Monitoring malaria vector densities and behaviours in Tanzania

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Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy

November, 2010

ABSTRACT

Malaria remains the most important parasite-related public health problem globally, with the majority of burden occurring in sub-Saharan Africa. Increased political and financial support has resulted in rapid scale-up of malaria prevention measures, so that disease burden has been substantially reduced in many African countries. However, behavioural change by malaria vector populations, so that a greater proportion of human exposure to bites occurs outdoors, threatens to undermine the impact of malaria control with existing front-line interventions such as insecticide treated nets (ITNs) and indoors residual spraying (IRS) because both act indoors. Also, progress towards lower transmission levels poses substantive entomological monitoring challenges because most standard methods fail to detect low levels of vector density and malaria transmission.

The overall goal of this study was to enhance understanding of the potential and limitations of ITNs for reducing malaria transmission by outdoor biting mosquitoes, and to develop a safe, sensitive, practical and effective malaria vector surveillance tool that enables sustained entomologic monitoring of intervention impact.

An existing mathematical model was adapted to examine the possibility that ITNs can achieve community suppression of malaria transmission exposure, even when mosquitoes avoid them by feeding on people while they are outdoors. Simulations indicated that ITNs may provide useful levels of community suppression of malaria transmission, even when outdoor biting rates exceed indoor biting rates and slightly more than half of bites occurred at times and places when using ITNs is not feasible. This suggests that ITNs should not be deprioritized as a malaria control tool simply because local vector species prefer to feed outdoors. Nevertheless, complementary interventions that target outdoor- and early-biting mosquitoes should be prioritized, especially for going beyond malaria control to achieve elimination.

Cross-over and Latin Squares experimental designs were used to compare the sensitivity of multiple trapping techniques for catching malaria vectors, under conditions of both high and low mosquito density, in rural Kilombero and urban Dar es Salaam, respectively. A new tent-style trapping device called the Ifakara Tent Trap was successfully developed and proved to be safe and more efficacious than any other commonly used alternative to human landing catch for catching *Anopheles gambiae s.l.* in the low transmission setting of urban Dar es Salaam. Its sampling efficiency appeared to be independent of vector density in a rural setting with high mosquito abundance but increased as mosquito densities decreased in an urban area of low mosquito density where it exceeded that of HLC at lowest densities. This density-dependence of the trap implies that this tool may have particular potential for monitoring malaria in low transmission settings. It was also demonstrated to be effective when used by unsupervised community members under programmatic conditions and it is currently the only technique used for routine adult mosquito surveillance by the Urban Malaria Control Programme of Dar es Salaam. However, it cannot be used to determine how bites upon humans are distributed between indoor and outdoor exposure components.

DECLARATION

None of the material contained in this thesis has been previously submitted for a degree in this or any other university. Chapters 2, 3, 4 and 5 have been published as papers in peer-reviewed journals in slightly different format from that presented here. The contributions of each of the various collaborators involved in each chapter are listed below:

Chapter 1: Introduction and literature review

Nicodem James Govella (NJG) wrote the entire chapter. Dr. Gerry F Killeen (GFK), edited the chapter.

Chapter 2: Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors.

NJG, Performed model parameterization and implementation, as well as comparison of various mosquito behavioral indices. He also drafted the manuscript. All of these were under the supervision of GFK who also drafted the revised equation. Fredros Oketch Okumu (FOO), contributed to model parameterization.

Chapter 3: A new tent trap for sampling exophagic and endophagic members of the *Anopheles gambiae* complex.

NJG developed both Ifakara Tent Trap formats and then designed and implemented mosquito sampling protocol in collaboration with the other authors. He also performed the data collection and analysis, interpreted the results and drafted the manuscript in consultation with the other authors. Prosper Pius Chaki (PPC), Yvonne Geissbuhler (YG), Fredros Oketch Okumu (FOO) and Khadija Kannady (KK) supported design and implementation of mosquito sampling protocols. Jacque Derek Charlwood (JDC) designed the Furvella trap and assisted with the field evaluation protocol. Robert Anderson (RA) contributed to the initial design of the Ifakara tent trap formats. GFK conceived the study and contributed to the development of Ifakara tent trap formats. He also supervised design and implementation of the mosquito sampling protocol, data analysis, interpretation of the results and drafting of the manuscript.

Chapter 4: Monitoring mosquitoes in urban Dar es Salaam: Evaluation of resting boxes, window exit traps, CDC light traps, Ifakara tent traps and human landing catches.

NJG designed and implemented mosquito sampling protocol in collaboration with other authors. He supervised the data collection, performed analysis, interpreted the results and drafted the manuscript in consultation with other authors. PPC and John Mpangile (JM), supported the design and implementation of the study. GFK supervised the design, implementation of the study, data analysis and edited the manuscript.

Chapter 5: An exposure-free tool for monitoring adult malaria mosquito populations.

NJG and Jason D. Moore developed an improved model of Ifakara Tent Trap (ITT model C) and GFK contributed to the design. NJG designed and implemented mosquito sampling protocol. He also supervised the data collection, performed analysis, interpreted the results and drafted the manuscript. GFK supervised the design, implementation of the study, data analysis and edited the manuscript.

Chapter 6: General discussion and Conclusions

NJG wrote and GFK edited this chapter

Signed:.....(Candidate)

Date:.....

TABLE OF CONTENTS

ACKNOWLEDGMENTS

The work presented here would not have been successful completed without the contribution of many people. Foremost acknowledgement goes to Dr. Gerry Killeen, my primary supervisor, who introduced me to this field of malaria mosquito research and control strategies. His scientific mentorship, commitment, friendship and generous supporter of my plan, with a lot of trust placed in me, allowed me to be independent in fulfilling my ambitions. Special thanks to Dr. Hilary Ranson and Dr. Louise Kelly-Hope, my secondary supervisors, for their constant professional support and personal encouragement that helped to maintain my personal motivation. Dr. David Malone and Dr. Gregor Devine assessed the progress and provided valuable inputs to my work. *Thanks a lot*. I thank Professor Steve Lindsay for constructive comments and for hosting me while I was registered at Durham University before transferring to Liverpool.

I sincerely thank my colleagues at Ifakara Health Institute: Prosper Chaki, Bernadette Huho, Yeromin Mlacha, Victoria Mwakalinga, Daniel Msellemu, Dr. Silas Majambere and Dr. Stefan Dongus for collaboration, friendship, and numerous inspiring discussions.

I would also like to express my appreciation to the residents of Kilombero Valley and Dar es Salaam for their tremendous cooperation throughout the study. Special thanks are due to all the mosquito catchers for their enormous commitment to this work and Architect Moses J Mollel for schematic drawings.

I am grateful for funding support from the Wellcome Trust (Research Career Development Fellowship 076806 awarded to Dr. Gerry F. Killeen), from the Bill & Melinda Gates Foundation (Malaria Transmission Consortium project grant 45114 coordinated by professor Frank Collins & Dr. Neil Lobo at Notre Dame University, as well as core salary support from the Ifakara Health Institute courtesy of our Director, Dr Salim Abdulla.

This thesis is dedicated to my wife Dorice and my son Moses for their love, support and patience, and to my parents and family for their wonderful support and encouragement during personally difficult situations.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ACT	Artemisinin Combination Therapies
CDC-LT	Center for Disease Control and Prevention Miniature Light Trap
CI	Confidence interval
DDT	Dichlorodiphenyltrichloroethane
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immunosorbent assay
GDP	Gross domestic product
GEE	Generalized estimating equations
I	Indoor
IRS	Indoor residual spraying
ITN	Insecticide-treated net
ITT	Ifakara Tent Trap
ITT-B	Ifakara Tent Trap design B
ITT-C	Ifakara Tent Trap design C
LLIN	Long lasting insecticide-treated net
NA	Not applicable
NE	Not estimable
NIMR	National Institute of Medical Research, Tanzania

O	Outdoor
OR	Odds ratio
P	Probability value (in statistical analyses)
PCR	Polymerase chain reaction
RB	Resting box
RBM	Roll back malaria
RR	Relative rate
RS	Relative sensitivity
SPSS	Statistical package for social sciences (Program for statistical analyses)
UK	United Kingdom
UMCP	Urban Malaria Control Program
WET	Window exit trap
WHO	World Health Organization

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 *Human malaria parasites*

Malaria is one of the most important diseases of human worldwide, and is caused by five species of protozoa of the genus *Plasmodium*. *Plasmodium falciparum, P. vivax, P. ovale* only infect human hosts (Singh *et al*. 2004) while *P. malariae* (Warrell and Gilles, 2002) and *P. knowlesi* (Jongwutiwes *et al*. 2004; Singh *et al*. 2004, Dondorp and Day, 2007) can both also infect monkeys. All *Plasmodium* parasites require two different host types to complete their life cycle: that is female anopheline mosquitoes and human beings. They are transmitted to humans almost exclusively through the bites of infected female *Anopheles* mosquitoes (Bruce-Chwatt, 1987; Warrell and Gilles, 2002; Greenwood *et al*. 2005). However, this blood parasite can also occasionally be transmitted through blood transfusion (Kitchen and Chiodin, 2006; Diop *et al*. 2009).

A mosquito can become infected when it bites a person whose peripheral bloodstream contains the sexual gametocyte form of the parasite which subsequently undergoes a sequence of development stages known as sporogony. Male microgametocytes and female macrogametocyte become mature gametes in the midgut of the mosquito and fertilization takes place when the former fuses with the latter. The zygote formed differentiates within one hour into a motile ookinete which penetrates the peritrophic matrix, and then the gut epithelium, to lodge itself under the basal lamina where it transforms to an oocyst. The oocyst grows and matures and then ruptures to liberate thousands of sporozoite form parasites which migrate and invade the salivary gland

where they finally become infectious to humans (Beier, 1998). When such an infectious mosquito takes its next human blood meal, sporozoites are injected into the bloodstream of that person and rapidily migrate to the liver, where they invade hepatocytes and develop into exo-erythrocytic schizonts. The schizont undergoes multiplication for between 6 and 15 days. The mature schizont ruptures, liberating thousands of merozoites into the bloodstream which then invade the red blood cells. While inside the red blood cells, merozoites digest haemoglobin to feed their development into trophozoites and then erythrocytic schizonts. After several divisions of the mature schizont, the infected red blood cell bursts and releases them into the bloodstream where they infect more fresh red cells. Progressive loss of both infected and uninfected red blood cells associated with increasing parasitemia result in bouts of clinical manifestation of the disease and also leads to indirect disease burden through anaemia and loss of resilience to a variety of co-infections (Warrell and Gilles, 2002).

After several cycles of erythrocytic schizogony, some merozoites differentiate into gametocytes rather than into schizonts, which can be ingested by female *Anopheles* and lead to another cycle of malaria transmission (Warrell and Gilles, 2002). Note that in *P. vivax* (Warrell and Gilles, 2002; Mueller *et al*. 2009) and *P. ovale* (Marquardt *et al*. 2000; Schmidt and Robert, 2000; Warrell and Gilles, 2002) sporozoites infecting the liver may develop into either schizonts or dormant hyponozoites which may remain inactive for long periods. While no particular biological factor is known to influence whether the sporozoites goes through either the active blood-stage or hyponozoite liver stage development pathway, it is speculated that this fate path results from genetic variations in the original inoculated sporozoites (Coatney, 1976; Miller *et al*. 1994), so that some give rise to schizonts that take longer to mature

(Coatney, 1976). Reactivation of hyponozoites may occur after weeks, months, or even years, unless destroyed by specific antimalarial drugs which target this cryptic liver stage is responsible for the relapses of disease in patients that otherwise appeared to have been cured (Coatney, 1976; Miller *et al*. 1994; Marquardt *et al*. 2000).

The severity of symptoms experienced during relapses is usually less severe than during the primary attack because acquired strain-specific immunity allows the patient to suppress parasite densities. However, even when the peaks of parasitemia are as high as during the primary attack, the patient may become tolerant to the fever-inducing effects of the parasites as a result of previous exposure (Miller *et al*. 1994; Molineaux *et al*. 2002).

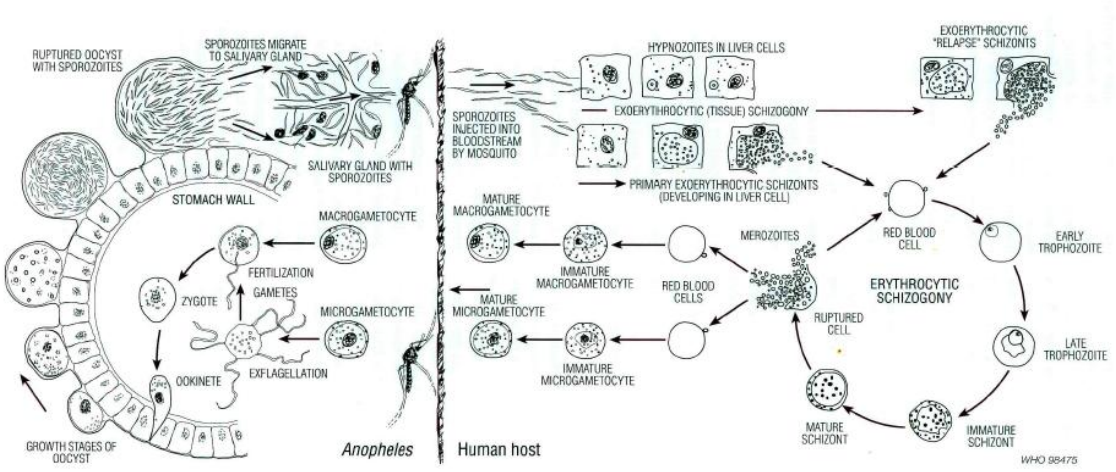

Figure 1.1.1: Life cycles of common human malaria parasites (reproduced with minor amendments, from Bruce-Chwatt's Essential Malariology 1993)

The common clinical symptoms of malaria include headache, abdominal discomfort, loss of appetite, vomiting, nausea, diarrhoea, chills, dry cough and fever. *P. falciparum* malaria is typically the most virulent of the human malaria parasites species and, if not treated early, severe complications such as jaundice, impairments of

consciousness (coma), splenomegaly and psychotic symptoms or convulsions can occur. Symptoms are typically most severe among individuals with little or no previous exposure or acquired immunity. Death may occur within days or even hours (Marsh *et al*. 1995; Schmidt and Robert, 2000).

1.2 *Burden and epidemiology of malaria*

Worldwide, an estimated 3.3 billion people are at risk of malaria with approximately 243 million cases and 863,000 deaths occurring in 2008. Africa alone accounts for 85% of cases and 89% deaths, with the remainder occurring mostly in South-East Asia and the Eastern Mediterranean (WHO, 2009).

In settings with stable, regular malaria transmission where repeated exposure leads to increasing immunity with age, deaths are concentrated in children under the age of five years (Rowe *et al*. 2006; WHO, 2009) and pregnant women (WHO, 2007; Desai *et al*. 2007; Largerberg, 2008). This is due to poorly developed or diminished immunity, respectively, in these population groups (WHO, 2007), thus making them more susceptible to severe anemia and death. Maternal malaria also increases the risk of spontenous abortion, low birth weight and premature delivery (Desai *et al*. 2007; Largerberg, 2008).

Plasmodium falciparum is the deadliest of the five species of human parasites, its distribution generally overlaps with *P. vivax*, which is the second most common species, around the tropics (Mueller *et al*. 2009). *P. falciparum* is responsible for

almost all cases of malaria in Africa (WHO, 2009; Mueller *et al*. 2009) and is the most dominant species in cross-sectional surveys of parasites prevalence globally (WHO, 2009; Mueller *et al*. 2009). The almost complete absence of *P. vivax* infections in African populations is explained by the high prevalence of an inherited trait of lacking the Duffy glycoprotein on the surface of red blood cells which is known to be essential for erythrocyte invasion by *P. vivax* merozoites (Mueller *et al*. 2009). Interestingly, lack of Duffy glycoprotein also prevents *P. ovale* and *P. knowlesi* from invading the red blood cells so these parasites are rare or absent across Africa (Schmidt and Robert, 2000).

Apart from the above direct health consequences, wherever malaria has occurred, it has always imposed the severest of impediments to economic development. Malaria is considered to be one of the major factors underlying poor economic growth in tropical countries particularly in sub-Saharan Africa (Carter and Mendis, 2002; Sachs and Malaney, 2002). For example, malaria is directly associated with high personal expenditure on treatment, preventions, diagnosis as well as government spending programmes for vector control, anti-malaria drug distribution and research (Sachs and Malaney, 2002). It is estimated that this disease accounts for an average slowing of economic growth rate of 1.3% annually (Sachs and Malaney, 2002) and all of the most malaria endemic countries suffer from extreme poverty (Figure 1.2.1).

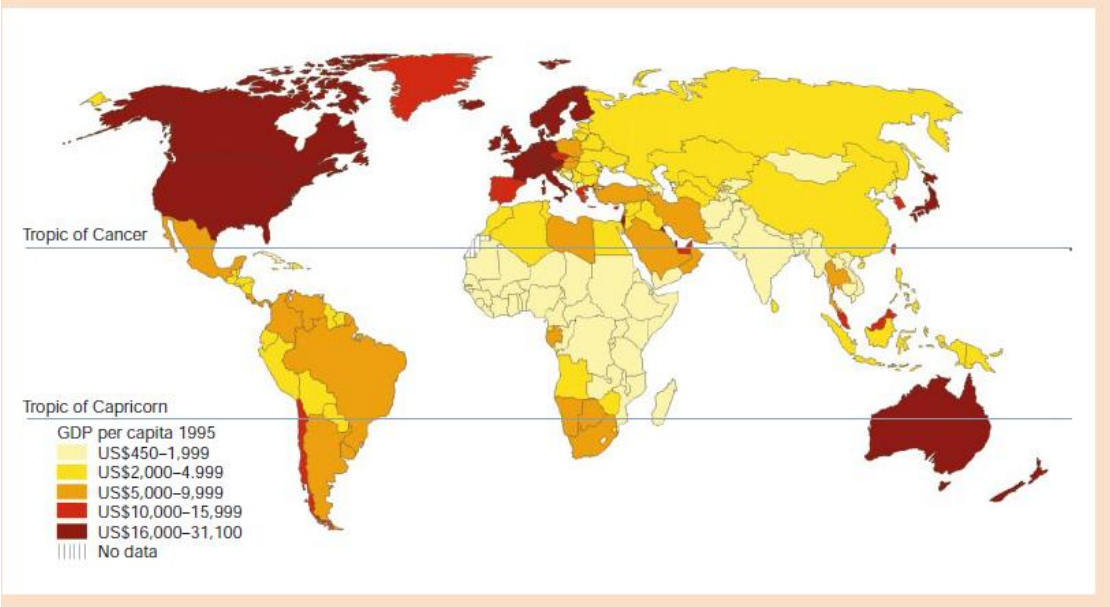

Figure 1.2.1: Global distribution of per capita GDP. Gobal pattern of income distribution is highly uneven, with average income levels significantly lower in the tropics (Sachs *et al* 2002)

1.3 *Global distribution of malaria*

The malaria burden is unevenly distributed globally. The burden is mostly focused upon tropical and subtropical regions (Hay *et al*. 2004; Kiszewski *et al*. 2004; Guerra *et al*. 2008; Hay *et al*. 2009; WHO, 2009). The primary reason for concentration of such high burden around the equator is generally due to favorable climatic conditions (Kiszewski *et al*. 2004 Guerra *et al*. 2008) while the particularly high endemicity in sub-Saharan Africa results from the presence of unusually anthropophagic malaria vectors that prefer human hosts as their source of blood (Bruce-Chwatt *et al*. 1966; Coluzzi, 1984; Gillies and Coetzee, 1987; Kiszewski *et al*. 2004), specifically mosquito species from the *Anopheles gambiae* complex and the *An. funestus* group (Coluzzi, 1984; Gillies and Coetzee, 1987; Kiszewski *et al*. 2004).

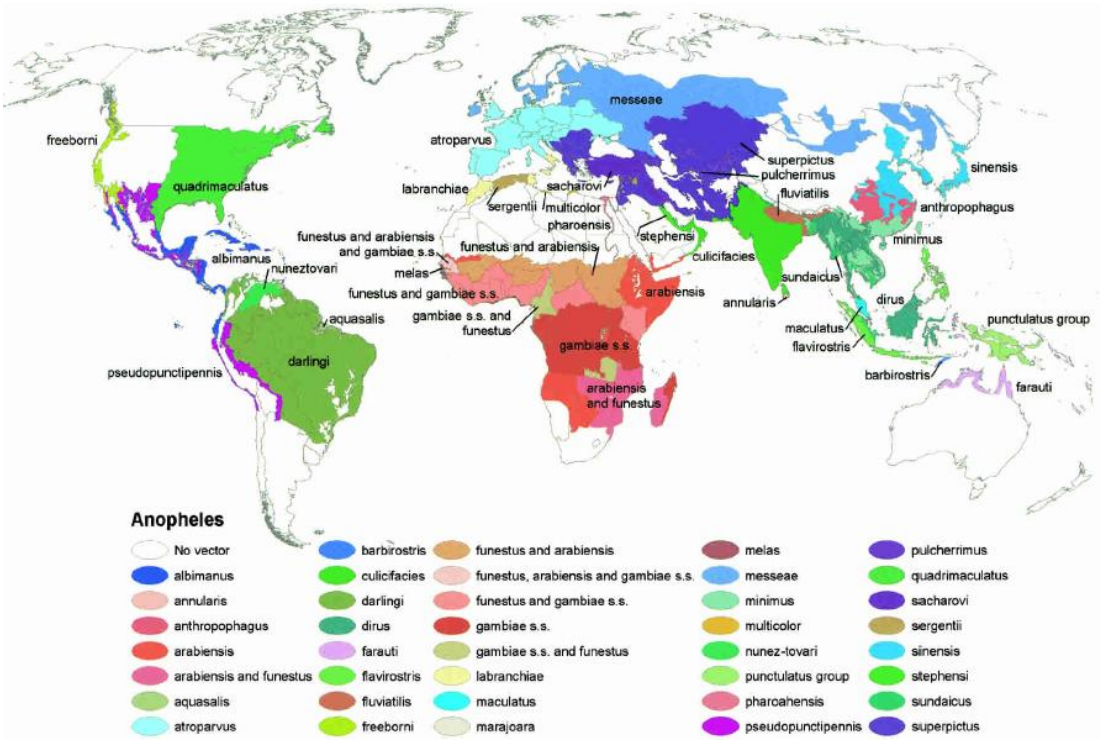

Figure 1.3.1: Global distribution of potentially important malaria vector *Anopheles* mosquito species (Kiszewski *et al*. 2004).

The remarkable human-biting habit some of the main African malaria vector intinsifies transmission, because a vector mosquito has to take at least two blood meals for transmission to occur: one from the infected person and one more when inoculating the sporozoite into another person (Beier, 1998). This is why essentially all process-explicit models of malaria transmission include the proportion of bloodmeals that come from humans as a squared term (MacDonald, 1957; Garrett-Jones, 1964a; Killeen *et al*. 2000a; Smith and McKenzie, 2004) and vector preference for human hosts is such a dominant determinant of malaria transmission intensity across the tropics. The differing seasonal patterns of population size fluctuation for *An. gambiae sensu lato* and *An. funestus* may also help explain why malaria transmission is so stable in sub-Saharan Africa. While the density of *An. gambiae s.l.* typically peaks during or soon after the rainy season (Gillies and DeMeillon, 1968), *An. funestus* that

has even greater preference for humans (Killeen *et al*. 2001; Kiszewski *et al*. 2004) typically persists and may even peak during the dry season (Gillies and DeMeillon, 1968; Smith *et al*. 1993; Minakawa *et al*. 2002; Mbogo *et al*. 2003; Kiszewski *et al*. 2004), thus maintaining transmission throughout the year.

1.4 *Role of climate in malaria transmission and distribution*

Humid and warm climates offer ideal conditions for mosquitoes to develop and survive (Bayoh and Lindsay, 2003; Guerra *et al*. 2008) to reach an age where they can transmit mature sporogonic stage-parasite (MacDonald, 1957; Garrett-Jones, 1964a; Garrett-Jones and Shidrawi, 1969; Guerra *et al*. 2008). The development of the parasite in mosquitoes is also temperature-dependent and differs for each *Plasmodium* species in terms of time from gametocyte ingestion to sporozoite invasion of the salivary glands (Beier, 1998). Although high temperatures create suitable condition for sporogony, arid conditions limit the development and the survival of mosquitoes (Guerra *et al*. 2008; Hay *et al*. 2009), so malaria transmission is generally highest where it is hot, wet and humid. These climatic factors largely explain why malaria transmission is limited to tropical and sub-tropical regions (Sachs and Malaney, 2002; Kiszewski *et al*. 2004; Snow *et al*. 2005; Guerra *et al*. 2008; Hay *et al*. 2009; WHO, 2009).

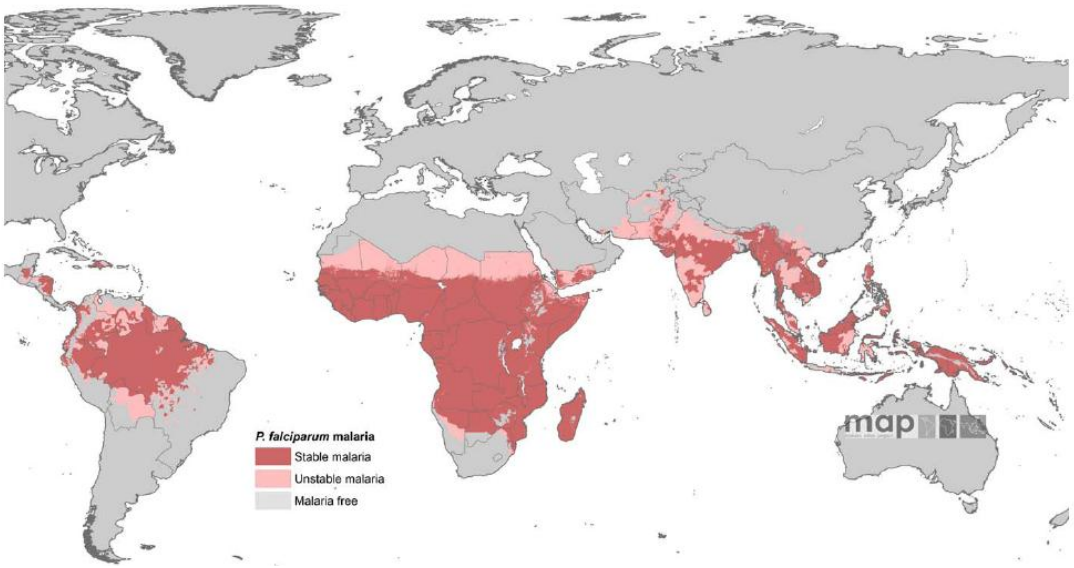

Figure 1.4.1: Estimated global distribution of *Plasmodium falciparum* malaria transmission stability (Snow *et al* 2008).

1.5 *Fine scale geographic heterogeneity of transmission*

Climate is a good predictor of where malaria occurs but substantial heterogeneity of exposure may occur between or even within towns or villages (Haddow, 1942; Ribeiro *et al*. 1996; Carter *et al*. 2000; Thomas and Lindsay, 2000; Robert *et al*. 2003; Ernst *et al*. 2006; Killeen *et al*. 2007b; Kirby *et al*. 2008). This implies that some people in a given population are far more exposed than others (Woolhouse *et al*. 1997; Smith *et al*. 2004; Mirghani *et al*. 2010) and these persons are responsible for stabilizing transmission (Woolhouse *et al*. 1997). Control programmes that target this small proportion of super-exposed people within a population are likely to be more effective in reducing transmission and associated disease burden than programmes that do not specifically target such high risk groups because these disproportionately contribute to the transmission reservoir of malaria (Woolhouse *et al*. 1997; Siri *et al*. 2010).

Although there are many contributing factors for such heterogeneity, some of the underlying reasons for such consistent micro-heterogeneity are well established. These include the distribution of human populations (Ribeiro *et al*. 1996; Thomas and Lindsay, 2000; Smith *et al*. 2004; Moffett *et al*. 2007), abundance and distribution of potential larval habitats (Trape and Zoulani, 1987; Smith *et al*. 1995; Thompson *et al*. 1997; Staedke *et al*. 2003; Minakawa *et al*. 2005; Mushinzimana *et al*. 2006; Protopopoff *et al*. 2009; Kulkarni *et al*. 2010) and demographic structure distribution of adult mosquito populations (Killeen *et al*. 2000b; Smith *et al*. 2004). As human populations are heterogeneously distributed, theoretical simulations show how adult mosquitoes tend to be heterogeneous and specifically concentrated around where more humans are found, even where larval habitat distribution is homogeneous (Smith *et al*. 2004). On other hand, young mosquitoes tend to disperse relatively short distances from their source breeding habitats while older mosquitoes have had a longer time to disperse further away from their source of origin (Service, 1993; Smith *et al*. 2004). The paradoxical observation of the occurrence of relatively higher risk of malaria with increasing distance away from the breeding source could be explained based on corresponding conclusion that this is where larger proportions of older mosquitoes are found (Smith *et al*. 2004), because for a mosquito to transmit the disease, it has to survive at least three feeding cycles (Beier, 1998). Note that the dispersal ranges of mosquitoes are generally determined by the availability of both bloodmeal and oviposition sites (Trape *et al*. 1992; Takken *et al*. 1998; Killeen *et al*. 2003). Where resources are scarce, mosquitoes can disperse for 5 kilometres or more (Takken *et al*. 1998), as opposed to few hundred metres (Trape *et al*. 1992; Thompson *et al*. 1997) where resources are abundant and closely associated.

Furthermore, host attractiveness varies considerably and is modified by co-inhabitation. As the number of occupants of a houses rise, the number of mosquito bites each person experiences rises (Haddow, 1942; Ernst *et al*. 2006; Killeen *et al*. 2007b; Kirby *et al*. 2008), possibly due to the increased attractiveness and range of the odour plume emanating from houses with large numbers of occupants (Mbogo *et al*. 1999; Takken and Knols, 1999; Okumu *et al*. 2010a).

Generally speaking, densities of malaria-transmitting mosquitoes rise and fall in response to rainy and dry seasons respectively. Although the number of mosquitoes surviving during the dry season is usually much lower, even these minimal vector densities may be sufficient to sustain malaria transmission (Beier *et al*. 1999; Gu *et al*. 2003), especially in and around perennial hotspot of vector proliferation and malaria transmission (Charlwood *et al*. 2000). Note that stable hotspot of malaria transmission that persist year after year on the coast of Kenya are clearly associated with persistent dry season transmission (Bejon *et al*. 2010). This crucial stabilizing role of dry season transmission may therefore allow supplementation of blanket coverage with standard prevention measures, such as insecticide-treated nets, with geographically targeted interventions directed at such perennial refugia which may dramatically enhance integrated strategies for control of both vectors and parasites (Griffin *et al*. 2010).

Because malaria transmission is highly variable over large areas and even upon very fine geographical scales (Woolhouse *et al*. 1997; Carter *et al*. 2000; Smith *et al*. 2005; Ernst *et al*. 2006; Smith *et al*. 2007; Bousema *et al*. 2010), high spatial and temporal resolution monitoring of exposure to transmission may be of critical importance in the

future as prerequisite for supplementing current front-line, ubiquitously distributed measures with geographically and perhaps seasonally targed complementary interventions.

1.6 *From control to eradication to apathy*

Before World War II, mosquito larval control strategies (source reduction and larviciding) were the main intervention options for suppressing malaria transmission (Bruce-Chwatt, 1987; Muturi *et al*. 2008). These strategies have a major advantage as they control mosquitoes while still in their immotile juvenile stages before they emerge and transmit disease. They have contributed significantly to controlling malaria for over 20 years in areas around Zambian copper mines and elimination of accidentally introduced, but well established, *An. gambiae sensu lato* from north east Brazil and the Nile valley of Egypt (Soper and Wilson, 1943; Shousha, 1948; Killeen *et al*. 2002a; Killeen *et al*. 2002b; Utzinger *et al*. 2002; Killeen, 2003). Also during the first half of the twentieth century, the applicatication of oil to water surfaces, and other larvicidal compound to the main water column of such larval habitats, effectively suppressed malaria transmission in parts of America, Asia, Europe and Israel (Bruce-Chwatt, 1987; Kitron and Spielman, 1989). However, this approach failed to reduce transmission in most majority rural tropical areas (Bruce-Chwatt, 1987). It should be understood here that the success of larviciding depends heavily on both timely location and treatment of all potential breeding habitats on daily or weekly (Killeen *et al*. 2002a; Killeen *et al*. 2006a). These requirements impose significant implementation challenges, especially with regard to extensive and often cryptic nature of breeding habitats that are preferred by African malaria vectors (Gillies and DeMeillon, 1968; Gillies and Coetzee, 1987).

When dichloro-diphenyl trichloroethane (DDT) was discovered in early 1940s (Bruce-Chwatt, 1984a; Bruce-Chwatt, 1987; Muturi *et al*. 2008), the focus shifted markedly from larval control to adult control with insecticide targeted at human houses (Bruce-Chwatt, 1987; Muturi *et al*. 2008). This dramatic shift in policy was based on the attractive idea that insecticides placed in and around houses would have greater impact than equivalent insecticide coverage of larval habitats (MacDonald, 1957; Garrett-Jones, 1964a). Even at modest coverage levels, insecticide targeted at human residences can result in dramatic suppression of transmission across entire community including those not covered due to repeatedly exposure of mosquitoes to insecticides during their life time (Killeen *et al*. 2007a). The malaria parasite requires at least eight days to complete sporogony, during which time the mosquito will usually feed at least three times. Therefore, reasonable coverage of houses with residual insecticides can results in dramatic reduction in the proportion surviving the multiple blood feeds required to reach an age at which they become capable of transmitting *Plasmodium* parasites (MacDonald, 1957; Garrett-Jones, 1964a; Garrett-Jones and Shidrawi, 1969). By comparison larval control has less dramatic effect with a more or less linear relationship between coverage and impact upon transmission (Killeen *et al*. 2006a) and usually difficult to implement (Killeen *et al*. 2002b; Killeen *et al*. 2006a; Vanek *et al*. 2006; Chaki *et al*. 2009).

The availability of cheap, effective insecticides such as dichloro-diphenyl trichloroethane (DDT) and antimalarial drugs such as chloroquine (Bruce-Chwatt, 1987; WHO, 2008) combined with oversimplified understanding of the biology of malaria transmission systems (MacDonald, 1957; Garrett-Jones, 1964a) led to the

Global Malaria Eradication Programme. By definition, eradication of any given pathogen means permanent reduction to zero of the worldwide incidence of infection (WHO, 2008). Achieving this ambitious goal for malaria depends on number of major prerequisites including: 1) fully understanding of the biology of disease vectors and parasite which often vary with epidemiological setting (Bruce-Chwatt, 1987; Ferguson *et al*. 2010), 2) availability of locally efficacious intervention options (WHO, 2008; Ferguson *et al*. 2010; Griffin *et al*. 2010), 3) long term commitment of both political and financial support from governments of all endemic countries and their overseas partners (WHO, 2008; Campbell, 2008; Feachem and Sabot, 2008), 4) major improvement of health systems (Abel-Smith and Rawal, 1992; McIntyre *et al*. 2006) and 5) broad social economic development (Sachs and Malaney, 2002).

This ambitious global programme was initiated by the World Health Organization (WHO) in 1955 and ended in 1969 without achieving its overall goal. It relied primarily on the strategy of ubiquitous application of DDT as an indoor residual spray to interrupt malaria transmission, combined with mass administration of antimalarial drugs to remove the reservoir of human stage-parasite (WHO, 1956; Gabaldon, 1969; Scholtens *et al*. 1972; Lepe, 1974; Bruce-Chwatt, 1987; Spielman *et al*. 1993; Aylward *et al*. 2000), regardless of geographical or epidemiological setting (Bruce-Chwatt, 1984b; Feachem and Sabot, 2008). Although this programme was initiated with a supposedly global agenda it excluded sub-Saharan Africa and Madagascar (WHO, 2008; Feachem and Sabot, 2008), even though this is where the majority of malaria burden occurs (Hay *et al*. 2004; Snow *et al*. 2005; Guerra *et al*. 2008; Hay *et al*. 2009; WHO, 2009). This region was not included because it was not considered to be technically feasible (Bruce-Chwatt, 1984b; Bruce-Chwatt, 1987; WHO, 2008).

Overall, this programme operated within very limited time frames and intervention options (Ferguson *et al*. 2010; Griffin *et al*. 2010). In hind-sight, it is perhaps not surprising that it fell far short of its local targets in many subtropical and tropical countries so the overall goal of global eradication was never achieved and malaria returned to areas where it had been previously eliminated when the programme ended (Harrison, 1978; Bruce-Chwatt, 1987; Najera, 2001; WHO, 2008; Feachem and Sabot, 2008). Other contributing factors to the collapse of this poorly-planned campaign included the increasing resistance of malaria vectors to insecticides (Soper, 1965; Molineaux and Gramiccia, 1980; Aylward *et al*. 2000) and of malaria parasites to drugs (Soper, 1965; Bruce-Chwatt, 1987; Aylward *et al*. 2000), increasing mosquito avoidance of indoor residual spraying (IRS) by feeding and resting more outdoors (Taylor, 1975; Ferguson *et al*. 2010; Griffin *et al*. 2010), the enormous logistical challenge any program of such large scales faces, and rising costs of residual insecticides (Bruce-Chwatt, 1987). The resulting loss of both confidence and support among donors and governments inevitably ended in rapid collapse of the programme and resurgence of malaria across the tropics in the 1970s and 1980s (Feachem and Sabot, 2008; Griffin *et al*. 2010).

Although this programme under-achieved simply because it over-promised, massive health benefits as a consequence of imperfect, but nevertheless impressive levels of control were accrued. During the immediate aftermath of the programme, WHO reassessed its strategy in 1969 and realized that the standards of existing health systems (Bruce-Chwatt, 1987; WHO, 2008) and real-world effectiveness of available intervention options (Molineaux and Gramiccia, 1980; Bruce-Chwatt, 1987; WHO, 2008) were not sufficient to eliminate the disease from areas of intense transmission

intensity, notably sub-Saharan Africa (Molineaux and Gramiccia, 1980; Gu *et al*. 2003; WHO, 2008; Griffin *et al*. 2010). Consequently, WHO soon lowered its target and extended its timelines indefinitely by changing its policy from eradication to sustained control (Molineaux and Gramiccia, 1980; WHO, 2008). The 22nd World Health Assembly suggested that alternative tools for malaria control should be developed specifically for areas where malaria eradication was proven unfeasible (WHO, 2008).

As neither financial resources nor technical support were forthcoming from the disillusioned international community, WHO recommended that each malaria-endemic country should commit itself to establishing antimalarial activities in accordance with its available human, technical, financial resources and maintain these activities until the disease no longer posed a major public health problem (Bruce-Chwatt, 1987; WHO, 2008). In fact many countries failed to effectively adopt this strategy and the only antimalarial activity retained was case management with antimalarial drugs (Bruce-Chwatt, 1987; WHO, 2008). In practice most developing countries were suffering from economic deterioration through the 1970s and 1980s (Bruce-Chwatt, 1987) and Africa was particularly badly hit as most countries struggled with newly-acquired independence.

1.7 *Malaria control in the modern era*

Prevention through vector control was revived as a priority on the global malaria control agenda when WHO launched a new programme, known as Roll Back Malaria

(RBM) (Dobson *et al*. 2000). RBM has since received growing political support, notably the Abuja declaration, signed in the year 2000 by the heads of states of most African countries, with overall goal of halving malaria mortality by 2010 (WHO, 2003a). The Abuja declaration also set the target that by 2015 malaria will no longer be a major cause of mortality or impediment to socio-economic development around the world. The highest priority interventions emphasized by RBM are 1) improved case management, 2) intermittent preventive treatment of pregnant women and 3) wide spread use of insecticides treated nets (ITNs) or, where appropriate, IRS (WHO, 2005b). The first trial in The Gambia, showing that ITNs could prevent childhood mortality (Alonso *et al*. 1991), paved the way for a series of randomized, controlled trials such as in The Gambia, Ghana, Kenya and Burkina Faso, areas with stable transmission in Africa. These trials demonstrated that ITNs can significantly reduce overall child mortality and associated anaemia in areas with high malaria transmission intensity (D'Alessandro *et al*. 1995; Binka *et al*. 1996; Nevill *et al*. 1996; Habluetzel *et al*. 1997). The consistent, positive results of these diverse trials restored confidence in interventions directed to kill the vector and their re-prioritization in malaria control agenda.

RBM policy has focused mainly on levering funding for free or highly-subsidized access to preventive and curative interventions, increasing support for national programmes to implement effective malaria control interventions nationwide, incorporating relevant and informative monitoring activities, engaging private sector and civil society stakeholders in scale-up of malaria control interventions and broaden investment in research to enable evidence-based policy formulation and implementation practice (WHO, 2005b).

Since the launch of RBM, national malaria control capacity has strengthened in endemic countries with progressively increasingly financial and technical support from international community (WHO, 2008). The cost required to deliver the basic minimum package of interventions prioritized by RBM is estimated to be around US$ 3.8 to US$ 4.5 billion per year globally (Kiszewski *et al*. 2007). While financial commitment from the international community has tremendously increased from US$ 0.3 billion in 2003 to around US$ 2 billion in 2009 (WHO 2009), this falls far short of addressing these needs fully (Kiszewski *et al*. 2007). Nevertheless, this wholesale expansion of the funding base is increasingly resulting in rapid scale-up of malaria control interventions such as ITNs or IRS, and the use of artemisinin-based combination therapies (ACTs), so malaria burden has dramatically declined in many African countries (Fegan *et al*. 2007; Sharp *et al*. 2007b; Battarai *et al*. 2007; Ceesay *et al*. 2008; O'Meara *et al*. 2008; Noor *et al*. 2009; WHO, 2009; Chizema-Kawesha *et al*. 2010; Okiro *et al*. 2010). In fact this wholesale alleviation of malaria burden in Africa has been so consistent that reduced rates of incidence of imported malaria are now being reported in Europe (Van Genderen *et al*. 2008).

The combination of new effective malaria control tools, improvement in social economic development in malaria endemic countries, increase global financial support, and recent decline in malaria burden across a wide range of epidemiological settings have inspired malaria communities to again consider the more ambitious goal of malaria eradication (Feachem and Sabot, 2008; Tanner and de Savigny, 2008). Although elimination of local transmission is possible with existing tools (LLIN, IRS and ACTs) in some areas with relatively low transmission (Mabaso *et al*. 2004; Sharp

et al. 2007a, WHO, 2009; John *et al*. 2009), it is considered extremely difficult or even impossible in high transmission intensity settings (Kleinschmeidt *et al*. 2009a; Ferguson *et al*. 2010) to even push prevalence down below the pre-elimination threshold of 1%. The Garki project in rural northen Nigeria, where transmission intensity, measured as the entomologic inoculation rate (EIR), ranged from 20 to 120 infectious bites per year, was implemented in the 1970s to evaluate the impact of indoor residual spraying with propoxur at 97-99% coverage per round and mass drug administration of sulfalene and pyrimethamine at 73-92% coverage. It showed that drastic reductions in malaria prevalence were attained but local elimination was not even approached (Molineaux and Gramiccia, 1980). One of the factors that contributed to incomplete vector suppression by IRS during the Garki trial was outdoor feeding (exophagy) and resting outdoor (exophily) behaviours (Table 1.7.1) of locally important vectors (Molineaux and Gramiccia, 1980).

More recently, some African malaria vector populations have been shown to feed extensively outdoor (Braimah *et al*. 2005; Pates and Curtis, 2005; Tirados *et al*. 2005; Oyewole and Awolola, 2006; Geissbühler *et al*. 2007). However, even those vector species which bite and rest primarily indoors (Gillies and DeMeillon, 1968; White, 1974; Gillies and Coetzee, 1987; Kiszewski *et al*. 2004), feed outdoors to some extent (Killeen *et al*. 2006b; Ferguson *et al*. 2010). On other hand *An. arabiensis* is know to prefer feeding on animals (zoophagy), (see table 1.7.1 for definition) cattle specifically, when this alternative host is available (White, 1974; Highton *et al*. 1979; Gillies and Coetzee, 1987). Even low levels of exophagy, exophily or zoophagy (Table 1.7.1) may substantially attenuate the impact of ITNs and IRS because this allows mosquitoes to obtain blood while avoiding fatal contact with insecticides (Pates

and Curtis, 2005; Geissbühler *et al*. 2007; Killeen and Smith, 2007). Monitoring of feeding and resting behaviours are therefore of critical importance for choosing and predicting realistically feasible levels of expected impact for vector control interventions. This is likely to become even more important as the extent of these behaviours may increase when strong selection pressure is applied through measures such as ITNs and IRs that specifically target resting and feeding upon humans inside houses.

Table 1.7.1.: Definition of mosquito behavioural choices

1	Exophagy: is a tendency for mosquitoes to prefer biting outside.
2	Endophagy: is a tendency for mosquitoes to prefer biting indoor
3	Exophily: is a tendency for mosquitoes to prefer resting outside
4	Endophily: is a tendency for mosquitoes to prefer resting indoor
5	Anthropophagy: is a tendency for mosquitoes to prefer feeding on human hosts
6	Zoophagy: is a tendency for mosquitoes to prefer feeding on animal hosts

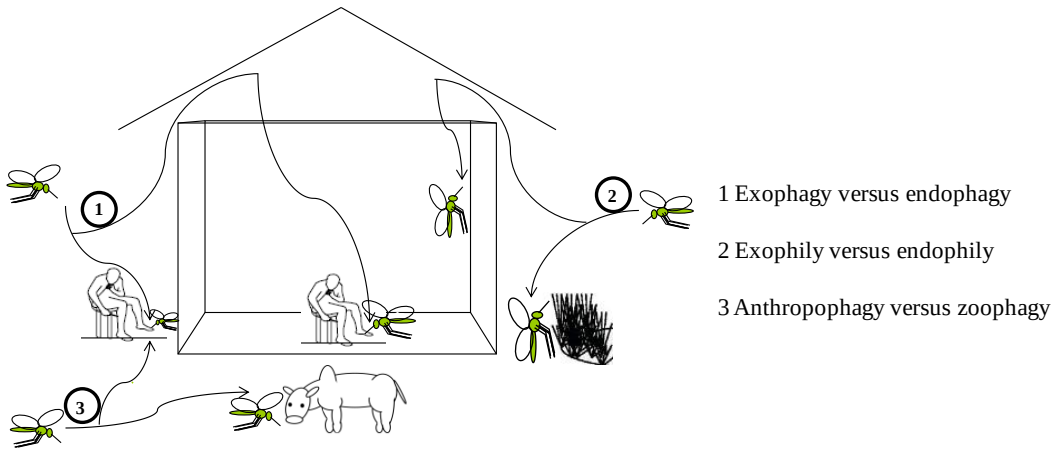

Figure 1.7.1: Mosquito behavioural choices

In places where local malaria vector populations are predominantly exophagic, exophilic or zoophagic, indoor-targeted interventions are therefore less effective. These mosquito populations could be controlled by attracting and killing adult female mosquitoes feeding outdoor using outdoor traps or insecticide dissemination stations (Knols *et al*. 2010; Okumu *et al*. 2010a), by applying insecticides to animals (Rowland *et al*. 2001) or by preventing their emergence as adults at source in larval habitats (Soper and Wilson, 1943; Shousha, 1948; Kitron and Spielman, 1989; Killeen *et al*. 2002b).

Larval control of *Anopheles* mosquitoes has long been neglected despite its historical success in eliminating transmission and associated disease burden as previously explained in section 1.6. This strategy is most likely to be effective in areas with low malaria transmission intensity where potential larval sources are relatively few, accessible and readily defined (Killeen *et al*. 2002a). Urban environments, which are generally characterized by lower malaria transmission intensity (Robert *et al*. 2003), relatively high numbers of inhabitants per square kilometer, better infrastructure and stronger institutional capacity, are likely to be best suited to larval control.

1.8 *Malaria control in Dar es Salaam*

Malaria control in Dar es Salaam, predominantly based on larval source management, began during the colonial period and continued after independence (Clyde, 1961a; Clyde, 1961b; Bang *et al*. 1975; Beck, 1977; Kilama, 1991) up to 1972 when adverse economic conditions, combined with rapid, poorly planned decentralization, led to deterioration of the health system (Kilama, 1994). In 1913, the German ordinance for

mosquito extermination was established. It provided legal authority to municipal authorities for the destruction of larval habitats such as vessels, tins, and ponds. The main interventions were source reduction measures such as as oiling water accumulations and drain construction. With this package of interventions, malaria vector populations in Dar es Salaam were reduced by approximately 90% (Kilama, 1991). The British government took over after World War I and sustained malaria control with drainage work, oiling of puddles, straightening of stream beds, ensuring that cattle were kept far from streams and swamps (Kilama, 1991) and were sometimes supplemented with adult mosquito control by spraying houses with DDT or dieldrin once these became available after World War II (Kilama 1991). In the 1960s, larviciding and environmental management were still maintained by a centralized vector control service well into the post-independence period up to 1972 (Bang *et al*. 1975; Bang *et al*. 1977; Kilama, 1991; Kilama, 1994). Thereafter, there was no maintenance of drains so water flow was blocked by silt, vegetation and waste, providing suitable habitat for mosquitoes (Castro *et al*. 2004). It remained so until 1983, when the Ministry of Health and Social Welfare (MoHSW) of Tanzania reformulated its malaria control policies, with priority given to integration of multiple complementary interventions, including vector control, chemotherapy, and monitoring of drug resistance. However translation of the vector control component of this policy into *de facto* practice took another 5 years.

In 1988, the government of Japan, through the Japan International Cooperation Agency (JICA), in collaboration with the government of Tanzania, launched an Urban Malaria Control Programme (UMCP) in Dar es Salaam and Tanga primarily focused on vector control. Chemical larviciding, indoor residual spraying, ultra low volume

space spraying of insecticides, ITNs and environmental management were all used. Polystyrene beads were also used to control *Culex* larvae in pit latrines and soakage pits (Maxwell *et al*. 1990; Chavasse *et al*. 1995; Castro *et al*. 2004). Apart from providing technical and operational expertise, this JICA-directed programme was also responsible for identification of larval habitat, distribution of equipment for malaria control, entomological surveillance and parasitological evaluation (Castro *et al*. 2004). Unfortunately this program was not sustained and it ended in 1996. Nevertheless, it provided a particularly good lesson of how successful malaria control programs depend not only on the interventions options and financial support available, but also on local managerial capacity and stakeholdership (Castro *et al*. 2004; Barat, 2006). The lack of integration with the City Council's institutional structures probably explains why this JICA-driven project was not sustainained in the long term (Castro *et al*. 2004).

More recently, in March 2004, the City Medical Office of Health for Dar es Salaam established a new pilot-phase Urban Malaria Control Programme (UMCP) which operates primarily through community-based implementation mechanisms, framed within a vertical management system (Mukabana *et al*. 2006; Fillinger *et al*. 2008; Chaki *et al*. 2009). The programme focuses on surveillance of malaria transmission by monitoring adult mosquito density, as well as cross-sectional prevalence of malaria infection, and intervenes mainly by supplementing national distribution systems for ITNs with biological larvicides *Bacillus thuringiensis var Israelensis* (Mukabana *et al*. 2006; Vanek *et al*. 2006; Geissbühler *et al*. 2007; Fillinger *et al*. 2008; Chaki *et al*. 2009; Geissbühler *et al*. 2009; Sikulu *et al*. 2009). Larvicides are applied on weekly basis to all potential breeding habitats observed by community-based staff assigned to

defined areas of approximately 0.6 km^2 (Mukabana *et al*. 2006; Dongus *et al*. 2007; Fillinger *et al*. 2008; Chaki *et al*. 2009). This weekly re-application cycle is due to the fact that *An. gambiae* complex mosquitoes, the dominant vectors in Dar es Salaam and most of Africa, can transform from egg to adult within one week or less (Gillies and DeMeillon, 1968; Gillies and Coetzee, 1987) so the adult mosquito surveillance system for this programme needs to report mosquito densities at correspondingly high spatial and temporal resolution. Such adult mosquito monitoring needs to detect and report coverage gaps almost fast as they occur, on such fine geographic scales as neighbourhoods, housing clusters and even individual plots (Dongus *et al*. 2007; Chaki *et al*. 2009) on a weekly basis (Killeen *et al*. 2006a; Fillinger *et al*. 2008).

Due to the need for such intensive and extensive monitoring of adult mosquito in response to larval control program, a total of 268 sentinel sites distributed randomly across the UMCP study area were chosen for the surveillance of biting densities once every four weeks. Outdoor human landing catch was chosen as the only sufficient sensitive technique for catching mosquitoes after it was demonstrated that other existing sampling methods were not adequately sensitive to monitor the low densities of malaria vector in this setting (Fillinger *et al*. 2008). Mosquito catchers were recruited from the local community in each neighbourhood of the study area on a paid but voluntary basis and each was responsible to catch mosquitoes in four sentinel sites within each neighbourhood as described elsewhere (Geissbühler *et al*. 2007; Fillinger *et al*. 2008).

1.9: *Common techniques for monitoring host-seeking Anopheles mosquitoes and malaria transmission in Africa*

While it can be difficult to catch sufficient numbers of infected mosquitoes to measure EIR in absolute terms where transmission intensity is low (Beier *et al*. 1999), vector biting density is in itself directly to EIR (Dye, 1986) and can act as a useful proxy of transmission for monitoring of impact of interventions, particularly larviciding which primarily targets vector densities rather than survival or infection rates (Killeen *et al.* 2000a; Killeen *et al*. 2000b). Here I review the various methods for trapping and monitoring malaria vectors mosquitoes that were available at the outset of this study and discuss their respective advantages and disadvantages:

Human Landing Catch (HLC)

The human landing catch consists of a volunteer exposing his/her legs and collecting the mosquitoes with an aspirator when they land on his/her legs (Figure 1.9.1) (WHO, 1975b; Service, 1977; WHO, 2002; Mboera, 2005; Geissbühler *et al*. 2007). This is the most direct method available for estimating human exposure to mosquito bites and obtaining samples of host-seeking, human-biting mosquitoes (Lines *et al*. 1991; Service, 1993; Davis *et al*. 1995; Mboera, 2005) and is therefore accepted as a gold standard (Service, 1977; Service, 1993).

Figure 1.9.1: Photograph illustrating the human landing catch method

Since mosquitoes are caught in the act of biting the human host (Lines *et al*. 1991; Service, 1993; Davis *et al*. 1995; Mboera, 2005), the number of mosquitoes caught can be considered to reasonably represent the human biting rate and the sample of mosquitoes obtained to have the same distribution of age, physiological status and infection status as those to which attack people at that time and place. Moreover, HLC can be performed both inside and outside the houses and therefore provides important information on when and where humans are exposed to mosquito bites, as well as the degree of exophagy of mosquito populations (Charlwood and Graves, 1987; Pates and

Curtis, 2005; Geissbuhler *et al*. 2006b; Oyewole and Awolola, 2006). Such information on the indoor and outdoor biting pattern of mosquitoes, have major implications for malaria epidemiology, both in term of host-vector contact and the choice of effective vector control strategy (Pates and Curtis, 2005).

Nonetheless, this technique has major drawbacks, some of which are severely limiting. It is extremely arduous, uncomfortable and labour intensive, requiring such intense supervision that it is difficult to sustain on large scales. Close supervision is required because the collector needs to not only remain awake but also constantly vigilant for the data to be reliable (Service, 1977; WHO, 1995; Mboera, 2005). This is especially difficult when sampling night-active African malaria vectors that prefer feeding at times when people like to sleep. An even greater concern arises from the fact that it inevitably increases the hazard of exposure of participants to mosquito-borne infections (Service, 1977; WHO, 1995; Mboera, 2005) which is difficult to justify on ethical grounds. One of the biggest sources of variation involved with the HLC is associated with human participants themselves who vary in their ability to attract (Lindsay *et al*. 1993; Takken and Knols, 1999) and catch landing mosquitoes (Service, 1977; WHO, 1995; Mboera, 2005).

Centers for Disease Control and Prevention miniature Light Trap (LT)

The most commonly-used alternative to HLC for sampling host-seeking African malaria vectors is the Centers for Disease Control and Prevention miniature Light Trap (LT) (Sudia 1962). The first field evaluation of this method for sampling adult African malaria mosquitoes was conducted in The Gambia (Odetoyinbo, 1969). The traps were

demonstrated to be effective, in term of numbers caught for both *Anopheles gambiae s.l.* and culicines. It was also noted that the catches increased when the LTs were placed close to hosts (Odetoyinbo, 1969) and subsequent experiments proved that sampling efficiency improved dramatically when placed beside human hosts protected by untreated bed nets (Garret-Jones and Magayuka, 1975). Since then, LTs have been placed indoor beside occupied bed nets as a successful standard practice (Maxwell *et al*. 1990; Lines *et al*. 1991; Mbogo *et al*. 1993; Githeko *et al*. 1994; Davis *et al*. 1995; Costantini *et al*. 1998; Mboera *et al*. 1998; Mathenge *et al*. 2004; Amusan *et al*. 2005; Mathenge *et al*. 2005). Although this trap has been reported to be less effective for sampling outdoor fractions of mosquito populations (Service, 1993), a recent study in the highlands of Kenya using LTs with an ultra-violet bulb has shown very promising results for sampling of malaria vectors both indoor and outdoor (Drakeley *et al.*, Personal Communication). The detailed behavioural processes that define how mosquitoes become attracted to, and consequently trapped by, LTs remain unknown. Nevertheless, it has been suggested that because host-seeking mosquitoes are denied access to the protected host within the net, but remain stimulated by emanating cues such as odour and body heat passing through it (Mathenge *et al*. 2002), these mosquitoes may persistently explore around the net and eventually or accidentaly be attracted to the trap itself.

Any alternative sampling method needs to be calibrated against the HLC to ensure that it provides representative information on the density, demographic status and infection rate of human-biting mosquitoes. LTs placed beside occupied nets have been evaluated in a range of different settings and exhibited varying degree of success. In two studies conducted in Tanzania, where LTs were hung beside an occupied,

untreated bed net, showed that they provided an efficient and unbiased estimate of human biting densities of *An. gambiae s.l.* populations (Lines *et al.* 1991; Davis *et al*. 1995). The LT has also been shown to provide a reliable estimate of biting rates in communities even where people sleep under treated bed nets (Magbity *et al*. 2002; Killeen *et al*. 2007b). However, in other studies the sampling efficiency of LTs have been demonstrated to be density-dependent (Hii *et al*. 2000; Magbity *et al*. 2002; Okumu *et al*. 2008) with age- and infection- related sampling biases for malaria transmitting mosquitoes (Githeko *et al*. 1994; Davis *et al*. 1995; Mboera, 2005). The reasons of such inconsistency in sampling efficiency of LTs remain unclear. It should also be noted that reliance upon electricity supply for recharging batteries limits the affordability and practicality of LTs, particularly in remote areas without a reliable power.

Bed net traps

Several bed net trap designs have been used to catch mosquitoes, using humans or animals as bait. These include the bed net with holes or suspending a large mosquito net from four poles placed vertically around a bed, leaving a gap of about 15cm between the floor and bottom of net to allow mosquito entry (WHO, 1975b; Service, 1977). The person acting as bait in the latter design may be confined within a protective inner net to prevent him or her from being exposed to mosquito bites. The biggest advantages of bed net traps over HLC are that they are not labour-intensive. These sampling techniques, however, have been found to be insensitive for sampling malaria vectors across range of epidemiological setting in Africa (Service, 1977).

More recently, an exposure-free bed net trap, known as the Mbita trap, was conceived primarily for sampling unfed, hungry, host-seeking malaria mosquitoes, based on observations of their behavior around human-occupied bed nets. It is conically-shaped, resembling a bed net made of cotton cloth with its circular upper part consisting of a netting funnel with a small inner aperture kept open by a small metal ring. These structural features allow the entrance of mosquitoes but limit their exit (Figure 1.9.2). The Mbita trap does not expose volunteers to mosquito bites, allows them to sleep throughout the sampling period, and requires neither skilled personnel nor electrical power (Mathenge *et al.* 2002).

Initial evaluation of Mbita trap was conducted using laboratory reared *An. gambiae s.s* released into a large-cage semi-field system (Mathenge *et al*. 2002) and then controlled field trials in Western Kenya (Mathenge *et al*. 2004). In both cases, the trap was reported to be relatively sensitive (Mathenge *et al*. 2002; Mathenge *et al*. 2005) and provided catches which were consistently proportional to those by HLC (Mathenge *et al*. 2004; Mathenge *et al*. 2005). The sporozoite rate observed for both *An. gambiae s.l.* and *An. funestus* was statistically indistinguishable from those observed with the HLC (Mathenge *et al*. 2004; Mathenge *et al*. 2005). However, other trials outside of Kenya, reported very poor performance in the highlands of Madagascar (Laganier *et al*. 2003) and in three sites in rural (Braimah *et al*. 2005; Okumu *et al*. 2008) and urban Tanzania (Fillinger *et al*. 2008).

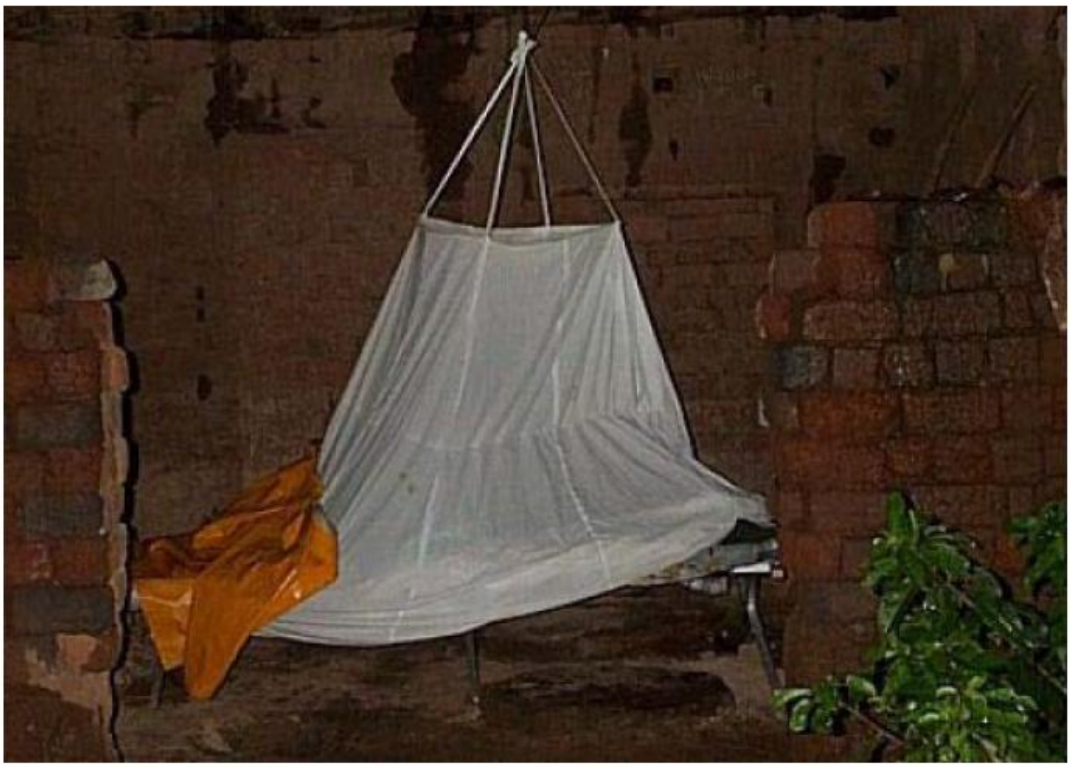

Figure 1.9.2: An Mbita trap as set up in a house in Madagascar. (Laganier *et al*. 2003)

1.10 *Common techniques for sampling resting populations of mosquitoes in Africa*

Samples of resting fractions of mosquito populations are essential to enable assessment of host-feeding patterns through blood meal analysis (Kay *et al*. 1979; Fontenille *et al*. 1997; Tirados *et al*. 2005; Lardeux *et al*. 2007; Mouatcho *et al*. 2007; Muriu *et al*. 2008; Lyimo and Ferguson, 2009). The proportion of blood meals that each mosquito species obtains from humans is a critical determinant of, not only transmission intensity (Garrett-Jones, 1964a; Garrett-Jones, 1964b; Dye, 1986), but also the efficacy of interventions targeted at humans or the houses they live in (Killeen and Smith, 2007; Killeen *et al*. 2007a; Le Menach *et al*. 2007). In addition, sampling resting mosquitoes also gives information about resting places, resting density, and seasonal changes in density (Kulkarni *et al*. 2006)

Direct collection of resting mosquitoes

The most common methods for sampling indoor-resting populations of mosquitoes involve either aspirating directly from accessible resting places a technique known as hand catch (Figure 1.9.3) (WHO, 1975b; WHO, 2002), or by pyrethrum spray collection (PSC) to "knock down" mosquitoes resting in the house collect them on the white sheets spread out on the floor (WHO, 1975b; WHO, 2002; Kulkarni *et al*. 2006; Odiere *et al*. 2007; Fornadel and Norris, 2008; Kweka *et al*. 2008). With the hand catch method, torches are always used to locate the indoor resting mosquitoes but these should not be very powerful to avoid disturbing them as they may escape by flying away (Service, 1993).

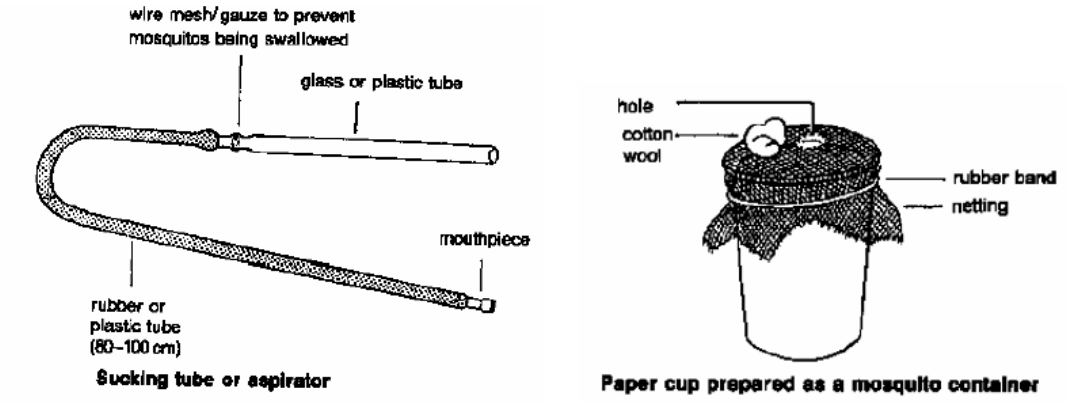

Figure 1.9.3: Aspirator and paper cup for hand catch collection. (WHO 2002)

While it is unlikely that the hand-catch method can collect more than a small proportion of mosquitoes resting in a given house, pyrethrum spray catch (PSC) is presumed to catch a much higher fraction from a well-closed room (WHO, 1995). Indoor resting sampling methods are often used to yield information on feeding pattern of mosquitoes because a large proportion of these are bloodfed but may also be used to

survey indoor resting densities and vector species composition (Service, 1977; Service, 1993; WHO, 2002; Kweka *et al*. 2008; Bayoh *et al*. 2010). However estimating human biting rates from indoor resting collections is likely to underestimate human exposure in houses with IRS or ITNs because mosquitoes are irritated or repelled by insecticide on nets or walls (WHO, 2005a; Diuk-Wasser *et al*. 2005). As a result most mosquitoes which feed in the house will rapidly exit (Chareonviriyaphap *et al*. 1997; Muenworn *et al*. 2006; Pothikasikorn *et al*. 2007) and rest outdoor (Muirhead-Thomson, 1960; Bogh *et al*. 1998; Quinones *et al*. 1998). Also, even in the absence of such irritant insecticides, some mosquito populations commonly leave houses soon after feeding (Kulkarni *et al*. 2006; Fornadel and Norris, 2008) and are consequently undersampled. This implies that such indoor resting sampling methods are not suitable for estimating human biting densities because they can underestimate the human biting rate and this underestimation can be exacerbated by common contol measures domestic vector. Furthermore, these techniques may also overestimate exposure when they sample mosquitoes that enter the house after feeding outside on other vertebrates. However, in places where malaria vectors are predominantly endophilic and sufficiently abundant, indoor resting collection may enable comparative estimation of malaria transmission. For example, this method has been successfully used to estimate transmission (mean number of infective bites per house occupant, based on freshly blood fed mosquitoes only) in the highlands of Burundi, where all vectors are highly endophilic due to relative low temperature outside (Protopopoff *et al*. 2007).

Exit traps

As mentioned above, some mosquito species tend to enter houses at night and bite and leave the house soon after feeding without resting indoors (Mboera, 2005; Pates and Curtis, 2005; Kulkarni *et al*. 2006; Fornadel and Norris, 2008). This fraction of pathogen-transmitting mosquitoes, together with those that do rest indoors but eventually leave to lay eggs, can be monitored by using exit traps placed over windows (WHO, 1975b; Hargreaves *et al*. 2003; Mouatcho *et al*. 2007; Sharp *et al*. 2007a; Ridl *et al*. 2008). Mosquitoes are trapped by window exit traps (WET) as they leave houses, thus allowing vector density to be monitored. Data and samples from WET provide information about exophilic versus endophilic resting behaviour, physiological and biodemographic status distributions of the mosquito population. This method may also be used to test the behavioural avoidance responses of malaria vectors to different insecticides sprayed on wall of houses or used to impregnate bed nets (Lindsay *et al*. 1989; Lindsay *et al*. 1991; Quinones *et al*. 1997). While the WET has been reported to be useful for monitoring malaria vector density trends in Southern Africa (Hargreaves *et al*. 2003; Mouatcho *et al*. 2007), Equatorial Guinea (Sharp *et al*. 2007a; Ridl *et al*. 2008), and for the vector of Japanese encephalitis in Korea (Chen and Chow, 1969), their sensitivity is likely to be highly affected by house design (WHO, 1995).

Outdoor resting traps

Some species of mosquitoes do bite and rest outside on vegetation and on surfaces in sheltered places such as the holes in rocks, culverts, animal burrows and stems of larger trees. Although outdoor collection can be conducted on these natural resting sites, man-made shelters constructed for this purpose have the advantage of providing

known places for concentrated, presumably more sensitive, sampling so that more representative samples can be collected for analysis (WHO, 2002). While PSC and simple hand-catch methods are both practically limited to sampling indoor resting mosquito populations, pit traps are the only widely used method for monitoring outdoor resting fraction of human malaria vector populations. The pit trap typically has quite limited sensitivity and also has major practical, logistic drawbacks, notably the physical hazard it presents to residents of the area (Odiere *et al*. 2007).

Clay pots have recently been assessed for collecting outdoor-resting *An. gambiae, An. funestus, An. arabiensis* and *Culex* species in Western Kenya (Odiere *et al*. 2007). The sampling sensitivity of pots was found to be better than pit shelters and equivalent to Colombian curtains, PSC and exit traps. However, these findings were not reproduced in northern Tanzania where they were found to have much lower sensitivity than that reported in Kenya (Van den Bijllaardt *et al*. 2009).

Resting boxes (RB) have also been used to sample mosquitoes since it was first observed that they tend to aggregate in dark, sheltered resting places during the day (Crans, 1989). Boxes are generally placed on the ground with the opening facing west to reduce the effect of direct sunlight during the early part of the day. In well-shaded areas, the exact direction of the open end becomes less important (Crans, 1989). While RB were found to be highly selective in sampling specific mosquito species in coastal areas of the United States of America (Crans, 1989), they have also shown potential for monitoring *Culex quinquefasciatus* and *Aedes aegypti* in urban Brazil (Barata *et al*. 2007). However, it has been shown that in many cases the number of female

mosquitoes collected by RB do not correlate well with those from HLC (Kay, 1982), possibly because they sample different components of the mosquito populations.

1.11 *Rationale of the study*

Previous studies in Dar es Salaam demonstrated that both *An. gambiae s.s* and *An. arabiensis* prefer to feed outdoors and, in case of the latter, feeding activity peaks in the early evening (Geissbuhler *et al*. 2007). Such outdoor-feeding preferences have also been recorded in other malaria endemic-settings across the tropics (Coluzzi *et al*. 1979; Pates and Curtis, 2005; Tirados *et al*. 2006; Van Bortel *et al*. 2010; Russell *et al*. 2011; Bugoro *et al.* 2011). However, the degree of community-level protection potentially provided by ITNs against malaria transmission by mosquitoes that bite earlier and outdoor had not been explicitly simulated or discussed in the literature at the outset of the study.

The Dar es Salaam Urban Malaria Control Programme requires both spatially- and temporally-intensive monitoring of adult mosquitoes to enable effective management of routine larvicide application activities. At the outset of this study, the human landing catch was found to be the only method sufficiently sensitive for surveillance and monitoring malaria vectors but this traditional method has major drawbacks, some of which are prohibitive. The most notable of these is that it increases exposure of catchers to mosquito-borne infections, which is ethically difficult to justify. HLC also requires intense supervision which is difficult to sustain on large scales.

1.12 *Goal and Objectives*

Goal

The overall goal was to assess how much the impact of ITNs can be attenuated by the outdoor human exposure to malaria vectors, as well as to develop and characterize new surveillance tools that enable sustained malaria vector control through effective monitoring, evaluation and optimization of impact upon mosquito populations.

Objectives

1. To determine the proportion of human exposure to malaria vectors which occurs indoors and can be directly prevented by using an ITN.

2. To develop and apply a novel simulation model of malaria transmission to assess how the community-level impact of ITNs might be attenuated by outdoor human exposure to malaria vector mosquitoes.

3. To develop and evaluate a safe, practical and affordable alternative to HLC that allows intensive and extensive monitoring of malaria vectors.

4. To evaluate the efficacy of this new trapping method compared with all the relevant alternative technologies to human landing catch.

Chapter 2 addresses objectives 1 and 2 by analyzing previously collected human and mosquito data (Geissbühler *et al.* 2007) and applying obtained summary parameters to adapted malaria transmission models. Chapters 3, 4 and 5 address objectives 3 and 4 by describing the development of three novel tent trap designs and comparing their efficacy and safety under semi-field and full field conditions. Chapter 6 provides an overview of the research presented in the previous chapters, discusses the implications of the results, and outlines areas for further investigation in the future.

CHAPTER 2

INSECTICIDE-TREATED NETS CAN REDUCE MALARIA TRANSMISSION BY MOSQUITOES WHICH FEED OUTDOORS.

This paper has been published in the

American Journal of Tropical Medicine and Hygiene 2010, 83(3): 415-419

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2.1 Abstract

Insecticide treated nets (ITNs) represent a powerful means for controlling malaria in Africa because the mosquito vectors primarily feed indoors at night. The proportion of human exposure that occurs indoors, when people are asleep and can conveniently use ITNs, is therefore very high. Recent evidence suggests behavioral changes by malaria mosquito populations to avoid contact with ITNs by feeding outdoors in the early evening. We adapt an established mathematical model of mosquito behavior and malaria transmission to illustrate how ITNs can achieve community-level suppression of malaria transmission exposure, even where mosquito evade them and personal protection is modest. We also review recent reports from Tanzania to show that conventional mosquito behavior measures can underestimate the potential of ITNs because they ignore the importance of human movements.

2.2 Introduction

Insecticide-treated nets (ITNs) represent a powerful means for controlling malaria in Africa(Lengeler, 2004). This is due to the fact that the principal malaria vectors, from the Giles *Anopheles gambiae* and *Anopheles funestus* species complexes (Gillies and DeMeillon, 1968; White, 1974; Gillies and Coetzee, 1987), primarily feed indoors at night (Gillies and DeMeillon, 1968; Pates and Curtis, 2005; Killeen *et al*. 2006b). Thus the proportion of human exposure which occurs indoors (π_i), when people are asleep and can conveniently use them, is very high (Figure 2.4.1 A, B, C and D). Such estimates of π_i which take into consideration the movement patterns of people are obtained in the field by weighting the observed indoor and outdoor biting rates at each

period of the night by the proportion of humans that are typically in these two compartments at that time (Killeen *et al*. 2006b; Geissbühler *et al*. 2007).

When reasonable levels of community-wide coverage are achieved, with approximately half of the population using them each night (Hawley *et al*. 2003; Killeen *et al*. 2007a), ITNs not only confer personal protection against infectious bites but can also reduce the survival, feeding frequency, feeding success and density of vector mosquito populations (Killeen and Smith, 2007; Killeen *et al*. 2007a). This finding means that ITNs not only prevent malaria in protected persons, but can also reduce the exposure of unprotected person by suppressing transmission across entire communities (Binka *et al*. 1998; Howard *et al*. 2000; Hii *et al*. 2001; Maxwell *et al*. 2002; Gimnig *et al*. 2003; Hawley *et al*. 2003).

Recent evidence suggests increasingly behavioral changes by malaria mosquito populations to avoid contact with ITNs by either feeding predominantly outdoors or in the early part of the evening (Charlwood and Graves, 1987; Braimah *et al*. 2005; Pates and Curtis, 2005; Oyewole and Awolola, 2006; Geissbühler *et al*. 2007). Such behavioral pattern can drastically reduce the level of personal protection conferred by ITNs for obvious reasons (Pates and Curtis, 2005; Geissbühler *et al*. 2007; Russell *et al*. 2011). These behavioral changes might have resulted from the selection of genetically inherited traits (Coluzzi *et al*. 1979) or, more directly, from phenotypic adaptation in response to increased coverage of ITNs or indoor residual spraying (Charlwood and Graves, 1987; Braimah *et al*. 2005; Pates and Curtis, 2005; Russell *et al*. 2011). Such intervention pressure may even be strong enough to cause changes in

species composition of vector populations by selectively eliminating the most susceptible species and leaving those which are less vulnerable (Gillies and Smith, 1960; Gillies, 1962; Gillies and Furlong, 1964; Gillies and DeMeillon, 1968; Odetoyinbo and Davidson, 1968; Lindblade *et al*. 2006). For instance, *An. arabiensis* Patton which is typically more exophilic, zoophagic and exophagic than its sibling species *An. gambiae* sensu stricto, already dominates malaria transmission in parts of western Kenya where widespread use of ITNs has progressively diminished the importance of *An. gambiae* s.s as the main malaria vector (Lindblade *et al*. 2006).

2.3 Methods

Although its commonly perceived that ITNs are ineffective against outdoor-biting mosquitoes based on conventional measures of mosquito behavior (Rubio-Palis and Curtis, 1992a; Pates and Curtis, 2005; Oyewole and Awolola, 2006), we adapt an established mathematical model of mosquito behavior and malaria transmission (Killeen and Smith, 2007; Killeen *et al*. 2007a) to examine the possibility that ITNs can achieve community-level suppression of malaria transmission exposure, even where mosquito evade them and personal protection is modest. We adapt an existing model (Killeen *et al*. 2007a) which was previously used to establish population-wide coverage thresholds levels of ITNs at which community-level protection is equivalent to or greater than personal protection (Killeen *et al*. 2007a). Specifically, we modify the model slightly to deal more realistically with vector populations that vary in terms of their feeding behaviors. The probability of mosquitoes surviving their eventual host attack (P_γ) is adjusted to account for the effect of ITN avoidance behavior, expressed as the proportion of normal exposure which would occur at times during which a human host would normally be under a net (π_i). This parameter can also be thought of

in simple terms as the maximum proportion of normal exposure, which is directly preventable through personal protection by using an ITN. The corrected probability of a mosquito surviving the eventual host attack, is calculated with the following modification of equation 13 of the original model (Killeen *et al*. 2007a), assuming that the proportion of all attacks that end in death is the sum of mortality probabilities for attacking protected and unprotected hosts, weighted according to the proportion of the availability of all hosts that they represent:

$$P_\gamma = 1 - \left(\frac{\mu_{h,p} A_{h,p} + \mu_{h,u} A_{h,u} + \mu_c A_c}{A_{h,p} + A_{h,u} + A_c} \right) = 1 - \left(\frac{(\mu_{h,p} \pi_i C_h + \mu_{h,u} (1 - \pi_i C_h)) A_h + \mu_c A_c}{A_h + A_c} \right)$$

The definitions of relevant terms in the model are shown in Table 2.3.1 and are consistent with a recent reformulation of this model (Okumu *et al.* 2010). Consistent with that revised formulation, the total host population availability parameters (A_h, $A_{h,p}$, $A_{h,u}$ and A_c) described here reflect the rate of attack of that particular host type per host-seeking mosquito per night, rather than that for successful blood feeding *per se*. The reduction in relative rate of exposure (RRE) to malaria transmission achieved by individual-level personal protection (ITN users), community-level protection (ITN non-users) and combined individual and community-level protection (ITN users) was estimated by fixing the additional mortality probability of mosquitoes encountering an ITN at (0.8) (Graham *et al*. 2005) and ITN coverage at the achievable level of 0.5, equivalent to 50% use as recorded in typical household surveys and specified by internationally agreed targets (Hawley *et al*. 2003; Killeen *et al*. 2007a). Otherwise, the model is formulated, parameterized, and applied exactly as previously described (Killeen *et al*. 2007a). The proportion of human exposure which occurs indoors was calculated as previously described (Killeen *et al*. 2006b).

Table 2.3.1: Parameter definitions (Okumu *et al.*, 2010)

P_γ	Mean probabilities of surviving eventual host attack
π_i	Proportion of human exposure to mosquito bites which occur indoors at times when they can be directly intercepted by using a net.
$\mu_{h,u}$	Mortality upon attacking an unprotected human
$\mu_{h,p}$	Overall mortality upon attacking a protected human
μ_c	Overall mortality upon attacking a cow
C_h	Coverage or usage rate of ITNs by humans
A_h	Total availability of all human hosts
$A_{h,u}$	Total availability of all human hosts unprotected by a net
$A_{h,p}$	Total availability of all human hosts protected by a net
A_c	Total availability of cattle

Note: Total availability for all humans (A_h), unprotected humans ($A_{h,u}$), protected humans ($A_{h,p}$) and cattle (A_c) are defined the rates at which a single host-seeking mosquito encounters and attacks all available hosts of that category (Okumu *et al.* 2010).

2.4 Results

Figure 2.4.1 E and F illustrates that less than half of all human exposure to *An. arabiensis* in urban Dar es Salaam, Tanzania (Geissbühler *et al*. 2007) occurs in times and places when using an ITNs is feasible ($\pi_i = 0.46$).

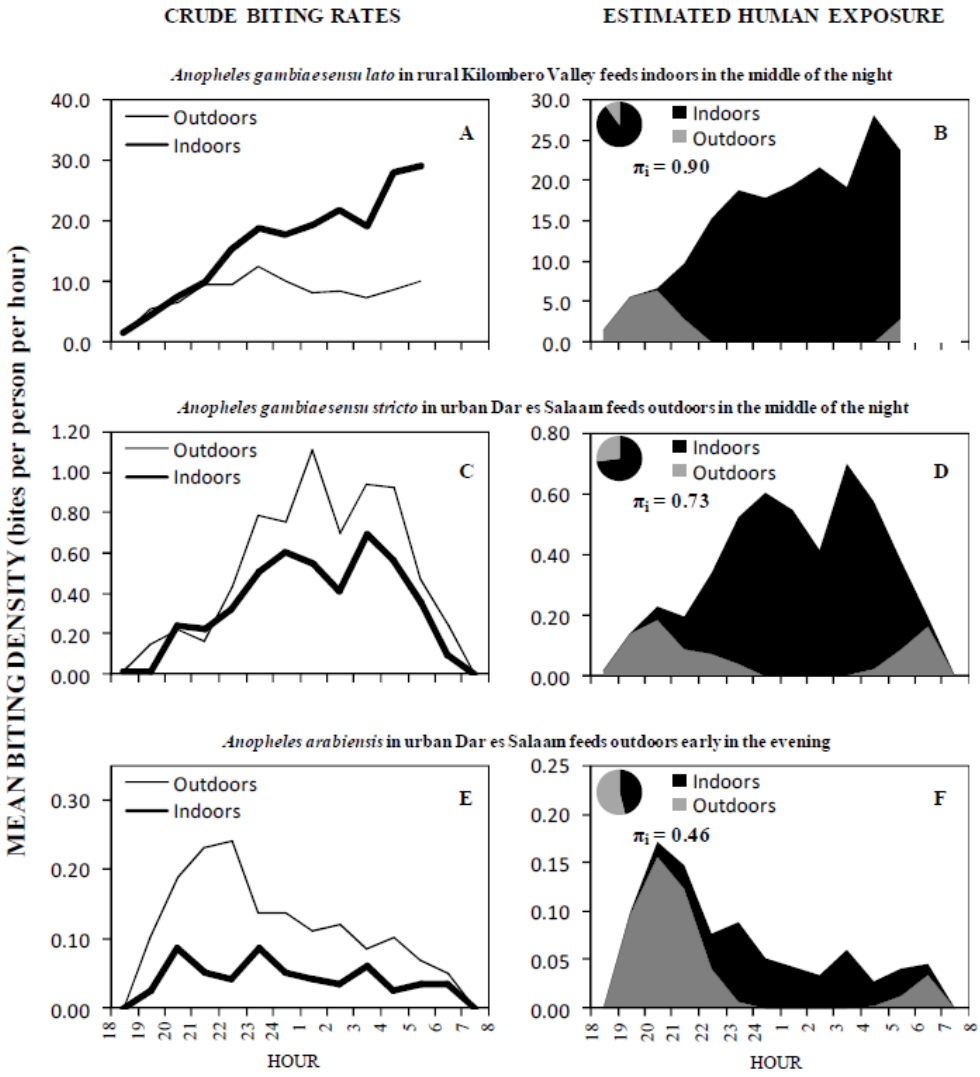

Figure 2.4.1: The crude behavioral profiles of three populations of malaria vectors in Tanzania (**A, C** and **E**) and the corresponding exposure profiles of the human populations exposed to them (**B, D** and **F**). The left panels plot crude behavioral profiles based on mean biting density of mosquitoes per hour, and the right panels represent human behavior-adjusted estimates of actual transmission exposure obtained by multiplying the mean biting density of mosquito in each hour and the proportion of humans present in the indoor and outdoor compartments.

Based on these published field data, simulations predict only a slight suppression in personal relative rate of exposure to transmission (RRE = 0.59), equivalent to a 1.7 fold reduction (Figure 2.4.2). However, much greater declines of exposure to transmission for ITN users (community plus personal protection; RRE = 0.19) and non users (community protection only; RRE = 0.32) are predicted at 50% community-wide coverage.

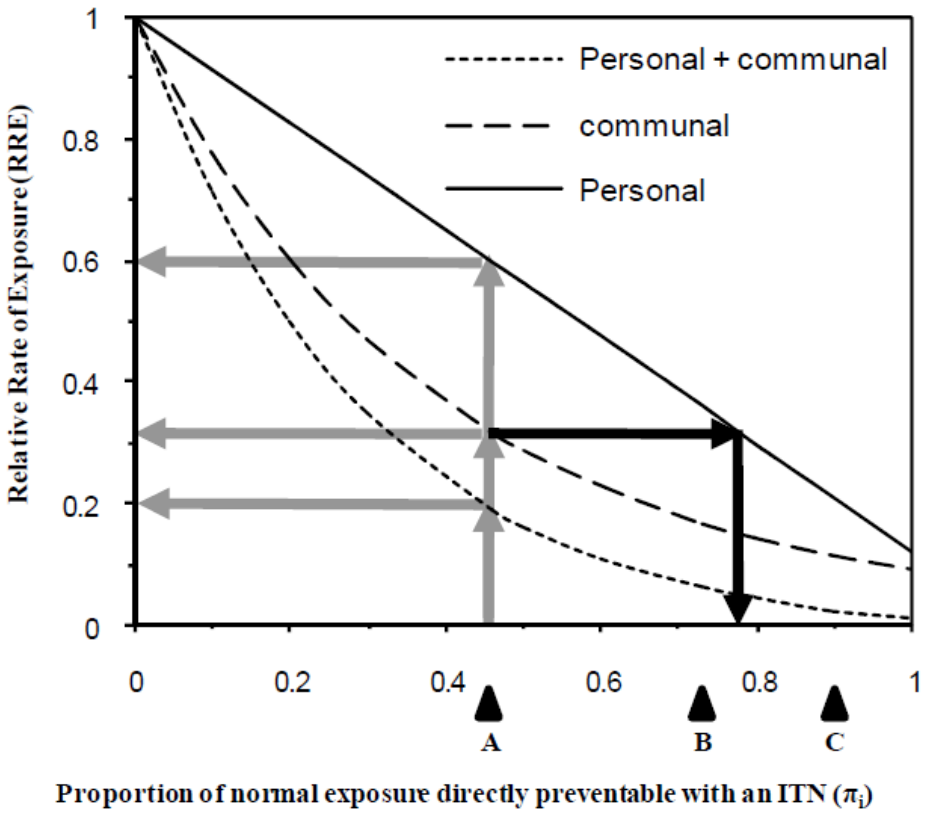

Figure 2.4.2: Simulated relationship between personal (users), community (non-users) and combined effect of personal and community (users) level suppression of malaria transmission exposure across a range values for the proportion of normal exposure for an unprotected individual occurring at times when insecticide-treated nets (ITNs) would be in use if they were available (π_i). **Arrows A**, **B** and **C** represent reported values of π_i for *Anopheles arabiensis and An. gambiae s.s* in urban Dar es Salaam (Geissbühler *et al*. 2007) and *An.gambiae* sensu lato in the rural Kilombero valley (Killeen *et al*. 2006b), respectively (Figure 2.4.1). Note that although here we present a scenario in which overall ITN coverage level is set at 50%, the degree of

personal protection against exposure is independent of coverage in the community at large.

Thus, even non-users receiving only community protection can expect 3.1 fold reduction of exposure to transmission while users enjoy a 5.3 fold reduction. Extrapolating this level of community protection horizontally across Figure 2.4.2 shows that this is equivalent to the personal protection provided when mosquitoes feed predominantly at times when most resident are indoors (π_i = 0.77). However, when mortality probability of mosquitoes encountering an ITN ($\mu_{h,p}$) is reduced to 50% as in (Killeen *et al*. 2007), suppression of exposure to transmission for both users and non-users is less impressive (RRE = 0.33, and 0.49), equivalent to 3 and 2 fold reduction respectively, but still offer reasonable level of protection.

Once reasonably high use rates are attained, community protection achieved is greater than personal protection because even very modest reductions of mosquito survival and feeding success per gonotrophic cycle result in much larger impacts upon proportion of mosquitoes surviving the multiple blood feeds required to reach an age where they can transmit mature sporogonic-stage parasites (MacDonald, 1957; Garrett-Jones, 1964a; Garrett-Jones and Shidrawi, 1969).

Conventional mosquito behavior measures (Henry and Gelfand, 1955; Krafsur, 1971; White, 1973; Rubio-Palis and Curtis, 1992a; Pates and Curtis, 2005; Oyewole and Awolola, 2006; Sungvornyothin *et al*. 2006) can underestimate the potential of ITNs because they ignore the importance of human movements (Stoddard *et al*. 2009)

indoors and outdoors. *An. gambiae* s.s. also prefers to bite outdoors in Dar es Salaam (Figure 2.4.1 C) (Geissbühler *et al*. 2007) but surveys of human malaria prevalence confirm that ITNs confer valuable personal protection and reduce infection risk by 23.6 % (95% confidence interval = 61.4 to 95.1 %, P= 0.016 (Geissbühler *et al*. 2009). This finding is due to the fact that because pearsons sleep indoors during peaks of mosquito activity, this location is where most human exposure occurs (π_i = 0.73; Figure 2.4.1 D), and can be prevented by using an ITN (Geissbühler *et al*. 2007).

Plotting π_i versus the proportion of mosquitoes which are caught indoors by conventional field methods (Figure 2.4.3) shows that in all cases, the latter consistently underestimates the former. Even for highly exophagic populations of mosquitoes, most bites (Figure 2.4.3) can be confined to times when most humans are indoors (Geissbühler *et al*. 2007) and possibly under a net. This approach can therefore underestimate the full potential of ITNs because it considers outdoor catches at times when they have little or no epidemiological relevance. Conversely, the proportion of mosquitoes that are caught at times during which most people are asleep can overestimate or underestimate π_i for exophagic and endophagic vectors, respectively, because outdoor catches during these period and indoor catches in the evenings and mornings are included (Figure 2.4.3).

However, the number of mosquitoes caught indoors during sleeping hours, expressed as a proportion of itself plus the number mosquitoes caught outdoors outside of sleeping hours closely matches formal estimates of π_i (Figure 2.2.3). Although the level of exophagy and endophagy of vector populations does influence the efficacy of ITNs for preventing malaria transmission, human movement patterns and the extent to

which vector activity patterns match them may often be more important. These examples from Dar es Salaam (Geissbühler *et al*. 2007) illustrates how two exophagic vector populations can avoid ITNs to very different extents because of differences in their peak times of activity and the degree to which these coincide with human behavioral patterns. In simple terms, it is more important that persons are asleep and can conveniently use an ITN when vector activity peaks than that the place they sleep is preferred by those mosquitoes.

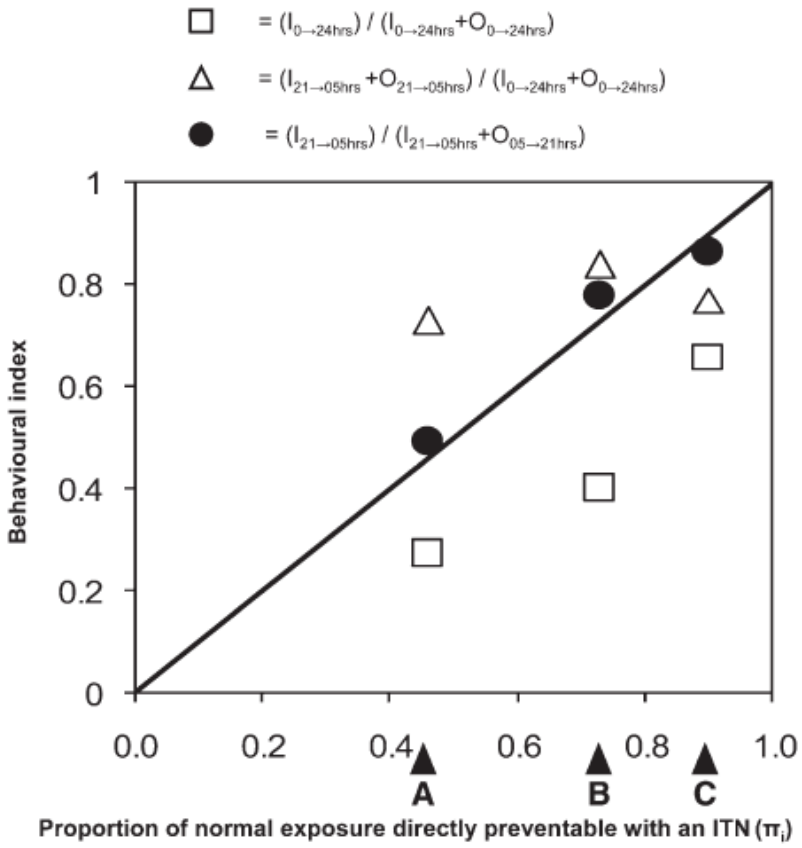

Figure 2.4.3: A graph of three crude behavioral indices for three populations of *Anopheles* in Tanzania compared with formal estimates of π_i, which is the maximum proportion of normal exposure which is directly preventable by using an insecticide-treated net. **Arrows A**, **B** and **C** represent reported values of π_i for *An. arabiensis and An. gambiae* s.s in urban Dar es Salaam (Geissbühler *et al*. 2007) and *An.gambiae* sensu lato in rural Kilombero valley (Killeen *et al.* 2006b), respectively (Figure 2.2.1). Open squares represent the proportion of mosquitoes which are caught indoors calculated by dividing the total catch indoors across all times ($I_{0\rightarrow24hrs}$) by total

catch occurring both outdoors ($O_{0\rightarrow 24hrs}$) and indoors ($I_{0\rightarrow 24hrs}$). The open triangles represent the proportion of mosquitoes which are caught at times when most humans are likely asleep, obtained by dividing the total catch occurring both indoor and outdoor from 21.00 hours to 05.00 hours ($I_{21\rightarrow 05hrs} + O_{21\rightarrow 05hrs}$) by total catch indoors and outdoors across all times ($I_{0\rightarrow 24hrs} + O_{0\rightarrow 24hrs}$). The filled circles represents a crude estimate of the proportion of exposure occurring indoor (π_i), obtained by dividing the total catch occurring indoor from 21.00 hours to 05.00 hours ($I_{21\rightarrow 05hrs}$) by itself plus the total outdoor catch from 05.00 hours to 21.00 hours ($O_{05\rightarrow 21hrs}$).

2.5 Discussion

We therefore caution that ITNs should not be automatically discarded as a priority vector control measure just because vector mosquitoes are observed to prefer feeding outdoors. Explicit estimates of π_i values for locally relevant populations should first be obtained in the field and the potential community-level benefits, which are rarely captured by standard survey designs, should be carefully considered. However, in some parts of south-east Asia (Van Bortel *et al*. 2010) and the Pacific (Bugoro *et al.* 2011) where this key parameter has been estimated, most human exposure to mosquito bites occurs outside houses and before bed time, a combination of complementary vector control tools that can be used at different periods of night might well be more effective. For instance, personal protection measures such as spatial repellents (Pates *et al*. 2002; Seyoum *et al*. 2003) may be required to protect against outdoor bites in the morning or early evening (Braimah *et al*. 2005; Trung *et al*. 2005; Sungvornyothin *et al*. 2006) but should only be considered a supplement to ITNs unless proven otherwise. If the equitable, population-wide benefits of community protection are ignored, potential opportunities for effective malaria control with a well-proven existing technology may be missed because the requirements for behaviorally-susceptible vector populations may be overestimated or overemphasized.

Perhaps the most obvious limitation of this study is the assumption that indoor and outdoor-biting mosquitoes within a given species constitute a single, homogenous population. Deviations from this assumption were demonstrated long ago in Africa where indoor and outdoor populations of *An. gambiae* and *An. arabiensis* proved to have non-random distributions of chromosomal inversion karyotypes (Coluzzi *et al.*, 1979). Furthermore, it should be remembered that most vectorial systems are composed of two or more distinct species which are differentially exposed to insecticide pressure as direct function of their propensity to enter or rest in houses (Molineaux and Gramiccia, 1980). Therefore, vector species or within-species sub-populations that account for a relatively small proportion of baseline transmission may dominate surviving residual vector population systems and represent the primary obstacle to malaria elimination. In the Solomon Islands, for example, historical IRS campaigns had a dramatic impact upon *An. punctulatus* and *An. koliensis* but far less upon *An. farauti* and contemporary campaigns combining IRS with ITNs have had negligible impact upon the latter species (Bugoro *et al*. 2011) This may also be the case in many parts of Africa where high coverage of IRS can dramatically alter vector population composition (Gillies and Smith, 1960; Gillies and Furlong, 1964; Gillies and DeMeillon, 1968; Bayoh *et al.* 2010; Russell *et al.* 2010), resulting in substantially lowered π_i values for these residual vectorial systems (Russell *et al*. 2011). Such increases in the proportion of outdoor biting by malaria vectors in response to wide-spread use of ITNs and IRS confirm that, these indoor target interventions will not be adequate to eliminate malaria in such settings (Griffin *et al*. 2010; Ferguson *et al*. 2010).

In Dar es Salaam and perhaps in other urban contexts, larval source management is perhaps the obvious option for controlling mosquitoes by preventing their emergence from aquatic habitats before they feed at all, regardless of whether they do so indoors or outdoors. Nevertheless, apart from the efficacy of insecticides or the accessibility of larval habitats, the success of larvicide application depends also on comprehensive detection and treatment of all potential larval habitats. Sensitive, practical and safe adult mosquito surveillance tools are required to allow intensive and extensive monitoring in order to detect intervention coverage gaps as rapidly as they arise.

CHAPTER 3

A NEW TENT TRAP FOR SAMPLING EXOPHAGIC AND ENDOPHAGIC MEMBERS OF THE *ANOPHELES GAMBIAE* COMPLEX

This paper has been published in the

Malaria Journal 2009, 8:157

Govella NJ, Chaki PP, Geissbuhler Y, Kannady K, Okumu FO, Charlwood JD, Anderson RA, Killeen GF.

3.1 Abstract

Introduction: Mosquito sampling methods are essential for monitoring and evaluating malaria vector control interventions. In urban Dar es Salaam, human landing catch (HLC) is the only method sufficiently sensitive for monitoring malaria-transmitting *Anopheles*. HLC is labour intensive, cumbersome, hazardous, and requires such intense supervision that is difficult to sustain on large scales.

Methods: Novel tent traps were developed as alternatives to HLC. The Furvella tent, designed in Mozambique, incorporates a CDC Light trap (LT) components, while two others from Ifakara, Tanzania (designs A and B) require no electricity or moving parts. Their sensitivity for sampling malaria vectors was compared with LT and HLC over a wide range of vector abundances in rural and urban settings in Tanzania, with endophagic and exophagic populations, respectively, using randomised Latin-square and cross- over experimental designs.

Results: The sensitivity of LTs was greater than HLC while the opposite was true of Ifakara tent traps (crude mean catch of *An. gambiae sensu lato* relative to HLC = 0.28, 0.65 and 1.30 for designs A, B and LT in a rural setting and 0.32 for design B in an urban setting). However, Ifakara B catches correlated far better to HLC ($r^2 = 0.73$, $P < 0.001$) than any other method tested ($r^2 = 0.04$, $P = 0.426$ and $r^2 = 0.19$, $P = 0.006$ for Ifakara A and LTs respectively). Only Ifakara B in a rural setting with high vector density exhibited constant sampling efficiency relative to HLC. The relative sensitivity of Ifakara B increased as vector densities decreased in the urban setting and exceeded that of HLC at the lowest densities. None of the tent traps differed from HLC in terms

of the proportions of parous mosquitoes (P ≥0.849) or *An. gambiae s.l.* sibling species (P ≥0.280) they sampled but both Ifakara A and B designs failed to reduce the proportion of blood-fed mosquitoes caught (Odds ratio [95% Confidence Interval] = 1.6 [1.2, 2.1] and 1.0 [0.8, 1.2], P = 0.002 and 0.998, respectively), probably because of operator exposure while emptying the trap each morning.

Conclusion: The Ifakara B trap may have potential for monitoring and evaluating a variety of endophagic and exophagic Afrotropical malaria vectors, particularly at low but epidemiologically relevant population densities. However, operator exposure to mosquito bites remains a concern so additional modifications or protective measures will be required before this design can be considered for widespread, routine use.

3.2 Introduction

A myriad of mosquito sampling techniques have been developed and the sensitivity with which they sample targeted mosquito species has been evaluated under an equally diverse set of field conditions (Service, 1977; Service, 1993). Effective mosquito traps are essential to monitor and evaluate malaria vector control programs (WHO, 2003b). Such information is vital to enable malaria control practitioners to optimize intervention strategies and tactics under practical conditions of operational programmes.

In the African context, sampling of malaria vectors relies almost exclusively upon trapping highly anthropophagic mosquitoes in and around houses, either directly

before or soon after feeding (Mboera, 2005). Aside from human landing catch (HLC), the most commonly used methods for sampling host-seeking African malaria vectors are Centers for Disease Control and Prevention miniature light traps (LTs) (Sudia 1962) placed beside occupied bednets. Another major strategy for trapping Africans malaria vectors exploits the tendency of these endophilic species to rest indoors after blood feeding (Pates and Curtis, 2005). Such indoor resting catches involve either aspirating directly from accessible resting places (WHO, 2002) or "knock down" with indoor pyrethrum spray onto white sheets where they are readily collected (WHO, 1975a; Kulkarni *et al*. 2006; Odiere *et al*. 2007). While LTs are relatively reliable (Odetoyinbo, 1969; Lines *et al*. 1991; Mathenge *et al*. 2004; Mathenge *et al*. 2005) and largely unaffected by the presence of insecticidal interventions (Magbity *et al*. 2002; Killeen *et al*. 2007b), methods which sample indoor-resting mosquitoes (Service, 1977) are unsuitable for many control programmes because they are adversely affected by the presence of insecticides on nets or walls (WHO, 2005a; Diuk-Wasser *et al*. 2005) which promote exit (Chareonviriyaphap *et al*. 1997; Muenworn *et al*. 2006; Pothikasikorn *et al*. 2007) and outdoor resting (Muirhead-Thomson, 1960; Bogh *et al*. 1998; Quinones *et al*. 1998). While exit traps placed in windows (WHO, 1975a) have proven useful for monitoring vector density trends in southern Africa (Mouatcho *et al*. 2007) and Equatorial Guinea (Sharp *et al*. 2007a), their efficiency is likely to be influenced by site and time-specific factors such as mosquito and human behaviours, as well as house design. These approaches may therefore also be unreliable for estimating representative, consistent and epidemiologically meaningful human-biting rates of vector populations.

Dar es Salaam in Tanzania is a typical, rapidly growing African city and has recently developed a large-scale programme for supplementing existing priority malaria prevention methods with systematic larviciding (Fillinger *et al*. 2008). The microbial larvicides (*Bacillus thuringensis var israelensis*) have little residual activity, necessitating weekly application and mosquito surveillance cycles (Fillinger *et al*. 2008). Unfortunately, none of the above mentioned trapping techniques nor a number of alternative method apart from HLC, proved sufficiently sensitive for routine mosquito surveillance. Initial attempts to use Mbita-design bednet traps (Mathenge *et al*. 2002; Laganier *et al*. 2003; Mathenge *et al*. 2005) indoors or outdoors, yielded only one *Anopheles gambiae sensu lato* over 181 full nights of sampling. The Centers for Disease Control and Prevention miniature light traps (LTs), pyrethrum spray catch and indoor aspirator catches all failed to catch significant numbers of *Anopheles*. In stark contrast, three nights of preliminary outdoor human landing catch (HLC) at one location yielded 136 *An. gambiae s.l.* and 30 other anopheline (Fillinger *et al*. 2008). It has since been shown through detailed behavioural studies that *Anopheles gambiae sensu stricto* and *Anopheles arabiensis* Patton are both predominantly exophagic in this highly urbanized environment (Geissbühler *et al*. 2007). Outdoor HLC was therefore undertaken as an interim monitoring and evaluation measure while alternative outdoor trapping technologies were developed (Fillinger *et al*. 2008). A major advantage of HLC is that mosquitoes are caught in the act of biting the human host (WHO, 1975b; Lines *et al*. 1991; Service, 1993; Mboera, 2005) so the sample obtained is assumed to be representative of the human biting rate. This enables estimation of EIR which is the average number of infective bites per person per unit time (Beier *et al*. 1999). Nonetheless, this technique has major drawbacks, some of which are prohibitive. It is extremely arduous and labour intensive, requiring intense supervision to the extent that is difficult to sustain on large scales. An even greater

concern arises from the fact that it inevitably increases the hazard of exposure of participants to mosquito-borne infections (WHO, 1975a; Service, 1977; Mboera, 2005) which is difficult to justify on ethical grounds. In this article we report the development and evaluation of new tent traps in both rural and urban settings in Tanzania with very different vector population densities and behaviours.

3.3 Methods

Study sites

The rural study site was Lupiro village in the Kilombero Valley, 40 km south of Ifakara (Killeen *et al*. 2007b) in Ulanga district, Morogoro region, Tanzania. This valley experiences extremely high *Plasmodium falciparum* malaria transmission with an EIR exceeding 600 infectious bites per person per year despite exceptionally high coverage with largely untreated bednets (Killeen *et al*. 2007b). The main malaria vectors are endophagic members of the *An. gambiae* complex (Killeen *et al*. 2006b).

The urban study site, Dar es Salaam is the largest city in Tanzania, situated on the Indian Ocean coast with lower transmission levels, have been proven accessible to control with larviciding and environmental management (Castro *et al*. 2004; Mukabana *et al*. 2006; Fillinger *et al*. 2008; Geissbühler *et al*. 2009). While the nocturnal biting cycle of *An. gambiae s.s.* is more or less consistent with that of classical reports (Gillies and DeMeillon, 1968), all members of the complex in Dar es Salaam have an unusual preference for outdoor feeding (Geissbühler *et al*. 2007) and biting activity of *An. arabiensis* peaks at about 10pm when many residents are often still awake and outdoors (Geissbühler *et al*. 2007). Such mosquitoes behavioural

patterns is not only the case in this setting but, also exist in other parts of malaria endemic countries (Coluzzi *et al*. 1979; Pates and Curtis, 2005; Tirados *et al*. 2006; Van Bortel *et al*. 2010)

In both sites, houses with open eaves were chosen in order to minimize the potential confounding effect of differences in house structure upon observations of feeding behaviour and trap efficiency. Nevertheless, we did use existing rather than standardized, purpose-built houses (often referred to as experimental huts) for these surveys so some differences between the two sites were unavoidable. In rural settings, houses were constructed of mud with thatched roofs while in Dar es Salaam, all had walls made with bricks and corrugated iron roofs.

Trapping methods

Furvella tent trap

The Furvella tent trap (Figure 3.3.1A) developed and tested by one of the authors (JDC) in Mozambique, is constructed from a dome shaped Eureka® sleeping tent with nylon taffeta body and floor surface. A standard LT with the light bulb removed is attached to the zip of the main tent door which is almost closed, leaving a 5cm gap (Figure 3.3.1A: X) for host odours to escape and mosquitoes to attempt entry. This trap is powered by a 6V battery kept inside the tent and mosquitoes are caught into the collection bag (Figure 3.3.1A: Y) through suction created by a rotating fan that is positioned near the tent entrance (Figure 3.3.1A: Z), which is in turn oriented away from the wind.

Ifakara tent trap

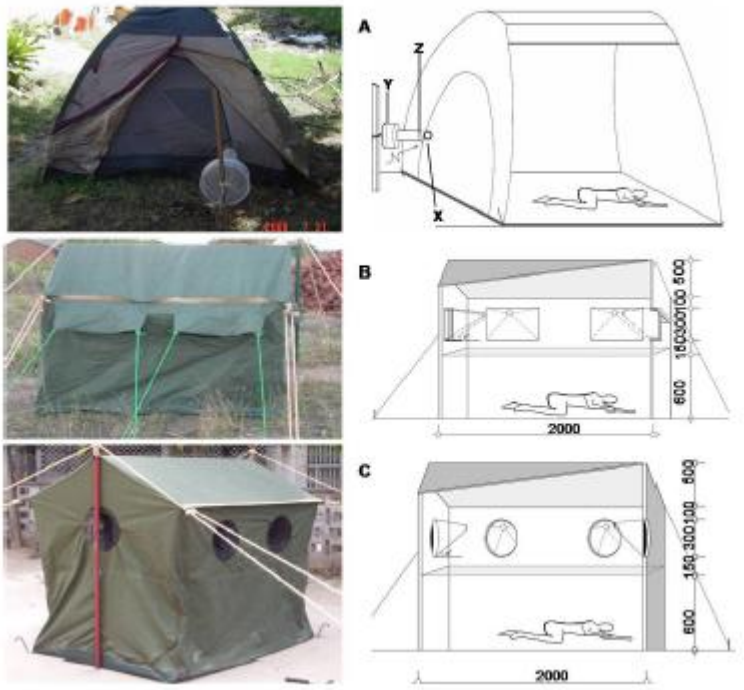

Figure 3.3.1: Furvella trap (A), Ifakara A tent trap (B), Ifakara B tent trap (C), with section drawing of each. The human occupant is protected from mosquito bites by a netting panel within the Ifakara A and B designs. For the Furvela trap, mosquitoes approach the small opening in the tent zipper (X) where they are drawn into the collection bag (Y) when they pass the CDC light trap entrance (Z), while in the Ifakara designs they enter through a funnel shaped entrances tilted upward. All dimensions in mm.

The Ifakara A and B tent traps (Figure 3.3.1B and C) are rectangular canvas boxes containing six funnel-like entrances for mosquitoes and inner small apertures tilted to an angle so that mosquitoes have to fly upward to enter the trap. Such baffled entrance structures are known to increase the probability that mosquitoes do not exit once inside traps (Service, 1976) and this was also found to be the case in this specific example during development. A layer of durable, Teflon-coated woven fibreglass netting between the entry funnels and the bait host allows the human participant to sleep while protected from mosquito bites. A zip bisecting the protective netting panel

enables the participant to aspirate mosquitoes from inside the trap. The trap floor is made of thick polyvinylchloride sheeting, which protects against rough substrates and surface water. The two traps differ only in the design of the entry points. Ifakara A used square shaped entrances that were partially covered by an over-hanging flap of canvas, while Ifakara B used completely exposed circular entrances (Figure 3.3.1B and C). These two designs, based on a prototype used previously to assess mosquito behaviour in the Kilombero valley (Anderson *et al*. 2000), were developed iteratively in Lupiro village where very high densities of *An. gambiae s.l.* allowed rapid assessment through a series of stepwise modifications.

Centers for Disease Control and Prevention miniature light traps (LTs)

CDC miniature light traps (model 512, John W. Hock Company, Gainesville, Florida USA) with 5 watt influorescent bulbs were each hung inside a house near an occupied, insecticide-free bednet with the top of the shield pan approximately 150 cm from the floor surface, placed at the end where the occupants feet lie and touching one side of the net (Mboera *et al*. 1998).

Human landing catch (HLC)

To conduct human landing catch, each adult male collector exposed his lower limbs and collected the mosquitoes when landing on his legs with an aspirator (Service, 1977). HLC was conducted by a single catcher at each station (site or house x indoor or outdoor position) for 45 minutes each hour, allowing 15 minutes break for rest. To obtain full hourly biting densities, the catches for each hour were therefore divided by

0.75 (Geissbühler *et al*. 2007). Collections were conducted both indoors and outdoors in accordance with the relevant experimental designs described below.

Experimental design

Experiment 1 (rural)

Three houses with three corresponding outdoor catching stations immediately beside them, approximately 5m away from a house, were selected. The Furvella, Ifakara A, and B tent traps were assigned to one of the three outdoor catching stations and rotated in order through 6 rounds of a 3 x 3 Latin square experiment design (Figure 3.3.2) so that we could directly compare each trap design with the LTs placed inside of all three houses as the reference method. The human subject assigned to each station remained fixed throughout the experiment in order to minimize the bias of differential individual attractiveness and particular locations and to combine these heterogeneities into single quantifiable source of variation. This experiment was carried out over 18 nights (8th November to 25th November 2006), during the short rains, constituting six full rotations of each of the three trap-site combination. Mosquitoes were collected by all methods from 19.30 to 05.30 h.

Experiment 2 (rural)

Experiment 2 was adapted from experiment 1 with slight changes. At one house, the pairing of the LT indoors with the Furvella tent trap outdoor was replaced by HLC, both indoors and outdoors (Figure 3.3.2) so that the Ifakara A and B tent traps could be compared with two reference methods. This experiment again relied upon a Latin square design and was implemented during the short rains (27th November to 14th December 2006) in similar fashion to experiment 1.

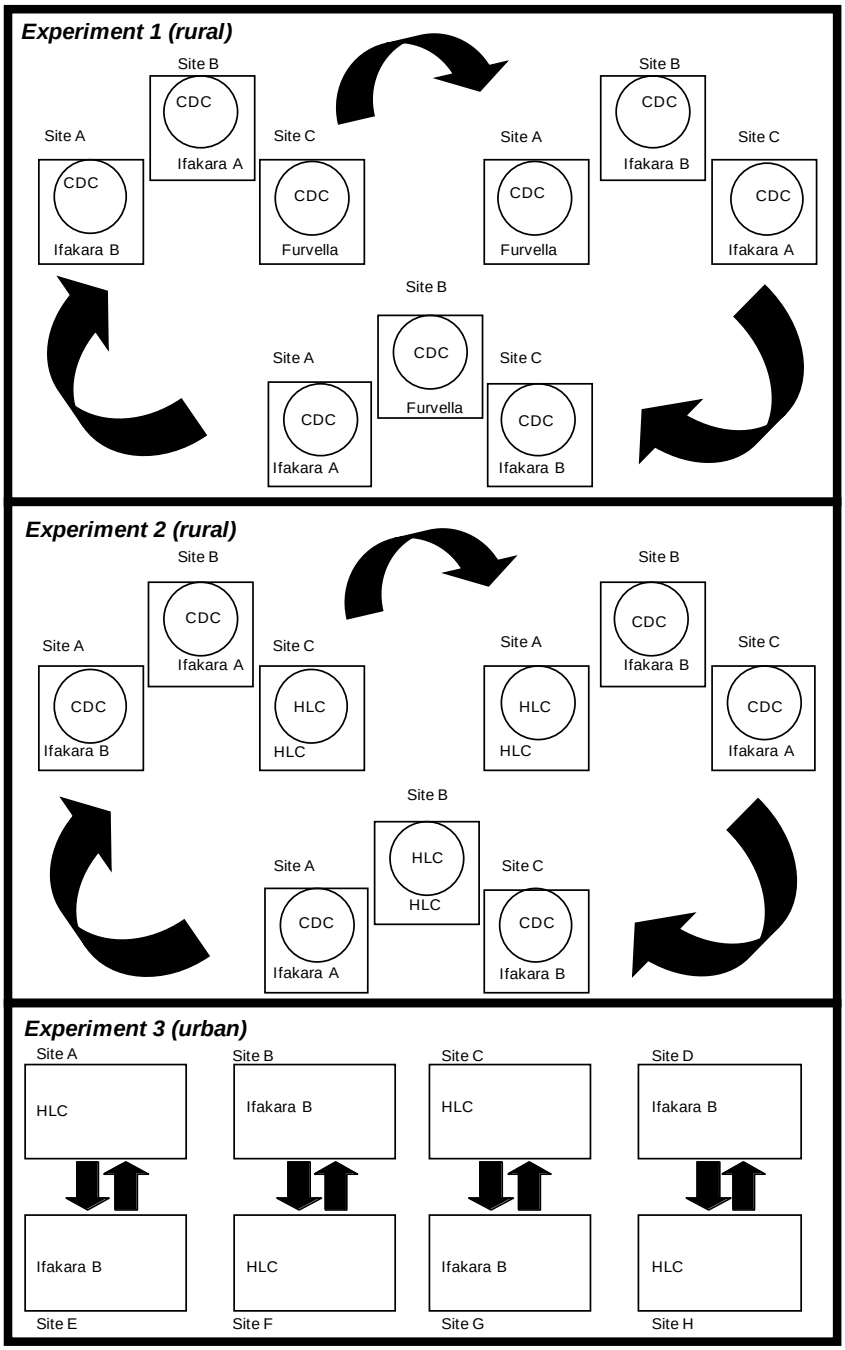

Figure 3.3.2: Schematic representation of a typical experimental design indicating three possible arrangements for one complete rotation in experiment one and two with cross over design in experiment three. Indoor and outdoor catching stations/sites are presented by circles and squares respectively.

Experiment 3 (urban)

In urban Dar es Salaam, Ifakara B traps were compared directly with HLC only. Four well-separated sites (>100m apart), each consisting of a pair of outdoor catching stations approximately 50m apart, were selected with each being associated with a nearby house approximately 5m away. Each catcher was allocated to and remained associated with a specific sampling station. On each experimental night, one participant at one of the two stations in each of the four sites conducted HLC while the other within the same site slept in an Ifakara B trap. The trapping techniques were swapped between the two stations within each site every night (Figure 3.3.2) from 23rd April to 21st June 2007 during the main rainy season and mosquitoes were collected by both methods from 19.30 to 05.30 h.This experiment proceeded for 41 nights with exception of one night during which data were discarded due to ants destroying some of the samples.

Processing of samples

Mosquitoes from all catches were sorted, counted and their abdominal status (unfed, part fed, fully fed, and gravid) classified directly in the field. The abdominal status was determined in order to test whether the alternative trapping methods, tent traps in particular, reduce the exposure of participant to mosquito bites. *Anopheles gambiae s.l., An. funestus*, and other anophelines were identified morphologically (Gillies and DeMeillon, 1968; Gillies and Coetzee, 1987) with the aid of a stereo-microscope and as many freshly caught specimens of *An. gambiae s.l.* as possible were dissected to determine parity (Detinova, 1962). All mosquito samples were stored in tubes with

desiccated silica for subsequent polymerase chain reaction (PCR) assay (Scott *et al*. 1993) to determine the sibling species of *An. gambiae* complex. Although these mosquitoes were also retained for sporozoite infection status determination, these samples were accidentally discarded following freezer failure before laboratory analysis could be completed. All culicines were counted, categorized as male or female and discarded.

Data analysis

Density-independent sampling efficiency

It is vital to measure whether the novel alternative sampling methods collects the same fraction of mosquito population as the reference method. However, the precise comparison between two sampling methods is generally difficult because errors exist in both methods and neither can be assumed to constitute a truly independent variable (Altman and Bland, 1983). Bearing this in mind, we decided to undertake a diverse series of analyses to check the consistency of outcomes based on the size of female *An. gambiae s.l.* catches. Low catches of *An. funestus* were obtained in all experiments so, although it is an important vector of malaria in Tanzania and elsewhere in Africa, we cannot report a rigorous evaluation of how well these traps sample this vector. All analyses were conducted using SPSS 15.0

We first aggregated catches of female *An.gambiae s.l.* by trap type and date in experiment 3 where multiple traps of the same type operated simultaneously, yielding consistently non-zero mean catches for each trap on each night. This is an important step as it eliminates the possibility of biasing analyses of sampling sensitivity with

logarithmically-transformed data which would otherwise have to be artificially converted to non-zero values by adding one (Smith, 1995).

Initially, simple Pearson correlation was applied using logarithmically transformed data ($\log_{10}(x)$) of female *An. gambiae s.l.* from each trap. This was then complemented by plots of the catches in alternative traps against the reference group using absolute catches. Subsequently, the dependence of the sampling efficiency of the alternative tent traps, relative to the LT or HLC reference method, upon vector density and experiment was evaluated by fitting the following model using generalised estimating equations (GEE).

$$y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \varepsilon$$

Where y is the relative sampling efficiency of the alternative technique on each night, estimated by dividing the alternative trap catch by that of the reference method, x_1 is the logarithm of the catch with the reference technique, x_2 is a categorical variable reflecting the identity of the experiment and β_o is the estimated intercept reflecting sampling efficiency at an infinitesimally low vector density as measured by reference method, while β_1 and β_2, are the estimated parameters reflecting the influence of x_1 and x_2 respectively. The catch of the alternative collection methods divided by the catch of the reference method on each experimental night was therefore treated as the dependent variable with a gamma distribution in all fitted models. Site and, where appropriate, station were treated as subject effects with experimental night distinguishing repeated measures. Initially, experiment and the log-transformed catch in the reference trap were included as factor and covariate variables, respectively, in a model fitted to the pooled data from all experiments relevant to that

alternative-reference method pairing. The influence of experiment was found to be significant in all cases so data from each experiment were then analyzed separately and the experiment term was removed from the model. If the influence of the log-transformed reference trap catch in such an experiment-specific initial model was not significant, indicating constant sampling efficiency across the range of vector densities within that experiment, this term was removed and the simplest model possible, with only an intercept, was fitted. The best-fit models for each experiment were then plotted and compared with the actual nightly catch data, plotted as recorded catches of the alternative collection methods divided by the recorded catch of reference methods against the catch of reference method using absolute catch numbers.

Distribution of parity, species and abdominal conditions among sampling techniques

The influence of collection method upon the distribution of parity, sibling species and abdominal condition of *An. gambiae s.l.* were analyzed by logistic regression, treating each as a binary outcome variable with experiment and trap design as independent categorical factors in the model.

The results of dissections, PCR species determination and visual inspections upon collection were expressed in a binary fashion as being parous versus nulliparous, *An. gambiae s.s.* versus *An. arabiensis* and partly or fully blood fed versus unfed, respectively

Ethical clearance and protection of human participants

Prior to any field work, research clearance was obtained from the institutional review board of Durham University in the UK, Ifakara Health Institute in Tanzania, and the Medical Research Coordination Committee of the National institute of Medical Research in Tanzania (Reference numbers NIMR/HQ/R.8a/Vol.IX/279 and 324). The written informed consents were obtained from all participants. These volunteers were screened weekly for malaria parasites and, when positive, offered the best medication available, namely artemisinin-lumefantrane, (Co-Artem®), free of charge.

3.4 Results

Crude relative sensitivity of tent traps and correlation with reference methods

The number of *Anopheles* trapped by each sampling method in each experiment is shown in Table 3.4.1. Based on the mean catch sizes described in Table 1, the Ifakara tent traps consistently caught fewer mosquitos than the reference LTs and HLC methods.

Table 3.4.1. Number of *Anopheles* mosquitoes caught by different techniques relative to human landing catch

Collection methods		Trap nights	Total catch	Mean catch	Relative sensitivity
			***Anopheles gambiae* s.l.**		
Furvella					
	Experiment 1	18	1306	72.6	NA
Ifakara A					
	Experiment 1	18	483	26.8	NA
	Experiment 2	18	429	23.8	0.28
Ifakara B					
	Experiment 1	18	1099	61.1	NA
	Experiment 2	18	1007	55.9	0.65
	Experiment 3	164	442	2.7	0.32
Light trap					
	Experiment 1	54	3736	69.2	NA
	Experiment 2	36	4008	111.3	1.30
HLC					
	Experiment 2	36	3081	85.6	NA
	Experiment 3	164	1398	8.5	NA
			Anopheles funestus		
Furvella					
	Experiment 1	18	2	0.11	NA
Ifakara A					
	Experiment 1	18	2	0.11	NA
	Experiment 2	18	2	0.11	0.28
Ifakara B					
	Experiment 1	18	3	0.16	NA
	Experiment 2	18	4	0.22	0.55
	Experiment 3	164	13	0.07	1.40
Light trap					
	Experiment 1	54	21	0.38	NA
	Experiment 2	36	24	0.68	1.70
HLC					
	Experiment 2	36	14	0.40	NA
	Experiment 3	164	8	0.05	NA

NA: Not applicable

Nevertheless, even these lower mosquito catches are encouraging, because these designs do not require electricity.

Comparing the quotient of variance divided by mean, shows that LTs and Furvella traps appeared to be less precise than HLC or Ifakara tent traps in the rural setting for sampling *An. gambiae s.l.* (Figure 3.4.1).

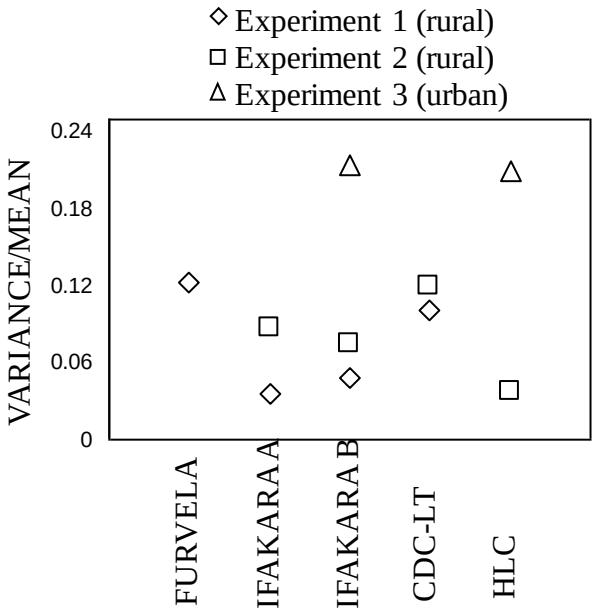

Figure 3.4.1: Illustration of the relative precision for different methods in sampling *An. gambiae s.l.* across different experiments**.**

The catches of the Ifakara B but not the Ifakara A trap were loosely correlated with those of the LTs (Table 3.4.2 and Figure 3.4.2). However catches by the Ifakara B design were correlated closely to those of the HLC gold standard. In fact this correlation was far stronger than that of the LTs in this study and at least matches any other previously reported evaluation of the LTs (Table 3.4.2 and Figure 3.4.3). In fact, examination of Figure 3.4.3 prompted us to restrict this linear correlation of data from pooled experiments to HLC catches of 10 per person per night or more because the relationship appears to be linear across both experiments within this range. This analysis restricted to reasonably high vector densities yielded even more encouraging results ($r^2 = 0.86$, $P < 0.001$).

Table 3.4.2: Correlation of numbers of female *An. gambiae* complex caught by alternative traps with reference collection methods, pooling data from all experiments in which simultaneously data for each pair was collected

	Alternative collection methods	versus CDC-light trap reference method		versus human landing catch reference method	
		r^2	P	r^2	P
This study	Furvella	**0.303**	**0.021**	NA	NA
	Ifakara A	0.008	0.590	0.04	0.426
	Ifakara B	**0.148**	**0.020**	**0.731**	**< 0.001**
	Light trap	NA	NA	**0.192**	**0.006**
Ref 14	Light trap	NA	NA	0.723	< 0.001
Ref 46	Light trap	NA	NA	0.409	< 0.001
Ref 48	Light trap	NA	NA	0.476	< 0.001
Ref 15	Light trap	NA	NA	0.521	< 0.001

NA: Not applicable

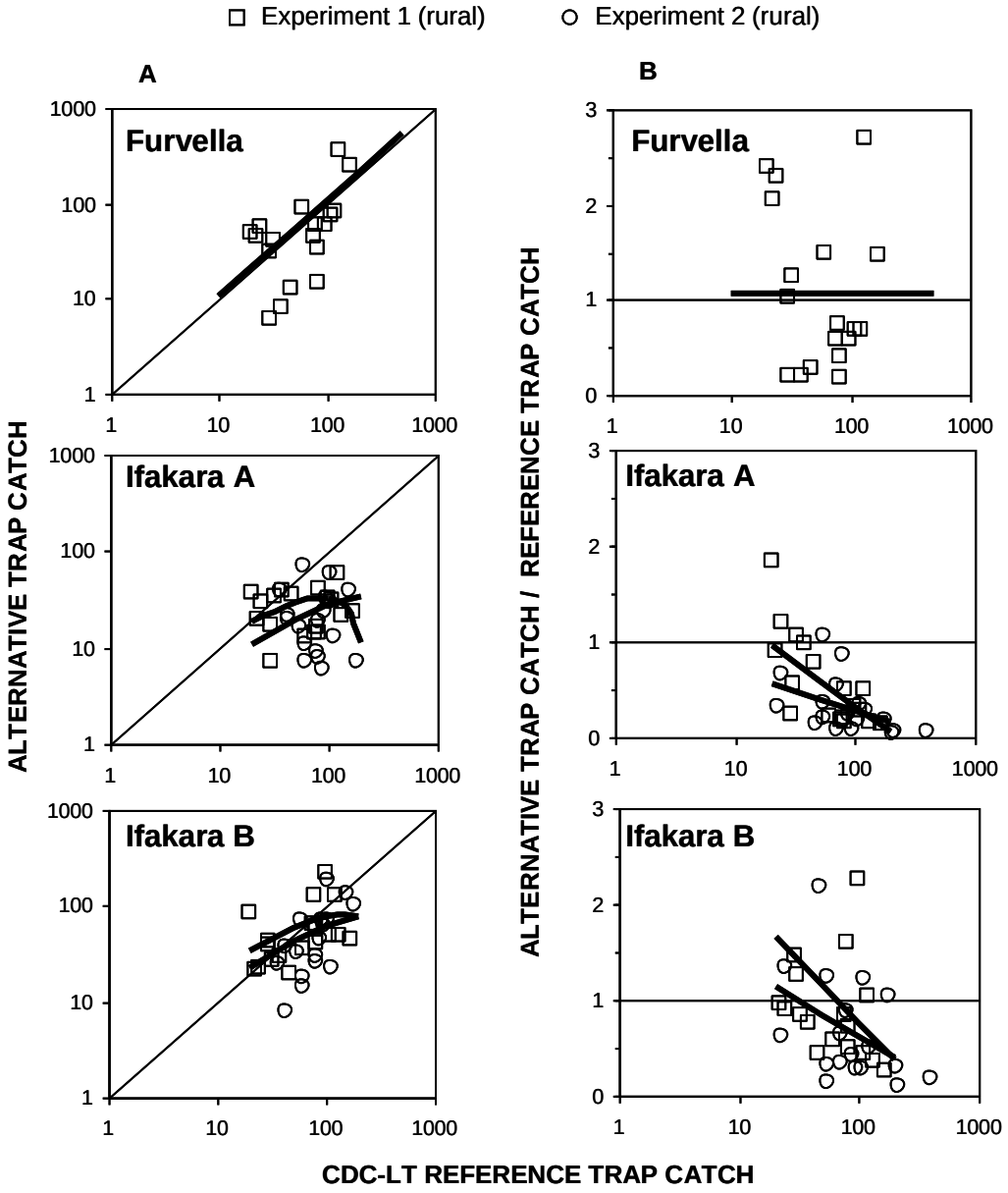

Figure 3.4.2: Correlation and density-dependence of alternative methods sampling efficiency, relative to the light trap reference method for catching *An. gambiae s.l.*. The correlation between the catches of *An. gambiae s.l.* in alternative methods and the light trap reference method is plotted using absolute catches is presented in the left hand panels with a thick line representing the best model fit. Right panels illustrate density-dependence by plotting the alternative method catches divided by corresponding catches in light traps against the absolute catches in the light trap.

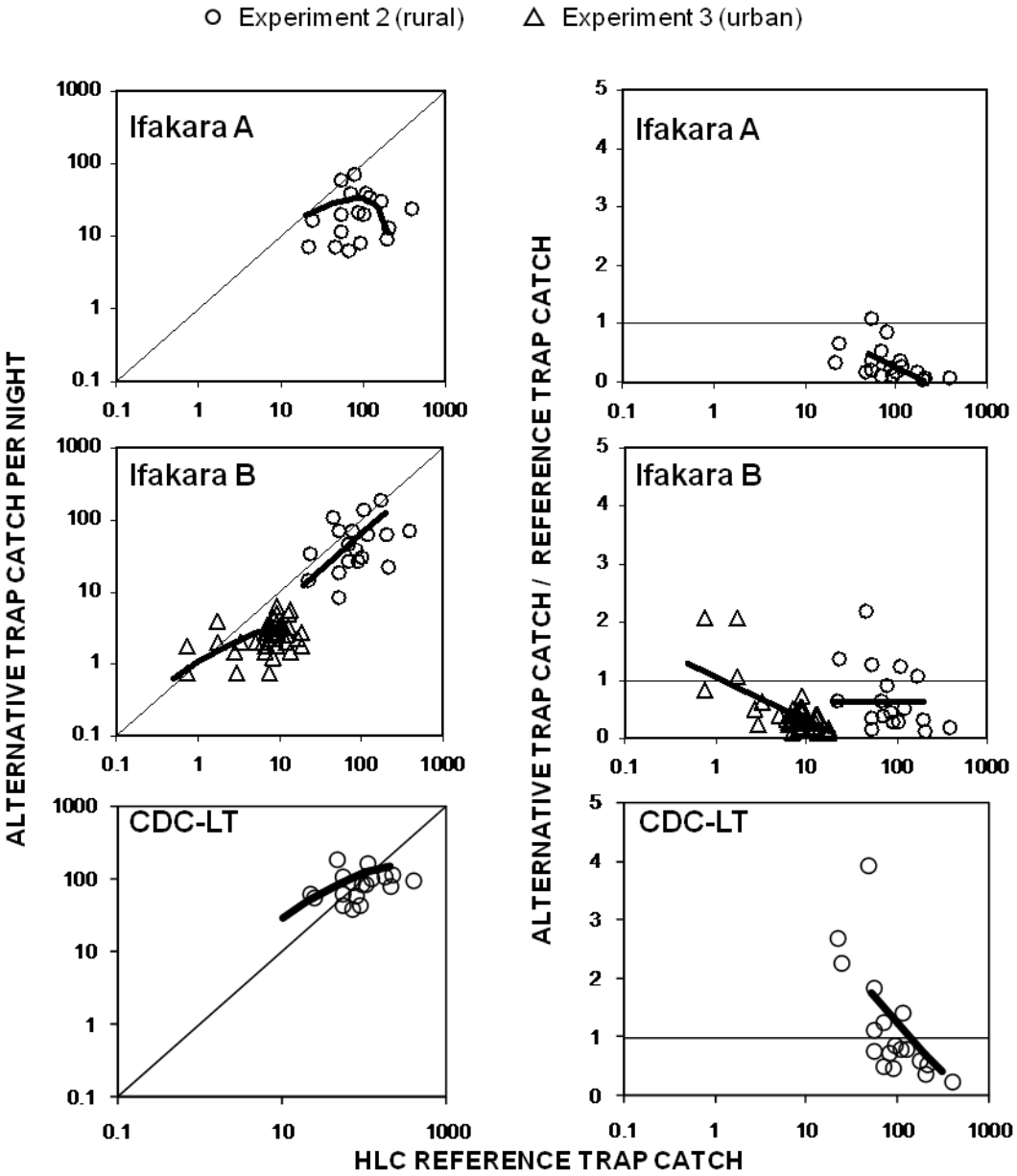

Figure 3.4.3: Correlation and density-dependence of alternative methods sampling efficiency, relative to human landing catch (HLC) gold standard reference method for catching *An. gambiae s.l.*. The correlation between the catches by alternative methods and HLC is presented in the left panels. Right panels illustrate density-dependence by plotting catches with alternative methods divided by corresponding catches by HLC against the absolute catches in HLC.

Density-dependence of trap sampling efficiency

Compared to the LT reference method, the Furvella trap was the only alternative method which showed density-independent sensitivity (Table 3.4.3 and Figure 3.4.2). Consistent with Figure 3.4.1, this method yielded catches which were imprecise but otherwise almost exactly equivalent to those of the LTs, suggesting their common components and mechanisms of action may result in similar sampling characteristics and dependence upon confounding factors. In contrast, both Ifakara A and B were clearly less sensitive at high vector densities when compared to LTs (Table 3.4.3 and Figure 3.4.2), but at low densities the Ifakara B design was at least as sensitive as the LT.

Similarly, only one instance of constant sampling efficiency was apparent when HLC was treated as the reference group. All alternative traps, with the exception of Ifakara B in experiment 2, proved to be more sensitive at low vector densities and decrease with increasing vector abundance (Table 3.4.3 and Figure 3.4.3). Although the sensitivity of the Ifakara B trap increased with decreasing vector density in experiment 3, it is noteworthy that, again this alternative exceeds the sensitivity of the reference method at the lowest vector densities. Given that the HLC is considered a more reliable gold standard than LT, these observations again strengthen the case that the Ifakara B trap is probably the most reliable, if not always the most sensitive, of the alternative traps evaluated here as surrogates of human exposure to malaria vectors.

Table 3.4.3: Density-dependence of relative sampling efficiency of alternative traps for *An. gambiae s.l.* by generalized estimating equations (GEE)

Alternative collection method		Parameter	Estimate [95%CI]	P
Versus CDC -light trap reference method				
Furvella				
	Experiment 1	Intercept	1.07 [0.70, 1.44]	<0.001
Ifakara A				
	Experiment 1	Intercept	2.12 [1.42, 2.82]	<0.001
		Log_{10}(CDC-LT)	-0.90[-1.22, -0.57]	<0.001
	Experiment 2	Intercept	1.05 [0.59, 1.51]	<0.001
		Log_{10}(CDC-LT)	-0.39[-0.57, -0.21]	<0.001
Ifakara B				
	Experiment 1	Intercept	3.31[1.17, 5.45]	0.002
		Log_{10}(CDC-LT)	-1.27[-2.35, -0.19]	0.021
	Experiment 2	Intercept	2.10[1.22, 2.99]	<0.001
		Log_{10}(CDC-LT)	-0.74[-1.09, -0.39]	<0.001
	Experiment 3	NA	NA	NA
Light trap				
	Experiment 2	NA	NA	NA
Versus human landing catch reference method				
Furvella				
	Experiment 1	NA	NA	NA
Ifakara A				
	Experiment 1	NA	NA	NA
		NA	NA	NA
	Experiment 2	Intercept	1.69[0.86, 2.52]	<0.001
		Log_{10}(HLC)	-0.71[-1.09, 0.33]	<0.001
Ifakara B				
	Experiment 1	NA	NA	NA
		NA	NA	NA
	Experiment 2	intercept	0.64[0.46, 0.81]	<0.001
	Experiment 3	Intercept	1.06[0.78, 1.33]	<0.001
		Log_{10}(HLC)	-0.75[-0.99, 0.50]	<0.001
Light trap				
	Experiment 2	Intercept	4.65[1.58, 7.71]	0.003
		Log_{10}(HLC)	-1.71[-3.243, -0.178]	0.029

Influence of trap design on the parity, species and abdominal status distribution

Table 3.4.4 compares the parity status distribution of *An. gambiae s.l.* sampled with the various alternative methods with that of the HLC gold standard. No significant differences were noted for any of the trapping methods. Although the raw data might suggest different parity rates in samples obtained with the various trapping methods, this arises from their differential distribution across experiments 1 and 2 which sampled populations with very different age structures (Table 3.4.4). The lack of differences between alternative methods and HLC suggests they all represent reasonable options for sampling mosquitoes to determine the age distribution, and therefore the infection status, of the host-seeking vector population. Furthermore, the sibling species composition of the *An. gambiae s.l.* revealed that *An. gambiae s.s.* and *An. arabiensis* were the only subspecies obtained from successfully (n = 3136) amplified specimens and LT was the only method which differed from HLC (Table 3.4.5). The LT oversampled *An. gambiae s.s.*

Over 89% of *An. gambiae s.l.* caught with each method over all experiments were unfed. This suggests that each method used in these experiments predominantly sampled host-seeking vectors. The proportions of mosquitoes caught with each method and in each experiment which were fully or partly blood fed are presented in Table 3.4.6. Both the Furvella and LT which rely on similar components and mechanisms, catch far fewer blood-fed mosquitoes than HLC. This confirms that these are indeed exposure-free methods which prevent the frequent occurrence of blood feeding upon the catcher before capture, as is inevitable when conducting HLC. The lack of

consistent differences between the Ifakara designs and HLC suggests that exposure does occur when sampling with this trap, most probably when the zip is opened in the morning and the operator aspirates from inside the trap chamber.

Table 3.4.4: The influence of trapping method and experiment upon the proportion of sampled *An. gambiae s.l.* which were parous, determined by binary logistic regression method

Variable	Parous (%)	OR [95% C.I.]	P
Trap type			
Furvella	22.2 (35/158)	0.89[0.55, 1.45]	0.849
Ifakara A	30.5 (68/2230	0.99[0.55, 1.45]	0.957
Ifakara B	30.4 (106/349)	1.00[0.73, 1.36]	0.999
Light trap	15.2 (141/930)	0.97[0.74, 1.27]	0.849
Human landing catch	41.0 (293/714	100[a]	NA
Experiment			
Experiment 1	23.6 (168/713)	0.51[0.39, 0.67]	<0.0001
Experiment 2	28.6 (475/1661)	100[a]	NA

Table 3.4.5: The influence of trapping method and experiment upon the proportion of sampled *An. gambiae s.l.* which were *An. gambiae s.s.* determined by logistic regression method

Variable	*An. gambiae s.s* (%)	OR [95%C.I.]	P
Trap type			
Ifakara A	8.2 (12/146)	0.71[0.39, 1.32]	0.28
Ifakara B	61.3 (234/382)	0.84[0.49, 141]	0.50
Light trap	14.3 (116/814	1.32[1.02, 1.71]	0.03
Human landing catch	32.9 (591/1794)	100[a]	NA
Experiment			
Experiment 2	11.9 (294/2471)	0.001[0.001, 0.002]	,0.001
Experiment 3	99.1 (666/672)	100[a]	NA

NA: Not applicable

Table 3.4.6: The influence of trapping method and experiment upon the proportion of sampled *An. gambiae s.l.* which were fully or part blood fed, determined by binary logistic regression method

Variable	Proportion fed (%)	OR [95%C.I.]	P
Trap type			
Furvella	1.53 (20/1306)	0.24[0.15, 0.39]	<0.001
Ifakara A	10.90 (47/429)	1.56[1.17, 2.10]	<0.002
Ifakara B	6.51 (166/2548)	1.00[0.80, 123]	0.998
Light trap	1.83 (142/7744)	0.32[0.25, 0.40]	<0.001
Human landing catch	32.9 (591/1794)	100[a]	NA
Experiment			
Experiment 1	3.10 (204/6624)	0.56[0.43, 0.72	<0.001
Experiment 2	3.74 (319/8525)	0.48[0.39, 0.58)]	<0.001
Experiment 3		100[a]	NA

NA: Not applicable

3.5 Discussion

The use of mosquito trapping techniques to estimate daily vector biting rates experienced by humans requires not only that such approaches are sufficiently sensitive, but also that sampling efficiency is known. The relative sampling efficiency of LT was found to be density-dependent with its efficiency decreasing at high vector densities. This finding supports other reports from areas of low malaria vector density in Kilifi on the coast of Kenya for *An. gambiae s.l.* (Mbogo *et al*. 1993) and in Papua New Guinea for *An. punctulatus* and *An. farauti* (Hii *et al*. 2000). In other studies, however, the relative sampling efficiency of LT has been found to be density-independent (Lines *et al*. 1991; Davis *et al*. 1995; Costantini *et al*. 1998; Magbity *et al*. 2002). Unlike the Kilifi study (Mbogo *et al*. 1993), no zero values were present in the aggregated data and no transformations other than logarithm were necessary.

Therefore, this density-dependence cannot be attributed to mathematical artifact (Smith, 1995) and appears to be a genuine property of the sampling device. Note that, our estimate of mean relative sensitivity for the LT differs from previous trials in the same Tanzanian village (Okumu *et al*. 2008) and re-analysis of that data revealed the same density-dependence for LT (Okumu *et al*. 2008). The apparently variable trapping efficiency of LT between and within studies may not necessarily be due to differences in statistical approach (Magbity *et al*. 2002) but rather to subtle and intensely heterogeneous factors which inevitably vary through space, time and investigation. Such essentially uncontrollable factors could include the positioning of the paired techniques, use of interventions such as bednets and insecticides, lunar phase, season, weather and house architecture. For instance, one study in Papua New Guinea (Hii *et al*. 2000) reported that the relative sampling efficiency of LT placed indoor was independent of outdoor *An. bancroftii* density changes and simultaneously density-dependent in relation to indoor vector abundance while the reverse trend was true for *An. longirostris*. One study in Africa (Magbity *et al*. 2002) noted some evidence that the presence of treated nets reduces the relative sampling efficiency of LT but this effect was slight and in other similar studies (Killeen *et al*. 2007b; Kirby *et al*. 2008) no effect could be demonstrated. Several previous studies concluded the LT to be free of age-related sampling biases (Lines *et al*. 1991; Faye *et al*. 1992; Costantini *et al*. 1998) consistent with our observations but not those of other reports (Rubio-Palis and Curtis, 1992b; Githeko *et al*. 1994). The difference between these studies might be partly explained by variability in both the position of LT relative to the floor (Mboera *et al*. 1998), the quality of net (Lines *et al*. 1991), and variability in sleeping behavior of net occupants (Charlwood *et al*. 1995).

Here the sampling efficiency of Ifakara B traps relative to HLC has been evaluated in two very different eco-epidemiological settings, where malaria transmission intensity ranges from less than one (Geissbühler *et al*. 2009) to over 600 (Killeen *et al*. 2007b) infectious bites per person per year and the vector species in question have clearly distinct feeding behaviors and activity patterns (Killeen *et al*. 2006b; Geissbühler *et al*. 2007). The sampling efficiency of the Ifakara B design appeared to be independent of vector density in the rural area with high vector abundance but appeared to increase at low densities in the urban setting, possibly reflecting reduced attentiveness of HLC catchers at low mosquito densities. Clearly none of these entomological techniques are precise, accurate or representative of true human biting rates but it is encouraging that the Ifakara B design correlates well to the HLC, given its lack of dependence on electricity, access to the inside of houses or intensive effort, plus its increased sampling efficiency at low densities. Also, the modest sampling efficiencies indicated by this crude analysis are sufficiently high to suggest these tent trap designs could be useful for extensive, sustained vector surveillance because of their lower cost and difficulty per trap night of sampling.

The proportion of fed mosquitoes caught in the tent traps is at least as high as for those caught by HLC. This implies that, either these traps act as resting shelters for freshly fed mosquitoes, or that the human bait actually does get bitten while aspirating mosquitoes. We have occasionally observed the latter process occurring in practice and suggest a relatively clear avenue for improvement to develop a design which truly is exposure-free and completely protects the user from exposure to mosquito bites. We conclude that the Ifakara B tent trap may be a valuable tool for large-scale surveillance, particularly in resource-limited settings if concerns

about operator exposure while collecting each morning could be overcome through modification or protective measures. However, because this new trapping method is primarily intended to replace both LTs and HLC for routine monitoring of African malaria vectors in large scale programmes such as the Dar es Salaam Urban Malaria Control Programme, there is an urgent need to first compare the efficacy of this new trapping technology with the most commonly used alternatives to HLC.

CHAPTER 4

MONITORING MOSQUITOES IN URBAN DAR ES SALAAM: EVALUATION OF RESTING BOXES, WINDOW EXIT TRAPS, CDC LIGHT TRAPS, IFAKARA TENT TRAPS AND HUMAN LANDING CATCHES

This paper has been published in the

Parasites & Vectors 2011, 4: 40

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4.1 Abstract

Background: Ifakara tent traps (ITT) are currently the only sufficiently sensitive, safe, affordable and practical method for routine monitoring host-seeking mosquito densities in Dar es Salaam. However, it is not clear whether ITT catches represent indoors or outdoors biting densities. ITT do not yield samples of resting, fed mosquitoes for blood meal analysis.

Methods: Outdoors mosquito sampling methods, namely human landing catch (HLC), ITT (Design B) and resting boxes (RB) were conducted in parallel with indoors sampling using HLC, Centers for Disease Control and Prevention miniature light traps (LT) and RB as well as window exit traps (WET) in urban Dar es Salaam, rotating them thirteen times through a 3 × 3 Latin Square experimental design replicated in four blocks of three houses

Results: The mean sensitivities of indoor RB, outdoor RB, WET, LT, ITT (Design B) and HLC placed outdoor relative to HLC placed indoor were 0.01, 0.005, 0.036, 0.052, 0.374, and 1.294 for *Anopheles gambiae s.l.* (96% *An. gambiae s.s* and 4% *An. arabiensis*) respectively and 0.017, 0.053, 0.125, 0.423, 0.372 and 1.140 for *Culex spp* respectively. The ITT (Design B) catches correlated slightly better to indoor HLC (r^2 = 0.619, P < 0.001, r^2 = 0.231, P = 0.001) than outdoor HLC (r^2 = 0.423, P < 0.001, r^2 = 0.228, P = 0.001) for *An. gambiae s.l.* and *Culex spp* respectively but the taxonomic composition of mosquitoes caught by ITT does not match those of the indoor HLC (χ^2 = 607.408, degrees of freedom = 18, P < 0.001).

Conclusion: The ITT (Design B) performed better than RB, WET and LT for surveillance of adult malaria vector densities but there is still uncertainty over whether the ITT best reflect indoor or outdoor biting densities.

4.2 Introduction

In urban Dar es Salaam, Tanzania the principal malaria vectors are species of *An. gambiae* complex and *An. funestus* (Geissbühler *et al*. 2007). *Culex sp*, also present in larger numbers (Geissbühler *et al*. 2007), causing appreciable nuisance and known to transmit Lymphatic Filariasis (Maxwell *et al*. 1990; Pedersen *et al*. 1999; Waynd *et al*. 2007; Bockarie *et al*. 2009; Hotez and Kamath, 2009). Human infection with *Wuchereria bancrofti* was generally thought to be increasing in urban African communities due to rapid urbanization coupled with inadequate sanitary facilities which provide ideal breeding habitats (Curtis and Feachem, 1981) for mosquitoes in the *Culex pipiens* complex (*Culex pipiens* L, and *Culex quinquefasciatus*,) (Smith and Fonseca, 2004),which is a major vector of lymphatic filariasis in South Asia, East Africa and Americas particularly in urban areas (Raghavan, 1957; Nathan, 1981; Janousek and Lowrie, 1989; Zhang *et al*. 1994; Pedersen *et al*. 1999; Service, 2004; Cantey *et al*. 2010). Although recent global effort to eliminate the filarial infections through mass drug administration (MDA) has reversed this trend to some degree (MacKenzie *et al*. 2009; Malecela *et al*. 2009a; Malecela *et al*. 2009b), it is becoming increasingly clear that elimination of filariasis transmission by MDA alone (Bockarie *et al*. 2009; Simonsen *et al*. 2010) is not enough, so vector control needs to be intergrated.

In its initial stages, routine monitoring of adult mosquito densities by the Dar es Salaam Urban Malaria Control programme (UMCP) was only possible with the laborious, uncomfortable and potentially hazardous human landing catch (HLC) for several years. This prompted development and evaluation of the Ifakara tent traps (ITT). A series of ITT designs have been tried and the B design has proven efficacious (Chapter 3) and effective (Sikulu *et al*. 2009) in terms of both number and species composition of mosquitoes caught. It is also cost-effective relative to other sampling methods in terms of cost per mosquito trapped (Sikulu *et al*. 2009). The B design exposed human subjects to mosquito bites while emptying the large trap chamber (Sikulu *et al*. 2009; Chapter 3) this model has since been modified to circumvent this problem, but the new design (C) was not available at the time of this study (Chapter 5). The ITT appears to be the most promising method for routine surveillance of biting densities of host-seeking mosquitoes in this setting and may be useful in a variety of African settings.

While monitoring host-seeking mosquito densities are an essential part of understanding disease, samples of resting mosquitoes (Pates and Curtis, 2005) are also required to enable assessment of host feeding patterns through blood meal analysis (Service, 1977; Lefevre *et al*. 2009; Lyimo and Ferguson, 2009). The proportion of blood meals that each vector species obtains from humans is a critical determinant of, not only transmission intensity, but also the efficacy of interventions targeted at humans or the houses they live in (MacDonald, 1957; Garrett-Jones, 1964a; Garrett-Jones, 1964b; Gillies and DeMeillon, 1968; Bruce-Chwatt, 1985; Killeen and Smith, 2007; Killeen *et al*. 2007a). Sample of resting mosquitoes for blood meal analyses are therefore important for selecting appropriate control strategies, particularly as vector

population composition may become dominated by zoophagic species once high coverage by insecticide-treated nets (Lindblade *et al*. 2006; Bayoh *et al*. 2010; Russell *et al*. 2010) or indoor residual spraying (Gillies, 1962; Gillies and Smith, 1960; Gillies and Furlong, 1964) is achieved. ITT and HLC both primarily sample host-seeking mosquitoes (Sikulu *et al*. 2009; Chapter 3) so either resting collection techniques (WHO, 1975b; WHO, 2003c; Kulkarni *et al*. 2006) or window exit traps (WET) (WHO, 1975b) are required to effectively characterize the feeding behaviours of vector mosquitoes that are relevant to intervention efficacy and selection.

The WET has been found useful for monitoring malaria vector density trends in Southern Africa (Hargreaves *et al*. 2003; Mouatcho *et al*. 2007), Equatorial Guinea (Sharp *et al*. 2007a) and for vectors of Japanese encephalitis (Chen and Chow, 1969) in Korea. However, their sensitivity is likely to be site-specific and strongly influenced by house design. Resting boxes were found to be highly selective in sampling specific mosquito species in coastal areas of the United States of America (Crans, 1989), but have also shown potential for monitoring *Culex quinquefasciatus* and *Aedes aegypti* in urban Brazil (Barata *et al*. 2007).

This article therefore presents an assessment of a number of mosquito trapping methods compared with HLC catches in Dar es Salaam, including the widely used Centers for Disease Control and Prevention miniature Light Trap (LT) and the WET design commonly used in programmatic contexts, in a rigorous formal comparison for the first time in this urban setting. We also assessed whether catches with the B design of the ITT best represent the indoor or outdoor fractions of mosquito populations

because, although this device is placed outdoors, it does resemble a small house and requires the mosquito to enter it so it may selectively sample indoor-biting mosquitoes.

4.3 Methods

Study site

This study was conducted at Mchikichini and Jangwani wards situated along the edge of Msimbazi River Valley in urban Dar es Salaam, the largest and most economically important city in Tanzania. The city is located at the shores of Indian Ocean coast with humid and hot climatic condition (NBS, 2003).

Dar es Salaam is also the home of the UMCP, a community-based vector control programme which primarily implements locally-supervised larviciding applied on a weekly basis at the neighbourhood level with vertical oversight from the city council (Sattler *et al*. 2005; Mukabana *et al*. 2006; Wang *et al*. 2006; Dongus *et al*. 2007; Geissbühler *et al*. 2007; Fillinger *et al*. 2008; Castro *et al*. 2009; Chaki *et al*. 2009; Dongus *et al*. 2009; Geissbühler *et al*. 2009; Sikulu *et al*. 2009). *An. gambiae* sibling species can grow from egg to adult in one week or less (Gillies and DeMeillon, 1968; Gillies and Coetzee, 1987) so the adult mosquito surveillance system for this programme needs to be not only affordable and practical (Sikulu *et al*. 2009), but also both spatially and temporally intensive to detect coverage gaps as they occur on such fine geographic scales as neighbourhoods, housing clusters and even individual plots (Dongus *et al*. 2007; Chaki *et al*. 2009) on a weekly or even daily basis (Killeen *et al*. 2006a; Fillinger *et al*. 2008). The need for sensitive adult malaria mosquito

surveillance in this setting is compounded by low levels of local malaria transmission and correspondingly sparse vector populations.

Transmission of malaria in urban Dar es Salaam is generally low with an entomologic inoculation rate of about one or less infectious bite per person per year (Fillinger *et al*. 2008), corresponding to the approximate limit of detection of malaria transmission by most entomological surveillance systems (Beier *et al*. 1999). Members of the *Anopheles gambiae* complex (*An. gambiae sensu stricto, An. arabiensis, An. merus*) and *An. funestus* are the primary malaria vector in this setting, with *An. gambiae s.s.* and *An. arabiensis* being most important (Geissbühler *et al*. 2007). While the nightly biting peak of *An. gambiae s.s.* in Dar es Salaam is consistent with that of classical reports (Gillies and DeMeillon, 1968), recent observations show that this vector species, together with *An. arabiensis*, tends to bite predominantly outdoors (Geissbühler *et al*. 2007).

Trapping methods

Resting boxes (RB)

Resting boxes made of cardboard (Figure 4.3.1) with one open end and black cotton cloth lined inside them (Sikulu *et al*. 2009), were each placed indoor in a room occupied by a person and outdoor in a shaded area. Mosquitoes caught were retrieved from the boxes using a hand-held aspirator from 8.00am to 9.00am on the morning following each sampling night.

Figure 4.3.1: Photographs of resting boxes. A and B illustrate how the boxes are made, C demonstrates how to install them and D demonstrates how to recover resting mosquitoes (Sikulu *et al*. 2009)

Window exit trap (WET)

Window exit traps are rectangular boxes made of a wooden frame covered in Teflon®-coated woven fiberglass netting, with a slit-shaped rectangular tilted wire opening at one side as a mosquito entrance (Figure 4.3.2A) and a sealable cotton sleeve aspirator inlet on the other side. The trap is first attached to a plywood sheet with screws and then the board plus trap combination is screwed to a house window frame (Figure 4.3.2B). Note that the edges of the plywood were wrapped with a foam seal to cover the gap between the board and the wall of the house, as well as protecting the wall from being scratched by the board. The traps were installed only to houses without intact screens or houses whose owners provided written informed consent to remove the screen under condition of being compensated with free installation of new screening at the end of the study. Mosquitoes were retrieved from the trap using hand-held aspirator through a sealable sleeve from 8.00am to 9.00am.

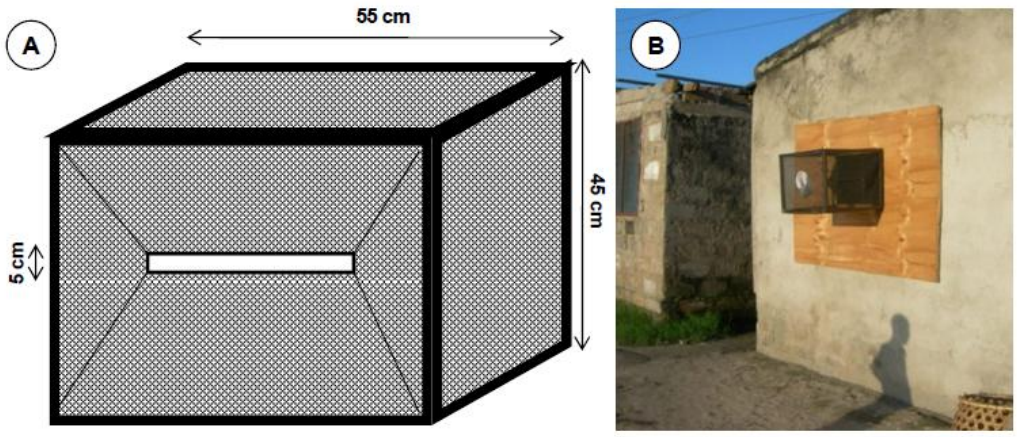

Figure 4.3.2: Window exit trap before fixing to a house window (**A**), and after installation (**B**)

Centers for Disease Control and Prevention miniature light traps (LT)

CDC miniature light traps (model 512) with influorescent bulbs (5 watt) were each hung inside a house near an occupied bed covered with either an untreated net or a long lasting insecticidal net (LLIN), with one block in each location assigned to the two types of nets to test for the effect of net treatment upon LT trap efficiency. The Permanet 2.0® brand of LLIN was used. The trap was hung approximately 150 cm from the floor surface and placed with the pan touching one side of the net at the end where the occupant's feet lay (Mboera *et al*. 1998).

Ifakara tent trap (ITT Design B)

The B design of the ITT was placed approximately 5m outside the house with a team member sleeping inside it and mosquitoes were collected in the morning as previously described (Sikulu *et al*. 2009; Chapter 3).

Human landing catch (HLC)

To conduct human landing catch, each adult male collector exposed his lower limbs and collected the mosquitoes with an aspirator when they landed on his legs (WHO, 1975b; Service, 1977; WHO, 2002; Mboera, 2005; Geissbühler *et al*. 2007). HLC was conducted by a single catcher at each station for 45 minutes each hour, allowing 15 minutes break for rest. To obtain full hourly biting densities, the catches for each hour were therefore divided by 0.75 (Geissbühler *et al*. 2007). Collections were conducted

both indoors and outdoors in accordance with the experimental design described below.

Experimental design

Four blocks (two from Mchikichini ward and the remaining two from Jangwani ward) of three houses each, with correspondingly matched outdoor catching stations about 5m away from each house were selected. Only houses with open eaves, distributed approximately 50m apart, were chosen. In each location, the two blocks were set up so that one block had all participants protected with untreated nets while those in the other slept under LLINs in order test whether the LLINs limits the house entry by mosquitoes.

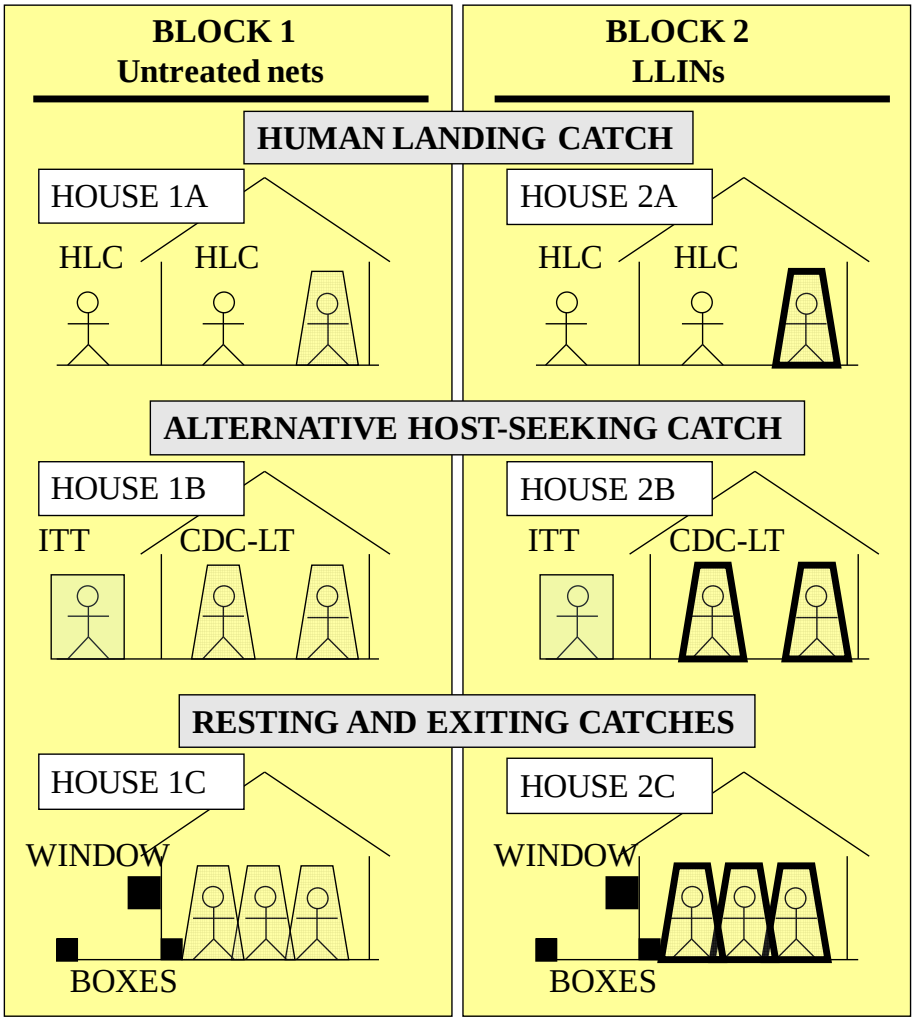

Figure 4.3.3: Schematic illustration of a typical night's experimental set up. Arrangement 1 as illustrated in figure 4.3.4) at one location with two blocks, one of which has occupants using untreated nets while the other has participants using long-lasting insecticidal nets (LLINs).

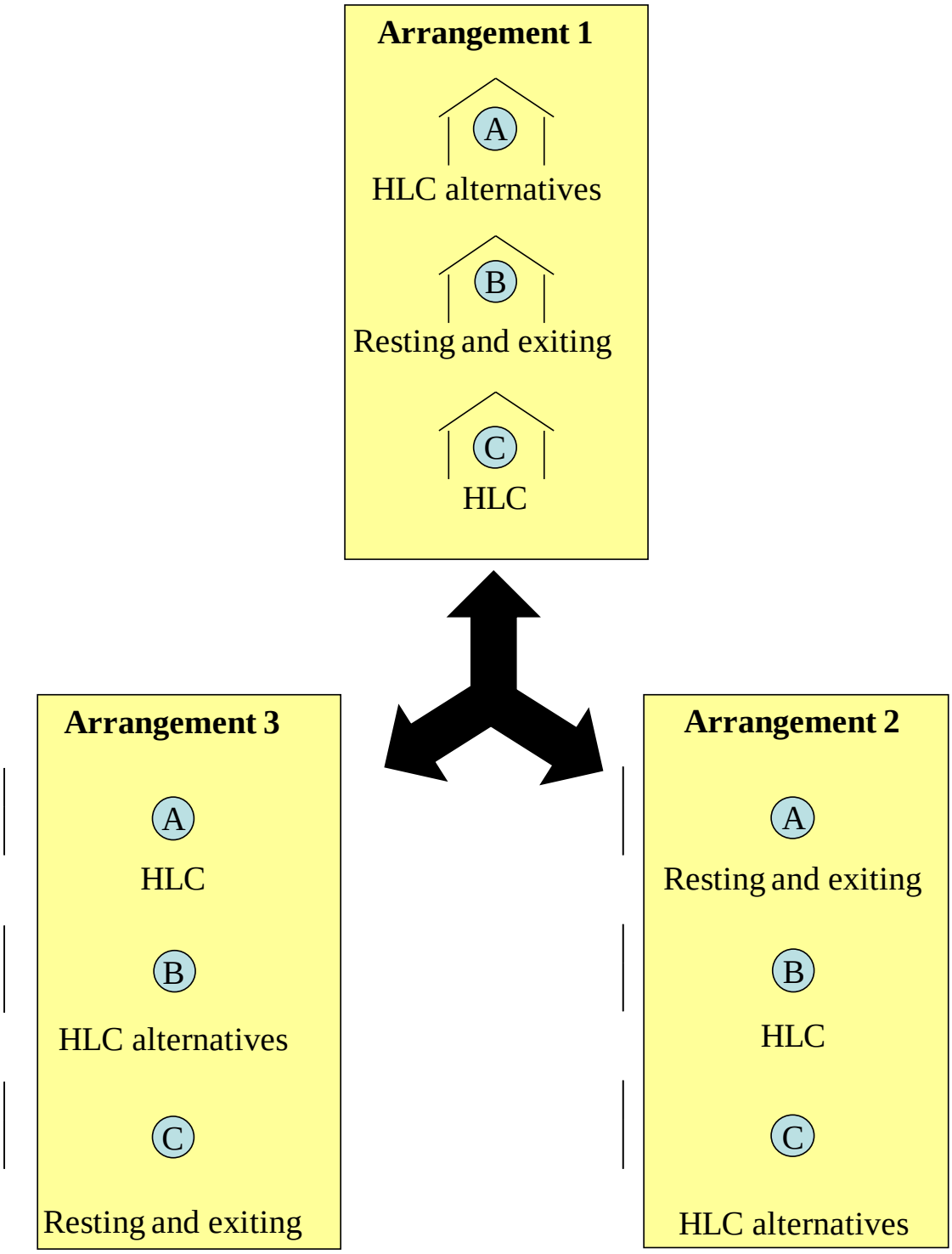

Figure 4.3.4: Schematic presentation of three possible arrangements of trapping methods rotated in order through the three houses in any given block. Note that the letters in blue circle represent the identifier within the block for each house, specified as 1A, 1B and 1C for houses in block 1 and 2A, 2B and 2C in for houses in block 2.

As described in the section entitled *Ethical clearance and protection of human participants*, these were planned based on the existing ownership of nets so that participants only experienced either no change or an increase in protection against mosquitoes and malaria: only participants lacking a net were provided with an untreated net and participants already owning a net of any description were provided with an Perma net 2.0® LLIN (Polyester treated with deltamethrin, developed by Vestergaard Frandsen A/S Kolding, Denmark) (Graham *et al*. 2005; Fettene *et al*. 2009; Tungu *et al*. 2010). The resting box, ITT design B, and HLC were placed in the corresponding respective indoor stations so that the combined indoor and outdoor stations at each house within each block could be considered to represent the conventional HLC gold standard, alternative host-seeking catching methods or methods for sampling resting and house-exiting mosquitoes, respectively (Figure 4.3.3). These indoor-outdoor combinations were rotated through all three houses of each block (Figure 4.3.4) for a total of thirteen complete rotations in 3 × 3 Latin Square experiment design. This experiment was conducted between 6th May and 2rd July 2008. Mosquitoes were collected from 19.00 to 08.00 h each night.

Processing of Samples

All *Anopheles* mosquitoes caught were sorted and morphologically identified (Gillies and Coetzee, 1987) with the aid of a stereomicroscope in the field. A total of 1180 *An. gambiae s.l* from all traps, were stored in tubes with desiccated silica for subsequent identification to sibling species level by polymerase chain reaction (Scott *et al*. 1993). All *Culex* were counted, categorized as male or female and discarded.

Data analysis

Sensitivity differences among trapping methods

Data analyses were computed using SPSS version 16.0 for Microsoft Windows (SPSS Inc., Chicago IL). Generalized estimating equations (GEE) were employed to assess the influence of trap type upon mosquito catches by treating house as subject variable with trap type-indoor/outdoor assignment combination and date as within-subject variables. Catches for female *An. gambiae s.l.* and *Culex spp*, were each treated as the dependent variable in separately fitted models. Normal distribution with a natural logarithm link function and exchangeable working correlation matrix were selected for these dependent variables. In the first place for fitting to the catches of *An. gambiae s.l.*, all trap types were included in the model, but yielded inestimable parameter values for both indoor and outdoor resting boxes so these two methods were thereafter removed from the fitted dataset.

The distribution of mosquito taxa among sampling methods and correlation of the catches

Trap type may affect taxonomic composition of mosquito catches (Okumu *et al.* 2010a) so the influence of trapping method upon the distribution of mosquitoes was analyzed by χ^2 test (Kirkwood, 1988). Comparison of multiple pair-wise Pearson correlation tests using logarithmically transformed data ($\log_{10}(x+1)$ for *An. gambiae s.l.* and $\log_{10}(x)$ for *Culex spp* of female catches aggregated by date was used to test whether the catches by the ITT best represent the indoor or outdoor biting catches.

The effect of net type on the indoor versus outdoor distribution of mosquitoes

The only method which yielded sufficient numbers of *An. gambiae s.l.* and for which both indoor and matching outdoor catches in the same house and night were available was HLC. Comparing the effect of LLINs versus untreated nets upon catches was therefore only possible for this particular method. All mosquitoes caught with HLC in a given house and on a particular night were either caught indoors or outdoors, hence the distributions of *An. gambiae s.l.* with regards to net type was analyzed by binary logistic regression, treating indoor versus outdoor catches of *An. gambiae s.l.* as binary outcome.

Ethical clearance and protection of human participants

Ethical approval was obtained from Institutional review board of Ifakara Health Institute in Tanzania (IHI/IRB/No. A50) and Medical Research Coordination Committee of the National Institute of Medical Research in Tanzania (NIMR/HQ/R. 8c/Vol. ii/03) and the Ethics Committee of the Liverpool School of Tropical Medicine in the UK (09.60). Written informed consent describing the potential risks and benefits of the study was obtained from all study participants before commencing the study and re-confirmed on each experimental night. Volunteers were screened for malaria parasites by microscopy during recruitment and on a weekly basis throughout the experiment. Those who were found malaria positive were offered treatment free of charge with Artemether-Lumefantrane (Co-Artem®, Roche, Basel, Switzerland), the recommended first-line treatment for malaria in the United Republic of Tanzania. The untreated net versus LLIN blocks were assigned so that no individual participant who already had a net was provided with an untreated net to replace it: participants lacking

a net were provided either an untreated net or an LLIN while individuals with an existing net, untreated or otherwise, were all provided with a free Perma net® 2.0 LLIN.

4.4 Results

Sensitivity of alternative traps relative to indoor human landing catch

The number of mosquitoes caught by each collection method is shown in Table 4.4.1. The RB, WET, and the LT caught far fewer *An. gambiae s.l.* than the indoor HLC reference method. ITT design B was the only alternative method that caught useful numbers of this vector complex (Table 4.4.1), with approximately one quarter the sensitivity of indoor HLC (Table 4.4.2). The LT, however, appeared relatively sensitive for sampling *Culex spp*, exceeding even the number caught by the ITT (Tables 4.4.1 and 4.4.2). All alternative trapping methods, with the exception of the outdoor HLC, sampled significantly fewer *An. gambiae s.l.* than the indoor HLC reference method (Table 4.4.2). The outdoor HLC, caught as many *An. gambiae s.l.* and significantly more *Culex spp* than the indoor HLC reference method (Table 4.4.2)

Table 4.4.1 Number of mosquitoes caught by different methods and crude estimates of sensitivity relative to indoor human landing catch

Collection methods	Trap night	Total catch	Mean catch	Relative sensitivity
Anopheles gambiae s.l				
Resting boxes indoor	156	6	0.038	0.01
Resting boxes outdoor	156	3	0.019	0.005
Window trap	156	21	0.135	0.036
CDC light trap	155	30	0.194	0.052
Ifakara tent trap	156	216	1.385	0.374
HLC outdoor	156	748	4.795	1.294
HLC indoor	156	578	3.705	NA
Anopheles funestus				
Resting boxes indoor	156	0	0	0
Resting boxes outdoor	156	0	0	0
Window trap	156	0	0	0
CDC light trap	155	0	0	0
Ifakara tent trap	156	1	0.006	0.158
HLC outdoor	156	19	0.122	3.210
HLC indoor	156	6	0.038	NA
Anopheles zeimanii				
Resting boxes indoor	156	4	0.03	0.017
Resting boxes outdoor	156	2	0.01	0.005
Window traps	156	2	0.01	0.005
CDC light traps	155	2	0.01	0.005
Ifakara tent traps	156	9	0.06	0.033
HLC outdoors	156	460	2.95	1.629
HLC indoors	156	283	1.81	NA
Culex spp				
Resting boxes indoor	156	293	1.878	0.017
Resting boxes outdoor	156	931	5.968	0.053
Window traps	156	2208	14.153	0.125
CDC light traps	155	7435	47.968	0.423
Ifakara tent traps	156	6585	42.212	0.372
HLC outdoors	156	20163	129.250	1.140
HLC indoors	156	17688	113.385	NA

NA = not applicable because this is a reference method

Table 4.4.2: Mosquito sampling sensitivity of alternative traps relative to the indoor human landing catch as determined using generalized estimating equations

Collection methods	RR [95%CI]	*P value*
Anopheles gambiae s.l.		
Resting boxes indoor	NE	NE
Resting boxes outdoor	NE	NE
Window exit trap	0.01 [0.002, 0.034]	< 0.001
CDC light trap	0.02 [0.009, 0.032]	< 0.001
Ifakara tent trap	0.26 [0.208, 0.330]	< 0.001
HLC outdoor	1.07 [0.851, 1.356]	0.549
HLC indoor	1.00*	NA
Culex spp		
Resting boxes indoor	0.02 [0.010, 0.026]	< 0.001
Resting boxes outdoor	0.07 [0.020, 0.274]	< 0.001
Window exit trap	0.11 [0.077, 0.166]	< 0.001
CDC light trap	0.50 [0.280, 0.893]	0.019
Ifakara tent trap	0.34 [0.256, 0.461]	< 0.001
HLC outdoor	1.17 [1.077, 1.278]	< 0.001
HLC indoor	1.00*	NA

RR = relative rate, CI = confidence interval, NE = not estimable
NA = not applicable because this is a reference method
* Reference value

***Sibling species composition of An. gambiae sensu lato*.**

Respectively, 96% (871) and 4% (41) of 912 (7, 10, 22, 94 and 779 sub sample from RB, WET, LT, ITT design B, and HLC respectively) successfully amplified specimens *of An. gambiae s.l.* were *An. gambiae s.s.* and *An. arabiensis*. This implies that the results presented here overwhelmingly reflect the response of *An. gambiae s.s.* to these traps.

Effect of sampling technique upon taxonomic composition of female mosquito and correlation of catches

An. gambiae s.l. (2.78%), *An. funestus* (0.05%), *An. ziemanni* (1.32%) and *Culex spp* (95.85%) were the only mosquitoes captured in this study. Trap type significantly

affected the composition of catches (χ^2 = 607.408, degrees of freedom = 18, P < 0.001). Apart from such an overall χ^2 all pair-wise χ^2 comparisons of either outdoor HLC or any of the alternative methods with indoor HLC proved significant (P≤ 0.0001). As illustrated in figure 4.4.1, the catches of *An. gambiae s.l.* and *Culex spp* by ITT correlated consistently slightly better with those of the indoor HLC (r^2 = 0.619, P < 0.001 and r^2 = 0.304, P = 0.001, respectively) than the outdoor HLC (r^2 = 0.423, P < 0.001 and r^2 = 0.228, P = 0.001, respectively).

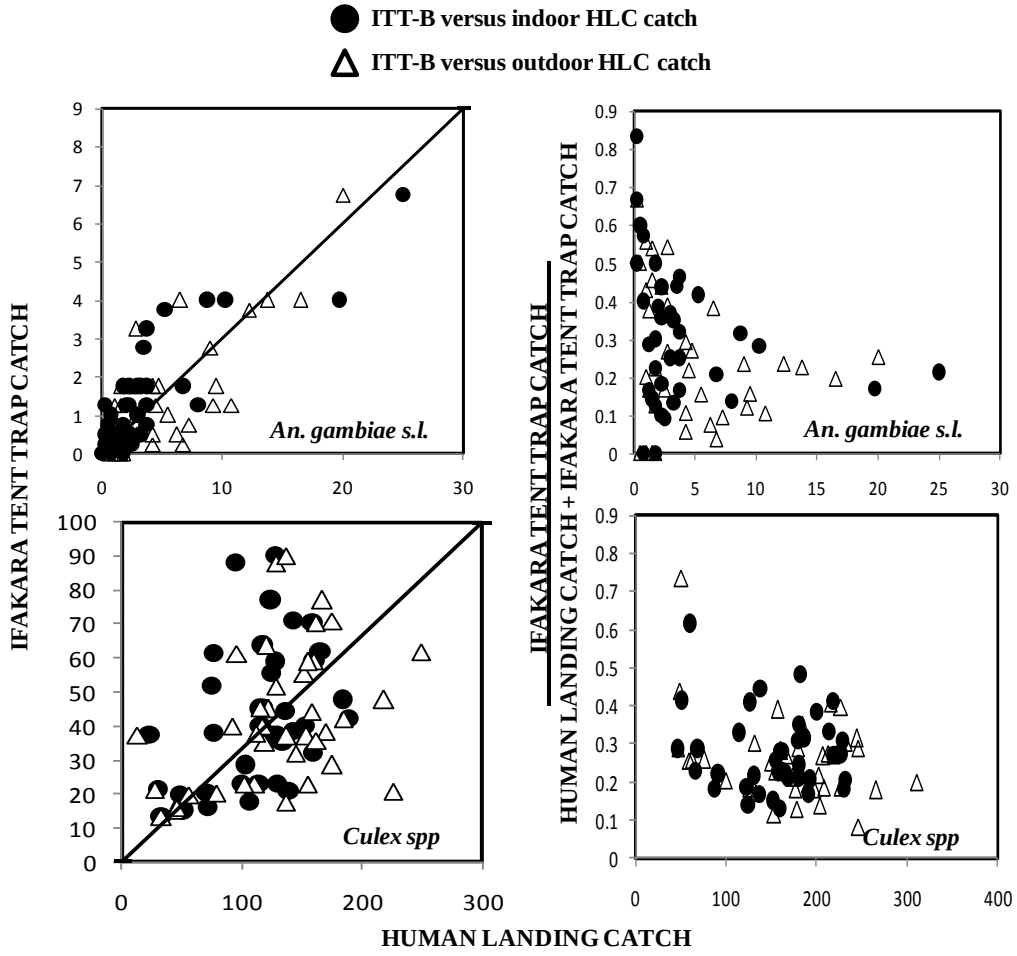

Figure 4.4.1: Correlation and density dependence of Ifakara tent trap (ITT design B) sampling efficiency relative to human landing catch (HLC). The correlation between the catches of *An.gambiae s.l.* and *Culex spp* with ITT and HLC plotted in absolute number is presented in left panels with complete equivalence depicted by the diagonal line. Right panels illustrate density dependence as catches in ITT divided by the sum catches of ITT and HLC against the absolute catches of the HLC reference method.

Effect of long-lasting insecticidal nets upon mosquito distribution

Human landing catch was the only method for which sufficient numbers of *An. gambiae s.l.* were caught to assess the impact of LLINs upon the relative fraction of mosquitoes found inside or outside houses. There was no significant difference in the proportion of *An. gambiae s.l.* caught indoors between LLINs and untreated bed nets houses (Table 4.4.3). This indicates that, in contrast with published trials (Dabire *et al*. 2005) these LLINs exerted little deterrence to stop mosquitoes from entering the house and provide no personal protection for non-users sharing the same house.

Table 4.4.3: The effect of treatment on proportion of *An. gambiae s.l.* sampled indoor and outdoor determined by binary logistic regression method

Categorical variables	*An. gambiae s.l.* caught indoor (%)	OR[95%CI)	P
Treatment			
Long lasting net	44.94 (333/741)	1.13[0.91, 1.41]	0.265
Untreated net	41.88 (245/585)	1.00*	NA

NA = not applicable because this is a reference group

OR = odds ratio; CI = confidence interval; 1.00* = reference value

4.5 Discussion

Sustained control of pathogen-transmitting mosquitoes requires sensitive and representative surveillance. This study compares a wide range of trapping methods, and demonstrates very poor performance of the RB, WET and LT for sampling adult malaria mosquitoes. This implies that such tools are not appropriate for surveillance and monitoring the impact of mosquito control measures in this urban setting where

the UMCP targets relatively sparse populations of *Anopheles* malaria vectors. These results also confirm the previous observational reports that the LT has very low sensitivity in this urban setting (Fillinger *et al*. 2008). This cannot be explained by the observation that *An. gambiae* is exophagic in this setting (Geissbühler *et al*. 2007) because the reference HLC method was also conducted indoors. While no particular explanation is obvious for such surprisingly poor performance by LT, we speculate that the light source from the LT, which is thought to play a vital role in attracting mosquitoes (Costantini *et al*. 1998), may have competed poorly for the attention of *Anopheles* in this highly illuminated (Miller *et al*. 1970), urbanized environment.

Some reports have suggested that the RB baited with urine odour are useful (Kweka *et al*. 2009) for surveillance of *An. arabiensis*, the most exophilic (White, 1974) sibling species of the *An. gambiae s.l.* complex (Gillies and Coetzee, 1987; Coetzee *et al*. 2000). However, this conclusion was neither supported by this study nor by a previous effectiveness evaluation in Dar es Salaam (Sikulu *et al*. 2009) which relied on unbaited RB. While these results are discouraging, it may be possible to improve the sensitivity of the approach by lining the boxes internally either with a sticky surface (Facchinelli *et al*. 2008) or a collapsible collection bag to maximize the catch size, because we observed that mosquitoes which entered the RB often escaped, particularly during retrieval.

Similarly, the weak performance by WET can be possibly partly explained by the architectural of the local houses. Most houses used in this study apart from having open eaves and lacking a ceiling, also had walls separating adjacent rooms which did

not reach the roof. It was therefore likely that many mosquitoes which entered a room fixed with WET exited via other rooms without a WET. Nevertheless, without such ready exits, there is also limited opportunity for mosquitoes to enter houses in the first place so there may be a fundamental limit to how efficacious such exit traps can be outside of experimental huts. Furthermore, variations in housing design are a normal feature of representative mosquito sampling so these disappointing results should be interpreted at face value until proven otherwise. It should also be noted that while this approach has been applied and advocated in a number of programmatic settings (Hargreaves *et al*. 2003; Mouatcho *et al*. 2007; Sharp *et al*. 2007a; Ridl *et al*. 2008), to our knowledge this is the first time the efficacy of this trapping method has been formally evaluated in comparison with HLC or other standard methods in typical residences rather than in experimental huts.

The correlation results obtained from this study indicated the catches from ITT relate better to those from the indoor rather than outdoor HLC but the taxonomic composition of female mosquitoes caught by ITT does not match those of the indoor HLC and re-analysis of data obtained from the previous study in rural setting (Chapter 3), yield contradictory correlation results that, although consistent with this study for *Culex spp* (r^2 = 0.452, P = 0.002 and r^2 = 0.260, P = 0.033 for ITT versus indoor and outdoor HLC respectively), the reverse was observed for the *An. gambiae s.l.* population consisting primarily of *An. arabiensis,* in that study (r^2 = 0.162 P = 0.098 and r^2 = 0.462, P = 0.002 for ITT versus indoor and outdoor HLC, respectively). It therefore remains unclear whether densities measured by ITT best reflect indoor or outdoor catches.

Consistent with our previous study of ITT evaluation (Sikulu *et al.* 2009; Chapter 3) it appears that this trap has potential for both research and routine programmatic surveillance applications. In addition to the *Anopheles* discussed in detail above, a large number of culicines were also captured with the ITT so this trap might also be used for surveillance of a diversity of other mosquito-borne pathogens, even though this was not a primary objective of this study. However, the observation of high proportions of blood fed mosquitoes in samples from this technique apparently associated with exposure of the user while empting the trap chamber remains a significant safety concern (Sikulu *et al*. 2009; Chapter 3). There is therefore an urgent need to first redesign this tool make it safe and more sensitive before used by unsupervised community-based staff conducting routine adult mosquito surveillance for the Dar es Salaam Urban Malaria Control Program.

CHAPTER 5

AN EXPOSURE-FREE TOOL FOR MONITORING ADULT MALARIA MOSQUITO POPULATION

This paper has been published in the

American Journal of Tropical Medicine and Hygiene 2010, 83(3): 596-600

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5.1 Abstract. Catches of *Anopheles gambiae* and *An. arabiensis* with the Ifakara Tent Trap-model B (ITT-B) correlate better to human landing catch (HLC) than any other method but fail to reduce the proportion of blood-fed mosquitoes caught, indicating users are exposed to bites during collection. An improved C model (ITT-C) was developed and evaluated by comparing with ITT-B both in semi-field and full-field conditions in southern Tanzania. The sensitivity of the ITT-C was approximately two times that of ITT-B: relative rate [95% confidence interval] = 1.92[1.53, 2.42], 1.90[1.48, 2.43] and 2.30[1.54, 3.30] for field populations of *An. arabiensis, Culex spp,* and *Mansonia spp,* respectively. The ITT-C caught 73% less blood-fed *An. arabiensis* than ITT-B in open field experiments and none in semi-field experiments, which confirmed that the C design is a safe trapping method. Validation of ITT-C by comparison with human landing catches and parasitological measures of human infection status may be necessary to confirm that this design produces consistent and epidemiologically meaningful results.

5.2 Introduction

In the drive to eliminate malaria, mosquito sampling measures are crucial to monitor changes in human exposure to infections and the effect of vector-control interventions (Service, 1977; WHO, 2003b; Mboera, 2005). However, existing monitoring methods for adult stages of the *Anopheles* vectors of human malaria all have significant limitations, particularly where densities of malaria-transmitting mosquitoes are low (Geissbühler *et al*. 2007; Fillinger *et al*. 2008; Kleinschmeidt *et al*. 2009b). This technology has become increasingly important as malaria control (WHO, 2000; WHO, 2005c; WHO, 2005d), elimination, and eradication (Feachem and Sabot, 2008) are

prioritized by policy makers and significant progress towards lower transmission levels are achieved (Battarai *et al*. 2007; Fegan *et al*. 2007; Sharp *et al*. 2007b; Ceesay *et al*. 2008; O'Meara *et al*. 2008; Kleinschmeidt *et al*. 2009b). Standard entomological methods often fail to detect (Beier *et al*. 1999) low levels of malaria transmission. Sensitive, scalable, safe and affordable tools are therefore required to achieve sustained and extensive monitoring of vector populations (Geissbühler *et al*. 2007; Geissbühler *et al*. 2009), so that control efforts can be managed and optimized.

A new device for sampling malaria vectors in Africa, called the Ifakara Tent Trap-design B (ITT-B), has recently been developed and evaluated as a means to catch malaria vector mosquitoes under conditions of both low and high mosquito densities in Tanzania (Chapter 3). The relative sensitivity of ITT-B increased as vector density decreased and exceeded that of human landing catches (HLC) at the lowest densities (Chapter 3) in urban Dar es Salaam. The ITT-B correlated better with human landing catches than any other tested method (Chapter 3), and is remarkably cost-effective under programmatic settings with minimal supervision (Sikulu *et al*. 2009). However, ITT-B failed to reduce the proportions of blood-fed mosquito caught relative to that observed in sample obtained by human landing catches (Sikulu *et al*. 2009; Chapter 3). The biggest disadvantage of the human landing catch method is the inevitable exposure of human participants to mosquito bites (WHO, 1975a; Service, 1977; Mboera, 2005). Thus, ITT-B operators may also have been exposed to mosquito bites (Sikulu *et al*. 2009; Chapter 3). Alternatively, these traps, may act as resting shelters for freshly fed mosquitoes, and both of these possibilities may cause blood-fed mosquitoes to be caught in the field.

This study reports an evaluation of the mosquito sampling properties of an improved C model of the Ifakara Tent Trap (ITT-C), compared with ITT-B to confirm that, this new version is comparably efficacious and successfully prevents operator exposure to mosquito bites.

5.3 Methods

Field study area. The field study was conducted in Lupiro village in the Kilombero River Valley in Tanzania. Detailed description of the area is found elsewhere (Killeen *et al*. 2007b), and the most recent study showed that *Anopheles arabiensis* is the dominant malaria vector in the area (Chapter 3). This location experiences high *P. falciparum* malaria transmission with an entomologic inoculation rate exceeding 500 infectious bites per person per year, in spite of high coverage with mainly untreated bed nets (Killeen *et al*. 2007b).

Semi-field study system. The semi-field system or screen house is an enclosed structure with walls of mosquito netting and a polyethylene roof located within the natural ecosystem of the target vector (Ferguson *et al*. 2008). The semi-field experiment was carried out within one of three 208 m^2 chambers of a screen house at the Ifakara Health Institute (Ferguson *et al*. 2008), in Kilombero District, south-east Tanzania (Killeen *et al*. 2007b).

Sampling methods. The Ifakara B and C traps were the only traps used. Although the ITT-B design has been described in detail (Chapter 3), ITT-C (Figure 5.3.1) differs

from this earlier prototype in that the netting panel lying between the entry funnels and the bait host is bisected into two compartments within the trap, which are 70cm apart. This enables a person in the process of collecting mosquitoes to stand up within the trap while protected from mosquito bites. In contrast, the B design requires the opening of the long zipper across the netting panel and aspiration from within the open trap chamber, thereby exposing the operator to bites. Also, there are two long (350mm) sealable cotton sleeves hanging from each trap chamber to enable operators to safely remove mosquitoes by using mouth aspirators while protected from bites. The two netting chambers, which the baffled entrance funnels lead into, are supported with two string braces to prevent them from sagging or collapsing. This structural feature is important as such sagging of the chambers down upon the occupant would increase the risk of contact with the human bait and thus exposure to mosquito bites. Although the baffled entrance funnels are held by strings suspended from the cross bar in the ITT-B, for the ITT-C they are maintained by wire bars with soft caps just outside of inner small apertures consisting of plastic rings sewn into each entry funnel, all of which are drawn tightly towards each other with a three-way elastic band tie. This feature smoothen the entry funnels and probably makes it easier for mosquitoes to enter the trap. For more detail see the appendix 1 illustrating on how to set up the ITT-C. Also available at www.ajtmh.org.

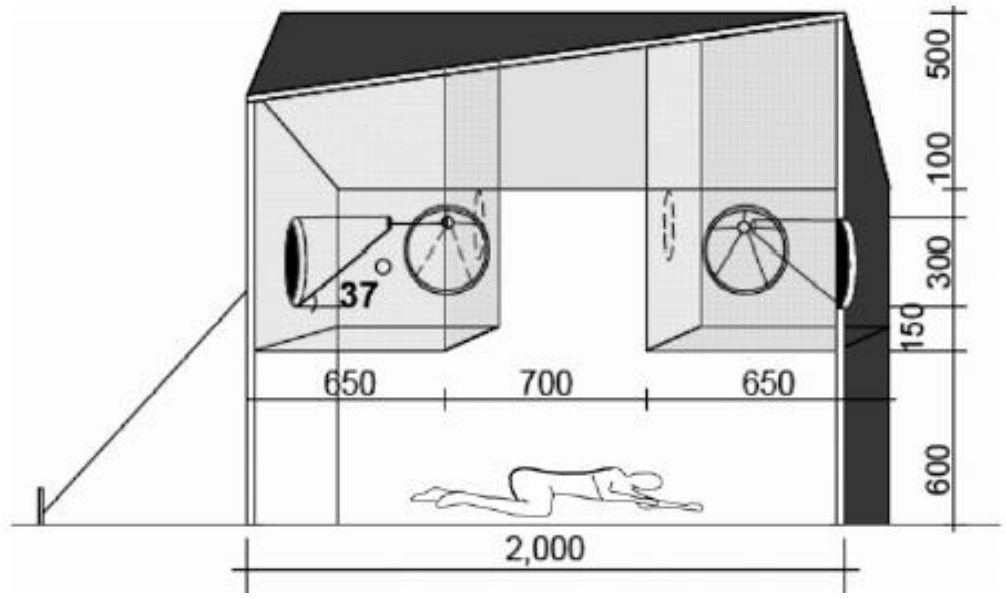

Figure 5.3.1: Ifakara Tent Trap-C design. The human occupant is protected from mosquito bites by two rectangular netting panels with the dotted circular point showing the position of the aspirator inlet though a sealable cotton sleeve. Mosquitoes enter through a funnel shaped entrances, each supported by a wire bar with soft caps just outside of the plastic rings, which form the inner small apertures of the funnel end. The three funnel apertures in each trap chamber are drawn tightly together with a three-way elastic band tie, which terminates in these wire bars that hold the ends of the funnels. All dimensions are in millimeters.

Experimental design: Open field. Four outdoor catching stations were selected approximately 50 meters apart and aligned about 100m from the main rice irrigation area on one side and approximately 15 meters from local houses on other side. Each collector was assigned to and remained associated with a specific sampling station throughout the experiment to control for the effect of differences in individual attractiveness and of a particular station. Two pairs of Ifakara B and C traps were allocated to all four catching stations and a cross-over experimental design was implemented in which each trapping method was exchanged between the two adjacent catching stations on each experimental night. This experiment was conducted for 10

nights (November 18-28, 2008), at a time when there was no rainfall. Mosquitoes were collected by both techniques from 8:00 PM to 7:00 AM.

Experimental design: Semi-field system. Two sampling stations approximately 16 meters apart inside a screen house (Okumu *et al*. 2010b), were established and each trap was placed in one of these stations. Two volunteers were recruited and each was assigned to and remained associated with a specific catching station. Traps were exchanged between positions on each experimental night for four nights by using a cross-over experimental design as described above. One hundred starved, insectary-reared, female *An. gambiae* sensu stricto were released from the central release point at 7:00 PM each night and mosquitoes were collected from 7:00 PM to 7:00 AM for four nights (November 29 to December 2, 2008).

Processing of samples. All anopheline mosquitoes caught were sorted and morphologically identified (Gillies and Coetzee, 1987) directly in the field. The abdominal condition of each female mosquito was classified as unfed, part fed, fully fed and gravid (Chapter 3). Sub-samples (179 of 344 and 227 of 714) from the ITT-B and ITT-C, respectively of *An. gambiae* sensu lato (members of this species complex are morphologically indistinguishable (Gillies and Coetzee, 1987)) were stored in tubes with desiccated silica for subsequent identification to sibling species level by polymerase chain reaction (Scott *et al*. 1993).

Data analysis.

Mean catch differences between sampling methods.

Although the goal of this study was to test whether the ITT-C is an exposure-free mosquito sampling method, it was also essential to confirm that it is as sensitive as the ITT-B. Using SPSS version 15 software (SPSS Inc., Chicago, IL), we applied generalized estimating equations to quantify the influence of trap design upon mosquito catches by treating station and date as subject and within-subject variables, respectively. The logarithmically transformed catches ($\log_{10}(x)$) for *An. gambiae* s.l., which appeared to be normally distributed, was treated as the dependent variable with an identity link function.

Influence of sampling technique upon blood-feeding status of trapped mosquitoes. Binary logistic regression analysis was used to test for differences in the distribution of abdominal status of mosquitoes from the *An. gambiae* complex caught in the two trap designs. We executed this test by treating abdominal status as a binary outcome, with each mosquito classified as being freshly blood fed (partly or fully) or not (unfed, gravid, semi-gravid), with trap design as an independent categorical factor in the model (Sikulu *et al*. 2009; Chapter 3).

Ethical clearance and protection of human participants. Prior to any field work, research clearance was obtained from the Ifakara Health Institute Ethics Review Committee and the Medical Research Coordination Committee of the National Institute of Medical Research in Tanzania (Reference numbers

NIMR/HQ/R.8a/Vol.IX/279 and 324). Informed consent was obtained in writing from all participants before initiation of the study and re-confirmed on each experimental night. These volunteers were screened for malaria parasites by microscopy during recruitment and after finishing the experiment. Those persons who were found to be malaria positive were offered treatment free of charge with Artemisinin-Lumefantrane (Co-Artem®; Roche, Basel, Switzerland) the recommended first-line treatment of malaria in Tanzania.

5.4 Results

The crude catch sensitivity of the ITT-C relative to the ITT-B. The crude mean sensitivity of the ITT-C for *An.gambiae s.l., Culex* spp, and *Mansonia* spp, relative to ITT-B are summarized in Table 5.4.1. The ITT-C consistently sampled about twice as many mosquitoes as the ITT-B for all three genera. This difference was significant for *An. gambiae s.l.*, the only malaria vector present in sufficient numbers, and for *Culex* spp and *Mansonia* spp (Table 5.4.2).

Table5.4.1: The number of mosquitoes trapped by the B and C designs of the Ifakara TentTtrap

Method	Night	*Anopheles gambiae s.l.*			*Culex* spp			*Mansonia* spp		
		Total	Mean	RS	Total	Mean	RS	Total	Mean	RS
Ifakara C	20	714	35.7	2.1	350	17.5	2.0	774	38.7	1.8
Ifakara B	20	344	17.2	NA	174	8.7	NA	441	22.1	NA

NA = not applicable because this is the reference method and RS = relative sensitivity

Sibling species composition of *An. gambiae s.l.* Of 366 successfully amplified specimens of *An. gambiae s.l.* caught in the field experiment, 97% (355) and 3% (11) were *An. arabiensis* and *An. gambiae* sensu stricto, respectively. This finding implies that *An. arabiensis* is the main malaria-transmitting vector in this locality. The results presented here relating to the *An. gambiae s.l.* species complex therefore overwhelming reflect the response of this particular sibling species to these traps.

Table 5.4.2: Mosquito sampling sensitivity of the Ifakara Tent Trap model C compared with the B design and evaluated by using generalized estimating equations and expressed as the relative rate at which mosquitoes are caught.

Taxon	Trap type	RR[95%C.I]	P
Anopheles gambiae s.l.			
	Ifakara C	1.92[1.53, 2.42]	< 0.001
	Ifakara B	1.00[a]	
Culex spp			
	Ifakara C	1.90[1.48, 2.43]	< 0.001
	Ifakara B	1.00[a]	
Mansonia spp			
	Ifakara C	2.30[1.54, 3.36]	< 0.001
	Ifakara B	1.00[a]	

RR = relative rate; CI = confidence interval and [a] = Reference value

Influence of trap design on the abdominal status distribution. The ITT-C caught 73% less blood-fed *An.gambiae s.l.* than ITT-B in the field and none were caught with

ITT-C in the semi-field experiment (Table 3). The observation that 6 fed specimens were caught with ITT-B in the semi-field experiment, even though all mosquitoes released were unfed, confirms that mosquitoes do feed upon users of the latter design. Although the difference in the proportion of blood-fed mosquitoes between the B and C designs in the semi-field system could not be estimated quantitatively using binary logistic regression (Table 5.4.3), they nevertheless differed significantly ($\chi^2 = 6.78$, d.f = 1, P = 0.009).

Table 5.4.3: The influence of trapping method on the proportion of *An. arabiensis* caught in the field and *An. gambiae s.s* recaptured in the semi-field system which was fully or partly blood fed as determined by binary logistic regression.

Experment	Trap Type	Proportionn fed (%)	OR[95% C.I.	P
An.. arabiensis in the field				
	Ifakara C	1.4 (10/703)	0.27[0.12, 0.60]	0.001
	Ifakara B	5.1 (17/336)	1.00[a]	NA
An. gambiae in the semi-field				
	Ifakara C	0.0 (0/190)	NE	NE
	Ifakara B	3.5 (6/171)	NE	NE

NA: Not applicable as this is the reference method.

NE: Not estimable

5.5 Discussion

We demonstrated that modifying the ITT-B improved this prototype beyond our primary target of preventing operator exposure from mosquito bites. The ITT-C sampled twice as many mosquitoes as the ITT-B, which suggests that it may yield mosquito catches more or less equivalent to that of the human landing catches based on previous comparisons of the latter two methods (Sikulu *et al*. 2009; Chapter 3). The reasons for a such improved sensitivity with ITT-C is not obvious but might be explained by increased airflow (Snow, 1987) caused by the 700-mm gap between the two netted chambers. The use of the elastic band tie which tightly extends and smoothens out the entry funnels, might also have contributed to this improved efficiency because it may make it easier for mosquitoes to enter the trap.

The high proportion of blood-fed mosquitoes caught with the ITT-B matches observations in previous studies (Sikulu *et al*. 2009; Chapter 3). The observation that this occurred even in a semi-field enclosure into which only unfed mosquitoes were introduced confirms that persons using this trap are exposed to mosquito bites. This exposure most likely occurs during removal of mosquitoes because of the need to open the long zipper bisecting the protective netting panel of the B design, as has been reported by field workers in previous evaluations (Sikulu *et al*. 2009). The observation that some fully and partially blood-fed mosquitoes from the field are trapped by the ITT-C, which appears to be essentially exposure-free in our semi-field experiment, suggests that these mosquitoes may have already fed when they entered the trap. These occasional specimens may have successful fed nearby and entered the ITT-C looking for either a second blood meal (Tirados *et al*. 2005) or shelter.

A pilot community-based surveillance system using ITT-B in urban Dar es Salaam has already proven to be representative, affordable and effective in term of both mosquito catch and species composition (Sikulu *et al*. 2009). Crucially, it was also found to be three times less expensive than human landing catches per vector mosquito caught (Sikulu *et al*. 2009). The ITT-C appears to have all of these advantages and is more sensitive and protects the users. It may therefore be a useful sampling tool for routine monitoring of adult malaria-transmitting mosquitoes under programmatic conditions, such as those experienced by the Urban Malaria Control Programme of Dar es Salaam (Mukabana *et al*. 2006; Geissbühler *et al*. 2007; Fillinger *et al*. 2008)

Any alternative mosquito sampling tool, apart from being safe and sensitive, must also yield epidemiologically representative estimates of human exposure to mosquito bites and pathogen transmission (Service, 1977). Because the human landing catch technique is still believed to be the most reliable method for estimating the human biting rate (WHO, 1975b; Lines *et al*. 1991; Service, 1993; Mboera, 2005), it may be necessary to validate the ITT-C by comparing it with this gold standard rather than the B design which preceded it. As previously suggested (Chapter 3), we recommend that the ITT-C and other potentially useful methods should ultimately be assessed in comparison with epidemiologic indicators of human infection so that the most meaningful entomologic approaches can be identified.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Further to the work presented in the previous chapters, here is presented a summary of how each objective was addressed, the immediate implications of the results, and remaining knowledge and technology gaps which should be prioritized for future research:

6.1 Insecticide-treated nets against outdoor biting mosquitoes

Understanding of where and when persons are most at risk and identification of high risk groups are useful for targeting interventions to achieve maximum impact. As malaria vector populations increase their preferences for biting outdoors, the personal protection conferred by an ITN will be reduced. However, as demonstrated in chapter 2, the conventional approach which is used to evaluate the appropriateness of ITNs, by simply considering the degree of exophagy of the vector population, underestimates the potential of this proven effective technology (Battarai *et al*. 2007; Fegan *et al*. 2007; Ceesay *et al*. 2008; O'Meara *et al*. 2008; Noor *et al*. 2009; WHO, 2009; Chizema-Kawesha *et al*. 2010; Okiro *et al*. 2010). This is because it neglects the importance of human behaviour as a fundamental determinant of the degree of human exposure to the vector and hence transmission of the pathogen. More realistic evaluations of how the impact of ITNs responds to changing mosquito behaviours also need to consider human behaviours and how these two phenomena overlap in times and space.

Also, the theoretical simulation described in chapter 2 indicates that much greater (three-fold) community suppression of risk of exposure to transmission can be achieved even when slightly more than half of bites occur in time and places when using ITNs is not feasible. This therefore suggests that ITNs should not be deprioritized as a vector control tool simply because locally important vector species are observed to prefer feeding outdoors.

However, as ITNs and IRS become more widespread, human risk of exposure to transmission is increasingly spread across the entire night so that much of it occurs outdoors and before bed time (Russell *et al.* 2011; Bugoro *et al.* 2011). While much of this change arises simply from personal protection while indoors, there is also substantial evidence that the remaining vector populations that survive these measures exhibit behaviours that are quite different to the original populations. This is due to the fact that the indoor biting species (Gillies and Smith, 1960; Gillies and Furlong, 1964; Gillies and DeMeillon, 1968; Taylor, 1975; Bayoh *et al.* 2010; Russell *et al.* 2011; Bugoro *et al.* 2011) and intra-species subpopulations (Molineaux and Gramiccia, 1980) will be more affected, leaving predominantly outdoor-feeding residual populations and subpopulations that maintain transmission. This such modifications of vector population composition and behavior is due to non-uniform exposure to ITNs or IRS and has now been reported several times from across Africa (Gillies and Smith, 1960; Gillies and Furlong, 1964; Gillies and DeMeillon, 1968; Molineaux and Gramiccia, 1980; Bayoh *et al.* 2010; Russell *et al.* 2011) and from the Solomon Islands (Taylor, 1975; Bugoro *et al.* 2011) over the last half century. In Africa, populations of highly anthropophagic, endophagic vector species such as *An. funestus* and *An. gambiae* can be dramatically reduced, typically leaving behind more robust

populations of their more zoophagic, exophagic sibling species. Historically, *An. funestus* has been replaced by *An. rivulorum* and/or *An. parensis* following the implementation of indoor residual spraying of insecticides on atleast three distinct occasions in South Africa, Kenya and Tanzania (Gillies and Smith, 1960; Gillies and Furlong, 1964; Gillies and DeMeillon, 1968). More recently, long term use of ITNs at wide coverage in western Kenya and south eastern Tanzania has resulted in changes in species composition in residual vector populations by progressively diminishing the importance of *An. gambiae s.s.* as the main malaria vector (Bayoh *et al*. 2010; Rusell *et al*. 2011). In the Solomon Islands, historical IRS campaigns appear to have eliminated *An. punctulatus* and *An. koliensis* on some islands but recent programmes combining ITNs and IRS have had little or no impact upon human biting rates by *An. farauti* (Bugoro *et al*. 2011). There is also increasingly evidence that these traditional vector control strategies will have little impact in some parts of Asia where human exposure to mosquito bites predominantly occurs outside houses and before bed time (Van Bortel *et al*. 2010). Outdoor biting of malaria vectors limit the impact of ITNs and IRS, so these front-line indoor-targetted interventions will not be adequate to eliminate malaria in (Griffin *et al*. 2010; Ferguson *et al*. 2010).

As such behaviours limit the effectiveness of these front-line intervention measures, and may even threaten to attenuate them as vector populations adapt to avoid them, complementary tools which target mosquitoes outside of houses and outside of sleeping hours will therefore be required to sustain and go beyond existing levels of malaria control and achieve elimination (Griffin *et al.* 2010; Ferguson *et al.* 2010). While possible options for such complementary measures include repellents, odor-baited traps, autodisseminated larvicides, insecticide-treated livestock, toxic sugar

baits and even genetically modified mosquitoes (Ferguson *et al.*, 2010), larval source management through either environmental management (Castro *et al.* 2004; Castro *et al.* 2009; Castro *et al.* 2010) or regular larvicide application (Geissbühler *et al.*, 2009) may have particular and immediate potential in urban Dar es Salaam.

Larval source management (Killeen *et al*. 2002a; Killeen 2003; Gu and Novak, 2006; Gu *et al*. 2006) is likely to be an effective complementary intervention for tackling early-biting and outdoor-biting mosquitoes in urban settings such as Dar es Salaam (Castro *et al*. 2004; Castro *et al*. 2009; Geissbühler *et al*. 2009; Castro *et al*. 2010) where larval habitats are relatively few, accessible and this area experiences low transmission intensity (Killeen *et al*. 2002a; Killeen *et al*. 2002; Robert *et al*. 2003). Also, because of the high population density of people in towns and cities, larval source management may be particularly cost-effective (Worrall, 2007) because more inhabitants are protected per square kilometer covered. However, larval source management through regular application of larvicides to aquatic habitats is intrinsically reliant upon careful, continuous performance management of large implementation teams based on feedback from monitoring systems for adult mosquito densities that operate at the high spatial and temporal resolution that ultimately determine success or failure on a day-to-day basis (Fillinger *et al.* 2008).

Furthermore, the need for practical mosquito traps for routine surveillance of mosquito populations and malaria transmission is broadly applicable and is by no means restricted to particular local scale programmes with specialist needs such as the UMCP in Dar es Salaam. As countries scales up the use of ITNs to achieve universal coverage (Killeen *et al*. 2007; WHO, 2007), it is essential that the density, composition and

transmission capacity of mosquito populations are monitored longitudinally as part and parcel of national surveillance systems in order to verify, quantify and optimize the impact of intervention programmes. Unfortunately, routine entomological transmission monitoring has not yet been implemented on national scales because of the limitations of existing entomological surveillance tools.

6.2 Efficacy and effectiveness of new surveillance methods

Efficacy evaluation entails experiments in which a technology or method is optimally deployed under well-controlled research conditions. In contrast, an effectiveness trial of a given technology or procedure is conducted by minimizing the influence of the researcher on how it is applied in practice by systems and personnel that are representative of sustainable implementation conditions. Here the evidence for efficacy of the ITT presented in chapters 3, 4 and 5 is discussed in the context of recent complementary effectiveness evaluations and the entomologic surveillance needs of vector control programmes for malaria and other mosquito-borne pathogens.

In this study a new tent-style trapping system called Ifakara tent traps (ITT) has been developed and the C design, in particular, represents a significant advance. This tent trap proven relatively efficacious in term of numbers, species composition and age distribution of *Anopheles gambiae s.l.*, by field experiments under conditions of both high and low mosquito densities. While over 96% of successfully amplified specimens of *Anophele gambiae s.l.* caught with ITT in a rural setting were *An. arabiensis*, the reverse was true for in urban Dar es Salaam where the vast majority 99% were *An. gambiae s.s.*. Furthermore, the sibling species composition of samples of this species complex caught with ITT in both the rural and urban settings proved to be

indistinguishable from those obtained with HLC. This suggests that these traps not only sample both *An. arabiensis* and *An. gambiae s.s.* populations but do so with very similar sensitivity (Chapter 3). Note that the utility of the ITT is not limited to sampling these sibling species of *An. gambiae s.l.* only, but also samples other species of mosquitoes including, *Culex spp, Mansonia spp*, *An. funestus*, and *An. ziemanni*, although the latter is undersampled when compared with indoor human landing catches. Like most traps (Torr *et al.* 2008), its sensitivity clearly is variable and is affected by both context and vector species (Chapter 3 to 5) so it is by no means a tool that can be readily applied to any situation in a ‘‘one-size-fits-all’’ manner. The true full potential of ITT will have to be assessed on a case- by-case basis. Nevertheless, it is currently the only feasible means to survey malaria vectors in the urban context it was developed for and it may well have a place in other surveillance systems in the future.

The parity status of *An.gambiae s.l.* sampled with ITT did not differ significantly with those obtained by HLC (Chapter 3), suggesting that it represents a reasonable option for sampling mosquitoes to determine the age distribution and infection status of the host-seeking vectors. Unfortunately, ITT was not tested to determine whether it representatively samples infected mosquitoes so such an evaluation is recommended for future studies.

The relative sensitivity of ITT increased as vector density decreased in urban Dar es Salaam and exceeded that of HLC at the lowest densities which is fortunate when one considers a future which malaria vector densities are expected to drop across the world

as vector control programmes are increasingly successful. Of the other alternatives to HLC that were evaluated, none proved to have any useful level of sensitivity in the context of urban Dar es Salaam. Resting boxes exhibited very poor sensitivity and proved quite impractical during both efficacy and effectiveness trials (Sikulu *et al*. 2009). Window exit traps also showed very poor sensitivity in this urban setting.

Based on these encouraging efficacy estimates from rural Kilombero and urban Dar es Salaam, the ITT is now being evaluated in Kenya, Zambia and Indonesia. Preliminary results from lowland rural Kenya are very encouraging for sampling both *An. arabiensis* and *An. gambiae* populations (Gimnig *et al*. Personal Communication) and the same is true for *An. funestus* in rural, lowland Zambia. In contrast, very poor sensitivity has been observed for *An. quadriannulatus* in the same Zambian setting (Sikaala *et al*. Personal Communication) and for all malaria vector species present in three diverse settings in Indonesia (Paraban *et al*. Personal Communication). The cause or causes for the ITT having consistently lower sensitivity than HLC, or even having negligible sensitivity in come cases, remain unclear. It may be that mosquitoes are less inclined to enter the trap than to attack a completely exposed host or that they leave after entering, or indeed both.

A small-scale pilot community-based surveillance system using ITT, HLC and Resting boxes (Sikulu *et al*. 2009) was conducted and evaluated in 12 of the 15 wards comprising the UMCP study area, which cover a surface area of 55 km^2 with a total population of 610,000 people (Fillinger *et al*. 2008). Community-based use of the ITT with no supervision from the research team proved the effectiveness of this new trap in

terms of number and species compositions of mosquitoes caught. Perhaps more importantly, it allowed more intensive sampling and was found to be more cost-effective than HLC in terms of cost per mosquito caught (Sikulu *et al*. 2009). The clear difference in the overall costs of ITT compared with HLC arises from the fact that surveillance of mosquitoes with ITT does not requires supervision while the HLC is so arduous that it inevitably needs intensive spot checks in the middle of the night for the resulting data to be reliable and meaningful.

The ITT-C is currently the only technique used for routine adult mosquito surveillance of UMCP in Dar es Salaam (Mukabana *et al*. 2006; Fillinger *et al*. 2008; Chaki *et al*. 2009) where it has been scaled-up to cover 620 sentinel sites distributed across 30 wards which cover an area of approximately 115 km^2 (Chaki *et al*. Personal Communication). This represents a spatial resolution of one trap-night sample per 0.2 km^2 every month and 0.8 km^2 every week. Such intensive and extensive monitoring of adult mosquito in response to larviciding programme is of critical important in identifying coverage gaps. These gaps normally occur within a narrow spatial resolution. In addition to this, the distribution of adult mosquito sampling points should more or less match to the assigned target areas of individual persons responsible for application of larvicides so as to assess their individual personal performance. This is due to the fact that, apart the efficacy of larvicides, the success of larviciding relies more on personal sensitivity of detection and treatment of all potential larval habitat (Chaki *et al*. 2009). Encouragingly, preliminary observations obtained from quality assurance surveys of this new system demonstrate that surveillance application of ITT by community-based staff without supervision has comparable sensitivity to that of the same tool in the hands of the research team for

catching both *An. gambiae s.l.* and *Culex* spp. This suggests that the ITT could be useful for intensive, extensive, sustained malaria vector surveillance in large scale programmes. Extensive, community-based surveillance with ITT has now been shown to have utility for identifying of malaria hotspot areas, with sites where even a single malaria mosquitoes was caught having significantly different age-prevalence profiles for human infection, characterised by higher prevalence rates early in life that then decline with age as a result of increasing exposure and immunity (Chaki *et al.*, Personal Communication). This implies that such surveillance with the ITT can be useful for mapping malaria hotspot areas and might therefore enable targeted control (Woolhouse *et al.* 1997; Carter *et al.* 2000) with supplementary measures such as larval control. It is also encouraging that it is now being evaluated alongside CDC-LT in term of its effectiveness when used through community-based systems on a pilot national scale as a means to monitor malaria and filariasis transmission in sentinel sampling clusters distributed across mainland Tanzania.

Despite all the advantages of the ITT, it nevertheless has important limitations as an entomological and epidemiological surveillance tool. It is impractical for indoor use and therefore unsuitable for surveying the proportion of human exposure to mosquito bites that occurs indoors. Unfortunately, monitoring trends in mosquito behaviours such as endophagy versus exophagy is still only possible with HLC (Charlwood and Graves, 1987; Pates and Curtis, 2005; Killeen *et al*. 2006b; Oyewole and Awolola, 2006; Geissbühler *et al*. 2007). With regard to mosquito behavioural changes and how this influences both transmission intensity and intervention efficacy, an alternative to

HLC for simultaneously and representatively measuring indoor and outdoor mosquito biting densities is urgently needed.

Furthermore, informal discussions with the ITT operators revealed that the trap is too heavy to be moved from one sampling site to another by one person. In the case of the UMCP, this problem was solved by supplying the operators with bicycles but reports from the highlands of Kenya indicate that this is not practical in settings with steep hills (Drakelely *et al.*, Personal Communication). Also, even the protective precautions suggested in the appendix may not fully prevent rain from entering the trap and poor compliance by operators has been reported from both rural, highland Kenya (Drakelely *et al*., Personal Communication) and urban Dar es Salaam (Chaki *et al.*, Personal Communication), with this problem being particularly notable in wet season in the latter case.

The fact that the trap almost exclusively catches unfed, presumably host-seeking, female mosquitoes can be both strength and weakness: while this is ideal for surveying human-biting mosquito densities, it also renders this trap unsuitable for surveying mosquitoes in other physiological states, particularly those which are gravid. ITT has also been found useful as a tool for assessment of host choice preferences of malaria vectors without depending upon catching already blood-engorged mosquitoes but rather offers host-seeking mosquitoes an equal choice between human and calf baits (Majambere *et al*., Personal Communication). Furthermore, recent advances in molecular analytical methodology now make it possible to identify the source of previous, fully-digested bloodmeals from host-seeking mosquitoes that otherwise

appear unfed (Fornadel and Norris, 2008) so the ITT may even be useful for surveying human blood indices as they occur in the field, even though very few mosquitoes with obvious blood meals are caught.

Despite these limitations, the trap is generally well ventilated, safe, sensitive and accepted by operators in Dar es Salaam. Ultimately, the goal of developing a technology that makes sensitive, affordable, exposure-free vector surveillance possible for the Dar es Salaam UMCP has been achieved. Beyond the Dar es Salaam UMCP, the ITT may also have potential for the routine monitoring of adult malaria-transmitting mosquitoes and vectors of other human diseases under programmatic conditions at national level in Tanzania and possibly in other African countries.

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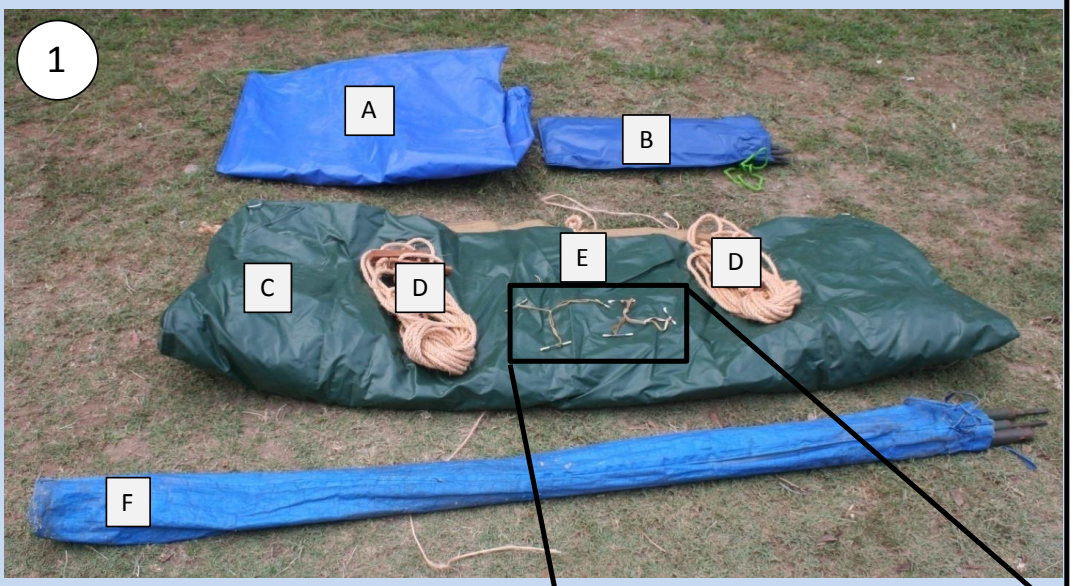
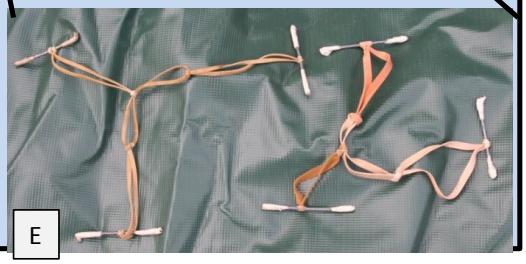
APPENDICES

Appendix 1: How to set up the Ifakara Tent Trap design C



Step 1: Open the trap bag (A) and make sure it contains a full set of 14 steel pegs (B), the main trap body (C), two apex guy ropes (D), two three-way elastic ties (E) and three interlocking metal bars (F)



Step 2: Lay out the trap body in a
Place with at least 4 meters of space on all sides and pin securely to the ground by driving the steel pegs through the metal rings on each corner. Make sure the metal pegs are driven into the ground at an angle facing away from the tent. Also make sure the pegs stretch the floor of the tent so that it is straight and flat.

Step 3: Insert the crossbar and then both vertical support bars. Erect the trap by raising the vertical bars which are wedged into the ground at the centre of the short ends of the trap. Then tie the trap body to the vertical support bars using the two pairs of laces on each end.

Step 4: Secure the 6 ropes by driving 6 steel pegs into the ground. At this stage, leave the guy ropes a little loose so that the trap does not get pulled and distorted to any particular side. Leave plenty of opportunity for tightening by leaving the wooden brace half way up the rope or less. Start with the corner ropes which should be placed at a 20 degree angle to ensure maximum stretching of the trap one tightened.

Step 5: Attach both apex guy ropes to the top of the vertical support pars using the wooden socket and peg out the ropes at an angle of 30 to 45 degrees to maximize the taughtness and balance of the trap.

Step 6: Tighten all the guy ropes in a balanced fashion by pulling through the wooden braces. Adjust so that that trap is as evenly stretched , balanced and taught as possible.

Step 7: Insert the elastic ties into the funnel O-rings through the zipper in the trap chamber and close the zipper afterwards. Draw the two trap chambers together and secure by tying the laces together. Ensure that the cotton sleeve is tied, zip the door shut and relax inside the trap until the following morning, at which stage mosquitoes in the trap chamber can be aspirated out through the sleeve .

Step 8: In case of rainfall, cover the trap roof with a plastic sheet which drops down to the level of the top quarter of the entry funnel. This prevents rain from entering the trap. Tie each edge of the plastic sheet to the guy rope with string to stop the sheet from being blown by high wind.