Sampling malaria vectors and other mosquitoes with the Ifakara tent trap and the standardized resting boxes in urban Dar es Salaam

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Declaration

I declare that this is my original work and has not been submitted for a degree in any other university.

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Dedication

I dedicate this thesis to my dad, Stephen Sikulu, my mum, Esther Sikulu and to my brothers and sisters who gave me an all round support and who always desired to see me excel in the academic field.

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Abbreviations and Acronyms

CDC-LT	Center's for Disease Control light trap
CORP	Community Owned Resource persons
EIR	Entomological inoculation rate
ELISA	Enzyme linked immunosorbent assay
GEE	Generalized estimating equations
HLC	Human landing catch
ITN	Insecticide treated nets
ITT	Ifakara tent trap
PCR	Polymerase chain reaction
PSC	Pyrethrum spray catches
s.l.	sensu lato
SRB	Standardized resting boxes
s.s	sensu stricto
TCU	Ten cell unit
UMCP	Urban Malaria Control Programme
WHO	World Health Organization

Abstract

The most reliable existing means to measure human biting rate is the human landing catch (HLC). Nonetheless, the HLC faces substantial limitations which necessitate development of alternative techniques to replace it. For example, it raises major ethical concerns because of the necessity to expose humans to vectors of malaria and a variety of other pathogens. This study developed a community-based cost-effective protocol for sampling malaria vectors and other mosquitoes. The protocol was used to determine the effectiveness of two alternative sampling methods, namely the Ifakara Tent Trap (ITT) and the Standardized Resting Boxes (SRB) in terms of the number of mosquitoes caught by each relative to the rigorously controlled HLC. Mosquitoes were collected once and three times every week by the HLC and the alternative methods respectively. Overall, the ITT, HLC and SRB caught 44,848 mosquitoes. The ITT, HLC and SRB caught 168, 143 and 46 *An. gambiae s.l.* as well as 26,315, 13,258 and 4,791 *Culex* species respectively. Regardless of the species sampled, the ITT was five times cheaper than the HLC per mosquito caught. A significant correlation between the numbers caught by HLC and ITT was observed for *An. gambiae s.l.* ($P<0.001$) and for *Culex* species ($P=0.003$). By comparison, there was no significant correlation between the catches with HLC and those of the SRB for *An. gambiae s.l.* ($P=0.195$). Neither ITT nor SRB exhibited any obvious density dependence for sampling the two species. This evaluation suggests that our protocol for using the ITT under programmatic condition is affordable and effective. However, it is recommended that the trap be evaluated further under conditions of routine surveillance at a full scale to fully establish the true effectiveness of this approach. On the other hand, the standardized resting boxes exhibited poor sensitivity to both species and are not recommended in this kind of setting.

CHAPTER 1

Introduction and Literature review

1.1 Malaria as a global burden

Malaria is a vector borne disease caused by single-celled parasites of the genus *Plasmodium* (Beier, 1998). Clinical cases of malaria are characterized by chills, headache, nausea, and periodic bouts of intense fever. It is transmitted from person to person by the infectious bite of female mosquitoes from the genus *Anopheles*. In Africa, the principal malaria vectors *An. gambiae* and *An. funestus,* are mainly anthropophagic, meaning that they prefer to obtain their blood meals from humans (Gillies & DeMeillon, 1968).

The current estimate from the World Health Organization (WHO) indicates that 247 million malaria cases and more than 880,000 deaths occurred in the year 2006 in which Africa alone represented 91 percent of the total deaths (WHO, 2008). The millions of reported cases and thousands of deaths caused by this disease are responsible for an estimated average loss of 1.3% of economic growth annually particularly in sub Saharan Africa (Sachs & Malaney, 2002). Although the disease poses a serious public health and economic threat globally, transmission is limited almost exclusively to developing countries, particularly in Sub-Saharan Africa and Southern Asia (Roll Back Malaria, 2005).

The United Republic of Tanzania is among the five countries listed in the 2008 WHO Malaria Report that comprised 50% of the total malaria cases in Africa. Others mentioned in the report are; Kenya, Nigeria, Democratic Republic of Congo and Ethiopia (WHO, 2008). Malaria has remained a major public health concern in Tanzania. Each year, the figures are estimated to be between 14 -18 million cases with 100,000-125,000 deaths. Of those deaths, 70,000- 80,000 occur in children less than 5 years of age (WHO-UNICEF, 2005).

1.2 Epidemiology of rural and urban malaria

The most direct index of malaria transmission intensity is the Entomological Inoculation Rate (EIR) defined as the frequency with which people are bitten by infective mosquitoes (Beier, *et al.,* 1999; Smith *et al.,* 2001) and measured as a product of estimates of the human biting rate and the sporozoite prevalence. More importantly, although it is represented as a function of the proportions of infected humans (Smith & McKenzie, 2004), it is affected mostly by mosquito emergence rate and their life time transmission potential than the infection among the human population (Killeen *et al.,* 2000). Also, it is shown that EIR directly relates to population density. Since population size is the denominator, higher human population densities in urban areas may reduce human biting rate due to high human population to mosquito population ratio (Killeen *et al.,* 2000; Smith *et al.,* 2004). Others include, increased pollution of breeding habitats, avoidance behaviour of mosquitoes due to presence of Insecticide treated nets (ITNs), window screens e.tc. in urban areas (Robert *et al.,* 2003).

It has been shown that rapid and unprecedented urbanization characterized by population increase with declining economies might have a profound implication for the epidemiology and control of malaria because the proportion of the African population living in urban areas is increasing (Keiser *et al.,* 2004). Currently, 24.6% (200 million people) of the African population are living in urban areas where they are at risk of contracting the disease and more than 50% of the overall African population will live in urban areas by the year 2030 (UN, 2002). Therefore, any progress directed towards control of malaria in the urban context will have considerable importance in this increasingly important population.

1.2.1 The history of urban malaria control

In Dar es Salaam, the history of urban malaria control dates back over 100 years. Urban Malaria Control Programme (UMCP) was a malaria control program that operated from 1988 to 1996, supported by, a bilateral agreement between the government of Japan and Tanzania. Its overall goal was to reduce malaria prevalence to the lowest possible level by encouraging the community to use personal protection measures and to improve their local environment. Later in 1992, when the WHO presented its global malaria control strategy, the UMCP expanded its objectives in accordance to WHO directives and included more integrated activities, this time including vector control (Castro *et al.,* 2004).

A new UMCP has been set up under the management of the City Council of Dar es Salaam, which operates primarily at the grassroots level through street health committees, based on a community-based system originally developed by one of the three municipal councils (Ilala) of the city. The program was expanded to 5 wards in each of the three municipalities (Kinondoni, Ilala and Temeke) as a community based pilot-scale program (Mukabana *et al.,* 2006). In 2004, the UMCP recruited and provided preliminary training to teams of Community Owned Resource Persons (CORPs) who performed weekly surveys of mosquito breeding habitats (Vanek *et al.,* 2006). Operational larviciding in three selected wards with *Bacillus thuringiensis var Israelensis* commenced in 2006 (Fillinger *et al.,* 2008). Currently, the UMCP has four major activities going on in all the three municipalities, namely larval control, larval surveillance, adult mosquito surveillance and household parasitological surveys. The overall goal is to strengthen the ability of the municipalities to deliver interventions already prioritized by the national control program and to compliment these with further interventions focusing on larval control (Fillinger *et al.,* 2008; Geissbühler *et al.,* 2008; Govella *et al.,* 2008; Mukabana *et al.,* 2006).

1.3 Monitoring and evaluation of malaria vectors

Despite the challenges that malaria presents, there are effective technologies to control and cure it. Many countries are renewing efforts to control the disease with an increasing global support enabling large-scale and sustained programmes to prevent it (Roll Back malaria, 2005). Preventing malaria by controlling mosquitoes is central to alleviating disease burden. For example, in Dar es Salaam, larviciding and environmental management have proven to be effective as vector control interventions (Castro *et al.,* 2004; Fillinger *et al.,* 2008; Mukabana *et al.,* 2006).

Monitoring and evaluation of malaria control interventions and their associated impact on malaria burden is essential for understanding progress, successes and challenges in any malaria control effort. In order to accurately estimate and manage the burden of disease and measure the trends in malaria transmission intensity, better survey instruments and methods are needed. The success of any vector control intervention results from the reduction of the rate of exposure to infection determined by trapping mosquitoes. Therefore, relatively accurate, safe, sensitive and reliable methods of vector collection are needed to monitor and evaluate malaria transmission (Service, 1977).

1.4 An overview of commonly used sampling techniques

1.4.1 The Human Landing Catch

Traditionally, entomological surveys of malaria vectors and transmission intensity have relied upon human bait as an attractant for the adult anopheline mosquitoes. The most widely accepted gold standard method is the HLC (figure 1) where people wait, often all night, to catch the mosquitoes that bite them. The other version of HLC involves 1-3 people acting both as baits and catchers as well as drop nets that descend at intervals to enclose the mosquitoes attracted to a stationary bait (Service, 1977). Mosquitoes caught by HLC are usually collected in the act of getting a blood meal from a bare human leg and feet so it is a very direct measure of the biting rate of the anthropophagic mosquitoes (Davis *et al.,* 1995). Such samples are reasonably assumed to be representative of the entire population of mosquitoes responsible for malaria transmission.

Nonetheless, the HLC faces substantial limitations which necessitate development of alternative techniques to replace it. It is difficult to supervise, unreliable, expensive, labor intensive and requires skillful catchers. It is also not representative of true human exposure because it is usually implemented by adult males who remain awake and seated all night. However, the most serious problem arises when human participants are at an increased risk of malaria infection (Service, 1977).

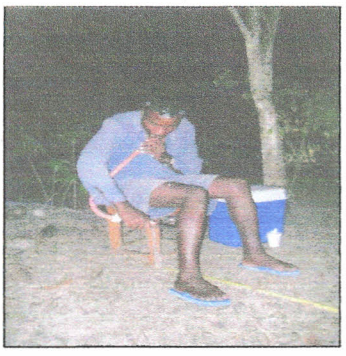

Figure 1: Photograph of HLC at one of the sampling locations in Dar es Salaam

1.4.2 Alternatives to the Human Landing Catch

Many other methods e.g. the CDC-LT, and the Mbita trap have been employed and evaluated as alternatives to HLC with varying degrees of success (Mathenge *et al.,* 2002; Reinert, 1989). Also, animals are sometimes employed as baits but these neither provide representative samples of the mosquitoes humans are exposed to, nor do they sample the highly anthropophagic species which are the most efficient vectors of malaria.

Nevertheless before any alternative tool to HLC as a means to evaluate human biting rate is implemented, the relative sensitivity with which mosquitoes are captured by the methods need to be quantified and calibrated against the HLC so that the data collected from it can be translated directly into estimates of human biting rate (Service, 1977).

1.4.2.1 The New Jersey Light Trap

The New Jersey Light Trap was developed in 1942 as a predecessor of CDC-LT. It provided the mosquito control community with a mechanical device capable of sampling host seeking mosquitoes. The trap was designed with the hope of maximizing adult mosquito catches while minimizing human labour and bias. At present, this trap remains a useful tool in mosquito surveillance but its design places certain restrictions on its use. Conventional usage requires electric current to power a trap that is expected to operate continuously at a single location for long periods of time. As a result the trap proved to be inefficient as a means to obtain rapid samples of mosquito populations, particularly in areas where electric current is difficult to access. Over the years, a variety of modifications intended to improve portability and enable live capture of specimens have been developed based upon this original design (Reinert, 1989).

1.4.2.2 The Centers for Disease Control Light Trap

In 1962, the CDC-LT was introduced specifically for arbovirus surveillance and other mosquito investigations which require short-term sampling in a given location. This trap attracts mosquitoes with white light and captures them with the down draft into a netting capture bag produced by a motorized fan attached to a six volt battery. Later on, the CDC-LT was combined with carbon dioxide in the form of dry ice. Although the dry ice baited CDC-LT is an efficient and reliable, surveillance tool for the surveillance specialist, it requires daily rebaiting with dry ice as well as electrical power and is therefore impractical in many contexts, particularly in the developing world (Reinert, 1989).

Since then, the CDC-LT has been tested in a number of efficacy trials in different eco-zones for sampling malaria vectors and other mosquitoes, sometimes with conflicting conclusions. For example, in The Gambia, a study concluded that CDC-LT could only be used for assessing the relative densities of different mosquito species at night but they could not be relied upon on assessing human biting rates (Odetoyinbo, 1969).

Later, it was found out that the mosquito sampling efficiency of the CDC-LT for measuring human biting rates of anthropophagic malaria vectors was greatly enhanced when they were placed next to untreated bed net occupied by a human bait (Garrett-Jones, 1975). This has consistently proven very specific for sampling host-seeking mosquitoes since most of the mosquitoes caught are unfed.

In several other studies, CDC-LT catches have been compared to HLC. In Tanzania, when light traps were hung beside an occupied untreated bed net they provided an efficient and unbiased estimate of human biting rate of *An. gambiae s.l.* populations (Davis et al., 1995; Lines *et al.*, 1991) A similar study in Kilifi on the coast of Kenya, concluded that their

efficiency was lower at a higher mosquito density (Mbogo *et al.,* 1993). It should be noted, however, that the density dependence observed in Kilifi may well have resulted from a mathematical artifact caused by artificially adding one to non-zero catches so that they can be logarithmically transformed for parametric analysis (Smith, 1995). Elsewhere in Kenya the *An. gambiae s.s* collections by the CDC-LT placed beside occupied nets did not differ from those of HLC but the two sampling methods differed significantly for *An. arabiensis* collections (Githeko *et al.,* 1994). Nonetheless, it has been shown that light traps can provide a reliable estimate of biting rates in communities where people sleep under treated bed nets (Killeen et al., 2007; Magbity *et al.,* 2002). In the Wosera area of Papua New Guinea, the mosquito sampling efficiency of CDC-LT hung adjacent to mosquito nets differed significantly from both indoor and outdoor human-bait collections with Anopheline species less frequent in the light traps than in the HLC. However, the light traps sampled older *An. punctulatus* and *An. farauti s.l.* more efficiently since the sporozoite positivity rates for both *Plasmodium falciparum* and *Plasmodium vivax* were significantly higher in the light trap collections than in either indoor or outdoor HLC (Hii *et al.,* 2000).

1.4.2.4 Exposure-free bed net trap (Mbita trap)

The exposure-free bed net trap known as the Mbita trap was developed for sampling host-seeking Afrotropical malaria vectors. The bed net trap does not expose people to potentially infectious mosquito bites and operates without electrical power or skilled personnel. This trap was developed to catch host-seeking mosquitoes (Mathenge *et al.,* 2002), specifically African malaria vectors.

A study conducted in west Kenya in both field and semi-field conditions, reported that catches of both the Mbita trap and the CDC-LT were directly proportional to HLC, regardless of mosquito density (Mathenge *et al.,* 2005; Mathenge *et al.,* 2004).The sporozoite

prevalence observed in samples caught with all three techniques did not differ significantly for either *An. gambiae s.l.* or *An. funestus.* It was concluded that the Mbita trap might be a promising tool for sampling malaria vector populations in this kind of setting (Mathenge *et al.,* 2005; Mathenge *et al.,* 2004). However, it has since been found to have very poor sensitivity in a range of settings that include the highlands of Madagascar (Laganier *et al.,* 2003), two rural sites in northern and southern Tanzania (Braimah *et al.,* 2005; Okumu *et al.,* 2008) and urban Dar es salaam (Fillinger *et al.,* 2008).

1.4.2.5. The Clay Pots

Clay pots have recently been evaluated for sampling outdoor-resting *An. gambiae, An. funestus, An. arabiensis* and *Culex* species in Western Kenya (Odiere *et al.,* 2007). The sampling efficiency of the pots was found to be better than pit shelters and equivalent to Colombian curtains, exit traps and the indoor pyrethrum spray samples. Although the clay pots proved to be effective, they also provided resting places for other animals like scorpions, lizards, spiders some of which are potential mosquito predators (Nelson & Jackson, 2006). In addition the proportion of adults resting in this man made shelters depend on the availability of alternative resting sites which varies according to location and changes seasonally (Service, 1977).

1.4.2.6 The Resting Boxes

Resting boxes have been similarly used to sample mosquitoes since the early days of malaria control when it was learned that a number of important malaria vectors congregated in diurnal resting places (Wayne, 1989). Boxes are generally placed on the ground with the open facing west to minimize the influence of direct sunlight during the early part of the day. In well shaded areas, the exact direction of the open end becomes less important (Wayne, 1989). It has been shown that female mosquitoes generally prefer larger and natural resting sites

over smaller and artificial resting sites, respectively (Burkett *et al.,* 2008) and that the numbers of mosquitoes collected do not correlate with bait collections in most cases (Kay, 1982).

In a separate study, cloth resting boxes or wicker resting baskets with a ceiling nets were utilized for sampling indoor resting malaria vectors in both field and green house conditions. These boxes performed better than hand collections but were 1/3 times as good as the Pyrethrum spray catches (PSR) (Harbison *et al.,* 2006). Such boxes can be used to recover indoor- resting mosquitoes where PSR could be expensive. However, their efficiency for sampling outdoor-resting samples of mosquitoes is yet to be determined.

1.4.2.7 The Ifakara Tent Trap (ITT)

A novel tent trap known as the ITT (figure 2) was developed to sample host-seeking mosquitoes. It comprises a rectangular canvas box containing six funnel-like entrances for mosquitoes and inner small apertures tilted to an angle so that mosquitoes fly upward into the trap. Such baffled entrance structures increases the probability that mosquitoes do not exit once inside traps. A panel of durable, Teflon-coated woven fibreglass netting is placed between the entry funnels and the bait host, allowing the human participant to sleep while protected from mosquito bites. Bisecting the protective netting panel, a zip enables the participant to aspirate mosquitoes while inside the trap. The trap floor is made of thick polyvinylchloride sheeting, which protects against rough substrates and surface water. (Anderson *et al.,* 2000). Two designs of this trap were developed iteratively in Lupiro village where very high densities of *An. gambiae s.l.* allowed rapid assessment through a series of stepwise modifications (Govella *et al.,* 2008). Earlier studies on the sampling efficacy of the ITT relative to HLC for *An. gambiae* complex were carried out in two different epidemiological and ecological settings in Tanzania: The Kilombero valley in the South East

of the country and urban Dar es Salaam on the Indian Ocean coast. In both settings the trap was found to be reasonably efficacious (Govella *et al.*, 2008).

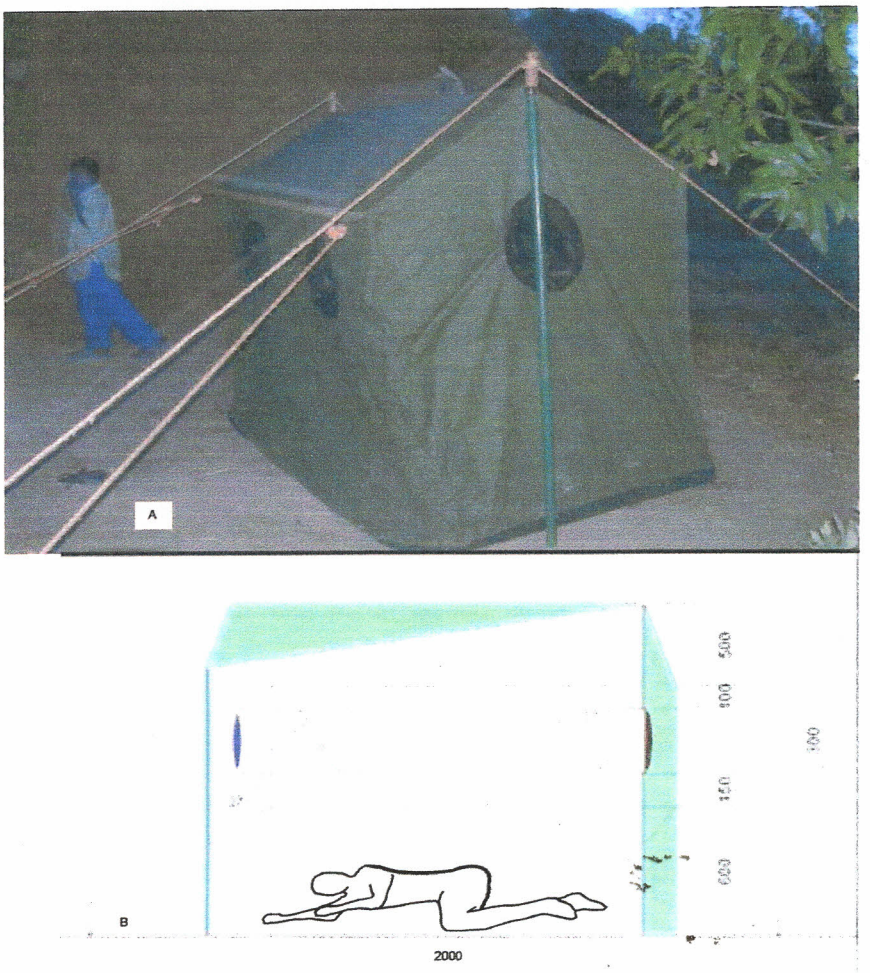

Figure 2: Photograph and diagram of the Ifakara tent trap. All measurements are presented in millimeters. The Trap works by enclosing a human operator but under unexposed conditions to malaria vectors as shown above.

1.5 Justification and significance of the study

The HLC technique is the most direct way of estimating human biting rate because mosquitoes are caught directly in the act of getting a blood meal. Nonetheless, apart from raising ethical concerns because of the necessity to expose humans to malaria transmitting mosquitoes, the technique is also prohibitively difficult to supervise, expensive and labour intensive. Alternative methods such as the CDC-LT and the Mbita trap have been evaluated as candidates to replace HLC as a surveillance tool in Dar es Salaam but both proved to have very low sensitivity which renders them essentially useless as a tool in this context where malaria vector mosquito densities are so low. (Fillinger *et al.,* 2008; Govella *et al.,* 2008) therefore, the HLC has remained the technique relied upon by UMCP in urban Dar es Salaam for monitoring and evaluation of regular larvicide application upon malaria transmission.

A new exposure-free tent trap called the ITT, has been developed for outdoor mosquito sampling in both rural and urban settings in Tanzania (Govella *et al.,* 2008). Like the Mbita trap, this trap does not expose people to potentially infectious mosquito bites and operates all night long without skilled personnel. Earlier studies on the efficacy of this trap were carried out at Kilombero valley, South East Tanzania and also in urban Dar es Salaam (Govella *et al.,* 2008). Field studies in both areas have established that the new trap is efficacious and could represent a viable alternative to HLC.

Standardized boxes are commonly used for sampling resting mosquitoes. Even though it is shown that catches of the boxes do not usually correlate with human bait collections (Kay, 1982), they have been shown else where in Kenya to be better than hand collections (Herbison et al., 2006).

This study therefore evaluated SRB's and ITT's potential for assessing vector population densities in Dar es Salaam relative to the human landing collections in an effort to replace the human landing catch.

1.6 Objectives

1.6.1 Broad objective

The study evaluated the sensitivity and specificity of the Ifakara tent trap and the Standardized resting boxes as alternative methods for sampling host-seeking mosquitoes under field conditions in Urban Dar es salaam.

1.6.2 Specific objectives

1. To develop a cost-effective protocol that will enable the community members of urban Dar es Salaam to trap record and submit samples of wild malaria vectors and other mosquitoes.
2. To measure the sensitivity of the ITT and SRB as tools for sampling mosquitoes relative to that of the HLC.
3. To compare the species composition, abdominal condition and infection status of mosquitoes caught using the ITT, HLC and SRB.

1.7 Research Hypothesis

There is no difference in size or composition between mosquito samples caught by the HLC and those caught by ITT and SRB.

CHAPTER 2

Materials and methods

2.1 Description of the study area

The study was carried out in Dar es Salaam where many other studies on malaria vectors had been carried out in the past (Fillinger *et al.,* 2008; Geissbühler *et al.,* 2007; Geissbühler *et al.,* 2008; Govella *et al.,* 2008; Mukabana *et al.,* 2006; Sattler, 2003; Sattler *et al.,* 2005; Vanek *et al.,* 2006). Dar es Salaam is a coastal city in Tanzania (6'46' S Latitude and 39'14 E Longitude) with approximately 2.7 million inhabitants living in an administrative region which covers a total area of 1400km^2. The city is divided into three municipalities; Temeke, Ilala and Kinondoni which collectively comprise 73 wards. Each ward is further subdivided into streets known as *mitaa (*singular *mtaa*) which typically comprise between 20 and 100 *mashina (*singular *shina*) or Ten Cell Unit (TCU). The TCU in principle, comprises a cluster of 10 houses with an elected representative known as *mjumbe* although in practice most TCUs include 20-30 houses and some may even exceed 100 (Dongus *et al.,* 2007). This study was based within the project area of the ongoing Urban Malaria Control Programme implemented by the Dar es Salaam City Council (Fillinger *et al.,* 2008; Mukabana *et al.,* 2006; Vanek *et al.,* 2006). The main project area includes five wards from each municipality with a total of 67 *mitaa*. Overall, this study area covers an area of 55 km^2 with a total population of 609,514 people (National Bureau of Statistics Tanzania profile, 2003). All houses involved in the ITT, SRB and the HLC experiments described here are located in these 15 wards (figure 4).The climate is warm and tropical, with temperatures averaging 27°C (80°F) and rainfall varying from 750 to 1,400 mm per year. Dar es Salaam has two rainy seasons; the short rains from late-October to early-December and the long rains from March to June. During the dry season temperatures often exceed 35°C.

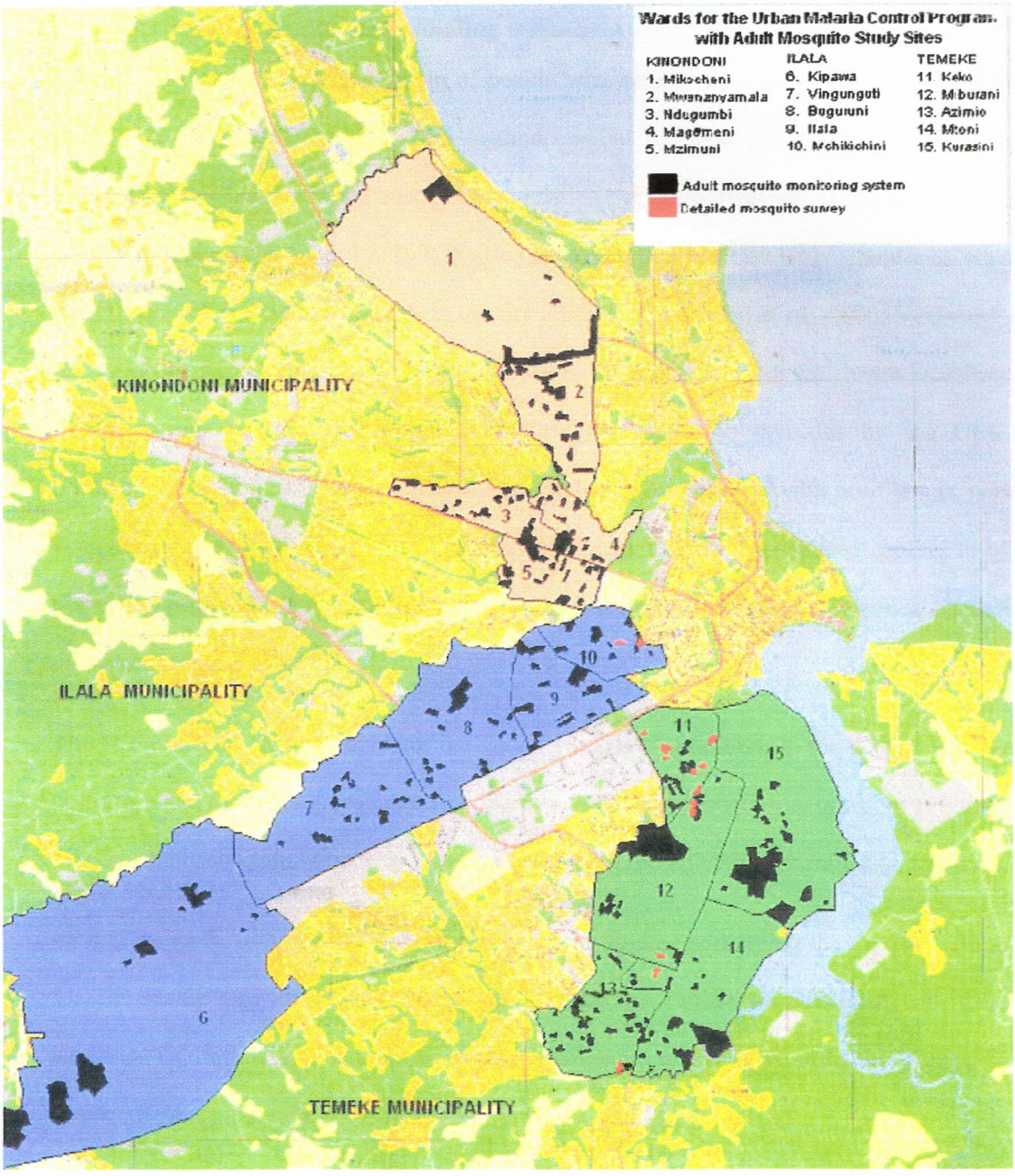

Figure 3: Map showing the 15 wards for urban malaria control program with the adult mosquito surveillance study sites. This particular study was carried out in 12 wards with the exception of Vingunguti, Keko and Buguruni as indicated by the key in the above map.

2.2 Overview of mosquito field sampling techniques used for this study

The HLC involved a person or team of people who sat out all night collecting mosquitoes coming to bite them (Figure 1). This method remains the most reliable and direct way of monitoring the human biting mosquito population which is relevant to transmission and control of malaria (Service, 1977). It is also considered the gold standard method with which the efficacy of new sampling tools is usually assessed (Mathenge *et al.,* 2005; Mathenge *et al.,* 2004). In this study, the existing system of human landing catches which was established as an interim platform for routine monitoring of mosquito densities by the UMCP (Geissbühler *et al.,* 2007) were utilized. The catchers were supplied with head lamps, paper cups and a cool box, sat all night long with bare legs to catch mosquitoes coming to bite them. The host-seeking mosquitoes were then sucked using a hand held mechanical aspirator as shown in figure 1.

The ITT enclosed a human operator but under unexposed conditions to the vectors who then acted as a bait to attract mosquitoes into the trap through the funnel-like openings (Figure 2). The mosquitoes caught in the trap were then recovered the following morning by aspiration.

SRB were made from ordinary cardboard boxes by folding and taping the top flaps (Figure 4). The inside was lined with a black cloth while the outside was wrapped with plastic sheeting. The former was to improve mosquito attraction into the box while the latter was to protect them against rain.

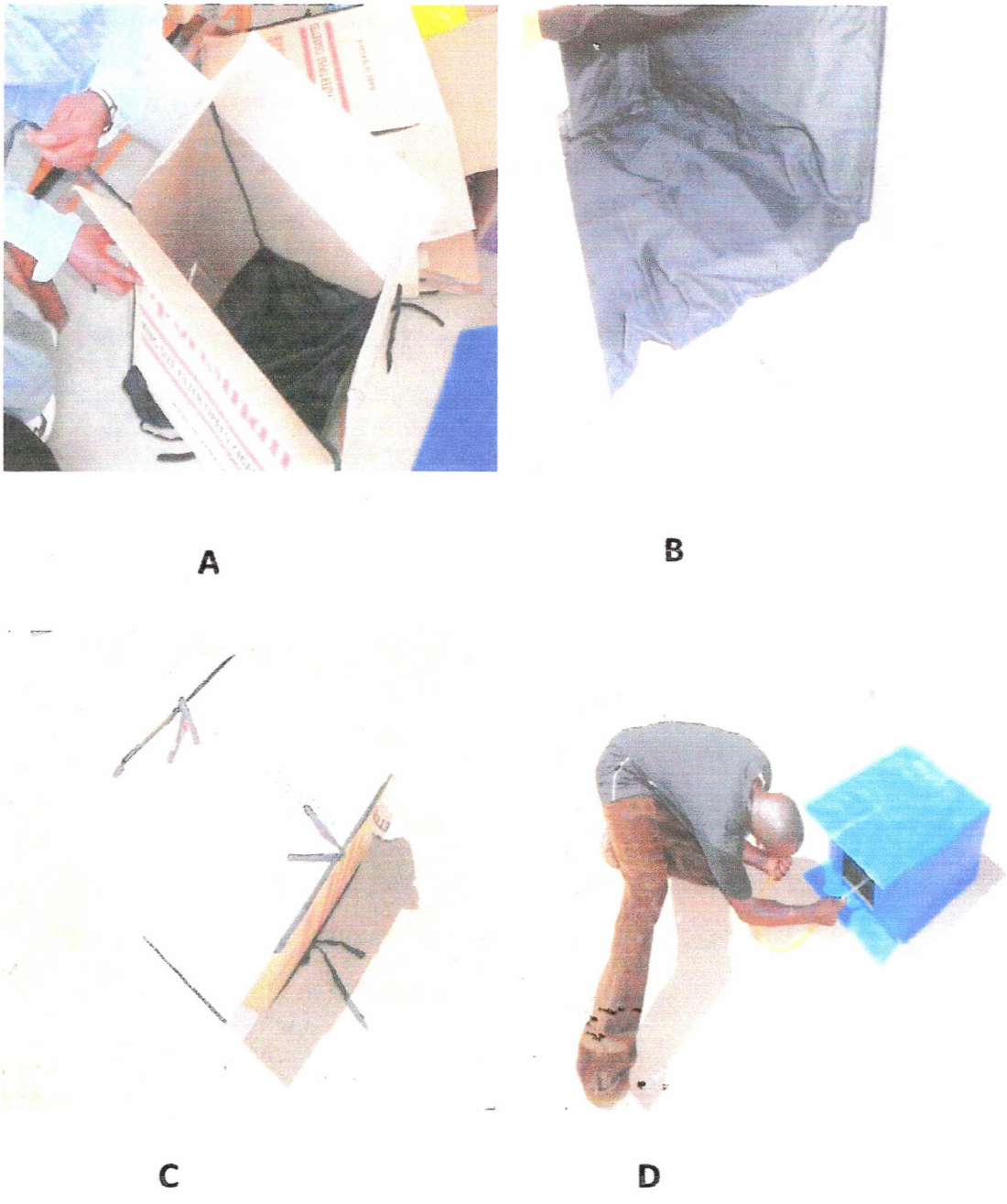

Figure 4: Photograph A and B shows how the boxes are made, C shows the way to install and D shows how to recover the resting mosquitoes.

2.3 Experimental design and selection of the sampling site for the ITT, HLC and SRB

The study was carried out in 12 wards in the study area for the Dar es Salaam Urban Malaria Control program with adult mosquito surveillance. One street in each ward was randomly selected for this study. In each street, four HLC sites already existed in four TCUs which were deliberately chosen to be well distributed across the street and as close to potential breeding habitats as possible. For each pre-existing HLC site, a nearby (100-300 meters) house was selected arbitrarily for both application of the ITT and the SRB. Therefore, in each street, 8 houses from different TCUs were used for the three sampling methods: 4 houses for the HLC and 4 houses for the ITT and the SRB, totaling 48 houses for the HLC and 48 for the combined the ITT and SRB methods, respectively.

2.4 Field sampling

2.4.1 Sampling with the Human Landing Catch

Direct catching by the human catchers/baits was conducted outdoors once a week in each street by one catcher working from 18.00 to 06.00 hours. Mosquitoes landing on the catcher's bare leg were carefully collected using hand-held aspirators and head lamps. The HLC was conducted for a period of 45 minutes every hour, allowing the catcher to have a 15 minutes break for snacks and rest. The mosquitoes caught each hour were placed in separate paper cups.

To reduce cheating, the catchers were obliged to record their mosquito catches for each hour and spot checks were conducted at arbitrary times of the night by a team of four supervisors. The supervisory team was provided with motorized transport so that they could visit any of the catchers operating each night at their discretion. The spot checks were also conducted in inconsistent and unpredictable manner so that the catchers were unable to guess the timetable

of these spot checks. The mosquitoes caught were collected the following morning and taken to the laboratory for processing.

2.4.2 Sampling with the Ifakara Tent Trap and Standardized Resting Boxes

A protocol for sampling malaria vectors and other mosquitoes using the ITT and SRB was developed to enable community members to trap record and submit malaria vectors without any night-time supervision and only occasional contact with programme staff. This protocol was used to evaluate the sensitivity of the ITT and the SRB relative to that of carefully controlled HLC as follows.

Prior to the supply of materials for ITT and SRB experiments, demonstration on the correct use of the two traps was made. The operators were supplied with small well-labeled containers filled with silica gel for storing and preserving the mosquitoes for as long as 1 week. In addition, a form for recording the results of the night's collection was issued to the operators. Data recorded on the form included the date, municipality, the ward, street, operator name and signature of the head of the household. All the operators returned to the laboratory all the mosquitoes collected and some of the materials supplied to them after one continuous week of mosquito sampling with only occasional supervision. Whether each night of sampling actually took place or not was verified by the household head's signature, and occasional spot checks by the supervisors. Later, the trap operators were supplied with bicycles to ease the transportation of the trap from one sampling site to the next. A human operator slept in the trap thrice a week at the same sampling site and then moved it to the next site the following week, following the rotation cycle of the HLC. In order to have paired comparisons between the HLC, the ITT and the SRB, the catches for both the ITT and the SRB were conducted at a sampling site close by to the HLC sampling site for that particular week. Therefore, each sampling site for ITT and SRB had a matching pair with each of the

HLC sampling sites. At the end of the month, the HLC and the SRB had rotated through all the four sampling sites in a paired manner with the HLC sampling sites located in each street. Mosquitoes trapped in the Ifakara tent trap were carefully aspirated using hand-held aspirators and placed into paper cups, once in the middle of each night (00.00-01.00) and then early in the morning the next day (05.00-06.00). Operators were allowed to choose at their own discretion which nights of every week they slept in the traps and what time they entered and left the trap under the condition that they recorded these dates and times. While still in paper cups, they were suffocated with cotton wool that had been soaked in petroleum ether. Preservation was done by transferring the suffocated mosquitoes into smaller silica gel-filled containers with a label indicating the ward, *mtaa*, site and day of collection. Cotton wool was placed on top of the silica gel to separate the mosquitoes from direct contact with silica gel. The mosquitoes were submitted to the laboratory for further processing at the end of each three day week on a pre-agreed day.

Resting boxes were installed nearby the trap in each street. The boxes were emptied between 06.00 and 08.00 in the morning of each working day using hand-held aspirators. Since experiments with ITT and SRB ran concurrently, suffocation, preservation and submission to the laboratory was done in exactly the same way and at the same time as those from the ITT.

2.5 Field processing and laboratory analysis

All the mosquitoes collected in the field by the HLC were taken to the laboratory and killed by suffocation with chloroform. For mosquitoes caught by ITT and SRB, this process was completed in the field by the trap operators who submitted their samples for identification and laboratory processing after a 1 week period of sampling. In the laboratory, they were identified morphologically using taxonomic keys (Gillies & Coetzee, 1987). Mosquitoes caught by the HLC were counted and identified the day after the catch whereas for mosquitoes caught by ITT and SRB, this process was undertaken as soon as the mosquitoes were submitted by the catchers at the end of the week.

All the mosquitoes were identified according to sex as males or females, morphologically as *An. gambiae s.l. An. funestus, An. coustani, Culex species,* or *Aedes*. The abdominal status was scored as gravid, fed or unfed for all the *An. gambiae s.l.* and for a manageable proportion of *Culex* species. It should be noted however that, mosquitoes in the semi gravid and part fed physiological status were considered gravid and fed respectively since the dry specimens in that state could not be clearly identified.

All *An. gambiae s.l.* caught by the three trapping methods were desiccated over silica gel and kept at room temperature until they were further processed. A wing or a leg of every *An. gambiae s.l.* mosquito caught was analyzed by PCR to identify the species within the *An. gambiae* complex (Scott, Brogdon, & Collins, 1993). Samples of DNA of *An. merus*, *An. gambiae s.s.,* and *An. arabiensis* were used as positive controls in each batch of reactions. Distilled water was used as negative control.

An enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody that recognizes a repetitive epitope on the circumsporozoite protein of *Plasmodium falciparum* was used to assess malaria sporozoite infection status in each individual *An. gambiae s.l.*

(Burkot, Williams, & Schneider, 1984). The combined head and thorax of each *An. gambiae s.l.* was homogenized in grinding solution and tested for the presence of this protein. Six negative controls and two positive controls were included on each microtiter plate. Negative controls consisted of the blocking agent alone while the positive controls consisted of synthetic peptide based on the sequence of amino acids found in the circumsporozoite protein of *Plasmodium falciparum.*

2.6 Ethical considerations

Informed consent was obtained from all the participants, namely the household owners and the mosquito catchers. All the activities conducted by UMCP including this survey are approved by the medical research coordination committee of the National Institute for Medical Research, Ministry of Health and Social Welfare, Government of Tanzania. No persons in high risks groups namely pregnant women were recruited to conduct these experiments. Moreover, thick and thin blood smears were taken from all the participants whenever they complained of fever to examine the presence of malaria parasites. When found positive, they were treated with Coartem® (Artemether Lumefantrine)

2.7 Data handling and analysis

All data handling and analysis was conducted with Microsoft Excel 2007® and SPSS 15.0® softwares. The only mosquito taxa considered for analysis were *An. gambiae s.l.* and the *Culex* species because these were the only ones for which sufficient numbers were collected throughout the study period. The overall catch and crude relative sensitivities reported here were calculated using the functions of the basic tables in SPSS.

To allow direct comparison with HLC conducted in the same area and in the same week, data was first aggregated by station and week, giving a total of 48 mean catches for matching station-week combinations over a period of 30 weeks. Prior to this analysis step, the numbers in each catch (x) were normalized by transforming to $\log_{10}$ [x+1] (Lines *et al.,* 1991). The relationship between catches by ITT or SRB and that of the HLC in the same week and the same station was initially assessed using simple Pearson's linear correlation method. Regression using generalized estimating equations was used to test for density dependence of the relative sampling efficiency of the ITT and SRB, methods relative to the sum of the ITT and the HLC. On several occasions, the ITT, HLC and SRB recorded zero values for *An. gambiae s.l.* mosquitoes even after aggregation so no logical comparison could be made and these data were discarded. Since divisions by zero gives infinite values, data for several week-site observations were aggregated and sorted by the sum of the catches for the traps (alternative plus the reference) with the mean of the two catches as the summary variables. The catch of the alternative collection methods divided by the catch of the reference method was treated as the dependent variable with a log link function and a gamma distribution for *An. gambiae s.l.* and a normal distribution for *Culex* species. The sum of the alternative and the reference methods was treated as a continuous independent variable in the model.

For the species composition, sporozoite incidence and the abdominal condition of the mosquitoes sampled by the different traps, binary logistic regression was used to check for any differences with the mosquito species collected by the three traps. Each outcome was treated as a binary outcome variable with trap design as an independent categorical factor in the model. The results of abdominal status, sibling species identity and sporozoite infection status were expressed as binary outcomes: fed versus non-fed (gravid and unfed), *An. gambiae s.s.* versus *An. arabiensis* and sporozoite positive versus sporozoite negative, respectively (Govella *et al.,* 2008).

CHAPTER 3

Results

3.1 Overall performance of the three sampling methods

A total of 44,848 mosquitoes were collected during the entire study period of 7 months. The composition of the sample was 98.9% *Culex* species, 0.8% *An. gambiae s.l.* 0.2% *Aedes* species and 0.1% *An. coustani* (table 2). The ITT, HLC and SRB accounted for 59%, 30% and 11% of the total number of mosquitoes caught respectively. The total catches of *Culex* species and *An. gambiae s.l.* are outlined in further detail in table 1.

Overall, more *An. gambiae s.l.* numbers were caught by the ITT than by the HLC or the SRB. The ITT and SRB caught between 0.35 and 0.15 times the number of *An. gambiae s.l.* respectively caught per night by the HLC. It is noteworthy that by applying the ITT for three nights in the same sampling site, its relative sensitivity per week matched the HLC for *An. gambiae s.l.* and exceeded it for *Culex* species. Both male and female mosquitoes of almost all the species sampled were found more frequently in the ITT than any other sampling method. It should be noted that male mosquitoes are just as useful an indicator of success or failure of a larval control programme even though they do not cause disease. Furthermore, male mosquitoes play an essential role in the life cycle of all mosquitoes and monitoring systems for genetic control strategies such as the release of sterile or genetically modified mosquitoes.

The results of this study show that the ITT was the most efficient method of collecting *An. gambiae s.l.* and *Culex* species. SRB, normally considered as the method of choice for recovering resting mosquito populations in different ecological settings was not productive in urban Dar es Salaam. Over the entire sampling period, the SRB caught only 46 and 4791 *An. gambiae* and *Culex* species, respectively

Table 1: A summary of the totals, means of *An. gambiae s.l.* and *Culex* species caught by the ITT, HLC and SRB

And the crude estimate of relative sensitivity *of* ITT and SRB relative to HLC

Collection method	Trap nights	*An. gambiae s.l.*					*Culex species*				
		Total catch			Mean catch[a]	Relative sensitivity[b]	Total catch			Mean catch[a]	Relative sensitivity[b]
		Male	Female	Total			Male	Female	Total		
ITT	606	33	135	168	0.27	0.35	8634	17689	26315	43.42	0.63
SRB	379	19	27	46	0.12	0.15	1786	3005	4791	12.64	0.18
HLC	195	0	143	143	0.78	1.00[c]	279	12979	13258	67.99	NA

[a]=Mean *An. gambiae s.l.* and *Culex species* caught per night.

[b]=Crude estimate relative to HLC, calculated by dividing the mean trap catch by the mean HLC. [c]=Reference trap.

3.2 Relationship between the catches from alternative sampling methods and the Human Landing Catches (HLC)

There was a significant correlation between the mean weekly numbers of female *An. gambiae s.l.* caught by the ITT and the HLC for both *Culex* species and *An. gambiae s.l.* in the 48 sampling sites (table 2). However, there was no correlation between the SRB and the HLC for *An. gambiae s.l.* even though a significant correlation existed for the *Culex* species.

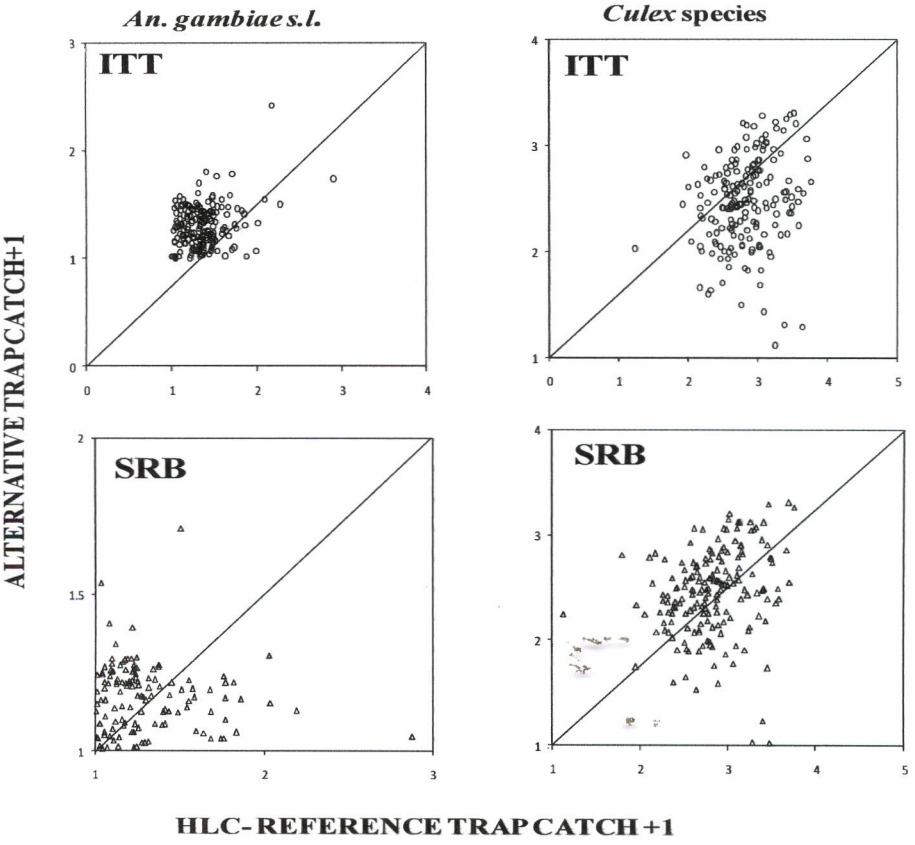

Figure 5: Relationship between the log of the mean weekly numbers of female *An. gambiae s.l.* and *Culex* species caught by the three sampling methods in 48 sampling stations over a period of 30 weeks in urban Dar es Salaam. All values (*X* or *Y*) are presented as $X + 1 + S$ or $Y + 1 + S$ where S a random number between 0 and 0.3 added to allow separation and visualization of otherwise identical data points

Table 2: Results from this and other studies evaluating the correlation between catches of female *An. gambiae s.l.* and *Culex species* obtained between the alternative traps and HLC.

		An. gambiae s.l.		*Culex species*	
Source	Alternative collection method	Versus HLC reference method			
		r^2	P	r^2	P
This study					
	ITT	0.104	<0.001	0.049	0.003
	SRB	0.115	0.195	0.167	<0.001
Other studies					
Govella *et al.*, 2007	ITT	0.731	<0.001	NA	NA
Mbogo *et al.*, 1993	CDC-light trap	0.409	<0.001	NA	NA
Magbity 2002	CDC- light trap	0.521	<0.001	NA	NA
Lines *et al.*, 1991	CDC- light trap	0.192	<0.001	NA	NA

CDC= Centre's for disease control

NA=Not applicable

3.3 Density dependence for the relative sampling efficiency of the ITT and the SRB

Both the ITT and SRB showed no density dependence for the relative sampling efficiency of *An. gambiae s.l.* and *Culex* species (table 3 and figure 6).

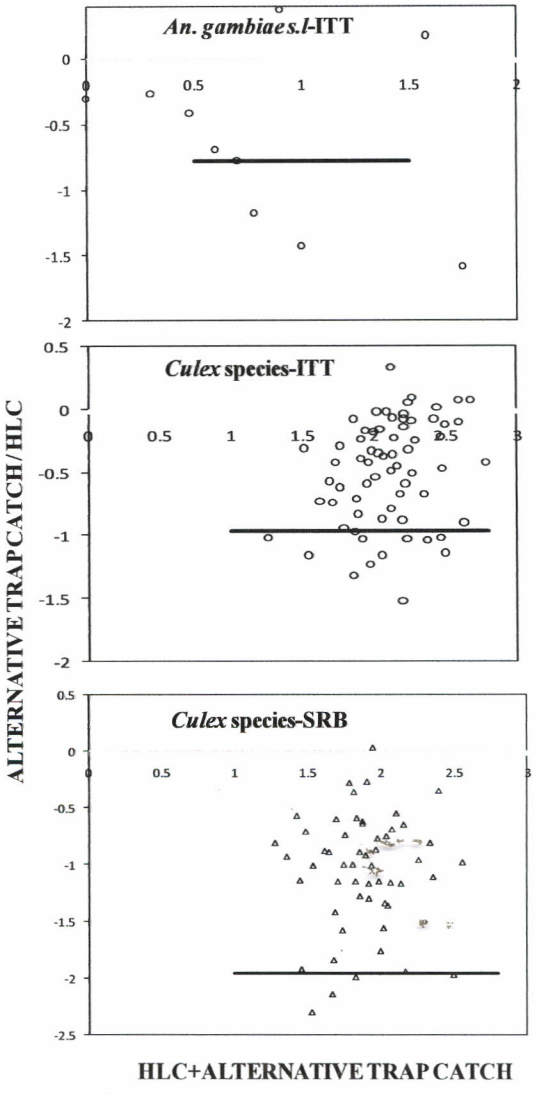

Figure 6: Density dependence for the relative sampling efficiency of the ITT, HLC and SRB for *An. gambiae s.l* and *Culex* species. Each point on X axis show the sum number of female *An. gambiae s.l. and Culex* species caught by the HLC and the alternative trap with several week-site observations. Solid lines depict the density-dependent sampling efficiency model.

Table 3: Regression analysis using generalized estimating equations (GEE) to determine density dependence relative sampling efficiency of the ITT and the SRB for sampling *An. gambiae s.l.* and the *Culex* species.

Species	Alternative method/HLC	Versus Alternative method+ HLC [a]		
		Parameter	Estimate [95%CI]	P
An. gambiae s.l.	ITT	Log_{10}(HLC+alternative)	NA	0.733[b]
		Intercept	-0.781 [-0.941, -0.621]	<0.001
Culex species	ITT	Log_{10}(HLC+alternative)	NA	0.096[b]
		Intercept	-0.969 [-1.282, -0.650]	<0.001
	SRB	Log_{10}(HLC+alternative)	NA	0.992[b]
		Intercept	-1.960 [-2.399, -1.521]	<0.001

[a] =Reference method

[b] = Not statistically significant and therefore not included in the model

NA=Not applicable

ITT=Ifakara tent trap

HLC=Human landing catch

SRB=Standardized resting boxes

3.4 Dependence of abdominal condition, sporozoite infection and species composition upon trapping method

3.4.1 Abdominal condition

Abdominal condition for *Culex* species and *An. gambiae s.l.* is presented in table 4. An abdominal condition was determined for 12776 *Culex* species and 305 *An. gambiae s.l.*. Abdominal condition varied significantly among the sampling methods. In this study we observed that unfed mosquitoes of both *An. gambiae s.l.* and *Culex* species comprised the majority captured by the three trapping methods. Notably, the SRB yielded the greatest proportion of blood-fed and gravid mosquitoes and far fewer unfed mosquitoes of both species than both the ITT and the HLC.

No significant difference was observed between proportions of fed *An. gambiae s.l.* captured by both the ITT and the HLC. However, a significant difference was observed between the proportion of fed mosquitoes caught by the SRB and the HLC for the same species. For *Culex* species, both the SRB and the ITT sampled a significantly higher number of fed mosquitoes than the HLC. One would expect a trap with a protective panel to yield less blood-fed mosquitoes than a human landing catch in which a certain amount of successful feeding is inevitable. The failure of the ITT to reduce the proportion blood fed suggests that; either exposure of the occupants may actually be occurring, probably during the collection process which necessitates opening of the long zipper which bisects the protective panel or the Ifakara tent trap actually samples resting samples of mosquito population.

Table 4: Abdominal condition for *An. gambiae s.l.* and *Culex* species scored by the ITT, HLC and SRB and the influence of each trap on the fed mosquitoes determined by binary logistic regression

Species	Variable	Fed		
An. gambiae s.l.	Trap type	Proportion	Odds [95%C.I]	P value
	ITT	0.08 [n=135]	1.38 [0.55, 3.42]	0.493
	SRB	0.37 [n=27]	9.53 [3.57, 25.47]	<0.001
	HLC	0.06 [n=143]	1.00[a]	NA
Culex species	ITT	0.06 [n=6661]	6.66 [4.86, 9.12]	<0.001
	SRB	0.21 [n=1351]	29.76 [21.52, 41.15]	<0.001
	HLC	0.01 [n=4975]	1.00[a]	NA

[a]=Reference method

n=Total number of mosquitoes

NA=Not applicable

ITT=Ifakara tent trap

HLC=Human landing catch

SRB=Standardized resting boxes

C.I=Confidence interval

3.4.2 Sporozoite infection

Although the SRB caught comparatively fewer mosquitoes compared to the HLC and the ITT, the *Plasmodium falciparum* sporozoite rate of *An. gambiae s.l.* was almost 4 and 6 times greater in the SRB than in both the ITT and the HLC respectively (table 5). Nonetheless, the sporozoite prevalence did not vary significantly between the mosquitoes sampled by the three trapping methods simply because the sample size was too small.

Table 5: The sporozoite prevalence for *An. gambiae s.l.* caught by the ITT, HLC and SRB and the influence of each trap on the number of *An. gambiae s.l.* that were sporozoite positive determined by binary logistic regression

Variable	Sporozoite prevalence		
Trap type	Proportion	Odds [95%C.I]	P
ITT	0.03 [n=110]	1.20 [0.24, 6.07]	0.825
SRB	0.12 [n=26]	5.08 [0.97, 26.56]	0.054
HLC	0.02 [n=132]	1.00[a]	NA

[a] =Reference trap

n=Total number of mosquitoes

HLC=Human landing catch,

ITT= Ifakara tent trap,

SRB=Standardized resting boxes.

NA=Not applicable

C.I=Confidence interval

Note: These results should however be viewed with great caution due to the small sample used.

3.4.3 Species composition

Of the 268 female *An. gambiae s.l.* analyzed by PCR for sibling species identification, a PCR success rate of 67.7% was obtained with 87 undetermined specimens (table 6). Of the successful amplifications, 83.3% were *An. gambiae s.s*, 15.5% *An. arabiensis* with the remainder being *An. merus*. Both *An. gambiae s.s and An. arabiensis* were taken by the three sampling methods but notably the SRB yielded comparatively fewer *An. arabiensis* while only the HLC recorded 2 *An. merus*. Analysis using binary logistic regression showed no significant difference in number of *An. gambiae s.s*. sampled by the ITT, HLC and SRB. Analysis of *An. merus* was not done due to low catches of this species. The evidence presented in table 6 suggests that the ITT is not biased towards sampling of *An. arabiensis*.

Table 6: *An. gambiae* complex sibling species composition for the ITT, HLC and SRB and the influence of each trap upon the proportion of *An. gambiae s.s*. sampled as determined by binary logistic regression

Variable	*An. gambiae s.s*		
Trap type	Proportion	Odds [95% C.I]	P
ITT	0.54 [n=110]	0.52 [0.23, 1.17]	0.115
SRB	0.48 [n=26]	1.80 [0.21, 15.12]	0.588
HLC	0.61 [n=132]	1.00[a]	NA

[a]=Reference trap, n=Total number of mosquitoes, C.I=Confidence interval, NA=Not applicable HLC=Human landing catch, ITT= Ifakara tent trap, SRB=Standardized resting boxes.

3.5 Evaluation of the protocol in terms of cost-effectiveness

Table 7 summarizes the initial cost per week per sampling station, running costs per week per sampling station, total cost and the cost of sampling one mosquito caught for both the ITT and the HLC. In all cases, the HLC was more costly than the ITT. For example, it was found out that weekly sampling with the HLC (1 night collection) in one sampling station was roughly equivalent in cost to three weeks of sampling with the ITT (nine nights collection) in three sampling stations. Also, regardless of the type of species sampled, ITT was 5 times cheaper than the HLC for every mosquito sampled. On the basis that the trap can handle more sampling nights than the HLC at an extremely lower cost, more sensitive measurements of biting density can be determined over a larger sampling area.

The fact that the ITT samples the vectors with minimal supervision and the HLC involves sampling in a highly controlled environment, is the primarily reason for the difference between their overall costs. Whereas HLC involved daily use of a vehicle to distribute the sampling materials to the respective sampling sites, spot checks as well as picking the vectors the next day, the ITT involved none of these. The other major differential cost associated with the HLC was the diagnosis and treatment of the HLC catchers in case of any reported fever. By comparison, the ITT requires little or no maintenance so after the initial outlay of purchase. It is remarkably affordable because it does not require skillful personnel, supervision or medical expenses.

Table 7: Comparative evaluation of cost effectiveness of the ITT and the HLC for weekly sampling of mosquitoes and for sampling a single mosquito

Type of cost	Item	Details	Quantity(ITT)	Quantity HLC	Total amount for HLC per week per site	Total amount for ITT per week per site
Initial set up costs	Traps	ITT	12	NA	NA	$2.10
		Car Maintained and depreciated over 5 years	NA	7.5kmx$0.50	$4.40	NA
		Bicycles maintenance and depreciation over 5 years	12	NA	NA	$0.37
	Collecting materials	Aspirators and torches	12	12	$0.25	$0.25
Total Initial set up cost					**$4.65**	**$2.74**
Recurring cost	Labour	Adult mosquito catchers	12	12	$5.00	$4.30

		Driver	1	NA	$0.80	NA
		Surveillance coordinators	NA	2	$2.40	NA
	Field work	Fuel and maintenance cost	NA	8kmx$0.5	$5.00	NA
	Office materials	Consumables			$1.48	$1.54
	Medical tests				$1.00	NA
	Miscellaneous cost				$2.55	$0.58
Total recurring cost					**$18.23** [a]	**$6.42** [a]
TOTAL COST [b]					**$22.88**	**$9.15**
OTHERS	Total number of mosquitoes collected		131[c] mosquitoes per catcher per week	68[c] mosquitoes per catcher per week	**$0.27**[d] **per mosquito caught**	**$0.05**[d] **per mosquito caught**

[a] =Total running cost per week per sampling site, [b] =Running cost plus initial cost [c]=Total number of all the mosquito caught (26541 for ITT and 13446 for HLC) divided by the number of sampling nights multiplied by the number of sampling nights in a week (606 for ITT and 195 for HLC): [d]=Cost of catching one mosquito calculated by dividing the total running cost per week per site by the total number of mosquitoes caught per week per site: NA=Not applicable.

Note: Traps, Bicycles and a car values depreciate in a five year period

CHAPTER 4

4.1 Discussion

This study investigated the effectiveness and affordability of community based approach for using the ITT and the SRB for sampling malaria vectors and other mosquitoes compared with rigorously scrutinized HLC. The ITT and the SRB were operated under representative and sustainable programmatic conditions with minimal supervision and in a real-world programmatic setting while the HLC was necessarily under intensely controlled research conditions.

Overall the ITT was far more cost-effective. On overall, no reported cases of staff anxiety related to the insecurity of working alone at night in a large city was noted which indicates that this trap could be a reliable, acceptable and sustainable tool for safe affordable community based surveillance of mosquito densities.

The SRB proved impractical and on several occasions they were either soaked by rain water or simply stolen. It also often proved difficult to retrieve the mosquitoes from the SRB. These problems, combined with their poor sensitivity and other logistical matters rule out the SRB out as a candidate tool for routine mosquito sampling in the city of Dar es Salaam. However, the higher sporozoite rate that was realized from the mosquitoes sampled by the boxes, suggests that the boxes attracted older and fed mosquitoes (Hii *et al.*, 2000).

The correlation results obtained from this effectiveness trial were slightly different from those of efficacy trials by others (Govella *et al.*, 2008; Lines *et al.*, 1991; Magbity *et al.*, 2002; Mbogo *et al.*, 1993). For example, the efficacy trials in particular that done by Govella *et al.*,2008 recorded a much stronger correlation between ITT and HLC, than seen in this study. This is most probably because of the very low vector densities in this setting and perhaps also

because this study was carried out under conditions that involved minimal supervision compared to the intensely controlled efficacy trials.

Another likely interpretation could be due to the fact that more sampling stations across a very heterogeneous environment were included in this study compared to the relatively few sampling stations for the efficacy trials listed in table 2.

Nonetheless, the significant positive correlation between the HLC and the ITT (table 2) suggest that this approach may be very useful in programmatic setting and provides a reasonably sensitive and accurate reflection of true mosquito biting densities. Apart from the use of window traps installed in existing houses and emptied by resident community-based workers for routine monitoring of indoor-residual spray progammes in southern Africa (Sharp *et al.*, 2007), no other effectiveness study of this kind has been reported for malaria vector trapping methods. The ITT not only represents an option for more accurate and representative human biting rate over a large sampling area, it is also practical and affordable to use in community based sampling schemes.

The SRB have been evaluated previously in terms of efficacy and found to correlate poorly with the HLC (Kay, 1982) and this study reinforces that view even though they were reported to be better for sampling indoor-resting malaria vectors in Kenya (Harbison *et al.*, 2006).

In earlier studies, it was found out that the proportion of adult mosquitoes resting in man-made shelters depended on the availability of alternative resting sites which varies according to location and changes seasonally (Service, 1977) and in a recent study that female mosquitoes prefers larger resting sites over smaller (Burkett *et al.*, 2008). SRB are therefore unlikely to be a useful alternative to the HLC for sampling host-seeking malaria vectors in urban Dar es Salaam, particularly under real-world programmatic operational condition.

One major disadvantage of the ITT often reported by the catchers was the fact that the trap was too heavy to be moved from one station to another by a single person. This problem was later solved by supplying the operators with bicycles. Also, occasionally the trap was reported to attract other insects but none of these were confirmed to be potential mosquito predators. Otherwise, the protocol was generally well accepted by the trap operators and appears to be easy enough for performance to be maintained with relatively modest incentives.

4.1 Conclusions and Recommendations

This seven month evaluation of the effectiveness of the two traps supports the use of the ITT under programmatic condition in the future. The trap has proven effective for routine vector sampling and is affordable to non-research programmes. It may therefore enable effective malaria control by making available data which allows programmatic failures to be identified and rectified. The ITT is reliable in the sense that it is easy to use, requires no skilled personnel and adequately reflects local mosquito densities. One concern remains the surprisingly high proportion of blood-fed mosquitoes caught, suggesting the design needs to be adapted to avoid human exposure during the empting process.

This evaluation suggests that our protocol for using the Ifakara tent trap under realistic programmatic condition is affordable and effective. I, however, recommend that the trap be evaluated in the longer terms and on full programmatic scales until the routine effectiveness of this approach in fully representative condition of practice is established. The SRB are not recommended in this setting for assessing the night time mosquito density. However, if modified to prevent escape of mosquitoes, further investigations done to justify the higher sporozoite rate observed in this study could be a good investment.

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