

A COMPARATIVE STUDY ON THE ECOLOGY OF TSETSE FLIES

(DIPTERA: GLOSSINIDAE) IN

THE WABE AND WALGA RIVER SYSTEMS

BY

FIKRU DESIE KOTYE

*A thesis submitted to the School of Graduate Studies of
Addis Ababa University in partial fulfillment of the requirements
for the Degree of Master of Science in Biology*

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April 2006

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ABSTRACT

The ecology of tsetse flies of the Wabe and Walga River Systems (southwest Ethiopia) was assessed and compared in the present study. Biconical flytraps baited with cow-urine were employed in both areas. No tsetse flies were detected in Wabe River System. However, di-species composition and bi-phasic diel activity of *Glossina morsitans* and *G. pallidipes* in Walga River System were determined.

The nature of fly abundance and distribution in relation to season and vegetation types were studied. As the dry season progressed, fly distribution was almost restricted to riverine vegetation, but in rainy and early dry season (August, September and December) fly distribution in riverine and bushy grassland vegetation was almost comparable. The infestation of flies in Borer (at Walga River System) was 24.7% and 26.5% for *G. morsitans* and *G. pallidipes*, respectively. There was no significant difference in proportion of infestation between the two fly species ($P>0.05$). Despite no flies were detected in Serite (at Wabe River System), cattle infection was observed at both study sites, and no significant differences were observed between proportions of infected cattle at the two sites ($P>0.05$).

One established fact from the mean packed cell volume (PCV) analysis was that even if the proportion of negative cattle was significantly higher than the proportion of positive cattle in both sites ($P<0.05$), however there was no significant difference between the mean PCV of negative and positive cattle at both sites. The infection in both flies and cattle was due to *Trypanosoma congolense*, except in Borer 25% of cattle infection was due to *T. vivax*.

Key words/phrases: *Trypanosomiasis, Glossina morsitans, Glossina pallidipes, Serite, Borer, fly infection rate, cattle infection rate.*

1. INTRODUCTION

Trypanosomiasis, a disease caused by trypanosomes (protozoan parasite), is of great economic and medical importance in Africa. African trypanosomiasis is confined to countries south of the Sahara. The area affected is an enormous block of the African landmass extending from approximately 14⁰ N to southern Angola at 29⁰ S, with continuous and pocket areas of infestation (Ford, 1970b). An estimated 10 million km² (extending to 38 countries), or nearly a quarter of the area of the African continent, is largely denied to cattle, other domestic animals as well as man because of trypanosomiasis (Ford, 1970a).

Human African trypanosomiasis, is exclusively transmitted by the tsetse flies (*Glossina* spp) while animal trypanosomiasis, which is transmitted mainly by tsetse flies could also be transmitted by other biting flies, such as Tabanids (Persoons, 1967).

Trypanosomiasis in domestic animals and malaria in humans have caused significant periodic movements of human settlement in Ethiopia from the 1960s up to the present day. These movements themselves are also likely to have influenced tsetse fly distribution. For example, *Glossina morsitans submorsitans* extended its range in southwest Ethiopia (Leak and Woudyalew, 1993), possibly by moving into areas where suitable habitat has been created by bush encroachment into previously cultivated land. Alternatively, climatic changes, resulting in a warmer climate, may have enabled tsetse flies to occupy land at higher altitudes than was previously possible.

Trypanosomiasis results in severe losses in productivity of domestic livestock due to poor growth, weight loss, low milk yield, reduced capacity for work, infertility and abortion. It also impairs the development of animal agriculture in zones which constitute 41% of the land but which carry only 26% of the ruminant population. The annual loss in meat production alone was estimated at US 5 billion dollar in 1984 (Murray and Gray, 1984). The number of cattle at risk of contracting tsetse fly-transmitted trypanosomes has been estimated at 46 million, in an area of about 8.7 million km². In Africa 80% of traction power is non-mechanized. A six-fold increase in agricultural output as a result of the availability of a draught ox to a family unit has been calculated (Mc Dowell, 1977).

Furthermore, the manure provided by livestock is essential for the production of food and cash crops and is a potential source of energy in the form of biogas.

In Ethiopia, an estimated area of 150,000-200,000 km² in the western, southern, southwestern low land areas, and the major river basins are reported to be infested by different tsetse fly species (Allsopp, 1997). As far as heads of cattle is concerned, Ethiopia stood in the first ten countries of the World and 1st in Africa. But the benefit the country gets from the sector is too low, for several reasons; of these trypanosomiasis (nagana) a vector (tsetse fly) born disease has got great significance, as it hampers agricultural productivity and economic development.

While eradication of trypanosomiasis remains an unrealistic goal for most of Africa, considerable effort has been invested in the control of this disease by the use of trypanocidal drugs, vector management and exploitation of genetic resistance exhibited by indigenous breeds. Even though the use of trypanocidal drugs is well established and represents the most adopted approach to control trypanosomes, there is a scope for increased use (Geerts and Holmes, 1998). Reports show increasing cases of trypanosome resistance to current drugs, both in individual cases and regionally, especially in East and West Africa (Clausen *et al.* 1992; d'Ieteron *et al.* 1997). There appears to be little hope for developing new trypanocidal drugs to benefit smallholder farmers in the short term. Given the actual or potential problem of drug resistance in many areas, drug usage clearly cannot be relied upon continuously as the sole method of trypanosome control.

According to the National Tsetse fly and Trypanosomiasis Investigation and Control Center (NTTICC), Ministry of Agriculture (MoA) (2001) trypanosomiasis control efforts using trypanocidal drugs are hindered by development of drug resistance. Moreover, drugs are costly to resource poor farmers. Thus, an integrated animal health management that comprises vector control and disease management is indispensable for human health improvement and poverty alleviation.

During the last few decades there has been a very great intensification of the attack against insect-borne disease, on an international scale as well as on a national base. There has also been a need for new and more precise quantitative data about vector composition, densities, distribution and behavior in order to place vector control programs on a much more scientific basis (Murihead-Thomson, 1968).

According to Abeshge Woreda Agriculture and Natural Resource Development and Protection Office (AWANRDPO, 2004), in one of the study area, Borer, Walga River System, ploughing by oxen traction is intercepted by nagana, that oxen live for one or two seasons only (if trypanocidal-drug treatment is given), otherwise they die and farmers are obliged to plough manually or they have to buy 'healthy' ox that will live and serve for short period of time. In Serite, Wabe River System, though the problem is not as severe as in the Walga River System, there is still an outbreak of the disease. Settlement in Borer is not more than 3 decades, but in Serite it approaches one century.

To this background, this study, therefore has the following objectives:

General objectives

- To study and compare the ecology (diversity, density, behavior, prevalence of trypanosomiasis) and importance of the tsetse flies in Borer and Serite.
- To develop, update information for control and development strategies.

Specific objectives

- To identify the species of tsetse flies and to determine their density and distribution in Borer and Serite.
- To determine activity pattern of tsetse flies in the study areas.
- To determine infection rate in tsetse flies.
- To determine trypanosome incidence rate in cattle.

2. LITERATURE REVIEW

2.1 Historical Account of Tsetse flies and Trypanosomiasis

Apart from two localities in the Arabian Peninsula, tsetse flies are found only in sub-Saharan Africa (Elsen *et al.*, 1990). Evidence of an earlier, wider distribution of tsetse fly, and of their evolutionary age, arose from the discovery of fossil flies in Florissant shales in Colorado, North America. Four fossil tsetse fly species found in those shales, and are from Oligocene period. Thus, it appears that tsetse flies originated at least 40 million years ago and inhabited parts of the United States of America. During that period, tsetse fly may have fed on giant terrestrial reptiles, and Cockerel, who described some of these fossils, suggested that tsetse fly may have had a role in the disappearance of some tertiary mammals in America (Cockerel, 1907, 1909, cited in Leak, 1999). The extinction of prehistoric horses and camels in North America might have been a result of tsetse fly-transmitted trypanosomiasis (Nash, 1969).

In 1590 Moroccan invaders complained of the terrible trials, which they had suffered in Sudan and announced that all their horses died of nagana and their cavalry had perished (Nash, 1969). In 1770, James Bruce of Kinnaird, in his Travels to Discover the Source of the Nile, had described a biting insect called the 'zimb' in Western Ethiopia; this could have been a tsetse fly or a stable fly. In 1830, two taxonomists described two species of tsetse fly from specimens brought back to Europe by travelers. The name tsetse fly is derived from the noise these flies make when flying. The name itself means 'fly' in Tswana, the language of the land formerly known as Bechuanaland (Botswana) (Nash, 1969).

Explaining the tsetse fly problem in a letter to Professor Westwood, Major Vardon wrote: 'In Botswana I have ridden up a hill and found tsetse fly increasing at every step, till at least forty or fifty would be on my horse at once. The specimens you saw cost me one of the best of my stud. He was stung by some ten or a dozen of them, and died in twenty days' (Nash, 1969).

In 1857 another explorer, David Livingstone, in his Missionary Travels, lost 53 oxen because of tsetse flies. In 1864, Kirk suspected the flies to cause a killing disease of animals. By 1880, clues to the understanding of the nagana problem are available for the first time (after Griffith Evans announced the discovery in India of a trypanosome later named *T. evansi*, in the blood

of horses and camels which are suffering from a disease known as 'surra'), but it was in 1895 that tsetse fly and trypanosomes became incriminated.

In 1894, while in the Zulu land, Surgeon Major Bruce, found and proved that trypanosomes are the cause of nagana, that the game forms a reservoir of a disease, and that the tsetse fly transmits it. Incidentally, Bruce introduced the name 'nagana'; it is a Zulu word, which signifies 'a state of depressed spirits' (Nash, 1969).

Later on, in 1902 Surgeon Ford discovered a trypanosome parasite in the blood of man (Langridge, 1976). In 1903, Castellani reported that he had found trypanosomes in the cerebro-spinal fluid of 70% of sleeping sickness cases in Uganda (Ford, 1971). Patton named the parasite as *Trypanosoma gambiense* in 1903 (Buxton, 1955; Nash, 1969; Ford, 1971). In 1909, Harsey voiced his suspicions that there were two different types of the human disease.

In 1912 Stephens and Fantham described a trypanosome from a patient in Rhodesia and named it *Trypanosoma rhodesiense*.

2.2 Parasites of African Trypanosomiasis

2.2.1 Classification of Trypanosomes

Researchers have observed and identified that trypanosomiasis is caused by flagellated protozoan parasite known as trypanosome (genus *Trypanosoma*) (Lloyd and Johnson, 1924).

Studies on the morphology of the parasites and the type of the disease they cause revealed that there were at least two distinct species of trypanosomes (*T. brucei gambiense* and *T. b. rhodesiense*) parasitizing man (cause sleeping sickness) and three other species of trypanosomes: *T. b. brucei*, *T. vivax*, and *T. congolense* parasitizing cattle (cause nagana). These parasites also showed marked difference in their virulence to their hosts. *T. simae* and *T. suns* affect pigs and other *Trypanosoma* spp also parasitize such animals like crocodiles, bats, rodents (Mulligan, 1970).

2.2.2 Life Cycle of Trypanosomes in Tsetse fly

In infected tsetse flies, the trypanosomes ingested with the blood meal undergo a cycle of development. They undergo a series of morphological changes, before assuming a form known as epimastigote. Eventually these give rise to metacyclic trypomastigotes, which infect the vertebrate host. Metacyclic forms then enter the mouthparts or salivary glands of the vector and get inoculated into the vertebrate host when the fly feeds (Mulligan, 1970). The development cycle of the trypanosomes within the tsetse fly varies according to the different sub-genera of trypanosomes.

The simplest development cycle occurs in the subgenus *Duttonella* where *T. vivax* is the main species. The trypanosomes ingested with the blood meal attach themselves to the wall of the fly's proboscis; and develop into the epimastigote form, before invading the hypopharynx and developing into the infective metacyclic form (Gardiner, 1989).

The *Trypanozoon* subgenus trypanosomes include *T. brucei brucei*, which infects wild and domestic animals, and *T. b. rhodesiense* *T. b. gambiense*, which infect humans. The cyclical development of *Trypanozoon* species in the tsetse fly takes place in the midgut and culminates in the metacyclic trypomastigote form in the vector's salivary glands. The metacyclic stage is infective for the mammalian host and, unlike the other developmental stages in the tsetse fly, these metacyclic trypanosomes possess a surface coat like that of bloodstream forms (Hoare, 1970).

Development of the trypanosomes of the sub-genus *Nanomonas*, namely *T. congolense* and *T. simae*, follow much the same sequence in the fore- and mid-gut regions as that of the sub-genus *Trypanozoon*. However, trypanosomes of the subgenus *Nanomonas* don't finally enter the salivary glands but become attached to the walls of the proboscis and develop first to the epimastigote stage and later to the infective metatrypanosome stage (Mulligan, 1970).

2.3 Classification and Biology of Tsetse fly

2.3.1 Classification

Tsetse flies are classified under one genus, *Glossina*, of the Family Glossinidae, Order Diptera. There are 31 species and subspecies identified in the genus at present. The classification of tsetse flies is based largely on morphological differences in structure of the genitalia. Three subgenera are identified based on these morphological differences and can also be broadly differentiated into three groups based on ecological characteristics (Mulligan, 1970; Jordan, 1993). The three sub-genera are *Morsitans*, *Palpalis*, and *Fusca*, and the naming is associated with the commonest species in each of the sub-genera.

The subgenus *Glossina* or the *Morsitans* group contains a total of 7-species and sub-species. Those of major economic importance are *G. morsitans* and *G. pallidipes* with *G. swynnertoni* and *G. austeni* being important in specific regions. The species of the *Morsitans* group are also called the savannah flies due to their preference to this environment. It is this fact that makes them the most important vectors as the African savannah is a vast area and the flies come into contact with man, livestock and wild game animals.

The sub- genus *Fusca* or *Austenina* are also referred to as forest flies. The 15 species and sub-species, which comprise this group, are mainly of no medical and veterinary importance. These large and more primitive flies feed on small mammals in forested areas. They have very little contact with man and his livestock due to their habitat. None of this group is a vector of sleeping sickness.

Flies of the sub-genus *Nemorhina* or *Palpalis* group are often called the riverine flies. There are 9 species and sub-species in this group, which are found in close association with local patches of dense vegetation along the banks of rivers and lakes in arid country, and also in dense, wet, heavily forested equatorial rainforest. Species of this group occur throughout much of western Africa. The principal hosts of these flies are reptiles especially monitor lizards and crocodiles. They will also bite ungulates. *G. palpalis* is the commonest and all species are vectors of trypanosomiasis.

2.3.2 Life Cycle of Tsetse fly

Tsetse flies have a very low rate of reproduction, closer to that of small mammals than to most other insects. During the life span a female can theoretically give birth to only a maximum of 8-10 offspring (in reality much lower), so tsetse flies are rather like human beings in that they make a large investment per offspring so that juvenile mortality is low; i.e., the maternal 'care' given to each larva enables a higher degree of survival of each offspring. The reproductive method of tsetse fly, in which the single egg hatches and develops to a third-stage larva in the uterus of the female fly where it is supplied with nutrients (from modified accessory glands), is adenotrophic viviparity.

Spermatogenesis only occurs during the puparial stage of males and ceases once the male fly has emerged (Curtis, 1968). The period of maturation is approximately 3 days for males, but females mature earlier. In most species, mating seems to take place on or near the host. There is no evidence of volatile sex pheromones being produced but females do have species-specific cuticular hydrocarbons, which induce a copulatory response in males of the same species. Mating takes an hour or two during which time a spermatophore is formed within the female's uterus using secretions from the male. Just before copulation ends, the male ejaculates sperm into spermatophore. Within the subsequent few hours, the sperm moves from the spermatophore up the paired spermathecal ducts into the paired spermathecae. These sperm serve the female throughout her life so she doesn't have to mate again (Pollock, 1970). Males are able to mate a number of times with different females.

Eggs develop sequentially in the female, alternating between the four ovarioles: after the female is about 9 days old, the first egg passes in to the uterus from one of the two ovarioles in her right ovary. After 9-10 days, there is the second ovulation from one of the two ovarioles in the left ovary, and so on. In the uterus the egg is fertilized by a sperm from the spermatheca (gained during earlier mating with a male) (Roberts, 1973a). After 3.5 days of development in the egg, the 1st instar larva breaks out of the egg case. The larva develops in the female's uterus by feeding on food from modified accessory glands. It passes through 2 moults to reach the 3rd instar and it is then larviposited (Roberts, 1973b).

The female finds a suitable place to lay the larva. In the wet season or wet regions such as rain forest, where there is general dampness everywhere, females tend not to concentrate their

larviposition in particular areas. However, in dry areas larviposition takes place mainly in well-shaded spots so that there is an aggregation effect in these places (Zdarek and Denlinger, 1993). The freshly laid free-living larva is fully fed, and after expelling the waste-products it gained while developing in its mother, it burrows into the soil where its skin hardens and blackens into a puparium and with the puparium metamorphosis takes place. The puparial period can range from 20 days (at 30⁰ C) to 47 days (at 20⁰ C). Development in the puparium is generally unsuccessful below about 17⁰ C and above about 32⁰ C. The entire life cycle from egg to adult usually takes about 30 days (Robinson *et al.*, 1985).

2.4 Distribution of Tsetse flies

According to Ford (1962), the southern limits of *Glossina* distribution in Africa lie north of a line drawn from Benguela, in Angola, to Durban, in South Africa. The northern limits are roughly a line from Dakar in Senegal across to Ethiopia and Mogadishu in Somalia on the east coast. In 1906 Carter recorded the presence of *G. tachinoides* in south Yemen in Arabian Peninsula, but no other reports confirmed this. It was therefore thought that tsetse fly in the Arabian Peninsula may have died out. However, *G. f. fuscipes* and *G. m. submorsitans* have since been detected in southwestern Saudi Arabia (Elsen *et al.*, 1990).

Balis and Bergeon (1970), Langridge (1976), and Ford *et al.*, (1976), indicated the presence of six species of tsetse fly in Ethiopia, which belong to the three groups (sub-genera).

A. The Fusca Group

These are *G. longipennis* and *G. brevivalpis*

1. *G. longipennis*, Corti 1895

First described in an insect collection made in 1895 along the Walmal River, Bale province. Also reported in the same year by Peel, who found it along the Daghatto River (a tributary of Wabe Shebele River) in the Ogaden. Ghidini reported this species to occur near Lake Abaya in 1938. Also Langridge (1976) showed the existence of this species in various parts of Gemugoffa and Keffa provinces. Later on its presence was verified by Ford *et al.* (1976); and Fuller (1978) reported *G. longipennis* actively biting at night.

2. *G. brevivalpis*, Newstead 1910

The only record of this species is from the lower part of the Omo River, Gemugoffa province. However, Langridge (1976) indicated that he was not able to find this species from the province or anywhere else in Ethiopia.

B. The Morsitans Group

Here again two species of this group, i.e. *G. morsitans ugandensis* and *G. pallidipes* have been reported from Ethiopia.

1. *G. m. ugandensis*, Vanderplank 1949

In Ethiopia this species was known (recorded) by the name *G. m. submorsitans* until 1971. First recorded by the Italian investigator di Damazzo from the Didessa Valley in Wellega province and near Lake Awassa in Arusi and Sidamo province in 1937. The same year Roetti found it along the Birbir, Chibise, and Berber-uaha River in Wellega and Keffa and on the Abobo River along the Sudan border in Illubabor province. In 1938, Caccavella confirmed the existence of this species at Awassa. Later work by Balis and Bergeon (1970), Langridge (1976) and Fuller (1978), indicated the existence of this species in various areas of Gojjam, Wellega, Sidamo, Shoa, Illubabor and Keffa provinces.

2. *G. pallidipes*, Austen 1903

In 1936, Moggridge recorded the presence of this species along the Wabe Shebele River. Rotti also recorded it along Didessa and Birbir rivers in Wellega and along the Akobo River (Illubabor) in 1938. Also in 1956 Ovazza found it along the Gojeb River in Keffa. Later on, Langridge (1976) mentioned the wide distribution of the species in Gemugoffa, Keffa, Shoa and Illubabor province.

C. The Palpalis Group

Langridge (1976) mentioned that two species of this group were recorded in Ethiopia; *G. tachinoides* and *G. fuscipes fuscipes*.

1. *G. tachinoides*, Westwood 1850

In 1839 Ghidini recorded this species on the Baro and Gilo Rivers (Illubabor). In 1956 Ovazza recorded this species along the Akobo River (Illubabor). Langridge (1976) mentioned that Balis and Bergeon had recorded this species along Didessa (Wellega) in 1970.

1. *G. fuscipes fuscipes*, Newstead 1910

First found by Brumpt in 1901 on the lower Omo River. In 1937 Ghidini recorded this species from L. Awassa in Sidamo and on the tributaries of the Akobo River in Illubabor province. Furthermore, in 1938 Rottei found it along the Didessa River in Wellega and Birbir River in Illubabor. Also Ovazza (1956) found this species on the Ghibe (Upper Omo) River.

According to Balis and Bergeon (1970), Langridge (1976), and Fuller (1978), this tsetse fly has been recorded around various streams and rivers in Gemugoffa, Sidamo, Wellega, Keffa, and Shoa Provinces.

2.5 Factors Affecting Tsetse fly Distribution

The general distribution of tsetse flies determined principally by climate and influenced by altitude, vegetation and the presence of suitable host animals (Ford, 1963).

2.5.1 Climate

It was recognized early on that climate was an important factor determining the distribution of tsetse fly, if not the factor controlling the basic pattern of distribution. The limit of distribution is closely correlated with the tropical savanna (summer rain) climate, which follows the 508mm annual isohyets. Climate, though dependent on latitude, is modified by altitude and of course has a great effect on the vegetation, which is vital for providing shade and maintaining a suitable microclimate for tsetse fly as well as a habitat for their vertebrate hosts. As a generalization, the tropical rainforest (equatorial) climate controls the habitats of the fusca and palpalis groups, and the surrounding woodlands are the habitat of the morsitans group (Leak, 1999). Altitude influences tsetse fly distribution through its effect on climate, particularly temperature. In Ethiopia, 1600m was considered the rough upper altitudinal limit to tsetse fly distribution (Langridge, 1976). Subsequently, however, *G. pallidipes* was found

at 1700m altitude, and *G. m. ugandensis* (*G. m. submorsitans*) at altitudes up to 2200m (Getachew and Teferi, 1984).

Desiccation of adult tsetse fly was at one time considered to be an important cause of death in natural tsetse fly populations although Bursell (1959, 1960) suggested that it was not the most important cause. Certainly, for the savanna (morsitans) tsetse fly species, Bursell's (1960) studies of fat and water content demonstrated that this was not the case. *G. palpalis*, in West Africa is in no danger of desiccation at 65% relative humidity, but at lower humidity, below 45% desiccation becomes an important cause of mortality. Nash (1933) demonstrated a negative correlation between saturation deficit and the apparent density of tsetse fly.

In northern Nigeria, when the mean relative humidity ranges between 43 and 57% in the late dry season, flies may suffer from desiccation and the longevity of *G. m. submorsitans* and *G. tachinoides* is then reduced (Nash, 1936). The dominant factor determining longevity appeared not to be humidity, although this was important, but maximum temperature, with which longevity was negatively correlated.

The concentration of *G. tachinoides* and *G. m. submorsitans* within denser vegetation during the late dry season in northern Nigeria is believed to be a response to increasing temperature (Nash, 1960). The lower temperature limit for evacuation to occur was 39⁰ C for both species. At normal temperatures, *G. morsitans* exhibits a positive reaction to light, if the temperature is raised sufficiently; the reaction of the flies is reversed and become strongly negative.

2.5.2 Effect of Human Activities

Apart of more 'natural' factors, the effect of humans has had an important influence on the distribution of tsetse flies. Most African countries, particularly those in tsetse fly infested areas, have low human population densities.

Study showed that up to two – thirds of the potential *G. m. submorsitans* population of Nigeria might have been suppressed by human activity. This is partly because humans scare away or kills potential hosts, and partly because of destruction of the vegetation forming the flies' habitat, associated with agricultural development. The expansion of the road network is one of the arguments on how humans have had an effect on the distribution and abundance of

tsetse fly (Jordan, 1986). The road network resulted in movement of local populations to newly established villages near commercial routes. Accompanying agricultural development may also have produced a drier climate and reduced shade, creating an unsuitable environment for tsetse fly (Popham, 1972).

Generally, *G. m. submorsitans* was thought to occur in areas with human population densities ranging from 0 to 15km⁻², occasionally in areas of 15-40km⁻² but never when the population exceeds 40km⁻² (Nash, 1948). In contrast to Nash's conclusion, *G. m. submorsitans* was found in 12 out of 19 districts in The Gambia with an average of more than 40 people km⁻² (Rawlings *et al.*, 1993). Distribution of *G. m. submorsitans* in The Gambia was associated with the presence of warthogs, no flies being found where warthogs were absent. Climate and vegetation in the Gambia are mediated by the river running the length of the country, which may result in conditions under which tsetse fly can survive at human population densities, which, in other localities, would result in their disappearance. Study showed that *palpalis* group flies, particularly *G. tachinoides*, are much less affected by human settlement, possibly because they are more able to adapt from a presence for feeding on wild mammals and reptiles to feeding mainly on humans and their domestic animals (Jordan, 1989).

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The Wabe and Walga Rivers are tributaries of Ghibe (Upper Omo) River, about 185 km southwest of Addis Ababa, found in Abeshge district (woreda), which is one of the districts of Gurage zone in Southern Nation, Nationalities and Peoples Region (SNNPR). As indicated in figures 1-3, Abeshge district is found at the extreme west of the zone and located between latitudes 8⁰30'N to 9⁰25'N and longitudes 37⁰45'E to 38⁰00'E (SNNPR Statistics and Demography Office, 2004; Ethiopian Mapping Agency, 1975).

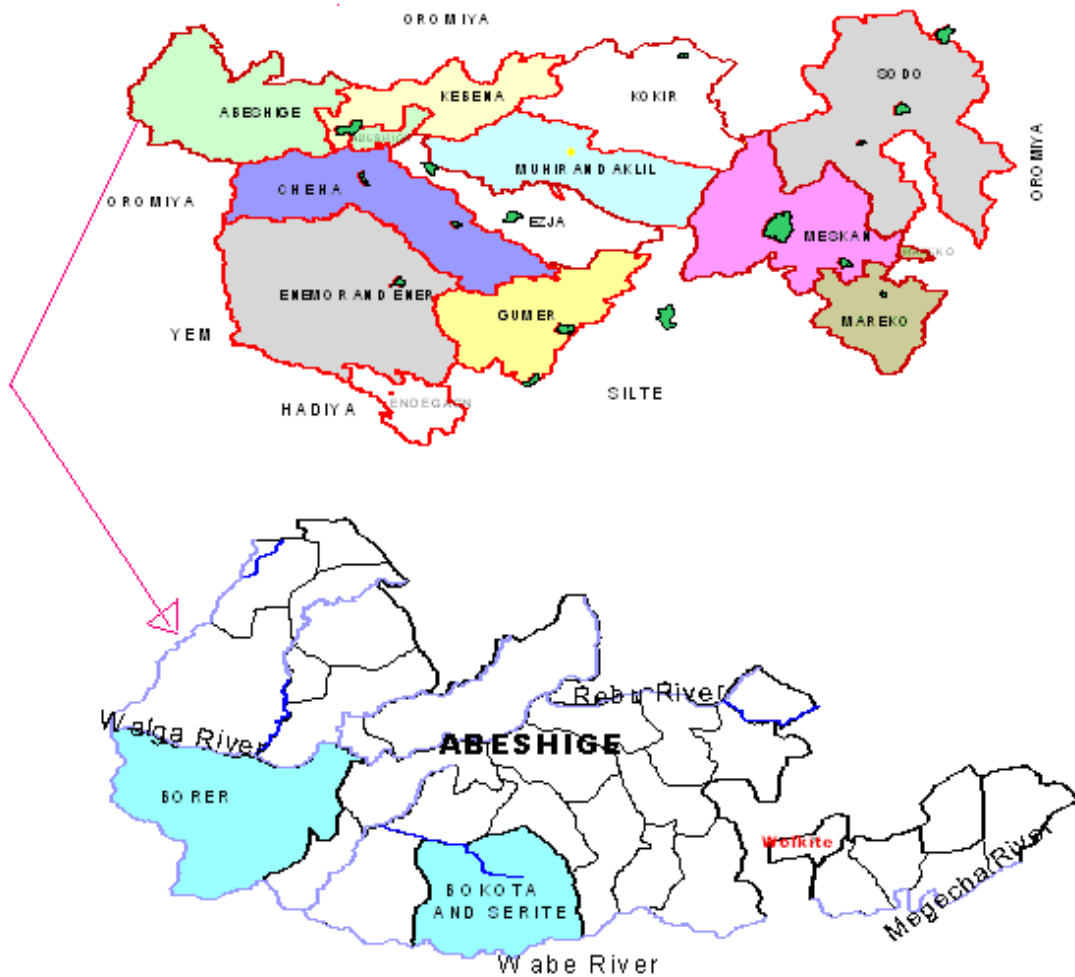
The total area of the district is about 615.6 km² with a population of about 82063 and the population density is assumed to be 133 km⁻², and its altitude ranges from 1060 to 1880 m.a.s.l. Population density of the project areas in Wabe and Walga River Systems is about 141 and 57 km⁻², respectively (AWANRDPO, 2004). According to AWANRDPO (2004) the land use of the district includes seasonal (annual) field crops 18637 ha, permanent (perennial) crops 36545 ha, forest and bush land 2073.5 ha, area occupied by construction (village) 1919.25 ha, grazing land 2391 ha, and others 27924 ha whereas the remaining 3111.75 ha are potentially cultivable and 1848.5 ha are uncultivable land.

Farmers in the district are principally engaged in livestock and crop farming activity. Statistical data of AWANRDPO (2004) showed that there are 30013 heads of cattle, 5828 goats, 713 sheep, 25249 poultry and 3219 equines and in the district farmers plough their crop field (land) mainly using draught power.

The mean annual temperature of the district is between 15⁰C and 37⁰C. The mean annual rainfall is 1294.2 mm (National Meteorological Services Agency, 2004). Climatically the district is classified as lowland (10%) and middle highland (90%) (AWANRDPO, 2004).

The natural vegetation in the study area is characterized by riverine vegetation, bushy-grass land and open grassland, Pratt *et al.*, 1966 (Fig. 4-7). The grass species include *Hyperrhenia* spp and the dominant tree species include *Acacia* spp and *Combretum* spp. One can find livestock in all vegetation types.

From observation and interviews with local people, the following are large wild mammals found in the study area: in Wabe River System: bush pig, warthog, bushbuck, Anubis baboon, and hyena; in Walga River System: warthog, Anubis baboon, bush pig, hyena, lion, hyrax, and porcupine.



Map of Gurage Zone & Abeshige Woreda

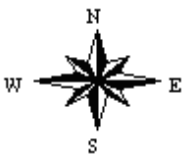
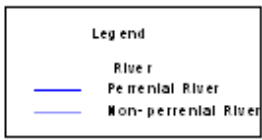


Figure 1. Map of Gurage zone and Abeshige woreda (Source: SNNPR Statistics and Demography Office, 2004).

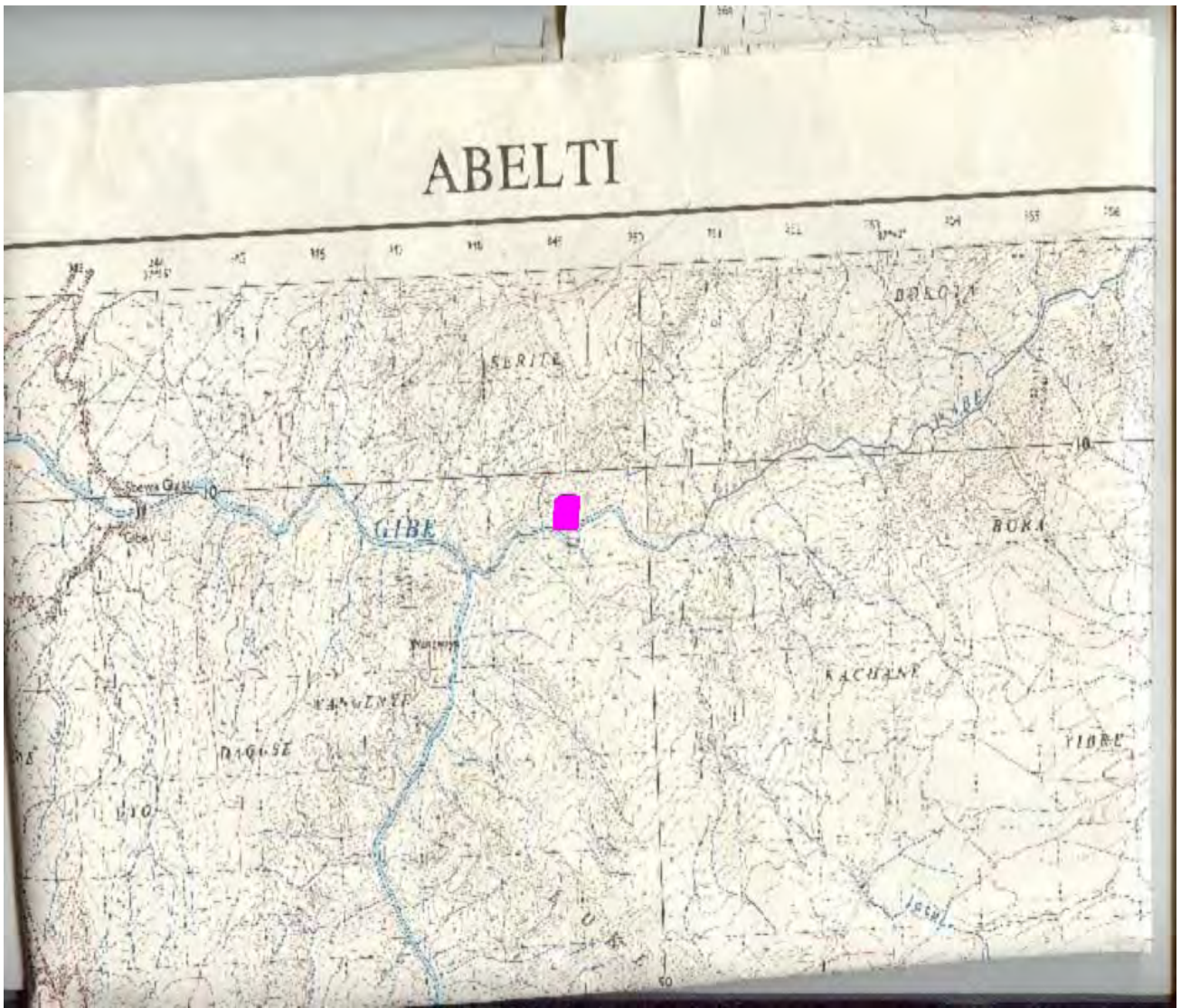


Figure 2. Map of the Study Site (Serite, Wabe River System) (Source: Ethiopian Mapping Agency, 1975).

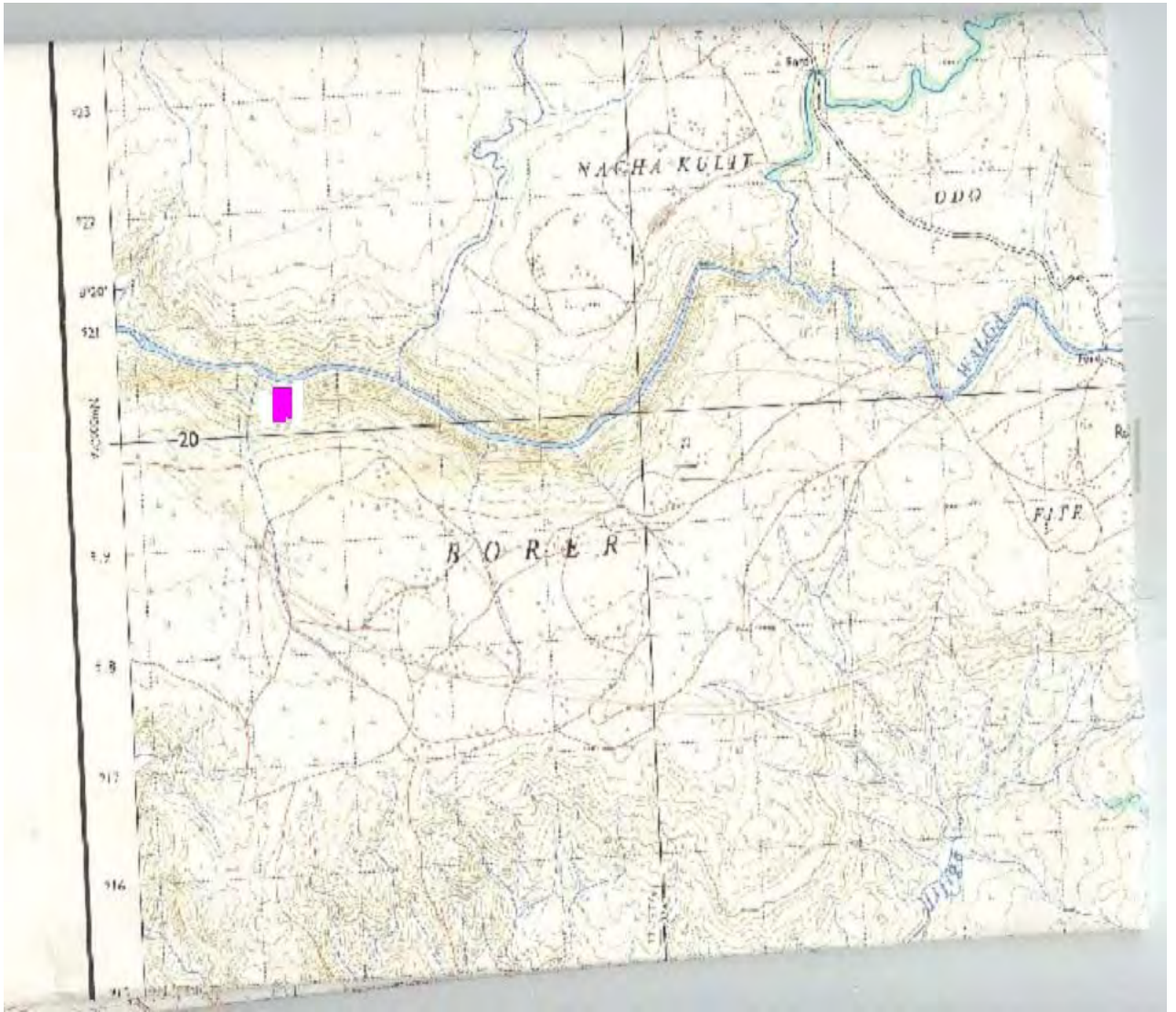


Figure 3. Map of the Study Site (Borer, Walga River System) (Source: Ethiopian Mapping Agency, 1975).



Figure 4. Trap deploying in open grassland (Borer, Walga River System)





Figure 5. Trap deploying in bushy grassland vegetation at Borer, Walga River System (top) and at Serite, Wabe River System (bottom).



Figure 6. Bushy grassland vegetation (top) and Riverine vegetation (bottom) at Borer, Walga River System.



Figure 7. Typical Riverine vegetation at Borer, Walga River System (top) and Serite, Wabe River System (bottom).

3.2 Trap Deploying and Sampling Procedures

Tsetse fly sampling was carried out in December-2004, March-2005, August-2005, and September-2005 for a period of 4 days in each month and in both study areas. Preliminary survey was carried out in August 2004 to select study sites and determine the field experimental layout in the area.

Species composition and monthly fly densities in the study site were determined by using biconical flytraps baited with cow urine following the methods of Owaga (1985). In each study site twelve biconical traps at 200 m interval were set along a transect 2.4 km long and Owaga and Challier (1985) sampling techniques were employed. The transect was laid down to pass through the three vegetation zones and set to include riverine, bushy grassland and open grassland vegetation types. At Borer (Walga River System) the transect was laid at latitudes $08^{\circ}19'N$ to $08^{\circ}20'N$ and longitudes $037^{\circ}32'E$ to $037^{\circ}33'E$, while that of Serite site (Wabe River System) was laid at latitudes $08^{\circ}15'N$ to $08^{\circ}16'N$ and longitudes $037^{\circ}37'E$ to $037^{\circ}39'E$. Collection was made twice a day, before 6:00 and after 18:00 hrs.

Diel activity pattern of tsetse flies in the study area was determined by using 12 biconical flytraps baited with cow urine for 12 hrs daytime in December 2004. Collection of flies was made by removing and examining cages at an hourly interval from 6:00 to 18:00 hrs during the daytime for four consecutive days in December. Diel activity pattern was expressed as average number of flies/trap/day for the 12 hr duration (Vanderplank, 1941).

3.3 Species Identification and Age Determination

The identification of the species of tsetse flies collected in the study area was done based on Mulligan (1970) and Jordan (1993). The distinguishing morphological features used in the identification of tsetse fly to the species level include color of dorsal surface of abdomen, color of hind tarsal segment and color of front tarsal segment (Annex 3a). The sex of collected tsetse flies was determined based on external morphology of genitalia (Jordan, 1993), (Annex 2).

The age of male flies was determined based on mean wing fray values as described by Jackson (1946), (Annex 3b). A wing was cut off each fly, placed on a slide, mounted and

examined under a low power compound microscope (4X); then wear on the trailing edge of the wing was compared with standard wing fray charts (Jackson, 1946).

3.4 Determination of Infection Rate in Tsetse fly

Dissection of tsetse flies and identification of trypanosome infections in tsetse fly was based on Lloyd and Johnson's technique (1924). Collected live flies were killed with chloroform in test tubes and dissected on a microscope slide after washing in 2% detergent-saline and removing the legs and wings.

Gentle forward traction on the head pulled out the salivary glands from the rest of the body. Using fine needles the proboscis was removed from the head capsule by lowering the basal bulb away from the head and spread out (unzipped) in saline solution and after placing cover slip the mouth parts were examined for trypanosomes using microscope (X 40 objective).

The salivary glands were later drawn through and then the head capsule discarded. The two glands were mounted on a slide in saline solution. After adding a cover slip the glands were examined under the microscope (X 40 objective) for trypanosomes.

Finally using needles or forceps the tip of the abdomen is pulled gently to withdraw the gut. Then the mid gut mounted separately on a compound microscope and examined for trypanosomes.

The type of trypanosome present was usually determined solely according to the locations within the fly in which they are found. Thus an infection in the proboscis alone is considered to be '*T. vivax*', infections in the proboscis and midgut '*T. congolense*', and an infection of the proboscis, midgut and salivary glands as '*T. brucei*' (Lloyd and Johnson, 1924).

3.5 Trypanosome Incidence in Cattle

To determine incidence of trypanosome infection in cattle, Buffy coat examination and packed cell volume (PCV) evaluation were the diagnostic techniques used (Murray *et al.*, 1977). Blood was collected in standard capillary tubes from 50 cattle (one ox per head farmer) at each study site by pricking the ear vein of cattle with sterile needle. The ends of capillary tubes were then sealed with putty and the sample in the tubes were centrifuged in a

micro-haematocrit centrifuge at 9000 rpm for 5-minutes. Following centrifugation, the PCV value of each sample was determined and recorded, then the Buffy coat layer was removed and examined on a slide under a compound microscope with 40X magnification to determine infection and identify trypanosome parasites.

The presence of trypanosomes in a wet preparation can be readily detected by the agitation they cause, and different species can be identified from their specific movement patterns (Hoare, 1970). *T. vivax* moves rapidly across the field of view in a lively manner, whereas *T. congolense* appears to be stuck to a red blood cell, occasionally moving in a sluggish manner to another red blood cell. *T. brucei* moves more freely than *T. congolense*, but much more slowly than *T. vivax*, and in a less directed manner, often going round in circles.

3.6 Data Analysis

Data on species composition, monthly variations in sex ratio, mean fly density with respect to vegetation type, season and altitude, variation in age distribution of male flies, infection rate in flies and incidence of trypanosomes in cattle were statistically tested for significance using Chi-square (χ^2) test and difference in mean PCV among infected and uninfected cattle was statistically tested for significance using t- test of SPSS package (SPSS 14.0).

4. RESULTS

4.1 Species Composition and Number of Flies in each Sex at Walga River System

Identification of a total of 438 adult tsetse flies collected during the study period revealed that two species (*Glossina morsitans* and *G. pallidipes*) were present in the Walga River System (Tables 1 and 2). *G. morsitans* comprised 45.2%, while *G. pallidipes* was 54.8%. Sex wise 36.4% of the *G. morsitans* collected were males, while 63.6% were females (Table 1). Similarly, 38.3% of the *G. pallidipes* collected were males and 61.7% were females (Table 2). There was no significant difference in proportion of male and female flies along seasons for both species ($\chi^2=0.436$, $0.90 < p < 0.95$ for *G. morsitans* and $\chi^2=5.974$, $0.10 < P < 0.25$ for *G. pallidipes*).

Table 1. Monthly variation in the number of male and female *G. morsitans* trapped at Walga River System in 2004/2005.

Number of	Months				
	Mar	Aug	Sep	Dec	Total
Male	11	14	22	25	72
Female	17	29	36	44	126
Total	28	43	58	69	198

$$\chi^2=0.436, 0.90 < P < 0.95, DF=3$$

Table 2. Monthly variation in the number of male and female *G. pallidipes* trapped at Walga River System in 2004/2005.

Number of	Months				
	Mar	Aug	Sep	Dec	Total
Male	9	27	33	23	92
Female	15	38	37	58	148
Total	24	65	70	81	240

$$\chi^2=5.974, 0.10 < P < 0.25, DF=3$$

4.2 Tsetse fly Population Density in Different Vegetations /Habitats at Walga River System

G. morsitans were recorded (collected) more in riverine vegetation and population density of *G. morsitans* varied significantly in the three vegetation types, with open grassland the least proportion. Also the proportion of *G. pallidipes* varied significantly and in decreasing order of density the habitats were bushy grassland, riverine and open grassland (Tables 3).

Table 3. Population density of *G. morsitans* and *G. pallidipes* in different vegetation types at Walga River System in 2004/2005.

Number of flies/trap/day	Vegetation type					
	Riverine	Bushy grassland	Open grassland	χ^2 (chi-square)	P-value	DF
<i>G. morsitans</i>	1.34	0.94	0.81	9.576	<0.01	2
<i>G. pallidipes</i>	1.30	1.48	0.97	6.975	<0.05	2

4.3 Tsetse fly Population Density in Different Seasons at Walga River System

Through out the seasons, population density of both *G. morsitans* and *G. pallidipes* varied significantly (Table 4). Population density of *G. pallidipes* was slightly larger than that of *G. morsitans*; they comprised 54.8% and 45.2% of the total fly catch, respectively. Table 4 showed that on the average the lowest and highest tsetse fly density was recorded in March (0.54) and in December (1.57), respectively, August (1.13) and September (1.34) in between.

Table 4. Population density of *G. morsitans* and *G. pallidipes* in different months at Walga River System in 2004/2005.

Number of flies/trap/day	Months						
	Mar	Aug	Sep	Dec	χ^2 (chi-square)	P-value	DF
<i>G. morsitans</i>	0.58	0.90	1.21	1.44	19.333	<0.005	3
<i>G. pallidipes</i>	0.50	1.35	1.46	1.69	31.033	<0.005	3

4.4 Altitudinal Distribution of Tsetse fly at Walga River System

Altitudinal distribution of both species of tsetse fly varied significantly ($P < 0.001$) and both species prevailed distribution at altitude less than 1500m (Table 5).

Table 5. Density of *G. morsitans* and *G. pallidipes* at Walga River System at different altitude range in 2004/2005.

Number of flies/trap/day	Altitude range						
	1451-1475	1476-1500	1501-1525	1526-1550	χ^2 (chi-square)	<i>P-value</i>	<i>DF</i>
<i>G. morsitans</i>	0.88	1.72	0.48	0.03	127.293	<0.001	3
<i>G. pallidipes</i>	1.08	2.08	0.59	-	58.675	<0.001	2

4.5 Age Structure of Tsetse fly at Walga River System

In Walga River System, except in March the age structure of male tsetse fly was not significantly vary with season and there was no significant difference in the number of male flies in each age group among different seasons; except for age category VI and III in *G. morsitans* and *G. pallidipes*, respectively. But, 48 (68.6%) and 74 (78.7%) of the male *G. morsitans* and *G. pallidipes*, respectively were in age category-III or more (Table 6).

Table 6. Wing fray category data for collected male flies.

Species of tsetse	Wing fray category	Number of males in each category				χ^2 , P-value, (DF=3)
		Mar	Aug	Sep	Dec	
<i>G. morsitans</i>	I	4	2	2	4	1.333, P<0.50
	II	-	4	3	3	3.600, P<0.25
	III	-	2	5	2	5.667, P<0.10
	IV	5	3	6	2	2.500, P<0.25
	V	-	2	4	5	5.250, P<0.10
	VI	2	1	2	7	7.333, P<0.05
	χ^2 , P-value (DF=5)	13.570, P<0.01	2.289, P<0.75	3.633, P<0.50	4.905, P<0.25	
<i>G. pallidipes</i>	I	-	3	3	2	3.000, P<0.25
	II	-	5	5	2	6.000, P<0.10
	III	-	6	7	7	6.800, P<0.05
	IV	3	4	7	6	2.000, P<0.50
	V	6	5	5	4	0.400, P<0.90
	VI	-	4	6	4	5.429, P<0.10
	χ^2 , P-value (DF=5)	21.000, P<0.001	1.220, P<0.90	2.091, P<0.75	4.917, P<0.25	

4.6 Activity Pattern (Diel Activity) of Tsetse flies at Walga River System

The diel (diurnal) activity of tsetse flies in the Walga River System showed two peaks for both species. The first peak occurred from 7: 00 to 8:00 hours, while the second occurred from 16:00 to 18:00 hours. The largest numbers were collected at 17:00 to 18:00 hours (Fig. 8).

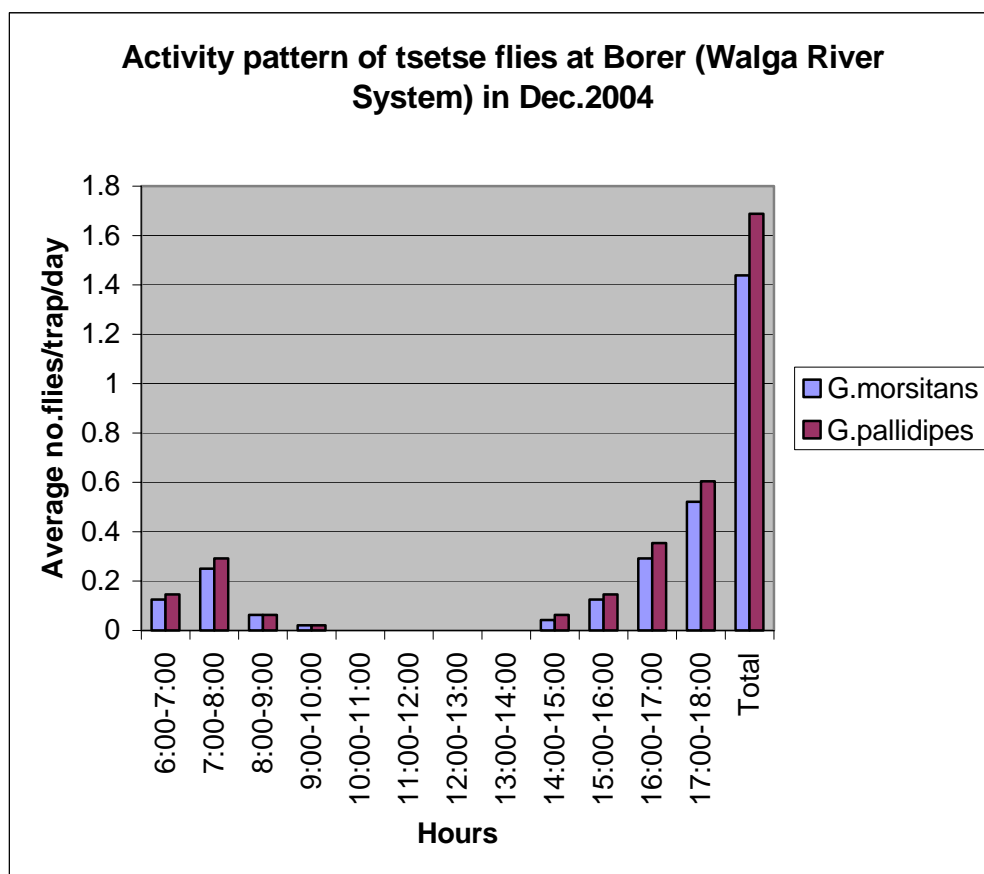


Figure 8. Activity pattern of tsetse flies at Borer (Walga River System) in December 2004

4.7 Trypanosome Infection Rate in Tsetse flies at Walga River System

Infection of tsetse flies (in Walga River System) with *Trypanosoma* spp was determined by dissection of 85 *G. morsitans* and 102 *G. pallidipes*. Of the 85 *G. morsitans* examined 21 (24.7%) and of the 102 *G. pallidipes* 27 (26.5%) were found infected with *Trypanosoma congolense*. There was no significant difference between the proportion of infected *G. morsitans* and *G. pallidipes* ($P > 0.05$) (Table 7).

4.8 Trypanosome Incidence in Cattle

Trypanosome incidence in cattle at both Walga and Wabe River Systems was determined by concentrated method (Buffy coat examination) of blood taken from 50 cattle at each sites. Of the 50 cattle examined at Borer (Walga River System), 12 (24%) were found positive for *Trypanosoma* spp, of which *T. vivax* and *T. congolense* comprised 25% and 75% of the infection, respectively. In Serite (Wabe River System), of the 50 cattle, 5 (10%) were found

infected with *T. congolense*. And the difference between proportions of infected cattle at the two sites was not significant ($P>0.05$) (Table 8).

PCV value, which indicated the state of anemia, is shown in Table 9.

Table 7. Infection rate in *G. morsitans* and *G. pallidipes* at Walga River System in 2004/2005 (number in parenthesis is percentage).

Number of tsetse flies	Species of tsetse fly		
	<i>G. morsitans</i>	<i>G. pallidipes</i>	Total
Infected	21 (24.7)	27 (26.5)	48 (25.7)
Uninfected	64 (75.3)	75 (73.5)	139 (74.3)
Total	85	102	187

$$\chi^2 = 0.011, 0.90 < P < 0.95, DF = 1$$

Table 8. Trypanosome incidence rate in cattle at Serite (Wabe River System) and Borer (Walga River System) in May 2005 (number in parenthesis is percentage).

Number of cattle	Location/Site		
	Serite (Wabe)	Borer (Walga)	Total
Infected	5 (10)	12 (24)	17 (17)
Uninfected	45 (90)	38 (76)	83 (83)
Total	50	50	100

$$\chi^2 = 2.551, 0.10 < P < 0.25, DF = 1$$

Table 9. Mean PCV of infected and uninfected cattle in study sites. Data are (Mean± SE)

Study site/Area	Mean PCV of infected cattle	Mean PCV of uninfected cattle	Significance at DF in parenthesis ($\alpha=5\%$)
Borer (Walga)	21.50±1.23	23.11±0.72	No significant (11)
Serite (Wabe)	21.80±1.65	26.04±0.65	No significant (4)

But at each site the proportion of negative cattle was significantly higher than the proportion of positive cattle at 5% level of confidence (Table 8).

5. DISCUSSION

In Walga River System both *G. pallidipes* and *G. morsitans* and one female and one male *G. fuscipes* were recorded. The present finding agrees with Langridge (1976) and Peregrine (1994) because there is close contact between the Ghibe and Walga River System and there is possibility of distribution of tsetse flies from the Ghibe River Valley to this tributary. The Ghibe River System, which has a direct contact with the Wabe and Walga Rivers (tributaries of Ghibe), is also known to harbor *G. pallidipes*, *G. fuscipes* and *G. morsitans* (Peregrine *et al.* 1994). Only 2 *G. fuscipes* was recorded in Walga River System; this might be brought by wind from the Ghibe River, usually this species is more restricted to large rivers such as Ghibe that harbor the favorable hosts (reptiles such as crocodiles). This species was less advanced to the tributaries might be because these rivers did not harbor the preferred hosts.

Even if traps were deployed in Wabe River System with similar vegetation, altitude range and study period of the Walga River System, no any tsetse fly was detected (entered the traps). But, trypanosome incidence rate in cattle was carried out and the result had been described in section 4.8. Even if it is one of the tributaries of Ghibe, no tsetse fly was recorded in Serite, Wabe River System. This might be due to the fact that Wabe River System was more disturbed than Walga River System and the human population density is 141 km⁻² and 57 km⁻² in the former and later areas, respectively. The second possible reason was that tsetse fly density might be too low to detect. The higher number of female *G. morsitans* (63.64%) and *G. pallidipes* (61.67%) caught at Borer (Walga River System) may be explained in relation to the fact that, the longevity of female tsetse flies is more on the average, than that of male tsetse flies (FAO, 1982 and Abebe *et al.*, 2005).

The study shows that, in Walga River System the distribution of *G. pallidipes* and *G. morsitans* in riverine and open grassland vegetation was almost similar in wet season, but as the dry season progresses fly distribution was almost restricted to the riverine vegetation. This showed that in wet and early dry season both riverine and open grassland habitats and at the end of the dry season or beginning of the rains riverine habitats were the best breeding grounds for the flies (this agrees with Nash, 1948). *G. pallidipes* was collected more from bushy grassland whereas *G. morsitans* from riverine vegetation; this agrees with Leak (1999), that *G. pallidipes* is more mobile species than *G. morsitans*.

Surveys in Walga River System showed that density of both species declined as the dry season progressed. This agrees with the previous work of Jordan (1965) in the northern Guinea Savanna zone of Nigeria. Here the climatic factor precipitation had seem more important factor than temperature; because average precipitation in early dry season (Nov - Dec), 30.9m.m was significantly higher than in late dry season (Jan - March), 13.4m.m; whereas average temperature in early dry season (32.15°C) was almost similar with late dry season (33.2⁰C) (Annex 1). Precipitation is known to affect puparial development directly and the eco-distribution of the fly and the mammalian hosts indirectly. The indirect effect is associated with the impact of precipitation on vegetation (Mulligan, 1970; Ford, 1971; FAO, 1982).

As the dry season progressed the distribution of tsetse flies in open grassland became less and less. In addition to climatic factors that affect vegetation growth, to my view the following factors might have some contribution:

- (i) the sorghum and maize fields which were adjacent to the open grass land , that might harbor some flies were harvested and cleared,
- (ii) in dry season livestock entered the grassland for grazing and the ground became more disturbed and unsuitable for flies to breed and rest,
- (iii) in dry season livestock reached the river for watering and flies have more chance to get and feed on livestock, then flight frequency to open grassland in search of vertebrate host might be reduced quite substantially.

In the present study traps were deployed at altitudes ranging from 1451 to 1836m in both river systems. But flies were found and collected in Walga River System only. Both species of tsetse fly prevailed altitude below 1500m. No tsetse fly detected (collected) above 1525m. According to Langridge (1976) the altitude limits for *G. morsitans* and *G. pallidipes* in Ethiopia is 1650m and 1700m, respectively, and Getachew (1983) reported *G. morsitans* in Fincha River Valley at altitude as high as 2200m. To my view the decrease in height limit might be due to: as altitude increased human population density increased and encroachment to fly's habitat could lead to disturbance (flies loose the ground or vegetation to breed and rest) and might forced the flies to limit their distribution in lower altitudes.

The age structure of male flies point out that both species of flies were in epidemiologically dangerous stages; because 68.6% of *G. morsitans* and 78.7% of *G. pallidipes* males were in age category-III or more and the longer an infected fly survives after becoming infective, the greater is its potential for transmission of trypanosomes (Leak and Rowlands, 1997); i.e. the risk of infection in the flies was high. To reach the infective stage trypanosome need development within the fly and usually takes 12-17 days and 5-13days in the case of *T. congolense* and *T. vivax*, respectively (Lambrecht, 1980). Comparatively *G. pallidipes* has more chance to play vector role than *G. morsitans*. In this study the age of female flies was not incorporated, because it demands expertise knowledge and could not be worked out.

In Walga River System the pattern of the diurnal (diel) activity of *G. morsitans* and *G. pallidipes* showed a biphasic-shape with a high early morning (07:00-08:00 hrs) and a late afternoon peak (17:00-18:00 hrs). And activity was too low in 10:00 to 13:00 hrs of the day (Fig 9). The activity of tsetse fly coincides with the activity of hosts (Nash, 1969). In the study area natural hosts were active at early morning and late afternoon; moreover, livestock passed the fly zone (habitat) to waterholes usually after 13:00 hr. In addition to host, light intensity and temperature were considered to affect the activity of tsetse flies. Most tsetse flies stop activity as darkness approaches (Jaenson, 1978). On the other hand, high temperatures of about 30°C and above seem to have a pronounced effect in reducing the mid day activity of *G. pallidipes* and *G. morsitans* in Walga River System, as has been observed by Jaenson (1978) for *G. pallidipes*.

The infection rate observed in the present study in *G. morsitans* and *G. pallidipes* was high, 24.7% and 26.5%, respectively and only *T. congolense* infection was found. This was slightly high compared with the infection rate reported for morsitans group in other places, which is normally in the range of 15-20% (Mulligan, 1970 and FAO, 1982) and quite high compared with 0.5% for *G. morsitans* in Fincha River Valley (Getachew, 1983). The infection rate in flies might not be representative of the population, as the number of dissected flies was not large enough, specially compared with Mulligan, 1970. The infection rate for tsetse fly in Serite (Wabe River System) could not be worked out (no any tsetse fly catch).

Trypanosome incidence rate in cattle was 10% and 24% at Serite and Borer, respectively. And relatively low compared with 17-35% and 33% incidence rate reported from Ethiopia by

Langridge (1976) and Getachew (1983), respectively. Those two sites showed no significant difference in proportion of infected cattle. The infection in cattle in both sites was caused mainly due to *T. congolense*; but in Borer 25% of the infection was found due to *T. vivax*, and this agrees with Wilson *et al.* (1974); reported that in East Africa *T. vivax* is generally less virulent than *T. congolense* and consequently cattle develop a tolerance to the former more easily than the latter.

The mean PCV value indicated that, even if the proportion of negative cattle was significantly higher than the proportion of positive cattle in both sites, but still there was no significant difference between the mean PCV of negative and positive cattle at both sites; this differs from what observed and reported by Rowlands *et al.*, 1993, at Ghibe valley, southwest Ethiopia.

The proportion of negative animals but became (grouped) anemic also confirmed the above, i.e. 60% and 79 % of the negative cattle at Serite and Borer, respectively were found anemic (with PCV less than 26). Cattle negative to the parasite but anemic might happen due to farmers give (inject) prophylactic and trypanocidal drugs to their cattle in both sites and the real infection rate would be more than observed.

Despite no tsetse fly was detected in Serite (Wabe River System) cattle positive for *T. congolense* were found which its transmission is reported mainly cyclical (Langridge, 1976). This could be either tsetse fly density was too low to detect or cattle had brought the infection from another area, specially at dry season cattle of Serite site usually graze in lower escarpment of Ghibe Valley which reported rich in *G. pallidipes* and *G. morsitans* (Peregrine *et al.*, 1994).

6. CONCLUSION

The present investigation has revealed the di-species composition of the Walga River System (specially the lower escarpment). Also there is negative correlation of human population density and intensity of encroachment with tsetse fly abundance. Altitude limits the pattern of distribution with respect to habitat in addition to seasons. This information could serve as vital input for control strategies/programs to determine when, where to launch and to expend minimum effort.

Furthermore, the bi-phasic (early in the morning and late afternoon) diurnal periodicity of the vector could be a relevant knowledge in indicating appropriate times of application of odor-attractants and insecticides. In addition this information could be helpful to determine when to drive cattle to water points and if possible to prepare and supply alternative water source that aimed to reduce fly-cattle contact.

The age structure and density of tsetse flies could be used as an input for further studies and would help to predict the future fly-population trends and risk of infection.

The infection rate in fly indicated that both *G. morsitans* and *G. pallidipes* as important vectors of trypanosomiasis in Walga River System; then reducing/controlling flies or cattle-fly contact could mean reducing the rate of cattle infection (besides treatment of cattle for trypanosomiasis).

The incidence rate in cattle and PCV analysis exhibited that cattle are highly affected by trypanosomiasis and deserve treatment. Despite farmers were treating their cattle the situation was not good. This lead us to question the potency of both prophylactic and trypanocidal drugs.

Once the details on tsetse fly ecology such as wild and alternative hosts, species diversity, density, abundance and behavior; and the risk of trypanosomiasis in a given area is determined, so environmentally sound trypanosomiasis and tsetse fly control and development strategies can easily be designed and practiced in tsetse fly and trypanosomiasis infested areas.

7. RECOMMENDATIONS

- In order to make the work more complete a year round ecological and entomological data must be developed. Moreover socioeconomic situation and the effect of human population density on tsetse fly and trypanosomiasis in the river systems have to be assessed.
- The trypanosomiasis situation in the study areas also demands to construct animal health station/post and to design mechanisms to supply appropriate drugs. Here inappropriate treatment together with drug-resistance would become great obstacle for livestock-based agricultural activity and development.
- Another area that needs attention is developing and organizing the history of cattle.
- Supplying (developing) alternative water source, to reduce fly-cattle contact.
- As dry season progresses tsetse flies retreat to riverine vegetation (habitat), then more effort needs to be employed to this habitat in control programmes.
- As much as possible farmers should not drive their cattle to water points in early morning and late afternoon (in dry season).
- Odor attractants and insecticides would be more effective if applied early in the morning and late afternoon.
- It is also important to integrate the out comes of the study with infrastructure of the area such as, as dry season progresses it is not only the fly density decreases and concentrated in riverine vegetation, but also this season is most suitable to have logistic and transport access to the sites.
- From my personal contact, there is high in and out of livestock (oxen) in the study area; then a sort of quarantine measure is recommended.

REFERENCES

- Abebe Hailemarim, Emiru Seyoum and Mark, V. (2005). Studies on the reproductive status, catch and age composition of the tsetse fly, *Glossina pallidipes* populations in the Nechsar National Park in Southern Ethiopia. *SINET: Ethiop. J. Sci.* **28(1)**: 51-60.
- Allsopp, R. (1997). Implementation of odor-bait techniques for the control of tsetse flies in eastern and southern Africa. *24th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*.
- AWNARDPO (2004). Statistical Information. Abeshge Woreda Agriculture and Natural Resource Development and Protection Office, Ethiopia (unpublished).
- Balis, J. and Bergeon, A. (1970). Etude sommaire de la repartition des Glossines dans l'empire d'Ethiopie. *Revue d'elevage et de Medicine veterinaire des Pays tropicaux* **23**:181-187.
- Bursell, E. (1959). The water balance of tsetse flies. *Transactions of the Royal Entomological Society of London* **111**:181-187.
- Bursell, E. (1960). Loss of water by excretion and defecation in the tsetse fly. *Journal of Experimental Biology* **37**:689-697.
- Buxton, P.A. (1955). *The Natural History of Tsetse flies*. Memoirs of the London School of Hygiene and Tropical Medicine, 10, Lewis, London.
- Carter, R.M. (1906). Tsetse fly in Arabia. *British Medical Journal* **2**:1393-1394
- Clausen, P. H., Sidibe, I., and Bauer, B. (1992). Development of multiple drug resistance of *Trypanosoma congolense* in zebu cattle under high natural tsetse fly challenge in the pastoral zone of Somorogouan, Burkina Faso. *Acta Tropica* **51**:229-236.

- Curtis, C. F. (1968). A possible genetic method for the control of insect pests with special reference to tsetse flies (*Glossina* spp). *Bulletin of Entomological Research* **57**:509-523.
- D'Ieteren, G.D.M., Coulibaly, L., Aste, P.A., Hecker, P.A.Krebs,H.A., Rawlands,G.J. Leak, S. G.A. and Nagda, S.M. (1997). Trypanocidal drug resistance in four regions of Cote d'Ivoire. Importance and possible impact on sustainability of integrated strategies for trypanosomiasis control. *In Proc. 23rd Meeting of the International Scientific Council for Trypanosomiasis Research and Control*, Banjul, the Gambia. Organization of African Unity. Scientific, Technical and Research Commission, Nairobi, Kenya.
- Ethiopian Mapping Agency (1975). *Map of Abelti*.
- Ethiopian Mapping Agency (1975). *Map of Tawula*.
- Elsen, P., Amoudi, M.A. and Leclercq, M. (1990). First record of *G. fuscipes* Newstead, 1910 and *G. morsitans submorsitans* Newstead, 1910 in south-western Saudi Arabia. *Annals of Tropical Medicine and Parasitology* **70**:281-287.
- FAO (1982). Tsetse fly Control Training Manual. **In:** *Ecology and Behavior of Tsetse fly*, pp. 90-101, (Pollock, J.N. ed.). Food and Agriculture Organization of the United Nations, Rome.
- Ford, J. (1962). Microclimates of tsetse fly resting sites in the Zambezi valley, southern Rhodesia. 9th meeting of ISCTRC, Conakry (1962).
- Ford, J. (1963). The distribution of the vectors of African pathogenic trypanosomiasis. *Bulletin of The World Health Organization* **28**: 653-669.
- Ford, J. (1970a). Recent information on changes in distribution of tsetse flies. *Joint WHO/FAO African trypanosomiasis information service*. TRYP/INF/70.41, 1-8.

- Ford, J. (1970b). The geographical distribution of *Glossina*. **In:** *The African Trypanosomiasis*, pp.274-297, (Mulligan, H. W., ed.). George Allen and Unwin, London.
- Ford, J. (1971). *The Role of Trypanosomiasis in African Ecology*. Clarendon Press, Oxford, 698pp.
- Ford, J., Makin, M. J. and Grimble, R. J. (1976). *Trypanosomiasis Control Program for Ethiopia*. Clarendon Press, Oxford, 469pp.
- Fuller, G. K. (1978). Distribution of *Glossina* in southwestern Ethiopia. *Bulletin of Entomological Research* **68**:299-302.
- Gardiner, P. R. (1989). Recent studies of the biology of *Trypanosoma vivax*. *Advances in Parasitology* **28**:229-316.
- Geerts, S. and Holmes, P. H. (1998). Drug management and parasite resistance in animal trypanosomiasis in Africa. *In Proc. 24th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, Maputo, Mozambique.
- Getachew Tikubet (1983). Studies on tsetse flies of the Fincha River Valley. *M. Sc. Thesis*, School of Graduate Studies, Addis Ababa University, Ethiopia.
- Getachew Tikubet and Teferi Gemechu (1984). Altitudinal distribution of tsetse fly in the Fincha River Valley, western part of Ethiopia (unpublished).
- Hoare, C. A. (1970). The mammalian trypanosomes of Africa. **In:** *The African Trypanosomiasis*, pp. 3-23, (Mulligan, H. W. ed.). George Allen and Unwin, London.

- Jackson, C.H.N. (1946). Male wing fray degrees. *Bulletin of Entomological Research* **37**:291-292.
- Jaenson, T.G.T. (1978). Reproductive biology of the tsetse fly *Glossina pallidipes* Austen (Diptera: Glossinidae), with special reference to mating behavior. *Acta Universitatis Upsalensis (Abstracts of Uppsala Dissertations from the faculty of Science)* 479:3-35.
- Jordan, A.M. (1965). Observations on the ecology of *G. morsitans submorsitans* in the northern Guinea savanna zone of northern Nigeria. *Bulletin of Entomological Research* **56**:1-17.
- Jordan, A.M. (1986). *Trypanosomiasis Control and African Rural Development*. Longman, London, 357pp.
- Jordan, A.M (1989). Man and changing patterns of the African trypanosomiasis. **In:** *Demography and Vector-borne Diseases*, pp.47-58, (Service, M.W.,ed.). CRC Press, Boca Raton, Florida.
- Jordan, A.M. (1993). Tsetse fly-flies (Glossinidae). **In:** *Medical Insects and Arachnids*, pp.333-388, (Lane, R.P. and Crosskey, R.W. eds). Chapman & Hall, London.
- Lambrechet, F.L. (1980). Ecological and physiological factors in the cyclical transmission of African Trypanosomiasis. *Insect science and its Application* **1**:47-54.
- Langridge, W.P. (1976). *A Tsetse fly and Trypanosomiasis Survey of Ethiopia*. Ministry of Overseas Development Report, MOD, London, 103pp.
- Leak, S.G.A. (1999). *Tsetse fly Biology and Ecology*. CABI Publishing, Wallingford, U.K. 568pp.

- Leak, S.G.A. and Rowlands, G.J. (1997). The dynamics of trypanosome infections in natural populations of tsetse fly (Diptera: Glossinidae); studied using wing-fray and ovarian ageing techniques. *Bulletin of Entomological Research* **87**:273-282.
- Leak, S.G.A. and Woudyalew Mulatu (1993). Advances of *G. m. submorsitans* and *G. pallidipes* along the Ghibe-river system in southwest Ethiopia. *Acta Tropica* **55**:91-95.
- Lloyd, L. and Johnson, W.B. (1924). The trypanosome infections of tsetse flies in northern Nigeria and a new method of estimation. *Bulletin of Entomological Research* **14**:265-288.
- McDowell, R.E. (1977). Ruminant products: more meat than milk. Winrock International Livestock and Training Centre, Marilton, Arkansas.
- Mulligan, H.W. (1970). *The African Trypanosomiasis*. Ministry of Overseas Development and George Allen and Unwin, London, 950pp.
- Murihead-Thomson, R.C. (1968). *Ecology of Insect Vector Populations*. Academic Press, London, pp.8-30.
- Murray, M. and Gray, A.R. (1984). The current situation on animal trypanosomiasis in Africa. *Preventive Veterinary Medicine* **2**:23-30.
- Murray, M., Murray, P.K., and McIntyre, W.I. (1977). An improved parasitological techniques for the diagnosis of African trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.* **71**:325-326.
- Nash, T.A.M. (1933). A statistical analysis of the climatic factors influencing the density of tsetse flies, *G. morsitans* Westw. *Journal of Animal Ecology* **2**:197-203.

- Nash, T.A.M. (1936). The relationship between the maximum temperature and the seasonal longevity of *G. m. submorsitans* Westw., and *G. tachinoides* in Northern Nigeria. *Bulletin of Entomological Research* **27**: 273-279.
- Nash, T.A.M. (1948). *Tsetse flies in British West Africa*. Colonial Office, HMSO, London, pp.1-75.
- Nash, T.A.M. (1960). A review of the African trypanosomiasis problem. *Tropical Diseases Bulletin* **57**:973-1003.
- Nash, T.A.M. (1969). *Africans' Bane: The Tsetse fly*. Collins, London, 244pp.
- National Meteorological Services Agency (2004). Climate of Ethiopia Series, Vol.1, No.1: *Rainfall*. Addis Ababa, Ethiopia, 134pp.
- NTTICC (2001). Annual Report. National Tsetse fly and Trypanosomiasis and Investigation and Control Center, Ministry of Agriculture, Ethiopia, 20pp (unpublished).
- Owaga, M.L.A. (1985). Observations on the efficacy of buffalo urine as a potent olfactory attractant for *Glossina pallidipes* Austen. *Insect Science and its Application* **6**:561-566.
- Owaga, M.L.A. and Challier, A. (1985). Catch composition of the tsetse fly, *Glossina pallidipes* Austen in revolving and stationary traps, with respect to age and sex ratio. *Insect Science and its Application* **6**:711-718.
- Peregrine, A.S., Woudyalew Mulatu, Leak, S.G.A. and Rowlands, G.J. (1994). Epidemiology of bovine trypanosomiasis in the Gibes valley, Ethiopia: multiple drug resistance and its effective control. *Kenya Veterinarian* **18(2)**:369-371.
- Persoons, C.J. (1967). The mechanical transmission of *Trypanosoma congolense* group by *Glossina morsitans* and *Stomoxys*. *East African Trypanosomiasis Research Organization Annual Report* 1966:52-53.

- Pollock, J.N. (1970). Sperm transfer by spermatophores in *G. austeni* Nestea. *Nature* **225**:1063-1064.
- Popham, E.J. (1972). The effects of local agriculture on the distribution of the species of *Glossina* in northern Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **66**:321-323.
- Pratt, D.J., Greenway, P.J. and Gwynne, N.D. (1966). A classification of East African Rangelands. *Journal of Applied Ecology* **3**:369-382.
- Rawlings, P., Ceesay, M.L., Wacher, T.J. and Snow, W.F. (1993). The distribution of the tsetse fly flies *G. m. submorsitans* and *G. palpalis gambiensis* (Diptera: Glossinidae) in The Gambia and the application of survey results to tsetse fly and trypanosomiasis control. *Bulletin of Entomological Research* **83**:625-632.
- Roberts, M.J. (1973a). The control of fertilization in tsetse flies. *Annals of Tropical Medicine and Parasitology* **67**:117-123.
- Roberts, M.J. (1973b). Observations on the function of the charioted and on egg hatching in *Glossina* spp (Diptera: Glossinidae). *Bulletin of Entomological Research* **62**:371-374.
- Robinson, M.W., Baker, P.S. and Finlayson, L.H. (1985). Influence of temperature changes on larviposition rhythm in the tsetse fly *Glossina morsitans*. *Physiological Entomology* **10**:215-220.
- Rowlands, G.J., Woudyalew Mulatu, Authie, A., d'Ieteren, G.D.M., Leak, S.G.A., Nagda, S.M. and Peregrine, A.S (1993). Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia. *Acta Tropica* **53(2)**:135-150.

SNNPR Statistics and Demography Office (2004). Statistical Information. Southern Nation and Nationalities Peoples' Region Statistics and Demography Office, Awassa (unpublished).

Vanderplank, F.L.(1941).Activity of *Glossina pallidipes* and the lunar cycle (Diptera). *Proceedings of the Royal Entomological Society of London* **16**:61-64.

Wilson, A.J.; Powis, J. and Davidson, C.R. (1974). A study in development of infections by different trypanosome species in cattle treated regularly with diminazene aceturate. ISCTRC, 14th Meeting.

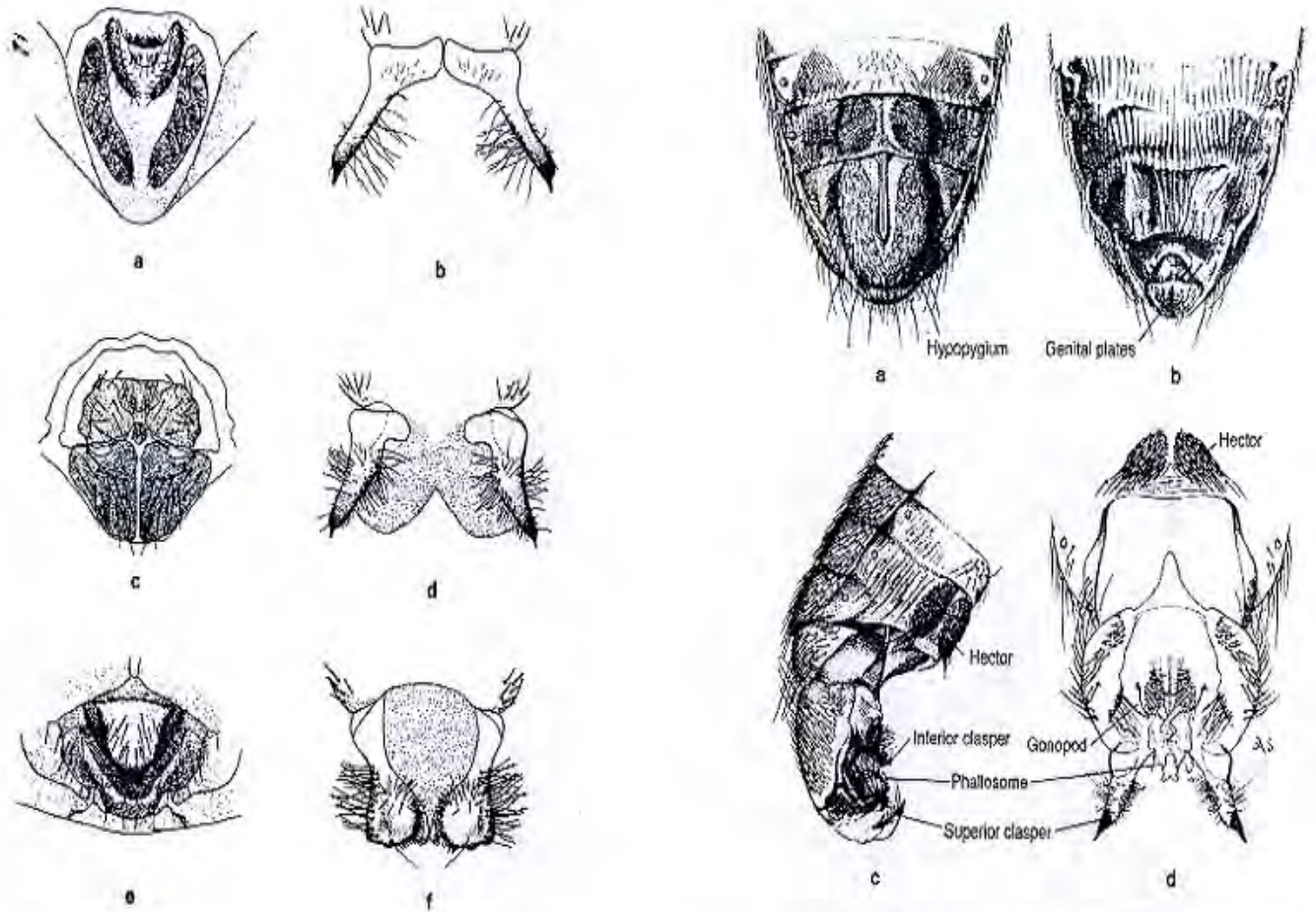
Zdarek, J. and Denlinger, D.L. (1993). Metamorphosis behavior and regulation in tsetse flies (*Glossina* spp) (Diptera:Glossinidae): a review. *Bulletin of Entomological Research* **83**:447-461.

ANNEX

Annex 1. Monthly maximum and minimum temperature (in °C) and rainfall in mm at Yaya Otona station, near the study area (Source: National Meteorological Services Agency, 2004).

MONTH	TEMPERATURE (in °C)		RAINFALL (in mm)
	Minimum	Maximum	
Jan	14.3	30.9	5.2
Feb	13.8	32.9	12.3
Mar	15.3	33.5	22.7
Apr	16.4	31.8	81.4
May	15.3	32.7	29.3
Jun	15.8	27.5	136.4
Jul	15.4	25.9	106.7
Aug	15.0	27.0	183.5
Sep	15.9	29.4	115.4
Oct	11.5	31.1	73.7
Nov	10.5	32.4	39.9
Dec	12.6	31.9	21.9

Annex 2. Genitalia of *Glossina* for subgeneric classification and sex identification (Jordan, 1993)



Distinguishing features of *Glossina* genitalia for subgeneric classification. (a) Female external armature – *fusca* group; (b) male superior claspers – *fusca* group; (c) female external armature – *palpalis* group; (d) male superior claspers – *palpalis* group; (e) female external armature – *morsitans* group; (f) male superior claspers – *morsitans* group.

Genitalia of *Glossina*. (a) Male external armature; (b) female external armature; (c) and (d) male genitalia, side and ventral views.

Annex 3. a) Tsetse species identification-abdominal and hind leg markings (Jordan, 1993).

b) Male tsetse wing fray degrees (Jackson, 1946).

