TSETSE FLY (DIPTERA: GLOSSINIDAE) SPECIES COMPOSITION, AGE STRUCTURE AND SEASONAL ABUNDANCE ALONG ESIA RIVER IN ITIRIKWA SUBCOUNTY, ADJUMANI DISTRICT, NORTH WESTERN UGANDA

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DECLARATION AND APPROVAL

DECLARATION

I hereby declare that this dissertation is my own original work and that it has never been submitted for the award of any degree in this University or any other institution of higher learning.

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APPROVAL

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DEDICATION

First of all, I would like to dedicate the success of this research to my parents for always supporting me financial and emotionally not to give up in my studies.

I also dedicate the success of this study to my supervisors for not giving up on me and ensuring that this study reached its logical conclusion.

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LIST OF ACRONYMS

WHO	World Health Organization
HAT	Human African Trypanosomiasis
UTCC	Uganda Trypanosomiasis Control Council
COCTU	Coordinating Office for Control of Trypanosomiasis in Uganda
SOS	Stamp Out Sleeping Sickness
AAT	Animal African Trypanosomiasis
NT	Non-teneral
T	Teneral
GIS	Geographical Information System
GPS	Global Positioning system
FTD	Flies per Trap per day
DRC	Democratic Republic of Uganda
PATTEC	Pan African Trypanosomiasis Eradication Campaign
SPSS	Statistical Package for social science

ABSTRACT

Background: Tsetse flies are vectors of African trypanosomes that present a threat to animal and human health on the continent. Controlling the vector therefore has the potential to improve agricultural productivity and human health in large parts of the continent. However, vector control requires reliable data on the actual species of the vectors and their dynamics. This study investigated the species composition, seasonal abundance and age structure of tsetse vectors along Esia River in Itirikwa Sub County in Adjumani district, northwest Uganda.

Methods: Tsetse surveys were conducted using biconical traps across 10 villages along Esia River in the dry (January 2023) and wet (May 2022) seasons. A total of 50 traps were deployed at a distance of at least 100m apart along riverbanks in each village, and checked after 72 hours. Trapped tsetses were identified using morphological keys, and data on entomological parameters assessed for the seasons. Age structure determination was carried out using wing fray analyses of flies.

Results: A total of 919 tsetse flies were captured (593 females and 326 males) in the 10 villages in both the dry and wet seasons. All the captured tsetse flies belonged to the *Glossina fuscipes fuscipes* subspecies. Average for all sites combined in both seasons was 3.205 ± 0.521 (Mean ± SE) with significant variations between villages ($p = 0.001$). Zoka (5.04 FTD) had the highest average fly abundance while the least was Lukwara (1.34 FTD). Generally more flies were caught during the wet season (n=618; mean FTD 4.11) than dry season (n=301; mean FTD = 2.225;p=0.031).

Overall, the difference in FTDs between the different vegetation types was not significant (p=0.542) though woodland appeared to have slightly higher number of catches than the other two vegetation types (forest and grassland). Estimated ages of the tsetse for the different villages ranged from 22-29 days for the wet season and from 18-25 days for the dry season implying that there were more old flies during the wet than dry season.

Conclusion and Recommendation

Glossina fuscipes fuscipes is the main vector for African trypanosomiasis along Esia River, with variable fly densities across villages and seasons. Given the relatively high FTDs in the area, regular surveillance and control should be instituted to reduce the incidence of African trypanosomiasis.

CHAPTER ONE

INTRODUCTION

1.1 Background

Tsetse flies belong to the genus Glossina and are vectors of African Trypanosomiasis, a parasitic disease caused by protozoa belonging to the genus *trypanasoma*. The diseases are called "sleeping sickness" (or Human African Trypanosomiasis, HAT) in humans and "Nagana" (or Animal African Trypanosomiasis, AAT) in animals. Transmission to both humans and animals is through the bites of infected tsetse fly that has acquired the infection from human or animals harboring the human pathogenic parasites (Wamwiri & Changasi, 2016). There are three main subgroups of tsetse flies depending on the environment they inhabit: riverine (palpalis), savannah (Morsitans), or forest-dwelling tsetse (fusca). All tsetse species are capable of transmitting human-infective trypanosomes. However, the major species involved in Human African Trypanosomiasis are the palpalis group specifically *G. palpalis, G. fuscipes* spp and *G. morsitans*.

African Trypanosomiasis (HAT) occurs in geographically delineated zones referred to as "foci". Such foci are often infested by sympatric species whereby one species is the predominant one (Mwanakasale & Songolo, 2011). Most people exposed to the vectors and the diseases are those who live in rural areas and depend on agriculture, fishing, animal husbandry or hunting for their livelihood.

Human African Trypanosomiasis takes two forms depending on the parasite involved: the chronic form *(T.brucei gambiense)* accounts for 97% of the reported cases found in 24 countries and the acute form due to *T. brucei rhodesiense* found in 13 counties accounts for 3% of the cases and infection (Organization & WHO Expert Committee on the Control and Surveillance of Human African Trypanosomiasis (2013: Geneva, 2013). Uganda for a long time harbored both forms of the disease but in separate foci. The chronic form was found in Maracha and Adjumani, Northwestern Uganda where the study area is found while the acute form is found in Tororo, South Eastern Uganda(Berrang-Ford et al., 2006). However, Uganda has now achieved elimination of the chronic form as a public health problem from the country (WHO, 2022). To sustain this gain, more surveillance and monitoring of the vectors and the parasites needs to be routinely carried out since HAT has always shown that it can rebound.

Other parasitic species and subspecies of the *Trypanosoma* genus are pathogenic to animals and cause AAT in both wild animals and domestic animals. AAT in domestic animals particularly cattle is a major obstacle to economic development of the affected rural areas due to its negative impact on livestock production ((Connor, 1994)). AAT causes both direct and indirect losses to livestock owners at herd level. The direct losses are due to mortality, morbidity, infertility and costs of trypanosomiasis control (drugs, chemicals, tsetse traps etc.). The indirect losses are due to the risk of the disease and these include: reduced food production due to reduced draught power, reduced livestock production levels due to restricted grazing and exclusion of ruminant production from tsetse infested areas (Alexandra P M Shaw et al., 2015).

Assessing the distribution and abundance as well as identification of trypanosomes species in tsetse populations is essential for understanding transmission dynamics, assessing human disease risk, and monitoring spatiotemporal trends and impact of control interventions of both AAT and HAT. Uganda eliminated gambiense sleeping sickness in 2022 (WHO 2023). However, cases of rhodesiense still continue especially in central and northeastern Uganda.

West Nile region, a traditional focus of gambiense HAT is therefore free of sleeping sickness following vector control campaign and continuous surveillance of the vectors and routine screening of the communities and treatment of affected victims in the endemic area led to successful eradication of the *Trypanosoma brucei gambiense* disease in west Nile region (J. R. Franco et al., 2024) But cases of AAT still remains. The hosting of the refugees in camps in the west Nile districts of Yumbe and Adjumani from South Sudan and Democratic Republic of Congo where *Trypanosoma brucei gambiense* is still existing puts the region at risk of re-introducing the diseases into the region if control measures are not put in place(Picado & Ndung'u, 2017).

West Nile region is currently hosting refugees from South Sudan and DRC, These refugees move with their livestock into the region without being subjected to screening for trypanosomiasis thus the unrestricted movement of humans and animals that might bring about a resurgence of the disease in the region. For this status quo of elimination to be sustained, and to reduce the burden of AAT in the area, there is need to provide periodical data on the status and distribution of the vectors and the disease incidence surveillance. This study aimed to assess the tsetse fly species composition, age structure and season abundance along Esia River in Itirikwa subcounty, Adjumani district, northwest Uganda. The result of

the study will help policy makes and humanitarian agencies in planning better control strategies in helping both the host and refugees together with their animals in controlling both the vector and trypanosomiasis in Itirikwa subcounty and Adjumani district at large.

1.2 Statement of the Problem

Tsetse flies transmit trypanosomes, which are unicellular parasites that causes sleeping sickness in Humans and Nagana in African livestock, resulting into Annual agricultural losses estimated in billions of dollars. In Uganda sleeping sickness especially the *Trypanosoma brucei gambiense* form is no longer a public health issue as declared by the WHO in 2022. However the parasites continue to have significant impact on livestock in Africa.

In Adjumani district tsetse flies continue to affect agricultural production through reduced animal draught power, reduced milk production and reduced conception rate leading to stagnated herd size. The community in Itirikwa sub county practice livestock farming and agronomy. The sub county serves as a food basket for the entire district due to high production yields in the area. The community relies on Esia River as source of water for livestock and household domestic chores, like washing clothes and bathing for communities that reside near the river. The communities in the area complain about tsetse fly bites whenever they go the riverbanks to bathe and wash clothes but don't know the kind of tsetse flies that are responsible for the bites. Besides, secondary reports from the district entomology office shows that the area is endemic for tsetse flies but no record of the specific species reported. Report from the Adjumani district veterinary office shows that the area has registered cases of Nagana in the cattle of the communities and have relied on use of trypanocidal drugs to manage the cases in their livestock but the disease continues to affect their livestock leading to drop in animal draught power for ploughing thus affecting agricultural production in the area.

The sub county is also home for the south Sudanese refugees who have been settled in the Mungula and Aliwara refugee camp. These refugees are coming from countries where sleeping sickness is still endemic. Human African trypanosomiasis (HAT) is endemic in south Sudan in the Eastern Mediterranean region and Foci of *Trypanasoma brucei gambiense* exist in the Eastern Equatoria region, a belt bordering Central African Republic, Democratic Republic of Congo and Uganda (Joja & Okoli, 2001).The sub county acts as wildlife corridor through which Elephants migrating from Nimule National Park in South Sudan to Murchison

National Park, Coupled with the movement of refuges with their livestock from South Sudan into Uganda and settling them in areas where there are tsetse flies, puts the community of Adjumani particular those in Itirikwa sub county and their livestock at risk of trypanosomiasis.

Trypanosomiasis control efforts have been largely determined and driven by observed disease outbreaks in the disease foci (Aksoy et al., 2017). The occurrence of the disease in an area cannot justify the types of control approach to be used without the knowledge of the vector spreading the diseases. It's not easy to accurately decide on control approach to use without having the knowledge about the tsetse fly's distribution and density pattern. This study assessed the tsetse fly species composition, age structure and seasonal abundance along Esia River in Itirikwa Sub-county in Adjumani district so as to assess the risk of both HAT and AAT in the district.

1.3 Objectives

1.3.1 General Objective

To assess the species composition, age structure and seasonal abundance of tsetse flies along Esia River in Itirikwa subcounty in Adjumani district to provide baseline data needed to assess disease risk and design effective control intervention.

1.3.2 Specific Objectives

1. To determine the tsetse fly species composition along Esia River in Itirikwa sub county in Adjumani district.
2. To determine seasonal variation in tsetse abundance along Esia River in Itirikwa subcounty in Adjumani district.
3. To assess the variation in abundance of tsetse flies with vegetation along Esia River in Itirikwa subcounty.
4. To determine the age structure of the male tsetse fly vectors along Esia River in Itirikwa subcounty.

1.4 Research questions

1. Which species of tsetse flies are found in villages along Esia River in Itirikwa Sub County?
2. What is the seasonal abundance of tsetse flies in villages along Esia River in Itirikwa Sub County?

3. What is the abundance of the tsetse flies in different vegetation types along Esia River?
4. What are the approximate ages of the male tsetse flies in different villages?
5. What is the variation in age of male tsetse flies with season?

1.5 Justification

Human African trypanosomiasis and Animal African Trypanosomiasis is a big threat to public health and constraint to agriculture sector. Although sleeping sickness is no longer deemed Public health issue in Uganda particularly in west Nile region, the parasites continue to have a significant impact on livestock in Africa. This is because it affects production in agriculture especially the livestock sector through decrease in milk production which is daily source of income and food for livestock farmers, affects productivity through affecting the reproductive potential of livestock through reduced conception rates, abortion, Morbidity and mortality. It also reduces animal draught power by making oxen weak and unable to plough which in turns affects acreage of land cultivated for agriculture production in communities that rely on animal draught power for cultivation, all these affects the livelihood of the community in Itirikwa sub county which is food basket of Adjumani district thus this if left unattended will in the near future bring food insecurity in the sub county and district at large. Therefore, this research will generate information and knowledge on tsetse fly species composition, age structure and seasonal abundance along Esia river in Itirikwa subcounty to help policy makers, investors, medical, veterinary and Entomological service managers in formulation and implementation of appropriate cost-effective control strategies. That will help restore and boost the production capacity of the community in the Itirikwa subcounty and Adjumani district at Large.

1.6 Scope of the Study

The study was conducted in Adjumani district Itirikwa subcounty, it was conducted in two seasons the wet season (May 2022), Reason being in this month chances of flooding are minimum and the risk of losing traps to flooding are low and dry season (January 2023) January being the peak month for dry season when most of the vegetative cover have been destroyed by bush burning thus no risk of traps getting burnt by wild fire. The study was conducted in 10 villages which included Ejunya, Oroguwa Aliwara in Mungula Parish chosen because of its close proximity to Esia River and their dependence on the river as source of water to for domestic chores, Livestock and others such as crop cultivation along the river banks. In Odu Parish villages chosen include Kolididi and Lukwara these villages are traverse

through by the river and the river is source of water for livestock and other domestic house chores that involves use. The community in this area during dry season also do brick laying along the river while in zoka Parish villages chosen include Gbayi, Zoka central, Oninyaraku, Ozugo East and Ozugo West these villages are also traversed through by Esia river and the community in this area graze their animal along the river banks during dry season and use the river as their main source of water for domestic work and agronomic practices like vegetable production, The community in zoka parish especially in Zoka central are also engaged in Lumbering and Charcoal burning. The study established tsetse species abundance and seasonal variation along Esia River in the 10 villages of Itirikwa Sub- County in Adjumani district

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

A tsetse fly (*Genus Glossina*) is a fly that appears greyish/ brownish in colour (Maudlin, 1999). It has one pair of wings and while at rest the wings overlap (i.e. the wings completely fold so that one wing rests directly on top of the other over the abdomen) (Figure 2.1). Several species and sub-species of *Genus Glossina* exist within sub-Saharan Africa. Species of glossina considered as vector of trypanasomiasis include:

1) *Glossina morsitans*
2) *Glossina pallidipes*
3) *Glossina longipalpis*
4) *Glossina palpalis gambiensi*
5) *Glossina fuscipes fuscies*
6) *Glossina longipennis.*



Figure 2.1: A tsetse fly at rest (Image reproduced from CDC website:

Source: https://wwwnc.cdc.gov/travel/diseases/african-sleeping-sickness-african-trypansosomiasis)

African trypanasomiasis occurs in geographically delineated zones referred to as "foci'. Such foci are often infested by sympatric species, whereby one species is the predominant on (Mwanakasale & Songolo, 2011). Flies pick up bloodstream parasites from their hosts: livestock, wildlife, and humans. Vectorial capacity describes the innate ability of a specific

fly species to acquire, mature, and transmit trypanosomes. Different tsetse species co-infesting the same habitat often have varying vectorial capacities for human-infective trypanosomes (Kohagne et al., 2010).

2.2 Tsetse Species

Tsetse flies can be grouped into three main subgroups depending on the environment they inhabit. These are riverine (palpalis), savannah (morsitans), or forest-dwelling tsetse (fusca) (Table 2.1). These species groups are distinguished based on the construction of the male genitalia (WHO Expert Committee on the Control and Surveillance of Human African Trypanosomiasis (2013: Geneva, 2013). The three groups of tsetse flies live in different types of habitats.

Table 2.1: Tsetse flies species and sub species

No.	**Species**	**Subspecies**
1	Savana	*Glossina morsitans*
		Glossina pallidipes
		Glossina longipalpis
		Glossina Swynnertoni
		Glossina Austeri
2	Riverine	*Glossina fuscipes fuscipes*
		Glossina tachinoides
		Glossina palpalis gambiensi
		Glossina morsitans submorsitan
3	Forest	*Glossina fusca*
		Glossina tabaniformis
		Glossina medicorum
		Glossina longipennis
		Glossina brevipalpis

According to Rogers (2003), the *palpalis* group (subgenus Nemorhina) occupies riverine habitats throughout Africa. With one exception (*G. longipennis*), the species of the *fusca* group (subgenus *Austenina*) are forest flies inhabiting either rain forests or isolated patches of forest, along with riverine forest in the savannah zones. The *morsitan* group prefers open woodland, forest edges and scattered thickets. The dominant sub-species in Uganda is *G. f.*

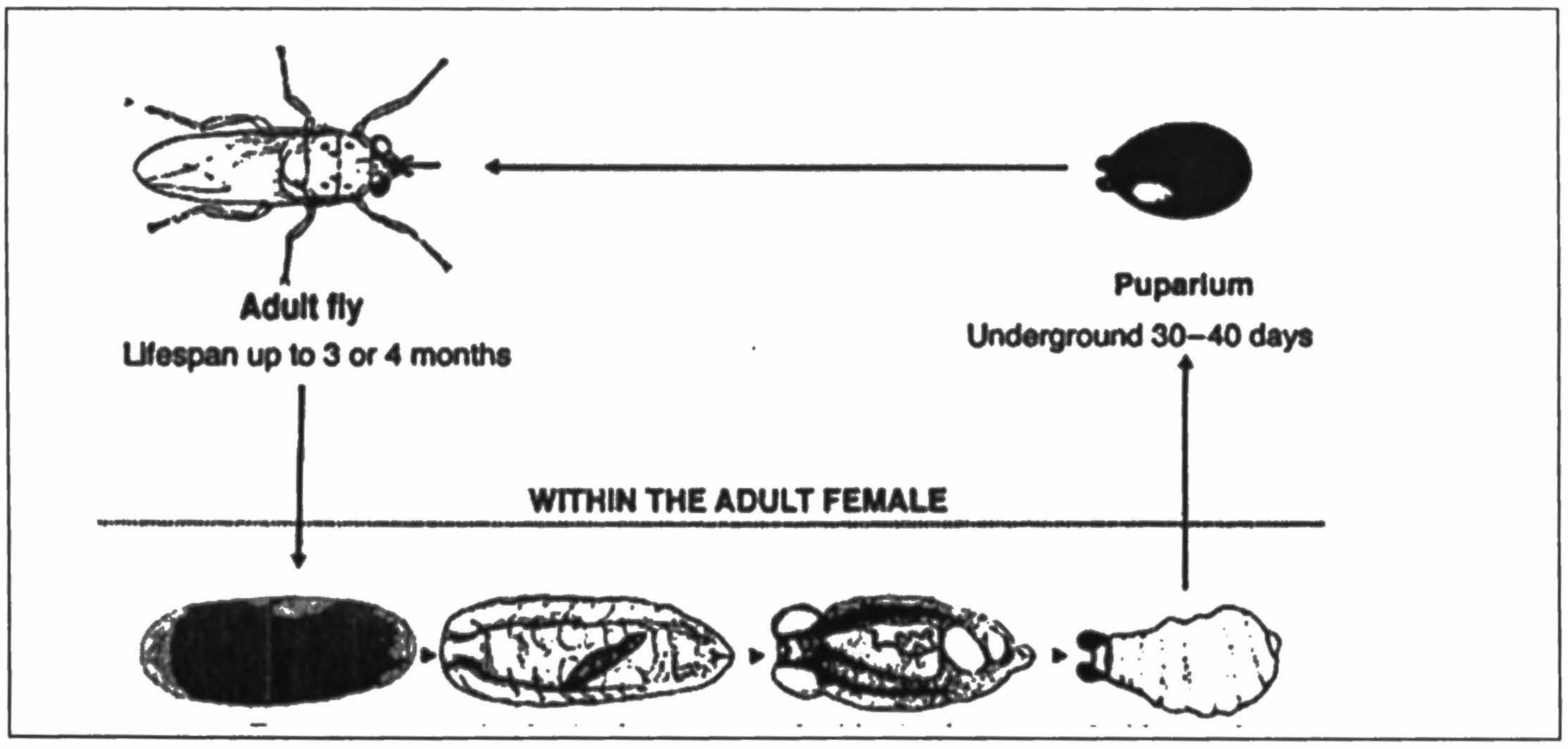
fuscipes and is part of the *Palpalis* group (A. P.M. Shaw et al., 2014). All species are capable of transmitting human-infective trypanosomes. However, the major species involved in human African Trypanosomiasis (HAT) transmission are the palpalis group tsetse, specifically *G. palpalis* spp and *G. fuscipes* spp.

2.3 Tsetse life cycle

Tsetse flies live in areas that provide ideal conditions for every stage of the life cycle. Precisely, tsetse flies live in habitats that provide shade for developing puparia and resting sites for adults (Rogers, 2003). Tsetse are characterized with a low reproductive rate, the female gives birth to one larva after one to nine days of egg deposition in the uterus. The larva burrows into the ground from where it pupates within a few hours without any feeding at all. The pupa transforms itself into a live adult fly from beneath the soil layer after 30 days (Maudlin, 1999).

The adult fly takes 12-14 days to mature in preparation for mating. Like most of insects, tsetse flies have a life cycle, which is constituted by four major stages (i.e. egg, larva, pupa and adult). The key difference is that for the tsetse fly, the egg is simply deposited and held in the uterus of the mother fly until it is converted into a larva. The larva is released from the mother fly and buries itself in the sub-soil. The process of converting from larva into pupa is done within the sub-soil. The pupa hatch into an adult and then exit the sub-soil onto the surface. This entire process takes close to 50 days in total (Maudlin, 1999).

Figure 2.2: showing Life Cycle of Tsetse fly



Source: Leak,1999.

2.4 Tsetse flies Abundance

Vector control programmes often make use of insect abundance and distribution data to plan interventions. Insect abundance refers to the measured population of insects in a unit area at a given timescale (Elama Ameh, 2011). The abundance of a target species is a fundamental ecological parameter and a critical consideration when making management and control decisions (Sileshi, 2007). Location of specific insect count data collected by entomologists during control and monitoring programmes acts as an informer on approximate levels of insect abundance in a given area. Low tsetse apparent density is usually associated with reduced transmission of trypanosomiasis, and *vice versa*. Unfortunately, in some situations due to a variety of factors at play, this assertion my not necessarily be correct (Tirados et al., 2020).

Sileshi 2007 asserts that; "there are many kinds and levels of decisions that need to be made based on insect abundance". For instance monitoring the performance of tsetse (*Glossina spp.*) control programmes depends largely on periodically measuring insect abundance. Such status usually determines the next steps.

2.5 Factors affecting tsetse flies abundance

2.5.1: Population pressure

Rising population levels in many parts of sub-Saharan Africa have caused increased land pressure, pushing more people into tsetse infested marginal areas. This immigration has led to: -

(i) Elimination of tsetse habitat, hence disappearance of tsetse flies and apparent elimination of the disease (Mwanakasale & Songolo, 2011).

(ii) Increased human-fly contact, leading to increased risk of contracting Trypanosomiasis (Rocque et al., 2001).

Simulation models have suggested that population growth will cause a decline of savannah and forest tsetse, with possible extinction in eastern and southern Africa (Esterhuizen et al., 2005) In these regions, tsetse populations have been confined to discrete habitats, with a high abundance in and around wildlife conservation areas such as game parks and reserves. Such conservation areas provide suitable conditions for tsetse survival and function as breeding sites. With increased human encroachment into protected areas, there is bound to be an increased disease risk at least in the initial period of settlement (Jones et al., 2013).

2.5.2 Climatic factors

Temperature

Generally, tsetse thrives best in areas with mean annual temperatures of 19 - 30°C. Temperatures below 19°C will slow down tsetse activity and general physiology; such unfavorable conditions will prevent the tsetse from flying and carrying out normal life functions. The tsetse will be unable to move to find a host for a blood meal, leading to its starvation (Lord et al., 2018). Under extreme temperatures of below 10°C, tsetse will die within 3 hours of exposure. Temperature regulates the ecology of insect communities. Overall, insects are very sensitive to climatic changes (Mboera et al., 2011)

Rainfall

Rainfall plays vital role in tsetse fly abundance; this is because it influences most of the environmental factors such as humidity, temperature and vegetative cover. Factors that affect the resting sites for adult tsetse flies, such as long-term changes in rainfall and temperature, can have a significant effect on the tsetse abundance, epidemiology and transmission of Trypanosomiasis ((Mboera et al., 2011). In Burkina Faso and Mali decreased rainfall and increased human density have been implicated in the contraction of previously documented tsetse habitat limits. Further, fragmentation of the tsetse habitat has important effects on fly population dynamics and has been shown to reduce tsetse apparent densities (Dennis et al., 2014).

2.5.3 Land use systems

Landscape features such as vegetation cover, livestock and human mobility are all important predictors of tsetse fly abundance and Human African Trypanosomiasis (HAT) incidence as they influence fly presence, density, and dispersal (Mweempwa et al., 2015). Social, cultural, and economic factors also affect outcomes in disease incidence (Rutto et al., 2013).

The land use systems such as crop farming, livestock rearing, fishing, hunting, charcoal burning and firewood collection increases the chances of flies' animal contact which provides the opportunity for the flies to get blood meal for egg production hence reproduction thus increasing tsetse abundance in areas with human and other animal activities than in areas with limited human activity (Reid et al., 2000).

2.5.4 Political instability

The resurgence of sleeping sickness in countries such as South Sudan, Angola, and the DRC has been attributed to political and civil unrest which has resulted in mass migration of populations into risk situations and the breakdown of traditional government support and disease control systems (Berrang-Ford et al., 2005) for example the case in Adjumani where over 100,000 South Sudanese refugees have been settled in Aliwara and Mungula settlement an area which is few kilometres from the Zoka forest reserve. This used to provide suitable habitats for tsetse flies. In many countries, disease surveillance and control activities are highly dependent on foreign aid, including nongovernmental aid agencies thus when war breaksup the already established facilities are destroyed disrupting the surveillance activities in such countries (Franco et al., 2012).

The reduction of foreign aid can affect control activities, leading to flare-ups of the disease. In the case of Democratic Republic of Congo (DRC), a dramatic upsurge in number of cases (up to 25,000 cases annually) (Organization & WHO Expert Committee on the Control and Surveillance of Human African Trypanosomiasis (2013: Geneva, 2013) was experienced when Belgian bilateral aid funding disease surveillance and treatment activities was terminated in 1990.

2.6 Epidemiology of Trypanosomiasis

Trypanosomiasis is a vector-borne disease transmitted to both humans and animals by the tsetse fly. The Trypanosomiasis occurs in 38 sub-Saharan African countries with an average of 15,000 human cases reported annually (Franco et al., 2014) and 70 million people at risk of contracting the infection (Shaw et al., 2014). Uganda reports approximately 500 cases of sleeping sickness annually and it is the only country that used to reporting the two forms of Human African Trypanosomiasis, the *gambiense f*orm in the northwest and the *rhodesiense* form in the southeastern Uganda before elimination of the gambiense form in 2022. (MOH and WHO, 2022)

The incidence of sleeping sickness has been declining over the years, falling from 26,000 cases reported in 2000 to less than 8,000 cases reported in 2012. Specifically, the number of *Gambiense* Human African trypanosomiasis (gHAT) and *Rhodesiense* African Trypanasomiasis (rHAT) cases reported to WHO in this period decreased by 75.9% and 87.9%, respectively (Franco et al., 2014) . This decrease is attributed to improved case detection and treatment and vector management ((Musaya et al., 2017).This improvement has

enable Uganda to achieve elimination of *Trypanasoma brucei* gambiense in 2022 although the Animal African trypanosomiasis and the *Trypanasoma brucei rhodesiense* form of the disease is still a challenge (WHO;2022).

2.7: The burden of Trypanosomiasis

2.7.1: Animal African Trypanosomiasis (AAT)

Animal African Trypanosomiasis commonly known as Nagana is one of the most significant constraints to livestock production in Africa (Shaw et al., 2014) . The disease is widely spread across the tsetse-infested territories of sub-Saharan Africa, with over 60million cattle at risk of Animal African Trypanosomiasis (AAT) infection in Africa (Shaw et al., 2014). The disease presents a number of symptoms, the common ones being; blood deficiency (Anaemia), intermittent fever, and edema, loss of weight, abortion and infertility among infected livestock (Okoth, 1991).

2.7.2 Human African Trypanosomiasis (HAT)

Human African Trypanasomiasis (Sleeping sickness), a tsetse transmitted disease, is associated with poor rural populations who primarily depend on their land and labour for livelihood (C Waiswa et al., 2006). The disease affects all age groups especially the most productive age group of 15-45 years and is 100% fatal if not treated (Organization & WHO Expert Committee on the Control and Surveillance of Human African Trypanosomiasis (2013: Geneva, 2013). This disease also affects a significant proportion of children. Unfortunately, many of these children even after successful treatment will usually have a considerable delay in their mental development, which will impact on their school results and professional performance (NSSCP report 2011). Infected individuals are weakened often for many years, which sinks them into further poverty, as they are unable to work.

The World Health Organization (WHO, 2012) estimates that in Africa 300,000 – 500,000 people are infected with sleeping sickness annually and according to a WHO report (2012), 70 million people are at risk of contracting the disease. With respect to Uganda, there is eminent danger that *T.b.rhodesiense* might overlap following the elimination of *Trypanosoma brucei gambiense* in the North western Uganda (Maudlin et al., 2009).

2.8 The Disease transmission cycle

The transmission cycle of gambiense-HAT (gHAT) is most commonly considered to be human-fly-human. It is thought that, in the presence of the vector, the long duration of gHAT

infection in humans is sufficient to maintain the transmission cycle. This forms the basis of the traditional screen-and-treat approach to gHAT control. A possible animal reservoir has been suggested in the epidemiology of gHAT, but its contribution to disease transmission remains unclear. Indeed, surveys conducted in gHAT foci indicate the presence of no or very few *Trypanosoma brucei gambiense* infections in livestock or wild animals (Farikou et al., 2010).

Additionally, it has been possible to locally eliminate *T. b. gambiense* transmission by treatment of the human reservoir alone, without recourse to animal targeted interventions. However, the involvement of animals in this cycle cannot be completely ruled out. It has been suggested that sustainable control of AAT is an indispensable approach towards achieving gHAT elimination in west and central Africa (Farikou et al., 2010),While the transmission of rHAT relies on the presence of vertebrate reservoirs comprising both domestic and wild animals and the cycle is typically animal-tsetse-animal/human. However, during epidemics when the number of infected persons is relatively high, the transmission cycle may follow the route human-tsetse-human (J. R. Franco et al., 2014).

In eastern and southern Africa, numerous wildlife species living in conservation areas serve to maintain the disease reservoir. As a result, some of the people infected do so in or around the game parks or reserves (Mohammed et al., 2013).

In the period 1990–2007, 49 non endemic cases were documented; principally tourists who were presumably exposed to tsetse bites in game parks in Kenya, Malawi, Tanzania, Uganda, Zambia, and Zimbabwe (Malvy & Chappuis, 2011).

Although wild animals are the key reservoirs in these game parks, investigations reveal low infection rates and low parasitaemia levels with human-infective trypanosomes. For instance, a recent study conducted within Zambia's Luangwa Valley found a paltry 0.5% *T. b. rhodesiense* infection prevalence in surveyed wildlife (Dennis et al., 2014).

Livestock in low endemicity foci exhibit a correspondingly low prevalence of *T. b. rhodesiense*, but this can be enough to cause a flare-up of the disease. The emergence of HAT in northern Uganda has been linked to the introduction of infected cattle from endemic southern areas in a restocking programme (Manangwa et al., 2016) and highlights the important role of veterinary policy in mitigating the effects of zoonotic disease (Malvy & Chappuis, 2011).

2.9 Disease and Vector Control options

Despite considerable investments towards the control and eradication of trypanosomiasis, tsetse and Trypanosomiasis still remains a major public health issue. The control of sleeping sickness hinges on two key aspects: disease control and vector control. Recent and ongoing improvements to these two aspects are contributing to attainment of the WHO target for disease elimination.

2.9.1 Integration of HAT Diagnosis and Treatment into National Primary Health Care Systems

The resurgence of sleeping sickness in countries such as Sudan, Angola, and the DRC has been attributed to political and civil unrest which has resulted in mass migration of populations into risk situations and the breakdown of traditional government support and disease control systems ((Berrang-Ford et al., 2005). In many of these countries, disease surveillance and control activities are highly dependent on foreign aid, including nongovernmental aid agencies (Maudlin et al., 2009). The reduction and cessation of foreign aid can affect control activities, leading to flare-ups of the disease. In the case of DRC, a dramatic upsurge in number of cases (up to 25,000 cases annually) was experienced when Belgian bilateral aid funding disease surveillance and treatment activities was terminated in 1990. The trend was reversed with the resumption of bilateral aid in 1998, and the subsequent continuation of large scale screening activities and treatment programmes (Pollock, 1992).

To reduce dependency on foreign aid for HAT control, endemic countries have formed the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) of the African Union to promote highest political level to fight the diseases and support member countries to take up ownership of the control process (J. R. Franco et al., 2014). Towards this, efforts have been made towards integration of disease diagnosis and treatment activities at government primary health care centers (Charles Waiswa & Wangoola, 2019)

2.9.2 Improved Diagnostic Tools

Substantial progress has also been made towards the development and routine application of improved diagnostic tools in endemic countries. These include incorporating the use of light-emitting diode fluorescence microscopy (Mitashi et al., 2015), Loop-Mediated Isothermal Amplification (LAMP) technique (Njiru et al., 2011), and individual Rapid Diagnostic Tests (RDTs)(Büscher et al.,2014) are currently being used as point-of-care tests(Musaya et al., 2017).

New techniques are being developed to shorten time-to-treatment for gHAT (Ndung'u et al., 2010), which effectively reduces the possibility of onward transmission. Another development towards improved HAT control has been compilation of disease distribution maps for HAT, taking advantage of the focal nature of the disease to compile comprehensive village-level maps of HAT distribution as an essential tool for disease control, research, and advocacy (Simarro etal, 2010) .

The HAT Atlas provides a valuable contribution towards informed decision-making for planning and monitoring of control activities and assessment of epidemiological trends as well as research activities.

2.9.3 Inclusion of Vector Management Strategies

Tsetse control plays a central role in controlling the zoonotic rHAT (Maudlin et al., 2009). However, epidemiologists now concur that vector control is required in the management of gHAT as well (Aksoy et al., 2017).Indeed, the implementation of vector control strategies along with medical interventions (screen and treat) in several gHAT foci, including Mandoul (Chad), northwestern Uganda, and Boffa (Guinea), has significantly reduced the incidence of new cases (Alexandra P M Shaw et al., 2015).

The necessity of vector control is also supported by the chronic nature of gHAT infection, with one case reportedly presenting as long as 29 years after the initial infection (Lindh et al., 2009).

In the presence of the vector, such an asymptomatic carrier may have an important potential role in disease transmission. There have been considerable advances made in the search for efficient and cost-effective control tools against riverine tsetse involved in the transmission of gHAT. This search has culminated in the development of so-called "tiny targets"(J. R. Franco et al., 2014). These insecticide-treated targets are much smaller (25 cm × 50 cm) than the traditional 1 × 1 m targets.

Tiny targets have proved to be quite effective for the control of riverine tsetse, specifically *G. fuscipes* spp. and *G. palpalis spp.*(Alexandra P M Shaw et al., 2015). In addition, they cost much less due to their smaller size and light weight, make them easily deployed by foot, or using bicycles and motorcycles (Alexandra P M Shaw et al., 2015). Further, tiny targets when mounted on sticks and fixed along rivers have proved to be effective in reducing tsetse density (Courtin et al., 2010)

2.9.4 Adoption of the "One Health" Concept in HAT Control

Increasingly, integrated strategies that use interdisciplinary study and action to address control of both HAT and AAT simultaneously are being promoted. This approach of controlling pests of veterinary importance that transmit zoonotic agents is an example of the "One Health" concept, where a single vector control technique mitigates the risk for transmission of two diseases. Indeed, the WHO specifically recommends that surveillance and control of rHAT should be coordinated with veterinary services in a "One Health" approach (WHO;2013). This strategy has been widely applied in both gHAT and rHAT foci to decrease tsetse density, thereby reducing human-tsetse contact and proving that simultaneous control of both AAT and HAT that has a bigger impact on disease incidence (Muhanguzi et al., 2014).

2.9.5 Vector Control in Protected Areas

In eastern and southern Africa, the distribution of tsetse flies is increasingly confined to protected areas such as game parks and reserves. These parks, due to their suitable vegetative cover and array of available host species, act as tsetse breeding sites and a large number of *Trypanasoma brucei rhodesiense* foci are associated with the wild animals in the game parks.

This situation has resulted in increased risk and incidence of rHAT infection among tourists and game park staffs (Neuberger et al., 2014) forcing some countries to implement tsetse control in game parks. In collaboration with tsetse control experts, wildlife authorities have instituted measures to reduce human-fly contact including aerial spraying, installation of impregnated traps, and targets/screens and spraying of vehicles on park exit (Manangwa et al., 2016). Such interventions may be successful if they are sustained for prolonged periods of time and should therefore be promoted.

2.9.6 Future Control Options

Genetic modification of the tsetse flies being one of the recent developments in vector control management. Arthopod research has revealed the presences of symbionts involved in the pathogenic organisms which can be manipulated to express foreign protein design to block pathogenic transmission (Aksoy et al., 2013).

The Para transgenic approach has been proposed as a strategy for inhibiting trypanosome survival, development, and maturation in tsetse and therefore interference with transmission of African sleeping sickness (Haines et al., 2013)]. The tsetse symbiont, *S. glossinidius*, has

been shown to influence vector competence, at least in some fly species. Belgian researchers have successfully genetically modified Sodalis to express antitrypanosomal genes that specifically target bloodstream parasites. This is a proof-of-concept that indeed the Sodalis bacterium is able to express and release a sufficient amount of active, functional, parasite-targeting compound (De Vooght et al., 2012) . This discovery provides a promising avenue in the fight against tsetse-transmitted Trypanosomiasis.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted in Adjumani district, in the West Nile region of Uganda,Adjumani lying on the Eastern side of river Nile (Figure 3.1). Adjumani district shares international border with South Sudan in the North, inter district border with Moyo in the North West, Arua and Yumbe in South West and Amuru South East. The District has 9 sub counties namely Adropi, Dzaipi, Ciforo, Ukusijoni, Ofua, Itirikwa, Pakele, Arinyapi, and 2 Town councils, Pakelle Town council and Adjumani Town council, 54 parishes and 206 villages. The district has a population of 232,813 Nationals (Uganda Bureau of Statistics, 2016) and 209,494 South Sudanese nationals (OPM, 2016).

Itirikwa Sub- County is one of the 9 sub-counties of Adjumani district. It's located in East constituency, consisting of 6 Parishes and 16 villages with total population of 47,100 people (UBOS, 2014), half of which are refugees settled mainly in Aliwara and Mungula Central in Mungula Parish. The major economic activities in the sub county is mainly agronomy with major crops being maize, cassava, sweet potatoes and vegetable production, livestock rearing mainly cattle and goats, brick laying along the River banks especially Esia river. The subcounty has the Zoka Forest Reserve towards the border with Amuru where charcoal burning and lumbering is taking place.

The Subcounty has two rivers (Esia and Itirikwa); the tributaries of these two rivers pour into Zoka River and finally into the Albert Nile River. Of the two rivers in the sub county, Zoka river is located within the reserve and not easily accessible due to the vegetation cover in the area. However, the vegetative cover along Esia River is riverine, forest, shrubs, savanna woodland and grassland and the river is used by communities for domestic and other uses hence partially playing a bigger role in the epidemiology of African Trypanosomiasis in the subcounty.

According to the record of the district Entomology Office, apparently no control is being deployed in the area compared to other sub counties in the district. This is because following the LRA insurgency in the year 1992 to late 2000, the sub county used to be a hideout for the rebels. Many of the locals in the area relocated to safer places like Ofua, Ciforo and Pakelle subcounty. However, following return of peace in the region and the subsequent creation of Itirikwa subcounty in 2013, the original natives of the area and other people with interest in

farming moved to the subcounty and started engaging in agronomic practices and livestock farming in the area. However, the challenge now is the reported presence and abundance of tsetse flies. Moreover Itirikwa subcounty act as the wildlife corridor where elephants migrating from Nimule National Park in South Sudan pass through Zoka forest reserve to Murchison national park. Coupled with the movement of refuges, the threat of Nagana and the possible resurgence of sleeping sickness in the area cannot be written off. Hence the need to carry out routine entomological and parasitological monitoring to inform interventions.

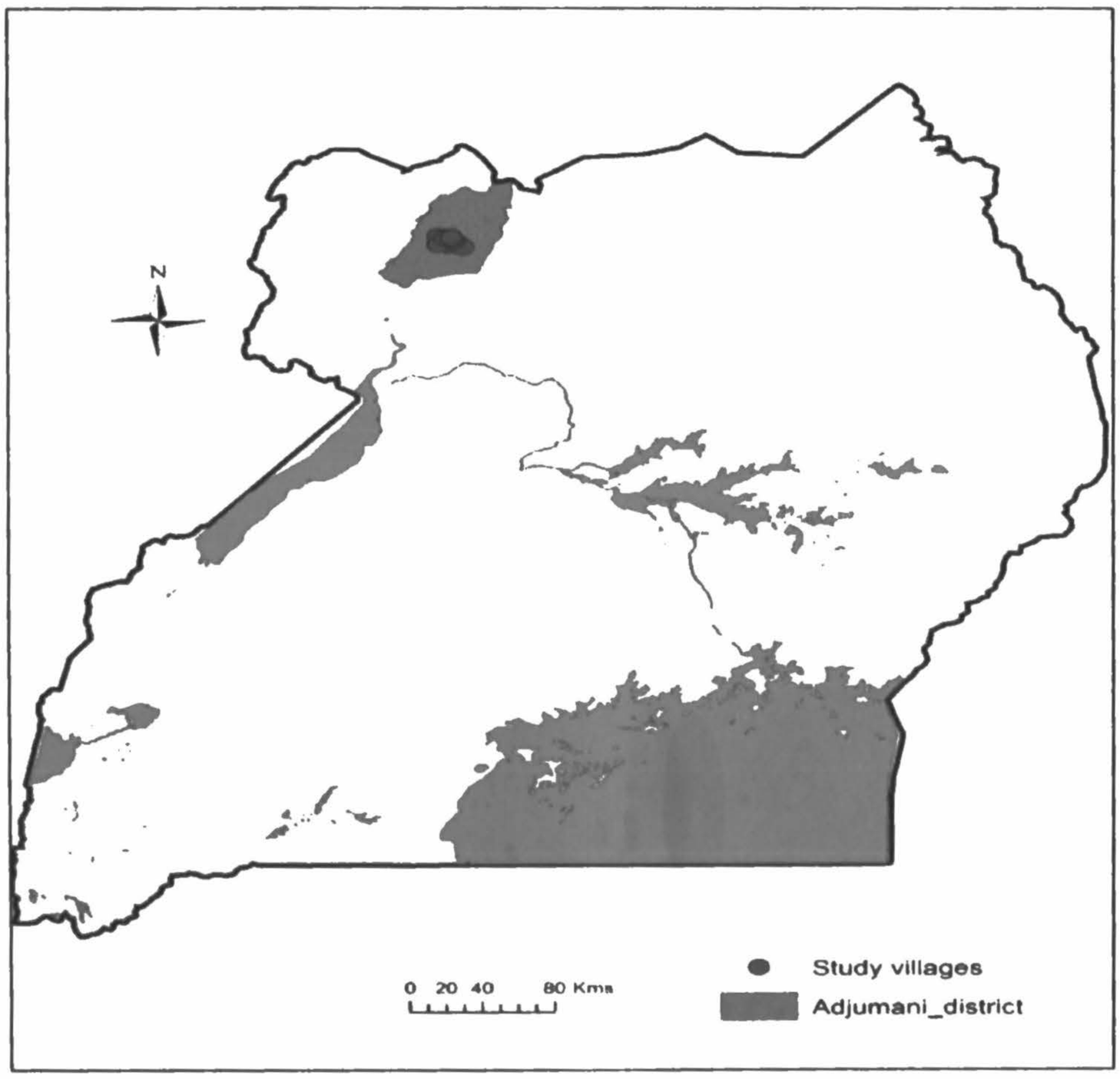


Figure 3.3: Map of Uganda showing the location of Adjumani district

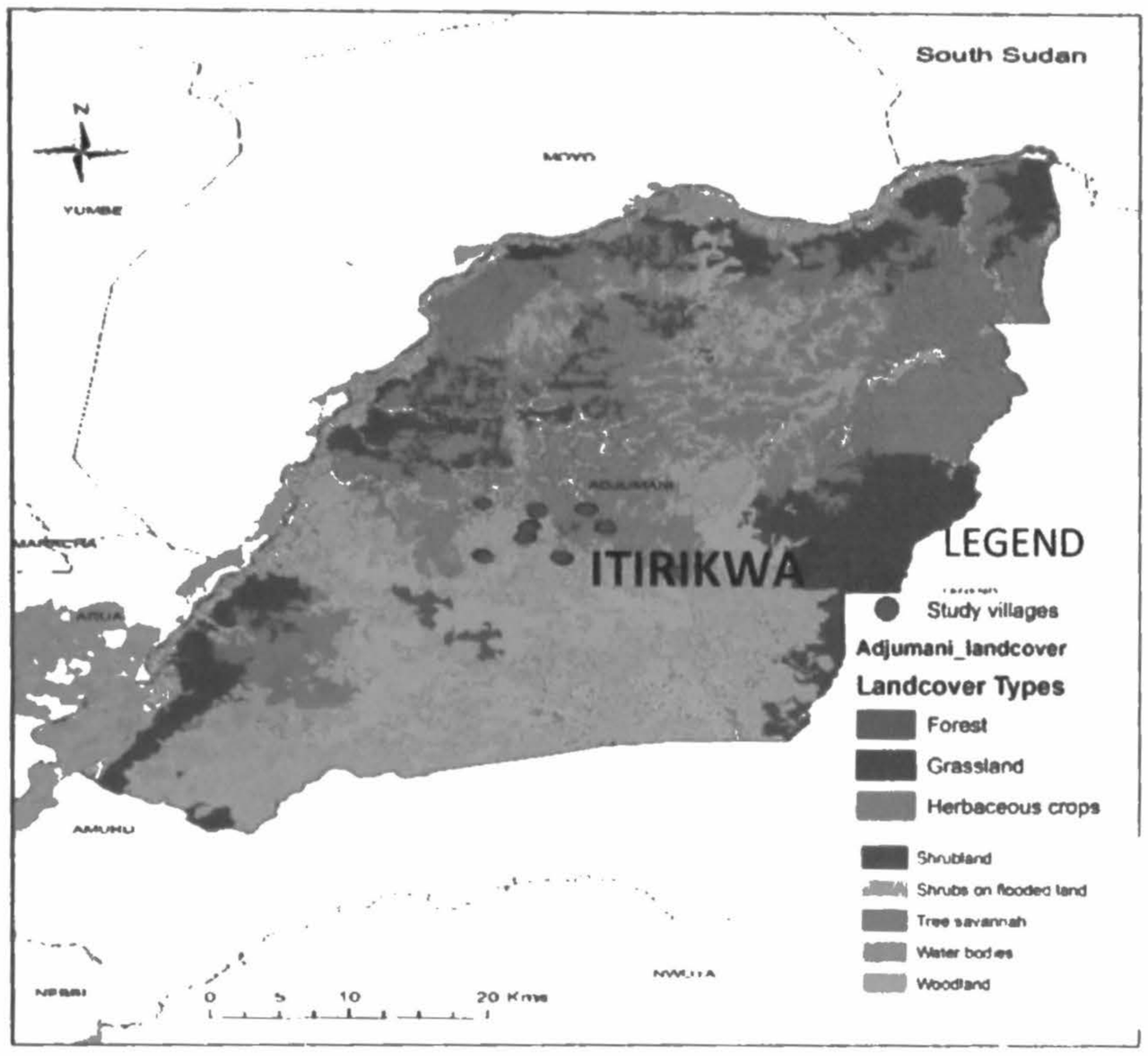


Figure 3.4: showing Map of Adjumani and Itirikwa subcounty



Figure 3.5: Figure 3.3 showing monitoring Trap and Esia river in kolididi village Itirikwa sub count.

3.2 Research Design

This research employed a longitudinal study design with the primary objective of establishing tsetse fly species composition, age structure and seasonal abundance along Esia river in Itirikwa subcounty Adjumani district. The study involved 10 villages in close proximity to Esia within the subcounty. Selection of the villages was based on information provided by the district veterinary department and District entomology office, as well as anecdotal reports of tsetse fly bites and associated trypanosomiasis case in the subcounty. Additionally, the choice of the Subcounty was based on the proximity to the zoka wild reserve which is migratory root

for the wild life especially the elephants moving from Nimule national Park to Murchison National Park and refugee settlement in the subcounty.

3.3 Sample size

Sample area comprised of the 10 villages in the 3 parishes of Itirikwa subcounty, the sample size consisted of 0.5km distance along the river for each village in the sample area. The vegetation cover in the villages was grouped into forest, Savana grassland and woodland. The vegetation cover was sampled to establish tsetse abundance in the different vegetation types along Esia River in Itirikwa subcounty

3.3.1 Selection of the trapping sites

Trapping sites in each village was chosen along the riverbank and each trap site is 100m from the next trap, vegetation around the trap site is cleared (2-3m) to make the traps more visible to the flies. Sites that are accessible where chosen while those with heavy vegetation and high risk of reptiles were not sampled.

3.3.2. Sampling tool

Sampling tool involved the use of 50 biconical Traps ((baited with acetone and phenol sachets) 5 traps deployed along the river in each village. Global position system (GPS) was used to georeferenced the location of the traps to be used in map generation using Global information system.Cool box with ice packs for transporting the tsetse to the field lab for identification and aging. Data survey data sheep for capturing the required information.

3.3.3 Sampling Procedure

10 villages along Esia river were randomly selected for tsetse sampling. Villages through which the river traverse were considered for sampling while those that the river does not pass through were left out.In each village 5 biconical traps were deployed 100 meters apart and each trap was georeferenced using Garmin etrex X20

3.3.4 Trap deployment

A total of 50 biconical traps were deployed in the 10 villages in two seasons. In each village, five traps were deployed along the riverbanks at a distance of at least 100metres. Each trap was georeferenced using Global positioning system (Garmin-etrex 20x) machine to incorporate in Geographical information system for Map development.

3.3.5 Sampling Frequency

Tsetse flies were harvested daily for 3 days (72 hours), for each season (Dry and Wet season) separately. Sorted by fly species, sex, and maturity stage (Teneral and non-teneral). The species of the tsetse fly was identified based on the habitat and their morphological features (FAO, 1992).

3.3.6 Collection of the tsetse flies

In each season the tsetse flies trapped are collected using the cones with label for the trap site number and village and vegetation type. At the field station flies sexed by observing the posterior end of the ventral aspect of the abdomen. The male flies were identified by their enlarged hypopygium in the posterior end of the abdomen. The blood meal level was used to group them as teneral for those that have not had their first blood meal and non-teneral for those that had their first blood meal. Age of the fly was estimated using the wing-fray technique (Leak et al., 2008).

3.3.7 Identification of the tsetse fly species

The collected flies were identified by sorting them into species, sex and age (nonteneral and teneral) categories (FAO training manual for tsetse control personnel 2000 Volume 4). The flies densities were counted and computed as the number of flies per trapping day (FTD) and also the number per species per trapping site. After sorting, the live flies were preserved for laboratory confirmation of the trypanosomes by dissection. Age estimation for both sexes of dissected nonteneral tsetse flies was also conducted concurrently using the wing fraying technique. These *Glossina* species were disiginated to one of the six age classes, in accordance to the observation of the fraying on the hind wing margin as described by Jackson (1946). Thereafter, wing fraying, the flie's ages were approximated using directions for estimating the average age of a sample of *Glossina* flies as described in the FAO training manual for tsetse control personnel volume four (FAO, 2000). The ages of tsetse flies determined by wing fraying were later grouped as "young tsetse" (WF1–2), "old tsetse" (WF 3–4) and "very old tsetse" (WF 5–6) for analysis. The nuisance biting flies count was determined concurrently using identification key in FAO Training Manual Vol. 1(1982a).

3.4 Apparent density determination for villages, Seasons and vegetation type

The apparent density, expressed as the number of flies per trap per day (FTD), was calculated by dividing the total number of tsetse flies captured ($\sum F$) by the product of the number of

functional traps used to catch the flies (T) and the number of days for which the traps were operational (D).

$$FTD = \frac{\sum F}{T} X D$$

FTD= Flies per day per trap

$\sum F$= summation of flies caught by the functional traps

T= Number of functional Traps

Using the above formulae apparent density for each village, season and vegetation type were calculated

3.5 Age determination of the tsetse flies

To estimate the age of the flies, the Mean wing fray value (MWFV) was calculated using the formulae, the flies were grouped under 1 to 6 wing fray categories depending on the level of fraying of their wing margins. Age for each season being determined using the formulae below.

$$MWFV = \frac{\sum(n * \alpha)}{N}$$

Where, n = number of flies in each category

α = coefficient attributed

N = number of flies in the samples.

3.6 Data Analysis

After the data has been sorted according to Species, Sex Teneral and Non teneral and wing fray category 1 to 6. Its entered into excel data sheet and transferred to Statistical Package for social science for analysis. Using statistical package for social science (SPSS) software Kruskal-Walli's test was used to compare the relative abundance (FTD) of tsetse flies among villages and among vegetation types in the study area. Student's t-test was used to compare the relative abundance between the wet and dry seasons, and between males and female fly abundance between dry and wet seasons. Level of significance was set at $p<0.05$. Kruskal-Wallis test is non-parametric test for testing whether samples originate from same distribution while Student-T test was used because population standard deviation for the tsetse flies is infinite and the distribution of the tsetse flies is unimodal and skewed.

3.6 Ethical Consideration

Ethical clearance to conduct the study was sought from Gulu University Research Ethics Committee. Permission for data collection was obtained from the district and the sub county authorities where the research was conducted.

CHAPTER FOUR

PRESENTATION, ANALYSES, AND INTERPRETATION OF DATA

4.1 Fly abundance along Esia River

A total of 919 tsetse flies were captured in both seasons from 50 traps deployed in 10 villages in Itirikwa sub-county, Ajumani district. Generally the number of female flies nearly doubled the number of male flies in both seasons (572 vs 347) (Table 4.3). Overall Zoka (5.04 FTD) had the highest fly abundance while the least was Lukwara (1.34 FTD) (Table 4.1). Average FTDs for all sites combined in both seasons was 3.205 ± 0.521 (Mean ± SE) with significant variations between villages (p = 0.001; Table 4.2).

Table 4.2: Entomological data on tsetse flies along Esia River (n=919)

Village			Wet Season					Dry season				
	X	Y	Traps	Male	Fem.	Tot.	FTD	Male	Fem.	Tot.	FTD	Overall FTD
Aliwara	3.9207	31.486	5	32	50	82	5.4 ± 0.61	13	24	37	4.45 ± 0.51	4.93[a]
Ejunya	3.1153	31.464	5	20	38	58	3.87 ± 0.86	4	11	15	0.75 ± 0.11	2.32[b]
Gbayi	3.1061	31.443	5	29	39	68	4.53 ± 0.31	11	17	28	2.1 ± 0.32	3.32[c]
Kolididi	3.1254	31.434	5	23	31	54	3.6 ± 0.46	8	16	24	2.4 ± 0.44	3[c]
Lukwara	3.1212	31.444	5	12	17	29	1.93 ± 0.21	4	11	15	0.75 ±0.15	1.34[d]
Oninyarak	3.1131	31.423	5	11	8	19	1.26 ± 0.29	11	19	30	1.5 ± 0.21	1.72[d]
Oroguwa	3.1156	31.475	5	22	43	65	4.3 ± 0.51	18	29	47	2.35 ± 0.33	3.33[c]
Ozugo East	3.1055	31.436	5	22	34	56	3.73 ± 0.41	18	22	40	2.3 ± 0.36	3.02[c]
Ozugo west	3.1131	31.431	5	26	43	69	4.6 ± 0.51	13	18	31	3.45 ± 0.42	4.03[a]
Zoka	3.9481	31.443	5	38	80	118	7.87 ± 0.81	12	22	34	2.2 ± 0.26	5.04[a]
Tot/mean												

*Means with the same letter are not significantly different and vice versa are true.

Table 4.3: Summary of ANOVA table showing partitioning of variation

Source of variation	DF	Type III SS	Mean Square	F-value	Pr>F
Village	9	154.23	42.35	39.32	0.001
Season	1	1.82	1.81	1.56	0.031

Table 4.4: Showing Tsetse species captured along Esia river

			WET SEASON		DRY SEASON		
VILLAGES	**Traps**	**species**	**M**	**F**	**M**	**F**	**Total**
ALIWARA	5	G.f.f	32	50	13	24	119
EJUNYA	5	G.f.f	20	38	4	11	73
GBAYI	5	G.f.f	29	39	11	17	96
KOLIDIDI	5	G.f.f	23	31	8	16	78
LUKWARA	5	G.f.f	12	17	4	11	44
ONINYARAKU	5	G.f.f	11	8	11	19	49
OROGUWA	5	G.f.f	22	43	18	29	112
OZUGO EAST	5	G.f.f	22	34	18	22	96
OZUGO WEST	5	G.f.f	26	43	13	18	100
ZOKA	5	G.f.f	38	80	12	22	152
TOTAL			**235**	**383**	**112**	**189**	**919**

4.2 Seasonal variation in fly abundance

Generally more flies were caught during the wet season (n= 618; mean FTD 4.11) than dry season (n=301; mean FTD = 2.225) flies (figure 4.1; Table 4.1). The difference in flies caught during the wet and dry season was significant (p=0.031; figure 4.1). Higher number of tsetse flies were trapped during the wet than dry season (t = 3.842, p = 0.01); similarly, significantly more females (383, p = 0.001) and males (n=235, p = 0.01) were trapped during the wet season compared to dry season).

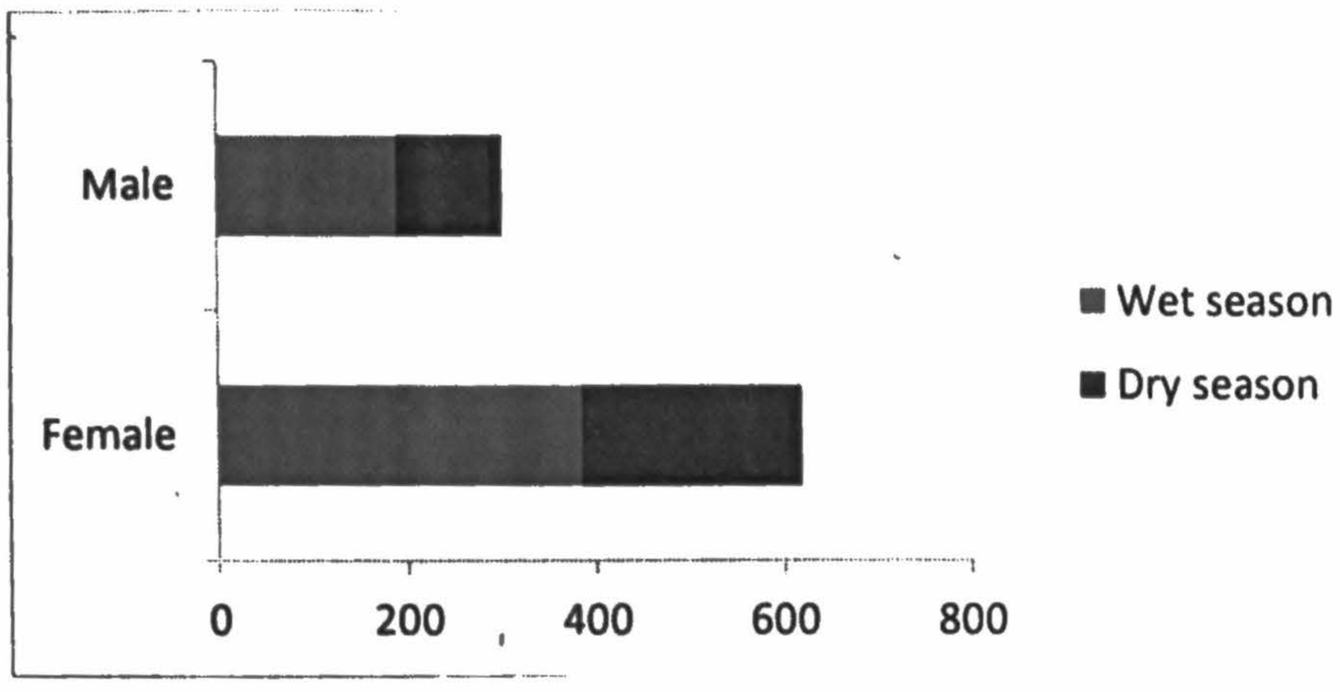


Figure 4.6: Variation in tsetse FTD with season along Esia River

4.3 Fly abundance and vegetation cover

Three different types of vegetation were observed across the villages along the river. These were forests, grasslands, and woodlands. Woodland was composed mainly of the full range of plant types including trees shrubs, herbs, grasses and some climbers. Grasslands, on the other hand, were dominated by cover grasses but lacked native plants, particularly trees and shrubs. Meanwhile forests consisted mainly wooded plants/trees growing together in disturbed or undisturbed conditions; This mainly occurred in Zoka Village. The average FTDs of tsetes flies from the different vegetation types are depicted in Table 4.4.

Table 4.5: Presentation of average FTDs by vegetation along Esia River

Vegetation type	No of traps	No of flies caught		FTD
		Male	Female	
Forests	12	82	125	4.93
Grassland	16	87	201	5.33
Woodland	22	178	246	5.62
Total	50	347	572	

*Data combines both dry and wet season catches

Overall, the difference in FTDs between the different vegetation types was not significant ($p=0.542$). However, woodland appeared to have slightly higher number of catches than the other two vegetation types although this was not significant (Figure 4.2).

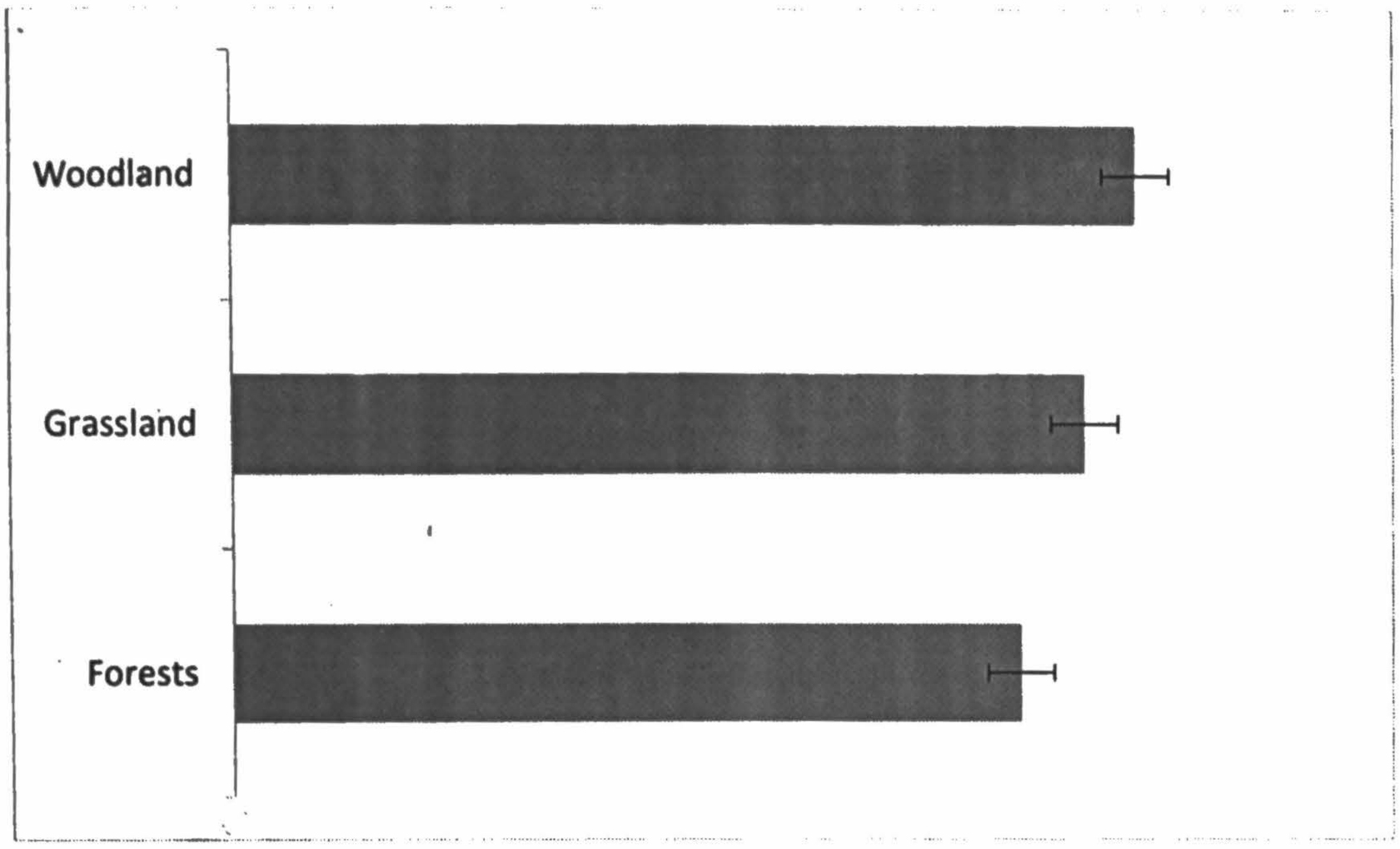


Figure 4.7: FTDs variation by vegetation type along Esia River

4.4 Age structure of the Male tsetse flies along Esia River

Analyses of the ages structure for the male tsetse flies data showed that most of the male flies captured fell in wing fray categories 2 and 3 in the dry season. This was followed by category 1, with yet fewer flies in categories 4, 5 and 6 (Table 4.4). The trend was similar for the wet season except that the wet season appeared to have more of the older Male flies (categories 4, 5 and 6) than the dry season (Figure 4.3). Estimated ages of the tsetse flies for the different villages ranged from 22-29 days for the wet season and from 18-25 days for the dry season, implying that there were more old flies during the wet than the dry season.

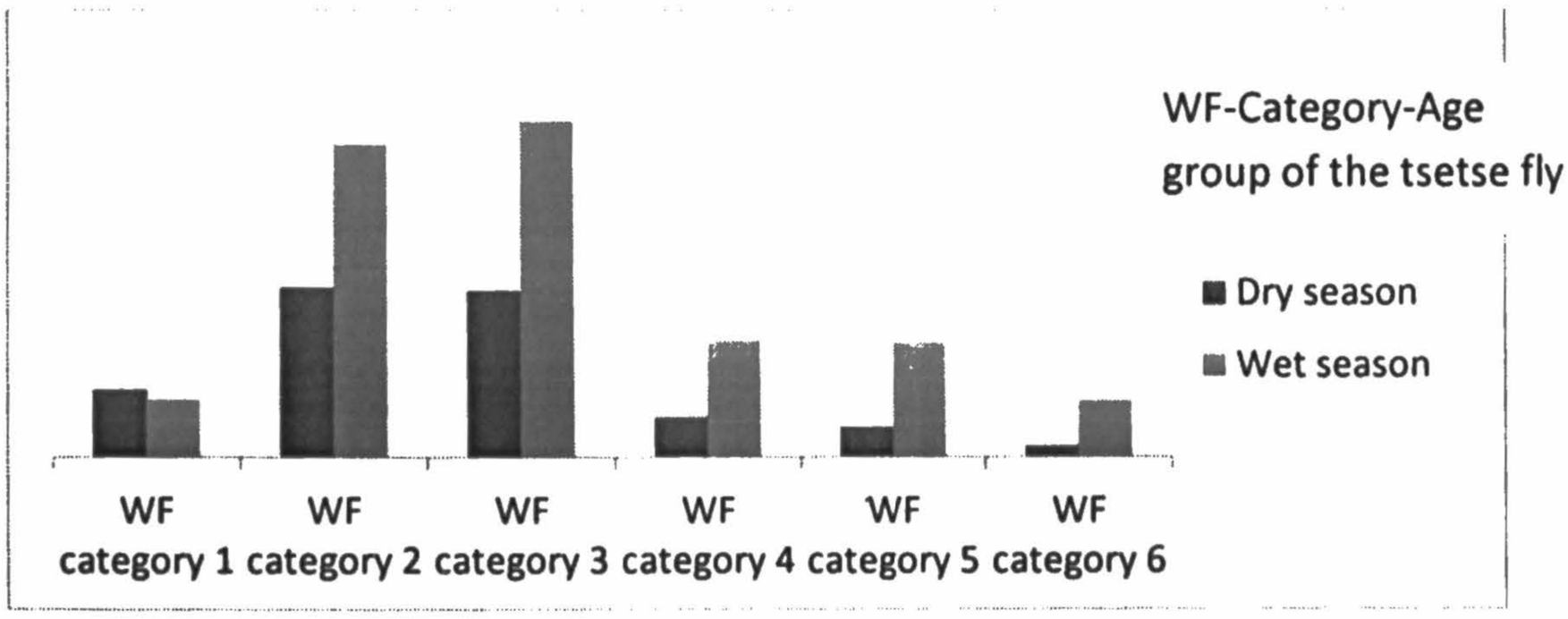


Figure 4.8: Wing fray Distribution of both female and male tsetse flies by Season.

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4.5 Age structure of the Male and Female tsetse flies along Esia River

The estimated age of both male and female flies during dry season ranged from 18days to 22days with tsetse flies found while in the wet season the estimate age of both female and male tsetse flies ranged from 22 to 29 days with Ozugo East and Ejunya having flies with 29 days of age while Aliwara village having the flies with age of 22 days. Hence there more older flies in the wet season than in the dry season as shown in the table 4.5 below.

Table 4.6: Age structure of flies along Esia River

Dry season								
Village	**Wing fray category**							
	1	**2**	**3**	**4**	**5**	**6**	**MWFV**	**Estimated age**
Aliwara	5	10	14	5	2	1	2.9	22 days
Ejunya	3	5	6	1	0	0	2.4	18 days
Gbayi	6	7	8	2	3	2	3.0	23 days
Kolidilidi	3	11	8	0	2	0	2.6	19 days
Lukwara	2	5	4	0	3	1	3.2	25 days
Oninyaraku	3	11	8	4	2	2	3.0	23 days
Oroguwa	4	22	15	3	3	0	2.6	19 days
Ozugo East	5	14	17	3	0	1	2.6	19 days
Ozugo West	5	8	12	4	2	0	2.8	21 days
Zoka	6	12	11	3	2	0	2.6	19 days
Wet season								
Village	**Wing fray category**							
	1	**2**	**3**	**4**	**5**	**6**	**MWFV**	**Estimated age**
Aliwara	3	27	31	10	7	4	2.9	22 days
Ejunya	4	18	16	8	10	2	3.6	29 days
Gbayi	8	22	17	10	8	3	3.5	27 days
Kolidilidi	1	20	18	4	7	4	3.4	26 days
Lukwara	2	8	14	1	2	2	3.3	27 days
Oninyaraku	1	6	7	2	2	1	3.4	26 days
Oroguwa	2	21	28	4	6	4	3.3	25 days
Ozugo East	6	14	18	6	8	4	3.7	29 days
Ozugo West	4	23	19	10	10	3	3.5	27 days
Zoka	5	35	41	18	11	8	3.5	27 days

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This study was conducted to assess the species composition, seasonal abundance and age structure of tsetse flies along Esia River in Itirikwa Subcounty in Adjumani district. The dynamics of tsetse population in the field is critical for determining which control strategy is appropriate for its control ((Berrang-Ford et al., 2006). Esia River is located in Itirikwa Sub-County, one of the 9 sub-counties of Adjumani District. The river plays an important role in the economic livelihoods of communities in the subcounty with livestock farming (goats and cattle rearing, piggery, and milk production) on the rise in Adjumani as a whole. However, secondary data and anecdotal reports highlight the presence of tsetse flies along the river, with apparently no control in the area compared to other sub counties like Ukusijoni, Ciforo and Dzaipi where tiny target deployment for tsetse control has been taking place(Charles Waiswa & Wangoola, 2019). Thus this study will help to provide data that can aid in the design of interventions.

The study revealed that *Glossina fuscipes fuscipes* is the only species of tsetse flies in the study area which agrees with a recent study conducted in West Nile (Opiro et al., 2021). The finding also corroborates the results of the habitat suitability prediction model of tsetse species in Uganda that depicts this area as being suitable for *G. f. fuscipes* (Lord et al., 2018). Besides, the sampling scheme where traps were placed along the Esia River meant that the expected species were going to be riverine, and *G. f. fuscipes* is currently the main riverine tsetse species in Uganda (Echodu et al., 2013). The subspecies is known to have poor waterproofing abilities and low water reserves, and as such are normally confined to hydrophytic habitats, such as forested patches along rivers and lacustrine environments (Enaro and Uro, 2020) an environment typical of where this study was conducted.

Data on tsetse abundance indicated that FTD for the different villages ranged from 1.34 to 5.04. This is comparable to studies conducted previously in this region,(Opiro et al., 2017), Angwech et al. (2015), in the nearby Nwoya District, and elsewhere in northern Uganda (Azabo et al., 2016). The implication is that tsetse density has not significantly fluctuated in the region despite some interventions such as the tiny target deployment (Bessell et al., 2021). These interventions probably worked but the true impact is hampered by

sustainability. Tsetse fly populations are known to always rebound whenever interventions are stopped before total eradication is achieved. Moreover, in the present work, it was found that tsetse densities varied across the different villages, in contrast to what was found previously in the region (Opiro et al., 2021). This is attributable probably to microclimatic variations and anthropogenic activities in the villages. As the trapping was being done, it was evident that changes in landscape and human activities had taken place within the different sites, affecting tsetse abundance to different extents in the different villages. Tsetse populations are especially known to decline or disappear as human populations grow and farmers clear fly habitat for cultivation (Reid et al., 2000). Humans therefore degrade terrestrial ecosystems, with such degradation taking the form of reduction in vegetation cover, particularly as a consequence of agriculture and other activities associated with human livelihoods such as Charcoal burning and Lumbering (Mwanakasale and Songolo, 2011). Moreover, 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). For instance, the low apparent density in the villages of Oninyaraku, Lukwara, Ejunya and Ozugo East is attributed to the human activity in these villages such as charcoal burning and lumbering in Oninyaraku village, and agronomic practices in Lukwara, Ejunya,Ozugo East and West. These human activities tend to disrupt the ecology of the flies thus contributing to their low apparent density.

Results of this study showed that season had a significant effect on tsetse abundance. The overall apparent density of flies during the wet season doubled that of the dry season, which corresponds with the general global trend of tsetse population dynamics (Mamoudou et al., 2008). During the dry season, most flies lack resting sites due to drying vegetation or bush burning and oviposition sites, which result into population decline. Moreover, higher temperatures decrease the survival of the flies, especially for *G. f. fuscipes* (Elama Ameh, 2011). This contrasts with conditions during the wet season where there is abundant habitat provided by the dense forest, bushy lands and grasslands which create favorable environment and protection for the flies, ensuring their optimum reproduction and survival rates (Rogers et all., 1994). The observed variation in seasonal abundance in this study is therefore probably due to the effect of optimum temperature on the normal physiological activity of tsetse flies during seasons in the different villages along the river belt of Esia River (Mamoudou et al., 2008).

Although there were fluctuations in tsetse density with season, one thing that remained rather consistent was the sex ratio. In all seasons and sites, female flies were always consistently higher than males in most traps along the river. This is similar to results from other studies in Uganda, for example, a survey in Mukono district, southeastern Uganda reported more females than males in all study months (C Waiswa et al., 2006). Opiro et al., (2021) study in West Nile also reported similar findings. Leak (1999) showed that in an unbiased sample, female would comprise between 70-80% of the mean population. For the case of this study, the probable explanation could be the longevity where by the female flies tend to live longer than the males, which makes them contribute to a higher proportion in sample than the males (Leak et al., 2008). Females are also known to be very prolific feeders compared to males; male flies feed every 3–4 days (Davies, 1977) whereas females take blood meals regularly due to their role in reproduction (Moloo, 1976), which increases their chances of getting trapped as they seek for blood meals.

Age structure analyses indicated that majority of the flies caught belonged to wing fray categories 2 and 3, which can essentially still be described as relatively young flies in comparison to those in the higher wing fray categories. Age structure determination is important in tsetse surveys because the longevity of the vector may be a limiting factor for the development of some trypanosomes species that require longer maturation times, such as is observed in *T. congolense* (Hargrove, 2020).

On the other hand, fly species differ in their infection rates and age at which tsetse become infective (Leak, 1999). The result of the wing fray analyses found in the present study is therefore instructive for control planning. For Itirikwa subcounty, the control technology should be those that are effective with young generation of flies which are mainly teneral and looking for mating and blood meals and can easily succumb to baits if effectively executed. Traps and tiny targets would be the best approach since young and hungry flies fly around looking for sources of food and mates, and hence higher chances of ending up in traps.

The study found that along Esia River, vegetation distribution does not have a substantial impact on abundance of tsetse, although woodland appeared to have slightly higher abundance. This is contrary to the general knowledge that vegetation plays a significant role in shaping tsetse habitat suitability and distribution patterns. However, tsetse flies prefer habitats with a heterogeneous vegetation structure, characterized by a mixture of open grasslands, woodland patches, and riparian vegetation(Garo & Aylate, 2023), hence this could be the reason why no particular vegetation in this study exhibited significantly higher

FTD than the other. Such environments offer diverse microclimates and suitable breeding grounds, contributing to increased tsetse populations. Moreover, seasonal fluctuations in vegetation also impact tsetse abundance. During the wet season, when vegetation is lush and abundant, tsetse populations tend to increase due to improved habitat conditions and higher availability of hosts (Vale, 1993). Conversely, during the dry season, vegetation becomes sparse, leading to a decline in tsetse populations as suitable habitats and host availability diminish.

5.2 Conclusions

The result of the study showed that tsetse species along Esia River Itirikwa subcounty is *Glossina fuscipes fuscipes* with comparatively high density. The density of this tsetse species varies with season, being higher in the wet than dry season but with a more ratio of females to males. This is because naturally female have long longevity than male tsetse flies (Clutton-Brock & Langley, 1997). The age structure analyses shows that the wet season had more male tsetse flies that are older compared to the dry season, perhaps an indication that Male *G. fuscipes fuscipes* experience better conditions for survival in the wet than in the dry season.

5.3 Recommendations

The tsetse density as reflected by FTDs in the studied area show that it is moderately high and comparable with other areas in Uganda. This implies that control should be instituted in the studied area, probably encompassing intensive deployment of traps or tiny targets along the river since the main species found in the area are riverine. This deployment can be done in both seasons; during the wet season, it will serve to suppress the tsetse population so that it reduces the disease burden in the area. However, intense deployment during the dry season could actually serve to considerably reduce and probably eradicate tsetse population in the area altogether because of the comparatively lower number during the dry season. For the case of Esia River, the absence of significant differences in *Glossina fuscipes fuscipes* abundance with vegetation could be attributed to the species' ecological adaptations, the predominant influence of host availability, and the interplay of various environmental factors.

5.4 Study limitations

The findings of this study, although providing valuable data, should be interpreted with caution. Firstly, the seasonal abundance data only considered just one of each of the dry and wet seasons. Because of the common climate variability these days, seasonal data should have been collected for more than one season each to give a better view of the actual

variation in abundance, an important parameter since seasons influence the climatic drivers that create fluctuations in tsetse fly populations.

This study also did not assess the trypanosome infection rates of the tsetse flies captured as well as animals, which could have given a complete picture of the disease burden in the study area. Future studies should therefore explore the trypanosome infection rates in tsetse flies and animals to indicate whether there's ongoing transmission of African trypanosomiasis and the relative risks it poses to both animals and humans in the study area.

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APPENDICES

Appendix 1: Tsetse Survey Data Sheet

Project name:
.....
.....

District: ....., County: .....Sub county: .....
Parish: .....

Season: ..... Month: .....Trap type:
.....

Village	Latitude	Longitude	Elev.	Human Activity	Veg type	Deploy date	Start time	Harvest date	End time	Species				Total	Rainfall	Others
										Gff		Others				
										M	F	M	F			

Key

Vegetation type (F = forested, W=wooded savanna, R=Riverine, G=grassland)
G.p = Glossina pallidipes
Gff = Glossina fuscipes fuscipes
FTD = Number of flies per trap per day
Other flies = Presence of other biting flies (Yes OR No)
Season = wet OR dry
M = Male, F= female
Elev. = elevation

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