Ecology of impala (*Aepyceros melampus*) and waterbuck (*Kobus ellipsiprymnus*) in Majete Wildlife Reserve, Malawi

By

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Declaration

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Abstract

Protected areas in Africa are under increasing pressure as the human population and their associated activities continue to rise. Habitat loss and fragmentation has led to the isolation of wildlife areas, which are commonly fenced to protect biodiversity and to reduce human-wildlife conflicts. As fencing impacts ecological processes, intense management is required to conserve biodiversity and prevent habitat degradation in these areas. Effective management and biodiversity conservation strategies depend on a good understanding of the ecological requirements and characteristics of dominant species.

African Parks is an example of an organization that has overcome many challenges to make an extraordinary contribution to conservation in Africa. After the decimation of most mammals by excessive poaching in Majete Wildlife Reserve (MWR), Malawi, the park underwent one of the largest reintroduction programmes on the African continent.

Of the 14 species and 2559 animals reintroduced, were 737 impala and 402 waterbuck, both of which are successful breeders and can compete vigorously for resources. Population abundance and density estimates were determined for a 140km^2 section of MWR using distance sampling methods on drive counts for 14 consecutive months (2013-2014). The data were analyzed in the software programme DISTANCE. Estimates indicated that post-reintroduction impala and waterbuck populations have increased significantly and displayed a preference for habitats adjacent to the perennial Shire and Mkulumadzi Rivers in the north-east of the reserve. Population control strategies needs to be implemented in the near future to curtail the impacts of habitat over-utilization by these two species and other ungulates.

An apt understanding of species behaviour in specific areas assists managers to develop management strategies. Baseline ecology for impala and waterbuck were determined using behavioural observations on drive counts, and waterhole counts. Overall, impala and waterbuck had similar ecology to other populations previously studied. However the impala lambing season occurred marginally earlier and waterbuck calving season peaked in May-June i.e. not in March and October as determined by other studies. Furthermore, it was established that impala and waterbuck adult males utilized waterholes more frequently than females. In addition, impala and waterbuck males displayed a preference for waterholes according to surrounding vegetation type. Managers should consider these trends when revising the artificial water point management for the reserve.

The foraging behaviour of impala and waterbuck were investigated in more detail. Stable isotope analysis of dung was used to estimate the graze and browse composition in these two ungulates'

diets. It was determined that impala are mixed feeders that readily shift from a high graze content in the wet summer season to relatively high browse content in the dry winter season. Waterbuck were typical grazers that were able to utilize browse species in more stressful environmental conditions. Contrary to a typical dietary overlap occurring in the dry season, impala and waterbuck have a dietary overlap in the wet, summer months when both species have a high graze species content in their diet.

MWR management required a better understanding of the ecology of impala and waterbuck post reintroduction to contribute toward management planning. Based on the information gleaned from the various studies conducted, both ungulates have successfully established themselves in MWR and their populations have significantly increased and require intensive management to prevent environmental degradation. Population management strategies should include the translocation of wildlife from MWR to other parks, as part of a national reintroduction programme in Malawi.

Opsomming

Bewarings gebiede regoor Afrika is toenemend onder druk weens 'n groeiende menslike bevolking, en hulle aktiwiteite wat lei tot die verlies en opbreek van natuurlike habitat en, dus, die isolasie van wildreservate. Hierdie wildreservate word omhein om konflik tussen mense en wild te verminder, en om biodiversiteit te beskerm. Omdat heinings sekere ekologiese prosesse ontwrig, is bestuursingryping nodig om omgewingsagteruitgang te verhoed. Effektiewe bestuurs- en bewaringsstrategieë is gefundeer op 'n diepliggende begrip van die ekologiese behoeftes en eienskappe van dominante spesies.

African Parks is 'n organisasie wat etlike uitdagings oorkom het en bygedra het tot natuurbewaring in Afrika. Nadat die meeste wild in Majete Wildreservaat (MWR) in Malawi deur stropers onwettig uitgejag is, is wild in die park hervestig tydens een van die grootste wildhervestigingsprogramme op die Afrika vasteland. Van die 14 spesies en 2559 diere wat hervestig is, was 737 rooibokke en 402 waterbokke. Beide spesies is geweldig kompeterend, en vermeerder maklik om gebruik te maak van beskikbare hulpbronne. Deur gebruik te maak van afstandsmetingsmetodes gedurende wildkykritte vir 14 agtereenvolgende maande (2013-2014) is bevolkingsdigthede vir 'n $140km^2$ gedeelte van die MWR vir hierdie twee spesies bepaal. Die sagtewareprogram DISTANCE is gebruik om data te analiseer. Beraminge dui daarop dat rooibok en waterbok bevolkings beduidend toegeneem het, en dat hulle die area langs die standhoudende Shire en Mkulumadziriviere in die noordoostelike gedeelte van die reservaat verkies. Daar word aanbeveel dat hulle getalle binnekort beheer word om te verhoed dat hulle impak die beskikbare habitat nadelig beïnvloed.

'n Gedetaileerde begrip van wild se gedragspatrone in sekere areas word benodig om bestuursstrategieë te ontwikkel. Rooibok en waterbok gedrag is aangeteken gedurende wildkykritte, en by watergate . Oor die algemeen tree rooibokke en waterbokke soos hulle eweknieë in ander studies op. Rooibokke lam egter effens vroeër, en waterbokke se kalfseisoen bereik hulle piek van Mei tot Julie m.a.w. nie in Maart en Oktober soos in vorige studies nie. Boonop het ons bevind dat volwasse rooibokramme en waterbokbulle watergate baie meer gereeld as rooibokeeue en waterbokkoeie besoek. Rooibokramme en waterbokbulle se voorkeur vir sekere watergate het afgehang van die omringende plantegroei. Bestuurders behoort hierdie tendense in ag te neem wanneer hulle kunsmatige watergate se posisionering in die toekoms hersien.

Weidingsgedrag van rooibokke en waterbokke is ook in meer besonderhede oorweeg. Stabiele isotope van mismonsters is geanaliseer om te bepaal hoeveel grasse en bossies in hulle dieët voorkom. Daar is vasgestel dat rooibokke 'n mengsel van grasse en bossies vreet, en gemaklik

oorskuif van meer grasse in die nat somerseisoen na meer bossies in die droë winterseisoen. Waterbokke het oorwegend grasse gevreet, maar hulle was daartoe in staat om bossies te vreet tydens ongunstige omgewingstoestande. Anders as in vorige studies het rooibokke en waterbokke se dieët in die nat somersmaande oorvleuel wanneer albei spesies meer gras geëet het.

MWR se bestuur het 'n meer volledige beskrywing benodig van rooibokke en waterbokke se bevolkingsdigthede en gedrag met die doel om die omgewing beter te bestuur. In hierdie studie het ons vasgestel dat beide boksoorte suksesvol hervestig is, dat hulle getalle beduidend toegeneem het, en dat intensiewe bestuurspraktyke binnekort benodig gaan word om omgewingsagteruitgang te voorkom. Ons stel voor dat hulle getalle bestuur kan word deur hulle vanuit MWR na ander parke te skuif in 'n nasionale hervestigingsprogram vir Malawi.

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Chapter One:

General introduction and thesis outline

1.1 Introduction

In most terrestrial ecosystems a diverse assemblage of herbivores has been maintained over time (du Toit & Cumming, 1999). Ecologists have investigated how these assemblages are maintained, considering the stable, coexistence of a large biomass of herbivores (Putman, 1996). The acquisition of nutrition and avoidance of predators are the main factors that contribute to the survival of animals (Cowlishaw, 1997; Kie, 1999; Orians, 2000). Secondary factors are access to water and shelter (Orians, 2000). Habitat selection may be influenced by vegetation type, water availability or substrate on a macro-scale; where the presence, absence or abundance of potential forage species will affect herbivore habitat selection at the landscape level (Druce, 2005). In order to successfully manage habitats to support wildlife populations, an understanding of the species' ecological requirements needs to be established (Dörgeloh, 1998, 2001; Kaunda, Mapolelo, Matlhahku & Mokgosi, 2002; Traill, 2004). Ecologists frequently conduct studies to assess habitat use from which they may deduce a species habitat selection and preference (Garshelis, 2000; Kaunda *et al*., 2002). Previous studies have shown that, within a number of species, individuals will choose which habitat to occupy and that there is an increase in the range of habitats used with an increase in population density (Kaunda *et al*. 2002). This phenomenon is called density-dependent habitat selection (Fretwell & Lucas, 1970; Kaunda *et al*., 2002) and could potentially have a strong effect on population dynamics and social organization, predominantly the distinctive distribution of males and females in space and time (Morris, 1988; Morris, 1992; Kaunda *et al*., 2002). Resource partitioning is the mechanism that facilitates the coexistence of species in a habitat, where resources may be selected to meet their requirements (McNaughton & Georgiadis, 1986). Studies are also conducted to determine the carrying capacity of the area, which is the number of animals, taking into account their habitat requirements that an area can support without having a detrimental effect on the environment (Vernier & Fahrig, 1996; Traill, 2003). By having a better understanding of the habitat requirements and behaviour of herbivores, wildlife managers may predict the distribution of herbivores (Dörgeloh, 2001) and their consequent effect on vegetation (van Aarde, Jackson & Ferreira, 2006).

Increasing human populations and anthropogenic activities, economic expansion, poverty, social and environmental human displacement, has a detrimental effect on residual protected and

wilderness areas (du Toit & Cumming, 1999; Norton-Griffiths, 2007; Somers & Hayward, 2011; Lindsey, Masterson, Beck & Romañach, 2012). In Africa, fences are commonly used as a conservation tool to retain wildlife within protected areas, (Lindsey *et al*., 2012). However, the increasing isolation and fencing of these natural and protected areas threatens the long-term success and survival of remaining wildlife populations (Noss, Csuti & Groom, 2006; Muths & Dreitz, 2008; Newmark, 2008). Ecological processes are impacted by the erection of fences around protected areas, as wildlife dispersal routes are disrupted and resources such as food, water and space are limited, creating an artificially closed system or ecological island (Macarthur & Wilson, 1967; Caughley, 1994; Hayward & Kerley, 2009; Albertson, 2010; Cumming 2010; Ferguson & Hanks, 2010). Various challenges that arise with the creation of these ecological islands include increased risk of inbreeding as gene flow between populations is disrupted (Caughley, 1994; Hayward *et al*., 2007; Cumming, 2010; Ferguson & Hanks, 2010). Additionally, density dependent population regulation is affected, resulting in environmental degradation and possible population crashes (Boone & Hobbs, 2004; Hayward & Kerley, 2008).

Based on non-equilibrial island biogeography theory (Brown, 1971) and species-area relationships, it is predicted that as more protected areas in Africa continue to become more isolated, the loss of species within reserves will be inversely proportional to the area of the reserve. (Newmark, 2008; Hanks, 2010). Thus various field studies have been conducted by ecologists to gain a better understanding of the consequences of the isolation of protected and natural areas (Newmark 2008). Disturbance island biology (Whittaker, 1998) stresses that natural and anthropogenic disturbances may have a significant impact on the persistence and turnover of wildlife within reserves, especially with with respect to edge effects (Newmark, 2008). Edge effects are the physical and biotic changes that vary in space and time, associated with artificial boundaries of fragments and may have negative impacts on biodiversity (Laurance, Mascimento, Laurance, Andrade, Ewers, Harms, Luizão & Ribeiro, 2007). Habitat management is widely understood to be an essential practice for the long-term maintenance of wildlife populations (Western & Pearl, 1989; Bibby, 1992; Kaunda *et al*., 2002). Intensive management of reserves is fundamental to the prevention of habitat degradation as a result of over-exploitation by herbivores (Hobbs & Huenneke, 1992; Bothma, 1995; Canter, 2008). The localized impacts on vegetation, altering vegetation composition and structure, may negatively, and in some cases, positively influence the biodiversity and habitat suitability for other species (Gordon *et al*., 2004; Mysterud, 2006).

The abundance and distribution of wildlife are also affected by disease. A classic example is the rinderpest virus outbreak that spread rapidly through sub-Saharan Africa, wiping out more than 90% of multiple ungulate populations after it was accidently introduced in the Horn of Africa in the

late 1880s via cattle (Plowright, 1982; Newmark, 2008). Another example is bovine tuberculosis, a bacterial disease that was transferred via domestic cattle to wildlife in the Kruger National Park, where buffalo are currently a reservoir host (Caron, Corss & du Toit, 2003; Newmark, 2008). Fences have also been used to prevent disease transmission between wild and domestic animal populations and to increase the protection of vulnerable and threatened species. The effects of fences erected in Namibia and in the rangelands of Botswana were devastating and were compounded by a series of drought years as wildlife access to water was cut off (Willimson & Mbano, 1988; Albertson, 1998; Mbwaia & Mbwaia, 2006; Newmark, 2008; Ferguson & Hanks, 2010). Another significant threat to wildlife populations is the bush meat trade (Fa, Garcia Yuste & Castelo, 2000; Lindsey, 2010; Somers & Hayward, 2011), especially when protected areas are within close proximity to human settlements where there is a lack of alternative protein sources (Brashares, Arcese, Sam, Coppolillo, Sinclair & Balmford, 2004; Newmark, 2008).

In the last century an increasing number of protected areas have been established in Africa, particularly since 1970, where the total area of terrestrial and marine protected areas has almost doubled (Newmark, 2008). Some of the principle factors that have led to the success of protected areas are the improved and increased number of courses at tertiary institutions in conservation management and ecology, the significant growth in ecological tourism in protected and natural areas, greater benefits to, and inclusion of, adjacent local communities in the management and protection of reserves, and better resources (Newmark, 2008). Ecotourism is an important source of revenue for game reserves where large herbivores are an attraction (Duffus & Dearden, 1990; Giannencchini, 1993; Ogutu, 2002; Canter, 2008). Conservation management in reserves aims to prevent the loss of biodiversity (Pelletier, 2006; Canter, 2008) by maintaining wildlife populations within (economic) sustainable limits (du Toit, 2002; Gordon *et al.* 2004). In numerous cases, large herbivores have proven to be a good source of revenue for game reserves through sustainable hunting (van der Waal & Dekker, 2000; Leader-Williams, Smith & Walpole, 2001; Canter, 2008). However encouraging it may be, that there are more protected areas, it is most important that these areas are optimally managed and have the capacity to sustain wildlife populations in the long-term in spite of threats from internal and external anthropogenic activities (Muths & Dreitz, 2008; Newmark, 2008).

The reintroduction of animals into protected areas where wildlife had become locally extint due to various anthropogenic effects, has become an effective tool in wildlife management (Kleiman, 1989; Griffith, Scott, Carpenter & Reed, 1989; Stanley-Price, 1991; Wolf, Griffith, Reed & Temple, 1996; Muths & Dreitz, 2008). Typically, non-government organizations are responsible for many

reintroduction programmes with the aim of protecting and restoring biodiversity. However, population extinction and growth is not a priority and therefore poorly understood. With the increase in reintroduction programmes, the need for knowledge and understanding of reintroduction preparations, reintroduced species and the assessment of reintroduction successes/failures is becoming more urgent (Sarrazin & Barbault, 1996; Armstrong & Seddon, 2007). Reintroduction may be defined as, "the establishment of a species to an area that was previously inhabited in an effort to advance the conservation of the species", (IUCN 1998; Sarrazin & Barbault, 1996). Reintroduction biology has become a recognized field of research as a result of the increasing number of reintroduction projects and publications over the past two decades. However most of the research has been descriptive in nature (Armstrong & Seddon, 2007; Seddon, Armstrong & Maloney, 2007). The success of a reintroduction programme should be measured by the successful release of animals, followed by their ability to reproduce and form a self-sustaining population (Dodd, 2005; Muths & Dreitz, 2008).

Conservation efforts, from a management perspective, typically achieve management objectives by manipulating systems rather than striving to understand how those systems work (Seddon, Armstrong & Maloney, 2007). This approach does not facilitate the accumulation of knowledge and understanding very well, especially as failed manipulations are not always documented (Seddon *et al*, 2007). The gap between field conservationists and scientists is a result of reintroduction programmes being management driven as opposed to research driven; especially as professional biologists have been poorly involved in past reintroduction efforts (Sarrazin & Barbault, 1996; Seddon *et al*., 2007). Resource managers and researchers need to work together and unite their efforts (Seddon, 2007) to address the acute need to augment the knowledge and understanding of reintroduction preparations, reintroduced species and reintroduction success assessments (Sarrazin & Barbault 1996). It is of high importance that monitoring and active management of reintroduced species is conducted, especially in a closed system, to ensure sustainability of the population and to establish the effect of the species on its environment and other species, post reintroduction. Resources contribute to population growth but when competing with other wildlife, resource competition may lead to population decline (Grover, 1997).

African Parks Pty (Ltd), a not-for-profit organization, works toward driving wildlife parks to be socially and economically viable, especially to the advantage of local communities, as they believe that this will contribute towards the survival of these protected areas against rivaling forms of land use. The primary source of funding for the establishment of such projects is received from various generous, private donors that value the protection and sound management of remaining wildlife

areas and social upliftment of surrounding communities. African Parks work in partnership with governments and local communities to rehabilitate, manage and finance protected areas throughout Africa. The first project taken on by African Parks was the rehabilitation of Majete Wildlife Reserve (MWR, hereafter), located in the Middle Shire Valley at the southern end of the Great Rift Valley in the southern region of Malawi.

The two study species, impala (*Aepyceros melampus*) and waterbuck (*Kobus ellipsiprymnus*), were chosen due to their suspected high population growth rate and vigorous resource competition with other herbivores in the reserve (Dorian Tilbury, pers.com.). Antelope consume browse (dicotyledonous tree and shrubs) and graze (monocotyledonous grasses) species that may be categorized according to their metabolic pathway, as C_3 and C_4 plants, respectively, (Cerling, Harris & Passey, 2003; Radloff, van der Waal & Bond, 2013). Stable isotope analysis may be used to estimate the contribution of C_3 and C_4 plants to animal diets (Hobson, 1999; Phillips, 2001; Phillips & Gregg, 2001), such as impala and waterbuck. The combined biomass of impala and waterbuck populations could have a detrimental effect on the reserves vegetation and possibly other herbivores if their numbers are left unchecked. Fieldwork efforts of this study were focused in the sanctuary, a 140km^2 area in the northeastern section of the reserve where initial reintroductions were made. At the start of the reintroduction programme, the sanctuary was fenced off, while the perimeter fence around the entire reserve was erected. Once the reserve fence was complete, the sanctuary fence was deconstructed, however, the movement of game out of this area was minimal. The sanctuary is now the ecotourism section of the reserve and wildlife occur at high densities, as they are attracted to the perennial source of water from two major rivers in the reserve.

Reintroduction and translocation programmes take individuals from natural populations or captive bred programmes (Sarrazin & Barbault 1996). There have been concerns that captive-bred animals are naïve and thus have a decreased ability to survive in the wild, whereas wild-to-wild translocated animals have been more successful in reintroduction efforts (Griffith et al. 1989; Wolf et al. 1996; Seddon et al. 2007). At present, MWR has a "no hunting" and "no culling" policy with the intention of relocating surplus animals to restock other protected areas within in Malawi. Thus, MWR will become a source of wild animals that will have better success of establishing themselves in a new environment post translocation.

In previous reintroduction programmes the monitoring period after reintroductions has been inadequately implemented and documented, despite being recommended by the IUCN (Sarrazin & Barbault, 1996). At MWR there is an opportunity to carry out thorough monitoring of reintroduced animals and the subsequent changes in vegetation. Distributions of animals may vary initially as

species adapt to and learn more about their new environment. Additionally, population dynamics will change as species establish themselves. It is important to know how the animals utilize natural resources so that the carrying capacity of the reserve during the most limiting period, can be calculated so that healthy populations of animals can be maintained without having any detrimental effects on the reserve. Findings from this research will be used to improve other reintroduction programmes and provide a better understanding of how pioneer populations establish themselves in a new environment. The reintroduction programme at MWR provides the opportunity to carry out "real-scale hypothetico-deductive experiments in ecology" (Sarrazin & Barbault 1996) that would generally be a challenge for ecologists on such a large-scale.

It is important to have a good understanding of the presence and distribution of wildlife within an area, to develop sound conservation strategies (Tolber, Carrillo-Percastegui, Leite Pitman, Mares & Powell, 2008). This thesis investigates habitat selection, dietary preferences and demography of impala and waterbuck post reintroduction to MWR.

1.2 Research goal and objectives

1.2.1 Goal

To propose management strategies for the impala and waterbuck populations in MWR, a closed system, by studying the basic ecology of the two species and determining/quantifying population growth post reintroduction.

1.2.2 Objectives and research questions

1. To estimate growth and structure of impala and waterbuck populations post reintroduction.
   a. What is the population size of impala and waterbuck in MWR?
   b. What are the population age/size structures (number of males, females, juveniles and calves)?
   c. When are the impala lambing and waterbuck calving seasons?

2. To establish the behaviour, preferred habitat and distribution of each species within the original sanctuary area.
   a. Which preferred habitats are utilized during the distinct wet and dry seasons?
   b. To what degree do the habitat selection patterns overlap between impala and waterbuck?
   c. What are the behavioural characteristics of each species?

3. To ascertain the basic dietary preferences of impala and waterbuck and how this may vary between wet and dry seasons.
    a. What is the isotopic composition of C_3 and C_4 biomass in their diets?
    b. Is there dietary overlap between wet and dry seasons?

4. Propose management recommendations for the impala and waterbuck populations.
    a. What management strategies need to be implemented for impala and waterbuck populations?
    b. Which studies should the local research group conduct to assist management strategies in the future?

1.3 Thesis structure

This master's thesis is divided into six chapters. Following the basic introduction to protected areas, herbivore research, reintroduction biology and aims of the study in Chapter One, Chapter Two elaborates on the study site and provides comprehensive background information on the two species and the methods used during the course of this study.

Chapters Three, Four and Five have been composed in the format of journal articles. As a result there is a degree of repetition and cross-referencing between chapters. In Chapter Three, I report on the population size, structure and distribution of impala and waterbuck in MWR, as estimated using several methods. Chapter Four describes the behaviour of impala and waterbuck populations in MWR and how it changes during the course of a year. Chapter Five investigates the diet of impala and waterbuck and how these change across the seasons, using stable isotopic analyses.

Chapter Six integrates the results and conclusions from Chapters Three, Four and Five, as well as considering the implications of these for the management of MWR. It concludes with a discussion as to how this study could have been improved, and suggestions for future research projects are given.

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Chapter Two:

Background: study site, study species and applied methods

2.1 Study area

Majete Wildlife Reserve (MWR, hereafter) is a 700km^2 fenced reserve located in the Middle Shire Valley at the southern end of the Great Rift Valley in the southern region of Malawi. The history of MWR started when the area surrounding Majete Hill was declared as a non-hunting area in 1951 due to the increasing Malawian population in the early 20th century that was placing pressure on the populations of larger wildlife such as elephant, waterbuck, buffalo and eland. As a result, the area that is now MWR, became a refuge for these animals. A game guard was designated to conduct patrols in the area in 1953 and monthly reports were sent to the District Commissioner in Chikwawa. In 1955, 500km^2 of land was gazetted establishing the Majete Game Reserve, as MWR was formerly named, in an effort to restrict elephants to this area (Morris, 2006). Majete Game Reserve was managed in accordance to the Malawi National Parks and Wildlife Act. MWR was extended northwards and eastwards in 1969 to include the Mkulumadzi River and a small area on the eastern bank of the Shire River (Sherry, 1989; Morris, 2006). A study conducted by Bell (1984) indicated that there were substantial wildlife populations including elephant, sable, kudu, warthog, waterbuck and several other species (Sherry, 1989). However, poor management, lack of finances and a poorly equipped anti-poaching law enforcement unit resulted in the depletion of most mammal species by the early 2000's.

The Malawian government entered into a public-private partnership (PPP) with African Parks on 28th March 2003, in which African Parks Majete (Pty) Ltd. was given the responsibility to rehabilitate, manage and develop MWR. Millions of US dollars have been spent in the last decade on infrastructure development, transport provision, fencing, equipment, administration fees, translocation of animals and more. An excess of US$2,000,000 was spent on an animal reintroduction programme where14 species were selected and a total of 2,559 animals were reintroduced over several years between "yearstart" and "yearend". Stock was sourced from South Africa and Zambia and from Lengwe and Liwonde National Parks in Malawi. Animals were initially released in the fenced sanctuary area (140 km^2) in the north-east of the reserve, while the establishment of the 142km fence was completed around the entire reserve. Under the custodianship of African Parks, MWR is now a 700km^2 fenced reserve that is a great tourist attraction, boasting the "Big Five". MWR currently has a "no hunting" and "no culling" policy with the intention of

relocating surplus animals to other parks and wildlife areas. Unfortunately, there is no scope for the expansion of MWR as high-density rural settlements and subsistence farming confine it. It is estimated that 140,000 people live around the reserve (Samuel Kamoto, pers. com.). In restoring and developing MWR, economic and social benefits have been created for local communities. The long-term financial viability of MWR depends on tourism, game sales and carbon funding, and if necessary, long-term donor funding.

Law enforcement has successfully reduced the incidences of illegal activities, such as grazing cattle in the reserve, cultivating crops, harvesting of various flora species, illegal fishing, hunting for bush meat and the carrying of illegal firearms. There is a close liaison with police and the judiciary system, resulting in arrests and subsequent convictions of offenders. Many weapons and hunting tools have been confiscated, such as gin traps, snare wire, muzzleloaders, shotguns, fishing line and spears.

The topography of the reserve is relatively gentle with undulations and several rocky outcrops and hills. The prevalent slope of MWR is from northwest to southeast with altitudes ranging from 900m to 150m close to the Shire River (Sherry, 1989; Macpherson, 2012). Pockets of relatively recent alluvial deposits overly the rock formations of Precambrian Basement Complex schists and gneisses (Sherry, 1989). According to a review of data from Geological Survey Bulletins conducted by Bell (1984), bands of quartz-schists and granulites and hornblende biotite gneiss are found in the Majete Escarpment area. The Kapichira Falls on the Shire River was formed by widespread dolerite formations such as dykes and sills (Sherry, 1989). The soil composition in MWR includes lithosols and shallow, stony, ferruginous soils, or lithosols with sandy or loamy soils of low fertility, and limited deposits of alluvial, more fertile soils are restricted to small areas along some rivers (Sherry, 1989). The soils are generally stony and shallow and therefore not suitable for cultivation.

The expected annual precipitation for MWR is 680-800mm in the east and 700-1000mm in the west, most of which occurs in the hot, wet season between November and March/April (Hall-Martin, 1972; Wienand, 2013). "Chiperoni" is the local name for the low cloud and drizzle that occurs between April and October as a result of south-easterly winds from the Mozambique Channel blowing moisture over the highlands of the Great Rift, including the mountain peak so named "Chiperoni", (Sherry, 1989; Morris, 2006). The mean annual temperature is 23.3°C. The lowest temperature recorded is 11°C and the highest is 45°C (Sherry, 1989; Wienand, 2013). The warmest month, October, has a mean temperature of 34°C and the cooler months of June and July have a mean temperature of 16°C. Three seasons were outlined by Hall-Martin (1972) for Lengwe National Park that lies just south of MWR, and are therefore relevant to MWR. These are: (*i*) hot,

wet season (November to March/April) in which most rainfall falls and relative humidity is high; (*ii*) cool, dry season (April to August) where there is no significant rain but relative humidity is high; (*iii*) hot, dry season (September to November) with no significant rain and lower humidity.

The Shire River is a major, perennial river that drains Lake Malawi and provides water throughout the year. It flows southwards, cutting through the north-eastern section of MWR and forms part of the boundary of the reserve with surrounding settlements and the hydropower station at Kapichira Falls. In the northern section is the Mkulumadzi River, which is another perennial river, but it is not as substantial. There are several non-perennial rivers in the reserve and flash floods may occur. At times water may be found deep in the sand in the dry season, as a result of reservoirs being formed in the riverbeds by barriers of rock (Sherry, 1989). There are 11 seasonal and perennial springs that are dispersed across the reserve. Borehole-fed artificial water points (AWPs) have been placed in several places in MWR to supplement the available natural water and stabilise surface water availability (Chamaillé-Jammes, Fritz & Murindagomo, 2007; Wienand, 2013). AWPs are important in increasing access to forage for wildlife during the dry season (Redfern, Grant, Gaylard, & Getz, 2005; Loarie, van Aarde & Pimm, 2009), thereby assisting population growth of species and for creating tourism opportunities (Shannon, Matthews, Page, Parker & Smith, 2009; Wienand, 2013).

The classification of vegetation in MWR has been revised several times (Sherry, 1989) and work is currently being conducted to improve classification and mapping (reserve manager, pers.com.). Vegetation types in MWR are influenced by soil type and depth. Based on previous work and other studies, Sherry (1989) defined the following vegetation types for MWR: riverine vegetation along larger river systems (*Kigelia africana*, *Lonchocarpus capassa* and *Euphorbia ingens*); low altitude (205-280m) mixed deciduous woodland (*Acacia spp.*, *Sclerocarya birrea* and *Sterculia spp.*); ridge-top (220-300m) mixed woodland (*Terminalia sericea*, *Diospyros kirkii* and *Diplorhynchus condactylcarpon*); medium altitude (230-410m) mixed deciduous woodland (*Brachystegia boehmii*, *Pterocarpus rotundifolius*, *Diospyrus kirkii* and *Combretum spp.*); and high altitude (410-770m) miombo woodland (*Brachystegia boehmii*, *Julbernardia globiflora*, *Burkea africana*, *Diplorhynchus condylocarpon and Pterocarpus angolensis*). This study was conducted in the original sanctuary area and not in the entire reserve due to physical, time and financial constraints.

2.2 Study species

2.2.1 Impala (*Aepyceros melampus*)

The impala, *Aepyceros melampus* (Lichtenstein 1812), is an ecotone (edge) ungulate (Estes, 1991) that has a widespread distribution in the north-east of southern Africa, extending through Central Africa to East Africa, reaching its northern most limits in central Kenya. (Stuart & Stuart, 2006). Impala are medium-sized, gracefully built and gregarious ungulates (Mooring, 1995; Skinner & Chimimba, 2005; Frost, 2014). Males have a shoulder height of 75-92cm and weigh between 53-76kg, while females have a shoulder height of 70-85cm and weigh 40-53kg (Estes, 1991). Males reach their mature height and weight at three years and four and a half years respectively, and females reach their mature height and weight at two years and three years respectively (Skinner & Chimimba, 2005).

The upper part of the body is chestnut-brown and is distinctly divided from the fawn band extending across the flanks, from behind the shoulders. The legs are also fawn coloured, but are lighter on the interior side of the leg and toward the hooves. The underparts are white, as are throat and chin, the narrow bands above the eyes, parts of the inner ear and under the tail. The rump has vertical, black lines that originate close to the base of the tail on either side, and tapers out down the back of the thigh. Other markings include a black, dorsal line along the hindquarters to the tip of the tail, black tip on the ears, a very dark patch on the forehead and a black tuft of hair on each fetlock, which overlies the metatarsal gland. Only the males have long, elegant, lyrate (S-curved) horns that measure 45-91.7cm. Horns have well pronounced ridges for two thirds of their length which even out as the horn tapers to a point (Estes, 1991; Skinner & Chimimba, 2005).

As an ecotone species, impala prefer woodland with minimal undergrowth and low to medium height grasslands on flat to moderately sloped landscapes. They often associate with woodland vegetation, such as *Acacia* and *Colophospermum mopane*, *Baikiaea*, *Combretum* and *Terminalia* woodlands. They are absent from montane regions as they do not favour the dense vegetation nor steeper gradients generally associated with such areas (Skinner & Chimimba, 2005). As a sedentary antelope, home ranges include a variety of vegetation types which are utilized at different seasons; upper slopes with good visibility and forage quality in the wet season, moving to drainage-line green belts during the dry season (Jarman, 1979; Estes, 1991).

The removal of nitrogenous waste due to the large crude protein intake in impala diet requires sufficient water intake. This demand for hydration, necessitates impala to stay within access to a

water source, especially during the drier periods (Fairall & Klein, 1984), thus impala are so called water dependent (Augustine, 2004; van Bommel, Heitkönig, Epema, Ringrose, Bonyongo & Veenendaal, 2006). A study conducted in the Kruger National Park by Young (1972), stated that impala remained within a distance of 8km from a water source and that half of the herds observed were within 1.6 km of water (Skinner & Chimimba, 2005). Impala are able to go without drinking for brief periods if there is a succulent food source available that may provide their essential moisture needs, in some circumstances a source of green vegetation (Estes, 1991; Skinner & Chimimba, 2005; Frost, 2014).

Impala have been described as intermediate mixed feeders (Hofmann, 1973, 1989; van Rooyen, 1992; Brashares & Arcese, 2002; Skinner & Chimimba, 2005; Cerling, Harris & Passey, 2003) as they are primarily grazers when grasses are green and have fresh growth, and browsers during the drier months (Estes, 1991). The extent to which they consume foliage or grass depends on the habitat occupied and the time of year (Azavedo & Agnew, 1968; Rodgers, 1976; Dunham, 1980; Ambrose & De Niro, 1986; van Rooyen, 1992; Pietersen, Meissner & Pietersen, 1993; Meissner *et al.*, 1996).

Impala utilize a wide range of grasses of which more common species that occur across their distributional range comprise an important part of their diet. These include *Digitaria eriantha* (finger grass), *Themeda triandra* (red grass), *Cynodon dactylon* (couch grass), *Panicum maximum* (buffalo grass), *Eragrostis* spp. and *Urochloa* spp. The proportion of theses grasses in the diet depends on the local availability and condition of vegetation due to the dry and wet times of the year (Wilson, 1975; Skinner & Chimimba, 2005).

Impala have been referred to as 'concentrate feeders' as they are able to select the most palatable and nutritious parts of plants (Frost, 2014). Browse substrates include leaves, fine twigs of shrubs and trees that can be green or dried up leaves on the ground, various forbs, young buds and wild fruit (Skinner & Chimimba, 2005). Impala forage on a varied list of browse species, depending on their distribution (Skinner & Chimimba, 2005). The young growth and twigs of *Acacia* spp. are frequently part of their diet. Impala will consume fine twigs and leaves of the following when available: *Combretum* spp., *Boscia* spp., *Grewia* spp., *Ziziphus* spp., *Maytenus* spp., *Dichrostachys* spp., *Commiphora* spp*., Terminalia* spp.; and dry fallen leaves of *Spirostachys africana* (tambotie), *Colophospermum mopane* (mopane) and *Combretum apiculatum* (bushwillow) during the dry season.

The ability to use monocotyledons (C_3 or graze species) and dicotyledons (C_4 or browse species) gives impala an unusually varied, abundant and reliable food supply, which enables them to lead a

sedentary existence and reach great densities (Estes, 1991). Impala thrive in areas where natural vegetation has degenerated and bush encroachment occurs as a result of the overgrazing (Augustine, 2004; Garine-Wichatisky, Fritz, Gordon & Illius, 2004; van Bommel, Heitkönig, Epema, Ringrose, Bonyongo & Veenendaal, 2006). As food availability and quality declines, impala spend more time foraging in a day and will travel greater distances in search of forage and water (Murray, 1982b). Although impala need to drink daily, they are able to subsist in drought conditions better than specialist species like sable antelope (*Hippotragus niger*) and roan antelope (*Hippotragus equinus*) due to the flexibility of their diet (Frost, 2014). Cerling *et al.* (2003) conducted a herbivore dietary study using stable isotope analysis in which it was determined that impala had the widest range of $\delta^{13}C$ values, with an approximated 52% C_4 contribution to their diet, indicating a mixed C_3-C_4 diet. Therefore impala are mixed feeders that are opportunistic and proportions of C_3 and C_4 in their diet may vary between individuals. The diet preferences of impala, as determined by previous studies are summarized in Table 2.1.

According to van Rooyen & Skinner (1989), the ratio of monocotyledons to dicotyledons in impala diet varied between sexes as a result of their social organization. As a result of territorial males defending their prime territories from other males in the time leading up to the dry season (autumn) the bachelors are forced to find forage in other areas. In their study they found that dicotyledons composed 31%, 48% and 49% of the diets of territorial males, females and bachelors, respectively. They determined that the territorial males spent time and energy, maintaining their prime territory and therefore had less time for selective feeding. In the region van Rooyen & Skinner (1989) conducted their study, bachelor males were pushed into surrounding koppies (small, isolated hills), where there are naturally fewer grasses, hence a higher percentage of dicotyledons in their diet (Skinner & Chimimba, 2005).

The time of year has an effect on the behaviour of these gregarious ungulates and how they are socially organised and distributed (Mooring, 1995; Skinner & Chimimba, 2005). Group sizes range from small herds of 6-20 individuals in the drier months when forage is less available, to gatherings of 50-100 in the wet and early dry seasons when forage is more abundant (Estes, 1991; Skinner & Chimimba, 2005). In general, the sexual segregation of ungulates refers to the separation of males and females outside of the breeding season (Ruckstuhl & Neuhaus, 2000, 2002, McKenzie & Hart, 1995) on different scales. On a spatial scale males and females have varying home ranges; on a habitat scale they will use different habitats in an area; on a dietary scale their foraging behaviours will differ; and on a social scale they form single-sex groups within a habitat (Mysterud, 2000; Ruckstuhl & Neuhaus, 2000, 2002)

The following designated group compositions may be used to describe the variable, social organization of impala (Estes, 1991):

i.   Bachelor herd: adult and immature males of which some adults are potential territorial males

ii.   Breeding herd: adult and immature females, immature males and a territorial male

iii.   Nursery herd: females and their young

iv.   Mixed herd: adult males and females and immature males and females

v.   Territorial males: lone adult males that compete for females and territory during the rut

During the rut the dominant male will defend a territory with a herd of several ewes and young and will not tolerate other rams in the area. Skinner & Chimimba (2005) describe impala as "short-day breeders" that mate within a limited period in autumn (Skinner *et al*., 1974; Skinner & Van Jaarsveld, 1987). In Southern Africa the daylight hours start to decrease in January prompting the testosterone levels in impala rams to rise, which causes their behaviour to be more aggressive and restless (Vincent, 1979; Skinner & Chimimba, 2005). Impala have a series of distinctive rutting sounds that are heard more frequently as fighting amongst the rams increase. Successful and dominant rams will establish a territory and scent-mark it by rubbing the vegetation with the glandular skin on their faces and foreheads (Jarman & Jarman, 1974; Skinner & Chimimba, 2005). Dominant rams intimidate, chase away and even fight with any trespassers in their territory, clearing out all non-juvenile males about 4-6 weeks before the mating period (Murray, 1982; Skinner & Chimimba, 2005). Around March hormone levels are high in the territorial rams and they start to build their harems of ewes by herding them together into groups (Fairall, 1972; Skinner & Chimimba, 2005). One year old females remain with their mothers and the yearling males are tolerated on the periphery of the breeding herd but young males generally join a bachelor herd after being expelled from the breeding herd by the dominant ram (Skinner & Chimimba, 2005). If an impala ewe leaves or wanders away from the herd the dominant ram will round her up and keep her close to the main herd. Males will vigorously compete for any ewe that wanders away from her herd. According to Fairall (1983), females cycle twice in a season, the first oestrus occurring in early May. The ram will constantly test each ewe for her readiness to mate (Skinner & Chimimba, 2005). If the female is receptive, the male will mount her for a very brief period (<10 seconds) several times in which copulation may occur. Successful copulation is followed by a snort or 'roar' from the male (Skinner & Chimimba, 2005).

The territorial rams vigorously defend their territories and harems of females from rival males using various vocalizations, displays of dominance, and if necessary they will engage in physical aggression. Typical behaviour in a territorial ram is the use of the alarm snort that becomes a long-

drawn-out bark as the ram gives chase. An impala holds a typical posture while roaring, with the head and nose directed forward and the tail extended. Roaring continues through the night as rams persist in defending their territories (Skinner & Chimimba, 2005). The territorial ram may deter rivals by lowering his horns and bobbing his head, engaging in horn clashing and head pushing if the rival is not successfully threatened. Generally impala do not have lengthy and intense contests and will separate and conclude with a roar, but some battles lead to acute injury and even death (Skinner & Chimimba, 2005). Territorial males may also bob their heads and lower their horns in conjunction with some roaring in a threatening manner while shepherding females (Skinner & Chimimba, 2005). This behaviour can be observed outside of the rutting season, but not to the same extent (Skinner & Chimimba, 2005).

The sexual cycle of males peaks in autumn (Skinner, 1971; Kerr, 1965) and drops to its nadir in spring (Skinner & Chimimba, 2005). A five-year study conducted by Murray (1982) suggested that the lunar cycle influenced the timing of the rutting season, peaking for a 20-day interval between full moons. Impala rams are often so consumed with mating and maintaining their harems and territories that they do not adequately forage and thus lose condition as they deplete fat reserves stored in their necks (Dunham & Murray; 1982). It is possible for a dominant ram to forfeit his harem and territory to a challenging ram that is stronger and in better condition. Dominant rams will give intimidating displays toward potential opponents, however some bachelor rams will be less intimidated if they have successfully mated with ewes before the arrival of the dominant male. The extended breeding season in East Africa leads to the maintenance of territories and sexual segregation for most of the year, however most mating occurs during the rainy season with a peak at the end of the rains (Estes, 1991). The gestation period of impala is 194-200 days and lambs are generally born between November and January, depending on region. For each area lambs are born within a certain period of each other (Fairall, 1971).

The peak lambing season lasts for about three weeks from late November to early December. Later lambing peaks in January could be a result of delayed conception because of a lack of nutrition, as seen near Messina, South Africa (Vincent, 1972; Skinner et al., 1977; Skinner & Chimimba, 2005). Ewes move away from the main herd to give birth to their lamb in thick vegetation. A ewe will have a single lamb, though there have been rare cases of twins recorded in East Africa (Kayanja, 1969). The female eats the afterbirth (Jarman, 1976) to remove any scent that may attract intruders, as the young lamb learns to coordinate its movements (Vincent, 1972). It will be able to follow its mother about one to two days after birth (Estes, 1991). A lamb remains hidden in dense cover for the first two days of its life, until it is stronger and able to follow its mother within the herd. For some

species, the synchronized birthing of young within a certain period reduces predation on newborns (Rutberg, 1987), however, the occasional birth out of season has been reported (Estes, 1991). Lambs initially stay close to their mothers but as they get older they will mingle with the rest of the herd and only return to their mother to suckle or for comfort after a disturbance. Female impala fat reserves are lowest over January and February when their energy requirements are the greatest for milk production (Skinner & Chimimba, 2005).

Smaller distinct groups of an older female, her daughters from previous seasons and their offspring, may form within sizeable herds of about 100 animals or more. These close-knit family units have a degree of independence from the main herd and will forage, drink and rest together. Females are able to conceive at just over two years of age. With an average lifespan of 15 years, females in the wild can produce 13 lambs with a 100% lambing record (Skinner, 1969; Skinner & Chimimba, 2005). Bachelor herds generally do not spend time in areas occupied by breeding herds so as to avoid disturbance especially during the rut as territorial males display aggressive behaviour toward other males. Members of bachelor herds may leave during the rut and re-join post rut and as a result bachelor herds are less cohesive than breeding herds (Skinner & Chimimba, 2005). Males are sexually mature at 13 months of age but they cannot yet claim and maintain territories. Males start to compete in the rutting season when they are older than three years (Kerr, 1965). In the southern parts of their distribution, only rutting males between the ages of 4.5 and 8.5 years, establish territories that range from five to eight hectares (Vincent, 1979; Murray, 1982; Skinner & Chimimba, 2005). The home ranges of breeding herds vary between 80-180ha in size, according to seasons and will overlap the territory of several males (Murray, 1982b; Skinner & Chimimba, 2005). Territorial males will accumulate females from these breeding herds (Skinner & Chimimba, 2005). Home ranges of animals in the same clan overlap by 73% and these animals congregate during the wet season when green grass is abundant. The home ranges of adjacent clans overlap by 4% and therefore rarely associate with each other. (Murray, 1981; Murray, 1982b; Skinner & Chimimba, 2005) Male home range sizes increase with age to 90 ha at 3-4 years, declining to 49 ha at 5-6 years (Murray, 1982b)

Impala are primarily diurnal but are nocturnally active at times. While active, impala move slowly through an area and are often seen deterring irritating flies by swishing their tails, stamping their feet and twitching their ears. During the warmer periods of the day they stand or lie down in the shade to rest. Impala may take this time to groom their coats removing ectoparasites such as ticks, using their lateral teeth (second and third incisors and the incisiform canine) of the mandible (McKenzie & Weber, 1993; Skinner & Chimimba, 2005). Impala are also known to use their hind

leg hooves to remove ectoparasites from the front of the body (Skinner & Chimimba, 2005). It has been suggested that the 'grooming clock', rather than the irritation of blood feeding ectoparasites, regulates the grooming sessions throughout the day so that parasites are removed before they are able to attach and blood-feed (Hart, Hart, Mooring & Olubayo, 1992; Mooring, 1995). The frequency of grooming sessions differs seasonally and geographically as ectoparasite densities rise and fall (Mooring 1995). Tick densities increase during the wet season and grooming will occur more often as a result (Mooring, 1995; Skinner & Chimimba, 2005). Impala also groom themselves more often in areas with greater densities of ticks, than they would in areas with a low tick density (Hart & Hart, 1992; Skinner & Chimimba, 2005). Impala lambs have a lower density of ticks compared to the adults as they groom themselves more frequently (Mooring & Hart, 1997a, 1997b; Skinner & Chimimba, 2005). Allo-grooming, occurs when an individual grooms another and it is important for the oral grooming of the head and neck region, as it is not possible for an individual to groom their own head and neck. Two individuals will stand head on and orally groom each other's head, ears and neck (Hart & Hart, 1992; Mooring, 1994; Skinner & Chimimba, 2005). Allo-grooming partners are chosen based on the frequency of interaction between age-mates in close proximity and not on kinship or dominance (Mooring & Hart, 1993; Skinner & Chimimba, 2005). Territorial males carry a heavier load of ticks than females and bachelor males because they do not groom themselves as frequently as their energy is primarily directed toward defending their territory during the rutting season (Mooring & Hart, 1995b; Mooring *et al*., 1996b; Skinner & Chimimba, 2005). Impala reduce their grooming efforts when oxpeckers (Buphagus spp.) co-occur in an area, as oxpeckers are "facultative tick-eaters" and are efficient at removing high densities of ticks from the head, ears and neck of impala (Mooring & Mundy, 1996; Mooring et al., 1996b; Skinner & Chimimba, 2005)

The vigilance of impala increases with predator density (Hunter & Skinner, 1998; Skinner & Chimimba, 2005). Impala carefully observe known intruders that are a fair distance away, but will move off slowly if the intruder moves in closer. If an intruder comes within the flight distance, impala may burst away in several directions in a display of agile leaps and jumps over imaginary obstacles, and then regroup to move together in one direction, bounding their way toward safety (Estes, 1991; Skinner & Chimimba, 2005). These elegant jumps can reach three meters in height and 10 meters length. The motion of kicking backwards and upwards with the hind legs when startled is thought to release a scent from the metatarsal glands under the black patch on the fetlocks (Kingdon, 1982; Skinner & Chimimba, 2005).

Young lambs are often preyed upon by spotted hyena (*Crouta crouta*), cheetah (*Acinonyx jubatus*), jackals (*Canis* spp.), and pythons (*Pythonidae* spp.). Impala have an alliance with baboons (*Papio*

cynocephalus) and monkeys (*Cercopithecus pygerythrus*) that serves as an anti-predation tactic. Baboons and monkeys have excellent eyesight and will alert surrounding animals of a potential threat approaching, such as a predator. Antelope such as impala use their keen senses to detect any potential danger and sound off an alarm call to warn others (Estes, 1991).

2.2.2 Waterbuck (*Kobus ellipsiprymnus*)

The waterbuck, *Kobus ellipsiprymnus* (Ogilby, 1833) is a large, robust antelope that derives its colloquial name from their association with water throughout their distributional range. There are two subspecies: *Kobus ellipsiprymnus ellipsiprymnus* and *K. ellipsiprymnus deffasa* (Rüppell, 1835). The defassa waterbuck are synonymous with common waterbuck. A study by Kingswood *et al.* (1998), determined that the common group and defassa group had a diploid number of 2n = 50-52 and 2n = 53-54, respectively. This could account for the variation in body markings (Kat, 1993). Common waterbuck occur in southern Somalia, the eastern regions of Kenya and Tanzania, Malawi, Mozambique, Zimbabwe, eastern and south-eastern Zambia, the Zambezi Region in Namibia, northern Botswana and northern South Africa (Skinner & Chimimba, 2005). The defassa waterbuck mostly occur west of the Great Rift Valley as their distribut extends from Ethiopia toward Senagal (Estes, 1990). The Muchinga escarpment and other unsuitable habitat in Zambia separate the subspecies, but their distribution does overlap in Kenya and north-eastern Tanzania where they have interbred. In comparison to fellow genus members, waterbuck lack inguinal pouches as found in lechwe, *Kobus leche* (rudimentary) and puku, *Kobus vardonii* (functional); and infra-orbital and pedal glands also found in puku.

Common waterbuck bulls have a shoulder height of 127cm and being one of the heaviest antelope, weigh between 198-262kg, while cows have a shoulder height of 117cm and weigh 161-214kg (Estes, 1991). Their pelage is coarse and shaggy and is white and grey, grizzled with darker colour hair that varies between a dark brownish-grey or greyish-brown. The most diagnostic feature of the common waterbuck is the broad, white ring that encircles the rump. Other markings include a white chin; the white collar on the throat and down the lateral part of the neck; the white patch above each eye that extends down to the muzzle; and a white band on the leg just above the hooves (Estes, 1991). Their tail is about 35cm long (Stuart & Stuart, 2006) and the colour is the same as the upper body (Skinner & Chimimba, 2005). Only the males have forward curving horns that are on average 75cm long. The horns are prominently ringed for about three-quarters of the length and smoothen toward the tips. It has been suggested that waterbuck have a poorly developed sense of smell. Waterbuck produce a nauseating, musky smelling substance that is secreted by diffuse sebaceous

follicles in the skin and creates an oily layer on their coats (Estes, 1991). This oily layer may act as further waterproofing in addition to their unusually thick pelage and even as an anti-predatory mechanism (Frost, 2014).

Waterbuck require an unusually high amount of water (Taylor, Spinage & Lyman, 1969), therefore their habitat preference is influenced by proximity to drinking water as well as the quality of grasses associated with savannah ecosystems, for example *Panicum maximum* (buffalo grass), which grows close to water (Melton, 1978). As a result waterbuck may have a patchy, ecotonal distribution (Estes, 1991). The need for a greater water intake is due to the consumption of mostly protein-rich grasses (Taylor *et al.*, 1969; Estes, 1991). Lamprey (1963) found that waterbuck kept within 1.8 km of open water in East Africa. In the Okavango Delta, waterbuck were found in wetlands and on floodplains close to the swamp. They have a varied range of habitat requirements but they can be pushed out of their ideal habitat by nyala (*Tragelaphus angasii*) as observed by Melton (1978) in Imfolozi Game Reserve, South Africa. Waterbuck, along with buffalo (*Syncerus caffer*) and plain's zebra (*Equus quagga*) do not over-exploit grazing areas, as impala, nyala and the common warthog (*Phacochoerus africanus*) tend to do (Melton, 1978). According to Taylor *et al.* (1969), waterbuck are more prone to dehydration in hot weather than the average domestic steer (*Bos* spp.). Waterbuck prefer dry ground but will go into water to elude predators and occasionally to feed (Estes, 1991).

Waterbuck have a preference for open habitats that have short to medium grasses for grazing (Wirtz & Kaiser, 1988; Traill, 2004; Gutbrodt, 2006). As they are primarily grazers they are more efficient in the digestion of fibre (Hofmann, 1973) and usually select the most abundant species present (Tomlinson, 1980b). Waterbuck have been described as selective feeders to a degree (Tomlinson, 1980; East, 1984; Melton, 1987; Gutbrodt, 2006), where they may select more nutrient rich grass species in the wet summer season that quickly lose their nutritive value and decrease in abundance toward the dry winter months. At this time waterbuck increase their consumption of alternative grass species that had relatively lower nutrient value in the summer months, but maintain their quality for a longer period in the winter months suggesting a shift towards a bulk feeding strategy as expected for an antelope with a heavy body mass (Gutbrodt, 2006). Waterbuck may browse species such as *Acacia tortilis* (umbrella thorn) and eat the fruits of *Sclerocayra birrea* (marula). Waterbuck select different vegetation species as the seasons change in the year (Herbert, 1972; Tomlinson, 1980b; Skinner & Chimimba, 2005). This variation in diet is a result of changing moisture, crude fibre and crude protein percentages of each vegetation species (Tomlinson, 1980b). Nutritious food is a limiting factor in winter especially when there is competition for the resource as Melton (1978) determined, when waterbuck were outcompeted by nyala and impala and calves died as a result of

poor nutrition and a high tick load. Waterbuck frequently select the following grass species: *Brachiara* spp., *Chloris* spp., *Cynodon dactylon*, *Digitaria* spp., *Heteropogon contortus*, *Panicum* spp. and *Themeda triandra*. Waterbuck select the following hydrophytic plant species: *Setaria* spp., *Hemarthria altissima*, *Cyperus* spp., *Phragmites* spp. and *Typha* spp. (Brynard & Pienaar, 1960; Child & Von Richter, 1969; Herbert, 1972; Hirst 1975; Melton, 1978 and Tomlinson, 1980b; Skinner & Chimimba, 2005). The diet preferences of waterbuck, as determined by previous studies are summarized in Table 2.1.

Waterbuck generally form small herds of 6-12 individuals, but larger congregations of about 30 individuals do occur (Skinner & Chimimba, 2005), usually during the summer months (Melton, 1978). As food availability is low and scattered during the drier months the waterbuck population fragments across the landscape to acquire adequate forage (Melton, 1978).

Home ranges of nursery herds may overlap the territories of several territorial and non-territorial males. Territory sizes may vary from 1.2-2.8 kilometers in diameter and do not overlap (Herbert, 1972). Tomlinson (1981) determined that the mean territory was 89.5 ha in Zimbabwe and Melton (1978) determined it was 66ha in KZN. Home ranges and territory sizes of waterbuck are dependent upon population density, habitat quality and the age and fitness of individuals (Estes, 1991). Females are often seen alone or in pairs but may form casual groups of five to ten individuals (Spinage, 1982; Estes, 1991). Dominant bulls try retain cows within territories when in rut but otherwise the cows and calves have freedom to move between territories. Bachelor bulls, cows and calves can form mixed herds of up to 30 individuals, though herds of nearly 70 individuals may be seen in highly favourable habitat. Waterbuck do not disseminate readily and may maintain their home range for years, yet herd compositions can change due to individuals joining and leaving herds freely. Waterbuck do not often display physical contact in the form of greeting or grooming (Estes, 1991). According to Spinage (1982), females are hostile toward male and female weaned calves, some of which stay close to older females while others detach from the herd to join floating spinster groups or become solitary (Estes, 1991).

Territorial males will push young males out of the herd when their horns emerge at eight to nine months old. Males from bachelor groups have a hierarchy according to seniority and dominance within each age class (Estes, 1991; Skinner & Chimimba, 2005). Sparring between males is common and may occur between individuals from different age classes (Estes, 1991). Maturation in waterbuck is slow but once adult males reach a strong physical condition at an age of five to six years, they establish territories that they maintain throughout the year (Spinage 1982; Skinner & Chimimba, 2005). Dominant bulls defend territories aggressively during the rut, though generally

tolerance is displayed towards non-threatening, trespassing bulls (Estes, 1991; Kingdon, 1997). A series of intimidating displays are used to defend territories such as the use of a 'proud' posture where the head is held high and the white markings on the throat and between the eyes are shown in a frontal display or the thickness of the neck is displayed in a lateral display. The directed lowering and shaking of the territorial male's head may threaten a trespasser. Submissive males acknowledge the territorial male's dominance by dropping their head and body. Waterbuck bulls participate in serious fights more often than other ungulates and combatants may die as a result. Tomlinson (1981) conducted a study in Lake McIlwaine, Zimbabwe, where he determined that bachelor herds avoided conflict with territorial males by inhabiting marginal areas far from the lake shoreline. In the dry season bachelor males will move into territories where highly nutritious, fresh grass growth occur after a recent fire event.

Waterbuck can breed throughout the year across their distributional range, though some studies have determined distinct birth peaks at certain time of the year. For example, the number of births peaks in October and again in February-March in the Kruger National Park, South Africa (Pienaar, 1963; Fairall, 1968; Skinner & Chimimba, 2005) and Melton (1983) determined that there was in increase in calving in February and March. A male will smell the vulva and urine of female to determine her reproductive status. After the urine of the female contacts the cupped philtrum of the male, the male will display flehmen, where the nose is pointed upwards, the head raised, lips are retracted and nose is wrinkled. During this act the male uses his olfactory organs to access the state of the female. An oestrus female's back will arch and the tail moved to the side. The male will rub his face and the base of his horns on the oestrus female's back in an act of courtship. If the oestrus female is receptive the male rests his head on her back or uses his forelegs to tap her hind legs and copulation may follow (Spinage, 1982) up to 10 times with the same male (Wirtz, 1983).

After a gestation period of 280 days (Spinage, 1982), the female withdraws from the herd to give birth in a concealed environment. Females generally give birth to a single calf though twins have been recorded. After eating the afterbirth, the mother leaves the calf to hide itself as it is susceptible to predation for two to four weeks. Calves are able to find their feet within half an hour and are able to outrun a man within a day. If discovered, calves readily bolt rather than remain motionless (Spinage, 1982; Estes 1991). The mother returns to nurse and clean the calf briefly several times a day, communicating with it using bleats and snorts to which the calf responds by alarm-trotting and stotting (Estes, 1991). Calves start to follow their mothers from three to four weeks old and the mother encourages the calf to do so by holding her tail up or out, a behaviour not common or known in other ungulates (Estes, 1991). Mothers stop lactating at 180-210 days and the calves are

weaned after 276 days (Spinage, 1982; Skinner & Chimimba, 2005). Waterbuck crèches are formed and are often relatively close to an adult herd to which they run to when under threat (Estes, 1991). In captivity waterbuck have a lifespan of 17 years and five months (Jones, 1993). As for males, females are relatively slow to mature and (rarely) conceive before the age of three years (Hanks, Stanley Price, Wrangham, 1969; Estes, 1991).

As with most grazers, waterbuck normally ruminate and rest between the two peak feeding times in the earlier hours of the morning and later hours of the afternoon to early evening, though short periods of foraging may occur according to the individual out of the peak time (Estes, 1991). Lactating females generally spend more time feeding than other females while territorial males feed less and rest more (Spinage, 1982; Estes, 1991). More data still needs to be gleaned on the nocturnal activities of waterbuck but it is suspected that they have another feeding peak and other short foraging bouts (Spinage, 1982; Estes, 1991).

Table 2.1 Summary of dietary preferences for impala and waterbuck determined by previous studies.

Reference	Impala	Waterbuck
Estes (1991)	Intermediate feeder. Predominantly a grazer when grasses are growing and green, at other times a browser of leaves, shoots, forbs and seedpods. Ability to consume monocots and dicots results in having highly varied diet, but more reliable food sources and they can adapt to different habitats.	Wide variety of grasses. Prefer short to medium length grasses that are protein rich; consume dicots for additional protein intake when green grass is scarce.
Kingdon (1997)	Primarily grazers in the wet season, grass content drops to 30% in dry season, browse shrubs, herbs, seeds and pods	Grazer, leaves of woody plant or fruit if grass is scarce
Gagnon & Chew (2000)	Browser-grazer intermediate; mixed feeder	Variable grazer, 84% monocots; 15 % dicots and 1% fruit
Cerling *et al*. (2003)	Mixed feeders Opportunistic feeders shifting between browse and graze species –largest range of carbon isotope values 52% grass	Grazer 70-95% grass.

It has been speculated that predators are deterred from preying on waterbuck due to the powerful, musky odour they emit from their skin, but this has been challenged. In some cases, waterbuck are more common prey items of predators such as lions (*Panthera leo*), crocodiles (*Crocodylus niloticus*) and hyena, than other herbivore species (Pienaar, 1969; Estes, 1991). Waterbuck hold an alert posture when vigilant and snorting, stotting, trotting, running into cover and even self-defense in males are all anti-predator behaviours.

2.3 Applied methods

2.3.1 Behavioural observations

The behaviour of organisms may refer to activites observed at a point in time or of those observed over a period of time. Altman (1974) described several methods that could be used to sample the spontaneous social behaviour of humans or other animals, such as ad libitum sampling, time sampling and one-zero sampling. Each sampling method can effect natural behaviour processes and therefore restrict what aspects can be studied. The observer is not able to constantly observe and capture data on the behaviour of each individual in the group and therefore has to be content with imperfect descriptions. It should also be considered that observer fatigue has an effect on the accuracy and detail of data captured. Scheduling of sample sessions may be predetermined to start according to times or when a specific behaviour is observed. There may also be no scheduling rule where observations are made spontaneously.

Ad libitum sampling is probably the most widely used method of recording behaviour in field studies. Observers typically record as much information as possible in the form of field notes, which could result in the observer making unconscious sampling decisions (Altman, 1974). Often this widely used technique is the one that is infrequently described in sampling methods. For ad libitum sampling, two assumptions are commonly made. These are: (i) that the probability of a behaviour being observed and recorded does not depend on the class of behaviour, so one behaviour will not significantly attract more attention than another, relative to the frequency of occurrence of each behaviour; (ii) behaviour does not depend on the age or the gender of individuals, thus behaviour may be juxtaposed across age and sex classes. Ad libitum sampling is often used in the primary quantification in field studies. It is uncommon and challenging to determine genuine differences between individuals, age-sex classes or behaviours when sampling ad libitum. Additionally, it is difficult to obtain all information on social behaviour even when employing the focal-animal technique where observers concentrate on an individual and their interactions as well as non-social behaviour (Altmann, 1974).

Time sampling was a systematic sampling method that developed in the 1920's to study the spontaneous behaviour of children (Olson, 1929; Altmann, 1974). This technique considered the presence or absence of a specific behaviour, as opposed to the frequency, therefore the state rather than an event is recorded at any time in the sampling period. These sampling periods were defined, short periods of time (Goodenough, 1928; Altmann, 1974). Each sample period occurs in succession of each other and records the behaviour of lone individuals or pairs of individuals.

One-zero sampling was used in the study of animals by Olson (1929). If a specific behaviour occurs within a time period a score of one is recorded and a score of zero is recorded if the behaviour does not occur. These scores may be tested by non-parametric tests such as the Mann-Whitney U Test. Correlational analysis may be conducted for different behaviour scores.

For this study, observed behaviour (activities) was recorded for impala and waterbuck populations to establish a baseline of their ecology in MWR, post reintroduction. However, there are several disadvantages in collecting data *ab libitum,* as was done for this study. In hindsight it did not produce sound data, which made statistical analysis challenging. Thus the results discussed in Chapter Four are descriptive rather than qualitative. For future behavioural studies, it would be beneficial determine more specific objectives and therefore develop clearer data collection and analysis protocols.

2.3.2 Distance Sampling

Distance sampling is referred to as such, as the distance of the observed animal from the point of observation is recorded and analysed in a software programme, DISTANCE 6.2 Release 1 (Thomas, Buckland, Rexstad, Laake, Strindberg, Hedley, Bishop, Marques & Burnhman, 2010) to estimate the number of animals including those not seen by the observer. Distance sampling relaxes the assumption that all animals are seen in a strip transect, thus transect widths may be greater, covering more of the total area. DISTANCE is a Windows-based computer package that may be used to design and analyse distance sampling surveys of wildlife populations (Thomas *et al*., 2010). The development of DISTANCE was based on a programme called TRANSECT (Burnham *et al*., 1980) by a team of programmers and investigators who continue to improve and develop the software. DISTANCE may be used to analyse distance data captured by line transects, point transects and cue-counts. Line transects may be conducted on the ground from a vehicle and on foot or aerially from an aeroplane.

The theory of distance sampling assumes that all animals occurring on the transect line or at the observation point, will certainly be seen and that the probability of detecting an animal diminishes as the distance from the observer's position increases (Buckland, Anderson, Burnham & Laake 1993; Buckland, Anderson, Burnham, Laake, Borchers & Thomas, 2001). Distance sampling also works on the assumption that there is a constant density of animals around the transect line or point and it generates an estimate of that density. It is preferable that animals are detected before their behaviour is altered by any disturbance from the observer. As distance sampling yields density estimates for poorly defined areas, it is best to determine the mean of estimates by randomly or systematically repeating distance samplings, for a defined area. Ideally, DISTANCE surveys should be designed so that parallel transects are randomly or systematically distributed throughout the study area. Segmented parallel lines or zigzag patterns may be used too. Using these designs will decrease the bias and improve the efficiency of the study (Thomas *et al*., 2010)

According to methodologies outlined by Buckland *et al*. (1993; 2001) and Thomas *et al*. (2010), the following factors should be considered when conducting distance sampling. Heterogeneous habitats should be divided and studied independently as the densities as well as the detectability of animals will vary in discrete habitats. Ideally, transects should be placed randomly over the study area, or systematically if fitting. The use of tracks and roads as transects may lead to biased estimates as animals may be attracted or deterred by such features. One should avoid physically deviating from the transect line and stay as close to it as possible to be certain that all animals are detected. The standard practice would be to have two observers, like the double-observer methods. The correction factor would be affected by "violations of the same assumptions of that method". When conducting aerial surveys the ground directly below the aircraft is obscured, therefore the ground parallel to the line of flight is regarded as the centre and it is assumed that detectability is ideal. The variability of results increases, as detections of individuals are not independent. For example: when a herd of animals is seen, the herd and not an individual is treated as a sample unit. The density of the population is determined by multiplying the average size of herds with the estimated density of the herd. Sample units should only be counted once which could be challenging if animals move into and out of view due to factors such as vegetation or topography. Animals may be observed directly or dung counts may be conducted as an indirect method of estimating the abundance or density of animals in the area, considering the decay and deposit rate of dung. The accuracy of the measured distance to the sampling unit is important in distance sampling, therefore it is preferable that more reliable tools such as measuring tapes and range finders are used wherever possible. When estimating distances it could be better to use two recording zones, where the sample unit is recorded as either being within or beyond a previously set distance. An advantage of this method is that

observers only need to be trained to estimate a fixed distance rather than estimate all distances as accurately as possible. However, this method is inevitably less precise than if more distinct measurements are taken. Alternatively, distances may by divided into several bands such as 0-10 m, 10-20 m, 20-30 m and so on. Five to 10% of observations are ignored, as the detectability of animals at greater distances is too low and could decrease the precision of estimates if included. It is best to remove these observations for analysis post data collection rather than predetermining that no data should be collected for distances greater than a chosen distance, particularly in multi-species analysis where cut-off points vary for each species. It is recommended that 60-80 animals should be observed to produce a more precise estimate.
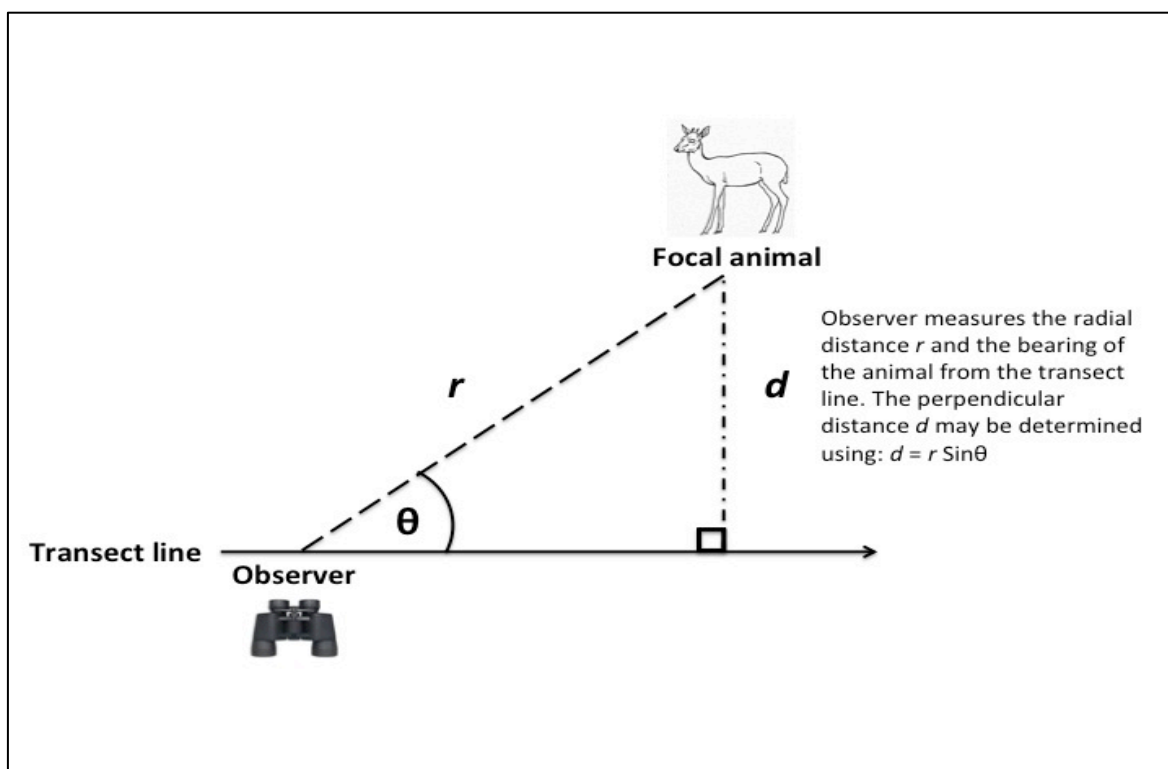
Figure 2.1 Diagram illustrating the radial distance *r* of the animal from the observer, and the angle *θ* from the transect line to the animal. The perpendicular distance *d* of the animal from the transect line may be calculated using *r* Sin*θ*. Adapted from Sutherland (1996).

Data is entered into the DISTANCE software package and variables such as the number of observers and area covered is considered in the setup of the project. DISTANCE has various analysis engines built into the programme that have different capabilities, two of which are discussed here (Thomas *et al.*, 2010). Conventional distance sampling (CDS) (Buckland *et al.*, 1999; 2001) models the probability of detection as a function of the observed distances from the transect line using a robust, semi-parametric method. Only one level of stratification is permitted and various methods are used to manage cluster size bias and variance may be estimated empirically or by using a non-parametric bootstrap. Multiple covariate distance sampling (MCDS)

is similar to CDS but includes more covariates in the detection function model and the observed distance (Thomas *et al*., 2010). The detection function in DISTANCE is the probability of detecting an animal given that it is at distance *d* from the transect line. The detection function is modelled using a robust key function and series expansion. Potential key functions to be selected are uniform, half-normal, hazard-rate and negative exponential. The options for series expansion are cosine, simply polynomial and Hermite polynomial. The measure of fit refers to how well data collected 'fits' a model and this may be measured using Akaike's Information Criterion (AIC), where the smaller the AIC the better the 'fit'. Other tests such as q-q plots, Kolmogorov-Smirnov test and the Cramér-von Mises test are also run within the programme. Models are run within an analysis engine and produce an estimated density and abundance for the area. Information gleaned from distance sampling may be used to predict future scenarios, conduct a risk assessment and support management in decision making.

For this study, distance sampling was used to determine the relative abundance estimate using a density estimate of impala and waterbuck populations in the sanctuary area of MWR. The average group (cluster) size that was encountered by observers was calculated and used to generate an estimated group size, thus accounting for individuals that were not seen by the observer. The study area was divided into three categories according to relative water availability and dominant vegetation types. Density estimates and thus abundance estimates were projected for each study area category.

Several factors may have had a negative effect on the results generated by the DISTANCE software programme. For example, transects were driven on established roads in the reserve due to the undulating terrain and vegetation, therefore transects were not parallel, nor random, as is ideal for distance sampling. The number of observers conducting counts was not constant, but an attempt to offset this affect was to pool data collected by single observers and multiple observers into different groups. In addition, observer fatigue played a role in the quality and consistency of data collected, as well as the inexperience of rotating observers. Using the DISTANCE software programme had many challenges and there are a variety of aspects that need to be understood when running analyses as well as learning how to best present your data.

The results from this study may be compared to those collected in a recent aerial survey of the entire reserve, and management staff and researchers may evaluate the effectiveness of using distance sampling in MWR.

2.3.3 Stable isotope analysis

Some elements have two or more naturally occurring stable isotopes, which have distinct masses. For example, carbon has two stable isotopes, ^{12}C and ^{13}C; as does nitrogen, ^{14}N and ^{15}N. Stable isotopes do not decay and therefore are not like radio isotopes (Crawford, McDonald & Bearhop, 2008). Stable isotope analysis is an important tool in studying systems when ascertaining fluxes of trace elements and cycling of nutrients, carbon and water. Stable isotope analysis, especially that of carbon, has become an established method in the dietary studies of animals (Smith & Epstein, 1971; Sponheimer, Robinson, Ayliffe, Passy, Roeder, Shipley, Lopez, Cerling, Dearing & Ehleringer, 2003; Parnell, Inger, Bearhop & Jackson, 2010), as animal tissues and excreta reflect the carbon and nitrogen isotope values of their diet (DeNiro & Epstein, 1981; Sandberg, Loudon & Sponheimer, 2012). The discrete, non-overlapping carbon isotope distribution observed across browse and graze species is uncommon, hence these carbon isotope signatures ($\delta^{13}C$) may be used to determine the relative contribution of different food sources to an animal's diet (Sponheimer *et al*., 2003; Phillips & Gregg, 2003). These results may be used to augment dietary classifications established in documented field observations (Cerling & Viehl, 2004; Crawford *et al*., 2008).

The relative abundance of stable isotopes varies naturally as they react differently in several environmental and physiological processes due to their differences in mass. This process is referred to as isotopic fractionation and can be used to predict changes in isotopic ratios in stable isotopic analysis (Crawford *et al*., 2008). Conventional methods of estimating the proportions of a source contributing to a mixture in stable isotope analysis used linear mixing models that are based on mass balance equations (Sandberg *et al*., 2012). A single isotope, two-source mixing model required two sources and the isotopic signature of one element, such as $\delta^{13}C$ (Balesdent & Mariotti, 1996; Phillips & Gregg, 2001) and a dual isotope, three-source mixing model requires three sources and isotopic signatures of two elements such as $\delta^{13}C$ and $\delta^{15}N$ (Phillips, 2001). The confidence intervals for these basic linear mixing models depend on the variability of the isotopic signatures of the mixture (Phillips, 2001; Parnell *et al*., 2010). Isotopic signatures are calculated for samples of each source and the mixture and used to establish the relative proportion of each source to the mixture (Phillips, 2001; Parnell *et al*., 2010). Linear mixing models are limited to ($n + 2$) sources, where *n* is the number of stable isotopes measured (Crawford *et al*., 2008). Other models such as IsoError (Phillips & Gregg, 2001), and IsoSource (Phillips & Gregg, 2003) were developed based upon the linear mixing models, but did allow for some uncertainties and variations and thus better suited for natural systems (Crawford *et al*., 2008; Parnell *et al*., 2010).

The standard expression of isotope ratios is in delta notation (δ) as parts per thousand or per mill ($^0/_{00}$) according to the equation below:

$$\delta^H X \ (^0/_{00}) = (R_{sample}/R_{standard} - 1) \times 1000$$

where the element is represented by X; H is the heavy isotope mass and R is the ratio of heavy to light isotope for element X (Craig, 1953; Crawford *et al.*, 2008; Crowley *et al.*, 2010; Sandberg *et al.*, 2012).

Therefore, carbon isotope ratios may be calculated using the following:

$$\delta^{13}C \ (^0/_{00}) = (R_{sample}/R_{standard} - 1) \times 1000$$

where, R_{sample} and $R_{standard}$ are the $^{13}C{:}^{12}C$ ratios of the sample and standard, respectively (Park & Epstein, 1959; O'Leary, 1981). The carbon isotope value of the Pee Dee Belemite (PDB), a Cretaceous marine fossil found in a marine formation in South Carolina was used as the original reference point for carbon isotopes (O'Leary, 1981; Sandberg *et al.*, 2010). The Vienna-Pee Dee Belemnite standard (VPDB) is now used as the PDB has been exhausted (Coplen, 1996; Cowley *et al.*, 2010, Sandberg *et al.*, 2012). The $\delta^{13}C$ value of PDB and VPDB is larger than most terrestrial biota, therefore plants and animals generally have a negative $\delta^{13}C$ value (Sandberg *et al.*, 2012). This equation may be used for other isotopes of light elements such as nitrogen, where the isotope values are calculated relative to ^{15}N compositions of atmospheric nitrogen (AIR) as the reference point (Cowley *et al.*, 2010; Sandberg *et al.*, 2012). $\delta^{15}N$ values for soil, plants, and animals are often greater than that of atmospheric nitrogen ($0^0/_{00}$).

The development of continuous-flow techniques to isotope ratio mass spectrometry (IRMS) lead to the high-precision measurements of isotopes, the ability to efficiently analyse a large number of samples and other advancements in the field (Brenna *et al.*, 1997; Crawford *et al.*, 2008).

A Bayesian stable isotope mixing model, SIAR (Stable Isotope Analysis in R) was developed by Parnell *et al.* (2010) and analyses data generated by IRMS. It has overcome some challenges faced by previous methods by incorporating several sources of variability and uncertainty such as differences in source isotope compositions and variation in fractionation effects. Thus more complex questions may be investigated (Sandberg *et al.*, 2012). The exploratory phase of stable isotope analysis is developing into one of hypothesis development and testing due to a better

understanding of isotopic processes and patterns. Further studies are necessary to gain even more understanding in some aspects of stable isotopes. For example, the causes of great variability in nitrogen isotope systems are not readily evident; therefore researchers should be cautious when trying to interpret data (Sandberg *et al.*, 2012).

The tropic enrichment factor (TEF), also referred to as fractionation or discrimination factors, is the isotopic difference between two substances and it is conventionally expressed using the Δ notation (Sponheimer *et al.*, 2003; Cowley *et al.*, 2010; Parnell, *et al.*, 2010). Fractionation is usually measured between the plants (source) and the faecal matter (product) or other organic tissues, such as hair or collagen, being tested ($\delta^{13}C_{hair/faeces} - \delta^{13}C_{diet}$) (O'Leary, 1981).

The fractionation of diet to faeces and hair has been studied extensively with the use of controlled-feeding (Jones *et al.*, 1979, 1981; Sponheimer *et al.*, 2003; Codron *et al.*, 2011; Codron *et al.*, 2012). Sponheimer *et al.* (2003) determined that the mean diet-faeces fractionation is -0.8‰, which is lower than the fractionation of -1.9‰ for similar animals on natural forages (Jones *et al.*, 1981). It is not fully understood why the values of diet-faeces fractionation are negative and not positive as would be supposed, but diet-faeces fractionation is relatively consistent and should not have a significant affect when determining dietary inferences (Sponheimer *et al.*, 2003). Diet-faeces fractionation in the diets of herbivores with a mixed diet may be more complicated due to the dissimilar digestibility of browse and graze species, but should not significantly bias field $\delta^{13}C$ values. (Jones *et al.*, 1979; Sponheimer, *et al.*, 2003; Codron *et al.*, 2011; Codron *et al.*, 2012). The soundness of using the stable isotope analysis of faecal matter to generate a representation of animal diet may be debated as only undigested forage is tested (Codron & Codron, 2008).

Plants may be categorized according to the photosynthetic pathway used to fix atmospheric carbon dioxide (CO_2). Most trees, shrubs, forbs and herbs are dicotyledons (dicots) that use the C_3 photosynthetic pathway and most grasses in the tropical regions are monocotyledons (monocots) that use the C_4 photosynthetic pathway (O'Leary, 1981; Cerling *et al.*, 2003; Codron *et al.*, 2005; Radloff *et al.*, 2013). For the C_3 pathway, ribulose bisphosphate carboxylase (RuBisCo) is the key carboxylating enzyme that fixes a three-carbon molecule (C_3) into CO_2 in the Calvin-Benson cycle (Park & Epstein, 1960; O'Leary, 1981; Farquhar, O'Leary & Berry, 1982; Sandberg *et al.*, 2012). For the C_4 pathway, a four-carbon molecule (C_4) is initially incorporated into CO_2 by the carboxylation of phosphoenolpyruvate (PEP carboxylase) before entering the Calvin-Benson cycle (O'Leary, 1981; Sandberg *et al.*, 2012). Past studies have demonstrated that C_3 photosynthesizing plants have lower $\delta^{13}C$ values than C_4 photosynthesizing plants. This is due to RuBisCo in the C_3

pathway discriminating against $^{13}CO_2$ and the presence of the Kranz anatomy in tropical (C_4) grasses that increases photosynthetic efficiency by concentrating CO_2 around RuBisCo thus inhibiting photorespiration (O'Leary, 1981, 1988; Sandberg *et al*., 2012).

Therefore $\delta^{13}C$ values are used as a distinguishing characteristic between C_3 and C_4 plants (Bender, 1968, 1971; Smith & Epstein, 1971; O'Leary, 1981; Codron *et al*., 2005). C_3 plants have $\delta^{13}C$ values between -23‰ and -31.5‰ (O'Leary 1981, 1988; Sandberg *et al*., 2012) and the $\delta^{13}C$ values of C_4 plants are generally between -11‰ and -14‰ (O'Leary, 1988; Codron *et al*., 2005; Sandberg *et al*., 2012). Environmental, dietary and physiological factors and their complex interactions have an effect on the $\delta^{15}N$ values of animal tissues and are not understood adequately (Sandberg *et al*., 2012). Relative to their diet, animal tissues are typically nitrogen enriched, however there is higher variability in diet-tissue nitrogen isotope discrimination (Martinez del Rio, 2009; Sandberg *et al*., 2012).

The diets of herbivores can include the leaves and fruit of dicotyledonous and monocotyledonous plants. Diet could be considered as a function of their dietary preferences which adjust to the change in the availability of vegetation species, as there is a trade-off between expending energy when foraging for preferred species versus the actual nutrition and energy gained by consumption (Sprent & McArthur, 2002; Radloff *et al*., 2013). Hofmann (1989) defined three major classes of herbivores depending on their feeding habits: (i) Grazers that primarily feed on grass; (ii) browsers that primarily feed on tree leaves, twigs and seasonal fruits, herbs and forbs; and (iii) intermediate feeders that feed on a mixture of grass and browse continually or change between grass and browse seasonally. Various conventional study methods have been used to investigate the diets of herbivores (Crawford *et al*., 2008). Commonly, field observations have been used to determine qualitative estimates of consumed forage (Talbot, 1962; Hofmann & Stewart, 1972; Sinclair, 1977; McNaughton & Georgiadis, 1986; Hofmann, 1989; Bodner, 1990; Gagnon & Chew 2000; Cerling *et al*., 2003), as well as the analysis of fecal matter and stomach contents (Field, 1972; Cerling *et al*., 2003). These methods have yielded much insight and interpretation of animal ecology, however there are limitations and biases that hinder further understanding (Putman, 1984; Webster *et al*., 2002; Crawford *et al*., 2008).

The use of stable carbon isotope analysis to gain a better understanding of diet preferences in mammals is founded on the carbon isotopic distinction (i.e. have significant differences in their $^{13}C:^{12}C$ ratios) between plants that use C_3 and C_4 photosynthetic pathways (Cerling *et al*., 2003). An advantage of stable carbon isotope analysis is that it can be evaluated quantitatively and it does not require direct observation and therefore is unbiased by it and it is better suited to statistical analyses

and model construction, however it does not provide taxonomic diet details as field studies do (Crawford *et al*., 2008). In past field observation studies, the percentage of dicotyledonous material in the diet of grazers and monocotyledonous material in the diet of browsers was frequently overestimated. An example of such is the study conducted by Cerling *et al*. (1999) on the diet of elephants. Looking at information from historical studies, it was thought that elephants were predominantly grazers but some studies indicated that browse was an important component in the elephants' diet.

Numerous animal tissue types may be used for stable isotope analysis, such as tooth enamel and bone (Ambrose & DeNiro, *et al*., 1986; Codron *et al*., 2005; Cowley *et al*., 2010), hair and faeces (Sponheimer *et al*., 2003) and even blood samples (Crawford *et al*., 2008). Hair and faeces samples could be considered the most practical as both can be more readily collected in the field from live animals (Sponheimer *et al*, 2003). Different animal tissues have independent turnovers; therefore they assimilate dietary and habitat information at different scales (Tieszen *et al*., 1983; Bearhop *et al*., 2003; Crawford *et al*., 2008). Tissues such as bones and teeth can be a long-term indicator of dietary intake for an animal, especially if the phenology of growth is known (Crawford *et al*., 2008). In an isolated sampling event, stable isotope information may be captured from various periods of time in an animal's life using various tissues (Crawford *et al*., 2008).

Faecal matter of animals has been shown to reflect their dietary intake sufficiently and typically has a $\delta^{13}C$ value that is 0.9‰ lower than $\delta^{13}C$ values of the diet (Sponheimer *et al*., 2003; Sandberg *et al*., 2012). The $\delta^{13}C$ values of animal fecal matter are an indication of the animal's short-term dietary intake. This is beneficial when examining short-term dietary variations. However, to determine a representation of diet in the long-term would require the collection of faecal matter over a protracted length of time (Sponheimer *et al*., 2003). The $\delta^{13}C$ values of bulk hair samples represent the animal's diet over a longer time period and assists in classifying a species according to general dietary type, such as grazer, browser or mixed feeder (Sponheimer *et al*., 2003).

For this study, dung samples were analysed to calculate the percentage of C_4 grasses in the diet of impala and waterbuck using the following linear mixing model equation for one isotope and two mixing sources:

$$\% \ C_4 \ \text{grass in diet} \ = \ \frac{(\delta^{13}C_{C3 \ plants} + \Delta\delta^{13}C - \delta^{13}C_{dung})}{(\delta^{13}C_{C3 \ plants} - \delta^{13}C_{C4 \ plants})} \ \text{x} \ 100$$

where, $\delta^{13}C_{C3\,plants}$ is the average isotopic value for C_3 vegetation, $\delta^{13}C_{C4\,plants}$ is the average isotopic value for C_4 vegetation, $\delta^{13}C_{dung}$ is the isotopic value of dung sample and $\Delta\delta^{13}C$ is the significance of discrimination between the source (vegetation in diet) and the product (dung), the value of which is accepted to be -0.9% for dung (Sponheimer *et al*., 2003; Codron & Codron, 2008; Radloff, 2008).

Cerling *et al*. (2003) recognized five classes of herbivores based on their carbon isotope compositions. These are (i) hypergrazers that have more than 95% C_4 grass in their diet; (ii) grazers that have 70-95% C_4 grass in their diet, (iii) mixed feeders that have more that 30% C_4 grass and more than 30% C_3 browse species in their diet, (iv) browsers that have 70-95% C_3 browse species in their diet, and (v) hyperbrowser or frugivore that have more than 95% C_3 browse or fruit in their diet.

Determining animal foraging preferences and movements is important to our understanding of physiological and behavioural processes and conservation actions (Crawford *et al*., 2008). The collection and analysis of isotopic data can contribute to the understanding of these processes (Sandberg *et al*., 2012). Carbon stable isotope analysis is an effective method of differentiating between grazing and browsing contents in the tropics and subtropics (Smith & Epstein, 1971; Cerling *et al*., 1999; Sponheimer *et al*., 2003; Sandberg *et al*., 2012) and it produces a quantitative description of animal diets (Crawford *et al*., 2008). The study site, MWR, falls within the tropics where most C_3 plants are dicotyledonous and C_4 plants are monocotyledonous grasses. The area is therefore in a model region in which to employ stable isotope analysis to study the diet of animals.

2.3.4 Waterhole counts

During periods when resources are scarce, wildlife are compelled to aggregate around these resources especially water in the dry season (Valeix, Chamaillé-Jamme & Fritz, 2007; Valeix, 2011; Hayward & Hayward, 2012). In African ecosystems wildlife managers may increase surface water availability by installing artificial water points as an intervention strategy (Smit, Grant, & Devereux, 2007). Rivers and artificial water points function as a junction of activity and have an effect on the distribution of herbivores on a landscape-scale during the dry season (Smit et al., 2007). Waterhole counts (monitoring) are best conducted at this time when there is little to no surface water remaining from the wet season in the surrounding areas. Herbivore species distribution is constrained as they are driven to use the same remaining perennial water resource, thus the dry season is an opportune time to count the number of animals in the area (Redfern *et al*., 2003; Smit et al., 2007; Valeix, 2011). Most herbivores acquire water during the day (Valeix *et al*., 2007; Valeix, 2011), hence most animal counts at waterholes are conducted over 12 hours from

morning to evening. Some observers may take advantage of the moonlight and continue observations over a 24-hour period for their studies (Crosmary, Valeix, Frizt, Madzikanda, Côté, 2012). It is important that animals visiting the specific waterhole are not deterred by human presence in the area and observers need to be mindful of their noise levels and movement, as it has been demonstrated that animals will avoid approaching a waterhole for up to six hours after humans have been there (Wakefield & Attum, 2006; Crosmary *et al.*, 2012; Hayward & Hayward, 2012). Observation points may vary for different waterholes as some have purposely built platforms or hides for tourism use, while others require observers to sit in a vehicle preferably from an elevated position on a hill. Observers make use of binoculars when necessary to confirm species identification, count the number of individuals and determine the sex and ages of individuals where possible. Additional data that could be recorded are weather conditions, state of waterhole, time of observation including the first and last drink times and other behaviors such as foraging observed around the waterhole. Contemporary methods of monitoring waterhole utilization include the use of remote cameras that could potentially collect significant scientific data (Wakefield *et al.*, 2008; Hayward & Hayward, 2012).

Animals that frequent waterholes in the dry season include buffalo (*Syncerus caffer*), eland (*Tragelaphus oryx*), elephant (*Loxodonata africana*), impala (*Aepyceros melampus*), waterbuck (*Kobus ellipsiprymnus*), and zebra (*Equus burchelli*) (Hayward & Hayward, 2012). Some biases of data collection should be considered when conducting waterhole counts such as the use of artificial water points by specific species. For example, it has been proposed that grazers may be more dependent on waterholes than browsers as the water content of grass falls below that of browse species, therefore grazers do not obtain enough moisture from their forage, particularly in the dry season (Taylor, 1969; Louw, 1984; Smit *et al.*, 2007). Another consideration is that the presence of elephants at a waterhole may or may not prevent other mammals from acquiring water, especially as competition for resources increases (Valeix *et al.*, 2007).

For each waterhole count event, the number of groups, number of individuals that are counted and relative abundance may be compared to other events that occur in other months or years at a waterhole (Valeix, 2011). Additionally, comparisons in species diversity, group numbers and demographics may be made between different waterholes. Using behavior observations it may be determined whether animals have random or specific drinking times and how some species may shift these times in response to other species present (Valeix *et al.*, 2007; Hayward & Hayward, 2012).

Information gleaned from waterhole counts may provide data on waterhole use, population sizes and dynamics as well as the behaviour of species and multi-species assemblage interactions (Valeix *et al.*, 2007; Hayward & Hayward, 2012). It is important to gain an understanding of how different species utilize artificial waterhole points and how species distribution is affected by artificial waterhole point locations in order to manage water sources and the effects of herbivore populations on surrounding habitat, as best as possible (Valeix, 2011).

For this study, waterhole counts were conducted to determine how impala and waterbuck used the artificial waterhole points, as well as what age and sex demographic were using these points. For this study the disadvantages of only conducting 12-hour waterhole counts are that the animals that drink water at night are not accounted for. This can be offset by the use of camera traps, but such a method has its own set of challenges, such as protecting the cameras from elephant damage, whilst placing it in a prime position next to the waterhole. The presence of humans could deter animals from utilizing the waterholes, thus affecting waterhole counts. Using camera traps and webcams would be beneficial; especially as technology and image analysis software programmes such as Timelapse Image Analyser are improving.

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Chapter Three:

An investigation of the distribution and size of impala (*Aepyceros melampus*) and waterbuck (*Kobus ellipsiprymnus*) populations in Majete Wildlife Reserve, Malawi

3.1 Abstract

It is fundamental to the management of protected areas that wildlife populations are monitored, especially in artificially closed systems. The monitoring of impala and waterbuck populations was required post reintroduction to Majete Wildlife Reserve, Malawi. Abundance estimates of impala and waterbuck were determined for a portion of the reserve by conducting distance sampling drive counts and analysing data in the DISTANCE software package. In addition sex and age structures were generated for both antelope populations. Twelve-hour waterhole counts were conducted on a monthly basis from June to December 2013 at several artificial water points in the dry season to determine the utilization of waterholes. Camera trap data from a long term monitoring project were used to supplement waterhole counts. Impala and waterbuck are water dependent species and occurred in areas associated within relatively close proximity to a water supply. For both species breeding herds were most frequently observed closer to the perennial rivers and adult males were the most frequent users of waterholes. The information gleaned from this study on the population size, structure and distribution of impala and waterbuck will contribute to management strategies for the reserve.

Keywords: distance sampling, distribution, impala, waterbuck, waterhole counts

3.2 Introduction

In Africa large herbivores have significant ecological and economic value (Cromsigt et al., 2009), but anthropogenic activities are a growing threat to wildlife populations (Newmark, 2008). This has led to the fragmentation and isolation of habitats (Noss *et al*., 2006; Muths & Dreitz, 2008). It is encouraging that since 1970 there has been a marked increase in the number of protected areas being established (WCMW, 2004; Newmark, 2008). However, it is crucial for conservation efforts that protected areas are well managed, despite the threats from internal and external anthropogenic activities and that the loss of biodiversity is curtailed (Martinez-Meyer, Peterson, Servin, & Kiff, 2006; Pelletier, 2006; Canter, 2008; Newmark 2008), by maintaining wildlife populations within ecologically sustainable limits (du Toit, 2002; Gordon *et al*. 2004). The reintroduction of species

into protected areas has become an effective tool in wildlife management (Kleiman, 1989; Griffith *et al*., 1989; Stanley-Price, 1991; Wolf *et al*., 1996; Muths & Dreitz, 2008). Reintroduction was defined by the IUCN (1987) as "the establishment of a species to an area that was previously inhabited in an effort to advance the conservation of the species", (Sarrazin & Barbault, 1996). The measurement of reintroduction success should be founded on the species' ability to reproduce and create a self-sustaining population post the initial reintroduction phase (Dodd, 2005; Muths & Dreitz. 2008), though there is a growing and acute need for greater knowledge and better understanding of reintroduction preparations, reintroduced species and reintroduction success assessment (Sarrazin & Barbault, 1996).

The distribution of wildlife is influenced by the basic need for water, shelter and forage, which may vary on spatial and temporal scales for different species (Hutchingson, 1957; Krebs 1985; Sinclair 1983; van Bommel et al., 2006). Resources contribute to population growth but when competing with other wildlife, resource competition may lead to population decline (Grover, 1997). Habitat selection may be defined as the process where individuals choose to occupy or make use of a selected area that provides essential resources for their survival from the available habitat (Morrison *et al*., 1998; Morris, 2003; Olivier, 2007; Canter, 2008). Animals may select vegetation types that provide the greatest gains to the animal's physiology, thus maximizing their gains from a specific habitat (Pullium & Danielson, 1991; Pulliam, 1998). It is essential to determine the varying impacts of different ungulates on ecosystems by studying their feeding behaviours, population growth and distribution (Ben-Shahar, 1991; Bodenstein *et al*., 2000; Gutbrodt, 2006). Vegetation is most impacted during the dry periods when resources are relatively limited in comparison to the wet season and species are competing for forage and water (Kie, 1999; Gaylard, Owen-Smith & Redfern, 2003; Parker, Barboza & Gillingham, 2009).

At times wildlife managers need to implement several intervention strategies to augment the natural resources. Such strategies are the provision of surface water, fire management, population manipulation and fencing of protected areas (Smit *et al*., 2007). Fences impact ecological processes in smaller, isolated protected areas as wildlife can no longer disperse, thus adding more pressure on the limited resources which leads to a greater need of intensive management and potential intervention strategies (Albertson in Ferguson & Hanks, 2010). It is essential to monitor wildlife populations and determine their effects on ecosystems as habitat quality may change quickly and the extent of habitat use may be affected by the availability, abundance and quality of resources as these factors influence the contribution of habitat to fitness (Boyce & McDonald, 1999).

Monitoring programmes were developed to estimate species populations but have become a good biodiversity conservation tool (Cromsigt et al., 2009). Ecologists face the challenge of estimating population sizes as accurately as possible and often base these estimates on samples of the population (Gibbs, 2000; Reid, 2005). Methods of monitoring larger mammal populations and their ecological requirements include, to mention but a few, drive counts, aerial counts, walking transects, waterhole counts, camera trapping and the collaring of individuals with satellite or GPS radio collars (Jachmann, 1991; Buckland 2001; Valeix, 2007; Cromsigt *et al*., 2009). It is of high importance that monitoring and active management of reintroduced species is conducted, especially in a closed system, to ensure sustainability of the population (Dörgeloh, 2001; Newmark; 2008) In addition, managers and researchers can make a contribution to the understanding of reintroduction biology by combining their efforts (Sarrazin & Barbault 1996; Seddon, 2007).

An isolated, 700km^2 protected area in southern Malawi, Majete Wildlife Reserve, underwent a reintroduction programme in which over 2500 animals were released. In 2003, 216 impala and 98 waterbuck were reintroduced into the temporarily fenced, 140km^2 sanctuary area of the reserve. In 2008 an additional 210 impala and 198 waterbuck were reintroduced outside the sanctuary area in the southern region of the reserve, followed by another 311 impala and 106 waterbuck outside the sanctuary area in 2010. A final total of 737 impala and 402 waterbuck were reintroduced (African Parks Majete management). The old sanctuary fence was removed in 2011, after the completion of the perimeter fence of the entire reserve. Majete Wildlife Reserve represents an example of a successful reintroduction programme that requires long-term monitoring.

Impala, *Aepyceros melampus* (Lichtenstein, 1812) are medium-sized antelope that are widely distributed from central Kenya in East Africa, to the north eastern regions of Southern Africa (Smithers, 1983; Estes, 1991; Skinner& Chimimba, 2005; Frost, 2014). Impala are considered to be an 'ecotone' ungulate that prefer to occupy the edges of open woodland, bordering grassland (Estes, 1991). In the wet summer months impala tend to spend more time in grasslands and have a higher grass relative to browse content in their diet when grass growth is fresh and nutrient rich. In the dry winter months impala are more common in woodland habitat and have a higher browse content in their diet as grass quality decreases (Azavedo & Agnew, 1968; Rodgers, 1976; Dunham, 1980; Ambrose & De Niro, 1986; van Rooyen, 1992; Pietersen et al., 1993; Meissner et al., 1996). Impala have a broad range of diet flexibility as they are mixed feeders that utilize a wide variety of vegetation, thus they are fierce resource competitors (Hofmann, 1973; Estes, 1991; van Rooyen, 1992; Gagnon & Chew, 2000; Brashares & Arcese, 2002; Cerling *et al*., 2003). As a result of high crude protein intake impala require sufficient water to expel nitrogenous waste, particularly in the

dry season (Augustine, 2004; van Bommel *et al.*, 2006; Canter, 2008). It is estimated that impala need 2.5 litres of water per day (du Toit, 2010) and generally remain within eight kilometres of a water source (Young, 1972a; Skinner & Chimimba, 2005).

Waterbuck, *Kobus ellipsiprymnus* (Ogilby, 1833) are large, robust antelope with a distribution range extending from southern Somalia, through eastern Kenya, Tanzania and Zambia, down to the northern regions of South Africa (Skinner and Chimimba, 2005). As the name suggests, waterbuck prefer habitats that are associated with natural water such as open habitats with short to medium grasses (Wirtz & Kaiser, 1988; Pérez-Barberia *et al.*, 2001; Traill, 2004; Gutbrodt, 2006). It is estimated that waterbuck require nine litres of water a day (Taylor *et al.*, 1969b; du Toit, 2010), which is due to the consumption of mostly protein-rich grasses (Taylor, Spinage & Lyman, 1969; Estes, 1991). Thus they are water dependent and their habitat preference is influenced by proximity to drinking water (Taylor *et al.*, 1969; Hofmann & Stewart, 1972). Waterbuck are classified primarily as grazers (Hofmann, 1973; Estes, 1991) but are able to consume some browse species in periods of low abundance of preferred grasses (Melton, 1978; Tomlinson, 1980; Estes, 1991).

The objectives of this study was to generate population estimates and to determine the distribution of impala and waterbuck populations in the sanctuary area of MWR post reintroduction, using distance sampling and waterhole counts. The fundamental hypothesis was that impala and waterbuck populations had increased post reintroduction and that their distributions would be determined by relative water availability. In addition the distributions of sexes and age classes for each species was captured. Information and understanding gained from this study will be used to aid MWR management to make sound decisions in the management of both species, as well as contribute to the overall management strategy for the reserve.

3.3 Methods

Study site

Majete Wildlife Reserve (MWR), lies at the southern end of the Great Rift Valley in the middle Shire Valley in south Malawi. The topography of the reserve is characterized by steeper hills with greater altitudes in the western region of the reserve that are cut into by river systems and more gentle slopes and lower altitudes in the eastern regions, toward the Shire River. There is a distinct rainfall period from November to early April. The expected annual precipitation is 680-800mm in the eastern region and 700-1000mm in the western region of the reserve (Wienand, 2013). Minor precipitation locally referred to as "chiperoni", occurs from April to October (Sherry, 1989; Morris, 2006). There are two perennial rivers in the reserve, the Shire River and the Mkulumadzi River, as

well as several seasonal rivers. Other natural sources of water are a number of seasonal and perennial springs as well as seasonal pools. A number of artificial water points that are borehole-fed have been installed in the reserve to supplement natural water sources, giving herbivores access to a greater area in which to forage in the dry, winter months. The vegetation of MWR varies from low to high altitudes in the reserve. Sherry (1989) defined the following vegetation types for MWR: riverine vegetation along larger river systems (*Kigelia africana*, *Lonchocarpus capassa* and *Euphorbia ingens*); low altitude (205-280m) mixed deciduous woodland (*Acacia spp.*, *Sclerocarya birrea* and *Sterculia spp.*); ridge-top (220-300m) mixed woodland (*Terminalia sericea*, *Diospyros kirkii* and *Diplorhynchus condactylcarpon*); medium altitude (230-410m) mixed deciduous woodland (*Brachystegia boehmii*, *Pterocarpus rotundifolius*, *Diospyrus kirkii* and *Combretum spp.*); and high altitude (410-770m) miombo woodland (*Brachystegia boehmii*, *Julbernardia globiflora*, *Burkea africana*, *Diplorhynchus condylocarpon and Pterocarpus angolensis*).

Historically, MWR lacked resources and funding and as a result was decimated of almost all mammals and was vulnerable to encroachment of settlements and agriculture. Since 2003 when an agreement was made between the Malawian Department of National Parks and Wildlife (DPNW) and African Parks Majete (Pty) Ltd., MWR has undergone one of Africa's largest reintroduction programmes where over 2559 animals were reintroduced; including 737 impala and 402 waterbuck. Animals were reintroduced in several phases over a six-year period. The initial animal reintroductions were made in a smaller fenced area of 140km^2 in the north-eastern region of the reserve and was referred to as the "sanctuary". Several years later the sanctuary fence was removed to allow the movement of animals into the rest of the reserve. Due to various limitations, this study was focused in the region in the original sanctuary area.

Distance Sampling

Established roads were used to conduct drive transects using the principles of distance sampling (Buckland, Anderson, Burnham & Laake, 1993) to determine the density of impala and waterbuck in the old sanctuary area of the reserve. Sampling generally began just before dawn and continued until shortly after sunset, with a rest period during the hottest part of the day. For most of the sampling period one observer was responsible for conducting the counts, but for some transects volunteers assisted in the observation and recording of data during the counts. Estimates improve with repetition of a transect count, thus transect counts were repeated on a monthly basis in drier regions towards the western side of the 'sanctuary' area and bimonthly in the eastern section. Repetition of transect counts were concentrated closer to the Shire River where densities of waterbuck and impala were presumed to be higher as both are water dependent species (reserve

manager, pers.com.). An average cruising speed of 15 kilometers per hour was maintained when searching for animals. Due to the nature of the vegetation in MWR, visibility of animals was generally less than 150m. Animals were initially sighted with the naked eye and binoculars were used when necessary to confirm species identification and to count and determine age and sex of group members. The bearing was measured with a compass and the observer, whom had a trained eye, estimated the radial distance to focal animal. Additional data recorded for distance sampling included: the date, time of observation, GPS position of observer, heading of vehicle, total distance covered, the total number of individuals seen, number of males and females in their respective age classes (lamb/calf, juvenile, sub-adult and adult) for each observation. It was also noted when no animals were sighted on a transect.

Waterhole Counts

There are several borehole-fed artificial water points (waterholes) in the reserve, most of which are run on solar energy. Eight 12-hour waterhole counts were carried out from June to December 2013, at four of the artificial water points in the sanctuary. Generally counts commenced at 06h00 and ended at 18h00 to observe and count the diurnal and crepuscular animal activity at waterholes (Ayeni, 1975; Valeix, 2007). Three to four observers were stationed in an elevated hide or viewing platform from which they could monitor animals within a 100m distance of the waterhole. For each waterhole count, observers noted the weather conditions (cloud cover, relative temperature, wind factor), species sighted, time of observation of individual or group, the size of the group, the gender and age of each group member and several behavioural observations (interspecific and intraspecific interactions) of species. Counts were only conducted during daylight hours due to resource limitations (such as the availability of observers) and visibility of the waterholes. This may have negatively influenced results, as animals make use of the waterholes at night and therefore would not have been included in counts.

Statistical analysis

Transects were categorized into one of three groups according to a combination of habitat type in which they occurred in and the relative proximity to a perennial water source such as the Mkulumadzi and Shire Rivers. This was done considering that one of the assumptions of the DISTANCE software is that wildlife densities are uniform throughout an area, thus heterogeneous habitats should be studied independently (Thomas, Buckland, Rexstad, Laake, Strindberg, Hedley, Bishop, Marques & Burnham, 2010). Set A included transects that were relatively close to perennial water sources with riverine vegetation. Set B included transects that had an intermediate proximity to a perennial water source and were in low altitude mixed woodland with ridgetop mixed

woodland. Set C included transects that generally had limited water availability and were in areas with a combination of low- & medium-altitude mixed woodland and ridgetop mixed woodland vegetation. A summary of transects conducted is outlined in Table A.1 in Appendix One. It was presumed that greater wildlife densities would occur closest to the perennial rivers and that densities would decrease along transects further away from the river to higher and drier regions of the reserve. The numerous data sets from 2013 and 2014 were run in the DISTANCE software, using the conventional distance sampling (CDS) model. Various tests including q-q plots, Kolmogorov-Smirnov test and Cramér-von Mises test are run within the DISTANCE software programme. Akaike's Information Criterion (AIC) was determined to measure how well data 'fitted' a model. The analysis engine produced density and abundance estimates and total population estimates for both species in the sanctuary area. Simple analyses were conducted in a data manager (Microsoft Excel, 2013) to determine the distribution and proportion of adults, sub-adults, juveniles and newborn to three month calves or lambs in the sanctuary and the use of waterholes by impala and waterbuck populations from waterhole count data.

3.4 Results

Distance Sampling

The DISTANCE software produced density and abundance estimates for impala and waterbuck populations in the sanctuary area for 2013 and 2014. Transects were grouped to make the data more manageable and to improve estimates for each area. Using the estimated density for each transect set in varying habitats and the area that they represent, relative abundances were calculated for impala and waterbuck in the sanctuary area of MWR.

Table 3.1 A summary of distance sampling results, using combined data collected in 2013 and 2014. The estimated abundances and densities (individuals per km^2) of impala and waterbuck for each of the transect groups according to perennial water availability and dominant vegetation type are supplied.

Transect Category According to Proximity to Perennial Water Source & Vegetation	Impala		Waterbuck	
	Abundance	Density per km^2	Abundance	Density per km^2
Set A: Close proximity to perennial water source; riverine vegetation	2884	78.13	815	22.07
Set B: Intermediate proximity to perennial water source; low altitude mixed woodland and ridgetop mixed woodland vegetation	1251	26.25	316	6.64
Set C: Limited water availability; combination of low- & medium-altitude mixed woodland and ridgetop mixed woodland vegetation	251	4.52	57	1.02
Total Estimate	**4385**		**1188**	

The distance sampling results for impala and waterbuck are summarized in Appendix One. Impala and waterbuck population estimates and their respective AIC values generated using isolated sets of data for 2013 and 2014 and those collected by a single observer, varied from each other (Table 3.2 and Table 3.2). Lower AIC values are an indication of how well the data 'fits' the model. Overall, the data collected by multiple users in 2014 'fit' the data model best. However, the shorter sampling period for 2014 should be taken into consideration.

Table 3.2 Summary of the estimated abundances and respective AIC values for impala in the sanctuary area of MWR. Data was collected by multiple and single observers in the three sets of transects according to perennial water availability and dominant vegetation.

Source	All Data 2013-2014		Multiple 2013		Multiple 2014		Single 2013-2014	
	Abundance Estimate	AIC	Abundance Estimate	AIC	Abundance Estimate	AIC	Abundance Estimate	AIC
Transect Set A	2884	2216.2	3885	1072.8	2808	124.9	2286	1016.3
Transect Set B	1251	1719.4	1694	785.3	1918	118.3	1161	791.0
Transect Set C	251	93.8	172	45.6	370	21.6	63	28.4
Total	4385		5752		5096		3510	

Table 3.3 Summary of the estimated abundances and respective AIC values for waterbuck in the sanctuary area of MWR. Data was collected by multiple and single observers in the three sets of transects according to perennial water availability and dominant vegetation.

Source	All Data 2013-2014		Multiple 2013		Multiple 2014		Single 2013-2014	
	Abundance Estimate	AIC	Abundance Estimate	AIC	Abundance Estimate	AIC	Abundance Estimate	AIC
Transect Set A	815	1968.7	1127	578.62	1377	209.3	785	1115.9
Transect Set B	316	930.5	343	267.46	790	133.3	221	531.8
Transect Set C	57	80.9	59	39.66	0	0	1062	80.9
Total	1188		1529		2167		1062	

The upper estimate of abundance for impala is 5752 individuals from 2013 data and the lower estimate is 3510 individuals from data collected by a single observer in 2013 and 2014 in the sanctuary area (Figure 3.2). For waterbuck, the highest estimate was 2167 individuals from 2014

data and the lowest was 1062 individuals from the single observer's data (Figure 3.3). In general, data collected by multiple observers produced greater abundance estimates.

The estimated cluster size for impala ranged from 7.60 to 17.36 for Set A transects; 4.85 to 7.63 for Set B transects; and 0.82 to 1.32 for Set C transects. Those for waterbuck ranged from 6.75 to 10.71 for Set A transects; 1.81 to 4.89 for Set B transects; and zero to 0.49 for Set C transects (Appendix One).
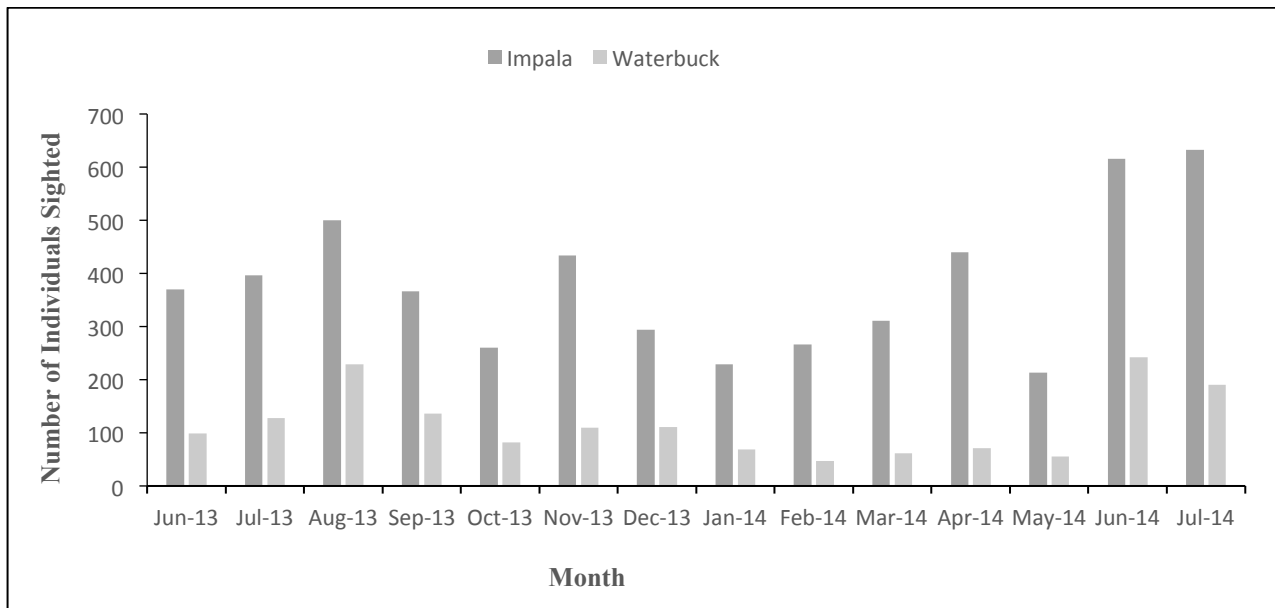
Figure 3.1 The total number of impala and waterbuck counted in a sampling event per month for the sanctuary area of Majete Wildlife Reserve.
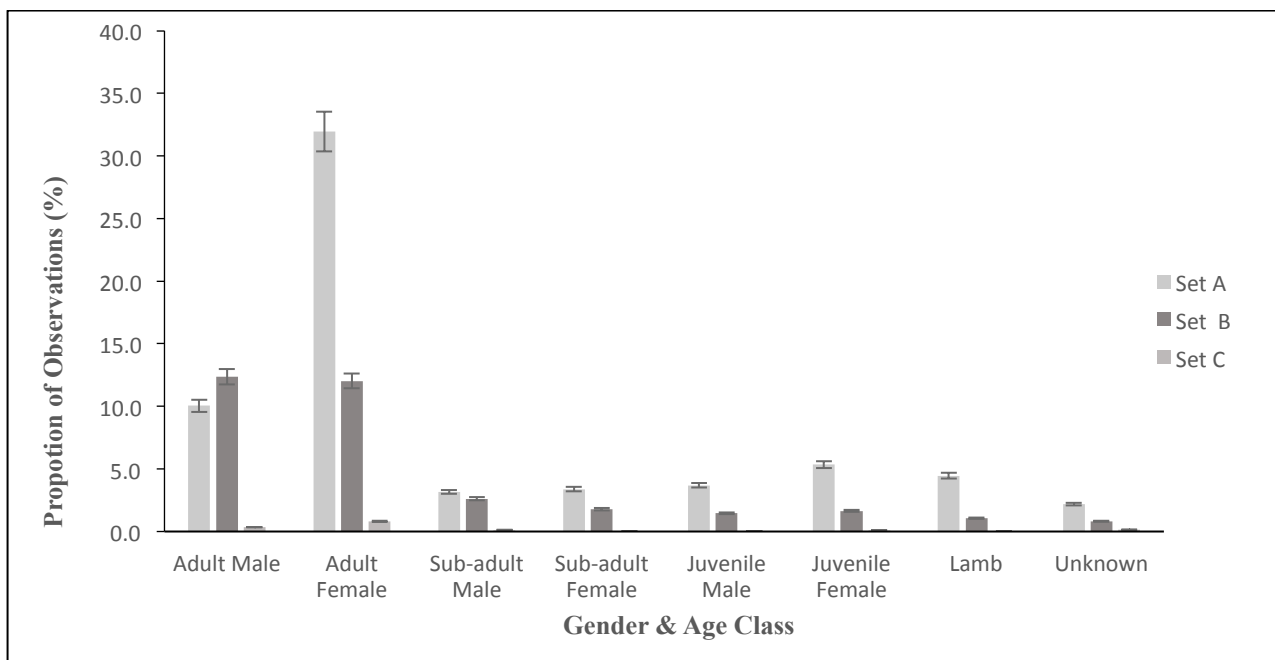
Figure 3.2 The proportion of impala observed according to age class and gender for transect sets A, B and C. Transect sets were grouped according to relative proximity to a perennial water source and the dominant vegetation types.

In Figure 3.1 the number of impala and waterbuck that were recorded in a sampling event per month, demonstrated that more impala were sighted than waterbuck. There are a few peaks and troughs in the number of sightings for both species, which fluctuate with the change in seasons. In general, there are fewer sightings of impala and waterbuck in the wet, summer months in comparison to the drier months.
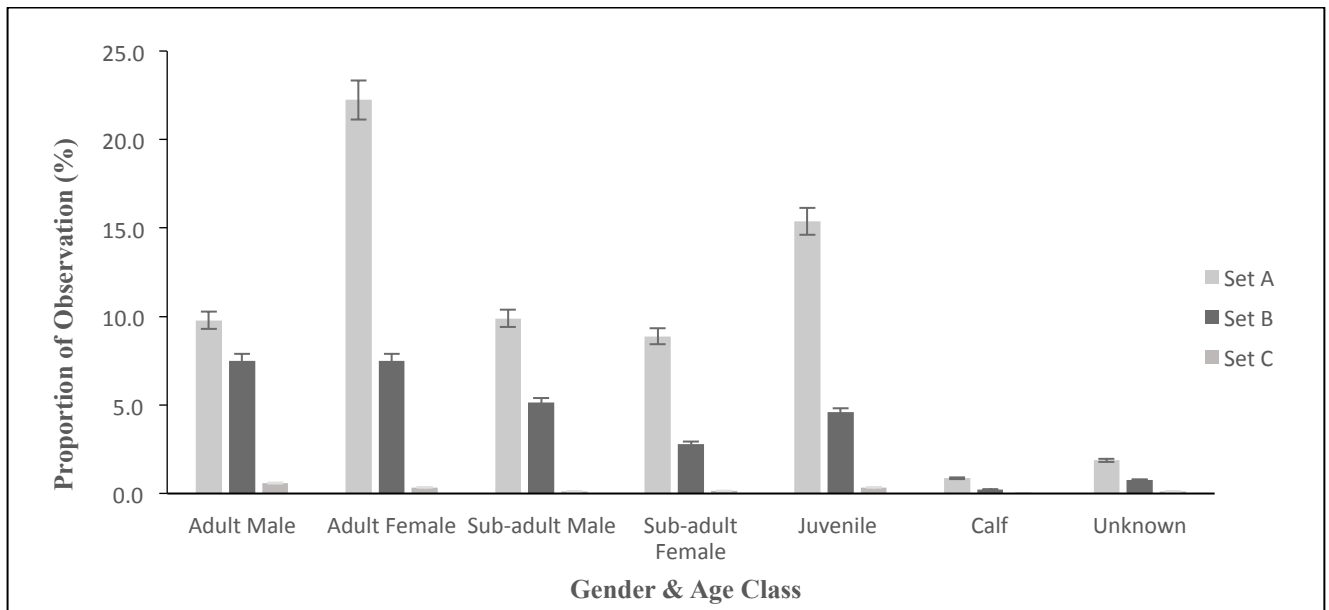
Figure 3.3 The proportion of waterbuck observed according to age class and gender for transect sets A, B and C. Transect sets were grouped according to relative proximity to a perennial water source and the dominant vegetation types.

Figure 3.4 The proportion of impala observed in the sanctuary area of Majete Wildlife Reserve according to age and gender.

The age and gender ratio were calculated and plotted for each set of transect sets A, B and C for impala populations (Figure 3.2) and for waterbuck populations (Figure 3.3). The proportion of individuals seen along transects in Set A suggests that impala and waterbuck favoured the riverine vegetation that was in relatively close proximity to a perennial water source.

Figure 3.5 The proportion of waterbuck observed in the sanctuary area of Majete Wildlife Reserve according to age and gender.

Histograms were generated to depict the proportion of adults, sub-adults, juveniles and young according to their gender for the sanctuary area for impala (Figure 3.4) and waterbuck (Figure 3.5). For waterbuck the male and female juveniles were grouped into one class as it was difficult to distinguish between male and female at that life stage. For impala there was a larger proportion of adults in comparison to other age classes, as there was for waterbuck to a lesser extent. For both species the greatest proportion of individuals sighted were adult females.

Waterhole Counts

Waterhole counts were conducted to supplement information regarding the distribution of impala and waterbuck. The total number of impala and waterbuck that visited each waterhole, as well as the gender and age class of individuals was determined. Table 4.2 summarizes the mean number of impala and waterbuck that visited each waterhole surveyed. For the Nakamba and Thawale waterholes, only seven and not eight complete waterhole counts were conducted due to very poor weather conditions.

Table 3.4 The mean number of impala and waterbuck counted over a period of seven months (June 2013 to December 2013) at four artificial waterholes in Majete Wildlife Reserve (n = the number of 12 hour counts for each waterhole).

Species	Mean number per species per waterhole				Mean number per species counted at four waterholes
	Heritage (n = 8)	Nakamba (n = 7)	Nsepete (n =8)	Thawale (n = 7)	
Impala	9.63	7.57	30.75	37.57	19.97
Waterbuck	17.25	10.43	16.25	0	10.65

The number of impala sighted at waterholes was irregular and varied between waterholes. More impala were observed at waterholes in August, November and December of 2013. Figure 3.6 illustrates that impala were generally sighted more frequently at Nsepete and Thawale waterholes and that in the dry months from July to September 2013, impala made greater use of the Heritage waterhole.
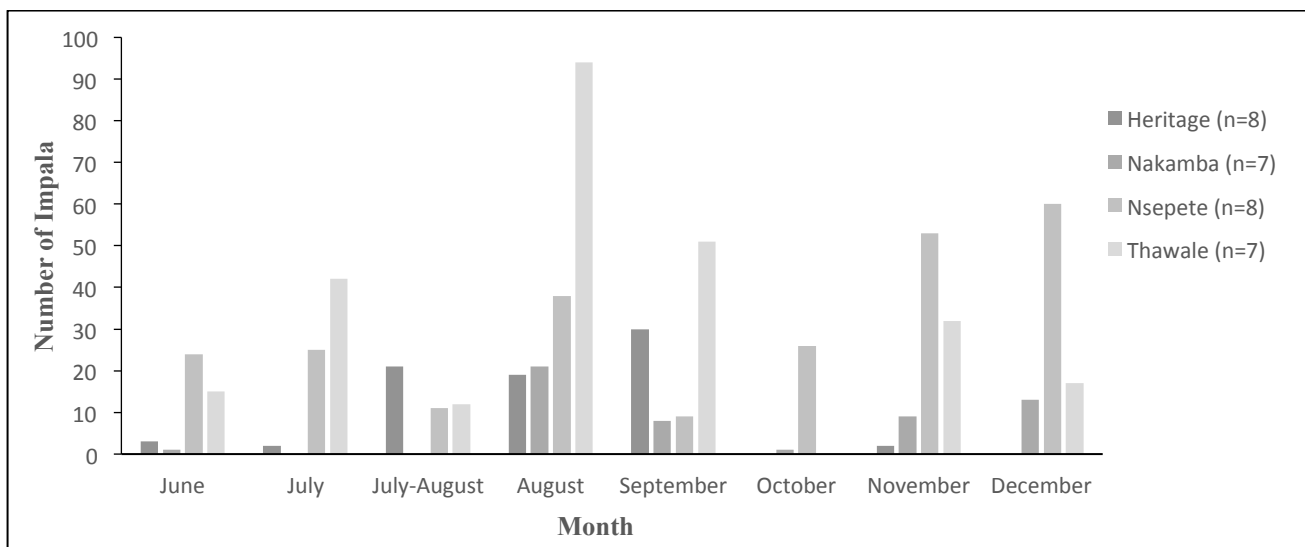
Figure 3.6 The number of impala observed at each waterhole for each month of the study in the sanctuary area of Majete Wildlife Reserve.

Waterbuck did not use all four waterholes uniformly. The most frequently attended waterhole was the Heritage waterhole followed by Nakamba. The use of the Nsepete waterhole increased in the early rainy season. Waterbuck were not observed at the Thawale waterhole for any of the waterhole counts in 2013. Bachelor herds and lone males were most commonly observed for both species, therefore adult males were the most frequent users of the waterholes.
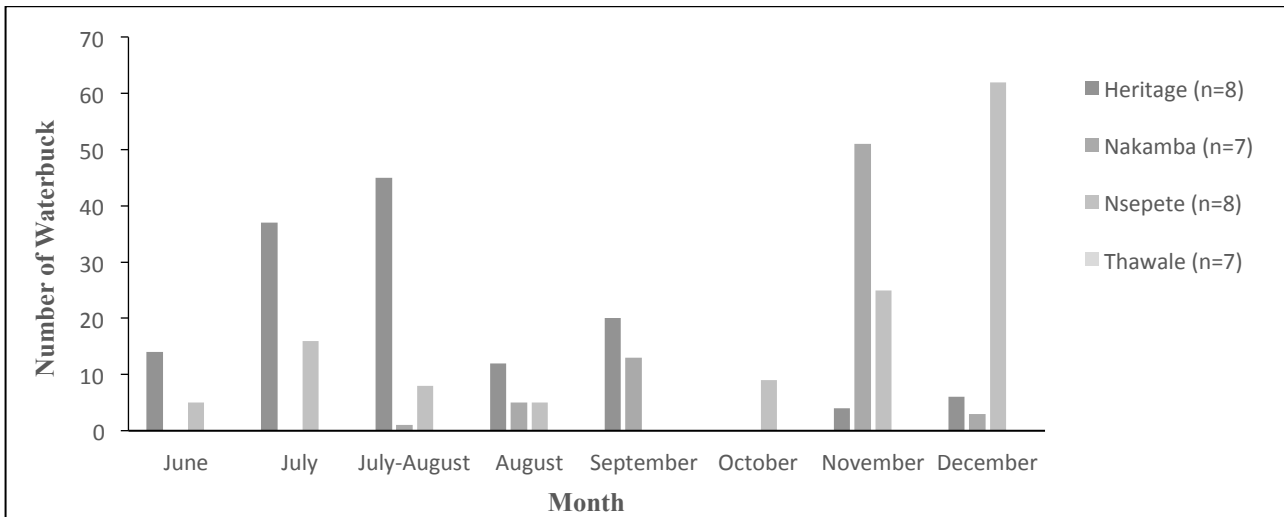
Figure 3.7 The number of waterbuck observed at each artificial water point for each month of the study in the sanctuary area of Majete Wildlife Reserve.

3.5 Discussion

The primary needs of animals are water, food and forage. The availability of these requirements vary on spatial and temporal scales and thus will influence the distribution of wildlife (Hutchingson, 1957; Krebs 1985; Sinclair 1983; van Bommel et al., 2006). Wildlife are able to make use of various habitats but will show preference for areas that not only cater to their needs, but will be to their maximum advantage for survival (Pullium & Danielson, 199; Morrison *et al*., 1998; Pulliam, 1998; Morris, 2003; Olivier, 2007; Canter, 2008). With increasing isolation and fencing of protected areas, the need for conscientious and persistent management of these areas becomes greater (Noss *et al*., 2006; Muths & Dreitz, 2008; Newmark, 2008). Ecological processes are affected when systems are artificially closed and numerous factors need to be considered, such as resource limitations, migration restrictions, inbreeding and environmental degradation (Macarthur & Wilson, 1967; Caughley, 1994; Boone & Hobbs, 2004; Hayward *et al*., 2007; Hayward & Kerley, 2009; Albertson, 2010; Cumming, 2010; Ferguson & Hanks, 2010). Post reintroduction monitoring of species is an important tool in closing the gap between research and management, as conservation managers are able to make more informed decisions regarding the management strategies for protected areas (Armstrong & Seddon, 2007; Muths & Dreitz, 2008).

The abundance estimates generated using data sets collected by multiple and single observers varied. According to the theory of distance sampling, this variation is due to the increased detection probability of an animal when there is more than one observer (Buckland *et al*, 2001; Thomas *et al*., 2010). An additional factor may be that these transects were conducted in the dry season when visibility of wildlife is greater, therefore more animals are seen. At a cursory glance this trend is

demonstrated by data collected on a monthly basis for 14 months where the number of impala and waterbuck observations decreased in the wet, summer months of November to February and increased in the dry, winter months of June to August. Although values vary between data sets, the distance sampling estimates for the densities of impala and waterbuck are generally greater in the riverine areas, close to the Mkulumadzi and Shire rivers. The density of impala decreases in the habitats that are further away from the perennial rivers, but not as steeply as waterbuck densities. In the slightly elevated areas toward the western side of the sanctuary where water was limited, the estimated density of impala diminished rapidly and waterbuck densities even more so. Thus this information supports the fact that impala and waterbuck are water dependent species and prefer habitat that is associated with natural water sources as demonstrated in previous studies (Taylor *et al.*, 1969; Hofmann & Stewart, 1972; Young, 1972a; Skinner & Chimimba, 2005; Augustine, 2004; van Bommel *et al.*, 2006; Smit *et al.*, 2007).

Population estimates of 1200 for impala and 700 for waterbuck were generated from an aerial survey for the entire reserve conducted by Macpherson (2012). It was estimated that the animal density is greater in the sanctuary area (0.11 animals/ha) than for other regions of the reserve (0.02 animals/ha). Macpherson (2012) reported that 74% of impala (±888) and 91% of waterbuck (±637) were observed in the 'Greater Matithi' area that is approximately 22500 ha (225km^2) and includes the 140km^2 sanctuary area and a portion of the higher terrain in the west. The population estimates from this study are far greater than those provided by previous counts. Both impala and waterbuck populations are thriving post reintroduction.

For impala and waterbuck, adult females were most commonly counted. This may be due to the selection of prime or most advantageous habitat as females need good quality forage when they are pregnant or lactating (Rooyen & Skinner, 1989; Skinner & Chimimba, 2005). For impala, more adults were seen than sub-adults and juveniles. This great proportion of adults may be due to impala having a life span of about 13 years and that they have a fast growth rate, reaching maturity relatively quickly. Young ewes are able to conceive at about two years of age (Skinner, 1969; Skinner & Chimimba, 2005). The sub-adult and juvenile age classes of waterbuck are better represented than those of impala, as waterbuck are relatively slow to mature. Young cows rarely conceive before three years of age and males generally only start to establish their territories at five to six years of age (Hanks, Stanley Price, Wrangham, 1969; Spinage 1982; Estes, 1991; Skinner & Chimimba, 2005). With regard to the estimated cluster size for impala and waterbuck, the overall trend may be a reflection of the sexual segregation as larger, breeding herds occupy the riverine

habitats and generally smaller bachelor herds and lone males occupy areas with less favourable habitat quality (Spinage 1982; Child & von Richter).

The impala's preference for the Nsepete and Thawale waterholes may be influenced by the surrounding vegetation of riparian thicket and low to medium mixed woodland, respectively. The two peaks in waterhole use may be attributed to the warm weather conditions and lower water content in forage species. Waterbuck were not observed at the waterhole counts at Thawale, and this may be due to waterbuck not having a habitat preference for the low to medium mixed woodland that is dominated by *Sclerocarya birrea* (marula). Instead waterbuck most frequently used the Heritage waterhole and Nakamba waterhole that are located in habitats that are associated with riverine vegetation. Impala and waterbuck males, are more frequent diurnal users of waterholes in MWR, thus indicating sexual segregation. However, it is possible that females are more frequent users of waterholes at night. The surrounding habitat may not be optimal for breeding females that clearly occupy the riverine areas from the drive count data. Therefore lone males and bachelor herds occupy less than prime habitat and avoid territories defended by territorial males (Tomlinson, 1981; Murray, 1982b; Skinner & Chimimba, 2005).

Conservation managers should have a basic understanding of the ecology of wildlife populations within protected areas that they are responsible for, so that they may make more relevant management decisions regarding the conservation of biodiversity (Bothma, 1995; van Rooyen *et al*., 2000; Newmark, 2008; Tolber *et al*., 2008). Results of this study revealed that impala and waterbuck populations are growing in MWR, thus indicating the success of their reintroduction. Impala and waterbuck were observed in greater densities in habitats associated with riverine vegetation. Population management strategies will need to be implemented in the future before there are any long-lasting detrimental impacts on the reserves ecosystem and biodiversity. Generally, hunting and culling should be the last solution that conservation managers should consider (Walker, Emslie, Owen-Smith, & Scholes, 1987), and as MWR has a strict no hunting and no culling policy, the MWR management will rely heavily on alternative strategies such as translocation to other protected areas.

Distance sampling is a useful tool in estimating population densities, however there were many challenges working in relatively thick bush with low visibility. The statistical analysis of distance sampling may be improved with the more frequent establishment of visibility factors for the various seasons and sections of the reserve. Ideally, distance sampling should be carried out along parallel transects, but this is a challenge when counducting drive transects along established roads, which

could lead to an overlap of study areas and thus double counts. Walking transects were conducted, but proved to be such a challenge that data was not included in this study. Barnes (2001) suggests that dung counts can provide equal or even better estimates of population abundance in systems with low visibility, thus dung counts could provide adequate population estimations (Cromsigt *et al.*, 2009). The waterhole count data only represented the diurnal activity of antelope, therefore there is a gap in the understanding of waterhole use. Camera traps may be a better method of monitoring waterhole use as data may be collected during the night and day, for extended periods of time. Camera traps present other challenges in 'Big Five' reserves, such as the effective placement of cameras at waterholes with the least risk of disturbance or damage by species such as elephant (*Loxodonta africana*).

In addition, it is important that population estimates and distribution of both species be considered for the rest of the reserve with the use of camera trapping, waterhole counts and an alternative method of monitoring may be the incorporation of dung sampling on patrols (Danielsen *et al.*, 2005; Cromsigt *et al.*, 2009) or dedicated walking transects. The dung sampling method may be most suited to the terrain, vegetation and shy behaviour of wildlife outside the tourism and sanctuary area.

3.6 Conclusions

Impala and waterbuck have successfully been reintroduced to MWR as both species are breeding and have established themselves in favourable habitats that are in close proximity to water, as they are water dependent ungulates. As the reserve is an artificially closed system, their populations need to be continually monitored and managed to prevent ecosystem degradation, as their optimal habitat is limited and inter-species competition increases with rapidly growing wildlife populations. Considering the population estimates generated for impala and waterbuck in the sanctuary alone, it is recommended that MWR management consider important conservation decisions before large herbivore populations negatively impact the reserves biodiversity.

3.7 Acknowledgements

3.8 References.

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Chapter Four:

Ecology and behaviour of impala (*Aepyceros melampus*) and waterbuck (*Kobus ellipsiprymnus*) in Majete Wildlife Reserve, Malawi

4.1 Abstract

It is important to have a sound understanding of the habitat requirements and behaviour of herbivores so that wildlife managers may predict their distribution and the consequent effect on vegetation and other species. Impala and waterbuck were successfully reintroduced into a fenced reserve in the Middle Shire Valley, Malawi. The behaviour of both species was studied on drive counts and at waterhole counts at artificial water points. Generally, these ungulates demonstated typical characteristcs as outlined in previous studies but the impala lambing and waterbuck calving season varied from findings of other studies. It was determined that the most prevalent demographic was that of adult females for waterbuck and impala. There was sexual segregation as females predominantly occupied the prime habitat, while males occupied a less advantageous habitat. Both species used the artificial waterhole points frequently, but demonstrated a different level of preference for each waterhole. Managers may take this into account when developing and improving the artificial water point management for the reserve.

Keywords: behaviour, impala, waterbuck, waterholes

4.2 Introduction

The long-term survival of wildlife populations and protected areas are under threat from the ever increasing human population and associated demands and impacts (du Toit & Cumming, 1999; Norton-Griffiths, 2007; Lindsey, Masterson, Beck & Romañach, 2012; Somers & Hayward, 2012). Biodiversity conservation is becoming more challenging as the fragmentation and isolation of protected areas increases (Noss, Csuti & Groom, 2006; Muths & Dreitz, 2008; Newmark, 2008). With various ecological processes being disrupted, conservation managers need to implement strategies to prevent degradation of the environment and resultant negative consequences on wildlife populations (Caughley, 1994; Hayward, O'Brien & Kerley, 2007; Cumming, 2010; Ferguson & Hanks, 2010). The principal factors that contribute to the survival of animals are the acquisition of adequate nutrition and other key resources (e.g. water) and predator avoidance (Cowlishaw, 1997; Orians, 2000). The management of wildlife is founded on the knowledge and understanding of the relationship between animals and their habitat (Melton, 1987; Ben-Shahar &

Skinner, 1988; Dörgeloh, 1998, 2001; Traill, 2003). Wildlife selects suitable habitats that are most beneficial to their survival, with various trade-offs between the costs and benefits of occupying an area (Kie, 1999; Parker, Barboza & Gillingham, 2009). It is important to determine habitat requirements and resource competition during the dry season, when resources are most limited (Sinclair 1975; Riney, 1982; Melton; 1987; Traill, 2004). Density-dependent habitat selection occurs when population densities increase, resulting in a broader range of habitats or resources being used by species, as individuals seek out alternatives (Fretwell & Lucas, 1970; Kauda, Mapolelo, Matlhahku, Mokgosi, 2002). This occurrence may influence population dynamics and social organization of ungulates (Morris, 1988; Morris, 1992; Kaunda *et al.*, 2002). However, some species co-exist in habitats due to a resource partitioning mechanism (McNaughton & Georgiadis, 1986). It is crucial to monitor wildlife populations to make significant contributions to management strategies for protected areas (Traill, 2003).

The provision of surface water to wildlife is an important management intervention (Traill, 2003; Smit, Grant & Devereux, 2007). Artificial water points (waterholes/AWP's) may be placed in protected areas to facilitate population growth and expand the area in which wildlife may forage, particularly during the dry season (Redfern, Grant, Gaylard & Getz, 2005; Loarie, van Aarde, Pimm, 2009; Wienand, 2013). It is beneficial to management to know which species are using which artificial waterholes as the potential impact on the surrounding vegetation and distribution of wildlife may be anticipated and assessed. In addition this knowledge will contribute to the installment and/or possible closure/shutdown of artificial waterholes and population manipulation in the future (Smith, Grant & Devereux, 2007; Wienand, 2013).

Impala, *Aepyceros melamupus* (Lichtenstein, 1812) are a medium-sized ungulate that are abundant within their range from the north-eastern regions of Southern Africa to Central and East Africa (Estes, 1991; Stuart & Stuart, 2006). As an ecotonal species impala prefer woodlands with minimal undergrowth and low to medium grasslands (Estes, 1991). Impala are resilient species that can readily shift their diet to incorporate browse or graze species to their advantage and fare better than other species in adverse conditions (Estes, 1991; Cerling *et al.*, 2003; Augustine, 2004; Garine-Wichatisky *et al.*, 2004; van Bommel, Heitkönig, Epema, Ringrose, Bonyongo &Veenendaal, 2006). With the ability to incorporate more grass into ther diets in the wet season and more browse in the dry season, impala are described as mixed feeders (Hofmann, 1973; van Rooyen, 1992; Gagnon & Chew, 2000; Brashares & Arcese, 2002; Cerling et al., 2003). However, with a high crude protein intake, especially in the dry season, impala need an adequate supply of water on a daily basis to assist in the removal of nitrogenous waste (Fairall & Klein, 1984), thus impala are

water dependent (Augustine, 2004; van Bommel *et al*., 2006). Impala require approximately 2.5 litres of water per day (du Toit, 2010) however, they are able to go without drinking if their essential moisture needs are met by the consumption of lush, green vegetation (Estes, 1991; Skinner & Chimimba, 2005; Frost, 2014).

Impala are a gregarious ungulate with a social organization that changes seasonally and influences their distribution and therefore dietary intake (Rooyen & Skinner, 1989; Skinner & Chimimiba, 2005). Generally group sizes range from six to 20 individuals in the drier months when forage species are limited, to herds of 50 to 100 individuals in the wet, summer months when forage is lush and plentiful (Estes, 1991;). Typically there is sexual segregation of males and females outside of the breeding season (Ruckstuhl & Neuhaus, 2000; 2002). Group compositions of impala include bachelor herds of mature and immature males; breeding herds of adult and immature females and a territorial male; nursery herds of females and their young; mixed herds of adult males and females with immature males and females; and territorial males that compete for females and territory during the rut (Estes, 1991).

In January, the days start to shorten in the southern hemisphere, which stimulates an increase in testosterone levels in impala males and their subsequent restless and more aggressive behaviour (Vincent, 1979; Skinner & Chimimba, 2005). Territorial males compete for and defend prime habitat and successful males will scent-mark their established territories by rubbing the glandular skin on their faces against the vegetation (Jarman & Jarman, 1974; Skinner & Chimimba, 2005). Territorial males will intimidate and chase away any trespassing males, especially as the breeding period approaches around March and harem herds of females are acquired (Murray, 1982; Skinner & Chimimba, 2005). During the rut various vocalizations are commonly heard as territorial males defend and maintain their territories and harem herds (Skinner & Chimimba, 2005). Males typically lose condition over this period, as there is less time to forage while safeguarding their females and territories from rival males (Dunham & Murray, 1982). Mating peaks toward the end of the rains and after a gestation period of 194-200 days, impala lambs are generally born between November and January (Fairall, 1971; Estes 1991). Impala ewes move away from the herd for several days to give birth and return once the lamb is able to follow (Skinner & Chimimba, 2005). Impala ewes coordinate the birth of their lambs within a few weeks as a defense mechanism that increases the overall survival rate of lambs.

Impala are primarily diurnal ungulates that have peaks of activity in the early morning and late afternoon when they move through an area and forage (Estes, 1991; Kingdon, 1997). In the warmer period of the day impala may rest by standing or lying down in the shade and are often seen ruminating. Impala may be observed grooming themselves and each other to remove ectoparasites such as ticks (McKenzie & Weber, 1993; Skinner & Chimimba, 2005). As an anti-predatory tactic

impala will survey their surrounds periodically for any sign of a threat and individuals in the herd will do so at different times to increase the overall surveillance of the area. Alarm calls may be used to warn others in the herd of the presence of a threat. Should an intruder come within the 'flight distance' impala disperse in several directions in a display of agile leaps and bounds, regrouping to move in one direction as they defend themselves from the threat (Skinner & Chimimba, 2005).

Waterbuck, *Kobus ellipsiprymnus* (Ogilby, 1833) are large, robust ungulates with an unusually shaggy coat. The common waterbuck (*K. e. ellipsiprymnus*) distribution ranges from the northern regions of South Africa, Botswana and Namibia, up towards Kenya and southern Somalia, in areas to the East of the Great Rift Valley (Skinner and Chimimba, 2005). Waterbuck are primarily grazers (Hofmann, 1973; Estes, 1991) that prefer open habitats with short to medium grasses and are in relatively close proximity to water (Taylor *et al*., 1969; Hofmann & Stewart, 1972; Wirtz & Kaiser, 1988; Traill, 2004; Gutbrodt, 2006). Waterbuck require an unusually high amount of water (Taylor *et al*., 1969b) with an estimated 9 litres of water needed per day (du Toit, 2010). The need for a greater water intake could be due to the consumption of mostly protein-rich grasses (Taylor, Spinage & Lyman, 1969; Estes, 1991). It has been recorded that waterbuck remain within 1.8 kilometres of water (Lamprey, 1963). Waterbuck are able to shift their diet to incorporate browse species in periods of environmental stress with a low abundance of preferred grasses (Melton, 1978; Tomlinson, 1980; Estes, 1991).

Waterbuck herds are not typical, distinct, cohesive units as individuals freely congregate and disband (Estes, 1991; Kingdon, 1997). Groups of six to 12 individuals form small herds and larger congregations of 30-70 individuals may be observed in areas where resources are plentiful (Skinner & Chimimba, 2005). Females are commonly seen alone or in pairs but casual groups of five to ten individuals may be observed (Spinage, 1982; Estes, 1991). Weaned waterbuck calves and juveniles commonly form groups as they are driven out of their natal herd by adults (Spinage, 1982, Estes, 1991). Waterbuck are slow to mature with males reaching their prime at five to six years old and females rarely conceive before three years of age (Spinage, 1982; Skinner & Chimimba, 2005; Kingdon, 1997; du Toit, 2010). Waterbuck are polyoestrus and do not have distinct breeding seasons, however calving peaks have been observed in October and again between February and March (Melton, 1983; Skinner and Chimimba, 2005). Females withdraw from the herd to give birth after a gestation period of 280 days (Spinage, 1982; Estes, 1991; Skinner & Chimimba, 2005) and stop lactating about 180-210 days afterwards so that calves are generally weaned by 276 days (Spinage, 1982; Skinner & Chimimba, 2005). Waterbuck do not typically interact physically for example in greeting or in grooming, but mothers will communicate with their young with a serious of bleats and snorts (Estes, 1991). Foraging activity peaks in the early morning and late afternoons

to early evening, and time is between is spent ruminating as with most grazers (Estes, 1991). Waterbuck are vigilant and regularly survey their environment for threats, running to cover when necessary and in some incidences, large bulls will engage in self-defence against predators.

In the recent past the number of reintroduction programmes have increased, as this strategy has become a valuable tool in wildlife management and biodiversity conservation (Kleiman, 1989; Griffith, Scott, Carpenter & Reed, 1989; Stanley-Price, 1991; Wolf, Griffith, Reed & Temple, 1996; Muths & Dreitz, 2008). Monitoring the success and challenges post reintroduction will contribute toward a better understanding of reintroduction biology and therefore the improvement of reintroduction strategies in the future (Sarrazin & Barbault, 1996; Armstong & Seddon, 2007; Muths & Dreitz, 2008).

The objective of this study was to examine the behavioural ecology of impala and waterbuck populations ten years after their reintroduction into Majete Wildlife Reserve. It was hypothesized that impala and waterbuck populations were well established in MWR and that their respective breeding and birthing seasons would be similar to that of other populations in southern Africa. Baseline data for daily activities such as foraging, resting and antipredatory behaviour was collected. The use of artificial waterhole points was also investigated to determine waterhole preference. Results were used to determine behaviour that was typical and that which may be specific to MWR and thus contribute towards reserve management strategies and potentially to a deeper understanding of reintroduction biology.

4.3 Methods

Study site

Majete Wildlife Reserve (MWR) is situated in the middle Shire Valley in the southern region of Malawi. A 142km long fence encloses the entire 700km^2 reserve that is surrounded by high-density rural settlements. The Shire River drains Lake Malawi and transects a small portion of the reserve in the north-east. The terrain is generally higher in the west of the reserve where river valleys cut through steep, undulating hills. The general altitude decreases and the gradient of the reserve decreases and flattens out toward the Shire River in the east. The Mkulumadzi River in the north of the reserve is a smaller perennial river than the Shire River. Other natural water sources include a number of springs and seasonal rivers and pools that are scattered throughout the reserve. Several (n = 9) borehole-fed artificial water points (AWPs) have been placed in the reserve to augment the perennial water supply and broaden the range that animals can forage in the dry season. The average annual precipitation is 680-800mm in the east and 700-1000mm in the higher western

regions. Most rainfall occurs between November and early April (Wienand, 2013), followed by less significant and light "chiperoni" precipitation from April to October (Sherry, 1989; Morris, 2006). The vegetation of MWR has been grouped into classes making it easier to describe and classify but the distribution of vegetation species is influenced by soil type and depth. The most prevalent vegetation types are: riverine vegetation along river systems, mixed deciduous woodlands varying from low to high altitudes and high altitude miombo woodland. MWR, originally gazetted in 1955, had not received the support and resources that a protected area requires. With encroaching, impoverished settlements and associated demands MWR was decimated of most of its mammal populations, including elephant and sable. Following a public-private partnership (PPP) agreement between the Malawian Department of National Parks and Wildlife (DNPW) and African Parks Majete (Pty) Ltd. in 2003, MWR underwent impressive infrastructure development and a large-scale reintroduction programme over several years. Initially a 140km^2 area, referred to as the "sanctuary", was fenced in the north-eastern region of the reserve, while the remainder of the perimeter fence was erected. The original sanctuary is also the tourism section of the reserve as there is an adequate source of water throughout the year as both the Shire and Mkulumadzi Rivers flow through this region and the road infrastructure is good. The first few mammal reintroduction events took place in the sanctuary, including 263 impala and 98 waterbuck. This sudy was focused within the sanctuary area

Behavioural Observations

In conjunction with determining population abundance and density estimates using distance sampling as another aspect of the larger study, *ab libitum* data (Altman, 1974) on the diurnal behaviour of waterbuck and impala was collected. Drive transects were conducted along established roads in the sanctuary. When impala or waterbuck were sighted, the observer stopped to record the required data, aided by the use of binoculars and a camera when necessary. Sampling generally began at first light and ended at dusk, with a rest period during the hottest part of the day. Data collected for each observation of impala and waterbuck included: species, group size, gender and size class structure of groups, GPS location, time of day, as well as information needed for distance sampling techniques, such as the radial distance of animal from observer and the total distance covered (Buckland, Anderson, Burnham, Laake, 1993; Buckland, Anderson, Burnham, Laake, Borchers & Thomas, 2001). The observer noted any behaviour that was displayed by any of the individuals in the group (Altman, 1974) including, foraging, drinking, resting, walking, running, leaping and grooming.

Waterhole Counts

Waterhole counts were conducted on a monthly basis from June 2013 to December 2013 at four borehole-fed AWP's within the original sanctuary area. Each count was executed over a 12-hour period; starting from 06h00 in morning and concluding at 18h00 in the evening. Toward the late dry season and summer months, counts started at 05h30 as the sun rose earlier as the summer solstice approached. Three to four observers were responsible for the data capture during waterhole counts. In the old sanctuary area, observations were made from a hide (Nakamba and Nsepete hides) or from a viewing area in tourism infrastructure (Heritage Centre and Thawale Lodge).
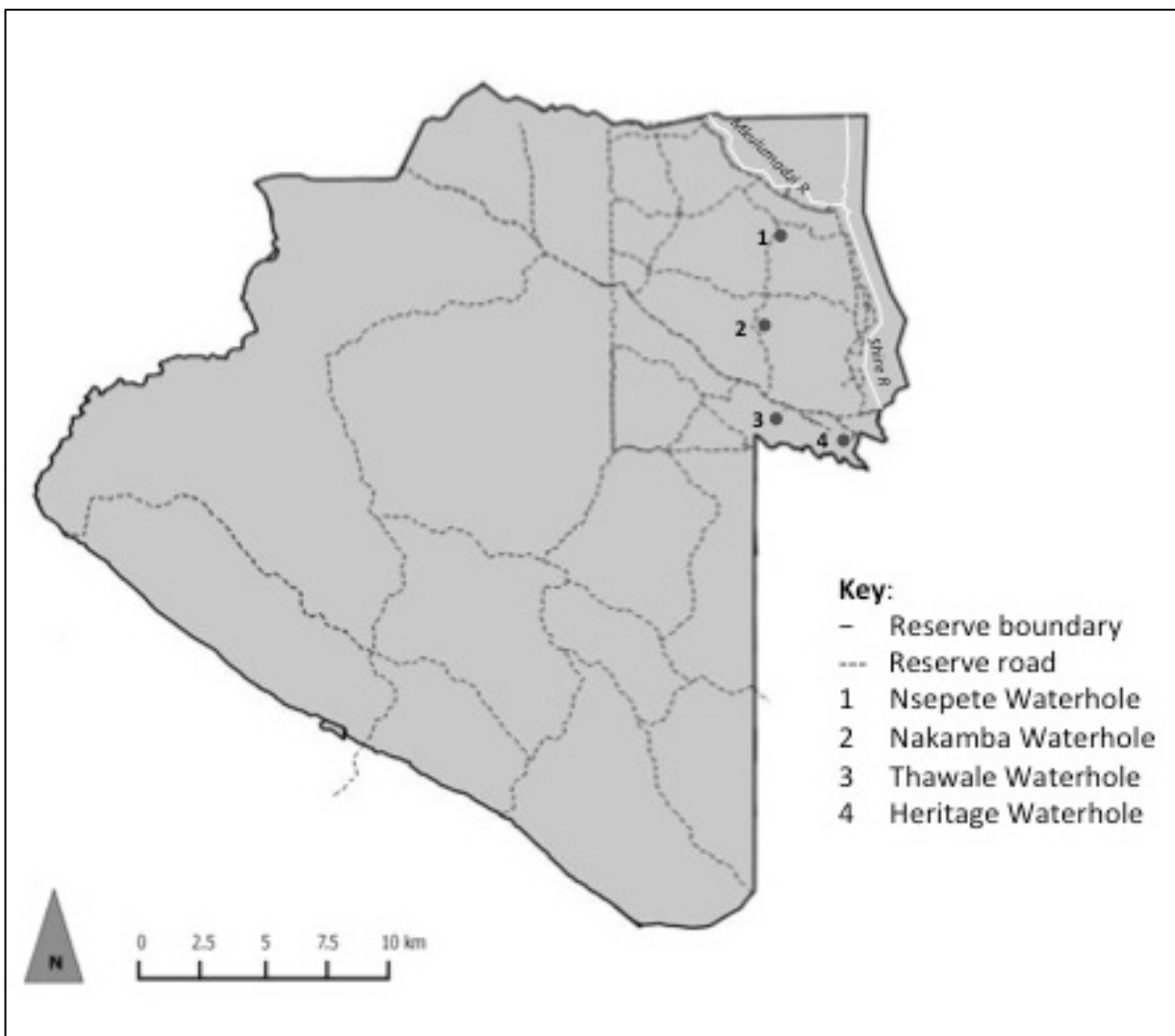
Figure 4.1 Location of the four borehole-fed waterholes in Majete Wildlife Reserve at which waterhole counts were conducted.

Observers remained as still and quiet as possible for the entire count so that animal activity was disturbed as little as possible by the presence of humans. Data was gathered for each individual or group of animals that were within 100m of the waterhole being observed. The gender and size class of each animal was recorded. In addition, the time that the animal or group of animals was first sighted, the first drink taken by an individual or member of a group, the time of last drink, as well

as the last time being sighted in the vicinity was recorded. Other behaviours such as intra- and inter-species interactions were also noted.

Statistical Analysis

Behavioural observations collected/recorded *ab libitum*, were sorted to determine the occurrence of various activities using the one-zero sampling principle (Olson, 1929; Altman, 1974) where the presence of a behaviour is recorded as a one and absence as a zero.

Behavioural activities were categorized based on categories outlined by Reid (2005):

Vigilant: aware with head up and neck upright

Feeding: browsing or grazing

Moving: walking, trotting, running, and leaping

Grooming: evident attempt to remove parasites

Territorial: rutting, sparring, territorial marking

Other: resting, ruminating, urinating or defecating

In addition to this the animals' response to the presence of the observer was also considered and reactions were categorized as follows: unaware, undisturbed, looked at observer, alarm called, walked away or ran away.

Waterhole count data was analyzed to determine the demography of waterbuck and impala that utilized artificial water points as well as their drinking patterns and behaviour associated around the waterhole. Tolerance of each other and other species while at the waterhole was determined by examining time overlaps and presence or absence of interactions between species (Valeix, Chamaille-Jammes & Fritz, 2007). The impala and waterbucks' utilization of the different artificial water points was compared to determine which, if any, of the waterholes were preferred, taking into account the surrounding vegetation.

4.4 Results

Drive count observations

Data gathered from distance sampling drive counts was used to determine the population structures for impala and waterbuck in the sanctuary area of MWR. It was determined that both waterbuck and impala densities were greatest in the riverine habitats (Table A.2 and Table A.3), in relatively close proximity to the Shire and Mkulumadzi Rivers; and that adult females were the most prevalent demographic group overall, as referred to in Chapter Three. As illustrated in Figure 4.2 a greater

proportion of impala adults (69%) were observed in comparison to sub-adults (11%), juveniles (13%) and lambs (6%).
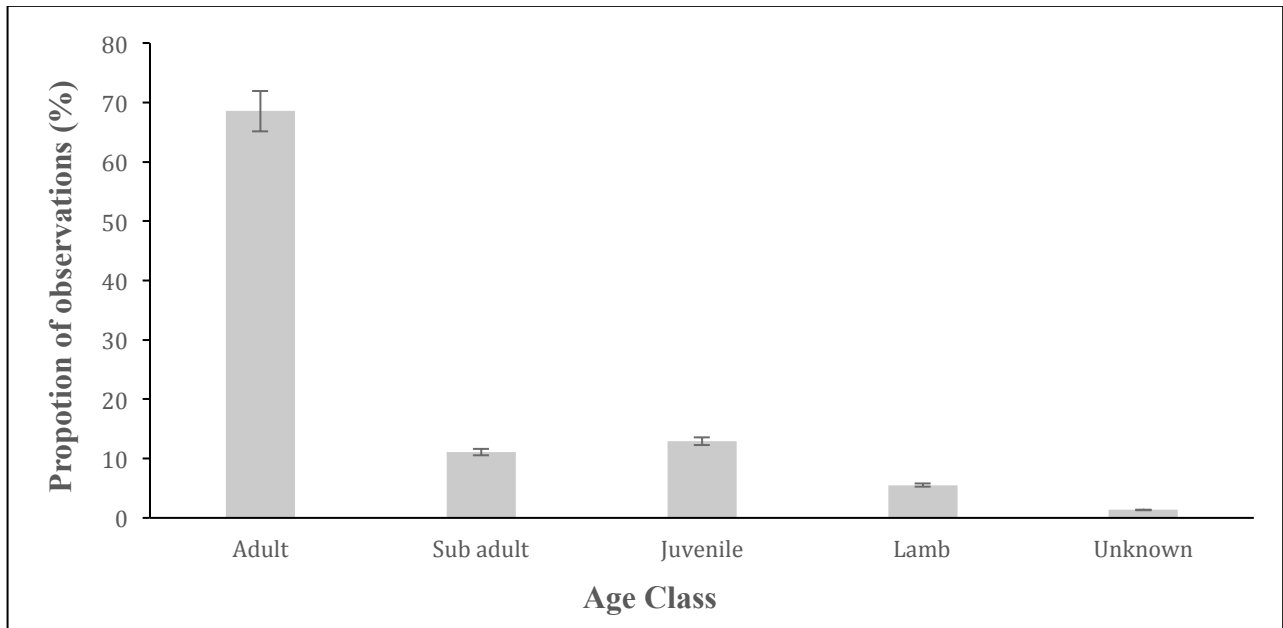
Figure 4.2 The proportion of adults, sub adults, juveniles, calves and unclassified impala observed from June 2013 to July 2014.

For waterbuck, adults were also the most commonly observed age class making up 48% of the observations, but sub-adults (27%) and juveniles (20%) were seen in greater proportions than those of impala. The proportion of waterbuck calves (1%) was lower than that of impala lambs.
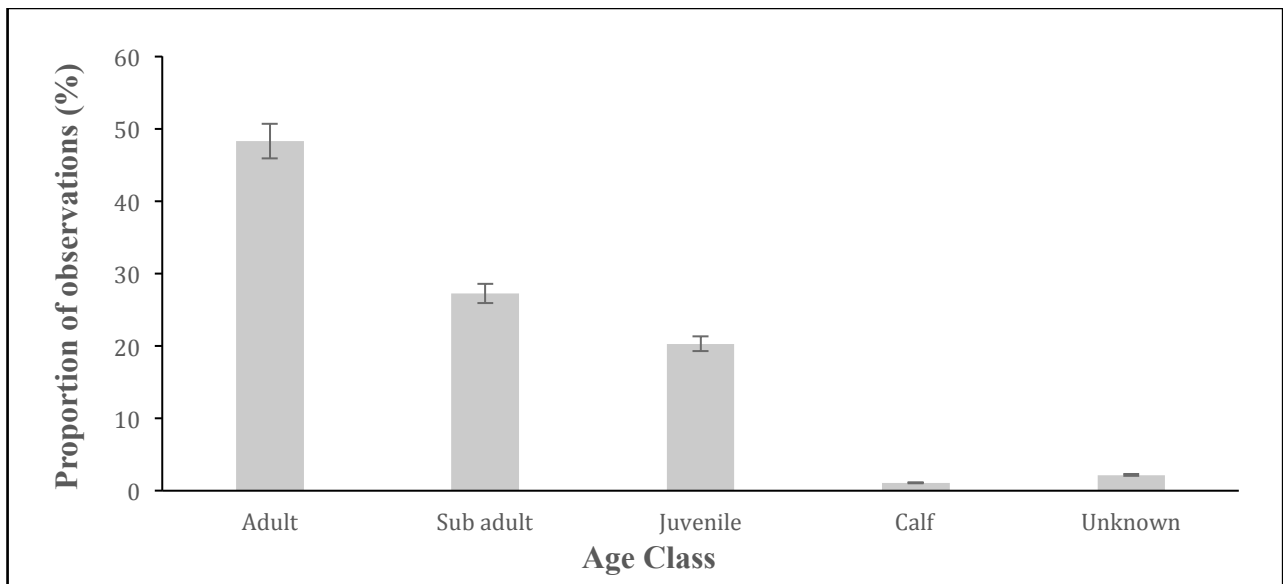
Figure 4.3 The proportion of adults, sub adults, juveniles, calves and unclassified waterbuck observed from June 2013 to July 2014.

The number of observed adults, sub-adults, juveniles and young for impala and waterbuck are illustrated in Figure 4.2 and Figure 4.3 respectively. For impala adults are more abundant than

individuals from other age classes (Figure 4.2). An increased number of sub-adults were observed between August and November 2013 and in June to July 2014 (Figure 4.4). Juveniles were observed in higher numbers between July and September 2013 and more prevalently in May to July 2014 (Figure 4.4). Lambs were first observed in November 2013 when the lambing season commenced.
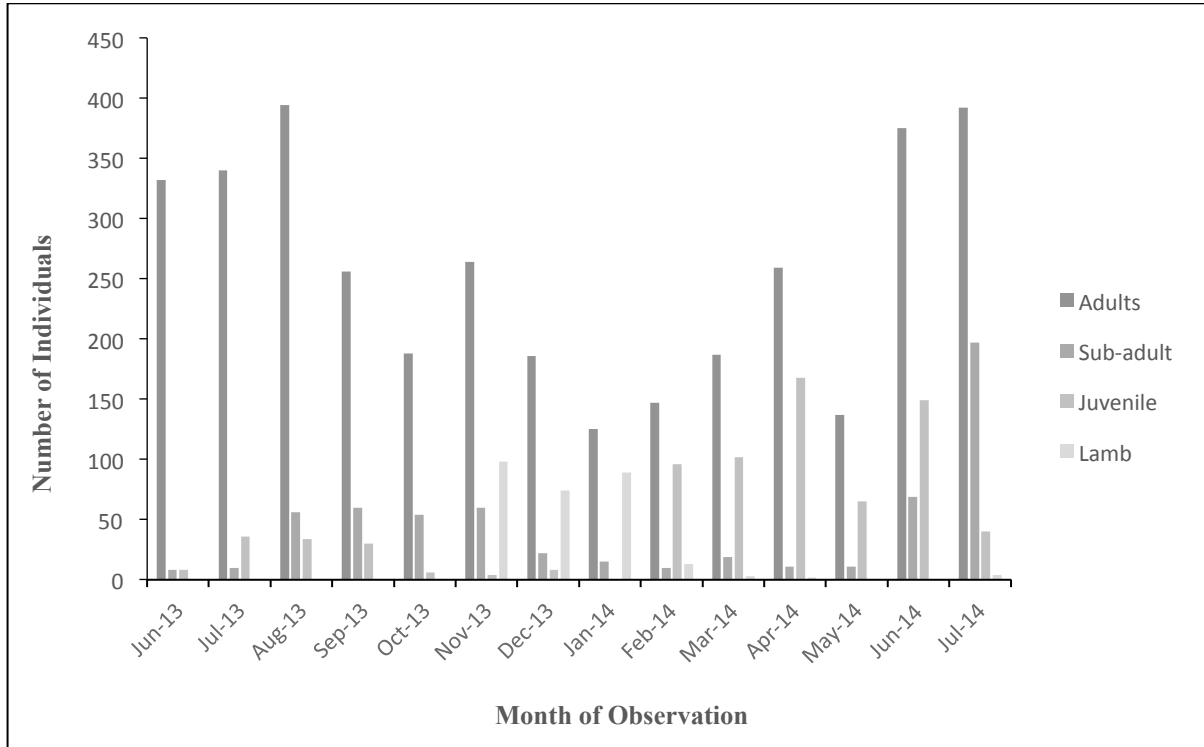
Figure 4.4 The number of impala sighted in the sanctuary area, according to age class.
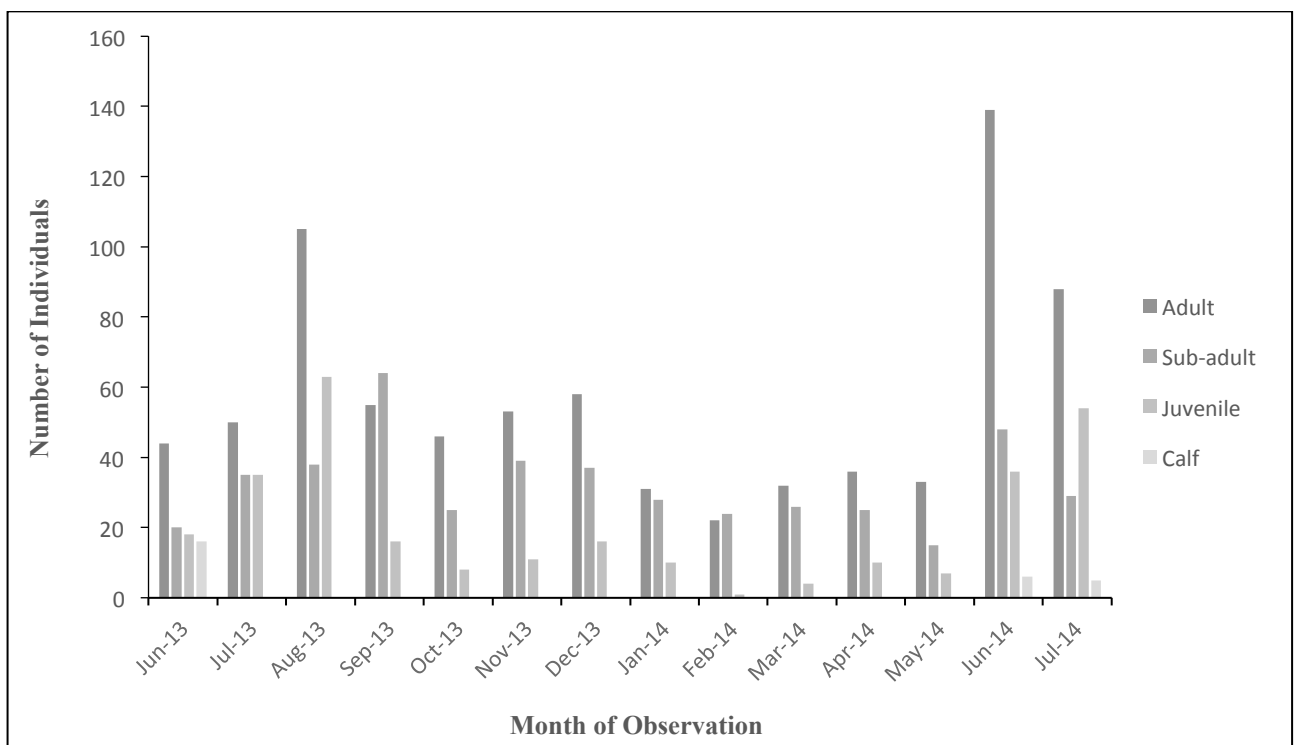
Figure 4.5 The number of waterbuck sighted in the sanctuary area, according to age class.

Waterbuck adults were sighted more often than other age classes (Figure 4.3). Young waterbuck calves were only recorded for June 2013 and in June to July 2014 (Figure 4.5). Generally there were fewer sightings of both species from September 2013 in the late dry season, to May 2014 in the late wet season; with the highest number of sighting occurring in June to August 2013 and June to July 2014 (Figure 4.4 and Figure 4.5).

Estimated group (cluster) sizes were generated from distance sampling (Table A.2 and Table A.3). For impala, cluster size estimates ranged from 0.82 in the least populated regions to 17.36 for the preferred habitat, close to the Shire and Mkulumadzi Rivers. The larger impala herds were observed between December 2013 and April 2014; the largest herd consisting of 51 individuals during the lambing season. Herd numbers decreased towards May 2014. Dominant males were associated with breeding herds all year round as very few observations were made where the dominant male was absent. For waterbuck, cluster size estimates ranged from 0.43 in the areas furthest from water and associated vegetation, to 10.71 in the riverine and low altitude mixed woodland habitat. The herd size for waterbuck fluctuated and dominant males were not always associated with breeding units. The larger congregations of waterbuck were not common but those recorded were mostly observed close to the Mkulumadzi River, the largest group consisting of 27 individuals.
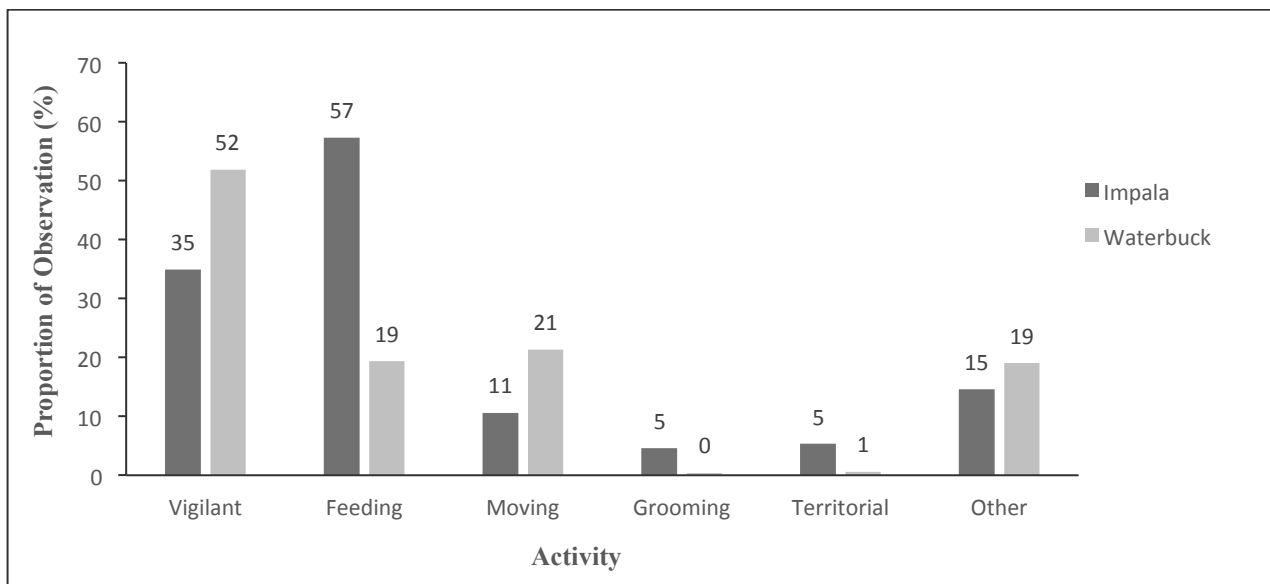
Figure 4.6 The proportion of observed diurnal behaviour of impala and waterbuck expressed as a percentage of all observations

The most commonly observed behaviours in impala were grazing, browsing and standing, while waterbuck were most often sighted just standing, grazing, walking and resting.
At a cursory glance, impala and waterbuck were commonly observed in the vicinity of baboons (*Papio cynocephalus cynocephalus*), nyala (*Tragelaphus angasii*), zebra (*Equus quagga*) and

warthog (*Phacochoerus africanus*), but infrequently with ungulates such as buffalo (*Syncerus caffer*), sable antelope (*Hippotragus niger*) and eland (*Taurotragus oryx*).

Waterhole count observations

All four waterholes were utilized more frequently by impala adult males compared to other demographic groups for impala. A greater number of impala were observed using the Nsepete and Thawale waterholes than the other two waterholes, which is represented in Figure 4.7.
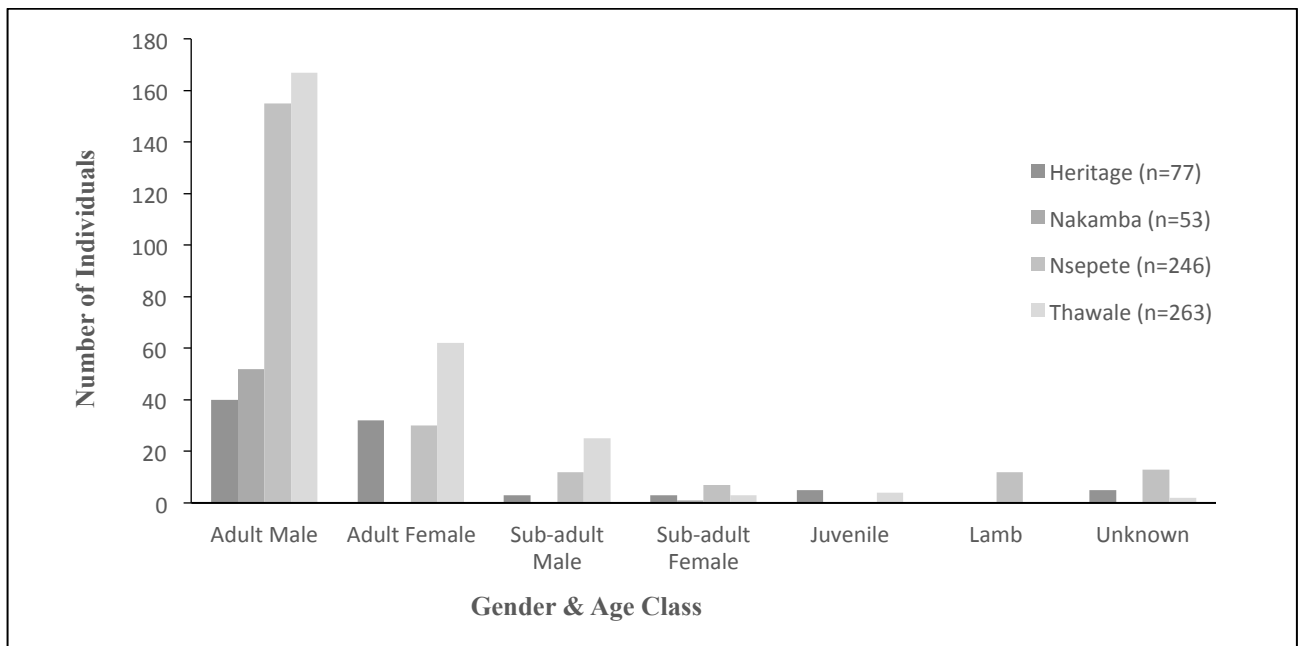
Figure 4.7 The number of impala according to gender and age class for each of the four artificial waterholes in the sanctuary area of Majete Wildlife Reserve.

Figure 4.8 The number of waterbuck according to gender and age class for each of the four artificial waterholes in the sanctuary area of Majete Wildlife Reserve.

Waterbuck were only observed at three of the waterholes as they did not utilize Thawale waterhole but they displayed a preference for the Heritage waterhole in the early dry season, and Nsepete and Nakamba waterholes in the late dry to early wet season (Figure 4.9). As for impala, adult males were the most common users of waterholes overall. The Heritage waterhole and surrounding habitat was utilized by adult females, male and female sub-adults and juveniles in comparison to Nakamba and Nsepete waterholes as illustrated in (Figure 4.7).

.
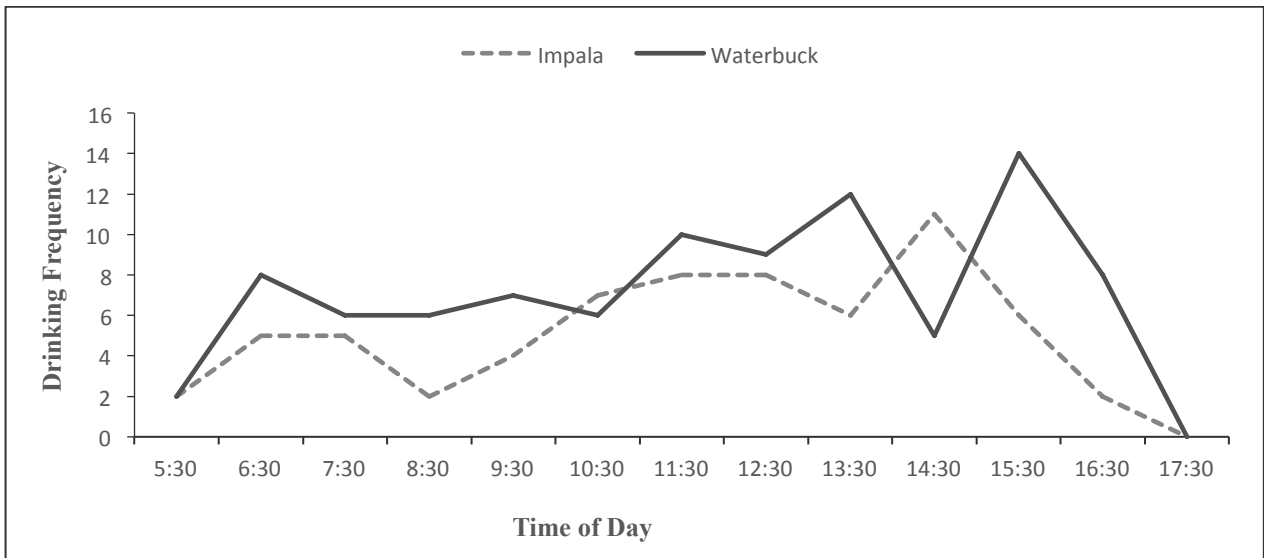
Figure 4.9 Impala and waterbuck drinking times observed at waterhole counts
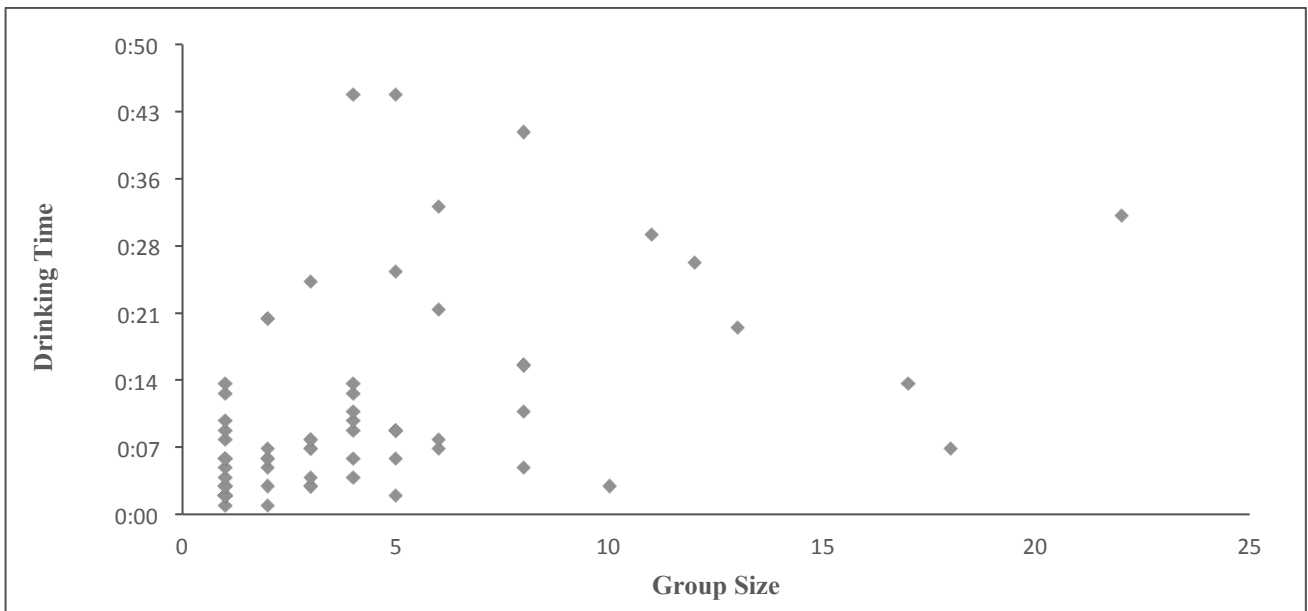
Figure 4.10 The time spent drinking (time between the first drink and last drink) in relation to the group size of impala

Drinking times from all counts were combined and plotted on a graph to illustrate the peak drinking times for impala and waterbuck (Figure 4.9). For impala there was a gradual increase in drinking activity toward midday with the highest peak between 14h00 and 15h00, after which there was a

decline in drinking until the waterhole count was concluded at 17h30There was a peak in waterbuck drinking activity between 06h00 and 07h00. Drinking activity continued throughout the morning with peaks around 11h30 and 13h30. There was a decrease in activity between 14h00 and 15h00, followed by another increase in activity between 15h00 and 16h00. Drinking activity declined steeply after 16h00.

By measuring the time between the first drink and the last drink of individuals and herds, the drinking time was calculated. In general, neither impala nor waterbuck spent more than 14 minutes drinking at the waterhole, although some lingered and spent time in the vicinity of the waterhole, resting or foraging.
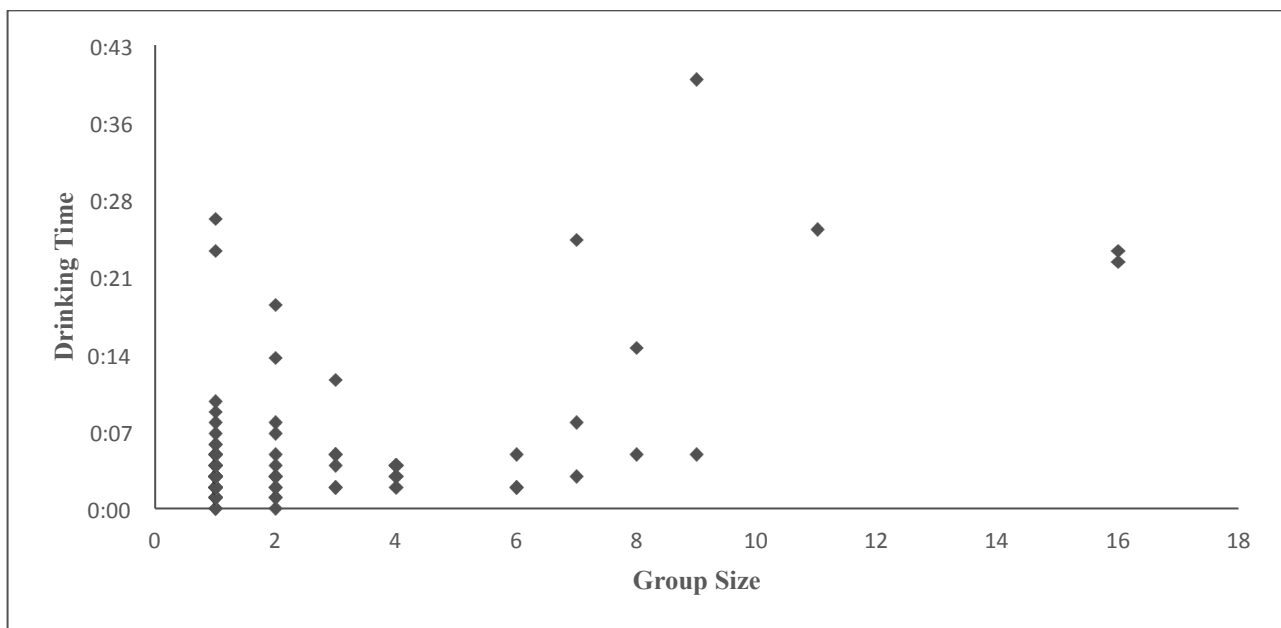
Figure 4.11 The time spent drinking (time between the first drink and last drink) in relation to the group size of waterbuck.

4.5 Discussion

Post reintroduction monitoring is valuable to conservation management and to augmenting an understanding of reintroduction biology (Muths & Dreitz, 2008). As part of a larger study, the basic behaviour of impala and waterbuck post reintroduction into MWR was investigated to contribute toward a better understanding of their specific requirements and preferences in the reserve.

Impala and waterbuck occurred in greater densities in areas closer to the Shire and Mkulumadzi Rivers, demonstrating a preference for a habitat that is dominated by riverine and low altitude mixed woodland vegetation. This preference is expected as previous studies have shown that both species are water dependent (Taylor *et al*., 1969; Hofmann & Stewart, 1972; Wirtz & Kaiser, 1988; Augustine, 2004; Traill, 2004; van Bommel *et al*., 2006). Impala have been described as an ecotone

species that utilizes grass in open areas and browse resources in open woodlands (Estes, 1991) and waterbuck prefer open habitats with short to medium grasses (Taylor *et al*., 1969; Hofmann & Stewart, 1972), thus the riverine and low altitude mixed woodland vegetation would best provide resources required for their survival.

The overall decrease in the number of waterbuck and impala observed in the wet season may be due to the increased foliage on vegetation, thus visibility into the lush bush is reduced as well as the wider dispersal of wildlife throughout the area as water availability is increased. Therefore, more sightings were made in the dry, winter months of 2013 and 2014 as vegetation foliage was sparse and wildlife was more concentrated in areas in close proximity to a perennial water source (Valeix, Chamaillé-James & Fritz, 2007). The common sightings of impala and waterbuck in the vicinity of nyala, zebra and warthog, may be an indication of an overlap in species' habitat preferences resulting in competition for resources or it may be a reflection of facilitation (Prins & Olff, 1998, Arsenault & Owen-Smith, 2002), however in depth data collection and statistical analysis is required to determine whether overlaps are random or not. Impala, waterbuck and baboons were often observed in the same area. Previous studies on baboons and impala have suggested that this co-occurrence is an anti-prdatory strategy as both species have keen senses and will warn each other if a threat is detected (Estes, 1991). Resource competition may be a disadvantage however; congregating in areas may be another anti-predatory strategy (Kie, 1999; Owen-Smith, Fryxell & Merrill, 2010).

Impala herds increased in number between November and December 2013 over the lambing season (Fairall, 1971; Estes 1991). However the unstable congregation of several family units joining to form herds of 100-200 individuals during the rainy season was not seen on such a scale, as other studies have demonstrated (Murray, 1981; Estes, 1991). Reduced herd sizes were observed towards May as resource abundance decreased and herds may have split, as well as juvenile males being expelled from herds by the dominant male during the rut (Murray, 1981). Previous studies have reported that dominant males are not always associated with breeding herds (Ruckstuhl, 2000, 2001, 2002; Mooring *et al*., 2005), however the absence of a dominant male from a breeding herd was rarely observed in MWR. As previously determined by other studies (Melton, 1978; Spinage, 1982; Estes, 1991; Kingdon, 1997), waterbuck formed family units that were not particularly cohesive as group numbers and compositions fluctuated throughout the year. The largest groups of waterbuck observed together were next to the Mkulumadzi River where natural water and associated vegetation was plentiful, thus able to sustain larger groups (Skinner & Chimimba, 2005).

Waterbuck calves were rarely seen in comparison to impala lambs. This may be due to waterbuck calves remaining hidden (termed "hiding out") for a three to four week period after their birth (Spinage, 1982; Estes 1991), whereas impala lambs join their herds when they are about three days old (Estes 1991). Impala form nursery herds (Estes, 1991) and are therefore more conspicuous than waterbuck that have less cohesive herds (Melton, 1978; Estes, 1991; Skinner & Chimimba, 2005). Impala typically drop their lambs within a few weeks of each other between late November and early December as part of an anti-predatory strategy (Vincent, 1972; Skinner et al., 1977b; Skinner & Chimimba, 2005). For MWR, the first lamb was sighted in late October, with most lambs dropping from mid-November to early December, therefore the lambing season in MWR is slightly earlier than indicated in pervious studies (Estes, 1991) The lambing season may shift to occur later with changes in environmental conditions, as it has been observed that females will only drop lambs as late as January if nutrition availability is low (Vincent, 1972; Skinner et al., 1977b; Skinner & Chimimba, 2005). Waterbuck are polyestrous and do not have a distinct breeding season, but calving peaks may be observed in some areas. A previous study conducted in the Kruger National Park, South Africa, demonstrated that waterbuck have calving peaks in October and again between February and March (Pienaar, 1963; Fairall, 1968; Melton, 1983; Skinner and Chimimba, 2005). However in MWR, calves were only observed in June 2013 and again in June to July 2014 thus they only have one clear calving peak for MWR from May to June, considering their hiding period after birth. It is possible that the calving period may occur sooner and that the post birth hiding period is longer than has previously been recorded. It would be valuable to monitor waterbuck and their calving periods, noting any changes, as populations increase together with an increase in resource competition. Another factor to consider would be the waterbucks' recovery after the trauma of being moved and reintroduced to MWR, as such stressful events may have an effect on reproduction (Parker, Barboza & Gillingham, 2009). As polyestrous mammals, waterbuck may have started breeding after a period once established in MWR, thus causing their calving period to be different when compared to other areas. In addition, the availability of nutrients in habitats varies seasonally and may also influence the timing of calving (Parker *et al.*, 2009).

Waterholes were situated to the west and southwest of the Shire River and were most commonly used by the male demographic of impala and waterbuck. Waterbuck made greater use of waterholes than impala throughout the day, except for a decline during the typically hottest time of day. The biggest peak in activity was in the mid-afternoon, after which waterhole use dropped toward dusk. This peak may be due to waterbuck replenishing their water reserves, as they are more prone to dehydration than domestic cattle (Taylor *et al.*, 1969). Overall, waterholes were more frequently used by impala and waterbuck in the late dry season when natural pans had dried up and the

moisture content of forage decreased, therefore unable to satisfytheir water requirements. With increased animal activity around waterholes in the dry season, surrounding vegetation is impacted by concentrated utilization and often over-utilization during the 'critical season' when water is limited (Sinclair, 1975; Riney, 1982; Traill, 2004; Valeix, Chamaillé-James & Fritz, 2007). As ungulates locally impact vegetation and therefore influence vegetation composition and structure, it is important that conservation managers implement strategies that prevent the degradation of habitat by over-exploitation (Behr & Groenewald, 1990; Bothma, 1995; van Rooyen *et al.*, 2000)

Typically, impala flee from a serious threat with an impressive display of leaps and bounds as they disperse in several directions (Estes, 1991; Skinner & Chimimba, 2010). However this behaviour was generally not observed in MWR. A potential reason for this is that the impala could be well habituated to human presence and chose to conserve energy by walking and only sometimes running away into deeper vegetation cover. In addition, predators were absent from the reserve until late 2011, with the exception of a remnant spotted hyena (*Crocuta crocuta*) population, thus the predation threats were initially very low for prey species in MWR and impala may have reduced their anti-predatory response as a result. It would be interesting to conduct a more detailed study on impala vigilance and anti-predatory behaviour in MWR and record possible changes in behaviour as predators such as lion (*Panthera leo*) and leopard (*Panthera pardus*) populations increase in size. Results from such a study could be beneficial to other reintroduction programmes thereby assisting researchers and conservation managers in project planning and implementation (Sarrazin & Barbault, 1996; Armstong & Seddon, 2007; Muths & Dreitz, 2008).

Various studies have been conducted in other areas to observe the grooming and allogrooming of impala (Mooring, 1995; Mooring, McKenzie & Hart, 1995). The greater the number of ticks in an area, the more frequently an individual will scratch and groom to remove these fitness-compromising ticks, although it has been argued that individuals have a "grooming clock" and that they do not have heavy parasite loads due a frequent habit of grooming (Mooring, 1995; Mooring *et al.*, 1995). In this study, impala and waterbuck were rarely observed grooming, therefore it may be suggested that MWR does not have a heavy tick load at this time. This may be an advantage for wildlife as they are less prone to mortality and declines in populations due to tick-borne diseases (Mooring, 1995).

In future, more effective behaviour observations may be conducted with the use of focal sampling, outlined by Altmann (1973) as this will provide quantifiable data, rather than simple descriptive data. Focal animals may also be used to conduct Point Centre Quadrats (PCQ) (Mitchell, 2001;

Canter, 2008) to gain a better understanding of forage selection from vegetation available to a species. Having effective monitoring practices in place will help conservation management to anticipate changes in biological systems. For example, the daily activities of ungulates are influenced by population size, availability of forage and day length. (Hamel & Côté, 2008). Typically, ungulates display peaks in foraging behaviour in the early mornings and evenings and ruminate during the day. If a feeding peak is observed during the warmer periods in the day it is an indication of environmental stress (Novellie, 1975; Parker *et al.*, 2009). As the availability of prime quality forage decreases ungulates spend a greater amount of time foraging (Kie, 1999; Owen-Smith *et al.*, 2010) as the survival of an individual is reliant on obtaining adequate food to fuel metabolism (Calder, 1983).

It is beneficial to conservation managers to have an adept comprehension of the fundamental requirements and interactions of wildlife so that they may improve management and conservation strategies of protected areas (Melton, 1978; Putman, 1996; Dörgeloh, 2001; Traill, 2004). Results from this study may be used to determine similarities and differences between the ecology of impala and waterbuck populations in MWR and those elsewhere, thus contributing to the understanding and management of local populations.

4.6 Conclusions

After the successful reintroduction and establishment of impala and waterbuck populations into MWR, it was determined that both species have a preference for habitats in relatively close proximity to perennial water. Both species are water dependant and utilized waterholes more frequently in the late dry season when water was a limiting factor. Their expanding populations require intensive management to prevent the deterioration of ecosystems and the subsequent loss of biodiversity.

4.7 Acknowledgements

4.8 References

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Chapter Five:

Stable isotopic analysis of the diet of impala (*Aepyceros melampus*) and waterbuck (*Kobus ellipsiprymnus*) populations in Majete Wildlife Reserve, Malawi

5.1 Abstract

Stable isotope analysis of herbivore faecal matter is a method used to determine the relative proportions of resources in the diet mixture of herbivores, thus provides a better understanding of herbivore diets. In this study, infrequently found distinct $\delta^{13}C$ signatures between C_3 browse and C_4 grasses occurring in subtropical savannas, were used to investigate the diets of impala and waterbuck in Majete Wildlife Reserve (Malawi). Results were examined and used to develop management's understanding of the needs of these two ungulates. For the wet and dry seasons of 2013, the diet of impala did not differ greatly, yet the species browsed more in the 2014 dry season. Waterbuck are clearly grazers, but diet is not that of an obligate grazer as expected. It is important to have an understanding of the dietary habits of herbivores, so that management may make more informed decisions with regard to potential population management strategies.

Keywords: browser, carbon-13, grazer, impala, stable isotope analysis, waterbuck

5.2 Introduction

Some elements have two or more naturally occurring stable isotopes, each with a distinct mass. For example, carbon has two stable isotopes, ^{12}C and ^{13}C; as does nitrogen, ^{14}N and ^{15}N (Crawford, McDonald & Bearhop, 2008). Stable isotopes are analysed using a stable light isotope ratio mass spectrometer, which compares the sample to a standard to generate an isotope ratio (O'Leary, 1981). Stable isotope analysis has been beneficial in studies that determine fluxes of trace elements and cycling of nutrients, carbon, and water. Developments in this field have led to stable isotope analysis, especially that of carbon, becoming an established method in the dietary studies of animals (Smith & Epstein, 1971; Sponheimer, Robinson, Ayliffe, Passey, Roeder, Shipley, Lopez, Cerling, Dearing & Ehleringer, 2003).

Conventional methods of stable isotope analysis used linear mixing models that are based on mass balance equations, to estimate the proportion of stable isotopes contributed to a mixture (product) from the source (Sandberg Loudon & Sponheiner, 2012). A single isotope, two-source mixing

model required two sources and the isotopic signature of one element, such as $\delta^{13}C$ (Balesdent & Mariotti, 1996; Phillips & Gregg, 2001). A dual isotope, three-source mixing model requires three sources and isotopic signatures of two elements such as $\delta^{13}C$ and $\delta^{15}N$ (Phillips, 2001).

Several methods and formulae were developed to overcome the limitations in early stable isotope analysis. The advancement of continuous-flow techniques to isotope ratio mass spectrometry (IRMS) lead to the high-precision measurements of isotopes, the ability to efficiently analyse a large number of samples (Brenna, Corso, Tobias & Caimi, 1997; Crawford et al., 2008). Parnell et al. (2010), developed a Bayesian stable isotope mixing model, SIAR (Stable Isotope Analysis in R) to analyse data generated by IRMS. It has overcome some challenges faced by previous methods by incorporating several sources of variability and uncertainty and thus allows for the investigation of more complex questions (Sandberg et al., 2012).

Photosynthesizing plants may be classified by the photosynthetic pathway utilised to fix atmospheric CO_2. Herbaceous and woody plants, such as trees, shrubs, forbs and herbs make up the group of C_3 photosynthesising plants and most grasses that occur in subtropical savannas belong to the C_4 group (O'Leary, 1981; Codron, Lee-Thorp, Sponheimer, Bond, de Ruiter & Grant, 2005). The photosynthetic pathways of these two groups differs in that ribulose bisphosphate carboxylase is the key carboxylating enzyme in the C_3 pathway (Park & Epstein, 1960; O'Leary, 1981), while CO_2 is incorporated by the carboxylation of phosphoenolpyruvate in the C_4 pathway (O'Leary, 1981). Past studies have demonstrated that C_3 photosynthesizing plants have lower $\delta^{13}C$ values than C_4 photosynthesizing plants, thus their respective $\delta^{13}C$ values do not overlap (Sandberg et al., 2012). This is because the ribulose bisphosphate carboxylase in the C_3 pathway discriminates against $^{13}CO_2$ and tropical C_4 grasses have greater photosynthetic efficiency due to the presence of the Kranz anatomy (O'Leary, 1981, 1988; Sandberg et al., 2012). Therefore $\delta^{13}C$ values are used as a distinguishing characteristic between C_3 and C_4 plants (Bender, 1968, 1971; Smith & Epstein, 1971; O'Leary, 1981; Codron et al., 2005).

C_3 and C_4 photosynthesising plants make up the forage for herbivores and can be generally referred to as browse (dicotyledonous trees and shrubs) and grass (monocotyledonous grasses) respectively (Hofmann & Stewart, 1972; Jarman, 1974; Cerling et al., 2003; Radloff et al., 2013). Herbivores may be classified according to the composition of grass and browse species in their diet (Hofmaan & Stewart, 1972; Cerling, Harris & Passey, 2003). It has been shown that animal tissues and excreta, such as hair and faecal matter (dung), reflect the carbon and nitrogen isotope composition of their diet (DeNiro & Epstein, 1981; Sponheimer et al., 2003; Codron et al., 2005a; Sandberg et al., 2012; Radloff et al, 2013). Therefore the relative contribution of different food sources to their

diets may be determined by using stable isotope analysis, for example mixing models (Hobson, 1999; Phillips, 2001; Phillips & Gregg, 2001). Stable isotope analysis has increasingly been used as a helpful tool in analysis of the diets of animals in tropical regions (Smith & Epstein, 1971, Sponheimer *et al.*, 2003; Parnell *et al.*, 2010).

Isotopic ratios are determined and corrected for fractionation during digestion, absorption and metabolism. The similarity in isotopic ratios of samples and sources, indicates the respective importance of food sources in their diet (DeNiro & Epstein, 1978; Phillips, 2001; Phillips & Koch, 2002). Gaining a better grasp on the factors that influence intra-species dietary variation and the range of adaptability of diet for each species can assist in interpreting the lack of compatibility between morphophysiological adaptation and their actual diet. Factors such as anthropogenic practices are increasingly affecting habitats by fragmentation and changes in composition, to which animals have to adapt (Wigley, Bond & Hoffman, 2009; Radloff *et al.*, 2013).

The classification of diet for herbivores based on stable isotope analysis estimates varies to an extent from previous classification proposals outlined by Hofmann (1989) and Hofmann & Stewart (1972), as it is only based on the differences of $\delta^{13}C$ values between C_3 and C_4 plants (Cerling *et al.*, 2003). No assumptions are made regarding forage quality, thus results are easily quantified and historic specimens may be analysed. However, results are limited to broad distinctions between C_3 and C_4 and cannot differentiate between C_3 browsers and C_3 frugivores for example (Cerling *et al.*, 2003).

Impala, *Aepyceros melamupus* (Lichtenstein, 1812) are an elegant, medium-sized antelope with a dominantly reddish-brown, sleek pelage, with lighter flanks, a white belly and characteristic black tufts of hair on their hind metatarsal glands (Smithers, 1983; Estes, 1991; Skinner& Chimimba, 2005; Frost, 2014). Impala have been described as mixed feeders (Hofmann, 1973; van Rooyen, 1992; Gagnon & Chew, 2000; Brashares & Arcese, 2002; Cerling *et al.*, 2003) and they are strong resource competitors as they have a broad range of diet flexibility, utilizing a wide variety of vegetation (Estes, 1991; Cerling *et al.*, 2003). Impala are water dependent (Augustine, 2004; van Bommel, Heitkönig, Epema, Ringrose, Bonyongo & Veenendaal, 2006; Canter, 2008) but will thrive in habitats that are degraded as a result of over-utilization and bush encroachment (Augustine, 2004; Garine-Wichatisky, Fritz, Gordon & Illius, 2004; van Bommel *et al.*, 2006).

Waterbuck, *Kobus ellipsiprymnus* (Ogilby, 1833) are large, robust antelope with a greyish, shaggy coat and conspicuous white ring on the rump (Smithers, 1983, Estes, 1991; Skinner& Chimimba,

2005). Waterbuck are classified as grazers (Hofmann, 1973; Estes, 1991). Waterbuck feed on several species of coarse grass that are not commonly consumed by other grazers (Frost, 2014). Occasionally, waterbuck will consume some browse species but this is more likely to occur in periods of low abundance of preferred grasses (Melton, 1978; Tomlinson, 1980; Estes, 1991). As waterbuck are dependent on water and need to drink on a daily basis, they remain relatively close to a water source (Taylor, Spinage & Lyman, 1969; Hofmann & Stewart, 1972).

Determining and understanding the extent of dietary flexibility of herbivores is important to developing and executing suitable management actions in conservation (Radloff, van der Waal & Bond, 2013). The aim of this study was to ascertain the basic dietary components of impala and waterbuck diets in Majete Wildlife Reserve, Malawi, using stable isotope analysis. The fundamental hypothesis was that impala would have a mixed diet of C_3 browse and C_4 grass, and that the reflection of these components will vary between dry and wet seaons. It was predicted that the waterbuck diet would reflect that of an obligate grazer, and that a higher component of C_3 browse would be observed in the dry season. The results and understanding gained will be considered when presenting conservation recommendations to the reserve management.

5.3 Methods

5.3.1 Study area

Majete Wildlife Reserve (MWR, hereafter) is a 700 km^2 fenced reserve located in the Middle Shire Valley in southern Malawi (S15°54'26.6"; E034°44'24.3"). Rocky outcrops are scattered across the relatively moderate topography of the reserve, with a gradual slope from the northwest to the southeast and the Shire River in the East. The soils are mostly lithosols, ferruginous soils or lithosols with sandy and loamy components (Sherry, 1989). The expected annual rainfall for MWR is between 680-1000mm (Wienand, 2013). Most rainfall occurs between November and April, with light precipitation, locally referred to as the "*chiperoni*", occurring in June to August (Sherry, 1989; Morris, 2006). There are a few perennial springs and two perennial rivers, the Shire River that drains Lake Malawi and the Mkulumadzi River; as well as several seasonal rivers and springs throughout the reserve. These natural water sources are supplemented by the installation of several borehole-fed artificial water points (AWPs). These AWPs are important as they extend the dry season forage areas of herbivores (Redfern, Grant, Biggs & Getz, 2005; Loarie van Aarde & Pimm, 2009; Wienand, 2013), stabilise water availability (Chamaillé-Jammes, Fritz, Murindagomo, 2007), and therefore facilitate species population growth. Vegetation is predominantly deciduous miombo savanna woodland, with *Brachystegia* species being dominant (Sherry, 1989). MWR was gazetted in 1955 but by 2003, had been decimated of most large game due to poaching and poor

management. MWR underwent one of Africa's biggest reintroduction programmes, after a Public-Private Partnership (PPP) agreement was made between the Malawian government and African Parks, Majete (Pty) Ltd. in 2003.

5.3.2 Faecal matter sample collection and lab analysis

To determine seasonal changes in the proportion of graze and browse for impala and waterbuck in MWR, representative vegetation samples and fresh faecal matter (dung) samples from impala and waterbuck were identified, collected and dried, over a period of 14 months. Dung and vegetation samples underwent stable isotopic analysis.

The $\delta^{13}C$ values were determined for the most prevalent grasses (C_4 plants) and browse (C_3 plants) species, according to unpublished vegetation studies conducted in MWR (Dorian Tilbury, pers comms). Less prevalent species will still be reflected in the diet as C_3 and C_4 plants have discrete $\delta^{13}C$ values in subtropical savannas. The $\delta^{15}N$ values for these specimens were also determined for this study, however these results are more complicated to interpret. Three distinct sample specimens were collected for each of the following five grasses: *Urochloa mosambicensis; Hyparrhenia rufa; Cynodon dactylon; Dactylonctenium aegyptium; Heteropogon contortus*; and three browse species: *Acacia tortilis; Dalbergia melanoxyln* and *Diplorhyncus conylocarpon*. The $\delta^{13}C$ values of these vegetation species were determined and used to act as average $\delta^{13}C$ values in a 1-isotope, 2-source mixing model, as they were amongst the most common forage species in MWR.

Twenty nine impala and 21 waterbuck dung samples, assumed to be from unique individuals, were collected in MWR from June 2013 to July 2014, so that the graze and browse proportions could be compared for both the wet and dry seasons. The seasons were defined as follows: Dry 2013 (June 2013 – November 2013), Wet 2013-2014 (December 2013 – May 2014) and Dry 2014 (June 2014-July 2014).

Samples were prepared for analysis by a technician at the Cape Peninsula University of Technology and analysed by a specialist technician at the Stable Isotope Analysis Unit at the University of Cape Town in May-June 2015.

All samples were completely dried at 60°C and prepared for analysis by being mill-ground through a 1mm sieve into a powder. Each weighed sample was placed separately in an automated elemental analyser (Carlo-Erba), where it was combusted and the CO_2 gas produced was transferred to the mass spectrometer (MAT 252 or DELTA XP) via a continuous flow-through inlet system. $^{13}C/^{12}C$ ratios are expressed as the delta (δ) notation in per mil (‰) relative to Vienna PeeDee Belemnite (VPDB) standard (Coplen, 1996; Radloff, 2008; Cowley *et al*., 2010; Radloff *et al*., 2013).

5.3.4 Statistical analysis

Isotopic ratios are expressed using the δ notation, relative to the Vienna-Pee Dee Belemnite standard (VPDB) using the following:

$$\delta^{13}C(^0/_{00}) = (R_{sample}/R_{standard} - 1) \times 1000$$

where R_{sample} and $R_{standard}$ are the $^{13}C{:}^{12}C$ ratios of the sample (dung) and standard (plant), respectively.

The average $\delta^{13}C$ for C_3 and C_4 plants was determined as $-29.09^0/_{00}$ and $-12.69^0/_{00}$ respectively, and used as end points. An estimate of %C_4 grasses in the diet of impala and waterbuck was calculated using the following dual-mixing model:

$$\% \ C_4 \text{ grass in diet} = \frac{(\delta^{13}C_{C3 \ plants} + \Delta\delta^{13}C - \delta^{13}C_{dung})}{(\delta^{13}C_{C3 \ plants} - \delta^{13}C_{C4 \ plants})} \times 100$$

where, $\Delta\delta^{13}C$ was the significance of discrimination between the vegetation (diet) and product (dung), the value of which was accepted to be $-0.9^0/_{00}$ for dung (Sponheimer *et al.*, 2003; Codron *et al.* 2005; Codron & Codron, 2008; Radloff, 2008).

The observed foraging behaviour data of impala and waterbuck were isolated from all the field observations. The incidence of browsing and grazing were expressed as a percentage of all observational entries from June 2013 to July 2014.

5.4 Results

Plant $\delta^{13}C$ values

The original and standard corrected $\delta^{13}C$ and $\delta^{15}N$ values that were determined for the vegetation samples are listed in Table 5.1. There was a difference between the $\delta^{13}C$ values of C_3 and C_4 plants. The standard corrected $\delta^{13}C$ values of the nine samples of the three C_3 browse species had a range of -26.0‰ to -30.9‰, and an average of -28.4‰ ($\pm$ 1.6). The $\delta^{13}C$ values of the 14 samples of the five C_4 grass species had a range from -10.3‰ to -13.4‰, and an average of -11.6‰ ($\pm$ 1.1).

Table 5.2 summarises the $\delta^{13}C$ and $\delta^{15}N$ values that were determined for the impala and waterbuck dung samples (product). The $\delta^{13}C$ values for impala ranged from -13.8‰ to -26.7‰ over the entire sampling period, and that of waterbuck ranged from $-14.2^0/_{00}$ to $-22.0^0/_{00}$ (or to $-28.8^0/_{00}$ if outlier was included).

For both Figure 5.1 and Figure 5.2 the $\delta^{13}C$ values of the C_3 plants are smaller than the C_4 values. $\delta^{15}N$ values of C_3 plants seem to be relatively close to each other while those of the C_4 plants were more widely distributed.

Table 5.1 $\delta^{13}C$ and $\delta^{15}N$ values $(^0/_{00})$ of C_3 browse and C_4 grass specimens used as a reference in the stable isotope analysis of the diet of impala and waterbuck in MWR, Malawi. The stanard corrected values were determined by laboratory technichians.

Species	C_3/C_4	$\delta^{15}N$	$\delta^{15}N$ (Std corrected)	$\delta^{13}C$	$\delta^{13}C$ (Std corrected)	C:N Ratio
C_3 Browse						
Acacia nigrenscens	C_3	3,26	3,68	-30,68	-30,20	11,85
Acacia nigrenscens	C_3	2,51	2,94	-30,00	-29,54	15,98
Acacia nigrenscens	C_3	3,44	3,86	-32,06	-31,54	18,06
Dalbergia melanoxylon	C_3	-0,35	0,10	-29,19	-28,75	12,61
Dalbergia melanoxylon	C_3	-0,64	-0,18	-30,25	-29,78	12,38
Dalbergia melanoxylon	C_3	0,03	0,48	-31,00	-30,51	14,12
Diplorhyncus cony3locarpon	C_3	0,61	1,06	-28,13	-27,72	21,84
Diplorhyncus conylocarpon	C_3	2,37	2,80	-27,10	-26,72	16,76
Diplorhyncus conylocarpon	C_3	1,24	1,68	-27,47	-27,08	19,45
C_4 Grass						
Cynodon dactylon	C_4	3,16	3,58	-14,32	-14,27	18,33
Cynodon dactylon	C_4	2,30	2,73	-13,85	-13,80	16,85
Cynodon dactylon	C_4	2,49	2,92	-14,50	-14,44	24,78
Dactylotenium aegyptium	C_4	1,66	2,10	-13,72	-13,68	24,24
Dactylotenium aegyptium	C_4	7,34	7,72	-12,87	-12,85	17,31
Dactylotenium aegyptium	C_4	5,37	5,77	-13,12	-13,09	26,53
Heteropogon contortus	C_4	-1,26	-0,80	-12,17	-12,17	21,75
Heteropogon contortus	C_4	0,86	1,30	-11,70	-11,72	25,31
Heteropogon contortus	C_4	-0,85	-0,39	-11,56	-11,58	32,75
Hyparrhenia rufa	C_4	-1,97	-1,50	-11,60	-11,62	34,01
Hyparrhenia rufa	C_4	-3,01	-2,53	-11,93	-11,94	40,68
Hyparrhenia rufa	C_4	-2,40	-1,93	-11,45	-11,47	29,09
Urochloa mosambicensis	C_4	0,82	1,27	-12,48	-12,47	19,77
Urochloa mosambicensis	C_4	0,73	1,17	-12,57	-12,56	16,25

C_3 and C_4 contributions to the diet of impala was similar in the Dry 2013 and Wet 2013-2014 seasons (Figure 5.1). There was an increase in the contribution of C_3 plants in the impala diet in the Dry 2014 season. Waterbuck diet consisted mostly of C_4 plants with a few deviants that consumed a fair proportion of C_3 plants (Figure 5.2). The estimated contribution of C_4 grasses to the diet of impala ranged from 19.88% to 98.8% ($\pm$ 10% error) (Codron *et al.*, 2007) and an average of 58.74% for the entire study period. The C_4 contribution estimates ranged from 44.90% to 80.88% (average 64.53%) for the 2013 dry season, from 49.09% to 98.80% (average 73.22%) 2013-2014 wet season, and from 19.88% to 48.11% (average 30.21%) for the 2014 dry season.

Table 5.2 $\delta^{13}C$ and $\delta^{15}N$ values (‰) of dung samples representing the diet of impala and waterbuck in the dry seasons of June to October 2013 and June to July 2014 and the wet season from November 2013 to May 2014, in MWR, Malawi. (The highlighted rows indicate the outlier for impala in the dry 2014 season and for waterbuck in the wet 2013/2014 season.)

| Species	Season	$\delta^{15}N$	$\delta^{15}N$ (Std Corrected)	$\delta^{13}C$	$\delta^{13}C$ (Std corrected)	C:N Ratio
Impala	Dry 2013	2,40	2,81	-19,58	-19,11	18,23
Impala	Dry 2013	1,45	1,88	-23,20	-22,63	25,78
Impala	Dry 2013	2,26	2,68	-19,43	-18,96	20,73
Impala	Dry 2013	2,32	2,73	-20,06	-19,58	19,88
Impala	Dry 2013	2,52	2,93	-19,46	-18,99	23,69
Impala	Dry 2013	2,02	2,43	-19,77	-19,29	24,40
Impala	Dry 2013	1,46	1,89	-17,14	-16,73	20,80
Impala	Dry 2013	2,18	2,59	-19,89	-19,40	17,66
Impala	Dry 2013	3,02	3,42	-17,55	-17,13	17,87
Impala	Dry 2013	2,71	3,12	-19,12	-18,66	21,80
Impala	Dry 2013	2,62	3,03	-21,87	-21,34	18,35
Impala	Dry 2013	2,40	2,81	-21,63	-21,10	18,92
Impala	Wet 2013/2014	1,73	2,15	-17,63	-17,21	18,46
Impala	Wet 2013/2014	1,42	1,85	-17,37	-16,95	24,14
Impala	Wet 2013/2014	1,77	2,20	-14,12	-13,79	24,25
Impala	Wet 2013/2014	1,69	2,12	-18,58	-18,13	19,13
Impala	Wet 2013/2014	2,23	2,64	-16,44	-16,05	21,34
Impala	Wet 2013/2014	2,28	2,69	-18,56	-18,11	27,04
Impala	Wet 2013/2014	2,12	2,53	-19,60	-19,13	21,60
Impala	Wet 2013/2014	1,05	1,49	-22,49	-21,94	26,91
Impala	Wet 2013/2014	1,15	1,59	-21,05	-20,53	26,82
Impala	Dry 2014	1,41	1,84	-24,81	-24,19	23,77
Impala	Dry 2014	1,10	1,54	-26,34	-25,68	21,51
Impala	Dry 2014	1,97	2,39	-22,66	-22,10	20,85
Impala	Dry 2014	0,49	0,94	-27,41	-26,73	27,88
Impala	Dry 2014	0,14	0,60	-15,25	-14,89	46,29
Impala	Dry 2014	1,92	2,34	-25,42	-24,79	19,85
Impala	Dry 2014	1,33	1,77	-26,85	-26,18	22,21
Impala	Dry 2014	0,90	1,34	-26,22	-25,57	27,71
Waterbuck	Dry 2013	0,86	1,30	-18,24	-17,80	28,88
Waterbuck	Dry 2013	2,07	2,48	-17,19	-16,78	26,36
Waterbuck	Dry 2013	2,06	2,48	-15,21	-14,85	25,13
Waterbuck	Dry 2013	3,51	3,90	-14,95	-14,60	23,97
Waterbuck	Dry 2013	2,30	2,71	-15,48	-15,11	24,60
Waterbuck	Wet 2013/2014	0,62	1,06	-21,85	-21,31	22,32
Waterbuck	Wet 2013/2014	1,42	1,85	-22,49	-21,94	19,84
Waterbuck	Wet 2013/2014	0,55	1,00	-15,12	-14,76	31,27
Waterbuck	Wet 2013/2014	0,69	1,14	-16,36	-15,97	25,76
Waterbuck	Wet 2013/2014	3,08	3,48	-29,54	-28,80	17,64
Waterbuck	Wet 2013/2014	1,10	1,53	-14,51	-14,17	34,24
Waterbuck	Dry 2014	2,58	2,99	-17,57	-17,15	25,89
Waterbuck	Dry 2014	3,64	4,03	-20,76	-20,26	22,44
Waterbuck	Dry 2014	1,69	2,11	-21,99	-21,45	34,05
Waterbuck	Dry 2014	0,86	1,30	-17,05	-16,65	29,94
Waterbuck	Dry 2014	1,34	1,78	-15,61	-15,24	31,50
Waterbuck	Dry 2014	1,83	2,25	-19,06	-18,60	22,96
Waterbuck	Dry 2014	1,42	1,85	-16,28	-15,89	28,33
Waterbuck	Dry 2014	0,07	0,53	-17,50	-17,08	38,69
Waterbuck	Dry 2014	-0,18	0,28	-15,61	-15,24	31,61
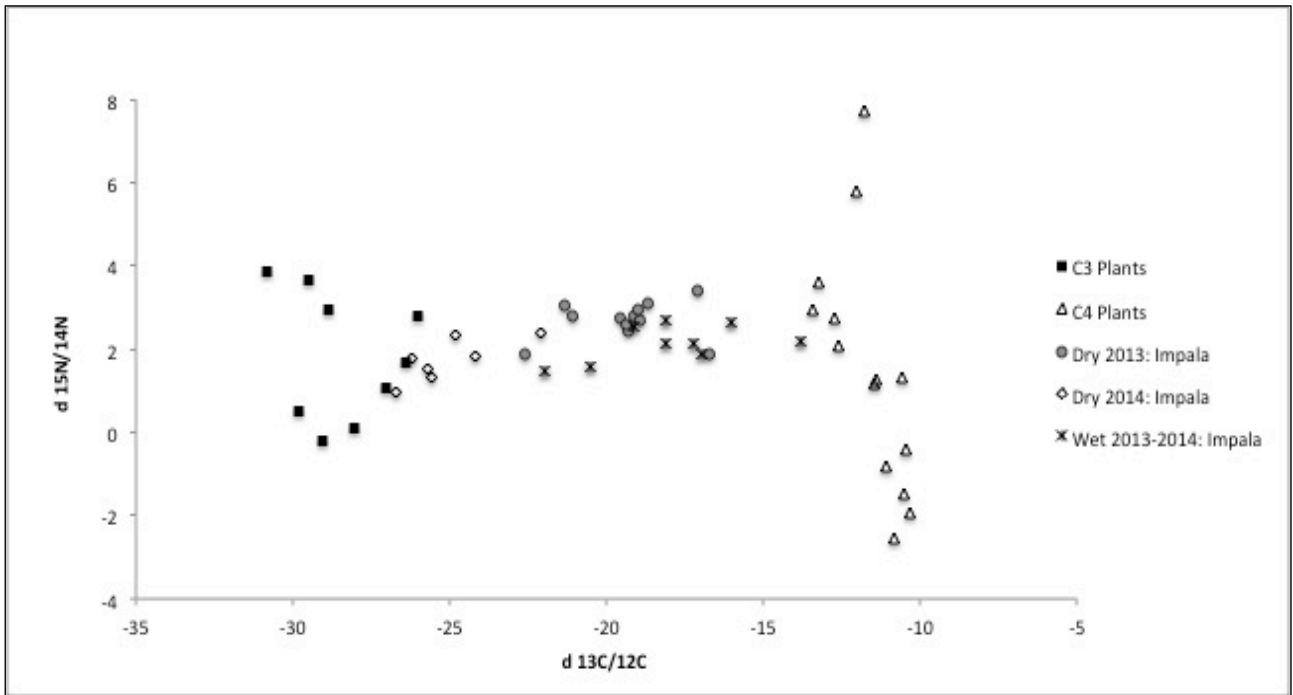
Figure 5.1 The isotopic values of carbon and nitrogen in the diet of impala (*Aepyceros melampus*) in the dry seasons of June to October 2013 and June to July 2014 and the wet season from November 2013 to May 2014 in MWR, Malawi.

For waterbuck, the C_4 contribution estimates ranged from 74.31% to 93.83% (average 86.35%) for the 2013 dry season, from 49.11% to 96.44% (average 75.36%) for the 2013-2014 wet season, and from 52.07% to 89.96% (average 76.13%), for 2014 dry season. Therefore the range of estimates for the entire study period of 13 months was 49.11% to 96.44% with an average of 78.61%.
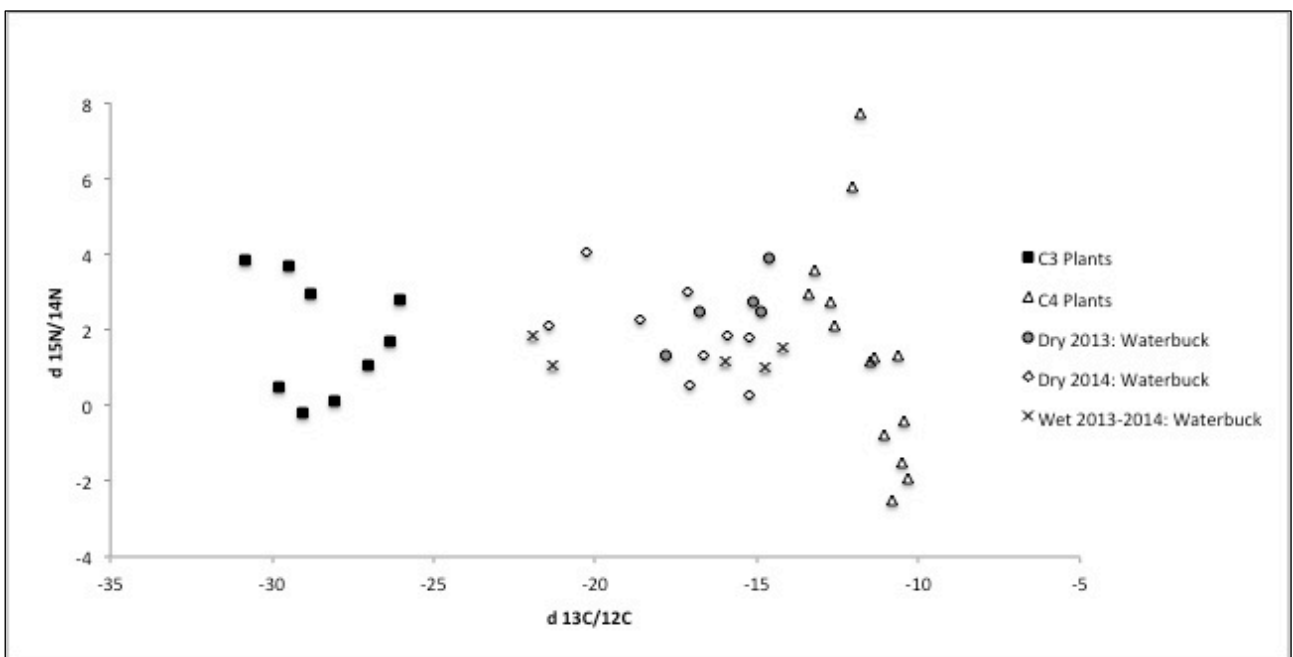
Figure 5.2 The isotopic values of carbon and nitrogen in the diet of waterbuck (*Kobus ellipsiprymnus*) in the dry seasons of June to October 2013 and June to July 2014 and the wet season from November 2013 to May 2014 in MWR, Malawi.
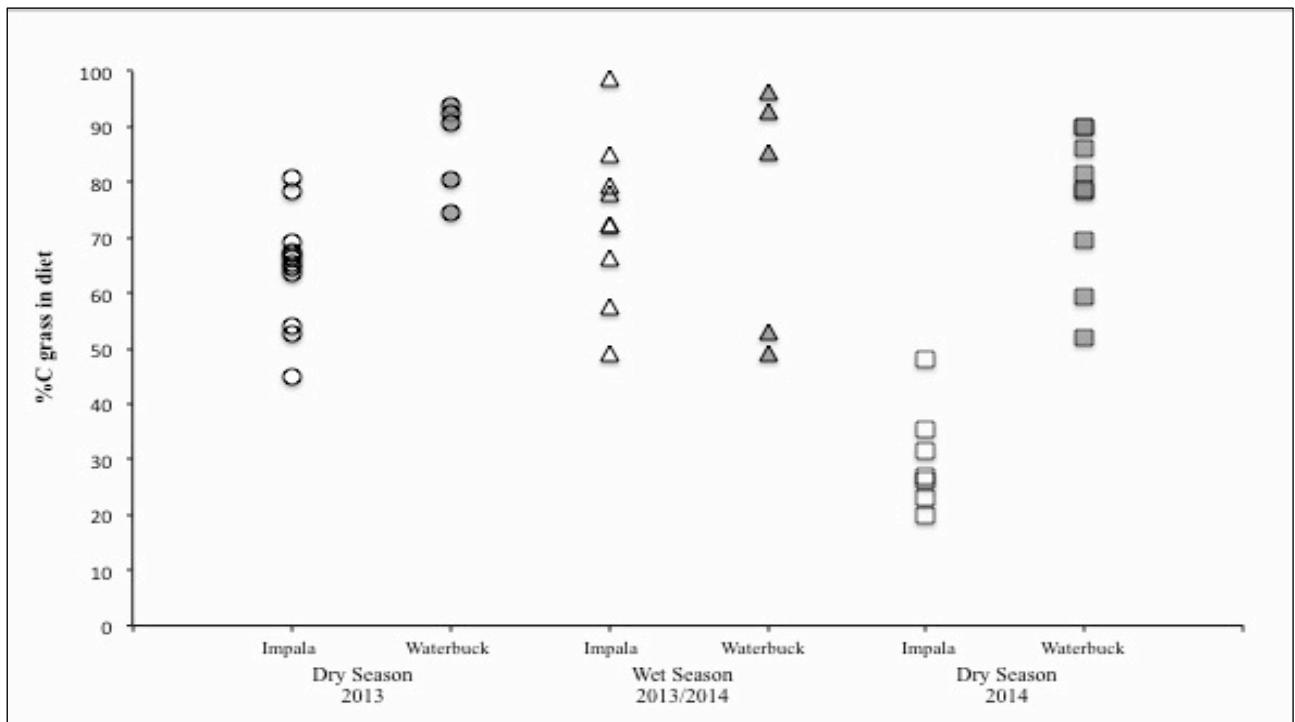
Figure 5.3 The proportion of C_4 grass in the diet of impala and waterbuck from MWR Malawi.

Figure 5.3 represents the percentage of C_4 grass in the diet. These values were derived from the linear mixing model used. The average %C_4 grass content in the impala diet was 64.53% (SD ± 10.1) in the dry season of 2013; 73.22% (SD ±14.7) in the wet season over 2013 to 2014; and 30.21% (SD ±9.4) in the dry season of 2014. Impala diet was similar between the dry season of 2013 and the wet season of 2013-2014, but there was a clear decrease in the amount of C_4 grass consumed in the dry season of 2014.

The average %C_4 grass content in the diet of waterbuck was 86.35% (SD ± 8.5) in the dry season of 2013; 75.36% (SD ±22.6) in the 2013-2014 wet season; and 76.13% (SD ±13.3) in the dry season of 2013. Waterbuck diet has a relatively high %C_4, the average occurring in the dry season of 2013. The average value for the 2013-2014 wet season was similar to that of the 2014 dry season. Overall, waterbuck clearly had a higher percentage of grass in their diet than that of impala. The diets of impala and waterbuck overlapped more in the 2013-2014 wet season as impala had increased %C_4, which was similar to that of waterbuck.

Table 5.3 provides a summary of the mean $\delta^{13}C$ values and average estimated %C_4 for impala and waterbuck between the dry and wet seasons. A significant change in diet was determined by calculating the differences of the average $\delta^{13}C$ values between seasons. If the difference was greater than 10%, then it was considered that there was a significant change in diet.

Table 5.3 Seasonal comparison for faecal $\delta^{13}C$ and estimated $\%C_4$ intake of impala (*Aepyceros melampus*) and waterbuck (*Kobus ellipsiprymnus*) in MWR, Malawi. Significant change in diet between the seasons was calculated by comparing average $\%C_4$ values.

Season	Property		Impala	Waterbuck	Significant diet change (>10% C_4)
Dry 2013 Impala: *n* = 12 Waterbuck: *n* = 5	$\delta^{13}C$ (‰)	Mean	-19.41	-15.83	**Dry 2013 and Wet 2013-2014:** Impala = No Waterbuck = Yes
		SD	1.66	1.39	
	$\%\,C_4$ in diet	Mean	64.53	86.35	
		SD	10.14	8.50	
Wet 2013-2014 Impala: *n* = 9 Waterbuck: *n* = 5	$\delta^{13}C$ (‰)	Mean	-17.98	-17.63	**Dry 2013 and Dry 2014:** Impala = Yes Waterbuck = Yes
		SD	2.41	3.71	
	$\%\,C_4$ in diet	Mean	73.22	75.36	
		SD	14.70	22.62	**Wet 2013-2014 and Dry 2014:**
Dry 2014 Impala: *n* = 7 Waterbuck: *n* = 9	$\delta^{13}C$ (‰)	Mean	-25.04	-17.51	Impala = Yes Waterbuck = No
		SD	1.66	2.19	
	$\%\,C_4$ in diet	Mean	30.21	76.13	
		SD	9.41	13.33	

For impala, there was not a large change in diet composition between the dry season 2013 and wet season 2013-2014 as the mean $\%C_4$ values were 64.5% and 73.2% respectively, therefore there was less than a 10% difference. The difference between the dry seasons of 2013 and 2014 (34.3%), and between the wet season of 2013-2014 and 2014's dry season (43.0%). There was a change in diet for waterbuck between the dry season of 2013 and the wet season 2013-2014 (11.0%) and between the dry seasons of 2013 and 2014 (10.2%), but only marginally so as $\%C_4$ differences were between 10% and 11%. There was little change in diet from the wet season 2013-2014 to dry season 2014 for waterbuck (0.8%).

5.5 Discussion

For this study, we only corrected the plant $\delta^{13}C$ values to dung equivalents as $\delta^{15}N$ values were of little use. This is because the current understanding of nitrogen isotopes is poor and the variation of nitrogen isotope values in plants and animals is more complex than that of carbon isotopes due to environmental factors affecting fractionation. Researchers have been cautioned to be careful when interpreting $\delta^{15}N$ values (Sandberg *et al*., 2012).

According to the isotopic classification of diets outlined by Cerling *et al* (2003), impala are considered to be mixed feeders, which supported previous proposals (van Rooyen, 1992; Brashares & Arcese, 2002; Gagnon & Chew, 2000). In this study, impala had no significant variability in diet

between the dry season of 2013 and the wet season of 2013-2014. Although the average %C_4 estimated increased from 64.53% in the dry season to 73.22% in the wet season, following the prediction that C_4 content of diet would increase in the wet season when fresh grass growth is nutritious and green (Estes, 1991). This minor shift in diet may be due to the grouping of months (ie: wet versus dry season) as well as an early start to the wet season. If there were no early rains, it would be expected that there would be a strong C_3 signature, indicating the greater consumption of browse as grasses would not be readily available or as nutritionally beneficial in drier conditions. As expected, impala had a higher browse content as the estimated %C_4 average decreased to 30.21% in the winter of 2014. There is a notable difference in estimated %C_4 between the dry seasons of 2013 and 2014. This may be due to samples only being collected in the early dry season of 2014, eliminating the likelihood of early rains increasing the availability of desirable C_4 grasses. However, there are no estimates in the dry season of 2013 that are as low as those in 2014 and this may be an indication of the early start to the wet season of 2013-2014 and thus faster drying out toward the dry season of 2014.

From this study we can confirm that impala are mixed feeders that have a seasonal shift in diet from predominantly grazing in the wet summer to browsing in the dry winter months when the nutrient content and availability of grasses decreases (Azavedo & Agnew, 1968; Rodgers, 1976; Ambrose & De Niro, 1986, Estes, 1991).

Previous studies have proposed that waterbuck are water dependent grazers that are able to consume foliage and fruits when grasses with adequate nutrition are scarce (Hofmann & Stewart, 1972; Kingdon, 1997; Gagnon & Chew, 2000; Cerling et al., 2003). Results of this study indicate that waterbuck are primarily grazers with an estimated average of 78.61% C_4 grass in their diet; thus the proportion of graze did not indicate a hyper-grazer according to classifications outlined by Cerling et al. (2003). There are two waterbuck samples that indicated a higher proportion of C_3 in the wet summer of 2013-2014. This was unexpected, especially as there was a significant gap in relation to the other samples of this sampling period that have estimated %C_4 values that are >80%. This could be due to the consumption of summer fruits or less common C_3 sedges of the Cyperaceae genera (Stock, W.D., Chuba, D.K, & Verboom, G.A. 2004; Sponheimer, Lee-Thorp, de Ruiter, Codron, Codron, Baugh & Thackeray, 2005) by these two individuals, which would indicate a higher C_3 content. An increased intake of C_3 browse in the dry season of 2014 was observed, which could indicate the lack of adequate grazing for waterbuck in the winter months. This may also be due to the overall lower availability of grasses in MWR, which is predominantly a mixed, miombo woodland. According to the average estimated %C_4 values for waterbuck, there is a marginally

significant change in diet between the 2013 dry season and 2013-2014 wet season and no significant difference in waterbuck diet between the 2013-2014 wet season and the 2014 dry season. This may be due to the two unexpected aforementioned results for the 2013-2014 wet season which indicate higher C_3 intake. If these two values were excluded there would be a clear dietary shift between high graze content in the wet summer season and a mixed C_3-C_4 content in the 2013 and 2014 dry seasons as waterbuck incorporate more browse to supplement their diet. There is a marginal difference between the dry seasons of 2013 and 2014, though the range of estimated %C_4 content in waterbuck diet is greater in the dry season, indicating that there was a greater intake of C_3 plants.

There was a degree in dietary overlap between impala and waterbuck, particularly in the 2013-2014 wet season where the average estimated %C_4 content was very similar. However, the specific C_4 grass species consumed could differ between the two ungulates. Dietary overlaps usually occur in the dry season when food is scarce (Ego, Mbuvi, & Kibet, 2003; Omphile, Aganga, Tshireletso & Nkele, 2004; Gutbrodt, 2006), but this was not the case in this study as the two ungulate species have different feeding strategies. Waterbuck, as anticipated, had a higher graze content than impala, as impala are selective feeders that shift more readily to browse species in the dry winter months when grass quality is poor. East (1984) proposed that waterbuck adopt an intermediate feeding strategy where they selectively feed on a few forage items and shift between bulk feeding and selective feeding strategies, depending on the season and region. In the dry season waterbuck shift to consuming grasses that are relatively nutrient rich but are not favoured in the wet season, as then there are other grasses that have a greater nutritional value but these rapidly decrease in abundance and nutritive value in the early dry season (Mowat, Fulkerson, Tossell & Winch, 1965; Prins & Beekman, 1989; Ben-Shahar & Coe, 1992; Gutbrodt, 2006). The higher proportion of browse may be expected in the dry months, as the remaining grasses may lack adequate nutritional value.

It has been proposed that grazers are not able to cope with the higher tannin and other allelochemical levels found in dicotyledenous plants (Owen-Smith, 1997; Sorensen & Dearing, 2003). However, there are studies that propose the contrary to this, stating that due to their dental and forestomach characteristics, grazers should be able to digest browse species more readily than browsers digest grasses (Clauss *et al*., 2003; 2010; Radloff, 2013). As waterbuck are able to browse in stressed environmental conditions, they compete with other herbivores for forage resources and water, therefore putting more pressure on the ecosystem. This is of great concern as the heavily water dependent waterbuck population in MWR is increasing and their home ranges are restricted to areas of the reserve where sufficient water is available, especially in the dry season.

Impala utilize a wide range of forage species that they exploit at different times of the year and they can thus better sustain themselves through stressful environmental conditions when compared to other species. The availability of water restricts the range of impala in the dry season (Fairall & Klein, 1984); therefore they may impact the vegetation relatively close to water along with other water dependent species.

Management planning should have an understanding of herbivore requirements and their responses to environmental changes, thus being able to make more sound management decisions and maintain habitat heterogeneity (Bothma, 1995; van Rooyen *et al.*, 2000; Canter, 2008). The impala and waterbuck populations of MWR have successfully established themselves post reintroduction, however it is vital to manage these populations, especially as they are part of a closed system and there is no option to expand the size of the reserve.

Several challenges were faced over the course of this study. For future studies on the diets of animals in the MWR it would be beneficial to make better use of vegetation maps. The different vegetation/habitat types are currently being updated for MWR (Craig Hay, pers.com.). Dung samples should be collected from respective herbivores from each vegetation type. When researchers are collecting dung samples, a description of vegetation type should be given along with dung identification, date and GPS co-ordinates as Codron & Codron (2008) did for their study. To ensure a better distribution of where samples are collected, it may be beneficial to divide the reserve in plots and systematically ensure that relevant samples are being collected from each vegetation type, as well as when no dung is found for certain herbivores, thus indicating the relative presence or absence of a species in an area. This will ensure that an adequate number of samples for each herbivore and for each sampling period will not be biased by collecting a majority of the required samples from a certain vegetation type and neglecting other vegetation types. For example, it may work out that most samples are collected from the areas along the perennial rivers in MWR where there are higher numbers of most herbivores and thus easier to locate dung samples, rather than in areas further away from the rivers and lower densities of herbivores and different vegetation compositions. It would be wise to consider the quality and relative abundance of key vegetation species versus the actual intake by ungulates. It could be recommended that in addition to stable isotope analysis, dietary compositions be determined by studying ungulate faecal matter using the microhistological technique (Stewart, 1967). In this method, vegetative remains may be identified to group or species level with the use of reference slides of local vegetation samples. Using this method will give researchers a better understanding of which vegetation species are being consumed and not just a broad indication of browse versus graze species intake. Further details of

this method may be referred to in Stewart (1967), de Jong *et al.* (1995), Suter *et al.* (2004) and Gutbrodt (2006).

5.6 Conclusions

The increase of habitat loss, fragmentation, isolation and fencing of protected areas has lead to the necessity of intensive management to preserve biodiversity and prevent the overuse and degradation of habitats. Impala and waterbuck populations in MWR need to be closely monitored to prevent overpopulation and significant detrimental effects on other herbivores and the ecosystem as a whole.

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Chapter Six:

Integrated discussion of results and conclusions with recommendations for wildlife management and suggestions for future research

6.1 Overview

The increasing herbivore population post-reintroduction to Majete Wildlife Reserve (MWR) will impact the ecosystem processes as demands for basic needs such as water and adequate forage increases. Impala and waterbuck were chosen as the study species as it was surmised by MWR management that their increasing numbers were (high) and would potentially have a significant impact on the vegetation as well as compete vigorously with other herbivore species. This study provided insight into the distribution and population estimate for impala and waterbuck in the original sanctuary area, as well as determining the approximate browse and grass contributions to their diet throughout the year. The findings of this study will contribute to the understanding of the baseline ecology of impala and waterbuck in MWR and aid the development of management strategies for herbivore populations and their impacts on ecosystem processes. This report summarizes the fundamental study findings and discusses management and future research recommendations.

6.2 Study findings

6.2.1 Chapter Three: Distribution and population estimates of impala and waterbuck

- Impala and waterbuck densities were greatest in areas that were in close proximity to the Shire and Mkulumadzi Rivers; dominated by riverine vegetation. Their densities were moderate in areas that were relatively close to perennial water sources, including four artificial water points (Heritage, Nakamba, Nsepete and Thawale waterholes); main vegetation types included a combination of low altitude mixed woodland and ridge-top mixed woodland. Densities of both species were lowest towards the western section of the original sanctuary area where perennial water availability was limited and the vegetation was a combination of low- to medium altitude mixed woodland and ridge-top mixed woodland.

- Impala abundance estimates for the original sanctuary area ranged from 3510 for data collected by a single observer to 5752 individuals for data collected by multiple observers. Waterbuck abundance estimates ranged from 1062 for data collected by a single observer to 2167 individuals for data collected by multiple observers. Both species are water dependent and tend to have a preference for habitat that is associated with natural water, especially waterbuck.

- Impala were more abundant than waterbuck and are observed more frequently, especially in the mid to late winter months when foliage is relatively sparse and animals tend to congregate around resources in times of environmental stress. The most common demographic observed for impala and waterbuck populations were adult females. The proportion of adult female impala of all impala sightings was greater than that of adult female waterbuck. For both species, adult females were the most prolific and clearly showed a preference for the riverine areas including the river loops, Mkulumadzi Road and Mendulo Road. Juveniles and lambs or calves were often associated with females in these areas. Adult and sub-adult males occurred in similar proportions in riverine areas and those associated with low altitude and ridgetop mixed woodlands, within moderate proximity to perennial water. Very few observations of all demographics were made in higher areas with a combination of low- to medium altitude mixed woodland and ridge-top mixed woodland that had limited perennial water sources.

- The use of waterholes by impala and waterbuck fluctuated; overall waterholes were used more frequently in the late dry season when seasonal water sources had dried up. Greater numbers of impala utilized Thawale and Nsepete waterholes than the Heritage and Nakamba waterholes. The Heritage and Nsepete waterholes were more frequently used by waterbuck than Nakamba waterhole, and the species were not observed using the Thawale waterhole during waterhole counts and were rarely captured by the camera trap stationed for the long-term monitoring of the waterhole.

6.2.2 Chapter Four: Basic behaviour of impala and waterbuck

- A greater proportion of impala adults were observed, relative to sub-adults, juveniles and lambs. This is due to impala's rate of maturity and average lifespan of 12 years. However, juvenile mortality needs to be investigated. Adult females were the most prevalent demographic that largely occupied habitats within close proximity to the Shire and

Mkulumadzi Rivers. Using drive count and waterhole count data, it was determined that habitats that were further away from the perennial rivers, but still had an adequate supply of water and forage, were primarily occupied by adult males. Thus there was a degree of sexual segregation.

- Contrary to other studies, impala herds did not congregate in vast numbers during the rains and dominant males were associated with breeding herds year round. The peak lambing season in MWR occurs in early to mid-November, a few weeks earlier than observed studies elsewhere.

- A large proportion of adult waterbuck were observed, relative to sub-adults, juveniles and calves, however proportions of sub-adults and juveniles were greater than those for impala. This was because waterbuck have a relatively slow rate of maturity, especially when compared to impala, therefore individuals remain in the juvenile and sub-adult age classes for a longer period. The riverine areas associated with the Shire and Mkulumadzi rivers are predominantly occupied by the largest demographic of adult females. Adult males were the most prevalent in adequate but less preferred habitat comprising of low altitude and ridgetop mixed woodland with relatively good access to water.

- Waterbuck formed herds that lacked cohesiveness and were not commonly observed in large numbers as determined in previous studies. In MWR waterbuck have a peak calving season from May to June which differed from other studies that suggested that waterbuck calving peaks occur in March and October although no discrete period has been strictly defined as waterbuck are polyoestrous.

- Impala utilized waterholes from early morning to mid-afternoon with a peak in drinking activity from noon to mid-afternoon, after which drinking activity declined rapidly. Waterbuck utilized waterholes from early morning with peaks of activity at midday and mid-afternoon with declines in activity in the early and late afternoon.

6.2.3 Chapter Five: Stable isotope analysis of impala and waterbuck diet

- Stable isotope analysis of vegetation samples from MWR provided distinct isotopic values of carbon ($\delta^{13}C$) for three browse (C_3) species and five graze (C_4) species. These values were used as a reference point for the stable isotope analysis of impala and waterbuck dung.

- Impala are mixed feeders that are capable of altering their diets between seasons. It was determined that impala diet had the highest C_4 content in the wet season of 2013 -2014 and a relatively large C_4 content in the dry season of 2013. For the dry season of 2014, the C_4 content dropped dramatically. Therefore impala consume a higher proportion of C_4 grass relative to C_3 browse in the wet season, and shift their diets in the dry season to incorporate more browse species.

- Waterbuck are primarily grazers that consistently have a relatively high proportion of C_4 grasses in their diet. However, waterbuck were observed supplementing their diet with C_3 browse species in the dry season

- Impala and waterbuck diets overlap in the wet season when grasses are abundant and more nutritious.

6.3 Conclusions and management recommendations

Conservation planning and protected area management benefit from cataloguing mammals in an area, such as determining the biodiversity and abundance of species, improving species distribution maps, comparing species habitat use between sites and determining the impact of anthropogenic activities on wildlife (Tobler, Carrillo-Percastegui, Leite Pitman, Mares, & Powell, 2008).

The impala and waterbuck populations have vastly increased since their reintroduction (as determined for the original sanctuary area in Chapter Three). The abundance estimates for the sanctuary using drive count data are much larger than the estimates of 1200 and 700 for impala and waterbuck respectively, generated for the entire reserve by the aerial count conducted by Macpherson in 2012. However, aerial counts have been reported to produce underestimates for animal abundance (Caughley, 1974; Caro, 1999; Jachmann, 2002; Gaidet Fritz, Messad, Mutake, & Le Bel, 2005; Cromsigt, van Rensburg, Etienne, & Olff, 2009) although aerial observations are one of the more effective animal population monitoring tools (Cromsigt *et al.*, 2009). Preliminary results from the aerial count conducted for MWR in September 2015 (research team, pers.com.) support the estimates determined using the distance sampling method (Buckland, Anderson, Burnham, Laake, Borchers, & Thomas, 2001; Thomas, Buckland, Rexstat, Laake, Strindberg, Hedley, Bishop, Marques & Burnham, 2010) on drive counts. Considering the growth in impala and waterbuck populations as indicated by generated estimates, it is important that reserve management prepare for the implementation of population control strategies in the future.

The habitat preference of impala and waterbuck were determined for the sanctuary area, and results presented, as seen in Chapters Three and Four. The preferred habitats of waterbuck and impala are primarily located along the Shire and Mkulumadzi Rivers. These habitats are limited and under great pressure from several "resident" ungulate species including nyala (*Tragelaphus angasi*) and warthog (*Ornithodoros moubata*). Vegetation composition and structures are altered (Morris, 1990) by the local impact of ungulates, thus the over-utilization of habitats can lead the loss of biodiversity (Vernier & Fahrig, 1996) and compromise the habitat suitability for other species (Bothma, 1995; van Rooyen *et al.*, 2000; Gordon Hester & Festa-Bianchet, 2004). The vegetation composition in the riverine areas has been a concern for MWR management. In 2012 a rapid alien vegetation assessment (RAVA) was conducted along the perennial riverbanks for MWR and removal strategies of these invasive alien species were implemented (reserve manager, pers.com.). RAVA's must be carried out periodically to assess the status of habitat due to the removal or encroachment of alien vegetation.

The secondary habitat preference for both species was the low-altitude mixed woodland areas that were relatively close to the aforementioned perennial rivers and are supplemented by four artificial water points. With the growing populations of most wildlife species in MWR, the competition for resources and the effects of density dependent habitat selection (Fretwell & Lucas, 1970; Kaunda Mapolelo, Matlhahku & Mokgosi, 2002) increases. As MWR is an isolated fenced reserve encompassed by high-density rural settlements, it is important that intensive management practices are executed to curtail the potential consequences of disrupting ecological processes such as wildlife dispersal, local wildlife movements and gene flow between populations (Caughley, 1994; Boone & Hobbs, 2004; Hayward, O'Brien, & Kerley, 2007; Hayward & Kerley, 2008; Cumming, 2010; Ferguson & Hanks, 2010).

The gender and age structures were determined for impala and waterbuck in the sanctuary area (Chapter Three and Chapter Four). Adult females where the most prolific demographic of impala and waterbuck populations and were the most prevalent in areas associated with the Shire and Mkulumadzi Rivers, thus their preferred habitat. It is a concern that the greater proportions of both populations are females that are capable of reproducing, as this would facilitate continued population growth at such impressive rates.

Considering that the fundamental survival strategies are to obtain nutrition and to avoid predators (Cowlishaw, 1997) factors that have contributed to population increases in MWR thus far are an adequate supply of forage and perennial water and the absence of predators, except for a few

remnant hyena (*Crocuta crocuta*) until 2011, when the first pair of leopard (*Panthera pardus*) was reintroduced. Since then, two more pairs of male and female leopards and three lion (*Panthera leo*) have established themselves in the reserve and have reproduced successfully (as of September 2015, lions = 5 and leopards = 10). At the time of this study, there were too few predators to have a significant impact on the herbivore assemblage in MWR. Future studies should be conducted on the tradeoffs made by foragers between energy gain and the risk of predation, once the predator numbers in MWR have increased. Interestingly, impala did not regularly display the elegant leaping behaviour that has been described to be typical anti-predatory behaviour of impala (Estes, 1991). Even though impala would move away from the observer, they tend to walk away slowly, trot or run away a short distance, with the odd alarm call given. Potential reasons for this are that impala may not feel as threatened by the observer's presence as they would be of a lion, or it may be due to the scarcity of predators in the past that impala have seemingly 'lost' this instinct. Impala may not leap so enthusiastically as a strategy to conserve their energy. With the increasing number of predators in MWR, this behaviour could be adopted more frequently. The low incidence of grooming behaviour, in which impala scratch and orally attempt to move ticks from their bodies, may be an indication that the prevalence of ticks in MWR is currently unsubstantial. This could be another advantage to the fitness of impala and waterbuck populations, as they are less prone to mortality due to tick-borne diseases (Mooring, 1995; Mooring *et al*., 1995)

The use of artificial water points to increase surface water availability (Chamaillé-Jammes, Fritz, & Murindagomo, 2007; Wienand, 2013) is widely disputed with growing concern with regard to the impact of more surface water availability on ecosystem processes, plasticity, stability, biodiversity, productivity and resistance to change. When planning the placement of waterholes it is important to consider habitat requirements and species dependence on water (Smit *et al*., 2007).

The stable isotopic analysis of impala and waterbuck diet (Chapter Five) confirmed that impala are mixed feeders that can shift their diets from predominantly grasses in the wet season to increase their intake of browse species in the dry season. Waterbuck are grazers that will include browse species when the nutritional value of grasses decline and are no longer sufficient. The positive and negative impact of ungulates on plant species richness and biodiversity relies upon factors such as grazing intensity (Hobbs & Huenneke, 1992; Mysterud, 2006) and nutrient availability (Proulx & Mazumder, 1998). It is important to consider the forage requirements of species to predict and manage their impact on vegetation communities. As various types of mixed woodlands dominate MWR, there may not be a sufficient amount of high quality grasslands, in close proximity to water that will support larger waterbuck populations in the future.

It is vital for the long-term conservation of biodiversity that the wildlife populations for the Namitsempha, Pende and Phwadzi areas are determined and managed. This may be done using various methods. One of the more appropriate methods for such areas that do not have extensive road infrastructure, may be the sampling of dung on walking transects or patrols as it has been reported that this method has yielded higher herbivore species richness and diversity estimates than observational counts conducted on foot (Cromsigt *et al*., 2009). Aerial censusing has been conducted over MWR and is probably the best method of animal censusing for this area, as demonstrated by the baseline data from an aerial count conducted from a helicopter in September 2015.

As MWR currently has a "no hunting" and "no culling" policy, wildlife populations require alternative management practices to maintain population numbers at sustainable levels. Recently African Parks signed another Public Private Partnership agreement with the Malawian government to take on the management of Nhotahkota Wildlife Reserve and Liwonde National Park. There is great potential for the relocation of some wildlife populations from MWR to Nkhotakota Wildlife Reserve. Programmes that restock protected areas with wild-to-wild translocated animals have been more successful than those that used captive-bred-to-wild translocations (Griffith, Scott, Carpenter & Reed, 1989; Wolf, Griffith, Reed & Temple, 1996; Seddon, Armstrong & Maloney, 2007).

Some of the principle factors that have led to the success of protected areas and projects are the improved and increased number of courses at tertiary institutions in conservation management and ecology, the significant growth in ecological tourism in protected and natural areas, greater benefits to and inclusion of adjacent local communities in the management and protection of reserves and better resources (Newmark, 2008). Considering this, African Parks Majete has made a valuable contribution to conservation; by facilitating research and supporting their local staff in various academic endeavors; successfully drawing local and foreign tourists to a 'Big Five' reserve; and for making a considerable contribution toward the social upliftment and environmental education of surrounding rural communities.

6.4 Future research recommendations

It is important that long-term monitoring of all wildlife in MWR is carried out and that results are used to aid management in the continual development and amelioration of conservation strategies and planning. A few recommendations should be considered when planning prospective research projects in MWR.

Behavioural observations should employ the method of focal sampling (Altman, 1974) where an individual animal is observed for a predetermined length of time from an unobtrusive distance. Efforts are dedicated to recording behavioural observations of an individual and that repetitions are conducted to ensure that several representatives from each age class and gender are included. Quantitative data is generated using the total amount of time in focal observation and the time spent in each activity, to determine the proportion of time the animal spent in each activity (Altmann, 1974; du Toit & Cumming, 1990; Reid, 2005). In addition a focal animal may be used to represent the group foraging behaviour (Altmann, 1974; du Toit & Cumming, 1990) and Point Centre Quadrats (PCQs) (Mitchell, 2001; Canter, 2008) may be used to confirm forage selection and determine relative vegetation density (Glover & Mitchell, 2001; Mitchell, 2001; Sparks Masters & Payton, 2002).

In addition to stable isotope analysis a detailed dietary study should be conducted to determine ungulate diet compositions by studying their faecal matter using the microhistological technique (Stewart, 1967). During the digestive process some fragments of the epidermis and cuticula of plants ingested by herbivores remain intact and may be identified to species or plant group level in the resultant dung, depending on the shape and distribution of epidermal cell forms (Stewart, 1967; Gutbrodt, 2006). Fresh plant material may be used to prepare reference slides of the entire leaf for all the principle grass (vegetation) species in the study area. Further details of this method may be referred to in Stewart (1967), de Jong Gill, Van Wieren & Burlton (1995), Suter, Suter, Krüsi & Schütz (2004) and Gutbrodt (2006). Using this method will give researchers and managers a better understanding of which vegetation species are being consumed and not just a broad indication of browse versus graze species intake.

6.5 Acknowledgements

6.6 References

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Appendix One

Supporting information for Chapter Three and Four

Table A.1 Summary of drive count transects in Majete Wildlife Reserve, Malawi.

Transect #	Transect name	Transect length (km)	Vegetation types covered by transect
1	Airfield	1.4	Low-altitude mixed woodland/cleared sward
2	B-Line Road	11.9	Low-altitude mixed woodland/Medium-altitude woodland/Riverine
3	Chimwala Road	6.0	Low-altitude mixed woodland
4	Chipembere	3.7	Riverine/Riparian thicket/Low-altitude woodland
5	Hall-Martin Road	1.5	Riverine
6	Kapichira Cul de Sac	0.6	Riverine
7	Main Road	1.2	Low-altitude mixed woodland
8	Masakala Road	2.8	Ridgetop-mixed woodland/Low-altitude mixed woodland
9	Mendulo Road	7.0	Low-altitude mixed woodland/Riverine
10	Milambe Road	4.2	Low-altitude mixed woodland/Riverine
11	Mkulumadzi Road	9.5	Riverine
12	Mphalapala Road	7.5	Low-altitude mixed woodland/Medium-altitude woodland/Riverine
13	Mvuu Loop	1.3	Riverine
14	Mwembezi Road	2.1	Riverine/Low-altitude mixed woodland
15	Namitsempha Road	11.1	Ridgetop-mixed woodland/Low-altitude mixed woodland/Medium-altitude woodland/Riverine
16	Njati Road	11.6	Low-altitude mixed woodland/Ridgetop-mixed woodland/Riverine
17	Nyala Loop	3.8	Riverine
18	Sefu Road	9.4	Ridgetop-mixed woodland/Low-altitude mixed woodland/Medium-altitude woodland
19	Shire Drive	1.5	Riverine
20	Thambo Road	3.4	Low-altitude mixed woodland/Riparian

Table A.2 A summary of distance sampling results for impala, using transects that were grouped into sets A, B and C, according to their relative distance from a perennial water source and major vegetation type. Data was analysed collectively and separately according to the number of observers responsible.

Impala	AIC	Average Cluster Size	Estimated Cluster Size	Estimated Density	Estimated Abundance
Set A Transects					
All Data	2216.23	7.25	10.78	78.126	2884
Multiple 2013	1072.82	6.06	17.36	105.26	3885
Multiple 2014	124.91	9.44	8.06	76.06	2808
Single Observer	1016.35	8.15	7.60	61.94	2286
Set B Transects					
All Data	1719.44	4.99	5.26	26.25	1251
Multiple 2013	785.30	4.66	7.63	35.55	1694
Multiple 2014	118.31	6.77	5.95	40.25	1918
Single Observer	791.00	5.02	4.85	24.35	1161
Set C Transects					
All Data	93.77	3.40	1.32	4.52	251
Multiple 2013	45.63	3.80	0.82	3.11	172
Multiple 2014	21.62	5.17	1.29	6.67	370
Single Observer	28.35	1.33	0.86	1.14	63

Table A.3 A summary of distance sampling results for waterbuck, using transects that were grouped into sets A, B and C, according to their relative distance from a perennial water source and major vegetation type. Data was analysed collectively and separately according to the number of observers responsible. Note that there were no waterbuck sightings for Set C transects in the early dry season monitoring period of 2014.

Waterbuck	AIC	Average Cluster Size	Estimated Cluster Size	Estimated Density	Estimated Abundance
Set A Transects					
All Data	1968.73	3.27	6.75	22.07	815
Multiple 2013	578.62	3.63	8.44	30.54	1127
Multiple 2014	209.31	3.48	10.71	37.31	1377
Single Observer	1115.93	3.03	7.13	21.26	785
Set B Transects					
All Data	930.48	2.79	2.38	6.64	316
Multiple 2013	267.46	2.88	2.50	7.19	343
Multiple 2014	133.30	3.39	4.89	16.58	790
Single Observer	531.79	2.57	1.81	4.63	221
Set C Transects					
All Data	80.94	2.09	0.49	1.02	57
Multiple 2013	39.66	2.50	0.43	1.06	59
Multiple 2014	0	0	0	0	0
Single Observer	80.94	2.09	0.49	1.02	57

Table A.4 Summary of total estimates for impala and waterbuck from all data sets.

Data Set	Total Impala Estimates	Total Waterbuck Estimates
All Data	4385	1188
Multiple 2013	5752	1529
Multiple 2014	5096	2167
Single Observer	3510	1062