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PMI VECTORLINK KENYA

ANNUAL ENTOMOLOGICAL MONITORING REPORT

OCTOBER 2017- SEPTEMBER 2018

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VECTORLINK KENYA ANNUAL ENTOMOLOGICAL MONITORING REPORT

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
Ace -1	Acetylcholinesterase 1 gene
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
EGC	Electrocuting Grid
ELISA	Enzyme-linked Immunosorbent Assay
FTT	Furvela Tent Trap
HDT	Host Decoy Trap
HBR	Human Biting Rate
HLC	Human Landing Catch
IRS	Indoor Residual Spray
KD	Knock Down
kdr	knockdown resistance gene
LLINs	Long Lasting Insecticidal Nets
NMCP	National Malaria Control Program
OLT	Outdoor CDC Light Trap
ILT	Indoor CDC Light Trap
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
VL	VectorLink
WHO	World Health Organization
ODK	Open Data Kit
GLMM	Generalized Linear Mixed Models

EXECUTIVE SUMMARY

In 2018, Actellic 300CS was sprayed in Migori County for the second consecutive year and for the first time in Homa Bay County. To monitor the impact of spraying on entomological indicators mosquitoes were collected in four sites within each sprayed county using pyrethrum spray catches (PSC), CDC light traps and window exit traps. Four sites were also monitored monthly in neighboring unsprayed Kisumu County. Insecticide resistance testing and cone bioassays to determine the residual life of Actellic 300CS were also conducted.

Insecticide resistance testing of *Anopheles arabiensis* from Migori, Homa Bay, Kisumu, Siaya and Bungoma counties showed full susceptibility to pirimiphos-methyl and resistance to both deltamethrin and permethrin. Pre-exposure to piperonyl butoxide (PBO) treated papers restored full susceptibility to deltamethrin and improved mortality rates to permethrin (but did not fully restore susceptibility). Full susceptibility to clothianidin was recorded within four days post-exposure to mosquitoes collected from Nyatike and Rongo sub-counties in Migori County and Bumula sub-county in Bungoma County. Cone bioassays using susceptible *An. gambiae* s.s. in houses sprayed with Actellic 300 CS showed at least seven months residual efficacy. Testing is ongoing.

Densities of *An. funestus* s.l. in Migori County were extremely low before the 2nd year of spraying, indicating year-round suppression by IRS conducted with Actellic 300CS in 2017. This was reinforced with the 2nd spray round in 2018, as densities remained low. In Homa Bay County, which received the first round of IRS in February 2018, the pre-spray period consisted primarily of *An. funestus* (83%), while *An. gambiae* s.l. was the most commonly collected anopheline species (76%) after IRS.

The indoor host seeking and resting densities of both *An. funestus* and *An. gambiae* s.l. were significantly lower in the IRS sites in Migori and Homa Bay counties compared to unsprayed sites in Kisumu County. In Kisumu County, *An. funestus* indoor biting rates were particularly high between June and August 2018, reaching a peak mean of >10 per light trap in August. The high densities of *An. funestus* in Kisumu County and successful control of this species in Migori and Homa Bay through IRS mean that potential future expansion to cover Kisumu County is likely to be highly effective.

Sporozoite rates in *An. funestus* were 3.8% (14/373) in Homa Bay County before IRS. Following IRS, few *An. funestus* were collected in sprayed sites and no sporozoites were detected. However, in Kisumu County, *An. funestus* was the primary vector species throughout the 12 month monitoring period, with a sporozoite rate of 2.3% (24/1,024). *An. arabiensis* biting rates were particularly high in Kisumu County between April and June, with a sporozoite rate of 0.9% (11/1,159).

An. gambiae s.l., which was identified as mostly *An. arabiensis* following molecular analysis, was the predominant vector species in Migori County before and after second round of IRS and in Homa Bay County after IRS. Only *An. arabiensis* was found with sporozoites after IRS with a mean of 1.3% (2/150) in Migori County and 0.6% (2/356) in Homa Bay County. This may be an indication that the species is the main driver of malaria transmission after IRS (albeit at a reduced rate).

A comparison of outdoor trapping methods indicated that outdoor CDC light trap (human baited) and Furvela Tent Trap showed the most potential for effectively sampling outdoor biting mosquitoes during routine surveillance. This will be important to gather more information on outdoor transmission.

Overall, IRS in Migori and Homa Bay counties resulted in reduced malaria vector densities and sporozoite rates compared with unsprayed Kisumu County. The greatest impact was on *An. funestus* populations which reached negligible densities having previously been the primary vector in these areas. To further drive reductions in malaria transmission in these counties, it will be necessary to expand IRS or other effective indoor control tools such as PBO LLINs to cover more counties in western Kenya. Additional control methods targeting zoophilic and exophilic *An. arabiensis*, which appear to sustain malaria transmission at a reduced rate in IRS sites, in combination with indoor vector control may also be needed.

INTRODUCTION

Malaria vector control chiefly depends on the use of long-lasting insecticidal nets (LLINs) and the application of indoor residual spraying (IRS). In Kenya, LLINs are mainly distributed through mass net campaigns and through routine distribution at antenatal clinics. In the eight counties of the lake endemic zone (Bungoma, Busia, Homa Bay, Kakamega, Kisumu, Migori, Siaya, and Vihiga), 54% of households were reported to have universal coverage with LLINs defined as 1 net per 2 people while 87% of households had at least one LLIN and net usage was at 67% in the general population (1). Strategic objective one of the Kenya Malaria Strategy (2009-2018) is "to have at least 80% of people living in malaria risk areas using appropriate malaria preventive interventions by 2018" (2). Application of IRS for malaria control in Kenya has not been as widespread as net distribution. IRS was recently reintroduced in parts of western Kenya, with two rounds (2017 and 2018) of spraying with Actellic 300 CS (pirimiphos-methyl) in Migori County and one round in Homa Bay County (2018). The combination of IRS and use of LLINs is expected to provide greater protection to the populations at risk within the Lake Victoria malaria endemic regions of western Kenya. The Lake endemic region of western Kenya has been identified as the highest burden area for malaria transmission nationally (3). Therefore, application of IRS with an effective insecticide is likely to cause rapid reduction in vector densities and malaria transmission.

Application of insecticide-based vector control methods are threatened by the rise and development of insecticide resistance in mosquito populations. While it is critical to use other classes of insecticide in IRS to preserve pyrethroids for LLINs, repeated application of the same insecticides should be avoided to prevent or delay the rise in insecticide resistance. The Kenya National Resistance Management Plan recommends rotation of insecticides with different modes of actions every two years (4). With repeat IRS in Migori County (2017 and 2018), it is critical to monitor susceptibility levels of the local vector populations to pirimiphos-methyl and clothianidin as well as other insecticides used in vector control, to assess their efficacy against the local vector population. As pyrethroid LLINs are still the primary vector control strategy in most counties of western Kenya, it is critically important to monitor pyrethroid resistance levels and intensity.

In western Kenya, *An. funestus*, *An. gambiae s.s.* and *An. arabiensis* are the main malaria vectors (6). Both *An. funestus* and *An. gambiae s.s.* have been previously reported to feed more readily on humans and rest indoors while *An. arabiensis* is reported to feed more frequently on cattle and rest outdoors (7-9). However, with increased presence and use of insecticide treated nets (ITNs) over the last 15 years, remarkable changes in local vector bionomics have been reported by different studies in the region. *An. funestus* were initially reduced to near elimination with the introduction of ITNs in the Asembo Bay area of western Kenya (10), but later, following development of pyrethroid resistance, re-emerged to be the predominant species (11). *An. gambiae* s.s. populations in the same region were reduced following widespread distribution of ITNs, with a concurrent relative increase in the proportion of *An. arabiensis (7)*. Elsewhere in the western Kenya highlands, increased frequency of *An. gambiae s.s.* feeding on cattle (12) and a shift in biting times of *Anopheles funestus* and *An. gambiae* s.l. populations due to increased ITN usage have been reported (13).

Following the first application of IRS with pirimiphos-methyl in Migori County in 2017, a remarkable reductions in populations of *An. funestus* were observed, but the impact on *An. arabiensis* was limited. Application of IRS and use of LLINs have been reported to have a lesser impact on *An. arabiensis* as the vector is reported to rapidly exit houses after entry (5). Therefore, with the observed persistence of *An. arabiensis* after application of IRS, it is important to monitor this species and consider alternative control strategies for the species which may be responsible for residual malaria transmission.

The current report covers entomological surveillance in Migori County where two rounds of IRS have been conducted, Homa Bay County, where IRS was conducted for the first time in 2018, and Kisumu County, which has remained unsprayed. Additional data is presented on the evaluation of outdoor mosquito trapping methods in Kisumu County, and insecticide resistance testing in counties where IRS was conducted plus unsprayed counties of Siaya and Bungoma. Collections in Migori County and two sub-counties in Homa Bay County occurred between October 2017 to September 2018 while collections at additional sites in Homa Bay and Kisumu counties started in December 2017. Objectives were to monitor malaria vector densities and behaviour in Migori and Homa Bay Counties before and after IRS, in comparison to control sites; to determine levels and mechanisms of insecticide resistance of local malaria vector populations; to determine decay rates of insecticide on the walls following IRS; and to evaluate outdoor mosquito trapping methods. VectorLink Kenya monitored mosquito vector indoor resting densities, exit rates, and biting location, time, and rates. Insecticide resistance monitoring was also conducted to inform decision making for IRS and LLINs while outdoor trapping methods were evaluated to guide the selection of a suitable trapping method for routine monitoring of outdoor mosquito populations. Results from this monitoring are intended to guide decision making by the NMCP and other development partners in the fight against malaria.

I. METHODOLOGY

1.1 SURVEILLANCE SITES

Routine entomological monitoring to evaluate the impact of IRS on vector mosquitoes was conducted in 12 sites, eight in sprayed areas and four in unsprayed areas (Table 1). In Migori County, monitoring was conducted in Rongo, Uriri, Suna West and Nyatike sub-counties, all of which received a second round of IRS with pirimiphos-methyl in February-March 2018. In Homa Bay County, monitoring was conducted in Homa Bay, Ndhiwa, Rachuonyo North and Rachuonyo South sub-counties, which received a first round of IRS in February-March 2018. The four control sites were Seme, Nyando, Muhoroni and Nyakach sub-counties in Kisumu County. All the sites are in the malaria hyperendemic region of western Kenya. For the current reporting period, sampling in existing sites began in October 2017 and collections in the two new sites in Homa Bay County and four new sites in Kisumu County started in December 2017. Insecticide resistance monitoring was conducted in eight sites in Migori, Homa Bay, Kisumu, Siaya, and Bungoma Counties (Table 1).

TABLE 1: LIST OF SURVEILLACE SITES WITH DETAILS OF ENTOMOLOGICAL DATA COLLECTED AT EACH SITE.

County	Sub-county	Sentinel Site Location	Intervention Status (existing or new site)	Data Collected at All Sites (Monthly)	Data Collected at Some Sites (Non-Monthly)
Migori	Rongo	Sumba	IRS (existing)	Monthly vector biting rates, resting densities, species, composition, sporozoite rates.	Insecticide resistance data, human landing catch during long rains (June) and monthly cone bioassay in Rongo and Nyatike sub-counties.
	Uriri	Ngiya	IRS (existing)		
	Nyatike	Sori-Karungu	IRS (existing)		
	Suna West	God Kwer	IRS (existing)		
Homa Bay	Homa Bay	Imbo	IRS (existing)	Monthly vector biting rates, resting densities, species composition, sporozoite rates.	Insecticide resistance data, human landing catch during long rains (June) and monthly cone bioassay in Homa Bay and Rachuonyo North sub-counties.
	Rachuonyo North	Kogweno Oriang'	IRS (new)		
	Ndhiwa	Ndhiwa	IRS (existing)		
	Rachuonyo South	Bonde	IRS (new)		
Kisumu	Nyando	Ahero	Control (new)	Monthly vector biting rates, resting densities, species, composition, sporozoite rates.	Insecticide resistance in Nyakach and Mhoroni sub-couties. Human landing catch during long rains (June) in Nyando and Nyakach subcounties. Outdoor trapping study in May in Masogo and Nyando sub-counties
	Seme	Kirindo	Control (new)		
	Nyakach	Sango Rota	Control (new)		
	Muhoroni	Masogo	Control (new)		
Siaya	Bondo	Bar Kanyango	LLINs	None	Insecticide resistance monitoring
Bungoma	Sirisia	Bitobo	LLINs	None	Insecticide resistance monitoring

1.2 Vector Density Surveillance

Pyrethrum spray collections (PSCs), indoor light traps, and window exit traps were used to monitor mosquito densities monthly. Each month, from October 2017 to September 2018, 18 houses were sampled in each site, ten by Centers for Disease Control and Prevention (CDC) light traps (CDC-LT), eight by PSC, and five by exit traps (paired with five PSC houses). Different houses were sampled every month. Prior to mosquito collection, a short questionnaire was administered to determine the number of people who slept in the house the previous night, whether the house was sprayed, whether nets were present in the house and some characteristics of the house including wall, roof and floor type and presence of open or closed eaves.

1.3 Pyrethrum Spray Collections

To monitor the numbers of indoor resting mosquitoes, 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 amplifiable 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 dead mosquitoes were collected from the sheets and transferred to the laboratory in a scintillation vial containing 70% ethanol.

1.4 CDC Light Trap

CDC light traps were used to monitor densities of host seeking mosquitoes inside houses. A single 12V CDC light trap was hung in each house in the sleeping area, approximately 1.5 m from the ground, adjacent to an occupied bed net. The traps were run from 06:00 pm and mosquitoes were collected at 07:00 am the next morning. Trapped mosquitoes were transferred into paper cups and transported to the laboratory for further analysis.

1.5 Window Exit Traps

Window exit traps (WET) were used to monitor proportions of mosquitoes that exit houses before morning. They were installed in the same houses in the evening before PSC were conducted the following morning. In each house sampled, a single exit trap was installed on a window in the sleeping area. The window trap was fitted in the window space with funnel shaped entry point facing the house and the trap was supported by an adjustable metallic stand from below. The trap was surveyed early the following morning. Trapped mosquitoes were collected using aspirators and were then placed into paper cups. The samples were taken to the laboratory for further analysis.

1.6 Mosquito Behaviour

Human landing catches (HLCs) were conducted to monitor mosquito biting behaviour in two sites in each of the 3 Counties (Migori, Homa Bay and Kisumu). In each site, HLCs were conducted in the same five houses each night for five consecutive nights. One volunteer sat outside and another inside a house with their trousers folded to knee length. They aspirated any mosquitoes landing on their exposed legs. Each house had a team of six collectors, with 2 collectors working six-hour shifts running from 5 p.m. to 11 a.m. the next morning. 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. The individual performing HLC provided written consent to participate in the study. They were tested for malaria infection seven days before collections began, and those that tested positive were treated. The collectors were placed on weekly malaria prophylaxis beginning seven days before collections began and continuing up to four weeks after the end of collections. Over the same period, the collectors were monitored for malaria infection. None were found to be malaria positive during and up to four weeks after HLCs were conducted. HLC is a very time-consuming exercise and requires prior malaria testing and initiation of HLC collectors who then need to be provided malaria prophylaxis one week before collections begin and need to be monitored for any malaria infection up

to four weeks after collection ceases. Therefore, it was not logistically feasible to perform the collections routinely. A one-off collection effort was considered sufficient to provide information on vector behaviour (biting times and location) while monthly density monitoring was performed by PSC and light trap.

1.7 OUTDOOR TRAPPING

Trapping of outdoor biting malaria vectors was conducted in Kakola Ombaka village, near Ahero rice irrigation schemes in Nyando Sub County, and Masogo village in Muhoroni Sub-County. Both sites are in Kisumu County, western Kenya. The primary objective was to determine which outdoor trapping method was most suitable for future monthly entomological monitoring (as currently only indoor trapping is conducted monthly). As HLC is considered the gold standard, the outdoor trap catching similar total numbers and species composition will be chosen for future use. Ease-of- use of the trap will be considered in selection. In each village, 5 houses, approximately 100m from each other were used per night with each trap being rotated nightly in a Williams Latin Square design (Table 2). Five collection methods were compared; HLCs, Outdoor CDC Light Traps (OLT), Furvela Tent Traps (FTT), Electrocuting Grids (EGC), and Host Decoy Traps (HDT) were used outdoors, with one collection method per compound per night. All outdoor collection methods were paired with a house that had an indoor CDC light trap installed in the bedroom next to an occupied bed net, which was used to calculate endophily rates by species. Outdoor collection methods were performed between 8-22m away from the house with indoor CDC-LT, with outdoor traps set up on flat, grassy ground. The distance varied by house as we avoided vegetation, cattle sheds, washing lines and muddy puddles surrounding the house. Each collection method requires a human volunteer. Therefore, both the volunteers and the collection methods were rotated between locations so that each volunteer and collection method appeared once at each location in each round (Table 2). To ensure complete rotation of volunteers and collection methods, each volunteer collected at one location for five consecutive nights before being moved to the next while the collection methods were rotated daily between locations in a similar fashion each week. The study was conducted for 25 nights, covering 5 weeks, with weekend breaks. Note that the HLC conducted in the mosquito behavior study was conducted as a separate exercise to the HLC in the outdoor trapping study and there was no overlap.

TABLE 2: A SAMPLE OF NON-RANDOM LATIN SQUARE ROTATION OF COLLECTION METHODS AND SLEEPERS.

	House 1	*House 2*	*House 3*	*House 4*	*House 5*
Night1	**HLC Sleeper A**	**CDC-LT Sleeper B**	**FTT Sleeper C**	**EGT Sleeper D**	**HDT Sleeper E**
Night2	**FTT Sleeper A**	**HDT Sleeper B**	**HLC Sleeper C**	**CDC-LT Sleeper D**	**EGT Sleeper E**
Night3	**HDT Sleeper A**	**FTT Sleeper B**	**EGT Sleeper C**	**HLC Sleeper D**	**CDC-LT Sleeper E**
Night4	**CDC-LT Sleeper A**	**EGT Sleeper B**	**HDT Sleeper C**	**FTT Sleeper D**	**HLC Sleeper E**
Night5	**EGT Sleeper A**	**HLC Sleeper B**	**CDC-LT Sleeper C**	**HDT Sleeper D**	**FTT Sleeper E**

HLC = Human Landing Catch, CDC-LT = CDC Light Trap, FTT = Furvela Tent Trap, EGT = Electrocuting Grid Trap, HDT = Human Decoy Trap

1.7.1 DESCRIPTION OF COLLECTION METHODS

The location for each outdoor collection method was marked in each compound to ensure consistency throughout the study. Outdoor collections were made approximately 8-22m away from the house in a cleared space. The distance to any animal enclosures was also documented. Outdoor collections were performed from 18:00hrs to 07:00hrs the following morning.

1.7.1.1 FURVELA TENT TRAP

The basic principle of the Furvela tent trap is that host odor and exhaled gases emanating from a gap, the diameter of a CDC trap, in the predominantly closed door of the tent, attract mosquitoes to the gap on the tent-door. Close to the gap, a CDC trap (without the light, lid or grid) is placed horizontally outside the tent, 2 to 3 cm from the opening in the door. On approach to the opening, the insects are sucked into the trap and held in the standard CDC trap conical collection bag (Figure 1). The suction from the fan effectively prevents any mosquitoes from entering the tent, even at very high densities, so that the sleeper is only exposed if the door is left open.

FIGURE 1: FURVELA TENT TRAP, SHOWING OPENING WITH CDC LIGHT TRAP (WITHOUT THE LIGHT) ATTACHED TO A SMALL OPENING.

1.7.1.2 HOST DECOY TRAP

The Host Decoy Trap exploits the blood-seeking behavior of mosquitoes by mimicking the sensory stimuli that a mosquito follows when searching for a person to bite. These include a visual stimulus, host odor and body temperature of warm-blooded hosts. These stimuli are incorporated into a trap that lures mosquitoes towards it and then captures them when they land. The trap was set as previously described (14, 15). Briefly, the host decoy trap is a cylindrical container filled with warm water, insulated with Styrofoam to prevent heat loss and regulate the surface temperature. The container is covered with a black jacket to provide visual contrast and a transparent sticky tape to which mosquitoes get stuck on landing. Host odor from a nearby occupied tent is exhausted using a fan, pushed through a pipe and vented close to the trap (Figure 2). Mosquitoes attracted to an odor source are induced to land upon the visually conspicuous, warm trap, where upon they get stuck. The stuck mosquitoes are recovered from the trap by dissolving the glue upon which they are stuck.

FIGURE 2: HOST DECOY TRAP, SHOWING HUMAN SLEEPING INSIDE TENT WITH TUBE TAKING HUMAN ODOR TO THE HEATING CYLINDER TRAP.

1.7.1.3 ELECTROCUTING GRIDS

These devices were originally developed to quantify the numbers of tsetse flies attracted to humans and wildlife hosts by placing electrocuting nets in an incomplete ring around the host species. The electrocuting grid is effectively invisible to tsetse and hence as they approach the host, tsetse inadvertently collide with it and are either killed or stunned, with the number caught outside and inside indicating their abundance and feeding success. Electrocuting grids (0.5 m high; 1 m wide) consist of vertical copper wires, 0.2 mm in diameter, 5 mm apart, 8 mm from each side of the net and spray painted black. The wires are intended to be invisible to nocturnal flying insects. Alternate wires were earthed or charged by a transformer with a direct current (DC) input (12 V; 3 A) and an output of 50 kV, pulsing at ~70 Hz. Insects killed or stunned after colliding with the grids were collected on a sticky panel placed under the electrocuting grid. A simple shelter was erected over a human volunteer to protect them from the rain. The human volunteer sat on a stool and four panels of electrocuting grid were arranged around the lower limbs of the human volunteer up to the knee level. The rest of the body was covered with untreated bed net attached to the top frame of the electrocuting grid. Mosquitoes attempting to access the volunteer through there were electrocuted and dropped on the sticky panel under the grind from where they were collected.

1.7.1.4 CDC-LIGHT TRAP (OUTDOORS)

CDC miniature light traps (CDC-LT) are commonly used indoors when hung next to a human host that is protected under a bed net. Several studies have demonstrated close correlation between the numbers of *Anopheles* mosquitoes caught by CDC-LT indoors compared to HLC. However, the evaluation of outdoor CDC-LT has been limited. The CDC-LT was hung outdoors at 1.5m above the ground next to an occupied, untreated bed net.

FIGURE 3: OUTDOOR CDC LIGHT TRAP, SHOWING HUMAN SLEEPING INSIDE AN UNTREATED BED NET, PROTECTED FROM RAIN BY A TARPAULIN.

1.7.1.5 HUMAN LANDING CATCH

The human landing catch (HLC) was used as the positive control 'gold standard' method for outdoor collections of human biting mosquitoes. A volunteer sat outside with their trousers folded to knee length and aspirated any mosquitoes landing on them. The individual performing HLC gave their consent and was tested for malaria and, if infected, was treated before collections started. The collectors were placed on weekly malaria prophylaxis beginning seven days before collections began and continuing up to four weeks after the end of collections. Over the same period, the collectors were monitored for malaria infection. No cases of infection were detected during and up to four weeks after the collection period.

1.8 INSECTICIDE RESISTANCE MONITORING (WHO TUBE TESTS)

Mosquito collections for insecticide resistance monitoring were performed between January and July 2018. Larval stages of *An. gambiae* s.l. were collected from Rongo and Nyatike in Migori County, Rachuonyo North and Homa Bay in Homa Bay County, Nyakach and Muhoroni in Kisumu County, Bondo in Siaya County and Bumula in Bungoma County. Collections were performed using larval dippers and sieves. *Anopheles* larvae were separated from the other aquatic organisms and sorted into different larval instars. The larval samples were maintained in a room with a portable space heater while in the field and were fed fish meal. Pupae developing from the larvae were collected daily and placed in pupal cups. The pupal cups were then introduced into paper cups labelled with the collection site and with a wet cotton wool soaked in 10% sugar solution. The emerging adults were trapped in the paper cups and sustained on the provided sugar pad. The emergent adults were marked with the date of emergence and raised to 2-5 days old adults for insecticide resistance tests.

Insecticide resistance status was assessed using the WHO test-tube bioassay using diagnostic concentrations of deltamethrin (0.05%), permethrin (0.75%), pirimiphos-methyl (0.25%), and clothianidin (2%). All papers (except for clothianidin) were prepared by the WHO collaborating center, University Sains Malaysia. The clothianidin dosage was determined based on internal testing conducted by Sumitomo which showed 2% w/v clothianidin to be a suitable diagnostic concentration for each treated filter paper. Clothianidin tests were conducted using filter papers prepared by VectorLink staff. Whatman® No.1 filter papers were treated with the diagnostic dose of clothianidin according to PMI African Indoor Residual Spraying (AIRS) project standard operating procedure 001. To prepare treated papers, 264mg SumiShield 50WG was dissolved in 20ml distilled water. A pipette was used to dispense 2ml of solution on each 12 by 15cm filter paper, resulting in a concentration of 13.2mg active ingredient clothianidin per paper. After 60 minutes exposure to clothianidin treated papers mosquitoes were transferred to a holding tube and mortality was monitored up to 7 days post exposure. Treated papers were tested within 24h of preparation.

All WHO bioassays were conducted with 2- to 5-day-old *An. gambiae* s.l. reared from collected larvae. At least 100 mosquitoes were exposed to each insecticide at a time in 4 replicates of 25 mosquitoes each. Knock-down was monitored every 10 minutes for 60 minutes. The samples were then transferred to a holding tube with cotton wool soaked in sugar solution and held for 24 hours. Mortality was scored 24 hours after exposure. Synergist assays were conducted by pre-exposing mosquitoes to WHO papers treated with piperonyl butoxide (4%) for one hour prior to exposure to pyrethroid treated paper for 60 minutes.

1.9 QUALITY OF SPRAY AND DECAY RATE OF INSECTICIDE ON THE WALL

Wall bioassays were conducted within two weeks of IRS and monthly thereafter using susceptible *An. gambiae* s.s. Kisumu strain colony mosquitoes. Ten houses were randomly selected in four clusters within the spray area (two in Migori County, two in Homa Bay County). Three cones were attached on the sprayed walls at different heights of 0.5m, 1m and 1.5m from the floor, each on different sides of the wall. Ten 2-5 days old susceptible *An. gambiae* s.s. Kisumu strain were introduced into each cone and exposed to the treated wall for 30 minutes. At the end of 30 minutes exposure, the samples were removed gently from the cone and placed into an appropriately labelled paper cup. The mosquitoes were given 10% sugar solution and then monitored for knock down after 30 minutes and 60 minutes and for mortality at 24-hours post exposure. A parallel control exposure was run on unsprayed surface (block board) close to each sprayed house. Relative humidity and temperature were recorded during the exposure and holding periods.

1.10 FUMIGANT EFFECT

To check the air-borne fumigant effect of the insecticide, a small cage 20 cm x 20 cm x 20 cm containing 10 insectary-reared *An. gambiae* was placed 1m away from the sprayed wall (i.e. mosquitoes did not contact the

sprayed walls). The mosquitoes were exposed for 30 minutes then transferred into paper cups and taken to an untreated holding room. Mortality was recorded 24 hours post exposure.

1.11 MOLECULAR ANALYSIS

All *Anopheles* mosquitoes collected (using all methods) were identified morphologically to species (16, 17). The physiological status was determined by observation of the abdomen and female mosquitoes were classified as either unfed, blood-fed, half gravid or gravid. Female mosquitoes were divided into three parts for additional laboratory analyses: head and thorax were used for determination of sporozoite infection by enzyme linked immunosorbent assay (ELISA) techniques (18), the abdomens of blood-fed and half-gravid females were kept for blood-meal host determination, and the legs and wings were used in polymerase chain reaction (PCR) assays to identify members of the *An. gambiae* s.l. complex and the *Anopheles funestus* s.l. group (19), and preserved for future genetic/molecular analysis. All mosquitoes morphologically identified as *An. gambiae* s.l. were analyzed by PCR for species identification while a random selection of 20% of *An. funestus* s.l. collected per month across all sites were initially analyzed for species identification in each month.

1.12 DATA MANAGEMENT

Field data was collected on tablets using open data kit software (ODK). The data collection interface was designed with buttons, drop down menus and data quality checks to limit entry errors in the field. Each house sampled was allocated a unique code. Collection devices containing mosquitoes from each house were marked with these numbers and the numbers were used to track the samples through all the laboratory procedures. Individual mosquitoes were labelled with pre-printed barcodes and linked to the field data by house code and study number. Additional tests on individual mosquitoes, including sporozoite ELISA and species identification by PCR, were linked by the unique barcode label. Data entry screens used drop down menus and automatic data checks to reduce errors. For data sharing, all data was merged into a single file and checked to ensure a proper merge. Personal identifiers were removed in the shared files used in analysis.

Analysis to determine the impact of IRS was done using R statistical software version 3.4.1. Data was fitted using Generalized Linear Mixed Effects Statistical Models (GLMMs) to describe effects of different treatment and collection period on mosquito catches. We used the package glmmTMB, which fits linear and generalized linear mixed models with various extensions, including zero-inflation. We used the package 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, period and intervention status as fixed effects while house was treated as a random effect. A binomial GLM model was used to analyse *Anopheles* species proportions between sprayed and unsprayed sites, before and after IRS. The same statistical package was used in comparison of mean catches between outdoor collection methods. Numbers of female *Anopheles* were assessed as a function of collection method as a fixed factor while house and day of collection were treated as random factors. To obtain the rates ratios (RR) and confidence intervals, we exponentiated the model coefficients.

2. RESULTS

2.1 MALARIA VECTOR SPECIES COMPOSITION AND SEASONALITY

A total of 6,190 *Anopheles* mosquitoes were collected by all trapping methods combined. Of these, 2,431 (39%) were *An. funestus* s.l., 2,735 (44%) *An. gambiae* s.l., 988 (16%) *An. coustani* and 36 (0.6%) *An. pharoenesis* (Table 3.) A total of 2,044 *An. gambiae* s.l. were analysed by PCR for species identification: 1,827 (89%) were *An. arabiensis* and 217 (11%) *An. gambiae* s.s. A total of 1,261 *An. funestus* s.l. were analysed by PCR and were all confirmed to be *An. funestus* s.s. Seventy-five (75) mosquitoes were non-amplified.

An. arabiensis was the predominant species in collections before and after the second round of IRS in Migori County (Figure 4). Densities of *An. funestus* s.l. in Migori County were extremely low before the 2nd year of spraying, indicating year-round suppression by IRS with Actellic 300CS. This was reinforced with the 2nd spray round, as densities remained low (Table 3).

In Homa Bay County, that received the first round of IRS in 2018, *An. funestus* was the predominant species and was collected at high densities before IRS (Oct 2017 to Feb 2018) in all collection methods (Table 3). After IRS, *An. arabiensis* was the predominant species, accounting for 76% of all collected *Anopheles,* compared with 16% before IRS (Figure 4). In Homa Bay County, other *Anopheles* species, chiefly *An. coustani,* accounted for 18% while *An. funestus* was just 6% of all *Anopheles* species after IRS (Figure 4).

In the non-intervention sites in Kisumu County, *An. funestus* and *An. gambiae* s.l. were captured in comparable proportions during the two periods, October 2017 to February 2018 and March 2018 to September 2018 marked as pre-spray and post spray respectively (Figure 5). While species composition was similar during the two periods, *An. funestus* and *An. gambiae* s.l. densities were particularly high from March to September 2018 in all collection methods (Table 3). These densities were far higher than in the sprayed counties of Migori and Homa Bay during the same period.

TABLE 3: MEAN NUMBER OF *ANOPHELES* MOSQUITOES PER TRAP-NIGHT BY DIFFERENT COLLECTION METHODS, BEFORE (OCT 2017 – FEB 2018) AND AFTER (MAR – SEP 2018) IRS.

County	Collection method	*An. funestus*		*An. gambiae* s.l.		*An. coustani*		*An. pharoensis*	
		Oct 2017-Feb 2018	Mar-Sep 2018	Oct 2017-Feb 2018	Mar-Sep 2018	Oct 2017-Feb 2018	Mar-Sep 2018	Oct 2017-Feb 2018	Mar-Sep 2018
Migori	Light trap	0.01±0.01	0.05±0.02	0.13±0.03	0.81±0.14	0.01±0.04	0.41±0.08	0±00	0.01±0.01
	PSC	0.04±0.02	0.01±0.01	0.16±0.07	0.08±0.02	0±00	0±0.00	0±00	0±0.00
	Window Exit Trap	0.01±0.01	0.01±0.01	0.05±0.04	0.05±0.03	0±00	0.02±0.02	0±00	0.01±0.01
Homa Bay	Light trap	1.78±0.33	0.14±0.04	0.2±0.05	1.66±0.25	0.01±0.01	0.46±0.14	0±00	0.03±0.02
	PSC	1.21±0.34	0.05±0.02	0.43±0.15	0.63±0.22	0±00	0.01±0.01	0±00	0±0.00
	Window Exit Trap	0.27±0.09	0.01±0.01	0.07±0.04	0.29±0.10	0±00	0.1±0.06	0±00	0±0.00
Kisumu	Light trap	0.66±0.45	3.41±0.54	0.34±0.08	4.65 ±0.58	0.3±0.17	2.46 ±0.82	0±00	0.08 ±0.03
	PSC	0.18±0.07	0.75 ±0.13	0.34±0.15	1.49 ±0.25	0±00	0.02 ±0.01	0±00	0±0.00
	Window Exit Trap	-	6.58±1.52	-	1.36±0.32	0±00	0.2±0.07	0±00	0.03±0.02

FIGURE 4: SPECIES COMPOSITION FOR MIGORI AND HOMA BAY COUNTIES PRE-IRS (OCTOBER 2017 TO FEBRUARY 2018) AND POST-IRS (MARCH TO SEPTEMBER 2018) COLLECTED BY CDC LT INDOORS, PSC AND WET.

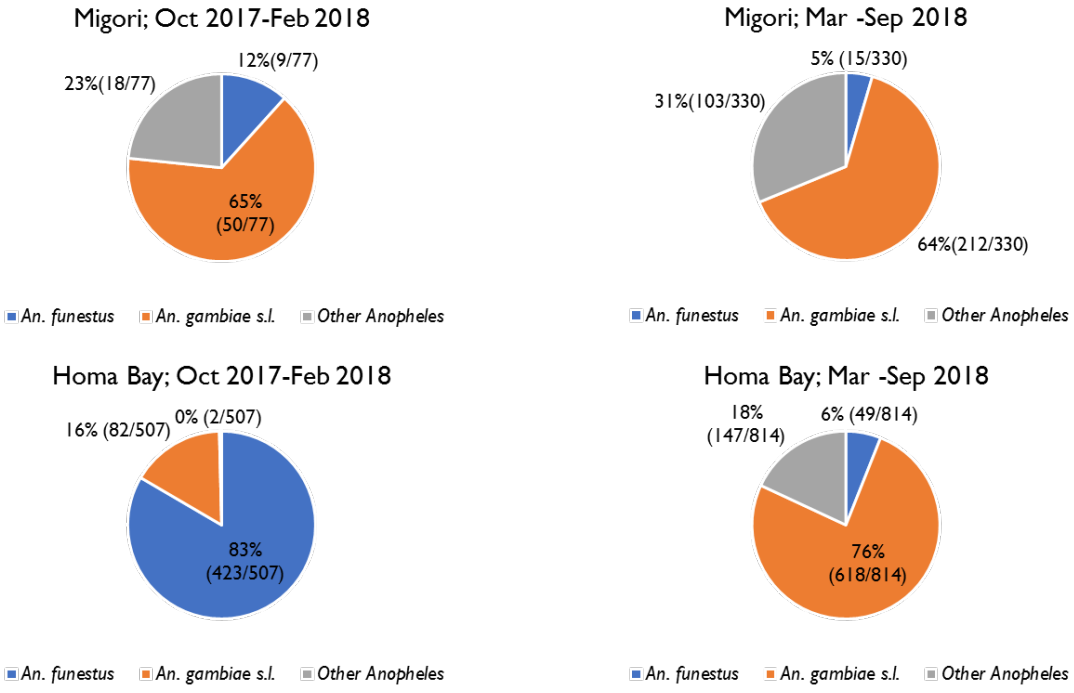


FIGURE 5: SPECIES COMPOSITION FOR ALL NON-IRS SITES IN KISUMU COUNTY (OCTOBER 2017 TO FEBRUARY 2018 AND MARCH TO APRIL 2018) COLLECTED BY CDC-LT INDOORS, PSC AND WET.

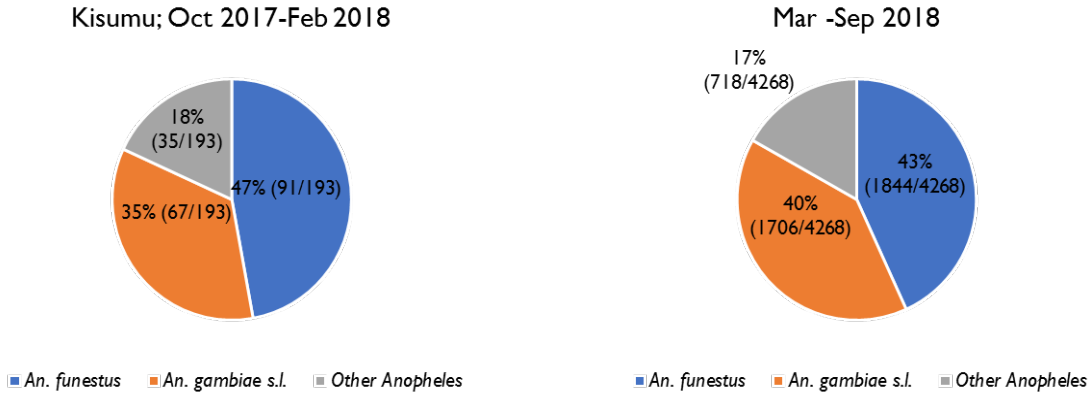


Figure 6 shows the monthly mean density per trap for *An. funestus* and *An. gambiae* s.l. in three counties, presented by collection method. The density of indoor host seeking and resting *An. funestus* in Migori County was particularly low throughout the collection period, before and after the second round of spraying.

The numbers of *An. funestus* (indoor biting and resting) were highest in Homa Bay County before IRS but declined substantially after spraying. Significantly lower densities of indoor host seeking *An. funestus* collected by light traps were observed in both Migori and Homa Bay counties following IRS as compared to unsprayed Kisumu County during the same period. In Kisumu County, *An. funestus* indoor biting rates were particularly high between June and August 2018, reaching a peak mean of >10 per trap in August.

The density of *An. gambiae* s.l. (mostly *An. arabiensis* according to PCR results) increased in CDC light trap collections after IRS in all sites. This trend is unexpected after IRS and may indicate that IRS had a greater impact on *An. funestus* than *An. arabiensis* populations. The peak in *An. gambiae* s.l. densities by CDC-LT was in May 2018 in all three counties. The densities of *An. gambiae* s.l. were also significantly lower in both IRS sites compared to the non-IRS sites. Similar reductions in *Anopheles* densities were observed in both PSC and window exit trap collections for both *An. funestus* and *An. gambiae* s.l., with significant reduction in densities of both species in the IRS sites compared to non-IRS sites (Table 4).

In general, mean PSC catch size was lower than by CDC light trap for both *An. gambiae* s.l. and *An. funestus*, a possible indication of mosquitoes exiting houses before dawn. However, the relatively low densities collected by window exit trap (Table 4) occurred most likely due to mosquitoes exiting through other routes, such as through eave spaces. The mean catch size of *An. gambiae* s.l. by PSC remained low in Migori but increased in Homa Bay after IRS (during the rainy season) and in the control site in Kisumu during the same period (Figure 6).

FIGURE 6: MONTHLY MEAN NUMBER OF INDOOR HOST SEEKING AND RESTING *ANOPHELES* MOSQUITOES PRE- AND POST-IRS IN MIGORI (2ND IRS), HOMA BAY (1ST IRS) AND KISUMU (NON-IRS) COUNTIES, PSC MEANS ARE ON A DIFFERENT SCALE FROM CDC LIGHT TRAP.

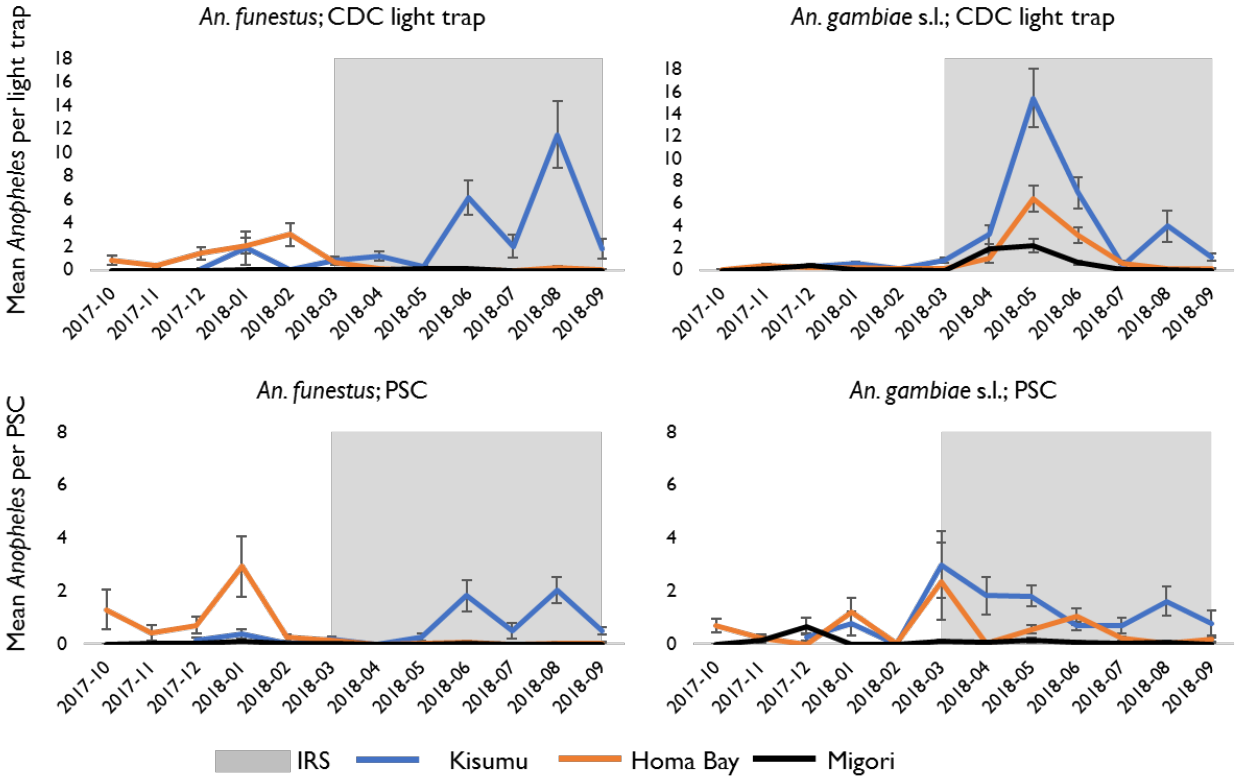


TABLE 4: COMPARISON OF POST-SPRAY MEANS OF AN. FUNESTUS AND AN. GAMBIAE S.L. IN CDC LIGHT TRAP, PYRETHRUM SPRAY COLLECTION AND WINDOW EXIT TRAP BETWEEN MIGORI AND HOMA BAY COUNTIES (IRS SITES) AND KISUMU COUNTY (NO-IRS).

Collection method	*Anopheles species*	*Category*	*Mean*	*RR (95% CI)*
CDC Light Trap	**An. funestus**	**Migori**	0.05	0.01 (0.00 - 0.03) ***
		Homa Bay	0.14	0.03 (0.01 -0.06) ***
		Kisumu	3.41	1
	An. gambiae s.l.	**Migori**	0.81	0.17 (0.00 - 0.26) ***
		Homa Bay	1.66	0.36 (0.24 - 0.53) ***
		Kisumu	4.65	1
PSC	**An. funestus**	**Migori**	0.01	0.02 (0.00 - 0.07) ***
		Homa Bay	0.05	0.06 (0.023 - 0.15) ***
		Kisumu	0.75	1
	An. gambiae s.l.	**Migori**	0.08	0.07 (0.02 - 0.13) ***
		Homa Bay	0.63	0.24 (0.13 - 0.44) ***
		Kisumu	1.49	1
Window Exit Trap	**An. funestus**	**Migori**	0.01	0.00 (0.00 -0.08) ***
		Homa Bay	0.01	0.00 (0.00 - 0.07) ***
		Kisumu	6.58	1
	An. gambiae s.l.	**Migori**	0.05	0.03 (0.01 - 0.11) ***
		Homa Bay	0.29	0.18 (0.07 - 0.43) ***
		Kisumu	1.36	1

(Significance codes: '***' <.001)

2.2 MALARIA VECTOR SPOROZOITE RATES

In Migori County, no sporozoite-infected *An. funestus* were detected either before (October 2017 – February 2018) or after (March –September 2018) the second round of IRS. However, a sporozoite rate of 1.3% (2/150) was observed in *An. arabiensis* post-IRS in the same county. In Homa Bay County, a sporozoite rate of 3.8% (14/373) among *An. funestus* was observed before spraying, while no sporozoite-infected *An. funestus* were detected post-IRS. Sporozoites were detected in *An. arabiensis* after IRS in Homa Bay with a sporozoite rate of 0.6% (2/356). In the control sites in Kisumu County, sporozoite infection was detected in both *An. funestus* and *An. arabiensis* during both monitoring periods and *An. gambiae* s.s. during the period March-September 2018 (Table 5).

TABLE 5: SPOROZOITE RATES PRESENTED BY *ANOPHELES* SPECIES ACCORDING TO IRS STATUS AND MONITORING PERIOD.

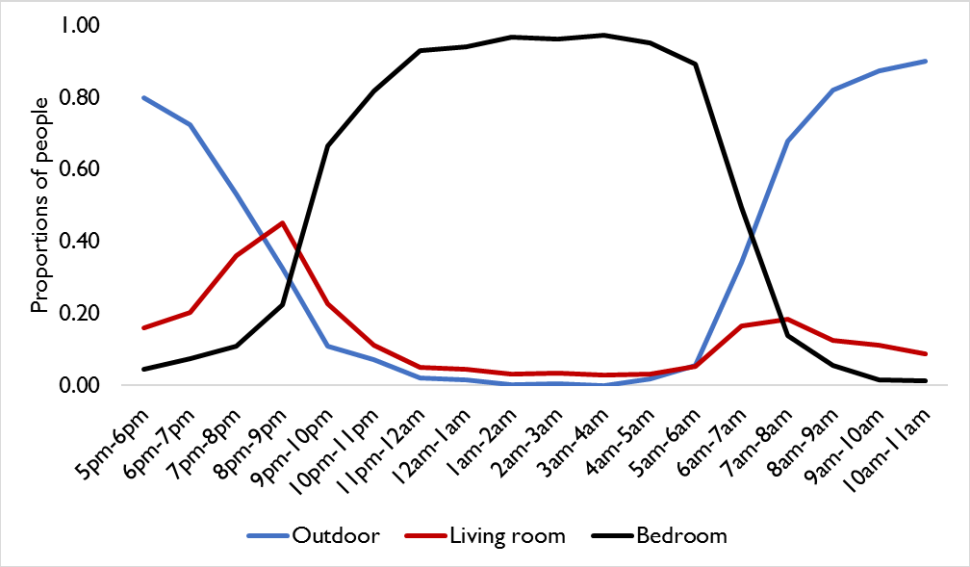
County & Spray Status	*Anopheles Species*	*Monitored period*	*No. of samples analysed*	*No. of sporozoite positive*	*Sporozoite rate % (95% CI)*
Migori (2nd Year IRS)	*An. funestus*	Oct 17 - Feb 18	9	0	0
		Mar - Sep 18	11	0	0
	An. arabiensis	Oct 17 - Feb 18	40	0	0
		Mar - Sep 18	150	2	1.3% (0.1-3.2)
	An. gambiae s.s.	Oct 17 - Feb 18	1	0	0
		Mar - Sep 18	10	0	0
Homa Bay (1st Year IRS)	*An. funestus*	Oct 17 - Feb 18	373	14	3.8% (1.8-5.7)
		Mar - Sep 18	34	0	0
	An. arabiensis	Oct 17 - Feb 18	60	0	0
		Mar - Sep 18	356	2	0.6% (0.1-1.3)
	An. *gambiae* s.s.	Oct 17 - Feb 18	1	0	0
		Mar - Sep 18	13	0	0
Kisumu (No IRS)	*An. funestus*	Oct 17 - Feb 18	88	4	4.5% (0.2-8.9)
		Mar - Sep 18	936	20	2.1% (1.2-3.1)
	An. arabiensis	Oct 17 - Feb 18	53	1	1.9% (0.1-5.5)
		Mar - Sep 18	1106	10	0.9% (0.3-1.5)
	An. gambiae s.s.	Oct 17 - Feb 18	5	0	0
		Mar - Sep 18	186	3	1.6% (0.1-3.4)

2.3 MOSQUITO BITING BEHAVIOUR

HLC was only conducted for a period of five nights during a period of high *Anopheles* densities as the aim was to determine vector biting times and location (indoor vs outdoor) only, not for longitudinal density monitoring. A total of 1, 974 *Anopheles* mosquitoes were collected by HLC; 1,821 (92%) in non-intervention (Kisumu) and 153 (8%) in intervention (Homa Bay) sites over five nights of trapping (50 person-nights per site). In the intervention sites, 49 (32%) *Anopheles* were collected indoors and 104 (68%) outdoors. In the non-intervention sites, most *Anopheles* were collected indoor 1,599 (88%) compared to 222 (12%) outdoor.

Figure 7 illustrates the proportion of residents in Homa Bay and Kisumu (combined) that were either outdoors, in the living room, or in the bedroom throughout the night. At the start of HLC collections, residents were mostly outdoors but we observed a steady decrease in the number of people outdoors with a proportionate increase in the number of people indoors between 5:00 pm and 10:00 pm. The number of people in the living room was highest at 9:00 pm and dropped rapidly while the numbers in the bedroom continued to rise until 12:00 am. Most of the residents were in their bedrooms between 12:00 am and 6:00 am. After 6am, the number of residents in the bedroom dropped rapidly, with a proportionate increase in the numbers outdoor between 6:00 am and 8:00 am. People that use LLINs correctly should be protected from the majority of indoor biting between midnight and 6am. However, *An. funestus* biting continued indoors during the morning (6-11am), during which time people are more likely to be unprotected by LLINs. While *An. gambiae* s.l. were not caught in significant densities during this 1-week period of HLC, they were an important vector throughout monthly surveillance and their biting behaviour should be studied in future.

FIGURE 7: PROPORTIONS OF PEOPLE IN HOMA BAY AND KISUMU (COMBINED) OUTDOORS, IN LIVING ROOMS AND BEDROOMS DURING THE HUMAN LANDING COLLECTION (HLC) PERIOD.



Bites by *An. funestus* indoors, in unsprayed areas were few between 5:00pm and 10:00pm. A steady increase in biting rates occurred through the night with a peak biting time occurring between 4:00 am and 8:00 am, corresponding to when most people leave their bedrooms. Outdoor biting rates in the non-intervention sites were low with smaller peaks of biting. Bites in intervention sites both indoor and outdoor were nearly zero. Most of the bites indoor were observed after 10:00pm when most of the people were in their sleeping rooms. (Figure 8).

Bites by *An. gambiae* s.l. indoors in the non-intervention sites were observed to begin by 7:00pm and increased steadily through the night with a peak occurring between 4:00am and 6:00am. Most of the biting by *An. gambiae* s.l were observed to occur when most people were indoor and a sleep. Outdoor biting by the same species were lower compared to indoor biting, with nearly similar biting trends. Bites by *An. gambiae* s.l in the sprayed areas were much lower both indoor and outdoor with no clear trend in biting pattern (Figure 9).

FIGURE 8: HOURLY INDOOR AND OUTDOOR BITING RATES OF *AN. FUNESTUS* IN IRS AND NON-IRS COUNTIES AND PROPORTIONS OF PEOPLE OUTDOORS, IN LIVING ROOMS AND IN BEDROOMS DURING THE HUMAN LANDING COLLECTION (HLC) PERIOD. BITES IN IRS AREA ON A DIFFERENT SCALE.

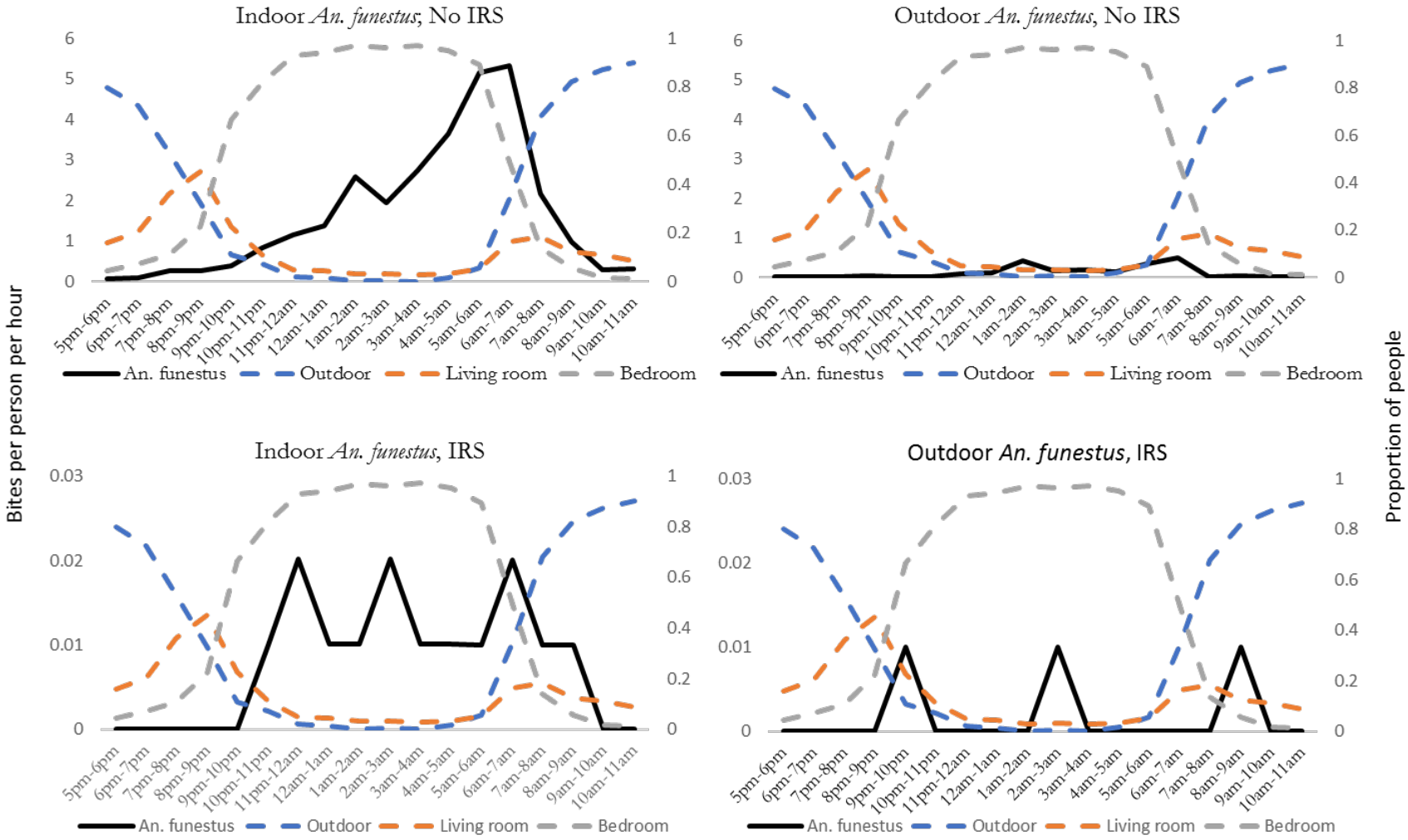
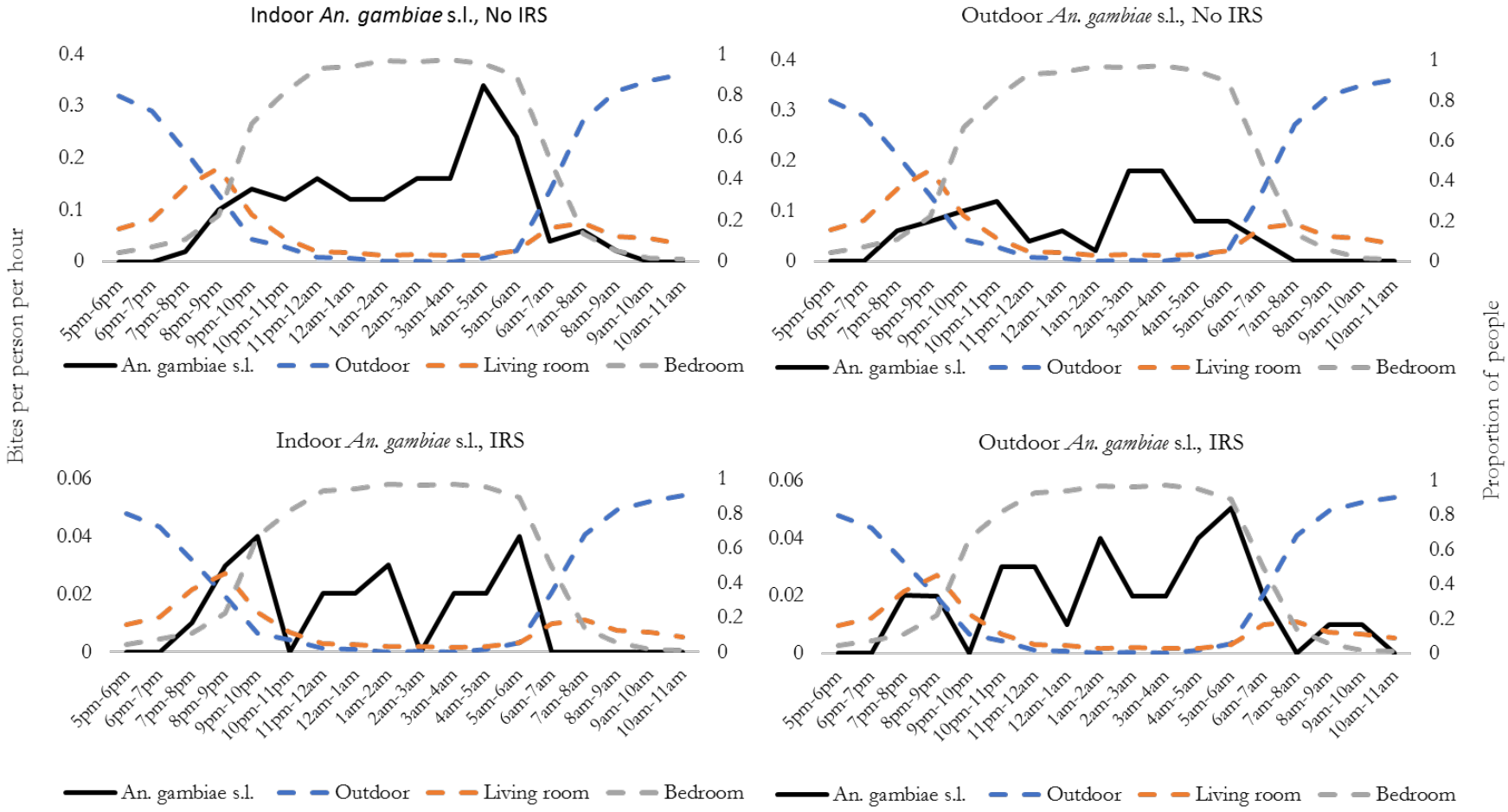


FIGURE 9: HOURLY INDOOR AND OUTDOOR BITING RATES OF *AN. GAMBIAE* S.L. IN IRS AND NON-IRS COUNTIES AND PROPORTIONS OF PEOPLE OUTDOORS, IN LIVING ROOMS AND IN BEDROOMS DURING THE HUMAN LANDING COLLECTION (HLC) PERIOD. BITES IN IRS AREA ON A DIFFERENT SCALE.



2.4 OUTDOOR TRAPPING COMPARISON

A total of 6,592 *Anopheles* and 29,045 *Culex* species were collected from five compounds in each of the two sites over 25 trapping nights. Of the collected *Anopheles*, the most abundant species were *An. gambiae* s.l., *An. coustani* and *An. funestus* (Table 6). Of the *An. gambiae* s.l., 1,519 were analysed by PCR for species identification and 1,503 (99%) were identified as *An. arabiensis* while 16 (1%) were *An. gambiae s.s.* Seven hundred and forty-one *An. funestus* s.l. were analysed by PCR and all were confirmed to be *An. funestus s.s.*

The indoor CDC LT collected a mean of 12.5 *Anopheles* per trap night. Comparatively, a mean of 20.8 *Anopheles* were collected per trap night by the most productive outdoor collection method, which was the outdoor CDC LT (Table 6). The 'gold standard' for outdoor trapping is HLC, however, the catch size was surprisingly low (despite close supervision through checks every 2h), catching a mean of just 7.8 *Anopheles* per trap night. HLC outdoors caught similar proportions of primary vector species, *An. gambiae* s.l. and *An. funestus*. However, all other outdoor trapping methods caught a greater proportion of *An. gambiae* s.l. than *An. funestus* (Table 6).

Sporozoite infections were detected in collections by indoor light trap 14/1786 (0.78%) and 4/201 (1.99%) in Kakola Ombaka and Masogo respectively, which are non-IRS sites in Kisumu County. From all outdoor traps, the number of *Anopheles* collected was far lower than by indoor CDC-LT. Therefore, it was more difficult to detect sporozoites due to smaller catch size. Sporozoite infection was observed in collections by the electrocuting grid 2/213 (0.87%) and Furvela tent trap 2/346 (0.58%) in Kakola Ombaka and 1/10 (10%) from HLC in Masogo (Table 6). Of all the sporozoite positive mosquitoes, 20 were *An. funestus* while 3 were *An. arabiensis.*

TABLE 6: NUMBERS OF *ANOPHELES* MOSQUITO SPECIES AND SPOROZOITE RATES BY DIFFERENT COLLECTION METHODS INDOOR AND OUTDOOR.

Collection site	Collection method	Anopheles Species					Sporozoite ELISA		
		An. funestus	*An. gambiae s.l.*	*An. coustani*	*An. pharoensis*	*Total*	*No. tested*	*No. positive*	*Sporozoite Rate*
Kakola Ombaka	**Indoor Light Trap**	779 (25.4%)	1,176 (38.4%)	966 (31.5%)	143 (4.7%)	3,064 (100%)	1,786	14	0.78%
	Human Landing Catch	37 (10.5%)	45 (12.7%)	263 (74.5%)	8 (2.3%)	353 (100%)	32	0	0.00%
	Furvela Tent Trap	171 (24.4%)	341 (48.6%)	165 (23.5%)	25 (3.6%)	702 (100%)	346	2	0.58%
	Host Decoy Trap	44 (12.5%)	256 (72.9%)	45 (12.8%)	6 (1.7%)	351 (100%)	214	0	0.00%
	Outdoor Light Trap	30 (3.1%)	89 (9.1%)	575 (58.8%)	284 (29.0%)	978 (100%)	287	0	0.00%
	Electrocuting Grid	67 (12.5%)	208 (39.0%)	239 (44.8%)	20 (3.7%)	534 (100%)	231	2	0.87%
Kakola Ombaka Sub-total (outdoor methods)		349 (12.0%)	939 (32.2%)	1,287 (44.1%)	343 (11.8%)	2,918 (100%)	1,110	4	0.36%
Masogo	**Indoor Light Trap**	157 (44.7%)	166 (47.3%)	13 (3.7%)	15 (4.3%)	351 (100%)	201	4	1.99%
	Human Landing Catch	13 (37.1%)	13 (37.1%)	8 (22.9%)	1 (2.9%)	35 (100%)	10	1	10.0%
	Furvela Tent Trap	54 (43.2%)	65 (52%)	6 (4.8%)	0 (0.0%)	125 (100%)	83	0	0.00%
	Host Decoy Trap	9 (34.6%)	17 (65.4%)	0 (0.0%)	0 (0.0%)	26 (100%)	11	0	0.00%

Collection site	Collection method	Anopheles Species					Sporozoite ELISA		
		An. funestus	*An. gambiae s.l.*	*An. coustani*	*An. pharoensis*	*Total*	*No. tested*	*No. positive*	*Sporozoite Rate*
	Outdoor Light Trap	6 (10.0%)	23 (38.3%)	23 (38.3%)	8 (13.3%)	60 (100%)	27	0	0.00%
	Electrocuting Grid	3 (23.1%)	7 (53.8%)	2 (15.4%)	1 (7.7%)	13 (100%)	3	0	0.00%
Masogo Sub-total (outdoor methods)		85 (32.8%)	125 (48.3%)	39 (15.1%)	10 (3.9%)	259 (100%)	134	1	0.75%

Overall species composition across all outdoor collection methods in Kakola Ombaka was 44.1% *An. coustani*, 32.2% *An. gambiae s.l.*, 12.0% *An. funestus,* 11.8% *An. pharoensis* (Figure 9). While in Masogo *An. gambiae* s.l. (48.3%) and *An. funestus* 32.8% formed the bulk of collections, with other species being *An. coustani* (15.1%) and *An. pharoensis* (3.9%) (Figure 10).

Anopheles species composition varied by collection method at both sampling sites. In Kakola Ombaka, *An. gambiae* s.l. and *An. coustani* formed the greatest bulk of the catch in most collection methods. *An. funestus* was highest in the Furvela tent trap and the indoor light trap while lowest in the outdoor light trap. *An. pharoensis* was most commonly collected in the outdoor light trap. In Masogo, collections were predominantly *An. gambiae* s.l. and *An. funestus*. *An. coustani* and *An. pharoensis* were lowest in catch across most collection methods except outdoor light trap which had the highest catch of *An. coustani* (Figure 11).

FIGURE 10 : *ANOPHELES* SPECIES COMPOSITION FROM ALL OUTDOOR COLLECTION METHODS AT TWO SAMPLING SITES.

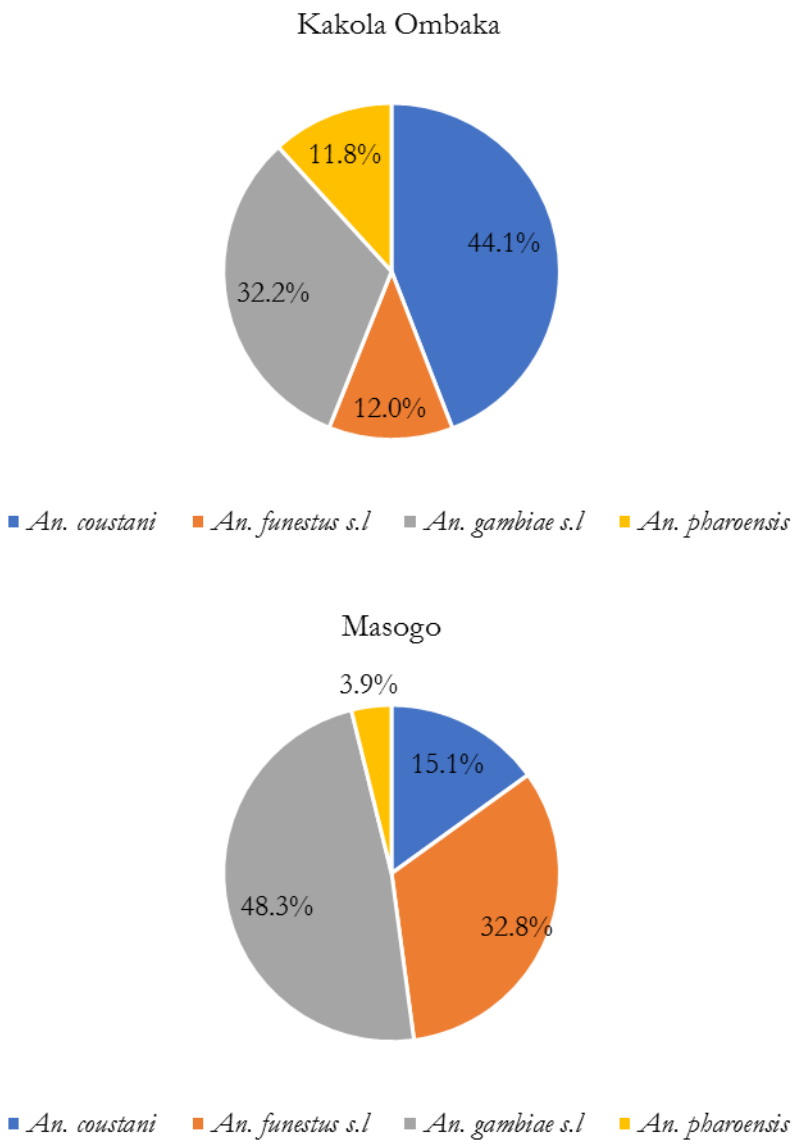


FIGURE 11: MEAN NUMBER OF *ANOPHELES* SPECIES PER TRAP PER NIGHT BY DIFFERENT COLLECTION METHODS

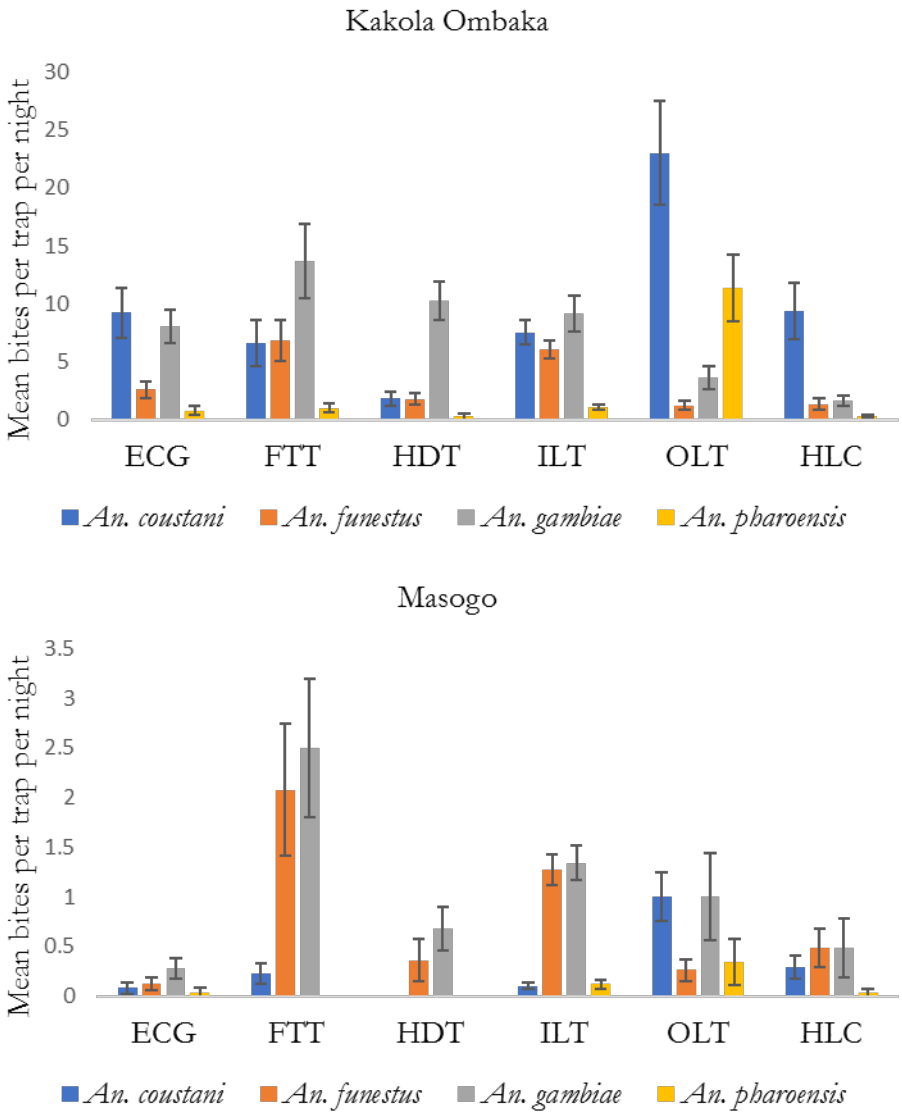


Table 7 summarizes the results from statistical comparison of catch size according to mosquito species and numbers collected in comparison with the 'gold standard' outdoor human landing catch. In Kakola Ombaka, all outdoor collection methods caught significantly more *An. gambiae* s.l. than outdoor HLC. Of the secondary vector species outdoor CDC-LT caught significantly more *An. coustani* and *An. pharoensis* than outdoor HLC. In Masogo the numbers caught were far fewer (as this site was away from flooded ground), however the general trends were similar (Table 7).

Operationally, the electrocuting grid was the most complicated to source parts, assemble and set-up in the field, making it the least suitable for routine surveillance. Preparing boiling water for the host decoy trap & transporting the long pipe was also problematic for use in routine surveillance. The two best choices for routine outdoor surveillance are either the Furvela tent trap or outdoor CDC-LT, with the final choice pending further analysis and discussion. In Kakola Ombaka, no significant difference in the number of *An. coustani* collected was observed for electrocuting grids and Furvela tent trap compared to HLC. HDT sampled significantly fewer numbers of *An. coustani* compared to HLC, while outdoor CDC light trap sampled significantly more of the species compared to HLC (Table 7).

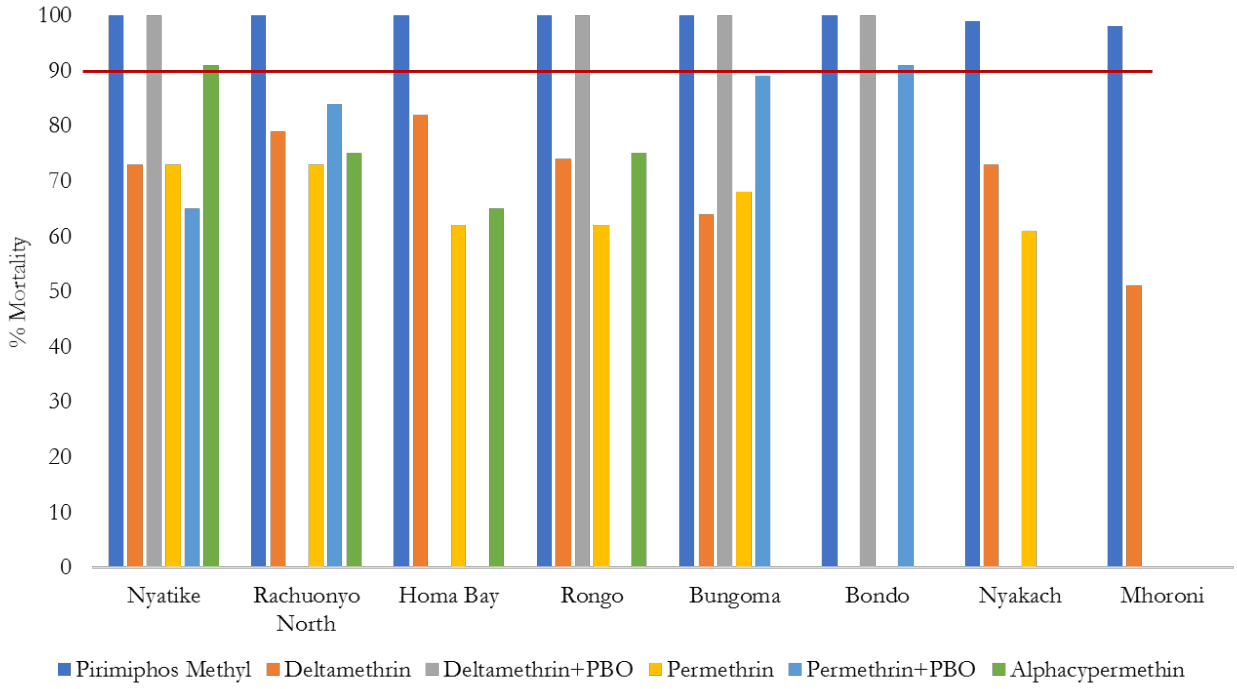
TABLE 7: COMPARISON OF NUMBERS OF *ANOPHELES* MOSQUITO SPECIES IN HLC AND DIFFERENT COLLECTION METHODS OUTDOOR.

Collection site	Anopheles species	Collection methods	Mean	RR (95% CI)	P Values
Kakola Ombaka	**An. funestus**	**Electrocuting Grid**	2.58	2.19 (0.98 - 4.87)	0.06
		Furvela Tent Trap	6.84	6.34 (2.84 - 14.18)	<.0001
		Host Decoy Trap	1.76	1.56 (0.68 -3.57)	0.29
		Outdoor Light Trap	1.20	1.02 (0.43 - 2.42)	0.97
		Human Landing Catch	1.32	1	1
	An. gambiae s.l.	**Electrocuting Grid**	8.00	4.98 (2.59 - 9.58)	<.0001
		Furvela Tent Trap	13.64	8.49 (4.41 - 16.33)	<.0001
		Host Decoy Trap	10.24	6.37 (3.30 - 12.29)	<.0001
		Outdoor Light Trap	3.56	2.22 (1.2 - 4.36)	0.02
		Human Landing Catch	1.61	1	1
	An. coustani	**Electrocuting Grid**	9.19	1.52 (0.79 -2.91)	0.21
		Furvela Tent Trap	6.60	0.73 (0.38 - 1.42)	0.36
		Host Decoy Trap	1.8	0.21 (0.10 - 4.15)	<.0001
		Outdoor Light Trap	23.00	3.26 (1.72 - 6.18)	0.0001
		Human Landing Catch	9.39	1	1
	An. pharoensis	**Electrocuting Grid**	0.77	2.69 (0.74 - 9.76)	0.13
		Furvela Tent Trap	1.00	3.78 (1.02 - 13.93)	0.05
		Host Decoy Trap	0.24	0.71 (0.15- 3.48)	0.67
		Outdoor Light Trap	11.36	56.56 (16 .41 - 194.95)	<.0001
		Human Landing Catch	0.29	1	1
Masogo	**An. funestus**	**Electrocuting Grid**	0.12	0.26 (0.06 - 1.13)	0.07
		Furvela Tent Trap	2.08	4.62 (1.70 -12.56)	0.003
		Host Decoy Trap	0.36	0.72 (0.23 -2.30)	0.58
		Outdoor Light Trap	0.26	0.54(0.15 - 1.90)	0.34
		Human Landing Catch	0.48	1	1
	An. gambiae s.l.	**Electrocuting Grid**	0.28	0.64 (0.21 - 2.01)	0.45
		FurvelaTent Trap	2.50	5.66 (2.36 - 13.57)	0.0001
		Host Decoy Trap	0.68	1.48 (0.55 - 3.94)	0.43
		Outdoor Light Trap	1.00	2.17 (0.84 - 5.63)	0.11
		Human Landing Catch	0.48	1	1
	An. coustani	**Electrocuting Grid**	0.08	0.27(0.05 - 1.33)	0.12
		Furvela Tent Trap	0.23	0.78 (0.25 - 2.40)	0.66
		Host Decoy Trap	0.00	0	1
		Outdoor Light Trap	1.00	3.37(1.38 - 8.28)	0.01
		Human Landing Catch	0.30	1	1

INSECTICIDE RESISTANCE TESTING

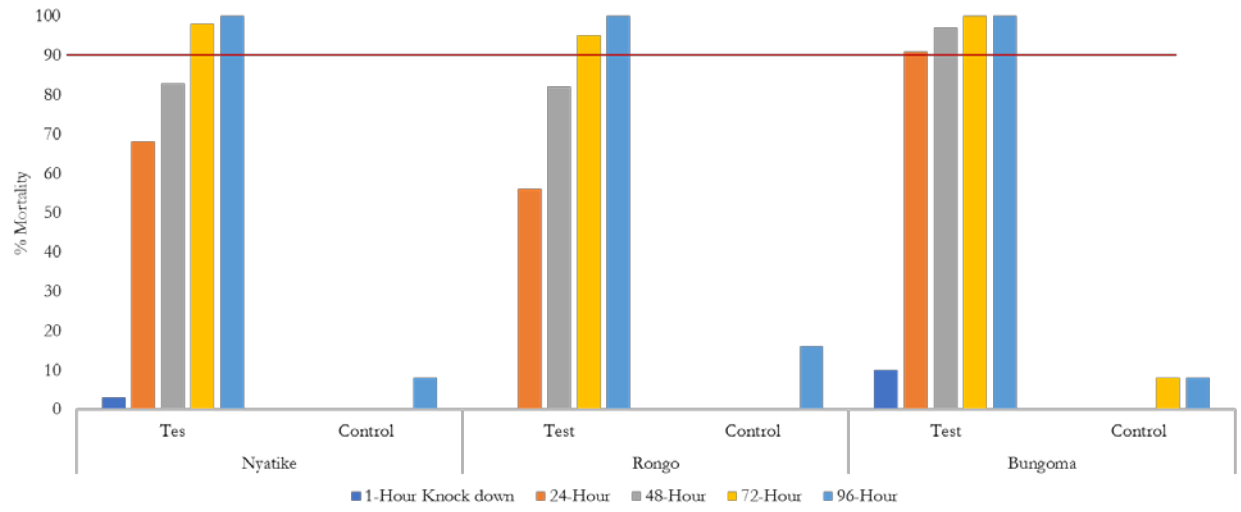
Susceptibility (>98% mortality) of wild *An. arabiensis* to pirimiphos-methyl was observed in all sub-counties where testing was conducted. Pyrethroid resistance (<90% mortality) was recorded for deltamethrin, permethrin and alphacypermethrin in all sites. Mortality rates were similar for permethrin, deltamethrin and alphacypermethrin, with mortality ranging between 55% and 80%. Full susceptibility to deltamethrin was restored following pre-exposure to PBO in Nyatike and Rongo (Migori County), Sirisia (Bungoma County) and Bondo (Siaya County). For permethrin, pre-exposure to PBO resulted in increased mortality in most sites, however full susceptibility was not restored (Figure 12). Bioassay testing with PBO was a late addition to the work plan and therefore could not be completed in all sites and with all pyrethroids.

FIGURE 12: 24-HOUR MORTALITY OF *AN. ARABIENSIS* FROM NYATIKE, RACHUONYO NORTH, HOMA BAY, RONGO, BUNGOMA, BONDO, NYAKACH AND MUHORONI SUB-COUNTIES FOLLOWING EXPOSURE TO DIFFERENT INSECTICIDES.



Full mortality of wild *An. arabiensis* to clothianidin was recorded after 96 hours (4 days) post exposure in tube tests. Knock-down rates were low (<20%) at one-hour post exposure, as expected, while >80% of mortality was achieved within 48h of exposure (Figure 13).

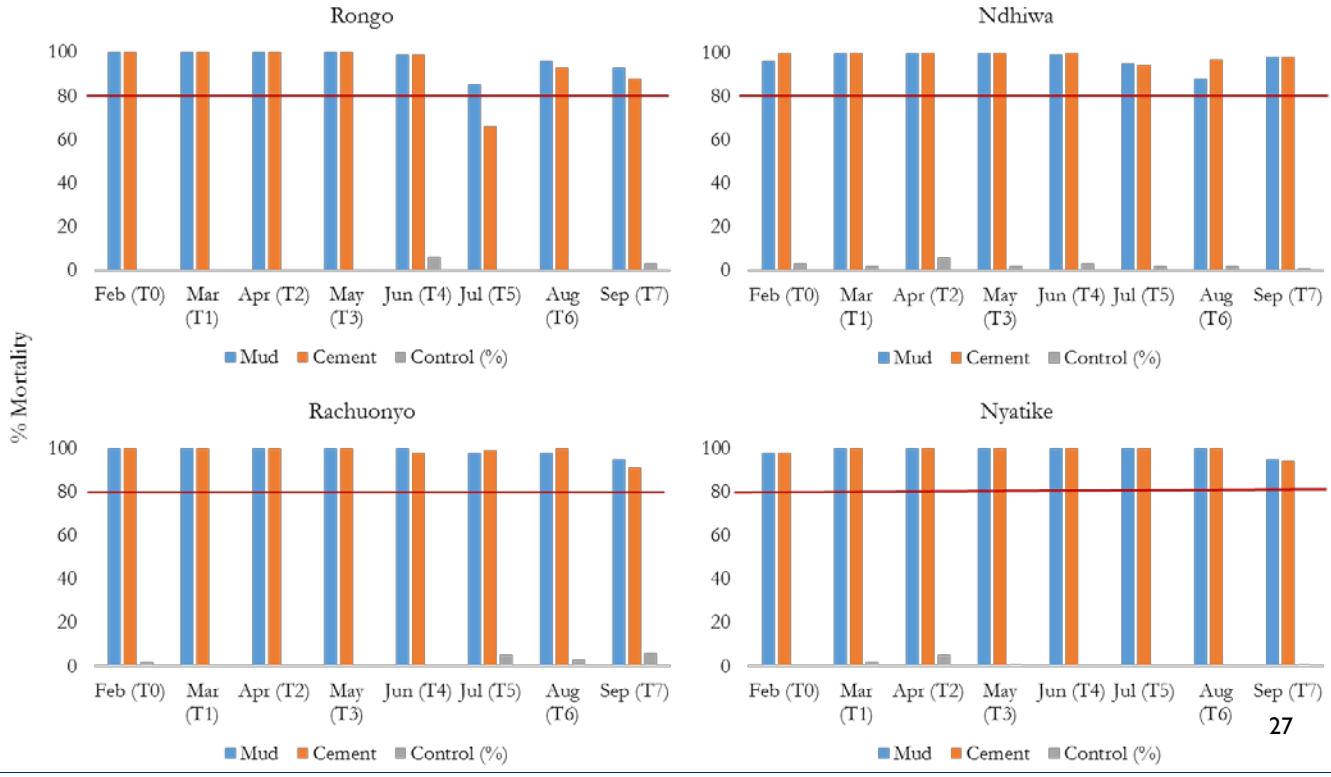
FIGURE 13 : 1-HOUR KNOCK-DOWN AND PERCENT MORTALITY OF *AN. ARABIENSIS* UP TO 96-HOURS (4 DAYS) POST EXPOSURE TO SUMISHIELD (CLOTHIANIDIN) TREATED FILTER PAPERS.



2.5 RESIDUAL DURATION OF ACTELLIC 300 CS

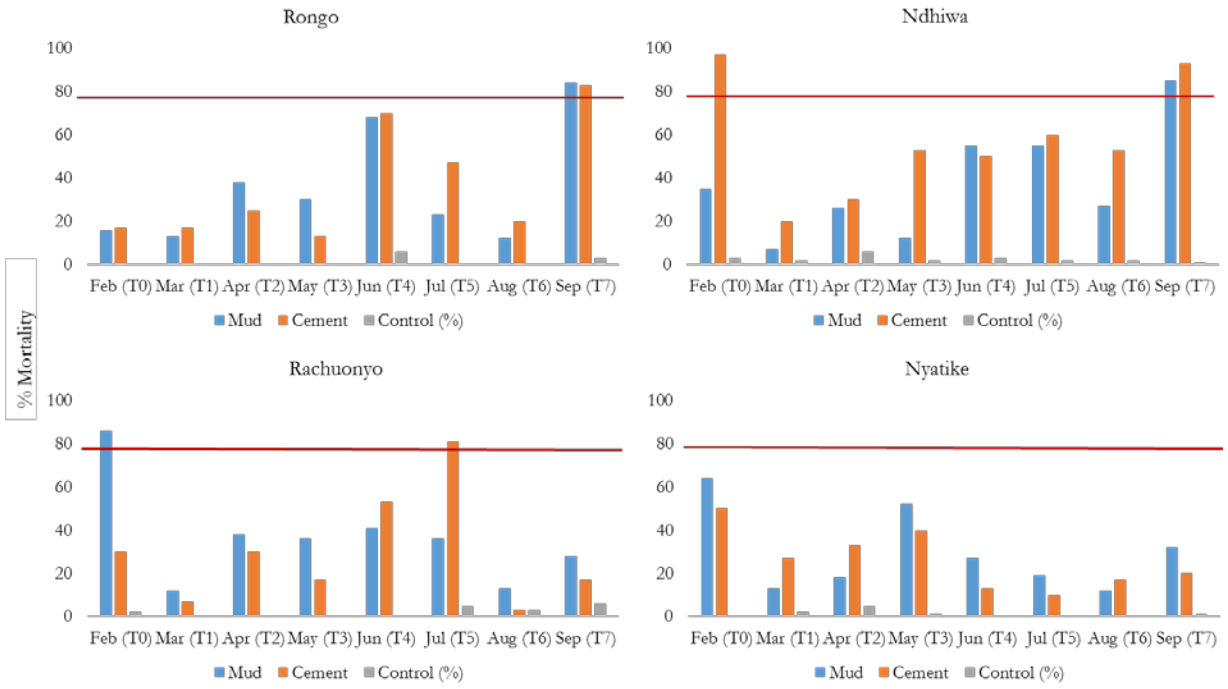
A total of 8,576 *An. gambiae* s.s. Kisumu strain were exposed to sprayed walls at different heights over seven months post-IRS (Annex, Table A-1). 100% mortality was observed in all sub-counties on both mud and cement walls up to four months after spraying. In the fifth month (July), mortality in Rongo sub-county was at 85% and 66% for mud and cement walls, respectively. However, mortality was observed to remain above 80% in the following months of August and September (Figure 14).

FIGURE 14: PERCENT MORTALITY OF SUSCEPTIBLE *AN. GAMBIAE S.S.* KISUMU STRAIN, 24 HOURS POST EXPOSURE BY MONTH IN EACH SUB-COUNTY.



A total of 2,853 susceptible *An. gambiae* s.s Kisumu strain were exposed in adult mosquito cages to test the fumigant effect of Actellic 300CS during a 30 mins exposure. Variable mortality rates of *An. gambiae* s.s. were observed. The highest fumigant effect was recorded within two weeks of spraying in February in Ndhiwa and Rachuonyo with an apparent rise in fumigant effect was observed between June and September in Rongo, Ndhiwa and Rachounyo sub-counties. Mortality in Nyatike sub-county was below 80% throughout the survey period (Figure 15).

FIGURE 15: PERCENT MORTALITY OF *AN. GAMBIAE* S.S. KISUMU STRAIN, 24 HOURS POST EXPOSURE IN FUMIGANT BIOASSAYS.



3. DISCUSSION

IRS with Actellic 300 CS in western Kenya was demonstrated to have a long residual efficacy and to be highly effective against indoor *Anopheles* mosquitoes, as demonstrated in other countries (20-22). Analysis of mosquito density data collected up to seven months after spraying shows a reduced risk of occurrence of both *An. funestus* and *An. gambiae* s.l. in sprayed sites compared to unsprayed sites post-IRS. This is in keeping with data from 2017 which demonstrated a significant reduction in *An. funestus* and *An. gambiae* s.l. densities for 12 months after the first round of IRS in Migori County. The greatest impact was on *An. funestus* which were reduced to near zero in both Migori and Homa Bay counties following IRS. The lowest risk was in Migori County due to the long duration of Actellic CS sprayed in 2017 which provided control through to February 2018 when the second round of spraying was conducted. The reduction of *An. funestus* populations in Homa Bay County was similar to results observed in 2017 following the first round of IRS in Migori County where *An. funestus* populations were reduced to almost undetectable levels (20). Population densities of *An. funestus* remained low after the second round of IRS. The absence of sporozoite infection in *An. funestus* both before and after the second round of IRS in Migori and after spraying in Homa Bay further demonstrates the effectiveness of IRS in transmission reduction.

An. gambiae s.l. (chiefly *An. arabiensis* according to PCR results), was the predominant species collected by CDC light traps and PSC collections after IRS. Even though our comparison of *An. gambiae* s.l. densities in sprayed and unsprayed sites showed lower densities indoors in sprayed sites, the vector population was not as dramatically reduced as was the case with *An. funestus*. The lower impact of IRS on *An. arabiensis* is most likely attributable to vector feeding and resting behaviour, with *An. arabiensis* known to feed more frequently outdoors on cattle (7-9) and spend less time indoors in contact with sprayed surfaces. *An. arabiensis* has been reported elsewhere to be less affected by indoor insecticide-based control methods such as LLINs and IRS since they often exit houses after entry and/or feeding (5).

Data from unsprayed sites demonstrated that *An. funestus* and *An. arabiensis* are the main drivers of malaria transmission in the absence of IRS (with a higher sporozoite rate in *An. funestus*). Biting by *An. funestus* in unsprayed sites was observed to peak in the late night until dawn, coinciding with the time when residents leave the protection of their bed nets. Biting was observed to continue until 11:00 am in the morning. We observed similar altered biting behaviour by *An. funestus* in other regions of western Kenya (20) and a survey in Senegal reported similar findings (23). As there is a large population of indoor resting *An. funestus* in Kisumu County, expanding IRS in the future to this area is likely to have a large impact on vector populations and malaria transmission as has occurred in Migori and Homa Bay counties.

An. arabiensis appears to sustain malaria transmission albeit at a reduced rate in IRS sites, as demonstrated by the presence of sporozoites in *An. arabiensis* after IRS in Migori and Homa Bay. When considering progress towards elimination it will be necessary to consider alternative control methods for *An. arabiensis* in combination with indoor vector control.

In an evaluation of outdoor collection methods, outdoor CDC light trap was observed to be most effective in collecting *An. coustani* and *An. pharoensis* which are considered secondary malaria vectors (24) and are more associated with outdoor activities. The trap also showed the highest variation in species composition of malaria vectors sampled, suggesting its suitability in collecting a wide spectrum of mosquito taxa outdoors. The use of a light next to a volunteer (host odour) under an untreated bed net might have provided additional attraction in the outdoor environment and attracted a greater diversity of mosquito species. Baiting the outdoor CDC light trap with a human is probably critical for attraction of truly host-seeking malaria vectors.

However, use of live hosts for outdoor CDC light trap may be unrealistic for routine entomological monitoring of outdoor mosquito populations. The technique may raise safety issues of the volunteer and would require night supervision to ensure compliance.

The Furvela Tent Trap on the other hand was observed to be most effective in sampling *An. funestus* and *An. gambiae* s.l. (mostly *An. arabiensis*), which contribute most to indoor malaria transmission in the region. *Anopheles* species composition in Furvela Tent Trap more closely mirrored the catches from indoor CDC light trap. As mosquitoes are attracted to the tent opening, it is possible that we may be catching mosquitoes that are 'house entering' on a smaller scale into the tent before they are collected into the CDC light trap. It is therefore possible that those mosquito species that exhibit indoor entry behaviours are more likely to access the tent trap than other traps that would require mosquitoes to land on them. The Furvela Tent Trap was the most practical outdoor trap and provided full protection of the human volunteer and can be easily deployed for routine collection. However, it remains questionable if the trap samples truly outdoor host seeking mosquitoes or whether the trap mimics a house, hence sampling similar vector species that would naturally enter human habitations.

Susceptibility of *An. arabiensis* to pirimiphos-methyl using WHO insecticide treated papers shows that Actellic CS can continue to be used for subsequent IRS campaigns. Susceptibility testing of *An. funestus* was not possible due to difficulty finding the vector species in sprayed areas. To prevent development of insecticide resistance however, Actellic 300CS needs to be used in rotation with other classes of insecticide according to the Kenya National Resistance Management Plan (4). We observed susceptibility of *An. arabiensis* to clothianidin within four days after exposures. Clothianidin may therefore be a potential alternative insecticide for rotation in IRS to delay the development of resistance. *An. arabiensis* showed resistance to deltamethrin, permethrin and alphacypermethrin. Pyrethroid resistance in *Anopheles* mosquitoes is widespread and threatens continued efficacy of pyrethroid LLINs. Pre-exposure of the test samples to PBO prior to deltamethrin exposure restored full susceptibility to the insecticide while the situation improved marginally for permethrin. A recent publication demonstrated that PBO long lasting nets in western Tanzania provide additional protection above pyrethroid nets against pyrethroid resistance *Anopheles* mosquitos (25). Our bioassay results suggest that PBO nets with deltamethrin are likely to be more effective than conventional pyrethroid LLINs in the control of pyrethroid resistant *Anopheles* mosquitoes in western Kenya.

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ANNEX

TABLE A-1: NUMBER AND PERCENT MORTALITY OF SUSCEPTIBLE *AN. GAMBIAE* S.S. KISUMU STRAIN 24 HOURS POST EXPOSURE BY MONTH AND SUB-COUNTY

Month	Sub county	Wall type	*Cone Bioassay*			*Fumigant Bioassay*		
			N	N Dead (24Hr)	% Mortality	N	N Dead (24Hr)	% Mortality
February	Rongo	Mud	210	210	100	70	22	31
		Cement	90	90	100	30	12	40
	Ndhiwa	Mud	180	173	96	60	21	35
		Cement	90	90	100	30	29	97
	Rachuonyo	Mud	198	198	100	66	57	86
		Cement	90	90	100	30	9	30
	Karungu	Mud	210	205	98	70	45	64
		Cement	90	88	98	30	15	50
March	Rongo	Mud	210	210	100	70	9	13
		Cement	90	90	100	30	5	17
	Ndhiwa	Mud	210	210	100	70	5	7
		Cement	90	90	100	30	6	20
		Mud	180	180	100	60	7	12
	Rachuonyo	Cement	90	90	100	30	2	7
		Mud	180	180	100	60	8	13
	Karungu	Cement	90	90	100	30	8	27
		Mud	180	180	100	60	23	38
April	Rongo	Cement	60	60	100	20	5	25
		Mud	150	150	100	50	13	26
	Ndhiwa	Cement	60	60	100	20	6	30
		Mud	180	180	100	60	23	38
	Rachuonyo	Cement	90	90	100	30	9	30
		Mud	150	150	100	50	9	18
	Karungu	Cement	90	90	100	30	10	33
		Mud	150	150	100	50	15	30
May	Rongo	Cement	90	90	100	30	4	13
		Mud	180	180	100	60	7	12
	Ndhiwa	Cement	90	90	100	30	16	53
		Mud	210	210	100	70	25	36
	Rachuonyo	Cement	90	90	100	30	5	17
		Mud	180	180	100	60	31	52
	Karungu	Cement	90	90	100	30	12	40
		Mud	180	178	99	60	41	68

Month	Sub county	Wall type	*Cone Bioassay*			*Fumigant Bioassay*		
			N	N Dead (24Hr)	% Mortality	N	N Dead (24Hr)	% Mortality
June	**Rongo**	**Cement**	90	89	99	30	21	70
		Mud	180	179	99	60	33	55
	Ndhiwa	**Cement**	60	60	100	20	10	50
		Mud	210	209	100	70	29	41
	Rachuonyo	**Cement**	90	88	98	30	16	53
		Mud	210	210	100	70	19	27
	Karungu	**Cement**	90	90	100	30	4	13
		Mud	180	153	85	60	14	23
July	**Rongo**	**Cement**	87	57	66	30	14	47
		Mud	174	166	95	51	28	55
	Ndhiwa	**Cement**	90	85	94	30	18	60
		Mud	153	150	98	50	18	36
	Rachuonyo	**Cement**	74	73	99	26	21	81
		Mud	210	210	100	70	13	19
	Karungu	**Cement**	90	90	100	30	3	10
		Mud	180	172	96	60	7	12
August	**Rongo**	**Cement**	90	84	93	30	6	20
		Mud	180	159	88	60	16	27
	Ndhiwa	**Cement**	90	87	97	30	16	53
		Mud	210	207	98	70	9	13
	Rachuonyo	**Cement**	90	90	100	30	1	3
		Mud	150	150	100	50	6	12
	Karungu	**Cement**	90	90	100	30	5	17
		Mud	150	140	93	50	42	84
September	**Rongo**	**Cement**	90	79	88	30	25	83
		Mud	180	176	98	60	51	85
	Ndhiwa	**Cement**	90	88	98	30	28	93
		Mud	150	142	95	50	14	28
	Rachuonyo	**Cement**	90	82	91	30	5	17
		Mud	150	143	95	50	16	32
	Karungu	**Mud**	150	143	95	50	16	32
		Cement	90	85	94	30	6	20