# SOME ASPECTS OF THE ECOLOGY, BEHAVIOUR AND VECTORIAL CAPACITY OF THE TSETSE FLY *Glossina austeni* Newstead.

BY

**MARY LUDVINE AKOTH OWAGA**

A THESIS SUBMITTED IN FULFILMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN KENYATTA UNIVERSITY

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# DECLARATION

1. I, Mary Ludvine Akoth Owaga, declare that this Thesis is my original work and has not been presented for a degree in any other university.

Signature 1. ..... Date 4th February 1992

We, Dr. R.O.Okelo, of the Department of Zoology, Kenyatta University,

Signature1. ..... Date 4th February 1992

and Dr. M.F.B. Chaudhury of Tsetse Physiology Unit, of the International Centre of Insect Physiology and Ecology [ICIPE]

Signature 2. ..... Date 4 Feb 1992

declare that this thesis has been submitted for examination with our approval as University supervisors.

# DEDICATION

To my family, especially my children, Mark, Genevieve and Martin, and my husband Captain Joseph Owaga.

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# SUMMARY

A study was undertaken in the south coast of Kenya, on the tsetse fly species, *Glossina austeni* Newstead. The objective was to investigate its population ecology, behaviour and vectorial capacity. The main aspects studied were: habitat requirements and density, responses to sampling devices and methods, such as traps and odours, activity pattern, and infection with trypanosome parasites.

Some aspects of the study, such as sampling and activity experiments, evaluation of density, dispersal and habitat requirements, and trypanosome infection rates, were conducted in the field. Others, such as assessment of the effect of temperature and relative humidity on activity and response to odours, and evaluation of the efficiency of *G. austeni* in picking up trypanosome parasites from infective blood meals, and in transmitting them to the next mammalian host, were conducted in the laboratory.

Sampling was carried out using five trap-types, the biconical, the NG2B, the Lancia (original), Lancia (modified) and the 4t traps, to determine whether *G. austeni* could be trapped in sufficient numbers, and which trap might be most suitable for routine sampling. Similarly, five odour sources were tested as baits: buffalo urine, cow urine, acetone, urine derived phenols and dry ice. Period of activity, and activity pattern as related to feeding, were determined by continuous trapping, using traps and stationary vehicle, and by laboratory observation in a flight chamber, where only olfactory, but no visual stimulant, was presented to the flies. To study the density and dispersal of *G. austeni*, the method of mark-release-recapture was used. By this method a sample of tsetse flies

was captured, marked with artist's oil paint, fed and released to mix with the wild population. Afterwards, several samples were taken in traps, and among the flies captured were some of the marked individuals. The locations and dates where the marked flies were captured were noted for the evaluation of dispersal. The density was later assessed using the recapture figures. The interaction between *G. austeni* and trypanosome parasites was studied by the determination of trypanosome infection rates in the field, and the efficiency with which the fly picks up trypanosome parasites from infective blood meals and transmits them to the next host, in the laboratory. These two aspects of the vector-parasite interaction constituted the vectorial capacity of *G. austeni.*

The results of the study show that *G. austeni* occur in relatively low densities in the Muhaka area of the south coast (Kenya), compared to a related but more wide-spread *species G. pallidipes.* Furthermore, the study showed that *G. austeni* was a resident species that did not disperse widely. It showed preference for forest and dense thicket. The evidence was that traps set on the forest floor consistently captured significantly more flies than those set on the fringes of the forest, or in open woodland. The effects of the different odour sources in attracting *G. austeni* to traps were generally poor, except those of dry ice and buffalo urine that increased the catches significantly ($P<0.05$). This poor response to odours was explained by the physical conditions prevailing at the coast, especially constant high relative humidities. The Lancia trap was found to be the most suitable for sampling *G. austeni*, but the other three types used also worked moderately well. The species was shown to be day-active,

flying about between 0600 h and 1830 h, but with peaks of increased activity in the morning (0700 to 1000 h) and in the afternoon 1400 to 1600 h), and a period of very low activity around midday. As regards the vectorial capacity, the evidence showed that *G. austeni* could be an important vector of trypanosome parasites. The rates of infection in the wild flies were higher than in two other *Glossina* species that ecologically co-exist with it in that area, namely, *G. pallidipes* and *G. brevipalpis*. In the laboratory, about 37% of the flies tested picked up infections from infective blood meals, but transmission from those with mature infections was 100%, i.e. all of them transmitted to the next host they fed on. However, about 50% of young (teneral) flies that had the infective feed as their first meal got infected but the infections did not mature and they were not able to transmit the parasites to the next host (hosts) they drew blood meals from. The habit of this species of staying in the forest and dense thickets restricts its importance as a vector to the settlements near such habitats, and to the cattle that are grazed near or in the forest during the dry seasons.

# CHAPTER I

# GENERAL INTRODUCTION

## 1.1 TAXONOMIC REFERENCE OF *Glossina*

Tsetse flies belong to the order Diptera and genus *Glossina*, Wieldeman. This genus was formerly in the family Muscidae (Newstead, 1911; Newstead *et al.* 1924; Patton, 1934;). However, since about 1954, it was placed in a monogeneric family, Glossinidae (Brues *et al.* 1954, Imms, 1957; Haeselbarth *et al.* 1966). Tsetse are of immence economic importance due to the fact that both sexes feed solely on vertabrate blood, and that in the course of their feeding they transmit pathogenic flagellates of the genus *Trypanosoma*, the causative germs of trypanosomiasis or sleeping sickness in humans and nagana in domestic stock. *Trypanosoma* spp. occur in the blood of some of the African wild ungulates, which are the natural hosts of tsetse (Geigy *et al.* 1967; Vanderplank, 1947; Ashcroft, 1959; Geigy *et al.* 1973). Bruce (1895) was the first to prove that trypanosomes are the cause of nagana, that game animals form a reservoir of the disease, and that the tsetse flies transmit it.

## 1.2 SCOURGE OF TSETSE AND TRYPANOSOMIASIS

The scourge of tsetse and trypanosomiasis is believed to be hindering agricultural advancement in over 40% of the African continent

(Buxton, 1955; Glasgow, 1963; Ford and Katondo, 1977), imposing a major constraint on human settlement and livestock production.

The disease incidence extends from latitude 14° North to 29° South of the equator, in 36 African countries, with either continuous or isolated areas of infestation in some regions (Ford, 1970; Anonymous, 1974; Kuzoe, 1991). The most notable effect of animal trypanosomiasis is the enormous losses in livestock which lead to shortage of dairy products and animal protein, as well as shortage of natural fertilizer for crop production.

The human form of the disease affects about 25,000 people each year (new cases), according to WHO estimates (Kuzoe, 1991), and poses a serious health problem to about 50 million people. The disease is potentially fatal. In its chronic form, the disease results in very poor health and low industrial and agricultural output, (Ford, 1971). This chronic form is found mainly in West and Central Africa. The Eastern African form, caused by *T.brucei rhodisiense*, is more virulent and kills faster as compared to its West and Central African counterpart, caused by *T. b. gambiense*. Trypanosomiasis has caused depopulation in the past, through death and migration of people to less infested areas, resulting in large areas being relinquished to tsetse and wild animals (Cockbill *et al.* 1963, Nash, 1969). Few drugs were developed against it around the middle of this century, but no new ones have come forth in the recent past, probably due to reluctance on the part of drug companies to invest in further research in that field.

## 1.3 DISTRIBUTION OF TSETSE FLIES (*Glossina*) IN AFRICA

The genus *Glossina* (plate 1) is presently limited to Africa south of the Sahara (Fig. 1.1), covering an area of about eleven million square km. (Nash, 1969). Outside Africa, four fossil species from Oligocene shales (35-40 million years), at Florrissant in Colorado, North America, were assigned to the genus (Ford, 1970; Glasgow, 1970) and a species believed to be *Glossina tachinoides* was recorded from southern Arabia by Carter (1906); but its continued presence there was never confirmed.

## 1.4 DISPERSAL AND HISTORICAL BACKGROUND OF *Glossina*

A number of authors have proposed theories about the possible dispersal routes in the evolution of *Glossina* (Machado, 1959, Bursell, 1957, and Evans, 1953). Ford (1970) gave a summary derived from a number of early authors such as Jeannel (1942) and Baker (1963). The geographical isolation that was responsible for speciation in tsetse had its origin in the Quaternary period (some 40 million years ago) (Ford, 1970). This lead to its separation into a number of phylogenetic branches, and the three presently recognised groups, *fusca*, *morsitans* and *palpalis* are the survivors of that process. Evans (1953) proposed an explanation for the existence of the Florissant fossils. His suggestion was that the expansion of the genus *Glossina* took place in two directions, during the evolution of the

order Diptera, in the Permian period (200 million years ago), that is, much earlier than the Quaternary period.

At that time the future continents were still joined, their surface being broken only by the inland seas. Diptera are supposed to have appeared in the great northern land mass (Pangaea) that later separated into Eurasia and North America. The early *Glossina* appeared in Central Eurasian landmass, and spread Westwards into the North American landmass (or Laurentia), and so reached and spread beyond the area in which Florissant shales now lie. Another branch spread into what is now Middle east, and into the southern part of the continental landmass Gondwana (later to form Africa). This became separated from the Western branch by a sea (Tethys sea) associated with east-west trough. The movement of the equator was accompanied by shifts in climates, followed by movement of flora and fauna associated with those climates. Evans (1953) thought that the Florissant species belonged to the western dispersal route that moved southwards with the equatorial tropics and entered Gondwana, perhaps in the neighbourhood of the present Gulf of Guinea, before the appearance of the Atlantic trough. Thus the invasion of Gondwana was two pronged, one from the west and the other from the east. The *fusca* group established itself around the Gulf of Guinea from where it spread out. The same general area served as the dispersal center for the *palpalis* group, that had derived from the eastern group and had moved westwards around the southern shores of the Saharan sea. The *morsitans* group arose in eastern Africa after isolation by the formation of the Rift Valley. A further isolation factor was created by a vast lake in what later became the basin of the Congo river.

This contributed to speciation in the original western line of *fusca* group, and the western fork of the eastern line of *palpalis* group.

### 1.4.1 The three main groups of *Glossina*

*Glossina* comprises 30 living species and sub species (Jordan, 1974). These are divided into three groups, distinguishable on the basis of male and female genital armatures (Newstead *et al.* 1924) but whose habitat requirements are also generally quite distinct (Jackson 1945a, 1953). These groups are *fusca*, *palpalis* and *morsitans*. The *fusca* group is believed to be the most primitive, and its members live mostly in the humid rain forest and gallery forest belt, except one member, *G. longipennis*, which has adapted to xerophylic thorny savannah and is now the only tsetse species whose pupa can survive under 0% relative humidity. Most members of the fusca group are relatively large in size, with *G. brevipalpis* having a wing length of 13.5mm, as compared to *G. austeni*'s 8mm. The *palpalis* group are mainly associated with vegetation near open water conditions, that is, lakes and rivers (riverine or reparanian vegetation) while members of the *morsitans* or savannah group are associated with deciduous thicket or savannah woodland, frequently far from permanent water.

The phylogeny of *Glossina* was an evolution, primarily to develop water proofing mechanisms (Bursell, 1958). The *fusca* group are the nearest in structure to the ancestral tsetse, which dwelt in the forests with a humid climate. The *palpalis* and *morsitans* groups

developed drought avoidance behavioural patterns, through the evolution of water proofing mechanisms. Bursell's (1958) studies on pupal ecology supports his claims. The puparia of *G. brevipalpis* and *G. fuscipleuris*, found in residual forests or humid secondary forest communities in Eastern Africa cannot tolerate enviroments with prolonged periods of less than 70% relative humidity. On the other hand, *G. swynnertoni* and *G. morsitans centralis* can complete development under 10% relative humidity. *Glossina morsitans submorsitans* needs 30% relative humidity; while *G. pallidipes* and *G. austeni* pupae have tolerance levels of between 40 and 50% relative humidity. The *palpalis* group living in habitats associated with lake and river edges avoid extreme climate by staying near water. However, there are two notable exceptions in Bursell's (1958) suggestion: *Glossina austeni* (of morsitans group), living in humid evergreen thicket and forest, and *G. longipennis* (of fusca group) being the best adapted to arid environment, with puparial shell and pupal skin that are less permeable to water than that of any other tsetse. Further more, *G. brevipalpis* lives in the same habitat as *G. austeni* and *G. pallidipes*.

### 1.4.2 *Glossina austeni*

*Glossina austeni* (Plate 1.2) is confined to Eastern Africa. Ford (1970) considers that this species extended its range within the East African coastal plain only after the abolition of slavery, when Arab farmers were forced to abandon their plantations and turn to commerce, leaving their farms to revert to secondary forest and thicket.

Bursell (1958) also postulated that *G. austeni*, having adapted itself to an arid environment, later made a secondary return to evergreen, comparatively humid, thicket habitats. Whatever the evolutionary history, the present distribution of *G. austeni* is a narrow 385 km stretch of humid and sub humid coastal zone. The northern limit of its range is Southern Somalia, and it extends southwards, through Kenya, Tanzania (including the Island of Zanzibar), Mozambique, to the northern part of the republic of South Africa (Nash, 1969), (Fig. 1.2, after Ford, 1970).

Among the morsitans group of tsetse, *G.austeni* is the least studied, from the ecological point of view, in comparison to *G. morsitans* and *G. pallidipes*, both of which have been subjects of most of the tsetse research in Eastern Africa since 1930s (for example, Williams, 1943; Jackson, 1933, 1937, 1939, 1945, 1948; Wilson, 1954; Bursell, 1957, 1963, 1970; Burtt, 1946). Ford (1970) listed this species among the economically important tsetse that have not been studied. The situation has not changed much since then. Most of the past works carried out on *G. austeni* were laboratory based. They included those on feeding habits in relation to larviposition (Nash *at al*, 1967), susceptibility to infection with *Trypanosoma brucei* (Ward, 1968), development of the thoracic flight musculature (Langley, 1970), maintenance in the laboratory (Curtis and Jordan, 1970), insemination potential (Pollock, 1970),laboratory maintenance (Jordan, 1972), blood intake and sexual maturation (Foster, 1976), pupal weights (Boyle, 1971) and chromosome analysis (Southern *et al.* 1972). However, Moggridge (1936, 1948, 1949) made observations on some ecological aspects, specifically studying seasonal

spread in Somaliland, night activiy at the Kenya coast, and climate in relation to *G. austeni*.

In all his studies, Moggridge used the fly round method of Potts (1930); that is, a team of catchers traversing a fixed path, and stopping at regular intervals to catch tsetse that come to them or to a bait oxen that may accompany them. This method captures mainly male tsetse, since they are more attracted to moving objects. In recent years, two independent attempts were made to sample *G. austeni* in Mozambique and Zanzibar by Takken (1984) and Dr. D.A Turner (1985 pers. comm.) respectively . Both workers failed to get this tsetse species to enter traps. In his three month study in Zanzibar, Turner (pers. comm.) used the biconical trap and 1-octen-3-ol as bait. He did not catch a single *G. austeni*, but on searching its probable breeding areas, he managed to locate its pupae under litter. Both Takken (1984) and Turner (pers. comm.) cited lack of suitable trappping device as the reason for failling to catch *G.austeni* in the field.

## 1.5 CONTROL OF THE VECTOR (*Glossina*)

Some control strategies were directed towards the control of the parasite (*Trypanosoma*), while others considered the vector. Vector control aims to reduce and/ or eliminate contact between the tsetse fly and humans (and between tsetse and cattle). Initially, this was achieved by resettling people and, therefore domestic stock, away from tsetse infested areas (Cockbill *et al.* 1963). As this became unac-

ceptable politically, the approach was shifted to the control and eradication of tsetse populations. Jordan (1976b, 1978), Finelle (1974), Dame and Jordan (1981), Mulligan (1970) and Allsopp (1984) have all reviewed some of the control methods that have been used at various times against the tsetse fly. These methods included attempts at extermination of favoured host species in tsetse infested areas (Baldry, 1964; Bursell, 1970); random clearance of woody vegetation in such areas including along the rivers (Nash, 1940; Jordan, 1974); and insecticide application (Baldry, 1971; Alsop, 1980). *Glossina morsitans* was drastically reduced in Zimbabwe by removal of elephants (*Loxodonta africanus*), buffaloes (*Syncerus caffer*), Kudu (*Strpsiceros imberbis*), bushbuck (*Tragelaphus scriptus*) and suids (Cockbill, 1960, 1967). Later it was shown that tsetse was capable of changing to other hosts in the absence of the favoured species (Cockbill, 1972). Jordan (1974) and Hursey and Whittingham (1985) have reviewed various control methods that have been used against trypanosomiasis in Africa.

### 1.5.1 Control of tsetse by vegetation clearance

Destruction of the vegetation rendered the tsetse habitats inhospitable for resting and breeding and could, therefore, eradicate the genus. But it was ecologically unacceptable, as it rendered the habitat inhospitable to humans also through much erosion and desertification. Moreover, it was very demanding in resources, due to regrowth of vegetation which necessitated repeated clearing. This method achieved some control in Zambia and Zimbabwe in the early

part of this century. The flies were not eliminated, as they changed their breeding habitats and hosts (Vale and Cumming, 1976). In Kenya in the 1960s large tracts of land around Lake Victoria, Alego location, as well as along the Kuja river, were cleared of tsetse in this way and people settled (Glover. 1963; Dr. R. Onyango, pers. comm).

### 1.5.2 Control of tsetse by insecticidal application

The method of insecticidal application acts only on adult flies, since the larvae and pupae are buried in the soil and are well protected. It provides the most reliable control for large scale campaigns, and has been widely used in Africa, with some measure of success, since the 1960s. Earlier, the use of insecticidal control was based on the application of persistent chemical compounds ( e.g. DDT or Dieldrin) to all vegetation types thought to be harbouring tsetse (Finelle, 1980; Robertson *et al.* 1972). These chemicals were originally applied using knapsacks sprayers (ground spray), but since 1970s, helicopters have been used in areas with rugged terrain, while fixed wing aircraft was used for extensive applications. The latter have also been used to apply ultra low volume (ULV) sequential aerosol in large scale applications in Botswana, Burkina Faso, Nigeria, Zambia and in Kenya, in the Lambwe Valley (Alsop, 1980; Hursey and Allsopp, 1983). This method works on the assumption that 100% of adult flies are killed at spraying time. Subsequent sprays, carried out at intervals of 9 days, then kill emerging flies before they can larviposit. The most commonly used insecticide for these ULV aerial applications is endosulfan. The advantages of this technique over the

ground spraying method using residual chemicals are threefold. First it is relatively less damaging to the environment; secondly, it is usually cheaper, and thirdly, it allows large areas to be treated in a relatively short time, with minimum staff and supervision.

Insecticide application methods have achieved successes in Nigeria (Davies 1964, 1979); Botswana (Davies, 1981); Cote d'Ivoire (Politzar and Cuisance, 1982) Kenya (Glover *et al.* 1960). However, eradication which is usually the goal in such treatments was not always achieved because of the problem of reinvasion or resurgence of the population in the treated areas (Cuisance *et al.* 1984; Allsopp, 1984). Financial constraints some times dictate that only sporadic applications be carried out (Alsop, 1980). Different methods have been used to reduce reinvasions. In Cote d'Ivoire and Zimbabwe, insecticidal barriers using dieldrin were employed (Hursey and Allsopp, 1984; Hursey and Whittingham, 1985).

The main drawback in the use of insecticidal control is the financial aspect. The cost of maintaing an effective barrier zone, for example, is too high and not economically feasible for many African Governments. Besides, buying of pesticides may require foreign exchange, which the African governments cannot afford. Environmental desruption by pesticides and the effect they have on non target fauna are problems that cannot be underrated. In view of the forgoing, insecticidal method of controlling tsetse can only be used as a temporary solution. Non chemical methods, for example, sterile male technique and trapping, are increasingly receiving priority and encouragement (Pant *et al.* 1977; Laveissiére and Couret, 1980; Offori, 1981).

### 1.5.3 Control of tsetse by sterile male technique

The use of sterile male techniques has been investigated and the method has been successfully employed in some countries in Africa (Dame and Schmidt, 1970; Jordan and Curtis, 1972; Williamson *et al.* 1983; and Takken *et al.* 1986). By this method mass rearing of tsetse has to be undertaken. The males are sterilised and released in the field to compete with the wild ones. Since a female tsetse is mated but once, if the sterile male finds a female before the field males, then that female will not be able to produce larvae. In this way, the population declines gradually and, provided there is no reinvasion, eventually dies off.

### 1.5.4 Trapping and integrated control approach

Traps have been used in tsetse work since 1930 when the first one was developed by Harris (1930). Their function was to attract tsetse to themselves and then capture them (tsetse). In this way traps could have an impact on the population, especially if many were employed. Harris trap was very successful in Zululand, and it was thought that eradication could be achieved by it (Nash, 1969). However, as the use of traps spread to other parts of Africa, it was realised that they were not efficient enough to be useful in control work. They were then mainly used in surveys, to detect the presence of tsetse (Swynnerton, 1933; Langridge, 1968; Glasgow and Potts, 1970), and for sampling in tsetse studies (Harley 1966, 1967; Moloo, 1973). With the desire to reduce the use of pesticides for control work, since

1980s, much attention has been paid to traps as a possible substitute for control by spraying of insecticides. Detailed studies were undertaken on the mode of function of traps, and this led to the development of simpler and more effective devices (Challier and Laveissiére, 1973; Hargrove, 1977; Vale, 1982a and 1982b; Flint, 1985; Brightwell *et al.* 1987).

In the last decade or so, host odours have been used with traps and targets, thus introducing another dimention, namely that of olfaction, as an added attractant to enhance the effects of shape and colour in attracting tsetse to traps. Detailed studies have been carried out on the efficiency of various odour sources as tsetse baits. These included carbon dioxide (Frezil and Carnevale, 1976); live animals and chemicals such as acetone ( Hargrove and Vale, 1979; Vale, 1979, 1980, 1981), and mammalian excretory products, such as urine, faeces and sweat (Owaga, 1984). Successfull studies of odour baits in different parts of Africa, have shown that this innovation is promising as a tsetse management tool, particularly for the savana tsetse (Vale and Hall, 1985; Owaga, 1985; Dransfield *et al.* 1986; Merot *et al.* 1988; and Kupper, 1988).

Simultaneous use of various control methods is gaining favour as a better means of eradication. In Nigeria eradication of *G. palpalis palpalis* was achieved in a 1,500 km$^2$ area of the central region with integrated use of the biconical trap, insecticide-impregnated targets and sterile male technique (Takken *et al.* 1986; Oladunmade *et al.* 1985). In that campaign, continuous removal-trapping using biconical traps reduced the target tsetse population by 90%, and the insecticide-impregnated targets controlled the population in the mar-

ginal habitat, while acting as efficient barriers preventing reinvasion of the control area. The sterile males released weekly at a ratio of 10 to 1 wild fly, achieved eradication in the control area. In Burkina Faso, elimination of *G. palpalis gambiensis* and *G. tachinoides* along 600 km of gallery forest in a pastoral area of Sideradougou, was achieved by use of deltamethrin-impregnated screens, in the dry seasons, followed by the release of sterile males of the two species in the rainy seasons (Cuisance *et al.* 1984; Cuisance *et al.* 1985).

## 1.6 CONTROL OF TRYPANOSOMIASIS

Attempts at controlling the trypanosome parasite directly were made using the methods of chemotherapy and chemoprophylaxis (Williamson J, in Mulligan, 1970 (Ed.); Nai'sa, 1971; Finelle, 1975; Challier, 1982); and trypanotolerant cattle (Roelants *et al.* 1987; Dolan *et al.* 1986).

### 1.6.1 Chemotherapy, drug intervention and prophylaxis

The use of drugs as a means of control is rather expensive and, by its very nature, has to be repetitive. Most of the drugs available for treatment of the disease can have unpleasant side effects (Finelle, 1975). Nevertheless, trypanocidal drugs have helped to maintain cattle in some areas, although under high challenge the frequency of prophylaction or curative interventions may rapidly lead to drug resistance (Finelle, 1975; Maclennan, 1975). There is increasing

evidence of development of strains of the parasite resistant to many of the drugs currently in the market (Kupper and Walters, 1983, Pinder and Authie, 1984)

### 1.6.2 Trypanotolerance

Some native breeds of cattle in Africa have been known to have high natural resistance to various strains of trypanosomiasis. Examples are N'dama and some Baule breeds in Central and West Africa (Roelants *et al.* 1987). The Orma Boran breed of East Africa was also found to be resistant (Roelants *et al.* 1987; Dolan *et al.* 1986). Cross breeding between resistant and sensitive animals, and the introduction of trypanotolerant cattle have had some degree of success (Dotoum, 1979; Leak *et al.* 1986; Dolan *et al.* 1986, Roelants *et al.* 1987). The development of a vaccine for immunization against trypanosomiasis has been illusive to scientists, but researchers have not dispaired and many laboratories, such as ILRAD and ILCA are still working on it (Anonymous, 1979).

## 1.7 STUDY AREA AND OBJECTIVES

### 1.7.1 Tsetse and trypanosomiasis in Kenya

In Kenya it is estimated that nearly 138,000 km$^2$ of the 570,000 km$^2$ land surface is tsetse infested, which is about 24% (Anonymous, 1974). There are eight *Glossina* species here, (Fig.1.3) *G. pallidipes*, *G. fuscipleuris*, *G. morsitans*, *G. fuscipes*, *G. longipennis*, *G.*

*brevipalpis*, *G. swynnertoni*, and *G. austeni*. (Anonymous, 1970). Of these, *G. pallidipes* is the most widespread in eastern Africa in general and Kenya in particular. It is also the most widely studied. In Kenya it has been studied in the field by various workers, particularly by Baldry (1972), Jaensen (1981), Etten (1981, 1982), Owaga (1981, 1984), Turner (1981, 1987), Snow (1982) and Tarimo *et al.* (1984). *Glossina morsitans* was a very important vector of human trypanosomiasis in Tanzania, Zambia and Zimbabwe in the 1960s, and most of the early tsetse work was modelled on this species and *G. swynnertoni* which occurred in close proximity with it in some areas in central Tanzania (Jackson, 1945a, 1945b, and 1953; Ford, 1970). *Glossina fuscipes* was thought to play an important role in the transmission of human sleeping sickness around the lake Victoria region in all the three East African countries. Consequently the East African Trypanosomiasis Research Organisation devoted some effort in its study, and to *G. brevipalpis* which coexisted with it in some areas (Harley 1965, 1967; Rogers, 1977). It is still the main transmitter of the disease in the Busoga area of Uganda, to this day (Lancien *et, al.* 1990). In recent years, KETRI, the successor of EATRO in Kenya, has undertaken some work on *G. longipennis* in the arid areas of Galana (Dr. E. Opiyo, pers. comm.), and ICIPE, through its ARPPIS programme, undertook further study on the ecology of *G. longipennis* (Kyorku *et al.* 1990). There is no record of any study on *G. fuscipleuris*.

The trypanosome parasites at the coast include species *Trypanosoma congolense* Broden, its relative *T. semiae*, *T. vivax* Ziemann, and *T. brucei brucei* . The two strains that cause human

sleeping sickness, *T. brucei rhodesience* Stephens and *T. brucei gambience* Dutton have, however, not been recorded in the coastal region. No attempts have been made to control tsetse at the coast in recent years. However, trypanocidal drugs, especially Berenil, are sometimes provided by the government veterinary officers to contain the disease (Snow, 1982).

### 1.7.2 Reason for choice of species

Among the eight tsetse species occurring in Kenya, *G. austeni* is one of the two that have not been studied in the wild; the other being *G. fuscipleuris*. Another reason for choosing *G. austeni* is that it is an important species in terms of proximity to human and domestic animal habitation, and of total surface area occupied in Kenya. Further more it occurs in association with *G. pallidipes* and *G. brevipalpis* over most of its range; although the latter two are found elsewhere in Eastern Africa other than the coast (Harley, 1967; Jaensen, 1980; Etten, 1981; Owaga, 1981) *G. austeni* does not associate with them inland. It would be interesting to find out why this species is confined to the coast. Moreover, its exact role in the transmission of trypanosomiasis is not known. It could be an important transmitter of the trypanosome parasites, or a non participant in the transmission cycle. A few attempts made to study *G. austeni* in the wild had not produced satisfactory results, apparently because it was untrappable, hence there was a challenge to find ways of sampling this species.

### 1.7.3 Description of study area

The study was conducted in Muhaka forest area (also known as Kambe by the local people) (Lat. 4° 20'S. Long. 39° 31'E, Alt. 30m) in the south coast of Kenya (Fig.1.4). But some samples were taken from Shimba Hills National Park which is about 20 km to the north of Muhaka forest (Lat. 4° 5' S Long. 39° 25' E, alt. 180m). This area was suitable as a typical habitat not only of *G. austeni*, but of an area where the three tsetse species, *G. brevipalpis*, *G. pallidipes* and *G. austeni* ecologically coexist. It was easily accesible and had human settlement so that it would be possible to assess whether the tsetse flies were actively transmitting trypanosomes to domestic stock. At the same time, it had wildlife which are the natural hosts. Physiographically, and according to Miller (1953, quoted by Ojany and Ogendo, 1979) this area falls under equatorial-type of climate. These authors (Ojany and Ogendo, 1979) called it coastal lowland tropical rain forest belt.

### 1.7.4 Climate

A number of factors control climate in any place. They are latitude, altitude, characteristics of the prevailing winds , distance from the sea or from any sizable water body and topography. These may interact with one another to influence climate; for example, prevailing winds are important in connection with topography in the form of relief barriers.

One factor that is important in the determination of seasonality is pressure belt (Ojany and Ogendo, 1979). Pressure belts generally shift with apparent movement of the overhead sun. The greatest insolation occurs directly below the overhead sun and creates the lowest pressure, or intertropical convergence zone, the point for convergence of winds and the creation of air masses. Throughout its length, width and depth, an air mass is uniform in temperature and humidity (Ojany and Ogendo, 1979). It, therefore, introduces its own particular climatic characteristics as it moves across a tract of country. The convergence air masses are associated with transportation of moisture, due to the cooling that takes place during the upwards movement of air over the area of low pressure. This causes condensation and results in precipitation.

Along the Kenya coast there are two distinct dominating air masses which control climate. From November to March there are the northseast trade winds (northeast monsoon winds) which are dry winds. By April the southeast monsoon winds set in. These will have travelled over a vast body of water in southern Indian ocean, and are the main source of rain. They persist with vigour and consistency until end of June and may go on to July. During this period coastal weather stations record over 20 rainy days per month especially in May. These winds get weaker around July - August and by Sept-October the north east winds start to form.

The mean maximum rainfall in Muhaka forest area is 1285mm (during this study it was 1235mm) and mean minimum 700mm, there are over 140 rainy days in a year. The seasons can loosely be divided into long rains, April to June, cool and dry season from July

to September, short rainy season, between October and December, and the hot dry spell from January to March.

### 1.7.5 Temperatures

There are two main factors that exert greatest influence on temperature; these are height above sea level or altitude, and aspect in relation to relief features. The coast zone (from 0 - 150 m above sea level) is one of the high temperature regions in the country, although sea breeze plays a very important moderating role. The mean maximum temperature is 34 - 36°C (during this study it was 36°C) and minimum 25 - 26°C. The diurnal range is generally small. The coolest months are July to August when afternoon temperatures average 25°C and night ones 20°C.

Similarly the relative humidity is high. Mean maximum is 85% and minimum 50%. RH is highest around dawn when temperatures are minimal and lowest around 1500 hours when temperatures are maximal. The longest hours of sunshine are in the drier months, during the southern summer.

### 1.7.6 Geomorphology

The Muhaka lowland forest and the surrounding areas are supported by Kambe limestone terra rossa soil (hence the area is sometimes called Kambe forest), reputed to be the most fertile under natural conditions at the coast (Ojany and Ogendo, 1979). It occurs in the form of coastal sandy soils, but some parts have evidence of shales

and lime stone. This is derived from Kambe shales and Shimba lime stones formation of the Triassic period (30,000,000m years ago) (Ojany and Ogendo, 1979).

### 1.7.7 Vegetation

Vegetation of woody plants and herbs commonly form layers or strata, and there is usually a characteristic species that dominate within the different strata. Plant communities are named after the dominant species. In Muhaka forest, the dominant species are *Brachylaena huillensis* O.Hoffm., *Steculia* sp. *and Chlorophora excelsa* (Welw.) Benth &Hook ( thus forming a *Brachylaena-Steculia-Chlorophora* vegetation), but there are many other species. Generally the vegetation consists of secondary forest and dense thicket. Tall trees, some of them 30 to 40 metres high and 100 to 300 cm in circumference, at breast height, occur within the forest (plate 1.3). Some of the species are typically coastal and do not occur elsewhere in the country. An example is *Brachystegia speciformis* which is the only Kenya representative of a genus known to be common in central Africa. Other species present are also found in other woodland areas in the country. For example, *Tamarindus indica* found on the fringes of the forest is wide spread in wooded grassland areas in central and Nyanza provinces (Dale and Greenway, 1961). There is practically no undergrowth within the forest where trees form a continuous canopy in most parts. Surrounding the forest is woodland and tree/grassland vegetation, where the main species of trees are *Hyphaene coriacea* Gaerth (plate 1.3c) and *Combretum aculeatum* Vent. Tree density

per 10 $m^2$ within the forest (for trees which are of 40-300 cm in circumference) is 3-7, but within this area there may be other smaller trees. The number of trees (of 40 cm or more in circumference) per 10 $m^2$, in the area surrounding the forest, is 0-2. The main grass species are: *Aristida keniensis, Cynodon dactylon, Panicum* and *Andropogon* spp. as well as various species of *Cyperus*. The immediate surrounding area has medium to dense human settlements with plantations of coconut palms, cashew nut and mango trees, in addition to cassava plantations.

### 1.7.8 Animal life

The forest is rich in fauna. Vertebrates include reptiles particularly snakes and several species of lizards. Mammal species, some of which are the natural hosts of *Glossina*, are bushback (*Tragelaphus scriptus*), grey duiker (*Cephalophus monticola*), red duiker (*Cephalophus adersi*), dikdik (*Rhynchotragus guentheri*), warthog (*Phacochoerus aethiopicus*), bushpig (*Potamochoerus porcus*), African hare (*Lepus capensis*), black and white colobus (*Colobus polykomos*), bushbaby (*Galago senegalensis*) and baboons (*Papio anubis*). Some of these, for example, bushpig, hare and bushbaby are rarely seen during the day as they are nocturnal. Most of the primates are tree dwellers and spend a good deal of their time 20 or so metres above the ground.

Insect fauna are abundant and a variety of species may be readily encountered. These include Orthopteran species such as the long horned grasshoppers *(Tettigoniidae)*, short horned grasshoppers,

*(Acrididae)*, bush crickets *(Gryllidae)*, and mole crickets *(Gryllotalpidae)*, some Hymenopteran species of wasps, bees and ants. For example, *Api mellifera* (a bee), *Oecophylla longinada* (red ants), *Polyrhachis* sp. (black ants) and *Myrmicaria natalensis* (safari ants). Some known predators of tsetse such as *Bombix* spp. (Bombilidae). Also, spiders *(Arachinida)* are represented by different species and several genera of moths and butterflies (Lepidoptera) are present.

The order Diptera is represented by biting flies, especially families *Tabanidae* and *Stomoxydae*, predators such as robber flies *(Assilidae)*, and juice feeders such as crane flies (*Tipulidae*), as well as the tsetse (Family *Glossinidae*), one of which is the subject of this study. *Glossinidae* is represented by three species that coexist along the Kenyan coast in general; *G. pallidipes* (of morsitans group), *G. brevipalpis* (of fusca group) and *G. austeni* (of morsitans group) (plate 1.1).

### 1.7.9 Domestic stock

The wooded grassland area surrounding the forest supports goats, a few sheep and cattle of the East African zebu breed. However, the number per homestead is low compared to the population of livestock encountered inland. This may partly be due to the problem of trypanosomiasis. The main source of dietic protein for the local people appear to be fish, and many of the indigenous people are fishermen.

## 1.8 OBJECTIVES OF THE STUDY

The general objectives of this study were to investigate some aspects of population ecology and behaviour of *G.austeni*, and its interaction with trypanosomes. Specifically, the research was undertaken to study the following aspects:

1) responses of *G.austeni* to traps and odours, with a view to identifying a sampling method for the species.
2) activity pattern in relation to feeding, both under field and laboratory conditions,
3) dispersal, distribution, seasonal fluctuations, and density of *G.austeni* in the Muhaka forest area
4) vectorial capacity of *G.austeni* with regard to*Trypanosoma congolense*, and
5) trypanosome infection rate in the field populations of *G. austeni* and the other two *Glossina* species which coexist with it.


3
1

Plate 1.1: THE THREE *Glossina* spp. WHICH COEXIST AT THE KENYA COAST

1. *Glossina brevipalpis* Newstead (x 9)
2. *G. pallidipes* Austen (x 7)
3. *G. austeni* Newstead (x 8)

*Glossina brevipalpis* is a representative of the fusca group of tsetse. Members of this group are typically large in size as compared to members of the other two groups. The wing length of *G. brevipalpis* is 12 to 13mm.

Both *G. pallidipes* and *G. austeni* belong to the morsitans group. *G. austeni* is one of the smallest in this group with wing length of 7 to 8mm. All the three species shown in the picture are found in Muhaka forest and Shimba Hills National Park.

Plate 1.2 : TSETSE IN THE PROCESS OF ENGORGI

*Glossina austeni* Newstead

a. Probing a cow for a blood meal (x8)

b. after engorgment (x8)

a

b

Plate 1.3: VEGETATION TYPES IN MUHAKA AREA

This plate shows different sections of Muhaka forest.

a. dense tall trees section that constitutes much of the forest

b. the northern edge of the forest

c. the eastern border is surrounded by almost pure stand of *Hyphaen coriacea* (mkoma, in Kiswahili). Leaves of this tr are used by the local people to weave baskets; fruits from it eaten by both human and the forest primates such as babo and monkeys.

a

b

c

Figure 1.1: DISTRIBUTION AND CLIMATIC LIMITS OF *Glossina*

Map of Africa showing the distribution and climatic limits of *Glossina* in the continent. The southern and internal isopleths, shown by solid black lines, indicate cold limits. The outer northern isopleth from the north Kenya coast to Senegal shows the hot limit. The dispersal centre of fusca and palpalis groups (Evans, 1953) is indicated by incomplete circle. The savana and rainforest areas are shown. (After Ford 1970)

Fig.1.2

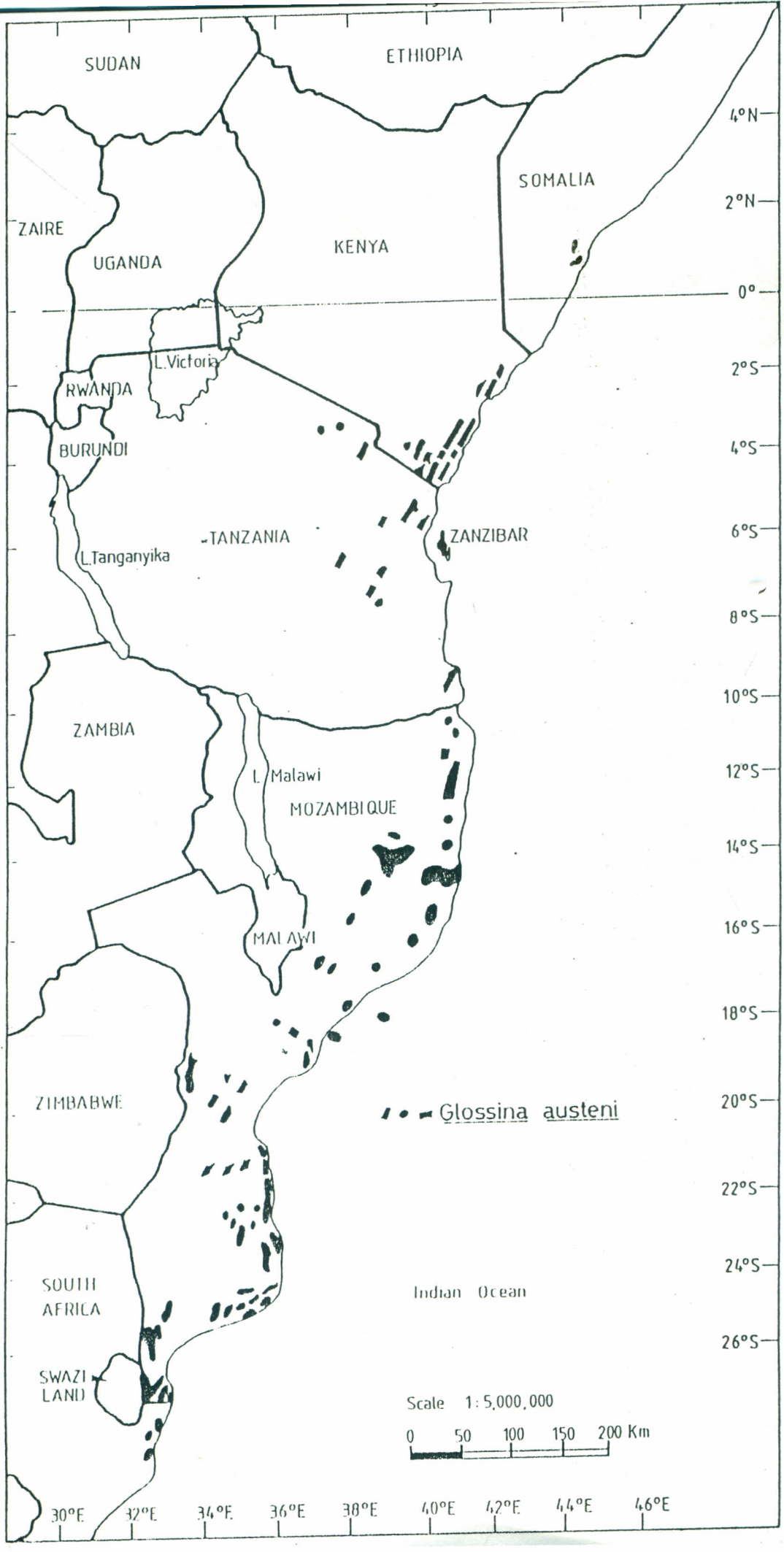

SUDAN
ETHIOPIA
SOMALIA
ZAIRE
UGANDA
KENYA
L.Victoria
RWANDA
BURUNDI
TANZANIA
ZANZIBAR
L.Tanganyika
ZAMBIA
L. Malawi
MOZAMBIQUE
MALAWI
ZIMBABWE
SOUTH AFRICA
SWAZI LAND
Indian Ocean
Glossina austeni
Scale 1:5,000,000
0 50 100 150 200 Km
4°N
2°N
0° EQU
2°S
4°S
6°S
8°S
10°S
12°S
14°S
16°S
18°S
20°S
22°S
24°S
26°S
30°E
32°E
34°E
36°E
38°E
40°E
42°E
44°E
46°E

Figure 1.2: DISTRIBUTION OF *G. austeni*

Map of the eastern region of Africa, showing the distribution of *Glossina austeni* along the eastern coast, from southern Somalia to the Republic of South Africa, including the Island of Zanzibar in the Indian ocean. The north and south latitudes as well as the east longitudes are shown for reference. (Ref. Ford and Katondo, 1977).

Figure 1.3: DISTRIBUTION OF *Glossina* IN KENYA

Map showing the distribution of eight species of *Glossina* in Kenya, with areas thought to contain tsetse but unsurveyed, and areas that are completely tsetse free. The latter consists mainly o the heavily populated western and parts of central areas of the co try.

(Ref. Anonymous, Kenya Atlas, 1970 Government Printers, Nair

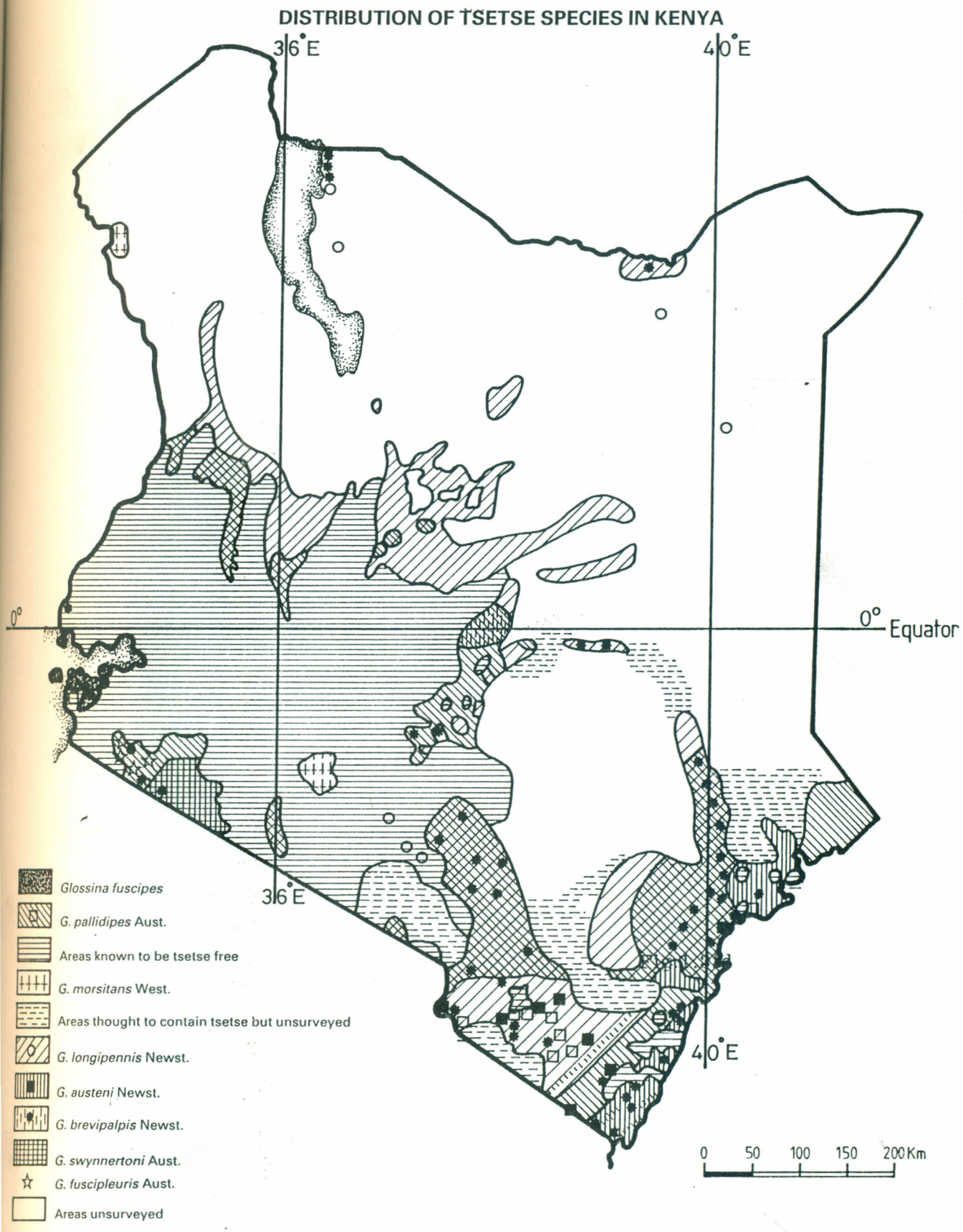

DISTRIBUTION OF TSETSE SPECIES IN KENYA
36°E
40°E
0°
0° Equator
36°E
40°E
0 50 100 150 200 Km
Glossina fuscipes
G. pallidipes Aust.
Areas known to be tsetse free
G. morsitans West.
Areas thought to contain tsetse but unsurveyed
G. longipennis Newst.
G. austeni Newst.
G. brevipalpis Newst.
G. swynnertoni Aust.
G. fuscipleuris Aust.
Areas unsurveyed

Figure 1.3

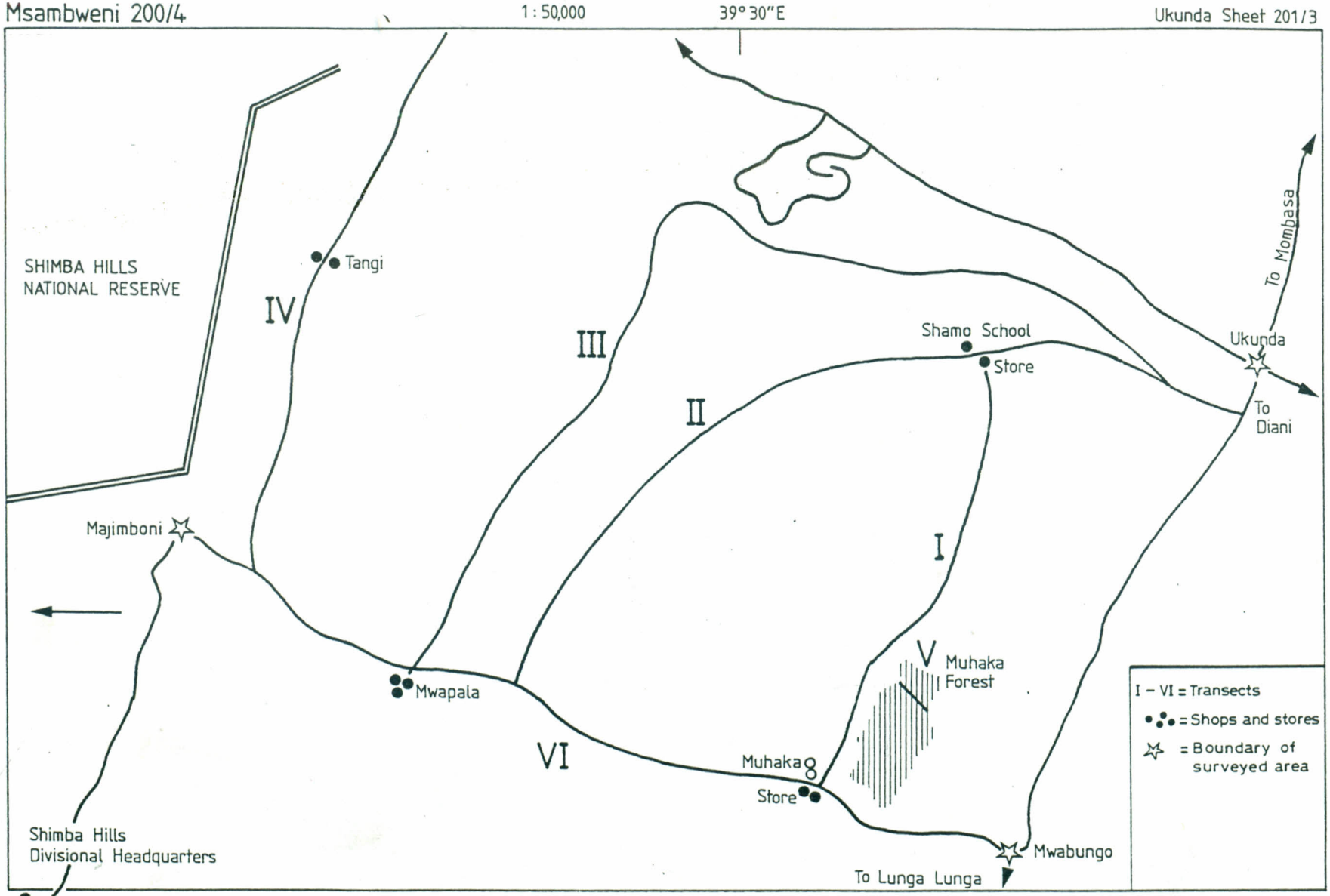



Fig. 1.4

Area surveyed in1989, from Muhaka Forest to Shimba Hills National Park

Survey of Kenya 1974

Figure 1.4: STUDY AREA, IN THE SOUTH COAST OF KENYA

Area surveyed from November 1988 to January 1989, from Muhaka forest to Shimba Hills National Park.
I-VI represents existing tracks that were used as transects.
Transects I to IV and VI yielded very few flies per week. Transect V was in Muhaka forest and was giving the highest numbers. It was, therefore, decided that the study should concentrate in Muhaka, with occasional samples being taken from Shimba hills (close to transect IV).

# Chapter 2

# RESPONSES OF *Glossina austeni* TO TRAPS AND ODOURS

## 2.1 INTRODUCTION

### 2.1.1 Sampling of tsetse

Sampling strategy for tsetse flies provides a contrast to that for other haematophagous Dipterous vectors such as mosquitoes, sand flies, and black flies, where only females are blood suckers. All *Glossina* spp., both male and female feed exclusively on vertebrate blood and both are vectors of the disease trypanosomiasis. Consequently, capture techniques for the genus have to take both sexes into account. Furthermore, sampling is the most important aspect in the study of tsetse, as all data relating to activity pattern, density, distribution, behaviour and interaction with trypanosomes are obtained by it. Early workers used various methods of capturing tsetse flies. These included the fly round (Potts, 1930; Jackson, 1949, 1953; Nash and Page, 1953; Moggridge, 1948, 1949; Ford *et al*, 1959; Glasgow, 1961; Harley, 1965), the use of stationary baits (Leggate and Pilson, 1961; Pilson and Leggate, 1962; Harley, 1965; Pilson and Pilson, 1967), use of traps (Harris, 1930; Swynnerton, 1933; Chorley, 1933; Morris and Morris, 1949; Morris, 1960; Moloo, 1973; Challier and Laveissiére, 1973) and capturing tsetse off vegetation (Nash, 1969; Weits and Glasgow, 1956).

### 2.1.2 Sampling by fly round method

The fly round was conceived by Potts (1930) specifically for tsetse species that were attracted to humans, for example, *G. morsitans*. A laid-out path for fly round passed through a range of vegetation types. Catchers carrying hand nets traversed the path, stopping at regular intervals to catch tsetse that came to them. For species less attracted to humans, for example *G. pallidipes* the method was modified to accomodate an ox in the company of catchers. Hand nets were then used to catch the flies off the ox.

Among the main limitations of this method was the deficiency of females in the sample. Male tsetse made up the bulk of the catch (over 90%) (Bursell, 1961, 1966; Phelps and Vale, 1978; Moggridge, 1948). Moreover, many of the males in the catch were not hungry, but appeared to follow moving objects probably as a part of their general search for females (Bursell, 1966). Another limitation was the high day to day fluctuation of catch size, probably attributed to lack of synchronization between the time of sampling and peak activity period of the fly. Moggridge (1936, 1948) seem to be the only author to have used this method for *G. austeni* in Somali and at the Kenya coast, respectively. The method now seems to have been largely abandoned.

### 2.1.3 Catching of tsetse off stationary bait animal

The use of stationary bait animal in sampling made it feasible to extend the period of catching to several hours or a whole day. That

way, any variables due to daily fluctuations in the activity cycle of the fly could be automatically dealt with. Moreover, both sexes of tsetse were captured almost in equal proportions. However, the method exposed both the catcher and the animal to the possibility of infective bites from tsetse. Moreover the catch varied according to the skill of the different catchers. The presence of humans was later shown to depress catch size, man being repellent to some tsetse species such as *G. pallidipes* (Vale, 1979 and 1982). The method is no longer popular for sampling.

### 2.1.4 Capturing tsetse off vegetation

In the past, the catching of tsetse off vegetation was carried out to study inactive flies, to obtain the replete section of the population to be used in the analysis of blood meal for the identification of the hosts (Etten, pers comm., Weitz, 1963; Glasgow in Mulligan, 1970); and to study the resting sites of different species of *Glossina*. Upto about 1950, all knowledge on tsetse was based on active flies, or the part of the population which was attracted to human or bait animals. The behaviour of tsetse at rest was not known, and most of the *fusca* species that are active only at night were still not studied. Nash 1969, related how he and Davey J.T. discovered *G. medicorum* by searching the vegetation in an area in Nigeria where the species had not been recorded. Bursell (1963, 1966) in describing the various phases of the hunger cycle and digestive stages in tsetse reported that tsetse in the first phase of the hunger cycle does not fly about much, but stays at rest among the vegetation. It is only after the

second phase when the fat bodies have been replenished that it starts to be active again. Hence this sampling method that is used with a specific category of flies in mind exploits the bias inherent in it for good use. There is no record in literature of this method having been applied on sampling of *G. austeni*.

### 2.1.5 Sampling by traps

Animal traps fall into two categories, those that catch at random and those that catch after having attracted animals in some way. Tsetse traps and trapping methods belong to the latter category. The three afore mentioned methods, of fly round, stationary bait and catching off vegetation, rely entirely on the skill of the catcher, so that samples obtained by them cannot be standardised. Traps, on the other hand, eliminate the human error in sampling since they are not manned. Traps have a number of other advantages over the other methods: they can be left out the whole day so that fluctuations in catches, due to wrong timing of the activity of the fly, are automatically taken care of; the problem of exposure to infective bites as in the case of human or animal baits, does not arise. Trap catches are often more representative of both sexes, hungry and not so hungry flies, and almost all age groups. Traps have demostrated the presence of tsetse when other methods failed (Thomson *et al.* 1961). In view of the fore going, traps are best placed as a sampling method for tsetse.

Tsetse traps were developed from the early catching methods of sticky panels or glue covered cloths that were used by Maldonado (1910, quoted in Mulligan, 1970) and Da Costa *et al.* (1915). How-

ever, the first real tsetse trap with a body and a collecting cage was that of Harris (1930). His traps were very successful against *G. pallidipes* and it was thought that they (the traps) could achieve complete eradication of that species.

Since Harris' time, trap designs have varied over the years according to the investigator, the ecology of the species to be studied, and the particular use for which the sample was intended. Harris' trap and most of its early successors, such as the trap of Morris and Morris (1949), were animal shaped and resembled hosts of tsetse. Other animal traps included Langridge's (1968) box screen and Moloo's (1973) awning screen skirt.

The biconical trap (Challier and Laveissier, 1973), is based on a different concept for attraction of tsetse. Like some of Swynnertone's (1933) devices, this trap attracts tsetse by colour contrast rather than by shape and form as was the case in animal traps. The authors of the biconical trap showed that the blue-white-black combination was the most effective for the riverine species, *G. palpalis*, for which the trap was originally designed (Challier *et al*, 1977).

Many workers have tested various colours for attractivity to various tsetse species (Lambrecht, 1973; Challier *et al*, 1977; Owaga, 1981, Dransfield *et. al.*; 1982; Jordan and Green, 1984; Green, 1990). All these workers showed that the various species favoured dark blue and black, except Lambrecht (1973) and Dransfield *et al.* (1982), who found that *G. morsitans* in Botswana and *G. morsitans submorsitans* in Nigeria, respectively, favoured white. Dean *et al.* (1969) and Jordan and Green (1984) went a step further and showe in laboratory experiments that blue was the most

attractive colour to the tsetse species *G. morsitans*, and that attractivity was highest at the spectoral wavelength of 400- 568nm. All the traps used in this study had royal blue material as the main part exposed when the trap was set.

Extensive use of odour baits in sampling tsetse started relatively recently, especially with the work of Vale and his co-workers in Zimambwe (Vale, 1980, 1983, 1984; Green, 1986; Warnes, 1990). Other work on odours were carried out in Kenya's Kajiado district (Owaga, 1984, 1985; Dransfield *et al.* 1986; Hassanali *et al.* 1986), and Somalia (Torr, 1989). In West African, not much success has been recorded with odour baits in trapping tsetse of the palpalis group that are found there, apart from that of Merot *et al.* (1988) for *G. tachinoides*. However, recently Cheke and Garms (1991) reported poor performance of odour baited traps for *G. palpalis palpalis* in Liberia.

## 2.2 MODE OF FUNCTION OF TSETSE TRAPS

In using traps emphasis is always laid on appropriate siting with regard to feeding ground of tsetse and vegetation, light and shade in relation to the sun and even resting places of tsetse hosts. *Glossina* have a tendency to fly towards dark surfaces and shaded cavities (Fuller and Mossop, 1929).

A tsetse trap consists of a body, an attractant and a catching device. In most cases the body is itself an attractant by the nature of its surface colour, or shape or both. In fly rounds, human/ or oxen, acted as the attractant and the hand net was the catching device. Later, traps were designed to simulate one or more of the attractive

elements in the animal hosts; colour, movement and odour. The catching device consists of a builtin no-return mechanism that is part of the trap, plus a holding cage. For example, electric screens in the case of electric traps (Hargrove, 1980), water with low surface tension in the case of water traps (Dransfield *et al.* 1982), and a small hole leading to the retaining cage in the case of many mechanical traps (Challier and Laveissière, 1973). Auxiliary equipment includes the support pole driven into the ground when the trap is in operation, and an anti predator device that may be a form of grease applied on the pole above ground level, to stop ants climbing up to attack the captured tsetse. Some traps may be hung from tree branches, in which case a pole is not necessary. Odour baits are generally placed in dispensers and used in conjunction with traps.

It is believed that adult *Glossina* are attracted, and come to traps when hungry. Their reaction would then be that of an insect in search of its host. A trap attracts tsetse when the insect makes visual contact with it. On approaching, it identifies an entrance and enters. It then finds itself in a very dark area except for a beam of light from the top. Due to the insect's photo positive behaviour, it is attracted upwards towards the light, and has to pass through a no-return passage into the fully lit top cage, from where it is taken when the trap is emptied.

## 2.3 OBJECTIVES OF STUDY

The main objectives of this study were: (a) to determine whether *G. austeni* is trappable or not. (b) if yes, to identify a trapping device or an appropriate trap-odour combination for sampling it. (c) to

determine the relative effects of visual and olfactory stimuli in attracting *G. austeni* to traps.

## 2.4 MATERIALS AND METHODS

### 2.4.1 Choice of sampling tool

Trapping studies were conducted between January 1989 and June 1991. The devices used were the biconical trap (Challier and Laveissier, 1973), the NG2B trap (Brightwell *et al.* 1987), the pyramidal trap (Lancien and Goutex,1989) models 1 and 2, (pyramidal 1 was made of fine cotton material, in France, pyramidal 2 was made of coarser local Jinja material) and two models of the 4t-traps, a modified biconical trap with attachments of blue or black strips measuring 80cm by 5cm and hanging from the base of the cones, (Owaga and Challier, 1985), (Plates 2.1 and 2.2). All the four traps had the royal blue cotton on the outer cover, white cotton mosquito netting on the upper portion, and black cotton in the interior, up to the part leading to the retaining cage. The pyramidal trap was different in that it had a wider area of black exposed, and all the four corners served as entrances Fig.2.3. All the devices have been successfully used for sampling various species of *Glossina*, belonging to the *morsitans* as well as the *palpalis* groups (Challier and Laveissiére, 1973; Brightwel *et al*, 1987; Lancien and Gouteux, 1989). The main difference between them is in their shape and appearance.

In addition to traps, the fly round method was tried out with one goat (three times) and later a black screen acting as a bait (three times), accompanying catchers. However, due to the very poor results

obtained, and the practical difficulties of using fly round in the forest, this method was abandoned during the initial stages of the work. To test *G. austeni*'s possible attraction to moving objects, a revolving screen, driven by a motor and a 12v battery, was attached to the biconical trap, and set up in the forest floor. This trap did not catch any fly after two days of operation, and its use was, therefore, discontinued. An experiment was also conducted using a light trap at night in the forest, between 1900 and 2300 hours for two days, the trap was set in its usual day time site and a spot light fitted inside. No tsetse was captured although other insects entered the trap, this method was also abandoned during the initial stages of the study.

### 2.4.2 Odour sources and dispensers

The traps were baited at various times with the following materials: buffalo urine (one to three months old), acetone, cow urine (one to three months old), buffalo urine (or cow urine) and acetone dispensed together near one trap but from separate containers, and carbon dioxide in the form of dry ice. Traps were also baited with the following phenols:

3-n-propylphenol,

4-cresol,

A mixture of 3-n-propylphenol and 4-cresol(12.3mg, 100mg respectively/150ml distilled water),

A mixture of 3-n-propylphenol and 4-cresol (6.5 mg, 200mg respectively/150 ml distilled water).

Dispensers consisted of a glass jar with a neck diameter of 6cm, a similar jar stoppered with a perforated lid (25 small holes), or a tin of similar size with similar perforations.

### 2.4.3 Catching methods

The relative efficiency of the traps was assessed both in conjuction with or without the olfactory stimulants. Field tests were performed in latin square designs involving treatments (traps or odour as the case may be), sites and days of test (Perry *et al*, 1980). The traps were placed 60 to 100 metres apart (depending on whether they were baited or not) within the forest floor, on the edge of the forest and on the transects.

To examine the relative efficiency of the various trap types without bait, the following latin square designs were adopted: 3 x 3 (3 by 3) or 5 x 5, depending on the number of traps to be compared, e.g. when comparing the biconical, the NG2B and the Pyramidal traps 3 x 3 was used, and when comparing the 4t-trap-black strips, 4t-trap-blue strips, the biconical, the pyramidal and the NG2B traps, 5 x 5 was used.

In comparing the relative efficiency of a number of baits (odours), only one trap type was used. Buffalo urine, acetone, cattle urine and buffalo urine with acetone were compared using five pyramidal or five biconical traps, one of which was an unbaited (control).

One hundred ml of buffalo or cattle urine was poured in a 300ml jar which was placed at the foot of the trap, 30cm from the trap pole.

Two Urine derived phenols of various dose rates were tested in a 5 x 5 latin square experiment, using five biconical traps. The traps were baited with:

a) distilled water (control),

b) 3-n-propylphenol (12.5mg/150 ml distilled water),

c) a mixture of 3-n-propylphenol and 4-cresol (12.5 mg and 100 mg respectively) in 150ml distilled water.

d) 4-cresol (100 mg/150 ml distilled water), and

e) a mixture of 3-n-propylphenol and 4-cresol (12.5 mg, 200 mg respectively) in 150ml distilled water.

Carbon dioxide was tested in the form of dry ice. 240g was placed in a jar of known weight (229g) and 6cm neck diameter. The jar, inside a plastic bag was in turn placed next to the trap pole. The emission rate was later calculated as 1.5g/minute.

To study the effect of odour on trap efficiency, and whether trap and odour effects could be compounded to produce greater catching power, a pair of each of the biconical, the NG2B and the pyramidal traps were tested in a 6 x 6 latin square design. One of each type was baited with the relevant odour source to be tested (one odour source at a time) and the other was unbaited.

Trapping was carried out between 0700 and 1900 h daily for 10 days each month (for daytime sampling). Night trapping was conducted between 1830 h and 0630 h and for the 24 h continuous trapping, the traps were left in operation from 0700 h to the following 0700 h. The starting position of each trap was randomly chosen at the start of the experiment, but on the following days, the trap positions were rotated in a clock wise direction. By the end of the 5 days

(in case of the 5 x 5), each of the 5 traps had operated in each of the 5 positions, thus completing one replicate of the latin square. Several replicates were completed for each experiment.

The traps were set at various times, on the edge of the forest, along a transect (cut within the forest floor (Fig. 2.1) measuring 240m long and 5.7m wide), under canopy conditions within the forest floor, and on a zig zag transect where no vegetation was cleared, and which had some sites in the forest and others on the edge of the forest. Preliminary results had shown that catches obtained from the edge of the forest, and from the cleared transect area were similar in size and composition, while trap yields from the forest floor were different.

Wind direction and approximate speed were assessed from time to time under canopy conditions and on the edge of the forest, in order to get an idea of the nature of the directional flow of the odour plume from the baits. To do this, a flint of cotton wool was mounted on a twig and dipped into hydrochloric acid (HCl). This was then held over an open jar containing ammonia (NH4). The ensuing smoke which shifted with the wind was sketched as in Fig.2.2 and the meanders measured using a piece of string.

## 2.5 LABORATORY BIOASSAY:

Laboratory observations were made in a flight chamber measuring 185cm by 50cm by 50cm made of perspex on four sides and a wire mesh on both ends. A fan was placed about 30cm from the wire mesh on one end. The area between the fan and the mesh was covered with

celophane sheet to reduce turbulence (Fig.2.3). The structure stood 64cm above ground level. Watson's filter paper No. 4 was used to dispense odour in the chamber. A rectangular piece (a triangular, or square may do just as well) of filter paper measuring 1.6 by 1.4cm was soaked in either buffalo urine or distilled water in which meta propylphenol (3-n-propylphenol) had been disolved (see 2.4.2 for dose). The filter paper was stuck at the end of 3.5 cm long, thin wire (0.5mm in diameter). This was then introduced between the mesh and the fan, on the wind ward side.

The tsetse flies used in the experiment were either obtained from field collected pupae, or they were first generation offspring of those emerging from field collected pupae. The flies were maintained in the laboratory under 12:12 LD, 25° C and relative humidity (RH) of 70%. Six hundred flies were tested under 55% RH, 25°C (designated dry air) and 500 under 75% RH, 25°C (designated wet air). Two odour sources were tested under 'dry' and 'wet' air conditions, buffalo urine and a mixture of the two phenols.

Non teneral tsetse flies were tested on their second and third days of hunger while tenerals were tested when they were 2 to 3 days old. The flies were placed in the chamber 3 hours before the commencement of the experiment. At the start of observation the fan was turned on (using remote control) at a speed of 0.3m/second, and left to run for 1 minute; then the odour source was introduced twice for one minute each, at intervals of 7 minutes. The fly's responses were observed and categorised as follows: (a) directed flight towards the source of odour followed by landing on or near the odour source,

(b) random flight (c) no flight. For analysis only directed flight accompanied by landing was considered as positive response.

## 2.6 STATISTICAL ANALYSIS OF DATA

Data for the catches were transformed to $\log_{10}(n+1)$ and subjected to an analysis of variance (Anova) and further comparison of means done using Duncan's multiple range test. When analysing the data, catches from the forest floor were treated differently, while those from the edge of the forest and the cleared transect area were pooled. Data from laboratory bioassay were treated in terms of proportions responding out of the total number tested as indicated in Table 2.6

## 2.7 RESULTS:

The results of trapping experiments are shown in Tables 2.1 to 2.5. The sex ratio in all catches, except those from phenol and $C0_2$ baited traps, were biased in favour of females (Table 2.1).

It was noted that the emission rate of the urine varied little from day to day, but that the mean rates of emission were two times greater at the edge of the forest than within the forest floor. The rates ranged between 0.2ml/h and 0.5ml/h on the forest floor; and 0.5ml/h and 1ml/h at the edge of the forest. The wind speed was also, at least, two times greater at the edge of the forest than on the forest

floor; the mean being 0.3m/sec - 0.4m/sec in the more open area near the edge, and 0.15m - 0.2m/sec within the forest floor.

Table 2.2 compares the effect of three odour sources, (a) buffalo urine, (b) acetone, and (c) buffalo urine and acetone dispensed together, on the biconical, the NG2B and the pyramidal trap catches. In both the biconical and the pyramidal traps, buffalo urine showed greater effect than either acetone or urine plus acetone ($P<0.05$), on female but not on male catches. Male catches were not significantly different from those in the unbaited trap. There was an indication of a depressing effect on catch size by acetone when dispensed together with urine.

Table 2.3 shows the results of a comparison between 5 trap types, each baited with either cow or buffalo urine, with one unbaited control. The pyramidal(1) gave the highest catch ($P<0.03$, $F = 3.68$, df = 4). The effects of various phenols on trap catches are shown in Table 2.4. Among them, only the meta propylphenol (3-n-propylphenol) showed significant effect on biconical trap catches ($P<0.05$, $F = 3.6$, df. = 4). Laboratory observations on responses to phenols revealed further information, it showed less response under high humidity conditions (Table 2.6), and this is relevant to the field situation. In comparison to unpublished results obtained for *G. pallidipes* in the same Muhaka area, there was no significant difference between the two species, with respect to response to the phenols.

The relative efficiency of carbon dioxide as an attractant for *G. austeni* is presented in Table 2.5. The difference in catches from baited and unbaited traps were significant ($P<0.05$, $F = 3.54$) in the biconical trap, ($P<0.03$, $F = 4.84$) in the NG2B trap, and not signifi-

cant in the pyramidal 2, (by this time pyramidal 1 traps were too wornout to be used). Carbon dioxide caused increase in catches of both male and females, unlike the urine bait which influenced female catches only.

The results of analysis of laboratory observations made under constant temperature (25°C) and two humidity regimes, relatively dry (RH 55%) and wet air (RH 75-80%) respectively, indicated greater level of response to odour when the air was dry than when it was wet Table 2.6). This confirms that the high coastal humidity may have an influence in reducing the effect of odours in attracting tsetse. Further more the smoke test (Fig.2.2) indicated that within the forest floor the wind movement was extremely low. The odour plume, like the smoke, was not easily swept away from the point of release to reach flies far away from the trap. This may have contributed to the ineffectiveness of the odours, as the range of attraction of odours is accentuated by wind movement and speed.

## 2.8 DISCUSSION:

The advantage of the latin square method used in the comparative experiments in the field is that all of the traps and odours are tested in each of the sites, thus taking account of the possible site differences. However, there are some basic assumptions to be made. One of them is that there are no differences between days. This, in fact, may not be true because small weather changes may result in important differences in fly availability to traps from day to day, thus introducing bias. However, replication of the sampling exercise several times may off set this bias.

From the reports of many researchers who have compared the performance of various trap types for different tsetse species

(Swynnerton, 1933; Jackson, 1933; Nash, 1930; Morris and Morris, 1949; Glasgow, 1970; Moloo, 1973; Hargrove, 1980; Etten, 1981; Mwangelwa *et al.* 1990; Cheke and Garms, 1991) it is evident that trap performance can be very variable, and that their effectiveness sometimes depends on the area, the species, and even the time of trapping. Perhaps it is not surprising, therefore, that *G. austeni* has been variously described as untrappable, in Zanzibar (Turner pers com) or trappable only in the forest floor by a sticky screen in Zanzibar (Madubunyi, 1990), or not amenable to trapping by the presently known tsetse traps in Mozambique (Takken, 1984 and pers comm.). The present study showed that *G. austeni* in the south coast of Kenya is trappable. The very fact that for at least two successive years, flies were captured in not less than ten traps, some of which were almost permanently in position (Fig. 2.1, trap sites, and Fig.4.4 on fluctuations in apparent density) confirms its trappability. More over the day to day apparent density as assessed, from records of continuous trapping, showed a pattern of fluctuation, commonly seen in trapping records of other trappable tsetse populations, such as *G. pallidipes* (Wilson, 1954; Nash, 1933; Parsons, 1954), in which the pattern of apparent density tends to change with the seasons. There are important factors to remember in connection with trapping of *G. austeni*: Firstly, the trap has to be in the right location to obtain high numbers, that is in the forest floor, or some dense thicket within the range of the species. This finding is in agreement with that of Madubunyi (1990), who in his two week study found that *G. austeni* could only be obtained from the forest floor in Zanzibar. Secondly the trap has to be properly sited, for example, it should not be fully under

sunshine all the time (under canopy is most favourable), and placing near animal tracks may result in higher catches. Thirdly one must not expect hundreds of flies per trap per day, because the species occurs in low densities. Catches per trap per day occur in one digit numbers, or at most tens (Tables 2.1 to 2.5).

The pyramidal trap (1) model, sent to me by Dr. J.Lancien (one of its inventors) performed better than model (2), catching significantly higher numbers of *G. austeni* than the other trap types (Table 2.3). Model (2) was made from coarser local Jinja material, it is possible that the difference in the texture of the royal blue and black materials made the difference in the catching efficiency of the trap.

In the laboratory study *G. austeni* responded more readily to host odour when the relative humidity was low, 55% or less (Table.2.6). Under wet air conditions (>75% relative humidity) there was less response to host odour, and more importantly, the hunger cycle was prolonged and peak response to host odour occurred on day four to five of the hunger cycle in females. This suggests the possibility of higher trap catches of hungry flies during periods of low relative humidity (r.h.). From the records of r.h. taken during studies of activity pattern, such periods of relatively low r.h. occurred daily between 1000 and 1500 hours, and the highest catches coincided with the beginning and the end of this period.

Jackson (1933) reported that *G. swynnertoni*, seems scarsely to become hungry at all during seasons when the evaporation rate was low. The finding of this study show that this statement applies to *G. austeni* as well. In reference to the high coastal humidity, he postulated that there would be difficulty in obtaining long enough catching

season at the coast. Another factor which may have affected the response of *G. austeni* to traps indirectly is either sunshine or radiation, by influencing activity. Although none of these two was measured systematically, observation notes made during studies of activity indicated higher catches on occasions when the sun was partially obliterated by clouds. Moreover, traps set under canopy, and not fully under sunshine captured more flies.

Previous studies had shown that the older urine was more effective as *G. pallidipes* attractant (Owaga 1985). Moreover, it was recently demonstrated at the ICIPE laboratories that microbial activity causing breakdown of pro-attractants (glucuronates and sulphates) continuously gives rise to the phenols in the process (Okech and Hassanali, 1990). The two month old urine was, therefore, preferred for this study. Even then, for *G. austeni* under coast conditions it appeared that when the air was relatively dry (could only be down to 60%s or rarely to within 50% at the coast) the radius of attraction of the odours was greater and the baited traps appeared to be more effective, capturing more tsetse than the unbaited ones. However, as the humidity rose, the effect of odour was much reduced, or nullified, but there was random activity of more tsetse, and the unbaited as well as the baited traps caught roughly equal numbers of tsetse. This cyclic pattern (of high - low - high humidity) which occurred on a daily basis, with highest relative humidity occurring in the early morning hours up to around 1000 h when it started to fall, only to rise again from about 1500 h, made the odour effect almost insignificant.

For this reason the best trap would be that which is both visually attractive to the fly and efficient in capturing all the tsetse that come to it. Among the trap models tested the pyramidal trap(1) (with

fine fabric) had the qualities nearest to these. It gave the highest catches most consistently, performing significantly better than the others when all were unbaited, and still better, though not always significantly so, when they were baited. Its performance was not affected much by the absence of odour baits. It is, however, noteworthy that the second version of this trap did not perform so well, and that the texture of the fabric used may be very important in the traps' efficiency. The biconical and the NG2B traps which are known to be less than 50% efficient for *G. pallidipes* but usually perform several times better with bait for that species under dry air conditions (Owaga, 1984; Brightwell *et al.* 1987), improved with the presence of bait under relatively dry air, but since this lasts only briefly at the coast, it did not make very significant difference to their performance. Hence, sometimes their performance was as good as that of the pyramidal, and at other times they captured fewer flies.

This study shows that the effect of odour baits on catches of *G. austeni*, at the coast was not as spectacular as those quoted for *G. pallidipes* in Zimbabwe by Vale (1980) or Nguruman, Kenya. But it is important to note that the effect on catches of *G. pallidipes* at the coast was also not spectacular. It has been shown that the climatic factors at the coast act to reduce the effect of odour baits. To deduce the role of high environmental humidity on emission rate of the bait, the figures obtained during this study, *viz* 0.2ml/to0.5ml/h, may be compared to those known from the semi arid conditions in south western Kajiado district (Owaga, 1985). The emission rate of buffalo urine within a thicket environment in Nguruman area, Kajiado district, was 2.6ml-3ml/h (Owaga 1985).

Nearly all the successful work reported earlier on odour baited traps were made far inland and away from the coast, for example, in

Rekomitjie in Zimbabwe (Vale, 1980; Vale and Hall, 1985; Hargrove, 1980), in arid area in Kajiado district, Kenya, (Owaga, 1984; Dransfield *et al.* 1986) and in Zambia. Recent studies reported from Liberia (Cheke and Garms, 1991), in a coastal situation, indicated poor performance by odour baited traps for *G. palpalis palpalis*, and Mwangelwa *et al.* (1990) studied *G. fuscipes* (a water side tsetse) in Rusinga Island, Kenya, and concluded that odours had no effect on trap catches of this species. Inference can also be made from the work of Torr (1989) in southern Somalia. He found that odour baits were almost ineffective for *G. pallidipes* in that area. His study site (some 50 km from the coast) is most likely under similar influence as the Kenyan coast.

With regard to sex ratio, it was noted, that other studies at the Kenyan coast, on other tsetse species, have observed a similar bias in favour of females in trap catches. Snow (1982) indicated catching more females than male *G. pallidipes* in biconical traps; Etten (1981) using Langridge trap in Mwalewa, 50 or so km south of Muhaka, also captured more females. Moloo (pers com ) capturing *G. brevipalpis*, *G. pallidipes* and *G. austeni* for laboratory rearing, in biconical traps experienced the same phenomenon. This is possibly explained by the presence of more females in the population (Glasgow, 1963).

It is concluded that *G. austeni* is trappable by all the trap types used in this study, but that the pyramidal trap is the best for sampling it as it, works equally well with or without bait. Among the odour baits, dry ice was the most effective, followed by buffalo urine. However, generally speaking, olfactory attractants did not drastically increase trap catches. The reason for this is probably climatic, especially the high relative humidity, and high temperatures in relation to sunlight, the responses of all the tsetse species in that area were affected by those conditions.

Table 2.1 Sex ratio of *G. austeni* captured in unbaited and baited traps.

| Trap | Bait | Sex ratio % female : male | Sig. level of sex ratio |
|---|---|---|---|
| Pyramidal | nil | 69 : 31 | |
| | urine (buffalo/cow) | 73 : 27 | P<0.05 |
| | acetone | 60 : 40 | F = 18.0 |
| Biconical | nil | 75 : 25 | |
| | urine (buffalo/cow) | 60 : 40 | not. sig. P<0.06 |
| | acetone | 70 : 30 | F = 15.1 |
| NG2B | nil | 79 : 21 | |
| | cow urine (buffalo/cow) | 67 : 33 | P<0.03 |
| | acetone | 72 : 28 | F = 30.5 |

Table 2.2 Catches of *G. austeni* from biconical and pyramidal traps baited with three different odour sources.

| Trap | Bait | Detransformed mean catches/trap/day female | male |
|---|---|---|---|
| Biconical | | | |
| | Unbaited trap | 2.1 | 1.4 |
| | buffalo urine | 4.0* | 1.7 |
| | buffalo urine/acetone | 1.9 | 1.3 |
| | acetone | 2.6 | 1.4 |
| | * = significant (P<0.05, F = 4.02, df = 3) | | |
| Pyramidal (1) | | | |
| | Unbaited trap | 2.3 | 1.4 |
| | buffalo urine | 6.0* | 1.6 |
| | buffalo urine/ acetone | 2.0 | 1.6 |
| | acetone | 1.8 | 1.2 |
| | * = (P<0.03, F = 3.7, df = 3) | | |

df = degrees of freedom

Table 2.3. Comparison of the performance of 5 different trap types, baited and unbaited in attracting and catching *G. austeni*, in Muhaka forest area.

| Trap | Detransformed mean catches/trap/day | | |
|---|---|---|---|
| | Bait | | no bait |
| | buffalo urine | cow urine | |
| NG2B | 2.0 *b* | 1.3 | 1.1 |
| Biconical | 2.2 *b* | 1.5 | 1.3 |
| Pyramidal | 6.6 *a* | 2.2 | 2.2 |
| 4t-black strips | 2.0 *b* | 1.5 | 1.6 |
| 4t-blue strips | 1.3 *b* | 1.3 | 1.5 |

Means not bearing the same letters are significantly different

($P < 0.03$, $F = 3.68$, $df = 4$)

Table 2.4 Relative effectiveness of urine derived phenols in attracting *G. austeni* to the biconical trap.

| Bait | Detransformed mean catch/trap/day | | female/male difference |
|---|---|---|---|
| | female | male | |
| Distilled water | 2.1 *b* | 2.0 | not sig. |
| 4-cresol (100mg/150 D.water) | 1.5 *b* | 1.3 | not sig. |
| 3-n-propylphenol(6.5mg) + 4-cresol (100mg)/150 water | 1.3 *b* | 1.4 | not sig. |
| 3-n-propylphenol(12.5mg) + 4-cresol(200mg)/150 water | 2.5 *ab* | 1.9 | not sig. |
| 3-n-propylphenol (12.5mg) in 150ml water | 3.0 *a* | 2.3 | not sig. |

means not bearing the same letter are significantly different ($P<0.05$, $F = 3.6$, $df=4$)

sig. = significant

fem = female, mal = male

Table 2.5. Effect of $C0_2$ on trap catches of *G. austeni* in Muhaka forest and Shimba Hils

| Traps | Bait | Detransformed mean catch | female/male difference |
|---|---|---|---|
| Biconical | $C0_2$ | 9.84 * | not sig. |
| | unbaited | 2.79 | |
| | * = sig. (P<0.05, F = 3.54) | | |
| NG2B | $C0_2$ | 11.02* | not sig. |
| | unbaited | 3.77 | |
| | * = sig. (P<0.03, F = 4.84) | | |
| Pyramidal 2 | $C0_2$ | 3.09 | |
| | unbaited | 1.71 | not sig. |
| | F = 2.5 | not sig. | |

data from Muhaka and Shimba Hills was pooled

Table 2.6. Responses of male and female *G. austeni* to buffalo urine and phenols under dry (55%) and wet (>75%) air in a flight chamber.

| Source of odour | Response of *G. austeni* | | | |
|---|---|---|---|---|
| | Female | | Male | |
| | non teneral | teneral | non teneral | teneral |
| Dry air (RH 55%) | | | | |
| Response at peak activity time | 62.5% | 86% | 70% | 100% |
| Response at low activity time | 0% | 15% | 16.7% | 0% |
| Wet air (RH 75-80%) | | | | |
| Response at peak activity time | 56% | 50% | 56.7% | 50% |
| Response at low activity time | 5% | 10% | 20% | 0% |

Table 2.6 Response of *G. austeni* to host odour.

(refer to chapt.3 for peak and low activity periods)

Generally, the response of *G.austeni* to host odour was significantly greater during peak activity periods than during low activity, ($P<0.001$, $F=30$, $df=10$

Response to host odour in teneral flies was significantly greater, under dry ($P<0.001$, $F=125.0$, $df=1$) than under wet air conditions.

Response of teneral males was greater than that of teneral females ($P<0.02$, $F=6.8$, $df=1$)

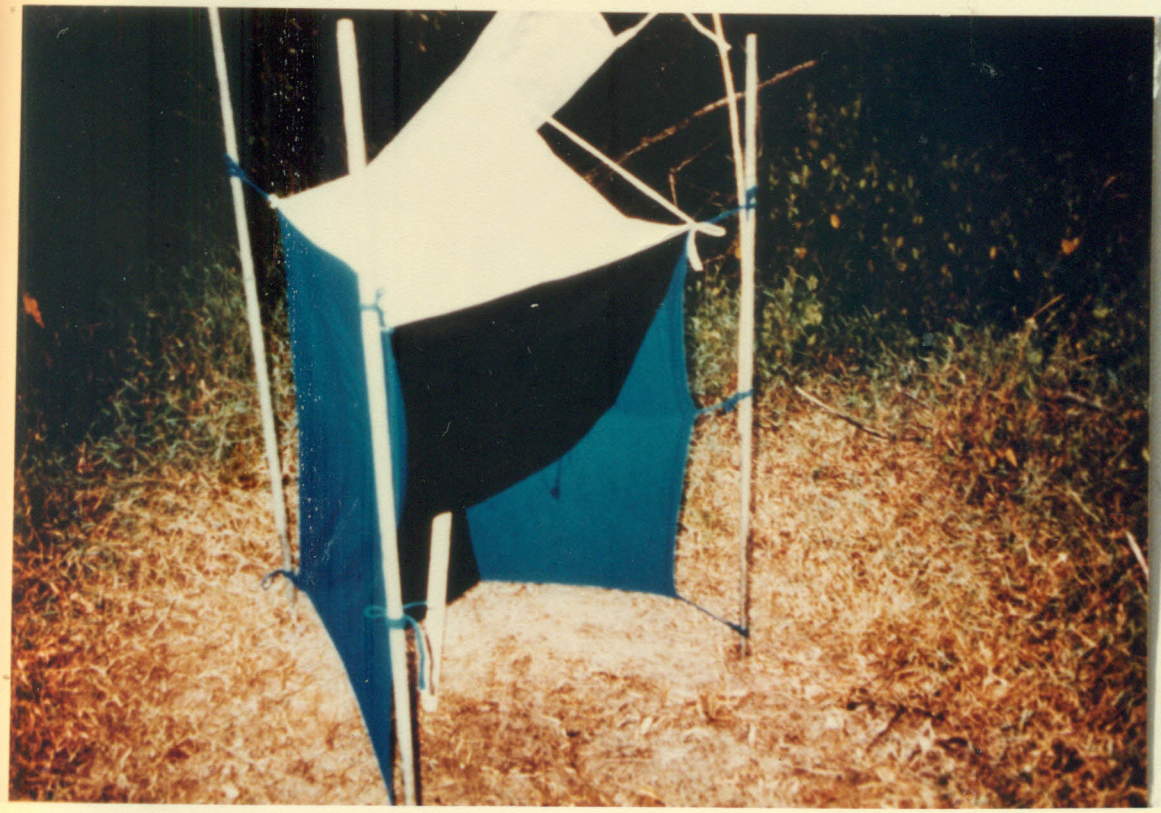
Response in non teneral flies was not significantly different.

Plate 2.1: TRAPS USED IN THIS STUDY

a. Biconical trap (Challier and Laveissiére, 1973) with an odo
bait dispenser near it. The trap is set on the forest floor

b. NG2B trap (Brightwel *et al*, 1987)

a



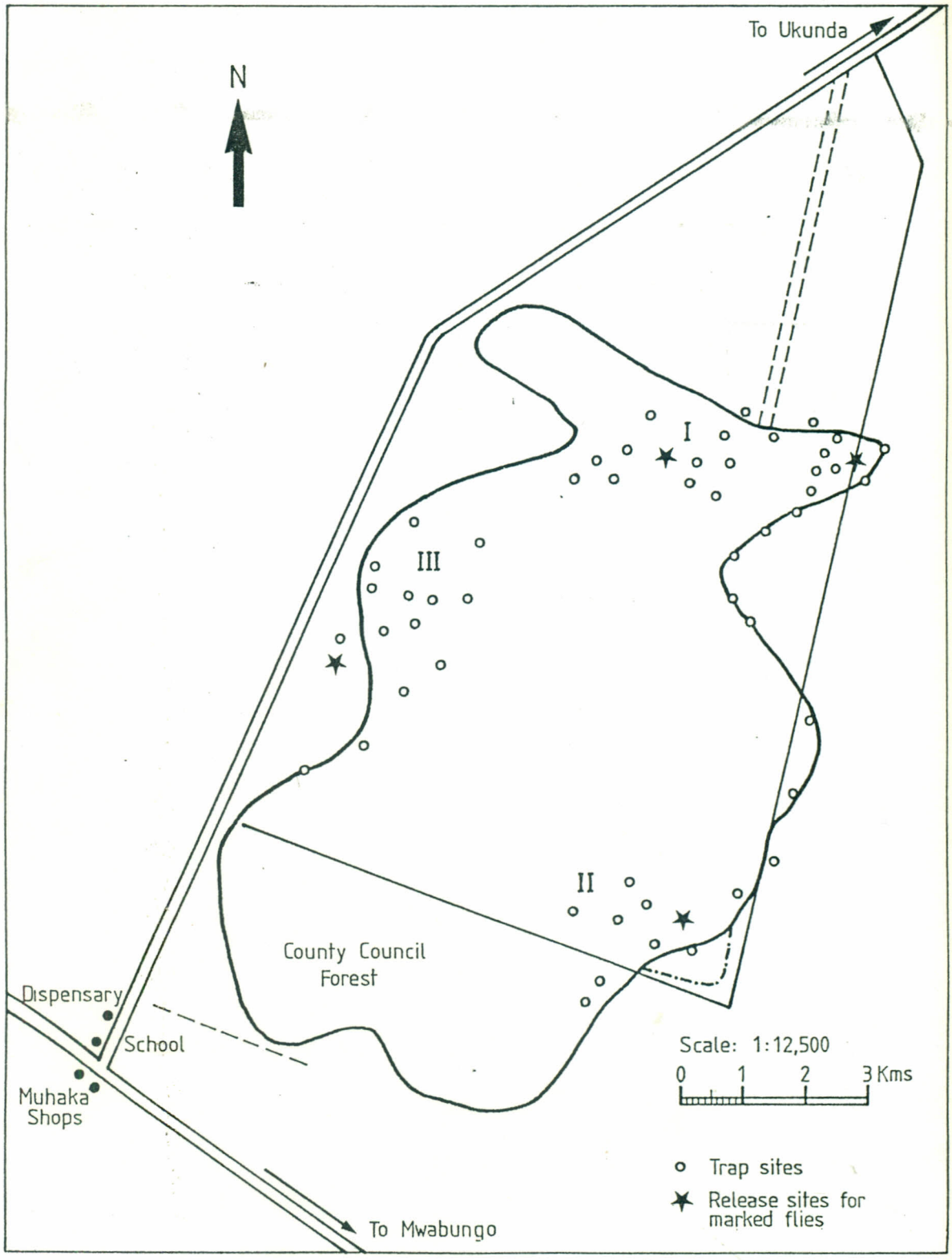
b

Plate 2.2: TRAPS USED IN THIS STUDY (continued)

a. The pyramidal trap (Lancien and Gauteux, 1989)
b. The 4t-trap with blue strips set on the forest edge.

a

b




Trapping sites within Muhaka Forest

Fig. 2.1

Figure 2.1 MUHAKA FOREST AREA

a. The general area of trap sites (marked I to III) used during the evaluation of the performance of the different traps and odours.

b. ★ = Release sites for marked flies (refer Ch.4)

Figure 2.2

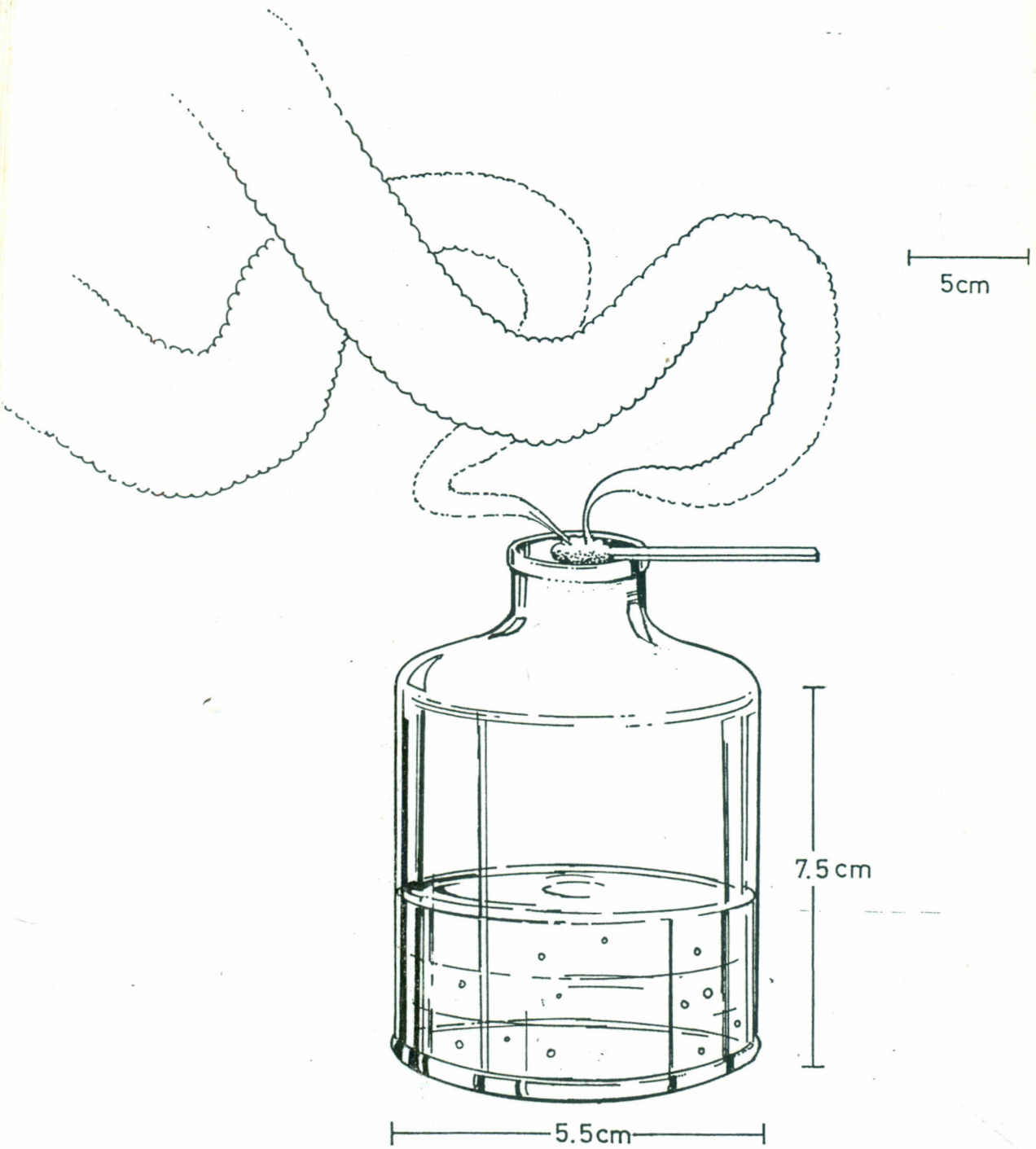


Figure 2.2:

EQUIPMENT USED TO MEASURE THE WIND SPEED AND DIRECTION

Ammonia solution is in the jar, a cotton bud dipped in Hydrochloric acid is held over the open jar. On contact with the fumes emanating from inside the jar, smoke is produced, the speed of which is measured with a string.

It was noted that the smoke changed direction almost every second with the changing of wind direction, creating many zigzags. But the smoke would eventually point to one general direction.

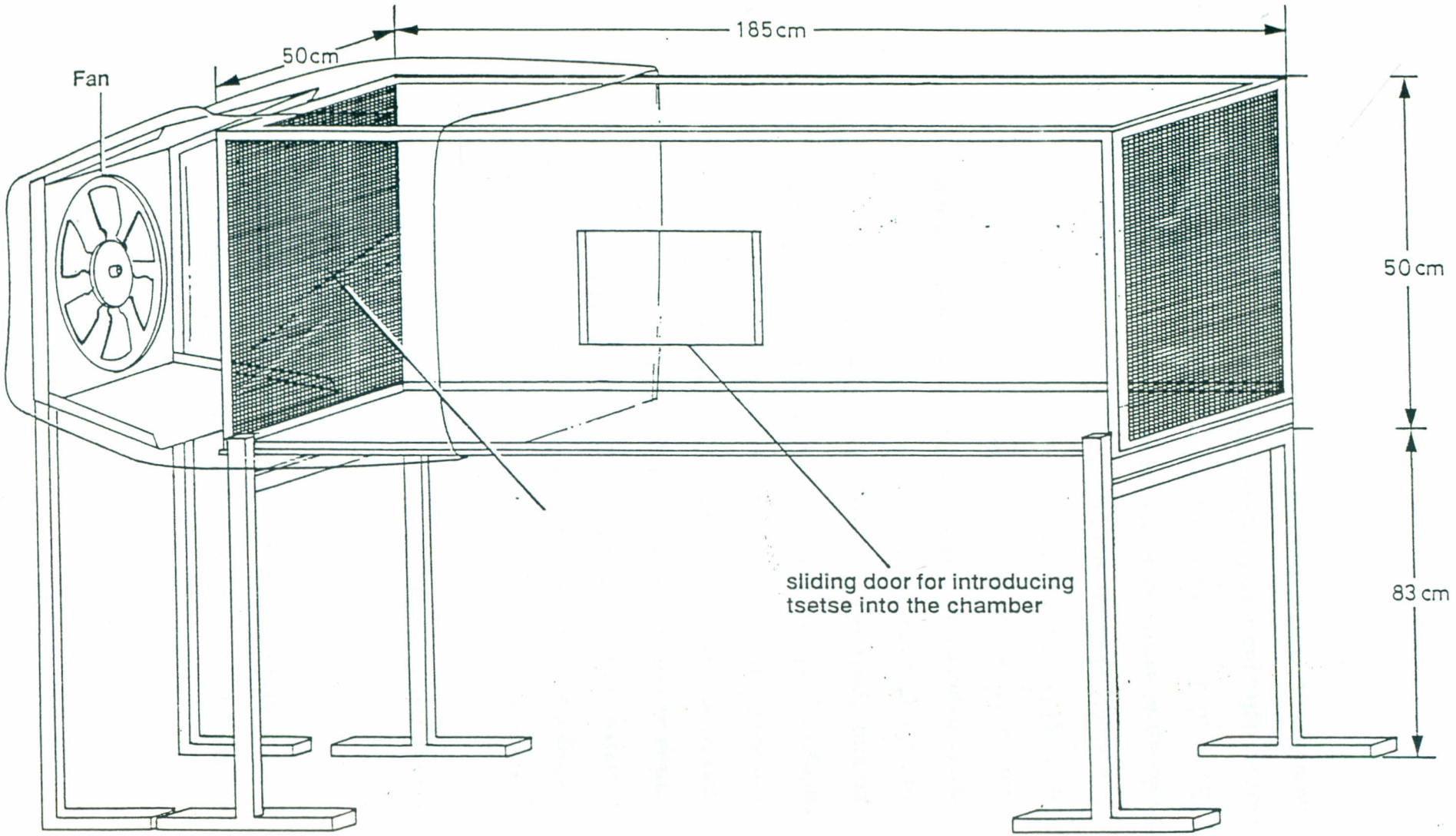
The speed with which the smoke was swept from the source was greater outside the forest than on the forest floor where the canopy effect broke the wind.

The relative influence of wind speed and direction on the emission rate of the odour in and outside the forest was deduced from these measurements.

Figure 2.3: FLIGHT CHAMBER USED IN THE STUDY

Flight chamber, measuring 185cm by 50cm by 50cm. It stood 83cm from the ground, and was made of perspex material, with a wire mesh on both ends (darker than the rest of the body in the Figure). The experimental flies were let in through the sliding door.

Fig. 2.3 Flight Chamber



# Chapter 3

# ACTIVITY PATTERNS AND FEEDING HABITS OF THE TSETSE FLY *Glossina austeni*, Newstead

## 3.1 INTRODUCTION

Insects restrict their activity to certain times of the diel cycle. Hence they may be referred to as diurnal (day-active), nocturnal (night-active), or crepuscular (twilight-active). The mechanisms controlling activity rhythms may be exogenous, that is, direct response to environmental changes; or endogenous, that is, controlled by an underlying circadian oscillations which are part of the physiological make up of the organism (Saunders 1976; Brady 1970; Crump and Brady 1979). Most activity rhythms in insects are a mixture of endogenous and exogenous components. The overt rhythm of activity being controlled by the endogenous oscillation is continuously modulated by the direct effects of the environmental cycles of light and temperature, particularly the abrupt changes in light intensity at dawn and dusk (Saunders 1976). The intrinsic and physiological oscillations controlling the endogenous aspects function as biological clocks, and important regular aspects of activity such as feeding, flight, mate seeking and oviposition (which are performed repeatedly) persist as rhythms.

### 3.1.1 Studies on activity rhythm

Activity rhythms have been studied in many insects such as Cockroaches (Lutz, 1932; Gunn, 1940; Harker, 1956; Roberts, 1960) *Drosophila* spp. (Roberts, 1956; Hardeland and Stange, 1971) and bees (*Apis mellifera*) (Spangler, 1972, 1973); crickets (*Acheta domestica*) ( Lutz, 1932). Saunders 1976 gave a summary of some of the insect families that have been fully investigated and shown to elicit rhythms of activity both as individuals and as populations. Among haematophagous Diptera, the most extensively studied are the mosquitoes (Nayar, 1967), ffor example, *Aedes* spp. (Jones *et al.* 1967; Taylor and Jones, 1969; Nayar and Sauerman, 1971), and *Anopheles* spp.(Jones, Ford and Gillet, 1966; Jones *et al.* 1967). But some tsetse species of the morsitans group have also been studied.

In laboratory investigations, artificial light/dark cycle can be created to provide clues to the physiological nature of the controlling mechanisms which have both endogenous and exogenous components. The endogenous nature of the rhythm is revealed when the organism is transferred from light/dark cycle (LD) into continuous dark (DD) or continuous light (LL) while keeping the other factors such as temperature constant. Under such experiments the endogenous oscillation controlling rhythmic activity reveals its natural periodicity, which in several insects deviate, but slightly from 24 h, so that the onset of peak activity appears earlier or later by a few minutes every day (Gunn, 1940; Harker, 1956; Roberts, 1960). This is the main evidence of endogenous controlling oscillator.

### 3.1.2 Activity rhythm in tsetse flies

In tsetse flies two sets of rhythmic flight activity appear to have been investigated, circadian pattern of spontaneous activity (Brady, 1970, 1972; Etten 1982), and a second type of activity which is usually studied under field conditions, measured by catches obtained either from traps ( Morris, 1960; Smith and Rennison, 1961; Jaensen, 1980; Etten, 1982; Turner, 1987; Mwangelwa *et al.* 1990), bait animal (Curson, 1924; Vanderplank, 1944; Leggate and Pilson, 1961; Smith and Renison, 1961; Pilson and Leggate, 1962; Harley, 1965; and Pilson and Pilson, 1967), fly round (Williams, 1943; Moggridge 1948, 1949; Ford *et al.* 1959) or motor vehicle (Jack, 1941; Jaensen, 1980; Etten, 1982). This type of activity may involve activation, attraction and/or spontaneous activity as well. The end result, which is total catch in a trap or net, does not allow a distinction between them. There is no way to tell which tsetse sighted the trap/bait in the course of their flight and which were 'activated' from the resting site by the presence of the bait/trap. It is also normally used synonymously with biting cycle in tsetse (Moggridge 1948; Turner 1987; Crump and Brady 1979). Brady (1974) reviewed 48 previous records on diel activity on *G. morsitans*, and found that about 50% of them showed clear V- shape, comparable to the pattern of sponteneous activity he obtained in laboratory studies. He concluded that at least 80% of the pattern of activity observed in the field was controlled by the endogenous circadian rhythm, and only 20% by the environmental physical factors.

### 3.1.3 Importance of study of activity in tsetse flies

The availability of tsetse flies for capture is a function of population density and flight activity, other factors being equal. This is well borne out in the reports given by Nash (1969). He related how a colleague and himself failed to catch *Glossina medicorum* in an area in Nigeria, using bait pigs or traps during day time, but when they resorted to searching the vegetation, they were able to capture the species in substantial numbers, revealing its presence in that area. *Glossina medicorium* is a dusk-dawn-active species, the distribution of which could only be studied by developing catching methods suited to its activity times. Neave (1912), Lewis (1942), and Power (1964) reported similar phenomenon for *G. longipennis*, stressing that during the day it was totally inactive, and could not be detected at all.

Activity pattern tells of the insects' habits from which it can be deduced whether the species is important with regard to trypanosome transmission or not. It is an important aspect of the ecology of a species. However, from the point of view of disease transmission, dawn-dusk-active species may only be important in areas where pastoralists herd their cattle to move to new pastures at night or very early in the mornings, e.g, among the Fulani of Nigeria or the East African pastoralists such as Samburu, Rendile, Boran and Maasai. It is in this connection that most of the day-active savana tsetse species such as *G. pallidipes* and *G. morsitans* have been considered of more economic importance than the forest fusca group, many of which are crepuscular (Ford, 1971). Flight activity in tsetse may be triggered by hunger (hence search for food), need for microclimate (hence

search for resting places), male's search for mates, or female's search for larviposition sites. The present study was directed mainly at activity in connection with feeding or search for hosts.

### 3.1.4 Activity and feeding in tsetse flies

Jackson (1933) and Bursell (1961) recognised 4 phases of hunger in tsetse which influence flight activity in relation to feeding. These are actually stages of digestion and reconstruction of fat reserves. Stage 1 was the gorged phase and comprised the period when blood meal was concentrated by excretion of water. This stage was characterised by complete inactivity when flies were not responsive at all. Stage 2 or replete phase started after partial digestion of blood meal and replenishment of fat stores had taken place. It was a phase of much spontaneous and random activity, but the flies did not respond to a host. During stage 3 (intermediate stage) activity continued at a high level and it was directed towards feeding. Stage 4 was a hungry stage, when any movement stimulated feeding reaction, i.e. flies were so hungry that they would feed on any vertebrate they could get. However,external factors such as temperature, and relative humidity influenced this behaviour. For example, during the hot seasons the rates at which food reserves were expended increased (Rajagopal and Bursell 1966), or light and temperature influenced the reponses of tsetse (Jack and Williams 1937, Turner 1987).

Brady (1970) defined 5 characteristics of the spontaneous activity in tsetse. Among them was an underlying circadian rhythm, activity influenced by increasing hunger, and sexual appetitive

activity that had earlier been suggested by Bursell (1961). In the field it is rather difficult to separate individual flies that fall in the different categories mentioned here. With the present state of the art, there is no selective trapping methodology for the various categories. Neither has there been selective methods to study age/sex related activity, but many field workers have concluded, from fly round data, that male tsetse are active most of the time while females are active only when hungry (Jackson, 1933, Ford *et al.* 1959; Bursell, 1961). Bursell (1961) described the behaviour of non-hungry males as being sexually appetitive, and found that such males spent more time in flight than females of corresponding hunger stage.

Little has been published on the biting cycle or activity pattern of *G. austeni* in the wild. The only report appears to be that of Moggridge (1949) who reported having captured highest numbers between dawn and 1000 h, using the fly round method of sampling. Crump and Brady (1979) observed the spontaneous activity of *G. austeni* under laboratory conditions at a constant temperature of 26.5 degrees Celcius, and found that the peak activity was in the afternoon; implying that *G. austeni* is predominantly an afternoon feeder.

The feeding habits of tsetse are usually inferred from the remains of blood meals recovered from individual flies, (Weits and Glasgow, 1956; Glasgow *et al.* 1958; Weitz, 1963; Snow and Boreham, 1979; Snow, 1984; Snow *et al.* 1988; Turner 1987). This is identified by serological methods and traced to the species the fly had taken blood from. By comparing the identity of the animals as revealed by

blood meal analysis with the list of the animals available in the area, it is possible to infer the preference of the fly species under study.

## 3.3 OBJECTIVES OF STUDY

This study of the activity pattern and feeding habits of *G. austeni* was made to (1) determine the environmentally modulated pattern of flight activity in the field populations of this species, particularly as it relates to host seeking. (2) study flight activity of hungry flies in response to host odour under controlled temperature and humidity conditions in the laboratory, in order to determine the influence of climate on the biting cycle of this species. (3) use the field and laboratory results to determine the proportions of the field activity pattern controlled by the endogenous rhythm and those controlled by environmental factors.

## 3.4 MATERIALS AND METHODS

Experiments were conducted under both field and laboratory conditions. Two methods were used in the field, the two hourly trap catch and continuous, 14 h, vehicle catch. Both were conducted within the forest floor. Eight traps were used (three biconical, three pyramidal and two NG2B traps). The vehicles used were a Subaru van that stands 183 cm high and 335 cm long, with six windows (3 on either side) measuring 113 cm from ground level, or a Land Rover that stands 190 cm in height and 420 cm in length, with six windows (3 on either side) measuring 130 cm from the ground level. Both

vehicles were white in colour. Laboratory observatons were carried out in the flight chamber described in Chapter 2, section 2.5, and Fig. 2.3.

### 3.4.1 Observations on activity of *G. austeni* in the field

Two hourly harvests were made from 8 traps starting at 0700 and ending at 1900 h. The traps were set 60 to 80 metres apart. Trap-cages were put in place at 0700 h, and the first two hourly harvest taken at 0900 h. Four traps were set along a cleared transect with the sites relatively open. The other four were set in isolated sites under canopy, where relative humidity during daytime was between 3 and 10% higher, and temperatures 1 to 5 degrees centigrade lower than that outside of the forest, or in the open transect.

For the vehicle catches, the vehicle was parked in a chosen position within the forest from around 0400 h to 1830 h, with two catchers sitting inside the vehicle and three of the six windows open (two on one side, one on the other). As the tsetse came into the vehicle the catchers captured them using hand nets. The time of capture was noted and the temperature and humidity readings were taken at hourly intervals, using a portable thermohygrometer (Cole-Palmer Instrument, model 3309-60, Chicago, Illinois). The two hourly observations were made for five days each month, for 9 months from November 1989, while the vehicle catches were made for four days each month for 8 months from February 1990. The vehicle catches were expressed in terms of catch per hour as a percentage of

the total day's catch, and the trap catches were expressed as catch per two hours as a percent of the total catch per day per trap.

### 3.4.2. Studies on activity of *G. austeni* in the laboratory

Tsetse flies used in laboratory experiments were either those emerging from field collected-pupae, or first generation offspring of those emerging from field collected pupae. They were maintaned in the laboratory under 12:12 hour light:dark cycle (LD), 25$^{o}$C temperature and 70% relative humidity.

Flight activity of the flies was observed directly at various times between 0600 and 1900 h, and under wet air (75-80% relative humidity) and relatively dry air (50-55% relative humidity) conditions. About forty flies were observed at each hour under each humidity regime. That is, 30 at 0600 h, another thirty at 0700 h, another thirty at 0800 h and so on. An assessment of the peak activity time under the given conditions, was then made for each category of flies (non teneral females, teneral females, non teneral males and teneral males) as indicated below.

Non-teneral flies were tested on their third day of hunger. Teneral flies were tested on their second and third days of life. This was because preliminary work had shown that flies tested on the first day of the hunger cycle did not fly about, nor respond to odour sources. Five flies from the same category were tested in the chamber at a time. They were let into the chamber at least three hours before the test. The odour source was introduced on the windward side and the flies' flight activity and/or response to odour observed (see also chapter 2, section 2.5).

### 3.4.3 Assessment of feeding habits and frequency

In order to determine the hosts of *G. austeni*, smears of the gut contents of captured flies which, by external appearance, were judged to contain some undigested blood were made on Watson's (No.4) filter paper. The source of the blood meal was later identified with the help of scientists at the Robert von Ostertag Institute, Berlin, Germany.

For the determination of feeding frequency, the mark-release-recapture method was used (Jackson, 1937, 1949; Randolph and Rogers, 1984). This was also part of a study to determine dispersal and population density, and the method of marking is described in chapter 4, section 4.5.

## 3.5 STATISTICAL ANALYSIS OF DATA

Activity pattern was measured in terms of the proportion captured at a particular time of the diel cycle, out of the total captured over the whole period (12 hours in the case of trap catches and 14 h in the case of continuous vehicle catches). Laboratory observations of activity were expressed in terms of the proportion of *G. austeni* responding to odour source at a particular time, out of the total tested at that time. This gave percentages of response for each of the twelve hours, and these, in turn, were plotted in the form of response against time.

## 3.6 RESULTS

### 3.6.1 On observation of activity of *G. austeni* in the field

The results of trapping experiments conducted to determine whether *G. austeni* is a nocturnal or a day-active fly are presented in Table 3.1. It is noted that daytime catch was significantly greater than night catch ($P<0.01$, $F = 12.8$, $df = 2$), and not significantly different from 24 h continuous catch. Most of the flies collected under night catch entered traps between 0600 and 0630 hours.

The activity pattern of males and females from the two hourly catches, are presented in Fig. 3.1 (1 to 3) showing the pattern of activity under canopy, open transect conditions and the mean of the two, respectively. *Glossina. austeni* was, generally, active throughout the day, from about 0600 h to 1800 h [Fig. 3.1 (1 to 3) and Fig.3.5]. The pattern of activity was bimodal with a peak in the morning and another one in the afternoon, and with a midday depression showing a period of less activity. For males, the morning peak was absent under canopy conditions [Fig. 3.1 (1)], where activity showed only the afternoon peak, and the build-up to the peak was more gradual. On the other hand, under the open transect or at the ecotone, a bimodal pattern of activity was evident, the morning peak emerging around 1100 h and the afternoon one between 1600 and 1700 h.

The diel activity obtained by continuous vehicle catch, and expressed in terms of hourly intervals is presented in Fig. 3.2, accompanied by saturation deficit (vapour pressure deficit) curve. From the Figure, a relationship is evident between activity and

saturation deficit, in the morning and late afternoon. However, around midday, the activity fell inspite of rising saturation deficit (Fig.3.2).

In the same Figure (3.2) the mean activity curve is plotted against temperature and relative humidity. The morning peak activity occurred during what seemed to be the optimum conditions of temperature and humidity interaction for *G. austeni*, when the temperature was about 30°C and relative humidity about 77%. However, a similar condition in the afternoon resulted in a much smaller peak, the bigger afternoon peak preceed it.

Figs. 3.3 and 3.4 show the mean activity results obtained on four days, two of highest and the other two of lowest activity in the field, respectivily. It is noted that on the days of high activity, the pattern was bimodal with a morning peak around 0700 to 0900 h and an afternoon peak around 1300 to 1500 h. On the second occasion (days of little activity) only one fly was captured on each of the two days (at 0815 h on the one and 1545 h on the other). There was no significant difference in the pattern of recorded climatic parameters, such as relative humidity, saturation deficit, and temperature on those contrasting activity days. However, notes made on other environmental attributes show that on both occasions of high activity, the sun was obliterated by clouds for most of the day. On one of the two days of highest activity, it drizzled the whole morning and part of the afternoon, but even when it stopped drizzling for a while, and there was some sunshine at 1340 h, the high activity continued and the afternoon peak became very prominent. Only three males were captured on each of the high activity days, and they were left out of

the graph shown. On the days of least activity there was sunshine throughout, with hardly any clouds being observed on the sky.

Activity of hungry tsetse responding to host odour in a flight chamber in the laboratory, in the absence of visual stimulus (trap), is shown in Fig. 3.5. The patterns of tenerals are shown side by side (Fig. 3.5, a), while that of non tenerals are also shown side by side in Fig. 3.5, b. There was no significant difference between the pattern of activity in tenerals and non tenerals. The activity curves of all the four categories of *G. austeni* (teneral males and females; and non teneral, males and females) indicated a bimodal pattern with a period of less activity around midday, thus confirming the results obtained in the field observations. The deppression in the activity curve was more marked in the tenerals than in the non tenerals.

### 3.6.2 Feeding frequency and host preference

The mark-release-recapture data provided some insight into the feeding cycle (interval) of *G. austeni*. Some of the flies marked and released without feeding were recaptured back in traps within 24 h, while among those fed before release 20 % of all recoveries were recaptured 3 days after release, and 40 % after they had been away for days divisible by three, such as 9, 12, 27, 66, and 90 (Table 3.2). This may indicate a three day feeding cycle. About 62% of the recaptures of February to June ( the release sights used were 10 to 60m from the edge of the forest) were captured in traps set at the ecotone, or along the cleared transect and they were all hungry with virtually no remains of blood visible through the abdomen, indicating

that they were in search of hosts. The flies released much deeper into the forest (100m or so inside) on the south west side were mostly recaptured within the forest floor, except about 20% which were recaptured in traps close to the edge.

The results of blood meal analysis of 30 samples collected from Muhaka and one from Shimba Hills, are shown in Table 3.3. The favoured hosts in Muhaka were warthog (60% and 21% of the total identified meal in female and male *G. austeni* respectively) and bushpig (33% and 57% of identified meals in females and males respectively). One male *G. austeni* had fed on bushbuck (7% of total identified *G. austeni* male meals), two fed on humans (14%) and one female fed on chicken (7% of total identified female *G. austeni* meals). The lone blood meal from a male fly identified from Shimba Hills had come from a buffalo. In comparison, ten out of 17 *G. brevipalpis* had fed on warthog, four on bushpig two on Felidae and Canidae (probably cat and dog), and a lone one from Shimba Hills, on buffalo.

## 3.7 DISCUSSION

The present study showed that *G. austeni* is a day-active tsetse. Moggridge (1948) reported catching this species, in fly rounds, at night, when flies approached the catchers to feed, sometimes during moonlight but also during dark night. Perhaps this happens from time to time especially during the dry periods. The main hosts of this species in Muhaka as shown in Table 3.3 are the pigs (bushpig and warthog). Among them bushpig is nocturnal, and in Muhaka they have the habit of getting out of the forest at around 1830 h, going into

the cassava plantations nearby, and returning by about 0500 to 0600 h. Bursell (1961) made the observation that the effects of temperature and relative humidity break down in the absence of light. Perhaps on days when the effects of light-temperature-relative humidy have been adverse, some flies may seek hosts at night. Be that as it may, the night activity would be occasional, just as Glasgow (1970) and Vanderplank (1948) reported spotting *G. pallidipes* feeding on moonlit and dark nights respectively, yet *G. pallidipes* is an established daylight species. The more, or less regular movement of bushpig in Muhaka seemed partly to coincide with the high activity observed in the fly around 0600-0700 h, which then drops temporarily only to start rising gradually again to a peak later in the morning. Traps placed along the known paths of bushpigs tended to catch more flies.

A number of workers have shown that various species of tsetse have a V- (or U)-shaped pattern of daily activity in the field (Nash, 1937; Bursell, 1957; Pilson and Pilson, 1967; Rowcliffe and Finlayson, 1982; Mwangelwa *et al.* 1990); although Etten (1982) reported observing only one peak, which was in the afternoon. This kind of pattern was construed to mean that the flies attacked hosts most intensely in the mornings and late afternoons. Further more, most of these workers have recognised the probability of a complex interaction of exogenous factors and endogenous programme being responsible for the modulation of the activity in tsetse (Mellanby, 1936; Bursell, 1961; Brady 1971). The U-shaped pattern of activity shown in the present study (Figs. 3.1 and 3.2), therefore, conforms to that known in other tsetse species of the morsitans group such as *G. pallidipes* (Jack and Williams, 1937; Bursell, 1957) and *G. morsitans*

(Brady, 1972; Brady and Crump, 1978). Brady (1972) showed experimentally that the U-shaped pattern reported from field studies also occurred in the laboratory and must, therefore, be at least partially and to a greater extent, the expression of an endogenous circadian rhythm.

One important question that arises is as to what extent is the field U-pattern due to the direct effect of the environment and to what extent is it due to endogenous control. Another point that may be raised is as to whether the effect exerted by the environment be greater in those tsetse species that inhabit climates with greater gradients of temperature range such as arid areas, than in those in places where the temperature gradients are lower such as coast conditions. For *G. morsitans*, Brady and Crump (1978) suggested that the U-pattern occurred indepentent of temperature and that the activity of *G morsitans* was positively correlated with temperature only in the mornings and evenings (upto 34°C). The U-shape was the outcome of a built-in (endogenous) programme.

In this study, a U-shaped pattern of activity was shown to occur both under field and laboratory conditions, where the temperature and relative humidity were constant (Figs. 3.1 and 3.2). However, in males from the two hourly trap catches from two different biotope types (ecotone and forest floor under canopy) the pattern differed. In the former area it was bimodal while in the later area it showed a gradual increase to one afternoon peak. However, the more detailed (and probably more accurate) continuous catch, shows a morning peak of activity as well as an afternoon one on the forest floor. This may partly be explained on the basis of the forest floor (where the

traps were set) being much cooler during mornings, whereas the vehicle made an opening in the vegetation.

The data shown on Figures 3.3 and 3.4 indicate that direct effect of the environment can influence the pattern of activity in *G. austeni*. High activity was recorded sometimes on days of exceptionally high humidity, and especially during drizzles. Jackson (1933) reported a similar phenomenon in *G. swynnertoni*, and noted that the main factors affecting variation in its appearance to catchers in fly round were metereological, far more than animal movement. " The species seemed scarcely to become hungry at all during seasons when the evaporation rate was low. Jack and Williams (1937) reported that the combined effects of temperature and light influenced the activity of *G. morsitans*, which became negatively phototactic at 32° C. This was confirmed by Pilson and Pilson (1967), but they said that in the field, any temperature above 30°C had a negative effect on flight activity of *G. morsitans*. In the present study, the light-temperature adverse effect seemed to occur from about 30°C if the humidity was low (well below 70%). On the other hand, all the three parameters, light, temperature and humidity were always more moderate in the forest floor than outside, explaining why there was more activity under canopy. Bursell's (1961) observation on the breakdown of temperature-humidity effect in the absence of light, may also help to explain why in this study, more flies were captured in traps when the sun was obliterated by clouds. Saturation deficit (Vapour pressure deficit) has been reported to influence activity of tsetse under field conditions (Takken pers com; Snow, 1981). In the present study, a correlation between saturation

deficit and activity was noticeable (Fig. 3. 2) in the mornings and late afternoons, but not during day time, when the midday depression in activity occurred inspite of rising saturation deficit. Perhaps when the saturation deficit is too high in the daytime it exerts a depressing effect on the activity. However, since the midday depression is persistent even under controlled laboratory conditions, it is reasonable to assume that this pattern is under the direct control of the endogenous programme.

Barrass (1968) showed that under constant temperature and humidity conditions in the laboratory, female *G. morsitans* showed greater activity. In this study, *Glossina austeni*, also, showed greater activity under constant conditions, and in relation to feeding. There was also greater response to host odour during conditions of dry (50-55% relative humidity) rather than wet air (75-80% r.h.) in the laboratory (refer Chapter 2, Table 2.6). Such increased activity (in search of host), during dry air conditions was also observed by Jackson (1949) in field populations of *G. swynnertoni* in Tanzania. And he suggested that it was due to increased hunger. Rajagopal and Bursell (1966) explained it by rapid depletion of fat reserves caused by dry air conditions. At any rate it is distinct from increase in random non-directed flight that may occur during high humidity, which is not dictated by hunger. Flies in the latter condition do not necessarily respond to host odour.

The activity of teneral flies could not be obtained by the direct observation in the field, as very few were captured. Their activity as observed in the laboratory, where only olfactory stimulus was presented, and the sexes tested separately, indicated a parallel

pattern to that of the non tenerals. In the tenerals, sexual appetitive behaviour could not have interfered with activity in response to feeding requirements. Vanderplank (1948), Bursell (1961), and Davis-Cole and Chaudhury (1990) indicated that teneral females of *G. morsitans* and *G. pallidipes* mate within five or so days of their life while the males become sexually mature after day seven or so (Vanderplank, 1948), so that at age 2-4 days their priority is a blood meal, and there is no sexually appetitive behaviour in them. This seemed to be so with *G. austeni* as well.

The non teneral males' unimodal pattern of activity observed within the forest floor, under canopy conditions, may not be an artifact, it does concur with the findings of Crump and Brady (1979) for *G. austeni* in the laboratory. Their results showed one peak of spontaneous activity (for males) in the afternoon. But then they did not present host (or any) odour, but tested spontenous activity, controlled by endogenous mechanisms. A similar pattern was obtained for *G. pallidipes* in the same area (Owaga, unpublished reports) in the absence of odour, but when host odour was introduced, a bimodal pattern of activity emerged. The unimodal peak, therefore, represents "non-host seeking activity" in males.

In view of the fact that similar patterns of female activity persisted both under constant laboratory and variable field conditions, it is reasonable to conclude that the main control is exerted by the endogenous programme and that the influence of the environmental conditions in modifying the pattern is little, and may occur only when these conditions are exeptionally adverse. The flies probably seek microclimates within the forest, and these do not change much, so that there is no need for the modification of the

endogenous programme. In males also the host seeking activity is similarly controlled, but due to mate seeking activity, and the habit of being more mobile and ranging widely, the pattern may vary a little, but not significantly from that of females; hence some secondary peaks may appear.

Feeding in tsetse can occur irregularly over a wide range of intervals. Glasgow (1961) reported between 1 - 15 days for *G. swynnertoni*. For *G. pallidipes* in the Lambwe valley, Kenya, Turner (1987) reported 3 to 5 days, while Etten (1982) reported about 4 days for *G. pallidipes* in Nkruman, southern Kenya, and 3 to 4 for the same species at the coast. Bursell (1966) found that *G. morsitans* fed with a range of from 10 to 80% of their full reserve load left.

In the present study, the indication from mark-release-recapture study was a cycle of roughly three days. A proportion of flies hunt for hosts around the edge and other open spaces within the forest, but the majority hunt under canopy in the forest. This is deduced from the recapture of fewer marked flies in traps set at the edge, and a greater proportion in traps set deep inside the forest. Also from the identified blood meals, many of which were from warthogs and bushpigs, but a few were from humans, chicken cats and dogs. These results also indicate that *G. austeni* is a rather sedentary species, which does not disperse very far (see Chapt. 6). Some of the main hosts of *G. austeni* e. g., bushpig, would be expected to lie in shady places, or holes in the forest since they are nocturnal, but others, e.g. bushbuck, may roam around the edge or other clumped bushes outside the forest. Bushbucks were encountered on 4 occasions during daytime on the edge of the forest and on the cleared transect,

while bushpigs were seen on ten occasions either after 1830 h getting out of the forest and heading for cassava farms, or between 0530 and 0600 h coming back into the forest.

The presence of chicken and human on the list of hosts fed on calls for comment. There are homesteads within a few metres of the forest; and on the eastern side, one such homestead is on the edge of the forest. Further more, women frequently gather fire wood within the forest. It was also noted that male *G. austeni* took more meals from warthog than bushpig and vice versa for female *G. austeni*. Perhaps males ranging further afield get in contact with the diurnal warthog more frequently in the relatively open spaces where it feeds. Warthogs were often encountered along the open transect during the rainy season, when there was lush grass on this cleared area. The list of hosts fed on (Table 3.3) is comparable to the only other such results on *G. austeni* (Weitz and Glasgow 1956). These authors analysed 187 samples of blood meal collected from Jahazi forest in Zanzibar. The animals present were listed as, bushpig, pigmy antelope (*Nesotragus moschatus*), duiker (*Cephalophus adersi*), leopard (*Panthera pardus*) and cattle (*Bovis indicus*). The results of the analysis showed that 88% had come from bushpig and 12% from cattle. They indicated that evidence of feeding on cattle was in flies captured on the fringes of the forest. In this study, the sample from Shimba Hills, where large mammals abound, was identified as having fed on buffalo. This indicates that the two suids (warthog and bushpig) need not be the preferred host where a buffalo, and probably other large mammals, are present. Further proof of this may be the fact that buffalo urine was relatively effective in attracting *G.*

*austeni* to traps while bushpig urine, was tested as bait for two consecutive days, but the trap did not capture any *G. austeni.*

From laboratory observations, it appeared that the rate of hunger and search for host in *G. austeni* may be slower, during rainy season (or high humidity and cloudy periods). The flies were not responsive to host odour, in the flight chamber, until they were in day four to five of the hunger cyle (when relative humidity was 70% or above) or day two to three of the hunger cycle (when humidity was 55% or lower). When left unfed for a week under high relative humidity conditions, the flies would still fly about actively after day 6 of hunger.

Table 3.1. A comparison of trap catches of *G. austeni* from three trapping regimes, day, night and continuous 24 h period, as a measure of activity time. (Duncan's multiple range test on the means)

| | Detransformed mean catch/trap/day | |
|---|---|---|
| | females | males |
| Day time | 6.1 *a* | 3.2 |
| Night time | 2.0 *b* | 1.5 |
| Continuous 24 h catch | 7.5 *a* | 4.2 |

Means not bearing the same letters are significantly different,

$P < 0.01$, $F = 12.8$, $df=2$

Anova test performed to compare the three regimes was very significant ($P<0.001$, $F = 15.9$), subsequently, Duncan's range test was performed on the means to determine which trapping regime gave significantly the highest catch.

Table 3.2 Recapture of marked *G. austeni*

| Days after release | % of recapture |
|---|---|
| 3 | 20% |
| 6, 9. 12] | |
| 27, 66, 90] | 43.3% |
| Other days | 36.7% |

From this table it may be deduced that *G. austeni* feeds roughly at three day intervals

Table 3.3 A list of possible hosts of *Glossina* in Muhaka forest and Shimba Hills

| Animals present (Muhaka) | % of total identified meal by *G. austeni* | |
|---|---|---|
| | female | male |
| Red duiker (*Cephalophus adersi*) | – | – |
| Grey duiker (*Cephalophus monticola*) | – | – |
| Bushpig (*Potamochoerus porcus*) | 33% | 57% |
| Warthog (*Phacochoerus aethiopicus*) | 60% | 21% |
| Bushbuck (*Tragelaphus scriptus*) | | 7% |
| Kirk's dikdik (*Rhynchotragus kirkii*) | – | – |
| Black and white monkeys (*Colobus polykomos*) | – | – |
| Baboons (*Papio anubis*) | – | – |
| Bushbaby (*Galago senegalensis*) | – | – |
| Human (*Homo sapiens*) | – | 14% |
| Chicken | 7% | – |
| Dogs (*Canis* sp.) | – | – |
| cats (*Felis domestica*) | – | – |
| reptiles (snakes, lizards) | – | – |
| (Shimba hills) | | |
| Sable (*Hippotragus niger*) | – | – |
| Elephant (*Loxodonta africana*) | – | – |
| Buffalo (*Syncerus caffer*) | – | 100% |
| Bushbuck | – | – |
| Bushpig | – | – |
| Warthog | – | – |
| Coke's hartebeest (*Alcelaphus buselaphus cokii*) | | – |
| Monkeys | – | – |
| Baboons | – | – |
| Reptiles | – | – |

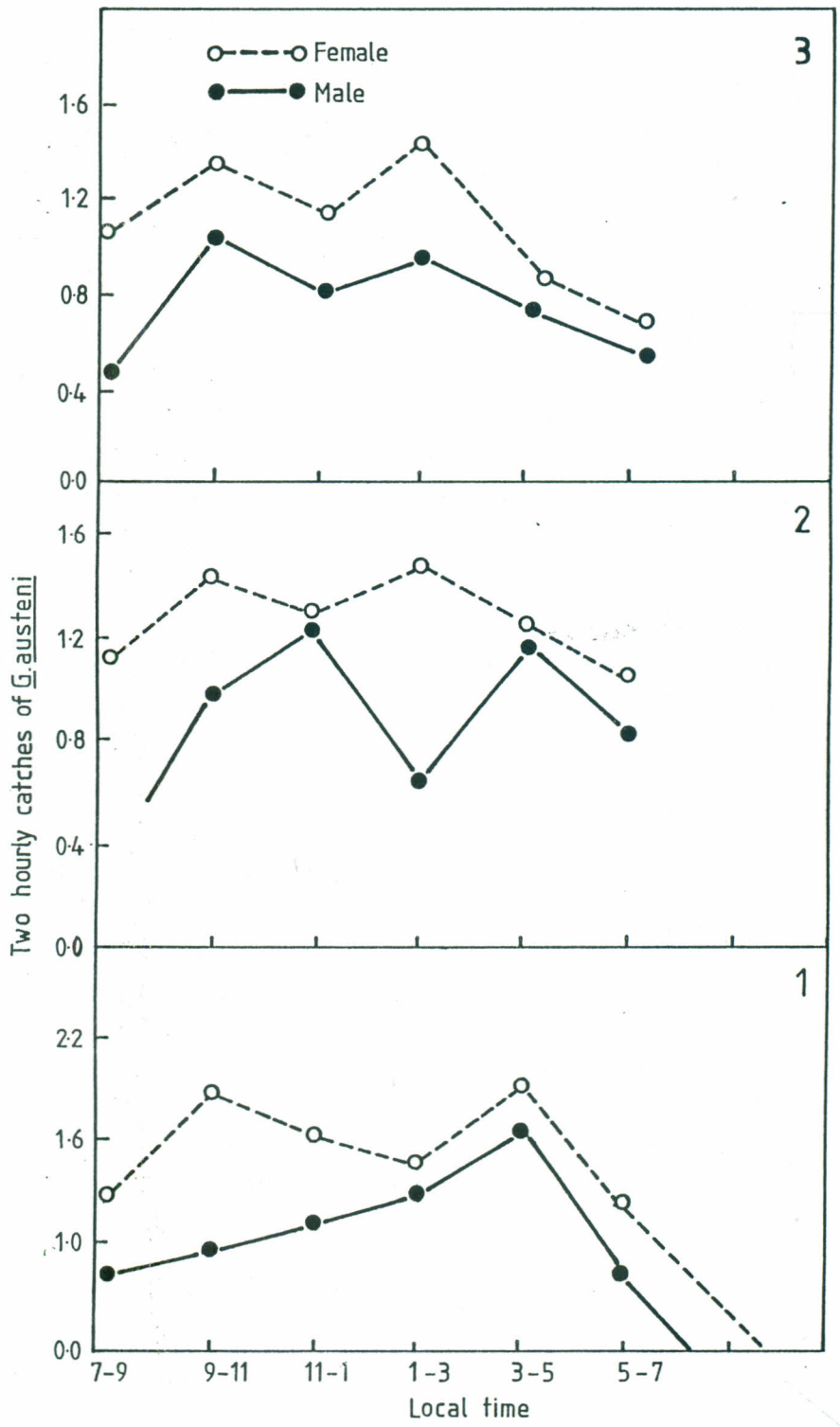
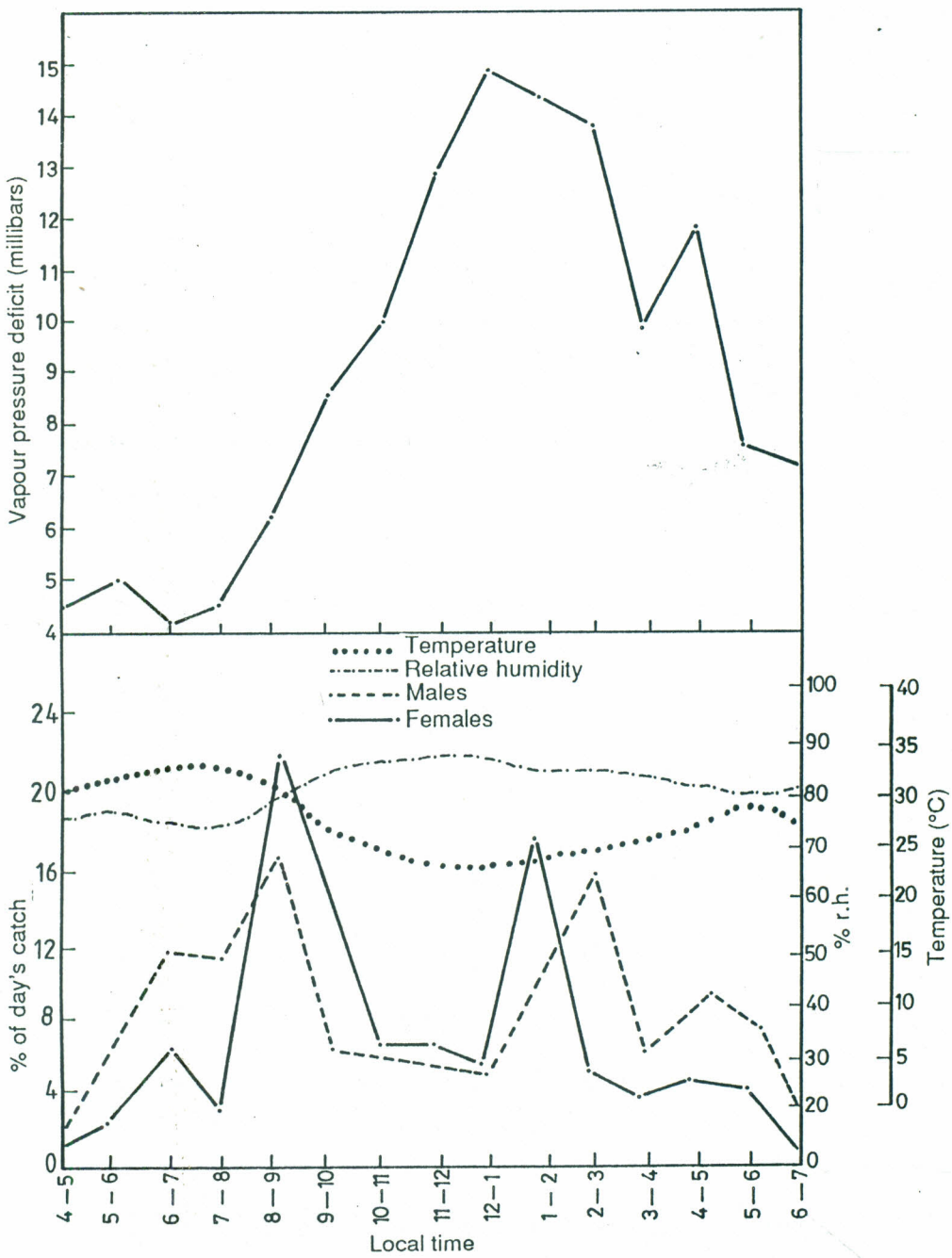

Female
Male
3
2
1
1·6
1·2
0·8
0·4
0·0
2·2
1·0
Two hourly catches of G.austeni
7-9
9-11
11-1
1-3
3-5
5-7
Local time

Fig. 3.1

Figure 3.1: ACTIVITY PATTERN OF MALE AND FEMALE *G. austeni*

Key.

1. on the forest fringes
2. Within the forest floor
3. mean activity from the two biotopes



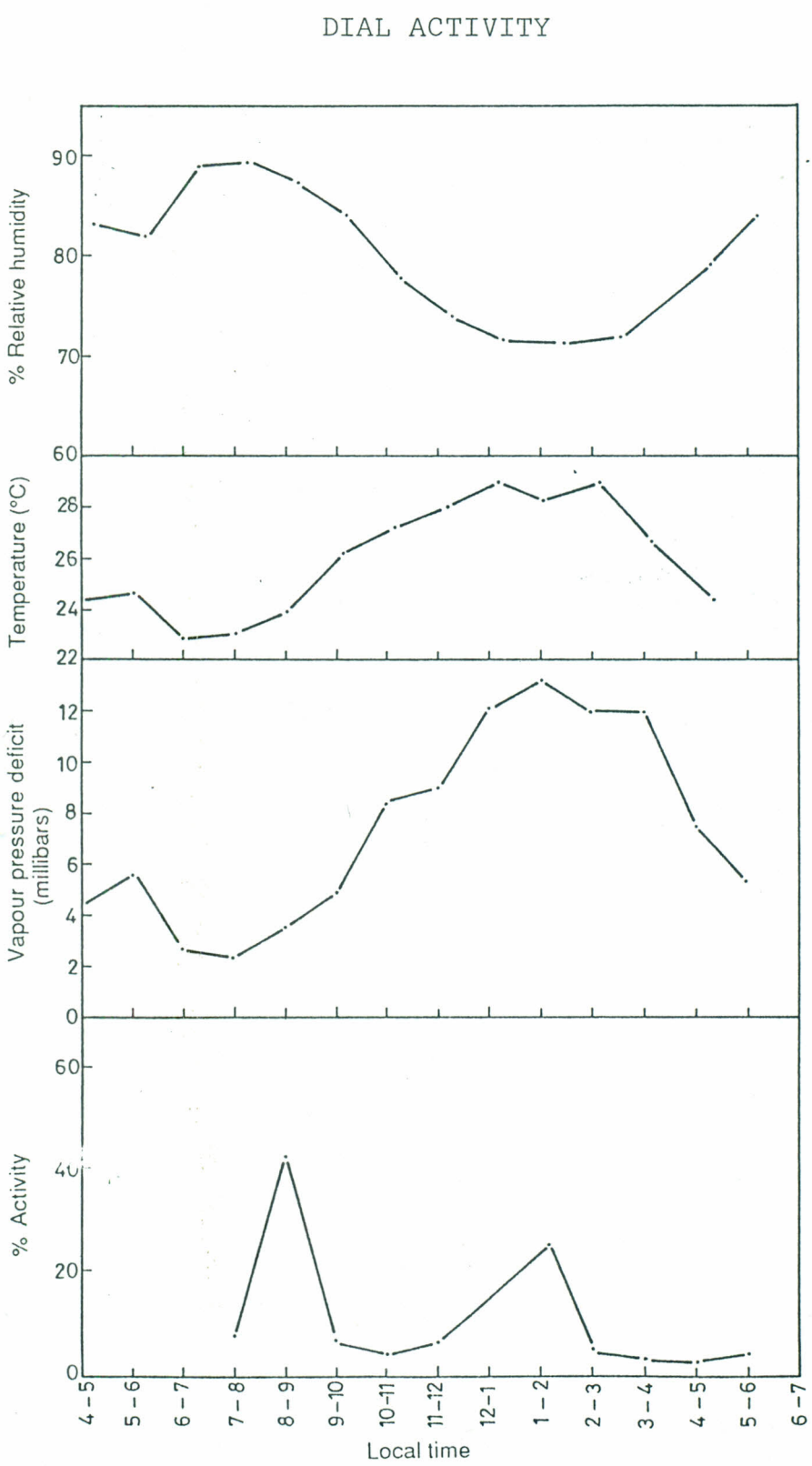

Activity of *G. austeni*, from stationary car catch, Feb. 1990–Feb. 1991

Fig. 3.2

Figure 3.2: DIEL ACTIVITY OF *G. austeni*

Hourly activity of *G. austeni* in relation to saturation deficit (vapour pressure deficit), humidity and temperature. There was some relationship between activity pattern and saturation deficit in the early morning and late afternoon, but not in the middle of the day when a lull in activity occurred inspite of rising saturation deficit.

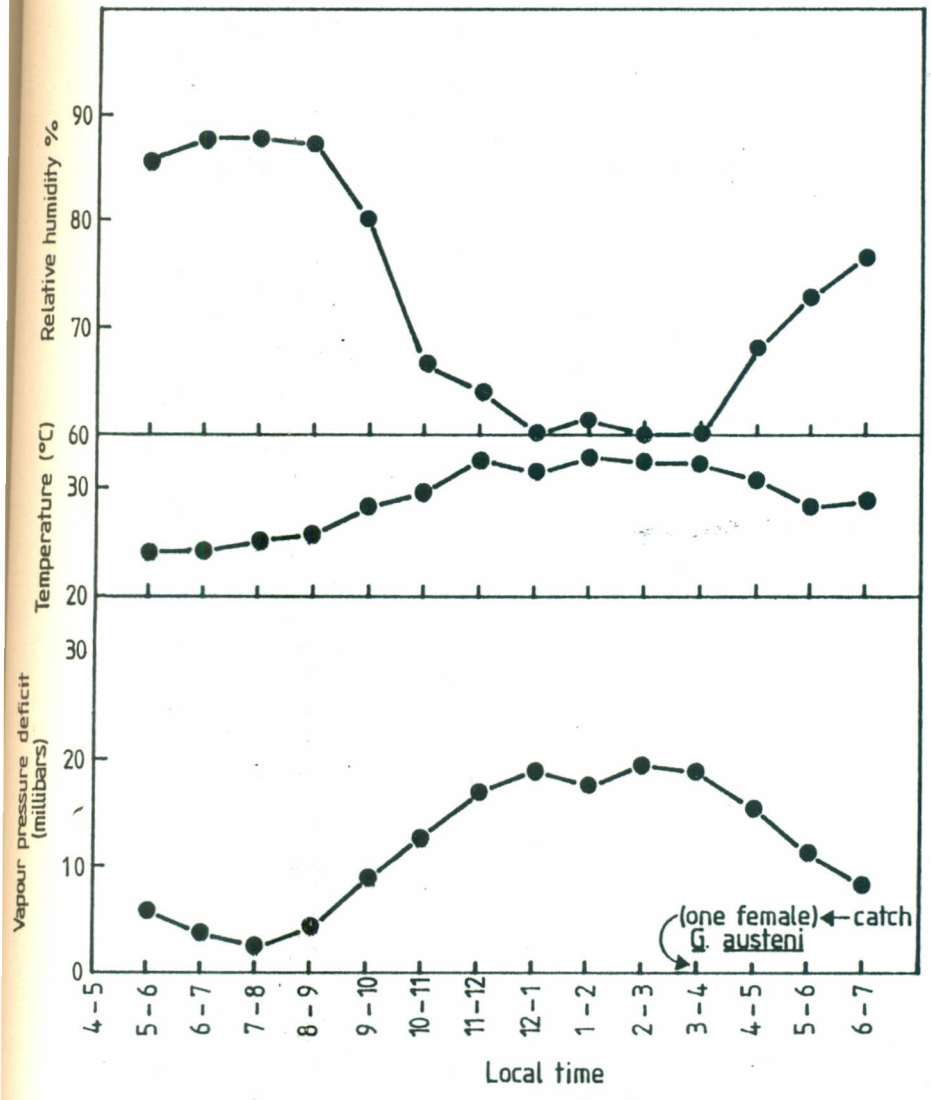
Figure 3.3


DIAL ACTIVITY
% Relative humidity
90
80
70
60
Temperature (°C)
28
26
24
22
Vapour pressure deficit (millibars)
12
10
8
6
4
2
0
% Activity
60
40
20
0
4-5
5-6
6-7
7-8
8-9
9-10
10-11
11-12
12-1
1-2
2-3
3-4
4-5
5-6
6-7
Local time

Figures 3.3: and 3.4: ACTIVITY IN RELATION TO CHANGES IN CLIMATIC PARAMETERS

Activity of *G. austeni* in relation to saturation deficit, temperature and relative humidity on two days of highest activity. Compare this with the pattern of these parameters on two days of lowest activity shown in Fig. 3.4, (when only one fly was captured on each occasion, throughout 14 hours of observation). The main difference between the two was lack of sunshine on the most productive days.

Figure 3.4




LACK OF ACTIVITY IN RESPONSE TO CLIMATIC PARAMETERS

Figure 3.5: ACTIVITY PATTERN OF *G. austeni* IN RESPONSE TO HOST ODOUR IN THE LABORATORY.

Key.

a. Non tenerals

b. Tenerals

There was no significant difference in the pattern of activity between the tenerals and non tenerals. But significant difference occurred between teneral males and females $P<0.01$, $F = 3.07$ with teneral males showing greater activity around 0800-1100 h and 1500-1700 h.

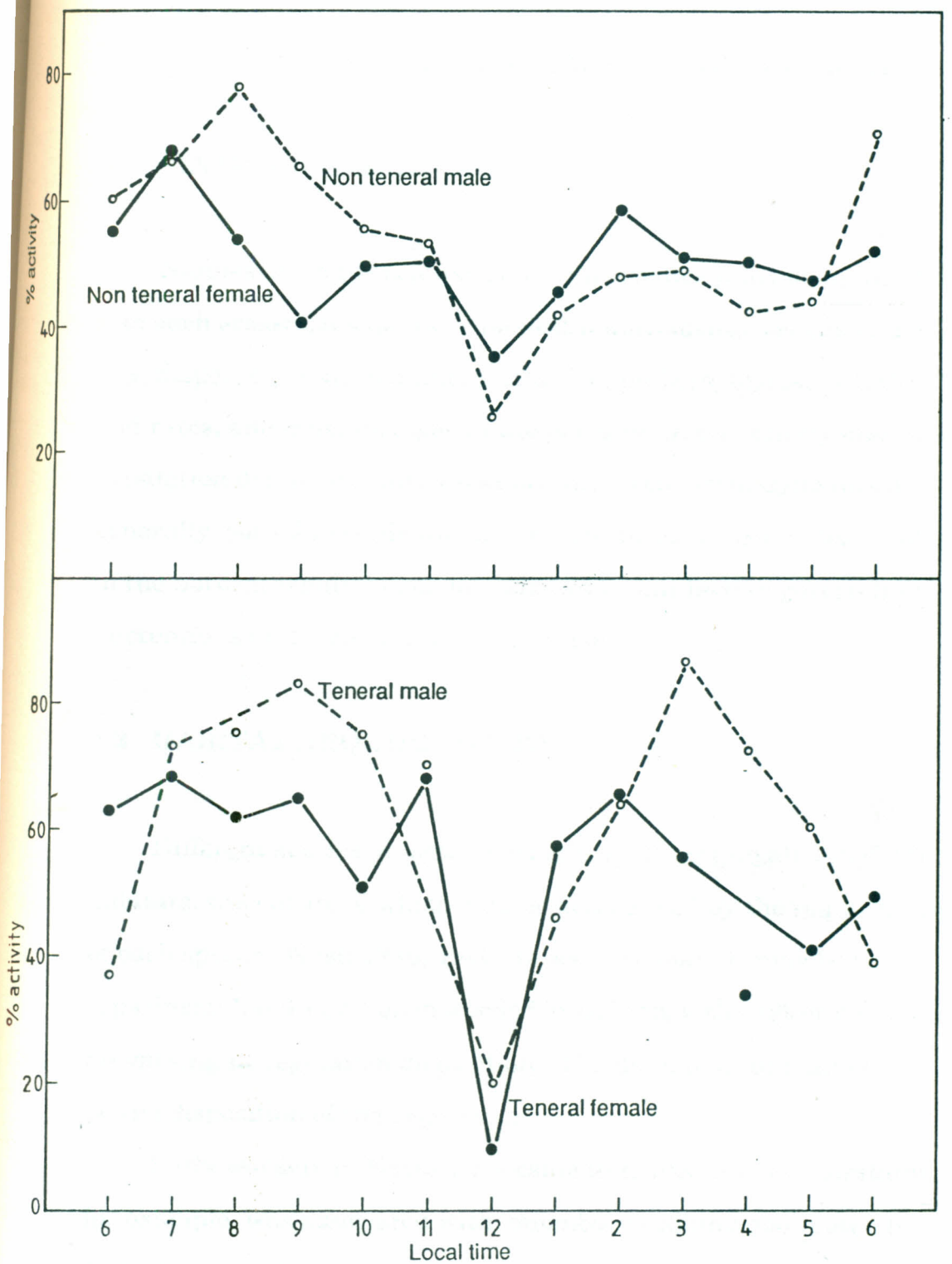

80
60
40
20
% activity
Non teneral male
Non teneral female
a.
Teneral male
Teneral female
b.
0
6
7
8
9
10
11
12
1
2
3
4
5
6
Local time

Fig. 3.5

# Chapter 4

# POPULATION CHARACTERISTICS OF *G. austeni*

## 4.1 INTRODUCTION

Studies on tsetse population characteristics provide an insight into such ecological aspects as habitat requirements, census or density, dispersals, seasonal fluctuations in numbers, mortality factors and rates, and other changes in the population, collectively known as population dynamics. Investigations into population dynamics are generally geared towards the understanding of the processes involved in the natural regulation of densities and what factors govern the fluctuations of the population in question.

## 4.2 HABITAT REQUIREMENTS

Different species of tsetse are more or less segregated in distinct habitats, the nature of which may be determined by the requirements of each species (Nash 1936, 1940; Jackson 1945a). It may be a reparinean habitat, an open wooded grassland, a thicket or a forest, all varying in vegetation disposition. The main determinant seems to be the disposition of the vegetation.

Early workers in East Africa came to realise that *G. morsitans*, for example, was associated with 'miombo' woodland dominated by *Isoberlina-Brachystegia* vegetation types, *G. fuscipes* with lake and river sides and *G. swynnertoni* with thicket (Jackson, 1945a; Nash, 1969). In his experiments to determine the underlying reasons for

such segregation, Jackson (1945a) introduced the two closely related species of East Africa, *G. morsitans* and *G. swynnertoni*, into each other's habitats. *Glossina morsitans* was in 'miombo' woodland formation, dominated by *Isoberlinia-Brachystegia* broad leaved deciduous woodland in Tanzania, while *G. swynnertoni* seemed to avoid this type of vegetation and was found in the thornbush dominated by *Acacia* and *Commiphora* communities. When he conducted fly round sampling several weeks later, he found that samples of *G. morsitans* recovered from the habitat of *G. swynnertoni* were, not only, lighter in weight, but were also physiologically inferior to their counterparts from the usual habitat of *G. morsitans*. Jackson (1945a) observed that the reason for this was not lack of food, as both habitats harboured plenty of game. His conclusion was that the eco-climate of the thornbush, which *G. swynnertoni* inhabited, was more severe than that of the miombo woodland which *G. morsitans* inhabited, because of less complete vegetation cover. *Glossina swynnertoni* was adopted to take more frequent meals in order to counteract these conditions.

In West Africa, Nash (1936), studying the habitat preference of *G. tachinoides* Westw. and *G. morsitans submorsitans* Newst., observed that *G. tachinoides* was somewhat more susceptible to very high temperatures than *G. m. submorsitans*, and that this contributed greatly to the difference in the late dry-season concentration habits of the two species. Barrass (1970) and Lambrecht (1973), in their study of sensitivity of tsetse to various colours, independently concluded that the sensitivity of *G. morsitans* to colour contrast reflected their habitat preference for edge vegetation where contrasts are greatest.

Vegetational correlations in respect of tsetse habits and habitats, inspired much of the early research, such that it led to the development of techniques of control by partial bush clearing (Glover *et al.* 1960; Glover, 1963; Dr. R. Onyango pers. comm.). Inspite of this, it has not been possible to find any common formula for control that fits all parts of the range of any species (Ford, 1970), mainly because the behaviour of one tsetse species may differ so much from one area to the next. Ford (1970) suggested another dimension to the habitat preference factor; he observed that the primary determinant of distribution of *G. pallidipes*, for example, is not its dependence upon thickets, but the attachment of bushbuck and a few other preferred animals to that type of habitat. At any rate, vegetation provides, perching and resting sites, shelter from adverse climates and predators as well as vantage points from which movement of potential host animals may be seen. Adult tsetse flies may use tree bark burrows, follen logs and fallen branches as shelter, or for larviposition (Pilson and Pilson, 1967). Another important factor that may control the limits of tsetse distribution is climate and/or temperature. This acts indirectly by reducing the size of the effective habitat (Ford, 1968); and is a vital factor in the ecology of *Glossina* (Nash, 1937).

## 4.3 POPULATION DENSITY

The numbers of tsetse obtained from catches by any sampling methods in the field do not necessarily give a true idea of the absolute census or density of the population per unit area. They merely give an activity/density index which is dependent, to a great extent, on density and also on activity (Jackson 1933). Very often it is desirable to know the density or census per unit area. Estimates of the total fly

population contained by marking a known number of flies and subsequently finding what proportion of the total population this forms (Lloyd, 1936; Jackson, 1937, 1939, 1949; Turner, 1987, Kyorku, 1990). In an isolated area where the delay between marking and recapturing is short, the change in the constitution of the populatig to birth and death of individual flies will not have had time to affect, appreciably, the estimates obtained. However, in a non-isolated area, there is the problem of movement of flies out of and into the area. The proportion of marked living flies will decrease to a point at which it will then remain constant, subject to steadfastness of the rates of births and deaths (Jackson, 1937, 1949.

### 4.3.1 Capture-recapture in animal density studies

The capture-mark-release-recapture method (capture-recapture) is an important tool used in the estimation of animal population densities. The method serves other purposes as well, for example, the assessment of feeding frequency (Turner, 1987) movement pattern and dispersal (Hargrove, 1981; Randolph Rogers, 1984) and study of resting sites (Scott, 1931; Jewell, 1956; Turner, 1982).

The capture-recapture method was first advocated by Petersen (1896) for the estimatation of growth and migration of fish. However, Lincoln (1930) was the first to use it to provide values of the total population size. He (Lincoln) estimated the population of ducks in North America. Subsequently, it was referred to as the Lincoln index. At about the same time, Jackson (1933) independently, had a similar idea to estimate tsetse numbers; and he used the same princi-

ple. In subsequent extension, Jackson (1937, 1939) introduced his 'negative and positive' methods to allow for the complications introduced by birth and death rates, and also discussed immigrations. Begon (1979) has summarised most of the subsequent methods which have been used by some of the workers to estimate populations of various animals since Petersen's or Lincoln's time, or to build population models. Among the works he summerised were those of Jackon (1937) (negative and positive methods), Jolly (1965) and Seber (1973). Begon (1979) asserts that 'even the most sophisticated models are directly descended from the simplest, which is the one advocated by Petersen (1896)'.

The principle of the capture-recapture method is as follows: A number of animals are captured, marked and released. When the marked individuals are thought to have mingled thoroughly with the unmarked, a further sample is captured and the proportion of the marked individuals in the recaptured sample is noted.

In the simplest Petersen's model (Lincoln's index), the estimate of the total population size is given by dividing the number of marked animals released by the proportion found to be marked in the recapture samples and multiplying the result by the total number (marked plus unmarked) captured.

$$\text{Lincoln's index,} = \frac{\text{Number marked} \times \text{Number captured}}{\text{Number of marked recovered}}$$

However, this simple concept assumes that no loss (death or emigration) has taken place between the time of marking and the time of

recapture, and that no 'gains' (births or immigration) have occurred during that time. It, therefore, rests on the assumption that all the animals marked and released on the first occasion represents the sum total of all marked animals available for recapture on the second occasion. Many of the alternative methods that have been used since Lincoln's time modify this assumption.

It is recognised that a proportion of the marked animals are subject to either death or emigration each day (loss). They are at risk of loss. But the marked at risk is really a sub population of the entire population in the area and behaves like it in every respect. The entire population is also subject to gains from birth and/or immigration, and this may dilute the proportion of the marked in relation to the entire population. The main interest is to estimate the size of a mobile population, and the strength of those processes which determine that size. Jackson (1937) extended his treatment of the mark-release-recapture data to allow for birth and death rates, advocating his 'negative and positive' methods. Further work by him (Jackson, 1939), took into account the effects of migration. Dowdeswell *et al.* (1940) applied the same method to Lepidoptera populations. Bailey (1951, 1952) reviewed these methods extensively and went further to develop analyses for calculating standard errors. Other workers who have modified the Lincoln index include Jolly (1965) and Manly and Parr (1968). They developed estimation of populations based on multiple recaptures. The various methods for handling the capture-recapture data may be grouped thus: Jackson's positive and negative methods, the Bailey's triple catch method, the Fisher-Ford method, the Jolly-Seber stochastic method and the Manly-Parr method. The

choice of any of these methods for application may depend on whether the underlying conditions have been met by the experiment.

### 4.3.2 Mark-release-recapture assumptions

There are some fundamental assumptions that are implicit in the mark-recapture method of estimating populations, and which should hold if the formula used is to give a suitable estimate of the density. These have been reviewed by Begon (1979) as follows:

1. All individuals in the population (marked or unmarked) inherently have an equal chance of being caught, i.e. the population is sampled at random without regard of the condition of individuals.
2. That all marks are permanent for the duration of the study and are noted correctly on recapture.
3. Being caught, handled and marked has no effect on an individual's subsequent chance of capture.
4. Handling has no effect on individual's chance of dying or emigrating; and all emigration is permanent and, therefore, essentially indistinguishable from death.
5. Individuals from different classes within the population will be sampled in the proportion in which they occur, i.e. a representative sample will be obtained

In addition to these general assumptions, each of the methods, listed above, is further based on a number of assumptions peculiar to it.

### 4.3.3 Seasonal changes in apparent density

Population fluctuation is seen in the light of numbers appearing for capture. Reduction in the number of individuals appearing might be caused by one of several factors, for example, unsuitable weather conditions for activity during the time of trapping, or real reduction in total numbers due to emigration or mortality. On the other hand, length of life may also affect density. Jackson (1941) observed that *G. morsitans* life in Kikoma area, Tanzania, was shortest during the dry, hot periods. An average male lived only for 2 weeks. During the rains and immediately after, life was much longer and an average male lived for 5 to 6 weeks, some of them reached 20 weeks. The actual birth rate possibly remained unchanged, but a rise in the death rate during the dry periods produced apparent drop in the birth rate. Moreover, when temperatures were lower, a longer time was spent in the early stages. Semi-starvation also caused abortion in the dry season. In non isolated populations, fluctuations may result from extensive dispersal to adjacent areas and this, in turn, might depend on climatic factors dictating when such dispersals or influx may occur. The flies may follow the movement of game hosts or leafy vegetation.

### 4.3.4 Mortality in tsetse populations

Mortality in tsetse populations is caused in two ways, by abiotic and biotic factors. The abiotic factors are mainly climatic e.g rainfall, temperatures, saturation deficit and so on. They act independently of the density of the population, and their effects are therefore, referred

to as density independent mortality (DIM). The biotic factors, on the other hand, act with increasing severity as the population density increases. They include predation, parasitism and competition (Snow 1981, and pers. comm.). Their effect is, therefore, rightly called density dependent mortality (DDM). In Muhaka-Shimba Hills area, Snow (1981) showed that the dynamics of *G. pallidipes* population was influenced partly by rainfall. Precipitation of between 50 and 200mm for 28 days, created optimum conditions, and the population invariably increased. With more or less amounts, the numbers declined due to high mortality, and these changes contributed greatly to the fluctuations in apparent density.

## 4.4 OBJECTIVES OF STUDY

The objectives of this study on population characteristics of *G. austeni*, were to, (1) determine habitat requirements of this species (2) estimate the density (3) longevity and birth rate. (4) study seasonal fluctuations and dispersal of the Muhaka area population. (5) A study of predation, as a mortality factor, requires devotion of much more time than could be afforded within the scope of this study, however, some observations were to be made concerning the predators encountered in trap cages

## 4.5 MATERIALS AND METHODS

### 4.5.1 Biotope preference of *G. austeni*

To determine the distribution of *G. austeni* within the two biotope-types, of forest and open woodland, and to assess biotope

preference, systematic recording of trap catches from ecotone, cleared transect and forest floor was undertaken. Subsequently the catches were analysed using an analysis of variance to determine whether the difference between the catches was significant. The means were then subjected to Duncan's range test to determine which of the areas was significantly favoured by the species. The data was transformed by the $\log_{10}$ transformation before being subjected to analysis.

### 4.5.2 Marking of *G. austeni*

Although many thousands of savanna tsetse are normally marked in order to be able to recapture a few (Jackson 1937, 1945a, Lloyd, 1936, Turner, 1987), the low density of *G. austeni* in the study area did not allow this. Consequently only about a hundred flies (sometimes more, other times less, depending on availability) could be marked at a time, in the hope that a reasonable proportion, of between 4% and 20% would be recaptured, just like in the case of woodland species (Jackson, 1933, 1937; Turner, 1987).

The flies were marked in four days each month for 9 months from February to November 1990. They were fed on lop-eared rabbit and then released back in the forest (except for the first two marking days of February 1990, when they were released unfed, in a central area). An artist's oil paints of various colours were used, taking a different colour each month and a different position on the fly's dorsal side of the thorax each day, (Fig. 4.1). This made it possible, on recapture, to identify the fly as to the day and month on which it was marked. For convenience, the position on the pre-scutum was re-

ferred to as north, that on the right and left sides of the scutum as east and west respectively, that on the central part as centre, and that on the scutellum as south (Fig. 4.1). The exercise was carried out using a fine point (wire like) of dry stem of a thin grass.

### 4.5.3 Estimation of population density, by Jackson's 1937 method

The method of analysis found most suitable for estimating the population size was Jackson's positive method (Jackson 1937, 1939), also reviewed by Begon (1979). The method is applicable when release is carried out on one occasion ( say, a day) followed by many occasions of recapture (say, six days, weeks or months). It allows variable mortality (loss) and recruitment (gains) in the population. On day **0**, $r_0$ individuals are caught, marked and released. On subsequent days (e.g. six days), $n_i$ individuals are captured of which $m_i$ are marked.

$q_i$ is the proportion of the day **i** sample that are marked:

$$q_i = \frac{m_i}{n_i}$$

Although there are losses from the population after the day **0** sample, this should not affect the proportion of the marked flies to either the

whole population, or future samples. It is assumed that marked and unmarked flies die and/or emigrate at the same rate. But there are additions to the unmarked portion of the population, and none to the marked portion. **qi** should, therefore, decline with time, as these gains dilute their proportion to the unmarked individuals in the population. Our interest is to estimate the population density, $N_0$, at day **0**, before the additions took place. According to the method of Jackson (1937) this is achieved by estimating the marked proportion of a hypothetical random sample taken on day **0**, $q_0$. This marked proportion in $q_0$ is the same as the proportion in the population. It is calculated using a birth (or gain) rate **b** per day in the population.

$$q_0 = \frac{r_0}{N_0} \qquad \text{therefore,} \qquad N_0 = \frac{r_0}{q_0}$$

In the present study, the four or five days of marking were pooled and considered as one week of marking, and the different sampling days of recapture pooled to weekly intervals (for six weeks). Owing to the fact that the number of individuals caught and marked at regular intervals vary from date to date, this method advocates the correction of all recaptures for numbers marked and size of the sample in which they are recovered. The correction is to give the number which should have been obtained per 100 marked in a sample of 100 flies caught on the recapture day. That is to correct the recaptures as if some constant number (100) had been marked, and the same

number caught on every recapture occasion. For example, supposing 503 flies captured in any week have 20 recaptures from some previous week in which 170 were marked. Then the number 20 may be corrected to a recapture of-

$$\frac{20 \times 100 \times 100}{503 \times 170} = 2.3389 \quad \text{(corrected recapture)}$$

This correction has been applied on all the recaptures from weeks one to six, after every monthly marking, and the corrected recapture figures are shown in Table 4.2. These corrected recaptures were then plotted against weeks after marking (Fig. 4.2). The corrected recapture figure for week $\mathbf{y_0}$ (call it **a**) (refer Fig. 4.2), is the figure which would have been obtained had we been able to recapture on the date of marking. To find **a**, we must first find **r** or the average ratio of each value of **y** (in this case total captures, marked and unmarked, per week) to the value preceding it, or of $\mathbf{y_1}$ to **a**. For example, the sum of the recaptures of the last four weeks of March (is 7.007), divided by the sum of the recapture of the first four weeks (11.969) is equal to 0.585.

Therefore,

$$\mathbf{r} = \sqrt{\frac{\mathbf{y3 + y4 + y5 + y6}}{\mathbf{y1 + y2 + y3 + y4}}} = 0.765$$

where **y** equals weeks of recapture.

Jackson (1937) argued that since the differences between the logarithms of the recaptures are more or less constant (Fig.4.2), and the survival curves decline in a geometrical progression, it follows that the figure for week *0* is in the same geometric series, and is calculable, as indicated in Fig.4.2. But the slope of the survival curves will be different for every week. Hence, supposing that **y1 y2 y3 y4 y5 y6** are the corrected recapture figures of the first six weeks after marking, and **a** the required figure for week *0*, then **a** is calculated thus:

$$\mathbf{a} = \frac{y1+y2+y3+y4+y5}{\mathbf{r}} - y1+y2+y3+y4$$

and

$$\text{population } \mathbf{N_0} = \frac{10{,}000}{\mathbf{a}}$$

In this case the estimate for the female population in Muhaka during the first week of March, for example, is found by:

$$\frac{6 \times 10{,}000}{150 \times 107} = 3.7383 \quad \text{(the corrected recapture)}$$

6 being the marked flies recovered, 150 the total recapture (marked and unmarked), and 107 the number of females originally marked in march, and 10,000 the correction factor. The population estimate was obtained by substituting the corrected recaptures for each of the six

weeks, $y1....y_6$ . The corrected recaptures for six weeks after each month of marking were calculated in a similar way.

Bailey (1951, 1952) and Begon (1979) have drawn attention to the detail that this standardisation (correction of recaptures) may not introduce enough 'weighting' in the calculation of density. They suggested using $\mathbf{m_i}$ (the number of marked flies recaptured) for this purpose, and that this may help one reach a more precise estimate, and calculate standard errors. A comparison is, therefore, hereby made (below) on the present data using weighted least squares and computing the figures for the slope of the decline of the proportion of the marked population with time, as well as the intercept and the values of $\mathbf{q_0}$ and $\mathbf{r_0}$ which are required to estimate the population.

### 4.5.4 Estimating density using weighted factors, method of Begon (1979)

As before, $\mathbf{r_0}$ = number caught marked and released on day **0**

$\mathbf{n_i}$ = total individuals caught on subsequent days of which

$\mathbf{m_i}$ = marked

$$q_i = \frac{m_i}{n_i}$$

$\mathbf{q_0}$ = the number taken on day **0** is unknown

and $\mathbf{N_0}$ = the population (to be estimated)

**b** = the birth rate

An average recapture rate was calculated for weeks after marking. To allow for different sample sizes on the different sampling occasions the recaptures are to be corrected. Begon (1979) gives the following formula for this correction:

$$\mathbf{In}\,(1\text{-}b) = \frac{\Sigma m_i\,(\mathbf{Inq_i}\text{-}\mathbf{In}\bar{q}_i)(i\text{-}\bar{i})}{\Sigma mi\,(i\text{-}\bar{i})^2}$$

$$\mathbf{Inq_i} = \mathbf{i(In(1\text{-}b))} + \mathbf{Inq_0}$$

(**In** symbolises log. to base e)

The logarithmic transformation serves to stabilize the variance and linearize the regression. The regression equation above, is of $\mathbf{Inq_i}$ on **i** (and both are known from the data, ($\mathbf{q_i}$ = proportion of marked flies in the population, and **i** the recapture period, in this case weeks). The regression constants **In(1-b)** and $\mathbf{In(q_0)}$ are to be computed, **b** (birth rate), and the slope can then be obtained and the line of best fit drawn as shown in Fig.4.3. $\mathbf{N_0}$ (the population at the begining of the exercise, before any additions took place) is then obtained, using the above equation. A test of significance may be performed thus; in any regression y = ax+b (where a=slope and b=y- intercept). In this test if the regression is significant then the indication is that the value of y (recaptures of marked flies) dwindled with increasing time period (weeks), and so the calculation of density which is really dependent on this relationship is justified.

### 4.5.5 Fluctuations in apparent density

Fluctuations in apparent density were estimated by monthly recording of arithmetic means of numbers captured in traps per day, from the different localities. This has been plotted in relation to the monthly rainfall in Muhaka (Fig. 4.4). In addition age grading of female *G. austeni* was carried out during the month of August 1989 (dry month) and May 1990 (wet month), to determine whether or not the number of tenerals were affected by dry and wet conditions. This is presented in Fig. 4.5.

## 4.6 RESULTS

The results of experiments conducted to determine habitat preference of *G. austeni* are presented in Table 4.1. There was significant difference between catches obtained from the forest floor and at the ecotone or cleared transect ($P<0.001$, $F = 17.67$, $df = 1$). This is an indication of a stronger affinity of *G. austeni* to the forest or dense thicket. Traps placed ten or so metres from the forest edge, in the open woodland hardly captured *G. austeni*, although they always captured *G. pallidipes*.

Table 4.2 gives the full list of the corrected recapture figures for the marked flies. The population estimate for the month of March 1990, using Jackson's (1937) method was as follows:- the value of **r** is equal to the square root ($\sqrt{}$) of the sum of the recapture values obtained during the last four weeks of recapture divided by the sum of the recapture values obtained during the first four weeks.

Therefore,

$y3+y4+y5+y6 = 3.665+1.575+1.126+0.641 = 7.007$

and $y1+y2+y3+y4 = 3.738+2.991+3.665+1.575 = 11.969.$

Therefore, $\sqrt{\text{ of }} \dfrac{7.007}{11.969} = 0.7651$

$$\mathbf{r} = 0.7651$$

$$\mathbf{a} = \frac{(y1+y2+y3+y4+y5)}{\mathbf{r}} - (y1+y2+y3+y4)$$

Therefore, $\mathbf{a} = \dfrac{(3.738+2.991+3.665+1.575+1.126)}{0.7651} - 11.969$

$$= 5.1467$$

The population estimate $= \dfrac{10{,}000}{\mathbf{a}}$

Therefore,

$$\frac{10{,}000}{5.1467} = \mathbf{1942.99} \text{ (the female population estimate)}$$

The estimates for the months of February May, June, and July were obtained in a similar manner, and are listed in Table 4.3. In February, during the first two days of marking the flies were not fed before release, and as a result many of them were recaptured in traps within the same or next day. They were still hungry and had to continue flying about in search of a blood meal. The recaptures did not spread

over six weeks and for this reason only four weeks of recapture were used. Table 4.4 shows the mean monthly sex ratio of *G. austeni* in the Muhaka area

### 4.6.1 Results of density obtained using weighted factors

The results obtained by the revised version of the method as given by Begon (1979), using the formula-

$$\ln(1-b) = \frac{\sum m_i (\ln q_i - \ln \bar{q}_i)(i-\bar{i})}{\sum m_i (i-\bar{i})^2}$$

are presented in Appendices 1–5, for the months of February to July. A summary of female densities is given on p. 163. It is noted that the estimates obtained by the two methods do not differ significantly.

### 4.6.2 *Glossina austeni* population fluctuation

Catches from monthly sampling in ten traps were pooled and the data used to find apparent density by calculating the arithmetic mean catch per trap per day (catch/trap/day). These were plotted to show monthly changes (fluctuations) in the population, and are summarised in Fig.4.4. Rising apparent densities (activity/density) were observed from second half of March to May, while June showed start of decline but still high apparent density. August figures were the lowest. There was a similar pattern of rising apparent density in

October-November, albeit at a lower level, both in 1989 and 1990. The two periods of high density coincided with the onset of rains (Fig.4.4). There was no proof of influx of *G. austeni* from else where, the age structure for females, obtained by ovarian age grading (Saunders 1962, revised by Challier 1965) in August 1989, and May 1990, showed more young (age category *0*) during the high density period (May) than during the low density one (August).

### 4.6.3 Predators of *G. austeni*

Table 4.5 shows a list of some of the arthropods, and non arthropods which were observed either devouring the flies, stunning or dragging them along. Some were seen chasing *G. austeni* within the cage. It is supposed that they could be predators of the other tsetse species as well, and at any rate could contribute to the overall loss in the population of *G. austeni*. Among the most frequent predators were two ants of the family Formicidae, the red ant (*Oecophylla longinoda*) and *Myrmicaria natalensis*. The other important group of predators was that of crickets, Gryllacrididae and Gryllidae and members of the robber flies, Asilidae. Lizards, like Geckos (*Hemidactylus mobula*) were also frequently encountered in cages taking tsetse.

## 4.7 DISCUSSION

The finding that *G. austeni* is a a dense thicket/forest species agrees with that of Madubunyi (1990), and Moggridge (1950), and may explain why Turner (pers. com.), placing traps in the wooded

grassland area adjacent to the Jahazi forest in Zanzibar did not catch any fly in three months. Madubunyi (1990) captured significantly higher numbers on the forest floor as compared to grassland in the same general area in Zanzibar. Moggridge (1950) noted that 75% of all the *G. austeni* he captured in a fly round exercise came from the heavily thicketed section of the round. According to him, the trend did not change whether it was dry or wet season. But the numbers from both areas increased during the 'humid' season. All of these results concur with Bursell's (1957) theory that this species reinvaded and readapted to life in the forest, abandoning the open grasslands. Cutting of transect through the forest created an edge effect as far as *G. austeni* captures were concerned. The conditions of wind, temperatures, relative humidity and sunlight in this area were so different from that of the forest floor that the flies apparently no longer perceived the area as forest floor but as open vegetation.

The recovery of marked males was very low (a total of 6 out of 175 marked in nine months) probably because much fewer were captured and marked in the first place (Table 4.2) but also because being more mobile, greater loss may have occurred and the chances of the remaining few sighting a trap became more remote. The recovery of females in most of the months, was considered reasonable and compared well in percentages, with those of other workers (Jackson, 1937; Turner,1987; Kyorku, 1990). The rate of recovery ranged between 5 and 14%. The recovery after every monthly marking spread over beyond six weeks. The oldest marked fly was recaptured after 4 months (having been marked as non teneral). The estimates of the female population may be subject to errors, mainly because of low recaptures during some months, and the pooling of the data, first

over a week of marking (regarding it as one unit) and then over many weeks (using only six weeks). However, the estimates give a trend over the months which agrees with the apparent density observed in catches per trap per day over the same period (Fig. 4.4). This agreement between two independent methods lends credibility to the results. February showed the lowest apparent density as it did the population size, and an increase was observed in both cases from March to May. This trend in fluctuation of density, also agrees with that shown for *G. pallidipes* in the same area by Snow (1981). The relationship between apparent density and change in the real population density, indicates that some of the components regulating the population are density dependent, and act with greater severity when the density is high. Such may be predators.

The data for August and September was not considered in estimating density for the simple reason that there were no recaptures. There are a few possible explanations for this, first, the release site during both months was the area referred to as II on the map (Fig. 2.1, Chap. 2). There were much less collecting traps on the fringes of the forest in that area, none in the central portion, and none in the part of the forest that belongs to the county council, but which is contiguous with the rest of the forest, leased to ICIPE for biological research. Since *G. austeni* is a resident species, the flies probably did not diperse as far north as the area with plenty of traps. This was an omission caused by the underestimation of the size of the southern portion of the forest (a fact that only came to attention later, during the preparation of the map). Secondly, the colours used were white (August), and black (September). These colours might have contributed to lack of recaptures, either by being too conspicuous and at-

tracting predators, or too dark to be noticed on recapture. However, this is not very likely, as orange and yellow appeared even more conspicuous than white, yet flies with those marks had been recaptured. The brown colour on *G. austeni* (which is itself brown) bore very close resemblance to it, and the recapture results were good.

It appears, from the reports of Madubunyi (1990) and the present study that *G. austeni* density may be generally low over its range. Madubunyi's catches of 169 individuals from 36 traps in 15 days, work out to 0.3 per trap per day. In Muhaka, during high density periods (March to May), the catch was about 12/trap/day within the forest floor (Fig.4.4), and 1-2/trap/day at the ecotone of forest and open woodland. During the low density period, the catch was down to 3/trap/day on the forest floor, and 0-1/trap/day at the ecotone. *Glossina austeni* (female) was found to be a resident species, which does not move very much, and not very far. The recaptures were in the same general area where the marked flies were released. Those released on the western side of the forest were never captured in the southen half and vice versa. Moreover, a reduction in numbers of this species could be noticed at a site on one side of the forest, through continuous trapping for two to three months, yet when the trap was moved barely 300 to 400 metres further from the old site into a new site, the catches per trap per day would be as high as in the old site (400 m away) before the two month long removal, indicating that the removal had not really affected the section of the population 400 metres away.

As for fluctuations in the population density, Buxton and Lewis (1934) found that young unfed (teneral) flies of *G. morsitans* lost more fat under drier conditions, but that fed, older flies, showed the oppo-

site effect. This meant that the death rate in tenerals was higher during the dry spells. This is very likely what happens in *G. austeni* population as well, especially to teneral males which were rare during the dry seasons, and the few encountered at that time appeared to be very weak. All of this is supported by the lowering of the population during the drier seasons, and the fact that the age structure curve shows a much reduced proportion of nulliparous (young females which have not yet larviposited) to parous (older) females during dry season. On the other hand, more nulliparous females during May would suggest higher emergence rate (and / or survival rate of this age grade) probably for reasons indicated by Buxton and Lewis (1934) and Jackson (1945a), of minimal death during periods of low evaporation or rainy conditions.

Table 4.1 Comparison of the effect of biotope type on catches of *G. austeni*, (Anova and Duncan's multiple range test).

Treatment = biotopetype (ecotone vs forest floor)

| ss | df | ms | F ratio |
|---|---|---|---|
| 1.842 | 1 | 1.842 | 17.670 *** |

Duncan's multiple range test for the means:

| ecotone | | forest floor | |
|---|---|---|---|
| male | female | male | female*** |
| 4.0 ± 1.1 | 4.0± 1.3 | 7.4 ± 1.3 | 17.5 ± 1.3 |

*** = Significant (P<0.001)

Table 4.2 Distribution of corrected recapture rates of *G. austeni*, in intervals of weeks after marking.

| Month of marking | Marked Female | male | Corrected recapture rates (total recaptured in parenthesis) 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|
| Feb. | 68 | 16 | 10.422 (127) | 4.456 (132) | 1.625 (181) | 1.313 (112) | 1.149 (128) | 1.021 (128) |
| Mar. | 107 | 37 | 3.738 (150) | 2.991 (125) | 3.665 (102) | 1.575 (178) | 1.126 (172) | 0.641 (161) |
| May | 81 | 21 | 1.794 (344) | 1.435 (258) | 1.371 (267) | 0.925 (180) | 0.744 (166) | 0.906 (118) |
| Jun. | 76 | 24 | 5.744 (252) | 5.395 (195) | 3.209 (246) | 3.256 (202) | 2.785 (189) | 1.926 (205) |
| July | 90 | 25 | 2.424 (258) | 2.189 (203) | 0.829 (198) | 0.572 (194) | 0.624 (178) | 0.949 (117) |
| Aug. | 84 | 16 | No marked flies recaptured | | | | | |
| Sept. | 120 | 20 | No marked flies recaptured | | | | | |
| Oct. | 75 | 7 | Too few recaptures, data not used | | | | | |

Key

fem = females

mal = males

Table 4.3 Estimated densities of female *G. austeni* for different months during the year 1990

| Month | Density |
|---|---|
| February | 539 |
| March | 1943 |
| May | 5282 |
| June | 1252 |
| July | 2919 |

There is a trend in the fluctuation of these densities (Table 4.3) which agrees with that recorded in monthly fluctuations in apparent density (refer to Fig. 4.4). February was a dry month. April and May were the wettest months, and the apparent density in both 1989 and 1990 was highest.

Table 4.4 Mean monthly sex ratio of *G. austeni* in Muhaka

| Month | Ecotone | | Forest floor | |
|---|---|---|---|---|
| | % | | % | |
| | male | female | male | female |
| January | - | - | 34.0 | 66.0 |
| February | 42.2 | 57.8 | 43.0 | 75.0 |
| March | 37.5 | 62.5 | 34.0 | 66.0 |
| April | 34.3 | 65.7 | 38.0 | 62.0 |
| May | - | - | 30.0 | 70.0 |
| June | 25.0 | 75.0 | 31.0 | 69.0 |
| July | 20.0 | 80.0 | 34.0 | 66.0 |
| August | 36.6 | 63.4 | 31.3 | 68.7 |
| September | 32.0 | 68.0 | 27.6 | 72.4 |
| October | 32.0 | 68.0 | 32.4 | 67.6 |
| November | 38.0 | 62.0 | 40.7 | 59.3 |
| December | 40.0 | 60.0 | 48.4 | 51.6 |

Table 4.5 Predators of *G. austeni* in Muhaka

| | |
|---|---|
| HYMENOPTERA: | |
| Formicidae | *Oecophylla longinoda* |
| | *Myrmicaria natalensis* |
| | *Polyrhachis* sp. |
| Sphecidae | *Trypoxylon* sp. |
| | *Chalybion* sp. |
| | *Bembix* sp. |
| Megachilidae | *Chalicodoma Grorocera* |
| | *felina* |
| Anthophoridae | *Anthophora torrida* |
| Bombiliidae | *Eurycarenus* sp. |
| DIPTERA | |
| Asilidae | *Leptogaster* sp. |
| | *Philonicus* sp. |
| ORTHOPTERA | |
| Gryllacrididae | |
| Grylotalpidae | |
| | *Gryllotalpa africana* |
| Gryllidae | *Gryllus* sp. |
| ARACHNIDA | |
| Araneae | |
| Vertabrate, REPTILIA | *Hemidactylus mobula* (gecko) |

Plate 4.1: TRAPS IN OPERATION AT SITES IN TWO DIFFERENT BIOTOPES

a. A biconical trap in operation at the grassed woodland / forest ecotone,

b. A section of forest floor.

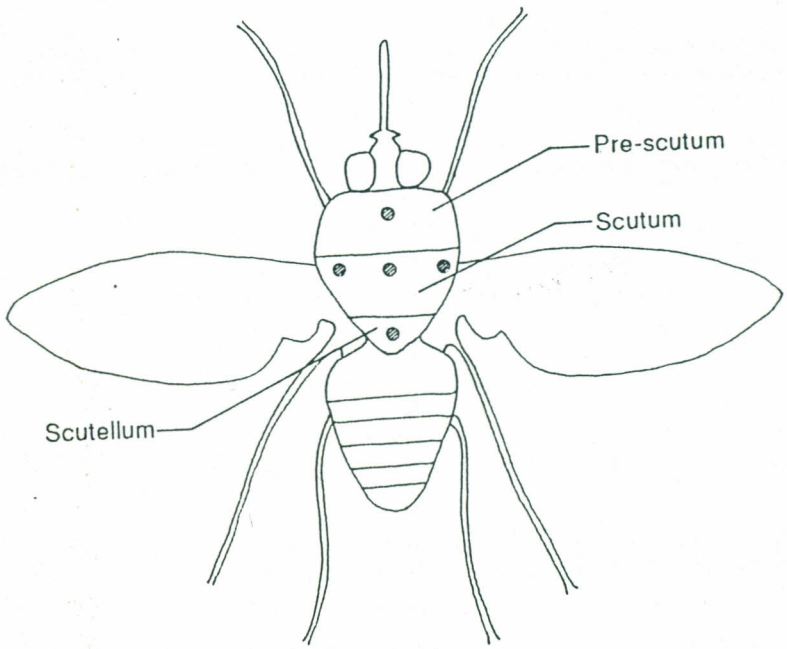
note: i. the area is not fully lit, due to canopy cover

ii. lack of ground cover.

a

b

Figure 4.1




Diagramatic representation of a tsetse showing parts marked — ⊗

Figure 4.1 Diagramatic representation of a tsetse fly, indicating positions marked on the thorax.

Figure 4.2 Corrected recaptures of marked *G. austeni*, plotted against six weeks following every monthly marking

The extrapolated point for recapture on week 0 is shown (x)

(Jackson's method, 1937)

Figure 4.3

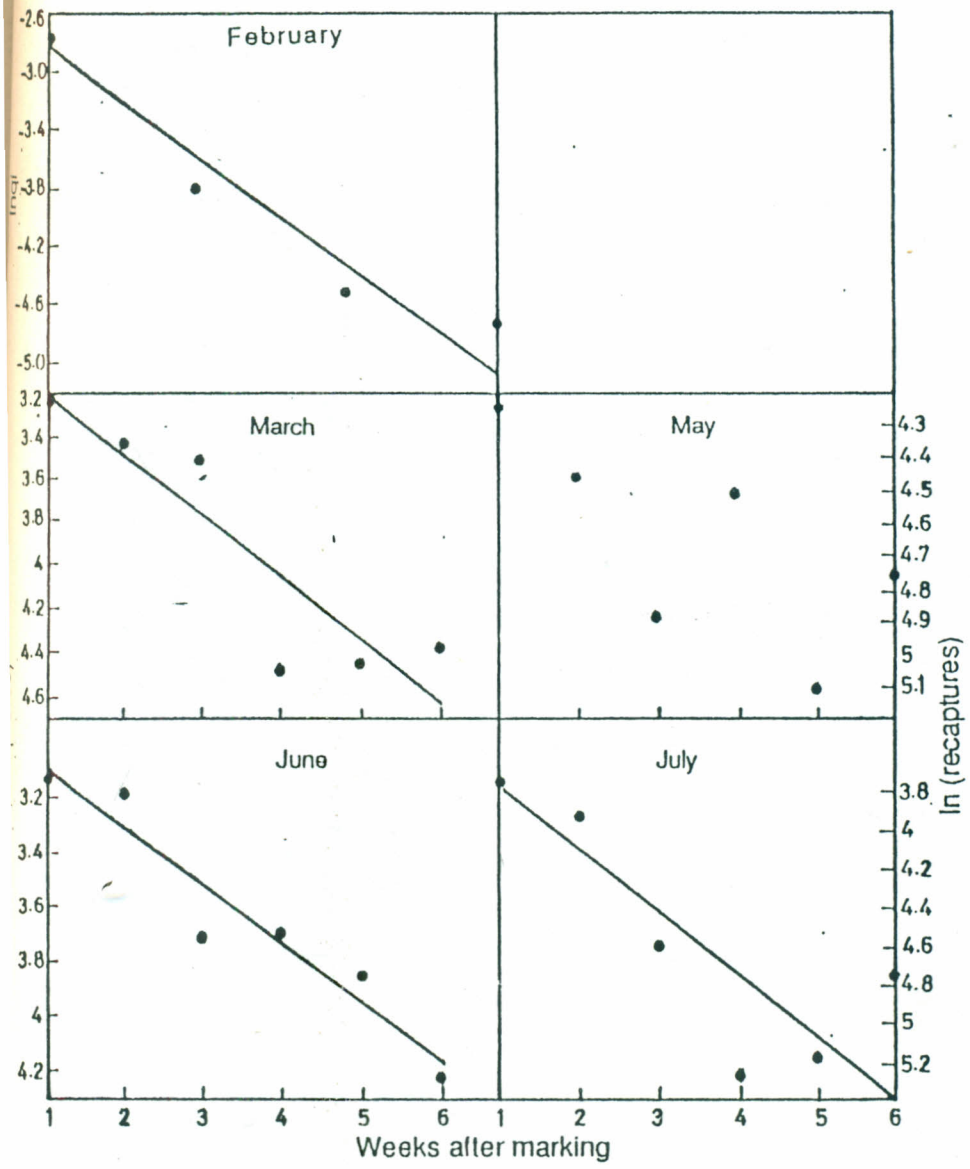

February
March
May
June
July
ln (recaptures)
Weeks after marking

Figure 4.3

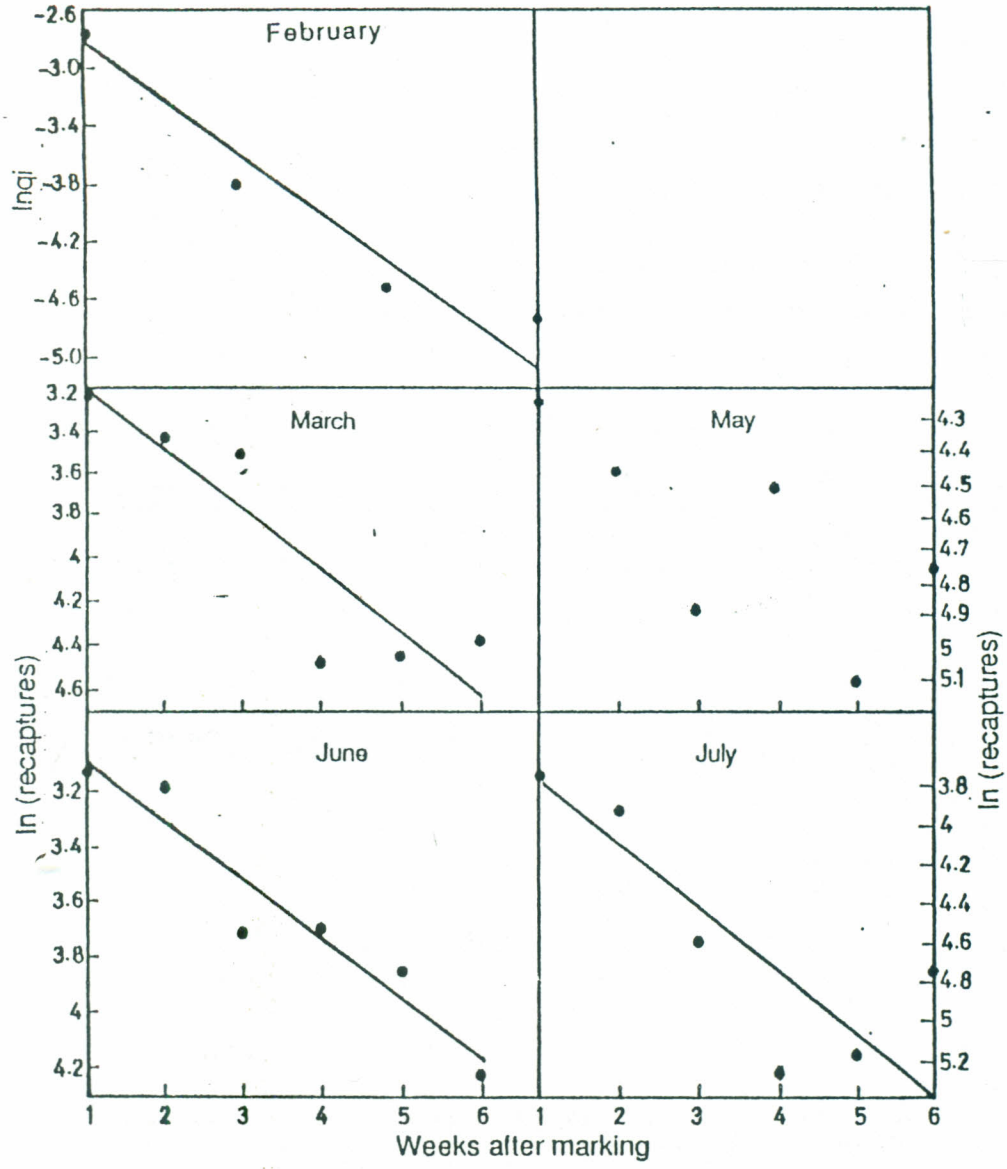

February
March
May
June
July
ln(recaptures)
ln (recaptures)
Weeks after marking
-2.6
-3.0
-3.4
-3.8
-4.2
-4.6
-5.0
3.2
3.4
3.6
3.8
4
4.2
4.4
4.6
4.3
4.4
4.5
4.6
4.7
4.8
4.9
5
5.1
3.2
3.4
3.6
3.8
4
4.2
3.8
4
4.2
4.4
4.6
4.8
5
5.2
1
2
3
4
5
6

Figure 4.3 Correlation of recapture rates of female *G. austeni* with weeks after marking

---

February recaptures, regression

y = -0.658-2.298

r = -0.902, Significantly ($P<0.05$). Female population- 539.2

March recaptures, regression

y = -0.281-2.935

r = -0.902 Significantly ($P<0.01$). Female population - 1985

May recaptures, regression

y = -0.122-4.234

r = -0.710 not significant . Female population - 5165

June recaptures, regression

y = -0.213-2.893

r = -0.961, Significant ($P<0.002$). Female population - 1446

July recaptures, regression

y = -0.270-3.638

r = -0.805 Significant ($P<0.05$). Female population - 2854

As the weeks advanced, the number of marked flies dwindled and where the regression is significant, the line fitted is a true measure of the relationship between x and y (in this case the decline in number of marked flies and weeks after marking. Therefore, there is justification in using it to estimate density (see appendices 1- 5).

Figure 4.4

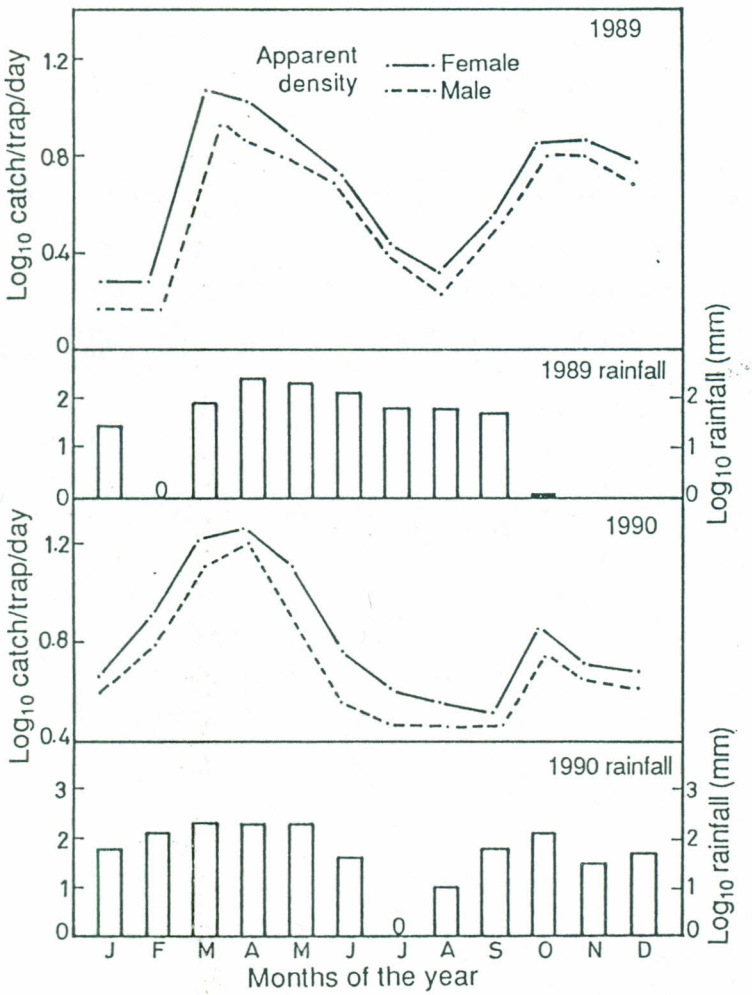

1989
Apparent density
Female
Male
Log10 catch/trap/day
1.2
0.8
0.4
0
1989 rainfall
2
1
0
Log10 rainfall (mm)
1990
1990 rainfall
3
J F M A M J J A S O N D
Months of the year

Figure 4.4 Monthly fluctuation in apparent density in 1989 and 1990.

Note that the apparent density of the two sexes run parallel and there is evidence of relationship between apparent density and rainfall.

Figure 4.5

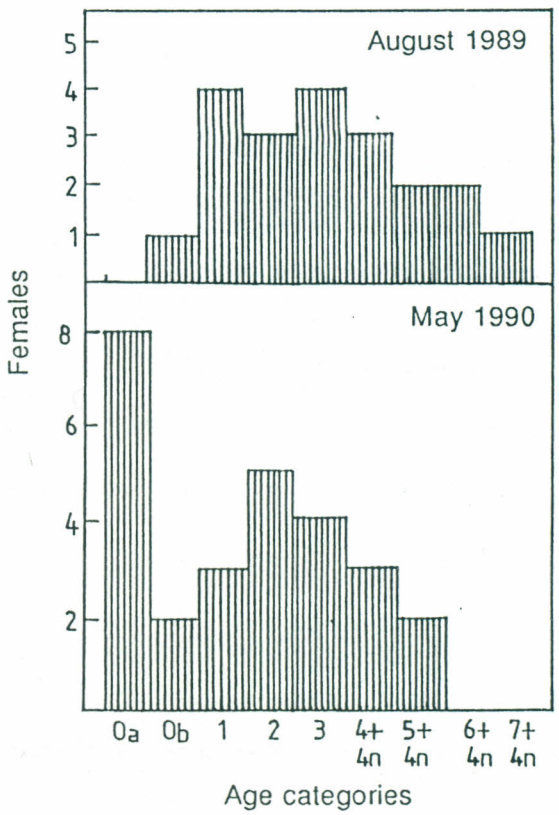

August 1989
May 1990
Females
5
4
3
2
1
8
6
4
2
0a
0b
1
2
3
4+ 4n
5+ 4n
6+ 4n
7+ 4n
Age categories

Figure 4.5 Age grades of mean samples of females *G. austeni* captured in three trap types in Muhaka, August 1989-1990.

Key

0a = nulliparous female, not yet mated

0b = nulliparous female, mated and carrying first or second instar larva.

4 + 4n *

5 + 4n

6 + 4n

7 + 4n

* These females may have ovulated more than 4 times, but due to the fact that only one scar is left after one or more ovulations one cannot be certain as to how many ovulations have taken place after the fouth. The normal practice since Challier (1965) is to designate categories four and above with a '+4n' as shown above,

# Chapter 5

# THE VECTORIAL CAPACITY OF *Glossina austeni* Newst.

## 5.1 THE GENUS *Trypanosoma*

Trypanosomes (*Trypanosoma* Gruby) are haemoflagellated protozoa of the order Kinetoplastida, many of which (especially members of subgenera *Trypanozoon*, *Nannomonas*, and *Duttonela* infect tsetse flies (*Glossina* Wied, Diptera) (Jordan 1976a).

The African wild ungulates which are the natural hosts of tsetse flies also habour the trypanosomes (Ashcroft, 1959; Moloo *et al.* 1971). Transmission, therefore, occurs through the blood meal. However, circumstances may arise (such as at watering places or during herding of cattle, or gathering of fire wood) in which tsetse flies may get to feed on humans or domestic stock. Transmission then takes place to these secondary or adventitious hosts and the disease may ensue.

## 5.2. TAXONOMY OF *Trypanosoma*

The genus *Trypanosoma* may be divided into two groups, based on the site of production of the metacyclic (infective forms) trypanosomes in the insect host, and the subsequent method of infection of the mammalian hosts (Vickerman 1976). These are the Stercoraria and Salivaria trypanosomes. Members of the Stercoraria group complete their developmental cycle in the hind gut of the insect host, and the metacyclic forms are present in the faeces. Transmis-

sion is by contamination. Trypanosomes in this group are basically non-pathogenic, except one member, *T. cruzi* which is the causative germ of Chaga's disease in human. The parasite is transmitted by triatomine bugs in Central and South America.

The Salivaria group of trypanosomes complete their developmental cycle in the mouth parts of the insect host, and the metacyclic forms are present in the salivary secretions. Transmission is mainly through the bite of infective vector, although mechanical contact transmission is known to occur as well (Lucas, 1955; Hoare 1972). The Salivaria trypanosomes are pathogenic to human and domestic animals. There are four subgenera within the Salivaria group (Hoare, 1972; Vickerman, 1976), *Pycnomonas* (found in wildlife, does not seem to be transmitted by tsetse to domestic animals), *Duttonella* (e.g. *T. vivax*), *Nannomonas* (e.g. *T. congolense*) and *Trypanozoon* (e.g. *T. brucei* group). Some *Trypanozoon* are found outside the tsetse belt and may be transmitted by mechanical means by other biting flies such as tabanid flies (Tabanidae) or *Stomoxys* (Muscidae) (Hoare 1972). *Trypanosoma evansi* is an example of this. However, in Kenya *T. evansi* is within the tsetse belt, in the North Eastern Province, and is mostly transmitted by biting flies. It is the causative agent of a disease of camels called surra, which may also affect horses, donkeys and domestic buffaloes and elephants in Asia (Willet, in 'the African trypanosomiasis' by Mulligan, 1970).

The nomenclature of trypanosomes and their relationship with allied flagellates have been reviewed by Hoare (1972) and Vickerman (1976). Trypanosomes are in:-

Phylum: Sarcomastigophora

Class: Zoomastigophorea

Order: Kinetoplastida

Sub order: Trypanosomatina

Family: Trypanosomatidae

Genus: *Trypanosoma*

### 5.2.1 Trypanosome infection in tsetse:

When tsetse ingests trypanosomes in the course of its feeding on an infected host, the parasites (trypanosomes) must adopt themselves to life inside the fly. The processes involved in this adaption, and subsequent development are more complicated in the case of *T. brucei* group than in the others (Baker, 1977; Jordan 1976a). For example, T. *vivax* simply attaches itself to the walls of the food canal formed by the labrum and labium, where they form compact colonies and multiply. The infective forms then migrate to the hypopharynx. *Trypanosoma congolense*, on the other hand, develops in the midgut, and migrates later to the food canal where it multiplies. The infective forms move to the hypopharynx (Nash, 1969). It is believed that the age of the fly at the time of the infective feed is important (Willet, 1966). In young flies the crop is still small and retains blood for a longer period allowing time for enzyme transformation to act on the trypanosomes (Langley 1975). The fact that the age of the fly at the time of the infective feed is important for the fly to become infective was also reported in experiments involving *T. brucei* and *G.*

*morsitans* (Jenni 1977; Otieno *et al* 1983). And Willet (1966) presented morphological evidence to support this. He showed that the peritrophic membrane in *G. austeni* and other *Glossina*, is usually incompletely developed in flies 24 hours old. This permits trypanosomes to reach the ectoperitrophic space readily in and near the proventriculus, or easily to penetrate the peritrophic membrane.

The duration from the time the fly ingests trypanosomes to the time it (fly) becomes infective seems to vary (Buxton, 1955; Hoare, 1972). *Trypanosoma congolense* was reported to take between 19 to 53 days in tsetse to develop into mature cyclic forms (Buxton 1955; Hoare 1972). These workers did not specify the tsetse species involved. On the other hand, Jenni (1977) obtained mature infection of *T. brucei* in *G. morsitans* in nine days.

Differences in susceptibility to infection have been known to occur between species of *Glossina* in the laboratory studies (Jordan 1976a) and in the field (Johnson and Lloyd, 1923; Harley and Wilson 1968). Individual flies also differ in their susceptibility to infection although the mechanism involved in susceptibility is still unknown (Jordan, 1976a). Many workers have noted that infected salivary glands are rarely found in the field by the dissection method (Ward and Bell 1971; Otieno 1978, 1983; Jordan 1974), and that when encountered the infection rates are usually extremely low. Ford and Leggate (1961) have discussed increases in trypanosome infection in tsetse of the morsitans group, in terms of distance from what they termed the *Glossina* equator, which lies at 7° South of the real equator. They showed that infections increased with rising latitude from the middle of the *Glossina* zone.

In general, the rates of infection in the fly may be highest with those trypanosomes which have the simplest cycle of development in the insect (e.g *Dutinnela* or *T. vivax* group) and lowest with those with the most complicated cycle of development (e.g.*trypoanozoon* or *T. brucei* group) (Jordan, 1976a; Willet, 1966ccs). Differences in rates of infection in the field may also be accounted for by such factors as temperatures, sampling method used to obtain tsetse and the type of host animal on which the fly fed (Ward, 1968; Harley, 1965; Jordan, 1965; Harley and Wilson 1968).

### 5.2.2 Identification of the parasites:

Trypanosomes are generally lanceolate in shape with the anterior end tapering to a point while the posterior may be rounded or pointed (Hoare 1972; Vickerman 1976). The parasites may be convoluted in different ways, but the flagellum and the undulating membrane follow the bands (Figs. 5.1 and 5.2). Of particular taxonomic importance to a species of trypanosome are: apparent shape and size of the kinetoplast, position of the kinetoplast in the body of the trypanosome, presence or absence of free flagellum and shape of the posterior end (Hoare 1972) Fig. 5.2. For example, the Nannomonas group generally lack free flagellum in both the mammalian and the insect hosts. In this group the kinetoplast is positioned sub terminal or marginal, and they are typically monomorphic, although Hoare (1972) observed some degree of polymorphism in *T. simiae*, which is a member of this group.

The most common method of examining trypanosome infection in tsetse flies is by the dissecion of the fly (Lloyd and Johnson 1924), but other methods exist ( e.g. salivation of the fly, whereby the tsetse is induced to deposit saliva on a warm glass slide which is then examined for the presence of the parasites). The various species of *Trypanosoma* look very similar (Fig 5.2) and can only be identified by careful study of their morphology in stained preparations. This is usually not practical in field studies. Lloyd and Johnson (1924) were the first to suggest that the position of trypanosomes in the organs of the infected tsetse might be an indication of their identity. The method was elaborated on by Duke (1933), and Fairbairn and Watson (1955). By this method, *T. vivax*, *T. congolense* and *T. brucei* may be shown to occur in the mouth parts; mouth parts and gut; and mouth parts, gut and salivary glands respectively. The strains that cause human sleeping sickness belong to the last group (*T. brucei*), while all three may cause nagana in domestic stock.

## 5.3. OBJECTIVES OF THE STUDY

The general objective of this study on the vectorial capacity of *Glossina austeni*, was to investigate its susceptibility to infection with trypanosomes in general and *T. congolense* in particular. Specific objectives were: (a) to determine the rate of infection in the field, with the three trypanosome species, (*T. vivax*, *T. congolense*, *T. brucei*), and (b) to determine whether or not *G. austeni* plays an important role in the transmission cycle of nagana along the Kenya coast, by studying its ability to transmit parasites from one mammal species to

another. Although sleeping sickness has not been recorded at the coast, the disease of livestock is very common and forms one of the major constraints in the livestock industry in this province.

Specifically, the study sought to establish two things:

1. The efficiency of this *Glossina* species in picking up and transmitting trypanosomes of the *T. congolense* group.

2. The relative rates of infection of *G. austeni* population with the various trypanosome species present at the coast; and how this compares with the infection in the other two ecologically co-existing tsetse species (*G. pallidipes* and *G. brevipalpis*). It was envisaged that such a comparison would enable an assessment of the relative importance of *G. austeni* in the transmission cycle in this area.

## 5.4. MATERIALS AND METHODS

A study was conducted in the laboratory to determine the efficiency with which *G. austeni* picks up trypanosomes from an infected host, and with which the parasites may develop into mature cyclic forms within the fly, and get transmitted to the next host (vectorial capacity). The dissection and examination of field specimens to provide evidence of *G. austeni* infectivity with trypanosomes in the wild, and the identity of the parasites involved, was carried out at the ICIPE Muhaka field laboratory.

### 5.4.1 Study of Trypanosome Infection in wild tsetse flies

To determine the infection of the three tsetse species with trypanosomes in the field, non teneral flies of all the three species were dissected to examine the mouth parts (hypopharynx and labrum), gut and salivary glands under a binocular dissecting microscope. These parts were then, placed on slides, covered with cover slips and, examined under a compound microscope to detect the parasites. All together 1210 *G. austeni* were examined (758 females and 492 males). For comparison, 1152 *G. pallidipes* (642 females, 510 males) and 755 *G. brevipalpis* (343 females, 412 males) were also examined. The dissections were performed on samples from Muhaka forest and Shimba Hills between January 1989 and May 1991, in ICIPE's Muhaka field laboratory.

### 5.4.2 Infecting *G. austeni* using an infected laboratory host

To determine the efficiency with which *G. austeni*, when exposed to infected blood meal, may pick up infections of *T. congolense*, the following experiments were carried out in the laboratory. The first generation of flies from field collected pupae were infected by feeding them on rats previously inoculated with *T. congolense* (IL 1180). This strain was obtained from the International Laboratory for Research on Animal Diseases (ILRAD), Nairobi. It had been derived, after one passage in mice, from a clone originally isolated from a lion in the Serengeti National Park, Tanzania (Olubayo *et al.* 1990). The para-

sites were inoculated into rats, which in turn were offered to tsetse for blood meal when they (rats) showed high parasitamia (at least 10 parasites per microscope field). This took between seven to twenty one days and probably depended on the immunological competence of the animals. The tsetse were placed on anasthetised rats (injected with 0.2ml of sodium pentobarbital sedative) and left to feed for 15 to 20 minutes in the dark. Afterwards the fed flies were separated and those that did not feed discarded. From the second day after the infective feed the flies were maintained on a normal, uninfected, rabbit as a precaution against mechanical transmission of trypanosomes from the proboscis of the recently fed fly. At the end of 21 days the flies might have harboured infective metacyclic trypanosomes in their mouth parts (Ward, 1968, waited for 7-14 days, for *T. brucei* to come up in mice). Salivation test was carried out at this stage (after 21 days) to determine if mature parasites had developed. Later (after the transmission experiments were complete) all the flies were dissected whether they had shown mature infection by the salivation method or not.

The experimental flies were fed 6 days a week, omitting Sundays. At the end of 21 days, salivation test was carried out, and those which showed infection were used in transmission experiment (see below) and later dissected. The others were dissected 30 days after the infective feed.

### 5.4.3 Effect of fly age on its susceptibility to infection by trypanosomes

In a series of experiments young *G. austeni* were segregated into three age-groups as follows: 24 h, 48h, and 72h (or over) old flies. The first two categories consisted of tenerals and the last non-tenerals. They were offered an infective meal to study the influence the age of the fly at infective meal may have on the establishment of the parasites in tsetse. When subsequent maintenance were completed the flies were dissected to check for the presence of trypanosomes.

### 5.4.4 Transmission of trypanosomes by *G. austeni*

Laboratory maintained tsetse flies whose infectivity had been confirmed by the salivation method, were fed on clean (non infected) mice to determine the flies efficiency in transmitting the parasites. Flies which did not show mature infection by the salivation method were also fed in this way, in case they had low parasitemia which could easily have been missed. The animals were then maintained for 30 days in the laboratory. From day 7 until day 30 (or until infection was confirmed, whichever, came first) blood smear was taken from the tail of the animal, and examined for the presence of trypanosome parasites. Those which showed positive results were recorded and discarded, the rest were discarded after 30 days.

### 5.4.5 Dissections of laboratory maintained tsetse flies

Before dissection, the flies were starved for a day so that the partially digested blood meal would not obsecure the dissection. The alimentary canal was removed and bathed in 0.85% saline solution, then transferred to a clean saline drop and loosely covered with a cover slip. This was then squashed gently before the slide was examined under the microscope. Similarly the hypopharynx and the labrum were dissected and examined.

## 5.5 RESULTS

The results of trypanosome infections in *Glossina austeni*, *G. pallidipes* and *G. brevipalpis*, in Muhaka and Shimba hills are presented in Table 5.1. In *G. austeni*, the mean infection rate in the two sexes was 10.4% in Muhaka and 15.1% in Shimba Hills. *Trypanosoma congolense* was the prevalent parasite in all the three *Glossina* species, in Muhaka. Its incidence, compared to that of *T. vivax*, was significantly higher ($P<0.01$, $F=11.1$, $df=1$). However, in Shimba Hills, the difference in the incidence of the two trypanosome parasites was not significant (Table 5.1). *Trypanosoma brucei*, was not encountered in *G. austeni*, although three incidences were encountered in *G. pallidipes* and one in *G. brevipalpis* from the Muhaka fly samples.

The mean infection rates in the three *Glossina* species were significantly different ($P<0.01$, $F=7.3$ $df=2$). *Glossina austeni* showed the highest mean infection with trypanosomes, and the inci-

dence was significantly greater in males than in females ($P<0.05$, $F=7.7$) (Table 5.1).

Trypanosome infection rate in *G. austeni* was shown to fluctuate between 2 and 20%, throughout the twelve months of the year (Fig.5.3). Higher infection rates were recorded during high levels of apparent density in *G. austeni*, and this, in turn, coincided with the rainy seasons in April-June and October-November (Fig. 5.3).

Table 5.2 shows the results of the laboratory experiments on the efficiency of *G. austeni* in picking up *T. congolense*. No significant difference was observed between teneral males and females (Table 5.2). However, significantly more of the young tenerals (24h) of both sexes showed greater susceptibility to infection than the older tenerals (48 h old). Moreover, tenerals of both sexes showed greater susceptibility than non tenerals ($P<0.01$).

The mean efficiency of *G. austeni* tenerals (24h old) in picking up infections was 56% the maturity rate of such infections was 23%, and the transmission rate among those with mature infections was 100% (Table 5.3). In non tenerals, on the other hand, the mean efficiency (of female and males) in picking up infections was 18.6% and the maturity and transmission rates were 100% in each case.

## 5.6 DISCUSSION

The finding that *T. congolense* is the prevelent parasite agrees with that of Tarimo *et al* (1984) who investigated trypanosome infection rates in *G. pallidipes* in five locations along the Kenya coast and

found that *T. congolense* was the most prevalent in that species at the coast.

The sampling method used may result in differences in infection rate between the sexes (Harley 1967, Owaga 1981). This is because some methods favour younger flies. Owaga (1981) recorded lower infection rates in samples of *G. pallidipes* obtained by slow moving vehicle, than in those obtained by Langridge traps, the former consisted mostly of young flies and the latter of older ones. Clarke (1969) observed higher infection in males than in females collected in fly rounds. This method is known to attract males of various ages, but only young females and not older ones. On the other hand, Leggate (1963) determined trypanosome infection in flies caught off a tethered bait (ox) and found no difference in infection between the sexes. Presumably, because all age groups of females and males approached the host for blood meal. In this study only the trapping method was used for sampling. Male and female flies of all age groups above teneral were captured, so that the capture method could not have been the reason for the difference between male and female infection rates in *G. austeni.*

In the laboratory study the results were in agreement with those of Ward (1968), for *T. brucei* in *G. austeni.* He reported that tenerals (24 h), having their first meal infected, showed higher rates of infection than both older non tenerals and older tenerals of 48-72 hours. With regard to the sexes, a difference was observed in non tenerals, with the males showing greater susceptibility than females (22.5% against 16.3%). This was, however, in line with the field results which showed that males had higher infection rates. In view of this

similar trend between field and laboratory results, it may be a fact in the interaction between *G. austeni* and *T. congolense*, that males are more susceptible.

The hosts of tsetse may be associated with different trypanosome species. Tsetse feeding on bovids are said to harbour high infections of *T. vivax*, whereas *T. congolense* is associated with both bovids and suids (Jordan 1964, Moloo *et al.* 1973). In Muhaka the hosts were mostly suids (bushpig and warthog), (Table 3.3, Chap. 3) and there were less *T. vivax* infections than *T. congolense* ones, whereas in Shimba Hills where the hosts were mainly bovids *T. vivax* infections were much higher (Table 5.1).

The results of the blood meal analysis (Table 3.3, Chap.3) also showed that the two *G. austeni* specimens that had fed on humans were males, and the one that had fed on the chicken was a female; indicating that both sexes sometimes wonder out of the forest to the fringes, and that they may encounter domestic animals from the nearby homesteads, and transmit the parasites to them.

The proportion of immature infections encountered in the field varied between the *Glossina* species, from, 3.8 % and 5.7% in female and male G. *austeni* respectively, to 3.1% and 3.3% in female and male *G. pallidipes* and 0.8% and 0.9% in female and male *G. brevipalpis*.

The difference in susceptibility to infection by male and female *Glossina* in laboratory studies reported by different workers are conflicting. Burtt (1946), Fairbairn and Culwick 1950), Clarke (1969) and Otieno *et al.* (1983) showed that males were more susceptible in

their respective studies. On the other hand, Duke (1933), Moloo *et al* (1973) and Harley (1971) reported that females were more susceptible in their laboratory studies. In this study a higher proportion of non teneral males than non teneral females picked up infections. Similarly, in the 24h teneral group, more males than females picked up infections although the number that maintained mature infections was the same. Some field workers have found higher infection rates in females than in males of *G. pallidipes* (Tarimo *et al* 1984; Bursell and Glasgow, 1958, 1960). While others, e.g. Okiwelu (1977), noted higher infection in males than in females of *G. morsitans* in Zambia. Leggate (1963) and Jordan (1964) observed no difference in infection rates between the sexes in *G. morsitans*. The difference between the sexes, therefore, may vary from area to area and species to species, and must partly depend on the interaction between the *Glossina* species in question and the various *Trypanosoma* species. Some *Glossina* are stronger immunologically and are relatively refractory to infections including those of trypanosomes (Dr. J. Stiles, pers. comm.; Robert and Gray (1972). In other reports females of *G. pallidipes* showed greater tolerance to insecticide exposure than their male counterparts (Golder *et al*. 1984). Such a difference in the degree of immunological competence in males and females, might apply to their susceptibility to trypanosomes.

Past work on infection of *G. austeni* with *T. brucei* in the laboratory showed that the age of tsetse at the time of the infective feed was the most important physiological condition of the vector for successful laboratory infection (Ward 1968). Young flies showed higher infections than older ones. Wijers (1958) reported similar results on

*T. brucei* in the tsetse species *G. palpalis*. It appears, therefore, that this factor is associated with, the requirements of that trypanosome species for successful establishment in a tsetse. The results of this study concurs with those of the two authors in that more tenerals picked up infections of *T. congolense* in the laboratory. However, it must be added that only half the number that had picked up those infections could transmit the parasites, the remaining half did not show mature infections, and so could not transmit to the next host. In the final analysis, the proportion that carried transmmitable parasites was still higher, though not significantly so, than that of non tenerals (Table 5.2).

Willet (1966) found that in older flies the peritrophic membrane appeared to be impenetrable except for a few rare trypanosomes which went through the mid and hind gut until they reached the open end of the peritrophic membrane. They then passed through the ectoperitrophic space to the proventricular region. He too was working with *T. brucei*, which as Baker (1977) and Jordan (1976a and 1976b) stated, undergo a more complicated process to adapt to life inside the fly and to develop and mature, than the other parasite species. Moreover, this would not explain why, having successfully established themselves in the teneral fly, so many should fail to reach maturity, whereas in non tenerals all should reach maturity. Probably the immunological competence of the teneral fly is higher. It appears from these results, that in nature *G. austeni* need not have an infected first meal to become infective with *T. congolense*, they may as well pick up infections later on in life from second, third or any other blood meal.

In this study, 56% of the teneral *G. austeni* became infected, but in 77% of these infections the parasites did not develop into mature cyclic forms (to be found in the hypopharynx) (Table 5.3). After 21, 30 or even 40 days (until they died) they remained immature. This meant that the flies could not (and they did not) transmit the parasites to the next host. The 23% of the tsetse that developed mature infections all transmitted trypanosomes to the mice after one feed (100% transmission). On the other hand 19% of the non teneral flies picked up the parasites, and they all developed cyclic forms which they were able to transmit to the mice (100% transmission).

It is concluded that *G. austeni* is an efficient transmitter of *T. congolense*. There was no evidence to show that it could be less efficient than *G. pallidipes*. On the contrary field results showed higher infections in this species than in both *G. brevipalpis* and *G. pallidipes*. This suggests that it is, at least, equally important compared to the latter, in the area, and probably more important than the former (*G. brevipalpis*). However, its being more restricted to the thicket and forest than *G. pallidipes* may reduce the chances of contact with domestic stock. It cannot, therefore, transmit trypanosomes as widely as *G. pallidipes* which is more adaptable in its habitat requirement, and is found in almost all habitat types, from semi desert to dense thicket (Anonymous, 1970). *Glossina austeni* may be important, in this respect, in areas where settlements are very close to the forest, as in Muhaka, and where cattle are grazed inside or close to the forests during the dry seasons.

Table 5.1 Trypanosome infection in three *Glossina* species in the south coast, Kenya

| Fly | No. examined | *T. congolense* | *T. vivax* | *T. brucei* | Total infection |
|---|---|---|---|---|---|
| Female (Muhaka) | | | | | |
| *G. austeni* | 640 | 6.0% | 3.1% | 0% | 9.1% |
| *G. pallidipes* | 525 | 5.9% | 2.8% | 0.3% | 9.0% |
| *G. brevipalpis* | 279 | 4.1% | 0.3% | 0.2% | 4.6% |
| Male (Muhaka) | | | | | |
| *G. austeni* | 378 | 7.4% | 4.2% | 0% | 11.6% |
| *G. pallidipes* | 313 | 5.6% | 1.7% | 0.2% | 7.5% |
| *G. brevipalpis* | 339 | 3.5% | 0.5% | 0.2% | 3.8% |
| Female (Shimba hills) | | | | | |
| *G. austeni* | 118 | 7.6% | 5.9% | 0% | 13.5% |
| *G. pallidipes* | 117 | 2.6% | 6.0% | 0.9% | 9.5% |
| *G. brevipalpis* | 64 | 1.6% | 1.6% | 0% | 3.2% |
| Male (Shimba hills) | | | | | |
| *G. austeni* | 74 | 2.8% | 13.9 | 0% | 16.7% |
| *G. pallidipes* | 101 | 1.0% | 4.0% | 1.0% | 6.0% |
| *G. brevipalpis* | 73 | 1.4% | 1.2% | 0% | 2.6% |

Table 5.1 Trypanosome infection rate in three *Glossina* species in Muhaka and Shimba Hills

The mean trypanosome infection in the three species was significantly different [ $P<0.01$, $F=7.3$ $df=2$ ], with the infection in *G. austeni* being the highest. The prevalence of *Trypanosoma congolense* in the south coast was, i. significantly higher than that of *T. vivax* in Muhaka [$P<0.01$, $F=11.1$ $df=1$]

ii. not significantly different from that of *T. vivax* in Shimba Hills, $p>0.25$.

The difference in the two localities was probably due to the preponderance of suids over bovids, as host, in Muhaka, and vise versa in Shimba Hills. It appeared that suids were relatively refractory to *T. vivax*.

Table 5.2 *Trypanosoma congolense* infection in laboratory treated *G. austeni*

| Age groups | No. treated | L.H.G. infection | L.G. infection |
|---|---|---|---|
| Females | | | |
| 1 | 82 | (20) 24.4% | (26) 31.7% |
| 2 | 77 | (8) 10.4% | (9) 11.7% |
| 3 | 86 | (14) 16.3% | 0 |
| Males | | | |
| 1 | 84 | (20) 23.8% | (36) 42.9% |
| 2 | 52 | (4) 8.0% | (4) 8.0% |
| 3 | 80 | (18) 22.5% | 0 |

L.H.G. = labrum, hypopharynx and gut infections

L.G. = labrum and gut infections (immature)

Age group, 1 = 24h old

2 = 48h old

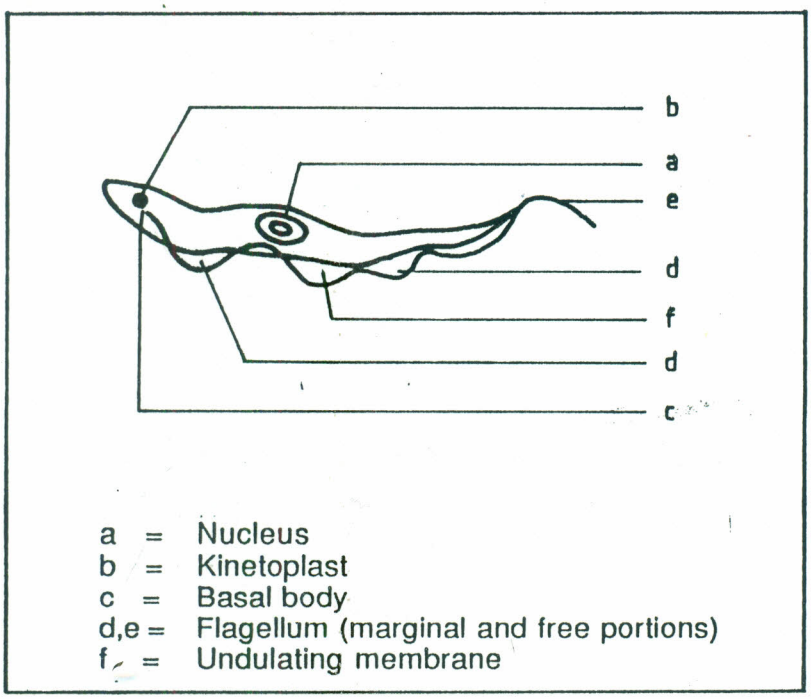
3 = 72h and older

Table 5.3 Rate of transmission of *T. congolense* by *Glossina austeni* to mice.

| *G. austeni* with mature infection | *G.austeni* used in transmission | No. of mice that became infected |
|---|---|---|
| Females, age | | |
| 1 | 20 | 20 |
| 2 | 8 | 8 |
| 3 | 14 | 14 |
| Males, age | | |
| 1 | 20 | 20 |
| 2 | 4 | 4 |
| 3 | 18 | 18 |
| *G. austeni* with labrum and gut infections (immature) | | |
| Females, age | | |
| 1 | 26 | 3 |
| 2 | 9 | 0 |
| males, age | | |
| 1 | 36 | 1 |
| 2 | 4 | 0 |

age 1 = 24h
2 = 48h
3 = 0ver 72h (non teneral)

Figure 5.1




General morphology of a trypanosome

Figure 5.1 **General morphology of a trypanosome**

Some of the diagnostic characters used to distinguish the different mammalian trypanosome species include, position and size of the kinetoplast in the body, presence or absence of free flagellum and shape of the posterior end. [Source - C.A. Hoare, 1970, in Mulligan H.W. (Ed. George Allen and Urwin Ltd. 1970)]

Figure 5.2 Three trypanosome species commonly found in tsetse flies at the Kenya coast.

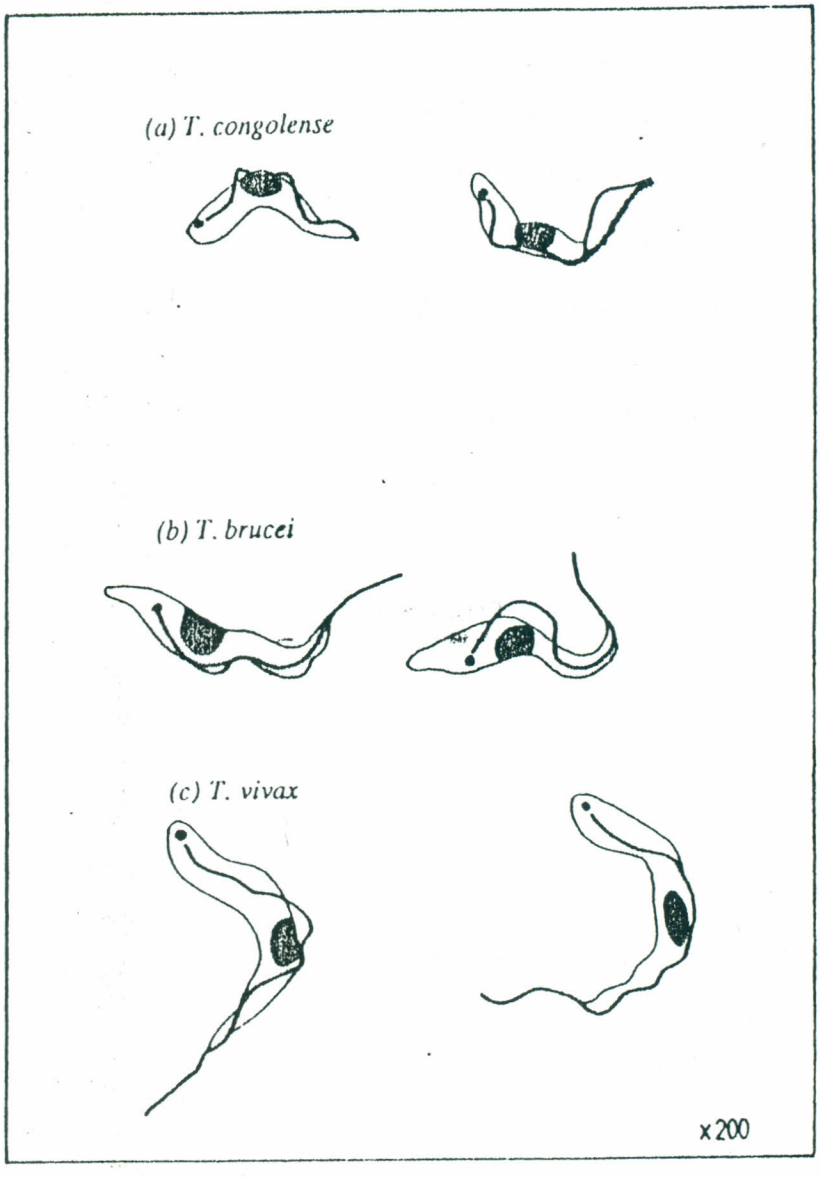
*T. congolense* - lacks free flagellum, position of kinetoplast is marginal

*T. brucei* - has a bluntly pointed posterior end, position of the kinetoplast is subterminal

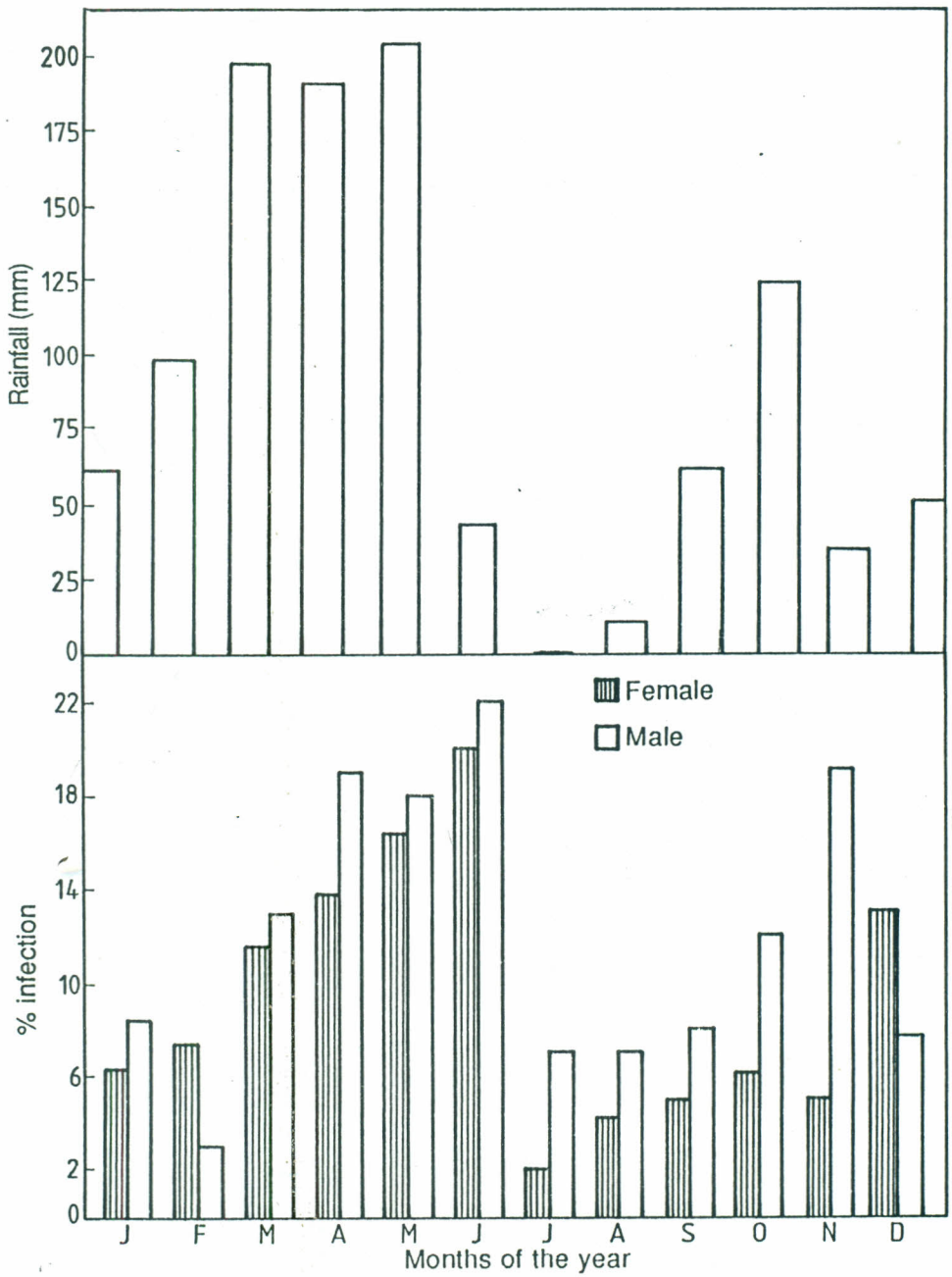
*T. vivax* - the position of the kinetoplast is almost terminal

[Source - C.A. Hoare, 1970, in Mulligan H.W.(Ed. George Allen and Urwin Ltd, 1970)]

Figure 5.2




Diagramatic representation of three trypanosome species found at the Kenya coast.




Trypanosome infection in *G. austeni* in Muhaka forest area (1989-1990)

Fig. 5.3

Fig. 5.3 Mean monthly trypanosome infection rates in *Glossina austeni* in Muhaka (1989 and 1990)

**a.** mean monthly rainfall in millimeters

**b.** mean monthly trypanosome infection in *G. austeni*.

Note. High infection coincides with high rainfall; the onset of high infection lags behind that of high rainfall by one month and ends one month later.

# Chapter 6

# GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE STUDY

## 6.1 GENERAL CONCLUSIONS

The main findings of this study were:

1. That *Glossina austeni* is a forest or /and dense thicket species, and is significantly associated with dense vegetation as opposed to woodland or more open vegetation.
2. That it occurs in relatively low densities compared to other tsetse of the same *morsitans* group, such as *G. pallidipes*.
3. *G. austeni* is a resident species, which does not disperse very widely.
4. It can be captured in sufficient numbers using traps, but due to high humidities found in the area of its range, odour baits generally do not make very significant difference in trap catches of this species, except for dry ice, and, to some extent, buffalo urine.
5. The Lancia trap was recomended as a suitable sampling tool, due to the fact that it works almost equally well with or without bait. But the biconical, the 4t and the NG2B traps can all obtain reasonable samples
6. *Glossina austeni* is a day-active species, and may be captured at any time between 0550 and 1800 h. However, its peak activ ity periods are around 0700-1100 h and 1400-1600 h
7. *G. austeni* is an important transmitter of trypanosome parasites.

In the south coast, Kenya, trypanosome infection rate in this species was significantly higher than in the other two *Glossina* species.

8. Trypanosome infection rate in *Glossina*, in the south coast, Kenya, was higher during the rainy season than during the dry seasons, this was so in *G. austeni* as well as in the other two *Glossina* species that are found there. The high rates coincided with periods of high density in the fly populations.
9. It was shown that a teneral *G. austeni* that takes infective blood (infected with trypanosomes) as its first meal, generally picks up trypanosome infections more readily than an older fly, although the latter may also get such infections.
10. In Muhaka area, in the south coast, Kenya, where large mammals do not occur, the main hosts of *G. austeni* are warthogs and bushpigs, and occassionally bushbuck. However, there was indication that where large mammals are present this need not be the case, and other mammal species such as the buffalo may play important roles as hosts. Domestic stock, including chicken may also be fed on.
11. *Glossina austeni* feeds at intervals of between three and five days. During dry conditions the feeding is more frequent due to rapid loss of fat reserves, but during wet, humid conditions the feeding interval is longer.
12. A number of arthropods may play some part in the regulation of *G. austeni* population. They include various families of Hymenoptera, such as Bombiliidae, Formicidae and Sphecidae; some Diptera, e.g. Asilidae (robber flies) and Orthoptera, such as Gryllacridae and Gryllidae (crickets).

13. The finding of this study, with regard to the three *Glossina* species, is that *G. pallidipes* is more widely distributed in more open areas, with the highest density occurring at the ecotone of dense vegetation and open woodland. *G. austeni*, on the other hand, prefer denser vegetation, and *G. brevipalpis* [a crepuscular species] also appeared to prefer dense vegetation; but in the evening (after 1830 h) when its activity period began, a lot of them were encountered outside the forest, flying low along paths. This habit and the activity time of this species may serve to separate it (ecological) from *G. austeni*, and to prevent direct competition.
14. It is speculated that the contact between *G. austeni* and domestic stock or humans may be greately minimised by avoiding grazing animals close to the forest and dense thicket, and by not building homes at the edge of forests, as is presently the case around Muhaka forest.

## 6.2 AREAS OF FUTURE STUDY

It was not possible during this study, to look into all aspects of the ecology and behaviour of *G. austeni*, in the limited time. Some ecological aspects require long term monitoring (for many years), to be able to get a full picture of what may be happening. Moreover, owing to the fact that this species has hardly been studied in the wild before, no one study could cover everything. It is, therefore, recom-

mended that the following aspects, that were touched on during this study could be continued to enable a fuller understanding of the ecology of *G. austeni* in the coastal area of Kenya:

1. Mortality factors acting on *G. austeni*, could be taken as a project on its own: for example both density dependent and density independent factors should be studied, and the role of predators quantified in terms of regulation of the population in relation to other mortality factors.
2. While there was no evidence that movement of *G. austeni* took place between Shimba Hills and Muhaka forest, occasionally (once in a month or two) few *G. austeni* were captured (usually one sometimes two) in the transects between the two areas (Fig. 1.4). It should be ascertained as to whether an ultra low density of *G. austeni* population exists around the homesteads, or whether aslow migration occurs from either Shimba Hills National Park or Muhaka. This could be done by marking flies in the park as well as in Muhaka, preferably tenerals, and then placing traps near homesteads in the settled area between Muhaka and Shimba Hills. It would take a long time to ascertain this due to the ultra low density involved, and because one may not catch flies every day, or even every week, but from the point of view of disease transmision, even one fly is important and is enough to spread the parasites. A similar study between Buda forest to the south (some 40 km away) and Muhaka, would further help clarify the importance of this species in the disease transmission to the local cattle. Around Lambwe valley in the western part of Kenya, it was found that *G. pallidipes* could

adapt to a 'peridomestic life', breeding and feeding around homes [Dr. L.H. Otieno, pers com). The proposed study would establish whether *G. austeni* can do the same, especially given the fact that most homesteads in the south coast have plantations, either of cashew nuts, mango or coconut palms or all three.

3. Use of electric nets around traps, both in the forest and outside it, and during dry seasons as well as wet seasons, would help establish the efficiency of various devices in terms of the proportion of *G. austeni* they catch out of the number that approach them. This would be important in developing a specific trap for the species. Once the device that attracts the highest number is known, one can go about modifying it to induce entry and so catch more. The finding of this study was that the pyramidal trap (Lancien trap) catches higher numbers. This may not necessarily mean that it attracts the highest number, as some traps may attract many tsetse but have poor landing inducement surface, thus resulting in poor catch. Sometimes this may be due to lack of suitable entry point or the colour /and /or tecture of the material at the entrance. A similar study with electric screens can be conducted using various odours. This can also institute a study project in itself. In addition one could construct an underground 'tunnel' within the forest and place various animals in it at different times, and a trap at ground level near the entrance. This would enable one to ascertain whether, in deed, *G. austeni* relies on olfactory cue to hunt or whether the high humidity condition which appeared to inter-

fere with its attraction to baited traps means that it may rely on other cues. According to the findings of the present study, there appeared to be some contradiction with what is widely believed, that forest species may rely more on olfaction than visual since they cannot see very far due to obstraction by foliage. Moreover, urine from bushpig, one of the main hosts, was ineffective as an attractant. It would be interesting to find out whether a live animal placed under ground, and therefore out of sight, would be detected through breath and the gases exuding from the body, and whether the flies would be attracted to a trap placed at such an entrance. The local people know how to trap bushpig and would possibly help in obtaining one for study.

4. Further comparative study needs to be conducted on the three *Glossina* species, to shed more light on their ecological separation strategy, if any, or determine if there is any competition between them. This may be more so in so far as G. brevipalpis and G. austeni are concerned, since both are forest dwellers. Such a competition might dictate that when the density of one rises that of the other falls. However, from the few observations made on *G. brevipalpis* and *G. pallidipes*, it appeared that their densities fluctuate at the same time as that of *G. austeni*, but this requires more detailed study.

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Appendix 1: Female population estimate for February 1990.

| wk (i) | 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| $r_i$ | 68 | | | | |
| $n_i$ | | 127 | 132 | 181 | 112 |
| $m_i$ | | 5 | 2 | 1 | 1 |
| $(i-\bar{i})$ | | -1.5 | -0.5 | 0.5 | 1.5 |
| $\mathbf{Inq_i}$ | | 1.178 | 0.159 | -0.56 | -0.775 |
| $\mathbf{Inq_i - Inq_i}$ | | -2.765 | -3.784 | -4.510 | -4.719 |
| $(i-\bar{i})^2 * m_i$ | | 18 | 0.75 | 0.5 | 2.25 |

$$\mathbf{In}(1-b) = \frac{\Sigma m_i (\mathbf{Inq_i - Inq_i})(i-\bar{i})}{\Sigma m_i (i-\bar{i})^2}$$

birth rate = 0.53
slope = 0.749
intercept = 2.071
proportion ($q_0$) = 0.126

Female population estimate for February = 539.2

Key:
wk (i) = weeks
$r_i$ = number marked
$n_i$ = number recaptured (marked+unmarked)
$m_i$ = number of marked recovered
$\bar{i}$ = mean of i

Appendix 2: Female population estimates for March 1990

| wk (i) | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| $r_i$ | 107 | | | | | | |
| $n_i$ | | 150 | 125 | 102 | 178 | 172 | 161 |
| $m_i$ | | 6 | 4 | 4 | 3 | 2 | 2 |
| $q_i = \frac{m_i}{n_i}$ | | 0.040 | 0.032 | 0.039 | 0.0169 | 0.0116 | 0.0124 |
| $i-\bar{i}$ | | -2.5 | -1.5 | -0.5 | 0.5 | | 1.52.5 |
| $\mathbf{Inq_i}$ | | -3.219 | -3.442 | -3.239 | -4.083 | -4.454 | -4.389 |
| $\mathbf{Inq_i}$-$In\bar{q}_i$ | | 0.7008 | 0.4777 | | | 0.5345 | 0.4687 |
| $(i-\bar{i})^2 * m_i$ | | 37.5 | 9 | | | 4.5 | 12.5 |

$$\mathbf{In}(1-b) = \frac{\Sigma m_i(\mathbf{Inq_i} - \mathbf{In\bar{q}_i})(i-\bar{i})}{\Sigma m_i (i-\bar{i})^2}$$

**b** (birth rate) = 0.25

slope = -0.2855

intercept = -2.9206

proportion ($\mathbf{q_0}$) = 0.0539

female population ($\mathbf{N_0}$) = $\frac{\mathbf{r_0}}{\mathbf{q_0}}$ = 1985

Appendix 3: Female population estimates for May 1990

| wk (i) | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| $r_i$ | 81 | | | | | | |
| $n_i$ | | 344 | 258 | 267 | 180 | 166 | 118 |
| $m_i$ | | 5 | 3 | 2 | 2 | 1 | 1 |
| $q_i = \frac{m_i}{n_i}$ | | 0.0145 | 0.0116 | 0.0075 | 0.011 | 0.0060 | 0.0085 |
| $(i-\bar{i})$ | | -2.5 | -1.5 | -0.5 | 0.5 | 1.5 | 2.5 |
| $\ln q_i$ | | -4.2315 | -4.4542 | -4.8941 | -4.5099 | -5.1120 | -4.7706 |
| $\ln q_i - \ln \bar{q}_i$ | | 0.4305 | 0.2079 | -0.2320 | 0.1522 | -0.4500 | -0.1086 |
| $(i-\bar{i})^2 * m_i$ | | 31.25 | 6.75 | 0.5 | 0.5 | 2.25 | 6.25 |

$$\ln(1-b) = \frac{\sum m_i (\ln q_i - \ln \bar{q}_i)(i-\bar{i})}{\sum m_i (i-\bar{i})^2}$$

birth rate (**b**) = 0.13

slope = -0.1448

intercept = -4.5552

$q_0$ = 0.01568

female population estimate for May $N_0 = \frac{r_0}{q_0} = 5165$

Appendix 4: Female population estimates for June 1990

| wk (i) | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| $r_i$ | 80 | | | | | | |
| $n_i$ | | 252 | 195 | 246 | 202 | 189 | 205 |
| $m_i$ | | 11 | 8 | 6 | 5 | 4 | 3 |
| $q_i = \frac{m_i}{n_i}$ | | 0.0437 | 0.0410 | 0.0244 | 0.0248 | 0.0212 | 0.0146 |
| $(i-\bar{i})$ | | -2.5 | -1.5 | -0.5 | 0.5 | 1.5 | 2.5 |
| $Inq_i$ | | -3.1316 | -3.1935 | -3.7136 | -3.6989 | -3.8556 | -4.2247 |
| $Inq_i - In\bar{q}_i$ | | 0.5048 | 0.4429 | -0.0773 | -0.0626 | -0.2193 | -0.5884 |
| $(i-\bar{i})^2 * m_i$ | | 68.75 | 18 | 1.5 | 1.25 | 9 | 18.47 |

$$In\,(1-b) = \frac{\Sigma m_i\,(Inq_i - In\bar{q}_i)(i-\bar{i})}{\Sigma m_i (i-\bar{i})^2}$$

birth rate (b) = 0.191 (19%)
slope = -0.212
intercept = -2.895
proportion ($q_0$) = 0.0553

female population $N_0 = \frac{r_0}{q_0}$ = 1446

Appendix 5: Female population estimates for July 1990

| wk (i) | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| $r_i$ | 90 | | | | | | |
| $n_i$ | | 258 | 203 | 194 | 198 | 178 | 117 |
| $m_i$ | | 6 | 4 | 2 | 1 | 1 | 1 |
| $(i-\bar{i})$ | | -2.5 | -5 | -0.5 | 0.5 | 1.5 | 2.5 |
| $\ln q_i$ | | -3.7610 | -3.9271 | -4.595 | -5.2678 | -5.1818 | -4.7622 |
| $\ln q_i - \ln \bar{q}_i$ | | 0.8215 | 0.6554 | -0. 0127 | -0.6853 | -0.5993 | -0.1797 |
| $(i-\bar{i})^2 * m_i$ | | 37.5 | 9 | 0.5 | 0.25 | 2.25 | 6.25 |

$$\ln(1-b) = \frac{\sum m_i (\ln q_i - \ln \bar{q}_i)(i-\bar{i})}{\sum m_i (i-\bar{i})^2}$$

birth rate (b) = 0.275
slope = -3.457
intercept = -3.4567
proportion ($q_0$) = 0.032

female population estimate for July = 2854