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2014-08-22

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THE PHYSIOLOGICAL STATUS OF THE TSETSE FLY, *GLOSSINA FUSCIPES FUSCIPES*, ATTRACTED TO DIFFERENT HOSTS AND CONTROL DEVICES AND ITS IMPLICATIONS FOR CONTROL OF HUMAN AND ANIMAL AFRICAN TRYPANOSOMIASIS

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A dissertation submitted to the faculty of Science, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Master of Science

Johannesburg, 2014

DECLARATION

I declare that this dissertation is my original work. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.



_____

(Signature of the candidate)

27th JANUARY 2014

ABSTRACT

Human African Trypanosomiasis (HAT) is transmitted by *Glossina* species and remains a serious health problem in Africa. Many aspects of control of the disease have been implemented throughout the years but vector control of tsetse flies has proven to be the most efficient long-term solution. Vector control interventions have been implemented for many tsetse species but relatively little is known about the behaviour of the riverine species, *Glossina fuscipes fuscipes*. Increased knowledge of this species would improve vector control interventions. This study aimed at: i) understanding the behaviour of tsetse flies around visual devices and odour baits; ii) understanding the behaviour of the flies with regard to human activities; iii) understanding the interaction between the nutritional status of tsetse flies and their attraction to various trapping devices (biconical traps and electric nets); and iv) establishing an age determination curve for field-caught flies. Results showed that visual targets were better attractants then odour-based ones and electric nets performed better than biconical traps. The sticky traps caught 10x more flies (males) than the stationary biconical traps. Sticky traps caught more young flies than the biconical traps which caught more old flies. An age curve was established for flies ranging from 1 day to 60 days old and the fluorescence-based age determination technique, using pteridine levels, has been shown to work for this species. Understanding the behaviour of tsetse flies around trapping devices should lead to improved trapping efficiency. The data gathered will be of importance in assisting with designing and running the Lake Victoria region

control operations planned by PATTEC and it will have application in *G. f. fuscipes* endemic regions in other parts of Africa.

DEDICATION

I dedicate this work to my dear wife Rosalind Wanjiru Ngari and our lovely children Robert Macharia Ngari and Samuel Murithi Ngari who supported me to grow in my academic career. My parents Anthony Njiru and Lucia Ngari who brought me up and made sure I joined school and equally to the almighty God for His unlimited favor.

ACKNOWLEDGEMENTS

I am immensely thankful to Prof. Mike J. Lehane, from the Liverpool School of Tropical Medicine (LSTM) for agreeing to be my main supervisor and providing scientific guidance throughout my Masters research. I appreciate the support obtained from Dr. Johan Esterhuizen who tirelessly guided and corrected me. I thank Prof. Steve Torr from LSTM for helping with the data analysis. I thank Prof. Maureen Coetzee, director of the Wits Research Institute for Malaria at the University of the Witwatersrand Johannesburg, who encouraged me, and also for being my university supervisor. I thank Bill & Melinda Gates Foundation USA, for funding this project through which I was able to carry out this research. Many thanks go to the International Centre of Insect Physiology and Ecology (ICIPE), my employer, for allowing me to study as I worked and use of their equipped laboratory facilities at the ICIPE-Thomas Odhiambo campus located at Mbita point in western Kenya. Special thanks go to the Tsetse team (Stanley Maramba (Insectary), Jeremiah Opiyo (Driver), William Owigo (Coxswain), James Kitui (Manga island), Philip Owigo (Kirindo), Dicken Orwa (Security), Martin Kirindo (Security), George Owino (Field work), Michael Muthee (Dissection and Laboratory duties) and Mercy Opiyo (Dissection) .They worked tirelessly with me in the field, laboratory and insectary. My sincere appreciation goes to my dear wife Rosalind Ngari who was full of moral support and encouragement during the hard times and took good care of the family when I was far away in the field collecting data so that I could succeed in this dignified task. Thanks to my two

sons Robert and Samuel for understanding their dad who kept pushing until something happened (PUSH).

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LIST OF ABBREVIATIONS

HAT	Human African Trypanosomiasis
AAT	Animal African Trypanosomiasis
NECT	Nifurtimox-eflornithine combination therapy
PATTEC	Pan African Tsetse Eradication Campaign
ICIPE	International Centre of Insect Physiology and Ecology
LSTM	Liverpool School of Tropical Medicine
WHO	World Health Organisation
MRR	Mark, release and recapture
RDW	Residual Dry Weight analysis
GPS	Global positioning system
RFU	Raw Fluorescent Units
G. f. fuscipes	*Glossina fuscipes fuscipes*
T. b. Gambiense	*Trypanosoma brucei gambiense*
T. b. Rhodesiense	*Trypanosoma brucei rhodesiense*
T. vivax	*Tryponosoma vivax*
T. congolense	*Tryponosoma congolense*
T. brucei	*Tryponosoma brucei*
G. f. quanzensis	*Glossina fuscipes quanzensis*
G. tachinoides	*Glossina tachinoides*
G. m. morsitans	*Glossina morsitans submorsitans*

G. p. gambiesis	*Glossina palpalis gambiesis*
G. pallidipes	*Glossina pallidipes*
G. pallicera	*Glossina pallicera*
G. nigrofusca	*Glossina nigrofusca*
G. f.f. M	*Glossina fuscipes fuscipes Male*
G. f.f. F	*Glossina fuscipes fuscipes Female*
G. palpalis	*Glossina palpalis*

APPENDIX

A Ethical approval on an exemption from Liverpool School of Tropical Medicine

B Ethical approval on an exemption from The University of the Witwatersrand

C Informed consent forms

D Data sheet for attraction of *G. f. fuscipes* to three commonly available hosts and two artificial devices.

E Data sheet on the effect of human movement on *G. f. fuscipes behaviour*.

F Data sheet for age determination through ovarian age grading and the pteridine technique

CHAPTER ONE

INTRODUCTION

1.1 The disease

Human African Trypanosomiasis (HAT) also known as sleeping sickness, is a disease caused by two protozoan parasites, *Trypanosoma brucei gambiense* (Gambian HAT) which is found to the west of the Rift Valley and *Trypanosoma brucei rhodesiense* (Rhodesian HAT) which is found to the east and south of the Rift Valley (Fig. 1.1, Tables 1.1, 1.2). The three sleeping sickness epidemics that we know about occurred in the early 20th century, the 1940's and again in the 1990's and each affected more than half a million people. Currently the number of cases reported to the World Health Organization (WHO) is about 10,000 per year, which the WHO privately estimates equates to about 30,000 total cases (Prof M. Lehane, pers. comm.). The disease occurs across the continent of Africa where 36 countries in Sub-Saharan Africa can be affected, with about 60 million people at risk (WHO, 2006).

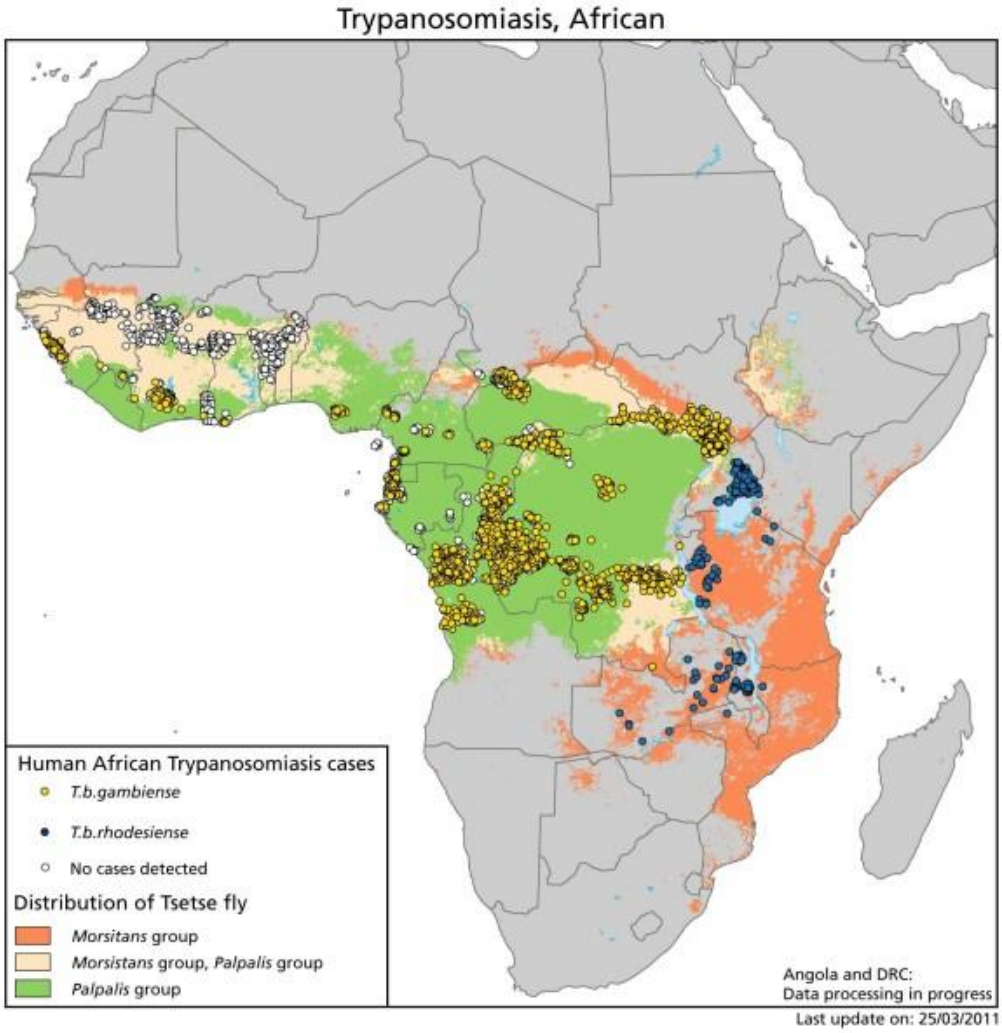


Figure 1.1 Africa map showing the distribution of cases of Human African Trypanosomiasis cases:

Trypanosoma brucei gambiense (Gambian HAT) found to the west of the Rift Valley and *Trypanosoma brucei rhodesiense* (Rhodesian HAT) to the east http://blog.wellcome.ac.uk/2012/03/01/developing-the-atlas-of-human-infectious-diseases/; accessed on 20th June 2012 (Wertheim, 2012).

1.2 The Tsetse fly and Trypanosome parasites life cycles

Tsetse flies are classified into one genus, *Glossina*, of the family Glossinidae and Order Diptera. Currently there are 33 species and sub-species recognised within

this genus. *Glossina* species are the only cyclical vectors of the protozoan parasites (Fig. 1.2) in the genus *Trypanosoma,* which cause both HAT and Animal African Trypanosomiasis (AAT = nagana) (Fig. 1.3, Table 1.3). The major vector of sleeping sickness caused by *T. b. gambiense* across Africa is *Glossina fuscipes fuscipes,* which is estimated to be responsible for the transmission of more than 90% of Gambian HAT cases (Omolo *et al*., 2009; Esterhuizen *et al*., 2011a, b) and is the focus of this study.

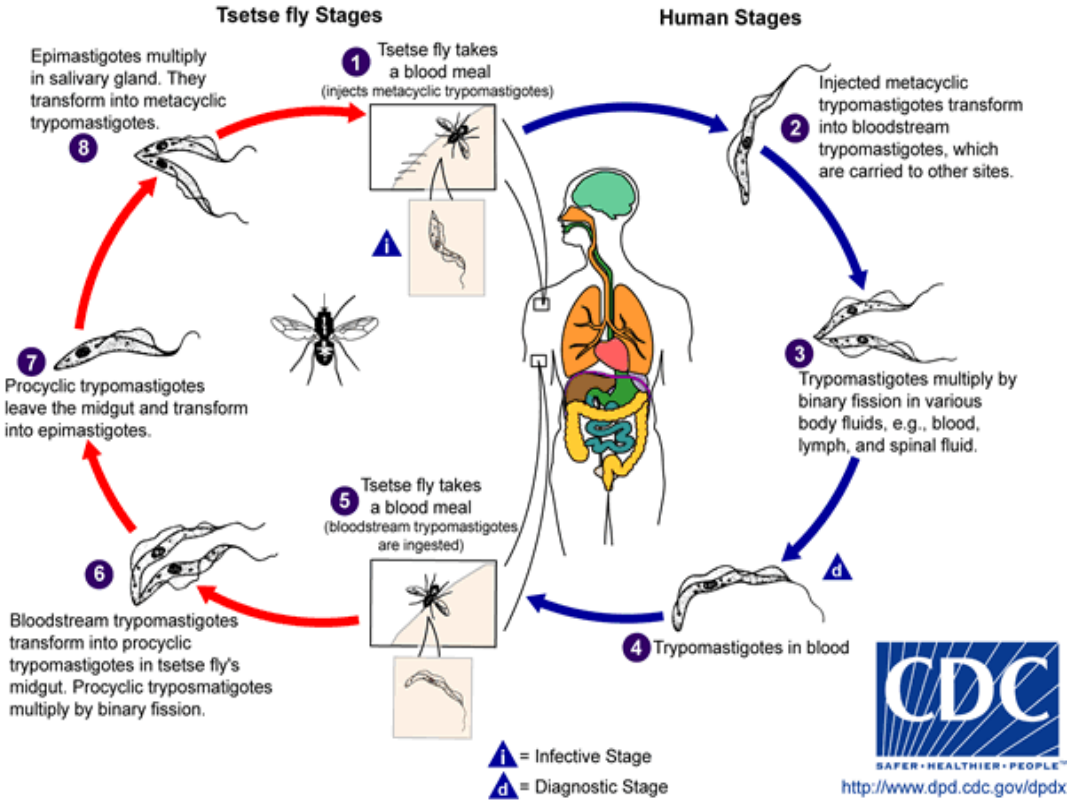


Figure 1.2 The life cycle of *trypanosomes*.

The cycle in a *Glossina ssp*. takes approximately 3 weeks before tsetse flies are infective to humans. Source: http://phil.cdc.gov/phil/details.asp?pid=3418 was accessed on 21st January 2014 and Leak (1998).

Table 1.1 Countries to the west of the Rift Valley with sleeping sickness (Gambian HAT Trypanosoma brucei gambiense) cases between 2000 and 2009.

Country	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**Total**
Central African Republic	988	718	572	539	738	666	460	654	1194	1054	7583
Chad	153	138	715	222	483	190	276	97	196	510	2980
Congo	111	894	1005	717	873	398	300	189	182	87	4756
Côte d'Ivoire	188	92	97	68	74	42	29	13	14	8	625
Equatorial Guinea	16	17	32	23	22	17	13	15	11	7	173
Gabon	45	30	26	26	49	53	31	30	24	14	328
Ghana	1	0	0	0	0	0	0	0	0	0	1
Guinea	52	72	132	130	95	94	48	69	90	79	861
Angola	4546	4577	3621	3115	2280	1727	1105	648	517	247	22383
Cameroon	27	14	32	33	17	3	15	7	13	24	185
D R Congo	16975	17322	13853	11481	10369	10269	8023	8162	7326	7183	110963
Nigeria	14	14	26	31	10	21	3	0	0	0	119
Sudan	1801	1919	3121	3061	1742	1853	789	469	623	376	15754
Uganda	948	310	604	517	378	311	290	120	198	99	3775
Total	**25865**	**26117**	**23836**	**19963**	**17130**	**15644**	**11382**	**10473**	**10388**	**9688**	**170486**

Source: Simarro *et al*. (2010).

Table 1.2 Countries to the east of the Rift Valley with sleeping sickness (Rhodesian HAT Trypanosoma brucei rhodesiense) cases between 2000 and 2009.

Country	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**Total**
Kenya	15	10	11	0	0	0	1	0	0	1	38
Malawi	35	38	43	70	48	41	58	50	49	39	471
Uganda	300	426	329	338	335	473	261	119	138	129	2848
Tanzania	350	277	228	113	159	186	127	126	59	14	1639
Zambia	9	4	5	15	9	7	6	10	13	4	82
Zimbabwe	0	0	0	0	0	3	0	0	0	3	6
Mozambique	0	0	1	0	1	0	0	0	0	0	2
Total reported	709	755	617	536	552	710	453	503	259	190	5086

Source: Simarro *et al*., (2010).

In addition to HAT, African Animal Trypanosomiasis has a huge impact on African agriculture. The major parasites infecting cattle are *Trypanosoma vivax, T. congolense* and *T. brucei brucei*. It is estimated that these diseases, commonly lumped together as "nagana", cause the loss of US$4.5Bn per year to African agriculture as estimated at the end of the 20th century (Budd, 1999).

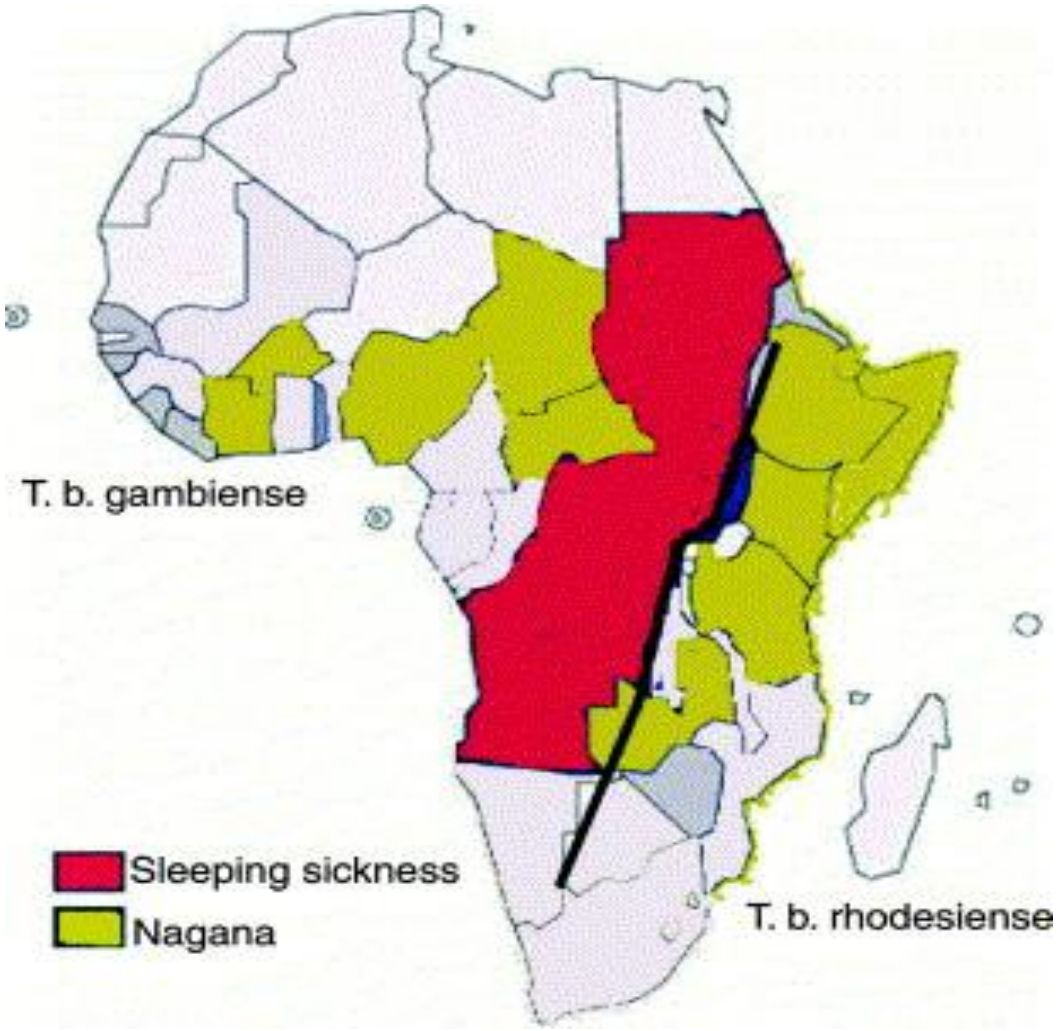


Figure 1.3 Distribution of sleeping sickness and nagana across Africa (Matovu *et al*., 2001).

Table 1.3 The African Animal Trypanosomiasis in domestic animals and their reservoir hosts.

Trypanosome species	Domestic animals affected	Reservoir hosts
T. congolense	Cattle, camels, horses, dogs, sheep, goats, pigs	Several groups of wild mammals
T. evansi	Camels, horses, dogs, domestic buffalo, cattle	Several wild mammals in Latin America
T. equiperdum	Horses, donkeys, mules	None known
T. vivax	Cattle, sheep, goats, domestic buffalo, horses	Several groups of wild mammals
T. simiae	Pigs	Wart hog, bush pig
T. godfreyi	Pigs	Wart hog
T. brucei gambiense	Human sleeping sickness; affect domestic animals	Several groups of wild mammals
T. brucei rhodesiense	Human sleeping sickness;	Several groups of wild mammals
T. brucei brucei	Horses, camels,dogs, sheep, goats, cattle, pigs	Several groups of wild mammals
T. theileri	Cattle, domestic buffalo	Various wild ruminants
T. ingens		

Source: http://www.fao.org/docrep/006/X0413E/X0413E02.htm accessed on 26 th June, 2012

The life cycle of tsetse involves the female adult producing a single egg (Fig.1.4) which after fertilization hatches to a first instar larva in the uterus. Through a process of development and moulting, this progresses to the third stage larva that the female deposits on suitable sandy soil. The larva burrows into the soft soil and hardens to form a puparium during which time it changes colour and harden from white to brown and finally black. A young fly (teneral) will emerge from the puparium after thirty to forty days depending on temperature. Tsetse females are capable of producing larvae every nine to ten days during a life span that can range up to four months depending on the climatic conditions. The reproductive system is described as adenotrophic viviparity (Leak, 1998).

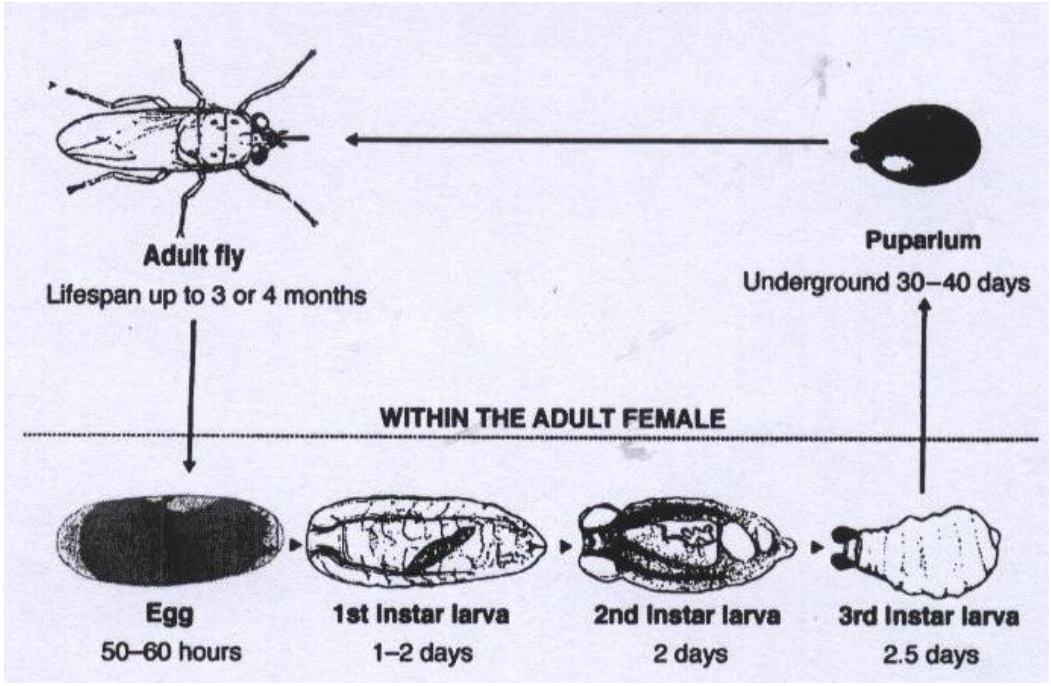


Figure 1.4 Life cycle of *Glossina spp*. (Source: Leak, 1998).

Within the adult female the egg takes 50 – 60 hrs and 1st to 3rd instar larva takes approximately 6 -7 days. The puparium takes 30 to 40 days (in the soft soil) and adult surviving for 3 to 4 months takes place in the environment. To differentiate male from female adult tsetse, sex determination is done by observing the tsetse fly genitalia where the presence of the hypopygium and hectors in males was used to differentiate males from females (Fig. 1.5). For a quick identification the female abdomen is flat/smooth while the male has protruding structures (Leak, 1998).

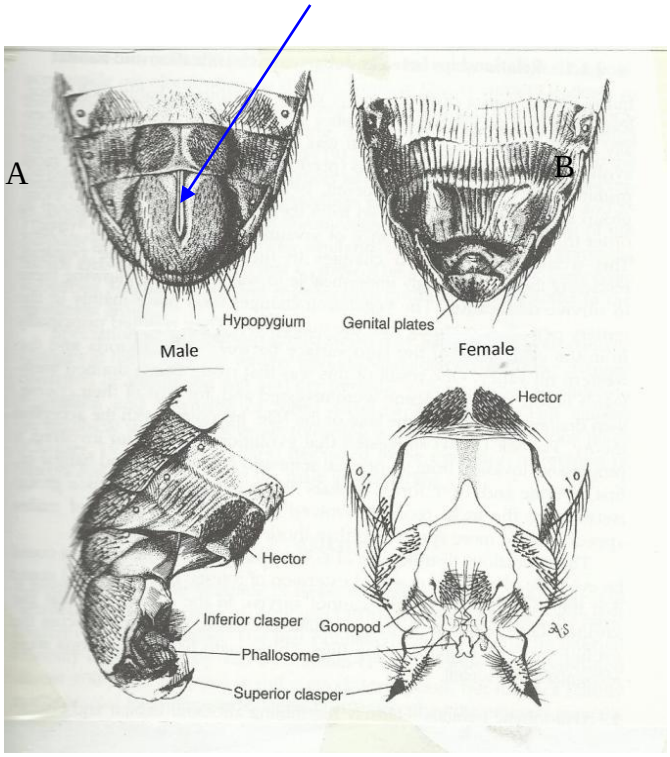


Figure 1.5 Differences between the posterior abdomens of male (A) and female (B) tsetse. Source: (Leak, 1998).

1.3 Epidemiology and treatment of Sleeping sickness

The two forms of HAT show different epidemiology`s leading to separate control approaches. In the Rhodesian form of the disease the effective reservoir of parasites is in wild or domesticated animals. Infected humans are not available to tsetse flies because they rapidly fall ill and retreat indoors. Consequently control of Rhodesian HAT relies on either vector control or reducing parasite numbers in animals by drug treatment which is, of course, only possible when the primary reservoir is in domestic animals (Table 1.3) (Lancien, 1991; Welburn *et al.*, 2001; 2006). Medical intervention using case detection and treatment cannot control Rhodesian HAT outbreaks and is only used for humanitarian reasons.

For Gambian HAT the reservoir is in humans. Consequently case detection and treatment when effectively delivered, can limit the number of parasites transmitted. This often results in the control of outbreaks of the disease (Simarro *et al*., 2008). This approach deals with the public health problem but is less satisfactory at an individual level as it leaves people open to contraction of a fatal disease because there are no prophylactic drugs or vaccines available. (Barret *et al*., 2004; Delespaux and Koning, 2007). Because of the very low numbers of HAT cases at present, it is becoming difficult to fund costly screen and treat programmes – the so-called penalty of success in an operation. In addition treatment of HAT relies on a dangerously small number of drugs (Brun *et al*., 2010). Despite the introduction of Nifurtimox-eflornithine combination therapy

(NECT), the drug melarsoprol is still used to treat second stage Rhodesian sleeping sickness and some Gambian sleeping sickness. It is a highly toxic drug resulting in 5-10% mortality from its use. There is one new drug, fexinidazole, being tested and this may be a significant breakthrough in treatment (Barrett, 2010). There is no vaccine and the prospects for developing one are slim. For all the above reasons we need as many new methods of combating HAT as possible.

1.4 Tsetse control devices

Tsetse flies are attracted to various hosts and collection devices, with the level of attraction differing between tsetse species and habitats, devices and hosts. For example, the savannah tsetse species *G. pallidipes* and *G. morsitans morsitans* are not attracted to small blue and black cloth screens (targets) (Fig. 1.6) (Hargrove, 1980; Vale, 1993; Torr *et al*., 2011), while *G. f. fuscipes* and several other riverine tsetse species are highly attracted by these small targets (Lindh *et al*., 2009; Esterhuizen *et al.,* 2011b). In addition, attraction and behaviour around a host or device is influenced by nutritional status and age of individual flies. A recently fed adult fly will most likely not be captured in a trap, because there is no need for the fly to risk moving around when it is still digesting a full blood meal. Similarly, an adult female fly, ready to deposit a larva, will probably not risk feeding because she needs to minimize risk and first safely deposit the larva.

Vector control has tremendous potential and can rapidly halt transmission of HAT (Gouteux and Artzrouni, 1996). Vector control is the only available means of protecting individuals from infection and is expensive and unaffordable, its cost

being estimated to US$ 2070/ km^2 / Year (Feldman and Hendrichs 2001; Torr *et al*., 2005; Torr *et al*., 2007; Shaw *at el* 2008),

Table 1.4 The cost of the potential methods for tsetse fly control

Technique	Cost US$/km^2/Year
Treating cattle (restricted application)*	20
Tiny Targets	<100
Conventional Traps / Targets	300
Ground Spraying	350
Aerial Spraying	500
Sterile Insect Technique	>800

*It is estimated 10 domestic animals per km^2.

As can be seen from Table 1.4 showing the cost of the potential methods for tsetse fly control, most methods are prohibitively expensive (e.g. SIT) or unpractical in many cases (e.g. insecticide treated cattle). Recently there has been a major effort to produce more cost effective bait strategies for control of riverine tsetse (Lindh *et al*., 2009; Omolo *et al*., 2009; Rayaisse *et al*., 2010; Esterhuizen *et al.*, 2011a, b). Conventionally, tsetse fly control is done with insecticide impregnated traps or targets, aerial spraying, insecticide treated cattle, ground spraying and sterile insect technique (Leak, 1998). Success in population reduction and elimination using traps and targets has been achieved in Zambia, Zimbabwe, Tanzania, Kenya, Burkina Faso and Ethiopia (Hargrove, 2003; 2005). These successes have been largely against Morsitans-group tsetse flies which are

of minor importance as vectors of HAT. Studies of the palpalis-group of tsetse which inhabit riverine habitats have shown that there are inter- and intra-specific differences (in the attraction of tsetse flies to the devices used, as well as differences in attraction to different hosts (Lindh *et al*., 2009; Esterhuizen *et al*., 2011a,b; Tirados *et al*., 2011; Rayaisse *et al*., 2011). For example, comparatively little information is available for *G. f. fuscipes (*main vector in the palpalis-group) on their behaviour around targets and traps.

The biconical trap (Fig 1.6) has conventionally been used (Okoth *et al*., 1991; Joja and Okoli, 2001) for control of *palpalis*-species. However, they are bulky and expensive resulting in an urgent need to reduce the size of devices so that they will be cheaper to make, easier to transport and easy to deploy. Tiny targets, using pthalogen blue cloth (25 x 25cm) with an adjacent flanking net (25 x 25cm) of black mosquito net have been proven to be more effective than conventional devices for control of *G. f. fuscipes* (Lindh *et al.,* 2009; Esterhuizen *et al*., 2011a). The use of the flanking net next to the tiny target improved the killing efficiency of the target by up to 80% (Lindh. *et al*., 2009; Esterhuizen *et al*., 2011a; Tirados *et al*., 2011; Rayaisse *et al*., 2011; 2012).

In comparison with the conventional target of $1m^2$, tiny targets use only one eighth ($1/8^{th}$) of the material and compared to the biconical trap it uses one twenty fourth ($1/24^{th}$) of the material. In a study around Lake Victoria, it was shown that the tiny target was significantly better in attracting and killing *G. f. fuscipes* than the biconical trap (Esterhuizen *et al*., 2011a).



Figure 1.6 Different trapping methods to collect tsetse flies.

1.5 Age grading

The epidemiological importance of insect vectors increase with their age because they have more chance of becoming infected and more time to mature that infection (Leak, 1998). Consequently age determination is a useful tool in the study of the epidemiological efficiency of vector populations (Detinova, 1968; Leak, 1998). Stable tsetse populations have high numbers of older females, while during control the age structure will change, with older females dying off and young flies emerging from pupae. Female tsetse flies can be age graded by dissection methods (Saunders, 1960; 1961; 1962) due to their polytrophic ovaries. However, this is a highly skillful operation and is open to problems with differences of interpretation between operators. In addition, it is not possible to

determine the age exactly beyond the fourth ovarian cycle (approximately 40 days and the female may live well over 100 days). Males have been age graded using wing fray techniques (Jackson, 1946) but this is also very variable particularly if flies have been trapped when wings become frayed rapidly in the holding device. Ideally a simpler, less subjective method could be used instead for females and would also be useful for male tsetse flies for which we do not currently have an acceptable technique (Lehane, 1985). One such method utilised metabolic waste products in determining the age of the tsetse fly *G. morsitans morsitans* (Lehane and Mail, 1985). Pteridines are nitrogenous waste products of metabolism and are stored in different parts of an insect's body and thus accumulate with time resulting in higher concentrations being found in mature insects (Mail *et al*., 1983). The techniques used here was similar to that described by Lehane and Hargrove, (1988) where newly emerged tenerals were marked, release in the nearby island and then recaptured for pteridine age determination (Tomic *et al*., 1996; Mail *et al*., 1983; Lehane *et al*., 1986; Langley *et al*., 1988; Robson and Crozier, 2009; Hayes and Wall. 1999). Nutritional status also influences tsetse behaviour, for example, for teneral flies, thoracic muscle development is the priority for blood meal utilization, while in adult flies it is used for fat storage, for flight energy (males and females) and larval production in females (Bursell, 1961; Rogers**,** 1978).

1.6 Aims and Objectives

The aims of this study were;

1. To investigate the behaviour of *G. f. fuscipes* and attraction to the tiny target and biconical trap, in comparison to three commonly available hosts: human, cattle and monitor lizard. This will add to our understanding of the behaviour of *G. f. fuscipes* around hosts and devices as well as providing a comparative efficiency of attraction of each device and natural host, and information on the killing efficiency of each device.
2. To determine the sex, age and nutritional status of flies attracted to the various baits and hosts listed above. This is crucial information in understanding how these devices will work in practice. To determine if the pteridine age determination technique can be applied to *G. f. fuscipes* and attempt to establish an age-curve for this species to assist in evaluation and interpretation of control operations.

The specific objectives were;

- To determine the attraction of *G. f. fuscipes* to three commonly available hosts, i.e. human, cattle and monitor lizard; as well as to two control devices, i.e. biconical trap and tiny target using ring of electric nets.
- To validate effect of electrocution on pteridine levels in *G. f. fuscipes*
- To determine the nutritional status of *G. f. fuscipes* attracted to different hosts and devices.
- To investigate the effect of human movement on *G. f. fuscipes* behaviour.
- To establish and calibrate an age curve for Pteridine age determination of *G. f. fuscipes* with field caught flies and colony flies of known age.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study site

The studies were carried out in western Kenya along the shores and islands of Lake Victoria (Fig. 2.1) between January 2012 to May 2013, based at Mbita Point 00˚25'49" E 34˚12'24" (Geographic positioning system (GPS, eTrex, USA) . The area has two rainy seasons, March to July (long rains) and October to mid-January (short rains) with average annual rainfall of 1,670 mm. The average minimum and maximum temperatures were 22^{o}C and 28 ^{o}C respectively while the average daily percentage minimum and maximum relative humidity ranged from 56.7% to 80.27% (HOBO® H8 Family data logger Onset Computer Corporation). The vegetation along the lake is green shrubs with a thick under cover of *Lantana camara* and other plants. Wild animals around the lake include monkeys, snakes, monitor lizards, rats, many different types of birds, crocodiles (few) and mongoose. The community in this area depend on fishing and there are many human settlements along the lake shore. There is considerable small-scale, subsistence farming activity in the area and domesticated animals include goats, donkeys, cattle and sheep.

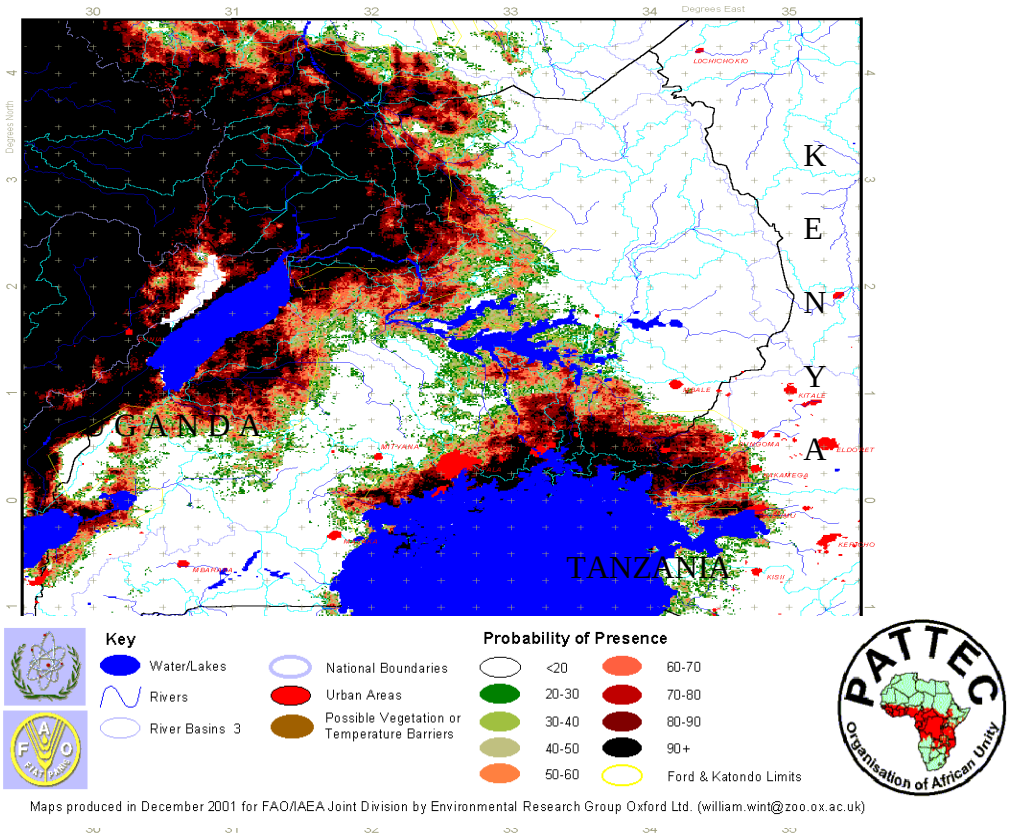


Figure 2.1 Distribution of *G. f. fuscipes* Source: William, (2008).

Laboratory studies were carried out at the International Centre of Insect Physiology and Ecology (ICIPE) at Mbita Point. The area around the ICIPE laboratories has a high density of *G. f. fuscipes*, but no sleeping sickness case has been reported here for more than 30 years. Indeed, no parasites could be found from over 1000 tsetse fly midgut dissections performed in 2010 (Prof. M. Lehane, pers. comm.).

Mark, release and recapture experiments and the effects of movement versus stationary target experiments on *G. f. fuscipes* were carried out on Small Chamaunga island (00˚25’53” E 34˚13’38”) which is approximately 0.5 km^2 in area (Fig. 2.2).



Figure 2.2 The Mbita area showing two experimental islands

Other collection sites included;
Big Chamaunga Island (00˚25’34” E 34˚13’58”) 800 meters offshore, Manga island (00˚21’14” E 34˚15’10”), Magare island (00˚20’48” E 34˚15’44”) Rusinga Island Lianda (00˚21’27” E 34˚13’15”), Karungu (Sori) lake shore (00˚25’56” E 34˚12’25”) and Nyagwethe Ungoye Icipe field station (00˚3644’56” E 34˚12’25”).

2.2 Ethical clearance

Ethical approval on an exemption basis was given by the Ethics committee of the Liverpool School of Tropical Medicine (Appendix A) and the University of the

Witwatersrand (Appendix B). Mbita point being an ideal place for carrying out this type of tsetse fly research because there is no risk for human exposure to trypanosomes hence no ethical issues involved. Despite this all the volunteers who participated in this study filled in participant informed consent forms. The originals are held by ICIPE. An example form is given in Appendix C.

2.3 Tsetse fly collection

Live flies were collected from the field using biconical traps (Fig. 2.6) for 3 hours (9.00 to 12.00 hrs) and were transported to the ICIPE tsetse holding facility (Fig. 2.3) using a dampened cool box containing a wet towel. The temperature in the holding facility was 25^0C-28^0C and relative humidity of 70-80%. The flies were sexed by observing the tsetse fly genitalia where the presence of the hypopygium and hectors in males was used to differentiate males from females (Fig. 1.5). All the male flies were killed as the female were fed on blood meals from goats at the holding facility and held for larval production in cages, with 25 to 30 flies/ cage. To determine whether the fly was fed or unfed, its abdomen was observed; some flies had their abdomen half fed or full. The pupae were held in plastic vials with netted tops until adults emerged (Fig. 2.4). These adults were used in laboratory experiments.



Figure 2.3 Tsetse holding facility



Figure 2.4 The holding rack for adult *G. f. fuscipes* in feeding cages

2.4 Determination of the attraction of *G. f. fuscipes* to three commonly available hosts: human, cattle and monitor lizard and two artificial devices.

This experiment was performed at Uyoga between 09:00 and 14:00 hrs for 30 days with six biconical traps deployed one meter from the lake shore and approximately 50 meters apart. This area was selected because of the high density of *G. f. fuscipes*, e.g. 20 to 50 flies/trap/day. Three live hosts (cow, human and monitor lizard), two inanimate control devices (visual targets), namely, a biconical trap and tiny electric target were compared as attractants for *G. f. fuscipes*. The experiment was set up with an incomplete ring of six electric nets according to the procedure developed by Vale (1974) and Torr and Hargrove (1998); Torr *et al.*, (2007a) with the treatments in the middle each day (Fig. 2.7 to 2.11). A randomized block design was used with five treatments and six replications (Table 2.1).

Table 2.1 Randomization of treatments versus block (set of treatments) with the days (D1-5).

Block	Biconical trap	Human	Electrified target (tiny target)	Cow	Lizard
1	D_1	D_2	D_3	D_4	D_5
2	D_4	D_5	D_2	D_3	D_1
3	D_5	D_4	D_1	D_2	D_3
4	D_2	D_1	D_3	D_4	D_5
5	D_3	D_2	D_4	D_5	D_1
6	D_5	D_3	D_1	D2	D_4

The treatments consisted of a cow (Fig. 2.7) (c. 300 kg) tethered to a stick in the ground in the middle of an incomplete ring of six electric nets and provided with fresh fodder grass. A medium–sized human (Fig. 2.8) (c. 65 kg) seated on a chair at the middle of the setup of the six incomplete electric nets and dressed in a white un-perfumed laboratory coat. A small monitor lizard (Fig. 2.9) (c. 5kg), contained in a holding cage of wire mesh measuring (40 x 40 x 90 cm). An electrified tiny target (Fig. 2.10) and standard biconical trap (Fig. 2.11). The data was tabulated (see Appendix D).

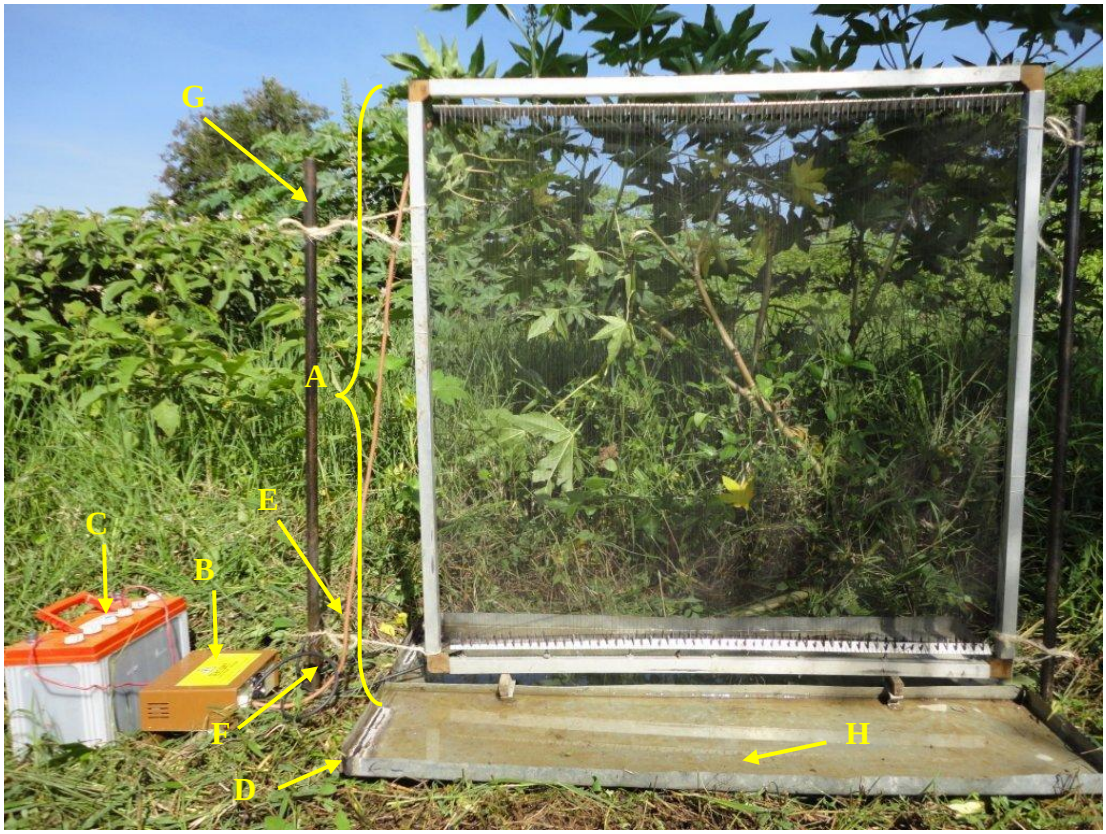


Figure 2.5 Electric grid (1x1 meter). (A) Electric net, (B) Spark box, (C) 12V battery, (D) Tray, (E) Positive wire, (F) Negative wire, (G) Supporting pole, (H) Soapy water 3-5cm deep in metal tray.

Six electric nets (Fig. 2.5) were put in a circle of 6m diameter to make a ring of electric nets (Vale, 1974; Torr and Hargrove, 1998) each separated by a gap of 1.5 meter. The electric nets were set up over a wide tray (120x60x3cm) filled with soapy water into which electrocuted flies fell. This was to avoid loss of flies from marauding ants before collection. All copper wires were coloured matt black using a black permanent marker pen so that flies did not see them as they approached the electric net. Location of the electrocuted tsetse flies at different positions and either inside or outside the ring of electric nets was recorded as fly collection was made. Each fly was stored for further analysis in labelled vials

referencing the position, date and treatment. The control device was the biconical trap (Fig. 2.6) which is the conventional collection tool for *G. f. fuscipes*, (Challier and Laveissiere, 1973; Laveissiere and Couret, 1980; Laveissiere *et al*., 1980; Green, 1994; Leak, 1998). A biconical trap stands approximately 1.6 meters high and is erected on a central iron pole. Flies enter the trap through holes in the blue cloth below and are attracted upwards by the light when they are finally trapped in the collection net seen at the very top of the device.



Figure 2.6 Biconical trap.

A cow was collected from the nearby village with permission of the owner and was tied in the centre of the incomplete ring of nets to a central pole with a rope

(Fig. 2.7). The animal was not sprayed with any insecticide during the experiment. The experimental design not only determined the absolute number of flies attracted but provided additional information on the directionality of flies in various physiological states.



Figure 2.7 Attraction of *G. f. fuscipes* to a cow

A human volunteer seated on a chair at the middle of the setup of the six incomplete rings of nets and dressed in a white un-perfumed laboratory coat (Fig. 2.8).



Figure 2.8 Attraction of *G. f. fuscipes* to a human

A monitor lizard was trapped and maintained in a cage and fed with fish or meat throughout the experiments. During the experiment the cage containing the monitor lizard was placed at the middle of the incomplete ring of nets (Fig. 2.9). During sunny days water was poured over the lizard and some vegetation was placed over the cage to provide shade and avoid excessive heating.



Figure 2.9 Attraction of *G. f. fuscipes* to a monitor Lizard

An electrified tiny electric target (blue target (25 x 25 cm) with a flanking net (25 x 25 cm) (Fig. 2.10) was placed at the middle of the 6 incomplete ring of nets to provide a visual attractant. In this experiment some of the flies attracted were electrocuted by the target itself and collected in its own tray.



Figure 2.10 Attraction of *G. f. fuscipes* to tiny electric target.

A biconical trap was placed at the middle of the incomplete ring of nets (Fig. 2.11). In this experiment some of the flies attracted were caught in the biconical trap.



Figure 2.11 Attraction of *G. f. fuscipes* to a biconical trap

2.5 The effect of human movement on *G. f. fuscipes behaviour*

In order to compare a moving versus a stationary target (Hargrove, 1991) for capturing *G. f. fuscipes*, the following design was adopted. Data was tabulated (Appendix E). Treatment 1: The experiment was carried out as from 9.30 hrs to 11.30 hrs, a human with a sticky panel (Fig. 2.13) attached to his back remained seated for 10 minutes.

Table 2.2 Randomization of starting points (A, B, C & D) versus sites against days.

	Site 1	Site 2	Site 3	Site 4
Day 1- 4	A1	B 2	C3	D4
Day 5 - 8	C5	A6	D7	B8
Day 9 -12	D9	B10	A11	C12
Day 13 -16	B13	D14	C15	A16
Day 17 - 20	A17	C18	B19	D20
Day 21 - 24	C21	A22	D23	B24
Day 25 - 28	D25	B26	A27	C28
Day 29 - 32	B29	D30	C31	A32
Day 33 - 36	A33			

Treatment 2: The human with the sticky panel attached walked slowly for 10 minutes along a specified circular path (Fig. 2.12). A geographic positioning system (GPS, eTrex, USA) was used to mark the four stationary point locations and the mobile distances covered from one stationary point to another recorded in meters along the circular path.

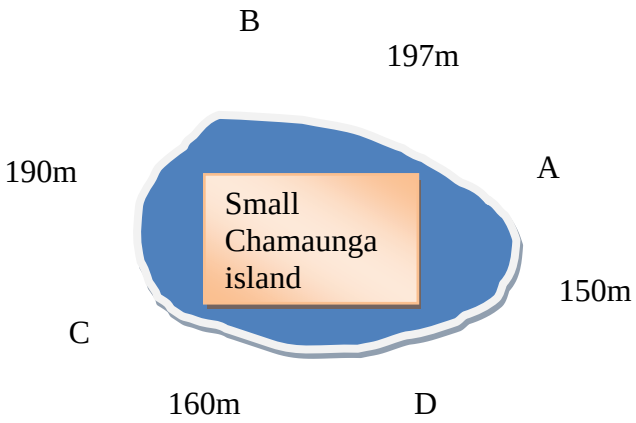


Figure 2.12 Sketch map of stationary and mobile positions

A black sticky panel (30 x 40 cm) was made with clear sticky film material using the method described by Kalcounis *et al.* (1992). The panel consisted of plywood covered with black cloth on both sides and clear sticky film (30 cm wide roll, Rentokil FE45, UK) on one side. Holes were made in the corners and fine galvanised wire was passed through to enable the panel to be held in position on the shoulders, sticky side out, as shown in (Fig. 2.13). Tsetse flies were attracted and caught on the sticky film. Flies were then picked off with forceps and transferred into individual vials which were labelled for location and date. These vials were transferred to the laboratory for analysis.



Figure 2.13 Human volunteer wearing the sticky panel

2.6. Determination of the nutritional status of flies attracted to different hosts and devices through residual dry weight analysis (RDW).

Live tsetse flies were collected from Manga Island and Karungu (Sori) along the lake shore using the biconical traps (Fig. 2.6) or the sticky panel method (Fig. 2.13) and were used for either pteridine or fat content analysis, depending on the availability of flies. Determination of fat contents and nutritional status of flies was carried out as described by Langley *et al*. (1990) and Green, (1990). This

procedure was carried out only for male flies since it has been shown by previous authors that the reproductive status of females interfered significantly with these analyses. The *G. f. fuscipes* males were immobilized by cooling to 4^0C. The wings and legs were cut off as close to the body as possible. The cut-off wings were tagged to a data sheet for future reference and analysis. A metal tray, subdivided into several wells , each contained a fly that had been weighed using a Sartorius model R200D analytical balance, with accuracy at 0.00001g and maximum capacity of 40/200 g (GMBH Gottingen made in Germany), numbered and recorded as "wt. 1". The tray was then placed in an oven at 70^0C for 8 hours. Silica gel was placed in each well to keep the flies dry during the second weighing process that was then recorded as "wt. 2" (see Appendix F).

Fat extraction was done in a fume hood. The silica gel was removed from the metal tray and chloroform was added to each well. The flies were covered with a clean wire mesh and a metal lid for 24 hrs, after which the chloroform extract (wash 1, to be re-used later) was collected and stored in a labelled container. The flies underwent a second and a third wash with fresh chloroform each time, while repeating the process as explained above. In all three sequential chloroform extractions, evaporation was prevented by covering the metal tray with parafilm.
After the 3^{rd} wash, the metal tray containing the flies was placed in the oven again at 70^0C for 8 hours for final drying while ensuring that the silica gel was replaced in the metal tray after the washing. Then, the final weight reading (wt. 3) was recorded. The percentage dry weight and fat content was analyzed using the formula below:

Water content = Wt. 1 (fresh fly) – Wt. 2 (dry weight with fat)

Fat content = Wt. 2 (dry weight with fat) – Wt. 3 (dry weight without fat)

Percentage dry weight= Wt. 2 (dry weight with fat) / Wt. 1 (fresh fly) x 100

Percentage fat = Wt. 2 (dry weight with fat) – Wt. 3 (dry weight without fat) / Wt. 2 (dry weight with fat) x 100

2.7 Age determination

2.7.1 Ovarian age grading

The ovarian age determination (dissection) in *G. f. fuscipes* was performed as described by Saunders (1960; 1961; 1962). The data were recorded on a datasheet (Appendix G).

2.7.2 The pteridine age determination technique

The pteridine age determination method for tsetse flies was used as described by Lehane and Mail (1985) and Mail *et al.* (1983). Live flies were immobilized by putting them in a fridge at 4^0C for 5 minutes, decapitated and analysed immediately. Fly heads were homogenized in 2ml buffer (described below) at room temperature using a 3ml homogenizer (potter-Elvehjem, Thomas Scientific, USA) composed of a glass potter and pestle. The homogenizer was rinsed with distilled water between the samples. The supernatant from the homogenate was transferred to an eppendorf tube and micro-centrifuged (Labnet prism, UK) for one minute at relative centrifugal force (G-Force), g = 1472. Two millilitres of supernatant were transferred to a plastic, UV compatible cuvette (Fisher

Scientific, Italy) and readings for Raw Fluorescent Units (RFU) were performed using a fluorimeter (Jenway 6280, UK) (Fig. 2.14) set at 450nm emission and 350nm excitation, gain (efficiency) was 55% and all experiments were standardized using a commercially bought fluorescent plastic block (Starna Ltd S4- Tetraphenylbutadiene).



Figure 2.14 The fluorimeter and centrifuge at the ICIPE laboratories, Mbita Point.

Since fluorescence is inversely proportional to temperature, readings were done at comparable temperatures every day. A buffer of pH10 was made through titration of glycine (ca. 11.5g/l) with 0.1N NaOH. After the buffer was standardized, a working solution of 500 ml was kept at room temperature because of the temperature effect on florescence mentioned above. The RFU results per batch of

12 samples were recorded, including the average readings for its standard S4 (Starna Ltd S4- Tetraphenylbutadiene) = (S4 initial + S4 final)/2 and the average for its buffer (B) readings = (B initial + B final)/2 in a tabulated manner. The readings were done in batches of 12 because of the gradual increase in RFU following homogenization. Consequently all RFU readings were carried out in a fixed time ±5 minutes after each homogenization. While measuring the fluorescence, the cuvette needed to be held on the top edge to avoid finger marks that affect the result of RFU. To standardise readings between batches, the following formula was used and data recorded (see Appendix H).

$$\left(\frac{Sample\ reading}{Average\ S4} - \frac{Average\ B\ reading}{Average\ S4}\right) X\ 1000$$

2.7.3 Mark release recapture

Flies were caught in the field and taken to the holding facility where they were fed on goats. Females were maintained in a cage of 25 females per batch and allowed to give birth to larvae. Flies of known age, hatching from the pupae, were given a blood meal after 6hrs. They were marked on their thorax with a date specific mark using Humbrol enamel paints of different colours and released on Small Chamaunga Island Those that were recaptured in biconical traps were used in pteridine age determination. To construct an age calibration curve of *G. f. fuscipes* males and females both wild and holding facility reared flies (lab based) were used to provide baseline data on the field population for comparison.

2.8 The effect of electrocution on pteridine levels in *G. f. fuscipes*.

Electric nets were used extensively in these studies, thus calling for the validation of the technique and its effects on electrocuted flies' pteridine levels. All flies were 24hrs old. Four groups of 24 flies each, were electrocuted and later removed from the water tray at different time intervals: 10 minutes (group 1), 1 hour (group 2), 2 hours (group 3) and 6 hours (group 4). Their heads were removed for pteridine age determination as described above and compared to 24 non-electrocuted control flies (flies from the same batch but were not put in water and not electrocuted).

2.9 Statistical analysis

ANOVA of log-transformed catches was used to assess whether different hosts attract significantly different numbers of tsetse flies and also to assess whether there were significant differences in the proportions of tsetse feeding on different hosts.

Two-way ANOVA was used to calculate the effect of human movement on *G. f. fuscipes* catch composition.

Comparison of RDW and fat content was performed by ANOVA as described by Green (1990) and Rogers (1978), and used to assess whether there were significant differences in the proportions of fed tsetse flies collected from the field.

The relationship between age and pteridine content was assessed according to Lehane and Mail (1985). Data analysis involved calculation of the age of *G. f. fuscipes*, through regression analysis as described in the equation ($y = a + bx$), y = pteridine level, b = slope of the regression line, a = pteridine levels of fly at birth; x = age of the fly. So re-arranging the equation we get $x = y - a/b$.

Regression analysis and ANOVA of log-transformed catches was used to assess whether electrocution had any effect on pteridine levels.

CHAPTER THREE

DETERMINATION OF THE ATTRACTION OF *G. f. fuscipes* TO THREE COMMONLY AVAILABLE HOSTS: HUMAN, CATTLE AND MONITOR LIZARD AND TWO ARTIFICIAL DEVICES

3.1 Introduction

The use of odour-baited targets, insecticide-treated cattle and improved persistence of insecticide deposits on targets has been used in control of tsetse flies (Vale *et al.*, 1988; Torr *et al.*, 1992; Vale *et al*., 1999). Studies that help us understand the behaviour of flies around baits may allow us to improve these control methods. Consequently, studies were carried out to determine the behaviour of *G. f. fuscipes* around various baits by use of an incomplete ring of nets as described by Vale (1974) and Torr and Hargrove (1998). Electric nets give valuable information on how tsetse flies behave in the vicinity of baits (Nigel and Brady, 1994). For example, through ring of nets experiments, it has been possible to enumerate the bias that the savannah tsetse species *G. pallidipes* and *G. m. morsitans* show in relation to small blue and black cloth screens (Vale, 1993; Hargrove, 1980; Torr *et al*., 2011) compared to *G. f. fuscipes* and other riverine tsetse species (Lindh *et al*., 2009; Esterhuizen *et al.*, 2011a). Consequently incomplete ring of electric nets experiments were used to enumerate the number of *G. f. fuscipes* that were visiting and departing from the various hosts. They

were also used to verify trapping efficiency. Knowledge gained about the vector's behaviour around hosts and devices will benefit improved understanding and control of these disease vectors. The results were used to interpret fly behaviour and the efficiency of conventional and new control devices for *G. f. fuscipes*.

3.2 Results

A total of 2812 *G. f. fuscipes* were collected over thirty days in the ring of nets experiment and of these, 78.1% (2197) were female flies. The attraction of the three live hosts and the two control devices to *G. f. fuscipes* is summarized in Table 3.1. A total of 1245 flies were caught from outside the ring of nets which provides an estimate of the number of flies attracted to the vicinity of the baits and intercepted before they got to the bait. The small blue electric target (tiny target) had the highest mean catches of 11.4 and 35.4 for male and female *G. f. fuscipes* respectively (Table 3.1) which is about 10% more compared with the biconical trap. It was also more attractive than the natural hosts, including the 'preferred' host of monitor lizard which attracted 13% less tsetse than the small electric net.

Table 3.1 Mean catches of tsetse from the outside surface of a ring of electric nets surrounding various natural and artificial baits.

	Bait	Mean outside catch	Transformed mean	Transformed SE
Males	Biconical	9.3	1.01	0.118
	Cow	7.6	0.94	0.042
	E-target	11.4	1.09	0.08
	Human	7.8	0.94	0.054
	Lizard	7.9	0.95	0.061
Females	Biconical	32.1	1.52	0.091
	Cow	20.6	1.33	0.09
	E-target	35.4	1.56	0.075
	Human	26.9	1.45	0.045
	Lizard	30.9	1.5	0.113

Although the results from the ANOVA of the catches from the outside of the ring (Table 3.2) showed that there were no significant difference between treatments, the data provided interesting information in terms of *G. f. fuscipes* host seeking behaviour, with apparent differences between male and female flies. For example, for male flies there was no difference in attraction to the three live hosts (Table

3.1), i.e. the cow (mean = 7.6), human (mean = 7.8) and the monitor lizard (mean =7.9). In contrast, for female flies the smallest host (lizard) attracted 33% (mean = 30.9) more flies than the largest host (cow, mean = 20.6), and 13% more flies than the human host (mean = 26.9).

Table 3.2 Analysis of Variance (ANOVA) of the *G. f. fuscipes* caught from the outside of the ring of nets.

	Source	DF	SS	MS	F	P
Males	Block	5	0.2036	0.04072	1.24	0.328
	Treatment	4	0.1053	0.02632	0.802	0.538
	Residuals	20	0.6567	0.03284		
	Total	29	0.9656	0.09988		
Females	Block	5	0.4695	0.09391	2.954	0.0372
	Treatment	4	0.1844	0.0461	1.45	0.2546
	Residuals	20	0.6359	0.03179		
	Total	29	1.2898	0.1718		

The number of fed flies caught from the inside surface of the ring of nets, expressed as a percentage of the total (fed + unfed) catch from the inside of the ring of nets, provided a relative estimate of the feeding efficiency. The catch from the inside of the ring of nets was used since it was assumed that flies had an

opportunity to feed – unlike those caught on the outside of the ring of nets. The number of blood fed tsetse were remarkably low (Table 3.3). Only one fed male and 30 fed females were caught from total catches of 997 and 1425, respectively. The low numbers precluded statistical analyses of the data but the results suggest that the feeding rates were higher for females than males.

Table 3.3 Numbers of tsetse caught from the inside of a ring of nets with various baits at the centre.

			Males		Females	
Bait	**Unfed Total catch	Total inside	Fed inside	Fed (%)	Fed inside	*Fed (%)
Biconical	264	69	0	0	4	1.2
Cow	315	256	0	0	10	1.8
E-target	299	58	0	0	5	0.9
Human	272	206	0	0	2	0.4
Lizard	275	225	1	0.4	8	1.8

*Feeding efficiency (Fed %) was calculated by expressing the number fed as a proportion of the total catch. **Catches were pooled totals obtained over six replicates.

In all the treatments except with the lizard there seemed to be more flies circling around and leaving the treatment, i.e. flies caught on the inside of the circle of

nets, rather than on the outside (which would signify approaching flies). These flies originally passed through the openings between the nets (50% of the circle, see Fig. 2.7 – 2.11) and presumably circled the treatment and were caught on their flight away. Interestingly, the two inanimate devices also collected fed flies, with the biconical trap attracting 4 fed females and tiny electric target 5 fed females.

Finally, the catch from the artificial baits, the small target and the biconical trap, expressed as a percentage of the combined catch from the bait and the inside of the ring of nets, provides an estimate of the device's efficiency (Table 3.4). The assumption is that those flies caught on the inside of the ring of nets had an opportunity of being caught by the target or trap but had evaded capture and were leaving the vicinity.

Table 3.4 Comparative efficiency of Biconical trap and E-target.

		Males	Efficiency		Females	**Efficiency
Bait	*Inside (i)	Bait (b)	(e, %)	*Inside (i)	Bait (b)	(e, %)
Biconical	69	13	15.9	264	13	4.7
E-target	58	14	19.4	299	33	12

*Total catches from the inside of the ring of nets (Inside catch, i) and artificial baits (Biconical trap or Tiny target) at the centre of the ring. **Efficiency is estimated as the catch from the bait (b) expressed as a percentage of b+i.

The total catches illustrate the general trend (Table 3.5). Overall the trap efficiency was low (10-20%), particularly for females approaching a biconical trap (5%). The data were subjected to logistic regression to assess the significance in capture efficiency for traps using R statistical software. The total catch from the inside of the ring of nets (i) and the bait (b) were specified as the binomial denominator, the catch from the bait (b) was the y-variable. The results (Table 3.5) showed that there was no significant effect of baits for males (i.e., the efficiency of the trap and target was not significantly different) but there was a significant difference in the efficiency of the trap and target for female tsetse. This indicates that the tiny target is more effective than a biconical trap as killing device for female tsetse due to a greater proportion of flies contacting the target and being killed.

Table 3.5 Comparative trap efficiency for male and female *G. f. fuscipes*.

	Source	DF	Deviance	Resid DF	Resid. Dev.	F	P
Males	NULL			11	29.75		
	Block	5	10.25	6	19.5	0.53	0.75
	Bait	1	0.1	5	19.4	0.026	0.88
Females	NULL			11	27.65		
	Block	5	7.41	6	20.23	1.09	0.46
	Bait	1	13.1	5	7.13	9.61	0.02*

ANODEV of proportion of tsetse caught by a trap or a target. Terms were added sequentially, with bait added last. A quasibinomial model with a logit link was fitted to the data using R statistical software. Analyses were carried out using R (R Development Core Team, 2008).

3.3 Discussion

The efficiency of the targets were analysed using the statistical package R. Since the catch from the trap is expressed as a proportion of the total catch (i.e. trap+ring of nets), analysis were carried out using a general linear model with a binomial rather than normal error structure. Preliminary analysis showed that the data were over dispersed and so the quasibinomial command was used. The analysis (Table 3.5) shows that there was no significant difference in the efficiency of the trap and e-net for males (15.9% vs. 19.4%) but the difference (4.7% vs. 12%) was significant (P=0.02) for the females.

The value of electric nets and especially employed as an incomplete ring of nets, lies in the knowledge gained about the vector's behaviour around hosts and devices. For example, the data clearly illustrated that the conventional control device (biconical trap) and tiny electric target attracted the highest number of *G. f. fuscipes*. On the face of it, this sounds very reassuring for control of *G. f. fuscipes*, but when looking at the data more carefully it is apparent that the biconical trap is actually performing poorly as a capturing device with only 35.62% of all flies being caught in this trap. Comparatively, the tiny electric target

accounted for almost twice the number of *G. f. fuscipes* killed. These data are supported by earlier findings in the same study area that showed that tiny electric targets caught about 8.6-37.5x more flies as a trapping device compared to biconical traps (Esterhuizen *et al.*, 2011a). As pointed out by others (Lindh *et al*., 2009; Omolo *et al*., 2009; Rayaisse *et al*., 2010; Esterhuizen *et al.*, 2011a,b; Torr *et al*., 2011), this has important cost implications for the use of insecticide impregnated small targets in the control of *G. f. fuscipes* and other riverine species. The tiny electric target uses only approximately 2.5% of the cloth needed to manufacture a biconical trap, plus it is easier to manufacture, easier to transport to the field and easy to deploy, thus reducing the costs of overall vector control.

In all treatments except with the lizard, most flies were circling around and presumably leaving the treatment, i.e. flies were caught on the inside of the circle of nets rather than on the outside (which would signify approaching flies). This indicated a high rate of movement around a host or device, without necessarily settling on it. In the case of male *G. f. fuscipes* this is partly explained by their attraction to hosts to find females to mate with. On the other hand, for female tsetse this is explained by their need to obtain a blood meal for egg and larvae development, although females may be circling the host for a considerable period before taking the risk of settling, thus getting killed on the nets as they circle. The attraction of the three live hosts to *G. f. fuscipes* provided further insight into the behaviour of this tsetse species which is well-known for feeding on monitor lizards Mohamed-Ahmed, (1998). In this study the small monitor lizard had higher catches than the 60x larger cow and the medium–sized human. This,

together with the high catches on the tiny target, illustrates the particular host-seeking behaviour of this species, presumably aimed at a preference for smaller hosts. The complacent nature of monitor lizards with almost no defensive behaviour, allows tsetse flies to blood feed undisturbed (*photographic evidence, J. Esterhuizen 2009*), (Fig. 3.1). In addition, the thick vegetation of the lizards' habitat necessitates a 'close-quarter' seeking behaviour and might explain the tsetse fly preference for these small reptile hosts.



Figure 3.1 Several *G. f. fuscipes* feeding on a monitor lizard, at big Chamaunga Island, Kenya. (Copyright J. Esterhuizen 2009).

This further illustrates how well the tiny targets are suited/ developed for the control of *G. f. fuscipes* with the flies being more attracted to small devices (Lindh *et al.,* 2009) and able to locate the tiny targets and be killed on them even in-between thick vegetation (Esterhuizen *et al.,* 2011a).

Although the human treatment had the lowest catches of *G. f. fuscipes* it still does show potential exposure risk to humans present in tsetse habitat. For example, the seated human attracted an average of 75 tsetse per day, only 22% and 29% less than the 'best' host, the monitor lizard and the control device, respectively. The relatively high catches of *G. f. fuscipes* on the cow shows that the use of insecticide-treated cattle might be a suitable solution in this area, if the cattle densities are high enough (Torr *et al.,* 2007 a, b). It also indicates a higher effect of vision-oriented host-seeking by *G. f. fuscipes* compared to the odour-and visual oriented savannah species where the presence of a cow's odour would increase catches 30x (Vale *et al.*, 1988; Torr and Hargrove, 1998; Torr *et al*., 2011). This again supports the idea of *G. f. fuscipes* having a close-quarter host seeking behaviour in the lakeshore environment.

CHAPTER FOUR

THE EFFECT OF HUMAN MOVEMENT ON *G. f. fuscipes* BEHAVIOUR

4.1 Introduction

Studies by Vale (1974) on *G. m. morsitans* and *G. pallidipes* in response to mobile and stationary baits showed that visual cues attracted more flies than odour cues. The outcome for *G. m. morsitans* and *G. pallidipes* showed that a stationary human had poor attraction compared to a mobile human and also more male flies compared to females were attracted to a mobile human than a stationary one. It was important to note that human odour was the most important factor although the erect posture of humans was in turn repellent. According to Torr and Hargrove, (1999), based on studies done in Zimbabwe where they compared tsetse behaviour around refuges, odour baited traps and baits using electric nets, they showed that there was a risk of communities living and working in the savannah areas being bitten by tsetse flies. Their findings were vital in the sense that flies caught trying to obtain a blood meal from humans were all young, although 1/3 were old enough to be capable of transmitting sleeping sickness. Tsetse control using baits was applied on the island of Principe between 1911 and 1914 where sticky targets carried on the back of plantation workers were used for the control of *G. p. palpalis* (Wall and Langley, 1990; Leak, 1998) and showed great success since its application on the island eliminated all potential vectors

during the study (Leak, 1998). In a similar way, sticky panels were used in this study to investigate the behaviour of *G. f. fuscipes* around humans as they work along the shores of Lake Victoria. The knowledge gained leads to a better understanding of sex and age at which *G. f. fuscipes* are main vectors, since the epidemiological importance of insect vectors increases with their age (Leak, 1998).

4.2 Results

4.2.1 Sticky panel collections

The sticky panel study on Small Chamaunga island collected 548 *G. f. fuscipes* in total (385 males and 163 females) over 33 days, with an average of 11 males and 5 females per day. The mobile sticky panel (i.e. with the person walking along a path) accounted for 90.4% (n=348) of the males and 73.6% (n=120) of the females collected. It is interesting to note that a larger percentage of females (26.4%, n=43) were caught on the stationary sticky panel, compared to only 9.6% of males (n=37), which clearly preferred the mobile sticky panel (Fig. 4.1).

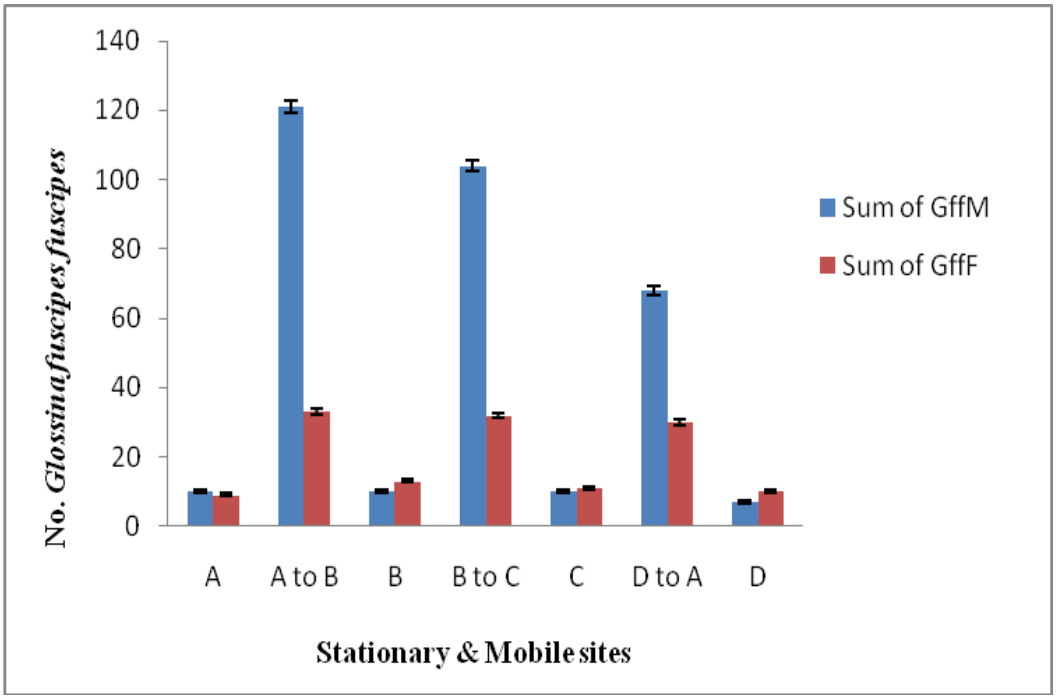


Figure 4.1 Catches of *G. f. fuscipes* from mobile sticky panels (human) and stationary sticky panel (human). GffM= *G. f. fuscipes* Male, GffF= *G. f. fuscipes* Female.

The results (Table 4.1) showed that for both male and female *G. f. fuscipes* there was a significant difference between the catches from mobile and stationary humans. The mean catch of tsetse flies from the stationary human was 0.9 males and 0.9 females per day compared to 8.9 and 2.6, respectively, for the mobile humans. The results are generally in accordance with the patterns seen with the Morsitans-group of tsetse flies: mobile humans caught more tsetses than stationary ones and the effect is particularly marked for male flies (Vale, 1974). For example, in the present study, there was a 10x difference in the catches for males compared to a ~2x effect for females between mobile and stationary

catches. For the Morsitans-group tsetse, the catches of males probably comprised both host- and mate-seeking tsetse whereas the females were flies in the feeding phase of behaviour only. It seems likely that this same phenomenon was occurring with *G. f. fuscipes*.

Table 4.1 Statistical data for catches of G. f. fuscipes from mobile and stationary humans.

Sex	Source	df	SS	MS	F	P
Males	Day	32	1.944	0.061	1.256	0.262
	Treatment	1	8.526	8.526	176.196	1.47E-14***
	Residuals	32	1.548	0.048		
	Total	65	12.018	8.635		
	Detransformed mean daily catches (transformed mean±SE)					
		Stationary	0.89	(0.28±0.047)		
		Mobile	8.9	(0.99±0.054)		
Females	Day	32	3.711	0.116	2.121	0.0185*
	Treatment	1	1.314	1.3137	24.033	2.64E-05***
	Residuals	32	1.749	0.0547		
	Total	65	6.774	1.4844		
	Detransformed mean daily catches (transformed mean±SE)					
		Stationary	0.88	0.27±0.047		
		Mobile	2.6	0.56±0.054		

4.2.2 Age composition of female flies collected by two different methods

The age of female *G. f. fuscipes* collected from a mobile host with a sticky panel and a biconical trap (Table 4.2) was investigated through dissections to assess their ovarian category. Flies were collected from Rusinga island (n=8), Manga island (n=117) and Karungu (n=16), (Sori) (n=28). The catches from all four sites were pooled and the results (Fig. 4.2) showed that 55% of tsetse flies from the sticky panel were in ovarian category 0 (within age 0 to 10 days) compared to just 15% from the trap.

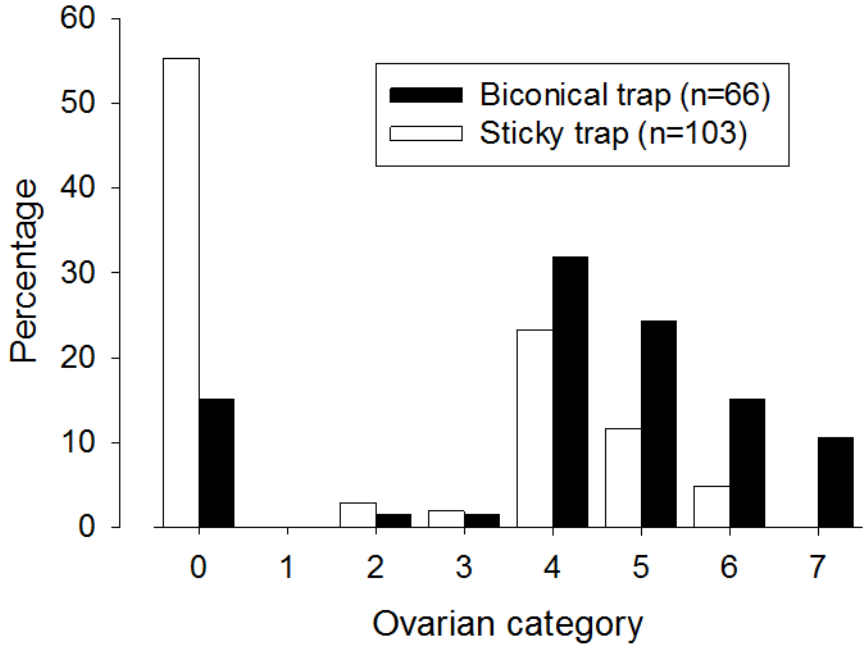


Figure 4.2 Percentage of *G. f. fuscipes* in different ovarian categories from a biconical trap or sticky panel.

A chi-squared test was used to analyse the statistical significance of difference in the composition of the two catches. The low number (<5 for both treatments) of tsetse females in ovarian categories 1-3 prevented using all eight ovarian categories for a 2 x *k*-column chi-squared test. The catches were therefore pooled into young (categories 0-3) and old (categories 4-7) flies to produce a 2x2 contingency table (Table 4.2) which was subjected to a chi-squared test. The catch difference there was a highly significant (P<0.001) with chi-squared value of 27.2.

Table 4.2 Contingency table of G. f. fuscipes from biconical traps and sticky panels classified as young (ovarian categories 0-3) or old (ovarian categories 4-7).

	Biconical	Sticky	Total
Young	12	62	74
Old	54	41	95
Total	66	103	169

These results suggest that the mobile bait attracted significantly younger females than the stationary trap and are in general accordance with those recorded for the Morsitans-group by Torr *et al*. (2012).

4.3 Discussion

A moving human is more attractive to *G. f. fuscipes* compared to a stationary human. More than 90% of the flies collected during this study were caught on the mobile sticky panel carried on the back of the research assistant, with three times more males than females caught on the mobile sticky panel, probably due to male tsetse mating behaviour. The male tsetse flies follow the moving host in search of females for mating, sometimes forming 'following swarms' (Langley and Hall, 1984; Boaz, 2006). Male *G. f. fuscipes* appears to be more risk-taking than female flies and will follow, approach and settle on a moving human host.

The mean ovarian age of female *G. f. fuscipes* collected from the mobile sticky panels and biconical traps showed that younger female flies (9-19 days) appear to be more significantly ($P<0.001$) attracted to the mobile bait, compared to older (30-50 days) females. These results are in agreement with those for *G. pallidipes* and the Morsitans-group where the stationary bait caught older females compared to males (Vale, 1974; Hargrove, 1991). The implication is that the risk of infection from exposure to female tsetse bites may be less for humans, because transmission potential increases with the age of the fly (Van den Abbeele *et al*., 2010). Despite this, the majority of *G. f. fuscipes* settling on a mobile human were male flies and although no determination of their age was done, it does suggest that the exposure risk of humans to *G. f. fuscipes* should not be underestimated in these lakeshore environments.

There is potential for sticky panels as in Principe island example that could be used as an additional or localized tool for tsetse control. The plantation workers succeeded in controlling *G. palpalis* between 1911 and 1914 on Principe island, (Wall and Langley, 1990; Leak, 1998). Clothing impregnated with insecticides is now becoming available on the international market (Maagan and Meshnick, 2011). These products do not irritate the human skin and it is tempting to consider the use of such insecticide-treated clothing as a modern twist on the past sticky panel technique for tsetse control.

These finding illustrate that exposure to *G. f. fuscipes* constitutes a high risk to humans along the lakeshore and islands of Lake Victoria, a situation probably applicable elsewhere in Africa where human activity is often focussed along the edges of water bodies.

CHAPTER FIVE

THE NUTRITIONAL STATUS OF *G. f. fuscipes* ATTRACTED TO A HUMAN HOST (STICKY PANEL) AND STANDARD COLLECTION DEVICE (BICONICAL TRAP)

5.1 Introduction

Adult tsetse flies take a blood meal that is converted into fat for flight energy and larval production in the case of females. The way in which a blood meal is utilised differs between teneral (newly emerged) and mature flies (Bursell, 1961; Rogers**,** 1978). In the teneral fly, thoracic muscle development is the priority for blood meal utilization while in older flies it is used for fat storage. In this study the nutritional status of male *G. f. fuscipes* attracted to different hosts and devices was studied using residual dry weight analysis (RDW) as determined by extraction of lipids using chloroform (Bursell, 1961; Langley *et al*., 1990; Green, 1990; 1994). Only male flies were used due to the high and variable fat content of female flies in different stages of nutrition and ovulation. In particular, the nutritional status of male flies attracted to mobile baits was compared to those attracted to stationary baits. Healthy flies are better disease vectors than unhealthy (hungry) flies and the knowledge gained from this study will contribute to better understanding of the tsetse population's nutritional status and the associated potential exposure risk of humans.

5.2 Results

The fat content obtained from 250 male *G. f. fuscipes* collected from either biconical traps (n=128) or a mobile sticky panel (n=122) were analysed (Table 5.1). The mean fat content of flies from the stationary biconical traps was 1.34 mg, significantly lower ($P<0.001$) than that collected from flies with the mobile bait mean fat content of 1.85 mg. The results showed that mobile baits attracted flies in better nutritional condition (higher fat content) than stationary baits, which attracted hungry flies only and thus the fat content was generally lower. In addition, the male flies collected from the sticky panel also showed a higher variance (±0.601 mg) in fat content compared to those from the biconical traps (±0.369 mg) suggesting that males coming to mobile baits comprised of flies seeking to feed (low fat content) or to mate (higher fat content).

5.3 Discussion

The Residual Dry Weight technique was used to determine the mean fat content of male flies collected from stationary biconical traps and from a mobile sticky panel. Considerable variation was seen between collected male flies that had a higher mean and a higher variance in fat content from the mobile bait, compared to those from the biconical traps (Table 5.1). This is in agreement with the suggestion that males coming to mobile baits are either seeking to feed (hungry) or to find females for mating (fed and healthy) and hence the catch comprises flies at various stages of hunger. This observation is also supported by the ring of nets study (chapter 3), which showed that few flies actually feed on a host, suggesting that flies are in good condition and are attracted to the host, but not necessarily for feeding. In general, the finding are again in accordance with results for Morsitans-group of tsetse flies (Torr and Hargrove, 1998) where mobile baits catch flies with a higher fat content (i.e., less hungry) than those from stationary baits.

Unfortunately, only male flies can be used for RDW analysis because of the varying fat contents of females (depending on stage of ovulation, egg and larva development). Nevertheless, the observations are interesting in terms of male tsetse fly behaviour, their host-seeking and subsequent human exposure risk. The data showed that better nourished male flies readily approached and settled on a moving human implying that, if they were infected, they would be good healthy

vectors. The high number of males caught on the mobile sticky panel suggests that they are not primarily interested in feeding, but rather in finding a mate; the so-called following-swarm often observed behind moving hosts and objects (Leak, 1998). In evolutionary terms, males in better condition will have the best chance for finding, following and feeding on a moving host, as well as the obvious chance to mate with females which are there for feeding and so exchange strong genes.

CHAPTER SIX

AGE DETERMINATION FOR *G. f. fuscipes* USING PTERIDINE ANALYSIS

6.1 Introduction

In tsetse control, it is important to understand the age structure of fly populations. The epidemiological importance of tsetse increases with their age because they have a higher likelihood of becoming infected and more time to mature that infection (Leak, 1998). Consequently age determination is a useful tool in the study of the epidemiological efficiency of vector populations (Leak, 1998). In natural conditions tsetse populations have older females, compared to tsetse population in a control operation, where the age structure will have younger females. Ovarian dissection has been used in age grading female tsetse flies (Saunders, 1961 and 1962), however, it is not possible to determine the age exactly beyond the fourth ovarian cycle. Males have been age graded using wing fray techniques (Jackson, 1946 ; Vale *et al*., 1976) but this is a questionable practice particularly if flies have been trapped when wings become frayed rapidly in the holding device. Ideally a simpler, less subjective method could be used instead for females and would also be useful for male tsetse flies for which we do not currently have an acceptable technique. In the recent past, many techniques have been developed for estimating the age for different species of Diptera, e.g. Wall *et al*., (1991) carried out detailed studies on ovarian development and

pteridine accumulation for age-determination in the blowfly *Lucilia sericata* while Lehane and Mail, (1985) determined the age of adult male and female *G. m. morsitans*. Mark release recapture was used to the study dispersal of G. *pallicera*, *G. nigrofusca* and *G. palpalis* in Ivory Coast by Gouteux *et al.,* 1982. In other studies by Lehane and Hargrove (1988) mark-release-recapture was used as a source of older flies to correctly determine their corresponding ages.

Electric nets are much more efficient in capturing riverine tsetse flies than traps (Esterhuizen *et al.*, 2011a,b), and provide important information on tsetse behaviour in the surrounding area of baits (Vale, 1974) because they were used extensively in the field study, the validation of the technique and its effects on electrocuted tsetse flies' pteridine levels was needed. This was to verify if tsetse flies collected from electric grids could reliably be used for pteridine analysis.

The knowledge gained from this study will improve understanding of tsetse ecology, interpret fly behaviour and the efficiency of conventional and new control devices for *G. f. fuscipes*. In addition, the establishment of a reliable age-curve for *G. f. fuscipes* which rely on pteridine analysis, will allow for accurate monitoring and understanding of the tsetse population's dynamics during vector control operations or research projects.

6.2 Results

6.2.1 Age determination with pteridine analysis of marked, released and recaptured *G . f. fuscipes*

The results (Fig. 6.1) showed that between 15 to 20 days old, female flies (red points) had a slightly higher RFU (Raw Fluorescence Units) value than males (blue points). Beyond this age period the RFU values of male flies was higher than females.

During the MRR the total flies released were 643, with 457 females and 186 males. The recapture rates were 25 /457 = 5.5% females and 12/186 = 6.4% males.

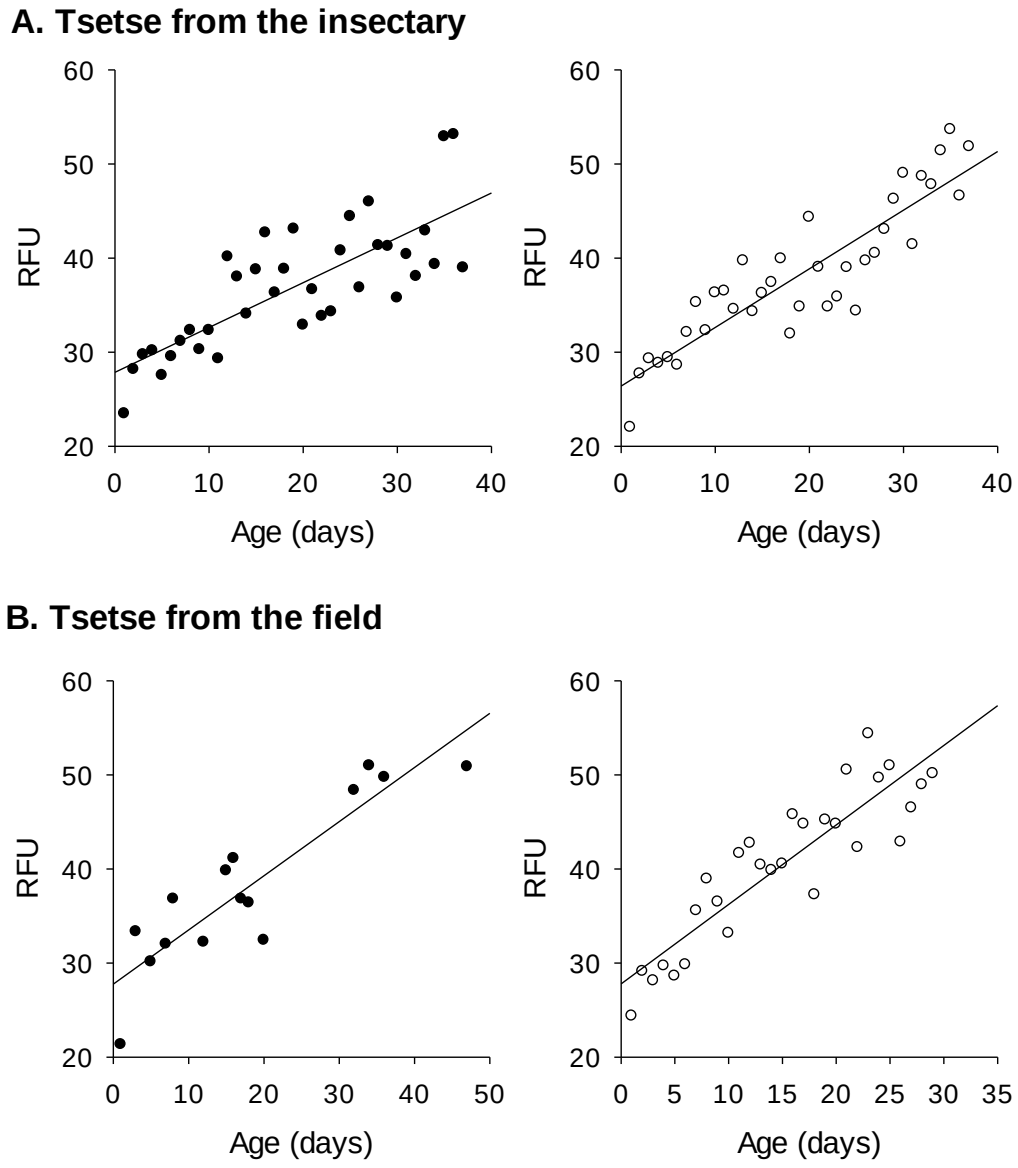


Figure 6.1 Scatterplot of age against raw fluorescence units (RFU) for male (●) and female (○) tsetse from the (A) insectary and (B) field.

The regression was carried out using the regression analysis function in Excel. The Excel sheet shows the ANOVA tables, R values, F-values and P-values as requested by the examiner. The Word file shows the graphs for each sex from the field and the insectary.

6.2.2 Pteridine and ovarian age determination of wild caught female *G. f. fuscipes*

Ovarian age of female flies was determined (age in days) and then compared with mean RFU values. More females were of age 4 (N=73) and 5 (N=79) days. The groups with a lower number of females were aged 2 (N=11) and 9 (N=1) days. The correlation value R^2=0.288 (Fig. 6.2) as seen on the graph shows a wide variation between RFU values, especially as flies gets older >40 days (Table 6.1). The RFU value obtained for age 9 days was done only for one fly and consequently the lower scale of the age graph cannot be interpreted properly.

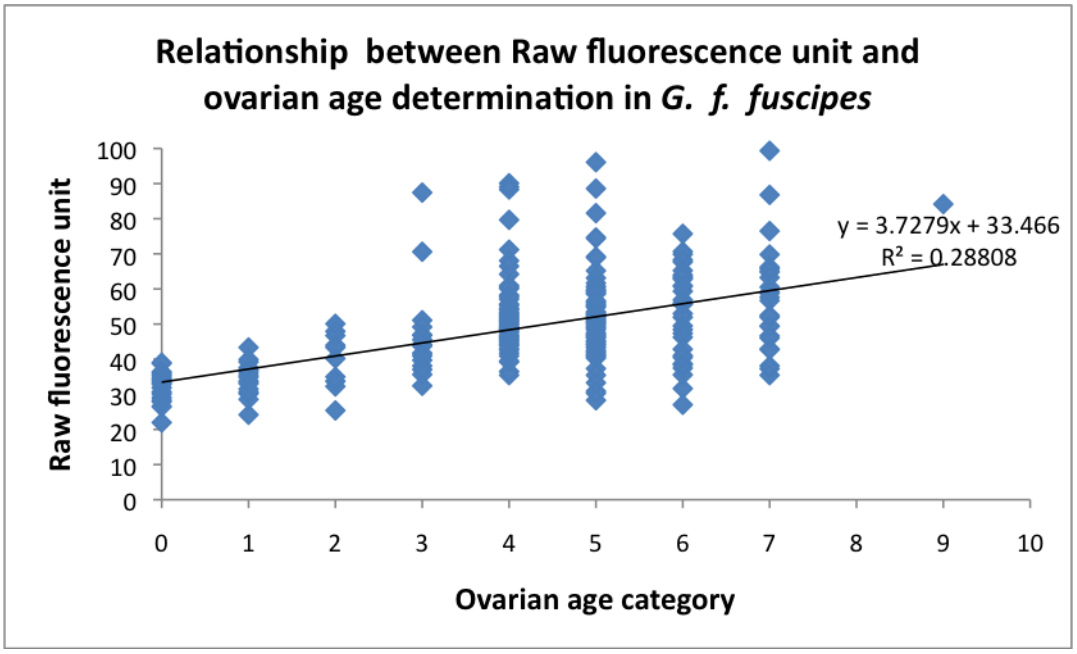


Figure 6.2 Relationship between raw fluorescence unit and ovarian age determination in *G. f. fuscipes*. (F=115, P<0.001 for regression)

Table 6.1 Relationship between raw fluorescence unit and ovarian age determination in G. f. fuscipes.

Ovarian age category	Mean RFU	SE	SD	N
0	32.5484	0.812601	4.063003	25
1	34.1135	0.995413	4.451621	20
2	39.304545	2.350772	7.796629	11
3	46.643889	3.077243	13.05564	18
4	51.176123	1.392598	11.89836	73
5	51.870253	1.340873	11.91794	79
6	53.894412	2.139598	12.47589	34
7	56.849615	3.046677	15.53507	26
8	84.16			1

Regression analysis was carried out to assess the statistical relationship between RFU and ovarian category. The ANOVA results (Table 6.2) showed a simple linear regression equation(y) = a + bx where y is RFU and x is ovarian category) and gave a good fit (r^2=0.95) to the data with the slope (b, t=22.1, P<0.0001) and the intercept (a, *t*=10.4, P<0.0001) both being highly significant. The general expression of the regression equation was: The standard errors (SE) for the intercept and slope are shown in brackets below each parameter.

RFU = 32.8 + 3.7 (ovarian category) (SE) (1.49) (0.36)

Table 6.2 ANOVA for the regression analysis of the relationship between RFU (y-variable) and ovarian category (explanatory variable).

Source	DF	SS	MS	F	P
Regression	2	17357.8	8678.9	1637.5	<0.001
Residual	6	31.8	5.3		
Total	8	17389.6	2173.7		

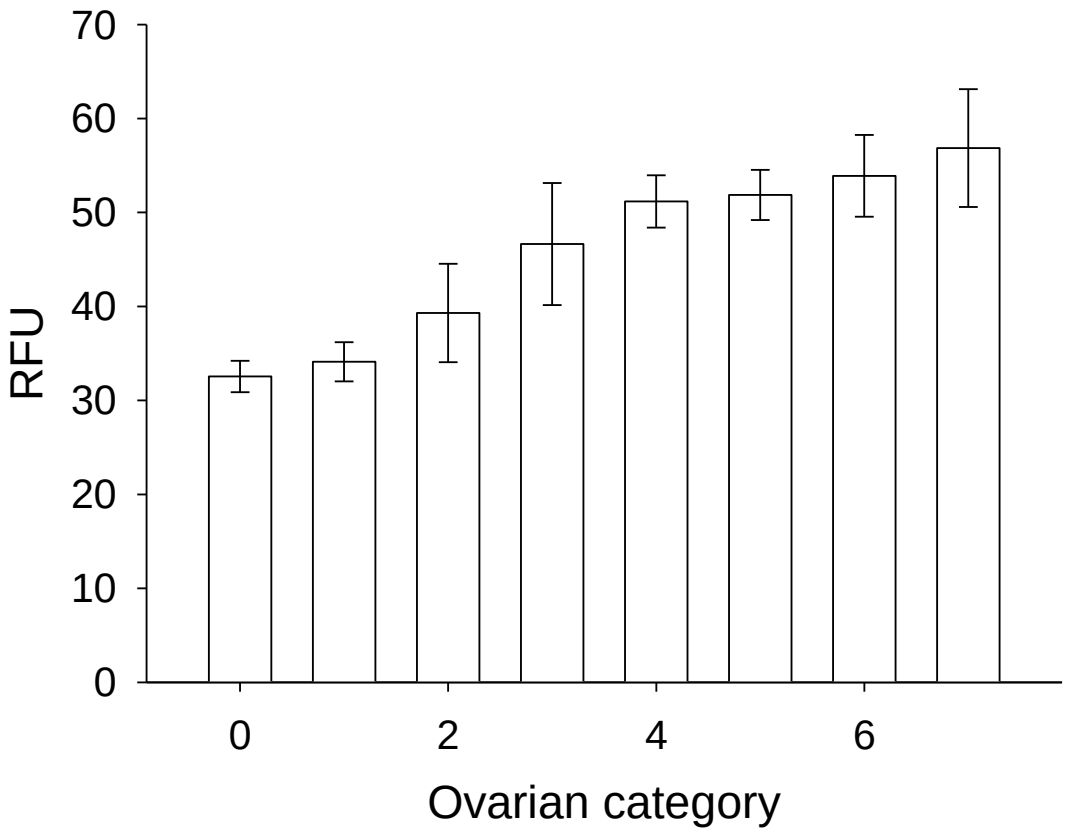


Figure 6.3 Pteridine and ovarian age determination of female field caught *G. f. fuscipes.* RFU (Raw fluorescence units)

From the results, ovarian age determination of female flies was done by comparing mean RFU values to age in days (Fig. 6.3). Age was considered from ovarian age category 0 to 8, the ovarian category was compared to pteridine age determination (RFU), in (Table 6.1). The graph (Fig. 6.2 and 6.3) show comparison of the pteridine and ovarian aging. Mean RFU (±95% confidence interval) against ovarian category. The fitted line has the function RFU= 32.8 + 3.71 × ovarian category (Fig. 6.2) where both parameters are significiant ($P<0.001$) and the SEs for the intercept and slope are 1.487 and 0.355, respectively. The RFU values compared both ovarian age groups (1-3 and 4-7) showed that the difference was statistically significant ($P<0.001$). The correlation value $R^2=0.288$ (Fig. 6.2) as seen on the graph shows a wide variation between RFU values as seen by the standard deviation values on (Table 6.1).

The RFU value obtained for age 9 was done only for one fly and therefore more RFU values for this age category are necessary to draw a cut off value for this age.

6.2.3 Determination of the effect of electrocution and exposure time on pteridine levels in 1 day old laboratory reared *G. f. fuscipes*

To determine the effect of electrocution on pteridine levels in teneral flies, 4 replicates of 24 flies of each sex were electrocuted and removed from the water tray at time intervals of 10 minutes, 1 hour, 2 hours and 6 hours. The RFU values

were recorded and compared with the non-electrocuted control flies (that were not left in water for different time interval since the test was on the effect of electrocution on pteridine level and time taken by a fly before it was aged using the technique (duration of exposure).

Table 6.3 Statistical results of the effects of electrocution, sex and duration of exposure to water on RFU using ANOVA.

Source	DF	SS	MS	F	P
Electrocution	1	19	19.3	1.129	0.2888
Sex	1	90	90	5.263	0.0223*
Time	3	1689	563.1	32.933	<2e-16***
Residuals	378	6463	17.1		
Total	383	8261	689.5		

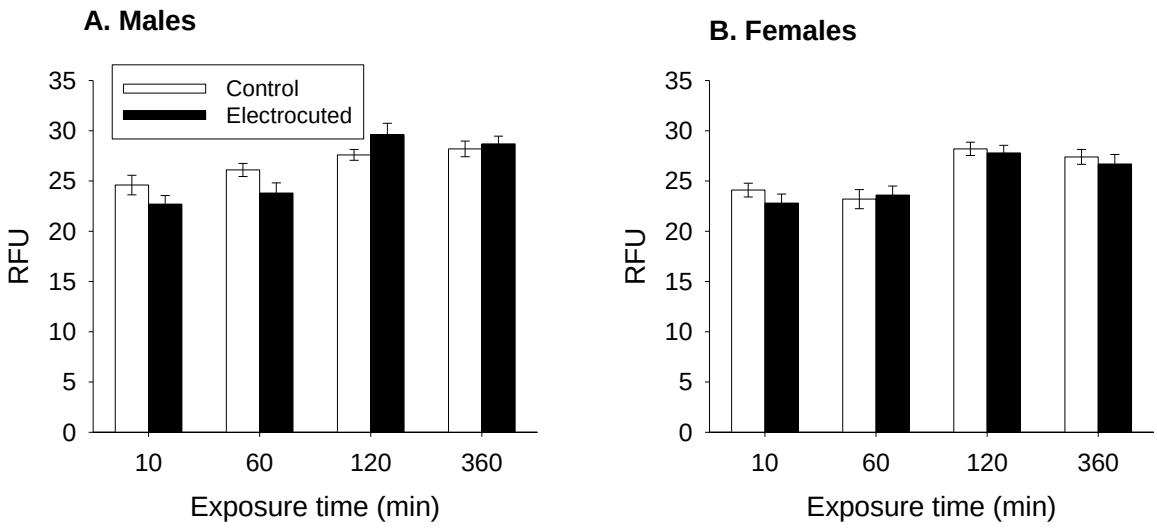


Figure 6.4 Mean RFU (SE) = Standard error for (A) male and (B) female tsetse electrocuted by an electric-net and left in a water tray for various periods post electrocution.

Statistical analysis of the RFU data showed that there was no effect of electrocution on the pteridine levels (Table 6.3), i.e., flies killed on the electric net and non-electrocuted flies showed no significant difference in pteridine levels. However, results did show that fly sex and the duration of exposure had significant effects on the pteridine levels between time intervals (Fig. 6.4) Although the differences between males and females was slight, it was significant ($P<0.05$) and the effect of increase with duration of exposure to water is also clear (Fig. 6.4). This showed that time from collection to processing in the laboratory is crucial, with flies being left in water for more than 2 hours having significantly higher pteridine levels, which in turn may influence correct age determination.

6.3 Discussion

My initial experiment was to determine that the pteridine method worked where freshly emerged flies from the pupae reared in the insect holding facility were used to determine pteridine levels (Fig. 6.1 and 6.2). At emergence (0-1 day) in both male and female flies the levels were between RFU 20-30. By 10 days of age this had risen to above 30 RFU. A similar RFU pattern in field caught flies (Fig. 6. 1 and 6.2) was established which supported the observed increase in RFU value with fly age. Emerging flies collected from the pupae of field caught flies could be maintained in the holding facility for periods up to about 30 days. These flies were used as a starting point for the construction of the calibration curve (Fig. 6.1 and 6.2) of wild flies. However, tsetse flies can live for more than 100 days (Lehane and Mail, 1985).

Mark-release-recapture flies were used as a source of older flies for completing the remainder of the calibration curve (Fig. 6.1). Despite considerable problems with retention of marks on the flies, we were able to recapture some older flies as seen in (Fig. 6.1). While the calibration curves look promising, more data from mark-release-recapture flies are required before a useful calibration curve is generated, as such the work is still in progress. In other studies, the R^2 values have been driven nearer to 90% (Lehane and Hargrove, 1988). The observed relationship seen in (Fig. 6.1) is a common observation for pteridine accumulation in field caught tsetse flies (Mail *et al*., 1983; Lehane *et al*., 1986; Robson and Crozier, 2009; Langley *et al*., 1988). Young flies do not come readily to hosts

(Lehane and Mail, 1985; Lehane and Hargrove, 1988 : Msangi and Lehane 1991), or in this case biconical traps, and so are under-represented in the age distribution curves obtained from field caught flies.

To determine effect of electrocution and exposure time on pteridine levels in *G. f. fuscipes*, showed that flies collected from electrocuting grids could reliably be used for pteridine analysis, provided that they were harvested within a 1-2 hour period. Results showed that the sex of the fly and the duration of exposure to water had significant effects ($P<0.05$) on pteridine levels, which may influence accurate estimation of age (Fig.6.4). In particular, the effect of increased duration in water post-electrocution is of concern, illustrating a significant difference in pteridine levels after 2 hours (Fig. 6.4). This information shows the importance of prompt processing of samples and was taken into consideration for establishing the age curve (Fig. 6.1) for *G. f. fuscipes* because many flies were collected in the field and kept for more than 2 hours before being analysed. Electric nets are much more efficient in capturing riverine tsetse flies than traps (Esterhuizen *et al.*, 2011a, b), and as such are important experimental tools for better understanding tsetse ecology and behaviour.

The outcome of my study is vital for control operations where electric nets are used for studying behaviour of tsetse flies, and I have proven that the electrocution does not affect pteridine levels significantly.

CHAPTER SEVEN

GENERAL DISCUSSION AND CONCLUSION

Tsetse flies are such resilient insects and efficient vectors, that despite more than a century of attempts at controlling or eradicating them and the disease, African Trypanosomiasis still plagues the continent. In the lake areas of central and east Africa, the major vectors are the *fuscipes*-group of tsetse, especially *G. f. fuscipes*, which occur over large areas with strong populations along the lakeshore and on the islands in Lake Victoria. Fortunately, sleeping sickness has not been present in this area for more than 3 decades. Studies done by Simarro *et al.* (2012) on the risk of African Trypanosomiasis in Kenya (2000 to 2009) concluded that there was no sleeping sickness around Lake Victoria (area chosen for the present study), which allowed for the safe investigation of these vectors in their natural habitats. There is a considerable lack of scientific literature on the tsetse fly species chosen for the present study, *G. f. fuscipes*. For example, its behaviour around hosts and control devices, as well as the physiological status of flies attracted to different devices and hosts are not as well known in comparison to morsitans-flies. The exposure risk to humans along the lakeshore, in terms of tsetse bites, was also unknown and is of great importance to the communities who live in these environments where there is a history of leeping sickness. Therefore, the aim of the study was to gather knowledge for improved understanding and control of these disease vectors.

Of particular interest were the attraction of hosts and the efficiency of conventional and new control devices for *G. f. fuscipes*. The best research tools to study this aspect of tsetse behaviour are electric nets, which were used in an intensive 30-day study, with an incomplete ring of nets arrangement (Fig. 2.7-2.11). The data provided interesting information in terms of host seeking behaviour and preferences, for example, male *G. f. fuscipes* do not show a preference for any of the live hosts, with little difference in mean catches between lizard, human and cow. In contrast, for female *G. f. fuscipes*, the smallest host (lizard) attracted 33% more flies than the largest host (cow), and 13% more flies than the human host. This work also showed that the tiny target is more effective than a biconical trap as killing device for female tsetse due to a greater proportion of flies contacting the target and being killed. This observation is well supported by research in the same area during development and field evaluation of the tiny targets (Esterhuizen *et al,* 2011 a, b). In addition, the tiny targets also proved to be more attractive to *G. f. fuscipes* than any of natural hosts, including the 'preferred' monitor lizard.

Due to the high human activity centred around the lakeshore and islands, e.g. fishing, washing etc. the exposure risk of humans to tsetse bites are of special concern. The relative attractiveness of stationary and mobile humans to *G. f. fuscipes* was investigated by employing sticky panels carried on the back. These results are in general accordance with those for *G. pallidipes* where the stationary bait caught older females compared to males (Hargrove, 1991; Vale, 1974). An implication of this is that risk of infection from exposure to female tsetse bites

may increase for humans because transmission potential increases with the age of the fly (Van den Abbeele *et al*., 2010). Despite this, the majority of *G. f. fuscipes* settling on a mobile human were shown to be male flies and although no determination of their age was done, it does suggest that the exposure risk of humans to *G. f. fuscipes* should not be underestimated in these lakeshore environments.

Data showed clearly that a mobile human is 90% more attractive to *G. f. fuscipes*, compared to a stationary human and conventional control device. There were clear differences in the sex ratios of catches, with 3x more male G. *f. fuscipes* than female, caught on the mobile sticky panel. This is likely the result of the male tsetse behaviour which follows hosts in search of females for mating (Boaz, 2006; Langley and Hall, 1984). The observation that female flies may prefer to take a low-risk approach and settle on the stationary human host, while male tsetse are more risk-taking and will follow, approach and settle on a moving human host (Torr and Hargrove, 1998; Torr *et al*., 2012), was evident for *G. f. fuscipes* as well.

In addition, the investigation into the ovarian age of female *G .f. fuscipes* collected from the mobile sticky panel and stationary, conventional control device, the biconical trap, showed significantly younger female flies (9-19 days) are attracted by the mobile bait, compared to older (30-50 days) females to the biconical trap. The nutritional status of male *G. f. fuscipes* varied significantly between stationary and mobile bait catches with male flies attracted to the mobile bait having a higher

mean fat content. This implies that flies caught on the mobile bait are potentially better vectors, candidate for infection since they lived longer and have better fat reserves.

Finally, a very specific gap in the current knowledge for control of tsetse flies was addressed: age determination. The goal was to establish a reliable age-curve for *G. f. fuscipes* using pteridine analysis and correlate it with field caught flies of known age. The pteridine age determination technique is a simpler, less subjective method (Lehane, 1985) and applies to both males and females. The results corresponded well with pteridine accumulation in other field-caught tsetse flies (Mail *et al*., 1983; Lehane *et al*., 1986; Robson and Crozier, 2009; Langley *et al*., 1988).A particular challenge was to obtain enough data points for young flies that do not readily come to hosts or devices (Lehane and Mail, 1985; Lehane and Hargrove, 1988; Msangi and Lehane, 1991) and so, they were under-represented in the age distribution curves obtained from field caught flies. This problem was addressed by using freshly emerged flies from the insect holding facility. Thus we can now use the pteridine analysis and the age curve to determine age of male and female tsetse in the field, which contributes to knowledge of their transmission potential because transmission efficiency also increases with age (Detinova, 1968: Leak, 1998). This finding will assist in future work with this tsetse species for rapid and easy age determination, information which is applied in various situations, e.g. to better understand tsetse population structures and the effectiveness of control operations.

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APPENDIX A: Ethical approval exemption from Liverpool.

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Professor M J Lehane
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

9 March 2009

Dear Professor Lehane

Re: Prospects for developing odour baits to control *Glossina fuscipes* spp., the major vector of human African trypanosomiasis

I can confirm that your study on developing odour baits to control Glossina fusicipes does not require ethical approval and has our Research Ethics Committee exemption.

Yours sincerely

Dr D Lalloo
Chair, Research Ethics Committee

APPENDIX B: Ethical approval exemption from Wits University

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical))
Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

Ref: W-CJ-130802-2 02/08/2013

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: **Prof M Coetzee, Mr B N Njiru (student no 283470).**

Project title: **The physiological status of the Tsetse fly, *Glossina fuscipes fuscipes*, attracted to different hosts and control devices and its implications for control of human and animal trypanosomiasis.**

Reason: This is a study using the Tsetse fly, *Glossina fuscipes fuscipes*. No humans are involved.

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2013 -08- 02
JOHANNESBURG

Professor Peter Cleaton-Jones
Chair: Human Research Ethics Committee (Medical)

copy: Anisa Keshav, Zanele Ndlovu, Wits Research Office

APPENDIX C: Informed consent forms

Participant Informed Consent Form
Confidential

Title of the project:

THE PHYSIOLOGICAL STATUS OF THE TSETSE FLY, *GLOSSINA FUSCIPES FUSCIPES*, ATTRACTED TO DIFFERENT HOSTS AND CONTROL DEVICES AND ITS IMPLICATIONS FOR CONTROL OF HUMAN AND ANIMAL AFRICAN TRYPANOSOMIASIS.

The general objective of this study is to investigate *G. f. fuscipes* physiology and behaviour, and to interpret and apply the findings to vector control and contribute to better management of Sleeping Sickness.

2.4 To use electric nets to determine the attraction of *G. f. fuscipes* to commonly available hosts, i.e. Human, cattle and monitor lizard; as well as to two control devices, i.e. biconical trap and Small target,

2.5 To investigate the effect of human movement on *G. f. fuscipes* behaviour by doing sticky panel studies on a moving and a stationary human going about their normal business in the area.

This information will lead to a better understanding of the biology and ecology of G. *f. fuscipes*, the major vector of HAT.

The conclusions will be interpreted in terms of application in large scale tsetse fly control operations.

A) There is high density of *G. f. fuscipes*, but no sleeping sickness cases

B) No fly will bite Human subject in the ring of net since 99% of Tsetse fly will be electrocuted

C) For the case of human movement and stationary human using a sticky panel, 99% will be trapped on the sticky material. No tsetse bites are expected to land on human Volunteer.

Around Mbita point on Lake Victoria has high density of *G. f. fuscipes*, but no sleeping sickness cases has been reported here for more than 2 years and no parasites could be found from over 1000 tsetse fly dissections done in 2010 (Lehane, personal communication).

I confirm I have read and understood my role in the experiment 2.4 & 2.5, for the above study. I had the opportunity to consider the information, ask questions that were answered satisfactorily.

I understand that participation in this study is voluntary and I am free to withdraw any time without giving reasons and without any penalties.

I understand the data collected during the study will be used and shared by the three institutions; Liverpool School of Tropical Medicine, University of the Witwatersrand and International Centre of Insect Physiology and Ecology (ICIPE),

I Mr/Mrs.....of ID NO.....

Do accept to participate in the above study as a Volunteer

Date.....Sign.....

Volunteer Witness

Name:.....

Date.....Sign.....

Experiment Co-ordinator

Name:.....Date.....Sign.....

APPENDIX D: Data sheet for attraction of *G. f. fuscipes* to three commonly available hosts and two artificial devices

Date	Exp Code	Day	Site	Treatment Code	Treatment	Device	Net no.	(in/out	Start Time	End Time	GffM	Males fed	GffF	Females fed	GffT	Month

APPENDIX E: Data sheet on the effect of human movement on *G. f. fuscipes* behaviour.

Date	Location	Exp Code	Day	Site	TreatmentCode	Treatment	Device	Start Time	End Time	GffM	GffF	GffT	weather	Person

APPENDIX F: Fata sheet for determination of the nutritional status of flies attracted to different hosts and devices through residual dry weight analysis (RDW).

Date	Collection Device	Fly N0.	Sex	Weight 1 (gm) Fresh Male Tsetse fly	Weight 2 (gm) after 8hrs at 70^{o}C (wt)	Weight 3 (gm) Dry wt -fat
		1				
		2				
		3				
		4				
		5				
		6				
		7				
		8				
		9				
		10				

APPENDIX G: Data sheet for ovarian age grading

Ref no.	Date	Collection site	Sex	Fed/unfed	ovarian sequence LoLiRiRo	Uterous content	Ovarian category	Estimated age	Device
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									

APPENDIX H: Data sheet for age determination through ovarian age grading and the pteridine technique

Date	No of G.f.f	Raw Fluorimetric unit	initial S4	final S4	Initial buffer	Final Buffer	Sex Description	Total S4	Mean S4	Total Buffer	Mean Buffer	Age units	No	Place of Origin	Device	RFU category