The transmission dynamics of parasites of the African elephant (*Loxodonta africana*) in the Okavango Delta: a coprological survey

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Abstract

Studies in a wide range of wild and domestic animals have shown that parasites can affect growth, reproduction and health.

Although, to date, there is little evidence to suggest that internal parasites of free-ranging African elephant are a significant cause of mortality or ill health, there are examples of high parasite loads and some pathology in nutritionally stressed free ranging African elephants suffering from drought conditions and lack of food.

A better understanding of parasites and their ecology, with respect to African free-ranging elephant population health is needed given the changing environment for these animals within an increasingly human dominated and modified landscape and increasing restriction on movement and diverse food resources.

A total of 397 faecal samples were collected from wild elephants in the Okavango Delta and subjected to parasitological analysis, either on fresh samples or after storage in 10% formalin. The samples were found to contain ova from nematode (prevalence 73.3%), fluke (23.4%) and coccidia (47.6%).

The following factors had a significant association between prevalence of certain parasites and burden in wild African elephants and age, sex, group dynamic, group size, month and year. Whether these risk factors are coincidental or significant drivers of parasite dynamics are not proven or disproven by this study. The strongest associations were towards: seasonal and animal concentration factors especially for coccidia, and significant differences in burden between browsers and grazers, findings which are consistent with parasite life cycles, and transmission dynamics.

A parallel study of seven captive elephants found a significant change in nematode egg density and coccidial oocyst prevalence over a three month period. Comparative morphological studies of fluke eggs, across a range of species were suggestive that those found in elephant faeces were representative of a host specific parasitism. However, whether other parasites were shared between elephant and other species was not conclusively determined.

Storage in formalin had a negative effect on parasite ova detection in faecal samples collected from wild elephants. A brief study on nematode-infected sheep faeces also suggested that formalin at high temperatures may have some limitations as a storage medium. Therefore alternative preservation methods should be considered in future investigations of natural parasite infections in free-living wildlife.

Parasite infection of wild elephants with coccidia, nematodes and trematodes was confirmed, and there was evidence that a variety of host and environmental factors influence the prevalence and burden. No evidence was presented to suggest these infections were pathological.

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Author's Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes. It has not been submitted for any other academic award or to any other University. Except where indicated by specific reference in the text, the views expressed are those of the Author.

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CHAPTER 1: INTRODUCTION

1.1 Study justification

Parasites have the ability to reduce reproductive success, body condition, and survival in their hosts (Irvine, 2006). The majority of data on elephant parasitism are from captive elephants, particularly the Asian species and research on wild elephant parasite fauna and their transmission dynamics is notably lacking. This study aims to determine the factors that influence parasite infections in elephants in the Okavango Delta, Botswana, as well as investigating whether there may be cross-species transmission of parasites between sympatrically grazing mammals.

1.2 The effect of parasites on their hosts

Parasites that cause host mortality, or negatively affect reproductive success are sometimes able to regulate host populations (Irvine, 2006). For example, parasites have been shown to influence population dynamics in snowshoe hares, *Lepus americanus*, Soay sheep, *Ovis aries*, and Svalbard reindeer (Irvine 2006). However, although a number of studies have found that gastro-intestinal nematodes have a negative effect on host body condition, it often cannot be proved that this is directly linked to mortality or decreased reproductive success. This appears to be the case in Dolphin-Union Caribou, *Rangifer tarandus* (Hughes et al., 2009), and red deer, *Cervus elaphus* (Irvine et al., 2006). As it has been found in the African elephant that mortality can be caused by parasite infection (Vitovec et al., 1984), it is possible that parasites might have a regulatory influence on elephant populations, although this is unlikely as there is a lack of evidence that serious pathology is caused by parasites in African elephants.

In addition to affecting host health and reproductive success, parasites can drive host behaviour. Some such behaviours are voluntary and may have evolved to minimise the effects of parasites: for example, dust bathing in African elephants protects against ectoparasites (Fowler and Mikota, 2006). The migration of Norwegian reindeer may be a parasite avoidance tactic: herds with post-calving migrations have a significantly reduced abundance of warble fly larvae than herds that remain on or near their calving territory (Folstad and Karter 1992). Furthermore, some parasites can cause involuntary host behaviour. It has been found that the Gordian worm, *Nematomorpha*, can influence the nervous system of its cricket host, to the extent that the cricket kills itself by jumping into water (Libersat et al. 2009). Parasites can influence their hosts in a plethora of different ways and further research is necessary to discover the ways in which internal parasites affect elephants at both individual and population levels.

1.3 Disease transmission between elephants and sympatric mammal species

A relatively recent but critical field of research investigates the transmission of disease between wildlife and livestock. Such research can help reduce the longstanding conflict between wildlife conservation and livestock farming. Although studies in the past have mainly focused on the transfer of disease from wildlife to livestock, progressive research has shown that livestock is often a source of infection for wildlife. The economic value of African game is increasing with the tourist trade, with the wildlife sector in Africa being worth $7 billion in 2005, and having an annual growth rate of 5% (Osofsky et al. 2005). With this exponential growth, there has been increased focus on trying to minimise the negative repercussions of integrating wildlife and livestock. Some research even suggests that the presence of wildlife may assist livestock to tolerate pathogens (Osofsky et al. 2005). However, there has been little research investigating whether disease may be spreading between wild elephants and proximate livestock and / or other wildlife.

Cross-host transmission has the potential to occur readily in sympatric species. The traditional method of preventing the spread of disease has been to eradicate wildlife from areas surrounding domestic livestock. A drastic example of this was an attempt to eliminate Rinderpest from an area in Malawi by shooting all of the wildlife. A detailed study of Rinderpest in the area at the time would have shown that cattle were in fact the reservoir hosts for these outbreaks, and the mass slaughter could have been prevented. The area is now Rinderpest free, but only due to recent research, and the subsequent vaccination of the resident cattle (Kingdon, 1982). Another favoured method for the prevention of disease is the use of veterinary control fences. Such barriers have been widely constructed in Botswana, Namibia, South Africa and Zimbabwe since the 1960s. They were originally constructed to prevent the spread of foot and mouth disease, but have been maintained to halt the spread of other diseases. However the effects of these fences are often disastrous, as they disrupt wildlife movement and can cause starvation and dehydration related mortality in many species. In northern Namibia, fences to prevent the spread of foot and mouth have been blamed for the unnecessary death of at least 200 buffalo in the Bushmanland area (Osofsky et al. 2005). This highlights the need for thorough research on disease transmission before control measures are put in place.

Parasite transmission between sympatric wild and captive elephants is also possible. The number of elephants kept in captivity in Africa is on the rise (IFAW, 2007), as they become an increasing tourist attraction. Collecting information concerning the transmission dynamics of elephant parasite fauna may allow the routines and conditions of captive elephant to be optimised to minimise disease risk to these as well as wild elephants.

Parasite transmission in captive elephants can also be minimised by the administration of drugs, including; fenbendazole, albendazole, ivermectin and mebendazole (Fowler and

Mikota 2006). This expensive process can be optimised by studying the lifecycles of infective parasites, and investigating the most favourable time to target parasites. However, in the case of African elephant parasites, this is challenging as so few lifecycles have been described in sufficient detail. An alternative method to drug administration that has been suggested for fluke control in elephants, is to control snail populations. As snails are the obligate intermediate hosts of most fluke species (Cotgreave and Forseth, 2002), controlling snail numbers and distribution may limit infection in elephants (Hammond, 1972). However, such control would be extremely difficult to implement and there would be little justification due to lack of evidence of extreme fluke-driven pathology in elephants.

Although parasites of wild mammals often have host adapted genotypes (Applebee et al. 2005), some parasites that infect elephants are known to be zoonotic, including *Cytosporidium*. It has been hypothesised that the high incidence of cytosporidiosis (18%) in school children and hospital patients in the Venda region near Kruger National Park (KNP), may be due to wildlife sharing the same pastures as humans and grazing animals (Abu Samra et al., 2011). It was found that wild elephants were highly parasitised, and that their highest *Cytosporidium* prevalence was next to the KNP fence. The authors speculate that this may be due to parasite transmission between humans, livestock and elephants in this area. However, further genotyping of *Cytosporidium* strains need to be carried out before this can be concluded (Abu Samra et al., 2011).

As parasite species are not limited to having adverse affects on elephant individuals, but may also have a wider effect on the ecosystem, this further enhances the justification for research to be carried out on these systems.

1.4 The parasite fauna of the African elephant

The work that has been carried out on the gastro-intestinal parasite fauna of African elephants has met with some limitations; opportunity for post mortems is infrequent and identification of parasite species by way of faecal egg investigations is difficult. However, with the advancement of technology such as DNA analysis, the categorisation and identification of parasites is becoming more achievable and reliable.

Recent research suggests that the the African savannah elephant, *Loxodonta africana africana* and the African forest elephant, *Loxodont africana cyclotis,* may be different species, rather then different subspecies (Kinsella et al. 2004). Although current work is beginning to differentiate between the two, previous parasite research has not always specified the subspecies studied (Kinsella et al. 2004). The majority of past research however is likely to have been conducted on savannah elephants, being the more numerous and accessible group, making up around 75% of all African elephants (Eggert et al. 2003). Forest elephants are only found in the Congo basin and in isolated populations in West Africa, and their forest habitat impedes observation and research (Blake et al. 2007).

Another potential factor that could be restricting the identification and classification of parasites is hybridisation between species. Although research is limited in this area, incidents of hybridisation have been identified between *Fasciola hepatica* and *Fasciola gigantica*, two parasites that have been reported in the African elephants (Norbury, 2008). As there has been little work on the fitness of hybrid parasites, how this may affect host species is largely unknown (Detwiler and Criscione, 2010).

More research has been conducted on the Asian elephant than the African elephant, as their captive population numbers over 16,000, thus validating the cost into veterinary research and facilitating study (Lei et al. 2012). Most of the detected parasites are

thought to be host specific at the species level (Fowler and Mikota 2006), with only the following species having been recorded in both African and Asian elephants: *Anoplocephala manubriata, Toxoplasma gondii, Fasciola hepatica, Protofascia robusta, Anoplocephalidae manubrata* and *Inofilaria pattabiramani*. This minimal overlap suggests that parasites have evolved to become highly environmentally and / or host specific in the 7.6 million years since the African-Asian elephant divergence (Rohland et al., 2007). Despite host specificity at species level, fluke, nematode and coccidia genera have all been found in African and Asian elephants (Fowler and Mikota 2006), and were found in a preliminary study of the African elephants of the Okavango Delta. However, due to the limited amount of work that has been carried out on the parasites of African elephants, life cycles are often unknown. Extrapolation from described lifecycles of similar parasites found in mammal hosts allow us an insight into possible transmission dynamics and requirements of the African elephant parasite fauna.

1.4.1 Fluke

Fluke species, *Protofasciola robusta,* has been found to cause African elephant mortality (Vitovec et al., 1984) by causing intestinal tissue damage and haemorrhages (Obanda et al. 2011), although this has only been reported on a couple of occasions. Other fluke species found in African elephants include *Fasciola hepatica*, and *Fasciola jacksoni,* the life cycles of which have been described: fluke eggs are discharged in the faeces from adult worms living in the bile duct or lung of the host (Fowler and Mikota 2006). These eggs require water for survival, and hatch into free swimming ciliated miracidia, a process which takes 10-12 days. The miracidia then penetrate the body of an intermediate host (usually a snail) and subsequently develop into; sporocysts, then redia, and finally into free swimming cercariae larvae, a process which takes 4.5-7 weeks (Fowler and Mikota 2006). Once these cercariae

have left the snail, they are free swimming for between a couple of minutes to a couple of hours, before attaching to a plant and developing into metacercariae, the infective stage for the definitive host (Fowler and Mikota 2006). They can remain on the plant for between a few days to a few weeks, depending on the level of surrounding moisture (Fowler and Mikota 2006). Similar lifecycles are hypothesised for other fluke species that parasitise elephants.

Fluke development is dependent on several factors including; moisture, temperature and oxygen levels. Temperature can affect the development of egg laying parasites in two ways; firstly, egg hatching usually only occurs once a critical temperature has been reached (over which the development rate tends to increase further), and secondly, temperature can affect the survival rate of hatched larval stages (Altizer et al. 2006). It has been found that eggs from *F. hepatica* and *F. jacksoni* will not develop at temperatures below 10 ^{o}C (Fowler and Mikota 2006), but above this, there is a direct positive correlation between temperature and development rate. However, at extreme temperatures, fluke eggs can be prone to desiccation (Suhardono et al. 2006). Environmental humidity is also an important factor in fluke development. *F. hepatica* eggs need to be surrounded by a surface film of water in order to hatch and avoid desiccation (Rowcliffe and Ollerenshaw, 1960).

It is therefore hypothesised that fluke levels in the Okavango Delta are high, as factors that limit development such as water deficiency and low temperatures are predominantly absent (Rowcliffe and Ollerenshaw, 1960).

1.4.2 Nematodes

Nematodes, the most frequently found elephant parasites (Fowler and Mikota 2006), are able to cause pathological lesions and haemorrhages in the bile ducts, intestines and liver of their host (Obanda et al. 2011).

The lifecycles of elephant infecting nematodes are hypothesised to be similar to those that parasitise domestic livestock (Fowler and Mikota 2006). The most common nematodes found in the African elephant are in the order Strongylidae, the lifecycles of which are generally as follows: eggs are expunged in the faeces, and hatch into L1 stages, remaining encased until conditions are suitable for hatching. 1-2 days post-hatching, the L1 becomes free living and starts to feed on microorganisms in the faeces. This is followed moulting to the L2 stage, and then to the infective L3 stage. Under optimum conditions, the L3 larvae will migrate from the dung onto surrounding vegetation within a week, where they are ingested by feeding host species. Once inside the host, development continues through L4 and L5 stages, and finally into the adult worm in the intestine or stomach (Fowler and Mikota 2006).

As is the case in fluke, nematode development can be affected by temperature, moisture and oxygen levels. Many nematode species have been found to require high environmental temperatures for development (Altizer et al. 2006). The high annual temperatures of the Okavango Delta, (the mean annual temperature is around 30°C (Bjorkvald and Boring, 2002), may aid rapid nematode development. However excessively high temperatures have been shown to lead to egg desiccation in some species of nematode (Wharton, 1979). Moisture is also important for the development and survival of helminth larvae; limited ground water can prevent transmission, and force larvae to migrate into the soil. On the other hand, an excess of ground water can wash away eggs and larvae, potentially obstructing transmission (Altizer et al. 2006).

Until more detailed lifecycles of nematode species have been compiled, how environmental conditions affect nematode development rates in the Okavango Delta are hard to predict.

1.4.3 Coccidia

Coccidia are single celled parasites that cause coccidiosis, an extremely common disease among ungulates and other mammals. However, no adverse clinical conditions have been linked to the presence of coccidia in elephants to date (Fowler and Mikota 2006).

The lifecycles of coccidia species are generally as follows: oocysts enter the small intestine of the host and four sporozoites are released. These invade the individual epithelial cells of the intestinal wall, and the sporozoites develop into trophozoites. Asexual division produces 6-8 nuclei per trophozoite, which develop into merozoites. These invade new cells and either form Type 1 or Type 2 meronts. Type 1 meronts invade more cells (self infection), while Type 2 meronts differentiate into either microgametes or macrogamete forms. The microgametes are released from the cells, fertilise macrogametes, and form zygotes. After meiosis, walls form around the resulting sporozites to form oocysts (Taylor et al. 2007), the infective parasite form.

Although coccidia oocysts generally require warm temperatures for sporulation to occur, excessive temperatures can be lethal (Sathyanarayanan and Ortega, 2006). In rabbit coccidia, *Eimeria perforans*, 33 ^{o}C is the optimum temperature for sporulation to occur, but at temperatures of 36 ^{o}C, many of the oocysts desiccate (Becker and Crouch, 1931). Moist conditions and oxygen also facilitate the sporulation of oocysts and outbreak of coccidiosis (Waldenstedt et al., 2001).

Insight into the lifecycles of parasites that are infecting African elephants is essential for being able to predict patterns in parasite transmission. Fluke, nematode and coccidia development are all affected by environmental conditions such as temperature, oxygen and moisture, and it is likely that the warm, wet conditions of the Delta are highly beneficial to

parasite development. Although the very high temperatures that can be reached here may cause developmental constraints.

1.5 Factors that may affect the parasite burden of African elephants

As discussed, environmental conditions, may be influencing development in parasite fauna that infect the African elephant. However, there are a number of other factors that may also influence the transmission dynamics of these species. These include inter-annual variance, as well as elephant age, sex, group size and group dynamic. Although this is not an exhaustive list of the potentially influencing factors, it includes the variables that have most commonly been found to influence parasite transmission in mammalian species (Cross et al. 2009), and are those that are investigated in this study. Other factors that are more challenging to research, such as individual genetics, are also likely to be playing a role in host-parasite dynamics, although further work is required to determine the extent of this role.

1.5.1 Age

The impact of host age on the parasite burden of wild mammals is influenced by many variables including; the rate of parasite induced mortality, the workings and extent of host immunity, parasite mortality and age dependent exposure (Cross et al. 2009).

An increase in elephant age may correlate with a build up of immunity against parasites, as is the case in horses. Young horses commonly have high strongyle burdens, whereas horses over the age of 15 are less heavily infected due to acquired immunity (Foster, 1937). On the other hand, high parasite burdens can be seen in elderly individuals of some species, due to a decline in the immune system with age (Masoro and Austad, 2010). It has been shown in wild Soay sheep that this weakening of immunity is partly due to significant differences in T-cell subsets and inflammatory markers at an advanced age (Nussey et al. 2012). A study on tree

swallows, *Tachycineta bicolour*, found that acquired T-cell mediated immunity declined with age, however acquired and innate humoral activity did not (Palacios et al. 2007). Even within the same host species, age can have contrasting effects on different parasite species. In the Saiga antelope, *Saiga tatarica*, *Nematodirus gazellae* parasite infection intensities declined with age, whereas *Marshallagia* spp. infections increased with age. This increase was suggested to be indicative of the relative unimportance of immunity in free-living populations, or due to a reduction in host condition with age. The decrease in parasite infections in *Nematodirus gazellae* with age may be linked to acquired immunity rather than parasite-induced host mortality. Furthermore, *Nematodirus* spp. penetrate deeper into the mucosa than other trichostrongyloid nematodes and may be more immunogenic as a consequence (Morgan et al. 2005).

There is a positive correlation between age and dominance in African elephants and the position that an individual holds in a hierarchical group has been found to affect parasite burdens in many species, including chimpanzees. In male chimps, high rank is correlated with high testosterone levels, which is linked to immunosuppression, and higher helminth burdens (Muehlenbein and Watts, 2010). Bull elephants also have hierarchies which have been found to affect hormone levels; the heightened hormonal state of musth can be inhibited in bulls by the presence of older, higher-ranking males (Poole et al. 1984). Female elephants have a female dominance hierarchy that is age-ordered but not nepotistic (Archie et al., 2010). However, little work so far has investigated the effect of rank on hormone levels, and in turn, whether this can affect parasite levels. The hierarchical position of African elephants can also affect the quality and quantity of vegetation and water consumed, as well as the degree of social interaction (Wittemyer et al. 2007). Social hierarchy could affect parasite burdens directly through hormone-linked immunosuppression or by affecting resource acquisition and hence infection pressure from trophically acquired parasites.

As elephants continue to grow throughout their lives, age can affect the height of the vegetation within trunk reach (Lee and Moss, 1986). This in turn may be affecting parasite exposure. In Queen Elizabeth National Park, Uganda, significantly more nematode larvae were found at ground level than at >20cm off the ground (Apio et al. 2006). This is expected, as nematode eggs are generally dropped at ground level in faeces. The survival of nematode larvae on vegetation is also heavily dependent on pasture humidity. As the distance from ground level increases, so does the evaporation rate, due to increased sunlight and wind, which leads to increased nematode desiccation (Apio et al. 2006). The negative correlation between nematode abundance and height of vegetation off the ground (Apio, et al., 2006) may result in older, and therefore taller elephants, having lower parasite contact rates.

Very young individuals are hypothesised to have a low or non existent parasite burden. Elephant calves do not ingest plant matter until they are at least a few months old (Smith, 1995), and are therefore not exposed to infectious parasite larvae that dwell in the vegetation. Suckling young may also have protection against parasites from antibodies produced in their mother's milk (Cross et al. 2009). The age at which elephants are weaned varies greatly. A calf aged 25 months can survive without suckling, although few individuals stop this early, with some calves having been reported to suckle till the age of 8 (Lee and Moss, 1986). It is also possible that milk produced by the mother only contains passive immunity for a short period of time. This is the case in deer mice, where passive immunity is found in the mother's milk for the first few weeks after giving birth, despite young suckling for longer (Theis and Schwab, 1992). On the other hand, there is the potential for disease exchange between mother and offspring through suckling (Roulin and Heeb, 1999). Suckling may also affect the mother's immune system; it has been found that during parturition and lactation in ewes, immunity to nematode infection is temporarily lost (Barger, 1993).

1.5.2 Sex

The sex of an individual has the potential to affect both exposure and resistance to parasites. In elephants, sexual dimorphism in seen in size, diet, social interactions, territory, immune response and spleen size (which is known to affect nematode infection), all of which have the potential to affect parasite load (Poulin, 1996).

A meta-analysis by Moore and Wilson (2002) found that in eight out of ten mammalian orders, males were more likely to be parasitised than females, and predominantly attribute this to sexual dimorphism in size. Larger individuals tend to ingest more food, present a larger target area for vectors, and have more internal body space for parasites (Cross et al. 2009).

Despite males eating more, female elephants have higher nutritional needs per unit body mass than males (Stokke and du Toit, 2000) and have been found to forage on higher quality food. Food quality has been found to influence parasite levels in many species for example, snow shoe hares (Krebs et al. 2001). If this is the case in elephants, then males could be hypothesised to have higher parasite levels due to both their increased food intake, and their ingestion of lower quality vegetation.

The different group dynamics of male and female elephants may also lead to differential parasite burdens between the sexes. Females remain in matriarchal herds for life, whereas males become semi-solitary at the onset of puberty, at around the age of 14 (Ganswindt et al. 2010). This may decrease the exposure that these post pubescent males have to parasites, as contact rate between hosts is often a critical factor influencing parasite spread (Ezenwa et al. 2006). However, small fission fusion societies of unrelated or related males will often form (Ganswindt et al. 2010), and males will continue to interact with matriarchal herds. Furthermore, the more solitary lifestyle of an adult bull allows them to cover an area much

larger than matriarchal groups, potentially increasing their exposure to parasites (Thurber et al. 2011).

Sex-specific internal factors may also influence parasite burden. Evidence suggests that estrogen, a typically female hormone, promotes immunity whereas androgen, a typically male hormone, has an immunosuppressive effect (Schalk and Forbes, 1997 and Zuk and McKean, 1996).Bull elephants have regular bouts of increased hormone levels (musth), which occurs after around 29 years of age (Hollister-Smith, et al. 2007). Musth is a competitive reproductive tactic that is preceded by elevated androgen levels (Ganswindt et al. 2010) and testosterone levels remain high throughout the musth period. A study in the Addo National Park showed that a low ranking, non musth bull had a plasma testosterone level of 3.48ng/ml whereas a musth bull had a plasma testosterone level of 19.80ng/ml (Hall-Martin and Van Der Walt, 1984). If testosterone does have an immunosuppressive effect in elephants, then males in musth may have a lowered resistance to parasites. Bulls in this elevated hormonal state also tend to eat less (Thurber et al. 2011), decreasing the probability of ingesting parasites. However, this reduction in nutrition could negatively impact condition which may increase stress levels (Ganswindt et al. 2010), potentially leaving bulls more susceptible to parasites. Furthermore, the increased energetic output of musth may also result in decreased immunity being seen over time (Thurber et al. 2011).On the other hand, bulls have to be in good condition in order to be able to enter musth, which could lead to a decreased parasite burden being observed in these bulls at the onset, or just prior to musth, as body condition and parasite burden are often negatively correlated (Thurber et al. 2011).

It is also hypothesised that stress predisposes elephants to increased endoparasitism (Fowler and Mikota 2006), but whether musth is a physiologically stressful condition is currently debated. Some research reports an increased glucocorticoid output and therefore increased stress during musth, whereas others have detected a reduced output (Ganswindt et al. 2010).

Another state to be considered when investigating sex specific parasite burdens is pregnancy, as this condition can increase host stress and lead to a rise in both progesterone and cortisol metabolite levels (Foley et al. 2001), which can affect immunity. This is seen in the European rabbit, *Oryctolagus cuniculus*, where pregnant females are especially susceptible to infection from the nematode, *Trichostrongylus retortaeformis* (Altizer et al. 2006). A*s* elephants have a gestation period of 22 months, hormonal changes during pregnancy could have a prolonged effect on parasite burden.

1.5.3 Group dynamic and size

Elephants form complex fission fusion matriarchal societies (Wittemyer and Getz, 2007) which are composed of around 6-40 elephants (Thurber et al., 2011) that live and travel together in close contact. As nematode burden is positively associated with population density in many mammal species (Arnenberg, 2002), group size and dynamic could exert a significant influence on the parasite burden of elephants. However, it could be suggested that the tendency to congregate around limited resources in the dry season, or raised islands of pasture during flooding, is a more important factor in parasite transmission than group size per se.

In the dry season, often a period of increased stress due to limited food, a positive correlation has been found in female elephants between group size and glucocorticoid levels (Ganswindt et al. 2010). An increase in glucocortoid levels can lead to a suppression of the immune system, such that larger group sizes could potentially lead to increased parasite levels, especially at stressful times of the year. The differential aforementioned living dynamics of male and female elephants is also likely to affect parasite burden, but once again, there are so many potentially influencing factors that this is hard to predict.

1.5.4 Seasonality

Seasonal factors such as rainfall, flooding, and temperature can have a significant effect on parasite dynamics in many species. Research has shown that the intensity of nematode infection in cattle and sheep can be predicted by seasonal variance in weather conditions (Altizer et al. 2006). However, current understanding of the extent of seasonal effects on elephant intestinal parasites is currently largely speculative, due to the lack of knowledge available on elephant parasite life cycles and effects of climatic factors on vital rates of free-living stages.

The elephants in this study were inhabitants of the Okavango Delta, a unique ecosystem in that, for most of the year, the area has exceptionally high water levels. The annual flood occurs when peak rains in the Angolan highlands arrive in the Delta from the Okavango River. The river enters Botswana at Mohembo, and it is between mid-March to mid-May when the peak flood occurs here. This flooding however, can take months to spread to other parts of the Delta, due to a minimal gradient and an extremely slow flow rate (McIntyre, 2007). On the NG26, the sample collection site, November to March is the rainy season, April to September is the flood season, and October constitutes the dry season.

In many areas of Africa, when rainfall and ground water levels are low, wildlife aggregate round water holes (Redfern et al. 2005), which can become hotspots for parasite transmission. However, as the Delta is relatively wet area all year round, with a mean annual rainfall of 6 $x10^9$ m^3 (Ramberg et al.2006) and 16 billion m^3 of water flowing into the Delta annually (Gieske 1996), forced aggregation around water holes rarely occurs and therefore extensive parasite hotspots are not predicted.

The distribution of critical resources such as food and water are often seasonal, and the diet of the elephant differs throughout the year. Grasses and herbs are generally preferred in rainy seasons whereas taller, wooded plants are more commonly consumed in dry seasons (Foley et al., 2001). As parasites tend to be most abundant at ground level (Apio et al., 2006), it could be hypothesised that the low level browsing habits of elephants in the rainy season may lead to a higher incidence of parasitism than in the dry season. However, it was found in a study in Namibia (Thurber et al., 2011) that strongyle infections in bull elephants were lower in years of higher rainfall. This was not predicted as elephants have more grass availabile in wetter environments, which should hypothetically increase parasite incidence. However, Thurber (2011) suggests that this increase in parasite burden in drier years could be due to increased nutritional stress, or due to larvae concentrating around the minimal food resources.

Seasonality can also influence the stress levels of the African elephant. Cortisol metabolite concentrations have been found to increase during dry seasons, a change at least partially induced by seasonal food limitation (Foley et al. 2001). A further study demonstrated that seasonality has an effect on glucocorticoid and progesterone levels in wild female elephants, although a study on bulls failed to find a relationship between season and androgen or glucocorticoid metabolite levels (Ganswindt et al. 2010). However, in an exceptionally long dry period, African elephants were found to have a 50% increase in androgen and glucocortoid levels (Ganswindt et al. 2010). Although the extent to which hormone fluctuation levels affect parasite burden in African elephants is widely unknown, it is likely that the two factors are linked.

A study in Queen Elizabeth National Park found that season had no effect on the prevalence or mean abundance of infectious ungulate nematode larvae on the pasture (Apio et al. 2006). Although unexpected, this was hypothesised to be due to the fact that, despite there being significant changes in precipitation between the seasons, there was no such change in relative

humidity, thereby maintaining a consistent microclimate throughout the year (Apio et al. 2006). With near permenant ground water in the Delta, it may be that similarly, a fairly consistent microclimate is maintained, and therefore seasonal difference in parasite burden may not be observed.

Many studies have reported nematode peaks in a range of host species during the wet season, including a study on sheep and goats in the Senegal (Vercruysse, 1983), goats in Zimbabwe (Pandey et al., 1994), chimps in Tanzania (Huffman et al, 1997), and donkeys in Ethiopia (Ayele et al. 2006). A study on small ruminants in Ethiopia found that fluke infections were significantly higher in the wet seasons than in the dry seasons (Sissay et al. 2007). This pattern was also found in cattle in Zambia (Phiri et al. 2005) and ruminants in Northern Nigeria (Schillhorn van Veen et al. 1980). Although previous research on a range of species, and in a range of areas often show a clear trend of nematode and fluke peaks during the rainy season, the unique ecosystem of the Delta makes it hard to hypothesise the effect of season on parasite prevalence in elephants and other Delta-dwelling species. It has also been found that, within the same host, different nematode species can have opposing peak abundance times. A study on small ruminants in The Gambia found that adult worms of *Trichostrongylus colubriformis* were recovered in high numbers in the mid-dry season, whereas *Haemonchus contortus* and *Strongyloides papillosus* were recovered in large numbers in the mid-wet season (Fritsch et al. 1993).

Observed relationships between season and parasite burden are likely to be strongly affected by lag times introduced by parasite maturation and longevity, as well as potential competition between parasites and fluctuation in immunity. As a result, seasonal peaks in parasite abundance might follow rather than coincide with optimum conditions for transmission.

1.5.5 Inter-annual variance

Variation in parasite burden may not only be seasonal, but may also differ from year to year. It has been found that the nematode *Trichostrongylus tenuis* causes very clear cyclical peaks in red grouse population abundance, which occur every 4-12 years, depending on their location (Altizer et al. 2006). These parasites are able to drive the red grouse population cycle as they reduce host breeding success, are distributed with a low degree of aggregation, and there are recruitment time delays into the adult population (Hudson et al. 1992). A wide scale study on the effect of multi-annual climate variation on this grouse population has shown that unexpected climate variance can cause a significant increase in nematode abundance and subsequently cause crashes in red grouse populations (Pascual and Bouma, 2009). However, evidence of such cyclic population crashes being caused by parasites is rare, and there is no current evidence that parasites have an effect on the population dynamics of elephants.

1.5.6 Individual genetics

The Major Histocompatibility Complex (MHC) is found in all mammals, and is crucial for protection against disease. High levels of genetic variance are observed at the MHC in vertebrates (Paterson et al. 1998). In Soay sheep it has been found that MHC allelic variation is significantly linked with immunity to intestinal nematodes (Paterson et al. 1998). A study on the MHC of elephants found moderate polymorphism and allelic diversity (Archie et al. 2010), suggesting that genome of individual elephants may play a significant role in regulating the parasite burden of elephants.

It is on the basis that individual genetics plays a large role in parasite susceptibility that the ‘immunocompetence handicap’ principle is founded. This asserts that females choose their mates based on costly male secondary sexual that signify parasite resistance in males (Folstad and Karter, 1992). A study on Asian elephants supports this theory: it was found that the tusk

length of male elephants had a significant negative correlation with intestinal parasite load (Watve and Sukumar, 1997). However, whether this correlation is found in the African elephant, and whether tusk length affects male sexual success and thus fitness, is currently unknown.

1.6 Conclusion and study aims

Although current knowledge on the internal parasitic fauna of the African elephant is limited, enough research has been conducted to conclude that elephants harbour a widespread and diverse range of species (Fowler and Mikota, 2006). This study seeks to investigate the factors potentially affecting parasite distribution in wild elephants in the Okavango Delta, including age, sex, group dynamic, group size, season and year. The access to captive elephants in the Okavango Delta was utilised to investigate the change in parasite levels in individuals over time, whilst collection of faecal samples from sympatric ungulate species allowed analyses to be carried out on whether elephants are sharing parasite species with other mammals (Chapter 3). Alongside these studies, an investigation into optimum methods for parasite ova storage was carried out (Chapter 4) in order to underpin future coprological studies of elephant parasites in remote areas.

CHAPTER 2: A COPROLOGICAL SURVEY OF PARASITES OF WILD AND CAPTIVE ELEPHANTS IN THE OKAVANGO DELTA

2.1 Introduction

The primary focus of this investigation was to determine the important factors that influence parasite infection in wild African elephants in the Okavango Delta. The effect of age, sex, group dynamic, group size, month, season and year on parasite infections were studied using data collected between 2008 and 2012. The analysis of parasites in faecal samples from a captive elephant herd was also carried out to investigate the variance in parasite infection over time within the same individual. With so little research having been carried out on the parasites of African elephants, especially on the factors that may be affecting burden and prevalence, directional research in this area is crucial.

2.2 Methods

2.2.1 Wild elephant faecal sample collection

Fresh faecal samples were collected from elephants on the NG26 concession in the Okavango Delta at a range of daylight hours (6am-7pm), between 12th November 2008 and April 11th 2012. Elephants were observed until they had defecated and had moved off to a safe distance. A sample was then taken, that was comprised of an inside and outside component of each dung bolus, to control for eggs having a heterogeneous distribution in the faeces. Only samples able to be collected within one hour of being dropped were taken. This was to control for rapid parasite egg hatching, bolus drying and consequential skew of results, as well as to avoid disturbance and dispersion by insects such as dung beetles. The dung beetle, *Onthophagus gazella,* can completely break down the structure of horse faeces in one night (English, 1979), and the dung beetle *Diastellopalpus quinquedens* is able to significantly

disperse infective L3 parasite stages from their dung patch (Gronvold et al. 1992). Parasite eggs could also be dispersed by passive transport, such as rainfall (Gronvold et al. 1992). The samples were placed in plastic bags and stored in a cooler box for transfer to the laboratory.

The following information was collected for each sample: the date of sample collection, and the age, sex, group size and group dynamic of the elephant collected from. Group dynamic data was categorised into two groups: Group One included all females and all males below the age of 15, while Group Two included all males above the age of 15. These two categories represent the differential group living dynamics of wild African elephants. Elephants were assigned an age based on a number of variables including, body size, tusk size and tusk, and general body condition. Many of the observed elephants could be matched to a previously compiled identification database by observing ear markings, tusks and tail hair.

For each faecal sample collected between 12th November 2008 and 20th January 2012, three grams were weighed out, stored in a 15ml storage pot and filled to the top with 10% formalin. These ('stored samples') were analysed anywhere between 1 and 15 months after collection. For the samples collected between 21st January and 11th April 2012 ('fresh samples'), three grams were also measured out, but were stored in a fridge and analysed in-situ within 24 hours of collection.

2.2.2 Captive elephant faecal sample collection

Abu Camp, where the research base was situated, is home to seven captive elephants, the Abu herd. The herd are kept in a Boma at night, but are allowed relatively free reign to wander in the bush during daylight hours. Although these elephants do have some degree of freedom, they are often taken to the same places to feed, drink, mud wallow and bathe. They are also ridden up to twice a day, with the same routes often being covered several times in a week.

This re-visitation of the same areas and the close contact that the Abu herd have in the Boma may increase the chance of parasite transmission and re-infection between the seven herd members. On the other hand, their partial segregation from wild elephants may reduce the probability of cross host transmission.

Faecal samples were collected from all seven herd members throughout the field study period (21st January to 11th April, 2012) at a range of daylight hours. All of these faecal samples were collected within one hour of being dropped, and were analysed within 24 hours of being collected. The change in nematode egg density (EPG) over the study period was monitored, as was the prevalence of coccidial oocysts and fluke eggs.

2.2.3 Detection of parasite ova: flotation test

Coccidial oocysts were identified by a modified McMaster method (MAFF 1986) using salt-sugar flotation solution. This method was used to detect the prevalence of coccidial oocysts in fresh and stored samples, as well as to detect the density of nematode eggs in fresh samples only (Table 2.1). 45ml of water were added to each three gram sample, mixed thoroughly and then sieved. Two centrifuge tubes were filled with an aliquot of the sieved solution, and placed the centrifuge for two minutes at 1500rpm (400g). The supernatant was then emptied and salt-sugar flotation solution (specific gravity of 1.28) was added to the remaining solid. The tubes were then inverted several times and a pipette was used to extract some of the mixed solution and place it in the chambers of a Fecpak slide (Fecpak Inc., New Zealand). This slide was used in preference to the standard McMaster slide because of the increased sensitivity, with one egg counted equating to 30 eggs per gram, compared to the McMaster count where one egg equates to 50 eggs per gram (Good et al. 2004). The slides were left for two minutes to allow the eggs time to float to the surface before being examined under 10x objective (100 x total magnifications) under a microscope. The prevalence of coccidial

oocysts was recorded and the number of nematode ova in each chamber was counted. The nematode eggs density in eggs per gram of faeces (EPG) was then calculated.

2.2.4 Detection of parasite ova: sedimentation test

As fluke eggs are too dense to float in salt-sugar solution, and it was found that the majority of nematode eggs did not float in salt-sugar solution after storage in formalin (Chapter 4), a sedimentation method (Happich and Boray, 1969) (MAFF, 1986) was used to assess both the fluke prevalence in fresh and stored samples, and to assess the nematode egg prevalence in stored samples. After the above flotation test had been carried out, the remaining suspension in the beaker (after some solution had been removed for centrifugation) was topped up with water to 200ml, mixed and poured into an inverse conical beaker. The beaker was left for three minutes to give the fluke eggs time to sink. The pipette was then used to obtain suspension from the very bottom of the jug the pipette contents were placed in the lid of a petridish and a drop of methylene blue was added. A graduated petridish was then placed bottom-down on top of the lid to create an even layer of sediment, and the whole examined under 4x zoom (40x total magnification) on a dissecting microscope. The prevalence of fluke and nematode eggs in this petridish was recorded.

Table 2.1: The methods used to analyse different parasite taxa in fresh and formalin stored elephant faecal samples

	Fresh samples	**Stored samples**
Flotation method	Coccidial oocysts Nematode ova	Coccidial oocysts
Sedimentation method	Fluke ova	Fluke ova Nematode ova

2.2.5 Egg morphology

If parasites were detected in high numbers in freshly analysed samples, then three grams from the original elephant dung samples were stored in formalin. These were transported to Bristol University where nematode ova were photographed using a digital microscope. The lengths and widths of detected fluke and nematode ova were measured. The width was taken as the widest part between the walls in the centre of the egg. The length was taken as a straight line from the top middle to the bottom middle of the egg. This was used for morphological comparison with eggs from faeces collected from sympatric grazer species (Chapter 4).

2.3 Statistical methods for wild elephant samples

2.3.1 Fresh sample analysis

Sixty-one fresh elephant faecal samples were analysed. The effects of age, sex, group dynamic, group size, month, and season on nematode egg density was investigated by linear multiple regression analysis in SPSS (v16), carried out on Log_{10} transformed nematode egg density.

The effects of age, sex, group dynamic, group size, month and season on coccidial oocyst and fluke egg prevalence was discerned using binary logistic regression analysis, again in SPSS (v16).

2.3.2 Stored sample analysis

A total of 397 stored elephant faecal samples were analysed: 336 of these were analysed after being stored in formalin, but the prevalence results for the above 61 fresh samples were also included in the analysis in order to obtain a larger dataset. To avoid confusion, this 'stored and fresh' sample combination will henceforth be referred to as 'stored samples'. The nematode egg data from 197 samples collected between 2008 and 2010 were not included in

the nematode egg prevalence analysis to avoid inconsistencies, as a different methodology was used to detect nematode eggs in these samples.

The effects of age, sex, group dynamic, group size, month, season, year and storage time on the prevalence of nematode eggs, fluke eggs and coccidial oocysts was investigated using binary logistic regression analysis carried out in SPSS (v16).

2.4 Statistical methods for Abu herd samples

A total of 79 samples were collected from the seven members of the captive Abu herd between mid-January and mid-April 2012. Whether there was a significant change in nematode egg density or a change in coccidial oocyst prevalence over the collection period was assessed using a two-tailed Pearson's correlation in SPSS (v16). The results from the individual elephant named Warona were excluded from the analysis as she was not found to have any parasites at any time in the study.

2.5 Results

All fresh wild elephant samples and all Abu herd samples were collected between 21st January and 11th April, 2012. All stored samples were collected between 12th November 2008 and 11th April 2012.

2.5.1 The distribution of fresh wild elephant faecal samples

Sample sizes for sex (Figure 2.1), age (Figure 2.2) and group size (Figure 2.4) had a fairly even distribution, but the following factors had visibly more skewed distributions: Group dynamics (Figure 2.3), month (Figure 2.5) and season (Figure 2.6).

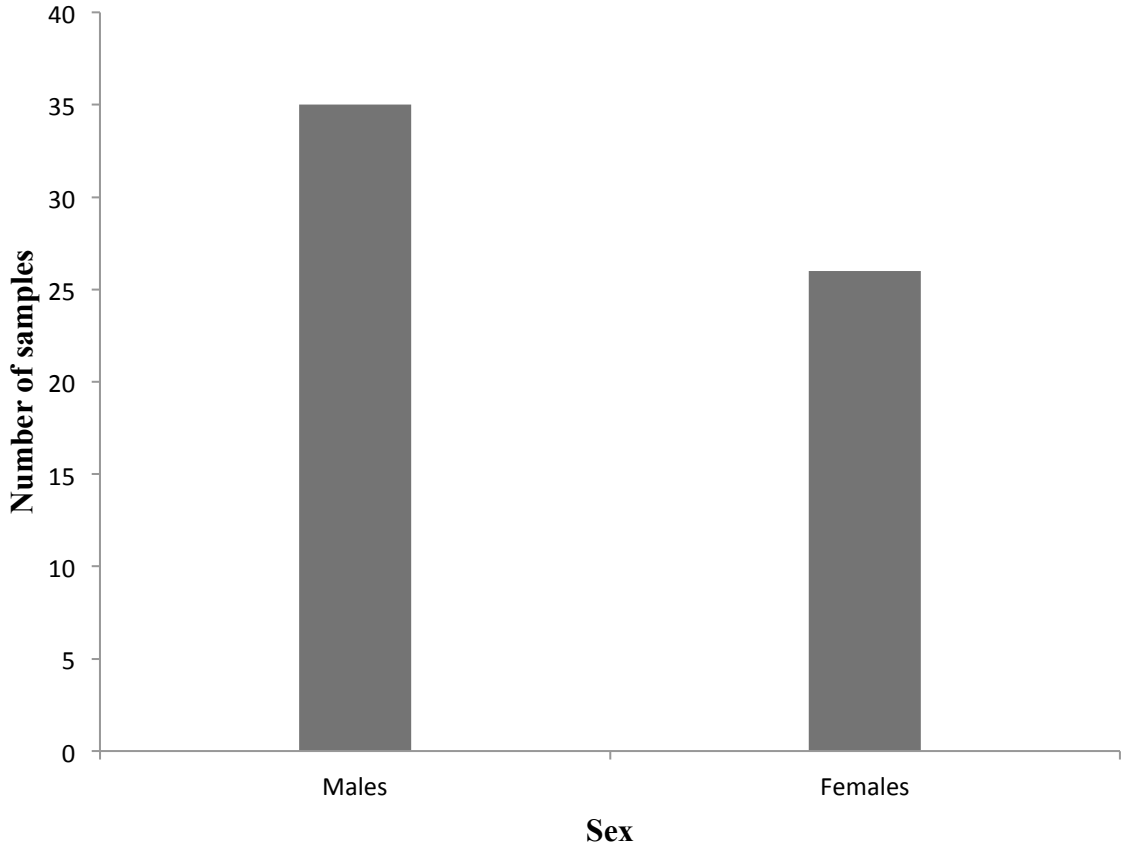


Figure 2.1: The distribution of fresh faecal samples from male and female wild elephants

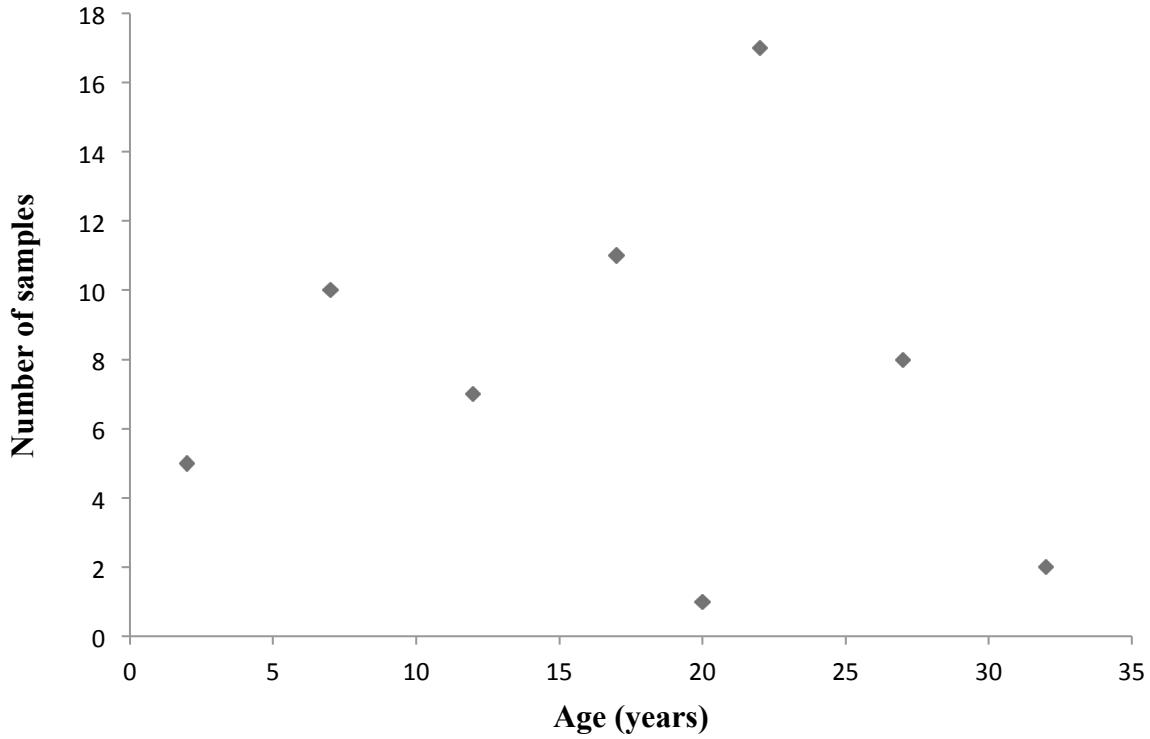


Figure 2.2: The distribution of fresh faecal samples from different ages of wild elephants

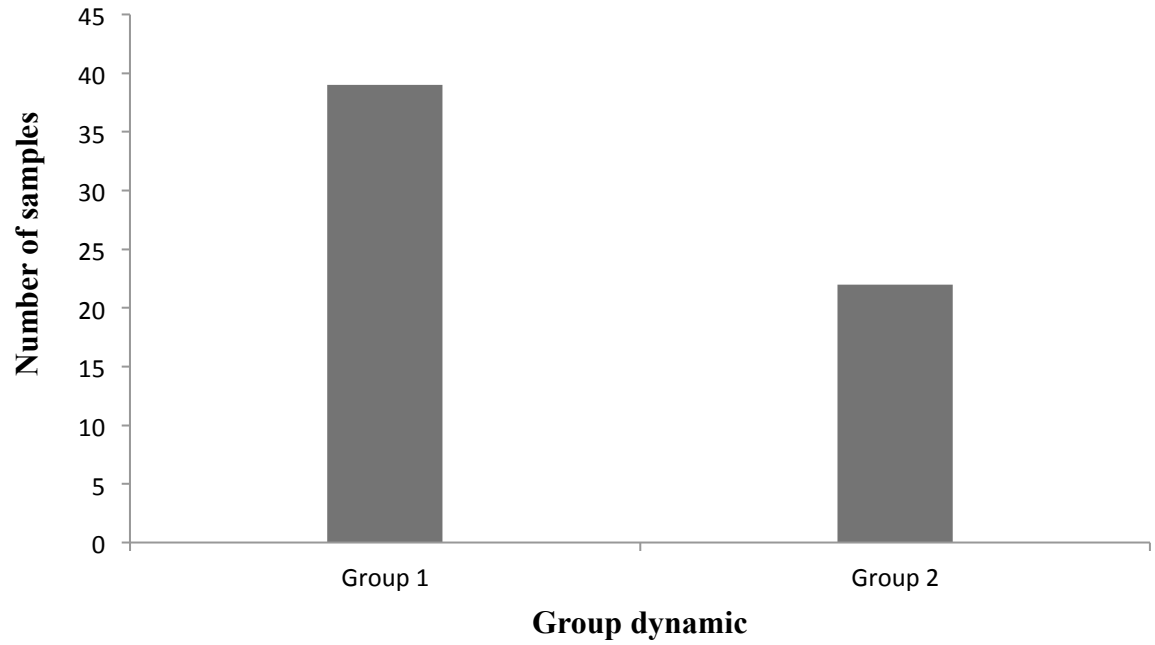


Figure 2.3: The distribution of fresh faecal samples collected from Group 1 and Group 2 wild elephants. Group 1 included all female elephants and / or male elephants under the age of 15. Group 2 included all male elephants over the age of 15.

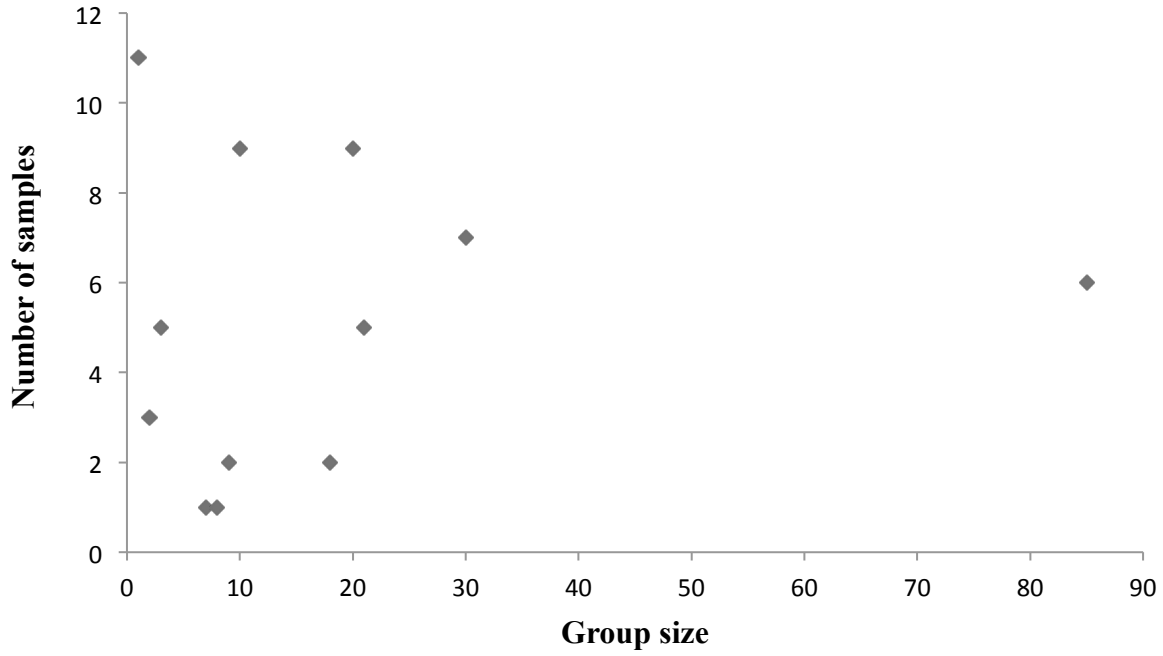


Figure 2.4: The distribution of fresh faecal samples collected from different group sizes of wild elephant.

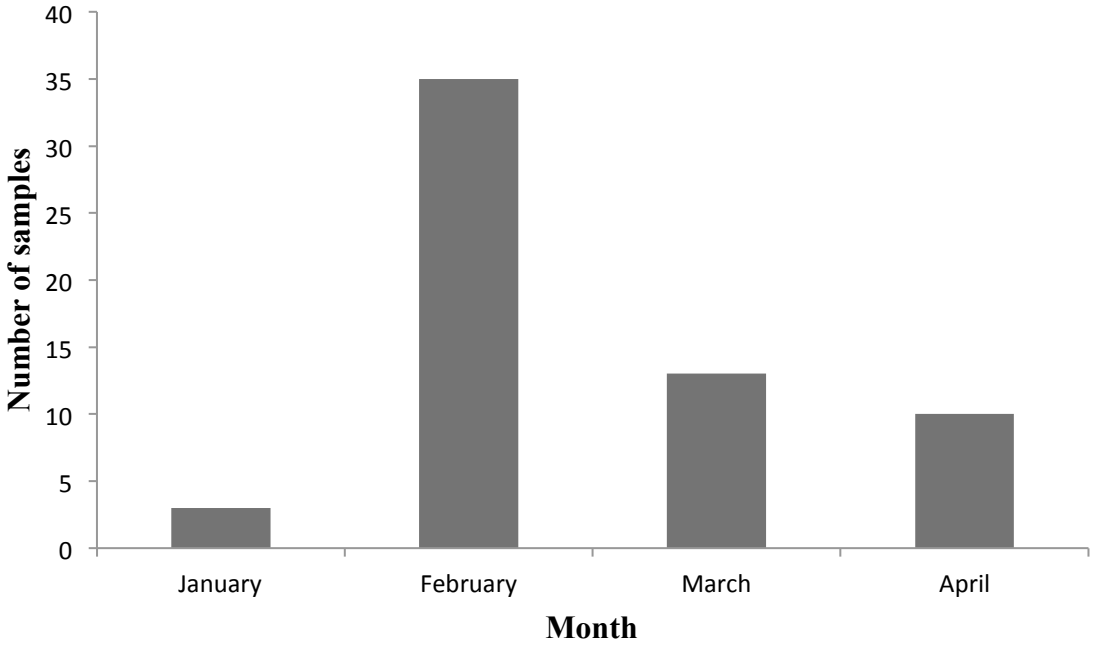


Figure 2.5: The distribution of fresh wild elephant faecal samples collected between January and April, 2012.

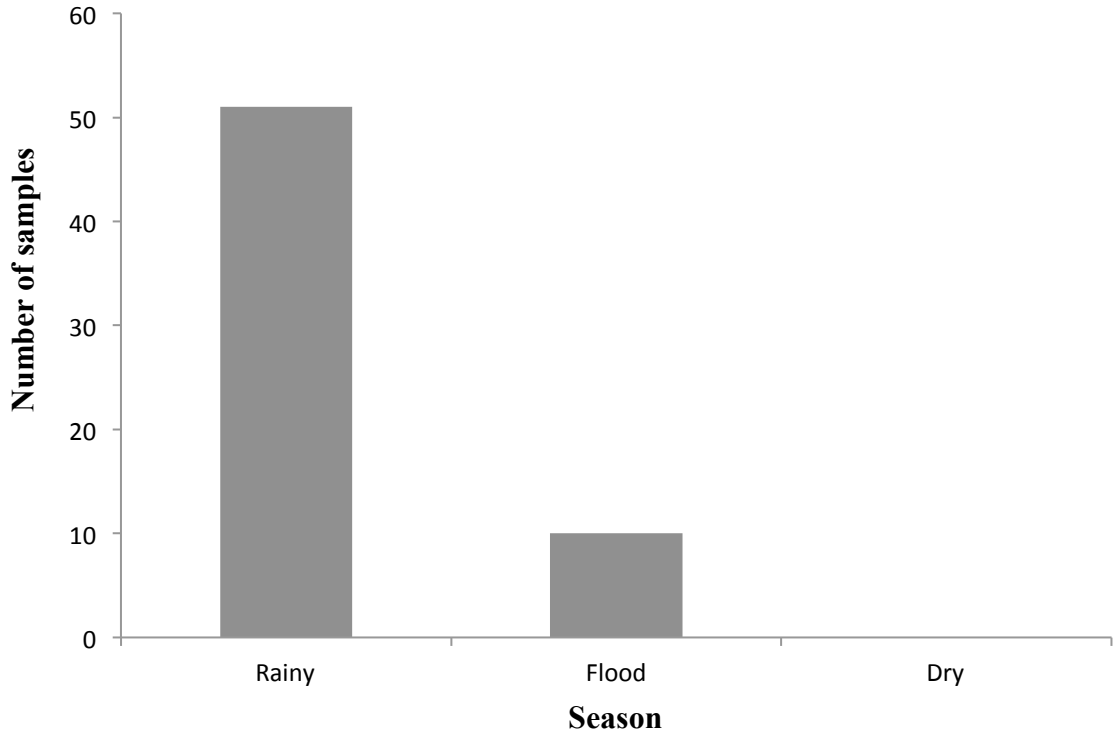


Figure 2.6: The distribution of fresh wild elephant faecal samples collected in the rainy, dry and flood seasons.

2.5.2 The distribution of stored wild elephant faecal samples

Sample sizes for age (Figure 2.8) and month (Figure 2.11) appeared to have a fairly even distribution, but sex (Figure 2.7), group dynamic (Figure 2.9), group size (Figure 2.10), season (Figure 2.12), year (Figure 2.13) and storage time (Figure 2.14) had a more skewed sample size distribution.

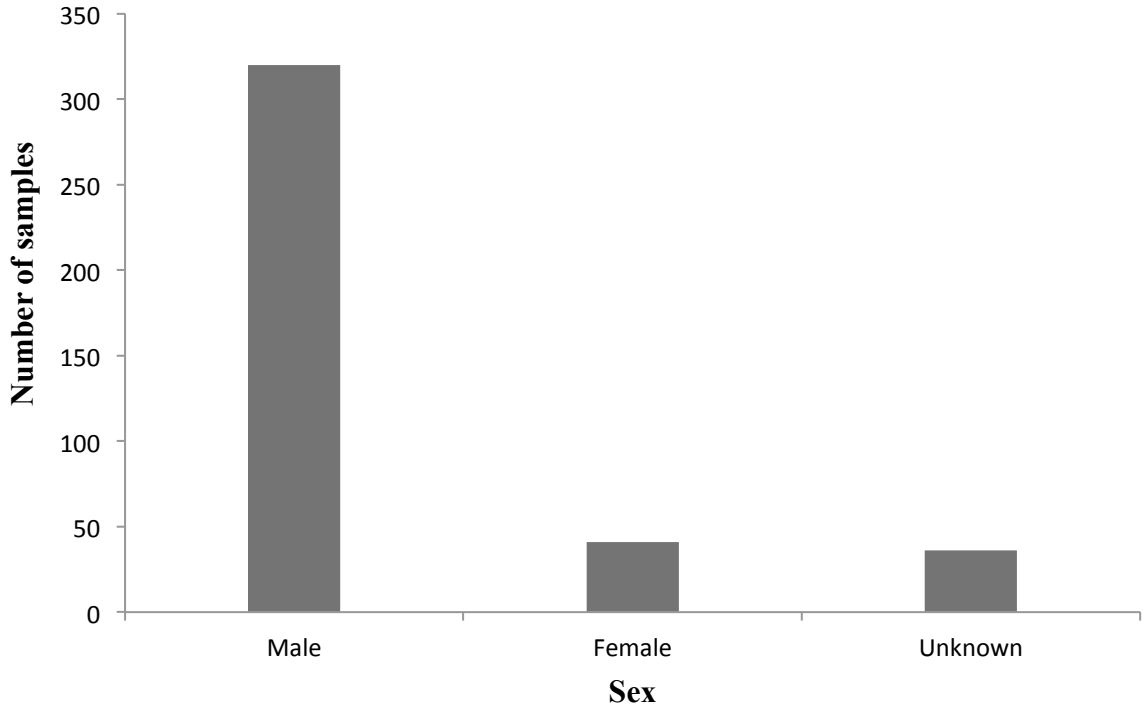


Figure 2.7: The distribution of stored faecal samples collected from male and female wild elephants as well as elephants of an unknown sex.

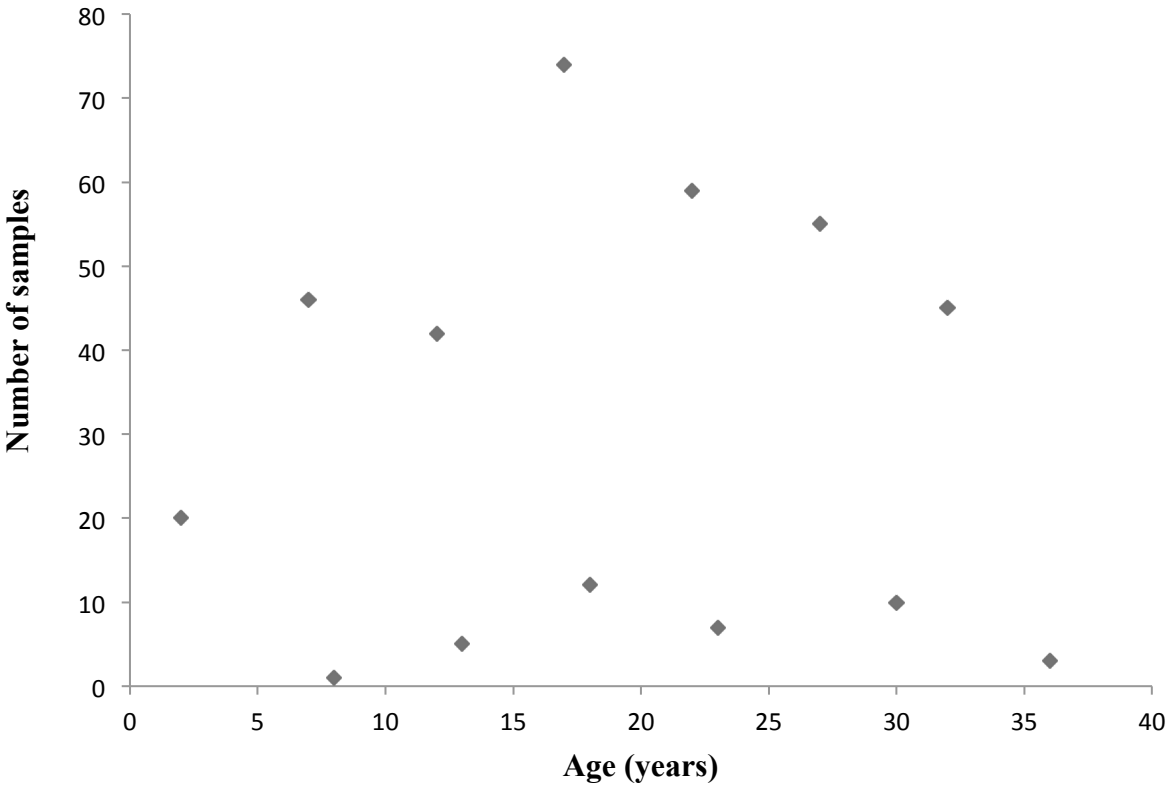


Figure 2.8: The distribution of stored faecal samples collected from different ages of wild elephant.

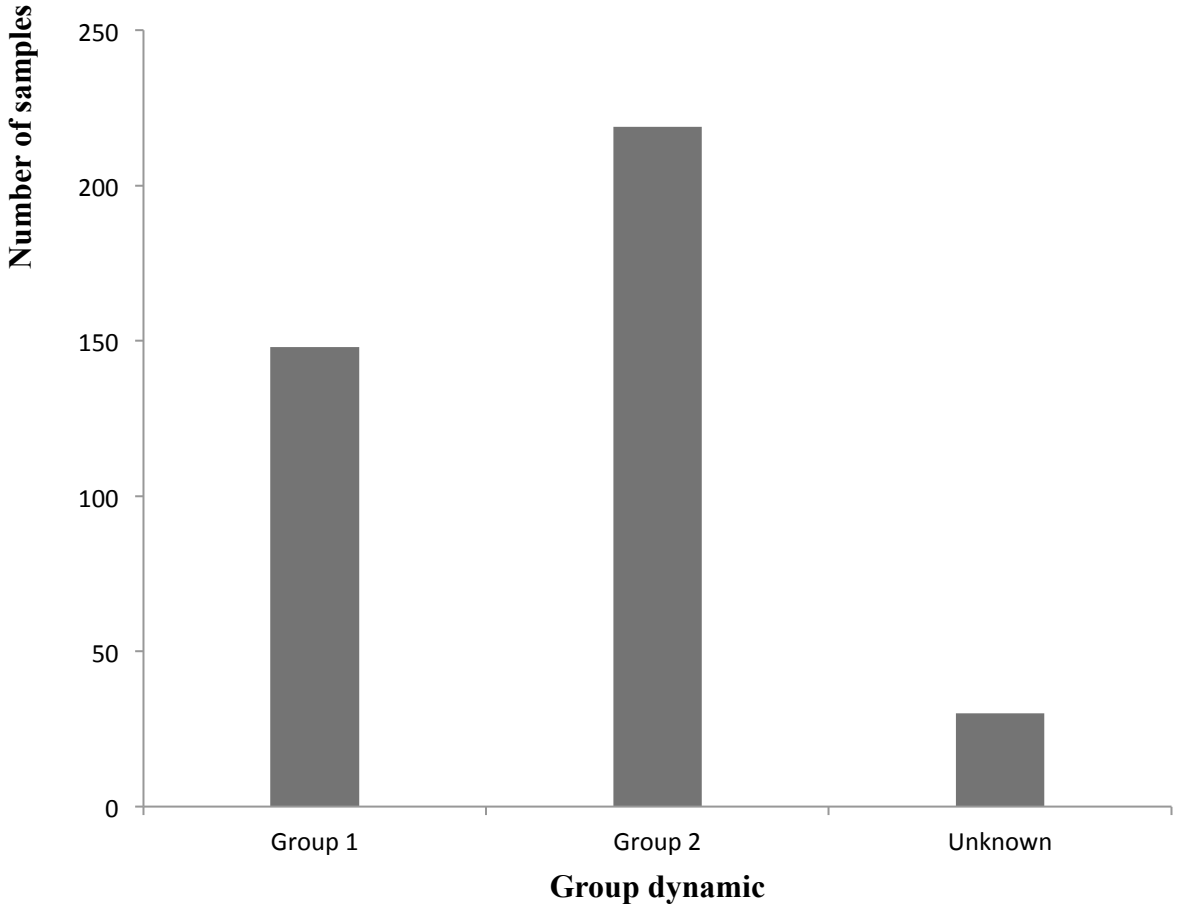


Figure 2.9: The distribution of stored faecal samples collected from Group 1 and Group 2 wild elephants, as well as elephants not assigned a group. Group 1 included all female elephants and / or male elephants under the age of 15. Group 2 included all male elephants over the age of 15.

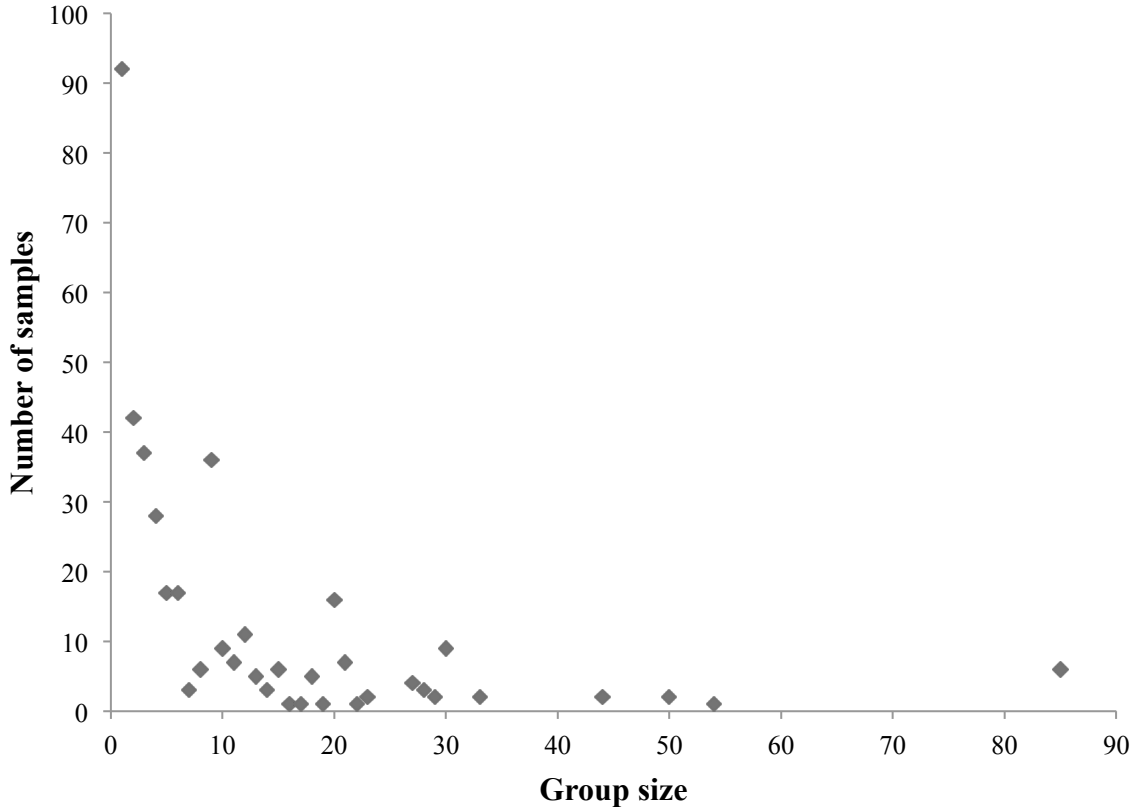


Figure 2.10: The distribution of stored faecal samples collected from different group sizes of wild elephant.

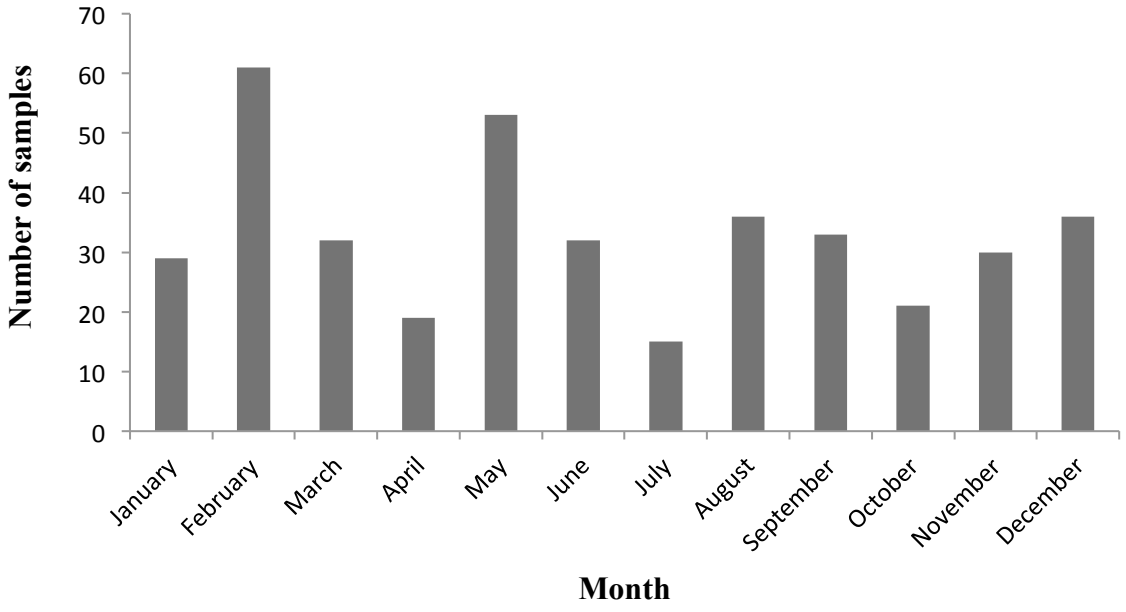


Figure 2.11: The distribution of stored wild elephant faecal samples collected in each month of the year, between 2008 and 2012.

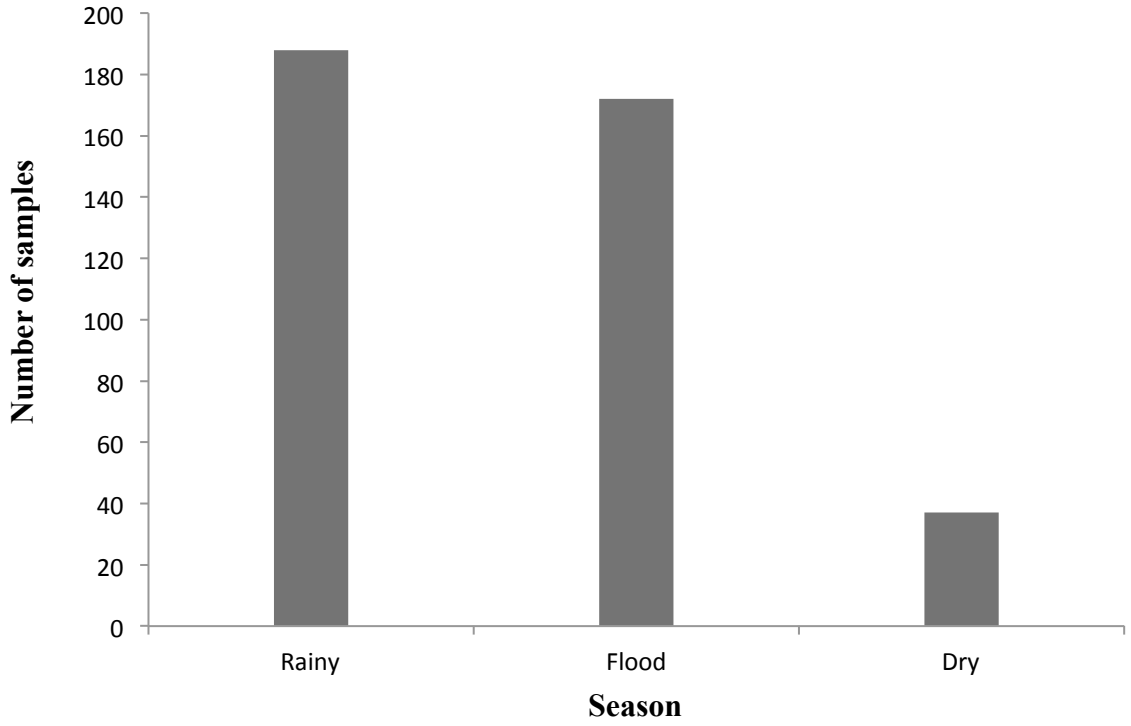


Figure 2.12: The distribution of stored wild elephant faecal samples collected in the rainy, flood and dry seasons of 2008-2012.

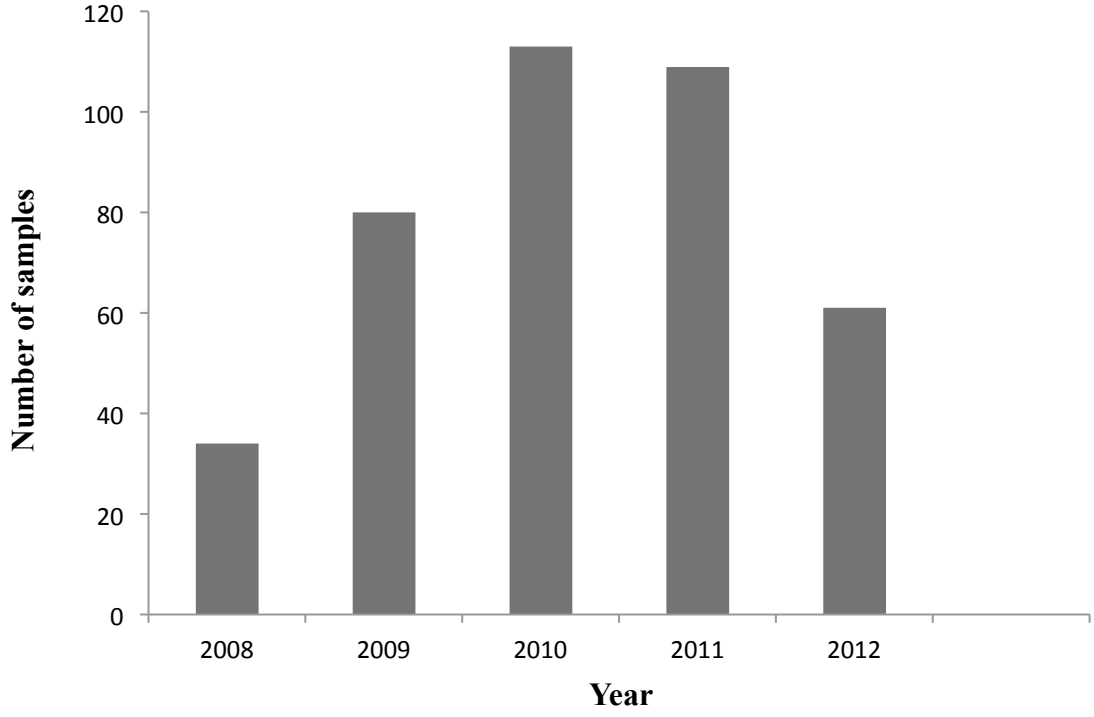


Figure 2.13: The distribution of stored wild elephant faecal samples collected between 2008 and 2012.

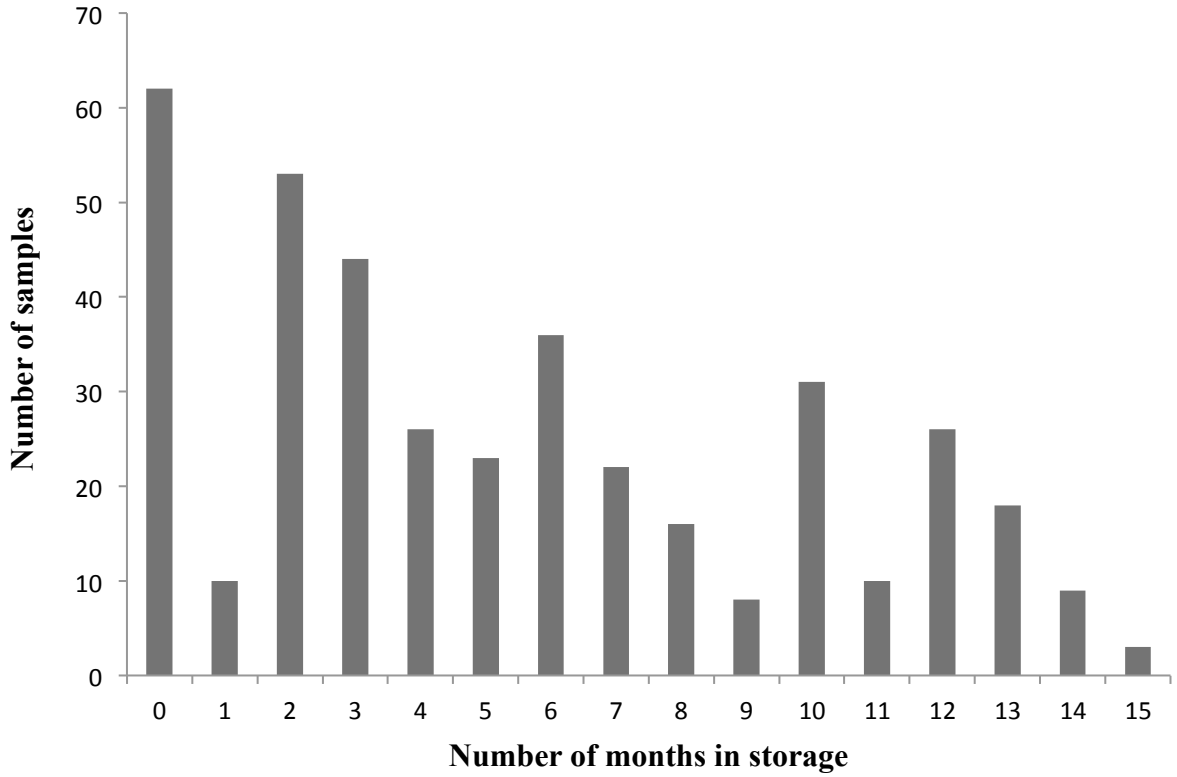


Figure 2.14: The distribution of wild elephant faecal samples stored in formalin for differing periods of time.

2.5.3 Analysis of fresh samples from wild elephants

2.5.3.1 Coccidial oocyst prevalence

In fresh wild elephant faeces, collected between mid-January and mid-April, 2012, coccidial oocysts were present in 68.9% of samples. Samples collected in the month of February were 152.301 times as likely as those collected in March to be infected with coccidial oocysts (logistic regression: $p = 0.004$) (Table 2.2, Figure 2.15).

Table 2.2: The significant factors from a binary logistic regression on the prevalence of coccidial oocyst in fresh wild elephant samples. Group size, the least significant factor, was removed from the analysis. The indicator month was March. The non significant factors in the analysis were age, sex, group size, group dynamic, season and year. B represents the value for predicting the dependent variable from the independent variable in the logistic regression equation. S.E. represents the standard errors that are associated with coefficients. Wald represents the Wald chi-square value. The df value lists the degrees of freedom for each of the tests of the coefficient. Sig. shows the obtained P value, and OR shows the odds ratios for the predictors.

	B	S.E.	Wald	df	Sig.	OR	95% Confidence Intervals for OR	
							Lower	Upper
Month			8.303	3	0.040			
February	5.026	1.744	8.303	1	0.004	152.301	4.989	4649.237

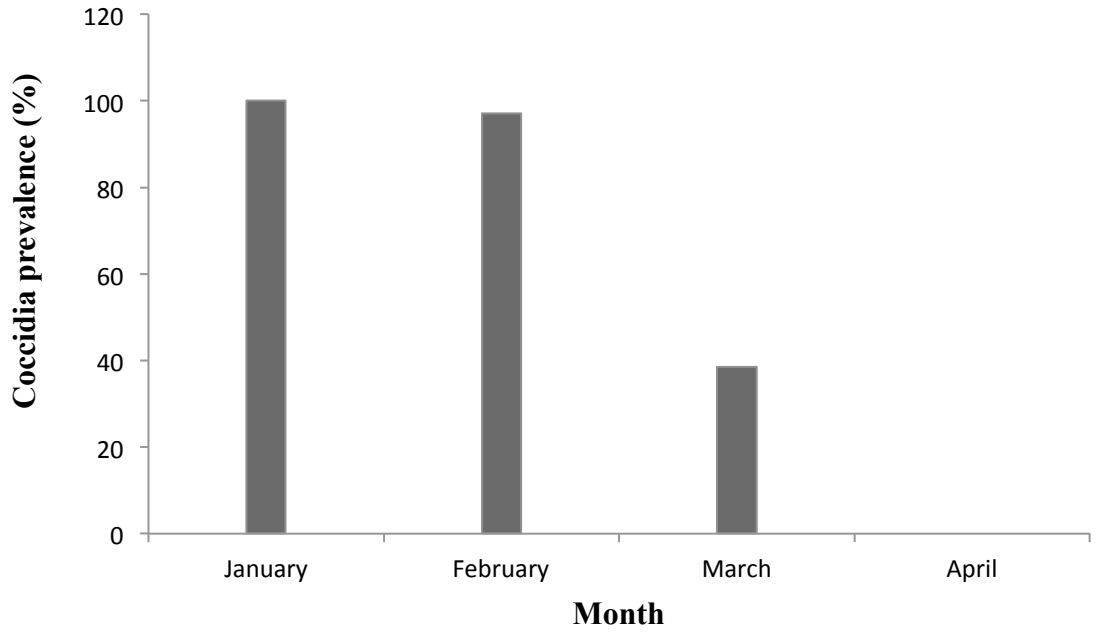


Figure 2.15: The prevalence of coccidial oocysts in male and female fresh wild elephant faecal samples.

2.5.3.2 Nematode egg density

Nematode eggs were present in 100% of fresh samples analysed, but in varying densities. It was found that samples from Group 1 (all females, and males that are below 15 years of age) had significantly higher nematode egg densities than those from Group 2 (males that are over the age of 15) (Multiple regression: t = -2.894, p = 0.005) (Table 2.3, Figure 2.16).

Table 2.3: The significant variables from a linear multiple regression on nematode egg density in fresh wild elephant faecal samples. The non-significant factors in the analysis were age, sex, group size, month, season and year. Unstandardized coefficients B shows the value of the numbers in the linear regression equation. The unstandardized coefficients Std. Error is the standard error for the coefficient. The Standardized Coefficients Beta measures the change in criterion. The t statistic is the coefficient divided by its standard error and the Sig. represents the P value.

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Intervals for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Group dynamic	-0.516	0.178	-0.595	-2.894	0.005	-0.874	-0.159

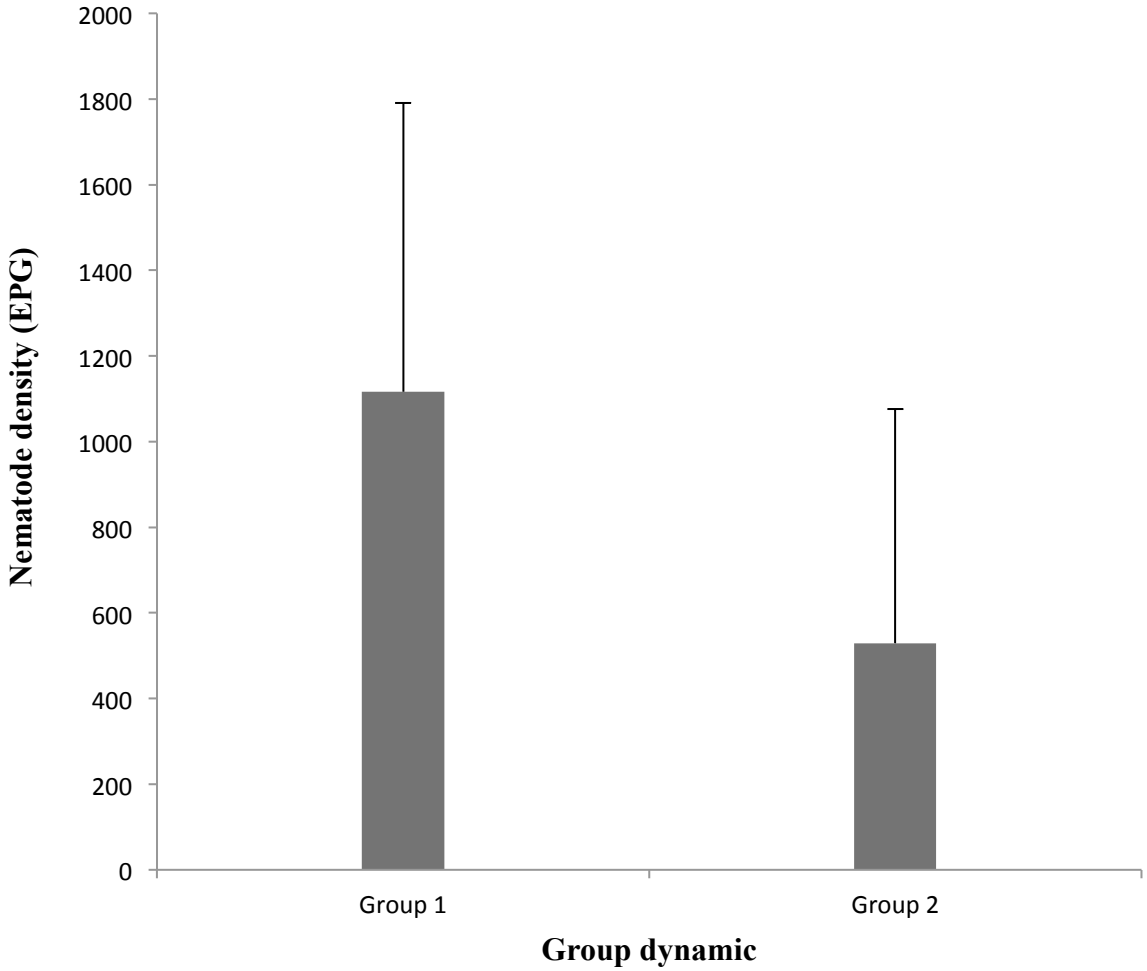


Figure 2.16: Nematode egg densities found in faecal samples collected from Group 1 and Group 2 wild elephants. Group 1 includes all female and elephants and / or male elephants under the age of 15, and Group 2 consists of male elephants over the age of 15. Error bars show the standard deviation. EPG = eggs per gram of faeces.

2.5.3.3 Fluke egg prevalence

Fluke eggs were present in 26.2% of fresh elephant faecal samples analysed. Samples from males were 0.057 times as likely as females to have fluke infections (binary logistic regression; $p = 0.046$) (Table 2.4, Figure 2.17).

Table 2.4: The significant variable from a binary logistic regression on the prevalence of fluke eggs in fresh wild elephant faecal samples. The least significant factor, season, was removed from the analysis. The other non-significant factors were age, group size, group dynamic, month, season and year. B represents the value for predicting the dependent variable from the independent variable in the logistic regression equation. S.E. represents the standard errors that are associated with coefficients. Wald represents the Wald chi-square value. The df value lists the degrees of freedom for each of the tests of the coefficient. Sig. shows the obtained P value, and OR shows the odds ratios for the predictors.

							95% Confidence Intervals for OR	
	B	**S.E.**	**Wald**	**df**	**Sig.**	**OR**	**Lower**	**Upper**
Male	-2.859	1.433	3.982	1	0.046	0.057	0.003	0.950

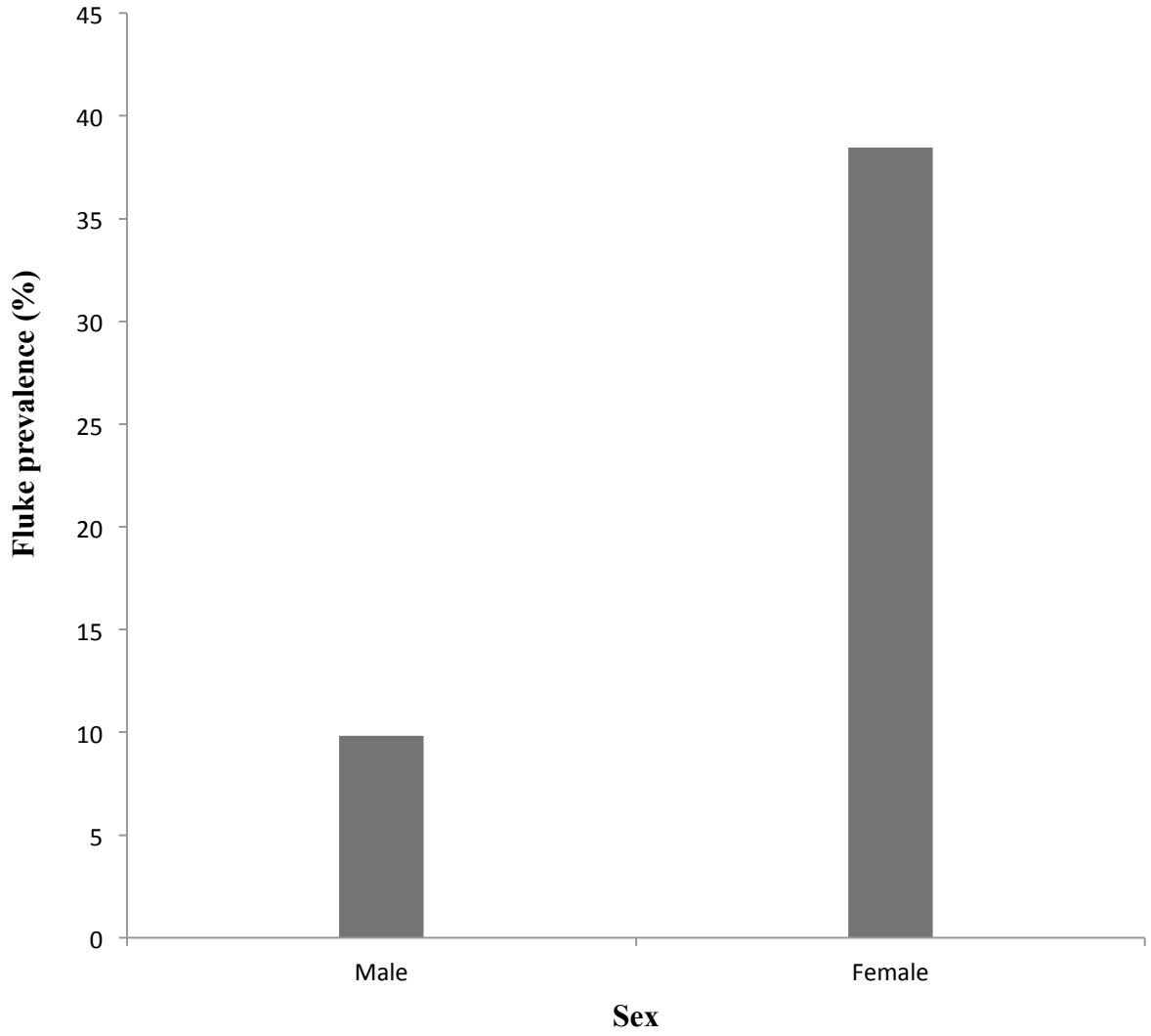


Figure 2.17: The average prevalence of fluke eggs in male and female fresh wild elephant faecal samples.

2.5.4 Analysis of stored samples from wild elephants

2.5.4.1 Coccidial oocyst prevalence

Coccidial oocysts were present in 47.6% of stored elephant faecal samples. Coccidial oocyst detection was 3.749 times as likely in males than in females (binary logistic regression: $p = 0.029$) (Table 2.5, Figure 2.18).

Coccidial oocyst detection was 20.202 times as likely in samples collected in January, than in samples collected in October (binary logistic regression: $p = 0.001$). Oocysts were also

76.902 times as likely to be found in samples collected in February than in samples collected in October (binary logistic regression: $p < 0.001$) (Table 2.5, Figure 2.19).

The average monthly coccidial oocyst prevalence between 2008 and 2012 was plotted alongside average daily maximum temperature, average rainfall and relative humidity in the Delta (Figure 2.20). Environmental data were collected by Okavango River Safaris, and averaged over 14 years (Conradie 2008). Flood and rainfall data collected by the Water Affairs Department of Botswana are also shown (Figure 2.21)

Compared to 2009 (the reference year), samples collected in 2010 were 4.082 times as likely to contain coccidial oocysts (logistic regression: $p < 0.001$), whereas samples collected in 2011 and 2012 were 0.186 and 0.080 times as likely to contain oocysts (logistic regression 2011: $p < 0.001$, 2012$p = 0.006$) (Table 2.5, Figure 2.22).

For every additional month of storage time, coccidial oocysts were 0.874 times as likely to be detected in faecal samples (binary logistic regression: $p = 0.011$) (Table 2.5) (Chapter 4).

Table 2.5: The significant variables from a binary logistic regression on the prevalence of coccidial oocysts in formalin-stored wild elephant faecal samples collected between 2008 and 2012 (397 samples). The least significant variable, group dynamic, was removed from the analysis. The other non-significant factors in the analysis were age and group size. B represents the value for predicting the dependent variable from the independent variable in the logistic regression equation. S.E. represents the standard errors that are associated with coefficients. Wald represents the Wald chi-square value. The df value lists the degrees of freedom for each of the tests of the coefficient. Sig. shows the obtained P value, and OR shows the odds ratios for the predictors.

							95% Confidence Intervals for OR	
	B	**S.E.**	**Wald**	**Df**	**Sig.**	**OR**	**Lower**	**Upper**
Sex			6.957	2	0.031			
Male	1.322	0.607	4.741	1	0.029	3.749	1.141	12.319
Month			35.946	11	0.000			
January	3.006	0.871	11.914	1	0.001	20.202	3.666	111.341
February	4.343	0.934	21.621	1	0.000	76.902	12.331	479.529
Storage time	-0.135	0.053	6.405	1	0.011	0.874	0.787	0.970
Year			43.882	4	0.000			
2010	1.407	0.399	12.458	1	0.000	4.082	1.869	8.916
2011	-1.786	0.506	12.444	1	0.000	0.168	0.62	0.452
2012	-2.525	0.910	7.702	1	0.006	0.080	0.013	0.476

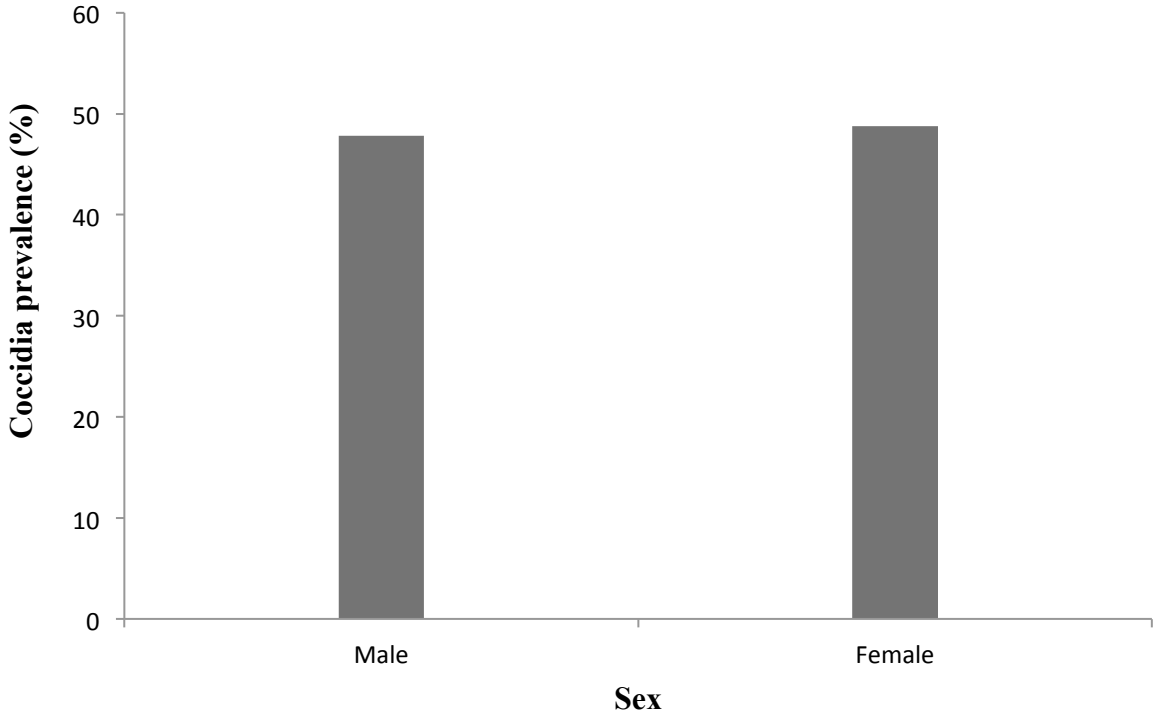


Figure 2.18: The prevalence of coccidial oocysts in male and female stored wild elephant faecal samples.

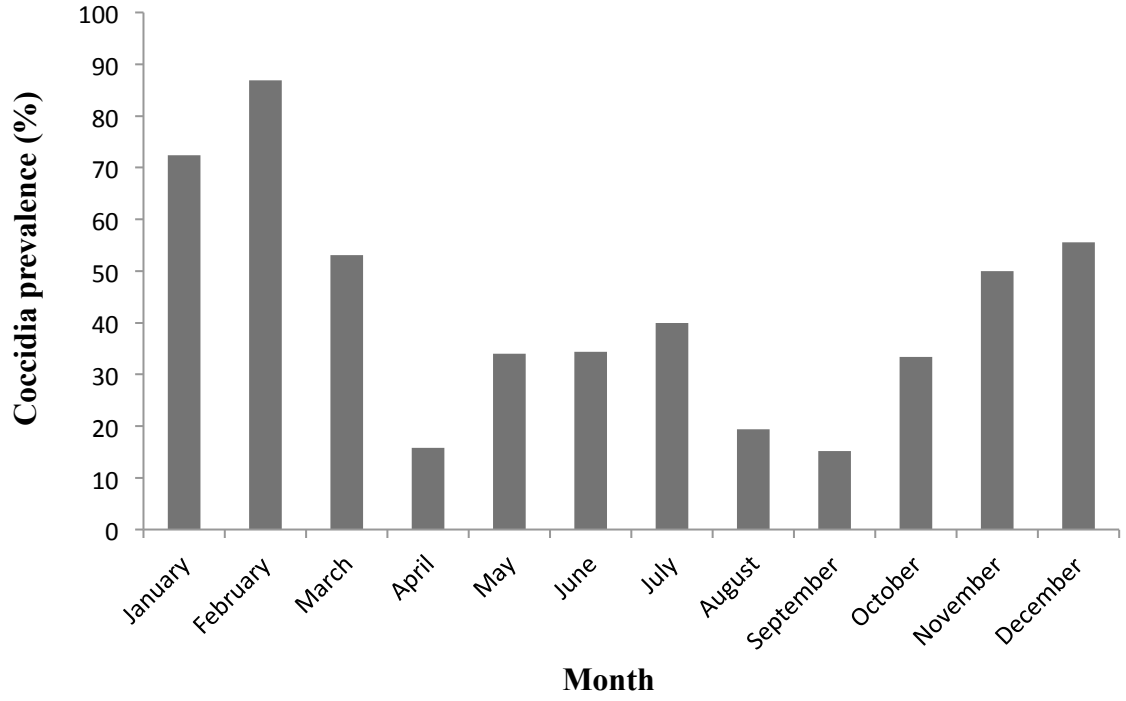


Figure 2.19: The prevalence of coccidial oocysts in stored wild elephant faecal samples detected in each month (2008 to 2012 combined).

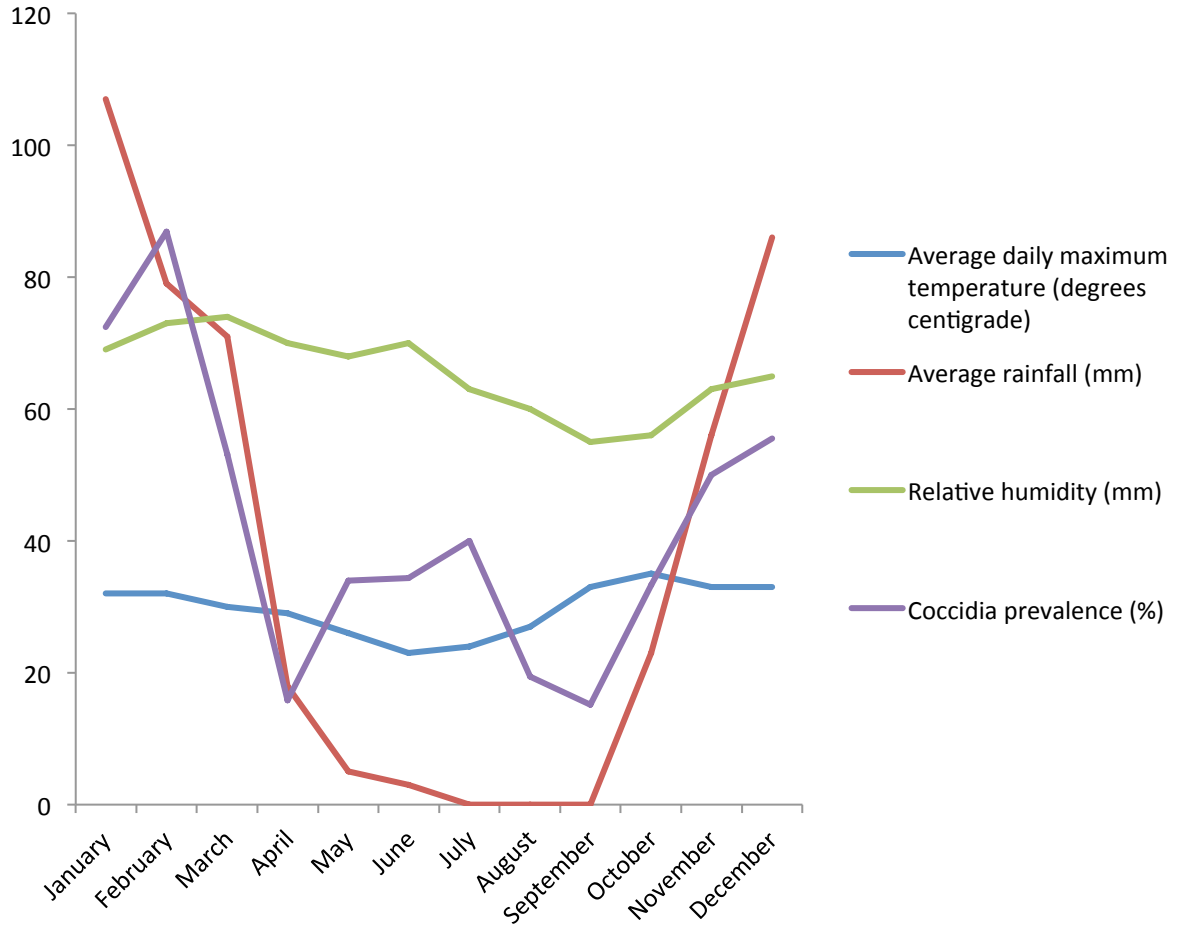


Figure 2.20: Average daily maximum temperature (^{o}C), average rainfall (mm) and relative humidity (%) each month, averaged from 14 years of data collected in Maun by Okavango River Safaris (Conradie 2008). The average coccidial oocyst prevalence (%) for each month, found in this study in samples in the Okavango Delta (collected between 2008 and 2012) has been added to this environmental data.

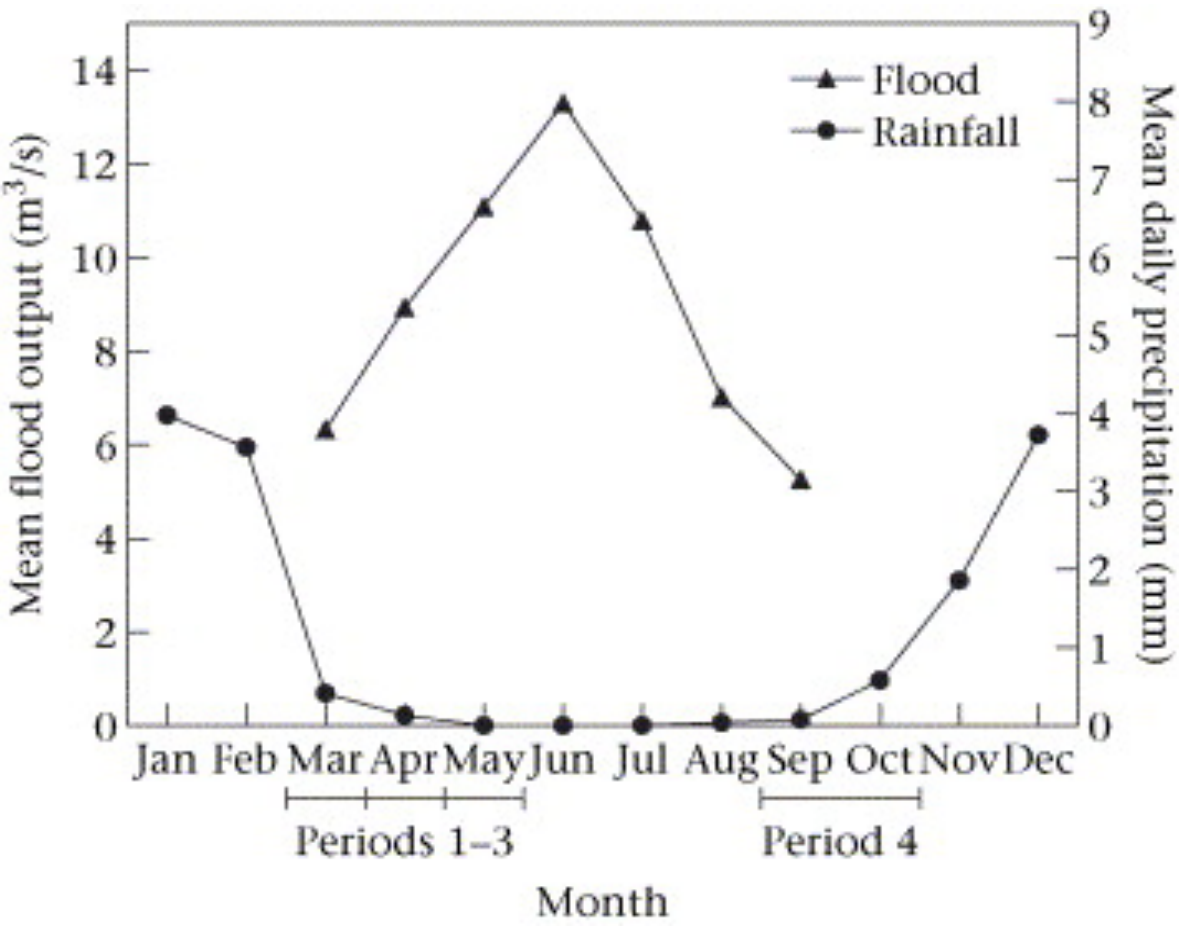


Figure 2.21: The mean flood output (m^3/s) and average daily precipitation (mm) in the Okavango Delta. The flood data was averaged from 13 years of data collected by the Water Affairs Department of Botswana. Rainfall data was collected by Beehner et al. between 1999 and 2003 (image taken from Beehner et al. 2005).

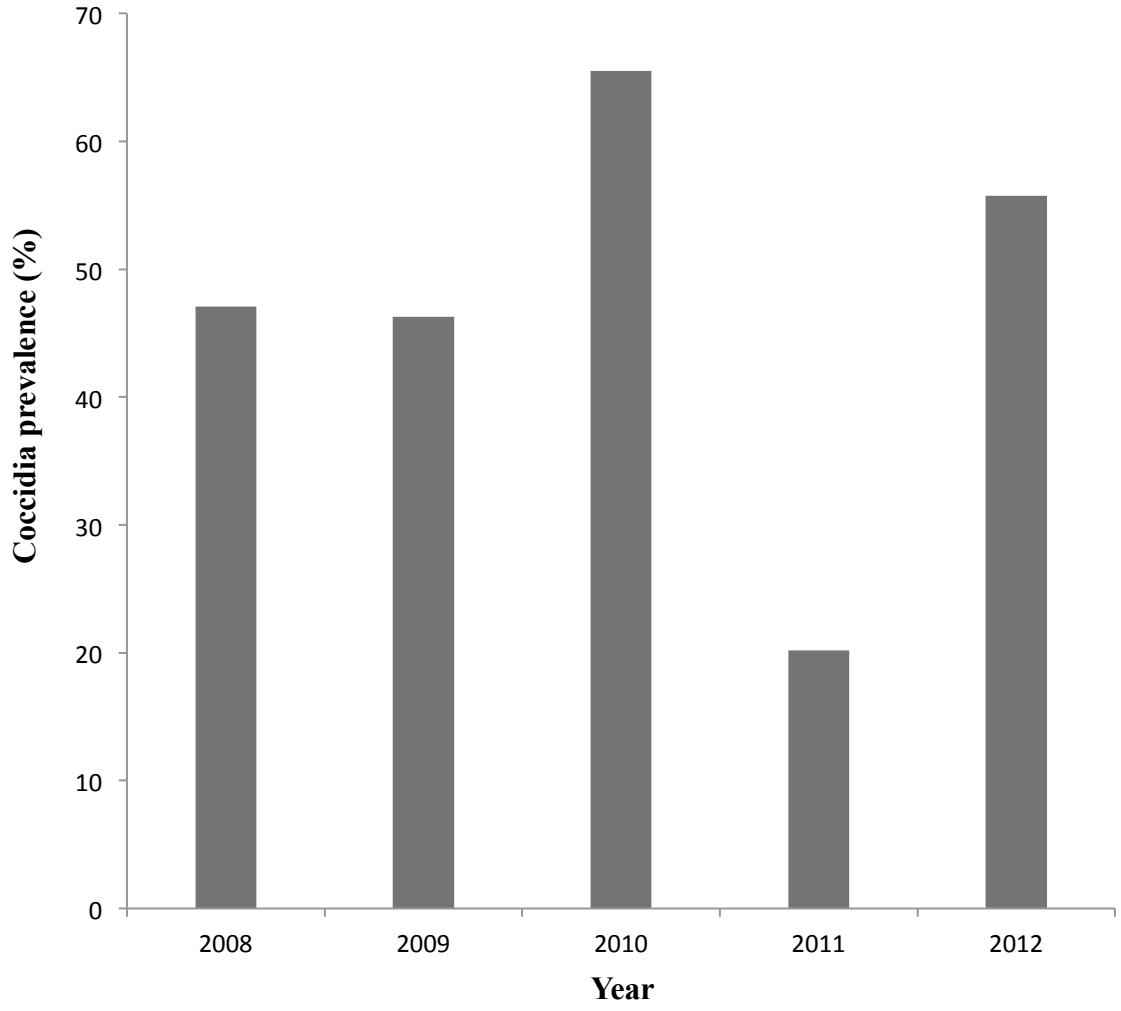


Figure 2.22: The prevalence of coccidial oocysts for the years 2008-2012 in stored wild elephant faecal samples.

2.5.4.2 Nematode egg prevalence

Nematode eggs were present in 73.3% of stored wild elephant faecal samples. It was found that for every increase in group size by one elephant, nematode eggs were 1.067 times as likely to be detected (binary logistic regression: $p = 0.035$) (Table 2.6, Figure 2.23). For every additional month of storage time, nematode eggs were 0.831 times as likely to be detected in samples (binary logistic regression: $p = 0.004$) (Table 2.6) (Chapter 4).

Table 2.6: The significant variables from a binary logistic regression on the prevalence of nematode eggs in formalin-stored wild elephant samples collected between 2008 and 2012 (200 samples). The least significant variables, month and year, were removed from the analysis. The remaining non-significant factors were age, sex and group dynamic. B represents the value for predicting the dependent variable from the independent variable in the logistic regression equation. S.E. represents the standard errors that are associated with coefficients. Wald represents the Wald chi-square value. The df value lists the degrees of freedom for each of the tests of the coefficient. Sig. shows the obtained P value, and OR shows the odds ratios for the predictors.

							95% Confidence Intervals for OR	
	B	**S.E.**	**Wald**	**df**	**Sig.**	**OR**	**Lower**	**Upper**
Group Size	0.065	0.031	4.438	1	.035	1.067	1.005	1.133
Storage time	-0.186	0.065	8.261	1	.004	0.831	0.732	0.943

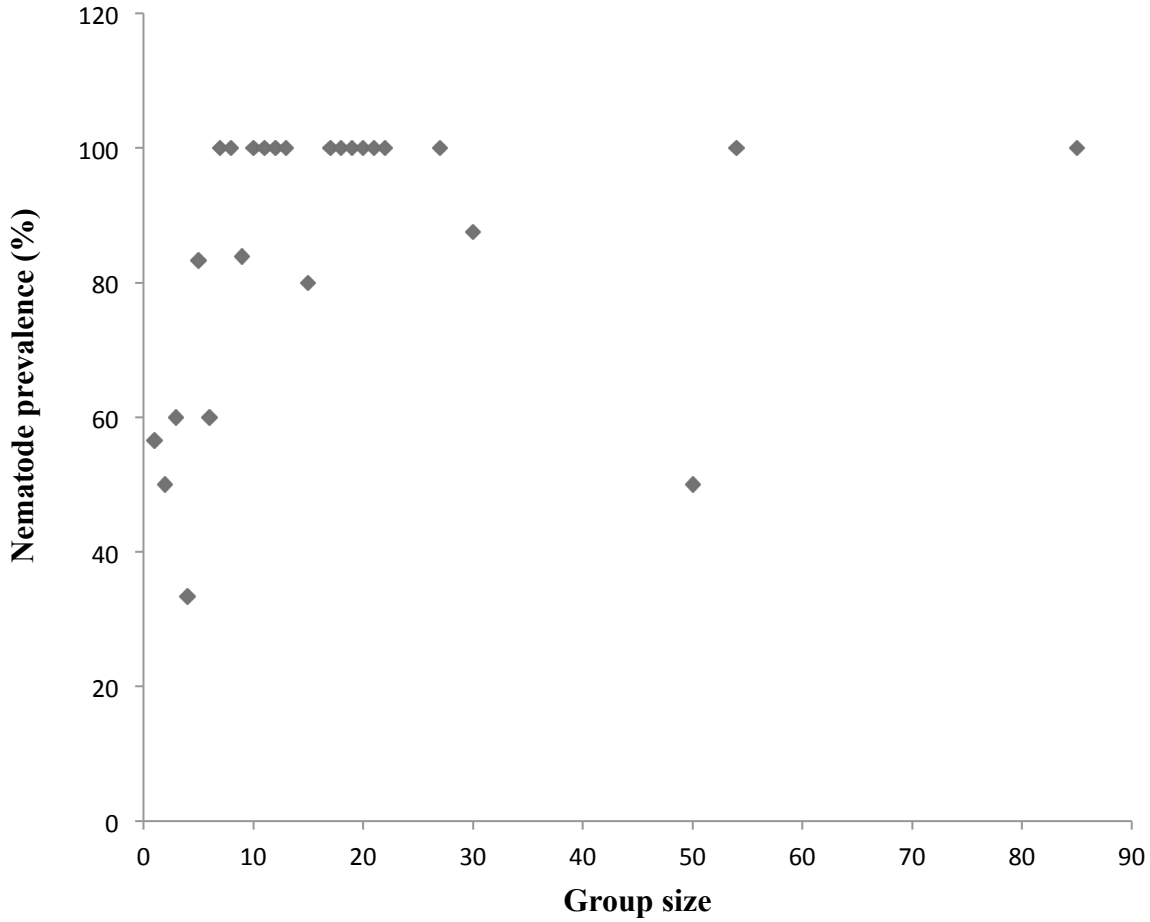


Figure 2.23: The prevalence of nematode eggs in stored faecal samples from wild elephants found in groups of different sizes.

2.5.4.3 Fluke egg prevalence

Fluke eggs were present in 23.4% of elephant faecal stored samples. Samples from 2010 were 5.084 times as likely to contain fluke ova than those from 2008 (binary logistic regression: p = 0.001). Samples from 2011 were 4.539 times as likely to contain fluke ova than those from 2008 (binary logistic regression: p = 0.002) (Table 2.7, Figure 2.24).

For each yearly increase in elephant age, fluke ova were 1.040 times as likely to be found in samples (binary logistic regression: p = 0.016) (Table 2.7, Figure 2.25). For every additional

month of storage time, fluke ova were 0.843 times as likely to be detected in faecal samples (binary logistic regression: p = 0.002) (Table 2.7) (Chapter 4).

Table 2.7: The significant variables from a binary logistic regression on the prevalence of fluke eggs in formalin stored wild elephant samples collected between 2008 and 2012 (397 samples).The least significant variable, group dynamic, was removed from the analysis. The other non-significant factors in the analysis were sex, group size, month and season. B represents the value for predicting the dependent variable from the independent variable in the logistic regression equation. S.E. represents the standard errors that are associated with coefficients. Wald represents the Wald chi-square value. The df value lists the degrees of freedom for each of the tests of the coefficient. Sig. shows the obtained P value, and OR shows the odds ratios for the predictors.

							95% Confidence Intervals for OR	
	B	**S.E.**	**Wald**	**Df**	**Sig.**	**OR**	**Lower**	**Upper**
Storage time	-0.171	0.056	9.207	1	0.002	0.843	0.755	0.941
Year			15.220	4	0.004			
2010	1.626	0.494	10.839	1	0.001	5.084	1.931	13.383
2011	1.513	0.494	9.420	1	0.002	4.539	1.728	11.924
Age	0.039	0.016	5.785	1	0.016	1.040	1.007	1.73

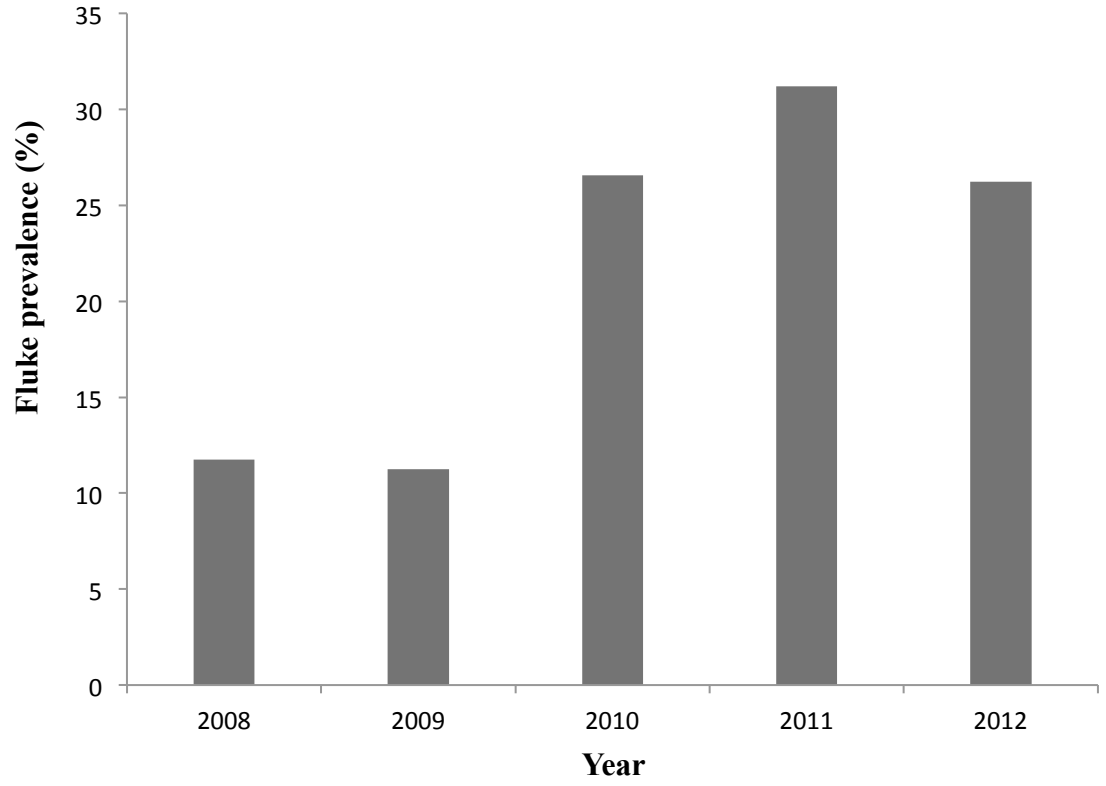


Figure 2.24: The prevalence of fluke eggs in stored wild elephants faecal samples collected between 2008 and 2012.

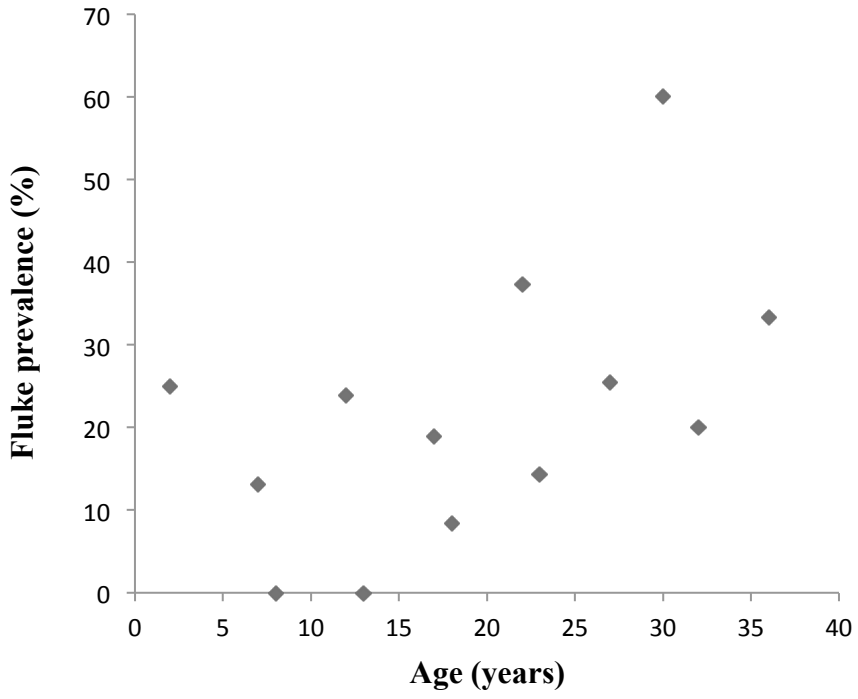


Figure 2.25: The prevalence of fluke eggs in stored faecal samples from different ages of wild elephant.

2.5.5 Abu herd

Samples collected from the captive Abu herd (Table 2.8) were taken between the 21st of January, and the 11th April, 2012.

Table 2.8: The age, sex, weight and height of the seven members of the captive Abu herd at the time of mid-study (March 2012).

Elephant	Age	Sex	Weight (kg)	Height (m)
Cathy	47 years	Female	3800	2.61
Sherini	25 years	Female	2680	2.44
Kitimetsi	17 years	Female	2070	2.43
Abu	5 years	Male	1000	2
Lorato	4 years	Female	800	1.9
Paseka	3 years	Female	550	1.5
Warona	3 months	Female	160	1

2.5.5.1 Coccidial oocyst prevalence

Between January and April, 2012, the coccidial oocyst prevalence in samples collected from the elephant named Abu (Table 2.8) significantly decreased (Pearson's correlation: $p = 0.043$, $pc = -0.569$, $n = 13$). Samples from the other members of the herd (Table 2.8) did not show a significant change (Pearson's correlation: Cathy; $p = 0.334$, $pc = -0.258$, $n = 16$, Kittimetsi; $p = 0.315$, $pc = -0.268$, $n = 11$, Lorato; $p = 0.631$, $pc = -0.164$, $n = 1$, Paseka; $p = 0.684$, $pc = -0.148$, $n = 10$).

2.5.5.2 Nematode egg density

There was no significant change in nematode egg density over the study period for Cathy (Pearson's Correlation: r = 0.129, p = 0.633, n = 16), Lorato (p = 0.341, r = -0.426, n = 7), Paseka (r = -0.178, p = 0.622,, n = 10) and Abu (r = 0.419, p = 0.154, n = 13) (Table 2.8). However, a significant increase in nematode egg density over the study period was found for Sherini (r = 0.727, p = 0.001, n = 16) (Figure 2.26) and Kittimetsi (r = 0.750, p = 0.008, n = 11) (Figure 2.27).

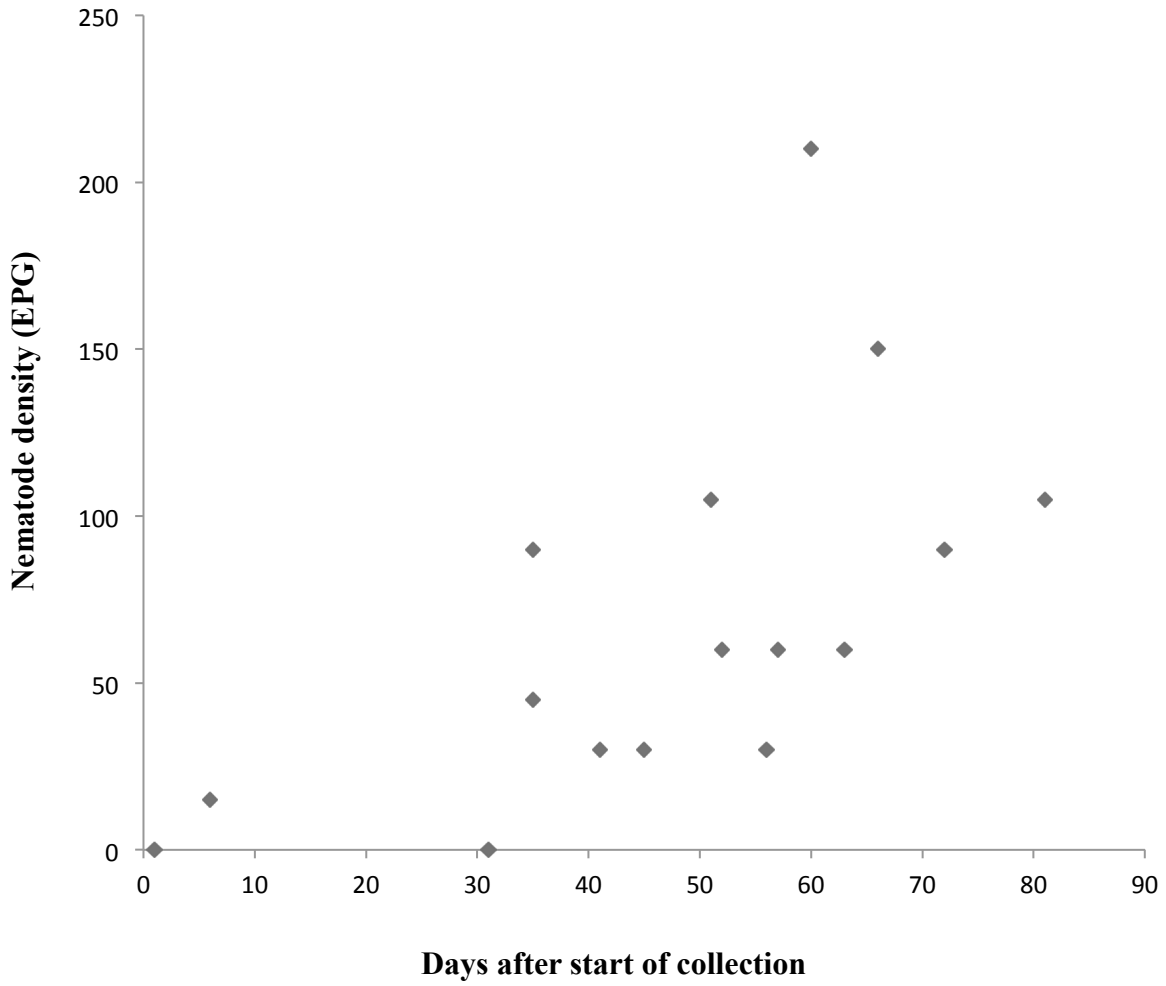


Figure 2.26: The nematode egg density found in samples colleted from Sherini between 21st January and 11th April 2012. EPG = eggs per gram.

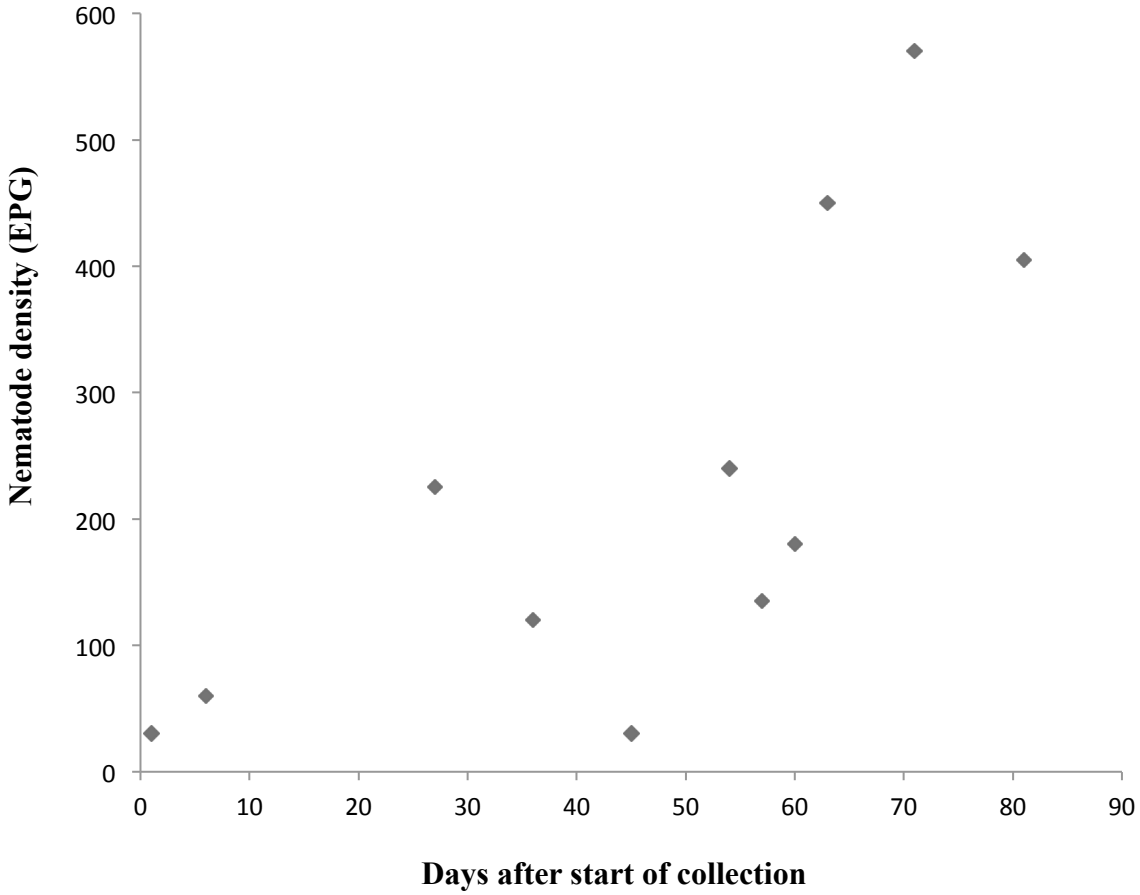


Figure 2.27: The nematode egg density found in samples collected from Kittimetsi between 21st January and 11th April, 2012. EPG = eggs per gram.

2.5.5.3 Fluke egg prevalence

The presence of fluke eggs was not detected in any of the samples collected from the Abu herd, at any point, over the period of study.

2.6 Discussion

Despite having to overcome difficulties with nematode egg preservation, a number of conclusive and revealing results were found. As predicted, a network of environmental and host-specific factors appear to be creating a combined influence on parasite presence and density in elephants in the Okavango Delta. Age, sex, group dynamic, group size, month and

year all had a significant effect on the transmission dynamics of elephant parasites in the Delta in some capacity.

While a male bias in coccidia infection in these elephants was found, a female bias in fluke infection was found. Fluke egg incidence also increased with age in formalin stored samples. The presence of coccidial oocysts was affected by both month and year, but these factors did not appear to affect nematode egg burden or fluke egg prevalence. Nematode infection increased with group size and was affected by the group dynamic, with lone bulls having higher nematode burdens than members of the matriarchal herd.

The change in nemataode ova density and coccidial oocyst prevalence in the Abu herd over the study period supplement the information collected from wild elephants (see Chapter 5), although these results have to be treated with caution as captive elephants do not have the same routine as wild elephants. Coccidial oocyst prevalence was significantly affected by month in one of the members of the Abu herd (Abu), and the nematode egg burdens found in Kittimetsi and Sherini changed over time. Fluke did not appear to be present in the samples from any member of the Abu herd throughout the entire study (see Chapter 5).

CHAPTER 3: ASSESEMENT OF PARASITE EGG DISTRIBUTION AMONG SYMPATRIC UNGULATE HOST SPECIES USING MORPHOMETRICS

3.1 Introduction

As the transfer of parasites between multiple host species is not uncommon (Hochberg and Holt, 1990), it is possible that some of the parasite fauna of elephants are also harboured by sympatrically grazing species (Chapter 1).

Despite the ability of elephants to reach a vegetation height of over five metres (Lessing, 2007), the browsing height of elephants is, for the most part, below two metres (Stokke and du Toit, 2000), and grass constitutes a large part of their diet (Tchamba and Seme, 1993). Therefore sympatric elephants and grazers share both food and water sources, presenting multiple opportunities for cross species parasite transmission. In this study, the morphometrics (length and width) of parasite eggs collected from elephant and grazer faeces in the Delta were compared. The finding of eggs similar in morphology may indicate the potential of transfer of parasites between sympatric species.

3.2 Method

Between 21st January and 11th April 2012, at a range of daylight hours, a total of 127 faecal samples were collected and analysed from: 41 impala, *Aepyceros melampus*, 10 greater kudu, *Tragelaphus strepsiceros*, 18 plains zebra, *Equus quagga*, 2 hippopotamus, *Hippopotamus amphibius*, 9 tsessebe, *Damaliscus lunatus*, 1 lechwe, *Kobus leche*, 4 buffalo, *Syncerus caffer*, 26 blue wildebeest, *Connochaetes taurinus*, 13 giraffe, *Giraffa camelopardalis*, 2 warthog, *Phacochoerus africanus*, 1 common reedbuck, *Redunca arundinum*, and 1 horse, *Equus ferus* from a nearby horseback safari camp. Grazers were observed from a distance until defecation, and faecal samples were only picked up once the animals had moved away. Samples were placed in plastic bags and taken to the laboratory for analysis. Three grams of each sample was analysed under a microscope within 24 hours of dropped. The same

flotation and sedimentation methods described in Chapter 2 were used to investigate the presence and density of parasite ova. A number of samples that were found to have parasites present were stored in formalin and brought back to Bristol where a digital microscope was used to capture images and to measure the length and width of detected parasite ova.

3.3 Statistical analysis

Nematode ova densities were compared between grazing and browsing species using a Mann Whitney-U Test, in order to investigate whether feeding height affected parasite levels, as this may indicate which animal species are at higher risk of infection.

3.4 Results

3.4.1 Comparing nematode ova densities between grazers

The grazers that had nematode ova present in faecal samples included; hippopotamus, warthog, reedbuck, tsessebe, wildebeest, impala, horse, lechwe, buffalo, zebra, kudu and giraffe (Figure 3.1). All of the above species are classified by their eating habit as 'grazers' except for kudu and giraffe, which are classified as 'browsers' due to their preference of eating at a higher vegetation height (Apio et al. 2006). The nematode egg distribution of both the grazers and the browsers were not normal (Shapiro Wilk test, grazers; $p < 0.001$, $df = 104$, statistic = 0.827. Browsers; $p < 0.001$, $df = 23$, statistic = 0.795). Browsers had a significantly lower average nematode density than grazers (Mann Whitney; $U = 412.500$, $p < 0.001$, (Figure 3.2).

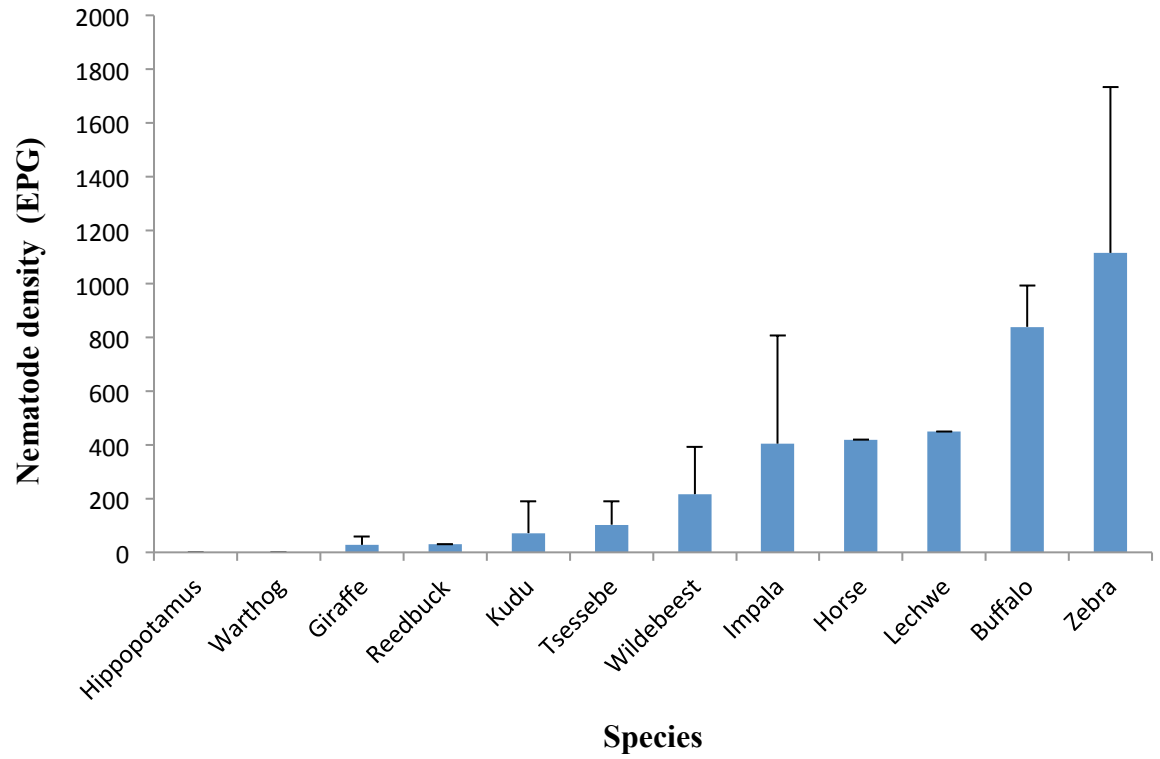


Figure 3.1: The average nematode egg densities found in 12 different grazer species. Error bars show the Standard Deviation. EPG = eggs per gram.

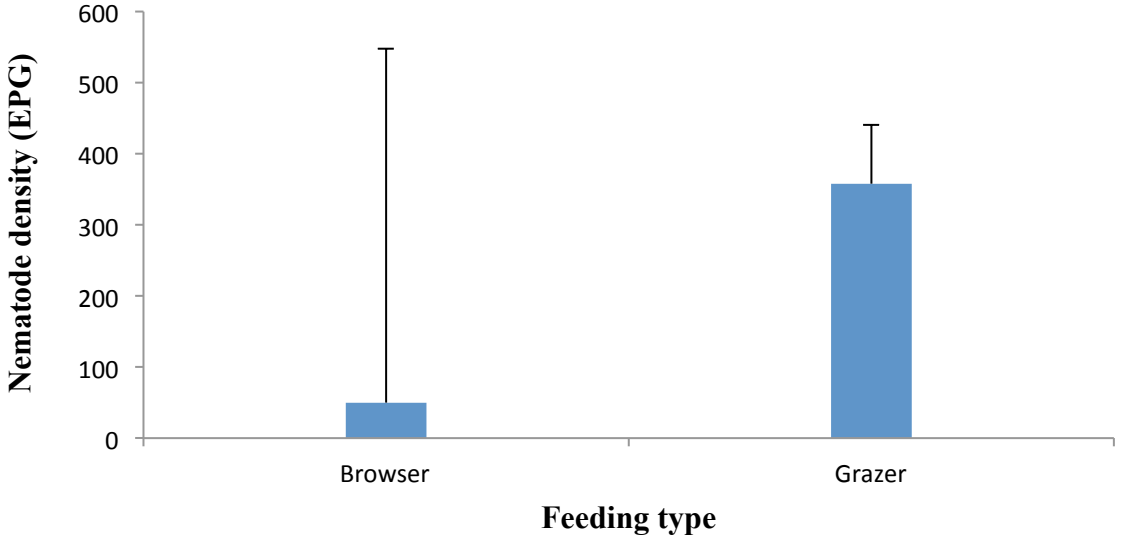


Figure 3.2: The average nematode egg densities found in grazing and browsing species. Error bars show the Standard Deviation. EPG = eggs per gram.

3.4.2 Comparing nematode ova from elephant and grazer samples

The average nematode egg length found in elephant faecal samples was 76.2 µm and the average width was 46.1 µm (Table 3.1). When egg widths were graphically plotted against lengths, there were no apparent separate clusters (Figure 3.3). There were no obvious visual differences between nematode eggs from elephants and nematode eggs from grazer species (Figures 3.4 and 3.5).

However, when nematode eggs from grazer species had the same morphological dimensions plotted, four clear clusters were present, suggesting that at least four different parasite species may be present in these grazers (Figure 3.6). When the data on elephant nematpde eggs were added to this graph, the majority appeared to fit in between the two middle clusters of the grazer nematode eggs, with partial overlap (Figure 3.7).

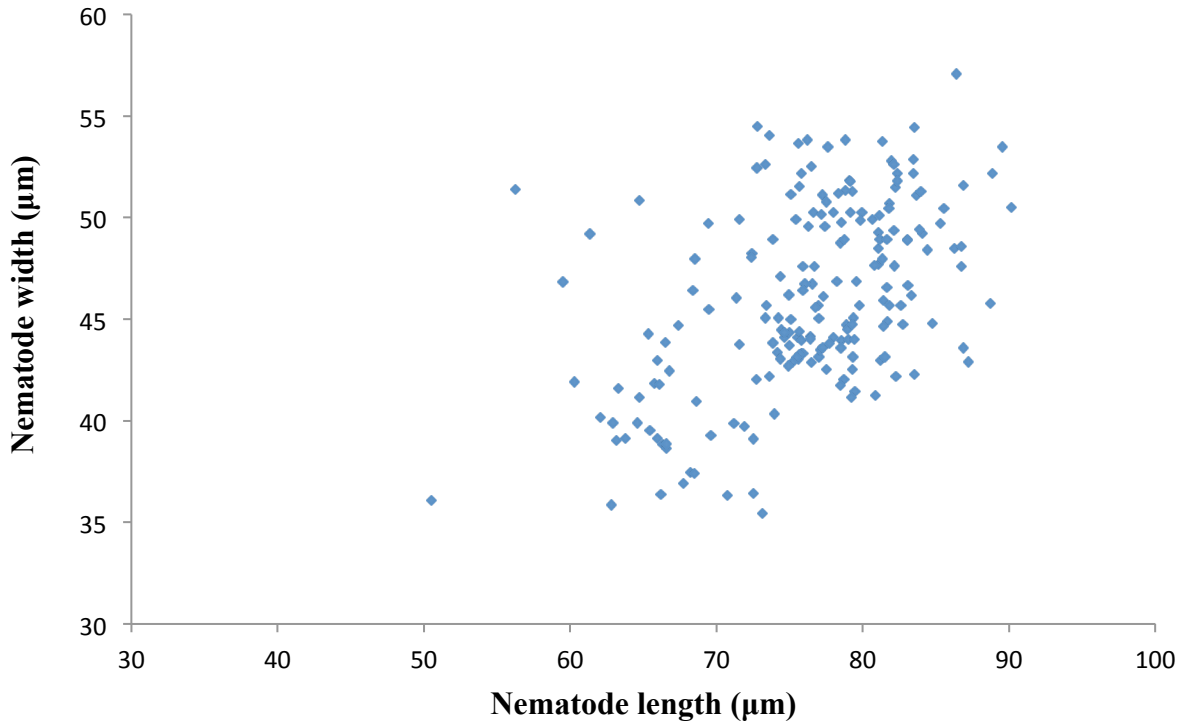


Figure 3.3: The dimensions (length and width) of nematode eggs found in wild elephant faecal samples.

Table 3.1: The average length and width of nematode eggs found in faecal samples in various mammal species found in the Okavango Delta.

Grazer species	**Average nematode length (µm)**	**Standard Deviation of nematode length**	**Average nematode width (µm)**	**Standard Deviation of nematode width**
Buffalo	70.1	2.5	39.4	0.5
Horse	90.3	7.8	51.8	3.8
Impala	71.5	30.3	41.1	16.6
Kudu	64.3	3.8	43.2	1.9
Reedbuck	79.4	15	46	7.2
Tsessebe	68.6	1.4	43.5	0.7
Wildebeest	59	15	34.8	10.6
Zebra	94.1	8.6	52.3	1.8
Elephant	72.6	6.7	46.1	4.6

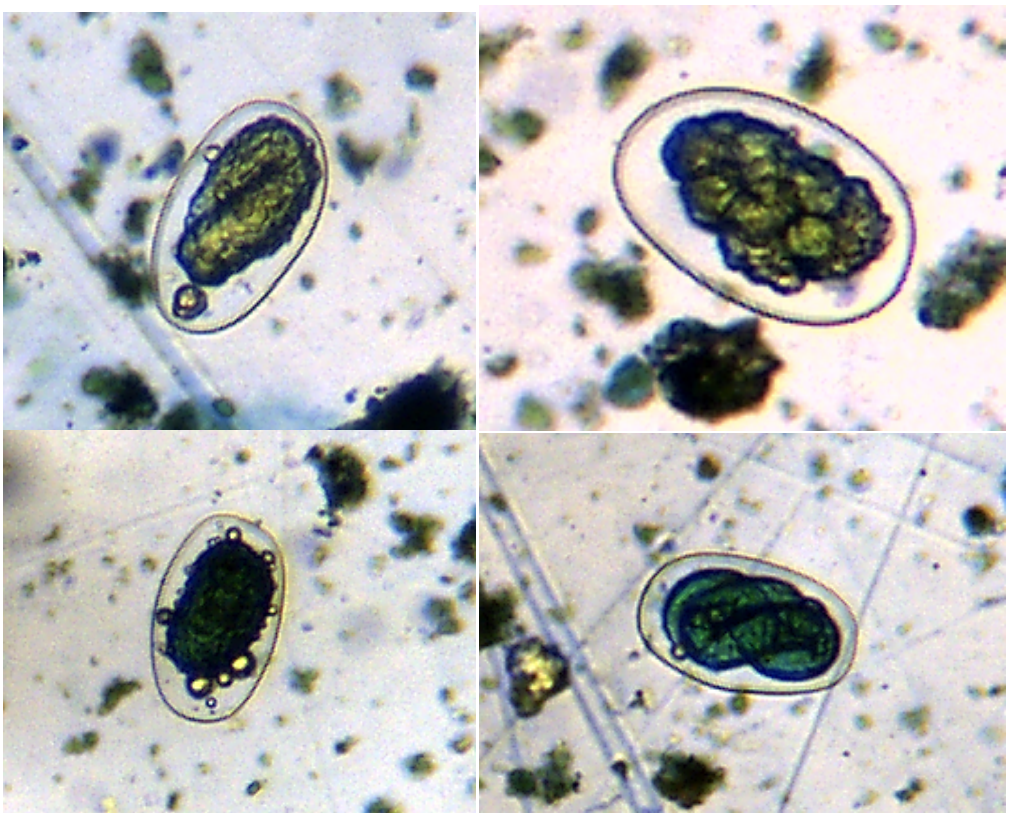


Figure 3.4: Images of nematode eggs found in wild elephant faecal samples collected from the Okavango Delta (not to scale). Images taken using a digital microscope. See Table 3.1 for average egg lengths and widths.

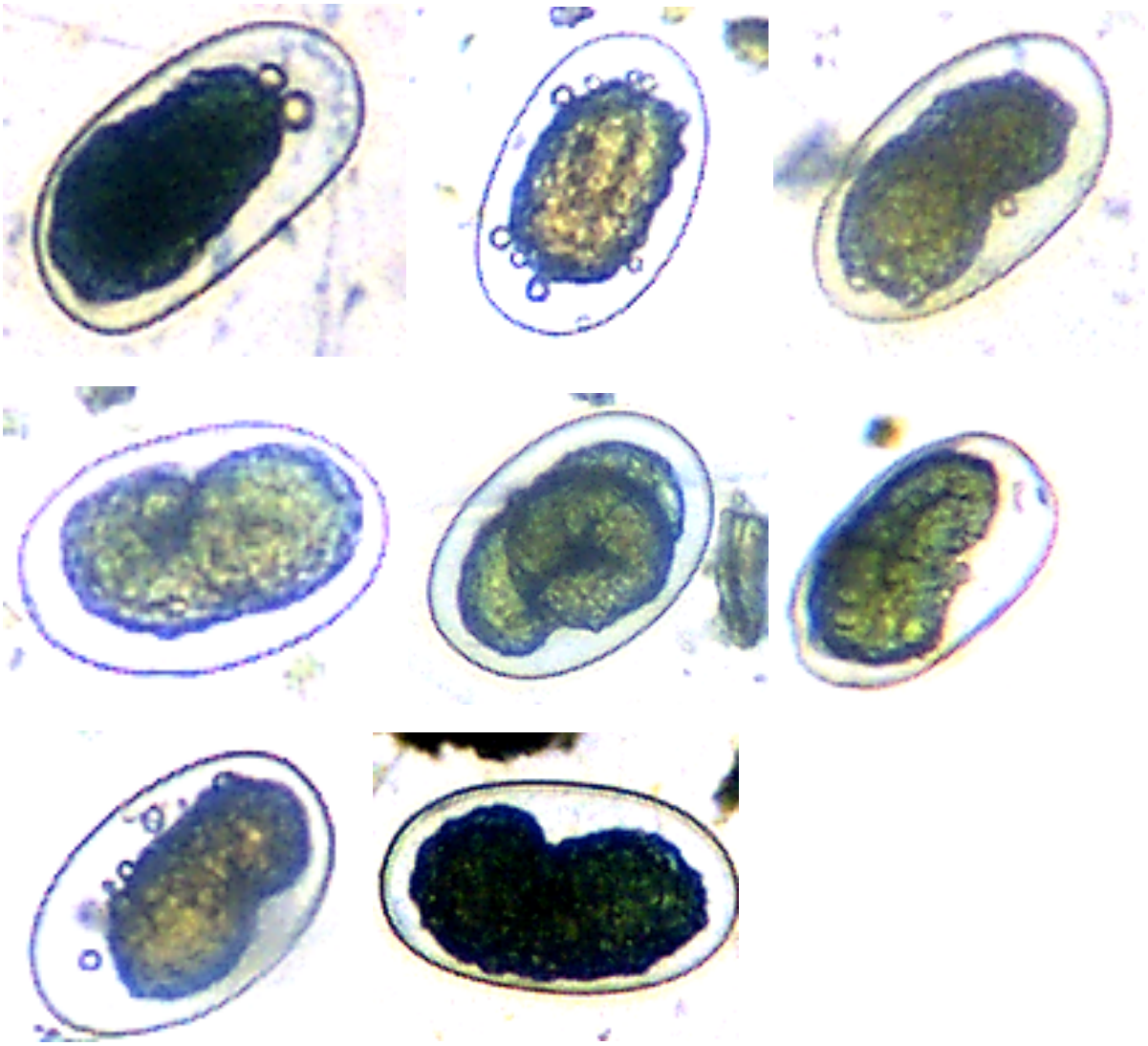


Figure 3.5: From top left across to bottom right, nematode eggs found in the faeces of: buffalo, impala, wildebeest, kudu, horse, tsessebe, zebra and reedbuck found in the Okavango Delta. Images taken using a digital microscope. See Table 3.1 for average egg lengths and widths.

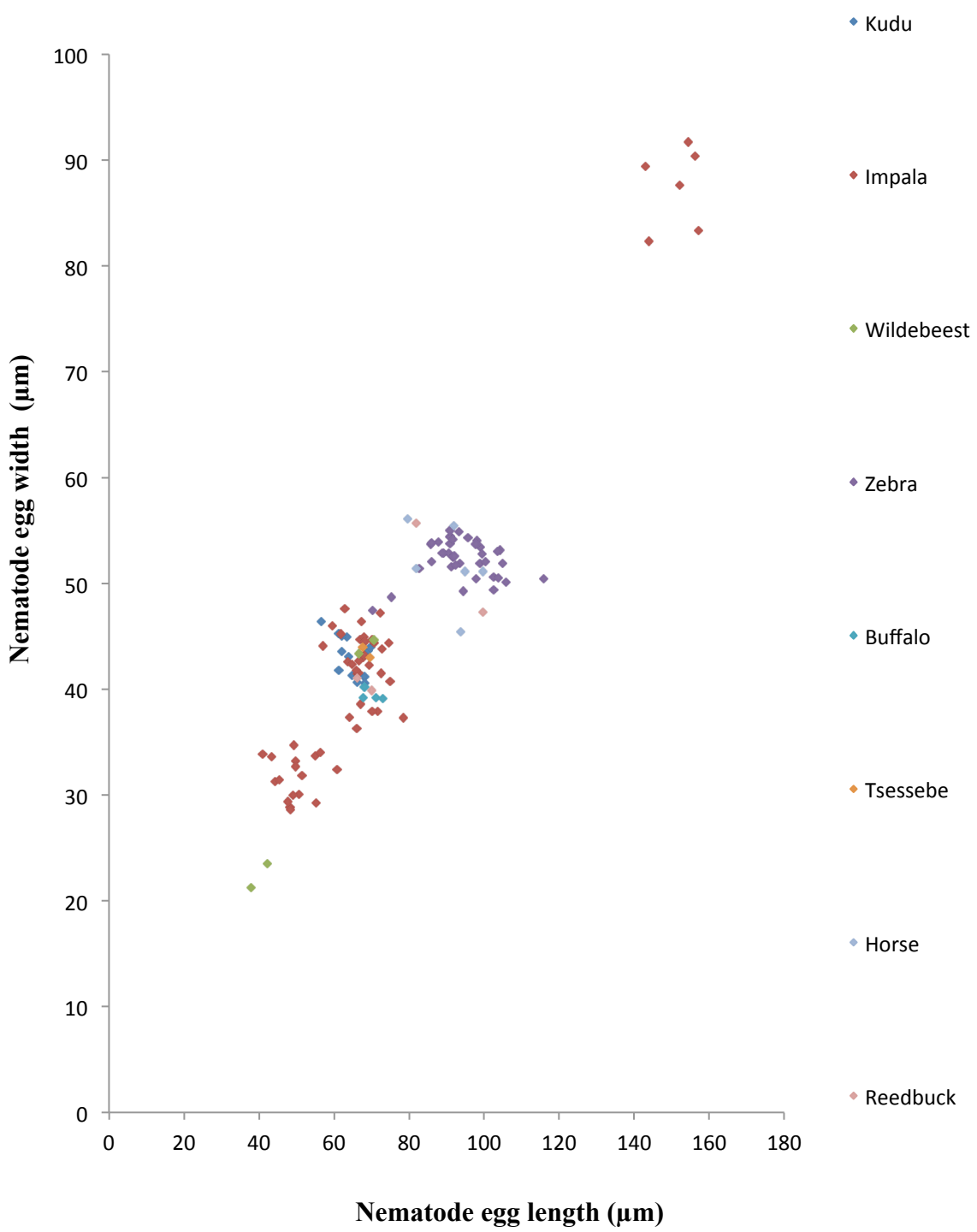


Figure 3.6: The dimensions (length and width) of nematode eggs found in grazer faecal samples.

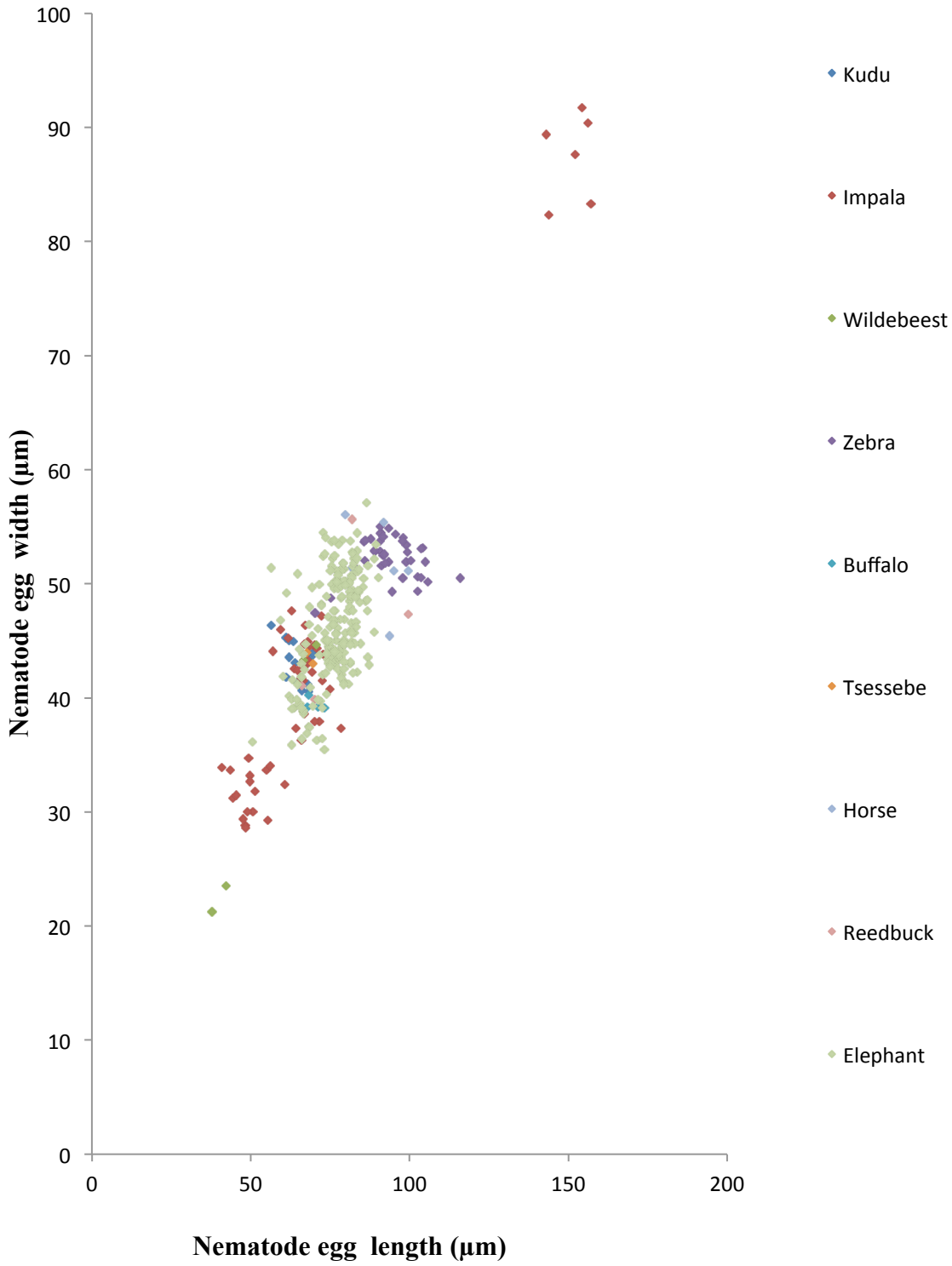


Figure 3.7: The dimensions (length and width) of nematode eggs found in and grazer faecal samples, with the addition of the dimensions of nematode eggs found in elephant faecal samples in order to visually compare egg morphology between different host species.

3.4.3 Comparing fluke ova from elephants and grazer samples

The average length of fluke eggs found in elephant faecal samples was 97.7 µm and the average width was 55 µm. There were no visible clusters when the egg widths were plotted against egg lengths (Figure 3.8). There were also no visible clusters when egg dimensions from grazer faecal samples were plotted (Figure 3.11). Although the majority of the fluke eggs found in grazer species looked morphologically similar (here named 'Type 1' fluke eggs), both the red lechwe and wildebeest faecal samples contained, in addition to 'Type 1' fluke eggs, fluke eggs that were visibly morphologically dissimilar (here named 'Type 2' fluke eggs). These eggs were large, golden brown in colour, and opaque. 'Type 1' eggs were smaller, lightly coloured and less opaque (Figure 3.13).

However, the fluke eggs found in grazer species were visibly morphologically different from those found in elephant species (Figure 3.9 and Figure 3.12). A graphical representation shows that the fluke eggs found in elephant samples are much smaller in length and width than those found in grazer samples (Figures 3.12 and Table 3.2). The fluke species from elephant faecal samples were very similar in morphology to *Protofasciola robusta* (Figure 3.10), a fluke species that has previously been found in African elephants, but has not been reported in any other species

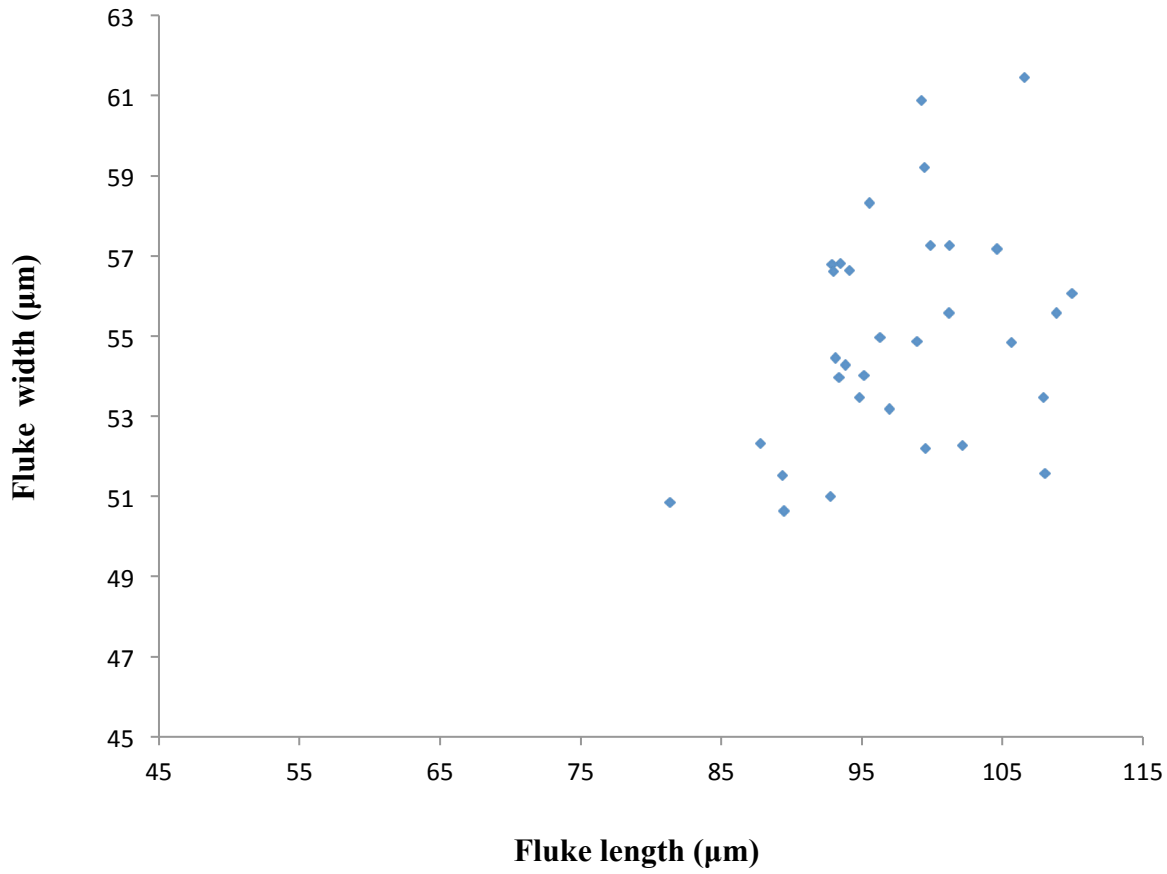


Figure 3.8: The dimensions (width and length) of fluke eggs found in faecal samples from wild elephants in the Okavango Delta.

Table 3.2: The average length and width of fluke eggs found in a range of mammal species in the Okavango Delta. ‘Type 1’ and ‘Type 2’ describe varying fluke egg morphologies. ‘Type 1’ eggs look visibly similar in all grazing species studied while ‘Type 2’ eggs describe a visibly different egg morphology found in red lechwe and wildebeest.

Grazer species	**Average fluke length (µm)**	**Standard Deviation of fluke length**	**Average fluke width (µm)**	**Standard deviation of fluke width**
Buffalo	147.3	18.8	83.7	12.6
Hippopotamus	131	4.9	69	1.9
Implala	156.5	9.5	88.7	3.3
Kudu	151.7	11.8	88.2	5.9
Red lechwe (Type 1)	128	11.3	72.5	10.6
Red lechwe (Type 2)	151	N/A	90.1	N/A
Reedbuck	124.4	9.9	67.3	5.7
Tsessebe	132.7	16.4	72.4	8.2
Warthog	122.7	10	72.8	5.2
Wildebeest (Type 1)	125.4	8.9	71	5.9
Wildebeest (Type 2)	137.4	14.5	82	6.5
Zebra	149.1	4.9	91	3.1
Elephant	97.7	6.8	55	2.8

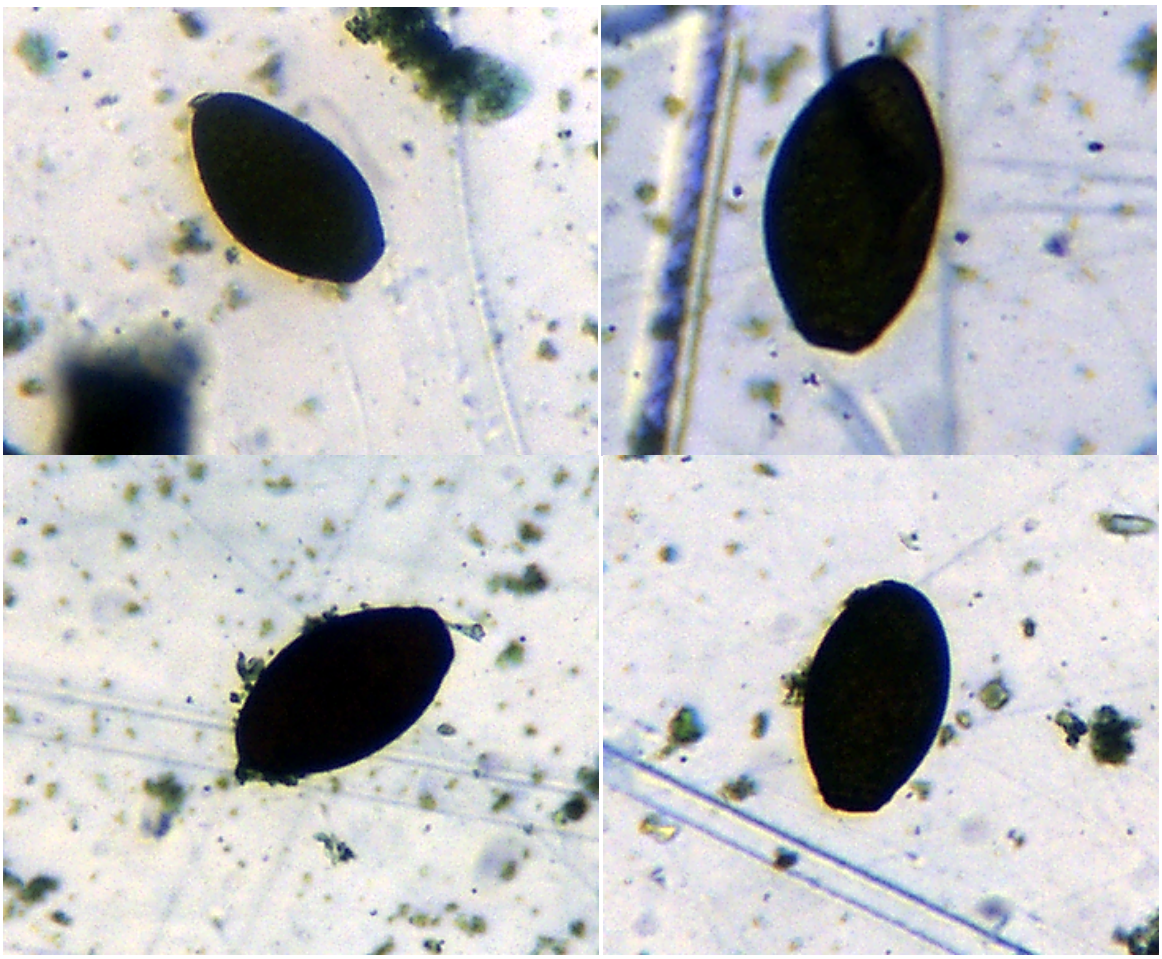


Figure 3.9: Images of fluke eggs found in elephant faecal samples in the Okavango Delta (not to scale). See Table 3.2 for average egg widths and lengths. Images taken using a digital microscope.

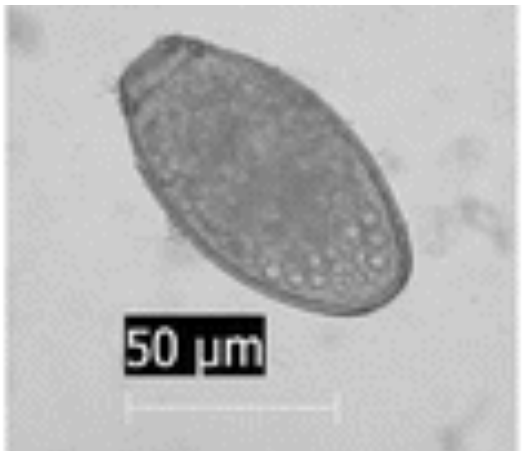


Figure 3.10: *Protofasciola robustra* fluke egg found in an elephant that had died from starvation in Kenya. Image reproduced from a report by Obanda et al. (2011).

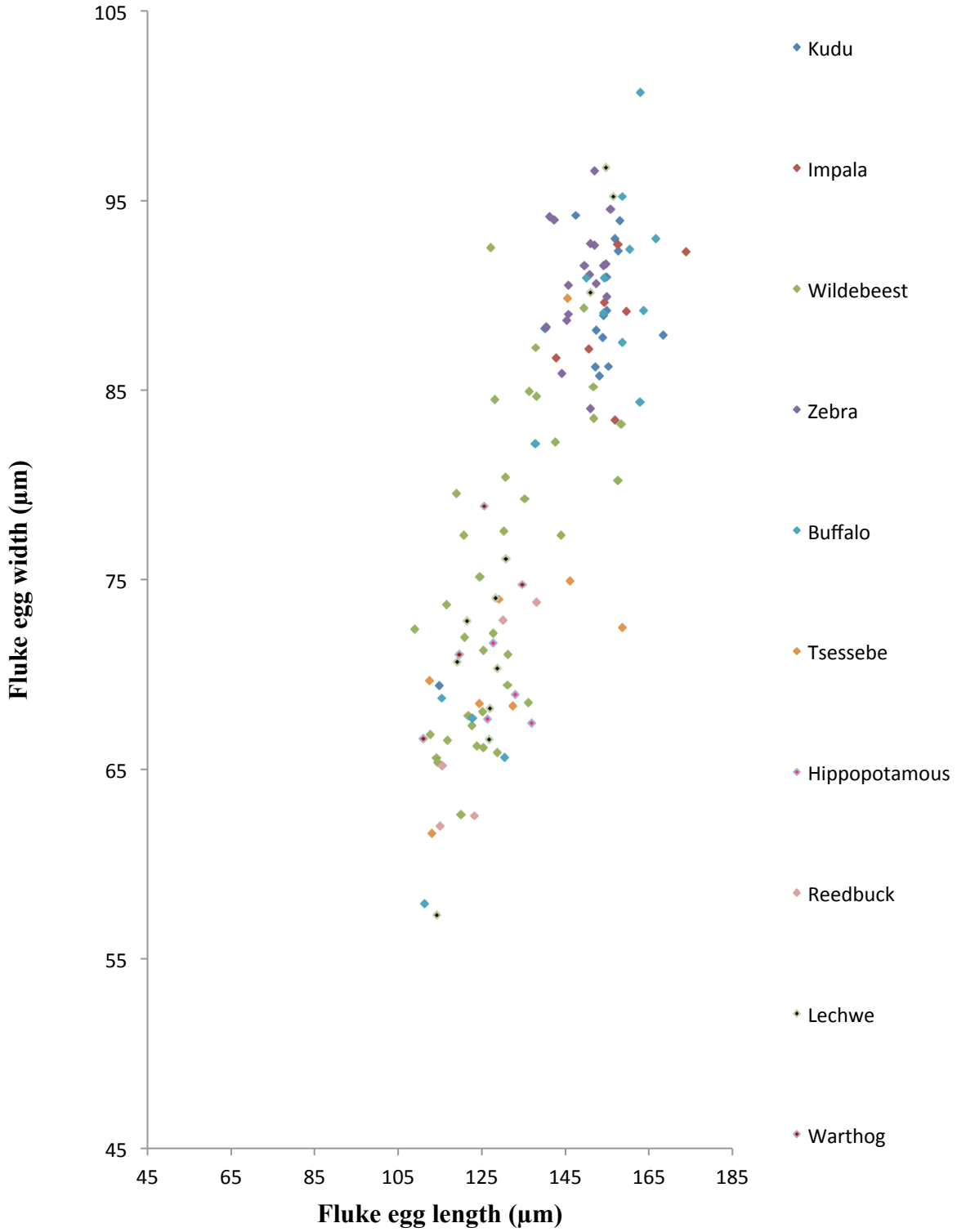


Figure 3.11: The dimensions (length and width) of fluke eggs found in grazer samples in the Okavango Delta.

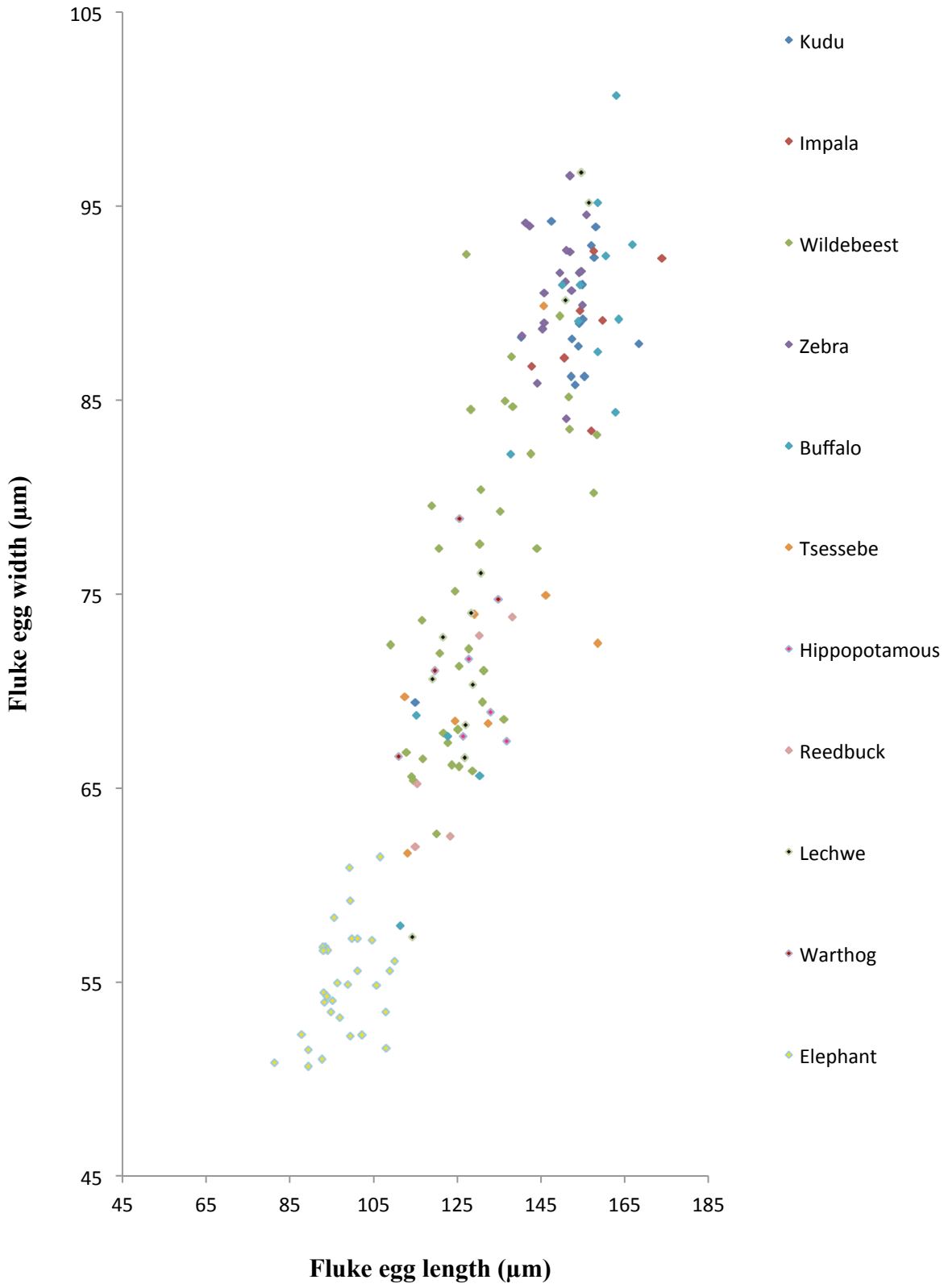
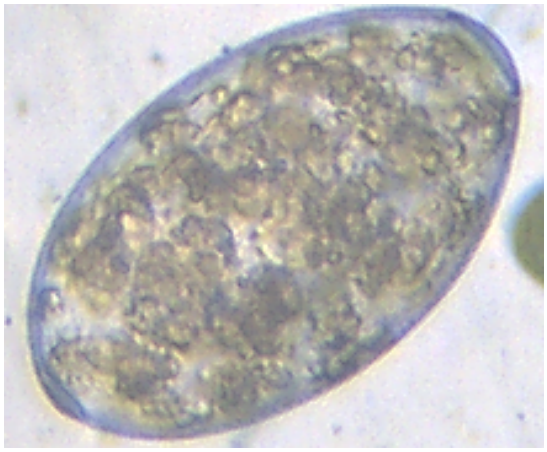
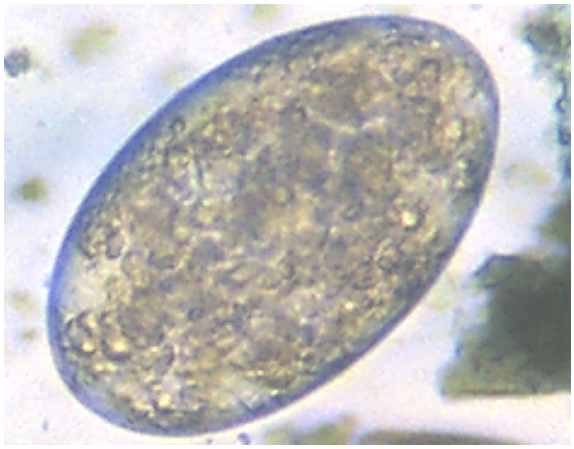
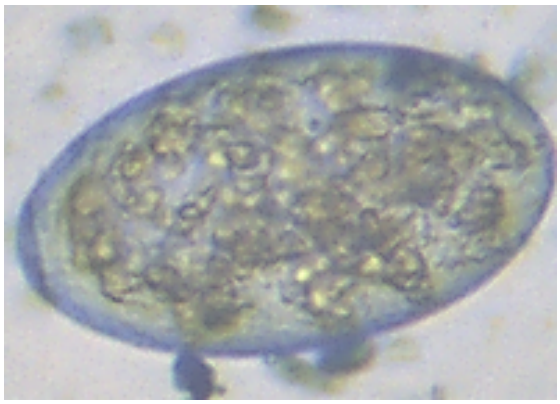
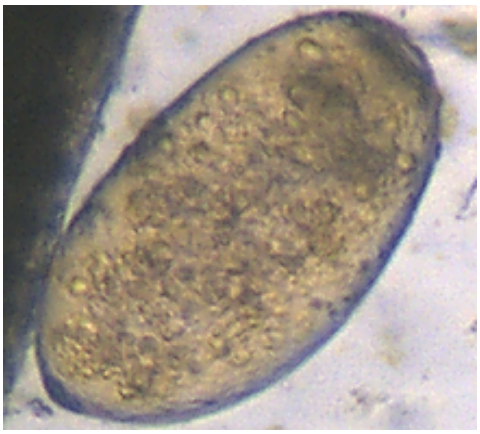
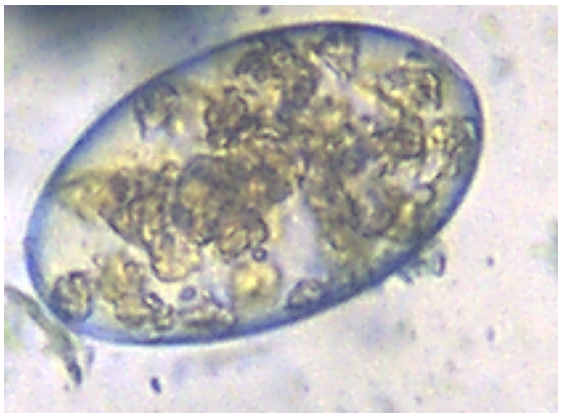
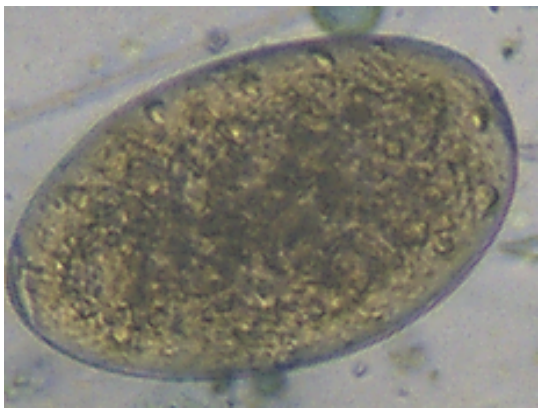
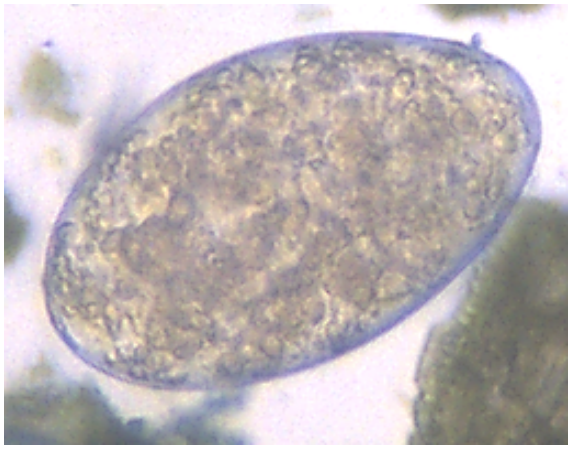
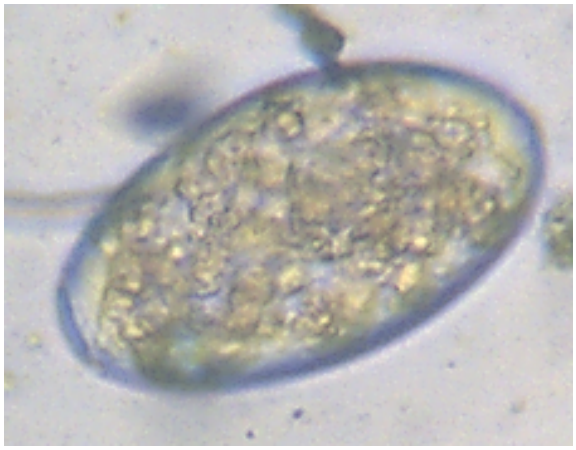


Figure 3.12: The dimensions (length and width) of fluke eggs from elephant and grazer faecal samples from the Okavango Delta.

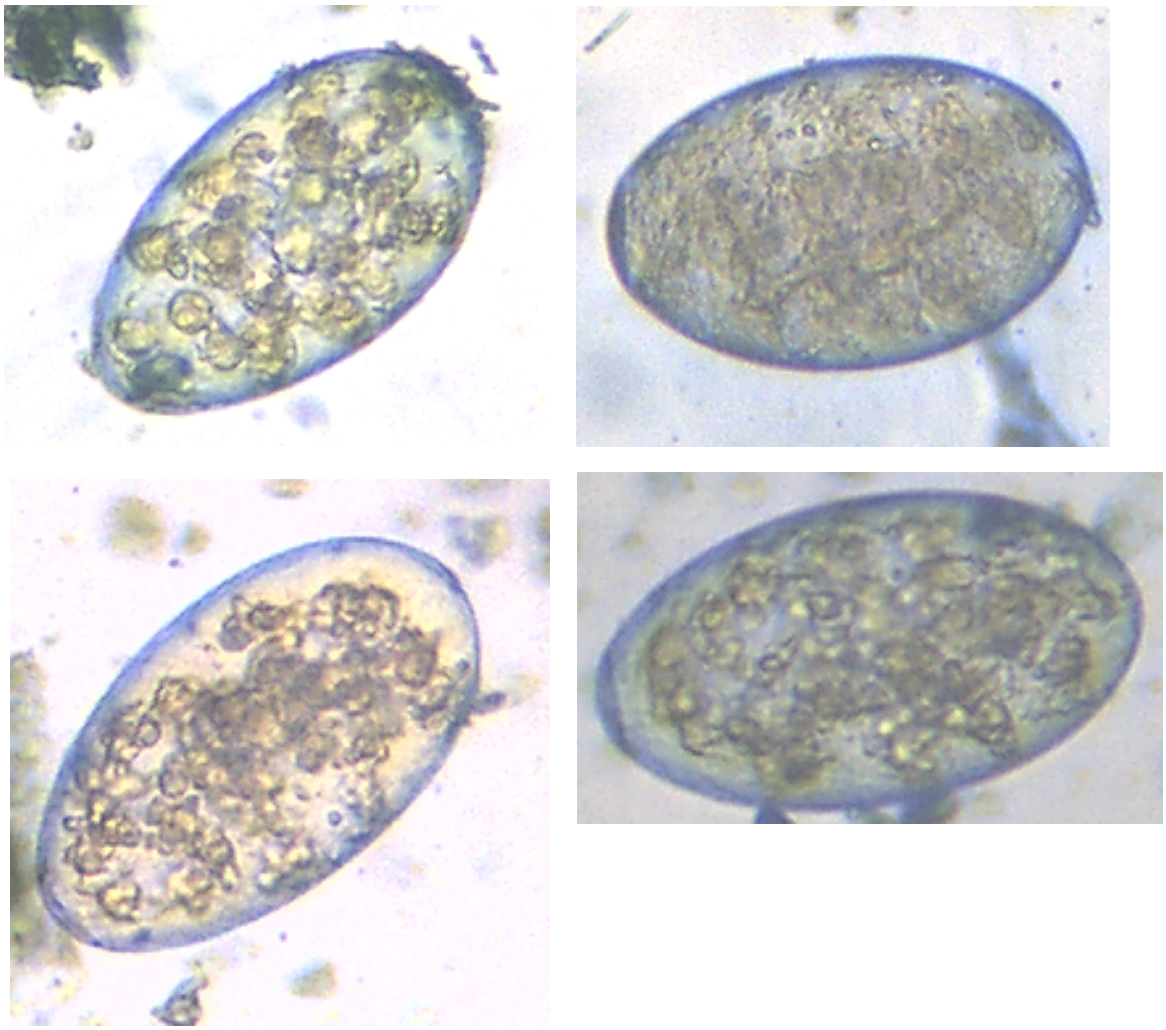


Figure 3.13: Going from top left across to bottom right, images of fluke eggs found in faecal samples from buffalo, impala, wildebeest (Type 1), wildebeest (Type 2), red lechwe (Type 1), red lechwe (Type 2), kudu, warthog, tsessebe, zebra, reedbuck, and hippopotamus. See Table 3.2 for average widths and lengths of eggs. 'Type 1' and 'Type 2' labels signify a visible difference seen in fluke eggs from the same species.

3. 5 Discussion

3.5.1 Comparing nematode egg densities between grazing species

Nematode eggs were found in the faeces of zebra, buffalo, lechwe, horse, impala, wildebeest, tsessebe, kudu, reedbuck and giraffe (Figure 3.1), but not in warthog or hippopotamus. However only one faecal sample was obtained for each of warthog and hippopotamus. Zebra

were found to have the highest average nematode burden, and this parallels with other research that suggests that nematode in zebra are diverse and numerous (Lichtenfels et al., 2008). The browsers (kudu and giraffe) which feed off raised bush and tree-like vegetation had a significantly lower average nematode burden than the grazers (zebra, buffalo, lechwe, horse, impala, wildebeest, tsessebe and reedbuck), which feed predominantly on grass. This finding is in agreement with previous research which found higher parasite levels at lower vegetation heights (Apio et al., 2006). However, due to the limited number of browsing species examined in this study, results have to be treated with caution.

3.5.2 Comparing grazer and elephant nematode ova

From egg dimension measurements, it is not clear whether elephants harbour one species of nematode that have a range of lengths and widths, or whether they harbour multiple infections of nematode that overlap in the measured dimensions (egg length and widthe) (Figure 3.3). There were no obvious visible differences when nematode eggs from different host species were compared (Figure 3.4 and Figure 3.5). When the nematode egg lengths and widths from grazer species were plotted against each other, four main clusters were observed. When the elephant nematode egg dimensions were added to this graph however, the majority of eggs fell between clusters, though with some overlap (Figure 3.7). This overlap suggest that elephants and grazers could be sharing nematode species although further work is necessary to prove this. This could include molecular analyses or comparisons of adult parasite fauna from *post mortem* examinations.

3.5.3 Comparing grazer and elephant fluke ova

Due to the lack of apparent morphological graphical clusters (Figure 3.8), it is hard to determine whether elephants harbour multiple fluke species, or whether they are infected by just one species that has variable egg lengths. This was the same within the grazer species,

where plotting egg dimensions did not reveal distinct morphological clusters. Although the majority of the fluke eggs found in grazer species were similar in shape, colour and size, wildebeest and red lechwe appeared to have an additional fluke egg type, which was more opaque than the other more common fluke egg, and was golden in colour (Figure 3.13). However, this fluke egg type was also morphologically very different to the fluke found in the elephants in this study.

However, unlike the nematode eggs, there were very clear morphological differences between the fluke eggs found in elephant species and the fluke eggs found in grazer species (Figure 3.12). The eggs were notably different in colour, opaqueness, shape and size (Figure 3.9 and Figure 3.13). The fluke eggs found in elephant species were much smaller in both length and width than any of the fluke eggs found from grazer species (Table 3.2).

The fluke eggs found in the elephant samples were very similar morphologically to *Protofasciola robustra* eggs, a fluke species that has previously been found in the duodenum of African elephants in Kenya (Figure 3.10). This fluke was linked to the cause of death by starvation in these Kenyan elephants (Obanda et al., 2011). However, further research is needed before being able to clarify that the fluke species found in these Delta elephants are *P. robustra*. In particular, morphological identification of adult specimens should be sought from opportunistic access to *post mortem* materials.

The fluke eggs found in the grazer samples were similar in morphology to those from *Fasciola* species. *Fasciola gigantica* is the most common liver fluke in domestic African ruminants, and these are also known to be prevalent amongst the wild grazers (Hammond, 1972). *Fasciola hepatica* has also been found in wild Africa mammals, although this is far less common (Hammond, 1972). Although studies have reported finding *Fasciola* species in

the Africa elephant, this did not seem to be the case in this study, where the eggs from elephant faecal samples did not resemble any *Fasciola* species.

Overall, the fluke data were far more conclusive than the nematode data, in that it can be said with almost certainty that the elephants sampled in the Okavango Delta did not harbour the same fluke species as any of the grazers studied. However, on the basis of similar morphology it seems that the grazer species may be sharing the same nematode species. The wide range in nematode egg dimensions suggests that a range of species are present, with overlapping egg sizes, such that separation of the eggs from different host species on the basis of morphology alone was not possible. Therefore this approach did not succeed in determining likely nematode overlap between species.

CHAPTER 4: EFFECTS OF STORAGE CONDITIONS ON RECOVERY AND ENUMERATION OF PARASITE OVA

4.1 Introduction

Prior to the field study described in Chapter 3, a pilot study was conducted in order to determine the optimum method of preserving nematode eggs collected in the field. This was also needed to evaluate the reliability of egg counts from previously stored elephant faecal material. It is very common in studies of wildlife in remote areas to store field collected faecal samples, or parasite eggs that have been extracted from faeces, in chemical preservatives, in order to enable transfer to the laboratory for further analysis. Critical evaluation of the effect of common storage methods on observed egg counts is therefore warranted.

Various research suggests that, despite its wide use as a storage medium, formalin may have some limitations. A study on nematode eggs in deer faeces (Foreyt, 1986) found that for long-term preservation, 10% formalin was the optimum method when compared to lower formalin concentrations, 70% ethyl alcohol and absolute ethyl alcohol. However, after storage in 10% formalin for 200 days, only half of the strongyle eggs originally present were detected by flotation. The same study also found that between days three and ten of storage, the rate of recovery of nematode eggs was less than 50%, possibly due to ion binding (Foreyt, 1986). Further material suggests that some faecal samples may lose their true egg count after only three weeks storage in formalin (Karki, 2008). In order to be able to rely on results collected from elephant faecal samples, an investigation into how formalin storage affects egg counts is paramount.

A number of different treatments were applied to sheep faeces to determine relative storage ability, and the use of salt and salt-sugar in flotation solutions for parasite ova detection was also compared. How storage time in formalin affects parasite recovery (nematode and fluke ova, and coccidial oocysts) in elephant faecal material was investigated, after completion of the fieldwork.

4.2 Method

4.2.1 Investigating a variety of faecal storage methods

Faeces from sheep artificially infected with one of two nematode species, *Haemonchus contortus* or *Teladorsagia circumcinta,* were collected from Morendun Research Institute, Edinburgh, UK. One gram aliquots of faeces were weighed out and the following treatments applied: 10% formalin solution at 25 °C, 10% formalin solution at 12 °C, 70% ethanol solution at 25 °C, 70% ethanol solution at 12 °C, anaerobic conditions at 25 °C, anaerobic conditions at 12 °C , vacuum packing at 25 °C, vacuum packing at 12 °C, and refrigeration. Anaerobic conditions were created by placing one gram of sheep faeces in a small storage container and slowly adding water, whilst mixing, until the container was full to the brim. All the air bubbles were removed before a seal-lid was carefully placed on the top, ensuring that there was no air space under the lid. Vacuum conditions were created by placing one gram aliquots of sheep faeces into a small polythene grip seal bags, and removing the internal air by hand as far as possible before sealing.

Four replicas of each treatment were carried out for each of *H. contortus* and *T. circumcincta* infected samples, and these were left for one week in their respective conditions. Faecel egg counts (FECs) were taken of fresh samples against which to later compare the FECs of the treated samples.

After one week of storage, 14ml of water was added to each sample, and the whole mixed thoroughly to break up and suspend the faecal material, which was then sieved through a tea strainer to remove coarse debris. Two centrifuge tubes were filled with the sieved suspension and placed in the centrifuge for two minutes at 1500rpm. The supernatant was then emptied out of the centrifuge tubes and salt flotation solution (saturated NaCl, specific gravity of 1.19) was added to one of the centrifuge tubes, and salt-sugar flotation solution (specific gravity 1.28) to the other. The tubes were then inverted several times to re-suspend the sediment, and a sample of each well-mixed solution was extracted using a pipette and placed in a McMaster slide (Cringoli, 2004). The slides were left for two minutes to allow the nematode eggs time to float to the surface, before being examined under the microscope at 100x total magnification. The number of eggs in both chambers was counted and the total number multiplied by 50 to obtain the EPG (Good et al. 2004). Analysis was carried out on the actual number of eggs counted using the following statistical methods in SPSS (v16): normality was assessed using a Kolmogorov-Smirnov Test and the effect of using different floatation solutions was assessed using a Mann-Whitney U Test. A Kruskal-Wallis test was carried out on the varying storage treatments to check for differences and a series of Mann-Whitney U Tests were carried out to compare egg counts after different storage methods using the fresh samples as the reference category.

4.2.2 Investigating the effect of formalin storage on elephant parasite ova detection

Elephant faecal samples were collected from the Okavango Delta between 12th November 2008 and 11th April, 2012, and were stored in formalin from between 0-15 months (Chapter 2). Ova from three parasite genera were found in these samples; coccidia, nematode and fluke. A total of 397 samples were analysed, and the prevalence of the above parasite genera were recorded. The results were analysed using the following statistical methods in SPSS (v16); normality was assessed using a Kolmogorov-Smirnov Test, and the affect of formalin

storage on parasite ova and coccidial oocyst prevalence was analysed using a binary logistic model. Accompanying storage time in the binary logisic models were variables that were recorded during sample collection, and include: elephant sex, age, group size, group dynamic, month, season and year.

4.3 Results

4.3.1 The use of salt and salt-sugar flotation solutions on nematode egg detection in sheep faeces

4.3.1.1 *Haemonchus contortus*

H. contortus egg counts were not normally distributed (salt; Kolmogorov-Smirnov statistic = 0.22, df = 40, $p < 0.001$, salt-sugar statistic = 0.213, df = 40, $p < 0.001$). There was no significant difference in nematode egg counts between salt and salt-sugar flotation solutions (Mann-Whitney U(40) = 764.5, p = 0.731).

4.3.1.2 *Teladorsagia circumcinta*

T. circumcincta egg counts were not normally distributed (salt; Kolmogorov-Smirnov statistic = 0.492, df = 40, $p < 0.001$, salt-sugar statistic = 0.357, df = 40, $p < 0.001$.) Nematode egg counts were significantly higher in salt-sugar flotation solution than in salt flotation solution (Mann- Whitney U (40) = 602.500, p = 0.027).

4.3.2 Optimum storage treatment to preserve nematode eggs in sheep faeces

4.3.2.1 *Haemonchus contortus*

H. contortus egg counts were not normally distributed (salt; Kolmogorov-Smirnov statistic = 0.22, df = 40, $p < 0.001$, salt-sugar statistic = 0.213, df = 40, $p < 0.001$).

There was a significant difference in the nematode egg counts found in faeces from artificially infected sheep, after being subject to different treatments (Kruskal Wallis, salt solution; $X^2 = 32.13$, df = 9, $p < 0.001$, salt-sugar solution; $X^2 = 29.691$, df = 9, $p < 0.001$) (Table 4.1 and Table 4.2).

When salt flotation solution was used the following treatments had significantly lower nematode egg counts than those from freshly analysed samples: ethanol at 25^{o}C, ethanol at 12^{o}C, vacuum at 12^{o}C and refrigeration. The treatments that did not have a significantly lower nematode egg count were: vacuum at 25^{o}C, formalin at 25^{o}C, formalin at 12^{o}C, anaerobic at 25^{o}C and anaerobic at 12^{o}C.

When salt-sugar flotation solution was used, the following treatments had significantly lower nematode egg counts than those from freshly analysed samples: ethanol at 25^{o}C, ethanol at 12^{o}C, formalin at 25^{o}C, vacuum at 25^{o}C, vacuum at 12 ^{o}C, and refrigeration. The treatments that did not have a significantly lower nematode egg count were: formalin at 12^{o}C, anaerobic at 25^{o}C and anaerobic at 12^{o}C

Table 4.1; The results of a Mann Whitney U Test (SPSS v16) that compared *Haemonchus contortus* egg counts from fresh sheep faeces to the nematode egg count detected after sheep faecal samples were stored for one week in varied treatments. The average fresh egg count was 36. The flotation solution used for egg detection was salt.

Treatment	**Average egg count after treatment**	**Mann Whitney Value**	**P Value**
Ethanol at 25 ^{o}C	0	0	0.014
Ethanol at 12 ^{o}C	0	0	0.014
Formalin at 25 ^{o}C	12	2	0.083
Formalin at 12 ^{o}C	48.5	6	0.561
Vacuum at 25 ^{o}C	12	2	0.083
Vacuum at 12 ^{o}C	2.5	0	0.02
Refrigeration	3	0	0.019
Anaerobic conditions at 25^{o}C	35	7	0.773
Anaerobic conditions at 12 ^{o}C	40	7	0.773

Table 4.2; The results of a Mann Whitney U test (SPSS v16) that compared *Haemonchus contortus* egg counts from fresh sheep faeces to the nematode egg count detected after sheep faecal samples were stored for one week in varied treatments. The average fresh egg count was 40.25. The flotation solution used for egg detection was salt-sugar.

Treatment	**Average egg count after treatment**	**Mann-Whitney (U) Value**	**P Value**
Ethanol at 25 ^{o}C	6	0	0.021
Ethanol at 12 ^{o}C	2	0	0.02
Formalin at 25 ^{o}C	11	0	0.019
Formalin at 12 ^{o}C	25.5	4	0.248
Vacuum at 25 ^{o}C	12.5	1	0.043
Vacuum at 12 ^{o}C	3.5	0	0.02
Refrigeration	3.5	0	0.02
Anaerobic conditions at 25 ^{o}C	28.5	5.5	0.468
Anaerobic conditions at 12 ^{o}C	49	6	0.564

Table 4.3: The mean rank generated from a Kruskal Wallis test on different storage treatments for *H. contortus* egg counts in both salt flotation solution, and salt-sugar flotation solution.

Treatment	**Mean Rank (salt)**	**Mean Rank (salt-sugar)**
Fresh	31	33.38
Ethanol 25^{o}C	6	13.38
Ethanol 12^{o}C	6	7.13
10% formalin 25^{o}C	21	20.25
10% formalin 12^{o}C	35.13	28.75
Refrigeration	31	20.13
Vacuum, 12^{o}C	11.25	9.25
Vacuum, 25^{o}C	11.25	9.25
Anaerobic, 25^{o}C	30.38	27.63
Anaerobic, 12^{o}C	32	35.88

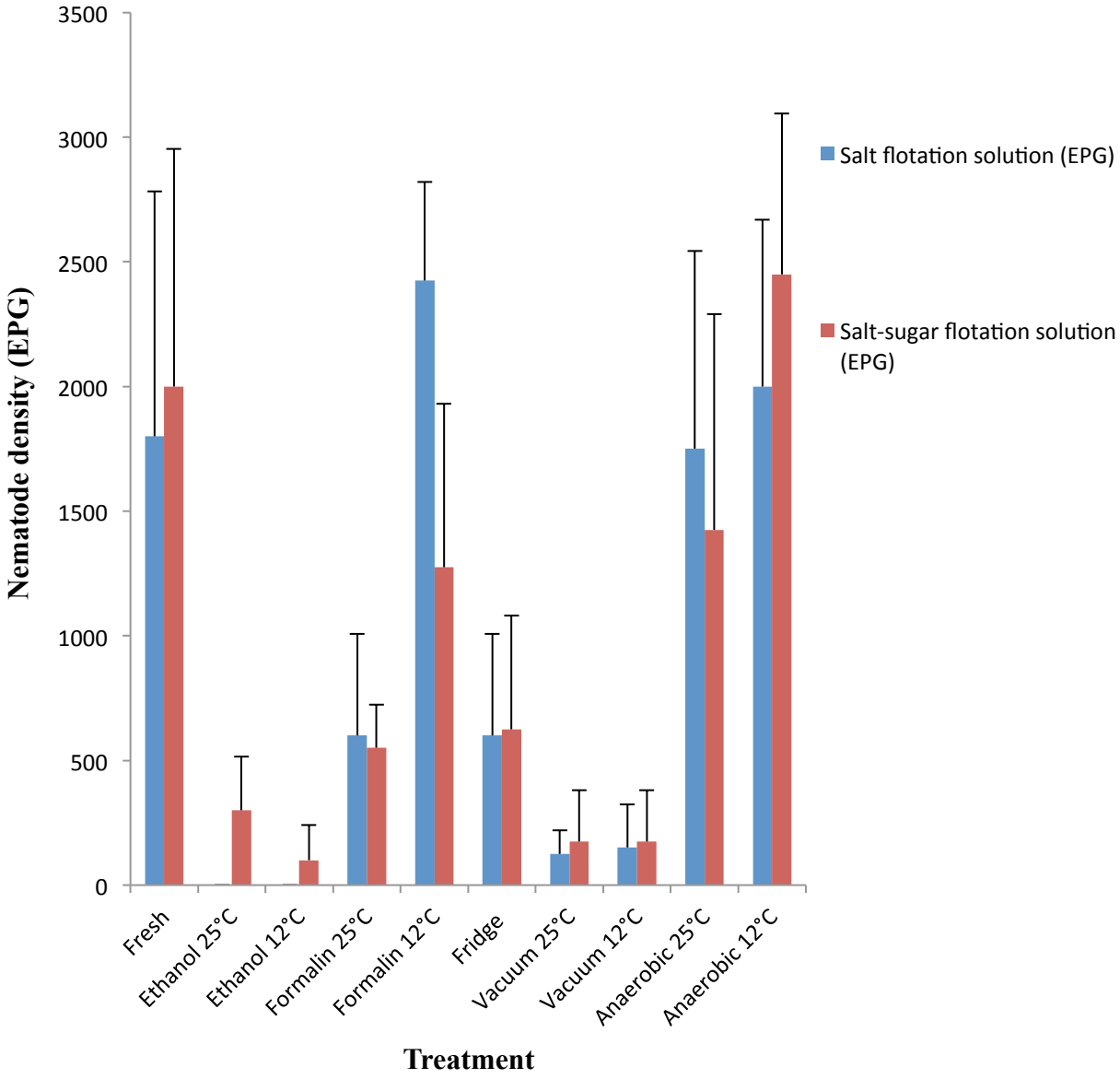


Figure: 4.1: The nematode egg count from sheep faecal samples artificially infected with *H. contortus* that underwent different treatments. Eggs counts from salt flotation and salt-sugar flotation solutions were compared. Error bars show the standard deviation. EPG = eggs per gram.

4.3.2.2 *Teladorsagia circumcinta*

The nematode faecal egg counts of *Teladorsagia circumcincta* were not normally distributed (salt; Kolmogorov-Smirnov statistic = 0.492, df = 40, $p < 0.001$, salt-sugar Kolmogorov-Smirnov statistic = 0.357, df = 40, $p < 0.001$). There was no significant difference in the nematode FEC found in faeces from artificially infected sheep, after having being subject to different treatments (Kruskal Wallis, salt; $X^2 = 10.538$, df = 9, $p = 0.309$, salt-sugar; $X^2 = 15.137$, df = 9, $p = 0.087$) (Figure 4.2).

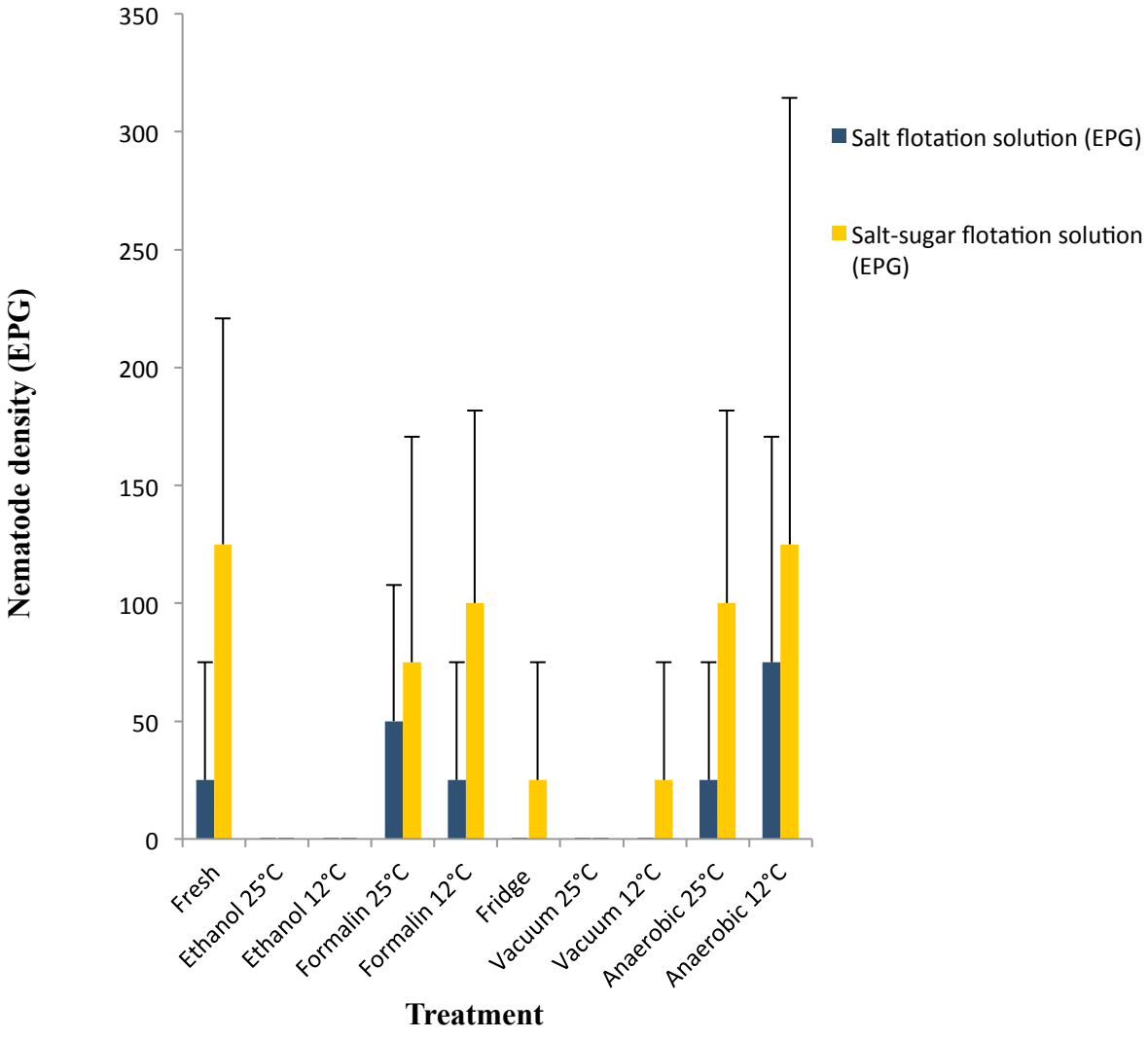


Figure 4.2: The nematode egg count from sheep faecal samples artificially infected with *T.circumcincta* that underwent different treatments. Egg counts from salt flotation and salt-sugar flotation solutions were compared. Error bars show standard deviation. EPG = eggs per gram.

4.3.3 The effect of storing elephant faecal samples in formalin on coccidial oocyst detection

Using logistic regression analysis, for every additional month of storage time, coccidial oocysts were found to be 0.874 times as likely to be detected in faecal samples (binary logistic regression: p = 0.011) (Table 4.4). The other variables in the model were age, sex, group dynamic, group size, month, season and year (Chapter 2).

Table 4.4; The output from a binary logistic regression on the effect of storage time (months) on coccidial oocyst prevalence. The other variables in the equation were sex, age, group size, group dynamic, month, season, and year. B represents the value for predicting the dependent variable from the independent variable in the logistic regression equation. S.E. represents the standard errors that are associated with coefficients. Wald represents the Wald chi-square value. The df value lists the degrees of freedom for each of the tests of the coefficient. Sig. shows the obtained P value, and OR shows the odds ratios for the predictors.

							95% Confidence Intervals for OR	
	B	S.E.	Wald	Df	Sig.	OR	Lower	Upper
Storage time	-0.135	0.053	6.405	1	0.011	0.874	0.787	0.970

4.3.4 The effect of storing elephant faecal samples in formalin on nematode ova detection

For every additional month of storage time, nematode ova were 0.831 times as likely to be detected in samples (binary logistic regression: p = 0.004) (Table 4.5). The other variables in the model were age, sex, group dynamic, group size, month, season and year.

Table 4.5: The output from a binary logistic regression on the effect of storage time (months) on nematode egg prevalence. The other variables in the equation were sex, age, group size, group dynamic, month, season, and year. B represents the value for predicting the dependent variable from the independent variable in the logistic regression equation. S.E. represents the standard errors that are associated with coefficients. Wald represents the Wald chi-square value. The df value lists the degrees of freedom for each of the tests of the coefficient. Sig. shows the obtained P value, and OR shows the odds ratios for the predictors.

							95% Confidence Intervals for OR	
	B	S.E.	Wald	Df	Sig.	OR	Lower	Upper
Storage time	-0.186	0.065	8.261	1	0.004	0.831	.732	.943

4.3.5 The effect of storing elephant faecal samples in formalin on fluke ova detection

For every additional month of storage time, fluke ova are 0.843 times as likely to be detected in faecal samples (binary logistic regression: $p = 0.002$) (Table 4.6, and Figure 4.3). The other variables in the model are age, sex, group dynamic, group size, month, season and year (Chapter 2).

Table 4.6: The output from a binary logistic regression on the effect of storage time (months) on fluke egg prevalence. The other variables in the equation were sex, age, group size, group dynamic, month, season, and year. B represents the value for predicting the dependent variable from the independent variable in the logistic regression equation. S.E. represents the standard errors that are associated with coefficients. Wald represents the Wald chi-square value. The df value lists the degrees of freedom for each of the tests of the coefficient. Sig. shows the obtained P value, and OR shows the odds ratios for the predictors.

							95% Confidence Intervals for OR	
	B	S.E.	Wald	Df	Sig.	OR	Lower	Upper
Storage Time	-0.171	0.056	9.207	1	0.002	0.843	0.755	0.941

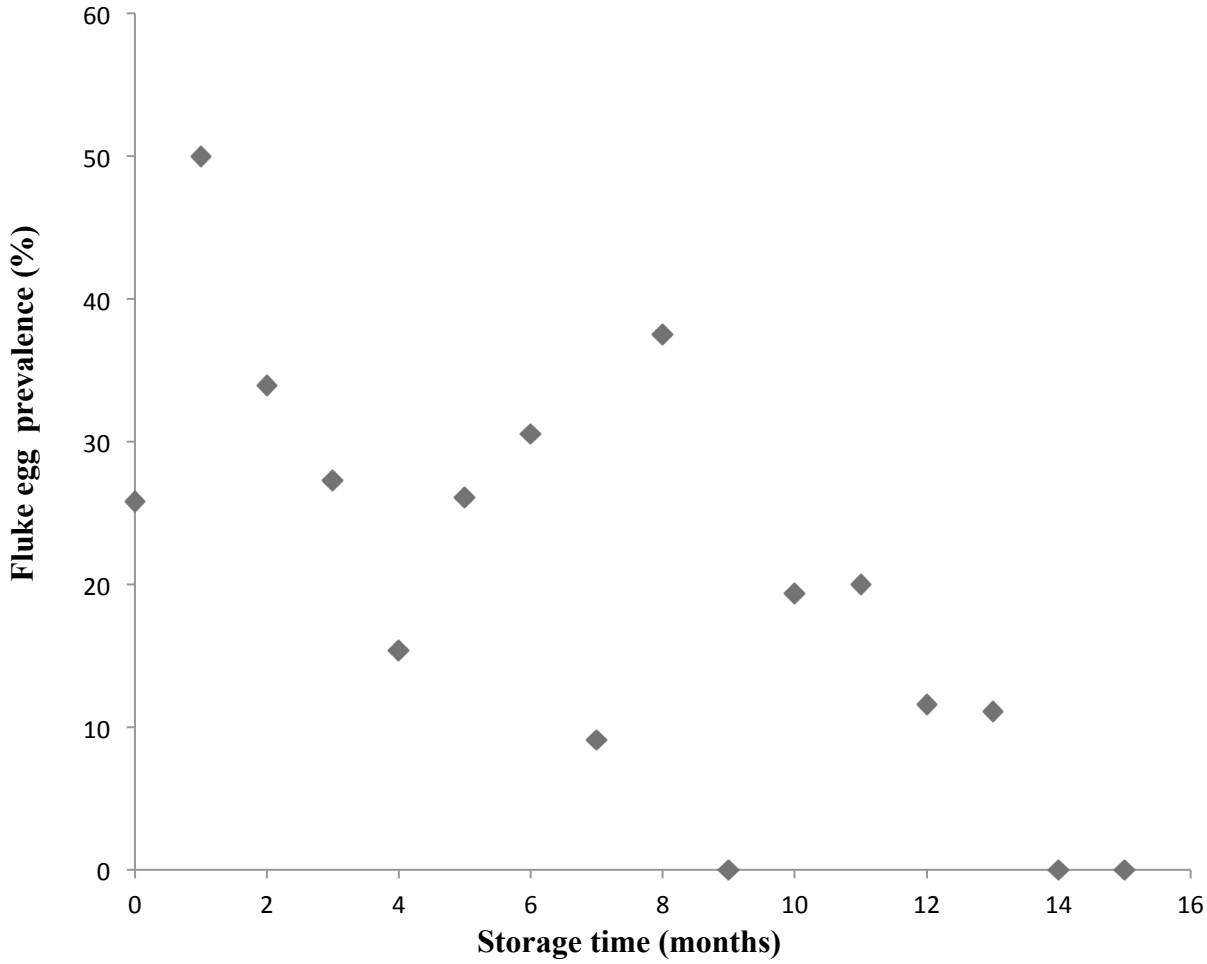


Figure 4.3: The effect of time in formalin on the average prevalence of fluke eggs, in wild elephants samples collected in the Okavango Delta between 2008 and 2012.

4.4 Discussion

4.4.1 Comparing salt and salt-sugar as flotation solutions

In the sheep faecal samples infected with *H. contortus* there was no significant difference in detected nematode egg density between counts obtained using salt and salt-sugar floatation solutions. However, in the samples infected by *T. circumcincta*, a significantly higher nematode egg count was found using salt-sugar flotation solution compared to the salt flotation solution. This suggests that the higher specific gravity of sugar-salt solution (1.28, compared to the 1.18 specific gravity of salt-solution) is aiding the flotation and therefore

detection of nematode eggs, either by allowing high density eggs that sink in salt-solution to float, or by speeding up the flotation procedure, and thereby allowing an increased detection of nematode eggs. This finding resulted in salt-sugar solutions being used throughout the fieldwork (Chapter 2) for the detection of nematode eggs in elephant faecal samples.

4.4.2 Comparing storage methods of sheep faecal samples

In sheep faecal samples infected with *T. circumcincta,* there was no significant difference in egg count between the ten different applied treatments. However there was a significant difference between applied treatments in samples infected with *H. contortus* (Figure 4.1).

In both the salt and salt-sugar flotation solutions, anaerobic conditions at 25^{o}C, anaerobic conditions 12^{o}C and formalin storage at 12^{o}C maintained fresh egg counts. This suggests that these conditions are suitable for short term storage of nematode eggs found in sheep faeces. Storage in 10% formalin at 12^{o}C had a higher mean ranking in Kruskal Wallis tests than storage in formalin at 25^{o}C for both salt and sugar flotation solutions (Table 4.1) suggesting that at this lower temperature of formalin, a more accurate egg count can be maintained. When using salt flotation solution, egg counts were also maintained after vacuum storage at 25^{o}C and formalin storage at 25^{o}C.

H. contortus egg counts after vacuum storage at 12^{o}C was significantly lower than egg counts from freshly analysed samples when either salt or salt-sugar flotation solution was used. However, this method could be improved upon by using mechanical vacuum packing, as it is unlikely that all air was completely removed from the sealable bags by hand, although the use of machines is not always practical in the field. However, a study on sheep faeces found that vacuum packing (using a domestic appliance to vacuum pack) could be used to store samples for up to 21 days without notably reducing the egg count, but not longer than this (Rinaldi et al. 2011). After storage in ethanol at both 12^{o}C and 25^{o}C, using either flotation solutions, no

eggs were detected after a week. This suggests that nematode egg storage in ethanol should not be carried out, even under controlled temperatures.

However, this study was limited, as only four samples were placed under each treatment, and due to the uneven distribution of nematode eggs in the faeces, a higher sample size would be necessary to obtain more accurate and conclusive results. Furthermore, storage in these treatments was only for one week and additional work would need to be carried out to see if the tested storage treatments have the same preservation ability over longer periods.

Further factors also need to be considered: for hand vacuum packing, fridge storage, and anaerobic storage, care needs to be taken to ensure that parasite eggs do not develop. The temperature requirements for egg hatching are species dependent, but hatching is generally halted at low temperatures, although hatching has been known to occur below 6 ^{o}C (Young et. al 1980). Sheep studies recommend keeping eggs at 4 ^{o}C or in anaerobic conditions, but these conditions are only reported to maintain egg counts for 7 days (Nielsen et al. 2009), a period often too short for field work requirements. A study on the methods of nematode preservation in horse faecal samples found that refrigeration was the optimum storage medium but also recommended anaerobic conditions. However, this study did find that anaerobic conditions did not prevent hatching of eggs in all cases (Nielsen et al. 2009). The results from these studies suggest that eggs found in faecal samples from differing host species may have different optimum storage methods, and perhaps further work is required in order to optimise egg preservation on a case by case basis.

4.4.3 Effect of formalin storage on egg and oocyst detection in elephant faecal samples

It was found that formalin at high temperatures is not an optimum storage medium for African elephant faecal samples. It was found that the majority of nematode eggs were not floating in salt-sugar solution after having been stored in formalin, and those that did float were generally disfigured. The prevalence of coccidial oocysts, nematode eggs and fluke eggs in samples significantly decreased with increased storage time in 10% formalin. This suggests that formalin may be damaging and altering the density of nematode and fluke eggs, as well as coccidial oocysts, and lowering their chance of detection.

There are a number of possible reasons why formalin did not sufficiently preserve nematode eggs from elephant faecal samples. It is advised that formalin stored samples are maintained at 4 ^{o}C (Karki, 2008), but this was not possible in the field and the samples were exposed to the temperature variances of the Okavango Delta where temperatures can exceed 41^{o}C (African Safari News, 2011) and fall below of 6 ^{o}C (Conradie, 2008). A number of studies have found that the storage of strongyles in 10% formalin does not maintain the nematode egg densities found in fresh samples (Foreyt, 1986 and Rinaldi et al. 2011), and it is possible that the nematode species which infect elephants are even more sensitive to formalin than nematode species previously studied.

Previous investigations into nematode species in African elephants have analysed fresh samples (Thurber et al. 2011) or samples stored in formalin but analysed by placing samples on slides, having been concentrated by using ethyl acetate sedimentation and Lugol iodine, rather than using flotation methodology (Kinsella et al., 2004). Therefore, if eggs from elephant parasite species are relatively sensitive to formalin storage, this may have previously gone undetected.

4.5 Conclusion

In conclusion, in order to obtain optimum nematode egg counts from parasite species infecting wild African elephants, fresh samples should preferably be examined. However, as this is not always possible then it is suggested that; 5% formalin could be used for sample storage as it is less damaging to protozoa than 10% formalin (Garcia 2005), and samples should be kept at a low constant temperature (Conradie, 2008). The results from this study suggest that even after a week, formalin storage at a high temperature (25 ^{o}C) can have a significantly detrimental effect on egg storage and detection in sheep faecal samples. However, it was also found that formalin storage at 12 ^{o}C is able to sustain *H. contortus* egg counts for a week, and therefore it is suggested that formalin could be used if storage temperatures are able to be controlled. The investigation also found that anaerobic conditions were successful for egg preservation and these could be an alternative storage treatment. However this may not be appropriate for long term storage, as it may allow egg hatching. Further work involving the treatment of elephant faecal samples with various storage methods and for varying lengths of storage time would be extremely useful in determining the optimum storage method, and thereby adding validation to future studies.

CHAPTER 5: DISCUSSION

The parasite prevalence and burden in African elephants are influenced by an intricate web of factors. Their importance however is difficult to determine as many factors may either be confounding, or working in synergy, and their effects may differ between parasite species. This was the case in this study, where it was found that the age, sex, group dynamic, group size, month and year all had a statistically significant association on parasite prevalence and density, but these effects were not standard across parasite genera.

Data collected from captive elephants allowed the short term fluctuations in detected parasite burdens in individuals to be explored, as well as providing data on the seasonal changes in parasite prevalence and density. The investigations did not exclusively focus on samples collected from elephants: the collection of faecal samples from grazers allowed a wider survey of parasites in the Okavango Delta to be carried out.

The unexpected sinking of nematode eggs from formalin stored elephant faecal samples led to an investigation into optimum storage methods, the results of which may be helpful for directing further studies.

5.1 The sample distribution of fresh wild elephant samples

Over the study, sample sizes for wild elephant sex and age had a fairly even distribution, but the following factors had more skewed distributions: Group 1 elephants had a notably higher number of samples than Group 2, although this was expected as the Group 1 bracket encompasses the majority of elephants (all females as well as males under the age of 15). The samples distribution for varying group sizes was fairly even and an outlier, at group size of 85, represents a one-off interaction with a very large herd where a high number of samples were collected. The skew of fresh sample size in month is due to the largest number of

research outings being carried out in February, and the apparent skew in season is explained by the majority of the research being carried out during the rainy season (70 research days in the rainy season, 11 research days in the flood season).

5.2 The sample distribution of stored wild elephant samples

Sample sizes for wild elephant age and month had a fairly even distribution, but sex, group dynamic and group size all had a skewed distributions. This is explained by the collection method. The majority of the faecal sample collection was carried out alongside a study that focused on bull elephants, and therefore a higher number of male samples were collected, a higher number of samples from Group 2 (this group dynamic bracket included only adult bulls) were collected and a skew towards lower group size was found, as post-pubescent male elephants are semi-solitary (Ganswindt et al. 2010). There appears to be a skew in seasonal data collection, but this is due to the dry season consisting solely of October, and as the average sample sizes for each month show, overall there was a fairly even collection throughout the study years. The difference in sample sizes between years is due to a number of factors, including varied time spent in the field, and the natural fluctuations of elephant movements (Loarie et al. 2009). The skew observed in storage time samples was due to the logistics of when samples were able to be collected, and when they were able to be sent to Bristol for anlaysis.

5.3 Significant factors affecting wild elephant parasite prevalence and burden

5.3.1 Sex

In stored samples, coccidial oocyst detection was significantly higher in samples from male elephants than from female elephants (Figure 2.18), however, in fresh samples, fluke ova

detection was higher in samples from female elephants than from males (Figure 2.17). Nematode levels were not found to be significantly affected by sex in fresh or stored samples.

5.3.1.1 Male bias in coccidia infection

Many mammal studies have found a male bias in parasitism (Schalk and Forbes, 1997), and these have been attributed to sexual dimorphism in behaviour or morphology, or by the effect of sex-specific hormones on the immune system (Zuk and McKean, 1996). In some species, female hormone, androgen, can stimulate both humoural and cell mediated immunity, whereas male hormone, testosterone, is believed to have an immunosuppressive effect (Schalk and Forbes, 1997). If this effect is present in elephants, then bulls in musth, a condition where their plasma testosterone levels rise significantly, would be hypothesised to have increased parasite levels. Unfortunately, as few musth bulls were encountered during the study, the effect of this heightened male hormonal state on parasite burden could not be investigated. However, Thurber's 2011 study in Namibia, found that musth had no significant affect on parasite burdens in bull elephants, suggesting that testosterone may not have a significant immunosuppressive effect in this species.

Males have high intra-specific competition for females, as cows are only sexually receptive for 3-6 days every 3-9 years (Hollister-Smith et al. 2007). Such competition has been found to lead to increased stress and consequently lower parasite resistance in many vertebrates (Zuk 1990, Esch et al. 1975). The extent of this competition is often location dependent: in areas exposed to elephant culling, a collapse in the normal social system is often observed and elephants can become very aggressive. In these cases, intra-specific mortality can account for 90% of deaths in the area, compared to only 6% in less stressed populations (Bradshaw et al. 2005). The area studied (NG26) had little poaching and minimal human

interference, so stress levels may not play a significant role in disease susceptibility in this case.

Non-hormone related sexual dimorphism such as group structure, range and diet in male and female elephants may also attribute to the observed pattern of male biased coccidia infections. The absence of the restrictions of herd living allows bull elephants to both interact with a number of different herds, and to travel long distances (Thurber et al. 2011), potentially increasing parasite contact. Elephants vary greatly in size, with males weighing two times the amount of females by the age of 25 (Lee et al. 1986). A positive correlation between host body weight and parasite burden has been reported in many species (Ezenwa 2006). Larger individuals can offer more space for parasites, increased target areas for vectors, and have a greater nutrition intake, therefore increasing the probability of parasite exposure (Cross et al. 2009).

Despite their larger size and greater trunk reach, it has been found that males do not feed any higher than cows and sub adults (Stokke and du Toit, 2000), and therefore both sexes have equal exposure to the high parasite levels that are found at low vegetation levels (Apio et al 2006). It has been found that when female elephants browse standing next to a family member, they fed at a significantly higher canopy level than when feeding alone, suggesting that feeding height is affected by intraspecific competition, rather than by sexual dimorphism (Stokke and du Toit, 2000).

5.3.1.2 Female bias in fluke infection

In a study by Kanyari et al. (2009), it was found that female livestock had a higher prevalence of trematode infection than males, and it is suggested this is due to the hormonal effects of pregnancy and parturition on parasite levels. However, female bias may have alternative

explanations in elephant, which are very different from livestock both genetically and in terms of the ecological systems in which they live.

It is suggested that further work investigating the association between hormone levels and parasite prevalence and burden may reveal some interesting patterns. This may help to determine the relative importance of different factors on parasite levels in the African elephant.

The different social systems of male and female elephants may affect the exposure of individuals to infectious larval stages. Females remain in matriarchal herds for life, and spend the majority of their time within four metres of another individual (Archie et al., 2006). These high contact levels could assist the transmission of fluke.

It is likely that a number of confounding variables are influencing parasite prevalence, but these factors may be of differential importance for different parasite species which could explain the pattern seen here. As there is little evidence of the deleterious effects of the investigated parasites, we do not know the extent of the selection pressures, if any, on the host. Although we can speculate, before we can truly decipher the effect that factors such as sex have on elephant parasite levels, it is necessary to further determine the relationship between the African elephant and its metazoan and protozoan parasites.

Duneau and Ebert (2012) suggest that differences between host sexes in traits such as morphology and hormone levels can impose selection on parasites, which could lead to parasite adaptations specific to the host sex most commonly encountered, as well as leading to differential expression of parasite traits depending on the host sex that they find themselves in. Host sex adapted parasites could contribute to differences between males and females in disease expression and prevalence. Further research on host-parasite relationships in the elephant is necessary to determine whether this theory may be applicable to this system.

5.3.2 Age

It was found in formalin stored samples that fluke egg incidence increased significantly with elephant age. However, age did not play a significant role on the incidence of coccidia or nematode.

The elephants in this study do not appear to be acquiring parasite immunity with age. Similarly, a study on wild elephants in Namibia found that within family groups, nematode burden increased with age (Thurber et al. 2011), and this was attributed to older elephants eating more, and therefore being exposed to a greater number of parasites. Young elephants may be receiving immunity from their mother's milk, and as elephants can suckle for up to eight years (Lee and Moss, 1986), this protection may enhance the observed pattern. Furthermore, senescence can have a negative impact on the immune system, and lead to increased parasite levels in elderly individuals (Masoro and Austad, 2010). It is only a recent innovation that senescence is not restricted to humans or domesticated species, and is in fact seen in a wide range of wild living species (Palacios et al. 2007).

An increase in fluke egg prevalence with age could also be caused by a combination of low infection related mortality rates, and failure of the elephant immune system to fully eliminate infection from the body. This is the case in Bovine Tuberculosis infections in the African buffalo, where hosts rarely recover from infections, and an increased parasite burden is seen with age (Jolles et al. 2005).

There could also be a number of unknown factors in the fluke lifecycle that have caused the observed increase in fluke ova detection. For example, the fluke species *Dicrocoelium dendriticum,* requires an ant for certain developmental stages, and alters the behaviour of infected ants, causing them to climb to the tops of vegetation during the day, and return to ground level at night (Carney, 1969). If the elephant fluke found in this study had a similar

lifecycle, then older and therefore taller elephants may have an increased exposure to ants that are dwelling in raised vegetation, during the day. Although this scenario is purely speculative, it makes the point that, as so little is known about the fluke species that infect elephants and their respective lifecycles, important factors may currently be overlooked.

5.3.3 Month

The Okavango Delta may provide a nurturing environment for some parasite species, as egg and larval development often require high temperature and high moisture levels (Altizer et al. 2006) and these remain relatively high all year round in the Delta.

The only parasite genera that was found to be statistically significantly affected by month was coccidia. In the fresh samples analysed from 2012, coccidial oocyst prevalence was significantly higher in Fehruary, than in March and oocysts were not present in any of the samples collected in April. In formalin stored samples, collected between 2008 and 2012, coccidial oocyst prevalence was significantly lower in the October months than in the months of January and February. October is one of the driest and hottest months of the year, with temperatures reaching 35 ^{o}C and above (Ramberg et al. 2006). Although coccidia oocysts require elevated temperatures for sporulation to occur, temperatures too high can lead to oocyst dessication (Becker and Crouch, 1931). Low levels of rain and ground water in October are also likely to be limiting the deveopment and transmission of oocysts (Waldenstedt et al., 2001). In contrast to the dry October, January has been found to have the highest average monthly rainfall in the Delta (Mendelsohn and Obeid, 2004), potentially explaining oocyst prevalence. The significantly higher detection of coccidial oocyst in elephant faeces during this month.

The data collected between 2008 and 2012 show a noted drop in coccidial oocyst prevalence between February and April (Figure 2.20). This drop coincides with a significant ebb in average rainfall, a correlation that has been found in many parasite studies (Vercuysse, 1983, Ayele et al. 2006, Sissay et al. 2007). The data also shows that parasite prevalence levels start to rise again in mid-April, coinciding with the arrival of the floods (Figure 2.21). The high ground water levels seen in April force elephants to congregate on the few remaining dry islands, increasing host density and thereby increasing the probability of parasite transmission (Arnenberg 2002). The oocyst prevalence remains high between May and July, coinciding with the time of the highest mean flood output levels (Figure 2.21). It should be noted that the ground water levels during the flood season are much higher than during rainy season.

The coccidial oocyst prevalence is seen to decrease again towards the end of July, coinciding with a drop in relative humidity levels and a rise in the daily maximum temperature (Figure 2.20). Very high temperatures can lead to oocyst dessication (Becker and Crouch, 1931), and decreased humidity levels can inhibit the transmission of oocysts (Waldenstedt et al., 2010). The coccidial oocyst levels rise again, coinciding with the arrival of the rains in September (Figure 2.21 and Figure 2.22).

It appears that rainfall is the best predictor of coccidial oocyst levels in the population over time (Figure 2.20), but that other environmental factors such as temperature and relative humidity may also be playing a part. Until more is known about the lifecycles of the parasites infecting wild elephants, it is hard to predict the effects that annual and climatic change will bring about.

5.3.4 Year

Wild elephant faecal samples from 2010 had a significantly higher coccidial oocyst prevalence than 2009. However, samples from both 2011 and 2012 had significantly lower coccidial oocyst prevalence than samples from 2009.

2010 received much attention for being a 'super flood' year, having one of the highest ground water levels seen in the Okavango Delta in 50 years (Ives, 2012). Rainfall can remain in the Delta system for over two years (Ives, 2012), and these extreme levels in 2010 were exacerbated by unusually high rainfall in 2009. These high water levels resulted in increased elephant herd aggregation on the remaining dry islands, thus increasing host density, an important factor in parasite transmission (Arnenberg 2002).

Relatively low flood levels were seen in 2012 (Ives 2012), allowing elephants increased access to dry land, therefore potentially decreasing host density and potentially explaining the observed low coccidial oocyst dessication. Coccidial oocyst levels in 2011 were significantly lower than those in 2009, despite the flood levels in 2011 being almost as high as those in 2010 (Ives 2012). This suggests that flood levels may not be the determining factor in predicting coccidial oocyst levels, and other factors such as humidity, rainfall, and temperature variances may be affecting coccidia transmission dynamiccs. Other factors that were not studied, for example, elephant movement patterns, may also have played a part in affecting coccidia spread and prevalence.

5.3.5 Group size

Elephant group sizes varied greatly in the study, ranging from between 2, to over 100 individuals. Overall, it was found that the prevalence of nematode infection increased with group size. This positive correlation has been found in a meta-analysis on population density

in mammals (Arnenberg 2002), and in a social species meta-analysis that investigated the relationship between contagious parasites and group size (Cote and Poulinb, 1994).

The rate that the environment is contaminated by parasite eggs is positively correlated with the number of parasitised individuals in the population (Thurber et al. 2011). As larger herds have an increased probability of including infected individuals, it would be expected that big herds have a high environment contamination rate, potentially resulting in higher parasite levels.

This host-density effect on parasite incidence may be exacerbated by the high water levels in the Delta, which force elephant group members to cluster together on dry 'islands'.

5.3.6 Group dynamic

Members of family groups (Group 1: females and males under the age of 15), had lower nematode egg density than males over the age of 15 (Group 2) in the fresh elephant faecal samples studied. Post pubescent males travel further distances than females and younger males (Thurber et al. 2011) although determining how this may affect their exposure to parasites would require further study, taking into account possible confounding variables such as age, stress and immunity which larger movement patterns could be an indicator for. Testosterone levels increase significantly once males leave the herd and remain high throughout their post-pubescent life (Mc Neilly et al. 1983). These high levels may be having an immunosuppressive effect, and causing the observed higher nematode egg density in post pubescent male elephants. This pattern was contradictory to that found in Thurber's study (2011), where members of the matriarchal group had a higher strongyloid burden than solitary bull elephants, suggesting that different environments can change the relative importance of influencing factors on parasite levels.

5.4 Parasites of the Abu herd

5.4.1 Coccidia

At the start of the captive herd sample collection (21st January 2012), the six members of the Abu herd (excluding the newborn Warona) were all infected with coccidia. However, from the 17th of March onwards, coccidial oocysts were no longer found in any of the faecal samples collected. However, the negative correlation between coccidial oocyst prevalence and number of days after the 21st January was only significant in one of the elephants, Abu. However, as sample sizes for herd individuals was fairly low, this could explain the absence in a significant result in the other members of the herd.

The results from Abu support the findings from wild elephants where, in the fresh samples, coccidial oocyst prevalence was significantly higher in February, than in March 2012. Coccidiosis was also absent from all the fresh wild samples collected in April as well as in all captive samples, suggesting that the prevalence of coccidiosis is seasonal, and drops between the rainy and flood seasons.

5.4.2 Nematode

Between 21st January and 11th April 2012, the nematode egg densities of Sherini and Kittimetsi significantly increased. There was no significant change in the nematode egg densities in any of the other captive herd members. No significant change was found in the nematode egg densities of wild elephants during the study period, but as the captive Abu herd do not have the same lifestyle as wild elephants, this is perhaps not surprising. The Abu herd individuals have much closer contact than wild elephants, as they are contained in a Boma at night, and all the individuals are generally encouraged to remain close to each other in the daytime. The Abu herd also frequently re-visit the same areas and bathing spots, so it is likely

that their parasite prevalence and patterns do not represent those of wild elephants. Their annual worming with Bimectin may also have disrupted the natural pattern of parasite transmission, although the elephants were not de-wormed in the year of study.

5.4.3 Fluke

No fluke eggs were found in any of the captive Abu herd at any stage during the study. As afore mentioned, due to snails being the obligatory secondary host for fluke development, fluke may be limited in the environment. As the Abu herd have restricted movements and their interactions with wild elephants are kept at a minimum (wild elephants are actively discouraged away from areas where the Abu herd graze and drink), this may explain the absence of fluke.

Furthermore, steps are taken to reduce parasite transmission in the Abu herd, including: clearing the dung daily from the Boma, and worming the herd in the January of each year with Bimectin. However, the herd was not wormed in the year of study so this may not explain the absence of fluke.

5.4.4 Warona observations

The youngest member of the Abu herd was a female calf, Warona, who was one month old at the start of the study (January 2012). Warona, suckled from the matriarch of the herd (Cathy), as well as from her mother (Sherini). Such behaviour may allow the calf to both gain extra milk, as well as possible immunological benefits (Roulin and Heeb, 1999, Cross et al. 2009). Although Warona's main source of nutrition was milk, towards the end of the study period she began to pick up plant material and put it to her mouth. However, this behaviour was only observed occasionally, and she would still rarely ingest the vegetation. Therefore it is likely that her parasite exposure was less than that for the rest of the herd. However, another

possibility is that Warona was host to parasite species, but the methodology used failed to pick up this presence, as can be the case, especially with low egg counts.

5.4.5 The morphological overlap of parasite species in elephants and grazers

The morphological investigation into nematode and fluke eggs found in elephant and grazer faecal samples was restricted due to the similarity of parasite eggs from different species. As parasite eggs are often alike in morphology, genetic analysis is often required in order to differentiate between species. Although it was not possible to conclusively decipher whether elephants and grazers were sharing nematode species in this study, this possibility cannot be ruled out due to the overlap of morphological data from the different host species. This overlap does, however, suggest that genetic investigations may indeed by worthwhile.

The data from fluke in these different host species, however, was more conclusive. Although it was impossible to determine whether the grazing species were sharing the same fluke species, it can conclusively be said that grazer species were not sharing fluke species with the elephants studied, due to their obvious difference in morphology. The fluke eggs found in elephant faeces were unique to the elephants, and were very distinctive. These eggs bore a close resemblance to eggs from *Protofasciola robustra*, a fluke known to infect African elephants, and the fluke eggs found in grazer faecal samples were morphologically similar to described Fasciola eggs, species of which are known to be found in African mammals. However further genetic or autopsical work is necessary to confirm this.

5.5 Limitations to faecal egg count methodology

There are a number factors that need to be taken into account when analysing the results of faecal egg counts: if no eggs are found in collected faecal samples, this does not guarantee that the host is free from infection, as eggs may be missed or parasites may be present in

immature or non identifiable stages. The number of eggs found in a sample does not necessarily correlate with the number of adult worms present in the host, as is often the case in nematode infection in cattle (Hansen and Perry, 1994). Factors that could disrupt the relationship between worm burden and egg count include; the presence of immature worms that are not yet producing eggs, regular fluctuations in faecal egg output, uneven distributions of eggs throughout the faeces, and host resistance reducing or inhibiting parasite egg production. The amount of faeces produced will alter the number of eggs per unit weight and some species of parasite will produce more eggs than others. The density of eggs may also differ, changing their ability to float and therefore their conspicuousness in faecal examinations (Good et al. 2004).

The faecal egg count methodology was further limited in this study due to the sinking in flotation solution of nematode eggs from elephant faecal samples after storage in formalin. This was unexpected but was overcome by changing the nematode detection methodology, although this meant that only parasite prevalence, rather than densities, could be studied for the stored samples. It was also found that increased time in formalin led to decreased detection for coccidia and fluke species. This led to the assumption that formalin may not be the optimum storage method for elephant faecal samples. The study on short term sheep faecal storage suggests that formalin may not function best as a preserving liquid at high temperatures. This may help to explain the observed pattern in ova detection in elephant faecal samples as, in this study, samples were stored at uncontrolled temperatures, some of which were very high.

5.6 Conclusion

It was found that a wide range of factors had a significant association with parasite presence and density in wild elephants in the Okavango Delta including: sex, age, group dynamic, group size, month and year. A significant effect of month on parasite prevalence was also found in captive elephants.

Although elephants of the Okavango Delta do not appear to be sharing the same fluke species as sympatric grazers, further genetic work is required to determine whether elephants and grazers are sharing the same nematode species.

The study on parasite ova found in both elephant and sheep faeces illustrated that 10% formalin has some limitations as a storage medium, and may not be the optimum long term preservative for eggs, especially at high storage temperatures.

The broad results of this study will provide a base from which further work can be directed, as well as providing an insight into the under-researched area of African elephant parasites in the unique ecosystem of the Okavango Delta.

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