SPECIES COMPOSITION AND DISTRIBUTION OF TSETSE FLIES (*GLOSSINA* SPECIES) IN KAMUKU NATIONAL PARK, BIRNIN GWARI, KADUNA STATE, NIGERIA

BY

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DECLARATION

I hereby declare that the work in the thesis entitled 'The Species composition and distribution of tsetse flies (*Glossina* species) in Kamuku National Park, Birnin Gwari, Kaduna State, Nigeria' has been performed by me in the Department of Biological Science under the supervision of Prof. C.G. Vajime, Dr. I.S Ndams and Dr. E. Kogi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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DATE

CERTIFICATION

This thesis entitled SPECIES COMPOSITION AND DISTRIBUTION OF TSETSE FLIES (*GLOSSINA* SPECIES) IN KAMUKU NATIONAL PARK, BIRNIN GWARI, KADUNA STATE, NIGERIA by OKOH, Kehinde Evelyn meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation

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DEDICATION

This Thesis is dedicated to my loving husband, Pastor Sunday Okoh for his understanding, encouragement and support throughout the period of the study.

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ABSTRACT

A study of the species composition and distribution of tsetse flies was conducted between January to December, 2007 at Kamuku National Park, Birnin Gwari Local Government Area, Kaduna State, using Biconical (Charlier and Laviessiere, 1973) and Nitse traps (Omoogun, 1994). Four traps each were placed for two days along five streams (i.e.Dagara, Kabungu Bungu, Kango Kabungu, Kuzomani and Kurishi) and the trap catches were harvested every day. Five hundred and two tsetse flies caught during the study period, differ significantly between streams. Dagara, Kabungu Bungu, Kango Kabungu, Kuzomani and Kurishi streams had 166 (33.1%), 33 (6.6%), 45 (9%), 41 (8.2%) and 217 (43.2%) flies respectively. *Glossina tachinoides* and *Glossina palpalis* were trapped in the area with one species *Glossina morsitans submorsitans* encountered during the preliminary studies only. Overall, *Glossina tachinoides* 309 (61.6%) dominated over *Glossina palpalis* 193 (38.4%), and in Kurishi, (98.2%), Kango Kabungu (97.8%) and Kabungu Bungu (93.9%) streams, while *Glossina palpalis* catches were more in Dagara (97%) and Kuzomani (61%) streams. Male tsetse flies were significantly higher than females (ratio 2:1), more teneral flies were caught than non-teneral. Tsetse catches were not significantly higher in the dry season than wet season and correlated positively with temperature and negatively with relative humidity. Overall apparent density of 0.1 fly per trap per day obtained in the study and for each species suggest a low density area; 0.2 fly per trap per day were obtained for both season. February had the highest fly density 3.0 while July had the least 0.2. The estimated age of male population was 11 days while females under ovarian category O with an approximate age of 0-10 days dominated. The Mean Hunger Stage (MHS) of

3.6 and 3.5 for *Glossina palpalis* and *Glossina tachinoides,* respectively, indicated hungry populations. Insemination rate (93.8%) was high whereas parity rate (25.8%) was low. Overall infection rate of 6.6% was high and infection due to *T. vivax* (5.2%) dominated followed by *T. congolense* (0.9%) while *T. brucei* (0.5%) was lowest. Infection rates were higher in *Glossina tachinoides* (9.4%) than *Glossina palpalis* (3.1%). The study has shown that *Glossina morsitans submorsitans* probably declined as a result of seasonal, vegetation and food factors; also that the high fly density observed in February is a significant month to carry out control. The presence of trypanosome infection in the park may constitute a public health risk to nomadic cattle and ecotourism in the park.

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CHAPTER ONE

1.0 INTRODUCTION

Tsetse flies are large biting flies from Africa which live by feeding on the blood of vertebrate animals. They are similar to other large flies such as housefly *Musca domestica*, but can be distinguished by two visually distinctive characters such as the forward projecting proboscis and the discal cell on the wing, shaped like a cleaver and referred to as the 'hatchet cell' which lies between the fourth and fifth wing veination (Buxton, 1955).

Tsetse flies belong to the genus *Glossina*, a name described by Wiedemann (1830) and monotypic family *Glossinidae*. They have existed in the modern morphological form for at least 34 million years since Fossil tsetse have been recovered from the Florissant Fossil Beds in Colorado (Cockerrel, 1917).

1.1 Tsetse Flies as Vectors of Trypanosomiasis

Tsetse flies are biological vectors of African Trypanosomiasis and are responsible for the transmission of Trypanosomes, the pathogenic agents of Human African Trypanosomiasis or sleeping sickness and Animal African Trypanosomiasis or Nagana in cattle (Boulanger *et al.,* 2002). They are obligatory blood feeders which transmit Trypanosomes either mechanically or cyclically during the processes of feeding. Tsetse- transmitted trypanosomiasis affect various vertebrate species including human, antelopes, horses, sheep, goats, pigs and cattle. The trypanosomes transmitted may also survive in wild animals such as crocodile and monitor lizards. A number of wild animals serve

as reservoir host of the parasite. The diseases have different distributions across the African continent and are therefore transmitted by different species of tsetse.

The Human African Trypanosomiasis also called sleeping sickness is caused by the trypanosomes of the *Trypanosoma brucei species* and is transmitted by the palpalis group species. The strain which infect humans are divided into two subspecies; the *Trypanosoma brucei rhodesiense* which causes the Rhodesian form of sleeping sickness in East Africa, and the *Trypanosoma brucei gambiense* which causes the Gambian form of sleeping sickness in West Africa. These two subspecies although morphologically undistinguishable, differ on the basis of their virulence and can be fatal if left untreated (Hoare, 1970; Kennedy, 2006).

African animal trypanosomiasis, nagana, is a disease complex caused by the tsetse fly-transmitted *Trypanosoma congolense, T. vivax,* or *T. brucei brucei* or simultaneous infection with one or more of these trypanosomes. They constitute a major constraint limiting the optimal utilization of land for agricultural (crop and livestock) production (Mahama, 2003).

1.2 Economic importance of Tsetse Flies

Tsetse fly and the diseases it transmits are one of the most severe medical and veterinary problems in Africa, infecting more than 500,000 people, killing 50,000 people and three million livestock annually (FAO, 2002; Okhoya, 2003); therefore, preventing the development of sustainable and productive agricultural systems (Vreysen, 2001). Tsetse fly and trypanosomiasis is widely

recognized as one of Africa's greatest constraints to socio-economic development, causing death, debility, diminished productivity in man and domestic animals, massive economic loss and reduction of revenue from tourism (Anon., 2004). The burden imposed by tsetse and trypanosomiasis problem continues to frustrate efforts and hamper progress in development activities, and remain one of the greatest causes of hunger, poverty and immense suffering to communities in Africa. Trypanosomiasis is the greatest cause of mortality, morbidity and low productivity in domestic animals, and severely diminished agricultural productivity (Anon., 2002).

The occurrence of tsetse flies in national parks and game reserves where natural fauna and flora are protected provide ideal habitat for the flies and the pathogenic trypanosomes, and as such, domestic livestock grazing around the periphery of parks are permanently exposed to the risk of trypanosomiasis and, if trypanosomes pathogenic to man occur in the local tsetse, staff and tourist frequenting the parks stand the risk of contracting sleeping sickness (Jordan, 1986).

In view of the problem stated above, a new initiative known as the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) was developed and a decision was passed by the African Heads of State and Government, urging member states (since tsetse-transmitted disease is trans-boundary) to collectively embark on the campaign in order to rid Africa of the threat and burden of tsetse-transmitted disease. The PATTEC initiative seeks to employ an area-wide approach and appropriate fly suppression methods, to

eradicate tsetse from areas of tsetse infestation, progressively and to ultimately create tsetse-free zones (Kabayo, 2002). This initiative was borne out of the successful elimination of tsetse from the Island of Zanzibar through the sterile insect technology (SIT) approach.

1.3 Tsetse Ecology

Tsetse flies are confined only to the subtropical and tropical regions of Africa from latitude 15^{o}N to 28^{o}S (Davies, 1977). The northern limit extends across the continent from Senegal in the West to Southern Somalia in the East. The southern limits are less well defined, but correspond closely to the northern edges of the Kalahari and Namibian deserts extending to South Africa (Jordan, 1986). The genus *Glossina* stretches across 10 million square kilometer, covering 37 countries of Africa, thus corresponding approximately to one-third of Africa total land area (Anon., 2002). They occupy a wide variety of vegetation types ranging from the semi-arid margins of the Sahel, through tropical rainforests, to the sub-tropical savannas (Ford and Katondo, 1977) depending on the climatic conditions of temperature, rainfall and vegetation type. In Nigeria, they cover all the five agro-ecological zones from latitude 4^{o} – 13^{o}N to longitude 2^{o} – 15^{o}E (Onyiah, 1997).

In the genus *Glossina* are about thirty four species and subspecies which have been divided into three distinct species group based on a combination of distributional, behavioral, molecular and morphological characteristics. These are the Morsitans group (subgenus: *Glossina* sensu stricto) which are mostly the

savanna flies and often referred to as the game flies. They are of significant economic importance because, they transmit animal trypanosomiasis; the Palpalis group (subgenus: *Nemorhina*) are mostly riverine flies, some are known to inhabit forest regions, they are also important transmitters of human trypanosomiasis; and the Fusca group (subgenus: *Austennia*) are mostly the forest flies which are of little economic importance (Davies, 1977).

Significantly, eleven out of the twenty two known species of *Glossina* endemic to Africa occur in Nigeria and have been reported to transmit the disease to susceptible host (Jordan, 1961). However, four of these species are major transmitters of the disease in Nigeria namely: *Glossina palpalis* and *Glossina tachinoides* (vectors of human trypanosomiasis). *Glossina morsitans submorsitans* and *Glossina longipalpis* (vectors of animal trypanosomiasis) (F.M.A, 1981; Onyiah, 1995).

1.4 JUSTIFICATION

Tsetse (*Glossina* spp*)* are the primary vector of animal and human trypanosomiasis in tropical Africa; and are a continuing threat to public health, livestock production, agricultural development and is also a major source of economic loss.

Tourism is arguably the world's largest and fastest growing industry, accounting for about five percent of the world's Gross National product and six percent of the employment (Glasson *et al*., 1995). Most governments encourage tourism for its ability to spread economic development and reduce inequalities

in income distribution by providing jobs (Pearce, 1988; Coccossis and Parpairis, 1995; Waheb and Pigram, 1997). Tourism is viewed by government as a catalyst for national and regional development bringing employment, exchange earnings, balance of payment advantages and important infrastructural developments benefiting local and visitors' alike (Glasson *et al*., 1995). Nigeria is also not left out in her quest to develop the Nation's tourism industry. In order to achieve this, National parks and their Governing Board established by Decree 46 of 1999 as amended in 2006 created seven National Parks. National park plays significant roles in science, research and educational development apart from acting as vehicles for the development of eco-tourism.

The threat to tsetse fly infestation exists in most national parks/wildlife park and is a major health risk to tourists coming to tropical Africa (Sabbah *et al*., 1997; Conway-Klaassen *et al*., 2002; Jelinek *et al*., 2002). Blumberg, (2005) reported two cases of East African trypanosomiasis acquired in Kasungu National Park in Malawi. Similar report was made by Moore *et al*., (2002) of an American tourist who after a trip to Africa was diagnosed as suffering from an infection of *Trypanosoma brucei rhodesiense*, an East African form of Trypanosomiasis. The continued and occasional importation of African trypanosomiasis to the United States by tourists and immigrants from high-risk areas was reported by Chretien *et al*. (2005). The work by Ndams, (1987) showed the occurrence of tsetse flies in Pandam Wildlife Park, in Plateau State, Nigeria.

The two types of African trypanosomiasis that exist namely Animal trypanosomiasis and Human trypanosomiasis are determined by the species of tsetse fly. The human trypanosomiasis poses a health hazard to human and will thus have adverse effect on tourism if not checked. This necessitates the study to ascertain the population structure of tsetse flies in Kamuku National Park The information that will be obtained from this study should serve as an input to the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) Programme.

1.4.1 Aim

To determine the species composition and the population structure of tsetse flies in Kamuku National Park.

1.4.2 Objectives

- To determine the tsetse fly species present,
- To investigate the seasonal density variations of tsetse fly in the park,
- To determine the sex, age structure, physiological and reproductive status and infection rates.

1.4.3 Hypotheses

- There is no difference in composition of tsetse species among the streams

- There is no difference in tsetse catches in relation to season
- There is no difference in the structure of the population in relation to sex, age, hunger stages, reproductive status and infection rates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Perspective

Tsetse flies and Trypanosomes were thought to probably have existed for 200 and 300 million years respectively (Pieter, 2002). The earliest recorded account of sleeping sickness came from the upper Niger during the 14th century when Ibn Khaldoun, an Egyptian historian wrote about the disease in his account of the history of North Africa. The next report came from Guinea in 1734 (Atkins, 1978). In West Africa, the disease that caused visible swollen lymph gland was known as winterbottom's sign after the description of Winterbottom in 1803. This sign which was readily recognized by slave traders who avoided trading and buying slaves with such signs. Forde and Dutton in 1902 found for the first time, a trypanosome infection in man and called it "trypanosome fever". In the same year, Castellani observed the presence of trypanosomes in cerebrospinal fluid taken from a sleeping sickness patient which he identified a year later as the cause of sleeping sickness (Pieter, 2002). The link between tsetse flies, the trypanosomes and the diseases was discovered in 1895 (Grev, 2002), by Bruce .D. who correctly recognized that trypanosomes were the causative agent of sleeping sickness transmitted to human by tsetse flies, and that "trypanosome fever" and "sleeping sickness" – both thought to be different diseases at the time – were in fact the same.

2.2 General Description of Glossina

Glossina species have been widely studied because of their economic importance as major transmitters of animal and human trypanosomiasis (Ndams, 1987).

Tsetse flies are robust insects measuring about 6-15mm in length. They are readily distinguished from other biting flies morphologically by the combinations of useful features such as widely separated compound eyes which are dark brown, and able to detect moving objects at 137meters (150yards) (Pollock, 1982), with forwardly projecting proboscis which sticks out horizontally from the front of the head (Jordan,1993). They differ from most biting flies and non cyclorrhaphous insects because when at rest, the wings are held over the other such that they overlap like the blade of a closed pair of scissors, thus revealing part of the abdomen (Davies, 1977). They also posses a characteristic wing veination where the discal medial cell of the wing is shaped like a butcher's cleaver (referred to as the 'Hatchet cell') and a distinctive row of branched hairs on the arista of the antenna (Service, 1980; Jordan, 1993).

Tsetse flies range in color from yellowish or grayish to dark or almost blackish-brown, sometimes there is a slight pink or sandy red tinge (Davies, 1977). The dorsal surface has a pattern of dark brown stripes and patches making the insect difficult to see when settled on tree bark, rock and soil. They have seven visible abdominal segments Pollock, 1982).

2.3 Habitat and Distribution of Glossina

The genus *Glossina* occurs over some 11 million km^2 of Africa. Its northern limit extends across the continent from Senegal in the west to southern Somalia in the east. This limit is at about 14^oN but in Somalia it is only about 4^oN. The northern limit corresponds closely to the southern edges of the Sahara and Somalia Deserts. The southern limit is less well defined. In the south west it ranges between $10^o$and 20^oS, corresponding closely to the northern edges of the Kalahari and Namibian Deserts, whereas in the south east, it is generally at about 20^oS but extends as far as 29^oS along the east African littoral (Jordan, 1986), with the mid pint of infestation at about 7^oS (Davies, 1977). In Nigeria, tsetse flies cover about 75% of the landmass from latitude 4^o to 13^oN to longitude 2^o to 15^oE, an area covering all the five agro-ecological zones of the country (Okam, 1988; Onyiah, 1997).

There are twenty two known species of Glossina which can be arranged into three distinct species groups based on habitat preference (Ford, 1970) and morphological differences in the construction of the male genitalia (Pollock,1982). These species groups are summarized below:

The Morsitans species group (Morsitans) these include seven species and subspecies namely:- *G. longipalpis; G. pallidipes; G. morsitans morsitans; G. morsitans submorsitans; G. morsitans centralis; G. swynnertoni; G. austeni.*

The Palpalis groups (Nemorhina) consists of a total of nine species and subspecies namely: - *G. palpalis palpalis; G. palpalis gambiensis; G. fuscipes*

fuscipes; G. fuscipes martini; G. fuscipes quanzensis; G. tachinoides; G. pallicera pallicera; G. pallicera newsteadi; G. calliginea.

The Fusca species group (Austenia) also consists of fourteen species and subspecies namely: - *G. Fusca Fusca; G. Fusca congolensis; G. nigrofusca nigrofusca; G. nigrofusca hopkinsi; G. fuscipleuris; G. haningtoni; G. schwetzi; G. tabaniformis; G. nashi; G. vanhoofi; G. medicorum; G. severini; G.brevipalpis; G. longipennis*.

The geographical distribution of tsetse flies is determined by temperature (with mean annual temperature of between 20^{o}C and 28^{o}C), relative humidity (ranging between 50% and 85%), rainfall; sunshine and vegetation cover (Glasgow, 1970). In higher altitudes of over 1800m (6000ft), tsetse flies are not found (Davies, 1977).

2.3.1 Habitat and Distribution of the Morsitans Group

The three *Glossina* subspecies of the morsitans group are exceptionally good vectors of trypanosomes; all the seven species are potential vectors of both human and animal trypanosomiasis. All species within this group inhabit the savanna woodlands that surround the two major blocks of lowland rainforests in Africa, and as such are referred to as 'game or savanna flies' (Davis, 1977). The distribution of tsetse flies in this group closely follows the distribution of wild animals and water sources. In wetter areas, the flies are observed to disperse more widely over the woodland, but in drier areas their movements are

restricted to the mesophytic vegetation of the watercourses, particularly during the severe dry season (Nash, 1937; Jordan, 1986).

In Eastern and Southern Africa where *Glossina morstans morsitans* is the primary vector of human and animal trypanosomiasis, the 'Miombo' woodland (Brachystegia – Jilbernardia) that extends from Mozambique to Tanzania, as well as the 'Mopane' woodlands (Colophospermum mopane) in Zambia and Zimbabwe are typical habitats. *Glossina morsitans centralis* dominate northwards from Botswana and Angola into southern Uganda. *Glossina morsitans submorsitans* have an east to west distribution from Ethiopia to Senegal in 'doka' woodland where the vegetation is dominated by *Isoberlinia doka* species, and can be sporadically found to occur in the southern Guinea savanna vegetation zone as well as the drier Sudan zone (Jordan, 1986). *Glossina swynnertoni* is restricted to a small area between Tanzania (Serengeti) and southern Kenya (Masaimara) where Acacia- commiphora vegetation can be found, with abundant wild life. *Glossina longipalpis* and *Glossina pallidipes* both have a much wilder range of possible habitats displaying versatility by existing in different vegetation types. *Glossina longipalpis* occurs in the narrow savanna belt just north of the rainforest in West Africa, from Guinea to Cameroon while *Glossina pallidipes* occurs in East Africa from Mozambique to Ethiopia over a relatively wide range of climatic and vegetation conditions. Finally, *Glossina austeni* occupies secondary shrub, thickets and islands of forests along the East African coast from Mozambique to Somalia. Its

distribution is discontinuous, rarely being found at altitudes over 200m or more than 250km inland from the coast (Jordan, 1986).

In Nigeria, the two morsitans groups species present are: - *Glossina morsitans submorsitans* found in the north where annual rainfall is as low as 635mm (25in) with a dry season of seven months and further south, an annual rainfall of 1400mm (55in) with a dry season of four months. *Glossina longipalpis* is found in the southern guinea and derived savanna zones in the west and a small localities in the east, with annual rainfall not less than 1150mm 45in) or more than 2300mm (90in) (Davies, 1977).

2.3.2 Habitat and Distribution of the Palpalis Group

Out of the nine species in the palpalis subgenera, only five *palpalis* and *fuscipes* subspecies are vectors of both human and animal trypanosomiasis. Although flies in this group are continuously found in the lowland rainforest, some are known to extend out of the savanna region particularly along rivers and streams. The habitat of the palpalis flies occurs mainly in the drainage systems leading to the Atlantic or the Mediterranean ocean, extending from the wet mangrove and rainforests along the coastal regions of West Africa to the drier savanna areas just north of the rainforests. The flies of the palpalis group are less tolerant to the wide range of climatic conditions of the savanna belt, and are therefore restricted to the ecoclimate of the water courses from where they derived their label as the 'riverine species'. Many of the palpalis species, such as the *Glossina palpalis palpalis* in Cote d'lvoire prefer peri-domestic

conditions and have been observed to maintain close association with villages (Baldry, 1980). Similarly, it is thought that the advancement of *Glossina tachinoides* in stern Cote d'lvoire and Togo have been attributed to intense agricultural development of the rapid human population growth around the plantation (Hendrick *et a*l., 1997).

In general, most of the palpalis group flies are less suited to desiccating conditions, and therefore survive in thick riverine forests with enough shelter from wind and heat. This is especially the case for the three fuscipes subspecies which are confined to hygrophytic habitats rarely far from open water lacustrine or riverine habitats. *Glossina tachinoides*, although typically a riverine species, were found in Northern Nigeria to extend into human-inhabited savanna woodlands during the wet season, displaying strong adaptation to peri-domestic habitats (Kuzoe, 1985; Ahmed, 2004).

2.3.3 Habitat and Distribution of the Fusca Group

With the exception of *Glossina brevipalpis* and *Glossina longipennis* all the tsetse flies in the Fusca group are found in West African forests. None of the species in the Fucsa group are vectors of human trypanosomiasis; however both *Glossina fusca* and *Glossina medicorum* are efficient vectors of trypanosomiasis to livestock (mainly *Trypanosoma vivax*) causing considerable economic burden.

Distribution of the Fusca group depends primarily on forest vegetation and climatic factors. With the exception of *Glossina longipennis*, most Fusca

group species inhabit moist, evergreen habitats either in riverine forests with savanna (such as *Glossina medicorum*) or in dense and wet rainforests (*Glossina tabaniformis* and *Glossina nigrofusca*). In stark contras to the rest, the *Glossina longipennis* species lives in one of the driest habitats inhabited by tsetse flies (Jordan, 1986). Due to its pupal adaptation to dry conditions, their primary habitats – consisting of dry deciduous acacia bush- are discontinuously spread throughout East Africa (Glasgow, 1963).

2.4 Reproduction of Glossina

Tsetse flies exhibit a form of reproduction known as adenotrophic viviparity (Hagan, 1951) because the egg and larva stages develop within the fly. The egg contains sufficient yolk for embryonic development and the larva in the uterus is nourished by special maternal organs. The consequences of viviparity are that only a small number of fully developed larvae can be produced, a free-larval stage is practically eliminated and both adults and immature stages are dependent upon the same source of food (blood) (Saunders, 1960).

2.4.1 Mating

Female tsetse flies mate within a day or so after emergence from their pupal case; mating which usually takes place near or on host animals (Pollock, 1982). Male and female generally meet when the female is about to take the first blood meal or is in the process of doing so (Jordan, 1986). The sexes remain in

copulo for an hour or two during which time a spermatophore is formed within the uterus of the female. Almost at the end of the period spent in copulo the male jerks vigorously and it is at this time that sperms are ejaculated into the spermatophore (Pollock, 1982). Within the next few hours, the sperms are slowly released from the spermatophore and move into the spermathecae, a paired golden-colored rigid structure connected by ducts to the anterior end of the uterus. Active and viable sperms can remain in the spermathecae throughout the life of the female fertilizing each egg as it is produced from the ovaries, thus allowing the female to breed throughout her life time (Davies, 1977). Female tsetse flies usually mat only once in their lives but some may mate more than once, males can mate several times. Older males are better able to mate successfully than very young ones (Pollock, 1982).

2.4.2 Egg Stage

The female tsetse fly has two ovaries, each of which has two ovarioles; eggs develop sequentially in the four ovarioles (Saunders, 1970) and are ovulated into the uterus at intervals of about 9 – 10 days, the first ovulation occurring when the fly is about 9 days old (Jordan, 1986). Each egg is fertilized immediately it enters the uterus by sperm from the spermathecae coming into contact with and penetrating the anterior part of the egg. The fertilized egg remains lying in the uterus for about four days, while development of the first instar larva takes place inside (Pollock, 1982). The age of wild-caught females can be determined through dissection technique involving ovaries examination

and counting the number of ovulations that have occurred (Saunders, 1962; Challier, 1965).

2.4.3 Larval Stages

The larva in *Glossina* passes through several stages or instars, as it grows. There are three larval instars in *Glossina*: the first, second and third instars. The larva has a mouth at the anterior end, and two posterior spiracles (Pollock, 1982). The intrauterine larva is supplied with nutrients in the form of 'milk' substance secreted from a modified accessory gland (Attardo *et al.*, 2006), grows rapidly and molts twice before larviposition

2.4.3.1 First Instar Larvae

The first instar larva develops within the egg and breaks out of the chorion using a sharp egg tooth. It grows to 1.8mm (*Glossina morsitans*) and last for one day (Pollock, 1982).

2.4.3.2 Second Instar Larvae

The second instar larva grows and develops rapidly lasting for two days and can reach the length of 4.5mm (*Glossina morsitans*). Each side of the posterior spiracles swells with small spines in between.

2.4.3.3 Third Instar Larvae

This also grows and develops rapidly. The third instar larva is white in color and has two conspicuous black respiratory lobes which are white at first, and later become black. The third instar lava last just over two days and grows to a length of 6.7mm (*Glossina morsitans*) (Pollock, 1982). The larva when fully developed is deposited (larviposited) on the ground at shady sites to prevent desiccation. The larva burrows itself rapidly in the soil to a depth of 1-5cm, depending on the species, the season and the soil type (Grev, 2002). Within an hour, the larva contracts to form a barrel-shaped puparium, darkens rapidly to black. After about four days, ecdysis occurs within the shell of the puparium and the true pupa is formed (Jordan, 1986).

2.4.4 The Pupa

The pupa is dark brown and rounded; at the posterior end are the polypneustic lobes, the shape of which helps to distinguish the tsetse pupa from the pupae of other flies. The pupa is slightly shorter than the larva. The pupal stage usually lasts about four to five weeks, depending on the temperature. Higher temperatures shorten the pupal period, lower temperatures lengthen the pupal period. Too high or too low temperatures cause death of the pupae (Pollock, 1982).

2.4.5 The Adult Fly

The adult fly emerges by expanding its ptilinum to burst open the end of the puparium, pushing itself out of the soil after which the ptilinum sink back into the head of the fly (Davies, 1977). At this stage, the body is very soft and the wings are small and crumpled (Pollock, 1982). The wing flatten out into their normal shape within a few minutes of birth by blood being forced into the veins, harden and is then capable of flight. The sex ratio on emergence is normally 1:1 (Jordan, 1986). The young fly before its first blood meal is called 'Teneral fly', the abdomen which appears whitish and semi-transparent with ptilinum everted when squeezed between fingers on the head. The non-teneral fly in contrast, are flies that have taken blood meal. They appear creamier yellow, the thorax is firmer and hard and the ptilinum is not easily everted (Pollock, 1982).

2.5 General Behavior of Glossina

The behavior, distribution pattern and density of a population of flies depends mainly on climate (temperature, humidity, sun, rain etc); vegetation (shade and shelter); wild animals(food); soil(breeding sites); predators of tsetse and human population (Davies, 1977).

2.5.1 Movement and Activity of Tsetse fly

The movement and dispersal of tsetse flies are related to the climate, the hunger stage of the fly and the sex of the fly (the males searching for mate and

the females actively searching for suitable larviposition sites) (Grev, 2002). The flies are active during the day spending only about 15 – 30 minutes each day in active flight. A single flight does not last longer than about 1½ - 2 ½ minutes and the speed of flight may be 3 -6 m/sec (Pollock, 2000). They are usually inactive during low temperatures and dull days; some species have been found flying in the moon light (Service, 1980).

Dispersal is higher in the wet period, during these periods they spread all over the savanna and have implications for transmission of African trypanosomiasis; such fly movement facilitate both the spread of the disease to new areas and their reintroduction to areas where it was previously under control. In drier periods, where unfavorable conditions prevail, they utilize places with dense vegetation close to water where suitable climatic condition exist (Ndams, 1987; Grev, 2002). The activity of tsetse flies during the daytime is mostly early morning or late afternoon. Females are only active for a few moments a day while mature males can be active up to 30 minutes a day (Grev, 2002).

2.5.2 Resting Sites

Resting sites may vary according to the time of the day or night, climate and season, species of tsetse fly, vegetation and resting places of host animals. During the hottest part of the day (usually early to mid-afternoon), the true resting sites are lowest down on tree trunks, and on the underside of shaded, fallen logs. At cooler times of the day, and in cooler seasons, the flies rest

higher up tree trunks, and on the underside of branches. At night some flies go up into the canopy of trees and rest on the leaves or twigs (Pollock, 2000).

2.5.3 Response to Host Animals

Response to host animals is usually by tsetse fly sense of smell and sight (Davies, 1977), from up to one hundred meters away, with larger host animals being more attractive to tsetse fly than smaller host animals (Pollock, 2000). Flies moves up-wind closer to the host animal when it smells the host. Tsetse fly land on a greater variety of host animals; flies that show the most attraction to the host animals are usually the most hungry flies in the population, while the non-hungry flies, particularly the males make up the 'following swarm'. A male in a following swarm may fly on to a virgin female as she comes for her first meal, and mate with her (Pollock, 2000).

2.6 Tsetse fly Population Dynamics

Tsetse populations are influenced mainly by density-independent factors such as temperature and humidity, which in turn depend on vegetation cover. Fly densities are determined by factors such as the availability of hosts or suitable habitats, which in turn are influenced by human activity.

According to Pollock, (2000) the density of a tsetse population in a given area is never very accurately known unless all the flies are caught, this could only be done on an island or in a very isolated woodland or thicket; even then the fact that more than half of the total population of tsetse fly in an area

are present below ground as pupae, makes the estimation of a tsetse population a difficult exercise. In some parts of Africa the fly population per square mile has been calculated, but in most of Nigeria, flies are not often evenly distributed over any area because of local differences in vegetation and climate, it is therefore difficult to determine the population this way (Davies, 1977).

In estimating tsetse population the term apparent density and true density are sometimes used. Apparent density is taken as the number of non-teneral males (savanna flies) caught in 900m (10,000 yards), which is a figure obtained from fly – round results in East Africa. This does not necessarily give information about the 'true density' (the number of flies per unit area) which may be heavy in a certain place indicating a hungry population rather than a dense one. The apparent density on the other hand may differ from time to time according to the availability of the flies, when conditions for catching are poor, the apparent density will be low even though the true density may be high (Davies, 1977). When traps are used, apparent density is defined as the number of flies per trap per day (F/T/D).

2.7 Transmission of the Disease

Tsetse flies transmit the protozoan parasite of the Genus *Trypanosoma*, the agents of human and animal trypanosomiasis in sub-Saharan Africa (Hu *et al.*, 2006). In West Africa, the human trypanosomiasis caused by T*rypanosoma brucei gambiense* is transmitted by *Glossina palpalis* and *Glossina tachinoides*, the disease is devastating and chronic. It is described as Gambian sleeping

sickness (Dutton, 1902; Abenga *et al.*, 2005), whereas animal trypanosomiasis caused by *Trypanosoma brucei brucei* is transmitted by *Glossina submorsitans* and *Glossina longipalpis.* In East Africa, both the human and animal trypanosomiasis caused by *Trypanosoma brucei rhodesiense and Trypanosoma brucei brucei* respectively are transmitted by *Glossina morsitans, Glossina pallidipes* and to some extent *Glossina palpalis* (Willet, 1970; Grev, 2002). The East African form described as Rhodesian form (Stephen and Fantham, 1910) is a more acute, lasting for weeks or months (Garcia *et al.*, 2006).

Trypanosomes exist as trypomastigote in blood and lymph in infected animals (Vreysen, 1995); they are slightly curved elongated protozoan measuring about 10 - 35µm with a single nucleus. Each possesses a single flagellum which originates near the posterior end of the body and extends forward to the body by an undulating membrane. Near the base of the flagellum is the dark-staining kinetoplast. The size and shape of the body, position of the nucleus and kinetoplast, length and form of the undulating membrane and flagellum are the diagnostic characters of various species of Trypanosomes (Jordan, 1986).

The form of trypanosomes in the tsetse fly can be identified to subgenus on morphological grounds and on their sites of development within the fly. In the subgenus *Duttonella* is the *Trypanosoma viva*; a group of trypanosomes with large terminal kinetoplast, distinct free flagellum and an inconspicuous undulating membrane. *Trypanosoma vivax* is a large (18 - 26µm long) monomorphic organism that is very active in wet – mount blood smears. Their

development within the tsetse fly is restricted to the proboscis (labrum or hypopharynx). In the subgenus: Nannomonas is the *Trypanosoma congolense* which is a small trypanosome with medium – sized marginal kinetoplast, no free flagellum, and a poorly developed undulating membrane. *Trypanosoma congolense* are hematic trypanosome found only in the blood vessels of the animal they infect. They develop within the proboscis (labrum or hypopharynx) and midgut of the tsetse fly. In the subgenus: Trypanozoon is the *Trypanosoma brucei brucei;* this group is an extremely polymorphic; while some trypanosome occur as short, stumpy organisms without flagella, others are found to be long slender organisms with distinct flagella, and there are intermediate forms that are usually flagellated (Moulton, 1976). These trypanosomes develop within the proboscis, midgut and salivary glands. However, when trypanosomes are found only in the labrum, they are regarded as immature form of all the subgenus mentioned, whereas if they are found in the labrum and midgut, it indicates immature form of the congolense group (Davies, 1977).

When trypanosomes are ingested by tsetse fly, they undergo a cycle of development within the fly. In the gut, they transform into the trypomastigote form and move into the mouthpart (labrum and/or hypopharynx) where they develop into the epimastigote form; they later reproduce by binary fission to produce the metacyclic form which is the infective form. This final binary fission takes place in the proboscis, midgut or salivary gland depending on the species of trypanosome (Jordan, 1986). However, transmission of trypanosomes can also occur from one mammalian host to the other in or on the mouthparts of

various species of biting flies e.g. Tabanus, Stomoxys, Chrysops etc including tsetse; this process is known as mechanical transmission (Jordan, 1986). This cycle of development varies in duration depending on the species of trypanosome, species of tsetse fly, temperature, reservoir host, age, sex. For *Trypanosoma vivax, Trypanosoma congolense* and *Trypanosoma brucei brucei*, it varies from 5 – 13 days at 22ºC - 29ºC; 12 -53 days and 11 – 60 days respectively (Davies, 1977; Jordan, 1986). Reports by various authors have showed that *Trypanosoma vivax* have the highest infection rate, followed by *Trypanosoma congolense* and then *Trypanosoma brucei brucei* (Onyiah, 1997; Omotainse *et al.*, 2000).

2.8 Control of Tsetse Flies

Attempts to control tsetse and trypanosomiasis date back nearly 100 years, employing a range of methods and approach. This range of methods have been developed and put into practice to keep the disease under control, some of them less good than others (Grev, 2002). The control strategies over the years have been directed against both the parasites and the vectors (Onyiah, 1997). The initial methods of tsetse control comprised of Hand-catching (Glasgow, 1970), Bush clearing (Steiner, 1964; Ford *et* al., 1970), Game destruction, Settlement of people (Davies, 1977) and the use of chemicals as insecticides (Davies, 1964). More recently, are the use of traps and screens (Challier and Laveissiere, 1973) and the Sterile Insect Technique (SIT). Other interventions aimed at eliminating the parasites have been by chemotherapy,

chemoprophylaxis and promotion of trypanotolerant breeds of cattle (Grev, 2002).

2.8.1 Control of Vector

2.8.1.1 Hand-catching

Hand-catching is the most ancient method of insect control and was first tried in about 1913 against *G. palpalis* in the Portuguese island Principe, and against *G. palpalis* (*G. fuscipes*) by the Germans on an island, Riamugasire, on Lake Victoria. Hand-catching is absolutely specific and has been found effective against some species, however they are more expensive than chemicals because it always require a large labour force especially if large areas were to be attacked, thus it fell into disuse (Glasgow, 1970).

2.8.1.2 Bush burning/Clearing

This method was performed based on the knowledge of the biology of tsetse fly, by cutting down dense vegetation, thus destroying both the adult fly and pupae due to decrease in humidity (Nagel, 1995). Bush clearing can be total or ruthless when all vegetation is totally cleared and partial when it involves the destruction of only a portion of the vegetation to render the environment unsuitable for tsetse fly. Partial clearing could be discriminate clearing when woody vegetation in known tsetse concentration sites is destroyed or selective where only certain components of the vegetation forming the fly habitat are removed, which may be removal of only the under storey, leaving the tall trees untouched or removing particular species of shrubs and trees (Jordan, 1986).

The history of the development and application of methods of partial clearing for tsetse control have been extensively reviewed by a number of authorities (Buxton, 1955; Ford *et al.,* 1970). In Nigeria, the record of partial clearing was described by Moiser (1912) in Geidam, Borno state when the population of *Glossina tachinoides* was controlled by vegetation clearing. Significant control was also achieved by Nash, (1940) on *Glossina tachinoides* at Gadau and Anchau in Kano state.

The major short-coming of these method lie in the limited size for which they can be economically deployed relative to the total size of tsetse affected area (Agyemang, 2001) since it requires economically unacceptable destruction of vast area of bush and forest. Bush clearing also results in soil degradation and deforestation and as such was no longer in use around 1970 (Nagel, 1995).

2.8.1.3 Game Destruction

The concept of game destruction was developed following the great rinderpest epizootic at the end of the nineteenth century, which resulted in the death of many game animals and thus the disappearance of tsetse flies and trypanosomiasis (Jordan, 1986). This method was used many years in Zimbabwe Zambia, Mozambique, Botswana and Uganda to eliminate a wide range of the population of savanna species of *Glossina* and trypanosomiasis due to its close association with game animal. McLennan, (1981) reported the ineffectiveness of game destruction on riverine species because they feed on other hosts besides wild game e.g. man, domestic animals, crocodiles and

reptiles. In West Africa, savanna species of tsetse population thrives on very low game densities and as such, this method of control against tsetse did not succeed in Nigeria. This method ceased because, animals migrated to area once cleared from larger mammals, making it possible for tsetse to recover, and also because it became unacceptable to public opinion (Grev, 2002).

2.8.1.4 Trapping Method

Trapping method for tsetse fly originated from the Island of Principe, where farm workers wore on their back a dark – colored piece of cloth, covered with glue, to reduce biting nuisance of the flies (Maldonado, 1910) along side with vegetation clearing and pig elimination to control tsetse on the Island (Da Costa *et al.,* 1916). The first real tsetse trap constructed by Harris was used to eliminate *Glossina pallidipes* from Zululand (Harris, 1938), and other various modified versions were introduced such as the Animal trap (Morris and Morris, 1949); Biconical trap (Challier and Laveissiere, 1973); Monoconical trap (Lancien, 1981); Nitse trap (Omoogun, 1994) etc. In Nigeria, Biconical and Nitse traps have been extensively used for tsetse sampling, ecological studies and control (Omoogun *et al.,* 1994; Dede *et al.,* 2005). Traps were initially made for control purposes but were later used for sampling and ecological studies due to their poor performance (Omoogun *et al.,* 1994).

The interest in controlling tsetse flies by trapping declined rapidly with the introduction of synthetic insecticides, but was renewed in the seventies, mainly due to increased public awareness of environmental pollution by

excessive use of insecticide (Grev, 2002). In West Africa, traps were widely used to control riverine species and was suggested to be better for community – based operations because local people can see flies being caught and killed, and can also see the catch decline as the operation progresses. Trapping method is simple, inexpensive, and harmless to the environment and is currently being used as an integrated part of the arsenal of tsetse control method where fly population is suppressed by trap and other conventional methods such as bait and target. However, reinvasion remains a reoccurring problem (Hargrove, 2002).

2.8.1.5 Use of Pheromones

Most work undertaken on pheromones (Langley *et al.,* 1975; Huyton *et al.,* 1980; Offor *et al.,* 1981; Carlson *et al.,* 1984) was based on the identification of the sex recognition pheromone, in the cuticle of female *Glossina* species which induces copulatory behavior in males of the same species, but not of other species, upon contact. This method aims at mass producing the compound(s) that constitute the sex recognition pheromones for the purpose of attracting flies to impregnated traps or screens to thus effect control, but because of the lack of volatility of these pheromones, they could not be exploited as attractant (Jordan, 1985).

2.8.1.6 Bait Technology

The potential of bait technology for the control of tsetse fly was appreciated in the first half of the 20th century. The strategy of the technology was to improve bait design (live or artificial) by the careful analysis of basic responses of tsetse to baits, and using the knowledge to improve the design of devices used in the field (Vale and Torr, 2004). Van den Bossche and De Deken, (2004) reported the use of artificial baits to control fly and reduce trypanosomiasis at lower cattle density and stressed that insecticide – treated cattle are more effective than stationary bait in area with higher cattle densities. The work of various authors (Ndams, 1987; Bossche, 1997; Brightwell *et al.*, 2001; Esterhuizen *et al.*, 2006) has suggested the efficiency of this method for the control of tsetse flies.

Even though improved traps and bait technology (targets) rapidly became the standard control method throughout Africa in late 1980s and 1990s, bait technology had pitfalls in its application which include tackling too small an area and the variability in costs and benefits relating to community – based action (Vale and Torr, 2004).

2.8.1.7 Use of Insecticides

The use of insecticides as control methods against tsetse fly commenced in the mid 1940s and is still today a major technique used in large scale (Grev, 2002), and almost all method now used depend on insecticide. Two chemical groups have been widely used in tsetse control: The organochlorides (DDT,

dieldrin, endosulfan):- DDT was the first chemical insecticides used against tsetse fly, after which dieldrin became popular because of its high lethal characteristics in more humid conditions. These two chemicals were then displaced by endosulfan for its toxicity and better solubility in spray solvent (Vreysen, 1995). DDT is cheap, has low mammalian toxicity, persists long in the environment and is effective against tsetse fly, while dieldrin on the other hand is expensive, has longer persistent rate than DDT (Davies, 1977). However, both fall victim of international ban due to their environmental side effect (Allsopp and Hursey, 2004). The spray of endosulfan against tsetse was reported to cause significant fish mortality (Douthwaite *et al.,* 1981).

The synthetic pyrethroids (deltamethrin, alpacypermathrin and beta-dyfluthrin) (Mangwiro *et al.,* 1999), are the most potent insecticides used against tsetse fly. Deltamethrin have been widely used for both spraying and for Impregnating traps and targets. The major disadvantages of the synthetic pyrethroids are their high cost (Vreysen, 1995).

In Nigeria, the use of insecticides to control tsetse fly started in 1955 (Davies, 1964) at Kamadugu Gana river system which later extended to Kiyawa-Jama'are Katagun system, both area lying within Kano, Borno and Bauchi States. Persistent insecticides (DDT) were applied from the ground on the tsetse resting sited by means of pneumatic knapsack pressure sprayers (Davies, 1964; Maclennam, 1967). Aerial spraying started in 1971 in the northern guinea vegetation zone, extended to the southern guineas savanna zone (Spielberger *et al.*, 1977). Spraying and re-spraying activities reclaimed large

area of land of about 399,551km² in the Sudan and northern guinea vegetation zones from tsetse fly (Bature, 1985). In Botswana, an area of 16,000km² in the Okavango Delta was reclaimed from *Glossina morsitans centralis* using aerial spraying with deltamethrin applied at 0.26 – 0.3g/ha and 12,000 deltamethrin-treated targets (Kgori *et al.,* 2006).

2.8.1.8 Sterile Insect Technique (SIT)

The application of the sterile male technique received considerable attention in the 1980s and is one method that seems feasible for the eradication of tsetse flies from the continent of Africa. Economic feasibility of which is grater in the area-wide approach (Feldmann, 2004). This approach was applied on an area-wide basis to eradicate the New World Screw worm *cochiiomyia hominivirax* in the U.S.A, Mexico and Central America. Since then very effective programmes integrating the SIT have been mounted against tropical fruit flies (Klassen and Curtis, 2005) and some species of tsetse flies on pilot trials at Lake Kariba, Zimbabwe (vs. *Glossina morsitans morsitans*), at Tanga, Tanzania vs. *Glossina morsitans morsitans*), in Burkina Faso (vs. *Glossina palpalis gambiense, Glossina tachinoides and Glossina morsitans submorsitans*) in Plateau state, Nigeria (vs. *Glossina palpalis palpalis)* and in Zanzibar, Tanzania (vs. *Glossina austeni*) (Feldmann, 2004). The success in Zanzibar demonstrated the technical feasibility of fighting the disease through the sterile insect technique approach (Kabayo, 2003)

The concept of sterile insect technique involves the mass production, sterilization – by exposure of males to short burst of gamma radiation from a cobalt-60 source (Okhoya, 2003) and sequential release of sterile males to the target species to compete with the wild male population. Mating between released sterile males and the wild females produces unviable progeny and the population is reduced over several generations to unsustainable levels (Abila *et al.*, 2003)

The obvious constraints of sterile insect technique are the high cons associated with mass rearing, low competitiveness of released sterile males (Whitten and Mahon, 2005), low reproductive rate and low rate of re-infestation (Feldmann, 2004). The technique which can only be employed realistically when density of target population is low is Impractical for use against high density population. It is therefore used only in an integrated approach with other control methods such as traps and targets (Jordan, 1986). However, sterile insect technique still remains an exceptionally promising pest control method in terms of efficacy and environmental compatibility (Nagel and Peverling, 2005).

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Kamuku National Park is located in Birnin Gwari Local Government Area of Kaduna State, bordered to the north by Kwiambana in Zamfara state and to the west by Kotankoro in Niger state. The park falls within latitude 10º 25´N to 11ºN and longitude 06º 30´E, and spans an area of 1,120sq.km, lying within the Southern Guinea savanna zone (Keay, 1953). It has an average annual rainfall of 1250mm with average temperature resembling that of the North Central Zone of the country. Dry season begins from November to April and the wet season from May to October. The hottest months are March and April, while the coldest months are December and January.

The vegetation is typically of the savanna woodland type, rivers and streams transverse the park giving rise to riverine forests and thickets along water courses. The park has varieties of flora and fauna forms ranging from plant species; such as *Isoberlina doka, Piliostigma thonningii, Vitex doniana, Raphia sudanica, Burassieus ethiopum, Phoenix reclinata, Elaeis guinansis, mimosa* etc, animal species detected from direct sighting, dung, and foot prints include baboons (*Papio papio*), roan antelopes (*Hippotragus eguinus*), elephant (*Loxodontan africana*), red flanked duiker (*Cephalophus rutilatus*), waterbuck (*Kobus defassa*), bushbuck (*Tragelaphus scriptus*), monkeys (*Ateles fuscieps robustes*) and Hymenoptera insects (butterflies, bees, wasps), tabanus, stomoxys, houseflies, tsetse flies etc.

The streams Dagara, Kabungu bungu, Kango kabungu, Kuzomani, and Kurishi surveyed are characterized by thick vegetation of tall and medium sized trees forming canopies with little light penetration, shrub and grasses extends about three to five meters on either side of the stream bank with localizes thickets along stream edges. Extensive pouching, grazing and bush burning activity particularly in the dry season leave the surface bare with scattered trees and shrub. All streams dries up completely in the dry season with the exception of permanent pools observed in Dagara and Kuzomani streams which serves as drinking points.

3.2 Preliminary Studies

A preliminary study of the area was undertaken in September, 2005 to ascertain the suitability of the area for the principal study.

A total of eighteen traps, fourteen Biconical (Challier and Laveissiere, 1973) and four Nitse (Omoogun *et al.*, 1994) traps were deployed for the purpose of the study. Traps were positioned at about 100 -150 meters apart for 48 hours in the following streams surveyed; Dagara - 4 biconical traps; Kabungu bungu - 2 biconical traps; Kango kabungu - 4 biconical traps; Kurishi - 4 biconical and 2 nitse traps; Kuzomani - 2 nitse traps while in Doka district, 2 biconical traps were installed at Mando river.

Flies caught were harvested daily and put in a cool box and brought to camp site for entomological analysis.

3.3 Tsetse Fly Population Sampling

Field investigations were carried out in both dry (November - April, 2007) and wet (May - October, 2007) season. The experimental design employed was the complete randomized block design where each stream represents treatment and the trapping points represents replicates or blocks.

Biconical (Challier and Laveissiere, 1973) and Nitse (Omoogun *et al.*, 1994) traps were used in this study. A total of twenty traps (10 biconical and 10 nitse) were used each month for one year, and four traps (2 biconical and 2 nitse) each were mounted along each stream bed or riverine vegetation suitable for tsetse fly in the dry season since the period has been shown by various workers to be notably characterized by tsetse concentration (Nash, 1937; Ford, 1971; Davies, 1977; Maclennan, 1981). In the wet season, traps were mounted along river banks and left for a period of two days (48 hours) and collected daily. The flies collected from each stream site and trapping points were labeled and conveyed in a cool box to the camp site for identification into species, separated by sex and into teneral and non-teneral, categorized by wing fray and grouped into different hunger stages. Tsetse flies were dissected to reveal the uterine content and trypanosome infection. Other information like the temperature and relative humidity of each trap point were recorded using the Whirling Hygrometer respectively (Davies, 1977).

3.4 Entomological Analysis

3.4.1 Species Identification

All tsetse flies caught were identified to species level according to the markings on the abdomen, hind leg and color of the thecal bulb of the proboscis (Davies, 1977). The *Glossina submorsitans morsitans* species was identified by the thecal bulb being dark brown, the 4^{th} and 5^{th} tarsal segments of the hind leg are black and the markings on the first segment of the abdomen is square shaped. For *Glossina palpalis* species, the thecal bulb is dark brown, all tarsal segments of the hind leg are black and the first segment of the abdomen is square shaped while in *Glossina tachinoides* they are triangular.

3.4.2 Teneral and Non-Teneral

Teneral flies identified were soft to touch; abdomen appears whitish with no dark area when held to light. Non-teneral flies on the other hand, were creamy white with dark area on the ventral surface of the abdomen when held to light (Pollock, 1982). Generally, teneral flies are those that have not feed on blood whereas non-teneral had fed.

3.4.3 Apparent Density Determination

The apparent density of fly was determined by dividing the total number of flies caught by the product obtained from the multiplication of the number of traps used by the trapping days.

3.4.4 Age Determination

3.4.4.1 Wing Fray Method

Wing fray method was used to determine the age of the male tsetse flies. The wings of the separated male tsetse flies were caught off the fly and placed on a drop of distilled water on a glass slide and examined through a low power dissecting microscope. The degree of wear and tear on the trailing edge of the wing were compared with the standard (plate 1) and recorded. The mean wing fray value was estimated by multiplying the number of wings in each category by the factor assigned to that category in accordance with the following system. For categories 1, 2 and 3, the factors are 1, 2 and 3, respectively; for categories 4 and 5 they are 4.4 and 5.5, and the factor for category 6 is 6.9. The sum of the products is divided by the number of wings examined and the estimated average age of the male flies was determined by comparing with standard (EATRO, 1955) plate 2.

3.4.4.2 Ovarian Method

Female flies' ages were determined by the ovarian method of Saunder, (1960) and Challier, (1965). The female flies caught after removing the wings and legs were placed dorsally on a slide containing a drop of 0.9% saline, the 6^{th} and 7^{th} abdominal segments was cut open using Borradiale needle. The reproductive system was pulled out to reveal the uterus and the contents (egg, 1^{st}, 2^{nd}, 3^{rd} instar larva, empty or aborted) was examined and recorded. The sequence of ovulation was examined by observing the position of the largest

ovariole in the ovaries; the different ovarian configurations recorded and the age then determined by comparing with the standard (plate 3).

3.4.5 Mating Scar and Insemination

The female tsetse fly caught were placed dorsally on a glass slide using a scissor-forceps and the 6^{th} segment of the abdomen were examined under a low power dissecting microscope for the presence of mating scar after which the extreme tip of the abdomen was removed and the reproductive system pulled out with a dissecting needle to reveal the ovaries and spermathecae. The spermathecae were gently cut off from the spermathecal duct and placed on a clean glass slide containing a drop of 0.9% saline, covered with a cover slip and was examined under a low power light microscope for a mass of sperm. And where the spermathecae appeared cleared and transparent they were regarded as not inseminated (Mellanby, 1936; Buxton, 1955)

3.4.6 Hunger Staging

All male flies caught were categorized into four hunger stages as described by Jackson (1933a; 1937). Stage 1: Gorged when abdomen was distended with red or blue-black blood. Stage 2: Replete when abdomen was observed to be slightly convex with no red or black color visible and two- thirds opaque. Stage 3: Intermediate when abdomen was slightly concave and a quarter to two- thirds was opaque. Stage 4: Hungry when abdomen was flattened and underside wrinkled. The Mean Hunger Stage (MHS) was

computed by multiplying the number of flies in stages 1, 2, 3 and 4 by 1, 2, 3, 4 respectively and dividing the sum of these numbers by the sum of the numbers of flies assigned to each of these stages. This gives, for the MHS, a figure between 2 and 4; a figure between 2 and 2.9 indicated a well fed population while a figure between 3 and 3.9 indicated a hungry population. Female flies were not hunger stages due to the development of larva which obscures the manifestation of the hunger cycle.

3.4.7 Trypanosomes Infection

Trypanosome infections were determined through dissection of the proboscis; midgut and salivary gland of non-teneral male and female flies caught in 0.9% saline and examined under x400 magnification. The proboscis bulb was held with fine forceps, and the head pulled off was mounted whole in a drop of saline. The labrum, labium and hypopharynx were separated with a dissecting needle, covered with a cover slip and the chitinous wall was examined under the microscope.

The midgut was revealed for examination by placing the fly dorsally on a slide and cutting the last two or three segments of the abdomen. The content of the abdomen was then squeezed out by placing a needle horizontally across the abdomen and pressing slightly downward; a cover slip was placed on the viscera and crushed gently by applying pressure on the cover slip.

The salivary gland was gently pulled out and placed on a slide containing a drop of saline solution by grasping the head of the fly with fine forceps and pulling it

gently away from the thorax, in a straight line. The salivary gland was then covered with a cover slip and the lumen of each gland was examined under the microscope (Pollock, 1982).

CHAPTER FOUR

4.0 RESULTS

4.1 Preliminary Observations

4.1.1 Composition of Glossina Species and Density

Table 1 is the preliminary results on tsetse composition and density which revealed three species namely; *G. palpalis, G. tachinoides* and *G. m. submorsitans*. Out of the 117 tsetse flies caught, 47.8% (56) were *G. palpalis*, 51.3% (60) were *G. tachinoides* and 0.85% (1) was *G. m. submorsitans.* The highest percentage of flies caught 50.42% (59) was from Dagara stream, while the lowest 0.85% (1) was from Kuzomani stream. Between these extremes the following catches were made: 21.37% (25) in Kango kabungu stream, 18.80% (22) in Kurishi stream and 8.55% (10) in Kabungu bungu stream while the lowest number caught 1 (0.85%) was from Kuzomani stream.

As shown in Table 1, an overall apparent density of 1.5 flies per trap per day (F/T/D) was observed. However among streams, fly densities varied from 3.0 F/T/D at Dagara stream, 1.3 F/T/D at Kabungu bungu stream, 1.6 F/T/D at Kango kabungu stream, 0.01 F/T/D at Kuzomani stream to 0.9 F/T/D at Kurishi stream. Similarly, apparent densities of 0.7, 0.8 and 0.1 F/T/D were observed for *G. palpalis, G. tachinoides* and *G. m. submorsitans* respectively. The proportion of male to female flies was skewed as more males in both *G. palpalis* and *G. tachinoides* were encountered. *G. m. submorsitans* was the least encountered species, being represented by a single female caught at Dagara stream.

TABLE 1: PRELIMINARY OBSERVATION ON TSETSE CATCHES AT KAMUKU NATIONAL PARK

Streams	Tsetse catches						% Total catches (♂+♀)	No of traps	Trapping days	App. Density (F/T/D)
	G. pal		G. tach		G.m.sub					
	♂	♀	♂	♀	♂	♀				
Dagara	32	22	01	03	00	01	59(50.42%)	5	4	3.0
Kabungu bungu	00	00	04	06	00	00	10(8.55%)	2	4	1.3
Kango kabungu	00	00	16	09	00	00	25(21.37%)	4	4	1.6
Kuzomani	00	01	00	00	00	00	01(0.85%)	2	4	0.1
Kurishi	00	01	12	09	00	00	22(18.8%)	6	4	0.9
Total (♂+♀)	**56(47.8%)**		**60(51.3%)**		**01(0.85%)**		**117**	**19**	**4**	**1.5**
App. Density	**0.7**		**0.8**		**0.01**					

4.2 Main Study Observations

A total of 502 flies were caught in the five streams, during the investigation in Kamuku National Park, from January to December, 2007. The highest number of flies caught 217 (43.2%) was in Kurishi stream, followed by these catches: 166 (33.1%) in Dagara stream, 45 (9.0%) in Kango kabungu stream, 41 (8.2%) in Kuzomani stream and 33 (6.6%) in Kabungu bungu stream Fig. 1. (Detail in Appendix 1)

4.2.1 Composition of Tsetse Catches at Kamuku National Park

4.2.1.1 Species Composition at Kamuku National Park

For the main study, two species of tsetse flies were encountered i.e. *G. palpalis* and *G. tachinoides. G. m. submorsitans* was not observed. Out of 502 tsetse flies caught, 38.4% (193) were *G. palpalis* while 61.6% (309) were *G. tachinoides* (Fig. 2).

Table 2 shows the proportion of these species caught in each of the five streams of the study area. Each species was represented in all five streams but the proportion of each species varied considerably among the streams. For example, out of 166 tsetse flies caught in Dagara stream, *G. palpalis* catches were 97% (161) higher than *G. tachinoides* 3% (5). Conversely, in Kurishi stream, *G. tachinoides* were 98.2% (213) more than *G. palpalis* 1.8% (4) out of 217 flies caught. Apart from Kuzomani steam which has a good proportion of *G. palpalis* 61% (25) with only 39% (16) *G. tachinoides* out of 41 flies caught, the remaining streams i.e. Kabungu bungu and Kango kabungu had poor

representation of 6.1% (2) and 2.2% (1) *G. palpalis* out of 33 and 45 flies caught respectively. *G. tachinoides* clearly dominated in both steams with 93.9% (31) and 97.8% (44) respectively (detail in appendix 2).

Table 3 shows the species composition of tsetse per month together with average of seasonal catches; both tsetse species were more abundant in the dry season than in the wet season. However, the average compositions of G. tachinoides for wet and dry season (32.7±10.1; 18.8±4.5) were greater than that of *G. palpalis* (21.3±10.1; 10.8±3.3).

Statistical analysis show a general non-significant different ($t = 1.97$, $df = 22$, $P>0.05$) between species. However, there was a significant difference ($\chi^2 = 411.7$, $df = 4$, $P<0.05$) between species in relation to streams. A non-significant difference ($\chi^2 = 0.43$, $df = 1$, $P> 0.05$) was observed between species in relation to season.

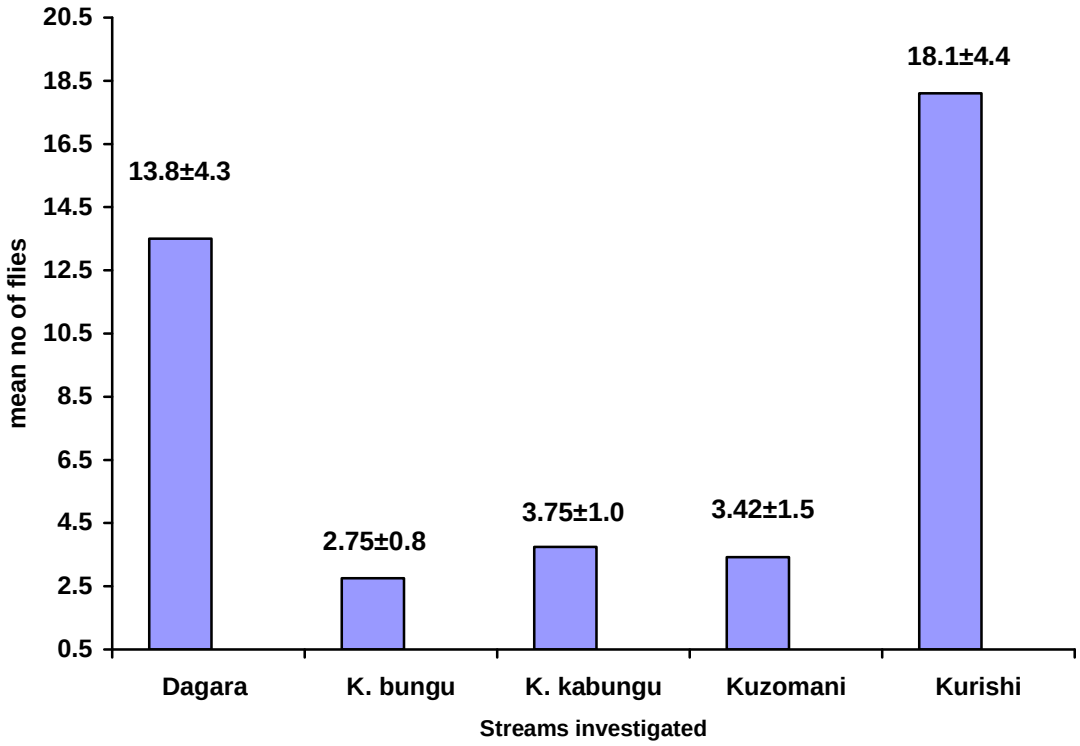


Fig. 1: Tsetse flies caught per stream

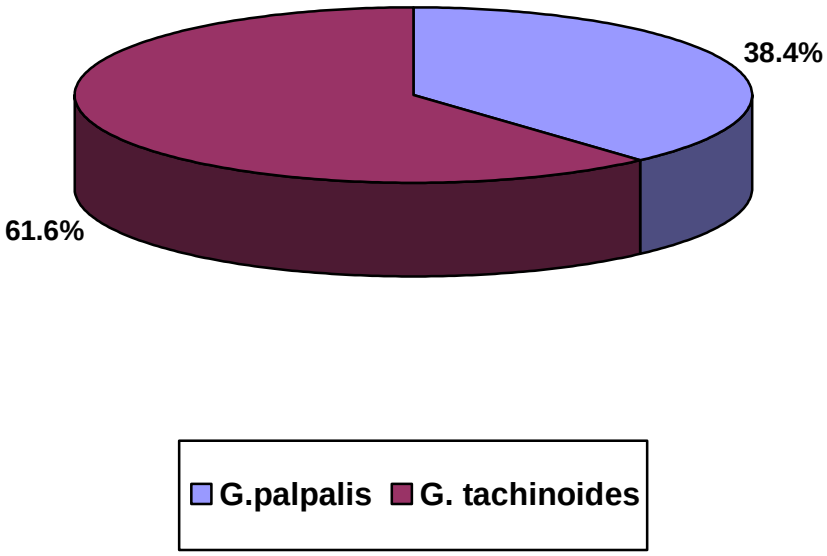


Fig. 2: Species composition at Kamuku National Park

Table 2: Tsetse species composition per stream

Species	No of flies caught per streams (%) Percentage and Mean ± SE									
	Dagara		**K.bungu**		**K. kabungu**		**Kuzomani**		**Kurishi**	
G. tachinoides	5 (3%)	0.4±0.3	31 (93.9%)	2.6±0.8	44 (97.8%)	3.7±0.9	16 (39%)	1.3±0.4	213 (98.2%)	17.8±4.3
G. palpalis	161(97%)	13.4±4.1	2 (6.1%)	0.2±0.1	1 (2.2%)	0.1±0.1	25 (61%)	2.1±1.2	4 (1.8%)	0.3±0.3
Total	**166**		**33**		**45**		**41**		**217**	

Table 3: Monthly Tsetse species composition

	Months												Average Seasonal catches	
Species	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**Dry**	**Wet**
G. palpalis	10	02	14	70	23	09	04	04	03	15	18	21	128 (21.3±10.1)	65 (10.8±3.3)
G. tachinoides	07	17	42	50	45	35	32	32	06	11	19	13	196 (32.7±7.0)	113 (18.8±4.5)

4.2.1.2 Sex Composition of Tsetse Flies caught

Table 4 shows in overall, a higher percentage of male tsetse 61.6% (309) than the females 38.4% (193) out of 502 flies caught in a ratio of 1.6:1.

Out of the 309 *G. tachinoides* caught, 64.4% (199) were males while 35.6% (110) were females, similarly for *G. palpalis,* 57% (110) were males while 43% (83) were females out of 193 flies caught. Therefore as expected, it was observed that in all streams surveyed, more male tsetse were caught than females except in Kabungu bungu stream where females were more than males Fig. 3 (see detail in Appendix 3)

With regard to seasonality, both male and female tsetse flies were observed to be greater in the dry season than in the wet season (Table 4).

Statistical analysis shows a significant difference ($t = 2.166$, $df – 22$, $P<0.05$) between sexes, and in relation to stream ($\chi^2 = 10.166$, $df = 4$, $P<0.05$), but a non-significant difference was observed between sexes in relation to species ($\chi^2 = 2.755$, $df = 1$, $P>0.05$) and season ($\chi^2 = 1.602$, $df = 1$, $P>0.05$).

4.2.1.3 Tenerality Composition of Tsetse Species

Table 5 presents a higher percentage of teneral flies 57.6% (289) than non-teneral flies 42.4% (213) in the overall 502 flies caught. Tenerality rates were higher in *G. tachinoides* 62.1% (192) than *G. palpalis* 50.3% (97). *G. tachinoides* had a higher percentage of teneral 62.1% (192) and non-teneral 37.9% (117) flies than teneral 50.3 % (97) and non-teneral 49.7% (96) *G.*

palpalis. However, teneral flies dominated in the dry season while non-teneral flies were dominate in the wet season.

Statistically, tenerality rate was not significant between species ($t = 1.725$, $df = 22$, $P>0.05$). However, a significant difference was observed between teneral and non-teneral flies in relation to species ($\chi^2 = 6.850$, df, = 1, $P<0.05$) and season ($\chi^2 = 4.748$, $df = 1$, $P<0.05$).

Table 4: Seasonal Sex Composition of Tsetse Species

	Sex	Months												%Total	Seasons	
		J	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**		Dry	Wet
Species *G. palpalis*	♂	9	40	8	7	3	3	0	9	11	11	8	1	110 (57%)	73(12.2±0.4)	37(6.2±1.9)
	♀	5	30	15	2	1	1	3	6	7	10	2	1	83 (43%)	55(9.2±4.7)	28(4.7±1.5)
G. tachinoides	♂	28	30	38	26	21	18	5	5	11	6	1	10	199 (64.4%)	133(22.2±5.6)	66(11±2.9)
	♀	14	20	7	9	11	14	1	6	8	7	6	7	110 (35.6%)	63(10.5±2.2)	47(7.8±1.8)
Total	♂	**37**	**70**	**46**	**33**	**24**	**21**	**5**	**14**	**22**	**17**	**9**	**11**	**309 (61.6%)**	**206(34.3±9.3)**	**103(17.2±2.8)**
	♀	**19**	**50**	**22**	**11**	**12**	**15**	**4**	**12**	**15**	**17**	**8**	**8**	**193 (38.4%)**	**118(19.7±6.5)**	**75(12.5±1.9)**

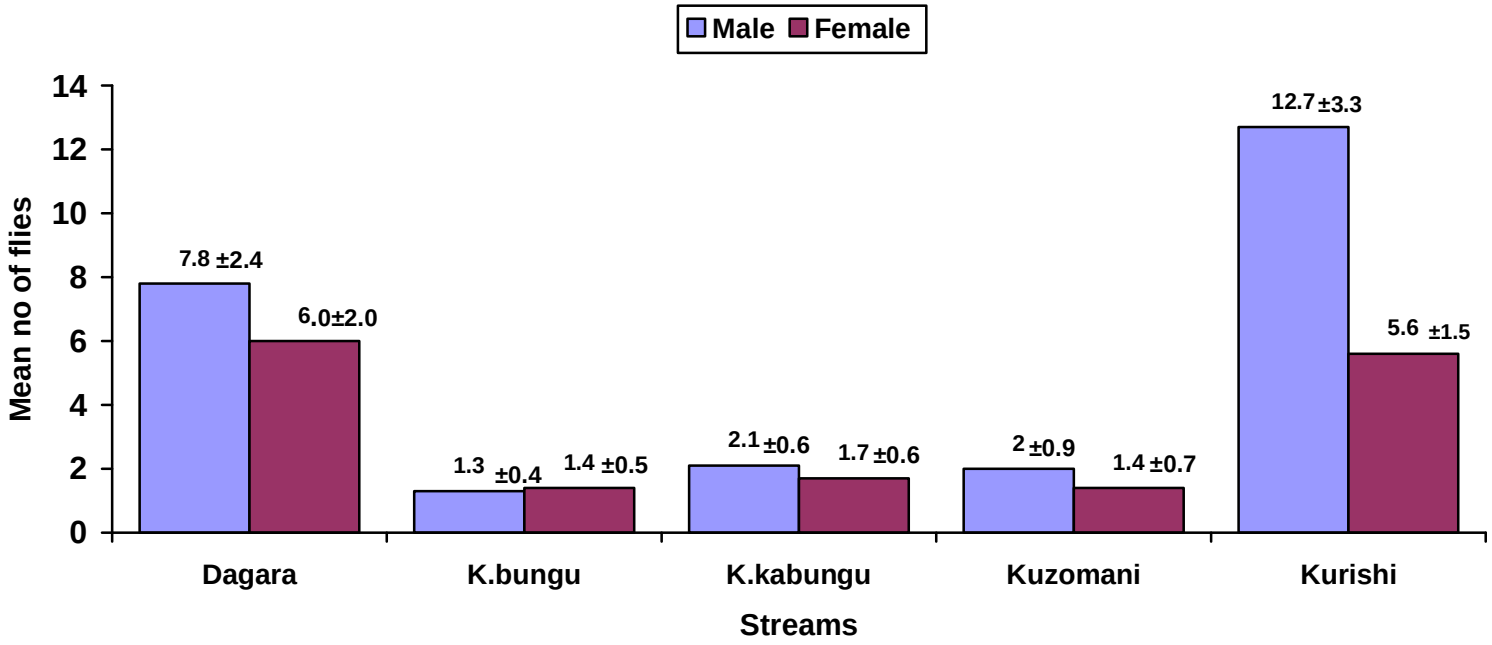


Fig. 3: Sex composition of tsetse catches per stream

Table 5: Tenerality composition of tsetse species according to seasons

	Species		Total	Seasons	
Tenerality	***G. palpalis***	***G. tachinoides***		**Dry**	**Wet**
Teneral	97 (50.3%)	192 (62.1%)	289 (57.6%)	37.2 ± 14.6	11 ± 4.2
Non-teneral	96 (49.7%)	117 (37.9%)	213 (42.4%)	16.8 ± 2.6	18.7 ± 4.6
Total	**193**	**309**	**502**		

4.2.2 Monthly Tsetse Abundance in relation to Temperature And Relative Humidity

Fig. 4 gives a summary of tsetse abundance in relation to temperature and relative humidity. At the onset in the dry season (i.e. November), a decrease in tsetse population (17 flies) was observed temperature was 26^{o}C and relative humidity was 73%; tsetse population increased later in subsequent months i.e. December and January and attained a maximum of 120 flies in February when temperature was highest 32^{o}C and relative humidity was lowest 14%. A decline featured in March (68 flies) and April (44 flies) as temperature decreased gradually and relative humidity increased.

Similarly in the wet season, as temperature decreased and relative humidity increased, tsetse population continued to decline reaching a minimum of 9 flies in July; this was followed by a build up of the population in August (26 flies) when temperature was lowest 25^{o}C and relative humidity was highest 92%. Also in subsequent months i.e. September and October, tsetse catches increased as temperature increased (28^{o}C) and relative humidity decreased (83%).

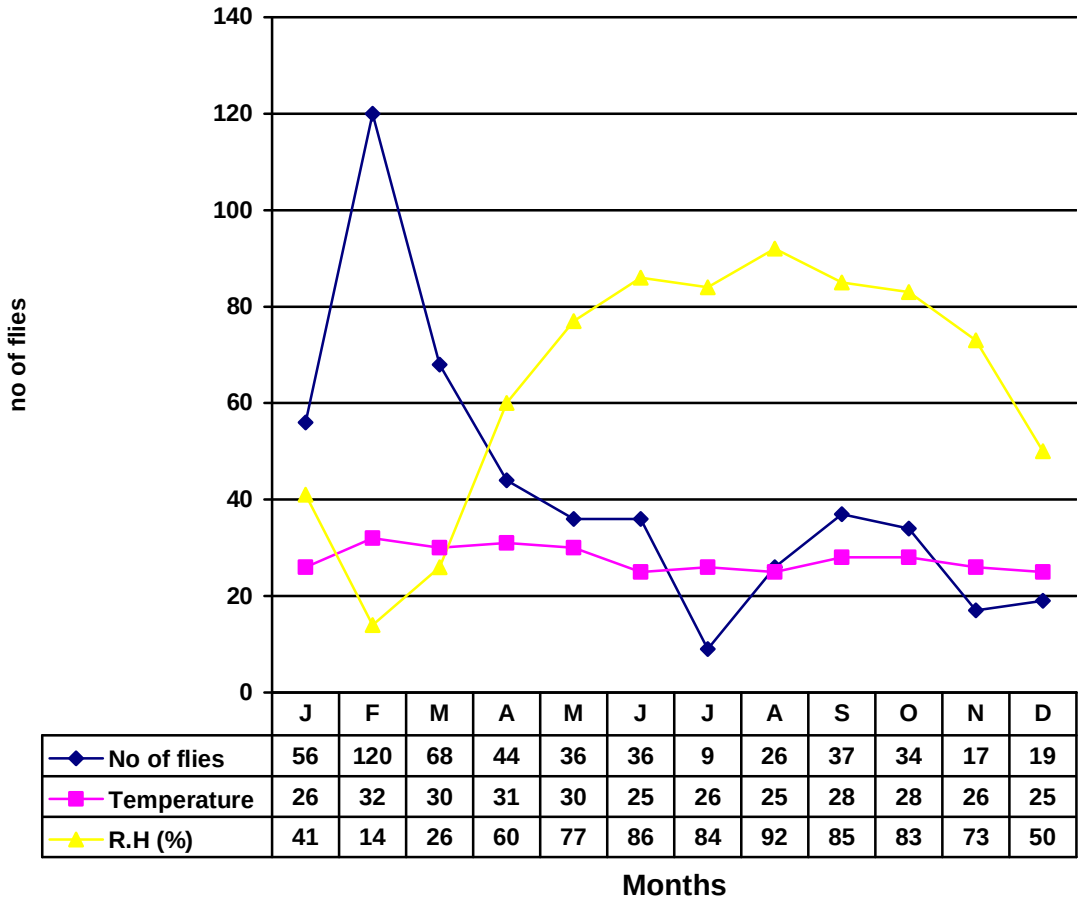
Statistically, tsetse abundance correlated positively with temperature ($R = 0.7038$, $df = 10$, $P < 0.05$) and negatively with relative humidity ($R = -0.7933$, $df = 10$, $P < 0.05$). In the wet season, no significant correlation with temperature ($R = 0.4222$, $df = 4$, $P > 0.05$) and relative humidity ($R = -0.2197$, $df = 4$, $P > 0.05$) was observed; however, in the dry season, relative humidity ($R = -0.8888$, $df = 4$, $P < 0.05$) correlated negatively while there was no correlation with temperature ($R = 0.7623$, $df = 4$, $P > 0.05$).

4.2.2.1 Seasonal Density Composition of Tsetse caught

Generally, the overall apparent density of 0.1 flies per trap per day (F/T/D) observed was low for all the months of the year (details in Appendix 4). For each month of the year the apparent density of flies summarized in Fig. 5 fluctuate considerably with a peak in February indicating three flies per trap per day (3 F/T/D).This figure declined to 0.2 fly per trap per day (0.2 F/T/D) in July. A second peak of 1.9 F/T/D was observed in September; this declined gradually to 0.5 F/T/D in December.

Table 6 shows an apparent density of 0.1 F/T/D for both *G. palpalis* and *G. tachinoides*. Similarly, an apparent density of 0.2 F/T/D was observed in both dry and wet seasons.

No significant difference was observed between seasons ($t = 1.532$, $df = 10$, $P>0.05$).



	J	F	M	A	M	J	J	A	S	O	N	D
No of flies	56	120	68	44	36	36	9	26	37	34	17	19
Temperature	26	32	30	31	30	25	26	25	28	28	26	25
R.H (%)	41	14	26	60	77	86	84	92	85	83	73	50

Fig.4: Monthly tsetse catches in relation to Temperature and Relative humidity

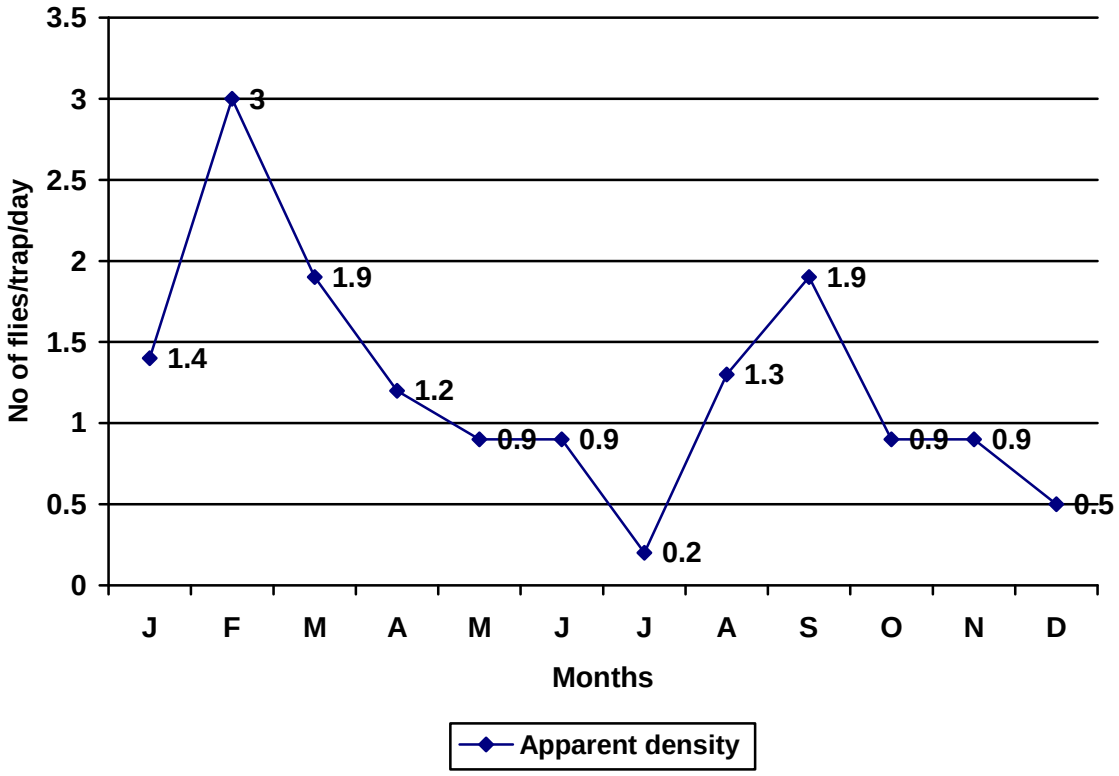


Fig. 5: Monthly densities of tsetse in Kamuku National Park

Table 6: Density of Tsetse Catches in Dry and Wet Season

Seasons	Species		App. density (F/T/D)
	G. palpalis	*G. tachinoides*	
Dry	128 (21.3±10.1)	196 (32.7±7.0)	0.2
Wet	65 (10.8±3.3)	113 (18.8±4.5)	0.2
Species app. density	**0.1 F/T/D**	**0.1 F/T/D**	

4.2.3 Age Composition of Tsetse Flies caught

4.2.3.1 Age Composition of Male catches by Wing Fray Method

Table 7 shows the Wing Fray data of age distribution of the total male catches. Out of 309 male tsetse flies caught, 110 flies were recorded for *G. palpalis* and 199 flies were recorded for *G. tachinoides*. The figure for *G. palpalis* was a Mean Wing Fray Value of 1.8 for both dry and wet season catches, with an estimated age of 12 days compared with *G. tachinoides* which had a Mean Wing Fray Value of 1.8 with an estimated age of 12 days in the wet season and a Mean Wing Fray Value of 1.4 with an estimated age that is less than 11 days in the dry season. A general Mean Wing Fray Value of 1.6 with an estimated age of 11 days was observed.

Male flies with Wing Fray category I was generally higher in both species and seasons than those with Wing Fray categories II, III and IV respectively (details in Appendix 5). No male fly was caught in Wing Fray categories V and VI.

Statistically, there was a significant difference in the age categories in relation to species ($\chi^2 = 10.037$, df = 3, $P<0.05$) and season ($\chi^2 = 15.083$, df = 3 $P<0.05$)

4.2.3.2 Age Composition of Female Tsetse by Ovarian Method

Table 8 presents the age structure of the female tsetse species caught. Of the total 193 female tsetse flies caught, 110 were *G. tachinoides* while 83 flies were *G. palpalis.* Out of 143 flies caught under category 0 (flies that had not

ovulated) 91 were *G. tachinoides* while 52 were *G. palpalis* and their estimated age were between 0 – 10 days. In category 1 (flies that had undergone one cycle of ovulation), 26 *G. palpalis* and 17 *G. tachinoides* was caught out of 43 flies and their estimated age was between 10 – 20 days. Under category 2 (flies that had undergone two cycle of ovulation) 5 *G. palpalis* and 2 *G. tachinoides* were caught out of 7 flies and their estimated age was between 20 -30 days. No flies were caught in other higher categories.

Statistically, there was a significant difference in the ovarian categories in relation to species (χ^2 =10.219, P<0.05)

Table 7: Seasonal age composition of male tsetse species

	Dry season		Wet season		Total
	G. pal	***G. tach***	***G. pal***	***G. tach***	
No of flies examined	73	133	37	66	309
MWFV	1.8	1.4	1.8	1.8	1.6
Estimated age (days)	12	<11	12	12	11

MWFV = Mean Wing Fray Value.

G. pal = Glossina palpalis

G. tach = Glossina tachionoides

Table 8: Age Composition of Female Tsetse Species by Ovarian Method

	Ovarian category			Total
	0	**1**	**2**	
No examined	143	43	7	193
G. palpalis	52	26	5	83
G. tachinoides	91	17	2	110
Approx. age (days)	0 – 10	10 – 20	20- 30	

4.2.4 Hunger Stages of Male Tsetse Species observed

Observations on hunger status of male tsetse flies (i.e. gorged, replete, intermediate and hungry) are presented in Appendix 6 with a summary in Table 9. The overall hunger stages composition of 117 male tsetse observed included 1 (0.9%) fly in the gorged stage, 7 (6.1%) flies in the Replete stage, 26 (22.6%) in the Intermediate stage and 81 (70.4%) flies in the Hungry stage. Out of 67 *G. tachinoides* flies observed, 5 flies were at the Replete stage, 15 flies were at the Intermediate stage and 47 flies were at the Hungry stage. Similarly, out of 48 *G. palpalis* flies observed, 1 fly was gorged, 2 flies were Replete, 11 flies were at the Intermediate stage and 34 flies were at the Hungry stage. The proportion of the various hunger stages were higher in the wet season than in the dry season, except for the Hungry stage which was higher in the dry season than in the wet season.

An overall Mean Hunger Stage of 3.6 was observed in the study. In the dry and wet season, the Mean Hunger Stage of 3.8 and 3.5 were observed respectively. *G. palpalis* had a Mean Hunger Stage of 3.6 while *G. tachinoides* had 3.5.

Statistical analysis showed a non significant difference in the hunger stages between species ($\chi^2 = 2.003$, df = 3, P>0.05) and season ($\chi^2 = 6.309$, df = 3, P>0.05).

Table 9: Seasonal composition of Mean Hunger Stages of male Tsetse species

	Dry		Wet		Total		Overall
	G. pal	***G. tach***	***G. pal***	***G. tach***	***G. pal***	***G. tach***	
No examined	24	31	24	36	48	67	115
MHS	3.8	3.8	3.5	3.5	3.6	3.5	3.6

G. pal = ***Glossina palpalis***

G. tach = ***Glossina tachinoides***

MHS = Mean Hunger Stage

4.2.5 Reproductive Status of Female Tsetse catches at Kamuku National Park

4.2.5.1 Uterine Contents of Female Tsetse catches

The age distribution of female tsetse in relation to their reproductive status is presented in Table 10. Overall, 143 out of 193 female flies were observed in category O (flies that had not undergone ovulation); Of the remaining 50 flies, 43 and 7 female flies in categories I and II respectively had undergone one and two cycles of ovulation; Within this group of 50 flies, 30 had eggs in-utero, 17 contained different stages of instar larvae while 3 had empty uteri as shown in Table 10.

In the dry season, 94 out of 118 female flies caught were observed in categories O; of the remaining 24 flies, 19 and 5 flies were in categories I and II respectively. Within this group of 24 flies, 14 had egg in-utero, 7 contained different stage of instar larvae while 3 had empty uteri. Similarly, in the wet season, 49 out of 75 female flies were in category O; while the remaining 26 flies contained 24 flies were in category I and 2 flies in category II. Within this group of 26 flies, 16 had egg in-utero while 10 contained different stages of instar larvae.

4.2.5.2 Parity and Insemination Rates of Female Tsetse

The overall parity rate composition of female tsetse catches showed a higher percentage of nullipars 74.1% (143) than pars 25.8% (50). *G. tachinoides* had a higher 82.7% (91) percentage of nullipars than *G. palpalis* 62.7% (52) as summarized in Table 11. In overall, the numbers of nullipars were higher in the

dry season than in the wet season, while parous flies were higher in the wet season than in the dry season.

Statistical analysis indicated a significant difference between nullipars and pars in relation to species (χ^2 = 4.950, df = 1 P<0.05) and season (χ^2 = 9.800, df = 1, P<0.05)

The insemination rate was generally high 93.8% (181) compared to non-inseminated flies 6.2% (12) out of 193 female flies examined. *G. tachinoides* had a higher insemination rate of 95.5% (105) out of 110 flies examined than *G. palpalis* 91.6% (76) out of 83 flies examined; non-insemination was higher in *G. palpalis* 8.4% (7) than *G. tachinoides* 4.5% (5). Inseminated flies were more in the dry season than in the wet season (Table 11)

No significant difference was observed between species (χ^2 = 1.244, df = 1, P>0.05) and season (χ^2 = 0.182, df = 1, P>0.05).

Table 10: Uterine Content of Tsetse Samples

Ovarian category	No of flies	Dry Uterine contents					No of flies	Wet Uterine contents				
		E	I	II	III	Emp		E	I	II	III	Emp
O	94	-	-	-	-	-	49	-	-	-	-	-
I	19	11	2	2	1	3	24	16	5	3	-	-
II	5	3	1	1	-	-	2	-	-	2	-	-
Total	**118**	**14**	**3**	**3**	**1**	**3**	**75**	**16**	**5**	**5**	**-**	**-**

E= Egg

I, II & III = 1st, 2nd & 3rd instar larvae

EMP = Empty uterus.

Table 11: Parity and Insemination rate composition

Season	Species	Parity rate		Insemination rate		Total
		Nullipars	Pars	Inseminated	non-inseminated	
Dry	*G. palpalis*	36	19	50	5	55
	G. tachinoides	58	5	60	3	63
Wet	*G. palpalis*	16	12	26	2	28
	G. tachinoides	33	14	45	2	47
%Total	***G. palpalis***	**52(62.7%)**	**31(37.4%)**	**76 (91.6%)**	**7 (8.4%)**	**83**
%	***G. tachinoides***	**91(82.7%)**	**19(17.2%)**	**105 (95.5%)**	**5 (4.5%)**	**110**
Overall		**143(74.1%)**	**50(25.8%)**	**181 (93.8%)**	**12 (6.2%)**	**193**

4.2.6 Infection Rates of Tsetse caught

An overall infection rate of 6.6% (14) was observed out of 213 flies examined. *G. tachinoides* had a higher infection rate of 9.4% (11) out of 117 flies examined than *G. palpalis* 3.1% (3) out of 96 flies. Infection rates were higher in males 5.2% (11) than females 1.4% (3); Table 12.

Infection with *T. vivax* 5.2% (11) was higher than infection with *T. congolense* 0.9% (2) and *T. brucei* 0.5% (1); Table 13

Table 12: Infection Rate in Tsetse Species

Species	No examined	Infection rate ♂	Infection rate ♀	Total
G. palpalis	96	1% (1)	2.1% (2)	3.1%(3)
G. tachinoides	117	8.5% (10)	0.9% (1)	9.4%(11)
% Total	**213**	**5.2% (11)**	**1.4% (3)**	**6.6%(14)**

Table 13: Trypanosome infection rates

Species	No examined	Type of Trypanosomes		
		T. vivax	***T. congolense***	***T. brucei***
G. palpalis	96	3	-	-
G. tachinoides	117	8	2	1
%Total	**213**	**11 (5.2%)**	**2 (0.9%)**	**1 (0.5%)**

CHAPTER FIVE

5.0 DISCUSSION

5.1 Composition of Glossina Species at Kamuku National Park

The preliminary study to estimate the apparent density of tsetse population in Kamuku National Park has shown that *G. palpalis, G. tachinoides* and *G. m. submorsitans* are sympatric within the park in low density of two flies per trap per day. The findings on co-existence of the three *Glossina* species particularly in game reserve and national park confirms earlier reports by Ndams, (1987); Omoogun, (1994) and Dankwa *et al.* (2000). The apparent densities of *G. palpalis* and *G. tachinoides* were one fly per trap per day respectively; while that of *G. m. submorsitans* was 0.01. The observation on *G. m. submorsitans* is an indication that a very low number of flies was caught (1) and as such must have affected the analysis.

During the main study, the number of flies caught at Kurishi, (217) and Dagara, (166) streams were significantly ($P<0.05$) higher than other streams. This may probably be due to local concentration of breeding sites along both streams; since these streams are characterized by dense vegetation of tall shaded trees and thicket (particularly at Kurishi stream) which provide suitable breeding locations for the flies.

Two *Glossina* species (*G. tachinoides* and *G. palpalis*) were identified as against the three encountered during the preliminary study with *G. tachinoides* being more abundant than *G. palpalis.* Similarly, the study has shown that *G. tachinoides* dominated in Kabungu bungu, Kango kabungu and

Kurishi streams while *G. palpalis* dominated in Dagara and Kuzomani streams. This may be associated with the differences in vegetation along the stream and probably due to the behavioral pattern of the fly, since *G. tachinoides* is known to inhabit thickets and areas with close-canopy vegetation, particularly where light penetration is minimal; whereas *G. palpalis* are found in vegetation of tall shaded trees where canopies slightly touches, to give sufficient light penetration. This observation is supported by the findings by Mellanby, (1936) who observed that *G. palpalis* perches in nature in flecks of sunlight at the boundary between shade and sun.

The observation made during this investigation on the absence of *G. m. submorsitans* could probably be due to the inefficiency of the sampling method employed to detect the fly and/or factors such as seasonal, vegetation and lack of food, resulting in a decline of the fly.

G. m. submorsitans are known to respond to bait oxen and moving object, therefore the use of Biconical and Nitse trap (designed to mimic the flank of a bovid which rotates gently on its axis through the action of the wind when fixed properly to indicate a moving object) should probably have enhance the visual attraction of *G. m. submorsitans* and their chances of being caught. Also, the only fly caught during the preliminary survey was in a moving vehicle, therefore moving through the park with a vehicle should probably have revealed the fly since they are known to follow moving vehicle. The observation of the inefficiency of Biconical and Nitse traps on *G. m. submorsitans*

contradicts the finding by Omoogun, (1994) who observed a better performance of both trap for *G. m. morsitans.*

The vegetation in the study area was usually dry and the woodland open in the dry season, except some few residual forests and thickets along streams; this makes the habitat unsuitable for *G. m. submorsitans* and may probably result in the migration of the fly to more suitable habitats. Even though the riverine and woodland vegetation are thick in the wet season, it is possible that dispersal could have been responsible for the absence of the fly due to high humidity. This observation is supported by the findings by Nash, (1937) who reported that *G. m. submorsitans* exhibits low density in the dry season due to high temperature which has drastic effect on the population.

The reduction of game animals in the study area, especially as a result of pouching activities may have accounted for the absence or decline of *G. m. submorsitans,* particularly due to lack of blood meal; and basically because they are game flies, they probably have followed the few game animals into the interior of the park. The observation supports the finding of Omoogun, (1991) and Hadis *et al*. (1995) who reported a decline in the morsitans group species and attributed it to increased human activities such as pouching which depleted the wild life population.

Overall, male tsetse flies caught were significantly higher than females with a sex ratio 1.6:1. This observation supports earlier finding that male tsetse respond more to stationary objects (Van Etten, 1981) and to traps, because trap catch the active segments of the population – activities of which could be

sexually oriented or as a result of variability in nutritional parameters (Jaenson, 1981).

As regards Tenerality, the significantly higher percentage of teneral flies than non-teneral flies in the dry season may be as a result of local concentration of flies and breeding sites along streams.

5.2 Tsetse Abundance and Seasonality Composition in relation to Temperature and Relative Humidity

The result on tsetse abundance in relation to temperature and relative humidity shows a significant ($P< 0.05$) positive correlation with temperature; which may be associated with increase in fly activity brought about, as a result of variability of nutritional status. This agrees with the finding by Nash, (1937) who observed that *G. tachinoides* were more active at about 29^{o}C and had maximum catches when temperatures were highest (28^{o}C– 31^{o}C). Similarly, the negative correlation observed between tsetse abundance and relative humidity may be due to low activity of flies; more so, because tsetse flies are more active in dry air than wet. This is supported by the finding of Bursell, (1957) and Mohamed-Ahmed *et al*. (1997).

The overall apparent density of 0.1 fly per trap per day was low indicating that the study area was a low density area (Davies, 1977). However, the high fly density of 3 flies per trap per day recorded in February during the dry season, may be due to increase in rate of emergence (since the larger number of flies caught were teneral flies) as a result of increase in temperature, which has been reported to shorten pupal period (Jaenson, 1981); and also due

to dry season concentration of flies into thickets and localized pools along riverine vegetations. This is in agreement with the finding of various authors (Nash, 1937; Baldry, 1969a; Davies, 1977 and Omoogun, *et al*., 1991). Similarly, the low fly density of 0.2 fly per trap per day observed in July during the wet season may be due to dispersal of flies away from riverine vegetation into the open woodland as a result of high humidity, thus reducing the number of flies visiting traps. The non significant difference observed between both seasons, even though the figure portrayed some differences may presumably be as a result of low density of flies which affected a thorough assessment of the statistical difference. This observation confirms the findings of other workers (Langride *et al.*, 1963; Ahmed, 2004).

5.3 Population Structure of Tsetse Species

Overall, the age composition of male tsetse catches in the present study has revealed a Mean Wing Fray Value of 1.6 and an estimated age of 11 days; while for the females, age category 0 dominated over age categories I and II amounting to 74% of the total females sampled (Table 8). This is an indication that the population was a relatively young and growing one, since no female flies were caught in the older categories and very few male flies (5) were caught under wing fray IV. Also, the high proportion of teneral flies encountered in this study may have accounted for the young population. A similar observation of young male population was made by Mohammed-Ahmed *et al.* (1993) and

concluded that excessive wing fray in tsetse are major factors that limits their longevity.

Results derived by analyzing the hunger stages of male populations of tsetse at Kamuku National Park revealed a Mean Hunger Stage of 3.6, *G. tachinoides* and *G. palpalis* having 3.5 and 3.6 respectively (Table 9). This implied that the population of tsetse flies at the park was a hungry one and that tsetse flies were hungrier in the dry season than wet season. This may presumably, be as a result of high metabolic rate associated with high temperature observed during the dry season, besides spontaneous flight activity have been demonstrated to be particularly, an expensive metabolic activity in tsetse (Hargrove, 1975b). More so, the reduction in host animal observed in the park may have resulted in the population being hungry.

The high insemination rate recorded and for both species was an indication that the sexes mated successfully, thus reflecting a healthy and viable population. The non-inseminated teneral female flies formed only about 6.2% of the total flies examined, and were probably trapped on their maiden flight. This observation agrees with the findings of other workers (Mohammed-Ahmed *et al.*, 1993; Omoogun, 1988).

Even though, insemination occurred efficiently, parity was low (25.8%) and may be due to the fact that younger flies were mainly encountered however, the presence of young pars and nullipars flies is an indication that the biotype constitutes a complete breeding habitat for both species, while the absence of old pars may be as a result of nutritional stress. Moreover, the frequency of

encounter of pregnancy stages in this study occurs in the order of egg, first, second and third instar larva, supporting the findings of other workers (Saunders, 1962; Randollph and Rogers, 1981; Mohammed-Ahmed and Dairri, 1987). The abortion rate was low (1.6%) and may have been due to the hunger state of the population.

According to Jordan (1974) and Davies (1977), the trypanosome infection rate in a population of tsetse may vary according to sex, age, species of trypanosome and tsetse fly, climate and hunger state of the fly etc. In this present study, the infection rates were higher *in G. tachinoides* than *G. palpalis*, male samples than females and in the wet season than dry season , even though the differences were not statistically significant ($P>0.05$).

Generally, the riverine species of *Glossina* have lower infection rate, however, the high infection observed in *G. tachinoides* may be due to the feeding habit of the fly since they feed on wide range of host animal and probably because, they are important transmitter of *T. vivax* particularly during the dry season when animals come to graze and drink around permanent pools thus allowing frequent host – fly contact.

It is apparent that male flies were more susceptible to trypanosome infection in this study, since infection rates were higher in males than in females, this may be due to the hungry population sampled, as hungry flies feed more often and have greater chances of becoming infected (Davies, 1977). Also, extreme starvation on tsetse have been reported by Kubi *et al.* (2006) to lower the developmental barrier for a trypanosome infection, thus enhancing their

ability to acquire trypanosome infection. More so, the increase in teneral flies in a population are of significant importance because, they are most likely to acquire and develop a mature, infective trypanosome infection.

The overall infection rate of 6.6% was high probably due to increased humidity associated with wet season. Increase in humidity results in fly dispersal into the open savanna which may bring about fly-host contact. The frequency of trypanosome infection with *T. vivax* dominated, followed by *T. congolense* and then *T. brucei*. This observation agrees with the findings of Owaga, (1981) who observed a higher trypanosome infection with *T. vivax* than *T. congolense* and no brucei infection. In contrast, Jamonneau *et al.* (2004) and Dagnogo *et al.* (2004) observed in *G. palpalis palpalis* a higher trypanosome infection with *T. congolense* followed by *T. vivax* and the least was *T. brucei*. The only 1 (0.5%) *T. brucei* encountered in this study agrees with Ahmed, (2004) who also observed one *T. brucei* in *G. palpalis palpalis*. This is significantly important because the detection of brucei – type infection in wild tsetse is a rare occurrence in Nigeria (Onah & Onyeka, 1985; Madubunyi, 1987; Kalu, 1991; Ahmed *et al.*, 2000). Although, this isolate was not characterized, its presence out of 14 infections recorded is an indication of a possible adventitious host.

5.4 Summary and Conclusions

The data presented above shows that tsetse catches differ significantly within streams and that *G. m. submorsitans* may probably have declined due to seasonal, vegetation and food factors, considering the very low density of flies observed during the preliminary study at Kamuku National Park. Seasonal result was non-significant even though tsetse catches were more in the dry season, with a peak in February. This is significant in controlling the flies. Also, tsetse catches correlated positively with temperature and negatively with relative humidity.

Evidence from reproductive, physiological status, ageing and parasitological data reflects a young, hungry and viable tsetse population, which can maintain itself and presumably impose a high trypanosomiasis challenge to nomadic cattle in the park during their annual dry season grazing. The presence of the brucei – type trypanosome infection, however low, is capable of supporting human trypanosomiasis in the park thus imposing a public health risk to tourist visiting the park

5.5 Recommendations

Further investigation of the whole park is suggested in order to understand the actual status of the *G. m. submorsitans* fly in the park, since the study area is in the savanna belt where morsitans group flies should ordinarily be found. Also, a broader investigation is required to ascertain the actual factor limiting the longevity of flies as older flies were not encountered. Even though

the study area was a low density one, the presence of tsetse and trypanosomes poses a health risk to villagers, rangers and tourist visiting the park. Therefore, control or eradication of tsetse is suggested, particularly in the month of February when fly emergence is high.

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APPENDIX 1: MONTHLY TSETSE CATCHES AT KAMUKU NATIONAL PARK STREAMS

Months	Streams					%
	Dagara	K. bungu	K. kabungu	Kuzomani	Kurishi	Total
Jan	14±2.2	02±0.5	00±0.0	03±0.5	37±3.7	56 (11.2%)
Feb	54±3.2	02±0.3	04±0.7	20±2.1	40±2.3	120 (23.9%)
Mar	23±2.2	00±0.0	08±2	00±0.0	37±3.3	68 (13.5%)
Apr	05±2.2	02±0.5	02±0.5	04±0.6	31±3.3	44 (8.8%)
May	03±0.5	01±0.3	02±0.3	03±0.8	27±4.4	36 (7.2%)
Jun	01±0.3	03±0.5	13±2.4	02±0.5	17±2.8	36 (7.2%)
Jul	01±0.3	00±0.0	03±0.5	03±0.8	02±0.3	09 (1.8%)
Aug	15±2.1	04±0.8	04±0.6	00±0.0	03±0.5	26 (5.2%)
Sept	17±1.7	10±1.7	03±0.5	01±0.3	06±1	37 (7.4%)
Oct	22±2.1	04±0.6	03±0.8	01±0.3	04±0.6	34 (6.8%)
Nov	08±1.7	03±0.5	01±0.3	02±0.3	03±0.8	17 (3.4%)
Dec	03±0.5	02±0.3	02±0.3	02±0.5	10±1.6	19 (3.8%)
% Total	**166(33.1%)**	**33(6.6%)**	**45(9%)**	**41(8.2%)**	**217(43.2%)**	**502**

APPENDIX 2: MONTHLY SPECIES COMPOSITION PER STREAM

Months	**Species per streams**										**Total**	
	Dagara		**K. bungu**		**K. kabungu**		**Kuzomani**		**Kurishi**			
	G. p	***G. t***	***G. p***	***G. t***	***G. p***	***G. t***	***G. p***	***G. t***	***G. p***	***G. t***	***G. p***	***G. t***
Jan	14	-	-	2	-	-	-	3	-	37	14	42
Feb	51	3	-	2	-	4	15	5	4	36	70	50
Mar	23	-	-	-	-	8	-	-	-	37	23	45
Apr	5	-	-	2	-	2	4	-	-	31	9	35
May	3	-	-	1	-	2	1	2	-	27	4	32
Jun	1	-	1	2	1	12	1	1	-	17	4	32
Jul	1	-	-	-	-	3	2	1	-	2	3	6
Aug	15	-	-	4	-	4	-	-	-	3	15	11
Sept	17	-	-	10	-	3	1	-	-	6	18	19
Oct	21	1	-	4	-	3	-	1	-	4	21	13
Nov	8	-	1	2	-	1	1	1	-	3	10	7
Dec	2	1	-	2	-	2	-	2	-	10	2	17
%	**161**	**5**	**2**	**31**	**1**	**44**	**25**	**16**	**4**	**213**	**193**	**309**
Total	**97%**	**3%**	**6%**	**94%**	**2%**	**98%**	**61%**	**39%**	**2%**	**98%**	**38%**	**62%**

APPENDIX 3: MONTHLY SEX COMPOSITION PER STREAMS

Months	Sex per stream										TOTAL	
	Dagara		K. bungu		K. kabungu		Kuzomani		Kurishi			
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Jan	9	5	1	1	-	-	2	1	25	12	37	19
Feb	31	23	2	-	4	-	11	9	22	18	70	50
Mar	8	15	-	-	6	2	-	-	32	5	46	22
Apr	3	2	-	2	1	1	4	-	25	6	33	11
May	2	1	-	1	2	-	2	1	18	9	24	12
Jun	1	-	1	2	5	8	2	-	12	5	21	15
Jul	-	1	-	-	3	-	1	2	1	1	5	4
Aug	9	6	3	1	2	2	-	-	-	3	14	12
Sept	11	6	4	6	1	2	-	1	6	-	22	15
Oct	11	11	3	1	-	3	1	-	2	2	17	17
Nov	7	1	1	2	-	1	-	2	1	2	9	8
Dec	2	1	1	1	1	1	1	1	6	4	11	8
Total	**94**	**72**	**16**	**17**	**25**	**20**	**24**	**17**	**150**	**67**	**309**	**193**

APPENDIX 4: APPARENT DENSITIES OF TSETSE CATCHES PER MONTH

Months	**Species caught**		**Total no of flies**	**Trapping days**	**No of traps**	**App. Density (F/T/D)**
	G.pal.	***G.tach.***				
Jan.	14	42	56	2	20	1.4
Feb.	70	50	120	2	20	3.0
Mar.	23	45	68	2	19	1.9
April	9	35	44	2	19	1.2
May	4	32	36	2	20	0.9
June	4	32	36	2	20	0.9
July	3	6	9	2	20	0.2
Aug.	15	11	26	1	20	1.3
Sept.	18	19	37	1	19	1.9
Oct.	21	13	34	2	19	0.9
Nov.	10	7	17	1	20	0.9
Dec.	2	17	19	2	20	0.5
Total	**193**	**309**	**502**	**21**	**236**	**0.1**

APPENDIX 5: SEASONAL AGE COMPOSITION OF MALE TSETSE CATCHES

Season	N	*G. palpalis* Wing fray categories						MWFV	Est. Age (days)	N	*G. tachinoides* Wing fray categories						MWFV	Est. Age (days)
		I	II	III	IV	V	VI				I	II	III	IV	V	VI		
Dry	73	32	27	11	3	-	-	1.8	12	133	90	37	6	-	-	-	1.4	<11
Wet	37	15	14	8	-	-	-	1.8	12	66	27	26	11	2	-	-	1.8	12
Total	**110**	**47**	**41**	**19**	**3**	**-**	**-**	**1.8**	**12**	**199**	**117**	**63**	**17**	**2**	**-**	**-**	**1.5**	**<11**

N = Number examined

MWFV = Mean Wing Fray Value

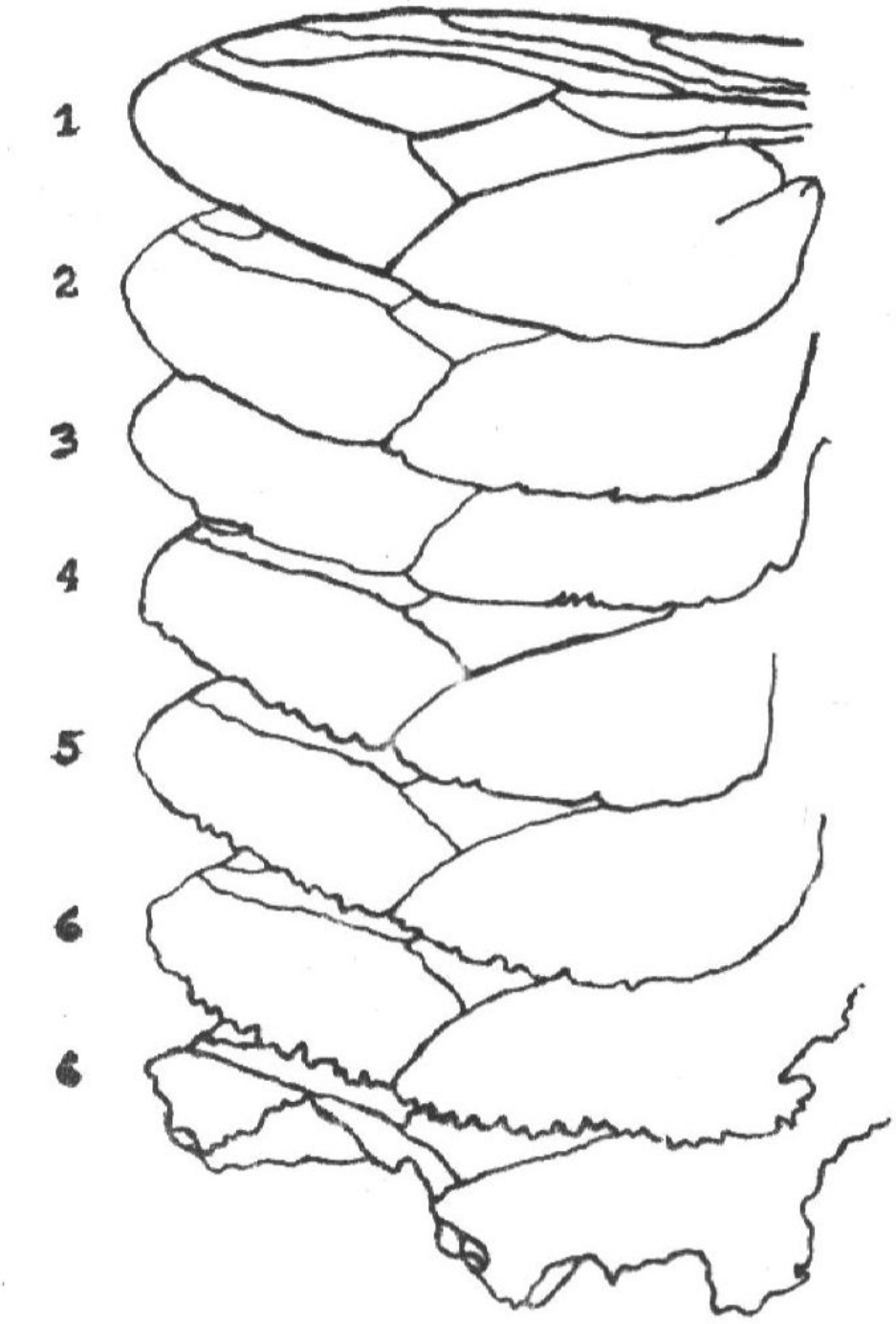
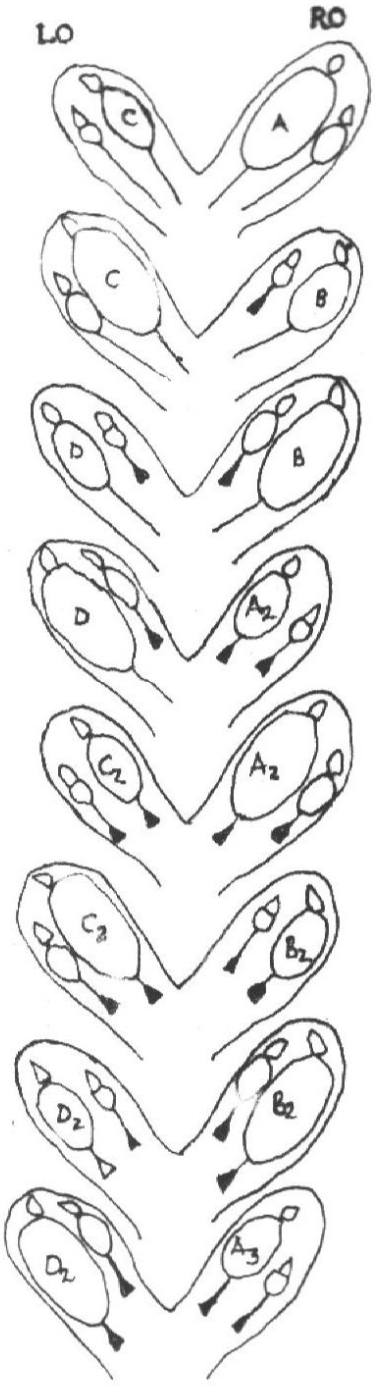


Plate 1: Wing Fray Categories (Baldry and Vander Vloedt, 1982)

MWFV.	Est.Age.	MWFV.	Est.Age.	MWFV.	Est.Age.	MWFV.	Est.Age.
1.6	11	2.8	21	3.9	31	5.1	41
1.8	12	2.9	22	4.0	32	5.2	42
1.9	13	3.0	23	4.2	33	5.3	43
2.0	14	3.1	24	4.3	34	5.4	44
2.1	15	3.3	25	4.4	35	5.5	45
2.2	16	3.4	26	4.5	36	5.6	46
2.3	17	3.5	27	4.6	37	5.8	47
2.4	18	3.6	28	4.7	38	5.9	48
2.6	19	3.7	29	4.8	39	6.0	49
2.7	20	3.8	30	5.0	40	-	-

Plate 2: Mean Wing Fray Value and Estimated Average Age in Days
(Baldry and Vander Vloedt, 1982)



a	b	c	d
0	4213	0	0–10
1	3142	1	10–20
2	2431	2	20–30
3	1324	3	30–40
4+	4213	4+4n	40–50
5+	3142	5+4n	50–60
	2431	6+4n	60–70
	1324	7+4n	70–80

Plate 3: Ovarian Method for Age Determination of Female Glossina RO=Right Ovary, LO=Left Ovary, a = age category, b = configuration, d = approximate age in days (Saunders and Phelps, 1970)