EFFICACY OF THE D-DESIGN IFAKARA TENT TRAP FOR SAMPLING MALARIA VECTORS IN AN AREA OF MASS LONG LASTING INSECTICIDAL BED NETS USE

By

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Registration number: I56/79048/2010

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Applied Parasitology of the University of Nairobi

DECLARATION

This is my original work and has not been presented for a degree in any other University.

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ACKNOWLEDGEMENT

DEDICATION

I dedicate this thesis to the men and women who work tirelessly hard to make health care better for everyone in the world and to my late parents Mr. and Mrs. Opondo.

I specially dedicate this to my late sister Mary Goretty Atieno. You endured all sufferings to make a better and brighter future. Rest in Peace dear sister.

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The quest to develop alternative mosquito surveillance tools that substitute for Human Landing Catches and that are effective, exposure free and easy to use has resulted in the development of a variety of traps. These include Centres for Disease Control miniature light traps, Ifakara Tent Traps, Mbita traps, Furvela tent traps and Back Pack Aspirators. With the global agenda currently mentored to eradicate malaria, effective sampling tools that can detect low mosquito vector densities and low transmission levels will be increasingly needed so that successful control programmes can monitor and manage their efforts to achieve local elimination.

Ifakara Tent Trap (ITT) was a promising outdoor mosquito sampling tool. The B and C designs of the ITT have previously proven to have acceptable sampling sensitivity levels in urban and rural Tanzania. However, they require a large amount of space to set up due to the ropes and pegs used to stretch them horizontally. In this study, the D design (ITT-D), modified from C design (ITT-C) and which uses a smaller space, was evaluated in parallel with ITT-C and CDC-LT in Lupiro in southern Tanzania where ITT-B and C had been earlier proven effective relative to CDC-LT or HLC.

Surprisingly, both ITT-C and ITT-D exhibited very poor sampling sensitivity relative to CDC-LT with relative rates, at 95% CI, 0.131 (0.119 – 0.144) and 0.044 (0.37 – 0.51) respectively. The mean catches for ITT-C, ITT-D and CDC-LT was 12.4 ± 18.77, 3.96 ± 0.424 and 90.5 ± 18.77 respectively. To understand the reason for this reduced efficiency of ITT-C, F1 generation of Lupiro *An. arabiensis* of 2011, laboratory reared *An. arabiensis* adults from Sakamaganga and Lupiro colonized in 2008 and 2009 respectively, were compared on their likelihood to enter ITT-C.

The wild mosquito populations in Lupiro of 2011 were less likely to enter ITT-C, relative rate 0.056 (95% CL 0.015 – 0.201, P< 0.001). Lupiro 2009 colony were more likely to enter ITT-C, relative rate 0.813 (95% CL 0.415-1.5010, P= 0.545). From time of development of ITT, time trend analysis of trap's sampling sensitivity from conducted studies showed declining trapping sensitivity.

This study showed that, in addition to other factors not investigated due to resource limitation, reluctance by Lupiro 2011 mosquito population to enter ITT-C appears to be a heritable trait passed to offspring. This reluctance to enter ITT-C appears to be associated with altered host-seeking patterns resulting from scale up of LLIN use in Kilombero Valley. It was thus concluded that ITT-D was not a better tool for sampling malaria vectors in this valley.

ACRONYMS

UMCP	Urban Malaria Control Programme
ITT	Ifakara Tent Trap
IVM	Integrated Vector Management
HLC	Human Landing Catch
BPA	Back Pack Aspirator
Bti	*Bacillus thuringiensis var israelensis*
IHI	Ifakara Health Institute
ITN	Insecticide Treated Net
IPT	Intermittent Presumptive Therapy
CDC-LT	Centres for Disease Control miniature light trap
EIR	Entomologic Inoculation Rate
ELISA	Enzyme Linked Immunosorbent Assay
MTC	Malaria Transmission Consortium
IRS	Indoor Residual Spraying
PCR	Polymerase chain reaction
NIMR	National Institute for Medical Research
LLIN	Long Lasting Insecticide Treated Net
LSM	Larval source management
PSC	Pyrethrum spray catch

Ss	sensu stricto
Sl	sensu lato
SFS	Semi field system
SPSS	Statistical Package for the Social Sciences
PASW	Predictive Analytics SoftWare

1.0 CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The human landing catch (HLC) technique for sampling mosquitoes has routinely been used over the years to sample host seeking mosquitoes for purposes of estimating biting rates and malaria transmission (Beier *et al.*, 1999; Hay *et al.*, 2000). The technique exposes human participants to bites from mosquitoes that might be infected with malaria or other vector borne disease agents (Lines et al., 1991). Hence, there has been a search for suitable replacements to HLC that are exposure-free and ideally do not require the user to stay awake all night (Govella *et al.*, 2009; Mathenge *et al.*, 2002).

Many trapping tools have been developed and evaluated in diverse settings of high and low vector density (Service 1977; Service 1993). The need for such tools is exacerbated by the inability of existing tools to sensitively sample malaria vectors that occur in low numbers and bite outdoor in urban areas (Geissbuhler et al., 2007). Urgency to develop these tools is hastened by the presently globally accepted goal to eliminate and eradicate malaria (Campbell and Steketee 2011; WHO 2010) which shall see various regions have a reduction in mosquito density and malaria transmission. Further, with urbanization occurring faster (Hay *et al.*, 2005; Keiser *et al.*, 2004), vector densities per person are lowered (Killeen *et al.*, 2000a; Robert *et al.*, 2003; Smith *et al.*, 2004). Therefore, without proper tools, monitoring malaria transmission will be increasingly difficult.

Malaria control efforts incorporating vector control strategies have achieved impressive reduction in mosquito populations (Magesa *et al.*, 1991; Robert and Carnevale 1991) and malaria related morbidity in humans (Gimnig *et al.*, 2003b; Lindsay *et al.*, 1989; Snow *et al.*, 1988). However, residual vector populations that remain after introduction of Long Lasting Insecticidal nets (LLIN) and Indoor residual Spraying (IRS) have altered feeding patterns, shifting from biting late in the night to early in

the evening (Charlwood and Graves 1987; Magesa *et al.* 1991; Russell *et al.,* 2011; Russell *et al.,* 2010).

It is hypothesized that at night, host-seeking female mosquitoes cannot forage on humans protected by an LLIN so female mosquitoes seek blood early in the evening while most humans are outdoor and fully exposed (Govella et al., 2010b). The reduction in density of malaria-transmitting mosquitoes and their change in host seeking behaviour make it difficult to measure and characterize malaria transmission by entomological means. This is because suitable tools for collecting mosquitoes, especially those that feed outdoor, are lacking (Govella et al., 2010a), particularly where vectors occur at low densities such as urban centres and arid areas.

Understanding mosquito host seeking and resting behaviour is essential to the development of sampling tools. Host-seeking mosquitoes may be attracted and lured into traps baited with mammals. Alternatively, mosquitoes which are seeking suitable resting locations may be attracted to natural or artificial shelters with or without such baits. Any change in host seeking/resting patterns further complicate development efforts of traps.

The Ifakara Tent Trap C design (ITT-C) was developed as an outdoor sampling tool and has been evaluated and applied to routine vector surveillance in urban Dar es Salaam, Tanzania (Govella *et al.* 2009; Govella *et al.* 2010a; Sikulu *et al.,* 2009). However, due to limited space in congested urban settings, its application has been limited because the ropes and pegs used to stretch it horizontally require a large amount of space. The ropes were thus replaced by an internal support frame to minimize space needed to set it up and a rain protection flap was added to the sides to make it more user friendly (figure 1). This modified trap was series D of the ITT and named the D design of the

Ifakara Tent Trap (ITT-D). We sought to determine whether sampling sensitivity of ITT-C was affected by the structural changes made to convert it into ITT-D.

Support frame   Rain protection flap   Entry cones   Rope
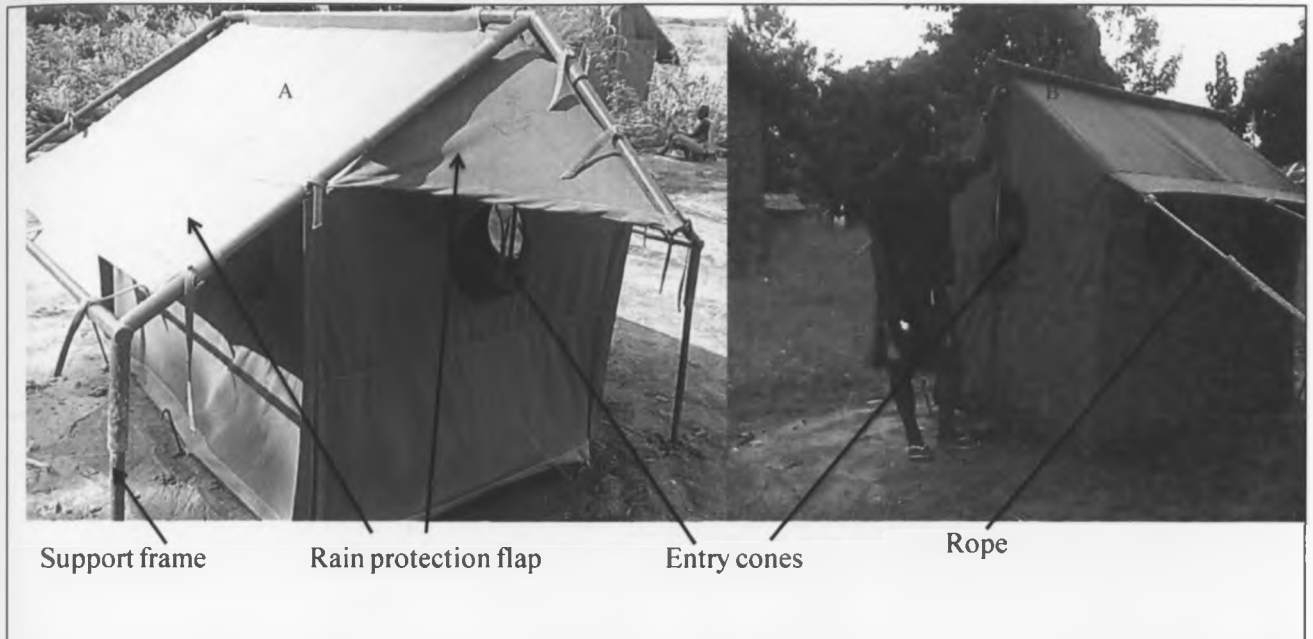
Figure 1: A- Ifakara Tent Trap D-design (left) with metal frame and extended rain protection flaps and B- Ifakara Tent Trap C-design (right) with ropes fully stretched

ITT-D was evaluated in parallel with ITT-C and Centres for Disease Control miniature Light Trap (CDC-LT) in the village of Lupiro which is situated in the Kilombero valley, southern Tanzania, an area where ITT-B and ITT-C have proven to be successful (Govella *et al.* 2009). From the year 2000, Kilombero valley community has benefited from subsidization and distribution of LLIN from public and private sector schemes (Killeen et al., 2007) so the valley presently boasts of 80% LLIN usage rates (Russell *et al.* 2010).

Evaluation of ITT-D led us to the surprising observation that ITT-C which initially worked in the valley had now greatly fallen in sampling efficiency. We therefore conducted experiments in an enclosed semi-field system to determine whether the reduced sampling efficiency of ITT-C was due to

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trap retention failure or mosquitoes refusing to enter the trap. We also sought to determine whether refusal to enter the trap was a heritable trait and whether it was associated to altered host seeking behaviour arising from successful LLIN scale up in the area.

1.2 Literature review

1.2.1 Background

The search for an effective tool for sampling malaria vectors began as early as 1902 when Ronald Ross developed a net trap with openings to permit mosquito entry but not exit (Bath 1931). This development came with the discovery that female *Anopheles* mosquitoes actively seek humans as a blood source to provide them with the protein that they need to develop their eggs. Further, female mosquitoes were attracted preferentially to human odor, as observed by the high density of mosquitoes that were trapped in detachable traps put in screened ventilators of laborers' barracks. Bath later concluded as follows:

"The mosquito trap is going to prove of great value in research work...I am strongly convinced of its practical value as an important anti-malaria measure and I believe that mosquito traps will be built into houses as an essential part of the screening on dwellings in unsanitated areas."

This marked the beginnings of mosquito sampling and development of malaria vector monitoring tools. At the time, accurately measuring the rate of malaria transmission was hampered by the lack of tools to undertake such exercises. Also, scarce knowledge about malaria vector ecology precluded adequate insight into vector population and transmission dynamics.

Various mosquito sampling tools have been developed and evaluated in different malaria transmission settings (Braimah *et al.*, 2005; Davis *et al.*, 1995; Githeko *et al.*, 1996; Govella *et al.* 2009; Krockel *et al.*, 2006; Mathenge *et al.* 2002; Okumu *et al.*, 2010a; Okumu *et al.*, 2008). The human landing catch (HLC) technique has traditionally been used for sampling host seeking mosquitoes because it is assumed to give an accurate measure of the actual biting activity of the vector upon humans (WHO 1975). Humans performing HLC (figure 2) usually expose their limbs and using an aspirator or collector tube, capture landing mosquitoes that are assumed to have come for a blood meal (Service 1993; Service and Townson 2002).

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Figure 2: Human subject collecting mosquitoes from his exposed limbs. The Human Landing Catch (HLC) is performed using an aspirator (A) and a torch (B).

Of the developed mosquito sampling tools, HLC still remains the gold standard method for estimating mosquito biting rate (Service 1977). However, it is impractical to use in large scale mosquito surveillance because it is extremely laborious and it necessitates a risk of exposure of volunteers to mosquitoes infected with malaria and other vector borne pathogens (Lines *et al.* 1991). These limitations together with the ethical concerns of HLC application have driven numerous efforts to develop alternative exposure-free tools that can be used under field conditions on a large scale and in a wide range of transmission settings (Govella *et al.* 2009; Okumu *et al.* 2010a).

These efforts to develop alternative sampling tools to HLC have seen development of various traps like the Centres for Disease control miniature Light Trap (CDC-LT) placed next to a bed net (Mboera *et al.,* 1998), the Mbita bed net trap, the Ifakara Tent Trap (ITT), and the Furvela Tent Trap and many other sampling methods. These have been designed and evaluated for particular mosquito species in

different settings, giving mixed results regarding their sampling efficiencies (Fornadel *et al.*, 2010; Govella *et al.* 2009; Laganier *et al.*, 2003; Maia *et al.*, 2011; Mathenge *et al.* 2002; Okumu *et al.* 2008).

The Mosquito Magnet-X® (MMX), a counter flow geometry trap (American biophysics Corporation ltd, North Kingstown, R.I, USA) baited with various mosquito attractants has been tested in various regions with varying degrees of success (Mboera *et al.*, 2000; Mukabana *et al.*, 2012; Njiru *et al.*, 2006; Okumu *et al.*, 2010b; Ritchie *et al.*, 2008) but its applicability for large scale surveillance is deterred by the cost of both the trap and bulk carbon dioxide it requires.

Pyrethrum Spray Catch (PSC) collections (Service 1977) and BPA (Bayoh *et al.*, 2011; Facchinelli *et al.*, 2008; Maia *et al.* 2011) have primarily been used to monitor resting mosquitoes. Their major setback as sampling tools is that they do not sample host-seeking adult females and outdoor resting mosquitoes are often thinly distributed across large spaces thus their utility as outdoor vector sampling tools is limited. Although initial trials of the Mbita bed net trap in western Kenya yielded encouraging results (Mathenge *et al.* 2002), when it was tested in southern Tanzania and Madagascar, it was found to have poor sampling sensitivity relative to HLC and CDC-LT (Laganier *et al.* 2003; Okumu *et al.* 2008).

Figure 3: CDC light trap (A), a technician using Back Pack Aspirator (B) and Mosquito Magnet-X® (C).

The poor sampling efficiencies and lack of consistency of these traps in low and high transmission settings with correspondingly low vector density have driven the need to develop more sensitive tools that can measure vector density and transmission in a diversity of settings, especially those where vector density occur at low levels (Govella *et al.* 2010a; Mbogo *et al.*, 1993). With the reignition of malaria eradication agenda (Alonso *et al.*, 2011; Tanner and Hommel 2010), rapidly growing (WHO and UN 2002) areas and regions in sub Saharan Africa are experiencing low malaria transmission and low vector density upon implementation of mosquito control programmes. This eradication agenda adds further urgency to this quest for improved monitoring and surveillance tools.

Vector control methods such as larval source management (LSM) and LLINs are now increasingly employed together with other malaria control strategies (WHO 2007; WHO 2010) to ensure low transmission levels. Integrated vector control combining two or more of these options sets the stage for subsequent progress towards malaria elimination (Kaneko *et al.*, 2000; Tanner and Hommel 2010).

Although current sampling tools often fail to detect and accurately quantify low densities of vectors and malaria transmission, these sparse mosquito populations are sufficient to mediate self-sustaining transmission (Beier *et al.* 1999; Gu *et al.*, 2003; Robert *et al.* 2003; Smith *et al.*, 2005). It is important to note that continuous monitoring, evaluation and adaptation of appropriate control programmes are fundamental to ensure progressive reduction of malaria transmission and ultimately elimination (De Savigny *et al.*, 2004; Mboera *et al.*, 2007; Rugemalila 2007).

A trap's success depends on local vector species composition and the variety of feeding and resting behaviours (Coetzee *et al.*, 2000; Pates and Curtis 2005) and no trap appears to be universally applicable to all vectors or settings. For example, the standardized CDC-LT (figure 3) has worked well in a wide range of transmission setting but failed to yield satisfactory results when compared with HLC in urban Dar es Salaam, Tanzania (Fillinger *et al.*, 2008; Govella *et al.*, 2011). This prompted the development of ITT in 2006 as an alternative exposure-free, sensitive, affordable and reliable sampling tool for large scale field surveillance.

The initial B design of ITT (ITT-B) compared well with HLC in its sampling power. Human occupants inside ITT-B at night are protected from bites of trapped mosquitoes by a netting layer that separates trapping chamber from the area occupied by human. Nevertheless, users still came into contact with trapped mosquitoes during emptying of nights' catches from the large trap chamber which was awkward to collect from. This is because the netting layer that separated humans had a long 2 metre zipper which had to be opened so that volunteer inserts his entire head and shoulders into the trap chamber while aspirating the trapped mosquitoes from the tent. An improvement of the trap to correct for the problem of exposure during aspiration was implemented and the trapping chamber was

split into two and a protective sleeve that prevents biting during aspiration inserted. This revised design was named the Ifakara Tent Trap design C (ITT-C).

The B and C designs of ITT which were developed and tested in Tanzania both in high (rural Kilombero valley) and low (urban Dar es Salaam) transmission zones have proven to be reasonably sensitive when compared with HLC (Sikulu *et al.* 2009) and CDC-LT (Govella *et al.* 2011). To erect the tent traps, both ITT-B and ITT-C utilize ropes and pegs that stretch them out horizontally. However, these ropes occupy a large footprint so that the tent stands out firmly. In densely populated urban settings, where space is limited, using the tent traps is difficult. In addition, occasionally when it rained, raindrops could spill into the tent trap making it unsuitable for use for humans. Modification by replacing the ropes with an internal support frame and addition of rain protection flaps has resulted in a D design ITT that now uses a smaller footprint and is easier to erect (figure 1).

1.2.2 Malaria transmission

Human malaria is an infectious disease that can be acute, chronic, mildly symptomatic or asymptomatic caused by five protozoan parasites from the genus *Plasmodium*. These five protozoa are *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmidium ovale*, *Plasmodium vivax* and *Plasmodium knowlesi*. The five parasites are all transmitted by female mosquitoes of the genus *Anopheles* (Guerrant et al., 2004). In Africa, the most important malaria vectors belong to the *Anopheles gambiae* complex, specifically *Anopheles gambiae sensu stricto* Giles and *Anopheles arabiensis* Patton as well as *An. funestus ss* within the *An. funestus* group (Gillies and De Mellion 1968; Takken and Knols 1999).

1.2.3 Life cycle of *Plasmodium* parasites

During feeding, the female *Anopheles* mosquito may ingest *Plasmodium* gametocytes circulating in the peripheral blood of an infected human host. Within the mosquito mid-gut, the gametocytes (female macrogamete and male microgamete) fuse to form a zygote that further develops into a motile ookinete. The ookinetes then migrate through the intestinal epithelium to develop into oocysts. Approximately six days after ingestion of gametes, the oocysts differentiate to contain numerous sporozoites. After approximately 12 days, the sporozoites are released into the haemocoele of the mosquito following the bursting of the oocysts. These sporozoites migrate to the salivary gland where they become infective to humans. (Guerrant *et al.* 2004). The parasite's development within the mosquito varies according to species and environmental factors and may take between 8 and 20 days to produce infective sporozoites (Beier 1998).
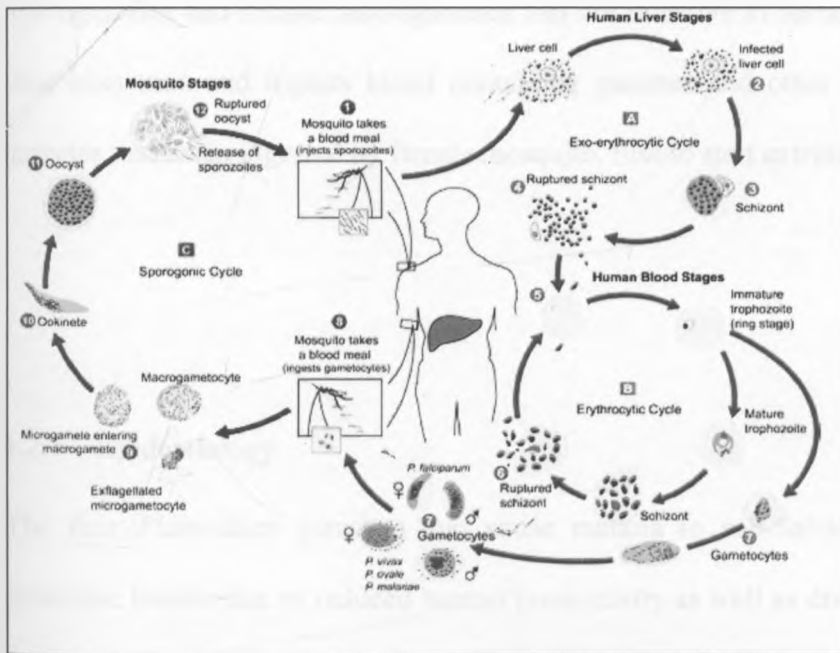
Figure 4: *Plasmodium falciparum* life cycle in human and mosquito (CDC)

The mosquitoes then remain infected for the remainder of their lifetime. During subsequent blood meals, the infected *Anopheles* mosquito discharges, into skin/adipose tissue, saliva containing sporozoites thereby effecting transmission to a new human host.

Inside the human host, the sporozoites quickly migrate through the blood to the liver in 2 to 30 minutes where they infect hepatocytes (liver cells). The sporozoites bind to the hepatocytes via receptor-ligand interactions. Further development inside the body varies according to the infecting *Plasmodium* parasites. Once inside, they develop into schizonts that later burst out from liver cells to release merozoites after approximately 6 days. Merozoites then invade red blood cells to form ring trophozoites that then mature into schizonts in 43 to 48 hours. The infected red blood cells with mature schizonts burst to release merozoites into the blood stream that re-infect other red blood cells to repeat the blood stage cycle approximately every two days. Some of the merozoites develop into male microgametes and female macrogametes that are infective to female *Anopheles*. A female *Anopheles* thus bites man and ingests blood containing gametes and other stages of the parasite. It is these gametes that when ingested by female mosquito, fuse to start extrinsic development phase

1.2.4 Epidemiology

The four *Plamodium* parasites that cause malaria in sub-Saharan Africa has resulted in heavy economic burden due to reduced human productivity as well as diversion of resources towards health care and malaria control (Breman *et al.,* 2004; Gallup and Sachs 2001). Malaria caused by *P. falciparum* has long been documented as the most virulent (Bass 1920). Malaria has caused more than one million deaths annually until recent scale up of effective interventions that has seen substantial reduction of transmission and approximately 665,000 deaths in 2010 (Murray *et al.,* 2012; WHO

2010). Although malaria transmission in Africa is heterogeneous with some different areas having high and low transmission, Africa still bears over 70% of the global malaria burden (WHO 2010).
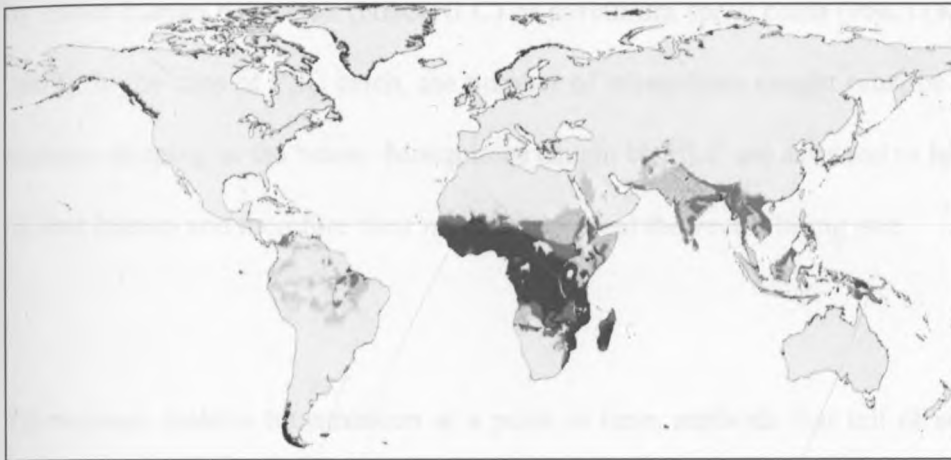
Figure 5: Map showing *P. falciparum* malaria distribution. Light green; hypo-endemic zone (childhood infection prevalence is less than 10%); medium green, meso-endemic zones (childhood infection prevalence is 11-50%); dark green, hyper/holo-endemic zones (>50%).

Successful transfer of *Plasmodium* parasites from one infected host to an uninfected and/or infected host by a mosquito results in transmission. Various methods of measuring the rate of malaria transmission have been used including incidence and prevalence rates. Malaria transmission can also be measured entomologically by expressing as a product of the vector biting rate upon humans and the proportion of mosquitoes infected with sporozoite-stage malaria parasites. This gives what is called the entomologic inoculation rate (EIR) that is still considered to be the most direct entomological measure of transmission (Beier *et al.* 1999).

Sporozoites can be detected in the female mosquito by dissecting the salivary gland and microscopically visualizing the sporozoites on slides (Service 1977). Alternatively, sporozoites can be detected by enzyme-linked Immunosorbent assay (ELISA) (Burkot et al., 1984) or polymerase chain reaction(Bass *et al.,* 2008) Estimation of vector biting rate requires capturing all adult female mosquitoes that have fed or are attempting to feed in a single night. Mosquito capturing can be done by either human bait catch (HBC/HLC) or pyrethrum spray catch (PSC) (Kelly-Hope and McKenzie 2009). In the case of PSC catch, the number of mosquitoes caught must be divided by the number of humans sleeping in the house. Mosquitoes caught by HLC are assumed to have come for a blood meal on that human and therefore their number represent the vector biting rate.

To measure malaria transmission at a point in time, methods that tell of seasonality of transmission typical of malaria is necessary to give insight of transmission dynamics. While rapid and appropriate treatment of clinical cases of malaria are shown to drastically reduce the number of deaths resulting from malaria, it is not thought that such strategies can in themselves alone reduce the prevalence of malaria in stable transmission areas in sub-Saharan Africa. This is because parasite clearance within a community is not instantaneous hence to use parasitological indices alone to measure transmission may not be sufficient to show seasonality of transmission (Smith et al., 1993).This calls for synergistic surveillance of malaria using entomological detection of parasites in mosquitoes and prevalence measurement in humans.

In the context of Africa where anthropophagic vectors can mediate EIR of more than 1000 bites per person per year, low transmission intensity refers to places with an EIR of less than 10 infectious bites per person per year and these typically occur in areas that are urban, arid or at high altitude (Hay *et al.* 2005; Mala *et al.,* 2011; Paaijmans *et al.,* 2009). These places are characterized by relatively low vector density and therefore low parasite prevalence in children below the age of five. Therefore, as a

result of less exposure of all age groups to *Plasmodium* parasites, children and adults are at an equal chance of experiencing clinical malaria due to poor immunity to malaria parasites.

On the contrary, high transmission zones are places that experience high malaria prevalence in children under the age of five with many cases of asymptomatic malaria infections throughout the year among older age group population and few febrile episodes. Such areas are also known as stable malaria transmission zones. The few febrile cases among adults could be due to well developed immunity (Hviid 2005) against malaria parasites resulting from the ever present asymptomatic *Plasmodium* infections within the population (Bejon et al., 2010).

Various factors determine malaria transmission intensity and the associated risk of exposure in an area: Temperature (Paaijmans *et al.* 2009) and therefore altitude (Garnham 1948), for example, affect malaria transmission with temperatures of between 27 and 31°C considered optimal for the development of both the parasite and the vector. During mosquitoes' host foraging activities in search of available blood meal sources, mosquitoes avoid travelling long distances (Killeen *et al.,* 2003; Midega *et al.,* 2007; Service 1997) by feeding on the most readily available hosts close to their breeding sites. Hence, malaria cases tend to be localized where humans live in proximity to mosquito breeding sites and reduce with increasing distance from the breeding sites both in urban and rural areas (Bejon *et al.* 2010).

The economic status of individuals in rural and urban areas can also result in difference in transmission rates. The economic status affects their power to acquire basic prevention measures like LLIN; mosquito-proofed houses or antimalarial drugs (Ogoma *et al.,* 2010; Sachs and Malaney 2002). Therefore, in urban areas, where individuals are less poor compared to rural areas, access to prevention measures reduce the transmission potential.

Another important factor determining malaria transmission is vector density. Studies have shown that as vector density increases, so does malaria transmission (Robert *et al.,* 1992; Staedke *et al.,* 2003; Thompson *et al.,* 1997; Trape *et al.,* 1992). This can be explained by the higher number of infectious bites experienced by people living in an area with high mosquito density. Nevertheless, other studies have found reducing malaria transmission with increase in vector density (Diuk-Wasser *et al.,* 2005; Muturi *et al.,* 2008). Reduced vector competence arising from competition of the many young vectors for available resources was given as the reason for this reverse observation.

1.2.5 Urban malaria

In the 20th century, Africa, as part of the developing world has rapidly urbanized (WHO and UN 2002) and is continuing to do so. This has affected the epidemiology of infectious diseases especially malaria. Presently, an increasing proportion of the continent's population lives in relatively low malaria transmission zones (Hay *et al.* 2005). Some authors have described an urban area as a geographical region whose boundaries are specified by a municipal/national government authority and contains one or more areas with a high concentration of businesses, housing, paved streets and roads, with a high population density, where agriculture is regulated by a municipal authority, and with a total population size that exceeds 15,000 people (Castro *et al.,* 2004).

Malaria transmission in urban areas is often described as relatively low compared to rural areas, typically with an EIR of less than 5 infectious bites per person per year which is nevertheless more than sufficient to maintain transmission (Beier *et al.* 1999; Gu *et al.* 2003; Smith *et al.* 2005). Many factors contribute to low malaria transmission such as pollution, construction and proper drainage that may reduce the quantity of available larval habitats. In addition, high level of mosquito avoidance by humans resulting from widespread use of window screens (Ogoma *et al.* 2010), LLINs (Govella *et al.*

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2010b) and other insecticides also contribute to low malaria transmission but perhaps the most important single factor is the higher ratio humans to mosquitoes reducing the overall biting rate per person (Killeen *et al.,* 2000b; Smith *et al.* 2004).

1.2.6 Vector behaviour

Aspects of the life cycle of mosquitoes that are important for developing sampling tools are their breeding sites, resting locations and foraging activities. For the purposes of vector control targeting adult mosquitoes and development of sampling tools for monitoring vector control interventions, it is necessary for ecologists, entomologists and epidemiologists to know what time of the day females engage in host-seeking and where they subsequently proceed to rest (Service 1982). This is because once these are known; vector control methods can be targeted to either of these behaviours. Thus, understanding vector behaviour and ecology is fundamental to vector control and sampling tool development (Takken and Knols 1999).

Two common approaches have been applied to most attempts to attract and trap adult mosquitoes: traps can act as sheltered resting sites or they can be baited with live humans or animals. Traps, such as Ifakara Tent Trap and Mbita bed net trap, allow consistent release of attractive cues through openings that permit mosquito entry but prevent exit.

The distribution of the Afro-tropical malaria vectors in sub-Saharan Africa varies from one locality to another (Coetzee *et al.* 2000; Gillies and De Mellion 1968) depending on local environments and biting patterns may vary within these localities. Therefore, importantly, focal characterization of host-seeking female *Anopheles* as to where, when and what they feed on, and where they rest, is necessary

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for the design of a sampling tool that can target either of these characteristic behaviours. Although it is difficult for any trap to attain better sampling sensitivity than HLC in diverse settings, attempts to develop such traps should evaluate generalizable applicability in varied settings (Davis *et al.* 1995; Laganier *et al.* 2003; Okumu *et al.* 2008).

The environment an organism inhabits affects its characteristic behaviors a lot. Mosquitoes that are exposed to varying environmental conditions will tend to have greater genetic variability as compared to those that are exposed to less environmental variables (Kassen 2002; Pombi *et al.*, 2008). For example, evolution of generalists and specialists mosquitoes depend on the presence of particular sources of blood meal. Those exposed to many sources of blood meal shall develop to be generalists as they will have many readily available options as compared to specialists that have one or limited number of blood meal sources (Lyimo and Ferguson 2009). Genetic mapping of laboratory reared mosquito colonies have shown reduction of genetic variability even in first generations (F1) after colonization (Norris *et al.*, 2001). This in turn may affect the resulting phenotypic behaviours of mosquitoes (Huho *et al.*, 2007; Ng'habi *et al.*, 2010). This may bring about bias during studies that seek to understand mosquito behavoiur.

1.2.6.1 Host seeking

During the feeding cycle of female malaria vectors, mosquitoes actively seek human hosts for a blood meal as a nutritional requirement for their egg development. Host-seeking is defined as the in-flight orientation of avid female mosquitoes toward a potential blood-meal host (Bowen 1991). While in the process of host-seeking, the vectors have been documented to fly as little as few metres to over 10 kilometres (Killeen *et al.* 2003; Midega *et al.* 2007; Service 1997; Thomson *et al.*, 1995) in search of available hosts upon which to feed on.

Host-seeking process in female mosquitoes starts with random flights then utilization of visual cues that direct them to objects. The mosquitoes then sense chemical compounds/cues called kairomones to detect the presence of their preferred hosts, whether human or animal (Mukabana *et al.,* 2002; Service 1993; Takken and Knols 1999). These cues emanate from the hosts' bodies and include body heat, carbon dioxide, and moisture and body odor. Mosquitoes then follow the cues up a concentration gradient in a host-seeking event until the host is located.

Once they reach the host, selection of biting location on the human occurs. Evidence suggests that the female anophelines approach the human host from the head region and then move downwards towards the feet (De Jong and Knols 1995). Convection currents along the human body and the odor coming from the feet might guide the mosquitoes to the feet in addition to skin temperature, moisture and presence of ecrine glands on the skin (Khan et al., 1969).

Anophelines preferentially bite legs and feet, and in some cases the head and forearms, to other body parts. Studies have shown that attractiveness of humans to the female anophelines vary with weight, malaria infection status, age, pregnancy and sometimes sex (Lindsay *et al.,* 2000; Mukabana *et al.* 2002; Mukabana *et al.,* 2007; Port *et al.,* 1980). Therefore, a trap that allows maximum release of these cues that mosquitoes follow will enhance sensitivity as many mosquitoes will be lured into it.

1.2.6.2 Resting

Female mosquitoes whether blood-fed or not, rest after periodic flights in search of available resources like resting locations, sugar source and vertebrate host. For an engorged blood-fed female mosquito,

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the female *Anopheles* usually rest and get ready for ovipositioning in sheltered corners and under furniture close to feeding locations as the eggs mature and gestate. After 2 to 3 days the female mosquitoes become gravid and then move and rest close to breeding sites. These sites are usually next to aquatic habitats as they await initiation of oviposition.

Members of the *An. gambiae* and *An. funestus* complexes primarily rest inside houses and outdoor in sheltered structures, containers and holes, under leaves and tall grass (Pates and Curtis 2005). Teneral mosquitoes that have just emerged from pupae usually rest under tall grasses and leaves near fresh water points from where they have emerged. *An. gambiae ss* were originally known to rest indoors, but presently they have been found to bite and rest outdoor in various regions in Africa (Braimah *et al.* 2005; Govella *et al.* 2010b; Reddy *et al.*, 2011; Russell *et al.* 2011).

1.2.7 Malaria control

Malaria control entails all measures undertaken in the effort to reduce the malaria burden within a society by targeting the *Anopheles* vectors and the *Plasmodium* parasite itself. Different strategies have been employed singly or synergistically to tackle either vector or parasite in the human population (Barat 2006). Controlling the vector or parasite alone cannot eliminate malaria transmission as the parasite exists in both human and vector hosts.

Governments and other organizations put increasing effort into controlling malaria to reduce the overall malaria-related disease burden and transmission, especially in parts of tropical sub-Saharan Africa where malaria remains a major health and economic concern. Malaria control recently received a boost when new objectives and goals were added by the Roll Back Malaria Programme: To reduce

preventable malaria deaths to near-zero; eliminate malaria in 8-10 countries by 2015 and eradicate malaria in the long term (WHO 2010).

1.2.7.1 Controlling *Plasmodium* parasite in human population

Targeting malaria parasites within the human population has thus far primarily relied upon chemotherapy with antimalarial drugs. As advised by World Health Organization (WHO), different countries prioritize locally-appropriate drug regimes depending on the type of drug against which *Plasmodium* parasites have not yet evolved resistance to (WHO 2010). Presently, Artemisinin-based Combination Therapy (ACT) is widely accepted and remains efficacious against uncomplicated and complicated *Plasmodium falciparum* malaria across large expanse of the globe (Adjuik *et al.,* 2004; Nosten and White 2007; Staedke *et al.,* 2001; von Seidlein *et al.,* 2000).

The WHO further recommends treatment of *Plasmodium vivax* malaria with chloroquine only in areas where it is efficacious and with appropriate ACT in areas it is resistant to chloroquine. A 14 day course with primaquine in addition to ACT or chloroquine is necessary for elimination of hypnozoites stage parasites and to avoid relapses in *P. vivax* malaria. Epidemiologically this is important to ensure interruption of transmission as the parasites will not present in the blood to be transmitted to anophelines that have come to feed.

Intermittent Preventive Treatment (IPT) is the administration of a full course of an effective anti-malarial treatment at specified time points to a defined population at risk of malaria, regardless of whether they are proven to be patently parasitaemic, with the objective of reducing the malaria burden in the specified target population. IPT for pregnant women (IPTp) typically involves two doses of

Sulfadoxine-primethamine (SP) given to expectant mothers during ante-natal care at the first noted movement of the fetus and the other dose a month later (Tutu et al., 2011). IPT for infants (IPTi) involve three doses of SP given to them during immunization procedures.

Vaccination against any infectious disease is known and documented to offer excellent protection against infectious diseases as seen in polio, measles and Diphtheria-tetanus-pertussis (Guerrant *et al.* 2004). In light of the success in disease control that vaccines have brought forth against other diseases, scientists in malaria field started to develop a vaccine that could confer protection to people at risk of contracting malaria. Thus, RTS,S vaccine, developed by scientists at GlaxoSmithKline Biological laboratories, is a recombinant protein that fuses a part of the *P. falciparum* circumsporozoite protein with the hepatitis B virus surface antigen (Moorthy et al., 2004). This is administered to humans together with an adjuvant to induce production of antibodies and T cells against the malaria parasite.

Recent results of phase IIb and first insight into phase III field trials have shown promising success with 50% protection against clinical and severe malaria in African children (Abdulla *et al.,* 2008; Bejon *et al.,* 2008; The RTS'S Clinical Trials Partnership 2011). This candidate vaccine together with ACT and vector control could be a major contributor to malaria reduction when combined with chemotherapy and vector control tools.

1.2.7.2 Controlling *Anopheles*, the malaria vector

Controlling *Anopheles* mosquitoes involves those activities that seek to reduce the vector population and/or minimize human-vector contact (Beier et al., 2008). Measures that suppress vector population at source involve application of larvicides to aquatic breeding sites or environmental management

(Walker and Lynch 2007) to target the juvenile stages of the mosquitoes. Larvicides can either be in chemical (insecticide) or biological in nature. While a number of chemical larvicides have proven efficacious historically (Walker and Lynch 2007), the bacterium *Bacillus thuringiensis var israelensis* has been shown to successfully reduce vector population in urban Dar es Salaam, Tanzania (Castro *et al.* 2004; Geissbuhler *et al.,* 2009), Eritrea (Shililu et al., 2003) and rural western Kenya (Fillinger and Lindsay 2006).

Alternatively, Indoor Residual Spraying (IRS) and/or personal or household protection against adult mosquitoes using insecticidal products in the form of LLIN have been proven to deliver massive reduction of transmission in a wide range of settings (Gimnig *et al.,* 2003a; Lindsay *et al.* 1989; Snow *et al.* 1988). Integrating these complementary control strategies, respectively targeting immature and adult stages, may well be required to eliminate transmission as one control method alone is not sufficient to interrupt transmission across a wide geographical area(Killeen *et al.* 2000a) as shown by malaria success stories (Barat 2006). Adapting appropriate vector control strategies based on measured biological characteristics of local vector populations is considered important in integrated vector management programme to realize the success of malaria vector control (Beier *et al.* 2008).

1.2.8   Implications of malaria control for sampling tool requirements

Models are tools that use mathematical concepts and language to describe a system. They illustrate possible outcomes of interest based on parameters and show how outcomes can be achieved and controlled (McLaughlin 1999). Models have predicted and illustrated that with implementation of integrated malaria transmission control by LLIN or IRS, larviciding and chemotherapy, very large reductions of transmission intensity can occur (Griffin *et al.,* 2010; Killeen *et al.* 2000a). These have been confirmed by field studies in various regions where remarkable reduction in malaria transmission

have been observed such as Zanzibar, western Kenya, Brazil and Asia (Aregawi *et al.*, 2011; Barat 2006; Bhattarai *et al.*, 2007; Fillinger *et al.*, 2009).

Vector control strategies have seen reductions in numbers of the malaria vectors, development of resistance to insecticides, altered host seeking and resting behaviours (Braimah *et al.* 2005; Govella *et al.* 2010b; Lefevre *et al.*, 2009; Pombi *et al.* 2008; Russell *et al.* 2011; Russell *et al.* 2010). In addition, also associated with land use and larval source management, is the adaptation of mosquitoes to occupy organically polluted aquatic habitats (Awolola et al., 2007).

Reduction in density of vector mosquitoes to very low levels, coupled with such altered behavioural characteristics makes it difficult to sample host-seeking mosquitoes (Govella *et al.* 2010a). The standardized CDC-LT, for example, failed to yield satisfactory results in urban Dar es Salaam when compared with HLC (Govella *et al.* 2011). Therefore, safe, sensitive and effective alternatives to HLC are urgently needed to aid in monitoring the density of malaria vectors as the world moves towards lower transmission levels.

An. gambiae resistance to pyrethroids that is resulting from the scaled up use of pyrethroids in the forms of LLINs and IRS has been hypothesized to be the cause of behavioural resistance of mosquitoes to alter host-seeking patterns. This could, over time, affect vector control and sampling practices that target this behaviour. In this study, we therefore look at the sensitivity of ITTD as well as the entry and exit characteristics of malaria vectors into and out of ITT-C and ITT-D in Lupiro village in Kilombero valley, south eastern Tanzania.

1.3 Problem statement

Quantifying outdoor biting malaria vectors that occur in low densities and mediate low but self sustaining transmission poses a challenge to develop better alternative sampling tools to HLC. Field evaluation of ITT-D compared with ITT-C showed far much poorer sampling efficiency. The evaluation also revealed reduced sampling sensitivity of ITT-C relative to CDC-LT than had been previously reported. It was therefore important to investigate why ITT-C had reduced in sampling sensitivity and further establish whether the reduced sampling efficiency arose from trap inefficiency or altered mosquito behaviour.

1.4 Justification and significance of the Research

The HLC method traditionally used to measure malaria transmission is labor intensive, requires a lot of supervision and skilled catchers, and more importantly, exposes users to infective mosquito bites. The CDC-LT has yielded mixed results, with success in some areas and failure in some areas of low transmission like urban Dar es Salaam, Tanzania (Govella *et al.* 2011). With the ongoing reduction of malaria transmission across sub-Saharan Africa expected to continue, measuring low transmission rates and low vector densities will require more sensitive exposure-free tools for sampling mosquitoes.

In addition, the present trends in vector behaviour preferring to bite outdoor rather than indoor further confront development of sampling tools and it is important to establish whether change in biting profile has any effect on the sensitivity of mosquito trapping tools. Development, of an alternative malaria vector sampling tool is necessary if accurate predictions of malaria transmission, specifically EIR, are to be achieved in all malaria-prone areas.

Data about the evaluation of ITT-D and mosquito behaviour effect on sampling tools will help governments, research institutes, researchers and policy makers by providing an insight to a better way of sampling mosquitoes for monitoring and surveillance purposes in low transmission levels.

1.5   Objectives

1.5.1   General objective

Evaluate efficacy of ITT-D and determine why and how the sensitivity of Ifakara Tent Traps has declined in Southern Tanzania, an area of mass LLIN use.

1.5.2   Specific objectives

a) Determine relative sampling sensitivity of ITT-D compared to ITT-C and CDC-LT

b) Determine whether recent apparent declines in ITT sensitivity arise from reduced mosquito entry or increased exit

c) Determine whether observed reluctance by present mosquito population in Lupiro village to enter ITT reflect a heritable trait that is expressed in captive insectary reared specimens

d) Establish sampling sensitivity time trend of ITT relative to CDC-LT or HLC from the time of its development in Kilombero valley, south eastern Tanzania.

1.6 Hypotheses

a) ITT-D will be a sensitive, cheap, exposure free, easy to use mosquito sampling tool relative to ITT-C and CDC-LT

b) Decline in ITT sampling sensitivity is brought about by reluctance of mosquitoes to enter trap and this behaviour is heritable

2.0 CHAPTER TWO: MATERIALS AND METHODS

2.1 Study site

The study was conducted in Lupiro village (8.385 S and 36.670 E) situated in the Kilombero valley, 40 kilometres south of Ifakara town in the Morogoro region of south eastern Tanzania. This valley historically experiences very high *Plasmodium falciparum* malaria transmission. Over the years, due to up-scale of LLINs use, EIR in this valley has significantly fallen from over 1000 infectious bites per person per year (ib/p/y) to 81 ib/p/y by 2008 (Russell *et al.* 2010; Smith *et al.* 1993) but this is still considered high enough to sustain malaria transmission. The village lies 300 metres above sea level and stands next to swampy rice fields. Annual rainfall is approximately 1200-1800mm with mean daily temperature range of 20-33° Celsius. The main malaria vectors belong to *Anopheles gambiae* complex primarily *An. arabiensis* (98%) and *An. gambiae ss* with small populations of *An. funestus*.

2.2 Semi field system

The Semi field system (SFS) in Ifakara Health Institute (IHI) in Ifakara, Morogoro region of south eastern Tanzania was a large, netting-enclosed, mesocosm in which vectors can fly freely, feed on natural plant and vertebrate host sources, and access realistic resting and oviposition sites. The IHI's SFS, is divided into three sections separated by transparent black netting thus referred to as a screen house (Ferguson et al., 2008). It is well enclosed to inhibit free entry into and/or exit of mosquitoes and other large insects.

The first section acts as an insectary where mosquito rearing activities take place. The second section, herein referred to as semi field system, is home to a self sustaining colony of free-flying *An. arabiensis (Ng'habi et al. 2010)* and imitates the conventional real world environment. The third

section is an enclosed free space for experimentation. It is in this third section that evaluation of mosquito entry into and exit out of ITT-C was done. The sampling efficiency of ITT-D was far much poorer hence it was not used in SFS experiments.

2.3 Laboratory rearing of mosquitoes

Mosquitoes were reared in the IHI screen house. At the initiation of the study, there were two *An. arabiensis* colonies present in the screen house. These were from Sakamaganga and Lupiro and had been separately colonized from the Kilombero valley and maintained since 2008 and 2009, respectively. The third set of mosquitoes was *An. arabiensis* obtained from larvae collected from breeding sites in Lupiro and reared to adulthood.

Larvae reared in the screen house and the ones collected and reared in Lupiro insectary were both fed on Tetramin® (Tetramin gmbH Germany) fish food and maintained at similar environmental conditions to harmonize rearing conditions. They were also provided with 10% glucose on cotton wool put on top of the cages and paper cups. This cotton wool would be replaced once it became dry. An air conditioned vehicle with its windows closed was used to transport three day old adults from Lupiro in a cage covered with a wet towel to the SFS in Ifakara.

The fourth source of mosquitoes was F1 generation adults of *An. arabiensis* reared from eggs of wild blood-fed adult female mosquitoes collected from inside houses and cattle sheds in Lupiro between August 20th and October 2011. Engorged adult female mosquitoes collected from Lupiro were placed in a netting cage and carefully transported to the SFS facility at IHI in Ifakara as described above. The mosquitoes were then transferred to small paper cups containing damp filter paper at the base for oviposition. After 7-10 days, eggs laid from each mosquito were transferred to labeled individual

plastic basins. Here, they were allowed to develop into larvae. Meanwhile, the parent mosquitoes were killed and taken to the IHI molecular laboratory for PCR identification. Only offspring of mothers confirmed by PCR to be *An. arabiensis* were allowed to develop further into adult mosquitoes for use in the SFS experiment.

All adult mosquitoes used in the SFS experiments were females aged between five and nine days old. These were thought to have mated and were mature to withstand environmental variables. Sampled mosquitoes for use in a day's experiment were starved of glucose six hours before the start of experiment and they were kept in the same place within the screen house to experience similar environmental conditions.

2.4   Study design

During this study, a series of individual experiments were done in Lupiro village, and the SFS at IHI in Ifakara. Starting from the first experiment, subsequent experiments were designed in response to problems and questions that arose from preceding experiments. To determine trapping sensitivity of ITT-D relative to ITT-C, a cross-over experiment was carried out in the Lupiro village. The observed inability of ITT-C to sample outdoor-biting mosquitoes and poorer performance of ITT-D led to the second and third experiments that sought to establish whether the reduced ITT-C sampling sensitivity was due to reduced entry of the mosquitoes into the trap or increased exit out of the trap. The fourth and fifth experiments thus sought to establish whether the reluctance of wild Lupiro mosquitoes to enter the trap was just a plastic response to environmental conditions or it was a heritable trait selected for by changed environmental factors such as LLIN usage.

2.4.1 Comparative Efficacy of ITT-D compared with ITT-C

A cross-over experiment (Ratkowsky *et al.*, 1992) using four ITT-D and four ITT-C was conducted for a period of ten days in Lupiro village. Eight sampling locations randomly selected from different homesteads that lay parallel, 70-100 metres apart along two transects were used. They were assigned numbers from one to eight. One row of homesteads was 20-30 metres from Lupiro River while the second row of houses was 100-120 metres from the Lupiro River. The traps were purposively assigned sampling locations in an alternating manner such that ITT-C alternated with ITT-D within and between rows.

Eight human volunteers were each randomly assigned one of the eight sampling locations where they remained fixed to for the rest of the experiment. Small pieces of paper were cut and labeled one to eight. They were rolled and shuffled then thrown on the ground. Volunteers were then asked one at a time to pick one ball of paper till all had picked. The number picked indicated the sampling site one was to be stationed. The two trap designs were swapped each night within pairs of trapping locations between rows for ten days.

Row 1	ITT-D	ITT-C	ITT-D	ITT-C
Row 2	ITT-C	ITT-D	ITT-C	ITT-D

Figure 6: Diagramatic presentation of cross-over experimental design. Arrows show how traps were swapped between pairs of trapping locations

Subsequently, after observation of reduced ITT-C sampling sensitivity, a Latin Square design (Saville and Wood 1991) experiment was conducted in Lupiro to determine whether reduced ITT-C sampling sensitivity in the field was due to mosquitoes leaving the trap once they had entered ITT or whether they were not getting into the trap in the first place. Eight new sampling locations were picked that occurred about 70-100 metres apart and along a single transect. Eight traps were then randomly assigned to those sampling locations. For the last ten days of this experiment, four CDC-LTs were placed inside houses that occurred between each pair of two tent traps such that they sandwiched the sampling locations.
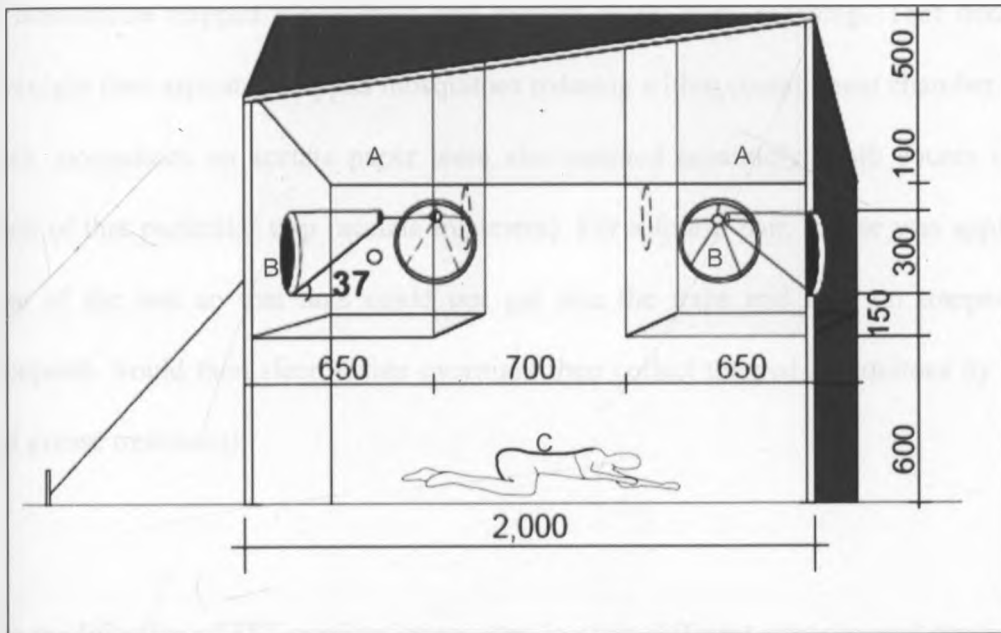
Figure 7: Schematic presentation of Ifakara Tent Traps common to ITT-C and ITT-D designs except for the ropes absent in ITT-D. A- Mosquito trapping/containment chamber. B- Mosquito entry cones. C- Tent occupant. Units are in millimeters.

In its standard mode of operation, ITT was designed to operate with one male human volunteer sleeping inside throughout the night. The volunteer then used an aspirator to collect trapped mosquitoes in the morning (standard sleep treatment). In this particular experimental set up, the standard method of operation of ITT was also modified such that a second pair of ITT-C and ITT-D had volunteers waking up every hour on the hour for 15 minutes to aspirate trapped mosquitoes each night (awake hourly treatment).

In the third pair, a colorless acetate coated with thin layer of rat glue (No Rat®, Kollant s.p.a., Italy), as described by Harris and others (2011), was hung inside each of the trapping/containment chambers to immobilize trapped mosquitoes and prevent them from escaping. Tent occupants slept inside overnight then aspirated trapped mosquitoes roaming within containment chamber by the morning and stuck mosquitoes on acetate paper were also counted separately. Both counts constituted the total catch of that particular trap (acetate treatment). For a fourth pair, grease was applied all round at the base of the tent so that ants could not get into the traps and feed on trapped mosquitoes. Tent occupants would then sleep inside overnight then collect trapped mosquitoes by the morning (Sleep and grease treatment).

The modification of ITT application resulted in eight different experimental treatments. Each of these treatments represented a distinct combination of trap design (ITT-C versus ITT-D) and application method (standard sleep, awake hourly, acetate, sleep and grease) rotated through the eight trapping locations for 24 nights, comprising three rounds of an 8 × 8 Latin Square design.

2.4.2 Assessing the ability of ITT-C to retain trapped mosquitoes

To establish the efficiency of the ITT for retaining trapped mosquitoes, a 3 × 3 Latin Squares design experiment was conducted in the IHI SFS screen house. Three groups of mosquitoes were used: *An. gambiae* sl, reared from larvae collected from Lupiro (Lupiro 2011), *An. arabiensis* colonized from Lupiro in 2009 (Lupiro 2009) and *An. arabiensis* colonized from Sakamaganga in 2008 (Sakamaganga 2008). Selected mosquitoes from the three groups of mosquitoes for use in a night's experiment were dusted with different fluorescent powder (Bioquip products.inc); Lupiro 2011 (blue), Lupiro 2009 (red) and Sakamaganga 2008 (green). Only ITT-C was used in this experiment as it had proven reasonably sensitive compared to HLC and CDC-LT (Govella *et al.* 2009; Govella *et al.* 2010a). In addition ITT-C was superior to ITT-D in the first cross over experiment described above in 2.4.1.

Three equidistant locations within the screen house were selected, to which three ITT-C traps were randomly assigned and fixed at each single location. Human volunteers were then randomly assigned to each of the traps. For each night of experimentation, twenty mosquitoes were selected from each group. They were dusted with different fluorescent powder and then each group put inside trapping chamber of a single ITT-C at 1910hrs. The volunteers would then get into the traps and sleep from 1920hrs to 0630hrs. In the morning, retained mosquitoes would be aspirated using a hand held aspirator and counted to different groups. Volunteers and mosquito groups were rotated in a 3 × 3 Latin Square design for nine days of three complete rounds.

2.4.3 Investigate heritability of trap entry traits

We assessed the tendency of three groups of five to nine day old *An. arabiensis* adults to enter into three ITT-C traps within the IHI screen house. Adults from Lupiro 2009 colony, Sakamaganga 2008 colony and adults reared from collected larvae from Lupiro were compared in terms of their tendency to enter the ITT-C. Twenty female adults from each source were starved of glucose for six hours before being used in a night's experiment. These five to nine day old adults from the three groups were released at the same time at 1910 hours outside ITT-C within the screen house. Tent occupants rotated through the three ITT-C traps in a 3 × 3 Latin Square fashion for nine days of the experimental period.

Mosquitoes trapped in the tents were aspirated from 0630hrs in the morning using a hand-held aspirator. The experiment was repeated a second time, replacing the group of mosquitoes reared from larvae with F1 generation adults reared from the eggs of blood-fed wild caught female *An. arabiensis* from Lupiro. F1 adults emerging from different mothers who were subsequently identified to species were put separately in netted cages. Up to 20 adult offspring from a single mother mosquito were used in a day's experiment. In five cases where less than 20 F1s were available, a minimum of ten were used.

2.4.4 Time trend analysis of relative trapping sensitivity of ITT-C in Kilombero valley

To visualize ITT's relative sampling power from the time it was developed, data from previous studies done in Kilombero valley that compared sampling sensitivity of ITT-C or ITT-B to CDC-LT or HLC were gathered from published papers (Govella *et al.* 2009) and unpublished works (table 8). Neither ITT-B nor ITT-C had been evaluated longitudinally in Kilombero valley. Further, at one particular time point, ITT was either compared to CDC-LT or HLC. Information on the relative sampling sensitivity with reference to either CDC-LT or HLC is not continuous.

Apart from a long layer of Teflon-coated netting in ITT-B that was split into two in ITT-C to reduce tent occupant's exposure to bites from collected mosquitoes, both ITT-B and ITT-C are similar in nature. Nevertheless, data for ITT-B and ITT-C compared to either CDC-LT or HLC were therefore pooled to obtain an, albeit discontinuous, time trend. Unpublished data was also provided to us by researchers who had used ITT-C or ITT-B in sundry, unpublished experiments in Lupiro or elsewhere in Kilombero valley.

For inclusion for analysis, these studies must have been done between 2006 (ITT development year) and 2011 and compared either ITT with either HLC or CDC-LT gold standard methods in the same time and place. Relative trapping sensitivity was calculated by dividing mean catch of alternative trap by mean catch of the reference trap.

2.5   Field and laboratory Identification of mosquitoes

All mosquitoes were morphologically identified using standard keys (Gillies and De Mellion 1968) at the entomology field station in Lupiro. *An. gambiae* complex mosquito samples stored with silica gel were taken to IHI molecular lab for PCR identification to sibling species (Scott *et al.,* 1993).

2.6   Data analysis

Statistical Package for the Social Sciences 16.0 (SPSS Inc, Chicago) was used for data analysis. Generalized Linear Models (GLM) with negative binomial or Poisson distributed dependent variable, both of which use logarithm link functions were used in the analysis of data collected. These GLM

functions were used to analyze the effect of different treatments upon counts of mosquitoes obtained from traps in both from field and SFS experiments. Generalized Linear Model with logistic outcomes was used to analyze binary data such as mosquito staying inside trap or exiting out of for the SFS experiments.

In the last experiment using F1s to assess whether reluctance to enter trap is heritable, the number of F1 adults used varied between 10 and 20 per day. To take care of the variations brought by this difference in number of mosquitoes used, number of mosquitoes caught was divided by the total number of mosquitoes released. These proportions were thus taken as a gamma distribution hence GLM with gamma distribution was used to analyze the number of mosquitoes caught by ITT-C in that experiment.

Correlation analysis was used to establish the relationships between ITT-D, ITT-C and CDC-LT catches. Density-dependent sampling efficiency was calculated by dividing the mean catch of alternative method by the reference method and correlating with the average catches per day of the reference method (CDC-LT) (Govella *et al.* 2009).

2.7   Ethical considerations

This study was approved by National Institute for Medical Research (Reference number NIMR/HQ/R.8a/Vol.IX/801). House hold heads whose houses were used to set up CDC-LT were informed, prior to commencement of the study, of the purpose of the study and that they were not at risk of any danger from the experiment after which they signed consent forms. The volunteers who slept inside ITT-C or ITT-D were not exposed to mosquito bites because the traps are exposure-free

and they were at no point exposed to risky experiment. The volunteers were further told that they were free to exit the study at any time without consequences.

3.0 CHAPTER THREE: RESULTS

The two field experiments comparing mosquito trapping sensitivity of ITT-D, ITT-C and CDC-LT in Lupiro village yielded *Anopheles* sp (n=5429), *Culex* sp. (n =4954) and *Mansonia* sp. (n=3822). Out of 150 *An. gambiae* specimens sent to the IHI molecular laboratory for PCR identification, all of the 138 specimens that were successfully amplified were confirmed to be *An. arabiensis*, corroborating evidence that *An. arabiensis* is the dominant malaria vector in Kilombero valley (Russell *et al.* 2011).
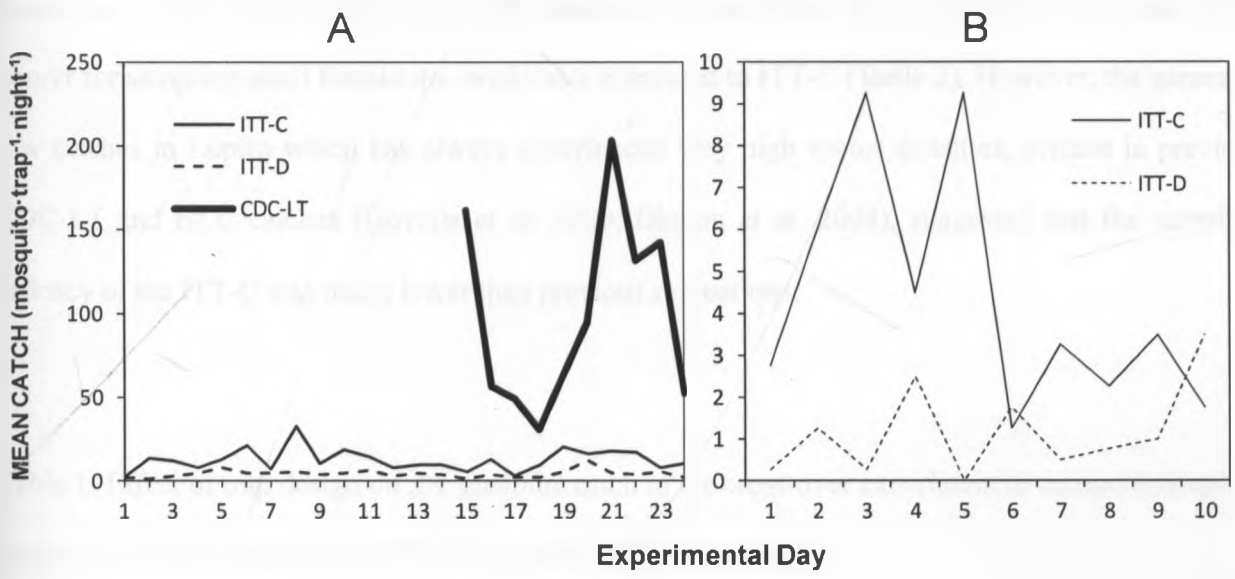
Figure 8: Mean catches of *An. arabiensis* caught per day using ITT-C, ITT-D and CDC-LT. Graph A shows daily mean mosquito catches during the Latin square design experiment. Graph B shows daily mean mosquito catches in the ten day cross-over experiment.

In the first nights of trapping, both ITT-C and ITT-D caught few mosquitoes. In contrast, CDC-LT caught many mosquitoes on the first night when it was introduced in the experimental setup with the usual levels of fluctuations that are normal in mosquito population sample (figure 8).

3.1 Crude catch estimates of field mosquito collections

In the ten nights of trapping using ITT-D and ITT-C during the comparative cross over experiment in the field, a total of 1745 mosquitoes were sampled by ITT-C and ITT-D. Of those, 12.7% (222) were *An. gambiae* sl, 0.1% (2) *An. funestus*, 12.2% (212) *Culex* sp. and 75% (1309) *Mansonia* sp. Blood fed mosquitoes were found only from the *An. gambiae* sl caught. This represented a small proportion, only 2.3% (5), of all caught *An. gambiae* sl.

Using GLM with Poisson distribution for analysis of count data, we found that ITT-D was much poorer for sampling adult female *An. arabiensis* compared to ITT-C (Table 2). However, the generally low catches in Lupiro which has always experienced very high vector densities, evident in previous CDC-LT and HLC catches (Govella *et al.* 2009; Okumu *et al.* 2008), suggested that the sampling efficacy of the ITT-C was much lower than previous evaluations.

Table 1: Effect of trap design on *An. gambiae* catch in the cross-over experiment to compare sampling sensitivity of ITT-D relative to ITT-C in Lupiro, Kilombero valley

Collection method	Trap nights	Total catch	Mean catch	Relative rate (95% CI)	P
ITT-C	40	175	4.38	1[a]	NA[a]
ITT-D	40	47	1.18	0.210 (0.139-0.317)	<0.001

NA[a]: Not applicable because it is the reference method

[a]: Reference method

P: Significance level

In the second experiment, 24 nights of trapping using ITT-C and ITT-D and ten days of trapping using CDC-LT gave a total of 12,464 mosquitoes. The ITT-C, ITT-D and CDC-LT collected 34.44% (4293), 23.44% (2922) and 42.11% (5249) mosquitoes respectively. Despite ITT-C and ITT-D being used for 24 nights to sample *An. arabiensis,* CDC-LT caught three times more *An. arabiensis* for the ten days that it was introduced into the experiment. All the three traps compared well in terms of the number of *Culex* spp and *Mansonia* spp sampled and in fact ITT-D and ITT-C sampled more culicines than CDC-LT.

The other anophelines caught by CDC-LT and ITT-C in Lupiro village were *An. coustani* and *An. squamosus* but these were very few and thus included in one group namely, other anophelines. *An. funestus* were only sampled by ITT-C and CDC-LT but again these were few in number. The three traps were compared on the number of mosquitoes sampled per taxon and table 3 below shows the proportion, per taxon, of number of mosquito species sampled by ITT-C, ITT-D and CDC-LT. Table 4 shows the relative sampling sensitivity of ITT-C and ITT-D for the period when CDC-LT was introduced into the experiment.

Table 2: Proportion of mosquitoes caught per taxon per trap by ITT-C, ITT-D and CDC-LT trap nights respectively in Lupiro, Kilombero valley.

Trap	N	*An. arabiensis* n (%)	*An. funestus* n (%)	*Culex* sp. n (%)	*Mansonia* sp. n (%)	Other Anophelines n (%)	*Aedes* sp. n (%)	Mean of total (95% CI)	P
ITT-C	96	1190 (22.93)	2 (66)	1899 (40.05)	1194 (47.71)	2 (22.22)	1 (50)	44.688 (31.814-57.56)	0.124
ITT-D	96	381 (7.32)	-	1580 (33.29)	960 (38.2)	-	-	30.417 (17.54 - 43.3)	0.124
CDC-LT	38	3622 (69.75)	1 (34)	1265 (26.66)	354 (14.09)	7 (77.78)	1 (50)	131 (111.08 – 151)	<0.001

N: Trap nights (sampling nights * number of traps)

CI: Confidence Interval

P: Significance level

Table 3: number of mosquitoes caught and relative sensitivity of ITT-D and ITT-C to CDC-LT for the last 10 days of the second field experiment when CDC-LT was introduced

Method	Trap nights	*An. arabiensis*			*Culex* spp			*Mansonia* spp		
		Total	mean	Relative sensitivity	Total	mean	Relative sensitivity	Total	mean	Relative sensitivity
ITT-C	40	475	11.88	0.1313	718	17.95	0.568	652	16.3	1.84
ITT-D	40	159	3.98	0.044	612	15.3	0.484	545	13.63	1.54
CDC-LT	40	3622	90.55	NA	1264	31.6	NA	354	8.85	NA

NA: Not applicable because it is the reference method

3.2 Treatment and trap design effect on sampling efficiency of ITT-C and ITT-D

Generalized Linear Model with Poisson loglink distribution was used to analyze effect of treatment and trap type on catch size by the traps. Table 4 below shows the effect of various treatments and trap design on catch size of mosquitoes. Although applying a line of grease round base of tent traps in 'grease and sleep' treatment improved the number of mosquito catch, this was not significantly different from mosquito catch in control 'standard sleep treatment'. Addition of sticky acetate paper in 'acetate' treatment improved the number of mosquito catch compared to catches in 'standard sleep treatment'. Intriguing results were observed in the 'awake hourly' treatment that caught very high number of adult mosquitoes when compared to all the other treatments.

In traps where sticky acetate paper was hung 'acetate treatment', there was observed improved retention capacity of traps. In this treatment, once mosquitoes had entered the traps, they would be immobilized by the sticky acetate paper but some would still be found roaming in the mosquito containment chamber. There was no difference in the number of mosquitoes found glued on the sticky acetate and found roaming the mosquito containment chamber when analyzed by chi-square test (χ^2 =33.425, degrees of freedom = 23, p=0.0739). Because tent occupants were stationary at sampling sites, and for the purposes of computing GLM modeling, effect of tent occupants and sampling sites was assumed to constitute a single combined source of variation

Table 4: Treatment and trap type effect on the relative sampling efficiencies of ITT-C and ITT-D. Mean catches are log-linked in the regression model.

Variable	Mean	Relative rate (95% CI)	P
Treatment			
Standard sleep	2.06	1[a]	NA[a]
Sleep and Grease	2.30	1.117 (0.891-1.402)	0.337
Acetate	3.16	1.535 (1.244-1.893)	<0.001
Awake hourly	15.21	7.379 (6.193-8.792)	<0.001
Trap Design			
ITT-C	6.76	1[a]	NA[a]
ITT-D	2.23	0.33 (0.293-0.372)	<0.001

NA: Not applicable because this is the reference method

[a]: Reference group

P: Significance level

3.2.1 Overnight hourly collection of mosquitoes in 'awake hourly' treatment traps

Mosquitoes were aspirated every night at every hour on the hour for 15 minutes in the 'awake hourly' treatment and recorded to have been caught at that hour. The mosquitoes caught were assumed to have been in the process of host-seeking when they were trapped. From early in the night, the number of mosquitoes caught per hour increased and peaked around the middle of the night then their numbers declined past midnight and started to peak again after four in the morning. Figure 9 below illustrates the general mosquito host-seeking events throughout the night as depicted by ITT-C and ITT-D.
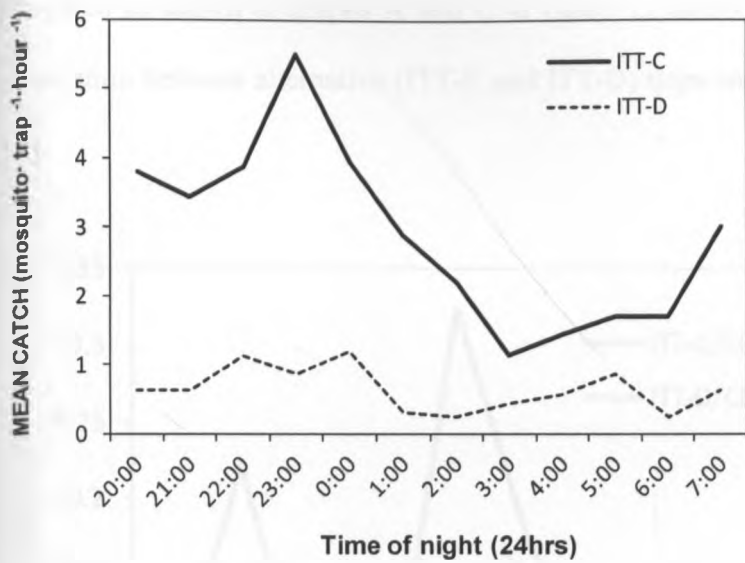
Figure 9: Hourly catches of *An. arabiensis* using ITT-C and ITT-D in Lupiro village, Kilombero Valley, Tanzania.

3.3 Density dependence sampling sensitivity of ITT-C and ITT-D

The number of mosquitoes caught by the three traps was used to analyze how dependent those traps were to changes in vector density through time. Mean daily catches by ITT-C and ITT-D were plotted relative to CDC-LT to see an overview of the effect of varying densities on the sampling power of ITT-C and ITT-D (figure 10). From figure 10, on nights that there was high number of mosquitoes caught by CDC-LT, relative sampling efficiency of both ITT traps would be seen dropping while as the number of mosquitoes caught by CDC-LT reduced, the relative sampling efficiency would be seen to rise.

We further assessed the density dependence relative sampling sensitivity of our two ITT traps by plotting the ratios of catches in alternative trap and reference trap versus absolute catches of reference trap (figure 11). The sampling sensitivity of the alternative traps reduced as CDC-LT catches

increased as shown in graphs A and C in figure 11 below. Correlation analysis showed only slight association between alternative (ITT-C and ITT-D) traps and the CDC-LT (Graphs B and D in figure 11).
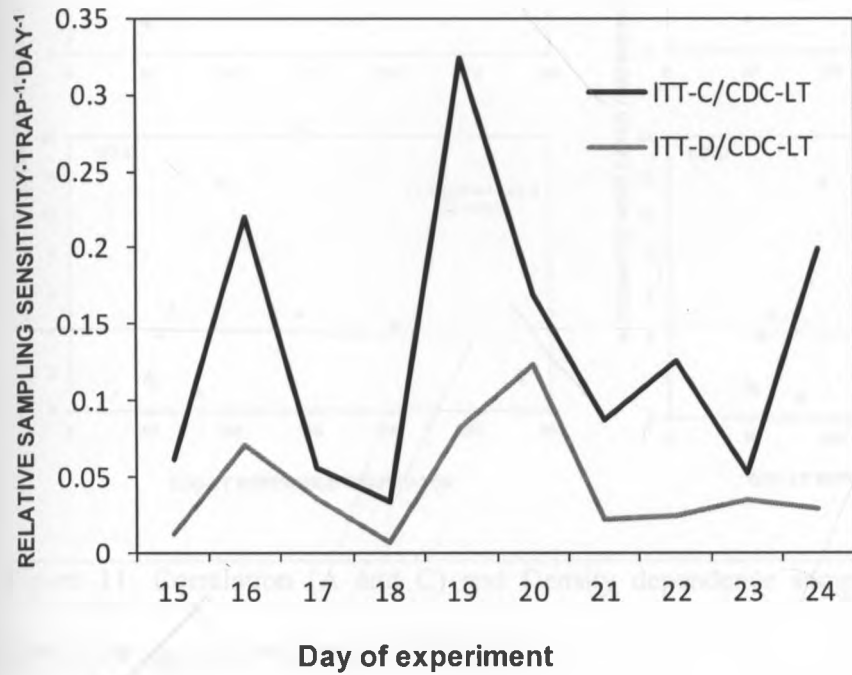
Figure 10: Daily sampling sensitivity of ITT-C and ITT-D per trap per day for the 10 days that they were compared in parallel. Relative sensitivity was got by dividing average catch per day in alternative trap by that of CDC-LT
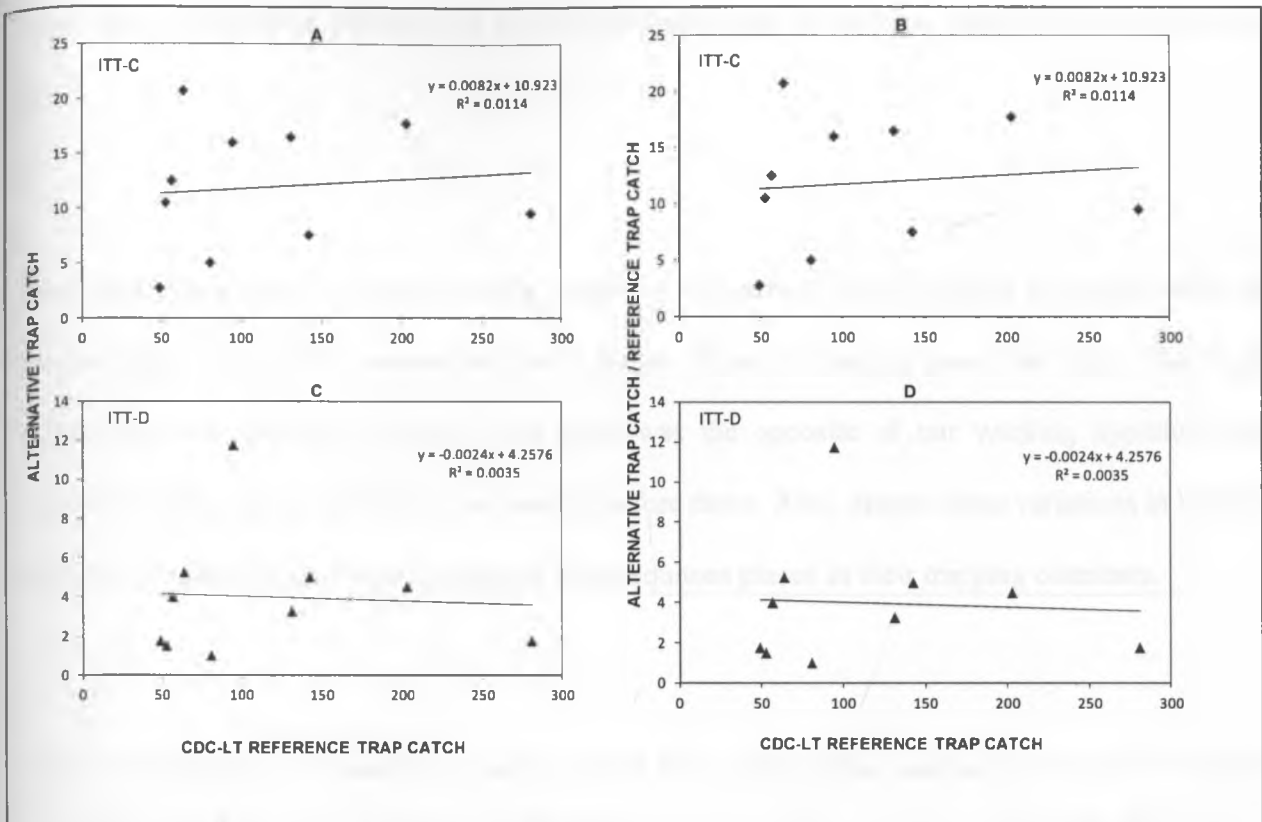
Figure 11: Correlation (A and C) and Density dependence sampling sensitivity (B and D) of alternative trap with reference to CDC-LT.

Following the observed reduced sensitivity of ITT-C in the field experiments, we tested the ability of ITT-C to retain trapped mosquitoes in a SFS environment. We also assessed the probability of mosquitoes to enter into ITT-C to establish whether the observed reduced sampling power of ITT-C was due to the trap itself or *An. arabiensis* of 2011 in Lupiro.

3.4   Mosquito retention capacity of ITT-C

To assess the probability of mosquitoes exiting ITT-C, three groups of mosquitoes; Lupiro 2011 (reared to adults from Lupiro larvae), Lupiro 2009 colony and Sakamaganga 2008 colony were compared in terms of their exit tendencies out of ITT-C after being placed in the trapping chamber.

There was no significant difference in the exit tendencies among the three groups of mosquitoes (table 5).

Once mosquitoes were put inside trapping chambers, it became difficult for them to escape outside and interestingly, Lupiro 2011 mosquitoes had a higher chance of staying inside the traps. This higher probability of mosquitoes staying inside traps was the opposite of our working hypothesis that mosquitoes were going inside trap and leaving before dawn. Also, despite these variations in retention efficacy, all tents retained three quarters of all mosquitoes placed in their trapping chambers.

Table 5: Probability of mosquitoes staying inside ITT-C with mean number of mosquitoes retained over night and the retention efficiency of ITT-C

Variable	Mean (absolute mosquito number)	Odds Ratio (95% CI)	P
Colony			
Sakamaganga 2008	14.11	1[a]	NA[a]
Lupiro 2009	14.22	1.029 (0.640-1.655)	0.905
Lupiro 2011	16.56	1.984 (1.182-3.331)	0.009
Tent			
1	14.89	1.314 (0.811-2.128)	0.268
2	16.22	1.898 (1.144-3.147)	0.013
3	14.96	1[a]	NA[a]

NA: Not applicable because it is the reference group

[a]: Reference group. CI: confidence interval

P: Significance level

3.5 Probability of *An. arabiensis* entering ITT-C

The three groups of adult mosquitoes Lupiro 2011 (reared to adults from Lupiro larvae), Lupiro 2009 colony and Sakamaganga 2008 colony were again compared in terms of their probability of entering the ITT-C in the Ifakara SFS screen house. When they were released outside tents within the screen house, using GLM with Poisson distribution function to analyze count data, Lupiro 2011 was less likely to enter trap when compared to Sakamaganga 2008 and Lupiro 2009 (table 6). The probability of Sakamaganga 2008 and Lupiro 2009 entering into ITT-C was not significantly different.

This test of the probability mosquitoes to entering into ITT-C was repeated, but this time F1 generation of wild caught blood fed female *An. arabiensis* from Lupiro 2011 was used instead of adults reared from wild-caught larvae. The F1 adults from lupiro 2011 were also reluctant to enter into ITT-C (table 7). This suggested that the reluctance to enter ITT-C is hereditary and passed to offspring.

During these semi field experiments, the colour of fluorescent powder applied on each group of mosquito was alternated daily to remove bias associated with one colour of fluorescent powder. This alternation was rotated daily in a 3 x 3 Latin Square design. In general, there was no observed effect of fluorescent powder on the number of mosquitoes caught by ITT-C.

Table 6: Probability of Lupiro 2011 (reared from wild caught larvae), Lupiro 2009 and Sakamaganga 2008 *An. arabiensis* groups to enter ITT-C

Colony	Mean (log-linked)	Odds Ratio (95% CI)	P
Sakamaganga 2008	2.07	1[a]	NA[a]
Lupiro 2009	2.2	1.062 (0.821-1.375)	0.645
Lupiro 2011	0.63	0.304 (0.207-0.446)	<0.001

NA: Not applicable because it is the reference group

[a]: Reference group. CI: confidence interval

P: Significance level

GLM Poisson distribution-log link analysis

Table 7: Probability of Lupiro 2011 (F1 from wild caught blood fed mosquitoes), Lupiro 2009 and Sakamaganga 2008 *An. arabiensis* groups to enter into ITT-C

Colony	Mean (log-linked)	Odds Ratio (95% CI)	P
Sakamaganga 2008	0.276	1[a]	NA[a]
Lupiro 2009	0.23	0.736 (0.543-0.996)	0.047
Lupiro 2011	0.065	0.234 (0.173-0.316)	<0.001
Fluorescent powder			
Red		1[a]	NA[a]
Blue		1.173 (0.866-1.587)	0.303
Green		1.105 (0.819-1.492)	0.513

NA: Not applicable because it is the reference method

[a]: Reference group. CI: confidence interval

GLM gamma distribution-log link analysis

3.6 Time trend analysis of trap effectiveness in Lupiro, Kilombero valley

Sampling sensitivity of ITT relative to CDC-LT or HLC (table 8) is seen to reduce from 2006 to 2011 as shown in figure 12 below. In 2006, ITT-B was compared to CDC-LT and HLC but we selected relative sensitivity with reference to CDC-LT for inclusion into this analysis. In 2009, ITT-C was

compared to HLC and relative sensitivity with reference to HLC was included. In 2011, we compared ITT-C to CDC-LT and relative sensitivity determined. There were no major changes in structure of ITT-B and ITT-C from the time of development to date therefore observed reducing sampling power of ITT-C could not be due to structural change. Thus, we excluded ITT-D as the fourth data point because its structural modification could have contributed to the observed low sampling power (table 8).

Table 8: Sources of data for time trend analysis of ITT sampling sensitivity

Published paper	unpublished	Experiment year	Trap	Crude catch	Mean catch	Relative sensitivity to CDC-LT/HLC
Govella 2009		2006	ITT-B	1007	55.944	0.50249501
		2006	CDC-LT	4008	111.33	NA
	Russell 2009	2009	ITT-C	280	7.4	0.164065271
		2009	HLC	12484	45.12	NA
	Opondo 2011	2011	ITT-C	1193	12.427	0.137240015
		2011	ITT-D	387	4.0313	0.044519602
		2011	CDC-LT	3622	90.55	NA

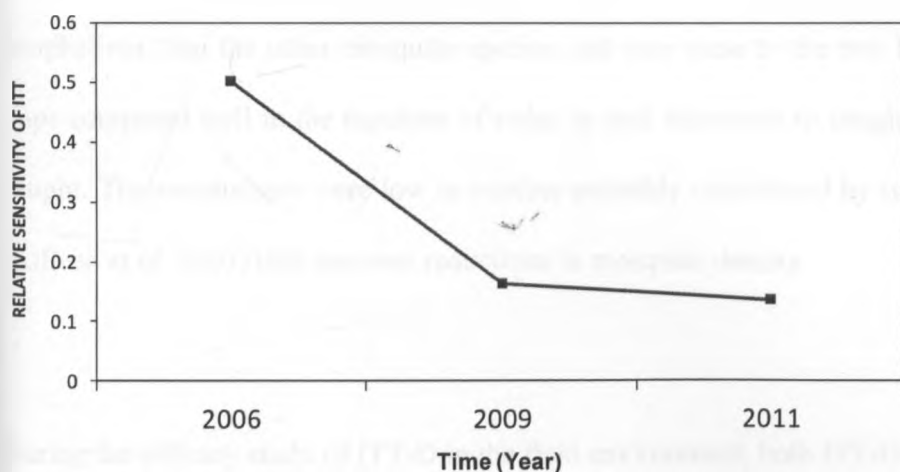NA: Not applicable because it is the reference trap

Figure 12: Sampling sensitivity of ITT relative to HLC and CDC-LT from 2006 to 2011

4.0 CHAPTER FOUR: DISCUSSION

During field evaluation of ITT-D relative to ITT-C and CDC-LT in Lupiro village, Kilombero valley, mosquito behaviour appeared to play an important role in determining the sampling sensitivity of ITT-C and ITT-D. In this village, from the year 2006 that ITT was developed and evaluated, *An. arabiensis* has been the dominant malaria vector with few populations of *An. gambiae* ss (Govella *et al.* 2009; Govella *et al.* 2010a).

Polymerase Chain Reaction identification of mosquitoes caught during our field experiments (99% *An. arabiensis*) imply the latter has since disappeared significantly giving way to *An. arabiensis* as the main malaria vector in Lupiro. It is this *An. arabiensis* that ITT-B and ITT-C were evaluated for in 2006 but also with *An. gambiae* ss present. Malaria vectors in this village and the larger Kilombero valley now prefer to feed early in the evening to late in the night (Russell *et al.* 2011). This is due to the successful LLIN usage in the valley that has also resulted in reduced malaria transmission (Russell *et al.* 2010).

All the traps caught anophelines, culicines and *Mansonia* sp mosquitoes. The CDC-LT caught many anophelines than the other mosquito species and vice versa to the two ITT series traps. All the three traps compared well in the numbers of *culex* sp and *Mansonia* sp caught but not on the *Anopheles* sp caught. The anophelines were low in number probably contributed by scale up of LLINs in the valley (Killeen *et al.* 2007) that has seen reductions in mosquito density.

During the efficacy study of ITT-D in the field environment, both ITT-D and ITT-C were far poorer in sampling power than previously observed with ITT-C in the same geographical region. It was difficult to discern the reason for this reduced ITT-C sampling power because species composition against

which all ITT have been evaluated remained unchanged. Also, ITT-C structure has not changed. A number of factors can contribute to the sampling power of sampling tools such as location of traps, set up of the trap, season, weather and individual host variability.

We did our evaluations in similar settings as initial evaluations of ITT-B and ITT-C. Therefore, this reduced ITT-C sampling sensitivity could probably be associated to behavioural adaptation of mosquitoes arising from the scale up of LLINs that has seen mosquitoes change from late night feeders indoor to feeding outdoor early in the evening where they encounter less insecticide exposure (Russell *et al.* 2011).

From the hourly catches in 'awake hourly' treatment in ITT-C and ITT-D, the mosquitoes caught that were assumed to be in the process of host-seeking were actively foraging from early in the night to the middle of the night and early morning typical of classical malaria vector behaviour (Pates and Curtis 2005).

Both ITT-C and ITT-D have entry cones on the sides of the traps as well as head and foot ends that permit air flow allowing odor from inside to get outside for host seeking mosquitoes to detect. The poorer performance of ITT-D compared to ITT-C may be explained by the presence of longer rain protection flap that could have interfered with airflow and that it could have made it difficult for mosquitoes to locate the entry cones. In addition, the flap might have directed odor plumes emanating from the tent downwards minimizing detection of odor by the flying host-seeking mosquitoes. Despite the poor sampling power of ITT-D, it increased user comfort by protecting tent occupants as well as mosquito entry cones from rain and stronger winds.

Comparing indoor against outdoor sampling tool is challenging in regard to the different populations that are likely to be sampled indoor and outdoor. In view of this, CDC-LT was still chosen to help in calibrating ITT-D as there is no any other outdoor sampling tool that has been successful in Kilombero valley. The hypothesis that mosquitoes were getting inside the traps and leaving before dawn was disapproved in SFS experiments that saw many mosquitoes retained inside traps when they were put in trapping chambers. It was therefore difficult for mosquitoes to escape once they had entered the ITT-C trapping chamber. This hypothesis was also disapproved by the modest increases in catch size arising from usage of sticky acetate to immobilize many trapped mosquitoes before they could escape out of ITT-C. Addition of sticky acetate improved the retention capacity of the traps.

In the Latin Square experimental design, low mosquito catches were recorded in all the treatments except in the 'awake hourly' treatment where occupants, aided with torch flash lights, aspirated mosquitoes every hour on the hour. This was an interesting observation that saw increased number of mosquitoes trying to enter ITT-C during the 15 minute aspiration events. Light coming from the flashlights used by the tent occupants during aspiration could have contributed to increased entry of mosquitoes into traps.

The responses of mosquitoes to light are influenced not only by spectral emissions characteristics but also by the intensity of light. Species may differ in their response to light sources at different distances from it. Others get attracted from much greater distances from the light source than other species but intense light can also repel mosquitoes that have approached near it (Roeder 1953). Light might act as a long range attractant but once the mosquitoes get closer to the source, short range cues start to determine where and which host to land on (Service 1977).

Our field experiments caught large numbers of unfed, presumably host-seeking female anophelines with few males. For the few blood fed female mosquitoes caught, we agree with the view that ITT probably acted as a resting site (Govella *et al.* 2010a) or that the mosquitoes might have been interrupted during feeding and were in the act of looking for a second blood meal source when they were trapped.

In addition, on a smaller scale the physiological changes (Thornton et al., 1976) that occurred during the oral aspiration activity may also contribute to this increase in number of mosquitoes. As argued in various studies (Mukabana *et al.* 2002; Takken and Knols 1999) about the effect of body odor on mosquitoes, the aspiration events probably could have led to increased carbon dioxide production, exhalation and sweat production leading to a stronger body odor.

The application of grease round some traps in bid to stop ants from eating mosquitoes did little to improve the sampling power of the traps. Therefore, in the field environment, low catch numbers can only suggest that the mosquitoes were not getting into the traps except in the ones that had volunteers waking up at the top of every hour to collect trapped mosquitoes. We later suggested that the mosquitoes were not readily getting into ITT-C or ITT-D unless with an additional stimuli as was the case for those using light periodically during aspiration events in the 'awake hourly' treatment.

The two laboratory reared groups of mosquitoes (Lupiro 2009 and Sakamaganga 2008) colonized when LLIN upscale in Kilombero valley was in its early stages, could easily get into ITT-C compared to mosquitoes in Lupiro in 2011. The LLINs and IRS are believed to contribute to phenotypic plasticity which result in changes in behaviour or environmental adaptation of malaria vectors (Pombi *et al.* 2008). Malaria vectors preference to now feed outdoor following LLIN/IRS upscale has been

observed in various regions of Africa (Awolola *et al.* 2007; Braimah *et al.* 2005; Lefevre *et al.* 2009; Reddy *et al.* 2011; Russell *et al.* 2011).

The ITT-C has been used for sampling mosquitoes in the Kilombero valley but now has a lower sampling sensitivity. This trap has not changed in structure therefore it was believed that the reduced sensitivity of the trap was as a result of altered host-seeking behaviour brought about by scale up of LLINs in the valley.

From table 8 progressive reductions in sampling power from 2006 to 2011 is seen. However, it was difficult to conclude with certainty that it was the mosquito behaviour that had resulted in the reduced sampling power of ITT-C. This was because longitudinal continuous data was lacking, during the period of LLIN scale-up, where ITT-C has been used to sample mosquitoes. In addition, the effect of LLINs on sampling tools is less known and behavioural study techniques that could tell the effect of environment on sampling tools were limited.

Mosquitoes maintained in laboratories or insectaries over time have a remarkably reduced genetic variability (Norris *et al.* 2001). Also, genetic diversity of a population depends on the degree of heterogeneity of the environment it inhabits (Kassen 2002). Colonies maintained in a homogeneous laboratory environment may not truly represent the behavioural traits of the original population as they might have lost them over time (Huho *et al.* 2007; Ng'habi *et al.* 2010) and laboratory reared mosquitoes start losing genetic variability within a single generation. Bias may be introduced during studies that look at change in mosquito behaviour when comparing laboratory reared mosquitoes and wild type mosquitoes.

It was not certain whether the behaviours exhibited by laboratory colonies relevant to this study were inherited faithfully and unmodified from original population or were adaptive traits resulting from the colonization process. Thus, laboratory reared Lupiro 2009 and Sakamaganga 2008 colonies would freely get into the traps compared to wild caught Lupiro 2011. But, since we could not go back in time to get earlier populations of Lupiro mosquitoes against which to compare the present Lupiro populations, laboratory reared ones were best available option. Rearing of eggs of wild 2011 *An. arabiensis* from Lupiro to F1 in the same insectary as other two colonies reduced the possibility of bias due to rearing environment while using F1 generation with the hope of retaining most of the wild traits of the present 2011 Lupiro mosquitoes.

Conclusion

The C and D designs of the Ifakara Tent Trap no longer appear to offer a viable alternative to human landing catch for catching mosquitoes outdoor in the Kilombero Valley. This reduced sampling efficacy of ITT-C appears to be associated with the scale up of LLINs in the valley that has seen mosquitoes change from late night feeders to feeding in the early evening (Russell *et al.* 2011; Russell *et al.* 2010). However, it remains unknown how host-seeking patterns can affect outdoor sampling tools.

Using behavioural study techniques like video recording, it may be useful to establish further how malaria vectors in Kilombero Valley have changed and how this impacts on sampling tool developments. In addition, studying and understanding the effect of mosquito host-seeking patterns on sampling tools was recommended. Studying the allele frequency of mosquito populations in the Kilombero valley longitudinally will shed light on how environment affects genetic variability hence behaviour of mosquitoes.

Modification of ITT to allow for more mosquito entry points by creating baffles could also be a point to consider in the development of future outdoor sampling tools. Further, the longer rain protection flap in ITT-D should be reduced a little but still be able to protect the tent occupant as well as entry cones from rain drops. This is to ensure user friendliness during mosquito sampling activities while increasing the ease with which host-seeking malaria vectors can locate the entry cones thus maximizing catch.

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