EVALUATION OF THE EFFECTIVENESS OF ADULT MOSQUITO SAMPLING METHODS IN THREE ECOLOGICAL HABITATS IN KWALE COUNTY IN SOUTH COAST, KENYA

By

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DECLARATION

Candidate

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

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SUPERVISORS

We confirm that the candidate carried out the work reported in this thesis under our supervision. This thesis has been submitted for examination with our approval as supervisors.

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DEDICATION

This work is dedicated to my parents, Asher Akal and Janet Betty Onyango, for their love, financial support and encouragement; and to my sisters Flavia and Barbara and brother Asher Junior for their moral support during my entire study.

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ABBREVIATIONS AND ACRONYMS

ACTs	Artemisinin-based combination therapies
CP	Clay pots
ELISA	Enzyme-linked immunosorbent assay
GEE	Generalized estimating equations
HBI	Human blood index
HLC	Human landing catches
IRS	Indoor residual spraying
ITNs	Insecticide treated nets
KEMRI	Kenya Medical Research Institute
KMIS	Kenya Malaria Indicator Survey
LT/N	Light traps alongside human occupied bed nets
LLINs	Long lasting insecticide nets
LF	Lymphatic filariasis
PSC	Pyrethrum spray catch
PK	Prokopack aspirator
SPSS	Statistical programme for social science
UBT	Urine baited traps
WHO	World Health Organization

ABSTRACT

Malaria and lymphatic filariasis transmission along the coastal strip of Kenya is endemic. The predominant mosquito vectors of human malaria include *Anopheles gambiae s.l.* and *Anopheles funestus.* The same mosquitoes transmit lymphatic filariasis with an added role for *Culex quinquefasciatus.* Current control strategies for anthropophagic mosquitoes largely involve methods that sample for adult mosquitoes inside human dwellings before they are implemented. Despite the intensive interventions, these vectors continue to elude the common forms of the adult mosquito control. At the coastal strip of Kenya, where interventions, LLINs and IRS are in place, the use of a single mosquito collection method will not be sufficient to achieve a representative sample of the vector populations in low mosquito densities. Hence, the need for effective mosquito sampling tools for vector surveillance. This research set out to identify the most effective mosquito sampling method for routine surveillance of malaria and lymphatic filariasis in coastal Kenya. The effectiveness of five collection methods: Light traps associated with a person sleeping under a net (LT/N), pyrethrum spray catches (PSC), prokopack aspirator (PP), clay pots (CP) and cow-urine baited traps (UBT) were evaluated in four villages representing three ecological settings during three surveys in south coast, Kenya. Female malaria vectors (*An. gambiae* s.l. and *An. funestus*) collected were tested for *Plasmodium falciparum* circumsporozoite protein and blood fed mosquitoes dissected for the identification of the blood meal sources by ELISA. A longitudinal regression analysis using the generalized estimating equations procedure in SPSS was used to compare mosquito counts of different categories. Kruskal–Wallis test was used to separately compare the performance of the sets of indoor and outdoor methods for each species. Of the five mosquito sampling methods evaluated, light traps were the most efficient for collecting female *An. gambiae* s.l and *An. funestus* mosquitoes, while the prokopack aspirator was most efficient in collecting *Cx. quinquefasciatus* and other culicines not known to transmit lymphatic filariasis. For the paired sets of indoor and outdoor methods, the indoor methods sampled significantly higher numbers of *Cx. quinquefasciatus* (Kruskal–Wallis $\chi^2 = 58.30$, $p<0.0001$) and *Culex spp*. (Kruskal–Wallis $\chi^2 = 228.89$, $p<0.0001$) than the outdoor methods. Overall, the infection rate was 1.78% with humans as the preferred blood meal source for all the tested species. The findings of this study have demonstrated that light traps remain a relevant tool for host-seeking mosquitoes. Prokopack aspirator has the potential for routine monitoring of indoor resting mosquitoes, and may be substituted for the more labour intensive and intrusive PSC. There remains lack of an efficient mosquito collection method for sampling the outdoor mosquitoes. Prokopack aspirator, pyrethrum spray catch, light traps, urine baited traps and clay pots should be evaluated further to contribute towards improving routine vector surveillance sensitivity in various habitats.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Entomological surveys associated with malaria and lymphatic filariasis prevention and control efforts require the sampling of adult mosquitoes (WHO, 1992; Service, 1993). Numerous mosquito sampling techniques have been developed and the sensitivity with which they sample targeted mosquito species has been evaluated under diverse ecological habitats (Service 1993). In Africa, this largely involves techniques that collect female mosquitoes inside human dwellings. Collections involving human bait such as human landing catches are unethical because they expose the human to bites from infected mosquitoes. Alternative sampling methods that do not expose human baits include light traps, pyrethrum spray catches (PSC), pit shelter, manual aspiration, Prokopack aspirator (Vazquez-Prokopec *et al.,* 2009; Maia *et al.,* 2011), clay pots (Odiere *et al.,* 2007), urine baited traps (Kweka *et al.,* 2009) as well as animal-baited traps (Mahande *et al*., 2007). The effectiveness of these methods varies, but their cost and practicality often prevent their acceptance in communities in disease endemic areas. Effective mosquito sampling methods are therefore essential to monitor and evaluate malaria vector control programs.

The most important outcome measures used for monitoring and evaluating of any malaria and LF control strategy is the biting density of adult female mosquitoes (Service, 1977). Human exposure to malaria and LF requires trapping host-seeking adult mosquito vectors to determine their densities and infection rates (Beier *et al*., 1999; Hay *et al.,* 2000). Despite its central role in disease epidemiology, sampling of

adult *Anopheles* remains problematic and all the available sampling methods suffer from significant drawbacks (Service, 1977). The gold standard for estimating malaria transmission rates based on entomological measures has been the human landing catches (HLC). However, this method has lost favour due to its labour-intensiveness and concerns about the increased exposure of collectors to mosquito bites and infection.

Many malaria research programmes in Africa use CDC light traps placed beside occupied bednets to sample host seeking adult mosquitoes in houses (Lines *et al.*, 1991; Shiff *et al*., 1995). This method requires regular access to electricity (to recharge the batteries) and is insensitive at low mosquito densities. Pyrethrum spray catch is commonly considered the gold standard method for collecting indoor resting mosquitoes. In as much as PSC reduces the overall nuisance from mosquitoes and other unwanted organisms, it is cumbersome because it requires the removal of furniture, food, cookware, animals and water from the dwellings. Additionally, residents have to vacate their houses in the early hours of the morning before spraying the insecticide. Pyrethrum spray collection samples mainly endophilic mosquitoes; underestimating mosquito populations that are more exophagic and exophilic (Mboera, 2005; Odiere *et al*., 2007). The prokopack aspirator is a new and effective tool for collecting both indoor and outdoor resting male and female mosquitoes. Light traps are moderately effective for collecting host seeking mosquitoes relevant in the transmission of disease (Okumu *et al*., 2008; Fornadel *et al.,* 2010). They may be a sensitive alternative to estimate human-biting activity of anopheline mosquitoes (Zaim *et al*., 1986; Davis *et*

al., 1995; Duo-quan *et al*., 2012) and are suitable for sampling endophagic malaria and LF vectors in Africa (Odetoyinbo., 1969; Lines *et al*., 1991; Mboera *et al*., 1998).

Effective sampling methods for the outdoor fraction of malaria and LF vectors is deficient despite the various attempts (Sikulu *et al*., 2009; Mahande *et al*., 2010; Govella *et al*., 2011). Clay pots have previously been explored in sampling mosquitoes both indoor and outdoor (Odiere *et al*., 2007; Bijllaardt *et al*., 2009). Clay pots are cheap and portable thus less cumbersome and collect mosquitoes both male and female and of all the three physiological stages (unfed, fed and gravid) of the female mosquito (Odiere *et al.,* 2007). Urine baited traps offer an alternative tool for sampling unfed, semi-gravid and gravid females (Kweka *et al.,* 2009; Mahande *et al.,* 2010). The objective of this study was to compare the effectiveness of LT/N, Prokopack, pyrethrum spray catch, clay pots and urine baited traps for sampling adult mosquitoes for routine vector surveillance of malaria and LF in various ecological habitats in coastal Kenya.

1.2 Statement of the problem

Current control strategies for anthropophagic mosquito vectors largely involve methods that sample for adult mosquitoes before control strategies such as insecticide treated nets (ITNs), long lasting insecticide nets (LLINs) and indoor residual spraying (IRS) are implemented. Even following these intensive intervention, anopheline vectors continue to elude common forms of domestic control such as bednets and insecticides (Geissbuhler *et al.,* 2007). In low mosquito densities where malaria interventions,

LLINs and/or IRS are in place, the use of a single mosquito collection method will not be sufficient to achieve a representative sample of mosquito population. Furthermore, the existing sampling methods require further evaluation both indoor and outdoor due to significant shortcomings to be able to improve on routine mosquito surveillance. In sub-Saharan Africa where *Anopheles gambiae* s.l. and *An. funestus* are the important malaria and lymphatic filariasis (LF) vectors, efficient mosquito collection tool(s) will be needed to be able to collect these vectors effectively, both indoors and outdoors, even at low densities. The long-term success of ongoing malaria control efforts based on mosquito bed nets (LLIN) and indoor residual spraying (IRS) is therefore dependent on continuous monitoring of mosquito vectors and thus the need for effective mosquito sampling tools.

1.3 Justification of the study

The current impetus for disease control through reduced contact between mosquito vectors and humans (ITNs and LLINs), vector control (IRS) and prompt treatment with artemisinin-based combination therapy (ACTs) have resulted in dramatic declines in malaria vectors (Bayoh *et al*., 2010; Mutuku *et al*., 2011) and in the number of malaria cases (Bhattarai *et al*., 2007, Ceesay *et al.,* 2008, O'Meara *et al*., 2008, Lee *et al*., 2010). These successful results have revived the idea of malaria elimination. However, many challenges such as insecticide (Kawada *et al.,* 2011; Mathias *et al*., 2011; Ranson et al., 2011; Trape *et al*., 2011) and drug resistance (Jambou *et al*., 2005; Dondorp *et al.,* 2009) and changes in peak mosquito feeding times remain (Geissbuhler

et al., 2007; Russell *et al*., 2011; Yohannes and Boelee, 2012). Hence, the current mosquito collection tools may not be sufficient for routine vector surveillance.

These challenges are confounded by other locality-specific barriers that hinder sustainable malaria reduction or elimination, including local heterogeneity in transmission patterns and variations in distribution and adoption of control measures (Tatem *et al*., 2010). Given these barriers, the establishment of a sustainable system for monitoring vectors is an essential long-term goal (Bockerie *et al*., 2009; Najera *et al*., 2011).

1.4 Research questions

a) What is the effectiveness of pyrethrum spray catch, prokopack mosquito aspirator and CDC light traps for sampling indoor adult mosquitoes?
b) What is the effectiveness of clay pots and urine baited traps for sampling outdoor adult mosquitoes?

1.5 Hypotheses

a) There is no difference in the effectiveness of pyrethrum spray catch, CDC miniature light traps and prokopack aspirator for sampling indoor adult mosquitoes in different seasons and ecological habitats in south coast, Kenya.
b) There is no difference in the effectiveness of clay pots and urine baited traps for sampling outdoor adult mosquitoes in different seasons and ecological habitats in south coast, Kenya.

1.6 Objectives of the study

1.6.1 General objective

To evaluate the effectiveness of five adult mosquito sampling methods in three ecological habitats in south coast Kenya

1.6.2 Specific objectives

a) To determine the effectiveness of pyrethrum spray catch, prokopack aspirator and CDC miniature light trap to sample indoor adult mosquitoes.
b) To establish the effectiveness of urine baited traps and clay pots for sampling outdoor adult mosquitoes

1.7 Significance and anticipated output

Malaria and LF occur as co-endemic infections affecting the same human populations and share common vectors. As a result, most communities are at prolonged risk of parasite infection. Current control strategies such as LLINs and IRS, for mosquito vectors largely involve methods that sample for adult mosquitoes. These control strategies decrease the densities as well as the parasite levels in both the mosquito vectors and the human hosts. Despite the effort, these vectors have continued to escape collection. Furthermore, toxicity and increasing ineffectiveness due to the development of insecticide and drug resistance has made them more unpopular to communities in endemic areas. Long term success of the ongoing malaria control efforts based on LLINs and indoor residual spraying is dependent on the continuous monitoring of the mosquito vectors hence an effective sampling method. A combination of two or more effective sampling methods can be used to replace unethical methods such as human

landing catches and improve on routine surveillance tools in various ecological habitats for the existing malaria and LF vectors.

CHAPTER TWO: LITERATURE REVIEW

2.1 Malaria and lymphatic filariasis co-infections and vectors

Malaria and lymphatic filariasis (LF) are significant causes of morbidity and mortality, with malaria notably being the most important global vector-borne disease hence a priority for control and elimination programmes (Molyneux and Zagaria, 2002; Zagaria and Savioli, 2002; WHO, 2005). Malaria and LF are co-endemic in many tropical and sub-tropical regions, such as Southeast Asia, including the western Pacific and in Africa, and are transmitted by a number of common *Anopheles* vector species (Reeder *et al.,* 2003; Muturi *et al.,* 2008). Co-infections with malaria and LF parasites in humans and mosquitoes (Burkot *et al.,* 1990; Ghosh and Yadav., 1995; Chandee *et al.,* 2003; Muturi *et al.,* 2006) are found in these endemic regions.

In sub-Saharan Africa, *Anopheles gambiae* s.l and *Anopheles funestus* complex are the important malaria and lymphatic filariasis vectors. In Kenya, both malaria and LF are endemic along the coast. In rural areas of the Kenyan Coast both diseases coexist in the same human populations and share common vectors namely *An. gambiae* s.l., *An. funestus* and *Culex quinquefasciatus* (Muturi *et al.,* 2006). Many communities in these areas are at continued risk of contracting and suffering morbidity associated with both diseases through mosquito transmission (Muturi *et al.,* 2006). Malaria is by far the most common mosquito-borne disease in the South Coast, Kenya. Therefore, effective adult sampling methods for routine monitoring of the vector densities is necessary.

Concomitant infections of malaria and LF in *Anopheles* vectors and humans are more likely to occur when the prevalence of both parasites is high (Manguin *et al*., 2010). Therefore, integrated control strategies targeting both diseases in areas with common vector species is recommended and hence entomological assessment of malaria and LF transmission. In the Kenya coast, *An. gambiae sensu stricto, Anopheles arabiensis* and *An. funestus* play the dual role in transmissions (Pedersen and Mukoko, 2002; Mbogo *et al*., 2003; Kasili *et al*., 2009). In addition, *Cx. quinquefasciatus*, initially considered an urban vector of LF, has also been shown to be an important vector in rural settings (Mwandawiro *et al*., 1997; Pedersen and Mukoko, 2002). Most communities are therefore at continued risk of infection and experience significant morbidity associated with both diseases.

2.2 Impact of adult mosquito vector control interventions

Large scale malaria prevention is achieved primarily through two major vector control interventions: indoor residual spraying (IRS) and the use of insecticide treated nets (ITNs) that target the adult mosquitoes. In confirmed cases of malaria, prompt treatment with Artemisinin-based combination therapies (ACTs) is recommended (World Malaria Report, 2009). ITNs and indoor residual spray (IRS) are the major tactics for the reduction of malaria morbidity and mortality by decreasing the levels of transmission in sub-Saharan Africa (Curtis *et al.*, 1999; Gu and Novak, 2009). Long-term success of the ongoing malaria control efforts based on mosquito bed nets and indoor residual spraying is therefore dependent on continuous monitoring of the

mosquito vectors hence the need for effective mosquito sampling tools (The malERA Consultative Group on Vector Control, 2011).

2.2.1 Impact of insecticide treated nets (ITNS) on mosquito vectors

Mosquito nets help keep mosquitoes away from people and greatly reduce transmission of malaria and LF and the resulting infections. Insecticide-treated nets (ITNs) are estimated to be twice as effective as untreated nets and offer greater than 70% protection compared with no net (D'Alessandro *et al*. 1995; Clarke *et al*., 2001). Insecticide treated nets excito-repellent properties prevent malaria transmission by killing and/or diverting mosquitoes away from the net user and the houses with the treated nets (Takken, 2002). In tropical Africa, different vector species vary substantially in host-seeking behaviours and consequently respond differently to use of ITNs (Curtis *et al.*, 1999; Gimnig *et al.*, 2003; Lindblade *et al.*, 2006). The effectiveness of ITNs differs by vector species for example, culicine mosquitoes have not been shown to be significantly affected by the use of ITNs (Bogh *et al.*, 1998; Lindblade *et al.*, 2006). Additionally, studies have shown that extensive use of ITNs could result in reduced susceptibility of *An. gambiae* s.l. to treated nets (John *et al.*, 2008).

The impact of ITNs upon the indoor resting densities of both *An. gambiae s.l.* and *An. funestus* has significantly reduced. Overall, there was a 71.5% reduction in the indoor resting densities of fed anopheline mosquitoes in intervention areas compared with the control areas (Gimnig *et al.*, 2003). Studies in the Gambia, Burkina Faso and coastal

Kenya have demonstrated reductions in indoor anopheline densities by more than 80% in the presence of ITNs or curtains (Mbogo *et al.,* 1996; Bogh *et al*., 1998; Cuzin-Ouattara *et al.,* 1999). Consequently, continuous monitoring of malaria and LF mosquito vectors for evaluation of the application of treated nets in the field using appropriate mosquito sampling methods is vital.

2.2.2 Impact of indoor residual spraying (IRS) on mosquito vectors

Indoor Residual Spraying (IRS) is a standardized control method that involves spraying a dilute solution of insecticide on the interior walls and roofs of houses and domestic animal shelters so as to kill indoor resting and flying females or repel mosquito vectors from entering the houses (WHO, 2006). Indoor residual spraying has been used in much of the world including Asia, the Pacific, Latin America and Africa, where its use has been limited to areas where malaria is prevalent (WHO, 2006). An advantage of IRS over ITNs is that much wider range of insecticide products (currently pyrethroids) can be used (Pluess *et al*., 2010). Indoor residual spraying suppresses malaria incidence by reducing both the vector densities and the transmission rates. However, the continuous use of insecticides has led to the development of resistance over several generations of major malaria and other disease vectors. Furthermore, indoor residual spraying only kills the endophilic fraction of the vector population leaving the exophilic mosquitoes unaffected, hence their continued effectiveness is not guaranteed and pause a threat to vector-borne diseases (Malaria Research Center, 2002; Sharmaa *et al*., 2005; WHO, 2006).

2.3 Adult mosquito sampling methods

Estimation of vector populations is imperative to understanding the dynamics of disease transmission and for evaluating the impact of subsequent control measures (Bruce-Chwatt, 1985). In the case of mosquito borne diseases, this is normally carried out by monitoring the resting and biting densities of vector mosquitoes using various sampling methods. In the African context, sampling of malaria vectors relies almost exclusively on trapping anthropophagic mosquitoes in and around houses, either directly before or soon after feeding (Mboera, 2005). Sampling adult stages of vectors of human diseases is an important and necessary process for estimating vector population densities, obtaining an adequate sample to measure the infection rate and quantifying the effect of interventions directed against vector populations (Odiere *et al*. 2007). Preference for any sampling method however depends on both its field efficiency and the characteristics of local vector populations.

2.3.1 Human landing catches

Human landing catch (HLC) has been considered the gold standard in mosquito sampling for surveillance and control programmes to estimate the infectivity rates, mosquito abundance and mosquito dynamics (WHO, 1975). Human landing catches and CDC-light trap placed besides occupied bed nets are reliable methods for trapping host seeking anthropophagic mosquitoes. Despite HLC being the most reliable and direct technique in catching anthropophagic mosquitoes, human landing catch exposes the catcher to the mosquito-borne infections and a variety of other pathogens (Service, 1977). It also requires intense supervision, and is rigorously restraining in terms of

affordability and sustainability. Therefore, many other methods such as the CDC light trap, Ifakara tent trap, woven baskets and Mbita-bed net trap (Harbison *et al.,* 2006; Okumu *et al*., 2008) have been employed and evaluated as alternatives to HLC, however, none has proved to be adequately efficient (Govella *et al.,* 2009). In northern Tanzania, in a comparison of oduor-baited traps, pit shelter, indoor resting collections and human landing catches in seasons of low mosquito densities and malaria transmission, odour-baited traps with different baits and pit shelters collected higher numbers of mosquitoes than the HLC (Kweka and Mahande, 2009). Human landing catch was compared to odour-baited entry traps for sampling malaria vectors both indoor and outdoor in Senegal. Human landing catch was more effective indoor and both were effective in sampling all four vectors - *An. gambiae s.s., Anopheles arabiensis, Anopheles funestus,* and *An. nili* (Dia *et al*., 2005). There is therefore, the need to improve or develop sampling techniques that are effective as alternatives to HLC for ethical mosquito surveillance in sampling mosquito borne diseases.

2.3.2 Indoor mosquito sampling by Pyrethrum spray collection (PSC)

A major strategy for trapping African malaria vectors exploits the tendency of endophilic species to rest indoors after blood feeding (Pates and Curtis, 2005). Such indoor resting catches are aimed at controlling the adult flying mosquitoes with indoor pyrethrum spray. The knocked down mosquitoes drop onto white sheets spread over the entire floor, bedding and furniture where they are readily collected (Gimnig *et al*., 2003; Kulkarni *et al*., 2006). This method is referred to as pyrethrum spray catch or pyrethrum spray collection (PSC) and targets the indoor resting adult mosquitoes

(Service, 1993). It is used to estimate the number of mosquitoes resting while digesting a blood meal prior to seeking oviposition sites (Nnoka *et al.,* 2008). Pyrethrum spray catches however sample indoor resting mosquitoes and tend to miss the mosquitoes that leave the houses after feeding, and may also include those that enter the houses after feeding outdoor (Mboera, 2005). In Nigeria, PSC method yielded a greater proportion of blood fed *An. gambiae,* and revealed that the adult vector species can selectively be controlled by insecticides applied at specific times (Aigbodion and Nnoka, 2008). Odiere *et al.,* (2007) showed that in Western Kenya the PSC method yielded a greater proportion of blood fed *An. gambiae* and relatively fewer gravid and unfed individuals, compared with clay pots. However, because PSC is biased to collect endophilic female mosquitoes it underestimates the outdoor mosquito population densities.

2.3.3 Indoor mosquito collections by CDC miniature light traps

Various designs and applications of light traps have been proposed and evaluated for sampling anthropophilic mosquito populations to estimate mosquito biting rates (Service, 1993). Of all light traps, the standard Centers for Disease Control (CDC) miniature light trap in Atlanta, GA has been found to be an efficient device for sampling endophagic malaria and filariasis vectors in Africa (Odetoyindo, 1969; Silver, 2008). The most favourable location of the light trap for sampling mosquitoes has been reported to be as close to the host(s) as possible, and its efficiency is greatly improved when the human bait is protected by a bed net (Maxwell *et al.,* 1990). This trap-bed net system is used to monitor vector populations and evaluate vector control interventions (Curtis, 1991; Shiff *et al.,* 1995).

Light Traps are relatively reliable and largely unaffected by the presence of insecticide through interventions such as IRS and ITNs, and provide information on the composition of the *Anopheles* fauna during periods of high mosquito densities (Joshi *et al.,* 1975; Lines *et al.,* 1991). However, it has been reported that light traps are biased towards sampling 2–2.3 times more *P. falciparum*–infected endophilic anopheline females than the human-landing catches due to higher parity and infection rates and hence may not provide a reliable cross-section of the host-seeking population (Mbogo *et al.,* 1993). Light traps have been used indoor to target endophilic mosquitoes; they are however influenced by the use of ITNs in households. This is because the mosquitoes are adversely affected by the presence of insecticides on nets or walls that promote the vectors' exit from the home (Service, 1993; World Malaria Report, 2005).

2.3.4 Indoor sampling by Prokopack aspirator

Battery-powered aspirators collect mosquitoes of both sexes and all physiological stages directly from their resting sites, allowing better estimations of richness (number of different mosquito species in a given area), abundance, sex ratio, age structure, and physiological condition of sampled populations (Silver, 2008). The prokopack aspirator is a relatively new sampling tool which when compared to the commonly used CDC-Backpack aspirator is more maneuverable, less expensive, easy to construct from locally available materials and compatible with the use of telescopic extension poles to access hard to reach locations (Vazquez prokopec *et al*., 2009). It has been shown to collect blood-fed mosquitoes (more than 6 times the proportion collected by the CDC-

BP) and is of importance in epidemiological studies of disease vectors (Vazquez-prokopec *et al*., 2009). This new mosquito collection tool has not been tested in Sub-Saharan African.

2.3.5 Outdoor mosquito sampling by Urine baited traps (UBT)

Trap-bait systems developed for tsetse flies have been used *en masse* for population suppression and disease transmission reduction (Torr, 1994; Willemse and Takken, 1994). Recently, similar attempts have been made to develop a trap-bait system for the main malaria vectors, *An. gambiae* s.l. (Kweka *et al*., 2009; Kweka and Mahande, 2009; Mahande *et al*., 2010). The essentials in construction of these traps include the 'attractant', the physical trap design, and trapping mechanism used. Unlike the physical trap design and trapping mechanism, the ‘attractant’ may vary with different vector species. Consequently, the ‘attractant’ chosen will most likely influence the efficiency of the trap.

The odour baited trap was previously used to evaluate the mosquitoes’ preference to host odour (Constantini *et al.,* 2001; Mahande *et al.,* 2007). Human odours, especially sweat, have been demonstrated as good ‘attractants’ for *An. gambiae* s.s. (Smallegange *et al.*, 2005; Njiru *et al*, 2006; Olanga, 2010) while bovine odours attract *An.arabiensis* (Kweka *et al*., 2009; Kweka and Mahande, 2009; Mahande *et al*., 2010). Cattle urine is an outstanding attractant for *An. arabiensis* (Kweka *et al*., 2009; Kweka and Mahande 2009). Decaying cattle urine has been shown to be a useful ‘attractant’ that makes

operationalization of box traps targeting *An.* a*rabiensis* easier and cheaper (Mahande *et al*., 2010).

Attractive odours increase trap efficiency as female mosquitoes mainly use odour in locating their hosts (Mathenge *et al.*, 2002; Kweka *et al.,* 2009). However, the usefulness of cattle urine in baited traps has not been investigated in different ecological habitats for *An. gambiae* s.s, *An. funestus* and *Culex quinquefasciatus.* This method has the advantage that it does not risk human volunteers who may be exposed to infection via a mosquito bite. It is also easily accessible locally and abundant in rural communities (Kweka *et al*., 2010). Literature on the time at which cow urine remains an 'attractant' after application is necessary to assist in determining re-treatment rates and improving on the trap efficiency.

2.3.6 Outdoor mosquito collections using clay pots

Clay pots have recently been explored as a method for sampling both indoor and outdoor mosquitoes (Odiere *et al*., 2007; Bijllaardt *et al*., 2009). Clay pots were tested alongside CDC light traps in an area with low malaria endemicity in northern Tanzania. The proportion of fed female anophelines was significantly higher than in the light trap whereas clay pots sampled different segments of mosquito populations (Bijllaardt *et al.*, 2009). Another study conducted using clay pots for point source application of fungi as a biological control agent targeting the malaria vectors *An. gambiae s.s.* and *An. funestus* showed that clay pots are attractive resting place for both sexes of *An. gambiae s.s.*, and hence an efficient tool for the application of entomophagic fungi against

mosquito vectors (Farenhorst *et al*., 2008). Thus, besides being a sampling tool, clay pots can be used to apply pathogens to male and female mosquitoes that enter the pots to rest.

This method is broad-spectrum, cheap and portable, and thus less cumbersome. Clay pots (AgREPOT) used for sampling the outdoor resting *An. gambiae, An. funestus, An. arabiensis* and *Culex* species of both sexes in western Kenya compared with PSC were significantly efficient and can be used as a tool to quantify variation in mosquito population densities outdoor (Odiere *et al*., 2007) in various ecological habitats.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

This study was carried out in south coast Kenya in Msambweni and Kwale districts, Kwale County. The districts border Tanzania to the south-west and the Indian Ocean to the East (Figure 3.1). The area is hot and humid year round with a range of annual mean temperatures of 23°C - 34°C and average relative humidity range of 60% - 80%. Altitude ranges from 0 to 464 meters above sea level. There are two rainy seasons; long rains (April to June) and the short rains (October to December) and a month hardly passes without some rains especially nearer to the coastline. The total annual precipitation varies from 900mm to 1500mm per annum along the coastal belt to 500–600mm inland.

The study area is located in an area with endemic malaria and LF. The predominant vectors for human malaria include *An. gambiae* s.l. and *An. funestus* which occurs year-round with peaks of population abundance coinciding with seasonal rains (Mbogo *et al.,* 2003; Mutuku *et al*., 2011). Lymphatic filariasis predominant vectors are also *An. gambiae* s.l. and *An. funestus* with an added role of *Cx. quinquefasciatus* (Bogh *et al*., 1998; Muturi *et al*., 2006). In the rural areas of the Kenyan Coast; malaria and LF parasites are known to coexist in the same vectors and human populations (Muturi *et al*., 2006). According to the 2009 census, the estimated human population for Msambweni and Kwale districts is 288,393 and 151,978 respectively (Central Bureau Statistics, 2009).

The ethnic communities in Msambweni and Kwale districts are predominantly the Digo and Duruma communities that constitute some of the nine sub-tribes of the Mijikenda of the Kenyan coast, with small proportions of the Kamba people and other communities especially in urban areas. The inhabitants of this area are peasants, who cultivate cassava, and maize for subsistence and cashew nuts, coconuts and mangoes as cash crops. They also practice livestock keeping and fishing for their consumption. These communities construct their houses using sticks from coconut trees or bamboo for a frame; walls are made of mud and thatch their roofs from palm tree leaves (*makuti*).

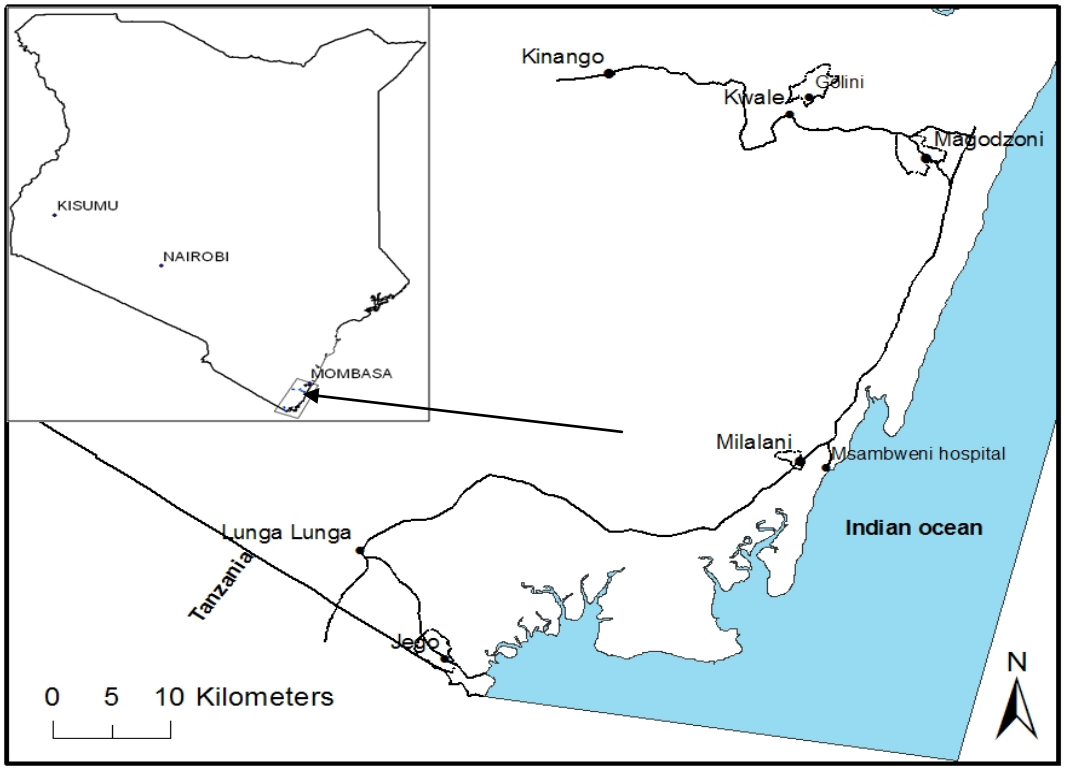

Figure 1: A map showing the location of the study area: Golini, Magodzoni, Milalani and Jego villages in Kwale County, South Coast Kenya

3.1.1 Study villages

Adult mosquito collections were done in Jego, Milalani, Magodzoni and Golini villages representing the coastal estuarine, coastal plain and coastal slope respectively. The selection of the villages was based on rainfall, elevation, relative humidity, distance from the sea and topography. Both Milalani and Jego villages have generally flat terrain and at comparable distances from the sea of (about 2 km. Jego village is characterized by aquatic habitats with higher salinity compared to other villages. Milalani and Jego villages were assigned to the coastal plain and estuarine habitats, respectively. The furthest village from the sea (Golini) has the widest elevation range. Temperature and rainfall decreased with increasing elevation while relative humidity (RH) increased with increasing elevation. Magodzoni and Golini villages were assigned to the coastal slope environment due to the topography and elevation changes.

3.1.1.1 Jego village

Jego village has a relatively flat terrain. It is about1.99km from the sea and has an elevation range of 4-26m. The average temperature is 28.2^0C (range 23.4-34.1^0C), rainfall 1384mm and the relative humidity averages 71.5% (range 54.8-92.7%). Almost half of the village has low lying areas with swampy environments. The drainage systems consist of the Umba River on the North West and a stream on the South East. The dominant vegetation cover is palm trees interspersed with shrubs and substantial rice fields. Households are mostly clustered along the main road which passes through the village

3.1.1.2 Milalani village

Milalani village also has a generally flat terrain. It is about 1.93km from the sea and an elevation range of 20-28m The average temperature, rainfall and relative humidity of this village is 27.8^{0}C (23.4-32.2^{0}C), 1214mm and 78.9% (range 64.5-100%) respectively. Water drains to several ponds and into Lukungwi stream on the South East. The vegetation cover is mainly palm trees with sizable rice fields. Households are randomly distributed and the communities in Milalani village practice low level subsistence cultivation of cassava and maize.

3.1.1.3 Magodzoni village

Magodzoni is characterized by gently rolling hills that are drained by several streams and the Mabu River. The distance from the sea is about4.62 km and has an elevation of 40-124m. The average temperatures are 26.7^{0}C ranging from 22.1^{0}Cto 30.5^{0}C and the relative humidity 77.7% ranging from 62.1% to 95.2%. Average rainfall in Magodzoni village is 846mm. The vegetation cover comprises of large grassland swaths on the West and palm, cashew nuts and mango trees on the East. Most households are situated on the eastern side and are clustered along the main roads.

3.1.1.4 Golini village

Golini village is characterized by gentle hills that are drained by two major streams with a distance of about16.35km from the sea and an elevation of 200-390m. The average temperature in Golini is 25.4^{0}C ranging from 21.2^{0}C to 29.2^{0}C, rainfall 846 mm and a relative humidity of 81.3% (range 66.0-94.5%). The area is heavily

dominated by palm trees with cashew nut trees and in close proximity to the Shimba hills national park. Households are clustered along the main roads.

3.1.2 Study design

Mosquito collections were done in three surveys; short rains (November-December 2010), dry season (March-April 2011) and the long rains (June-July 2011). Three equitably dispersed mosquito collection clusters of ten houses were randomly selected in each of the four villages. The criteria used for the selection was purposive. Adult mosquitoes were collected indoor using pyrethrum spray catches, prokopack aspirator and CDC light traps whereas outdoor cow-urine baited traps and clay pots techniques were used. Each mosquito collection survey consisted of bi-weekly collection intervals (Table 1).

The sampling methods were rotated among the selected houses in each village such that all the five methods were deployed once in each cluster. The sampling procedure was such that in the first cluster of ten houses of each village, PSC was paired with a clay pot and used to sample for mosquitoes indoor and outdoor, respectively. The night prior to the mosquito collection morning, the clay pots were set up outside the ten PSC houses. In the second cluster of ten houses, mosquitoes were sampled using prokopack aspirator paired with urine baited traps for sampling indoor and outdoor mosquitoes, respectively. Similarly, the urine bait traps were set up outside the ten prokopack aspirator houses the night prior to the mosquito collection morning. Five CDC light traps alongside bednets were then used to sample mosquitoes indoor in the last cluster

of ten houses (Table 1). Mosquitoes were collected from the same houses in each mosquito collection survey and each house was used for PSC only once per week due to the residual activity in the insecticide that is used for PSC.

Table 1: The experimental schedule used for setting up mosquito collection methods

Week	Village	Day	Cluster 1	Cluster 2	Cluster 3
One	A	1	LT/N	PK+UBT	PSC+ CP
	B	2	PK+ UBT	PSC+ CP	LT/N
	A	3	PSC+CP	LT/N	PK+ UBT
Two	B	1	LT/N	PK+ UBT	PSC+ CP
	A	2	PK+ UBT	PSC+ CP	LT/N
	B	3	PSC+ CP	LT/N	PK+ UBT

KEY
LT/N- light traps along human occupied mosquito nets
PK- Prokopack aspirator
UBT- Cow urine baited trap
PSC- Pyrethrum spray catches
CP- Clay pots

3.2 Adult mosquito collections

Pyrethrum spray catch, prokopack aspirator and CDC miniature light traps were used to trap adult mosquitoes indoor in each collection morning. Clay pots and urine baited traps were used to trap outdoor adult mosquitoes the night prior to the morning mosquito collections were carried out.

3.2.1 Indoor mosquito sampling using CDC light traps

In every mosquito trapping night, mosquitoes were collected from 10 randomly selected houses from a single collection cluster. In each house, light traps were hanged a meter off the ground and approximately150 cm (Figure 2) from an occupied mosquito net which may or may not have been treated with insecticide. These traps were positioned at the head side of the bed. Five light traps were switched on at 1800 hrs and switched off at 0600 hrs the next morning. Mosquitoes were then collected in the morning. Live mosquitoes were aspirated into clearly labeled paper cups and transported to the laboratory for further processing.

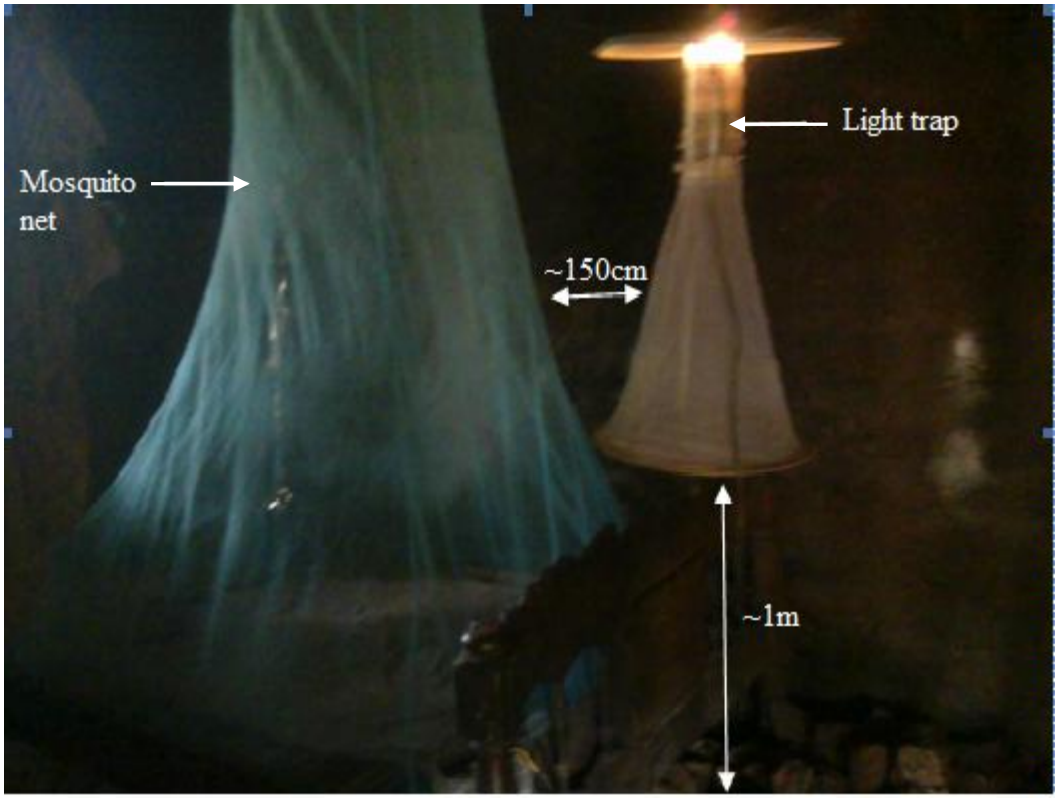

Figure 2: A photograph of a light trap set alongside a mosquito net.

3.2.2 Pyrethrum spray catches indoor method

Pyrethrum spray catch was carried out in 10 houses during each mosquito collection morning once per cluster in each village. White sheets were laid on the entire floor and over the furniture within all the rooms of each house. White sheets facilitate visibility of the knocked down mosquitoes. The doors and windows of the houses were shut then the rooms sprayed with 50ml of 10% permethrin dissolved in 5litres of kerosene as described by Gimnig *et al*. (2003). Briefly, a collector outside the house sprayed around the eaves with insecticide to prevent the mosquitoes inside the houses from escaping and another collector sprayed the roofs and the walls inside the house. The houses were then closed for 10-15 minutes. The white sheets were removed from all the rooms of the houses and the knocked down mosquitoes collected using forceps. Knocked down mosquitoes for each room were recorded and then transferred onto moist filter paper inside labeled petri dishes indicating the date and house number. The same procedure was repeated for all the ten houses and collected mosquitoes put in a cool box and transported to the laboratory for further processing.

3.2.3 Indoor mosquito sampling by prokopack aspirator

The prokopack aspirator used was powered by a 12V battery (Figure 3A). Similar to PSC, indoor resting mosquitoes were collected from 10 houses in each village using the prokopack aspirator in one mosquito collection cluster per collection night. Mosquitoes were aspirated from their resting and hiding places; under the bed, thatched roofs, upper regions of mud walls, dark and damp corners/areas, ceilings, the cracks and crevices in the walls as described by Maia *et al* (2011). In brief, the walls and ceilings in each

room of the ten houses were systematically aspirated using a progressive down and upward movement along its entire length. The open end of the plastic collection container (Figure 3B) was covered before switching off the prokopack aspirator to prevent the mosquitoes from escaping. Small cotton wool swabs were moistened with ethyl acetate to kill mosquitoes and other arthropods to ensure that the mosquitoes that were collected were not preyed upon. A different collection cup was used for each house and labeled accordingly. The collected mosquitoes were then transferred to the laboratory for further processing.

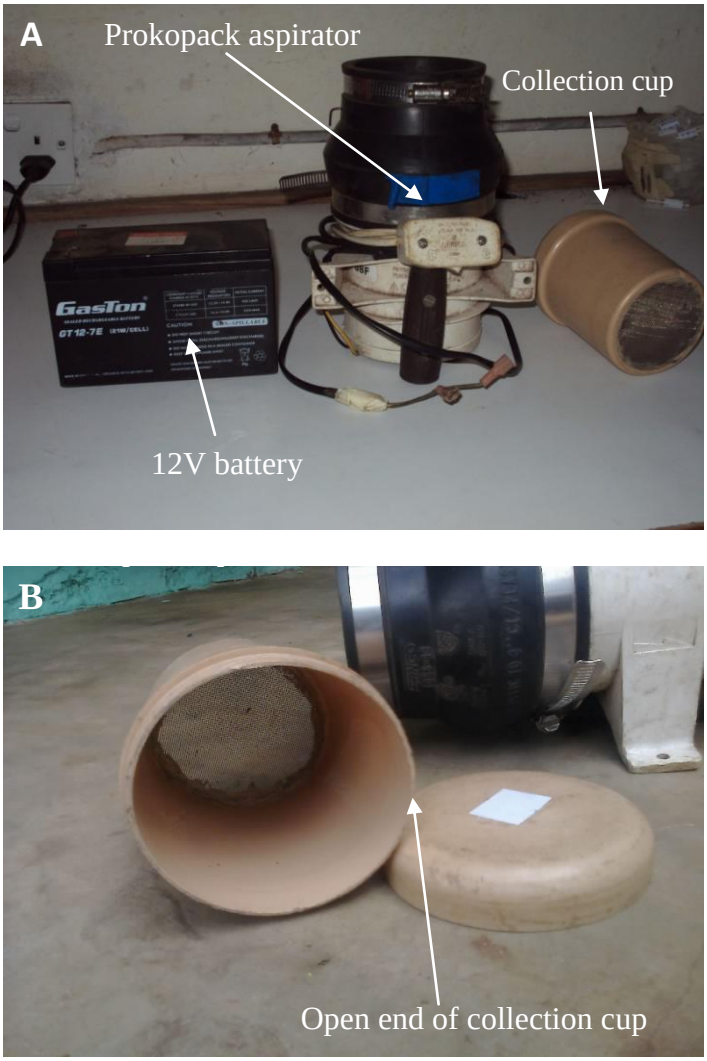

Figure 3: (A) Picture of the prokopack aspirator, 12V battery and plastic collection cup; (B) shows a picture of the open end of the collection cup

3.2.4 Outdoor mosquito collection by clay pots

Outdoor mosquito collections by clay pots were paired with indoor collections by PSC. The clay pots were locally constructed using clay and water measuring approximately 0.5 m in diameter and an opening of 20 cm width (Figure 4). The pots were principally used for storing water in the homes and traditional rituals such as "*kifudu*" by the communities in the study area. Each pot had a 2-cm hole at the center of the base of the pot rendering them useless for storage of water.

Three clay pots were set up outdoor near each of the 10 mosquito collection houses representing a single cluster (30 clay pots per collection night) during the short rains from November to December 2010. Only one clay pot was set up in each house during the dry season from March to April 2011 and the long rains from June to July 2011. The pots were set adjacent to the bedrooms at 1800 hrs and left overnight until 0600 hrs early morning. The mosquitoes were then collected using netted cages that were placed over the open end of the pot and secured by the collector. The pots were then lifted to expose the open end to the light so as to agitate the resting mosquitoes inside. The pots were then blown at the bottom into the small hole causing the mosquitoes to take to flight and into the netted cages.

Figure 4: A picture of a clay pot used to sample adult mosquitoes outdoor

3.2.5 Urine baited outdoor traps

During the short rainy season survey, 3 cow urine baited traps were set up outdoor per house (30 traps per collection trap night). The size of the urine baited traps were 25cm by 23cm by 15cm, constructed from cardboard and completely covered with a polythene paper to make it water proof. The entire inner part of the trap was lined with a black cotton cloth soaked in 7 day old cow urine to enhance trap efficiency. During the dry season and long rains surveys traps made from plastic buckets measuring approximately 0.5 m in length and width and 2 ft in height were placed on the ground at a 30^0 angle sideways facing the mud house (Figure 5). Only a single trap per house per mosquito collection night was set up during the dry and long rain seasons (10 traps per

collection night). Urine was collected by a washing basin from adult female Zebu cows (*Bos indicus*) of the same herd in Msambweni area. Black cotton cloth was soaked in fresh cow urine daily for seven days before the experiments. The soaked cotton clothing materials were placed at the bottom and held in place by a metal rod. The bucket was lined with a netting material that aided recovering mosquitoes from the trap (Figure 5A). Mosquitoes were collected from the urine baited traps between 0600 hrs and 0730 hrs. Collected mosquitoes were then transported to the Msambweni District hospital laboratory for identification and further processing.

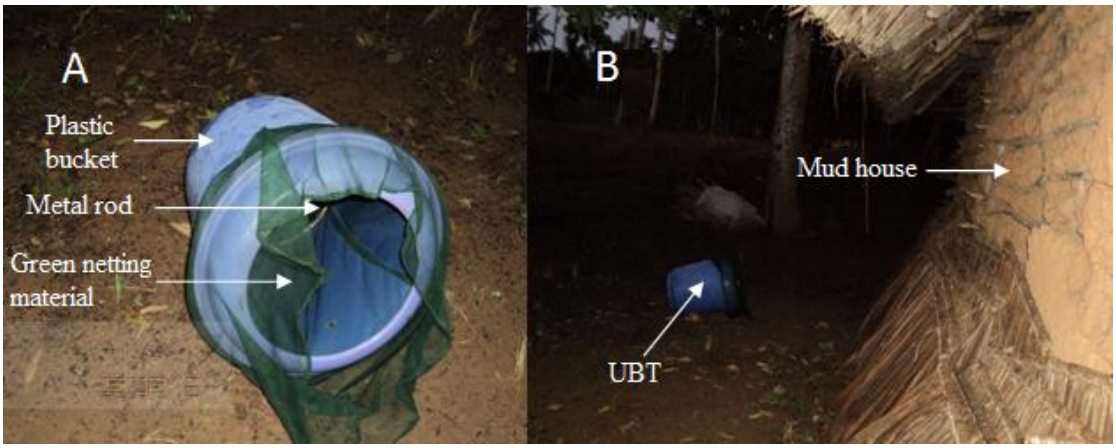

Figure 5: Cow urine baited trap used for sampling outdoor mosquitoes. (A) shows the different parts of the trap, (B) shows the urine baited trap set outside a traditional Digo mud house

3.3 Mosquito processing

All the collected mosquitoes were transported in cool boxes to the laboratory at the Msambweni District hospital. Live mosquitoes were killed using ethyl acetate, classified according to their species and sex and counted. Female mosquitoes were classified according to their abdominal conditions as blood fed, unfed or gravid. *An. gambiae* s.l, *An. funestus* and *Cx. quinquefasciatus* were identified morphologically as

described by Gillies and De Meillon, (1968) and Gillies and Coetzee, (1987) for each of the five collection methods. The other culicines collected were identified to the genus level as *Culex* species but the abdominal status was recorded.

All mosquito samples were subsequently dried over silica gel granules at room temperature and stored in -20°C freezers after completely desiccating to prevent decay. The heads and the thoraces of a portion of all female malaria vectors (*An. gambiae s.l* and *An. funestus)* were dissected and tested for *Plasmodium falciparum* circumsporozoite protein by enzyme linked immunosorbent assay (ELISA) technique (Wirtz *et al.,* 1987). Briefly, the heads and thoraces of *An. gambiae s.l* and *An. funestus* females were ground in 50µl of NP-40. The volume was then adjusted by adding 200µl of blocking buffer. The infected mosquitoes were then identified using ELISA technique. The abdomens of the blood fed and half fed *An. gambiae s.l, An. funestus* and *Cx. quinquefasciatus* were separated from the heads and thoraces, legs and wings to determine the host blood meal (Beier *et al*., 1988). In summary, the abdomens were ground in 50 µl of phosphate-buffered saline (PBS) with subsequent addition of 950 µl of PBS and then stored at -20°C. Blood meals were identified by a direct enzyme-linked immunosorbent assay (ELISA) using anti-host (IgG) conjugates (Kirkegaard and Perry, Gaithersburg, MD) against human, bovine and goat. All blood meal samples were screened simultaneously for human, bovine and goat antibodies.

3.4 Data management and analysis

Data analyses were computed using SPSS version 17.0 for Microsoft windows (SPSS Inc, Chicago IL). Mosquito counts collected by different traps were compared between the different villages and seasons. A longitudinal regression analysis using the generalized estimating equations (GEE) procedure in SPSS, version 17.0 was used to compare counts of mosquito species and sex. Analyses were determined for females of *An. funestus, Cx. quinquefasciatus* and other culicines. House was treated as a subject because mosquito count data were amassed by household. Mosquito collection traps, village and season were treated as both within-subject and between subject factors.

In the longitudinal regression analysis, the Wald statistic tested for the significance of the main effects of sampling methods (df =4), seasons (df =2) and villages (df =3). Comparisons of individual sampling methods were done with the LT/N as the reference method against which the Prokopack aspirator, pyrethrum spray catch, urine baited raps and clay pots methods were tested. Similarly, the long rains were the reference for seasons and Golini was the reference for village.

Kruskal–Wallis test was used to separately compare the effectiveness of the paired sets of indoor and outdoor methods for each species; Prokopack aspirator was paired with UBT and PSC with clay pots. Kruskal–Wallis test was also used to compare the relative efficiency of prokopack aspirator and PSC in collecting indoor resting mosquitoes.

3.5 Ethical clearance

The study involved the use of pyrethrum spray for collecting mosquitoes inside the houses. Individual verbal and written consent was sort from every head of the households. Ethical clearance was obtained from Kenya National Ethical Review committee (protocol approval number KEMRI/RES/7/3/1 amended in 2010), Ministry of Health (MOH) and from Kenyatta University before the onset of this research.

CHAPTER FOUR: RESULTS

4.1 Overall performance of the mosquito sampling methods

A total of 5,852 mosquitoes were sampled during the short rains, dry season and long rains. The numbers of mosquitoes collected per sampling method constituted 2,618 (44.4%) by Prokopack aspirator, 1,861 (31.6%) by pyrethrum spray catch and 1,237 (21%) by LT/N for the indoor sampling. For the outdoor sampling methods, the clay pots yielded 120 (2%) mosquitoes whereas the urine baited traps caught 60 (1%) (Table 2). The species composition was 1.9% *An. gambiae* s.l., 5.8% *An. funestus,* 3.8% *Cx. quinquefasciatus,* 88.5% *Culex* species, 0.3% *Aedes* spp. and 0.6% *Mansonia* spp. *Anopheles coustani* was collected by the LT/N. *Aedes* species were mainly sampled from the urine baited traps and clay pots.

The mean mosquito density per house was 0.06 for *An. gambiae* s.l, 0.17 for *An. funestus,* 0.12 for *Cx. quinquefasciatus* and 1.45 for *Culex spp,* respectively. Of the 1,587 houses in which mosquito sampling was carried out, at least a mosquito was caught in 1.4%, 7.6%, 6.7% and 32.0% for *An. gambiae* s.l., *An. funestus, Cx. quinquefasciatus* and *Culex* species, respectively.

Table 2: Diversity of mosquitoes sampled by different trapping methods in south coast Kenya

	Trap nights	***An. gambiae s.l***	***An. funestus***	***Cx. quinquefasciatus***	***Culex spp***	**Total**
Short rains (November-December 2010)						
PCS	120	0	14	19	772	805
Prokopack	120	0	12	23	1323	1358
LT/N	54	3	126	0	333	462
Clay pots	360	1	32	1	44	78
UBT	360	0	20	0	5	25
Total		**4**	**204**	**43**	**2477**	**2728**
Dry season (March-April 2011)						
PCS	120	2	8	7	184	201
Prokopack	120	4	11	32	380	427
LT/N	51	85	11	6	237	339
Clay pots	120	2	2	0	3	7
UBT	120	3	0	0	2	5
Total		**96**	**32**	**45**	**806**	**979**
Long rains (June-July 2011)						
PCS	120	0	25	48	782	855
Prokopack	120	0	14	79	737	830
LT/N	42	10	53	7	329	399
Clay pots	120	0	6	1	24	31
UBT	120	5	0	2	22	29
Total		**10**	**104**	**137**	**1894**	**2144**

The paired sets of indoor and outdoor methods performed differently for the various mosquito species. The Prokopack aspirator captured similar numbers of *An. gambiae* s.l as UBT. Similarly, the total PSC catches were comparable to those of clay pots. For *An. funestus*, the indoor methods, Prokopack aspirator (Kruskal–Wallis $\chi^2 = 8.28$, $p<0.01$) and PSC (Kruskal–Wallis $\chi^2 = 3.87$, $P<0.05$) captured significantly more female

mosquitoes than their corresponding outdoor methods (UBT and clay pots, respectively). The indoor methods sampled significantly higher numbers of *Cx. quinquefasciatus* (Kruskal–Wallis $\chi^2 = 58.30$, $p<0.0001$) and *Culex spp.* (Kruskal–Wallis $\chi^2 = 228.89$, $p<0.0001$) than the outdoor traps. Overall, while the Prokopack aspirator was the best for collecting *Cx. quinquefasciatus* and *Culex spp*, LT/N were effective than all the other methods in sampling *An. funestus* and *An. gambiae* s.l. in all villages and seasons.

Light traps alongside human occupied bed net, pyrethrum spray catch and Prokopack aspirator trapped 97% of the total number of mosquitoes collected while the urine baited traps and clay pots trapped only 2.9%. Light traps collected *An. funestus* and *An. gambiae s.l* females and nearly 4 times more females than males (Figure 6). Prokopack aspirator collected the most number of males (58%) indoor. Clay pots (2%) collected twice the number of mosquitoes of both sexes compared to the urine baited traps (1%) outdoor. Prokopack aspirator collected nearly twice as many males than females. Overall, all sampling methods collected more males (52%) than females (48%) except for the LT/N (Figure 6).

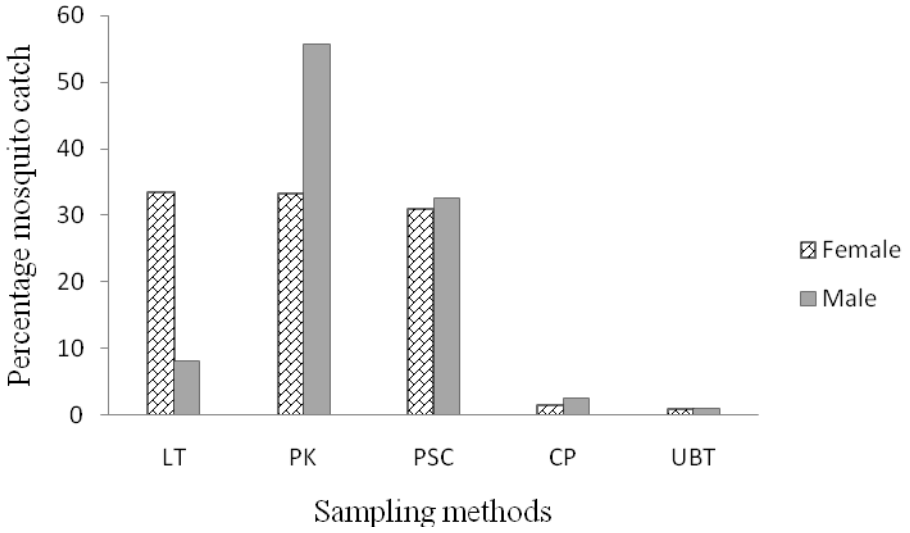

Figure 6: Mean percentage of male and female mosquitoes collected by each sampling method in south coast Kenya.

LT- CDC miniature light trap, PSC-Pyrethrum spray catch, Pk-Prokopack aspirator, UBT-Urine baited traps, CP- Clay pots.

The abdominal status of the females varied with species and the trapping method. Light traps alongside human occupied bed nets collected 87% of unfed female mosquitoes of *An. gambiae* s.l., *An. funestus*, *Cx. quinquefasciatus* and other *Culex* species compared to PSC, prokopack aspirator, UBT and clay pots. Prokopack and PSC were consistent in trapping the blood fed and gravid females for all species. UBT and clay pots were not consistent in trapping a specific abdominal status (Figure 7). For *An. gambiae s.l*, LT/N collected mostly unfed females while Prokopack and PSC caught only blood fed mosquitoes. Blood fed, unfed and gravid *An. funestus* mosquitoes were collected by PSC, Prokopack aspirator, UBT and clay pots except for the LT/N that mainly collected unfed (Figure 7). *Culex quinquefasciatus* collected by clay pots were all blood fed whereas UBT caught gravid females for *An. funestus, Cx quinquefasciatus* and other

culicines except for *An. gambiae s.l.* Outdoor methods were not consistent in trapping mosquitoes of a specific abdominal condition (Figure 7).

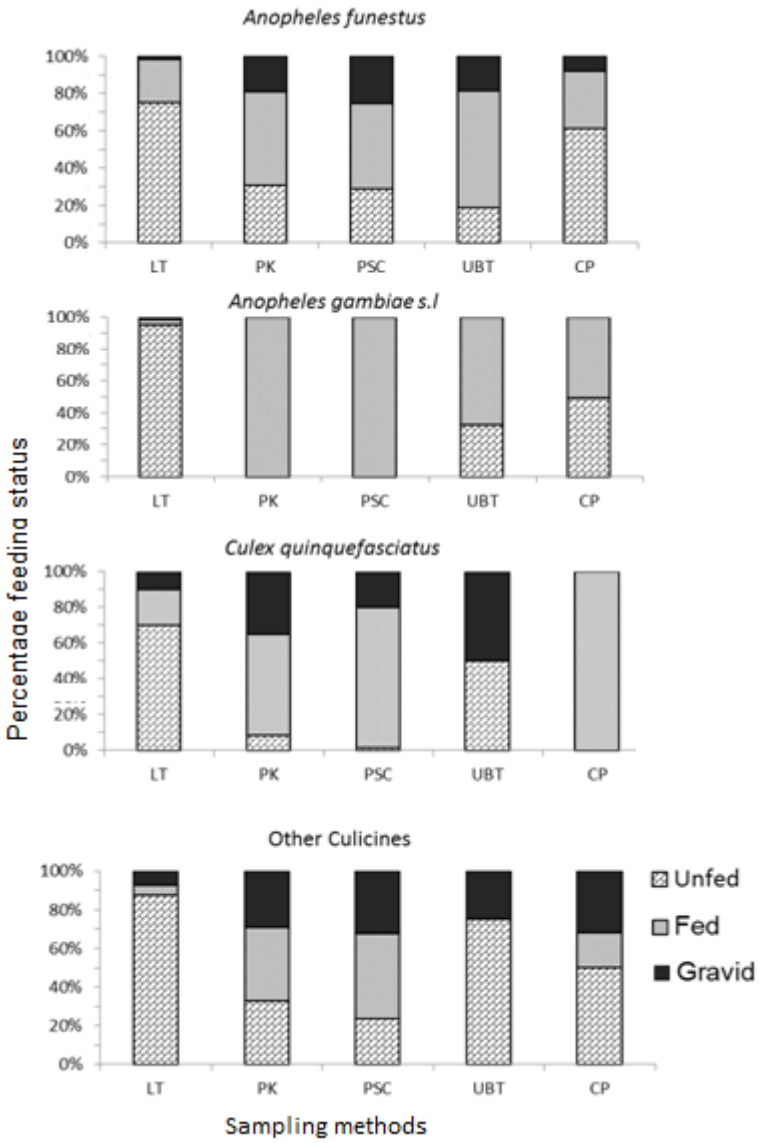

Figure 7: Proportions (%) of *An. funestus, An. gambiae s.l, Cx. quinquefasciatus* and other culicine females in three physiological categories (blood fed, gravid and unfed) sampled by five sampling methods in south coast Kenya.

LT- miniature light trap, PSC-Pyrethrum spray catch, Pk-Prokopack aspirator, UBT-Urine baited traps, CP- Clay pots

4.2 Comparative effectiveness of sampling methods

Light traps alongside human occupied mosquito nets, urine baited traps and clay pots collected more *Anopheles* mosquitoes (6.1%). PSC and Prokopack aspirator were similar in sampling both *An. gambiae s.l* and *An. funestus* but the Prokopack aspirator was effective in sampling *Cx. quinquefasciatus* and other culicines (75%) , especially males (Figures 8A and B). The outdoor methods collected few (4 mosquitoes) or no *Cx. quinquefasciatus* (Figures 8A and B; Table 3). Mosquito sampling method was significantly associated with sampling effectiveness in trapping female *An. funestus* ($P<0.01$). Light traps alongside mosquito nets was the most effective mosquito trapping method for *An. funestus*, trapping four times as many *An. funestus* as the other four trapping methods combined (Figures 8A and B; Table 3). Mosquito population densities were extremely low in most villages and across all seasons.

From this study 77.3% of *An. gambiae* s.l mosquitoes were collected during the dry season by LT/N. Out of the total number of *An. gambiae* s.l. (110) collected, 85 were captured during a single trapping night by three CDC light traps in three different houses in Jego village in the dry season. *An. gambiae* s.l densities were extremely low in most villages and in all seasons. LT/N remained the most effective trapping method for *An. gambiae* s.l., even without the outlier houses (Figures 8A and B; Table 3).

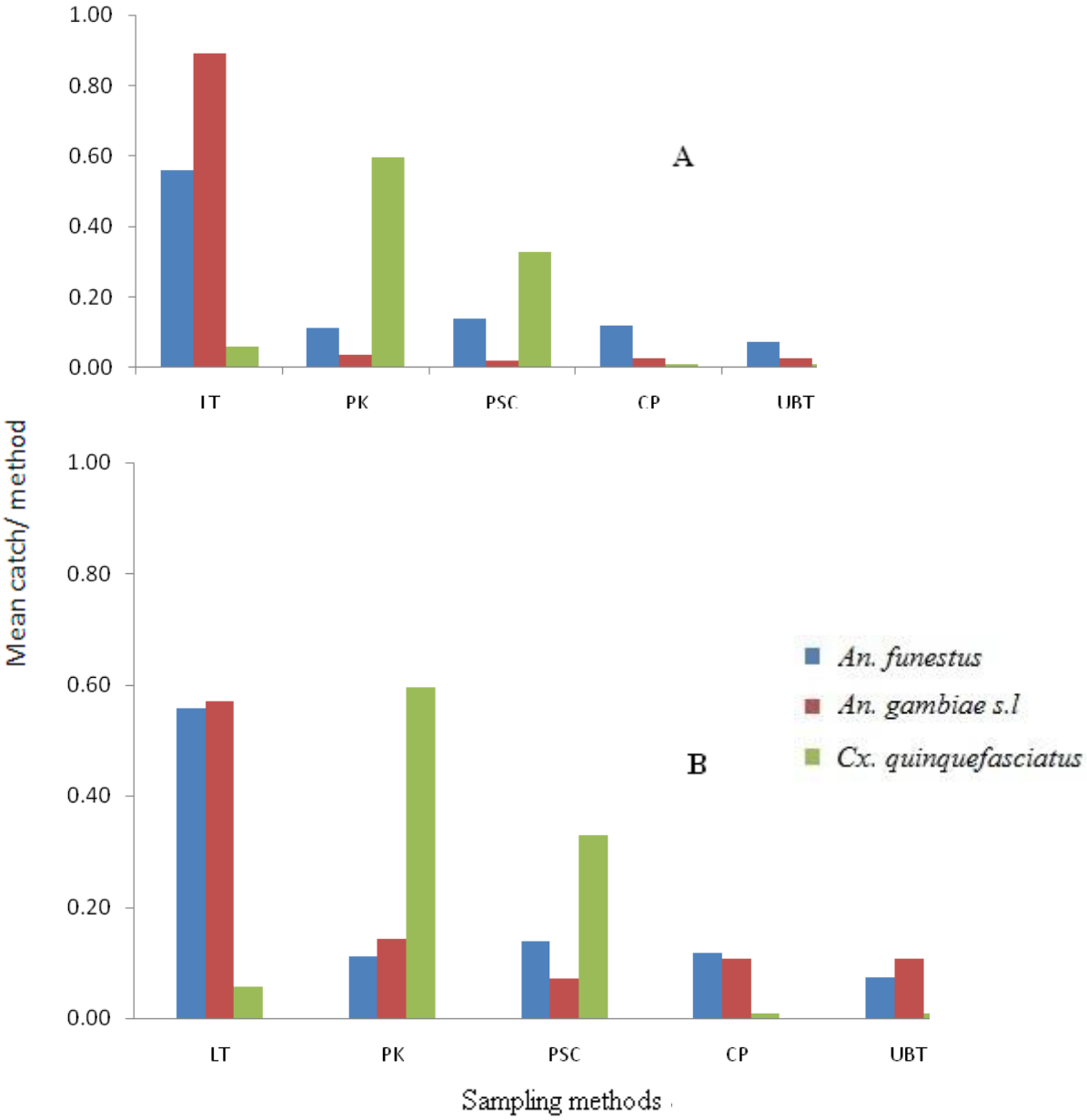

Figure 8: Mean number of mosquitoes collected using five sampling method; Mosquito count mean calculation in figure 8A includes the outlier houses and 8B did not include them.

LT-CDC miniature light trap, PK- Prokopack aspirator, PSC- Pyrethrum spray catch, CP- Clay pots, UBT- Urine baited traps.

The *An. funestus* mean catch of 0.60 were significantly higher in the short rains (P<0.01) and 0.61 for *Cx. quinquefasciatus* in the long rains seasons (P<0.01) collected by LT/N and Prokopack aspirator, respectively. *An. gambiae* s.l was substantially low in the rainy seasons as shown in Figures 9 C and D. Unlike in the dry season, when all sampling methods recovered *An. gambiae s.l*, few or no *An. gambiae s.l* were collected by most of the sampling methods during either the short or long rainy seasons. *An. gambiae* s.l and *An. funestus* showed comparable fluctuations in the mean catch in all seasons collected by all traps. *Cx. quinquefasciatus* on the other hand had minimal variation between the short rains and the dry season and peaked during the long rains season (Figures 9 C and D, Table 3). Significantly more *An. funestus* (Wald = 84.59, P<0.01) and *Culex spp* (Wald = 249.8, P<0.01) were collected by all the traps during the rainy seasons compared to the dry season (Figures 9 C and D, Tables 3 and 4).

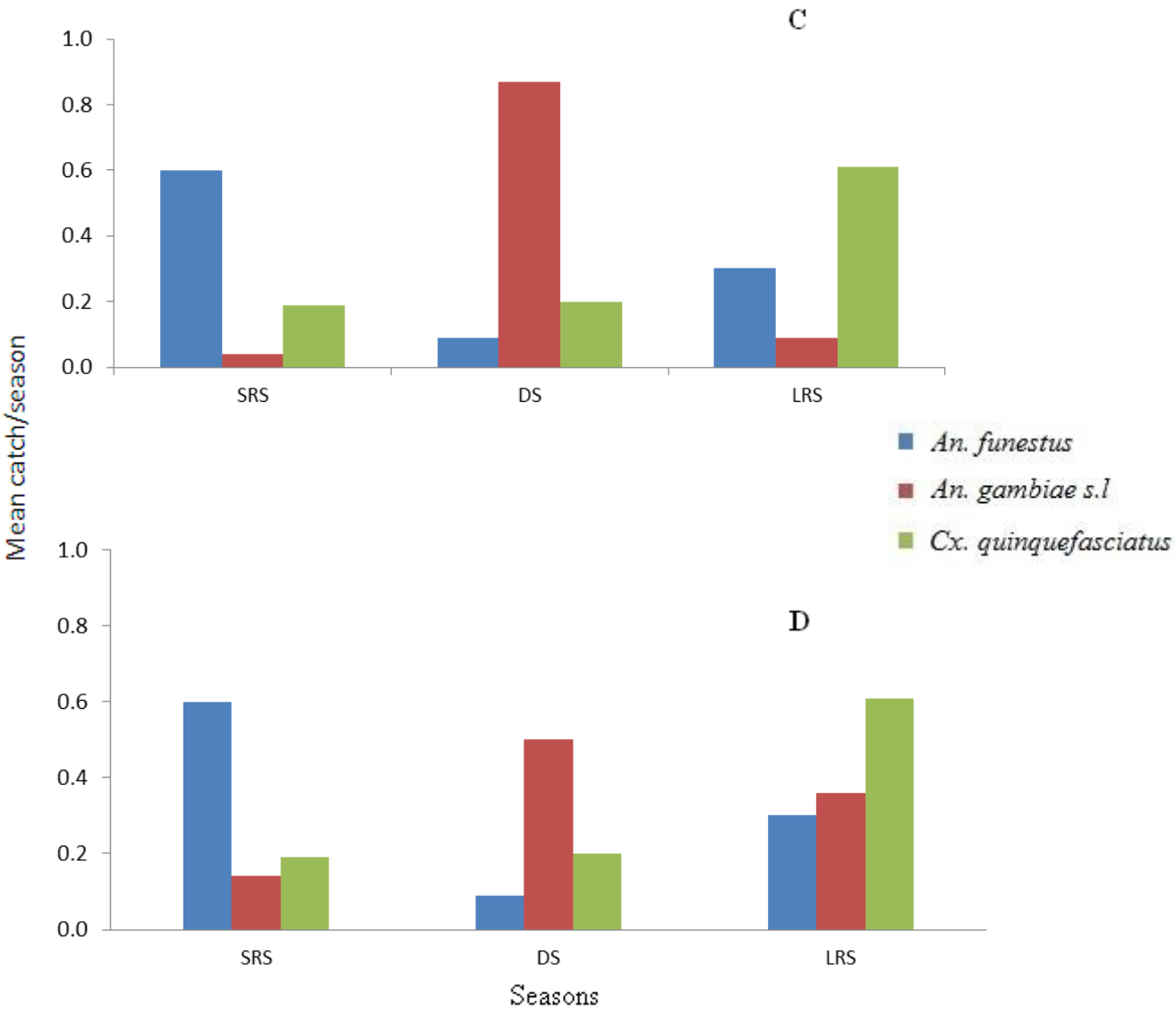

Figure 9: Mean number of mosquitoes collected during the short and the long rains and the dry season; Mosquito count mean calculation in figure 9C includes the outlier houses and 9D did not include the outlier houses.

SRS- Short rains season, DS- Dry season, LRS- Long rains season

Anopheles funestus, An. gambiae s.l and *Cx. quinquefasciatus* were collected in all the four villages (Jego, Milalani, Magodzoni and Golini) by all sampling methods. *An. gambiae* s.l had the highest mean catch of 0.85 in Jego village whereas *An. funestus* and *Cx. quinquefasciatus* were abundantly collected from Milalani village with a mean of 0.59 and 0.36 respectively (Figures 10E and F). The bulk (85/110) of *An.gambiae* s.l. were collected during a single trapping night by three CDC light traps in three different houses in one village (Jego) during the dry season. Light traps were the most efficient trapping method for *An. gambiae s.l* on all other nights. Magodzoni had the highest catch of *Cx. quinquefasciatus* (outliers) and highest mean numbers of *An. gambiae* s.l excluding outliers (Table 3). Very few or no (0.04) *An. funestus* and *An. gambiae* s.l were collected from Golini village by any of the sampling methods (Figures 10E and F). For all the species collected, Milalani and Jego villages had the highest mosquito densities of 51% and 37% respectively then Magodzoni (7%) and last Golini village (5%) (Figures 10E and F).

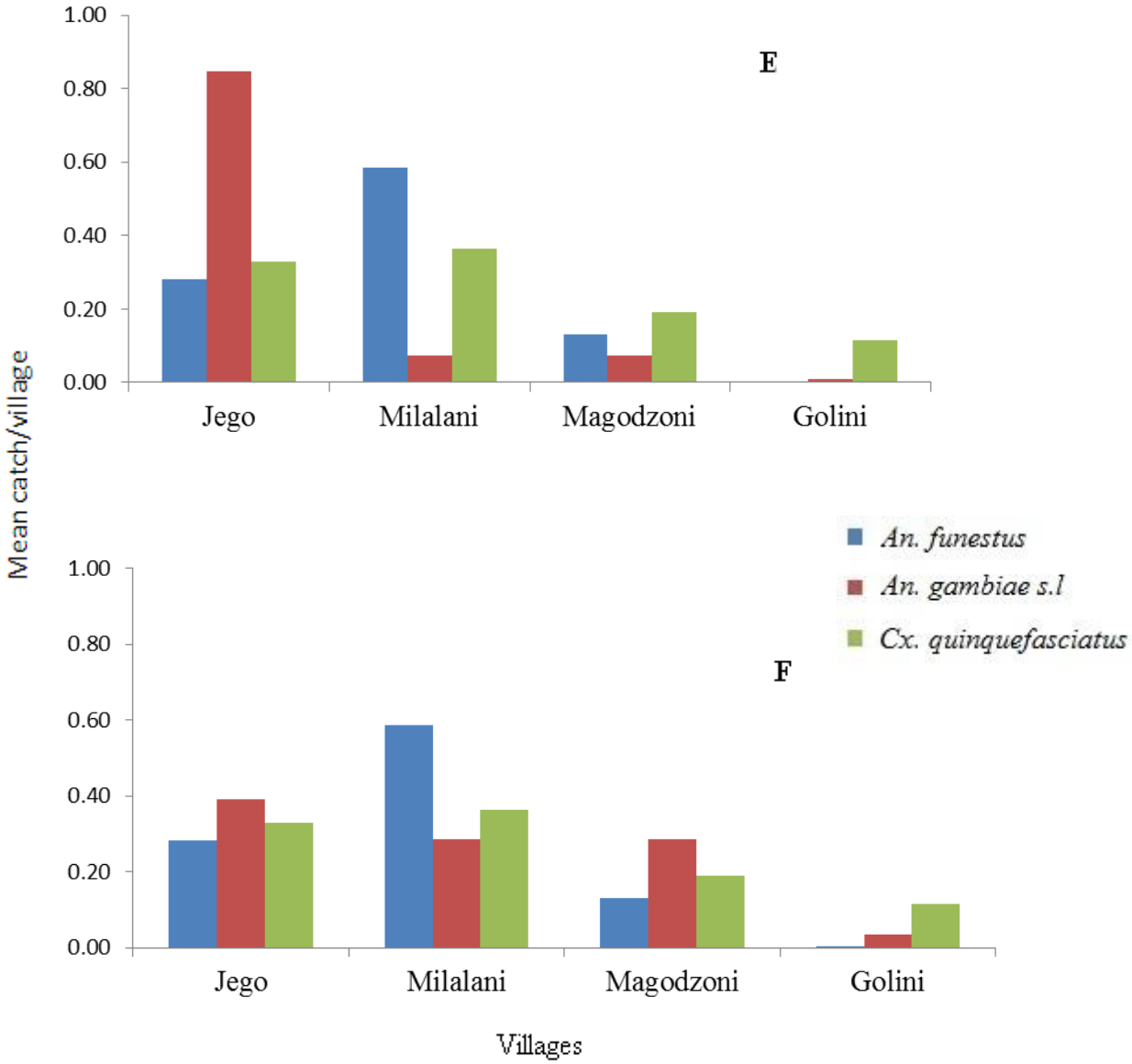

Figure 10: Mean number of mosquitoes collected in four villages; Mosquito count mean calculation in figure 10E includes the outlier houses and 10F did not include the outlier houses.

Table 3: Longitudinal regressions using generalized estimating equations of *An. funestus* females and *Cx. quinquefasciatus* females by five sampling methods in four villages during three seasons in south coastal Kenya, November 2010-July 2011

	Species	Wald statistic[a]	Parameter estimate[b]	Lower and upper CI
***An. funestus* Females**				
Sampling methods	Urine baited trap	84.591**	-2.323	-3.231 (-1.414)
	Clay pot		-2.122	-2.863 (-1.381)
	Prokopack		-1.799	-2.342 (-1.256)
	PSC		-1.466	-2.003 (-0.928)
	Light trap			-
Seasons	Short rains season	16.546**	-0.047ns	-0.481 (0.387)
	Dry season		-1.285	-1.931 (-0.638)
	Long rains season			-
Villages	Jego	21.012**	3.917	1.964 (5.869)
	Milalani		4.158	2.208 (6.108)
	Magodzoni		3.520	1.538 (5.502)
	Golini			-
***Cx. quinquefasciatus* Females**				
Sampling methods	Urine baited trap	52.457**	-1.991	-3.528(-0.454)
	Clay pot		-2.515	-4.582(-0.448)
	Prokopack		1.463	0.773(2.152)
	PSC		0.854	0.118(1.589)
	Light trap			-
Seasons	Short rains season	21.032**	-1.121	-1.667(-0.575)
	Dry season		-0.751 ns	-1.223(-0.278)
	Long rains season		-	-
Villages	Jego	3.255**	0.432 ns	-0.129(0.993)
	Milalani		0.130 ns	-0.460(0.720)
	Magodzoni		0.060 ns	-0.587(0.707)
	Golini		-	-

Analysis for both male and female *An. gambiae* s.l. and *An. funestus* and *Cx. quinquefasciatus* males was not performed due to small numbers sampled. ns - not significant; * -$P < 0.05$; **,- $P < 0.01$; *** - $P < 0.001$.

Table 4: Longitudinal regressions using generalized estimating equations of other culicine mosquitoes collected by five sampling methods in four villages during three seasons in south coast Kenya from November 2010 to July 2011

	Species	**Wald statistic[a]**	**Parameter estimate[b]**	**Lower and upper CL**
Culex spp. male and female				
Sampling methods	Urine baited trap	249.809**	-3.427	-4.254(-2.600)
	Clay pot		-2.608	-3.030(-2.185)
	Prokopack		0.187 ns	-0.145(0.518)
	PSC		-0.092 ns	-0.494(0.311)
	Light trap		-	-
Seasons	Short rains season	38.843**	0.182 ns	-0.175(0.539)
	Dry season		-0.797 ns	-1.134(-0.460)
	Long rains season		-	-
Villages	Jego	191.427**	1.057	0.577(1.538)
	Milalani		1.427	1.032(1.822)
	Magodzoni		-0.569 ns	-0.984(-0.154)
	Golini		-	-
***Culex* spp. Males**				
Sampling methods	Urine baited trap	155.154**	-1.006	-2.404(0.391)
	Clay pot		-0.369 ns	-1.481(0.743)
	Prokopack		2.554	1.465(3.642)
	PSC		2.038	0.886(3.190)
	Light trap		-	
Seasons	Short rains season	10.105**	-0.016 ns	-0.633(0.602)
	Dry season		-0.870 ns	-1.440(-0.301)
	Long rains season		-	-
Villages	Jego	54.163**	1.402	0.697(2.107)
	Milalani		1.273	0.647(1.900)
	Magodzoni		-0.171 ns	-0.778(0.436)
	Golini		-	-
***Culex* spp. Females**				
Sampling methods	Urine baited trap	205.209**	-4.345	-5.195(-3.496)
	Clay pot		-3.193	-3.756(-2.630)
	Prokopack		-0.551 ns	-0.848(-0.253)
	PSC		-0.519 ns	-0.839(-0200)
	Light trap		-	
Seasons	Short rains season	25.858**	0.191 ns	-0.104(0.486)
	Dry season		-0.516 ns	-0.796(-0.236)
	Long rains season		-	
Villages	Jego	201.957**	0.709 ns	0.316(1.103)
	Milalani		1.201	0.858(1.544)
	Magodzoni		-1.068	-1.477(-0.659)
	Golini		-	

Analysis for both male and female *An. gambiae* s.l. and *An. funestus* and *Cx. quinquefasciatus* males was not performed due to small numbers sampled. ns - not significant; * -$P < 0.05$; **,- $P < 0.01$; *** - $P < 0.001$

4.3 Mosquito infection rates

A total of 336 female anopheline mosquitoes were tested for the presence of *P. falciparum* circumsporozoite protein by ELISA. The overall sporozoite infection rate was 1.78% (6/336). The infection rates for *An. funestus* and *An. gambiae* s.l were 1.94% (5/257) and 1.26% (1/79) respectively. Chi-square statistics did not show significant differences in infection rates by village, collection method, season or species (Table 5).

Light traps alongside human occupied bet net collected 3 infected *An. funestus* and 1 *An.gambiae s.l.* while PSC collected 2 infected *An. funestus* mosquitoes. The single infected *An. gambiae* s.l. collected by the LT/N was trapped during the dry season in Jego village. In the short rainy season, four of the five infected *An. funestus* were collected and only a single infected *An. funestus* mosquito during the long rainy season. The infected mosquitoes were evenly distributed across Jego, Milalani and Magodzoni villages (two infected mosquitoes in each village). There were no infected mosquitoes collected by PSC, Prokopack, LT/N, UBT and clay pots from Golini Village. Light traps alongside human occupied bet net were effective in collecting 2 infected *An. funestus* and 1 infected *An. gambiae s.l.*

Table 5: Mosquito infection rates in Kwale county, south coast Kenya

Species	**No. of anopheles tested**	**No. of infected mosquitoes**	**Infection rates (%)**
An. funestus	257	5	1.94
An. gambiae s.l	79	1	1.26
Total	**336**	**6**	**1.78**

4.4 Mosquito blood meal sources

Of the total number of mosquitoes (n=434) tested for blood meal, 67% were ELISA positive and 33% were negative. Humans constituted of 54% of the blood meal sources for *An.gambiae s.l, Cx. quinquefasciatus* and other culicine mosquitoes with an exception of *An.funestus* and 10% constituted non-human hosts including cattle and goats (Table 6). The distribution of the tested *An. funestus* by village was 14, 45 and 13 fed mosquitoes were collected from Jego, Milalani and Magodzoni respectively. Blood fed *An. funestus* collected from Jego and Milalani were by UBT, PSC, Prokopack aspirator, clay pots and LT/N while fed *An. funestus* from Magodzoni were by LT/N, Prokopack and PSC. *Anopheles funestus* in Jego village representing 51% fed on cattle; 43% of *An. funestus* that fed on bovine were from two houses adjacent to each other where relatively large herds of cattle were kept. In Milalani, 25% and 42% *An. funestus* had cattle/goat and human feeds respectively while feeds of 33% mosquitoes were ELISA negative. On average, 7% households owned cattle and 11% owned goats. *Anopheles funestus* that fed on cattle/goat (9/11) were collected from two houses also with herds of both cattle and goats. A half (50%) of the tested blood fed *An.funestus* collected by UBTs had fed on cattle.

Table 6: Mosquito blood meal sources in Kwale county, south coast Kenya

Species	**Number of mosquitoes tested**	**Human (%*)**	**Cattle+Goats (%*)**	**[+]Mixed blood meals (%*)**	**ELISA negatives (%*)**
An. gambiae s.l.	4	2 (50)	0 (0)	0 (0)	2 (50)
An. funestus	72	17 (24)	25 (35)	3 (4)	27 (37)
Cx. quinquefasciatus	101	68 (67)	2 (2)	0 (0)	31 (31)
Culex spp.	257	147 (57)	14 (6)	11 (4)	85 (33)
Total	**434**	**234 (54)**	**41 (10)**	**14 (3)**	**145 (33)**

*Percentage of total number of tested mosquito
[+]Mixed blood meals included human blood mixed with either that of cattle or goat.

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study assessed mosquito sampling effectiveness of Centers for Disease Control and Prevention miniature light traps, Prokopack aspirator, Pyrethrum spray catch, urine baited traps and clay pots focusing on their comparative evaluations in Kwale County, an area with low Anopheles mosquito densities (Mutuku et al., 2011). Light traps were the most effective for sampling female *An. gambiae* s.l and *An. funestus*, while the Prokopack aspirator was the most effective in collecting *Cx. quinquefasciatus* and *Culex* spp. The results demonstrated that LT/N collected more host seeking *An. funestus* and *An. gambiae* s.l females than PSC and prokopack aspirator indoor. These species have been reported to be major malaria vectors in coastal Kenya (Mbogo *et al*., 2003; Mutuku *et al*., 2011) an indication that LT/N can be useful for monitoring malaria transmission.

In related studies, Fornadel *et al*. (2010) reported that in Zambia CDC miniature light traps have shown equally high sampling efficiencies from locations with comparable ecological conditions and are also fairly good for sampling endophagic malaria and LF vectors in Africa (Odetoyinbo., 1969; Lines *et al*., 1991; Mboera *et al*., 1998). These findings are also supported by other studies that showed that light traps may be a sensitive alternative to estimate human-biting activity of anopheline mosquitoes (Zaim *et al*., 1986; Davis *et al*., 1995; Duo-quan *et al.,* 2012). On the other hand, Overgaard *et al*. (2012) have shown that the light traps are likely not the best tool for collecting host-

seeking mosquitoes even though in this study LT/N were effective than PSC, Prokopack aspirator, urine baited traps and clay pots in sampling host seeking female anophelines. Light traps were inferior to HLC in Dar es Salaam, Tanzania where mosquito densities are comparable to those in the current study area (Govella *et al.*, 2011). The performance of the light trap has been reported to be dependent on mosquito abundance, especially at low densities (Mbogo *et al.,* 1993; Overgaard *et al* 2012). The results obtained from this study suggest that light traps will continue being useful in mosquito surveillance for monitoring the malaria and LF vectors, *An. gambiae* s.l and *An. funestus*. *Anopheles arabiensis*, which comprised the larger proportion of the *An. gambiae* s.l population in the study area, is known to be anthropophilic.

Prokopack aspirator was shown to be efficient in trapping *Cx. quinquefasciatus*, an important vector for LF in the study area (Pedersen and Mukoko, 2002; Rwegoshora *et al*., 2005). Prokopack aspirator recorded relatively higher efficiencies for male *Cx. quinquefasciatus* and *Culex* species and is thus suitable for vector control programmes involving larval control and/or use of sterile male mosquitoes. The female *An. gambiae* s.l collected by Prokopack and PSC were mainly fed implying that *An. gambiae* s.l mosquitoes are habitually highly anthropophagic, endophilic and endophagic. These behaviour patterns have also previously been reported in other studies (Fontenille *et al*., 1997; Oyewole *et al*., 2005; Russell *et al*., 2010). Additionally, female mosquitoes are slow in flight when fully engorged hence the fed status of the indoor-captured mosquitoes or the number of mosquitoes resting for a blood meal digestion (WHO, 1975). The Prokopack aspirator was also shown to be a useful tool in collecting female

Cx. quinquefasciatus than PSC and light traps indoor. This suggests that Prokopack could potentially be useful in monitoring lymphatic filariasis vectors with high infection and transmission rates in Kenya. Pyrethrum spray catch method is arduous, time consuming and a bother to the house owners. It is often used to catch fed and gravid indoor-resting females and indeed tends to miss the mosquitoes that leave the house after feeding and includes those entering the house after feeding outdoor (Mboera, 2005; Odiere *et al*., 2007; Ndiath *et al*., 2011). Furthermore, rural houses are difficult to completely seal off due to large eaves and large cracks on the mud walls hence mosquitoes escape before knock down. However, Prokopack is easy to assemble, cheap and more maneuverable increasing coverage of mosquito sampling as compared to the PSC method. The observation in this study suggests that Prokopack can be used in place of PSC that is cumbersome in collecting blood fed and gravid female mosquitoes.

The intensive trapping effort across three seasons, using five different traps in this study, demonstrated that malaria and LF vectors densities remained low in all the four villages. The anopheline densities reported here were lower than those reported by a recent study in south coast Kenya (Mutuku *et al.*, 2011). These low mosquito counts and sparse distribution prevented meaningful comparisons between and among the several trapping methods, especially for the two outdoor methods. Clay pots and urine baited traps had low sampling efficiencies which may have been constrained by the resting and passive mosquitoes. Outdoor methods target resting passive mosquitoes that are either endophagic or exophagic and exophilic. This observation may also be

explained by environmental conditions. During the study period, the weather was characteristic hot, humid and windy. A previous study conducted by Bijllaardt *et al.* (2009) that used clay pots both indoors and outdoors in northern Tanzania attributed poor catch outdoors to dry and hot weather which may also be the case in this study. On the other hand, western Kenya where rainfall is seasonally bimodal with the long rains from March through May and the short rains from November to December, clay pots were efficient in sampling malaria vectors (Odiere *et al*., 2007).

High mosquito densities for *An. funestus, Cx. quinquefasciatus* and *Culex* species corresponded to the rainy seasons. The mosquito densities were collectively high in space and time especially for *An. gambiae s.l* and *Cx. quinquefasciatus*. Although Milalani village recovered slightly more mosquito densities than Jego village, the differences were not significant which is implied by the commonly observed trends of a decrease in mosquito densities collected with increasing altitude (Bodker *et al*., 2003; Kurlkarni *et al*., 2006; Ndenga *et al*., 2006) and increasing distance from the sea. This trend was observed for *An. gambiae s.l, An. funestus, Cx. quinquefasciatus and Culex spp* in all sampling methods in this study.

Anopheles mosquitoes tested for infection with *P. falciparum* indicated a significantly low infectivity rate. Similar to other mosquito sampling evaluation studies have shown that collection methods had no effect on the sporozoite rates (Mathenge *et al*., 2004; Ndiath *et al*., 2011). Previous adult mosquito sampling evaluation studies by Sikulu *et al*. (2009) have reported infection rates that were too low for a meaningful analysis and Fornadel *et al*. (2010) demonstrated no infections. The observation that low infection

rates in the mosquitoes and the absence of association with sampling method is in contrast to previous studies by Davis *et al*., (1995) and Mbogo *et al*., (1993).This may be due to an increased insecticidal interventions (Lindblade *et al*., 2006; Bayoh *et al*., 2010; Mutuku *et al*., 2011) in the current study area.

There was a dramatic decline in the Human blood index of 0.24 in this study as compared to a study conducted by Mutuku *et al.* (2011) in 2009/2010 that had a HBI of 0.94. The extended use of ITNs has been associated with the feeding behavioral change of important malaria vectors of Africa; *An. gambiae s.l* and *An. funestus* (Charlwood and Graves, 1987; Geissbuhler *et al*., 2007; Russell *et al*., 2011). Lindblade *et al.* (2006) and Magesa *et al*. (1991) demonstrated that LLINs and ITNs significantly reduce human-vector contact due to intervention pressure as well as the malaria vector populations and as a result these species are compelled to look for alternative hosts. The high numbers of mixed blood meals consisting of cattle or goat blood in this study suggests that the mosquito vector may have fed on alternative host. The differences in HBI between the two studies may also be attributed, at least in part, to differences in experimental designs. The few and unevenly distributed alternative hosts may explain the concentration of non-human vertebrate blood meals in few houses. Some (33%) of the blood meals were ELISA negatives. It is possible that the mosquitoes fed on other non-human vertebrate hosts other than bovine and goat.

5.2 Conclusions

1. Centre for Disease Control and Prevention miniature light traps alongside human occupied bed nets were the most effective method in sampling *Anopheles* species indoors. Prokopack aspirator was effective in sampling *Cx. quinquefasciatus* and *Culex* species. Prokopack aspirator and CDC light traps are both potential for indoor sampling of malaria and lymphatic filariasis vectors in south coast Kenya.
2. Clay pots and urine baited traps are unsuitable for outdoor sampling of malaria and LF vectors in coast Kenya.
3. There was no association of the low infection rates and mosquito sampling methods. In low mosquito densities, many mosquitoes should be collected in order to detect any infections. Species vectors prefer alternative hosts due to intervention pressure of insecticides; ITNs, LLINs and IRS.

5.3 Recommendations

1. Centre for Disease Control and Prevention miniature light traps alongside human occupied mosquito nets could be used as an alternative for human landing catches to estimate the human–biting activity of *Anopheles* species. Prokopack aspirator should be used to replace labor intensive and intrusive PSC. There should be further evaluation of the indoor adult sampling methods through longitudinal comparisons.
2. There should be intensified research for effective and practical outdoor adult mosquito sampling methods in different ecological settings.

3. Adult mosquito sampling methods alongside intervention strategies should be combined to monitor and evaluate malaria vector control programs in endemic areas.

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