

**DUST BATHING BEHAVIOURS OF ELEPHANTS, ZEBRAS
AND WILDEBEEST AND THE POTENTIAL RISK OF
INHALATIONAL ANTHRAX IN ETOSHA NATIONAL PARK**

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ABSTRACT

Bacillus anthracis is a soil-borne bacterium and the causative agent of the disease anthrax which commonly infects herbivorous animals. The disease is transmitted via three routes of infection: ingestion, cutaneous or inhalation of spores. This study investigated the seasonality of dust bathing as a possible contributor to inhalational anthrax in zebra, blue wildebeest and African elephants, in relation to the seasonality of anthrax mortalities in Etosha National Park. Most anthrax cases are observed during the wet season, although elephant anthrax mortalities occur primarily during the dry season. The study was conducted from May 2013 to July 2014 focusing on the three herbivorous host species that dust bathe (African elephants, *Loxodonta africana*; plains zebra, *Equus quagga*; and blue wildebeest, *Connochaetes taurinus*). The objective was to determine the dust bathing behavioural patterns (seasonality and age and sex of individuals) and to correlate that with anthrax cases in the park to assess whether the three study species may be at risk of inhalational anthrax through dust bathing. Motion triggered cameras were positioned at dust bathing sites of zebras and wildebeest, while elephants dust bathing behaviour was studied through observations at four selected waterholes. Given that mud bathing is also a potential means of soil contact by elephants their mud bathing behaviours were included in the study. Polymysin-lysozyme-EDTA-thallos acetate (PLET) agar was used to grow and quantify *B. anthracis* spores concentration from surface soil at dust bathing sites.

The findings showed that zebra dust bathed significantly more in the dry seasons than in the wet season. There was no significant difference among the seasons in dust

bathing by wildebeest. Elephant dust bathing behaviours showed no relationship with maximum daily temperature. Mud bathing on the other hand increased with temperature, However the intensity of both mud and dust bathing by African elephants did not increase as temperature increased.

To investigate whether dust bathing contributes to inhalational anthrax, 83 dust bathing soils were screened for *B. anthracis* only 2 were found positive with low concentrations of 20 and 10 spores/g.

Firstly as a result of poor relationship between the seasonality of dust bathing and the timing of anthrax mortalities, secondly the low to no spore counts of *B. anthracis* in dust bath sites and, thirdly the finding that carcass sites are unlikely to become future dust bathing locations, it is unlikely for dust bathing behaviours to serve as an important risk for inhalational anthrax for herbivores in Etosha National Park.

Key words: *Bacillus anthracis*, anthrax, dust bathing, mud bathing, Etosha National Park

All maps and graphs in this thesis are produced by the author unless otherwise stated.

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ABBREVIATION

| | |
|------|-----------------------------------|
| AF | Adult female |
| AM | Adult male |
| AU | Adult unknown |
| °C | degree Celsius |
| DBS | Dust bathing site |
| Db | dust bath |
| EDTA | Ethylendiaminetetraacetic acid |
| EEI | Etosha Ecological Institute |
| EF | Oedema factor |
| ENP | Etosha National Park |
| FAO | Food and Agriculture Organisation |
| GPS | Geographical positioning system |
| J | Juvenile |
| LD | Lethal dose |
| MID | Minimum infectious dose |
| PA | Protective antigen |

| | |
|------|--|
| PLET | Polymixin-lysozym-EDTA-thallos acetate |
| RCF | Relative centrifugal force |
| SA | Sub-adult |

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DECLARATION

I, Zoe R Barandongo, 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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Zoe R Barandongo

CHAPTER 1: INTRODUCTION

1.1 Orientation of the study

Anthrax is a fatal disease of mammals caused by *Bacillus anthracis*. The pathogen is a soil-borne, gram-positive, rod-shaped bacterium that forms spores which persist in the environment (Coker *et al.*, 2003). The disease is primarily of herbivorous livestock and wildlife species (Friedman & Yabuku, 2012). Carnivores may also become infected. However, they are generally less susceptible to anthrax with disease related illness and death being rarer than herbivores even though they can feed on *B. anthracis*-contaminated carcasses (Bellan *et al.*, 2012; Fiedman & Yabuku, 2012). Human infection with *B. anthracis* is typically zoonotic (Dey, Hoffman & Glomski, 2012), meaning it is a disease which is transmitted from animals to humans (World Health Organisation (WHO), 2008). Anthrax can manifest in three forms: pulmonary anthrax (through inhaling *B. anthracis* spores), gastrointestinal anthrax (through ingestion) and cutaneous anthrax (through skin). Of the three forms, cutaneous anthrax is most common among humans but least deadly, while pulmonary anthrax is least common but is the deadliest (WHO, 2008).

During the late 19th century and early 20th century, anthrax was one of the foremost causes of uncontrolled mortality in livestock and wildlife (Hugh-Jones & De Vos, 2002). However, with the successful development of antibiotics, the effective live-stock vaccine by Stern in 1937 and the implementation of quarantine regulation,

there has been a decline in anthrax mortalities in livestock (de Vos & Turnbull, 2004). Due to the practical challenges of vaccinating free-living wild animals, anthrax remains ecologically important in free-ranging wildlife in several parts of the world (Magwedere, Hemberger, Hoffman & Dziva, 2012).

Herbivorous species that come in contact with anthrax spores and die as a result include plains zebra (*Equus quagga*), kudu (*Tragelophus strpsiceras*), African elephants (*Loxodonta africana*), blue wildebeest (*Connocheates taurinus*), springboks (*Antidorcas marsupailis*), roan antelope (*Hippotragus equinus*) and other large mammals in southern Africa (Clegg *et al.*, 2007 and Turner *et al.*, 2013).

Anthrax infections are sporadic and it is known as a seasonal disease with climatic factors such as rain recognised as important triggers of outbreaks (Turner *et al.* 2013). Anthrax related mortalities in grazing herbivores have been assumed to primarily be transmitted through the ingestion of *B. anthracis* spores through contact with contaminated soil (Nicholson, 2002) or vegetation (Turner *et al.*, 2014) although dust bathing has been postulated to be a possible cause of inhalational anthrax for some species (Turner *et al.*, 2013, Mackintosh, Haigh & Griffin, 2002). Bison (*Bison bison*) in Canada are an example of animals that are assumed to contact anthrax spores through wallowing, sometimes referred to as dust bathing (Dragon, Rennie & Elkin, 2001).

From the herbivorous species that are infected by *B. anthracis*, African elephants are known to take dust baths (Rees, 2002), as are zebras (Yahya, 2004). Wildebeest have also been observed dust bathing by previous researchers in Etosha National Park, thus they were selected for this study.

The dust bathing sites of zebra are commonly found near waterholes or along game paths. This was also personally observed for wildebeest. These dust bathing sites are characterised by bare open patches of soil devoid of vegetation (Carnaby, 2010). Figure 1 shows an example of a dust bathing sites for zebras.

Zebras, wildebeest, African elephants and springbok are the main host of anthrax in Etosha National park (ENP). Etosha Ecological Institute (EEI) initiated recording mortalities in 1976 as a result of anthrax in ENP in the host species. Surveillance of the disease indicate that the seasonality in the park with deaths in zebra and blue wildebeest peaks towards the end of the wet season (March –April) while African elephant mortalities from anthrax peak towards the end of the dry season (October–November) (Lindeque &Turnbull 1994; Beyer *et al.*, 2012; Turner et al., 2013). This is shown in Table 1.

Table 1: Anthrax cases of study species in Etosha National Park from 1975 – 2013 (Etosha Ecological Institute, 2014). The hot-wet season counts were excluded in data analysis for African elephants.

| Species | Hot-wet | Cool-dry | Hot-dry | Total cases |
|-------------------|---------|----------|---------|-------------|
| Zebra | 754 | 168 | 108 | 1 030 |
| Blue wildebeest | 159 | 95 | 45 | 299 |
| African elephants | 78 | 88 | 170 | 336 |

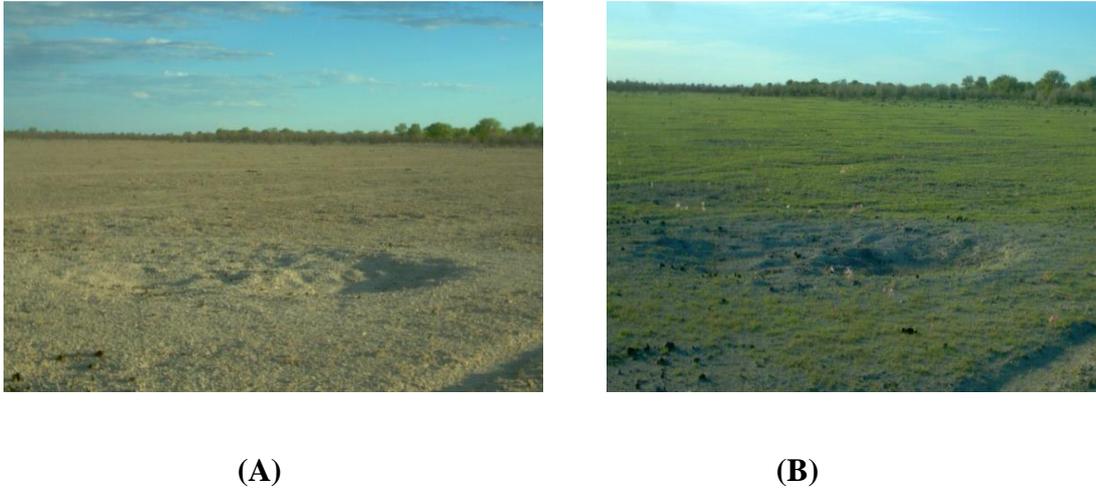


Figure 1: A zebra dust bathing site. The left panel (A) indicates the site during the dry season and the right panel (B) is the same dust bathing site during the rainy season.

Elephants do not visit these defined dust bathing sites, but rather dust bathe at seemingly random sites where it is possible for them to scoop soil. Elephants also get potential soil contact through mud bathing, because elephants at waterholes with mud bathe, but not the other species (personal observation).

Animals may dust bathe for a good scratch and to relieve themselves of the irritation of parasites, such as ticks and fleas which may come off as the animal rubs its body against the ground and rolls on its sides and back (Carnaby, 2010). During dust bathing, the disturbed soil creates clouds of dust which may carry air-borne microbial particles. If *B. anthracis* spores are present in the soil, it may be possible for dust bathing animals to inhale the anthrax bacterium. This hypothesis has not been explored or tested before for any animal species, to the best of our knowledge on the basis of published evidence.

In April 2012 a dead zebra was found by researchers in ENP on a dust bathing site in the Okaukuejo area and was confirmed to have died of anthrax. This incident was the inspiration for this study: to understand if such dust bathing sites (and others like it) would expose dust bathing individuals to potentially inhale lethal doses of *B. anthracis*. Herbivorous wildlife and livestock are generally thought to acquire gastrointestinal anthrax from ingesting the bacterium with food or water. This study evaluated whether inhalational anthrax may also be important to the ecology and epidemiology of anthrax in dust bathing species in Etosha National Park.



Figure 2: A zebra that died of anthrax on an active dust bathing site in April 2012. Photo credit: Gabriella Flacke.

To explore the role of dust bathing for anthrax transmission, this research will 1) investigate the ecology and seasonality of dust bathing behaviours of zebra, wildebeest and elephants in ENP and 2) survey dust bathing sites for *B. anthracis* to determine the possibility of inhaling *B. anthracis* spores during dust bathing.

1.2 Problem Statement

The inhalational route of anthrax infection has been well documented for human infection (e.g., Collier & Young, 2003). However, this has not been thoroughly studied for wildlife or domestic animals. A study by Turnbull *et al.* (1998) on the airborne movement of anthrax spores from carcass sites due to wind disturbance showed that spores may be carried by wind through the air before being re-deposited on the ground. However, the concentrations of spores aerosolized by wind are low. This demonstrated that aerosolising of *B. anthracis* spores from more active soil disturbance than wind, such as dust bathing, could lead to greater exposure to the pathogen, and hence, transmission of inhalational anthrax. It has been hypothesized that under dry conditions, water movements concentrate anthrax spores into low lying areas of the landscape and that animals may contract anthrax when they utilise these sites during activities such as dust bathing (Hugh-Jones & Blackburn, 2009; Dragon, Bader, Mitchell & Woollen, 2005). The present study investigated the potential role of dust bathing behaviours in the transmission of anthrax through the inhalational route in selected herbivores. This will fill a gap in the knowledge regarding transmission of anthrax in ENP and provide important information about

the potential links between dust bathing and inhalational anthrax for dust bathing animal species in other systems.

1.3 Objectives of the study

- (a) To determine the seasonality of dust/mud bathing behaviours of zebras, wildebeest and elephants.
- (b) To determine whether seasonal peaks in dust/mud bathing behaviour correlate with peaks in anthrax cases for zebras, wildebeest and elephants.
- (c) To document the age-sex of dust/mud bathing herbivores.
- (d) To determine whether *B. anthracis* spores are present in dust bath sites of herbivores.

(Note: Mud bathing behaviours were investigated only for elephants)

1.4 Research hypotheses

- (a) (i) Zebra, wildebeest and elephants predominantly dust bathe during the hot dry season when the soil is dry and soft and temperatures are high.

- (ii) Elephants mud bathe during the hot-dry season when temperatures are high.

- (b) There is no significant positive correlation between the seasonal numbers of anthrax cases and seasonal number of dust baths of zebras and wildebeest because their mortalities occur mostly during the wet season. In elephants, there is a significant positive correlation between the seasonal numbers of anthrax cases and dust and mud baths because anthrax elephant mortalities have been mostly observed during the dry seasons.

- (c) Male adults and sub adults dust/mud bathe significantly more often than adult females or juveniles in all three species, because male adults move around mostly in solidarity.

- (d) Soils at dust bathing sites contain significantly low or no concentrations of *B. anthracis* spores since *B. anthracis* is rarely found outside of known carcass sites (Lindeque & Turnbull, 1994).

(Note: Mud bathing behaviours were investigated only for elephants)

1.5 Significance of study

Dust bathing has been suggested as a possible behaviour that may put some species, like elephants, at risk of contracting anthrax (Turner *et al.*, 2013), yet there is

no empirical evidence on natural exposures to inhalational anthrax in domestic or wild animals. There is also very little information on the ecology of dust bathing behaviours, let alone on how these behaviours may influence contact with soil-borne pathogens. Thus, this study investigated the ecology and epidemiology of anthrax through dust bathing and whether this behaviour puts herbivores at risk of inhalational anthrax. Information on inhalational anthrax will enable a broader understanding of disease transmission that will be important for the management of anthrax outbreaks.

1.6 Study limitations

One challenge of this study was the recording of the dust bathing behaviours and *B. anthracis* exposure risk for elephants. This species does not use clearly defined dust bathing sites as do zebra and wildebeest, but have been observed dust and mud bathing around water points (W. Kilian and G. Shatumbu, personal communication, 2013). Therefore, elephants dust and mud bathing behaviour were monitored by observations at waterholes, not camera traps which were used to continuously monitor zebra and wildebeest dust bathing sites. Elephants also used the Okaukuejo study area only during the cool dry and hot dry seasons and dispersed into inaccessible areas during the wet season (W. C Turner, personal communication, 2013). Therefore the dust bathing behaviour of elephants could not be monitored year round. Hence, in this study, recording of dust and mud bathing behaviour of elephants was conducted only during the dry season, comparing how temperature

affects their behaviours. It was further a challenge to collect soil samples where elephants dust or mud bathe to test for *B. anthracis*, because of concerns for personal safety working around elephants, as well as efforts not to disturb tourists or other animals visiting the waterholes.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Anthrax has been known for millennia, the earliest record of the disease is thought to be from the Bible in the book of Exodus chapter 8 and 9 referring to the fifth and six plague of Egypt (Smith *et al.*, 2000; Hugh-Jones & De Vos, 2002; De Vos & Turnbull, 2004). The multi-host pathogen *B. anthracis* has caused mortalities to animals both livestock and wildlife. Humans also contract the disease directly or indirectly (WHO, 2008). Therefore anthrax is of concern for animal conservation, wildlife management, and public health. The anthrax bacillus became the subject of ground-breaking 19th century microbiological studies of infection and prevention of diseases by R. Kock and L. Pasteur (Schuch & Fischetti, 2009) because of its impact on livestock production. *B. anthracis* is amongst the list of pathogens that could be used as bioweapons (Johnson, 2006).

Bacillus anthracis is a soil-borne pathogen that characteristically has two life forms, the vegetative form within a host and the non-vegetative endospore form in the environment. These endospores do not undergo any metabolic activity and are highly resistant to radiation, chemicals, desiccation and heat (Edwards, Clancy & Baeumner, 2006). Anthrax spores have the ability to remain viable and infectious in the environment for years until coming into contact with a susceptible host and initiating a new cycle of the disease (Dragon *et al.*, 2001). The process of sporulation

is important for the survival of *B. anthracis* and for epidemiological and ecological studies.

There are three modes through which the pathogen can infect its host. The primary mode of infection in animals is thought to be through ingestion of *B. anthracis* spores, and secondly through cutaneous means by biting flies (Hugh-Jones & Blackburn, 2009). The possible inhalation of spores by animals while grazing close to dusty soils (WHO, 2008) or through dust bathing (Turner *et al.*, 2013) has been suggested but no studies have been conducted to test for the role of inhalational anthrax in the epidemiology of this disease.

This literature review thus focuses on the bacterium *B. anthracis*, the disease anthrax and its transmission in herbivores and dust bathing behaviours in herbivores.

2.2 Dust bathing

Animal behaviour is an important mechanism which contributes to the way animals adapt to different environmental conditions. Dust bathing has been a subject of much research in avian species (Van Rooijen, 2005; Roll, Levrino & Briz, 2008; Jensen, 2009), with little focus on large mammals. Literature on dust bathing in avian species is more on the behaviour and hen housing, however dust bathing has not been thoroughly studied as an exposure to soil-borne pathogens.

Asian elephants have been found to dust bathe in response to high temperature in order to reduce the heat load (Rees, 2002). Elephants also frequently cover their body with mud to help keep them cool for long periods of time and get rid of flies, which

irritates during their movements especially during the monsoon period (Joshi, 2009). Animals may also dust bathe to get rid of ectoparasites as they rub their body against the soil particles or they may dust bathe as a skin care mechanism (Carnaby, 2010). Avian species not only dust bathe to remove ectoparasites but dust bathing also serves to remove dirt and excess oil from the feathers and to improve structures of feathers (Jensen, 2009). Once a suitable site for dust bathing is chosen, animals use it until eventually all the vegetation is removed (Van Rooijen, 2005).

Generally, fine soils such as sand or peat are preferred as substrates for dust baths (Van Rooijen, 2005). Dust bathing in hens starts with lying down and pulling loose substrate close to its body by rubbing itself on the substrate and then shaking its wings and body to toss the soil on its back and work it through the feathers (Jensen, 2009). Asian elephants dust bathe by pinching soil with the side of their trunks and throwing it onto their body (Rees, 2002). By definition of Reinhardt (1985), dust bathing by bison involves the animal lying on the ground and rolling towards its dorsum (Copperedge & Shaw, 2002). Different species have different ways of dust bathing and perhaps do so for different benefits with a common factor of a behaviour that brings the animal's body in close contact with soil.

In regards to animal age, seasonality and gender, older hens dust bathe more intensively (Roll, Levrino & Briz, 2008). Dust bathing in hens takes place early afternoons, on average for about 30 minutes every 2 – 3 days (Van Rooijen, 2005; Jensen, 2009). Bison dust bath aggressively during the summer and is practiced mostly by adult bulls and cows (Copperedge & Shaw, 2000).

Dust bathing causes disturbance in the soil that generates a cloud of dust. A study conducted by Turnbull et al., (1998), showed that *B. anthracis* could be isolated from aerosolised soils from carcass site by mechanically aerosolising carcass site soil using cyclone filters. This positively indicated that the *B. anthracis* spores can be aerosolized. However, no studies have looked at how dust bathing behaviours may relate to exposure to soil borne pathogens.

2.3 Background on anthrax and *B. anthracis*

Bacillus anthracis is the causative agent of anthrax. It is a gram-positive, rod-shaped, aerobic, and immobile spore-forming bacterium that is closely related to several *Bacillus* species, such as *B. cereus* and *B. thurengiensis* (Vahedi, Moazeni, Kianizadeh & Mahmoudi, 2009). The bacterium is classified under Family Bacillaceae (Fasenella, Galente, Garofola and Jones, 2008). The virulence of *B. anthracis* is distinguished by the presence of two plasmids, pXO1 and pXO2 (Koehler, 2009). The pXO1 plasmid is known as the toxin plasmid because it encodes for the lethal factor (LF) and the oedema factor (EF), two virulence factors that bind to the protective antigen (PA) and work together to kill the host cells (Pilo & Frey, 2011; WHO, 2008). The pXO2 plasmid encodes for the poly- γ -D-glutamic capsule which protects the bacterium from phagocytosis (Pilo & Frey, 2011; WHO, 2008). The host eventually dies of septicaemia (WHO, 2008). The vegetative cells

are released into the environment after death, sporulate, and after contact with a new host, the cycle repeats.

Early literature (Van Ness, 1971; Kaufmann, 1990), argued that *B. anthracis* could replicate in the soil. This has subsequently changed and it is believed that *B. anthracis* is found in its non-vegetative form and not to have replication taking place in the environment (WHO, 2008). Nevertheless, there is growing evidence of activities of *B. anthracis* outside an animal host. Dey, Hoffman & Glomski, (2012), demonstrated that *B. anthracis* interacts with soil microbes such as soil dwelling amoeba. Salie & Koehler (2006) also found vegetative *B. anthracis* cells in the rhizosphere of grass plants stating that *B. anthracis* can respond to germinants in the rhizosphere of plant seedlings in a way that is similar to *Bacillus cereus*. *Bacillus anthracis* life cycle is generally known to have the vegetative bacillus sporulating after host death and remaining in environment soils for years until encountering its next host (Schuch & Fischetti, 2009). It is often described as a short term vegetative burst in an infected host alternating with long periods of spore form in the environment until the disease is re-established in a new host.

Bacillus anthracis spores can persist in the environment and most literature agrees that anthrax is a seasonal disease in animals (Clegg *et al.*, 2007; Hampson *et al.*, 2011; Chikerema, Matope and Bhebhe, 2012). Thus, it is unlikely that pathogen concentrations are changing seasonally, but rather the means by which animals contact the pathogen is changing seasonally.

Anthrax outbreaks tend to have seasonal signals within a particular locality, although the seasons in which most cases occur vary from locality to locality. The seasonality variations from locality to locality were reviewed by Hampson *et al.*, (2011)

Anthrax seasons are reported to be characterised by hot-dry weather which stresses animals to reduce their innate resistance to infections thus allowing low doses of spores to be infective (Hugh-Jones & Blackburn, 2009). However anthrax is not only limited to hot-dry weather. Anthrax outbreaks occur during the wet season in localities such as Etosha National Park in Namibia (Turner *et al.*, 2013) and Lake Mburo National Park in Uganda (Wafulu, Patrick & Charles, 2008). In Etosha National Park, these wet season anthrax mortalities occur in plains ungulates (plains zebra, springbok and blue wildebeest) (Turner *et al.*, 2013) In Mburo National Park, they occur in plains zebra (Wafulu, Patrick & Charles, 2008).

The mechanisms behind why seasonality occurs and why it varies among species and localities remain unknown (Turner *et al.*, 2013).

Infection of hosts can occur through any of the three routes as shown Figure 3 below depending in the mode of contact. Spores are generally shed upon death when scavenging takes place and blood is spilled onto the environment (WHO, 2008). Scavenging is not the only way in which spores are released into the environment. Bloating of unscavenged carcasses may also be sufficient to rupture the skin of the animal depending on the temperature and skin thickness (Bellan, Turnbull, Beyer and Getz, 2013).

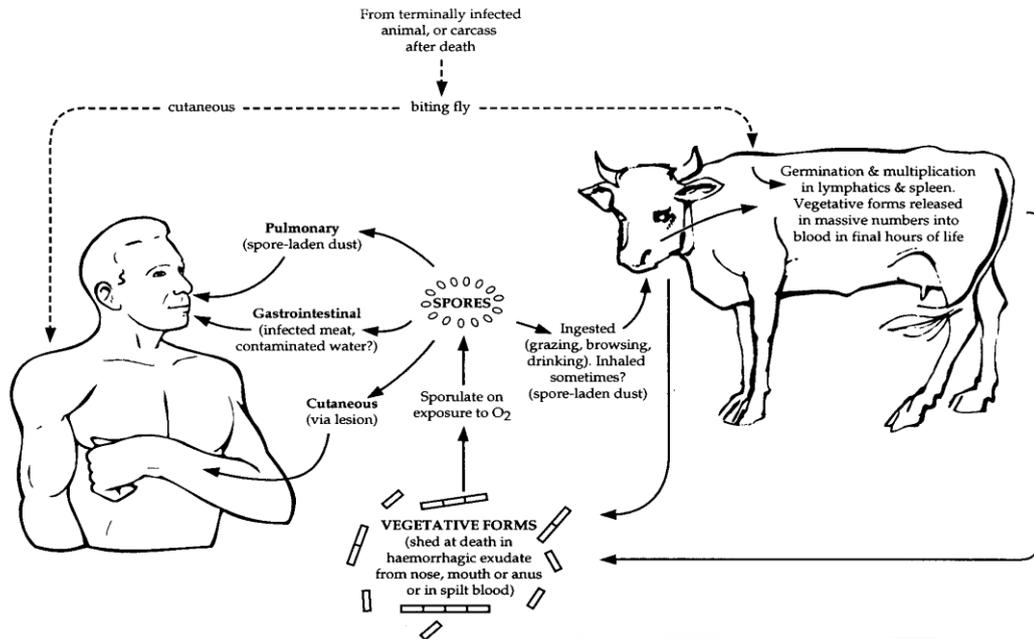


Figure 3: The infectious cycle of anthrax. Contact with anthrax spores by humans is known in all three forms. The image also shows that animals primarily contact anthrax through ingestion of spores and that the inhalational route remains unexplored (Figure reproduced from WHO, 2008).

2.4 Contact with *Bacillus anthracis*

In contrast to herbivores, carnivores are generally more resistant to anthrax, producing antibodies to protect them from lethal infections as they can frequently feed on infected carcasses (Bellan *et al.*, 2012). However they may still die on occasion from anthrax infections (WHO, 2008). Herbivores are thought to be poorly

resistant to *B. anthracis* toxins seen that deaths of animal once infected to lethal dose occur within few days to two weeks following a lethal dose (WHO, 2008). Low antibody titres against anthrax have been observed in herbivores, but these are considerably lower than those in carnivores and likely indicate the exposure to sublethal doses of the pathogen (Cizauskas, Bellan, Turner, Vance and Getz, 2014; Bellan *et al.*, 2013). A recent study on antibody response against anthrax by herbivores in ENP found that zebra have more measurable anti-PA antibodies titres in the wet season compared to the dry season, indicating that exposures rates to *B. anthracis* differ between seasons (Cizauskas *et al.*, 2014). Cizauskas *et al.*, (2014) further concluded that zebra in ENP may become infected with sublethal doses of *B. anthracis* and survive. Inhaled spores are also assumed to remain in ungerminated form in lungs for months after uptake (WHO, 2008).

Transmission in herbivores largely occurs indirectly as a result of exposure to spores rather than animal -to- animal transmission (Hampson *et al.*, 2011). *Bacillus anthracis* has been confirmed in a wide range of herbivores and causes mortalities in herbivorous species. Anthrax transmission and outbreaks can be distinguished between those affecting primarily grazing herbivores and browsing herbivores. For example, grazing herbivores like plains zebra (*Equus quagga*) and blue wildebeest (*Connochaetes taurinus*) are assumed to get anthrax through ingesting soil containing *B. anthracis* spores when feeding on heavily utilised short grasses or herbs (Clegg *et al.*, 2007). Recent research has shown that these species are attracted to anthrax carcass sites for grazing (Turner, Kausrud, Krishnappa, Cromsigt, Ganz, Mapaure, Cloete, Havarua, Küsters, Getz and Stenseth, 2014). However, for

browsing herbivores, for example, greater kudu (*Tragelaphus strepsiceros*) or white tailed deer, anthrax infections have been linked to large number of blowflies, which after feeding on infected carcasses rest on nearby trees and shrubs, defecating and regurgitating *B. anthracis* onto the vegetation (Braak & de Vos, 1990; Clegg *et al.*, 2007).

The site of death of an infected animal remains the area with the highest *B. anthracis* spore contamination, and the most likely site for future infections (Dragon *et al.*, 2005). Not only do these sites have high counts of *B. anthracis* spores of about 10^5 to 10^8 spores per gram (spg) (Bellan *et al.*, 2013), but they also attract herbivores (Turner *et al.*, 2014). Animals such as zebra, wildebeest and springbok are up to 4 times more likely to graze at sites where zebras died of anthrax than grassland control increasing their risk of exposure to *B. anthracis* sites (Turner *et al.*, 2014).

2. 5. Anthrax in Namibia

Sporadic anthrax outbreaks were recorded across Namibia since the 1870s (Ebedes, 1976). Between the periods of 1920 to 1971, about 1541 anthrax cases were confirmed on White-owned farms (Ebedes, 1976). However, livestock were regularly vaccinated against the disease (Ebedes, 1976).

Etosha Ecological Institute (EEI) in Etosha National Park (ENP), under the Ministry of Environment and Tourism (MET), in 1976 initiated electronically documenting anthrax mortalities including mortalities due to other causes such as old age, predation etc. Anthrax cases continue to occur outside ENP.

Anthrax has been recorded in both livestock and wildlife, in communal farming communities and national parks. Although ENP perimeters are fenced, animals, especially elephants do occasionally break through the fence. (Beyer *et al.*, 2012). This therefore promotes spread of anthrax between ENP and farms located in close vicinity to the park. For instance Beyer et al., (2012) isolated GT13 strains of *B. anthracis*, from a farm abutting the ENP, which was closely related to GT6 which could be an indication of the spread.

Mortality due to anthrax now regularly occurs in domestic and wild animals in the Caprivi region and also occasionally affects humans. In 2004 anthrax was recorded to kill 11 wild animals in the Caprivi area that caused a spread to livestock in which 9 cattle mortalities were reported (Shigwedha, 2004). During this outbreak the Ministry of Agriculture, Water and Forestry took protective measures by vaccinating all cattle (Shigwedha, 2004). More recently during January 2013 an anthrax outbreak was reported in the Oniipa Constituency in the Oshikoto region (Windhoek Staff Reporter, 2013). Two people died of anthrax during the outbreak while other received treatment (Windhoek Staff Reporter, 2013).

The Directorate of Veterinary Services of the Ministry of Agriculture, Water and Forestry started a mass livestock vaccination campaign in the area (Kleinhans, 2013).

2.6 Host susceptibility to *B. anthracis*

Host susceptibility to *B. anthracis* depends on host species, strains and the route of infection (Yadav, Pradhan, Kapoor, Bangar, Burzynski, Prow & Levin, 2011). Some animals such as rats are very sensitive to *B. anthracis* toxin but are difficult to infect by spores, while others such as guinea pigs are more resistant to toxins yet can be killed with few spores (Yadav *et al.*, 2011). The required amount of spores to cause an infection in animals was found to be dependent on the route of entry. Inhalation LD₅₀ (the dose required to kill half of the exposed population) in guinea pigs is about 16 650 – 40 000 spores while that of ingestion exceeds 10⁸ Spores (WHO, 2008), thus fewer spores are required for inhalation than ingestion route of infection

The minimum infectious dose (MID) estimate of *B. anthracis* is largely unknown for wildlife (Hugh-Jones and de Vos, 2002). Even though MID can be experimentally established, a MID of *B. anthracis* in a controlled laboratory setting is difficult to relate to exposures that herbivores are likely to encounter naturally (WHO, 2008). The inhalation MID and (LD₅₀) by anthrax in wildlife is highly unknown. In the 1940's the British biological warfare established that the aerosol MID for sheep was 3.5 x10⁴ spores and that the dose needed to ensure lethal infections via the oral route in sheep and horses and cattle was 5 x10⁸ spores (WHO, 2008).

In conclusion the *B. anthracis* pathogen and the disease anthrax have been studied for years, yet much remains unknown. It is well knowledgeable that *B. anthracis* is

transmitted through three modes in human hosts (Who, 2008). On the other hand, the transmission of anthrax in animal host has only been confirmed to occur through ingestion of spores while grazing (Turner *et al.*, 2013) and cutaneous route through biting flies (Hugh-Jones & Blackburn, 2009). The possibility for herbivorous mammals to inhale *B. anthracis* spores has been in suggestion (Turner *et al.*, 2013). Seen that dust bathing creates clouds of dust while rolling (Coppedge & Shaw, 2002), rubbing their bodies (Jensen, 2009) or throwing soil onto their bodies while dust bathing (Rees, 2002), therefore illustrating the importance of this study.

CHAPTER 3: MATERIALS AND METHOD

3.1 Study area

The study was carried out in Etosha National Park (ENP) in the north-central part of Namibia as shown in Figure 4. The park covers an area of about 22,915km² (Cizauskas *et al.*, 2014), of which about 4,410km² makes up the salt pan, that contains no woody plants and accumulates water only during the rainy season. The park has 63 artificial and natural water points (de Beer *et al.*, 2006).

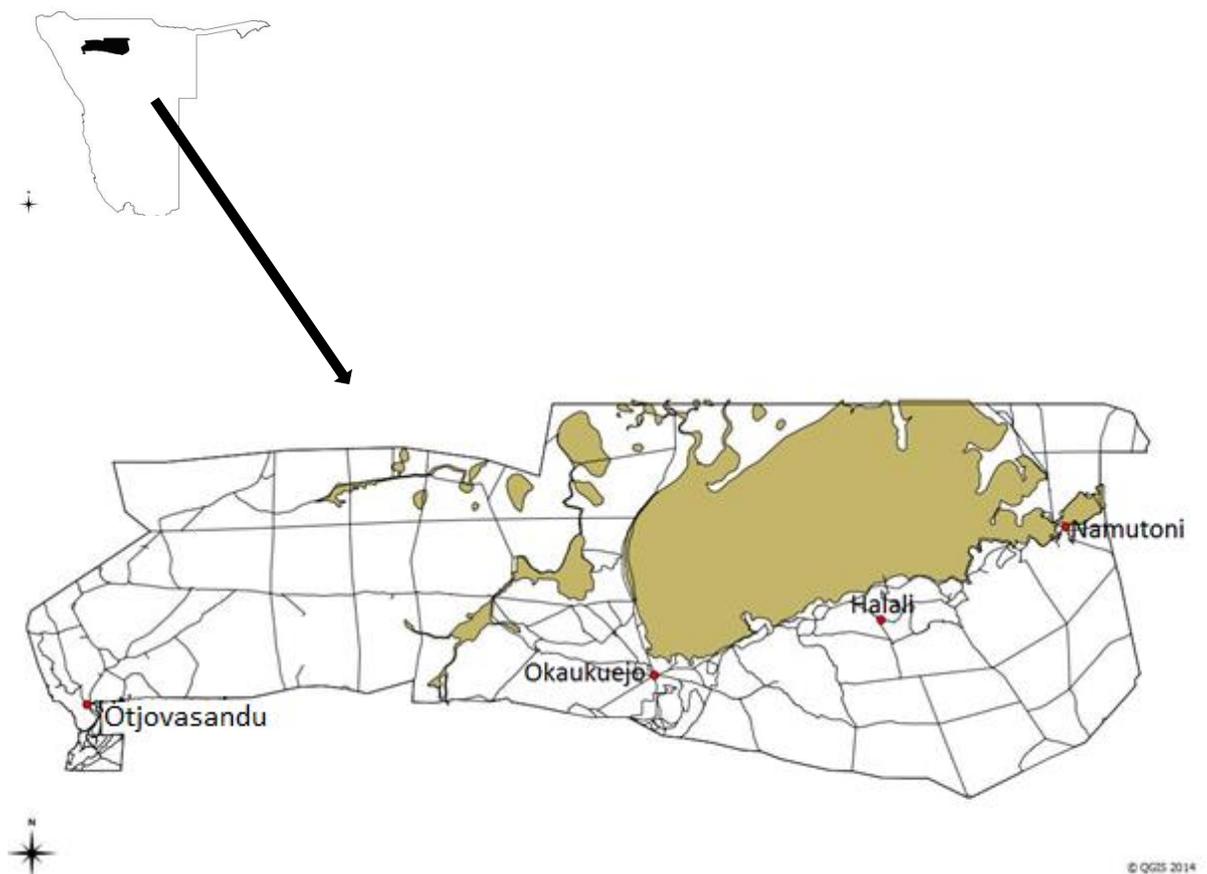


Figure 4: Map of Etosha National Park showing the boundaries, camps, salt pan and the road network.

Three seasons are recognised in ENP. These are hot-wet (January – April); cool-dry (May – August) and hot-dry (September - December) (Turner *et al.*, 2013; Lindeque, 1991). Rainfall in ENP occurs from November to April, in which January and February are the wettest months of the rainy season (Turner *et al.*, 2013) as shown in Figure 5 below.

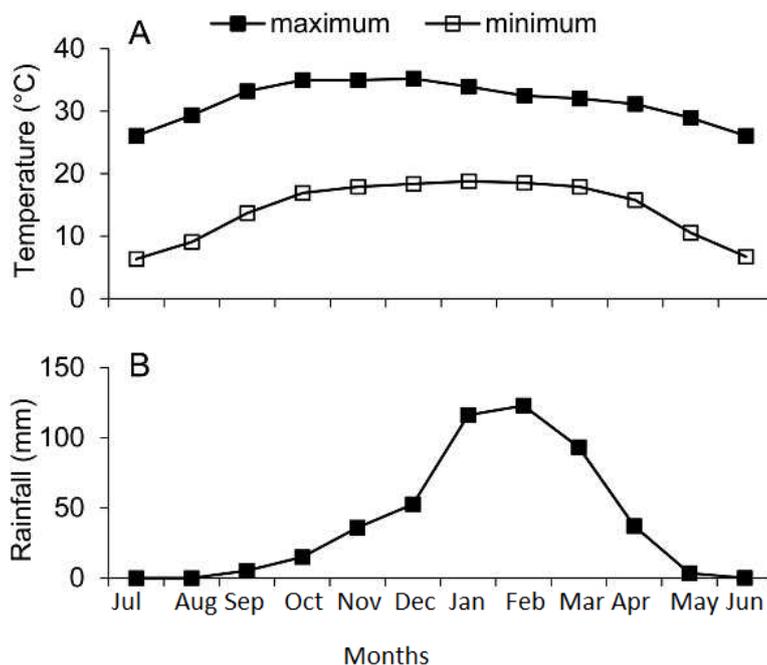


Figure 5: (A) The mean monthly minimum and maximum temperatures recorded at Okaukuejo station in central ENP from Jan 1975 – Jan 2004, (B) Mean monthly rainfall seasons during 2005 – 2011 rainfall seasons recorded at Okaukuejo (source Turner *et al.*, 2013).

Mean monthly maximum temperatures vary from 25°C to 35°C and the mean monthly minimum temperatures vary from 6°C to 18°C in winter and summer respectively (de Beer *et al.*, 2006).

The soils in the park are categorised into nine major soil classes according to the Food and Agriculture Organisation (FAO) soil nomenclature (Beugler-Bell & Buch, 1997). The major soil types in ENP according to Beugler-Bell & Buch (1997) are thus areonossols, calcisols, cambisols, fluvisols, leptosols, regosols, solonchaks, solonetz and vertisols. Woody plants found in ENP include *Acacia ssp.*, *Colophospermum mopane*, *Boscia foetida*, *Combretum apiculatum*, *Commiphora ssp.*, *Grewia ssp.*, *Terminalia prunioides*, *Terminalia sericea* and *Ziziphus mucronata* (Le Roux, Grunow, Morris, Bredenkamp & Scheepers, 1988; de Beer et al., 2006).

Apart from zebra, wildebeest and elephants (the three study species), ENP is home to a number of herbivores such as springboks (*Antidorcas marsupialis*), black-faced impalas (*Aepyceros melampus petersi*), red hartebeests (*Alcelaphus caama*), giraffes (*Giraffa camelopardalis*), kudu (*Tragelaphus strepsiceros*), elands (*Taurotragus oryx*), Gemsboks (*Oryx gazelle*), rhinos (*Rhinocerotidae*), Steenboks (*Raphicerus campestris*) and damara dik dik (*Madoqua kirki*). Carnivores such as lions (*Panthera leo*), hyenas (*Hyaenidae*), cheetahs (*Acinonyx jubatus*) and leopards (*Panthera pardus*) are also found in ENP (Berry, 1997).

3.2. Dust bathing behaviours of plains zebra (*Equus quagga*) and blue wildebeest (*Connochaetes taurinus*) and African elephant (*Loxodonta africana*)

3.2.1 Criteria for camera trap site selection

Study sites were selected based on the limitation of certain factors. Fuel for driving almost every third week to and fro camera sites is costly. Thus the area chosen was restricted to be between Okaukuejo and Halali which are approximately 75km apart. Sites to put up camera were found by driving around the park between Okaukuejo and Halali in search for either zebras or wildebeest dust bathing. This was because locations of dust bath sites were initially known. This thus limited sites to those visible from the road and not obscure sites. However this was also for the safety of personnel's during sampling and changing of camera trap batteries because it is restricted to drive off-road in the park. Once a site was located, a camera was set up.

3.2.2. Use of camera traps to record dust bathing behaviour in zebra and wildebeest

Dust bathing sites of zebras are well defined, characterised by a bare open patch of soft soil devoid of vegetation (Carnaby, 2010). Wildebeest have similar dust bathing sites except they differ from zebra in the structure of the dust bath site (personal observation). The site of a zebra dust bath is a flat patch of fine soft soil whereas those of wildebeest tend to have a small depression in the ground, such that these

sites can hold water during the rainy season. In this study five, zebra and three wildebeest sites were selected for observation with camera traps. These sites were identified by either observing zebras or wildebeest dust bathing or by identifying features such as foot prints or faeces around zebra or wildebeest dust bath site. Cameras were placed 12 m from the centre of the dust bath site; a pole was placed into the ground and the camera case was attached to the pole 1.2 m above the ground as shown in Figure 6.

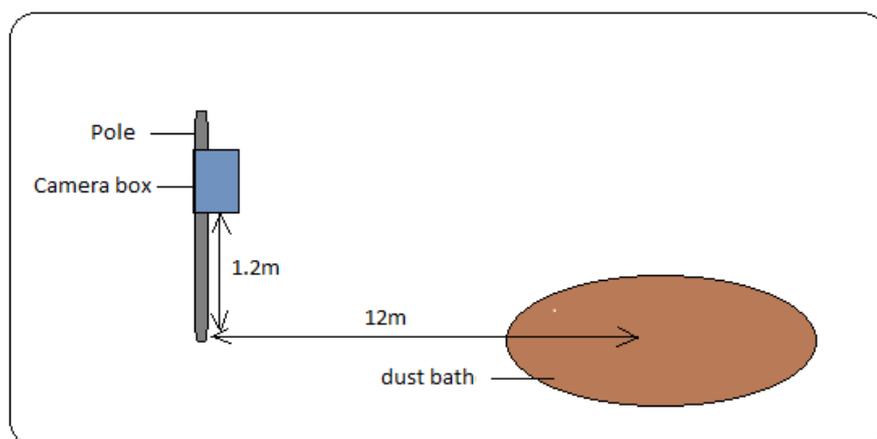


Figure 6: The setup of the camera traps at dust bathe sites.

Reconyx PC800 HyperFire Professional high output covert IR and RC 55 RapidFire color IR camera traps were used in this study. The cameras were programmed to capture ten pictures at one second intervals per trigger/movement sensed, with continuous retriggers in the case of additional movements sensed. Cameras were monitored after every three weeks for RC RapidFire color IR cameras and after every month for Reconyx PC800 HyperFire Professional high output cameras. This was because of the life span of the batteries that were used. The cameras were designated as shown in Table 1 and Figure 7 showing their locations on the map of ENP. One

camera was fitted at the dust bath of the anthrax positive zebra (Figure 2), five cameras were placed at additional zebra dust baths, and three cameras were placed at wildebeest dust baths.

Camera 12-036 was set up where a zebra died of anthrax in 2012 and used as a test to see whether animals re-use a dust bathing site after an animal died of anthrax on the site. As this is quite a rare event, there are no replications of dust bathing sites with anthrax carcasses, thus there was only a single carcass dust bathing site monitored.

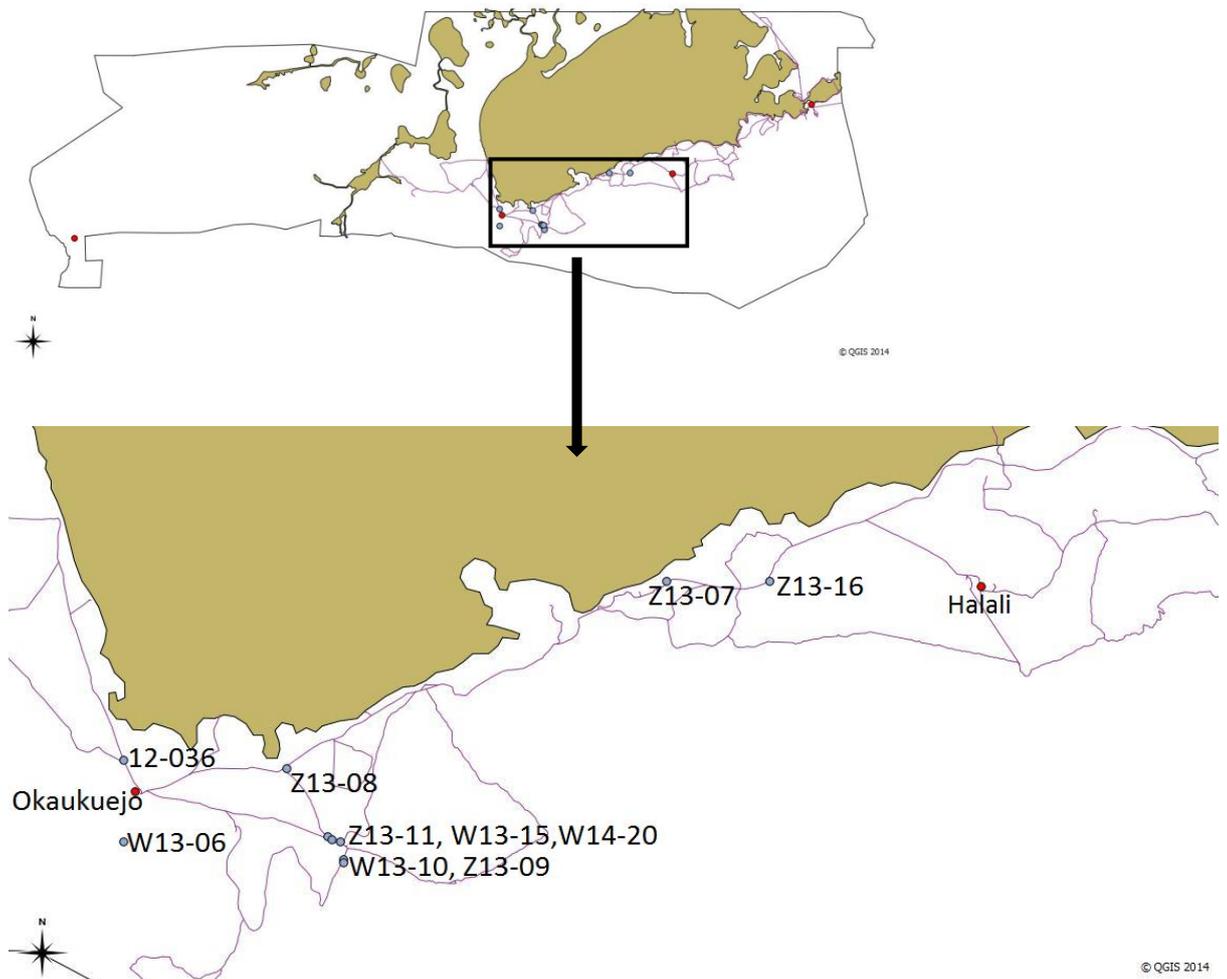


Figure 7: The map of ENP indicating the location of the camera traps in the vicinity of Okaukuejo and Halali for the dust bathing behavioural study of zebra and wildebeest.

A camera trap data management program was designed by Y. Krishnappa to download and store photographs from data cards, and to extract behavioural data on dust bathing behaviours from the photographs and metadata from the photographs (this software was further developed and published: Krishnappa and Turner, 2014). After loading the pictures into the camera trap data management program, the following data were obtained from the photo metadata: time, date and temperature at which the camera was triggered. The species which triggered the motion sensor was recorded manually by going through each group of ten photos and marking the species present with a checkbox. Every motion trigger was additionally assessed to determine if any dust bathing occurred or if any dust that could be inhaled was visible.

Where possible the age and sex of individual animals observed dust bathing was recorded. Sex and age were defined in three categories: adult male, adult female, and juvenile. These coarse-scale age and sex classifications were due to the difficulty of identifying age and sex accurately from the photographs. The features that distinguished male from female in both species was the presence of a male reproductive organ (penis sheath). In a case where that was not visible different features were used. Such as in the case of zebras the alternative distinction used was the thickness of the black skin in the anus/vulva area. Females have a thicker area of black skin than males. In the case of adult wildebeests, the facial colouring was used. The male wildebeest have an all-black mark on the front of their face whereas the female have a triangular section of brown mark (also visible in all young ones). Both males and females have horns. Juveniles have horns that are short on length and

straight while sub- adults have slightly curved and long horns than juveniles. Adult horns are fully grown, well curved and resemble parentheses.

In both wildebeest and zebra, juveniles (young ones) have slender bodies and shorter in height compared to both adults and sub-adults. However differentiating between sub-adult and adults were a challenge thus they were all considered as adults.

3.2.3 Dust and mud bathing behaviours in African elephant, (*Loxodonta africana*)

The elephant behavioural observations were carried out during the cool-dry and part of the hot-dry season from May 2013 - October 2013 and May 2014 to July 2014. Unlike the zebra and blue wildebeest, preliminary observations indicated that elephants do not have specific sites for dust bathing that could be monitored using camera traps. Waterholes were the choice for elephant dust bathing behavioural studies for several reasons. First, personal observations indicated that zebra and wildebeest predominantly had dust bathing sites in close proximity to waterholes (although some dust bath sites were also found in localities with no waterholes in close proximity). Assuming that animals choose to dust bath at similar locations on the landscape, waterholes were used as the focal areas at which to study elephants. Second, elephants are not found at high densities in Etosha. Therefore, it can be

difficult to locate elephants by driving. Working at waterholes that are known to be frequented by elephants made it easier to locate individuals to observe. Finally, conducting observations at waterholes allowed additional behavioural observations of elephant mud bathing behaviours, another source of potential soil contact by elephants

Four waterholes (Okaukuejo; Ombika; Olifantsbad and Rietfontein) were selected in the study area based on elephant preference for these waterholes (G. Shatumbu, personal communication, March, 2013) (Figure 8). The preference is likely to be due to the proximity of these waterholes to wooded mopane habitats. The waterholes were also located in the study area between Okaukuejo and Halali.

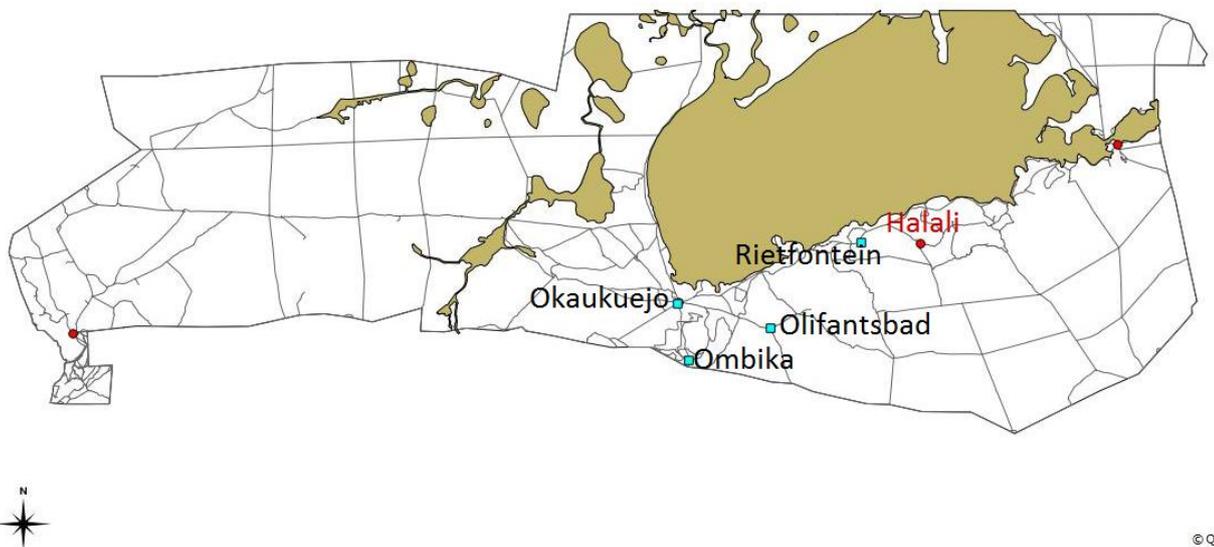


Figure 8: The four waterholes at which elephant dust and mud bathing behaviours were studied.

Elephant dust bathing observations were carried out from 12h00 to 16h00 at each waterhole. The choice of these times was based on the time of the day when elephants have been observed to visit waterholes most frequently to drink water (G. Shatumbu, personal communication, April, 2013). Night times were excluded due to the park regulations against driving after sunset. On each observation day, the following information was recorded: the arrival time of elephants, the elephant group type and number of individuals upon first group arrival.

The elephants were classified into three groups: Bulls (males only), cows (females only), and mixed groups (both male and females). In elephants, primary sex distinction in adults was done by observing the sexual reproductive organs. Infants in the group were regarded as juveniles and they were considered to be those below belly height of an adult. This was concluded from pre-observations done before the initiation of the study. Shoulder heights are considered a reliable indicator of age of African elephants (Della Rocca, 2007), and thus were used to differentiate sub-adult from adults. The Matriarch (biggest and tallest female elephant in the group) was identified and any elephant near her shoulder height and above were regarded as adults and those below her shoulder height but above her belly height were regarded as sub-adults.

Additionally to the elephants at the waterhole, any bathing activity by individuals was recorded. The information documented was the time at which an individual dust bathed or mud bathed, the age (juvenile, sub-adult or adult) and sex (male or female) of the bathing individual and the number of times soil or muddy water was thrown onto the body using the trunk. Maximum daily temperatures from sampling days

were obtained from the Namibia Weather Network website (www.namibiaweather.info) using data of Etosha Safari Lodge located 29.2km from Okaukuejo.

3.3 Isolation of *Bacillus anthracis* from dust bath soil

3.3.1 Sampling of soil from dust bath sites

In order to determine the presence or absence of *B. anthracis* in areas where animals dust bathe, surface soil was collected from dust bathing sites of the three study species. For zebras and wildebeest, soil sampling sites were either those where individuals were observed dust bathing, or from clearly identifiable dust bath sites without animals present, identification of the species using the site was done based on visible foot prints or faeces at the dust bath site. Surface soil where elephants were observed dust bathing was also sampled when it was possible to do so. Sampling of soil from dust bath sites was carried out during the period from March 2013 – July 2014. Sampling was done at 52 zebra dust bathing sites, 20 wildebeest dust bathing sites, 2 sites which were used by both zebra and wildebeest, 7 elephant dust bathing sites and 3 unknown dust bathing sites (the species using those sites could not be determined). For zebra and blue wildebeest sites, soil was collected by scooping surface soil of about 1cm – 2 cm deep with a spoon starting in the centre moving out towards the marginal ends of the dust bath in a cross section and diagonally as illustrated in Figure 9. This protocol was designed by the Z. Barandongo with the aim of exclusively collecting surface soil. Soil samples to

screen for *B. anthracis* at elephant bathing sites were collected where elephants left markings of their trunks when scooping soil for dust bathing as shown in Figure 10. Sampling was done along the markings (shown in Figure 10) left by the elephant's trunks during dust bathing. Collected soil samples were approximately 70 g per site.

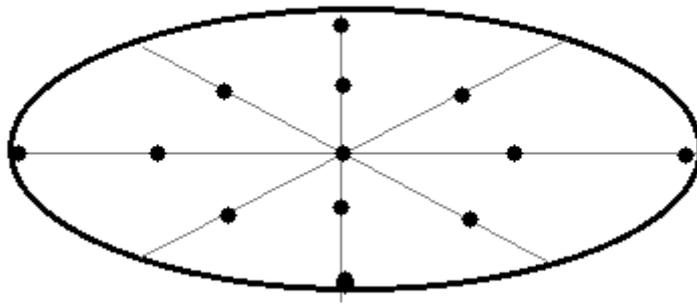


Figure 9: Demonstration of how sampling was done at wildebeest and zebra dust bath sites. The dots indicate the areas on which soil was scooped using a single sterile spoon for each sites. The lines indicate the transect lines from the centre of the dust bath site.



Figure 10: Ground markings (indicated by the arrows) left by the trunk of an elephant that took a dust bath. Those markings were the guide lines used for sampling soil.

The collected soil was placed in a sterile WhirlPark® bag and labelled by giving the date of collection and the identity of each site as described in Table 2, and the GPS location of the site recorded. The samples were stored at 4°C in a walk-in fridge at Ministry of Environment and Tourism (MET): Etosha Ecological Institute (EEI) until cultured on growth media to quantify the amount of *B. anthracis* per gram of soil.

3.3.2 Preparation of media to culture *B. anthracis* from soils collected at dust bath sites

The preparation of Polymixin-lysozyme-ethylenediaminetetraacetic acid (EDTA)-thallous acetate (PLET) agar and soil samples to be cultured were done in the anthrax laboratory at EEI. According to WHO (2008), Polymixin-lysozyme-ethylenediaminetetraacetic acid (EDTA)-thallous acetate (PLET) agar of Knisely (1966) is the best selective (3 – 5 spores/g of soil) media for the isolation of *B. anthracis* from clinical materials or environmental samples heavily contaminated with other bacteria. The combination of EDTA and thallous acetate cations permits the growth of *B. anthracis* strains but generally inhibits growth of other *Bacillus* species (Dragon & Rennie, 2001).

The working bench was disinfected using 10% Sodium hypochlorite (NaClO). Selective (PLET) culture media was prepared by adding 78 g of brain heart infusion agar, 0.45 g (EDTA) and 1 ml thallous acetate into 1.5 L distilled water in a 2 L Erlenmeyer flask. The solution was heated using a hot plate stirrer (PC-351) until all

particles dissolved whilst being stirred using a magnetic stirrer. The solution was thereafter autoclaved for 45 mins at 125 °C and 15 psi and terminal cooling was done for 1 hour to ensure solution was at 50 °C as that was the required temperature at which antibiotics could be added. The agar was removed from the autoclave and 400 µl of lysozyme and 400µl of polymyxin B sulphate solutions were added to the agar using sterile micropipette tip for each solution and the mixture was gently mixed by swirling. Using a sterile 25 ml pipette, 20 ml of the PLET agar was transferred into 90 mm petri plates, whilst avoiding forming bubbles. Once completed the agar plates were left on the bench to solidify and stored in a 4 °C refrigerator until ready for use.

3.3.3 Plating of soil samples

The protocol for plating the soil samples was adapted from the protocol used by Ganz et al, (2014) with a few minor changes. The 0.1% peptone water in Ganz's protocol was replaced with sterile distilled water, the dilution series were reduced and the incubation period was only done for 48 hours instead of 48 hours and 98 hours. After incubation at 37 °C for 36–48 hours, the colonies of *B. anthracis* are 2–3 mm (WHO, 2008). The detailed protocol that was followed is outlined below.

In total 84 dust bath soil samples were collected and each was analysed as individual samples. The sampled dust bath soils were homogenised by shaking and inverting the whirlpark bags then 5 g of soil was placed into a 50 ml falcon tube. Weighing and

homogenising of the soil sample, were done wearing protective goggles and 3m 8210 particulate respiratory mask and a laboratory coat. The soil was then suspended in 45ml of 0.1% sodium pyrophosphate and vortexed for ten minutes to discharge and loosen spores from the soil particles (Ganz et al., 2014). After discharging the soil particles, the mixture was centrifuged at 0.3x 1000 rcf for 2 minutes to keep spores in suspension but allow the soil to settle. The supernatant was then transferred to a new 50ml falcon tube and centrifuged at 3.0 x 1000 rcf for 15 minutes to pellet any spores. After centrifuging for the second time, the supernatant was discarded and later autoclaved with the trash from the lab. The pellet was re-suspended in 5 ml sterile distilled water. It was then briefly vortex to get spores in suspension after which 1.0 ml was transferred to 1.5 ml Eppendorf tubes.

Ten fold dilutions of the mixture were made by transferring 100µl of the suspension to another Eppendorf tube preloaded with 900 µl of sterile distilled water using sterile micropipette tips. Dilutions of 10^{-1} and 10^{-2} were prepared. From each dilution sample and the undiluted sample, 100µl was plated onto PLET agar by spreading gently using disposable sterile spreaders for each sample. Sterile distilled water was used as a negative control, of which 100µl was plated using the same techniques as the experimental samples. A suspension of live spores of an uncapsulated non-virulent strain of *B. anthracis* (Stern 34F2) was used as a positive control to assist in morphological identification of *B. anthracis* colonies by diluting it to 10^{-5} and then also plating it out. The culture plates were incubated at 37 °C and were read after 48 hours.

3.3.4 Identification of *B. anthracis* and biochemical confirmation of suspected colonies

According to Dragon & Rennie (2001), *B. mycoides* MU711/84, *B. thuringiensis* QC12093, *B. subtilis* 1A289 strains formed colonies on PLET. However conclusions were still focused around PLET being the best media for isolation of *B. anthracis*. *Bacillus anthracis* generally produces off-white colonies that have irregular edges and a rough “ground glass” appearance (Koehler, 2009).

To confirm colonies of *B. anthracis*, each colony that was identified as *B. anthracis* based on morphology described above and one colony from the positive control were picked after 96 hours of incubation and sub-cultured on PLET by using the streaking technique. Penicillin G disc and 10 µl of gamma phage were added to the bacterial streak. Penicillin G inhibits the growth of *B. anthracis* by forming an inhibition zone around the microbial growth while λ-phage lyses it (World Organisation for Animal Health, 2012).

3.4 Data analysis

The data were analysed using IBM SPSS (version 20). Normality test for data were done using the Shapiro-Wilk test ($P > 0.05$). The null hypothesis of the test claims that data are normally distributed if the p-value is more than 0.05. Any data that has a p-value of less than 0.05 is therefore not normally distributed. In cases where data

were not normally distributed, non-parametric tests were conducted. Given how little is known about dust bathing behaviours of these species, basic summary data are presented from the camera trap study to demonstrate which species use the different camera sites and in what proportion based on the total number of triggers of the camera by a species. Even though camera-monitored sites were assigned to zebra or wildebeest based on which species was observed using the sites when the cameras were placed, we summarized data from the camera from each of the three study species as a test of how species-specific these dust bath sites really are.

To assess how the dust bathing behaviours of zebra and wildebeest varied with season, Kruskal – Wallis and Mann – Whitney test were used. Using separate statistical tests for each species, the dependent variable was the average number of dust baths per day averaged at a monthly scale. The independent variable was a categorical variable for season, where the months of data were group into the three seasons (hot-wet (January – April), cool-dry (May – August) and hot-dry (September to December)). The amount of photo data collected varied considerably among cameras. They were placed for different lengths of time and there are gaps from when cameras were not recording data, due to factors such as batteries going flat, cameras knocked over by animals etc. In addition, the number of animal visitations to these sites varied, as did the frequency of dust bathing by the target species at a particular site. Given all these potential sources of variation among sites and over time, analysing these data based on simple numbers of dust baths observed could result in biased results and interpretation. In particular gaps in the data had to be accounted for because it is essential to distinguish between a true zero, of no dust

bathing occurring on a day, from a false zero, where no dust bathing is recorded because the camera was not functioning. Data gaps in the time series of each camera were assessed and the number of days of data per month calculated for each camera and month of data. Thus a month could end up with fewer than 30 or 31 days. Daily average dust baths per month were then calculated for each camera site from the number of dust baths observed and the number of data per month. These averages were calculated by determining days of data per month for each camera trap (five cameras for zebra and three cameras for wildebeest). Only days in which the cameras were functional (i.e., cameras did not have flat batteries, etc) were considered as days of data. Dust baths per day were calculated using the following formula. This was done for each month.

$$\text{dust baths per day in month } x = \frac{\text{total number of dust baths observed in month } x}{\text{total days of data in month } x}$$

This was calculated separately for each camera and each month of available data. The total dust baths per month were the sum of dust baths observed at each camera. If a month had no days of data recorded at a site (e.g., the camera was not functioning for a whole month or if the camera was not on the site for a full calendar year), any month with zero days of data at a site were excluded from data analysis.

Elephant dust and mud bathing was evaluated at the scale of the daily observations, calculating the proportion of individuals observed during a day's sampling period that dust bathed. These were calculated by dividing the number of individuals dust or

mud bathing by the total number of elephants observed on that day. Linear regression were used to determine whether changes in daily maximum temperature (as an index of seasonality) had any effect on the proportion of individuals dust and mud bathing by African elephants. The intensity of elephant dust and mud bathing was evaluated at the scale of the daily observations. The intensity of dust and mud bathing was determined by counting the number of times an individual elephant threw mud or dust onto its body. Linear regression was used to determine whether there was any correlation between daily maximum temperature and dust/mud bathing.

A contingency table was used to determine if there were seasonal associations between numbers of anthrax mortalities and dust bathing behaviours for the three study species. The anthrax mortality and dust and mud bathing counts were grouped into hot-wet, cool-dry and hot-dry categories. This was carried out separately for each species. The anthrax mortalities since 1975 – 2013 from the EEI data base were used. These data have been recoded throughout the park.

It was a challenge to analyse age-sex patterns of the dust bathing species due to the difficulty of assigning individuals to age and sex from the photos. The sample populations were thus small for data analysis therefore the age-sex of dust bathing zebra and wildebeest are presented as descriptive data.

Chapter 4: Results

In this chapter the results on the ecology of dust bathing behaviours of zebra, blue wildebeest and African elephants are presented. The chapter also presents results of the study on the presence or absence of *B. anthracis* spores at dust bathing sites of the three study species, and the seasonal relationship between dust/mud bathing and anthrax mortalities observed in ENP.

4.1 The ecology of dust bath sites

The camera traps mounted at zebra and wildebeest dust bath sites were triggered a total of 43,760 times, collecting 437,600 photographs over a combined total of 1693 days (Table 2). These eight cameras recorded a total of 271 motion triggers of zebra dust bathing, 26 triggers of wildebeest dust bathing, and none of elephant dust bathing. These dust bath triggers represented 0.6% of zebra triggers, 2.0% of wildebeest triggers and 0% of elephant triggers. The three species seem to only rarely share dust bath sites: only once did a zebra dust bathe at one of the wildebeest sites and only once did a wildebeest dust bathe at a zebra site, and elephants never used the sites of either species despite being observed at half of the sites.

Table 3: Summary of the camera data for the dust bathing observational study of zebra and blue wildebeest dust bathing sites.

EB = zebra, CT = blue wildebeest, LA = African elephant and db = dust bathing.

| Camera ID | Total triggers | Days of data | Total EB triggers | Total CT Triggers | Total LA triggers | Total number of db by EB | Total number of db by CT | Total number of db by LA |
|------------------|-----------------------|---------------------|--------------------------|--------------------------|--------------------------|---------------------------------|---------------------------------|---------------------------------|
| Z13-07 | 3 530 | 68.99 | 1 494 | 633 | 6 | 49 | 0 | 0 |
| Z13-08 | 3 299 | 370.86 | 827 | 10 | 0 | 199 | 0 | 0 |
| Z13-09 | 2 398 | 298.45 | 847 | 204 | 0 | 18 | 1 | 0 |
| Z13-11 | 624 | 145.12 | 150 | 42 | 0 | 3 | 0 | 0 |
| Z13-16 | 802 | 186.48 | 124 | 4 | 1 | 1 | 0 | 0 |
| W13-06 | 4 333 | 295.79 | 586 | 347 | 57 | 0 | 5 | 0 |
| W13-10 | 1 586 | 244.9 | 357 | 54 | 1 | 1 | 16 | 0 |
| W13-15 | 188 | 82.8 | 10 | 35 | 1 | 0 | 4 | 0 |

These cameras were all placed at dust bathing sites in the dry season when the sites were actively in use. When the rains came, the sites were overgrown with grasses. Zebras did not return to any of the five monitored dust bath sites as the next dry season approached. Wildebeest on the other had re-used one of the dust bathing sites, W13-10, after the rainy season but not the other two sites.

The dust bath site on which a zebra died of anthrax (12-036; Figure 2) was monitored for 20 months after the death of the animal using a motion sensing camera trap, however the site was never used again as a dust bath. Prior to this animal's death the site had soft fine soil suitable for dust bathing, but after death body fluids released from the carcass were churned into the soil and compacted by scavenger activity. The soil at this site remained firmly compacted and hard two years after death.

4.2 Dust bathing behaviours of zebra

The monthly average of the number of dust baths/day for zebra that were captured using camera traps at five dust bathing sites are shown in Appendix 1. The average monthly number of dust bath triggers recorded per day across the five zebra dust bath sites are presented in Figure 11.

The number of triggers by zebras varied among the five study sites. From the five cameras, Z13-07 (S19.03608°, E16.27114°) had the most triggers, of which 1,494 were by zebras only. From these 1494 zebra triggers, 49 were triggers in which

zebras were observed taking dust bathes (Table 2). Camera Z13-16 (S19.03551° E65.34004°) had the least number of triggers with 124 zebra triggers. Out of the 124 zebra triggers 3 were of dust bathing zebras (Table 2). In total 10, 653 triggers (camera triggers consisting of any species that captured by camera, such as zebra, wildebeests, springbok, gemsboks jackals etc.) were recorded, however from that total only 3, 442 (32.3%) were triggers by zebra only. From those 3, 442 zebra triggers, 270 (7.8%) were of dust bathing triggers by zebras.

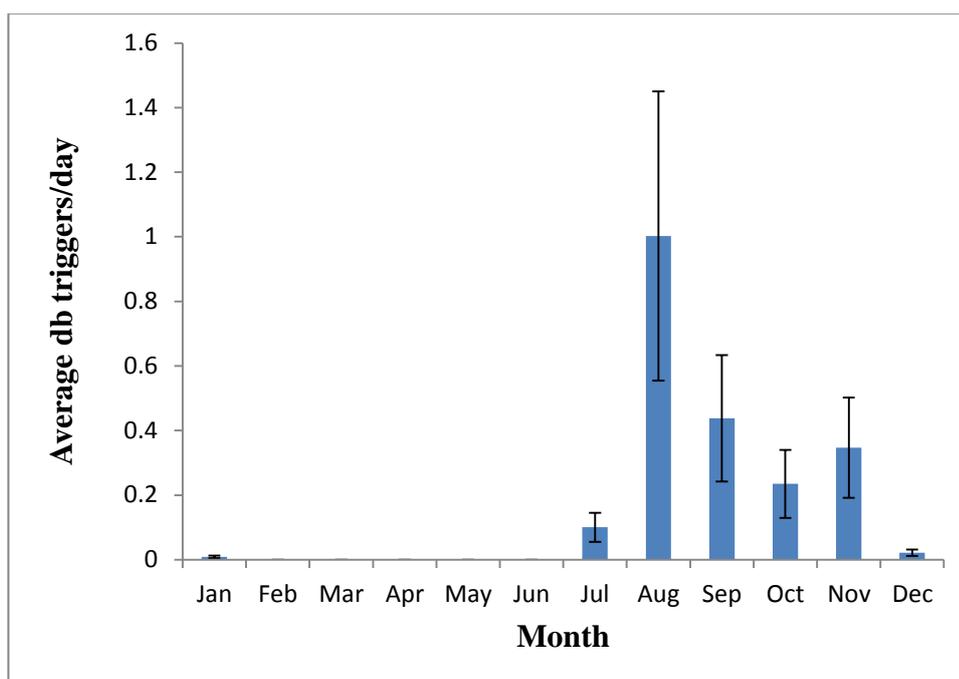


Figure 11: The average number of dust bath triggers per day by zebra in each month, calculated from 5 dust bath site ($N = 5$). The error bars indicate standard error of the mean (\pm SEM).

The monthly number of dust bath triggers per day were not normally distributed ($W=0.6792$; $p = 0.001$). Mann–Whitney test was used to test whether there was any

statistical difference in the number of dust bath triggers per day among the seasons in pairwise comparisons. The test revealed that there was no significant difference between the cool-dry and the hot-dry ($z = -0.787$ and $p = 0.232$). However, comparing hot-dry with hot-wet also using the Mann - Whitney test showed that there was a significant difference in the number of dust bath triggers per day between the two ($z = -0.231$ and $p = 0.01$), where fewer dust baths occurring in the hot-wet season than in the cool dry season.

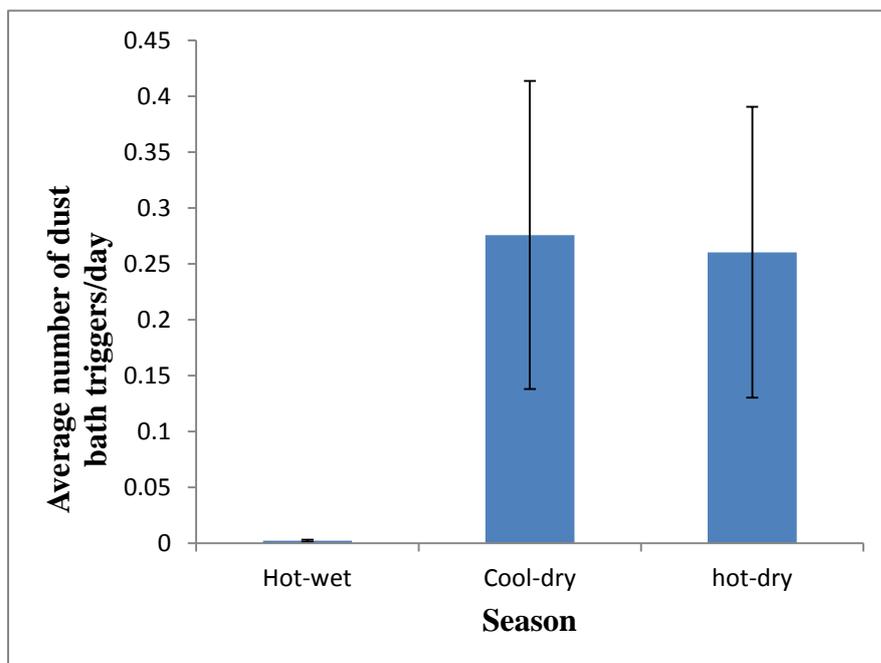


Figure 12: Seasonality of dust bathing by zebras. Seasonal averages of the monthly number of dust bath triggers per day by zebras were calculated from the five cameras. The months were grouped into the three seasons (hot-wet = January – April; cool –dry = May – August; and hot – dry = September – December based on Turner *et al.*, 2013). The error bars indicate mean standard error (\pm SEM).

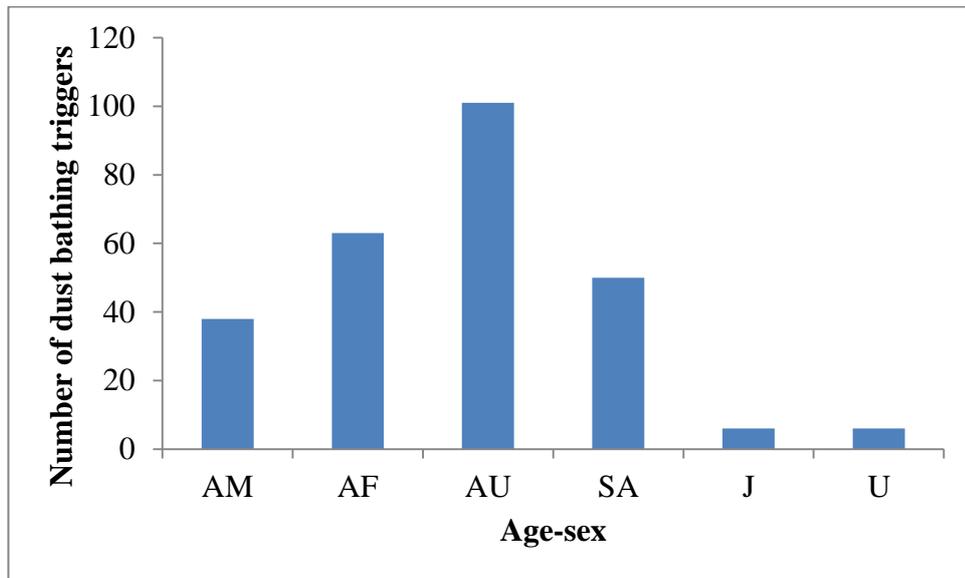


Figure 13: Number of dust bathing triggers in different age-sex classes recorded for zebra in which the individual dust bathing was assigned to the following demographic groups: AM refers to adult male, AF = adult female, AU = adult unknown sex, SA = sub-adult, J = Juvenile and U = unknown. $N = 264$

In the present study, it was difficult to determine the sex animals from the photographs, Statistical test were not carried out to test for significant differences for patterns of sex of dust bathing zebra because the sex of majority of zebra were not determined, hence recorded as unknown (Figure 13). However the trends in Figure 13 suggest that adults dust bath more than sub-adults and juveniles. Figure 13 further point to observation that sub-adults dust bath more than juveniles. This nonetheless would require factors such as age and sex ratios in a population to give more clarity on observation data.

4.3 Dust bathing behaviours of wildebeests

The monthly average number of dust baths/day for wildebeest that were captured using camera traps at three dust bathing sites are shown in Appendix 2.

A total of 6, 107 camera triggers were recorded by the three wildebeest cameras. This total included triggers by any species (such as wildebeest, zebra, springboks, gemsboks, jackals etc.) that were captured by the cameras. From that total (6, 107), only 436 were triggers by wildebeest and out of the 436 only 5.7% were triggers in which wildebeest were observed dust bathing (Table 2).

The camera in which most wildebeest triggers were observed was camera W13-06 (S19.16617°, E15.90927° in Okaukuejo area), of which 347 out of 4, 333 triggers were of wildebeests. From the 347 triggers, 5 triggers were of dust bathing wildebeest. The camera in which the least wildebeest triggers were observed was W13-15 (S19. 20685°, E16. 04516° in Gemsbokvlakte area) of which 35 out 188 triggers were of wildebeests and from the 35 wildebeest triggers, 2 were of dust bathing wildebeest. Camera W13-10 (S19.22454°, E16.05538° in Gaseb area) had a total of 1, 586 triggers (triggers by various species), of which 54 were triggers by wildebeest only. From the 54 wildebeest triggers, 16 triggers were of dust bathing wildebeests.

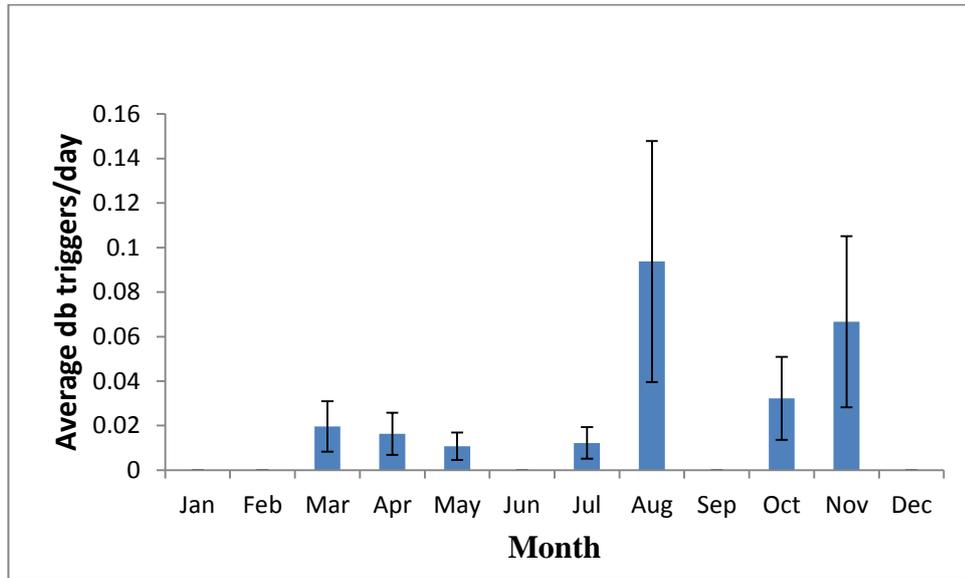


Figure 14: The average number of dust bath triggers per day by blue wildebeest in each month calculated from 3 dust bath sites ($N = 3$). The error bars indicate standard error of the mean (\pm SEM).

The monthly averages of dust bath triggers recorded per day at the three blue wildebeest dust bath sites (Figure 14) were not normally distributed ($W = 0.741$; $p = 0.002$), therefore a Kruskal–Wallis test was used to determine whether there was a seasonal difference in dust bathing for wildebeest.

A Kruskal-Wallis test showed that there was no significant difference among the three seasons (hot-wet, cool-dry and hot-dry: $\chi^2 = 0.269$, $df = 2$, $p = 0.87$ $\alpha = 0.05$; Figure 15).

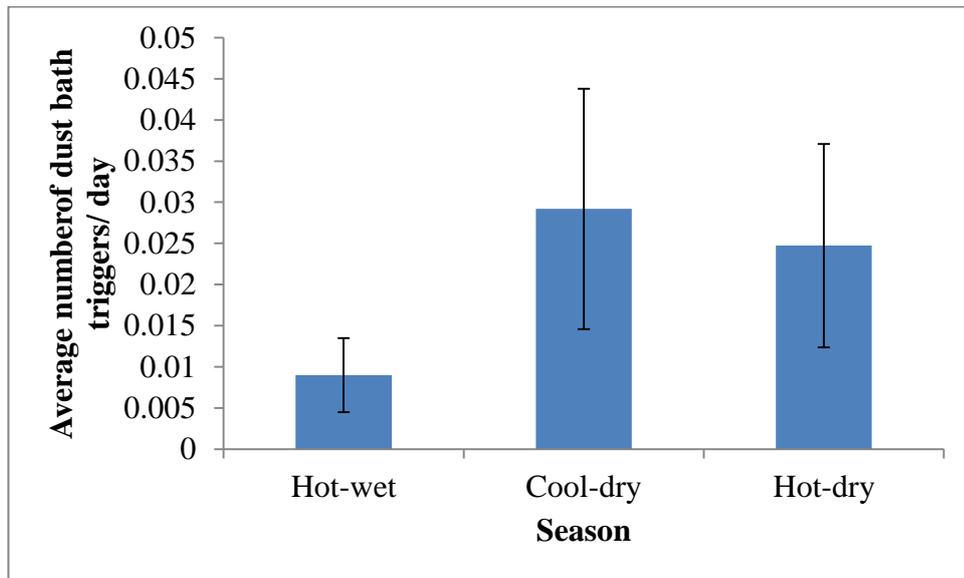


Figure 15: Seasonality of dust bathing by wildebeest. Seasonal averages of the monthly number of dust bath triggers per day by wildebeest were calculated from the three cameras. The months were grouped into the three seasons (hot-wet = January – April; cool –dry = May – August; and hot – dry = September – December based on Turner *et al.*, 2013). The error bars indicate mean standard error (\pm SEM).

Although wildebeest were observed dust bathing throughout the year, the age and sex of dust bathing wildebeest were not statistically analyzed. This was because the number of dust bathing by wildebeest were only 28 in total for the four groups of age-sex (AM = adult male, AF = adult female, SA = sub-adult and J = juvenile) including a fifth group for unknown (Figure 16).

Juvenile wildebeest were not observed dust bathing, while adult males, adult females and sub-adult wildebeest were observed dust bathing. Adult males were observed

dust bathing more than the adult females and sub-adults, and the adult females were observed dust bathing more than the sub-adults. These data are presented in Figure 16.

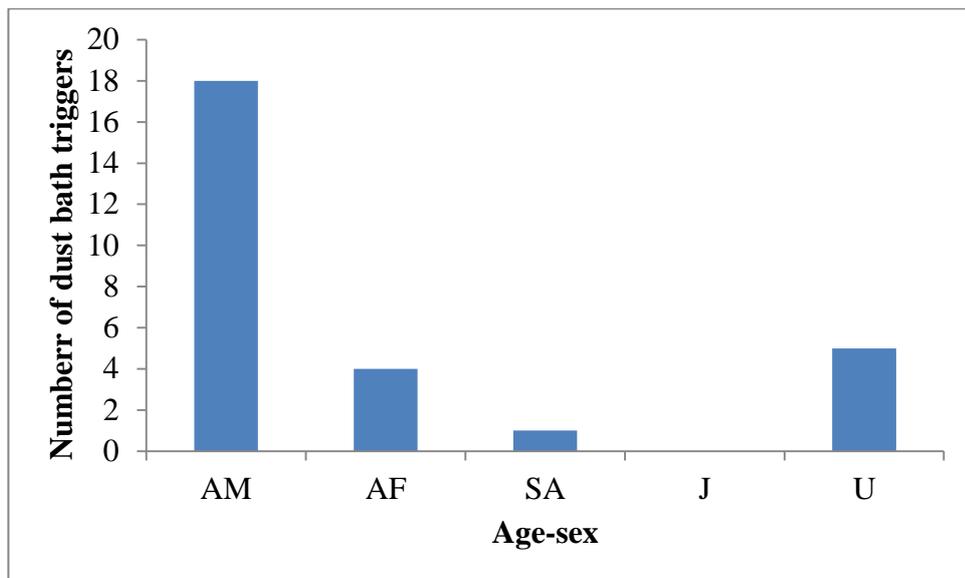


Figure 16: Number of dust bathing triggers in different age-sex classes recorded for wildebeest in which the individual dust bathing was assigned to the following demographic groups: AM refers to adult male, AF = adult female, SA = sub-adult, J = Juvenile and U = unknown. $N = 28$

4.4 Dust and mud bathing behaviours of African elephants

There were a total 27 days of waterhole observations at which elephants were observed (does not include days where no elephants were observed). This is because during the initiation of the study, days in which elephant did not turn up at the study waterholes were not recorded thus total days of observation trails are unknown. On these days, a total of 285 elephants were observed of which 35 elephants (12.3%) were observed dust bathing and 30 elephants (10.5%) mud bathing.

Elephant dust and mud bathing proportions were evaluated on a daily basis. These were calculated by dividing the number of individuals dust or mud bathing by the total number of elephants observed on that day.

There was no statistically significant relationship between the proportion of elephants dust bathing (i.e., the number of individuals observed dust bathing/total number of individuals observed during an observation period) and maximum daily temperature (linear regression: $R^2 = 0.0092$, $t = -0.43$, $p = 0.671$ and $N = 27$ days of observation; Figure 17). On the other hand, there was a statistically significant relationship between the proportion of individuals mud bathing and temperature (linear regression: $R^2 = 0.21$, $t = -2.29$, $p = 0.03$ and $N = 27$ days of observation; Figure 18), whereas maximum daily temperature increased, the proportion of elephants mud bathing increased.

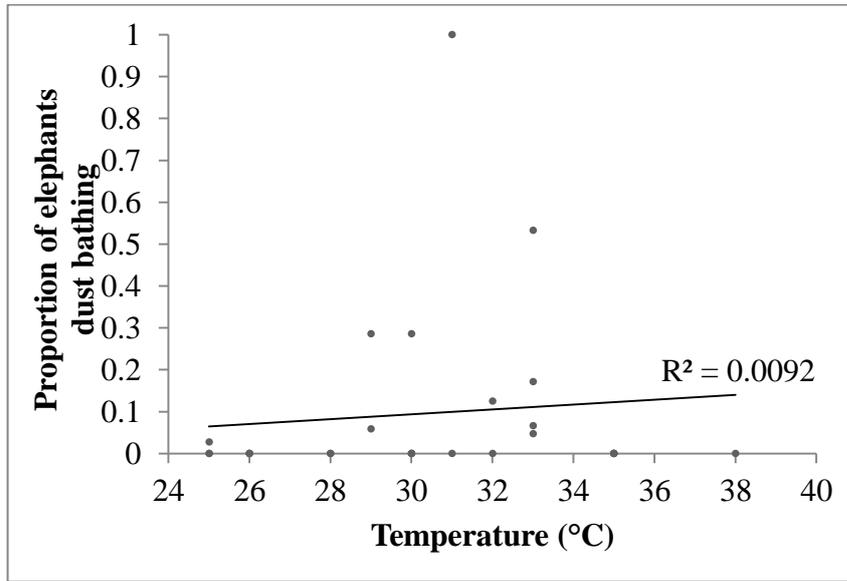


Figure 17: The relationship between maximum daily temperature (degrees Celsius) and the proportion of African elephant dust bathing in Etosha National Park. N days of data = 27.

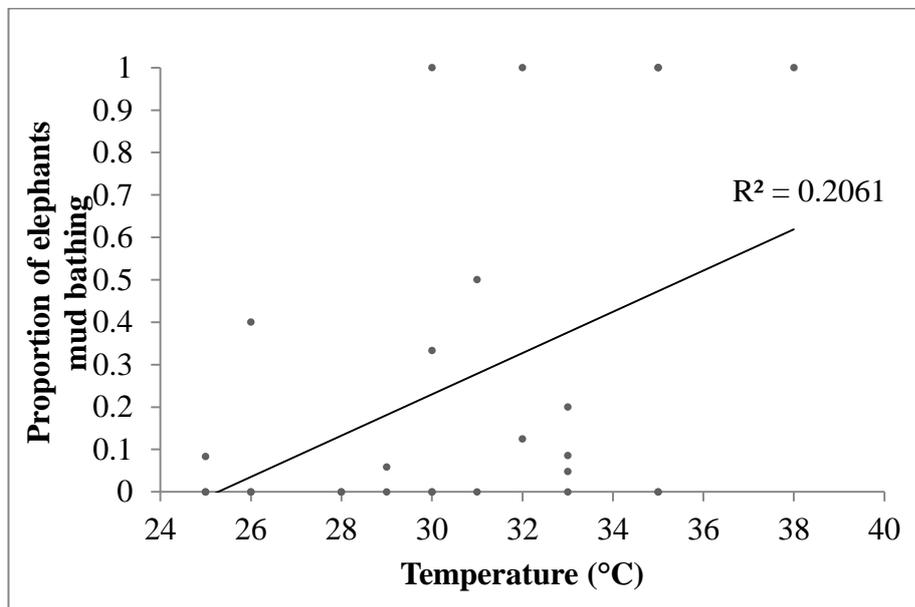


Figure 18: The relationship between maximum daily temperature (degrees Celsius) and the proportion of African elephant mud bathing in Etosha National Park. N days of data = 27.

The intensity of dust bathing (i.e., the number of times an elephant threw dust on itself while dust bathing) showed no statistically significant relationship with maximum daily temperature (linear regression: $R^2 = 0.037$, $t = 1.61$, $p = 0.116$ and $N = 34$; Figure 19). There was also no significant relationship between the intensity of mud bathing (the number of times an elephant threw mud on itself while mud bathing) and maximum daily temperature (linear regression: $R^2 = 0.0283$, $t = -0.18$, $p = 0.985$ and $N = 30$; Figure 20).

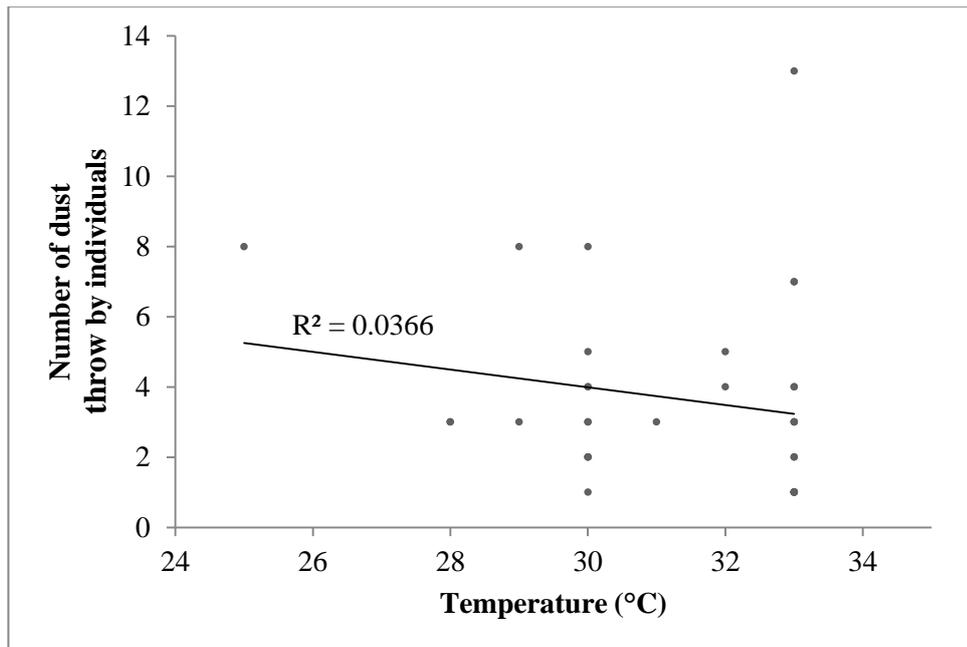


Figure 19: Intensity of dust bathing by African elephants. Intensity was defined as the number of times a dust bathing individual grabbed dust with its trunk and threw it on its body. $N = 35$ elephants.

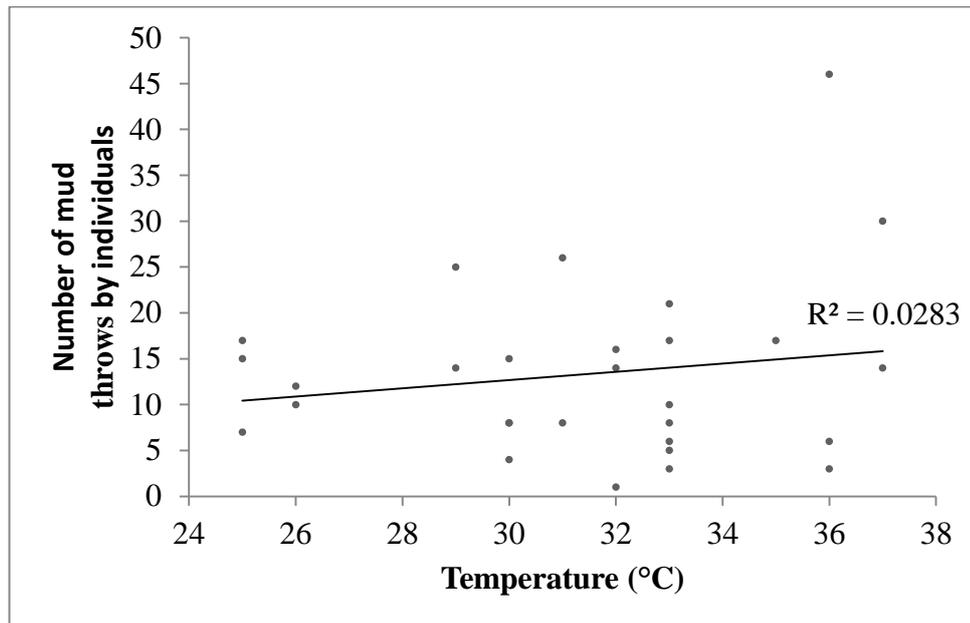


Figure 20: Intensity of mud bathing by African elephants. Intensity was defined as the number of times a mud bathing individual grabbed mud with its trunk and threw it on its body. $N = 30$ elephants.

A Kruskal-Wallis test was used to determine whether the proportion of dust bathing was the same among the age-sex groups of elephants. The same was done for mud bathing. The proportions of dust and mud bathing are presented in Appendix 5. The test showed that there was no significant difference in dust bathing among the age-sex groups ($\chi^2 = 3.295$, $p = 0.384$, $df = 3$, $\alpha = 0.05$; Figure 21) and also no significant difference in mud bathing ($\chi^2 = 5.023$; $p = 0.170$, $df = 3$, $\alpha = 0.05$; Figure 22).

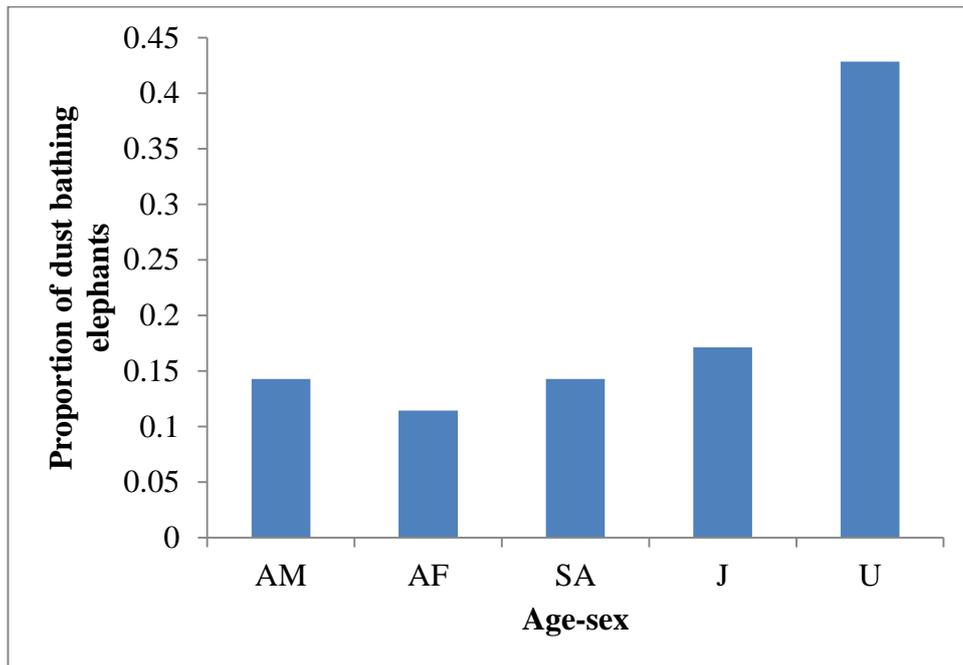


Figure 21: proportion of dust bathing of African elephants in terms of age-sex class groups. AM refers to adult male, AF = adult female, SA = sub-adult, J = Juvenile and U = unknown. The proportion was calculated by taking total elephants dust bathing per age-sex group / 35 (the total number of elephants observed dust bathing).

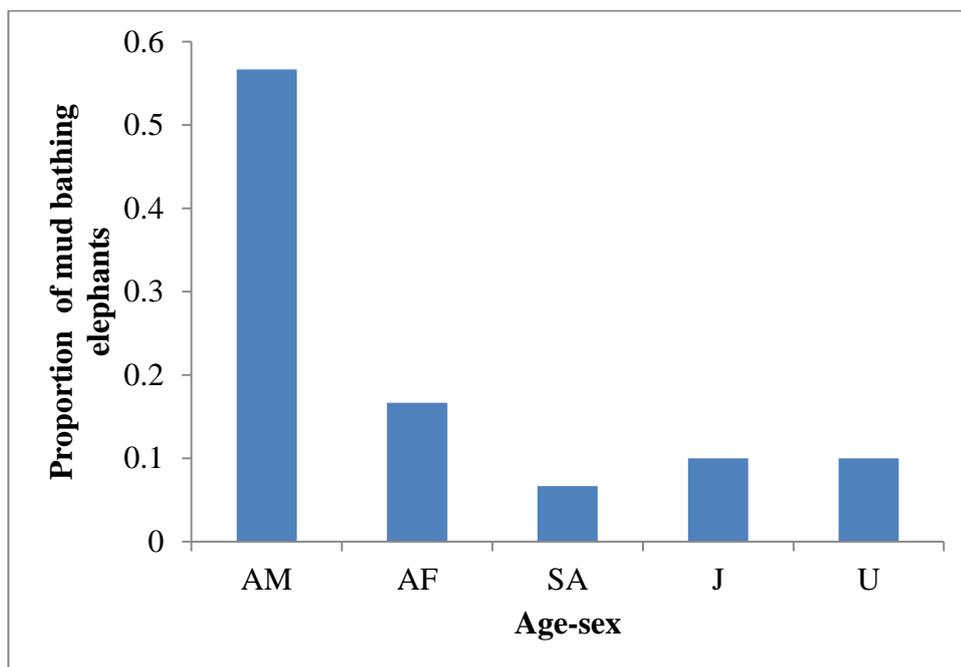


Figure 22: proportion of mud bathing behaviours of African elephants in terms of age-sex class groups. AM refers to adult male, AF = adult female, SA = sub-adult and J = Juvenile. The proportion was calculated by taking total elephants mud bathing in an age-sex group / 30 (total number of elephants mud bathing)

4.5 Seasonal relationship between dust/mud bathing and anthrax mortalities

The anthrax mortality data of the three study species from the year 1975 – 2013 obtained from Etosha Ecological Institute (EEI) are shown in Table 3. A contingency table analysis was performed to test whether the seasonality of dust bathing and anthrax mortalities in Etosha National Park correlate (Table 4).

Table 4: Results of contingency table analyses comparing the number of bathing behaviours observed seasonally and the number of anthrax mortalities in ENP (1975 – 2013) seasonally for the three study species.

| | X^2 | df | P |
|---------|-------|------|---------|
| LA (Db) | 22.7 | 1 | <0.0001 |
| LA (Mb) | 6.45 | 1 | 0.009 |
| EB | 455 | 2 | <0.0001 |
| CT | 14.3 | 2 | 0.001 |

*Note: LA (Db) = African elephant dust bathing, LA (Mb) = African elephant mud bathing, EB = Zebra and CT = Wildebeest. The wet season was excluded for LA analysis.

In all species, the p -values were less than 0.05. Therefore, there was a significant difference between the seasonal timing of bathing behaviours of the three study species (zebra, wildebeest and elephants) and the seasonal anthrax mortalities in Etosha National Park. Thus there was no seasonal correlation between the timing of anthrax mortalities and dust bathing behaviours in ENP.

4.6 Isolation of *B. anthracis* from dust bathing sites

A total of 83 dust bathing sites were analysed for the presence of *B. anthracis*. Of these sites, 51 were utilized by zebra, 20 by wildebeest, 7 by African elephants, 2 by a combination of zebra and wildebeest, and 3 sites by unknown species.

Only 2 (2.41%) of the sites were positive for *B. anthracis* spores (locations shown in Figure 23). These positive sites were one zebra dust bathing site with 20 CFU/g and a site used by both zebra and wildebeest with 10 CFU/g. The remaining 97.59% of

dust bathing sites were negative for *B. anthracis*. The dust bath site where the zebra died of anthrax was used as a positive control site and had a count of 2.59×10^5 CFU/g.

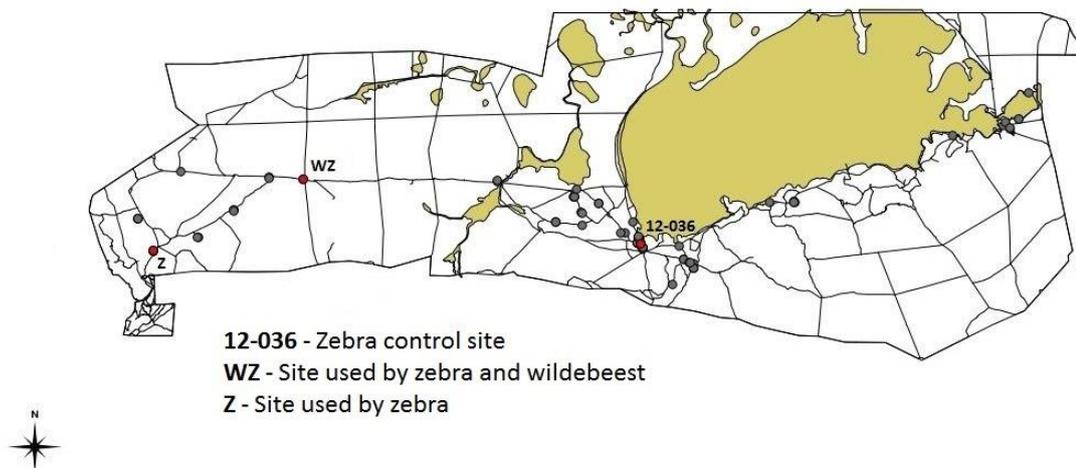


Figure 23: Map of Etosha National Park indicating the location of the three positive sites in red. The two positive dust bath sites are labelled WZ and Z on the map; the anthrax positive zebra carcass site (12-036) which served as a positive control is also shown.

Chapter 5: Discussion

The goals of this study were to (i) assess the ecology of dust bathing behaviours of zebra, blue wildebeest and African elephants, and (ii) to determine whether dust bathing may put any of these species at risk for inhalational anthrax. This chapter discusses the findings of this study.

5.1 Seasonality of dust bathing and mud bathing

The research hypothesis in the present study stated that zebra, wildebeest and elephant dust bath primarily during the hot dry season, when the soil is dry and soft and temperatures are high. There was no significant difference for any of the three species in the amount of dust bathing done in the cool-dry season versus the hot-dry season. Thus temperature alone was not a significant predictor of dust bathing in the dry season. The elephant dust and mud bathing studies were only conducted during the dry seasons, so dust bathing behaviours during the wet season remains unknown. During the wet season elephants did not visit the waterholes at which the behavioural studies were conducted.

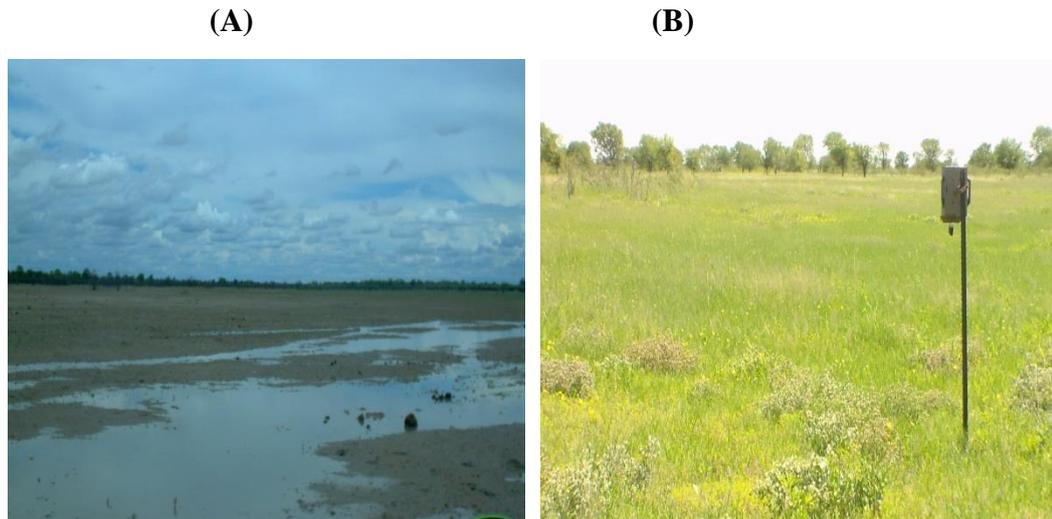


Figure 24: Dust bath sites during rainy seasons. Panel (A) shows how a dust bath site looks after rainfall. Panel (B) shows vegetation covering a camera-monitored dust bath site during the wet-season.

Zebras were recorded dust bathing only rarely during the wet season, and significantly less than in the dry seasons. On the other hand, wildebeests showed no significant difference in the amount of dust bathing among these seasons. During the dry season, the dust baths contained fine, powdery soil. However, after rainfall, these soils became hard with clumped soil particles. Rainfall also caused dust bathing sites to be overgrown with vegetation (Figure 24). This indicates one possible reason for zebra dust bathing occurring more often during the dry season, since vegetation made the site unsuitable for dust bathing. Moreover none of the five dust bathing sites mounted with motion sensing cameras were re-used by zebra after the wet season. Instead zebra opened up new sites for dust bathing the following year. This indicates that dust bathing sites may be ephemeral for zebra, closing up each wet season and

opening up in new areas as the next dry season approaches. Due to the ephemeral nature of these sites, the camera-based monitoring may not have fully captured the seasonality of dust bathing for this species. Zebra were observed dust bathing at other non-monitored sites during the wet season (personal observation), therefore the seasonal differences in dust bathing by this species may not be as dramatically different as appears from the camera data.

This, on the other hand, was different among wildebeest. The study revealed that wildebeest dust bathe in all three seasons in ENP. There was no statistically significant difference in the proportion of dust bathing camera triggers per day among the three seasons. Only one of the wildebeest sites was overgrown with vegetation after the rainy season; two of them were used during the wet season. Although a relatively small number of sites were monitored, this provides evidence that wildebeest, unlike zebra, re-used their dust bath sites from the previous year. This may explain why wildebeest dust bathing sites had deeper depressions in the soil whereas zebra sites were flat to the ground (personal observation).

In regards to animals dust bathing to get rid of ecto-parasites as they rub their bodies against the soil particles (Carnaby, 2010), the finding of these study raises another question when addressing the epidemiology of wild life diseases. It may be debatable whether wildebeest may re-take up ectoparasites that they shed during dust bathing seen that they re-use their dust bathing sites. Zebras on the other hand change their dust bathing sites yearly thus the effect of re-uptake of ecto-parasites would be short term per year.

There was no significant relationship between the proportion of individuals dust bathing and maximum daily temperature for elephants. Neither was there any significant relationship between the intensity of dust bathing (number of throws of soil by an elephant onto its body) and temperature. Thus elephant dust bathing in Etosha National Park was not temperature dependent. This is however different from the findings of Rees (2002) who reported that dust bathing in captive Asian elephants increased as maximum daily temperatures increased and there was a clear correlation between the amount of time spent dust bathing and the daily maximum temperatures once it exceeded approximately 13°C. In Etosha, both mud and dust bathing were recorded approximately from 25°C - 38°C at waterholes. The difference between these two studies could be due to various factors. First elephants were not observed throughout the entire day in Etosha National Park. Second the Etosha elephants are free ranging in the wild thus the possible times to observe them were limited. Free ranging elephants also spend most of their time foraging thus reducing the amount of time for social activities (Shannon, Page, Mackey, Duffy & Slotow, 2008). Another possible factor could be that animals in groups stay and travel with the group (Wittmeyer, Getz, Vollrath & Douglas-Hamilton, 2007), thus the freedom to dust bath while others are initiating departure from the waterhole are restricted. This, however, does not apply to bulls travelling by themselves. Captive elephants are restricted in their natural movement (Vanitha, Thiyagesan & Baskaran, 2010) and therefore this may not be a very good test of elephant natural behaviours.

Mud bathing significantly increased as daily maximum temperatures increased, However there was no significant relationship between the intensity of mud bathing

(number of throws of mud onto the body) and daily maximum temperature. The increase in mud bathing by elephants with increasing temperature suggest that mud bathing serves as a cooling mechanism for the African elephants.

5.2 Demography of dust (or mud) bathing individuals

The hypothesis for the demography of dust bathing individuals was that male adults and sub adults dust/mud bathe significantly more often than adult females or juveniles in all three species. Zebras and elephants of all ages and both sexes were observed dust bathing but not for wildebeest. Juveniles of wildebeest were not observed dust bathing. Although it was difficult from cameras to accurately assess the age and particularly the sex of individuals, adults of both sexes in wildebeest and zebra were observed dust bathing. It may be possible that adult males dust bath proportionally more than adult females, given that populations tend to have more females than males (Berger & Gompper, 1999). In order to address this with confidence more data would need to be collected. The proportion of elephant dust bathing whose age and sex were unknown also makes it a challenge to test for dust bathing patterns between adult males and females. Although it is possible that adult males dust and particularly mud bathe more than the adult females, seen that adult females have the responsibility of taking care of young ones and mostly visit waterholes in groups.

5.3 Dust bathing and the risk of inhalational anthrax

In the current study the research hypotheses stated (i) that there is no significant positive correlation between the seasonal number of anthrax cases and seasonal number of dust baths of zebra and wildebeest because their mortalities due to anthrax occur mostly during the wet season, and (ii) that there is significant positive correlation between the seasonal number of anthrax cases and dust or mud baths seen that elephant mortalities are mostly observed during the dry season.

The results showed that there were no significant correlation for all three studied species. The dust bathing behaviours had no seasonal correlations with the occurrences of anthrax mortalities.

Anthrax cases in Etosha National Park have a seasonal peak. Throughout the years of anthrax surveillance in ENP, death in zebra and blue wildebeest have had strong peaks in the late wet season (March – April) (Turner *et al.*, 2013). On the other hand comparing the percentage dust bathing per season showed extremely low counts of dust bathing, with only 1.14% of all observed zebra dust bath triggers occurring during the wet season (total dust bath triggers recorded = 3; Appendix 4). Compared to zebra, fewer dust bath triggers were observed for wildebeest, and 21% (6 out of 28 CT dust bath triggers; Appendix 4) occurred in the wet season, there was no significant difference among the seasons in dust bathing by wildebeest.

The dust and mud bathing behaviours of African elephants were tested against increase in maximum daily temperature. Anthrax mortalities of African elephants

peak in the late dry season (October –November) when maximum temperatures range between 30°C to 40°C and represent the hottest period of the year (Turner *et al.*, 2013). These temperature patterns can be seen in Figure 5. Nevertheless elephant dust bathing was not dependent on daily maximum temperatures.

For free ranging elephants, this information only provides evidence of how elephants dust bathe when near watering points during the dry season, since elephants were not monitored on 24 hour basis or during the wet season. It is possible that more elephant dust bathing may have taken place in locations where they are not readily visible to humans. Regarding the dry season however, given the expected reasons for dust bathing activities (e.g, temperature regulation), this behaviour should more often occur during the hottest part of the day when sampling was conducted, not the other times of the day.

Whether or not dust bathing was linked to the timing of anthrax for these species, it was also hypothesized that soils at dust bathing sites would contain low or no concentrations of *B. anthracis* spores. This is because *B. anthracis* is rarely found in soils outside of anthrax carcass sites (Lindeque & Turnbull, 1994). As expected, the study demonstrated that only 2.41% of soils at dust bathing sites (2/83 samples) contained *B. anthracis* and only at very low concentrations.

The zebra dust bathing site (12-036) on which a zebra died of anthrax provides evidence that a carcass site is very unlikely to become a dust bath site. Although this represents anecdotal evidence because only a single dust bath carcass site was available for study, this dust bath site was not used again for dust bathing after the death of the zebra on it in 2012. When an animal dies and the carcass is scavenged,

the gut contents and blood are released into the soil and churned up by scavenging animals. As a result, the soil at a carcass site can become hardened and resist even plant growth, a feature of a carcass site described by W. C. Turner as the “gut cement” (personal communication, W. C. Turner, 2014). Although in this study a single carcass site was observed, in a study of more than 50 zebra carcass sites, this gut cement was observed at all of the sites, a pattern that persists for at least four years after death, and none of these monitored sites developed into dust bath sites (personal communication, W. C. Turner, 2014). This hardened soil observed at scavenged carcass sites indicates that it is very unlikely that an anthrax carcass site would become a dust bath site. Both zebra and wildebeest show attraction to grazing at anthrax carcass sites up to 1.5 years after death (Turner *et al.*, 2014), providing evidence that both these species are contacting the pathogen by grazing at anthrax carcass sites. This puts both these species in greater contact with the pathogen than if they contacted infectious sites by chance. Therefore this is credible that these species are getting gastrointestinal anthrax through grazing (Turner *et al.*, 2013) and not inhalational anthrax through dust bathing.

Unlike zebra and wildebeest, elephants were not found to be attracted to *B. anthracis* contaminated carcass sites for grazing (Turner *et al.*, 2014), thus it is not yet evident that elephants may be contracting gastrointestinal anthrax. How elephants contact the *B. anthracis* pathogen still remains unknown. The timing of elephant anthrax mortalities which is toward the late dry season (Turner *et al.*, 2013; Hampson *et al.*, 2011) provided a suitable hypothesis that dust bathing might be a possible factor

contributing to contact with the pathogen. However results from this study imply that is is unlikely the case.

It is possible that concentrations of *B. anthracis* could be moved away from carcass sites to other areas more likely to become future dust bath sites. This includes local movements such as wind (Turnbull *et al.*, 1988) water runoff or fecal deposition or movement by spore adherence to animal fur or feathers during scavengers (Dragon *et al.*, 2002). However, these movements of spores would be perhaps highly diluted concentrations (such as 160 cfu (2.1×10^{-4} cfu/l of air) aerosolized air sampled *B. anthracis* spores that were found over a distance of 6m from the centre of the sample site by Turnbull *et al.*, (1998)) and thus unlikely to be significant for transmission of *B. anthracis*, even for inhalational anthrax which has considerably lower lethal doses.

Chapter 6: Conclusion and recommendation

6.1 Conclusion

It has been found that many herbivores in Etosha National Park are likely to acquire anthrax through ingestion of *Bacillus anthracis* spores (Turner *et al.*,2014), in particular for plains zebra blue wildebeest and springbok. However, this link between foraging and anthrax was not found to be the case for African elephants. For management purposes it is of great importance to know all possible ways of infections, particularly if species differ in their infection pathways. Thus the objective of this study was to determine the dust bathing behaviour of herbivores species (zebra, blue wildebeest and African elephants) to assess the possibility that they may inhale anthrax spores while dust bathing as suggested in several studies such as Dragon, Rennie & Elkin (2001); Mackintosh, Haigh & Griffin (2002) and Turner *et al* (2013).

The study showed that dust bathing activities by zebra occur predominantly during the dry season. However, with dust bath soils becoming hardened and dust baths being overgrown with vegetation in the wet season, other locations are used for dust bathing (personal observation). Sex-age proportions showed no difference in dust bathing behaviours among zebra. Blue wildebeest dust bathed both in the dry and wet seasons. There was also no difference in the dust bathing behaviours of blue wildebeest between the adult males, adult females and sub-adult; although no

juvenile was observed taking dust baths during the study. African elephants were found to mud bath in response to increase in daily temperature but dust bathing was not temperature dependent.

Microbiological screening of dust bathing sites that were found during the study period showed that dust bathing is unlikely to contribute to transmission of anthrax through the inhalation of *B. anthracis* spores. This however does not rule out the possibility of animals inhaling anthrax spores through other means such as when walking near old carcass sites.

The results of this present study may be useful to assist the Ministry of Environment and Tourism or other relevant institutions in Namibia such as farmers on understanding the epidemiology of anthrax in the areas where the disease is endemic. Beyond Namibia, this study also provides knowledge on both dust bathing behaviours of large herbivorous mammals (zebra, wildebeest and elephants in particular). In search of anthrax transmission mechanisms, this information can be used to assess the transmission of anthrax by identifying dust bathing sites and microbiologically screening them for the presence of anthrax, especially in Northern Canada where it is known that Bison species wallow (roll in mud or water) (Coppedge & Shaw, 2000) and are also susceptible to anthrax (Dragon *et al.*, 2005).

This may help with time management in control of the disease and give a start point on which areas to give more focus on in disease controlling. As it is known that anthrax cases occur in ENP, this study together with past research conducted in ENP provides information on how the anthrax susceptible host animals may or may not

contract the pathogen. This in future may possibly contribute in the formulation of guidelines in surveillance and control of this enzootic disease.

6.2 Recommendation

In follow-up to the current study, a long term study on elephant dust and mud bathing behaviours in localities at and apart from waterholes will give more insight on the dust bathing behavioural studies of elephants. This would require access to off road locations in protected conservancies to observe elephants around the clock and in all seasons. Carrying out the study in a smaller reserve or private game reserve where elephants are still free-ranging, yet more accessible to regular observations throughout the day and year is another way around the problem. In the case of zebra and wildebeest behavioural studies, it will be an advantage to do a long term study with more camera sites in which camera locations are changed to newly used sites after rainfall if the current sites are no longer in use.

Additionally in the search to understand transmission of anthrax to animals through inhalation of spores, it will be useful to conduct a new study in which the concentration of *B. anthracis* spores on animal walking paths near or on anthrax carcass sites can be mechanically aerosolised and quantified.

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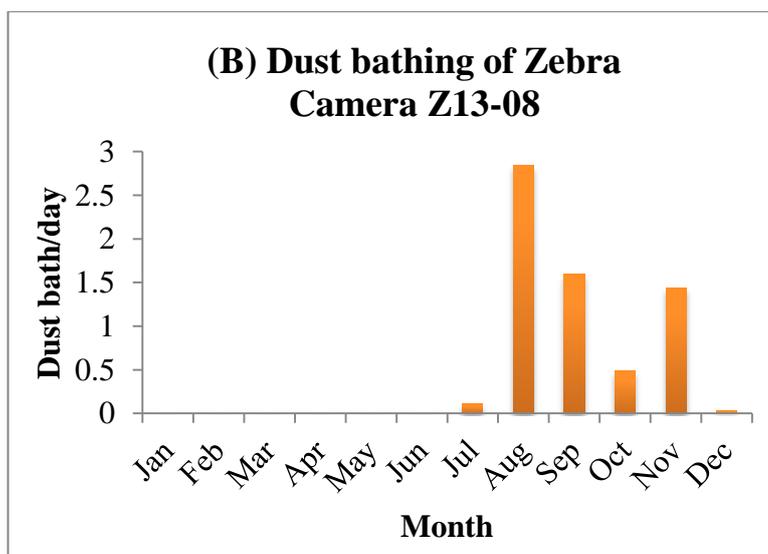
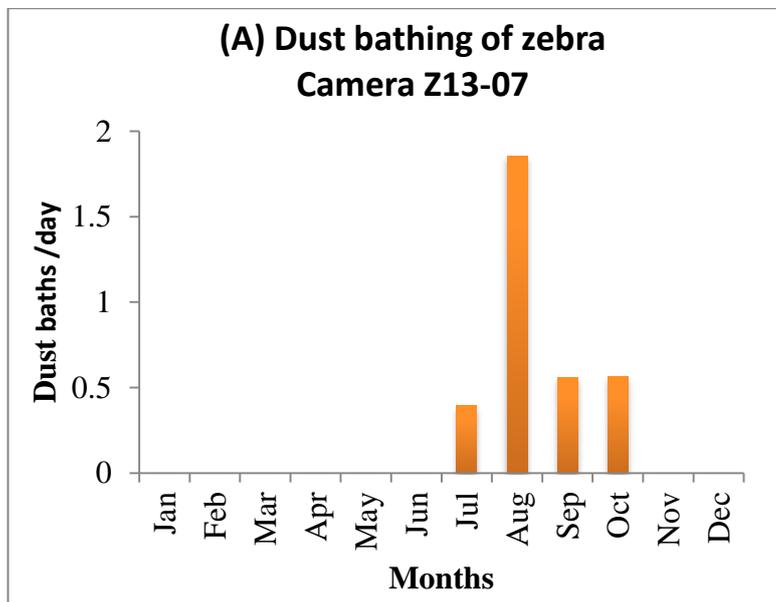
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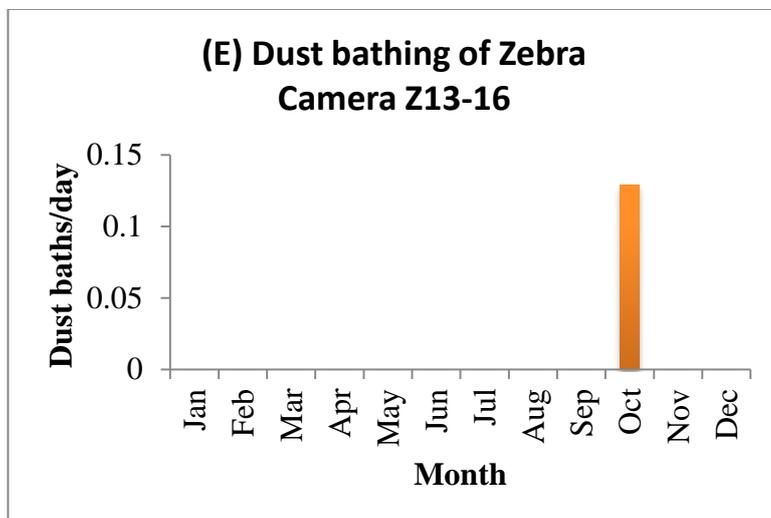
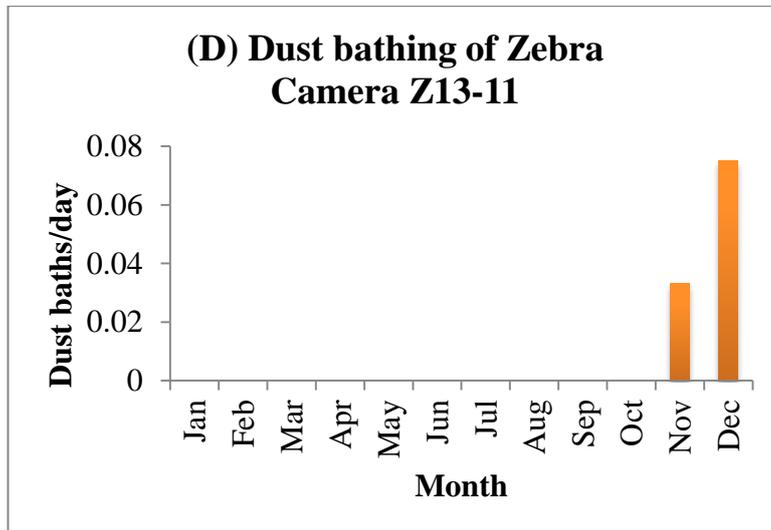
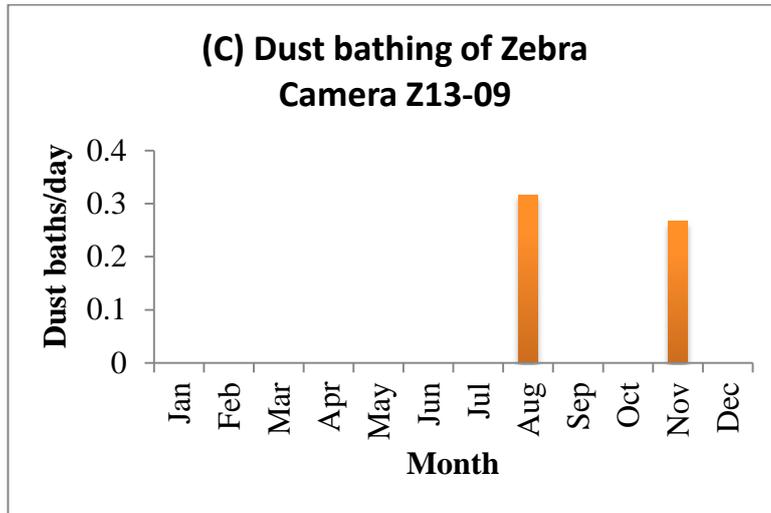
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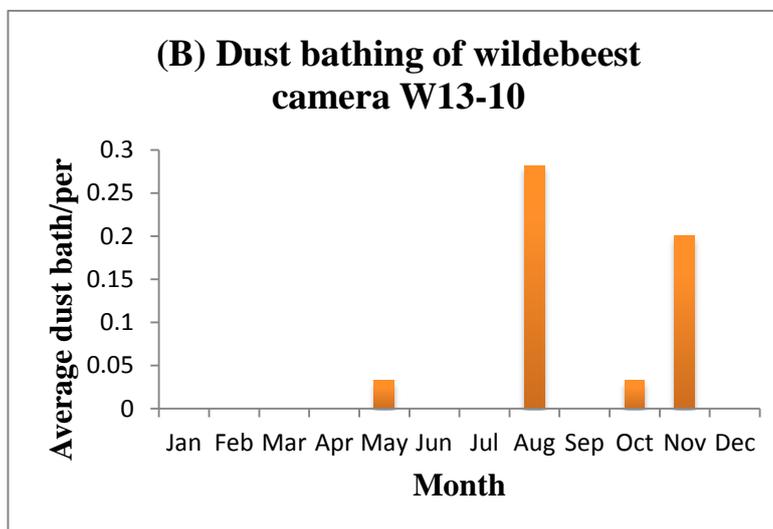
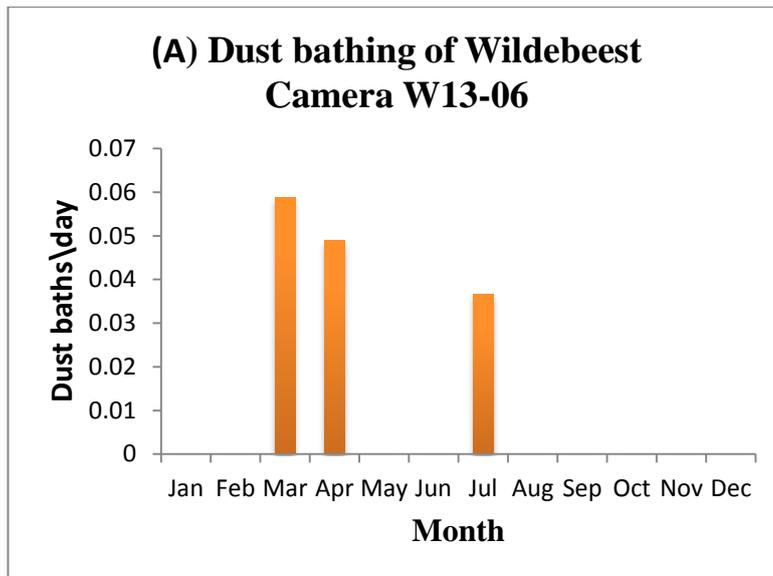
Appendices

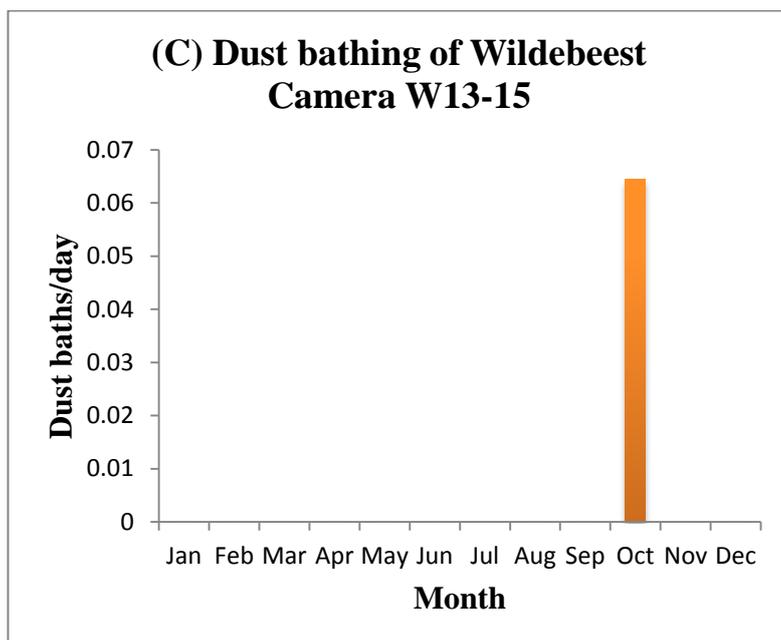
Appendix 1: A, B, C, D, and E indicate the monthly average triggers per day of dust bathing zebras at the five sites where cameras were set up for the behavioural study.





Appendix 2: A, B and C indicate the monthly triggers per day of dust bathing by wildebeest at the three sites where cameras were set up for the behavioural study.





Appendix 3: The table below shows the number of dust baths (DB) (mud bath (MB) in B) observed during the study and the anthrax mortalities (ANP). (A and B) are for African elephant, (C) is zebra data and (D) is for blue wildebeest. The anthrax mortality counts are from the year 1975 – 2013 was obtained from EEI 2014.

(A)

| Row Labels | AM | AF | SA | J | U | Grand total |
|--------------------|-----|----|-----|----|----|-------------|
| DB (LA) | 5 | 4 | 5 | 6 | 15 | 35 |
| ANP (LA) | 99 | 41 | 109 | 48 | 39 | 336 |
| Grand total | 104 | 45 | 114 | 54 | 54 | 371 |

(B)

| Row Labels | AM | AF | SA | J | U | Grand total |
|--------------------|-----|----|-----|----|----|-------------|
| MB (LA) | 17 | 5 | 2 | 3 | 3 | 30 |
| ANP (LA) | 99 | 41 | 109 | 48 | 39 | 336 |
| Grand total | 116 | 46 | 111 | 51 | 42 | 366 |

(C)

| Row Labels | AM | AF | SA | J | U | Grand total |
|--------------------|-----|-----|-----|----|-----|-------------|
| DB (EB) | 38 | 63 | 50 | 6 | 107 | 264 |
| ANP (EB) | 320 | 351 | 133 | 43 | 183 | 1030 |
| Grand total | 358 | 414 | 183 | 49 | 289 | 1293 |

(D)

| Row Labels | AM | AF | SA | J | U | Grand total |
|--------------------|-----|-----|----|----|----|-------------|
| DB (CT) | 18 | 4 | 1 | 0 | 5 | 28 |
| ANP (CT) | 111 | 97 | 25 | 25 | 41 | 299 |
| Grand total | 129 | 101 | 26 | 25 | 46 | 327 |

Appendix 4: Contingency table analysis for the seasonal correlation of dust (and mud) bathing and anthrax mortalities observed from 1975 – 2013 in ENP obtained from the EEI database. Table (A) is analysis for blue wildebeest, (B) is for zebra, (C) is for dust bathing elephants and (D) is represents that of mud bathing elephants.

(A)

| Season | Anthrax | DB (CT) | Grand total |
|--------------------|---------|---------|-------------|
| Hot-wet | 159 | 6 | 165 |
| Cool-dry | 95 | 11 | 106 |
| hot-dry | 45 | 11 | 56 |
| Grand total | 299 | 28 | 327 |

(B)

| Season | Anthrax | DB (EB) | Grand total |
|--------------------|----------------|----------------|--------------------|
| Hot-wet | 754 | 3 | 757 |
| Cool-dry | 168 | 137 | 305 |
| hot-dry | 108 | 124 | 232 |
| Grand total | 1030 | 264 | 1294 |

(C)

| Season | Anthrax | DB (LA) | Grand total |
|--------------------|----------------|----------------|--------------------|
| Cool-dry | 88 | 26 | 114 |
| hot-dry | 170 | 8 | 178 |
| Grand total | 258 | 34 | 292 |

(D)

| Season | Anthrax | MB (LA) | Grand total |
|--------------------|----------------|----------------|--------------------|
| Cool-dry | 88 | 17 | 105 |
| hot-dry | 170 | 12 | 182 |
| Grand total | 258 | 29 | 287 |

Appendix 5: Proportion of dust bathing (DB) and mud bathing (MB) of elephants.

| AGE&SEX | Ave_pro-Db | Ave_pro_Mb |
|--------------------|-------------------|-------------------|
| AM | 0.124716553 | 0.39047619 |
| AF | 0.020408163 | 0.207142857 |
| SA | 0.075 | 0.04047619 |
| J | 0.157692308 | 0.1 |