DETECTION OF *LEPTOSPIRA* AND SEASONAL PREVALENCE OF FLEAS COLLECTED FROM SMALL MAMMALS IN MUKWE CONSTITUENCY, KAVANGO EAST REGION OF NAMIBIA

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Abstract

Biotic and abiotic factors can alter the abundance and community composition of mammals and in turn that of associated parasites. The aim of the study was to measure the impact of climatic variables (temperature and relative humidity) on mammal populations and on the population dynamics of associated micro- and ecto-parasites. For this, the current study described prevalence and diversity of pathogenic *Leptospira*, the etiological agent of leptospirosis, and fleas, arthropod vectors of medical importance in mammals sampled over a period of 11 months in the Kavango East region of Namibia.

In total, 121 small mammal hosts were examined for *Leptospira* using a molecular method (Multilocus sequence typing method) and yielded an overall prevalence rate of 9.9%. However, only a single *Leptospira* species could be identified as *Leptospira kirschneri*, on one host species (*Saccostomus campestris*). Throughout the study period, almost all mammal species harboured the same flea species, which included *Cryptonella numae* (1.8%), *Pulex irritans* (43.0%), *Pariodontis riggenbachi riggenbachi* (12.9%), *Synosternus caffer* (3.9%) and *Xenopsylla* species (38.3%).

Using the Kruskal-Wallis test, the monthly population fluctuation of fleas varied significantly on *M. natalensis* (χ^2=29.440, df=10, P=0.001), *S. pratensis* (χ^2 =30.521, df=10, P=0.001) and *S. campestris* (χ^2=32.681, df=10, P=0.0001). However, no significant difference in the number of fleas per month was found for *G. leucogaste*r (χ^2=10.831, df=10, P=0.371). The Pearson correlation test revealed that fleas abundance on small mammal hosts was weakly positively correlated with temperature (r=0.3, df=9, N=11, P=0.310) and strongly positively correlated with relative humidity (r=0.6, df=9, N=11, P=0.109), this same comparison for fleas showed a weak positive correlation (r=0.4, df=9, N=11, P=0.224). Lastly, there was a very strong positive correlation (r=0.8, df=9, N=11, P=0.0058) between average abundance of small mammals and average abundance of fleas. Therefore, climatic variables (temperature and relative humidity) were found to have an influence on the abundance of small mammals and fleas.

List of Publication(s)/Conference(s) Proceedings

Presentation of the thesis result at a small mammals conference in **Arusha, Tanzania**

1. Title: Prevalence of *Leptospira* and fleas in small mammals from Mukwe constituency, Kavango East Region of Namibia

   Presented by: Ms. Saima Kapia (University of Namibia)

2. Prevalence of haemoparasites on small mammals in Fallow land fields within Mukwe constituency, Kavango East Region, Namibia.

   Author of the presentation: Ms. Saima Kapia (University of Namibia)

   Presented by: Dr. Seth J. Eiseb (University of Namibia)

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1. Title: Prevalence of haemoparasites on small mammals in fallow-lands within Mukwe Constituency, Kavango East Region, Namibia

   Author of the presentation: Ms. Saima Kapia (University of Namibia)

   Presented by: Dr. Seth J. Eiseb (University of Namibia)

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List of Abbreviations and/or Acronyms

µm:	Micrometre
° :	Degree
°C:	Degree Celsius
‘:	Minute
“:	Second
cDNA:	Double stranded DNA synthesised from a single RNA
CRVOI:	Centre de Recherche et de Veille sur les Maladies Emergentes dans l'Océan Indien
df:	Degrees of freedom
DRC:	Democratic Republic of Congo
E:	East
FAFLP:	Fragment length polymorphism analyses
g:	Gram
h:	Hour
H’:	Diversity index
i.e.:	In this instance
ITCZ:	Inter Tropical Convergence Zone

K-S:	Kolmogorov-Smirnov test
Kg:	Kilogram
KEr:	Kavango East region
Km2:	Kilometre square
LPS:	Lipopolysaccharide
MET:	Ministry of Environment and Tourism
mm:	Millimetre
MLST:	Multi Locus Sequence Typing
MLVA:	Multilocus variable number of tandem repeats analyses
NSA:	Namibia Statistics Agency
NPC:	National Planning Commission
PCR:	Polymerase Chain Reaction
P :	Probability
r:	Correlation coefficient
rDNA:	Ribosomal deoxyribonucleic acid
S:	South
Sp.:	Refers to a single species
Spp.:	Refers to two or more species
SPSS:	Statistical Software Package for Social Sciences

SASSCAL:	Southern African Science Service Centre for Climate Change and Adaptive Land use
StopRats:	Sustainable Technologies to Overcome Pest Rodents in Africa Through Science

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Dedication

I dedicate this thesis to my parents Elizabeth Martin and Tomas Kapia for laying and initiating the foundation of my education and for being my pillars of strength. I also dedicate this work to my beloved Aunt Saara Helvi Shaanika and my siblings: Bertha Kapia, Aron-Jocks Kapia, Michelle Lucky Kapia, Elizabeth Ndapandula Ndahambelela Kapia and Henny-Namawe Kapia for their prayers, patience and encouragement.

Declaration

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Date……..

Chapter 1

1. Introduction

1.1 Diversity of symbiotic interactions

Leung and Poulin (2008) defined symbiosis as the relationship between two or more organisms of different species living in close physical proximity, and includes mutualism, commensalism and parasitism (Martin and Schwab, 2012). In a mutualistic relationship, both organisms benefits from their co-existence. Benefits can be, for example, increased fitness of the host organism provided with nutritional needs (Martin and Schwab, 2013; Duron *et al*., 2014) or protection against pathogen infections (Teixeira *et al*., 2008), and, in return, the host can provide shelter (Leung and Poulin, 2008) and/or several other biological functions.

Alternatively, the interaction may involve one species gaining benefits while the other species is not significantly affected (commensalism). Parasitism is the last form of symbiosis, where by one organism (symbiont) benefits at the cost of the other organism (host) (Martin and Schwab, 2013). This association may have negative effects on life expectancy, reproduction and other general traits of the host (Krasnov *et al*., 2004; Bazen-Leon *et al*., 2013).

Parasites may live in or on the body or cells of the hosts, which are often harmed to some extent by the association (Krasnov *et al.*, 2004; Poulin, 2007). Thus, parasites depend partially or fully on their hosts for survival and reproduction (Bazen-Leon *et al*., 2013) while playing important roles in the biology of their hosts by influencing the dynamics of host communities. Parasites can be of importance from a medical and/or veterinary perspective (Bazen-Leon *et al*., 2013) because of their ability to be

harmful pathogen or carry pathogens. The diversity of these organisms (parasites) includes microbial organisms (viral, prokaryotic or eukaryotic), which can be pathogenic to their hosts (Bazen-Leon *et al*., 2013) arthropod ectoparasites that are key partners as biological vectors of a variety of arthropod borne infectious agents potentially pathogenic for humans and domestic animals (Minard *et al*., 2013; Guernier *et al*., 2014; Dieme *et al*., 2015).

Over evolutionary time, the pathogens and hosts have evolved mechanisms to cope or avoid adverse effect on their coexistence (Combes, 2001). However, the results between them may differ depending on the following factors concerning the host; pathogen concerned, age, gender, diet and the physical environmental effects (Bush, Fernandez, Esch and Seed, 2001). According to Combes (2001), one of the end effects of parasitic pathogens on the host can occur when the parasite influences the host genome, which then results in phenotype expression. Another effect occurs when there is DNA exchange between the hosts and micro parasites, which have been increasingly reported (Fort *et al*., 2012) and is best exemplified between bacterial mitochondria and eukaryotic cells (Bush *et al*., 2001). In addition, pathogens have been reported to alter the physiology and the behaviour of their host in a manner that favours the survival of the pathogenic parasites (Bush *et al*., 2001). Further on pathogens avoid host immune response by avoiding detection or by copying (mimicking) the host cell macromolecules or making their own antigens with the host macromolecules (Fort *et al*., 2012). Consequences of pathogens on the host also include direct suppression of the host immune response or apoptosis (programmed cell death) once the pathogens have invaded the host body (Luder, Gross and Lopes,

2001). Alternately, they can induce or inhabit intercellular survival like the case between e.g. *Cryptosporidium parvum* and *Taxoplasma gondii* (Luder *et al*., 2001).

In response to this, the host counteracts to invading pathogens by firstly, resistance and secondly via immune response that result in infectivity or response that leads to the death of the pathogens.

Mammals and associated parasites: complex interactions strongly influenced by biotic and abiotic parameters.

The study hereafter describes the three components that were studied in the frame of the project: pathogenic *Leptospira*, fleas and their mammal hosts.

Pathogenic *Leptospira*

Leptospirosis is an emerging infectious disease that is caused by gram-negative bacteria in the genus *Leptospira* (Vijayachari *et al.*, 2008; Brown *et al*., 2011). Leptospires can either be pathogenic (having the potential to cause disease in animals and humans) or non-pathogenic (free-living and generally considered not to cause disease). Leptospirosis is considered as the most common bacterial zoonosis in the world (Smythe *et al*., 2002; Bharti *et al*., 2003; Machang'u, Mgode and Mpanduji, 2012; Guernier *et al*., 2014; Dieme *et al*., 2015; Gomard *et al*., 2016). The disease causes over one million cases per year, of which over 10,000 severe cases require hospitalization (Viayachari *et al*., 2008; Costa *et al*., 2015). All mammals are prone to infection by the bacteria. However, domestic animals and rodents are the source of infection to humans, both directly and indirectly (Holt, Davis and Leirs, 2006; Villanueva, Ezoe and Baterna, 2010).

Tropical and subtropical countries shoulder the heaviest burden of leptospirosis (Bharti *et al*., 2003), most probably because *Leptospira* excreted by reservoir animals in the environment (grass, surface water, muddy and swampy area) meet the warm

and humid conditions likely conducive to bacterial maintenance (Vijayachari *et al*., 2008; Gomard *et al*., 2016; Balassiano, Vital-Brazil, Ramos, Timm and Pereira, 2017). Although the disease has been poorly investigated in continental Africa, an increasing body of studies suggests that leptospirosis severely affects Africa and threatens global public health (Gomard *et al*., 2014; Mgode, Mbugi, Mhamphi, Ndanga and Catterill, 2014; Dobigny *et al*., 2015).

Currently, Lipopolysaccharide (LPS) is the major antigen used in serological classification of the bacteria. Pathogenic leptospires are distinguished from one another based on the antigen since each serovar has a unique antigen make-up. Two stains of leptospires are said to belong to different groups if after cross absorption with adequate amount of heterologous antigen, more than 10% of homologous titre regularly remain in at least one of the two tested serovars in repeated test. Serovars have different degree of host specificity (Holt *et al.*, 2006). Obiegala *et al*. (2016) reported that in Germany rodents were tested positive for *L. kirschneri*, in that study, the bacteria did not affect rodents since they are the reservoir host but infection resulted in diarrhoea and dehydration in end host such as humans (dead end hosts because, these are species that do not significantly participate in the natural cycle of the bacteria). A molecular classification has been more recently developed and is increasingly used by the scientific community (Ahmed *et al*., 2010). Using the sequence of one or a limited number of housekeeping genes, the genus *Leptospira* can be divided into 10 pathogenic genospecies. It has been recently shown that some *Leptospira* are strictly specific to their hosts, in which case *Leptospira* species diversity in a given environment is strongly influenced by the local community composition of mammals (Dietrich *et al*., 2014; Gomard *et al*., 2016).

Moreover, rodents have been reported to be efficient reservoirs of pathogenic *Leptospira* (Smythe *et al*., 2002; Desvars *et al*., 2011) because of their high diversity, opportunistic life history, and high reproductive potential and periodically high densities in most environmental settings. Rodents have been further reported to be prolonged carriers of the bacteria, because of their ability to shed it throughout their life span without developing clinical symptoms (Desvars *et al*., 2011).

Leptospira interrogans is often reported as the species infecting humans and it is associated with murid rats (sub-family Murinae, which includes common house hold rats and mice and they live in close association with humans) but recent investigations report that other species may be dominant in other environmental settings (Bharti *et al*., 2003; Dietrich *et al*., 2014). The diversity of leptospires bacteria in a given area is influenced by the local geography, climate, interaction with other species and human activities (Bharti *et al*., 2003), as well as aspects such as pH, temperature, nutrients and other sympatrically occurring organisms (Gomard *et al*., 2016).

Fleas

Fleas are diverse and highly adapted group of insects that inhabit the external body surface of warm-blooded animals (Hubbart Jachowsi and Eads, 2010; Bazan-Leon *et al.,* 2013). They are placed in the order of Siphonaptera (Krasnov *et al*., 1997; Krasnov *et al*., 2004; Shihepo, Eiseb & Cunningham, 2008; Hubbart *et al*., 2010; Bazan-Leon *et al*., 2013). The order is composed of 34 genera and for which about 110 species are recognized from Southern Africa (Segerman, 1995; Shihepo *et al*., 2008). Fleas are wingless insects that suck blood from warm-blooded animals (Shihepo *et al*., 2008). However, they are mostly abundant and diverse on small burrowing mammals such as rodents (74%) (Krasnov *et al*., 2004; Shihepo *et al*., 2008).

In addition, they are vectors of a number of arthropod borne pathogens that cause the following diseases: Lyme, Ehrlichiosis, tapeworm infections, Rocky Mountain spotted fever, cat scratch fever and plague (Meerburg, Singleton and Kijlstra, 2009). Plague is an important threat in certain portions of the world to public health, as it can re-emerge in some areas after dormant periods, such as Madagascar, Democratic Republic of Congo (DRC), Mozambique, Uganda and Tanzania, North and South America, Central Asia and South East Asia (Makundi *et al*., 2008).

Fleas are found on animal hosts, where their abundance and species composition differ depending on the host species distribution, host habitat, season and insect stage, and can often be found in the host's nest or burrow system (Krasnov *et al*., 1997, 2004; Makundi *et al*., 2015). Small mammals nest material is important because of the ectoparasite communities that can be found in the burrows, depending on the material of the burrow. With the aid of radio tracking on small mammals, the burrow ecology of *Parotomys brantsii* (Whistling rat) indicated that normally, without anti parasitic treatment, they stay in a single burrow for 2 days (Roper, Jackson, Conradt and Bennett, 2002). Once, the anti-parasitic treatment is applied to the rats, they were reported to stay longer in one burrow chamber. From this study, movement from one burrow to another within a single burrow system was found to be influenced by ectoparasites mainly flea species *Xenopsylla eridos* (Roper, Jackson, Conradt and Bennett, 2002). Since small mammals should have a burrow that protect it from predators and provide protection against extreme environmental elements such as temperature, this result in burrows that foster for parasite prevalence and transfer of this parasite from one host to another. That in turn influences the frequency of the burrow usage resulting in them either staying for a while or longer in a burrow (Wells, Lakim and Pfeiffer, 2006).

In some cases, certain species of fleas tend to only occur on certain host families or genera and have mechanisms or adaptations to cope with host immune response or other biological factors (Krasnov *et al*., 2004; Makundi *et al*., 2015). Generally, fleas can locate potential host animals by detecting warmth, air movement, shadow, vibration or the odour of their host (Makundi *et al.*, 2015).

The occurrence of certain fleas on a wide range of host species is usually advantageous within a specific habitat to the flea because, it enables the flea to have a lot of different host to choose. Movement of the host as burrow visitation of the same or different species can facilitate dispersion of the fleas (Laudisoit *et al*., 2009). The spatial distribution of fleas has been the subject of a number of studies but still much of the abiotic and biotic variables shaping the distribution of these insects are currently unknown (Krasnov *et al*., 2004).

Small mammals

Small mammals are defined by several authors based on body weight: Dutton (1995) defined them as non-flying mammal species that weight 1 kg; Fleming (1979), defined them as those mammalian species that weight less than 5 kg when adult; Delany and Happold (1979) & Linzey and Kesner (1997) referred to small mammals as those mammalian species with maximum body weight of up to 120g or below 200g (Linzey and Kesner, 1997). Herein, the present study follow the definition by (Linzey and Kesner, 1997) that defines small mammals as those non-flying mammalian species with a maximum weight of 200g, when adults this gives both rodents and shrew an equal chance to be included in this study.

Ecologically, small mammal species occupy a wide range of habitat, where they can be solitary, occurring in pairs or social (Kingdon, 1997; Skinner and Chimimba, 2005). In terms of diet, they comprise species that are omnivorous or specialized few

species that are insectivorous (Fiedler, 1994; Kingdon, 1997). Due to their small size, both flying and non-flying mammalian species have been considered as small mammals, despite both groups having notable anatomical and ecological differences. The small mammal diversity includes rodents and shrews.

Mulungu *et al.* (2008) stated that within different ecosystems small mammals serve numerous ecological functions. Firstly, they are consumers, they feed on several organisms both plants and animals in the ecosystem and secondly, they can be prey to carnivores or omnivorous mammals, avian and reptilian predators. They are further efficient seed dispersers and important habitat bio-indicators, as their population dynamics respond to changing environmental and habitat shifts (Hoffmann and Zeller, 2005; Mulungu *et al*., 2008).

Apart from the beneficial effects of small mammals within ecosystems, they are also efficient disease transmitters to humans, because of their high reproductive potential that ensure high densities in different environmental settings. Small mammals have the ability to invade human dwellings and become problem animals as they contaminate their food, damage personal belongings and the agricultural fields (Mulungu *et al*., 2015). Where they threaten global food security because of the damage that they cause to plants and stored food grains (Mdangi *et al*., 2013; Swanepoel *et al*., 2017).

Small mammals are a highly diverse group, with the order Rodentia being the largest living of extant mammals and composed of over 2000 species (Kingdon, 1997; Wilson and Reeder, 2005). Within the order, a considerable diversity exists and it ranges from typical rodents to larger mammals. These different types of Rodentia are characterised by rootless and ever-growing upper and lower incisors, followed distally

by an open gap between the teeth (diastema), then premolars and finally molars (Wilson and Reeder, 2005). In addition, in general members of the order are highly adaptable and in many cases with broad distributions. For instance, *Mastomys natalensis* is a pest occurring in agricultural fields of Eastern and Southern Africa (Mulungu *et al*., 2015). They also play an important role as reservoir hosts of several human and animal pathogens (Krasnov *et al*., 2004; Katakweba *et al*., 2012).

The distribution of species is directly related to both biotic and abiotic factors (Massawe *et al*., 2012). For instance, Hoffmann and Zeller (2005) explained that within the Nama Karoo, vegetation cover influenced the abundance of small mammals due to disturbance caused by overgrazing in communal farms by domestic animals. Additional biotic factors include aspects such as competition, predation and diseases. Abiotic factors include habitat fragmentation, modification, loss and changes associated with climatic conditions (Massawe *et al*., 2012). Since, all this factors aid in the distribution of parasites and other animals. The present study aims to investigate the diversity as well as the temporal and spatial dynamics of a tripartite pathosystem composed of (i) ecto-parasites (fleas) and (ii) micro-parasites (i.e. *Leptospira* spp.) of medical importance in association with (iii) their small mammal hosts.

1.2 Statement of the problem

Studies on the seasonal patterns of fleas in Namibia are limited, as is the detection of *Leptospira spp*., and these data are currently either missing or scarce in other Southern African countries. Increase in occurrence of leptospirosis and flea-borne diseases in developing countries have encouraged entomologists to investigate the

role of transmission of diseases between wild and domesticated animals, as well as establish links to human diseases. The investigation of disease dynamics involves several scientific disciplines including mammalogy, entomology, microbiology, sociology and epidemiology. Such multi-disciplinary investigations aim at linking human health to that of the environment and led to an innovative scientific framework known as One Health Concept (Wallace *et al*., 2015).

The present study investigated the role of abiotic environmental factors such as temperature and relative humidity on the abundance and species diversity of each investigated biological partner (because they can cause diseases to other animals *Leptospira* directly and fleas indirectly) i.e. small mammals, fleas and pathogenic *Leptospira*. In addition, there is a lack of awareness among farmers about rodent borne-diseases. In developing countries, malaria has attracted more attention than other fever causing diseases and large financial resources have been invested in research associated with this disease. Leptospirosis is rarely diagnosed in African hospitals despite suggestions that the magnitudes of infections might be greater than appreciated (Bharti *et al*., 2003). The irregular fever complaints from patients visiting health facilities that do not respond to anti-malaria drugs implicate other possible causes of these health problems. Different aspects of a lack of awareness by medical personnel, particularly physicians, of other locally circulating zoonotic diseases, clearly calls for the need of other field investigations to provide new and updated information.

1.3 Objectives of the study

1.3.1 Broad objective

The study aimed to investigate the diversity as well as the temporal and spatial dynamics of a tripartite pathosystem composed of ecto- (arthropods) and micro- (pathogenic bacteria) parasites of medical importance in association with their small mammal host within the Mukwe Constituency of the Kavango East Region (Namibia).

1.3.2 Specific objectives

1) To determine the diversity of pathogenic *Leptospira spp*. infecting the small mammal communities in the Kavango East region.

2) To compare the abundance, composition and diversity of fleas on small mammals in the Kavango East Region.

3) To determine which climatic variables (temperature and relative humidity) influence the abundance of fleas and host species within the Mukwe constituency of the Kavango East region.

1.4 Hypotheses of the study

1) Different small mammal species harbour dissimilar species of pathogenic *Leptospira* (Dietrich *et al*., 2014). Therefore, small mammals in this study area are expected to harbour different *Leptospira* strains.

2) The abundance, composition and species diversity of fleas on different host species is influenced by aspects of season and microclimate (Laudisoit *et al*., 2009). Therefore it is hypothesised that, the abundance, composition and diversity of fleas is expected to be higher during dry months then during the wet months, because of the optimal conditions during these months for fleas survival and exchange within and between host species (Laudisoit *et al*., 2009; Hang'ombe *et al*., 2012; Guernier *et al*., 2014; Makundi *et al*., 2015).

3) Fleas have been reported to be sensitive to climatic factors such as temperature and relative humidity; therefore, this will influence their seasonal abundance on the different host species (Laudisoit *et al*., 2009; Hubbart *et al*., 2010; Makundi *et al*., 2015).

1.5 Significance of the study

The result on pathogenic *Leptospira* spp. obtained from this study has constituted a critical form of baseline information on the different species of this bacteria occurring within the Kavango East region and Namibia at large. The present study is important from a human health perspective as leptospirosis incidence has been increasing in developing countries because of a lack of awareness amongst community members and hospital personnel or lack of laboratory confirmation for this disease (Bharti *et al*., 2003; Holt *et al*., 2006; Pappas *et al*., 2008; Costa *et al*., 2015).

Additionally, information on the diversity of pathogenic *Leptospira* spp. within a given area is important to determine the source and route of infection to humans in order to control outbreaks (Kathryn *et al*., 2015). Since there is a lack of awareness of the disease in Namibia at large, symptoms that might result from leptospirosis or flea borne diseases such as murine typhus may be misdiagnosed with other diseases such as malaria, which receives notably greater attention from the Namibian Government (Smythe *et al*., 2002; Bharti *et al*., 2003; Holt *et al*., 2006; Vijayachari *et al*., 2008; Kathryn *et al*., 2015).

Therefore, this calls for enhanced detection and diagnosis capacities at the level of health facilities in rural regions. Hence, this project tackled questions of both medical and veterinary importance. Leptospirosis has been reported to have significant impact among adult males, as well as people working in the fields of agriculture, waste disposal, forestry and butchery, with incidence rates peaking after periods of high rainfall (Gratz, 1997; Watt, Jongsakul and Sattinout, 2008; Meerburg, Singleton and Kijlstra, 2009).These different work activities constitute the largest industry in Mukwe Constituency of the Kavango East region at 60.3% of the working population (Namibia Statistics Agency, 2014).

According to the Namibia Statistics Agency (2011), the Kavango region is one of the most densely populated regions of the country at five individuals/km^2 as compared to the national average of three individuals/km^2. The region is further characterized by 71% of the population living in rural areas, and more than the majority use thatch grass as roofing material and mud or clay for house construction; hence, these people are prone to small mammal infestations. Furthermore, agricultural activities are the main source of livelihood and survival for these populations. Therefore, if indeed pathogenic *Leptospira* sp. are present in the small mammals of the Kavango region, then information needs to be made available to the relevant authorities to understand this possible threat to public health.

Cases of bubonic plague outbreak in Algeria and Tanzania indicate that the disease may re-emerge in unpredictable patterns in the same or neighbouring areas because of small mammal population expansion after prolong period of silence (Makundi *et al*., 2008), therefore it is critical to monitor the host and flea populations in and around known plague foci regions namely: Ohangwena and Oshikoto region in Namibia (Shihepo *et al*., 2008).

Small mammals threaten health and food security in Namibia and other African countries because of the damage that they cause before the harvest (eating seeds), during (damaging the crops) and after the harvest (feeding on stored grains). Additionally, small mammals damage personal belongings and they contaminate food source in the house with their urine and faeces. Thus, they may serve as a source of infection of rodent borne diseases to humans due to their ability to live in close proximity with humans.

1.6 Limitations of the study

This study only concentrates on small mammal host species of fleas and *Leptospira* found on fallow lands previously used as crop fields, but the study could have collected and compared the samples from the natural forest and the currently used crop fields in order to monitor if population dynamics and species composition of fleas and host are different in these environments of Mukwe Constituency, Kavango East region. However, this could not be done because the study was a subset of a larger project that focused on small mammals in fallow land fields. Moreover, fallow fields are sites where both commensal and wild rodents continually interact. Therefore, this increased the chance of capturing both commensal and wild small mammal species.

Obtaining fleas from animals that are being collected influences trap success over at least short or moderate periods, therefore it would have been advantageous to collect fleas from captured, marked and released host animals to study flea diversity across a broader period. This could not be done because the study had to answer several objectives including some objectives concerning *Leptospira* that required sample of organ (Liver) from the host species that could only be obtained by dissecting the host.

People from the community within the Mukwe Constituency are known to eat small mammals as a source of protein. Based on previous mammalogists working in this region, local people recognize small mammal traps and therefore they took more than 40% of the traps for their own use and these traps had to be continuously replaced.

1.7 Delimitation of the study

In this study, small mammals were defined as those non-flying mammalian species that weight less than 200g when adults. The traps that were used gave both rodents and shrews an equal chance of being encountered during the sampling however, they are not from the same order. Rodents are from the Order Rodentia while shrews are from the Order Eulipotyphla. Therefore the term 'small mammals' was used in order to refer to both of them.

Since this study was a subset of the bigger StopRats project, which involved collecting different samples from single host in order to answer several objectives of this study and the bigger project (StopRats), the study could only sample individuals from fallow land fields. Because, it is the site were both wild and commensal rodents interact therefore, the study could include host species from both sites by focusing on fallow land fields.

In spite of increasing incidence of Leptospirosis in developing countries and of the climatic conditions mainly temperature and rainfall in the region that are conducive for leptospiral maintenance, there is currently no information regarding this zoonotic disease in Kavango and Namibia at large, so all pathogenic *Leptospira* species were screened in the sample using a previously described RT-PCR protocol (Smythe *et al*., 2002).

Chapter 2

2. Literature Review

2.1 *Leptospira*

2.1.1 General aspects of *Leptospira*

Leptospires are spirochetes in the Order Spirochetes, Family Leptospiraceae (Vijayachari *et al*., 2008). Leptospires, belonging to the genus *Leptospira*, are helically coiled slender bacteria that are motile by the use of flagella (Bharti *et al*., 2003). They are aerobic with an optimum temperature of about 30°C (Bharti *et al*., 2003; Vijayachari *et al*., 2008; Desvars *et al*., 2011). Leptospirosis is a broadly distributed zoonosis across the world (Bharti *et al*., 2003; Vijayachari *et al*., 2008; Ahmed, Anthony and Hartskeerl, 2010; Desvars *et al*., 2011; Gomard *et al*., 2016).

There are two species of *Leptospira* based on phenotypic characters, namely; *L. interrogans* (pathogenic) and *L. biflexa* (non-pathogenic) (Vijayachari *et al*., 2008) and each of these "species" has several serovars characterized by the composition of their surface antigens. All members of the genus are morphology similar, being 0.25 x 6-25 µm in size and 0.1-0.2 µm in diameter (Bharti *et al*., 2003). *Leptospira* exhibit three types of movement rotation around a central axis, progressive movement and circular motion by means of flexion (Baharti *et al*., 2003).

Molecular approaches of *Leptospira* such as Fluorescent amplified fragment length polymorphism analysis (FAFLP) does not depend on bacterial sequence thus, they can be used on wide range of experiments because, it is important for outbreak investigation and to group isolates concerning their geographic origin. However, this method have some draw backs as it requires high quality reagents, no contamination

in the samples and a high concentration of DNA, this method is not useful for determining e.g the host origin of the serovar/species (Nalam *et al*., 2010). A second molecular approach is the multilocus sequence typing method (MLST), which is the golden molecular method approach as it allows for more reliable, consistent data that can be shared through open access databases. However the success of this method relies on the success of PCR amplification (Ahmed *et al*., 2006; Ko and Picardeau, 2009; Nalam *et al*., 2010). The last method is the multilocus variable number of tandem repeats analyses (MLVA), this method is not mostly applied beyond *L. interrogans* therefore, it lacks flexibility.

2.1.2 Typical life cycle of pathogenic *Leptospira*

The bacteria enter into the host body through damaged skin, mucous membrane and conjunctiva membrane. Once it enters the host body, they start to reproduce asexually by binary fusion, since the bacteria reproduce asexually all *Leptospira* strains are genetically the same (no genetic diversity within an infected host) and no additional serovars are added. Apart from reproduction, respiration of the bacteria also starts to take place because of the optimum conditions (temperature) in the host body.

The bacteria survive in the host body because it changes its surface antigen to the host environment (Nalam *et al*., 2010; Haake and Levett, 2015). Therefore, it is difficult for the host immune response to detect the bacteria; following this, the bacteria spread throughout the host body. Reservoir host (those not showing clinical symptoms but shed the bacteria) maintain the bacteria in the wild. Rats are the main reservoirs because they shed the bacteria in large amounts and they have a short life span, therefore the bacteria have no lasting effect on the population (Haake and Levett,

2015). Reservoir hosts are further non-migratory, territorial animals and incidental host are the main cross boundary transfer of the bacteria from one place to another. Therefore, infection to incidental host e.g. humans occurs from reservoirs such as rats and their domestic animals. It may result in illness that eventually leads to death of incidental hosts (humans and most domestic animals) (Haake and Levett, 2015).

2.1.3 Epidemiology and host association

Leptospirosis is a bacterial disease that affects many groups of mammals including, humans, dogs, cattle and goats (Machang'u *et al*., 1997; Machang'u *et al*., 2004; Holt *et al*., 2006; Vijayachari *et al*., 2008; Obiegala *et al*., 2016). *Leptospira* live and reproduce asexually in the kidney by binary fusion but in acute situations, it can also be found in all other body fluids and organs such as the brain, genital tract, eyes, blood and milk (Bharti *et al*., 2003). The source to human infection is either by direct or indirect contact with contaminated urine, soil or water (Bharti *et al*., 2003; Holt *et al*., 2006; Vijayachari *et al*., 2008). Principal known reservoirs, that is to say animals that shed via their urine viable *Leptospira*, include rodents, domestic animals pigs, swine, cattle, goats and dogs (Vijayachari *et al*., 2008; Desvars *et al*., 2011).

Generally, most strains are associated with one or more mammalian host species. However, a particular mammalian species may serve as the primary host for multiple *Leptospira* strains in geographically separate populations. For example, introduced populations of the small Indian mongoose (*Herpestes auropunctatus*), was reported to harbour several strains of *Leptospira* in different regions of the world name: Hawaii and Porto Rico, serovar *iterohaemorrhagiae* and *jules* in Jamaica, serovar *iterohaemorrhagiae* (Bharti *et al*., 2003). Based on these findings, the Indian mongoose serve as a host to different serovars of *Leptospira* in different areas.

Leptospirosis originates from both wild and domestic animals; however, known taxa of wild animal hosts are not properly studied and many details still need to be resolved (Dietrich *et al*., 2014). Holt *et al*. (2006) further stated that there is limited information as to whether there is competition between serovars within a host, and if infection and immunity is passed on from one generation to another (Holt *et al*., 2006).

Several authors have proposed that high rainfall is linked to increased primary productivity, leading to augmentation in rodent abundance, and increased number of epizootic and human cases of Leptospirosis; however, support for this is not always found (Mgode *et al*., 2014). Cases of the disease have been reported in countries/locations such as Nicaragua, El Salvador, Rio de Janeiro (Brazil), and Orissa and Mumbai (India). Most of these cases were related to events such as cyclones or hurricanes (Bharti *et al*., 2003; Vijayachari *et al*., 2008). In addition, the disease is significantly more prevalent in tropical countries than in temperate because of the longer survival period of the *Leptospira* in warm humid environments (Bharti *et al*., 2003; Vijayachari *et al*., 2008; Dietrich *et al*., 2014; Mgode *et al*., 2014).

On islands in the southwestern Indian Ocean, the disease is prevalent on La Réunion, Mayotte and islands in the Seychelles Archipelago (Guernier *et al*., 2014). In Africa, reported cases of leptospirosis include the countries of Benin, Ivory Coast, Kenya, Madagascar, Mali, Senegal, South Africa, Tanzania, Uganda and Zimbabwe (Gratz, 1997; Frean, Rossouw and Trataris, 2012; Saife *et al.,* 2012). Gratz (1997) reported that on the African continent, host species that were recorded positive (presence in the kidney and shedding through the urine) include *Arvicanthis niloticus*, *Cricetomys gambianus*, *Mastomys* spp., *Mus musculus, Rattus norvegicus* and *R. rattus*.

In a study by Dietrich *et al*. (2014), it was pointed out that Madagascar endemic small mammal species harboured different species of *Leptospira* than introduced rodents. Malagasy endemic rodents (Subfamily Nesomyinae) and tenrecs (Family Tenrecidae) were carriers of *L. kirschneri* and *L. borgpetersenii* group B species, while the introduced small mammal species had *L. interrogans* and this likely because of evolutionary forces, because mainland species had a different evolutionary pressure compared to introduced species. Therefore, this gave rise to host species harbouring different leptospire strains based on their geographic origin. Data from austral Africa are scarce and most *Leptospira* identifications are based on immunological approaches. Due to the absence of similarity between serological and molecular classifications, only molecular data allows identifying the actual infecting *Leptospira* species and these data are currently mostly missing in Southern Africa countries (Dietrich *et al*., 2014). Brown *et al*. (2011) stated that there is also lack of awareness of the disease and its transmission among people who work in environments that render them at risk of contracting the disease, such as agriculture, sewage, forestry and butchery.

2.2 Fleas

2.2.1 Flea morphology

According to Mullen (2009), fleas belonging to the Order Siphonaptera, are of medical and veterinary importance, particularly those of families Pulicidae, Ceratophyllidae, Leptopsyllidae and Vermipsyllidae and occasionally Histrichopsyllidae and Rhopalopsyllidae, as they feed on humans and domestic animals. Currently there are 35 genera of fleas known in Southern Africa (Segerman, 1995; Shihepo *et al*., 2008).

Unlike other insects, fleas are laterally compressed to ease movement on host and to allow the parasite to hide in spaces such as ears, between toes and under limbs. They are efficient parasites because they can occur on a wide range of host species and they lack or exhibit little host specificity (Laudisoit *et al*., 2009). These aspects provide a clear mechanism for dispersal, however flea dispersal is assisted by introduction of fleas by non-native species thus resulting in fleas to colonize a wide range of hosts and occurring in geographically larger regions than the distribution of the host type.

Fleas are classified into different groups based on molecular and morphological differences. The body of the flea consists of three principal regions: the head, thorax and the abdomen. Depending on the species, eyes, genal and pronatal combs might be present. All the adult flea species are dependent on a blood meal and they do so with an elongated mouthpart that is used for feeding on the host. They are further composed of three pairs of legs and the hind pair of legs is specially adapted for jumping. Adult fleas are 1-3 mm long, they can live for up to 100 days (Patel and Forsythe, 2008), both male and female fleas mate on the host (Patel and Forsythe, 2008; Hubbart *et al*., 2010).

2.2.2 The flea life cycle

Depending on environmental parameters such as temperature and relative humidity, the life cycle of fleas can range from weeks to months. The flea has a complete metamorphosis and its start with reproduction by means of sexual reproduction. Female unmated flea will only be able to lay unviable eggs (eggs that will not result in the development of the larvae) which, later serve as food source for the developing larvae. Lawrence and Foil, (2002) and Zason-Aiken, Gregory and Shop, (1997) further explained that, the flea has evolved this strategy of laying unfertilized eggs in order to ensure that the developing larvae has food to eat and to reduce cannibalism

by eating on unfertilized eggs (Hsu, Hsu and Wu, 2002). A mating flea together with a blood meal from the host will result in viable eggs (eggs that will hatch and produce larvae) (Zakson-Aiken *et al*., 1996; Hsu and Wu, 2000), the fleas further, increase their chances by mating with different partners in order to have a high number of fertilized eggs.

Once the eggs are laid on the host, they later detach into the host's nest or surrounding environment (Patel and Forsythe, 2008; Hubbart *et al*., 2010) were further development takes place. The fertilized eggs hatch after five days depending on the prevailing climatic conditions because fleas are susceptible to low relative humidity (less than 50%) and higher temperature, following this, the larvae undergo three stages. At the initial phases, the larvae are non-parasitic and they feed on organic matter, dried excreta of adult fleas and cannibalistically (eating fleas eggs and other younger larvae) (Lawrence and Foil, 2000; Hsu *et al*., 2002; Krasnov *et al.,* 2004; Patel and Forsythe, 2008; Hubbart *et al*., 2010). At the last larvae stage, they transform into pupae, which is enclosed in a cocoon where they undergo transformation into the adult stage. This latter stage lasts about 2 weeks but is influenced by temperature and availability of the host. The pupae will only transform into adult fleas if the prevailing conditions (temperature, relative humidity and availability of host) ensures survival (Patel and Forsythe, 2008; Hubbart *et al*., 2010).

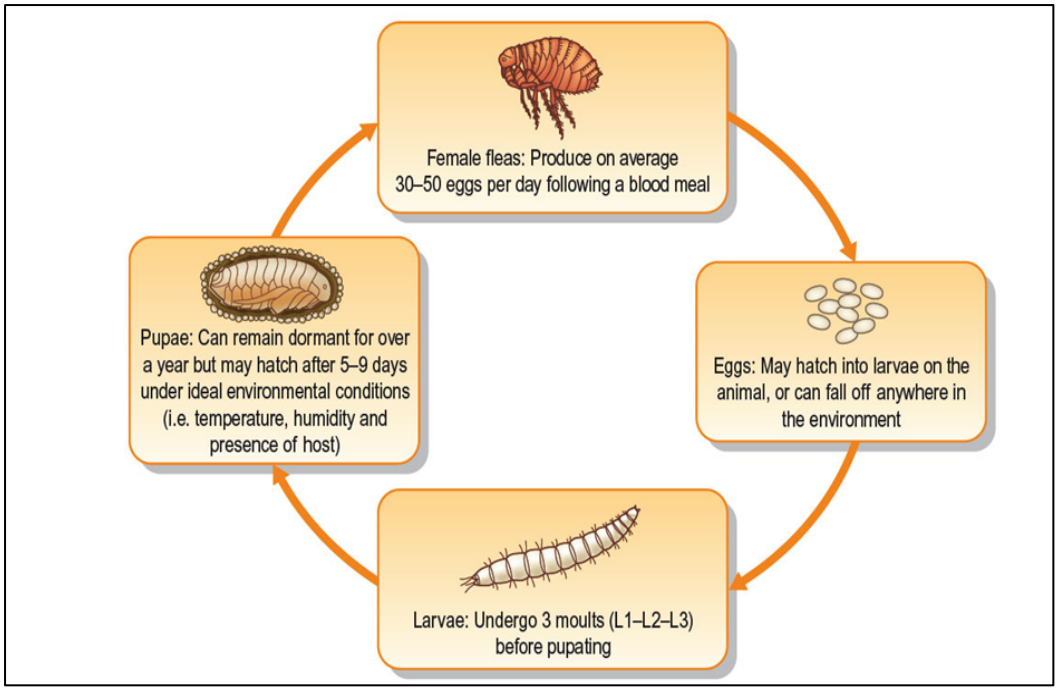

Figure 1: An example of a life cycle of a flea (Patel and Forsythe, 2008)

2.3 Small mammal flea hosts

The first case of plague in Southern Africa was reported in 1930 in northern Namibia (Shihepo *et al*., 2008). The rodent species that were found in the area were *Rhabdomys pumilio*, *Mastomys* spp. and *Gerbilliscus leucogaster* (Shihepo *et al*., 2008). However, there is a gap in knowledge concerning rural household activities that includes woman, men and the children. This, this gap has been a reason for high mortality rate in other African countries such as Tanzania where the house hold activities and sleeping arrangement in rural houses, were the contributing factor to the high mortality of plague in woman and children (Davis *et al*., 2006). Since, they sleep on the floor therefore they had a higher exposure to fleas compared to men.

A study conducted in the southern part of Namibia (Gellap-Ost and Nabaos, Keetmanshoop District) indicated that there was exclusive association between an

elephant shrew host species, *Elephantulus intufi*, with the parasite flea *Macroscelidopsylla albertyni* (Eiseb, 2002). However, *Xenopsylla trifaria* and *Xenopsylla piriei* commonly occurred on most small mammal species captured at two farms investigated in the southern part of Namibia (Eiseb, 2002). Eiseb, (2002) further mentioned that the fleas species in the study are showed seasonality. The flea abudance was high in August and low in February and May. In selected haitats around Windhoek, Namibia, (Mfune, Kangombe and Eiseb, 2013) reported the following small mammals species in the city *G. leucogaster*, *Thallomys nigricauda*. *R. pumilio* and *E. intufi*. In this study *G. paeba* (61.1%) had the highest prevalence while *E. intufi* had the lowest (0%)

Hieronimo *et al*. (2014) reported in Tanzania, vegetation cover and plant species diversity were projected to directly influence prevalence (number of host infested divided by the total number of host animal examined, multiplied by a 100) and species diversity, as well as intensity of fleas associated with small mammals (total number of individual ectoparasites divided by the host animal infested). In addition, land use was found to influence the abundance of fleas, being higher on fallow lands and lower during the wet season. The flea species that showed the most pronounce variations were *Xenopsylla brasiliensis* and *Nosopsyllus sp.* (Hieronimo *et al*., 2014).

Within the Kavango East region of Namibia, Katakweba *et al*. (2012) reported that two rodent species, *Rhabdomys pumilio* and *Gerbilliscus leucogaster*, were found positive for antigens against *Y. pestis*. In this study, one positive individual was found in the austral winter and the other in the austral summer (Katakweba *et al*., 2012). However, two specimens are not a sufficient sample size to draw definitive conclusions and a larger sampling and further screening of *Y. pestis* from fleas is needed to understand aspects of the epidemiology of this disease in Namibia.

In addition, Shihepo *et al*. (2008) reported that there was one species of flea belonging to the genus *Xenopsylla* on the host *Saccostomus campestris* recorded in irrigated agricultural fields. Therefore, one of the objectives of the study was to provide additional information on flea species found in fallow land (previously used crop fields), especially those closer to homesteads over a 11-month period and to further document local small mammals species in addition to those recorded by (Katakweba *et al*., 2012).

Uusiku (2007) conducted a study on the seasonal occurrence of fleas and other ectoparasites at Waterberg Plateau Park and the result indicated that there was a seasonal change of ectoparasites based on host sex species and month. Kapia (2003) results indicated that the number of host influenced flea species composition at the Neudamm Agricultural Farm. Kapia (2013) further attributed the low encounter of small mammals of small mammals during winter (May and June) to low vegetation cover. Vegetation cover is needed by the host species as it provides some protection against predators (Eiseb, 2002). Results from Karuaera (2011), showed contradicting results. The study suggested that bush encroached and non-bush encroached area did not have significant effect on the abundance of small mammals at the Neudamm agricultural farm.

Another type of variable that influences the distribution of fleas is climate, specifically rainfall, relative humidity and temperature. Makundi *et al*. (2015) explained that fleas in Tanzania usually form a strong association with the host, because the host provides a place to live and reproduce. Therefore, flea community composition on a given host is a direct result of the host it's occurring on and the distribution of the host within a particular habitat (Laudisoit *et al*., 2009). Another factor that influences the number and composition of fleas is the abundance of the

host species within a habitat. Highly abundant flea species will correspond to high abundance of hosts because of a greater chance of fleas to find a host (Laudisoit *et al*., 2009).

A recent study carried out on La Réunion Island, Indian Ocean, showed a strong structuration of flea distribution with flea infestations restricted to the drier leeward coastal area of the island (Guernier *et al*., 2014). Most interestingly, this sharp geographical distribution coincided with the occurrence of murine typhus, only known from the leeward coastal area of the island. In Tanzania, it was found that there was a seasonal peak in cases of plague, specifically during rainy season from December to February (Davis *et al*., 2006). Thus, as shown by these different studies, prediction of flea occurrence based on season and/or land cover is quite controversial and currently there is a lack of information about the conditions favouring flea abundance and/or diversity in Tanzania. It has been further proposed that geographic range of rodents might increase because of rodent outbreaks in new areas (Makundi *et al*., 2008). Rodent outbreaks can be defined periods when populations of rodents within a certain area increase exponentially following conditions that favours or aid in their survival and reproduction (Leirs, Sluydts and Makundi, 2010).

One of the conditions that aid in rodent population outbreaks is high amount of rainfall early in the rainy season, as this allows the rodents to have an extended breeding season and higher supply of food thus leading to high occurrence. Periods of high amounts of rainfall after long dry periods are also known to cause outbreaks. Another contributing factor is highly accessible food for rodents in homesteads and agricultural fields compared to natural forests as this result in high numbers of rodents in homesteads and agricultural fields (Leirs *et al*., 1996). Apart from this two factors predation pressure, diseases and social structures are also some of the factors that lead

to exponential increase of rodents in an area. The results of population outbreak are food loss and disease outbreaks and on a small scale (homesteads), personal belongings are damaged.

In Uganda, Moore *et al*. (2015) reported that densities of *Crocidura* spp. and *Arvicanthis niloticus* were positively correlated to rainfall and harvest of millet and maize within the plague foci area and negatively correlated with temperature. The associated flea diversity was found to be strongly and positively correlated with rainfall amount on the host species (*M. natalensis*, *Crocidura* spp. and *A. niloticus*). While in the eastern African savannah, intensity of flea infestation was affected by body mass and season (Young *et al*., 2015).

Adler *et al*. (2001) and Wells *et al*. (2011) reported that in Southeast Asian forests, flea diversity is low because of the warm conditions and low abundance of small mammal hosts. Adler *et al*. (2001) further reported one species of flea infesting a single species of rodent in lowland forest and higher species richness of both rodents and fleas in highland forest. Wells *et al*. (2011) reported that niche partitioning of diurnal tree shrews was low compared to species in the low land of the montane forest in Asia.

Kim *et al*. (2010) have studied aspects of the seasonality of fleas in Korea, their results indicate a lack of small mammal infestation during the summer (June) and fall (August to September). In contrast, flea infestation rates were higher in the late winter months, with the exception of one species, *Ctenophthalmus congeneroides*, which showed high infestation from spring summer and fall. In California, Hubbart *et al*. (2010) reported that different types of land use were correlated to high abundance of

fleas at different times of the year. More precisely: 1) overall abundance of fleas was higher in summer and winter in grazing areas where fertilizer was not applied, 2) the less disturbed site, where pesticide were not applied and no grazing took place, had high abundance of the flea *Hoplopsyllus anomalus* in winter: and 3) at the agricultural site, flea abundance was high in summer.

Chapter 3

3 Materials and methods

3.1 Map of the study site

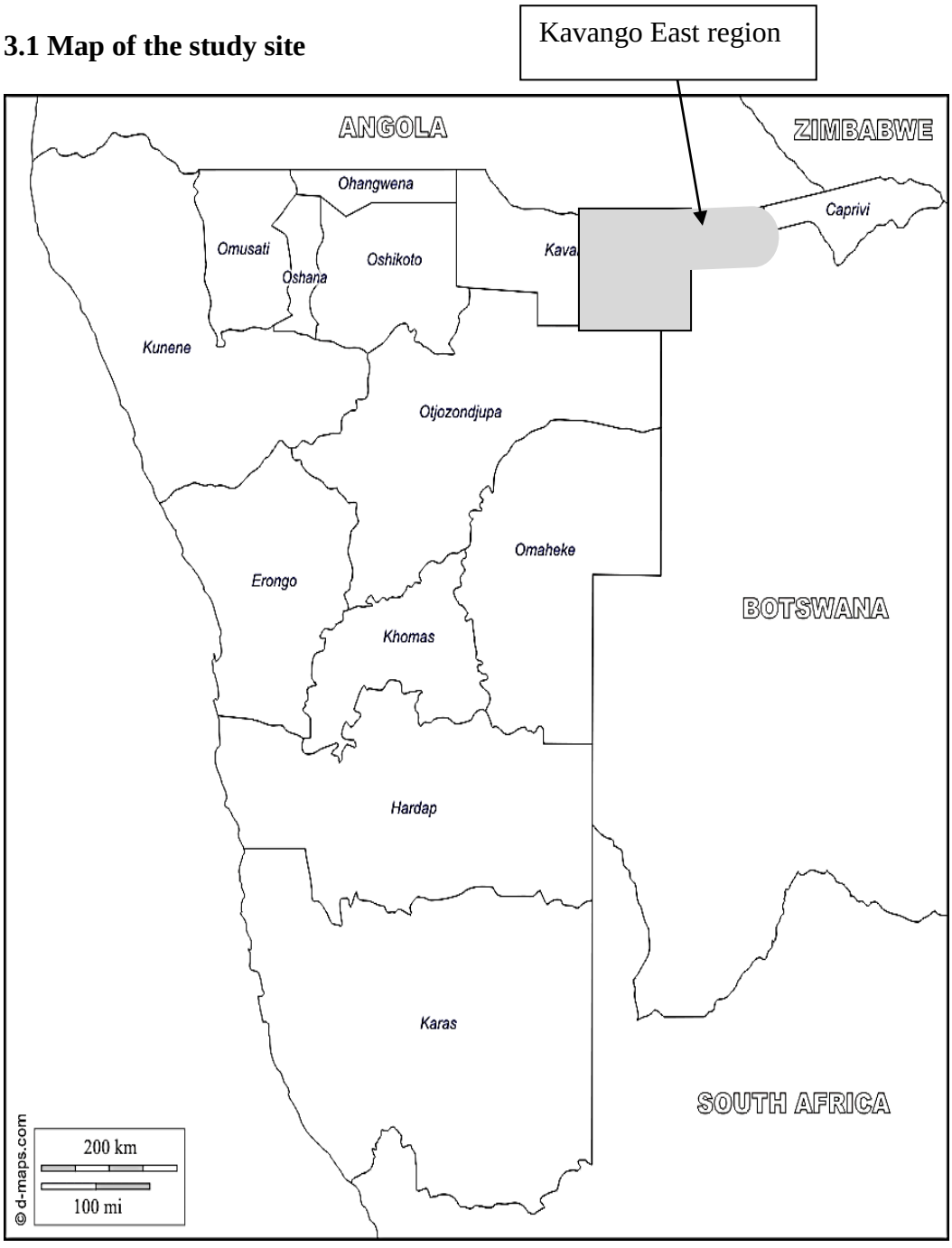

Figure 2: Map of the study site (Kavango East region) in north eastern Namibia in relation to neighbouring countries (d- maps, 2017).

3.2 Description of the study site

The sampling was conducted near the villages of Kake (021°30’43.8”E, 018°05’29.3”S) and Andara (021°26’23.9”E, 018°03’54.0”S). The Kavango East region has borders joining to Angola in the north and Botswana in the southeast. Locally, it borders the following regions: Zambezi (east), Otjozondjupa (south), Oshikoto (west), and Ohangwena (northwest) (Mendelsohn & Obeid, 2006).

3.3 Climate

3.3.1 Rainfall

The region has a semi-arid climate (Mendelsohn & Obeid, 2003; NPC, 2007), with two major influences on the weather patterns within the region: 1) one is the Inter-Tropical Convergence Zone (ITCZ), which moves north in winter (cool and dry air) and south in summer (clouds, moisture or rain) to northern Namibia and 2) The other aspect are belts of temperate high pressure cells, one off the Namibian coast in the Atlantic Ocean and the other in Botswana, which influences the regional weather patterns by bringing cool and dry air, resulting in no rain.

The average rainfall in the region is around 500-600 mm/year, which occurs mostly from October to March. The region has wet summer months (November to March). and dry winter periods (June to September). However, the amount of annual precipitation varies amongst years (Mendelsohn & Obeid, 2003; NPC, 2007; MET, 2011). The rainfall distribution within the region is highest (more than 550 mm) towards the north eastern part of Namibia and decreases progressively towards the south and west (MET, 2011) (Figure 3).

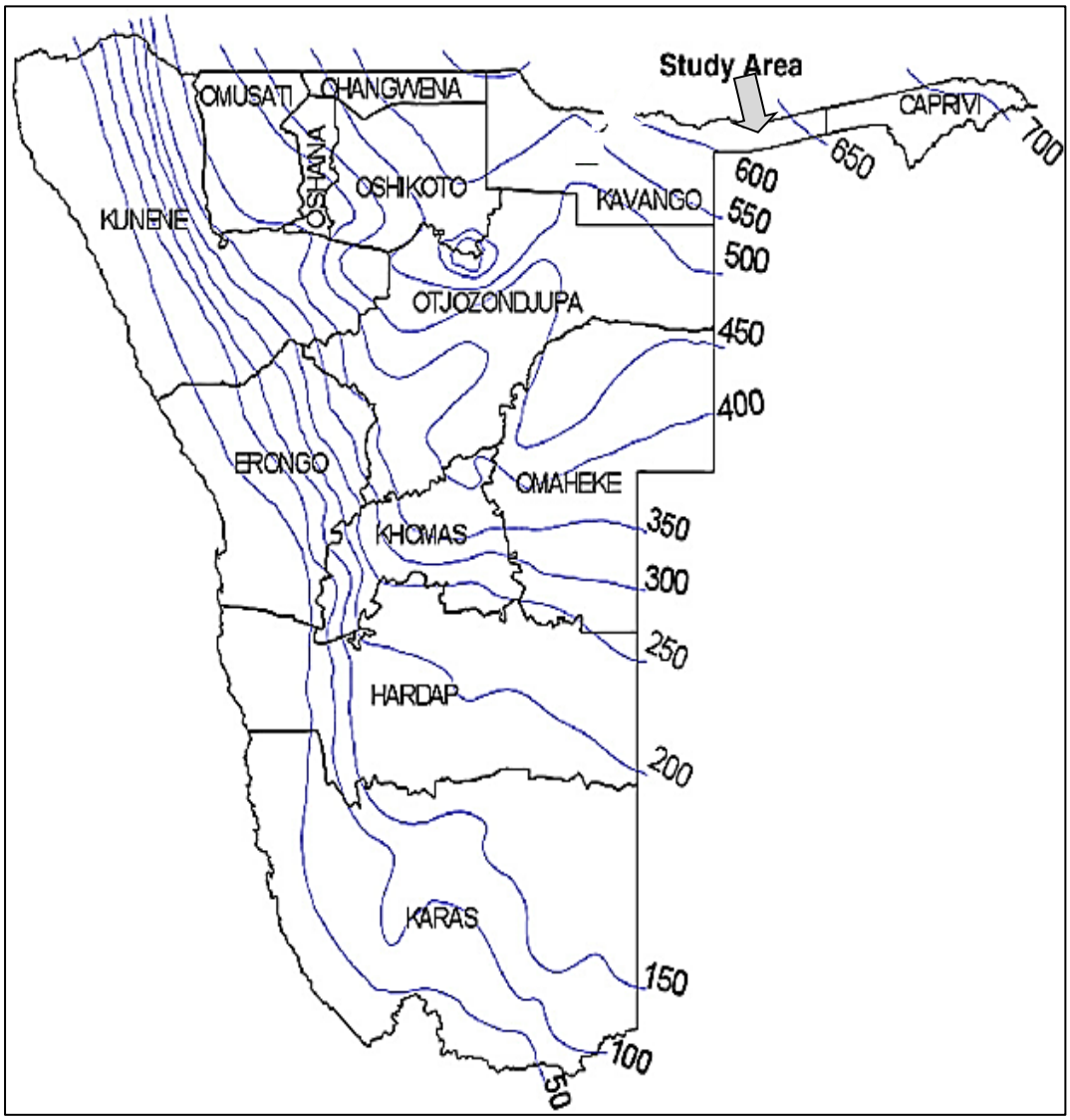

Figure 3: Rainfall distribution patterns in Namibia (Stohbach and Peterson, 2007)

3.3.2 Temperature, relative humidity and evaporation

The annual average daily temperature in the region is 22.4ºC, and during the winter cold season, the minimum temperature at night can drop to below 10ºC (Mendelsohn and Obeid, 2003). The coolest months are June and July, while the warmest month is October.

The high relative humidity (37–69%) occurs between October and March (Mendelsohn & Obeid, 2003). Mean annual evaporation is about 1900 mm per year which is four times higher than the amount of rainfall received in the region; the principal factors contributing to this are the sparse cloud cover, the regular winds, and the climate that is mostly hot and dry (Mendelsohn and Obeid, 2003; NPC, 2007).

3.4 Soils

Dominant soil type is the Kalahari sands, which are fine wind-blown sands (arenosols) that hold little moisture, have high water run-off and low organic and minerals content (Mendelsohn and Obeid, 2006; NPC, 2007). Fluvisols are soil types found along rivers that consist of a mixture of clay, silt and fine sand (Mendelsohn & Obeid, 2006). This type of soil is deposited during periods of flooding. A third type of soils is referred to as anthrosols, which are not associated with river margins and are formed after repetitive ploughing. Other types of regional soil types include Solonetz and Calcisols, which generally occur in the Kavango East region and are low in fertility except in dry riverbeds, also known as “Omuramba” (Stohbach and Petersen, 2007).

3.5 Flora, fauna and human livelihoods

The vegetation in the region is diverse with close to 87 distinct species belonging to 88 families thus far recognized (Mendelsohn and Obeid, 2003). Vegetation types alternates between Kalahari woodlands and stripes or patches of grassland. There is a difference in terms of species composition and community structures between the woodlands but in general, the dominant species include kiaat (*Pterocarpus angolensis*), teark (*Baikiaea plurijuga*), syringa (*Burkea africana*), silver terminalia (*Terminalia sericea*), mangetti (*Schinziophyton rautanenii*), false mopane (*Guibourtia coleosperma*), camelthorn (*Vachellia erioloba*), marula (*Sclerocarya birrea*), weeping wattle (*Peltophorum africanum*) and small palm (*Hyphaene petersiana*). The dominant grass species is *Eragrostis pallens*, which is used for making roof thatching.

Wild fires that burn most of the standing biomass frequently affect the vegetation in the region and leaving little for decomposition (Strohbach and Petersen, 2007; MET, 2014). Another problem that affects the vegetation in the region is bush encroachment. Because of the dominant agricultural practice in the region, which involves farming in one area and abandoning the area after several years, opportunistic invasive species usually dominate the fallow land areas because they are better competitors than the native species (MET, 2014).

The region has great variety of wildlife that is highly concentrated in the national parks (Mendelsohn and Obeid, 2003) and free roaming domestic animals such as goats, cattle, chicken, dogs and cats. People in the region practice mostly subsistence farming with crops and livestock (Mendelsohn and Obeid, 2003; NPC, 2007; MET, 2011) whereas crop yields are often very low. Commercial irrigation farming activity is also common in the region. Other types of subsistence activities include local fisheries and wood crafting (Mendelshohn and Obeid, 2006; MET, 2011).

3.6 Field trapping of small mammals

Trapping of small mammals was conducted in fallow lands (previously used crop fields) at the villages named, Andara and Kake, Mukwe Constituency, Kavango East region. The two sampling sites are about 6 km apart and one sampling area (Kake) was situated less than 10 m from village homesteads. At each sampling site, 100 traps placed in a straight line at 10 m intervals were used for trapping. Trapping at each sampling site was carried out over three nights each month for a period of 11 months.

Sherman® traps (75 mm x 234 mm x 75mm) were baited with a mixture of oats and peanut butter and left open in the late afternoon (16h00) and checked the following morning before (08h00). During the morning trap check, all the traps were removed from the sites and those with live animals were separately placed in a box and transported to the field laboratory. This procedure helped to ensure that the fleas were still on the body of the host when handled in the field.

3.7 Weather data

The temperature and relative humidity data were obtained from the Bagani weather station. The station is located ±seven km from the study site. The temperature and relative humidity recordings were recorded per hour for each day. Small mammals are active in the late afternoon 16h00 to early morning 08h00. Therefore, the average noon temperature was calculated adding the maximum and minimum temperatures (°C) divided by two. From this result, the monthly noon temperature was calculated from the total number of trap nights. The average relative humidity was calculated for each trap night and on a monthly basis. From these calculations, the following results were obtained:

Months	November	Decemer	January	February	March	May	June	July	August	Septembe	October
Relative humidity (%)	53.1	57.9	78	47	54	61	56	40	35	30	27.5
Temperature (°C)	26.1	26.2	22.5	26.8	25.5	16.6	13.9	17.7	21.6	24.4	28.3

3.8. Laboratory processing

Each animal was euthanized with a piece of cotton wool soaked in chloroform within separate "zip-lock" plastic bags to avoid the mixing of ectoparasites between the hosts. The sacrificed individual was subsequently removed from the bag, placed on a white tray, and brushed vigorously from front to back with a fine toothbrush to remove all the ectoparasites. The ectoparasites were collected from the tray using fine pair of forceps and preserved in a small vial with a solution of 70% ethanol.

3.8.1 Flea identification

Flea samples were transferred from 70% ethanol into distilled water for 60 minutes and then to 15% potassium hydroxide for 3 days and dehydrated in a series of different concentrations of ethanol (50%, 70%, 95% and absolute for 60 minutes each). The individual fleas were mounted on a microscopic glass slide using Canada Balsam and its diluting agent xylene.

Based on their morphological features and a compound binocular microscope, a dichotomous key was used to identify flea ectoparasites to the level of genus and species (Segerman, 1995).

3.8.2 Small mammal collections and identification

The following information was recorded for all host animals: the sampling date, the district, the region and each dead individual was allocated a unique number that was

written on a piece of label and tied on the right hind leg of the dead animal with a thread.

Additional recordings were: (1) sex of the animal was determined, whether male or female based on the presence of a testis or vagina. (2) Reproductive status of the host was determined, for the female: the sex conditions were further classified into two groups: pregnant or not pregnant and if the females was found to be pregnant, then the number of embryos were counted on left and right side of the uterine horns. If there were no embryo present, then the embryo scars were counted and recorded on each side of the uterine horns for those females that were at post reproductive stage. Males captures were categorized into two groups based on the conditions of the testis; testis that were scrotal and testis that were not scrotal.

The following body measurements were taken from the host animal: body mass (for females including the foetuses), the length of the tail from the end of the chordate to the tip of the tail. While the ear was measured on the longest side (vertically), the hind leg measurement included the hind foot including the claws, the head-body measurements were taken from the tip of the nose to the end of the chordate bone.

Based on this information the small mammals, specifically rodents, were identified to the levels of genus and species using the identification key developed by Monadjem *et al*. (2015) for all species known to occur in southern African countries. Afterwards the host were deposited in 15% formaldehyde for 24 hours, before transferring them into 70% ethanol and the preserved specimen were transported to the University of Namibia (UNAM), Biological Sciences Department for further studies.

3.8.3 Detection of *Leptospira*

Obtaining kidney samples

Kidney samples were collected immediately after euthanizing the animal at the field laboratory and marked with the field catalogue number of the small mammal specimen. Samples were obtained via dissection, specifically with a scissor and then removing the kidney with forceps. Between the collections of samples from different individuals, the dissecting equipment was cleaned with soap and later disinfected with bleach. The sampled kidneys were stored in a vial containing 70% ethanol for preservation and later transferred to a refrigerator for storage before shipped to the CRVOI laboratory on La Réunion.

Obtaining the DNA samples and *Leptospira* detection

This procedure was done at the Centre de Recherche et de Veille sur les Maladies Emergentes dans l'Océan Indien (CRVOI) [Centre for Research and Surveillance of Emerging Diseases in the Indian Ocean], La Réunion, France by the staff members. The following procedure was followed: for each kidney sample, total nucleic acids were extracted using the Biorobot EZ1and EZ1 Virus Mini Kit version 2.0 (Dietrich *et al*., 2014; Gomard *et al*., 2016). Using GoScript Reverse transcriptase (Promega, Madison, WI), reverse transcription was performed on the total nucleic acid and molecular detection was carried out on cDNA with a Real Time-Polymerase Chain Reaction (RT-PCR) using specific fluorescent probes for pathogenic *Leptospira* and targeting the 5′ end of the 16 S encoding gene (Gomard *et al*., 2016).

Leptospira identification

Leptospira were identified to species level by sequencing a portion of the *secY* gene, a highly polymorphic housekeeping gene, which is commonly used for the determination of members of the *Leptospira* genus (Gomard *et al*., 2016). *Leptospira* phylogenies were constructed using two additional genes, adk and rrs2, both markers used in combination with *secY* in previously described multilocus sequence typing (MLST-scheme). In each reaction PCR mixture constrained 12.5 µL of GoTaq hot start green master mix two times (Promega, Madison, W1), one µL (1mm) of each primer, 8.5 µL of nuclease free water and two µL of cDNA. The PCR conditions consisted of an initial denaturation step at 95 °C for five minutes followed by 45 cycles at 94 °C for 30 seconds, 52 °C-56°C for 30 seconds and 72 °C for one minute and a final elongation step of seven minutes at 72 °C. PCR products were visualised under UV light after electrophoresis on a 2% agarose gel stained with one times gelred™ (Biotium inc) and sequenced on both strands through direct sanger sequencing (Genoscreen, Lille, France) using the same amplification set (Gomard *et al*., 2016),

3.9 Data analyses

3.9.1 *Leptospira*

The two sample sites were used for replicates as each site individually hat very low data. Therefore, results from both sites were added together for all analyses in the study. Documenting for pathogenic *Leptospira* species was done by associating each *Leptospira* strain with the host animal. In addition, the overall prevalence of all pathogenic *Leptospira* on all the host species was calculated. Prevalence and percentage of host animals that tested positive for pathogenic *Leptospira* serovars were calculated using the following formulae:

$$\frac{Positive\ host\ animal}{Total\ tested\ animal} \times 100$$

3.9.2 Fleas

Abundance of fleas

A statistical software package for SPSS 23.0 windows was used for flea data manipulation. For monthly abundance of fleas, the data were first tested for normality using the Kolmogorov-Smirnov (K-S) test. Six nights per sampling month was used as a replicate, while months and host species were used as factors. Kolmogorov-Smirnov test was used in accordance with the sample size that was recorded in this study. Shapiro-wilk test is more suitable for sample size that are between 30 to 40 (Ghasemi and Zahediasl, 2012).

Results from the Kolmogorov-Smirnov test indicated that the data was not normally distributed. Therefore, a non-parametric alternative Kruskal Wallis test was used to examine if the ranks from the population were the same. These tests were used herein

as they do not assume normal distribution, they allow for comparisons of three or more groups.

Similarity index for fleas

To determine if the species composition of fleas differ between small mammal hosts, the Jaccard similarity coefficient according to Sneath and Sokal (1973) was used to assess the degree of community (i.e. host species) overlap. This was done to determine if individual host species harbour the same or different flea species throughout the study period. The Similarity Index between hosts *i* and *j* is given by:

$$Sij = \frac{a}{(a+b+c)} \times 100$$

Where 'a' is the number of flea species present in both hosts *i* and *j*, 'b' is the number of flea species present in *i* but not in *j* and 'c' is the number of flea species present in *j* but not in *i*. The coefficient is out of 100, the closer the coefficient is to 100, then the higher the two communities (small mammals) harbour the same species of fleas and a lower coefficient indicates that the two communities harboured different species of fleas. This index is based on the species richness within a community (Mulungu *et al*., 2008).

Species diversity

To determine if the diversity of fleas on the different rodent species was significantly different, species diversity of fleas was calculated using the Shannon-Wiener index of biological diversity. This index measures the degree of uncertainty within a community (i.e. host species). Calculating this diversity index involves two components: the number of different genera or species present, which is referred to as

species richness and the relative abundance (dominance or evenness) of individual taxonomic units within a community (Magurran, 2004). The degree of uncertainty within a community increases with an increase in a community having unique species or a high species evenness. Thus, the index gives information about the rarity and commonness of the taxonomic units within the community. The index was measured using the following formula:

$$H' = -\sum_{j=1}^{R} pi \ln pi$$

Where H' is the diversity index or the degree of uncertainty, pi is the proportion of individuals belonging to the *ith* species and *ln* is the natural log.

Small mammal data manipulation

Small mammals trapping success was calculated using the following formula for each month:

$$\text{Trap success (\%)} = \frac{Total small mammal captured for one month}{100\, trap set\ per night x\, 6 trap night per month}$$

Correlation between climatic variables and abundance of fleas and small mammals

The data was first tested for normality with the Kolmogorov-Smirnov (K-S) test. This test revealed that relative humidity was normally distributed in this study area df=11, P=0.200. Temperature also showed a normal distribution df=11, P=0.200. However, the average number of fleas for all species grouped together did not show a normal distribution df=11, P=0.001.

According to Salkind and Neil (2011), a correlation test reveals the relationship between two variables. It gives an indication of how one variable changes in relation to another variable and the result is expressed as r_{xy}. To test if there was a correlation between the climate variables (temperature and relative humidity) and abundance of fleas on small mammals, a Pearson correlation was used (Burdess, 2010). The test therefore, measures the strength of two variables which are given by the coefficient 'r' and the result is always between +1 and −1, with r =1 indicating a very strong positive or direct correlation, r =0 indicating no correlation and r=−1 indicating a very strong negative or indirect correlation (Burdess, 2010; Salkind and Neil, 2011).

Chapter 4

Research ethics

Before the study commenced a permit from the Ministry of Environment and Tourism was obtained (Permit Number: 2048/2015). Following this, the King (Fumu) of the Hambukushu Traditional Authority, Headmen of the two villages namely Kake and Andara and the owners of the field study sites were consulted for permission to conduct the study for a period of 11 months. Further, community members were also informed about the research.

Chapter 5

Results

The field data were collected from November 2014 to October 2015. The study was conducted over 66 trap nights and 212 small mammal hosts were recovered. The results were collected from two fallow fields (Andara and Kake). This result represents both sites grouped together as a representation of Mukwe constituency.

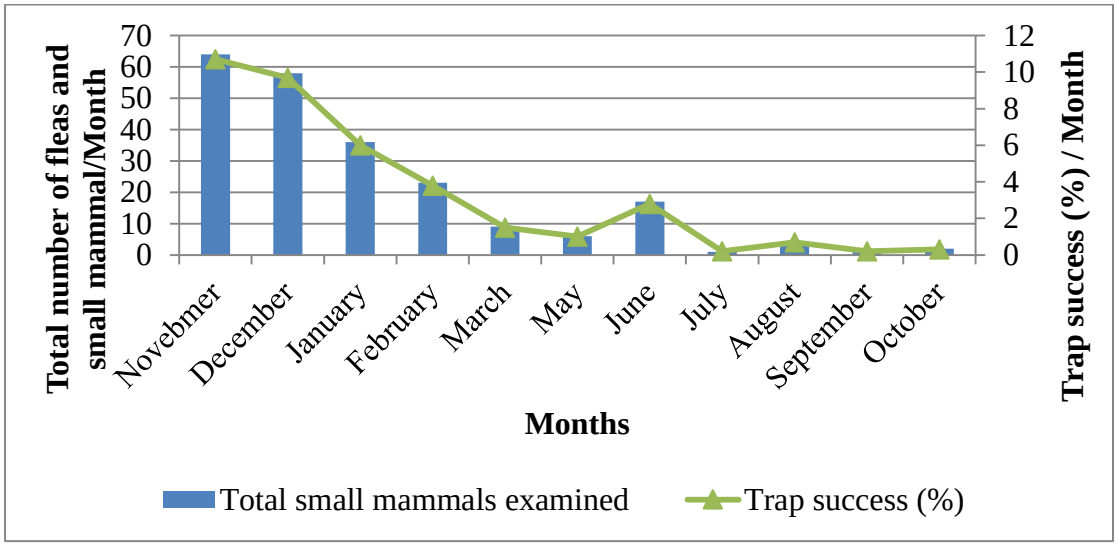

Figure 4: Number of small mammals captured and frequency cumulative curve of all small mammal species grouped together at the two study sites.

Based on the cumulative frequency curve in Figure 4, more the trap success was the highest in November (10.7%) and lowest from July (0.17%). The highest number of small mammals were captured in November (64) and lowest from July (1).

Table 1: Number of small mammal host species examined for fleas from November 2014 to October 2015 in Mukwe Constituency, Kavango East Region.

Small mammals species			Number of small mammals captured each month from November 2014 to October 2015										
Scientific name	**Common name**	**November**	**December**	**January**	**February**	**March**	**May**	**June**	**July**	**August**	**September**	**October**	**Total**
Aethomys chrysophilus	Red veld rat	1	3	0	0	0	0	0	0	0	0	0	**4**
Elephantulus intufi	Bushveld elephant shrew or bush veld sengi	0	1	0	2	0	2	1	0	1	0	0	**7**
Gerbilliscus leucogaster	Bushveld gerbil	4	3	4	4	1	2	7	1	3	1	2	**32**
Mastomys natalensis	multimammate mouse	54	42	22	6	6	3	9	0	0	0	0	**142**
Saccostomys campestris	Pouched mouse	1	2	6	10	2	0	0	0	0	0	0	**21**
Steatomys pratensis	Fat mouse	4	6	7	0	0	0	0	0	0	0	0	**17**
Total		**64**	**57**	**39**	**22**	**9**	**7**	**17**	**1**	**4**	**1**	**2**	**223**
Trap success (%)		10.7	9.5	6.5	**3.7**	1.5	1.17	2.8	0.17	0.67	0.17	0.33	**36.8**

Based on Table 1: *M. natalensis* was the dominant species (n=142) the small mammal host that were recorded at the study site were, *A, chrysophilus* and *E. intufi* had the lowest numbers (n=4 and n=7) respectively.

Most of the host species were captured in November, while July and September had the lowest number of host species recovered. Additionally, the trap success was highest in November and lowest (less than 1%) as from July to October.

5.1 *Leptospira*

5.1.1 Prevalence of pathogenic *Leptospira* spp. infecting small mammal communities in the Kavango East region

Table 2: Prevalence of *Leptospira* in small mammal species in Kavango East region of Namibia.

Small mammal species	Number of tested specimens	Number of positives *Leptospira* infections	Percentage of positive (%)
Aethomys chrysophilus	4	0	0.0
Elephantulus intufi	2	1	50.0
Gerbilliscus leucogaster	8	1	12.5
Mastomys natalensis	85	5	5.9
Saccostomus campestris	10	3	30.0
Steatomys pratensis	12	2	16.7
Total	**121**	**12**	**9.90**

Using PCR and molecular identification method, from all the host species that were examined, there was an overall prevalence rate of 9.9% of *Leptospira* infection. *M. natalensis* had the highest abundance compared to all the other small mammal species but it had one of the lowest prevalence rates to *Leptospira* infection. The prevalence of *Leptospira* was further recorded to be high in *Elephantulus intufi* (50%) and lowest in *Aethomys chrysophilus* (0%) (Table 2).

5.1.2 Pathogenic *Leptospira* identification in host species in the study area

Due to low bacteria load, 11 out of 12 positive samples could not be confirmed. Following this, reverse transcriptase PCR on the 12 previous positive samples were performed, in order to increase the detection rate but it did not work. Only one positive out of total (127) tested samples could be further analysed, five different

sequences of this positive corresponded to *Leptospira kirschneri*. That was found on a female adult host species, *Saccostomus campestris* that was captured in March 2015.

5.2 Fleas

In total 432 fleas belonging to five species were recovered, while the host species were dominated by *M. natalensis* and *G. leucogaster* 64% and 14%, respectively.

Table 3: Total fleas recovered during the study in Mukwe Constituency Kavango East region.

Fleas species	**Total fleas recovered**	**Relative abundance (%)**
Cryptonella numae	8	1.8
Pariodontis riggenbachi riggenbachi	56	12.9
Pulex irritans	186	43.0
Synosternus caffer	17	3.9
Xenopsylla spp.	166	38.3
Total	432	

According to Table 3, the dominant flea species in the study was *P. irritans* and *Xenopsylla spp*. with 43% and 38% respectively.

Table 4: Number of total small mammals captured and hosts sex ratio during each month at the study site.

Months	November	December	January	February	March	May	June	July	August	September	October
Number of small mammals examined	64	58	36	23	9	6	17	1	4	1	2
Number of host infested	62	51	31	20	8	3	12	1	4	1	2
Percentage of host infested (%)	97	88	86	87	89	50	71	100	100	100	100
Total number of fleas	183	25	97	70	7	6	13	1	4	12	6
Ratio of male to female host	36;28	25;33	13;23	13;10	3;6	2;4	3;14	1;0	3;1	0;1	2;0

July to October had the highest infestation percentage (100%), while, May had the lowest percentage of infestation by fleas (50). Additionally, the highest number of fleas were recovered in November and July had the lowest number of fleas (n=1). According to Table 4, the sex ratio of male to female was high from November and it decreased progressively while the female to male ratio was increasing. The trap success per month was recorded to be high in November and low in July

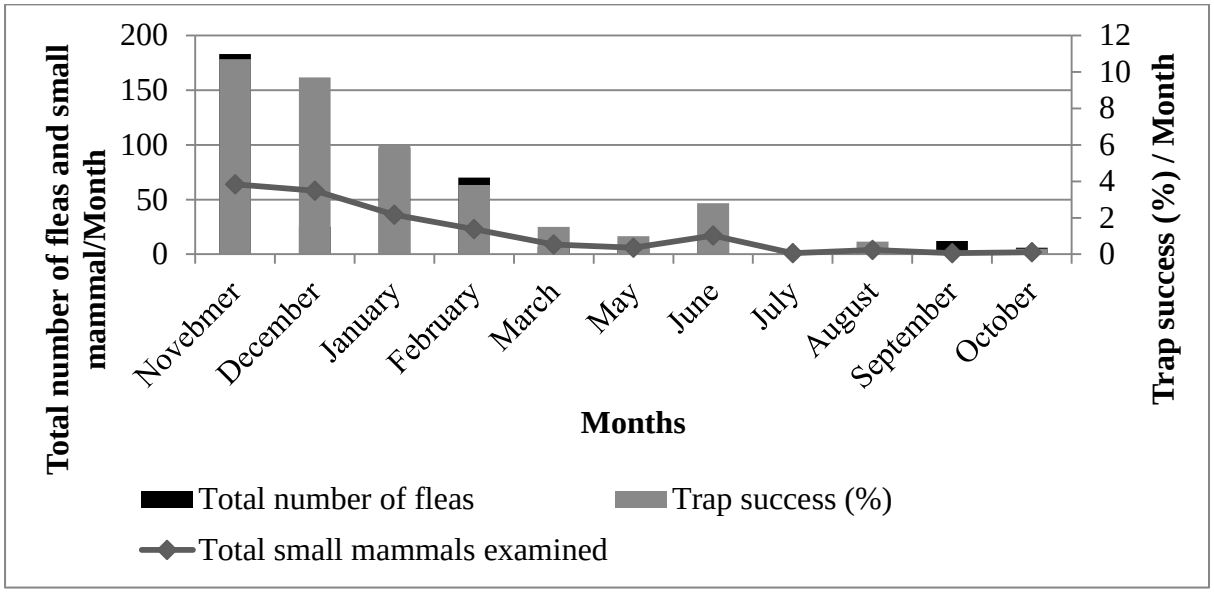

Figure 5: Trap success (%) in relation to total number of small mammals and fleas examined per month in Mukwe Constituency of the Kavango East region.

The trap success ranged from 0.2 to 10.7 % per month (Figure 5) and the overall trap success was 36.8%. The number of small mammals examined for fleas differed during the study period, with November having the highest number of small mammals (n=64), fleas (n=183) and trap success (10.7%). In contrast, July had the lowest total number of captured hosts, fleas and trap success (0.2%).

5.2.1 Abundance of fleas on host species

Generally, all the host small mammal species harboured the same type of flea species except for *A. chrysophilus* and *E. intufi* (Figure 6).

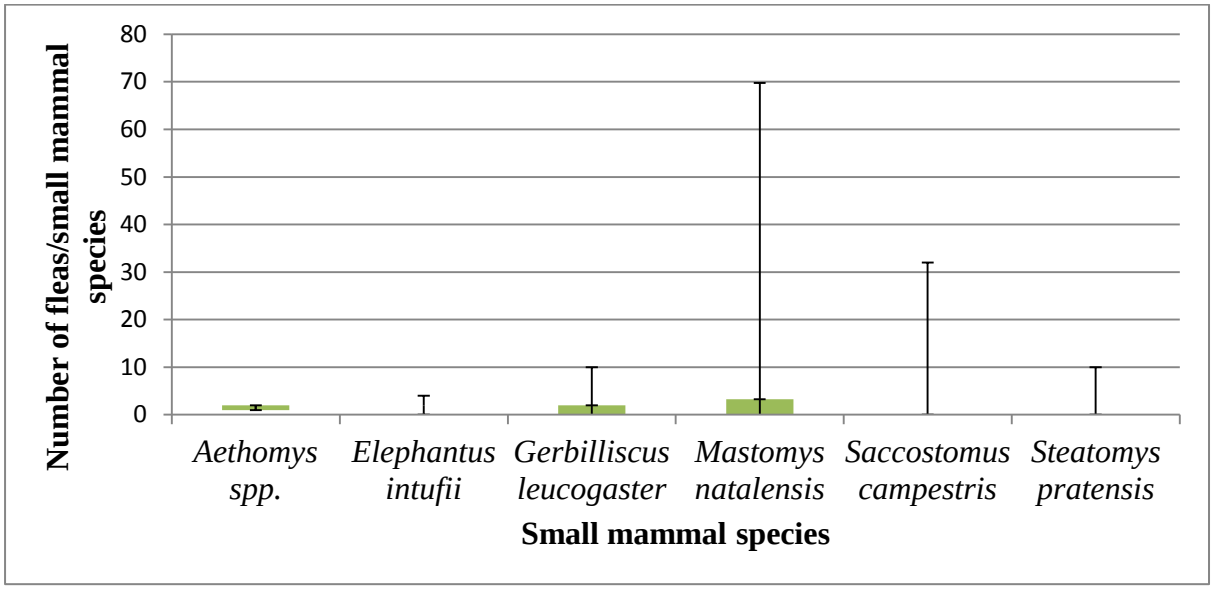

Figure 6: Median number of fleas on host species during the study period, in Mukwe Constituency of the Kavango East Region.

According to the Kolmogrov-Smirnov test, P=0.001, since the data was not normally distributed. The Kruskal-Wallis H test, there was no significant difference in the median number of fleas on different small mammal host species (χ^2=10.306, df=5, P=0.067).

5.2.2 Flea population fluctuations on small mammals in Mukwe constituency of the Kavango East region.

Generally, the abundance of different flea species on *M. natalensis* was high in November and low from May to September. The overall abundance of fleas per month entire study period at the two study sites combined.

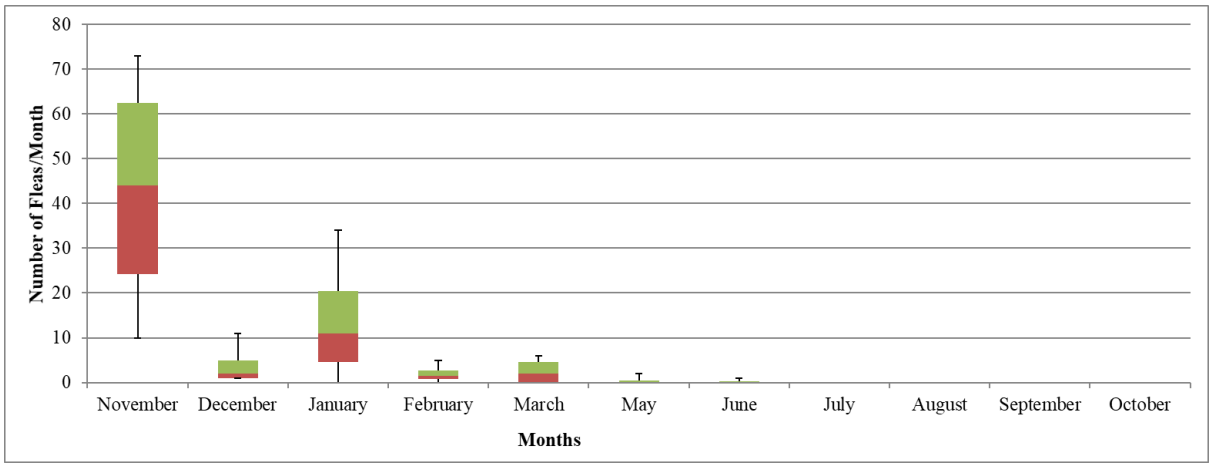

Figure 7: Median number of fleas on host species *Mastomys natalensis* from November 2014 to October 2015 (excluding April 2015).

November had the highest interquartile range (50% representation of the data). The median number of fleas was observed to vary with each month. November had the highest median about 45 fleas while, the lowest median was recorded from May to October with about one flea and nothing for the other months respectively. The box plots are skew towards the top for each month indicating a non-normal distribution. Kruskal-Wallis H test showed that there was a statistically significant difference in the flea population fluctuation on *M. natalensis* from January to December (χ^2=29.440, df=10, P=0.001).

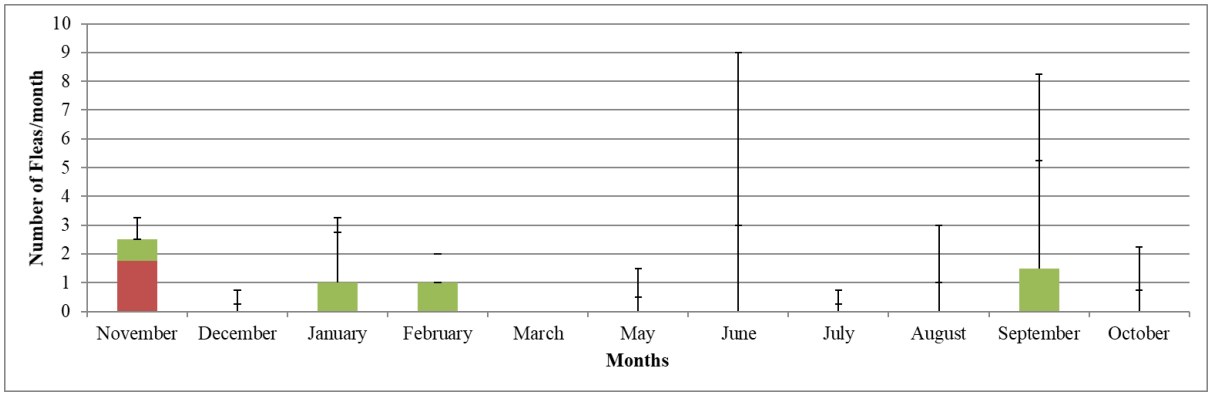

Figure 8: Median number of fleas on host *Gerbilliscus leucogaster* from November 2014 to October 2015 (excluding April 2015).

The total number of fleas collected is 59, the median number of fleas per month is skew towards the top. The median number of fleas per month was the highest in November 2 fleas. There was one species recorded in December, May, June, July, August and October. Kruskal-Wallis H test showed that there was no significant statistical difference in flea population fluctuations in the host species *G. leucogaster* from January to December (χ^2=10.831, df=10, P=0.371).

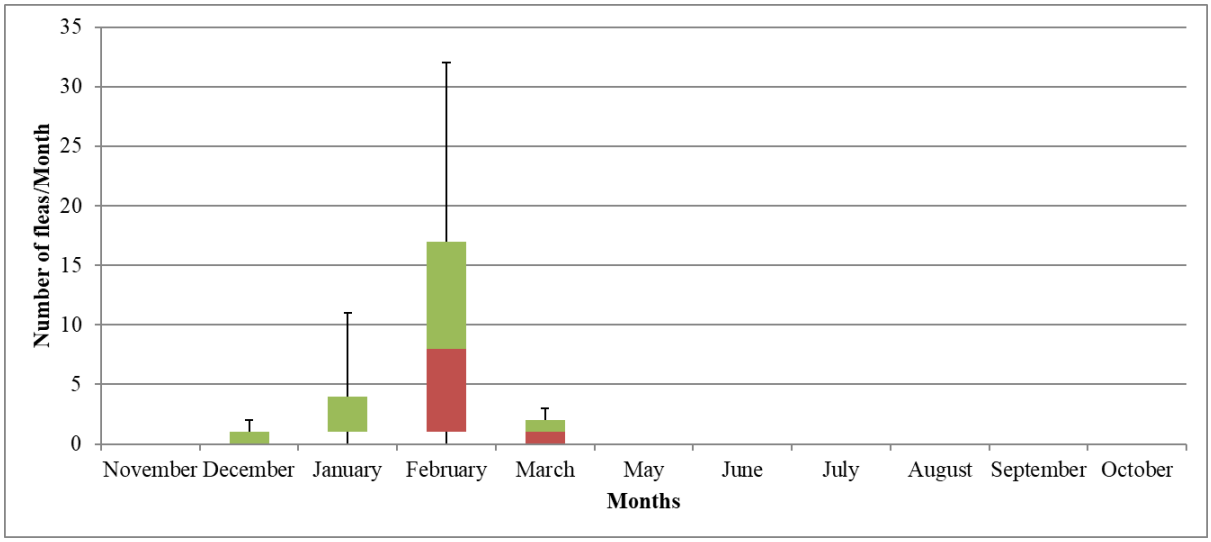

Figure 9: Median number of fleas on *Saccostomus campestris* from November 2014 to October 2015 (excluding April 2015).

The total number of fleas on the host species (all flea species grouped together) was 84 fleas. The highest median number of fleas per month was recorded in February 7 fleas/month, lowest from March and no fleas recorded as from May. A Kruskal-Wallis H test showed that there was a statistically significant difference in the flea population fluctuations on host species *S. campestris* from November to October (χ^2=32.681, df=10, P<0.0001).

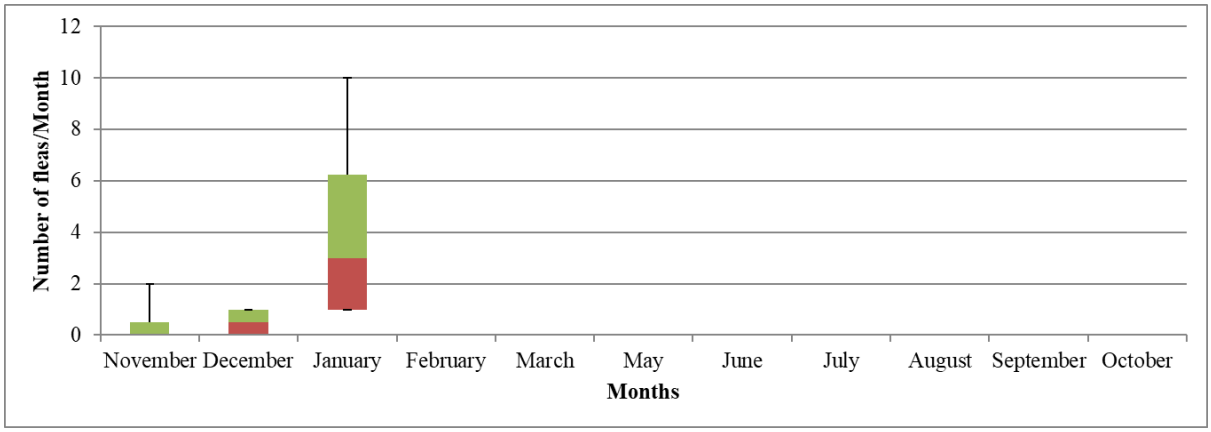

Figure 10: Median number of fleas on host *Steatomys pratensis* during the study period from November 2014 to October 2015.

The overall number of fleas recorded from the host species (all species grouped together) was 21, the highest median was recorded in January with 3 fleas per month while, the rest of the month from February did not have any fleas recorded. Kruskal-Wallis H test showed that there was a statistically significant difference in flea population fluctuations on host species *Steatomys pratensis*. from November to October (χ^2=30.521, df=10, P=0.001).

5.2.3 The composition of fleas on small mammal species in Mukwe constituency of the Kavango East Region and diversity

Table 5: Jaccard's community similarity coefficient (S_{ij}) values for fleas on different small mammals species

	1	2	3	4	5	6
1. Mastomys natalensis	1					
2. Aethomys chrysophilus	0.84	1				
3. Gerbilliscus leucogaster	1	0.97	1			
4. Saccostomus campestris	1	0.87	0.92	1		
5. Steatomys pratensis	1	0.68	1	0.93	1	
6. Elephantulus intufi	0.94	0.67	0.77	0.61	0.89	1

Generally, all the small mammal species had similarity index of above 0.5 and they all had the same kind of species. Results from the Jaccard's similarity coefficients indicated a high (more than 50%) similarity coefficient for all small mammal species trapped during the study period from January to December (excluding April 2015) at the two study sites (Table 5).

Table 6: Distribution of flea species on small mammals at the two study sites.

Fleas	Small mammals (Host)					
	Aethomys chrysophilus	*Elephantulus intufi*	*Gerbilliscus leucogaster*	*Mastomys natalensis*	*Saccostomus campestris*	*Steatomys pratensis*
Cryptonella numae	-	-	-	-	X	-
Pulex irritans	X	X	X	X	X	X
Pariodontis r. riggenbachi	-	X	X	X	X	X
Synosternus caffer	-	-	X	X	X	X
Xenopsylla spp.	X	-	X	X	X	X

Excluding *E. intufi* and *A. chrysophilus*, all the species had the same distribution of flea species at the study site. Almost all small mammal species had closely supporting the same flea species except for *A. chrysophilus* and *E. intufi* (Table 6).

5.2.4 Flea diversity on small mammal species at the two study sites

The diversity index for the different host species revealed that all the flea species diversity were in the same range.

Table 7: Flea species diversity (H') on small mammals at the two study sites.

	Host species composition					
Fleas Species	*Aethomys spp.*	*Elephantulus intufi*	*Gerbilliscus leucogastr*	*Mastomys natalensis*	*Saccostomus campestris*	*Steatomys pratensis*
Cryptonella numae	0	0	0	0	8	0
Pulex irritans	1	2	37	141	28	7
Pariodontis riggenbachi riggenbachi	0	1	4	39	3	10
Synosternus caffer	0	0	3	11	1	1
Xenopsylla species	1	0	15	100	47	3
Species diversity (H')	**0.64**	**0.7**	**1.08**	**1.12**	**1.1**	**1.14**

The table shows the number of fleas that were recovered from the host species at the two study sites. *M. natalensis* had the highest number of fleas (144), While *Aethomys* spp. Had the lowest number of fleas (2). However, the diversity did not differ from each other despite the high abundance of fleas on *M. natalensis*. According to the Kruskal-Wallis test, species diversity was not significantly different on host species at the two study sites (χ^2=5.00, df=5, P=0.416). Since, the actual diversity index were used instead of the means of the diversity index, this indicates that the diversity index were not different from each other among the host species.

5.3 Correlations between temperature and flea abundance on small mammals at the two study sites.

Fleas are very sensitive to high temperature (Laudisoit *et al*., 2009), in this study we investigated if the average temperature had any influence on the fleas in the study sites from November 2014 and October 2015.

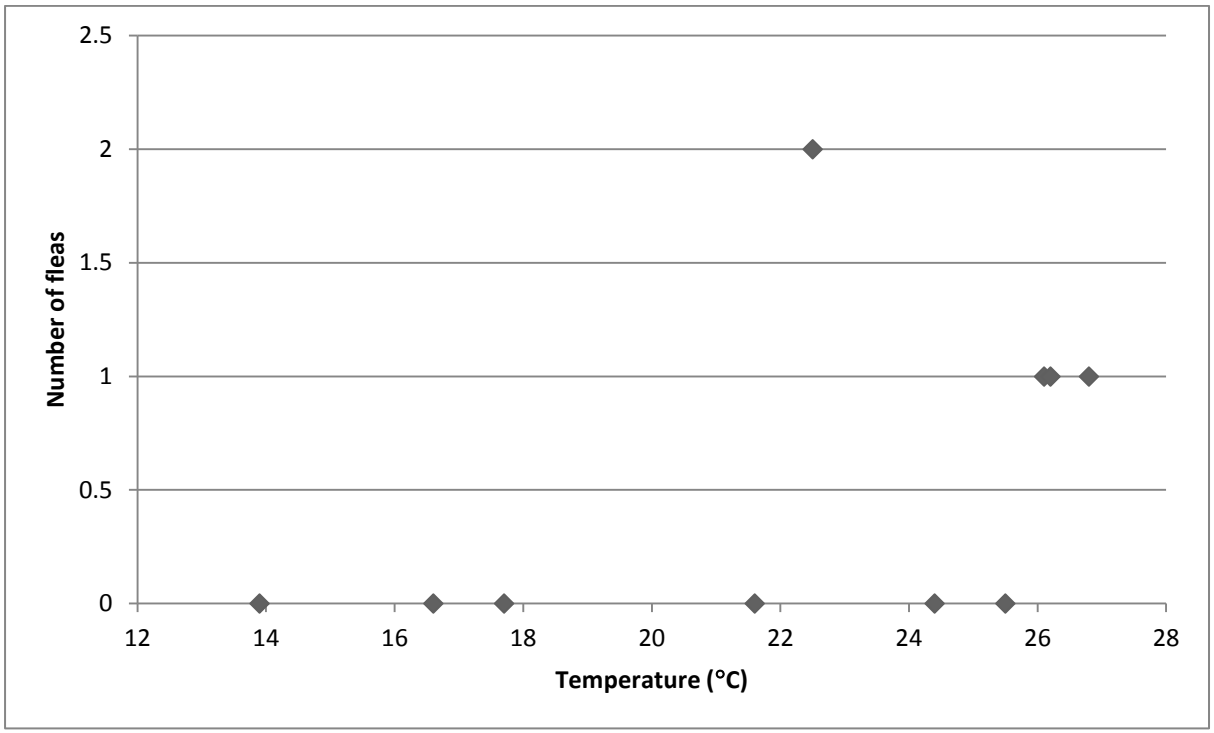

Figure 11: Correlation between temperature (°C) and the median number of fleas (all species grouped together) at the two study sites.

Based on Figure 11, there was a weak positive correlation (r=0.34, df=9, n=11, p=0.310) recorded between temperature (°C) and the median number of fleas from November 2014 to October 2015 (excluding April 2015).

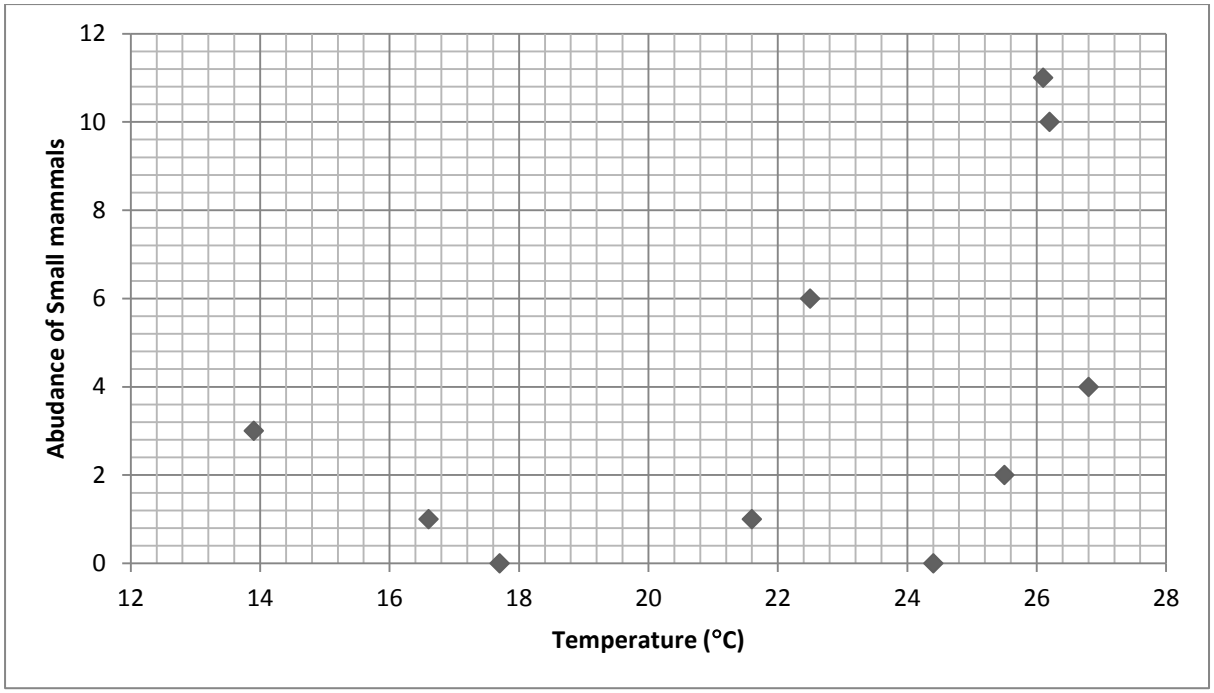

Figure 12: Relationship between small mammal abundance and temperature (°C) at the two study sites.

On the basis of Figure 12, there was a positive correlation recorded between the abundance of small mammals (all species grouped together) and temperature ($r=0.3$, $df=9$, $n=11$, $p=0.35$).

5.4 Correlation between relative humidity, flea abundance, and small mammals at the two study areas.

All the flea species grouped together, and all small mammals species abundance grouped together, the following results were obtained for this study, in relation to relative humidity and abundance of fleas.

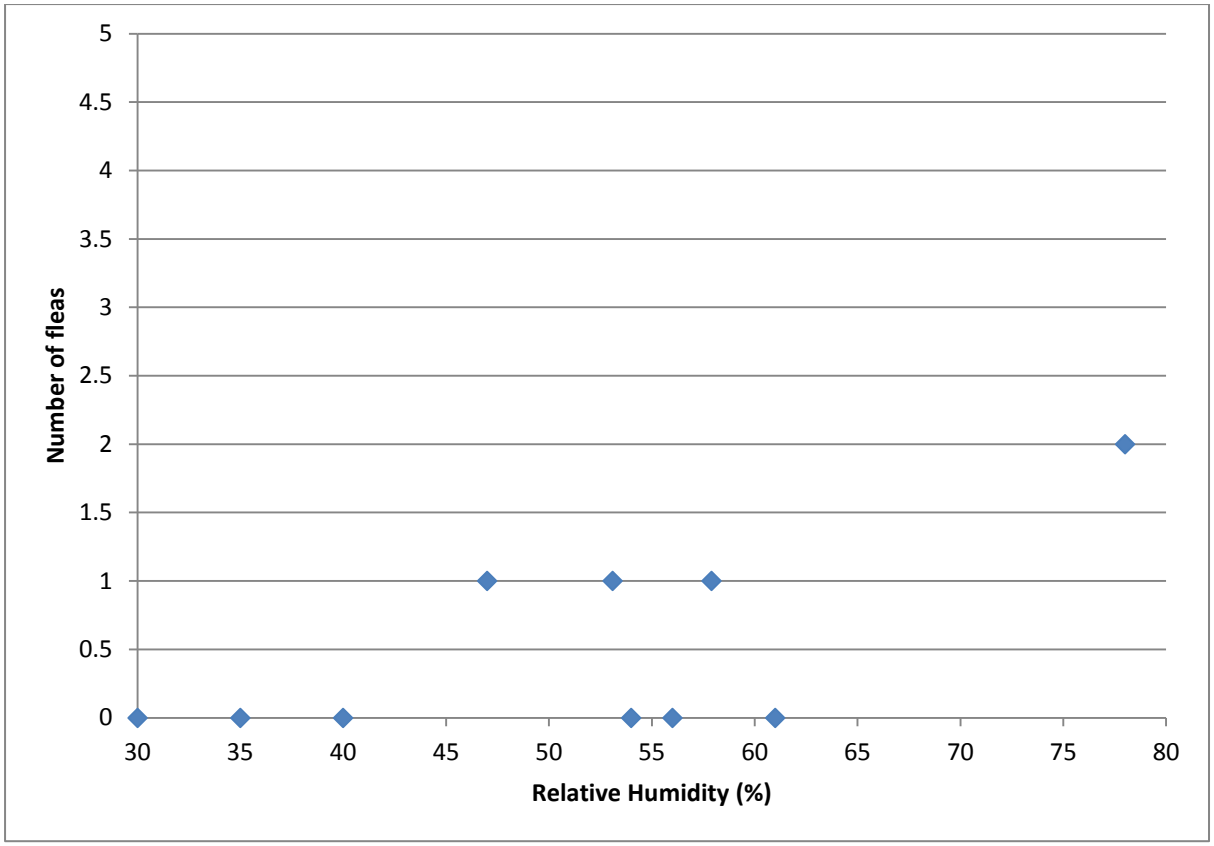

Figure 13: Relationship between relative humidity (%) and median number of flea at the two study sites.

Based on Figure 13, there was a weak positive correlation observed between the median number fleas (all species together) and relative humidity (r=0.4, df=9, n=11, p=0.224).

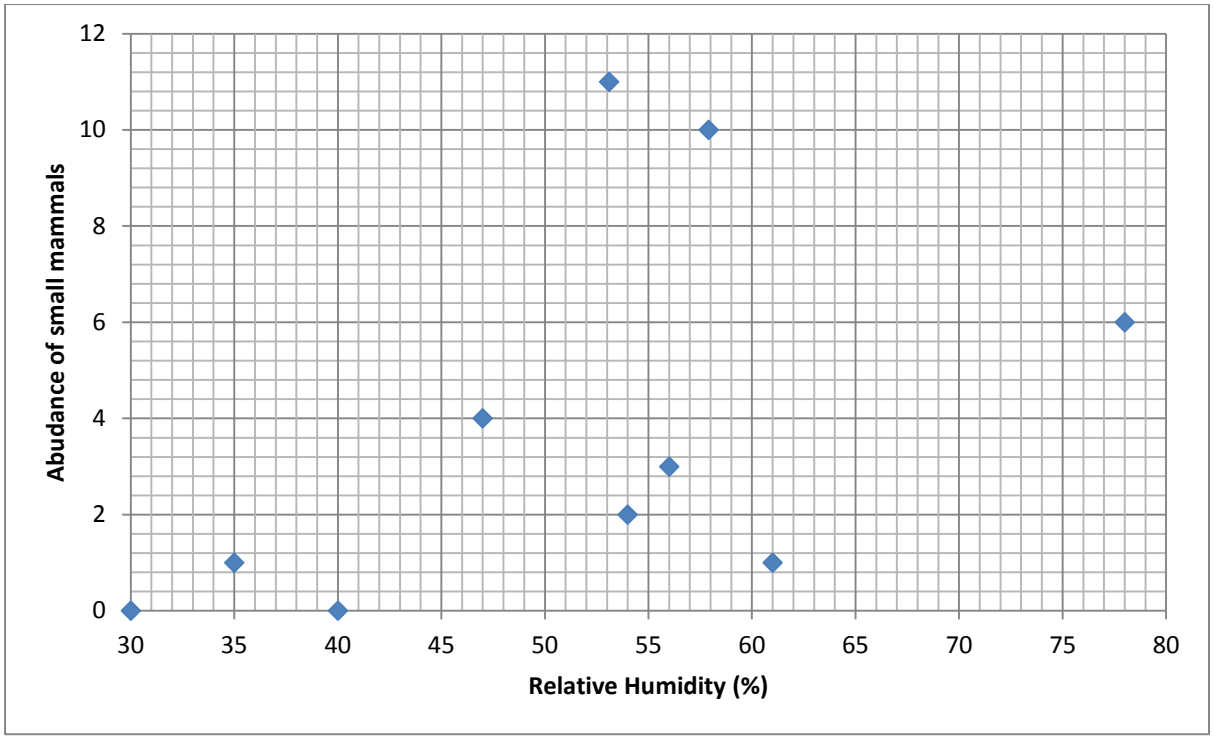

Figure 14: Relationship between relative humidity (%) and abundance of small mammals at the two study sites.

There was a positive correlation (r=0.6, df=9, n=11, p=0.109) observed between relative humidity and the average abundance of small mammals (all species grouped together) from November 2014 to October 2015 (excluding April 2015).

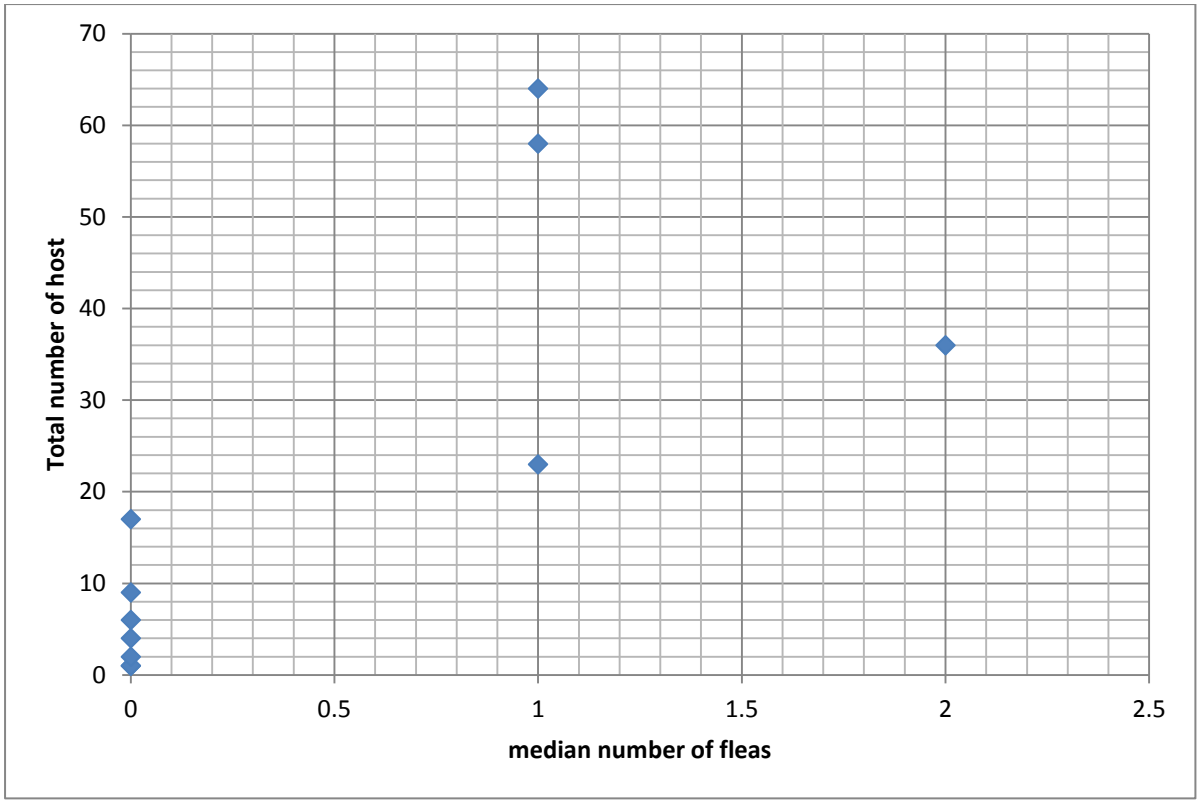

Figure 15: Relationship between total number of host and median number of fleas from the two study sites.

There was a positive correlation (r=0.8, df=9, n=11, p=0.005821) between average abundance of host species (all species grouped together) and average abundance of fleas (all species grouped together).

Chapter 6

Discussion

The small mammal species composition in Mukwe Constituency is similar to those found in the plague foci namely Oshikoto and Ohangwena regions of Namibia. *Mastomys natalensis* and *Gerbilliscus leucogaster* were the most dominant species in the region throughout the year. This study documents the first encounter of *E. intufi* and *Aethomys sp*. in Mukwe Constituency of the Kavango East region. However, in this study *Rhabdomys pumilio* was not encountered although Katakweba *et al*. (2012) recorded it in the previous study. Mammals that were recorded in this study are similar to those recorded by Shihepo *et al*. (2008) and Katakweba *et al*. (2012). Indicating that these species are widely distributed in different agricultural habitats and fallow fields within the Mukwe constituency.

6.1 Pathogenic *Leptospira*

For the first time, information on *Leptospira* is documented in the Kavango East region and Namibia at large. Currently, in Namibia nothing is known about the actual impact of Leptospirosis, particularly on the carrier small or large mammal. In sub-Saharan countries, peridomestic rodents have been reported to be potential carriers of zoonotic diseases (Gratz, 1997) since, they frequently go into homesteads in search of food and in the process, they contaminate stored food with infected urine. On several occasions, species of small mammals such as *Mastomys natalensis* were recorded to move from fallow land fields into people's houses, according to a radio telemetry study done by Monadjem *et al*. (2011). These rodents can act as carriers of the bacteria from small mammal communities to other animals including humans when they come into homesteads. Incidence of transmission from animals to humans has, also, been documented to be related to rainfall, livestock holding and farming activities (Vries *et al*., 2014). Farming activities, such as crop farming, constitutes the main livelihoods

that local people depend on for survival in the Kavango East region and other northern regions of Namibia (NSA, 2011). Therefore, this puts them at risk of contracting pathogenic *Leptospira*. However, prevalence of the bacteria could not be included in this study because this study main objective was to document the small mammal host prevalence.

The overall prevalence of *Leptospira* from the examined small mammal species in the study regions was 9.9%, and ranged from 0 to 50 % between the different host species (Table 2). Such prevalence rates have been reported to vary considerably throughout Africa and other continents, such as in Benin reported to be 19.0% and 7.0% in southeast Asia, while in south-eastern Africa it was reported to be 11,0% (Houemenou *et al*., 2013; Cosson *et al*., 2014). Data from Morogoro, Tanzania, indicate that presence of rodents and insectivores in an area could serve as indicators of possible presence of *Leptospira* in an area, because they feed on insects that are occurring in dung of herbivores. Thus, they serve as carriers of the bacteria from large herbivores to other animals (Mgode *et al*., 2005). Rodents were further reported to be reservoirs of *Leptospira* in different environmental settings and areas of the world such as Asia and Africa (Houemenou *et al*., 2013; Cosson *et al*., 2014; Vries *et al*., 2014).

Insectivores such as *E. intufi* mostly feed on insects found in wet or moist soil or dung of herbivores (Rathbun & Rathbun, 2006). *Leptospira* survival is higher in those conditions because, of the optimal conditions for the survival of the bacteria leading to a higher prevalence of *Leptospira* in host species *E. intufi* in comparison to *M. natalensis* that was recorded to be the most abundant with low *Leptospira* prevalence as observed in (Table 2). Similar results were also recorded in Germany where a high prevalence of *Leptospira* was recorded in shrews compared to rodents (Mayer-Scholl *et al*., 2014) this might be attributed to the survival of the bacteria in animal dung.

At our study sites, *M. natalensis* was the most abundant species but it had a low prevalence of *Leptospira* compared to its abundance. Similar results were also reported by Mgode *et al*. (2015) for a variety of small mammal species, and this difference was suggested to be associated with different foraging behaviour of various small mammal groups (rodents and shrews).

Cosson *et al*. (2014) explained that the variation between the different host species could be attributed to their different habitat requirements or behaviour. The three main *Leptospira* spp. present on the African continent based on DNA extracts from rodent kidneys are *L. borgpetersenii*, *L. interogans* and *L. kirschneri* (Allan *et al*., 2015; Houemenou *et al*., 2015). From this study, *L. kirschneri* was the only species identified and other samples could not be identified due to low bacterial load. Obigegala *et al*. (2016) stated that human cases that are caused by this bacterium are scarce. However, it was reported to cause the following symptoms in dogs: diarrhoea, exhaustion and dehydration and in humans, it might cause unspecific illness that may include flu like symptoms thus it might be undiagnosed or overlooked with other fever causing illness. Since dogs and humans are mobile animals and they constantly move from one place to another these organisms may serve as carrier of the bacteria to other places as they move from one place to another. Based on the climatic conditions and the host species *Saccostomus campestris* that was reported positive with the bacteria suggest that this bacterium might be present in the Zambezi, Ohangwena and Kavango West region due to rodent population outbreak. Additionally, outbreaks might also serve as a possible trigger for *Leptospira* outbreaks in the neighbouring regions.

6.2 Fleas

The ectoparasite community is comprised mainly of *P. irritans* and *Xenopsylla* spp (Table 3). In total, 221 small mammals were captured with an overall trap success ranging from 0.2 to 10.7 % per month (Figure 5). Despite the below average rainfall amount recorded at the study site and elsewhere in the country, this seem to have little effect on the small mammals capture rate in comparison to similar trap success in similar habitats in Tanzania. The overall trap success in fallow land fields within Mukwe constituency of the Kavango East region was 36.8% (Figure 5), similar trap success rate were recorded in disturbed habitats on Mount Kilimanjaro in Tanzania (Mulungu *et al*., 2008).

The highest trap success in the region was recorded from November to March, which constitutes the first period of trapping from late 2014 to early 2015. The rainy season in the Kavango East region coincides with the above period (Mendelsohn and Obeid, 2003; MET, 2011). Many continental African small mammal species have been reported to be opportunistic animals, which includes eastern and southern African countries (Makundi *et al*., 2007; Mulungu *et al*., 2008). Therefore, they take advantage of the favourable conditions during the rainy season associated with denser vegetation cover and reproduce, which leads to higher densities. Conditions like this offers optimal conditions for survival of the litters. This in turn explains higher trap success and recovery of the fleas at the study sites from November to March (Figure 5).

The coldest period in the region occurs in June while the hottest is in October. The combination of population cycling and the physical removal of small mammals via trapping during the first few months of the study, lead to lower densities of small mammal populations during the period of lower temperatures.

According to Laudisoit *et al*. (2009) ectoparasites are subjected to changes in the environment, these changes can be increased or decreased by the following parameters: temperature, relative humidity and rainfall. In this study, the host small mammals were more abundant from November to March, a period of high small mammal densities, and their associated fleas (Figure 15). This can be attributed to the high vegetation density that occurs shortly after the rainfall, because vegetation serves as food source for the rodents (Massawe *et al*., 2012). Massawe *et al*. (2012) reported that rodents breeding season of rodents was associated with rainfall and cultivation of agricultural fields. Rainfall further aid in softening the earth for rodents that use ground burrows for rodent species that mostly use this type of burrow.

The dry period had low population density of the rodent population and their associated fleas (Figure 15), because of low survival chance of small mammals that is caused possibly by the following conditions: inadequate food source, low quality food and increased competition for resources within the habitat (Massawe *et al*., 2012). In addition, since the study was conducted in the fallow land fields, which were situated closer to homesteads, during the dry season small mammals can move from the fields into the homesteads in search of food (Monadjem *et al*., 2011) and move back during the day, leading to the low encounter during the study period.

Small mammals and their associated parasites can transmit more than 60 different types of diseases, e.g. bubonic plague that is caused by the bacteria *Yersinia pestis*. Makundi *et al*, (2008) stated that fallow land fields are sites where wild and commensal rodents continuously interact. This interaction and the continuous movement of commensal small mammals often from the field into the homesteads could contribute to contamination of the food source by urine or faeces. Once this occurs human can be infected with rodent borne zoonosis. Other route of infection includes handling contaminated rodent tissues or other animal tissues.

During the first few months of the field study, removal of animals had little effect on small mammal population density, probably associated with immigration through birth and local dispersal (Laudisoit *et al*., 2009). Removal of animals in the study area started in November and December, which coincides with rainy season and small mammal reproduction and in other words host abundance.

6.2.1 Composition of fleas and small mammal host

This study was conducted in the fallow agricultural fields, a habitat type that serves in portions of Sub-Saharan Africa for small mammals that are agricultural pests (Makundi *et al*., 2008; Mulungu *et al*., 2008), including native and introduced species. Fallow fields, serve a refuge and interaction between wild and commensal small mammals often occurs in such settings. Lofty (2015) explained that small mammal interactions in human modified habitats associated with, for example, deforestation, bush fire and other types of disturbance, is often correlated with higher than typical high parasite loads. This could explain the high parasite load of the species that were recovered in this study (Table 3).

Burning and deforestation are common agricultural activities that are practised for agricultural land acquisition in the Mukwe Constituency and in other African countries. This type of activity has been linked with increasing small mammal populations in human habitats, where food resources can often be plentiful. Moreover, Massawe *et al*. (2005) mentioned that agricultural activities lead to an increase in population densities of *M. natalensis*, which are opportunistic therefore, it was mostly abundant throughout the study period as the area was frequently disturbed by foraging lager herbivores (e.g. cattle) (Figure 5) and (Table 4).

Host species in fallow fields have a high chance of harbouring the same flea species as the regular disturbance of these area favours the survival of a few flea species. This in turn results in multiple parasites occurring on a single host individual and yielding similar

diversity index of fleas between the host species and a high similarity index (closer to one) as shown in Table 5.

Another contributing factor to a high similarity index between host species can be attributed to the type of host. Makundi *et al*. (2015) stated that burrow visitation by the same or different small mammal species can facilitate exchange of fleas between host species of the same or different species. *Saccostomus campestris G. leucogaster* do not dig their own ground burrows and occasionally use those of other species (Kingdon, 1997; Skinner and Chimimba, 2005; Mfune *et al*., 2013). This behaviour aid in acquisition of different fleas from different ground burrows that the host is visiting because often flea larvae do not develop into an adult flea until a host is available.

Animals such as *E. intufi* are territorial with confined movements within a home range of 0.25-0.6 ha (Kingdon, 1997; Skinner and Chimimba, 2005; Rathbun & Rathbun, 2006; Rathbun, 2015), the aggregation consist of same sex (female) which is protected by one male. Therefore, this species is proportionately more limited to being exposed to fleas (Rathbun, 2015), as it does not share the burrows with other individuals apart from itself and one juvenile at a time thus resulting in few species of fleas encountered on the host (Table 6). Another contributing factor to the low flea population on this host can be attributed to the low encounter during trapping using the Sherman live traps. Similar observation were reported in Ethiopia, were Sherman live traps were reported to have low trap success of shrews compared to pitfall methods because they are limited to enter such devices (Bantihun and Bekele, 2015). Mfune *et al*., (2013) also reported no fleas recovered from *E. intufi* from selected habitats in Windhoek. The low prevalence as attributed to the strong dirty smell that could be possibly from the diet of species as it feeds on insects in dungs of larger herbivores (e.g. cattle). However, reported that the species was reported to harbour other flea species in

the Southern part of Namibia (Eiseb, 2002) this might be attributed to the difference in the species composition of fleas that were reported in this study.

Krasnov (2008) explained that a single host species could harbour different flea species because these ectoparasites may prefer different portions of the host's body such as the head, hind legs, between toes, ears, etc. Therefore, this could contribute to high similarity index amongst the host species as those observed in Figure 5.

6.2.2 The population fluctuations of fleas on small mammal species at the study sites.

Different types of parasites interacting with host communities can be influenced by various factors associated with the host and in the local environment. At our study sites, *Mastomys natalensis* was the dominant host species compared to other small mammals living in fallow fields (Table 2). The associated high population density lead to an increased chance of higher parasite loads because, they had a higher chance of being exposd to parasites compared to other species like *E. intufi*. Based on Table 4 and Figure 6, most host species that had high parasite loads were trapped during the same period (November to January), when the sampling commenced and when the low rainfall amount was received in the study site. This lead to a high population density of small mammals after rainfall, which lead to a high flea load (Eiseb, 2002). In Tanzania, Laudisoit *et al.* (2009) reported that flea numbers were significantly higher during the dry season then the wet season. This is contrary to the results presented in the current study (Figures 7 to 11). The difference was attributed to the fact that most fleas were recorded during the rainy season, however the rainy season did not really have normal amount of rainfall recorded.

The rainfall amount that was recorded in the study site occurred over few days with an average of 64.5 mm/year compared to the average in the region, which usually occur in the range of 500-600mm/year. This can affect the host and flea populations in two ways: firstly,

this has resulted in a rapid increase in the host population and in turn, the fleas after the low rainfall received in the study area (Table 4). Due to the ideal conditions for the flea larvae survival, which has a high chance of host encounter and the dry soil that increase the survival of other flea life stages (larvae and pupae) thus increasing the abundance of adult fleas on the host as recorded in Table 3.

According to Laudisoit *et al*. (2009), seasonality of fleas is common in areas with little or strong seasonality, the Kavango East region is subject to seasonality that is characterised by warm wet, warm dry and dry cold. Since, flea population dynamics may be influenced on host species by the weather this can be an explanation for the result that were observed in Figures 7-11. However, some flea species may not have high overall abundance on the host because of the fur structure or anti-parasitic behaviour of the host, such as grooming. While abiotic factors like temperature and relative humidity may influence the abundance of the host and the fleas have been reported to be sensitive to both temperature and relative humidity, because high temperature and low humidity result in low capture rate of the host and low recovery of the parasites as recorded in Figures 12-15). Laudisoit *et al*. (2009), further reported elevation gradient to result in flea species replacing one another. *Xenopsylla* spp. and *P. irritans* were the common flea species in anthropogenic habitats and they have been reported to be important vectors of plague in several African countries (Hang'ombe *et al*., 2012). *P. irritans* is a human flea, however, in the study the flea was reported to occur on commensal and wild rodents. This might be attributed to the fact that fallow fields are sites were commensal and wild rodents continually interact. The dominant host species in this study (Table 1) *M. natalensis* is a semi commensal rodents and it continually move from the field into the nearby homestead and back to the field. Therefore, as it moves from one habitat to another it might take along the flea that is associated with humans (*P. irritans*). This species was also reported on different wild rodents at the Waterberg Plateau Park (Uusiku,

2007). Habitats like where the study was conducted in the Mukwe constituency are fallow fields were in certain cases less than 10 m away from human habitations. Therefore, small mammals and their associated ectoparasites are constantly in contact with local human inhabitants. Furthermore, during the night or dry season, these small mammals enter into houses to seek or look for food (Lofty, 2015).

G. leucogaster and *M. natalensis* are small mammals that can be common in fallow fields and have similar dietary needs during different seasons (Hang'ombe *et al*., 2012). However, in this study, *M. natalensis* was distinctly more common throughout the study, perhaps because it tolerates the ecological conditions of the fallow fields, as compared to the other species like *E. intufi* and *G. leucogaster*. *M. natalensis* is a pioneer species and conditions such as low rainfall and frequent field disturbance by humans and grazing animals does not seem to strongly affect this species (Hang'ombe *et al*., 2012). Female *M. natalensis* have been found in Tanzania carry embryos of different paternities in breeding season to have a high number of fertilized eggs (Borremans *et al*., 2013).

Frequent disturbance by herbivores influences the kind of small mammal communities that will be found in the area, which is another contributing factor in the stage of community succession of the fallow land. Early stage of succession (as in this study), will support more r-selected species such as *M. natalensis* that has been reported to occur in similar environmental settings in other African countries such as Tanzania.

Further, habitat use is also another factor that can influence the diversity and abundance of small mammals. In southern Namibia, Hoffmann and Zeller (2005) reported that large herbivores change the structure and species composition of the habitat and in overgrazed areas there was a lower abundance of small mammals associated with reduced vegetation cover. In the present study, fallow fields are also used for goat and cattle grazing.

Species like *Steatomys pratensis* were only recorded in the first period of the study, from November to January, as members of this genus collect and store food when it is available and during the hot season rarely exit their burrows, hence, the low encounter rates of this species in the hot season (Figure 10) (Skinner and Chimimba, 2005).

Based on data collected in California, Hubbart *et al.* (2010) explained that periods between on host and off host differ for different parasites and host. Fleas on ground squirrels were recorded to be high during the hot dry months when fleas and all other stages of fleas life cycle are less susceptible to dying because the soil has less moisture and survival of the larvae are increased. This observation was not observed in this study because of the low rainfall during the rainy season therefore most parasites were recorded during the rainy season (Figures 7-10) on different host species that were captured during the study.

6.2.3 Correlation between climatic variables (temperature, relative humidity) and flea and small mammal abundance at the study sites

Burrowing small mammals have tunnel systems that are infested by fleas of different life-cycle stages or eggs as both the host and the parasites have the same environmental preferences (Krasnov, 2008). It has been proposed that areas with warmer tropical conditions have lower flea diversity (Adler *et al*., 2001) and this might explain the low flea diversity in southeast Asia rain forest and South Vietnam (Adler *et al*., 2001; Lakim and Beaucournu, 2011). However, places like Mukwe constituency with strong seasonality are expected to have higher diversity that is correlated to the climate because, different conditions are needed for the survival of different life stages of the fleas.

Extrapolating from data presented in Figure 11, there was a positive correlation observed between abundance of fleas and temperature (r=0.3. This observation is contrary to data from Uganda reported on by Moore *et al*. (2015), where flea abundance was negatively correlated

with temperature. This divergence could be attributed to the difference in the species composition of the host encountered in the two countries and habitat types this study recorded small mammals that are pest in most agricultural crop fields. In addition to this, soil composition (soil type, how the soil particles are packed) was reported to influence the abundance of adult fleas occurring on burrowing host mammals, as this is related to aspects of moisture and temperature in the burrow systems and these environmental aspects are important for the development of immature stages of fleas (Moore *et al*., 2015).

The climatic conditions, rainfall and other weather patterns (relative humidity and temperature) in the region of our study sites did not follow normal yearly patterns. Certain aspects associated with our interpretations may be called into question more specifically; the average annual rainfall in the region is normally from 500-600 mm (Stohbach and Petersen, 2007). According to SASSCAL weather information and data from the Metrological Weather Office, the overall rainfall amount during the study from November 2014 to October 2015 (excluding April 2015), was 65.4 mm over 11 months. This was by far the lowest amount of rainfall that the region had received. Natural events such as drought facilitate disease outbreaks, as small mammals are obliged to move towards human habitations in search of food (Makundi *et al*., 2008).

In areas with strong seasonality, such as the Mukwe constituency, abundance of fleas will change in relation to environmental factors such as temperature and rainfall (Lofty, 2015). Low temperatures in winter (June and July) will influence flea abundance in different ways; firstly, it will interrupt the flea life cycle by decreasing or increasing the generation time and by decreasing the probability of finding a host, which will dampen flea reproductive cycles. The results of this current study indicate positive correlation between abundance of fleas and abundance of small mammals. In summer a high abundance of small mammals are foraging in the field thus increasing the chance of fleas to find a host but in winter, there is low or no

host species available for the fleas because its dry and they move to homesteads and other areas in search of food. The below average amount of rainfall (less than 500 mm per year) that was recorded over the study period, most of which fell over a few days during the months of November and December 2014, the population of fleas increased after a few days when the small mammals abundance was high (Figure 4).

Massawe *et al.* (2012) reported that rainfall influences the breeding season of sub-Saharan small mammals and is directly associated with population cycling. This is presumably associated with the availability of food and nesting sites. Apart from weather conditions, there are other factors that may influence the distribution of fleas, such as soil properties, specifically conditions that enhance flea reproductive cycles and survival (Mullen and Durden, 2009). However, in the current study, there was a positive correlation between abundance of small mammals and relative humidity. When low or no amount of relative humidity was recorded, this notably resulted in low abundance of small mammals because; at low humidity, there is an increase in search for food by the small mammals thereby increasing the chance to be occupied by the fleas. At high relative humidity, this indicates possible presence of rainfall, and when there is rainfall it also indicates a possible presence for high abundance of food from plant material available thus, they usually feed in a smaller home range size as compared to when it is dry.

The population fluctuation of small mammals is influenced by many factors, including relative humidity and temperature, a high percentage of relative humidity will indicate a possible presence of rain, and this result in soft soil for small mammals to dig their burrows. Fleas where positively correlated with abundance of small mammals because they provide the fleas with a place to live and reproduction however, time during which they occurred on host differed depending on the host species concerned of the fleas species and their behavioural activities of the host species (Borremans *et al*., 2013).

Another reason for low small mammals encountered can be attributed to the behavioural activities of the small mammals in the area, because of the below average rainfall that was recorded during the rainy season. Low rainfall amounts could not support high vegetation cover; this in turn resulted in low small mammal abundance from December.

Chapter 7

Conclusions

Results from this study confirm that *Leptospira* is present in the small mammal community of the Kavango East region of Namibia and they further affirm that the bacterium (*Leptospira*) is distributed world widely. Most of the host species except *Aethomys* spp were tested positive for *Leptospira*, therefore presence of *Leptospira* was found in 12 small mammals of five different species, but due to low bacteria load, confirmation and identification of the *Leptospira* species was only possible for one specimen that was identified as *Leptospira kirschneri* on host species *S. campestris*. Hence, it is possible that a higher diversity of pathogenic *Leptospira* may be sheltered by mammals in Namibia, which calls for an investigation through complementary methods such as *Leptospira* culture.

There was no significant difference in the abundance of fleas on the different small mammal host. Because of frequent disturbance of fallow agricultural lands by animals and humans, some small mammal species, such as *M. natalensis*, are notably more abundant at our study sites than others like *E. intufi*. The species composition of fleas consisted of *Cryptonella numae*, *Pulex irritans*, *Pariodontis riggenbachi riggenbachi*, *Synosternus caffer* and *Xenopsylla* species. There was no significant difference in the species diversity and distribution of fleas on host species.

All the identified flea taxa showed measureable population fluctuations, as a function of seasonality. The only exception was *G. leucogaster* that did not show any significant difference in flea abundance during the course of the study. In general, most flea species showed high rates of prevalence during the period from November to March. In this study there was a positive correlation recorded between flea abundance and environmental temperature. There was a positive correlation between flea and host abundance and relative humidity.

Chapter 8

Recommendations

The researcher recommends that the information from this study be given to the Ministry of Health and Social Services, since the results from this study confirmed the presence of the bacteria *Leptospira* in the small mammal communities of the Mukwe Constituency (Kavango East Region). However, one confirmed species is not large enough sample size to draw definitive conclusions on *Leptospira* in the region. The next step should be the initiation of an awareness rising within the communities. The high rainfall amount that will be received in this region following the drought period when the study was conducted. The awareness should tackle everyone in the community from the community members to the health professionals in the region, local community members need to be informed about another possible fever causing bacteria, so that they are aware of the signs and symptoms of the disease. Additionally, more data is needed on the population dynamics of the host small mammal populations and fleas populations on the diferent host, as this will increase our understanding on *Leptospira* epidemiology and fleas population dynamics in Namibia. Therefore, further research should focus on different environmental settings such as agricultural fields (rice and crop field), abattoir and natural forest in order to document the

different strains of the bacteria and fleas diversity in small mammal communities and to better understand the route of transmission of the bacteria from the small mammal communities.

It will be important to include a wide variety of potential host mammal species, including wild and domestic animals, to better understand the different bacteria present and their role in human health issues. Additionally, the next step is to include human screening of *Leptospira*, especially during the rainy season, which based on data from other African countries is the period of highest prevalence. Further studies should also include the use of pitfall buckets as trapping technique in addition to the method in this study and the use of live trap to capture small mammals. The Kavango East, West and the Zambezi region are all in the northern eastern part of the country that receives the high amount of rainfall annually because of the similar climatic conditions. Further studies should be carried out in all other neighbouring regions (Kavango West, Zambezi, Otjozondjupa and Ohangwena in order to include a larger geographic region in the country.

Population outbreak of small mammals has been linked to different factors including rainfall, based on information from (Leirs *et al*., 1996), there is a possibility of small mammal population outbreak in Mukwe constituency. This study was conducted in the dry period and following the dry period, high amount of rainfall shall be expected in this part of the country. Therefore, it is highly recommended the community members to engage in pest management strategies in order to reduce the loss from small mammal infestation, and transmission of rodent borne disease and the parasites that they carry (i.e fleas). Farmers can further all work together to start trapping at the same time and store their harvest in rodent proof containers such as a metal drum.

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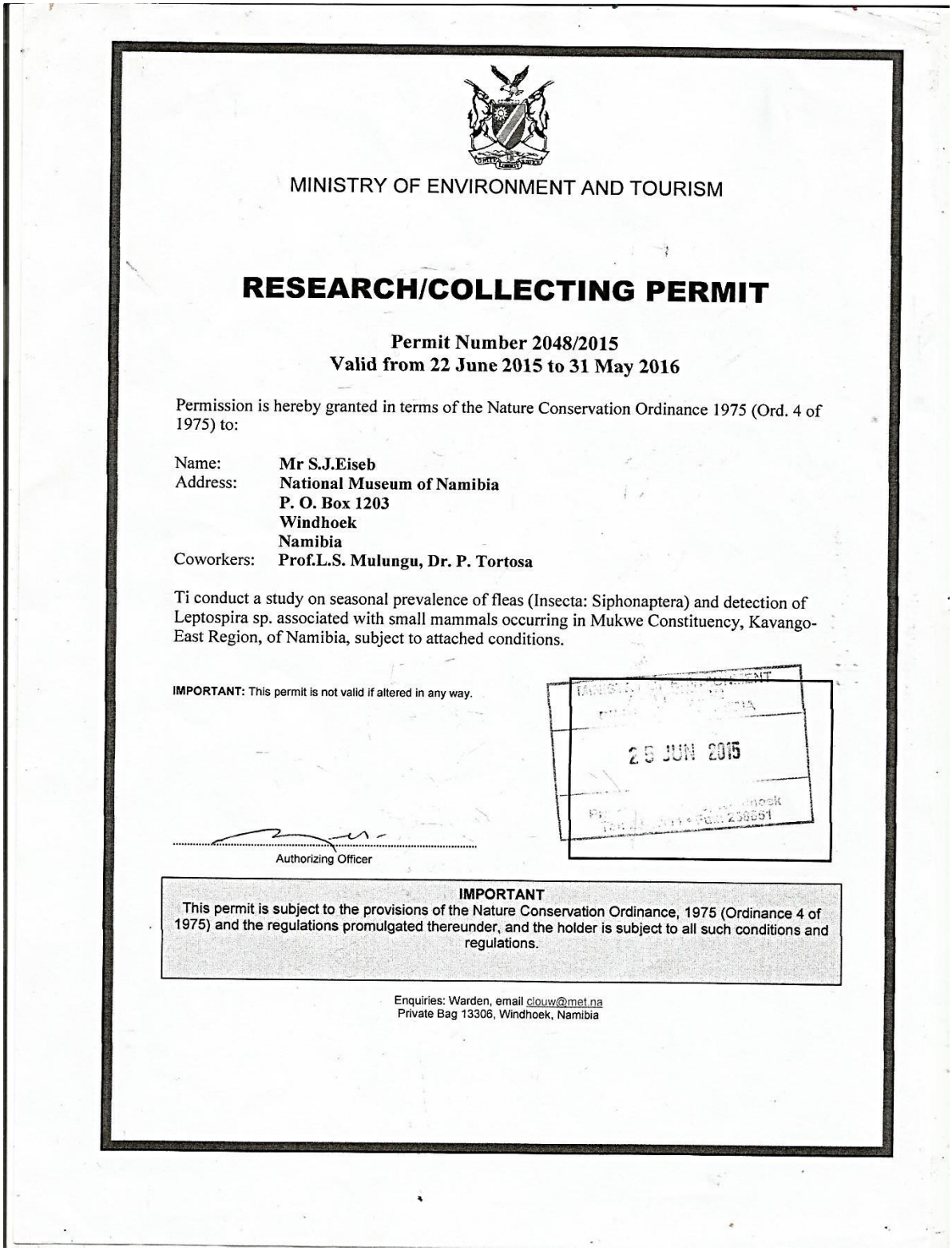
Appendices



MINISTRY OF ENVIRONMENT AND TOURISM

RESEARCH/COLLECTING PERMIT

Permit Number 2048/2015
Valid from 22 June 2015 to 31 May 2016

Permission is hereby granted in terms of the Nature Conservation Ordinance 1975 (Ord. 4 of 1975) to:

Name: **Mr S.J.Eiseb**
Address: **National Museum of Namibia**
P. O. Box 1203
Windhoek
Namibia
Coworkers: **Prof.L.S. Mulungu, Dr. P. Tortosa**

Ti conduct a study on seasonal prevalence of fleas (Insecta: Siphonaptera) and detection of Leptospira sp. associated with small mammals occurring in Mukwe Constituency, Kavango-East Region, of Namibia, subject to attached conditions.

IMPORTANT: This permit is not valid if altered in any way.

25 JUN 2015

Authorizing Officer

IMPORTANT
This permit is subject to the provisions of the Nature Conservation Ordinance, 1975 (Ordinance 4 of 1975) and the regulations promulgated thereunder, and the holder is subject to all such conditions and regulations.

Enquiries: Warden, email clouw@met.na
Private Bag 13306, Windhoek, Namibia

Recording sheet for captured small mammals host

Recording sheet small mammals host specimen				
Field number:			Date:	
Species:				Age:
Measurements (mm)				
Total length:		Head Body:		Tail length:
Hind foot:		Ear:	Weight(g):	
Ectoparasites collected: Yes/No				
Sex:				
Female:		Vagina: perforated/not		
Embryo :	Left	Right		
Ovulating:	Yes	No		
Lactating	Yes	No		
Male				
Testis condition:		Scrotal/Abdominal		
Epidermis condition:		con/not con		

Sample recording sheet for ectoparasites collected

Ectoparasite recording sheet			
Host field number			
Number of	Fleas	Ticks	Mites
Flea field number			
Sex of flea			
Family	Genus	Species	

Table 8: Total number of small mammal species recovered during the study period

Small mammals species	Number of small mammals captured each month from November 2014 to October 2015											
Scientific name	**January**	**February**	**March**	**May**	**June**	**July**	**August**	**Septembe**	**October**	**Novembe**	**December**	**Total**
Aethomys chrysophilus	0		0	0		0	0	0	0	1	3	**4**
Elephantulus intufi	0	2	0	2	1	0	1	0	0	0	1	**7**
Gerbilliscus leucogaster	4	4	1	2	7	1	3	1	2	4	3	**32**
Mastomys natalensis	22	6	6	3	9	0	0	0	0	54	42	**142**
Saccostomys campestris	6	10	2	0	0	0	0	0	0	1	2	**21**
Steatomys pratensis	7	0	0	0	0	0	0	0	0	4	6	**17**
Total	**39**	**22**	**9**	**7**	**17**	**1**	**4**	**1**	**2**	**64**	**57**	**223**

Table 9: Total number of fleas recovered per month during the study period

Fleas recovered	January	February	March	May	June	July	August	September	October	November	December	**Total**
Cryptonella numae	0	0	8	0	0	0	0	0	0	0	0	8
Pariodontis riggenbachi riggen	27	24	6	4	13	1	0	12	3	78	14	182
Pulex irritants	17	2	2	4	0	0	0	0	0	31	4	60
Synosternus caffer	2	0	1	0	0	0	0	0	0	13	1	17
Xenopsylla spp.	51	36	6	0	0	0	4	4	0	60	5	166
Total	97	62	23	8	13	1	4	16	3	182	24	**433**