UNIVERSITY OF KWAZULU-NATAL

Influence of human-associated tsetse habitat degradation on tsetse fly (Diptera: Glossinidae) populations and prevalence of infection with trypanosomes in North-Eastern Zambia

By

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As the candidate's supervisor, I have ~~/ have not~~ approved this thesis for submission

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DEDICATION

Dedicated to:

The memory of the late John Silutongwe (1951 to 2013) and late John Mashili (1965 to 2019), the two technicians that diligently undertook the tsetse dissections and associated microscopy in the field – work that was indispensable to the study.

PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Biological Sciences, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, South Africa. The research was financially supported by the University of KwaZulu-Natal and the Department of Veterinary Services in Zambia.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

Signed: **Prof. S. Mukaratirwa**

Date: 21 July 2021

DELARATION 1: PLAGIARISM

I, Kalinga Sageon Chilongo, declare that:

(i) the research reported in this thesis, except where otherwise indicated or acknowledged, is my original work;

(ii) this thesis has not been submitted in full or in part for any degree or examination to any other university;

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a) their words have been re-written but the general information attributed to them has been referenced;

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PUBLICATIONS FROM THESIS AND MY CONTRIBUTIONS

My role in each publication is indicated below. The * indicates corresponding author.

1. **Chilongo, K.,** Manyangadze, T., *Mukaratirwa S. (2020). Human Settlements and Spatial Distribution of Wing Vein Length, Wing Fray Categories and Hunger Stages in *Glossina morsitans morsitans* (Diptera: Glossinidae) and *Glossina pallidipes* (Diptera: Glossinidae) in Areas Devoid of Cattle in North-Eastern Zambia. *Journal of Medical Entomology,* **20(9)**: 1-9

I herewith declare that I was responsible for collection of the field samples of tsetse flies, examination and tests on the samples, literature research on tsetse well-being and human-associated tsetse habitat degradation. I further declare that I played the major role in the analysis of the data and discussion of the results, drafting of the manuscript and effecting changes as suggested and discussed with co-authors, and in responding to the comments received from the manuscript reviewers.

2. **Chilongo, K.**, Manyangadze, T., *Mukaratirwa S. (2021). Human-associated scarcity of hosts for tsetse flies (Diptera: Glossinidae) is related to an increase in prevalence of trypanosome infection in flies in North-Eastern Zambia. *Tropical Animal Health and Production*, **53**:305

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Signed: **Kalinga Sageon Chilongo**

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ABSTRACT

African trypanosomiasis is among the most important parasitic diseases of livestock and humans caused by several species of trypanosomes, and the disease occurs in 36 countries in sub-Saharan Africa. Human African Trypanosomiasis (HAT) causes a considerable public health burden on rural populations, and Animal African Trypanosomiasis (AAT) is an important constraint to livestock production and full utilization of land for agricultural production, such that if not controlled the disease can induce important losses through limiting crop production and access to land, and diminishing income from meat, milk and other livestock products, consequently resulting in poverty. In Zambia, approximately 40% of the country's land is tsetse-infested and the infestation in the Luangwa valley is among the most important with respect to occurrence of both human and animal trypanosomiais. In affected areas, occurrence of trypanosomiasis in humans and in livestock normally correlates with the prevalence of trypanosome infection in tsetse flies. Laboratory studies have shown that among the major factors that affect such trypanosome infection in tsetse flies, is occurrence of stress in tsetse flies. Occurrence of stress in wild tsetse fly populations is associated with unfavourable environmental conditions for the flies, and this is usually a consequence of tsetse habitat degradation. In many parts of Zambia's eastern tsetse belt, human-associated degradation of the tsetse habitat has been on the increase over the last decades. This suggests that research to determine the effects of such human-associated tsetse habitat degradation, on tsetse populations and prevalence of trypanosome infection in the tsetse population in the area, could provide some insights into the epidemiology of trypanosomiasis in the area.

In this study undertaken in three sites, Mpika, Lundazi and Rufunsa sites, in north-eastern Zambia (in parts of the eastern tsetse belt), the objectives were, to determine and measure (i) variation in size, age and hunger stages in tsetse flies and (ii) variation in prevalence of trypanosome infection in the tsetse flies, with increase in distance away from the edge into the inner parts of tsetse belt, and in relation to the distribution of human settlements; and (iii) to detect, assess and evaluate the contribution and importance of existing agricultural and other forms of ecosystem utilization, to tsetse-habitat degradation in the three sites.

Three study sites were selected based on level and pattern of human settlement, i.e. Mpika and Rufunsa sites with human settlement concentrated at or close to the edge of the tsetse belt, and Lundazi site with human settlement evenly distributed from the edge into the innermost parts of the tsetse belt. Samples of two species of tsetse flies found in the sample sites, i.e. *Glossina morsitans morsitans* and *G. pallidipes*, were collected and (i) size, age and

hunger stage in the tsetse flies were recorded and assessed with reference to distance away from the edge of the tsetse belt; (ii) variation in prevalence of trypanosome infection in the tsetse population in the study sites, with reference to distance away from the edge of the tsetse belt, and in relation to distribution of human settlements; and (ii) key land-use and socio-economic factors in the human settlements, with reference to human-associated tsetse habitat degradation in the study sites.

Trapping of the tsetse flies was done in defined sample points, identified with use of a Global Position System (GPS) unit, in the transect line, with use of the Black-screen fly round (BFR) and Epsilon traps. From the sampled flies, the following were recorded; species of fly, sex, body size, age and hunger stage (as indicators of levels of occurrence of stress), and screening for trypanosome infection using microscopy and the loop-mediated isothermal amplification (LAMP). A semi-structured questionnaire was administered in each of the settlements within our study location, and national land cover maps for the years 2000 and 2010, produced by the country's Forest Department, were used to estimate vegetation cover change during the period 2000 to 2010 in each of the sites.

Regression models were applied to determine and measure the level of association of the distance from the edge into the inner parts of the tsetse belt with; size, age and hunger stages of tsetse samples, and prevalence of trypanosome infection in the tsetse flies. In each settlement, data were collected on key land-use and socio-economic factors that may be linked to human-associated habitat degradation and changes in the vegetation cover during the period 2000 and 2010, was calculated in QGIS.

The results showed that in the Mpika and Rufunsa sites, the number of *Glossina morsitans morsitans* tsetse flies caught increased along with the increase in distance from the edge into the inner parts of the tsetse belt. This was also associated with increase in the body size ($p < 0.0001$ in both sites), and reduction in the age ($p < 0.001$ in each site) and the hunger stages ($p < 0.0001$ in both sites), and reduction in the prevalence of trypanosome infection ($p = 0.024$ and $p = 0.012$ in the case of all sub-species of trypanosomes tested for in the Mpika and Rufunsa sites respectively; and $p = 0.013$ and $p = 0.025$ in the case of only nannomonas sub-species in the two sites, respectively). The level of vegetation cover change was insignificant in each of the sites, such that it was unlikely to have had any significant impact on the quality of the tsetse habitat in each of the sites. In the Mpika and Rufunsa sites, human activities associated with access to resources might have had significant effect on the distribution of wild mammals that served as tsetse hosts in the area, such that numbers (of wild mammals) were low in locations that were close to the settlements and high in locations that

were furthest from the settlements – giving rise to a gradient of increasing levels of availability of tsetse hosts with increase in distance away from the human settlements. This same trend was observed with regard to the distribution of body size of the flies, age, hunger stages, and prevalence of nannomonas and trypanozoon trypanosome infection, in *G. m. morsitans* in the Mpika and Rufunsa sites. This was indication that (in the Mpika and Rufunsa sites) increase in the levels of availability of tsetse hosts was associated with increase in levels of tsetse well-being – in turn associated with increase in levels of tsetse habitat quality.

With regard to the findings in the Lundazi site (where human settlement was evenly distributed in transect line), the absence of any such variation (in each of the respective attributes in *G. m. morsitans*) with distance from the edge of the belt, could be taken as supportive to the reason indicated above as the likely basis for the existence of a gradient of reducing levels of tsetse habitat degradation in the Mpika and Rufunsa sites. In the case of *G. pallidipes*, the results showed no variation in the respective features in the tsetse flies, with increase in distance from the edge of the tsetse belt, and factors such as the relatively fewer numbers of the species caught, and a large proportion of the transect length not having registered any catch of the species, in each site in the study, likely contributed to this outcome.

ACKNOWEDGEMENTS

Foremost, I wish to profoundly thank my supervisor and mentor, Professor Samson Mukaratirwa, for effectively guiding me through the study programme. The journey was long and with many hurdles, but his wisdom, tolerance, commitment, focus, patience and persistence saw him effectively provide the necessary guidance and support that led to this fruitful conclusion. I am highly indebted to him for this achievement. In the same vein, I also greatly thank Dr Tawanda Manyangadze, for providing valuable guidance and input into the two published manuscripts.

Sincere gratitude to my employers, the Ministry of Fisheries and Livestock in Zambia, through the Department of Veterinary Services, for funding the tsetse survey work that facilitated collection of the tsetse samples and undertaking of the questionnaire in the study sites. I salute members of the field teams in the study, for their dedication and commitment to the assignments undertaken, and for the good job done.

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There are many others not mentioned above who provided support in one way or another, I wish to express sincere gratitude to them all.

OUTLINE OF THESIS

The thesis consists of a total of 5 chapters. Each chapter contains its own references section. Chapters 2 and 3 were published in peer-reviewed journals.

Chapter 1 is an introduction and literature review on tsetse-transmitted trypanosomiasis, and factors in the transmission of the disease, particularly environmental factors, in relation to occurrence of stress in tsetse flies. The rationale for the study, justification, aims and objectives of the study, are presented.

Chapter 2 consists of a published manuscript titled cited as follows: **Chilongo, K.,** Manyangadze, T., Mukaratirwa S. (2020). Human Settlements and Spatial Distribution of wing vein Length, wing fray categories and hunger stages in *Glossina morsitans morsitans* (Diptera: Glossinidae) and *Glossina pallidipes* (Diptera: Glossinidae) in Areas Devoid of Cattle in North-Eastern Zambia. *Journal of Medical Entomology,* 20(9**)**: 1-9

Chapter 3 consists of a published manuscript cited as follows: **Chilongo, K.**, Manyangadze, T., Mukaratirwa S. (2021). Human-associated scarcity of hosts for tsetse flies (Diptera: Glossinidae) is related to an increase in prevalence of trypanosome infection in flies in North-Eastern Zambia. *Tropical Animal Health and Production*, **53**:305

Chapter 4 is a study on the "Human-associated activities related to tsetse habitat degradation in areas devoid of cattle in North-Eastern Zambia"

Chapter 5 provides synthesis of the findings of the study, significance, research gaps and suggestions for future studies, in respect of human-associated tsetse habitat degradation, occurrence of stress in tsetse flies, and tsetse-transmitted trypanosomiasis.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Tsetse-transmitted African trypanosomiases are a complex of diseases of man and livestock, collectively referred to as trypanosomiasis (Holmes, 2013). The disease results from infection with one or more species of extra-cellular protozoan parasites called trypanosomes that are transmitted by flies of the genus *Glossina* (tsetse flies) (Leak, 1999). The disease affects an estimated 9 million km^2 covering 39 countries in sub-Saharan Africa (Mattioli et al, 2016). Trypanosomiasis takes a variable course depending on the factors associated with both the hosts and the parasites, but is characterized in most instances by intermittent fever, anaemia, loss of body condition, reduced body condition, reduced productivity and, often high mortality (Holmes, 2013; Alsan, 2015).

Transmission of the disease to humans and livestock is dependent on occurrence of trypanosome infection in tsetse flies, and thus understanding factors that affect trypanosome infection in tsetse flies is important (Holmes, 2013). Findings by Kubi et al. (2006) and Akoda et al. (2009) indicate that in reared *Glossina morsitans morsitans* (*G.m. morsitans*) tsetse flies, occurrence of stress, particularly starvation, significantly increases the level of susceptibility of the flies to infection with *Trypanosoma congolense* and *T. brucei brucei* trypanosomes.

Noting the importance of occurrence of stress in tsetse flies in the transmission of trypanosomes to the flies, the study investigated, (i) occurrence of stress and trypanosome infection in *G. m. morsitans* and *G. pallidipes* tsetse flies, and (ii) occurrence of human-associated tsetse habitat degradation, and evaluated existence of any relationships among these in the eastern tsetse belt in north-eastern Zambia.

The quality of a tsetse habitat is defined by the level of availability of key environmental conditions that are critical to the well-being and survival of tsetse flies. Climate, vegetation cover and sources of blood meals (hosts), and changes that give rise to sub-optimal levels of these (in a tsetse infested area) are associated with occurrence of stress in the affected tsetse flies (Leak, 1999; Hargrove, 2004). Of these factors, human activities tend to have significant effect of vegetation cover change and availability of vertebrate hosts (sources of blood meals), mainly through clearing of vegetation for agricultural land use; building material for habitation, source of energy, and for many other uses including, hunting down and/or scaring away vertebrate animals (Reid et al, 2000; Saunders et al, 2012). Human-associated ecosystem degradation has over the years been a major factor in the occurrence of tsetse habitat degradation in Zambia, particularly in the eastern tsetse belt in north-eastern Zambia. This is

closely associated with the distribution of areas of concentration of human settlements at interface with wildlife (Mwanakasale and Songolo, 2011; Anderson et al, 2015). Levels of human-associated ecosystem degradation tend to be highest in locations that are close, than in location that are far away, from areas of concentration of human settlements (Reid et al, 2000). In a tsetse belt, the edge or limit of the tsetse belt represents a transitional zone (in space) from the area with suitable, to that with unsuitable, environmental conditions for tsetse habitation (Robinson et al, 1997).

In this study, against the background indicated, locations that were at the edge of the tsetse belt were looked at as potentially providing the least suitable conditions for tsetse survival and well-being, than those in the innermost parts of the tsetse belt. This formed the basis for investigating occurrence of stress and trypanosome infection in tsetse flies along a transect line that ran from the edge into the innermost parts of the tsetse belt. Noting the importance of human-associated activities in occurrence of tsetse habitat degradation, and presence of cattle as a factor in tsetse habitat quality, selection of study sites was done with due consideration of the distribution of human settlements and also absence of cattle.

The findings of the study are reported in chapters 2, 3 and 4. Chapter 2 presents findings on occurrence of stress in the tsetse flies, and chapter 3 provides the findings on prevalence of trypanosome infection along the transect line, in the three sites. In chapter 4, the outcomes of the investigation on the influence of human activities, in relation to the findings reported in chapters 2 and 3, are presented. The contents in chapters 2 and 4 have since been published in peer-reviewed journals.

1.2 Justification of the study

Considering the importance of prevalence of trypanosome infection in tsetse flies in the epidemiology of tsetse-transmitted trypanosomiasis, it follows that understanding of factors that affect trypanosome infection in tsetse flies are also important. Considerable research has been done on effects of tsetse habitat degradation on tsetse flies, but not much has focused on the subject in the context of areas with a gradient of increasing or reducing levels of human-associated tsetse habitat degradation, and in relation to occurrence of stress and prevalence of trypanosome infection in affected tsetse populations.

Kubi et al. (2006) reported that tsetse flies starved for periods of 3 to 7 days were more susceptible to trypanosome infection, that non-starved tsetse flies, and this was attributed to occurrence of nutritional stress in tsetse flies. This study, contributes to gaining insights into

human-associated tsetse habitat degradation and how it may affect the epidemiology of tsetse-transmitted trypanosomiasis in particular areas.

1.3 Aim of the study

To contribute to the understanding on, the influence of human-associated tsetse habitat degradation on tsetse fly (Diptera: Glossinidae) populations and prevalence of infection with trypanosome in North-Eastern Zambia

1.4 Objectives of the study

The following were the specific objectives;

a) To determine and measure variation in size, age and hunger stages in tsetse flies, with increase in distance away from the edge into the inner parts of tsetse belt, and in relation to the distribution of human settlements, in the Mpika, Lundazi and Rufunsa sites study sites in north-eastern Zambia.

   H_o: No change occurred in size, age and hunger stages in tsetse flies, with increase in distance from the edge into the inner parts of the tsetse belt, and in relation to the distribution of human settlements, in the Mpika, Lundazi and Rufunsa sites in north-eastern Zambia.

b) To determine and measure variation in prevalence of trypanosome infection in the tsetse flies, with increase in distance away from the edge into the inner parts of tsetse belt, and in relation to the distribution of human settlements, in the Mpika, Lundazi and Rufunsa sites study sites in north-eastern Zambia.

   H_o: No change occurred in the prevalence of trypanosome infection in tsetse flies, with increase in distance from the edge into the inner parts of the tsetse belt, and in relation to the distribution of human settlements, in the Mpika, Lundazi and Rufunsa sites in north-eastern Zambia.

c) To detect, assess and evaluate existing agricultural and other forms of ecosystem utilization, and their contribution and importance in tsetse-habitat degradation in the three sites.

H_o: Existing agricultural and other forms of human ecosystem utilization did not have any effects on tsetse habitat quality in the Mpika, Lundazi and Rufunsa sites in north-eastern Zambia.

1.5 Literature Review

1.5.1Tsetse-transmitted trypanosomiasis

African Animal Trypanosomiasis (AAT), also referred to as Nagana, is mainly caused by *Trypanosoma brucei brucei*, *T. congolense* and *T. vivax* transmitted by tsetse flies (Holmes, 2013). AAT impacts negatively on rural development, directly and indirectly, through its negative effects on livestock production and productivity and on access to land resources for agriculture (Kabayo, 2002; Franco et al, 2014). The economic loss attributed to the impact of the disease on livestock-agriculture production in sub-Saharan Africa is estimated at US$ 4.5 billion per year (Mattioli et al, 2016).

Trypanosomiasis in humans, commonly referred to as sleeping sickness (SS), is caused by *Trypanosoma brucei gambiense*, found in west and east Africa, and the disease manifests in the chronic form and *T. brucei rhodesiense,* found in east and southern Africa, with cases taking the acute form (Jordan 1986). Both forms of the disease have devastating effects on the host resulting in death (Simarro et al, 2008). It has been reported that over 60 million people, of whom the majority live in rural areas, are at risk of getting infected with Human African Trypanosomiasis (HAT) (Holmes, 2013). Occurrence and transmission of tsetse-transmitted trypanosomiasis involves interactions among the parasites, tsetse flies and wildlife hosts of the trypanosomes, susceptible livestock, and/or humans (Leak, 1998; Holmes, 2013).

1.5.2 Trypanosomes

Trypanosomes are flagellated protozoan parasites that cause trypanosomiasis in humans and mammalian hosts. The organisms belong to the order Kinetoplastida, Family Trypanosomatidae, and the genus *Trypanosoma* (Hoare, 1972). They are extracellular parasites mostly found in the blood circulation of their mammalian hosts, and there are several species of the parasites that are pathogenic to domestic animals (Holmes, 2013). In Zambia, and specifically in the eastern tsetse belt, the most prevalent species of trypanosomes are *T. congolense* followed by *T. brucei brucei*, and *T. vivax* in that order (Simukoko et al, 2007).

Tsetse flies are the only biological vectors of African animal and human trypanosomes, although it is also known that other biting insects may also transmit trypanosomes mechanically

(Desquesnes and Dia, 2003). In cyclic transmission, after a tsetse fly feeds on a trypanosome-infected host, the ingested parasites (in blood meal) undergo a developmental cycle within the insect vector, and this entails substantial morphological, biochemical and physiological transformations in the parasite whilst in the tsetse fly, to the final metacyclic stage that is infective to a new mammalian host (Leak, 1999; Peacock et al, 2012). Once inoculated into the mammalian host, through the bite of a tsetse, the metacyclic trypanosomes undergo development and multiplication at the site of infection, and eventually trypomastigote forms of the parasites are released into the blood circulation via the lymphatic system. In the mammalian host, the parasites constantly change their surface antigenic coat, the variable surface glycoprotein (VSG), to escape the host's immune system – and this is a major challenge with regard to development of a vaccine against the disease (Holmes, 2013).

1.5.3 Tsetse flies

1.5.3.1 Morphological features and classification

Tsetse flies belong to the phylum Arthropoda, class Insecta, order Diptera, family Glossinidae, and genus *Glossina*. There are three features that are unique to the flies, i.e. (i) wings that fold one on top of the other in scissors-like manner, (ii) presence of a hatchet-shaped cell in each wing, and (iii) a forward pointing and conspicuous proboscis (Buxton, 1955; Mulligan, 1970). Based largely on morphological differences in the structure of the genitalia, tsetse flies are classified into three groups or subgenera, namely fusca, morsitans and palpalis (Leak, 1999).

Classification at species level is based mainly on morphological differences in particular body parts, for example presence of black rings in the legs, length of scutellar bristles, length of hairs on the third antennal segment, among other features (Buxton, 1955). There are currently 31 documented species and sub-species of tsetse flies (Leak, 1999).

1.5.3.2 Life Cycle

Tsetse flies reproduce by adenotrophic viviparity, i.e. giving birth to live offspring nourished within the female parent by secretions from highly modified accessory glands and born at an advanced stage of development (Benoit et al., 2015). The female tsetse fly is fertilized only once during the course of its reproductive life, and the sperm is stored in the spermatheca from which sperm is released at every ovulation, facilitating production of one full-grown larva every 9 to 10 days. The larvae pupates within 1-2 hours after being deposited

into the ground, and the adult fly emerges after a pupal period that varies according to temperature, usually about 30 days at 24°C (Buxton, 1955; Leak, 1999).

Nutritional needs for all the non-adult stages in the life cycle is provided through blood meals taken by the female parent, and this indicates the importance of access to blood meals by females - for the survival and well-being of the female parents and the non-adult stages in the life cycle of tsetse flies (Hargrove, 2004; English et al, 2016)

1.5.3.3 Distribution

Tsetse flies infest about 10 million km^2 of sub-Saharan Africa, and the distribution of different tsetse groups is related to their habitat preferences (Leak, 1999). Species belonging to the fusca group occur in the dense, lowland rain forests of mainly West and West-central Africa, whilst the species of the palpalis group are basically forest-dwellers, and mostly found in the riverine vegetation of West Africa (Buxton, 1955; Leak, 1999). The distribution of species of the morsitans group often corresponds with the distribution of wild hosts in the savanna areas of West, East and southern Africa -in areas ranging from moist savanna woodlands, the margins of the forests, to dry savanna woodlands near the margins of the deserts (Buxton, 1955; Robinson et al, 1997; Leak, 1999).

In Zambia, the following five species and sub-species have been reported; *G. m. morsitans*, *G. m. centralis*, *G. pallidipes*, *G. brevipalpis* and *G. fuscipes*. The most prevalent and important vectors are *G. m. morsitans* and *G. m. centralis*. In the eastern tsetse belt, the most prevalent and important is *G. m. morsitans* (Robinson et al, 1997; Van den Bossche, 2001).

1.5.4 Impact of trypanosomiasis on animal and human health

The impact of a trypanosome infection on animal and human health depends on the susceptibility of the host and the pathogenicity of the trypanosome species involved in the infection (Van den Bossche, 2001; Holmes, 2013). Wild animals usually do not show severe clinical signs but can carry trypanosome species including *T. b. rhodesiense* and hence constitute important reservoirs of trypanosomes (Anderson et al, 2015). In susceptible domestic animals, particularly livestock such as cattle, the disease may be acute, but chronic forms of the infections are more common (Van den Bossche, 2001). The host-parasite interaction produces extensive pathology and severe anaemia, and affected animals lose condition and productivity is severely affected (Kabayo, 2002). Trypanosomiasis is often fatal and, at the

herd level, its negative impact is literally on all aspects of production and productivity of affected livestock species (Jordan, 1986; Shaw, 2004). Mortelmans (1984) estimated that if trypanosomiasis did not exist in sub-Saharan Africa, the affected areas could carry three to five times more livestock.

Trypanosomiasis in humans occurs in two stages, as follows; Stage 1 (the haemolymphatic phase) which is characterized by non-specific symptoms such as headaches and bouts of fever (in the absence of active sleeping sickness surveillance, this phase generally goes undiagnosed); Stage 2, the neurologic phase, occurs when the parasite crosses the blood-brain barrier, which leads to sleep cycle disruptions, paralysis, progressive mental deterioration, behavioural changes, and this ultimately results in death in the absence of treatment (Kennedy, 2008).

Tsetse flies are mostly found in wildlife zones, and therefore the risks of a person getting infected depends on human movements (associated with human activities) that tend to facilitate tsetse-human contact (Mwanakasale and Songolo, 2011). Animal trypanosomiasis (in livestock) mostly occurs in human-wildlife interface zones, or in areas previously occupied by wildlife that remain tsetse infested. In sub-Saharan Africa, the human population continues to increase significantly and this is associated with increased demand for land and land resources for livelihoods (Vinya et al, 2012; Brandt et al, 2017). In Africa, as it is worldwide, it is the rural populations that directly dependent on natural resources the most, for their livelihoods (e.g. agriculture, forest resources, and hunting), and this entails that it is rural populations that are most exposed to the effects and impacts of both human and animal trypanosomiasis (Jordan, 1986; Leak, 1999 Anderson et al, 2015).

1.5.5 Factors influencing ability of tsetse fly to transmit trypanosomes

Several factors are known to play a role in cyclic transmission of trypanosomes, and these are related to the tsetse fly, the trypanosomes and the environment (Leak, 1999).

1.5.5.1 Factors related to trypanosomes

Trypanosomes are grouped into three subgenera, i.e. Duttonella (Chalmers 1908), Nannomonas (Hoare, 1964) and Trypanozoon (Luhe 1906) based on their site(s) of development in the tsetse fly. In Jordan (1986) and Leak (1999), these differences among the subgenera are described as follows; development of Nannomonas trypanosomes (such as *T. congolense*) and trypanozoon trypanosomes (such as *T. brucei brucei*) partly takes place in the mid gut of the tsetse fly. Duttonella trypanosomes complete their development cycle within the

tsetse proboscis, and hence, are not affected by the tsetse immune system. Table 1 summarizes the site(s) of development of trypanosomes in the tsetse fly.

Table 1. Pathogenic trypanosomes and site of development in tsetse flies (adapted from (Jordan, 1986)

Subgenus	Species	Site of development in the tsetse fly
Duttonella (Chalmers 1908)	*T. vivax* (Ziemann 1905)	Proboscis only
Nannomonas (Hoare 1964)	*T. congolense* (Broden 1904) *T. simiae* (Bruce 1912)	Mid gut and proboscis
Trypanozoon (Luhe 1906)	*T. brucei brucei* (Plimmer and Bradford 1899) *T. brucei gambiensis* (Dutton 1902) *T. brucei rhodesiensis* (Stephens and Fantham 1910)	Mid gut and salivary glands

1.5.5.2 Factors related to the tsetse fly

Ability to transmit trypanosomes generally differs among the morsitans group (savanna species), the palpalis group (riverine species) and the Fusca group (forest species). Of the three groups, the species that belong to the morsitans group, except *G. austeni*, are efficient vectors of all trypanosomes(Leak, 1999). Species of the palpalis group are generally poor vectors except with respect to certain strains from West African which can transmit *T. vivax* trypanosomes, and *G. f. fuscipes* which are important vectors of the human trypanosomes. Tsetse flies of the Fusca group appear to be efficient vectors of *T. congolense* and *T. vivax*, but are poor vectors of trypanozoon trypanosomes (Leak, 1999). This variation in ability to transmit trypanosomes could have association with host preference among the groups of tsetse flies (Holmes, 2013).With regard to the sex of tsetse flies, female flies tend to have higher infection rates, but this is however, to some extent, associated with the fact that females live longer than males and therefore have a greater chance of feeding on an infected host and, picking up and maturing an infection (Mulligan, 1970). In terms of interpretation of observations on infection rates in tsetse flies, it is important to take into consideration the sampling bias of the tsetse sampling methods and/or devices (Vale, 1978).

In relation to trypanosome development in the tsetse fly, the capacity or level of effectiveness of the tsetse fly's immune system, during the time that ingested nannomonas

and/or trypanozoon trypanosomes are in the midgut, determines whether or not infection establishes.

1.5.5.3 Environment-related factors

The main factors associated with environmental conditions are, climate (particularly temperature), vegetation cover, and availability of hosts. Temperature affects both the pupa and adult stages of tsetse flies, such that higher than optimum temperatures tend to facilitate increased tsetse susceptibility to trypanosome infection (Leak, 1999; Akoda et al, 2009). Vegetation cover is critical as habitat for vertebrate hosts, and also provides the necessary shelter for tsetse flies against unfavourable weather conditions associated with occurrence of stress (Akoda et al, 2009). As indicated previously, vegetation cover as a critical component of tsetse fly habitat provides shelter for tsetse flies and also for vertebrate hosts. Non availability of hosts entails longer feeding intervals for the affected tsetse flies (i.e. to a high proportion of tsetse flies staying hungry for periods much longer than normal) and this increases prospects for starvation in affected tsetse flies (Brady 1992; Leak, 1999). Considering that starvation in tsetse flies depresses tsetse immunity and as such enhances prospects for trypanosome infection in affected tsetse flies, it follows that factors that diminish the quality of the critical environmental conditions for tsetse well-being and survival, affect prospects for trypanosome infection in tsetse flies (Kubi et al, 2006). Occurrence of stress is also associated with manifestation of detrimental changes in body size and in the proportion of young and/or old flies, with time, in affected tsetse populations (Brady 1992; Leak, 1999).

1.5.6 Human-associated tsetse habitat degradation

Ecosystems that serve as tsetse habitats also provide land resources for humans, and the associated human activities tend to give rise to deterioration of ecosystem function and capacity (Reid, 2000; Mwanakasale and Songolo, 2011). The nature and level of negative impact of human activities on a tsetse habitat is closely associated with the distribution of human settlements in the ecosystem, the human population size (population density) in the settlements, and the nature of the human activities (Reid, 2000; Vinya et al, 2012, Anderson et al, 2015).

With regard to distribution of human settlements in ecosystems that serve as tsetse habitat, the level of human-associated degradation of tsetse habitat tends to be highest in locations that are closest to human settlements, and lowest in location that are furthest from settlements (Reid et al, 2000; Lhoest et al, 2020). This is largely because land resources that

are in close proximity (to settlements) are much more readily accessible by the population, such that it is usually only after there is depletion of the land resources located close by, do people undertake to access the land resources further away from the settlements (Vinya et al, 2012, Lhoest et al, 2020). Furthermore, the rate of depletion of land resources in an ecosystem tends to be directly proportional to the human population density in the settlements located in or close to the ecosystem (Reid et al, 2000).

The nature of human activities in the ecosystems, the nature of land use and other human activities aimed at obtaining benefits from the resources provided by the ecosystem, are critical determinants of the nature and level of human-associated ecosystem degradation (Vinya et al, 2012, Revermann et al, 2018; Lhoest et al, 2020). Removal of natural vegetation cover for agricultural crops, for use as building material, for charcoal and fuel wood, leads to reduction in the vegetation cover that tsetse flies need for shade and shelter from extreme temperatures, and which also serves as habitat for vertebrate animals that serve as sources of blood meals for tsetse flies (Leak, 1999; Reid et al, 2000; Hargrove, 2004). Furthermore, wild animals in these ecosystems, , are usually also illegally hunted down by the humans for meat and for other benefits, and due to scarcity of wildlife, contact with humans as alternative hosts is high (Reid et al, 2000; Mwanakasale and Songolo, 2011; Anderson et al, 2015).

It may therefore be taken that where human settlement exists in a tsetse belt, the aspects of human activities indicated above tend to play an important role in determining the nature and magnitude of occurrence of human-associated tsetse habitat degradation, and consequently on the nature and magnitude of its effects on tsetse populations in affected areas.

1.6 References

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CHAPTER 2. EFFECTS OF HUMAN SETTLEMENTS AND SPATIAL DISTRIBUTION OF WING VEIN LENGTH, WING FRAY CATEGORIES AND HUNGER STAGES IN *GLOSSINA MORSITANS MORSITANS* (DIPTERA: GLOSSINIDAE) AND *GLOSSINA PALLIDIPES* (DIPTERA: GLOSSINIDAE) IN AREAS DEVOID OF CATTLE IN NORTH-EASTERN ZAMBIA

Chilongo K, Manyangade T, Mukaratirwa S (2020). Effects of human settlements and spatial distribution of Wing Vein Length, Wing Fray Categories and Hunger Stages in *Glossina morsitans morsitans* (Diptera: Glossinidae) and *Glossina pallidipes* (Diptera: Glossinidae) in areas devoid of cattle in North-Eastern Zambia, *Journal of Medical Entomology* **20(9)**, 1-9.

2.1 Abstract

Effect of human-associated habitat degradation on tsetse populations is well established. However, more insights are needed into how gradual human encroachment into tsetse fly belts affect tsetse populations. This study investigated how wing vein length, wing fray categories and hunger stages, taken as indicators of body size, age and levels of access to hosts, respectively, in *G. m. morsitans* and *G. pallidipes*, varied along a transect from the edge into inner parts of the tsetse belt, in sites that had human settlement either concentrated at the edge of belt or evenly distributed along transect line, in north-eastern Zambia. Black-screen fly round and Epsilon traps were used in a cross-sectional survey on tsetse flies at three sites, following a transect line marked by a road running from the edge into the inner parts of the tsetse belt, per site. Two sites had human settlement concentrated at or close to the edge of the tsetse belt, whilst the third had human settlement evenly distributed along the transect line. Where settlements were concentrated at the edge of tsetse belt, increase in distance from the settlements was associated with increase in wing vein length and, a reduction in the proportion of older, and hungry, tsetse flies. Increase in distance from human settlements was associated with improved tsetse well-being, likely due to increase in habitat quality due to decrease in effects of human activities.

Key words: Tsetse flies, *Glossina morsitans morsitans*, Tsetse fly belt, settlements, human activities, Tsetse habitat degradation, transect, stress factors, Zambia

2.2 Introduction

Tsetse flies infest an estimated 8.7 million km^2 and affect 38 countries in sub-Saharan Africa where they transmit human African trypanosomosis (HAT) (sleeping sickness) and animal African trypanosomiasis (AAT) (Kabayo 2002, Holmes 2013, Alsan 2015). The Eastern tsetse fly belt of Zambia, which is largely associated with the Luangwa Valley, is among the most affected areas in the country with regard to occurrence of both AAT and HAT (Anderson et al. 2011, Munangandu et al. 2012).

For a tsetse population to survive and sustain itself, it is critical that the flies are able to adequately reproduce and, have adequate access to blood meals, and to shelter from adverse climatic conditions (Leak 1999, Hargrove 2004). All stages of the tsetse life cycle are exclusively reliant on blood meals as the source of nourishment, indirectly through the female parent in the case of the larva and pupa stages (Hargrove 2004, English et al. 2016). This indicates the critical importance of availability and access to sources of blood-meals, i.e. hosts and capacity of tsetse flies to seek and find these hosts (Hargrove 1999, Van den Bossche and Hargrove 1999, Hargrove 2001). Because tsetse flies have no capacity to internally regulate body temperature, they need to find shelter whenever the ambient temperatures are too high or too low (Leak 1999, Hargrove 2004). With regard to reproduction, it is vital for females to adequately provide for the nourishment of the larvae and to find larviposition sites that provide appropriate micro-environmental conditions for development of pupae to ensure successful pupation (Hargrove and Brady 1992).

Reduction in tsetse habitat quality diminishes the level of availability of the key environmental conditions necessary for the survival of tsetse flies, and entails prospects for occurrence of stress and consequently mortality in affected tsetse population (Reid et al. 2000, Schowalter 2012, Anderson et al. 2015). Occurrence of stress in tsetse flies could with time give rise to particular changes in the characteristics of tsetse population including, change in the body size of tsetse flies, change in the proportions of young and/or old flies, and to longer feeding intervals (i.e. a high proportion of tsetse flies staying hungry for periods much longer than normal) (Brady 1992; Leak, 1999).

In large and continuous tsetse belts, particularly those associated with protected wildlife conservation areas, such as the Eastern tsetse belt in Zambia, reduction in tsetse habitat quality tends to manifest starting from the edges of the tsetse belt that constitute the interface with human settlements and hence human activities (Anderson et al. 2015). As such, in the absence of a physical tsetse fly barrier, the edge (or limit) of a tsetse belt usually marks a zone of transition from favourable to unfavourable environmental conditions for tsetse flies (Robinson et al. 1997, Reid et al. 2000, Schowalter 2012).

Human settlements are associated with human activity, and human activity is a major factor in degradation of natural ecosystems (Reid et al. 2000). In this regard, the higher the human population density, the higher the level of human-associated ecosystem degradation tends to be (Reid et al. 2000, Sala et al. 2000, Anderson et al. 2015). In terrestrial ecosystems, such degradation largely takes the form of reduction in vegetation cover, particularly as a consequence of agriculture and other activities associated with human livelihoods (Mwanakasale and Songolo 2011, Timberlake and Chidumayo 2011). Humans also negatively affect tsetse habitat quality through their direct impact on availability of animals, by scaring away and/or killing wild animals that serve as sources of blood-meals (Leak 1999). Thus, where tsetse flies exist, an increase in human population density and activity usually leads to a decline in the tsetse fly population density (Leak 1999, Reid et al. 2000, Mwanakasale and Songolo 2011, Ducheyne et al. 2009). Such observations have led to the argument that human-associated negative change in habitat quality could eventually result in disappearance of tsetse flies all together (Reid et al. 2000). However, human activity may also affect tsetse habitat quality favourably through keeping of livestock species such as cattle that serve as good hosts for tsetse flies (Van Den Bossche and Staak 1997). This indicates that tsetse habitat quality can be assessed based on the characteristics of the tsetse flies including wing body size, age, availability and access to hosts.

In view of this background, our study investigated how the distribution of wing vein length (as indicator of body size), wing fray categories (as indicator of age), hunger stages (as indicator of level of relative availability of hosts or relative level of access to hosts), and ovarian age categories, in *Glossina m. morsitans* and *G. pallidipes,* varied with increase in distance from the edge of the tsetse belt, and along a transect line, into the inner parts of the tsetse belt. This was undertaken in three sites that were devoid of cattle, two with the human population concentrated at or close to the edge of the tsetse belt, and the third with human population relatively evenly distributed along the transect line, in the eastern tsetse belt in north-eastern Zambia.

2.3 Materials and Methods

2.3.1. Study areas and design

The study was undertaken in three sites located in Lundazi, Mpika and Rufunsa districts respectively (Figure 1). The sites were selected based on absence of cattle, location and concentration of human settlements in relation to the edge of the tsetse belt, and presence of a road that ran from the edge into the inner parts of the tsetse belt, marking a transect line from the edge to a point located 15km, 45km and 46km inside the tsetse belt. With human settlements at and beyond the edge (outside) of the tsetse belt, the transect line (road) was taken to represent a potential gradient of increasing habitat quality, i.e. from the edge into the innermost part of the tsetse belt. In the Lundazi and Mpika sites, the last point on the transect line was located at the edge of the North Luangwa National Park (NLNP), and at the edge of the South Luangwa National Park (SLNP), respectively. In the Rufunsa site, the point that marked the end of the road, i.e. at the camp for wild life protection personnel (Wild life Scouts) in the area, well inside the Luano game management area (GMA), marked the last sample point in the site. In each site, the approximate edge of the tsetse belt was determined based on the maps by Evison and Kathuria (1982). From the approximate edge of the tsetse belt, the length of the transect line measured 15km, 45km and 46 km in the Rufunsa, Mpika and Lundazi sites respectively (Figure 2 and 3). The location of human settlements in relation to the edge of the tsetse belt, human settlements and wildlife conservation areas, was as indicated in Figure 2.

In all the sites, the transect line was such that the start point (at the edge of the tsetse belt) fell in location that was at the highest altitude (above sea level), and the last point was at the lowest altitude, i.e. in the transect line. Each of the transect lines ran from a point (at edge of tsetse belt) located either in the plateau area or in the transition zone from the plateau to the valley areas, to a location that fell in or close to the valley floor. The difference in altitude between the start point and end point was 1485m and 675m in the Mpika site, 805m and 561m in the Lundazi site, 928m and 786m in the Rufunsa site.

Among the criteria used to select the study sites was absence of a cattle population, and this was intended to eliminate presence of cattle as a factor in habitat quality in the respective sites, considering the importance of cattle (where they exist in a tsetse infested area) as hosts for tsetse flies (Van Den Bossche and Staak 1997). For each of the sites, information was collected from the district veterinary office on the distribution of cattle owning households in the veterinary camps that covered parts of the eastern tsetse belt in the district – i.e. through

the veterinary assistants that manned the respective camps. It was this information, largely based on registers of cattle owners and their location (per veterinary camp), and in combination with the other criteria indicated above, that guided the selection of the particular sites. The map in Figure 1 was created with application of QGIS (version 3.4 (Madeira)).

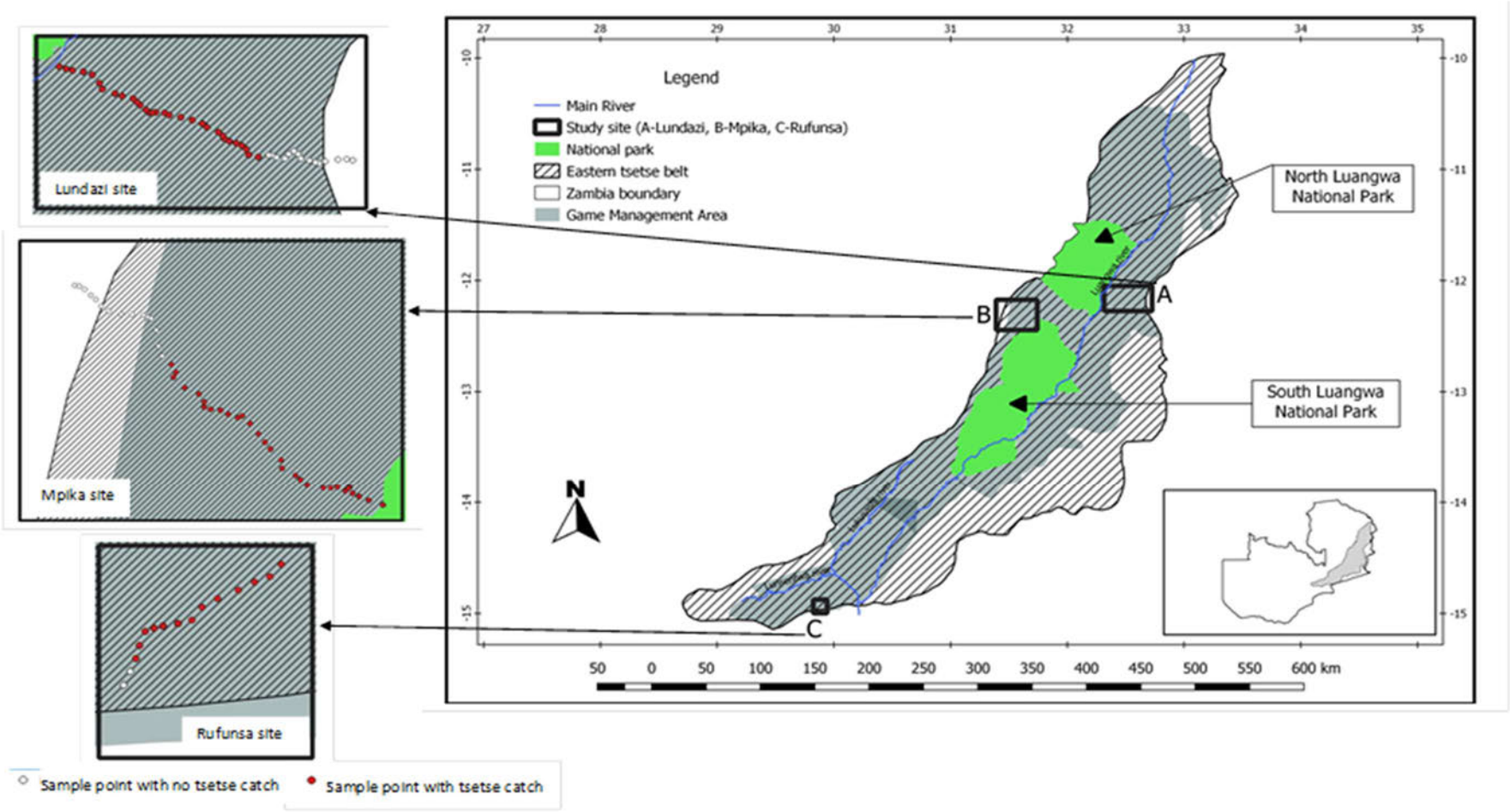

Figure 1. Location of the study sites and sample points in relation to the tsetse belt and wildlife conservation areas in north-eastern in Zambia

2.3.2 Collection of tsetse samples

Collection of samples of tsetse flies in each transect line was undertaken as indicated in Figures 2 and 3. Two methods of tsetse sampling techniques, the Black-screen man fly round (BFR) and the Epsilon trap, were used and deployed as indicated in Figure 3. The BFR was applied based on the method described by Potts (1930) except that it involved a pair of operators that carried a 1.5m x 1.0m whole-black fabric screen, with the pole carried horizontally on their shoulders and the screen hanging from a pole between them and kept vertical by weighting with a second horizontal pole at the bottom. In addition, Methyl Ethyl Ketone (Butanone) and 1-octen-3-ol (Octenol) were used as odour attractants, with the butanone dispensed through 500ml brown bottles at about 200mg/hour, and the octanol dispensed through 250µm thick polythene sachets with evaporation surface measuring 50mm (length) x 50mm width x 2 (sides) (Shereni, 1984; Vale and Hall, 1985; Willemse, 1991). The length of each BFR and the relative location of the traps in the transect line, were as indicated in Fig. 3. The pair of BFR operators stopped after every 100m for about 2 minutes to capture any tsetse flies that alighted on the screen, on the ground and on surrounding vegetation. Each BFR was walked two times during the day, in the morning (between 06:00 and 09:00) and in the afternoon (between 16:00 and 18:30), i.e. over a period of 2 to 3 hours per BFR, considering that tsetse flies are most active (e.g. hunting for hosts) in the hours just before and/or just after sunrise, and just before and/or just after sunset, when the temperatures are usually ideal for them to do so, i.e. neither too high nor too low (Leak, 1999). The start and end points of each BFR were marked in advance through brazing of marks on trees located on road-side, and so were points located every 500m from the starting point, with use of a vehicle odometer. An Epsilon trap was deployed (set up) at a point that was located as indicated in Fig. 3, but was deployed (set up) at a point that was located 5m to 10m away from the BFR sample point that was located in the middle of the road – i.e. the location that served as the sample point for both the BFR and the epsilon trap – the points for which GPS coordinates were recorded as such. In each trap site, the trap was deployed at least two days before the BFR was conducted in the particular sample point in the transect line – and a trap remained in each site for a period of about 20 hours – i.e. from about 14:00hrs on day 1 to about 10:00hrs the following day (on day2).

Samples were aggregated to ‘Sample points’ in the transect line each having covered a 1 km stretch of each BFR, and also to a ‘Section’ of the transect line that covered a stretch equivalent to one BFR (3 km) in the Rufunsa site (due to the shorter length of the transect line

in the site), and two BFRs (6km) in the Lundazi and Mpika sites – with the first 'sample point' and first 'section' located closest to the edge of the tsetse belt - and the last located furthest away from the edge of the tsetse belt.

Three BFRs were undertaken during any one sample collection session, i.e. with three pairs of operators (i.e. six operators). The same team of operators undertook deployment of Epsilon traps and collection of samples from the traps. Each pair of operators was led by a Tsetse Control Technician (TCT) or Tsetse Control Assistant (TCA) that had many years of experience with field application of the two tsetse survey methods. Two well trained technicians identified tsetse catches to species and sex, and undertook dissections to establish ovarian categories, on site. Examination of the samples for wing fray categories and for measurement of wing vein length was undertaken much later after the field sample collection assignments.

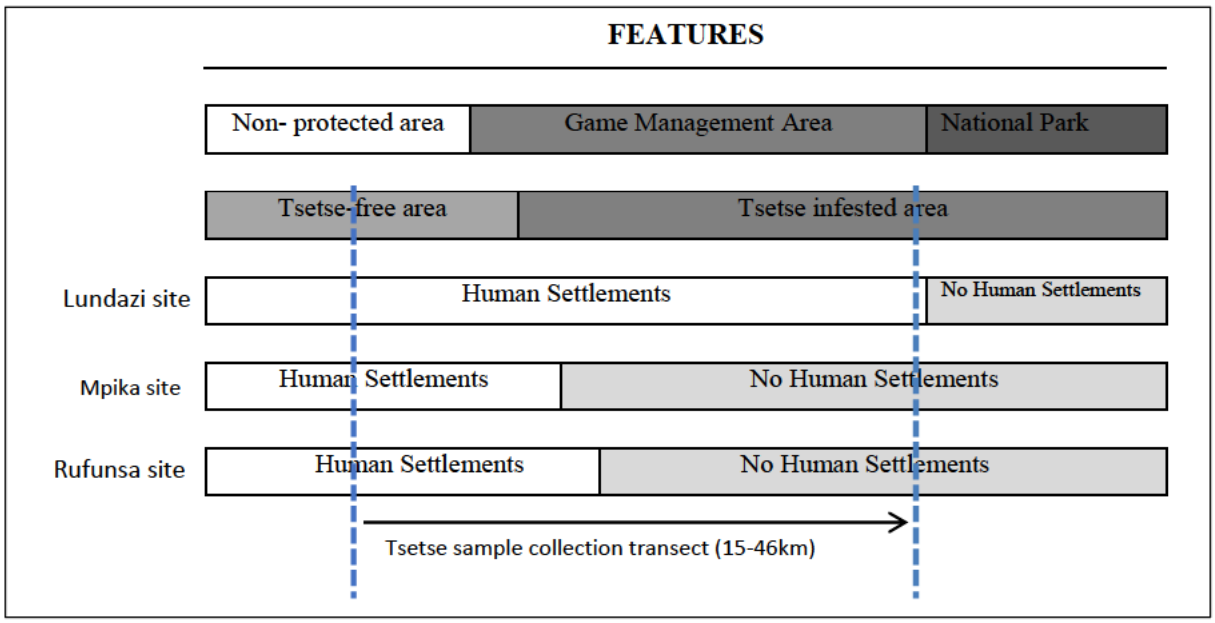

Figure 2. Location of sample collection transect line in relation to tsetse belt, human settlements and wildlife conservation areas, in the Lundazi, Mpika and Rufunsa sites in north-eastern Zambia

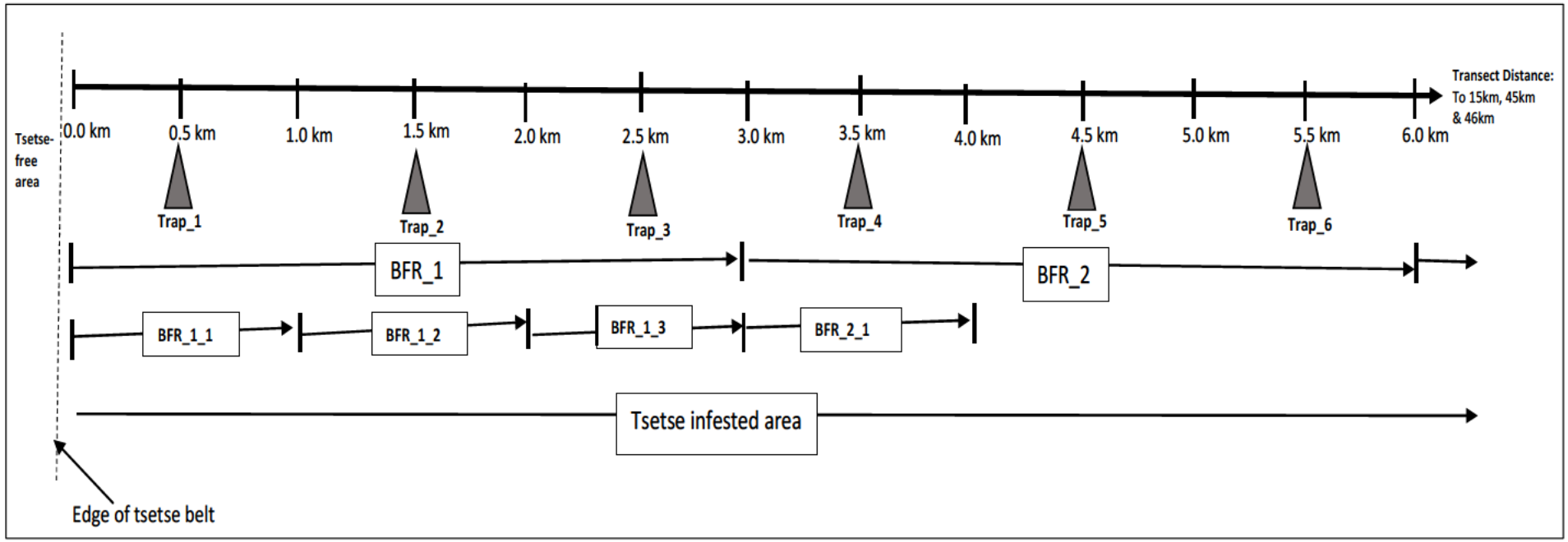

Figure 3. Relative location of Black-screen fly rounds (BFRs) and traps in tsetse sample collection transect line in the Lundazi, Mpika and Rufunsa sites in north-eastern Zambia

2.3.3. Examination of tsetse fly samples

Measurement of wing vein length, examination/assessment for wing fray categories, hunger stages, ovarian age categories, were undertaken with application of the procedures described by the FAO (1982). In the procedures; (i) levels of wing fray (level of damage to the wings) falls in six (06) categories (i.e. categories 1 to 6) – with category 1 having the least damage and category 6 having the most damage to the wings; (ii) Hunger stages, based on the appearance of the abdomen, ranging from stage 1 (newly and fully fed tsetse flies) to stage 4 (hungry tsetse flies); and (iii) Ovarian categories, based on the state of each of the fours ovarioles in relation to release of ova, ranging from ovarian category 0 (newly emerged adult) to category 7 or higher for tsetse flies that are 70 to 80 days old or older – noting that this is the highest category that can be determined in a straight forward manner through the method (Leak, 1999). Wing vein length, wing fray categories and ovarian categories have been applied by several authors, e.g. Allsopp (1985) and Hargrove et al.(2019).

2.4 Data analyses

The linear regression model was used to determine and measure association between wing vein length and increase in distance from the edge of the tsetse belt. The proportional ordinal logistic regression (POLR) model was applied to determine and measure association between wing fray categories, hunger stages and ovarian age categories, and increase in distance from the edge of the tsetse belt. This was done in R version 3.6.2, and specifically with use of the MASS package in the case of the POLR (R Core team (2016).

For each of the species of tsetse flies, the analyses were carried out on data for the categories ‘males & females’, ‘males only’ and ‘females only’. 95% confidence intervals were considered in all the estimates reported, and a p-value of less than or equal to 0.05 was considered statistically significant.

2.5 Results

2.5.1. Tsetse fly samples collected

A total of 1,731 tsetse samples were collected, 1279 *G. m. morsitans* (846 males and 433 females) and 452 *G. pallidipes* (206 males and 246 female). In each of the transect lines, the first tsetse fly was caught at a distance of 2.5km, 14.5.km and 18.5km from start point of the transect line (i.e. from the estimated edge of the tsetse belt) in the Rufunsa, Lundazi and Mpika sites respectively. The distribution of the two species and sexes of the tsetse flies in

relation to the two sample collection methods, study sites, and sections of transect lines, was as shown in Table 2. As shown in the table, the first two sections of the transect lines, in all the sites, recorded none or very few *G. pallidipes*. Most of the tsetse flies, particularly male *G.m. morsitans*, were captured in the BFRs. In the last two sections, Epsilon traps captured relatively more *G. pallidipes* than did BFRs. Tsetse sample collection was undertaken over a period of 2 weeks in the Rufunsa site and, 3 weeks per site in the Lundazi and Mpika sites, during the period September to December 2012. Samples collected were made up of two species, *Glossina m. morsitans* and *G. pallidipes*.

2.5.2 Wing Vein Length

The results were as shown in Figure 4 (distribution among sections of each transect line) and in Table 3. In the case of *G. m. morsitans* in the Lundazi and Rufunsa sites, increase in distance by 1 km was associated with an increase in wing vein length by 0.003 (0.002 - 0.005) ($p < 0.0001$), and by 0.005 (0.002 -0.007) ($p < 0.0001$) in 'males + females'; by 0.004 (0.002 - 0.005) ($p < 0.0001$) and by 0.005 (0.003 - 0.007) ($p < 0.0001$) in 'males only'; and, by 0.004 (0.002 - 0.005), $p < 0.0001$) and by 0.005 (0.003 - 0.007) ($p < 0.0001$) in 'females only', in the two sites respectively. No association between wing vein length in *G. m. morsitans* and increase in distance was observed in the Lundazi site. With regard to *G. pallidipes*, only in the Rufunsa sites was association observed between increase in distance and wing vein length, with 1 km increase in distance associated a 0.007 (0.001- 0.014) increase in wing vein length in 'males + females' ($p = 0.013$).

2.5.3 Wing Fray Categories and Hunger Stages

The outcomes were as shown in Figures 5 and 6, and in Table 4. In the case of *G. m. morsitans* in the Rufunsa and Mpika sites, increase in the distance was associated with a reduction in the likelihood of having an increase, and not a reduction, in wing fray categories and hunger stages, as follows;

(a) Wing fray categories:

In 'males + females', 1 km increase in distance was associated with a 4.1% (2 – 6%) and 8.5% (4.1 – 12.7%) reduction in the likelihood of an increase, against a reduction, in wing fray categories in tsetse flies caught, in the Mpika and Rufunsa sites respectively. Similarly for 'males only', the increase in distance was associated with a 4.6% (2.1 – 7.2 %) and 8.1% (2.8 – 13.0%) reduction in 'males only', and a 5.3% (1.8 – 8.7%) and 7.0% (2.0 – 18.5%) reduction

in the likelihood of an increase, as opposed to reduction, in wing fray category in 'females only' in tsetse flies caught, in the two sites respectively.

(b) Hunger stages:

Increase in distance by 1 km was associated with a reduction in the likelihood of finding tsetse flies with high levels of hunger stage, and not low levels of hunger stage, by 5.4% (3.4 – 7.5%) and 9.3% (4.9 – 13.4%) in 'male + females'; by 4.8% (2.2 – 7.2%) and 7.8% (2.7 – 12.8%) in 'males only'; and by 4.1% (0.4 – 7.6%) and 10.6% (2.0 – 18.5%) in 'females only', in the two sites respectively.

For *G. pallidipes*, in all the three sites, there was no association between increase in distance from the edge of the tsetse belt and, wing fray categories and hunger stages. Similarly, with regard to ovarian categories in both species, the model indicated no relationship with increase in distance from the edge of the tsetse belt.

Results suggested that in the Mpika and Rufunsa sites, and for *G. m. morsitans* tsetse flies, increase in distance from the edge into the inner parts of the tsetse belt was associated with increase the proportion of flies with larger body size, and with reduction in the proportion of older flies (and as such an increase in the proportion of younger flies). The results further suggested that increase in this distance was associated with reduction in the proportion of hungry flies, in turn suggesting an increase in the proportion of non-hungry flies of the species. In the Lundazi site, the results suggested that there was no relationship between increase in the distance indicated and, the distribution of wing vein length, wing fray categories, and hunger stages. Similarly, in the case of all the three sites looked at collectively, the outcomes suggested non-existence of a relation between increase in the particular distance and the distribution of wing vein length, wing fray categories, and hunger stages in the species.

Table 2. Number of samples of tsetse flies collected per section of transect distance in the Lundazi, Mpika and Rufunsa sites in north-eastern Zambia

Site/Section	Number of tsetse fly samples – Black-screen Fly rounds							Number of tsetse fly samples - Epsilon Traps						
	Gmm_M	Gmm_F	Gmm_F_Ova	Gp_M	Gp_F	Gp_F_Ova	**Total**	Gmm_M	Gmm_F	Gmm_F_Ova	Gp_M	Gp_F	Gp_F_Ova	**Total**
Lundazi:														
Section 1	19	11	7	0	0	0	**30**	6	9	5	0	0	0	**15**
Section 2	45	16	9	2	1	1	**64**	15	12	6	4	6	3	**37**
Section 3	26	9	4	5	3	2	**43**	11	10	8	3	8	3	**32**
Section 4	21	14	6	9	5	4	**49**	5	18	8	9	16	10	**48**
Section 5	46	20	12	8	7	4	**81**	21	10	4	7	18	13	**56**
Total	**157**	**70**	38	**24**	**16**	11	**267**	**58**	**59**	**31**	**23**	**48**	**29**	**188**
Mpika:														
Section 1	28	10	8	0	0	0	**38**	9	7	4	0	0	0	**16**
Section 2	65	22	15	0	0	0	**87**	14	17	12	0	0	0	**31**
Section 3	49	18	11	8	7	5	**82**	17	15	11	11	18	10	**61**
Section 4	104	42	28	35	23	14	**204**	39	31	8	30	41	24	**141**
Total	**246**	**92**	**62**	**43**	**30**	**20**	**411**	**79**	**70**	**35**	**41**	**59**	**34**	**249**
Rufunsa*:														
Section 1	59	18	0	1	1	0	**79**	15	11	0	2	1	0	**29**
Section 2	25	10	0	1	3	0	**39**	9	13	0	2	6	0	**30**
Section 3	51	22	0	6	9	0	**88**	21	16	0	14	21	0	**72**
Section 4	91	25	0	28	18	0	**162**	35	27	0	21	34	0	**117**
Total	**226**	**75**	0	**36**	**31**	0	**368**	**80**	**67**	0	**39**	**62**	0	**248**

Gmm - *Glossina m. morsitans*; Gp - *G. pallidipes*; M - male; F – female; Ova – Ovarian dissection; *Ovarian dissections not done in the site

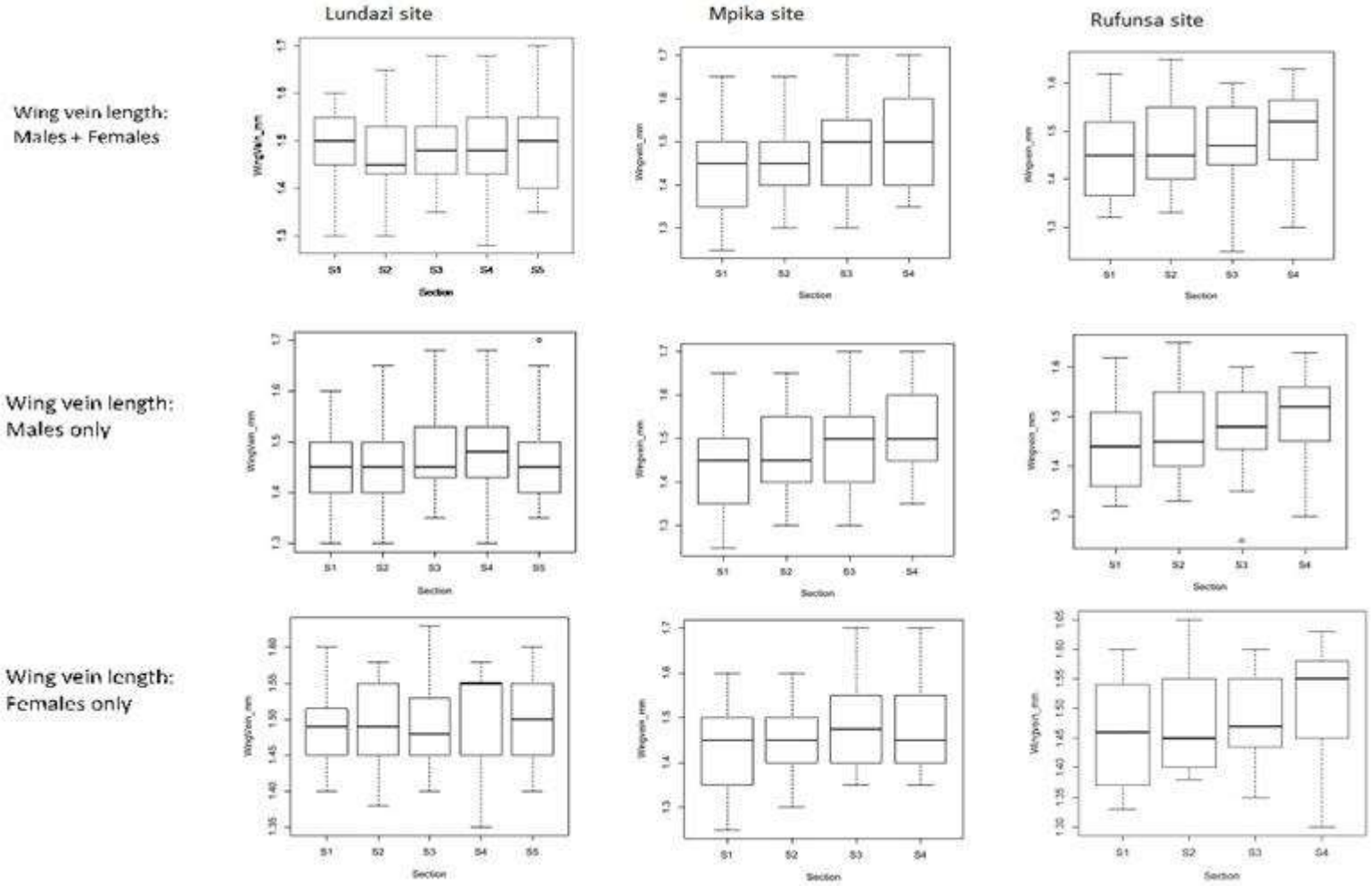

Figure 4. Boxplots: Distribution of Wing vein length in samples of *G. m. morsitans* among sections of the transect distance in the Mpika, Lundazi and Rufunsa sites in north-eastern Zambia.

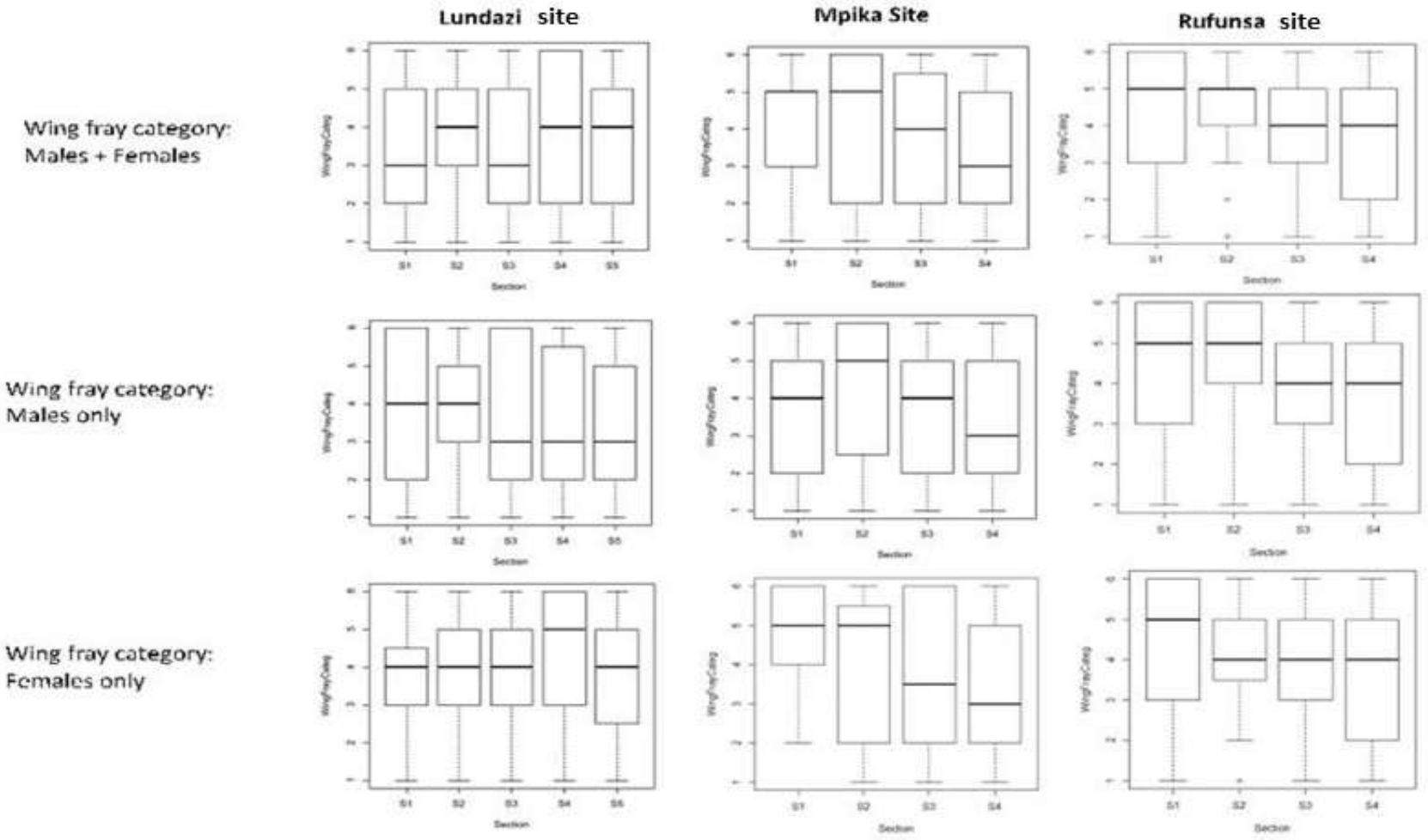

Figure 5. Boxplots: Distribution of wing fray categories in samples of *G. m. morsitans* among sections of the transect distance in the Mpika, Lundazi and Rufunsa sites in north-eastern Zambia

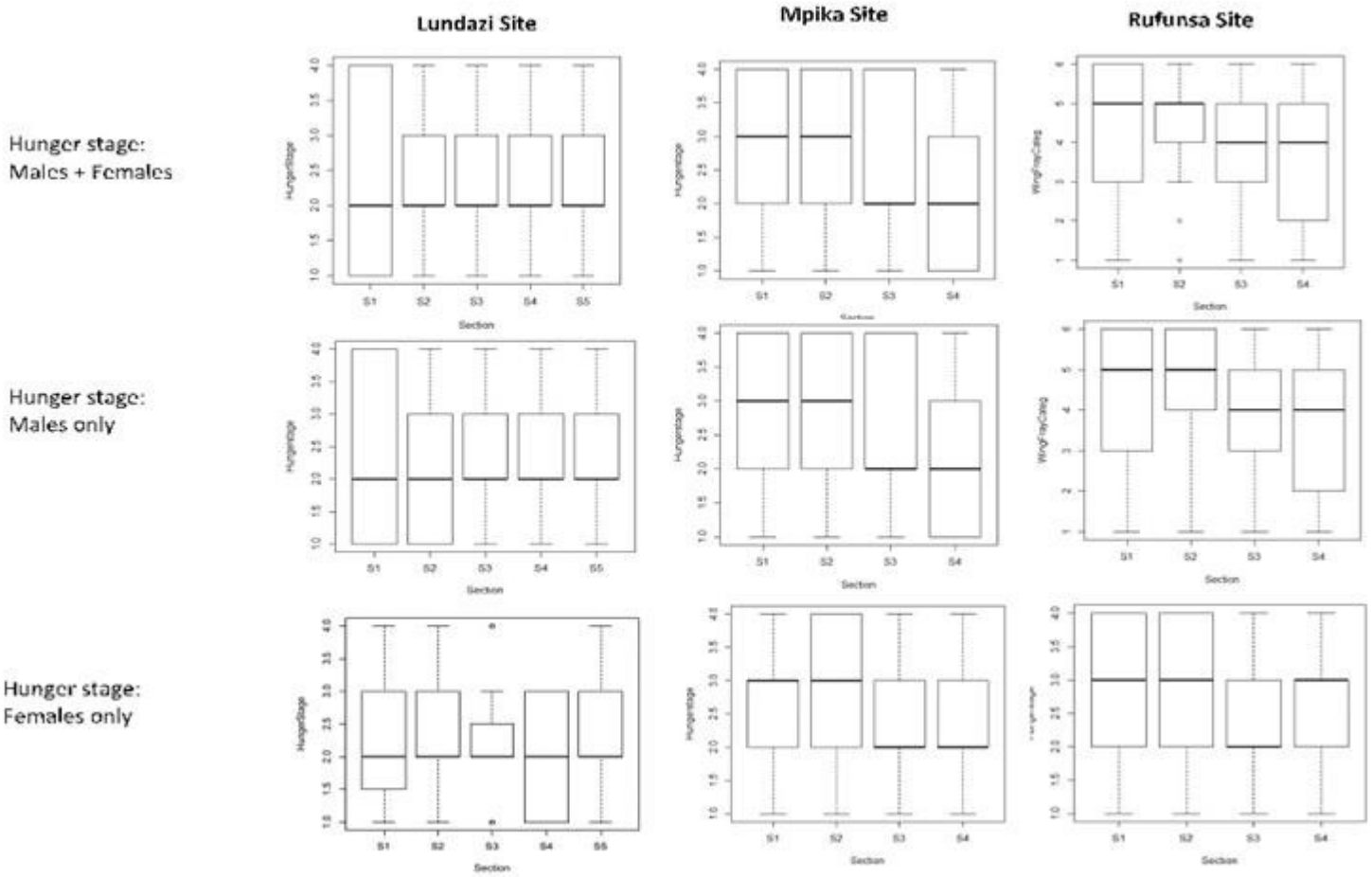

Figure 6. Boxplots: Distribution of Hunger stages in samples of *G. m. morsitans* among sections of the transect distance in the Mpika, Lundazi and Rufunsa sites in north-eastern Zambia.

Table 3. Variation of wing vein length with increase in distance away from the edge of the tsetse belt in samples of *Glossina m. morsitans* and *G. pallidipes* in the Lundazi, Mpika and Rufunsa sites in north-eastern Zambia

	LUNDAZI				MPIKA				RUFUNSA			
	G. m. morsitans			*G. pallidipes*	*G. m. morsitans*			*G. pallidipes*	*G. m. morsitans*			*G. pallidipes*
	Males+ Females	Males only	Females only	Males+ Females	Males+ Females	Males only	Females only	Males+ Females	Males+ Females	Males only	Females only	Males+ Females
Coefficient	0.0005	0.001	0.0003	0.002	0.003	0.004	0.002	0.003	0.005	0.005	0.006	0.007
Confidence interval	-	-	-	-	0.002 - 0.004	0.002 - 0.005	0.0005 - 0.004	-	0.002 - 0.007	0.003 - 0.007	0.002 – 0.01	0.001 - 0.014
p-value	0.283	0.086	0.669	0.133	< 0.0001	< 0.0001	0.010	0.167	< 0.0001	< 0.0001	0.004	0.013

Table 4. Results (PORL regression test): Distribution of Wing fray Categories, Hunger Stages and Ovarian Categories in tsetse flies in relation to increase in distance away from the edge of the tsetse belt in the Lundazi, Mpika and Rufunsa sites in north-eastern Zambia

	Wing fray categories				Hunger stages				Ovarian categories	
	G. m. morsitans			*G. pallidipes*	*G. m. morsitans*			*G. pallidipes*	*G. m. morsitans*	*G. pallidipes*
Site	**Males+ Females**	**Males only**	**Females only**	**Males+ Females**	**Males+ Females**	**Males only**	**Females only**	**Males+ Females**	**Females**	**Females**
Lundazi site:										
Coefficient	- 0.005	- 0.005	0.003	- 0.031	- 0.015	- 0.008	- 0.009	- 0.033	- 0.004	- 0.014
p-value	0.648	0.738	0.876	0.249	0.166	0.526	0.526	0.231	0.880	0.767
Mpika site:										
Coefficient	- 0.042	- 0.048	- 0.054	- 0.038	- 0.056	- 0.056	- 0.042	- 0.034	- 0.039	- 0.074
Odds Ratio	0.959	0.954	0.947	-	0.946	0.952	0.959	-	-	-
Confidence Interval	0.939 - 0.980	0.928 - 0.979	0.913 - 0.982	-	0.925 - 0.966	0.928 -0.978	0.924 - 0.996	-	-	*
p-value	< 0.001*	< 0.001*	0.003*	0.260	< 0.0001*	< 0.0001*	0.028*	0.303	0.117	0.162
Rufunsa site#:										
Coefficient	- 0.088	- 0.084	- 0.073	- 0.094	- 0.097	- 0.082	- 0.112	- 0.096	-	-
Odds Ratio	0.915	0.919	0.930	-	0.907	0.922	0.894	-	-	-
Confidence Interval	0.873 - 0.959	0.870 - 0.972	0.815 -0.980	-	0.866 - 0.951	0.872 - 0.973	0.815 - 0.980	-	-	-
p-value	< 0.001*	0.003*	0.011*	0.118	< 0.0001*	0.004*	0.016*	0.114		

*Significant result; # No Dissection for ovarian categories undertaken in the site

2.6 Discussion

This study indicated that in the Mpika and Rufunsa sites, increase in distance away from the human settlements was associated with an increase in the proportion of larger tsetse flies, reduction in the proportion of older flies and, reduction in the proportion of hungry tsetse flies. This was in agreement with observations that have been made in the past on effects of habitat degradation on the short- and long-term well-being and age structure of tsetse populations (FAO, 1982, Hargrove 1994, Leak 1999). No association was observed between increase in this distance and the distribution of wing vein length, wing fray categories and hunger stages in *G. m. morsitans* in the Lundazi site, where human settlement was relatively evenly distributed from the edge to the innermost parts of the tsetse belt. This further suggested that the nature of the distribution of human settlements had influence on the distribution of wing vein length, wing fray category and hunger state in *G. m. morsitans*, in the three sites, and also that of wing vein length in *G. pallidipes* in the Rufunsa site.

In sampling tsetse populations, as applied in this study, the number of tsetse flies caught per unit effort, e.g. per fly round and/or per trap per day, and the characteristics of the samples collected, are affected to varying degrees by the type of sampling method (Leak, 1999). This is largely due to variation in the nature of response of different categories of tsetse flies (e.g. species, sex, age, etc.) to particular sampling devices or methods (Vale and Phelps 1978). In this regard, literally all available tsetse sampling tools or methods have some level of biasness (Vale 1974, Vale and Phelps 1978, Vale and Hall 1985, Leak 1999). With use of the two methods having been implemented by the same field teams in each transect line, it may be assumed that the biasness or systematic error of each of the methods and/or devices was consistent (constant) throughout the length of each transect lines, such that any variation in the numbers caught per sample point and the characteristics of the samples collected, with increase in distance along each of the transect lines, may be attributed to other factors and not to the biasness and/or weaknesses of the sampling methods or devices.

In the same vein, temporal variation in weather conditions over a three-week period within the month of September in the Mpika site, and within the month of October in the Lundazi site, and, over a period of two weeks within the month of November in the Rufunsa site, was unlikely to have affected the outcome of the study - considering that comparison of wing vein, wing fray categories and hunger stages in the tsetse fly samples was made among sample points in the transect line per site and not among the sites. Considering that section 1 in the Lundazi site, and sections 1 and 2 in the Mpika, did not record any catch of *G. pallidipes*, and the number of flies of the species recorded was relatively low per section and per transect,

it follows that this was likely to have negatively affected the analyses. This could in turn explain the inability of the model to detect any relationship between increase in distance (from age of tsetse belt) and variation in wing vein length, wing fray categories and hunger stages (except in the case of the Rufunsa site) among samples of *G. pallidipes* in the study. In the Rufunsa site, the number of *G. pallidipes* samples collected was relatively higher than in the Lundazi and Mpika sites, thus giving test results that were similar to that obtained in the case of *G. m. morsitans* samples; where the number of samples of the species was relatively higher than in the other two sites-i.e. where the results were similar to those obtained for *G. m. morsitans*

Tsetse infested areas that are least exposed to human activity are known to be subjected to the least levels of tsetse fly habitat deterioration, and the rate of decline in habitat quality tends to be dependent on the nature and pattern of manifestation of the human-associated factors involved (Reid et al. 2000, Schowalter 2012, Anderson et al. 2015, Matawa et al. 2019). Considering the difference in the relative proximity to the human settlements, among the sample points in the transect line in the Mpika and Rufunsa sites, this association between level of exposure to human activities, and occurrence of tsetse habitat degradation, could in turn explain the observed association between increase in distance (away from the human settlements) and distribution of body size, age and hunger state in *G. m. morsitans,* in the two sites. In a study undertaken in parts of the eastern tsetse fly belt in Zambia by Ducheyne *et al.* (2009), tsetse apparent density was highest in areas that had low levels of human-associated habitat fragmentation, and was lowest where the level of such habitat fragmentation was high. This indicates that increase in human activities in tsetse infested areas, usually associated with proximity to human settlements, give rise to a reduction in tsetse fly population density, and this is usually a consequence of reduction in tsetse habitat quality (Reid et al. 2000, Ducheyne et al. 2009). This association between human activity, habitat quality and tsetse population density could explain the relatively lower numbers of tsetse flies captured in the first section of the transect lines (located closest to human settlements) and also the observed distribution of body size, age and hunger state in *G. m. morsitans* in the study.

As indicated earlier, the well-being and survival of tsetse flies is dependent on habitat quality with regard to adequate availability of key needs, mainly, suitable climatic conditions, vegetation cover for shelter during adverse weather conditions and, adequate access to sources of blood meals (Hargrove 1988, Hargrove 2004). Thus, suboptimal levels of these requirements, or their elimination, in a tsetse infested area, will diminish the well-being of tsetse flies thereby giving rise to increased levels of occurrence of stress and density

independent mortality in the affected tsetse population (Hargrove 2004, Van den Bossche et al. 2010). Considering that higher levels of effects of human activity are associated with higher levels of degradation of tsetse habitat (Reid et al. 2000, Ducheyne et al. 2009), it may be taken that in the Mpika and Rufunsa sites, the level of habitat degradation was highest in locations that were closest to the human settlements, and lowest in locations that were furthest away from the human settlements. In turn, this could indication of a higher prospect for exposure to stress for tsetse flies that inhabited locations closest to the human settlements, than in tsetse flies that inhabited locations that were further away from the human settlements.

Occurrence of stress in tsetse flies is associated with exposure to conditions that are below the optimum requirements for the tsetse flies, particular with regard to detrimental exposure to unfavourable weather conditions such as significant reduction or disappearance of vegetation cover and lack of access to blood meals as a consequence of human activities (Leak 1999, Hargrove 2004). Nutrition in adult tsetse flies is directly dependent on access to sources of blood meals (i.e. hosts) such that a significant reduction in the supply of hosts in an area entails diminished prospects for tsetse flies to find hosts, resulting in starvation associated stress in affected tsetse flies (Van den Bossche and Hargrove 1999, Hargrove 2001). Occurrence of stress has the potential to result in serious negative implications for the survival, distribution, reproduction and vectorial capacity of the individual tsetse flies (Reid et al. 2000, Hargrove 2004, Akoda et al. 2009).

Nutrition (supply of energy fat reserves) and general well-being of the non-adult stages in the life cycle (i.e. the larva and pupa stages), and as such that of the teneral stage of the adult, is wholly dependent on the nutrition and well-being of the female parent, and as such occurrence of nutritional or any other kind of stress in the female parent has serious negative implications for the survival and fitness of these particular stages in the life cycle of the tsetse fly (Leak 1999, Hargrove 2004, English et al. 2016) . For this reason, diminished levels of nourishment in females entails diminished nourishment for the larva and pupa stages, resulting in newly emerged adults that are smaller in body size and with diminished levels of fat reserves – and thus with reduced prospects for survival. Consequently, an adult tsetse fly is most vulnerable to stress, and hence to mortality, during the period before accessing its first blood meal, due to low fat reserves and underdeveloped wing muscles, resulting in limited capacity for flight to find hosts and/or to escape predators. This indicates that adverse deterioration in tsetse habitat quality tends to have the most negative impact (particularly in terms of mortality) on newly emerged teneral adults (Van den Bossche and Hargrove 1999, Hargrove et al. 2018). The size of individual adult tsetse flies is determined at the time of emergence from the pupal

stage, i.e. it does not change thereafter, and as such body size in tsetse flies provide an indirect measure of levels of exposure to factors such as nutritional stress and mortality in the parent and/or earlier generations of a tsetse population (Leak 1999, Van den Bossche and Hargrove 1999). Against this background, it may be taken that variation in body size, age structure, and levels of access to hosts, in a tsetse population, could serve as indicator of variation in levels of exposure to unfavourable environmental conditions in the existing population and, in the parent and/or earlier generations of the tsetse population (Leak 1999, Van den Bossche and Hargrove 1999, Reid et al. 2000, English et al. 2016).

When habitat degradation diminishes availability of sources of blood meals, tsetse flies are deprived of their blood meal sources due to reduced tsetse-host contact and hence an increase in feeding intervals (Hargrove and Brady 1992, Loder 1997, Leak 1999). This could explain the higher likelihood in this study of finding hungry *G. m. morsitans* flies in locations that were close to the edge of the tsetse belt, and hence within or close to human settlements, than in locations that were far away inside the tsetse belt, in the Mpika and Rufunsa sites. This could similarly explain the relatively larger proportion of smaller sized and older *G. m. morsitans* flies in locations that were close to human settlements (at or close to the edge of tsetse belt) than in locations that were located further away inside the tsetse belt. Such outcomes, as observed in the locations that fell within and/or close to the edge of the tsetse belt, and also within or close to human settlements, may be associated with occurrence of stress in one or more of the parent generations of the tsetse flies (Hargrove 1999, Leak 1999, Van den Bossche and Hargrove 1999).

Areas with similar environmental characteristics in the context of environmental conditions, and hence habitat quality, tend to offer similar prospects for existence, and hence for survival of tsetse flies, and this forms the basis for models that have been applied to predict tsetse presence and distribution (Robinson et al. 1997, Rogers 2000, Cecchi et al. 2008). In the study area, increase in distance away from the edge (into the inner parts of the tsetse belt), also entailed transition from the high altitude locations (plateau areas) towards the low lying (low altitude) locations in the Luangwa valley (in the Lundazi and Mpika sites) and in the Luano valley (in the Rufunsa site). Altitude affects tsetse well-being and hence distribution indirectly, through changes on climatic factors (Leak, 1999). The difference in altitude, in the study area, is associated with increase in mean temperatures and associated change in climatic conditions and related agro-ecological conditions that in turn influence the distribution of human settlements and the nature of human activities (Reid *et al*. 2000; Anderson *et al*, 2015). Thus, similar kinds and levels of human activity tend to have similar impact on the environment (Reid

et al. 2000; Tilman, 2001; Schowalter, 2012), and this could relate to the situation that was obtaining in the Lundazi site where human settlements were distributed relatively evenly from the edge to the innermost section of the transect line. As such, it may be taken that in the particular site, the effects of the human settlement were likely to have also been evenly distributed along the transect line.

Although it had been envisaged (in the study) to have dissections for ovarian age categories (ovarian ageing) undertaken in each of the study sites, this could not be undertaken in the Rufunsa site due to budgetary constraints at the time of sample collection in the area. Further, owing to the challenge of keeping tsetse samples adequately fresh from the time of collection (in the BFRs and in the traps) to the camp site for such dissections to be performed, only a relatively small proportion of females were dissected for ovarian categories (ovarian aging). This entailed that where tsetse dissection did get done (in the Rufunsa and Mpika sites), a relatively large proportion of the sample points had no female flies dissected. Similarly, samples of *G. pallidipes* were considerably few and with most collected far inside the tsetse belt, in comparison to samples of *G. m. morsitans*. These factors could explain the inability of the model to detect associations with regard to ovarian categories and *G. pallidipes*.

2.7 Conclusion

We conclude that in the Mpika and Rufunsa study sites in the eastern tsetse belt in north-eastern Zambia, increase in distance from the edge into the inner parts of the tsetse belt was associated with increase in the proportion of bigger sized flies and, reduction in the proportion of old flies and that of hungry flies, in *G. m. morsitans*. The findings suggested existence of a gradient of increasing tsetse habitat quality along the transect line from the edge into the inner parts of the tsetse belt. Considering that human settlement was concentrated at and close to the edge of the tsetse belt in the two sites, this suggested that such a gradient in habitat quality could in turn have been due to existence of a gradient of reducing levels of human-associated tsetse habitat degradation, in each of the two sites. This likely explanation for the findings in the Mpika and Rufunsa sites, was further supported by the absence of any such gradient in the Lundazi site where human settlement was evenly distributed along the transect line. The findings could potentially have implications on prospects for shrinking of the tsetse belt, and also on the distribution of the risk of transmission of trypanosomiasis, in sites/areas of this kind.

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CHAPTER 3: HUMAN-ASSOCIATED SCARCITY OF HOSTS FOR TSETSE FLIES (DIPTERA: GLOSSINIDAE) IS RELATED TO INCREASE IN PREVALENCE OF TRYPANOSOME INFECTION IN THE FLIES IN NORTH EASTERN ZAMBIA

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3.1 Abstract

Occurrence of nutritional stress (due to depletion of fat reserves) in tsetse flies, associated with inadequate levels of access to blood meals, enhances susceptibility of the flies to trypanosome infection. Thus, in a tsetse infested area, a spatial gradient of reducing tsetse habitat quality is potentially a gradient of increasing prospects for occurrence of stress in tsetse flies. This study investigated prevalence of trypanosome infection in *Glossina morsitans morsitans* and *G. pallidipes* along a transect line hypothesised to represent such a gradient, in relation to the edge of the tsetse belt and distribution of human settlements. This was undertaken in three sites located in Lundazi, Mpika and Rufunsa districts respectively, in north-eastern Zambia. Human settlement was concentrated at the edge of the tsetse belt in the Mpika and Rufunsa sites, and evenly distributed along transect line in the Lundazi site. Tsetse fly samples were collected using black-screen fly rounds and epsilon traps. Detection of trypanosome infection was by dissection and microscopy in Lundazi and Mpika sites, and Loop-mediated Isothermal Amplification (LAMP) test in Rufunsa site. Multiple logistic regression models were applied to determine whether the following factors; 'change in distance from edge of tsetse belt', 'tsetse sampling method' and 'sex of tsetse fly', had effect on 'prevalence of trypanosome infection' in the tsetse flies. Only 'increase in distance from the edge of tsetse belt' for *G. m. morsitans* was significantly associated with 'prevalence of trypanosome infection' in the flies, in the Mpika and Rufunsa sites. Distance was associated with reduced likelihood of infection with 'One or more subgenera of trypanosomes' and with 'Nannomonas trypanosomes', in the case of 'All sites collectively', 'Lundazi and Mpika sites collectively', Mpika site alone, and Rufunsa site alone. Per site, increase in distance entailed reduced prospects for Trypanozoon infection, but only in the Mpika and Rufunsa sites. We conclude that in the Mpika and Rufunsa sites, increase in distance from human settlements entailed reduced likelihood of trypanosome infection, likely due to reducing tsetse habitat

degradation, increasing availability of hosts, and hence increasing levels of nutrition and fat reserves, thus enhancing tsetse immunity against trypanosome infection.

Keywords Tsetse flies Stress Hosts Fat reserves Immune system Trypanosome infection Habitat degradation Human settlements

3.2 Introduction

Tsetse-transmitted trypanosome infections in humans and livestock occur across an area of 8.7 million km^2 in sub-Saharan Africa and are responsible for Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT) (Holmes 2013). The veterinary and medical importance of tsetse flies is centered on their capacity to transmit trypanosomes that cause trypanosomiasis (Leak 1999). In a tsetse population, the proportion of trypanosome-infected flies, in both spatial and temporal context, is an important factor in the epidemiology of the disease (Leak 1999; Roditi and Lehane 2008). This further emphasizes the importance of factors that diminish or enhance susceptibility of tsetse flies to trypanosome infection (Rotureau and Van Den Abbeele 2013). Several factors influence occurrence of trypanosome infection in tsetse flies, and these include factors inherent to tsetse flies and to trypanosomes, and environmental factors, that either enhance or diminish prospects for occurrence of trypanosome infection in tsetse flies (Leak 1999; Haines 2013). After a tsetse fly has fed on trypanosome-infected blood, it is the interactive net effect of factors such as species and genetic variation in tsetse flies and in trypanosomes, and environmental factors, that determine whether or not infection occurs (Leak 1999; Aksoy et al. 2014). Concurrent infection in tsetse flies, with the endosymbionts *Wigglesworthia glossinidia*, *Sodalis glossinidius* and *Wolbachia* spp. (Aksoy et al. 2014), effect of lectins in the tsetse mid-gut (Peacock et al. 2012) and, nutrition and fat reserves in tsetse flies (Kubi et al. 2006) have been reported to play a part in the susceptibility of tsetse flies to trypanosome infection. Level of fat reserves in tsetse flies are a key determinant of capacity of tsetse immune system to prevent trypanosome infection, such that low fat reserves diminish the tsetse immune system capacity to prevent trypanosome infection (Haines 2013). All stages of the tsetse life cycle depend on blood meals for nutrition - directly for adults and indirectly (through the female parent) in the other stages. In turn, the level of access to sources of blood meals (i.e. to hosts) is dependent on environmental factors – i.e. tsetse habitat quality (Hargrove 2004; English et al. 2016).

According to Roditi and Lehane (2008), tsetse flies with a weak or under-developed immune system tend to be the most vulnerable to trypanosome infection. In studies by Kubi et al. (2006), reared *G. m. morsitans* flies starved over periods of three to seven days displayed significant increase in susceptibility to infection with *Trypanosoma congolense* and *T. brucei brucei* – and this was indication that starvation enhanced prospects for trypanosome infection in tsetse flies. Variation in prevalence of trypanosome infection may also be affected by factors such as tsetse species, sex (largely because females live longer), age (at time of first infective feed) and trypanosome prevalence in the preferred hosts – and may thus be affected by tsetse sampling methods (Leak 1999; Peacock et al. 2012; Odeniran et al, 2019).

n wild tsetse populations, depletion of fat reserves is a consequence of poor nutrition associated with significant reduction in levels of access to hosts – an indicator of deterioration in tsetse habitat quality (Hargrove 2004). As such, variation in the temporal and spatial distribution of key factors that determine tsetse habitat quality, such as hosts, could in turn affect the distribution levels of tsetse well-being such as levels of fat reserves in a tsetse population (Leak 1999, Hargrove 2004). Tsetse habitat degradation is usually closely associated with human activities, in turn linked to presence of, and/or proximity to human settlements (Mwanakasale and Songolo 2011). Increase and distribution of human settlements and human population density, and the nature and levels of associated human activities, determine the nature, magnitude and distribution of human-associated tsetse habitat degradation (Reid et al. 2000; Hargrove 2004; Mwanakasale and Songolo 2011). In and around the eastern tsetse belt in Zambia, increase in distance away from the tsetse belt entailed increase in distance away from the low altitude areas that were associated with very low annual rainfall and very low human population density (i.e. in the Luangwa and Luano valleys) and flies (Anderson et al. 2015). A large proportion of the valley areas serve as wildlife conservation areas (i.e. as national parks (NPs) and game management areas (GMAs), such that an increase in distance from these valley areas entails increase in human population density and agricultural activity (Mwanakasale and Songolo 2011; Anderson et al. 2015). Hence, in the absence of a physical tsetse fly barrier, the edge (or limit) of a tsetse infested area could be considered as a zone of transition from favourable to unfavourable environmental conditions for tsetse well-being and survival (Robinson et al, 1997). This suggests that tsetse habitat quality increases from the edge of the tsetse belt going towards the innermost parts (of the tsetse belt).

Human activities degrade tsetse habitat quality through their negative effects on vegetation cover (through agriculture and other forms of land use) and also on limiting

availability of animals on which tsetse flies feed – i.e. through scaring away and/or killing of wild animals (Leak 1999; Reid et al. 2000). The level, intensity and distribution of human-associated habitat degradation is usually directly linked to the spatial distribution of human settlements and population density, in relation to the relative location and extent of the tsetse belt (i.e. the tsetse habitat) (Reid et al. 2000). Tsetse habitat degradation tends to be highest in places located closest to areas of concentration of human settlement, and lowest in areas located furthest away (Reid et al. 2000; Mwanakasale and Songolo 2011; Anderson et al. 2015).

Studies on human-associated tsetse habitat degradation, in the context of possible effect on tsetse flies as vectors of trypanosomes are limited. In this study, based on the background given above, it was hypothesised that a transect line running from the edge into the inner parts of the tsetse belt, represented a gradient of increasing tsetse habitat quality. The study investigated the prevalence of trypanosome infection in the tsetse flies within the transect line, in three sites in which human settlement was either concentrated at the edge of the tsetse belt or was evenly distributed along the transect line.

3.3 Materials and Methods

3.3.1 Study sites, design and collection of tsetse samples

The study sites and the collection of tsetse samples were as described in 2.2.1.

3.3.2 Detection of pathogenic trypanosomes in the tsetse samples

Tsetse fly samples collected from the Mpika and Lundazi sites were subjected to dissection and examination by microscopy for detection of trypanosome infection (on site) following the procedure described by the Food and Agriculture Organisation of the United Nations (FAO 1986). The procedure is based on the differences in the sites in which trypanosomes of the different subgenera (i.e. Nannomonas, Trypanozoon and Duttonella) develop in the tsetse digestive system. Duttonella trypanosomes (e.g. *T. vivax*) develop and mature within the proboscis, Nannomonas trypanosomes (e.g. *T. congolense*) develop in the mid gut and mature in the proboscis, and, Trypanozoon trypanosomes (e.g. *T. brucei brucei*) develop in the mid gut and mature in the salivary glands (Haines 2013).

For each tsetse sample, based on this procedure, a sample with trypanosomes detected only in the mid gut was taken to have only immature forms of trypanosomes. Where trypanosomes were detected in the mid gut and also in the proboscis, it was taken to be infection with Nannomonas and/or Duttonella trypanosomes, whilst infection in the mid gut and also in

the salivary gland was taken to be infection with Trypanozoon trypanosomes and, infection in the proboscis only was taken to be one with only Duttonella trypanosomes (Haines 2013). This facilitated categorisation of the tsetse samples in the context of 'Infected with one or more subgenera of trypanosomes', 'infected with Nannomonas trypanosomes', and 'infected with Trypanozoon trypanosomes' – considering the importance and role of the mid gut in relation to manifestation of the tsetse immune system, and effects on Nannomonas and Trypanozoon trypanosomes.

In the Rufunsa site, the tsetse samples were preserved in Eppendorf tubes (with silica gel) and later screened for trypanosome infection using the Loop-mediated Isothermal Amplification (LAMP), following the procedure described by (Kuboki et al (2003) – dissection and microscopy (on-site) could not be undertaken in the site. The outcome of the test on each sample was deemed 'presence or absence of trypanosome infection', and presence was in the form of the species of trypanosomes detected. The information on the species of trypanosomes (in the infected tsetse flies) allowed for grouping of the trypanosome infected flies into those infected by the trypanosome subgenera, Nannomonas, Trypanozoon and Duttonela.

3.3.3 Data Analysis

The analyses were undertaken in R version 3.6.2 (R Core team, 2016) with application of multiple logistic regression models, and use of the lme4 package in the case of the mixed-effect logistic regression model. For each of the species of tsetse flies, the predictor variables were 'Distance from edge of the tsetse belt', 'Method of tsetse sampling applied' and 'Sex of the tsetse fly' – and the response variable being 'Infection with trypanosomes'.

For 'All the sites collectively', the mixed-effect logistic regression model was applied to determine and measure association between each of the predictor variables and each of three categories of 'Infection with trypanosomes', i.e. 'Infection with one or more species of trypanosomes', 'Infection with Nannomonas trypanosomes' and 'Infection with Trypanozoon trypanosomes'. The analyses also factored-in the difference in the trypanosome detection methods - i.e. Microscopy only on samples from the Lundazi and Mpika sites, and the LAMP only on samples from the Rufunsa site. Consequently, 'Prevalence of trypanosome infection in the tsetse samples' was looked at in respect of, 'All the three sites collectively', 'the Lundazi and Mpika sites collectively' and, 'each of the sites on its own'. In the estimates, 95% confidence intervals were considered, and a *p*-value of less than or equal to 0.05 was taken to be statistically significant.

3.4 Results

A total of 1,731 flies (1279 *G. m. morsitans* and 452 *G. pallidipes*) were collected, and 1158 (66.9%) of these were examined/tested for trypanosome infection - i.e. 776 (67%) *G. m. morsitans* and 382 (33%) *G. pallidipes*. The prevalence of trypanosome infection in *G. m. morsitans* was 11.7%, 14.5% and 16.5% in the Lundazi, Mpika and Rufunsa sites respectively. In *G. pallidipes*, the prevalence in each of the three sites was 3.7%, 6.0% and 8.9% respectively (Table 5). The first sample of *G. m. morsitans* was recorded at distances of 14.5km, 18.5 km and 2.5 km from the estimated edge of the tsetse belt, in the Lundazi, Mpika and Rufunsa sites respectively. In the case of *G. pallidipes*, the first sample was recorded at distances of 23.5km, 30.5km and 5.5 km, in the Lundazi, Mpika and Rufunsa sites respectively.

In the mixed effects logistic regression model (for each of the predictor variables), the unadjusted odds ratio (UOR) and adjusted odds ratio (AOR) were either similar or identical, and as such only the AOR are reported. In both species of tsetse flies and in all the sites, 'method of tsetse sampling' or 'sex of the tsetse flies' had no association with prevalence of trypanosome infection in the tsetse samples. Tables 6 and 7 present the outcomes of the respective analyses.

In the model, 'distance from edge of the tsetse belt' was associated with reduction in the likelihood of 'prevalence of trypanosome infection in the tsetse flies', but only in *G. m. morsitans*, and only in the Mpika and Rufunsa sites. In the case of 'all the sites collectively', increase in the distance was associated with reduction in the likelihood of finding a tsetse fly 'infected with one or more subgenera of trypanosomes' ($p<0.001$); 'infected with Nannomonas trypanosomes' ($p<0.001$); and 'infected with Trypanozoon trypanosomes' ($p<0.001$). With regard to 'Lundazi and Mpika sites collectively' (i.e. where microscopy was applied), increase in the distance entailed a reduction in the prospects of finding a tsetse fly that was, 'infected with one or more subgenera of trypanosomes' ($p<0.05$) and, 'infected with Nannomonas trypanosomes' ($p<0.05$) – with no association detected in relation to 'prevalence of Trypanozoon trypanosomes'.

Taking each site in isolation, increase in the distance in the Mpika site was associated with reduction in the likelihood of finding a tsetse fly, 'infected with one or more subgenera of trypanosome' ($p<0.05$), 'infected with Nannomonas trypanosomes' ($p<0.05$) and, 'Infected with Trypanozoon trypanosomes' ($p<0.05$). In the Rufunsa site, the association was such that increase in the distance entailed reduction in the chance of finding a tsetse fly that was, 'infected with one or more subgenera of trypanosomes' ($p<0.05$), 'infected with Nannomonas trypanosomes' ($p<0.05$), and 'infected with Trypanozoon trypanosomes' ($p<0.05$). In the

Lundazi site, there was no association between increase in the distance and prevalence of trypanosome infection in the tsetse samples. Similarly, distance from the edge into the inner parts of the tsetse belt had no association with prevalence of trypanosome infection in the case of *G. pallidipes* samples in all the sites.

Table 5. Number of *Glossina m. morsitans* and *G. pallidipes* flies collected and prevalence of trypanosome infection in the tsetse flies in the Lundazi, Mpika and Rufunsa sites in north-eastern Zambia

Site	No. of tsetse flies	No. of tsetse flies examined/tested (%)	No. infected with one or more subgenera of trypanosomes (%)	No. infected with Nannomonas trypanosomes (%)	No. infected with Trypanozoon trypanosomes (%)
G. m. morsitans:					
Lundazi	344	128 (37.2)	15 (11.7)	15 (11.7)	7 (5.5)
Mpika	487	200 (41.1)	29 (14.5)	23 (11.5)	12 (6.0)
Rufunsa	448	448 (100)	74 (16.5)	69 (15.4)	53 (11.8)
Total	**1279**	**776 (60.7)**	**118 (15.2)**	**107 (13.8)**	**72 (9.3)**
G. pallidipes:					
Lundazi	111	81 (73.0)	3 (3.7)	3 (3.7)	3 (3.7)
Mpika	173	133 (76.9)	8 (6.0)	8 (6.0)	3 (2.2)
Rufunsa	168	168 (100)	15 (8.9)	15 (8.9)	6 (3.6)
Total	**452**	**382 (84.5)**	**26 (6.8)**	**26 (6.8)**	**12 (3.1)**

Table 6. Determination and measurement of effects of 'distance from edge of tsetse belt', 'method of tsetse sampling', and 'sex of tsetse flies' on trypanosome infection in *Glossina m. morsitans* and *G. pallidipes* in the Lundazi, Mpika and Rufunsa sites

Response variable	Predictor variable	*G. m. morsitans*: All sites collectively		*G. m. morsitans* : Lundazi & Mpika sites collectively		*G. pallidipes*: All sites collectively	
		AOR[a]	p-value	AOR	p-value	AOR	p-value
Infected with one or more subgenera of trypanosomes	Distance	0.9457(0.9300 - 0.9616)	5.60E-09[b]	0.9512(0.9120 - 0.9921)	1.99E-02[b]	0.9789 (0.9510 - 1.0077)	1.49E-01
	Method: BFR	1	-	1	-	1	-
	Trap	0.9326 (0.6070 - 1.4330)	7.50E-01	1.0753(0.5396 - 2.1420)	8.36E-01	0.6646 (0.3026 - 1.4597)	3.09E-01
	Sex: Female	1	-	1	-	1	-
	Male	1.0638 (0.6950 - 1.6383)	7.81E-01	1.1338(0.5722 - 2.2648)	7.12E-01	0.5298 (0.2299 - 1.2208)	1.36E-01
Infected with Nannomonas trypanosomes	Distance	0.94 45(0.9281 - 0.9612)	1.88E-09[b]	0.9427 (0.8924 - 0.9960)	3.56E-02[b]	0.9789 (0.9510 - 1.0077)	1.49E-01
	Method: BFR	1	-	1	-	1	-
	Trap	1.1023 (0.7118 - 1.7072)	6.62E-01	0.8755(0.3911- 1.9598)	7.46E-01	0.6646 (0.3026 - 1.4597)	3.09E-01
	Sex: Female	1	-	1	-	1	-
	Male	1.2055 (0.77687– 1.8755)	4.16E-01	0.9699 (0.4538 - 2.0726)	9.37E-01	0.5298 (0.2299 - 1.2208)	1.36E-01
Infected with Trypanozoon trypanosomes	Distance	0.9278 (0.9060 - 0.9502)	7.66E-09[b]	0.9558 (0.9016 - 1.0132)	1.29E-01	0.9827(0.9412 - 1.0260)	4.28E-01
	Method: BFR	1	-	1	-	1	-
	Trap	1.4284 (0.8521- 2.3948)	1.70E-01	1.7892 (0.6898 – 4.5830)	2.25E-01	1.7647(0.4595 - 6.7018)	4.11E-01
	Sex: Female	1	-	1	-	1	-
	Male	1.0042(0.5887 - 1.7128)	9.8E-01	0.9686 (0.3707 – 2.5306)	9.48E-01	0.4490 (0.1177 - 1.7144)	2.42E-01

[a]AOR = adjusted odds ratio
[b]Indicate that the data is significant ($p < 0.05$)

Table 7. Determination and measurement of effects of 'distance from edge of tsetse belt', 'method of tsetse sampling', and 'sex of tsetse flies' on trypanosome infection in *Glossina m. morsitans* in the Lundazi, Mpika and Rufunsa sites respectively

Response variable	Predictor variable		*G. m. morsitans*: Lundazi site		*G. m. morsitans*: Mpika site		*G. m. morsitans*: Rufunsa site	
			AOR[a]	p-value	AOR	p-value	AOR	p-value
Infected with one or more subgenera of trypanosomes	Distance		0.9616 (0.8776 - 1.0272)	2.42E-01	0.9406 (0.9022 - 0.9982)	2.45E-02[b]	0.9196 (0.5077 - 0.9840)	1.15E-02[b]
	Method:	BFR	1	-	1	-	1	-
		Trap	1.1665 (0.3481 - 3.4588)	7.88E-1	0.9751 (0.3830 - 2.2245)	9.46E-01	0.9026 (0.5097 - 1.5581)	7.18E-01
	Sex:	Female	1	-	1	-	1	-
		Male	1.0292(0.3510 - 3.4236)	9.67E-01	0.9673 (0.4378 – 2.2576)	9.36E-01	1.1241 (0.6474 - 2.0094)	6.84E-01
Infected with Nannomonas trypanosomes	Distance		0.9616 (0.8976 - 1.0272)	2.49E-01	0.9321 (0.8865 - 0.9806)	1.30E-02[b]	0.9232 (0.8608 - 0.9899)	2.46E-02[b]
	Method:	BFR	1	-	1	-	1	-
		Trap	1.2344 (0.2485 - 4.8773)	7.88E-01	0.8869 (0.3142 - 2.2733)	8.23E-01	1.0301 (0.5781 - 1.7931)	9.18E-01
	Sex:	Female	1	-	1	-	1	-
		Male	2.0705 (0.4831 - 14.3806)	9.60E-01	0.9410 (0.3935 - 2.4276)	8.87E-01	1.2061 (0.6823 - 2.2063)	5.29E-01
Infected with Trypanozoon trypanosomes	Distance		1.0254(0.9328 - 1.1371)	6.08E-01	0.8996 (0.8205 - 0.9715)	1.12E-02[b]	0.9027 (0.8337 - 0.9764)	1.26E-02[b]
	Method:	BFR	1	-	1	-	1	-
		Trap	1.1588 (0.1587- 5.8725)	8.66E-01	2.6642 (0.7785 - 8.9907)	1.08E-01	1.1752 (0.6180 - 2.1759)	6.13E-01
	Sex:	Female	1	-	1	-	1	-
		Male	1.1523 (0.2414 – 8.1890)	8.68E-01	0.8481 (0.2529 - 2.9882)	7.71E-01	1.0301 (0.5505– 2.0022)	9.28E-01

[a]AOR = adjusted odds ratio
[b]Indicate that the data is significant ($p < 0.05$)

3.5 Discussion

The findings in the study suggested that in the particular parts of the eastern tsetse belt in north-eastern Zambia, increase in distance away from the edge, into the inner parts of the tsetse belt, reduced the likelihood of finding trypanosome-infected *Glossina m. morsitans* tsetse flies. However, the results also indicated that this held true only in the Mpika and Rufunsa sites, i.e. not in the Lundazi site. In the Lundazi site, the even distribution of human settlements, from the edge into the inner sections of the tsetse belt, likely entailed absence of variation in key aspects of tsetse habitat quality such as availability of hosts. *Glossina m. morsitans*, were the most important vectors of trypanosomes, and, *Trypanosoma congolense* and *T. brucei* trypanosomes (belonging to Nannomonas and Trypanozoon sub-genera respectively) were the most important species of trypanosomes with regard to occurrence of both the animal and human forms of trypanosomiasis in the eastern tsetse belt in Zambia (Robinson et al, 1997; Van den Bossche 1997; Anderson et al 2015). In the Lundazi and Mpika sites, relatively few *G. pallidipes* flies were collected, with the first catch of the species recorded at distances that were much further away from the edge of the tsetse belt i.e. in comparison with the locations that recorded the first catch of *G. m. morsitans* flies. This was in line with the record that puts *Glossina m. morsitans* as the most widely distributed species in the eastern tsetse belt, extending well into the highly settled plateau areas, and puts *G. pallidipes* as largely restricted to the innermost parts of the tsetse belt particularly along drainage lines (Robinson et al. 1997). Further, the BFR was a much less effective sampling method for *G. pallidipes* (Vale 1993). These factors are likely to have contributed to the observed low numbers of *G. pallidipes* samples collected and to the observed distribution of the samples of the species in each of the transect lines.

Looking at the results with regard to 'all sites collectively' and 'Mpika and Lundazi sites collectively', it may be taken that the nature and level of association in the Mpika and Rufunsa sites, i.e. between 'distance from the edge of the tsetse belt' and each of the three categories of 'trypanosome infection in tsetse flies', likely masked the absence of such association in the Lundazi site. Further, in the case of 'infection with Trypanozoon trypanosomes' in the 'Lundazi and Mpika sites collectively', it was likely that absence of association in the Lundazi site was such that it masked the association observed in the case of the Mpika site alone. This may also have been affected by the relatively lower prevalence of Trypanozoon infections in the samples in all the sites, in comparison to Nannomonas trypanosomes.

The observed reduction in the likelihood of finding trypanosome-infected *G. m. morsitans*, with increase in distance from the edge of the tsetse belt, was likely to be a consequence of a gradient of reduction in levels of one or more environmental factors that enhanced susceptibility of *G. m. morsitans* to trypanosome infection, and this was in agreement with the observations made in reared tsetse flies by Kubi et al. (2006).

Considering the relationship among, tsetse habitat degradation, occurrence of nutritional stress and depletion of fat reserves in tsetse flies, and tsetse immune system function (Roditi and Lehane 2008; Hargrove 2004), it follows that an area with a spatial gradient of reducing levels of tsetse habitat degradation, as observed in our study, could entail existence of a spatial gradient of reducing levels of tsetse exposure to nutritional stress and to depletion of fat reserves (Leak 1999; Hargrove 2004). In turn, a gradient of reducing levels of nutritional stress and thus of reducing prospects for depletion of fat reserves, could entail a gradient of increasing levels of tsetse immune system and capacity to prevent trypanosome infection – i.e. to a gradient of reducing levels of tsetse susceptibility to trypanosome infection (Kubi et al. 2006).

Increase in distance away from an area of concentration of human settlement, i.e. from an area with high human population density, to areas with sparse or no human settlement at all, is usually associated with reduction in human-associated tsetse habitat degradation (Reid et al 2012). This relationship between human settlements and habitat degradation is the likely explanation for the results obtained in this study. In the Mpika and Rufunsa sites (where human settlement was concentrated at the edge of the tsetse belt), it was likely that there existed a gradient of increasing habitat quality from the edge into the inner parts of the tsetse – likely associated with decreasing levels of human-associated habitat degradation – particularly reduction in availability of tsetse hosts. In the Lundazi site (where human settlement was uniformly distributed along the transect line), it was unlikely that increase in the distance (from the edge into the inner parts of the tsetse belt) was associated with any significant change (gradient) in tsetse habitat quality.

In this regard, the findings in this study suggest that within a tsetse belt, spatial variation in levels of tsetse habitat degradation, particularly the kind that is associated with human activities, could give rise to spatial variation in the prospects for manifestation of stress such as that associated with a significant reduction in sources of blood meals (i.e. hosts) (Leak 1999; Hargrove 2004). In turn, this could entail spatial variation in levels of fat reserves in tsetse flies, and hence variation in levels of tsetse susceptibility to trypanosome infection - and consequently to spatial

variation in the prevalence of trypanosome infection in affected tsetse infested areas (Kubi et al. 20).

We conclude that in the Mpika and Rufunsa sites, where human settlement was concentrated at the edge of the tsetse belt, increase in distance from the edge into the inner sections of the tsetse belt, entailed a gradient of reducing likelihood of finding a *G. m. morsitans* tsetse fly infected with Nannomonas and Trypanozoon trypanosomes. Further, we conclude that this was likely also a gradient of reducing levels of human-associated tsetse habitat degradation, particularly in the context of increasing levels of availability of tsetse hosts. Absence of such a gradient in prevalence of trypanosome infection in the flies in the Lundazi site, where the distribution of human settlement was even in the transect line, further supported this conclusion. As such, the particular gradient also represented that of increasing levels of nutrition and fat reserves in the tsetse flies, and consequently increasing levels of tsetse immune system capacity to prevent trypanosome infection in the tsetse flies.

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CHAPTER 4: HUMAN-ASSOCIATED FACTORS RELATED TO TSETSE HABITAT DEGRADATION IN AREAS DEVOID OF CATTLE IN NORTH-EASTERN ZAMBIA

4.1 Abstract

In the study presented in chapter 2, the findings showed an improving gradient of habitat quality from the edge into the inner parts of the tsetse belt, in the Mpika and Rufunsa study sites. These findings entailed need to establish the likely explanation for the observed gradient in tsetse habitat quality. The quality of a tsetse habitat is determined by levels of availability of ideal climatic conditions, vegetation cover, and tsetse hosts that support the survival and well-being of tsetse flies. An ideal tsetse habitat provides optimum levels of climatic conditions, vegetation cover, and tsetse access to hosts. Cattle are good tsetse hosts, and presence of cattle in a tsetse infested area entails good availability of hosts for the tsetse population in the particular area – and absence of such livestock species entails tsetse dependence on wild hosts for blood meals. In an earlier study, the findings indicated existence of a gradient of increasing tsetse habitat quality from the edge into the inner parts of the tsetse belt in the Mpika and Rufunsa sites, and this suggested that climatic and/or environmental conditions were less favourable for tsetse flies at the edge of the tsetse belt, and improved with increase in distance from the edge into the inner parts of the tsetse belt. This part of the study investigated land use and human-associated factors that could affect availability of hosts, as the potential reasons for the gradient in habitat quality in the two sites – considering that climatic conditions tend to be similar over vast areas and were known to be favourable for tsetse flies in the whole tsetse belt. In each site, vegetation cover change during the period 2000 to 2010 was calculated, based on land cover maps of the country as of 2000 and 2010 –considering that the data in chapter 2 was collected in 2012. Through a questionnaire survey, data were collected from communities living in the study areas on land use and ecosystem resources accessed/used at each site. Results indicated that minimal loss of vegetation cover had occurred during the period 2000 to 2010 ranging from 0.3%, 0.2% and 0.3% in the Mpika, Lundazi and Rufunsa sites respectively. This suggested that loss of vegetation cover was unlikely to explain the gradient in habitat quality in the two sites. Although results showed that communities obtained a variety of ecosystem resources from locations away from their settlements, it was also evident

that locations close to the settlements were more readily accessed for the purpose, than locations that were further away. Another finding was that wild animals were more likely to be found in locations that were far away from human settlements. It was concluded from the study that in the Mpika and Rufunsa sites, levels of tsetse access to blood meals (to hosts) was lowest in locations that were closest, and highest in locations that were furthest, from the human settlements – supporting the observed gradient of increasing tsetse habitat quality - from the edge into the inner parts of the tsetse belt in the two sites. The findings indicate the importance of host availability as a factor in tsetse habitat quality and degradation.

4.2 Introduction

Human beings undertake a variety of activities on land with the intention of obtaining products and/or benefits from land resources, and these activities are generally referred to as 'land use' (Reid et al, 2000; Tilman, 2001). Components of land that are of benefit to humans directly or indirectly are referred to as land resources (FAO, 1995). Human basic needs that include food, water, fuel and other forms of energy, medicines, clothing and shelter are in limited supply and, consequently, increase in human population and greater aspirations for development have led to land resources becoming increasingly scarce (FAO, 1995; Tilman, 2001). Scarcity of land resources leads to human encroachment into areas designated for wildlife conservation and that are usually also tsetse infested (Anderson et al, 2015). Human encroachment into such areas is associated with occurrence of detrimental consequences for the affected ecosystems that serve as habitats for wild life in general, and also for tsetse flies (Reid et al, 2000; Mwanakasale and Songolo, 2011).

With regard to quality of ecosystems as tsetse habitats, the key determinants are vegetation cover, temperature, and availability of wild vertebrate animals that serve as sources of blood meals for tsetse flies (Hargrove, 2004). In ecosystems that serve as tsetse habitats, a significant detrimental change in the level of availability of appropriate climatic conditions, vegetation cover, and vertebrate hosts, leads to manifestation of stress and related consequences for the affected tsetse population (Reid et al., 2000; Hargrove, 2004; Ducheyne et al., 2009).

In the components of the study presented in chapter 2 (Chilongo et al, 2020) and chapter 3 (Chilongo et al, 2021), the findings indicated existence of a gradient of reducing levels of occurrence of stress, and reducing prospects for prevalence of trypanosome infection, in the tsetse

flies, from the edge into the inner parts of the tsetse belt, in the Mpika and rufunsa sites (Chilongo et al, 2020; Chilongo et al, 2021). It was concluded the observed gradient of reducing levels of occurrence of stress in the tsetse flies, in the two sites, was likely associated with a gradient of increasing tsetse habitat quality - from the edge into the inner parts of the tsetse belt (Chilongo et al, 2020). This follow up component of the study was undertaken to establish the factors that contributed to the observed gradient of increasing tsetse habitat quality in the Mpika and Rufunsa sites.

All the study sites were tsetse infested, and were located in the eastern tsetse belt where climatic conditions were known to be suitable for tsetse flies as described by Rogers (1979) and Robinson et al (1997). For this reason, climatic factors were unlikely to have contributed to the existence of the noted gradient of increasing tsetse habitat quality in the Mpika and Rufunsa sites. All the sites did not have a cattle population, and this entailed that the tsetse flies in the area depended on wild hosts for their blood meals. Hence, focus was put on assessing changes in the level of vegetation cover during the period 2000 to 2010, and other factors that could affect availability of vertebrate hosts in each of the sites.

4.3 Materials and Methods

4.3.1 Study area

As described in 2.2.1.

4.3.2 Determination of change in vegetation cover during the period 2000 to 2010 in the study site

Considering that the questionnaire data (Table 9) was collected in the year 2012, it was taken that information on land cover change during the period 2000 to 2010 could provide helpful indication of the level and nature of vegetation cover change that could have occurred in each study site during the 10-year period before 2012. Land cover data (raster maps) covering the whole country for the years 2000 and 2010 were readily available under the Forestry Department in the Ministry of Lands Natural Resources and Environmental protection (MLNREP) (MLNREP, 2016). For this reason, the particular land cover raster maps were obtained from the Forestry Department (Forestry department, 2016) and used to determine land cover change during the period 2000 to 2010, in each site.

Land cover data for each site, were extracted from raster land cover maps indicated above, for the years 2000 and 2010. The land cover classes in the maps were as shown in Table 8. From the maps indicated above, sub land cover maps (raster maps) that covered only the defined area of interest in each of the study sites, was extracted with application of QGIS (version 3.4 (Madeira)) as indicated in Figure 7.

Table 8. Land cover classes in the 'Determined deforestation rate in Zambia' maps (Forestry department, 2016)

Class code	Description
1	Forest land
2	Grassland
3	Cropland
4	Wetlands
5	Settlements
6	Other land

4.3.3 Questionnaire survey on land use and ecosystem resources in the study sites

A semi-structured questionnaire was designed and administered to a sample of household heads in each of the human settlements in the study sites. The questions were framed in such a way as to collect information on use of the ecosystem resources at household and community levels, and also the nature, availability and access to the land resource in each of the sites.

Prior to implementation of the questionnaire, the community was informed through the local/traditional leadership about personnel from the department of Veterinary Services (2 to 3 workers per site, including the Veterinary Assistant responsible for the particular area) were to visit their residences on specified days, to talk to heads of households about issues concerning the problem of tsetse flies in the area. In each site, administering of the questionnaire started with the residence(s) that were located at the start of the transect line indicated in chapter 2, and following the transect road systematically in the direction of the inner parts of the tsetse belt. The enumerator (interviewer) visited each of the houses (residences) that were visible from the transect road, i.e. he/she moved from one house to the next and, interviewed the head of the household or the spouse

where one of these was present at the time of the visit. The questions/attributes (and context) in the questionnaire, and the response options per question, were as indicated in Table 9.

Table 9. Questions and response options in questionnaire survey undertaken in the Mpika, Lundazi and Rufunsa sites

Attribute ID	Attribute (Question)	Response options
1	Period lived in the area (years)?	1a. Less than 5 years
		1b. 5 to 10 years
		1c. 11 to 20 years
		1d. More than 20 years
2	Reason for decision to live/settle in the area?	2a. Need for More land
		2b. Need for better quality land
		2c. Both 2a and 2b
		2d. Ancestral home
3	Size of land (area) cultivated per season (acres)?	3a. Less than 5
		3b. between 5 and 10
		3c. between 10 and 20
		3d. 20 or more
4	Main crops grown (list)	
5	Frequency of clearing virgin land for new crop fields	5a. Every year/season
		5b. After 2 to 5 years
		5c. After at least more than 5 years
6	Use of chemical fertlisers	6a. Yes, 6b. No
7	Ruminant livestock: Number of goats kept?	7a. None
		7b. Less than 5
		7c. 5 to 10
		7d. 11 to 20
		7e. More than 20
8	Was grazing resource for ruminant livestock (goats) adequate?	
9	Source of energy for cooking and heating (indicate)	
10	Source of energy for cooking and heating: was there likelihood that the source/supply could get depleted in the near future?	10a. Yes
		10b. No
		10c. Not able to tell
11	Resources obtained from the forested areas (indicate)	
12	Sightings of antelopes and/or other similar kind of wild animals in the area?	12a. Yes/Common
		12b. No/rare
13	If 'No/rare' in Q12, how far away would one likely find/see such wild mammals?	13a. Far (at least an hour's walk)
		13b. Very far (more than an hour's walk)
14	Did tsetse flies exist in the area	14. Yes, 14b. No

4.4 Data analysis

4.4.1 Vegetation cover change during the period 2000 to 2010 in the study sites

The extracted land cover raster maps for 2000 and 2010, for each site, were analysed for vegetation cover change during the period 2000 to 2010. The process was undertaken using overlay operations in QGIS (version 3.4 (Madeira)), and procedure followed is shown in Figure 7. In the calculation, the change of interest was that from 'Forest land' to any of the other classes.

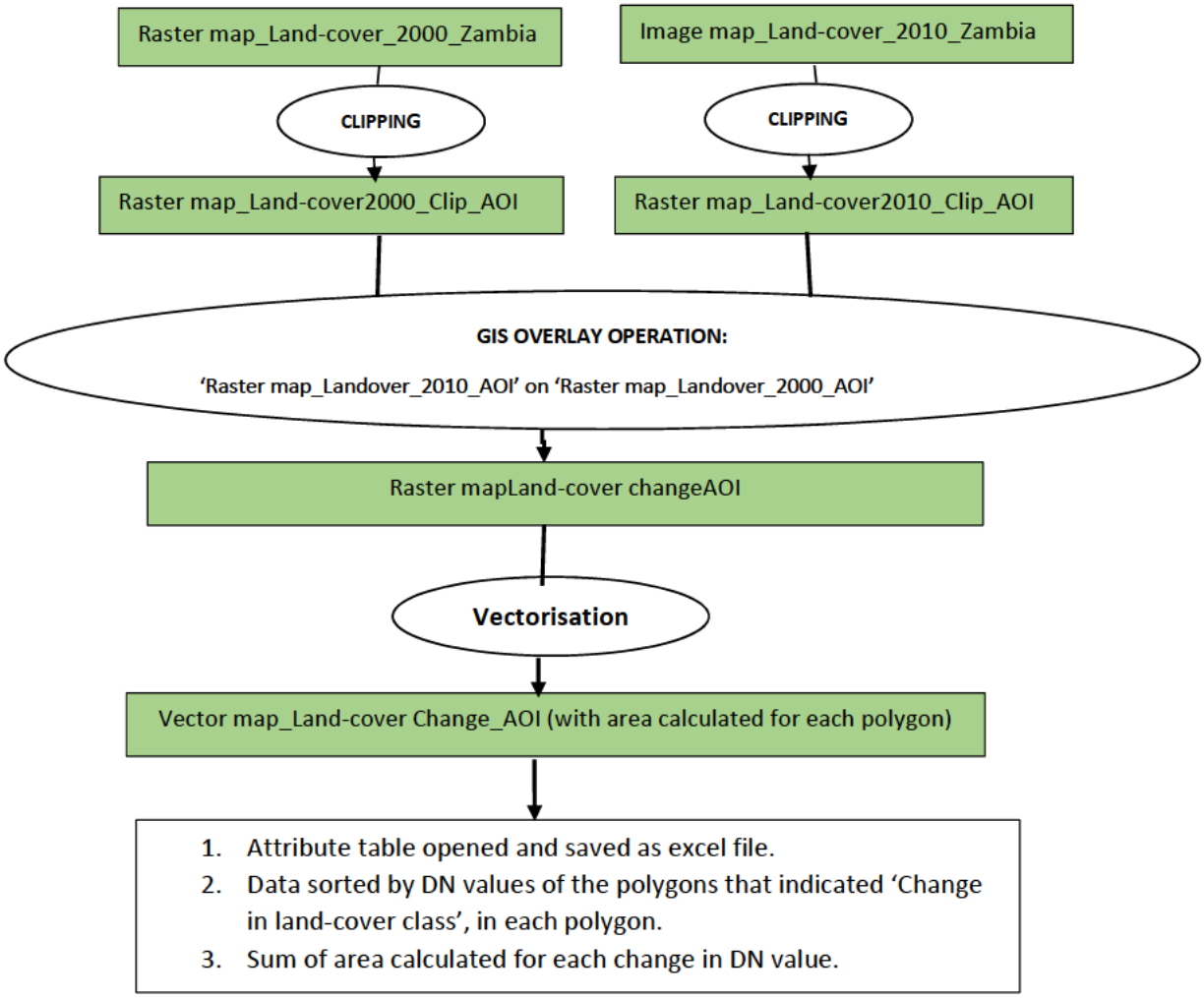

Figure 7. Procedure used to determine land cover change per site in the study area in north-eastern Zambia (adapted from Lillesand and Kiefer (2000)).

4.4.2 Questionnaire survey data

Questionnaire data were entered and stored in a table in Microsoft Excel. Calculations were made to establish the proportions (%) of respondents that gave particular responses to each of the questions/attributes, in each site. Cluster graphs were created that indicated the distribution of particular responses in each sites and among the three sites. In six of the questions/attributes (in the questionnaire), the response per question/attribute was identical/similar for all the respondents within and among the sites – as shown in Table 12. In the statistical analysis, the null hypothesis that there was no difference in the proportion of respondents that gave particular responses to each of the questions in the questionnaire, among the Mpika, Lundazi and Rufunsa sites, was tested with application of the chi-square in R version 3.6.2 (R Core Team, 2016).

4.5 Results

4.5.1 Vegetation cover change in sample sites between 2000 and 2010

The nature and magnitude of land cover and land cover change between 2000 and 2010, in the rectangular area that covered each site (Chilongo et al, 2020), was as shown in Tables 10 and 11. In 2000, the area under 'Forest' was 5582.7km^2, 2687.8km^2 and 171.6km^2 for Mpika, Lundazi and Rufunsa sites, respectively, and this represented 85%, 74% and 70.6% of the total area in the respective sites. The area under 'Forest' in 2010 was 5560.4 km^2, 2695.6 km^2 and 171.0 km^2 in the in the Mpika, Lundazi and Rufunsa sites, which represented 84.7%, 74.6%) and 70.4% of the total area in the three sites respectively. On the other hand, in 2010, the area under 'Cropland' in the Mpika, Lundazi and Rufunsa sites was 22.3 km^2, 7.8 km^2, 0.6 km^2 which was 4.2%, 1.4% and 5.4% of the total area in each of the three sites, respectively. The magnitude of reduction (change) in land cover from 'Forest' to 'Crop land', as a proportion of the total area in each site in 2010, was by 22.3 km^2 (0.34%) in the Mpika site, by 7.8 km^2 (0.22%) in the Lundazi site, and by 0.6 km^2 (0.24%) in the Rufunsa site. The largest magnitude of reduction in forest cover was in the Mpika site, followed by the Rufunsa site, with the least reduction in the Lundazi site.

Table 10. Land cover change from 2000 to 2010 in the Mpika, Lundazi and Rufunsa sites in north eastern Zambia

Land cover Type	2000						2010					
	Mpika		Lundazi		Rufunsa		Mpika		Lundazi		Rufunsa	
	km²	%	km²	%	km²	%	km²	%	km²	%	km²	%
Forest land	5582.7	85.0	2695.6	74.8	171.6	70.6	5560.4	84.7	2687.8	74.6	171.0	70.4
Grassland	686.8	10.5	767.9	21.3	57.7	23.7	686.8	10.5	767.9	21.3	57.7	23.7
Cropland	274.1	4.2	52.2	1.4	13.7	5.6	296.4	4.5	60.0	1.7	14.3	5.9
Wetlands	13.3	0.2	15.1	0.4	0.0	0.0	13.3	0.2	15.1	0.4	0.0	0.0
Other land	7.8	0.1	73.9	2.0	0.0	0.0	7.8	0.1	73.9	2.0	0.0	0.0
Total	6564.7	100.0	3604.6	100.0	243.0	100.0	6564.7	100.0	3604.6	100.0	243.0	100.0

Table 11. Land cover change from 2000 to 2010 as proportion of total land cover in 2010 in the Mpika, Lundazi and Rufunsa sites in north eastern Zambia

DN	Change Description	Land cover change as proportion of total land cover in 2010					
		Mpika		Lundazi		Rufunsa	
		km²	%	km²	%	km²	%
11	Forest to Forest	5560.4	84.7	2687.8	74.6	171.0	70.4
13	Forest to Cropland	22.3	0.34	7.8	0.22	0.6	0.24
22	Grasslands to Grassland	686.8	10.4	767.9	21.3	57.7	23.7
33	Cropland to Cropland	274.1	4.2	52.2	1.4	13.7	5.4
44	Wetlands to Wetland	13.3	0.2	15.1	0.4	0.0	0.0
66	Other lands to Other land	7.8	0.1	73.9	2.1	0.0	0.0

4.5.2 Questionnaire survey on land use and ecosystem resources

The number of respondents was 42, 57 and 34 in the Mpika, Lundazi and Rufunsa sites, respectively. The proportions of respondents that gave particular responses to each of the questions in the questionnaire, were as shown in Figures 8 and 9. In the case of responses that were identical or similar, in all the sites, the responses were as indicated in Table 12.

In all the sites, the proportion of respondents that had lived in the respective areas for less than 5 years was relatively low, 0%, 18% and 24 % in the Lundazi, Rufunsa and Mpika sites respectively. More than 60% of the respondents in the Mpika site had lived in the area for a period of 5 to 20 years, with 14% having lived in the area for more than 20 years. In the Rufunsa and Lundazi sites, 44% and 93% of the respondents had lived for more than 20 years respectively. In the Mpika site, more than 90% settled in the area in search of more and/or better-quality land for farming, and 76% of these cultivated between 5 and 20 acres per year. All respondents in the Lundazi and Rufunsa sites were in the area because that was their ancestral land, and 74% to 93% of these, respectively, cultivated less than 5 acres per year. As shown in Table 12, the list of the main crops grown in the three sites was literally identical in the three sites.

With regard to the rate at which new crop fields were cleared/established, 10% and 14% indicated doing so every year, in the Mpika and Rufunsa sites, and none did so in the Lundazi site. In all the sites, 29% to 64% cleared new field every 2 to 5 years, and 26% to 71% did so every after more than 5 years. All the respondents did make use of chemical fertilizers in their crop farming.

Ownership of ruminant livestock was such that 55%, 62% and 95% of the respondents in the Mpika, Rufunsa and Lundazi sites, respectively, did not own any ruminant livestock, and 26%, 5% and 24% owned less than 5 goats in the respective sites. Furthermore, 12% in the Mpika site, and 6% in the Rufunsa site, owned between 5 and 10 goats, and only in the Rufunsa site did 3% indicate owning more than 20 goats. All the respondents indicated that there was no challenge with regard to availability of the grazing resource in their respective areas, and that they did not foresee any challenges with availability of the resource in the near future.

With regard to source of energy for cooking and heating, all respondents used firewood and charcoal, with 81% in the Mpika site, 96% in the Lundazi site, and 74% in the Rufunsa site, indicating that they did not expect to face a shortage of the resource any time soon. In all the sites, the other main resources obtained from the communal areas were as listed in Table 14 - the list

included building materials (mainly trees, reeds and grass), honey, fish, wild fruits, herbal medicines, mushrooms, mice and edible caterpillars. Only in the Lundazi area did 95% of the respondents indicate seeing wild mammals such as antelopes in their routine activities/movements in the area. All respondents were affirmative about presence of tsetse flies in their areas.

The outcomes of the chi-square tests, per question, were as indicated in Table 13. The test results indicated that the differences observed in the cluster graphs were significant, with the p-value of less than 0.001, in the case of each of the following questions/attributes: (i)'period lived in the area'; (ii) 'reason for decision to live/settle in the area'; (iii) size of land cultivated', 'frequency of clearing virgin land for new crop fields'; (iv) number of goats kept'; (v) 'likelihood of depletion of source of fuelwood/charcoal' and (vi) sighting of antelopes and similar animals in area. Only in the case of the case of 'if antelopes and other similar animals sighted, how far away from the human settlements were the animals sighted?', applicable to only the Mpika and Rufunsa sites, was there no significant difference in the proportion of respondents that gave each of the two possible responses, in the two sites.

Table 12. List of questions/attributes on which respondents provided identical or similar responses

Attribute ID	Attribute/Question	Response (in each site)
4	Main crops grown	List included almost all of the following: Mainly: Maize, beans, groundnuts, Soya beans, sweet potatoes, cassava, sunflower, millet, pumpkins, tomatoes & vegetables in gardens.
6	Use of chemical fertilisers	All respondents indicated 'Yes'
8	Whether grazing resource for ruminant livestock (goats) was adequate	All respondents indicated 'Yes'
9	Source of energy for cooking and heating	All respondents indicated 'Fuelwood and charcoal'
11	Other resources obtained from the ecosystem	List included almost all of the following: Building materials, firewood, herbal medicines, honey, fish, mice, wild fruits, edible caterpillars, mushrooms, mice.
14	Whether tsetse flies existed in the area	All respondents indicated 'Yes'

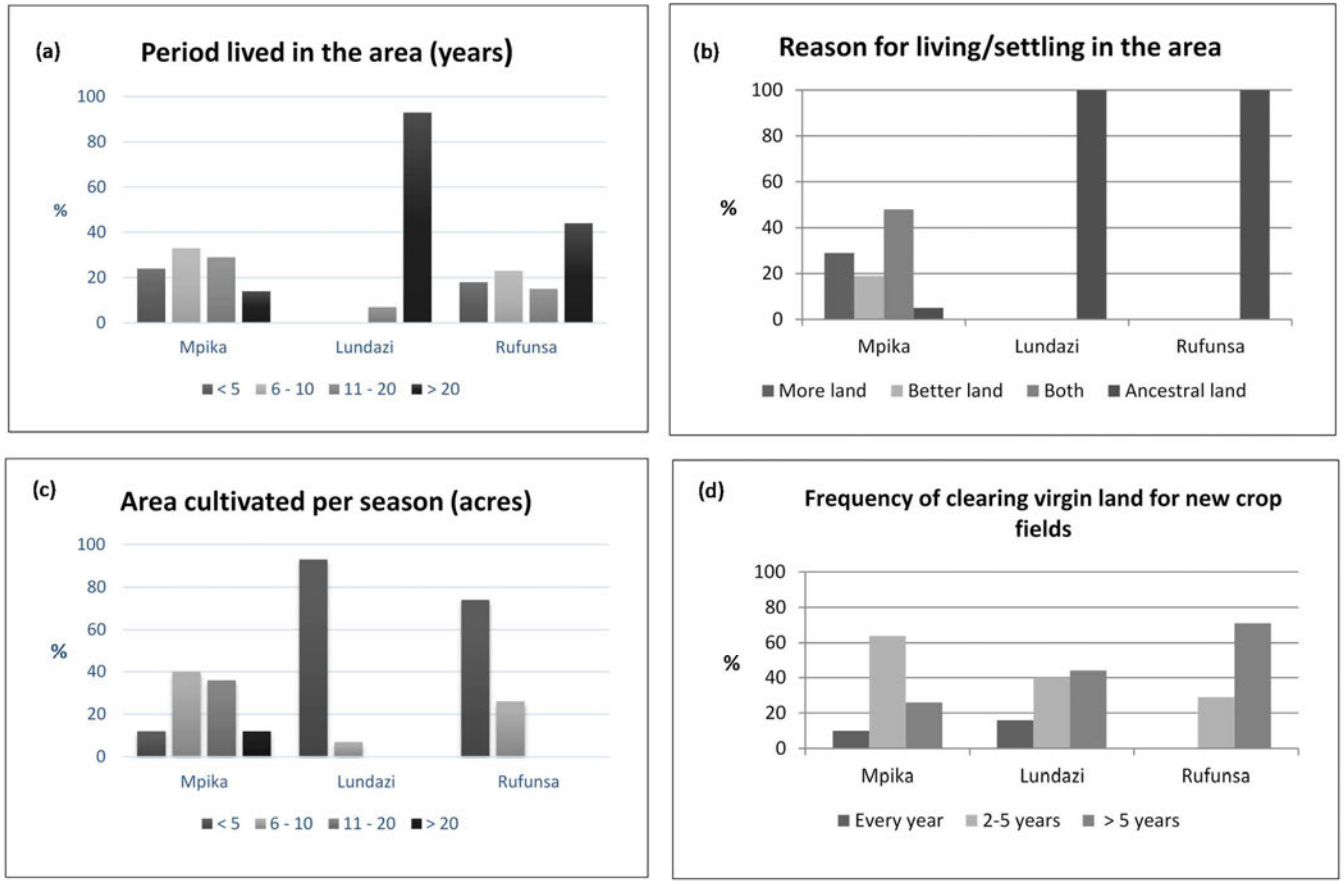

Figure 8. Proportion of respondents on (a) period of residence in the area, (b) reason for decision to settle/live in the area, (c) size of crop fields cultivated per season, and (d) frequency of clearing new crop fields, in the Mpika, Lundazi and Rufunsa sites

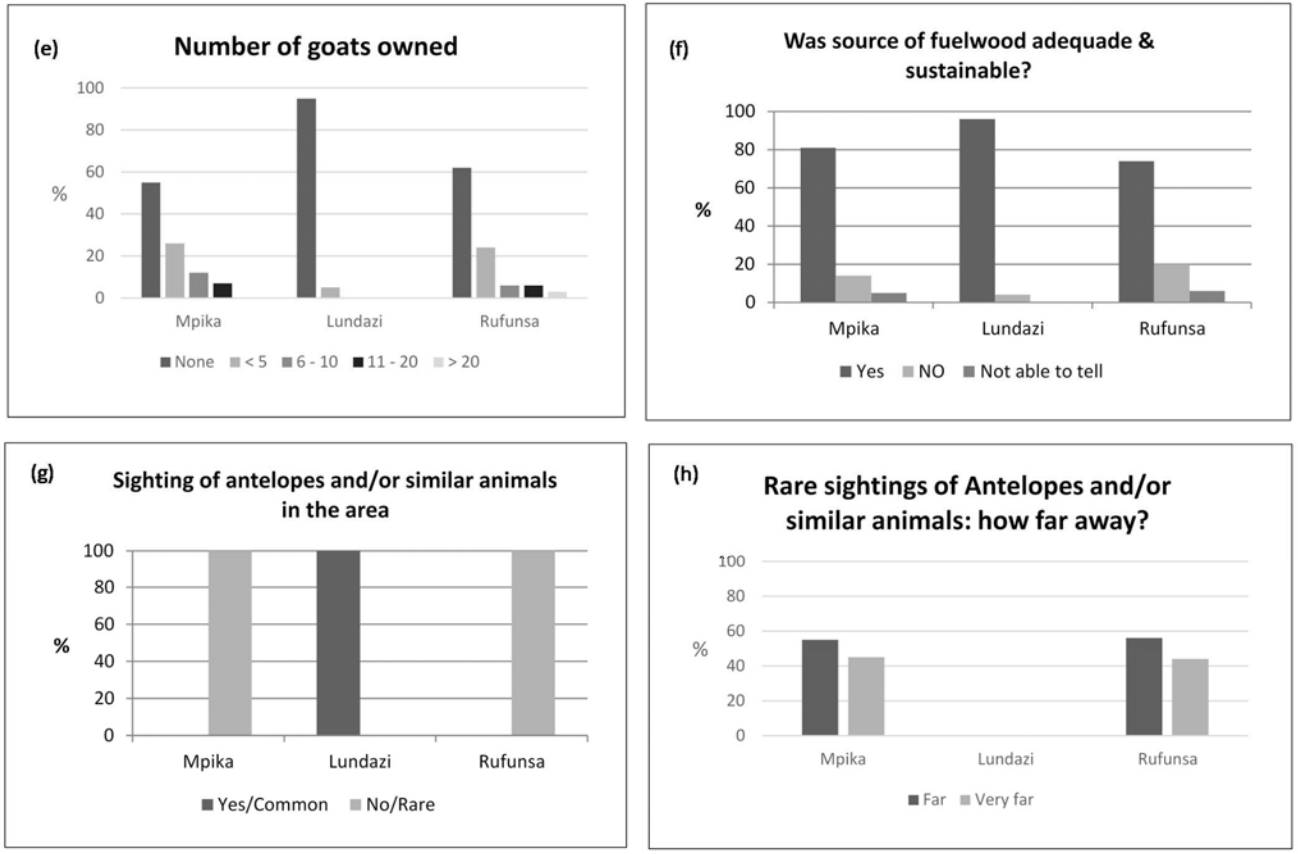

Figure 9. Proportion of respondents on (e) rearing of goats, (f) likelihood of depletion source of energy for cooking and heating, (g) sighting of antelopes and similar vertebrate wild animals, and (h) how far away from settlements sighting of antelopes and other similar vertebrate wild animals occurred, in the Mpika, Lundazi and Rufunsa sites.

Table 13. Results: chi-square test of the hypothesis that there was no difference in the proportion of respondents that gave particular responses to each of the questions (in the questionnaire) among the Mpika, Lundazi and Rufunsa respondents

	Responses to attributes/question:	x^2	df	p-value
1	Period lived in the area	133.51	6	< 0.0001*
2	Reason for settling/living in the area	279.12	6	< 0.0001*
3	Size of area cultivated per season	178.69	6	< 0.0001*
4	Frequency of clearing a new crop field	51	4	< 0.0001*
5	Number of goats reared	52.28	8	< 0.0001*
6	Source of energy for cooking and heating: Is shortage likely in the near future?	19.87	4	< 0.0001*
7	Sightings of antelopes and/or similar wild animals in the area	295	4	< 0.0001*
8#	Sightings of antelopes and/or similar wild animals in the area - how far away from settlements?	0	1	1

*Difference in proportions significant at $p = < 0.05$

#Comparison of only the Mpika and Rufunsa sites – the question was not application to the Lundazi site

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4.6 Discussion

Changes in ecosystem function and consequently ecosystem productivity, is usually a consequence of significant negative external impacts such as those caused by human activities (Reid et al, 2000; Vinya et al, 2012). Understanding the nature of such external impacts is of importance, particularly in the context of how these impacts may be mitigated (Chidumayo, 1987; Vinya et al, 2012).

The findings from this study show that the proportion of the area under crop fields, in each of the sites, was relatively very small in relation to that under forest cover. The

communities practiced subsistence farming, as shown by the type of crops they were growing (IAPRI, 2016). In the Lundazi and Rufunsa sites, this may also have been attributed to that households lived in the respective areas largely because that was their ancestral land and were not prepared to move to virgin land.

In the Mpika and Rufunsa sites, 40 to 50 percent of the residents had moved to new areas during the last10 years. The move resulted in the opening up of new crop fields leading to the observed land cover change from 'forest' to 'cropland', noting that agricultural encroachment is a significant cause of reduction in forested land in sub-Saharan Africa (Saunders et al, 2012). In addition, in all the three sites, the change may also have been associated with the expansion of existing crop fields, although the rate was very low. Another contribution to the vegetation cover change was likely due to use of firewood and charcoal as the only source of energy for cooking and heating, in all the three sites (Chidumayo and Gumbo, 2013).

The low level of vegetation cover change from 'forest' to 'cropland', and the high proportion of 'forest' in each of the sites, as at 2010, suggests that vegetation cover change was unlikely to be the explanation, or the major reason, for the observed existence of a gradient of increasing tsetse habitat quality from the edge into the inner parts of the tsetse belt, in the Mpika and Rufunsa sites.

In the Mpika and Rufunsa sites, sighting of wild mammals such as antelopes in the area, was unknown, one could only see wild animals of the kind if one traveled to locations further away from the settlements. Although wild animals were not listed by communities as source of meat, it was established that poaching activities were a major challenge in the tsetse belt (DNPW, 1998).

In the Mpika and Rufunsa sites, the human population was concentrated in the human settlements, with literally no other human settlements in the inner parts of the tsetse belt. According to Reid et al (2000) and Ducheyne et al (2009), areas of high human population density are associated with higher levels of human-associated environmental degradation, than areas that are uninhabited or sparsely populated. This generally suggested that human-associated ecosystem degradation was likely to be highest in locations that were close to the settlements – than in locations that were furthest from the settlements inside the tsetse belt.

In the Mpika and Rufunsa, places that provided the necessary ecosystem resources were located in the inner parts of the tsetse belt. Locations that were more distant from the settlement were likely accessed largely for resources that could not be found in locations that were in close proximity – and this likely included cases where particular kinds of resources (e.g. plants that

served as sources of herbal medicines, reeds for building and for making mats, wild fruits, natural beehives for honey, edible caterpillars, and mushrooms) became depleted in locations that were close to the human settlements. This suggested that levels of associated human activities, and their effects on the ecosystem, were likely greater in places that were close, than in locations that were far from the settlements. In the Mpika and Rufunsa sites, this was the likely nature of the observed gradient of reducing levels of human-associated tsetse habitat degradation, from the settlements into inner parts of the tsetse belt.

In the Lundazi site, the even distribution of human settlement from the edge into the innermost parts of the tsetse belt, likely entailed a similar (even) distribution of the effects associated with the human activities in relation to use/access to ecosystem resources in the area. The observed absence of a gradient of increasing tsetse habitat quality from the edge into the inner parts of the tsetse belt, in the Lundazi site, could be explained by this pattern of human settlement in the area - in relation to distribution and effects of human activities in the ecosystem.

4.7 Conclusion

Considering that climatic conditions in the whole study area were suitable for tsetse flies, and vegetation cover change (between years 2000 and 2010) was extremely low in all the study sites, it is concluded that it was a gradient of increasing levels of availability of hosts, from the edge into the inner parts of the tsetse belt, that could best explain the findings in the Mpika and Rufunsa sites.

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CHAPTER 5: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

In the study, the aim was to contribute to the understanding of the influence of human-associated tsetse habitat degradation on tsetse fly (Diptera: Glossinidae) populations and prevalence of infection with trypanosome in North-Eastern Zambia. Tsetse flies infest about 277,000 km^2 (38%) of Zambia's land area, with tsetse-transmitted trypanosomiasis among the most important diseases of livestock in the country (DVS, 2019). Furthermore, prevalence of the disease in humans remains a significant challenge in the affected rural/remote areas in the country (DVS, 2019). The disease largely affects economically vulnerable communities in rural and remote parts of the country, where livelihoods are largely dependent on subsistence agriculture (Anderson et al, 2015; Mulenga et al, 2020). The eastern tsetse belt in north-eastern Zambia is among areas most affected by the tsetse and trypanosomiasis problem (Anderson, 2015; DVS, 2019).

In rural communities, human activities associated with livelihoods are largely centred on utilisation of the available land/ecosystem resources, mainly through subsistence agriculture (FAO, 1995; Reid et al, 2000). In a tsetse belt, agricultural activities, demand for building materials and, for fuelwood and charcoal, are known to diminish natural vegetation cover (Reid et al, 2000; Vinya et al, 2012; Chidumayo and Gumbo, 2013). However, communities obtain much more resources from these ecosystems, and these include mushrooms, honey, fish, medicinal plants, and edible caterpillars (De Cauwer et al, 2018). Very limited work has gone into how livelihood associated human activities may affect tsetse habitat quality, particularly in relation to potential implications on tsetse flies and on occurrence of trypanosomiasis – and this was the focus of this study.

Outbreaks of the human form of trypanosomiasis, sleeping sickness, is known to occur in particular foci - villages or settlements (Mwanakasale and Songolo, 2012). Factors in occurrence of such outbreaks may be linked to the nature of the interactions among humans and human activities, tsetse habitat quality and, tsetse flies and trypanosomes. The outcomes of this study bring out some helpful insights into the nature of these interactions.

Manifestation of stress in tsetse flies is associated with occurrence of environmental conditions that are detrimental to tsetse survival and well-being (Hargrove, 2004), and this is often linked to human – associated degradation of tsetse habitat (Reid et al, 2000). This component of the study investigated occurrence of stress in samples of tsetse flies collected

following a transect line that ran from the edge into the innermost parts of the tsetse belt, in relation to distribution of human settlements, in three sites in north-eastern Zambia.

The selection of study sites that had human settlement concentrated either at the edge of the tsetse belt (the Mpika and Rufunsa sites), or evenly distributed along transect line (Lundazi site), facilitated detection and measurement of any association between occurrence of stress in tsetse flies and distribution of human settlements. In the evaluation of occurrence of stress in the tsetse flies, in the transect line, use of tsetse body size, age and hunger stages in the tsetse flies as indicators of occurrence of stress, was a useful indirect practical field approach (Bursell, 1960; Leak, 1999). However, measurement of residual dry weight in tsetse samples is considered a more accurate and direct way of measuring for occurrence of stress in tsetse flies (Van den Boscche, 1997).

The findings led to the conclusion that there existed a gradient of reducing levels of occurrence of stress in the tsetse flies, from the edge into the inner parts of the tsetse belt, in the Mpika and Rufunsa sites where human settlement was concentrated at the edge of the tsetse belt (Chilongo et al, 2020). This finding formed the basis for the follow up component of the study that is detailed in chapter 4, aimed at establishing the possible reasons for existence of the observed gradient in occurrence of stress in the tsetse flies in the two sites, in relation to the distribution of human settlements.

Considering that measurement of residual body weight, where feasible, is a more direct and more accurate measurement of occurrence of stress, particularly nutritional stress, in tsetse flies, it would be of benefit if future research work applied this method of measuring occurrence of stress in tsetse flies. Future studies could also make use of more effective and less biased tsetse sampling methods, such as electric screens (Vale, 1993). Further, it would have been helpful, in the study, to also undertake blood meal analysis on the tsetse samples, for identification of the sources of blood meals in the transect line in relation to the objectives of the study. This is another area of research recommended for the future.

In view of the observed existence of a gradient of reducing levels of occurrence of stress in tsetse flies, from the edge into the inner parts of the tsetse belt in the Mpika and Rufunsa sites (Chilongo et al, 2020), follow up investigations were necessary. One of the two follow up studies evaluated the potential implications of this gradient on prevalence of trypanosome infection in the study sites. In studies by Kubi et al (2006) and Akoda et al (2009), occurrence of stress in tsetse flies was associated with increase in the susceptibility of *G. m. morsitans* tsetse flies to infection with *T. b. brucei* and *T. congolence* trypanosomes. This study was in part intended to investigate if the phenomenon as observed in the reared flies may occur in wild

populations of the tsetse flies, in relation to occurrence of stress in tsetse flies. The findings indicated that, in the Mpika and Rufunsa site, increase in distance from the edge into the inner parts of the tsetse belt was associated with reduction in the prevalence of trypanosome infection in *G. m. morsitans*. It was concluded that the observed gradient reducing levels of occurrence of stress, also represented a gradient of reducing prospects for prevalence of trypanosome infection, in the tsetse flies, in the two sites (Chilongo et al, 2021).

However, it may be helpful to also look at other factors that could possibly play a role in occurrence of the observed gradient of reducing prospects for prevalence of trypanosome infection in the tsetse flies, in the Mpika and Rufunsa sites. One example is the possible role of endosymbionts as factors in the occurrence of trypanosome infection in tsetse flies (Mbewe et al, 2015), and this entails potential benefits of studies in future that could investigate this possibility. Considering the outcomes of the study indicated, it would have been of benefit if tsetse blood meal analyses were also undertaken, as the information would have helped to get insights into the nature of any variation in the sources of the blood meals in the tsetse hosts in the transect line in relation to the observed gradient. For this reason, it was recommended that future research on the subject includes work on blood meal analysis along such a transect line – as this could provide helpful insights into the nature of the distribution of vertebrate hosts in relation to occurrence of nutritional stress in tsetse flies (Leak, 1999; Hargrove, 2004; Muturi et al, 2011).

In findings reported by Chilongo et al (2021), the Mpika and Rufunsa sites, locations that were closest to human settlement had the highest prevalence of trypanosome infection in tsetse flies. What would happen if a significant number of cattle were to be introduced in the human settlements, how would this affect the observed gradient of reducing prospects for trypanosome infection? Research work on this aspect, in future, could help answer these questions, noting that presence of cattle is a major factor in the quality of a tsetse habitat, as noted by Van den Bossche and Staak (1997).

In the third component of the study, the aim was to identify the major human-associated tsetse-habitat-degrading factors that could explain the observed existence of a gradient of reducing levels of occurrence of stress, in the Mpika and Rufunsa sites, as indicated by Chilongo et al (2020). Climatic factors in the eastern tsetse belt in Zambia are known to be favourable for tsetse survival and it was thus unlikely that variation in climatic factors could explain the observed gradient (Robinson et al, 1997). For this reason, focus was put on factors that were known to degrade tsetse habitat quality in the eastern tsetse belt, specifically depletion of vegetation cover and reduction in presence/availability of vertebrate animals that serve as

tsetse hosts (Reid et al, 2000; Mwanakasale and Songolo, 2012, Vinya et al, 2012; Anderson et al, 2015). As indicated in chapter 4, this was done by establishing the level of loss of vegetation cover during the period 2000 and 2010 (noting that field sample/data collection in the study sites was undertaken in 2012), and also through a questionnaire on use of the land/ecosystem resources in each site (Chilongo et al, 2020; Chilongo et al, 2021).

In the findings, there was minimal loss of vegetation cover that had occurred between the years 2000 and 2010, and this indicated that loss in vegetation was unlikely to explain the observed gradient in tsetse habitat quality. Through the questionnaire survey, the findings indicated that human activities associated with access and use of ecosystem resources, were the likely major factor involved. These activities were associated with access to ecosystem resources that included honey, mushrooms, building materials, medicinal plants, fish, mice, and edible caterpillars. Demand for these ecosystem resources in rural communities, is documented by Arnold and Townson (1998). It was taken that most of these human activities were more likely to take place in locations that were close to the human settlements, than in locations that were far away from the settlements (Reid et al, 2000). As a consequence, wild vertebrate tsetse hosts were unlikely to stay in locations that were close to the human settlements, and were more likely to be found in locations that were least frequented by humans and far away from the human settlements (Reid et al, 2000; De Cauwer et al., 2018).

Poaching is known to be a major challenge in the wildlife conservation areas located in the eastern tsetse belt (Jackmann and Billiouw, 1997; DNPWS, 1998). There was a likelihood that illegal hunting of vertebrate wild animals, particularly for meat, was among the human activities that took place in the ecosystem in each of the study sites. Considering that poaching is a crime, the respondents in the questionnaire were not expected to indicate vertebrate animals among the resources accessed in the ecosystems. Information on poaching activities may have been obtained from authorities responsible for wildlife protection and management in the respective areas. For this reason, it is important to include in future research information from authorities on levels of occurrence of poaching at study sites. Furthermore, modern methods of estimating the distribution and abundance of wild vertebrate animal species, including the known tsetse hosts in the areas, could help in the assessment of how the distribution of particular species may be related to human activities.

It was concluded that in the Mpika and Rufunsa sites, where human settlement was concentrated at the edge of the tsetse belt, there existed a gradient of reducing levels of occurrence of stress in *G. m. mositans* tsetse flies, from the edge into the inner parts of the tsetse belt. Furthermore, it was concluded that this gradient of reducing levels of occurrence of

stress in tsetse flies, also represented a gradient of reducing prevalence of trypanosome infection in the tsetse flies, and that the gradient of reducing levels of occurrence of stress also represented a gradient of increasing levels of availability of vertebrate animals that serve as tsetse hosts, noting that vertebrate animals are inclined to keep away from areas with a high level concentration of human settlements (Reid et al, 2000). Finally, it was concluded that the intensity of human access/use of ecosystem resources was highest in locations closest to settlements, and lowest in location that were furthest from the settlements, and that this led to the noted gradient of increasing prospects for presence of vertebrate hosts.

With regard to future research, it is recommended that studies of a similar nature, on occurrence of stress in tsetse flies, also looked at measurement of tsetse body fat content, which is a direct and more accurate measure of occurrence of nutritional stress in tsetse flies. With regard to prevalence of trypanosome infection in tsetse flies, in relation to the transect line, investigations on possible effects of endosymbionts, and also sources of blood meals, could help to bring out helpful new knowledge. In the case of factors in human-associated tsetse habitat degradation, it would be of value to gain more insights into possible occurrence of non-agricultural human activities such as poaching, that may have direct negative effects on availability of vertebrate tsetse hosts (Jackmann and Billiouw, 1998; Reid et al, 2000).

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