

**Impact of changed feeding behaviour of *An. funestus* on
malaria transmission in southern rural Tanzania**

by

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DECLARATION

I, **SALUM AZIZI** declare that, with the exception of references to other people's work which I have duly acknowledged, the work described in this research report was carried out by me. This report, either in whole or in part, has not been submitted before for any degree or examination at any other university.

A handwritten signature in dark ink, appearing to be 'Salum Azizi', written in a cursive style.

February 2012

ABSTRACT

In Tanzania both *Anopheles funestus* and *An. gambiae s.l.* play a role as major vectors of malaria. Different species exist in the *An. funestus* group and the *An. gambiae* complex and play different roles in disease transmission. For malaria vector control programmes knowledge of vector species and their behaviour is key. A recent report on increased exophagy of *An. funestus* in southern rural Tanzania as a response to increased use of insecticide treated bed nets raised the question of whether there was misidentification of species and/or behavioural insecticide resistance playing a part. The present study used molecular tools to identify the species and determine human biting rates indoors and outdoors along with development and field evaluation of a new tool for sampling malaria vectors which is more effective than human landing catches.

The results showed that the majority (96.2%) of the *An. funestus* group that were collected were *An. funestus s.s.* by PCR assay. Also, the exophagic proportion (45.9%) of *An. funestus* was lower than the endophagic proportion (54.1%), similar to other places in Africa, although in this study the difference was insignificant when untreated bed nets and treated bed nets were used. In addition, there was significant outdoor biting activity early in the evening that could lead to the malaria transmission cycle being unaffected by ITNs. The new trap, “Sticky Bucket Trap”, caught considerably fewer mosquitoes (14.2%) than that caught by human landing catches (85.8%), with statistical significance of $P > 0.05$. These results imply that the sticky bucket trap is not a suitable substitute for human landing catches and some modifications are needed to make it more effective. Whereas indoor and outdoor

proportions insignificant difference in feeding preference imply that both indoor and outdoor interventions should be used to control this vector.

DEDICATION

To my wife Asia,
and
my lovely children
Mu'aadhi and Thuwaybah

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I thank my supervisor Prof Maureen Coetzee for her academic guidance and support, helping me to sharpen my research proposal that eventually turned out to be this productive research report. Prof Maureen had been very nice to me, smiling and always ready to help when I show up for research report review and corrections regardless of her ever busy schedule.

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TABLE OF CONTENTS

Declaration	ii
Abstract	iii
Dedication	v
Acknowledgements	vi

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background	1
1.2 Burden of malaria in Tanzania	1
1.3 Malaria transmission in the Kilombero valley	2
1.4 Behaviour and roles of different members of the <i>An. funestus</i> group in malaria transmission.....	2
1.5 The impact of intensive insecticide application on malaria vector population structure and behaviour.....	3
1.6 The scope of available sampling techniques for measuring human exposure to malaria transmission	7
1.7 Aim of this Study	8
1.8 Specific objectives.....	8

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study design	9
2.2 Description of the study site.....	9
2.3 Mosquito collection	11
2.4 Morphological identification.....	14
2.5 Species identification by PCR.....	14
2.6 Sporozoite rate determination.....	16
2.7 Data analysis.....	16

CHAPTER THREE

RESULTS

3.1 Species composition of malaria vectors at the study site.....	18
3.2 Sporozoite rate of <i>An. funestus</i>	19
3.3 Biting profile of <i>An. funestus</i> at the study site under the pressure of ITNs.....	21
3.4 Results for HLC and SBT.....	26

CHAPTER FOUR

DISCUSSION

4.1 Indoor and outdoor species composition of different malaria vectors.....	28
4.2 Sporozoite rates of <i>An. funestus</i>	28
4.3 The feeding (biting rates) behaviour of <i>An. funestus</i> when ITNs are in use and when not.....	29
4.4 Effectiveness of SBT against that of HLC.....	31

4.5 Conclusion.....	32
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REFERENCES.....	33
-----------------	----

LIST OF FIGURES

Fig 2.1: Map showing Kilombero valley and the study site	10
Fig 2.2: Typical shamba house	11
Fig 2.3: HLC in operation	13
Fig 2.4: Sticky Bucket Trap in operation.....	13
Fig 2.5: Schematic diagram of the Sticky Bucket Trap.....	14
Fig 3.1: PCR results for <i>An. funestus</i> identification.....	19
Fig 3.2: Indoor and outdoor hourly biting profile of <i>An. funestus</i> in vicinity of untreated bednets	22
Fig 3.3: Indoor and outdoor hourly biting profile of <i>An. funestus</i> in vicinity of treated bednets.....	23
Fig 3.4: <i>An. funestus</i> biting activity before sleeping time.....	25
Fig 3.5: <i>An. funestus</i> biting activity after waking time.....	25
Fig 4.1: Biting pattern of <i>An. funestus</i> group from 1997 to 2009.....	31

LIST OF TABLES

Table 3.1: Species composition indoors and outdoors by PCR	18
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Table 3.2: Sporozoite rates of <i>An. funestus</i> indoors and outdoors in the vicinity of untreated bed nets.....	20
Table 3.3: Sporozoite rates of <i>An. funestus</i> indoors and outdoors in the vicinity of treated bed nets.....	20
Table 3.4: The effect of bed net treatment on endophagic and exophagic proportions of <i>An. funestus</i>	24
Table 3.5: Comparison of HLC and SBT on catching <i>An. gambiae s.l.</i>	26
Table 3.6: Comparison of HLC and SBT on catching <i>An. funestus</i> group.....	26
Table 3.7: Comparison of HLC and SBT on catching <i>An. coustani</i> complex	27

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Malaria is one of the major killing diseases in tropical regions, sub-Saharan Africa being affected the most with 90% of malaria related deaths (Greenwood and Mutabingwa, 2000). The epidemiology of malaria results from the interactions of vectors, parasites, humans, physical environment and socio-economical situations. Malaria is transmitted only by mosquitoes of the genus *Anopheles*, where about 40 out of 400 species in the genus are implicated in transmission worldwide (Service, 1993).

1.2 Burden of malaria in Tanzania

Malaria is a serious health problem in Tanzania, accounting for most deaths and 33% out-patient attendance and hospital admissions. This information is based only on those who attended the health facilities to seek medical care, whereas most of the cases are believed to occur at home, and go unreported (Snow *et al.*, 1999). The major parasite is *Plasmodium falciparum* which accounts for over 90% of all infections. To overcome this situation emphasis was placed on early case management by readily available chloroquine, which had successful results in reducing the proportion of infected mosquitoes between the 1930s and 1970s, but malaria transmission increased again between the 1980s and 1990s as chloroquine resistance became widespread (Mboera *et al.*, 2001).

The majority of Tanzanians (28 million people) still live in malaria endemic areas, 1.3 million people live in epidemic areas whereas only 0.6 million people live in low risk areas (MARA/ARMA: <http://www.mrc.ac.za/maract>).

1.3 Malaria transmission in the Kilombero valley

Kilombero is one of the districts in Tanzania where the burden of malaria in some of its villages (e.g. Idete) is so high that the Entomological Inoculation Rate (EIR) was estimated to be over 2000 infective bites per year. Studies conducted in 1990s revealed malaria prevalence of greater than 80% (Alonso *et al.*, 1991; Kitua *et al.*, 1996). However, an increased use of bednets has resulted in the mean EIR being reduced to 81 infectious bites per person per year (ib/p/y), an 18 fold decrease from the previous rate. Nevertheless, transmission is still high and it needs continuous harmonized vector control programmes and case detection and management in order to further reduce transmission and prevent a return to previous levels of malaria.

1.4 Behaviour and roles of different members of the *An. funestus* group in malaria transmission

This group consists of at least nine species distinguishable by morphological, cytotaxonomic and molecular means, namely: *Anopheles funestus* s.s. Giles, *An. vaneedeni* Gillies and Coetzee, *An. confusus* Evans and Leeson, *An. leesoni* Evans, *An. rivulorum* Leeson, *An. aruni* Sobti, *An. parensis* Gillies, *An. fuscivenosus* Leeson and *An. brucei* Service.

Different members of the *An. funestus* group express different degrees of vectorial competence and therefore their involvement in transmitting the disease varies. Apart from *An. funestus*, which is the most efficient vector of malaria within the group, it is only *An. rivulorum* which has been reported to act as a minor vector in Tanzania (Wilkes *et al.*, 1996). All remaining members of this group are zoophilic (preferring feeding on animals) (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987) and have not been reported as vectors of malaria. *Anopheles funestus* s.s. is responsible for the high malaria endemicity in large parts of Sub-Saharan Africa. It possesses both a high degree of susceptibility to the most virulent human malarial parasite, *Plasmodium falciparum*, and also has a high survival rate which allows the malaria parasite to complete its sexual reproduction and development to the infectious stage. *Anopheles funestus* breeds in permanent water bodies which accounts for its role in dry season malaria transmission (Charlwood *et al.*, 2000). Since it also displays endophilic (indoor resting) and endophagic (indoor biting) characteristics, host contact rates tend to be very high which accounts for its increased capacity for malaria transmission.

1.5 The impact of intensive insecticide application on malaria vector population structure and behaviour

Malaria control becomes practical if the behaviour component of the vector is taken into consideration, because different approaches are applied to control mosquitoes with different behaviours. In Africa, where *An. gambiae* and *An. funestus* are the major malaria vectors, indoor residual house spraying (IRS) and insecticide treated bed nets (ITNs) provide the most effective tools for reducing the entomological inoculation rate (Killeen *et al.*, 2000) and hence malaria transmission.

Indoor house spraying against malaria vectors is effective when mosquitoes rest indoors (endophilic behaviour). By resting indoors the mosquito picks up a lethal dose while resting on a surface which is sprayed with an effective insecticide and eventually dies. Malaria control through IRS becomes problematic when mosquitoes alter their behaviour to outdoor resting in response to increased IRS or ITNs using insecticides that have an excito-repellency effect. Examples include: 1950s malaria control through IRS failed in southern Java due to an increased exophilic proportion of *An. sundaicus*. A naturally endophilic *An. fluviatilis* was in the 1970s found to largely rest outdoors and enter houses only in search of blood, thus rendering the IRS programme useless (Gunasekaran *et al.*, 1994). In addition, many malaria vector mosquitoes exhibit variable resting behaviour and an increase in the exophagic proportion may be favoured because they are less affected by indoor interventions.

Insecticide treated bed nets are effective in controlling malaria transmission when the major malaria vectors bite at hours when most people are in bed. Changes in biting behaviour, for example when most biting occurs outside, would jeopardize malaria control even in a community which is covered by bed nets. This effect has been experienced in Tanzania (Njau *et al.*, 1993) and Kenya (Mbogo *et al.*, 1996) where *An. gambiae* was reported to have increased outdoor biting after the introduction of insecticide treated bed nets.

Malaria vector control in Tanzania is largely based on the use of insecticides. Initially insecticides were applied in the form of indoor residual house spraying. This was conducted from 1955 – 1959 as a pilot study in Pare, northeast Tanzania, with huge success in reducing

malaria transmission (Bradley *et al.*, 1991). Unfortunately, high financial and man-power needs led to the discontinuation of the IRS operations. However, there is a current revival of IRS campaigns in Zanzibar and Kagera which became feasible under collaboration with international organizations, starting in 2006 and 2007. In addition, in many regions malaria vector control is focused on using insecticide treated nets which has a similar impact to IRS but is easier to implement (Curtis *et al.*, 1998). The distribution of ITNs was mainly carried out through special programmes. One such programme, based on social marketing of nets and insecticides namely KINET, was initiated at Kilombero in 1997 (Schellenberg *et al.*, 2001). The result of this programme was an enormous reduction of malaria prevalence from 46% to 26% after an expansion of ITN usage from 10% to 61% (Abdulla *et al.*, 2001). This went along with a reduction of mosquito feeding frequency, life span and density (Killeen and Smith, 2007).

Major vectors of malaria in this area are *Anopheles gambiae sensu lato* and *An. funestus*. In some areas the population structure of *An. gambiae s.l.* is recorded as having changed from predominantly endophilic to predominantly exophilic members of the group as a result of intensive ITN usage. Also, the feeding preference is reported to have substantially increased outdoors for *An. gambiae* and *An. funestus* (Pates and Curtis, 2005; Killeen *et al.*, 2006; Russell *et al.*, 2011). *Anopheles funestus* is mainly endophagic (indoor feeder) and anthropophilic (prefers human blood) (Gillies and Coetzee, 1987) and has consistently remained so in a number of places over decades. Examples include: Kenya (Githeko *et al.*, 1994; Kamau *et al.*, 2003), Nigeria (Oyewole *et al.*, 2007) and Cameroon (Tanga and Ngundu, 2010). It is also almost exclusively endophilic, preferring to rest inside human

habitations than anywhere else. These intrinsic characteristics (endophagy, endophily and anthropophily) have rendered its control by chemical insecticides through IRS and ITNs feasible in many parts of Africa, leading to its reduced number, elimination and/or replacement by other exophagic species. Such cases include elimination of *An. funestus* in South Africa in the 1950s by DDT spraying and again in 2001 (Hargreaves *et al.*, 2000), the decline of *An. funestus* in Western Kenya (Bayoh *et al.*, 2010) and replacement of *An. funestus* by *An. rivulorum* in Uganda (Aboul-Nasr, 1970) and South Pare, Tanganyika (Gillies and Smith, 1960). However, this insecticide pressure has not been sufficient to change the endophagic behaviour of *An. funestus* even in a closely watched experiment assessing ITN efficacy in Cameroon (Chouaibou *et al.*, 2006).

The shift of a proportion of *An. funestus* from endophagy to exophagy recently reported in Tanzania (Russell *et al.*, 2010; Russell *et al.*, 2011) is therefore of great interest, and has serious implications for vector control programmes using IRS and ITNs. Initiation of and continuous close monitoring of this behaviour pattern would need to become an integral part of the control programme.

There are a number of proposed reasons for this change in biting behaviour pattern, ranging from misidentification of species, replacement of endophagic by exophagic members of the *An. funestus* group and insecticide resistance. In Tanzania four members of the *An. funestus* group exist (Temu *et al.*, 2007) and in the same area where *An. funestus* preference for exophagy has been reported studies have relied on morphological identification of members of this group (Ogoma *et al.*, 2010). There is thus the chance that an exophagic member of the

group is present and has been mistaken for *An. funestus* in the reported exophagy case. The case of molecular misidentification of other anophelines to *An. funestus* has also been reported in other parts of Africa (Kent *et al.*, 2006).

Insecticides have been reported in some studies to induce altered resting behaviour of some malaria vectors (Potikasikorn *et al.*, 2005; Chouaibou *et al.*, 2006). It has been reported that insecticide resistant mosquitoes exhibit lower flight activity than susceptible ones (Rowland, 1990). The possibility that insecticide behavioural resistance is also playing a role warrants investigation.

1.6 The scope of available sampling techniques for measuring human exposure to malaria transmission

Human landing capture (HLC) remains the gold-standard for measuring human exposure to malaria vectors (Lines *et al.*, 1991; Davis *et al.*, 1995). A number of technical and ethical problems face application of HLC ranging from a need for constant alertness, close supervision, skills for capturing mosquitoes, increased risks of infectious bites, and being cumbersome and labour intensive. All of these problems affect its reproducibility and reliability. While indoor resting collections may be a practical alternative to HLC, increased application of insecticides for vector control compromises this technique due to exito-repellent and killing effects of insecticides (WHO, 2005). Therefore traps have been proposed as an alternative although evaluation of most traps has had unsuccessful results (Mboera *et al.*, 2005; Okumu *et al.*, 2008).

1.7 Aim of this Study

The overall goal of this study was to investigate the extent and consistency of reported exophagic behaviour of *An. funestus* and whether it rests outdoors and the impact it has on malaria transmission in an area of rural Tanzania.

In this study I also evaluated a novel sticky bucket trap which is simple, inexpensive, requires little supervision, is exposure-free, less laborious, and more effective than HLC and requires very little skill in catching mosquitoes.

1.8 Specific objectives

The objectives of this study were:

- To determine and compare species composition of indoors and outdoors caught mosquitoes belonging to the *An. funestus* group using polymerase chain reaction (PCR)
- To determine infectivity using ELISA and compare sporozoite rates of indoor and outdoor collections of the *An. funestus* group
- To determine and compare human biting rates between indoor and outdoor collections of the *An. funestus* group
- To compare the effectiveness of a new trap (sticky bucket trap) with that of Human Landing Catches

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study design

A cross-sectional, descriptive and prospective study was carried out in southern rural Tanzania from May to June 2011. A 4X4 Latin Square Design was adopted

Inclusion criteria; Only *Anopheles* mosquitoes were used for the purpose of the study:

- Only female *Anopheles* were checked for sporozoites by ELISA test, those falling into the *An. funestus* group were further analyzed to species level by PCR
- Houses with and without interventions were included

2.2 Description of the study site

The study was carried out in Njagi in south-eastern Tanzania (7°44'-9°26'S, 35°33'-36°56'E), 300m above sea level (Fig. 2.1). The village receives rain from November to May and the dry season is from June to October. Annual rainfall ranges from 1200 to 1800mm and annual mean temperatures are 20-32°C.

High malaria transmission prevails despite high coverage of treated bed nets (Killeen et al., 2007). *Anopheles arabiensis*, *An. gambiae* s.s. and *An. funestus* are the major malaria vectors in this area (Charlwood et al., 1997) and during the dry season *An. funestus* becomes the most important malaria vector (Charlwood et al., 2000).

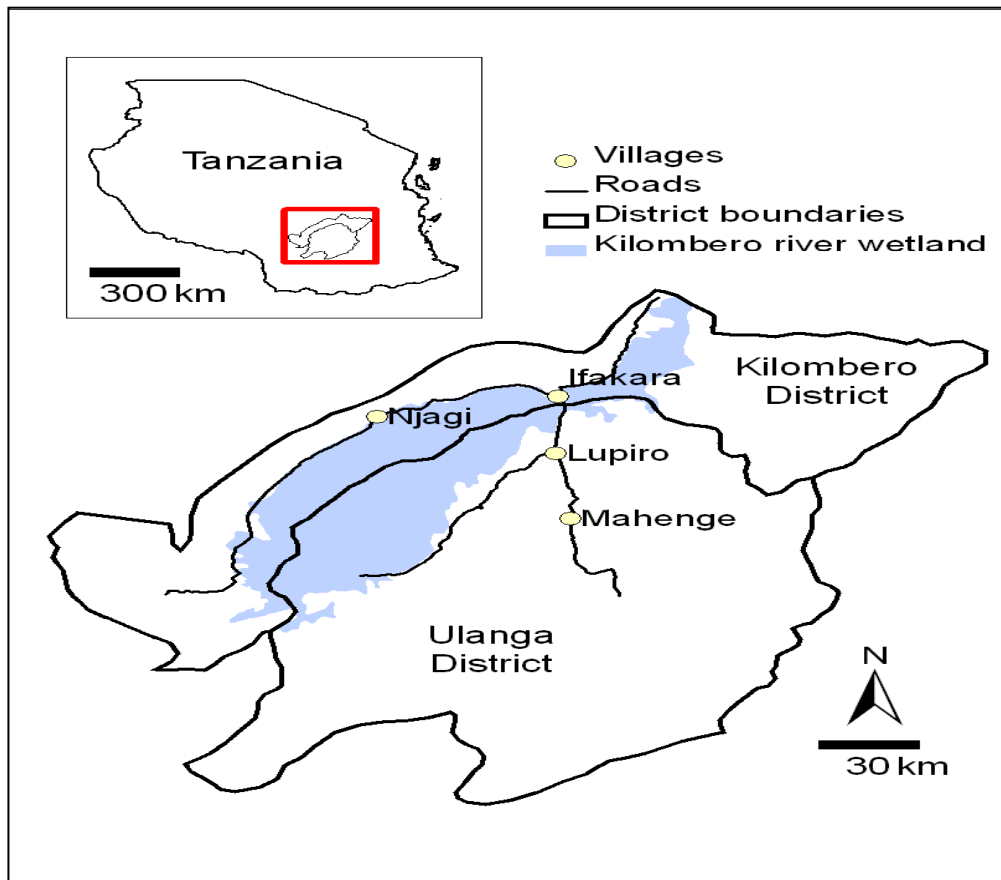


Fig 2.1. Map showing Kilombero valley and the study site (Russell *et al.*, 2011)

A large proportion of the villagers are subsistence farmers (mainly of rice) who get exposed to maximum mosquito populations during the growing and harvesting periods while living in the temporary farm (shamba) houses (Fig. 2.2). This is because the majority of shamba houses are poorly constructed and do not prevent malaria vectors entry. For example, they generally lack insect-proof wire mesh on windows, ceiling boards, curtains and have large eaves (Fig 2.2).



Fig 2.2. Typical shamba house.

Along with malaria, the people in the Kilombero Valley are also faced with other infectious vector-borne diseases such as River Blindness (onchocerciasis`), trachoma and elephantiasis (filariasis). There is no vector control programme for these diseases, instead they are controlled through mass drug administration which is mainly Mectizan for river blindness (Kilosa Focus 2001, unpublished report), trachoma through zithromax in the style of a ‘district wide approach’, and ivermectin for filariasis.

2.3 Mosquito collection

All the mosquitoes/materials were sourced through routine on-going entomological surveillance by the Malaria Transmission Consortium (MTC) project of the Ifakara Health

Institute. Mosquitoes resting inside houses and outdoors from resting boxes were collected by using a back pack-aspirator from 0700am to 0830am. The indoor and outdoor human biting rates of *An. funestus* group were estimated from human landing capture (HLC) and Sticky Bucket Traps (SBT). HLC was conducted by one pair of adult male technicians sitting with their legs exposed and using hand aspirators to capture mosquitoes which came to bite (Silver, 2008) (Fig. 2.3). SBT was conducted by a pair of adult male technicians, who sat with their bare legs inside an exposure-free bucket and their upper parts protected with intact untreated bed nets. The bucket has a wire mesh on its sides treated with rat glue which traps each landing mosquito (Figs 2.4 and 2.5). Trapped mosquitoes were then removed from the sticky traps by gently touching mosquito legs with a mini paint brush dipped in petrol. This method did not affect the PCR identifications. Mosquitoes caught from both SBT and HLC were put in cups that were labelled with time and place where they were caught. Both HLC and SBT collections were done from 06:00pm to 07:00am for 16 days in 4 village houses selected at random, including houses with interventions (ITNs) and without interventions. A 4X4 Latin Square design was adopted for this study to minimize confounding factors. The design for 4 houses included 8 male volunteers in which 2 were used in each house per night, one was positioned inside and the other outside. Two houses used HLC technique and other two houses employed SBT technique. On the first day one volunteer took position inside and the other outside houses with individual treatments (untreated or treated bed nets) and trapping technique (HLC or SBT). On the second day volunteers changed position, maintaining other conditions. On the third day, mode of treatment was changed while the position of volunteers was maintained. On the fourth day the positions of volunteers were again swapped while maintaining mode of treatment. After

every 4 days this cycle is complete for one trapping technique for that particular house with those volunteers, so the cycle for all volunteers for all the houses and different trapping techniques was followed in the same course. Mosquitoes were collected for 45 minutes of each hour throughout the night allowing 15 minutes resting each hour.

In the morning, collections were done from 2 resting boxes inside and outside the house by back-pack aspirator. Collected live mosquitoes from SBT, HLC, resting boxes and indoor resting collections were then killed with chloroform and stored in 1.5ml eppendorf tubes with silica gel.



Fig 2.3 HLC in operation



Fig 2.4 Sticky Bucket Trap in operation

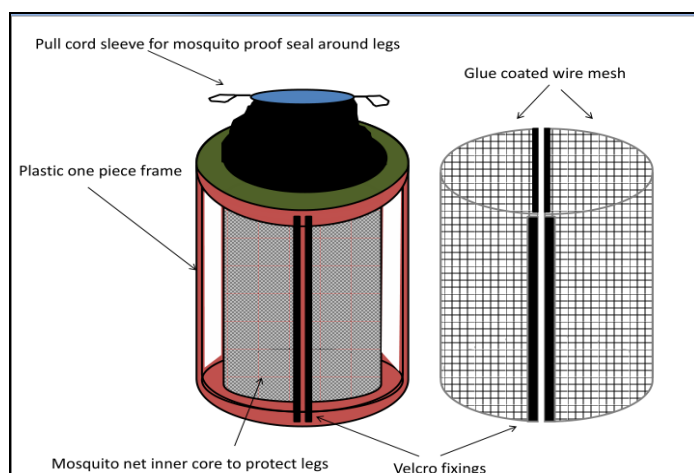


Fig 2.5 Schematic diagram of Sticky Bucket Trap

2.4 Morphological Identification

Collected anopheline mosquitoes were identified morphologically by using taxonomic keys (Gillies and De Meillon 1968; Gillies and Coetzee, 1987). All mosquitoes that were morphologically identified as members of the *An. funestus* group were further identified to species level by Polymerase Chain Reaction (PCR) and checked for the presence of *Plasmodium falciparum* sporozoites using ELISA.

2.5 Species determination by PCR

Further identification of members of the *An. funestus* group to species level by PCR method was done according to methods described in Koekemoer *et al.* (2002). DNA was extracted from each mosquito leg by methods described in Collins *et al.* (1987). DNA pellets were resuspended in 200µls of 1XTE buffer and 0.5 µl of DNA was used in a 25 µl PCR reaction mixture comprising: 2.9 10XPCR Buffer, 1.5mM MgCl₂, 200µM of each dNTP, 8.25pmol

of primer for the universal (UV), *An. funestus* (FUN), *An. vanedeeni* (VAN), *An. rivulorum* (RIV), *An. parensis* (PAR) and *An. leesoni* (LEE) primers and 0.5 units of thermostable taq DNA Polymerase.

Primer name	Sequence (5' to 3')	T _m (°C)
UV	TGT GAA CTG CAG GAC ACA T	55.34
FUN	GCA TCG ATG GGT TAA TCA TG	52.4
VAN	TGT CGA CTT GGT AGC CGA AC	58.0
RIV	CAA GCC GTT CGA CCC TGA TT	58.8
PAR	TGC GGT CCC AAG CTA GGT TC	60.5
LEE	TAC ACG GGC GCC ATG TAG TT	60.2

The following amplification conditions were applied: initial hot start at 94°C for 2min, denaturation at 94°C for 30 seconds for 40 cycles, annealing at 50°C for 30 seconds, extension at 72°C for 40 seconds and final extension at 72°C for 10 minutes. Negative controls consisted of extraction buffer with no DNA and master mix with no DNA template while the positive controls comprised DNA from previously identified mosquitoes provided by Vector Control Reference Unit of the National Institute for Communicable Diseases, Johannesburg. 12.5 µls of PCR products were electrophoresed and visualized by Gel Logic 100 Imaging System.

2.6 Sporozoite rate determination

All PCR-identified female mosquitoes were analyzed to determine the sporozoite infection. The head and thorax were dissected from the mosquitoes and crushed in Phosphate Buffered Saline (PBS) (pH 7.4) and tested to detect circumsporozoite proteins using enzyme-linked immunosorbent assays (ELISA) described in Burkot *et al.* (1984). Circumsporozoite proteins were extracted by grinding the mosquito thorax with 50µl of grinding buffer (adding 500µl of nonidet into 100ml of Blocking Buffer (BB)) at pH 8.0. 96-well ELISA plates were then coated with 50µl of antibody (Mab) solution (4µg/ml) and incubated for 1 hour at room temperature. Then ELISA plates were aspirated and filled with 50µl of blocking buffer and incubated for 1 hour at room temperature. 50µl of mosquito homogenate were then added per well, where 2 wells and seven wells in the last row on the ELISA plate were added with positive and negative controls respectively. The plates were then incubated at room temperature for 2 hours and then aspirated and washed twice with washing buffer (PBS-Tween). 50µl of Mab-peroxidase conjugate (0.025µg/50µl BB) and incubated at room temperature for 1 hour. ELISA plates were then washed with washing buffer 4 times and 100µl of ABTS substrate A and B (1:1) incubated at room temperature at dark for 30 minutes to 1 hour. Results were scored visually and with aid of an elisa plate reader at 405nm. All samples with Optical Density above the calculated cut off point were scored as positive, and confirmed by repetition.

2.7 Data Analysis

- Species composition and sporozoite rates indoors and outdoors were compared by Chi square cross tabulation descriptive analysis, using PASW Statistics 18, Release

version 18.0.0 (SPSS, Inc., 2010, Chicago, IL, www.spss.com) where -

Sporozoite rate = number of infected mosquitoes/ total number of mosq caught X 100

Indoor sporozoite rate = number of infected mosquitoes indoor/ total number of mosq caught indoor X 100

Outdoor sporozoite rate = number of infected mosquitoes outdoor/ total number of mosq caught outdoor X 100

- Biting rates were determined by indoor and outdoor collection based on host-seeking methods (HLC and SBT, which catches host seeking mosquitoes). Comparison of indoor and outdoor biting rates were compared by independent t-test using the MATLAB built in function (t-test2 (x,y))
- Effectiveness (based on number of mosquito caught) between HLC and SBT were compared by independent t-test (MATLAB), comparison between two methods for indoor catchment and outdoor catchment were done separately.

CHAPTER THREE

RESULTS

3.1 Species composition of malaria vectors at the study site

A total of 2403 wild anophelines were collected over a period of 16 days from May to June. *Anopheles gambiae s.l* was the dominant group, followed by *Anopheles coustani* and *Anopheles funestus* group respectively. *Anopheles gambiae s.l* comprised 87.5% (2103) of the total collection, *An. coustani* 6.7% (161) while *An. funestus* group was 5.8% (139). PCR identification of the *An. funestus* group (Fig. 3.1) showed that *An. funestus s.s* constituted 96.2% of all amplified specimens (n=110) and *An. lesoni* constituted 3.8% (Table 3.1). There was no difference in species composition indoors and outdoors, $P>0.05$.

Table 3.1 Species composition indoors and outdoors by PCR

Species	Indoors	Outdoors
<i>An. funestus</i>	59 (55.7%)	47 (44.3%)
<i>An. lesoni</i>	2 (50%)	2 (50%)

Figure 3.1 shows a typical agarose gel of samples of *An. funestus* after PCR amplification and electrophoresis.

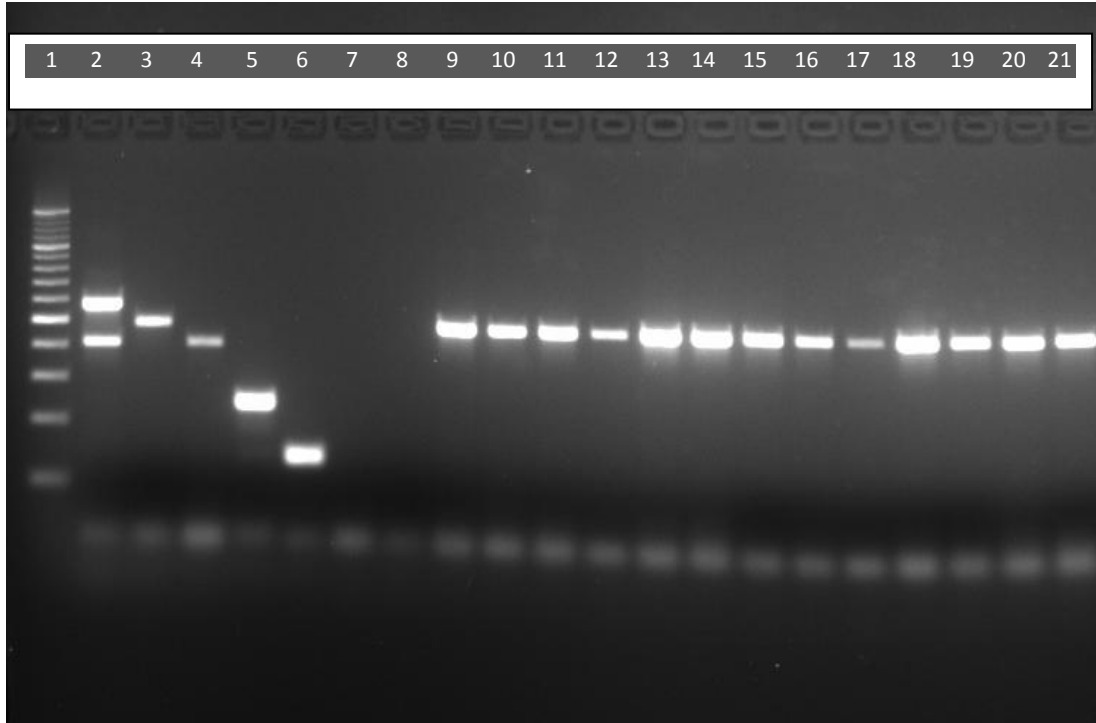


Fig. 3.1. PCR results for *An. funestus* identification.

Lane 1 = Molecular weight marker; lanes 2-6 = positive controls: *An. vanedeeni*, *An. funestus* s.s., *An. rivulorum*, *An. parensis* and *An. lesoni* respectively; lanes 7 & 8 = negative controls; lanes 9-21 = wild *An. funestus* s.s.

3.2 Sporozoite rate of *An. funestus*

ELISA tests were conducted on specimens collected outdoors and indoors, separately for houses that contained untreated and treated bed nets.

Table 3.2 Sporozoite rates of *An. funestus* indoors and outdoors in the vicinity of untreated bed nets

		Number caught	
Indoor collection	Elisa results	Positives	0
		Negatives	39
		Total	39
Outdoor collection	Elisa results	Positives	0
		Negatives	32
		Total	32

Table 3.3 Sporozoite rates of *An. funestus* indoors and outdoors in the vicinity of treated bed nets

		Number caught	
Indoor collection	Elisa results	Positives	1
		Negatives	34
		Total	35
Outdoor collection	Elisa results	Positives	0
		Negatives	29
		Total	29

Sporozoite rates indoor and outdoor when untreated bed nets were used were 0% and there was no difference ($p>0.05$).

However in the vicinity of treated bed nets:

Sporozoite rate indoors = number of positives/total collected indoors = $1/35 \times 100 = 2.9\%$

Sporozoite rate outdoors = number of positives/total collected outdoors = $0/61 \times 100 = 0.0\%$

From the above tables, the sporozoite rate was higher indoors than outdoors but this was not statistically significant: 1.439 (95%CI .320-6.477), $p>0.05$.

3.3 Biting profile of *An. funestus* at the study site under the pressure of ITNs

The effect of bed net treatment was evaluated for the endophagic and exophagic proportions of *An. funestus* (Fig. 3.2, Table 3.4). None of the factors significantly affected the indoor/outdoor proportion of *An. funestus*.

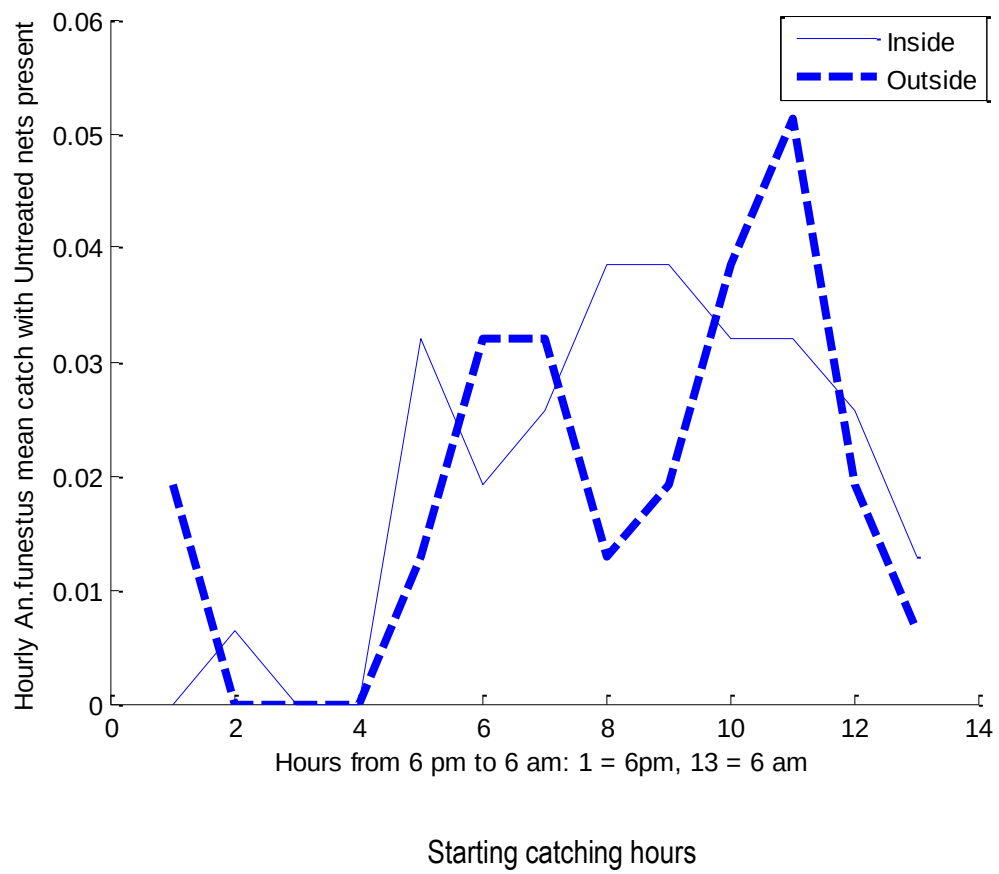


Fig. 3.2. Indoor and outdoor hourly biting profile of *An. funestus* in vicinity of untreated bed nets

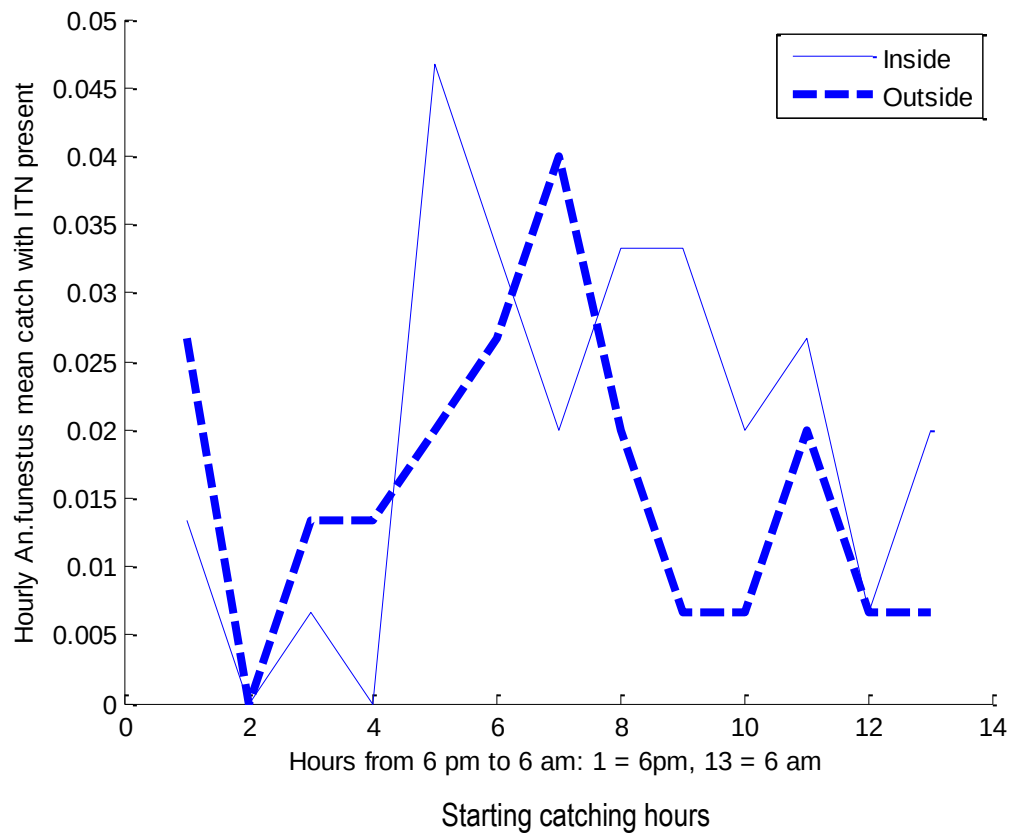


Fig. 3.3. Indoor and outdoor hourly biting profile of *An. funestus* in vicinity of treated bed nets

Table 3.4. The effect of bed net treatment on endophagic and exophagic proportions of *An. funestus*.

Condition	Place of catchment		Total caught (N)
	Indoor	Outdoor	
When untreated bed nets were used in house	54.9% (39)	45.1% (32)	100% (71)
When LLINs were used in house	53.1% (34)	46.9% (30)	100% (64)

The collections from houses with untreated bed nets were not significantly different from those houses where LLINs were present in affecting indoor/outdoor proportions: 1.597 (95%CI, 0.709-3.598), $P > 0.05$.

The figures below show biting activity before and after sleeping hours for *An. funestus*.

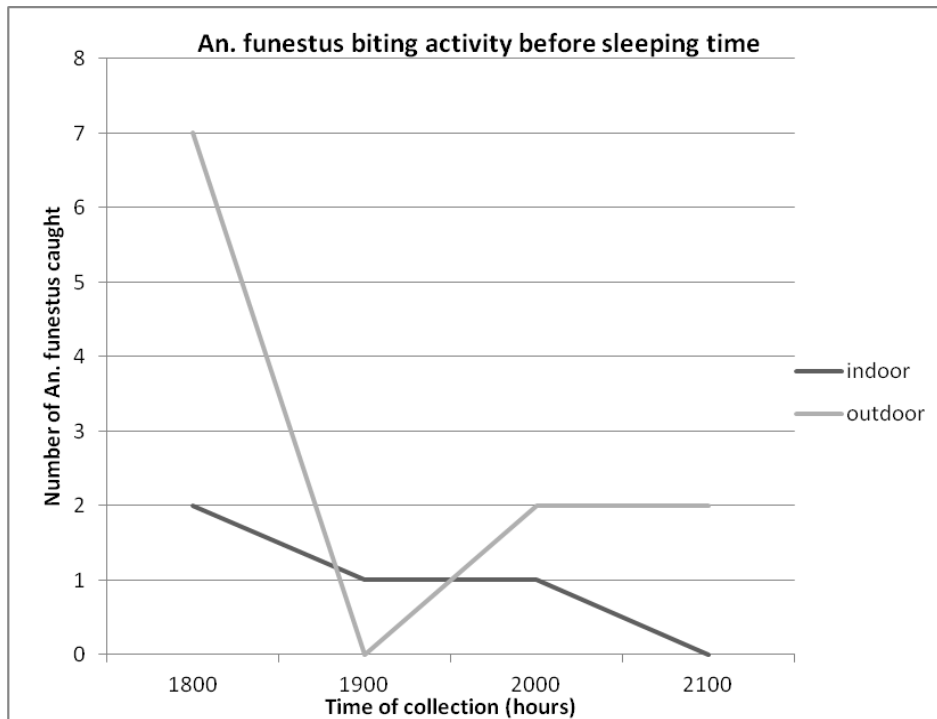


Figure 3.4 *An. funestus* biting activity before sleeping time

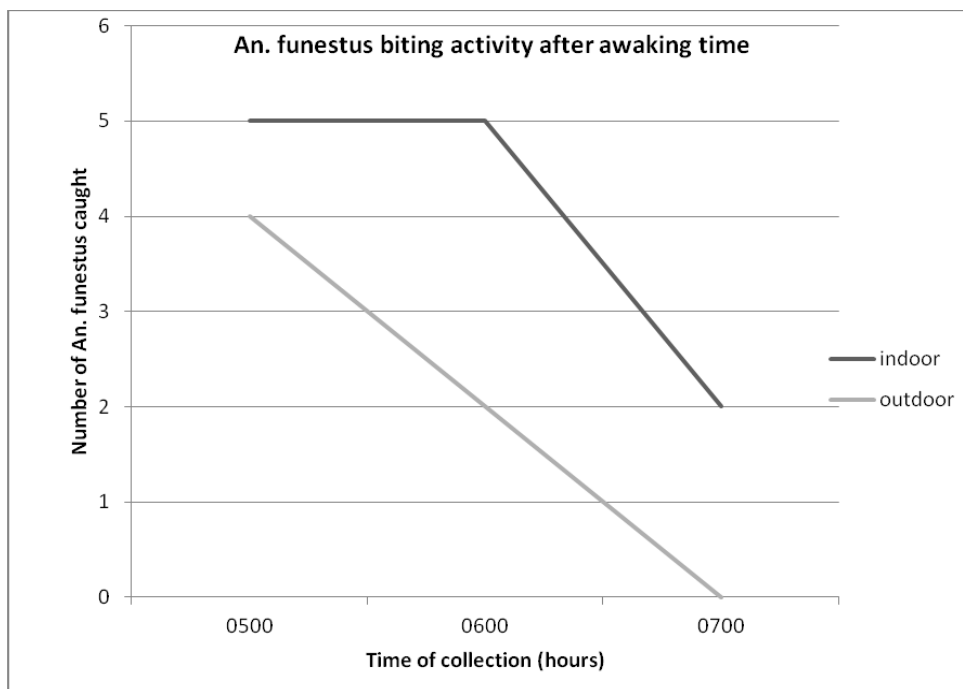


Figure 3.5 *An. funestus* biting activity after waking time

3.6 Results for HLC and SBT

The following (Tables 3.5 - 3.7) are the results of anopheline species (*An. gambiae s.l.*, *An. funestus* group and *An. coustani* complex) caught by the two techniques, human landing catch and Sticky Bucket Trap. It is clear from the results that the sticky bucket trap did not perform nearly as well as human landing catches and cannot be used to replace this standard technique.

Table 3.5. Comparison of HLC and SBT for catching *An. gambiae s.l.*

Technique used	Number	Percent
HLC	2008	95.5%
SBT	95	4.5%
Total	2013	100%

P = 0.03, d.f= 1, Relative rate = 0.591 (95% CI_{0.368 - 0.949})

Table 3.6. Comparison of HLC and SBT for catching *An. funestus* group

Method	Untreated bed net in use		Treated bed net in use	
	Indoor	Outdoor	Indoor	Outdoor
HLC	36	31	37	30
SBT	2	0	4	8
Statistical	p=2.3028e-005	p=9.0560e-006	p=3.0863e-004	P=0.0145
significance	CI _{0.0170-0.0461}	CI _{0.0159-0.0409}	CI _{0.0123-0.0416}	CI _{0.0038-0.0341}

Table 3.7. Comparison of HLC and SBT for catching *An. coustani* complex

Technique used	Number caught	Percent
HLC	134	83.2%
SBT	27	16.8%
Total	161	100%

P = 0.000, d.f= 1, Relative rate = 0.658 (95% CI _{0.544 - 0.796})

CHAPTER FOUR

DISCUSSION

4.1 Indoor and outdoor species composition of different malaria vectors

The two groups of species containing the major African malaria vectors were found. *Anopheles gambiae* s.l. (87.5%) was the dominant group with *An. funestus* (5.8%) being relatively scarce, these results are similar to previous studies (Killeen et al., 2006) in the area. This implies that *An. funestus* is the third important malaria vector after *An. gambiae* s.s. and *An. arabiensis* which were the majority. Likewise results from this study indicate that species composition within the *An. funestus* group was not different indoors and outdoors. 96.2% of the *Anopheles funestus* group were found to be *An. funestus* s.s. with only 3.8% *An. lesoni*. These results are similar to previous studies in 2009 (Russell et al., 2011) and also PCR results done in March 2010 (Okumu et al unpublished data) for 233 specimens which revealed that 96.6% were *An. funestus* s.s. while 3.4% were *An. rivulorum*. These results suggests that there is neither major species replacement from *An. funestus* s.s. in the study area nor misidentification and that *An. funestus* s.s. remains the dominant member of this group in this area.

4.2 Sporozoite rates of *An. funestus*

Sporozoite rates indoors and outdoors were not different although high biting peaks indoors (hourly mean biting of 0.05 and 0.033 when a house does not have ITNs and 0.006 and

0.047 when ITNs are in use) at a time when people are indoors and biting peaks outdoors (0.014 mean biting when a house has ITNs) when people are awake implies that there is high risk for human-vector contact during relevant times for disease transmission . The plasmodium sporozoite rates of 2.9% found for *An. funestus* when ITNs were utilized is not very different from previous studies in this area, 3.4% in 1997 (Charlwood *et al.*, 1997), 5.4% in 2000 (Charlwood *et al.*, 2000), 2.3% in 2010 (Mboera *et al.*, 2010). This presence of high infectivity (2.9% for *An. funestus*) indicates that *An. funestus* is an important vector for malaria transmission in the area. However, other areas such as Mkuzi district, northern Tanzania reported higher sporozoite rates of 24% (Kweka *et al.*, 2008).

4.3 The feeding (biting rates) behaviour of *An. funestus* when ITNs are in use and when not

Results from this study are consistent with the report (Russell *et al.*, 2011) of increased exophagy where in 2009 the indoor proportion of *An. funestus* is reported to have dropped to 50.5% from 100% in 1997 and 76.1% in 2004 as a consequence of increased ITN usage. In this study I have also found that, before the nominal sleeping time the indoor exposure to *An. funestus* was 2.6% and the outdoor exposure 7.4%. On the other hand, the proportion biting after waking time was 7.9% indoors and only 3.94% outdoors. This indicates that people are at higher exposure risk outdoors in the early evening due to outdoor biting activities. It was generally found that the endophagic proportion was only marginally higher than the exophagic proportion regardless of whether treated or untreated bed nets were being used. These results are not consistent with other reports from various parts of Africa where the endophagic proportion of *An. funestus* is normally reported as very high, usually over 55%

(Oyewole et al., 2007). This stability in behaviour in other settings could be attributed to the type of insecticide used (whether it has excito-repellent nature), ecological settings and insecticide application activities (including agriculture) and other genetically driven intrinsic features which could be restricted to specific localities. It has been suggested that treated bed nets have a higher protective capacity than untreated bed nets by lowering the density of malaria vectors (D'Alessandro *et al.*, 1995) and the repellent nature of pyrethroid formulations on bed nets leads to fewer mosquitoes entering the rooms (Chandre *et al.*, 2000). However, for *An. funestus* in the present study, untreated bed nets were not significantly different from LLINs in affecting indoor/outdoor proportions of mosquitoes.

One unusual observation was found in the hourly outdoor biting profile where, *An. funestus* exhibited multiple biting peaks (>2) when LLINs were present. This may be due to the repellency effect of the pyrethroids on the nets (Govella *et al.*, 2010) where repellency could contribute to disturbed feeding or host seeking activity of mosquitoes. The previous study carried out in this area (Russell *et al.*, 2011) shows in figure 4.1 only one biting peak for both indoor and outdoor collections.

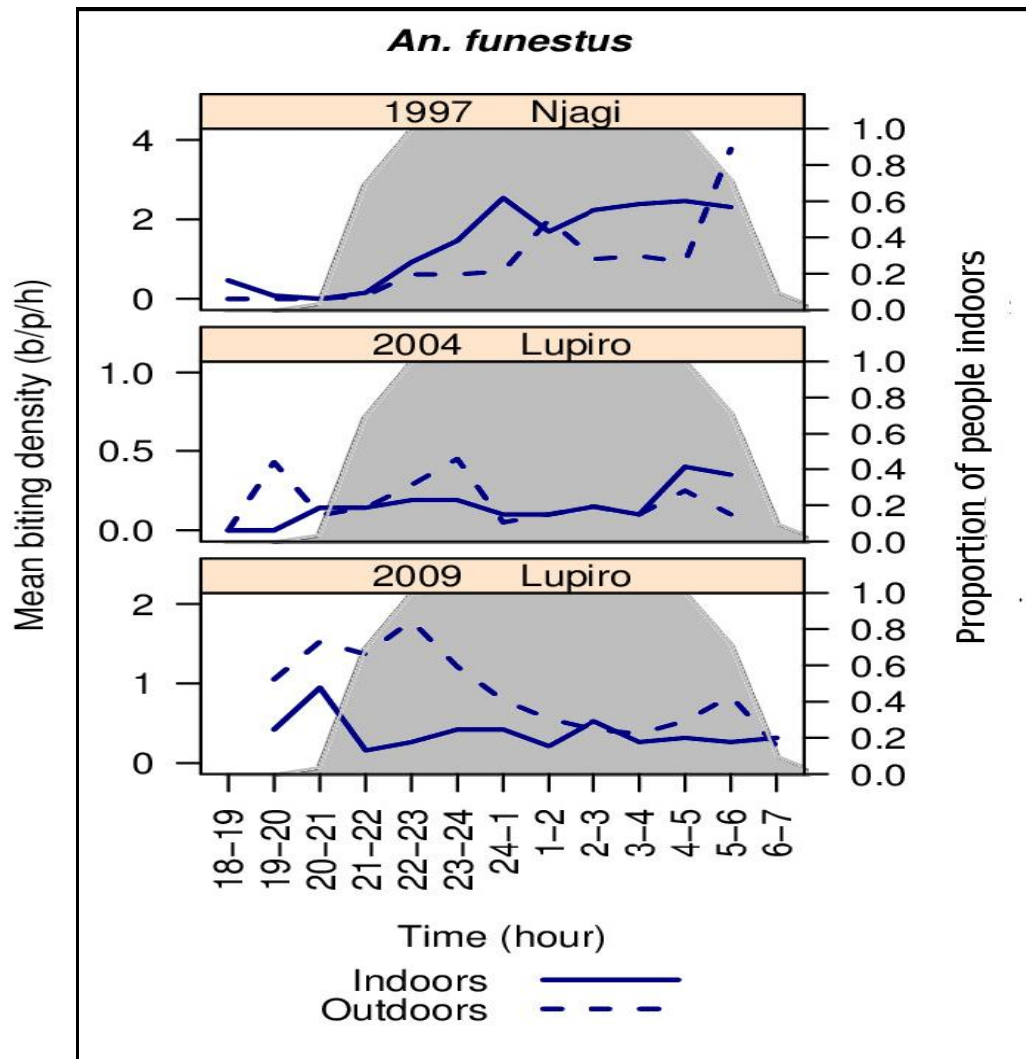


Fig 4.1 Biting pattern of *An. funestus* from 1997 to 2009 (Russel *et al.*, 2011)

4.4 Effectiveness of SBT against that of HLC

From this study it was observed that the sticky bucket trap always caught markedly less mosquitoes than human landing catches.

4.5 Conclusion

In this study *An. funestus* behaviour was only found to be modified (relative to fig 4.1) in the hourly biting pattern but the indoor and outdoor feeding proportions were not significantly different. This means the level of exposure indoors and outdoors is similar. The observed biting peak outdoors during waking hours could be responsible for malaria transmission even if most houses utilize treated bed nets and this will require outdoor vector interventions to compliment the indoor interventions. It is concluded that an increased exophagic proportion of *An. funestus* biting at hours when people are still awake, has an impact on malaria transmission in this area of rural Tanzania. The use of ITNs in this area as the major vector control intervention has little or no effect on bites at a time when people are not under bed nets. The application of outdoor interventions like use of repellents, larviciding and traps could be used to supplement ITNs in Tanzania. Also, the sticky bucket trap is not a suitable substitute for human landing catches and some modifications are needed to make it more effective.

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