruthers, 1995).

Some of the earliest and only work done on the reproductive ecology of amphibia in Southern Africa was that of B.I. Balinsky (1969). More extensive reproductive research has been done on the genera *Xenopus* (see Berk, 1938; Kalk, 1960; Du Plessis, 1966), probably due to their importance as a laboratory animal, and *Pyxicephalus* (Balinsky & Balinsky, 1964; Kok *et al*., 1989; Cook, 2001), probably due to their charismatic nature. At the time, it was already well established that breeding occurred during specific seasons; and the importance of local weather conditions on reproductive activity had been observed. In his work, Balinsky (1969) focussed on trying to understand firstly, the factors that controlled the beginning and end of the breeding season, determining the time range when spawning may occur; and secondly, the specific factors that were responsible for the actual onset of spawning. Important for the present

discussion, are his summarised findings on the observed breeding habits of the Transvaal Highveld amphibians. He divided them into three discrete ecological classes. The first are the "*breeders in temporary rain pools,"* whose breeding season is restricted to spring and summer, between October and mid-February, when depressions are temporarily filled with rain water, making suitable breeding sites available. For such species, it seems logical to assume that rainfall is the primary activator of reproductive activity, given that the sooner spawning occurs within these newly established water bodies after sufficient rain, the longer tadpoles have to complete metamorphosis before the temporary water body dries up. The second class is the "*breeders in semi-permanent and permanent bodies of water"* (not necessarily excluding breeding in temporary rain pools) whose breeding season starts earlier than that of the first class and also continues through the summer, between August and mid-February. The third and final class are the "*breeders in permanent waters such as large dams, rivers and swamps"* whose breeding season is not restricted to spring and summer; reproductive activity was observed in autumn and winter as well, and they may possibly display continuous reproduction throughout the year (Balinsky, 1969).

An additional, important outcome of Balinsky's work was the statistically significant finding that species occurring in the same area may respond very differently to rainfall. Whilst the majority of species he observed revealed a significant positive correlation between spawning and rainfall, one species, *Bufo regularis* [now *Amietophrynus gutturalis*], showed no correlation at all. This observation implies that the trigger for reproductive activity and spawning is not necessarily alike in different species. Moreover, this trigger may even vary within a species where members occupy different geographic locations; such as populations of *Xenopus* in the Transvaal Highveld versus those of the Western Cape. In fact, spawning in *A. gutturalis* was observed in the Highveld in the early weeks of spring, prior to any rainfall; this may be attributed to their breeding sites in semipermanent or permanent waters, and thus the need for spawning to coincide with rainfall and temporarily filled rain pans is nonexistent (Balinsky, 1969).

3.1.2 The function of anuran vocalisation in the reproductive process

The details of reproductive ecology and activity would not be a point of investigation or discussion if it were not for the ability of males and females of a particular animal species to meet. Mechanisms have evolved in all animal species that allow potential sexual partners to find one another, recognise one another and finally copulate in order complete the sexual cycle. Such mechanisms have been termed the *'specific mate recognition system' (SMRS)*, and they ensure matings occur between biologically similar species, i.e. "conspecific matings" (Passmore, 1978). Due to the function of SMRS to bring biological species together, it can be readily assumed that these systems are species specific, vary greatly between different

species, and consist of a unique signal that yields a unique response. Furthermore, each species specific SMRS can be viewed as an adaptation that has evolved over time, and thus it is a stable and consistent feature within a species (Passmore, 1978).

In the case of anurans, this SMRS takes the form of acoustic signals, and members from this order can be easily heard and identified by the human ear (Passmore, 1978). In fact, frogs and toads are more commonly heard than seen, and the sound of amphibian choruses are readily associated with the arrival of spring and summer rains (du Preez & Carruthers, 2009). Although there has been much debate regarding the function of mating calls in the overall reproductive process in anurans, a review by Salthe & Mecham (1974) of experimental evidence provided conclusive proof and verification of the significance of the mating call. Therefore, it is commonly accepted that "the call of the male anuran is a beckoning note directed toward the female of the same species so that she may locate him and reproduction be effected," (Axtell, 1958). Balinsky (1969), too, made reference to the link between vocalisation and breeding, and he utilised frog calls as indicators of breeding and mating seasons.

When the vocalisations of anurans are investigated in more detail, it becomes apparent that they actually produce four different types of calls: (1) *aggressive or spacing calls* when rival males get too close; (2) *release calls* produced by females to indicate the end of amplexus once eggs have been laid and males when they are mistaken for a female and accidently seized by another male; (3) *distress calls* produced by both sexes when molested or captured by a predator and possibly to warn other anurans of the danger; and finally and most importantly for this study, (4) *advertisement calls*. These are the most common type of calls and can be heard in abundance at breeding sites. Each species has a unique, species specific advertisement call that is produced by males and aimed to lure a conspecific female close enough that mating can occur. The female's hearing is attuned only to her species specific advertisement call, preventing an erroneous response to the wrong call and the unnecessary expenditure of energy in tracking down the caller (du Preez & Carruthers, 2009). Males, too, can gain information from these calls by locating breeding sites in their vicinity, and determining their position within the chorus relative to rival males. Advertisement calls are especially useful and reliable for species identification by observers in the field because there is minimal variation within a particular species, even if populations of that species occupy widely different geographical ranges. Since it is these vocalisations that attracts conspecific mates to one another - and thus delineates their species - in nature, it is obvious how and why these can be used as reliable identification tools by interested observers (Passmore & Carruthers, 1995).

Within a breeding chorus, numerous different species can often be identified sharing the same water resource, and the synchronous calling of males in sometimes massive aggregations can

understandably seem rather chaotic. However, there is some structure to such a breeding chorus. The first line of simplification is purely the structural differences of advertisement calls of different species, as discussed above, and these can be defined in terms of frequency separation, call pulse rate, call duration and frequency modulation. Such structural differences are so characteristic of the species, and so accurate, that two species are often taxonomically separated purely based on differences in call. The second line of simplification is species' preferences in call sites, which tend to be similar within species but vary considerably between species. Call sites may be chosen based on elevation, position in or distance from water, resulting in the spatial polarisation of a habitat. Finally, different species occupying the same breeding site may be active at different times. This may refer to different times in the breeding season (e.g. early spring or late spring) or different times within one night, where, for example, some species exhibit peak activity in the early evening while others only begin calling at midnight (Passmore & Carruthers, 1995).

3.1.3 Nutrient flow and productivity in the Okavango Delta

The wealth of any ecosystem is only as rich as its carrying capacity. Therefore, abiotic factors such as nutrients and rainfall determine the final biomass that an ecosystem can sustain. On a superficial level, the Okavango Delta may seem somewhat nutrient deficient when the quantity of nutrients entering it is evaluated. This results from the Kalahari sand substrate in the catchment area, which not only consists mainly of quartz from which little can be dissolved, but has also been leached for an extensively long period of time; and in addition, dense vegetation not only slows water flow as it enters the alluvial fan, but also filters out any possible nutrients that may have arrived there. Incidentally, these features explain the crystal clear waters that are so characteristic of the Okavango Delta. However, despite a lack of research into nutrients and their cycling, the fact that the Delta supports ten times the volume of large mammal biomass than is expected for the area, based on carrying capacity comparisons with other regions in Africa, indicates there is much more nutrient availability than the superficial level suggests (Mendelsohn *et al.,* 2010).

Carbon, nitrogen and phosphorus are the three most essential nutrients for biotic growth, but precisely how sufficiently large quantities of these and other nutrients have arrived in the Delta is still open to debate. It has been speculated that there are possibly five nutrient sources:

- The Okavango River: the large volumes of water which this river brings to the region compensates for its significantly small nutrient concentrations.
- Aerosols: the local weather brings with it vast quantities of dust, often containing large amounts of nutrients, that settles locally.

- Dissolved out of soil particles: although a possible source, contributions are more than likely minimal due to the nature of quartz-rich sandy soils.
- Microbes: nutrients are transformed and made available to higher trophic levels by microorganisms such as bacteria, blue-green algae and mycorrhizae.
- Larger mammals: the faecal matter of larger animals such as elephants and impala brings nutrients from elsewhere to be incorporated into the Delta"s nutrient load.

Although nutrients can be lost through processes like fire, leaching, trapping beneath islands and ingestion followed by emigrations of large mammals, it is still largely believed that the Okavango Delta is a nutrient sink, with more nutrients being collected than is lost (Mendelsohn *et al.,* 2010). Globally, inland deltas are known as massively productive ecosystems resulting from the water, nutrients and organic carbon periodically made available to adjacent, terrestrial environments by the annual floods. Such floodplains and seasonally inundated water bodies may be key areas of biodiversity and production, and provide important water-land ecotone habitats (Høberg *et al.,* 2002).

The nutrient cycle of the Okavango Delta is a complex process, but a summary is attempted. Much of the nutrient loss from the Delta is only temporary; they are removed from the system and stored mostly in living plants, dead organic matter and detritus. Nutrients are returned to the system when consumed by herbivores and deposited in faeces; when insects break down dead animals or animal dung and are then themselves eaten; when living plants die as a result of receding flood water or channel switching and decompose; or when organic matter is burnt or otherwise broken down by bacteria and fungi. The most remarkable recycling and release of nutrients, however, occurs when dry floodplains and seasonal swamps are inundated by the annual flood, which then triggers a series of production events (Mendelsohn *et al.,* 2010).

3.1.4 Hypothesis and objectives

Amphibian breeding behaviour is driven by the hydrology of the ecosystem: It is predicted that amphibian breeding behaviour in the Okavango Delta is not controlled by rainfall alone, but that the flood pulse results in opportunistic, uncommon breeding behaviour and strategies.

In order to test this hypothesis, several objectives were formulated:

- Using available literature, determine the known breeding seasons of all the observed species in this study.
- Monitor amphibian breeding in the rain and dry seasons using the following indicators: calls, adult matings, spawn and tadpoles.

3.2 METHODOLOGY

3.2.1 Literature review of breeding seasons for Okavango Delta species

A desktop study of known breeding seasons for all species recorded in this study (as listed in Table 2) was completed. Information from Balinsky (1969), Auerbach (1987), Passmore & Carruthers (1995), Channing (2001) and du Preez & Carruthers (2009) was pooled and summarised. In combination, these sources provided the most comprehensive review of breeding seasons for Southern African amphibian species.

Despite a thorough investigation of the literature, it was concluded that information on breeding biology and seasons for the relevant species was sparse and often lacking for individual species. It was thus decided to assess breeding information at genus level (rather than at species level) and combine this with any additional information provided by individual species accounts.

The investigation revealed that there is little standardised terminology regarding the classification of species into discrete breeding classes, with reference to their breeding seasons. To avoid ambiguity and confusion, the following breeding classes were thus defined for the present study, principally based on the “ecological classes” defined by Balinsky (1969):

- *Explosive Breeders:* relates to the first ecological class; breeding coincides with heavy rain.
- *Prolonged Summer Breeders:* relates to the second ecological class; breeding is associated with the rainy season.
- *Continuous Breeders:* relates to the third ecological class; breeding is not necessarily initiated or dependant on rain.

3.2.2 Observation of breeding indicators under natural conditions

Prior to initiation of this portion of the study, several preliminary surveys were conducted between November 2008 and March 2009 in order to assess feasibility of this section"s objectives and finalise sampling techniques. Official monitoring of breeding seasons and field observations (i.e. study period for this part of the study) were carried out during the period July 2009 to June 2010. Within this period, several observations were carried out every month (barring three months as explained below), usually spanning over at least two weeks of every month. The number of surveys conducted during official field trips was 26 in July 2009 and 38 in November 2009; and ranged from one to seven per month for additional ad-hoc sampling.

No surveys were conducted in August, October and December of 2009 for two reasons. For August and October, random listening exercises made it clear that only a limited number of species were calling prior to and during this period and no additional species were heard calling throughout this time. This observation was confirmed by a single acoustic survey in September 2009 that did not yield any additional, active species. The second reason, specifically for December 2009 and also influencing the number of surveys that could be conducted during ad-hoc sampling, was the result of limiting logistical factors that included: full time employment for periods outside official field trips, exceptionally busy periods in the work scenario that did not allow time for sampling and a lack of vehicles available to conduct field work.

Field observations were based on the techniques used by Balinsky (1969). Information on breeding was collected using the following indicators:

- Amphibians in amplexus [definition: “Sexual embrace of amphibians. The grasping by the male of the female"s body usually with the forelimbs but in some species by adhesion” (du Preez & Carruthers, 2009)];
- Observation of spawn;
- Collection of tadpoles using dipnet surveys as explained in the previous chapter;
- Monitoring of vocalisation using acoustic encounter surveys as explained in the previous chapter.

In August 2010, all unidentified tadpole specimens and vocalisation recordings were analysed at the North-West University, Potchefstroom Campus. Tadpoles were identified using a Nikon SMZ 445 dissecting microscope and the field key to tadpole genera in du Preez & Carruthers (2009). Where possible, identification was done to species level using tadpole descriptions present in individual species accounts as well as through comparison with voucher specimens present in the AACRG collection. This, however, proved extremely difficult due to a lack of tadpole knowledge in the available literature, and therefore supported the earlier decision to assess breeding seasons only at the genus level, where tadpole identification was accurate and reliable.

For the purpose of this section, the Okavango Delta climate was divided into two seasons. The first was termed *summer* and occurs from the beginning of November to the end of February. This is the rainy season within which the region receives the majority of its annual rainfall; and also coincides with low flood water levels (the minor flood). The second season was termed *winter* and occurs from the beginning of March to the end of October. This is the dry season with generally no rain falling over this period; and it coincides with the major flood where water levels are highest (see Chapter 1 for clarity on minor and major flood pulses).

3.2.3 Assessment of breeding classes and seasons for Okavango Delta species

All the data gained from breeding indicator observations were combined and analysed. Using the dates (months) associated with these observations, each genus (and therefore the species within) was assigned to a breeding class based on when amplexus, spawn, tadpoles or calls were observed. Although all indicators were monitored and utilised for this assessment, dipnet and acoustic surveys proved to be the most reliable and consistent, and thus they were the only indicators employed in further analyses.

Abundance data was used to monitor breeding activity throughout the year, and to determine when each class was most active. For this assessment, all acoustic and dipnet data for all genera belonging to one breeding class was combined and ranked. The number of tadpoles for each class collected every month was counted, then ranked and the average calculated; similarly, the average number of calling adults per month was calculated. Both the number of tadpoles and number of calling adults were ranked according to the following abundance rank:

Rank 1 = 1 – 2
Rank 2 = 2 – 5
Rank 3 = 6 – 10
Rank 4 = 11 – 20
Rank 5 = 21 and over

These two figures (average number of tadpoles and average number of calling adults) were then summed together to provide an overall abundance average per month for the duration of the study. This summed total provides accurate insight into activity level since the two indicators used focus on different stages in life history (tadpoles and adults) and thus there was no possibility of overlap in collected data.

3.2.4 Comparisons and differences in literature based and present study breeding class composition

A comparison of genus assignment to breeding classes was done in order to determine how the composition changed between literature knowledge and current observations, and to determine exactly which genera were assigned to a different breeding class in this study. Since observations of breeding activity for one genus only was nominal, maximum abundance of acoustic and dipnet collections were used to indicate the level of breeding activity.

For each genus, this was achieved by investigating the number of tadpoles and the number of calling adults observed for each dipnet and acoustic survey in a month. The maximum

abundance per month for each of these surveys was then ranked according to the above abundance rank.

3.2.5 Correlations of breeding activity with rainfall and flood pulse

Regression methods were used to illustrate any correlation, for each breeding class, between breeding activity and rainfall, or breeding activity and flood pulse. The overall abundance averages for each month calculated in 3.2.3 were used in these analyses. Approximate, mean monthly rainfall figures and flood water level figures were taken from Mendelsohn *et al.* (2010). Flood figures correspond to mean monthly flows (in million cubic meters) measured at the top of the Panhandle, and these flows require approximately four to six weeks before reaching the present study area.

3.3 RESULTS

3.3.1 Literature review of breeding seasons for Okavango Delta species

The information on the breeding seasons of the relevant species is summarised in Figure 19. A total of 12 genera including 29 amphibian species were recorded during the present study. Every genus was assigned to a breeding class, and then the number of species of every genus was summed to provide the percentage composition below.

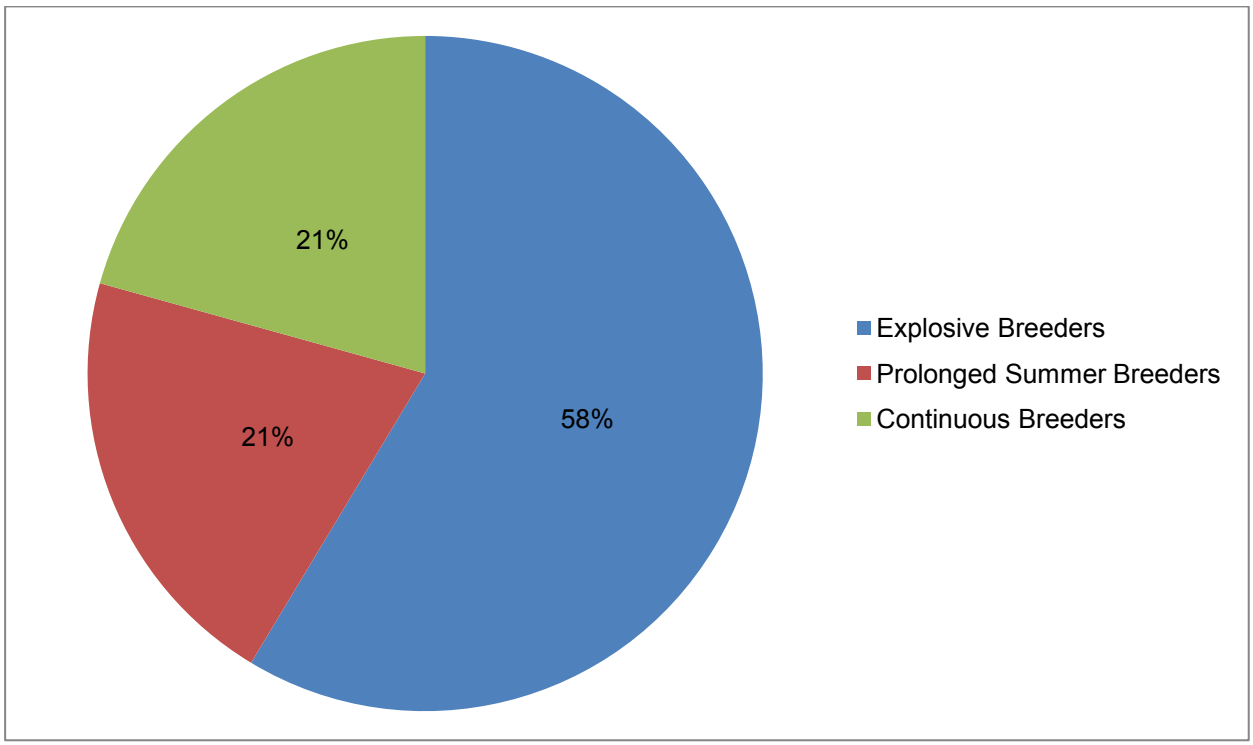

Figure 19: Classification of species recorded in the present study into discrete breeding classes based on available literature.

Eight genera including 17 species are known explosive breeders, resulting in this being the dominant class. Genera included are *Breviceps, Chiromantis, Hemisus, Kassina, Phrynomantis, Ptychadena, Pyxicephalus* and *Tomopterna*. A further 2 genera including 6 species, namely *Amietophrynus* and *Xenopus*, are known prolonged summer breeders. The final 2 genera also including 6 species, *Phrynobatrachus* and *Hyperolius*, are known continuous breeders.

There was some difficulty in the classification of *Phrynobatrachus* into a breeding class due to conflict in the available literature, where its members have been identified as both summer and continuous breeders. The consistent information that *P. parvulus* breeds continuously under favourable conditions, and that *P. mababiensis* calls throughout the year, has led to the present classification of the genus into the continuous breeding class.

Confusion around the classification of *Amietophrynus* was also created by the information in Channing (2001) that *A. gutturalis* "breeds throughout the year over much of its range but is seasonal in the south." However, the location of the present study area in Southern Africa, and the general trend of other species in this genus as prolonged summer breeders, resulted in the present classification of the genus as a whole.

3.3.2 Breeding classes of Okavango Delta species

The results of species assignment to a particular breeding class are summarised in Figure 20.

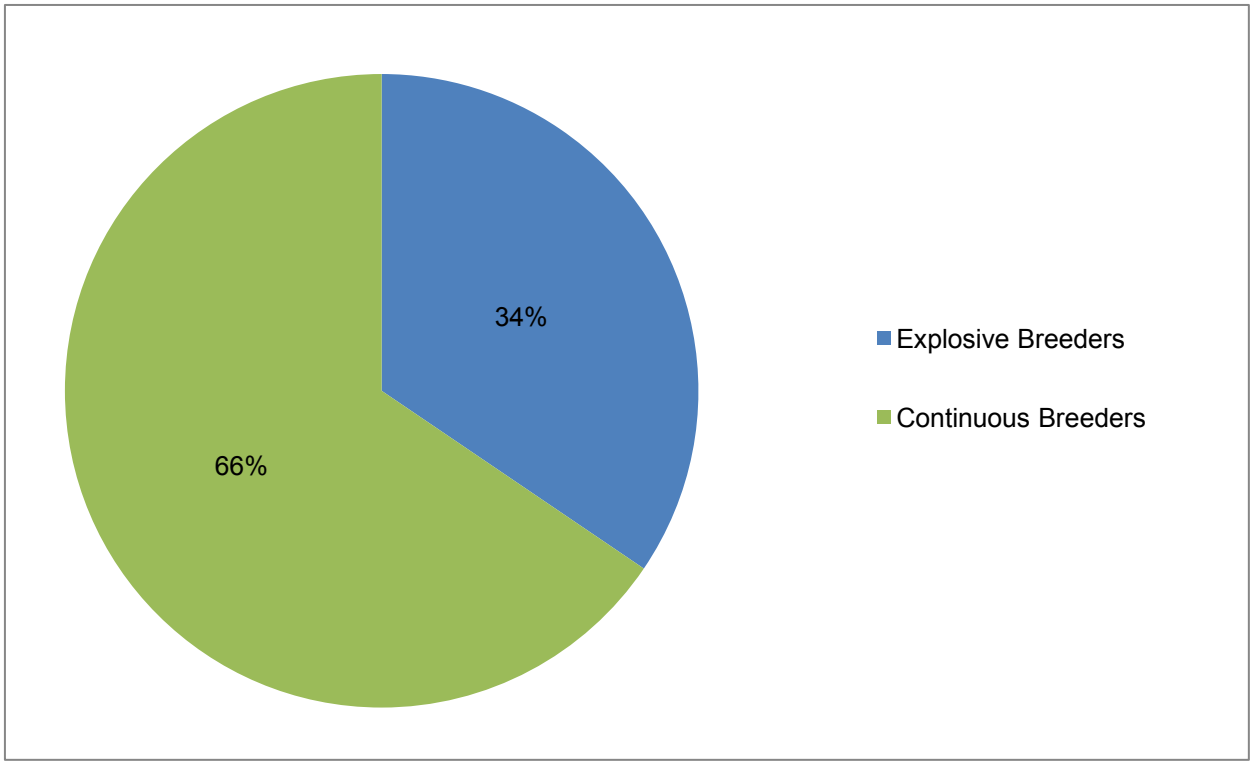

Figure 20: Classification of species recorded in the present study into discrete breeding classes based on current observations.

Five genera including 19 species were classed as continuous breeders; genera included are *Amietophrynus, Hyperolius, Phrynobatrachus, Ptychadena* and *Xenopus.* The remainder, 7 genera including 10 species, were classed as explosive breeders, and these included *Breviceps, Chiromantis, Hemisus, Kassina, Phrynomantis, Pyxicephalus* and *Tomopterna.* None of the genera, therefore, were classed as prolonged summer breeders since those who did indicate breeding during August and September also displayed it for some or all of the other winter months, and thus they were classified as continuous breeders.

The level of breeding activity was calculated per month for each of the breeding classes determined above, using average abundances for acoustic and dipnet surveys. The results are summarised in Figure 21.

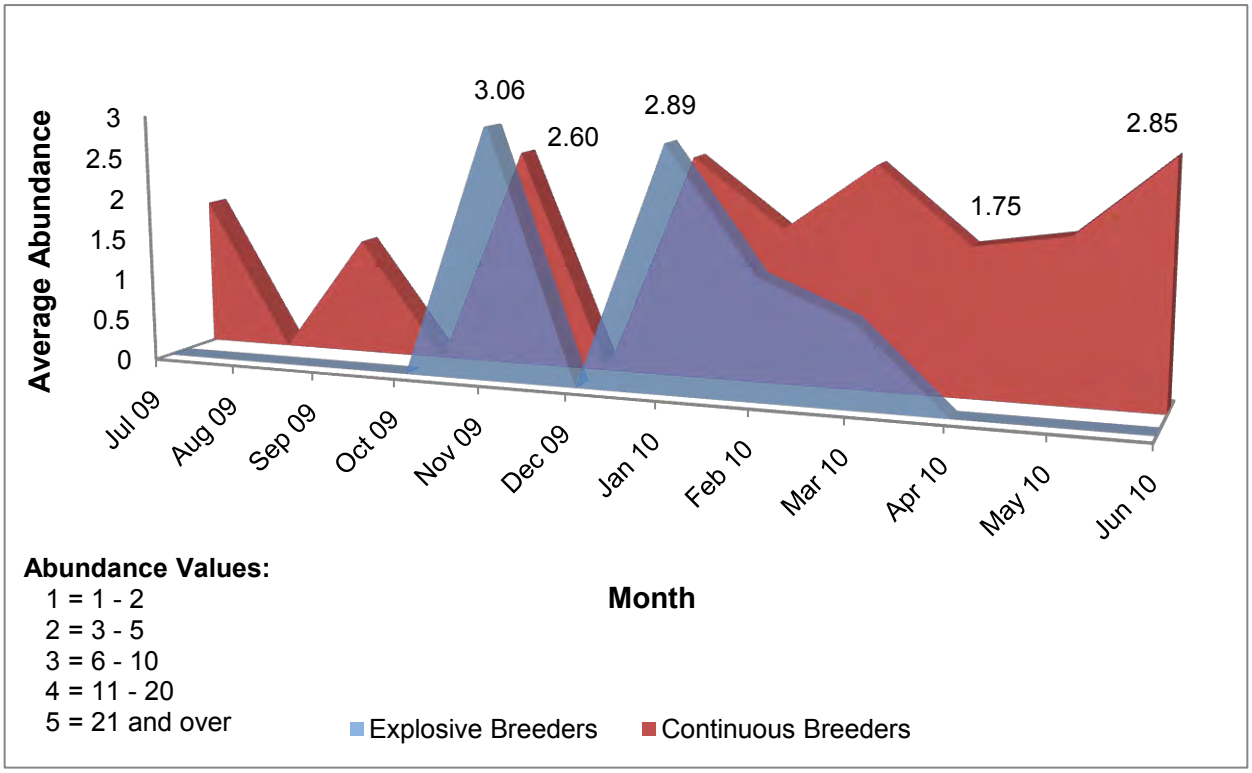

Figure 21: Average breeding activity [acoustic (# of calling adults) and dipnet (# of tadpoles) collections combined] for each breeding class for the period July 2009 - June 2010.

The results suggest that there are marked differences in both breeding season, and peak breeding activity, between the two classes. Explosive breeding activity was only observed from November 2009 to March 2010. Peak activity levels were observed in November (3.06) and January (2.89), then gradually tapering to zero by April. Zero activity for this class observed in December is explained by the lack of sampling during that month.

Breeding activity for continuous breeders appears different from the first class in that it seems to extend over the entire year. For each observation plotted on the graph, the average abundance value calculated received contributions from both acoustic and dipnet values (no month had a zero value for either of the indicators) and thus there was no biased data or misleading breeding indication from tadpoles that have prolonged development. Peak activity was recorded in June (2.85), but high levels of activity began in November (2.60) and fluctuated between 1.75 and 2.85 from November through to July. The zero values for December, August and October are all due to a lack of sampling.

3.3.3 Comparisons and differences in literature based and present study breeding class composition

Figures 19 and 20 clearly show that there are changes in the composition of breeding classes when the results from the present study are compared with that of known literature. Most obviously, prolonged breeders drop from 21% to 0%, with both genera *Amietophrynus* and *Xenopus* instead classed as continuous breeders. The removal of the large genus *Ptychadena* from explosive breeders and its assignment to the continuous breeders results in a 24% decrease of the former class. And finally, the addition of these three genera to the continuous breeding class resulted in a considerable 45% increase in that class - increasing from 21% to 66% - rendering it the dominant class.

Breeding activity and periods of peak activity for each genus that changed breeding classes are graphically displayed in Figures 22 to 24. These representations indicate why this change of breeding class occurred. All the zero values in August, October and December result from a lack of sampling.

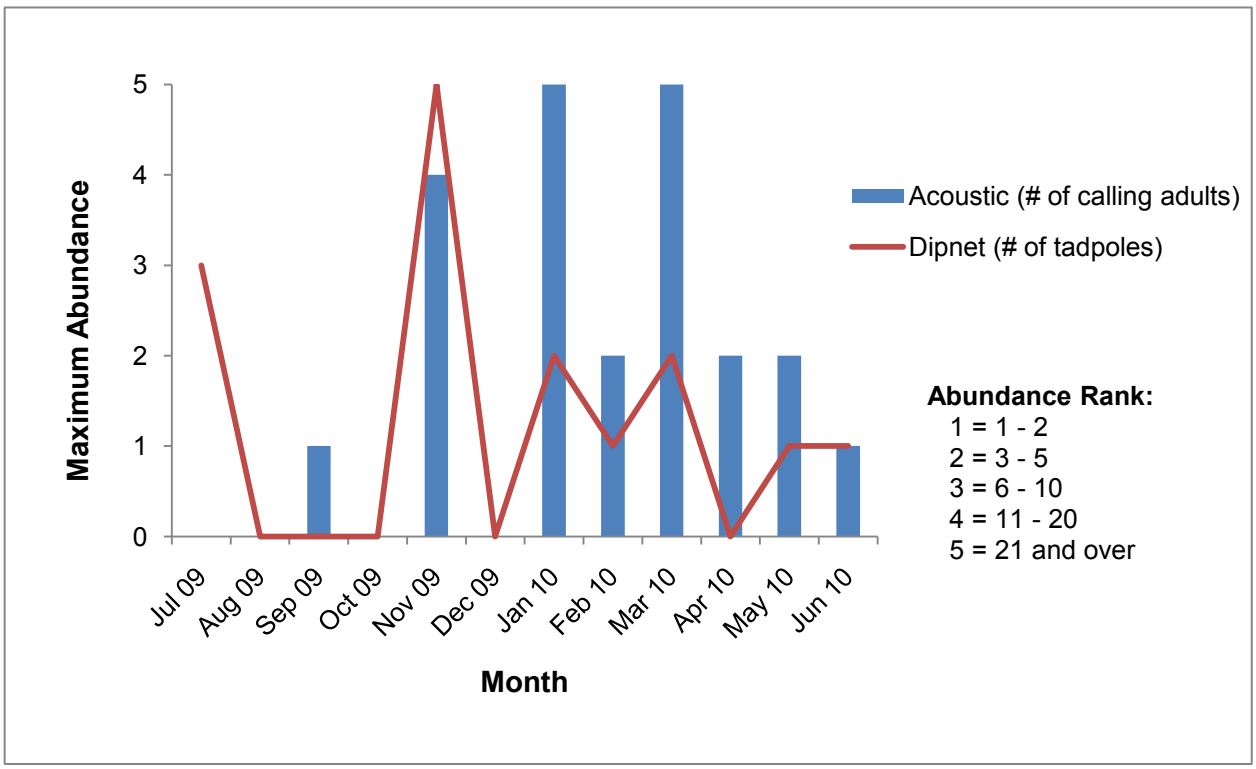

Figure 22: Breeding activity for *Ptychadena* over the period July 2009 - June 2010.

Strong evidence was found that at least some species of *Ptychadena* breeds throughout the year. Peak acoustic abundance was observed in January (5.00) and March (5.00), while peak dipnet abundance was observed in July (3.00) and November (5.00). This breeding activity differs considerably from the expected activity times of explosive breeders, and it was subsequently classed a continuous breeder.

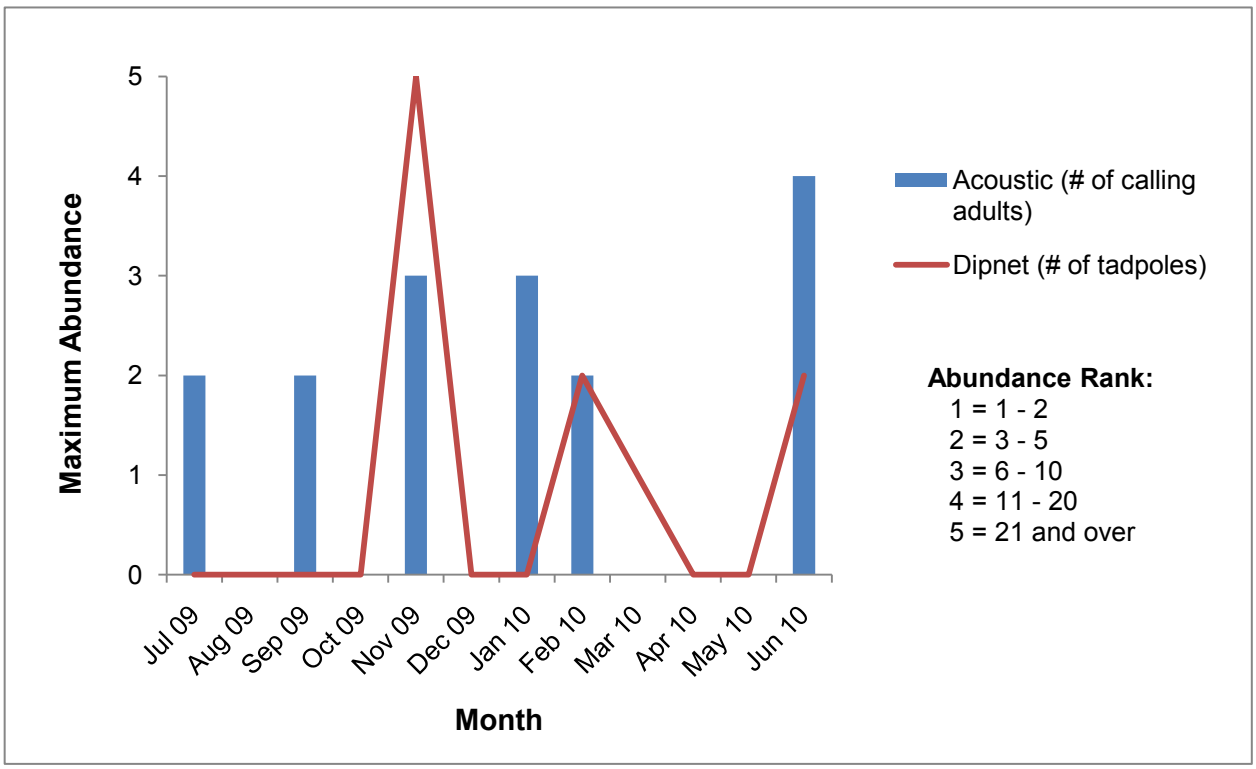

Figure 23: Breeding activity for *Amietophrynus* over the period July 2009 - June 2010.

Amietophrynus displayed breeding activity in June and July which falls outside the prolonged summer breeding period, and therefore it was classified as a continuous breeder. Peak dipnet abundance was observed in November (5.00) while peak acoustic abundance was observed in June (4.00). No breeding activity was recorded in April and May.

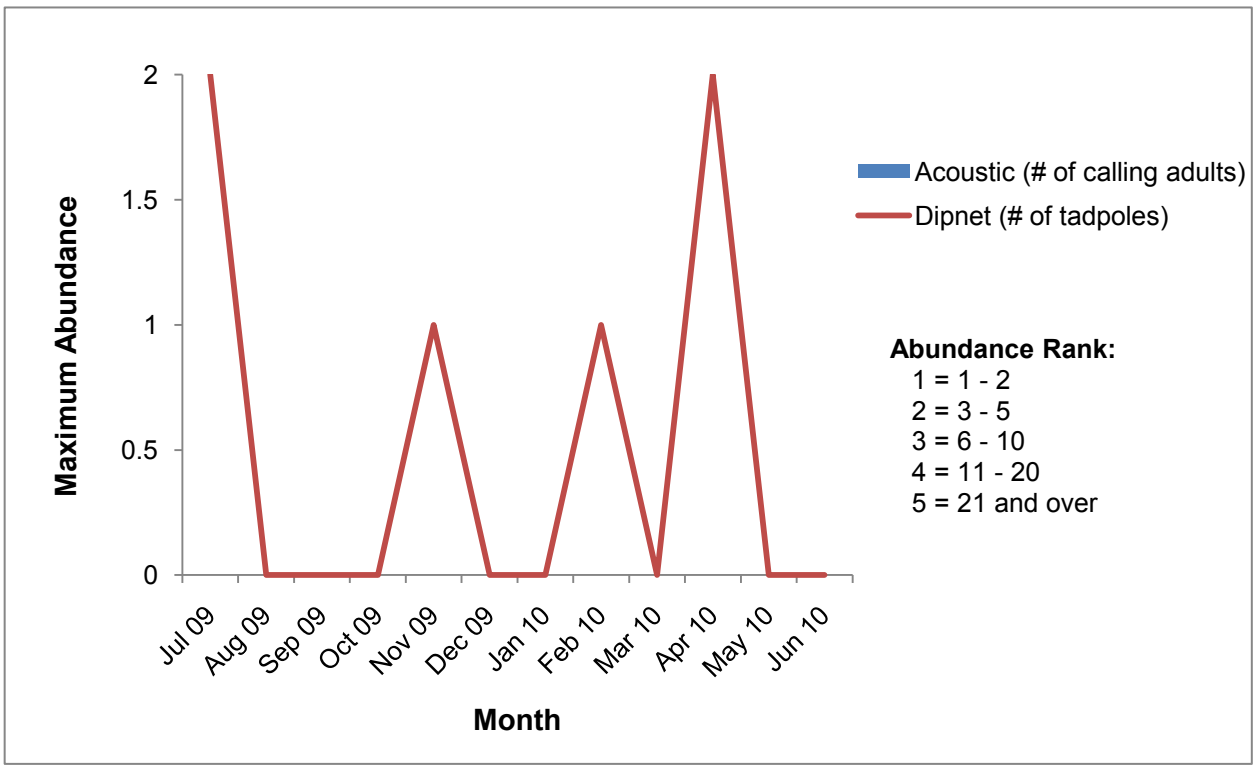

Figure 24: Breeding activity for *Xenopus* over the period July 2009 - June 2010.

Xenopus displayed breeding activity in April and July which renders the genus a continuous breeder. These were also the months when peak dipnet abundance was observed, a maximum abundance value of 2.00 in both cases. No breeding activity was recorded in September, January, March, May and June. Species belonging to this genus produce a soft vocalisation underwater that is difficult to hear by observers in the field. This explains the zero acoustic abundance throughout the year, and thus it is not a reflection of calling males.

3.3.4 Correlations of breeding activity with rainfall and flood pulse

In order to discover any possible trends in breeding activity, rainfall and flood pulse were investigated as possible drivers for breeding. Figures 25 and 26 are the resultant scatter plots.

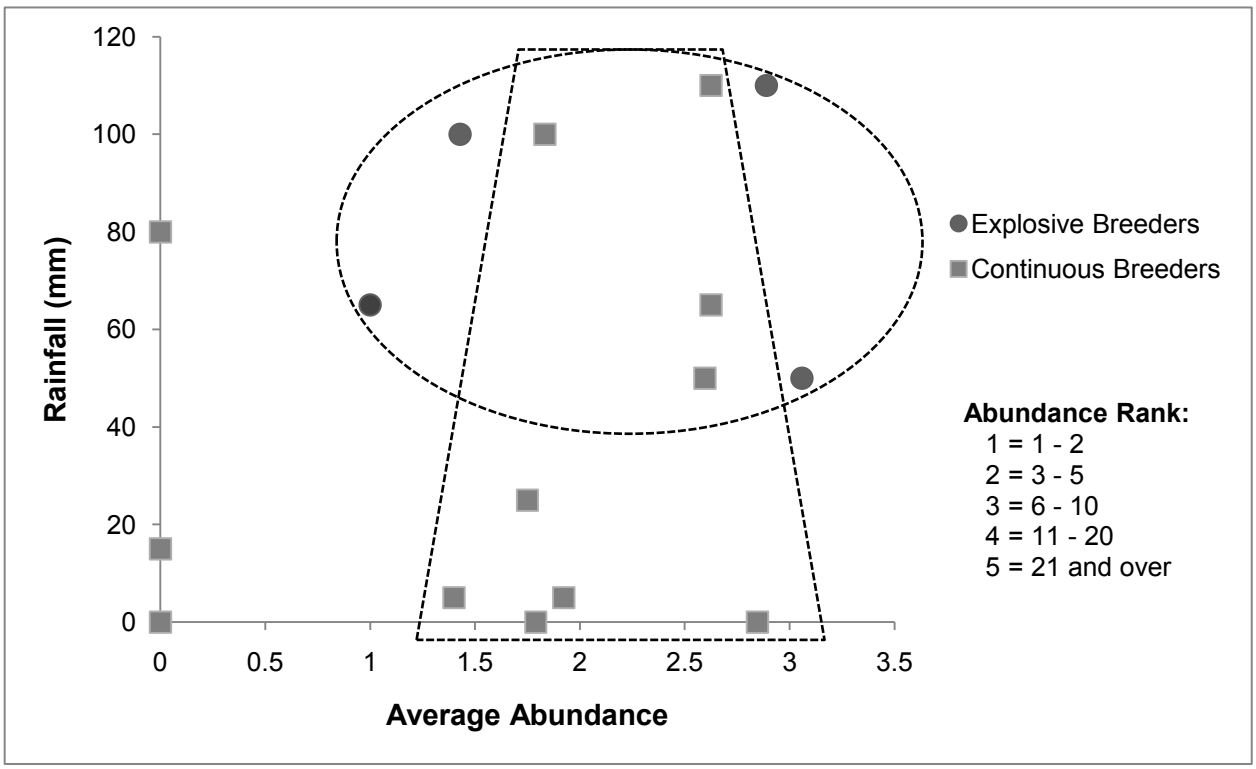

Figure 25: Breeding activity for each breeding class compared with rainfall.

Two discrete clusters of breeding activity are evident. The first (oval shape) encompasses all the average monthly, explosive breeding observations [note however that it also contains some continuous breeding observations]. This cluster is grouped and occurs only at high rainfall levels, above 50mm. The second cluster (trapezoid shape) encompasses all the continuous breeding, average monthly observations. It ranges from 0 to 120mm of rainfall and seems scattered with less dependence on rainfall.

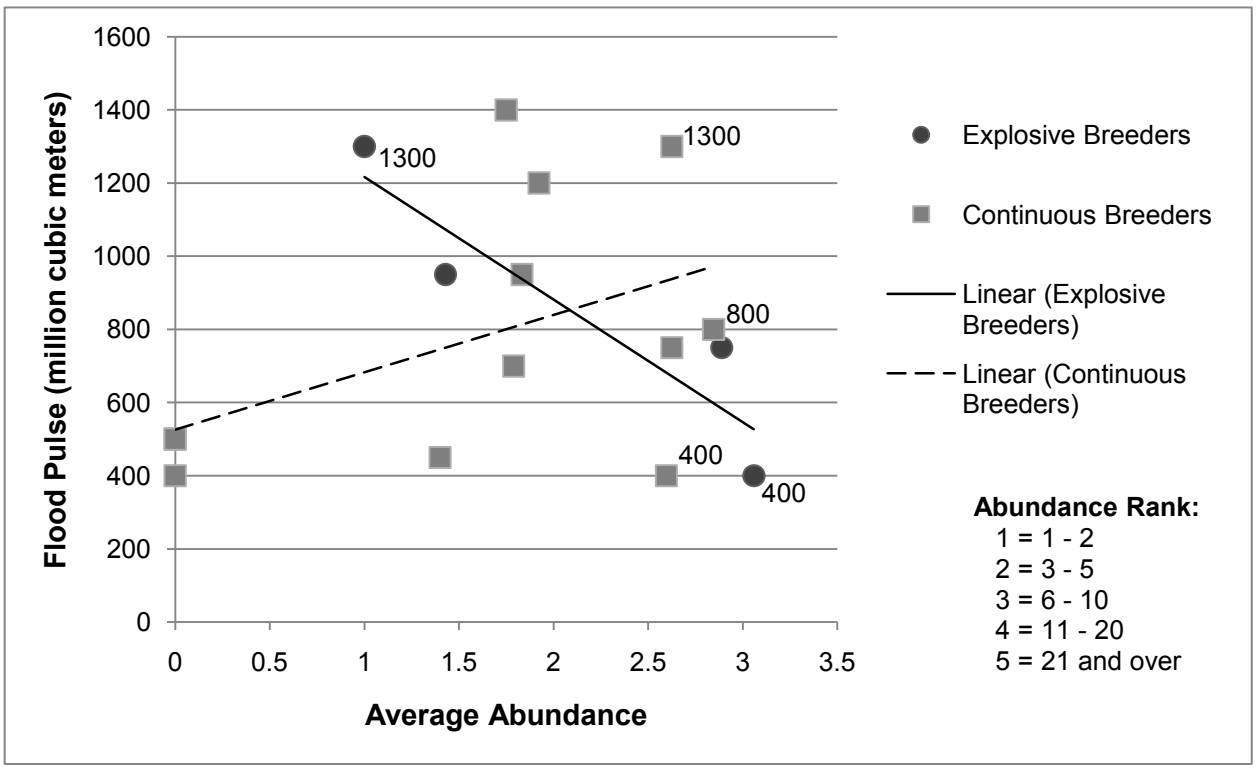

Figure 26: Breeding activity for each breeding class compared with flood pulse.

Two very different trends in the breeding response of each class to flood pulse are displayed. Explosive breeding activity is negatively correlated with increasing water flow levels. Peak activity is recorded at 400 million m³ while lowest activity is recorded at 1300 million m³. Continuous breeding activity, on the other hand, shows a positive correlation, and breeding activity increases as water flow levels increase. However, it should be noted that peak activity levels occur both at high (1300 million m³), intermediate (800 million m³) and low (400 million m³) water flow levels.

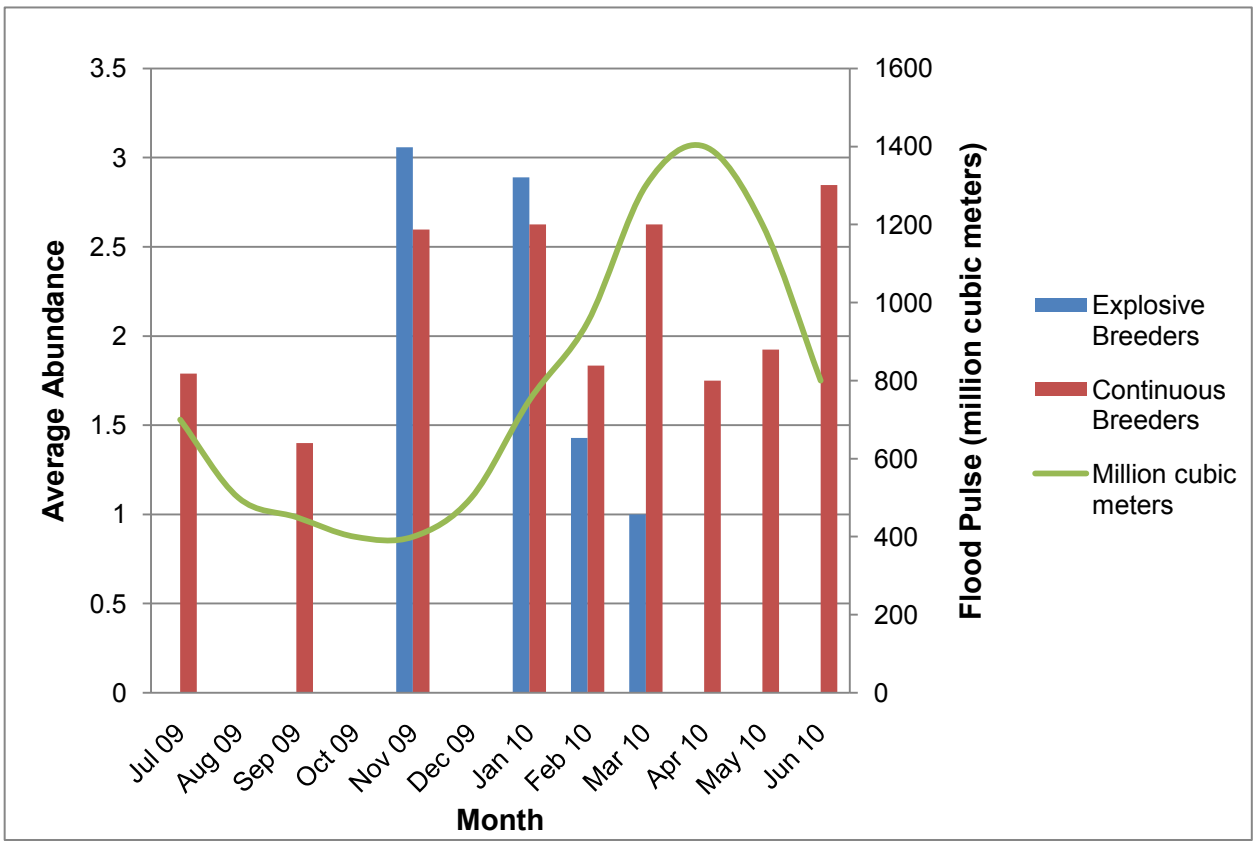

Figure 27: Breeding activity for each breeding class and flood pulse water flow levels for 2009 – 2010.

When flood pulse and average abundance were superimposed over the study period, it seems apparent that peak breeding activity for explosive breeders (in November and January) occurred prior to the arrival of the annual flood around March (Figure 27). Their activity gradually subsided as the flood water levels increased, and then stopped as the flood reached its peak in April. Continuous breeders, on the other hand, seemed to have two peaks of breeding activity. The first between November and March, when flood levels were lowest and gradually began to rise; the second and highest (2.85) in June, a short time after the flood had reached its peak in April.

3.4 DISCUSSION

3.4.1 Comparisons of breeding behaviour: expected versus actual

It has already been well established in the literature that anuran reproductive patterns, both globally and locally in Southern Africa, are largely controlled and initiated by rainfall, with temperature and other climatic factors contributing to a lesser extent (Balinsky, 1969; Auerbach, 1987; Duellman & Trueb, 1994; Passmore & Carruthers, 1995; Channing, 2001; du Preez & Carruthers, 2009). Based on this, it was expected that the breeding behaviour of species of the Okavango Delta would correspond to approximately 58% explosive breeders and 21% for each of the prolonged summer breeders and continuous breeders. The results of actual observations, however, proved considerably different from what was expected, with the explosive breeding class decreasing substantially, the prolonged summer breeding class falling away completely, and the continuous breeding class increasing dramatically by 45%. Such marked differences solicit further investigation into the breeding behaviour of the two breeding classes that occur there, as well as that of individual genera in order to determine precisely which taxa exhibit differences, and what the possible drivers for this unexpected breeding behaviour may be.

The genera recorded as explosive breeders in this study displayed typical explosive breeding behaviour, with breeding activity only observed between November 2009 and the end of March 2010. In fact, simply the presence of all the species belonging to these genera, as adults not showing signs of breeding, was also only observed during this period, barring one observation of a juvenile *Hemisus guineensis* and one of a juvenile *Pyxicephalus edulis* in April 2010. Calling adults of *Breviceps, Chiromantis, Hemisus* and *Kassina* were first observed in November, with observations of *Chiromantis* foam nests (housing eggs) and *Kassina* adults in amplexus also first seen in this month. *Hemisus* tadpoles were already observed in November, while *Chiromantis* and *Kassina* tadpoles were recorded in January. *Breviceps* do not have a free-swimming larval stage and metamorphs emerge directly from the egg mass (du Preez & Carruthers, 2009), but no metamorphs were observed. Although *Tomopterna* was first heard calling in January, their tadpoles were recorded in November, and thus it can be assumed that they were active from November at least. The final two genera, *Pyxicephalus* and *Phrynomantis,* were only recorded in January and the first week in February. From all these results it is evident that these seven Okavango Delta genera are typically explosive breeders and their behaviour is consistent with the literature and that of other populations in the species‟ range.

Such breeding behaviour is expected when the life histories and habits of these genera are examined more closely. The species belonging to *Breviceps, Hemisus, Pyxicephalus* and *Tomopterna* are all fossorial, characterised by digging and burrowing, and spend most of their time underground, only emerging to breed with the rains. *Hemisus* and *Breviceps* even lay their eggs in underground chambers. *Pyxicephalus* species have the most pronounced fossorial behaviour of all: they spend majority of their lives enclosed in a cocoon approximately one meter underground and emerge only in the peak of the rainy season after very heavy downpours. They have the shortest breeding season of all the genera concerned, and they can spend several years buried if rainfall conditions are not favourable (Balinsky, 1969; du Preez & Carruthers, 2009). *Kassinas* require relatively moist environments where they inhabit concealed sites some distance from water (du Preez & Carruthers, 2009), and they have been known to inhabit mole rat burrows out of the breeding season (Eloff, 1952). *Chiromantis* and *Phrynomantis* also spend the non-breeding season in hibernation; the former is arboreal and finds shelter in trees or cool buildings, while the latter seeks refuge in holes and crevices in logs, tree trunks and rocks, as well as having been observed to burrow (Carruthers, 1995; du Preez & Carruthers, 2009).

From the above accounts, it is clear that all species belonging to the explosive breeding class are adapted to survive in dry and arid conditions by means of hibernation. Consequentially, they display typical explosive breeding behaviour by only emerging after heavy rainfall, as observed in this study and according to the literature. Their reproductive strategy is aimed to exploit temporary water bodies that appear after the rains and a major adaptation is swift larval development to ensure metamorphosis into the adult form occurs before temporary water bodies dry out. It thus follows that rainfall is likely the major initiator of breeding activity, so that spawning occurs as soon as water is available to ensure maximum time for larval development and metamorphosis, and therefore maximum breeding success (Balinsky, 1969).

It was the reclassification of the genera *Ptychadena*, *Amietophrynus* and *Xenopus* that resulted in the 45% increase in the continuous breeding class. Although only three out of the twelve genera were reclassified, they include 13 out of the 29 species and thus result in the dramatic class shift. Each genus is discussed individually below:

***Ptychadena*:** This genus was reclassified from explosive to continuous breeders. Wells (1977) acknowledged that these species may breed over several months, but that breeding was mostly explosive in nature consisting of several peaks within the wet months, and that breeding activity was frequently stimulated by rainfall. Du Preez & Carruthers (2009), too, refer to the explosive breeding strategy displayed by these species, even though they continue to be active throughout most of the year. As expected, acoustic activity and several visual encounters of adults were observed throughout the year; this is consistent with the literature. However,

findings from the dipnet surveys portray an unexpected breeding strategy for species occurring in the Okavango Delta. Not only were tadpoles observed throughout the dry, winter months from April to August, but the second highest collection of tadpoles was recorded in July 2009. For those species where information is available, metamorphosis is completed within four to nine weeks (du Preez & Carruthers, 2009); it thus follows that mating was still taking place at the end of April and later for those with shorter development time. This confirms that the genus is not only active, but also actively breeds in the non-rainy season, and this implies that rainfall is not the sole driver of breeding.

***Amietophrynus*:** This genus was reclassified from prolonged summer breeders to continuous breeders. This change was the result of peak acoustic activity in June as well as tadpoles observed in the same month. Although this extension of breeding season contradicts the majority of literature, it is not completely unexpected since Channing (2001) recorded continuous breeding for *A. gutturalis* in the northern part of its range, and Balinsky (1969) recorded spawning in *A. gutturalis* prior to the onset of the first spring rains. Again, it follows that rainfall may not necessarily be required for the onset of breeding activity. The fact that species belonging to this genus are known to breed in both permanent and semipermanent water bodies that are available regardless of rainfall, suggests that their breeding is not dependant on rain as there is no need for spawning with the onset of the rain and rapid larval development (Balinsky, 1969).

***Xenopus*:** This genus also changed breeding classes, from prolonged summer breeders to continuous breeders. Although there were no acoustic encounters to indicate breeding, the highest tadpole collections were recorded in April and July, indicating breeding in the dry winter months. This is not unexpected since Balinsky (1969) observed an extended breeding season in *X. laevis,* lasting six and a half months, which was the longest of all Highveld species. Again, breeding is not necessarily correlated with rainfall.

For all three genera, breeding was evident in the summer rainy season, as expected, with tadpole collections peaking in November for *Ptychadena* and *Amietophrynus*; however, it can be concluded that breeding is not restricted to these months. The results imply that there may be several breeding peaks for these genera over the course of a year, and that these peaks are not consistently associated with rainfall. It appears as though these genera are opportunistic breeders, utilising other environmental variables for the commencement of breeding activity. Balinsky (1969), too, refers to the variety of mechanisms that seem to influence spawning, and that these mechanisms not only vary between species, but may even vary between different geographical populations within the same species.

3.4.2 Mechanisms responsible for anuran breeding activity in the Okavango Delta

The Okavango Delta is unique in that it has two wet seasons with high biological productivity: the first is standard and occurs in the summer resulting from rainfall; the second is unusual and occurs in the winter resulting from the annual flood. Ramberg *et al.* (2006) reports that many of the mammal species occurring in the region are winter breeders, even though they are stringent summer breeders in other parts of the species‟ range in Southern Africa. They suggest this breeding strategy has evolved to exploit higher biological production resulting from the flood pulse in winter. Furthermore, they suggest this evolution has a genetic basis, not merely a behavioural one, since mating occurs prior to the flood and offspring are born simultaneous to flood arrival, thus indicating that flood arrival is not the trigger for reproduction.

With this in mind, correlations of breeding activity with rainfall and flood pulse were investigated. When breeding activity was compared with rainfall, two discrete clusters were evident. The explosive breeding genera exhibit a clear correlation with rainfall, breeding only when 50mm or more rain has fallen. This corresponds with the very definition of explosive breeders and their need for temporary water bodies (see Figure 28) within which to spawn and where tadpoles develop (Balinsky, 1969; du Preez & Carruthers, 2009). The second cluster of continuous breeding is clearly not affected by rainfall and there is no correlation between the two. They exhibit breeding activity throughout the rainfall range, breeding from zero to 120mm of rainfall; in fact, peak abundance for this class was actually recorded at zero rainfall. This is easily explained by the fact that continuous breeders frequently breed in permanent water bodies which are present regardless of rainfall (Balinsky, 1969).

Figure 28: Tadpoles of explosive breeders observed in a temporary rainwater pool in November 2009.

When breeding activity is compared with flood pulse, however, a very different situation emerges. In contrast to rainfall, explosive breeders now exhibit a negative correlation with flood water levels while a positive correlation exists for continuous breeders. Since explosive breeding activity is highly dependent on rainfall, this negative correlation to flood pulse is expected and not regarded as extraordinary. In the case of continuous breeders, however, a positive correlation indicates that some underlying environmental factor associated with the flood may act as an alternative mechanism that initiates breeding in this class; and it may explain the opportunistic breeding behaviour observed in the genera *Ptychadena, Amietophrynus* and *Xenopus.* Although not common, such opportunistic breeding behaviour in amphibians is not unknown in Southern Africa. Balinsky (1969) observed spawning in *Rana angolensis* in April, May, August and September in addition to its summer breeding, and it should be noted that this species is also permanently associated with water.

It can therefore be concluded that the breeding activity of some amphibian species occurring in the Okavango Delta is controlled not only by rainfall, as is the case for the majority of Southern African taxa occurring in summer rainfall areas, but also by some additional mechanism possibly related to the flood pulse that result in opportunistic breeding in winter (Figure 29A and

B). The question then, is what mechanisms could be responsible for opportunistic breeding in the Okavango Delta?

A:

B:

Figure 29A and B: Spawn of continuous breeders observed in shallow floodplains in July 2009.

3.4.3 Flood pulse influences on amphibian breeding strategies

In the course of his extensive research on the common frog *Rana temporaria* and its breeding strategies, Savage (1961) formulated the *algal hypothesis* as an explanation for the onset of spawning activity. He agrees that there are numerous factors such as light, temperature, rainfall, altitude and humidity that may influence spawning initiation, but he suggests that these factors act indirectly, rather than having a direct effect on spawning (Savage, 1961). His hypothesis suggests that breeding is initiated and controlled by the growth of algae in water bodies, which in turn is dependent on a variety of environmental factors. He proposes that adult frogs are likely drawn to specific breeding sites as a result of the pungent smell released by algae, and that they choose to spawn in ponds with high algal concentrations since this is an important food source for tadpoles (Savage, 1961; Balinsky, 1969). He concludes that although the right physiological state of a frog is essential for spawning to occur, his observations have led him to believe that it is actually the state of the breeding site that controls breeding activities. In other words, breeding is controlled by changes in the environment rather than changes in physiology. In his work, Savage found many parallels between the behaviour of frogs and the behaviour of algae; and thus, despite not being universally accepted due to difficulties in empirical testing, his observations do fit and support his hypothesis (Savage, 1961).

The algal hypothesis obviously does not apply to explosive breeders in Southern Africa that spawn shortly after heavy rainfall and prior to any algal growth, but may be of importance to continuous breeders that spawn in permanent water (Balinsky, 1969). In the Okavango Delta, it may be argued that this hypothesis is also generally applicable, with a few alterations due to factors unique to that ecosystem.

Figure 27 displays the relationship between flood pulse and breeding activity for both breeding classes. It is clearly visible that there is a second breeding peak for continuous breeders in June, and this follows shortly after the peak in flood water levels in April. It should be noted that the water levels displayed in this figure were gained from readings at the top of the Panhandle, and this flood water takes approximately four to six weeks to reach the present study area. Therefore, the flood water peak in April would arguably have occurred around mid May in the area where breeding activity was recorded, reducing the time lapsed between flood and breeding peak to less than four weeks. This implies that some underlying environmental condition(s) associated with the flood pulse may initiate a second wave of breeding activity in amphibians of the Okavango Delta.

Høberg *et al. (*2002) investigated the effect of the annual flood on aquatic productivity in the Delta and the accompanying food web dynamics in the floodplain ecosystem. The production

events following the arrival of the flood as follows: firstly, as nutrient deficient flood waters enter the floodplains from the channels and permanent swamp, where primary productivity is characteristically low, they are rapidly enriched with nutrients from various sources. Also, dormant microbes in the soil become active once submersed, and thus add even more nutrients to the water (Mendelsohn *et al.,* 2010). This is followed by an almost instantaneous surge of primary production and large blooms of phytoplankton and other autotrophic organisms. Next follows massive populations of zooplankton, such as cladocerans, whose densities are highest along floodplain and seasonal swamp shorelines (Høberg *et al.,* 2002). The eggs of zooplankton lies dormant in dry sediments, hatch with the arrival of the flood and congregate in the shallow waters on the peripheries where there is maximum nutrient release, abundant food in the form of phytoplankton, algae and bacteria, and where they can find shelter to avoid predators (Mendelsohn *et al.,* 2010). Finally, the heterotrophs flourish: fish and other predators such as insects take advantage of the increased production, feeding intensively on zooplankton, and they in turn provide food for countless higher predators such as birds, frogs, reptiles and mammals (Høberg *et al.,* 2002). Many fish species migrate into the newly inundated areas to spawn, and this is synchronised with the availability of abundant zooplankton on which the fish fry (larvae) feed; it is generally accepted that there is a correlation between fish breeding times and zooplankton abundance (Høberg *et al.,* 2002; Mosepele *et al.*, 2009). The data collected by Høberg *et al. (*2002) therefore concludes zooplankton production is a large contributor to the success of various fish species in the Okavango Delta, and that periodically inundated floodplains and seasonal swamps are essential hotspots of biological production.

Anuran larvae, or tadpoles, are generally classed as specialised herbivores that obtain food by filter-feeding (Duellman & Trueb, 1994); however, it has been suggested that they are better described as opportunistic omnivores or detritivores. In any event, they filter various sized, suspended particles from the water by opening their mouth and creating currents that carry these particles into the buccal cavity. Gut content analyses have revealed a great number of items ingested by tadpoles, some of which include detritus; bacteria; unicellular organisms (e.g. protists); algae (e.g. diatoms); plant and fungal material; small animals (e.g. cladocerans, insects and water mites); faecal and carrion fragments; anuran eggs and other tadpoles (McDiarmid & Altig, 2000). It thus appears as though the floodplain and seasonal swamp habitats of the Delta provide ample feeding opportunities and options for amphibian larvae. From this it can be speculated that, just as fish preferentially spawn in the production hotspots of newly inundated areas, so frogs and toads will take advantage of the increased productivity that the flood pulse brings and follow similar, opportunistic patterns of breeding.

Therefore, it can be reasonably concluded that the opportunistic breeding behaviour observed in the continuous breeding amphibians of the Okavango Delta may be the result of increased

biological production associated with the arrival of flood waters in previously dry zones. Indeed, observations from the present study do support this hypothesis. Dipnet sampling during the winter, peak flood months proved most successful when observers concentrated sampling efforts in the shallow, well vegetated floodplains at the head of advancing flood waters (Figure 30). This concentration of tadpoles in these areas could likely be in response to high concentrations of zooplankton and other food sources that occur there, as well as the additional advantage of shelter and protection from predators among the dense vegetation.

Figure 30: Highly successful dipnet sampling site in recently inundated floodplains in July 2009.

From the results of this study, it is concluded that amphibian breeding behaviour in the Okavango Delta is indeed driven by the hydrology of the ecosystem. Although this conclusion does not apply across the range of amphibians that occur there, the insight gained from this study emphasises the unique nature of the Okavango Delta ecosystem. There is much opportunity for further research in the region, which may include:

- Further investigation into the opportunistic mating strategies of amphibians with a potential for long term monitoring to determine how annual variation in flood extent may affect breeding and reproduction.
- Evolutionary based studies to determine whether speciation may eventually occur resulting in new, endemic species for that region, and how the rate of speciation may be affected by the increased breeding opportunities.
- There is much scope for studies based purely on the genus *Ptychadena* since they are abundant throughout the year; with specific emphasis on vocalisation behaviour as well as the description of tadpoles and breeding behaviour since knowledge is lacking for many of the species belonging to this genus.

CHAPTER 4:
AMPHIBIAN CHYTRID SURVEY IN THE OKAVANGO DELTA

4.1 INTRODUCTION

4.1.1 A history and review of amphibian chytrid fungus

As mentioned previously, rapid and dramatic declines in amphibian populations worldwide have caused much concern amongst scientists and naturalists alike over recent decades. Observable declines began in the 1970s, but severe statistics have emerged since the 1980s, with at least nine and up to 122 amphibian species becoming extinct since that time. 435 Species have been classed as "rapidly declining" by Stuart *et al.* (2004). Of these, almost 50% of losses are "enigmatic declines," which means that declines occur in seemingly pristine habitats resulting from causes not altogether understood (Stuart *et al.*, 2004). However, the emerging infectious disease chytridiomycosis (also known as amphibian chytrid fungus), caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), has been identified as a possible, major causal factor (Berger *et al.*, 1998; Daszak *et al.*, 1999; Collins & Storfer, 2003).

Chytridiomycosis is a non-hyphal zoosporic fungus first described by Longcore *et al.* (1999) that develops within the thickened, superficial epidermis of an animal, specifically in the skin of the feet, hind legs and ventral abdomen (Longcore *et al.,* 1999; Bosch *et al.*, 2007). Infections are associated with keratinised body part regions: therefore, it affects the mouth parts of tadpoles or alternatively, the skin of metamorphs and adults. Mass mortality, however, has mainly been observed in post-metamorphic specimens. It is speculated that the fungus causes death in these specimens through severe impairment of cutaneous respiration and osmoregulation (Voyles *et al*., 2009). An alternative hypothesis holds that anurans absorb toxins produced and released by the fungus (Berger *et al.*, 1998). The disease is spread by aquatic zoospores, and a range of effects in infected populations have been observed, from zero impact to mass mortality and even extinction.

A review of literature by Daszak *et al.* (2003) confirmed that amphibian declines are best explained by chytridiomycosis, and that the spread of the disease is likely attributed to anthropogenic introductions into novel regions. This argument is further supported by the publication of Skerratt *et al.* (2007), which concluded that chytridiomycosis is the primary cause

of enigmatic amphibian declines and extinctions. Furthermore, they also present evidence that spread of the disease is likely due to the *„spreading pathogen hypothesis'* which states that (1) the fungus is extremely pathogenic, virulent and transmissible, responsible for population declines and extinctions; (2) the fungus is spreading; and (3) it is present during population declines and mass die-offs. The impact of this disease on amphibian populations have proven catastrophic: within four to six months of its introduction into a novel site, losses of up to 50% of species and 80% of individuals have been reported. Compounding environmental and anthropogenic factors may also be adding to the severity of the disease, with global climate change possibly providing ideal conditions for Bd to thrive; the commercial trade in wildlife probably responsible for spreading the fungus to new areas; and pollution increasing amphibian vulnerability to disease and pathogens (Mendelsohn *et al.,* 2006).

Epidemiological studies on the origin and spread of Bd were done by Weldon *et al.* (2004). They found that Bd is widely spread throughout Africa, common from specimens of Ghana, Kenya, South and West Africa; and the first wild anuran specimen positive for Bd dates back to 1938, from a collection in South Africa. Chytrid fungus is present on all continents that amphibians inhabit, and the authors propose that Bd originated in Africa and was spread to other countries worldwide from South Africa, most likely due to the global trade and movement of amphibians: they proposed the *novel pathogen hypothesis.* They speculate that *Xenopus laevis* was a probable natural carrier since the species does not die or become diseased when infected by the fungus. In addition, many wild specimens of this species were captured and exported subsequent to the discovery in 1934 that they could confirm pregnancy in humans, and their accidental escape or discarding of the water within which they lived may have resulted in transmission of the disease to other local amphibians in the country of importation. Introduction of chytrid fungus into novel host populations without a naturally evolved immunity, as is present in many African amphibian species, could explain the appearance and disastrous effects of chytridiomycosis in new regions.

An alternative hypothesis is provided by James *et al.* (2009), whose work focussed on the DNA sequences and population genetics of chytridiomycosis. Their results also support the novel pathogen hypothesis of Weldon *et al.* (2004), based on low genetic variation of the disease and the extensive dispersal of closely related strains. However, although their results do not point to any specific source population, they speculate that the disease may have originated from eastern North American bullfrog populations that contain sufficient heterozygosity to provide all the allelic variation thus far collected for all global strains of the disease.

Despite the fact that some species display no negative effects when exposed to the disease, many display severe declines and even extinction, resulting in the description of

chytridiomycosis in the *Amphibian Conservation Action Plan* as „the worst infectious disease ever recorded among vertebrates in terms of the number of species impacted, and its propensity to drive them to extinction,"" (Fisher & Garner, 2007; Gascon *et al.*, 2007). In light of these facts, conservation efforts must be swift and aggressive, with a particular emphasis on research into the control and management of the disease (Skerratt *et al.* 2007). Fisher & Garner (2007) stress the importance of active measures aimed at controlling the emergence and spread of amphibian chytrid fungus. They highlight threat abatement plans that have been successful, and prompt all countries to implement similar strategies; either to control and limit the spread of the disease, or to control potential threats that may result in new introductions, especially in current disease-free regions.

4.1.2 Current status of amphibian chytrid fungus in the Okavango Delta

Over the last decade, several chytrid surveys have been carried out in Central, Eastern and South Africa, specifically in the Democratic Republic of Congo, Uganda, Tanzania, Kenya and South Africa; incidentally, the results of these studies all confirmed the presence of Bd in the respective sampling areas (Weldon *et al.,* 2004; Moyer *et al.*, 2006; Goldberg *et al.*, 2007; Greenbaum *et al.*, 2008; Kielgast *et al.*, 2010). An assessment of amphibian chytrid fungus, however, has not been undertaken in the Okavango Delta region in the past. However, due to the widespread distribution of Bd across the rest of the African continent, it is highly likely that Bd is present in the ecosystem, or at least that it is extremely vulnerable to Bd introduction.

In this section of the study, the hypothesis and objectives as follows:

- Amphibian chytrid fungus is a threatening process to frogs in the Okavango system.
- To establish the Bd status and its prevalence in populations of amphibians for each locality.
- To report any possible amphibian die-offs.

4.2 METHODOLOGY

4.2.1 Amphibian collection and sampling effort

Amphibian collections were conducted as specified in Chapter 2 with the goal of collecting a minimum of 20 specimens per sampling site, as this provided a sufficiently large sample size for Bd. A sample size of 20 is required to say, with 95% confidence, that Bd is absent from an area given an expected prevalence of 15% (DiGiacomo & Koepsell, 1986). Any adult specimens collected during sampling sessions were stored in individual plastic bags until swabbing occurred at the end of the session or at the field lab; between handling specimens, gloves were changed or hands thoroughly washed and dried. Prevention of disease contamination between specimens was considered a high priority; therefore, precautionary measures were put in place to avoid any possible false positive results. Collection of Bd occurred for the duration of the study in order to collect as many samples from as many species as possible.

4.2.2 Procedure for Bd Swabbing

2. Wearing fresh gloves, a specimen was removed from its bag and firmly held upside down so that the ventral surface of the animal was exposed. See Figure 33 at the end of the chapter.
3. A sterile, cotton swab was removed from its housing; careful caution was taken to ensure the swab did not become contaminated. The swab was rolled, and gently but firmly scraped over the belly, inside hind legs, feet and webbing between toes ten times; no part of the animal that may have come into contact with the ground was left un-swabbed.
4. The cotton bud was carefully placed back in its labelled housing and allocated a collection number. Again, care was taken to ensure the cotton bud did not come into contact with anything that may have caused disease contamination.
5. If the specimen was not being used for the voucher specimen collection, it was returned to its plastic bag for storage until its later release at the original site of capture.
6. As soon as possible, the swabs were refrigerated and stored at a temperature of 4°C at the North-West University until they could be analysed.
7. Swabs were analysed at the National Zoological Gardens (NZG), Pretoria, South Africa. Qiagen®[3] DNeasy®[4] blood and tissue kit was used according to the manufactures‟ instructions to isolate DNA from the swab samples. The presence and quantity of the fungus was determined by using real-time PCR TaqMan standard curve assay (Boyle *et al.* 2004).

[3] Qiagen®is a registered trademark of Beckman Instruments, Inc
[4] DNeasy® is a registered trademark of Beckman Instruments, Inc

4.3 RESULTS

4.3.1 Sampling effort

A total of 249 swab samples were collected between 28 November 2008 and 14 May 2010. Increased activity of adults during the warm, rainy season resulted in the majority of collections being obtained in summer: only 3 swabs were collected in winter (March to October). There were no observations of any amphibian mass die-offs during the course of this study.

Of the 12 genera, 29 species recorded during this study, Bd samples were collected from specimens across 11 genera and 25, possibly 26, species as shown in Figure 31.

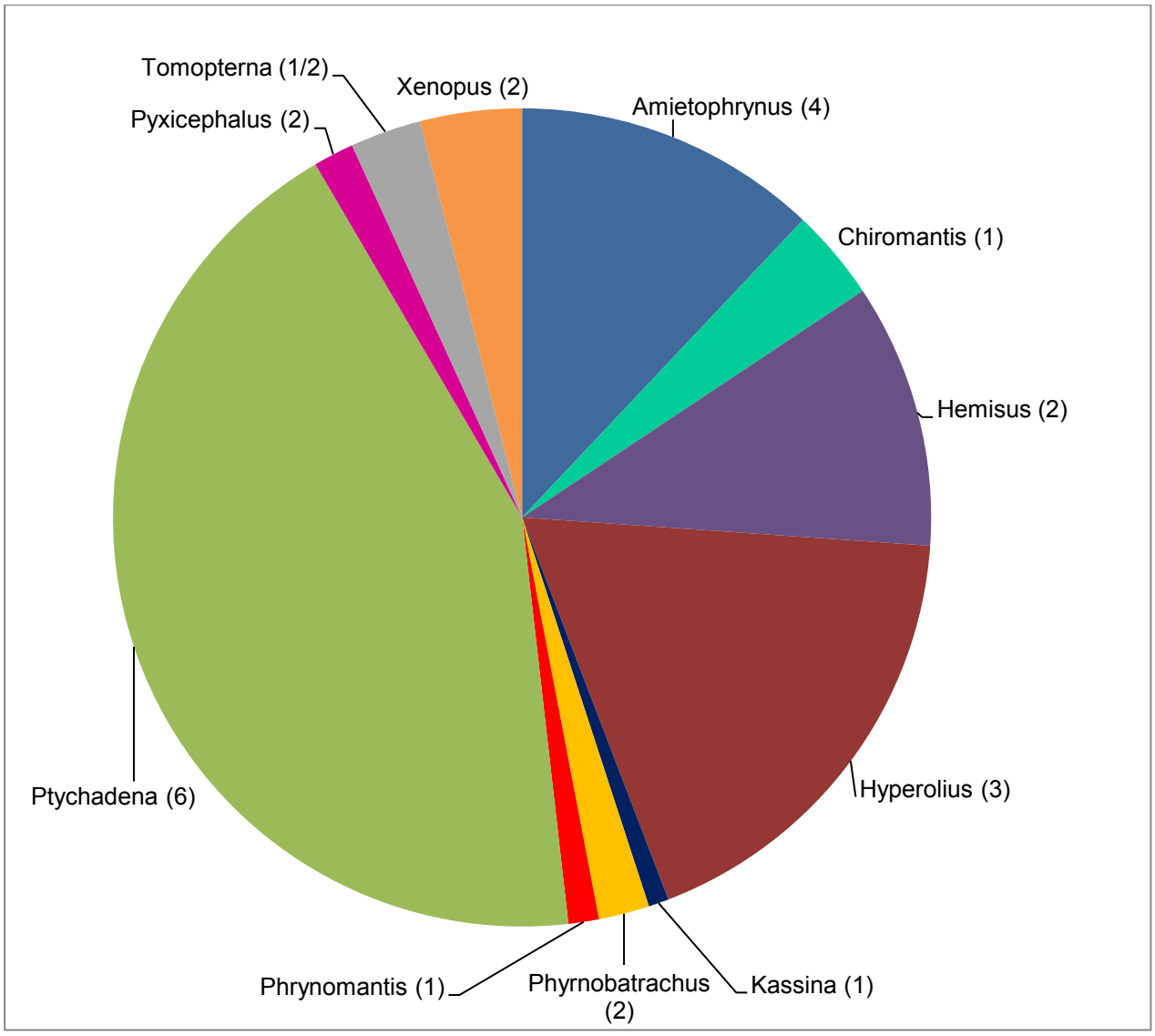

Figure 31: Division of total collected chytrid samples for each genus (number of species belonging to each genus in parentheses).

Swab samples were not obtained from three, possibly four, species: (1) *Breviceps adspersus adspersus,* (2) *Phrynobatrachus natalensis,* (3) *Ptychadena mossambica* and possibly (4) *Tomopterna cryptotis or T. tandyi.* Bd swabs were possibly not collected from one of the two species belonging to the *Tomopterna* genus; these species are morphologically indistinguishable in the field since species can only be separated on the basis of genetics or vocalisation. Therefore, it was not possible to assert whether chytrid samples were obtained from only one, or both, species. A large majority of samples (108 swabs) were obtained from the genus *Ptychadena*, which is consistent with the high number of species (6) belonging to this genus. This was followed by *Hyperolius* with 45 swabs and *Amietophrynus* with 30. The least number of swabs was obtained from *Kassina* with only 2 swabs, followed by *Phrynobatrachus* with 3 and then *Pyxicephalus* with 4 swabs.

4.3.2 Status of Bd in the amphibians of the Okavango Delta

In total, 43% of samples were collected from Xigera, 26.9% from Mombo and the remaining 30.1% from Kwedi. Figure 32 gives an indication of the relatively even geographical distribution of collected samples in each locality across the study area, and the number of samples that were collected at each sampling site. In order to provide a more robust data from which more accurate inferences could be made regarding the status of Bd in the study area, sampling sites that were geographically close enough to allow for amphibian dispersal between the sites were grouped; these sites were often connected by water corridors. For the present section, therefore, Mombo had 7 chytrid sampling sites, while Xigera and Kwedi each had 5 sites. Within each of these sampling sites, the number of swabs collected across all species, fell in the range of 2 to 39 swabs for Xigera; 1 to 24 for Mombo; and 0 to 39 for Kwedi.

The results from the swab analyses have, thus far, not tested positive for Bd. Forty nine (19.68%) swabs are still awaiting analysis, 1 swab did not contain enough DNA to yield any result, and the remaining 199 swabs, or a total of 79.92% of total samples, tested negative for amphibian chytrid fungus.

It can be concluded, with 95% confidence, that chytrid is absent from at least 47.06% of sampling sites. This equates to seven sites with sample size above 20, and one sample site with a sample size of 18, only marginally below the minimum sample size. It is thus concluded, with relative certainty, that Bd is absent from the present study area. This is supported further by the fact that each study locality contained at least 2 sampling sites where sample size was sufficiently large (over 20); and thus the absence of Bd from each study locality can be concluded with relative confidence.

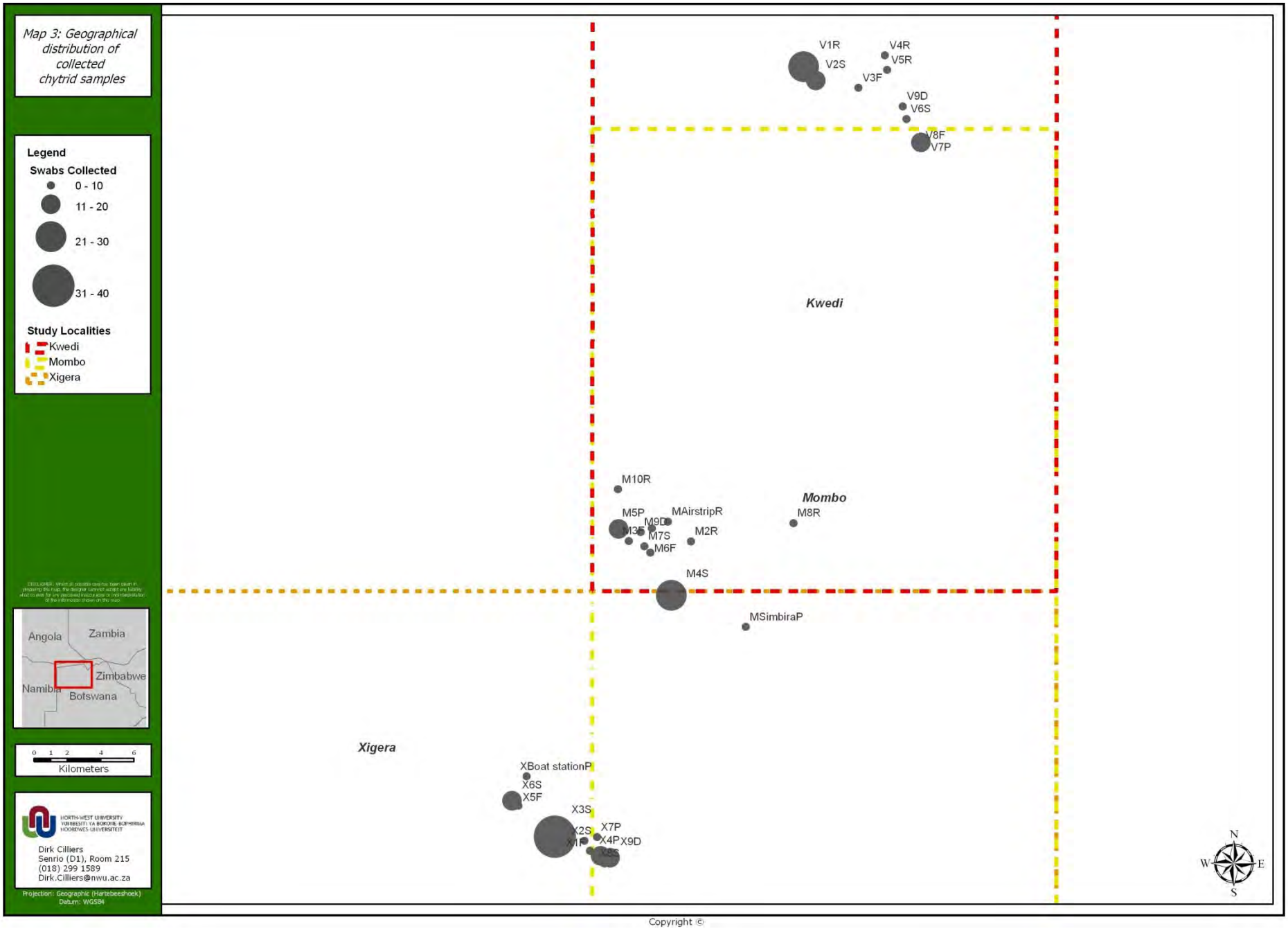

Figure 32: Geographical distribution and quantity of collected chytrid samples.

4.4 DISCUSSION

4.4.1 Absence of amphibian chytrid fungus from the Okavango system

The degree of coverage of chytrid sample collection both geographically and taxonomically in three different localities implies that the results accurately reflect the status of Bd for this part of the Okavango Delta, and it is concluded that Bd appears to be absent from the study area. Based on an expected 15% prevalence level, it can be concluded with confidence that chytrid is absent from nearly half of sampled sites, and together with a lack of even one positive result, this conclusion is acceptable. Based on recent, comparable studies that did not detect Bd, it appears that the current sample sizes are sufficiently large to allow for this accurate deduction. The best comparison, based on expected prevalence level, is provided by Weldon *et al.* (2008): they collected a minimum of 20 samples per site for ten localities based on an expected 10% prevalence level. Richards *et al*. (2008) collected five specimens from each of 17 localities, no prevalence level stated; and Lehtinen *et al.* (2008) collected 20 specimens from one locality, again with no prevalence level given. Both of these studies, however, inferred chytrid absence from their results. In addition to this, samples were collected from a large majority of the amphibian species present in the Delta, thus the possibility that collections were obtained from certain species that may be resistant to the disease is unlikely. It is thus reasonable to conclude that Bd is either absent from the system, or alternatively, is only present in a very low level of prevalence which has remained undetected thus far.

A need for further sampling to corroborate the current results must, however, be emphasised. Despite a lack of Bd detection for all collected samples, Chestnut *et al.* (2008) could not infer Bd absence in Alaska due to low sample size that dramatically reduced confidence level, between one and seven specimens per sample site. In the present study, several sites did not have a sufficiently large sample size. In fact, several of the sites had a sample size less than seven. Therefore, the implication from the well sampled sites that Bd appears absent must be substantiated further through additional chytrid collection.

Two hypotheses may be proposed to account for the apparent lack of this disease in the region. Firstly, Bd may not yet have spread to or reached the Okavango Delta and the nature of the Delta as a mosaic of island and wetland habitats could have inhibited the dissemination of the disease from the surrounding mainland towards the interior. However, positive Bd results have been obtained from the Kasane area to the north-east of the Delta (C. Weldon, unpublished data). This area has intermittently been connected to the Delta via a waterway called the Selinda spillway (Mendelsohn *et al.*, 2010), and thus it follows that the Okavango system may

have been exposed to Bd in the past. In addition, the widespread distribution of the disease over other parts of the African continent suggests it is unlikely that the region has never been exposed to amphibian chytrid fungus (Weldon *et al.*, 2004). Perhaps an explanation is given by the fact that although Bd is dispersed through aquatic zoospores that may travel long distances in water currents, the chance contact of a zoospore with a host when they are present in such low concentrations, in masses of water, is rare. Also, the Delta is surrounded by mostly desert country, thus dispersal of adult individuals, possibly carrying the disease, and their coming into close contact with another amphibian to transmit the disease is uncommon (Piotrowski *et al.*, 2004). However, frog to frog transmission seems a more likely means of spread than water medium mediated spread in the Okavango Delta.

The second hypothesis relates the absence of Bd due to unsuitable ecological conditions that do not allow the disease to survive or flourish. Several studies have been dedicated to exploring the environmental conditions within which the disease seems to thrive or diminish, and factors such as temperature, altitude, pH, rainfall and nutrient availability have all been investigated (Piotrowski *et al.*, 2004; Bosch *et al.*, 2007; Pounds *et al.*, 2006;
et al., 2007; Kriger & Hero, 2008). Investigation of ecological factors within the Okavango Delta was beyond the scope of the present study, but such factors should be scrutinised in detail if a cause for the absence of chytrid is to be found.

A novel hypothesis that is unique to the Okavango Delta may relate to the exceptional purity of the Delta"s water: to put this in perspective, the Delta"s water is said to be 40 times better than that classed as *acceptable water quality* by the government. This purity results from three system processes: (1) extremely high rates of water infiltration due to the permeability of the substrate; (2) the constant, lateral movement of water from floodplains to beneath islands from where water transpires through island vegetation and salts concentrate below islands; (3) hyper-saline groundwater below islands gradually become denser than the water beneath it and subsequently sinks to much deeper levels (Mendelsohn *et al.*, 2010). It could be speculated that this consistent and aggressive removal of water from the Okavango Delta ecosystem may simultaneously remove any substances or organisms, such as chytrid zoospores, suspended in it as well. This intense filtration would likely reduce zoospore load that may influence water mediated transmission, but it would not completely remove Bd from the system since frog to frog transfer would still be a major spreading factor. In fact, Mendelsohn *et al.* (2010) state that only 6% of dissolved material entering the Delta actually leaves via outflow, the remainder is removed by the above processes. It thus follows that the above speculation deserves further attention and research.

4.4.2 Protection of the Okavango Delta's amphibian communities

The Australian Government has been a prominent leader in combating the disease in that country. Their *Department of the Environment and Heritage* classed Bd as a "Key Threatening Process" in 2002, and finalised their "Threat Abatement Plan" in 2006, resulting in massive funding and sponsoring from the government for research and projects related to chytridiomycosis (Skerratt *et al.*, 2007). The Australian threat abatement plan aims to prevent further introductions of Bd into regions currently disease free, and minimise Bd impact on already infected populations (Fisher & Garner, 2007). For Bd free countries, Fisher & Garner (2007) specifically highlight the following sections (1) reduce risk of importation; (2) reduce risk of release of Bd; (3) reduce risk of release of infected amphibians; and (4) limited spread upon introduction. Unfortunately, such actions have been slow or lacking in numerous other countries around the world, likely due to a lack of emphasis on the significance of Bd and its potential threats (Skerratt *et al.*, 2007). However, recent efforts to highlight these threats appear to be having an effect, with progressively more conservation strategies emerging worldwide. For example, authorities in Madagascar have this year (2010) agreed to the development of a *National Bd Monitoring Early Detection Plan,* due for implementation in 2011 (C. Weldon, per. comm.)

Regardless of the fact that the Okavango Delta is apparently Bd free for the time being, amphibian chytrid fungus remains a potential threat to the Okavango system. Factors that leave the Delta exposed and vulnerable to new infection include: (1) positive Bd results nearby and proximity of the disease to the region; (2) human inhabitation around the Delta periphery with little control over the import and export of biological material; (3) intense tourism with all its associated support structures means a constant movement of people and material into the interior of the Okavango system, and (4) the nature of the Okavango Delta, with its vast network of connected swamps and channels, is conducive for spreading an aquatic born disease. Lips *et al.* (2006) monitored the behaviour of amphibian chytrid fungus upon arrival at a Bd free site in Panama and its effect on local amphibian diversity. They describe its effect as "epizootic," spreading like an epidemic wave, resulting in extensive amphibian die-offs and local population extinctions. Therefore, a risk assessment is urgently needed to establish the vulnerability of the Okavango Delta to Bd introduction; and management plans must be formulated and implemented, aimed at preventing introduction and controlling the spread of the disease should introduction occur.

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In order to achieve the abovementioned goals, further knowledge and research is first and foremost required, guided by strategies proposed in Weldon *et al.* (2008). This includes:

- Further assessment of the current status of Bd through additional sampling, to ensure the disease is indeed absent from the entire geographical region.
- Establishment of long term monitoring sites so that Bd status can be continually and closely monitored, and any amphibian die-offs observed and investigated.
- Investigation of the effect of Bd on all Okavango Delta amphibian species, possibly through exposure to Bd isolates of nearby infected populations through ex-situ challenge experiments, to determine the likely response of local populations to infection and whether infection will have little impact on populations as in other parts of Southern Africa.
- If further Bd investigation continues to yield negative results and the conclusion that Bd is indeed absent from the system, the reasons for its absence should be explored. Both ecological conditions in the regions should be analysed, as well as the evolution of the Delta system and its amphibian communities.

Further knowledge is essential to create strategies and management plans that are effective. Meanwhile, education and information dissemination to local stakeholders is essential to warn of this potential threat, not only to the Delta"s amphibian biodiversity, but the region"s biodiversity as a whole. Such guidance could be focussed on tourist operators, their guides, staff and guests; local wildlife authorities; local communities around the Delta"s periphery; academic institutions and nature enthusiasts in general.

Figure 33: Swabbing of *Ptychadena subpunctata* specimen.

CHAPTER FIVE:
CLOSING DISCUSSION

5.1 Final review of present study outcomes

The overall goal of this study was to gain knowledge on the amphibians of the Okavango Delta in order to contribute to the global information base of this vertebrate class. This information could then be used to guide conservation management strategies of amphibians in order to achieve optimum results. In light of the fact that the last detailed assessment of amphibians in the region was completed more than two decades ago, and that amphibians have since become the most threatened vertebrate class, this evaluation was both timely and necessary.

It was reasonably concluded that species richness for the Okavango Delta is 29, which has increased the richness observed by Auerbach in 1987 by one species, but is four species below the expected richness quoted by Ramberg *et al.* in 2006. Species diversity throughout the region appears to be driven by available habitat types, rather than the degree of isolation of a particular island and the physical barriers to dispersal that may exist between an island and its source area. The effect of isolation is likely less pronounced due to the dynamic nature of the system, and the constantly changing landscape of aquatic and terrestrial habitats. This finding implies that should this faunal group become threatened in the future, conservation strategies should be aimed at the preservation of habitat diversity, especially those favoured by amphibians and necessary for their survival.

The uncommon and opportunistic breeding behaviour observed for several amphibian species in the Okavango Delta was a novel finding. Research into anuran breeding behaviour in Southern Africa has been minimal over recent decades, but it is generally accepted that breeding coincides with rainfall in summer rainfall regions. The outcome of this portion of the study contradicts this notion, and highlighted the opportunistic behaviour exhibited by some species when environmental conditions were favourable. Several species appeared to take advantage of increased biological production stimulated by the arrival of the annual flood; therefore, they displayed a second breeding peak in winter. This finding adds emphasis to the unique nature of the Okavango system, and supports the exceptional biological productivity highlighted by Ramberg *et al.* (2006) as a key feature of the Delta. It also highlights the fact that the uniqueness of this flood pulsed system drives associated unique behavioural adaptations by animal inhabitants of the Delta.

The disease survey of chytridiomycosis marks the first of its kind for this region. This widespread and sometimes deadly disease was not found in the Delta; an unexpected result when compared with its presence in Botswana and its greater distribution over the African continent. The factors responsible for the absence of Bd are currently unknown, but several hypotheses have been proposed, including: (1) isolation of the Delta as a whole, limiting the dispersal of amphibians to and from surrounding areas; (2) unfavourable ecological conditions in the region; and (3) the constant and aggressive removal of water, with any suspended material, from the system. Characteristic of Bd upon its introduction into a previously disease free area is its ability to spread as an epidemic wave, resulting in mass mortality and even population extinction. Therefore, despite the current lack of Bd in the Delta, it remains under threat, and the current findings thus have major implications for the conservation of the Okavango Delta. It has highlighted the vulnerability of the Okavango"s amphibian fauna, and calls for urgent research and management plans to be implemented so that the possible adverse effects of Bd may be avoided.

5.2 Conservation of the Okavango Delta

The Botswana government is commendable in its drive to protect the country"s natural resources, and in setting aside large stretches of land for conservation and tourism. This effort has proved advantageous for the country"s economy; international tourism and associated tourism investment; local residents whom have benefitted financially; and in drawing naturalists worldwide to the region. It has supported the notion that natural resources can be utilised sustainably whilst contributing to economic growth and development. In fact, the Ramsar boundary encompasses a significant portion of country; making it one of the largest Ramsar sites worldwide (Mendelsohn *et al.*, 2010).

However, despite all these conservation efforts, the Okavango Delta remains threatened, and Mendelsohn *et al.* (2010) provide a detailed analysis of these potential threats with suggested mitigation strategies. Within the Delta and including its immediate surroundings are a number of internal threats:

- Chemical pollution should be consistently monitored due to its adverse and long-lasting effects. There are many origins of chemical pollutants, including villages, lodges, tourism operations and boats. Also, pesticides used in tsetse fly eradication have already had considerable negative effects on biodiversity. Similar impacts should be avoided, or at least better controlled, in the future.
- Plant and animal species exotic to the region and invasive should be carefully monitored and controlled. They are often accidentally introduced and cause major disruptions to the ecosystem, with widespread and long lasting effects. Already

problematic species include the water weed *Salvinia molesta*, the burweed *Xanthium stramonium* and the syringa *Melia azederach.*

- Clearing of channels for ease of boat routes and to provide permanent water to surrounding villages has caused damage in the past, and remains a constant threat. Such activities alter the very processes that drive the dynamic nature of the Delta system. Over time, this may cause the size of the alluvial fan to shrink and lower biological productivity.
- The effect of booming elephant populations in Northern Botswana remains a heated debate, with much evidence suggesting a devastating loss of riverine forest and woodland in some areas. If such losses begin to occur on islands, this could result in the Delta becoming saline in the long term.

The Delta is also under threat externally from surrounding countries, specifically Angola and Namibia, where there has been little emphasis on environmental conservation and the benefits of tourism as a national income generator. Threats highlighted by Ramberg *et al.* (2006) and Mendelsohn *et al.* (2010) include:

- Water extraction from the Okavango River in Namibia for agricultural, domestic and industrial use. In times of drought, there have been plans for the construction of pipelines to transfer water to the centre of the country; this remains a concern. Reduced flow into the Delta may impact on key system processes and reduce the extent of flooding.
- In Angola, construction of up to 16 hydro-electrical power plants along tributaries of the Okavango has been suggested; fortunately, plans are yet to be finalised.
- Low output subsistence farming in Namibia and Angola will likely increase as populations grow, adding pressure to an already fragile ecosystem and resulting in long term loss of productivity and biodiversity in these areas.
- The possibility of large scale irrigation in the Angolan catchment may seem attractive for the commercial production of sugar cane, maize, rice and biofuel products. In combination, such water extraction may seriously impact water inflow into the Delta. In addition, and equally worrisome, is the run-off of fertilisers, pesticides, herbicides and other toxins associated with farming into the water entering the Delta.
- Increased erosion as a result of irrigation and farming projects may increase the sediment and clay load of river water, which could ultimately destroy the freshwater ecosystem.
- Finally, further global threats that may influence the functioning and protection of the Okavango system include climate change; politics; fluctuating food and energy prices; a vulnerable and unpredictable tourism industry, especially during times of economic downturn and global recession; and tectonic forces within the Delta depression.

From the above discussion, it is clear that although the Okavango Delta is currently a pristine wetland, receiving protection from various interested parties and stakeholders, its situation remains precarious. Therefore, it is essential that there are comprehensive and effective monitoring policies in place that constantly scrutinize the system‟s biotic and abiotic components. For the purpose of such monitoring projects, anurans are valuable resources because they are biological indicators that signify the health of wetland and river systems (du Preez & Carruthers, 2009). Some features that make them useful bio-indicators - crucial for any environmental deterioration assessment or conservation management project - include:

- A permeable skin that constantly and easily absorbs water, and therefore any potentially harmful substances dissolved in it;
- They can be easily monitored due to their presence in most environments, and are readily observed, either visually or acoustically;
- Many tadpoles are bottom feeders and adults, too, consume soil and plant material. Therefore, they are vulnerable to food contaminants such as heavy metals and chlorinated compounds;
- Amphibians are especially sensitive to habitat loss since they generally have highly specific habitat requirements and resultant patchy distributions, rendering any isolated populations extremely vulnerable to extinction;
- The hormone-driven processes of tadpole development and metamorphosis may be disrupted by exposure to external, alien hormones;
- Any drastic temperature changes and extremes may negatively affect amphibian biology and behaviour;
- Their need for both terrestrial and aquatic habitats makes them sensitive to changes in either environment;
- Their position in the food web as both predator and prey species means they influence a much wider ecological range.

(du Preez & Carruthers, 2009.)

The focus of the present study was on the amphibians of the Okavango Delta, for many an inconspicuous vertebrate class that can be easily overlooked, but whose presence is an integral part of most ecosystems. This faunal group is only a small constituent of the unique Okavango Delta ecosystem, but it may aid in the preservation of one of the world‟s last remaining, pristine regions. “The Delta of the Okavango River means different things to different people: a wonderland to some, a wetland or wildlife paradise to others, and for many it provides for wealth and welfare” (Mendelsohn *et al.*, 2010). Most of all, in modern times, it suggests the possibility that our world‟s natural wonders can indeed continue to exist.

ANNEXURE I: Amphibian species previously recorded in the Okavango Delta by Auerbach (1987) and du Preez & Carruthers (2009), the expected species list and the actual species list for the present study.

Key:

X - Recorded in the study area: 18.75° - 19.50° latitude and 22.50° - 23.00° longitude.

S - Recorded in the Okavango Delta, but not in the study area: 18.50° - 20.00° latitude and 22.00° - 24.00° longitude.

O - No records in the Okavango Delta

√ - Expected species for the Okavango Delta.

Family	Species	Common Name	du Preez & Carruthers, (2009)	Auerbach, R.D. (1987)	Expected species list for the Okavango Delta.	Species actually recorded during the present study.
Breviceptidae	*Breviceps adspersus adspersus*	Bushveld/Common Rain Frog	S	S	√	X
Bufonidae	*Amietophrynus gutturalis*	Guttural Toad	S	S	√	X
	Amietophrynus lemairii	Lemaire's Toad	S	S	√	X
	Amietophrynus maculatus	Flat-backed Toad	S	S	√	X
	Amietophrynus poweri (garmani in Auerbach)	Western Olive Toad	S	S	√	X
	Poyntonphrynus kavangensis	Kavango Pygmy Toad	S	O	√	O
Hemisotidae	*Hemisus guineensis*	Guinea Shovel-Nosed Frog	S	S	√	X

	Hemisus marmoratus	Mottled Shovel-Nosed Frog	S	S	√	X
Hyperoliidae	*Hyperolius benguellensis*	Bocage's Sharp-nosed Reed Frog	S	X	√	X
	Hyperolius nasutus	Long Reed Frog	S	X	√	X
	Hyperolius parallelus	Angolan Reed Frog	S	X	√	X
	Kassina senegalensis	Bubbling Kassina	S	S	√	X
Microhylidae	*Phrynomantis bifasciatus*	Banded Rubber Frog	S	S	√	X
Phrynobatrachidae	*Phrynobatrachus mababiensis*	Dwarf Puddle Frog	S	S	√	X
	Phrynobatrachus natalensis	Snoring Puddle Frog	S	S	√	X
	Phrynobatrachus parvulus	Small Puddle Frog	S	S	√	X
Ptychadenidae	*Hildebrandtia ornata*	Ornate Frog	S	O	√	O
	Ptychadena anchietae	Plain Grass Frog	S	S	√	X
	Ptychadena guibei	Guibe's Grass Frog	S	S	√	X
	Ptychadena mascareniensis	Mascarene Grass Frog	S	S	√	X
	Ptychadena mossambica	Broad-banded Grass Frog	S	S	√	X
	Ptychadena oxyrhynchus	Sharp-nosed Grass Frog	S	S	√	X
	Ptychadena subpunctata	Speckled-bellied Grass Frog	S	S	√	X
	Ptychadena taenioscelis	Dwarf Grass Frog	S	S	√	X
Pipidae	*Xenopus laevis*	Common Platanna	S	O	√	O
	Xenopus meulleri	Müller's Platanna	S	S	√	X

	Xenopus petersii	Peters's Platanna	S	S	√	X
Pyxicephalidae	*Pyxicephalus adspersus*	Giant Bullfrog	S	S	√	X
	Pyxicephalus edulis	African Bullfrog	S	S	√	X
	Tomopterna cryptotis	Tremolo Sand Frog	S	S	√	X
	Tomopterna krugerensis	Knocking Sand Frog	S	O	√	O
	Tomopterna tandyi	Tandy's Sand Frog	S	O	√	X
Rhacophoridae	*Chiromantis xerampelina*	Southern Foam Nest Frog	S	S	√	X
TOTALS			**33**	**28**	**33**	**29**

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