**The ecology of *Glossina fuscipes fuscipes* (Diptera: Glossinidae) and its responses to 4-Methylguaiacol and specific compounds in waterbuck odour**

By

**Njelembo Joshua Mbewe**

Submitted in partial fulfilment of the requirements for the degree

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# DECLARATION

I declare that the dissertation, which I hereby submit for the degree of Doctor of Philosophy in Entomology at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at this or any other tertiary institution.

Signature:

Student name: Njelembo Joshua Mbewe

Month Year: November 2018

## DEDICATION

I dedicate this thesis to my parents: Webby J. Mbewe and Florence N. Mbewe; my wife Matildah Mulenga Mbewe; my children: Dalitso W. Mbewe, Chiluba N. Mbewe and any other to come; my siblings: Dingiswayo M. Mbewe, Shlezzippi T. Mbewe and Mwangala Z. Mbewe; and my niece Bwalya R. Samwenda

# SUMMARY

**The ecology of *Glossina fuscipes fuscipes* (Diptera: Glossinidae) and its responses to 4-Methylguaiacol and specific compounds in waterbuck odour**

by

**Njelembo Joshua Mbewe**

Supervisor:          Prof. C. W. W. Pirk
                     Department of Zoology and Entomology
                     University of Pretoria
                     South Africa

Co- supervisors:     Dr A. A. Yusuf
                     Department of Zoology and Entomology
                     University of Pretoria
                     South Africa

                     Prof. B. Torto, Dr R. K. Saini and Dr J. Irungu
                     International Centre of Insect Physiology and Ecology
                     Kenya

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                     Apparent fly densities, Fly size, Wing shape,
                     Geometric morphometrics, Sampling, Host seeking,
                     Repellents, Release rates, Western Kenya

Tsetse flies (Glossinidae) are important biological vectors of trypanosomes, the protozoan parasites that cause Nagana and sleeping sickness. They are distinguished into three taxonomic groups; morsitans, palpalis and fusca. Morsitans and palpalis group tsetse species are the most important vectors of both nagana and sleeping sickness. Control methods of nagana and sleeping sickness that target the vector all exploit particular aspects of tsetse biology. So far none of the methods can be

considered as a silver bullet as they are usually used in a variety of complementary combinations; allowing for development of other methods to complement the already existing ones. Use of repellents is one such method that has been developed and shown to reduce levels of nagana in cattle, transmitted by tsetse from the morsitans group. However, these repellents had not been evaluated against tsetse species from the palpalis group, hence the need to also evaluate these repellents against tsetse from the palpalis group. Herein, studies were carried out in western Kenya on four islands (Big and Small Chamaunga, Manga and Rusinga) of Lake Victoria which harbour *Glossina fuscipes fuscipes* an important vector from the palpalis group in order to understand the ecology of this vector and its responses to known synthetic and natural repellents. On two of the islands (Big Chamaunga and Manga), an intervention previously undertaken between 2011 and 2013 reduced fly densities from over 3 flies per trap per day to less than 1 fly per trap per day. Thus, the recovery of fly densities and the population structure of *G. f. fuscipes* on the islands were first assessed. Since tsetse species in the palpalis group usually occur at lower densities compared to those from the morsitans group, apart from the standard biconical trap, a more efficient sampling tool is required in order to capture any effect on the fly catches due to the candidate repellent. The small targets previously shown to attract and kill more tsetse was modified and its efficiency compared to those of biconical traps. Furthermore, the responses of *G. f. fuscipes* to the known repellents (4-methylguaiacol and specific compounds from waterbuck odour) were assessed in biconical traps and sticky small target for their use as baits.

A before and after intervention study was undertaken to assess the recovery of fly densities and the populations structure of *G. f. fuscipes* on the islands while randomised block design experiments were used to evaluate sampling tools and

responses of *G. f. fuscipes* to 4-methylguaiacol and specific compounds in waterbuck odour. Using wing geometric morphometric analyses the population structure of *G. f. fuscipes* was determined. Whilst the effects of trapping devices and responses of flies to repellents were evaluated using generalised linear models. Results indicates that tsetse population densities on the islands had recovered to pre-intervention levels and the flies that recovered were smaller in size indicating that vector control does have an effect on fly size. Sticky small targets caught seven times more *G. f. fuscipes* than biconical traps. Furthermore, when4-methylguaiacol or specific compounds in waterbuck odour were dispensed from trapping devices, catches of both sexes of *G. f. fuscipes* was significantly reduced by between 17 – 29% overall (*P*<0.05). Thus, indicating their efficacies as potent repellents. Following these findings, there are needs for studies to understand the mechanism behind the effect of vector control on fly size as it may guide future control strategies. Sticky small targets should also be evaluated for their cost effectiveness as alternative sampling tools to biconical traps. Further studies to assess the potential of 4-methylguiacol and specific compounds in waterbuck odour dispensed near hosts to protect from bites of *G. f. fuscipes* are also required.

# PUBLICATIONS AND PRESENTATIONS FROM THIS WORK

**Peer reviewed journal articles**

1. Mbewe, N.J., Saini, S.K., Torto, B., Irungu, J., Yusuf, A. A., Pirk, C., (2018) **Effects of vector control on the population structure of tsetse (*Glossina fuscipes fuscipes*) in western Kenya.** Acta Trop. 179 (1-9) http://doi.org/10.1016/j.actatropica.2017.12.015

2. Mbewe, N. J., Saini, R. K., Torto, B., Irungu, J., Yusuf, A.A., Pirk, C.W.W., (2018) **Sticky small target: an effective sampling tool for tsetse fly *Glossina fuscipes fuscipes* Newstead, 1910.** Parasit. Vectors 11:268. Available from: https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-018-2840-6

**Conference presentations**

*Oral*

1. Mbewe, N.J., Saini, R.K., Torto, B.T., Irungu, J.I., Yusuf, A., & Pirk, C. (2017) **Responses of *Glossina fuscipes fuscipes* 4-methylguaiacol and specific compounds in waterbuck body odour at stationary visual attractive devices.** Oral Presentation: 34th International Scientific Council for Trypanosomiasis Research and Control. September 11-15, 2017. Livingstone, Zambia

*Poster*

1. Mbewe, N.J., Saini, R.K., Torto, B.T., Irungu, J.I., Yusuf, A., & Pirk, C. (2017) **Effects of vector control on the population structure of tsetse (*Glossina fuscipes fuscipes*) in western Kenya. Poster Presentation:** 34th International Scientific Council for Trypanosomiasis Research and Control. September 11-15, 2017. Livingstone, Zambia

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AAT | Animal African trypanosomiasis |
| ANOVA | Analysis of variance |
| AOR | Adjusted odds ratio |
| AT | African trypanosomiasis |
| CI | Confidence interval |
| CS | Centroid size |
| DALYs | Disability adjusted life years |
| gHAT | Gambian human African trypanosomiasis |
| HAT | Human African trypanosomiasis |
| Icipe | International Centre of Insect Physiology and Ecology |
| OR | Odds ratio |
| PATTEC | Pan African Tsetse and Trypanosomiasis Eradication Campaign |
| PDMS | Polydimethylsiloxane |
| PW | Partial warps |
| rHAT | Rhodesian human African trypanosomiasis |
| RW | Relative warps |
| SNK | Student Newman Keuls |
| SPME | Solid phase micro-extraction |
| UOR | Un-adjusted odds ratio |
| USD | United States Dollar |
| WRC | Waterbuck repellent compounds |

# STRUCTURE OF THESIS

This thesis is partly based on manuscripts that have either been published or in preparation. Consequently, there are some repetition or overlap between chapters.

**Chapter 1**

General Introduction

**Chapter 2**

Mbewe, N.J., Saini, S.K., Torto, B., Irungu, J., Yusuf, A. A., Pirk, C., (2018) **Effects of vector control on the population structure of tsetse (*Glossina fuscipes**

*fuscipes*) **in western Kenya.** Acta Trop. 179 (1-9)

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**Chapter 3**

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https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-018-2840-6

**Chapter 4**

Mbewe, N.J., Saini, R.K., Irungu, J.I., Yusuf, A., Pirk, C. & Torto, B., (2017)

**Responses of *Glossina fuscipes fuscipes* 4-methylguaiacol and specific**

**compounds in waterbuck body odour at stationary visual attractive devices.**

(Manuscript in prep)

**Chapter 5**

General conclusions and recommendations

## ETHICS STATEMENT

The author, whose name appears on the title page of this dissertation, has obtained for the research described in this work, the applicable research ethics approval.
The author declares that he has observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Research and the Policy guidelines for responsible research.

Signature:

Student name: Njelembo Joshua Mbewe
Month Year: November, 2018

# ACKNOWLEDGEMENTS

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## CHAPTER 1      General Introduction

**Abstract**

Tsetse flies are differentiated into three taxonomic groups: morsitans, palpalis and fusca. Flies from the morsitans and palpalis groups are the main cyclical vectors that transmit trypanosomes, the parasites that cause African trypanosomiasis (AT) in humans and livestock. AT has a devastating impact on human and animal health often leading to death and large economic losses. The disease can be managed by targeting the parasite or vector. Targeting the parasite involves the use of trypanocidal drugs and, of late, proposals to use genetically modified tsetse symbionts to produce trypanolytic agents in the vector have gained momentum. Control methods that target the vector all exploit particular aspects of tsetse biology but none of these so far can be considered as the silver bullet and so they are used in a variety of complementary combinations. This has allowed for development of other vector control methods that add to and complement the already existing methods. The use of repellents is one such method which has been developed and shown to reduce fly catches and animal AT levels transmitted by tsetse from the morsitans group by over 80%. There is a need to evaluate this method for tsetse from the palpalis group as it could offer individual protection against the vector and opportunities for development of novel integrated AT control strategies.

Key words: Tsetse; Tsetse control methods; Palpalis group; Repellents

# CHAPTER 1

## Background and scope

Tsetse flies (*Glossina spp*) infest a total area of about 10 million km$^2$ in sub-Saharan Africa [1]. They are cyclical vectors that transmit trypanosomes, the parasites that cause African trypanosomiasis (AT). In humans, AT is known as human African trypanosomiasis (HAT) or sleeping sickness while in animals it is called animal African trypanosomiasis (AAT) also known as nagana. HAT is in two forms. The chronic form that accounts for more than 98% of the cases caused by *Trypanosoma brucei gambiense* and found mainly in central and western Africa [1,2]. The acute form common to east and southern Africa accounts for less than 2% of the cases caused by *T. brucei rhodesiense* [1,2]. It is estimated that, between 8.5 and 55 million people are at risk of contracting the acute and chronic forms of HAT respectively [3,4]. HAT is among the neglected tropical diseases (NTDs), however, due to increased commitment by stakeholders, surveillance and availability of drugs in the recent past (from the year 2000 onwards), the number of cases have declined from about 300,000 to less than 7, 000 annually [3,4].

AAT is a disease complex mainly caused by *T.brucei brucei, T. congolense* and *T. vivax* in cattle and *T. simiae* in pigs. About 50 to 70 million animals are at risk of contracting AAT of which between 10-30% could become infected and exhibit clinical signs [5,6]. It causes about 3 million deaths in cattle annually and depresses agricultural productivity in terms of reduced calving rates, poor growth rates, lower milk yields and reduced draught power [6]. This represents a severe constraint to development in areas affected with AAT because productive livestock is considered a prerequisite to significantly improve agriculture in order to alleviate food insecurity and poverty [1]. The overall annual direct and indirect losses attributed to AAT are estimated at about US$4.75 billion [1].

This chapter highlights the current commonly used methods and those under or recently developed to control African trypanosomiasis with emphasis on tsetse control methods in relation to the vector biology. It also highlights the limitations of these control methods and proposes a way forward.

**Tsetse fly biology**

**Identification and taxa**

Tsetse flies belong to the taxonomic order Diptera and family Glossinidae. Two features distinguish them from other Dipterans. The first feature is the discal cell of the wing which is like a cleaver and is referred to as the hatchet cell. The other feature is the presence of secondary branches in the hairs of the arista on the antenna [7]. A total of 31 species and subspecies of tsetse are known, out of which eight to ten species are of veterinary and human sanitary importance [1]. The species are divided into three sub genera: the Austenina (Fusca group), Glossina (Morsitans group), and Nemorhina (Palpalis group). This division is based on the morphological features of the adult genitalia, host preference and habitat [1,8].

The species from palpalis group (subgenus Nemorhina) are associated with riverine habitats [1]. In west Africa, *Glossina tachinoides, Glossina palpalis palpalis* and *Glossina palpalis gambiensis* from this group are the main important vectors of AAT [1]. Tsetse flies from the palpalis group are also important vectors of HAT in central Africa (*G. fuscipes* subspecies) as they are opportunistic feeders and have shown flexibility by tolerating high degree of disturbance in landscape [1,9]. *G. fuscipes* subspecies have a wide distribution throughout the Democratic Republic of Congo, DRC, and neighbouring countries extending to the eastern shore of Lake Victoria

[10]. The species is separated into three subspecies which include: *G. fuscipes fuscipes, G. fuscipes quanzensis* and *G. fuscipes martini.* The subspecies *G. fuscipes fuscipes* and *G. fuscipes quanzensis* are responsible for transmission of over 90% of the reported gambiense HAT cases while *G. palpalis* subspecies are implicated in transmission of about 8% of the cases [2,11].

The morsitans group (subgenus: *Glossina sensu stricto*) also referred to as savannah species are restricted to savannah woodlands and their distribution and abundance is often related to that of wildlife [1,12]. Of these, *Glossina morsitans* subspecies and *Glossina pallidipes* are the most important species transmitting AAT and HAT in east and southern Africa. Species within the morsitans group unlike those in the palpalis group are very sensitive to disturbances in landscape and, their density decreases when human population exceeds a density of five people per km$^2$ [9]. Species of tsetse flies from the fusca group (subgenus: Austenina) which occur in low densities are of little or no economic importance and are mainly confined to habitats in and along thick forests like the rain forest of Africa [1,10]. However, *Glossina brevipalpis* is of restricted importance and found in forest islands in east Africa often that are near water courses whereas *Glossina longipennis* inhabits the more arid regions [1].

**Haematophagy and host seeking behaviour**

Both sexes of tsetse flies feed primarily on blood. In the act of piercing the skin and drawing blood from the host, the flies may transmit or pick up trypanosomes. During feeding, blood is prevented from clotting by the anticoagulant that is contained in the fly's saliva which helps the trypanosomes to stay alive throughout the digestive process and later develops into mature metacyclic trypomastigotes which are

infective to the vertebrate host [7]. It is estimated that only about 10% of the wild flies encounter trypanosomes during their adult life [13,14].

Tsetse flies locate their hosts by olfaction and sight [7]. A study has shown that there is a general conservation of chemosensory gene families that mediate olfaction across tsetse species [15]. Tsetse flies usually demonstrate feeding preference to particular vertebrates regardless of the vertebrates' abundance in the tsetse habitat. The differential vertebrate host preference has been attributed to particular of combination of compounds found in vertebrate odours. Some of these compounds could be repellent or attractive [16–19]. Tsetse flies generally fly up towards the source when they detect an attractive odour from a host in close proximity [20]. These odours are said to serve as long range cues, whilst they mainly use sight to make the final approach [7]. Lindh and others [21] have shown that tsetse flies are attracted to particular wavelengths of blue reflected light (3%, 29% and 20% reflectance at UV 360nm, 460nm and 520nm respectively). Whereas blue (phthalogen and royal) attracts tsetse, black promotes a settling response [22]. Fly size, is among key indicators of the population structure in tsetse flies, and is an important factor that influences fly mobility during host location [23–25]. Larger flies are considered to have greater mobility and probability of finding a host to feed on than smaller ones [25]. The host range depends on the tsetse fly species and may include mammals, reptiles and avians. However, the flies do not always feed on all vertebrates in their environment. For example commonly encountered animals like zebra and wildebeests are not fed on while Waterbuck and Impala are rarely fed on when encountered in the wild [7].

**Reproduction**

Tsetse exhibits a viviparous reproductive biology. During mating, male flies transfer a spermatophore containing sperms at the exit of spermathecal ducts in the uterus of a female [7]. The sperms then make their way to the spermathecae through the ducts where they are stored and remain active for the entire life of the female fly [7]. When ovulation takes place, sperms are released from the spermathecae and fertilisation takes place in the uterus [7]. The zygote sequentially develops into first, second and third instar larva in the uterus. The larval instars feed on the milk secretions from the milk glands of the mother until they are fully grown. Female tsetse flies give birth to live offspring one at a time which burrows into the ground and pupate [7]. Pupal development is solely temperature dependent with higher and lower temperatures shortening and lengthening the pupal period respectively [7]. The male flies that emerge have fully functional spermatozoa and capable of successfully inseminating a female fly under laboratory conditions every 2 – 3 days [1,26,27]. However, females usually mate once but polyandry has been observed in wild *G. f. fuscipes* populations [28]. Once mated, a female fly can produce the first larva after 18-20 days of emerging and thereafter every 9 – 10 days for the rest of its life [7]. Due to the maternal care given by female tsetse, mortality among offspring is low.

**Symbionts**

Tsetse flies habour symbionts which are essential for provision of nutrient, fecundity and immunity [29]. Owing to complete haematophagy throughout their adult life, tsetse flies rely on a primary endosymbiont *Wigglesworthia glossinidia* to supplement nutrients that lack in their diet [14,30]. *Wigglesworthia* is also essential for immune maturation during development of tsetse [31]. Over millions of years

*Wigglesworthia* has coevolved with tsetse and consequently all natural populations habour this obligate mutualist [32]. It is found inside the cells of the midgut bacteriome organ and outside cells in mother's milk secretions [29].

Though not obligate, tsetse can habour a facultative commensal *Sodalis glossinidius* in and outside cells of various tissue such as the haemolymph, milk glands and midgut [29,33]. Its density is known to vary according to species and is associated with susceptibility of tsetse to trypanosome infection [34,35].

Tsetse flies can also habour the parasitic *Wolbachia pipientis* exclusively in the germ line tissue [29,36]. Infections with *Wolbachia* can cause reproductive anomalies in hosts; cytoplasmic incompatibility (CI) being the most important [37]. CI mediated by *Wolbachia* occurs when an infected male mates with a female that is not infected or has a different strain [29,38].  Apart from CI some *Wolbachia* infections can be of benefit by providing supplement nutrients and improving host fitness, longevity and resistance to pathogens [29,39–41]. In the tsetse host *Wigglesworthia* and *Sodalis* are transmitted vertically from mother to the offspring through milk, while *Wolbachia* is transmitted via ovum (trans-ovum) [22,26].

**Control of African trypanosomiasis**

Various methods are available and some proposed to manage African trypanosomiasis. These methods target either the parasite or vector (Figure 1.1). Due to the parasites' developmental cycle it can be targeted either in the vector or vertebrate host. The vector on the other hand can be targeted in its natural habitat and or when it wants to take a blood meal from a host.

**Trypanosomes targeted control**

Successful management of African trypanosomiasis cases are mainly dependent on detecting the trypanosomes in vertebrate hosts. The main method targeting trypanosomes affecting humans involves monitoring and surveillance for cases and treating same [6]. In HAT, determining whether the infection is in the early or late stage is important because the treatment regimens are different. In the early stage, trypanosomes can be detected in blood or lymph smears and this is common for infections attributed to *T.b. rhodesiense* which are usually related to high parasitemia [6]. Infections due to *T. b. gambiense* are attributed to low parasitemia in the early stage, making diagnosis difficult on blood or lymph smears. Consequently, card agglutination trypanosomiasis test (CATT) an antibody test has been developed for *T. b. gambiense* infections [6]. The late stage is determined by detection of trypanosomes and or high level of white blood cells in the cerebrospinal fluid (CSF) [6].
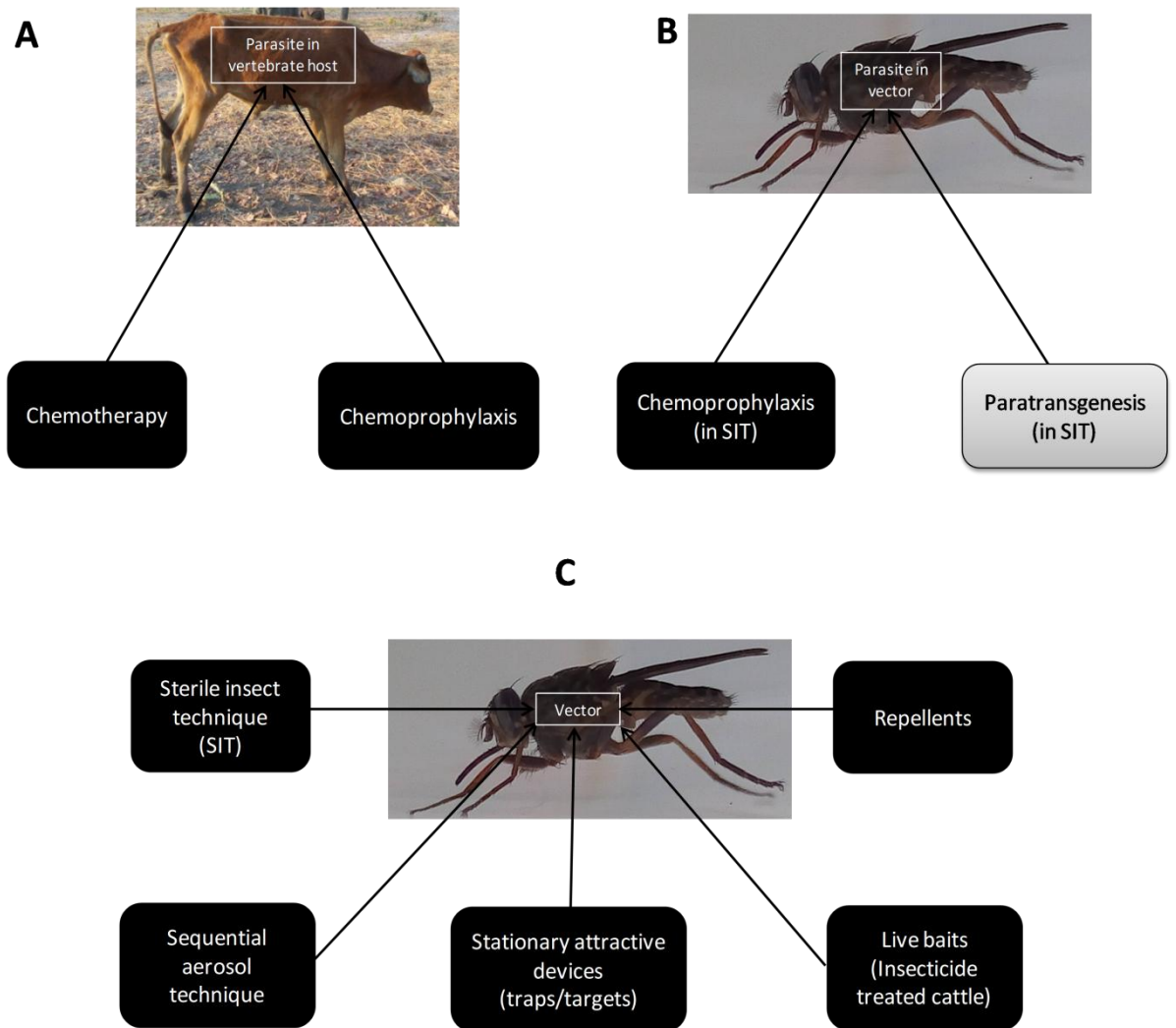
Figure 1.1: Current methods (in black) in use and under development (in grey) for control of African trypanosomiasis. A and B represents control methods that target the parasites in the vertebrate host and vector respectively. C represents vector targeted control methods.

In Rhodesiense HAT (rHAT), the early stage is treated with the drug suramin while for Gambiense HAT (gHAT) suramin or pentamidine can be used [42]. The drug melarsoprol is used to treat the late stage of rHAT while melarsoprol or eflornithine sometimes combined with nifurtimox can be used to treat late stage gHAT [42]. One problem with these drugs is their side effects, which could include anaphylactic shock, renal failure, hypotension and post treatment encephalopathy when administered [6,42].

AAT is mostly diagnosed by detecting trypanosomes in wet blood films, blood smears or buffy coat preparations using a microscope. However, these methods are not very sensitive and some infections are missed [43,44]. Indirect methods such as measuring anaemia status by determining the packed cell volume in endemic areas could be a good indicator of AAT and can be used in combination with microscopy to increase chances of detecting a positive case [43,44]. More sensitive methods for AAT diagnosis are molecular based such as amplification of trypanosome DNA using polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP).

Using trypanocidal drugs, AAT can be treated or prevented once a diagnosis is made. The chemo therapeutic drug diminazene aceturate is used to cure AAT, while isometamidium chloride is used as a prophylaxis. However these drugs are mostly administered by untrained farmers leading to misuse [12], which has contributed to the development of drug resistance [45]. Isometamidium chloride is also administered to sterile male tsetse before they are released in the target operation area to give them temporary refractoriness to trypanosome infections [46].

A proposed method which is still under development is the use of tsetse symbionts which provides an opportunity for using a paratransgenesis approach to produce tsetse that are refractory to trypanosome infections [47,48]. *Sodalis* has been identified as an ideal candidate for genetic modification to deliver trypanolytic agents because it lives closely to trypanosomes in different tsetse tissues such as the midgut, salivary glands and haemolymph [48]

**Vector (Tsetse) targeted control**

Control methods that target the vector remain the most desirable ways of containing African trypanosomiasis [49,50]. All vector control methods exploit particular or combined aspects of tsetse biology. Tsetse flies' longevity, mobility and frequent feeding enable them to be highly efficient vectors [51]. Nevertheless, their low rate of population growth exposes them to population decline and even extinction with small increases in mortality if their population is isolated. Of late models that predict tsetse distribution have been applied to aid in planning of vector control interventions [52].

Currently, the four acceptable and "environmentally friendly" methods of managing tsetse fly populations include: spraying ultralow volumes of non-residual insecticides using the sequential aerosol technique (SAT), attractive stationary devices (traps and targets), live baits, and sterile insect technique (SIT) [1]. So far, all these methods have shown some limitations (Table 1.1) and are used in a variety of combinations to complement each other [53]. Avoidance of host-vector contact using repellents is another method that has shown promising results [19,54].

In SAT, aircrafts with fixed wings or helicopters are used to apply a spray of ultra-low volume non- residual insecticides such as deltamethrin (0.35%(w/v)) [55] above tree canopies in 5-6 sequential spraying cycles with gaps of between 16-18 days

Table 1.1: Advantages of commonly used tsetse control methods and some of their limitations unrelated to cost of operations

| Tsetse control method | Advantages | Limitations | References |
|---|---|---|---|
| Sequential aerosol technique | 1. Rapid<br>2. No known long lasting negative environmental effects<br>3. Can achieve both suppression and elimination of tsetse population | 1. Most effective when used in flat savannah habitat if fixed winged aircrafts are used<br>2. Insecticides have to be sprayed during the period of temperature inversion | [1,56] |
| Stationary attractive devices | 1. Relatively simple tools | 1. Requires period maintenance<br>2. Efficiency is affected by tsetse dispersal<br>3. Can only achieve tsetse population density suppression | [1,25] |
| Insecticide treated cattle | 1. No maintenance problems<br>2. Less prone to thefts | 1. High treatment frequency with insecticide<br>2. Only effective where cattle is main source of blood meals<br>3. Residues of insecticide in dung<br>4. High cattle densities are required<br>5. Can only achieve tsetse population density suppression | [1,57,58] |
| Sterile insect technique | 1. No adverse effects to non target insects<br>2. Can achieve both suppression and elimination of tsetse populations | 1. Is effective only when target tsetse population density is low<br>2. Low reproduction potential make rearing of tsetse a challenge | [1] |

depending on the temperature [59]. Rotary atomisers driven by air or electrically running at a speed of 16,000 rpm are used to produce insecticide aerosol droplets of 30-40µm in size [60]. SAT exploits the reproduction cycle and aims to kill all adult flies in the first cycle through tsetse flies contacting the insecticide mist directly. The subsequent cycles kill all flies that emerge before the females can drop their first larvae. The application of the insecticide is done during periods of temperature inversion and targets the vector in its natural habitat.

Targets and traps (stationary attractive devices) kill the tsetse flies when they have tarsal contact with insecticide on a target panel [61] and heat or starvation after entering a non-return cage of a trap [1,62]. Stationary attractive devices exploit attraction of tsetse to the colours blue/ black and aim to exert 2-3% extra daily mortalities on female flies in the natural population [1]. The performance of targets to control tsetse flies could be affected by the dispersal of the flies, which is partly determined by some population structure parameters such as fly size [25]. In most cases the use of targets as the only method of control results in suppression and not eradication of flies, thus recovery of fly populations once the intervention is discontinued. Traps are more efficient when impregnated with insecticides because only about 20% of the flies attracted get trapped [1,63]. Studies have shown that small targets that are 16 times smaller than the large blue-black (1-1.7m$^2$) target were cost-effective and reduced apparent densities of *G. f. fuscipes* drastically by over 90% [2,64]. However, for the morsitans group of tsetse, such small targets were not as efficient as the larger ones [65].  Traps and targets are also used as sampling tools in ecological and evaluation studies. Use of a particular sampling tool will depend on the efficiency required to obtain the objectives of a study; for example a more efficient sampling tool is required for studies with an aim of determining presence of

tsetse in an area than when its presence is already confirmed. In relation to the unit area of material used, small targets attract and kill more *G. f. fuscipes* than biconical traps [64]. However, this attractive ability of the small targets has not been exploited for sampling purposes.

Live bait technique exploits the host seeking behaviour of both male and female tsetse flies. Livestock is treated with an insecticide and in an attempt to take a blood meal from cattle or other treated livestock, tsetse flies pick up lethal doses of the insecticide on ventral tarsal spines and are killed [1,49].

SIT targets the vector in its natural habitat and takes advantage of the mating behaviour of tsetse flies as females usually mate once in their lives [7]. Thus, the technique relies on the release of a large number of sterile males in relation to wild males in order to out-compete them for mating with the females from the natural population [1]. The mating of a sterile male insect with a virgin wild female does not give rise to offspring [66]. In the subsequent generations, the ratio of sterile to wild males increases and the efficiency of the technique increases at low population densities [1,67].

More recently, the use of tsetse repellents to prevent the vectors from coming into contact with the host has been developed as a control method targeting tsetse [68]. Earlier studies had shown that pentanoic acid and guaiacol reduced the efficiency of traps for *G. pallidipes* [69]. Through research 4-methylguaiacol, a synthetic analogue of guaiacol [70] and naturally occurring repellents (waterbuck repellent compounds, WRC) isolated from waterbuck (*Kobus defassa*) which is a non-preferred host in tsetse habitats, were identified to be involved in differential attraction of vertebrate hosts/non-hosts [18,19]. The intentional use of repellents has attracted interest because these methods may be appealing as they are modern variation to the

traditional approach of using smoke as a repellent to prevent bites from insects [19,71]. The repellent, 4-methylguaiacol, has been shown to reduce the number of bites from tsetse belonging to the morsitans group on cattle by 80% [70]. On the other hand the WRC, were also shown to reduce the feeding efficiency of *G. pallidipes* on cattle by about 96% [19]. A trial undertaken in Shimba Hills area of Kenya (mostly infested with *G. pallidipes*) has shown that repellents can reduce AAT levels by over 80% [68]. In those trials, the WRC were dispensed from a repellent collar placed around the neck of cattle [68]. Most of the published works on repellents for tsetse flies has mainly been made on fly species from the morsitans group even though tsetse flies from the palpalis group are also important vectors of AT [20].

**Rationale of the study**

Targeting the tsetse fly for control of AT is the most desired way because it does not involve dealing with the complexities of reservoirs like it is with targeting the parasite. However, all available and commonly used methods of tsetse control have their limitations; leaving room for improvement and development of other methods that could be complementary to those in existence.

In western Kenya, on the eastern shores and some islands of Lake Victoria, several studies on the biology and control of *G. f. fuscipes* have been undertaken [2, 72–74]. On Big Chamaunga and Manga Islands, densities of *G. f. fuscipes* were drastically reduced by over 90% from 3.9 and 28.2 flies/trap/day to less than 0.1 and 1 fly/trap/day respectively during a tsetse control intervention trial using tiny targets between June 2011 to October 2013 [2]. Further, host location, an aspect of tsetse biology exploited when targets are used for AT control could be influenced by the

fly size which is an element of the tsetse population structure. After the control intervention trial there was no information on vector densities and how the new generation of *G. f. fuscipes* had been affected. Thus, there was the need to assess recovery and structure of tsetse populations on Big Chamaunga and Manga Island three years after the control intervention was discontinued in order to unveil any changes.

 Furthermore, tsetse flies from the palpalis group are known to occur at relatively lower densities compared to those from the morsitans group [20]. As a consequence, apart from the standard biconical trap, a more efficient sampling tool for *G. f. fuscipes* was needed to catch large enough numbers of tsetse flies in order capture any effect in fly catches due to the candidate repellents. Therefore, it was proposed to modify the small target into a sampling tool by covering it with a sticky transparent film since it had been previously shown to attract and kill more *G. f. fuscipes*; hence the need for its assessment for effectiveness as a sampling device. The repellents currently dispensed from collars placed on necks of cattle provide a mobile tsetse control technology and demonstrate effectiveness in reducing AAT levels transmitted by *G. pallidipes* a tsetse fly belonging to the morsitans group [68]. With some habitats harbouring more than one species of tsetse flies, protection is required against the different tsetse species that a potential host is exposed to. Thus, an effective tsetse repellent will need to repel the least sensitive tsetse species in such habitats. Tsetse flies from the palpalis group are considered as the least responsive to host odours [20]. With the observation that chemosensory genes that mediate olfaction are generally conserved across species, among them *G. pallidipes* and *G. f. fuscipes* [15], there was a need to evaluate the repellent technology against tsetse flies from the palpalis group more so that flies in this group are responsible for

transmission of AAT and about 98% of HAT [2]. *G. f. fuscipes* in the subspecies of *G. fuscipes* is the most widely distributed and an important vector of AT. Assessment of its responses to the known tsetse repellents could give insights on potential repellent compounds that could be evaluated for protection of hosts from bites.

**Aim of the study**

The main aim of this study was to undertake ecological studies on *G. f. fuscipes* in order to assess the: influence of a previous vector control intervention on its population structure in the study area; sticky small target as a tool for its sampling; and its responses to candidate repellents that repel tsetse species from the morsitans group. Information on this could guide the planning, monitoring and development of future tsetse and trypanosomiasis control strategies. Further, information from this study could give insights into some aspects at play during tsetse control intervention using targets that lead to suppression and not eradication of tsetse fly population. Such information could be most useful to workers involved in tsetse control strategies under the African Union's Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) which aims to eradicate tsetse flies and African trypanosomiasis.

**Thesis organisation and structure**

**Chapter one** of this thesis provides a general introduction reviewing literature on AT control with a focus on aspects of the vector biology that have been exploited for control and sampling of tsetse flies. **Chapter two** assesses the recovery of the fly populations and whether vector control intervention had an effect on the population

structure that recovered after the intervention was discontinued. **Chapter three** evaluates the modified small target that is covered by a transparent sticky film as a sampling tool for *G. f. fuscipes* and determines its efficiency in comparison to the biconical trap as well as the influence of its blue and black colour panels on the landing response of female and male flies. In **Chapter four** the responses of *G. f. fuscipes* to 4-methylguaiacol and WRC at the biconical traps and sticky small targets were determined. In particular, the effective and optimal release rates of 4-methylguaiacol and WRC were determined at the stationary visual attractive devices as well as the relative contribution of the individual constituents of WRC to its overall repellency. **Chapter five** is the general conclusion and looks at the key findings from all the data chapters and recommends future research directions.

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## CHAPTER 2  Effects of vector control on the population structure of tsetse (*Glossina fuscipes fuscipes*) in western Kenya

**Abstract**

Dispersal of tsetse affects performance of targets during vector control. Fly size, one of the indicators of population structure usually obtained from wing measurement, is among the determinants of displacement rates. Although recovery of tsetse in previous intervention areas has been widely reported, the population structure of tsetse that recover is rarely evaluated despite being associated with displacements rates. Previously, intervention trials had reduced tsetse densities by over 90% from >3 flies/trap/day to <1fly/trap/day on Big Chamaunga and Manga islands of Lake Victoria in western Kenya. In this study, we assessed the recovery in densities of *Glossina fuscipes fuscipes* on the two islands and evaluated the effects vector control might have on the population structure. A before and after intervention study was undertaken on four islands of Lake Victoria in western Kenya; Small and Big Chamaunga, Manga and Rusinga Islands, two of which tsetse control intervention had previously been undertaken. Three years after intervention average *G. f. fuscipes* catches in biconical traps were estimated on each island. Wing centroid size (CS) (a measurement of fly size) and shape, indicators of the population structure of flies from the four islands were compared using geometric morphometric analyses. CS and shape of available female but not male tsetse wings obtained before the intervention trial on Big and Small Chamaunga islands were compared with those from the same islands after the intervention trial. *G. f. fuscipes* apparent density on the previous intervention islands were >9 flies/trap/day. Irrespective of sex, wing shape did not isolate tsetse based on their islands of origin. The fly size from Big and Small Chamaunga did not differ significantly before intervention trials (*P*=0.728).

However, three years after the intervention flies from Big Chamaunga were significantly smaller than those from Small Chamaunga ($P<0.003$). Further, there was an increase in the divergence of wing morphology between flies collected from Big Chamaunga and those from Small Chamaunga after tsetse control. In conclusion, even though populations are not isolated, vector control could influence the population structure of tsetse by exerting size and wing morphology differential selection pressures. Therefore, we recommend further studies to understand the mechanism behind this as it may guide future vector control strategies.

 **Key words:** Dispersal; apparent tsetse densities; Recovery; Fly size; Centroid size; Wing shape; Geometric morphometrics

**Introduction**

Tsetse flies (*Glossina* species) are important cyclical vectors of protozoan parasites, trypanosomes which cause animal and human African trypanosomiasis [1]. Animal African trypanosomiasis (AAT) is mainly caused by *Trypanosoma brucei brucei, T. congolense* and *T. vivax* with 50 to 70 million cattle at risk [2]. Direct and indirect losses due to AAT in the agricultural sector are estimated at about USD 4.75 billion in sub Saharan Africa [1,3]. On the other hand, human African trypanosomiasis (HAT) is caused by *T. brucei rhodesiense* in eastern and southern Africa and *T. brucei gambiense* in central and western Africa [4]. Whereas Rhodesian HAT (rHAT) is acute and usually causes death within weeks, Gambian HAT (gHAT) is chronic and infections can last as long as 29 years [5,6]. In fact, it has been suggested that the chronic carriers harbouring low levels of *T. brucei gambiense* which is undetectable by conventional diagnostic techniques are the ones who sustain gHAT foci [7]. HAT has an impact of 1.59 million disability adjusted life years (DALYs) with about 8.5 and 55.1 million people at risk of rHAT and gHAT [8,9].

Tsetse flies are distinguished into three taxonomic groups based on their habitat, host preference and morphology of the external genitalia [1]. These taxonomic groups include morsitans, palpalis and fusca. Of the three taxonomic groups, palpalis and morsitans are of economic importance as they transmit most of the cases of AAT and HAT [1,10,11]. In the palpalis group, *Glossina fuscipes* subspecies which include but not limited to *G. f. fuscipes* and *G. f. quanzensis* are responsible for transmission of over 90% of HAT cases while, *G. palpalis* subspecies and *G. tachinoides* mainly transmit AAT in central and western Africa [10–12]. From the morsitans group, *G.*

*morsitans* subspecies and *G. pallidipes* are responsible for transmission of both AAT and HAT in eastern and southern Africa [1].

Tsetse flies from the palpalis group (subgenus Nemorhina), are associated with riverine habitat and wetlands as well as lowland rain forest [1]. Species within the palpalis group are opportunistic feeders and have shown flexibility by tolerating high degree of disturbance in landscape [1,13]. Among *G. fuscipes* subspecies, *G. f. fuscipes* are the most widely distributed with ranges spanning from northern Democratic Republic of Congo, DRC, and its neighbouring countries extending through to the eastern shore of Lake Victoria [10,14]. However, insect species do not generally inhabit their geographic space in a uniform manner but strategically arrange themselves according to needs such as, reproductive, dispersion, availability of food resources, adaptation to local conditions and survival to treatments which may give rise to structuring in populations occupying same or separate geographical space [15–18]. This structuring could result in subpopulations with phenotypic and genetic variation [17]. In medical entomology, it is important to quantify existing exchange of individuals among subpopulations and to give information on the population isolation status and structure as these may have consequences on epidemiology and control of vector borne diseases [19]. Thus, the use of a fast and low cost tool of morphometrics is critical in population structure studies.

Morphometrics, defined as "an interwoven set of largely statistical procedures for analysing variability in size and shape of organs and organisms" focuses on variation, its description in terms of parameters and relation to extrinsic factors of organs and organisms under study [20]. Previous studies on population structures of tsetse have shown a strong correlation between morphometrics results with methods that are based on genetics and cuticular hydrocarbons [17,21–23].

Several methods of managing African trypanosomiasis exist. These include screening and curative treatments in humans and prophylactic and curative treatments with trypanocidal drugs in animals [1]. Other methods include promotion of trypanotolerant cattle and suppression or eradication of the vector, the tsetse fly [1]. However, controlling the vector is considered the most desirable way of managing African trypanosomiasis [1,24,25] but, in the absence of area-wide control interventions covering biologically relevant areas and targeting isolated tsetse populations, re-invasion is commonly reported [21,23,26]. Some vector control techniques such as the use of targets exploit the host seeking behaviour which to a larger extent depends on the dispersal of the tsetse fly [27]. Among the factors that influence displacement rates is fly size, with the displacement potential increasing as fly size increases [27]. Fly size is one of the indicators of tsetse population structures and can be obtained from wing measurement [21,28]. Inter-species variation in tsetse fly size has been associated with differences in displacement rates, responses to attractive and repellent odours, availability to tiny or large targets, persistence and landing responses [27,29–31]. Interestingly, the fly size of tsetse populations that recover in previously controlled/suppressed areas are rarely reported. Environmental conditions such as temperature in a living organism's habitat have a direct effect on its size [19]. However, size in insects has shown high heritability values and can be selected for experimentally to produce subpopulations that are genetically distinct for size an indication that it could have trans-generational effects [19,32–34].

Wing shape, another indicator of population structure, is a more stable trait than size and less influenced by environmental changes [18,19]. It is strongly determined by genes and is a polygenic trait [19,35–37].

In Western Kenya on some islands of Lake Victoria, where *G. f. fuscipes* thrives along the shores of the lake, densities of the flies were reduced drastically by over 90% on the islands of Big Chamaunga (June 2011 to December 2012) and Manga (January 2012 to October 2013) from 3.9 and 28.2 flies/trap/day to <0.1 and <1 fly/trap/day respectively during a tsetse control intervention trial using tiny targets [12]. The tiny targets were deployed at densities of 20/km on Big Chamaunga and 10/km on Manga. Therefore, in this study, which was carried out three years later, we assessed the recovery of fly densities on Big Chamaunga and Manga Islands. We also evaluated the impact of vector control on the population structure of *G. f. fuscipes* using wing geometric morphometrics.

**Materials and methods**

**Study area**

*G. f. fuscipes* were captured from the following Islands on Lake Victoria in western Kenya: Small Chamaunga (surface area of about 0.2 km$^2$), Big Chamaunga (surface area of about 0.2 km$^2$), Manga (surface area of about 1 km$^2$) and Rusinga (surface area of about 43 km$^2$) (Figure 2.1) [12,38]. These Islands were selected as study sites based on the fact that there was both anecdotal and documented evidence on studies previously undertaken on *G. f. fuscipes* [11,12,38,39].
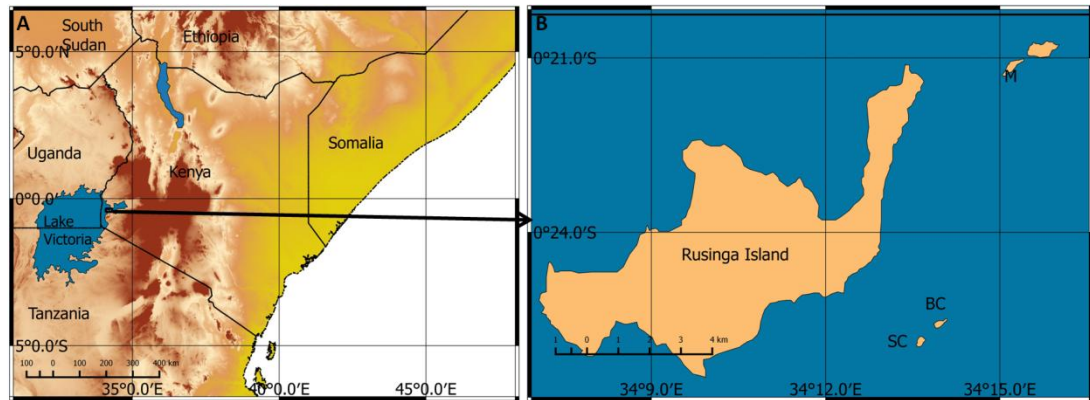
Figure 2.1: (A) Study area in western Kenya. (B) Islands on Lake Victoria where the study was undertaken: BC is Big Chamaunga; SC is Small Chamaunga; and M is Manga.

The islands' vegetation consists of a mixture of *Aeschynomene eraphroxylon*

(freshwater mangrove), *Dombeya* spp. and *Lantana camara* [12]. Whereas Manga

and Rusinga Islands are inhabited by humans, Big and Small Chamaunga Islands are

not. Tsetse fly populations mainly take their blood meals from *Varanus niloticus*

(monitor lizard) and *Hippopotamus amphibius* (common hippopotamus) but can also

feed on cattle and humans on the inhabited Islands [12].


**Study design, sample collection and wing preparation**

A before and after intervention study design [40] was undertaken on Big

Chamaunga, Manga, Rusinga and Small Chamaunga islands. Between June 2011 to

December 2012, during the tsetse control intervention using tiny targets Small

Chamaunga served as the control island (non-intervention) for Big Chamaunga [12].

Un-baited biconical traps [41] were used to catch tsetse for the periods before and

after the intervention. For the period before the tsetse control intervention using tiny

targets, we used samples collected between April 2010 and May 2011 [12]. From

those only female wings of *G. f. fuscipes* caught on Big Chamaunga and Small

Chamaunga were available. Three years after tsetse control intervention male and

female flies were collected for three days during the months of March and April

2016. A total of 35 biconical traps (8 on Small Chamaunga, 9 on Big Chamaunga, 6 on Manga and 12 on Rusinga) were set in *G. f. fuscipes* suitable habitat within a meter from the lake shore at minimum and maximum distances of about 50 and 4000 meters apart respectively on the four islands. Flies collected were sorted and thereafter sexed according to the island they were collected on. All fly wings collected before and after intervention that were intact and had the 8 landmarks of interest (Figure 2.2) for morphometric measurements were selected. The wings were mounted between microscope glass slides. To avoid asymmetry bias [42] only one side of the pair of wings was mounted.
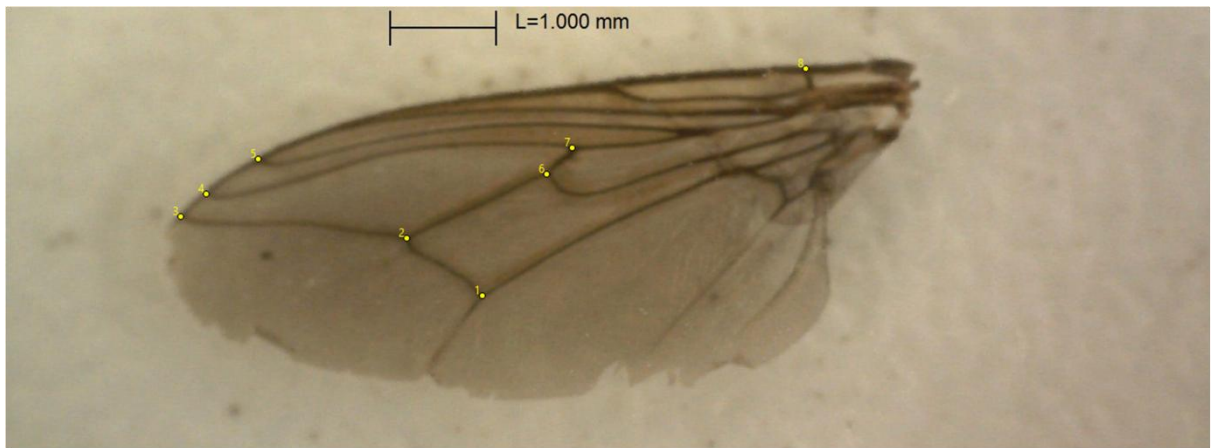


Figure 2.2: Eight landmarks and order of their collection from male and female right wings.

**Wing morphometric measurements**

A total of 1,986 right wings for both male and female flies (Table 2.1) mounted on glass slides from Big  Chamaunga, Manga, Rusinga  and Small Chamaunga  Islands were photographed using a Dino-Lite digital microscope  (AnMo Electronics Corporation, Taiwan) at a magnification of  ×34, image size of 1,280 × 1,024 pixels and 96 dots per inch.  Scaling of the image in pixels to millimetres was done and thereafter eight land marks defined as junctions of wing veins were collected using

the COO module of Collection of landmarks for identification and characterisation
(CLIC) software [43] ( Figure 2.2). To avoid individual bias, all measurements were
taken by the same person. The data was then formatted in TET module and the wing
shape (partial warps, PW) and size (centroid size, CS) [43] variables were generated
in MOG module of CLIC software [19].

Table 2.1: Number of *G. f. fuscipes* wings used from island and time of collection

| Island | Before intervention | | After intervention | |
|---|---|---|---|---|
| | No. Female | No. Males | No. Female | No. Males |
| Big Chamaunga | 89 | N/A | 162 | 226 |
| Manga | N/A | N/A | 126 | 151 |
| Rusinga | N/A | N/A | 317 | 276 |
| Small Chamaunga | 92 | N/A | 291 | 256 |

N/A represents not available

**Statistical analyses**

Daily tsetse catches (n) from biconical traps were normalised using a $\log_{10}(n+1)$
transformation and detransformed apparent densities were reported. A negative
binomial regression was performed to measure any associations between fly catches
and the status of human habitation on the Islands. A test for association between sex
of flies with Islands where they were caught from and human habitation status were
performed using Fisher's exact test. Differences between the overall proportion of
male and female flies were tested using a Student's t-test. Analysis of variance

(ANOVA) and Bonferroni tests were used for multiple comparisons of mean CS of groups (according to the islands) for each sex. A multiple linear regression was used to model CS for females collected on Big and Small Chamaunga with tsetse control status as an explanatory variable while controlling for the island and time of collection of flies (either before or after control). There were a total of 12 PW as shape variables and the principal components of these (relative warps, RW) were used as input for discriminant analysis of the groups of flies from the four islands. A cross validation procedure was undertaken to determine the success of discriminant analysis in assigning specimen to groups whereby each individual after being omitted from the initial calculation of the discriminant factors and introduced as supplementary data. CS variation was regressed against the first two discriminant functions to estimate its contribution to their variation [23]. The residue allometry was approximated by a multivariate regression with CS and PW as the independent and dependent variables respectively. For this, statistical significance was estimated by 1,000 permutations [23,44]. Procrustes distance matrix was used to build a neighbour joining tree in order to illustrate divergence of wing shape among the group of flies from the islands. Statistical software used for analyses were R [45], PAD and COV modules of CLIC [43]. *P* values of $< 0.05$ were considered statistically significant.

**Results**

**Fly densities and sex structure after intervention**

A total of 3,367 flies were caught of which 1,599 were males and 1,768 females from 35 trapping sites. The overall apparent fly densities (number of flies/trap/day)

on the islands were as follows: Big Chamaunga 9.2 (95%CI:8.4-9.9); Manga 22.7 (95%CI: 22.1-23.3); Rusinga 25.6 (95%CI:25.3-25.8) and Small Chamaunga 24.8 (95%CI: 24.3-25.2).The highest apparent fly density for females (14.0; 95%CI: 13.7-14.2) was recorded on Rusinga and that for males (12.7; 95%CI: 12.3-13.1) on Small Chamaunga. Big Chamaunga recorded the lowest for both sexes (Figure 2.3). The total apparent fly density on Big Chamaunga was significantly different from those of the flies on Small Chamaunga, Manga and Rusinga islands (ANOVA, df104, F=5.94, P<0.001). There was no significant difference in fly catches between human un-habited and human inhabited islands (Catch index=1.1; 95%CI: 0.8 -1.5; *P*>0.05).
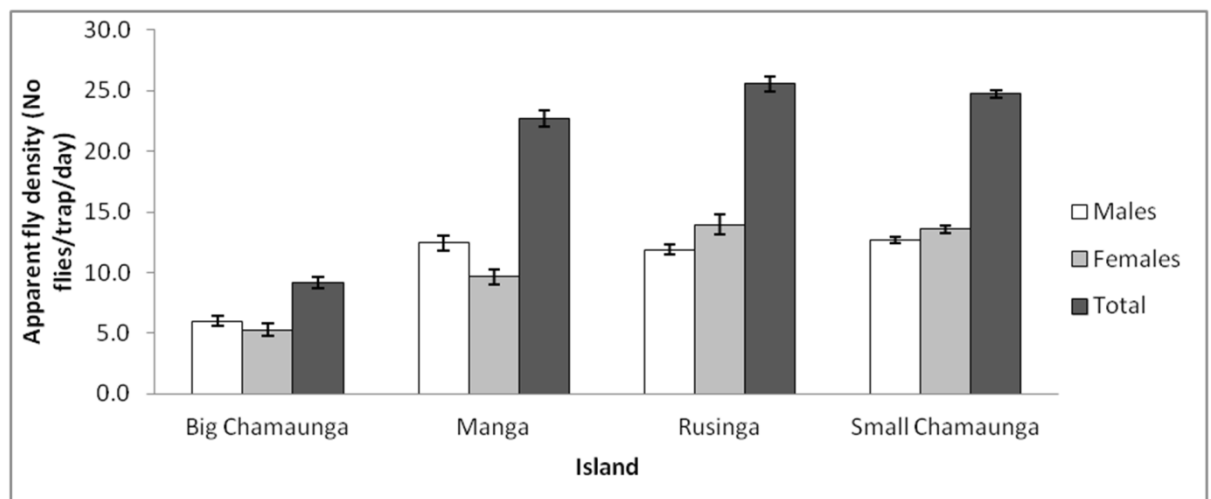


Figure 2.3: Detransformed apparent *G. f. fuscipes* density on Big Chamaunga, Manga, Rusinga and Small Chamaunga Islands.

The overall proportion of females (52.5%; 95%CI: 50.8-54.2%) was significantly higher than those of males (47.5%; 95%CI: 45.8-49.2%) with *P*< 0.01. Higher male catches were only observed on Big Chamaunga Island (Table 2.2). A test of association using Fisher's exact test showed a statistically significant association between sex of the flies and the islands (*P* < 0.05). However, when sex and human

habitation status were tested using Fisher's exact test, no significant associations were observed ($P>0.05$).

Table 2.2: Proportion by sex of *G. f. fuscipes* and its association with island

| Island | n | Number of males (%;95%CI) | Number of females (%;95%CI) |
|---|---|---|---|
| Small Chamaunga | 882 | 402 (45.6; 42.3-48.9) | 480 (54.4; (51.1-57.7) |
| Big Chamaunga | 615 | 324 (52.7; 48.7-56.6) | 291 (47.3; 43.4-51.3) |
| Manga | 530 | 261 (49.2; 45.0-53.5) | 269 (50.8; 46.5-55.0) |
| Rusinga | 1340 | 612 (45.7; 43.0-48.3) | 728 (54.3; 51.7-57.0) |
| Total | N= 3367 | 1599 (47.5; 45.8-49.2) | 1768 (52.5; 50.8-54.2) |
| P-value | 0.016 | | |

CI: Confidence interval

**Wing morphometrics before and after intervention**

Male tsetse wings from Big and Small Chamaunga for the period before tsetse control intervention were not available for comparison with those collected during the period after intervention because only female wings for that period were preserved. Before tsetse control intervention, there was no significant difference ($P>0.003$ after Bonferroni correction) between mean CS of female tsetse flies collected from Big and Small Chamaunga (Figure 2.4; Table 2.3). However, 3 years after tsetse fly control intervention on Big Chamaunga, the mean CS for female flies significantly differed between Big and Small Chamaunga with $P<0.003$ after

Bonferroni correction. CS data for female flies caught on Big and Small Chamaunga Island before and after tsetse control were combined and subjected to a multiple linear regression. The results showed that tsetse control intervention significantly lowered CS of females by an average of 0.07mm (95% CI: 0.02 – 0.12mm; $P<0.01$) while accounting for island and time of collection (either before or after intervention) of the flies.
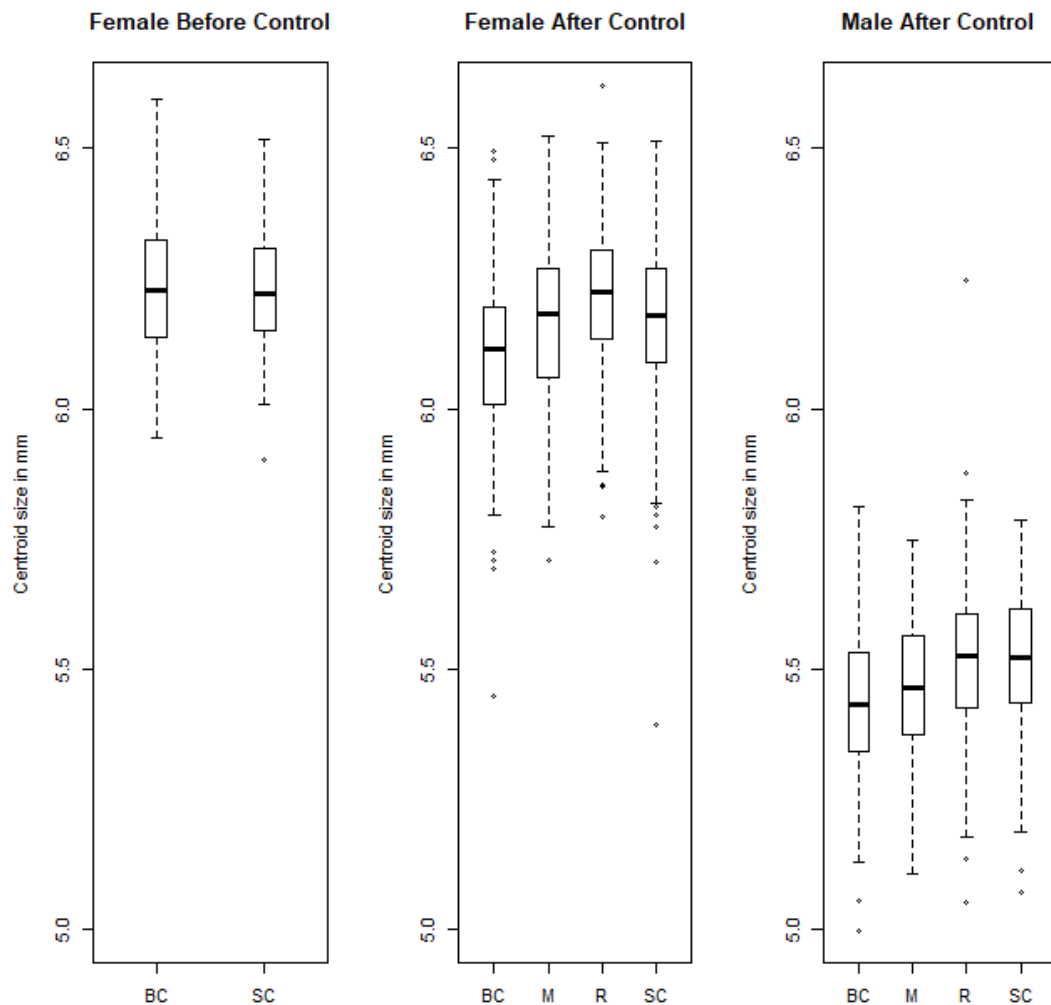


Figure 2.4: Wing centroid size distribution of *G. f. fuscipes* by location. BC, SC, M and R, stand for Big Chamaunga, Small Chamaunga, Manga, and Rusinga islands respectively. The boxes indicate the 25th and 75th percentiles; the solid line in the box shows the median while the capped bars are the 10th and 90th percentiles; data points outside these limits are shown as circles.

Table 2.3: Wing size comparison between groups of male and female *G. f. fuscipes* from Small Chamaunga, Big Chamaunga, Manga and Rusinga Islands

| Group 1 | Group 2 | Absolute difference in mean CS between groups (P-value) | |
| --- | --- | --- | --- |
| | | Female | Male |
| BC | M | 0.06 (**0.000**) | 0.03(0.02) |
| BC | R | 0.12 (**0.000**) | 0.08 (**0.000**) |
| BC | SC | 0.07 (**0.000**) | 0.08(**0.000**) |
| BC | BCB | 0.13 (**0.000**) | N/A |
| BC | SCB | 0.13 (**0.000**) | N/A |
| M | R | 0.06 (**0.000**) | 0.05 (**0.001**) |
| M | SC | 0.01 (0.728) | 0.05 (**0.000**) |
| M | BCB | 0.07 (**0.000**) | N/A |
| M | SCB | 0.06 (**0.002**) | N/A |
| R | SC | 0.05 (**0.000**) | 0.00 (0.849) |
| R | BCB | 0.02 (0.305) | N/A |
| R | SCB | 0.01 (0.538) | N/A |
| SC | BCB | 0.07 (**0.000**) | N/A |
| SC | SCB | 0.06 (**0.000**) | N/A |
| BCB | SCB | 0.01 (0.728) | N/A |

CS denotes centroid size. All *P*-values <0.003 and <0.008 for females and males respectively are significant (in bold) after Bonferroni correction. N/A= Not applicable, BC=Big Chamaunga, M= Manga, R=Rusinga, SC=Small Chamaunga, BCB= Big Chamaunga before intervention, SCB= Small Chamaunga before intervention.

As an explanatory variable, time of collection whether before or after intervention

significantly affected CS with female flies collected after intervention having CS

which were smaller by 0.06mm (95%CI: 0.02 –0.09mm; $P<0.001$). Island of collection (Big or Small Chamaunga) did not have any significant effect on CS ($P>0.05$).

With regard to the four islands the CS of female and male flies caught after tsetse control intervention (in March to April 2016)significantly varied according to the island (ANOVA, $df_{905}$, F=18.96, $P<0.001$ and $df_{892}$, F=23.7, $P<0.001$ respectively). Males were smaller than females (Figure 2. 4). The mean CS for males from Big Chamaunga and Manga were significantly smaller than those from Small Chamaunga and Rusinga ($P<0.008$ after Bonferroni correction (Table 2.3) while that for females from Big Chamaunga were significantly smaller than those from Manga, Rusinga and Small Chamaunga ($P<0.003$ after Bonferroni correction ;Table 2.3). The CS of female flies collected on Rusinga island and those collected from Big and Small Chamaunga before control were not significantly different ($P>0.003$ after Bonferroni correction).

Discrimination among groups (according to island) in the morphospace defined by the first two discriminant functions derived from the shape variables were projected without evidence of separation irrespective of sex and whether the flies where collected before or after tsetse control intervention (Figure 2.5). CS for female flies contributed 1.3% and 6.3% to the variation of the first and second discriminant factors while for males it contributed 6.2% and 0.1% to the first and second discriminant factors. The residue allometry which was estimated by a multivariate regression of PW on CS was significant for both females and males ($P<0.001$). Discriminant analysis based on Mahalanobis distance resulted in assignment of *G. f. fuscipes* ranging from 28% to 42% for females and 32% to 52% for males (Table 2. 4).
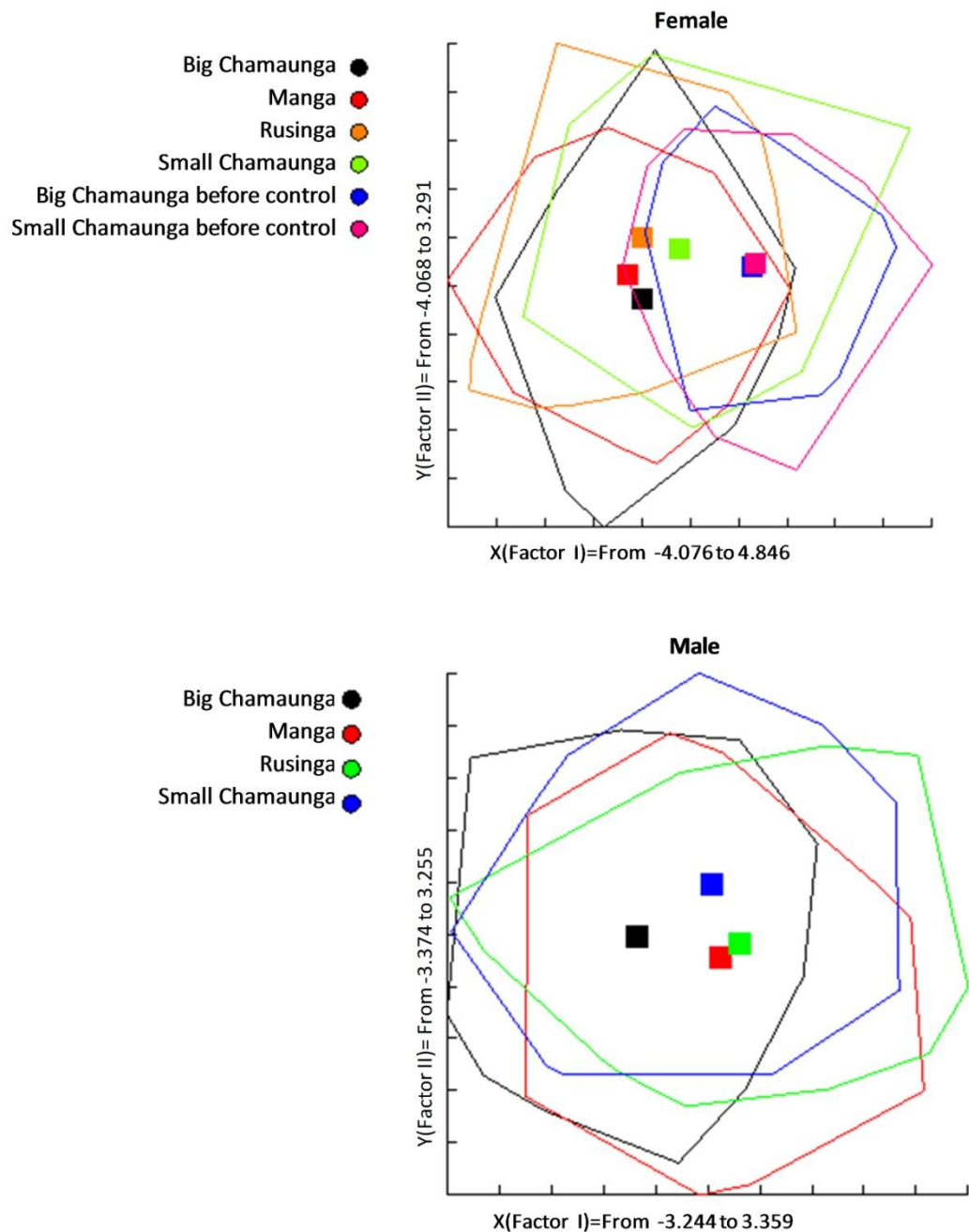
Figure 2.5: Plots of *G. f. fuscipes* wing shape for females and males in the morphospace.  The X-axis is first discriminant factor and the Y-axis is the second discriminant factor. Both discriminant factors account for 93% and 94% of variation for females and males respectively.

Neighbour joining tree derived from Procrustes distances analysis produced two

clusters representing female populations collected before and after tsetse control

intervention on Big Chamaunga (Figure 2.6). The wing morphology of female

populations collected from Big and Small Chamaunga Islands before the tsetse

control intervention were less divergent than after tsetse suppression on Big

Chamaunga (Figure 2.6).
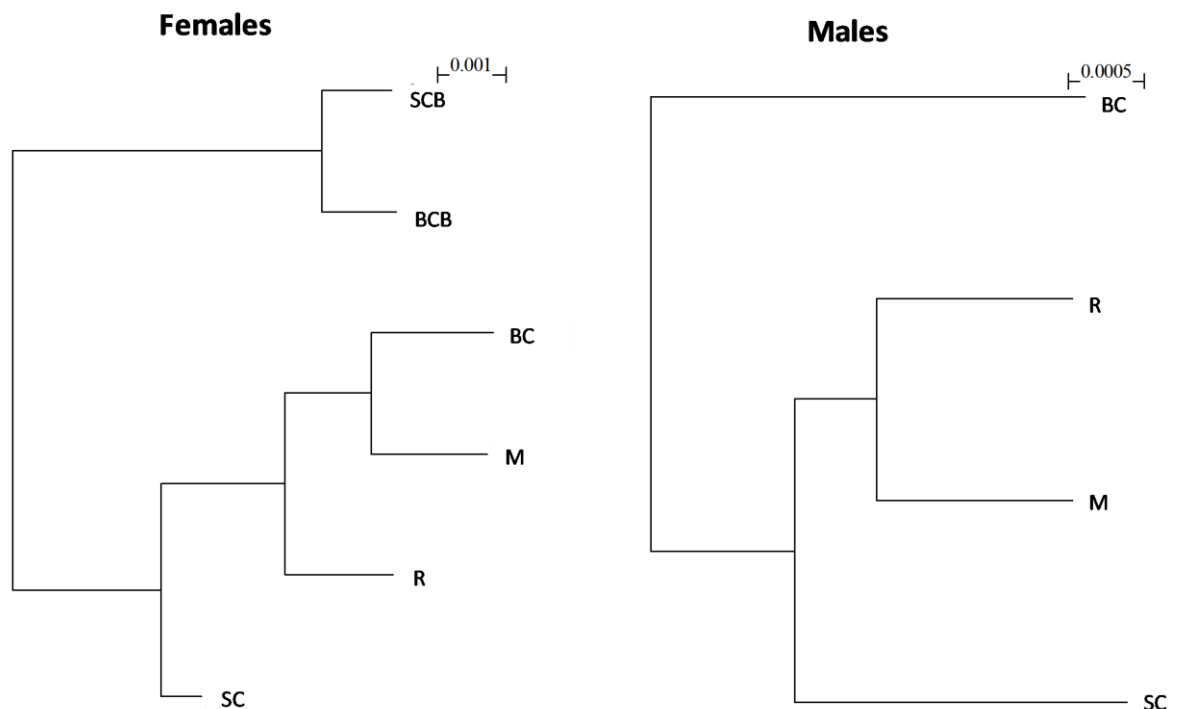


Figure 2.6: Phenetic trees of female and male *G. f. fuscipes* derived from Procrustes distances. BC=Big Chamaunga, M= Manga, R=Rusinga, SC=Small Chamaunga, BCB= Big Chamaunga before intervention, SCB= Small Chamaunga before intervention.

The female population from Manga, another island where tsetse control intervention

was undertaken were grouped with those from Big Chamaunga (Figure 2.6). The

male population collected on Big Chamaunga was the most distant from those

collected on Small Chamaunga, Rusinga and Manga Islands (Figure 2.6).

**Discussion**

This study clearly demonstrated the absence of separation of *G. f. fuscipes* groups on

the four islands as evidenced from the morphospace of the first two discriminant

factors which was supported by the observed reclassification rates. It also

demonstrated the divergence of wing shape, an indication of population structuring

of *G. f. fuscipes* on the islands, as seen from the phenetic tree derived from Procrustes distances. CS derived from wing measurement can be used as an estimate for adult insect body size [18,19]. In tsetse, fly size is among the factors associated with displacement rates, with larger flies having a higher displacement potential than smaller flies [27]. Displacement rates affect performance of targets [27]. In the recent past, preliminary trials to determine the number of tiny targets required to reduce the population of *G. f. fuscipes* by more than 90% were undertaken on Big Chamaunga (2011 to 2012) and Manga (2012 to 2013) [12]. During these trials, tiny targets deployed at 20/km and 10/km reduced the apparent fly densities from 3.9 and 28.2 flies/trap/day to less than 0.1 and 1 flies/trap/day on Big Chamaunga and Manga respectively [12]. When mean fly size of the available females obtained prior to tsetse control intervention were compared, there was no significant difference in size between those from Small and Big Chamaunga. Three years after tsetse control intervention on Big Chamaunga, the flies that recovered had a significantly smaller mean size than those from Small Chamaunga. This suggests that vector control could have had an influence on the observed smaller size of tsetse that recovered after control intervention. Furthermore, the observed lack of significant difference in size of females from Rusinga Island with those from Big and Small Chamaunga before the suppression of flies seems to supports this. It is possible that with a higher density of tiny targets more of the larger tsetse whose mobility and displacement potential is higher [27,31] and had a higher chance of encountering the killing devices were eliminated more than the smaller flies. Killing the larger flies could have exerted an increased selection pressure for smaller flies paving way for a new generation of smaller flies once tsetse control intervention was ceased. This could have led to the observed smaller size in tsetse that recovered and were caught on Big

Chamaunga compared to Small Chamaunga and the other island. Apart from density dependant factors, the lower mobility and displacement rate of smaller flies [27,31] and their reduced chance of encountering targets (thereby increasing their chance of survival) could be among the factors that explain why the use of targets alone as a tsetse control method has rare reports of successful elimination of tsetse populations [1,46]. Probably the use of targets only could achieve more successes in elimination by incorporating strategies in the tsetse control approach that also aim at killing the smaller flies that do not encounter targets. However, despite previous control on Manga Island the size of female flies caught were not significantly different with those from Small Chamaunga Island. The probable explanation could be that a target density of 10/km on Manga did not exert a strong pressure enough for selection of smaller female flies compared to a density of 20/km on Big Chamaunga. Even though insect size is a reversible character and can vary due to but not limited to environmental factors, population density and diet, it has often shown high heritability estimates [19,32]. Some studies have shown that insect size can be experimentally selected for to produce subpopulations that are genetically distinct [33,34]. This can take place through a process referred to as "genetic assimilation" whereby a phenotypic trait initially expressed as a response to some environmental factor is taken over by the genotype through selection such that it is found even when the environmental factor is absent [47]. Through the concept of "genetic assimilation" it is asserted that phenotypic plasticity could acquire evolutionary significance [19]. Thus [19] cautions against excluding the possible trans-generational effects of size. Wing shape shows strong genetic determinism and is a good indicator of population structure of insects [18,23,43]. The observed increase in divergence of wing shape (Figure 2.6) between female *G. f. fuscipes* population

collected on Big Chamaunga and those from Small Chamaunga before and after tsetse control intervention on the former supports our assertion that vector control could lead to population structuring. Further, the phenetic tree differentiated into two clusters clearly separating female populations collected before and after the tsetse control intervention, could be an indication of the influence of environmental elements on the population structure over time. The phenetic tree for male *G. f. fuscipes* clearly separated the population collected on Big Chamaunga from the other three islands. However, due to the unavailability of male samples for the period before tsetse suppression intervention, it is not possible to ascertain whether vector control and environmental elements could have the same effect as it has on wing shape of the female population.

When determining the effect of tsetse suppression on size using females from Big and Small Chamaunga, our results could have been biased by the effect of environmental elements over time (before and after control) on the size. However, this confound was addressed during statistical analysis by accounting for it in the multiple linear regression. The multiple linear regression showed an increased size in the absence of tsetse control using tiny targets. Further, with only female wing samples available for the period before control using tiny targets, it is possible that our results for males could be different from those of females. Nevertheless the observed size for both female and male collected on Big Chamaunga after control were significantly smaller compared to Small Chamaunga and Rusinga islands where no tsetse suppression intervention was undertaken. This could be an indication that the factors that influence size in both female and male tsetse could be the same. However, further investigations are needed to ascertain this.

In other dipteran vectors, size has been associated with fecundity, longevity and blood volume intake, all factors that affect epidemiology of vector borne diseases [48–51]. With the observed intra-population variation of tsetse fly size in our study, further studies are needed to investigate the fecundity, longevity and nutritional status in the area. Furthermore, there is also need to investigate how fly size could influence the epidemiology of African trypanosomiasis.

Our results indicate that, the tsetse populations on Big Chamaunga and Manga islands have recovered from the previously reported apparent densities of less than 0.1 and 1 fly/trap/ day after their suppression during trials using tiny targets to control *G. f. fuscipes* (2011 - 2012 and 2012 - 2013 respectively) [12] to 9.2 and 22.7 flies/trap/day respectively. Recovery could be due to suppressed population growing back to pre-suppression levels or re-invasion from neighbouring areas [46]. The significantly smaller size of both females and males collected three years after tsetse control intervention on Big Chamaunga suggests that recovery in this case could have been mainly due to the suppressed population growing back. The apparent fly density on Big Chamaunga was significantly lower compared to the other islands. This could be explained by the higher level of suppression of densities to <0.1 flies/ trap day on Big Chamaunga compared to <1 fly/trap/day on Manga [12]. Even so, the rate of recovery on Big Chamaunga was much higher (approximately 91.2 times) compared to that of Manga (approximately 22.7 times). Further as observed elsewhere [46], the recovery in density of *G. f. fuscipes* even after their suppression by over 90% also brings us to the realisation that as long as flies are not completely eliminated we should be wary of the constant threat of fly populations recovering either due to re-invasion from neighbouring and/or growing back in density to pre-suppression levels in previously control areas. Additionally, the apparent fly

densities on the other islands were 2.4 to 2.8 fold higher than that of Big Chamaunga.

The lack of significant association between *G. f. fuscipes* catches and sex with human habitation status seems to support the observation by Van den Bossche et al (2010) that palpalis group tsetse species are able to tolerate high degree of disturbance in their ecological niche [13]. The adaptive capacity of palpalis group tsetse species has been attributed to their capability to utilise microclimatic niches and ability to feed on hosts they encounter first [1,13,53]. Although both sexes of tsetse emerge from pupae approximately in equal numbers, females live longer than males in their habitat [54]. As a result, field population of flies comprise of more females than males. In addition, the catching methods and physiological state of tsetse are also known to influence the proportion of females in field catches [1]. For example were as fly rounds catch more males than females, traps tend to catch more females than males [1].These among others could be among the reasons for the observed significantly higher proportion of female flies than males in our study.

**Conclusion**

The study showed that no separation of populations of *G. f. fuscipes* from Big Chamaunga, Small Chamaunga, Manga and Rusinga Islands was evident based on wing shape; vector control could induce the diminishing of fly size and divergence of wing morphology in tsetse that recover. Therefore an investigation to understand how this happens is recommended as it may guide future tsetse control strategies. Additionally we recommend further studies on the effect of fly size on the vectorial capacity of tsetse as it could give more insights into the epidemiology of African trypanosomiasis in previous intervention areas where recovery of populations has

occurred. Furthermore, given the recovery of tsetse population densities on islands where their densities were previously suppressed (Big Chamaunga and Manga) we recommend sustained area wide tsetse control interventions and those that target isolated populations to prevent population recovery. We further emphasise on the need to undertake population structure studies as part of baseline for both trials and full scale vector control interventions as they may be a reference to assist in determining whether population recovery in previous intervention areas are due to re-invasion from neighbouring areas or the population growing back from suppressed to pre-suppression levels. Additionally we recommend land use planning and utilisation through integration of crop and livestock farming for increased agricultural production in cleared areas after tsetse fly control. This could contribute towards recovering from the lost agricultural potential due to AAT and alleviating food insecurity, hunger and poverty in Africa.

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# CHAPTER 3    Sticky small target: an effective sampling tool for tsetse fly *Glossina fuscipes fuscipes* Newstead, 1910

**Abstract**

Background: Small targets comprising panels of blue and insecticide treated black netting material each 0.25m $\times$ 0.25m have been shown to attract and kill *Glossina fuscipes fuscipes* Newstead 1910 (Diptera: Glossinidae) thereby reducing its population density by over 90% in field trials. However their attractive ability has not been fully exploited for sampling purposes. Therefore in this study we assessed the effectiveness of using sticky small targets as sampling tools for *G. f. fuscipes* in western Kenya. We also determined the influence of colour on the landing response of female and male flies on sticky small targets.

Methods: Using a series of randomised block experiments, the numbers of tsetse flies caught with sticky small targets were compared with those caught with biconical traps. A negative binomial regression was used to model fly catches. Odds ratios as measures of association between the landing response on the blue or black panel of the sticky small target and the sex of flies were obtained from a multiple logistic regression.

Results: The results showed that sticky small targets caught 13.5 and 3.6 times more female and male tsetse flies than biconical traps ($Z$=9.551, $P$<0.0001 and $Z$=5.978, $P$<0.0001 respectively). Females had a 1.7 times likelihood of landing on the black panel than males ($Z$=2.25, $P$=0.025).

Conclusion: This study suggests that sticky small targets are an effective sampling tool for *G. f. fuscipes.* Therefore, we recommend the use of sticky small targets to complement other devices used for sampling *G. f. fuscipes* in observational and experimental investigations.

Key words: Riverine tsetse flies; Small targets; Sampling; Behaviour; Density; Surveillance

**Background**

Human African trypanosomiasis (HAT), also referred to as sleeping sickness is transmitted by tsetse flies harbouring the mature protozoan parasites, *Trypanosoma brucei rhodesiense* and *T. brucei gambiense*, as they take a human blood meal causing Rhodesian and Gambian HAT respectively [1,2]. Tsetse flies from the palpalis group which occupy riverine habitats have been implicated in the transmission of over 90% of HAT cases caused by *T. brucei gambiense* in central and west Africa [3,4]. So far, there is no vaccine and the main intervention for Gambian HAT (gHAT) is the use of case detection and treatment programmes [4]. The method aims to clear the parasite in a substantial proportion of the human population so that even when bitten, the vector will not pick up any trypanosomes for further transmission [1]. However, infected flies can still transmit new gHAT infections to humans [4]. Further, the chronic nature of gHAT allows for silent carriers harbouring low parasite levels undetectable by conventional diagnostic methods to sustain transmission foci [5–7]. Thus, interventions that target the vector further reduce the risk of new infections from occurring [4,8]. For tsetse fly control, targets are simple, cheaper and easier to maintain than traps [9]. Insecticide treated targets usually greater or equal to $1.0 \times 1.0$ m in size are effective control tools for the savannah tsetse fly, *G. pallidipes* Austen and *G. morsitans morsitans* Westwood [10]. Additionally, behavioural studies that aimed at developing cost effective targets for the main vectors of gHAT, *G. fuscipes* subspecies and other riverine species, show that even after reducing the target size 16 times, the alighting response was about 40-55% on the small target, which is comparable to large targets [9]. Further studies found that a small target with a black cloth panel and netting material

each 0.25×0.25 m in dimensions caught more than twice the number of flies than the biconical trap, and replacing the black with a blue cloth panel having the same dimensions slightly increased the catches [9]. The small targets are highly efficient in reducing tsetse fly densities (by over 90%) and easier and cheaper to deploy than large targets [4,9,11].   Apart from being exploited for killing the tsetse fly, small targets could also be exploited for sampling purposes of *G. f. fuscipes* and other riverine species.

Some ecological aspects critical for control of tsetse flies, especially riverine species, include fly movement and spatial occupation within their restricted  habitat and can only be studied with adequate sampling techniques [8]. Tsetse fly sampling during studies in the form of observations and experiments are important for planning and monitoring tsetse fly control interventions [8]. In the last four decades, traps that attract and guide tsetse flies into a non-return cage have been developed to serve as sampling or control tools [12]. Although traps have biases and interpretation of catches should be done taking into account these biases; the higher proportion of females to males is close to the natural sex ratio of the tsetse fly population [8,13,14].

Sticky panel traps have been mainly used to hold tsetse flies that are killed or stunned by other sampling tools, such as electric screens [13]. They have also been used to sample *G. austeni* a tsetse fly species from the morsitans group (as was the case of the eradication of *G. austeni* in Zanzibar with sterile insect technique, SIT) [13,15,16]. Further, some studies have used traps and targets covered with sticky material to assess the efficacy of these as landing devices [17,18].

The ability of a sampling tool to catch tsetse flies particularly at low density is important as it could give information on residual populations and lead to appropriate action for planning and monitoring control interventions. A previous study showed that the small targets comprising of black and netting panels (each 0.25×0.25m) caught over twice more *G. f. fuscipes* than the biconical trap [9]. Therefore, in this study we applied a sticky film to the small target having a black and blue panel without any nettings, and compared its performance as a sampling tool to the biconical trap which is commonly used for sampling *G. f. fuscipes* and other riverine tsetse flies. We also assessed the influence of colour on the landing response of male and female *G. f. fuscipes* on the sticky small target.

**Materials and methods**

**Study area**

The study was undertaken in the month of February 2017 on Big and Small Chamaunga Islands on Lake Victoria in western Kenya (Figure 3.1). On these islands, *G. f. fuscipes* is the only tsetse fly species found and mainly inhabits the area along the lake shore [4,9,19,20]. Study site selection was based on the fact that *G .f. fuscipes* populations on these islands were well documented and high numbers of flies are present for meaningful experimentation [4, **Chapter 2**]. These islands are not inhabited by humans and vegetation on the lake shore consists of a mixture of fresh water mangroves (*Aeschynomene eraphyroxylon*), tropical hydrangea (*Dombeya spp.*) and tickberry (*Lantana camara*). Monitor lizards (*Varanus niloticus*) are the main hosts for tsetse fly populations in the area [4].
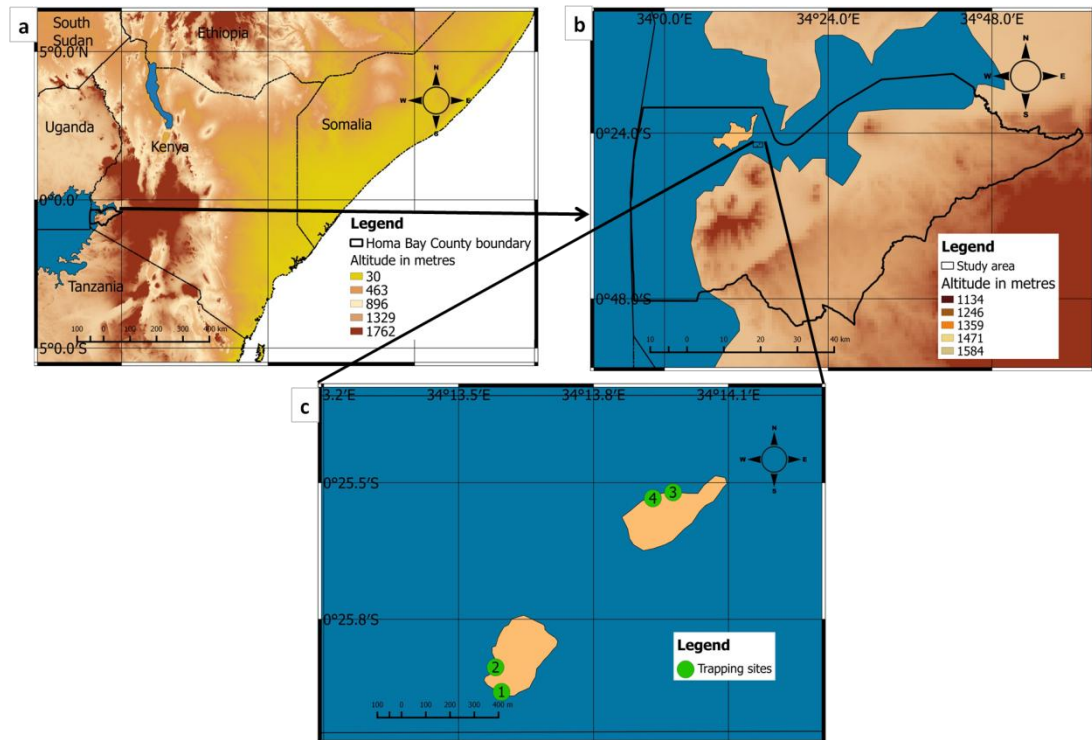
Figure 3.1: (a) Study area in western Kenya. (b) Location of study area in Homa bay County. (c) Trapping sites on Big and Small Chamaunga Islands

**Sampling tools**

Tsetse flies were caught using biconical traps [21] and sticky small targets [9,22]. The small target comprised of blue and black panels made from cotton cloth each $0.25 \times 0.25$ m in size and thus making it $0.25 \times 0.50$ m in dimension. A board $0.25 \times 0.50$ m in dimension of plywood was placed in between two small targets and fastened using staples. The board with the fastened targets was then covered with a transparent sticky film (Luminos 4 adhesive rolls-ungridded: Rentokil Initial supplies, Liverpool, UK) to make sticky small targets (Figure 3.2). The sticky material on the target was not changed over the experimental period. Both sampling tools were not baited with any odour. The biconical traps had a radius of 0.40m at its widest point, and a height of 1.30m.
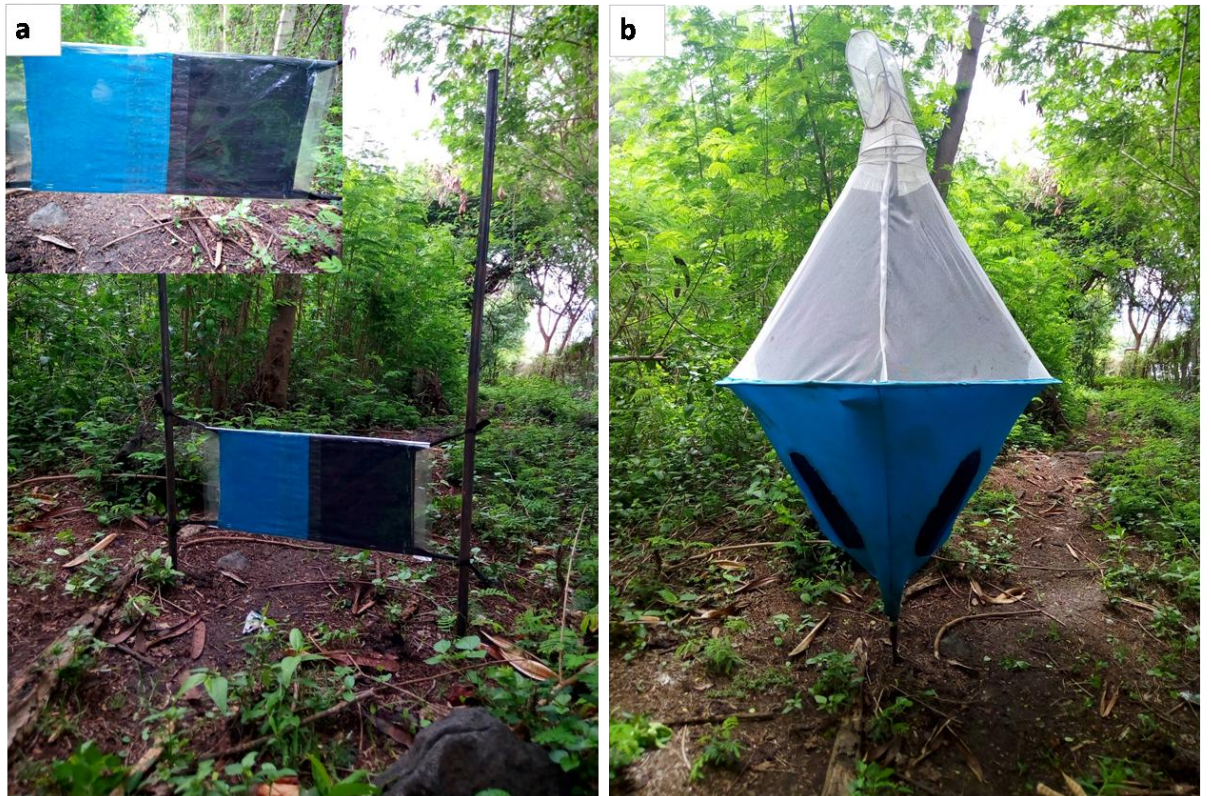
Figure 3.2: Sampling tools. (a) Sticky small target and (b) Biconical trap

**Study design, sample collection and analyses**

Field experiments with the traps and sticky small targets were undertaken for 4 h between 08:00 and 12:00 h local time when *G. f. fuscipes* are most active [3,20], after which the flies collected in the non-return cage of the biconical trap and those on the sticky small target were sexed and counted. On the sticky small target, flies that landed on the blue or black panels were carefully removed using forceps and placed in separate storage containers colour coded according to the part of the target that they landed on. The experiments were conducted over a period of eight days at four sites. Sites one and two were on Small Chamaunga and sites three and four on Big Chamaunga (Figure 3.1c). On the first and second day, experiments were carried out at two sites and from the third to eighth day, the experiments were undertaken at all four sites.

The overall tsetse fly catches from biconical traps and the sticky small targets were compared using 14 replicates of randomised block design experiments, each comprising of two adjacent days at a site as different blocks [23]. The sampling tools as treatments were randomly allocated to the days within the blocks and sites were at least more than 100m apart [3].  A nested survey in the randomised block design was used to collect data on the landing response of female and male *G. f. fuscipes* on the blue or black panel of the sticky small target according to the method developed by Vale [24].

All statistical analyses were carried out using R version 3.2.5 [25]. Tsetse fly catches from the sampling tools were modeled using a negative binomial regression to determine the catch index of the sticky small target with the biconical trap as a reference while taking into account the block and day of study. The effect display (for detransformed means) of treatments in the negative binomial model was obtained using the '*effects*' package in R [26]. Associations were tested using Fisher's exact test. A *Z*-test was used to test for difference in proportions. Fly catches on the sticky small targets were further analysed using a multiple logistic regression to measure the association between the sex ratios of flies that landed on the blue or black panels while accounting for trap site and the day of the study. All estimates reported are accompanied by 95% confidence intervals and *P*-values less than 0.05 were considered statistically significant. All maps were created using QGIS version 2.10.1-Pisa.

**Results**

**Characteristics of fly catches**

A total of 441 flies were collected, 58 (13%; 95%CI: 10-16%) were caught in biconical traps while 383 (87%; 95%CI: 84-90%) were caught by the sticky small targets. The overall catches for the females and males were 290 (66%; 95%CI: 61-70%) and 151 (34%; 95%CI: 30-39%) respectively. The catches for the females in the biconical traps were 24 (41%; 95%CI: 28-54%) and those for the males were 34 (59%; 95% CI: 45-72%). The flies caught on the sticky small targets comprised of 266 (69%; 95%CI: 65-74%) females and 117 (31%; 95%CI: 26-35%) males. Overall, there was an association between fly sex and site of collection (Fisher's exact test: $P$<0.016, (OR: 0.46; CI: 0.25 – 0.83)). The total number of females caught at all four sites was higher than those of males (Table 3.1).The flies caught on Small and Big Chamaunga were 209 (47%; 95%CI: 42-52%) and 232 (53%; 95%CI: 48-57%) respectively and their proportions were not significantly different (Z-test, $Z$ =-1.55, $P$=0.121).

Table 3.1: Fly catches at the four sites according to sex of *G. f. fuscipes*.

| Island | Site no | Male | | Female | | Total | |
|--------|---------|------|------|--------|------|-------|------|
| | | n | % (CI) | n | % (CI) | n | % (CI) |
| Small | 1 | 42 | 30 ( 22-38) | 97 | 70 (62-78) | 139 | 32 (27-36) |
| Chamaunga | 2 | 34 | 49 (37-61) | 36 | 51 (39-63) | 70 | 16 (12-19) |
| Big | 3 | 35 | 39 (29-49) | 55 | 61 (51-71) | 90 | 20 (17-24) |
| Chamaunga | 4 | 40 | 28 (21-36) | 102 | 72 (64-79) | 142 | 32 (28-36) |
| | Total | 151 | 34 (30-39) | 290 | 66 (61-70) | 441 | 100 |

CI: confidence interval

**Comparison of sampling tools**

Overall, the sticky small targets caught more *G. f. fuscipes* than the biconical traps (Table 3.2). Detransformed means were 3.2 (95%CI: 2.3-4.4) for the biconical trap compared to 23.3 (95%CI: 20.6-26.4) for the sticky small target (Table 2). The negative binomial regression showed that the sticky small target significantly caught 7.2 (95%CI: 5.3-10.1) times more flies ($Z= 12.226$, $P<0.0001$) than the biconical trap after taking into account the variation due to the day of the experiment and the block. While according to sex,  the sticky small target significantly caught 13.5 and 3.6 times more females and males respectively compared to the biconical trap ($Z=9.551$, $P<0.0001$ and $Z=5.978$, $P<0.0001$ respectively).

**Landing preference**

Overall, from 383 flies that landed on the sticky small targets, 169 (44%; 95%CI: 39-49%) landed on the blue panel while 214 (56%; 95%CI: 51-61%) landed on the

black panel. Overall, 108 (41%, 95%CI: 35-47%) female flies landed on the blue

panel and while 158 (59%, 95%CI: 53-65%) landed on the black panel. For male

flies, 61 (52%, 95%CI: 43-61%) landed on the blue panel while 56 (48%, 95%CI:

39-57%) landed on the black panel. From all the flies that landed on the blue panel

64% (95%CI: 56 – 71%) were females and 36% (95%CI: 28 – 43%) were males. For

flies that landed on the black panel 73% (95%CI: 68 – 80%) were females while

26% (95%CI: 20 – 32%) were males. There was a statistically significant association

between proportion of flies that landed on the blue or black panels with sex of the fly

(Fisher's exact test: $P<0.044$, (OR: 1.59; 95%CI: 1.02-2.47)). A multiple logistic

regression showed that females had a 1.71 significant increase in the likelihood of

landing on the black compared to the blue panel of the sticky small target than males

and the increase could be as low as 1.07 and as high as 2.74 at 95% confidence

interval while holding the trap site and day of study constant ($Z=2.25$, $P<0.025$). The

trap site and day of study did not show any significant effect on the landing response

(Table 3.3).

Table 3.2: Means and indices of catches obtained from negative binomial model for female and male *G.f. fuscipes*

| Treatment | Females | | | Males | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Catch (CI) | Catch index (CI) | *P* -value | Catch (CI) | Catch index (CI) | *P* -value | Catch (CI) | Catch index (CI) | *P* -value |
| Biconical trap (control) | 1.1 (0.6-1.9) | 1 | | 1.9 (1.2-3.1) | 1 | | 3.2 (2.3-4.4) | 1 | |
| Sticky small target | 15.1 (12.8-17.8) | 13.5 (8.2-24.4) | <0.0001 | 6.9 (5.2-9.1) | 3.6 (2.4-5.6) | <0.0001 | 23.3 (20.6-26.4) | 7.2 (5.3-10.1) | <0.0001 |

CI, Confidence Interval;

Table 3.3: Association between proportions of *G. fuscipes fuscipes* that land on blue or black panel of sticky small target with sex, site and study day number

| Explanatory variable, total (N=383) | UOR (CI) | P-Value | AOR ( CI) | P-Value |
|---|---|---|---|---|
| Sex | | | | |
| Male | 1 | - | 1 | - |
| Female | 1.59 (1.02-2.47) | 0.04* | 1.71 (1.07-2.74) | 0.025* |
| Site | | | | |
| 1 | 1 | - | 1 | - |
| 2 | 0.47 (0.24-0.90) | 0.023* | 1.13 (0.36-3.58) | 0.838 |
| 3 | 1.02 (0.57-1.83) | 0.931 | 1.30 (0.53-3.20) | 0.570 |
| 4 | 1.11 (0.68-1.83) | 0.664 | 1.13 (0.63-2.01) | 0.679 |
| Study day | | | | |
| 1 | 1 | - | 1 | - |
| 2 | 0.33 (0.11-0.95) | 0.040* | 0.30 (0.08-1.15) | 0.079 |
| 3 | 0.92 (0.44-1.90) | 0.822 | 0.84 (0.37-1.92) | 0.684 |
| 4 | 1.13 (0.48-2.66) | 0.780 | 0.98 (0.39-2.43) | 0.964 |
| 5 | 1.85 (0.86-4.01) | 0.117 | 1.90 (0.82-4.41) | 0.135 |
| 6 | 0.53 (0.20-1.44) | 0.215 | 0.48 (0.16-1.41) | 0.182 |
| 7 | 0.97 (0.43-2.18) | 0.949 | 0.82 (0.34-1.98) | 0.653 |
| 8 | 1.23 (0.51-2.95) | 0.648 | 1 | - |

UOR, unadjusted odds ratio; AOR, adjusted odds ratio; CI, Confidence Interval; * Statistical significance at $P<0.05$

**Discussion**

The present study revealed that sticky small targets are more efficient sampling tools for *G. f. fuscipes* than the biconical traps which are the most commonly used sampling tools for the species. In another study, blue/black targets that were 1.0×1.5m in dimension covered with sticky film caught an average of about 4 to 6 times more *G. f. fuscipes* than biconical traps [27]. The results from our study are comparable with the sticky small targets of dimensions 0.25×0.50m catching 7 times more flies than the biconical traps suggesting that they could be an alternative and more effective sampling tool for *G. f. fuscipes.* Furthermore, the higher proportion of females compared to males caught on the sticky small target is more representative of the sex ratio in the population [13] which also makes this sampling tool more useful for ecological studies. Previous versions of a sticky trap for tsetse flies were comprised of a coloured metal, wooden or cloth screen coated with sticky substance [13]. These have not been popular with workers due to the difficulty in collecting and handling the flies and the poor condition of the flies in subsequent processing such as counting and dissections [13]. As opposed to the sticky substances coated on the screens of the earlier sticky traps, the transparent sticky film used in this study did not present any difficulties in collecting, handling and counting the flies. Additionally, all the flies collected on the sticky small target were easily distinguished by sex, an indication that the samples were in good condition. This is consistent with observations from other studies that used the transparent sticky film on targets to sample other tsetse fly species [22]. However, further studies are needed to assess whether samples collected from sticky small targets could be used for dissections and molecular biological studies. Additionally, with the current

knowledge, sticky small targets could do particularly well in circumstances requiring highly efficient sampling for *G. f. fuscipes* such as determining its distribution limits, mark-release-recapture experiments and monitoring of residual populations. All these are important in the planning and implementation of tsetse fly control interventions.

Studies on the cost effectiveness of small targets indicate that they use 1/24th of the material in the biconical trap and are much easier to deploy [9,28]; thus their use in control of *G. f. fuscipes* could substantially increase field cost-effectiveness by a factor of 10 [9,30]. In our study we recommend the use of the small targets covered with sticky material for sampling as they significantly caught more *G. f. fuscipes* than biconical traps. Although further cost effectiveness studies need to be undertaken, the additional costs of the sticky material and its advantages as seen in the current study to sample fly populations and carry out ecological studies could justify the slight increase in costs particularly when monitoring tsetse fly populations during control interventions.

In order to address variations, other than those due to the variables of interest, we used randomised block design experiments, where treatments were randomly assigned to experimental units in a block. This randomisation ensured that confounds were controlled for. Further, residual confounding was addressed during statistical analysis by accounting for the block and day the experiments were undertaken. Additionally, for assessment of the influence of the colour on the landing response of *G. f. fuscipes* according to sex, confounds were addressed during analysis by accounting for the site and day of the study in the multiple logistic regression.

Our results are also consistent with earlier studies which showed that a larger proportion of *G. f. fuscipes* land on the black panel of the target than the blue panel [27]. Similar landing behaviours have also been reported for species belonging to the morsitans group such as *G. m. morsitans* and *G. pallidipes* [24,30]. The observation that females show a preference to land on the black than on the blue panel supports earlier practices of impregnating the black panel of the target with insecticide to kill females [24]. As an alternative to insecticides, bio-control agents such as entomopathogenic fungi have been shown to be efficacious against *G. f. fuscipes* and can be horizontally transmitted from infected to non- infected tsetse flies [31,32].  As indicated in our results, the most likely point of first contact for male flies is the blue panel of the small target, thus it could be impregnated with a bio-control agent. In this case, the male flies having a preference to land on the blue than black panel of the small target could be contaminated and serve as carriers to deliver the bio-control agent to females during mating. With the male fly mating more than once in its lifetime; the bio-control agent would be transferred to each female it mates with [8]. Likewise, though to a lesser extent, unmated females that are contaminated with the agent could also transfer it to other males. The bio-control agent impregnated small targets can be used in an integrated manner either concurrently or sequentially with those treated with insecticide. However, studies to determine the feasibility and cost effectiveness of such integrated methods would be required. Notwithstanding, exploiting landing preference of male *G. f. fuscipes* on the blue panel in the manner suggested could provide an opportunity for more target based integrated riverine tsetse fly control strategies.

**Conclusion**

Sticky small targets significantly caught more flies than biconical traps suggesting that they are more efficient sampling tools for *G. f. fuscipes.* Our study also showed male *G. f. fuscipes* preferred to first land on the blue part of the small target; a behaviour that could be exploited for disseminating biological control agents in populations for tsetse control.

CHAPTER 3

## Funding

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# CHAPTER 4        Responses of *Glossina fuscipes fuscipes* to 4-methylguaiacol and specific compounds in waterbuck odour at stationary visual attractive devices

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**Abstract**

A blend of compounds (pentanoic acid, guaiacol, delta-octalactone and geranylacetone) identified in waterbuck (*Kobus defassa*) body odour referred to as waterbuck repellent compounds (WRC) and a synthetic repellent 4-methylguaiacol have previously been shown to repel tsetse from the morsitans group. However, these repellents have not been evaluated on riverine tsetse. In this study we evaluated the effect of these repellents on catches of *Glossina fuscipes fuscipes,* a palpalis group tsetse species, at stationary visual attractive traps. We also assessed the effect of removal of individual constituents of WRC on catches of *G. f. fuscipes.* Randomised block design experiments were conducted in western Kenya on some islands of Lake Victoria to compare catches of *G. f. fuscipes* in the absence/presence of the known repellents. The catches for each treatment were modeled using a negative binomial regression to determine their effect on trap catches. In the presence of 4-methylguaiacol and WRC (released at $\approx 1.4$mg/h and $\approx 2$mg/h respectively) catches of both sexes of *G. f. fuscipes* were significantly reduced by 22% ($P<0.001$) and 33% ($P<0.001$) respectively at biconical traps. Further through subtractive assays, only the removal of geranylacetone from WRC significantly increased the fly catches (by 1.8 times; $P <0.001$) compared to the total blend of WRC. We conclude that WRC and 4-methylguaiacol reduce fly catches of *G. f. fuscipes* attracted to stationary visual traps and suggest that they may be broad spectrum repellents for *Glossina* species. We recommend further studies to investigate the effects of these compounds on reduction of *G. f. fuscipes* attracted to human and animal hosts.

Key words: Tsetse fly; Repellent; Riverine tsetse; waterbuck repellent compounds.

**Author summary**

Tsetse flies are divided into 3 taxonomic groups: morsitans, palpalis and fusca. Flies from the *morsitans* and *palpalis* groups are the main vectors of trypanosomes that cause human and animal African trypanosomiasis. The chemical 4-methylguaiacol and compounds (pentanoic acid, guaiacol delta-octalactone and geranylacetone ) identified in waterbuck (*Kobus defassa*) body odour, a known non-preferred host of tsetse, have been shown to repel *morsitans* group species *G. pallidipes* and significantly reduce levels of animal African trypanosomiasis. However, these repellent compounds have not been evaluated against other groups of tsetse, for example those in the palpalis group. Here, we show that these compounds and some of their blends significantly repelled *G. f. fuscipes* one of the important vectors belonging to the palpalis group at visual attractive devices. This could be an indication of an evolutionary relationship of responses to the same chemical compounds by tsetse flies from different taxonomic groups and provides information for future studies that will evaluate the effect of these repellent compounds to reduce host-vector contact as a control method for human African trypanosomiasis (sleeping sickness) in its foci. It also justifies assessment of responses of palpalis group species to other compounds which are known to repel morsitans tsetse species.

**Background**

Tsetse flies (Diptera: Glossinidae) are exclusive blood feeders and biological vectors of African trypanosome, the parasites that cause human and animal African trypanosomiasis [1]. They find their vertebrate hosts through olfactory and visual cues [2]. Beyond its visual range, the fly is activated by the odour from the host and orients upwind following the odour plume until it comes near the host where visual cues of colour, shape and size may elicit a landing response [2–5].  It is during a blood meal from a host that an infected fly transmits the parasites that cause African trypanosomiasis [6]. However, not all vertebrates found in tsetse habitats are fed on [7,8].  The differential preference of vertebrate hosts has been attributed to particular or a combination of compounds found in the vertebrate's body odour which could either attract or repel tsetse [2,4,8–10].  Consequently, research on identification of repellents to break the host-tsetse fly contact as a method of control against African trypanosomiasis (AT) has been ongoing since the 1970s [11].  Pioneer work on repellents showed that, humans are poorly attractive to *Glossina pallidipes* and *G. morsitans morsitans* which are tsetse species that belong to the morsitans group [2]. Lactic acid in human odour was identified as the component responsible for this repellency [12]. Since then, a number of synthetic and naturally occurring repellent compounds have been identified [9–11,13–15]. Among these are; acetophenone and 4-methylguaiacol shown to reduce catches of *G. pallidipes* by 69% and 80% respectively [11,13], and delta-nanolactone which reduce *G. pallidipes* catches by 76% when used in attractant odour baited traps [15].

Furthermore some naturally occurring tsetse repellents found in the body odour of waterbuck (*Kobus defassa*) a non-preferred host were identified [7,8]. These

compounds were 15 in total and comprised of straight chain carboxylic acids ($C_5$-$C_{10}$), phenols (guaiacol and carvacrol), 2-alkanone homologues ($C_8$-$C_{12}$), geranylacetone and Delta octalactone [8,10]. A blend of all these compounds was found to significantly reduce catches of *G. pallidipes* at traps baited with odour attractants by 97% [10]. The blend of these compounds was reduced to a five blend component (consisting of pentanoic acid, hexanoic acid, guaiacol, delta-octalactone and geranylacetone,) [10] and then finally to a four blend component comprising of pentanoic acid, guaiacol, delta-octalactone and geranylacetone) referred to as waterbuck repellent compounds (WRC) which has been shown to reduce levels of animal African trypanosomiasis transmitted by *G. pallidipes* [16].

Even though much has been done on repellents for important tsetse species belonging to the morsitans group, important tsetse species that belong to the palpalis group which are also responsible for human and animal African trypanosomiasis transmission in central and western Africa have received less attention. The important tsetse species belonging to the *palpalis* group include *G. fuscipes* subspecies, *G. palpalis* subspecies and *G. tachinoides*. Palpalis group tsetse species account for over 95% of transmissions of all human African trypanosomiasis (HAT) cases [17,18]. Among these, *G. fuscipes* subspecies with *G. f. fuscipes* having the widest distribution are predicted to account for about 90% of transmissions in the HAT foci of west Africa [17–19]. Though they are opportunistic blood feeders, tsetse from the *palpalis* group have shown preferences to vertebrate hosts as observed from blood meal analysis [7,20]. For example, monitor lizards were consistently found to be the main hosts in Central African Republic, Kenya and Uganda, while ruminant hosts were fed on by less than a percentage of the flies [7].

With evidence that some compounds in non-preferred hosts' body odour are repellent to tsetse species belonging to the *morsitans* group, it is possible that these could also be repellent to *palpalis* group tsetse; more so that previous studies have shown a general conservation of chemosensory gene families across five tsetse species which include *G. austeni, G. brevipalpis, G. pallidipes, G. m. morsitans* and *G. f. fuscipes* [21–24]. Here, we evaluated the effect of WRC and 4-methylguaiacol released at different rates on catches of *G. f. fuscipes* at stationary traps. We also assessed the effect of removal of individual constituents of WRC on trap catches.

## Materials and Methods

### Study Area

The experiments were carried out on Small Chamaunga (latitude -0.431°, longitude 34.227°; surface area of about $0.2km^2$), Big Chamaunga (latitude -0.426°, longitude 34.227°; surface area of about $0.2km^2$), Manga (latitude -0.353°, longitude 34.253°; surface area of about $1km^2$) and Rusinga (latitude -0.358°, longitude 34.218°; surface area of about $43km^2$) islands of Lake Victoria in western Kenya [**Chapter two**,18,25] from April 2016 to October 2017. The islands exclusively harbor *G. f. fuscipes* and are extensively described by [18,25,26].

### Capture devices and test compounds

Tsetse catches were made by biconical traps [27] and sticky small targets [**Chapter three**]. All compounds were 98-99% pure and sourced from ChemSampCo, LLC. The compounds: pentanoic acid (PA), guaiacol (GU), delta-octalactone (DO) and geranylacetone (GE) were blended in similar proportions (3:2:3:1) as found in

waterbuck odour [8,10,16]. All individual compounds and blends of repellent compounds from waterbuck and 4-methylguaiacol were dispensed from sealed polythene sachets with 0.125 mm thick walls, 50mm × 75mm in width and height placed next or underneath the catching devices [28]. The release rates were obtained by measuring the difference in masses between freshly prepared sachets and their masses after been used in field experiments.

## Experiments

### Effective release rates of WRC and 4-methylguaiacol

Different release rates for the WRC and 4-methylguaiacol were achieved by varying the number of dispensers between one, two and four sachets per baited traps with the un-baited traps serving as controls. The number of sachets containing the compounds that effectively reduced both male and female catches of *G. f. fuscipes* at biconical traps were placed at sticky small targets as traps to assess if the effect was similar as that observed for biconical traps.

### Effect of removal of individual constituent from WRC

A series of subtractive assays to achieve blends without one constituent of WRC (Table 4.1) were prepared in sachets. The blends that resulted from subtractive assays (two sachets), WRC (two sachets) and individual constituent (one sachet) of WRC served as treatments with the control being a biconical trap alone. Further, two sachets, each containing blends that resulted from subtractive assays, WRC and individual constituents were subjected to the same field conditions and the weight of each dispenser taken every 24 hours for 3 days to determine differences in their release rates. In order to confirm the release of all compounds from the polyethene

sachets, samples with WRC or their blends that resulted from subtractive assays were subjected to GC-MS analyses after headspace extraction using a pre-cleaned (through thermal desorption at 250°C for 30 minutes to remove any contaminants) polydimethylsiloxane (PDMS) solid phase micro extraction (SPME) fibre (Supelco, Bellefonte, USA). This was done by placing each sachet with blends in a 700 ml bottle covered with aluminium foil tightly bound with two rubber bands. A hole was then made at the centre of the aluminium cover using the SPME holder and the PDMS fibre exposed to the headspace without it touching the sample for 5 minutes before it was retracted into its protective sheath. Thereafter, the fibre was injected into the injection port of an 7890B Agilent gas chromatograph (Agilent Technologies, Wilmington, DE) coupled to an Agilent mass spectrometer (MSD 59977A) (Agilent Technologies, Wilmington, DE) fitted with a split-less injector (250°C) to desorb the trapped volatiles for 2 minutes. The separation of compounds were done on an Agilent HP-5 MS capillary column (30m × 0.25mm id × 0.1 μm film thickness) using the following temperature programme: 35°C for 5 minutes, then raised at 10°C/min to a final temperature of 280°C and held for 10.5 minutes. Helium was used as the carrier gas at a constant flow rate of 1 ml/min. The compounds were detected using the electron ionisation mode (70eV; Ion source 230°C; quadrupole 150°C; mass scan range, 30-350 amu).

Table 4.1: Various treatments of subtractive assays of WRC, WRC and individual constituents at biconical traps

| Serial Number | Treatments |
|---|---|
| 1 | WRC |
| 2 | WRC *minus* pentanoic acid |
| 3 | WRC *minus* guaiacol |
| 4 | WRC *minus* δ-octalactone |
| 5 | WRC *minus* geranylacetone |
| 6 | No repellent |
| 7 | Pentanoic acid |
| 8 | Guaiacol |
| 9 | Delta octalactone |
| 10 | Geranylacetone |

**Experimental design and analyses**

Experiments where biconical traps were used ran from 08:00 to 18:00 hrs [29] while

those that used the sticky small target as the trapping device  ran from 08:00 to 12:00

hrs during the period when *G. f. fuscipes* is most active [26]. The treatments were

incorporated into a series of randomised block design experiments comprising

groups of near or adjacent days at a site as different blocks [30]. Treatments were

randomly allocated to days within these blocks. All statistical tests were done with R

version 3.2.5 [31]. A negative binomial model was used to measure the effect of

various treatments on the fly catch while taking into account the block and

experimental day. The detransformed means (effects display) of treatments in the

negative binomial regression was obtained using the "*effects*" package in R [32].

Analysis of variance (ANOVA) and Student Newman Keuls (SNK) test were used

for multiple comparisons of average release rates from single sachets of the

individual constituents of WRC; resultant blends from subtractive assays and WRC.

Statistical significance was considered at α less than 0.05.

**Results**

**Effective release rates of WRC and 4-methylguaiacol**

A total of 8,267 flies were collected comprising of 3,576 males and 4,691 females.

For WRC, the overall detransformed means of flies collected in the control

(biconical trap only) were higher than those collected from biconical traps with

varying number of repellent dispensers as treatments (Fig. 4.1).
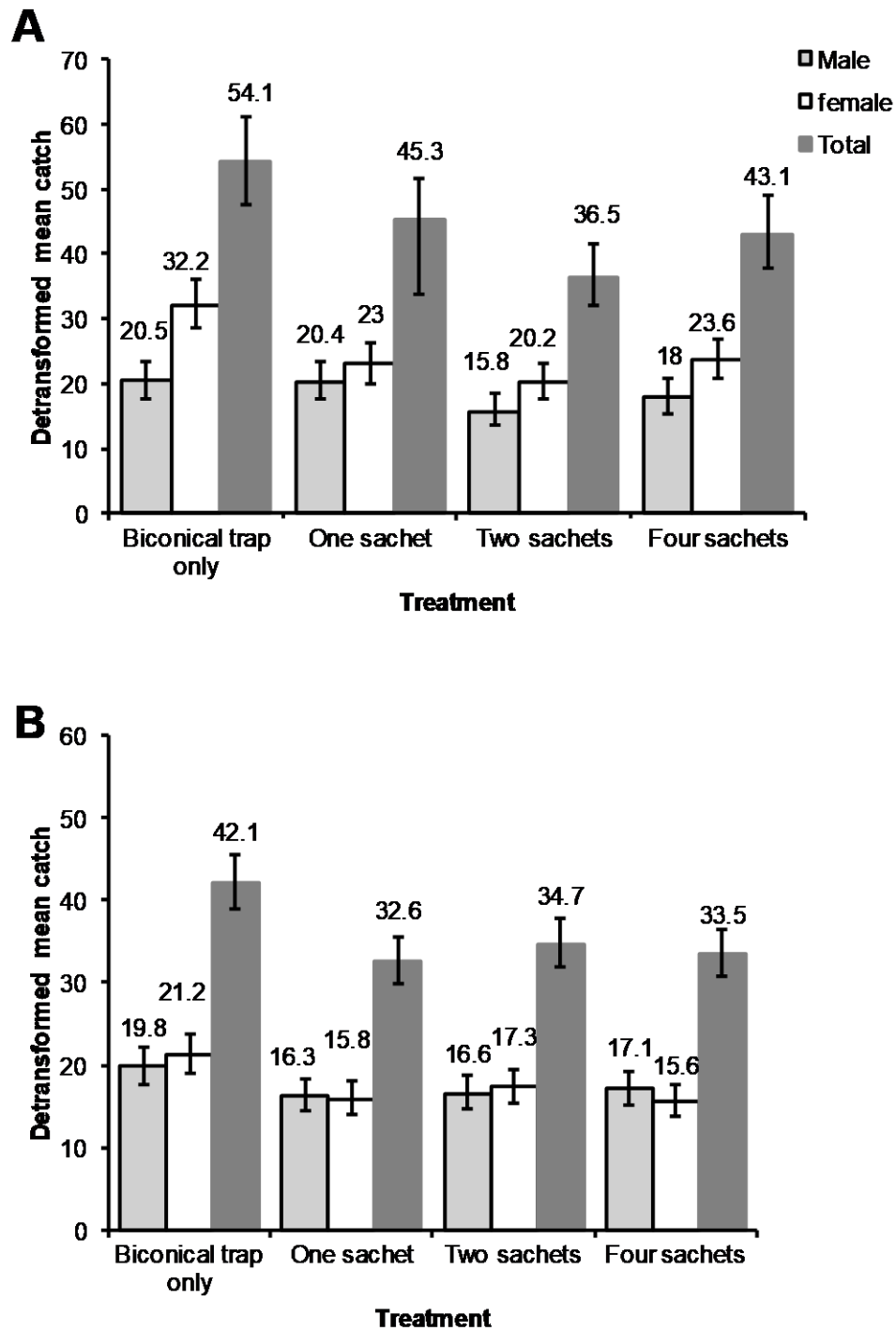
**A**



**B**



Figure 4.1: Detransformed means of *G. f. fuscipes* catches at biconical traps with varying numbers of dispenser of the 4-component blend of WRC (A) and 4-methylguaiacol (B).

The catch for both sexes of *G. f. fuscipes* was significantly reduced when WRC was dispensed from two sachets at a biconical trap by 23% (95%CI: 6 – 37%; *P*<0.05) and 37% (95%CI: 25 – 47%; *P*<0.001) respectively and overall by 33% (95%CI: 20 – 44%; *P*<0.001). However, when WRC was dispensed from one or four sachets at biconical traps, only the female catches were significantly reduced (Fig. 4.2A). The average release rate of WRC from each polythene sachet was about 0.83mg/h (95%CI: 0.65 – 1.01). Dispensing 4-methylguaiacol from one and two sachets significantly reduced catches of male *G. f. fuscipes* by 18% (95%CI: 3 – 30%; *P*<0.05) and 16% (95%CI: 2 – 28%; *P*<0.05) respectively while those of females were reduced by 25% (95%CI: 12 – 36%; *P*<0.001) and 19% (95%CI: 5 – 30%; *P*<0.01). Overall, when 4-methylguaiacol was dispensed from one and two sachets, the reduction in catches were 22% (95%CI: 13 – 31%; *P*<0.001) and 18% (95%CI: 8 – 26%; *P*<0.001) respectively. However, dispensing 4-methylguaiacol from four sachets only reduced female catches significantly (26%; 95%CI: 14 – 33%) (Fig. 4.2B).
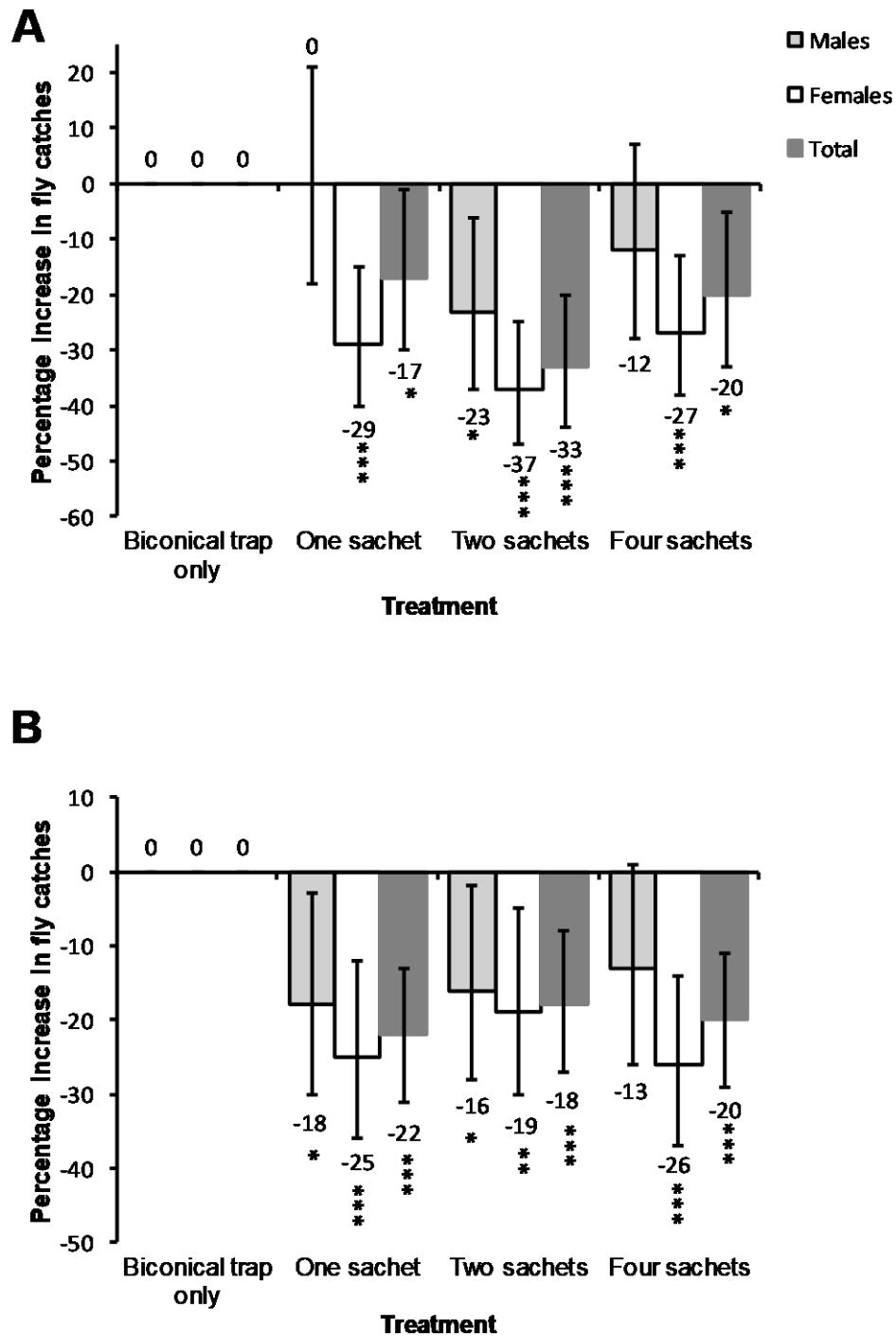
Figure 4.2: Percentage reduction in catches in the presence of varying numbers of dispenser of the 4-component blend of WRC (A) and 4-methylguaiacol (B) at biconical traps. *$P<0.05$, **$P<0.01$ and ***$P<0.001$

Two sachets of WRC and a sachet of 4-methylguaiacol dispensed from sticky small

targets to test if catches of both male and female *G. f. fuscipes* could be significantly

reduced when a different trapping device was used showed lower detransformed means (Fig. 4.3A) and significant reductions in catches of both sexes of *G. f. fuscipes* compared to the control (Fig. 4.3B). The average release rate from each polyethene sachet of 4-methylguaiacol was 1.40 mg/h (standard error: ±0.05).
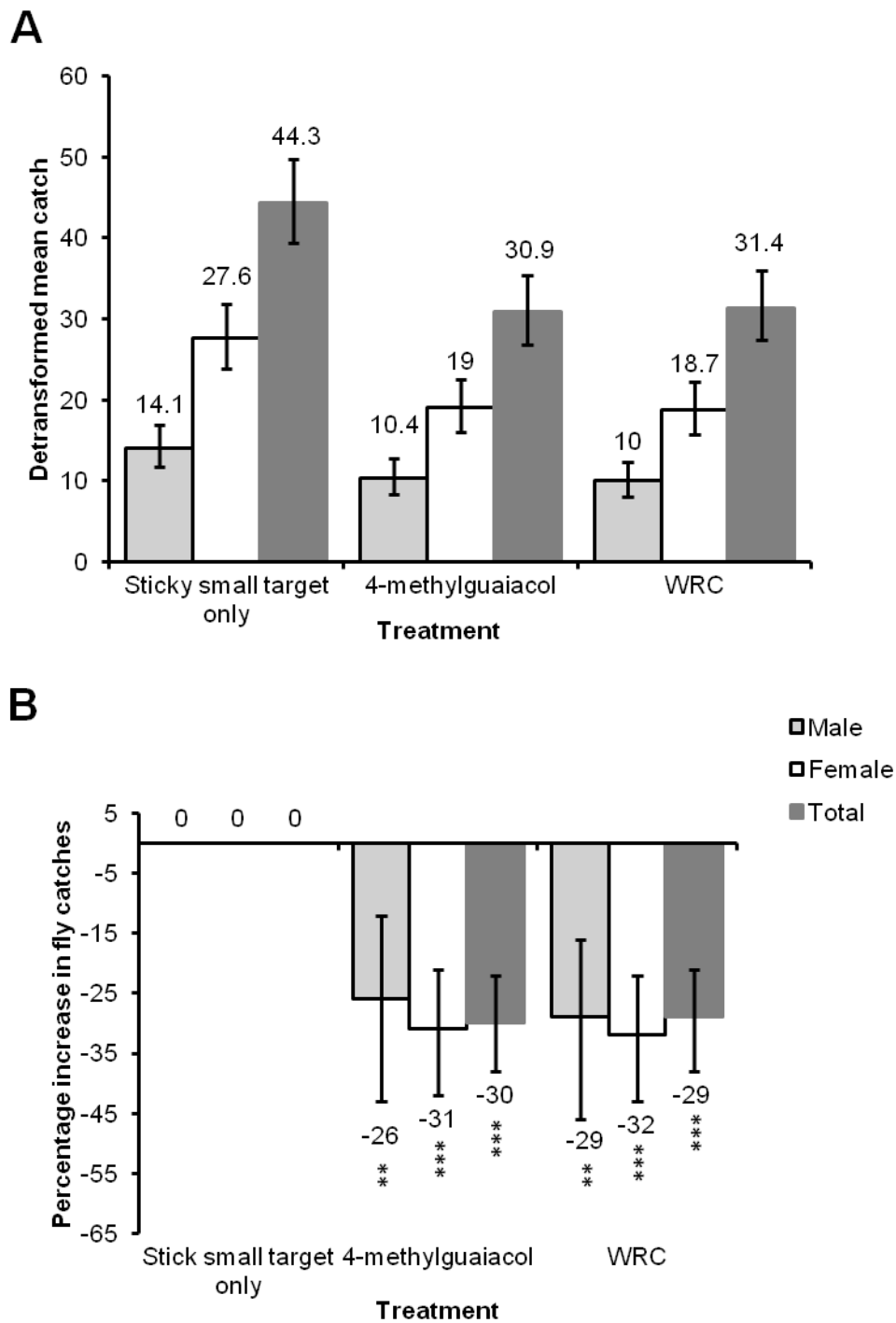


Figure 4.3: Detransformed means (A) and percentage reduction in catches (B) of *G.*

*f. fuscipes* at effective release rates of the 4-component blend of WRC and 4-methylguaiacol at sticky small target. *P<0.05, **P<0.01 and ***P<0.001

**Effect of removal of individual constituent from WRC**

During these experiments, a total of 5,489 *G. f. fuscipes* were caught comprising of 2,923 males and 2566 females. Apart of geranylacetone, the removal of pentanoic, guaiacol or delta octalactone from WRC did not lower the overall detransformed mean daily catches of *G. f. fuscipes* and were not significantly reduced compared to WRC at biconical traps (P>0.05; Fig 4.4A – D and Fig. 4.5A – D). Dispensing WRC without geranylacetone from two sachets significantly increased the catch of male and female *G. f. fuscipes* by 1.76 times (95%CI: 1.36 – 2.29 times; *P<0.001*) and 1.71 times (95%CI: 1.31 –2.25 times; *P<0.001*) compared to WRC.
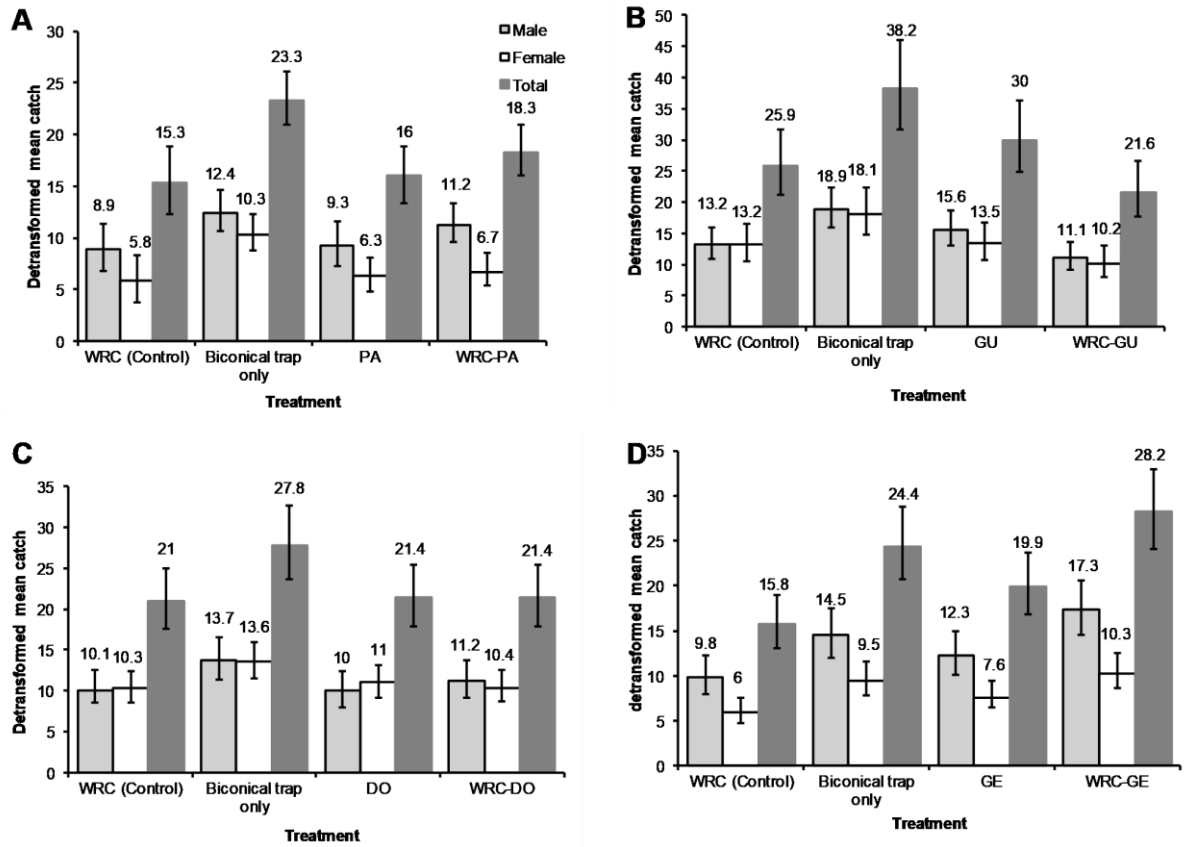
Figure 4.4: Fly densities of WRC without pentanoic acid (A), guaiacol (B), Delta octalactone (C) and geranylacetone (D). PA, GU, DO and GE represent pentanoic acid, guaiacol, Delta octalactone and geranylacetone respectively. Bars signify 95% confidence interval of the mean.
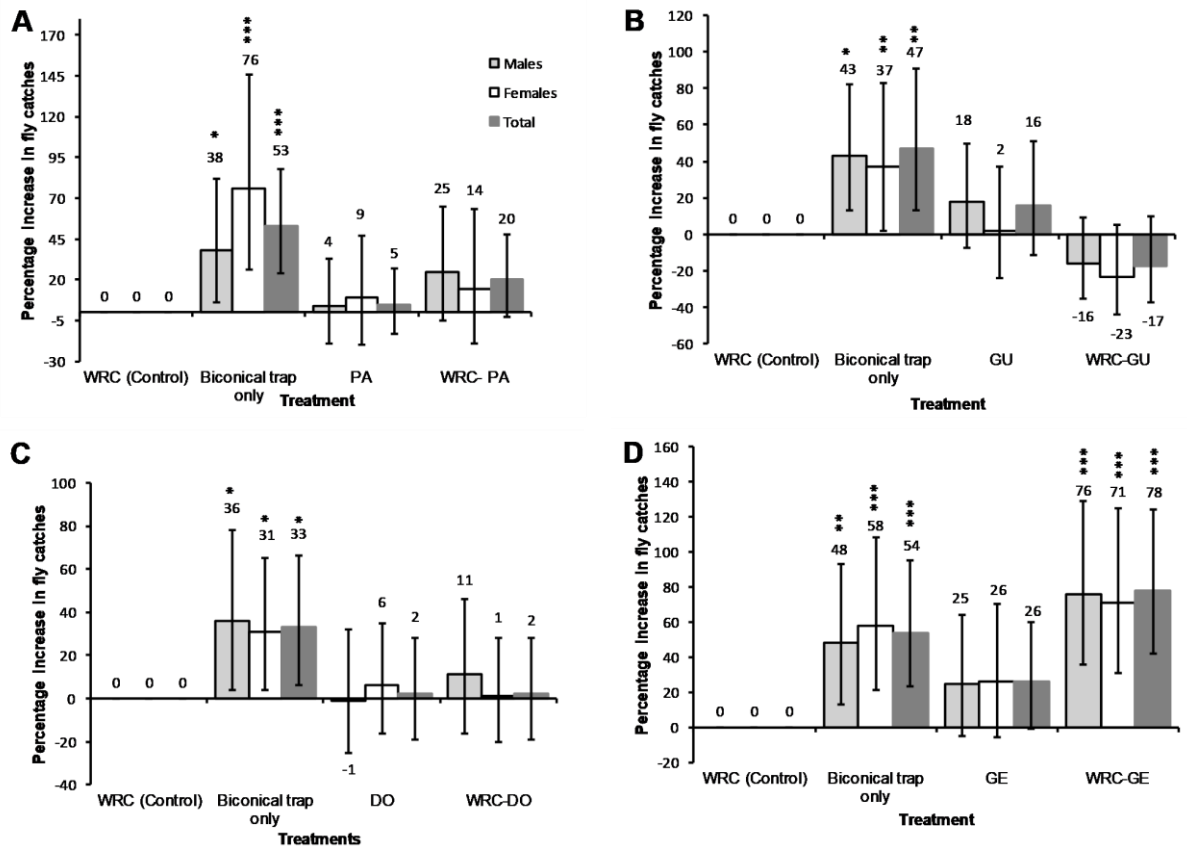
Figure 4.5: Percentage reduction in catches of WRC without pentanoic acid (A), guaiacol (B), Delta octalactone (C) and geranylacetone (D). PA, GU, DO and GE represent pentanoic acid, guaiacol, Delta octalactone and geranylacetone respectively. *$P<0.05$, **$P<0.01$ and ***$P<0.001$

Samples of sachets containing WRC and WRC without a particular constituent subjected to GC-MS confirmed that all the individual constituents were dispensed from the polyethene sachet dispensers as volatiles (Fig. 4. 6). From single sachets of individual constituents, Delta-octalactone had the lowest release rate (0.26 mg/h; 95%CI: 0.08 – 0.44), while pentanoic acid had the highest (3.83 mg/h; 95%CI: 2.29 – 4.01) (Table 4. 2). For single sachets of the blends, WRC without pentanoic acid had the lowest whilst WRC without Delta octalactone had the highest release rate (Table 4.2). There were significant differences in the release rates from single sachets of the different blends and individual constituents of WRC (ANOVA, $df_{44}$, F=175.81, $P<0.001$). The overall release rate in mg/h of WRC without pentanoic

acid or guaiacol was not significantly different from those of WRC (SNK: $P>0.05$;

Table 4.2). However, when Delta octalactone or geranylacetone were removed from

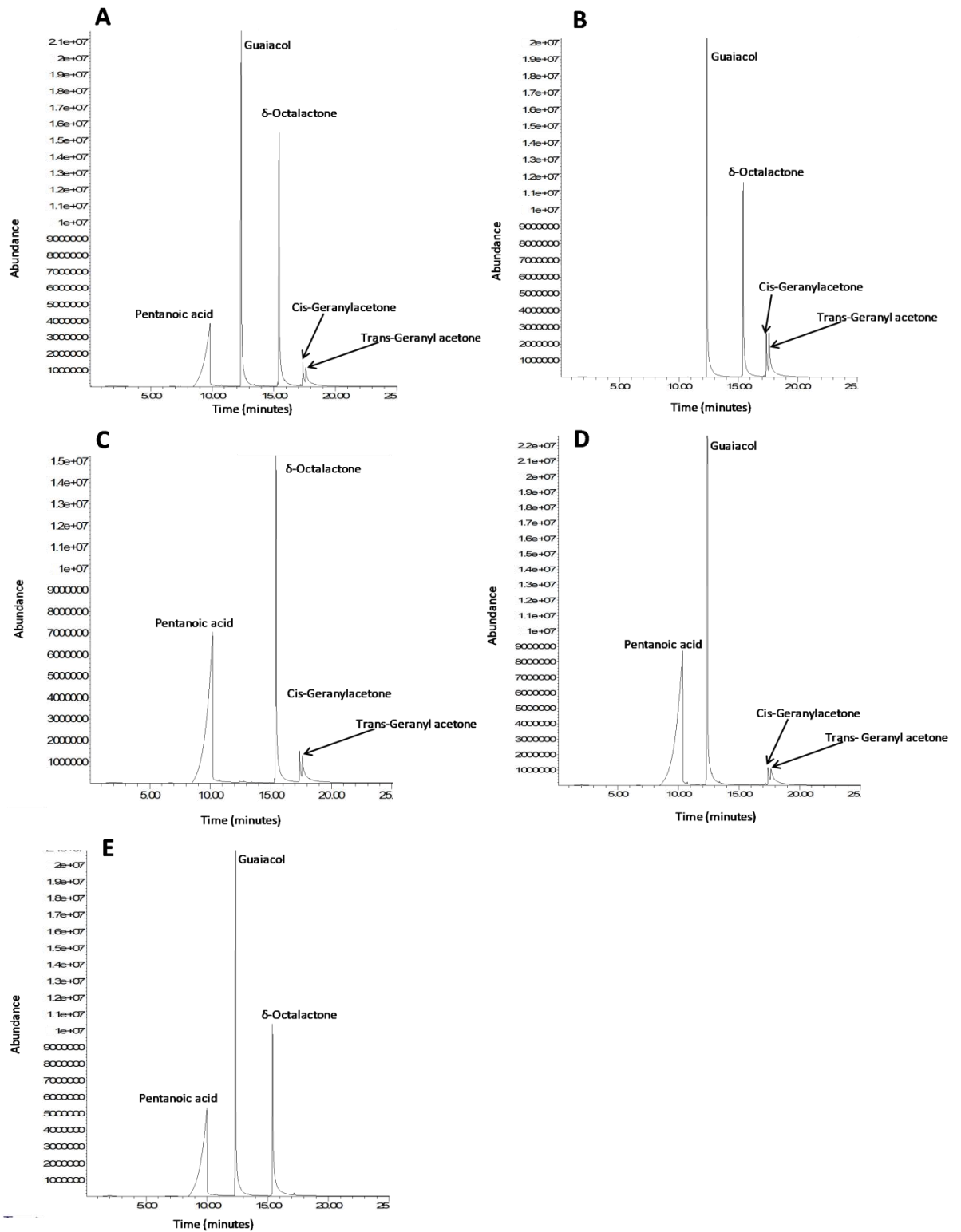WRC, release rates differed significantly (SNK: $P<0.05$; Table 4.2).

Figure 4.6: Total ion chromatograph of: WRC (A), WRC without pentanoic acid (B), WRC without guaiacol (C), WRC without Delta octalactone (D) and WRC without geranylacetone (E)

Table 4.2: Average release rates of the repellent compounds from waterbuck

| Repellent compounds | Average release rates (95% CI) n=6 |
|---|---|
| WRC | 0.83 (0.65 - 1.01)[f] |
| WRC without pentanoic acid | 0.80 (0.62 - 0.98)[f] |
| WRC without δ-octalactone | 3.08 (2.90 - 3.26)[b] |
| WRC without geranylacetone | 1.48 (1.30 - 1.66)[e] |
| WRC without guaiacol | 1.07 (0.89 - 1.24)[f] |
| Pentanoic acid | 3.83 (2.29 – 4.01)[a] |
| δ-octalactone | 0.26 (0.08 – 0.44)[g] |
| Geranylacetone | 1.75 (1.57 – 1.93)[d] |
| Guaiacol | 2.47 (2.89 – 2.64)[c] |

CI is 95% confidence interval. Average release rates with the same super script letter are not significantly different

**Discussion**

Assessing the responses of tsetse from the palpalis group to compounds and natural odours that repel flies from the morsitans group is important as it may lead to development of novel control methods that could reduce host-vector contact particularly in areas with low density and infection rates in tsetse populations, such as those of the HAT foci in West Africa. In this study, we report responses of *G. f. fuscipes,* a tsetse species from the palpalis group, to 4-methylguaiacol and WRC at stationary visual attractive traps. We observed that both male and female catches of *G. f. fuscipes* in biconical traps were reduced at particular dispensing rates of WRC

(2 dispensers approximately 2.0 mg/h) and 4-methylguaiacol (1 or 2 dispensers approximately 1.4 and 2.8 mg/h respectively), suggesting that they are true repellents as defined by Dethier et al. [33]. The repellency of WRC and 4-methylguaiacol were confirmed by the reduction of catches even at sticky small targets. We also observed a differential sex response when WRC was dispensed from a single or four sachets (approximately 1.0 or 4.0 mg/h respectively) with only female catches being reduced. A similar observation with 4-methylguaiacol was made with only female catches reducing when it was dispensed from four sachets (approximately 5.6 mg/h). Related differential sex responses have been reported with *G. pallidipes,* a tsetse fly from the morsitans group, to constituents of human odour where the effect was greater for females than males [2]. This provides support to a study that showed that there is a general conservation of chemosensory gene families across five tsetse species that includes *G. f. fuscipes* and *G. pallidipes* [21]. The reduction in *G. f. fuscipes* catches of ~33% by WRC we observed is less than ~84% reported for *G. pallidipes* [10]. This variation could be due the differences in formulation of WRC, where in our case, hexanoic acid was not added as a constituent. However, the repellency of hexanoic acid was reported not to be significantly different from that of pentanoic acid for *G. pallidipes* [10]. Additionally, the use of a trap baited with odour attractants as the control (reference) in the previous study [10]  whereas in this study the use of traps without odour attractants as controls could explain the differences in reduction in fly catches. However, the reduction in *G. f. fuscipes* catches of ~22% by 4-methylguaiacol is also less than ~70% reported for *G. pallidipes* at traps without odour attractants [11]. This could be an indication that *G. f. fuscipes* exhibits weaker responses to odours than *G. pallidipes.* This is consistent

with other reported observations that tsetse flies in the palpalis group show markedly weaker responses to host odours compared to those from the morsitans group [5]. Our results also indicate that when geranylacetone is removed from WRC; the catch of the resultant blend (pentanoic acid, guaiacol and delta-octalactone) was increased by 1.8 fold showing less potency in repellency. This suggests that geranylacetone may be playing an important role to the overall repellent effect of WRC to *G. f. fuscipes.* Interestingly, fly catches at traps with the blends that result after removal of pentanoic acid, guaiacol or delta-octalactone from WRC did not significantly differ with those from traps with WRC. Additionally, the catches at traps with the individual constituents did not also significantly differ from those of WRC. These results suggest that the individual constituents could substitute WRC as a repellent at biconical traps. Despite being dispensed from sachets of relatively consistent measurements, WRC without delta-octalactone or geranylacetone had significantly higher release rates compared to WRC clearly indicating that in blends, the relative diffusion of different constituents across the walls of the polyethene sachet dispensers and subsequent evaporation from the surface could be affected by their size, structure and proportion [10]. This is further supported by the observed significant variation in release rates of the individual constituents of WRC.

Even though our results have shown that *G. f. fuscipes* can be repelled by synthetic and allomonal compounds from water buck odour at traps, conclusions cannot be drawn as to whether a similar effect can be seen in the presence of vertebrate hosts. Torr and others [13] have previously shown that the repellent compound guaiacol could show potency at traps but was ineffective at protecting hosts from tsetse bites [13]. Therefore, we recommend further studies that will focus on evaluating the

effect of various blends and individual compounds shown to be repellent in our study in the presence of hosts.

In conclusion, the study showed that at specific release rates WRC and 4-methylguaiacol reduced catches of both sexes of *G. f. fuscipes* in un-baited traps, an indication that they are true repellents. It also showed that sex of *G. f. fuscipes* could play a role in its responses to repellents. Additionally, WRC without pentanoic acid, guaiacol or delta-octalactone had the same repellency to *G. f. fuscipes* as WRC at biconical traps. Furthermore, individual WRC constituents: pentanoic acid, guaiacol, geranylacetone and delta-octalactone repel *G. f. fuscipes* just as well as WRC at biconical traps. Therefore, we recommend further studies to evaluate these repellents in the presence of hosts as it may lead to the development of novel control methods especially in HAT foci.

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## CHAPTER 5      General conclusions and recommendations

In this thesis, the preceding data chapters report the results of ecological studies on *G. f. fuscipes'* population structure, sampling tools and responses to candidate repellents. The population structure was determined using wing geometric morphometric analyses of flies collected before and after a tsetse control intervention trial on Big Chamaunga and Manga islands of Lake Victoria in the study area. This formed a baseline for subsequent studies on the sampling tools and responses of *G. f. fuscipes* to candidate repellents.  The key elements necessary for consideration during experimentation such as fly densities were determined more so that a previous intervention had reduced fly populations by over 90% in some parts of the study area. Further, the use of sticky small targets as sampling tools during experiments to assess the responses of *G. f. fuscipes* to candidate repellents were evaluated against the standard biconical traps. Additionally the landing response of females and males on the blue and black panels of the sticky small target was also evaluated. Responses of *G. f. fuscipes* to candidate repellents in the field were also assessed at biconical traps and sticky small targets.

The proceeding section of this chapter summarises the key findings and implications from the preceding data chapters on the ecological aspect of tsetse flies. Based on this, recommendations and future research directions are made.

Changing the natural habitat of tsetse fly, *G. f. fuscipes,* by adding vector control devices such as small targets could induce the diminishing of size and divergence of wing shape in fly populations that recover after the intervention **(Chapter two)**. The performance of targets as a control tool is affected by the tsetse fly displacement

rates and other density dependent factors [1]. Fly size plays an important role in displacement rates, with the displacement potential increasing as fly size increases; hence larger flies that displace more have a higher chance to encounter and be killed by the targets [1]. With much of the movement of tsetse flies attributed to host seeking for a blood meal, the feeding success is negatively correlated to relative densities [2]. At high relative densities more movement is expected and the chances of tsetse to encounter and be killed by the targets increases. Thus, during a vector control intervention, it is possible that on the one hand, larger flies that displace more and are at a higher risk of encountering the killing devices were eliminated more than smaller flies; on the other hand, as the relative density reduces, the feeding success increases and the fly movement is expected to lessen, thereby reducing further the chances of smaller flies to encounter and be killed by the targets. The results of this complex interaction of ecological elements of tsetse flies could be the observed small size of *G. f. fuscipes* that recovered after a control intervention using small targets as evidenced in **Chapter two**. The observed smaller size and wing shape divergence of tsetse that recovered after the control intervention with small targets could be a demonstration of complex interaction of the different elements of tsetse ecology. It is possible that the vector control intervention exerts a selection pressure for smaller flies which displaces less than the bigger flies giving rise to a new generation of small flies that recovered after the intervention was stopped. This could be among the reasons why vector control using targets has not been successfully used to eliminate tsetse populations to date. Perhaps if the diminishing size of flies during vector control is taken into consideration, specific intervention that targets the smaller flies could greatly increase the chances of elimination of

tsetse flies. One already existing method for such is SIT. Releasing sterile males that are larger compared to the wild males could increase their mating chance as they are more mobile thus increasing the efficiency of the control method. Investigations to understand how vector control using targets induces the diminishing of tsetse fly size and divergence of wing shape need to be undertaken. This may give an insight on how vector control using targets could be improved and made more effective, thus guide future tsetse control strategies. The average size of the female flies was larger than the males, another factor that could play an important role in targeting the female fly population when targets are used for control.

Furthermore, sticky small targets are more efficient for sampling of *G. f. fuscipes* than biconical traps indicating that they could be used as alternative sampling tools. Elements of tsetse ecology such as displacement rates, relative densities and associated factors and their interactions can only be studied with adequate sampling tools [2]. Traps have mostly been used as sampling tools where tsetse flies are attracted to the vicinity by the blue colour of the trap, enter it and are guided into a non-return cage. However, only 20% of the flies that are attracted enter the trap [3]. In the morsitans tsetse species the attraction of the flies to the vicinity is enhanced by placing attractant odour at the trap thereby increasing the fly catches. *G. f. fuscipes* and some of the other tsetse fly species from the palpalis group are not as responsive as species from the morsitans group to known attractants. Therefore, development of more efficient traps for *G. f. fuscipes* relays on exploiting the visual cue. As shown in **Chapter three** it was observed that a small target covered with a transparent sticky film caught more *G. f. fuscipes* than the biconical trap. This could be attributed to the fact that all flies that landed on the sticky small target were caught.

Furthermore, the higher likelihood of females landing on the black panel of the target than the blue compared to males and vice versa could be exploited for delivery of bio-control agents that target particular sex of the tsetse fly as discussed in **Chapter three.** The higher number of flies caught by the sticky small target than the biconical trap indicates that it could be an alternative and a more efficient sampling tool for *G. f. fuscipes* than the biconical trap. Particularly that it caught more females than males which represents the sex ratio in the natural population, makes it ideal for ecological studies. Such an efficient sampling tool offers an opportunity to effectively monitor and evaluate tsetse fly control interventions that aim for either suppression or eradication of populations. With most palpalis group species occurring at relatively low densities compared to tsetse flies from the morsitans group, the sticky small target being more efficient than the biconical trap for *G. f. fuscipes* could be used for experiments that require large fly catches in order to achieve reliable and valid results and overcome challenges associated with treatment effects. Thus where *G. f. fuscipes* densities are low, the use of the sticky small target could reduce the number of replicates thereby reducing the cost of the experiments. In addition, although *G. f. fuscipes* caught on sticky small targets were in relatively good condition, studies that assess these for other processes like dissections and molecular based techniques are required. Additional studies to establish the cost effectiveness of sticky small targets as sampling tools are also required as they could influence the acceptability of the tool.

Additionally, 4-methylguaiacol and WRC reduce catches of *G. f. fuscipes* at un-baited biconical traps and sticky small targets. Host location by tsetse flies is facilitated by olfactory and visual cues [2]. Olfactory cues play a major role in long

range location while visual cues play a major role in short range host location and landing [4]. Particular compounds or their combination from host odour have been implicated in attraction or repellency of tsetse; hence the observed host preference behaviour of tsetse [5,6]. Specific compounds isolated from waterbuck body odour when dispensed at traps have shown allomonal properties against morsitans tsetse species [7]. In addition 4-methylguaiacol, a synthetic analogue of guaiacol a known repellent, has also shown repellent properties against morsitans tsetse species. In **Chapter four**, 4-methylguaiacol and WRC reduced fly catches of *G. f. fuscipes* a tsetse fly species from the palpalis group at sticky small targets and biconical traps. The observed reduction of fly catches in the presence of specific compounds from waterbuck body odour at biconical traps and sticky small targets could be an indication of the ability of *G. f. fuscipes* to avoid particular vertebrate hosts found in its habitat. The reduction in fly catches was more prominent in females at varying release rates of repellents compared to males. This could also be an indication of the important role chemical sensing plays in host seeking by *G. f. fuscipes* especially the fact that most movements of tsetse are associated with the need for a blood meal [2]. Further, the observed reduction in catches of *G. f. fuscipes* in the presence of 4-methylguaiacol and specific compounds in waterbuck odour indicates that they are true repellents. Interestingly individual constituents of the WRC are just as repellent as the complete blend of the four compounds: pentanoic acid, guaiacol, Delta octalactone and geranylacetone (**Chapter four**). Additionally, the blend without geranylacetone seems to be more attractive than the repellent. However the observed repellency at the stationary visual attractive devices does not necessarily mean that the compounds could protect hosts from bites of *G. f. fuscipes* [9]. Therefore, there is

need to further evaluate 4-methylguaiacol and specific compounds in waterbuck odour for protection of human and animal hosts against bites from *G. f. fuscipes.* This could lead to development of novel control strategies against African trypanosomiasis as it would provide insights in the prospects of developing personal protection technologies against the vector which are currently lacking for HAT. Certainly, personal protection technologies would come in handy in reducing the number of HAT cases that are reported as a result of visits to tsetse infested areas especially game parks [8] which attract tourist. Presence of the vectors in these areas poses great threats to the tourism industry, thus affecting economic activities and development in these areas. However, high tsetse densities or bite rates as well as high trypanosome infection rates in the vector could lower the effectiveness of repellents in protecting animal hosts in particular [9,10]. This could limit their use to areas that have low tsetse challenge and infection rates for protection of cattle. On the other hand repellents could be very effective in protecting humans against HAT, as bite rates and human infective trypanosome rates in tsetse populations are relatively low [10]. As an addition to the existing control methods targeting tsetse, repellent technology offers promise for an individual protection tool against AT for hosts as well as, an opportunity to develop novel tsetse control strategies such as those that use a "push-pull" mechanism and other integrated disease control approaches. In the case of "push-pull" mechanism, the repellent could "push" the tsetse flies away from a potential host while attractive baits treated with insecticides "pull" and kill them.

In conclusion, the studies undertaken have revealed complex changes that occur to tsetse populations subjected to control interventions using targets thereby possibly

allowing the survival of residue population that could repopulate a previous vector control intervention area. This insight could help in improving target based tsetse control methods to make them more efficient by targeting the residue population of smaller flies that displace less and are less likely to encounter the targets. The absence of separation between the island populations facilitates the possibilities of reinvasion. This supports calls for undertaking of ecological studies that determine the population structure in intervention and surrounding area prior to implementation of control intervention in order to plan for protection of tsetse fly controlled areas (11). Furthermore, a more efficient sampling tool for *G. f. fuscipes* which could be an alternative to the biconical trap and the influence of the colours blue and black on the landing response of female and male flies are unveiled in the studies. This could pave way for effective monitoring of tsetse control interventions and development of sex specific biological control strategies. The studies also affirm that *G. f. fuscipes,* a tsetse fly subspecies from the palpalis group could be repelled by known repellents at stationary visual attractive devices. This could be an opportunity to develop personal protection technologies for palpalis group, tsetse species that are repellent based

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