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THE PMI VECTORLINK PROJECT ZAMBIA PROJECT

ANNUAL ENTOMOLOGY REPORT AUGUST 2020-JULY 2021

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Abt Associates | 6130 Executive Blvd | Rockville, Maryland 20852
T. 301.347.5000
abtassociates.com

THE PMI VECTORLINK PROJECT ZAMBIA ANNUAL ENTOMOLOGY REPORT AUGUST 2020 – JULY 2021

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ACRONYMS

CDC	(U.S.) Centers for Disease Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
GF	Global Fund
GPM	Global Precipitation Measurement
GRZ	Government of the Republic of Zambia
HBR	Human Biting Rate
HLC	Human Landing Catch
IRR	Incidence Rate Ratio
IRS	Indoor Residual Spraying
ITN	Insecticide-treated Net
LSM	Larval Source Management
NMEC	National Malaria Elimination Centre
NMEP	National Malaria Elimination Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	(U.S.) President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SOP	Standard Operating Procedure
WHO	World Health Organization

EXECUTIVE SUMMARY

Zambia implements indoor residual spraying (IRS) and insecticide-treated net (ITNs) distribution as its main malaria vector control interventions. The U.S. President's Malaria Initiative (PMI) VectorLink Project, funded by the U.S. Agency for International Development (USAID) and implemented by Abt Associates, supports the implementation of both interventions in Zambia. From September 29 to November 18, 2020, VectorLink Zambia conducted its 2020 IRS campaign in all nine districts in Eastern Province (which has since been divided into 14 districts in total), three districts in Copperbelt Province, and three districts in Luapula Province using SumiShield and Fludora Fusion insecticides. The project sprayed 648,952 structures out of 672,620 structures found by spray operators, resulting in 97% spray coverage. In addition, VectorLink provided technical assistance to the NMEP at the national level for planning, coordinating, implementing, and monitoring of the 2020/2021 insecticide-treated net (ITN) mass campaign along with enhanced planning and implementation support at the provincial and district levels in four PMI focus provinces—Eastern, Luapula, Muchinga, and Northern. Between November 2020 and April 2021, the NMEP together with its partners distributed 2,101,403 ITNs, including 1,619,376 standard pyrethroid nets and 482,027 permethrin+piperonyl butoxide (PBO) nets across these four provinces.

Entomological monitoring associated with the 2020 IRS campaign included vector surveillance and insecticide resistance monitoring, assessment of the quality of spray, and insecticide residual efficacy. Vector surveillance to assess the impact of IRS was conducted from August 2020 to June 2021 in 14 sentinel sites, including four IRS sites and four control sites across the three provinces where IRS was supported by PMI VectorLink. In addition, for historical reasons and to provide additional support for the national entomological surveillance strategy, PMI VectorLink supported entomological monitoring in two sites in Central Province, two sites in Luapula Province, and two sites in Copperbelt Province—one IRS site sprayed by the Government of the Republic of Zambia (GRZ) and one control site in each province. Mosquitoes were collected using pyrethrum spray catches (PSCs) and human landing catches (HLCs). Baseline data were collected in August and September 2020 and post-intervention data collections started in October 2020 and were conducted monthly or bi-monthly[1]. Spray quality was assessed 24 hours after IRS at seven sprayed sites supported by PMI VectorLink, and three sprayed sites supported by GRZ. Five of the PMI VectorLink sites were subsequently followed by monthly assessments of the insecticide decay on walls. Insecticide susceptibility tests were conducted in the 14 sites between December 2020 and May 2021 using World Health Organization (WHO) tube tests or U.S. Centers for Disease Control and Prevention (CDC) bottle assays.

Data from August 2020 to June 2021 indicate that *Anopheles funestus* s.l. was the most abundant mosquito (53.8% of 135,004 mosquitoes), while *An. gambiae* s.l. made up 8.1% of the total number of mosquitoes collected. The overall indoor resting density of *An. funestus* s.l. was significantly lower at the IRS sites compared to the non-IRS sites (2.6 versus 7.5 vectors per house) and reduction in density was observed at sprayed sites after IRS (4.1 to 2.2 vectors per house) while a slight increase was observed post- IRS at the control sites (6.7 to 7.6 vectors per house). In contrast, the overall density of *An. gambiae* s.l. was higher at the IRS sites (0.46 versus 0.33 vectors per house) and post-IRS density was also higher than pre-IRS density at the IRS sites (0.53 versus 0.21 vectors per house). At the IRS sites, the average human biting rate of *An. funestus* s.l. indoors and outdoors reduced from 39.2 bites per person per night (b/p/n) before IRS to 23.4 b/p/n after IRS, while there was an increase at the non-IRS sites (30.3 to 43.6 b/p/n). Overall biting rates for

[1] The initial plan to conduct monthly collections in all seven districts was updated in October 2019 based on recommendations from a field visit by the CDC Entomology backstop for Zambia. It was determined together with PMI that, based on available funding, monthly collections should be done in three districts (one in each of the three provinces supported by PMI). Collections would be done every other month in the other four districts.

An. gambiae s.l. increased after IRS at both IRS and the control sites. Reduction in parity rate—a desirable outcome of IRS which suggests vectors are not surviving long enough to transmit malaria—was observed post-IRS for both *An. funestus* s.l. and *An. gambiae* s.l. in Eastern and Copperbelt Provinces. There were also less sporozoite positive *An. funestus* s.l. at the sprayed sites compared to the control sites, which corroborates the reduced parity observed.

The majority (99.4%) of the *An. funestus* s.l. vectors collected during the reporting period were *An. funestus* s.s., with 0.5% *An. vaneedeni* and 0.2% *An. parensis*. The majority (99.2%) of *An. gambiae* s.l. were *An. gambiae* s.s. with 0.8% *An. arabiensis*. The mean number of *Plasmodium* parasite infective bites received per person per month (the entomological inoculation rate, or EIR) from *An. funestus* s.l. was lower at the sprayed sites compared to the control sites in five out of the seven districts monitored, while that of *An. gambiae* s.l. was lower in four out of the six districts with valid data. Despite the higher *An. gambiae* s.l. biting rates observed in some sprayed districts, the low sporozoite rates observed at the sprayed sites resulted in overall low EIRs (0-1.06 infective bites/person/month). The absolute EIR values for *An. funestus* s.l. at the sprayed sites ranged from zero to as high as 40 infective bites per person per month. signaling the need to consider the deployment of additional interventions to supplement IRS in the affected areas. We found the human blood index for both *An. funestus* s.l. and *An. gambiae* s.l. at sprayed and control sites; specifically, most of the vectors fed on humans than on alternative hosts in the environment. Thus, vector control interventions targeting the interruption of human-vector contact continue to be an appropriate strategy.

In all houses and on both surface types (mud and cement), we observed 100% mortality of *An. gambiae* s.s. 48 hours post-exposure in the five districts sprayed with Fludora Fusion. In the two districts sprayed with SumiShield, 100% mortality was achieved 120 hours after exposure in most of the houses, while the remainder of the houses attained at least 96% mortality. These findings signify a high quality of spraying by the majority of spray operators in the 2020 campaign in the respective districts. As of August 2021, based on longitudinal data collected on the effectiveness of the two insecticides deployed in the 2020 IRS campaign on sprayed surfaces, the effective duration of the two insecticides is at least 10 months.

An. funestus s.l. and *An. gambiae* s.l. were fully susceptible to clothianidin and chlorfenapyr in all provinces where the products were tested (Luapula, Eastern, Central, and Copperbelt). There was a mixture of full susceptibility and suspected resistance to dichlorodiphenyltrichloroethane (DDT) in *An. funestus* s.l. vector populations in Luapula and Copperbelt Provinces and full susceptibility in *An. gambiae* s.l. populations in Eastern Province. There is confirmed resistance to pyrethroid insecticides in Luapula, Eastern and Copperbelt Provinces. Due to the continued widespread resistance to pyrethroid insecticides and the need to conserve pyrethroids for use on ITNs, the current strategy of not deploying pyrethroids for IRS remains valid. The results from synergist assays suggest the presence of oxidase-based metabolic resistance mechanisms among vector populations in Luapula and Copperbelt Provinces based on restoration of susceptibility after exposure to a synergist.

Despite vector reductions seen after IRS, vector numbers remain persistently high. Therefore, we recommend revisiting the vector control strategy in Zambia around potential co-deployment of vector control interventions. Consideration should be given to integrated vector management wherein all malaria transmission zones are targeted for ITNs while IRS is deployed only in high transmission zones, whenever this is effective and practical. Larval source management (LSM) could be considered for deployment in some well-characterized and LSM-receptive focal areas to target vectors that do not frequent the indoor environment and to complement existing vector control interventions. Due to the continued resistance of local vectors to pyrethroid insecticides, we recommend continuing to transition away from standard pyrethroid-only ITNs to the deployment of PBO or next-generation nets with dual active ingredients (that is, pyrethroid plus a pyrrole or pyriproxyfen) in areas where ITNs are the major vector control intervention.

1. INTRODUCTION

Malaria is endemic to Zambia and is transmitted by the *An. gambiae* and *An. funestus* groups of mosquitoes, with the main vector species being *An. gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s. Transmission is stable, with a seasonal peak associated with the rainy season from November to May and peak parasite prevalence occurring towards the end of the transmission season in April to June. Indoor residual spraying (IRS) and insecticide treated nets (ITNs) are the primary vector control interventions implemented in Zambia by the Zambian National Malaria Elimination Program (NMEP). From September 29 to November 18, 2020, the U.S. President's Malaria Initiative (PMI) VectorLink Project supported IRS in 15 districts in three provinces: Eastern (all nine districts; which are currently divided into 14), Copperbelt (the three rural districts), and Luapula (three districts), targeting 629,255 structures using a clothianidin-based insecticide. VectorLink Zambia sprayed 648,914 structures out of 672,581 structures found, resulting in an overall spray coverage of 97%. PMI, through its implementing partners, also supported the 2020/2021 ITN mass campaign through technical assistance at the national, provincial, and district levels and procurement of 1.7 million standard ITNs for Luapula, Northern, and Muchinga Provinces and 372,000 piperonyl butoxide (PBO) ITNs for Eastern Province.

Entomological surveillance is a key component of IRS programming, providing information on the impact of IRS on malaria vector density and behavior in geographic areas where IRS has occurred compared to non-IRS areas. PMI has provided financial and technical support to the NMEP and district health offices for IRS and entomological surveillance activities since 2008. The support was provided through prior PMI IRS programs and transitioned to PMI VectorLink starting in 2018. VectorLink Zambia supports the NMEP through routine entomological surveillance and generates data on key entomological indicators including malaria vector species composition, density, feeding behavior, feeding habits, and parity rate in seven districts. In addition, VectorLink Zambia conducts insecticide susceptibility tests, assesses the quality of spray during the IRS campaign, and monitors the duration of efficacy of the insecticide on the walls after IRS. These data guide the NMEP and other stakeholders on vector control decision making, including insecticide selection, IRS programming, and insecticide resistance management.

This report covers the period August 2020 to July 2021 and is linked to the 2020 IRS campaign. It presents all entomological monitoring activities conducted by PMI VectorLink Zambia and discusses the implications of the results obtained. During the reporting period, entomological monitoring activities were suspended for one month (July 2021) as a risk mitigation measure due to the third wave of the COVID-19 pandemic in Zambia. Vector surveillance activities resumed in August 2021 and the data collected will be reported in the 2021/2022 annual report.

Table 1 below outlines the entomological indicators covered in this report (PMI Technical Guidance FY2022)[2].

[2] PMI Technical Guidance FY 2022 https://d1u4sg1s9ptc4z.cloudfront.net/uploads/2021/03/pmi-technical-guidance-fy2022-1.pdf

Table 1: Entomological Indicators by Collection Method and Frequency of Collection

Indicator	Collection Methods	Frequency	Parameters measured
Vector species composition and abundance	PSC, HLC	Every 1-2 months*	Number and relative proportion of mosquito species captured
Indoor resting density	PSC	Every 1-2 months*	Number of mosquitoes collected per house
Vector feeding location	HLC	Every 1-2 months*	Indoor and outdoor biting rates: Nightly human biting rates - number of mosquito bites per person per night
Vector feeding time	HLC	Every 1-2 months*	Indoor and outdoor biting rates: Hourly human biting rates- number of mosquito bites per person per hour
Sporozoite rate	HLC	Every 1-2 months*	Proportion of mosquitoes with sporozoites
Entomological Inoculation Rate	HLC	Every 1-2 months*	Number of infectious bites an individual is exposed to in a given time period: Product of biting rate and sporozoite rate
Human/animal blood indices	PSC	Every 1-2 months*	Human blood index: Portion of mosquito blood meals taken on humans versus animals
Parity rate	HLC	Every 1-2 months*	Percentage of vectors that are parous
Spray quality assurance	Insectary colony mosquitoes	Once per year, within 48 hours of spray	Percentage mortality up to seven days
Residual efficacy monitoring	Insectary colony mosquitoes	Monthly[1]	Percentage mortality up to seven days
Insecticide susceptibility	Larval and adult collections	Once per reporting period[a]	Percentage mortality at 24 hours or at seven days, depending on insecticide type

HLC=Human Landing Catch, PSC=Pyrethrum Spray Catch; [1]Conducted monthly after spray campaign until mortality below 80% for two consecutive months.

*Data were collected monthly during the reporting period in three districts (Nchelenge, Mambwe and Lufwanyama, bimonthly from August 2020 to April 2021 and monthly thereafter at the other four districts (Milenge, Katete, Serenje, and Chililabombwe).

[a]Tests conducted between December 2020 and May 2021.

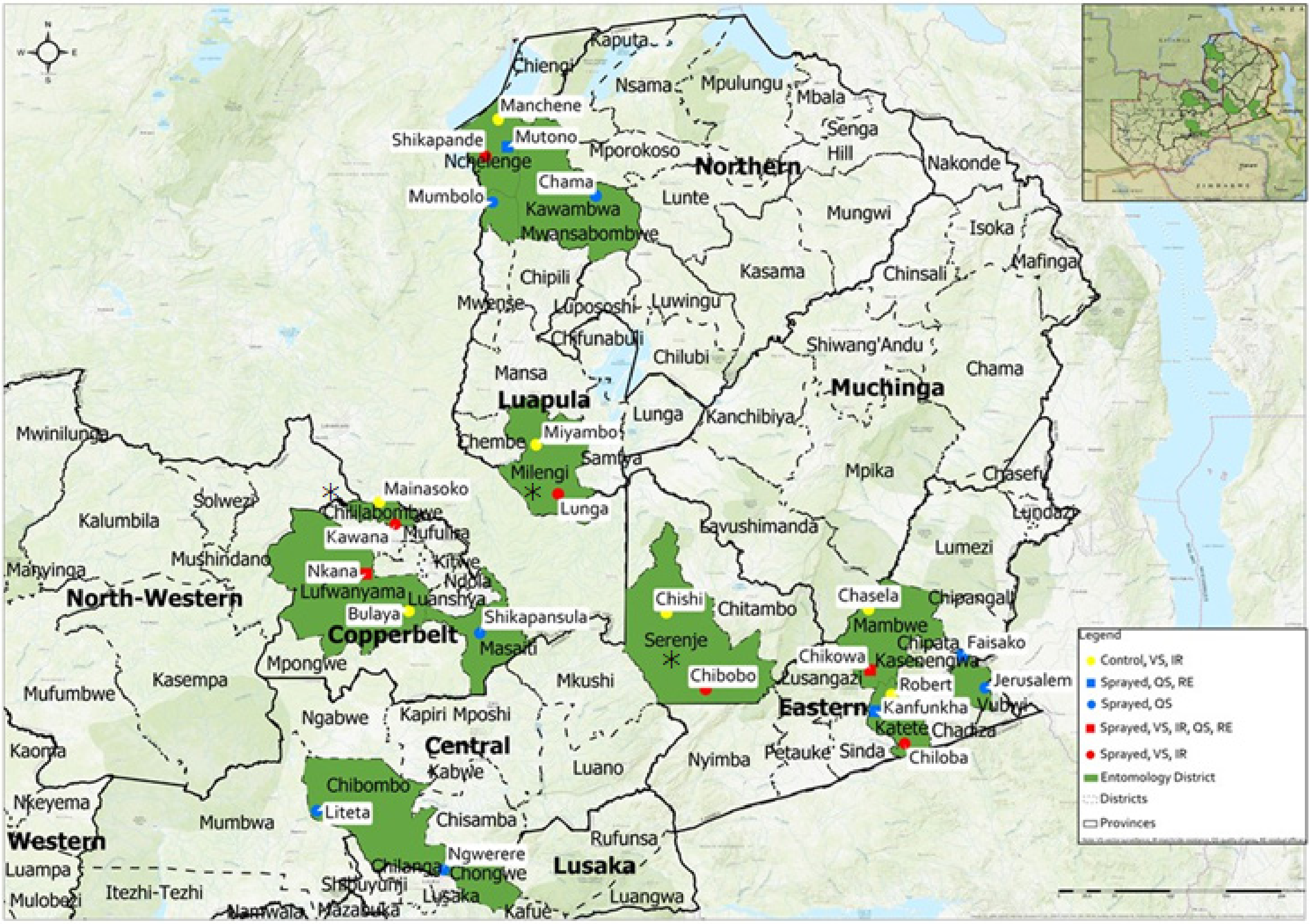
2. MATERIALS AND METHODS

2.1 MONITORING SITES

From August 2020 to June 2021, VectorLink Zambia conducted malaria vector surveillance and insecticide resistance monitoring activities in 14 sentinel sites in four PMI-supported IRS districts (Nchelenge, Mambwe, Katete, and Lufwanyama) and three non-PMI supported IRS districts (Milenge, Chililabombwe and Serenje). Quality of IRS was assessed in seven districts (Nchelenge, Kawambwa, Mambwe, Chipata, Katete, Masaiti and Lufwanyama) in October 2020 during the IRS campaign, while monthly monitoring of the residual efficacy of the insecticide on the walls was conducted in five districts (Nchelenge, Mambwe, Chipata, Katete, and Lufwanyama). Insecticide resistance testing was conducted in the 14 sentinel sites for the main insecticides currently deployed in Zambia and other potential IRS insecticides. Entomological monitoring activities were suspended for the month of July as COVID-19 risk mitigation precaution occasioned by the intensity of the third wave of the pandemic in Zambia.

VectorLink Zambia conducted IRS in four of the intervention sentinel sites (Shikapande in Nchelenge District, Chikowa in Mambwe District, Chiloba in Katete District, and Nkana in Lufwanyama District) in October 2020. The Government of the Republic of Zambia (GRZ) conducted IRS in the other three intervention sites (Lunga in Milenge District and Kawama in Chililabombwe District in November 2020, and Chibobo in Serenje District in December 2020). Fludora Fusion was sprayed at all PMI-supported sites except Chikowa in Mambwe District which was sprayed using SumiShield 50WG. In the non-PMI supported sites, SumiShield 50WG was sprayed in Kawama-Chililabombwe while dichlorodiphenyltrichloroethane (DDT) was sprayed in Chibobo-Serenje and Lunga-Milenge. Figure 1 below is a map showing the location of all entomological monitoring sentinel sites in their respective districts.

Figure 1: Geographical Locations of PMI-Supported Entomological Monitoring Sites in Zambia (August 2020-July 2021)



Note: * GRZ Districts, VS-vector surveillance, IR-insecticide resistance, QS-quality of spray, RE-residual efficacy

A site is a cluster of households and is typically a single village or a continuous string of villages within a catchment area of the district. The control (unsprayed) sites were selected as the nearest available unsprayed cluster to the corresponding sprayed cluster. The clusters selected as control sites were usually not targeted for IRS due to factors such as hard-to-reach areas and sparsely distributed houses. Control sites were at least two kilometers from any sprayed structures. In line with the current national malaria strategy, unsprayed sites were provided with ITNs. Further details of the monitoring sites according to the activities conducted are shown in Table 2.

Table 2: Entomological Monitoring Sites

<table>
<tr><th>Province</th><th>District</th><th>Health Facility Catchment Area</th><th>Sentinel Site (Village)</th><th>Spray Status (Distance to Nearest Sprayed Community)</th><th>Percent of Households Targeted for IRS by PMI/VL in 2020*</th></tr>
<tr><td colspan="6">Vector Surveillance and Insecticide Resistance Monitoring</td></tr>
<tr><td rowspan="4">Luapula</td><td rowspan="2">Nchelenge</td><td>Lushiba</td><td>Shikapande</td><td>Sprayed with Fludora Fusion</td><td>100%</td></tr>
<tr><td>Kafutuma</td><td>Manchene</td><td>Non-sprayed control (3km)</td><td>0%</td></tr>
<tr><td rowspan="2">Milenge</td><td>East Seven</td><td>Lunga</td><td>Sprayed with DDT</td><td>100% (by GRZ)</td></tr>
<tr><td>East Seven</td><td>Miyambo</td><td>Non-Sprayed control (7km)</td><td>0%</td></tr>
<tr><td rowspan="4">Eastern</td><td rowspan="2">Mambwe</td><td>Chikowa</td><td>Chikowa</td><td>Sprayed with SumiShield</td><td>100%</td></tr>
<tr><td>Chikowa</td><td>Chasela</td><td>Non-Sprayed control (6km)</td><td>0%</td></tr>
<tr><td rowspan="2">Katete</td><td>Katiula</td><td>Chilowa</td><td>Sprayed with SumiShield</td><td>100%</td></tr>
<tr><td>Kamphambe</td><td>Robert</td><td>Non-Sprayed control (10km)</td><td>0%</td></tr>
<tr><td rowspan="2">Central</td><td rowspan="2">Serenje</td><td>Chibobo</td><td>Chibobo</td><td>Sprayed with DDT</td><td>100% (by GRZ)</td></tr>
<tr><td>Chibobo</td><td>Chishi</td><td>Non-Sprayed control (5km)</td><td>0%</td></tr>
<tr><td rowspan="4">Copperbelt</td><td rowspan="2">Lufwanyama</td><td>Nkana</td><td>Nkana</td><td>Sprayed with Fludora Fusion</td><td>100%</td></tr>
<tr><td>Bulaya</td><td>Bulaya</td><td>Non-Sprayed control (4km)</td><td>0%</td></tr>
<tr><td rowspan="2">Chililabombwe</td><td>Kawama</td><td>Kawama</td><td>Sprayed with Fludora Fusion</td><td>100% (rural/peri-urban)</td></tr>
<tr><td>Kawama</td><td>Mainasoko</td><td>Non-Sprayed control (6km)</td><td>0%</td></tr>
<tr><td colspan="6">IRS Quality Assurance (QA) and Insecticide Residual Efficacy Monitoring</td></tr>
<tr><td>Luapula</td><td>Nchelenge</td><td>Lushiba</td><td>Shikapande</td><td>Sprayed with Fludora Fusion</td><td>100%</td></tr>
<tr><td>Eastern</td><td>Mambwe</td><td>Chikowa</td><td>Chikowa</td><td>Sprayed with SumiShield</td><td>100%</td></tr>
<tr><td>Eastern</td><td>Chipata</td><td>Namseche</td><td>Margazine (QA only)</td><td>Sprayed with SumiShield</td><td>100%</td></tr>
<tr><td>Eastern</td><td>Katete</td><td>Kafunkha</td><td>Kafunkha</td><td>Sprayed with SumiShield</td><td>100%</td></tr>
<tr><td>Copperbelt</td><td>Masaiti</td><td>Chilese</td><td>Shikapansula (QA only)</td><td>Sprayed with Fludora Fusion</td><td>100%</td></tr>
<tr><td>Copperbelt</td><td>Lufwanyama</td><td>Nkana</td><td>Nkana</td><td>Sprayed with Fludora Fusion</td><td>100%</td></tr>
<tr><td>Copperbelt</td><td>Chililabombwe</td><td>Kawama</td><td>Kawama</td><td>Sprayed with Fludora Fusion</td><td>100% (rural/peri-urban)</td></tr>
</table>

*In practical terms, 100% indicates that 100% of households in the local community around the operational sites were targeted.

2.2 LONGITUDINAL MONITORING OF MALARIA VECTOR DENSITY AND BEHAVIOR

Vector surveillance was conducted at two sentinel sites (one sprayed and one unsprayed) in each of the seven districts using pyrethrum spray catch (PSC) (Standard Operating Procedure (SOP) 03/01)[3], and human landing catches (HLCs) (SOP 02/01) (see Table 3). Adult mosquitoes were collected from all sites from August 2020 to June 2021 either monthly (for sites in Nchelenge, Mambwe, and Lufwanyama) or bimonthly at the sites in the other four districts (Milenge, Katete, Serenje, and Chililabombwe) up to April 2021 and then monthly thereafter.

Entomological monitoring to assess the impact of IRS on malaria vectors started the same month the intervention sites were sprayed (October 2020 for sentinel sites in PMI-supported districts, November 2020 for Chililabombwe and Milenge, and December 2020 for the sites in Serenje).

Table 3: Adult Mosquito Collection Methods for Vector Surveillance

Method	Time	Frequency*	Sample
PSC	4:00 a.m. to 6:00 a.m.	Monthly or once every two months (in some districts)	15 houses per site
HLC	6:00 p.m. to 8:00 a.m.	Monthly or once every two months (in some districts)	Four houses, four consecutive nights per house, indoor and outdoor

*In Milenge, Katete, Serenje, and Chililabombwe, collections were done every other month from August 2020-April 2021 and then monthly from May-July 2021. In Nchelenge, Mambwe, and Lufwanyama, collections were monthly throughout the work plan period.

2.2.1 PYRETHRUM SPRAY CATCHES

At each of the 14 sentinel sites, 15 houses (five distinct houses per day over three consecutive days) were identified for sampling indoor-resting mosquitoes between 4:00 and 6:00 a.m. in each collection month. Collections were done in the same 15 houses throughout the data collection period, except in a few cases where the house owner was absent, and the nearest available house was recruited for that day. Before the PSCs were performed, all occupants were asked to vacate the house without disturbing the resting mosquitoes. Pressurized 300ml spray cans of Raid (SC Johnson & Son S.A. Ltd) were used to knock down the mosquitoes. Raid contains the pyrethroids tetramethrin 0.2% w/w, prallethrin 0.04% w/w, imiprothrin 0.034% w/w, and the synergist piperonyl-butoxide (PBO) 1.15% w/w. Mosquitoes were collected by PSC following the procedures on SOP 03/01.

The following parameters were measured from PSC at each sentinel site: species composition, indoor resting density, and vector abdominal status.

2.2.2 HUMAN LANDING CATCHES

Four houses were selected for HLCs at each of the 14 sentinel sites. HLCs were used to monitor mosquito feeding behavior. At each site, mosquitoes were collected indoors and outdoors in each house for four consecutive nights during each collection month to yield 16 person-nights indoors and 16 person-nights outdoors per site per month. The same houses were used each time throughout the surveillance period. Community-based mosquito collectors trained on the HLC technique participated in the collections and worked in pairs—one collector was seated indoors and another seated outdoors (within five meters of the front of the house) from 6:00 p.m. to 1:00 a.m. The pair was replaced by another pair of collectors from 1:00 to 8:00 a.m., meaning four collectors per house per night participated in collections from 6:00 p.m. to 8:00 a.m.

[3] Complete SOPs can be found here: https://pmivectorlink.org/resources/tools-and-innovations/

Mosquitoes were collected by the human landing catches following the procedures on SOP 02/01. All community-based collectors involved in the HLCs were provided malaria chemoprophylaxis with Deltaprim (pyrimethamine and dapsone). In addition, the temperature of each collector was checked using infra-red thermometers and a short questionnaire on COVID-19 symptoms was administered. Collectors that were experiencing fever or any other COVID-19 symptom, or had been in recent contact with someone with COVID-19, were not allowed to participate as a risk mitigation measure.

The following parameters were measured from the HLCs at each sentinel site: species composition, human biting rate (HBR), vector feeding behavior (time and location of biting), parity rate, sporozoite rate, and entomological inoculation rate (EIR).

2.3 QUALITY ASSURANCE OF IRS AND MONITORING INSECTICIDE RESIDUAL EFFICACY

Cone bioassays (SOP 09/01) using a susceptible *An. gambiae* s.s. Kisumu strain were conducted once during the month of the IRS campaign to confirm the quality of spray and monthly thereafter to assess the residual efficacy of the insecticides on the walls. This was performed in the PMI-supported entomological surveillance sites, and therefore does not provide data on the quality of spraying in the two Global Fund (GF)/GRZ program areas where we conduct entomological surveillance.

Quality of spray was done at the seven sites in PMI-supported IRS program districts, namely: Mutono Village (Nchelenge District), Chama Village (Kawambwa District), Kafunkah Village (Katete District), Shikapansula Village (Masaiti District) and Nkana Village (Lufwanyama District) sprayed with Fludora Fusion, and Chikowa Village (Mambwe District) and Jerusalem Village (Chipata District) sprayed with SumiShield during the 2020 IRS campaign. Based on a request from the National Malaria Elimination Centre (NMEC), we also conducted quality of spray checks at three GF/GRZ supported districts that were sprayed with DDT: Mumbolo (Mwansabombwe District in Luapula Province), Ngwerere (Chongwe District in Lusaka Province), and Liteta (Chibombo District in Central Province).

At each site, six sprayed houses—three mud and three cement—were randomly selected for bioassays. In addition, two unsprayed, control houses—one mud and one cement—were used as negative controls (See Table 4). When control houses were not available, an untreated surface such as a mud brick or a cement brick carried by the field technicians was used for the purpose. A total of 42 houses were involved in the quality assurance activity in the PMI-supported districts—18 houses in the SumiShield sprayed areas and 24 houses in the Fludora Fusion sprayed areas. Cone bioassays were conducted 24 to 48 hours after spraying and within two weeks of the spray campaign (T0) to gauge quality of spray. In each house, 30 susceptible, 3–5-day-old, unfed, female *An. gambiae* s.s. Kisumu strain mosquitoes were exposed to the walls in replicates of 10 per cone.

Table 4: Quality Assurance and Insecticide Residual Efficacy Activities

Activity	Frequency	Sample
Quality assurance of IRS	Once within 24-48 hours of spraying during the first two weeks of the campaign	Eight houses per site (sprayed: three mud and three cement; unsprayed: one mud and one cement)
Monitoring of insecticide decay rate on walls	Monthly, until exposed mosquito mortality falls below 80% for two consecutive months	Eight houses per site (sprayed: three mud and three cement; unsprayed: one mud and one cement)

Longitudinal monitoring of the insecticide decay rate on walls after IRS was done in 30 houses (six houses each in Mambwe and Chipata where SumiShield was sprayed, and six houses each in Nchelenge, Katete, and Lufwanyama Districts where Fludora Fusion was used). The cone bioassays were repeated monthly.

The cone bioassays were conducted following the procedures on SOP 09/01. A replicate of 10 mosquitoes was placed in a paper cup one meter above the floor of each house and about 0.1 meter from the sprayed wall to assess the fumigant (airborne) effect of the insecticide. The number of mosquitoes knocked down after 30 minutes and 60 minutes and the number dead after every 24-hour holding period were recorded up to seven days. When the mortality of the control was between 5-20%, corrected mortality was determined using Abbot's formula.

Fumigant effect refers to the release of the insecticide from the sprayed wall into the air (airborne) which produces a lethal effect on mosquitoes flying inside the house or resting on other (non-sprayed, insecticide-free) surfaces in the house. Monitoring of fumigant effect has been a part of PMI VectorLink's bioassay procedures since the deployment of pirimiphos-methyl due to documented airborne effect of this insecticide. The procedure was extended to the new neonicotinoid insecticides to determine if these new products also exhibit the fumigant effect. Data from multiple countries has indicated some level of airborne effect of these products; the consensus is to continue monitoring to obtain adequate data on the duration of this phenomenon.

2.4 INSECTICIDE RESISTANCE MONITORING

Susceptibility of *An. funestus* s.l. and *An. gambiae* s.l. mosquitoes to the insecticides used in IRS or ITNs, DDT (an organochlorine), clothianidin (a neonicotinoid insecticide) and in ITNs deltamethrin and alpha-cypermethrin (pyrethroids) was assessed at sites in all entomological monitoring sentinel districts. A new product chlorfenapyr (a pyrrole insecticide) awaiting WHO prequalification for IRS was also tested. Given the susceptibility of the mosquitos shown to DDT at some sites in Zambia, the GRZ deployed DDT in specific areas of the country during the 2020 IRS campaign. Clothianidin is the main active ingredient in the two chemicals used for IRS by VectorLink Zambia in 2020 (SumiShield and Fludora Fusion); Fludora Fusion also contains deltamethrin. Pirimiphos-methyl (an organophosphate) was also tested in a few sites; we did not prioritize it this year because we have many years of data showing susceptibility, and it was not deployed in the 2021 IRS campaign.

2.4.1 WHO SUSCEPTIBILITY TESTS

WHO susceptibility tests (SOP 06/01) were performed on 2-5 day-old unfed adult *An. funestus* s.l. and *An. gambiae* s.l. mosquitoes collected from the 14 surveillance sentinel sites. The mosquitoes were sampled either as larvae or pupae collected from larval habitats and reared to adults or wild unfed female mosquitoes collected from houses using battery-operated CDC backpack and Prokopack aspirators. The mosquitoes were exposed to diagnostic doses of various insecticides using insecticide-impregnated papers, as described by WHO guidelines. Susceptibility of *An. funestus* s.l. and *An. gambiae* s.l. to clothianidin 2.0% (a neonicotinoid), DDT 4.0% (an organochlorine), and deltamethrin 0.05% (a pyrethroid), pirimiphos methyl 0.25% (an organophosphate) were tested in select sentinel sites.

The exposure time was 60 minutes, after which mosquitoes were transferred into the holding tubes and provided with 10% sugar solution. For the clothianidin tests, mortality was recorded after 24 hours, and again at 48 hours and 72 hours while, for the other insecticides, mortality was recorded after 24 hours only. Mortality for clothianidin-exposed mosquitoes is recorded over a longer period due to the slow-acting nature of the insecticide on mosquitoes. The sugar solution was changed daily during the holding periods. Susceptibility tests were done from December 2020 to May 2021.

Clothianidin papers used in the susceptibility tests were locally impregnated following procedures developed by the PMI VectorLink project. In this procedure, Whatman® No. 1 filter papers measuring 12 cm by 15 cm were treated with the diagnostic dose of clothianidin (2% w/v) which is 13.2 mg active ingredient per paper, equivalent to 734 mg ai/m^2. Firstly, 26.4 mg of SumiShield 50WG (containing 50% clothianidin as active ingredient) was suspended in two milliliters of distilled water and the resulting suspension (containing 13.2mg ai) was shaken well before pipetting it onto the filter paper. After drying overnight, the filter papers were stored in aluminum foil at 4°C in the fridge. Papers were freshly prepared for each test. Control papers were prepared by pipetting two milliliters of distilled water on the Whatman® No. 1 filter paper. With the availability of technical grade clothianidin and a new protocol[4], future susceptibility tests of this product will involve the use of CDC bottle assays.

2.4.2 CDC Bottle Assays

CDC bottle assays were used to assess the susceptibility status of *An. funestus* s.l. and *An. gambiae* s.l. to chlorfenapyr (100 µg) at some sites. The standard CDC bottle assay procedures were followed (SOP 04/01); the exposure time was 60 minutes and the mortality was recorded one hour, 24 hours, 48 hours, and 72 hours after exposure. The bottles were coated each month with technical grade chlorfenapyr supplied by BASF at the NMEC laboratory and transported to the field in compartmentalized cardboard boxes for the assays. Each bottle was used a maximum of three times and were returned to Lusaka for cleaning and reuse.

2.5 Laboratory Analysis

Mosquitoes collected by HLCs were killed using cotton wool soaked in ethyl acetate[5] to enable pre-laboratory handling. Live *Anopheles* mosquitoes in paper cups were placed in an airtight container containing the soaked cotton wool and were preserved on silica gel prior to laboratory analyses[6]. Identified vectors were counted according to house number (in case of PSC samples) and by house number, location, and hour of collection (for HLC samples). The abdominal status of all female *Anopheles* collected by PSC were categorized as either unfed, blood-fed, or gravid. All collected *Anopheles* mosquitoes were preserved in 1.5ml Eppendorf tubes with silica gel desiccant. A hole was pierced in the cap of the tube and the tubes were kept in transparent Ziploc bags also containing silica gel and stored at the NMEC laboratories in Lusaka. A sub-set of preserved *An. funestus* s.l. and *An. gambiae* s.l. from sprayed and unsprayed sentinel sites were processed to: 1) identify the sibling species and the source of the blood meal (blood-fed samples only) using polymerase chain reaction (PCR[7,8], and 2) detect circumsporozoite proteins of *Plasmodium falciparum* sporozoites[9] using Enzyme-Linked Immunosorbent Assays (ELISAs)[10]. *An. gambiae* s.l. samples that were resistant to pyrethroids were analyzed by PCR for the presence of the kdr allele.

2.6 Data Presentation and Statistical Analysis

Database. The DHIS2-based VectorLink Collect instance for entomological data management was used in Zambia for the first time in 2020. PMI VectorLink Home Office staff remotely trained and supported VectorLink Zambia entomology technicians and database managers on updated data workflows, including field paper collections, technical reviews, data entry, data cleaning, and analytics, to support the generation and use of high-quality entomological data.

Starting in 2020, all entomological data collected in Zambia was managed within VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results. All results presented here were downloaded as data tables directly from the VectorLink Collect platform except the laboratory data which was derived from the locally maintained molecular laboratory database. By the end of 2021, stakeholders including NMEP and PMI will have ongoing access to these results dashboards to support timely decision-making. Additionally, the NMEP, through the recently

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[4] https://pmivectorlink.org/resources/tools-and-innovations/

[5] Note: Standard protocols and Safety datasheets are followed when using ethyl acetate

[6] Coetzee, M. Key to the females of Afrotropical Anopheles mosquitoes (Diptera: Culicidae). Malar J 19, 70 (2020)

[7] Scott JA, Brogdon WG, Collins FH: Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain-reaction. Am J Trop Med Hyg. 1993, 49: 520-529.

[8] SOP for blood meal PCR adapted from 2016 Methods in Anopheles Research Manual (2015 Edition) Chapter 8.3 Molecular identification of mammalian blood meals from mosquitoes.

[9] The reagent was obtained through BEI Resources, NIAID, NIH: *Plasmodium falciparum* Sporozoite ELISA Reagent Kit, MRA-890, contributed by Robert A. Wirtz.

[10] Wirtz RA, Zavala F, Charoenvit Y, et. Al. (1987): Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, Andre RG: Comparative testing of monoclonal antibodies against Plasmodium falciparum sporozoites for ELISA development. Bull World Health Org., 65: 39-45.

formed Entomology Data Management Committee, will receive the raw data on a regular basis for hosting on a yet-to-be-determined database platform.

Mosquito Collection Data. Data obtained from PSC were used to determine the indoor resting density (the average number of mosquitoes per house per night) and the abdominal status of the vectors (proportion of vectors that are gravid), while data from HLCs were used to estimate the human biting rate (mean number of mosquitoes collected per person per night) and vector parity rate (proportion of parous vectors). Indoor resting densities, human biting rates, and parity rates are presented with standard errors or 95% confidence intervals to compare variations between IRS and non-IRS sites. Biting times are presented as averages of hourly human bites from each of the monthly/bimonthly HLC efforts. To determine the impact of IRS on sibling species composition, human blood index, Sporozoite rate and EIR, data was categorized into pre-IRS period (August-September or October or November 2020 depending on month of spray in the different districts) and post-IRS (October, November, or December through June 2021 and transmission indicators between these two periods were compared.

Rainfall Data. Rainfall data is based on the Level 3 Global Precipitation Measurement (GPM) mission's Integrated Multi-satellite Retrievals of GPM data obtained from the Giovanni online data system, developed and maintained by the U.S. National Aeronautics and Space Administration Goddard Earth Sciences Data and Information Services Center[11]. The following were the GPS boundaries (user bounding box) used for each of the districts to obtain the area averaged merged satellite-gauge precipitation estimates for each month: Nchelenge District (28.3582,-9.7358,29.2179,-8.8476), Milenge District (28.7641,-12.472,29.573,-11.2996), Mambwe District (31.5023,-13.8327,32.5043,-12.9759), Katete District (31.449,-14.4233,32.3172,-13.7847), Serenje District (29.8071,-13.9302,31.429,-12.0005), Lufwanyama District (26.8413,-13.3908,28.3292,-12.3289), and Chililabombwe District (27.4992,-12.4636,28.0234,-12.2204).

Collection Periods (Months Relative to IRS Implementation). Given that not all districts were sprayed at the same time (for instance, Serenje was sprayed in December 2020 while the other districts were sprayed in October and November 2020), data in the graphs that combine districts are presented by number of months relative to the month of IRS implementation (e.g., T-1 is one month before IRS, T+1 is one month after IRS) instead of calendar months (see Table 5). This allows for comparison between and across districts.

Table 5: Month and Year for Collection Period (Months Relative to IRS) for Each District (August 2020-February 2021)

Collection period (months relative to IRS)	Luapula Province		Eastern Province		Central Province	Copperbelt Province	
	Nchelenge District	Milenge District	Mambwe District	Katete District	Serenje District	Lufwanyama District	Chililabombwe District
T-3	-	-	-	-	Sep-20	-	-
T-2	Aug-20	Sep-20	Aug-20	-	-	Aug-20	Sep-20
T-1	Sep-20	-	Sep-20	Sep-20	Nov-20	Sep-20	Oct-20
T-0	Oct-20	Nov-20	Oct-20	-	Dec-20	Oct-20	Nov-20
T+1	Nov-20	-	Nov-20	Nov-21	Jan-21	Nov-20	-
T+2	Dec-20	Jan-21	Dec-20	-	-	Dec-20	Jan-21
T+3	Jan-21	-	Jan-21	Jan-21	Mar-21	Jan-21	-
T+4	Feb-21	Mar-21	Feb-21	-	-	Feb-21	Mar-21
T+5	Mar-21	-	Mar-21	Mar-21	May-21	Mar-21	-
T+6	Apr-21	May-21	Apr-21	-	Jun-21	Apr-21	May-21
T+7	May-21	Jun-21	May-21	May-21	-	May-21	Jun-21
T+8	Jun-21	-	Jun-21	Jun-21	-	Jun-21	-

11 https://giovanni.gsfc.nasa.gov

Statistical Analysis. To determine the impact of IRS on entomological indicators, we performed negative binomial regressions with random effects for overall and district-level data, and fixed effect for site-specific data using house numbers or site names as the repeated measure to explain changes in entomological parameters measured in sprayed sites compared to unsprayed sites and during the period before IRS compared to the period after IRS. We considered five main parameters: 1) number of indoor resting vectors, 2) number of gravid vectors, 3) number of human biting vectors, 4) number of indoor versus outdoor bites, and 5) number of parous vectors, with separate analyses for *An. funestus* s.l. and for *An. gambiae* s.l.

3. RESULTS

Results from all entomological monitoring activities conducted during the period August 2020 to June 2021 are presented below. Vector surveillance by HLC and PSC were conducted bimonthly as well as monthly from August 2020 to June 2021 in the sentinel districts to assess vector species composition, density, and behavior. The 2020 IRS campaign by PMI VectorLink began in October 2020, and thus baseline vector surveillance data was collected in August and September 2020, and post-IRS data was collected from October 2020 to June 2020. Due to the third wave of COVID-19 in Zambia, entomological monitoring activities were suspended for July 2020 and no HLCs or PSCs were done in that month (the planned end date for vector surveillance in the 2020/2021 reporting period). Restrictions imposed on the number of staff that can work in the laboratory at NMEC (a COVID-19 mitigation measure) affected the proposed schedule for processing the mosquito samples with fewer samples analyzed than targeted at the time of reporting. Residual efficacy monitoring commenced in October 2020 and continued monthly through August 2021 (except for July, when all entomological monitoring activities were suspended due to increased COVID-19 cases across the country). Cone bioassays conducted in August 2021 provide insecticide residual efficacy data at 10 months post-IRS. Insecticide resistance tests were performed from December 2020 to May 2021.

3.1 LONGITUDINAL MONITORING OF VECTORS

3.1.1 SPECIES COMPOSITION

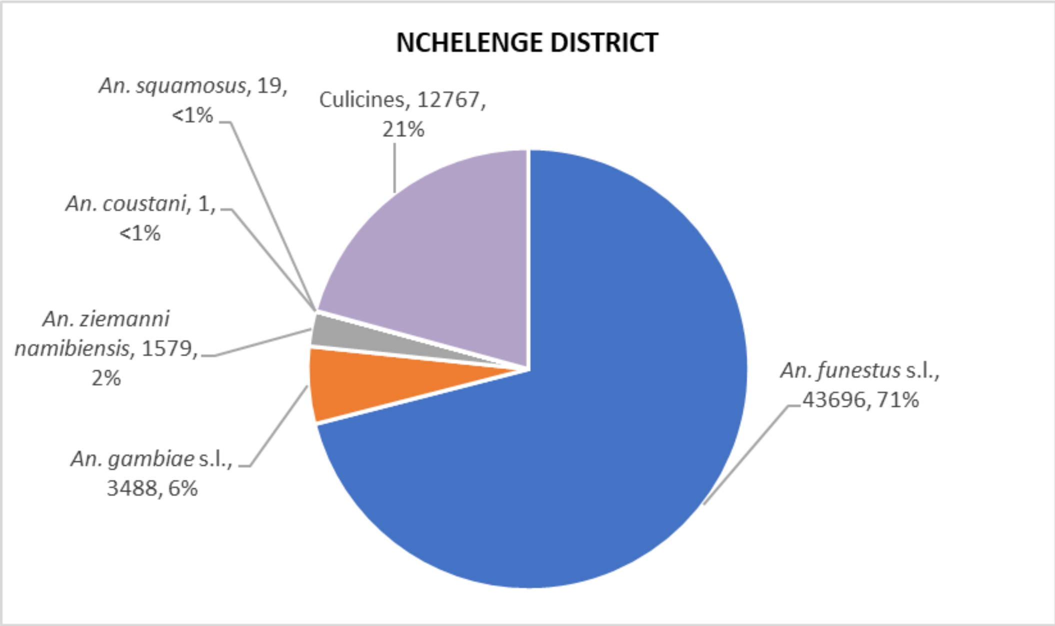
A total of 135,004 mosquitoes were collected by HLC and PSC during the reporting period. *An. funestus* s.l. was the most abundant (53.8%), followed by culicines (18.6%), *An. ziemanni namibiensis* (14.9%), *An. gambiae* s.l. (8.1%), and *An. tchekedii* (3.0%). Other species (*An. coustani*, *An. maculipalpis*, *An. squamosus*, *An. rufipes*, *An. argentiolobatus*, *An. gibbinsi*, *An. pretoriensis,* and *An. tenebrosus*) accounted for 1.6% of the total collected.

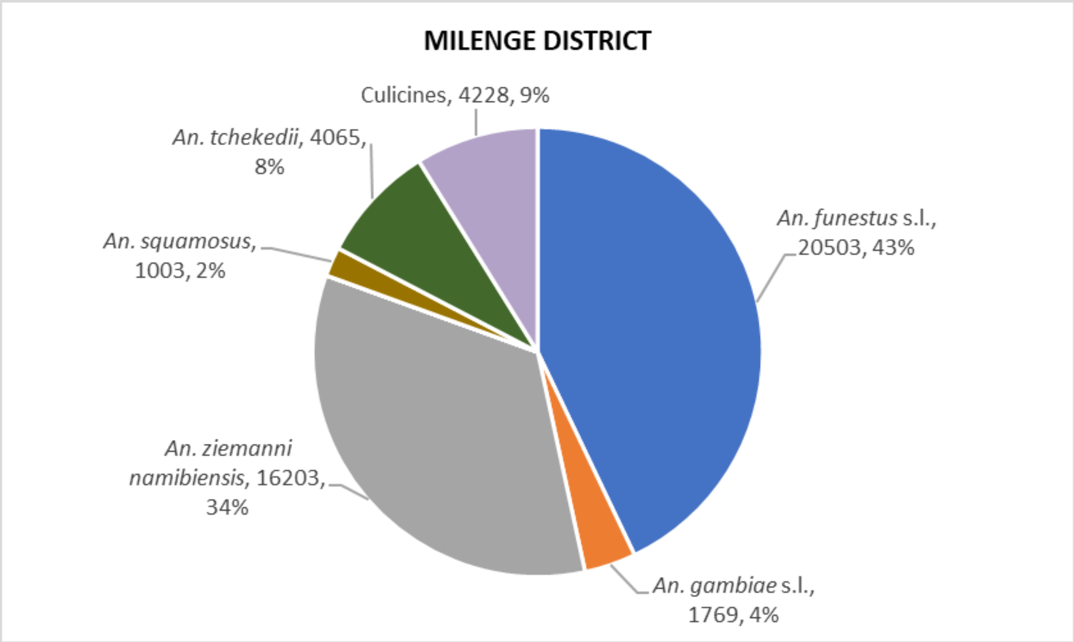
Out of the 83,644 primary vector complexes collected, *An. funestus* s.l. accounted for 86.9% (72,663), while *An. gambiae* s.l. accounted for 13.1% (10,981). The distribution of the different species varied according to district. District level species composition grouped by province are presented in Figure 2A-D.

In Luapula Province, *An. funestus* s.l. was the predominant species among the two primary vectors (*An. funestus* s.l. constituted 92%, and *An. gambiae* s.l. 8%). There was a high presence of *An. ziemanni namibiensis* in Milenge District (34% of all *Anopheles* collected) (Figure 2A). In Eastern Province, among the two primary vectors, *An. gambiae* s.l. was the predominant species in Mambwe District (84%), while *An. funestus* s.l. was the predominant species in Katete District (96%). There was notable presence of *An. coustani* in both districts in Eastern Province. Among the primary vectors in Central Province, *An. funestus* s.l. (96%) was the predominant species; *An. ziemanni namibiensis* constituted 14% of all mosquitoes collected and *An. squamosus* constituted 10% (Figure 2C). In Copperbelt Province, there was slightly more *An. funestus* s.l. (64%) with a substantial presence of *An. gambiae* s.l. (36%). There was a notable presence of *An. ziemanni namibiensis* in both districts in Copperbelt Province; comprising 15% of all mosquitoes collected in Lufwanyama District and 5% of mosquitoes collected in Chililabombwe District (Figure 2D). Details of the numbers and types of mosquitoes collected by the different collection methods in each sprayed and unsprayed sentinel site are provided in Annex A.

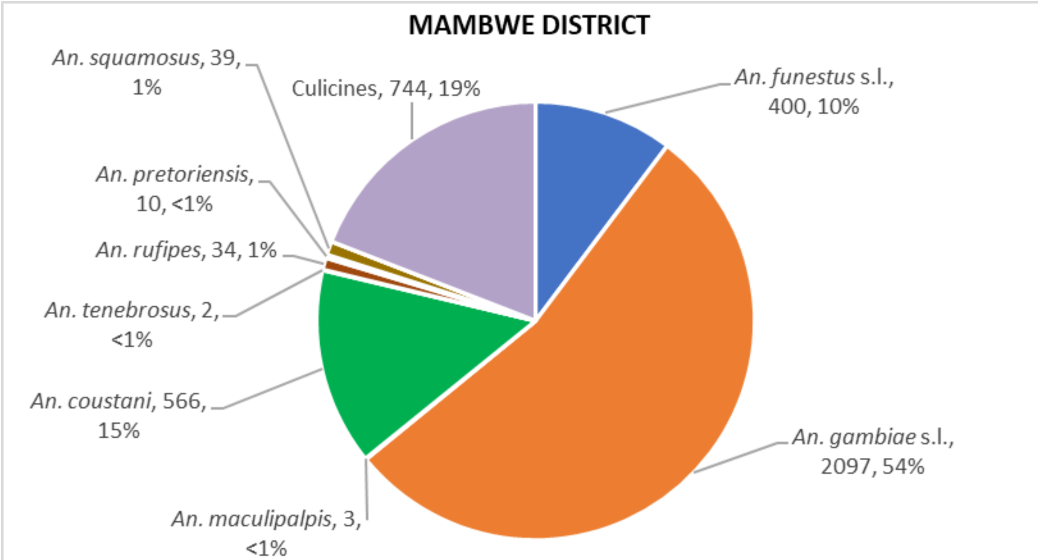
Figure 2: Species Composition by Province and District (August 2020-June 2021)

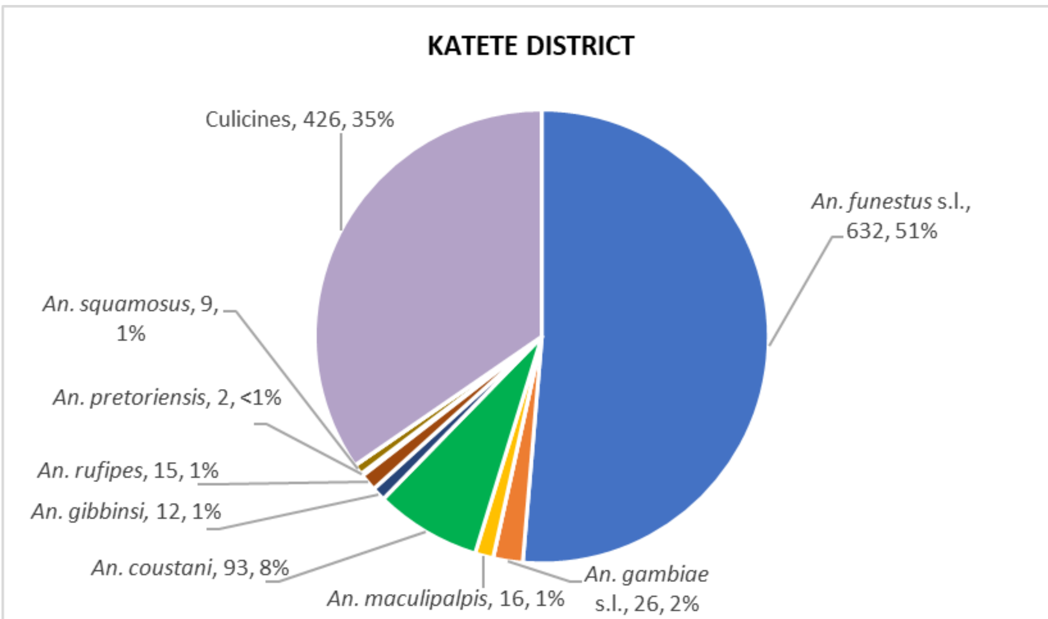
2A: Luapula Province: Nchelenge and Milenge Districts



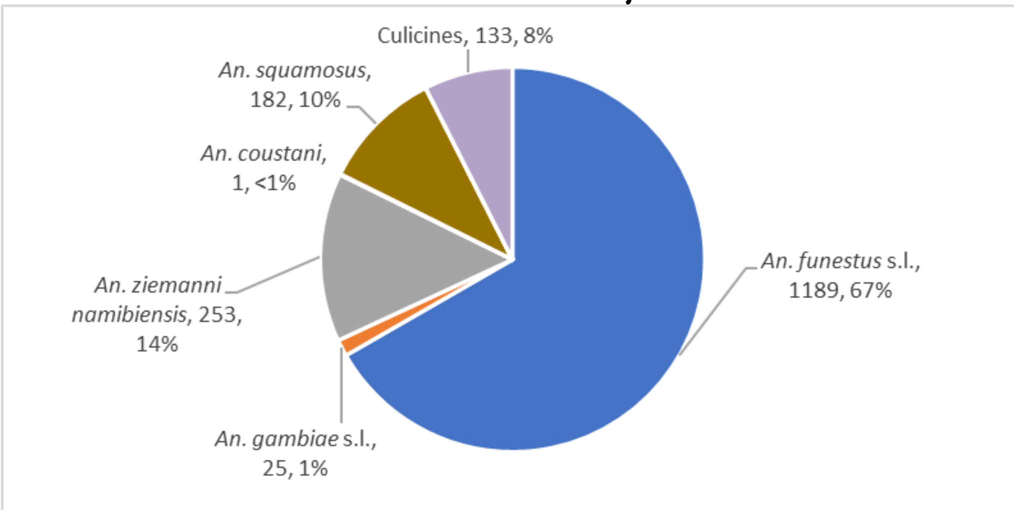


2B: Eastern Province: Mambwe and Katete Districts

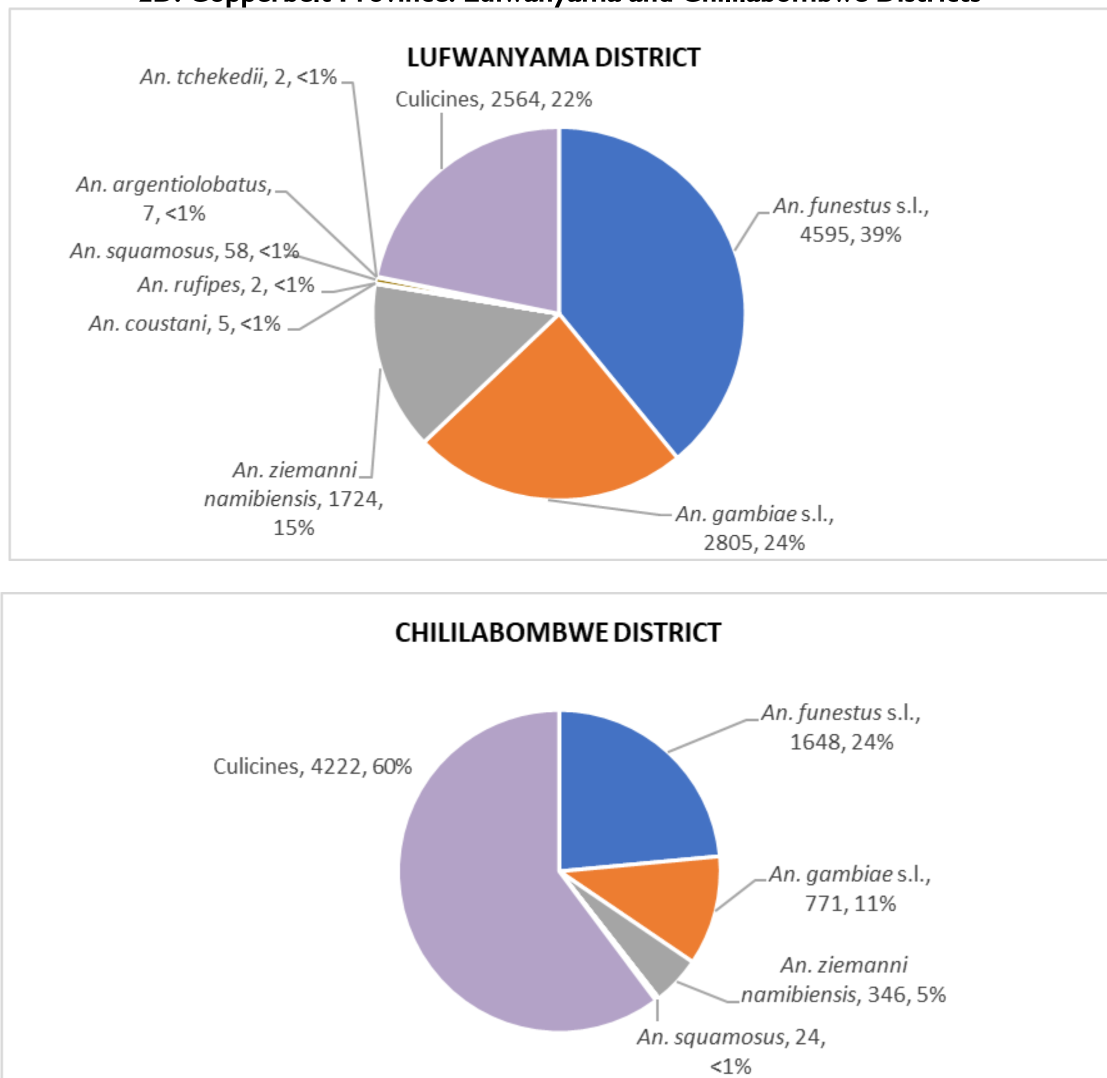




2C: Central Province: Serenje District

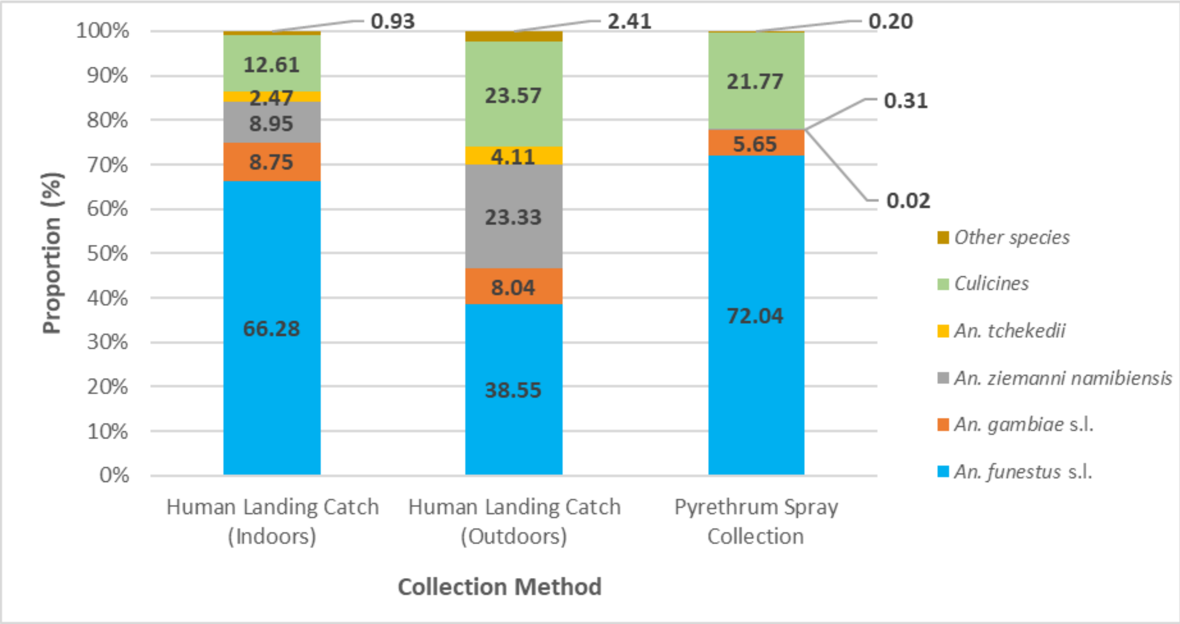


2D: Copperbelt Province: Lufwanyama and Chililabombwe Districts



The species composition by collection method is displayed in Figure 3. All 13 different Culicidae collected over the reporting period were found in the HLC collections, while only eight were found in the PSC collections. The proportion of *An. funestus* s.l. was higher in the indoor collections—PSCs (72%) and indoor HLCs (66%)—compared to outdoor HLC (39%). *An. gambiae* s.l. did not show a marked difference between outdoor and indoor collections (ranging from 6-8%). Higher numbers of other *Anopheles* species were collected outdoors compared to indoors; 29.8% in the outdoor HLC collections compared to 12.4% in the indoor HLC collections and 0.5% using PSCs. Approximately 70% of these non-vector *Anopheles* species were collected outdoors. A total of 74,039 (89%) of the primary vectors were collected from HLCs and 9,605 (11%) were collected from PSCs. Annex B includes the total number of primary vectors collected by site and collection method.

Figure 3: Species Composition across Sites by Collection Method (August 2020-June 2021)

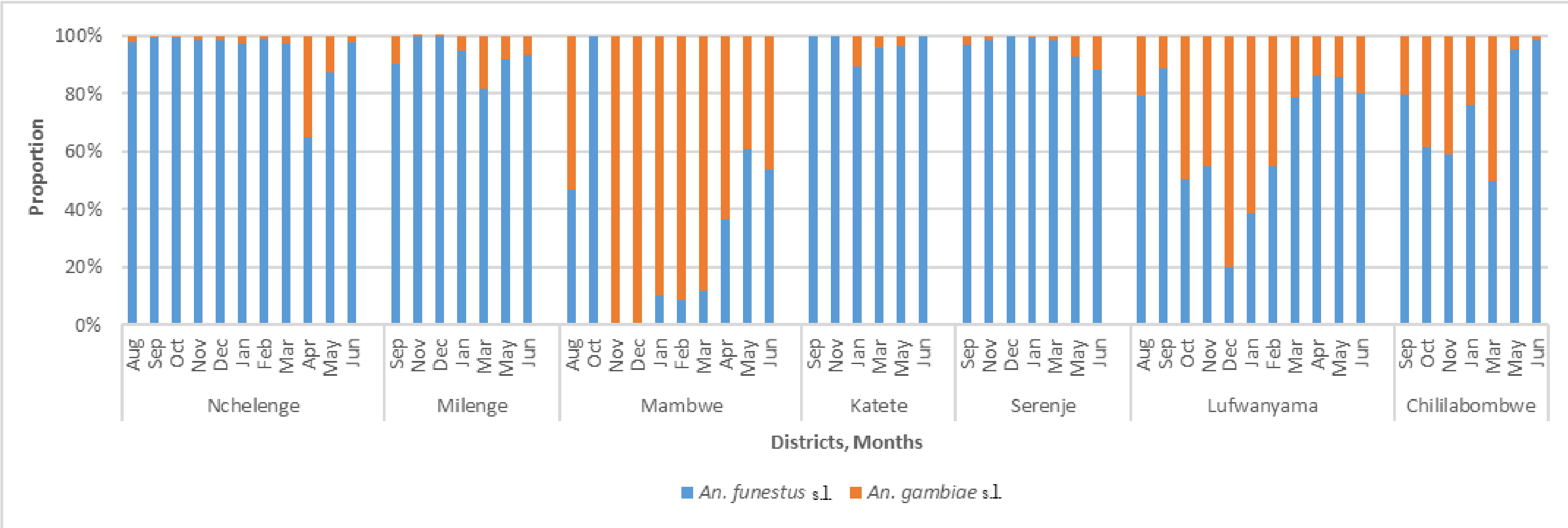


Other species collected by HLC-Indoors include: *An. squamosus* (0.65%), *An. coustani* (0.23%), *An. rufipes* (0.02%), *An. gibbinsi* (0.01%), *An. maculipalpis* (0.01%), *An. pretoriensis* (0.01%), *An. argentiolobatus* (0.01%), and *An. rufipes* (0.01%). Other species collected by HLC-Outdoors include: *An. squamosus* (1.46%), *An. rufipes* (0.06%), *An. coustani* (0.84%), *An. maculipalpis* (0.02%), *An. gibbinsi* (0.01%), *An. pretoriensis* (0.01%), and *An. tenebrosus* (0.003%). Other species collected by PSC include *An. squamosus* (0.17%), *An. coustani* (0.02%), and *An. rufipes* (0.01%).

Figure 4 shows monthly relative abundance of the two primary vector species in each of the sentinel districts. *An. funestus* s.l. was the predominant malaria vector in all districts except Mambwe in the Eastern Province where *An. gambiae* s.l. was the most common species collected. Mambwe in Eastern Province and Lufwanyama and Chililabombwe in the Copperbelt Province were the districts with the highest proportions of *An. gambiae* s.l. Monthly distribution of this species in these districts indicate a trend of increasing numbers from the start of the rainy period extending into the peak rainy months around (November to February).

Both primary vectors were collected from sprayed and unsprayed sites, however, more *An. funestus* s.l. were collected from unsprayed sites (62.2%) than sprayed sites (37.8%), while the reverse was true for *An. gambiae* s.l. with higher proportion from the sprayed sites (69.6%) compared to unsprayed sites (30.4%).

Figure 4: Monthly Variations in the Relative Proportions of *An. funestus* s.l. and *An. gambiae* s.l. by District (August 2020–June 2021)



3.1.2 INDOOR RESTING DENSITY OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY PSC

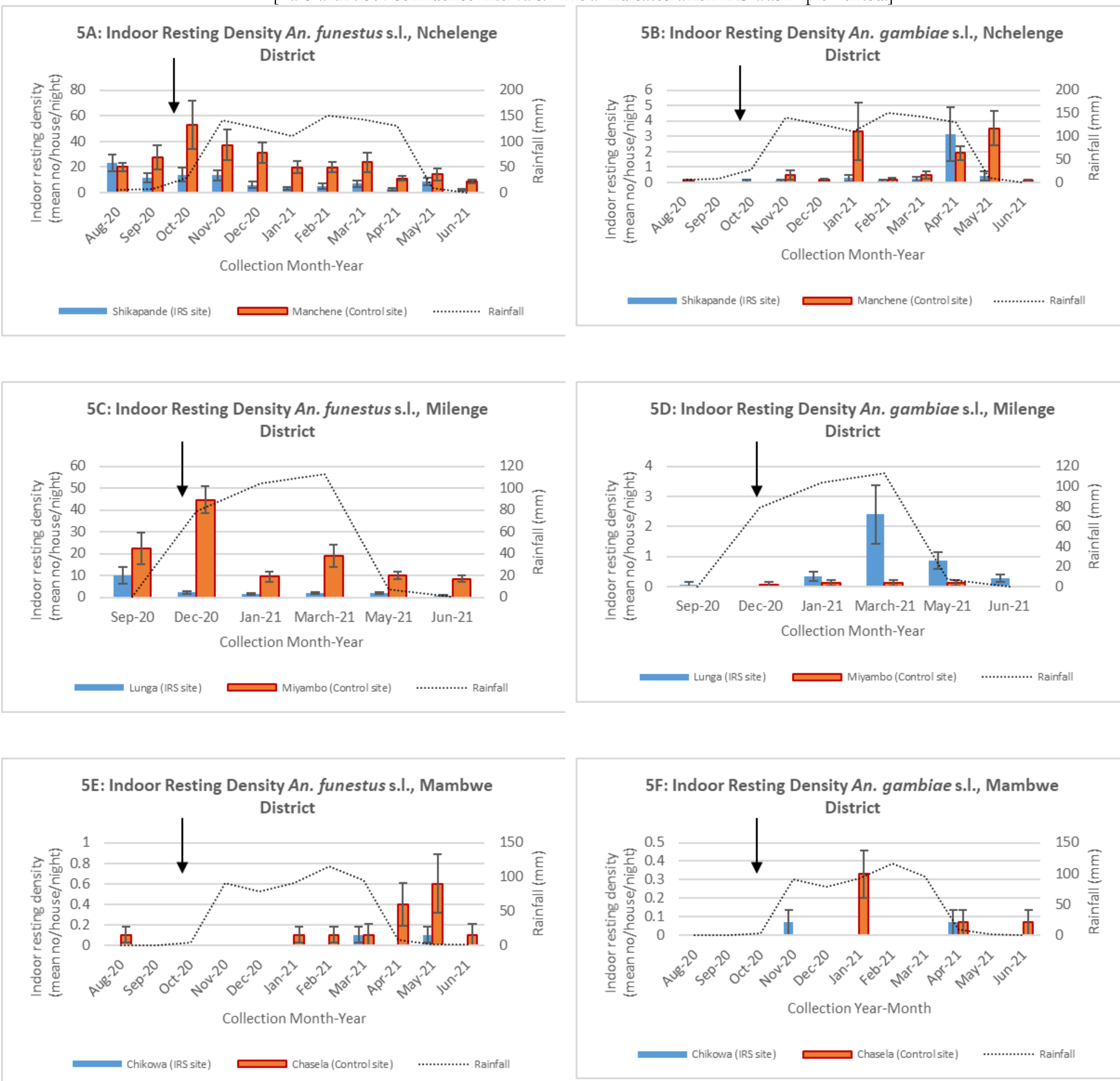
Overall indoor resting density of *An. funestus* s.l. was significantly lower at the combined sprayed sites with 2.6 vectors per house compared to the combined control sites with 7.5 vectors per house [incidence rate ratio (IRR) 0.63, p<0.001)]. A reduction in *An. funestus* s.l. density was observed at sprayed sites after IRS (4.1 to 2.2 vectors per house) while a slight increase was observed at the control sites (6.7 to 7.6 vectors per house). *An. gambiae* s.l. overall density was similar at the combined sprayed sites, 0.46 vectors per house compared to the combined control sites of 0.33 vectors per house (IRR 1.36, p=0.06). Post-IRS *An. gambiae* s.l. mean densities were significantly higher at the sprayed sites (0.53 versus 0.21 vectors per house, IRR 2.61, p<0.001) as well as the control sites (0.39 versus 0.07 vectors per house, IRR 4.27, p<0.001). Overall, indoor resting density increased by 2.5-fold increase at the sprayed sites compared to a 5.6-fold increase at the unsprayed control sites. Detailed output of statistical analyses of the impact of IRS on indoor resting density are presented in Annex C-I.

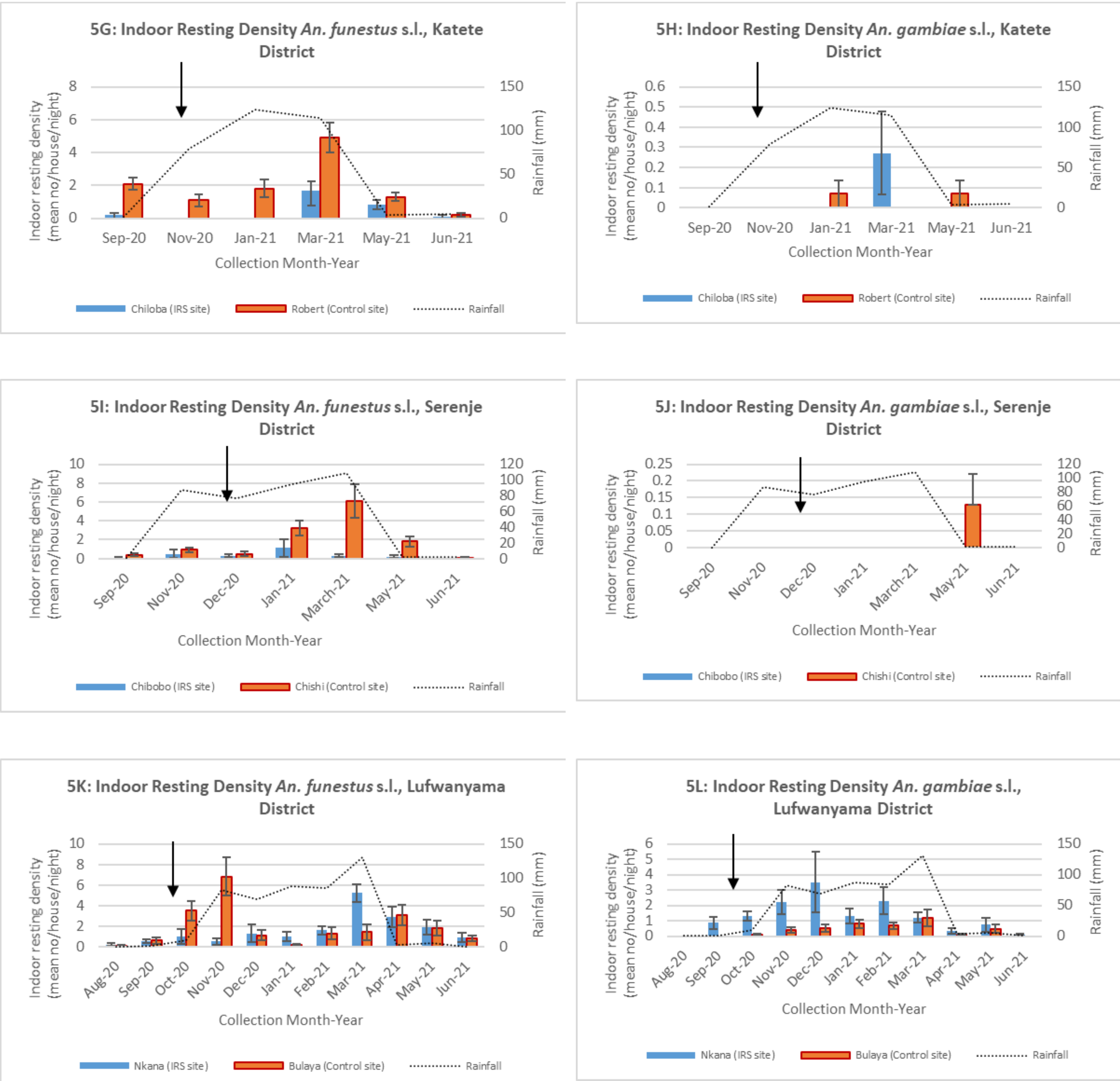
Figure 5 below is a panel of figures showing the indoor resting densities for both *An. funestus* s.l. and *An. gambiae* s.l. vectors at sprayed and unsprayed sites in each of the seven districts with monthly rainfall.

At district level, there were fewer indoor resting *An. funestus* s.l. vectors before and after IRS at the sprayed sites compared to the control sites in six of the seven districts (Nchelenge District-Figure 5A, Milenge District-Figure 5C, Mambwe District-Figure 5E, Katete District-Figure 5G, Serenje District-Figure 5I, and Lufwanyama District-Figure 5K). The differences between mean densities of sprayed and control sites were statistically significant at p=0.05 in five of the six districts (Nchelenge, Milenge, Mambwe, Katete and Serenje). *An. funestus* s.l. vector densities were higher at the sprayed sites compared to control sites in Chililabombwe District-Figure 5M, but the difference was not significant. Post-IRS mean *An. funestus* s.l. indoor resting densities were reduced to pre-IRS levels or lower at two of the seven IRS sites (Shikapande in Nchelenge District (17.3 to 6.9) and Lunga in Milenge District (10.1 to 1.7). Densities also reduced in some control sites including Miyambo in Milenge District (3.3 to 1.6), Robert in Katete District (2.1 to 1.9), and Mainasoko in Chililabombwe District (3.3 to 1.6). The reductions in Shikapande, Lunga, Miyambo, and Mainasoko were all statistically significant. *An. gambiae* s.l. indoor resting densities were lower in sprayed sites compared to control sites in only three of the seven districts (Nchelenge District-Figure 5A, Mambwe District-Figure 5F, and Serenje District-Figure 5J) and the reductions were statistically significant at p=0.05. Post-IRS mean *An. gambiae* s.l. indoor resting density was lower than pre-IRS in Kawama in Chililabombwe District only (0.8 to 0.67 vectors per house) and the reduction was not statistically significant (IRR 0.81, p=0.384). At all other sites, *An. gambiae* s.l. densities either remained the same or increased after IRS.

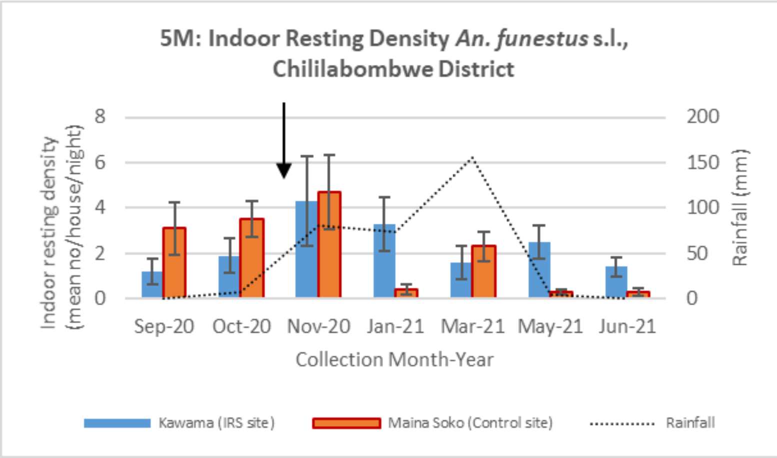
Figure 5: *An. funestus* s.l. and *An. gambiae* s.l. Indoor Resting Density Across Sites (August 2020-June 2021)

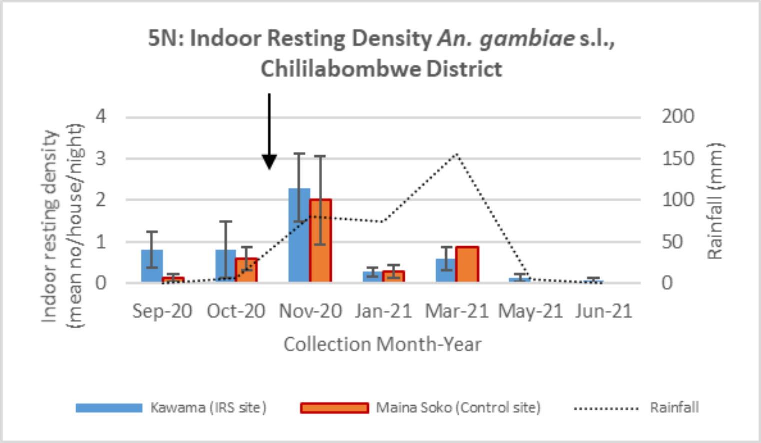
[Bars with 95% confidence intervals. Arrow indicates when IRS was implemented.]





5G: Indoor Resting Density An. funestus s.l., Katete District
Indoor resting density (mean no/house/night)
Rainfall (mm)
Collection Month-Year
Sep-20
Nov-20
Jan-21
Mar-21
May-21
Jun-21
Chiloba (IRS site)
Robert (Control site)
Rainfall
5H: Indoor Resting Density An. gambiae s.l., Katete District
Indoor resting density (mean no/house/night)
Rainfall (mm)
Collection Month-Year
Sep-20
Nov-20
Jan-21
Mar-21
May-21
Jun-21
Chiloba (IRS site)
Robert (Control site)
Rainfall
5I: Indoor Resting Density An. funestus s.l., Serenje District
Indoor resting density (mean no/house/night)
Rainfall (mm)
Collection Month-Year
Sep-20
Nov-20
Dec-20
Jan-21
March-21
May-21
Jun-21
Chibobo (IRS site)
Chishi (Control site)
Rainfall
5J: Indoor Resting Density An. gambiae s.l., Serenje District
Indoor resting density (mean no/house/night)
Rainfall (mm)
Collection Month-Year
Sep-20
Nov-20
Dec-20
Jan-21
March-21
May-21
Jun-21
Chibobo (IRS site)
Chishi (Control site)
Rainfall
5K: Indoor Resting Density An. funestus s.l., Lufwanyama District
Indoor resting density (mean no/house/night)
Rainfall (mm)
Collection Month-Year
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Mar-21
Apr-21
May-21
Jun-21
Nkana (IRS site)
Bulaya (Control site)
Rainfall
5L: Indoor Resting Density An. gambiae s.l., Lufwanyama District
Indoor resting density (mean no/house/night)
Rainfall (mm)
Collection Month-Year
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Mar-21
Apr-21
May-21
Jun-21
Nkana (IRS site)
Bulaya (Control site)
Rainfall





3.1.3 Abdominal Condition of *An. funestus* s.l. and *An. gambiae* s.l. Collected by PSCs

Abdominal condition (whether the vector is unfed, fed, or gravid) was determined for a total of 8,690 *An. funestus* s.l. (2,181 from sprayed sites and 6,509 from control sites) and 699 *An. gambiae* s.l. (410 from sprayed sites and 289 from control sites) collected indoors by PSCs. Overall, the proportions of fed and gravid *An. funestus* s.l. mosquitoes were 74.7% and 6.8% in the sprayed sites and 79.4% and 10.2% in the control sites, respectively, while the proportions of fed and gravid *An. gambiae* s.l. were 91.5% and 2.2% in the sprayed sites and 91.3% and 1.0% in the control sites, respectively. There were slightly fewer gravid *An. funestus* s.l. vectors at the sprayed sites compared to the control sites, while there were more gravid *An. gambiae* s.l. vectors at the sprayed sites compared to the control sites. However, the difference in both cases were not statistically significant (IRR 0.96, p=0.877 and IRR 1.42, p=0.84, respectively).

Figures 6 and 7 show the abdominal status (proportions of unfed, fed, and gravid) *An. funestus* s.l. and *An. gambiae* s.l. mosquitoes from sprayed and control sites during the reporting period. After IRS, there were consistently fewer gravid *An. funestus* s.l. vectors at the sprayed sites compared to the control sites for most of the period. There were however more gravid *An. gambiae* s.l. vectors at sprayed sites compared to the control sites. There was no overall reduction in gravid *An. funestus* s.l. or *An. gambiae* s.l. vectors at the sprayed sites after IRS compared to the period before IRS. See detailed statistical output in Annex C-II.

Figure 6: Abdominal Condition of *An. funestus* s.l. in Intervention and Control Sites Before and After IRS (August 2020-June 2021)

[Arrow indicates the time IRS was implemented]

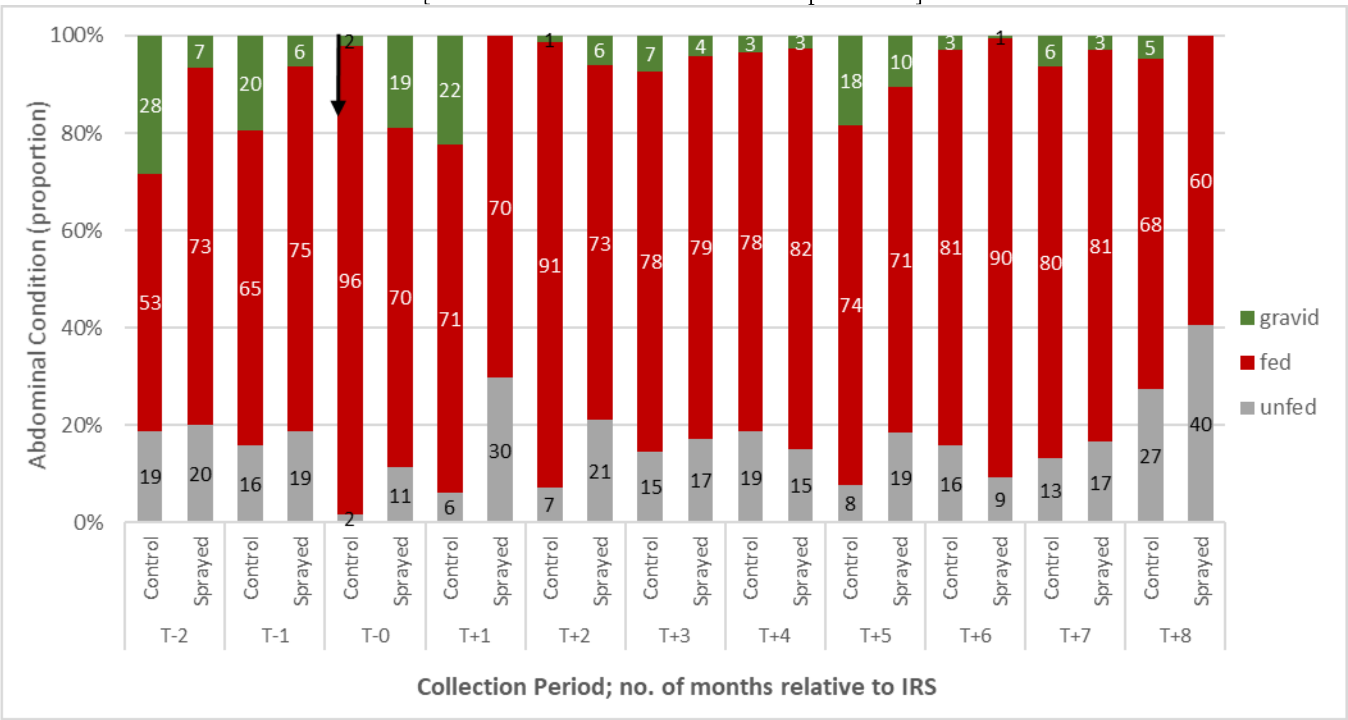
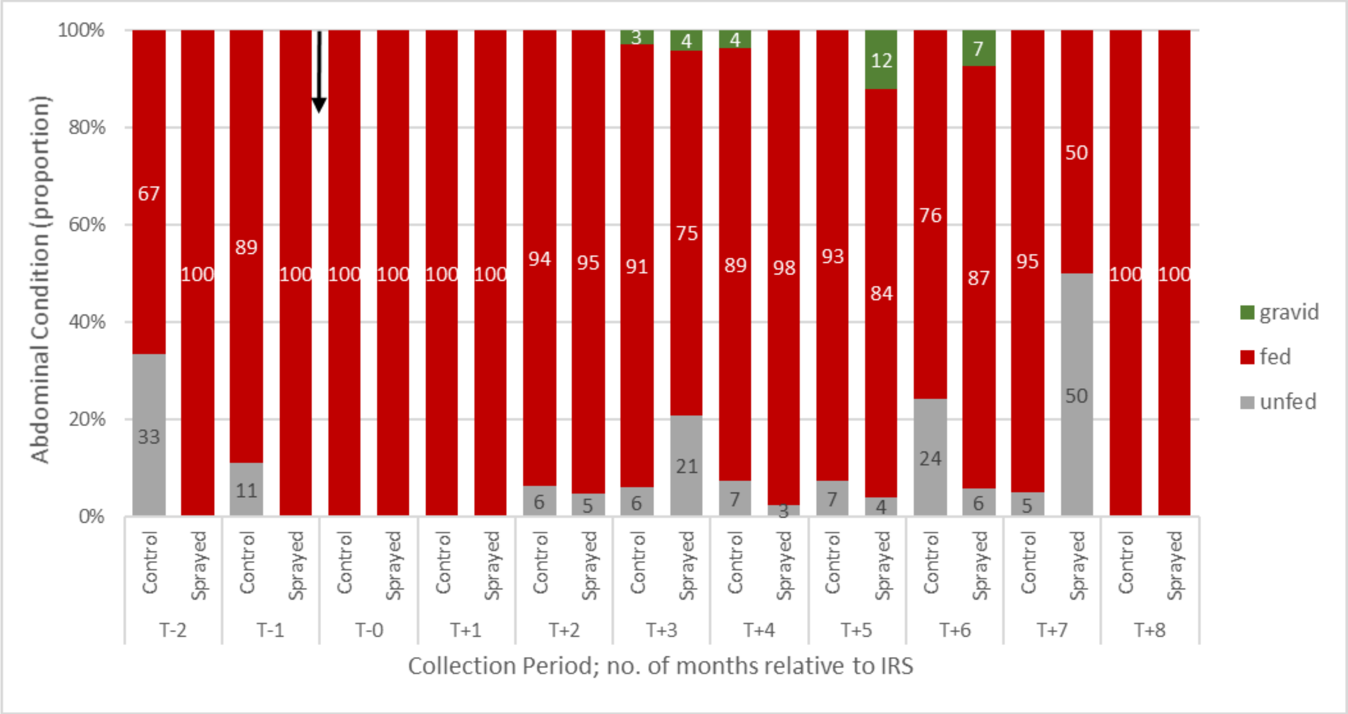


Figure 7: Abdominal Condition of *An. gambiae* s.l. in Intervention and Control Sites Before and After IRS (August 2020-June 2021)

[Arrow indicates the time IRS was implemented]



3.1.4 HUMAN BITING RATES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY HLC

The indoor and outdoor HBR of *An. funestus* s.l. and *An. gambiae* s.l. in the IRS and control sites are presented in Figure 8. There were overall fewer bites from *An. funestus* s.l. at the combined IRS sites compared to the combined control sites (from 40.9 to 26.6 bites per person per night, or b/p/n), though this was not statistically significant. Reduction in *An. funestus* s.l. HBR was observed at sprayed sites after IRS (39.2 to 23.4 b/p/n), while an increase was observed at the control sites (30.3 to 43.6 bites). The overall biting rate of *An. gambiae* s.l. at sprayed sites (7.7 b/p/n) was higher than control sites (3.2 b/p/n). There were more *An. gambiae* s.l. bites after IRS than before IRS at combined sprayed sites as well as combined control sites.

Statistical significance was observed for the *An. gambiae* s.l. post-IRS increase at the control sites (p=0.05); the differences in all other cases were not statistically significant (see detailed statistical output in Annex C-III). There were fewer *An. funestus* s.l. bites at the sprayed sites compared to the control sites in three of the seven districts (Milenge District-Figure 8C, Serenje District-Figure 8I, and Chililabombwe District-Figure 8M). The differences were statistically significant in all three districts Milenge (p<0.001), Serenje (p<0.001), and Chililabombwe (p=0.03). *An. funestus* s.l. biting rates were higher at the sprayed sites compared to control sites in Nchelenge, Mambwe, Katete, and Lufwanyama Districts (Figures 8A, 8E, 8G, and 8K, respectively); the differences in all cases were not statistically significant.

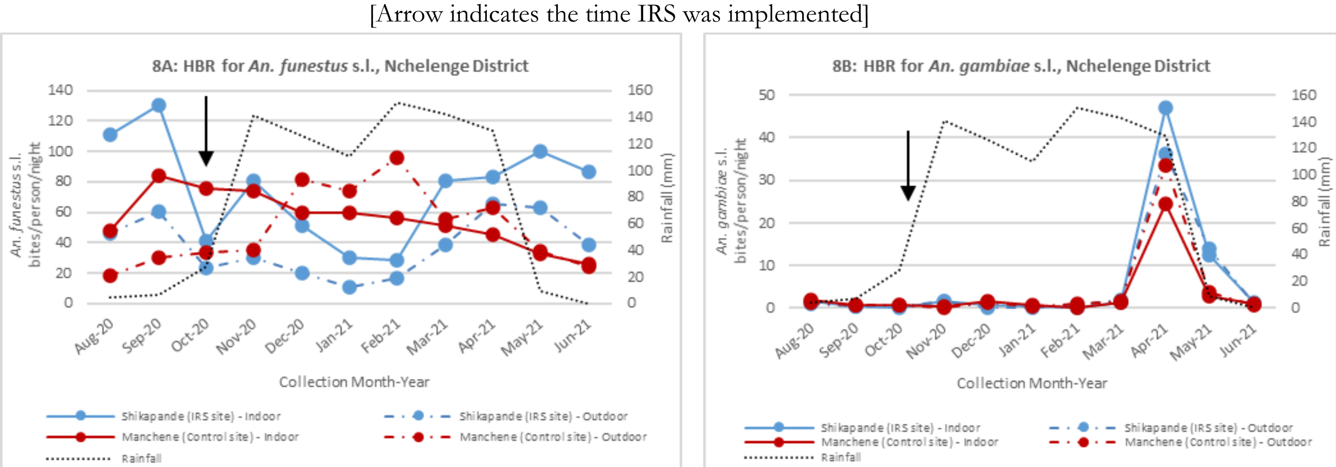
Post-IRS *An. funestus* s.l. biting rates reduced to pre-IRS levels or lower at three of the seven IRS sites (Shikapande in Nchelenge District (173.9 to 98.7 b/p/n, p<0.001), Lunga in Milenge District (43.2 to 15.2, p<0.001), and Nkana in Lufwanyama District (36.9 to 8.5 b/p/n, p=0.53). Biting rates increased above pre-IRS levels at the other four sprayed sites and all control sites, with only one site having a statistically significant increase (Chibobo, sprayed site in Serenje District, 0.2-2.2 b/p/n, p=0.03).

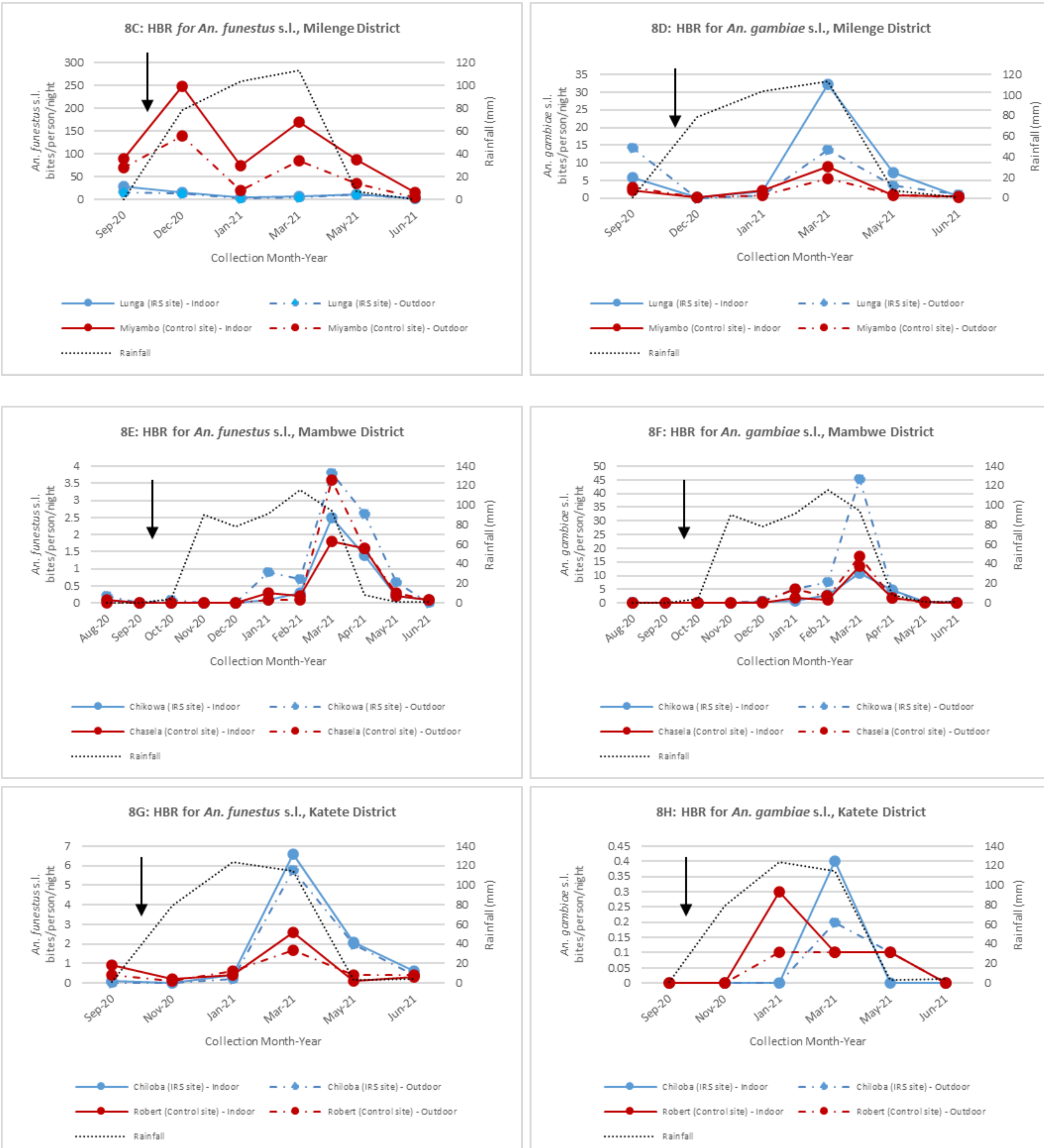
An. gambiae s.l. biting rates in sprayed sites were lower than control sites in two of the seven districts [Serenje (0.2-01 b/p/n) and Lufwanyama (12.8-1.6 b/p/n)], and higher at the sprayed sites in Nchelenge (7.3-11.3b/p/n), Milenge (4.2-13.5 b/p/n), Mambwe (4.2-7.7 b/p/n), and Chililabombwe District (2.8-2.9 b/p/n). *An. gambiae* s.l. biting rates in both control and sprayed sites were similar in Katete (0.1-0.1 b/p/n).

Post-IRS *An. gambiae* s.l. biting rates were lower than pre-IRS in two sprayed sites [Lunga in Milenge District (20.19 to 12.20 b/p/n) and Kawama in Chililabombwe District (3.81 to 2.59 b/p/n)] and one control site [Miyambo in Milenge District (5.13 to 4.03 b/p/n)]. Biting rates post-IRS were higher than pre-IRS at all other sites including the five sprayed sites of Shikapande-Nchelenge District, Chikowa-Mambwe District, Chilowa-Katete District, Chibobo-Serenje District, and Nkana-Lufwanyama District together with their accompanying control sites and the control site in Chililabombwe District.

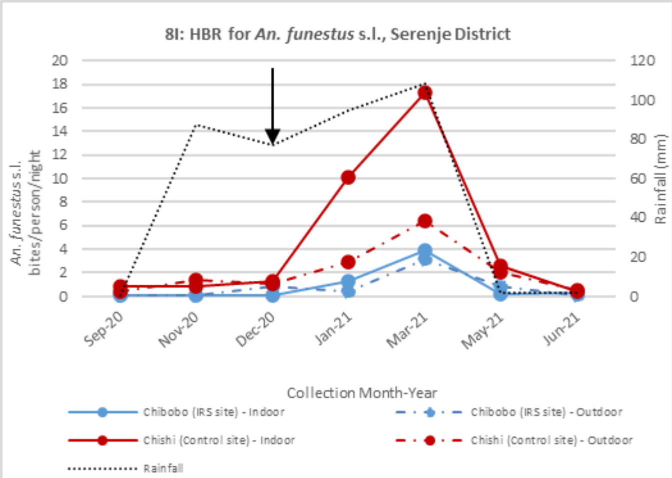
Figure 8: Human Biting Rates of *An. funestus* s.l. and *An. gambiae* s.l. (August 2020-June 2021)

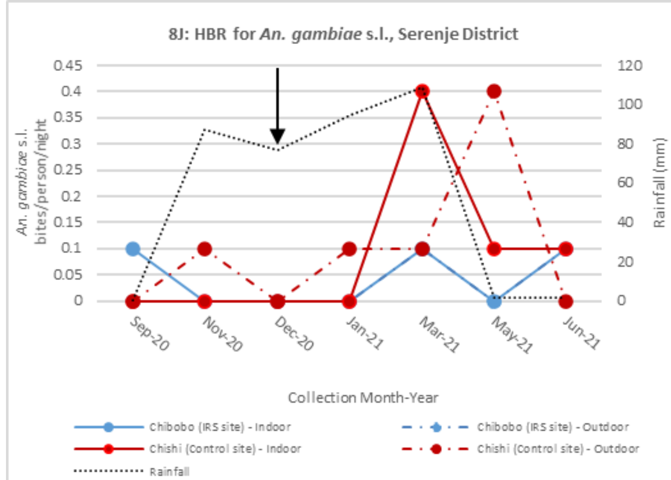
[Arrow indicates the time IRS was implemented]

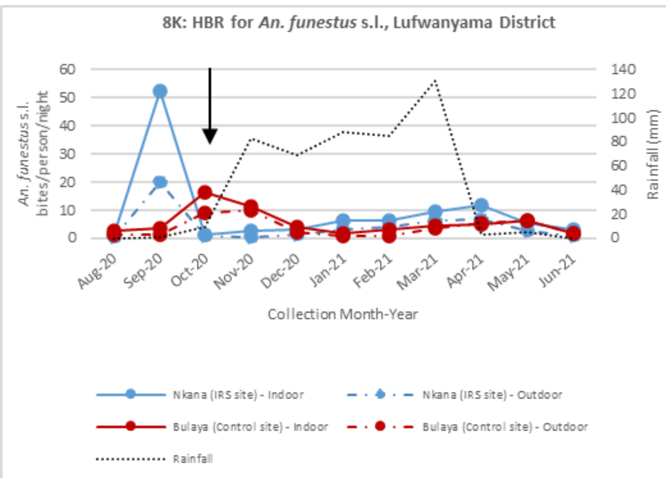


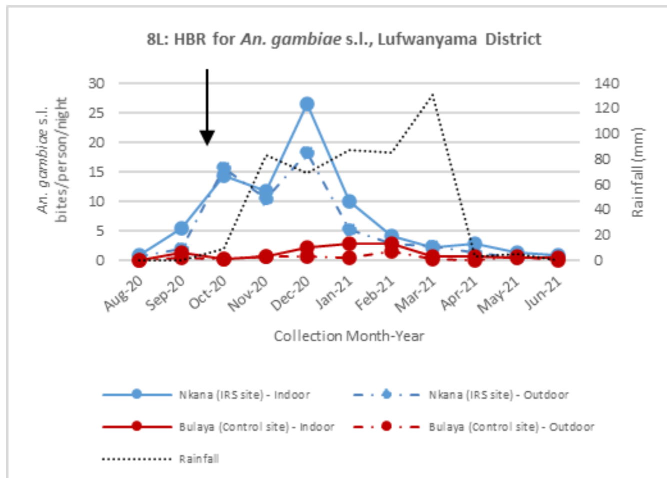


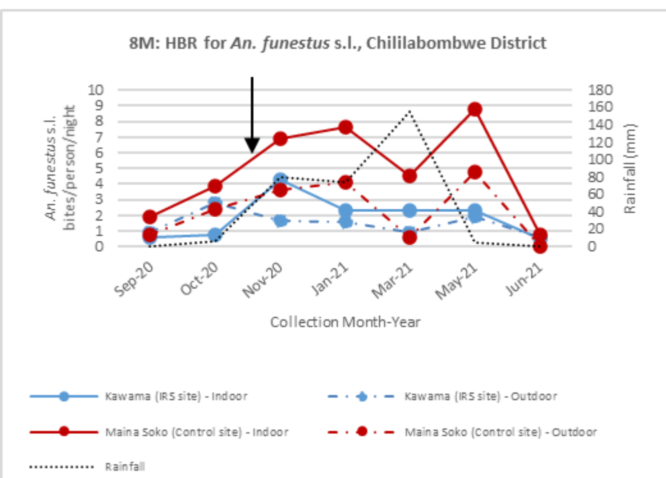
8C: HBR for An. funestus s.l., Milenge District
An. funestus s.l. bites/person/night
Rainfall (mm)
Collection Month-Year
Sep-20
Dec-20
Jan-21
Mar-21
May-21
Jun-21
Lunga (IRS site) - Indoor
Lunga (IRS site) - Outdoor
Miyambo (Control site) - Indoor
Miyambo (Control site) - Outdoor
Rainfall
8D: HBR for An. gambiae s.l., Milenge District
An. gambiae s.l. bites/person/night
Rainfall (mm)
Collection Month-Year
Lunga (IRS site) - Indoor
Lunga (IRS site) - Outdoor
Miyambo (Control site) - Indoor
Miyambo (Control site) - Outdoor
Rainfall
8E: HBR for An. funestus s.l., Mambwe District
An. funestus s.l. bites/person/night
Rainfall (mm)
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Mar-21
Apr-21
May-21
Jun-21
Collection Month-Year
Chikowa (IRS site) - Indoor
Chikowa (IRS site) - Outdoor
Chasela (Control site) - Indoor
Chasela (Control site) - Outdoor
Rainfall
8F: HBR for An. gambiae s.l., Mambwe District
An. gambiae s.l. bites/person/night
Rainfall (mm)
Collection Month-Year
Chikowa (IRS site) - Indoor
Chikowa (IRS site) - Outdoor
Chasela (Control site) - Indoor
Chasela (Control site) - Outdoor
Rainfall
8G: HBR for An. funestus s.l., Katete District
An. funestus s.l. bites/person/night
Rainfall (mm)
Sep-20
Nov-20
Jan-21
Mar-21
May-21
Jun-21
Collection Month-Year
Chiloba (IRS site) - Indoor
Chiloba (IRS site) - Outdoor
Robert (Control site) - Indoor
Robert (Control site) - Outdoor
Rainfall
8H: HBR for An. gambiae s.l., Katete District
An. gambiae s.l. bites/person/night
Rainfall (mm)
Collection Month-Year
Chiloba (IRS site) - Indoor
Chiloba (IRS site) - Outdoor
Robert (Control site) - Indoor
Robert (Control site) - Outdoor
Rainfall

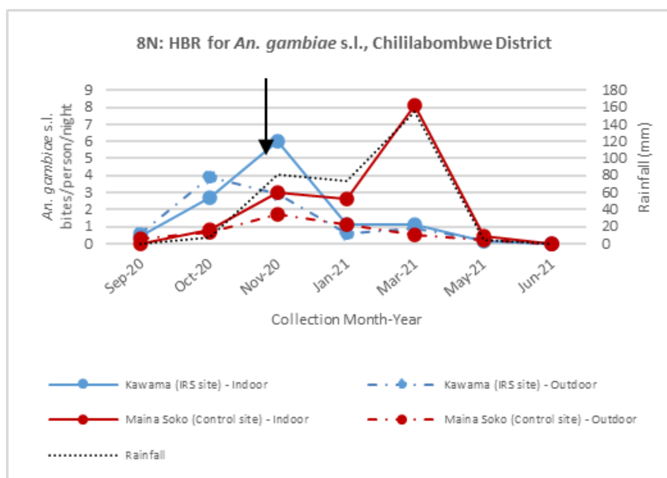

8I: HBR for An. funestus s.l., Serenje District
An. funestus s.l. bites/person/night
Rainfall (mm)
Sep-20
Nov-20
Dec-20
Jan-21
Mar-21
May-21
Jun-21
Collection Month-Year
Chibobo (IRS site) - Indoor
Chibobo (IRS site) - Outdoor
Chishi (Control site) - Indoor
Chishi (Control site) - Outdoor
Rainfall


8J: HBR for An. gambiae s.l., Serenje District
An. gambiae s.l. bites/person/night
Rainfall (mm)
Sep-20
Nov-20
Dec-20
Jan-21
Mar-21
May-21
Jun-21
Collection Month-Year
Chibobo (IRS site) - Indoor
Chibobo (IRS site) - Outdoor
Chishi (Control site) - Indoor
Chishi (Control site) - Outdoor
Rainfall


8K: HBR for An. funestus s.l., Lufwanyama District
An. funestus s.l. bites/person/night
Rainfall (mm)
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Mar-21
Apr-21
May-21
Jun-21
Collection Month-Year
Nkana (IRS site) - Indoor
Nkana (IRS site) - Outdoor
Bulaya (Control site) - Indoor
Bulaya (Control site) - Outdoor
Rainfall


8L: HBR for An. gambiae s.l., Lufwanyama District
An. gambiae s.l. bites/person/night
Rainfall (mm)
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Mar-21
Apr-21
May-21
Jun-21
Collection Month-Year
Nkana (IRS site) - Indoor
Nkana (IRS site) - Outdoor
Bulaya (Control site) - Indoor
Bulaya (Control site) - Outdoor
Rainfall


8M: HBR for An. funestus s.l., Chililabombwe District
An. funestus s.l. bites/person/night
Rainfall (mm)
Sep-20
Oct-20
Nov-20
Jan-21
Mar-21
May-21
Jun-21
Collection Month-Year
Kawama (IRS site) - Indoor
Kawama (IRS site) - Outdoor
Maina Soko (Control site) - Indoor
Maina Soko (Control site) - Outdoor
Rainfall


8N: HBR for An. gambiae s.l., Chililabombwe District
An. gambiae s.l. bites/person/night
Rainfall (mm)
Sep-20
Oct-20
Nov-20
Jan-21
Mar-21
May-21
Jun-21
Collection Month-Year
Kawama (IRS site) - Indoor
Kawama (IRS site) - Outdoor
Maina Soko (Control site) - Indoor
Maina Soko (Control site) - Outdoor
Rainfall

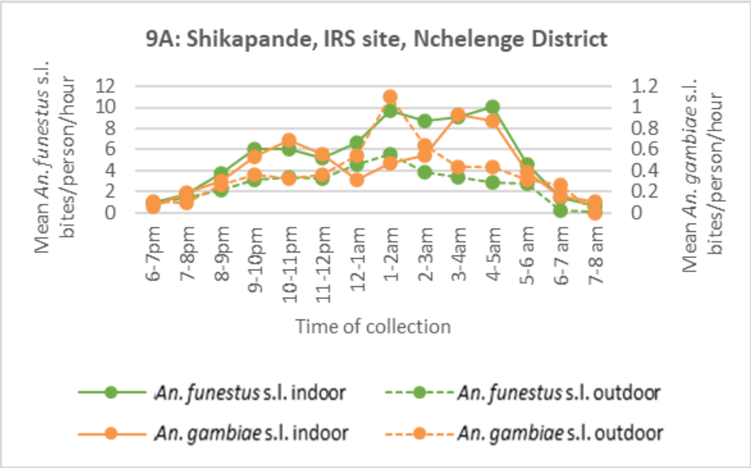
3.1.5 *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. FEEDING LOCATION AND BITING TIME

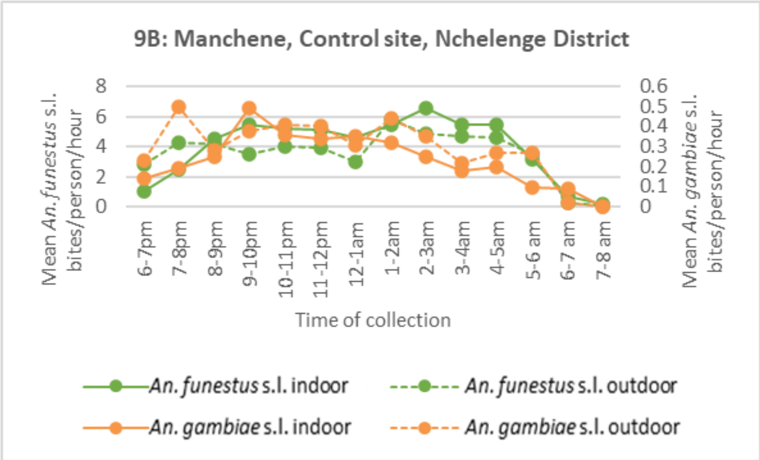
The feeding location (indoors or outdoors) and biting times for *An. funestus* s.l. and *An. gambiae* s.l. mosquitoes for all sentinel sites are presented in Figure 9. There was more indoor biting than outdoor biting for both *An. funestus* s.l. and *An. gambiae* s.l. in all districts except Mambwe. Indoor *An. funestus* s.l. bites were significantly higher than outdoor bites in Nchelenge District only (65.2 versus 43.5 b/p/n, IRR 0.55, p=0.01). At the site level, only the two sites in Mambwe District, Chikowa and Chasela, had more outdoor bites than indoor bites for both *An. funestus* s.l. and *An. gambiae* s.l. One additional site—Manchene in Nchelenge—had more outdoor than indoor *An. gambiae* s.l. bites. All other sites had more biting indoors than outdoors. The difference was statistically significant for *An. funestus* s.l. at three sites: Mainasoko in Chililabombwe District (4.9 versus 2.3 b/p/n, IRR 0.49, p=0.02), Miyambo in Milenge District (114.2 versus 58.8 b/p/n, IRR 0.60, p<0.001), and Shikapande in Nchelenge District (74.8 versus 37.5 b/p/n, IRR 0.47, p=0.005), and for *An. gambiae* s.l. at one site, Bulaya in Lufwanyama District (1.13 versus 0.47 b/p/n, IRR 0.42, p=0.01). See statistical output in Annex C-IV.

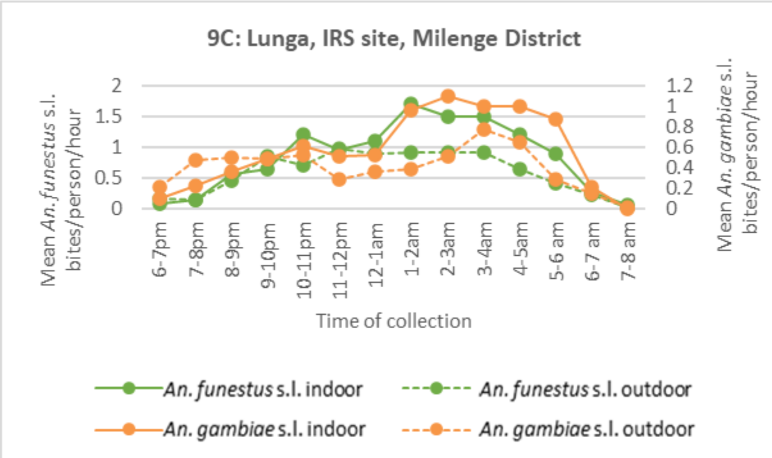
The biting trend was mainly unimodal at sites with high vector numbers (more than five bites/person/hour), peaking generally between 12 a.m. and 4 a.m. (Fig. 9A-D). A weak bimodal peak was observed for *An. gambiae* s.l. in Chasela in Mambwe District, with one peak in the early evening around 9-10 p.m. and one late at night around 1-2 a.m. (Figure 9F). For areas with low vector numbers, we observed multiple peaks throughout the night. In Lufwanyama District, the level of biting during the late-night period was sustained until morning at both the IRS and control sites.

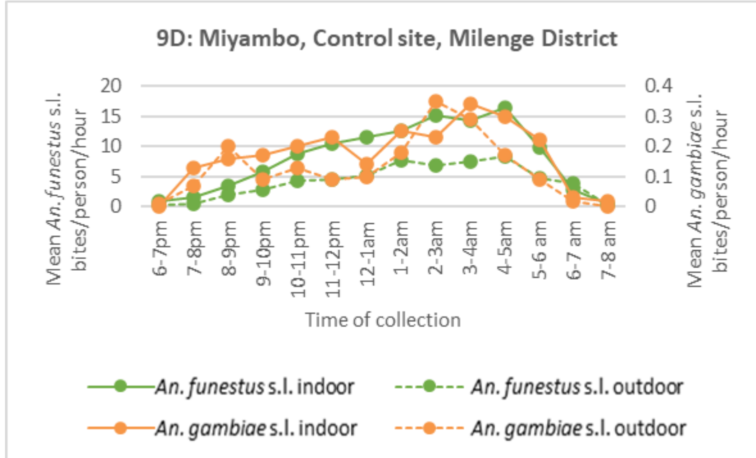
Figure 9: *An. funestus* s.l. and *An. gambiae* s.l. Biting Times and Location by Site (August 2020-June 2021)

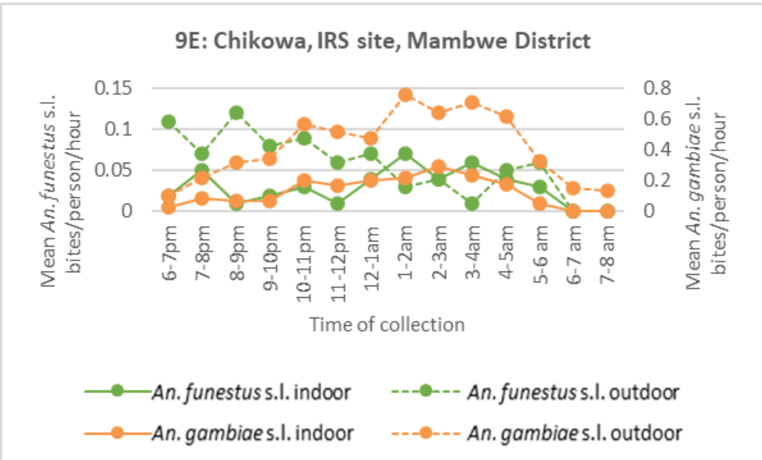
[Primary Axis = *An. funestus* s.l.; Secondary Axis = *An. gambiae* s.l.]

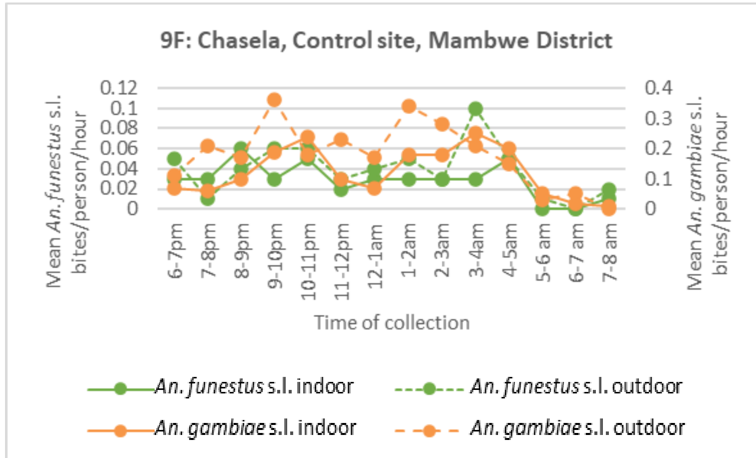


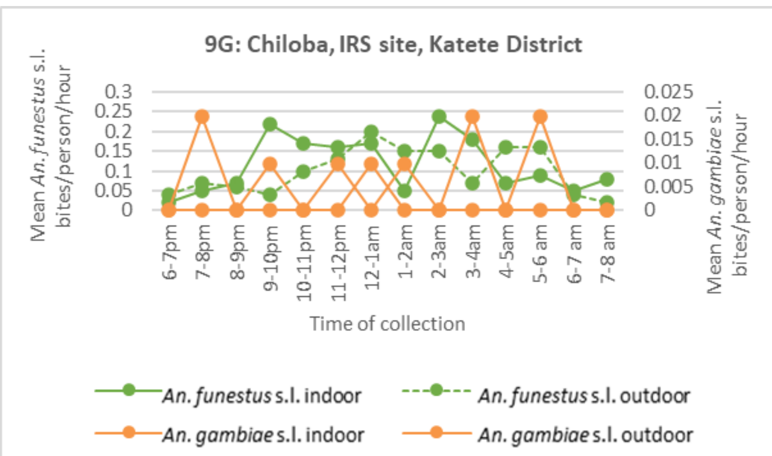


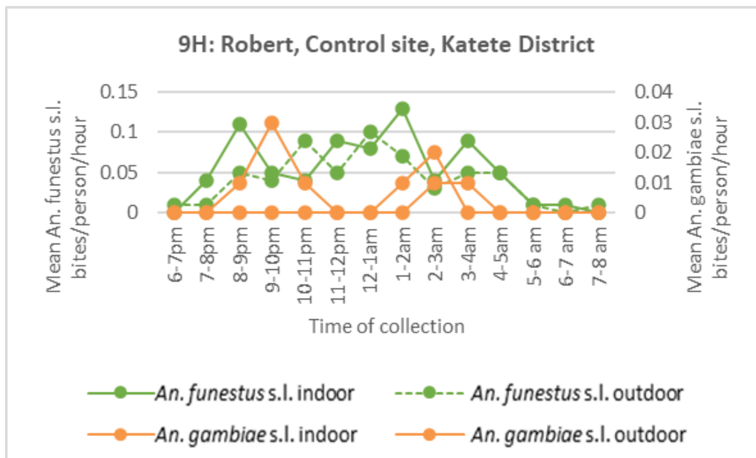

9C: Lunga, IRS site, Milenge District
Mean *An. funestus* s.l. bites/person/hour
Mean *An. gambiae* s.l. bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor

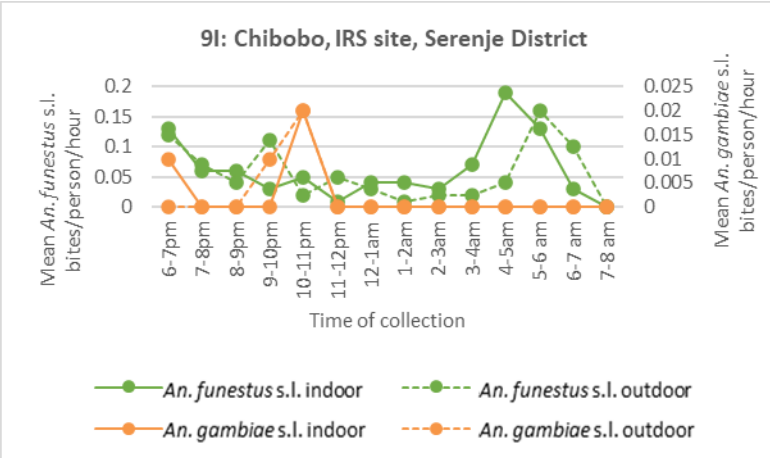

9D: Miyambo, Control site, Milenge District
Mean *An. funestus* s.l. bites/person/hour
Mean *An. gambiae* s.l. bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor

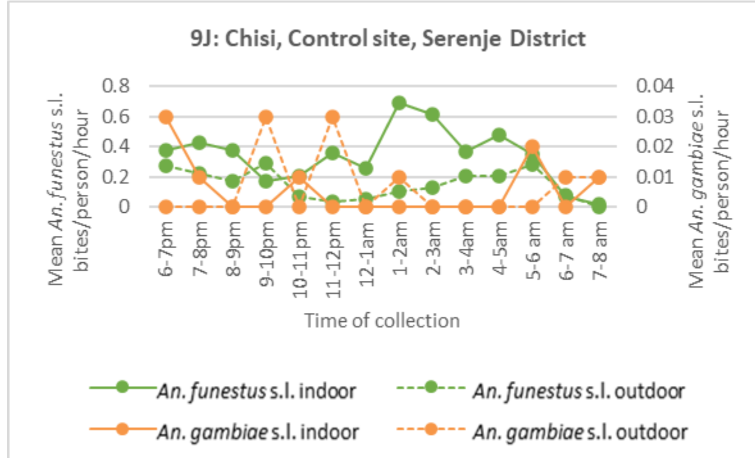

9E: Chikowa, IRS site, Mambwe District
Mean *An. funestus* s.l. bites/person/hour
Mean *An. gambiae* s.l. bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor

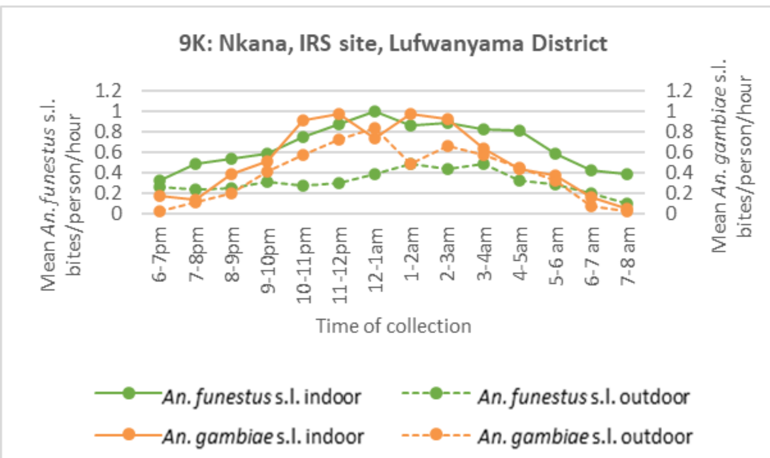

9F: Chasela, Control site, Mambwe District
Mean *An. funestus* s.l. bites/person/hour
Mean *An. gambiae* s.l. bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor

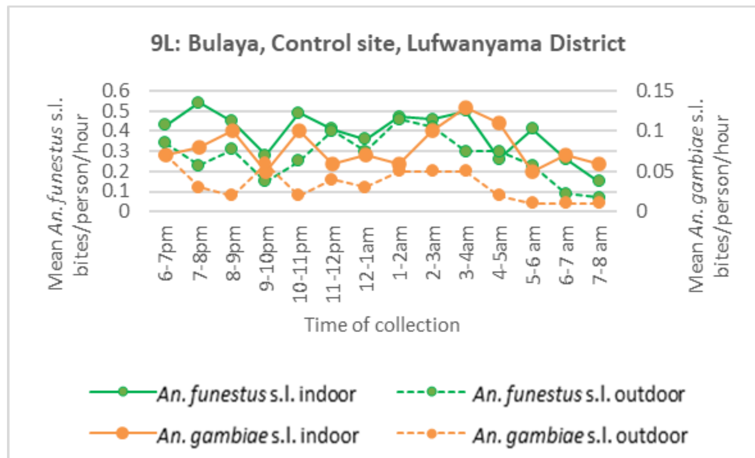

9G: Chiloba, IRS site, Katete District
Mean *An. funestus* s.l. bites/person/hour
Mean *An. gambiae* s.l. bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor

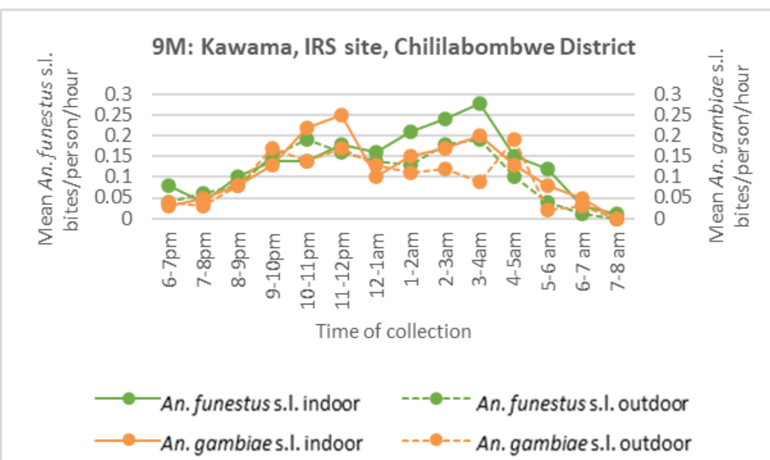

9H: Robert, Control site, Katete District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor

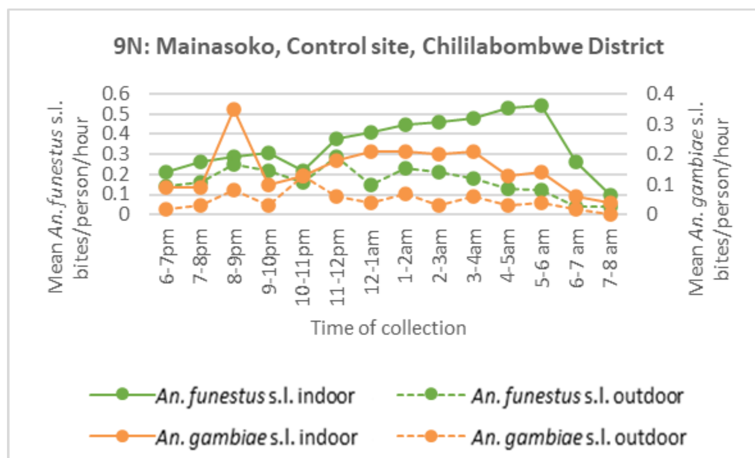

9I: Chibobo, IRS site, Serenje District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
6-7pm 7-8pm 8-9pm 9-10pm 10-11pm 11-12pm 12-1am 1-2am 2-3am 3-4am 4-5am 5-6 am 6-7 am 7-8 am
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor


9J: Chisi, Control site, Serenje District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
6-7pm 7-8pm 8-9pm 9-10pm 10-11pm 11-12pm 12-1am 1-2am 2-3am 3-4am 4-5am 5-6 am 6-7 am 7-8 am
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor


9K: Nkana, IRS site, Lufwanyama District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
6-7pm 7-8pm 8-9pm 9-10pm 10-11pm 11-12pm 12-1am 1-2am 2-3am 3-4am 4-5am 5-6 am 6-7 am 7-8 am
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor


9L: Bulaya, Control site, Lufwanyama District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
6-7pm 7-8pm 8-9pm 9-10pm 10-11pm 11-12pm 12-1am 1-2am 2-3am 3-4am 4-5am 5-6 am 6-7 am 7-8 am
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor


9M: Kawama, IRS site, Chililabombwe District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
6-7pm 7-8pm 8-9pm 9-10pm 10-11pm 11-12pm 12-1am 1-2am 2-3am 3-4am 4-5am 5-6 am 6-7 am 7-8 am
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor


9N: Mainasoko, Control site, Chililabombwe District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
6-7pm 7-8pm 8-9pm 9-10pm 10-11pm 11-12pm 12-1am 1-2am 2-3am 3-4am 4-5am 5-6 am 6-7 am 7-8 am
Time of collection
An. funestus s.l. indoor
An. funestus s.l. outdoor
An. gambiae s.l. indoor
An. gambiae s.l. outdoor

3.1.6 PARITY RATES

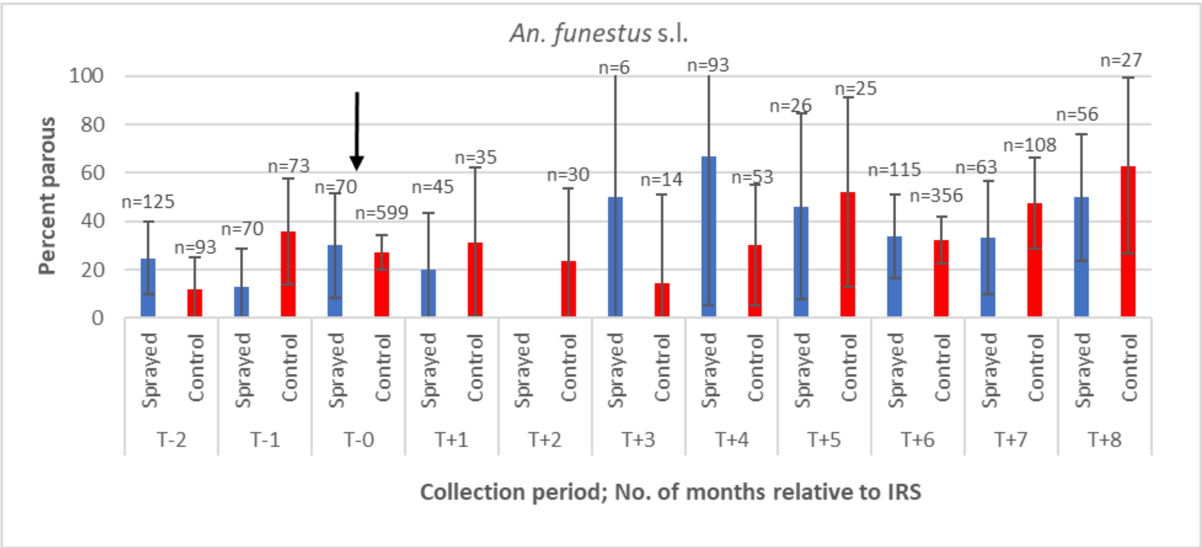
A total of 3,668 unfed female *An. funestus* s.l. and 1,788 *An. gambiae* s.l. collected by HLCs were examined for parity status (SOP 10/01) during the reporting period. Overall parity rates for *An. funestus* s.l. and *An. gambiae* s.l. were 33.4% and 41.6% respectively. *An. funestus* s.l. parity rate at combined sprayed sites was 33.6% (496/1474) and at combined control sites was 33.5% (735/2194). While for *An. gambiae* s.l. parity rate was 37.0% (474/1280) at combined sprayed sites and 53.1% (270/508) at the combined control sites. Mean parity for *An. funestus* s.l. before and after IRS at the sprayed sites were 32.7% versus 33.9% and at the control sites were 31.7% versus 33.8% while parity rates for *An. gambiae* s.l. were 39.1% versus 36.7% at the sprayed sites and 13.3% versus 54.4% at the control sites, respectively.

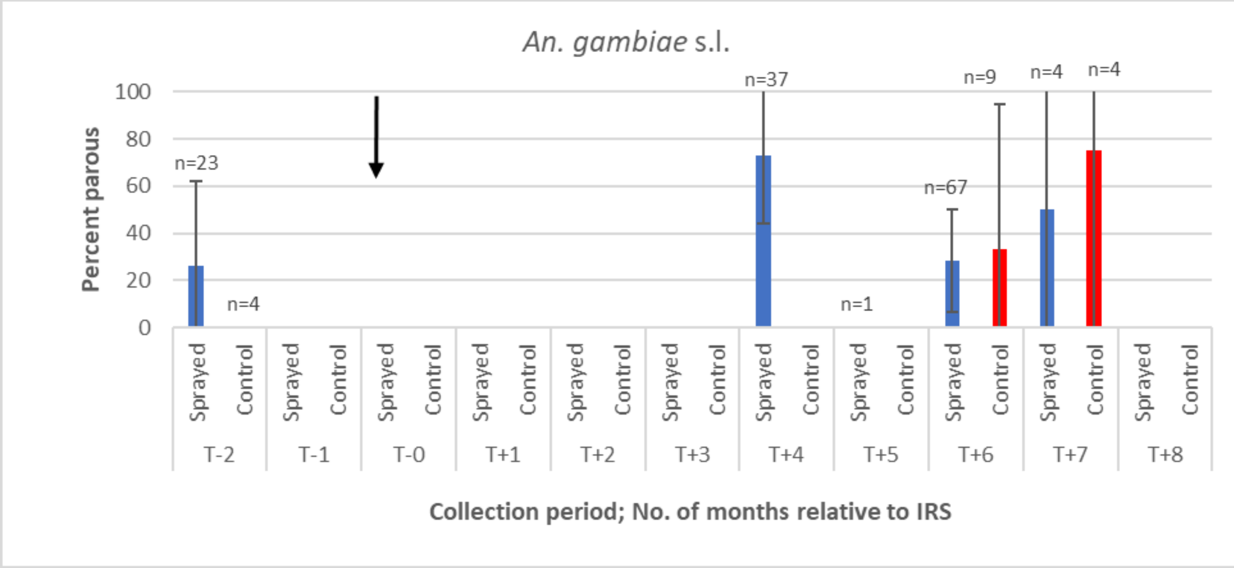
Although there seem to be no impact on parity when data for all sites were combined, we saw some significant impact when the data was broken down into provinces, districts, or sites. Figure 10 is a panel of monthly parity rates for *An. funestus* s.l. and *An. gambiae* s.l. comparing sprayed and control sites for each of the months before and after IRS. All districts from the same province have been combined in this presentation. Serenje District (Central Province) has been excluded from this analysis because the vector numbers collected are not adequate for pre- and post-IRS comparisons. When data was aggregated at the provincial level, we observed no positive impact on *An. funestus* s.l. or *An. gambiae* s.l. parity rates in Luapula Province. In Eastern Province, we observed fewer parous *An. funestus* s.l. and *An. gambiae* s.l. vectors at sprayed sites compared to control sites 44.0% versus 60.6% and 42.8% versus 61.3% respectively. There was reduction in parous mosquitoes during the post-IRS period compared to the period before IRS for both *An. funestus* s.l. or *An. gambiae* s.l. (43.6% versus 66.6% and 42.6% versus 62.5% respectively). In Copperbelt Province, parity rate was similar between combined sprayed and combined control sites for both *An. funestus* s.l. and *An. gambiae* s.l., however when broken down into the period before and after IRS, there were less parous *An. funestus* s.l. and *An. gambiae* s.l. vectors after IRS compared to before IRS at the sprayed sites (24.6% versus 50.0% and 19.3% versus 40.0% respectively). At the district level, positive impact of IRS on parity rate was observed in all districts with statistically significant reductions observed in Katete District (27.4% fewer *An. funestus* s.l. $p=0.05$), Mambwe District (27.1% fewer *An. funestus* s.l. $p=0.07$, 30% fewer *An. gambiae* s.l. $p<0.001$) and Lufwanyama District where there was reduction in parous *An. funestus* after IRS [53% reduction in *An. funestus* s.l. ($p=0001$) and 61% reduction in *An. gambiae* s.l. ($p<0.001$)]. See Annex C-V for statistical output of comparisons of vector parity between sprayed and control sites as well as pre-IRS and post-IRS periods.

Figure 10: Parity Rates of *An. funestus* s.l. and *An. gambiae* s.l. in Sprayed and Control Sites in Each Province By Number of Months Relative to IRS (August 2020-June 2021)

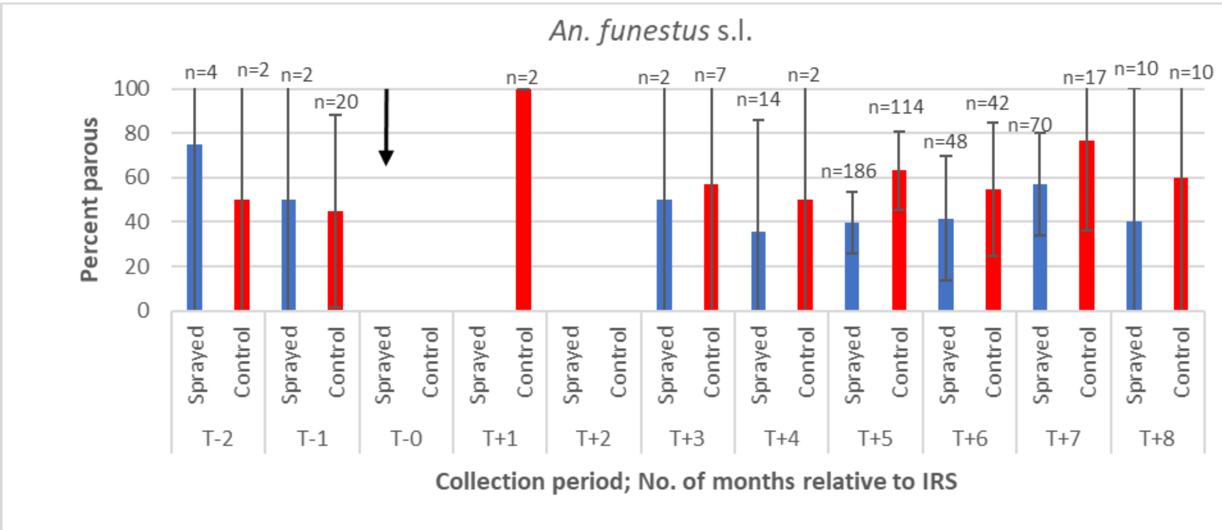
[Bars with 95% confidence intervals. n= total samples examined]

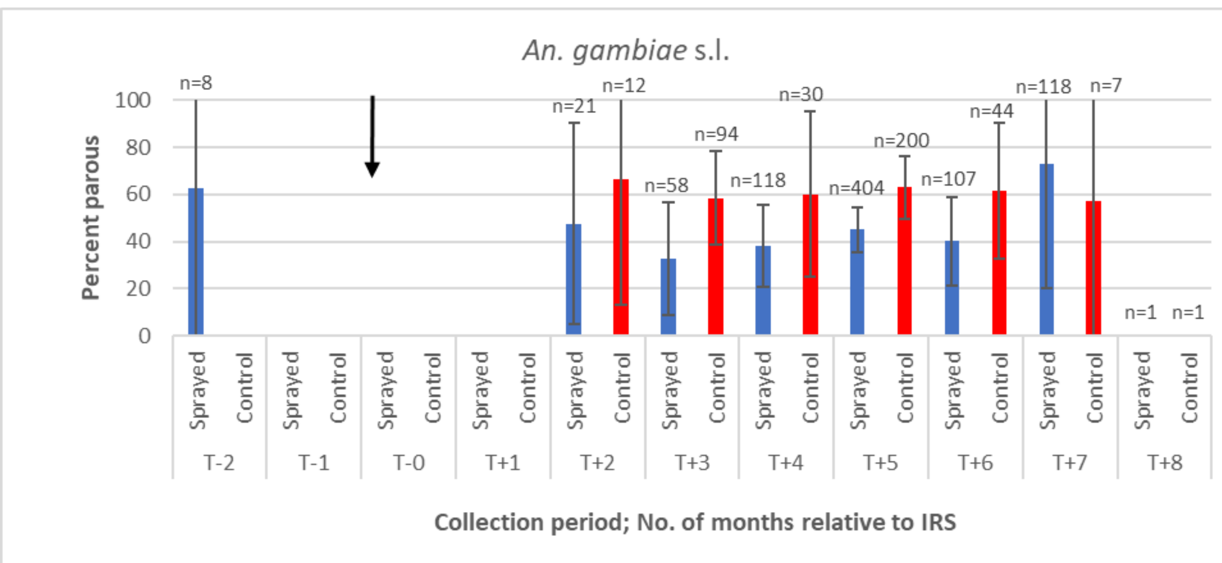
10A: Luapula Province: Parity Rates of *An. funestus* s.l. and *An. gambiae* s.l. in Sprayed and Control Sites



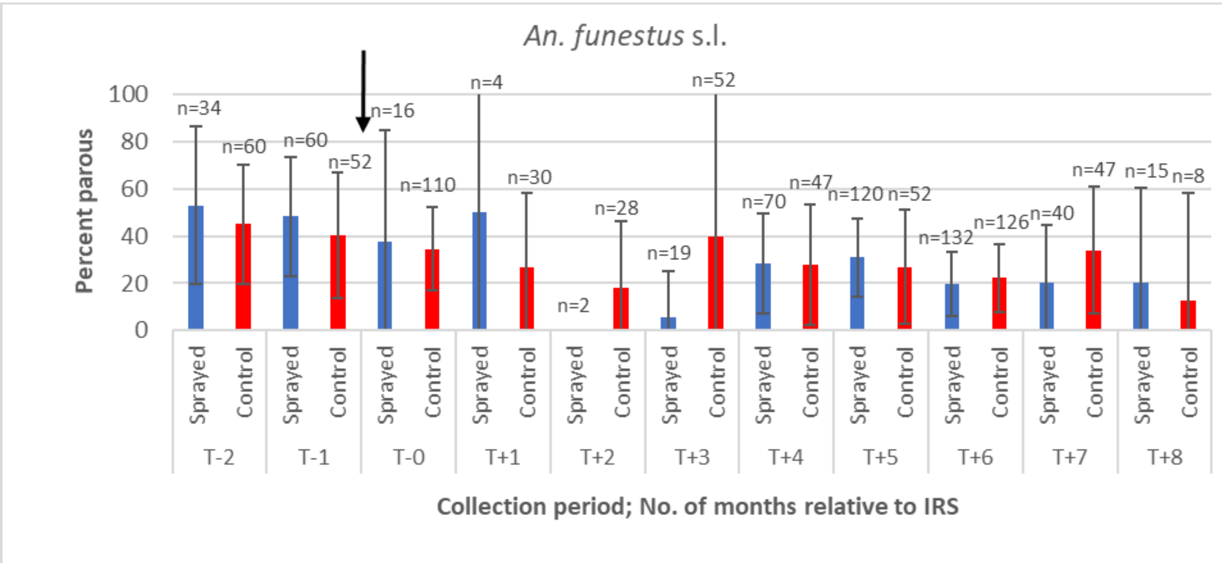


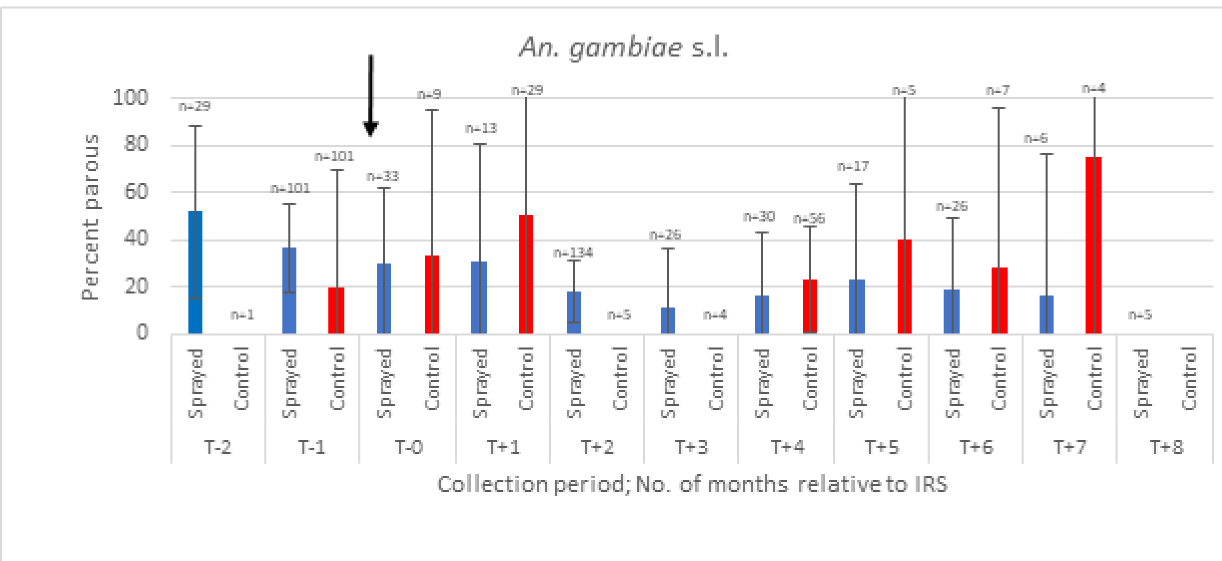
10B: Eastern Province: Parity Rates of *An. funestus* s.l. and *An. gambiae* s.l. in Sprayed and Control Sites





10C: Copperbelt Province: Parity Rates of *An. funestus* s.l. and *An. gambiae* s.l. in Sprayed and Control Sites





3.2 LABORATORY RESULTS

Limited access to the laboratory at the NMEC due to COVID-19 restrictions continued to hinder the progress in sample analysis. We planned to clear the backlog and achieve a two-month lag time between sample collection and laboratory processing, but this has not been achieved. We have a four-month lag time and the data presented here is based on the samples analyzed to date 64% of the 1,554 samples targeted for PCR analysis, more than double (2.6 times) the 2,515 samples targeted for ELISA analysis, and 29% of the 560 samples targeted for blood meal source determination (2020 work plan targets).

3.2.1 PCR IDENTIFICATION OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. SPECIES AND KDR ALLELES

Of the 402 *An. gambiae* s.l. and 1,520 *An. funestus* s.l. tested by PCR, 263 and 695 successfully amplified, respectively. There has been some improvement in specimen amplification rate since the 2019/20 annual report due to some of the changes effected to optimize the laboratory process—amplification for *An. gambiae* s.l. increased from 32% to 65% and *An. funestus* s.l. increased from 31% to 46%.

Almost all of the *An. gambiae* s.l. that amplified were *An. gambiae* s.s. (99.2%) the remainder being *An. arabiensis* (0.8%) while most *An. funestus* s.l. that were tested successfully were *An. funestus* s.s. (99.4%) with few *An. vaneedeni* (0.4%) and *An. parensis* (0.1%). Table 6 below shows the distribution of the different molecular species of *An. gambiae* s.l. and *An. funestus* s.l. vectors by district for the period August 2020 to May 2021. *An. vaneedeni* was found in Lufwanyama District (Copperbelt Province) while *An. parensis* was found in Katete District in Eastern Province. Out of 24 alpha-cypermethrin resistant *An. gambiae* s.l. samples from Katete District tested for the presence of *kdr*, none were positive for either East or West Africa *kdr* alleles.

Table 6: Molecular Identification of *An. gambiae* s.l. and *An. funestus* s.l. Collected from Sentinel Districts (August 2020-May 2021)

District	*An. gambiae* s.l.			
	Total tested	Total amplified	*An. gambiae* s.s.	*An. arabiensis*
Nchelenge	34	30	30	0
Milenge	17	12	12	0
Mambwe	6	2	1	1
Katete	1	1	0	1
Lufwanyama	211	136	136	0
Chililabombwe	133	82	82	0
Total	**402**	**263**	**261**	**2**
		% of Total Amplified	**99.2**	**0.8**

District	*An. funestus* s.l.				
	Total tested	Total amplified	*An. funestus* s.s.	*An. vaneedeni*	*An. parensis*
Nchelenge	476	208	208	0	0
Milenge	256	166	166	0	0
Mambwe	9	1	1	0	0
Katete	139	58	57	0	1
Lufwanyama	266	72	69	3	0
Chililabombwe	374	190	190	0	0
Total	**1,520**	**695**	**691**	**3**	**1**
		% of Total Amplified	**99.4**	**0.4**	**0.1**

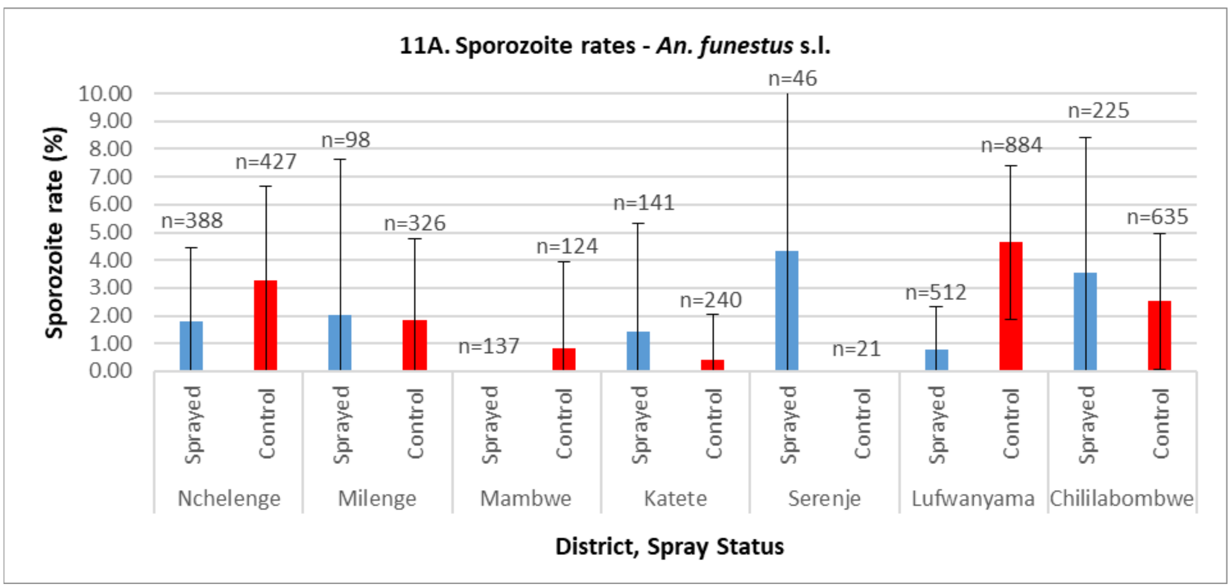
3.2.2 SPOROZOITE INFECTIVITY RATES AND ENTOMOLOGICAL INOCULATION RATES (EIRS)

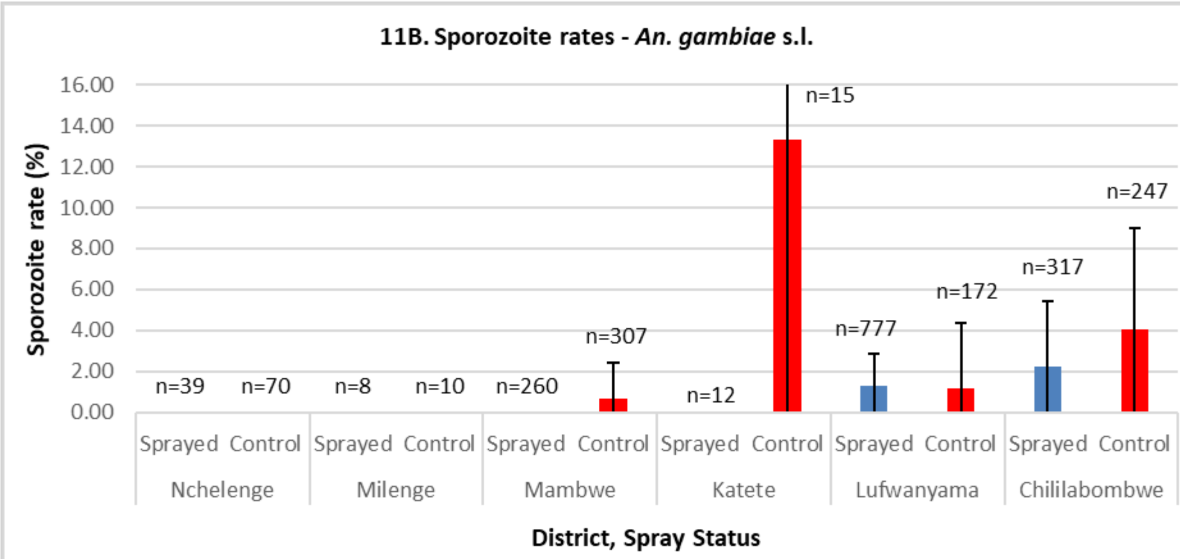
A total of 2,235 *An. gambiae* s.l. and 4,204 *An. funestus* s.l. collected from both sprayed and control sites were tested for *Plasmodium* circumsporozoite proteins. The sporozoite rate for the two species were 1.48% and 2.47%, respectively. Sporozoite rates were lower at the combined sprayed sites compared to the combined control sites; 1.620% versus 2.97% for *An. funestus* s.l. and 1.20% versus 1.95% for *An. gambiae* s.l., respectively. At district level, *An. funestus* s.l. sporozoite rates were lower at sprayed sites compared to control sites in Nchelenge, Mambwe and Lufwanyama Districts, while *An. gambiae* s.l. sporozoite rates were lower in Mambwe, Katete and Chililabombwe Districts. No sporozoite positive *An. gambiae* s.l. vectors were detected in Nchelenge and Milenge Districts. (Fig 11A and 11B).

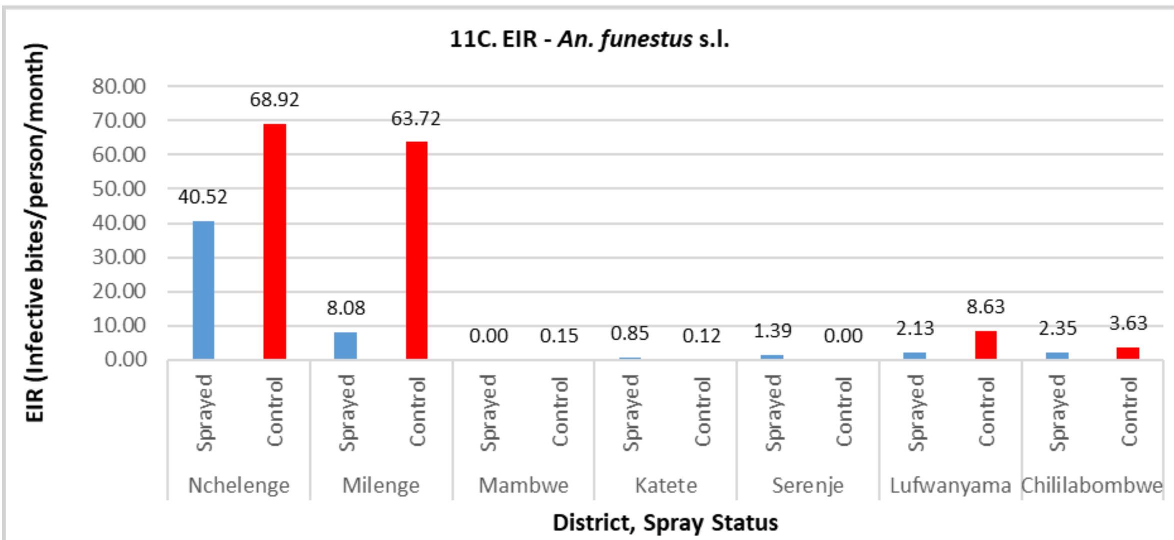
The average EIR for *An. funestus* s.l. was lower at the sprayed sites compared to the control sites in five of the seven districts (Nchelenge, Milenge, Mambwe, Lufwanyama and Chililabombwe) while that for *An. gambiae* s.l. was lower in four districts (Mambwe, Katete, Lufwanyama and Chililabombwe). No *An. gambiae* s.l. infective bites were detected in Nchelenge and Milenge Districts. No sporozoite tests were performed on *An. gambiae* s.l. samples in Serenje and therefore EIR for this species was not determined for this district (Figures 11C and 11D).

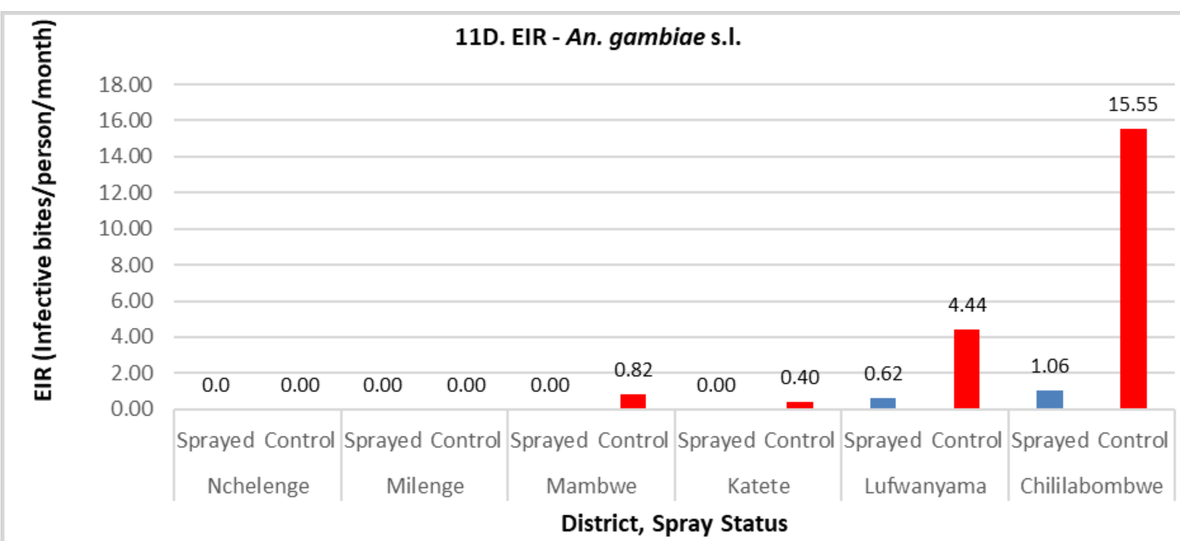
Figure 11: *An. funestus* s.l. and *An. gambiae* s.l. Sporozoite Infection Rates (A and B) and Entomological Inoculation Rates (C and D) at Sprayed and Control Sites By District And Spray Status (August 2020-June 2021)

[Bars with 95% confidence intervals. n=total sample examined. Note that figures on the bars for 11C&11D are EIR values]




11B. Sporozoite rates - An. gambiae s.l.
Sporozoite rate (%)
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
n=39
n=70
n=8
n=10
n=260
n=307
n=12
n=15
n=777
n=172
n=317
n=247
Sprayed Control Sprayed Control Sprayed Control Sprayed Control Sprayed Control Sprayed Control
Nchelenge
Milenge
Mambwe
Katete
Lufwanyama
Chililabombwe
District, Spray Status


11C. EIR - An. funestus s.l.
EIR (Infective bites/person/month)
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
40.52
68.92
8.08
63.72
0.00
0.15
0.85
0.12
1.39
0.00
2.13
8.63
2.35
3.63
Sprayed Control Sprayed Control Sprayed Control Sprayed Control Sprayed Control Sprayed Control Sprayed Control
Nchelenge
Milenge
Mambwe
Katete
Serenje
Lufwanyama
Chililabombwe
District, Spray Status

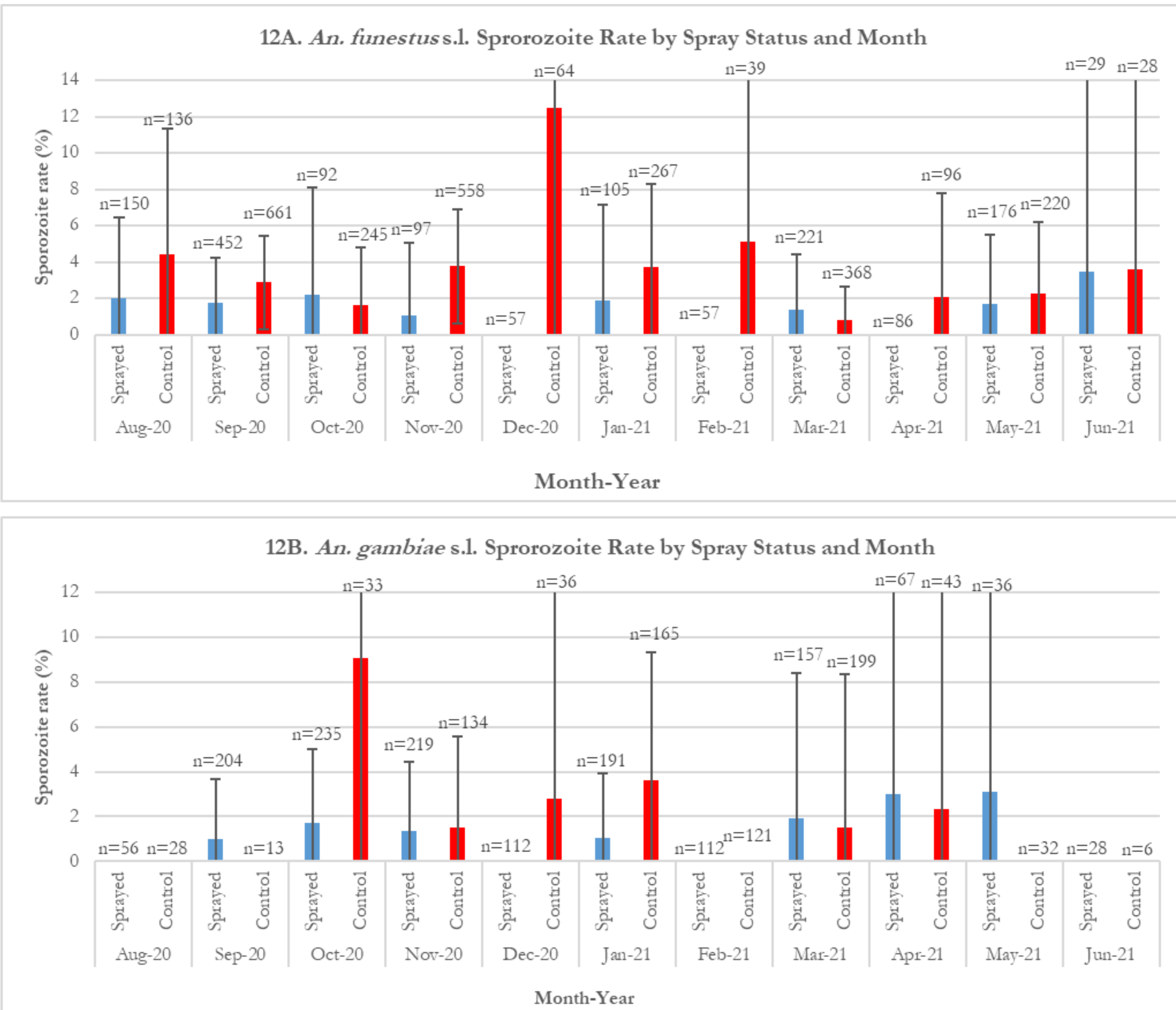

11D. EIR - An. gambiae s.l.
EIR (Infective bites/person/month)
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
0.0
0.00
0.00
0.00
0.00
0.82
0.00
0.40
0.62
4.44
1.06
15.55
Sprayed Control Sprayed Control Sprayed Control Sprayed Control Sprayed Control Sprayed Control
Nchelenge
Milenge
Mambwe
Katete
Lufwanyama
Chililabombwe
District, Spray Status

The number of molecular species tested and number positive, along with a breakdown of numbers tested, numbers positive, and EIR for indoor and outdoor *An. funestus* s.l. and *An. gambiae* s.l. before and after IRS, are provided in Annex D. Post-IRS EIRs were lower at the sprayed sites compared to the control sites indoors as well as outdoors., while for *An. gambiae* s.l. EIR at sprayed sites was higher after IRS compared to before IRS at the sprayed sites.

Sporozoite infection rates by collection month for each vector species are shown in Figure 12. December was the peak sporozoite infection month for *An. funestus* s.l. vectors while October was the peak for *An. gambiae* s.l. vectors. At the sprayed sites, sporozoite rates for *An. funestus* s.l. were below pre-IRS values for up to seven months after IRS and up to four months after IRS for *An. gambiae* s.l. Note that no weighting was done by either vector density or sporozoite rates. Some districts contributed more than others to the total vectors tested each month.

Figure 12: *An. funestus* s.l. and *An. gambiae* s.l. Sporozoite Infection Rates By Spray Status and Month of Collection (August 2020-June 2021)

[Bars with 95% confidence intervals. Arrow indicates the time IRS was implemented, n= total sample examined]

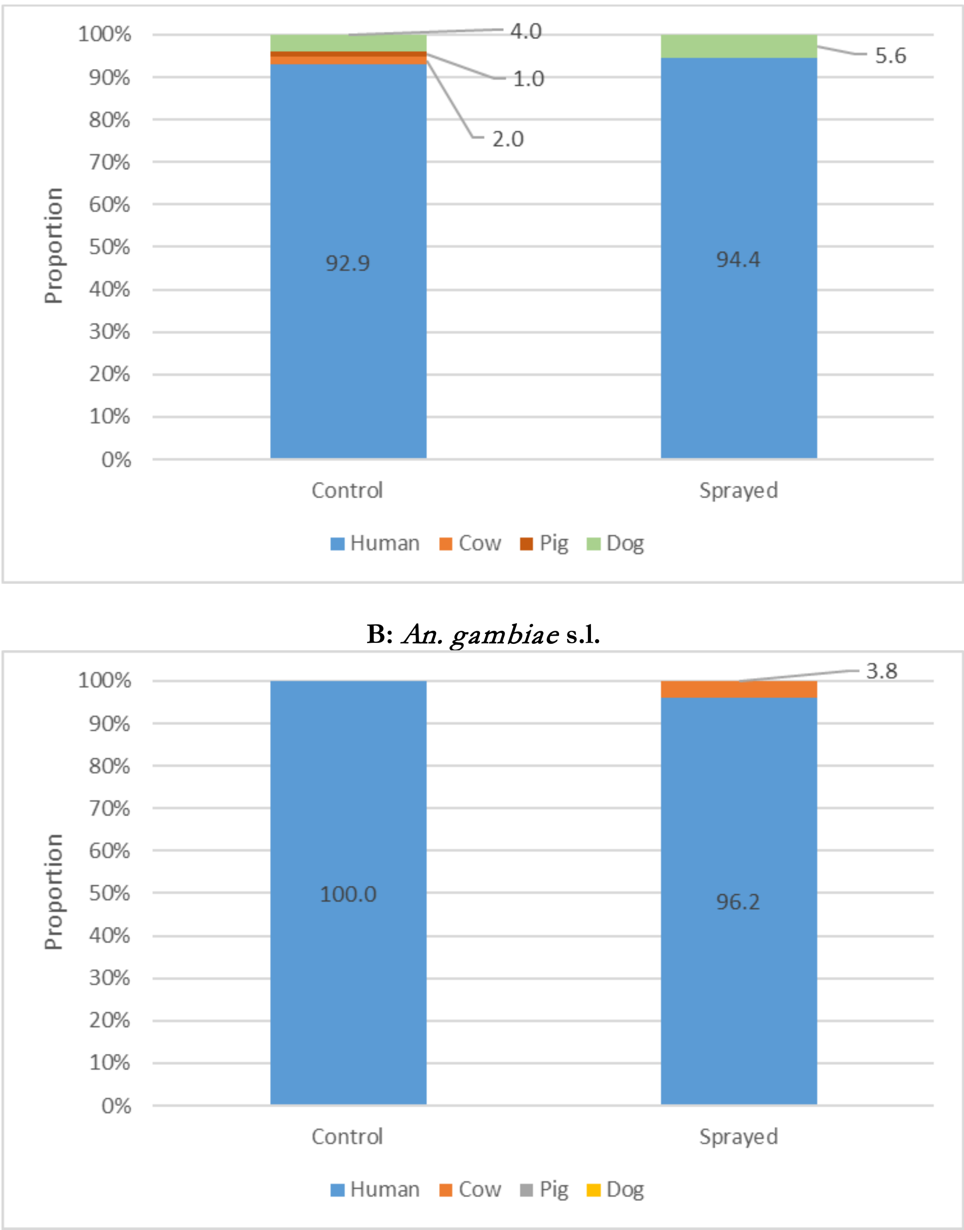


3.2.3 BLOOD MEAL SOURCES

Out of the 117 blood meals identified from fed *An. funestus* s.l. vectors, 93.2% were from humans followed by 4.3% from dogs, 1.7% from cows and 0.9% from pigs. Out of the 43 blood meals identified from fed *An. gambiae* s.l. vectors, 42 (97.7%) were from humans and one (2.3%) was from cow. When blood meal sources were grouped into control and intervention sites, the human blood index for *An. funestus* s.l. was slightly higher in the combined sprayed sites (94.4) compared to the combined control sites (92.9) (Figure 13A) and that for *An. gambiae* s.l. was also higher at control sites (100%) compared to the sprayed sites- 96.2% (Figure 13B). This finding suggests that, in the entire region, the majority of vectors resting indoors obtain their blood meals from humans.

Figure 13: Sources of Blood Meal for *An. funestus* s.l. and *An. gambiae* s.l. Vectors from Indoor Resting Collections (August 2020-April 2021)

A: *An. funestus* s.l.



3.3 QUALITY ASSURANCE OF IRS AND MONITORING OF INSECTICIDE DECAY RATE

3.3.1 QUALITY ASSURANCE

Cone bioassays were conducted in a total of 42 treated houses (21 mud and 21 cement houses) and 14 control (unsprayed) houses (seven mud and seven cement) in seven districts where VectorLink Zambia conducted IRS during the quality of spray determination at the start of the 2020 IRS campaign. In all, 1,260 susceptible *An. gambiae* s.s. mosquitoes (Kisumu strain) were exposed to treated walls in seven districts. All mosquitoes exposed to walls sprayed with Fludora Fusion were dead after the 24-hour holding period, except for one house in Katete where 100% mortality occurred after 48 hours (Table 7). Knockdown after 60 minutes was 98.3% in Nchelenge, 98.9% in Kawambwa, 86.1% in Katete, and 100% in Lufwanyama and Masaiti.

Table 7: Mortality of Kisumu Susceptible Strain of *An. gambiae* s.s. after Exposure to Walls Sprayed with Fludora Fusion in October 2020

District	Wall Type	House Code	*An. gambiae* s.s. Kisumu strain				
			No. of females exposed	% Knockdown observed 30 mins post-exposure	% Knockdown observed 60 mins post-exposure	% Mortality observed after 24 hours	% Mortality observed after 48 hours
Nchelenge	Mud	1	30	100.0	100.0	100.0	-
		2	30	96.7	100.0	100.0	-
		3	30	96.7	100.0	100.0	-
	Cement	4	30	60.0	93.3	100.0	-
		5	30	50.0	96.7	100.0	-
		6	30	100.0	100.0	100.0	-
Kawambwa	Mud	1	30	96.7	100.0	100.0	-
		2	30	96.7	100.0	100.0	-
		3	30	86.7	96.7	100.0	-
	Cement	4	30	100.0	100.0	100.0	-
		5	30	86.7	100.0	100.0	-
		6	30	93.3	96.7	100.0	-
Katete	Mud	1	30	70.0	90.0	100.0	-
		2	30	70.0	93.3	100.0	-
		3	30	73.3	90.0	100.0	-
	Cement	4	30	86.7	93.3	100.0	-
		5	30	60.0	83.3	100.0	-
		6	30	53.3	66.7	90.0	100.0
Lufwanyama	Mud	1	30	100.0	100.0	100.0	-
		2	30	100.0	100.0	100.0	-
		3	30	100.0	100.0	100.0	-
	Cement	4	30	100.0	100.0	100.0	-
		5	30	100.0	100.0	100.0	-
		6	30	100.0	100.0	100.0	-
Masaiti	Mud	1	30	100.0	100.0	100.0	-
		2	30	100.0	100.0	100.0	-
		3	30	100.0	100.0	100.0	-
	Cement	4	30	100.0	100.0	100.0	-
		5	30	100.0	100.0	100.0	-
		6	30	100.0	100.0	100.0	-

Mosquitoes exposed to walls sprayed with SumiShield had a slower mortality, with 80% mortality occurring at 24 hours for seven out of the 12 houses assessed and 100% mortality occurring at 48 hours for five out of 12 houses (Table 8). By the end of the observation period (120 hours post-exposure), eight out of 12 houses attained 100% mortality in Chipata and Mambwe Districts. Knockdown after 60 mins was 25.6% in Chipata and 46.7% in Mambwe. Overall, 38 out of 42 houses monitored during the PMI VectorLink IRS campaign in 2020 attained 100% mosquito mortality at the end of the observation period. This translates to about 90% of spray operators performing high spray quality.

Table 8: Mortality of Kisumu Susceptible Strain of *An. gambiae* s.s. after Exposure to Walls Sprayed with SumiShield in October 2020

District	Wall Type	House Code	*An. gambiae* s.s. Kisumu strain							
			No. of females exposed	% Knockdown observed 30 mins post-exposure	% Knockdown 60 mins post-exposure	% Mortality after 24 hours	% Mortality after 48 hours	% Mortality after 72 hours	% Mortality after 96 hours	% Mortality after 120 hours
Chipata	Mud	1	30	6.7	33.3	86.7	96.7	96.7	100.0	-
		2	30	0.0	10.0	70.0	80.0	86.7	93.3	96.7
		3	30	0.0	6.7	83.3	90.0	96.7	96.7	100.0
	Cement	4	30	30.0	60.0	96.7	96.7	96.7	100.0	-
		5	30	13.3	16.7	33.3	53.3	56.7	70.0	96.7
		6	30	10.0	26.7	96.7	100.0	-	-	-
Mambwe	Mud	1	30	43.3	56.7	80.0	100.0	-	-	-
		2	30	16.7	50.0	90.0	100.0	-	-	-
		3	30	40.0	66.7	96.7	100.0	-	-	-
	Cement	4	30	30.0	30.0	56.7	56.7	70.0	85.7	96.5
		5	30	56.7	60.0	66.7	73.3	80.0	85.7	96.5
		6	30	13.3	16.7	76.7	100.0	-	-	-

We conducted quality of spray in three GF/GRZ-supported districts—Mwansabombwe (Luapula Province), Chongwe (Lusaka Province), and Chibombo (Central Province). All three districts were sprayed with DDT. There was high quality of spraying by the spray operators monitored in all three districts with 100% post-exposure mortality of susceptible *An. gambiae* s.s. vectors in 15 out of the 18 houses (nine mud and nine cement) checked for spray quality (Table 9).

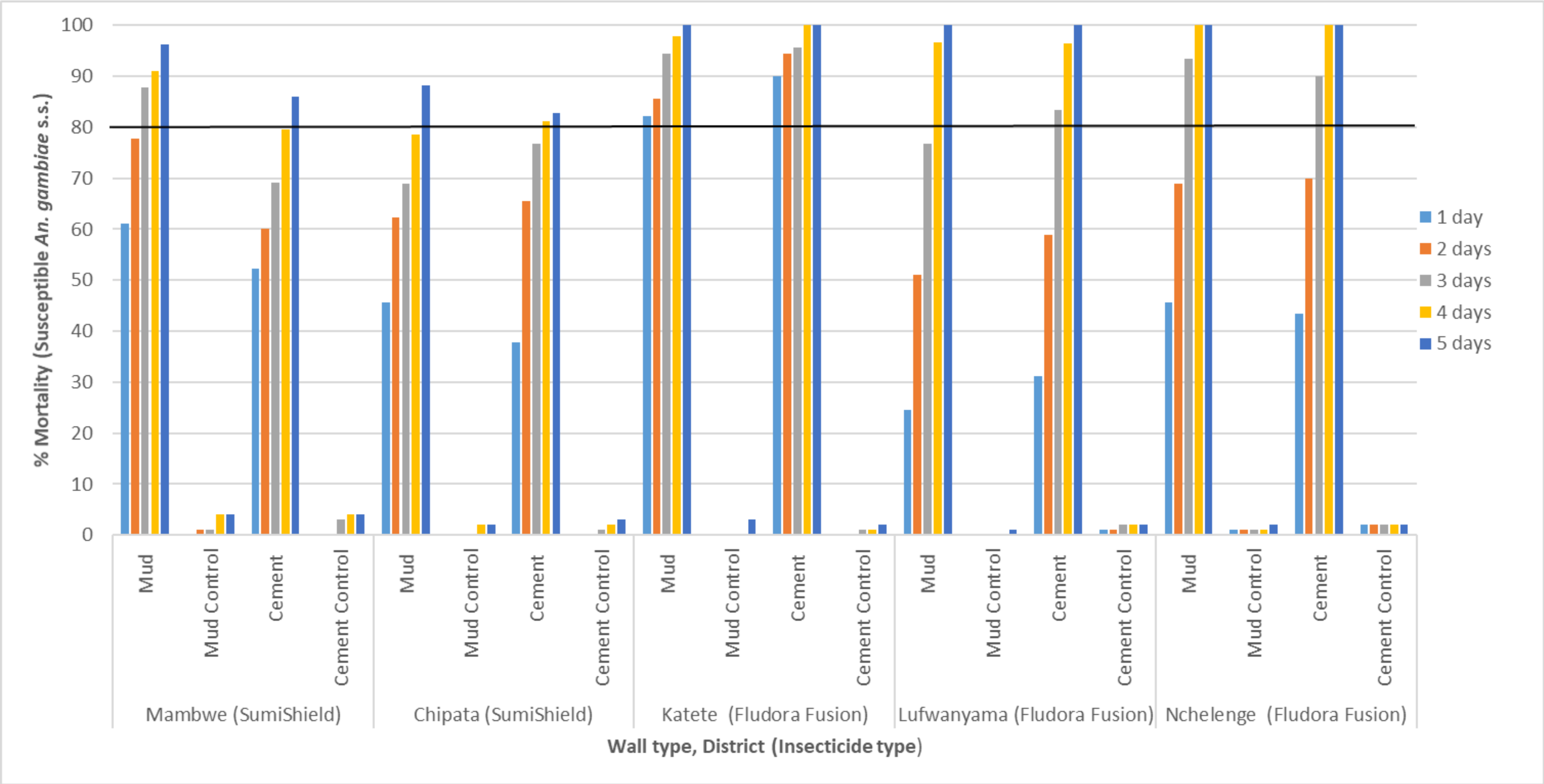
Table 9: Quality of Spray at Three GF/GRZ supported Districts Sprayed with DDT (November 2020 IRS Campaign): Mortality of Kisumu Susceptible Strain of *An. gambiae* s.s. after Exposure to Sprayed Walls

District	Wall Type	House Code	*An. gambiae* s.s. Kisumu strain			
			No. of females exposed	% Knockdown observed 30 mins post-exposure	% Knockdown 60 mins post-exposure	% Mortality after 24 hours
Chibombo	Mud	1	30	6.7	70.0	100.0
		2	30	23.3	76.7	100.0
	Cement	3	30	100.0	100.0	100.0
		4	30	90.0	93.3	100.0
		5	30	93.3	80.0	100.0
		6	30	96.7	96.7	100.0
Mwansabombwe	Mud	1	30	70.0	96.7	100.0
		2	30	36.7	63.3	90.0
		3	30	53.3	90.0	100.0
	Cement	4	30	53.3	80.0	93.3
		5	30	73.3	90.0	100.0
		6	30	50.0	96.7	100.0
Chongwe	Mud	1	30	50.0	83.3	100.0
		2	30	15.6	65.6	96.9
		3	30	36.7	73.3	100.0
	Cement	4	30	60.0	56.7	100.0
		5	30	76.7	100.0	100.0
		6	30	3.3	20.0	100.0

3.3.2 Insecticide Decay Rate

Monthly cone bioassays were conducted in five of the seven PMI-supported districts to monitor the residual efficacy of the insecticides on the walls. Figure 14 shows mortality at 120 hours of exposed and control mosquitoes by wall type and site at 10 months post-IRS (residual efficacy data for August 2021). Note that bioassays were not conducted in July 2021 due to COVID-19 restrictions. Both SumiShield and Fludora Fusion were effective 10 months post-IRS at all five sites (more than 80% mortality at 120 hours post-exposure for both insecticides on mud and cement walls at all sites). Control mortality was below 20% in each case, and corrected mortality was calculated using Abbot's formula for the sites where control mortality was between 5-20%.

Figure 14: Mortality of *An. gambiae* s.l. Kisumu Strain to SumiShield and Fludora Fusion 10 Months Following the October 2020 IRS Campaign



Note: The black line indicates the 80% minimum mortality threshold for insecticide efficacy; the rate of insecticide decay is measured according to when the mosquito mortality falls below 80% for two consecutive occurrences.

3.4 INSECTICIDE RESISTANCE MONITORING

An. funestus s.l. and *An. gambiae* s.l. were fully susceptible to clothianidin 2%, chlorfenapyr (100 µg/bottle), and pirimiphos methyl 0.25% at all sites tested. Susceptibility to clothianidin (>98% post exposure mortality) among *An. funestus* s.l. populations was determined at 48 hours for two sites and at 24 hours at all other sites investigated, while among *An. gambiae* s.l. populations susceptibility was determined at 24 hours for all sites tested. Susceptibility to chlorfenapyr (>98% post exposure mortality) was determined at 72 hours for one site, 48 hours for three sites and at 24 hours at all other sites tested. A mix of resistance profiles for DDT 4% (susceptible, possible, and confirmed resistance) were observed for *An. funestus* s.l. in Luapula and Copperbelt Provinces while there was full susceptibility among *An. gambiae* s.l. populations in Eastern Province. There was resistance (possible or confirmed) among *An. funestus* sl. and *An. gambiae* s.l. vector populations to all pyrethroid insecticides tested (alpha-cypermethrin 0.05%, deltamethrin 0.05%, and permethrin 0.75%) in Luapula and Copperbelt Provinces (Figures 15A and 15C). There was full susceptibility to deltamethrin among the *An. gambiae* s.l. vector populations at the single site tested in Eastern Province (Robert, Katete District-Figure 15B). There was full susceptibility to pirimiphos-methyl at the sites tested in Luapula and Eastern Provinces. *An. funestus* s.l. vectors at the two sites in Serenje District in Central Province were susceptible to chlorfenapyr.

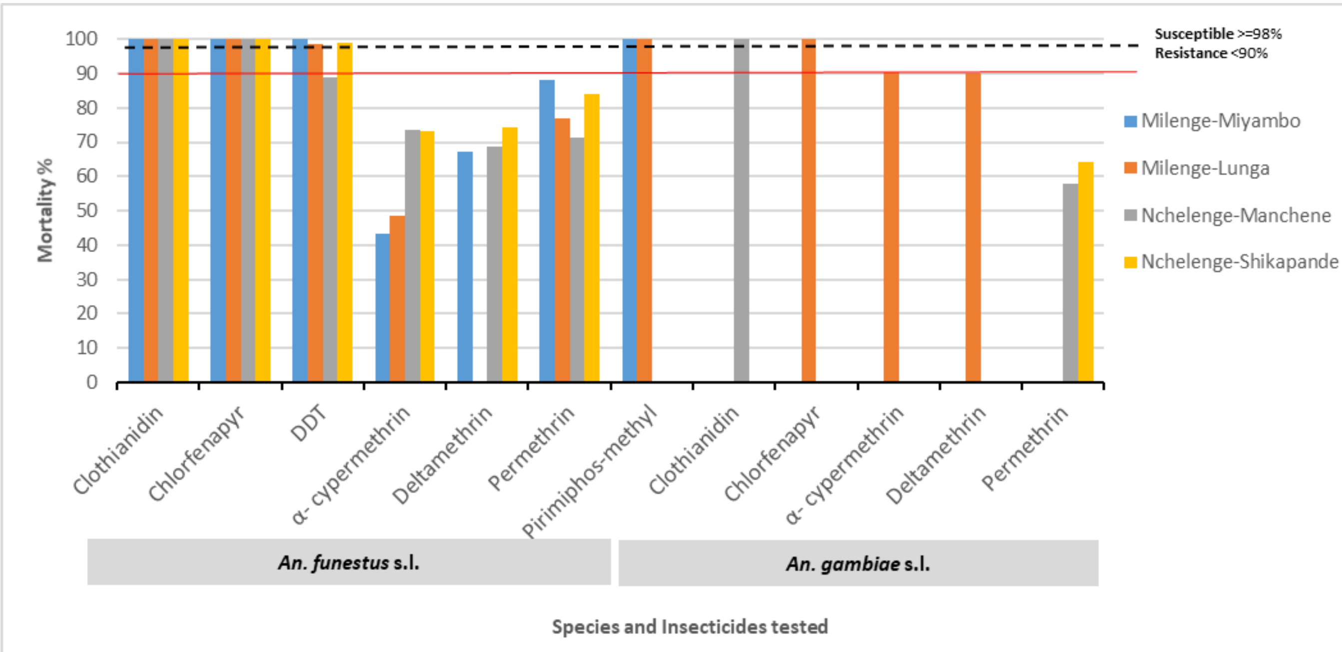
Mortality in all control tests (non-insecticide-treated papers or untreated bottles) were below 20%; corrected mortality using the Abbott formula was used for all assays in which control mortality was between 5-20%. Exposed mosquito mortality of 98% (shown by the top dotted line) or above indicates susceptibility, while mortality below 90% (shown by the bottom line) indicates confirmed resistance. Mortality between the two is indicative of possible resistance. Annex E contains a table of the insecticide susceptibility test results conducted from December 2020 to May 2021 for both species.

Full or partial susceptibility was restored among pyrethroid resistant mosquitoes in Luapula Province (Figure 16A) and Copperbelt Province (Figure 16B) by the pre exposed of resistance vectors to the synergist PBO. This suggests that metabolic resistance together with other additional resistance mechanisms may be present in these provinces.

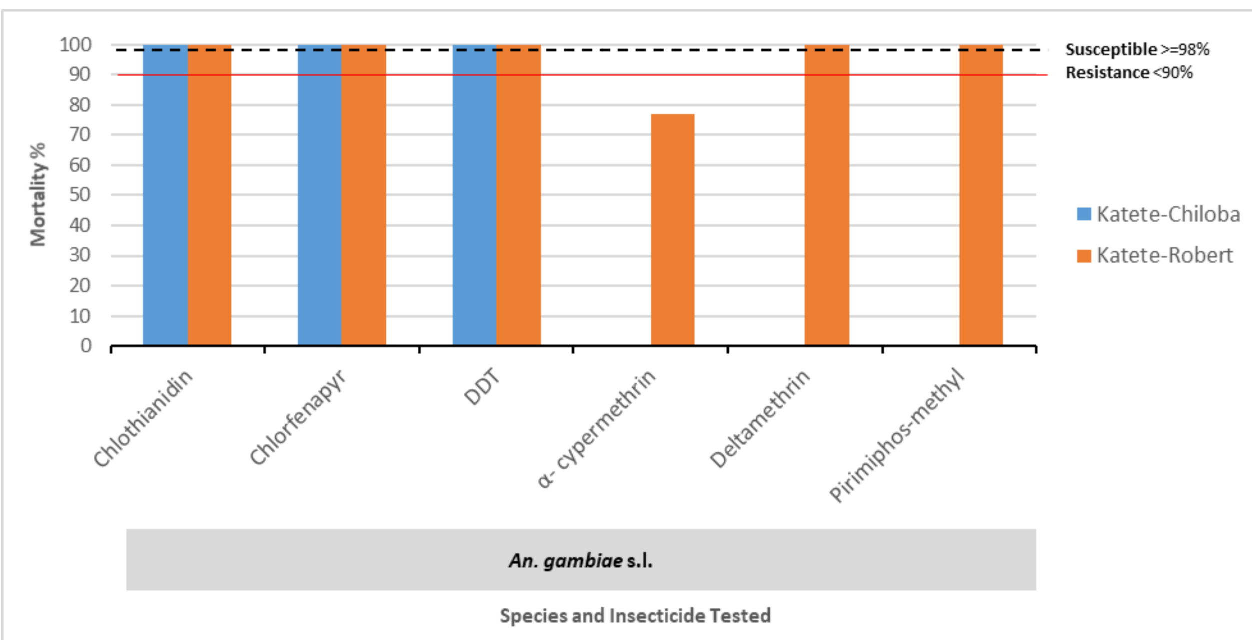
Figure 15: Insecticide Susceptibility Profile for *An. funestus* s.l. and *An. gambiae* s.l. by Province (December 2020-June 2021)

[Mortality reported at a maximum of 48 hours for clothianidin, 72 hours for chlorfenapyr, and 24 hours for DDT, alpha-cypermethrin, deltamethrin, permethrin, and pirimiphos-methyl.]

15A: Luapula Province: Insecticide Susceptibility Profile for *An. funestus* s.l. and *An. gambiae* s.l.



15B: Eastern Province: Insecticide Susceptibility Profile for *An. gambiae* s.l.



15C: Copperbelt Province: Insecticide Susceptibility Profile for *An. funestus* s.l. and *An. gambiae* s.l.

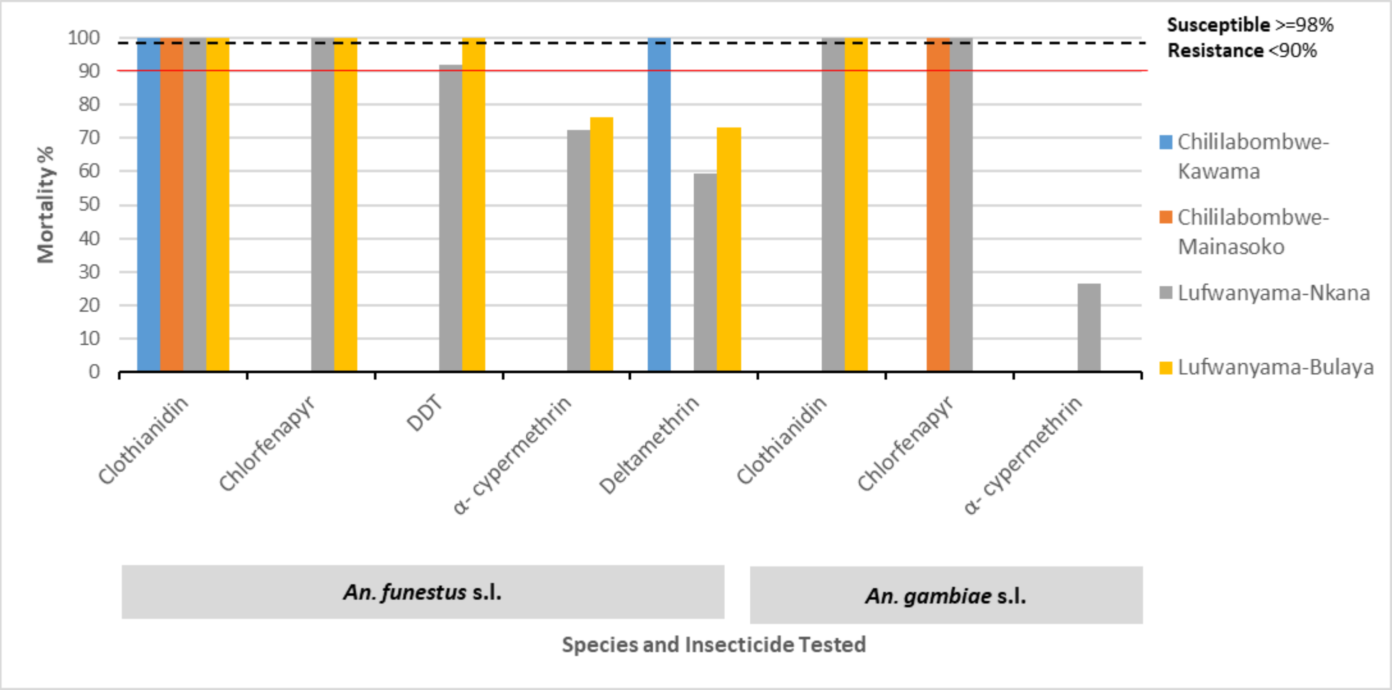
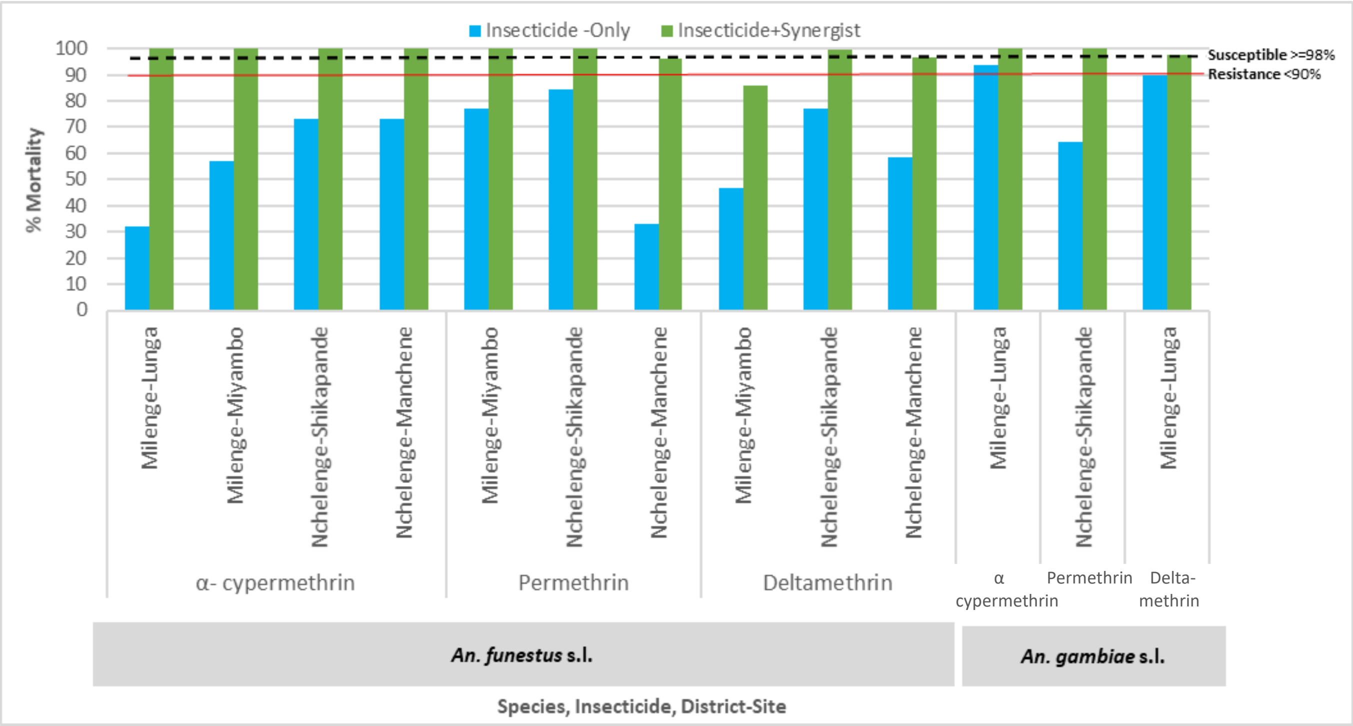


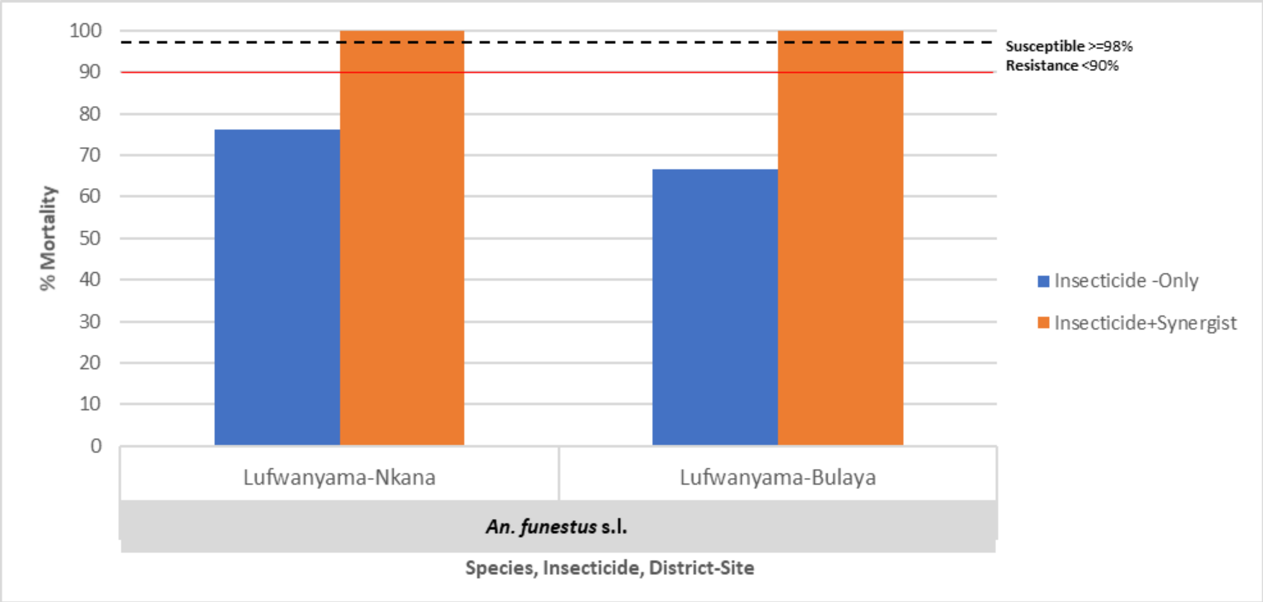
Figure 16: PBO Synergist Assays for *An. funestus* s.l. and *An. gambiae* s.l. by Province (December 2020-June 2021)

[Mortality reported at 24 hours.]

16A: Luapula Province - PBO Synergist Assay for *An. funestus* s.l. and *An. gambiae* s.l.



16B: Copperbelt Province: PBO Synergist Assays for *An. funestus* s.l.



4. DISCUSSION

4.1 SPECIES COMPOSITION AND VECTOR DENSITY

An. funestus s.l. remains the predominant *Anopheles* species and predominant malaria vector at most of the surveillance sites. *Anopheles* species diversity observed during this surveillance period was similar to previous years with a significant presence of *An. ziemanni namibiensis* in Luapula Province and some presence in Copperbelt Province. Though there is relatively high abundance of *An. ziemanni namibiensis*, in our vector collections, the role of this species as a malaria vector is not fully known as we have not found any sporozoite infection among the samples we have screened so far. All 13 different mosquito species identified from the sentinel sites during the reporting period were found in the HLC collections; there was less species diversity in the indoor resting collections.

Of the two main malaria vectors in the region, *An. funestus* s.l. remains dominant over *An. gambiae* s.l. with an overall proportion of 86.9%, which is similar to what was observed in 2019-2020 and 2018-2019 periods (87.9% and 87.6% respectively) [12, 13]. The relative proportion of both species at sprayed sites relative to control sites this reporting period (2020-2021) was similar to the 2019-2020 reporting period. A higher proportion of *An. funestus* s.l. was observed at control sites (62.2% in 2020-2021, 56% in 2019-2020), while a higher proportion of *An. gambiae* s.l. were observed at the sprayed sites (69.6 % in 2020-2021 and 58% in 2019-2020). *An. funestus* s.l. vector numbers were highest in the two districts in Luapula Province. This trend of high *An. funestus* s.l. vector numbers have been reported in Luapula previously and has been attributed to the formation of marshes and other water bodies from the Luapula River in many parts of the province which creates more stable habitats that are good for *An. funestus* s.l. *An. funestus* s.l. was the predominant species in Luapula and Central Provinces. *An. gambiae* s.l. vector numbers relative to *An. funestus* s.l. were highest in Mambwe District in Eastern Province, followed by Lufwanyama and Chililabombwe Districts in Copperbelt Province. There was a noticeable influence of time of year to the relative proportions of the two vector species in Mambwe, Lufwanyama, and Chililabombwe Districts where there was substantial presence of both species. Higher *An. gambiae* numbers were observed at the start of the rainy season compared to the dry season which saw increase in the proportion of *An. funestus* s.l. This relates well with the preference of *An. gambiae* s.l. for transient pools of water (rain pools) that are abundant at the start of the rainy season, as opposed to *An. funestus* s.l. which prefers more stable habitats which linger through the dry season.

There were fewer indoor resting *An. funestus* s.l. vectors at sprayed sites compared to control sites for most of the surveillance districts (six out of seven) and fewer human bites (four out of seven). This outcome is an improvement from the 2019 campaign where reductions in vector numbers were found only at five out of the seven districts and human biting rates at three out of the seven districts. Post-IRS reductions in indoor *An. funestus* s.l. densities were maintained in one site in Luapula Province and one site in Eastern Province. Post-IRS biting rates were reduced to pre-IRS levels or lower in three sprayed sites during this reporting period compared to only one sprayed site last year. Post-IRS reductions in *An. funestus* s.l. human biting was maintained in both sites in Luapula Province. Indoor resting densities are a better measure of IRS impact than biting rates. Where biting rates remain high in IRS sites, it is envisioned that most of those biting are younger mosquitoes – first-time biters with lower risk of transmitting malaria. Differences in the biting rates at the baseline makes comparisons of impact between district difficult. For example, Nchelenge and Lufwanyama had the highest baseline indoor biting rates of *An. funestus* that were substantially reduced following IRS.

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[12] The President's Malaria Initiative (PMI)/VectorLink Project. *Zambia 2018-2019 Entomology Annual Report.* Rockville, MD. The PMI VectorLink Project, Abt Associates.

[13] The President's Malaria Initiative (PMI)/VectorLink Project. *Zambia Annual Entomology Report (June 2019-August 2020).* Rockville, MD. The PMI VectorLink Project, Abt Associates.

However, the post-IRS biting rates in these two districts were higher than districts such as Serenje and Chililabombwe where biting rates actually increased following IRS. The district-level variations in vector numbers reflect either a lack of impact of the intervention at some of the districts or differences in the landscape and ecological characteristics between the IRS and control sites in these districts, most notably, the IRS sites located closer to disproportionately more potential vector habitats than the control sites. There was little or no impact on indoor resting and human biting *An. gambiae* s.l. vector populations, an outcome similar to the findings last year. We observed increases in *An. gambiae* s.l. vector density at both sprayed and control sites. However, the increase at the sprayed sites (two-folds) were far less than that at the control sites (five-fold). There is usually a seasonal increase in *An. gambiae* s.l. just after IRS coinciding with the onset of the rainy season. IRS was probably responsible for the modulated increase observed at the sprayed sites.

We note that the reductions in vector numbers are far less compared to reports from other countries e.g., Kenya[14], where one round of IRS reduced *An. funestus* s.l. numbers by 88%. In the same region, ITNs alone reduced *An. funestus* s.l. populations to near extinction[15], though the vector made a comeback over time probably due to pyrethroid resistance. In Ghana, two years after the shift from pyrethroid insecticides to pirimiphos-methyl in northern Ghana with seven years of IRS, transmission intensity (entomologic inoculation rates) was reduced to undetectable levels even though biting rates were over 10 bites per person during peak vector abundance[16]. However there has been sustained reductions in some districts in Zambia. Post-IRS indoor resting vector numbers were maintained or reduced below pre-IRS levels in Milenge and Mambwe Districts for *An. funestus* s.l., and in Serenje and Mambwe Districts for *An. gambiae* s.l., while post-IRS biting rates were maintained at or reduced below pre-IRS levels in Nchelenge and Milenge Districts for *An. funestus* s.l. and in Serenje, and Katete districts for *An. gambiae* s.l. Generally low *An. funestus* s.l. biting rates (less than two bites per person per night) were maintained for most of the post-spray period at the sprayed sites in Mambwe, Katete, and Serenje Districts, while low *An. gambiae* s.l. biting rates were maintained in Serenje, Katete, and Chililabombwe Districts. Based on these findings, the most concerning districts with little or no reduction in vector numbers after IRS are Nchelenge and Lufwanyama. It is however noteworthy that both districts had the highest baseline indoor biting rates of *An. funestus* s.l. that were substantially reduced following IRS. Milenge District responds well to IRS with indoor densities below two vectors per house, though biting rates there remain high, averaging more than six bites per person per night. It is worth mentioning that an IRS experimental hut study in Benin[17] found that, even though cone bioassay mortality of >80% was maintained on walls against wild-caught, resistant *An. gambiae* s.l. vectors for up to nine months after spraying with Fludora Fusion or a clothianidin-alone product, mortality rates of wild free-flying pyrethroid-resistant *An. gambiae* s.l. that entered the treated huts declined progressively to less than 40% after the first four months. It is unclear to what extent this outcome may explain the high vector numbers seen after IRS with Fludora Fusion and SumiShield in Zambia. This lack of further reduction in numbers in most districts is consistent with findings since 2017 showing a stagnation of vector densities in the area. *An. funestus* s.l. indoor densities reduced from highs of 10-11 vectors per house in 2015 and 2016 to highs of 3-6 vectors per house from 2017 to 2020. There has been no significant and sustained further reduction from these figures for almost four years. For *An. gambiae* s.l., indoor densities slightly increased from highs of 0.5 and 0.1 vector per house in 2017 and 2018 to 1.7 and 1.2 vectors per house in 2019 and 2020. Similarly, *An. funestus* s.l. indoor biting rates from highs of 39-50 bites/person/night in 2015-2016 has stagnated between highs of 14-37 bites/person/night since 2017 and *An. gambiae* s.l. biting rates increased from highs of 5-6 bites/person/night in 2016-2017 to highs of 4-18 bites/person/night in past three years. (See Annex with monthly trends in indoor vector densities and human biting rates from 2015 to 2021. Note that this data should be interpreted with caution as some of the districts were replaced with new districts at certain points during the period which may account for some year-to-year variations in overall vector numbers). A recent report on impact of IRS in Nchelenge District, Luapula Province, described only moderate decreases in

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[14] Abong'o et. al. Scientific Reports 10(1):4518 (2020)

[15] Gimnig *et al. American Journal of Tropical Medicine and Hygiene* 68, 115–120 (2003).

[16] Coleman *et al. Malar J (2017) 16:324*. DOI 10.1186/s12936-017-1971-0.

[17] Fongnikin *et al. Parasites and Vectors*, 13(466), (2020)

indoor vector abundance and suggested that a more comprehensive package of interventions is needed to effectively reduce the malaria burden in such settings18[1].

4.2 VECTOR BITING BEHAVIOR

There was more biting indoors than outdoors for both *An. funestus* s.l. and *An. gambiae* s.l. in six out of the seven districts (the exception being Mambwe District which had more outdoor bites). In addition, one site in Nchelenge had more *An. gambiae* s.l. bites outdoors than indoors. More indoor biting has been reported in previous years and used to strengthen the case for the use of indoor vector control strategies that require vectors to enter dwellings (such as IRS and ITNs). Even though indoor bites were relatively more than outdoor bites, we have observed substantial outdoor biting at all sites with no statistically significant differences between the two feeding locations for either *An. funestus* s.l. and *An. gambiae* s.l. Whether the outdoor biting contributes to residual malaria transmission and how this limits the impact of current vector interventions (ITNs and IRS) is a relevant question that requires investigation so that vector control approaches can be instituted targeting the outdoor environment[19,20]. For now, the only WHO- and PMI-approved vector intervention that targets outdoor biting mosquitoes is larval source management. Deployment of larval source management however requires certain criteria to be met, including areas of low transmission (that is, approaching pre-elimination or elimination) and where larval habitats are few, fixed, and findable. Other tools that target outdoor vectors include attractive toxic sugar baits, housing improvements, and topical and spatial repellents, but these are still under development and are not currently available for programmatic deployment.

A discernable unimodal peak in human biting was observed at sites with high vector numbers such as Luapula Province, while at most of the other sites, there were several small peaks throughout the night. A bimodal peak was observed for *An. gambiae* s.l. at one site in Mambwe District (Eastern Province), the first at 9-10 p.m. and the second at 1-2 a.m. Most of the human biting in Luapula Province by both *An. funestus* s.l. and *An. gambiae* s.l. occurred late at night when people were likely asleep. In Lufwanyama District in Copperbelt Province, biting was sustained until morning indicating a possible risk of late morning biting which can also be a source for residual transmission as residents are usually at home at that time.

4.3 VECTOR ABDOMINAL STATUS, PARITY RATES, SPECIES IDENTIFICATION BY PCR, SPOROZOITE RATES, EIR AND HUMAN BLOOD INDEX

Gravid vectors. The proportion of gravid *An. funestus* s.l. mosquitoes were lower at the combined sprayed sites relative to the combined control sites and also during the overall post-IRS period relative to the pre-IRS period. This is similar to the observations last year though the differences observed this year were not statistically significant. The desired reduction of gravid *An. gambiae* s.l. mosquitoes post-IRS was not observed; the proportion gravid was higher at the combined sprayed sites and combined post-IRS period. However, the proportion of gravid mosquitoes in both sprayed and control sites are generally low. Fewer gravid mosquitoes are a crude indication of younger vector populations, which is a desired outcome of vector control interventions.

Parity. There were no overall significant differences in *An. funestus* s.l. and *An. gambiae* s.l. parity rates when data from all sprayed sites were pooled and compared to pooled data from all control sites. However, when aggregated by province we observed significant positive effects on parity in Eastern and Copperbelt Provinces. We observed significantly lower proportion of parous mosquitoes in Eastern Province at sprayed sites relative to control sites and during the post-IRS period compared to the period before IRS. In the

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[18] Hast *et. al.* Am J Trop Med Hyg. 2021 Feb; 104(2): 683–694. DOI 10.4269/ajtmh.20-0537.
[19] Mario H Rodriguez, *The Journal of Infectious Diseases*, Volume 223, Issue Supplement_2, 1 May 2021, Pages S55–S60, https://doi.org/10.1093/infdis/jiaa582
[20] Sougoufara, S. *et. al. Parasites Vectors* **13,** 295 (2020). https://doi.org/10.1186/s13071-020-04170-7

Copperbelt Province, there was less parous *An. funestus* s.l. and *An. gambiae* s.l. vectors after IRS compared to before IRS at the sprayed sites. The reductions were statistically significant in Lufwanyama District but not in Chililabombwe District. This positive effect on parity was sustained throughout the post-IRS period (up to eight months) in both Eastern and Copperbelt Provinces and rates did not return to the pre-IRS levels. In Luapula Province, there was little to no effect on parity rates. During the previous reporting period (2019-2020), post-IRS parity rates were assessed four months after IRS, due to suspension of activities because of the COVID-19 outbreak in Zambia. There was sustained impact on parity among both *An. funestus* s.l. and *An. gambiae* s.l. vectors during that period (this significant and encouraging finding was submitted and accepted as a poster presentation at the 2022 ASTMH meeting: "Evaluating The Entomological Impact Of The 2019 PMI-Supported IRS Campaign In Zambia On Malaria Transmission Parameters" Poster Number: 1177). Observations this year (with up to eight months of post-IRS data) indicate that the impact of IRS on parity can be sustained for up to 8 months. Parity rates are monitored to determine the age structure of a vector population. The presence of parous mosquitoes is indicative of an older vector population and an increase in the likelihood of malaria transmission because the vectors have survived long enough for the parasite to complete the sporogonic cycle and develop into the infective stage within the mosquito. A decrease in parity rates implies a reduction in the average longevity of the vectors which reduces the ability of the vector to transmit malaria and is the desired outcome for vector control interventions such as IRS and ITNs.

Species identification by PCR. Among the *An. gambiae* s.l. vectors that successfully amplified, 99.2% were *An. gambiae* s.s. and 0.8% were *An. arabiensis*. In the last reporting period (2019-2020), *An. gambiae* s.s. made up 71% of successfully tested samples and *An. arabiensis* made up 29%. The *An. arabiensis* was detected in Eastern Province. Most of the *An. funestus* s.l. samples (99.4%) were *An. funestus* s.s. with a few *An. vaneedeni* and *An. parensis*. Last year we reported a high presence of *An. rivulorum* among the *An. funestus* s.l. population. During the analysis of samples from this reporting period we had cause to perform quality checks on the *An. rivulorum* samples from last year and discovered that there was misidentification of *An. funestus* s.s. as *An. rivulorum*. In the laboratory analysis last year, the Koekemoer PCR protocol was used (in error) to interpret gels that were run with the Wilkins PCR primers. The band sizes differ based on the primer sequences used. All stored photos of the gels from last year laboratory analysis were re-examined and the bands interpreted using the correct protocol. All samples previously identified as *An. rivulorum* were correctly re-identified as *An. funestus* s.s. We also gave 107 randomly selected samples previously identified as *An. rivulorum* to PATH laboratory for independent re-run of species identification PCR and all 65 samples that successfully amplified after one PCR run were identified as *An. funestus* s.s. These results validate the outcome of the gel reinterpretation exercise where all samples misidentified as *An. rivulorum* were re-identified as *An. funestus* s.s. Thus, *An. rivulorum,* in direct contradiction of what was suggested in the addendum to the PMI VectorLink Zambia 2018-2019 Annual Entomology Report and the PMI VectorLink Zambia 2019-2020 Annual Entomology Report is not currently of any significance in malaria transmission in our entomological monitoring sites in Zambia.

Sporozoite rates and EIR. The *Plasmodium* parasite sporozoite rates were higher among *An. funestus* s.l. than *An. gambiae* s.l. populations. Sporozoite rates were lower in sprayed sites compared to control sites for both species. This was an improvement from last year where sporozoite rates for *An. gambiae* s.l. were higher at sprayed sites than control sites. After aggregating data from all IRS sites and that from all control sites, the number of *An. funestus* s.l. infective bites received per month was lower at the IRS sites compared to the control sites but was slightly higher for *An. gambiae* s.l. at the intervention sites compared to the control sites. EIR was reduced after IRS at the sprayed sites while we observed an increase in EIR at the control sites. The reduction in the number of infective bites observed for *An. funestus* s.l. is an indication of a desired outcome of IRS in the area. Reduction in the number of infective bites means a reduction in transmission intensity even in a situation with high vector biting rates. The human blood index was more than 90% for both *An. funestus* s.l. and *An. gambiae* s.l. at combined sprayed and combined control sites indicating that local vectors mostly bite humans rather than other animals thus targeting intervention at the human domicile continues to be an appropriate strategy.

The establishment of the PMI VectorLink supported molecular laboratory space at the NMEC has resulted in improvements in the timing of reporting laboratory indicators. The laboratory processes (PCR and ELISA) continue to be optimized with the assistance of an established molecular laboratory within the same premises that affiliated to PATH, one of the PMI VectorLink partners. The COVID-19 restrictions imposed at the NMEC facilities continue to limit the volume of samples that we can process and has slowed down our ability to clear or significantly minimize the backlog of samples.

4.4 QUALITY OF THE 2020 IRS SPRAY

In the five districts sprayed with Fludora Fusion, we observed 100% mortality of *An. gambiae* s.s. 48 hours post-exposure in all houses and on both surface types (mud and cement). In the two districts sprayed with SumiShield, 100% mortality was achieved in eight out of the 12 houses tested, while the remainder of the houses attained at least 96% mortality. These findings signify a high quality of spraying by the majority of spray operators in the 2020 campaign in the respective districts.

4.5 DURATION OF EFFICACY OF SUMISHIELD AND FLUDORA FUSION

SumiShield and Fludora Fusion were effective on both mud and cement walls with duration of efficacy of at least 10 months. This long duration of efficacy is an encouraging observation as communities in areas with year-round transmission can be protected by IRS, as the insecticide will persist long enough to cover the entire transmission season. It is important to point out that in some places like Nchelenge where vector surge and associated peak transmission lasts from March through September it may be necessary to shift the IRS implementation timetable to coincide with the start of this period. However, Zambia may be faced with a crucial decision as to whether to continue using these clothianidin based products for IRS or rotate to another active ingredient as deployment of this product has surpassed the two years rotation strategy in the national insecticide resistance management and mitigation plan in many districts by the 2021 IRS campaign. Currently, the only viable active ingredient to rotate to is pirimiphos methyl, which has been out of use for at least three consecutive years in most districts and no resistance has been detected among the local vectors. However, pirimiphos-methyl has a short duration that may require at least two spray rounds in a year. A new IRS insecticide product Sylando® 240SC with the active ingredient, chlorfenapyr, has potential for rotation if it obtains WHO pre-qualification listing. This product has been reported to show 7-10 months of residual efficacy on cement walls in experimental hut trials[21] and we have observed full susceptibility to the active ingredient for both *An. funestus* s.l. and *An. gambiae* s.l. in all sites. If a new product is not available, Zambia may have to continue the use of clothianidin-based products in some districts for the fourth year in most districts and for the fifth year in about three districts, raising concerns of the onset of insecticide resistance.

4.6 INSECTICIDE SUSCEPTIBILITY

An. funestus s.l. and *An. gambiae* s.l. were both fully susceptible to clothianidin and chlorfenapyr in Luapula, Eastern and Copperbelt Provinces. There was susceptibility to pirimiphos methyl in Luapula and Eastern Provinces. Based on this and past reports, both vectors are susceptible to clothianidin, chlorfenapyr and pirimiphos methyl in all four provinces monitored by VectorLink Zambia (Luapula, Eastern, Central and Copperbelt). We found a mix of full susceptibility and possible resistance to DDT among populations of either species in Luapula, Eastern and Copperbelt Provinces. A mixture of full susceptibility, possible resistance and confirmed resistance was reported in our 2019/2020 annual report. Use of this product must be considered at the district level based on where susceptibility is reported and any other environmental factors. We observed widespread pyrethroid resistance among vector populations in Luapula, Eastern, and Copperbelt Provinces. Thus, the current strategy of not deploying pyrethroid for IRS remains valid. During the reporting period, the target insecticides (clothianidin, chlorfenapyr, alpha-cypermethrin, and deltamethrin)

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[21]Ngufor, C., Fongnikin, A., Hobbs, N. *et al.* Indoor spraying with chlorfenapyr (a pyrrole insecticide) provides residual control of pyrethroid-resistant malaria vectors in southern Benin. *Malar J* 19, 249 (2020). https://doi.org/10.1186/s12936-020-03325-2

were tested in all provinces except Central due to low mosquito numbers. We tested chlorfenapyr at the two sentinel sites in Central Province and found *An. funestus* s.l. vectors to be fully susceptible.

Synergist assay results indicate the use of oxidase-based metabolic resistance mechanisms by local *An. funestus* s.l. and *An. gambiae* s.l. vectors in Luapula Province and among *An. funestus* s.l. in the Copperbelt Province to avoid mortality caused by pyrethroid insecticides. The partial restoration of susceptibility observed at some of the sites means that additional resistance mechanisms may also be at play. Effectiveness of nets against malaria vectors may be improved in areas with widespread resistance if nets containing the PBO synergist or dual active ingredient net are deployed. Zambia should consider transitioning fully to these new net types (PBO-nets and the dual-active ingredient nets e.g., Interceptor G2) due to the widespread resistance to pyrethroids. In the scenario where clothianidin based insecticides are planned for use in 2022, the dual-active ingredient net should be used and where the chlorfenapyr product becomes available and is used for IRS, then the PBO ITNs or pyriproxyfen ITNs should be used. Intensity assays (to measure intensity of pyrethroid resistance) and synergist assays should be conducted in areas where PBO ITNs will be deployed to provide evidence-based justification for the deployment of the nets.

5. CONCLUSIONS AND RECOMMENDATIONS

This section presents the key findings and implications for each of the indicators monitored, followed by recommendations. See Table 10 for a summary. Note that PMI-supported entomological monitoring is implemented in four of the 10 provinces in Zambia (Eastern, Central, Copperbelt, and Luapula) and these are the provinces considered in this section. Only one district (Serenje) is monitored in Central Province, and it may not be fully representative of the province with respect to entomological and malaria indices.

Species Composition

An. funestus s.l. remains the most abundant of the two primary malaria vectors in Luapula and Central Provinces, while in Eastern Province, *An. gambiae* s.l. was the predominant species in Mambwe District and *An. funestus* s.l. was predominant in Katete District. There was substantial numbers of *An. gambiae* s.l. vectors in the Copperbelt Province though *An. funestus* s.l. was more abundant. Species composition information is important for determining the appropriateness of interventions (IRS and ITNs) in different parts of the countries. Usually, data obtained from a few districts is extrapolated to the provincial level for decision-making.

- When decisions on the deployment of vector control tools are taken based on the predominant primary vector species in an area, those targeting *An. funestus* s.l. can be broadly applied to Luapula and Central Provinces. In Eastern and Copperbelt Provinces, vector control strategies targeting both species should be applied at the provincial level. Where available, district-level species composition information may be used to determine applicability of relevant strategies to certain districts.

Vector Abundance

There were fewer indoor resting and human-biting *An. funestus* s.l. vectors at the sprayed sites compared to the control sites throughout the reporting period. Post-IRS reductions in indoor resting density were maintained in Luapula and Eastern Provinces, while reductions in human biting were maintained in Luapula and Copperbelt Provinces. These results indicate that IRS had an overall positive impact on *An. funestus* s.l. numbers but the reductions are probably not adequate for a sustained impact on malaria transmission. Overall, there were more *An. gambiae* s.l. vectors at the sprayed sites after IRS indicating little or no impact on *An. gambiae* s.l. vector numbers. *An. gambiae* s.l. vector densities are usually low at most of our surveillance sites where they are present. The marginal impact on vector density at sprayed sites has been observed since 2017, indicating a stagnation of vector numbers in the region. This scenario necessitates consideration of the co-deployment of the main vector interventions (IRS and ITNs) or deployment of complimentary vector control interventions such as larval control, house screening and spatial repellents where these are feasible, to further reduce vector numbers below the current levels.

- We support the current PMI-sponsored evaluations of added benefits of co-deployment of IRS with next-generation ITNs. If there is a positive outcome from these investigations, we recommend these interventions in areas with high vector abundance e.g., Luapula Province.
- We recommend the deployment of PBO ITNs or IRS and other supplementary interventions such as larval control (in localities where this is feasible and recommended) to maintain the low numbers or to further reduce the numbers in areas with relatively higher densities in Eastern and Copperbelt Provinces.

Biting Behavior

Most biting by both *An. funestus* s.l. and *An. gambiae* s.l. occurred late at night (between 10 p.m. to 5 a.m.) when people are likely asleep, thus both ITNs and IRS can be good interventions in this region. We note an extension of the late-night biting into the morning hours in Lufwanyama when people are awake. Substantial outdoor biting occurred at many of the monitoring sites and was more than indoor biting at two sites in Eastern Province.

- We recommend an extension of vector collections up to 10 a.m. in Lufwanyama District to investigate the possibility of morning biting. This should be accompanied by human location/sleeping behavior surveys to quantify the risk of human exposure to bites indoors and outdoors throughout the night.
- Identify areas where community-based larval source management is feasible and consider its implementation as a complementary intervention to target vectors that bite outdoors and do not necessarily enter houses to be exposed to the insecticides on walls or in nets. Areas suitable for LSM—that is, with few, fixed and findable larval habitats—can be identified through larval surveys and mapping. This will be proposed in the next work plan.

Parity

There were slightly fewer gravid *An. funestus* s.l. and *An. gambiae* s.l. vectors at the sprayed sites compared to the control sites, an indication of a reduction in older mosquitoes.

Parity rate reduction by IRS was observed for both *An. funestus* s.l. and *An. gambiae* s.l., with fewer parous vectors biting people after IRS than before IRS, in Eastern and Copperbelt Provinces. This reduction was observed throughout the post-IRS period or up to eight months after IRS. Parity was not reduced after IRS in Luapula Province. It is speculated that the timing of IRS implementation may be too early given the late surges in vector abundance and transmission peaks in Nchelenge, a district typical of the environmental conditions that prevail in this province. Reduction in parity rates is an indication that the vectors are not surviving long enough to complete the *Plasmodium* parasite's sporogonic cycle and therefore are unlikely to transmit malaria.

Reduced number of parous vectors after IRS at the sprayed sites was the main impact of IRS observed. The indoor resting density or biting rates might increase at the intervention sites due to natural seasonal increases of the vector populations which would have been higher in the absence of IRS. However, parity provides a more apparent determination of impact. Reductions in older mosquitoes, which are more likely to transmit disease, is the desired outcome of insecticide-based vector control interventions.

- The lack of impact on parity in Luapula Province supports the earlier recommendation that a new strategy may need to be piloted, such as the co-deployment of IRS with SumiShield and PBO ITNs to determine the potential of co-deployment for possible use to reduce vector abundance. Since the mosquito surges and associated transmission peaks in Nchelenge District, Luapula Province extends from March-September, timing of IRS just before these surges may be more effective than IRS conducted in September/November. This maybe applicable to Milenge District as well with similar low-lying swampy environment

Molecular Species, Sporozoite Rates, and EIR

Almost all *An. gambiae* s.l. tested by PCR were *An. gambiae* s.s. and *An. funestus* s.l. were *An. funestus* s.s. Due to the correct re-identification of samples that were identified as *An. rivulorum* in the 2019-2020 survey, and the absence of this species in the 2020-2021samples, we report that *An. rivulorum* is not a potential major vector in this area. Sporozoite rates were lower at the sprayed sites relative to the control sites for both *An. funestus* s.l. and *An. gambiae* s.l. At the sprayed sites, the EIR was lower for *An. funestus* s.l., and slightly higher for *An. gambiae* s.l. The absolute values for EIR at the sprayed sites (approximately 10 and 3 infective bites per person per month for *An. funestus* s.l. and *An. gambiae* s.l. respectively) is enough to maintain high malaria transmission in an area. There was high human blood index for both *An. funestus* s.l. and *An. gambiae* s.l. at sprayed and control sites, that is, majority of the vectors fed on humans and less so on alternative hosts in the environment. Vector control interventions targeting the interruption of human-vector contact continues to be an appropriate strategy for the fight against malaria at these sites.

- Additional interventions on top of vector control interventions, especially those with potential to reduce the transmission of the parasite from humans to the vectors such as prompt diagnosis and treatment of all positive cases is required in the high EIR scenarios observed.

Residual Efficacy

The high mosquito mortalities observed at most houses tested immediately after spraying in 2020 indicates that majority of spray operators performed a good quality of spraying at homes during the campaign.

The residual efficacy of SumiShield and Fludora Fusion on walls after IRS is at least 10 months. The long duration of activity of these clothianidin-based insecticides means that one spray round should suffice to cover the malaria transmission season in Zambia.

- Noting that local vectors remain susceptible to clothianidin-based insecticide products, we recommend continued use of this product for IRS into 2022 with due consideration of the national resistance management plan.

Insecticide Resistance

An. funestus s.l. and *An. gambiae* s.l. were fully susceptible to clothianidin and chlorfenapyr in all three provinces tested. There was a mixture of full susceptibility and suspected resistance to DDT in *An. funestus* s.l. vector populations in Luapula and Copperbelt Provinces and full susceptibility in *An. gambiae* s.l. populations in Eastern Province. There is confirmed resistance to pyrethroid insecticides in Luapula, Eastern and Copperbelt Provinces. There is also presence of oxidase-based metabolic resistance mechanisms among vector populations in Luapula and Copperbelt Provinces.

- We recommend pirimiphos-methyl (Actellic CS) if resources are available to carry out two rounds of spray in the year to cover the long transmission season in the country.
- We also recommend the deployment of clothianidin-based products for IRS with due consideration to the national resistance management plan and chlorfenapyr when it becomes available hopefully in the not-too-distant future and when vectors are still susceptible to it.
- The deployment plans for DDT should be based on district level information on vector susceptibility and consideration should be given to a mosaic approach at the provincial level where some districts deploy DDT while others deploy other insecticide classes. This is applicable to all three provinces (Luapula, Copperbelt and Eastern).
- In the case of the pyrethroids, we support the current insecticide resistance management plan that excludes the use of pyrethroids for IRS and recommend that pyrethroids should not be used in IRS at this time.
- Due to the continued resistance of local vectors to pyrethroid insecticides in some areas, we recommend the transition to next generation ITNs including PBO nets (that is, nets with pyrethroid plus the synergist piperonyl butoxide), dual active ingredients nets (that is pyrethroid, plus the pyrrole chlorfenapyr) and pyrethroid plus the insect growth regulator pyriproxyfen in select areas, especially as/when the ITNs resume their role as the major vector control intervention in the country, as currently planned for 2023 and beyond.

Finally, vector abundance in the region were not greatly reduced post-IRS, which may be due to the natural seasonal rise of vector populations, which would have been higher in the absence of IRS. However, the reduction in number of parous vectors seen in the majority of districts—that is, in older mosquitoes which are more likely to transmit malaria after IRS at the sprayed sites—is an indication of a desired impact of the intervention.

Table 10: Summary of Key Findings and Vector Control Recommendations by Province

Indicator	Luapula Province	Eastern Province	Central Province	Copperbelt Province
Species Composition	*An. funestus* s.l. predominant. ▶ Can use *An. funestus* s.l. to represent the province when known predominant species is needed for decision-making.	A mix of *An. funestus* s.l. and *An. gambiae* s.l. ▶ Consider use of both *An. funestus* s.l. and *An. gambiae* s.l. to represent the province when predominant species is needed in decision making. May need district-level species composition to determine applicability of relevant strategies.	*An. funestus* s.l. predominant ▶ Can use *An. funestus* s.l. to represent the province when known predominant species is needed for decision-making.	A mix of *An. funestus* s.l. and *An. gambiae* s.l. ▶ Consider use of both *An. funestus* s.l. and *An. gambiae* s.l. to represent the province when predominant species is needed for decision-making.
Vector Abundance	Post-IRS reduction in *An. funestus* s.l. indoor density and human biting rates. More *An. gambiae* s.l. vectors at the sprayed sites after IRS ▶ IRS had an overall desirable impact on *An. funestus* s.l. numbers, but reductions likely inadequate for sustained impact on malaria transmission. Recommend IRS or PBO nets and (if there is a positive outcome from the PMI supported operational research) the co-deployment of IRS with PBO or dual AI ITNs.	Post-IRS reduction in *An. funestus* s.l. indoor density ▶ IRS had an overall positive impact on *An. funestus* s.l. numbers. Overall reduced numbers seen. Little or no impact on *An. gambiae* s.l. vector numbers. Recommend IRS or PBO nets and larval control at selected sites.	Positive impact on *An. funestus* s.l. and *An. gambiae* s.l. numbers. ▶ Overall reduced numbers seen at sprayed site. Recommend IRS or PBO ITNs.	Post-IRS reduction in *An. funestus* s.l. HBRs ▶ Positive impact of IRS on *An. funestus* s.l. numbers. Little or no impact on *An. gambiae* s.l. vector numbers. Recommend IRS or PBO nets and any complementary methods such as house screening to further reduce vector numbers. Note that while house screening can be applied anywhere, Copperbelt may be highly suitable as it is highly urbanized with stronger commercial development.
Biting Location	Indoor biting higher than outdoor biting at most sites. Substantial outdoor biting at all sites. ▶ Consider complementary interventions to target outdoor biting vectors such as larval control, and spatial repellents where recommended and feasible.			
Biting Time	Most biting occurred late at night. ▶ Both IRS and ITNs are appropriate interventions.	Most biting occurred late at night. ▶ Both IRS and ITNs are appropriate interventions.	Most biting occurred late at night. ▶ Both IRS and ITNs are appropriate interventions.	Late night and morning hour biting. ▶Both IRS and ITNs are appropriate interventions. Recommend extension of vector collections up to 10 a.m. in Lufwanyama District accompanied by human sleeping behavior surveys to quantify the risk of human exposure at each collection time.
Parity Rates	No reduction in parity rates after IRS ▶ Not a desired outcome of IRS. Consider supplementary vector control strategies for the province.	Reduction in parity rates after IRS ▶ Desired outcome of IRS achieved.	Insufficient data collected	Reduction in parity rates after IRS ▶ Desired outcome of IRS achieved.
HBI	Very high human biting by mosquitoes ▶ Targeting intervention at the human domicile continues to be an appropriate strategy			

<table>
<tr><th>Indicator</th><th>Luapula Province</th><th>Eastern Province</th><th>Central Province</th><th>Copperbelt Province</th></tr>
<tr><td>Sporozoite Rates</td><td colspan="4">Lower sporozoite rates at sprayed sites relative to control sites for both An. funestus s.l. and An. gambiae s.l. Reduction in An. funestus s.l. sporozoite rates after IRS at the intervention sites while there was increase in sporozoite rates at the control sites. Reduction in An. gambiae s.l. sporozoite rates at both sprayed and control sites. ▶ Additional interventions required to reduce sporozoite rates.</td></tr>
<tr><td>EIR</td><td colspan="4">An. funestus s.l. EIR lower at sprayed sites (vs. control sites) and post-IRS (vs. pre-IRS). An. gambiae s.l. EIR slightly higher at the sprayed sites for EIR still high enough to sustain malaria transmission. ▶ Additional interventions required to reduce the transmission in the high EIR scenarios observed in some districts.</td></tr>
<tr><td>Insecticide Residual Efficacy</td><td>At least 10 months of residual efficacy of clothianidin products on walls after IRS ▶ Duration of efficacy adequate to cover malaria transmission season.</td><td>At least 10 months of residual efficacy of clothianidin products on walls after IRS ▶ Duration of efficacy adequate to cover malaria transmission season.</td><td>No residual efficacy site in Central Province, so no data collected.</td><td>At least 10 months of residual efficacy of clothianidin products on walls after IRS ▶ Duration of efficacy adequate to cover malaria transmission season</td></tr>
<tr><td>Insecticide Susceptibility</td><td>Susceptibility: clothianidin & chlorfenapyr (An. funestus s.l. and An. gambiae s.l.), pirimiphos-methyl (An. funestus s.l.)
Susceptibility & possible resistance: DDT (An. funestus s.l.)
Confirmed resistance: alpha-cypermethrin, deltamethrin, permethrin (An. funestus s.l.), permethrin (An. gambiae s.l.)
Possible resistance: alpha-cypermethrin, deltamethrin (An. gambiae s.l.)
▶ Can deploy clothianidin-based products, chlorfenapyr (when available), or pirimiphos-methyl for IRS. Can deploy DDT for IRS at district level. Transition to next generation ITNs - use dual-active ingredient nets where clothianidin based insecticides are planned for IRS and use PBO nets if the chlorfenapyr product becomes available and is used for IRS.</td><td>Susceptibility: clothianidin, chlorfenapyr, deltamethrin, DDT, pirimiphos-methyl (An. gambiae s.l.)
Confirmed resistance: alpha-cypermethrin (An. gambiae s.l.)
▶ Can deploy clothianidin-based products, chlorfenapyr (when available), DDT, and pirimiphos-methyl for IRS. Transition to next generation ITNs - use dual-active ingredient nets where clothianidin based insecticides are planned for IRS and use PBO nets if the chlorfenapyr product becomes available and is used for IRS,</td><td>Susceptibility: chlorfenapyr (An. funestus s.l.)
▶ Can deploy chlorfenapyr for IRS (when available). Transition to next generation ITNs - use dual-active ingredient nets where clothianidin based insecticides are planned for IRS and use PBO nets if the chlorfenapyr product becomes available and is used for IRS,</td><td>Susceptibility: clothianidin, chlorfenapyr (An. funestus s.l. and An. gambiae s.l.)
Susceptibility & possible resistance: DDT (An. funestus s.l.)
Confirmed resistance: alpha-cypermethrin (An. funestus s.l. and An. gambiae s.l.)
Susceptibility & confirmed resistance: deltamethrin (An. funestus s.l.)
▶ Can deploy clothianidin-based products, chlorfenapyr (when available) for IRS. Can deploy DDT for IRS at district level. Transition to next generation ITNs - use dual-active ingredient nets where clothianidin based insecticides are planned for IRS and use PBO nets if the chlorfenapyr product becomes available and is used for IRS</td></tr>
</table>

Annex A: Culicidae Collected in Sprayed and Control Sites by Collection Method (August 2020-June 2021)

District	Village	Status	HLC Indoors												
			An. funestus s.l.	*An. gambiae* s.l.	*An. ziemanni namibiensis*	*An. maculipalpis*	*An. coustani*	*An. tenebrosus*	*An. gibbinsi*	*An. rufipes*	*An. pretor-iensis*	*An. squamosus*	*An. argentio -lobatus*	*An. tchekedii*	*Culicines*
Nchelenge	Shikapande	Sprayed	13,167	1,073	157	0	1	0	0	0	0	0	0	1	300
	Manchene	Control	9,782	572	336	0	0	0	0	0	0	10	0	0	2,701
Milenge	Lunga	Sprayed	1,118	764	2,932	0	0	0	0	0	0	157	0	951	912
	Miyambo	Control	10,966	231	1,138	0	0	0	0	0	0	90	0	517	606
Mambwe	Chikowa	Sprayed	73	319	0	0	20	0	0	0	0	0	0	0	211
	Chasela	Control	68	299	0	0	69	0	0	8	3	16	0	0	55
Katete	Chilowa	Sprayed	156	6	0	2	23	0	0	3	1	2	0	0	54
	Robert	Control	73	7	0	5	17	0	3	3	0	2	0	0	121
Serenje	Chibobo	Sprayed	96	3	4	0	0	0	0	0	0	22	0	0	9
	Chishi	Control	535	8	65	0	0	0	0	0	0	49	0	0	27
Lufwanyama	Nkana	Sprayed	1,645	1,295	104	0	4	0	0	0	0	8	0	0	656
	Bulaya	Control	953	198	390	0	1	0	0	0	0	19	4	1	717
Chililabombwe	Kawama	Sprayed	209	184	6	0	0	0	0	0	0	0	0	0	644
	Mainasoko	Control	549	239	189	0	0	0	0	0	0	13	0	0	481
		TOTAL	**39,390**	**5,198**	**5,321**	**7**	**135**	**0**	**3**	**14**	**4**	**388**	**4**	**1,470**	**7,494**

District	Village	Status	HLC Outdoors												
			An. funestus s.l.	*An. gambiae* s.l.	*An. ziemanni namibiensis*	*An. maculi-palpis*	*An. coustani*	*An. tene-brosus*	*An. gibbinsi*	*An. rufipes*	*An. pretoriensis*	*An. squam-osus*	*An. argentio-lobatus*	*An. tchekedii*	*Culicines*
Nchelenge	Shikapande	Sprayed	6,606	912	380	0	0	0	0	0	0	2	0	0	1,654
	Manchene	Control	8,723	718	704	0	0	0	0	0	0	7	0	0	8,010
Milenge	Lunga	Sprayed	787	535	4,542	0	0	0	0	0	0	350	0	1,401	1,248
	Miyambo	Control	5,647	173	7,567	0	0	0	0	0	0	405	0	1,193	1,352
Mambwe	Chikowa	Sprayed	141	1,034	0	0	310	2	0	2	0	5	0	0	360
	Chasela	Control	94	436	0	3	164	0	0	23	7	18	0	0	102
Katete	Chilowa	Sprayed	133	4	0	1	32	0	1	6	1	1	0	0	52
	Robert	Control	57	3	0	8	21	0	8	3	0	4	0	0	136
Serenje	Chibobo	Sprayed	88	3	23	0	0	0	0	0	0	8	0	0	9
	Chishi	Control	236	9	157	0	1	0	0	0	0	83	0	0	46
Lufwanyama	Nkana	Sprayed	753	959	182	0	0	0	0	0	0	14	0	0	442
	Bulaya	Control	676	82	1,043	0	0	0	0	2	0	17	3	1	544
Chililabombwe	Kawama	Sprayed	165	145	7	0	0	0	0	0	0	0	0	0	648
	Mainasoko	Control	261	71	141	0	0	0	0	0	0	11	0	0	296
		TOTAL	**24,367**	**5,084**	**14,746**	**12**	**528**	**2**	**9**	**36**	**8**	**925**	**3**	**2,595**	**14,899**

District	Village	Status	PSC												
			An. funestus s.l.	*An. gambiae* s.l.	*An. ziemanni namibiensis*	*An. macul-ipalpis*	*An. coustani*	*An. tenebrosus*	*An. gibbinsi*	*An. rufipes*	*An. preto-riensis*	*An. squa-mosus*	*An. argenti-olobatus*	*An. tchekedii*	*Culicines*
Nchelenge	Shikapande	Sprayed	1,443	63	1	0	0	0	0	0	0	0	0	0	22
	Manchene	Control	3,975	150	1	0	0	0	0	0	0	0	0	0	80
Milenge	Lunga	Sprayed	279	59	16	0	0	0	0	0	0	0	0	0	19
	Miyambo	Control	1,706	7	8	0	0	0	0	0	0	1	0	3	91
Mambwe	Chikowa	Sprayed	2	2	0	0	3	0	0	1	0	0	0	0	11
	Chasela	Control	22	7	0	0	0	0	0	0	0	0	0	0	5
Katete	Chilowa	Sprayed	41	4	0	0	0	0	0	0	0	0	0	0	27
	Robert	Control	172	2	0	0	0	0	0	0	0	0	0	0	36
Serenje	Chibobo	Sprayed	38	0	0	0	0	0	0	0	0	0	0	0	13
	Chishi	Control	196	2	4	0	0	0	0	0	0	20	0	0	29
Lufwanyama	Nkana	Sprayed	257	208	1	0	0	0	0	0	0	0	0	0	123
	Bulaya	Control	311	63	4	0	0	0	0	0	0	0	0	0	82
Chililabombwe	Kawama	Sprayed	244	74	0	0	0	0	0	0	0	0	0	0	1903
	Mainasoko	Control	220	58	3	0	0	0	0	0	0	0	0	0	250
		TOTAL	**8,906**	**699**	**38**	**0**	**3**	**0**	**0**	**1**	**0**	**21**	**0**	**3**	**2,691**

ANNEX B: *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. BY MONTH, SITE, AND COLLECTION METHOD (AUGUST 2020-JUNE 2021)

Month, Year	District	Site	Status	*An. funestus* s.l.				*An. gambiae* s.l.			
				Number collected by Indoor HLC	Number collected by Outdoor HLC	Number collected by PSC	Monthly Total Collected	Number collected by Indoor HLC	Number collected by Outdoor HLC	Number collected by PSC	Monthly Total Collected
Aug-20	Nchelenge	Shikapande	Sprayed	1,779	738	346		26	15	0	
		Manchene	Control	768	296	299		31	23	1	
	Mambwe	Chikowa	Sprayed	1	3	0		0	8	0	
		Chasela	Control	2	0	1		0	0	0	
	Lufwanyama	Nkana	Sprayed	18	7	3		16	9	0	
		Bulaya	Control	41	23	2		0	0	0	
	Chililabombwe	Kawama	Sprayed	0	0	0		0	0	0	
		Mainasoko	Control	0	0	0	**4,327**	0	0	0	**129**
Sep-20	Nchelenge	Shikapande	Sprayed	2,087	961	172		5	7	0	
		Manchene	Control	1,349	488	416		11	9	0	
	Milenge	Lunga	Sprayed	452	239	152		95	228	1	
		Miyambo	Control	1,429	1,118	335		33	49	0	
	Mambwe	Chikowa	Sprayed	0	0	0		0	0	0	
		Chasela	Control	0	0	0		0	0	0	
	Katete	Chilowa	Sprayed	2	0	3		0	0	0	
		Robert	Control	15	6	32		0	0	0	
	Serenje	Chibobo	Sprayed	1	2	1		1	0	0	
		Chishi	Control	15	7	6		0	0	0	
	Lufwanyama	Nkana	Sprayed	835	321	8		88	32	13	
		Bulaya	Control	55	23	9		20	7	0	
	Chililabombwe	Kawama	Sprayed	10	14	18		7	9	12	
		Maina Soko	Control	30	13	47	**10,671**	0	4	2	**633**
Oct-20	Nchelenge	Shikapande	Sprayed	660	379	208		1	5	1	
		Manchene	Control	1,208	531	789		11	12	0	
	Mambwe	Chikowa	Sprayed	0	1	0		0	0	0	
		Chasela	Control	0	0	0		0	0	0	
	Lufwanyama	Nkana	Sprayed	24	15	15		230	250	20	
		Bulaya	Control	263	142	53		4	3	1	
	Chililabombwe	Kawama	Sprayed	13	45	29		43	63	12	
		Mainasoko	Control	62	38	52	**4,527**	13	11	9	**689**
Nov-20	Nchelenge	Shikapande	Sprayed	1,293	475	202		27	25	1	
		Manchene	Control	1,177	567	557		8	2	7	
	Milenge	Lunga	Sprayed	0	0	0		0	0	0	
		Miyambo	Control	3,983	2,226	0		2	2	0	
	Mambwe	Chikowa	Sprayed	0	0	0		0	0	1	
		Chasela	Control	0	0	0		0	1	0	

Month, Year	District	Site	Status	*An. funestus* s.l.				*An. gambiae* s.l.			
				Number collected by Indoor HLC	Number collected by Outdoor HLC	Number collected by PSC	Monthly Total Collected	Number collected by Indoor HLC	Number collected by Outdoor HLC	Number collected by PSC	Monthly Total Collected
	Katete	Chilowa	Sprayed	0	0	0		0	0	0	
		Robert	Control	3	1	17		0	0	0	
	Serenje	Chibobo	Sprayed	2	1	8		0	0	0	
		Chishi	Control	14	22	13		0	1	0	
	Lufwanyama	Nkana	Sprayed	43	8	8		189	167	33	
		Bulaya	Control	181	161	102		13	9	6	
	Chililabombwe	Kawama	Sprayed	69	27	65		96	46	34	
		Maina Soko	Control	111	57	71	**11,464**	48	27	30	**775**
Dec-20	Nchelenge	Shikapande	Sprayed	813	317	88		12	3	0	
		Manchene	Control	951	1,308	463		25	21	2	
	Milenge	Lunga	Sprayed	265	230	34		1	0	0	
		Miyambo	Control	0	0	669		0	0	1	
	Mambwe	Chikowa	Sprayed	0	0	0		7	15	0	
		Chasela	Control	0	0	0		3	9	0	
	Serenje	Chibobo	Sprayed	2	14	5		0	0	0	
		Chishi	Control	21	17	8		0	0	0	
	Lufwanyama	Nkana	Sprayed	47	23	20		426	292	53	
		Bulaya	Control	62	35	16	**5,408**	35	12	8	**925**
Jan-21	Nchelenge	Shikapande	Sprayed	487	176	50		7	3	4	
		Manchene	Control	949	1,178	294		10	5	49	
	Milenge	Lunga	Sprayed	70	28	23		31	12	5	
		Miyambo	Control	1,194	311	141		35	12	2	
	Mambwe	Chikowa	Sprayed	2	14	0		13	80	0	
		Chasela	Control	5	2	1		31	82	5	
	Katete	Chilowa	Sprayed	6	3	0		0	0	0	
		Robert	Control	6	9	27		4	1	1	
	Serenje	Chibobo	Sprayed	21	7	16		0	0	0	
		Chishi	Control	162	46	48		0	1	0	
	Lufwanyama	Nkana	Sprayed	101	52	15		162	83	20	
		Bulaya	Control	27	10	3		44	8	12	
	Chililabombwe	Kawama	Sprayed	36	25	50		18	9	4	
		Maina Soko	Control	121	66	6	**5,788**	42	18	4	**817**
Feb-21	Nchelenge	Shikapande	Sprayed	448	271	75		2	10	1	
		Manchene	Control	902	1,529	294		3	15	2	
	Mambwe	Chikowa	Sprayed	4	11	0		47	123	0	
		Chasela	Control	3	2	1		16	38	0	
	Lufwanyama	Nkana	Sprayed	102	67	24		65	44	34	
		Bulaya	Control	46	15	20	**3,814**	47	26	10	**483**
Mar-21	Nchelenge	Shikapande	Sprayed	1,288	616	103		23	28	3	
		Manchene	Control	823	881	352		21	27	7	
	Milenge	Lunga	Sprayed	100	72	30		515	220	36	
		Miyambo	Control	2,701	1,351	285		141	90	2	
	Mambwe	Chikowa	Sprayed	40	61	1		172	726	0	
		Chasela	Control	28	57	2		213	274	0	
	Katete	Chilowa	Sprayed	105	92	25		6	3	4	
		Robert	Control	42	27	74		2	1	0	
	Serenje	Chibobo	Sprayed	62	49	5		1	2	0	
		Chishi	Control	276	103	92		6	1	0	
	Lufwanyama	Nkana	Sprayed	152	99	78		36	40	18	
		Bulaya	Control	73	58	21		13	4	18	
	Chililabombwe	Kawama	Sprayed	37	14	24		18	15	9	
		Maina Soko	Control	72	10	34	**10,415**	130	8	13	**2,846**

Month, Year	District	Site	Status	*An. funestus* s.l.				*An. gambiae* s.l.			
				Number collected by Indoor HLC	Number collected by Outdoor HLC	Number collected by PSC	Monthly Total Collected	Number collected by Indoor HLC	Number collected by Outdoor HLC	Number collected by PSC	Monthly Total Collected
Apr-21	Nchelenge	Shikapande	Sprayed	1,328	1,050	41		751	578	47	
		Manchene	Control	727	1,012	166		391	535	29	
	Mambwe	Chikowa	Sprayed	22	41	0		75	69	1	
		Chasela	Control	25	26	6		31	30	1	
	Lufwanyama	Nkana	Sprayed	186	107	43		46	23	5	
		Bulaya	Control	78	83	46	**4,987**	9	2	1	**2,624**
May-21	Nchelenge	Shikapande	Sprayed	1,605	1,011	126		197	222	6	
		Manchene	Control	519	550	216		47	60	52	
	Milenge	Lunga	Sprayed	172	172	29		115	59	13	
		Miyambo	Control	1,410	561	148		11	16	2	
	Mambwe	Chikowa	Sprayed	3	10	1		5	9	0	
		Chasela	Control	3	5	9		4	2	0	
	Katete	Chilowa	Sprayed	33	32	12		0	1	0	
		Robert	Control	2	7	19		1	1	1	
	Serenje	Chibobo	Sprayed	3	13	3		0	0	0	
		Chishi	Control	41	33	27		1	6	2	
	Lufwanyama	Nkana	Sprayed	90	40	29		21	8	11	
		Bulaya	Control	102	101	27		7	11	7	
	Chililabombwe	Kawama	Sprayed	36	31	37		2	3	2	
		Mainasoko	Control	141	77	5	**7,491**	6	3	0	**914**
Jun-21	Nchelenge	Shikapande	Sprayed	1,379	612	32		22	16	0	
		Manchene	Control	409	383	129		14	9	1	
	Milenge	Lunga	Sprayed	59	46	11		7	16	4	
		Miyambo	Control	249	80	128		9	4	0	
	Mambwe	Chikowa	Sprayed	1	0	0		0	4	0	
		Chasela	Control	2	2	2		1	0	1	
	Katete	Chilowa	Sprayed	10	6	1		0	0	0	
		Robert	Control	5	7	3		0	0	0	
	Serenje	Chibobo	Sprayed	5	2	0		1	1	0	
		Chishi	Control	6	8	2		1	0	0	
	Lufwanyama	Nkana	Sprayed	47	14	14		16	11	1	
		Bulaya	Control	25	25	12		6	0	0	
	Chililabombwe	Kawama	Sprayed	8	9	21		0	0	1	
		Mainasoko	Control	12	0	5	**3,771**	0	0	0	**146**

ANNEX C: STATISTICAL OUTPUT

Negative Binomial Regressions Comparing *An. funestus* s.l. and *An. gambiae* s.l. Vector Numbers, Abdominal Condition, and Parity between Sprayed vs. Control Sites, and Pre- vs. Post-IRS (August 2020-June 2021)

I. Indoor Resting Density - Vectors Collected by PSC

Site	Comparison	*An. funestus* s.l.				*An. gambiae* s.l.			
		Mean [First group]	Mean [Second group]	Random effects IRR*	p-value	Mean [First group]	Mean [Second group]	Random effects IRR*	p-value
All	Control v Sprayed	7.5	2.6	0.63	0.00**	0.33	0.46	1.36	0.06
ALL-Sprayed	Pre-IRS v Post-IRS	4.1	2.2	0.81	0.09	0.21	0.53	2.61	0.0006**
ALL-Control	Pre-IRS v Post-IRS	6.7	7.6	1.08	0.46	0.07	0.39	4.27	0.0002**
Nchelenge	Control v Sprayed	24.1	8.7	0.32	0.00**	0.91	0.38	0.44	0.06
Milenge	Control v Sprayed	19.0	3.1	0.17	0.00**	0.08	0.66	8.36	0.00**
Mambwe	Control v Sprayed	0.1	0.01	0.09	0.00**	0.04	0.01	0.28	0.13
Katete	Control v Sprayed	1.9	0.5	0.34	0.005**	0.02	0.04	2.38	0.5095
Serenje	Control v Sprayed	1.9	0.4	0.20	0.00**	0.02	0	N/A	N/A
Lufwanyama	Control v Sprayed	1.9	1.6	0.86	0.42	0.38	1.26	3.20	0.000**
Chililabombwe	Control v Sprayed	2.1	2.3	1.14	0.56	0.55	0.70	1.29	0.42
Shikapande-Sprayed	Pre-IRS v Post-IRS	17.3	6.9	0.39	0.000**	0	0.47	N/A	N/A
Manchene-Control	Pre-IRS v Post-IRS	23.8	24.1	1.00	0.93	0.03	1.10	33.69	0.00**
Lunga-Sprayed	Pre-IRS v Post-IRS	10.1	1.7	0.16	0.000**	0.07	0.77	12.43	0.01**
Miyambo-Control	Pre-IRS v Post-IRS	22.3	18.3	0.82	0.001**	0	0.09	N/A	N/A
Chikowa-Sprayed	Pre-IRS v Post-IRS	0	0.01		N/A	0	0.01	N/A	N/A
Chasela-Control	Pre-IRS v Post-IRS	0.0	0.2	4.75	0.13	0	0.05	N/A	N/A
Chiloba-Sprayed	Pre-IRS v Post-IRS	0	0.5	0.21	0.09	0	0.05	N/A	N/A
Robert-Control	Pre-IRS v Post-IRS	2.1	1.9	0.91	0.65	0	0.03	N/A	N/A
Chibobo-Sprayed	Pre-IRS v Post-IRS	0.3	0.4	0.87	0.72	0	0	N/A	N/A
Chishi-Control	Pre-IRS v Post-IRS	0.6	2.4	4.20	0.000**	0	0.03	N/A	N/A
Nkana-Sprayed	Pre-IRS v Post-IRS	0.4	1.8	5.00	0.000**	0.43	1.44	3.34	0.00**
Bulaya-Control	Pre-IRS v Post-IRS	0.4	2.2	6.25	0.000**	0	0.47	N/A	N/A
Kawama-Sprayed	Pre-IRS v Post-IRS	1.6	2.6	1.61	0.003**	0.80	0.67	0.81	0.3840
Maina Soko-Control	Pre-IRS v Post-IRS	3.3	1.6	0.49	0.000**	0.37	0.63	1.77	0.09

*For IRR, the reference group is "control" or "pre-intervention period". Two asterisks indicate statistical significance at 0.05%. N/A means no p-values obtained because two sites had the same value or one site had two zero values

II. Abdominal Condition - Vectors Collected by PSC

Site	Comparison	*An. funestus* s.l.				*An. gambiae* s.l.			
		Mean proportion gravid [First group]:	Mean proportion gravid [Second group]:	Random effects IRR	p-value	Mean proportion gravid [First group]:	Mean proportion gravid [Second group]:	Random effects IRR	p-value
All	Control v Sprayed	10%	6%	0.96	0.877	1%	2%	1.42	0.84
ALL-Sprayed	Pre-IRS v Post-IRS	5%	7%	0.77	0.60	0%	2%	N/A	N/A
ALL-Control	Pre-IRS v Post-IRS	22%	7%	0.36	0.004**	0%	1%	N/A	N/A
Nchelenge	Control v Sprayed	11%	7%	0.80	0.60	0%	10%	N/A	N/A
Milenge	Control v Sprayed	9%	6%	1.69	0.63	14%	0%	N/A	N/A
Mambwe	Control v Sprayed	55%	50%	0.95	0.96	14%	0%	N/A	N/A
Katete	Control v Sprayed	40%	44%	1.14	0.67	50%	75%	1.50	0.73
Serenje	Control v Sprayed	5%	0%	N/A	N/A	N/A	N/A	N/A	N/A
Lufwanyama	Control v Sprayed	1%	2%	1.12	0.91	0%	0%	N/A	N/A
Chililabombwe	Control v Sprayed	0%	0%	N/A	N/A	0%	0%	N/A	N/A
Shikapande-Sprayed	Pre-IRS v Post-IRS	4%	9%	1.07	0.92		10%	N/A	N/A
Manchene-Control	Pre-IRS v Post-IRS	16%	9%	0.48	0.14	0%	0%	N/A	N/A
Lunga-Sprayed	Pre-IRS v Post-IRS	12%	0%	N/A	N/A	0%	0%	N/A	N/A
Miyambo-Control	Pre-IRS v Post-IRS	41%	1%	0.02	0.000**		14%	N/A	N/A
Chikowa-Sprayed	Pre-IRS v Post-IRS	0%	50%	N/A	N/A	N/A		N/A	N/A
Chasela-Control	Pre-IRS v Post-IRS	0%	57%	N/A	N/A	N/A	14%	N/A	N/A
Chiloba-Sprayed	Pre-IRS v Post-IRS	33%	45%	1.34	0.77	N/A	75%	N/A	N/A
Robert-Control	Pre-IRS v Post-IRS	50%	37%	0.72	0.40	N/A	50%	N/A	N/A
Chibobo-Sprayed	Pre-IRS v Post-IRS	0%	0%	N/A	N/A	N/A	N/A	N/A	N/A
Chishi-Control	Pre-IRS v Post-IRS	5%	5%	0.17	0.22	N/A	N/A	N/A	N/A
Nkana-Sprayed	Pre-IRS v Post-IRS	0%	1%	N/A	N/A	0%	0%	N/A	N/A
Bulaya-Control	Pre-IRS v Post-IRS	0%	0%	N/A	N/A	0%	0%	N/A	N/A
Kawama-Sprayed	Pre-IRS v Post-IRS	0%	2%	N/A	N/A	0%	0%	N/A	N/A
Maina Soko-Control	Pre-IRS v Post-IRS	0%	1%	N/A	N/A	N/A	N/A	N/A	N/A

*For IRR, the reference group is "control" or "pre-intervention period". Two asterisks indicate statistical significance at 0.05%. N/A means no p-values obtained because two sites had the same value or one site had a zero value or no value (-)

III. Human Biting Rates - Vectors Collected by Human Landing Catch

Site	Comparison	*An. funestus* s.l.				*An. gambiae* s.l.			
		Mean [First group]	**Mean [Second group]**	**Random effects IRR***	**p-value**	**Mean [First group]**	**Mean [Second group]**	**Random effects IRR***	**p-value**
All	Control v Sprayed	40.9	26.6	0.51	0.38	3.2	7.7	0.85	0.51
ALL-Sprayed	Pre-IRS v Post-IRS	39.2	23.4	1.27	0.41	3.4	8.8	1.23	0.563
ALL-Control	Pre-IRS v Post-IRS	30.3	43.6	1.38	0.244	1.1	3.8	2.21	0.05**
Nchelenge	Control v Sprayed	105.1	112.3	1.17	0.32	7.3	11.3	1.06	0.81
Milenge	Control v Sprayed	173.1	19.8	0.11	0.00**	4.2	13.5	1.57	0.12
Mambwe	Control v Sprayed	0.9	1.2	1.45	0.24	4.2	7.7	1.64	0.09
Katete	Control v Sprayed	1.4	3.01	0.85	0.60	0.1	0.1	0.68	0.54
Serenje	Control v Sprayed	6.9	1.6	0.34	0.00**	0.2	0.1	0.35	0.07**
Lufwanyama	Control v Sprayed	9.3	13.6	0.96	0.87	12.8	1.6	2.91	0.01**
Chililabombwe	Control v Sprayed	7.2	3.3	0.64	0.03	2.8	2.9	1.01	0.97
Shikapande-Sprayed	Pre-IRS v Post-IRS	173.9	98.7	0.51	0.00**	1.66	13.42	1.34	0.52
Manchene-Control	Pre-IRS v Post-IRS	90.7	108.4	1.11	0.59	2.31	8.44	0.92	0.86
Lunga-Sprayed	Pre-IRS v Post-IRS	43.2	15.2	0.31	0.00**	20.19	12.20	0.35	0.02**
Miyambo-Control	Pre-IRS v Post-IRS	159.2	175.8	0.79	0.53	5.13	4.03	1.01	0.99
Chikowa-Sprayed	Pre-IRS v Post-IRS	0.1	1.5	3.08	0.13	0.25	9.34	4.25	0.05**
Chasela-Control	Pre-IRS v Post-IRS	0.1	1.1	4.13	0.17	0	5.10	N/A	N/A
Chiloba-Sprayed	Pre-IRS v Post-IRS	0.1	3.6	4.7	0.14	0	0.13	N/A	N/A
Robert-Control	Pre-IRS v Post-IRS	1.3	1.4	0.76	0.56	0	0.13	N/A	N/A
Chibobo-Sprayed	Pre-IRS v Post-IRS	0.2	2.2	3.92	0.03**	0.03	0.06	1.71	0.65
Chishi-Control	Pre-IRS v Post-IRS	1.8	8.9	1.86	0.11	0.03	0.20	4.8	0.1
Nkana-Sprayed	Pre-IRS v Post-IRS	36.9	8.5	1.23	0.53	4.53	14.65	1.54	0.21
Bulaya-Control	Pre-IRS v Post-IRS	4.6	10.3	1.45	0.27	0.87	1.76	5.44	0.02**
Kawama-Sprayed	Pre-IRS v Post-IRS	2.6	3.7	1.75	0.09	3.81	2.59	0.49	0.07
Maina Soko-Control	Pre-IRS v Post-IRS	4.5	8.3	1.33	0.40	0.88	3.53	1.29	0.55

*For IRR, the reference group is "control" or "pre-intervention period". Two asterisks indicate statistical significance at 0.05%. N/A = no estimated computed either because two sites had the same value or one site had two zero values.

IV. Indoor Versus Outdoor Human Biting Rates - Vectors Collected by Human Landing Catch

		An. funestus s.l.				*An. gambiae* s.l.			
Site	**Comparison**	**Mean [First group]**	**Mean [Second group]**	**Random effects IRR***	**p-value**	**Mean [First group]**	**Mean [Second group]**	**Random effects IRR***	**p-value**
All	Indoor v Outdoor	23.3	14.5	0.57	0.24	3.1	3.1	1.04	0.93
ALL-Sprayed	Indoor v Outdoor	19.7	10.3	0.49	0.29	4.4	4.3	1.09	0.88
ALL-Control	Indoor v Outdoor	27.0	18.6	0.63	0.50	1.9	1.8	0.91	0.87
Nchelenge	Indoor v Outdoor	65.2	43.5	0.55	0.01**	4.7	4.6	0.96	0.94
Milenge	Indoor v Outdoor	62.9	33.5	0.60	0.41	5.2	3.7	1.20	0.71
Mambwe	Indoor v Outdoor	0.4	0.7	1.60	0.56	1.8	4.2	2.42	0.55
Katete	Indoor v Outdoor	1.2	0.99	0.73	0.65	0.1	0.0	0.54	0.19
Serenje	Indoor v Outdoor	2.8	1.4	0.55	0.43	0.0	0.1	1.08	0.89
Lufwanyama	Indoor v Outdoor	7.4	4.1	0.46	0.14	4.3	3.0	0.63	0.52
Chililabombwe	Indoor v Outdoor	3.4	1.9	0.63	0.20	1.9	1.0	0.66	0.32
Shikapande-Sprayed	Indoor v Outdoor	74.8	37.5	0.47	0.005**	6.10	5.18	0.84	0.85
Manchene-Control	Indoor v Outdoor	55.6	49.6	0.67	0.17	3.25	4.08	1.16	0.81
Lunga-Sprayed	Indoor v Outdoor	11.6	8.2	0.60	0.29	7.96	5.57	1.25	0.63
Miyambo-Control	Indoor v Outdoor	114.2	58.8	0.60	0.000**	2.41	1.80	0.94	0.81
Chikowa-Sprayed	Indoor v Outdoor	0.4	0.8	1.95	0.52	1.81	5.88	3.32	0.487
Chasela-Control	Indoor v Outdoor	0.4	0.5	1.25	0.87	2	2.49	1.46	0.881
Chiloba-Sprayed	Indoor v Outdoor	1.6	1.4	0.8	0.89	0	0.04	0.67	0.530
Robert-Control	Indoor v Outdoor	0.8	0.6	0.78	0.16	0	0.03	0.43	0.22
Chibobo-Sprayed	Indoor v Outdoor	0.9	0.8	0.92	0.94	0.03	0.03	1.00	1.000
Chishi-Control	Indoor v Outdoor	4.8	2.1	0.48	0.30	0.07	0.08	1.1	0.809
Nkana-Sprayed	Indoor v Outdoor	9.3	4.3	0.41	0.18	7.36	5.45	0.67	0.50
Bulaya-Control	Indoor v Outdoor	5.4	3.9	0.65	0.31	1.13	0.47	0.42	0.01**
Kawama-Sprayed	Indoor v Outdoor	1.9	1.5	1.02	0.97	1.64	1.29	0.95	0.871
Mainasoko-Control	Indoor v Outdoor	4.9	2.3	0.49	0.02**	2.13	0.63	0.36	0.11

*For IRR, the reference group is "Indoor". Two asterisks indicate statistical significance at 0.05%.

V. Vector Parity Rates - Vectors Collected by HLC (Human Landing Catches)

Site	Comparison	*An. funestus* s.l.				*An. gambiae* s.l.			
		Mean Proportion Parous [First group]	**Mean Proportion Parous [Second group]**	**Random effects IRR***	**p-value**	**Mean Proportion Parous [First group]**	**Mean Proportion Parous [Second group]**	**Random effects IRR***	**p-value**
All Sites	Control v Sprayed	33.5%	33.6%	0.95	0.71	53.2%	37.0%	0.85	0.47
All Sprayed Sites	Pre-IRS v Post-IRS	32.7%	33.9%	1.03	0.81	39.1%	36.7%	0.69	0.06
All Control Sites	Pre-IRS v Post-IRS	31.7%	33.8%	1.07	0.64	13.3%	54.4%	2.69	0.17
Nchelenge	Control v Sprayed	30%	33%	1.08	0.70	40%	56%	1.39	0.58
Milenge	Control v Sprayed	31%	26%	0.90	0.65	29%	39%	0.67	0.63
Mambwe	Control v Sprayed	59%	43%	0.73	0.07	62%	43%	0.69	0.0000**
Katete	Control v Sprayed	62%	45%	0.72	0.05**	40%	57%	1.43	0.68
Serenje	Control v Sprayed	-	46%	N/A	N/A	-	-	N/A	N/A
Lufwanyama	Control v Sprayed	28%	34%	1.24	0.35	23%	27%	1.18	0.62
Chililabombwe	Control v Sprayed	32%	28%	0.86	0.35	30%	25%	0.73	0.43
Shikapande-Sprayed	Pre-IRS v Post-IRS	22%	41%	1.86	0.02**	50%	57%	1.14	0.87
Manchene-Control	Pre-IRS v Post-IRS	28%	32%	1.11	0.76**	0%	44%	N/A	N/A
Lunga-Sprayed	Pre-IRS v Post-IRS	8%	29%	3.80	0.06	21%	42%	1.20	0.85
Miyambo-Control	Pre-IRS v Post-IRS	8%	32%	3.54	0.04	0%	50%	N/A	N/A
Chikowa-Sprayed	Pre-IRS v Post-IRS	75%	42%	0.56	0.33	63%	42%	0.68	0.39
Chasela-Control	Pre-IRS v Post-IRS	50%	60%	1.19	0.86	-	62%	N/A	N/A
Chiloba-Sprayed	Pre-IRS v Post-IRS	50%	45%	0.72	0.77	-	57%	N/A	N/A
Robert-Control	Pre-IRS v Post-IRS	45%	66%	1.50	0.264	-	40%	N/A	N/A
Chibobo-Sprayed	Pre-IRS v Post-IRS	100%	-	N/A	N/A	-	-	N/A	N/A
Chishi-Control	Pre-IRS v Post-IRS	55%	33%	0.61	0.32	-	-	N/A	N/A
Nkana-Sprayed	Pre-IRS v Post-IRS	48%	27%	0.57	0.13	25%	29%	1.14	0.75
Bulaya-Control	Pre-IRS v Post-IRS	44%	24%	0.54	0.04**	0%	24%	N/A	N/A
Kawama-Sprayed	Pre-IRS v Post-IRS	51%	24%	0.48	0.001**	47%	18%	0.38	0.000**
Maina Soko-Control	Pre-IRS v Post-IRS	42%	30%	0.70	0.09	29%	30%	1.05	0.95

*For IRR, the reference group is "control" or "pre-intervention period". Two asterisks indicate statistical significance at 0.05%. N/A = means no estimate computed either because two sites had the same value or one site had a zero value or no value (-).

Annex D: Sporozoite Rates and EIR (August 2020-June 2021)

I: *An. funestus* s.l. and *An. gambiae* s.l. Collected Indoors and Outdoors at Sprayed and Control Sites Before and After IRS

Species	Location	Time	Intervention sites					Control sites				
			# Tested	# Positive	Sporozoite Rate	Biting Rate	*EIR	# Tested	# Positive	Sporozoite Rate	Biting Rate	*EIR
An. funestus s.l.	Indoors	Pre-IRS	535	12	0.02	32.5	21.86	688	18	0.03	23.74	18.63
		Post-IRS	347	2	0.01	16.6	2.87	509	23	0.05	27.74	37.60
	Outdoors	Pre-IRS	253	2	0.01	14.55	3.45	374	8	0.02	12.69	8.14
		Post-IRS	277	1	0.00	9.31	1.01	322	11	0.03	20.02	20.52
	Both In/Out	Pre-IRS	788	14	0.02	47.03	25.07	1,062	26	0.02	36.43	26.76
		Post-IRS	624	3	0.00	25.93	3.74	831	34	0.04	47.76	58.62
	TOTAL		**1,413**	**17**	**0.01**	**26.60**	**9.61**	**1,893**	**60**	**0.03**	**40.90**	**39.89**
An. gambiae s.l.	Indoor	Pre-IRS	462	9	0.02	1.75	1.02	65	3	0.05	0.68	0.95
		Post-IRS	322	3	0.01	5.00	1.40	220	2	0.01	2.14	0.58
	Outdoor	Pre-IRS	252	0	0.00	2.32	0.00	70	1	0.01	0.65	0.28
		Post-IRS	342	5	0.01	4.79	2.10	218	2	0.01	2.05	0.57
	Both In/Out	Pre-IRS	714	9	0.01	4.07	1.54	135	4	0.03	1.34	1.19
		Post-IRS	664	8	0.01	9.79	3.54	438	4	0.01	4.19	1.15
	TOTAL		**1,378**	**17**	**0.01**	**7.70**	**2.85**	2.85	**8**	**0.01**	**3.20**	**1.34**

*EIR – mean number of infective bites per person per month
Note that no weighting was done by either vector density or sporozoite rates. Some districts contributed more than others to the total vectors tested each time period presented.

II: Sporozoite Rates for Molecular Species of *An. funestus* s.l. and *An. gambiae* s.l. by District

District	Molecular Species	Total Tested	Number Positive
Nchelenge	*An. funestus*	150	3
	An. gambiae s.s.	30	0
Milenge	*An. funestus*	166	3
	An. gambiae s.s.	12	0
Mambwe	*An. gambiae* s.s.	1	0
	An. funestus	1	0
	An. arabiensis	1	0
Katete	*An. funestus*	57	1
	An. arabiensis	1	0
	An. parensis	1	0
Lufwanyama	*An. funestus*	136	2
	An. gambiae s.s.	69	5
	An. vaneedeeni	3	0
Chililabombwe	*An. funestus*	190	2
	An. gambiae s.s.	82	3

ANNEX E: INSECTICIDE SUSCEPTIBILITY TEST RESULTS (DECEMBER 2020-MAY 2021)

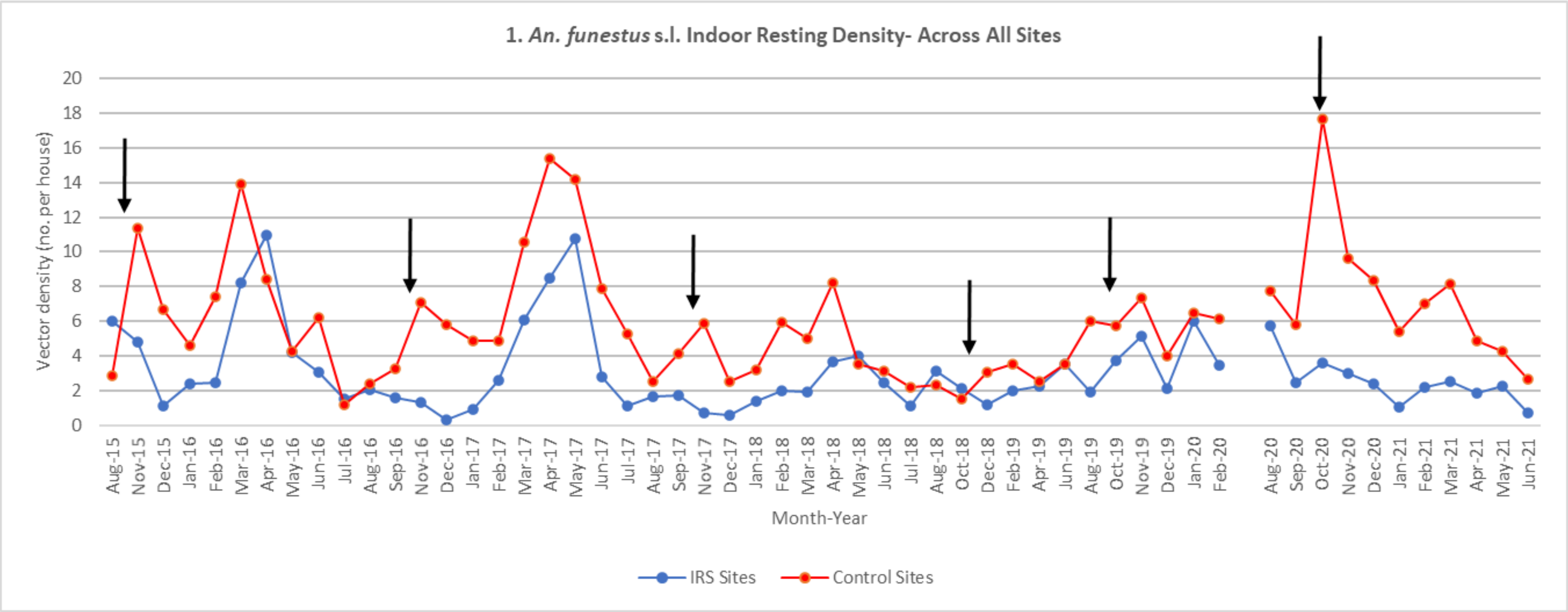
Chemical	Species	District, Sentinel Site	Intervention Status	# Exposed	% Mortality after 24 hours	% Mortality after 48 hours	% Mortality after 72 hours	Interpretation
Clothianidin (2%)	*An. funestus* s.l.	Lufwanyama, Bulaya	Control	41	100	N/A	N/A	Susceptible
		Lufwanyama, Nkana	Sprayed	61	100	N/A	N/A	Susceptible
		Milenge, Lunga	Sprayed	61	100	N/A	N/A	Susceptible
		Nchelenge, Manchene	Control	52	97.9	100	N/A	Susceptible
		Milenge, Miyambo	Control	67	100	N/A	N/A	Susceptible
		Nchelenge, Shikapande	Sprayed	110	97	100	N/A	Susceptible
		Chililabombwe, Kawama	Sprayed	33	100	N/A	N/A	Susceptible
		Chililabombwe, Mainasoko	Control	43	100	N/A	N/A	Susceptible
	An. gambiae s.l.	Lufwanyama, Bulaya	Control	10	100	N/A	N/A	Susceptible
		Lufwanyama, Nkana	Sprayed	23	100	N/A	N/A	Susceptible
		Milenge, Lunga	Sprayed	9	100	N/A	N/A	Susceptible
		Nchelenge, Manchene	Control	11	100	N/A	N/A	Susceptible
		Nchelenge, Shikapande	Sprayed	6	100	N/A	N/A	Susceptible
		Katete, Chilowa	Sprayed	100	100	N/A	N/A	Susceptible
		Katete, Robert	Control	100	100	N/A	N/A	Susceptible
Chlorfenapyr (100ug)	*An. funestus* s.l.	Serenje, Chibobo	Sprayed	11	100	N/A	N/A	Susceptible
		Serenje, Chishi	Control	18	100	N/A	N/A	Susceptible
		Chililabombwe, Kawama	Sprayed	18	100	N/A	N/A	Susceptible
		Chililabombwe, Mainasoko	Control	28	100	N/A	N/A	Susceptible
		Lufwanyama, Nkana	Sprayed	20	100	N/A	N/A	Susceptible
		Milenge, Lunga	Sprayed	79	100	N/A	N/A	Susceptible
		Nchelenge, Manchene	Control	119	95.8	100	N/A	Susceptible
		Milenge, Miyambo	Control	129	100	N/A	N/A	Susceptible
		Nchelenge, Shikapande	Sprayed	202	95.5	100	N/A	Susceptible
	An. gambiae s.l.	Chililabombwe, Mainasoko	Control	12	100	N/A	N/A	Susceptible
		Lufwanyama, Nkana	Sprayed	40	100	N/A	N/A	Susceptible
		Katete, Chilowa	Sprayed	100	88	94	100	Susceptible
		Katete, Robert	Control	100	91	98	N/A	Susceptible

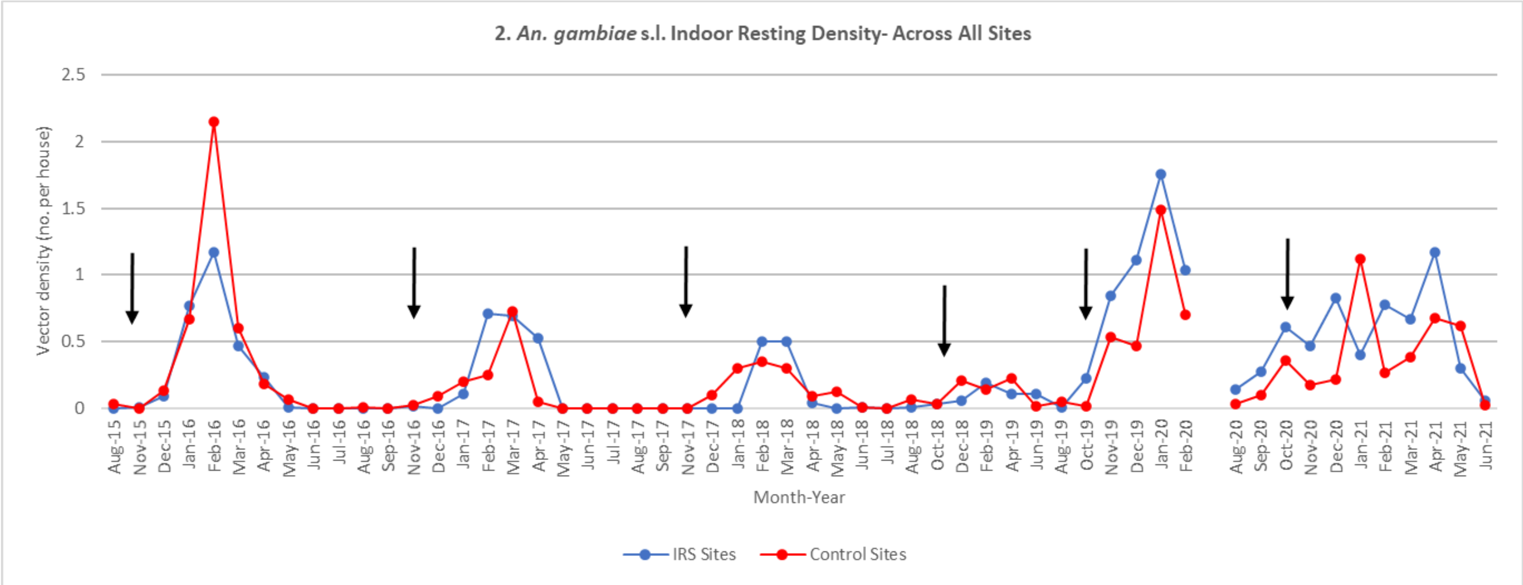
Chemical	Species	District, Sentinel Site	Intervention Status	# Exposed	% Mortality after 24 hours	% Mortality after 48 hours	% Mortality after 72 hours	Interpretation
		Milenge, Lunga	Sprayed	38	100	N/A	N/A	Susceptible
DDT 4%	*An. funestus* s.l.	Lufwanyama, Bulaya	Control	10	100	N/A	N/A	Susceptible
		Milenge, Lunga	Sprayed	125	98.4	N/A	N/A	Susceptible
		Nchelenge, Manchene	Control	56	88.9	N/A	N/A	Confirmed resistance
		Milenge, Miyambo	Control	77	100	N/A	N/A	Susceptible
		Lufwanyama, Nkana	Sprayed	37	91.9	N/A	N/A	Probable resistance
		Nchelenge, Shikapande	Sprayed	93	98.8	N/A	N/A	Susceptible
	An. gambiae s.l.	Katete, Chilowa	Sprayed	80	100	N/A	N/A	Susceptible
		Katete, Robert	Control	100	100	N/A	N/A	Susceptible
Alpha-cypermethrin 0.05%	*An. funestus* s.l.	Lufwanyama, Bulaya	Control	21	76.2	N/A	N/A	Confirmed resistance
		Milenge, Lunga	Sprayed	150	48.7	N/A	N/A	Confirmed resistance
		Nchelenge, Manchene	Control	64	73.4	N/A	N/A	Confirmed resistance
		Milenge, Miyambo	Control	199	45.5	N/A	N/A	Confirmed resistance
		Lufwanyama, Nkana	Sprayed	18	72.2	N/A	N/A	Confirmed resistance
		Nchelenge, Shikapande	Sprayed	41	73.2	N/A	N/A	Confirmed resistance
	An. gambiae s.l.	Milenge, Lunga	Sprayed	43	90.5	N/A	N/A	Probable resistance
		Lufwanyama, Nkana	Sprayed	90	26.7	N/A	N/A	Confirmed resistance
		Katete, Robert	Control	100	77	N/A	N/A	Confirmed resistance
Deltamethrin 0.05%	*An. funestus* s.l.	Lufwanyama, Bulaya	Control	37	73	N/A	N/A	Confirmed resistance
		Chililabombwe, Kawama	Sprayed	15	100	N/A	N/A	Susceptible
		Nchelenge, Manchene	Control	120	68.8	N/A	N/A	Confirmed resistance
		Milenge, Miyambo	Control	63	67.3	N/A	N/A	Confirmed resistance
		Lufwanyama, Nkana	Sprayed	27	59.3	N/A	N/A	Confirmed resistance
		Nchelenge, Shikapande	Sprayed	100	74.3	N/A	N/A	Confirmed resistance
	An. gambiae s.l.	Milenge, Lunga	Sprayed	57	90	N/A	N/A	Probable resistance
		Katete, Robert	Control	100	100	N/A	N/A	Susceptible
Permethrin 0.75%	*An. funestus* s.l.	Milenge, Lunga	Sprayed	37	88.1	N/A	N/A	Confirmed resistance
		Nchelenge, Manchene	Control	26	71.5	N/A	N/A	Confirmed resistance
		Milenge, Miyambo	Control	38	77.1	N/A	N/A	Confirmed resistance
		Nchelenge, Shikapande	Sprayed	84	84.2	N/A	N/A	Confirmed resistance
		Nchelenge, Manchene	Control	15	58	N/A	N/A	Confirmed resistance
		Nchelenge, Shikapande	Sprayed	9	64.4	N/A	N/A	Confirmed resistance
Pirimiphos-methyl (0.25%)	*An. funestus* s.l.	Milenge, Lunga	Sprayed	22	100	N/A	N/A	Susceptible
		Milenge, Miyambo	Control	58	100	N/A	N/A	Susceptible
	An. gambiae s.l.	Katete, Robert	Control	60	100	N/A	N/A	Susceptible

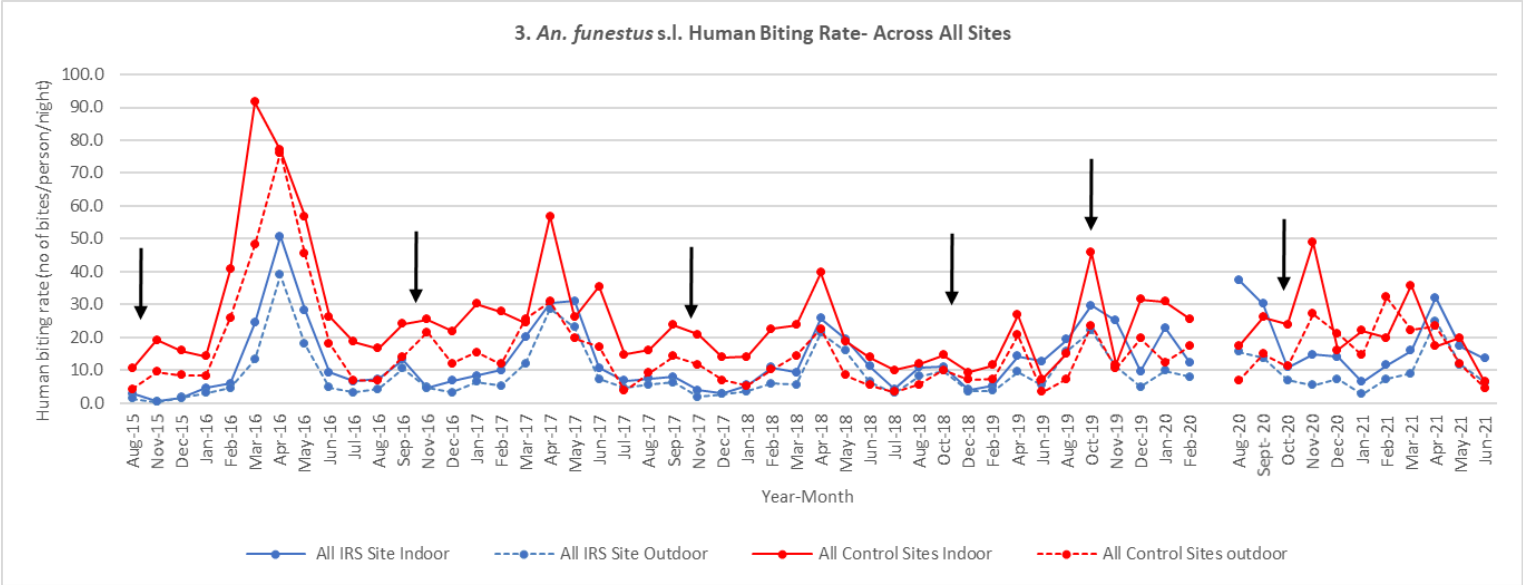
Key: <90% mortality (confirmed resistance), 90-97% mortality (probable resistance), and ≥98% mortality (susceptible). N/A = Not applicable.

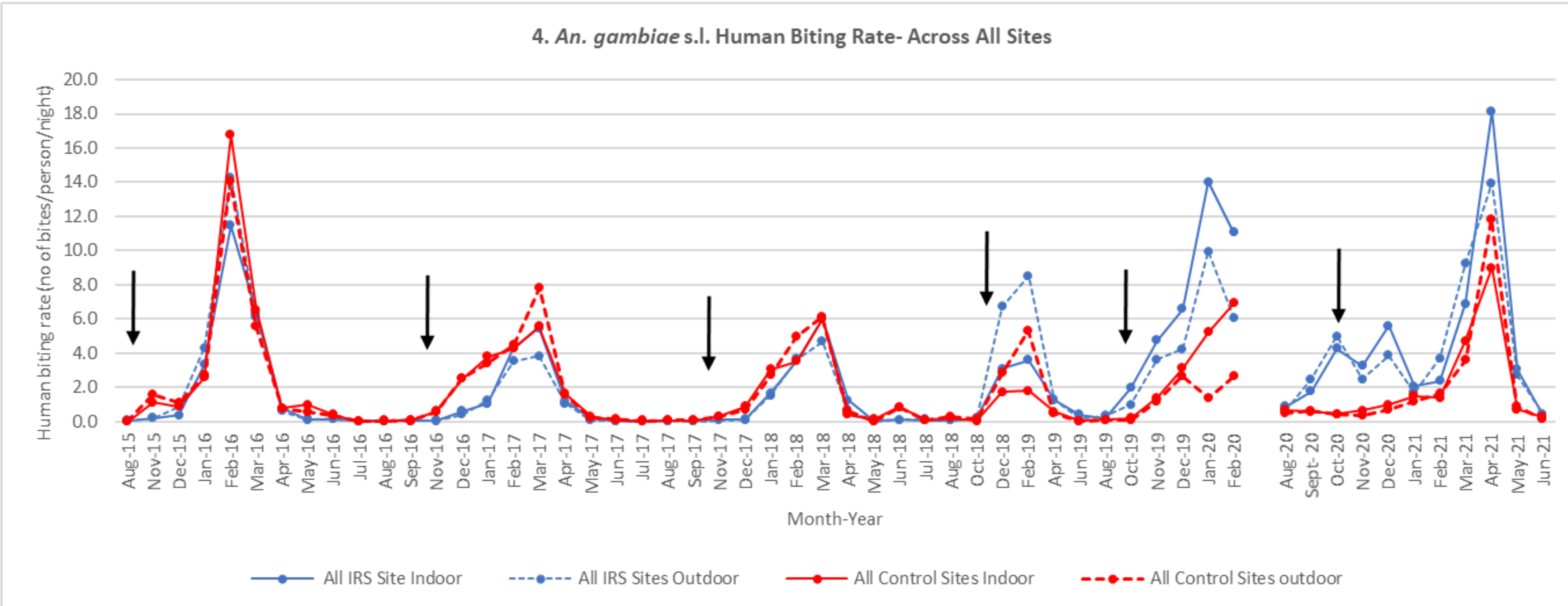
ANNEX F: TRENDS IN INDOOR RESTING DENSITIES AND HUMAN BITING RATES FOR *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. ACROSS ALL SITES 2015 -2021*

[Arrow indicates when IRS was implemented.]




2. An. gambiae s.l. Indoor Resting Density- Across All Sites
Vector density (no. per house)
Month-Year
IRS Sites
Control Sites


3. An. funestus s.l. Human Biting Rate- Across All Sites
Human biting rate (no of bites/person/night)
Year-Month
All IRS Site Indoor
All IRS Site Outdoor
All Control Sites Indoor
All Control Sites outdoor



*Note that some districts were replaced at certain points during the period. Here is a list of districts for each reporting period:

2015/2016: Mwense, Milenge, Kasama, Isoka, Katete, Serenje
2016/2017: Mwense, Milenge, Kasama, Isoka, Katete, Serenje
2017/2018: Mwense, Milenge, Kasama, Isoka, Katete, Serenje
2018/2019 Mwense, Milenge, Kasama, Isoka, Mambwe, Katete, Serenje
2019/2020: Nchelenge. Milenge. Mambwe, Katete, Serenje, Lufwanyama, Chililabombwe
2020/2021: Nchelenge. Milenge. Mambwe, Katete, Serenje, Lufwanyama, Chililabombwe