U.S. PRESIDENT'S MALARIA INITIATIVE

THE PMI VECTORLINK PROJECT ZAMBIA PROJECT

ANNUAL ENTOMOLOGY REPORT AUGUST 2021-JULY 2022

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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 (ITN) 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 28 to November 10, 2021, VectorLink Zambia conducted its 2021 IRS campaign across 21 districts—14 in Eastern Province, four in Copperbelt Province, and three in Luapula Province. The project used SumiShield 50WG (the neonicotinoid, clothianidin) in Kalulushi, Lufwanyama, Masaiti, and Mpongwe districts in Copperbelt and Nchelenge district in Luapula Province. Fludora Fusion (clothianidin and deltamethrin) was used in all 14 districts in Eastern Province (Chadiza, Chasefu, Chipangali, Chipata, Kasenengwa, Katete, Lumezi, Lundazi, Lusangazi, Mambwe, Nyimba, Petauke, Sinda, and Vubwi) and in Chiengi and Kawambwa districts of Luapula Province. The project sprayed 717,351 structures out of 738,659 structures found by spray operators, resulting in 97% spray coverage and protected 3,032,558 people, including 416,039 children under 5 years and 94,511 pregnant women.

Entomological monitoring associated with the 2021 IRS campaign included vector surveillance and insecticide resistance monitoring, assessment of the quality of spray, and monitoring insecticide residual efficacy. Vector surveillance to assess the impact of IRS was conducted from August 2021 to June 2022 in 14 sentinel sites, including four IRS sites and four control sites across the three provinces where IRS was supported by 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 2021 and post-intervention data collections started in October 2021 and were conducted monthly or bi-monthly[1]. Spray quality was assessed 24 hours after IRS at seven sprayed sites supported by PMI VectorLink. Monthly assessments of the insecticide residual efficacy on walls followed in five of the PMI VectorLink sites. Insecticide susceptibility tests were conducted in the 14 sites between December 2021 and May 2022 using World Health Organization (WHO) tube tests or U.S. Centers for Disease Control and Prevention (CDC) bottle assays.

PMI-supported entomological monitoring data from August 2021 to June 2022 indicate that *Anopheles funestus* s.l. was the most abundant mosquito (61.4% of 116,851 mosquitoes), while *An. gambiae* s.l. made up 13.5% of the total number of mosquitoes collected. The overall indoor resting density of *An. funestus* s.l. was lower at the IRS sites compared to the non-IRS sites (2.8 versus 5.1 vectors per house) and reduction in density was observed at sprayed sites after IRS (3.2 vectors per house pre-IRS to 2.6 vectors per house post-IRS) while a slight increase was observed post-IRS at the control sites (4.2 vectors per house pre-IRS to 5.4 vectors per house post-IRS). In contrast, the overall density of *An. gambiae* s.l. was higher at the IRS sites compared to control sites (0.59 vectors per house pre-IRS versus 0.54 vectors per house post-IRS), and post-IRS density was also higher than pre-IRS density at the IRS sites (0.7 versus 0.13 vectors per house). At the IRS sites, the average human biting rate of *An. funestus* s.l. indoors and outdoors reduced from 33.2 bites per person per night (b/p/n) before IRS to 27.5 b/p/n after IRS, while there was an increase at the non-IRS sites (29.4 to 35.3 b/p/n). Overall biting rates for *An. gambiae* s.l. increased after IRS at both the combined IRS sites (8.4 versus 1.5 b/p/n) and the combined control sites (9.1 versus 0.7 b/p/n). Reduction in parity rate—a desirable outcome of IRS which suggests vectors are not surviving long enough to transmit malaria—was observed post-

[1] Monthly collections from August 2021 to April 2022 at all sites and bimonthly collections at PMI Vectorlink supported sites only from June 2022 according to the 2022 approved work plan.

IRS for both *An. funestus* s.l. and *An. gambiae* s.l. in Luapula and Eastern Provinces. There were less sporozoite positive *An. funestus* s.l. at the sprayed sites compared to the control sites, which corroborates the reduced parity observed.

The majority (99.1%) of the *An. funestus* s.l. vectors collected during the reporting period were *An. funestus* s.s., with 0.9% *An. leesoni*. 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. and from *An. gambiae* s.l. was lower at the sprayed sites compared to the control sites in six out of the seven districts monitored. The absolute number of malaria infective bites per person per month at the sprayed sites was as high as 45 bites for *An. funestus* s.l. and 14.1 bites for *An. gambiae* s.l. This signals the need for the deployment of additional interventions to supplement IRS in the affected areas. We found very high human blood index (>99%) for both *An. funestus* s.l. and *An. gambiae* s.l. at sprayed and control sites; specifically, most of the vectors fed on humans rather than 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. 24 hours post-exposure in all seven districts where quality of spray was evaluated at the time of the 2021 IRS campaign. These findings signify a high quality of spraying on all sprayed surfaces that were evaluated. As of August 2022, based on longitudinal data collected on the residual efficacy of the two insecticides deployed in the 2021 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, and Copperbelt). There was a mixture of full susceptibility and confirmed resistance to dichlorodiphenyltrichloroethane (DDT) in Luapula Province, full susceptibility in Eastern Province and susceptibility and probable resistance in Copperbelt Provinces. 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 Eastern 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 the use of supplementary vector control measures in such areas. Consideration should be given to integrated vector management wherein all malaria transmission zones are targeted with either ITNs or IRS, with available supplementary methods such as LSM and house screening deployed when 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 support the transition away from standard pyrethroid-only ITNs to the deployment of piperonyl butoxide (PBO) nets and potentially new 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). VectorLink Zambia conducted its 2021 IRS campaign from September 28 to November 10, 2021, in support of the National Malaria Elimination Program (NMEP), Ministry of Health in 21 districts—14 in Eastern Province, four in Copperbelt Province, and three in Luapula Province. The project sprayed 717,351 out of 738,659 structures found by spray operators, resulting in 97% spray coverage. The project used SumiShield 50WG (the neonicotinoid, clothianidin) in Kalulushi, Lufwanyama, Masaiti, and Mpongwe districts in Copperbelt and Nchelenge district in Luapula Province. Fludora Fusion (clothianidin and deltamethrin) was used in all 14 districts in Eastern Province (Chadiza, Chasefu, Chipangali, Chipata, Kasenengwa, Katete, Lumezi, Lundazi, Lusangazi, Mambwe, Nyimba, Petauke, Sinda, and Vubwi) and in Chiengi and Kawambwa districts of Luapula Province. VectorLink Zambia supported the 2020/2021 mass ITN campaign through technical assistance in planning of the campaign and training of staff. Other ITN activities supported by the project include an ITN durability monitoring baseline study in Nyimba and Serenje districts, an ITN misuse study in Luapula, Northern, and Muchinga Provinces, and technical assistance to school-based distribution in four districts in 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 2021 to June 2022 and is linked to the 2021 IRS campaign. It presents all entomological monitoring activities conducted by PMI VectorLink Zambia and discusses the implications of the results obtained.

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

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

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

Indicator	Collection Methods	Frequency	Parameters measured
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 by adult female vectors per person per unit time: 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 five days
Residual efficacy monitoring	Insectary colony mosquitoes	Monthly[1]	Percentage mortality up to five days
Insecticide susceptibility	Larval and adult collections	Once per reporting period[a]	Percentage mortality at the end of holding periods.

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 from August 2021 to March 2022 in 7 districts (Nchelenge, Milenge, Serenje, Lufwanyama, Chililabombwe, Katete and Mambwe) and bi-monthly from April 2022 to June 2022 in four districts (Nchelenge, Lufwanyama, Katete and Mambwe)

[a]Tests conducted between December 2021 and May 2022.

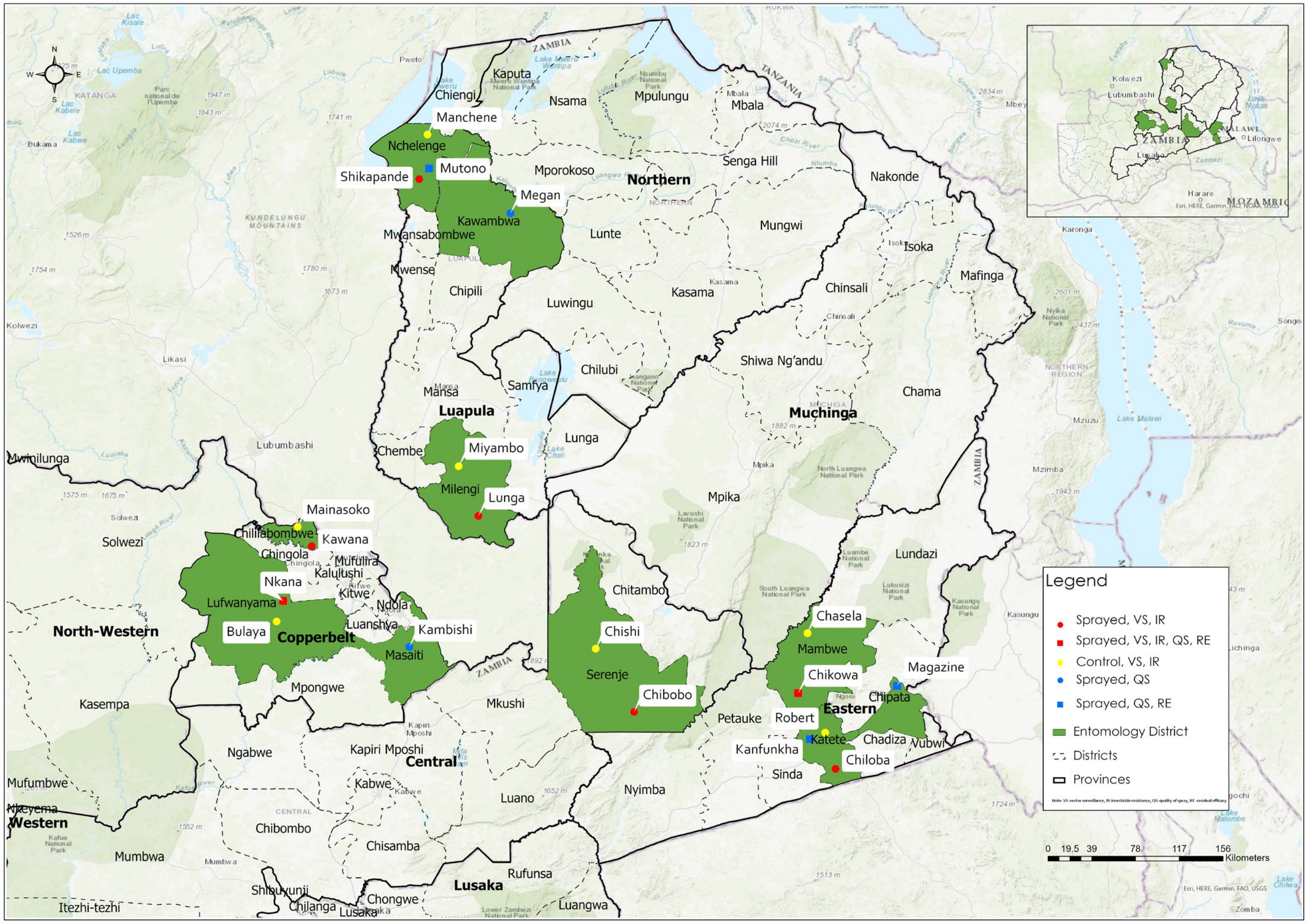
2. MATERIALS AND METHODS

2.1 MONITORING SITES

From August 2021 to June 2022, 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 September/October 2021 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 for both IRS and ITNs and other potential IRS insecticides.

VectorLink Zambia conducted IRS in September/October 2021 in four of the intervention sentinel sites [using SumiShield 50WG in Shikapande (Nchelenge District) and Nkana (Lufwanyama District) and Fludora Fusion in Chikowa (Mambwe District) and Chiloba (Katete District)]. The Government of the Republic of Zambia (GRZ) conducted IRS in the other three intervention sites [using DDT in August 2021 in Lunga (Milenge District) and in October 2021 in Chibobo (Serenje District) and Fludora Fusion in September 2021 in Kawama (Chililabombwe District)]. 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 2021-July 2022)



Note: 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 during the 2020/2021 mass campaign. Four sites received PBO nets, two sites received standard ITNs, and one site did not receive any nets during the last mass campaign in 2020/2021. Further details of the monitoring sites according to the activities conducted are shown in Table 2.

Table 2: Entomological Monitoring Sites

Province	District	Health Facility Catchment Area	Sentinel Site (Village)	Spray Status (Distance to Nearest Sprayed Community)	Percent of Households Targeted for IRS by PMI/VL in 2021*
Vector Surveillance and Insecticide Resistance Monitoring					
Luapula	Nchelenge	Lushiba	Shikapande	Sprayed with SumiShield	100%
		Kafutuma	Manchene	Non-sprayed control (3km); received standard ITNs in 2020	0%
	Milenge	East Seven	Lunga	Sprayed with DDT	100% (by GRZ)
		East Seven	Miyambo	Non-sprayed control (7km); received standard ITNs in 2020	0%
Eastern	Mambwe	Chikowa	Chikowa	Sprayed with Fludora Fusion	100%
		Chikowa	Chasela	Non-sprayed control (6km); received PBO nets in 2020	0%
	Katete	Katiula	Chilowa	Sprayed with Fludora Fusion	100%
		Kamphambe	Robert	Non-sprayed control (10km); received PBO nets in 2020	0%
Central	Serenje	Chibobo	Chibobo	Sprayed with DDT	100% (by GRZ)
		Chibobo	Chishi	Non-sprayed control (5km); received PBO nets in 2020	0%
Copperbelt	Lufwanyama	Nkana	Nkana	Sprayed with SumiShield	100%
		Bulaya	Bulaya	Non-sprayed control (4km); received PBO nets in 2020	0%
	Chililabombwe	Kawama	Kawama	Sprayed with Fludora Fusion	100% (rural/peri-urban)
		Kawama	Mainasoko	Non-sprayed control (6km); no nets received in 2020	0%
IRS Quality Assurance (QA) and Insecticide Residual Efficacy Monitoring					
Luapula	Nchelenge	Kashikishi	Mutono	Sprayed with SumiShield	100%
	Kawambwa	Megan	Megan (QA only)	Sprayed with Fludora Fusion	
Eastern	Mambwe	Chikowa	Chikowa	Sprayed with Fludora Fusion	100%
	Chipata	Namseche	Margazine	Sprayed with Fludora Fusion	100%
	Katete	Kafunkha	Kafunkha	Sprayed with Fludora Fusion	100%
Copperbelt	Masaiti	Kambishi	Kambishi (QA only)	Sprayed with SumiShield	100%
	Lufwanyama	Nkana	Nkana	Sprayed with SumiShield	100%

*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 2021 to April 2022 monthly and then bimonthly till June 2022 in four sites (Nchelenge, Lufwanyama, Katete, and Mambwe).

Entomological monitoring to assess the impact of IRS on malaria vectors started within 1-3 weeks after the intervention sites were sprayed in each site (October 2021 for sentinel sites in Nchelenge, Mambwe, Katete, Serenje, Lufwanyama and Chililabombwe and in September 2021 for the sites in Milenge).

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

*Collections were done monthly at all sites from August 2021 to April 2022, thereafter collections continued bi-monthly at sites in Nchelenge, Mambwe, Katete and Lufwanyama only

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.

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

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

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 SPRAY QUALITY ASSESSMENT 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 three Global Fund (GF)/GRZ program areas where we conduct entomological surveillance.

Quality of spray was assessed at the seven sites in PMI-supported IRS program districts, namely: Mutono Village (Nchelenge District), Nkana village (Lufwanyama) and Kambishi Village (Masaiti District) sprayed with SumiShield, and Chikowa Village (Mambwe District), Margazine village (Chipata), Kafhunka village (Katete) and Megan village (Kawambwa) sprayed with Fludora Fusion during the 2021 IRS campaign.

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 the 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 as control)
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 as control)

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

The cone bioassays were conducted following the procedures on SOP 09/01. In each house, cohorts of 10 mosquitoes were exposed on walls at 0.5m, 1m and 1.5m above the floor. 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.

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 chlorfenapyr (pyrrole) alpha-cypermethrin, deltamethrin and permethrin (pyrethroids) was assessed at sites in all entomological monitoring sentinel districts. Clothianidin is the main active ingredient in the two chemicals used for IRS by VectorLink

Zambia and GRZ (Government of the Republic of Zambia) in 2021 (SumiShield and Fludora Fusion); Fludora Fusion also contains deltamethrin.

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 (Centers for Disease Control) 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 DDT 4.0% (an organochlorine), and deltamethrin 0.05% (a pyrethroid) 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. Mortality was recorded after 24 hours for all insecticides tested. The sugar solution was changed daily during the holding periods. Susceptibility tests were done from December 2021 to June 2021.

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) and clothianidin (4 µg/ml) at some sites. Clothianidin tests were done using a new protocol. In this procedure, 250ml glass Schott bottles (or equivalent) were treated with the diagnostic dose of clothianidin which is defined by WHO as 4 µg AI/bottle for *An. funestus* s.l. and *An. gambiae* s.l. by firstly adding 4 mg of technical grade clothianidin in 100ml of acetone/Mero solution, creating a stock solution of 40µg/ml. 10ml of clothianidin stock solution (40 µg/ml) was diluted with 90ml of 800 ppm acetone/Mero to make a working solution of 4µg/ml. A 250 ml glass bottle was coated with 1ml of the clothianidin working solution (4 µg/bottle) using a pipette according to the standard VectorLink bottle assay SOP 4/01. Control bottles were treated using a solution of acetone/Mero mixture. The exposure time was 60 minutes, and the mortality was recorded at one hour and at 24 hours after exposure. The bottles were coated each month with technical grade chlorfenapyr supplied by BASF and with technical grade clothianidin supplied by Bayer at the National Malaria Elimination Centre (NMEC) laboratory and transported to the field in compartmentalized cardboard boxes for the assays. Each bottle was used a maximum of three times and was returned to Lusaka for cleaning and reuse.

2.5 Laboratory Analysis

Mosquitoes collected by HLCs were killed using cotton wool soaked in ethyl acetate[4] 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[5]. 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[6,7],

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

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

[6] 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.

[7] 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.

and 2) detect circumsporozoite proteins of *Plasmodium falciparum* sporozoites[8] using Enzyme-Linked Immunosorbent Assays (ELISAs)[9]. *An. gambiae* s.l. samples that were resistant to pyrethroids were analyzed by PCR for the presence of the west and east kdr alleles[10,11].

2.6 Data Presentation and Statistical Analysis

Database. The DHIS2-based VectorLink Collect instance for entomological data management was used for entry and management of all field data collected during the reporting period. 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. The NMEP, through the recently formed Entomology Data Management Committee, will receive the raw data on a regular basis for hosting on the recently developed NMEC DHIS2 (District Health Information Software Version 2) Ento module.

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 for Milenge and August-September 2021 for all other districts) and post-IRS (September in Milenge and October through June 2022 for all other districts) and transmission indicators between these two periods were compared.

Rainfall Data. Rainfall data presented here was extracted from the World Food Program data visualization platform: https://dataviz.vam.wfp.org/seasonal_explorer/rainfall_vegetation/visualizations. The primary data sources are CHIRPS gridded rainfall dataset produced by the Climate Hazards Group at the University of California, Santa Barbara and the MODIS NDVI CMG data made available by NOAA-NASA. CHIRPS stands for Climate Hazards Group InfraRed Precipitation with Station data. CHIRPS is a 35+ year quasi-global rainfall dataset. Spanning 50°S-50°N (and all longitudes), starting in 1981 to near-present, CHIRPS incorporates 0.05° resolution satellite imagery with in-situ station data to create gridded rainfall time series for trend analysis and seasonal drought monitoring. CHIRPS data is available at 5- and 10-day accumulations. During rainfall data extraction for each district, Zambia was selected as country followed by the specific province and then the district and the rainfall data downloaded as CSV file.

Collection Periods (Months Relative to IRS Implementation). Given that not all districts were sprayed at the same time (for instance, Milenge was sprayed in September2021 and the other districts were sprayed in October), 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.

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

[9] 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.

[10] Martinez-Torres D et al. (1998) Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae s*.s. Insect Mol Biol 7:179-184

[11] Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH (2000) Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. Insect Mol Biol 9:491-497

Table 5: Month and Year for Collection Period (Months Relative to IRS) for Each District (August 2021-February 2022)

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-2	Aug-21	-	Aug-21	Aug- 21	Aug-21	Aug-21	Aug-21
T-1	Sep-21	Aug -21	Sep-21	Sep-21	Sep-21	Sep-21	Sep-21
T-0	Oct-21	Sep- 21	Oct-21	Oct-21-	Oct -21	Oct-21	Oct-21
T+1	Nov-21	Oct -21	Nov-21	Nov-21	Nov-21	Nov-21	Nov- 21
T+2	Dec-21	Nov-21	Dec-21	Dec-21-	Dec-21	Dec-21	Dec-21
T+3	Jan-22	Dec- 21	Jan-22	Jan-21	Jan-21	Jan-22	Jan-22
T+4	Feb-22	Jan- 22	Feb-22	Feb-22-	Feb- 22	Feb-22	Feb-22
T+5	Mar-22	Feb- 22	Mar-22	Mar-22	Mar- 22	Mar-22	Mar-22
T+6	Apr-22	Mar-22	Apr-22	Apr- 22-	-	Apr-22	-
T+8	Jun-22	-	Jun-22	June-22	-	June-22	-

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 2021 to July 2022 are presented below. Vector surveillance by HLC and PSC were conducted monthly from August 2021 to April 2022 in seven sentinel districts. In May 2022, the frequency of collections shifted to every other month in four districts only (Nchelenge, Mambwe, Katete and Lufwanyama). Between May-July 2022, collections were only conducted in June 2022 in these four districts. The 2021 IRS campaign by PMI VectorLink began in late September 2021, and thus baseline vector surveillance data was collected in August and September 2021, and post-IRS data was collected from October 2021 to June 2022. Residual efficacy monitoring commenced in September/October 2021 and continued monthly through August 2022. Cone bioassays conducted in August 2022 provide insecticide residual efficacy data at 10 months post-IRS. Insecticide resistance tests were performed from December 2021 to May 2022.

3.1 LONGITUDINAL MONITORING OF VECTORS

3.1.1 SPECIES COMPOSITION

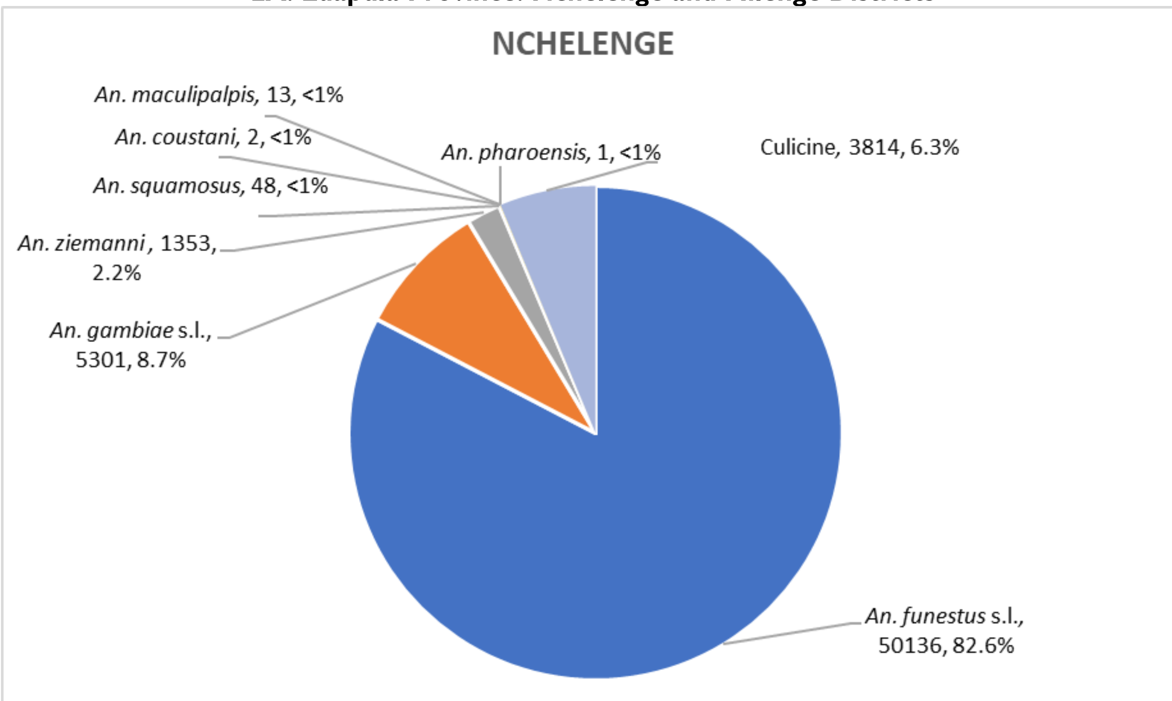
A total of 116,851 mosquitoes were collected by HLC and PSC during the reporting period. *An. funestus* s.l. was the most abundant (61.4%), followed by culicines (15.7%), *An. gambiae* s.l. (13.5%), *An. ziemanni namibiensis* (6.1%), and *An. tchekedii* (1.9%). Other species (*An. coustani*, *An. maculipalpis*, *An. squamosus*, *An. rufipes*, *An. argentiolobatus*, *An. gibbinsi*, and *An. tenebrosus*) accounted for 1.4% of the total collected.

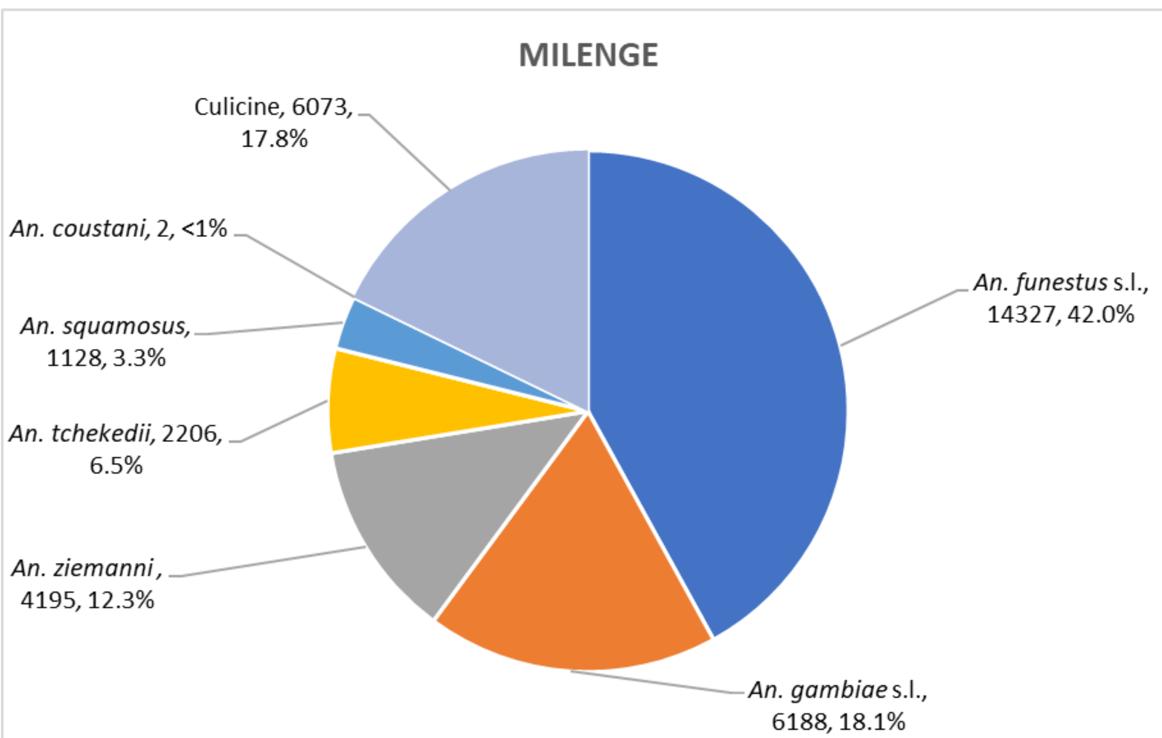
Out of the 87,587 primary vector complexes collected, *An. funestus* s.l. accounted for 82% (71,803), while *An. gambiae* s.l. accounted for 18% (15,784). The distribution of the different species varied according to district. District level species composition grouped by province are presented in Figure 2A-D.

In Luapula Province, *An. funestus* s.l. was the predominant species among the two primary vectors (*An. funestus* s.l. constituted 85%, and *An. gambiae* s.l. 15%). There was a high presence of *An. gambiae* s.l. and *An. ziemanni namibiensis* in Milenge District (18.1% and 12.3% of all mosquitoes collected respectively) (Figure 2A). In Eastern Province, among the two primary vectors, *An. gambiae* s.l. was the predominant species in Mambwe District (95%), while *An. funestus* s.l. was the predominant species in Katete District (92%). There was notable presence of *An. coustani* in Katete District in Eastern Province (10%). Among the primary vectors in Central Province, *An. funestus* s.l. (97%) was the predominant species; (Figure 2C). In Copperbelt Province, there were more *An. funestus* s.l. (71%), with a substantial presence of *An. gambiae* s.l. (29%). There was a notable presence of *An. ziemanni namibiensis* in Lufwanyama District in Copperbelt Province, comprising 14.3% of all mosquitoes collected (Figure 2D). Annex A contains details of the number and types of mosquitoes collected by the different collection methods in each sprayed and unsprayed sentinel site.

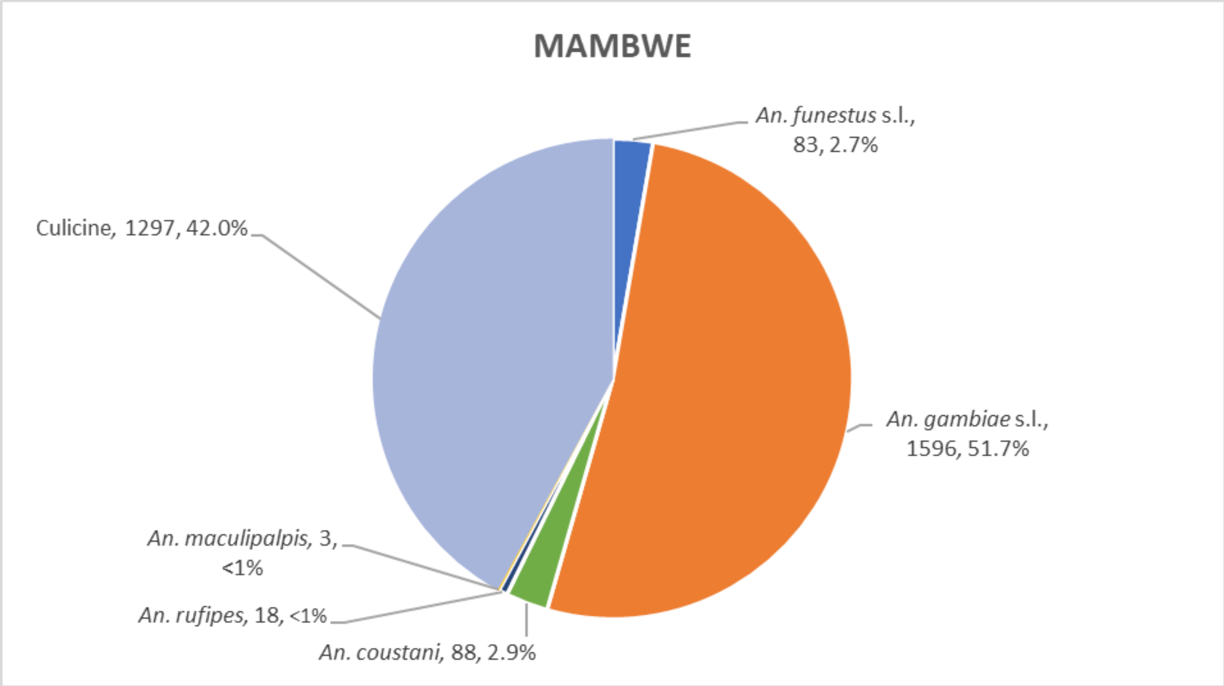
Figure 2: Species Composition by Province and District (August 2021-June 2022)

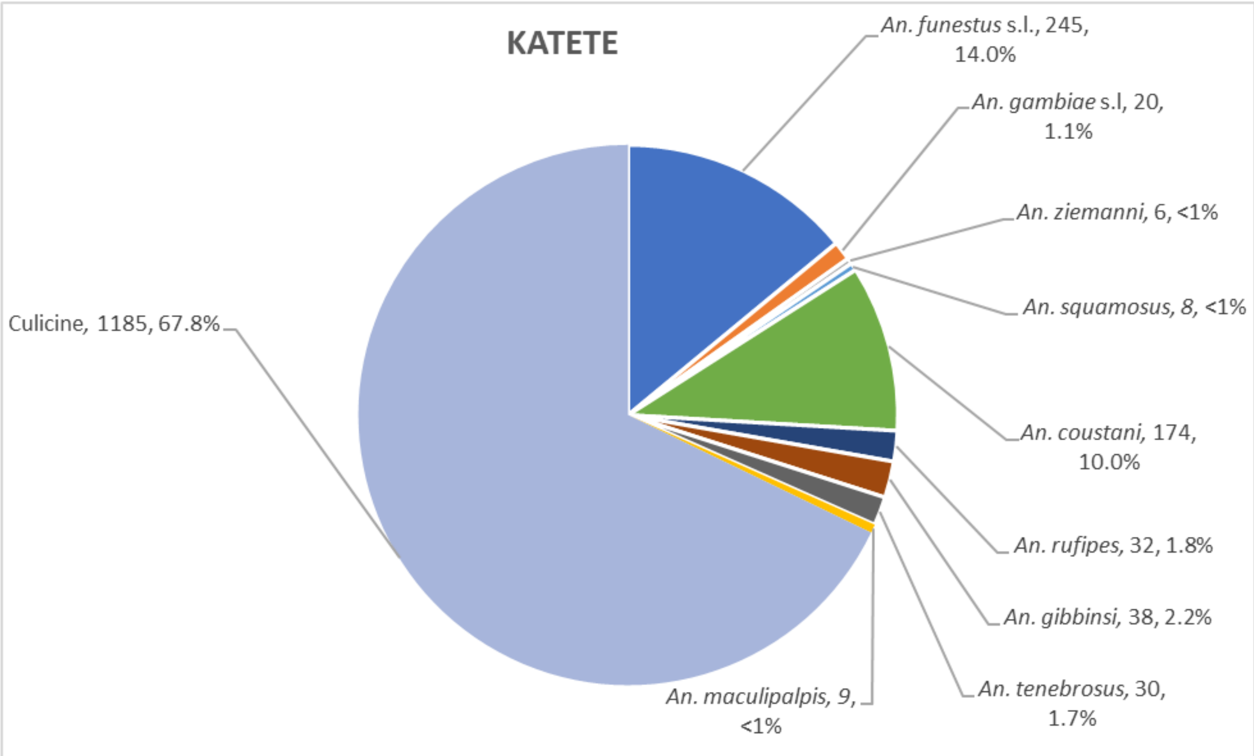
2A: Luapula Province: Nchelenge and Milenge Districts



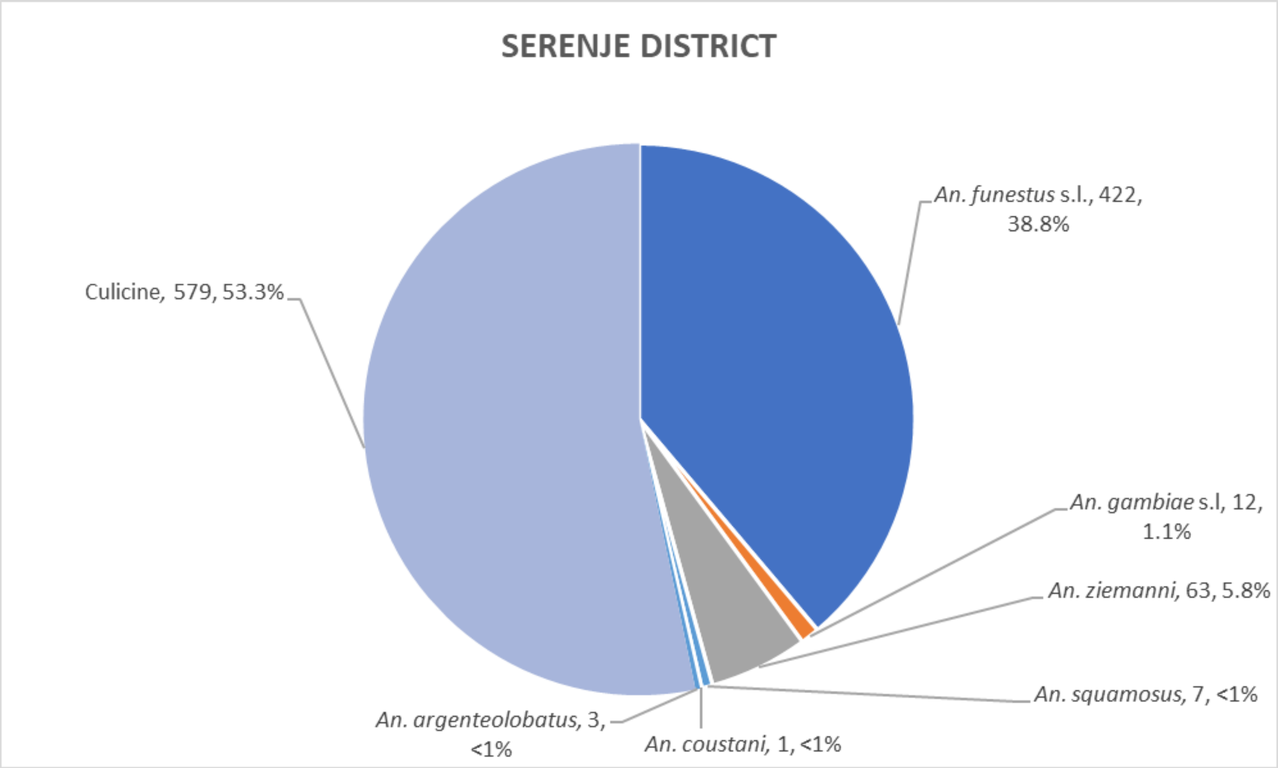


2B: Eastern Province: Mambwe and Katete Districts

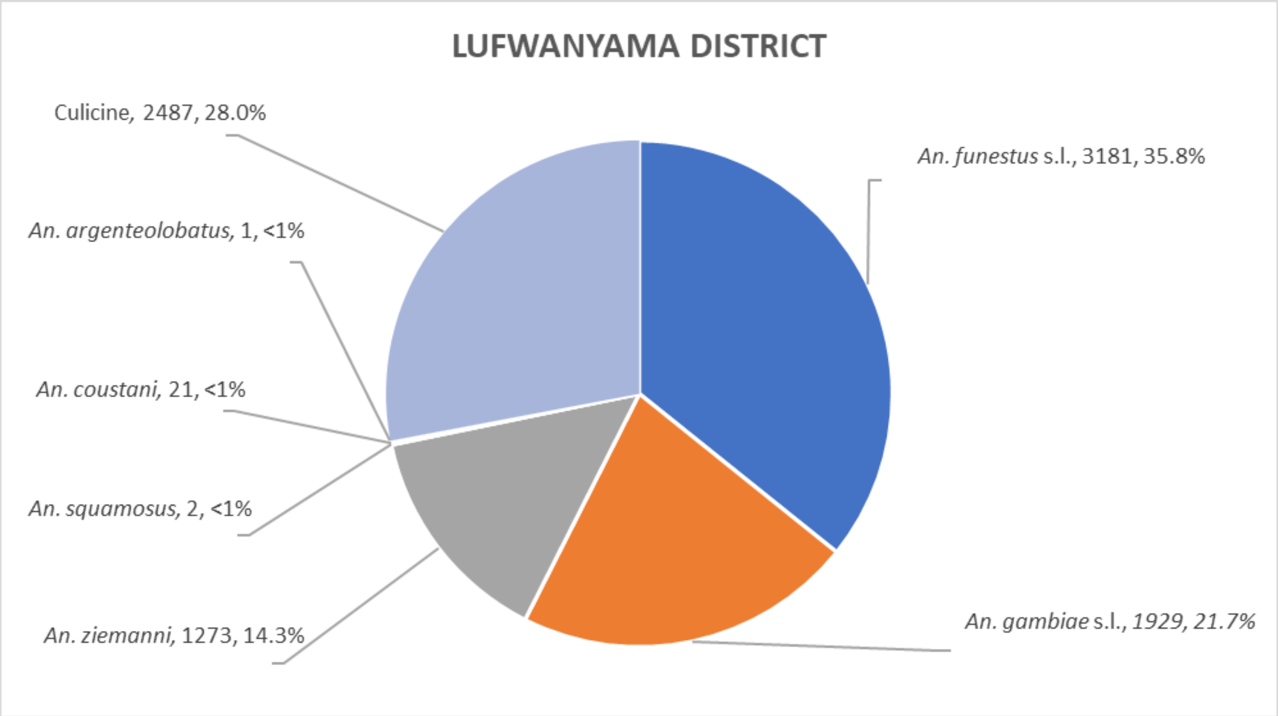


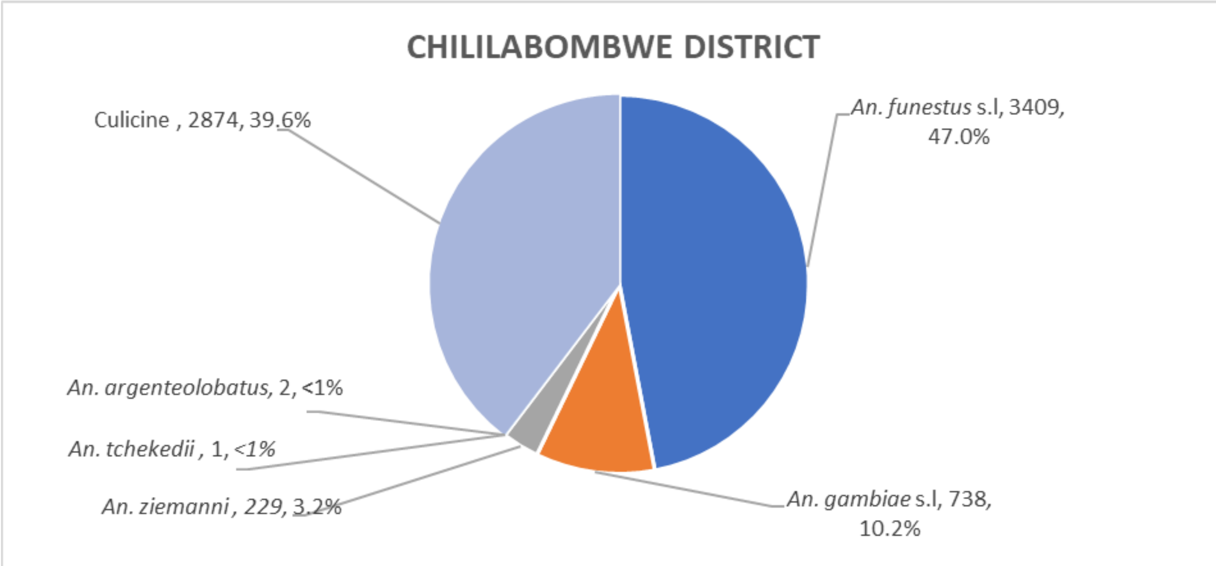


2C: Central Province: Serenje District



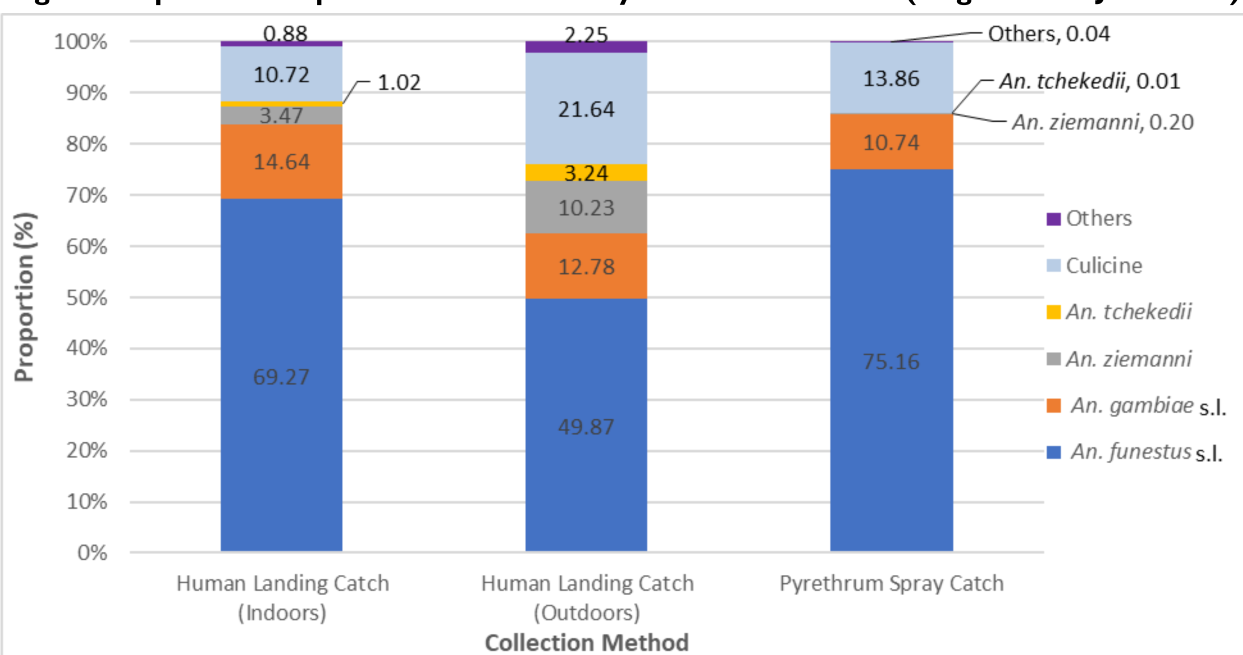
2D: Copperbelt Province: Lufwanyama and Chililabombwe Districts





The species composition by collection method is depicted in Figure 3. All 11 different Culicidae collected over the reporting period were found in the HLC collections, while only six were found in the PSC collections. The proportion of *An. funestus* s.l. was higher in the indoor collections— indoor HLCs (69.9%) and PSCs (75.2%)—compared to outdoor HLC (51.0%). There was no marked difference between the proportion of *An. gambiae* s.l. collected indoors/outdoors (ranging from 10.5-14.8%). Higher percentages of other *Anopheles* species were collected outdoors compared to indoors; 13.8% in the outdoor HLC collections compared to 4.5% in the indoor HLC collections and 2.1% using PSCs. A total of 78,940 (90.1%) of the primary vectors were collected from HLCs and 8,647 (9.9%) 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 2021-June 2022)

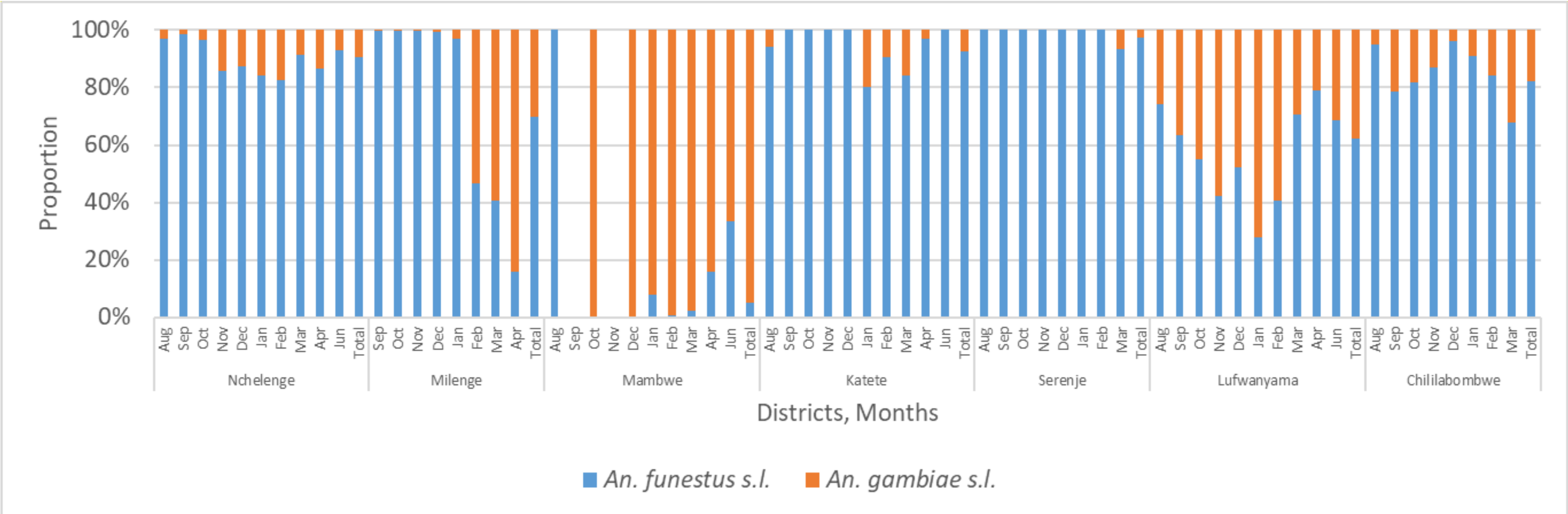


Other species collected by HLC indoors included *An. squamosus* (0.53%), *An. coustani* (0.22%), *An. rufipes* (0.04%), *An. gibbinsi* (0.04%), *An. maculipalpis* (0.02%), *An. argentiolobatus* (0.01%), and *An. tenebrosus* (0.02%). Other species collected by HLC-Outdoors include *An. squamosus* (1.77%), *An. rufipes* (0.05%), *An. coustani* (0.33%), *An. maculipalpis* (0.03%), *An. gibbinsi* (0.03%), *An. argentiolobatus* (0.002%), and *An. tenebrosus* (0.04%). Other species collected by PSC included *An. squamosus* (0.03%) and *An. rufipes* (0.01%).

Figure 4 shows monthly relative abundance of the two primary vector species *An. funestus* s.l. and *An. gambiae* s.l. in each of the sentinel districts. *An. funestus* s.l. was the predominant malaria vector in all districts except Mambwe in Eastern Province where *An. gambiae* s.l. was the most common species collected. In Milenge District, there was a shift from predominantly *An. funestus* s.l. to *An. gambiae* s.l. from February up to April (immediately after peak rainfall). In Lufwanyama District, where 37.7% of the total collected were *An. gambiae* s.l., the species was dominant in the months of November, January, and February coinciding with the peak rainy period.

Both primary vectors were collected from sprayed and unsprayed sites, however, more *An. funestus* s.l. were collected from unsprayed sites (55.5%) than sprayed sites (44.5%), while similar numbers of *An. gambiae* s.l. were found at both sprayed sites (49.0%) and control sites (51.0%).

Figure 4: Monthly Variations in the Relative Proportions of *An. funestus* s.l. and *An. gambiae* s.l. by District (August 2021–June 2022)



3.1.2 Indoor Resting Density of *An. funestus* s.l. and *An. gambiae* s.l. Collected by PSC

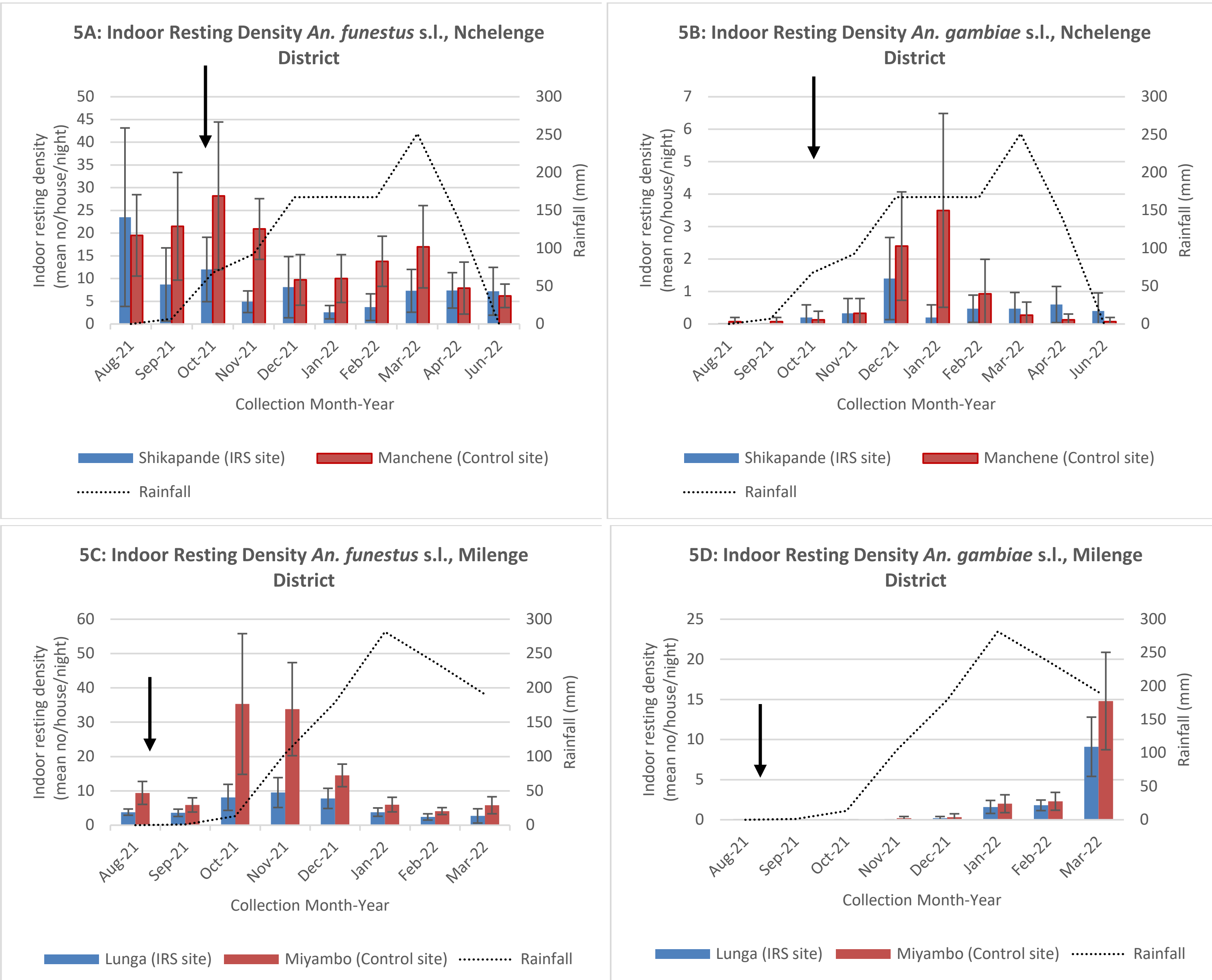
Overall indoor resting density of *An. funestus* s.l. was significantly lower at the combined sprayed sites with 2.8 vectors per house compared to the combined control sites with 5.1 vectors per house [incidence rate ratio (IRR) 0.51, p<0.01)]. A reduction in *An. funestus* s.l. density was observed at sprayed sites after IRS (3.2 to 2.6 vectors per house) while an increase was observed at the control sites (4.2 to 5.4 vectors per house). *An. gambiae* s.l. overall density at the combined sprayed sites, 0.59 vectors per house, was not different from that at the combined control sites 0.54 vectors per house (IRR 1.07, p=0.764). Post-IRS *An. gambiae* s.l. mean densities were significantly higher at the sprayed sites (0.70 versus 0.13 vectors per house, IRR 22.9, p<0.001) as well as the control sites (0.67 versus 0.03 vectors per house, IRR 5.07, p<0.001). Overall, *An. gambiae* s.l. indoor resting density increased by 4-fold at the sprayed sites compared to a 25-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.

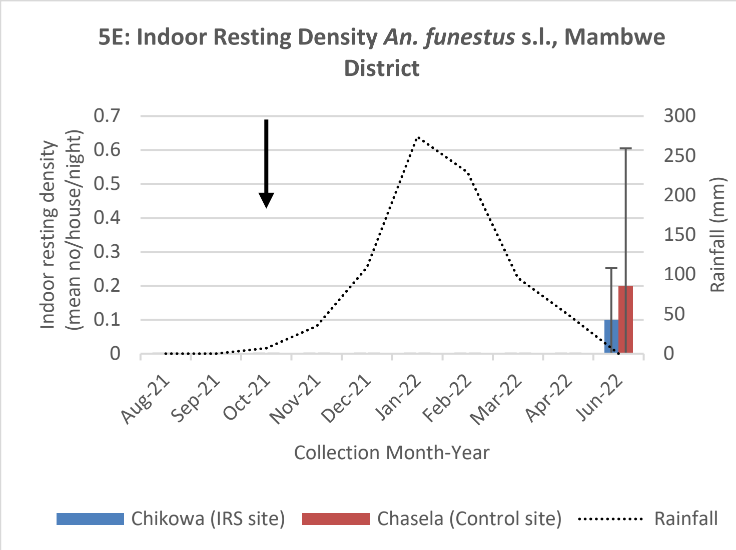
Figure 5 below is a panel of figures showing the indoor resting densities for both *An. funestus* s.l. and *An. gambiae* s.l. vectors at sprayed and unsprayed sites in each of the seven districts with monthly rainfall.

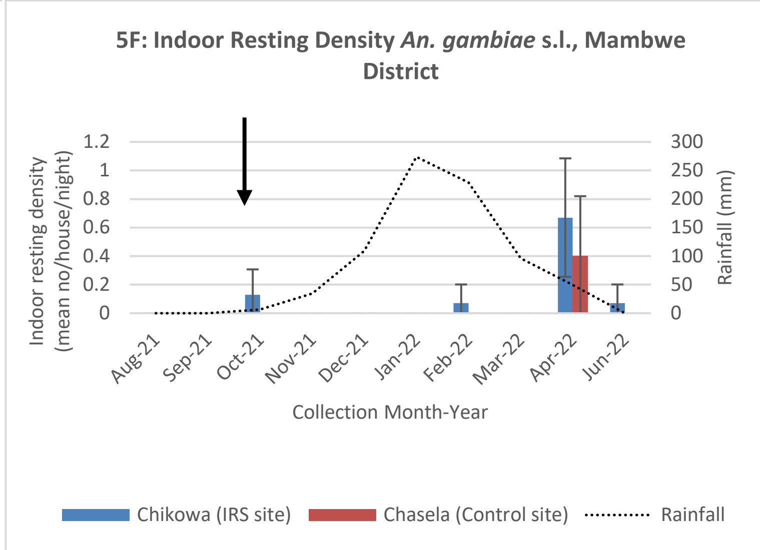
At district level, there were fewer indoor resting *An. funestus* s.l. vectors at the sprayed sites compared to the control sites in six of the seven districts (Nchelenge District-Figure 5A, Milenge District-Figure 5C, Mambwe District-Figure 5E, Katete District-Figure 5G, Serenje District-Figure 5I, and Lufwanyama District-Figure 5K). The differences between mean densities of sprayed and control sites were statistically significant at p=0.05 in four of the six districts (Nchelenge, Milenge, Katete, and Serenje). *An. funestus* s.l. vector densities were significantly higher at the sprayed sites compared to control sites in Chililabombwe District-Figure 5M. 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 (16.1 to 6.7) and Chilowa in Katete District (0.07 to 0.01). Densities remained the same or increased at all control sites except Manchene in Nchelenge District (density reduced from 20.5 to 14.2). Only the reductions in Shikapande and Manchene were statistically significant. At sites where *An. funestus* s.l. densities increased after IRS, the increases were up to 5.6 folds at the control sites but only up to 3.2 folds, at the sprayed sites. *An. gambiae* s.l. indoor resting densities were lower in sprayed sites compared to control sites in four of the seven districts (Nchelenge, Milenge, Katete and Serenje Districts) and the reductions were statistically significant for only Milenge District (p=0.04). Post-IRS mean *An. gambiae* s.l. indoor resting densities either remained the same or increased after IRS at all sprayed and control sites. Similarly, the increases in *An. gambiae* s.l. densities were up to 13.9 folds at the control sites but only up to 3.0 folds, at the sprayed sites.

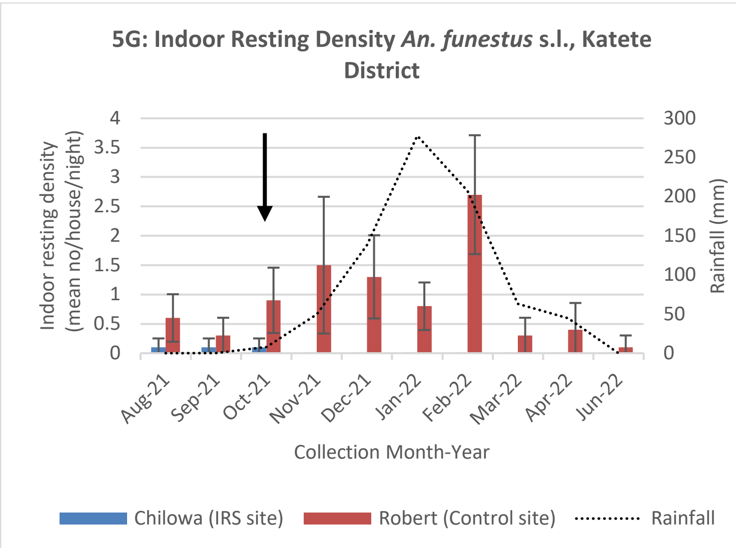
Figure 5: *An. funestus* s.l. and *An. gambiae* s.l. Indoor Resting Density Across Sites (August 2021–June 2022)

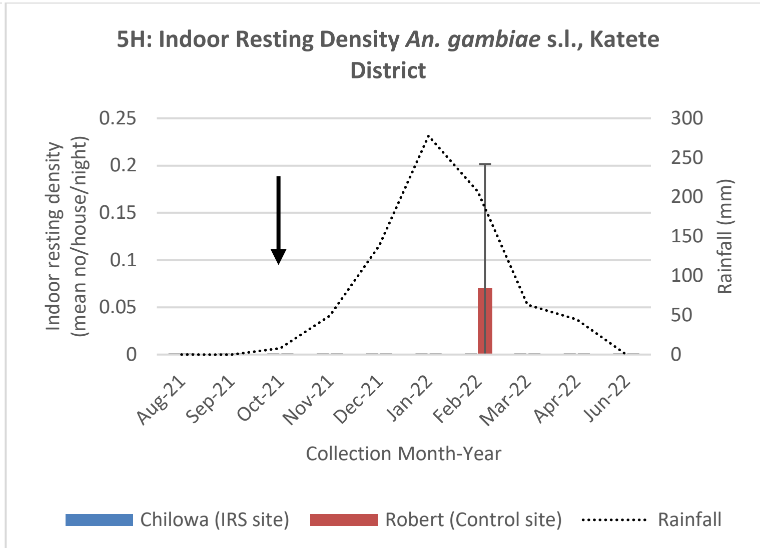
[Bars with 95% confidence intervals. Arrow indicates when IRS was implemented.]

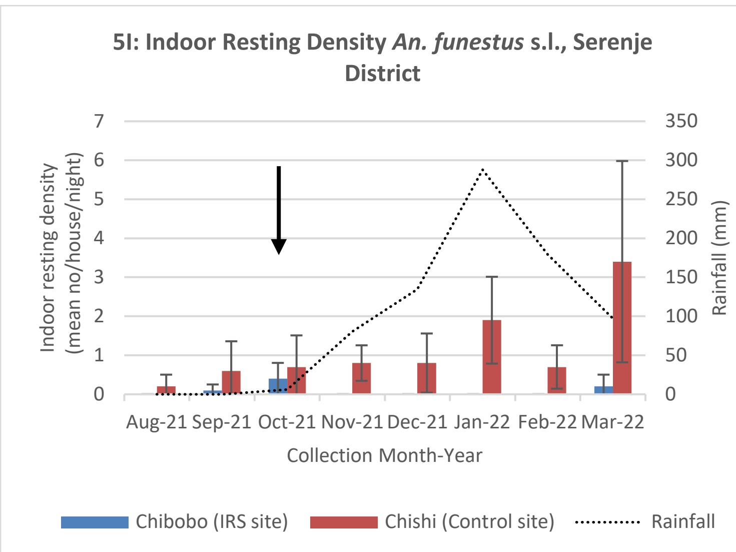


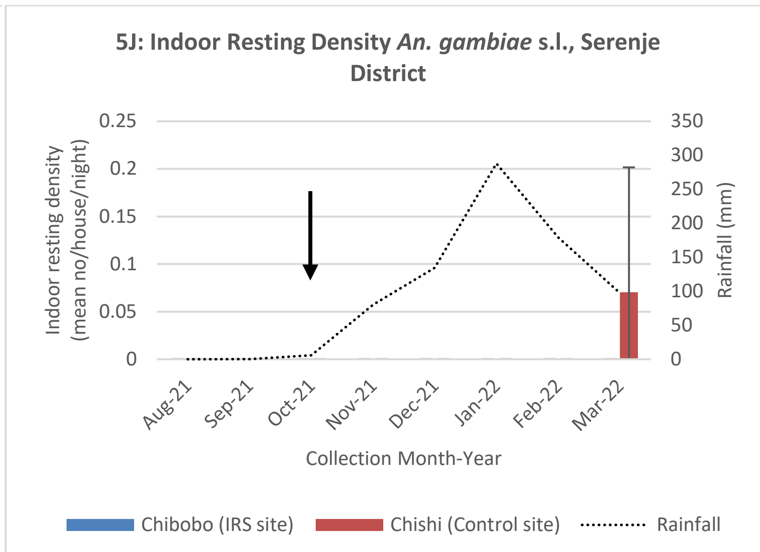

5E: Indoor Resting Density An. funestus s.l., Mambwe District
Indoor resting density (mean no/house/night)
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Rainfall (mm)
0 50 100 150 200 250 300
Aug-21 Sep-21 Oct-21 Nov-21 Dec-21 Jan-22 Feb-22 Mar-22 Apr-22 Jun-22
Collection Month-Year
Chikowa (IRS site)
Chasela (Control site)
Rainfall

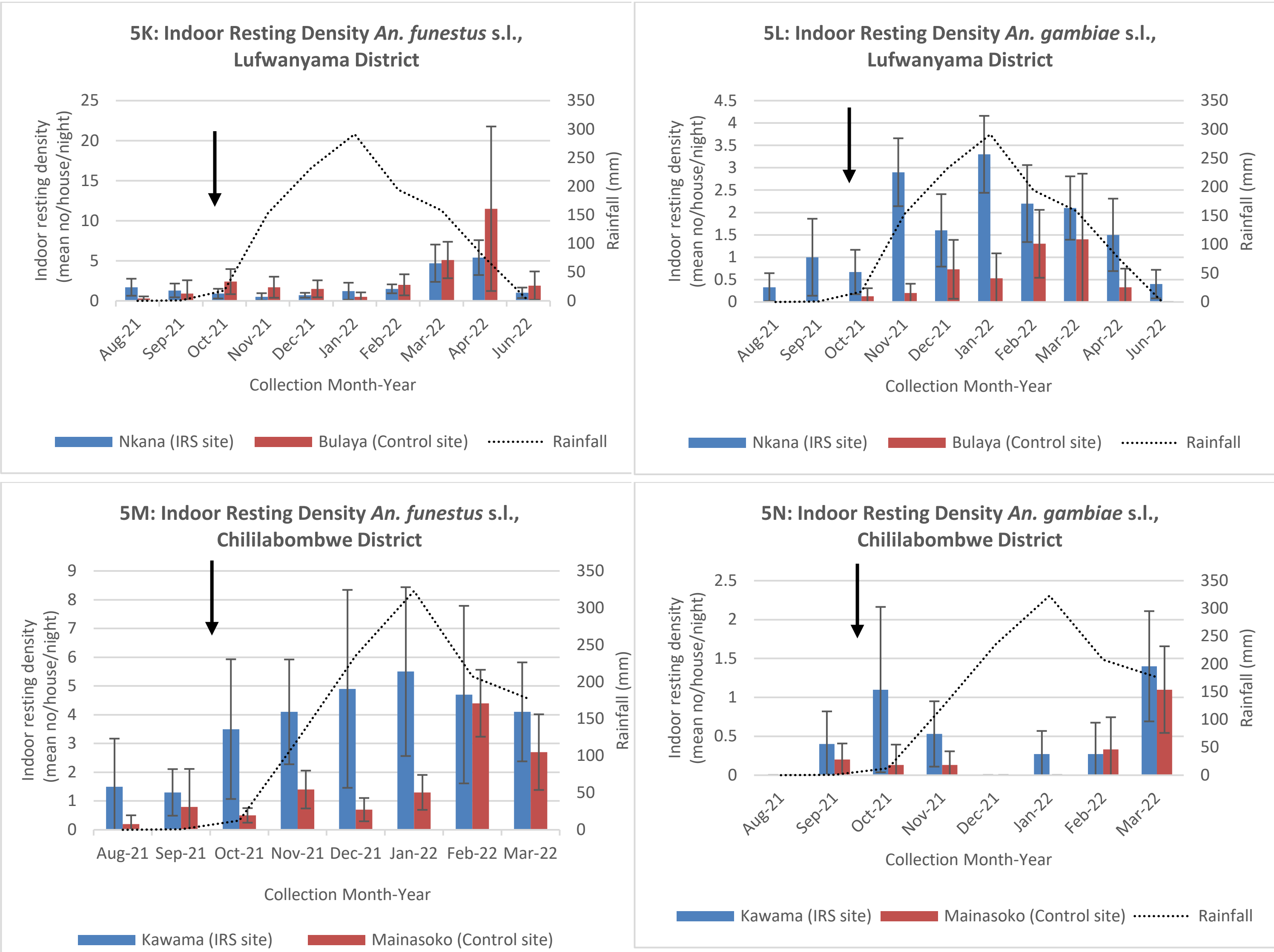

5F: Indoor Resting Density An. gambiae s.l., Mambwe District
Indoor resting density (mean no/house/night)
0 0.2 0.4 0.6 0.8 1 1.2
Rainfall (mm)
0 50 100 150 200 250 300
Aug-21 Sep-21 Oct-21 Nov-21 Dec-21 Jan-22 Feb-22 Mar-22 Apr-22 Jun-22
Collection Month-Year
Chikowa (IRS site)
Chasela (Control site)
Rainfall


5G: Indoor Resting Density An. funestus s.l., Katete District
Indoor resting density (mean no/house/night)
0 0.5 1 1.5 2 2.5 3 3.5 4
Rainfall (mm)
0 50 100 150 200 250 300
Aug-21 Sep-21 Oct-21 Nov-21 Dec-21 Jan-22 Feb-22 Mar-22 Apr-22 Jun-22
Collection Month-Year
Chilowa (IRS site)
Robert (Control site)
Rainfall


5H: Indoor Resting Density An. gambiae s.l., Katete District
Indoor resting density (mean no/house/night)
0 0.05 0.1 0.15 0.2 0.25
Rainfall (mm)
0 50 100 150 200 250 300
Aug-21 Sep-21 Oct-21 Nov-21 Dec-21 Jan-22 Feb-22 Mar-22 Apr-22 Jun-22
Collection Month-Year
Chilowa (IRS site)
Robert (Control site)
Rainfall


5I: Indoor Resting Density An. funestus s.l., Serenje District
Indoor resting density (mean no/house/night)
0 1 2 3 4 5 6 7
Rainfall (mm)
0 50 100 150 200 250 300 350
Aug-21 Sep-21 Oct-21 Nov-21 Dec-21 Jan-22 Feb-22 Mar-22
Collection Month-Year
Chibobo (IRS site)
Chishi (Control site)
Rainfall


5J: Indoor Resting Density An. gambiae s.l., Serenje District
Indoor resting density (mean no/house/night)
0 0.05 0.1 0.15 0.2 0.25
Rainfall (mm)
0 50 100 150 200 250 300 350
Aug-21 Sep-21 Oct-21 Nov-21 Dec-21 Jan-22 Feb-22 Mar-22
Collection Month-Year
Chibobo (IRS site)
Chishi (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 7,566 *An. funestus* s.l. (2,653 from sprayed sites and 4,913 from control sites) and 1081 *An. gambiae* s.l. (563 from sprayed sites and 518 from control sites) collected indoors by PSCs. Overall, the proportion of gravid *An. funestus* s.l. mosquitoes were 9.6% and 11.5% in the sprayed and control sites, respectively, while the proportions gravid *An. gambiae* s.l. were 10.1% and 24.3% in the sprayed and control sites, respectively. There were fewer gravid *An. funestus* s.l. and *An. gambiae* s.l. vectors at the sprayed sites compared to the control sites. The differences in mean proportions gravid were statistically significant for *An. gambiae* s.l. (IRR 0.51, p=0.0123) but not for *An. funestus* s.l. (IRR 0.78, p=0.1212).

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 only two periods (T+1 and T+3) with fewer gravid *An. funestus* s.l. vectors at the sprayed sites compared to the control sites while there were fewer gravid *An. gambiae* s.l. for most of the period after IRS (four out of the seven post-IRS months). 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 2021–June 2022)

[Arrow indicates the time IRS was implemented]

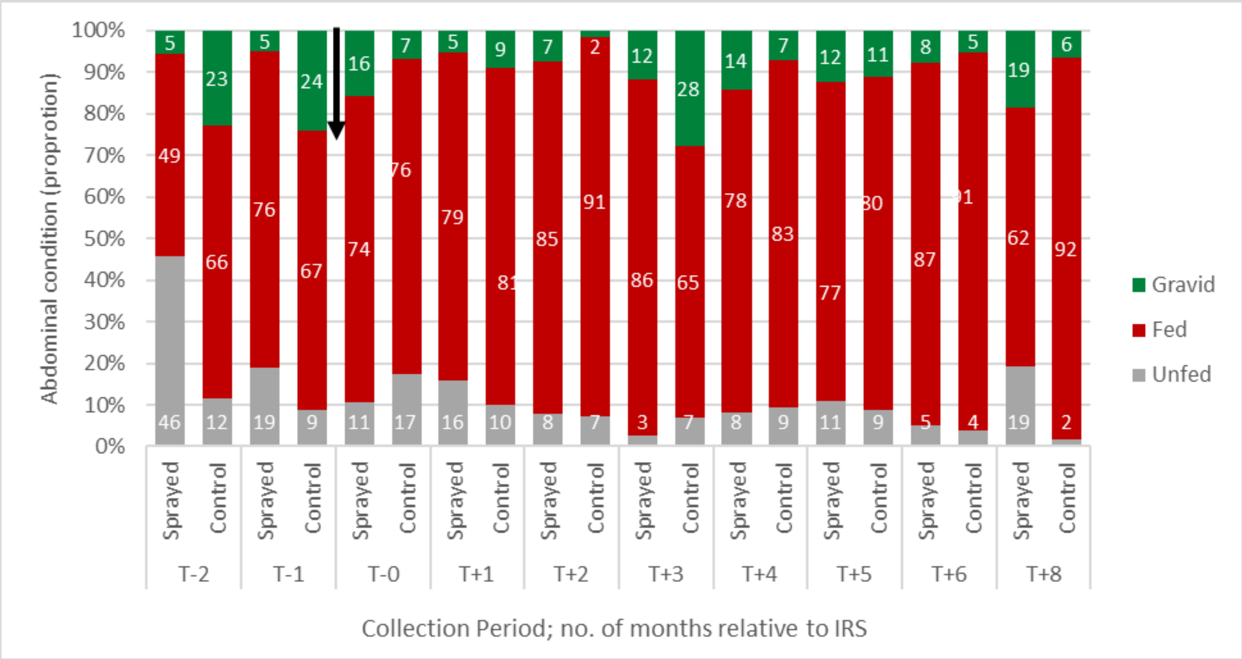
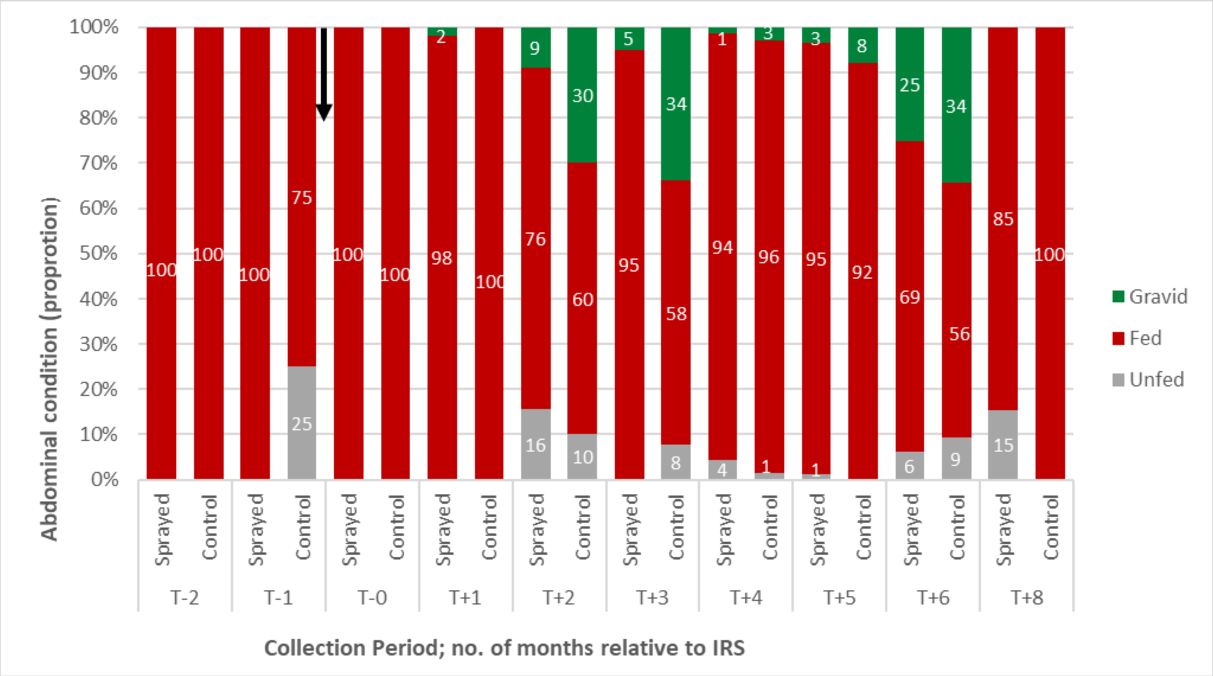


Figure 7: Abdominal Condition of *An. gambiae* s.l. in Intervention and Control Sites Before and After IRS (August 2021–June 2022)

[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 34.1 to 28.6 bites per person per night, or b/p/n; the difference was not statistically significant IRR 0.95, p=0.912). A statistically significant reduction in *An. funestus* s.l. HBR was observed at sprayed sites after IRS (33.2 to 27.5 b/p/n, IRR 0.76 p<0.001), while an increase was observed at the control sites (29.4 to 35.3 bites, not statistically significant). The overall biting rate of *An. gambiae* s.l. at sprayed sites (7.0 b/p/n) was slightly lower than control sites (7.4 b/p/n). There were significantly more *An. gambiae* s.l. bites after IRS than before IRS at combined sprayed sites (8.4 versus 1.5 b/p/n, p<0.001) as well as combined control sites (9.1 versus 0.7 b/p/n, p<0.001).

There were fewer *An. funestus* s.l. bites at the sprayed sites compared to the control sites in five of the seven districts (Nchelenge District Figure 8A, Milenge District-Figure 8C, Katete District Figure 8G, Serenje District-Figure 8I, and Chililabombwe District-Figure 8M). [p-values]. *An. funestus* s.l. biting rates were higher at the sprayed sites compared to control sites in Lufwanyama District (Figures 8K,) The differences were statistically significant in four districts Milenge (p<0.001), Katete (p<0.001), Serenje (p<0.001), and Chililabombwe (p=0.015). *An. funestus* s.l. biting rates were higher at the sprayed sites compared to control sites in Mambwe District (no significant difference p=0.91) and Lufwanyama District (significant difference p=0.028) (Figures 8E and 8K respectively).

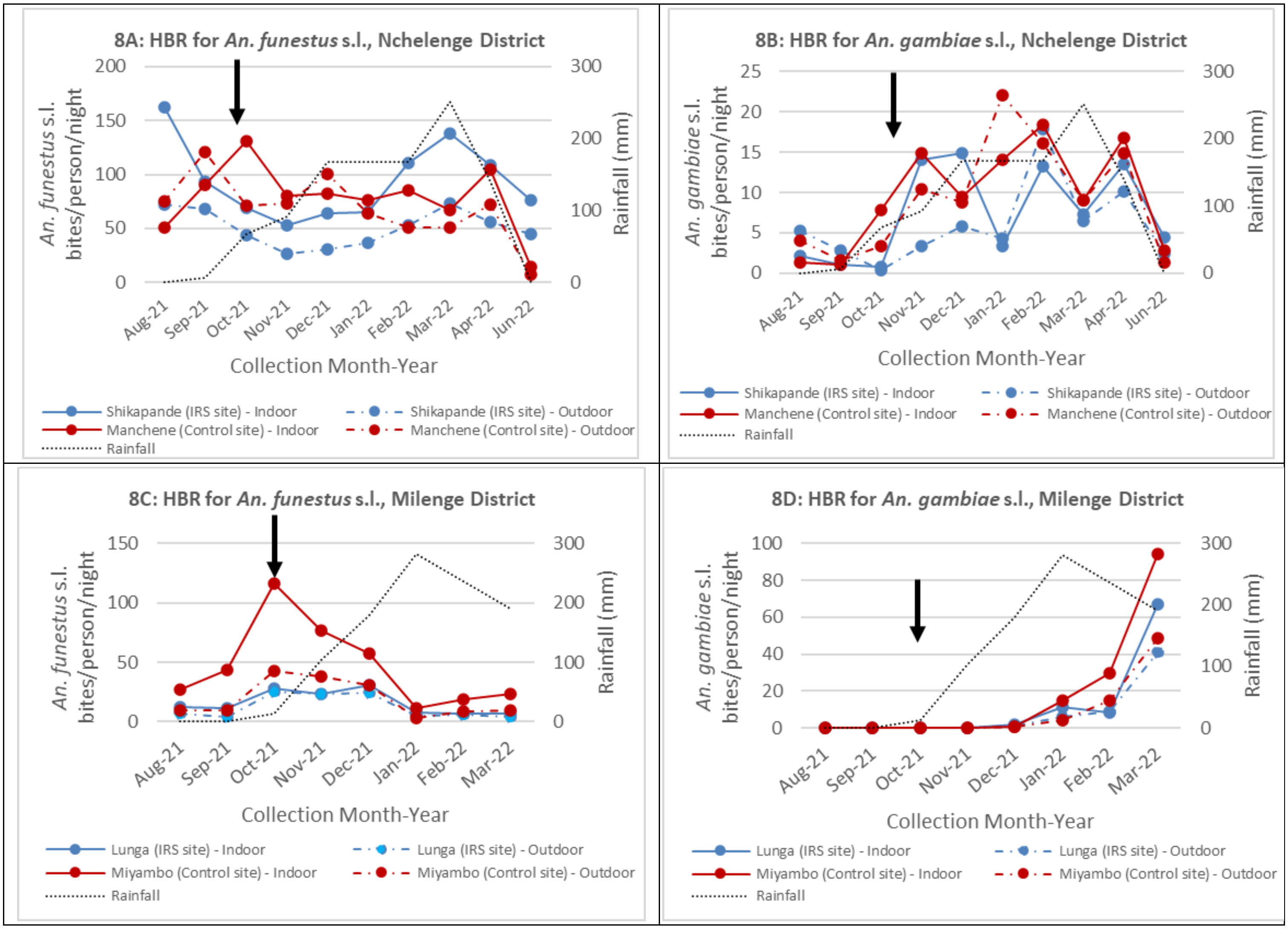
Post-IRS *An. funestus* s.l. biting rates were significantly lower than pre-IRS rates at two of the seven IRS sites (Shikapande in Nchelenge District (197.5 to 130.8 b/p/n), and Chiloba in Katete District (0.13 to 0.05 b/p/n), while it was significantly higher for the remainder of the sprayed sites. The increases in *An. funestus* s.l. biting rates at the control sites ranged from 0.74 folds (Chasela, Mambwe District) to 9.7 folds (Mainasoko, Chililabombwe District) while at the sprayed sites it ranged from 0.38 folds (Chiloba, Katete District) to 11.33 folds (Chibobo, Serenje District).

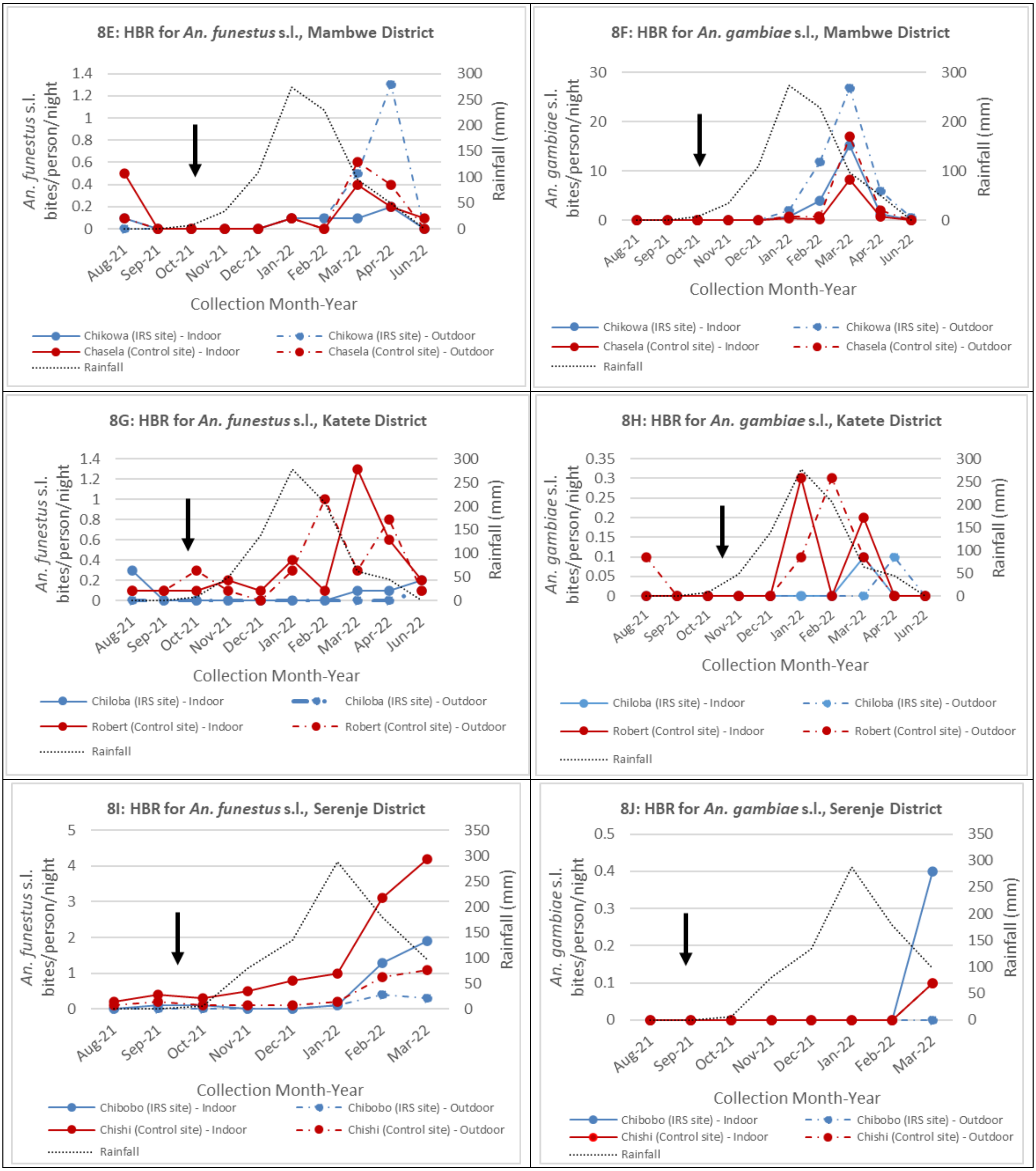
An. gambiae s.l. biting rates in sprayed sites were lower than control sites in four of the seven districts: Nchelenge (13.3 versus 18.7 b/p/n), Milenge (18.4 versus 26.13), Katete (0.02 versus 0.1), and Chililabombwe (1.77 versus 3.31). The differences were significant at p=0.05 in Milenge, Katete, and Chililabombwe).

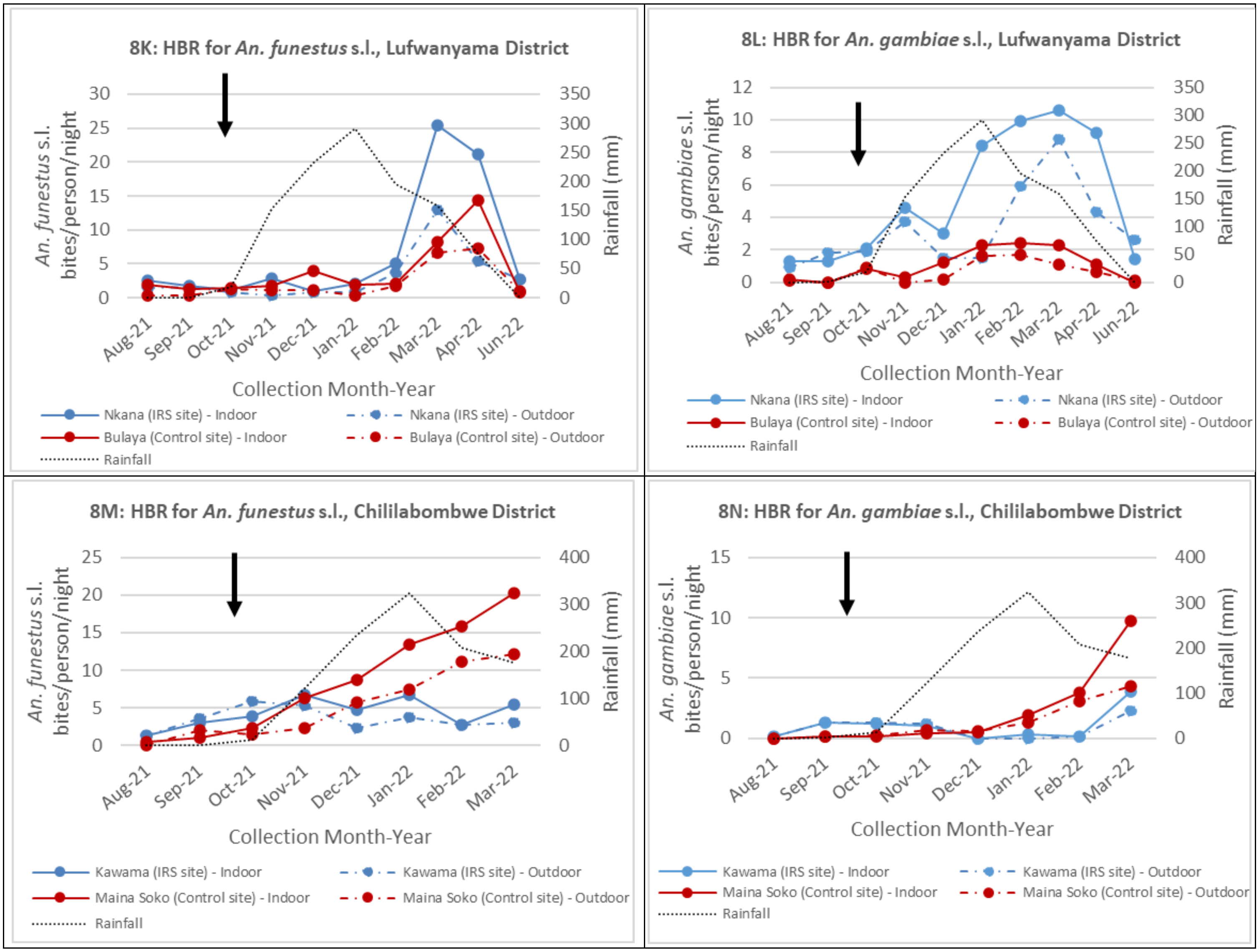
Post-IRS *An. gambiae* s.l. biting rates were higher than pre-IRS at all sprayed and control sites (at p=0.05) except at sites where it was not possible to calculate IRR (both sites in Mambwe District and Serenje District and the sprayed site in Katete District. The observed increases in *An. gambiae* s.l. densities after IRS at the control sites ranged from 0.04 folds (Chishi, Serenje District) to 497.7 folds (Miyambo, Milenge District) while at the sprayed sites, it ranged from 0.02 (Chilowa, Katete District) to 350.8 folds (Linga, Milenge District) (see detailed statistical output in Annex C-III).

Figure 8: Human Biting Rates of *An. funestus* s.l. and *An. gambiae* s.l. (August 2021-June 2022)

[Arrow indicates the time IRS was implemented]




8E: HBR for *An. funestus* s.l., Mambwe District
An. funestus s.l. bites/person/night
Rainfall (mm)
Collection Month-Year
Aug-21 Sep-21 Oct-21 Nov-21 Dec-21 Jan-22 Feb-22 Mar-22 Apr-22 Jun-22
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)
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)
Collection Month-Year
Aug-21 Sep-21 Oct-21 Nov-21 Dec-21 Jan-22 Feb-22 Mar-22
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)
Collection Month-Year
Chibobo (IRS site) - Indoor
Chibobo (IRS site) - Outdoor
Chishi (Control site) - Indoor
Chishi (Control site) - Outdoor
Rainfall



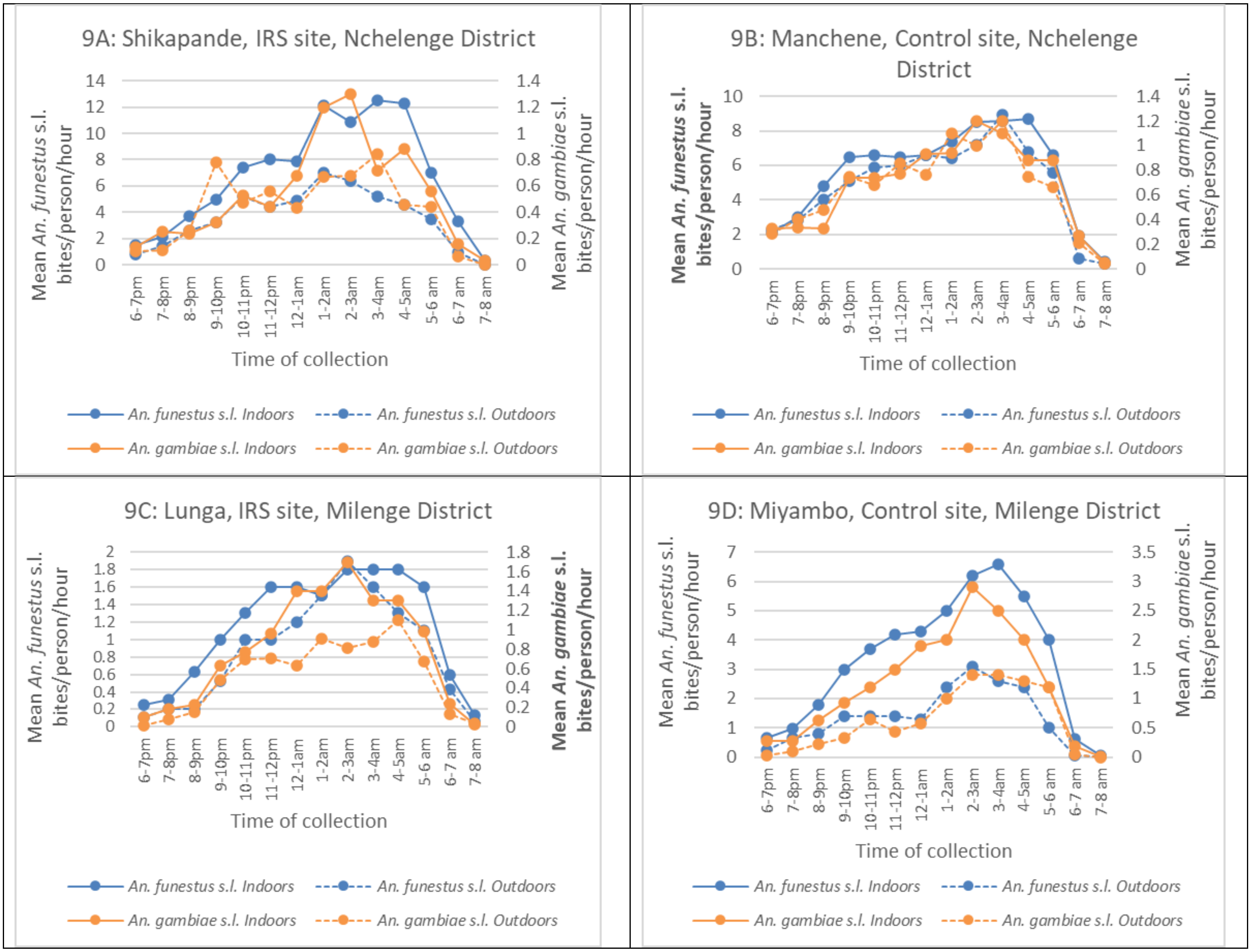
3.1.5 *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. FEEDING LOCATION AND BITING TIME

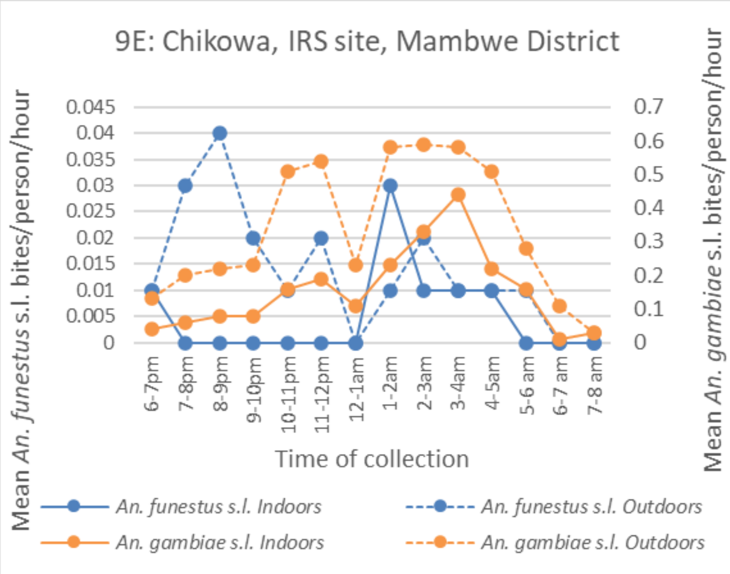
The feeding location (indoors or outdoors) and biting times for *An. funestus* s.l. and *An. gambiae* s.l. mosquitoes for all sentinel sites are presented in Figure 9. There was more indoor biting than outdoor biting for both *An. funestus* s.l. and *An. gambiae* s.l. in all districts except Mambwe. In Mambwe, there were more outdoor bites for both species (0.2 versus 0.1 b/p/n for *An. funestus* s.l., p=0.2937, and 3.4 versus 1.5 b/p/n for *An. gambiae* s.l., p<0.0001). Indoor *An. funestus* s.l. and *An. gambiae* s.l. bites were significantly higher than outdoor bites in four districts (Nchelenge, Milenge, Lufwanyama, and Chililabombwe). At the site level, only one site (Chikowa in Mambwe District) had more outdoor than indoor *An. funestus* s.l. bites (p<0.0001), while four sites had more outdoor than indoor *An. gambiae* s.l. bites; the difference was statistically significant in two sites (Chasela p=0.008 and Chikowa p<0.0001, both in Mambwe District) and not statistically significant in Robert-Katete District or Miyambo-Milenge District. All other sites had more biting indoors than outdoors. The differences were statistically significant for *An. funestus* s.l. at eight out of the 13 sites and for *An. gambiae* s.l. at four out of eight sites. See statistical output in Annex C-IV.

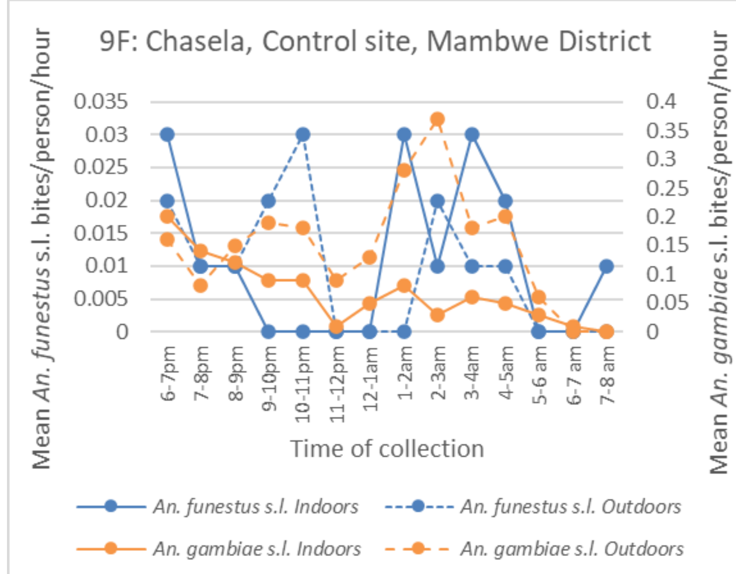
and one late at night around 1-3 a.m. (Figure 9E, F, I, and J). In Katete District, we observed multiple peaks throughout the night.

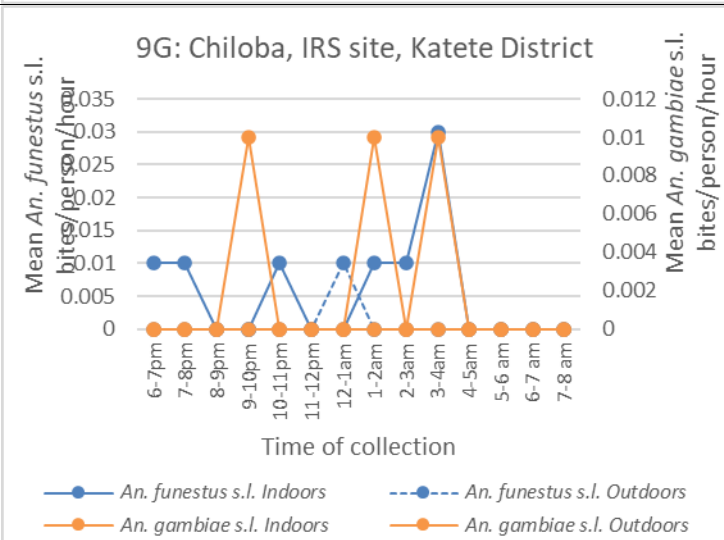
Figure 9: *An. funestus* s.l. and *An. gambiae* s.l. Biting Times and Location by Site (August 2021-June 2022)

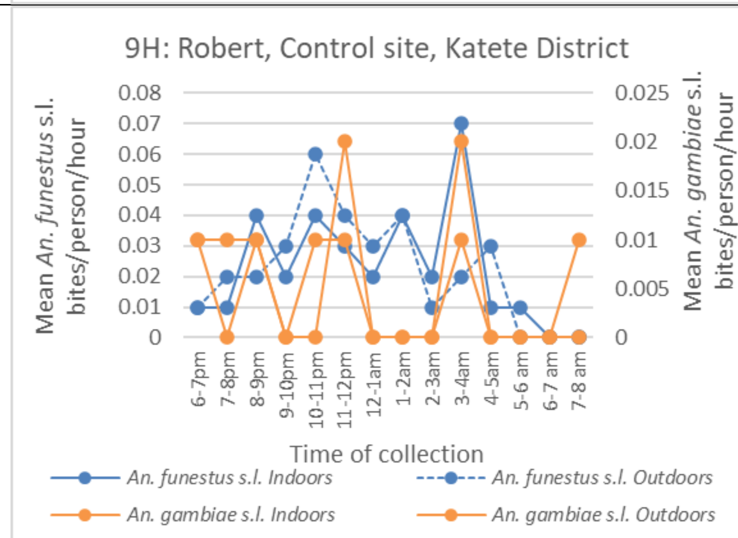
[Primary Axis = *An. funestus* s.l.; Secondary Axis = *An. gambiae* s.l.]

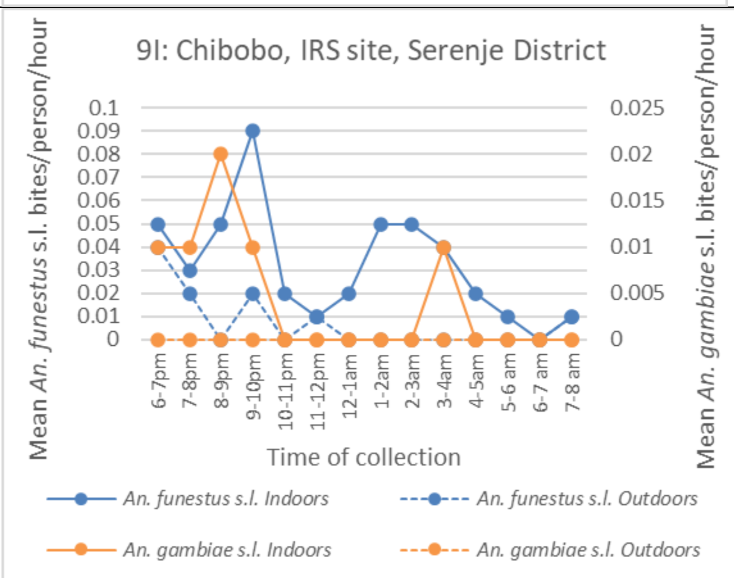


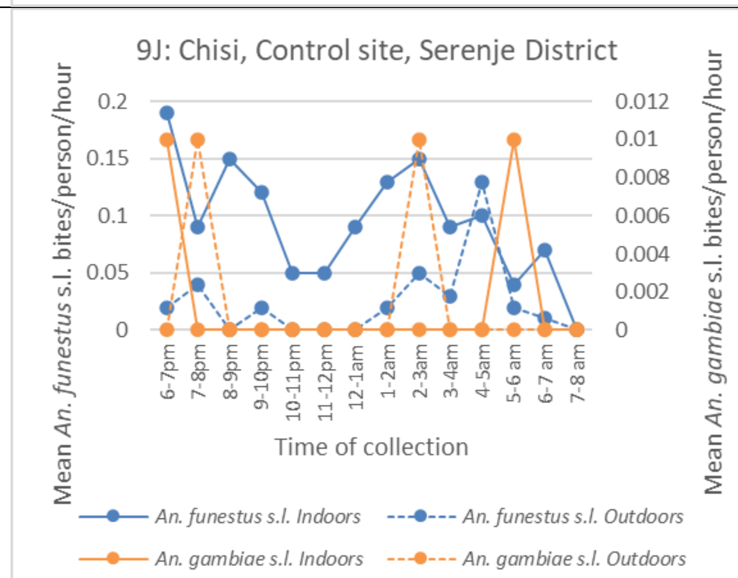

9E: Chikowa, IRS site, Mambwe District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
6-7pm 7-8pm 8-9pm 9-10pm 10-11pm 11-12pm 12-1am 1-2am 2-3am 3-4am 4-5am 5-6 am 6-7 am 7-8 am
Time of collection
An. funestus s.l. Indoors
An. funestus s.l. Outdoors
An. gambiae s.l. Indoors
An. gambiae s.l. Outdoors

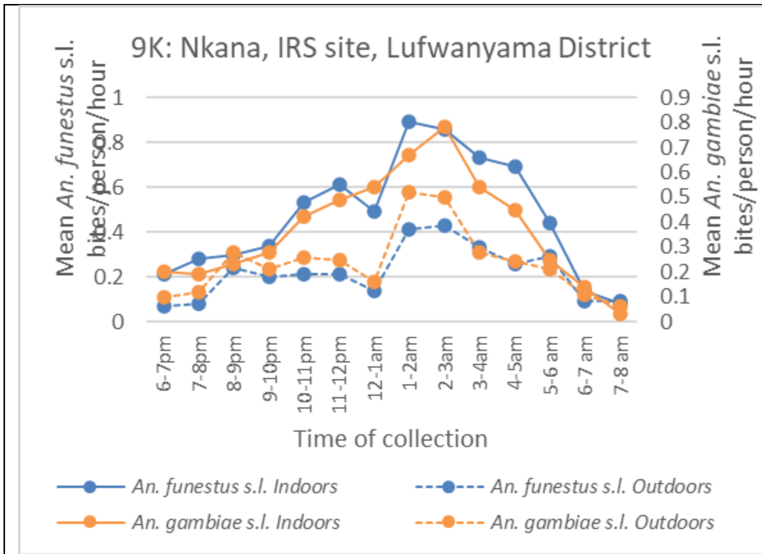

9F: Chasela, Control site, Mambwe District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
6-7pm 7-8pm 8-9pm 9-10pm 10-11pm 11-12pm 12-1am 1-2am 2-3am 3-4am 4-5am 5-6 am 6-7 am 7-8 am
Time of collection
An. funestus s.l. Indoors
An. funestus s.l. Outdoors
An. gambiae s.l. Indoors
An. gambiae s.l. Outdoors

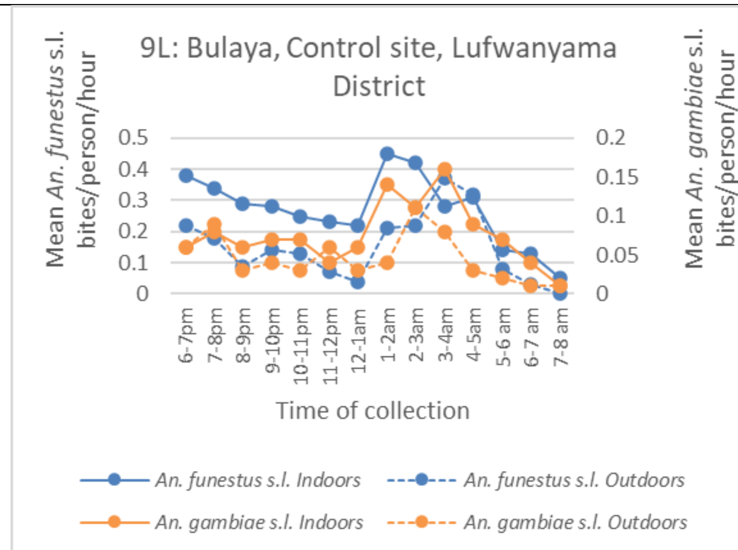

9G: Chiloba, IRS site, Katete District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
6-7pm 7-8pm 8-9pm 9-10pm 10-11pm 11-12pm 12-1am 1-2am 2-3am 3-4am 4-5am 5-6 am 6-7 am 7-8 am
Time of collection
An. funestus s.l. Indoors
An. funestus s.l. Outdoors
An. gambiae s.l. Indoors
An. gambiae s.l. Outdoors

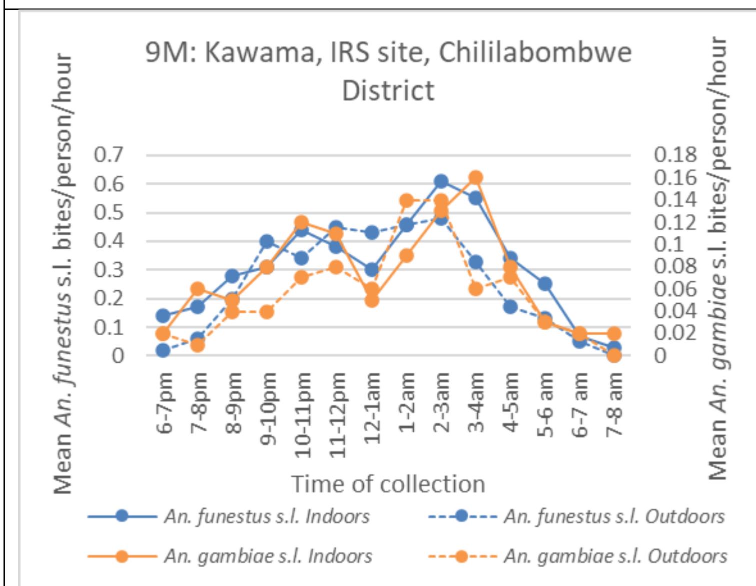

9H: Robert, Control site, Katete District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
6-7pm 7-8pm 8-9pm 9-10pm 10-11pm 11-12pm 12-1am 1-2am 2-3am 3-4am 4-5am 5-6 am 6-7 am 7-8 am
Time of collection
An. funestus s.l. Indoors
An. funestus s.l. Outdoors
An. gambiae s.l. Indoors
An. gambiae s.l. Outdoors

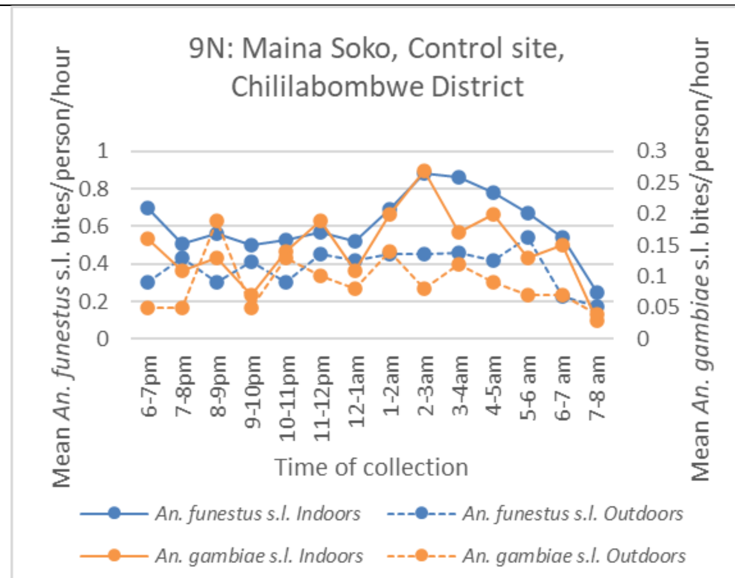

9I: Chibobo, IRS site, Serenje District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
6-7pm 7-8pm 8-9pm 9-10pm 10-11pm 11-12pm 12-1am 1-2am 2-3am 3-4am 4-5am 5-6 am 6-7 am 7-8 am
Time of collection
An. funestus s.l. Indoors
An. funestus s.l. Outdoors
An. gambiae s.l.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


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. Indoors
An. funestus s.l. Outdoors
An. gambiae s.l. Indoors
An. gambiae s.l. Outdoors


9K: Nkana, IRS site, Lufwanyama District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
0
0.2
0.4
0.6
0.8
1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
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. Indoors
An. funestus s.l. Outdoors
An. gambiae s.l. Indoors
An. gambiae s.l. Outdoors


9L: Bulaya, Control site, Lufwanyama District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
0
0.1
0.2
0.3
0.4
0.5
0
0.05
0.1
0.15
0.2
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. Indoors
An. funestus s.l. Outdoors
An. gambiae s.l. Indoors
An. gambiae s.l. Outdoors


9M: Kawama, IRS site, Chililabombwe District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
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. Indoors
An. funestus s.l. Outdoors
An. gambiae s.l. Indoors
An. gambiae s.l. Outdoors


9N: Maina Soko, Control site, Chililabombwe District
Mean An. funestus s.l. bites/person/hour
Mean An. gambiae s.l. bites/person/hour
0
0.2
0.4
0.6
0.8
1
0
0.05
0.1
0.15
0.2
0.25
0.3
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. Indoors
An. funestus s.l. Outdoors
An. gambiae s.l. Indoors
An. gambiae s.l. Outdoors

3.1.6 PARITY RATES

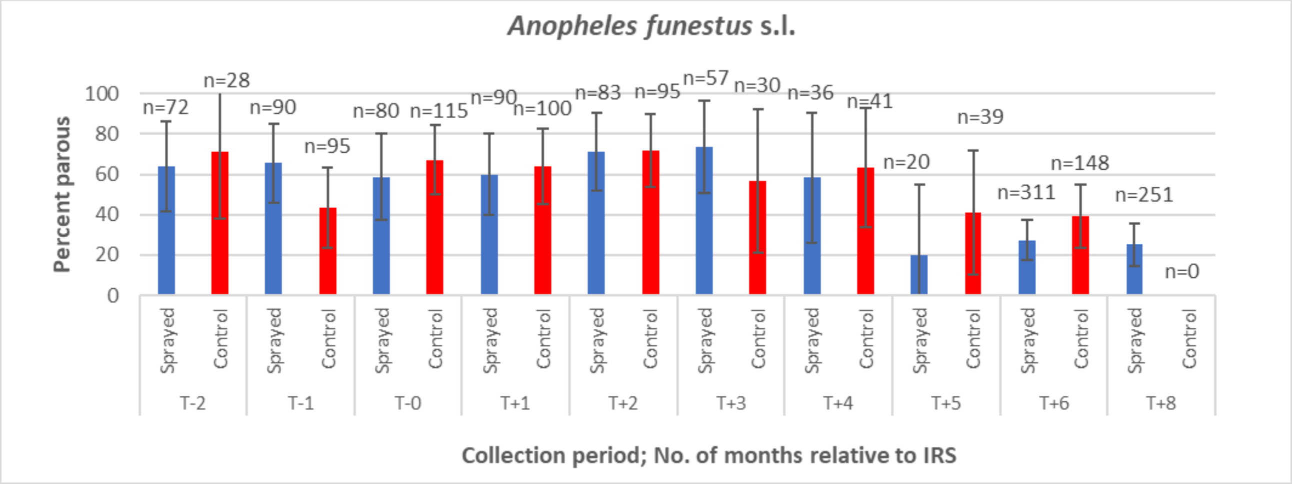
A total of 2,894 unfed female *An. funestus* s.l. and 1,545 *An. gambiae* s.l. collected by HLCs were examined for parity status (SOP 10/01) during the reporting period. Overall parity rates for *An. funestus* s.l. and *An. gambiae* s.l. were 42.3% and 39.6%, respectively. *An. funestus* s.l. parity rate at combined sprayed sites was 39.7% (638/1607) and at combined control sites was 45.6% (587/1,287). While for *An. gambiae* s.l. parity rate was 45.1% (395/1065) at combined sprayed sites and 45.2% (217/480) at the combined control sites. Mean parity rate was significantly lower at the combined sprayed sites compared to the combined control sites for both species (*An. funestus* s.l. - IRR 0.82, p=0.017; *An. gambiae* s.l. - IRR 0.76, p=0.0102). Mean parity for *An. funestus* s.l. was significantly lower after IRS compared to before IRS at the combined sprayed sites (50.0% versus 39.4%; IRR 0.65; p<0.0001) while mean parity was increased after IRS at the combined control sites (52.5% versus 54.3%, p=0.721). Mean parity for *An. gambiae* s.l. was significantly higher after IRS at the combined sprayed sites 13.1% versus 36.2% IRR 3.02 p=0.0436) but significantly lower at the combined control sites 100% versus 47.2% IRR 0.48, P<0.0001.

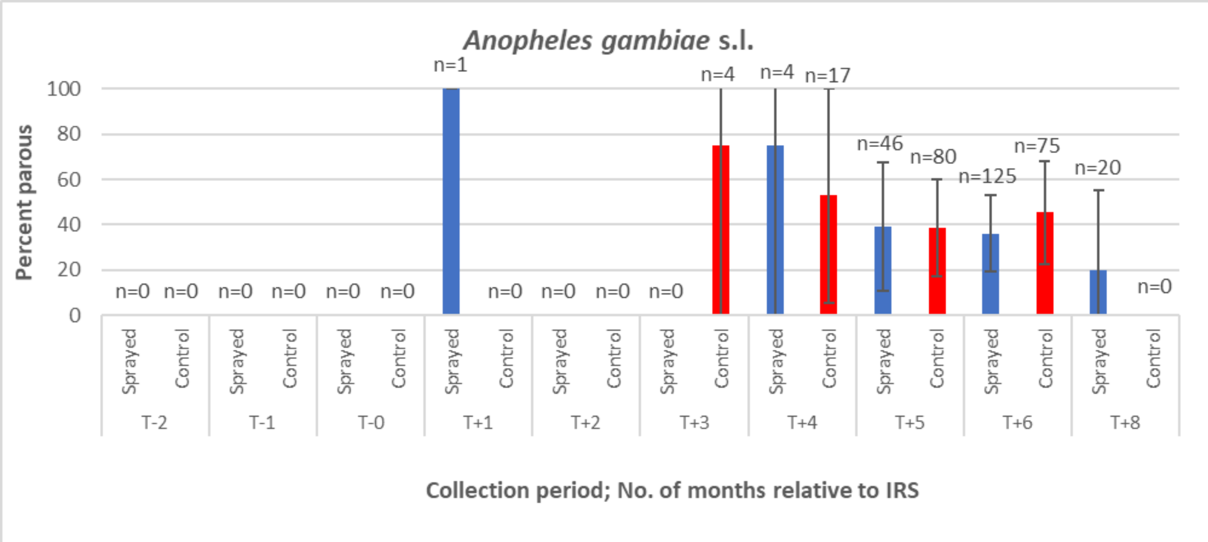
Figure 10 is a panel of monthly parity rates for *An. funestus* s.l. and *An. gambiae* s.l. comparing sprayed and control sites for each of the months before and after IRS. All districts from the same province have been combined in this report. Serenje District (Central Province) has been excluded from 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 fewer parous *An. funestus* s.l. and *An. gambiae* s.l. vectors at sprayed sites compared to control sites in Luapula Province (44% versus 56% and 36% versus 44% respectively) and in Eastern Province (55% versus 72% and 48% versus 57% respectively). In Copperbelt Province, the overall proportion of parous vectors was similar between combined sprayed and combined control sites for *An. funestus* s.l. (29.6 versus 30.9%) and *An. gambiae* s.l. (26.0 versus 25.2%). Mean *An. funestus* s.l. parity rates were significantly lower after IRS at three sprayed sites Shikapande and Lunga in Luapula Province and Kawama in Copperbelt Province). The numbers of *An. gambiae* s.l. examined were insufficient to compare pre- versus post-IRS for most sites. See statistical output in Annex C-V.

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 2021-June 2022)

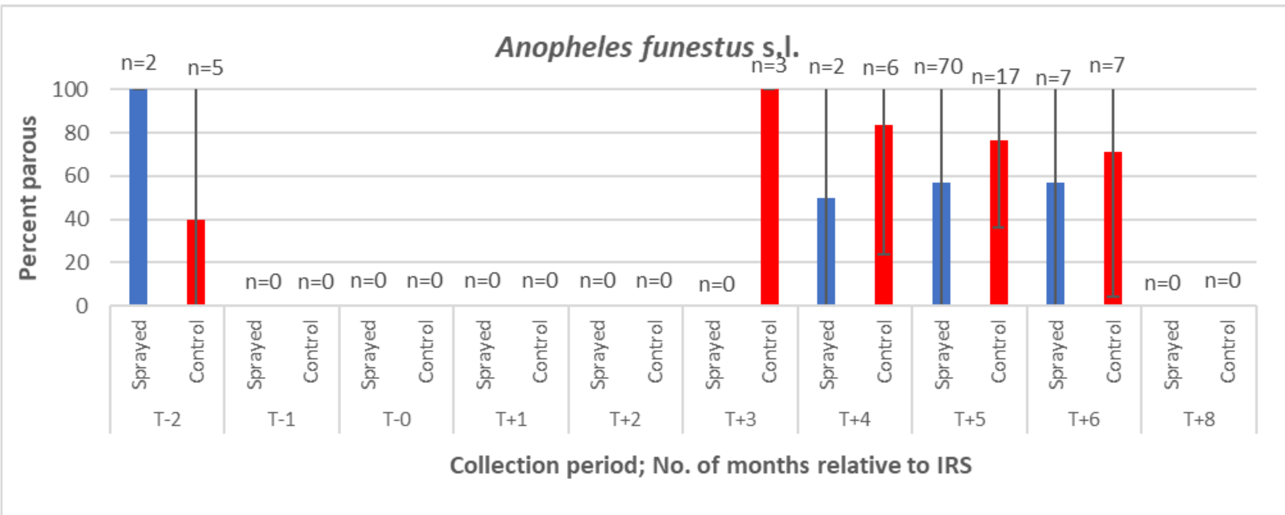
[Bars with 95% confidence intervals. n= total samples examined]

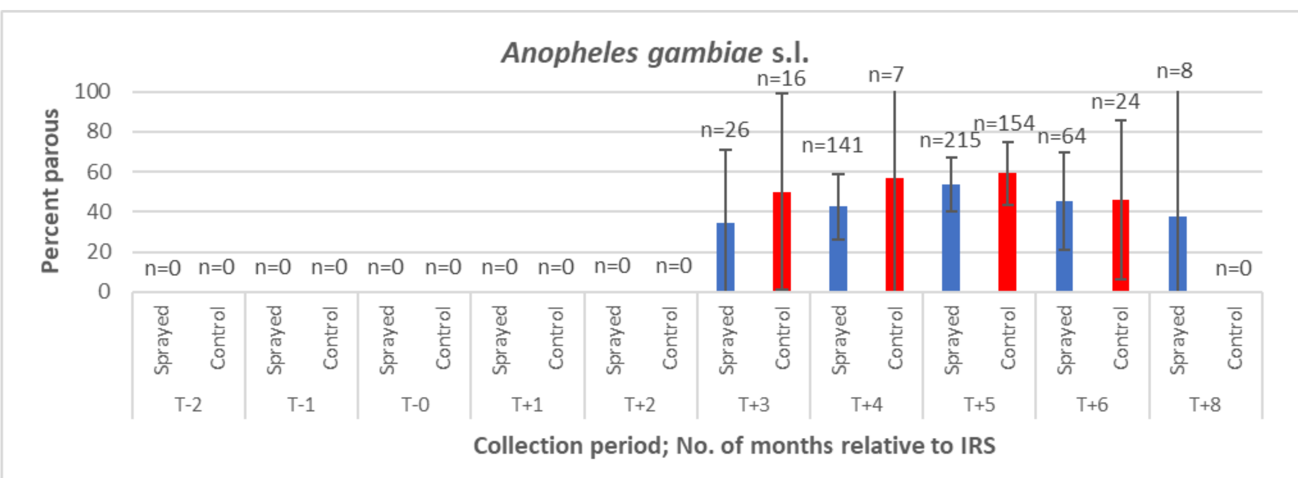
10A: Luapula Province: Parity Rates of *An. funestus* s.l. and *An. gambiae* s.l. in Sprayed and Control Sites



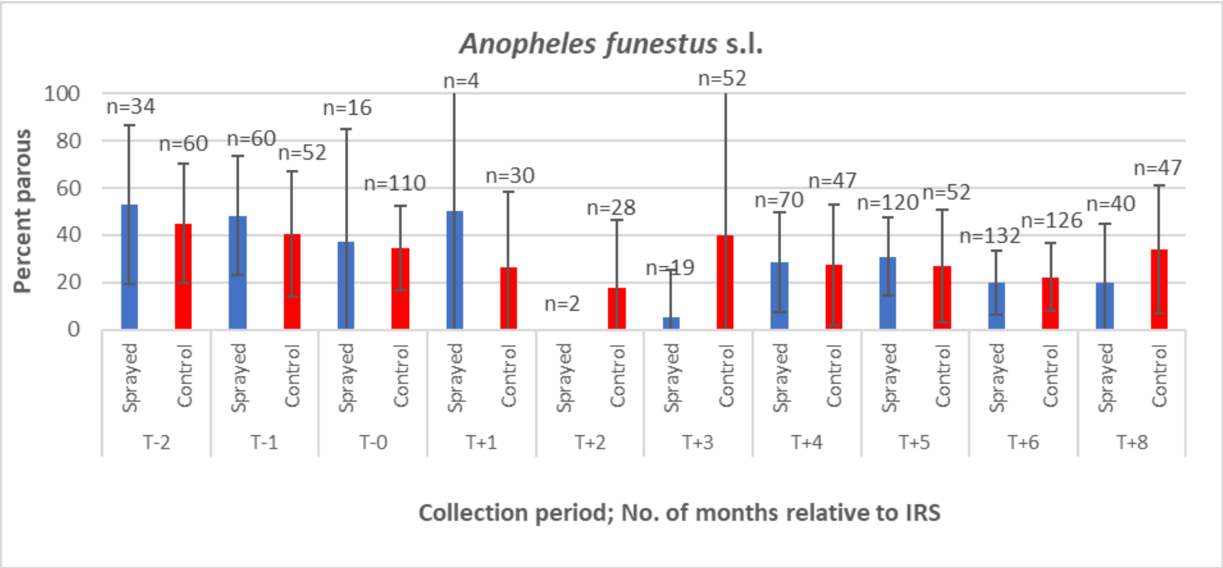


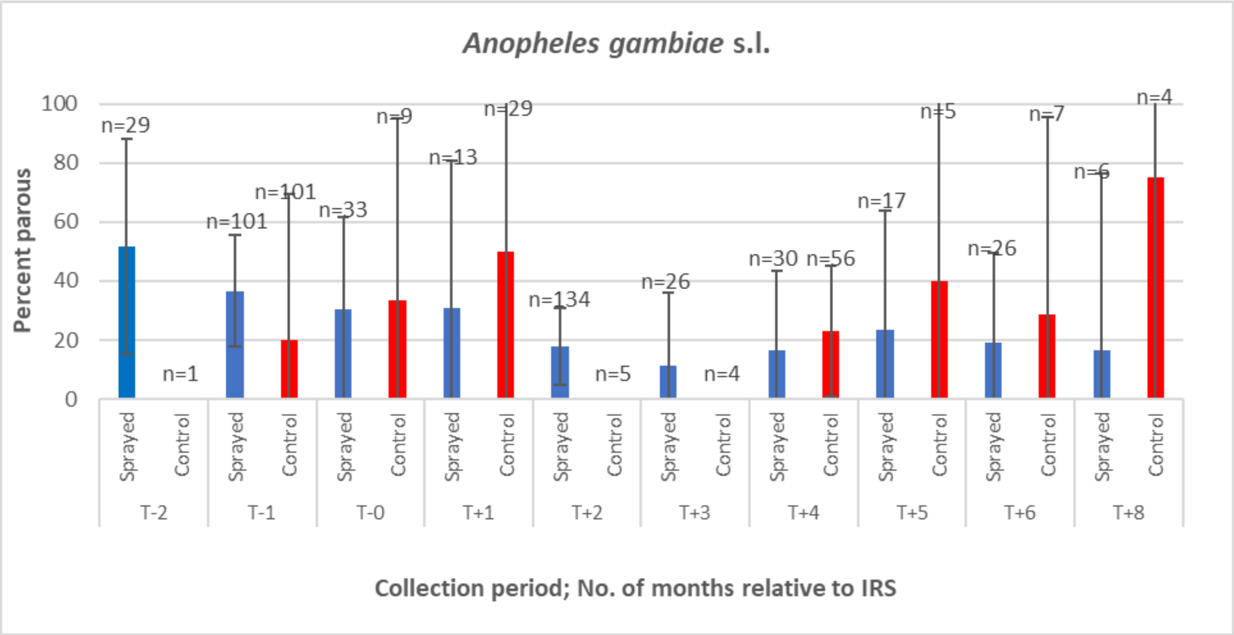
10B: Eastern Province: Parity Rates of *An. funestus* s.l. and *An. gambiae* s.l. in Sprayed and Control Sites





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





3.2 Laboratory Results

VectorLink staff were responsible for the analysis of all samples, and there is no longer the need to split samples for analysis by other parties. We have been able to analyze samples within the projected 2-month time lag from the date of sample collection. Data presented here includes samples analyzed up to June 2022 collections. PCR was successfully done on a total of 1630 samples for species ID and 165 samples for bloodmeal while sporozoite ELISAs were done on a total of 9,524 samples.

3.2.1 PCR Identification of *An. gambiae* s.l. and *An. funestus* s.l. Species and *kdr* Alleles

Of the 1,379 *An. funestus* s.l. and 674 *An. gambiae* s.l. tested by PCR, 1,250 and 648 successfully amplified, respectively. There has been a marked improvement in specimen amplification rate since the 2019/20 and 2020/21 annual reports due to some of the changes effected to optimize the laboratory process—amplification for *An. funestus* s.l. increased from 31% in 2020 to 46% in 2021 and to 91% this reporting period, while the *An. gambiae* s.l. amplification rate increased from 32% in 2020 to 65% in 2021, and to 96% this reporting period. VectorLink laboratory staff are working with the NMEP in implementing these optimized protocols.

The majority of *An. funestus* s.l. that were tested successfully were *An. funestus* s.s. (99.1%) with a few *An. leesoni* (0.9%). Most of the *An. gambiae* s.l. that amplified were *An. gambiae* s.s. (71.9%); the remainder were *An. arabiensis* (29.1%). Table 6 shows the distribution of the different molecular species of *An. gambiae* s.l. and *An. funestus* s.l. vectors by district for the reporting period. *An. leesoni* was found in Luapula and Copperbelt Provinces while *An. arabiensis* was found in Copperbelt and Eastern Provinces.

Table 6: Molecular Identification of *An. gambiae* s.l. and *An. funestus* s.l. Collected from Sentinel Districts (August 2021-June 2022)

	An. funestus s.l.			
District	Total tested	Total amplified	*An. funestus* s.s.	*An. leesoni*
Nchelenge	552	542	532	10
Milenge	220	217	217	
Serenje	71	55	55	
Lufwanyama	189	144	143	1
Chililabombwe	347	292	292	
Total	**1,379**	**1,250**	**1,239**	**11**
		% of Total Amplified	**99.1**	**0.9**
District	*An. gambiae* s.l.			
	Total tested	Total amplified	*An. gambiae* s.s.	*An. arabiensis*
Nchelenge	91	91	91	
Milenge	88	88	88	
Mambwe	180	180	1	179
Lufwanyama	259	238	236	2
Chililabombwe	56	51	50	1
Total	**674**	**648**	**466**	**182**
		% of Total Amplified	**71.9**	**28.1**

A total of 71 *An. arabiensis* samples (22 pyrethroid resistant and 49 pyrethroid susceptible) were tested for the presence of *kdr*-east and *kdr*-west alleles. No *kdr*-east or *kdr*-west alleles were detected among the numbers that successfully amplified (46 and 56, respectively). A total of 14 *An. gambiae* s.s. samples (1 pyrethroid resistant and 13 pyrethroid susceptible) were tested. None amplified for the *kdr*-east tests while all amplified for *kdr*-

west tests, and both *kdr*-east and *kdr*-west alleles were absent. Note that we are currently optimizing these processes using modified procedures by Huynh Lynn.[12]

3.2.2 Sporozoite Infectivity Rates and Entomological Inoculation Rates

A total of 6,600 *An. funestus* s.l. and 2,895 *An. gambiae* s.l. collected from both sprayed and control sites were tested for *Plasmodium* circumsporozoite proteins. The sporozoite rate for the two species were 1.95% and 1.35%, respectively. Sporozoite rates were lower at the combined sprayed sites compared to the combined control sites; 1.12% versus 2.59% for *An. funestus* s.l. and 0.51% versus 2.32% for *An. gambiae* s.l., respectively. At the district level, sporozoite rates for *An. funestus* s.l. and *An. gambiae* s.l. were lower at sprayed sites compared to control sites in all seven surveillance districts (note that no sporozoite positives were found in sprayed and control sites for *An. funestus* s.l. in Serenje and *An. gambiae* s.l. in Katete). This is a marked improvement over last year when only three districts had lower sporozoite rates for *An. funestus* s.l. and *An. gambiae* s.l. at the sprayed sites compared to the control sites (Fig 11A and 11B).

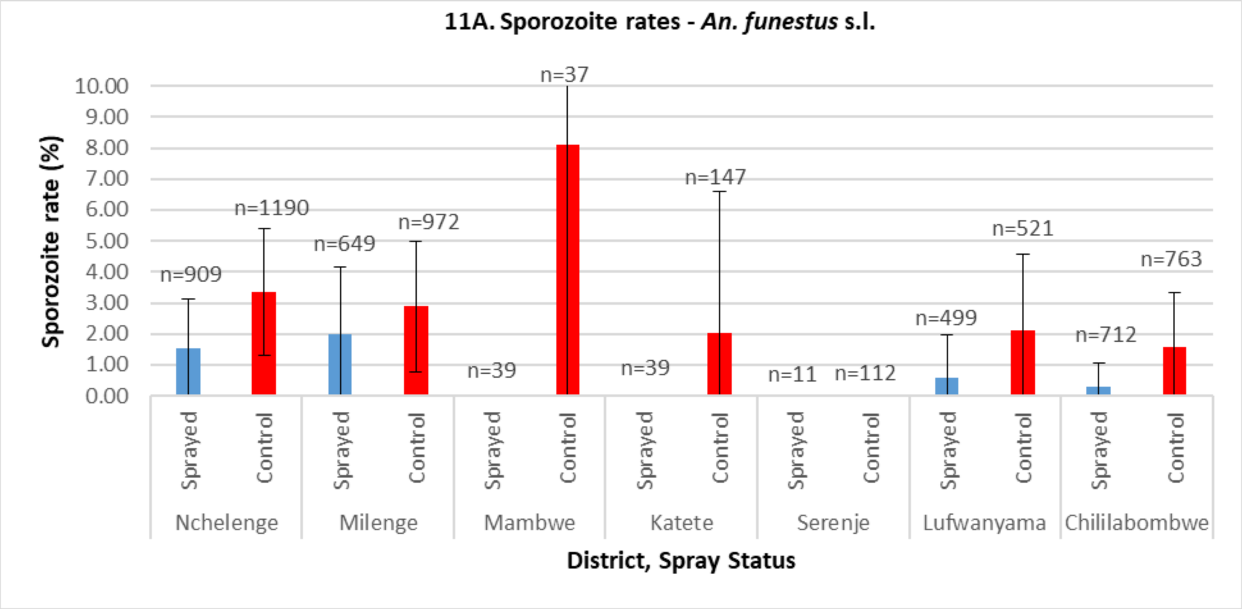
Overall, average monthly EIRs for the two primary vectors were lower at the sprayed sites compared to the control sites: 9.7 versus 27.1 infective bites per person per month for *An. funestus* s.l. and 1.1 versus 5.1 infective bites per person per month for *An. gambiae* s.l. At the district level, EIRs were lower at the sprayed sites in six of the seven districts (with Serenje having no sporozoite positive *An. funestus* s.l. mosquitoes in both the sprayed and control sites and Katete District with no sporozoite positive *An. gambiae* s.l. at both sprayed and control sites. Average EIRs for *An. funestus* s.l. ranged from 0 to 68 infective bites per person month at sprayed sites and from 0 to 153 at control sites, while average EIRs for *An. gambiae* s.l. ranged from 0 to 5.7 infective bites per person month at sprayed sites and from 0 to 14 at control sites (Figures 11C and 11D).

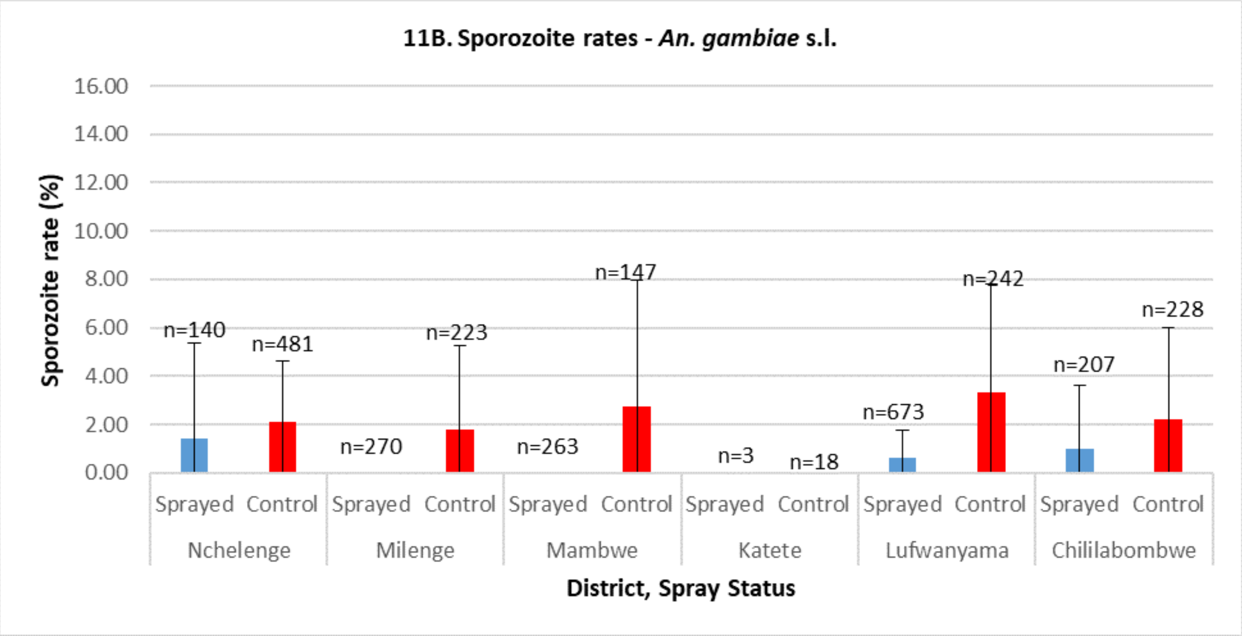
A total of 1,010 of the most abundant non vector *An. ziemanni* (150 from Chililabombwe District, 360 from Lufwanyama District and 500 from Milenge District) were tested by ELISA (in batches of 10 specimens per test) for the presence of *Plasmodium falciparum* sporozoites. All specimens were negative for *P. falciparum* sporozoites.

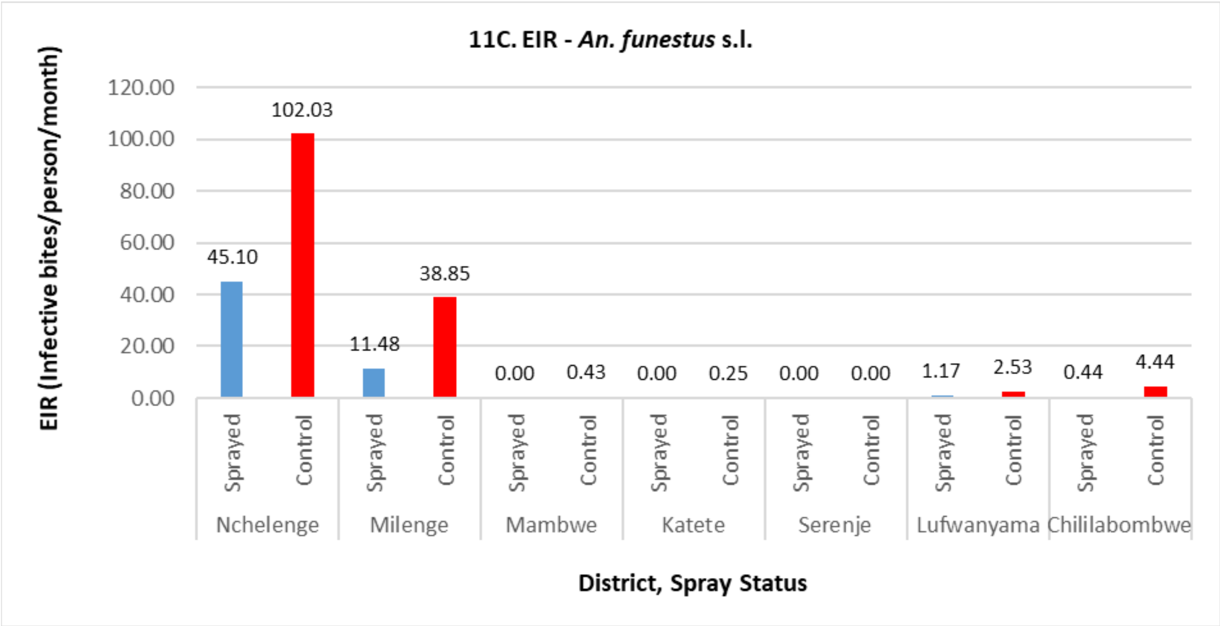
[12] Huynh LY, Sandve SR, Hannan LM, Van Ert M, Gimnig JE (2007) Fitness costs of pyrethroid insecticide resistance in *Anopheles gambiae*. In: Annual Meeting of the Society for the Study of Evolution, Christchurch, New Zealand

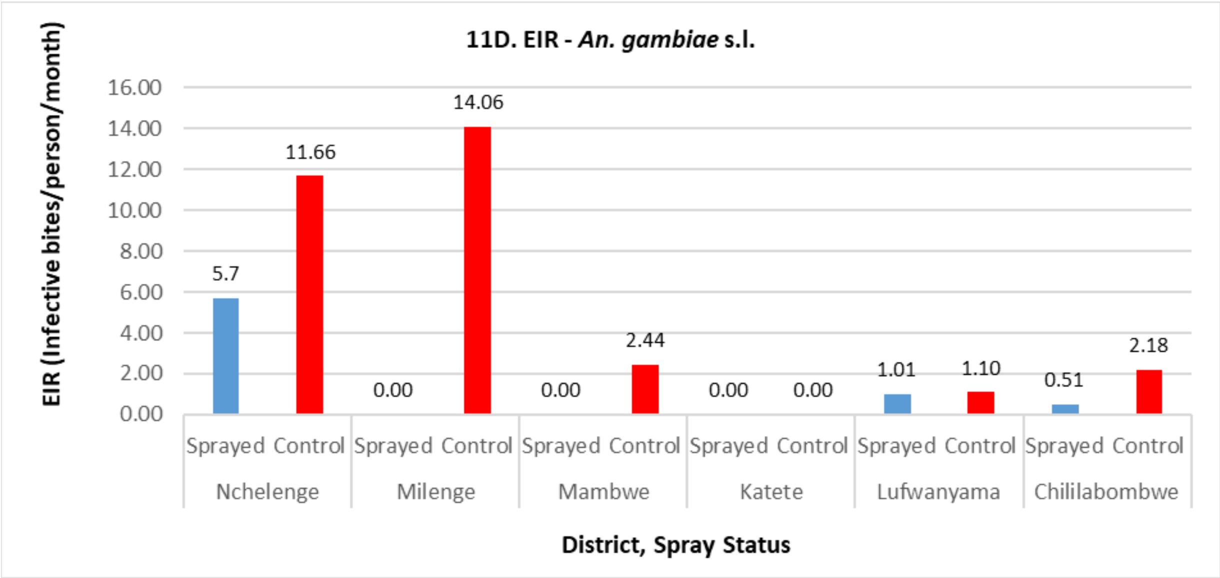
Figure 11: *An. funestus* s.l. and *An. gambiae* s.l. Sporozoite Infection Rates (A and B) and Entomological Inoculation Rates (C and D) at Sprayed and Control Sites by District and Spray Status (August 2021-June 2022)

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





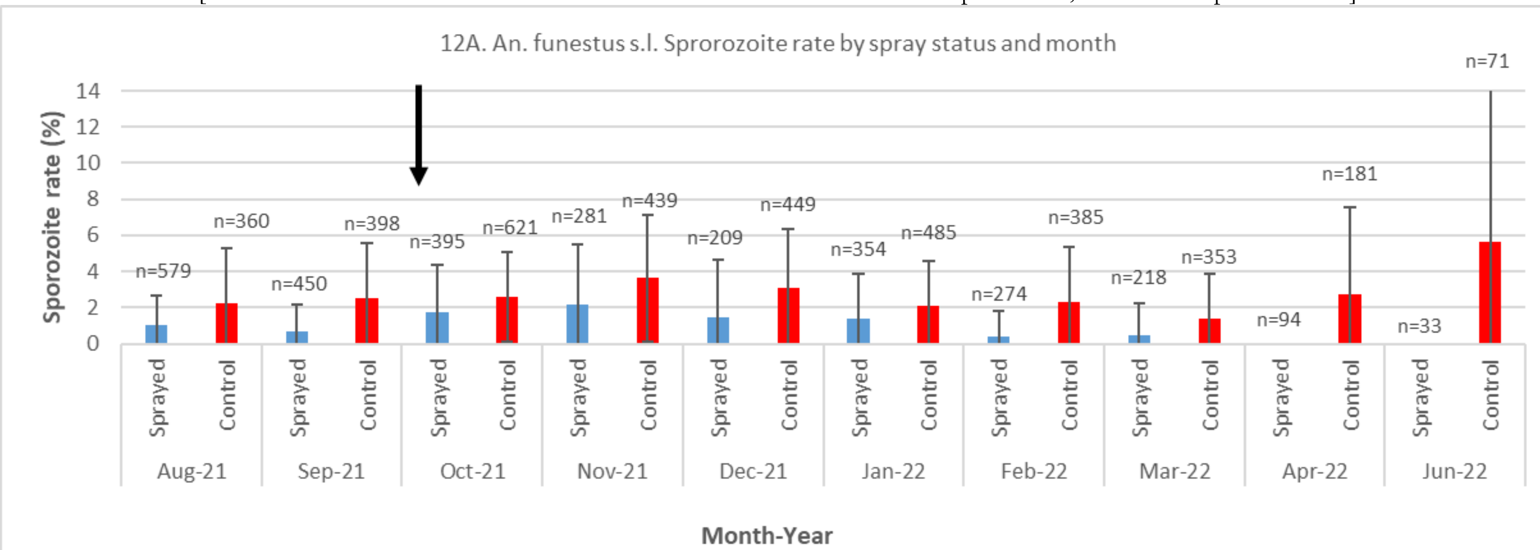


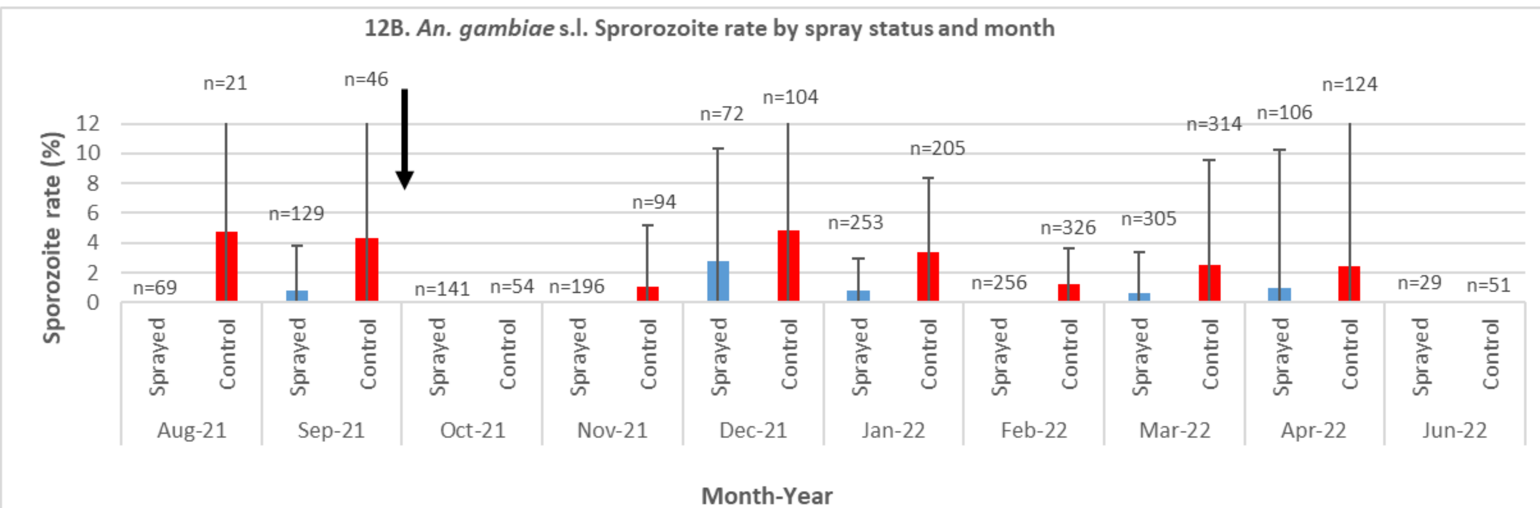


Sporozoite infection rates by collection month for each vector species are shown in Figure 12. November was the peak sporozoite infection month for *An. funestus* s.l. vectors at the sprayed sites (2.1%) while November and June were the peaks at the combined control sites (3.6% and5.6% respectively. The two highest peaks in sporozoite rates for *An. gambiae* s.l. vectors occurred in Dec (2.78%) and February (1.23%) at the sprayed sites while at the control sites they occurred in August (4.76%) and December (4.81%). Monthly sporozoite rates for both *An. funestus* s.l. and *An. gambiae* s.l. were lower at the sprayed sites compared to the control sites throughout the reporting period. Note that some districts contributed more than others to the total vectors tested each month and the between district variation in sporozoite rates were not accounted for in the calculations of the monthly sporozoite infection rates.

Figure 12: *An. funestus* s.l. and *An. gambiae* s.l. Sporozoite Infection Rates by Spray Status and Month of Collection (August 2021-June 2022)

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



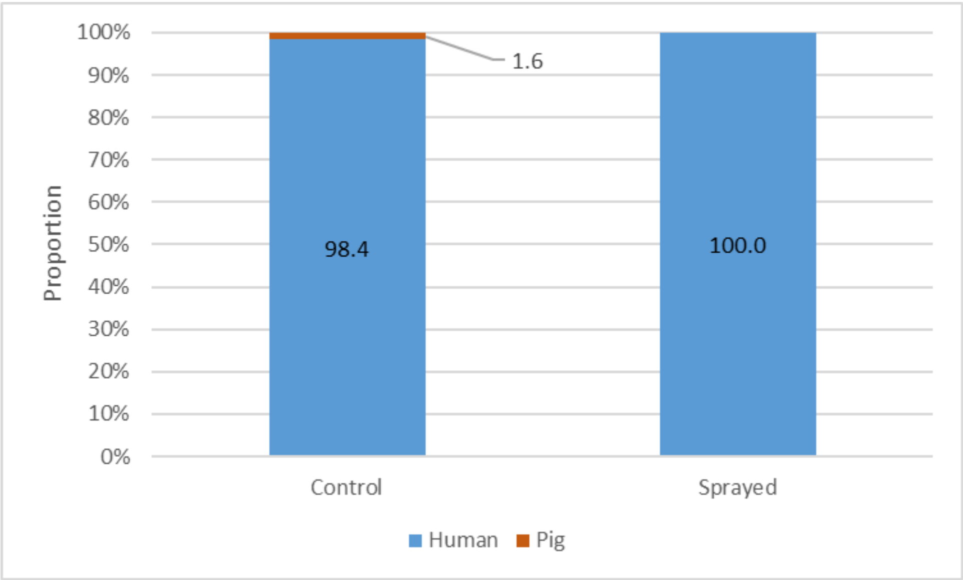


3.2.3 Blood Meal Sources

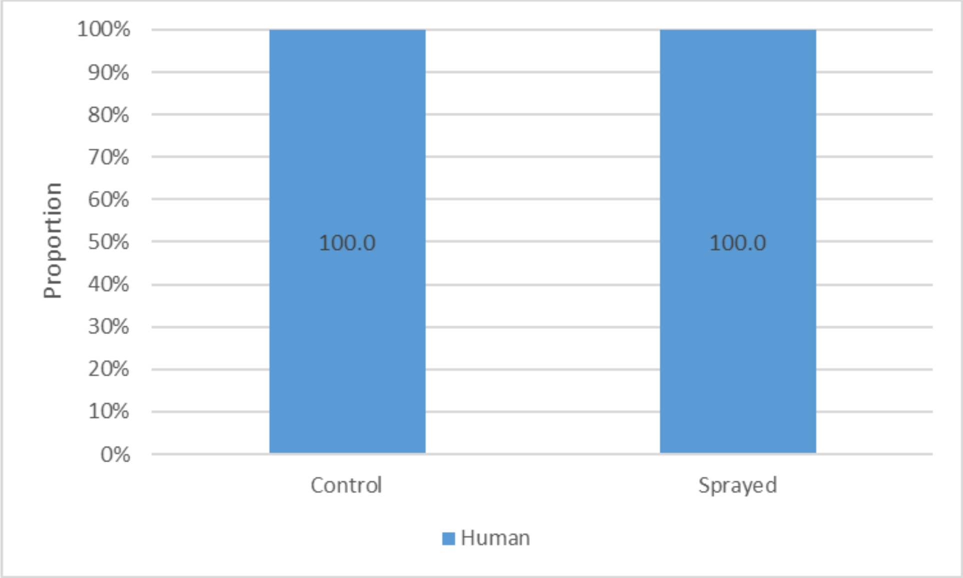
Out of the 123 blood meals identified from fed *An. funestus* s.l. vectors, 98.4% were from humans while a single mosquito was fed on pig. All 42 blood meals identified from fed *An. gambiae* s.l. were from humans (Figures 13A and 13B). This finding suggests that, in the entire region, most vectors resting 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 2021-June 2022)

A: *An. funestus* s.l.



B: *An. gambiae* s.l.



3.3 QUALITY ASSURANCE OF IRS AND MONITORING OF INSECTICIDE DECAY RATE

3.3.1 QUALITY ASSURANCE

A total of 42 sprayed houses and 14 unsprayed controls were used in cone bioassay tests to determine quality of the 2021 IRS spray campaign in seven districts where VectorLink Zambia conducted IRS. In all, 1,260 susceptible *An. gambiae* s.s. mosquitoes (Kisumu strain) were exposed to treated walls in seven districts at T0. All mosquitoes exposed to walls sprayed with either Fludora Fusion or SumiShield were dead after the 24-hour holding period. Assessment of mortality after 24 hours was not necessary because all mosquitoes were dead after 24 hours. Knockdown after 60 minutes was 100% for all houses and wall types sprayed with Fludora Fusion, except for one cement house in Kawambwa District and one cement house in Mambwe District where knockdown was 96.7% in each case. In houses sprayed with SumiShield, knockdown after 60 minutes ranged from 20% to 96.7% (see Table 7).

Corrected mortality was calculated for the three instances where control mortality was greater than 5%. Control mortality for each assay conducted was less than 20% which obviated the need to repeat any of the assays.

Table 7: T0 Mortality of Kisumu Susceptible Strain of *An. gambiae* s.s. after Exposure to Walls Sprayed with Fludora Fusion or SumiShield in September/October 2021

Insecticide sprayed during IRS	District	Wall Type	House Code	*An. gambiae* s.s. Kisumu strain			
				No. of females exposed	% Knockdown 30 mins post-exposure	% Knockdown 60 mins post-exposure	% Mortality after 24 hours
Fludora Fusion	Kawambwa	Mud	1	30	63.3	100.0	100.0
			2	30	80.0	100.0	100.0
			3	30	86.7	100.0	100.0
			4	30	93.3	100.0	100.0
		Cement	5	30	40.0	96.7	100.0
			6	30	70.0	100.0	100.0
	Mambwe	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	96.7	96.7	100.0
	Chipata	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	93.3	100.0	100.0
			5	30	93.3	100.0	100.0
			6	30	100.0	100.0	100.0
	Katete	Mud	1	30	96.7	100.0	100.0
			2	30	90.0	100.0	100.0
			3	30	100.0	100.0	100.0
		Cement	4	30	83.3	100.0	100.0
			5	30	100.0	100.0	100.0
			6	30	100.0	100.0	100.0
SumiShield	Nchelenge	Mud	1	30	26.7	86.7	100.0
			2	30	46.7	93.3	100.0
			3	30	66.7	96.7	100.0
		Cement	4	30	53.3	90.0	100.0
			5	30	43.3	83.3	100.0
			6	30	40.0	93.3	100.0

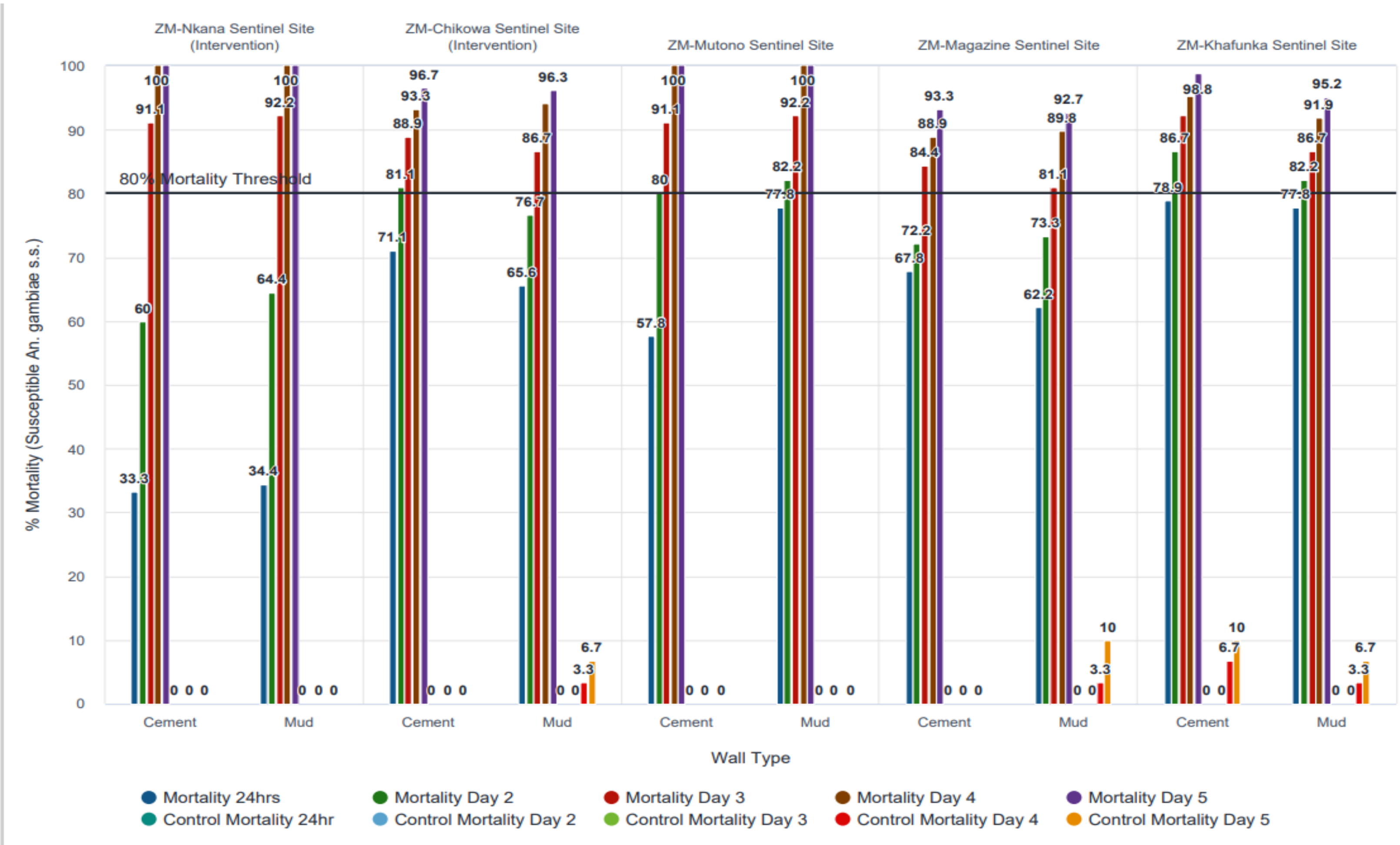
Insecticide sprayed during IRS	District	Wall Type	House Code	*An. gambiae* s.s. Kisumu strain			
				No. of females exposed	% Knockdown 30 mins post-exposure	% Knockdown 60 mins post-exposure	% Mortality after 24 hours
	Lufwanyama	Mud	1	30	46.7	50.0	100.0
			2	30	30.0	40.0	100.0
		Cement	3	30	23.3	56.7	100.0
			4	30	43.3	53.3	100.0
			5	30	36.7	40.0	100.0
			6	30	26.7	50.0	100.0
	Masaiti	Mud	1	30	30.0	33.3	100.0
			2	30	23.3	30.0	100.0
			3	30	30.0	33.3	100.0
		Cement	4	30	23.3	36.7	100.0
			5	30	36.7	40.0	100.0
			6	30	10.0	20.0	100.0

3.3.2 Insecticide Decay Rate

Monthly cone bioassays were conducted in five of the seven districts where quality of spray assessed 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). 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). Corrected mortality was calculated using Abbot's formula for the three cases where control mortality was between 5-20%.

Figure 14: Mortality of *An. gambiae* s.s. Kisumu Strain to SumiShield and Fludora Fusion 10 Months Following the September/October 2021 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

Vector susceptibility data is presented by province for the insecticides tested in Figures 15A-C. *An. funestus* s.l. and *An. gambiae* s.l. were fully susceptible to clothianidin 4 µg/bottle, chlorfenapyr (100 µg/bottle), and pirimiphos methyl 0.25% at all sites tested. Susceptibility to chlorfenapyr (>98% post exposure mortality) was determined at 48 hours for one site and at 24 hours at all other sites investigated. Susceptibility to clothianidin (>98% post exposure mortality) was determined at 24 hours for all sites tested. There was a mix of resistance profiles for DDT 4%; *An. funestus* s.l. was resistant to DDT at one site in Luapula Province, susceptible at four sites in Luapula and the two sites in Copperbelt, while *An. gambiae* s.l. was susceptible to DDT at the two sites in Eastern Province with possible resistance at one site in Copperbelt Province. There was confirmed resistance to at least one of the pyrethroids tested (alpha-cypermethrin 0.05%, deltamethrin 0.05%, permethrin 0.75%) in Luapula, Copperbelt and Eastern Provinces among *An. funestus* sl. and *An. gambiae* s.l. vector populations. There was a mixture of the three susceptibility profiles (susceptible, possible resistance, and confirmed resistance) for each of the different pyrethroids tested. There was full susceptibility to pirimiphos-methyl at the sites tested in Luapula and Copperbelt Provinces.

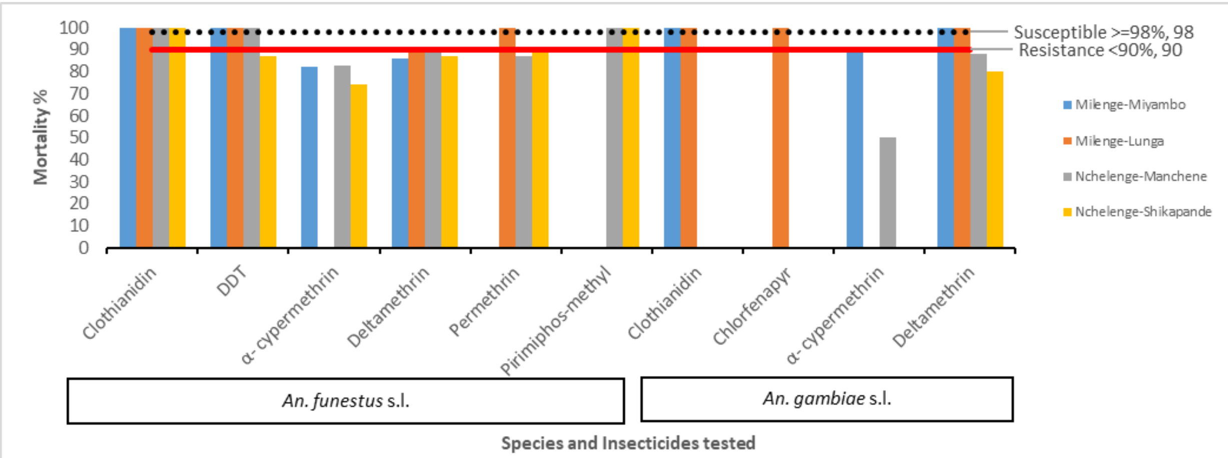
Mortality in all control tests (non-insecticide-treated papers or untreated bottles) were below 20%; corrected mortality using the Abbott formula was used for all assays in which control mortality was between 5-20%. Exposed mosquito mortality of 98% (shown by the top dotted line) or above in Figure 15 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 2021 to May 2022 for both species.

Full susceptibility was restored among pyrethroid resistant mosquitoes in Luapula Province (Figure 16A) and Eastern Province (Figure 16B) when pre exposed to the synergist PBO. This suggests that metabolic resistance mechanisms may be present in these provinces.

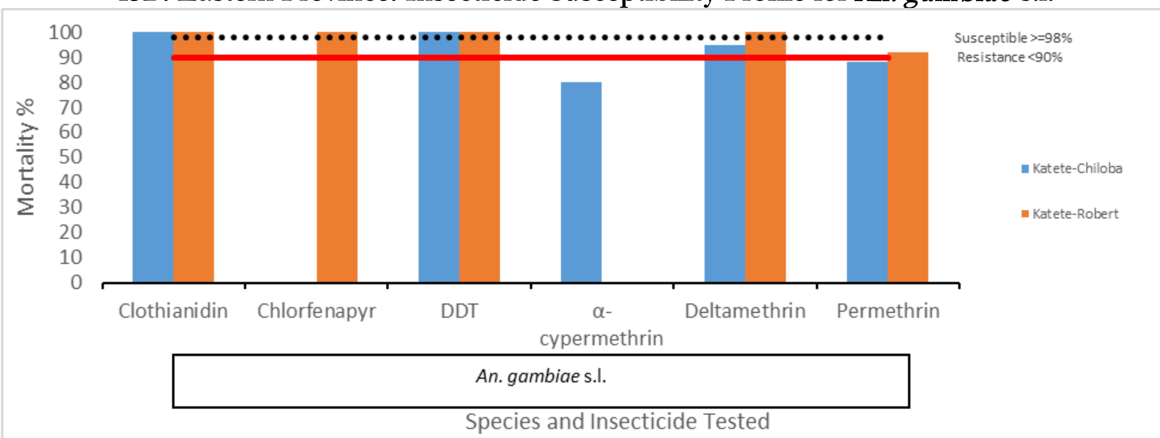
Figure 15: Insecticide Susceptibility Profile for *An. funestus* s.l. and *An. gambiae* s.l. by Province (December 2021-May 2022)

[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.

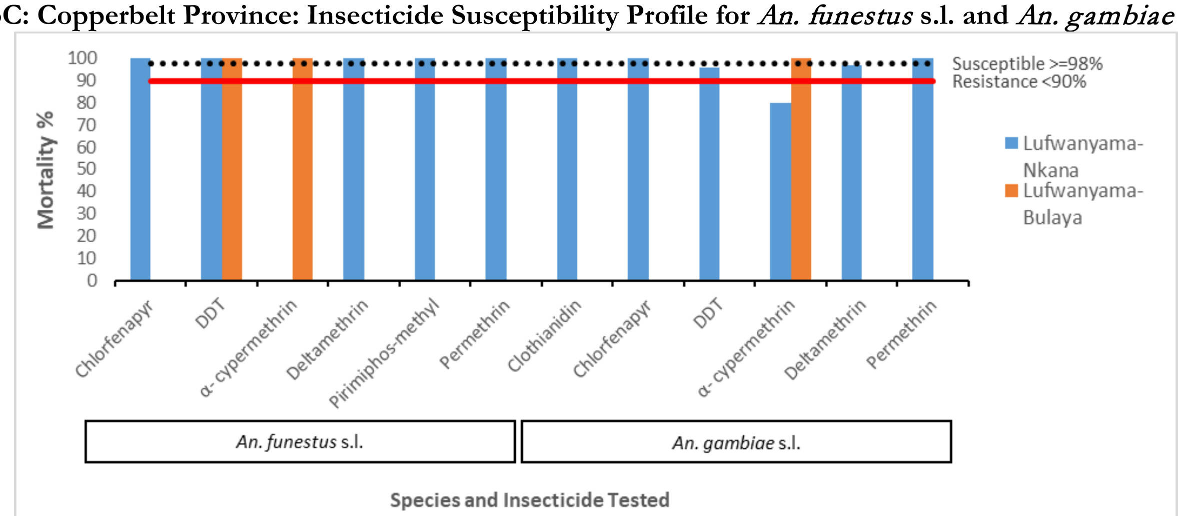
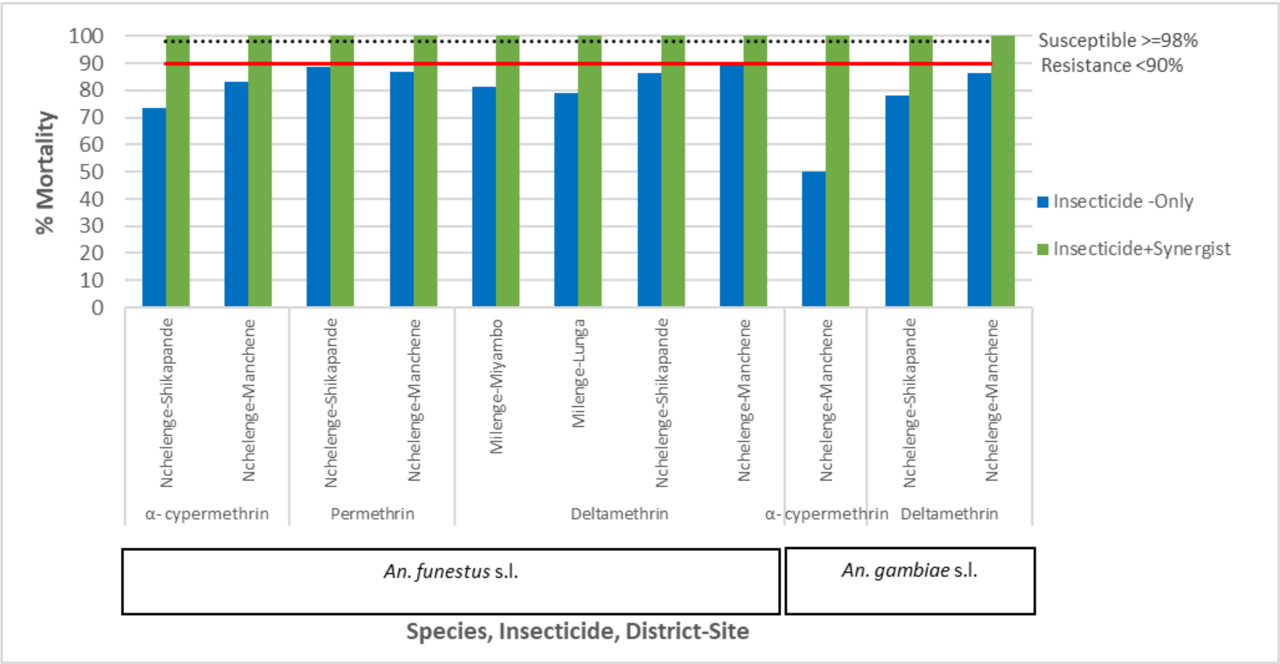


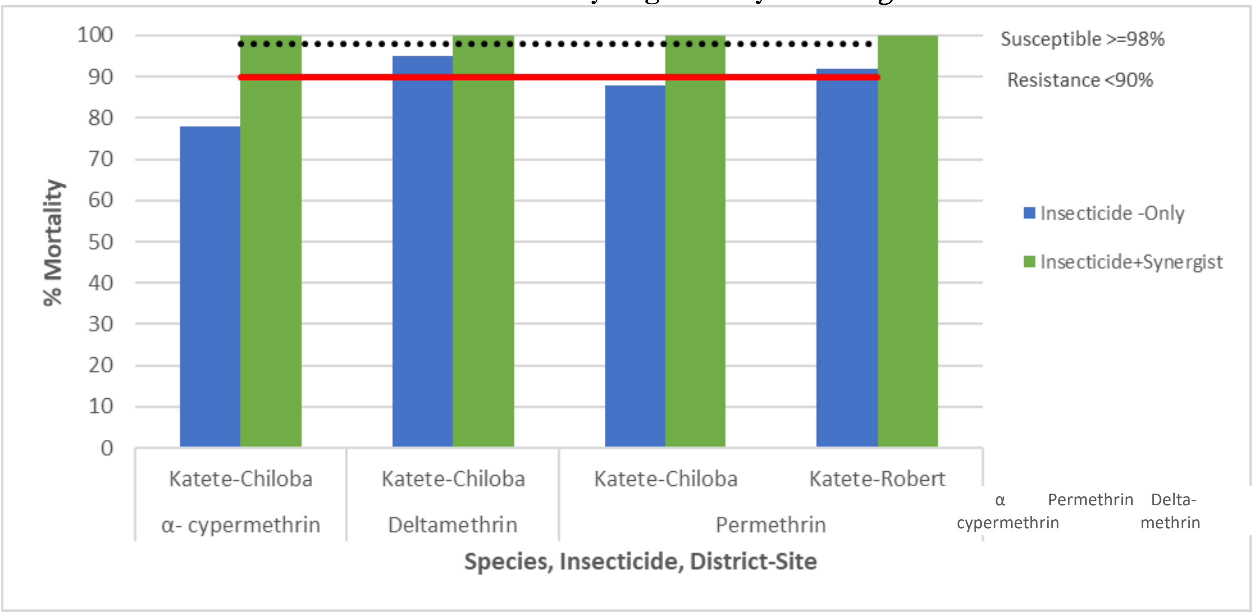
Figure 16: PBO Synergist Assays for *An. funestus* s.l. and *An. gambiae* s.l. by Province (December 2021-May 2022)

[Mortality reported at 24 hours.]

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



16B: Eastern Province: PBO Synergist Assays for *An. gambiae* 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. The diversity of *Anopheles* species observed during this surveillance period is like previous years with a significant presence of *An. ziemanni namibiensis* and *An. tchekedii* in HLC collections. Despite the abundance of these species in our vector collections, their role in malaria transmission is not fully known as we have not found any sporozoite infection among the samples we have screened so far including the 1,010 *An. ziemanni* specimens we screened this year. All 11 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 82.0%, which is similar to what was observed in the 2020-2021, 2019-2020, and 2018-2019 periods (86.9%, 87.9% and 87.6% respectively)[13,14,15]. The relative proportion of both species at sprayed sites relative to control sites during this reporting period (2021-2022) was similar to the previous two annual reporting periods. A higher proportion of *An. funestus* s.l. was observed at control sites (55.5% this year, 62.2% in 2020-2021, and 56% in 2019-2020). In previous reports, there were higher proportions of *An. gambiae* s.l. at sprayed sites (69.6% in 2020-2021 and 58% in 2019-2020) while this year we found similar proportions at sprayed and control sites. There was a high proportion of *An. gambiae* s.l. in Milenge during this reporting period (18.1%) compared to the last reporting period (4%). This shift was first noticed in January 2022 when the numbers of *An. gambiae* s.l. collected changed from single digits to triple digits which continued up to April 2022 (the end of the collection period in the district). *An. funestus* s.l. remains the predominant species in Nchelenge, Chililabombwe and Serenje districts while *An. gambiae* s.l. is the predominant species in Mambwe district. *An. gambiae* s.l. vector numbers relative to *An. funestus* s.l. were highest in Mambwe District in Eastern Province, followed by Lufwanyama in Copperbelt Province and Milenge in Luapula Province. There was a noticeable influence of time of year to the relative proportions of the two vector species in Milenge District where there was substantial presence of both species. Higher *An. gambiae* numbers were observed during peak rainy season (Jan-Mar) compared to the dry season were mostly collected *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 and human biting at sprayed sites compared to control sites for most of the surveillance districts (six out of seven and five out of seven respectively). This outcome is a slight improvement from the 2020 campaign where reductions in vector density were found in six districts, but human biting reduced in only four districts. Post-IRS reductions in *An. funestus* s.l. indoor densities and human biting rates 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 two sprayed sites during this reporting period compared to three sprayed site last year. The degree of increase in indoor vector numbers after IRS was highest at the control sites compared to the sprayed whereas the degree of increase in human biting was highest at the sprayed

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

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

[15] The PMI VectorLink Zambia Project, Annual Entomology Report. August 2020-July 2021. Rockville, MD. The PMI VectorLink Project, Abt Associates Inc.

sites compared to the control sites. This indicates an impact on indoor resting mosquitoes but not on human biting. IRS was probably responsible for the modulated increase in indoor resting mosquitoes observed at the sprayed sites. 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 districts difficult. 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. Another difficulty with interpretation of vector numbers is the differences between the pre-IRS and post-IRS periods. The pre-IRS period (2-months) is shorter than the post-IRS period (7 months) and the pre-IRS period coincides with dry season and low vector numbers while the post-IRS period coincides with the rainy period with naturally higher mosquito numbers. There was some impact on indoor resting and human biting *An. gambiae* s.l. vector populations, in Nchelenge and Milenge an improvement on the findings last year where we observed increases in *An. gambiae* s.l. vector density at both sprayed and control sites. There is usually a seasonal increase in *An. gambiae* s.l. just after IRS coinciding with the onset of the rainy season.

Low *An. funestus* s.l. and *An. gambiae* s.l. biting rates (less than one bite per person per night) were maintained throughout the post spray period in Katete. There were more bites during the 2020-2021 reporting period with such low biting rates; Mambwe, Katete and Serenje for *An. funestus* s.l. and Serenje, Katete, and Chililabombwe for *An. gambiae* s.l. Based on these findings, Katete is singled out as the district with the most impact of IRS on vector numbers. It is worth mentioning that an IRS experimental hut study in Benin[16] 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 2021. There has been no significant and sustained further reduction from these figures for almost five 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 four years. (See Annex E with monthly trends in indoor vector densities and human biting rates from 2015 to 2022. 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 indoor vector abundance and suggested that a more comprehensive package of interventions is needed to effectively reduce the malaria burden in such settings[17].

4.2 VECTOR BITING BEHAVIOR

There was more biting indoors than outdoors for both *An. funestus* s.l. and *An. gambiae* s.l. in five out of the seven districts (the exception being Mambwe District which had more outdoor bites for both species and Katete with more outdoor bites for *An. funestus* s.l.). 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). Last year there were more indoor than outdoor bites in six out of the seven

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[16] Fongnikin *et al. Parasites and Vectors*, 13(466), (2020)
[17] Hast *et. al.* Am J Trop Med Hyg. 2021 Feb; 104(2): 683–694. DOI 10.4269/ajtmh.20-0537.

districts monitored. Even though indoor bites were more than outdoor bites, we have observed substantial outdoor biting at all sites this year similar to what was observed last year. 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[18,19]. For now, the only WHO-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. We are currently in the process of conducting a LSM feasibility study in Eastern Province including in Katete District, one of our ento monitoring sites.

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. Weak bimodal peaks were observed in Mambwe and Katete. Most of the human biting by both *An. funestus* s.l. and *An. gambiae* s.l. occurred late at night when people were asleep. In Lufwanyama District in Copperbelt Province, the early morning biting that was reported in 2020-2021 was not observed during this reporting period, there was a downward trajectory of the number of bites received for both species from 3 a.m. till 8 a.m. when collections ended.

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. and gravid *An. gambiae* s.l. mosquitoes were lower at the combined sprayed sites relative to the combined control sites during the reporting period. This was the same observation reported last year where fewer gravid mosquitoes were found at the combined sprayed sites compared to the combined control sites. There were fewer gravid *An. gambiae* s.l. at the sprayed sites relative to the control sites for most of the post-IRS period while the desired reduction of gravid *An. funestus* s.l. mosquitoes at sprayed sites relative to control sites was observed in only a few months after IRS. Last year, we reported reduced gravid mosquitoes during most of the post-spray period for *An. funestus* s.l. but not for *An. gambiae* s.l. Overall, the proportion of gravid mosquitoes was higher at the sprayed sites after IRS compared to the period before IRS for both species. Fewer gravid mosquitoes are a crude indication of younger vector populations, which is a desired outcome of vector control interventions.

Parity. Overall, there were fewer parous *An. funestus* s.l. at the sprayed sites compared to the combined control sites while there were similar rates for *An. gambiae* s.l. This is an improvement from last year when there were no overall significant differences in parous *An. funestus* s.l. between sprayed and control sites. Parity rates for *An. funestus* s.l. at combined sprayed sites during the post IRS period was lower compared to the period before IRS. Parity rates at the combined control sites were either higher or remained the same after IRS. When aggregated by province we observed positive effects on parity in Luapula and Eastern Provinces but not in Copperbelt Province. Last year we reported significantly fewer parous *An. funestus* s.l. and *An. gambiae* s.l. vectors after IRS compared to before IRS at the sprayed sites in Copperbelt Province. 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

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18 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

19 Sougoufara, S. *et. al. Parasites Vectors* **13,** 295 (2020).

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. More than 99% of the *An. funestus* s.l. samples were identified as *An. funestus* s.s. while *An. gambiae* s.l. vectors were split between *An. gambiae* s.s. (71.9%) and *An. arabiensis* (28.1%). Last year most of the *An. gambiae* s.l. (99%) were identified by PCR as *An. gambiae* s.s.

Sporozoite rates and EIR. The *Plasmodium* parasite sporozoite rates were higher among *An. funestus* s.l. than *An. gambiae* s.l. populations, a similar observation during the last reporting period. Sporozoite rates remained were lower in sprayed sites compared to control sites for both species as was the case last year. This trend was observed for every month throughout the reporting period this year. After aggregating data from all IRS sites and that from all control sites, the number of *An. gambiae* s.l. and *An. funestus* s.l. infective bites received per month was lower at the IRS sites compared to the control sites. This is an improvement from last year when lower infective bites were observed for *An. funestus* s.l. but not for *An. gambiae* s.l. The reduction in the number of infective bites observed this year for both species 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 (Enzyme linked Immunosorbent Assay)) continue to be optimized and work plan targets can now be increased.

4.4 QUALITY OF THE 2021 IRS SPRAY

All 42 houses monitored during the PMI VectorLink IRS campaign in 2021 attained 100% mosquito mortality 24 hours after exposure to sprayed walls. This translates to 100% of assessed spray operators performing high spray quality. This was slightly different from the 2020 IRS campaign where two teams were retrained, as a precautionary measure, because at least one member of each team did not attain 100% mosquito mortality at the end of the observation period in two SumiShield sprayed 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. Zambia continues to be faced with the 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 2023 IRS campaign. An abstract submitted by VL Zambia on use of insecticide resistance management plan by national program in the selection of IRS insecticides has been accepted as a poster at the 2022 American Society of Tropical Medicine & Hygiene (ASTMH) Annual Meeting. It raises awareness on the dwindling of alternative insecticides for IRS that are available for malaria programs. Currently, the only available active ingredient to rotate to is pirimiphos methyl, which has been out of use for at least four 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[20] 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

[20] 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

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 at all sites tested in Luapula, Eastern and Copperbelt Provinces. There was susceptibility to pirimiphos methyl in Luapula and Copperbelt 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, possible resistance, and confirmed resistance to DDT among populations of either species in Luapula, Eastern, and Copperbelt Provinces; comparable results were obtained in 2020/2021 and 2019/2020 report. The use of this product must only be considered at the district level based on where susceptibility is reported, and any other environmental requirement fulfilled. Like 2020/2021, pyrethroid resistance was confirmed 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) were tested in all provinces except Eastern due to low mosquito numbers.

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. gambiae* s.l. in Eastern Province to avoid mortality caused by pyrethroid insecticides. Similar observation was reported last year in Luapula and Copperbelt. 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 is currently transitioning to these new net types (PBO nets) due to the widespread resistance to pyrethroids. 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 8 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 Central and Copperbelt 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 were substantial numbers of *An. gambiae* s.l. vectors in the Lufwanyama district in Copperbelt Province and in Milenge District in Luapula Province. Species composition information is important for determining the appropriateness of interventions (IRS and ITNs) in various parts of the country. 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. Post-IRS reductions in indoor resting density and human biting rates were maintained in Luapula and Eastern Provinces. These results indicate that IRS had the overall desired effect on *An. funestus* s.l. numbers in the two provinces 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 typically 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 evaluation of current national approach to vector intervention with a view of developing comprehensive strategies that will reduce vector numbers in these communities.

- 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 of malaria vectors in Eastern Province or to further reduce the numbers in areas with higher densities in Luapula 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 asleep, thus both ITNs and IRS can be good interventions in this region. Substantial outdoor biting occurred at many of the monitoring sites. Although there is very little or no outdoor sleeping in the communities where the collections were done, it will be good to investigate the contribution of outdoor biting to malaria transmission in the communities by conducting human sleeping behavior and net use studies alongside vector biting behavior surveys.

- A PMI supported larval source management feasibility study in Eastern Province is currently at the preparatory phases. LSM as a complementary intervention will target vectors that bite outdoors and do not necessarily enter houses to be exposed to the insecticides on walls or in nets.

Parity

There were 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 Luapula and Eastern Provinces but not in Copperbelt 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.

The 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 Copperbelt Province and the low parity reductions observed creates the need for a deliberation on the national approach to vector control in this province including use of supplementary vector control interventions where practical and feasible.

Molecular Species, Sporozoite Rates, and EIR

Almost all *An. funestus* s.l. tested by PCR were *An. funestus* s.s. while *An. gambiae* s.l. were either *An. gambiae* s.s. or *An. arabiensis*. Sporozoite rates were lower at the sprayed sites relative to the control sites for both *An. funestus* s.l. and *An. gambiae* s.l. The absolute values for EIR at the sprayed sites (approximately 10 infective bites per person per month for *An. funestus* s.l. and 1.1 for *An. gambiae* s.l. respectively) are enough to maintain high malaria transmission in an area. There was a high human blood index for both *An. funestus* s.l. and *An. gambiae* s.l. at sprayed and control sites, that is, most 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 in all houses tested immediately after spraying in 2021 indicates that spray operators performed an excellent 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 2023 with consideration of the national resistance management plan.

Insecticide Resistance

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

- We recommend the continued deployment of clothianidin-based products for IRS with consideration to the national resistance management plan.
- 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. For ITNs, we support the addition of synergists or other insecticide classes to the pyrethroids.
- Due to the continued resistance of local vectors to pyrethroid insecticides in some areas, we support the transition to new ITN types, including PBO nets (that is, nets with pyrethroid plus the synergist piperonyl butoxide), and in addition recommend dual active ingredients nets (that is pyrethroid, plus the pyrrole chlorfenapyr) and pyrethroid plus the insect growth regulator pyriproxyfen in select areas, especially as ITNs resume their role as the primary vector control intervention in the country, as per the 2022-2026 Zambia National Malaria Elimination Strategic Plan.

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 most 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 8: 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. Most of *An. funestus* were *An. funestus* s.s. while most of the *An. gambiae* s.l. were *An. gambiae s.s.* ▶ 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. Most of *An. funestus* were *An. funestus* s.s. while most of the *An. gambiae* s.l. were *An. arabiensis*. ▶ 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. All *An. funestus* s.l. were *An. funestus* s.s. ▶ Can use *An. funestus* s.l. to represent the province when known predominant species is needed for decision-making.	*An. funestus* s.l. dominant but sizeable presence of *An. gambiae* s.l. Last year there was no outright dominant species. Most *An. funestus* were *An. funestus* s.s. and most *An. gambiae* s.l. were *An. gambiae* s.s. ▶ Consider the 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. This situation was similar to the last reporting period. Recommend IRS or PBO nets. National program to conference on current vector control approach.	Post-IRS reduction in *An. funestus* s.l. indoor density and human biting rates. ▶ 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. National program to conference on current vector control approach. The results are similar to what was reported last year.	Positive impact on *An. funestus* s.l. and *An. gambiae* s.l. numbers. These findings were similar to what was reported last year. ▶ Overall reduced numbers seen at sprayed site. Recommend IRS or PBO ITNs.	No Post-IRS reduction in vector numbers though some reductions in *An. funestus* s.l. IRD and HBRs (Human Biting Rates) at sprayed sites compared to control sites. In the last reporting period, we observed post-IRS reductions in *An. funestus* s.l. HBRs. ▶ Some 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 supplementary 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. National program to conference on current vector control approach.
Biting Location	Similar to 2020-21, indoor biting is higher than outdoor biting at most sites with 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. ▶ IRS and ITNs are appropriate interventions. Last year, we reported some early morning biting in the Copperbelt, but it was not observed this year.			

Indicator	Luapula Province	Eastern Province	Central Province	Copperbelt Province
Parity Rates	Significant reduction in parity rates after IRS ▶ Desired outcome of IRS achieved. This is an improvement from last year when we did not observe a reduction in parity rates after IRS.	Overall fewer parous mosquitoes at sprayed sites compared to control sites. ▶ Desired outcome of IRS achieved. Impact on parity rates has been consistent for the past two years in Eastern Province.	Insufficient data collected. Same as previous year.	Similar proportions of parous vectors at sprayed and control sites▶ Not a desired outcome of IRS. Consider supplementary vector control strategies for the province. The performance on parity rate was better last year compared to this year. where we observed fewer parous vectors at the sprayed sites.
HBI	Extremely high human biting by mosquitoes. ▶ Targeting intervention at the human domicile continues to be an appropriate strategy.			
EIR	*An. funestus* s.l. and *An. gambiae* EIRs were lower at sprayed sites (vs. control sites) and post-IRS (vs. pre-IRS). This is an improvement on last year for *An. gambiae* s.l. where EIR was slightly higher at the sprayed sites. The absolute values of EIRs are still high enough to sustain malaria transmission. ▶ Additional interventions required to reduce the transmission in the high EIR scenarios observed in some districts.			
Insecticide Residual Efficacy	At least 10 months of residual efficacy of clothianidin products on walls after IRS. ▶ Duration of efficacy adequate to cover malaria transmission season.	At least 10 months of residual efficacy of clothianidin products on walls after IRS. ▶ Duration of efficacy adequate to cover malaria transmission season.	No residual efficacy site in Central Province, so no data collected.	At least 10 months of residual efficacy of clothianidin products on walls after IRS. ▶ Duration of efficacy adequate to cover malaria transmission season.
Insecticide Susceptibility	• Susceptibility: clothianidin, chlorfenapyr, and pirimiphos-methyl • Confirmed resistance and susceptibility: DDT (*An. funestus* s.l.) • Confirmed resistance: alpha-cypermethrin (possible resistance last year), deltamethrin, permethrin (*An. funestus* s.l.), alpha-cypermethrin and deltamethrin (*An. gambiae* s.l.) ▶ Can deploy clothianidin-based products, chlorfenapyr (when available) for IRS. Can deploy DDT for IRS at district level. Transition to new ITN types - use dual-active ingredient nets or PBO nets.	• Susceptibility: clothianidin, chlorfenapyr, DDT (*An. gambiae* s.l.) • Confirmed resistance: alpha-cypermethrin and permethrin. Possible resistance: deltamethrin (*An. gambiae* s.l.) ▶ Can deploy clothianidin-based products, chlorfenapyr (when available), DDT, and pirimiphos-methyl for IRS. Transition to new ITN types - use dual-active ingredient nets or PBO nets.	• Susceptibility: chlorfenapyr (*An. funestus* s.l.) ▶ Can deploy chlorfenapyr for IRS (when available). Transition to new ITN types - use dual-active ingredient nets or PBO nets.	• Susceptibility: clothianidin, chlorfenapyr, pirimiphos-methyl • Susceptibility and possible resistance: DDT (*An. funestus* s.l.) • Confirmed resistance: alpha-cypermethrin and probable resistance to deltamethrin (*An. gambiae* s.l.) ▶ Can deploy clothianidin-based products, chlorfenapyr (when available) for IRS. Can deploy DDT for IRS at district level. Transition to new ITN types - use dual-active ingredient nets or PBO nets.

ANNEX A: CULICIDAE COLLECTED IN SPRAYED AND CONTROL SITES BY COLLECTION METHOD (AUGUST 2021-JUNE 2022)

District	Village	Status	HLC Indoors											
			An. funestus s.l.	*An. gambiae* s.l.	*An. ziemanni*	*An. maculipalpis*	*An. coustani*	*An. tenebrosus*	*An. gibbinsi*	*An. rufipes*	*An. squamosus*	*An. argenteolobatus*	*An. tchekedii*	Culicine
Nchelenge	Shikapande	Sprayed	15,040	1,193	7	0	0	0	0	0	1	0	0	337
	Manchene	Control	12,516	1,522	266	0	0	0	0	0	7	0	0	342
Milenge	Lunga	Sprayed	2,035	1,428	858	0	1	0	0	0	268	0	506	1,102
	Miyambo	Control	5,977	2,242	198	0	0	0	0	0	18	0	73	508
Mambwe	Chikowa	Sprayed	9	342	0	0	4	0	0	0	0	0	0	595
	Chasela	Control	21	152	0	2	9	0	0	9	0	0	0	118
Katete	Chilowa	Sprayed	10	2	2	0	39	10	3	0	1	0	0	430
	Robert	Control	50	7	0	7	60	0	18	16	1	0	0	139
Serenje	Chibobo	Sprayed	55	7	14	0	0	0	0	0	2	3	0	182
	Chishi	Control	166	2	18	0	1	0	0	0	3	0	0	68
Lufwanyama	Nkana	Sprayed	1,052	828	35	0	8	0	0	0	0	0	0	360
	Bulaya	Control	602	169	452	0	2	0	0	0	0	0	0	683
Chililabombwe	Kawama	Sprayed	555	127	12	0	0	0	0	0	0	0	0	453
	Mainasoko	Control	1,096	263	99	0	0	0	0	0	0	2	0	736
		TOTAL	**39,184**	**8,284**	**1,961**	**9**	**124**	**10**	**21**	**25**	**301**	**5**	**579**	**6,053**

District	Village	Status	PSC											
			An. funestus s.l.	*An. gambiae* s.l.	*An. ziemanni*	*An. maculipalpis*	*An. coustani*	*An. tenebrosus*	*An. gibbinsi*	*An. rufipes*	*An. squamosus*	*An. argenteolobatus*	*An. tchekedii*	Culicine
Nchelenge	Shikapande	Sprayed	1,283	61	0	0	0	0	0	0	0	0	0	20
	Manchene	Control	2,321	118	0	0	0	0	0	0	1	0	0	22
Milenge	Lunga	Sprayed	627	190	1	0	0	0	0	0	0	0	0	46
	Miyambo	Control	1,722	294	0	0	0	0	0	0	2	0	0	141
Mambwe	Chikowa	Sprayed	1	14	0	0	0	0	0	0	0	0	0	64
	Chasela	Control	3	6	0	0	0	0	0	0	0	0	0	43
Katete	Chilowa	Sprayed	3	0	2	0	0	0	0	0	0	0	0	90
	Robert	Control	134	1	0	0	0	0	0	1	0	0	0	29
Serenje	Chibobo	Sprayed	10	0	9	0	0	0	0	0	0	0	0	53
	Chishi	Control	136	1	1	0	0	0	0	0	0	0	0	88
Lufwanyama	Nkana	Sprayed	284	239	1	0	0	0	0	0	0	0	0	135
	Bulaya	Control	418	70	0	0	0	0	0	0	0	0	0	63
Chililabombwe	Kawama	Sprayed	445	59	6	0	0	0	0	0	0	0	1	475
	Mainasoko	Control	179	28	0	0	0	0	0	0	0	0	0	126
		TOTAL	**7,566**	**1,081**	**20**	**0**	**0**	**0**	**0**	**1**	**3**	**0**	**1**	**1,395**

District	Village	Status	HLC Outdoors											
			An. funestus s.l.	*An. gambiae* s.l.	*An. ziemanni*	*An. maculipalpis*	*An. coustani*	*An. tenebrosus*	*An. gibbinsi*	*An. rufipes*	*An. squamosus*	*An. argenteolobatus*	*An. tchekedii*	Culicine
Nchelenge	Shikapande	Sprayed	8,024	937	172	1	2	0	0	0	11	0	0	1,352
	Manchene	Control	10,952	1,470	908	11	0	0	0	0	28	0	0	1,741
Milenge	Lunga	Sprayed	1,560	931	1,382	0	1	0	0	0	488	0	887	2,036
	Miyambo	Control	2,406	1,103	1,756	0	0	0	0	0	352	0	740	2,240
Mambwe	Chikowa	Sprayed	31	755	0	1	51	0	0	1	0	0	0	363
	Chasela	Control	18	327	0	0	24	0	0	8	0	0	0	114
Katete	Chilowa	Sprayed	1	1	2	0	34	20	0	0	4	0	0	378
	Robert	Control	47	9	0	2	41	0	17	15	2	0	0	119
Serenje	Chibobo	Sprayed	12	0	13	0	0	0	0	0	2	0	0	158
	Chishi	Control	43	2	8	0	0	0	0	0	0	0	0	30
Lufwanyama	Nkana	Sprayed	489	525	57	0	7	0	0	0	2	0	0	245
	Bulaya	Control	336	98	728	0	4	0	0	0	0	1	0	1,001
Chililabombwe	Kawama	Sprayed	451	100	16	0	0	0	0	0	0	0	0	612
	Mainasoko	Control	683	161	96	0	0	0	0	0	0	0	0	472
		TOTAL	**25,053**	**6,419**	**5,138**	**15**	**164**	**20**	**17**	**24**	**889**	**1**	**1,627**	**10,861**

ANNEX B: *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. BY MONTH, SITE, AND COLLECTION METHOD (AUGUST 2021-JUNE 2022)

Month, Year	District	Site	Status	*An. funestus* s.l.				*An. gambiae* s.l.			
				# collected by Indoor HLC	# collected by Outdoor HLC	# collected by PSC	Monthly Total Collected	# collected by Indoor HLC	# collected by Outdoor HLC	# collected by PSC	Monthly Total Collected
August 2021	Nchelenge	Shikapande	Sprayed	2,591	1,148	353		34	83	0	
		Manchene	Control	814	1,207	292		20	66	1	
	Milenge	Lunga	Sprayed	200	105	57		1	0	0	
		Miyambo	Control	426	153	141		1	0	0	
	Mambwe	Chikowa	Sprayed	2	0	0		0	0	0	
		Chasela	Control	8	2	0		0	0	0	
	Katete	Chilowa	Sprayed	4	0	1		0	0	0	
		Robert	Control	1	1	9		0	1	0	
	Serenje	Chibobo	Sprayed	0	0	0		0	0	0	
		Chishi	Control	3	1	3		0	0	0	
	Lufwanyama	Nkana	Sprayed	40	23	26		20	15	5	
		Bulaya	Control	30	4	4		3	1	0	
	Chililabombwe	Kawama	Sprayed	21	21	23		2	2	0	
		Mainasoko	Control	8	1	3	**7,726**	0	0	0	**255**
September 2021	Nchelenge	Shikapande	Sprayed	1,497	1,083	130		17	45	0	
		Manchene	Control	1,440	1,924	323		16	26	1	
	Milenge	Lunga	Sprayed	184	59	54		0	0	0	
		Miyambo	Control	700	150	89		1	1	0	
	Mambwe	Chikowa	Sprayed	0	0	0		0	0	0	
		Chasela	Control	0	0	0		0	0	0	
	Katete	Chilowa	Sprayed	0	0	1		0	0	0	
		Robert	Control	1	1	4		0	0	0	
	Serenje	Chibobo	Sprayed	2	0	1		0	0	0	
		Chishi	Control	6	3	9		0	0	0	
	Lufwanyama	Nkana	Sprayed	29	26	19		21	29	15	
		Bulaya	Control	21	4	14		0	0	0	
	Chililabombwe	Kawama	Sprayed	50	58	19		21	20	6	
		Mainasoko	Control	16	34	12	**7,963**	1	1	3	**224**

Month, Year	District	Site	Status	*An. funestus* s.l.				*An. gambiae* s.l.			
				# collected by Indoor HLC	# collected by Outdoor HLC	# collected by PSC	Monthly Total Collected	# collected by Indoor HLC	# collected by Outdoor HLC	# collected by PSC	Monthly Total Collected
October 2021	Nchelenge	Shikapande	Sprayed	1,111	702	180		12	6	3	
		Manchene	Control	2,099	1,141	423		125	55	2	
	Milenge	Lunga	Sprayed	442	406	122		4	2	0	
		Miyambo	Control	1,862	684	530		0	1	0	
	Mambwe	Chikowa	Control	2	4	0		0	0	2	
		Chasela	Sprayed	1	0	0		0	0	0	
	Katete	Chilowa	Control	4	1	1		0	0	0	
		Robert	Sprayed	19	13	14		33	30	0	
	Serenje	Chibobo	Control	22	21	6		12	14	0	
		Chishi	Sprayed	62	95	10		19	21	0	
	Lufwanyama	Nkana	Control	39	24	13		1	3	10	
		Bulaya	Control	0	0	36		0	0	2	
	Chililabombwe	Kawama	Sprayed	0	0	53		0	0	16	
		Mainasoko	Control	0	0	7	**10,149**	0	0	2	**375**
November 2021	Nchelenge	Shikapande	Sprayed	848	416	74		224	52	5	
		Manchene	Control	1,284	1,164	314		238	166	5	
	Milenge	Lunga	Sprayed	373	373	143		4	3	0	
		Miyambo	Control	1,228	602	507		4	4	3	
	Mambwe	Chikowa	Control	3	1	0		0	0	0	
		Chasela	Control	8	2	0		0	0	0	
	Katete	Chilowa	Sprayed	47	6	0		74	59	0	
		Robert	Control	29	19	23		5	0	0	
	Serenje	Chibobo	Sprayed	107	85	0		16	19	0	
		Chishi	Control	101	38	12		6	11	0	
	Lufwanyama	Nkana	Sprayed	0	0	8		0	0	43	
		Bulaya	Control	0	0	26		0	0	3	
	Chililabombwe	Kawama	Sprayed	0	0	61		0	0	8	
		Mainasoko	Control	0	0	21	**7,923**	0	0	2	**954**
December 2021	Nchelenge	Shikapande	Sprayed	1,016	481	122		237	93	21	
		Manchene	Control	1,322	1,604	146		150	140	36	
	Milenge	Lunga	Sprayed	498	386	117		29	24	3	
		Miyambo	Control	913	486	217		9	11	5	
	Mambwe	Chikowa	Sprayed	0	0	0		0	1	0	
		Chasela	Control	1	0	0		0	0	0	
	Katete	Chilowa	Control	13	2	0		0	0	0	
		Robert	Sprayed	14	12	20		48	24	0	
	Serenje	Chibobo	Control	63	17	0		19	3	0	
		Chishi	Sprayed	77	39	12		0	0	0	
	Lufwanyama	Nkana	Control	139	93	11		8	9	24	
		Bulaya	Control	0	0	23		0	0	11	
	Chililabombwe	Kawama	Sprayed	0	0	74		0	0	0	
		Mainasoko	Control	0	0	10	**7,928**	0	0	0	**905**

Month, Year	District	Site	Status	*An. funestus* s.l.				*An. gambiae* s.l.			
				# collected by Indoor HLC	# collected by Outdoor HLC	# collected by PSC	Monthly Total Collected	# collected by Indoor HLC	# collected by Outdoor HLC	# collected by PSC	Monthly Total Collected
January 2022	Nchelenge	Shikapande	Sprayed	1,042	582	39		53	68	3	
		Manchene	Control	1,214	1,019	150		224	354	52	
	Milenge	Lunga	Sprayed	121	74	57		182	99	24	
		Miyambo	Control	176	45	90		242	69	30	
	Mambwe	Chikowa	Sprayed	2	1	0		13	30	0	
		Chasela	Control	1	1	0		5	12	0	
	Katete	Chilowa	Control	7	5	0		4	2	0	
		Robert	Sprayed	2	1	12		0	0	0	
	Serenje	Chibobo	Control	16	3	0		0	0	0	
		Chishi	Sprayed	34	12	29		134	24	0	
	Lufwanyama	Nkana	Control	30	6	18		37	25	49	
		Bulaya	Sprayed	108	61	7		5	0	8	
	Chililabombwe	Kawama	Control	215	119	83	**5,382**	31	20	4	**1,803**
		Mainasoko	Sprayed	1,775	843	19		212	286	0	
Feb-22	Nchelenge	Shikapande	Control	1,373	818	56		294	258	7	
		Manchene	Sprayed	108	94	207		136	147	14	
	Milenge	Lunga	Control	298	137	36		479	242	27	
		Miyambo	Sprayed	1	1	61		63	190	34	
	Mambwe	Chikowa	Control	0	0	0		3	11	1	
		Chasela	Control	1	16	0		0	5	0	
	Katete	Chilowa	Sprayed	20	7	0		0	0	0	
		Robert	Control	49	14	40		0	0	1	
	Serenje	Chibobo	Sprayed	82	59	0		158	94	0	
		Chishi	Control	34	27	10		39	27	0	
	Lufwanyama	Nkana	Sprayed	44	43	23		1	2	33	
		Bulaya	Control	253	179	30		61	49	20	
	Chililabombwe	Kawama	Sprayed	0	0	70		0	0	4	
		Mainasoko	Control	0	0	66	**6,894**	0	0	5	**2,903**
March 2022	Nchelenge	Shikapande	Sprayed	2,203	1,168	110		117	104	7	
		Manchene	Control	1,080	810	255		144	144	4	
	Milenge	Lunga	Sprayed	109	63	41		1,072	656	136	
		Miyambo	Control	374	149	87		1,506	775	222	
	Mambwe	Chikowa	Sprayed	1	8	0		244	430	0	
		Chasela	Control	7	9	0		132	272	0	
	Katete	Chilowa	Sprayed	2	0	0		2	0	0	
		Robert	Control	21	5	4		3	1	0	
	Serenje	Chibobo	Sprayed	30	4	3		7	0	0	
		Chishi	Control	67	17	51		2	2	1	
	Lufwanyama	Nkana	Sprayed	406	208	70		170	140	31	
		Bulaya	Control	131	105	77		36	18	21	
	Chililabombwe	Kawama	Sprayed	86	49	62		63	36	21	
		Mainasoko	Control	325	195	41	**8,433**	155	68	16	**6,758**

Month, Year	District	Site	Status	An. funestus s.l.				An. gambiae s.l.			
				# collected by Indoor HLC	# collected by Outdoor HLC	# collected by PSC	Monthly Total Collected	# collected by Indoor HLC	# collected by Outdoor HLC	# collected by PSC	Monthly Total Collected
April 2022	Nchelenge	Shikapande	Sprayed	1,744	893	111	6,767	216	162	9	1,340
		Manchene	Control	1,665	1,148	118		267	238	2	
	Mambwe	Chikowa	Sprayed	3	21	0		20	95	10	
		Chasela	Control	3	6	0		12	32	6	
	Katete	Chilowa	Sprayed	1	0	0		0	1	0	
		Robert	Control	10	13	6		0	0	0	
	Lufwanyama	Nkana	Sprayed	338	87	81		147	68	23	
		Bulaya	Control	229	117	173		18	9	5	
June 2022	Nchelenge	Shikapande	Sprayed	1,213	708	108	2,638	71	38	6	267
		Manchene	Control	225	117	93		44	23	1	
	Mambwe	Chikowa	Sprayed	0	0	1		2	9	1	
		Chasela	Control	2	0	3		0	0	0	
	Katete	Chilowa	Sprayed	3	1	0		0	0	0	
		Robert	Control	3	1	2		0	0	0	
	Lufwanyama	Nkana	Sprayed	43	43	15		23	42	6	
		Bulaya	Control	13	16	28		0	1	0	

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 2021-June 2022)

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	5.12	2.77	0.51	0.0013**	0.54	0.59	1.07	0.7641
ALL-Sprayed	Pre-IRS v Post-IRS	4.17	5.37	1.14	0.42	0.03	0.67	5.07	<.0001**
ALL-Control	Pre-IRS v Post-IRS	3.23	2.65	0.78	0.355	0.13	0.7	22.9	<.0001**
Nchelenge	Control v Sprayed	15.47	8.55	0.55	0.0165**	0.79	0.41	0.51	0.0754
Milenge	Control v Sprayed	14.35	5.23	0.36	<.0001**	2.45	1.58	0.65	0.0446**
Mambwe	Control v Sprayed	0.02	0.01	0.34	0.4283	0.04	0.09	2.33	0.1337
Katete	Control v Sprayed	0.89	0.02	0.02	<.0001**	0.01	0	N/A	N/A
Serenje	Control v Sprayed	1.13	0.08	0.07	<.0001**	0.01	0	N/A	N/A
Lufwanyama	Control v Sprayed	2.79	1.89	0.68	0.0981	0.47	1.59	3.41	<.0001**
Chililabombwe	Control v Sprayed	1.49	3.74	2.51	<.0001**	0.23	0.5	2.13	0.0148**
Shikapande-Sprayed	Pre-IRS v Post-IRS	16.1	6.67	0.42	0.005**	0	0.51	N/A	N/A
Manchene-Control	Pre-IRS v Post-IRS	20.5	14.22	0.69	0.0385**	0.07	0.97	14.44	0.0006**
Lunga-Sprayed	Pre-IRS v Post-IRS	3.8	5.43	1.43	0.0538	0	1.81	N/A	N/A
Miyambo-Control	Pre-IRS v Post-IRS	9.4	15.06	1.6	0.0154**	0	2.8	N/A	N/A
Chikowa-Sprayed	Pre-IRS v Post-IRS	0	0.01	N/A	N/A	0	0.12	N/A	N/A
Chasela-Control	Pre-IRS v Post-IRS	0	0.03	N/A	N/A	0	0.05	N/A	N/A
Chiloba-Sprayed	Pre-IRS v Post-IRS	0.07	0.01	0.13	0.0895	0	0	N/A	N/A
Robert-Control	Pre-IRS v Post-IRS	0.43	1.01	2.33	0.0014**	0	0.01	N/A	N/A
Chibobo-Sprayed	Pre-IRS v Post-IRS	0.03	0.1	3	0.269	0	0	N/A	N/A
Chishi-Control	Pre-IRS v Post-IRS	0.4	1.38	3.44	0.0186**	0	0.01	N/A	N/A
Nkana-Sprayed	Pre-IRS v Post-IRS	1.5	1.99	1.33	0.31	0.67	1.83	2.74	0.0072**
Bulaya-Control	Pre-IRS v Post-IRS	0.6	3.33	5.56	0.0153**	0	0.58	N/A	N/A
Kawama-Sprayed	Pre-IRS v Post-IRS	1.4	4.53	3.24	<.0001**	0.2	0.6	2.99	0.0312**
Mainasoko-Control	Pre-IRS v Post-IRS	0.5	1.82	3.64	0.038**	0.1	0.28	2.78	0.0258**

*For IRR (Incidence Rate Ratio), 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.			
		Proportion Gravid [First group]	Proportion Gravid [Second group]	IRR	P value	Proportion Gravid [First group]	Proportion Gravid [Second group]	IRR	P value
All	Control v Sprayed	12.98	9.17	0.78	0.1212	11.43	6.01	0.51	0.0123
All-Control	Pre-IRS v Post-IRS	18.87	12.06	0.69	0.0602	0	11.89	N/A	N/A
All-Sprayed	Pre-IRS v Post-IRS	8.81	9.24	1.25	0.5369	0	6.46	N/A	N/A
Nchelenge	Control v Sprayed	13.3	12.83	1.01	0.9155	11.94	9.2	0.51	0.0668
Milenge	Control v Sprayed	7.5	10.66	1.4	0.2017	19.25	13.32	0.74	0.2649
Mambwe	Control v Sprayed	100	0	N/A	N/A	25	4.55	0.43	0.5358
Katete	Control v Sprayed	46.31	66.67	1.59	0.2683	0	N/A	N/A	N/A
Serenje	Control v Sprayed	2.13	0	N/A	N/A	0	N/A	N/A	N/A
Lufwanyama	Control v Sprayed	4.33	3.4	0.85	0.8056	3.03	3.44	1.45	0.7397
Chililabombwe	Control v Sprayed	8.13	6.98	0.74	0.609	5.56	1.79	0.48	0.5988
Shikapande -Sprayed	Pre-IRS v Post-IRS	10.2	13.57	1.59	0.3261	N/A	9.2	N/A	N/A
Manchene -Control	Pre-IRS v Post-IRS	19.43	11.58	0.57	0.0127**	0	12.79	N/A	N/A
Lunga -Sprayed	Pre-IRS v Post-IRS	0	12.22	N/A	N/A	N/A	13.32	N/A	N/A
Miyambo -Control	Pre-IRS v Post-IRS	0	8.54	N/A	N/A	N/A	19.25	N/A	N/A
Chasela -Control	Pre-IRS v Post-IRS	N/A	100	N/A	N/A	N/A	25	N/A	N/A
Chilowa -Sprayed	Pre-IRS v Post-IRS	100	0	N/A	N/A	N/A	N/A	N/A	N/A
Chikowa -Sprayed	Pre-IRS v Post-IRS	N/A	0	N/A	N/A	N/A	4.55	N/A	N/A
Robert -Control	Pre-IRS v Post-IRS	77.78	40.76	0.5	0.0173**	N/A	0	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	0	2.38	N/A	N/A	N/A	0	N/A	N/A
Nkana -Sprayed	Pre-IRS v Post-IRS	6.67	2.78	0.22	0.1501	0	3.83	N/A	N/A
Bulaya -Control	Pre-IRS v Post-IRS	0	4.69	N/A	N/A	N/A	3.03	N/A	N/A
Kawama -Sprayed	Pre-IRS v Post-IRS	4.17	7.48	1.83	0.6017	0	2.08	N/A	N/A
Mainasoko -Control	Pre-IRS v Post-IRS	0	8.66	N/A	N/A	0	6.67	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	34.09	28.64	0.95	0.9124	7.35	7.01	0.95	0.8692
ALL-Sprayed	Pre-IRS v Post-IRS	29.37	35.3	1.07	0.822	0.65	9.06	11.18	<.0001**
ALL-Control	Pre-IRS v Post-IRS	33.17	27.48	0.76	0.0003**	1.49	8.41	4.88	<.00001**
Nchelenge	Control v Sprayed	146.68	144.15	0.98	0.93	18.7	13.31	0.72	0.1846
Milenge	Control v Sprayed	65.49	28.09	0.43	<.0001**	26.13	18.43	0.71	0.0242**
Mambwe	Control v Sprayed	0.24	0.25	0.96	0.91	2.99	6.86	2.27	0.0152**
Katete	Control v Sprayed	0.61	0.07	0.11	0.0002**	0.1	0.02	0.19	0.0053**
Serenje	Control v Sprayed	1.63	0.52	0.32	0.003**	0.03	0.05	1.75	0.3926
Lufwanyama	Control v Sprayed	5.86	9.63	1.64	0.0287**	1.67	8.46	5.07	<.0001**
Chililabombwe	Control v Sprayed	13.9	7.86	0.57	0.0158**	3.31	1.77	0.54	0.0111**
Shikapande-Sprayed	Pre-IRS v Post-IRS	197.47	130.82	0.64	<.0001**	5.59	15.24	2.71	0.0356**
Manchene-Control	Pre-IRS v Post-IRS	168.28	141.27	0.84	0.5475	4	22.38	5.59	<.0001**
Lunga-Sprayed	Pre-IRS v Post-IRS	19.06	29.38	1.54	0.0256**	0.06	21.05	336.9	<.0001**
Miyambo-Control	Pre-IRS v Post-IRS	36.19	69.68	1.93	0.0013**	0.06	29.86	429.6	<.0001**
Chikowa-Sprayed	Pre-IRS v Post-IRS	0.06	0.3	4.71	0.0734	0	8.57	N/A	N/A
Chasela-Control	Pre-IRS v Post-IRS	0.31	0.23	0.73	0.2775	0	3.74	N/A	N/A
Chiloba-Sprayed	Pre-IRS v Post-IRS	0.13	0.05	0.44	0.0302**	0	0.02	N/A	N/A
Robert-Control	Pre-IRS v Post-IRS	0.13	0.73	5.81	0.0011**	0.03	0.12	3.75	0.0587
Chibobo-Sprayed	Pre-IRS v Post-IRS	0.06	0.68	10.83	0.0153**	0	0.07	N/A	N/A
Chishi-Control	Pre-IRS v Post-IRS	0.41	2.04	5.03	<.0001**	0	0.04	N/A	N/A
Nkana-Sprayed	Pre-IRS v Post-IRS	3.69	11.12	3	<.0001**	2.66	9.91	3.73	<.0001**
Bulaya-Control	Pre-IRS v Post-IRS	1.84	6.87	3.72	<.0001**	0.13	2.05	16.44	<.0001**
Kawama-Sprayed	Pre-IRS v Post-IRS	4.69	8.92	1.9	0.0073**	1.41	1.9	1.35	0.0125**
Mainasoko-Control	Pre-IRS v Post-IRS	1.84	17.92	9.72	<.0001**	0.06	4.4	70.33	<.0001**

*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

Site	Comparison	*An. funestus* s.l.				*An. gambiae* s.l.			
		Mean [First group]	**Mean [Second group]**	**IRR**	**P value**	**Mean [First group]**	**Mean [Second group]**	**IRR**	**P value**
All	Indoor v Outdoor	19.13	12.23	0.64	0.0022**	4.04	3.13	0.77	0.0367**
All-Control	Indoor v Outdoor	19.95	14.15	0.71	0.153	4.25	3.1	0.73	0.1154
All-Sprayed	Indoor v Outdoor	18.32	10.32	0.56	<.0001**	3.83	3.17	0.83	0.1739
Nchelenge	Indoor v Outdoor	86.11	59.3	0.69	0.0592	8.48	7.52	0.89	0.5754
Milenge	Indoor v Outdoor	31.3	15.49	0.5	<.0001**	14.34	7.95	0.55	0.006**
Mambwe	Indoor v Outdoor	0.09	0.15	1.66	0.2937	1.54	3.38	2.19	<.0001**
Katete	Indoor v Outdoor	0.19	0.15	0.8	0.5421	0.03	0.03	1.11	0.8492
Serenje	Indoor v Outdoor	0.86	0.21	0.25	<.0001**	0.04	0.01	0.22	0.0448
Lufwanyama	Indoor v Outdoor	5.17	2.58	0.5	<.0001**	3.12	1.95	0.63	<.0001**
Chililabombwe	Indoor v Outdoor	6.45	4.43	0.69	0.0472**	1.52	1.02	0.67	<.0001**
Shikapande -Sprayed	Indoor v Outdoor	94	50.15	0.53	<.0001**	7.46	5.86	0.79	0.4803
Manchene -Control	Indoor v Outdoor	78.23	68.45	0.88	0.6279	9.51	9.19	0.97	0.8849
Lunga -Sprayed	Indoor v Outdoor	15.9	12.19	0.77	0.0293	11.16	7.27	0.65	<.0001**
Miyambo -Control	Indoor v Outdoor	46.7	18.8	0.4	<.0001**	17.52	8.62	0.49	0.0513
Chikowa -Sprayed	Indoor v Outdoor	0.06	0.19	3.43	0.0704	2.14	4.72	2.21	<.0001**
Chasela -Control	Indoor v Outdoor	0.13	0.11	0.86	0.7416	0.95	2.04	2.15	0.0089**
Chilowa -Sprayed	Indoor v Outdoor	0.06	0.01	0.1	0.0567	0.01	0.01	0.5	0.327
Robert -Control	Indoor v Outdoor	0.31	0.29	0.94	0.8576	0.04	0.06	1.29	0.707
Chibobo -Sprayed	Indoor v Outdoor	0.43	0.09	0.22	0.0081**	0.05	0	N/A	N/A
Chishi -Control	Indoor v Outdoor	1.3	0.34	0.26	0.0003**	0.02	0.02	1	0.2064
Nkana -Sprayed	Indoor v Outdoor	6.58	3.06	0.47	<.0001**	5.18	3.28	0.63	<.0001**
Bulaya -Control	Indoor v Outdoor	3.76	2.1	0.56	0.0436**	1.06	0.61	0.58	0.0016**
Kawama -Sprayed	Indoor v Outdoor	4.34	3.52	0.81	0.65	0.99	0.78	0.79	0.0991
Mainasoko -Control	Indoor v Outdoor	8.56	5.34	0.62	<.0001**	2.05	1.26	0.61	<.0001**

*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.			
		Proportion Parous [First group]	Proportion Parous [Second group]	IRR	P value	Proportion Parous [First group]	Proportion Parous [Second group]	IRR	P value
All	Control v Sprayed	53.96	41.78	0.82	0.017**	47.74	34.38	0.76	0.0102**
All-Control	Pre-IRS v Post-IRS	52.51	54.3	1.04	0.721	100	47.22	0.48	<.0001**
All-Sprayed	Pre-IRS v Post-IRS	50.01	39.37	0.65	<.0001**	13.1	36.23	3.02	0.0436**
Nchelenge	Control v Sprayed	61.34	51.83	0.81	0.0096**	59.44	24	0.57	<.0001**
Milenge	Control v Sprayed	58.35	57.84	1	0.9876	37.09	50.69	1.21	0.0469**
Mambwe	Control v Sprayed	67.42	38.89	0.74	0.1437	55.65	45	0.84	0.0034**
Katete	Control v Sprayed	80.56	100	1.33	<.0001**	22.22	N/A	N/A	N/A
Serenje	Control v Sprayed	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Lufwanyama	Control v Sprayed	44.49	29.31	0.69	<.0001**	43.16	25.45	0.68	0.0008**
Chililabombwe	Control v Sprayed	37.28	23.45	0.72	<.0001**	47.56	15.83	0.39	<.0001**
Shikapande -Sprayed	Pre-IRS v Post-IRS	62.85	46.97	0.58	<.0001**	N/A	24	N/A	N/A
Manchene -Control	Pre-IRS v Post-IRS	53.43	65.72	1.15	0.3503	N/A	59.44	N/A	N/A
Lunga -Sprayed	Pre-IRS v Post-IRS	65.28	56.83	0.83	<.0001**	N/A	50.69	N/A	N/A
Miyambo -Control	Pre-IRS v Post-IRS	60.42	57.96	1.16	0.4761	N/A	37.09	N/A	N/A
Chikowa -Sprayed	Pre-IRS v Post-IRS	N/A	38.89	N/A	N/A	N/A	45	N/A	N/A
Chasela -Control	Pre-IRS v Post-IRS	66.67	67.71	1.59	0.0158**	N/A	55.65	N/A	N/A
Chilowa -Sprayed	Pre-IRS v Post-IRS	100	100	N/A	N/A	N/A	N/A	N/A	N/A
Robert -Control	Pre-IRS v Post-IRS	0	87.88	N/A	N/A	N/A	22.22	N/A	N/A
Chibobo -Sprayed	Pre-IRS v Post-IRS	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Chishi -Control	Pre-IRS v Post-IRS	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Nkana -Sprayed	Pre-IRS v Post-IRS	33.93	28.07	0.89	0.6449	18.33	26.68	1.45	0.2742
Bulaya -Control	Pre-IRS v Post-IRS	50	43.65	0.8	0.2478	100	40.69	0.37	<.0001**
Kawama -Sprayed	Pre-IRS v Post-IRS	30.77	20.87	0.69	0.0016**	0	26.39	N/A	N/A
Mainasoko -Control	Pre-IRS v Post-IRS	33.33	37.59	0.84	0.3574	N/A	47.56	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 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	626	6	0.01	21.33	6.13	431	15	0.03	13.34	13.92
		Post-IRS	1,091	18	0.02	17.55	8.69	1,981	58	0.03	21.63	19.00
	Outdoors	Pre-IRS	363	3	0.01	11.85	2.94	256	2	0.01	16.03	3.76
		Post-IRS	778	5	0.01	9.93	1.91	1,074	22	0.02	13.66	8.40
	Both In/Out	Pre-IRS	989	9	0.01	33.17	9.06	687	17	0.02	29.37	21.80
		Post-IRS	1,869	23	0.01	27.48	10.15	3,055	80	0.03	35.30	27.73
		TOTAL	**2,858**	**32**	**0.01**	**28.64**	**9.62**	**3,742**	**97**	**0.03**	**34.09**	**26.51**
An. gambiae s.l.	Indoor	Pre-IRS	110	1	0.01	0.56	0.15	33	0	0.00	0.20	0.00
		Post-IRS	760	6	0.01	4.67	1.11	699	22	0.03	5.29	4.99
	Outdoor	Pre-IRS	88	0	0.00	0.93	0.00	34	3	0.09	0.46	1.21
		Post-IRS	598	1	0.00	3.74	0.19	573	6	0.01	3.77	1.18
	Both In/Out	Pre-IRS	198	1	0.01	1.49	0.23	67	3	0.04	0.65	0.88
		Post-IRS	1,358	7	0.01	8.41	1.30	1,272	28	0.02	9.06	5.98
		TOTAL	**1,556**	**8**	**0.01**	**7.01**	**1.08**	**1,339**	**31**	**0.02**	**7.35**	**5.11**

*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	Sporozoite rates (%)
Nchelenge	*An. funestus*	532	8	1.5
	An. leesoni	10	0	0.0
	An. gambiae s.s.	91	1	1.1
Milenge	*An. funestus*	217	2	0.9
	An. gambiae s.s.	88	0	0.0
Mambwe	*An. gambiae* s.s.	1	0	0.0
	An. arabiensis	179	0	0.0
Serenje	*An. funestus*	55	0	0.0
Lufwanyama	*An. funestus*	113	2	1.8
	An. leesoni	1	0	0.0
	An. gambiae s.s.	226	3	1.3
	An. arabiensis.	2	0	0.0
Chililabombwe	*An. funestus* s.s.	291	4	1.4
	An. gambiae s.s.	18	1	5.6
	An. arabiensis	1	0	0.0

ANNEX E: INSECTICIDE SUSCEPTIBILITY TEST RESULTS (DECEMBER 2021-MAY 2022)

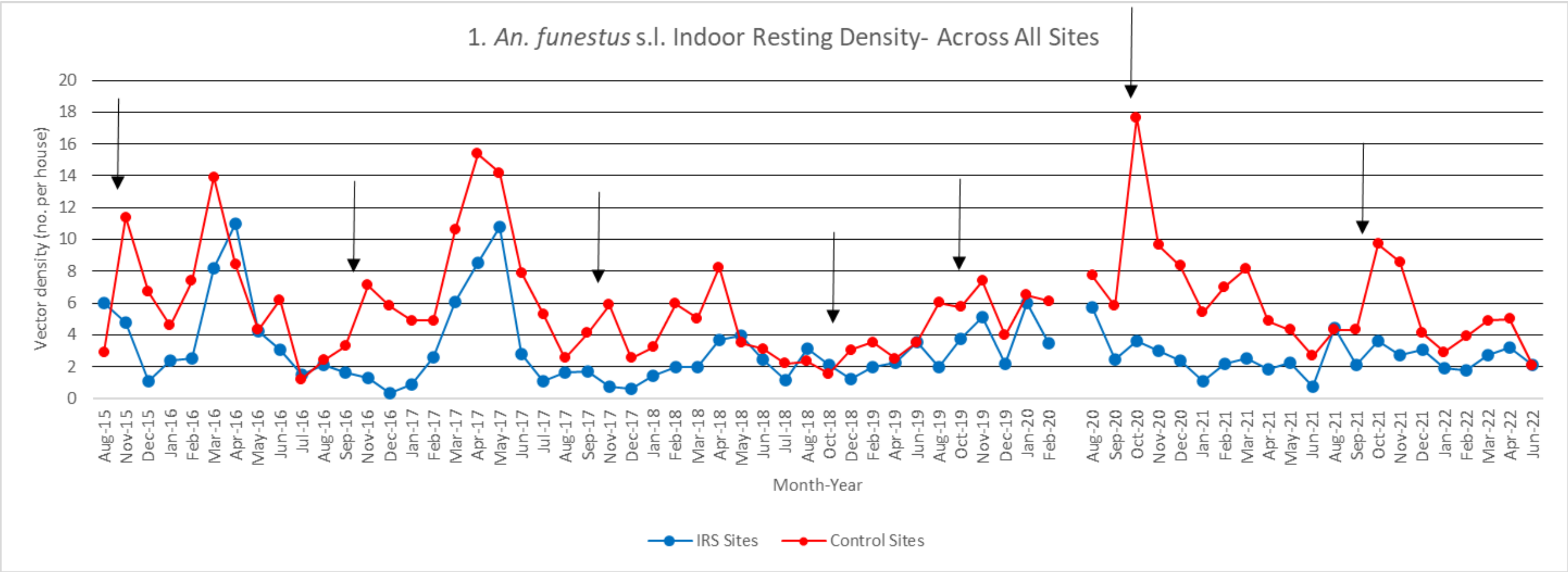
Chemical	Species	Province, District, Sentinel Site	Intervention Status	# Exposed	% Mortality after 24 hrs	% Mortality after 48 hrs	% Mortality after 72 hrs	Interpretation
Alpha-cypermethrin 0.05%	*An. funestus* s.l.	Copperbelt, Lufwanyama, Bulaya	Control	17	100	N/A	N/A	Susceptible
		Luapula, Milenge, Miyambo	Control	17	82	N/A	N/A	Confirmed Resistance
		Luapula, Nchelenge, Manchene	Control	36	83	N/A	N/A	Confirmed Resistance
		Luapula, Nchelenge, Shikapande	Sprayed	136	74	N/A	N/A	Confirmed Resistance
	An. gambiae s.l.	Copperbelt, Lufwanyama, Nkana	Sprayed	35	80	N/A	N/A	Confirmed Resistance
		Copperbelt, Lufwanyama, Bulaya	Sprayed	50	100	N/A	N/A	Susceptible
		Eastern, Katete, Chilowa	Sprayed	200	80	N/A	N/A	Confirmed Resistance
		Luapula, Milenge, Miyambo	Control	35	91	N/A	N/A	Possible Resistance
		Luapula, Nchelenge, Manchene	Control	8	50	N/A	N/A	Confirmed Resistance
Chlorfenapyr (100 ug)	*An. funestus* s.l.	Copperbelt, Lufwanyama, Nkana	Sprayed	20	100	100	100	Susceptible
	An. gambiae s.l.	Copperbelt, Lufwanyama, Nkana	Sprayed	50	98	98	98	Susceptible
		Eastern, Katete, Robert	Control	100	97	100	100	Susceptible
		Luapula, Milenge, Lunga	Sprayed	40	100	100	100	Susceptible
Clothianidin (2%)	*An. funestus* s.l.	Luapula, Milenge, Lunga	Sprayed	37	100	N/A	N/A	Susceptible
		Luapula, Milenge, Miyambo	Control	10	100	N/A	N/A	Susceptible
		Luapula, Nchelenge, Manchene	Control	30	100	N/A	N/A	Susceptible
		Luapula, Nchelenge, Shikapande	Sprayed	45	100	N/A	N/A	Susceptible
	An. gambiae s.l.	Copperbelt, Lufwanyama, Nkana	Sprayed	75	100	N/A	N/A	Susceptible
		Eastern, Katete, Chilowa	Sprayed	80	100	N/A	N/A	Susceptible
		Eastern, Katete, Robert	Control	80	100	N/A	N/A	Susceptible
		Luapula, Milenge, Lunga	Sprayed	125	100	N/A	N/A	Susceptible
		Luapula, Milenge, Miyambo	Control	113	100	N/A	N/A	Susceptible

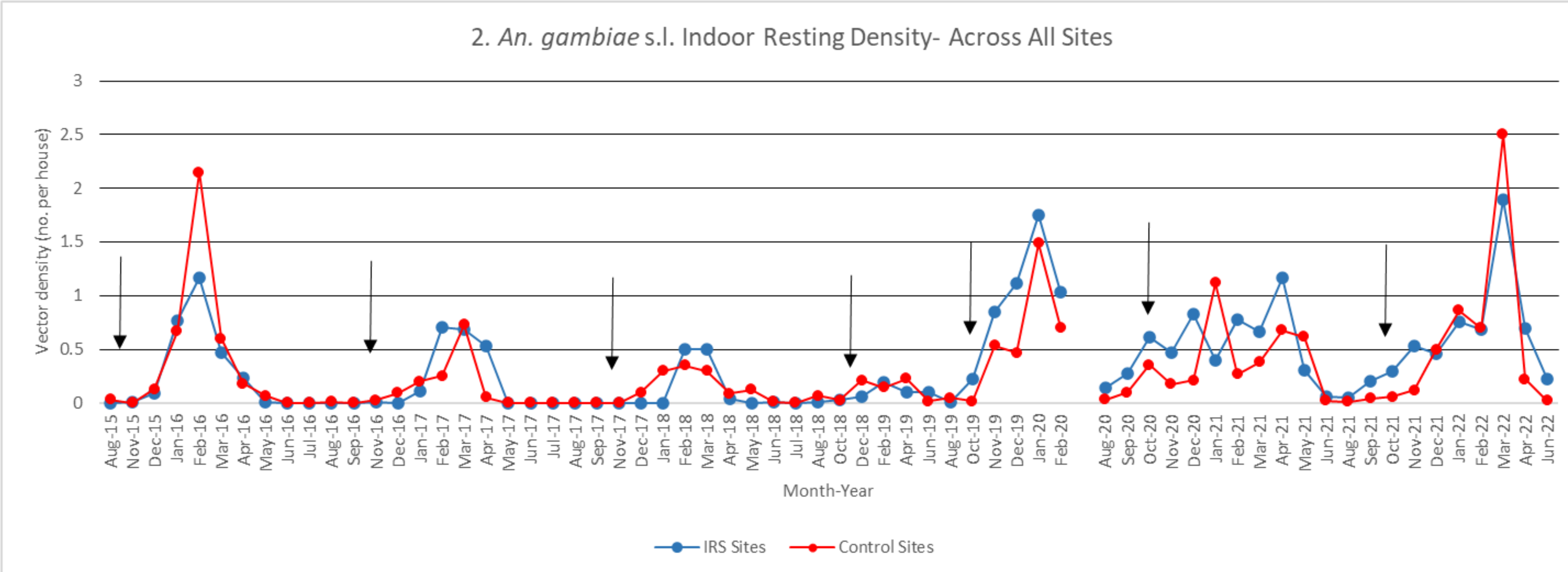
Chemical	Species	Province, District, Sentinel Site	Intervention Status	# Exposed	% Mortality after 24 hrs	% Mortality after 48 hrs	% Mortality after 72 hrs	Interpretation
DDT 4%	*An. funestus* s.l.	Copperbelt, Lufwanyama, Bulaya	Control	25	100	N/A	N/A	Susceptible
		Copperbelt, Lufwanyama, Nkana	Sprayed	75	100	N/A	N/A	Susceptible
		Luapula, Milenge, Lunga	Sprayed	43	100	N/A	N/A	Susceptible
		Luapula, Milenge, Miyambo	Control	43	100	N/A	N/A	Susceptible
		Luapula, Nchelenge, Manchene	Control	10	100	N/A	N/A	Susceptible
		Luapula, Nchelenge, Shikapande	Sprayed	75	87	N/A	N/A	Confirmed Resistance
	An. gambiae s.l.	Copperbelt, Lufwanyama, Nkana	Sprayed	50	96	N/A	N/A	Possible Resistance
		Eastern, Katete, Chilowa	Sprayed	40	100	N/A	N/A	Susceptible
		Eastern, Katete, Robert	Control	40	100	N/A	N/A	Susceptible
Deltamethrin 0.05%	*An. funestus* s.l.	Copperbelt, Lufwanyama, Nkana	Sprayed	16	100	N/A	N/A	Susceptible
		Luapula, Milenge, Lunga	Sprayed	78	90	N/A	N/A	Possible Resistance
		Luapula, Milenge, Miyambo	Control	43	86	N/A	N/A	Confirmed Resistance
		Luapula, Nchelenge, Manchene	Control	98	90	N/A	N/A	Possible Resistance
		Luapula, Nchelenge, Shikapande	Sprayed	182	87	N/A	N/A	Confirmed Resistance
	An. gambiae s.l.	Copperbelt, Lufwanyama, Nkana	Sprayed	36	97	N/A	N/A	Possible Resistance
		Eastern, Katete, Chilowa	Sprayed	80	95	N/A	N/A	Possible Resistance
		Eastern, Katete, Robert	Control	100	100	N/A	N/A	Susceptible
		Luapula, Milenge, Lunga	Sprayed	12	100	N/A	N/A	Susceptible
		Luapula, Milenge, Miyambo	Control	25	100	N/A	N/A	Susceptible
		Luapula, Nchelenge, Manchene	Control	16	88	N/A	N/A	Confirmed Resistance
		Luapula, Nchelenge, Shikapande	Sprayed	10	80	N/A	N/A	Confirmed Resistance
Permethrin 0.75%	*An. funestus* s.l.	Luapula, Milenge, Lunga	Sprayed	25	100	N/A	N/A	Susceptible
		Luapula, Nchelenge, Manchene	Control	46	87	N/A	N/A	Confirmed Resistance
		Luapula, Nchelenge, Shikapande	Sprayed	36	89	N/A	N/A	Confirmed Resistance
	An. gambiae s.l.	Copperbelt, Lufwanyama, Nkana	Sprayed	17	100	N/A	N/A	Susceptible
		Eastern, Katete, Chilowa	Sprayed	100	88	N/A	N/A	Confirmed Resistance
		Eastern, Katete, Robert	Control	100	92	N/A	N/A	Possible Resistance
Pirimiphos-methyl 0.25%	*An. funestus* s.l.	Copperbelt, Lufwanyama, Bulaya	Control	112	100	N/A	N/A	Confirmed Resistance
		Copperbelt, Lufwanyama, Nkana	Sprayed	95	100	N/A	N/A	Confirmed Resistance
		Luapula, Nchelenge, Manchene	Control	44	100	N/A	N/A	Confirmed Resistance
		Luapula, Nchelenge, Shikapande	Sprayed	48	100	N/A	N/A	Confirmed Resistance

Key: <90% mortality (confirmed resistance), 90-97% mortality (possible 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 2015-2022*

[Arrow indicates when IRS was implemented. The data gap between February 2020 to August 2020 was a result of project activity restrictions occasioned by the COVID-19 outbreak.]





2. An. gambiae s.l. Indoor Resting Density- Across All Sites
Vector density (no. per house)
3
2.5
2
1.5
1
0.5
0
Aug-15
Nov-15
Dec-15
Jan-16
Feb-16
Mar-16
Apr-16
May-16
Jun-16
Jul-16
Aug-16
Sep-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
Jul-17
Aug-17
Sep-17
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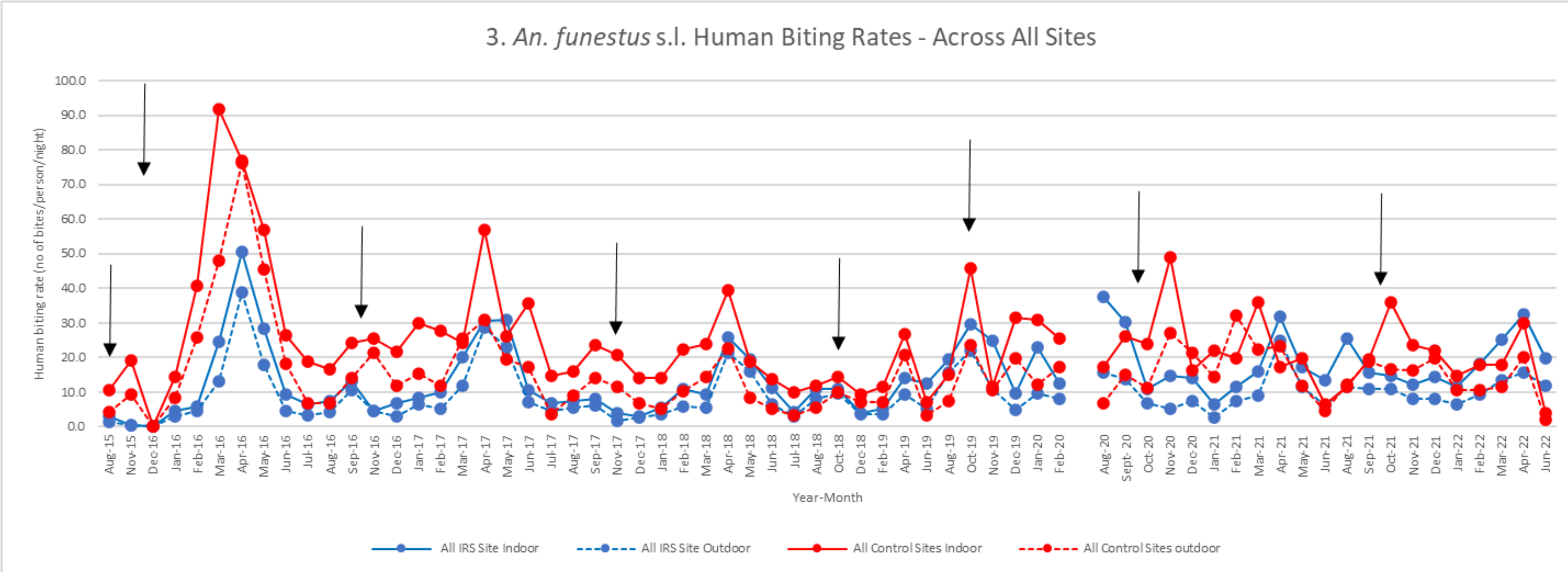
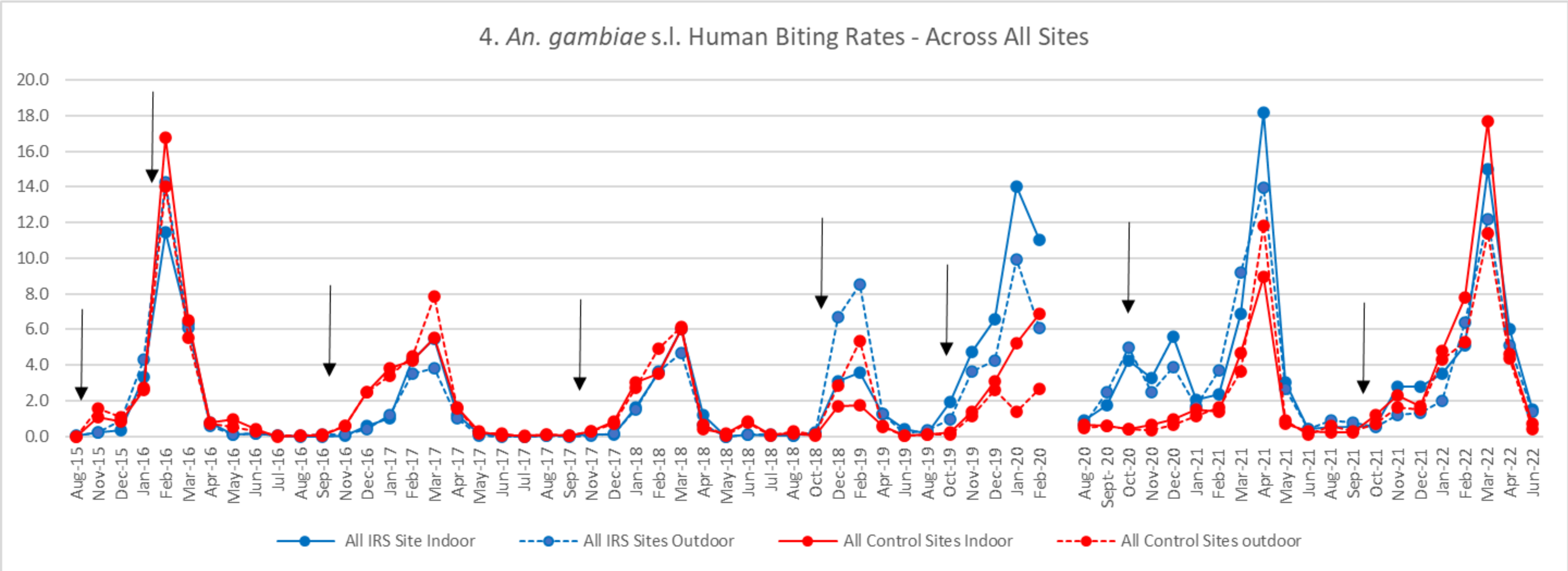
Feb-21
Mar-21
Apr-21
May-21
Jun-21
Aug-21
Sep-21
Oct-21
Nov-21
Dec-21
Jan-22
Feb-22
Mar-22
Apr-22
Jun-22
Month-Year
IRS Sites
Control Sites


3. An. funestus s.l. Human Biting Rates - 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 and the insecticides used for IRS. DDT was used in GRZ supported districts only:

2015/2016: Mwense, Milenge, Kasama, Isoka, Katete, Serenje (Organophosphate-Actellic)
2016/2017: Mwense, Milenge, Kasama, Isoka, Katete, Serenje (Organophosphate-Actellic)
2017/2018: Mwense, Milenge, Kasama, Isoka, Katete, Serenje (Organophosphate-Actellic)
2018/2019 Mwense, Milenge, Kasama, Isoka, Mambwe, Katete, Serenje (Organophosphate-Actellic and Clothianidin)
2019/2020: Nchelenge. Milenge. Mambwe, Katete, Serenje, Lufwanyama, Chililabombwe (DDT and Clothianidin)
2020/2021: Nchelenge. Milenge. Mambwe, Katete, Serenje, Lufwanyama, Chililabombwe (DDT and Clothianidin)
2021/2022: Nchelenge. Milenge. Mambwe, Katete, Serenje, Lufwanyama, Chililabombwe (DDT and Clothianidin)