

**SEASONAL FORAGING BEHAVIOUR OF THE PLAINS ZEBRA AND THE
AFRICAN ELEPHANT IN RELATION TO THE OCCURRENCE OF ANTHRAX
IN ETOSHA NATIONAL PARK, NAMIBIA**

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ABSTRACT

It is generally assumed that herbivores acquire gastrointestinal anthrax by ingesting spores, hence information on foraging behaviour of ungulates can be a fundamental component in understanding epidemiology of anthrax. This study investigated seasonal foraging behaviour in relation to the timing of anthrax mortalities in zebras (wet season, anthrax peak) and elephants (dry season, anthrax peak) in Etosha National Park. Zebra diet composition and foraging efficiency was determined using video playback of focal animals and elephant diet quality and soil ingestion was studied using faecal analysis. The results show that zebras forage more intensively in the wet season, taking on average 3.5 bites per step in wet season versus 1.8 bites in the dry season. Nineteen grass species and six shrub species were consumed by zebras in the study areas (Okaukuejo and Halali) plains and zebras tended to eat short grass species in the wet season and tall grass species in the dry season. Elephant faecal crude protein (CP), phosphorus (P) and crude fiber (CF) was significantly higher in the wet season (CP 5.2%, P120mg/100g and CF 34%) than the dry season (CP 4.3%, P 80.1mg/100g and CF 49.5%) and the amount of soil in elephant faeces was significantly higher in the wet season (4.9%) than the dry season (0.8%). It was concluded that foraging behaviour can be a powerful tool for understanding host interactions and contact with *B. anthracis* spores in the environment; however an understanding of anthrax transmission built around foraging behaviour alone will remain speculative until we consider other aspects of the host-*B. anthracis* interaction.

Key words: African elephant, *Bacillus anthracis*, diet composition, diet quality, foraging efficiency, Etosha National Park, plains zebra.

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DEDICATION

This thesis is dedicated to my parents deep down in the rural areas. Even though they hardly understood what I was doing they never waived their moral support towards my research.

DECLARATION

I Zepee Havarua, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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CHAPTER 1

INTRODUCTION

1.1 General introduction

Anthrax is a highly virulent disease caused by the bacterium, *Bacillus anthracis* and is an important zoonotic disease in livestock and wildlife nearly worldwide (Hugh-Jones and de Vos, 2002). Although immunization has led to a global decline in livestock anthrax cases in many parts of the world, anthrax still remains enzootic in many national parks and even in some game ranching areas (Hugh-Jones and de Vos, 2002). Anthrax causes mass mortality in herbivores in Etosha National Park (ENP) (Appendix 2), but there are differences in the incidence of anthrax related deaths in elephant and other species (Lindeque and Turnbull, 1994). There is some evidence to suggest that incidence of anthrax in elephant peak during the dry season while in plains ungulates, mainly zebras, deaths due to anthrax peak during the wet season (Appendix 4). The occurrence of peak anthrax outbreaks among Etosha plains ungulates in the rainy seasons differs from those in other localities in Africa (Lindeque and Turnbull, 1994). For example, in Kruger National Park (South Africa), Lake Manyara National Park (Tanzania) and Luangwa National Park (Zambia), peak anthrax mortalities occur during the dry summer seasons (Prins and Weyerhaeuser, 1987 and De Vos, 1990). This unique timing of anthrax in Etosha provides excellent opportunities to establish baseline knowledge on anthrax epidemiology and to gather basic data on the causes of anthrax in this system.

While many studies have sought to understand the causes and seasonality of anthrax outbreaks in endemic localities (Van Ness, 1971; Ebedes, 1976; Braack and de Vos, 1990; Lindeque and Turnbull, 1994), many basic aspects of anthrax ecology in

Etosha remain unresolved (Lindeque *et al.*, 1996). An older competing hypothesis is that the peak anthrax incidence in many ecosystems occurs during hot and dry times of the year (Van Ness, 1971). Over the course of the dry season, the height and biomass of grass is reduced, this prompts animals to graze close to the ground as they feed on short and/or on tall dry spiky grasses (WHO, 2008). Since *B. anthracis* is apparently non-invasive (a lesion is required for the initiation of infection), thus it is alleged that spiky grass and grit from the soil produce gastrointestinal lesions and if soil and/or grass are contaminated with *B. anthracis* spores, foraging may enable infection to occur during dry seasons (WHO, 2008). This hypothesis does not hold in Etosha where peak anthrax incidences in plains ungulates occur during wet seasons when fresh green grasses are abundant. In addition, the dry spiky grass hypothesis is rather difficult to explain in Etosha where anthrax incidence in dry seasons occurs mainly in elephants, at times when Etosha elephants are known to browse (Versveld *personal communication*). Moreover, Etosha elephants utilize Etosha plains in association with other species during dry seasons and the dry spiky grass hypothesis does not explain the isolated mortality in elephants.

The traditionally held 'incubator area' hypothesis suggests that *B. anthracis* spores are concentrated in low-lying areas during rainfall events and animals are subsequently exposed to contaminated soil/grass during the dry season (Van Ness, 1971). The 'incubator area' hypothesis does not explain anthrax seasonality patterns in Etosha, since anthrax occurs in both wet and dry seasons (e.g. Etosha zebras, springboks and wildebeests anthrax mortalities occur in the wet season, whilst mortality in elephants occur in the dry season). Nevertheless, enzootic sites of Etosha are flat plains with no low lying areas for water to concentrate on during dry seasons.

In southern Africa the most accepted hypothesis, although only for browsers, is that flies feeding off positive anthrax carcasses deposit *B. anthracis* spores on leaves of nearby trees and shrubs through regurgitation (Braack and de Vos, 1990). Browsing animals become infected after they browse on these contaminated leaves. Blowflies select horizontally arranged resting sites, such as the leaves of shrubs or trees in preference to vertically arranged sites, such as grass stalks and vertical branches (Braak and de Vos, 1990). Hence, browsers in these systems have higher exposure to *B. anthracis* than the grazers. In Etosha flies are insignificant in the transmission of *B. anthracis* spores (Ebedes, 1976). Grazers form 98.3% of all confirmed anthrax cases in the park, while browsers only make up 1.68% of the total cases (ENP mortality database 1976-2010).

Earlier studies focusing on anthrax ecology within Etosha National Park have led to the belief that waterhole/gravel pits resulting from excavation of gravel for roads maintenance are related to the incidence of anthrax (Ebedes, 1976; Berry, 1981). In contrast, Lindeque and Turnbull (1994) found very poor microbiological association between waterholes/gravel pits and the abundance of *B. anthracis* spores and concluded that waterhole/gravel pits are not a source of anthrax infection in Etosha.

Therefore in Etosha other factors such as survival of *B. anthracis* outside the host, and seasonal changes in host susceptibility, movement patterns and foraging behaviour may play a larger role in contributing to anthrax outbreaks. This study was part of a larger Etosha National Park anthrax ecology research project through the University of California at Berkeley. The larger project assesses seasonal anthrax occurrence using factors such as the survival of *B. anthracis* outside the host, host movement patterns, immunology and genetics of elephants, zebras, springbok, jackal and vultures. The parent

project aims to achieve the following objectives: 1) To determine the structure of the soil microbial community, including *B. anthracis* and characterise any changes that may contribute to the seasonality of anthrax epizootics, 2) To determine whether primary hosts in this system exhibit variation in genes that may influence susceptibility to *B. anthracis*, 3) To investigate seasonal changes in host condition and immunity in relation to potential stressors including nutrition, reproduction, and lactation cycles and gastrointestinal parasite infections to determine if seasonal nadirs in host condition correlate with timing of anthrax outbreaks, 4) To understand how scavenging affects the environmental distribution of *B. anthracis*.

Despite a complex series of proposed inter-related components that may influence the timing of anthrax outbreaks in Etosha, it is generally assumed that herbivores become infected by ingesting spores (Smith *et al.*, 2000; Regoes *et al.*, 2003). This study investigated how seasonal changes in host foraging behaviour in different seasons affects the timing of infection and mortality of elephants and zebras in Etosha. Natural foraging environments offer plenty of foraging resources to herbivores, but there is immense variation in the quality and quantity of these resources (Launchbaugh, 1999). The herbivore's challenge is to acquire sufficient nutrients to evade starvation and produce viable offspring, in spite of variation in resources quality and quantity (Distel *et al.*, 1994). Herbivores generally succeed in adapting to changes in forage quality and quantity by using a variety of foraging strategies (Parker *et al.*, 2009). African elephants (*Loxodonta africana*) mainly graze during wet seasons when grazing foliage is abundant, but increase consumption of trees, shrubs and roots during dry seasons to meet their energy requirements (van Soest, 1996; Cerling *et al.*, 2006). Zebras are predominantly

grazers and feed on fresh growth during wet seasons and increase their bite rate to meet daily needs, while during the dry season zebras survive on larger amount of foods with low nutrient concentrations by processing them quickly (Moehlman, 2003). Although herbivores generally succeed in adapting to seasonal changes in food quality and quantity (Owen-Smith, 1993), they may become infected with parasites while searching for and/or consuming food (Fenton *et al.*, 2002; Hutchings *et al.*, 2003; Hall *et al.*, 2007b). Again, herbivores use their adaptive foraging strategy to mitigate parasite infection by avoiding foraging in patches contaminated by faeces (Ezenwa, 2004). Although mammalian herbivores may adjust their foraging strategies according to an element of risk, there is strong doubt that foraging strategies exist which allow animals to avoid infectious diseases such as anthrax and botulism where spores are ingested while foraging (Bednekoff, 2007). This limitation in herbivores adaptive foraging strategies may expose them to pathogens such as *Bacillus* and *Clostridium* spores that can be ingested while foraging. Therefore herbivore foraging ecology may play a critical role to their contact with the pathogen in the environment and establishment of the infection by completing the cycle of infection from the environment to the host.

In Etosha it is proposed that carcass sites (where positive anthrax victims died) form the main link between susceptible hosts and new anthrax cases (Lindeque and Turnbull, 1994). It is proposed that grazing animals acquire the disease by ingesting spores when grazing over sites where previous victims of anthrax died and deposited the spores (Turner *unpublished data*). If a carcass site serves as an enzootic site, then variation in foraging efficiency (number of bites per step) could alter the probability that zebras will ingest a lethal dose of *B. anthracis* spores while foraging. It is proposed that

elephants in the Etosha National Park may be predisposed to anthrax because of nutritional stress and possible oral lesions caused by eating *Acacia* species during the dry season (Berry, 1993). Contact with *B. anthracis* spores is most likely through grazing behaviours when hosts feed at ground level than when browsing. Seasonal changes in diet selection may possibly explain seasonality of anthrax outbreaks in Etosha National Park. Using broadly applicable aspects of foraging ecology *diet selection* (kind of plants consumed and quality) and *foraging efficiency* (number of bites per step taken within a feeding patch), we constructed hypotheses for anthrax transmission as a function of foraging ecology.

The purpose of this study was to assess host foraging behaviour which may be a fundamental component in determining *B. anthracis* exposure by herbivores and advance our general understanding of natural anthrax transmission.

1.2 Statement of the problem

Even though anthrax has been known for centuries, little is known about the disease (Hugh-Jones and de Vos, 2002). Anthrax has caused massive death in both wildlife and livestock worldwide (WHO, 2008). Limited insight into a disease that can result in decline in population or possible extinction of species poses a serious problem to conservation. Ebedes (1976) reported that anthrax was responsible for >50% of the total recorded deaths in the Etosha National Park and, between 1966 and 1974; a total of 1 635 animals from 10 different species (cheetah, eland, elephants, gemsbok, giraffe, kudu, ostrich, springbok, wildebeest and zebra) were recorded as dying from anthrax. Mortalities in the Etosha National Park continue to occur annually in mammalian species.

To date, confirmed anthrax cases from two species (zebra and elephant) total at 1 277 animals (ENP mortality data base 1975-2010).

The problem in Etosha is that elephants contract anthrax during dry seasons in Okaukuejo plains, while zebras contract anthrax in the same place but during wet seasons (Appendix 4). This unique anthrax outbreak and the impact of climate change that might change the dynamics of anthrax and other infectious diseases pose a great challenge to conservation in Etosha. Therefore scientific research is needed to understand the causes and seasonality of anthrax outbreaks in Etosha, if we want to curtail the spread of this disease. It was proposed that factors such as survival of *B. anthracis* outside the host, sexual segregation, seasonal changes in host immunity and host foraging behaviour may play a major role in contributing to anthrax outbreaks in Etosha (Lindeque and Turnbull 1994). This study was part of a larger Etosha anthrax ecology research project, which assessed the survival of *B. anthracis* outside the host, movement patterns and seasonal changes in zebras and elephants immunity. Therefore one basic aspect of anthrax ecology to be assessed was the foraging behaviour of hosts which may be critical to their contact with the pathogen in the environment.

1.3 Objectives of the study

- (a) To determine if seasonal changes in zebra foraging efficiency may influence the likelihood that zebras ingest a lethal dose of *B. anthracis* spores while foraging.
- (b) To determine if seasonal variation in zebra diet composition may influence potential contact with *B. anthracis* in the soil while foraging.
- (c) To determine seasonal differences in the quantities of soil ingested by elephants as a means of estimating potential exposure of Etosha elephants to *B. anthracis* in the soil.
- (d) To determine seasonal variation in elephant nutrition (using faecal samples) as a stressor factor that may relate to the seasonality of anthrax outbreaks in Etosha's elephants.

1.4 Hypotheses of the study

(a) During the wet season when grass is abundant, zebra will have higher bites per step within feeding sites than during the dry season. This will increase their likelihood of ingesting a lethal dose in the wet season than in the dry season while foraging at infectious feeding sites.

(b) Zebras will predominantly graze but the consumption shrubs increases during dry seasons when fresh green grass is not available; hence zebras will have higher contact with the ground and to *B. anthracis* in the soil when grazing than when browsing.

(c) Elephants ingest appreciable quantities of soil during dry seasons than wet seasons by uprooting shrubs. Ingesting substantial amounts of soil while in enzootic sites during dry seasons may expose elephants to soil contaminated with *B. anthracis*.

(d) The diet quality of elephants will be higher in the wet season than the dry season. During the dry season plant nutrient contents are reduced due to an increase in fiber content. Low diet quality in the dry season can result in nutritional stress which may affect their immunity, making them more susceptible to *B. anthracis*.

1.5 Significance of the study

There is limited knowledge on the role that herbivore foraging behaviour plays in disease transmission and this study introduces a first look at seasonal changes in the foraging behaviour of elephant and zebra in relation to seasonal anthrax outbreak patterns in the endemic zone of Etosha. This study can also lead to an expanded methodology for determining specific pathways of infection in wildlife and widen our understanding of anthrax epidemiology under natural conditions. This study has implications for understanding food habits of wild herbivores and will form a basic requirement for the efficient management of rangeland resources.

CHAPTER 2

LITERATURE REVIEW

2.1 Transmission of anthrax in herbivores

The genus *Bacillus* contains the most common organisms isolated from the soil (Saile and Koehler, 2006), and anthrax has featured in records since ancient times but little is known about the disease (Turnbull, 2008b). Much of the literature addresses the ubiquitous nature of *B. anthracis* as a soil-borne bacterium and many of these studies recognize the environmental constraints on long-term survival and the replication of *B. anthracis* (van Ness, 1971; Dragon and Rennie, 1995; Smith *et al.*, 2000; Coker 2003). While early literature argued that *B. anthracis* could replicate in the soil (e.g. van Ness 1971; Kaufmann, 1990), the current literature supports that *B. anthracis* only replicates in the animal host and can then survive long periods of dormancy in soil (Smith *et al.*, 2000; Dragon and Rennie, 2001). Central to the persistence of *B. anthracis* in the area is the ability of *B. anthracis* to form long lasting, highly resistant spores (Dragon and Rennie, 1995). Endospores are able to withstand environmental stresses such as desiccation, ionizing and ultraviolet radiation, chemicals and severe temperature, pH, and pressure (Gould, 1977; Roberts and Hitchins, 1969). Host mortality introduces spores into the environment when fatal septicaemia or scavenging animals expose vegetative *B. anthracis* cells to the external environment where sporulation takes place (Kaufmann, 1990).

The epidemiology of anthrax is centred on the lethal dose needed for infection to occur and the mode on which the animals are exposed to *B. anthracis* spores under

natural environments (Dragon and Rennie, 1995). The minimum *B. anthracis* infectious dose (MID) estimates are largely unknown for wildlife (Hugh-Jones and de Vos, 2002). In spite of that De Vos (1990) and de Vos and Scheepers (1996) recorded 100-250 spores of a strain isolated from a kudu in the Kruger National Park which consistently resulted in death from anthrax when administered parenterally in impala, while the oral infection with the same strain in these animals required approximately 15 million spores to induce death. In a study on which 50 pigs were given 10^7 - 10^{10} doses of spores in feed containing grit the majority of pigs showed clinical signs with recovery, while two pigs died of confirmed anthrax after being given 1.6×10^7 spores (Redmond *et al.*, 1997). Even though the MID of *B. anthracis* are experimentally established, it's challenging to relate experimental lethal doses to the level of environmental contamination that these herbivores are likely to encounter naturally. Firstly, the susceptibility of animals to natural infections does not correlate with their susceptibility to experimental infection (Hass, 2002). For example, experimentally, cattle, horses and donkeys appear to possess a far greater degree of natural resistance than sheep and goats, but under natural conditions the former species are affected much more commonly than small stock (Hass, 2002). Secondly, the exorbitant lethal doses needed experimentally to infect herbivores are unlikely to be encountered by these herbivores in natural environments (WHO, 2008). Urgent questions facing ecologists in this regard is how herbivores get infected with such high dose in nature and whether such high doses are really encountered under natural conditions (WHO, 2008).

In nature, anthrax can be transmitted in various routes as a cutaneous, pulmonary (inhalational), or gastrointestinal infection (Cherkasski, 2004; Turnbull *et al.*, 2008b).

Cutaneous infection occurs when *B. anthracis* infects the host through lesions, but this is the most common form in humans and may give rise to a local oedema that develops into a necrotic lesion and/or progress to a systemic infection (Turnbull *et al.*, 2008b). In herbivores *B. anthracis* may be transmitted mechanically by vectors such as biting flies, non-biting flies and other arthropods including mosquitoes and ticks (Greenberg, 1973). Although biting flies as vectors of anthrax can transmit the disease to horses (Akmerov *et al.*, 1982), biting flies are epidemiologically insignificant in the spread of anthrax in southern Africa (Braak and de Vos, 1990). However in North America and the foothills of the Himalayas, wildlife and livestock anthrax outbreaks are associated with seasonal tabanid activity and above normal numbers of biting flies (Hugh-Jones and de Vos, 2002). Non-biting flies can act as carriers of *B. anthracis* (Hugh-Jones and de Vos, 2002), but they generally do not play an important role in the epidemiology of the disease for grazing animals, but are an important source of infection for browsers (Braak and de Vos, 1990). Ticks collected from terminally ill animals have been found to contain *B. anthracis*, but they are probably less effective in transmission of the disease as interhost transfer for adult ticks is rare (Akmerov *et al.*, 1982). Direct animal-to-animal transmission of anthrax is also believed to occur very rarely (WHO, 2008). Although it is possible for herbivores to get infected with *B. anthracis* by pica/osteophagia (licking and consuming bones) from animals that died of anthrax, this is thought to be of little consequence (WHO, 2008).

Pulmonary or inhalational anthrax manifests from breathing in endospores suspended in the air (Turnbull *et al.*, 2008b). This method of infection was also referred to as 'wool sorter's disease' since its exposure was often associated with people handling

contaminated wool and hair (Van Ness, 1971). Pulmonary anthrax is the most fatal form in humans with 75% of all infections ending in death (Turnbull *et al.*, 2008b), but natural cases of pulmonary anthrax in herbivores are poorly defined (Turnbull, 1998). Garstang and Macko (1993) suggest that *B. anthracis* spores are larger in size and they tend to attach themselves to objects making infection through inhalation less effective for herbivores under natural conditions. In an effort to determine the extent of pulmonary anthrax in Etosha National Park, Turnbull *et al.* (1998) sampled air 6-18 m downwind from old anthrax carcass sites. The sampled carcass sites had contamination levels of 2×10^4 to 1.6×10^6 cfu/gram. Turnbull *et al.* (1998) found the highest aerosol concentration to be approximately 2 spores per 100 litres of air. They concluded that the dilution effect of wind spread is too great for wind to contribute greatly to the transmission of *B. anthracis*.

Gastrointestinal anthrax results from the ingestion of *B. anthracis* and this is believed to be the common form of anthrax infection in herbivores (Joseph and Lawrence, 2002). It is generally assumed that herbivores acquire gastrointestinal anthrax by ingesting spores together with food and water (Regoes *et al.*, 2003). Although artificial and natural water reservoirs have been found to be contaminated with *B. anthracis*, the role that water sources play in transmission of anthrax in herbivores is far from clarified (Lindeque and Turnbull 1994). Lindeque and Turnbull (1994) detected no *B. anthracis* spores in water samples collected from gravel pits, natural fountains or natural pans in Etosha National Park. Lindeque and Turnbull (1994) ruled out the possibility that waterholes are a source of anthrax infection in Etosha. This may provide

strong evidence to support the general assumption that anthrax transmission rate in herbivores is fundamentally influenced by foraging ecology.

The understanding of natural anthrax transmission in herbivores requires a synthesis of component processes acting at multiple scales and levels of biological organization (WHO, 2008). In spite of complex and inter-related processes in natural transmission in herbivores, there are strong suggestions that contact with *B. anthracis* in the environment and the completion of the cycle of infection from the environment to the host is largely influenced by host foraging behaviour (Klemm and Klemm, 1959; Van Ness, 1971; Dragon and Rennie, 1995; Clegg *et al.*, 2007; and Wafula *et al.*, 2007). Even in the earliest known reference to anthrax epidemics, the Egyptian cattle died of a carbuncular disease whose description matched that of anthrax (Klemm and Klemm, 1959). The Egyptian cattle that grazed in the vegetation rich flood plains of the Nile died, but the livestock of the Israelites, which were forced to graze the dry, sparsely vegetated highlands above the river, were spared (Klemm and Klemm, 1959). Dragon and Rennie (1995) suggested that anthrax outbreaks in bison in Wood Buffalo National Park were influenced by bison foraging habits. The prolonged drought caused a wilting of vegetation; forcing bison to graze closer to soil that is potentially infected with spores (Dragon and Rennie, 1995). Dragon and Rennie (1995) further suggested that, although other conditions possibly account for increased susceptibility of bison to anthrax; altered grazing behaviour has played a more important role in exposing bison to *B. anthracis*.

Most epizootics of anthrax in African wildlife have occurred in the dry season (Hugh-Jones and de Vos, 2002). An outbreak of anthrax in Mago National Park, Ethiopia occurred in successive years both in the dry season (Ramachandran *et al.*, 1988). The

anthrax outbreak in impala in Lake Manyara National Park, Tanzania occurred at the end of the dry season (Prins and Weyerhauser, 1987). The epidemic in hippopotamus in Luangwa Valley, Zambia took place in the dry season and abated after the onset of the rainy season (Turnbull *et al.*, 1991). These peak anthrax outbreaks in African wildlife during dry seasons can be largely explained by traditionally held hypotheses of 'incubator area' and 'dry spiky grass' hypotheses (Van Ness, 1971). The 'incubator area' and 'dry spiky grass' hypotheses proposed that animals are forced to graze dry spiky grass close to the ground surface during dry seasons in low lying areas where spores have been concentrated during wet seasons. Furthermore, de Vos (1990) related anthrax outbreaks in African wildlife during dry seasons to seasonally altered foraging habits. de Vos (1990) claims that the poor condition of the range during dry seasons forced herbivores to feed on heavily utilized short grass and may contract anthrax through ingesting soil containing the spores.

In Queen Elizabeth and Lake Mburo National Parks, Uganda, the massive outbreak of anthrax in hippopotamus was linked to hippopotamus foraging behaviour (Wafula *et al.*, 2007). In Kruger National Park, South Africa, browsers have a higher exposure to anthrax than the grazers (Braak and de Vos, 1990). These discrepancies between species in Kruger National Park (KNP) have been explained in terms of differences in host feeding habits (Braak and de Vos, 1990). In Kruger the browsers are exposed to higher risk from contaminated browse by blowflies (mainly *Chrysomya albiceps* and *Chrysomya marginalis*), which after feeding on an anthrax infected carcass excrete large numbers of *B. anthracis* on the nearby foliage by regurgitating (Braak and de Vos, 1990). Blowflies form the main link between the carcass and the new cases

during an epidemic in this system (de Vos and Bryden, 1998). In addition, both roan (*Hippotragus equinus*) and sable (*Hippotragus niger*) antelope are found in the KNP but mortality in the former is very significantly higher than for the latter (Braak and de Vos, 1990). The difference appears to be not in susceptibility but from altered foraging behaviour. When grazing is sparse roan switches to browsing and as a result has much higher exposure to blowfly contaminated browse (de Vos and Bryden, 1998). In Malilangwe Wildlife Reserve, Zimbabwe, a massive anthrax outbreak in wildlife badly affected browsers with death of almost all the reserve's estimated 500 kudu (*Tragelaphus strepsiceros*) while nyala (*Tragelaphus angasi*) and bushbuck (*Tragelaphus scriptus*) suffered losses of approximately 68% and 48% of their populations, respectively (Clegg *et al.*, 2007). Similar to KNP the massive outbreak in Malilangwe was attributed to blowflies that put the browsers at high risk of exposure to *B. anthracis* where they formed 78% of all anthrax cases while grazers accounted for only 22% (Clegg *et al.*, 2007). In Malilangwe, zebras (grazer) accounted for only 2% of the anthrax mortalities and no wildebeest or elephant mortalities were reported (Clegg *et al.*, 2007). In Kruger National Park zebras are only noted among the miscellaneous anthrax cases in the park (de Vos, 1990).

In contrast, in Etosha National Park, Namibia, the species most affected by anthrax is the plains zebra (grazer) which accounts for 49.5% of all confirmed anthrax cases while kudu (browser) accounting for 0.5% of all total cases in the park. In Etosha it is proposed that previous carcass sites form the main link between the hosts and new anthrax cases (Lindeque and Turnbull, 1994). Despite the fact that spores are known to persist in the environment for long periods of time, it is very difficult to isolate *B.*

anthracis spores from an 'anthrax area' except from the immediate vicinity of animals that have died of the disease (Dragon *et al.*, 2004). Turner (*unpublished data*, 2010) isolated *B. anthracis* spores from grasses sampled at anthrax positive sites in Etosha and found the number of spores to be significantly higher at a spot where body fluids and gut contents soaked into the soil. This implies that herbivores in Etosha may be continuously exposed to low levels of anthrax spores through foraging, but high numbers of anthrax spores are only likely to be encountered at sites of anthrax mortalities. This may put grazers at higher risk by grazing at sites where previous anthrax positive victims died. This shows that the probability that Etosha grazers and other herbivore species contract anthrax depends on their encounters with carcass sites. However, the rate at which mammalian hosts encounter carcass sites and contract anthrax may include both behavioural (movement and grazing patterns) and physiological (stress and immunology) factors, in addition to genetic and environmental factors (WHO, 2008). This study was part of a larger Etosha National Park anthrax ecology project, which focuses on factors that may influence seasonality of anthrax outbreaks in Etosha.

With a handle on such factors, the present study investigated the role of foraging behaviour in relation to the timing of anthrax in Etosha's zebras and elephants. Preliminary data from camera traps set at anthrax positive carcass sites in Etosha have shown that grazers do encounter these sites while grazing (Turner *unpublished data*, 2011). Mammalian herbivores usually become infected with *B. anthracis* when they ingest sufficient numbers of spores in soil or on vegetation (WHO, 2008). So given an encounter with carcass sites the probability of an individual host to ingest a lethal dose depends on the amount of forage consumed within that site. This study followed the

examples of Underwood (1982), Murray (1991), Shipley et al. (1996) and Owen-Smith (2002) that used bites per step as an index to measure the amount of food eaten. Many infectious diseases exhibit seasonality, including anthrax in wildlife and livestock (Smith *et al.*, 2000), and resources of host often fluctuate seasonally (Altizer *et al.*, 2006). If transmission depends upon foraging ecology, fluctuation in resources may create pronounced yet predictable variability in transmission of *B. anthracis* to the host. Abundant, green vegetation in wet seasons may allow grazers to take more bites per step at a single location, increasing the chance of ingesting a lethal dose of *B. anthracis* if foraging at a carcass site. As in many species, foraging efficiency (bites per step) of ungulates increases with food density (wet season) but then slows as density becomes low (dry season) (Murray, 1991, Shipley *et al.*, 1996). This assumption would forecast higher prevalence of infection at higher food densities as a result of increased foraging efficiency at single location. Moreover, contact with the pathogen in the soil is more likely when a host grazes close to the ground surface than when browsing on trees or shrubs. Therefore seasonal changes in host foraging behavior may play a critical role in influencing the contact with the pathogen in the ground and establishment of the infection.

2.1.1 Foraging ecology of zebra

2.1.1.1 Foraging efficiency

Mammalian herbivores adopt foraging strategies to optimize nutritional trade-offs against restrictions imposed by seasonal changes in forage nutritional quality. The foraging efficiency of ungulates may also be influenced by factors such as the time of day, temperature, season, vegetation type and reproductive status (Wobeser, 2006; Neuhaus

and Ruckstuhl, 2002). Herbivores increase their foraging efficiency to acquire sufficient nutrients at times when nutrient quality is high. Herbivore may increase their foraging efficiency by altering their foraging behaviour such as bite rate and foraging time (Black and Kenney, 1984). The decisions made by animals at the level of the bite have important consequences to the herbivores nutrient intake and survival (Spalinger and Hobbs, 1992). Herbivores increased their bite rate when forage on high quality food patches to get sufficient nutrients while reducing their rate of travel (Shipley *et al.*, 1996). A highly concentrated pulse of nutrients released in to the soil from ungulate carcasses (Ben-Shahar and Coe, 1992) can improve vegetation quality or quantity and this may attract and encourage grazers to forage longer at these sites. Ben-Shahar and Coe (1992) studied foraging behaviour of zebra and wildebeest and nutrients in grasses and noted a positive correlation between intake and increased nutrients content of grasses. Zebras are non-selective grazers (Moelhman, 2003), however, they establish preferred feeding patches and they will direct their movement towards the selected foraging path (Brooks and Harrins, 2008). Using data from collared zebras, Brooks and Harrins (2008) found zebras to direct their movement to preferred feeding patches up to 3.7 km, well beyond their visual and possibly their olfactory perceptive range. Brooks and Harrins (2008) argued that zebras may have used their cognitive ability to remember the location of preferred patches from previous foraging bouts. By using their spatial memory and directing their movement to preferred patches, zebras may improve their foraging efficiency by reducing search time, increasing time within high-quality patches, reducing walking distance and reducing the energetic costs of foraging (Laca, 1998; Howery *et al.*, 2000). Once encountering a preferred grazing patch, zebras decreased the rate of travel from an

average 1.1 km/h to 0.3km/h and this was associated with an increased mean residence time within the patch (Brooks, 2005). Higher food densities during wet seasons may allow grazers to increase feeding time per foraging patch and the number of bites taken per step (Murray, 1991; Howery *et al.*, 2000). Blackburn (2006) suggests that limited home ranges of 109.6 or fewer hectares on a West Texas ranch was associated with repeated anthrax outbreaks, suggesting animals in the vicinity of anthrax-positive carcasses were likely to remain in the area and therefore feed in the area. The concentrations of zebras in enzootic zones of Okavuejo plains during the wet seasons may increase the probability to encounter carcass sites while foraging. Brooks (2005) found that Makgadikgadi zebras express a preference for swards of high tuft density and high biomass during the dry season, for which they travelled a greater than expected distance.

2.1.1.2 Diet composition

Diet selection is a complex behavioural act that is influenced by several factors (Belovsky, 1986). Physiological condition, degree of hunger, topography, competition from other animals and present and past grazing experience all influence which and how much of individual plant species are consumed (Wobeser, 2006). Contemporary diet selection theories assert that food preference and aversions are based on experiences within the life of the animal, where herbivores form dislikes for foods when consumption is followed by negative gastro-intestinal consequences (Bernays and Lee, 1988). In a similar manner, preferences are formed for foods when their consumption is followed by positive digestive feedback from protein or energy or cessation of illness (Green and Garcia, 1971).

Zebras are predominantly grazers but will occasionally browse and feed on herbs (Moelhman, 2003). In the Kruger National Park, Smuts (1975) from direct observation on feeding, listed 50 species of grasses utilised, detailing the parts of the grasses eaten, whether inflorescence, leaf, sheath or stem. The plains zebra (*Equus quagga*) is considered one of Africa's most adaptable and successful grazers (Estes, 1991). Zebras are primarily grazers and have dental adaptations for feeding on grasses (Moelhman, 2003). Zebras have a strong, sensitive, mobile upper lip which they use to push the herbage between their incisor teeth which then cut it free (Stewart and Stewart, 1970). The primary forage eaten by zebra in Kruger National Park is *Themeda triandra*, other grasses consumed include: *Cymbopogon plurinodis*, *Heteropogon contortus*, *Setaria neglecta*, and *Enneapogon scoparius* (Grobler, 1983; Nowak, 1999). Zebra shifted from *Andropogon greenwayi* in the wet season to consumption of *T. triandra* and a tall grass, *Hyparrhenia fillipendula*, during the dry season (McNaughton, 1984). Bodenstein *et al.*, (2000) noticed that both the zebra and the blue wildebeest browsed on the leaves of *Grewia* sp., probably as a nutritive supplement during the dry season. Such browsing correlated with the period when the grass had low phosphorous and protein percentages.

Zebra are the most abundant non-ruminant species among the large mammalian grazers, and their diet contains substantial quantities of bulk particularly stems, while highly lignified tissues are minor components of the diets of the ruminant (Bell, 1971; Stewart and Stewart, 1970; Field, 1971). Zebra usually feed on short grasses and will feed on almost any grass species provided it is short and in the young growing stage (Field, 1971).

Similar to other equines, zebras feed with their lips along the soil surface and prefer areas with short grass (Moelhman, 2003). This foraging strategy of preferring short grasses and grazing closer to the ground may put zebras at higher risk to contract anthrax through ingesting soil containing *B. anthracis* spores. Imologhome (*unpublished data*) found zebra in Etosha to ingest more soil in wet seasons than dry seasons. Anthrax mortalities in Etosha zebras coincided with the movement of zebras during wet seasons into the short grass plains of Okaukuejo (enzootic site). The zebra mortality in Lake Mburo National Park, Uganda, was associated with lush short grass at the beginning of the rains (Wafula, 2007).

2.1.2 Foraging ecology of elephants

2.1.2.1 Diet quality.

Mammalian herbivores use various foraging strategies to cope with seasonal changes in forage quality and quantity. The foraging ecology of elephants is unique in terms of its broad nature of the diet and the spatial extent of the effective foraging zones *ca.* 1.0 m below ground (feeding on roots and tubes) to >5.0 m above ground (Cordon *et al.*, 2006). These are foraging parameters not replicated within the niche of any other extant mega-herbivore taxa (Owen-Smith, 1982). The dynamic nature of African elephant foraging ecology has led to its description as a keystone species on seed dispersal, energy flow in ecosystems, nutrient cycling and on vegetation dynamics and structure (Western, 1989). The African elephant is also a generalist, mixed feeder with the ability to adjust the quality of forage ingested by varying the type of forage (browse to grass) ingested (Cerling *et al.*, 2006), as well as plant part (even to the point of uprooting woody species

to access roots; Stokke and du Toit 2000, Shannon *et al.*, 2006), to maximize nutritional intake. Williamson (1975) has shown that, in the Wankie National Park, Zimbabwe, 87 browse species, 42 grass species and 36 herb species are eaten by elephants. The ratio between the types of food varies both with their availability, quality and the season, and elephants consume more grass in a season of good rainfall (Ruggiero, 1992). Among the many species of browse plants eaten, mopane (*Colophospermum mopane*) ranks high and elephants also prefer to browse on re-growth from previously damaged trees (Anderson and Walker, 1974). The bases of perennial grass stems are normally richer in carbohydrates and this forms part of elephant diet during dry seasons (Anderson and Walker, 1974). The elephant trunk which is a distinct feature of the species is used in conjunction with the tusk to break down substantial branches to get at the nutritious fresh outer twigs and leaves (Stokke and du Toit, 2000). They also pluck bundles of grass by curling the end of the trunk around the stems thus making the elephant an efficient mixed feeder in maximizing energy intake. Ruggiero (1992) studied elephant foraging behaviour using seasonal variation in feeding time, quality of browsing and grazing forage and weight per trunkful to estimate forage intake and gross assimilation efficiency. Ruggiero (1992) found elephants to generally select the most nutritious and palatable of the plants available. Forage selection may be more clearly related to secondary properties of the plant rather than to its strict nutritional suitability (McNaughton, 1981). Jachmann and Bell (1985) also attributed elephants' avoidance of certain species to the presence of secondary compounds, but few data are available both on the occurrence of these chemicals in plants, and on their effects on mammalian consumers (Owen-Smith, 1982).

Elephants are more challenged in terms of attaining sufficient nutrients particularly in dry seasons when forage materials are more fibrous. Unlike ruminants, their digestive system does not have blocking structures that limit the rate of passage of material through the gut (van Soest, 1996). Their retention time of ingesta is as short as 14 h compared to 70-100 h in ruminants even for coarse materials (Clauss *et al.*, 2003). Short passage time results in limited time spent on digestion resulting into insufficient digestion of cell wall materials per unit time.

Therefore large herbivores with hindgut fermentation (e.g. African elephant) utilize lower-quality forage by maximizing throughput rate (Stokke and du Toit, 2000). Elephant bulls utilized more grass longer into the dry season than cows because their larger body size, hence larger mouthfuls allows a greater rate of mass intake that compensates for a lower density of cell soluble (Stokke and du Toit, 2000). Larger herbivores have the advantage of increased digestive capacity (mean retention) and therefore can use large quantities of lower-quality forage (van Soest 1996 and Clauss *et al.*, 2003).

Even though, elephants are adapted to lower-quality forage, they do experience nutritional stress if they are cannot attain sufficient nutrients from the available forage. Viljoen *et al.* (2008) found seasonal nutritional stress in elephants to cause increased stress levels in the dry season. Nutritional stress may modify elephants resistance and cause them to be in a weakened immunological state during dry seasons. Elephant breeding-herd fecal stress hormone levels were higher in the dry season than in the wet season in the southern Kruger National Park (Viljoen *et al.*, 2008). Dust bathing usually

creates larger dust clouds and this may greatly increase elephant's chances of exposure to anthrax spores especially during dry summer months.

2.1.2.2 Geophagy in the African elephant.

Given the nature of food resources on which herbivores forage coupled with their feeding behavior, avoidance of soil ingestion while foraging seems difficult to attain. Soil ingestion in mammalian herbivores may function as a dietary supplement for mineral deficiency (Holdø *et al.*, 2002; Mills and Milewski, 2007), or to alleviate gastrointestinal disorders (Krishnamani and Mahaney, 2000; Houston *et al.*, 2001). The detrimental effects of some lethal elements (if over-dose) and environmental contaminants found in ingested soil by mammalian herbivores may outweigh the benefits of soil ingestion in herbivores. This makes soil ingestion an important means of exposure to environmental contaminants and soil-borne pathogens. Increased environmental pollution and restriction of animal ranges into fenced protected areas has aggravated the impact of soil ingestion in wildlife. Wildlife may ingest substantial amounts of soil while feeding either deliberately or inadvertently. Deliberate soil ingestion or geophagy is seasonal, and is most common in ungulates in arid areas and probably results from deficiency of sodium (Kreulen and Jager, 1984; Ruggiero and Fay, 1994). Herbivores may also ingest soil inadvertently while foraging i.e. grazers ingest dried mud on grass and soil attached to roots.

Geophagic behaviour of mammalian hosts has been reported by many authors (e.g. Klaus *et al.*, 1998; Houston *et al.*, 2001), but cattle and elephants are among the larger herbivore mammals most extensively studied for their geophagic habits (Mahaney *et al.*, 1996; Abrahams *et al.*, 1999). Most studies on lick chemistry show that lick soils

consumed by African elephants have high concentrations of trace elements than the surrounding soils. Previous studies on soil-eating behaviour by elephants, other ungulates and primates have shown that they select soil with a high concentration of sodium and other mineral nutrients that may be deficient in the diet (Izawa *et al.*, 1990; Moe 1992; Ruggiero and Fay, 1994). Elephants may also eat soil to avert gastrointestinal disturbances such as diarrhoea, nausea and vomiting. Elephants in Ngorongoro consumed soil containing up to 35% kaolin. The pharmaceutical properties of kaolin are well known: it adsorbs toxic substances from the alimentary tract and increases the bulk of faeces (Wade, 1977). It has long been employed in the treatment of gut disorders associated with alkaloid poisoning and food poisoning in both human and veterinary medicine (British Pharmacopoeia, 1985, 1993). Kaolin clays are used in animal feeds, partly as binding agents, but also because of their effect in preventing diarrhoea and mycotoxicosis (Loughbrough, 1993). Analyses of the proportion or quantities of soil ingested by elephants will be a useful measure to estimate the risks of exposure of elephants to infectious diseases such as anthrax where spores can be ingested with contaminated soil.

2.3 Determination of herbivores diet composition and quality.

Procedures used for estimating the botanical composition and diet quality of herbivore's diets include direct animal observation, stomach analysis, fistula sampling, and faecal analysis (Holechek, 1982).

Direct animal observation requires minimal time and equipment inputs but accuracy and precision are problems, particularly with wild animals, as they are often

difficult to locate and approach closely enough for accurate observation (Ruggiero, 1992). Diet quality can be determined by direct field observation based on the assumption that herbivores select and consume the nutritious plants or plant parts (Owen-Smith 1993). Direct field observations in determining diet quality are tedious and it is also difficult to assess the exact proportions of each plant species consumed because of variation in bite size. However these problems are reduced or eliminated with tame or habituated animals particularly in conservation areas (Ruggiero, 1992). Species identification and quantification of how much of a plant was consumed are important limitations associated with direct observation procedure (Wobeser, 2006). Quantitative information from direct observation has been obtained from the number of bites and feeding time approaches (Wobeser, 2006). When the feeding time approach is employed, time spent feeding on each species is quantified and assumed to be proportional to the importance and the quality of the species in the diet (Bjugstad *et al.*, 1970). Additional factors influencing the accuracy and precision of the direct observation procedure include observer bias and/or development stage of the individual.

Stomach analysis will provide information on what species are being consumed and gives an indication of relative proportions consumed (Brown, 1961). The main disadvantage of this procedure is that it involves sacrifice of animals and, therefore is restricted primarily to livestock with large populations (Chippendale, 1962). Other disadvantages are that differential destruction of forage species during digestion alters the proportions of the food items consumed (Chippendale, 1962; Talbot and Talbot, 1963) and the location of consumption cannot be determined. There may also be considerable

difficulty in identification of partially digested food items (Anderson *et al.*, 1965; Brown, 1961).

Fistula methods are accurate but are difficult to use with wild animals. In addition they are costly and require considerable time. The oesophageal fistula is preferable to the rumen fistula because it provides more accurate information and requires less labour (Brown, 1961). Fistulation is more direct but is invasive, costly and an ethically questionable technique for assessing diet quality (Westoby *et al.*, 1976) and esophageal fistulation is impractical for free-ranging animals. Moreover, it is unsuitable for dietary phosphorus assessment because of salivary phosphorus (Westoby *et al.*, 1976).

Faecal analysis is a powerful tool which has been mostly used for studying mammalian diets (Anderson *et al.*, 1965). Faecal analysis has several unique advantages which account for its popularity as a research tool. It does not interfere with the normal habits of the animals and it permits practically unlimited sampling (Anderson *et al.*, 1965). It places no restriction on animal movement and it can be used to compare the diets of two or more animals at the same time (Brown, 1961). More importantly it is a feasible procedure to use when studying secretive and/ or endangered species (Brown, 1961). Accuracy is a problem because forage species passed in the faeces are often not proportional to those consumed and preference indices cannot be accurately assigned because where the food was consumed cannot be determined (Anderson *et al.*, 1965). Faecal indices of diet quality, such as those using CP and P have shown to be useful tools. Plant identification from faeces is both tedious and time consuming and destruction of some plant species may occur during sample preparation (Brown, 1961).

Given the limitations on which procedure were possible with the species under study, this study used direct observation with the aid of video play-back technique (Zebra) and faecal procedures (Elephant) to study foraging efficiency and diet composition. Zebras in Etosha are habituated to vehicles thus make direct observation by filming them convenient for determining foraging efficiency diet composition; while in Etosha it is difficult to directly observe large number of foraging elephants, as they mostly forage in inaccessible areas. Therefore faecal analysis was deemed to be the most suitable procedure for determining the diet quality of elephants.

CHAPTER 3

METHODOLOGY

3.1 Study site

The study was carried out from December 2009 to November 2010 at Etosha National Park, in northern Namibia between 18° 30'–19° 30' S and 14°–15°–17° 10' E (Figure 3.1). Etosha National Park extends about 300 km east-west and 65–110 km north-south, encompassing an area of about 22,915 km² of which 5,104 km² is a salt pan. The Etosha pan receives water from all directions with major drainage channels in the form of omurambas (dry rivers) and oshanas (seasonal wetlands) from the north and north-east. Three seasons are recognised in Etosha National Park namely hot-wet (March– April), cool-dry (May– June) and hot-dry (August–September) (le Roux *et al.*, 1988). Rainfall is strongly seasonal and there is a rainfall gradient from east to west, with Namutoni receiving an average of 441 mm, Halali 399 mm, Okaukuejo 383 mm and Otjovasandu 304 mm (Mendelsonhn *et al.*, 2000). The vegetation of Etosha National Park is classified into four major zones saline deserts with a dwarf savanna fringe, mopane (*Colophospermum mopane*) savanna, mountain savanna and karstveld and tree savanna and woodlands (le Roux *et al.*, 1988).

The focal area for the study was the Okaukuejo and Halali plains which surround the Etosha pan and both have extensively grazed short grassland and dwarf shrub savanna. The dominant grasses in terms of cover in this habitat are *Enneapogon desvauxii*, *Aristida adscensionis* and *Eragrostis nindensis*, *Eragrostis porosa*, *Choris virgata*, *Urochloa brachyura*, *Eragrostis enchinochloidea*, *Tragus racemosus* *Monelytrum luederitzianum* and the dominant dwarf shrubs are *Leucosphaera bainesii*,

Cyathula hereroensis, *Monechma tonsum*, *M. genistifolium* and *Petalidium englerianum* (le Roux *et al.*, 1988).

A complete survey of mammals in Etosha has been done, and regular censuses and distribution surveys of the large herbivores have been undertaken since the late 1960s. Zebras in central Etosha are concentrated on the Okaukuejo plains during the wet season while in the dry season they migrate to the Halali plains. Concentrations of elephants occur during dry seasons around the perennial watering points in Okaukuejo section, but most elephants disperse north and westwards during the wet season (Zidon, *personal communication* 2009).

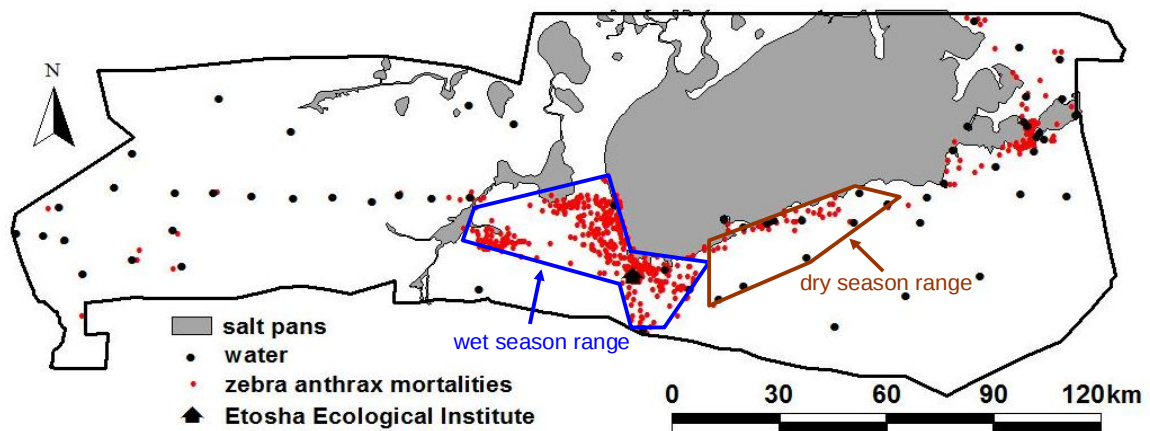


Figure 3.1. Distribution of zebra anthrax mortalities in Etosha 1974-2008, and the areas of zebra concentration in wet and dry seasons. Zebra migrate between the Okaukuejo plains in the wet seasons and Halali plains in the dry seasons. Whilst elephants are in okaukuejo plains during dry seasons and they migrate to the western side of the park during wet seasons.

3.2 Study design -Zebra foraging efficiency and diet composition.

Three general seasons were considered for sampling the hot-wet (March-April), the cool-dry (May-June) and the hot-dry (August-September). Five sites of *ca.* 30-ha each in the Okaukuejo and in Halali plains were selected based on habitat types with range distance of 15-30 km apart (Figure 3.2). Habitat types were classified using le Roux *et al.* (1988) vegetation classification model in Etosha, thus grassland savannas, grassland, high and low tree savannas, and shrub savannas. Road transects were set using permanent tourist roads from Grootvlei to Reitfontein to ensure optimal coverage of the study area (Okaukuejo-Halali). Research has shown that activity levels of African ungulates decreases above 32 °C, including eland (Lewis, 1977), giraffe (Leuthold and Leuthold, 1978), impala and blesbok (Klein and Fairall, 1986). Therefore, daily maximum temperature was recorded from Okaukuejo weather station using a maximum and minimum thermometer.

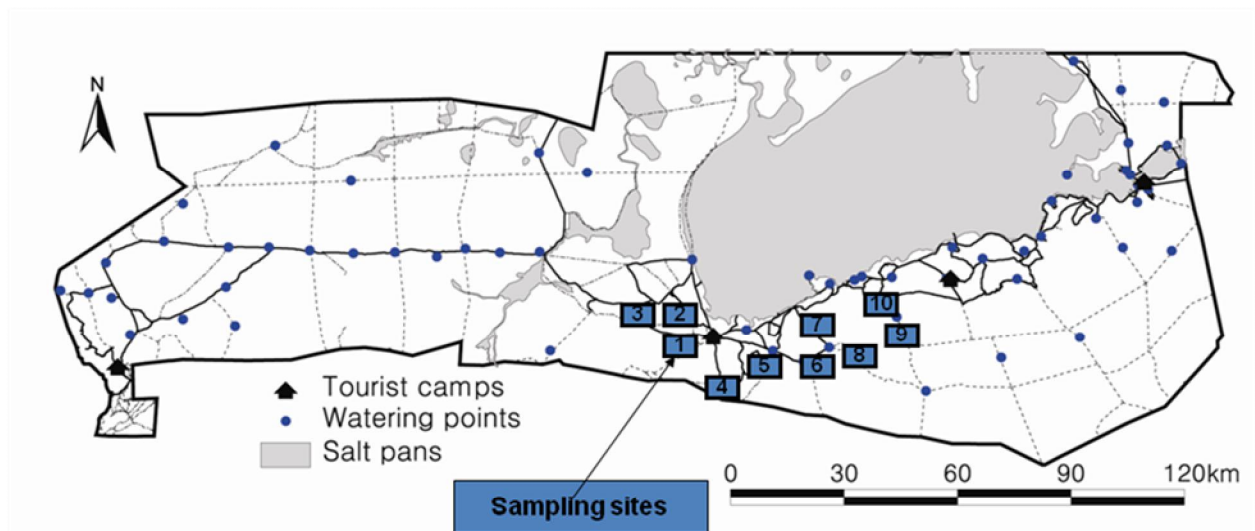


Figure 3.2. Etosha map showing selected sampling sites within the study area, five sites near Okaukuejo (1-5) and five near Halali (6-10).

3.2.1 Zebra focal animal sampling

The method of instantaneous sampling of focal animals was used (Altmann, 1974). A focal animal is a selected individual from a population where its specified actions are recorded during a predetermined sample period (Altmann, 1974). In this study focal animals were selected by choosing the closest animal to the observer at the beginning of data collection. Group sizes of more than 10 individuals were excluded due to the difficulty to sustain visual contact with focal animals within a large group. If no zebras were available at scheduled site for sampling during that day another un-sampled site was chosen depending on the distribution of zebras. Focal animals were observed during a feeding bout using a 5 minutes focal animal sampling period (as used by Neuhaus and Ruckstuhl, 2002). A total of 360 focal animals were observed, with an average of six individuals per site per month. The observation was spread over two periods of the day, morning (06:00–12:00 hours) and afternoon (13:00–18:00 hours).

The reproductive status of females and males was determined by direct observation. Females were considered to be lactating when they nursed a foal. Females without a foal were classified as not lactating. In general, although reliable evidence is difficult to come by, the incidence of anthrax in wild animals seems to be slightly biased towards adulthood (WHO, 2008); hence only adult animals were sampled. Focal animals were classified as adult on the basis of relative size and pelage, while focal animals sex were assessed on the basis of genitalia (Smuts, 1975).

3.2.2 Zebra foraging efficiency

In this study foraging efficiency was defined as the number of bites per step and the time spent feeding by zebras while foraging within a feeding patch. *Feeding patch* was defined using a similar definition to Owen-Smith (2002) as the area the feeding focal animal could reach without moving its fore-feet. *Feeding* was defined as biting, chewing and swallowing, using a similar definition to Novellie (1978). Feeding began when the focal animal lowered its head searching/taking the first bite and ended when the animal raised its head, as used by Southwell (1987) and Clarke *et al.* (1995). In this study *bite* was defined after Watson and Dawson (1993) as a quick jerky movement of the head of the feeding animal associated with the removal of plant tissue with the mouth (teeth). A *step* was counted as a single forward movement by one of the front legs; subjects usually encounter a new potential feeding patch with each step (Underwood, 1982).

It is difficult to observe a foraging animal's bite by visual inspection in wild animals. The rate at which bites are removed also precludes determining bite rate in real time (Griffiths *et al.*, 2005). The simplest approach is to extract information on bite rate from a video record of the feeding activities (Griffiths *et al.*, 2005). Hence, focal animals were filmed from a parked vehicle using a monopod-mounted video camera during daylight hours, in the morning (6:00-12:00) and afternoon (13:00-18:00) active feeding times. Distance to animals did not exceed 100 m to avoid observer error (Shahar and Coe, 1992), and a range finder was used to measure the distance between the focal animal and the observer. Each focal animal was filmed for a maximum of 5 min feeding sample. The feeding bout included time spent feeding and other non-feeding activities such as movements between food patches, standing looking around and grooming actions and

social interactions. Back at the office the number of bites, number of steps, feeding, standing and walking time were recorded by playing back video sequences of each focal animal feeding bout.

3.2.3 Zebra diet composition

Species composition of consumed plants was determined by playing back focal animal video footage while at the feeding site and locating where the focal animal had been foraging. Signs such as exudation of sap, crushed tissue and fresh clippings were used to judge if a plant species had been consumed (Owen-Smith, 2002). Plant parts that were plucked and masticated as the animal lifted its head was observed to aid in identification of plant that was consumed. A specimen of each species consumed at each feeding site was collected for identification once the focal animal had moved into safe distance to reduce disturbance. Plant samples were collected by cutting off a branch from woody plants or by uprooting a specimen. In addition, photographs of sampled plant species were taken to aid in the identification process. Sampled plant specimens were preserved using a plant press and later identified to species level with aid of identification keys. Identification of all plant species were verified at the Botanical Research Institute.

3.3 Study design - Elephants faecal sampling.

Elephant faeces were collected from two different regions of Etosha. In the same Okaukuejo and Halali study area used for zebra, elephant faecal samples were collected to assess seasonal differences in elephant nutrition and soil ingestion. In addition to the seasonal study, faecal samples were collected from known elephant bulls at a waterhole in north-eastern Etosha (Mushara waterhole) in the dry season. The work on known

elephant bulls was done in collaboration with Dr. Caitlin O'Connell-Rodwell from Stanford University who conducts research on the dominance hierarchy of elephant bulls at Mushara waterhole. Known elephant bulls were identified based on distinctive features i.e. broken tusks, torn ears and scars.

Elephant faecal samples for the seasonal study were collected weekly by visiting waterholes in early morning hours during the sampling period for a dry (June-November) and wet (January-May) season. This was done to minimise collecting multiple samples from the same animal per day, as elephants rarely visit more than one waterhole in a day. Elephant faecal samples were also collected opportunistically from road transects set using permanent tourist roads. Elephant dung piles were examined to determine how recently they had defecated. Elephant faeces become completely dry 2-3 days after being deposited (Holdø *et al.*, 2002). Fresh elephant dung was collected for nutritional and silica/soil analysis. Dung boluses were carefully broken apart by hand and faecal matter was collected from the inner core of a bolus to avoid soil contamination.

As with zebras only adult animals were sampled. The circumference of the dung bolus was used to estimate approximate age of individual elephants. An adult African elephant has dung bolus circumference of 13.96–15.42 cm (Morrison *et al.*, 2005). A seasonal change in the fiber content of the diet causes the bolus produced by an individual to vary in fiber content (bulk density), but the size of the bolus remains consistent in circumference and thus depends on anal size (Morrison *et al.*, 2005). The largest bolus was selected for measurement per sampled dung pile; a dung pile was defined as any number of boli in a clump or mound at about 1-m distance from any other elephant dung (Gonthier, 2007).

For the known elephant bulls at Mushara waterhole faecal samples were collected during the dry season only (June-July 2009 and 2010) from known male individuals of different age classes. A comprehensive study has been done on these individuals' social interactions; dominance hierarchy and their ages are known. Faecal samples from elephant were collected by observing individuals defecating at a waterhole where observers had camp for the entire sampling period.

Known elephants were separated into four broad size categories on the basis of shoulder height, which was used as an index of age, with the smallest (one-quarter size) elephants categorized as the youngest, and the largest (full size) elephants the oldest. Shoulder height was measured on all elephants included in this study using a TruPulse 200 Rangerfinder (Laser Technology Inc, 2000) at a fixed distance (80 m) and location (the source of fresh water) as well as shoulder position (perpendicular to the observation tower). These data were then compared for accuracy against a fixed object positioned at the same spot with incremental measurements taped onto the object and visible from measurement distance.

All faecal samples were placed in a paper bag and oven dried at 60°C for 48 hours and then taken to the Ministry of Agriculture, Water and Forestry laboratory and Analytical laboratory in Windhoek for analysis. A total of 87 faecal samples were collected, 50 faecal samples from unknown individuals in Okaukuejo/Halali area and 37 samples obtained from 13 known individuals at Mushara waterhole in North-eastern Etosha. Only two seasons wet (January-May) and dry (June-September) were considered for elephants based on the availability of elephants in the study area.

3.3.1 Elephant diet quality

Elephant diet quality was analyzed using faecal analysis (Stewart and Stewart, 1970; De Boer *et al.*, 2000; Holdø *et al.*, 2002). Elephant faecal samples ($n = 87$ weighing ± 100 g each) were collected in sampling months from waterholes, road transects and from known elephants observed defecating. For nutrient analysis, samples were analyzed for crude protein, crude fibre and phosphorus. Total nitrogen and phosphorus were measured after Kjeldahl destruction (Bradstreet, 1954). Crude protein content was calculated from the nitrogen content by multiplication of 6.25 (Udén *et al.*, 2005). Crude fiber was determined by boiling the samples in neutral detergent reagent which dissolves the cell contents, leaving the crude fiber. Crude fiber residue was expressed as a percentage (without subtracting residual ash) in per cent of dry matter (Udén *et al.*, 2005). Only two seasons wet (January-May) and dry (June-September) were considered for elephants based on the availability of elephants in the study area.

3.3.2 Ingestion of soil by elephants

Using faecal samples collected carefully to avoid soil contamination (as discussed in section 3.3), a method for estimating the concentration of silicates in faeces was used to analyze the proportion of soil ingested by elephants. The method used was modified and adopted from the incineration acid wash (ICW) procedures for determining the crude silica content of soil (Beyer *et al.*, 1994 and Wong *et al.*, 1988). The ICW is based on the removal of organic material by high temperature incineration and dissolving inorganic materials in concentrated acid, leaving a residue of acid-insoluble inert silicates. Grasses contain silica up to 4% for structural support (Neethirajan *et al.*, 2009); hence recorded

amounts of silica above 4% level indicate ingestion of soil or soil contaminated forage (Neethirajan *et al.*, 2009). Oven dried faecal samples were ground with a Wiley mill to fit through a 1 mm mesh. From each 100g faecal collection a ground 1 to 2g sub-sample was extracted and oven dried in crucibles overnight at 105°C, and then incinerated in a muffle furnace at 800°C for 1 hour. The ash was then cooled in a desiccator, covered with 6M HCL for 12 hours, then filtered under vacuum on to Whatman 541 filter pads in a Millipore apparatus. The residue of inert silicates was then left to dry to constant weight under desiccation at 50°C. This procedure was performed by laboratory technicians at the Ministry of Agriculture, Water and Forestry.

3.4 Anthrax surveillance

Anthrax incidence was obtained from Etosha anthrax ecology research project database. For each carcass found observers recorded the sex, age (via dental assessment) and spatial location, assessed possible causes of death and sampled for *B. anthracis* diagnostic testing. For diagnosis of *B. anthracis*, samples were taken by swabbing the nasal cavity of carcasses or other parts of the body if the skull was not available. The swabs were sent to Central Veterinary Laboratory in Windhoek and the University of Hohenheim (Stuttgart, Germany) for diagnosis by selective culture and polymerase chain reaction (PCR).

3.5 Data analysis

Statistical analyses were conducted using JMP v4. The differences was considered statistically significant at $\alpha = 0.05$ for all the tests. In the results, means are reported $\pm$ standard error. Before analysis, the data were tested to ensure that they met the assumption that they are normally distributed.

The number of bites per step (total number of bites/total number of steps) in the five minute sampling intervals were log transformed (Log_{10}) to approximate a normal distribution. A two-way analysis of variance (ANOVA) procedure was used to determine how the dependant variable bites per step taken by zebras is affected by the independent variables season, time of the day, temperature and vegetation type. The independent variable season was taken as an ordinal variable and encompassed three seasons hot-wet, cold-dry and hot-dry seasons. The data used for analysis included a total of 354 focal animals ($n = 123$ for wet-hot season; $n = 120$ for cold dry season and $n = 111$ for hot-dry season). Time of the day was divided into two session morning (6:00-12:00) and afternoon (13:00-18:00). Research has shown that activity levels of African ungulates decrease above 32 °C (Lewis 1997), therefore for analysis temperature was rated as higher (>32 °C) or lower (<32 °C). In the analysis vegetation type was coded as grassland savannas (A), grassland (B), high and low tree savannas (C) and shrub savannas (D). For each 5-minute sampling interval time spent foraging, standing and walking was calculated. Standing time comprised non-grazing actions which terminated feeding and these were grooming, nursing young one, standing-inactive, scratching, urinating, vigilance and defecating. Walking time included non-grazing actions that also terminated feeding and these were running and moving with head up above the shoulder. Zebra were

expected to walk more during the dry season than the wet season in search of food and it was also expected that female's zebras would forage for longer than males because of high energy demand from lactation or pregnancy. Time spent feeding once encountering a feeding patch can be limited by forage type which affects chewing and swallowing time. Foraging, standing and walking time was not normally distributed. Therefore Kruskal-Wallis test was used to test for differences in grazing, standing and walking time, between season and sex. Zebra diet composition was analyzed by performing Chi-Squared tests on the number of grasses consumed versus non-grass species consumed per season.

The proportion of silica in elephant faeces was not normally distributed and the Kruskal-Wallis test was used to test for significant difference between the proportion of soil ingested by elephants and the season. Only two seasons wet (January-May) and dry (June-November) were considered for elephants based on the availability of elephants in the study area. To assess seasonal differences in soil ingestion and nutritional variation a total of 50 faecal samples for Okaukuejo and Halali elephants comprised of 25 faecal samples per season were included in the analysis. To assess how elephant dominance hierarchy influences the quality of diet consumed and proportion of soil ingested, 37 faecal samples from 13 Mushara elephant bulls were included for analysis. A t-test was used to assess relationship between the season and the quality of diet utilized by Okaukuejo elephants. T-test was also used to assess the relationship between season and quality of diet utilized by Halali elephants. The Kruskal-Wallis test was used to test for difference between the quality of diet utilized by Mushara bulls and bull age ranking in the dominance hierarchy. Kruskal-Wallis test was also used to test for difference between

quality of diet utilized by Mushara bulls and physiological state (in musth or not). Diet quality was represented by percentage faecal crude protein (%CP), percentage faecal crude fiber (%CF) and faecal Phosphorus content (mg/100g).

CHAPTER 4

RESULTS

4.1 Foraging efficiency of zebra

The mean bites/step taken by zebras was significantly higher in the wet season than during the drier months May to November (hot-wet versus cold dry; $t=-4.59$, $P < 0.001$; cold-dry versus hot-dry; $t=-5.38$, $P < 0.001$, Figure 4.1). Time of the day (morning and afternoon), daily maximum temperature ($>32\text{ }^{\circ}\text{C}$ or $< 32\text{ }^{\circ}\text{C}$) and vegetation type had no significant effects on bites/step taken by zebras (Two Way ANOVA, time of the day: $F=0.093$, $P = 0.759$; temperature: $F = 0.119$, $P = 0.730$; vegetation: $F = 1.720$, $P = 0.162$; Appendix 1).

Temperature was significantly related to the number of bites/step if the variable season was not included in the model ($t=-6.72$, $P < 0.001$). Either variable could be used to describe seasonal variation, however this study was designed around specific seasons, hence the variable season was retained in the model. The seasonality of temperature variation during the study is shown in (Figure 4.2).

There was no significant difference in the number of bites/step taken by males and non-lactating females (males, $n = 144$) versus non-lactating females ($n = 90$) $t=1.46$, $P = 0.067$), but the difference between lactating females and non-lactating females was significant lactating females ($n = 120$) versus non-lactating females, $t=1.026$, $P < 0.023$; Figure 4.3). There was also a significant difference ($t=-6.07$, $P < 0.001$) between lactating females and males on the number of bites/step taken while foraging.

The time spent feeding by zebras increased significantly across the three seasons (hot-wet season; $3.38\text{ min} \pm 0.014$; cold-dry season; $3.50\text{ min} \pm 0.062$ and hot-dry season;

3.71 min $\pm$ 0.034. There was no statistically significant difference between seasons on time spent standing by zebras per feeding sample (hot-wet season: 3.72 min $\pm$ 0.087; cold-dry season: 3.74 min $\pm$ 0.078; hot-dry season: 3.78 min $\pm$ 0.092; Kruskal-Wallis test, $df= 2$, $P= 0.606$), however there was a significant difference between seasons on time spent walking (hot-wet season: 2.78 min $\pm$ 0.024; cold-dry season: 3.20 min $\pm$ 0.048; hot-dry season: 3.78 min $\pm$ 0.062; Kruskal-Wallis test, $df= 2$, $P<0.030$).

The study reveal that there was no significant difference in time spent grazing, standing, or walking between male and female zebras of different reproductive classes (Kruskal-Wallis test, grazing: $Z= -1.37$, $P= 0.171$, standing: $Z= 1.52$, $P= 0.128$ and walking: $Z= 1.67$ $P= 0.069$).

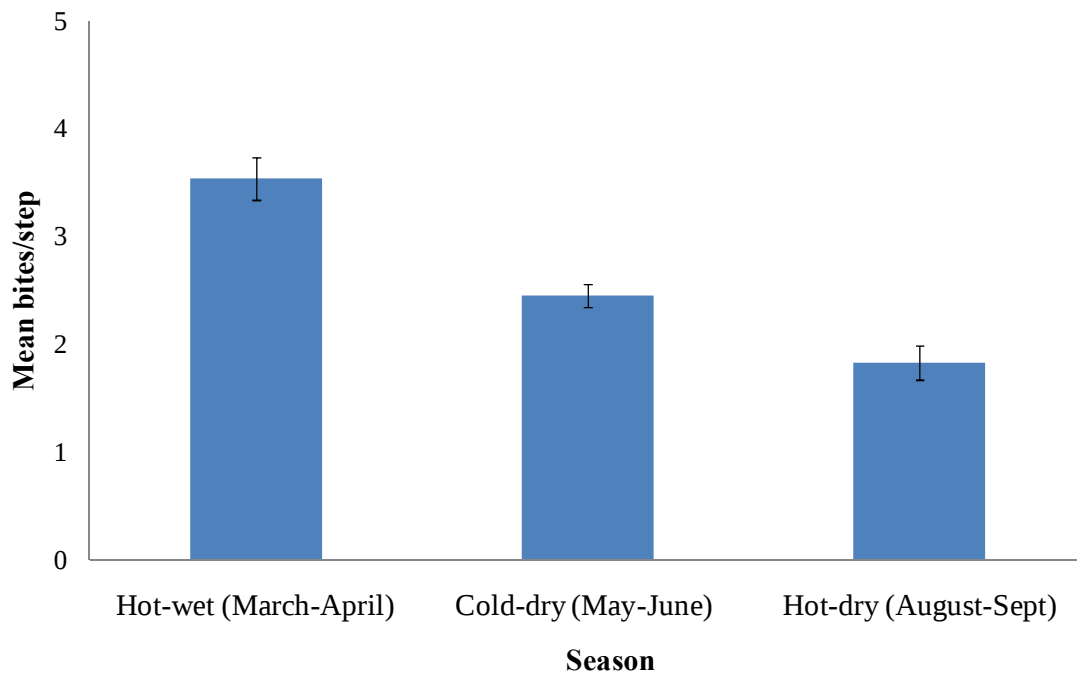


Figure 4.1. Mean of bites/step taken by plains zebras while foraging during different seasons. **Note:** Error bars are standard error of the mean.

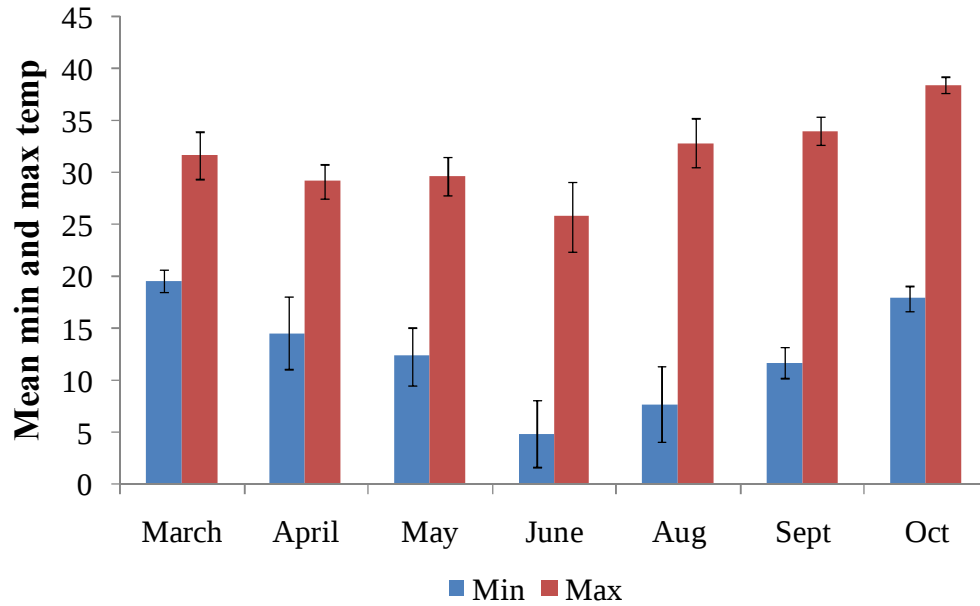


Figure 4.2. Seasonality of temperature (mean min and max) during sampling month; temperature was only recorded during the sampling days from Okaukuejo weather station. **Note:** Error bars are standard error of the mean.

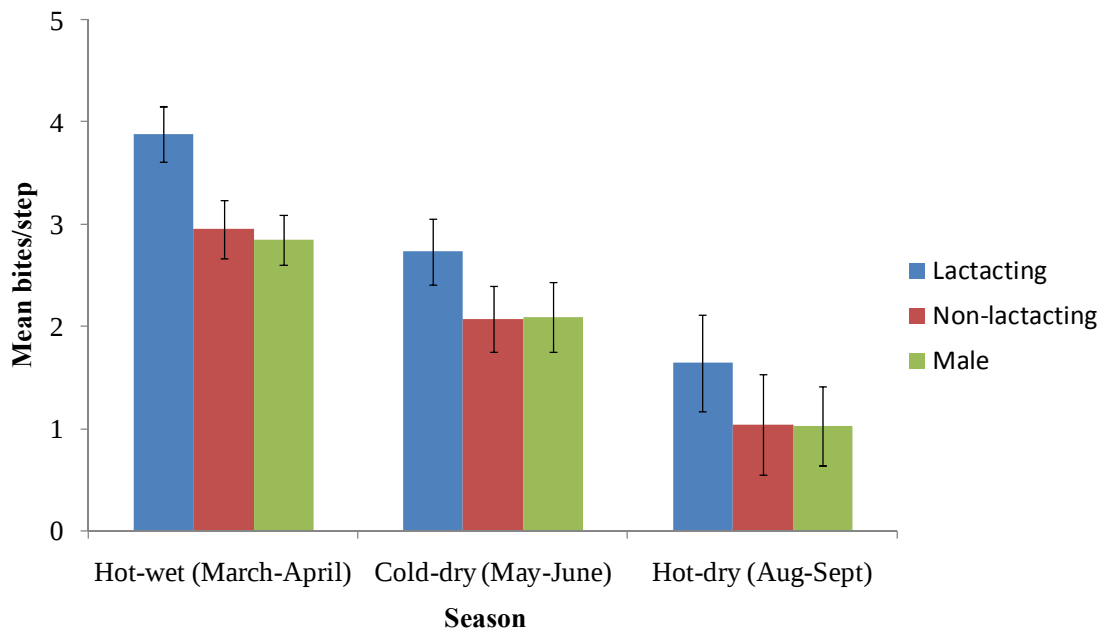


Figure 4.3. Mean of bites/step taken by zebras of different reproductive status in each seasons. **Note:** Error bars are standard error of the mean.

4.2 Diet composition of zebra

Nineteen grass species were recorded in the feeding sites used by zebras on the Etosha plains (Halali and Okaukuejo plains) (Table 4.1). Two grass species contributed most of the diet of zebra, *Enneapogon desvauxii* and *Eragrostis nindensis*. *Enneapogon desvauxii* and *Eragrostis nindensis* were the most favoured grass species throughout the year, being most always grazed when present in feeding sites. *Urochloa brachyura*, *Chloris virgata*, *Setaria verticillata* and *Cenchrus ciliaris* were also generally acceptable throughout the year, but their consumption slightly increased in the dry season along with other tall grasses with more biomass such as *Monelytrum luederitzianum*, *Fingerhutia africana*, *Enneapogon cenchroides* and *Heterpogon contortus* which were highly represented during the hot-dry season. Six shrub species were recorded in the feeding sites used by zebras on the Etosha plains (Halali and Okaukuejo) (Table 4.2). It appeared that zebras consume more shrubs *Leucosphaera bainesii*, *Cyathula hereroensis*, *Catophractes alexandri* and *Petalidium englerianum* in the dry season. However, this pattern was not statistically significant ($\chi^2=12.72$, $P= 0.076$, Figure 4.4).

Table 4.1. Grass species composition grazed by zebras in Etosha plains (Okaukuejo and Halali) presented as percentages of total grass selection.

Grass species	Total (n)	Seasons		
		Wet-hot	Cold-dry	Hot-dry
<i>Antheophora schinzii</i>	12	2.5	4.6	2.5
<i>Aristida adscensionis</i>	12	1.7	3.1	5.4
<i>Aristida congesta</i>	13	0.8	3.8	5.9
<i>Cenchrus ciliaris</i>	25	10.5	6.1	4.5
<i>Chloris virgata</i>	20	3.3	7.7	6.7
<i>Enneapogon cenchroides</i>	20	5.0	3.5	6.5
<i>Enneapogon desvauxii</i>	79	29	23.8	11.5
<i>Eragrostis enchinocloidea</i>	10	1.7	0.8	5.8
<i>Eragrostis nindensis</i>	34	17.1	11.5	7.7
<i>Eragrostis porosa</i>	23	4.1	7.6	7.5
<i>Eragrostis trichophora</i>	9	3.3	1.1	2.5
<i>Eragrostis sabinae</i>	10	5.0	2.3	0.8
<i>Fingerhutia Africana</i>	9	0.0	0.8	5.8
<i>Heterpogon contortus</i>	7	0.0	1.5	4.2
<i>Moneltytrum luederitzianum</i>	11	1.7	2.3	5.0
<i>Setaria Verticillata</i>	13	2.5	7.2	6.2
<i>Tragus spp</i>	8	3.3	2.3	1.2
<i>Triraphis purpurea</i>	7	2.5	0.8	2.8
<i>Urochloa brachyura</i>	28	6.0	9.2	7.5

Note: N= total number of times the plant species was observed as consumed.

Table 4.2. Shrubs species composition consumed by zebras in Etosha plains (Okaukuejo and Halali) presented as percentages of the total shrub selection.

Grass species	Total (n)	Seasons		
		Hot-wet (March-April)	Cold-dry (May-June)	Hot-dry (Aug-Sep)
<i>Catophractes alexandri</i>	20	23.4	23.5	24.1
<i>Cyathula hereroensis</i>	10	12.1	13.2	15.3
<i>Leucosphaera bainesii</i>	17	38.5	39.0	43.0
<i>Monechma genistifolium</i>	8	12.2	12.0	3.3
<i>Monechma tonsum</i>	6	4.4	2.3	3.3
<i>Petalidium englerianum</i>	8	9.4	10.0	11.0

Note: N= total number of times the plant species was observed as consumed.

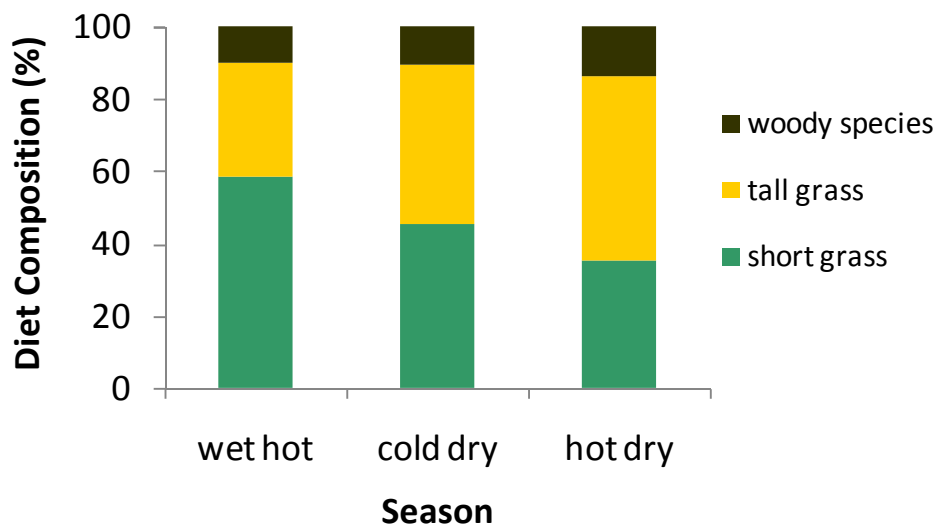


Figure 4.4. The proportion (%) of grass and shrub species consumed by zebras in each season in Etosha plains.

4.3 Soil ingestion in elephants

The amount of silica in elephant faeces was significantly higher in the wet season than the dry season (Kruskal-Wallis test; $Z=-4.202$, $P<0.001$, Figure 4.5). There was no significant difference between elephants of different age/size classes and the proportion of soil ingested (Kruskal-Wallis test; $df= 3$ $P= 0.972$), nor was there a significant difference between dominance rank and the amount of soil ingested (Kruskal-Wallis test; $df= 3$ $P= 0.864$).

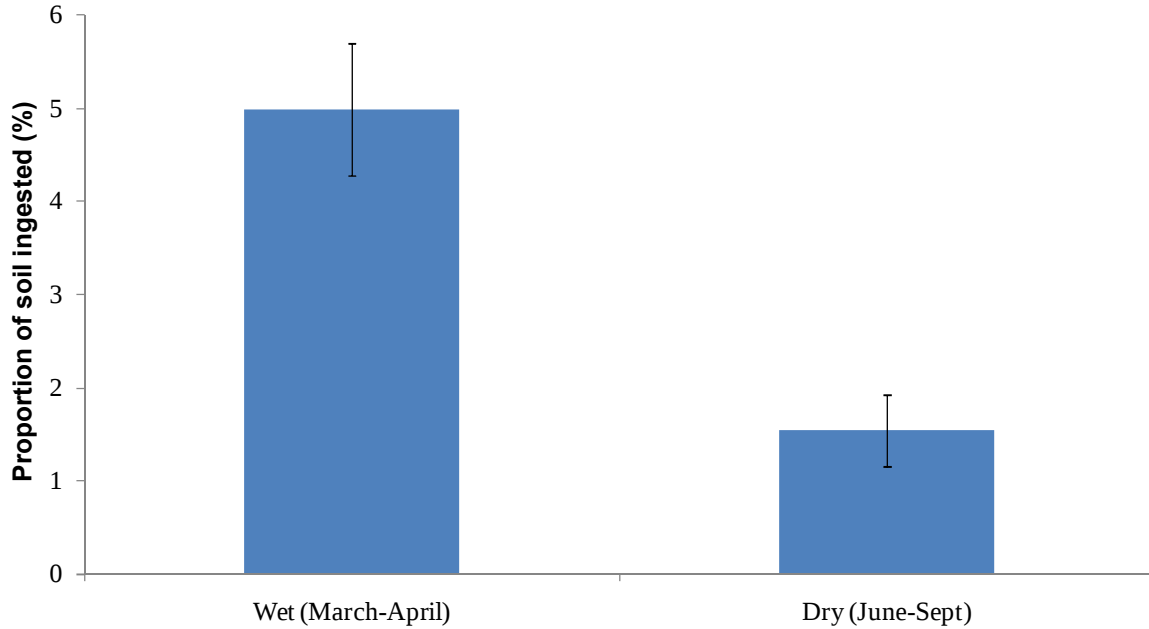


Figure 4. 5. The proportion of soil ingested by elephants during the wet and dry season in Etosha. **Note:** Error bars are standard error of the mean.

4.4 Elephant diet quality

Seasonal patterns

The crude protein content of elephant faeces was significantly higher in the wet season than the dry season ($t=-5.04$; $P<0.001$; Figure 4.6a). There was a significant difference in elephant phosphorus between the wet and the dry season ($t=-5.27$, $P<0.001$; Figure 4.6b). The crude fiber of elephant faecal was significantly higher in the dry season than the wet season ($t=-5.08$, $P<0.001$, Figure 4.6c).

Age and dominance patterns.

There was no significant difference in the faecal crude protein (CP) content of elephants of different age/size classes (1/2 size CP mean: 4.6%, $n=2$; 3/4 size CP mean: 4.5% $n=5$; full size CP mean: 4.6%, $n=6$; Kruskal-Wallis test; $df=3$ $P=0.063$). The present found no significant difference in the faecal crude fiber (CF) content of elephants of different age/size classes (1/2 size CF mean: 59.6%; 3/4 size CF mean: 58.2%; full size CF mean: 55.2%; Kruskal-Wallis test; $df=3$ $P=0.405$). No significant difference was found in faecal phosphorus (P) of elephants of different age/size classes (1/2 size P mean: 77.5 mg/100g; 3/4 size P mean: 78.3 mg/100g; full size P mean: 83.9mg/100g; Kruskal-Wallis test; $df=3$ $P=0.754$).

The difference between the rank in the dominance hierarchy and faecal nutrients contents (CP, CF and P) was insignificant (Kruskal-Wallis test; CP, $df=3$ $P=0.445$; CF, $df=3$ $P=0.554$; and P, $df=3$, $P=0.665$). Although elephant bulls in musth show a decrease in faecal nutrients content (CP, CF and P) this difference was insignificant (Kruskal-Wallis test; CP, $df=1$, $P=0.090$; CF, $df=1$, $P=0.735$, $df=1$, $P=0.499$; $n=3$ musth bulls).

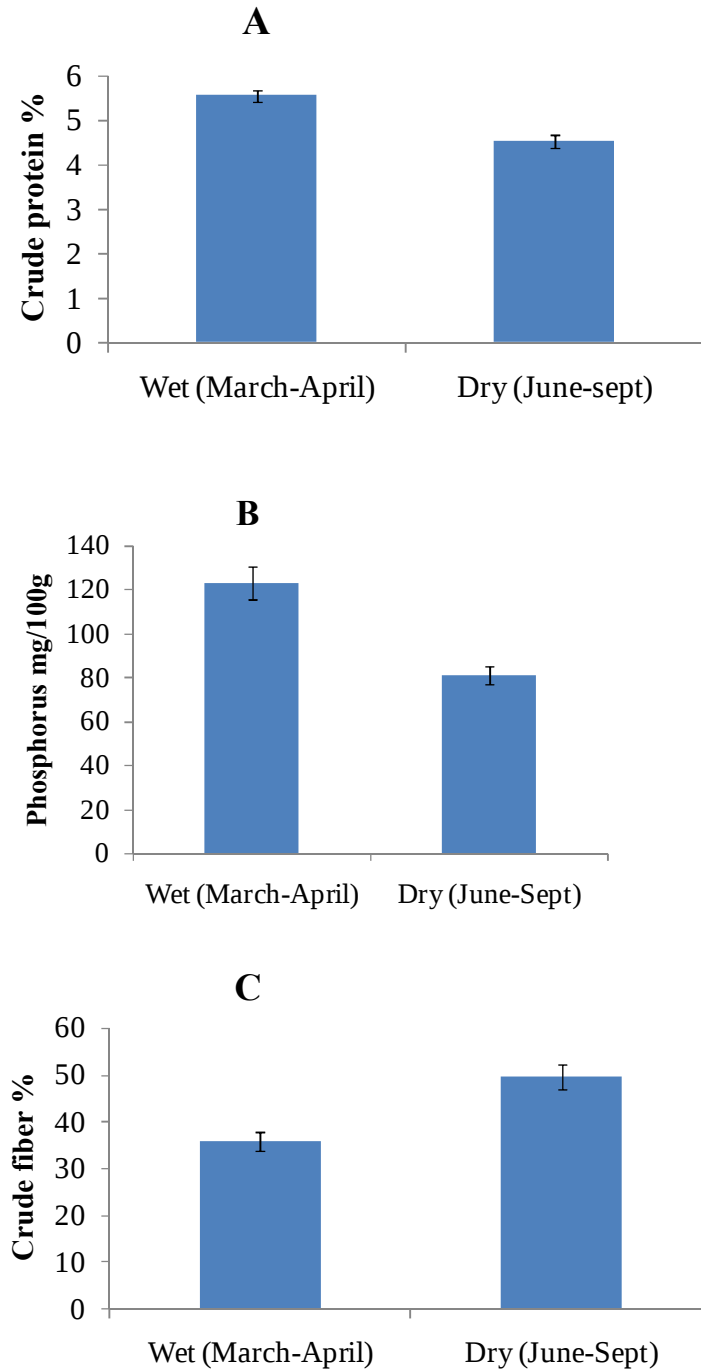


Figure 4.6. Seasonal changes in the quality of the diet of elephants in Etosha plains measured as percentage (%) faecal crude protein (A), mass of phosphorus (mg/100g faecal) and percentage (%) faecal crude fiber. **Note:** Error bars are standard error of the mean.

CHAPTER 5

DISCUSSION

5.1 Foraging efficiency

This study has revealed that zebras had increased foraging efficiency in the wet season compared to the dry seasons (Figure 4.1). Observed differences in the number of bites/step taken by foraging zebras can be explained by seasonal changes in zebra's foraging strategy to maximize forage intake. This seasonal change in the number of bites/step taken by zebras may allow zebras to increase forage intake when available biomass/quality increased. The decisions made by animals at the level of the bite have important consequences to the herbivore's rate of nutrient intake (Black and Kenney, 1984; Spalinger and Hobbs, 1992) and thus its fitness. An ungulate's rate of travel affects its encounter rate with food resources (Shipley *et al.*, 1996), and is directly linked with its optimal energy intake (Murray, 1991). Ungulates therefore, vary their rate of travel according to the quality of the available sward (Murray, 1991). Slower rates of travel indicate the availability of high-quality resources, requiring fewer steps between bites, thereby enabling ungulates to maintain a high instantaneous intake rate (Murray, 1991; Shipley *et al.*, 1996). Faster rates of travel indicate lower-quality resources, forcing ungulates to take more steps between each bite and increasing search rate and frequency of search between selected grazing sites (Shipley *et al.*, 1996). This foraging strategy is consistent with present study results where zebras have taken more bites/step during wet season with high-quality resources than dry season.

This study observation has shown that lactating females take significantly more bites/step than males or non-lactating females (Figure 4.3). The present study results are

similar to the finding of Neuhaus and Ruckstuhl (2002) on plain zebras in Etosha. They reported that lactating females took more bites per minute than either non-lactating females or males. Sundaresan *et al.* (2007) found that lactating female Grevy's zebras use more areas with greener but shorter grass more often than both bachelor groups and non-lactating females. Specific nutrient demands of lactation may drive lactating females to have a higher bite rate and may seek forage of higher quality than males and non-lactating females. While lactating females of some species may compensate by reducing metabolic expenditures (e.g. by reducing locomotor activities, Miller *et al.*, 2006; fasting, Mellish *et al.*, 2000; entering periodic torpor, Racey and Speakman, 1987) or by increasing metabolic efficiency (Mellish *et al.*, 2000), females of most species are expected to increase nutrient intake during lactation (Bunnell and Gillingham, 1985). However, zebras have a high degree of stability and cohesion within family groups with synchronized activity budget. Increased bite rate may be the only option for lactating female zebras to meet their higher energetic and nutritional needs while synchronizing their behaviour and movements with those of the rest of the group (Neuhaus and Ruckstuhl, 2002).

A number of studies on ungulates have confirmed that the time of day, vegetation type and temperature often influence foraging efficiency (Wobeser, 2006). However, this was not the case with zebras in Etosha. Twine (2002) reported that zebras in Suikerbosrand Nature Reserve, South Africa, to maintain a high feeding activity throughout the day, including the hottest period and have probably evolved a high heat tolerance, necessitated by the need to sustain high food passage rate through the gut. Furthermore, zebras are bulk forage grazers and can maintain a less restricted foraging

efficiency (bites/step) when grazing on short or tall swards. Foraging efficiency is a function of body size, mouth and tooth dimensions, stomach structure, digestive capabilities and foraging behavior. This study design did not allow for round-the clock observations on the daily activity of zebras, we only observed activities of foraging zebras. Therefore these factors may affect the overall activity budgets of zebras, but not necessary how they forage.

The results of the present study show that zebras forage for longer per 5 minute feeding sample in dry season than wet season, longer feeding time is correlated with grazing of tall dry grass in the study area. As a larger and unselective grazer feeding on coarser grass, the cropping rate of zebra is limited by its ability to handle (masticate, moisten and swallow) forage. Since chewing and cropping are two competitive processes, it seems likely that the forage intake of zebra can only be limited by handling time. There was no significant difference in time spent grazing, standing or walking by males and lactating or non-lactating females. Neuhaus and Ruckstuhl (2002) found similar results where activity budgets between male and female zebras when grazing, standing or walking were insignificant. These results are consistent with the high degree of stability and synchronization of activity budgets within zebra groups. It has been suggested that in species with a relatively high degree of sexual size dimorphism, males and females have such different activity budgets that it would be energetically difficult for them to stay in the same group, and indeed these species show sexual segregation (Neuhaus and Ruckstuhl, 2002). However, both male and female zebras manage to stay in the same group without sexual segregation. This might be due to similarity in body size in both

male and female zebras, thus making it energetically possible for both sexes to stay in the same group (Neuhaus and Ruckstuhl, 2002).

In Etosha it is proposed that herbivores contract anthrax by foraging at carcass sites where previous victims of anthrax died. If carcass sites serve as the primary source of infection, then the probability that an herbivore contracts anthrax depend on the timing and frequency of their encounters with carcass sites and their foraging intensity at these sites. Preliminary data from camera traps set at carcass sites to monitor herbivory at these sites showed that zebras do encounter and forage at these sites. Moreover, Brooks and Harris (2008) reported zebras to have a well-defined spatial awareness of patch location and can orient their movements towards high-quality patches within their heterogeneous sward resources. The use of direct movement towards high-quality patches implies that Etosha zebras may frequently forage at carcass site (high-quality patches), particularly during wet seasons when they are concentrated in the enzootic area around Okaukuejo. If herbivores enter such a patch (carcass site) the chance of infection will depend upon the number of bites per step (foraging efficiency) taken at that site. An animal that takes more bites/step is more likely to contact *B. anthracis* at a carcass site than one that takes fewer bites/step. Okello (2002) found that zebras have a bite size of 1.93g/bite in wet season, and Turner (*unpublished data*) found spore counts as high as 435,000 spores/g on grass. Therefore our results of zebras taking on average 3.5 bites per step while foraging in the wet season compare to 1.8 bites per step in hot-dry seasons show that zebra have more chance to ingest sufficient numbers of spores while forging in the wet season than in the dry season if they forage at a carcass site. Since animals forage more intensively at nutrient hot spots and carcass sites form nutrient hot spots (Ben-shahar and Coe, 1992),

we suggest that bites/step values at or above our mean value (3.5 bites/step) would likely occur at carcass sites. If zebra were to forage so intensively at a carcass site, they are likely to have a higher exposure to *B. anthracis* than if they foraged less intensively. This then implies that zebras are likely to have the highest exposure to *B. anthracis* during wet seasons, which matches the infection patterns observed. This may in part explain the seasonality of anthrax outbreaks in Etosha where zebra anthrax mortalities peak in wet seasons.

In many species in different ecosystems adult males are more likely to die of anthrax, either as a result of reproductive stressors and/or behavioural differences between males and females (WHO, 2008). The present study observed lactating female zebras to have significantly more bites/step than both males and non-lactating females. This implies that lactating females may have higher risk than both non-lactating females and males to be in contact with the pathogen while foraging. In spite of that, there is no sex difference observed in anthrax occurrence in Etosha zebra. The answer to this is beyond the scope of this study, however ongoing assessment by Berkeley Anthrax Research Project on immunity, reproductive and stress hormones their role and inter-relation in anthrax outbreak may aid in understanding anthrax outbreak in zebras in Etosha.

5.2 Zebra diet selection

Zebras are known to utilize a wide variety of plant species, but invariably a few species are most important in terms of biomass eaten (McNaughton, 1984). Analysis of forage selection by zebras shows exploitation of a large variety of the species in the study area. On the Etosha (Okaukuejo and Halali) plains the total number of plant species consumed

was 19 grass species and 6 shrubs species. In the study in Timbavati area of the Northern Province Lowveld, South Africa, Bodenstein *et al.* (2000) found relatively the same numbers (21 grass species) of consumed plant species by zebras and wildebeest in both wet and dry seasons. In the present study, the diet of zebra was dominated by two grass species namely *Enneapogon desvauxii* and *Eragrostis nindensis* (Table 4.1). Etosha zebras selectively grazed these grass species throughout the year, but increased the consumption of these species during the wet season when they were more available. This result is similar to that of Grobler (1983) and Nowak (1999) who found the diet of zebra to consist of the major grass species in Kruger National Park.

The observed increase in consumption of woody species in the dry season than the wet season was not significant. Zebras are predominantly grazers with a diet composed of 90-92% C4 plants (Sponheimer *et al.*, 2003), and they may not significantly alter the diet to browsing, even though they browse more during dry season to compensate for grass poor quality. This corresponds with work done by Bodenstein *et al.* (2000), their result show that both the zebras and the blue wildebeest browsed on the leaves of *Grewia* spp., probably as a nutritive supplement in Timbavati area, South Africa. In this study zebras showed an increase in grazing of tall grass species during dry season. This finding is in accordance with the survival strategies of zebras during the dry seasons to consume larger quantities of forage with poor quality and process them quickly (Moehlman, 2003). Indeed Sinclair (1982) found that in the dry season in the Serengeti, zebra preferred areas with the tallest grass. Increased consumption of *Aristida* spp by zebras in this study, a genus of grasses rated as highly unpalatable, shows how much zebras opt for biomass over quality during dry seasons. Grobler (1983) and Nowak (1999) found *Heteropogon*

contortus to be one of the dominant grass species in zebra diet during wet season in Kruger, but *H. contortus* was of less relevance in Etosha zebras during the wet season.

Zebras' preference for short grasses during the wet season increases their contact with the ground surface. This behavior can put them at a higher risk for contact with soil-borne pathogens that can be consumed while foraging, such as *B. anthracis* spores. The finding in this study showed that zebras prefer short grass species (*Enneapogon desvauxii*, *Eragrostis nindensis* and *Tragus spp*, Table 4.1) during wet seasons than during dry seasons and may put zebras at a higher risk of exposure to *B. anthracis* in the soil during wet seasons than dry seasons. This is supported by results showing that zebras in Etosha ingest significantly more soil in wet seasons than dry seasons (Imologhome, *unpublished data*). Outside of Etosha, there is evidence in multiple systems for a link between herbivory and anthrax exposure (Dragon, 1995; Wafula *et al.*, 2007). An anthrax outbreak in zebra in Queen Elizabeth National Park and Mburu National Park was associated with lush short green grasses (Wafula *et al.*, 2007). Grazing of short wilted grasses close to the ground surface may have contributed to the timing of anthrax outbreaks in bison in west Canada (Dragon 1995). In addition, the same foraging habits were proposed to contribute to hippopotamus susceptibility to anthrax in Queen Elizabeth National Park and Mburu National Park (Wafula *et al.*, 2007). Moreover, studies have shown that the risk of gastrointestinal parasite infections increase with grazing close to the ground compared with browsing shrubs and trees (Apio *et al.*, 2006).

5.3 Elephant soil ingestion

There are probably many benefits to ungulates from soil consumption (Kreulen and Jager, 1984; Kreulen, 1985; Klaus *et al.*, 1998). Krishnamani and Mahaney (2000) suggested different benefits from geophagy in primates which include supplementary dietary minerals and aid the digestion of some fibrous and hard consumed materials. Soil ingestion may function to supplement dietary mineral deficiencies (Holdo *et al.*, 2002, Mills and Mileswski, 2007), alleviate gastrointestinal disorders (Mahaney 2000; Houston *et al.* 2001) or detoxify unpalatable foods (Houston *et al.*, 2001; Mee *et al.*, 2005). However, increased environmental pollution and the risk of ingesting soil-borne bacteria with soil is high due to restrictions of wildlife into fenced area, making soil ingestion an important means of exposure.

Previous studies on soil-eating behaviour by elephants, other ungulates and primates have shown that they select soils with a high concentration of sodium and other mineral nutrients that may be deficient in their diet particularly during the dry season (Izawa *et al.*, 1990; Moe, 1992; Ruggiero and Fay, 1994). The present study indicated that Etosha elephants ingest more soil during wet seasons than dry seasons (Figure 4.5). This coincided with the time when there was a high quality diet available for Etosha elephants, as supported by our nutrition data (Figure 4.6). This might suggest that Etosha elephants ingest soil inadvertently by ingesting soil attached to grass roots or soil on grass splashed over by rain. Nevertheless, there is some evidence that animals ingest more soil during the wet season not just for nutrient supplement, but to buffer the digestive system in the wet season when highly digestible grasses became available. It was hypothesized that soil ingestion is a means of elephants exposure to *B. anthracis* in the soil during the

dry season. It was hence expected that elephants would ingest more soil during the dry season than the wet season. Unlike zebra, there was no link between the seasonal timing of elephant anthrax in Etosha and aspects of their foraging ecology. Therefore Etosha elephants may be exposed to *B. anthracis* spores in the soil during the dry season through other behaviours not addressed in this study, such as dust bathing. Elephants have a sensitive skin and they may dust bathe more in the dry season to protect themselves from sun burn. This behaviour may exposure elephants at risk for inhalational anthrax during the dry hot season. However further research would be required to investigate the mode of anthrax transmission in Etosha elephants especially the role of inhalational anthrax.

5.3 Elephants diet quality

The quality of diet of elephants fluctuated with seasonal variation in resources in Etosha plains. During the wet season, African elephants largely feed on grass, which is generally higher in digestibility and dietary quality than browse at this time of year, but switch to browse during the dry season when grass becomes fibrous and less nutritious (Cerling *et al.*, 2006). The diet during the wet season is therefore expected to be of higher quality than dry season diet, this was the case as revealed in the present study (Figure 4.6). There was a significant difference in the quality of the diet (faecal crude protein, crude fiber and phosphorus) between the wet season and the dry season. This fluctuation in forage quality consumed has also been observed in east African elephants (Ruggiero, 1992), and Asian elephants (Xiaobao *et al.*, 2006). Elephant crude protein and phosphorous in the diet of elephants from the southern Kruger National Park also showed a dramatic increase from the dry to wet season (Codron *et al.*, 2006). Elephant damage to trees in western Zimbabwe during dry seasons was found to correlate positively with leaf calcium,

magnesium, potassium, and protein (Holdo, 2003). Protein and phosphorus content is higher during wet seasons when there is new re-growth than during the dry season, while crude fiber is higher during the dry season when plants have matured and increased lignin content. Therefore seasonal fluctuation in elephant diet quality may be explained in terms of seasonal variation in resources. Indeed, Woolley *et al.* (2008) found elephants of Pilanesberg National Park showed, South Africa, to drop in body condition during the dry season, but had excellent body condition in the wet season. During the dry season, excessive protein intake is also undesirable for elephants because nitrogen excretion requires more water, which may be in short supply (Sukumar, 1989). Looking for fiber-rich plants such as *Acacia* may be critical for elephants to achieve a proper ratio of protein to fiber and to avoid constipation during the dry and hot season (Xiaobao *et al.*, 2006).

Generally large herbivores tend to ingest a diet lower in quality than smaller herbivores, trading-off quality against quantity, as the benefits of ingesting abundant forage resources outweigh the costs of searching for forage of higher nutritional return which are usually rare in the environment (Demment and van Soest, 1985; Owen-Smith, 1988). Small herbivores are generally more selective feeders, maximizing quality in response to digestive capacity constraints brought about by small body size and higher relative energy demands (Demment and van Soest, 1985). The influence of body size and nutritional requirement could apply to both between species differences (Gordon and Illius 1994, Robbins *et al.*, 1995), as well as to intraspecific differences between juveniles and adults (Munn *et al.*, 2006) and different sexes of mature individuals within the same species (Shannon *et al.*, 2006a). Results of the study by Woolley *et al.* (2009) showed

that elephant's body size influences foraging strategies and quality of diet. The present study showed no difference between diet quality and elephants of different body size. This may be due to small sample size (1/2 size $n=2$; $\frac{3}{4}$ size $n=3$; full size $n=6$). This study faecal sampling from known individual was collected in dry season only and forage resources may be more homogenous in the dry season than the wet season and there might be larger differences in nutrition by age/dominance in the wet season. This may be the reason for no difference in age/dominance in this study.

Musth is a physiologic state of sexual maturity in bulls manifested by observable characteristics including urine dribbling, temporal gland secretions and heightened sexual and aggressive activity (Poole, 1999; Ganswindt *et al.*, 2005). Bulls in musth are typically more restless and eat and drink less than non-musth bulls, and this may create a negative energy balance (Ganswindt *et al.*, 2005). With this possibility, it is expected that bulls in musth will have lower faecal nutrient content than non-musth bulls. Results of the present study however, showed no significant differences in faecal nutrient content (CP, CF and P) between musth and non-musth bulls. This may be due to the small sample size of bulls in musth ($n=3$), because musth is a rare event and bulls in musth do not defecate as often as non-musth bulls (Ganswindt *et al.*, 2005).

We speculate that poorer dietary quality is among some of the many possible explanations for the higher elephant mortality due to anthrax in dry season in Etosha. Ruggiero, (1992) found nutritional stress to contribute to poor body condition in Gounda elephant, central Africa, during dry season and the immunity of animals in poor body condition may be compromised.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In Etosha it is proposed that herbivores in Etosha acquire *B. anthracis* spores when grazing over sites where previous victims of anthrax died and deposited the spores. The objective of this study therefore, was to investigate the role of foraging ecology in anthrax seasonality outbreaks in zebra and elephant in Etosha National Park.

Analyses of zebra foraging efficiency indicate that zebras took more bites per step in the wet season than dry season. This led to the conclusion that zebra may be more susceptible to ingest a lethal dose of spores in the wet season than the dry season when they forage less intensively.

In the present study zebra diet selection showed that zebras predominantly grazed throughout the year. Although zebra browse more during the dry season, the difference was not significant. The results indicated that zebra preferred short grass species during the wet season and tall grass species in the dry season. This may put zebras at a higher risk of being in contact with *B. anthracis* in the soil in wet season when grazing on short grass close the ground surface than during the dry season when grazing on tall grass and browsing on trees and shrubs.

Elephants significantly ingested more soil in the wet season than the dry season. Hence the present study found no link between the seasonal timing of elephant anthrax in Etosha and aspects of their foraging ecology. The conclusion drawn from this was that Etosha elephants may be exposed to *B. anthracis* spores in the soil during the dry season through other behaviour not addressed in this study, such as dust bathing.

Elephant faecal crude protein, crude fiber and phosphorus was significantly higher in the wet season, when there was more re-growth available than during the drier months. It is concluded that low dietary quality stress is among some of many possible explanations for the higher elephant mortality due to anthrax in dry season in Etosha.

All in all, this study prediction built around foraging ecology alone will remain speculative until we consider other aspects of the host-*B. anthracis* interaction. Anthrax outbreak and timing in herbivores include complex and inter-related components: host physiological status, immunological status, age, movement patterns, foraging behaviour and environmental conditions. Thus, anthrax transmission rate is challenging to craft in nature and surely hard to explain in terms of simple ingesting of spores model. However, with these caveats in mind, our main message is that basic tenets of zebras and elephants foraging ecology can produce more powerful understanding of hosts interaction and contact with *B. anthracis* spores in the environment.

6.2 Recommendations

Beyond the obvious insights gained into the role or the relation between foraging behaviour and anthrax outbreak in herbivores, are the implications of the present study results for understanding outbreaks dynamics that have a strong foraging-dependent transmission rate (e.g. worm and botulism infection in mammals, ticks vectors that wait for hosts in vegetation and poisonous plants infections in herbivores, etc). Further, by understanding how foraging behaviour influences the outbreaks of diseases, we may be able to effectively curtail the spread of such diseases with measures that modify where and when animals forage. These measures can be a rotational grazing system, fencing of enzootic sites (e.g. carcass sites); luring of animals to 'safe areas' by controlled burning, and addition of artificial water sources or supplements feeding.

The study results on elephants (mega-herbivore) and zebras (heavy-grazer) foraging behaviours can provide important information which management can apply to predict overgrazing and changes in vegetation structures and composition. Information on the zebra's diet selection and elephant's diet quality may help to identify factors by which these animals select preferred feeding areas and gain vital understanding into observed seasonal distribution of these species in Etosha. Our findings can be used to assess the management implications to a species when conditions of weather, food abundance or food quality are altered especially due to climate change. These effects need to be considered in population management planning, particularly for threatened species where assumptions and predications need to be made at the cohort level rather than a broad species level. It is also important to increase scientific knowledge of the dynamic interactions between animal species and their environment to counteract unexpected

changes in environmental conditions. Foraging information is required to achieve optimal productivity in both wildlife and livestock production by selecting types of grazing/browsing animals compatible with the forage resource.

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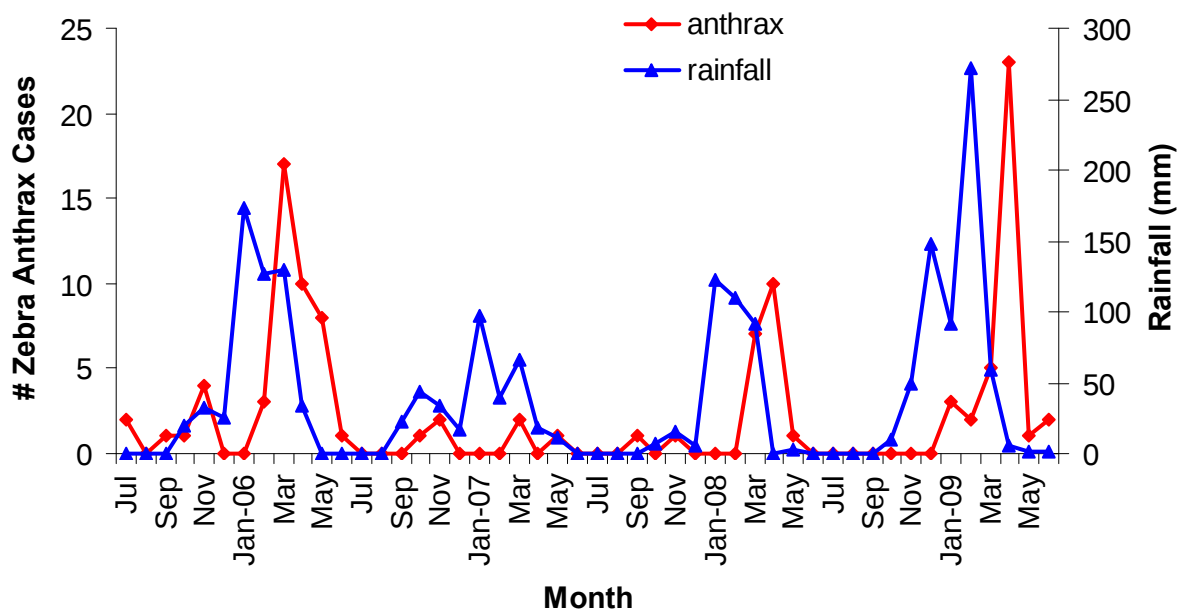
Appendix 1. Comparison of difference of the factors that affected bites per step taken by zebras during wet-hot, cold-dry and hot-dry seasons in Etosha plains.

Source	Nparm	DF	SE	P
Season	1	1	0.039	0.001
Time of the day	1	1	0.018	0.759
Temperature	1	1	0.027	0.730
Vegetation type	3	3	0.042	0.162

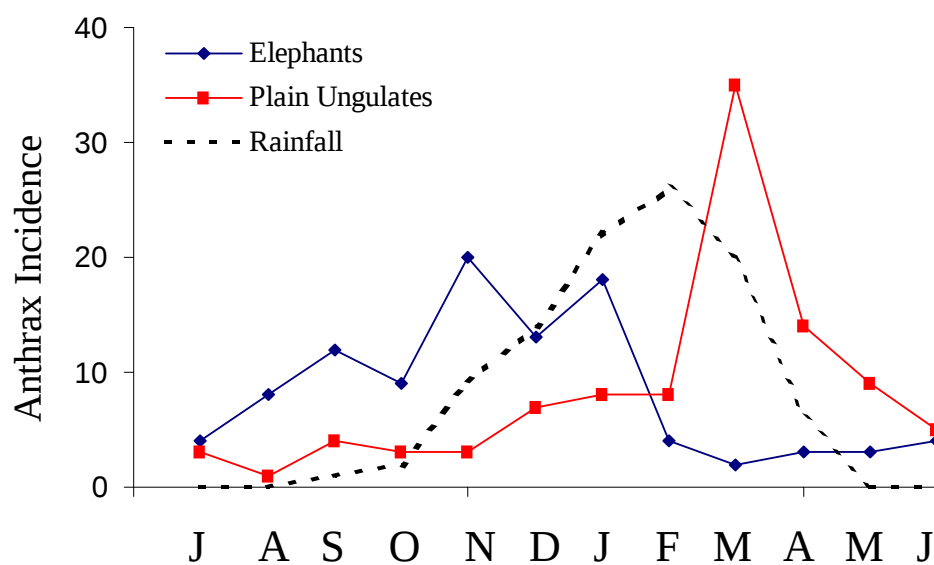
Appendix 2. Anthrax cases in herbivores in Etosha National Park, 1975-2010

Species	Anthrax cases
Plains zebra	944
Elephant	333
Wildebeest	294
Springbok	283
Gemsbok	20
Greater kudu	10

Black rhino	9
Giraffe	7
Red hartebeest	3
Eland	2
Impala	1



Appendix 3. Monthly rainfall and anthrax cases in zebra within the enzootic site (Okaukuejo) in Etosha National Park.



Appendix 4. Monthly rainfall (2009) and anthrax cases in elephant and plains ungulates within the enzootic site (Okaukuejo) in Etosha National Park.