Malaria vector surveillance in the context of enhanced vector control in western Kenya

A thesis submitted in accordance with the requirements of Liverpool School of Tropical Medicine for the degree of Doctor in Philosophy by

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ABSTRACT

This thesis describes a series of entomological studies designed to assess malaria vector surveillance in the context of enhanced vector control and altered vectorial system. As malaria vector control efforts are scaled-up and supplemented with new ones, vector populations decline, and behavioural adaptations arise that make surveillance by traditional methods and systems difficult and less informative.

The first study assessed the impact of indoor residual spraying (IRS) on the local mosquito population and malaria transmission in a region with high bednet coverage and wide-spread pyrethroid resistance in malaria vectors. Mosquito collections were performed by pyrethrum spray catch (PSC), light trap and human landing collection (HLC). Secondly, a comparison of mosquito surveillance by supervised entomology teams and community-based sampling approach was performed in a region with low mosquito numbers and high bednet coverage. The last study evaluated a novel sampling tool, Host decoy trap (HDT), for collection of outdoor host-seeking mosquitoes.

IRS was associated with 88% ($p<0.001$) and 93% ($p<0.001$) reduction in the population of *An. funestus* in the intervention areas compared to non-intervention areas as measured by light trap and PSC respectively. Reduction in the numbers of *An. arabiensis* in PSC was 69% ($p=0.006$), while no significant difference was detected with light traps ($p=0.05$). After IRS, *An. arabiensis* become dominant, 86% and 66% in PSC and light traps respectively while human-biting rates by *An. funestus* reduced to undetectable levels. No sporozoite infections were detected in the sprayed areas post-IRS and malaria test positivity among febrile patients within IRS areas was lower post- compared to pre-IRS by 44%, 65.03% and 47.42% in Rongo, Uriri and Nyatike health facilities respectively. Community-based sampling collected approximately 90% fewer Anopheles in indoor CDC light trap compared to supervised mosquito sampling schemes. Similar monthly trends in mosquito numbers and sporozoite infection rates, were observed in indoor light trap, outdoor light trap and prokopack aspiration indoor by community-based collectors. In evaluation of HDT, cattle baited trap (HDT-C) collected a nightly mean of 43.2 (26.7-69.8; 95% CI) *Anopheles*, compared to 5.8 (4.1-8.2; 95% CI) in HLC, while human baited, (HDT-H) collected 0.97 (0.4-2.1; 95% CI), significantly fewer than the HLC. The proportion of *An. gambiae* was highest in HLC (0.55 ±0.05) followed by HDT-H (0.20 ± 0.09) and least in HDT-C (0.06 ± 0.01).

A single application IRS with pirimiphos-methyl resulted in near elimination of *An. funestus* and a corresponding reduction in malaria test positivity rates among out-patients. Community-based mosquito surveillance offered prospects for extensive, multiple mosquito sampling, but substantially underestimated mosquito numbers. The addition of low cost devolved supervisory system is recommended to enforce compliance and improve data quality. The HDT, on the other hand, offered the prospect of a system to monitor and potentially control *An. arabiensis* and other outdoor-biting mosquitoes more effectively.

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ABBREVIATIONS

LLIN	Long-lasting insecticidal net
IRS	Indoor residual spray
RDT	Rapid diagnostic kit
GMAP	Global Malaria Action Plan
ANC	Antenatal Clinic
MCH	Mother Child Health
NMCP	National Malaria Control Program
HLC	Human Landing Catch
CDC	Center for Disease Control and Prevention
EIR	Entomological Inoculation rate
CO_2	Carbon dioxide
LT	Light Trap
PSC	Pyrethrum Spray catch
WET	Window Exit Trap
ITN	Insecticide Treated Net
ITT	Ifakara Tent Trap
MM-X	Mosquito Magnet X
HDT	Host Decoy Trap
BG-HDT	Biogent Host decoy trap
HDT-C	Host decoy trap - cattle
HDT-H	Host decoy trap - human
CBC	Community-based collector
PMI	Presidents Malaria Initiative
RRR	Ration of Rate Ration
WHO	World Health Organization
PDF	Portable Document Format
ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase chain reaction
OPK	Open Data Kit
GLMM	Generalized Linear Mixed Model
IRR	Incident Rate Ratio
ARIMA	Auto-Regressive Integrated Moving Average
KEMRI	Kenya Medical Research Institute
CI	Confidence interval
TPR	Test Positivity rates
GPS	Global Positioning System
GlmmADMB	Generalized linear mixed model Automatic Differentiation Model Builder
HBI	Human Blood Index

1 CHAPTER ONE: CONTEXT AND LITERATURE REVIEW

1.1 Background and Rationale

1.1.1 Malaria prevalence and elimination

A substantial decline in global malaria morbidity and mortality has been realized following the scale-up and use of LLINs, IRS and prompt treatment of malaria cases. New malaria infections have declined globally within the past fifteen years by an estimated 37% with an overall drop in global malaria deaths by 60% [1]. However, the disease has been observed in many endemic zones to be a resilient ecological system with a strong ability to resist elimination despite the sustained implementation of control methods. The most recent world malaria report showed no progress in reducing global, malaria cases between 2015 and 2017 [2]. While countries have been successful in achieving rapid improvements in malaria control, the burden is still unacceptably high, particularly in underserved parts of rural Africa [3] where just fifteen countries in the sub-Saharan Africa and India account for almost 80% of global malaria burden and 75% of deaths [1]. In Kenya, the Lake Victoria malaria-endemic region has the most intense malaria transmission [4] and is the most important source of the disease nationally [5]. In the most recent surveys from this region, malaria prevalence was at 26.7% by microscopy and 42.4% by RDT[6].

The global community has embraced an ambitious plan for scaling up malaria control that progresses towards country-by-country towards regional elimination and ultimately global eradication [7]. Enhanced vector control with scale-up of long-lasting insecticidal nets (LLINs) and increased coverage with indoor residual spraying (IRS) in combination with new control strategies have been recommended for progression towards malaria elimination [8]. The control strategies are needed to reduce the reservoir of infection, the time that a person or mosquito is infectious, and the rate at which transmission spread [9]. To achieve these, an in-

depth understanding of local epidemiology of malaria parasite, vector bionomics, transmission patterns, effective surveillance and vigilance systems, among other things are needed [10]

1.1.2 Malaria vector species

There are approximately 70 *Anopheles* species that have the capacity to transmit human malaria globally and 41 of these are considered primary vectors of malaria [11-13]. In Africa, there are seven species in the *Anopheles gambiae* complex, five of which have been identified to be effective malaria vectors: *An. gambiae sensu stricto (s.s.), An. arabiensis, An. merus, An. melas* and *An. coluzzii*. The remaining primary vectors species in Africa are *An. funestus, An. nili, An. moucheti* [11]. Six *Anopheles* species have been identified to transmit *P. falciparum* causing malaria in Kenya, three of the six, *An. funestus, An. gambiae* and *An. arabiensis* are primary vectors in western Kenya [11]. Data on malaria vector species distribution in Kenya are aligned with surveillance sites associated with medical research institutions in western Kenya and in the coastal region (Figure 1.1) [11].

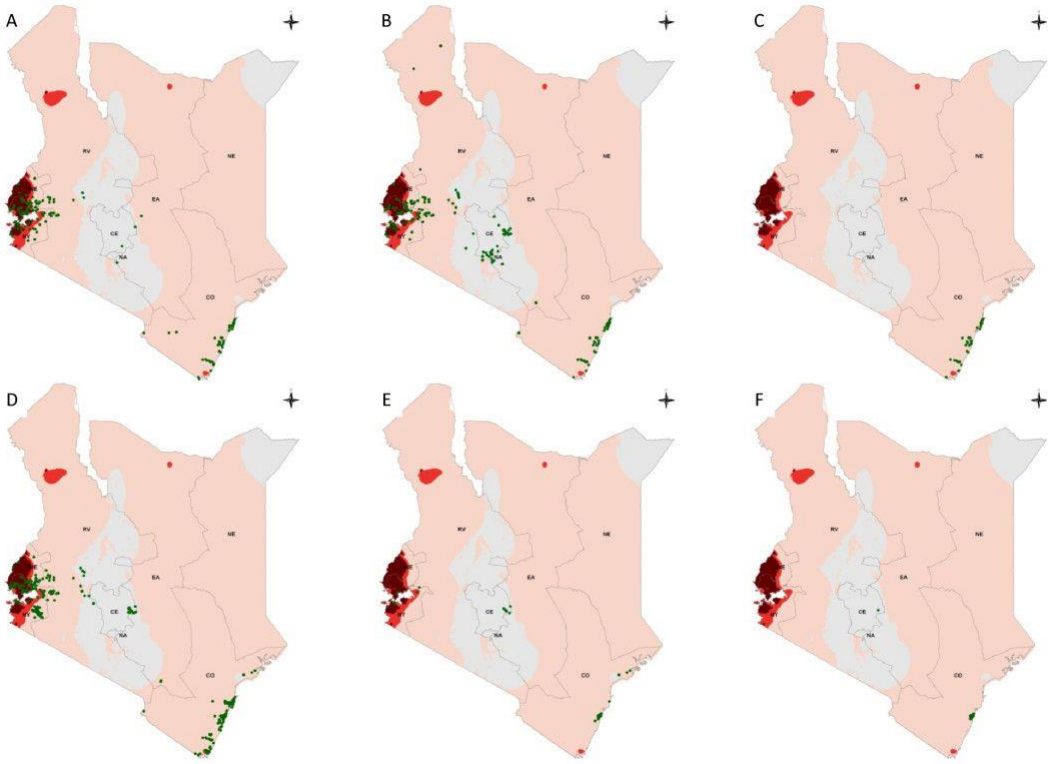

Figure 1.1: Map of Kenya showing the distribution of spatially unique survey sites for a) *An. gambiae* **b)** *An. arabiensis*, **c)** *An. merus*, **d)** *An. funestus*, **e)** *An. pharoensis*, **f)** *An. nili*. (The different dots colours represent survey sites for different *Anopheles* species). **Source:** Okara *et al*., 2010 [11]

1.1.3 Vectorial capacity

Vectorial capacity is a measure of the efficiency of a local vector population in potentially transmitting the parasite [14]. The vectorial capacity of an individual mosquito species depends on a number of factors including vector density, distribution, longevity, host-seeking behaviour, host-choice, and the vector's ability to survive and thrive in close connection with human habitation [15]. In Africa, primary malaria-transmitting mosquitoes, *An. gambiae s.s,* and *An. funestus*, are exquisitely adapted to enter houses and feed on people [16]. In western Kenya, these vectors are closely associated with human habitation [17-21]. Studies investigating host selection in the same region have reported *An. funestus* and *An. gambiae* to have taken their blood meals almost exclusively from humans, highlighting one factor

contributing to their high vectorial capacity, with *An. arabiensis* feeding on both cattle and humans [17, 21-23]. *An. arabiensis* has been reported to bite both indoors and outdoors [20, 24] and feeds almost indiscriminately on both humans and cattle [17, 22, 23] whenever collected indoor. However, when collected outdoors, the species has been observed to feed almost exclusively on cattle [23]. *An. gambiae* and *An. funestus* on the other hand, rest more indoors [18, 19, 25] and bite more indoors [24, 26] and feed almost exclusively on humans [17, 22, 23]. Consistently, high sporozoite positivity has been reported in *An. gambiae* and *An. funestus* with low sporozoite rates in *An. arabiensis* [21]. Table 1.1 below is a summary of epidemiologically relevant behaviour of different *Anopheles* species reported in western Kenya.

Table 1.1: Summary of epidemiologically relevant behaviours of malaria vector species in Kenya

Vector species	Site and Year	% Indoor Resting	% Indoor biting	% Outdoor biting	Blood meals	Reference
An. arabiensis	Ahero, 1989-1990		75.6	24.4		Githeko *et al.*, [20]
	Asembo 2009		54.1	45.9		Bayoh *et al.*, [24]
	Asembo 2011		63.3	36.7		
	Ahero, (Indoor resting)				46.98 % human 47.84% cattle	Githeko *et al.*, [23]
	Ahero (Outdoor resting)				0% human 98.92% cattle	
	Asembo, 2010	16.2			51.3% human 48.7% cattle	McCann *et al.*, [22]
	Asembo, 2011	37.0			35.6% human 64.4% cattle	
	Asembo	99.0			65% cow blood meals	Bayoh *et. al.*, [17]
	Asembo 1994-1994	5.8				Gimnig et al., [18]
An. funestus		87.0				Atieli *et al.*, [27]
	Ahero, 1989-1990		92.3	7.7		Githeko *et al.*, [20]
	Asembo, 2009		69.7	30.3		Bayoh *et*

	Asembo, 2011		67.3	32.7		*al.,* [24]
	Ahero (Indoor resting)				92.55% Human 20.21% cattle	Githeko *et al.,* [23]
	Asembo, 2010	75.2			97.5% human	McCann *et al.,* [22]
	Asembo, 2011	37.9			97.5% human	
An. gambiae	Asembo, 2010				75.5% human 24.5% cattle	
	Asembo, 2011				94.5% human 5.5% cattle	
	Vihiga 2011-2013				26.5% Human 8.2% bovines 2.0% goats 51.1% Mixed	Ndenga *et al.,* [28]
	Asembo 2010	1.0			70% human	Bayoh *et. al.* [17]
	Asembo 1994-1997	94.2				Gimnig at el., [18]
An. gambiae s.l		84.0				Atieli *et al.,* [27]
	Miwani, 1889-1990		65.5	34.5		Githeko *et al.,* [20]
	Asembo, 2011		62.3	38.7		Bayoh *et al.,* [24]
	Miwani, (indoor resting)				74.05% human 26.58% cattle	Githeko *et al.,* [23]
	Miwani, (outdoor resting)				3.33% human 6.67% cattle 90.00% unknown	

1.1.4 Entomological inoculation rate (EIR)

The entomological inoculation rate (EIR) is a measure of exposure to infectious mosquitoes. It is interpreted as the number of infective bites received by an individual during a season or annually [29].

EIR = *MaS*.

The human biting rate (*Ma*) is the number of vectors biting an individual over a fixed period of time. '*M'* is the human blood-feeding rate and is calculated as the number of mosquitoes that feed on humans divided by the total number of blood-fed mosquitoes, '*a'* equals the average number of persons bitten by one *Anopheles* in one day. The sporozoite rate *(S)* is the

fraction of vector mosquitoes present and biting that are considered infectious, i.e. *Anopheles* with sporozoites in their salivary glands [30, 31]. Light traps, HLC and bed net traps catch host-seeking mosquitoes, representative of the vectors which would have bitten humans [29, 31] and are useful in collecting mosquitoes for calculation of EIR.

1.1.5 Malaria vector control

The current malaria vector control strategies rely mainly on the use of LLINs and IRS [32]. Both interventions use insecticides which are either incorporated in the net fabric for LLINs [33] or applied to wall surfaces in the case of IRS [34]. The insecticides mainly act as a killing agent, when mosquitoes land on the treated surfaces and pick a lethal dose which in turn kills them [35, 36]. Whereas some insecticides have exito-repellant properties [37] causing either deterrence or rapid exiting of mosquitoes from the immediate presence of the insecticide. In addition to the insecticidal effect, nets also create a physical barrier limiting human-mosquito contact [33]. Both LLINs and IRS are limited to mostly indoor applications, hence providing control against indoor biting and resting mosquito populations.

1.1.5.1 Long-Lasting Insecticidal Net (LLINs)

The international community developed a Global Malaria Action plan (GMAP), with the aim of scaling up malaria interventions for impact, sustained control and subsequent elimination [7]. This resulted in the scale-up of LLINs and IRS to populations at risk in the past two decades. It was envisioned that sustaining control over time with appropriate interventions would substantially reduce malaria, to cease to be a major source of deaths worldwide [7]. In Kenya, there has been a scale-up of LLINs over the years. Distribution of nets in the country began in late 2001 with the sale of subsidized conventional nets bundled with insecticide treatment (deltamethrin) through rural retail shops [38]. The following years saw the start of the distribution of subsidized nets through antenatal clinics (ANC) to pregnant mothers and

children under 5 years [38]. In the year 2006, the government initiated a mass net distribution campaign achieving coverage of 58% of houses with at least one ITN and 28% with more than one net in the malaria-endemic regions [38]. A global strategy of ensuring universal coverage with ITNs for all persons at risk was adopted in 2009. However, a survey in the following year showed ITN coverage to be one net for every five people at risk [39]. Between the years 2008 and 2011, routine distributions of LLINs was provided through ANC and Mother-Child Health (MCH) clinics. The following period between 2011 and 2015 saw a series of LLIN distribution campaigns mainly in endemic and epidemic-prone zones of the country [40]. Reports from the 2015 National Malaria Indicators Survey indicates that majority of nets (69%) were accessed through routine distribution channels, mass net distribution campaign, other distribution campaigns, or distribution by government, clinical, and faith-based health facilities. The other 21% of the nets come from supermarkets or retail shops, while the rest were obtained either from friends and relatives or the households could not disclose the source. The highest concentration of LLINs showed coverage of 86.8% of houses with at least one and 60.2% of houses with more than one net in the lake endemic regions of western Kenya [6].

1.1.5.2 Indoor residual spraying (IRS)

IRS is the application of long-acting chemical insecticides on the walls and roofs of all houses and domestic animal shelters in a given area, in order to kill the adult vector mosquitoes that land and rest on these surfaces [34]. Unlike LLIN distribution, the application of IRS in Kenya has been at a relatively low scale in a few counties with an interruption between the years. Between 2005 and 2007 spraying was initially focused in 12 epidemic-prone counties and three endemic counties as an epidemic response measure following appropriate signals from an early warning system [39]. In 2008 and 2009 it was used for vector control to reduce the burden of malaria in the lake endemic zones in two

districts. Between 2010 and 2012, blanket IRS was conducted in the Homa Bay, Migori and parts of Kisumu counties, in the lake endemic region of western Kenya (Figure 1.2). However, spraying was interrupted between 2013 and 2016 due to a lack of registered non-pyrethroid insecticide in the country following widespread pyrethroid resistance in the vector population in the region. The national malaria strategy has prioritized IRS for malaria-endemic counties with additional support for capacity building and focal IRS in epidemic-prone counties [40]. In 2017, IRS with pirimiphos-methyl was re-introduced in Migori county [41].

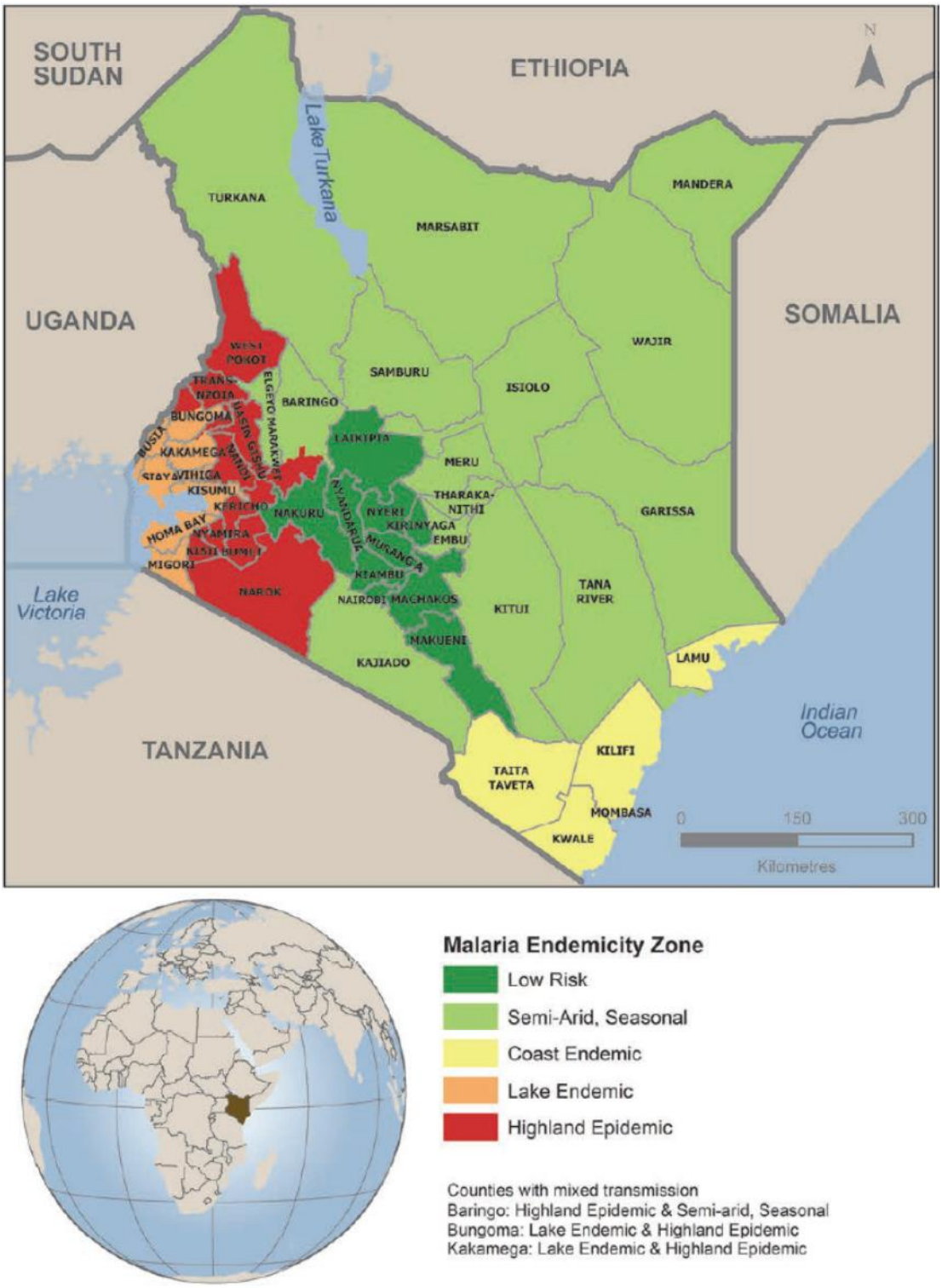

Figure 1.2: A map of Kenya showing Counties with different levels of malaria transmission. Source: Kenya Malaria Indicator Survey Report 2015 [6]

1.1.6 Impact of LLINs and IRS on malaria vector bionomics

1.1.6.1 Vector density

LLINs and IRS work principally by killing mosquitoes that come into contact with the treated surfaces, deterrence of house entry or causing increased exiting of mosquitoes from the immediate presence of the interventions [42, 43]. A community randomized ITN trial in Asembo bay western Kenya, observed high net coverage to be associated with a community-wide suppression of mosquito populations and reduction of sporozoite rates [18, 19]. The greatest decline in vector populations due to indoor based interventions has been mostly reported on endophilic and anthropophagic vector species. In western Kenya, the introduction of bed nets reduced *An. funestus* populations to near extinction [19] while sustained use of ITNs over a 10-year period resulted in a marked decline of *An. gambiae* populations to near absence [17, 44]. A similar observation was made on the Kenyan coast, where a diminishing role of *An. gambiae* in malaria transmission was reportedly associated with high bed net coverage [45]. Implementation of IRS has also been associated with the elimination of *An. funestus* following effective spray campaigns in South Africa, Mauritius and the Pare/Taveta area of Tanzania/Kenya [46, 47].

A combination of ITNs and IRS using insecticides with divergent yet complementary properties have been suggested to have enhanced household-level protection [48]. Literature review of household surveys in Bioko, Equatorial Guinea, Zambezi, and Mozambique reported a reduced risk of infection in those protected by both interventions [49]. In Tanzania, a combination of ITNs and IRS was reported to result in 84% reduction in vector population density relative to ITNs alone [50] and a significant added protection from combining IRS and ITNs compared to ITNs alone [51].

1.1.6.2 Vector species composition and distribution

The impact of LLINs and IRS differs between malaria vector species depending on feeding and resting orientations. Early reports of the effectiveness of permethrin-treated nets suggested changes in mosquito population to be either behavioral or due to changes in vector composition [52]. There existed three major vector species in western Kenya in considerable proportions before the bed net era [53]. However, marked changes in vector species composition have been reported with the introduction and sustained ITN use. Indoor *An. funestus* population in Asembo Bay, western Kenya, was reduced to near elimination by the introduction of ITNs in the 1990s [18]. The species has been known to be more susceptible to insecticides than members of the *An. gambiae* complex [54]. However, a recent rise in the population of *An. funestus* was reported from surveys conducted between 2010 and 2011[21], a change that the authors suggested was associated with the development of pyrethroid resistance in the vector species. Changes in populations of *An. gambiae s.l.* in the same region have also been reported. A decline in the population of the more anthropophilic and endophilic *An. gambiae* with a proportionate rise in the indoor population of the more zoophilic and exophilic *An. arabiensis* was observed with the increased use of bed nets [17]. Similar results were observed in Southeast Zambia where proportional decline of *An. quadriannulatus* and an increase in *An. arabiensis* population was reported following IRS with pirimiphos-methyl [55]. Elsewhere in Tanzania, LLINs and ITNs treated with pyrethroids were observed to be more effective at killing *An. gambiae* and *An. funestus* than *An. Arabiensis* [56]. While these changes in vector species composition were associated with implementation of either ITN or IRS, it remains unclear to what extent universal coverage with LLINs in combination with IRS would affect the vector species composition and distribution. Such changes in vector species composition have a direct impact on malaria transmission since the vectors differ markedly in their ability to transmit malaria. There is a chance that a decline in the population of a primary vector species may reveal a less-known

species whose presence might have been masked by the previously dominant primary vector species. Such changes in vector species composition impose new challenges in vector control and surveillance if differences in feeding and resting behavior exist between the different species.

Distribution of *Anopheles* species is influenced by geographically related environmental factors and habitat characteristics [57]. A study in western Kenya highlands suggested that locations, where habitats were repeatedly observed, had a significant relationship with the distribution of adult mosquitoes. The study further observed that houses with greater proximity to streams had more abundant mosquitoes [58]. A separate survey conducted in the Lake region of western Kenya observed that the distance of larval habitat to the nearest house and substrate type were significantly associated with the relative abundance of *An. gambiae* [57]. Elsewhere, agricultural lands and forest fragmentation were observed to significantly increase the probability of finding mosquitoes [59].

Distribution of mosquitoes and the risk of malaria transmission is affected by human activities leading to the creation of standing water pools [60, 61]. A study in Ethiopia observed malaria transmission and mosquito distribution to be affected by wind profile, marginal pools, temperature and shoreline locations [61]. Elsewhere in Suda, the larval stage of most mosquito species was significantly positively correlated with temperature and turbidity of the water [62]. Whereas, at the Kenya coast, high temperatures, water salinity, dissolved solids, and canopy cover, were among the important factors influencing the development and abundance *An. merus* larvae [63]. Also, habitats with floating debris and emergent plants were key predictors of presence of *An. merus* larvae [64]. Larval habitat type and temperature are therefore key factors that determine productivity of larval habitats, while wind speed and direction and location of human dwellings determine the distribution of adult mosquitoes.

Changes in vector species composition and distribution have been observed to result in different regions due to a number of changing environmental factors. With the dynamic ecological systems, including urbanization, changes in land use, deforestation, climatic changes, and enhanced vector control efforts, remarkable changes in vector species composition and distribution are anticipated. These factors can either reduce or increase malaria transmission. Therefore, with a scale-up of vector control methods, it is imperative to have an effective vector surveillance system, to effectively understand the local vector species composition and distribution.

1.1.6.3 Changing vector behaviour

LLINs work principally to prevent human-vector contact by providing a physical barrier and an insecticidal effect on mosquitoes that land on the treated nets [33]. IRS, on the other hand, makes inside walls of houses lethal for mosquitoes that rest on them [34]. The insecticide used in both LLINs and IRS may have an irritant and/or repellent effect [35, 65] that causes mosquitoes to leave the immediate presence of the treated surfaces. These properties of LLINs and IRS have been associated with behavioural adaptation in mosquitoes defined by one or a combination of the following traits: a natural or insecticide-induced avoidance of contact with treated surfaces indoor and early exit from them; feeding upon humans when they are active and unprotected outdoors; feeding upon animals thereby limiting contact with insecticides targeted indoor and; resting outdoors, away from insecticide-treated nets, walls and roofs [66, 67].

While behavioral modifications that facilitate avoidance or circumvention of insecticides may be emerging in mosquito populations [66-69], the phenomenon is less frequently reported [69] and the data are sparse and less convincing [70]. In Senegal, *An. funestus* was reported to bite during the day after prolonged use of LLINs [71], while in western Kenya highlands,

both *An. funestus* and *An. gambiae* were observed to feed indoor, early before people went under the protection of the bed nets [72, 73]. Elsewhere at the Kenyan coast, the use of permethrin-treated nets was associated with increased outdoor biting, which the authors associated with either behavioural modifications or changes in vector species composition [52]. There is some evidence that mosquitoes, like other insects, can learn and adapt their behaviour in response to environmental cues [74]. However, it remains unclear whether the reported behavioural changes are emerging adaptations in the strict sense or they are cases of behavioural resilience of mosquito sub-populations that persist once vulnerable populations are controlled [66, 75, 76]. These changes in malaria vector behaviour present a challenge to the current malaria control strategies requiring new control tools.

1.1.6.4 Challenges to the current malaria interventions

There is growing evidence that the current major vector control methods, LLINs and IRS are insufficient to achieve malaria eliminations [8, 77, 78]. Their implementation is restricted mostly to indoor application hence targeting only a section of the vector population while a range of challenges including insecticide resistance, incomplete coverage, changing vector behavior and species composition, funding gaps and political unrest are all setbacks to the successful implementation of these interventions.

Insecticide resistance in mosquitoes against pyrethroid-based insecticides is widely reported in malaria-endemic zones [79-86]. Insecticide-resistant mosquitoes evolve mechanisms that enable them to withstand the toxic effect of the insecticides used in bed nets and on walls for IRS. Early genetical and biochemical studies of insecticide resistance showed that single major semi-dominant genes and a limited number of enzymes and structural nerve proteins encoded by these genes were involved, however, recent advances in resistance detection now allow for measurements of genotype frequencies for some of these resistance mechanisms [79]. Insecticide resistance in mosquitoes can be as a result of mutation in the target protein

(target site insensitivity), a lower penetration or sequestration of the insecticide, or increased biodegradation of the insecticide due to enhanced detoxification activities (metabolic resistance) [87]. A range of metabolic and site insensitivity mechanisms, including esterases, cytochrome P450s and GSTs combined with AChE and sodium channel target site insensitivity has been positively associated with resistance in *Anopheles* mosquitoes [88]. Over-expression of the different enzymes associated with rapid breakdown of insecticide [89-91] and target site allelic variants are widely reported in different mosquito populations [83, 84]. Additionally, insecticide penetration assays have been shown to significantly lower amounts of insecticide in resistant strains than in the susceptible mosquito strains [92]. Reduced susceptibility of mosquitoes to insecticides has been feared to compromise the effectiveness of the current pyrethroid-based intervention [80, 93-95]. Accordingly, the global community formulated a basis for coordinated action against insecticide resistance, to preserve the current vector control methods [96]. This resulted in the development of a global plan for insecticide resistance management [97] with each county required to develop policies to guide the use of insecticide-based intervention for insecticide resistance management. The Kenya National Malaria Control Program (NMCP) adopted an insecticide resistance management strategy restricting the use of pyrethroid insecticides to nets only while using non-pyrethroids in rotation [98]. Insecticide resistance monitoring, therefore, forms an integral part of any entomological surveillance plan to advise on vector control.

Behavioural resistance is defined as any modification to mosquito behaviour that facilitates the avoidance or circumvention of insecticide-based interventions indoor [67]. The trait is mostly expressed by changes in biting time and location, outdoor resting, changes in blood meal host and early exiting. There is growing evidence of these behavioural modifications in malaria vectors with scale-up of interventions [28, 67, 71, 73, 99]. Behavioural modification in malaria vectors is a major challenge to malaria elimination in endemic areas since it is the

driver for residual malaria transmission [66, 99]. Consequently, elimination of malaria requires interventions that target changing vector behavior as an urgent priority to sustain the gains made in reducing malaria morbidity and mortality [69, 70].

1.1.6.5 Vector surveillance

Knowing what vector numbers there are in a region, their physiological status, behaviour, and ecology are fundamental to understanding the risk of diseases, future threats and formulating methods of control and monitoring [100]. Thus, surveillance is critical in elucidating vector-host interaction and processes that contribute to diseases transmission. Also, surveillance is critical for the evaluation of disease control programs, to monitor the operational aspects of the program and measure impact or process indicators to ensure that the activities are yielding the desired results in moving the program towards achieving its operational goals [10]. The measurement of human exposure to malaria vectors requires trapping of malaria vectors to determine their biting density and infection rate [101-103].

1.1.7 Vector sampling methods

Mosquito surveillance methods vary in their application based on the physical location of trapping and the indicators being monitored. The collection methods depend mainly on either host-seeking or resting behaviour of mosquitoes. Therefore, the entomological parameters being studied and the behavior of the mosquito species being sampled determine the choice of a method [104]. The trapping techniques used to estimate human-biting rates need to be sufficiently sensitive, and the sampling efficiency must be known [105]. Furthermore, the techniques must be standardized to enable comparisons between studies [104]. However, estimation of a calibrating factor even for some of the most standardized trapping methods such as CDC light traps has been challenging [106-108]. Consequently, comparison of trap efficacy between different trapping techniques, in different settings remains a major challenge [108] for vector biologists. Given the weaknesses of different mosquito sampling

methods, coupled with changing vector bionomics and variations in trapping techniques across different settings, the use of a single mosquito collection method may not be sufficient to provide epidemiologically meaningful entomological data. The various aims of mosquito collection should, therefore, be considered to select a suitable combination of trapping techniques for a given vector population [109]. Additionally, the ultimate choice of collection methods for operational surveillance should be driven by trap efficacy and scalability. For instance, operational estimation of EIRs, high overall capture rates and scalability allowing for intensive sampling are likely more important than perfect precision with regard to HLC [108]. A study in Zambia further identified the need to specifically evaluate sampling methods based on their ability to selectively trap either host-seeking, exiting or resting mosquitoes, and to compare them with sufficient sensitivity relative to absolute house entry or host attack rates [107].

1.1.7.1 Human landing catches

Human landing catch (HLC) is the traditional 'gold standard' method for measuring human-biting rates in any mosquito population [12]. Human landing catch collections involve persons sitting with their lower legs exposed and collecting mosquitoes that land on them (Figure 1.3). The technique is suitable for both indoor and outdoor application and collectors are able to record the time when mosquitoes are collected, hence providing mosquito hourly biting rates at each trapping location.

Figure 1.3: A picture of a volunteer performing human landing catch (HLC) outdoor.

Even though HLC is considered to provide the most relevant measure of human biting rates, it is labour intensive and dependent on a wide range of environmental factors, sites, individual attractiveness to mosquitoes [110, 111] and skill of the catchers. HLC also raises ethical concerns due to exposure of the catchers to potentially infectious mosquito bites. However, a study in western Kenya reported HLC to be simple, elegant, and a powerful tool and the most direct measure of mosquito biting rates [112]. To address the ethical concerns, the study demonstrated that providing the collectors with chemoprophylaxis, Malarone, lowered the incidence of malaria by 96.6% as compared with non-collectors [112]. While the provision of malaria chemoprophylaxis is demonstrated to be protective against malaria infections in HLC collectors, the risk of infection with arboviruses in regions where local mosquito populations sustain transmission of such diseases still raises ethical concerns.

HLC is not however easily scalable, and unsupervised collection by community-based teams is not attainable. Also, it is operationally difficult to measure the amount of actual biting experienced by HLC collectors and proportions of mosquitoes that actually land and are missed by the collectors. Consequently, alternative vector sampling methods that give an

improved measure of the human-biting rates of a mosquito population are required. Evaluation of a new trapping technique that combined odour and visual stimuli with a thermal signature in the range equivalent to human body temperature was observed to sample ten times more *Anopheles* mosquitoes [113] and seven times more *An. arabiensis* [114] compared to HLC. The trap described as a "host decoy" [113] showed the potential to improve mosquito sampling with the possibility of replacing HCL. Mosquito electrocuting trap (MET) has also been demonstrated to be a human exposure free, highly sensitive tool that accurately quantifies epidemiologically relevant metrics of mosquito biting densities, with potential to replace HLC [115-117]. Nonetheless, despite its shortcomings, HLCs still remains the most suitable method for estimating human biting rates [109]

1.1.7.2 CDC-Light trap

CDC light traps are used in adult mosquito surveillance. Developed by the Center for Disease Control and Prevention (CDC), the portable traps are battery powered with a motorized fan, light bulb, and mosquito collection cup. The trap can be used with CO_2 to mimic exhaled gasses from mammals. Mosquitoes attracted to the traps by either light or CO_2, are drawn in at the top and forced downward by the fun into the collection net where they cannot escape. Malaria transmitting mosquitoes are nocturnal, therefore, traps are typically deployed at dusk and collected at dawn the following day.

Mosquito surveillance and monitoring require accurate sampling techniques based on the behavior and ecology of the target species [118]. Light traps have been evaluated for monitoring mosquitoes both indoors and outdoors. When deployed indoors, the optimum location for sampling house-visiting mosquitoes has been reported to be as close as possible to the host (Figure 1.4), with improved catching efficiency when the trap is installed at the foot of an occupied bednet [119, 120]. From an epidemiological point of view, the use of light-trap + bednet combination is an approach that is more meaningful than using light trap

alone because the trap functions more efficiently when placed near where mosquitoes approach a sleeping human [104]. Outdoor deployment of light traps is not commonly used in surveillance particularly in regions people mostly spend time indoor at night. However, a need to concurrently undertake indoor and outdoor vector surveillance to better understand residual transmission is recommended in a study [121]. Furthermore, a study evaluating indoor and outdoor CDC light traps in Thai-Myanmar border observed the outdoor traps to collect higher frequency of outdoor mosquito species, indicating its usefulness in targeting mosquitoes that would otherwise not go inside houses.

Figure 1.4: A picture of CDC light traps next to an occupied bed net.

CDC - light traps have been recently observed by several studies to be the most effective alternative to HLC [107-109]. However, the trap presents several weaknesses that may reduce their performance and affect the comparability of data across different surveillance settings. Trapping efficacy of the light traps is affected by factors such as trap position, height, and nearness to an occupied bed net [119], therefore the optimization of its application across

different settings is needful. Results on relative sampling efficiency of the light trap are mixed, some studies reported reduced efficacy at high vector densities [105, 122] while other studies found that trap efficacy was density-independent [108, 120, 123]. The presence of ambient light sources has been associated with low catch numbers [124], thus the use of light traps during full moon nights and in well-lit neighbourhood reduce catch rates. Furthermore, mosquitoes collected from light traps are usually unfed [107, 118] since the traps preferentially sample host-seeking females. This potentially hinders studies designed to study arboviruses or collection of blood-fed mosquitoes for analyses of host selection. An important operational limitation is that light traps require a continuous recharge of batteries that might be challenging for surveillance in rural communities, particularly where electricity is not readily available [107]. Also, light traps have been found to capture mosquitoes with higher sporozoite rates as compared to those from human bait catch thus leading to an overestimation of EIR [119, 122]. Consequently, for effective vector population sampling, a combination of light traps with an additional technique is recommended [109, 124].

To increase trap efficacy, light traps are sometimes baited with CO_2 or other olfactory signals. However, a study in Kenya reported CO_2 –baited CDC-LT to have trapped significantly higher numbers of *Culex* species but the numbers of *An. arabiensis* and *An. funestus* did not differ between baited and non-baited traps [118]. Similarly, in a study comparing collection methods for mosquitoes infected with the Japanese encephalitis virus, a dry ice-baited CDC-LT collected significantly fewer mosquitoes than the other traps [125]. The addition of dry ice to CDC-LT for CO_2 production is not commonly used in routine sampling due to the cost of dry ice and its limited availability. Furthermore, in community vector surveillance with CDC-LTs, CO_2-baited traps would make application logistically unrealistic. Unbaited CDC-LTs have been demonstrated to perform equally well or better than CO_2 baited traps, hence most suitable for community routine vector surveillance.

1.1.7.3 Pyrethrum spray-catches (PSC)

The pyrethrum spray catch (PSC) is a technique designed to sample indoor resting mosquitoes which involve the use of insecticide to rapidly knock down mosquitoes which are then collected on white sheets spread on the floor and over furniture[19]. Pyrethrum spray-catches is an indoor collection technique and provides an estimate of the mean house resting density in a given area. However, it may not give a good estimate of EIR due to the fact that sampling of indoor-resting mosquitoes tends to miss the mosquitoes that leave the house immediately after feeding and may include those that enter after feeding outdoors on another host [104]. Therefore, it is not possible to get a direct estimate of the per-capita human biting rate from PSC collections. The procedure has also been reported to be labor-intensive and intrusive [109] making it unsuitable for wide-scale sustained routine vector surveillance. With widespread pyrethroid resistance, it remains unclear to what extent resistance lowers the efficacy of PSC as a sampling tool.

PSC has been used routinely, either singly or in combination with other collection methods, to assess the impact of IRS [126, 127], or ITNs [19] on the local mosquito population. A comparison of the number and characteristics of mosquitoes sampled by HLC, light traps and PSC in Senegal, observed that the diversity of mosquito species to be minimal in PSC compared to light trap and HLC. Also, light trap collections correlated much closely with HLC while PSC yielded significantly lower catch sizes [128].

1.1.7.4 Motorized aspirators

Indoor resting mosquitoes have traditionally been collected by mouth aspirators. The procedure is slow, labor-intensive and depends on the expertise of the individual collector hence is not suitable for routine vector surveillance [129]. Battery-powered aspirators (Figure 1.5) reduce the level of skill and motivation needed by the operator due to the large sampling radius and sanction and therefore offers better sampling compared to mouth aspiration [130].

The most commonly used mechanical aspirators are CDC backpack aspirator and prokopack aspirator. A study in Tanzania evaluated the two sampling tools and found prokopack to be better than the CDC backpack aspirator since it can be assembled using simple, low-cost and easily attainable materials [131]. The authors further recommended longitudinal comparisons of prokopack aspiration with pyrethrum spray with associated mosquito density measurements from human landing catch to calibrate it against, in order to understand the merits of the prokopack aspiration as a mosquito monitoring tool [131]. In other studies, backpack aspiration was observed to be more effective than sticky resting box catches in sampling indoor resting mosquitoes in the Kilombero Valley, Tanzania [132]. Similarly, in Burkina Faso, backpack aspiration was observed to perform better than sticky resting boxes for collection of mosquitoes indoor [133]

Figure 1.5: Pictures of battery-powered aspirators, Indoor and Back-pack aspirators

1.1.7.5 Window Exit Trap (WET)

Window exit traps are used to trap mosquitoes that exit a house through the windows (Figure 1.6). Mosquitoes enter and exit houses mainly through windows, doors, and eaves. While

indoor entry is associated mainly with host-seeking and resting, exit of mosquitoes from houses may result from other factors such as attempts to escape the presence of interventions indoor, a quest for blood meal elsewhere or outdoor resting locations. Therefore, the window exit trap is useful in determining the proportion of mosquito that exit houses after entry. The trap is useful in the determination of the effect of IRS and ITNs on the movement and feeding of mosquitoes. Furthermore, it is useful in the determination of residual effects of the insecticides as indicated by mortality rates of mosquitoes recovered from the trap.

WETs were observed to perform moderately well in western Kenya with pooled relatives catch rates of 52% for *An. gambiae s.l* and 49% for *An. funestus* compared to indoor HLC [108]. Elsewhere, the performance of WETs has been very poor, suggesting that the technique is not appropriate for surveillance and monitoring of the impact of mosquito control [106, 107]. In both studies, it was observed that mosquitoes were likely to exit through other rooms without the WETs and the open eaves. A comparison of WET and CDC-LTs in experimental huts in Tanzania showed similar numbers of mosquitoes in both traps when the experimental huts were fitted with net baffles to allow entry but prevent the exit of mosquitoes via the eaves [134]. This suggests that WETs are effective when other exit routes from the house are blocked. More detailed evaluations of WETs in different types of houses and environmental settings are necessary for understanding when and where the use of WET is reliable [108].

Figure 1.6: A picture of a window exit trap

1.1.7.6 Resting traps

Resting traps such as pot traps, box trap, and pit shelters are commonly applied for outdoor collection. Clay pots have been previously reported to be more effective in sampling outdoor resting *An. gambiae*, *An. arabiensis*, *An. funestus*, and *Culex spp*. of both sexes in rural western Kenya. These were demonstrated to perform better than Pit shelters and were comparable to Colombian curtain exit traps and indoor pyrethrum spray samples in return of numbers of mosquitoes [135]. In contrast, other studies have reported pot traps and box traps to yield very few malaria vectors when used either indoors or outdoors [108, 109]. Resting boxes have been separately reported to perform poorly in sampling indoor or outdoor mosquitoes [106, 107]. The poor sensitivity of resting boxes is most likely explained by the fact that they represent a small proportion of the total suitable resting surface area available to mosquitoes indoors [107]. In Kilombro Valley, Tanzania, resting bucket trap performed much better than the sticky resting box trap in sampling outdoor resting mosquitoes [132]. A separate investigation in Burkina Faso similarly observed the daily catch sizes of mosquitoes in Sticky Resting box to be lower than that of traditionally used indoor and outdoor resting

collection approaches. However, unlike the other traps, the Sticky Resting Box could be set up to collect mosquitoes passively over at least one week [133], hence being suitable in situations where traps are not monitored daily. The resting behaviour of *Anopheles* mosquitoes and catch sizes in resting traps was observed to be affected by the presence of host and mosquito feeding orientations. In southern Tanzania, *An. arabiensis* were generally found in Resting Boxes stationed in cattle sheds where livestock was present, and inside houses when absent [136]

Sampling of outdoor resting vector population is a lot more challenging since the mosquitoes are dispersed across a large environment with numerous potential resting places. Currently, there exist no sufficiently efficient mosquito collection methods for large scale sampling of outdoor mosquitoes, particularly those that are blood-fed [109]. Different studies investigating outdoor mosquito trapping methods have reported varying levels of success in terms of efficacy of the techniques used. Such differences are likely to result from a number of factors such as methodology, environmental factors, and variation in vector species composition and behavior. Clay pots showed great potential for not only outdoor vector monitoring but also as a vehicle for delivery of insecticides for vector control in western Kenya [135]. While this study showed a level of success with pots, another study in the same area [108], and at the Kenyan coast [109] show dismal performance with pots. In the study at the Kenyan coast, pots were deployed at 1900 hours the night before the collection morning [109], with the expectation that mosquitoes would go inside and rest after the night blood meal. However, observation from the field indicates that the clay pots need to be stationed outdoor for several days before collection begin. This would give mosquitoes time to locate the pots and begin resting in them. Additionally, study pots meant for mosquito trapping should be uniquely designed to make them useless for the local community use hence limiting human interference that would otherwise drive away resting mosquitoes.

Furthermore, for effective outdoor sampling, the pots need to be moist, cool, well shaded and dark inside to provide a conducive environment for resting mosquitoes. *Anopheles* vectors that transmit malaria are always associated with human habitation whether they bite indoor and rest outdoor or keep strictly to the outdoor environment. Deployment of suitable outdoor traps such as Clay pots stationed close to houses is ideal for outdoor trapping.

1.1.7.7 Ifakara Tent Traps (ITT)

The Ifakara tent traps (Figure 1.7) are rectangular canvas boxes containing six funnel-like entrances for mosquitoes and inner small apertures tilted to an angle so that mosquitoes have to fly upward to enter the trap. A layer of durable, Teflon-coated woven fiberglass netting between the entry funnels and the bait host allows the human participant to sleep while protected from mosquito bites. Bisecting the protective netting panel, a zip enables the participant to aspirate mosquitoes from inside the trap. The trap floor is made of thick polyvinyl chloride sheeting, which protects against rough substrates and surface water [105]. The Ifakara tent trap is designed to replace the HLC by providing an exposure free method of mosquitoes biting a human [137, 138]. The Ifakara trap has been reported to be effective in collecting adult mosquito vectors in trials conducted in Tanzania [105, 106, 138], Zambia [107] and western Kenya [108]. The trap has been observed to correlate well with HCL with increased sampling efficiency at low densities [105]. It has the potential for both research and routine programmatic surveillance applications. However, it remains unclear whether densities measured by ITT best reflect indoor or outdoor catches [106]. When used in community-based monitoring, ITT was reported to be the most cost-effective and epidemiologically relevant way to monitor adult malaria vector mosquitoes and safer than HLC [139]. However, ITT exhibited relative low rates of capture per night of sampling compared with HLC [107, 139] and is observed to be bulky, making it difficult to move between sampling location [107]. Nonetheless, ITT offers great potential for sustained

community-based vector surveillance and requires additional evaluation in different geographical settings and vector species composition for effectiveness.

Figure 1.7: A picture of Ifakara Tent Trap

1.1.7.8 Suna trap

The Suna trap, named after the Dholuo word for mosquito, consists of five main components (Figure 1.8); a funnel and ventilator section, carbon dioxide release pipe, perforated plastic base, netting catch bag, hanging tripod and conical plastic cover. When the trap is connected to a 12-volt power supply the ventilator rotates, sucking air up through the funnel at a rate of 3.1 m/s, thus opening the funnel shutter gate. As air circulates under the conical cover of the trap, volatiles from a synthetic chemical blend of attractants are released from the nylon strips suspended from the hanging tripod. The odour-saturated air is forced out of the trap through holes in the plastic base at a rate of 0.5 m/s. This generates a flow of attractants, which are carried away from the trap. In addition, a plume of CO_2 diffuses from the CO_2 release pipe, mimicking the breath of a host. In effect, the combination of odours and CO_2 forms

a human surrogate. Mosquitoes encountering these odours fly upwind towards the trap and, when they are in close proximity to the funnel, they are sucked into the trap through the ventilator. Inside the trap, the mosquitoes are contained in the catch bag. When the power supply is turned off, the shutter gate automatically drops to a closed position due to a weighting mechanism and mosquitoes are unable to escape. Mosquitoes caught inside the trap die due to dehydration and lack of food [140].

Figure 1.8: Cross-sectional schematic view of the Suna trap [140].

Suna trap has been described as a monitoring tool for trapping host-seeking mosquitoes as well as an intervention tool against *An. gambiae* house entry [140] (Fig 1.9). The catch of mosquitoes from a Suna trap was is comparable to that from a CDC light trap and MM-X trap when used to sample *An. gambiae* inside a human-occupied house under semi-field conditions. The trap was also found to be effective in sampling mosquitoes outdoor, and the use of a synthetic blend of attractants negates the requirement of human bait [140]. Since

only a single report of the evaluation of the Suna trap against other traps exist, additional studies are needed to evaluate the trap in different geographical setting and mosquito species composition.

Figure 1.9: A picture of Suna Trap used in the collection of outdoor host-seeking mosquitoes

1.1.7.9 Host Decoy Trap (HDT)

To improve surveillance and sampling of vectors, to reach the goal of sampling technology that is economical, universally accepted, and produces data that can be interpreted with confidence, particular attention must be given to the response of the vector to either the host or trap [100]. Traps that lure actively host-seeking female mosquitoes are most useful for surveillance in the face of declining vector density [108]. The use of carbon dioxide and skin emanations to locate hosts is the basis for many traps used [117, 140-142] for vector surveillance. Human Decoy Trap (HDT) is a new design of trap that combines host odour and visual stimuli with a thermal signature in the range equivalent to human body temperature to lure and trap host-seeking mosquitoes [113]. When compared to HLC, the trap caught almost ten times more *Anopheles* mosquitoes [113] with comparable results presented in chapter three of this thesis. In the previous surveys, the trap was used with hot water to provide heat

and a live host (e.g., human) in a tent to produce natural odours useful in attracting mosquitoes [113, 114]. Further improvement on the tap to provide a stable heating system and source of host odour are needed to improve efficiency and enable scalability. Otherwise, HDT offers the prospect of a system to monitor and potentially control *An. arabiensis* and other outdoor-biting mosquitoes more effectively [114].

1.1.8 Community-based (CB) vector surveillance

In western Kenya, low indoor vector densities are frequently observed in field surveys under the widespread implementation of LLINs. Recently, reports of changing vectorial systems in the region have been published [72, 73]. These observed changes are attributable to the sustained use of LLINs. As the global community braces itself for malaria elimination, characterized by additional control tools, it is anticipated that vector density will be reduced further with a proportionate decrease in malaria transmission. Such a possible reduction in vector densities will increase the challenge of collecting enough vector numbers for entomological evaluations. The challenge of entomological monitoring under declining transmission levels and dwindling vector density scenario is enormous and requires greater sensitivity in the surveillance tools and sampling design. Therefore, the National Malaria Control Programs (NMCPs) presently face the challenge of monitoring declining transmission levels mediated by dramatically altered residual vectorial systems with greater sensitivity than ever before [139]. Consequently, with advancements in regional and global malaria elimination, it is important to establish a vector surveillance system that will be easily scalable, cost-effective and sustainable. A community-based (CB) vector surveillance system offers such potential.

Traditionally, entomological vector surveillance has been designed and evaluated for research purposes with close supervision from expert scientists and technicians with very few reports of application through community-based platforms [139]. Conventional longitudinal entomological monitoring strategies rely operationally upon trained specialist technical staff managed centrally usually by academic or research institutions, so they are usually limited in both their geographic scope and the frequency of sampling at any survey location [143]. This design has been reported to be impractical and unsustainable to implement on a large scale to be able to detect residual transmission that persists in the population or hotspots of low transmission following massive control effort [139]. Additionally, the cost of implementing adult mosquito surveillance through conventional terms of specialist entomologists have been suggested to be prohibitive in impoverished African countries [139, 144]. Therefore, under enhanced vector control, with dramatically altered vectorial systems, supervised vector surveillance would become even more challenging and expensive, hence, a need for a devolved surveillance system.

The community-based approach to mosquito control and surveillance has been implemented in Dar as Salaam, Tanzania under Urban Malaria Control Programme (UMCP) for larval control. Modestly-paid community members, known as Community-Owned Resource Persons (CORPs) performed surveys of larval habitats and larviciding [145]. While the implementation of routine larviciding in African cities showed considerable potential for sustained, rapidly responsive, data-driven and affordable applications [145], the level of coverage achieved by the CORPs at the start of the Dar es Salaam trial were insufficient to enable effective suppression of malaria through larval control [146]. This was possibly due to a lack of accessibility of habitats in the urban settings because the majority of the compounds were fenced for security reasons [147]. To overcome the challenges of low coverage by the CORPs in larval surveillance and control, further operational research was

recommended to develop surveillance systems that are practical, affordable, effective and acceptable for implementation of community-based vector management [146]. Accessibility to closed compounds and improved sensibility with which the CORPs sought for larval habitats was deemed necessary to improve coverage and performance by the community teams [147]. Additionally, a community-driven larval control and surveillance in Africa can only be established through long-term programs which are stably financed and allow for operational teams and management infrastructures to mature by learning from experience [145]. A review of larval source management for controlling malaria underscored the possibility of LSM being effective in most settings where adequate coverage of larval habitats can be achieved [148]. A community-based approach for LSM presents a greater potential for achieving the required coverage for larval control.

1.1.9 Study rationale

Sustained use of LLINs and application of IRS in western Kenya have contributed immensely to changes in the local mosquito populations, characterized by reduced mosquito densities [17-19], changes in vector species compositions [17, 21], increases in exiting behaviour [149], alteration in biting time [73] and host selection [28]. These changes in the biology of malaria-transmitting mosquitoes have been witnessed in the face of ongoing malaria transmission [2, 6] and make entomological monitoring difficult. An increased presence of intervention results in greatly depleted mosquito numbers requiring more sensitive tools and efficacious surveillance systems to monitor the residual vector populations [139]. It is hypothesized that as the current interventions are scaled up to universal coverage and supplemented with new strategies [150, 151], the local vector populations will be depleted further and entomological monitoring will become more challenging. It will therefore be difficult to obtain enough mosquito data by the traditional collection approaches for epidemiologically meaningful decisions. Furthermore, current mosquito collection methods

have individual trap weaknesses [152] that may be exaggerated through alterations to the vectorial system and become uninformative when used in isolation to monitor vector populations. The results presented in this thesis demonstrate the use of a combination of conventional mosquito sampling approaches, implemented under the supervision of expert entomology technicians to evaluate the impact of enhanced vector control with a combination of IRS and LLINs. In addition, a community-based surveillance scheme against the conventional sampling approach by supervised technicians for longitudinal entomological monitoring in a region with high bed net coverage and low mosquito numbers is evaluated. Finally, a novel sampling tool, HDT, which incorporates host odours, heat and a visual cue was evaluated against the HLC in sampling of outdoor biting mosquitoes.

1.1.10 Aims and Objectives

The aim of this thesis was to assess malaria vector surveillance in the context of enhanced malaria control in western Kenya.

Specifically;

1.0. To evaluate the impact of indoor residual spraying with pirimiphos-methyl (Actellic 300CS®) on entomological indicators of transmission and malaria test positivity rates in Migori County, western Kenya.

1.1. *Hypotheses 1*: IRS with pirimiphos-methyl is highly effective in reducing entomological indicators of transmission.

1.2. *Hypotheses 2*: Reduction of in entomological indicators of transmission is associated with the corresponding reduction in test positivity rates following IRS with pirimiphos-methyl

2.0. To evaluate a community-based vector surveillance system for routine entomological monitoring under low malaria vector densities and high bednet coverage in western Kenya.

2.1. Hypotheses 1: Unsupervised community-based vector surveillance is robust enough to estimate the same vector density and species composition as supervised teams.

2.2. Hypotheses 2: Community-based surveillance scheme tracks similar seasonal entomological variation compared to supervised surveillance team.

3.0. 3.0. To evaluate Host Decoy Tap (HDT) for the collection of outdoor host-seeking malaria vectors.

3.1. Hypotheses 1: Host Decoy Trap is highly effective for sampling outdoor host-seeking mosquitoes of all taxa in a region with high LLIN coverage.

2 CHAPTER TWO: IMPACT OF INDOOR RESIDUAL SPRAYING WITH PIRIMIPHOS-METHYL (ACTELLIC 300CS®) ON ENTOMOLOGICAL INDICATORS OF TRANSMISSION AND MALARIA TEST POSITIVITY RATES IN MIGORI COUNTY, WESTERN KENYA

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Impact of indoor residual spraying with pirimiphos-methyl (Actellic 300CS®) on entomological indicators of transmission and malaria case burden in Migori County, western Kenya

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Abstract.

Background. Indoor residual spraying (IRS) of insecticides is a major vector control strategy for malaria prevention. We evaluated the impact of a single round of IRS with the organophosphate, pirimiphos-methyl (Actellic 300CS®), on entomological and parasitological parameters of malaria in Migori County, western Kenya in 2017, in an area where primary vectors are resistant to pyrethroids but susceptible to the IRS compound.

Methods and Findings. Entomological monitoring was conducted by indoor CDC light trap, pyrethrum spray catches (PSC) and human landing collection (HLC) before and after IRS. The residual effect of the insecticide was assessed monthly by exposing susceptible *An. gambiae* Kisumu strain to sprayed surfaces in cone assays and measuring mortality at 24 hours. Malaria case burden data were extracted from laboratory records of three health facilities within the spray area and two adjacent unsprayed areas. IRS was associated with

reductions in *An. funestus* numbers in the intervention areas compared to non-intervention areas of 88% with light traps (risk ratio [RR] 0.12, 95% CI 0.07-0.21, p<0.001) and 93% with PSC collections (RR=0.07, 0.03-0.17, p<0.001). The corresponding reductions in the numbers of *An. arabiensis* collected by PSC were 69% in the intervention compared to the non-intervention areas (RR=0.31, 0.14-0.68, p=0.006), but there was no significant difference with light traps (RR=0.45, 0.21-0.96, p=0.05). Before IRS, *An. funestus* accounted for over 80% of *Anopheles* mosquitoes collected by light trap and PSC in all sites. After IRS, *An. arabiensis* accounted for 86% of *Anopheles* collected by PSC and 66% by CDC light trap in the sprayed sites while the proportion in non-intervention sites remained unchanged. No sporozoite infections were detected in intervention areas after IRS and biting rates by *An. funestus* were reduced to near zero. *Anopheles funestus* and *An. arabiensis* were fully susceptible to pirimiphos-methyl and resistant to pyrethroids. The residual effect of Actellic 300CS® lasted ten months on mud and concrete walls. Malaria case counts among febrile patients within IRS areas were lower post- compared to pre-IRS by 44%, 65.03% and 47.42% in Rongo, Uriri and Nyatike health facilities respectively.

Conclusions. A single application of IRS with Actellic 300CS® in Migori County, an area with susceptible vector population provided ten months protection and resulted in the near elimination of the primary malaria vector *An. funestus* and a corresponding reduction of malaria case count among out-patients. The impact was less on *An. arabiensis*, most likely due to behavioral avoidance of sprayed surfaces.

Introduction. Over the last two decades, malaria control has been scaled up throughout sub-Saharan Africa with an emphasis on the distribution of long-lasting insecticidal nets (LLINs), targeted application of indoor residual spraying (IRS), and improved diagnostics and case management. As a result, the burden of malaria has declined substantially with a 40% reduction in incidence and a 50% reduction in prevalence between 2000 and 2015. While LLINs contributed an estimated 68% of the decline in malaria prevalence, IRS was responsible for 13% [153].

The efficacy of insecticide-treated nets was demonstrated in a series of cluster randomised, controlled trials [19, 154, 155]. Formal randomised controlled trials of IRS have also demonstrated the efficacy of IRS [50, 156]. Furthermore, there is a long history of programmatic implementation of IRS in many settings of the world which resulted in reduced malaria burden and even elimination in some settings [157]. The use of both LLINs and IRS for malaria control has a direct impact on mosquito bionomics. LLINs and IRS have multiple effects on mosquito populations which may result in reduced malaria transmission including: reduced indoor *Anopheles* densities [18, 19, 158], shifts in vector species composition [17], changes in the time and location of mosquito biting [35, 71, 73], and changes in host selection [28], and increases in early exophily [149].

In western Kenya, vector control has included universal coverage of LLINs through periodic mass campaigns and routine distribution to high-risk groups as well as IRS in specifically targeted areas. The first mass LLIN distribution occurred in 2006 and targeted children <5 years of age. Additional distributions aiming for universal coverage occurred in 2011 and 2014 leading to 54% of households in the lake endemic zone having one LLIN for every two residents [6]. The region also bears the highest malaria burden nationally [4-6].

Implementation challenges facing LLINs include incomplete coverage [2, 6, 25, 159], widespread pyrethroid resistance [83, 84, 93, 160] and possibly changing vector behaviour [71].

IRS in western Kenya was based exclusively on pyrethroids until 2012 [39]. However, spraying was interrupted between 2013 and 2017 due to widespread pyrethroid resistance in local malaria vector populations and the lack of a registered, non-pyrethroid insecticide in the country. In response to widespread pyrethroid resistance, the Kenyan National Malaria Control Programme (NMCP) developed an insecticide resistance management strategy involving the rotation of different non-pyrethroid classes of insecticides used in IRS every two years in endemic and epidemic-prone areas where 80% or more households own one or more LLIN [98]. This is in accordance with the global insecticide resistance management strategy aimed at delaying the rise and spread of insecticide resistance to new classes of insecticide while preserving pyrethroids for use in bednets [97]. In 2017, IRS was re-introduced using a microencapsulated formulation of pirimiphos-methyl (Actellic 300CS®). The insecticide has been reported to be effective against pyrethroid-resistant *Anopheles* mosquitoes [161-163] and has a relatively long residual effect on sprayed wall surfaces, of up to twelve months [161, 162, 164].

Given the high cost of IRS and the moderate coverage of LLINs in western Kenya, it was important to determine the impact of IRS with an organophosphate, Actellic 300CS, against a background of moderate to high coverage of pyrethroid LLINs [6] in an area of extensive pyrethroid resistance [84] to guide the implementation of vector control interventions. Therefore, we evaluated the impact of IRS with Actellic 300CS®on pyrethroid-resistant *Anopheles* mosquitoes and malaria cases in Migori County, western Kenya.

Methods.

Study Sites

Entomological monitoring was conducted in 12 villages in Migori (-1.0667 S; 34.4667 E) and Homa Bay (-0.5396 S; 34.4565 E) Counties from July 2016 to February 2018. Six IRS intervention sub-counties were in Migori County, and six control sub-counties were in neighbouring Homa Bay County (n=4) and unsprayed areas of Migori County (n=2) (Fig. 2.1). The residents in the study area are mainly of the Luo ethnic group and are subsistence farmers with a few growing cash crops such as sugar cane and tobacco. Residents mostly live in small houses, clustered into family social units called compounds. The region has bimodal peaks of rainfall with the long rains between April and June and short rains in October and November. The Lake Victoria region of western Kenya is malaria endemic; the most recent Malaria Indicator Survey in 2015 documented a malaria prevalence of 27% by microscopy. Though 87% of households own at least one LLIN and 60% own more than one LLIN, only 54% of households have an adequate number of nets, defined as one LLIN for every two residents [6]. *Anopheles funestus*, *An. arabiensis* and *An. gambiae* are the main malaria vectors in the region [11, 21].

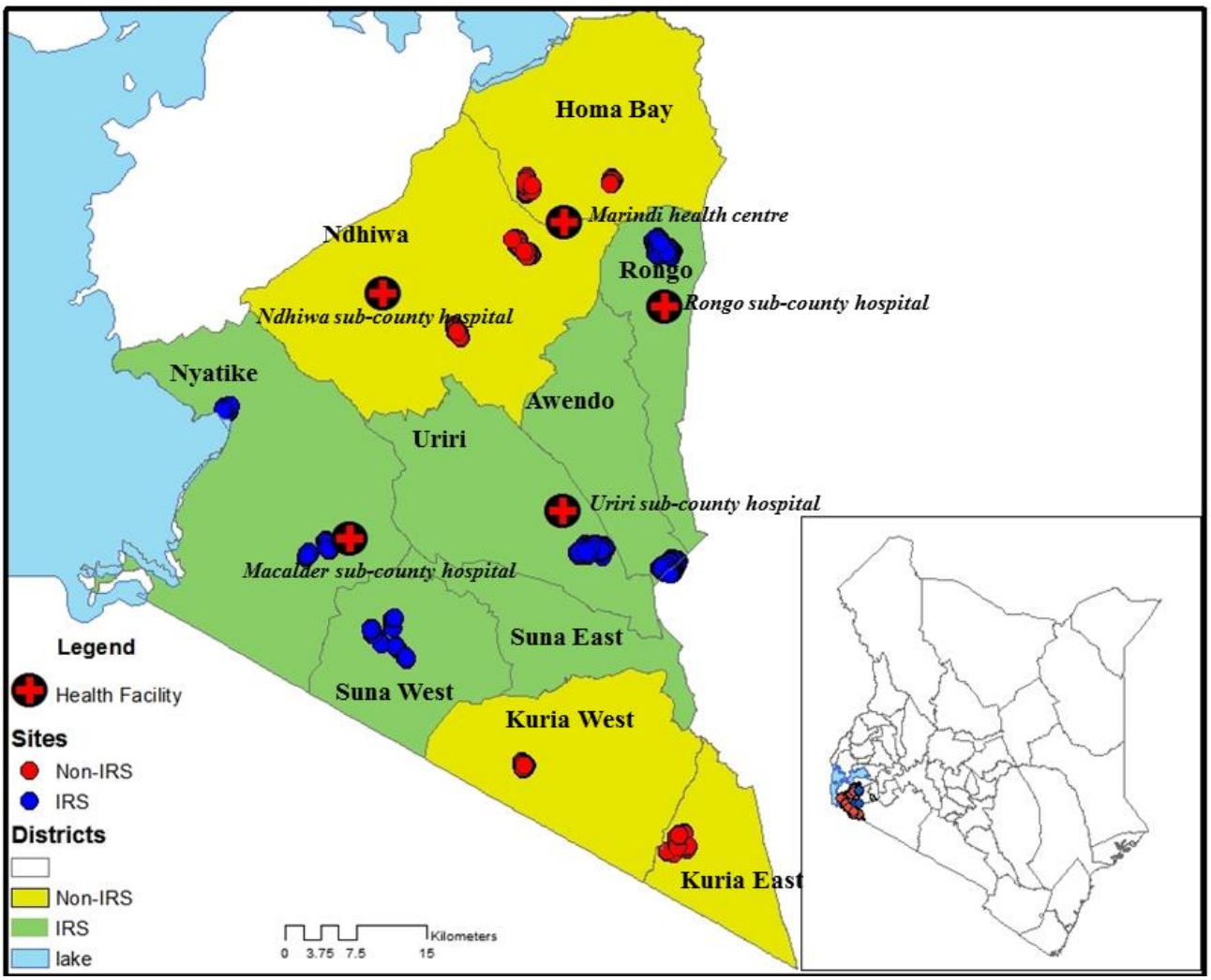

Figure 2.1: Map of Kenya, showing study sites in western Kenya with the names of sub-counties. Yellow shading represents non-intervention sites and red dots represent sampled houses. The green shading is the intervention site with the blue dots representing sampled houses.

IRS campaign. IRS was conducted in February-March 2017. A total of 212,029 houses in Migori County were sprayed representing coverage of 97.7% of houses sprayed against houses found. The campaign covered a population of 906,388 people, including 16,932 pregnant women and 127,157 children below five years of age [165].

Vector surveillance. Vector surveillance was conducted in the twelve villages from July 2016 to February 2018. Houses were randomly selected in each village every month for mosquito collections by PSC and indoor CDC light trap (CDC-LT). Household information

including roof type, wall type, open or closed eaves, the presence of nets, number of people that slept under a net the previous night and those that did not, and the presence of cattle were collected on a tablet computer. The mosquito density for each method was expressed as the mean number of mosquitoes caught house per collection visit.

Indoor-resting mosquitoes were collected between 07:00 and 11:00 by PSC in five houses per site per month. PSCs were done by laying white sheets on the floor and over the furniture within the house. Two collectors, one inside the house and another outside, sprayed around the eaves with 0.025% pyrethrum emulsifiable concentrate mixed with 0.1% piperonyl butoxide (supplied by the Kenya Pyrethrum Board) in kerosene. The collector inside the house then sprayed the roof space. The house was closed for 10-15 minutes after which knocked-down mosquitoes were collected from the sheets and transferred to the laboratory in scintillation vials containing 70% ethanol.

Indoor host-seeking mosquitoes were collected by CDC-LT in 10 houses per site once per month. A single 12-volt CDC-LT was hung in each house in the sleeping area, approximately 1.5 meters from the floor, adjacent to an occupied bed net owned by a member of the household. The traps were run from 18:00 to 07:00 the following morning. The trapped mosquitoes were transferred into paper cups and transported to the laboratory for further analysis.

Human landing catches (HLC) were used to assess biting time and location (indoor vs outdoor) of the local vector population before and after spraying. HLC was done during the short rains pre-IRS in November 2016, and after the long rains in June 2017. Collections were performed at six sites used for routine surveillance, two in non-IRS areas and four IRS

areas. In each site, five houses were selected, and collections were performed for five consecutive nights in each house once before and after IRS.

During HLC, one volunteer sat outside within 5 meters from the house, and another sat inside the house in the living room. Collectors kept their trousers folded to knee length and aspirated any mosquitoes landing on their lower legs. Each house had a team of six collectors, each working in pairs during one of three six-hour shifts running from 17:00 to 11:00 the next morning. Collections were performed for 45 minutes, and the collectors rested for 15 minutes in each collection hour. The collectors recorded the location of members of the household observed at the end of each hour as either outdoor, in the living room, or in the bedroom. Collected mosquitoes were separated by time and location of collection and sustained on a 10% sugar solution before being transported to the laboratory for analysis. Estimation of exposure of individuals to bites by *An. funestus* was performed using models previously described by Seyoum et al., [166].

Persistence of insecticidal activity on sprayed walls. To assess the persistence of insecticidal activity on sprayed walls following IRS, WHO cone bioassays [167] were conducted each month using laboratory-reared, 2-5 day old, non-blood fed susceptible colony of *An. gambiae* Kisumu strain. Mosquitoes were exposed in 10 randomly selected sprayed houses, seven with mud walls and three with cement walls, in each of four sub-counties in Migori county. Exposures were performed monthly in the same houses at three heights (0.5 m, 1 m, and 1.5m) from the floor for 30 minutes, on three different walls of the living room of each sprayed house. A control cone with ten mosquitoes was set on an unsprayed plywood board outside of each sprayed house in a shaded area close to the house. Temperature and relative humidity were recorded at every house where mosquitoes were exposed.

Insecticide resistance monitoring. WHO insecticide susceptibility tests were performed in Rongo, Nyatike, Awendo and Uriri sub-counties in Migori County (IRS sites) and Homa Bay and Ndhiwa sub-counties in Homa Bay County (no IRS). Larval stages of *An. gambiae* s.l. were collected from Homa Bay, Ndhiwa, Rongo and Nyatike sub-counties. The collected larvae were raised to three-day-old adults before testing. Adult *An. funestus* were also collected by hand aspiration inside houses for insecticide resistance tests as larvae were difficult to find. Collections were performed in Homa Bay and Ndhiwa, Rongo, Awendo and Uriri sub-counties before IRS. However, after IRS few adult mosquitoes were found in Rongo, Awendo and Uriri sub-counties, so no *An. funestus* s.l. were available for testing from these areas.

Insecticide resistance status was assessed using the WHO diagnostic concentrations of deltamethrin (0.05%), permethrin (0.75%), pirimiphos-methyl (0.25%) and alpha-cypermethrin (0.05%). All papers were prepared by the WHO collaborating centre, Universiti Sains Malaysia. The WHO bioassay was done using 2- to 5-day-old *An. gambiae* s.l. emerging from collected larvae or by direct exposure of field-collected adult *An. funestus* since these were difficult to collect as larvae and raise in the lab. At least 100 mosquitoes (four replicates of 25) of each species were exposed to each insecticide per sub-county. The samples were then transferred to a holding tube, provided with cotton wool soaked in 10% sugar solution and held for 24 hours. Mortality was scored 24 hours after exposure.

Mosquito species identification, sporozoite infection and blood meal identification. All *Anopheles* collected were identified morphologically to species using the keys of Gillies and DeMeillon or Gillies and Coetzee [168, 169]. The physiological status was determined by observation of the abdomen to classify female mosquitoes as either blood-fed, gravid, half

gravid or unfed. Female mosquitoes were dissected into three parts for various procedures: heads and thoraces were used for determination of *Plasmodium falciparum* sporozoite infection by enzyme-linked immunosorbent assay (ELISA) using the MR4 Methods in *Anopheles* Research adapted from Wirtz et al. [170, 171]; the abdomens of blood-fed females were used to determine the source of mosquito blood meals by targeting cytochrome b protein using a multiplexed PCR protocol [172], with slight modifications. The legs and wings were used in PCR analyses to identify to species level members of the *An. gambiae* species complex and *Anopheles funestus* group [173]. All mosquitoes morphologically identified as *An. gambiae* s.l. and of 20% of randomly selected *An. funestus* s.l. from all collections per month, were analyzed by PCR each month. This approach was done due to the greater number of *An. funestus* collected and based on previous studies in the area showing that *An. gambiae* and *An. arabiensis* are found in sympatry, while *An. funestus* s.s. was the only member of the species group routinely collected [11, 21]. To determine the local mosquito population age structure, parity dissection was performed on live females from CDC-LT using MR4 Methods in *Anopheles* Research[171].

Health facility surveillance. Health facility laboratory data were collected from Rongo, Uriri, and Macalder sub-county hospitals within Migori County (IRS) and Marindi health centre and Ndhiwa sub-County hospital in Homa Bay County (No IRS). The facilities were chosen based on proximity to entomological surveillance sites, availability of health records and catchment area as falling within either IRS or non-IRS area. Febrile cases were tested by health facility staff using light microscopy as part of routine health care and data were recorded in registers provided by the Kenya Ministry of Health. Data were abstracted from laboratory registers of the selected health facilities for the period from January 2015 until June 2018. Each page of the register was photographed using a smartphone camera, and the

photographs converted to PDF files using CamScanner-Phone PDF creator, (INTSIG Information Co., Ltd). To ensure confidentiality, the column containing the patient's name was covered when taking the photograph. The PDF copies were then printed and filed.

Data management and analysis. Field entomological data collection used Open Data Kit software (ODK) run on tablets with an interface designed to limit data entry errors. Data entry screens used drop-down menus and automatic data checks to reduce errors. Each house sampled received a unique code and a study number. Individual mosquitoes were placed in Eppendorf tubes labeled with pre-printed barcodes and linked to the field data by house code and a unique study number. Results of additional testing, including sporozoite ELISAs, species identification by PCR and blood meal analysis, were linked to individual mosquito by the unique barcode label. Individual patient records including included date of testing, age, gender, village, clinical diagnosis, test performed, and test results from scanned copies of health facility registers were entered into a Microsoft Access database.

Data analysis was performed using R statistical software version 3.4.1 or SAS version 9.4. The risk ratio (RR) was used to assess the statistical significance of differences in mosquito densities pre and post IRS, between intervention and non-intervention sites. Data were fitted using Generalized Linear Mixed Effects Statistical Models (GLMMs). Since the data were over-dispersed, we used the package Generalized Linear Mixed Models using Template Model Builder (glmmTMB) or PROC GLIMMIX, to fit negative binomial distribution models for the analysis of mosquito numbers. The mean numbers of *An. gambiae* and *An. funestus* were assessed as a function of the period of collection (before or after IRS) and intervention status (sprayed or non-sprayed) as a fixed effect, while village was treated as a random effect. To analyse the association between household characteristics and vector

abundance, the numbers of female *Anopheles* were assessed as a function of different house characteristics including net use, eave type and presence of cattle with or without IRS as a fixed effect, while the village was treated as a random effect. Model selection was done by backward elimination of variables with *P*-value larger than 0.05 from the full model. To obtain the risk ratios (RR) and confidence intervals, we exponentiated the model coefficients. Models were adjusted for reported net use, the presence of open eaves, and the presence of cattle in the compound. A test of interaction was performed to compare differences in estimates of mosquito numbers between the period of mosquito collection and intervention status [174]. Conditional estimates of the change in mosquito densities pre- and post-IRS conditional on the IRS or non-IRS County were generated. A chi-squared test was used to analyse the distribution of different house characteristics between intervention and non-intervention sites. A test of proportion was used to assess the probability of occurrence of individual *Anopheles* species of all collected female *Anopheles* mosquitoes, before and after IRS in intervention and non-intervention sites for each trapping method. A binomial GLM model was used to analyse sporozoite rates (proportion of sporozoite ELISA tests that are positive of all tested samples), parity rates (proportion of parity dissections that are parous of all dissected female mosquitoes) and human biting rates between intervention and non-intervention sites, before and after IRS and proportions of the types of mosquito host blood meals. The proportion of sporozoite positive tests of all tested samples were assessed as a function of collection period and intervention status. Table 2.1 below is a summary of different statistical models fits for the different statistical analysis.

To detect changes in numbers of malaria cases before and after IRS within each health facility Auto-Regressive Integrated Moving Average (ARIMA) analysis was performed. Data from each facility was analysed using the "Time Series Analysis" (TSA) [175] and

"Alternative Time Series Analyses" (aTSA) [176, 177] packages in R to determine the number of malaria test positive cases by both malaria RDT and microscopy per facility per month. The ARIMA model was derived by observation of the autocorrelation and partial-autocorrelation functions to determine the most parsimonious solution of the "order" (p), "differencing" (d), and "moving-average" (q) parameter values. The model was then regressed on the absence (prior to) or presence of IRS in the village to estimate the value of the number of positive malaria cases prior to, and during the period of IRS.

Table 2.1: Summary of different statistical model fits used in data analysis.

Trait	**Response variable**	**Fixed effect**	**Random effect**	**Mosquito species**	**Data**	**Distribution**
Abundance	Mean number per house per night	Period (Per-, Post-IRS)	Village	*An. arabiensis* and *An. funestus*	Light trap no IRS, Light trap IRS, PSC no IRS, PSC IRS	Negative binomial
Abundance	Mean number per house per night	Net use, Eaves, Cattle	Village	*An. arabiensis* and *An. funestus*	PSC, Light trap	Negative Binomial
Sporozoite rate	Proportion of sporozoite positive tests of all tests performed	Period (Per-, Post-IRS) Status (IRS, No-IRS)	-	*An. arabiensis* and *An. funestus*	CDC light trap and PSC combined	Binomial
Parity rate	Proportion of parous sample of all dissected mosquitoes	Period (Per-, Post-IRS) Status (IRS, No-IRS)	-	All *Anopheles* species	CDC light trap and PSC combined	Binomial
Host blood meal type	Proportion of a host blood meal type of all blood meal types	*Anopheles* species (*An. funestus, An. arabiensis*)	-	Blood mean types (Human, Cow, Goat, and Pig)	Blood meal analysis dataset	Binomial

Ethical considerations. The study was approved by the Kenya Medical Research Institute/ Scientific and Ethics Review Unit (KEMRI/SERU), number 2776 and by CDC through a reliance agreement with KEMRI/SERU (CDC IRB 6728). Individuals participating in HLC gave informed consent. They were screened for malaria before the start of the study and treated if positive. Collectors were placed on mefloquine malaria prophylaxis, (Mephaquin, Acino Pharma AG, Switzerland) one week before collections began, with repeat doses once every week through the collection period, until four weeks after collections ended. During routine mosquito collections, verbal consent was sought from the household head to use CDC-LT and PSC in their compound. All methods were performed in accordance with relevant guidelines and regulations.

Results

Vector species composition and seasonality. A total of 10, 838 *Anopheles* mosquitoes were collected by all methods combined in both intervention and non-intervention sites. Morphologically, 79.21% were identified to be *An. funestus* (N=8585), 19.14% *An. gambiae* s.l. (N=2074), 1.50% *An. coustani* (N=163), 0.09%, *An. rufipes* (N=10), 0.04% *An. paroensis* (N=4) and 0.02% *An. maculipalpis* (N=2). A sub-sample of 4091 *An. funestus* were analyzed by PCR for species identification and confirmed to be *An. funestus s.s.* Similarly, a total of 1,061 *An. gambiae* s.l were analyzed by PCR for species identification, 98.69% were confirmed to be *An. arabiensis* (N=1,045) while 1.51% *An. gambiae* (N=16).

The mean number of *An. funestus* and *An. gambiae s.l.* found in indoor CDC-LT and PSCs are presented by IRS status and period (pre- or post-IRS) in table 2.2. The number of each species of mosquito collected by the two different methods was compared using negative

binomial regression models incorporating IRS status, period, an interaction between IRS status and period, net use, the presence of open eaves and the presence of cattle on the compound (Appendix 1-4). For all models except for the *An. gambiae* s.l. collected by PSC, the interaction term was statistically significant indicating a differential effect of the period based upon the IRS status. Conditional estimates of the effect of period controlling for IRS status with associated Chi-squared test statistics are provided in Table 2.2. The number of *An. funestus* collected in light traps in intervention sites were significantly lower in the post-IRS compared to pre-IRS period (RR=0.12, 95% CI: 0.07-0.19, P<0.001). No significant difference in the mean number of *An. funestus* was observed in the non-intervention sites between pre- and post-IRS (IRR=0.98, 95% CI: 0.69-1.38, p=0.899). A statistically significant difference-of-differences between the period of mosquito collection and intervention status was observed based on the statistically significant interaction term (RR=0.12, 95% CI: 0.07 – 0.21) (Appendix 1). From PSC collections, significantly fewer numbers of *An. funestus* were observed in both IRS and non-IRS sites in the post-IRS period compared to pre-IRS period (RR=0.04, 95% CI: 0.02-0.07, p<0.001). The number of *An. funestus* in the non-IRS area also declined but the conditional difference between pre-IRS and post-IRS was not statistically significant (RR=0.64, 95% CI: 0.41-1.00, p=0.052). A statistically significant difference-of-differences was observed between period of mosquito collection and intervention status post-IRS indicating a stronger decline in the IRS sites compared to the non-IRS sites (RR=0.06, 95% CI: 0.03-0.13, p<0.001) (Appendix 2).

The mean numbers of *An. arabiensis* collected in indoor CDC-LTs in both intervention and non-intervention sites increased in the post-IRS compared to pre-IRS period with a statistically significant increase in the non-IRS sites (IRS sites: RR=1.39, 95% CI: 0.78-2.47, p=0.266; non-IRS sites: RR=3.06, 95% CI: 1.59-5.92, p=0.001). The conditional estimates

are provided in Table 1 although the interaction term was not significant (RR=0.45, 95% CI: 0.2-1.01, p=0.052) (Table 1) indicating the increase was not statistically greater in the non-IRS sites than the IRS sites (Appendix 3).

The mean numbers of *An. arabiensis* collected by PSC in the intervention sites were not significantly different in the post-IRS compared to pre-IRS period (RR=0.60, 95% CI: 0.33-1.09, p=0.093). For the non-IRS areas, the number of *An. arabiensis* collected by PSC increased although not significantly (RR=1.64, 95% CI: 0.87-3.09, p=0.123). Although no significant difference in the mean numbers of *An. arabiensis* was observed pre- and post-IRS in either the IRS or the non-IRS sites, a statistically significant difference-of-differences was observed between the period of mosquito collection and intervention status indicating a significant difference between the IRS and non-IRS areas after IRS implementation (RR=0.36, 95% CI: 0.16-0.82, p=0.015) (Appendix 4).

Table 2.2: Comparison of mean numbers of *An. funestus* and *An. arabie*nsis collected indoors by CDC-LTs and PSCs pre- and post-IRS in intervention and non-intervention areas. Risk ratios of post- versus pre-IRS periods conditional on intervention status are also provided for each species and collection method. See Appendix 1-4 for the full model

Anopheles Species	Collection Method	IRS Status	Level	Mean	Risk Ratio	Lower CL	Upper CL	X^2	P-value
Anopheles funestus	Light trap	IRS	Post Spray	0.05	0.12	0.07	0.19	82.03	**<0.001**
			Pre-Spray	0.45	Ref				
		Non-IRS	Post Spray	0.88	0.98	0.69	1.38	0.02	0.899
			Pre-Spray	0.92	Ref				
	PSC	IRS	Post Spray	0.04	0.04	0.02	0.07	102.48	<0.001
			Pre-Spray	0.99	Ref				
		Non-IRS	Post Spray	1.05	0.64	0.41	1.00	9.16	0.052
			Pre-Spray	2.05	Ref				
Anopheles arabiensis	Light trap	IRS	Post Spray	0.19	1.39	0.78	2.47	7.47	0.266
			Pre-Spray	0.10	Ref				
		Non-IRS	Post Spray	0.21	3.06	1.59	5.92	24.53	0.001
			Pre-Spray	0.05	Ref				
	PSC	IRS	Post Spray	0.24	0.60	0.33	1.09	0.63	0.093
			Pre-Spray	0.52	Ref				
		Non-IRS	Post Spray	0.41	1.64	0.87	3.09	5.20	0.123
			Pre-Spray	0.27	Ref				

The mean number of *An. funestus* collected by CDC-LT and PSC in both intervention and non-intervention areas varied by month, with the highest numbers collected during the short rainy season before IRS (Nov-Dec 2016) and during the long rainy season in the unsprayed area (March-June 2017) (Fig. 2.2). After IRS, the mean numbers collected by both CDC-LT and PSC in the intervention areas remained low, with no seasonal variation throughout the study period. The mean number of *An. arabiensis* collected by either method was lower compared to *An. funestus* with little monthly variation before and after IRS. No clear difference was observed in the seasonality of *An. arabiensis* before and after IRS (Fig. 2.2).

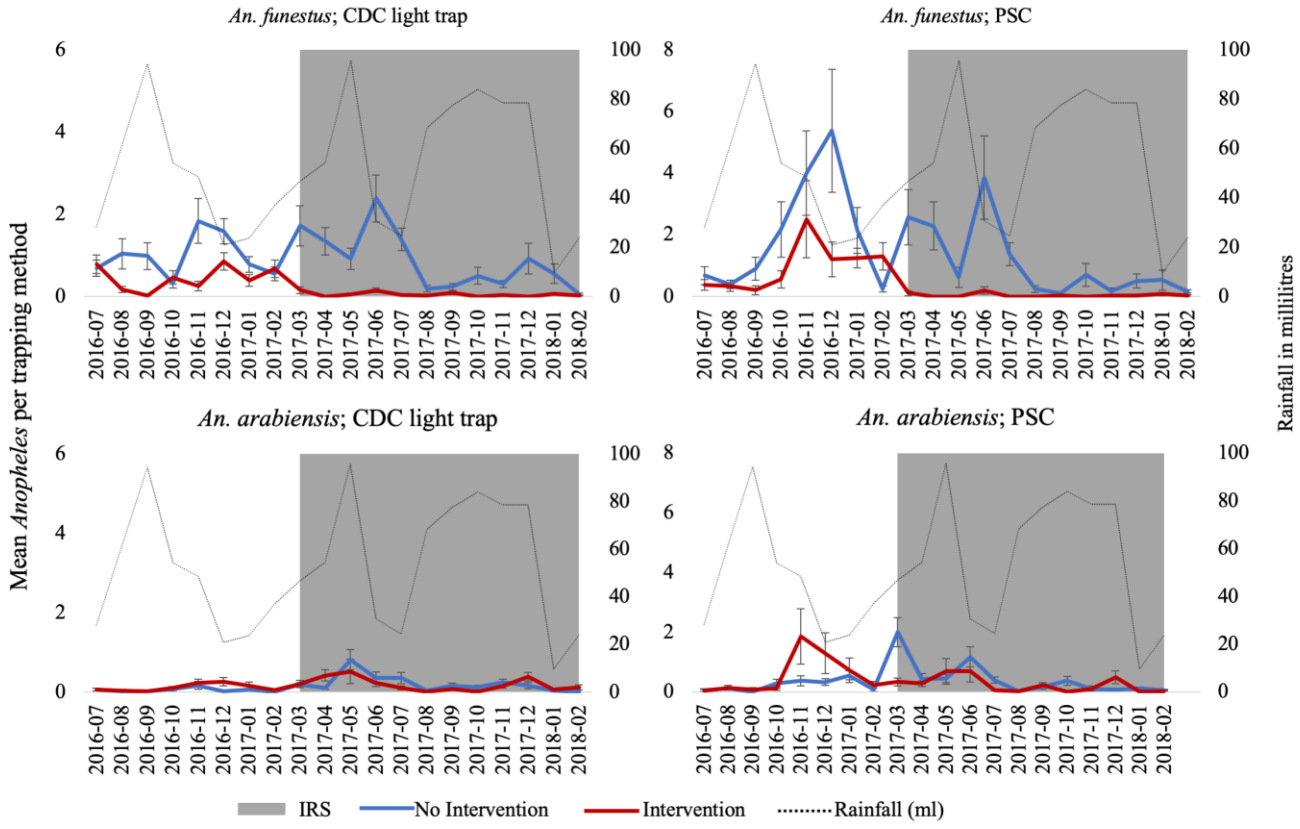

Figure 2.2: Mean monthly density (means ± std errors) of indoor host-seeking and resting *Anopheles* mosquitoes before and after IRS in sprayed and unsprayed areas. The grey shade shows period under IRS. The primary scale shows *Anopheles* density while the secondary scale shows rainfall in millilitres.

Significant differences in *Anopheles* species composition were observed in intervention areas before and after IRS. Significantly high proportions of *An. funestus* were observed in non-intervention sites compared to *An. arabiensis* and other *Anopheles* species, X^2=1204.3, df=2, P<0.001 and X^2=1094.6, df=2, P<0.001, before and after IRS respectively. Whereas, in the intervention sites, significantly high proportions of *An. funestus* were observed before IRS, X^2=441.4, df=2, P<0.001, after IRS, *An. arabiensis* become the most dominant species X^2=144.3, df=2, P<0.006. Similarly, from PSC collection, significantly high proportions of *An. funestus* were observed in non-intervention sites before and after IRS, X^2=1253.3, df=2, P<.0.001 and X^2=821, df=2, P<0.001 respectively. In the intervention site, *An. funestus* most dominant in proportion X^2=360.5, df=2, P<0.001 before IRS, however, after IR, *An. arabiensis* become the most dominant species X^2=254.2, df=2, P<0.001. (Fig. 2.3).

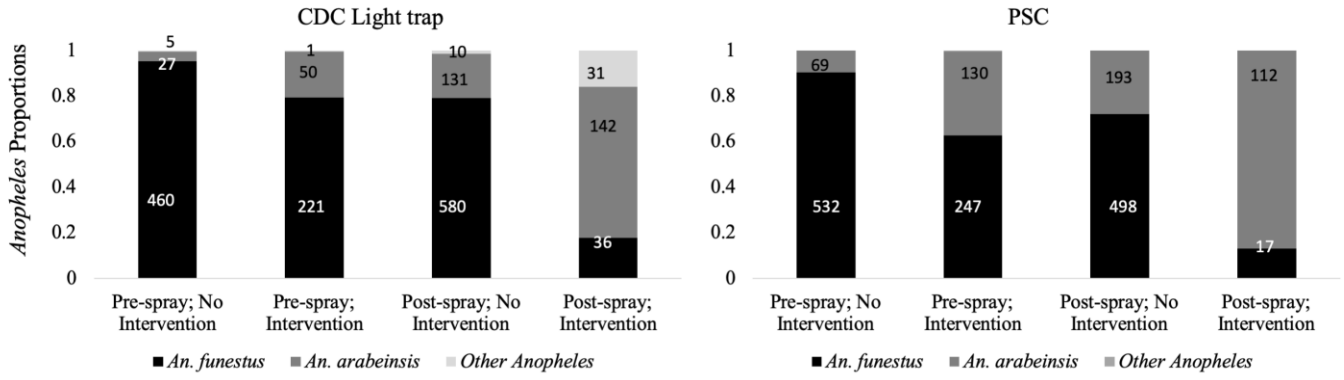

Figure 2.3: Proportions of *Anopheles* species by CDC light trap and PSC before and after IRS in sprayed and unsprayed regions.

Insecticide decay rate and insecticide resistance monitoring. Mortality rates of susceptible *An. gambiae* s.s females were over 80% up to 10 months post-IRS (Supplemental Fig. 1). Using WHO bioassays both *An. funestus* (Supplemental Fig. 2a) and *An. arabiensis* (Supplemental Fig. 2b) were fully susceptible to pirimiphos-methyl and bendiocarb but resistant to the pyrethroids, deltamethrin, permethrin, and alpha-cypermethrin.

Factors affecting *Anopheles* mosquito numbers. Analysis of household characteristics during the baseline period indicated there was, no significant difference in the distribution of different roof types between intervention and non-intervention sites (X^2 = 3.76, df=2, P=0.15). However, there was a significant difference in the distribution of wall types between the intervention and non-intervention sites (X^2 = 258.52, df=5, P<0.0001). There were more houses with brick, cement, mud, and painted cement wall in the intervention sites compared to non-intervention sites, whereas, houses with plastered mud walls were more common in the non-intervention site. Distribution of the different eaves types was significantly different between intervention and non-intervention sites (X^2=10.19, df=2, P=0.01). Houses with open and closed eaves were more common in the intervention sites compared to non-intervention sites, while a slightly higher proportion of houses with partially open eaves were observed in the non-intervention sites versus intervention sites. Similarly, distribution of cattle and net use in houses within the intervention and non-intervention sites were significantly different, (X^2=19.98, df=1, P<0.0001) and (X^2= 30.66, df=2, P<0.0001) respectively. Higher proportion of houses had cattle in the non-intervention sites versus intervention sites. Additionally, higher proportion of households in the non-intervention sites used bednets compared to intervention sites. No significant difference in the proportion of households that reported cooking indoors in intervention and non-intervention sites, (X^2=0.13, df=1, P=0.71) (Table 2.3).

Table 2.3: Comparison of different house characteristics between intervention and non-intervention sites.

Categorical variable	**Categories**	**Number in intervention (%)**	**Number in non-intervention (%)**	X^2	**df**	**P value**
Roof type	Grass thatch	100 (4.15)	121 (5.28)	3.76	2	0.15
	Iron Sheet	2310 (95.81)	2169 (94.63)			
	Tiles	1 (0.04)	2 (0.09)			
Wall type	Brick	93 (3.86)	54 (2.36)	258.52	5	<0.0001
	Cement	238 (9.87)	62 (2.71)			

	Mud	392 (16.26)	289 (12.61)			
	Painted Cement	201 (8.34)	34 (1.48)			
	Plastered mud	1477 (61.26	1837 (80.15)			
	other	10 (0.41)	16 (0.70)			
Eaves	Open	287 (11.90)	240 (10.47)	10.19	2	0.01
	Partially open	1624 (67.36)	1642 (71.64)			
	Closed	500 (20.74)	410 (17.89)			
Cattle kept	Yes	1628 (67.52)	1685 (73.52)	19.98	1	<0.0001
	No	783 (32.48)	607 (26.48)			
Cook in the house	Yes	645 (26.75)	625 (27.27)	0.13	1	0.71
	No	1766 (73.25)	1667 (72.73)			
Net use	All under net	1467 (61.64)	1539 (68.16)	30.66	2	<0.0001
	some under net	357 (15.00)	229 (10.14)			
	Non under net	556 (23.36)	490 (21.70)			

Table 2.4 presents data showing modelled estimates of the effect of net use, open eaves, and presence of cattle in the compound on the indoor occurrence of *An. funestus* and *An. arabiensis* in sprayed and unsprayed houses, measured by CDC-LT and PSC collections. For *An. funestus*, significantly fewer were collected by light traps in houses with completely closed eaves (RR=0.68, 95% CI: 0.48-0.96, p=0.030) while significantly more were collected from houses where cattle were kept on the compound (RR=1.62, 95%CI: 1.22-2.13, p=0.001). No other comparisons were statistically significant. By PSC, there were again significantly more *An. funestus* collected in households were cattle were kept on the compound (RR=1.63, 95% CI: 1.12-2.35, p=0.010). There were significantly more *An. funestus* in houses where some but not all residents used a net the previous night compared to houses where no one used a net (RR=2.02, 95% CI: 1.13-3.59, p=0.017). No other comparisons were statistically significant.

From light trap collections, closed eaves were associated with significantly lower numbers of *An. arabiensis* (RR=0.57, 95% CI: 0.33-0.96, p=0.033) while significantly more *An.*

arabiensis were collected in houses where some but not all residents of the household used a net the night before compared to houses where no one used a net (RR=2.17, 95% CI: 1.02-4.62, p=0.045). The number of *An. arabiensis* collected by PSC also was significantly lower in houses with closed eaves compared to those with open eaves (RR=0.34, 95% CI: 0.18-0.67, p=0.002). No other comparisons were statistically significant.

Table 2.4: Model estimates comparing the mean number of indoor *An. funestus* and *An. arabiensis* collected, by collection type, eave type, net use, and presence of cattle in intervention and non-intervention areas. Models include terms for IRS status, pre/post spray period and an interaction term. Risk Ratio is the probability of the occurrence of mosquitoes under the different house parameters (Table 1). See Appendix 1-4 for full models.

Species	Collection Method	Parameter	Level	Risk Ratio	Lower CL	Upper CL	t-value	P-value
Anopheles funestus	Light Trap	Net Use	All under net	1.11	0.77	1.6	0.576	0.565
			Some under net	1.2	0.77	1.86	0.816	0.415
			None under net	Ref				
		Eaves	Closed	0.68	0.48	0.96	-2.174	**0.030**
			Partially open	0.84	0.56	1.27	-0.817	0.414
			Open	Ref				
		Cattle	Yes	1.62	1.22	2.13	3.395	**0.001**
			No	Ref				
	PSC	Net Use	All under net	0.96	0.61	1.5	-0.187	0.852
			Some under net	2.02	1.13	3.59	2.383	**0.017**
			None under net	Ref				
		Eaves	Closed	0.8	0.5	1.3	-0.889	0.374
			Partially open	1.08	0.64	1.83	0.291	0.771
			Open	Ref				
		Cattle	Yes	1.63	1.12	2.35	2.583	**0.010**
			No	Ref				
	Light Trap	Net Use	All under net	1.95	0.99	3.84	1.94	0.052
			Some under net	2.17	1.02	4.62	2.008	**0.045**
			None under net	Ref				
		Eaves	Closed	0.57	0.33	0.96	-2.131	**0.033**
			Partially open	0.78	0.43	1.42	-0.814	0.416
			Open	Ref				
		Cattle	Yes	1.33	0.89	1.98	1.383	0.167

Anopheles arabiensis			No	Ref				
	PSC	Net Use	All under net	1.61	0.9	2.87	1.594	0.111
			Some under net	1.85	0.89	3.84	1.652	0.099
			None under net	Ref				
		Eaves	Closed	0.34	0.18	0.67	-3.165	**0.002**
			Partially open	0.60	0.32	1.13	-1.583	0.114
			Open	Ref				
		Cattle	Yes	1.53	1.00	2.34	1.944	0.052
			No	Ref				

Sporozoite infection rates. Sporozoite infection rates in *Anopheles* mosquitoes were determined in intervention and non-intervention sites before and after IRS. Before IRS, 4.8% (48/1,000) of *An. funestus* were sporozoite positive in non-intervention sites compared to 2.2% (10/447) in the intervention sites whereas for *An. arabiensis*, sporozoite positivity rate was 2.8% (10/357) in non-intervention sites and 1.5% (3/192) in the intervention sites before IRS. Sporozoite infection rates for both species combined were not significantly different between intervention and non-intervention sites before IRS (RR=0.27, 95% CI: 0.06 -1.26, P = 0.09). After IRS, sporozoite infections were detected only in the non-intervention sites, where 3.5% (40/1,132) of *An. funestus* and 3.3% (22/643) of *An. arabiensis* were positive. (Fig. 2.4).

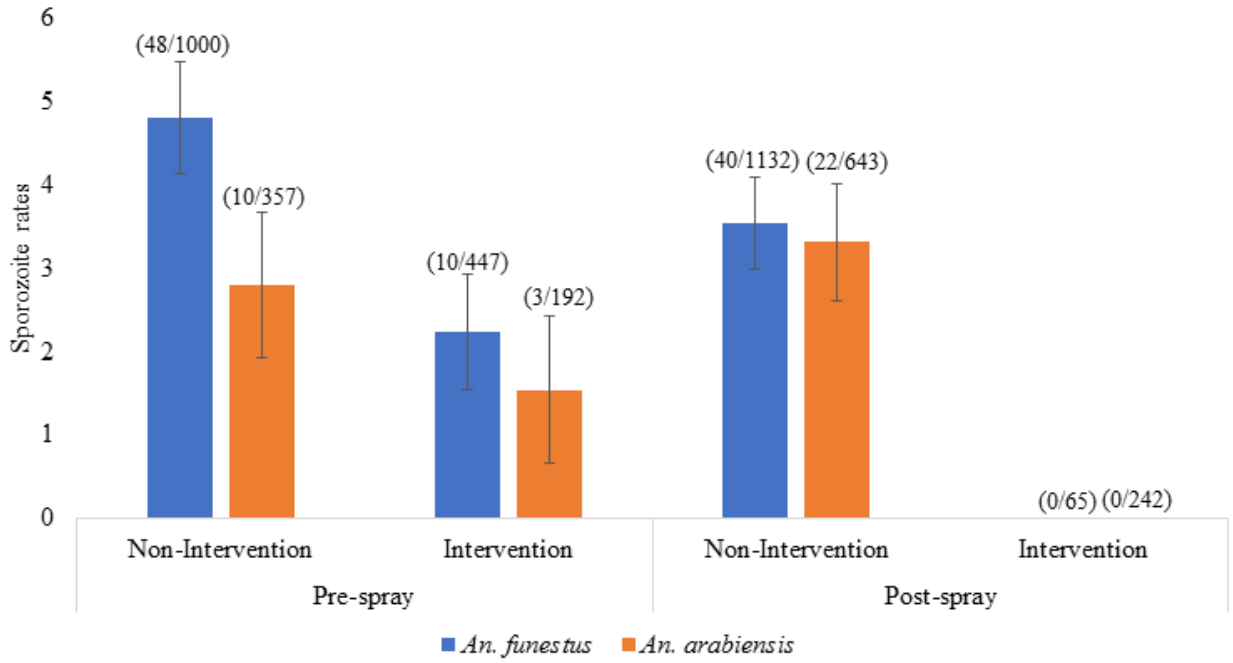

Figure 2.4: Sporozoite rates (proportions ± std errors) in *An. funestus* and *An. arabiensis* in sprayed and unsprayed areas, pre- and post-IRS.

Parity rates. High parity rates were observed in *Anopheles* collected in both non-intervention and intervention sites before IRS, 83% (24/29) and 78% (7/9) respectively. The parity rates were not statistically different between intervention and non-intervention sites before IRS (RR=0.06, 95% CI: 0.01-5.26). After IRS, the rates fell to 67% (22/33) in the non-intervention sites while *Anopheles* numbers were extremely low in the intervention sites post-IRS in the intervention sites with only 4 mosquitoes examined and one parous. No significant difference in parity rates was observed post-IRS (IRR=0.01, 95% CI: 0.00 – 5.76).

Vector biting behaviour. We estimated the exposure of humans to the risk of mosquito bites based on their observed behaviour and time and location of *An. funestus* biting. The numbers of *An. arabiensis* were insufficient to be included in the analysis. Over 70% of people within the study area were observed to be outdoors at 17:00, the beginning of mosquito collection. The number of people outdoors declined steadily over time with an increase in the number of

individuals observed indoors, either in the living room (indoors not asleep) or in the bedroom (indoors and in bed). Over 90% of the people were observed to be indoors and in bed between 23:00 and 05:00 (Fig. 2.5). In both intervention and non-intervention sites, before IRS, exposure to *An. funestus* was estimated to occur mostly, although not exclusively, indoors, late at night when people were asleep (Fig. 2.6 a and b). In the post-IRS period, no change in the estimated exposure to bites by *An. funestus* was observed in the non-intervention sites (Fig. 2.6c). However, in the intervention sites, the risk of exposure to mosquito bites was nearly zero post-IRS (Fig. 2.6d). The relative proportion of bites by *An. funestus* increased both indoors and outdoors at dawn (05:00 am -08:00 am), corresponding to the time when most individuals woke up. Low levels of biting continued until 11:00 am when collection ceased.

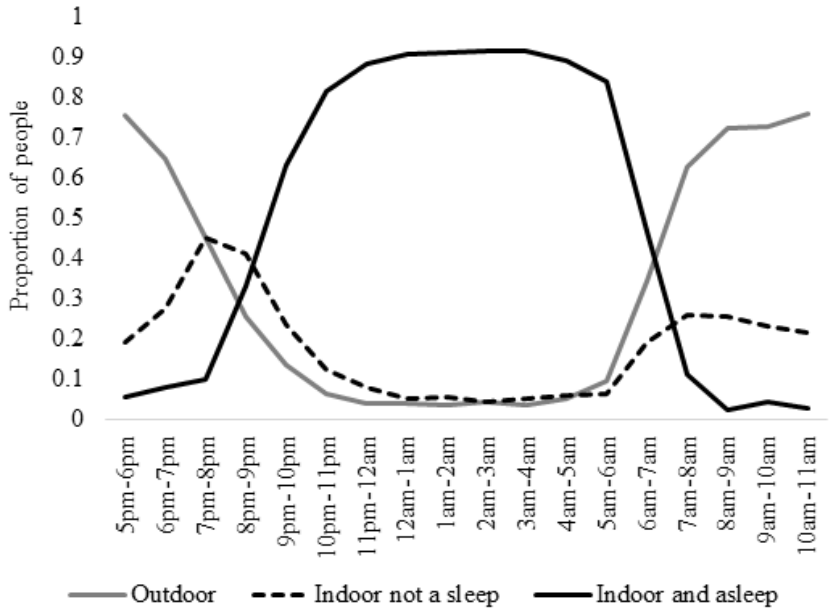

Figure 2.5: Proportion of people within the study area at a different location during Human Landing Catch collection

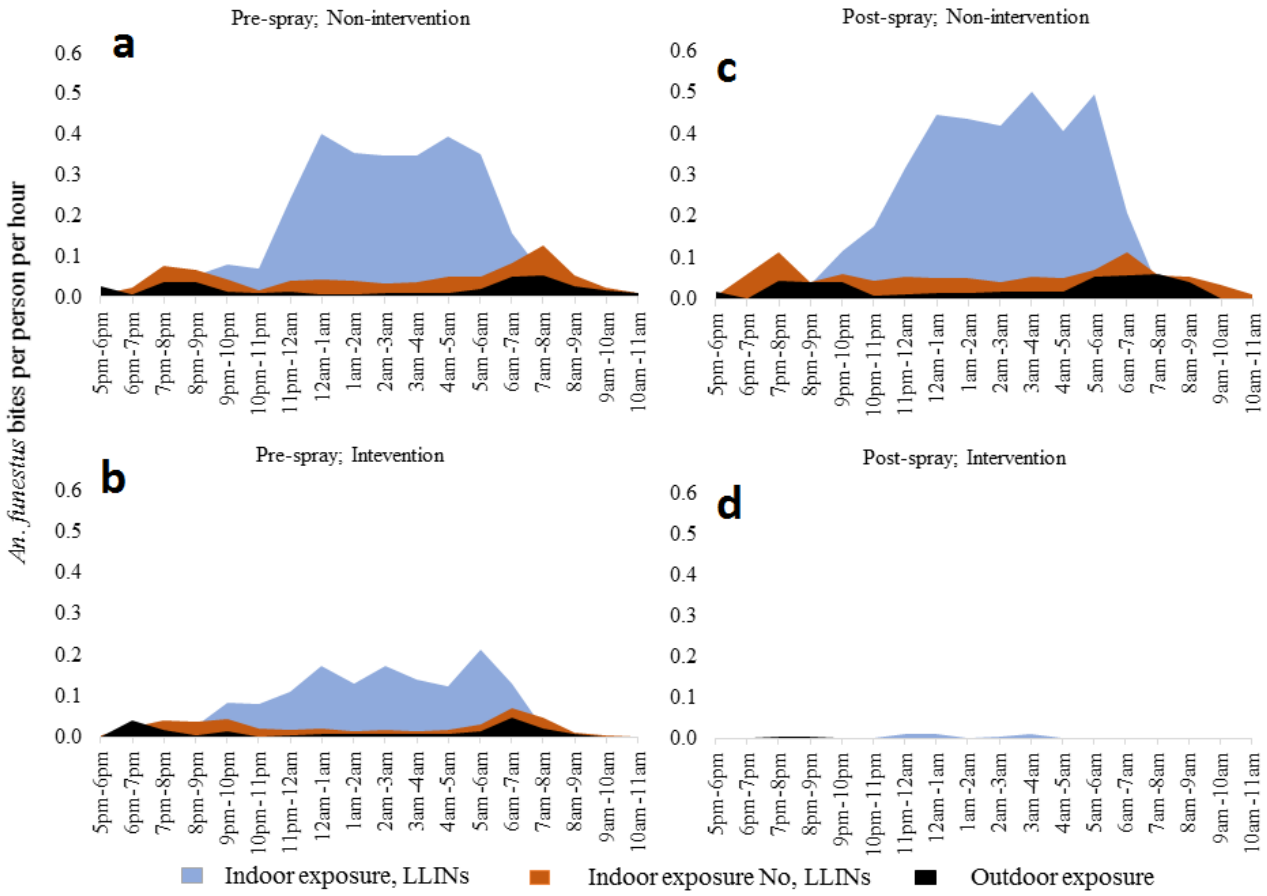

Figure 2.6: Profiles of biting by *An. funestus* experienced by the human population in intervention and non-intervention sites before and after IRS. The black area represents biting that occurs outdoors, the dark red represents biting that occurs indoors when people are away from their bed nets and the blue represents biting that occurs while people are asleep.

Biting rates by *An. arabiensis* were substantially lower compared to those of *An. funestus*. In both intervention and non-intervention sites, before IRS, exposure to *An. arabiensis* was estimated to occur indoor, late at night when most people were asleep (Fig. 2.7a and b). In the post-IRS period, no change in estimated exposure to bites by *An. arabiensis* was observed in both intervention and non-intervention sites (Fig. 2.7c and d). The risk of outdoor exposure to *An. arabiensis* bites in the non-intervention sites post-IRS were observed to increase in the evening (6:00 pm to 9:00) pm and at dawn (6:00 am to 8:00) am. No extended morning (up to 11:00 am) biting by *An. arabiensis* was observed.

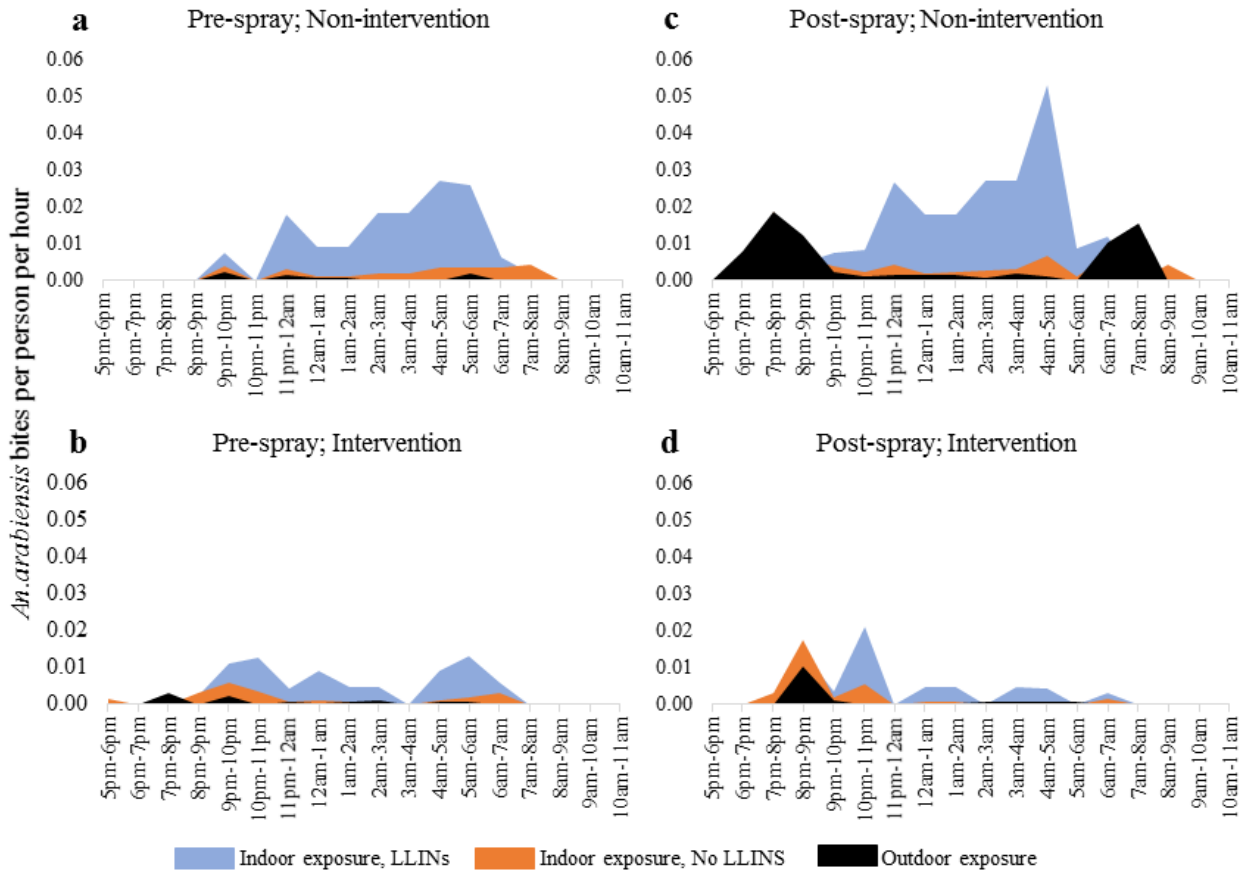

Figure 2.7: Profiles of biting by *An. arabiensis* experienced by the human population in intervention and non-intervention sites before and after IRS. The black area represents biting that occurs outdoors, the orange colour represents biting that occurs indoors when people are away from their bed nets and the blue represents biting that occurs while people are asleep.

Blood meal type. Blood meal analysis using mosquitoes collected by PSC was conducted on 236 fed *Anopheles* mosquitoes, 151 *An. funestus* and 85 *An. arabiensis*. *An. funestus* fed mostly on humans 52.3% (79/151), followed by cattle 40.4% (61/151), goat 3.3% (5/151), pig 0.7% (1/151) and mixed-blood meals 3.3% (5/151, 2 human/cow, 2 human/goat and 1 human/pig). *An. arabiensis* had fed mostly on cattle blood 70.6% (60/85), followed by pig 12.9% (11/85), human 9.4% (8/85), goat 4.7% (4/85) and mixed-blood meal, human/goat 1.9% (1/85) (Fig. 2.8).

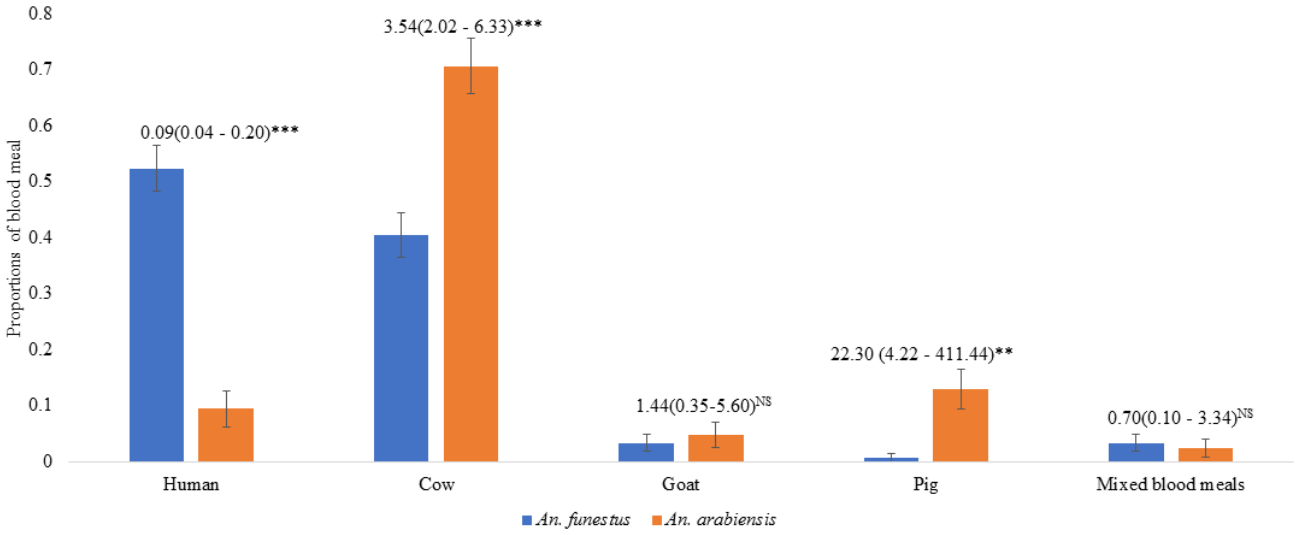

Figure 2.8: Comparison of mammalian host blood meal type (proportions ± std errors) between *An. funestus* and *An. arabiensis* (Numbers tested; *An. funestus*- 61 cow, 5 goats, 79 humans, 1 pig and 5 mixed and *An. arabiensis* – 60 cows, 4 goats, 8, humans,11 pigs and 3 mixed).

The insecticide decay rate and insecticide resistance monitoring. Mortality rates of susceptible *An. gambiae* s.s females were over 80% up to 10 months post-IRS (Fig 2.9). In WHO bioassays both *An. funestus* (Fig 2.10a) and *An. arabiensis* (Fig 2.10b) were fully susceptible to pirimiphos-methyl and bendiocarb but resistant to the pyrethroids, deltamethrin, permethrin, and alpha-cypermethrin.

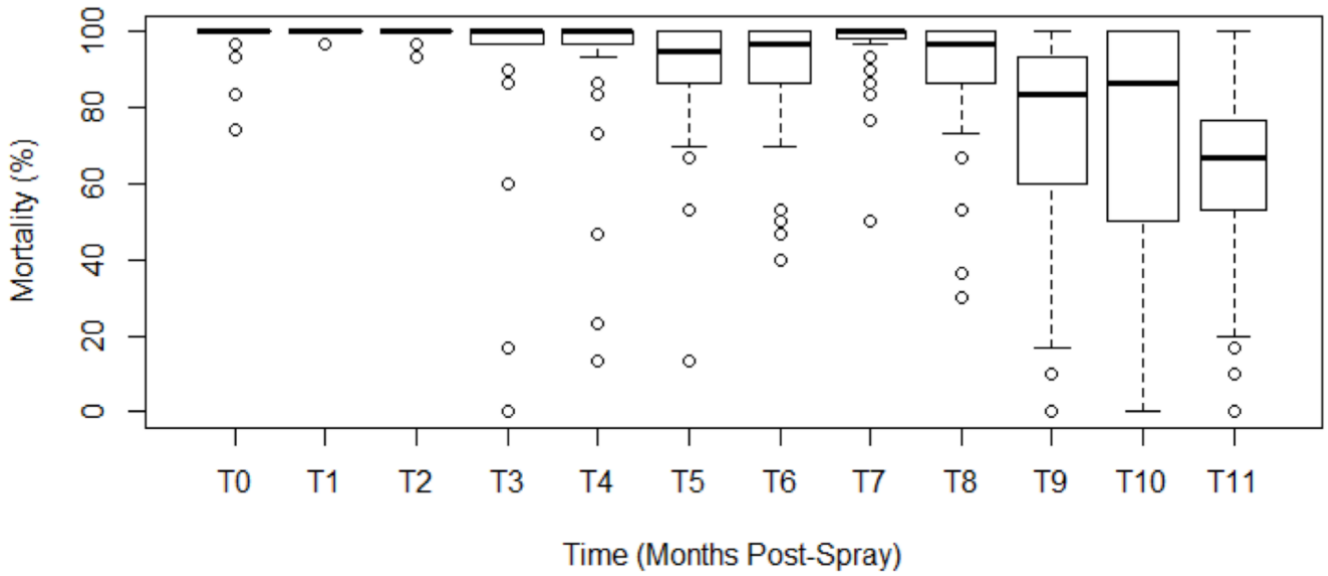

Figure 2.9: 24-hour mortality rates of susceptible *An. gambiae* exposed to sprayed walls over eleven months post-IRS. Distribution of mortality rates by box-whisker plots showing median values and interquartile ranges.

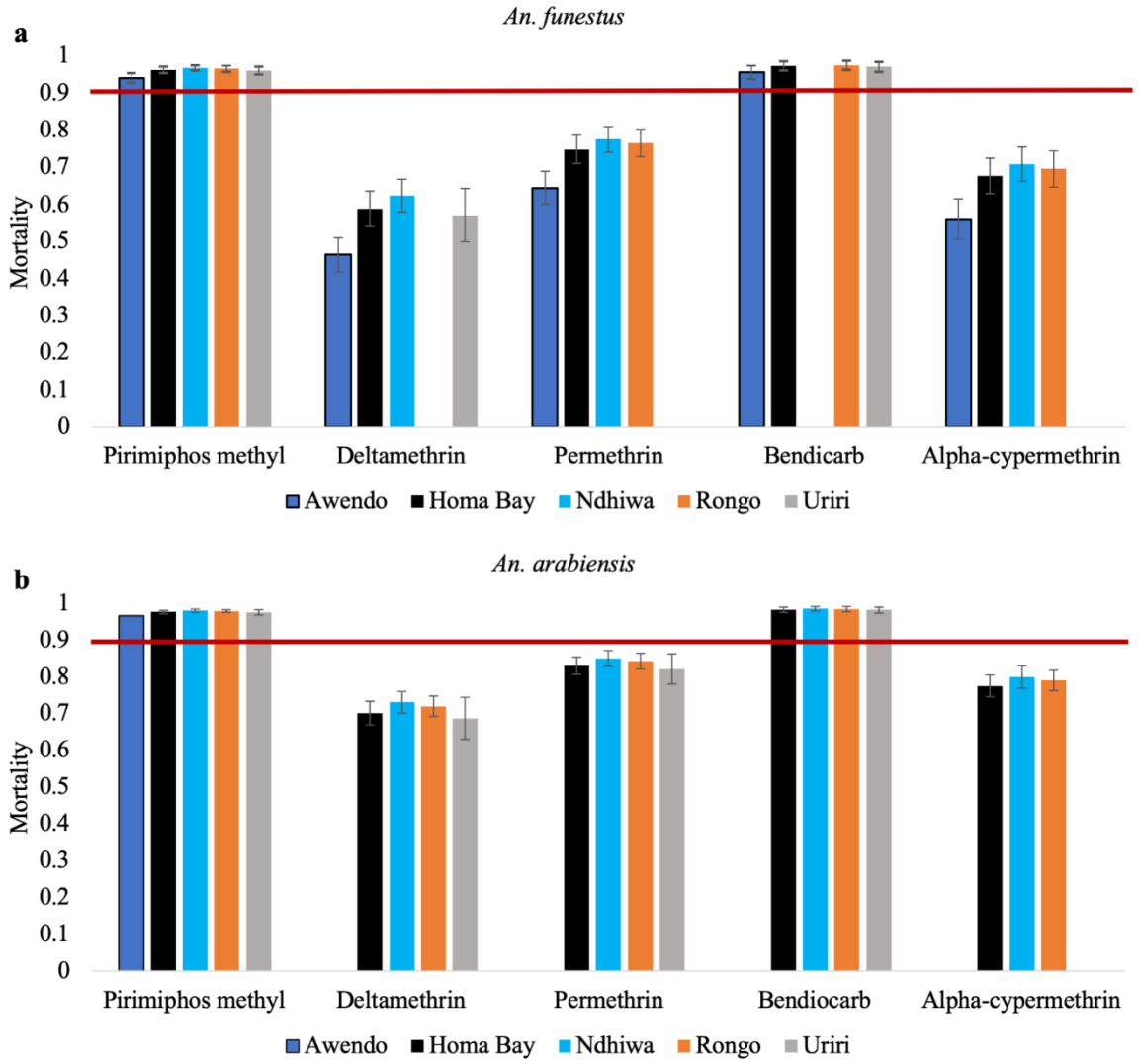

Figure 2.10: 24-Hour mortality (proportions ± std errors) of *An. funestus* and *An. arabiensis* following exposure to pirimiphos-methyl, deltamethrin, permethrin, bendiocarb, and alpha-cypermethrin in WHO susceptibility test.

Malaria case count. A total of 137,972 laboratory test records from patients attending the out-patient departments were extracted from the five health facilities. For the two-year period before IRS (January 2015 – February 2017), malaria test positive proportions were similar at 33.2% (18,036/54,404) in intervention and 33.3% (12,920/38,835) in non-intervention sites respectively. For the post-IRS period (March 2017 – May 2018), the test positivity rates were 30.4% (6,347/20,882) in the non-intervention sites and 20.6% (4905/23,851) in the intervention sites.

ARIMA analysis of malaria case counts for health facilities within IRS areas showed a reduction in malaria cases at the facilities post-IRS. Estimated mean monthly malaria cases in Rongo sub-county hospital dropped by 44% from 323 cases per month before IRS to 178 after IRS [mean difference = -142; 95% CI: -236 to -48; P = 0.003]. A similar reduction in mean monthly malaria cases with 65.0% drop from 301 before IRS to 78 cases after IRS [mean difference = -196; 95% CI: -345 to -47; P = 0.01] was observed in Uriri sub-county hospital. In Nyatike sub-county hospital, the mean monthly malaria cases dropped by 47.4% from 118 cases before IRS to 72 after IRS [mean difference = -56; 95% CI: -123 to 11; P=0.1]. For the two health facilities within non-IRS sites, no significant changes in malaria case counts were observed post-IRS, [Ndhiwa hospital: mean difference= -82; 95%CI: -230 to 65; P = 0.3; Marindi hospital: mean difference = 9.3; 95%CI: -132 to 151; = 0.9]. A plot of positive malaria cases over time, before and after IRS, showed a decline in the number of cases detected at facilities within sprayed areas compared to those in unsprayed regions (Fig. 2.11).

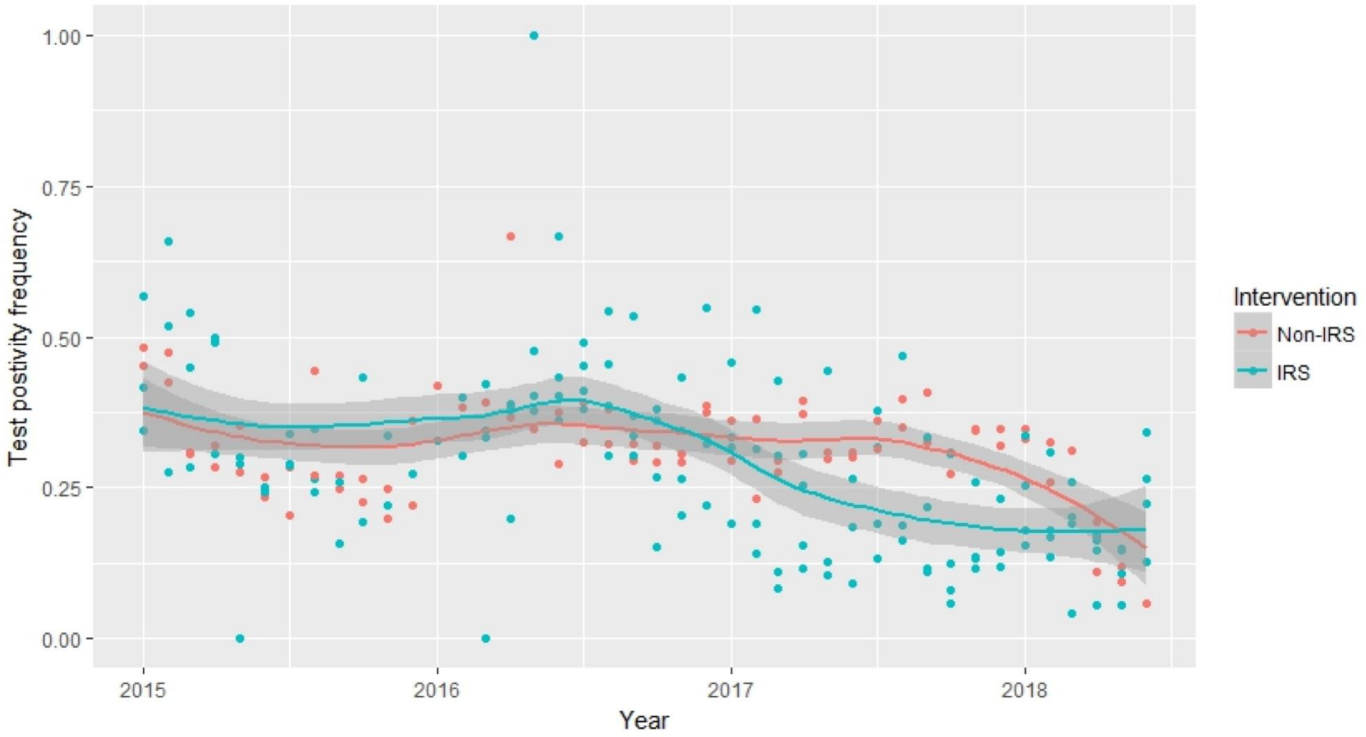

Figure 2.11: Health facility laboratory test positivity rates among febrile out-patients in Homa Bay (Non-IRS) and Migori (IRS) covering two years pre-IRS and over one-year post-IRS. Each point represents the proportion of malaria test positivity cases per facility per month.

Discussion

Our results demonstrate a significant reduction in *An. funestus* indoor resting densities, biting rates and sporozoite infections, as well as a decline in malaria test positivity rates and case counts at health facilities after one round of Actellic 300CS® IRS in Migori County, western Kenya. Human biting rates and sporozoite infections in *Anopheles* mosquitoes are the most direct entomological measures of malaria infection risk. We observed moderate biting and sporozoite rates in both intervention and non-intervention sites before IRS and the unsprayed sites after IRS. However, after IRS, *An. funestus* biting rates were nearly zero, and no sporozoite infections were detected post-IRS. Susceptibility tests confirmed that the major vector species, *An. arabiensis* and *An. funestus,* were both resistant to pyrethroid insecticides but were susceptible to pirimiphos-methyl (Actellic 300CS).

Similar reductions in *An. funestus* populations to near elimination were observed in the Asembo Bay area of western Kenya following the scale-up of pyrethroid-treated nets [18] although *An. funestus* later returned as the primary malaria vector in the region presumably due to the development of pyrethroid resistance [21]. The complete elimination of *An. funestus* following effective IRS campaigns has been reported in South Africa, Mauritius and the Pare/Taveta area of Tanzania and Kenya [46, 47]. *An. funestus* is particularly sensitive to effective indoor insecticides and has previously been reported to be highly endophilic and anthropophilic [20, 23, 24], traits that increase the level of exposure of the species to treated surfaces. Contrary to these earlier reports, we observed 52.3% and 40.4% human and cow blood respectively in *An. funestus*. This is a much higher degree of zoophily than commonly assumed for *An. funestus.* Since the samples used for host blood meal analysis were collected by PSC, the results presented here suggest a case of outdoor feeding and indoor resting by the species. Consequently, despite the high zoophily observed, this species is still exposed to toxic walls during either feeding or resting resulting in the high population reduction. Additional investigations are however needed to understand the dynamics in host selection by *An. funestus* in the study area.

In contrast, *An. arabiensis* indoor resting densities, human-biting rates, and sporozoite infection rates all reduced only marginally in sprayed areas post-IRS. With the decline in *An. funestus*, *An. arabiensis* became the predominant vector species in the sprayed areas. IRS had a limited impact on the population of *An. arabiensis,* despite full susceptibility to pirimiphos-methyl in WHO susceptibility tests. This lesser impact of IRS on *An. arabiensis* is therefore unlikely to be due to insecticide resistance but may be attributable to the behaviour of this species. Blood-meal host analysis showed that *An. arabiensis* fed more frequently on cattle than humans, unlike *An. funestus* that fed more frequently on humans. This finding is

supported by a previous study in western Kenya that reported *An. arabiensis* fed predominantly on cattle (65% of blood meals on cattle; 22% mixed bovine/human; 13% human) [17]. Furthermore, results from a deterministic model, developed using data from Kilombero, Tanzania, suggested that *An. arabiensis* fed outdoors on both humans and cattle and rapidly exited houses without fatal exposure to insecticidal nets or IRS [99]. A recent trap evaluation in western Kenya observed over sevenfold more *An. arabiensis* collected by cow odour compared to human odour outdoor [114]. Therefore, it is likely that a significant population of *An. arabiensis* rests predominantly outdoors and feeds primarily on cattle, but occasionally bites humans and transmits malaria, albeit less efficiently than the more anthropophilic vector *An. funestus*. Therefore, it is possible that the population of *An. arabiensis* collected indoors by light traps and PSC represents only a proportion of a larger outdoor population. These factors may explain the lesser impact of IRS on *An. arabiensis*.

IRS with Actellic 300CS® had a prolonged residual activity of at least ten months post-IRS, as measured by wall bioassays. As spraying was conducted in February, the insecticide provided protection throughout the periods of highest malaria transmission during the long (April-June) and short (October-November) rainy seasons. Similar prolonged residual activity of Actellic 300CS® and control of pyrethroid-resistant mosquitoes have been reported in other countries [164]. This long residual efficacy of Actellic 300CS® makes the insecticide particularly useful in providing all-year-round protection with just one spray round each year.

Biting by *An. funestus* in the intervention and non-intervention areas before IRS and non-intervention areas after IRS occurred mostly indoors late at night corresponding to the period when most people were indoors and in bed. Late night, indoor biting by *An. funestus* has been previously reported, dating back to the pre-bed net era. For instance, in 1975, 94% of *An.*

funestus were observed to bite after midnight, with another a peak in the hours before dawn in Kano plain of western Kenya [53]. Similar late night, indoor biting was observed in the same study area in 1996 [20] and more recently the vector species have been reported to persistently bite indoors, late in the night despite high coverage in insecticide-treated nets [24]. While all these studies observed a biting peak at dawn, the collections were ceased at 07:00 hours. With the extension collections to monitor *An. funestus* biting in the morning, we observed extended biting until 11:00 hours. Similar findings of day-biting *An. funestus* in the presence of LLINs have been recently reported in Senegal [71] and Benin [178]. This seemingly emerging biting behaviour in *An. funestus* not previously investigated may potentially undermine the effectiveness of LLINs as people may be exposed to mosquito bites while away from the protection of their bed nets. However, one round of IRS with Actellic 300CS® substantially reduced the number of *An. funestus* collected and it was not possible to detect biting either indoors or outdoors in the sprayed areas post-IRS.

Notable differences were observed in distribution of different wall types, eave types, presence of cattle and net use between the intervention and non-intervention sites. While these factors potentially affect the occurrence of mosquitoes indoor, only eave type, net use and presence of cattle were associated with significant differences in mosquito numbers. Significantly fewer *An. funestus* and *An. arabiensis* were collected by light traps in houses with closed eaves. Similar results were observed for *An. arabiensis* collected by PSC. Open eaves are known to be the main route for indoor entry of *Anopheles* mosquitoes [35, 179] and blocking them has been demonstrated to be effective in preventing *Anopheles* house entry [16]. Closing eave spaces or deploying vector control tools in these spaces may present an additional intervention to the current vector control tool kit for reducing the indoor occurrence of mosquitoes in addition to IRS and LLINs. The presence of cattle in the

compound was associated with increased numbers of *An. funestus* in both light trap and PSC collection. Cattle corralled within the compound possibly contribute to increased attraction of mosquitoes which in turn increases the risk of indoor entry, especially for endophilic and endophagic species such as *An. funestus*. On the contrary, for exophilic and endophagic mosquito species such as *An. arabiensis*, cattle corralled outdoor, have been demonstrated to provide zooprophylaxis as a control strategy for malaria [180]. Consequently, the effect of cattle outdoor on indoor mosquito occurrence is likely dependent on vector species. The use of bednets on the other hand has been effectively associated with reduced vector numbers indoor [18, 19, 35, 36, 45, 52, 149, 181]. Notwithstanding the differences in the distribution of house-associated risk factors between intervention and non-intervention sites, the greatest reduction in *Anopheles* population was observed in the intervention sites, post-IRS, an indication that spraying was the main factor associated with decline in the vector population. Reductions in malaria cases at the health facilities within sprayed areas post-IRS provided further evidence of the impact of a single round of IRS on malaria transmission. Health facility-based surveys of malaria cases in febrile patients have been useful as part of a rapid analysis of changes in local malaria epidemiology [182-185]. Malaria infection is highly correlated with febrile cases reported at the health facilities [184]. Furthermore, a systematic review of febrile illness over 20 years in sub-Saharan Africa reported a dramatic reduction in the proportion of fevers associated with *Plasmodium falciparum* malaria [186]. Consequently, reductions in malaria cases likely contribute considerably to the reductions in febrile illnesses presenting at health facilities. The use of routine Health Management Information System (HMIS) data to evaluate malaria control interventions [185] however surfers from incompleteness in reporting and variation in the utilization of the health system [187]. We extracted data from the primary records, health facility laboratory registers and observed a reduction in confirmed malaria cases in health facilities within sprayed areas post-

IRS, with no change in the number of cases detected at the facilities in control regions. Reduction in *An. funestus* densities, sporozoite rates and man-biting rates coupled with reduced malaria cases following one round IRS with pirimiphos-methyl provide compelling evidence of the effectiveness of IRS in malaria transmission reduction when implemented with an effective insecticide to which mosquito populations are susceptible.

Conclusion. IRS with pirimiphos-methyl was highly effective for the control of indoor biting and indoor resting, pyrethroid-resistant *An. funestus* and resulted in substantially reduced numbers of this primary vector species coupled with reduced malaria cases. Due to the long residual effect of pirimiphos-methyl, it was possible to achieve year-round protection with a single round of IRS. Sustaining these gains is a priority for the Kenya NMCP and development partners and IRS should continue to be implemented to sustain the impact on *An. funestus*. However, there was less of an impact of spraying on *An. arabiensis* populations, likely due to behavioural avoidance. Additional control measures are needed to control outdoor biting and resting *An. arabiensis*.

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collection and sample analysis, especially, Benjamin Oloo, Tobias Odongo, Amos Wabwile, Celestine Wekesa and Mercy Nduta.

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Competing interest

The authors declare no competing interests.

Disclaimer. The findings and conclusions in this report do not necessarily reflect the official position of the U.S. Department of Health and Human Services or the U.S. Centers for Disease Control and Prevention. The use of trade names is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services or the U.S. Centers for Disease Control and Prevention.

Data availability

All data generated or analysed during this study are included in this published article and its Supplementary Information files.

3 CHAPTER THREE: EVALUATION OF COMMUNITY-BASED VECTOR SURVEILLANCE SYSTEM FOR ROUTINE ENTOMOLOGICAL MONITORING UNDER LOW MALARIA VECTOR DENSITIES AND HIGH BEDNET COVERAGE IN WESTERN KENYA.

Evaluation of community-based vector surveillance system for routine entomological monitoring under low malaria vector densities and high bednet coverage in western Kenya.

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3.1 Abstract

Vector surveillance is critical for tracking progress of disease control efforts. The monitoring of mosquito populations is usually carried out by supervised teams of trained technicians. However, as interventions are scaled up, mosquito populations are depleted and new behavioural adaptations in mosquito populations arise. These changes make traditional surveillance challenging. Alternatively, a community-based sampling scheme is proposed to be more effective for longitudinal entomological monitoring. To evaluate this, community-based sampling and supervised mosquito sampling schemes were compared. The community-based sampling scheme was conducted in eighteen clusters of villages using indoor CDC light trap, outdoor CDC light trap, and prokopack aspiration indoor. Charging of light traps batteries was done locally using solar panels while collected data were transmitted daily to the project server and mosquito samples were collected bi-weekly for laboratory analysis. Parallel collections by supervised teams were conducted for one year by indoor light trap, prokopack aspiration indoor and pyrethrum spray catch (PSC) in the same houses within two weeks after collections by community-based teams. Results from a community-based sampling scheme showed similar trends in mean monthly catch sizes for *An. gambiae* s.l. and *An. funestus* and 3% sporozoite infection rates in all three collection methods. Both outdoor light traps and prokopack aspiration indoor caught significantly fewer *Anopheles* of all species compared to indoor light trap. The proportions of different *Anopheles* species were

similar in all collection methods for *An. gambiae* s.l., *An. funestus* and *An. coustani*. Community-based and supervised sampling schemes showed similar monthly trends in indoor light trap and aspiration collection for *An. gambiae* s.l. Mean catch sizes were significantly lower in community-based sampling scheme compared to the supervised system for all *Anophelines* collected by the light trap. Also, significantly lower catch sizes of *An. funestus* were observed in prokopack aspiration indoor by community-based compared to the supervised system, whereas no significant differences were realized for *An. gambiae* s.l and *An. coustani*. Community teams overestimated the numbers of *An. funestus* by a factor of six compared to identification by experienced technicians. Unsupervised community-based mosquito surveillance by indoor CDC light substantially under intimated the mosquito population compared to quality-assured collection by supervised teams. Adoption of low-cost, devolved supervision with spot check is necessary to enforce compliance with proper installation of indoor light traps.

3.2 Background

Monitoring of mosquito populations for densities, species composition, population structure, insecticide resistance status and sporozoite infection are important in the evaluation of the effectiveness of different vector control strategies. Presently, long-lasting insecticidal nets (LLINs) and indoor residual spray (IRS) are the main malaria vector control strategies. Both are commonly applied indoor and affect mosquito populations by reducing not only population densities [18, 19, 73] and composition [17, 68] but also alter vector behaviour [28, 71, 73, 149]. To detect these changes for effective monitoring of control operations, an evaluation framework is required. However, these changes in malaria vectors bionomics make entomological monitoring particularly difficult. As the vector numbers decline, more frequent and intense sampling is required to collect sufficient mosquito numbers to make an entomological decision. Consequently, in enhanced vector control scenario, many national malaria control programs (NMCPs) are presented with a problem of entomological monitoring with greater sensitivity, under greatly altered vectorial systems [139].

Traditionally, entomological surveillance has been reliant upon closely supervised, well-trained, centrally managed monitoring teams. The teams make routine travels to the collection sites, at times with over-night stays for mosquito sampling. This approach to entomological monitoring is usually limited in geographic scope and frequency of sampling at any survey location [143]. It has been reported to be impractical to implement on a large scale to detect residual transmission [139]. Additionally, the cost of implementing adult mosquito surveillance through conventional terms of specialist entomologists have been suggested to be prohibitive in African countries [139, 144]. Therefore, with enhanced vector control and dramatically altered vectorial systems, supervised vector surveillance is envisioned to become even more challenging and expensive, highlighting the need for a

devolved surveillance system. A more cost-effective approach may be to develop a community-based system [139].

Devolved systems that adapt cost-effective trapping methods to a local, longitudinal application by resident community-based teams represent an attractive alternative [139, 143, 144]. This strategy is anticipated to be affordable and sustainable on a large scale [143, 144, 188] and allows for more intensive sampling of each cluster in terms of trap-nights conducted over the whole study period [143]. However, the implementation of vector surveillance through a community-based system relies on a suitable choice of trapping method that is logistically relevant, cost-effective and generates epidemiologically useful data. Besides, an evaluation framework for data validation of collections by community-based teams is necessary.

While community-based entomological monitoring is largely reported to be cost-effective, previous studies evaluating its effectiveness have been faced with several challenges that limit its validation and implementation. A study in Tanzania found that the Ifakara Tent Trap (ITT) was less sensitive at high mosquito density [106] and this was worsened when used through a community-based system [139]. A separate survey in Zambia implemented community surveillance with CDC light traps [143]. A major challenge with using light traps was the need for regular recharging of batteries [107]. The authors failed to indicate how this challenge was overcome with the community approach. In the same study, attempts to validate data by community-based teams through comparison with collections by supervised teams failed since the community-based teams were aware that they were being evaluated. It was suspected that collection through community-based schemes improved during the visits for supervised collections [143]. Consequently, the evaluation of the community-based trapping scheme for data validation was observed to be a major problem. We, therefore, evaluated a community-based vector surveillance system for routine entomological

monitoring under low malaria vector densities and high bednet coverage in western Kenya. The study aimed to assess the community-based sampling in estimating vector species composition and seasonality compared to sampling by supervised team and to monitor temporal distribution of vector densities and species composition between the two sampling schemes in western Kenya.

Methods

Study area and populations. The community-based vector surveillance system was implemented in Asembo (-0.18139 S; 34.38552 E) and Uyoma (-0.316667S;34.3167E) communities of Rarieda sub-county, Siaya County in western Kenya. The region has been part of the KEMRI/CDC Health and Demographic Surveillance System (HDSS) for nearly three decades. Community prevalence of malaria among children <5 years has declined from over 70% in 1997 to around 40% in 2008 with entomologic inoculation rates (EIRs) dropping from >150 to <20 infectious bites per person per year over a similar period. However, since 2008, population parasite prevalence has remained at 40% despite the continued implementation of LLINs. Indoor *Anopheles* densities have remained low with recent collections by PSC reporting an average of 0.5 mosquitoes per house. The main malaria vector species in this region are *An. funestus*, *An. arabiensis* and *An. gambiae s.s*

Study design. Eighteen clusters were designated in the study area. Each cluster was ~4km in diameter and centered on the house of the collector. In each cluster, 60 houses were randomly selected for mosquito collection (Figure 3.1). Each primary collection house was assigned two replacement houses that were to be used for mosquito collection if the primary houses become unavailable. Each of the 60 houses was sampled once every month by indoor light trap, outdoor light trap, and prokopack aspiration indoor.

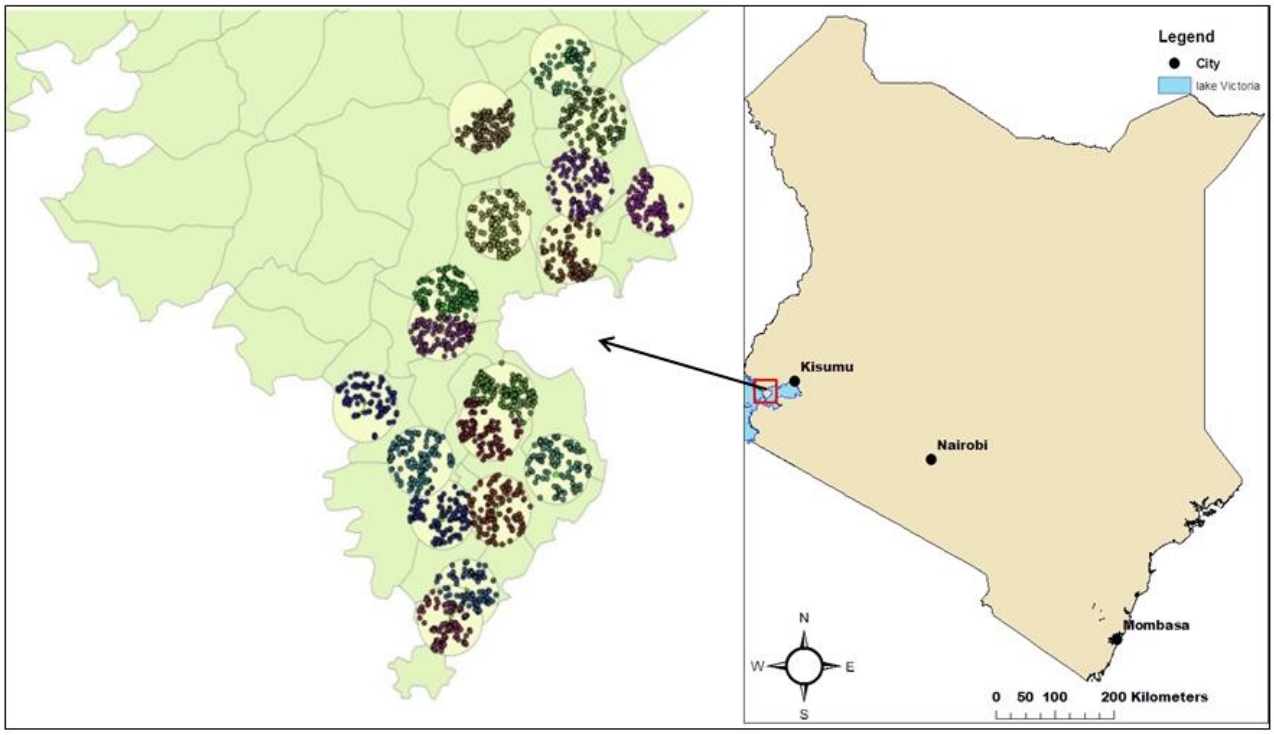

Figure 3.1: A map of Kenya showing the study area with the clusters and randomized compounds.

Selection and training of community-based collectors. A mixed approach was used in the identification and recruitment of the community-based collectors (CBCs). For recruitment, the person had to be (i) a resident of the community, (ii) have a personal means of transport preferably a bicycle or a motorcycle, (iii) be either a community health worker or have prior participation in mosquito collection, (iv) be able to operate a mobile device for data collection and transmission and (v) live in a house with a tin-roof for installation of solar panels. The CBCs were identified through local health facilities if community health volunteers (CHVs) were recruited or through the local administrative authorities if the CHVs were not available.

The CBCs were trained in mosquito collection techniques using CDC light trap and prokopack aspiration indoor. Additional training included basic mosquito identification using

morphological features to differentiate between *Anopheles* mosquitoes and *Culicine* species and between female and male mosquitoes. The collectors were also trained on capture and transmission of entomological and household data using Open Data Kit (ODK) software on an Android mobile device. Other training included: operation of a solar charging system for charging of light trap batteries and tablets, administration of questionnaire and consenting process. All training included practical demonstration and field practice covering a period of five days. After the initiation of mosquito collection, support training was provided to the collectors on an as-needed basis.

Building and installation of the solar charging system. Eighteen solar charging units were assembled by a local engineer within Kisumu town. Each unit was composed of four solar panels attached to a lockable metallic frame. Three of the solar panels were connected to charge controllers (SolarTech) with each charge controller connected to a 12 V rechargeable battery. The fourth solar panel was connected to a Universal Serial Bus (USB) cable for charging the tablet (Figure 3.2).

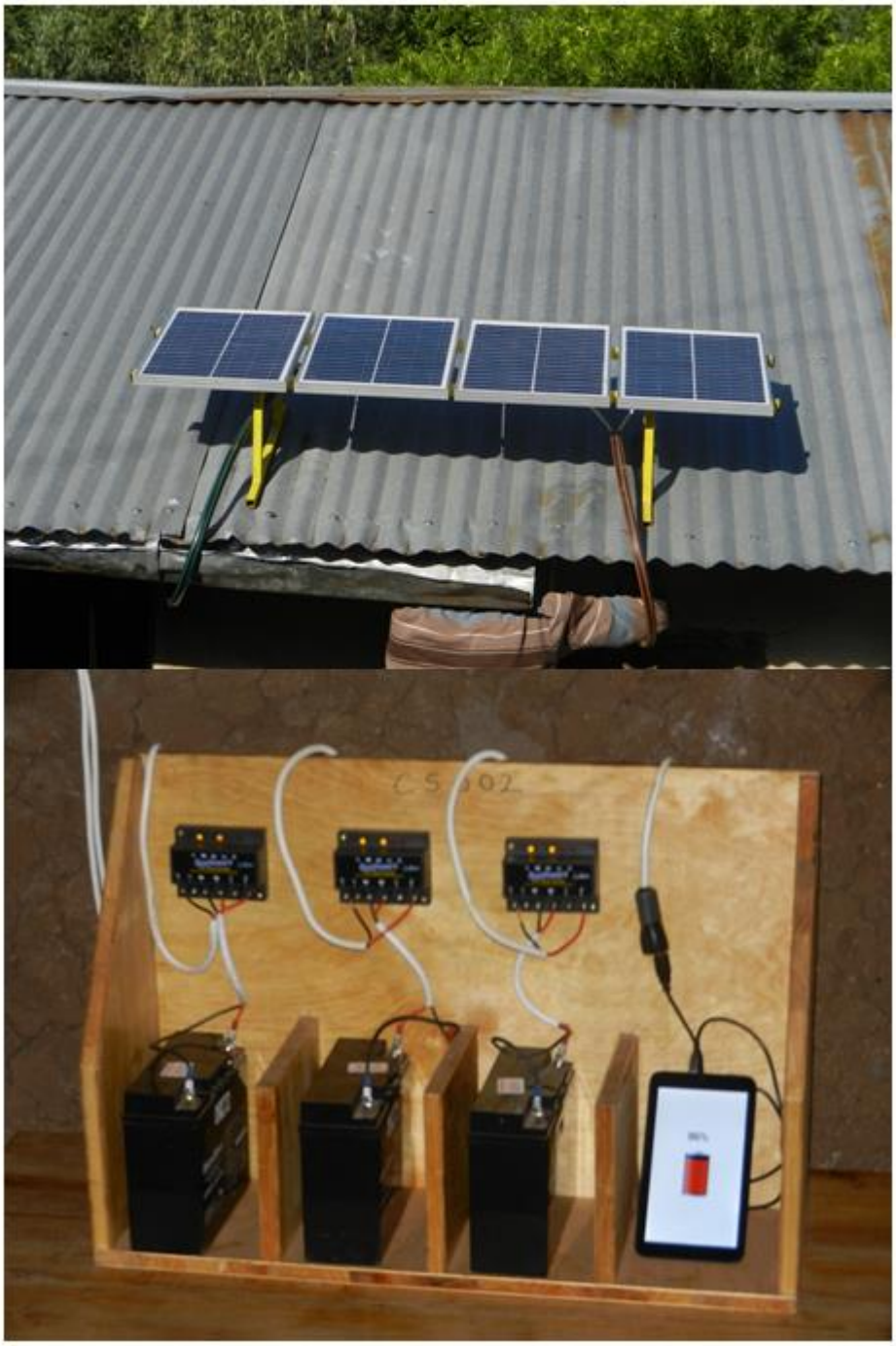

Figure 3.2: A picture of four solar panels attached to a metallic frame mounted on a roof and charging station showing batteries connected to charge controllers and an Android tablet connected to a USB charging port.

During installation, the set of solar panels on a metallic frame was attached on top of a tin-roofed house belonging to the CBC. The frame was attached to the roof with screws from

inside the house while the screws holding the individual panels on the frame were blocked with a metallic plate to prevent theft. Cables from the solar panels were passed under the iron-sheet roof to connect to the charging station within the house.

Equipment and material. Each CBC was issued with, a solar charging unit, three 12 V rechargeable solar batteries with terminals modified to connect two light traps at the same time, six CDC light traps, three with 5 m long connecting cables for outdoor installation and the rest with 2 m cable for indoor trapping, a prokopack aspirator and three collection cups for indoor mosquito collection, an adult mosquito cage, a mouth aspirator, three paper cups, a pair of forceps and Petri dishes for transferring and holding collected mosquitoes. Other equipment included, a tablet (Nexus 7) for collection and transmission of data, data forms and consent forms, Additionally, set of scintillation vials with 70% ethanol for the preservation of collected mosquitoes were provided to the collectors bi-weekly while the tablets were loaded with data bundles for internet connectivity monthly.

Consenting. Written consent was obtained from the randomized houses by the CBCs under the supervision of the project staff. Each collector obtained consent from households within his/her cluster. During consenting, the first 60 primary houses were targeted, however, in cases of a refusal or a completely missed household, the collector then contacted the first replacement household for consent with the second replacement household being contacted only if the first two houses were unavailable. Consent from the 60 households was completed before collection began. Additional consents were sought during the collection period in instances where householders withdrew their consent.

Mosquito collection and processing. Mosquito collection by indoor /outdoor CDC light trap and prokopack aspiration indoor was conducted in three houses nightly for five consecutive nights each week, meaning that each of the 60 consented houses was sampled once every

month. Indoor CDC light traps were set in the sleeping area next to an occupied bed net at about 1.5m from the floor. Outdoor traps were placed within 5m from the house, suspended at 1.5m from the ground on either a tree, pole or immediately under the roof. Both traps were run from a single 12V battery. The traps were operated from 18:00 h to 07:00 h the following morning. After the removal of the light traps in the morning, the collector performed collection using an indoor aspirator.

During mosquito collection, the collector administered a brief questionnaire to collect information on household characteristics, including roof type, wall type, presence of eaves, presence of bed nets and net use, presence of cattle and numbers of people that slept in the house over the collection period. The location of each house was recorded using a GPS on every visit. The household information was collected on an Android tablet using ODK Collect and was automatically sent to a cloud server.

The community-based collectors processed the mosquitoes, recording whether *Anopheles* or *Culicine*. Within each genus, numbers of male and female mosquitoes were recorded, and the females classified by physiological status as either fed, unfed, gravid, or half-gravid. Numbers of mosquitoes in each of these categories were recorded on a form, and the data subsequently entered into the tablet and transmitted to the cloud server. All mosquitoes were preserved together in 70% ethanol in a scintillation vial. Each vial was labeled with the date of collection, collection method, and house code. The collectors were instructed to record and preserve any insect which they thought to be a mosquito.

The preserved mosquitoes and completed data forms were collected from the field every two weeks for further laboratory processing. Once in the lab, trained entomology technicians repeated the sorting process. All mosquitoes in the genus *Anopheles* were further identified to species/complex level using morphological features [189].

Monitoring of light trap battery charging cycles. The light trap batteries were charged daily and their charge status recorded at the beginning and end of every charging session. Light indicators on charge controllers provided information on the charge status of each battery as either fully charged, half charged or empty. A barcode label on each battery was scanned and charge status was captured on an Android tablet. In addition to charge monitoring, the CBCs reported daily any fault in the solar charging unit, lost or broken items or needed supplies.

Parallel surveillance by trained entomology technicians. Parallel collections by trained entomology technicians were conducted in eight of the eighteen clusters sampled by the CBCs. The eight clusters were selected based on mosquito densities from the community-based collections, three clusters with highest densities, three with lowest and two with median mosquito numbers. The Collections were conducted in the same houses as the CBCs within two weeks after the community teams. Data transmitted by the CBCs were downloaded to provide details of households already visited by the community teams. The parallel surveillance teams visited the same houses, without contacting the CBCs. In every cluster, parallel surveillance was conducted by indoor CDC light trap and prokopack aspiration indoor in ten houses visited by CBCs. Additionally, seven neighbouring households, not visited by CBCs were sampled by Pyrethrum Spray Collection (PSC) each month by the parallel teams. The CDC light traps were run from 18:00 h to 07:00 h the following morning in the sleeping area next to an occupied bed net. After removal of light traps in the morning, indoor resting collections were performed by indoor aspirators in the same houses.

PSCs were conducted early in the morning by laying white sheets on the floor and over the furniture within the house. Two collectors, one inside the house and another outside, sprayed

around the eaves with 0.025% pyrethrum emulsifiable concentrate mixed with 0.1% piperonyl butoxide in kerosene. The collector inside the house then sprayed in the roof space. The house was closed for 10-15 minutes after which knocked down mosquitoes were collected from the sheets and transferred to the laboratory in a scintillation vial containing 70% ethanol.

Laboratory analysis. All *Anopheles* mosquitoes were transported to the lab and identified to species level morphologically [190, 191]. The abdominal status as determined by observation of the abdomen and scored as either fed, unfed, gravid or half gravid. Female mosquitoes are divided into three parts for various procedures; head and thorax are used for determination of sporozoite infection rate by enzyme-linked immunosorbent assay (ELISA) techniques [192], the abdomen of blood-fed females were kept for blood-meal host determination and the remainder of the specimen are used in polymerase chain reaction (PCR) analysis to identify species within the *An. gambiae* s.l. and the *Anopheles funestus* s.l. complexes [173] and for future molecular genetic analysis. All mosquitoes morphologically identified as *An. gambiae s.l.* were identified to species by PCR. For *An. funestus s.l.,* only 20% of *An. funestus s.l.* were identified by PCR [193].

Data management and analysis. Data collection was performed using ODK Collect, designed with an interface to limit entry errors. A list of houses including house code and the household name was synchronized with the household characteristics form to restrict the collectors within randomized houses only. For every house sampled, the house code was unique, and each collection effort was uniquely identified by a combination of house code, collection method, and collection date. At the end of each collection, each collection cup, paper cup or light trap bag containing samples was labeled with the combination of variables

to distinguish between different collections. The combination of date, collection method, and house code was used to track the samples through laboratory processing.

During morphological identification of the mosquitoes, a unique barcode was given to each individual mosquito. The mosquito code was used to relate different parts of the same mosquito through various laboratory procedures including, species identification by PCR, and analysis of sporozoite infection by ELISA procedure and blood meal analysis. The individual mosquito code was used in relating results from the different laboratory procedures to the primary individual mosquito file while the date of collection, house code and collection method were used to link individual mosquito data to the household characteristic data from the field.

Data analysis was performed using R statistics version 3.4.1. Data were fitted using Generalized Linear Mixed Model Statistics (GLMMS) to measure the mean abundance of *An. gambiae* complex, *An. funestus* and *An. coustani* per trapping night between community-based and supervised teams and between different trapping methods. Since the data were over-dispersed, Generalized Linear Mixed Models using Template Model Builder (glmmTMB) was used to fit negative binomial distribution models for the analysis of mosquito numbers. The female *Anopheles* mosquito numbers were assessed as a function of sampling scheme (community-based or supervised) and collection method (indoor light trap, outdoor light trap, and prokopack aspiration indoor) as a fixed effect, while house was treated as a random effect. To obtain the rate ratios (RR) and confidence intervals, the model coefficients were exponentiated. Chi-Squared test was used to test for correlation in *Anopheles* mosquito catch for each species between community-based and supervised sampling. A test of proportions was used to assess the probability of occurrence of *An. gambiae*, *An. funestus* and *An. coustani* and sporozoite infection of all tested female *Anopheles* mosquitoes by trapping method. Binomial GLM model was also used to analyse

the proportion of *Anopheles* mosquitoes identified by community-based collectors compared to expert entomology technicians of all collected mosquitoes and the proportions of light trap batteries at different levels of charge status after every charging cycle. The rate of misidentification of *Anopheles* mosquitoes by community-based collectors was determined as a ration of identification by the community-based team and expert entomology technicians.

3.3 Results

Community-based collections

A total of 14,563 *Anopheles* mosquitoes were collected from 89,706 collection efforts by all collection methods combined as implemented by community-based collectors and data verified by expert entomology technicians. Of these, 6,149 (42%) were *An. gambiae* s.l., 6,481 (45%) *An. funestus*, 1930 (13%) *An. coustani* and 3 (0.02%) other *Anopheles*. Of the *An. gambiae* s.l., 2,045 mosquitoes were analyzed by PCR for species identification, 1,539 (75%) were identified as *An. arabiensis* and 506 (25%) *An. gambiae* For *An. funestus*, 1,399 were analyzed by PCR and were all confirmed to be *An. funestus s.s.* (Table 3.1)

Table 3.1: Summary of numbers of *Anopheles* species by morphological and Polymerase Chain Reaction (PCR) identification for different collection methods by community-based collectors

Collection method	**Morphological identification**					**PCR Identification**				
	An. gambiae s.l	***An. funestus***	***An. coustani***	**Other *Anopheles***	**Total**	***An. arabiensis***	***An. gambiae s.s.***	***An. funestus s.s.***	**Non-amplified**	**Total**
Indoor light trap	3058	3068	838	1	6965	764	280	686	58	1788
Outdoor light trap	1292	1428	452	2	3174	348	104	281	34	767
Prokopack aspiration indoor	1799	1985	640	0	4424	427	122	432	33	1014
Total	**6149**	**6481**	**1930**	**3**	**14563**	**1539**	**506**	**1399**	**125**	**3569**

Similar monthly trends of mean *An. gambiae* s.l. and *An. funestus* were observed in indoor CDC light trap, outdoor light trap and prokopack aspiration indoor throughout the study

period. The highest peaks of mosquito numbers were associated with periods of short and long rains in the study area. Between August 2015 and December 2016, *An. funestus* were observed to have a delayed peak and the numbers remained high compared to *An. gambiae* s.l. following periods of high rainfall. However, the numbers of *An. funestus* caught dropped at the beginning of 2017 and remained low through the study period with no seasonal variation as was observed with *An. gambiae* s.l (Figure 3.3)

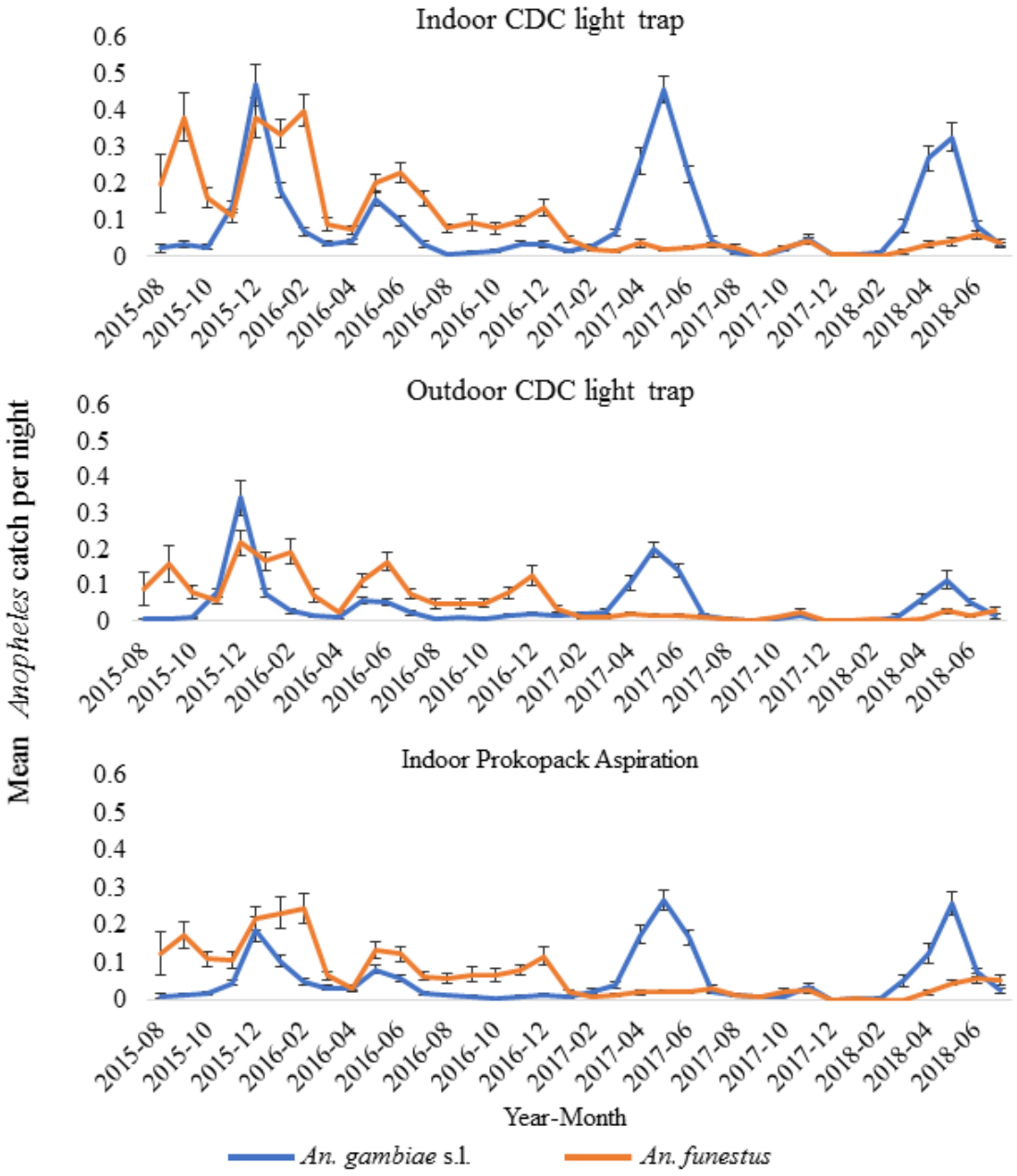

Figure 3.3: Monthly mean (means ± std errors) *An. funestus* and *An. gambiae* s.l. catch per night by indoor CDC light trap, outdoor CDC light trap, and prokopack aspiration indoor.

Sporozoite infections were detected in both *An. funestus* 149/3678 (4%) and *An. gambiae* s.l. 30/2153 (1%). Significantly higher sporozoite infection rates were observed in the indoor light trap as compared to the outdoor light trap, (X^2=29.08, df=1 P<0.001). Similarly, sporozoite infection rates were significantly higher in indoor light trap collections compared to prokopack aspiration indoor, (X^2=14.83, df=1, P=0.0001). Whereas, no significant

difference in the proportions of sporozoite infections were observed between prokopack aspiration indoor and outdoor light trap collections (X^2=2.40, df=1, P=0.12). (Figure 3.4).

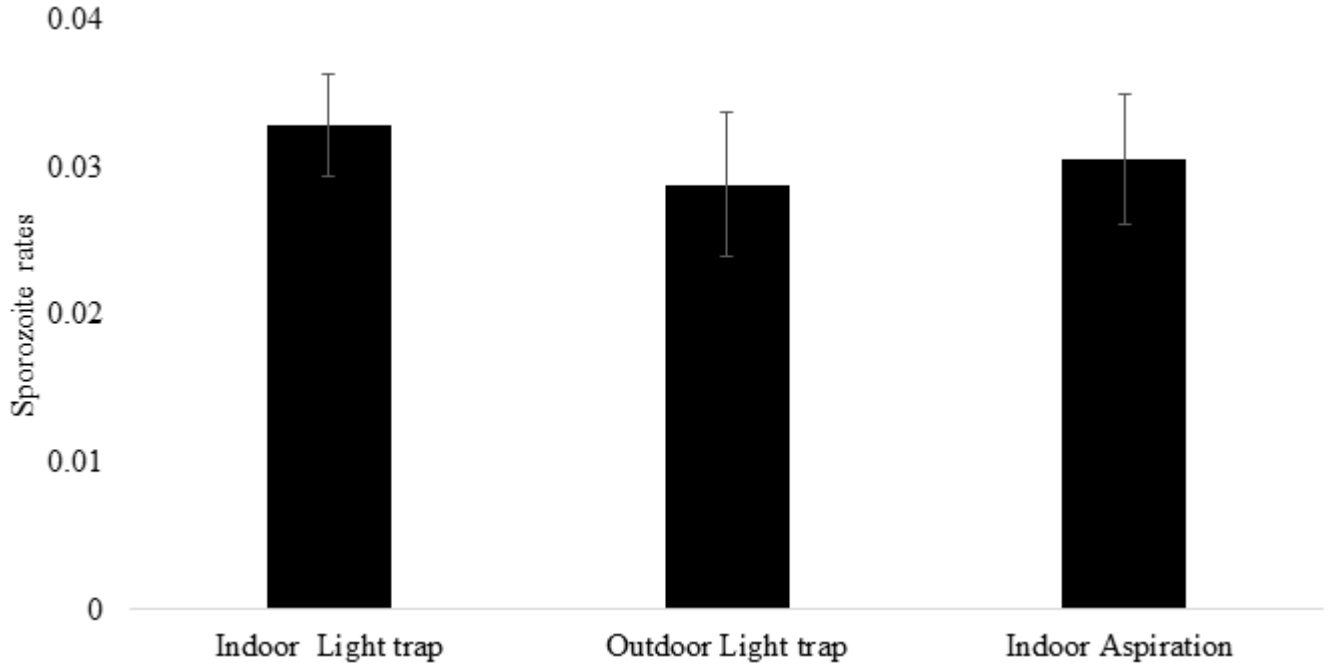

Figure 3.4: Sporozoite infection rates (proportions ± std errors) in *Anopheles* mosquitoes collected by indoor CDC light trap, outdoor CD light trap, and prokopack aspiration indoor.

The mean catches of *An. gambiae* s.l., was significantly lower in outdoor CDC light trap and prokopack aspiration indoor compared to indoor CDC light trap [RR=0.5; (95% CI: 0.4 – 0.5); P<.001] and [RR= 0.6; (95% CI: 0.6 – 0.7); P<.001] respectively. Similarly, the numbers of *An. funestus* caught by outdoor CDC light trap and prokopack aspiration indoor were significantly lower compared to indoor CDC light trap, [RR=0.5; (95% CI: 0.5 – 0.6); P<.001] and [RR = 0.6; (95% CI: 0.6 – 0.7), P<.001]. The numbers of *An. coustani* were also significantly lower in outdoor CDC light trap and prokopack aspiration indoor compared to indoor CDC light trap, [RR = 0.1; (95% CI: 0.05 0.07); P<.001] and RR = 0.1; 0.5 – 0.08, P<.001 (Figure 3.5A). From indoor light trap collections, there was no significant difference in the proportion of *An. gambiae* compared to *An. funestus, (*X^2= 0.02, df=1, P=0.88). Whereas, both *An. gambiae* and *An. funestus* were significantly higher in proportion compared to *An. coustani*, (X^2=1754.7, df=1, P<0.0001) and (X^2=1767.8, df=1, P<0.0001)

respectively. In outdoor light trap collections, there were significantly more *An. funestus* compared to *An. gambiae*, (X^2=11.9, df=1, P=001). *Both An. gambiae and An. funestus* were significantly higher in proportions compared to *An. coustani*, (X^2=556.7, df=1, P<0.0001) and (X^2=719.9, df=1, P<0.001) respectively. From indoor prokopack aspiration, there were significantly more *An. funestus* compared to *An. gambiae* (X^2=15.8, df-1, P=0.0001). Highly significant differences were observed in the proportions of both *An. gambiae* and *An. funestus* compared to *An. coustani*, (X^2=759, df=1, P<0.0001) and (X^2=917, df=1, P<0.0001) respectively (Fig. 3.5B).

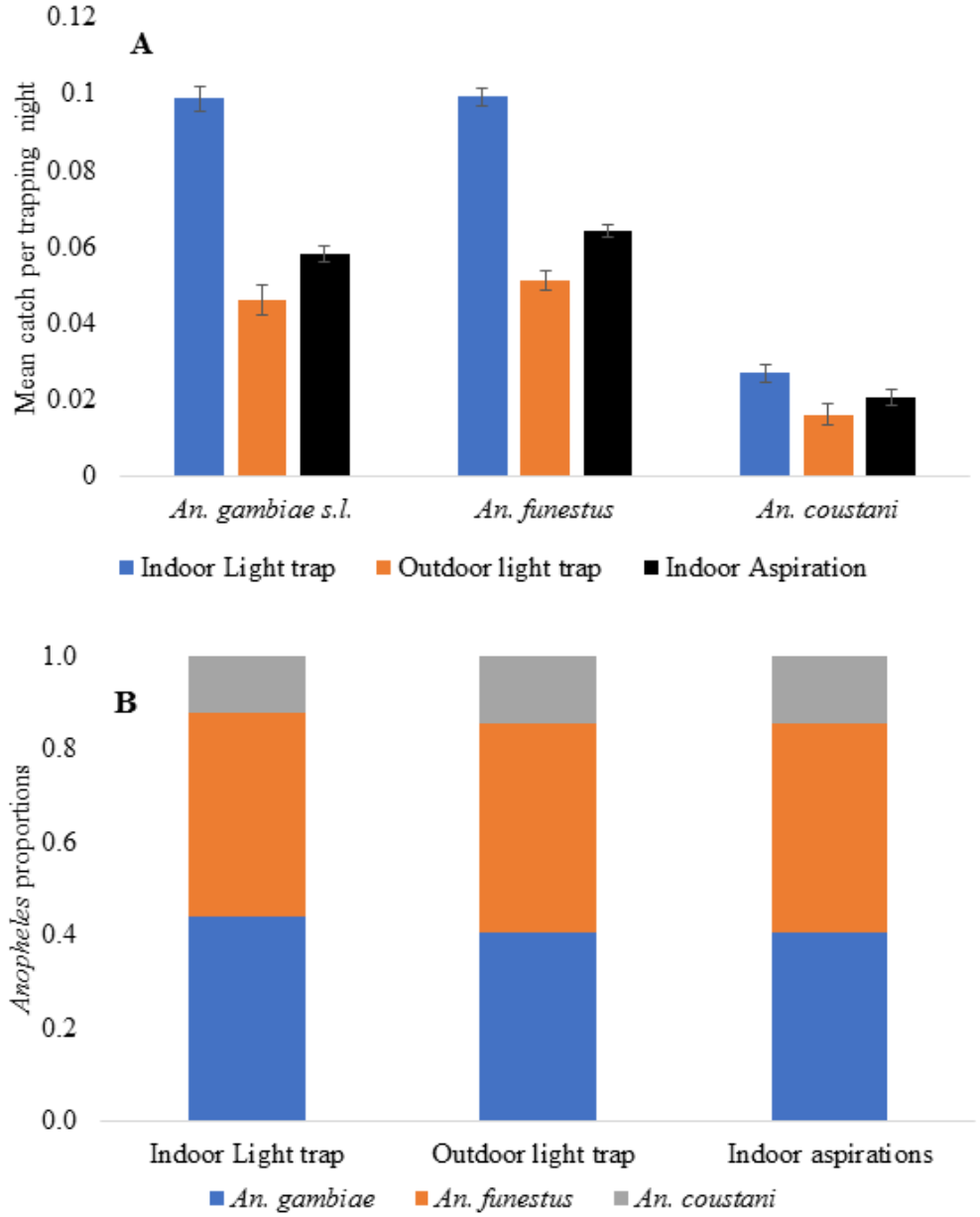

Figure 3.5: (A) Comparison of mean catch (mean ± std errors) of *An. gambiae* s.l., *An. funestus* and *An. coustani* by indoor CDC light trap, outdoor CDC light trap, and aspiration; (B) Comparison of proportions of *Anopheles* species by collection method.

Comparison of community and parallel surveillance

A total of 4,910 collection efforts, were conducted by both community and supervised teams in the same houses over a twelve-month period collecting 2,050 *Anopheles* mosquitoes. The supervised teams made 1,024 collection efforts by CDC light traps and 1, 017 by aspiration, while community-based collectors conducted 1,437 and 1432 collections by light trap and aspiration respectively. Figure 3.6 shows a comparison in the mean monthly catch of

Anopheles species by indoor CDC light trap and prokopack aspiration indoor between the community-based collector and supervised teams. From indoor CDC light trap collections of *An. gambiae* s.l, April to June marked the period of high mosquito collection by both community and supervised teams while the catch sizes in the rest of the month remained low. The mean catch of *An. funestus* was low in community-based collections with no evident seasonal variation while supervised collections showed increased catch sizes between May and September. From prokopack aspiration indoors, trends in mean monthly catch sizes between community-based and supervised collectors were similar for *An. gambiae* s.l. but different for *An. funestus*.

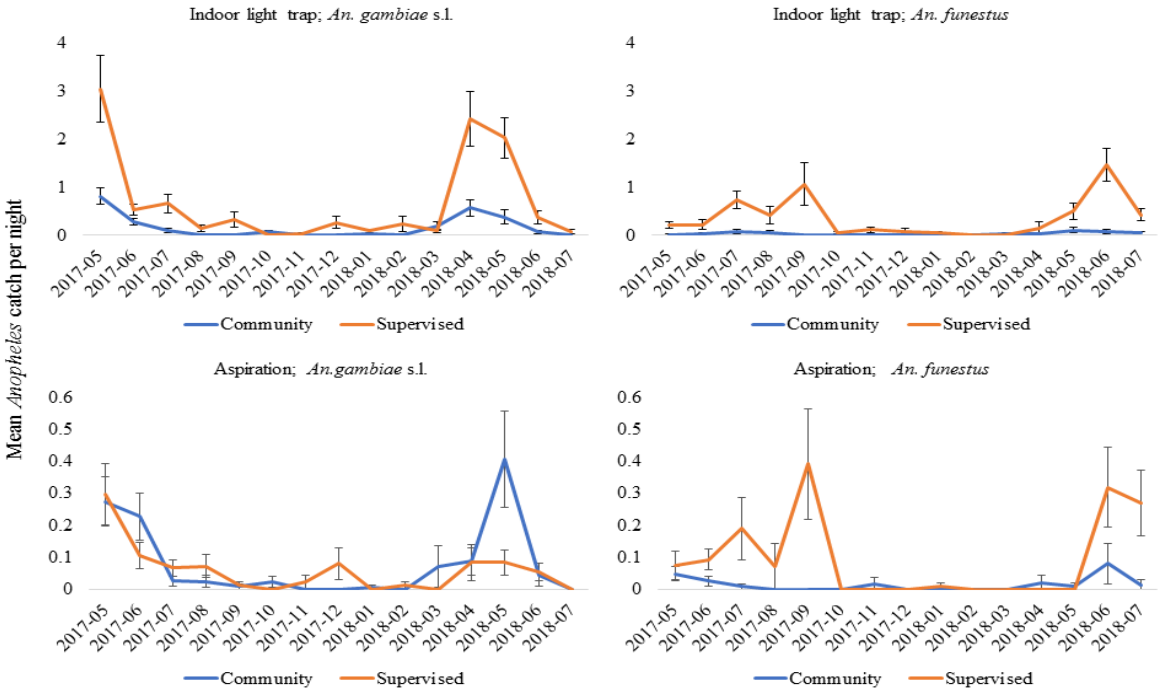

Figure 3.6: Comparison of mean monthly catch sizes (means ± std errors) of *An. gambiae* s.l. and An. funestus between community-based and supervised collection by indoor CDC light trap and aspiration.

Community-based collectors caught 80% fewer *An. gambiae* s.l. compared to supervised teams, [RR=0.2; (95%CI: 0.17 – 0.32); P<0.001]) by CDC light traps. Similarly, the mean abundance of *An. funestus* and *An. coustani* collections by community teams were 90% and 80% lower compared to supervised teams for, [RR=0.1; (95%CI: 0.07-0.16); P<0.001] and [RR=0.2; (95%CI: 0.08-0.44); P<0.001] respectively. From indoor prokopack aspiration, no significant difference in the mean catch of *An. gambiae s.l.* and *An. coustani* were observed between community-based collectors and supervised teams. However, significantly fewer, *An. funestus* (90%) were collected by community teams compared to supervised collections, [RR=0.1; (95%CI: 0.05-0.23); P <0.001] (Table 3.2).

Table 3.2: Comparison mean *An. gambiae* s.l. and *An. funestus* catch by indoor CDC light trap and aspiration between community-based collectors and supervised collectors.

Collection method	*Anopheles* species	Study	Mean	RR (95% CI)	X^2	*p* values
Indoor CDC Light Trap	*An. gambiae* s.l.	Community	0.18	0.2(0.17 - 0.32)	72.77	<0.001
		Supervised	0.70	1		
	An. funestus	Community	0.03	0.1(0.07 -0.16)	104.39	<0.001
		Supervised	0.39	1		
	An. coustani	Community	0.01	0.2(0.08 - 0.44)	22.63	<0.001
		Supervised	0.05	1		
Prokopack aspiration indoor	*An. gambiae* s.l.	Community	0.09	1.4(0.85 – 2.26)	2.00	0.16
		Supervised	0.06	1		
	An. funestus	Community	0.02	0.1(0.05 – 0.23)	23.69	<0.001
		Supervised	0.10	1		
	An. coustani	Community	0.003	1.4(0.19 – 10.41)	0.12	0.73
		Supervised	0.002	1		

Comparison mosquito identification by community teams and trained entomology technicians

Community-based collectors overestimated the proportions of *Anopheles* mosquitoes by an average factor of six though out the study period (Figure 3.7). A statistical analysis of difference in genera (*Anopheles* or *Culex*) identification by the two teams indicates that the community teams identified significantly more *Anopheles* species compared to entomology

technicians, [RR= 1.8; (95%CI:1.7 -1.8); P<.001]. For *Culicine* species, the community teams identified significantly fewer numbers compared to entomology technicians, RR =0.7; 95%CI 0.6 – 0.7), P<.001.

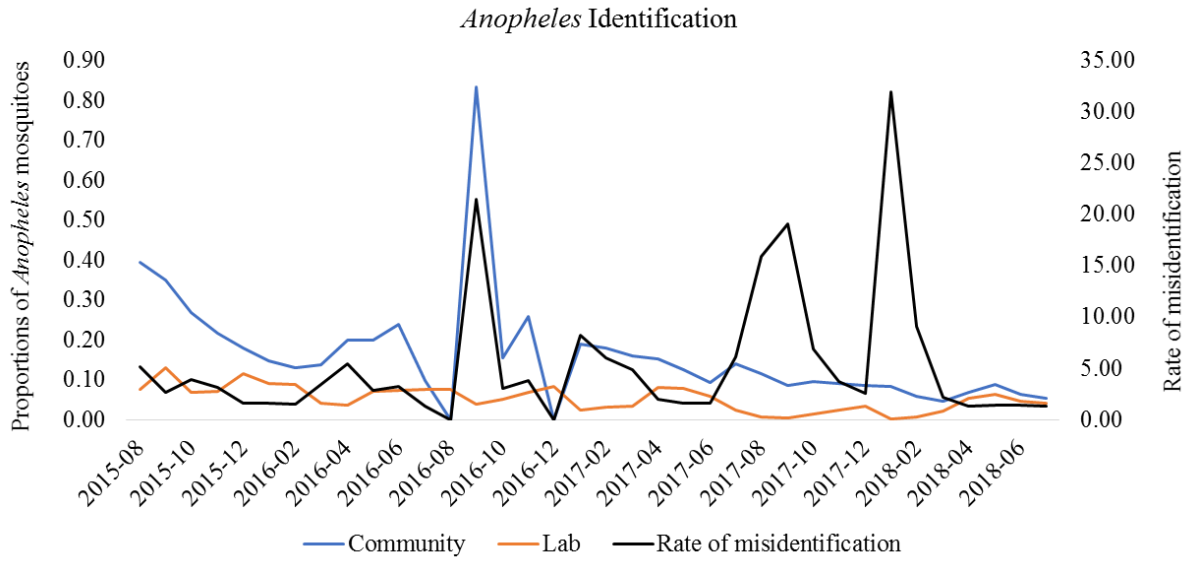

Figure 3.7: Proportion of *Anopheles* mosquitoes identified by community-based collectors and confirmed by expert entomology technicians in the lab. The rate of misidentification of *Anopheles* by community-based collectors is shown on the secondary axis

The figure in Appendix 1 shows battery charge status at the beginning of each charging session following over-night trapping the previous day and at the end of the charging session, before setting the traps again. At the beginning of each charging session, over 60% of the light trap batteries were indicating averagely 75% of charge status. The proportion of batteries showing the low charge status of 25% and below increased between April and December 2016, about 8 months after the start of the survey. At the end of the charging session, the charge status was 100% for over 60% of the batteries. All the first bunch of batteries was replaced in February 2017, 18 months from the start of the survey.

3.4 Discussion.

Community-based collections by use of CDC light traps without supervision by professionals caught substantially fewer, 80% less *Anopheles* mosquitoes than supervised collections while

the catch sizes in indoor prokopack aspiration were largely comparable between the two sampling schemes for *An. gambiae* s.l. and *An. coustani*. Community-based collectors were therefore observed to underestimate mosquito numbers by light trap collections when compared with supervised collections. This is contrary to a report from a previous entomological survey that observed community-based sampling scheme to be more effective compared to supervised collection [143]. Compared to the supervised sampling scheme, community-based sampling showed no seasonality with monthly mean values being consistently low across the year. Consequently, community-based sampling by light traps was not useful in tracking *Anopheles* seasonality and underestimated densities by 80% compared to supervised collections. However, setting of light traps outdoor in the peri-domestic environment by community-based teams demonstrated similar monthly trends in mosquito numbers and sporozoite infection rates as indoor light trap collections performed by the same team. Even though previous studies reported community-based sampling scheme to be more affordable for longitudinal entomological surveillance, enabling multiple, intense sampling over a large geographical area, at the same time [139, 143, 194], its improvements are required for optimization.

A community-based mosquito sampling scheme using Ifakara Tent Trap (ITT) in Tanzania was observed to be the most cost-effective and epidemiologically relevant way to monitor adult malaria vector populations [139]. While another study in Zambia reported community-based collections using light traps to be more effective than centrally supervised sampling scheme [143]. However, both studies recognized challenges with the validation of data collected by community teams. The study in Tanzania observed that ITT has limited sensitivity at high mosquito density and this was exacerbated when used in community sampling scheme possibly due to poor compliance [139]. On the other hand, with the use of light traps in Zambia, it was suspected that collection by community teams improved during a

visit by supervised teams for quality-assured data collection due to prior knowledge of such visits [143]. Consequently, validation of data collected by community teams has been considered a major public health concern. To overcome these challenges, unsupervised community-based sampling scheme was implemented using CDC light trap which has been previously reported to be effective for large-scale vector sampling in western Kenya [108]. The use of mobile-based data collection and transmission system was valuable in keeping the two surveillance teams independent from each other as the supervised team was able to trace the houses sampled by community-based teams without contact with the latter group. Furthermore, mobile data collection and transmission provided a unique opportunity to remotely monitor activities of the community-based teams. The use of light traps through a community-based surveillance system, validated by independent quality assurance data collection demonstrated the potential of unsupervised devolved entomological surveillance and associated challenges.

The observed difference in *Anopheles* catch sizes in light trap collections between the two sampling schemes is suggestive of another case of poor compliance. The differences in catch sizes are presumably due to inconsistency in trap location by the community teams. For best performance, the indoor light trap should be at an approximate height of 1.5m from the floor, at the foot-side of an occupied bednet [119]. However, the installation of light traps in the sleeping area is usually considered intrusive by some households and at times requires explanation by the collector before consent is granted. Otherwise, the households would more readily offer to have the light traps installed elsewhere in the houses other than the sleeping area. We suspect that the community-based collectors might have failed to gain access to the sleeping areas hence installing the traps in other rooms. Thus, a possible lack of access to the sleeping areas by the community teams for light trap installation, contributed to the small catch size when compared to the collection by supervised teams. Additionally, the

community-based sampling scheme was faced with another challenge arising from possible fatigue by households due to repeated sampling from the same houses over time. While this challenge was potentially overcome by the provision of a list of replacement houses in cases where primary houses withdrew consent, cases of some community-based collectors visiting certain houses two to three times in a month instead of just a single collection were observed. The collectors possibly resorted to sample repeatedly from more receptive households while avoiding those that resisted. It may be useful for future studies adopting community-based sampling scheme to consider letting the collectors sample from all houses within the study site other than restricting them to a set of few selected houses which limits their options in cases consents are withdrawn. Also, it is recommended the community-based sampling scheme be integrated with low-cost, devolved supervision to provide spot checks on compliance with light traps installation standards and support with challenges of any arising resistance in the community.

Community-based mosquito sampling schemes have been reported to be a lot cheaper compared to conventional sampling by supervised teams. While the costs for implementation of the community-based sampling were not collected in this study, data from previous studies have demonstrated its cost-effectiveness. A survey in Zambia reported the cost of sampling a single specimen of *An. funestus* to be $141.2 and $5.3 for quality assured and community-based light trap collections respectively [143]. Whereas, in Tanzania, the cost of sampling a specimen of *An. gambiae* s.l. was approximated at $608.1 and $119.1 for quality assured and community-based Ifakara Tent Trap collections respectively [139]. Therefore, quality assured collections by supervised teams cost 26.6 times more in the Zambian study and 5.1 times more in the Tanzanian study compared to the community-based sampling demonstrating the cost-effectiveness of community-based sampling for entomological monitoring.

Collection of malaria vectors outdoors is becoming increasingly necessary with the increased use of indoor-based vector control tools [2] and possible associated behavioral modifications in mosquitoes, characterized by increased outdoor activities [99, 195-198]. Ability to trap *Anopheles* mosquitoes outdoors within 5m from the houses using an unbaited light trap is of particular interest. Outdoor trapping through community-based trapping scheme was able to track monthly vector densities and measure sporozoite infection rates with similar trends and rates as indoor, albeit at low densities. While there is a chance that the mosquitoes might have been intercepted on their flight path into the nearby houses the data shows the necessity of targeting outdoor mosquito. Additionally, an outdoor collection using CDC light traps through community-based teams is perceived to be easier to implement compared to setting the same traps indoors. While monitoring of outdoor vector population through a community-based sampling scheme was perceived to be logistically easier, the numbers were much lower outdoor compared to indoor collections by community-based teams, and substantially lower compared to indoor collection by supervised teams. Consequently, unbaited outdoor light traps may not be epidemiologically informative in monitoring mosquito populations. It is, however, important to note that catch size in outdoor light traps in the peri-domestic environment are more likely to be affected by ambient light sources [124].

Community teams consistently misidentified *Anopheles* mosquito species from their collection. In attempts to distinguish between *Anopheles* and *Culex* species using morphological features, the teams reported more *Anopheles* than there were in each collection. A review of the morphological identification by a team of experienced entomology technicians observed six-fold fewer *Anopheles* mosquitoes compared to identification by community-based teams. The rate of misidentification did not improve throughout the study period as no additional training on identification was provided.

3.5 Conclusion

Unsupervised community-based mosquito surveillance by indoor CDC light traps substantially underestimated the mosquito population compared to quality-assured collection by supervised teams. While the community-based sampling scheme is potentially cost-effective with concurrent sampling in several locations, it is still faced with challenges of low compliance. It is recommended that community-based sampling approaches be integrated with devolved low-cost supervision with spot checks to enforce compliance. The use of solar panels to charge light trap batteries and mobile data collection and transmission system provides a sustainable system for routine, daily entomological monitoring in rural Africa.

4 CHAPTER FOUR: EVALUATION OF HOST DECOY TRAP FOR COLLECTION OF HOST-SEEKING MALARIA VECTORS IN A REGION WITH HIGH BEDNET COVERAGE OF WESTERN KENYA

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Cattle odour is a powerful attractant for exophagic malaria vectors

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4.1 Abstract

Background: As currently implemented, malaria vector surveillance in sub-Saharan Africa occurs indoors, targeting endophagic and endophilic mosquitoes, leaving exophagic (outdoor blood feeding) mosquitoes unrepresented. We evaluated the recently developed Host Decoy Trap (HDT) and compared it to the gold standard, Human Landing Catch (HLC), in a 3x3 Latin square study design outdoors in western Kenya. HLCs are favoured because they elicit a more natural range of *Anopheles* biting-behaviour compared to other sampling tools, and therefore, in principle, provide the most reliable profile of the biting population. The HDT incorporates the main host stimuli that attract blood meal seeking mosquitoes and can be baited with the odours of live hosts.

Results: Mosquito numbers and species diversity varied significantly between HLCs and HDTs baited with human (HDT-H) or cattle (HDT-C) odour, revealing important differences in behaviour of *Anopheles* species. In the main study in Kisian, the HDT-C collected a nightly mean of 43.2 (26.7-69.8; 95% CI) *Anopheles*, compared to 5.8 (4.1-8.2; 95% CI) in HLC, while HDT-H collected 0.97 (0.4-2.1; 95% CI), significantly fewer than the HLC. Significantly higher proportions of *An. arabiensis* were caught in HDT-Cs (0.94 ± 0.01) and HDT-Hs (0.76 ± 0.09) than in HLCs (0.45 ± 0.05) per trapping night. The proportion of *An. gambiae* was highest in HLC (0.55 ±0.05) followed by HDT-H (0.20 ± 0.09) and least in HDT-C (0.06 ± 0.01). An unbaited HDT placed beside corralled cattle overnight caught mostly *An. arabiensis* with proportions of 0.97 ± 0.02 and 0.8 ± 0.2 in presence and absence of cattle respectively, and a mean of 10.4 (2.0-55.0) *Anopheles*/night near cattle, compared to 0.4 (0.1-1.7) in unbaited HDT away from the host, indicating that the HDT can be effective without the need for directed odour.

Conclusions: The capability of HDTs to combine host odours, heat and visual stimuli to simulate a host provides the basis of a system to sample human- and cattle-biting mosquitoes. The trap caught a large number of cattle-host seeking malaria vectors outdoor but did not give a reliable estimate of human exposure reflected by HLC. The HDT offers the prospect of a system to monitor and potentially control *An. arabiensis* and other outdoor-biting mosquitoes more effectively.

Key words: *Anopheles*, *An. arabiensis*, *An. gambiae*, vector behaviour, host, odour, mosquito trap, exophily

4.2 Introduction

Sustained use of long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) have reduced malaria infection prevalence by half between 2000 and 20015 with LLINs and IRS contributing 68% and 11% of this decline respectively [153]. Significant changes in vector populations have also been observed with sustained implementation of LLINs [17, 199, 200]. Both interventions, however, are limited to indoor application and are therefore biased towards indoor resting ('endophilic') and feeding ('endophagic') mosquitoes leaving those that feed and rest outdoors such as *Anopheles arabiensis* and *An. culicifacies* untargeted [99]. Sustained use of LLINs and IRS may also select for outdoor resting ('exophily') and feeding ('exophagy') in mosquito populations [195, 198, 201], day-time feeding [71] and a shift towards non-human hosts ('zoophagy') such as cattle [28]. It is now recognized that mosquito populations that feed and/or rest outdoors play an important role in the maintenance of malaria transmission [195]. Accordingly, there is a pressing need for better methods to control and monitor these species.

Methods for sampling adult mosquitoes often exploit host-oriented behaviour. For instance, the use of the human landing catch (HLC) or placement of CDC-light traps adjacent to a

human under a bednet [108] relies on the attraction of mosquitoes to their host [202-204]. Hitherto, research to develop devices to attract malaria mosquitoes have focused largely on human odours. Identification of the chemicals present in human odour has led to the development of blends of artificial odours [141], which have been used with MMX [205] and Suna [140] traps to sample and/or control [206] *An. gambiae* sensu lato. However, the design of some of these traps, such as light traps, are dependent on actively aspirating mosquitoes via a fan, thereby limiting catch efficacy, as odours induce only part of the behavioral sequence that leads a mosquito to a host [207]. Artificial odour blends in isolation do not fully mimic the range of physical and visual stimuli that attract mosquitoes to natural hosts, particularly those that most influence their close-range orientation behaviour [208-210].

However, laboratory studies have begun to quantify synergistic effects between olfactory, visual and thermal cues on mosquito behaviour during host location [209, 211]. These developments can contribute to more effective ways to measure vector-host contact, particularly in outdoor environments, where HLCs remain an important means of sampling, despite exposing collectors to mosquito bites and data quality relying on individual collector skill [210]. A recent study showed that exploitation of the responses of mosquitoes to the heat produced by hosts may be a potent tool for monitoring and/or controlling outdoor-biting species of mosquito. The Host Decoy Trap (HDT), which combines natural human odour, visual stimuli, and a thermal signature equivalent to human body, caught between two and tenfold more *An. coluzzii* (*An. gambiae* sensu lato) outdoors than a field technician performing HLC [113], even though *An. coluzzii* is generally considered a primarily endophagic and endophilic species.

In East and Southern Africa, *An. gambiae* sensu stricto and *An. arabiensis* and *An. funestus* are important vectors of malaria. *An. arabiensis* feeds mostly outdoors on humans and cattle

[20, 23, 212] while *An. gambiae* and *An. funestus* mostly feed indoors on humans [20, 23]. In western Kenya we tested the relative performance of HDTs baited with either natural human (HDT-H) or cattle (HDT-C) odours against HLC, to attract and trap outdoor biting mosquitoes and assessed whether natural host odours might provide a better basis for systems to monitor and control exophagic and zoophagic vectors of malaria.

4.3 Methodology

Study area

The study was conducted in Kisian village (0.0749° S, 34.6663° E), near the Kenya Medical Research Institute Centre for Global Health Research (KEMRI-CGHR) in Kisumu County, and in Orego village (0.6167° S, 34.55°E), Homa Bay County, western Kenya, in May and June 2017. Western Kenya is malaria endemic with transmission occurring throughout the year. The region has two wet seasons, March to June and October-December, corresponding to periods of highest malaria transmission. Residents are of Luo ethnic group practicing small-scale mixed crop-livestock farming. *Anopheles funestus*, *An. arabiensis* and *An. gambiae* are the main malaria vectors in the study area. The region has high reported rates of LLIN usage (>85% of households with at least one net) [6].

Mosquito collection methods

Host Decoy Trap (HDT). A standardized HDT was manufactured by the University of Greenwich and Biogents AG (BG-HDT version) using the same principles as the prototype described in Hawkes *et al*. [113]. It consists of a watertight lay-flat plasticized aluminum foil container similar to packets of single-use fruit juice drinks, insulated with layers of polystyrene held in a collapsible cylindrical bucket (height 36 cm, diameter 38 cm), around which a black fabric jacket is secured using hook and eye strips. The watertight bag is filled with ~15 l of water heated to ~80°C, which is sufficient to maintain surface temperature across the fabric jacket of 30 - 40°C for at least 12 hours. The watertight bag is insulated with

a layer of styrofoam to prevent rapid heat loss so that the water temperature is 30 - 45ºC by morning. The bucket is closed with a transparent polyethylene plastic cover to protect the interior from rain. This unit provides high contrast visual stimuli and human-equivalent thermal stimuli to induce close-range attraction and landing behaviour in host-seeking mosquitoes. A transparent adhesive plastic sheet (FICS film, Barrettine Environmental Health, Bristol, UK) covers the circumference of the trap (Figure 26A) to catch mosquitoes as they land. In contrast, the original Host Decoy Trap (O-HDT) consisted of a metal cooking pot or plastic barrel/container (~ 40 l), with 15-20 l hot water. The container was insulated with toweling material to maintain the surface temperature at 30-40ºC. A black fabric "jacket" was sewn to fit over the insulating material to provide a strong visual contrast against the background.

To provide natural host odours, two tents made from canvas supported by a metal frame, each measuring 2.0 m high × 2.0 m square were used to house odour baits (Figure 27A). One tent was assigned to a cow and another to a human volunteer throughout the study period. Tents were aerated and rotated between the trapping sites each night. A 12V fan (Biogents AG) connected to a 10m length of PVC tubing (10 cm diameter) was placed inside the tent (Figure 27B). The other opening of the tube was covered with untreated mosquito netting and placed ~10 cm from the base of the HDT unit, thus venting host odours from the tent around the trap at approximately 2000 l/min (Figure 27C). Carbon dioxide produced by both cow and human baited tents was measured at the pipe outlet using a CO_2 meter (EGM-4, PP Systems, MA, USA).

In principle, mosquitoes following odour plumes emanating from the end of the PVC tube see the HDT and approach it. They then encounter the warmth of the trap's surface, whereupon they land and become stuck to the transparent adhesive sheet (Figure 26A). At the end of the

sampling period, a thin plastic sheet of transparent polyethylene wrap (cling film/food wrap) was laid on the surface of the adhesive sheet, sandwiching trapped mosquitoes between the two sheets (Figure 26B). Using a razor blade, the sheets were cut and removed from the HDT and mosquitoes were later removed from the sheets in the laboratory using Romax Glue Solvent (Barrettine Environmental Health, Bristol, UK).

Figure 4.1: Mosquitoes collected by Host Decoy Traps (HDT). (A) A section of the HDT showing trapped mosquitoes stuck to clear adhesive sheet. (B) Trapped mosquitoes recovered from HDT by removing the adhesive sheet from the trap and covering it with a layer of thin plastic food wrap before species identification in the laboratory.

Whole host odours were used to attract mosquitoes to HDTs. Four cows, each weighing 150 - 200 kg were used individually to provide natural odours in the experiment. Each cow was used for six consecutive nights before being replaced (Figure 2). Eight field assistants working in pairs conducted the experiments, with each pair participating for six consecutive nights before being replaced. The field assistants worked in two shifts (6:00 pm -12:00 am and 12:00 am to 7:00 am.), changing places each night to perform either an outdoor HLC or sleeping in the tent to provide human odour for the HDT-H.

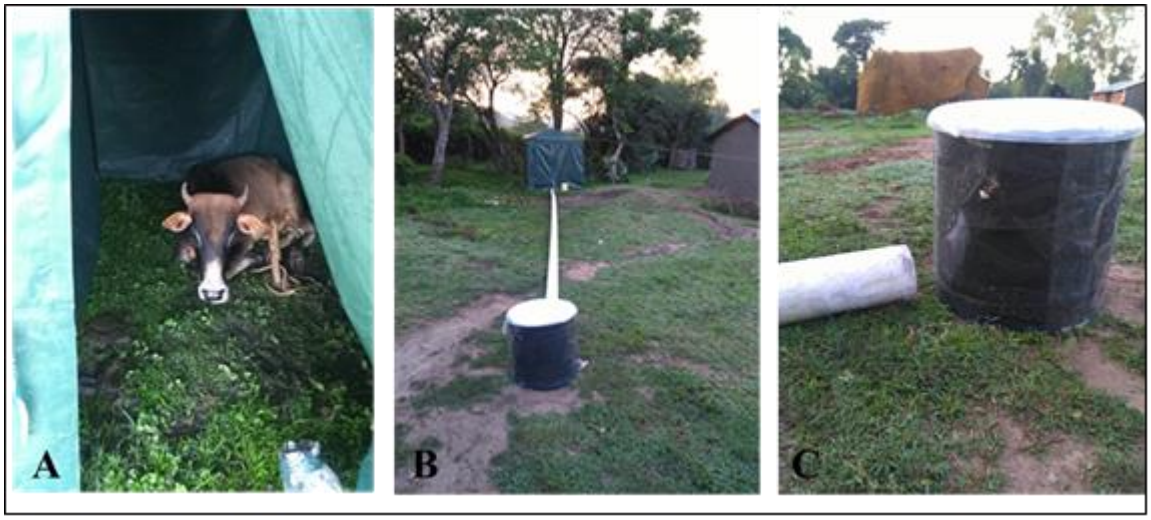


Figure 4.2: Host Decoy Trap (BG-HDT) set up. (A) Cow tethered inside tent provides natural host odour and carbon dioxide for baiting HDT. (B) Experimental set-up showing host-occupied tent, PVC pipe (fan inside pipe directs host odour to trap) and HDT. (C) Pipe opening releases host odour within 10 cm of the HDT. Visual stimuli of the dark trap and warmth of water-filled trap induce mosquitoes to land on the clear adhesive sheet covering the dark surface of the trap.

Human Landing Catch (HLC). Field assistants performing HLCs sat outside with their trousers folded to knee height and caught mosquitoes landing on their exposed lower limbs using a mouth aspirator. Collections were performed for 45 min and the collectors rested 15 min in each collection hour. Collected mosquitoes were placed in paper cups and were sustained on a 10% sugar solution before transportation to the laboratory for analysis.

Species identification and parasite detection. Mosquitoes were sorted to subfamilies to separate *Anopheles* from culicine species. In each subfamily, mosquitoes were further separated by abdominal status as either fed, unfed, gravid or half gravid. All *Anopheles* mosquitoes were identified morphologically to species [190, 191] and then placed singly in 1.5 ml micro-centrifuge tubes for further laboratory analysis. This involved species identification by PCR for *An. gambiae* s.l. [173] and *An. funestus* s.l. group of species [213]

and enzyme-linked immunosorbent assay (ELISA) for the detection of sporozoite infections [192].

Experiment 1: Comparison of catches from HDTs and HLCs

We investigated the host choices of outdoor-biting malaria vectors using the BG-HDT, baited with either human or cattle odour, and to compare these catches with the HLC. Our null hypothesis was that an HLC and the HDTs baited with a cow (HDT-C) or human (HDT-H) odour would catch equal numbers of mosquitoes with the same species composition in an outdoor peri-domestic environment. A replicated Latin Square experimental design of collection methods × sites × nights was conducted. Collection sites were 100 m from each other. The experiment was carried out twice, first (May 2017) in Kisian village, Kisumu county, and subsequently (June 2017) in Orego village, Homa Bay County. Collections ran from 18:00 h to 07:00 h for 24 nights in Kisian village and 12 nights in Orego village.

Experiment 2: Catches from un-baited HDT

In the second experiment, we tested whether mosquitoes would be attracted to an unbaited BG-HDT (i.e. operated without any host odours released from the tent) placed within 5 m of a corralled herd of cattle. The main aim was to determine whether dispersed host odour is sufficient to attract mosquitoes close enough to the HDT to induce them to land on the warm, visually conspicuous trap. Two pairs of neighbouring compounds in Kisian village were chosen for this study, each ~100 m apart. Within each pair, approximately 10 cattle were present in one compound and absent in the other. The BG-HDT (excluding tent and pipe used to deliver odours in Experiment 1) was placed next to the corralled cattle herd or in the centre of the compound where cattle were absent. Trapping was performed for six consecutive nights in each pair of compounds between 18:00 h and 07:00 h.

Experiment 3: Trap validation – does the BG-HDT catch similar abundance and species composition as the original trap?

In Experiment 3, we tested whether the commercially produced BG-HDT performed as well as the original proof of concept trap used in Hawkes *et al.* [113], with an additional reference HLC, with respect to mosquito species composition and abundance. A 3 × 3 Latin Square was conducted in Kisian, comparing the BG-HDT and the original version (O-HDT), both baited with human odour as described in Experiment 1, with the exception that small one-person tents were used. A protocol describing how to make the original HDT using commonly available materials is provided online at http://dx.doi.org/10.17504/protocols.io.n95dh86. This experiment was completed over 24 nights in May-June 2017.

Data analysis. The analysis was done using R statistical software version 3.4.1. Data were fitted using Generalized Linear Mixed Effects Statistical Models (GLMMs) to describe the effects of collection method on mosquito catches. Since the data was over-dispersed, we used the package glmmADMB [214] to fit negative binomial distribution models for the analysis of mosquito numbers. The numbers of female *Anopheles* mosquitoes were assessed as a function of collection method as a fixed effect, and collection sites and days were treated as random factors. A binomial GLM model was used to analyse the distribution of each *Anopheles* species of all collected *Anopheles* per trapping method. The proportion of each *Anopheles* was assessed as a function collection method. A pairwise comparison of means of *Anopheles* species between different trapping methods done by Turkey's test.

Ethics. The study was approved by the Kenya Medical Research Institute/ Scientific and Ethics Review Unit (KEMRI/SERU), number 2776 and by CDC through KEMRI/SERU (CDC IR 6728).

4.4 Results

Overall, a total of 1,807 *Anopheles* and 22,222 culicine mosquitoes were collected in Experiments 1, 2 and 3 combined, confirming outdoor-biting occurs for all the main malaria vector species in the study areas; *An. arabiensis*, *An. gambiae*, *An. funestus* and *An. coustani* (Table 4.1).

Table 4.1: Numbers of *Anopheles* and culicine species collected by different treatments for each experiment

		Anopheles species						Culicine species					
Experiment	Treatment	Fed	Gravid	Half gravid	Unfed	Male	Total	Fed	Gravid	Half gravid	Unfed	Male	Total
Exp. 1 (Kisian, n=24 nights)	HDT-C	1	0	1	1011	0	1013	4	1	1	8610	25	8641
	HDT-H	0	0	1	23	0	24	2	0	1	605	22	630
	HLC	21	0	2	120	5	148	47	6	5	1686	0	1744
Exp. 1 (Homa Bay, n=12 nights)	HDT-C	1	0	0	124	0	125	0	0	0	246	0	246
	HDT-H	0	0	0	9	0	9	0	0	0	26	0	26
	HLC	7	0	1	8	1	16	0	1	6	9	2	18
Exp. 2 (n = 6 nights)	Cattle Present	41	3	6	86	0	136	570	1	33	2793	1	3398
	Cattle Absent	0	0	0	7	0	7	0	0	0	122	1	123
Exp. 3 (n = 24 nights)	O-HDT	0	0	0	90	0	90	7	0	0	3089	31	3127
	BG-HDT	1	0	0	119	0	120	2	0	0	2721	9	2732
	HLC	4	0	0	111	4	119	19	32	30	1558	9	1648
Total		76 (4.2)	3 (0.2)	11 (0.6)	1708 (94.5)	10 (0.6)	1807	651 (2.9)	41 (0.2)	76 (0.3)	21465 (96.1)	100 (0.4)	22333

Experiment 1: Comparison of catches from HDTs and HLCs

We compared proportions of *Anopheles* species with respect to total anopheline numbers, between an HLC and HDT baited with either cow or human odour. The proportions varied

417 according to the trapping method and field location (Figure 3). From HDT-C collections, *An.*
418 *arabiensis* were the highest in proportion of all *Anopheles* species caught in both Kisian and
419 Homa Bay; 0.94 ± 0.01 and 0.57 ± 0.05, respectively. *Anopheles gambiae s.s.* were only
420 collected in Kisian, 0.06 ± 0.01, while both *An. funestus* and *An. coustani* were only collected
421 in Homa Bay at 0.04 ± 0.02 and 0.38 ± 0.04, respectively (Figure 28A). Collections by HDT-
422 H were equally dominated by *An. arabiensis* at both sites, 0.76 ± 0.1 in Kisian and 0.82 ±
423 0.12 in Homa Bay. *Anopheles gambiae* s.s. was at a proportion of 0.2 ± 0.1 in Kisian while
424 0.18 ± 0.12 of *An. coustani* were collected in Homa Bay (Figure 28B). Comparable
425 proportions of *An. arabiensis* were collected by HLC in both Kisian and Homa Bay, 0.45 ±
426 0.05 and 0.46 ± 0.09 respectively. The highest proportion of *An. gambiae,* 0.55 ± 0.05 was
427 collected by HLC in Kisian, while 0.43 ± 0.09 *An. funestus* were collected in Homa Bay
428 (Figure 28C).

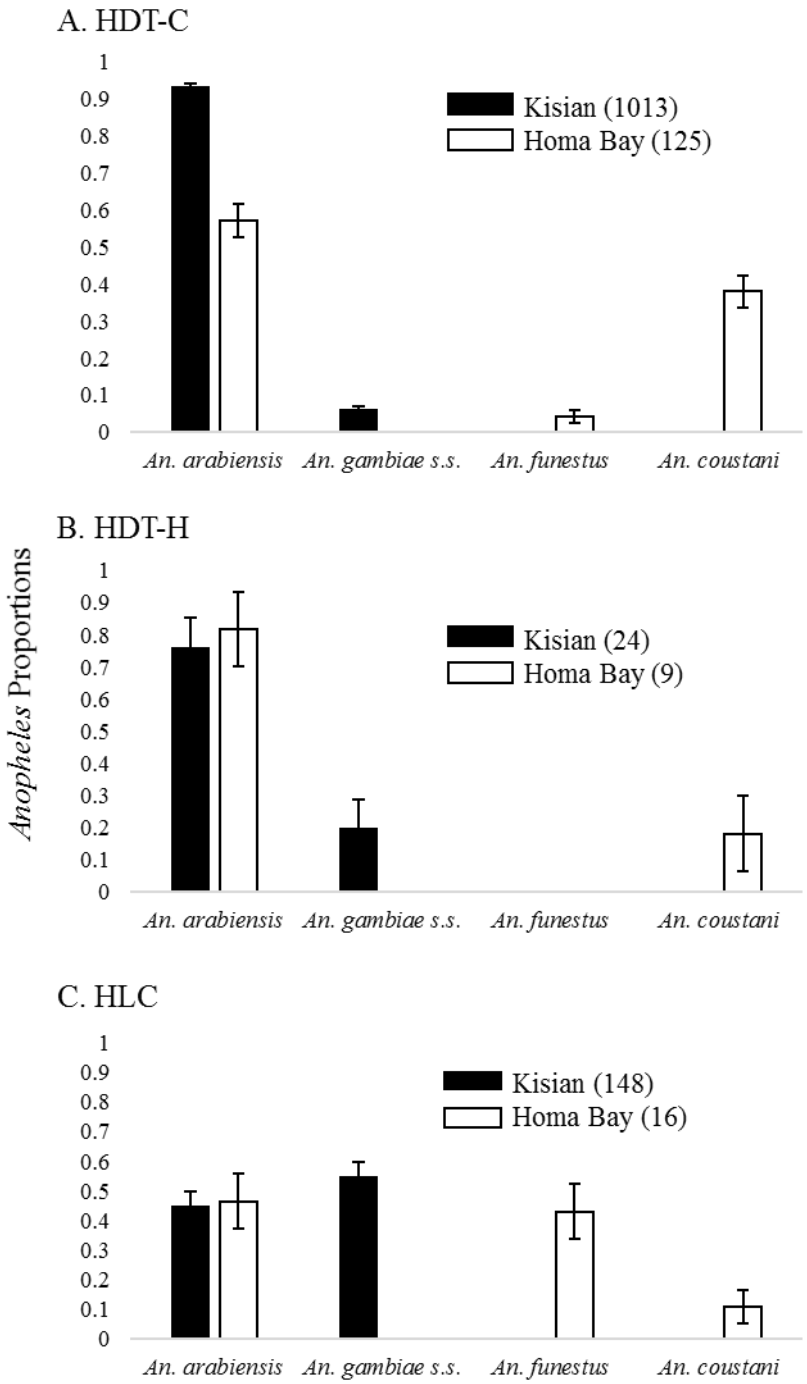


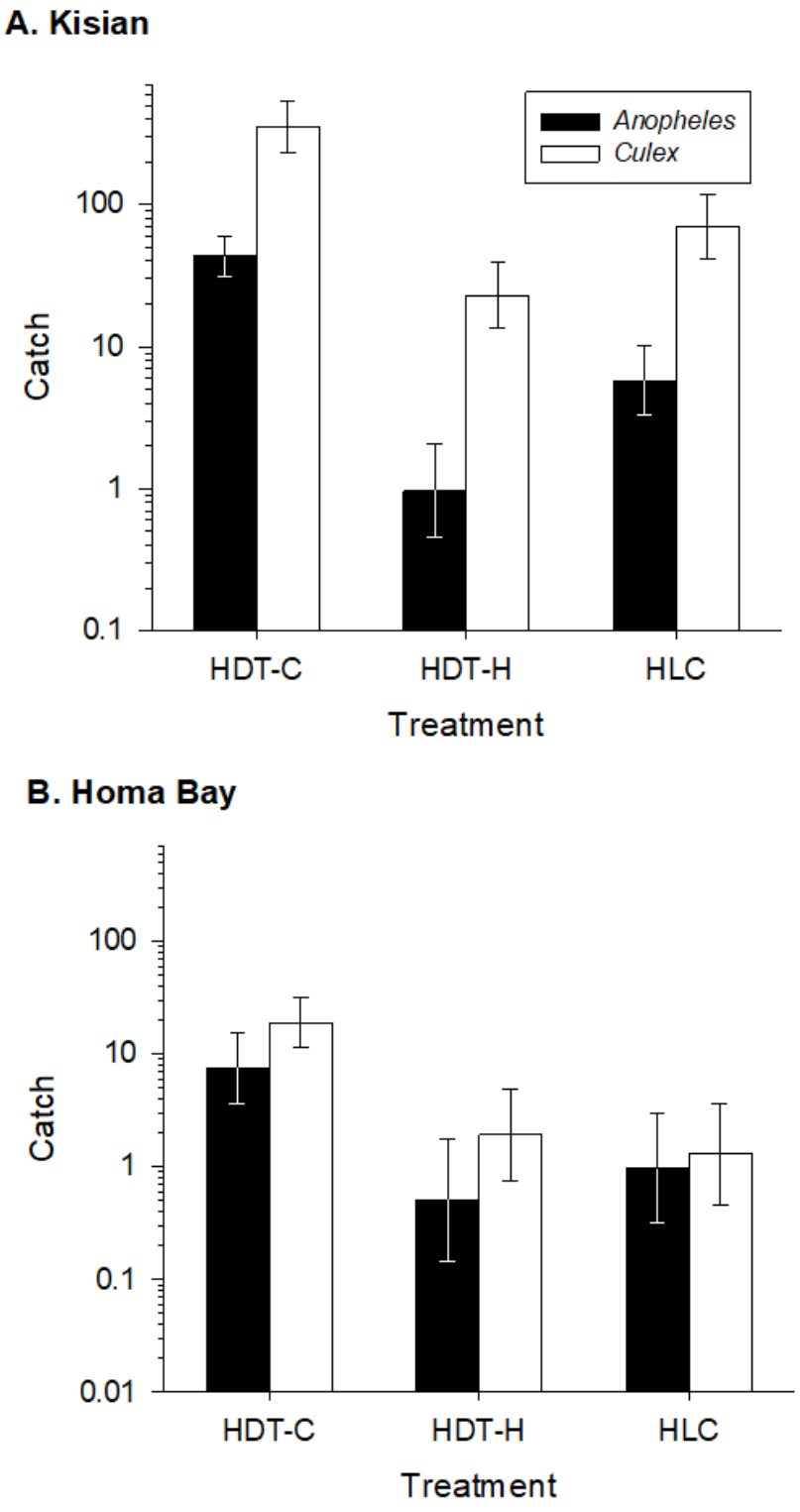
Figure 4.3: Relative species composition (proportions ± std errors) of *Anopheles* mosquitoes from three outdoor trapping methods (cattle-baited HDT (HDT-C), human-baited HDT (HDT-H) and human landing catch (HLC) traps in Kisian) in Kisian and Homa Bay, western Kenya (Experiment 1). Numbers in key show total catch of *Anopheles* caught in Kisian (n=24 nights) and Homa Bay (n=12 nights).

In Kisian, significantly higher proportions of *An. arabiensis* were found in HDT-C compared to HDT-H ($z = -2.8$; $P = 0.01$), and in HDT-H compared to HLC ($z = -2.5$; $P = 0.03$). A

significant difference in proportions of *An. arabiensis* was observed between HLC and HDT-C ($z = -12.4$; $P < 0.001$). Significantly higher proportions of *An. gambiae* were observed in HLC compared to HDT-C ($z = 12.5$; $P < 0.001$), HLC compared to HDT-H ($z = 2.7$; $P = 0.02$) and HDT-H compared to HDT-C ($z = 2.3$; $P = 0.05$). Only 2 *An. funestus* were collected by HDT-C in Kisian, hence no analysis was performed on the species.

In Homa Bay, there was no significant difference in the proportion of *An. arabiensis* caught by the collection methods. Significantly higher proportions of *An. funestus* were collected in the HLC compared to HDT-C ($z = 4.8$; $P < 0.001$). No *An. funestus* were collected by HDT-H. *Anopheles coustani* was sampled by all collection methods. HDT-C collected significantly higher proportions of *An. coustani* compared to HLC ($z = -2.66$; $P = 0.03$), while no significant differences were found between HDT-C and HDT-H or between HLC and HDT-H.

Mosquito abundance in Kisian village, differed dramatically by trap type. The HDT-C collected a nightly average of 43.2 (26.7-69.8; 95% CI) *Anopheles*, compared to 5.8 (4.1-8.2; 95% CI) in HLC ($z = -8.99$, $P < 0.001$), while HDT-H collected 0.97 (0.4-2.1; 95%CI), significantly fewer *Anopheles* than the HLC ($z = -6$, $P < 0.001$). A similar pattern was observed in mean nightly catch of culicine species. These were significantly higher in HDT-C with a mean of 349.6 (208.5-586.3; 95% CI) compared to 70.5 in HLC (46.5-106.7; 95% CI), ($z = -10.1$, $P < 0.001$), while the HDT-H collected 22.9, the fewest culicine mosquitoes (13.6-38.8; 95% CI), significantly less than the HLC ($z = -7.05$, $P < 0.001$; Figure 29A).



458

459 Figure 4.4: Nightly outdoor catches (mean ± std errors) of *Anopheles* spp. and *culicine* spp.
460 mosquitoes from cattle-baited HDT (HDT-C), human-baited HDT (HDT-H) and human
461 landing catch (HLC) traps in Kisian (n=24 nights) and Homa Bay (n=12 nights), western
462 Kenya (Experiment 1). Data are plotted on a logarithmic y-axis.

The overall abundance of *Anopheles* in Homa Bay showed a trend of significantly higher numbers of mosquitoes in HDT-C, compared to the other methods. Here, a mean of 7.5 (2.8-19.9) *Anopheles* were collected by HDT-C each night, compared to 1.0 (0.4 -2.3) in HLC, ($z = 5.31$, $P < 0.001$). However, no significant difference was found between catches in HLC and HDT-H with a mean of 0.5 (0.1 – 2.1), ($z = -1.26$, $P = 0.21$). As in Kisian, a significantly higher mean number of culicine mosquitoes, 18.9 (7.5 – 47.3), were also collected by HDT-C each night in Homa Bay, compared to 1.3 (0.7-2.6) in HLC ($z = 6.61$, $P < 0.001$; Figure 29B).

Both cattle- and human-baited HDTs exclusively collected unfed female *Anopheles* (97.4%) while fed *Anopheles* accounted for 17% of HLC samples (Table 2). Sporozoite infection rates were 1.4% (9/635) in HDT-C, 5.5% (1/18) in HDT-H and 0.9% (1/111) in HLC.

The mean amount of CO_2 was 1298.3 ± 39.5 ppm in the cattle tent and 532.9 ± 56.1ppm in the human tent, which means effectively, 2.44 times more CO_2 was released from the HDT-C trap than the HDT-H trap. However, there were ~ 44 times more *Anopheles* and ~14 times more culicines in the HDT-C than in the HDT-H.

Experiment 2: Catches from un-baited HDT

Unbaited BG-HDTs were placed either next to a herd of corralled cattle or in a compound with no cattle present. Despite lacking a dedicated odour source, traps in this experiment still captured *Anopheles* mosquitoes outdoors. The traps collected mostly *An. arabiensis,* the proportions of 0.97 ± 0.02 and 0.8 ± 0.2 in the presence and absence of cattle, respectively, were not significantly different. However, the HDT collected a mean of 10.4 (2.0-55.0) *Anopheles* each night in the presence of cattle versus 0.45 (0.1-1.7) when cattle were absent ($z = -3.81$; $P = 0.0001$). A significantly higher mean number of culicine mosquitoes were collected in the presence of cattle, 314.5 (70.0-1412.3) versus 3.83 (1.4 – 10.5) in compounds without cattle ($z = -6.92$, $P < 0.001$; Figure 30). No sporozoite positive *Anopheles* were

detected in Experiment 2, however, 30% of *Anopheles* mosquitoes in the HDT next to cattle were blood-fed, which may reflect partial blood meals on the available cattle.

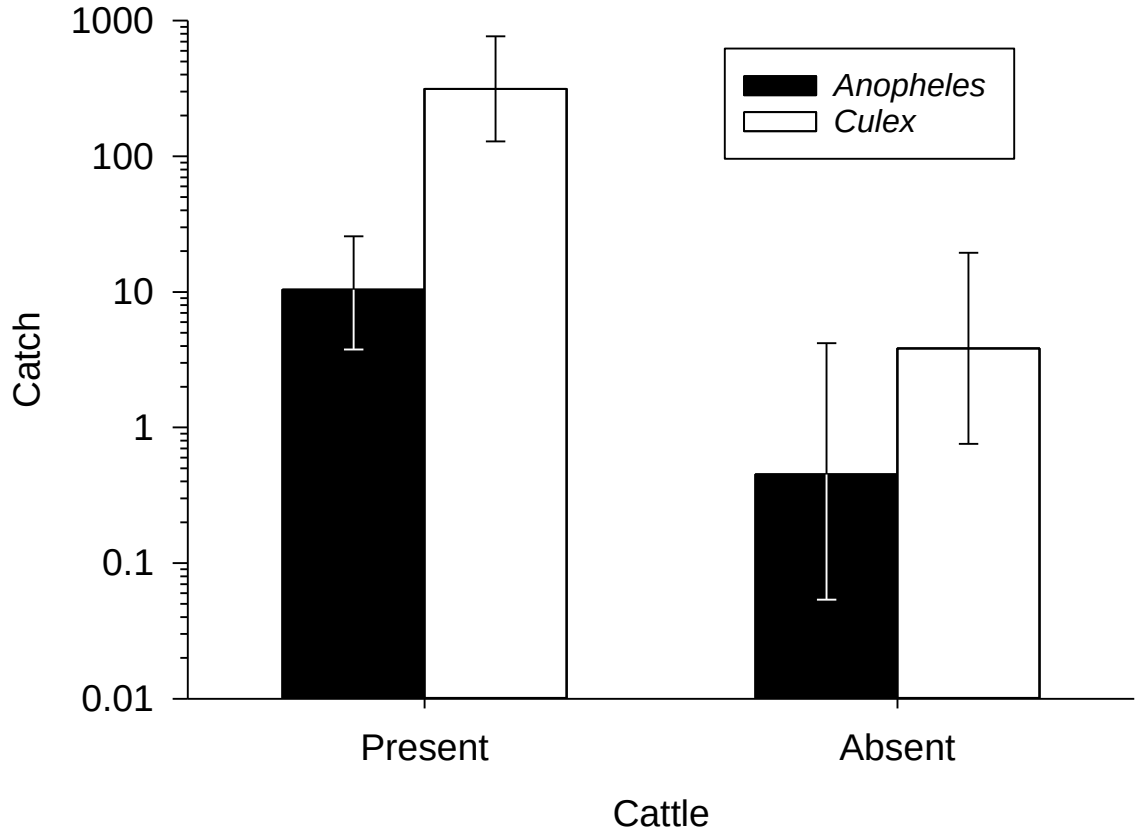
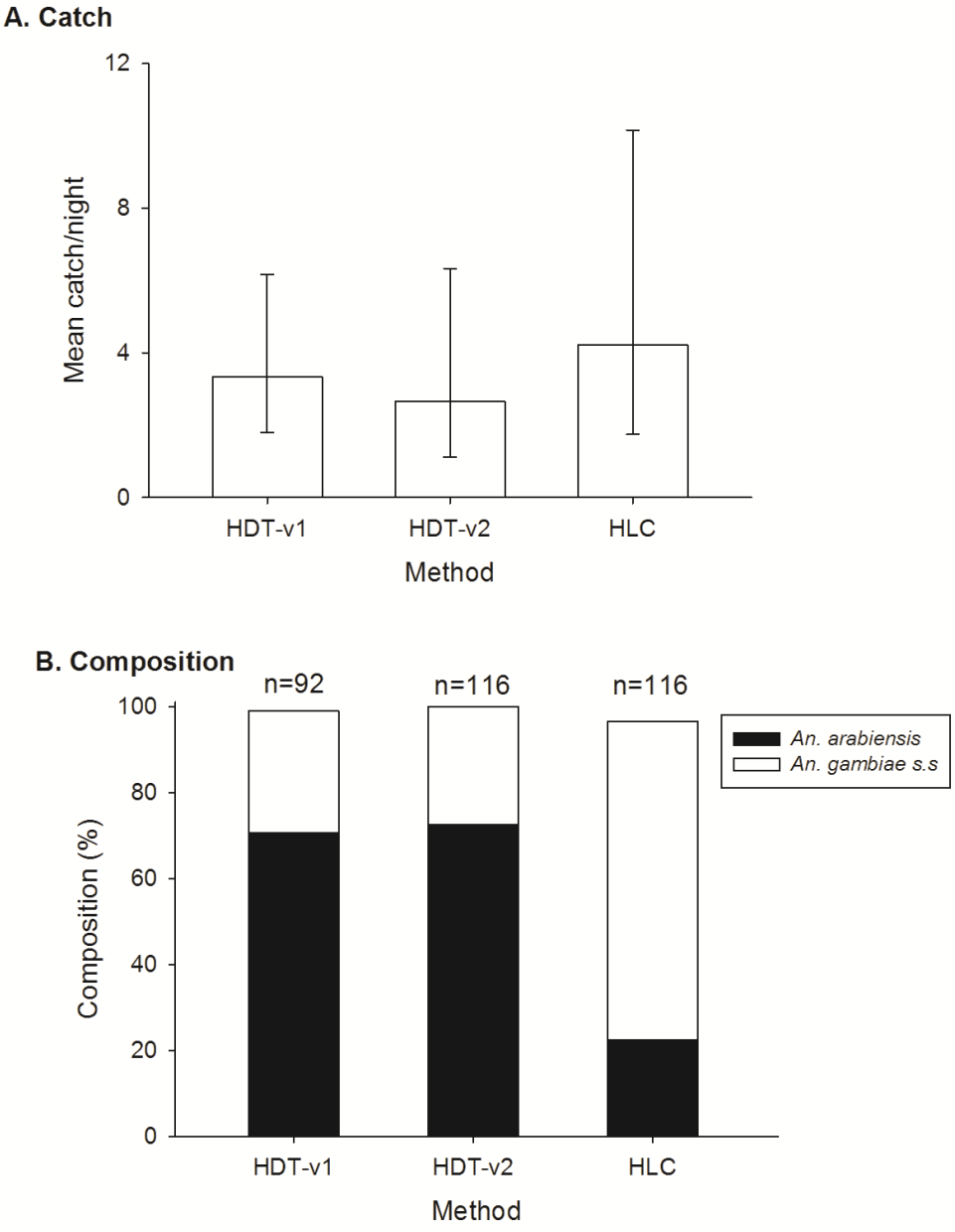


Figure 4.5: Comparison of mean (± std errors) catches by Host Decoy Traps in the presence or absence of cattle in Kisian, western Kenya. Mean nightly outdoor catch (n=6 nights/site for each treatment) of *Anopheles* spp. and *culicine* spp. mosquitoes (Experiment 2). Data are plotted on a logarithmic y-axis.

Experiment 3: Trap validation – does the BG-HDT catch similar abundance and species composition as the original trap?

We compared the commercial BG-HDT produced by Biogents and the O-HDT, the original proof of concept version, alongside a standard HLC. We found no statistical difference ($z = -0.73$, $P = 0.46$) in the mean nightly outdoor catch of *Anopheles* between the commercial BG-

HDT, which caught 3.33 (1.4-8.0), and the original version made using locally available materials, which caught 2.66 (1.1-6.5) per night (Figure 31). There was also no significant difference in mean nightly *Anopheles* catch between the commercial BG-HDT and HLC (4.21 (2.2-7.9; z = -0.74, P = 0.46). The commercial BG-HDT and O-HDT caught near identical proportions of *An. arabiensis* (72% and 69% of specimens, respectively; z = -0.5, P = 0.86).



506

507 Figure 4.6: Nightly outdoor catches (mean ± se; n=24 nights) of *Anopheles* mosquitoes with
508 the original Host Decoy Trap (HDT-v1), the BG-HDT (HDT-v2) and the human landing
509 catch (HLC), in Kisian, western Kenya (Experiment 3).

510

511

4.5 Discussion

Our results demonstrate that the HDT baited with cattle odour is a highly efficient method of sampling outdoor biting anophelines, with a cattle-baited HDT catching consistently more *Anopheles,* mainly *An. arabiensis,* than the HLC. Overall, the cattle-baited HDT caught over seven times more *Anopheles* than HLC outdoors. This suggests that HDTs may be useful both for collecting large numbers of mosquitoes outdoors, but also for elucidating mosquito host choice. Our ability to trap mosquitoes when placed in the presence of cattle outdoors demonstrates how the HDT trap could be deployed as a passive monitoring device for use in outdoor peri-domestic environments. The HDT incorporates sensory stimuli used by host biting mosquitoes to locate their next blood meal and is a potentially significant development in the science of mosquito sampling, particularly in outdoor environments. We recommend further improvement of the trap with the development of artificial odours that mimic a full arrange of host-associated odours to be used in combination with other mosquito host stimuli for malaria vector surveillance.

The number of *Anopheles* caught in HDT-H was significantly lower than HLC in the Kisian experiment while no significant difference was observed between the two methods in Homa Bay. In the initial development of the trap, HDT-H caught significantly more *Anopheles* overall than the HLC [113]. In the current study, local vector populations are composed of *An. gambiae*, *An. arabiensis*, *An. funestus* and *An. coustani*, whereas *An. coluzzii* is overwhelmingly dominant in the area of Burkina Faso where the first evaluation of HDT took place. Given that Experiment 3 confirmed the original prototype used in Burkina Faso [113] showed similar catch abundance and composition to the BG-HDT deployed in experiments 1 and 2, the observed difference in HDT performance is likely a result of species differences in response to the trapping methodology. The effect of different CO_2 concentrations in the cattle

and human tents on the respective HDT catches demonstrated that there is a non-linear relationship between CO_2 and attractiveness to mosquitoes, which merits further research.

Anopheles arabiensis dominated the catches by HDT-C, illustrative of the species behavior with reference to feeding location and host choice. Previous studies in western Kenya have largely associated *An. arabiensis* with cattle feeding, and outdoor biting with occasional feeds on humans both indoors and outdoors [17, 20, 23, 44]. Even though the overall catch of *An. arabiensis* was low in both HDT-H and HLC, the vector species composed a considerable proportion of *Anopheles* trapped by the two methods at both sites, indicating the likelihood of feeding on humans outdoors. Earlier investigations of *An. arabiensis* biting behavior in western Kenya found that outdoor resting *An. arabiensis* d did not feed on humans at all, whereas those caught resting indoors had a human blood index (HBI) of 0.23 [23]. A similar observation was reported in northern Tanzania, where odour from cattle attracted 90.3% *An. arabiensis* compared to 9.7% were attracted to human odour [215]. In Ethiopia, evaluation of the blood-feeding behavior of *An. arabiensis* using host-baited sampling methods showed that this species fed preferentially on humans over cattle outdoors, but with a preference for cattle-biting outdoors over human-biting indoors [212, 216]. These studies illustrate the diversity of feeding behaviour of *An. arabiensis,* which makes it particularly difficult to control them by LLINs and IRS.

Human-baited traps, HDT-H, and HLC caught the largest proportions of *An. gambiae* While earlier studies investigating host selection reported the species to feed more frequently on humans indoors [17, 20, 23, 44], there is a recent report of an unusually high frequency of animal and mixed-blood meals in *An. gambiae* [28] and a shift in biting time [73] in regions with high bed net coverage in western Kenya highlands. These observations suggest possible behavioral modification in the presence of bed nets. While our data is unable to confirm any

of these observations, we recommend further studies to determine the current contribution of *An. gambiae* to malaria transmission both indoors and outdoors in the lake endemic regions of western Kenya, following previous reports of the historical population decline of the species with associated with the introduction of bed nets [217].

Additional control tools that target outdoor-biting vector populations are needed to supplement LLINs and IRS [195, 218]. Zooprophylaxis by keeping cattle around houses has been suggested as a strategy to protect humans from malaria [215]. Classical zooprophylaxis (without insecticides) may not have a significant impact on the malaria vectorial capacity of *An. arabiensis* [216] in regions where the vector bites both humans and cattle. Indeed, the presence of cattle may result in the proliferation of the species and sustain outdoor transmission. However, treating cattle with insecticides or endectocides, such as ivermectin, may be a viable strategy [219]. A recent evaluation of endectocide administration to local Zebu cattle under semi-field conditions in western Kenya showed a significant reduction in survival of *An. arabiensis* of up to 21 days post-treatment [220]. Furthermore, a field evaluation of topical formulations of eprinomectin against *An. arabiensis* in western Kenya showed a 38% reduction in indoor resting densities of the species within one-week post-treatment [221]. The HDT is suitable for sampling outdoor-biting vectors under such treatments, and therefore, could be a valuable method for monitoring the impact of the next generation of control interventions that target malaria vectors, including a periodic assessment of host preference. The numbers of *An. arabiensis* collected and killed each night by the HDT also raises the question of whether the concept of host decoys can be developed as a behaviour-based vector control tool, similar to the Suna trap [206] or to the lethal targets used to lure and kill tsetse vectors of trypanosomes [222].

583 The implementation of HDT was faced with a few limitations. The trap required hot water to
584 regulate the surface temperature between 30-40ºC throughout the night. Boiling water every
585 day was logistically challenging, and regulation of surface temperature was affected by
586 weather conditions, leading to greater heat loss in colder nights. Use of live hosts for natural
587 odours and the need to exhaust the odours from a tent to the trap added to the logistical
588 challenges in implementation of the trapping technique. The HDT was also observed to
589 poorly estimate human exposure compared to HLC. While the HDT provided the basis of a
590 system to sample host-seeking mosquitoes outdoor, it requires development of an internal
591 heating system regulated at bodily temperature and an artificial odour source to avoid use of
592 live hosts in order to optimize its performance and enable scalability. Additional tests are
593 necessary to optimize the trap against HLC for collection of anthrophilic mosquito vectors.

594 **4.6 Conclusion**

595 The HDT, which combines odours, heat, and a visually-conspicuous stimulus to simulate a
596 host, provides the basis of a system to sample human- and cattle-biting mosquitoes. The
597 cattle-baited HDT is particularly effective for *An. arabiensis*, an important vector of malaria
598 which feeds, in part, outdoors on cattle and is, therefore, not efficiently sampled or controlled
599 by standard methods. However, it did not give a reliable estimate of human exposure as
600 reflected by HLC. The HDT offers the prospect of a system to monitor and potentially control
601 *An. arabiensis* and other outdoor-biting mosquitoes more effectively. To achieve a practical,
602 standardized system, the use of artificial host odours to replace the natural odours used in this
603 and previous studies of the HDT should be explored.

604 **Declarations**

605 **Ethics approval and consent to participate**: The study was approved by the Kenya Medical
606 Research Institute/ Scientific and Ethics Review Unit (KEMRI/SERU), number 2776.

Individuals participating in HLC were duly consented, screened, and treated for malaria if positive and were placed on malaria prophylaxis, Mephaquin (Acino Pharma AG, Switzerland) over the collection period until four weeks after collection ended. Additional consent was sought for use of cattle in the tent and trapping within the compound.

Consent for publication

This publication is done with consent from the Director Kenya Medical Research Institute (KEMRI)

Availability of data

The datasets used during the current study are available from the corresponding author on a reasonable request.

Competing interest

The authors declare they have no competing interests

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Authors' contributions

BA, FMH, and SJT conceived the study. BA, FMH, SJT, XY, NFL, and MD participated in the design of the experiment. BA, SJT, FMH, XY, and MO performed the experiment. BA, SJT, MD, and FMH analysed the data. FMH, GG, and GM developed the BG-HDT. BA,

FMH, GG, MD, SJT, JG, FK, AS, NL, EO, and SM wrote and reviewed the manuscript. All authors read and approved the final manuscript.

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5 CHAPTER FIVE: DISCUSSION AND CONCLUSION

Discussion and Conclusion

5.1 Summary and conclusion

The overall goal of the study was to evaluate malaria vector surveillance under enhanced disease control scenarios and reducing indoor mosquito densities. It was hypothesized that as vector control strategies are scaled up, mosquito numbers decline, and surveillance by centrally managed teams become less effective compared to a devolved system. The use of a combination of sampling tools is more effective in evaluating the impact of vector interventions. While trapping technique that exploits mosquito host-oriented behaviour to attract and trap mosquitoes are more effective for malaria vector sampling. A series of studies were conducted to evaluate these hypotheses. The impact of IRS on local mosquito populations in a region with high LLIN coverage was evaluated by PSC, light trap and HLC collections, implemented under the supervision of expert entomology technicians. Supervised collections by expert entomology technicians and unsupervised collection by community-based teams were compared for effectiveness. While HDT was evaluated against HLC for collection of outdoor host-seeking mosquitoes.

IRS with pirimiphos-methyl was highly effective in reducing population densities of *An. funestus*, sporozoite rates, and test positivity rates in the sprayed sub-counties. *An. funestus* was considerably the main malaria vector in the region. Due to its close association with human habitations, resting indoors and feeding more frequently on humans, the vector species were most affected by spraying. Reduction of the overall *An. funestus* populations resulted in a corresponding fall in sporozoite rates, parity rates, and malaria test positivity rates. IRS is effective in achieving rapid malaria transmission reduction. Sustenance of these gains remains a major priority for the NMCP. A robust surveillance system for both entomological indicators and disease prevalence are key in tracking progress towards sustained vector control and malaria eliminations. However, with the critically reduced vector

densities observed post-IRS in the sprayed areas, indoor mosquito collection by PSC and indoor CDC light trap implemented by centrally managed teams become less informative and unsustainable. A robust, low-cost, devolved surveillance and supervision systems are considered more effective in tracking vector populations under an altered vectorial system following a successful implementation of control effort.

An. arabiensis were only marginally affected by the IRS. The species has been previously reported to be more exophilic and zoophilic, hence associated more with outdoor feeding and resting. In the evaluation of HDT, a large number of *An. arabiensis* were collected outdoors with cow odour which is an indication of the existence of this species in large numbers outdoors. It may be that the numbers of *An. arabiensis* usually collected in indoor traps are, but a small proportion of the large outdoor population sustained on cattle hosts. This possibly explains the low impact of IRS reported on the species. Outdoor feeding and/or resting populations of mosquito have been reported in other studies to sustain malaria transmission following successful control of indoor vector population by IRS and LLINs [66, 75, 76, 99]. The monitoring of outdoor malaria vector populations is therefore critical in understanding changes in the local vector populations to advise on suitable complementary control strategies.

Community-based sampling scheme bears a potential for sustainable, multiple sampling in several locations at the same time. However, the catch sizes in the indoor light trap were observed to be substantially lower in the community-based sampling scheme compared to supervised collections. The community-based sampling approach has been reported in other surveys to be more cost-effective compared to supervised sampling scheme [139, 143]. However, the sampling approach was observed to suffer from low compliance by community-based collectors. A similar observation has been made previously in two different

surveys evaluating community-based sampling scheme [139, 143]. It is recommended that community-based sampling scheme be integrated with low cost, devolved supervisory system.

Host Decoy Trap was more effective in collecting outdoor host-seeking *An. arabiensis* compared to HLC. The trap which combines essential stimuli used by mosquitoes to locate their blood meal hosts showed great potential for improving the science of mosquito sampling. The trap could be used as a passive outdoor sampling tool by placing it next to outdoor hosts. As the mosquitoes approach their hosts outdoors, the nearby trap simulates a host due to the visual contrast, heat and is presumably in a plume of host odours from nearby natural hosts. The mosquitoes are then induced to land on the trap whereupon they get stuck on the sticky surface. The HDT offers the prospect of a system to monitor and potentially control *An. arabiensis* and other outdoor-biting mosquito populations. An improvement of the trap's heating system is a major requirement in enhancing its trapping efficacy and scalability.

Pirimiphos-methyl is highly effective in controlling pyrethroid-resistant mosquito populations. The insecticide was shown to be highly potent in killing mosquitoes in the cone assay up to 11 months post-spray. The long residual life of the insecticide makes it suitable for providing all-year-round protection with a single round of spraying. The availability of new non-pyrethroid insecticides for malaria vector control presents new opportunities for managing the rise and spread of insecticide resistance in mosquitoes and achieving rapid transmission reduction in malaria-endemic Africa.

5.2 Study limitations

Limitations identified in the series of studies presented in this thesis range from inefficiencies in trap design to methodological issues in the study implementation. Several of the limitations

712 are already identified and discussed in the respective chapters and are highlighted in this
713 section.

714 From the introduction chapter, inefficiencies in trap design that reduce their sampling
715 efficacy coupled with variations in trap applications across different settings make
716 standardization of mosquito trapping methods difficult and comparison of data impossible.
717 Individual mosquito collection methods exploit either mosquito host-seeking or resting
718 behaviour. Historically, entomological monitoring has been focused on the use of indoor
719 mosquito collection tools such as CDC light traps and PSC for the evaluation of different
720 intervention strategies. As these interventions are scaled up, changes in vectorial systems
721 characterized by increased outdoor biting and resting, altered species composition and
722 declining numbers emerge and the application of a single monitoring tool does not provide
723 adequate entomological information. Therefore, the use of a combination of mosquito
724 monitoring tools, targeting both indoor and outdoor malaria vectors is recommended under
725 enhanced vector control. Unfortunately, there are currently no outdoor sampling tools that are
726 easily scalable for longitudinal entomological monitoring.

727 Other than challenges with implementation of the current monitoring tools, traditional
728 mosquito sampling strategies also present limitations for entomological monitoring. With
729 reduced mosquito numbers under enhanced vector control, more intense and frequent
730 sampling has become necessary to collect sufficient mosquito numbers for decision making.
731 Traditional entomological monitoring with centrally managed teams, therefore, becomes less
732 cost-effective as they are limited by geographical coverage and intensity of sampling. A
733 devolved mosquito monitoring framework with community-based teams is recommended for
734 sustainable longitudinal monitoring.

735 Some limitations were also identified with combined implementation of CDC light trap, PSC
736 and HLC for evaluation of the impact of IRS described in Chapter two. Longitudinal
737 monitoring of vector densities was conducted indoors only. While the IRS was successful in
738 reducing the indoor host-seeking and resting densities of *An. funestus*, there was a marginal
739 impact on *An. arabiensis* population. Since the local *An. arabiensis* population is known to
740 feed mostly on cattle and rest outdoor, an outdoor trapping method would have been
741 extremely useful in providing a quantitative measure of the impact of IRS on mosquitoes
742 feeding and/or resting outdoors. Results presented in Chapter two of the thesis showed that
743 the IRS has had a relatively small impact on *An. arabiensis* population. The low impact was
744 attributable to a known behavioural adaptation of the species, based on previous studies [17,
745 23] However, data generated in the context of IRS would have provided more compelling
746 evidence of species' behavioural adaptation that makes it less susceptible to indoor based
747 interventions such as IRS.

748 In the study design of the IRS trial (Chapter two), sampling was conducted before and after
749 IRS, at intervention and non-intervention sites. Even though sampling was conducted with
750 the same intensity and frequency in both intervention and non-intervention sub-counties, the
751 regions were not matched in terms of vectors numbers. *Anopheles* densities were significantly
752 lower in the intervention area compared to non-intervention sub-counties prior to the use of
753 IRS. Also, the pre-IRS sampling period was shorter compared to the post-IRS period. Even
754 though the results provide strong evidence for the impact of IRS on *An. funestus*, stronger
755 evidence would be provided by a randomized controlled trial. The differences between
756 intervention and non-intervention sub-counties did not obscure the large impact spraying had
757 on the local mosquito population. However, there is a chance that such differences in study
758 arms may mask the impact of intervention so that it is not detected. That said, it is important
759 to note that implementation of vector surveillance under programmatic roll-out of

intervention is more likely to be faced with unmatched study arms since it is difficult to determine vector distribution prior to the control operation.

The individual mosquito trapping methods used in the evaluation of IRS have inherent limitations that might have affected the results. PSC was implemented with pyrethrum mixed with butoxide. While the synergist would be useful in tackling pyrethroid resistance in mosquitoes, it is unclear to what extent PSC is a useful sampling tool with widespread pyrethroid resistance in mosquitoes. For instance, it is not known if using pyrethroid in PSC selects for pyrethroid resistance. The use of CDC light traps, on the other hand, is affected by competing light sources if used outdoors, especially where light is the only attractant that brings mosquitoes to the trap.

In the evaluation of community-based sampling strategies with light trap collections being made outdoors, the impact of competing light sources and the effect of full moon nights on catch sizes was not accounted for. Light traps have been reported to work better when installed at the foot-side of an occupied bed net. Possible variations in trap installation by different collectors may have contributed to variations in catch sizes. Also, installing a single light trap in a house with several sleeping areas may not provide a correct measure of host-seeking densities per household. Other sleepers who at times may not be under bednet preferentially attract more mosquitoes which then reduced catch sizes in the trap installed next to an occupied bednet. Similarly, human landing collections by community members are faced with several limitations ranging from differential attractiveness between collectors, level of expertise in catching mosquitoes and personal motivation. While attempts were made to limit these limitations by providing training, constant supervision and moving collectors between different collection locations and shifts, it remains a major challenge to overcome

these individual sampling weaknesses. The limitations were not accounted for in the data analysis and it is unknown to what extent these affect trap catch sizes.

In demonstrating the impact of IRS on malaria transmission, test positivity rates were used. This involved collecting malaria test data from laboratory registers in selected health facilities. While this was a quick and less costly way of obtaining malaria test data, it was faced with cases of data gaps and missing data due to poor records management at the facilities. In addition, changes in malaria test positivity rates are considered an imprecise matrix for measuring changes in disease prevalence in a population since it is affected by a number of factors such as seasonality, changes in cases of other febrile illnesses, facility catchment area and quality of health services offered among other factors. Tracking disease incidence in a population or by following a cohort provides better information on changes in population disease prevalence. Whereas a significant decline in malaria test positivity rates was associated with the IRS, the limitations to this approach are recognized.

In Chapter Three, the unsupervised community-based sampling scheme was observed to be affected by low compliance in the installation of indoor light trap that potentially resulted in low trap catch sizes. It is suspected that the collectors at times did not set the traps next to an occupied bednet as recommended. Collections by the community-based teams were restricted to randomly-selected houses, with every house being sampled once every month for three years, some houses withdrew consent over time and the collectors tended to sample repeatedly from more friendlier houses. While working with randomly-selected houses was initially thought to enable tracking of the community-based collectors, it turned out to limit them as houses withdrew consent. It is recommended that future studies implementing community-based entomological surveillance should include a low cost devolved supervisory

scheme and the collectors should be able to sample from all houses in the community in a systematic manner.

In Chapter Four, the Host Decoy Trap required regulation of surface temperatures between 30ºC-40ºC throughout the night. The surface temperature was maintained by hot water introduced into the trap at boiling point. While the surface temperature was always at least 30ºC by the morning of the trapping night, maintaining the temperature by boiled water was logistically challenging. This limits the possibility of rolling out the trap on a large scale. Also, heat loss is likely to be high in colder nights and during wet weather, which may affect trap performance. The trap was evaluated with natural host odours which involved placing a live host inside a tent while exhausting odours from it. While this worked well for small scale trapping experiments, placing live hosts in tents for large scale sampling would be logistically challenging. HDT showed great success in sampling outdoor host-seeking mosquitoes with potential for controlling outdoor mosquito populations. However, an internal heating system and a reliable source of host odours need to be incorporated into the trap to enable scalability.

5.3 Recommendations for future research

Tackling residual malaria transmission in sub-Saharan Africa is an urgent need in the progress towards malaria eliminations. However, this depends largely on understanding dynamics in malaria vector behaviour with scale-up of indoor-based malaria interventions. The smaller impact of IRS on *An. arabiensis* coupled with the large catch size of the species outdoor with HDT using cattle odour raises fundamental questions about the epidemiological role of this species. Characterizing outdoor *An. arabiensis* population, their resting and feeding behaviour and assessing possible control strategies are opportunities for further research.

Sampling of outdoor host-seeking malaria vectors by HDT provides new opportunities for understanding the outdoor mosquito populations. However, the trap requires improvement involving the development of an internal heating system and a sustainable odour source to replace boiling of water and the use of a live host respectively. These open new research opportunities to improve and evaluate HDT for trapping efficacy and scalability in regions with different malaria vector populations.

Previous studies assessing community-based sampling scheme identified challenges of low compliance by the community-based collectors. A similar limitation to this sampling approach was identified in the present study. It is recommended that future studies implementing community-based vector surveillance to incorporate a low-cost devolved supervisory system to improve compliance. However, the generation of new data on the cost-effectiveness of supervised community-based sampling scheme is necessary.

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Appendix 1. Model table for *Anopheles funestus* collected in light traps. Conditional estimates for the effect of pre- versus post-spray conditional on IRS status are also included.

Parameter	Level	Risk Ratio	Lower CL	Upper CL	t-value	P-value
Intercept	Interceptor	0.4	0.2	0.8	-2.841	0.013
Period	Post Spray	0.98	0.69	1.38	-0.127	0.899
	Pre Spray	Ref				
Status	Intervention	0.64	0.28	1.45	-1.063	0.288
	Control	Ref				
Period*Status	PostSpray*Intervention	0.12	0.07	0.21	-7.513	<0.001
	PostSpray*Control	Ref				
	PreSpray*Intervention	Ref				
	PreSpray*Control	Ref				
Net Use	All under net	1.11	0.77	1.6	0.576	0.565
	Some under net	1.2	0.77	1.86	0.816	0.415

	None under net	Ref				
Eaves	Closed	0.68	0.48	0.96	-2.174	0.030
	Partially open	0.84	0.56	1.27	-0.817	0.414
	Open	Ref				
Cattle	Yes	1.62	1.22	2.13	3.395	0.001
	No	Ref				
Period (Conditional on IRS)	Post Spray	0.12	0.07	0.19	-8.615	<0.001
	Pre Spray	Ref				
Period (Conditional on non-IRS)	Post Spray	0.98	0.69	1.38	-0.127	0.899
	Pre Spray	Ref				

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Appendix 2. Model table for *Anopheles funestus* collected by pyrethrum spray catches. Conditional estimates for the effect of pre- versus post-spray conditional on IRS status are also included.

Parameter	Level	Risk Ratio	Lower CL	Upper CL	t-value	P-value
Intercept	Interceptor	0.72	0.27	1.94	-0.704	0.493
Period	Post Spray	0.64	0.41	1.00	-1.945	0.052
	Pre Spray	Ref				
Status	Intervention	0.54	0.17	1.72	-1.047	0.296
	Control	Ref				
Period*Status	PostSpray*Intervention	0.06	0.03	0.13	-7.094	<0.001
	PostSpray*Control	Ref				
	PreSpray*Intervention	Ref				
	PreSpray*Control	Ref				
Net Use	All under net	0.96	0.61	1.5	-0.187	0.852
	Some under net	2.02	1.13	3.59	2.383	0.017
	None under net	Ref				
Eaves	Closed	0.8	0.5	1.3	-0.889	0.374
	Partially open	1.08	0.64	1.83	0.291	0.771
	Open	Ref				
Cattle	Yes	1.63	1.12	2.35	2.583	0.010
	No	Ref				
Period (Conditional on IRS)	Post Spray	0.04	0.02	0.07	-9.289	<0.001
	Pre Spray	Ref				
Period (Conditional on non-IRS)	Post Spray	0.64	0.41	1	-1.945	0.052
	Pre Spray	Ref				

Appendix 3. Model table for *Anopheles arabiensis* collected in light traps. Conditional estimates for the effect of pre- versus post-spray conditional on IRS status are also included.

Parameter	Level	Risk Ratio	Lower CL	Upper CL	t-value	P-value
Intercept	Interceptor	0.03	0.01	0.07	-7.351	<0.001
Period	Post Spray	3.06	1.59	5.92	3.335	0.001
	Pre Spray	Ref				
Status	Intervention	1.79	0.55	5.76	0.972	0.331
	Control	Ref				
Period*Status	PostSpray*Intervention	0.45	0.2	1.01	-1.942	0.052
	PostSpray*Control	Ref				
	PreSpray*Intervention	Ref				
	PreSpray*Control	Ref				
Net Use	All under net	1.95	0.99	3.84	1.94	0.052
	Some under net	2.17	1.02	4.62	2.008	0.045
	None under net	Ref				
Eaves	Closed	0.57	0.33	0.96	-2.131	0.033
	Partially open	0.78	0.43	1.42	-0.814	0.416
	Open	Ref				
Cattle	Yes	1.33	0.89	1.98	1.383	0.167
	No	Ref				
Period (Conditional on IRS)	Post Spray	1.39	0.78	2.47	1.112	0.266
	Pre Spray	Ref				
Period (Conditional on non-IRS)	Post Spray	3.06	1.59	5.92	3.335	10
	Pre Spray	Ref				

Appendix 4. Model table for *Anopheles arabiensis* collected by pyrethrum spray catches. Conditional estimates for the effect of pre- versus post-spray conditional on IRS status are also included.

Parameter	Level	Risk Ratio	Lower CL	Upper CL	t-value	P-value
Intercept	Interceptor	0.12	0.04	0.38	-3.954	0.001
Period	Post Spray	1.64	0.87	3.09	1.543	0.123
	Pre Spray	Ref				
Status	Intervention	1.16	0.3	4.44	0.214	0.831
	Control	Ref				
Period*Status	PostSpray*Intervention	0.36	0.16	0.82	-2.445	0.015
	PostSpray*Control	Ref				
	PreSpray*Intervention	Ref				
	PreSpray*Control	Ref				
Net Use	All under net	1.61	0.9	2.87	1.594	0.111
	Some under net	1.85	0.89	3.84	1.652	0.099
	None under net	Ref				
Eaves	Closed	0.34	0.18	0.67	-3.165	0.002
	Partially open	0.60	0.32	1.13	-1.583	0.114
	Open	Ref				
Cattle	Yes	1.53	1.00	2.34	1.944	0.052
	No	Ref				
Period (Conditional on IRS)	Post Spray	0.60	0.33	1.09	-1.681	0.093
	Pre Spray	Ref				
Period (Conditional on non-IRS)	Post Spray	1.64	0.87	3.09	1.543	0.123
	Pre Spray	Ref				

5.5 Appendix 5: Proportion of light trap batteries at different charge status at the beginning and end of charging each session