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# THE PMI VECTORLINK PROJECT ZAMBIA

## ANNUAL ENTOMOLOGY REPORT

## JUNE 2019 - AUGUST 2020

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# THE PMI VECTORLINK PROJECT ZAMBIA ANNUAL ENTOMOLOGY REPORT

## JUNE 2019 – AUGUST 2020

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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 |
| **GRZ** | Government of the Republic of Zambia |
| **GPM** | Global Precipitation Measurement |
| **HBR** | Human Biting Rate |
| **HLC** | Human Landing Catch |
| **IRS** | Indoor Residual Spraying |
| **ITN** | Insecticide-treated net |
| **NMEC** | National Malaria Elimination Centre |
| **NMEP** | National Malaria Elimination Program |
| **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 distribution of insecticide-treated nets (ITNs) 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. VectorLink Zambia supported the 2019 IRS campaign from October 2 to November 30, 2019, using two clothianidin-based insecticides (SumiShield and Fludora Fusion) across 20 districts in Luapula, Eastern, and Copperbelt Provinces. The project sprayed a total of 536,983 structures out of 598,732 structures found by spray operators in targeted districts, resulting in a coverage rate of 90%.

Entomological monitoring associated with the 2019 IRS campaign included vector surveillance and insecticide resistance monitoring, assessment of IRS quality of spray, and insecticide residual efficacy. Vector surveillance to assess the impact of IRS was conducted from June 2019 to February 2020 in 14 sentinel sites, including five IRS and five control sites across the three provinces where IRS was supported by VectorLink. In addition, for historical reasons and to provide addition support for the national entomologic surveillance program, VectorLink supported an additional two sites in Central Province and two sites in Luapula Province—one IRS site sprayed by the Government of Zambia (GRZ) and one control site in each. Mosquitoes were collected using pyrethrum spray catches (PSCs) and human landing catches (HLCs). Baseline data were collected in June and August 2019 and post-intervention data collections started in October 2019 and were conducted monthly or bi-monthly[1]. Spray quality was assessed 24 hours after IRS at seven sites supported by VectorLink, followed by monthly assessments of the insecticide decay on walls at five sites. Insecticide susceptibility tests were conducted in the 14 sites between December 2019 and April 2020 using the World Health Organization (WHO) tube tests.

Data from June 2019 to February 2020 indicate that *Anopheles funestus* s.l. was the most abundant (45,637, or 59.7%) *Anopheles* species collected. A total of 6,238 *An. gambiae* s.l. were also caught (8.2%). *An. funestus* s.l. and *An. gambiae* s.l. indoor resting densities increased immediately after IRS at both IRS and non-IRS sites. *An. funestus* density increased from 3.7 to 5.1 vectors per house at the IRS sites and from 5.7 to 7.4 vectors per house at the non-IRS sites. However, reductions in *An. funestus* s.l. indoor densities were observed at both IRS and non-IRS sites two months after IRS to 2.2 and 4.0 vectors per house per day, respectively. At the IRS sites, the average human biting rate of *An. funestus* s.l. indoors reduced from 19.3 bites per person per night before IRS to 9.7 bites per person per night two months after IRS. Outdoors, mean *An. funestus* s.l. human biting rates reduced from 15.6 bites per person per night before IRS to 4.8 bites per person per night two months after IRS, while human biting rates increased after IRS at the control sites. Overall biting rates for *An. gambiae* s.l. increased after IRS at both IRS and the control sites. *An. arabiensis* was the main vector among the *An. gambiae* s.l. populations while *An. rivulorum* was the predominant species in the *An. funestus* group. Reduction in parity rate was observed for both *An. funestus* s.l. and *An. gambiae* s.l. after IRS compared to before IRS, an indication that, as desired, the vectors are not surviving long enough to transmit malaria. There were more sporozoite positive *An. funestus* s.l. at the control sites compared to the IRS sites. The predominance shown by *An. rivulorum* and the sporozoite infectivity rate observed indicates that this species should be considered as an important malaria vector in the area.

There was 100 percent mortality of susceptible *An. gambiae* s.s. mosquitoes exposed to walls sprayed with SumiShield and Fludora Fusion insecticides at the time of the 2019 IRS campaign (T0) in all seven districts

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

(Nchelenge, Mambwe, Chipata, Katete, Masaiti, Lufwanyama, and Chililabombwe). The observed mortality implies good spray quality during the campaign. As of August 2020, based on longitudinal data collected of the effectiveness of the two insecticides deployed in the 2019 IRS campaign on sprayed surfaces, the effective duration of both insecticides is 10 months.

*An. funestus* s.l. and *An. gambiae* s.l. were susceptible to clothianidin, chlorfenapyr, and pirimiphos-methyl. Both species showed susceptibility and resistance at different locations to dichlorodiphenyltrichloroethane (DDT) and bendiocarb. *An. gambiae* s.l. were fully susceptible to deltamethrin while *An. funestus* s.l. were resistant or suspected to be resistant to deltamethrin.

Based on these results, there are several overarching recommendations for the way forward. Consideration should be given to integrated vector management wherein all malaria transmission zones are targeted for ITNs while IRS is deployed only in high transmission zones, where effective and practical. Larval source management could be considered for deployment in some well-characterized focal areas to target vectors that do not frequent the indoor environment and as a complement existing vector control interventions. If faced with the decision to either rotate to a susceptible shorter duration insecticide such as pirimiphos-methyl or continue the deployment of a clothianidin-based insecticide for another year, the latter should be considered for IRS. Due to the continued resistance of local vectors to pyrethroid insecticides, we recommend deployment of piperonyl butoxide (PBO) nets or nets with dual active ingredients (that is, pyrethroid plus a pyrrole) 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) is one of the primary vector control interventions of the Zambian National Malaria Elimination Program (NMEP). From October 2 to November 30, 2019, VectorLink conducted IRS in 20 districts in three provinces (Luapula, Eastern, and Copperbelt), targeting 597,625 structures using a clothianidin-based insecticide. A total of 536,983 structures were sprayed out of 598,732 structures found by spray operators in the targeted districts, accounting for a coverage rate of 90%.

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. The U.S. President's Malaria Initiative (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 the Africa Indoor Residual Spraying (AIRS) Project starting in 2014 and transitioned to 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 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 June 2019 to August 2020 and is linked to the 2019 IRS campaign. It presents all entomological monitoring activities conducted by PMI VectorLink Zambia and discusses the implications of the results obtained. The COVID-19 pandemic affected some of the entomological monitoring activities during the reporting period. Entomological monitoring activities were suspended from March to July 2020. With clearance from the NMEP and PMI, VectorLink Zambia resumed insecticide residual efficacy monitoring in April 2020, given the importance of this data in evaluating the clothianidin-based insecticides (SumiShield and Fludora Fusion) used during the 2019 campaign. Insecticide resistance monitoring was also allowed to continue at sites where it was possible to obtain mosquito larvae from larval habitats without entering households for adult mosquito collections. Vector surveillance activities resumed in August 2020 and the data collected will be reported in 2020/2021.

Table 1 below outlines the entomological indicators covered in this report. Indicators are delineated as basic or advanced per PMI's 2019 Technical Guidance[2].

[2] PMI Technical Guidance (August 2019) https://www.pmi.gov/docs/default-source/default-document-library/tools-curricula/pmi-technical-guidance.pdf?sfvrsn=20.

**Table 1: Basic and Advanced Entomological Indicators by Collection Method and Frequency of Collection**

| Indicator | Collection Methods | Frequency | Parameters Measured |
|---|---|---|---|
| **Basic Indicators** | | | |
| Vector species composition | PSC, HLC | Every 1-2 months* | Percentage of mosquito species captured |
| Vector abundance | PSC, HLC | Every 1-2 months* | Indoor resting density and human biting rate |
| Seasonality | PSC, HLC | Every 1-2 months* | Indoor resting density and human biting rate |
| Insecticide susceptibility | Larval and adult collections | Once per year | Percentage mortality at 24hrs or at seven days, depending on insecticide type |
| Spray quality assurance | Insectary colony mosquitoes | Once per year, within 48 hours of spray | Percentage mortality at seven days |
| Residual efficacy monitoring | Insectary colony mosquitoes | Monthly[1] | Percentage mortality at seven days |
| Vector feeding time | HLC | Every 1-2 months* | Hourly human biting rates |
| Vector feeding location | HLC | Every 1-2 months* | Indoor and outdoor biting rates |
| **Advanced Indicators** | | | |
| Sporozoite rate | HLC | Every 1-2 months* | Percent positive for sporozoites |
| Entomological Inoculation Rate | HLC | Every 1-2 months* | Product of biting rate and sporozoite rate |
| Parity rate | HLC | Every 1-2 months* | Percentage parous |

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 in all sites monthly from June to October 2019. Starting in November 2019, data were collected monthly at three districts only (Nchelenge, Mambwe, and Lufwanyama), while collections occurred every two months at the other four districts (Milenge, Katete, Serenje, and Chililabombwe).

# 2. MATERIALS AND METHODS

## 2.1 MONITORING SITES

VectorLink Zambia conducted entomological surveillance activities from June 2019 to February 2020 and insecticide resistance monitoring activities from December 2019 to May 2020 in 14 sentinel sites in five PMI-supported IRS districts (Nchelenge, Mambwe, Katete, Lufwanyama, and Chililabombwe) and two districts that were sprayed by the GRZ (Milenge and Serenje). Each district consisted of two sentinel sites—one sprayed (sites sprayed with either SumiShield, Fludora Fusion, or DDT in the 2019 IRS campaign) and one unsprayed (control site with no IRS)—to enable direct comparison.

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.

IRS quality of spray checks were done at seven sprayed sites at the start of the 2019 IRS campaign in October 2019. Longitudinal monitoring of the duration of residual efficacy of the insecticides on walls was conducted at five of the seven sprayed sites from November 2019 to August 2020. Further details of the monitoring sites according to the activities conducted are shown in Table 2. Figure 1 shows the location of the sentinel sites in their respective districts.

**Table 2: Entomological Monitoring Sites**

| Province | District | Health Facility Catchment Area | Sentinel Site (Village) | Spray Status (Distance to Nearest Sprayed Community) | Percent of Local Households Targeted for IRS by PMI/VL in 2019* |
|---|---|---|---|---|---|
| **Vector Surveillance and Insecticide Resistance Monitoring** | | | | | |
| Luapula | Nchelenge | Lushiba | Shikapande | Sprayed with Fludora Fusion | 100% |
| | | Kafutuma | Manchene | Non-sprayed control (3km) | 0% |
| | Milenge | East Seven | Lunga | Sprayed with DDT | 100% (by GRZ) |
| | | East Seven | Miyambo | Non-Sprayed control (7km) | 0% |
| Eastern | Mambwe | Chikowa | Chikowa | Sprayed with SumiShield | 100% |
| | | Chikowa | Chasela | Non-Sprayed control (6km) | 0% |
| | Katete | Katiula | Chilowa | Sprayed with SumiShield | 100% |
| | | Kamphambe | Robert | Non-Sprayed control (10km) | 0% |

| Province | District | Health Facility Catchment Area | Sentinel Site (Village) | Spray Status (Distance to Nearest Sprayed Community) | Percent of Local Households Targeted for IRS by PMI/VL in 2019* |
|---|---|---|---|---|---|
| Central | Serenje | Chibobo | Chibobo | Sprayed with DDT | 100% (by GRZ) |
| | | Chibobo | Chishi | Non-Sprayed control (5km) | 0% |
| Copperbelt | Lufwanyama | Nkana | Nkana | Sprayed with Fludora Fusion | 100% |
| | | Bulaya | Bulaya | Non-Sprayed control (4km) | 0% |
| | Chililabombwe | Kawama | Kawama | Sprayed with Fludora Fusion | 100% (Rural/Peri-urban) |
| | | Kawama | Mainasoko | Non-Sprayed control (6km) | 0% |
| **IRS Quality Assurance (QA) and Insecticide Residual Efficacy Monitoring** | | | | | |
| Luapula | Nchelenge | Lushiba | Shikapande | Sprayed with Fludora Fusion | 100% |
| Eastern | Mambwe | Chikowa | Chikowa | Sprayed with SumiShield | 100% |
| Eastern | Chipata | Namseche | Margazine (QA only) | Sprayed with SumiShield | 100% |
| Eastern | Katete | Kafunkha | Kafunkha | Sprayed with SumiShield | 100% |
| Copperbelt | Masaiti | Chilese | Shikapansula (QA only) | Sprayed with Fludora Fusion | 100% |
| Copperbelt | Lufwanyama | Nkana | Nkana | Sprayed with Fludora Fusion | 100% |
| Copperbelt | Chililabombwe | Kawama | Kawama | Sprayed with Fludora Fusion | 100% (Rural/Peri-urban) |

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

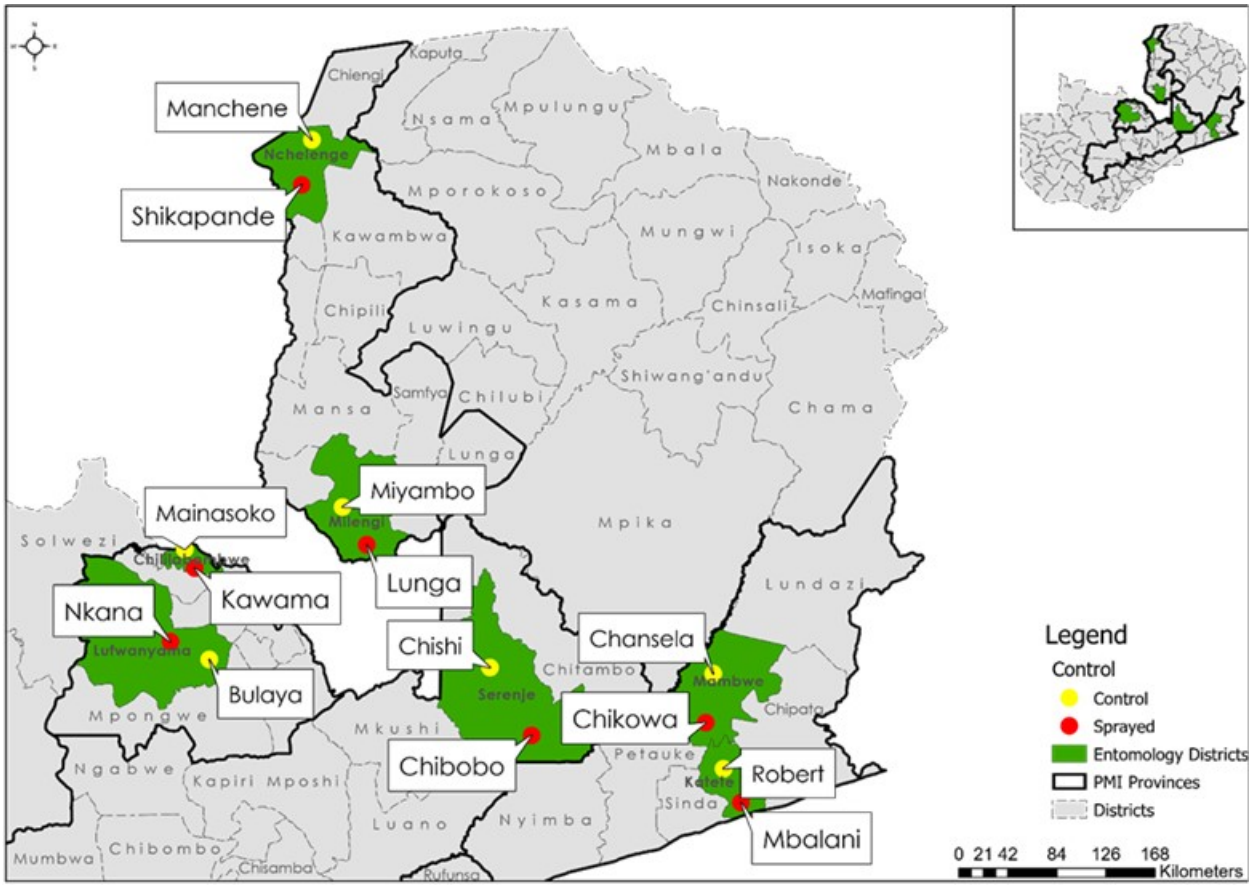



**Figure 1: Geographical Locations of PMI Supported Entomological Sentinel Sites in Zambia**

## 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. Adult mosquitoes were collected from all sites once every two months from June to October 2019 and then monthly at three districts (Nchelenge, Mambwe, and Lufwanyama) while bimonthly collections continued at the other four districts (Milenge, Katete, Serenje, and Chililabombwe). The initial plan to conduct monthly collections in all seven districts was revised in October 2019 based on recommendations from the field visit by the U.S. Centers for Disease Control and Prevention (CDC) Entomology backstop for Zambia. As a result and based on available funding, starting in November 2019, the project continued conducting monthly collections in three priority sentinel sites (one in each of the three provinces supported by PMI) and shifted collections in the other four districts to every other month. Vector surveillance did not occur in some districts in February 2020 due to the gassing incidents and risk of mob violence in parts of Zambia, and was subsequently suspended in all districts in March 2020 due to the COVID-19 pandemic. Pyrethrum spray collection (PSC) (SOP 03/01)[3], and HLCs (SOP 02/01)[4] were the collection methods employed in the vector surveillance activities (see Table 3).

[3] SOP 03/01. Pyrethrum Spray Catch, January 2020.
[4] SOP 02/01. Indoor and Outdoor Human Landing Catch (HLC), January 2020.

Entomological monitoring to assess the impact of IRS on malaria vectors started four months prior to the implementation of IRS at the intervention sites except for the site in the GRZ-supported district of Serenje which was sprayed in December 2019 (all other sites were sprayed in October 2019).

**Table 3: Adult Mosquito Collection Methods for Vector Surveillance**

| Method | Time | Frequency* | Sample |
|---|---|---|---|
| PSC | 4:00 a.m. to 6:00 a.m. | Every 1-2 months | 15 houses per site, five houses per day |
| HLC | 6:00 p.m. to 8:00 a.m. | Every 1-2 months | Eight houses, two consecutive nights per house, indoors and outdoors |

*The initial plan to conduct collections every other month in all seven districts was revised in October 2019 based on recommendations from a field visit by the U.S. Centers for Disease Control and Prevention (CDC) Entomology backstop for Zambia. It was determined together with PMI that based on available funding, monthly collections should be done in three districts (one in each of the three provinces supported by PMI

### 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 a.m. 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. A pyrethroid-based insecticide product, Raid (SC Johnson & Son S.A. Ltd), in pressurized 300ml spray cans was 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 1.15% w/w. The eaves, windows, and other openings in the house were sprayed followed by the interior and roof of the house. Ten minutes after spraying, all mosquitoes knocked down by the insecticide were collected using white sheets placed on the floor before spraying.

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

Eight 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 two 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 volunteers trained on the HLC technique served as the collectors 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 am. The pair was replaced by another pair of volunteers from 1:00 to 8:00 a.m., meaning four volunteers per house per night participated in collections from 6:00 p.m. to 8:00 a.m.

During collection, each collector sat on a small chair and exposed their legs from the ankle to the knee (collectors wore long sleeved shirts to cover their arms). When a mosquito landed on their legs, they used a flashlight to locate the mosquito and a mouth aspirator to collect it and carefully transfer it into paper cups labelled with the hour of collection, site name, and house ID. For each hour of collection, the volunteers collected mosquitoes for 50 minutes and took a 10-minute break. During breaks, the collectors swapped positions—that is, the outdoor collector moved indoors and the indoor collector relocated outdoors as a mitigation measure against collector bias. All community-based volunteers involved in the HLCs were provided malaria chemoprophylaxis with Deltaprim (pyrimethamine and dapsone).

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).

### 2.2.3 Laboratory Analysis-Advanced Indicators

Mosquitoes collected by HLCs were killed using cotton wool soaked in formalin to enable pre-laboratory handling. *Anopheles* mosquitoes collected by PSC and HLC were identified morphologically by species using the Gilles and Coetzee 1987 identification key[5], and 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 PCR[6,7], and 2) detect circumsporozoite proteins of *Plasmodium falciparum* sporozoites using Enzyme-linked Immunosorbent Assay (ELISA)[8].

## 2.3 Data Presentation and Statistical Analysis

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 advanced indicators sibling species composition, human blood index, Sporozoite rate and EIR, data was categorized into pre-IRS period (June-August-October 2020) and post-IRS (October 2019-February 2020) and transmission indicators between these two periods were compared.

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 four main parameters: number of indoor resting vectors, number of gravid vectors, number of human biting vectors, and number of parous vectors with separate analyses for *An. funestus* s.l. and for *An. gambiae* s.l.

## 2.4 Quality Assurance of IRS and Insecticide Decay Rate

Cone bioassays (SOP 09/01[9]) using susceptible *An. gambiae* Kisumu strain mosquitoes were conducted during the IRS campaign to confirm the quality of spray in seven districts: Nchelenge, Mambwe, Chipata, Katete, Masaiti, Lufwanyama and Chililabombwe. Subsequently, cone bioassays were conducted monthly to assess

[5] Gillies MT and Coetzee M. 1987. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region). South African Institute for Medical Research, 55: 33–81.
[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.
[8] 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.
[9] SOP 009. Wall Cone Bioassay of Sprayed Surfaces for Quality Assurance and Residual Efficacy Monitoring of IRS, January 2020.

the residual efficacy or decay rate on the insecticide on the walls in five districts (Nchelenge, Mambwe, Katete, Lufwanyama, and Chililabombwe). Details of the bioassay activities are presented in Table 4. 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. When control houses were not available, an untreated surface carried by the field technicians was used for the purpose. A total of 42 houses were involved in the quality assurance activity—18 houses in the SumiShield sprayed areas and 24 houses in the Fludora Fusion sprayed areas. Cone bioassays were conducted 24 to 48 hours after spraying and within two weeks of the spray campaign (T0) to gauge quality of spray. Longitudinal monitoring of the insecticide decay rate on walls after IRS was done in 30 houses (six houses each in Mambwe and Katete where SumiShield was sprayed, and six houses each in Nchelenge, Lufwanyama, and Chililabombwe districts where Fludora Fusion was used). The cone bioassays were repeated monthly until mosquito mortality dropped below 80% for two consecutive months.

The cone bioassays were conducted according to standard test procedures. The cones were placed on the treated walls at three locations: 0.5m, 1m, and 1.5m above the ground. Ten female *An. gambiae* s.s. Kisumu strain mosquitoes were introduced per cone and exposed for 30 minutes. A fourth replicate of 10 mosquitoes was placed in a paper cup one meter above the floor and about 0.1 meter from the sprayed wall to assess the fumigant (airborne) effect of the insecticide. After 30 minutes of exposure, the mosquitoes were transferred to insecticide-free paper cups supplied with 10% sugar solution. The cups were then placed in plastic buckets covered with a damp napkin to create favorable humidity for the mosquitoes and held for up to seven days. The number of mosquitoes knocked down after 30 minutes and 60 minutes and the number dead after every 24-hour holding period were recorded up to seven days. When the mortality of the control was between 5-20%, corrected mortality was determined using Abbot's formula.

Fumigant effect refers to the release of the insecticide from the sprayed wall into the air (airborne) which produces a lethal effect on mosquitoes flying inside the house or resting on other (non-sprayed, insecticide-free) surfaces in the house. Monitoring of fumigant effect has been a part of VectorLink's bioassay procedures since the deployment of pirimiphos-methyl due to documented airborne effect of this insecticide. The procedure was extended to the new neonicotinoid insecticides to determine if these new products also exhibit the fumigant effect. Data from multiple countries has indicated some level of airborne effect of these products[10]; the consensus is to continue monitoring to obtain adequate data on the duration of this phenomenon.

**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 campaign at seven sites | Eight houses per site (sprayed: three mud and three cement; unsprayed: one mud and one cement) |
| Monitoring of Insecticide Decay rate on walls | Monthly, until exposed mosquito mortality falls below 80% for two consecutive months at five sites | Eight houses per site (sprayed: three mud and three cement; unsprayed: one mud and one cement) |

[10] https://www.pmi.gov/resource-library/partner-reports [Rwanda End of Spray Report, 2020; Zimbabwe End of Spray Report, 2020; Ghana End of Spray Report, 2019; Mozambique Entomological Monitoring Annual Report, 2019]

## 2.5 INSECTICIDE RESISTANCE MONITORING

Susceptibility of *An. funestus* s.l. and *An. gambiae* s.l. mosquitoes to the insecticides used in IRS, DDT (an organochlorine), deltamethrin (a pyrethroid), SumiShield 50WG (a new insecticide formulation from Sumitomo Chemical—with the neonicotinoid insecticide clothianidin as the active ingredient), and in ITNs (deltamethrin) was assessed at sites in all entomological monitoring sentinel districts. A new product chlorfenapyr (a pyrrole insecticide) awaiting WHO prequalification for IRS was also tested. Given the susceptibility of the mosquitos shown to DDT at some sites in Zambia in 2018, the GRZ deployed DDT in specific areas of the country during the 2019 IRS campaign. Clothianidin is the main active ingredient in the two chemicals used for IRS by VectorLink in 2019 (SumiShield and Fludora Fusion), Fludora Fusion also contains deltamethrin. Other insecticides including pirimiphos-methyl (an organophosphate) and bendiocarb (a carbamate) were also tested in instances where this was possible.

### 2.5.1 WHO SUSCEPTIBILITY TESTS

WHO susceptibility tests (SOP 06/01)[11] were performed on 2-5 day-old unfed adult *An. funestus* s.l. and *An. gambiae* s.l. mosquitoes collected from the 14 surveillance sentinel sites. The mosquitoes were sampled either as larvae or pupae collected from larval habitats and reared to adults or wild unfed female mosquitoes collected from houses using battery-operated CDC backpack and Prokopack aspirators. The mosquitoes were exposed to diagnostic doses of various insecticides using insecticide-impregnated papers, as described by WHO guidelines. Susceptibility of *An. funestus* s.l. and *An. gambiae* s.l. to clothianidin 2.0% (a neonicotinoid), DDT 4.0% (an organochlorine), and deltamethrin 0.05% (a pyrethroid), pirimiphos methyl 0.25% (an organophosphate) were tested in select sentinel sites.

The exposure time was 60 minutes, after which mosquitoes were transferred into the holding tubes and provided with 10% sugar solution. For the clothianidin tests, mortality was recorded after 24 hours, and again after two, three, four, five, six, and seven days while, for the other insecticides, mortality was recorded after 24 hours only. Mortality for clothianidin-exposed mosquitoes is recorded over a longer period due to the slow-acting nature of the insecticide on mosquitoes (24 hours, 48 hours, and 72 hours). The sugar solution was changed daily during the holding periods. Susceptibility tests were done from December 2019 to April 2020.

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

### 2.5.2 CDC BOTTLE ASSAYS

CDC bottle assays were used to assess the susceptibility status of *An. funestus* s.l. and *An. gambiae* s.l. to chlorfenapyr (100 µg) at some sites. The standard CDC bottle assay procedures were followed[12]; the exposure time was 60 minutes and the mortality was recorded 1 hour, 24 hours, 48 hours, and 72 hours after exposure. The bottles were coated each month with technical grade chlorfenapyr supplied by BASF at the NMEC laboratory and transported to the field in compartmentalized cardboard boxes for the assays. Each bottle was used a maximum of three times and were returned to Lusaka for cleaning and reuse.

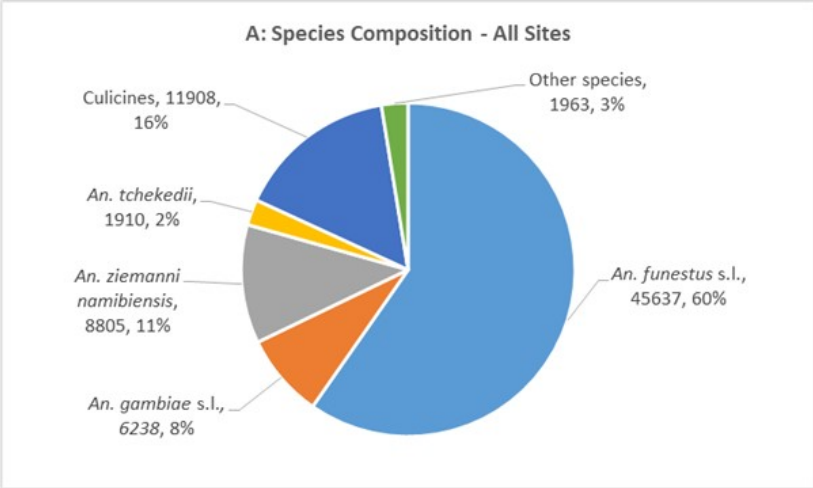
[11] SOP 06/01. Insecticide susceptibility test, intensity, and synergist assay using WHO test kits, January 2020.
[12] SOP 04/01 Susceptibility testing, resistance intensity and synergist assays using the CDC bottle bioassay, January 2020.

# 3. RESULTS

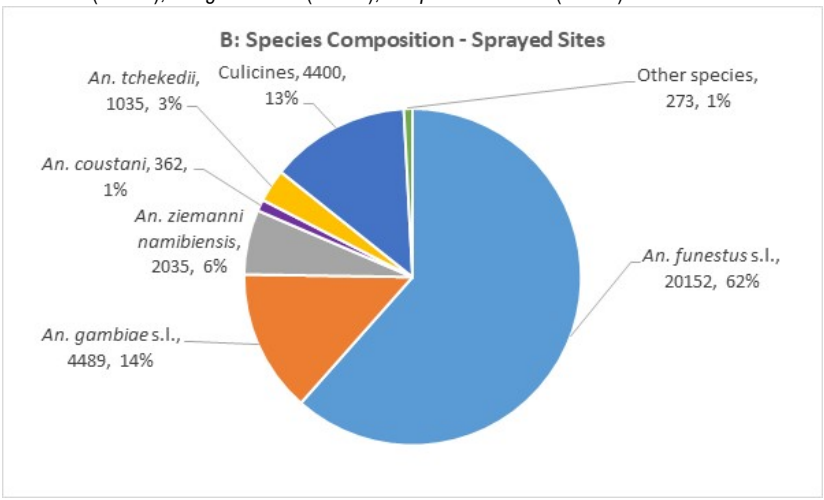
Results from all entomological monitoring activities conducted during the period June 2019 to August 2020 are presented below. Vector surveillance by HLC and PSC were conducted bimonthly as well as monthly from June 2019 to February 2020 in the sentinel districts to assess vector species composition, density, and behavior. The 2019 IRS campaign began in October 2019, and thus baseline vector surveillance data was collected in June and August 2019, and post-IRS data was collected from October 2019 to February 2020. Due to the COVID-19 pandemic, entomological monitoring activities were suspended in March 2020 and no HLCs or PSCs were done from March to June 2020 (the planned end date for vector surveillance in the 2019/2020 reporting period). Laboratory analysis for measuring advanced entomological indicators on the 2019/2020 mosquito samples commenced in March 2020 after completing the 2018/2019 backlog mosquito samples. Restrictions imposed on the number of staff that can work in the laboratory at NMEC (a COVID-19 mitigation measure) affected the schedule for processing the 2019/2020 mosquito samples with fewer samples analyzed at the time of reporting. Residual efficacy monitoring commenced in October 2019 and continued monthly up to August 2020 except for March when the activity was temporarily halted due to COVID-19. Insecticide resistance tests were performed from December 2019 until April 2020. Tests in March and April 2020 were restricted to areas were test mosquitoes could be obtained from larval habitats due to restrictions on house entry for adult collection as a result of COVID-19.

## 3.1 SPECIES COMPOSITION

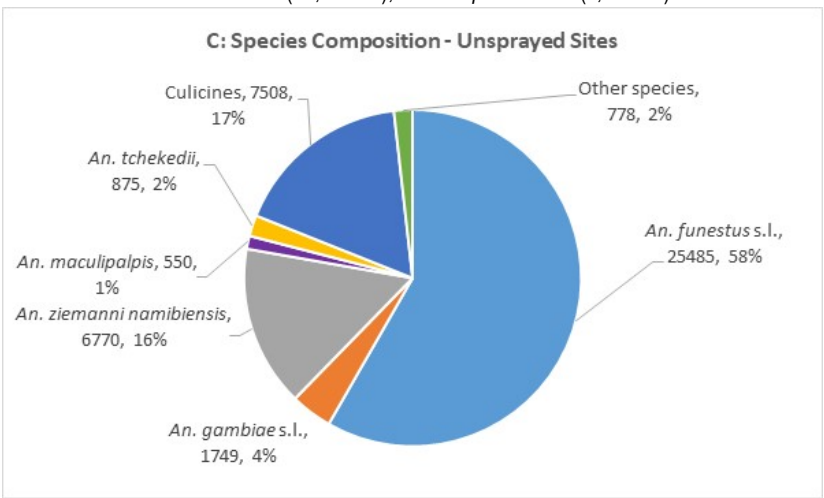
A total of 76,461 mosquitoes were collected by HLC and PSC during the reporting period. *An. funestus* s.l. was the most abundant (59.7%), followed by culicines (15.6%), *An. ziemanni namibiensis* (11.5%), *An. gambiae* s.l. (8.2%), and *An. tchekedii* (2.5%). Other species (*An. coustani*, *An. maculipalpis*, *An. squamosus*, *An. rufipes*, *An. argentiolobatus*, *An. gibbinsi*, *An. pretoriensis,* and *An. tenebrosus*) together accounted for 2.6% of the total collected (Fig. 2A). Figures 2B and 2C display mosquito species composition at the sprayed and unsprayed sites, respectively. The proportion of *An. funestus* s.l. was 61.5%, at the sprayed sites and 58.3% at the unsprayed sites, while that of *An. gambiae* s.l. was 13.7% at the sprayed sites compared to 4.0% at the unsprayed sites. Details of the numbers and types of mosquitoes collected by the different collection methods in each sprayed and unsprayed sentinel site and mosquito species composition by district are provided in Annex 1 and 2, respectively.




Other species: *An. coustani* 627 (0.82%), *An. maculipalpis* 611 (0.80%), *An. squamosus* 371 (0.49%), *An. rufipes* 169 (0.22%), *An. argentiolobatus* 71 (0.09%), *An. gibbinsi* 45 (0.06%), *An. pretoriensis* 40 (0.05%) and *An. tenebrosus* 29 (0.04%)



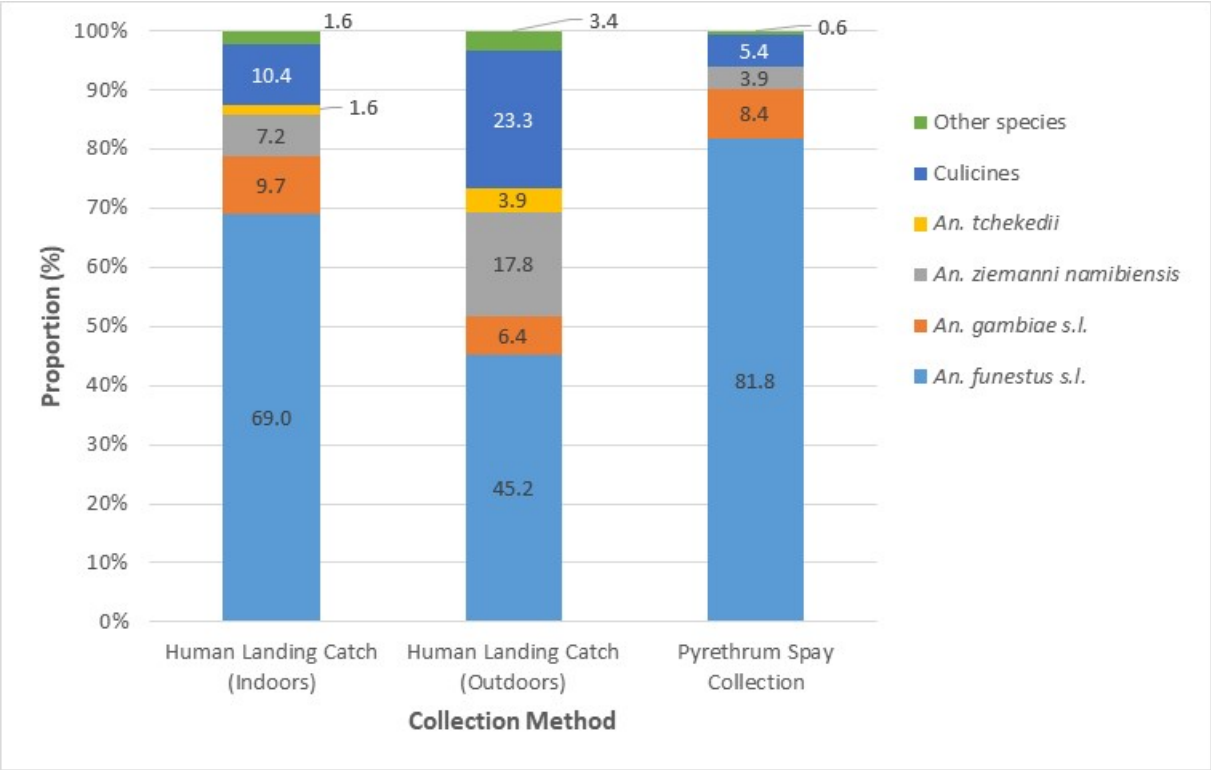

Other species: *An. squamosus* (95, 0.29%), *An. rufipes* (73, 0.22%), *An. maculipalpis* (61, 0.19%), *An. gibbinsi* (26, 0.08%), *An. tenebrosus* (13, 0.04%), and *An. pretoriensis* (5, 0.02%).




Other species: *An. squamosus* (276, 0.63%), *An. coustani* (265, 0.61%), *An. rufipes* (96, 0.22%), *An. argentiolobatus* (71, 0.16%), *An. pretoriensis* (35, 0.08%), *An. gibbinsi* (19, 0.04%), and *An. tenebrosus* (16, 0.04).

**Figure 2: Species Composition of Mosquito Samples Collected from All Sites (A), Sprayed Sites (B), and Unsprayed Sites (C) (June 2019-February 2020)**

The species composition by collection method is displayed in Figure 3. All 13 species collected in the region over the reporting period were found in the HLC collections, while only 10 of the 13 species were found in the PSC collections. Those not found in PSC collections were *An. maculipalpis*, *An. squamosus,* and *An. argentiolobatus*. The proportion of *An. funestus* s.l. and *An. gambiae* s.l. was higher in the indoor collections (PSCs or indoor HLCs) compared to outdoor collections (outdoor HLCs). This reflects the more endophilic tendency of the primary malaria vector species in the area. There were also more non-vectors or secondary vector complexes in the outdoor collections compared to the indoor collections; 48.4% in the outdoor HLC collections compared to 20.8% in the indoor HLC collections and 9.8% in the PSC collections.




Other species collected by HLC-Indoors include: *An. maculipalpis* (0.73%), *An. coustani* (0.57%), *An. tenebrosus* (0.03%), *An. gibbinsi* (0.06%), *An. rufipes* (0.19%), *An. pretoriensis* (0.04%), *An. squamosus* (0.45%), and *An. argentiolobatus* (0.06%).
Other species collected by HLC-Outdoors include: *An. maculipalpis* (1.02%), *An. coustani* (1.24%), *An. tenebrosus* (0.05%), *An. gibbinsi* (0.03%), *An. rufipes* (0.23%), *An. pretoriensis* (0.07%), *An. squamosus* (0.61%), and *An. argentiolobatus* (0.14%).
Other species collected by PSC include: *An. coustani* (0.07%), *An. tenebrosus* (0.02%), *An. gibbinsi* (0.17%), *An. rufipes* (0.33%), and *An. tchekedii* (0.02%).

**Figure 3: Species Composition Across Sites by Collection Method (June 2019-February 2020)**

Out of the 51,875 primary vector complexes collected, *An. funestus* s.l. accounted for 88% (45,637), while *An. gambiae* s.l. accounted for 12% (6,238). Ninety percent of the vectors (46,636) were collected from HLCs (both indoors and outdoors) while 10% (5,239) were collected from PSCs. Figure 4 shows monthly relative abundance of the two primary vector species in each of the sentinel districts. *An. funestus* s.l. was the predominant malaria vector in all districts except Mambwe in the Eastern Province and Lufwanyama in the Copperbelt Province where *An. gambiae* s.l. was the most common species collected. Monthly distribution of the two species show a trend of higher *An. funestus* s.l. before the peak rainy period (June-November) and more *An. gambiae* s.l. during and after the peak rainy period (December to February) The total number of the two primary vector species complexes collected at sprayed and unsprayed sites by month and collection method are presented in Annex 3. Both primary vectors were collected from sprayed and unsprayed sites, however, more *An. funestus* s.l. were collected from unsprayed sites (56%) than sprayed sites (44%), while more *An. gambiae* s.l. were collected in sprayed sites (58%) compared to unsprayed sites (42%).

The collection effort during the entire surveillance period was 2,432 (68%) for HLCs and 1,140 (32%) for PSCs. For each collection month and at each sentinel site, teams collected from 32 houses for HLCs (16 houses indoors and 16 houses outdoors) while the sample size for PSC at each site each month was 15 houses. Though the number of sampled households by HLCs was twice that of PSCs, the total vectors collected by HLC was about nine times that collected by PSC. Among those collected by PSCs, *An. funestus* s.l. accounted for 91% while *An. gambiae* s.l. accounted for 9%. Among those collected by HLCs, 88% were *An. funestus* s.l. and 12% were *An. gambiae* s.l.

*An. funestus* s.l. vector numbers were highest in Milenge district in Luapula Province (107 per collection effort) followed by Nchelenge district in Luapula Province (66 per collection effort). The number of *An. funestus* s.l. per collection effort was lowest in Mambwe district in Eastern Province (0.41 per collection effort) and Serenje in Central Province (0.42 per collection effort). The other districts ranged from 1.6 to 6.5 vectors per collection effort. Overall, *An. gambiae* s.l. vector numbers were highest in Lufwanyama district (13.4 per collection effort) followed by Nchelenge (3.1 per collection effort) and Mambwe district (1.7 per collection effort), and were lowest in Serenje (0.07 per collection effort). The other districts had between 0.1 to 0.7 vectors per collection effort.

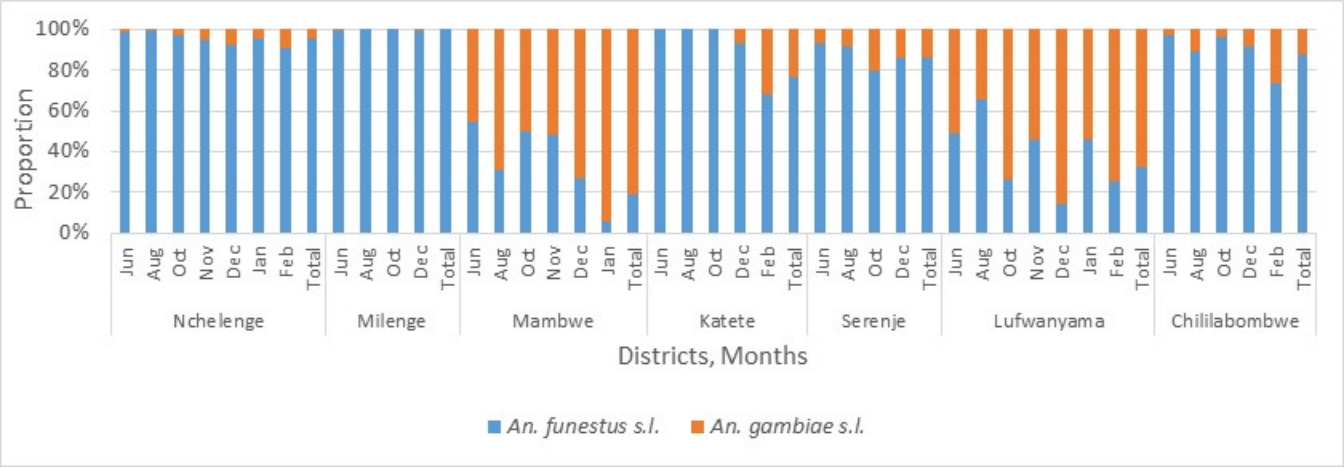



**Figure 4: Relative Proportion of *An. funestus* s.l. and *An. gambiae* s.l. by Month and District (June 2019-February 2020)**

## 3.2 Vector Density and Behavior

### 3.2.1 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. increased immediately after IRS at both IRS and non-IRS sites from 3.7 to 5.1 and 5.7 to 7.4 vectors per house respectively, though reductions were observed at both sites two months after IRS 2.2 and 4.0 vectors per house respectively (Fig. 5A). A similar trend was observed for *An. gambiae* s.l. where no reduction was seen at the IRS sites until four months after IRS. There was a slight reduction at the control sites two months after IRS and then at four months (5B). Given that not all districts were sprayed at the same time—for instance, Serenje was sprayed in December while the other districts were sprayed in October—graphs that combine districts present data according to the time relative to the month of IRS (e.g., T-1 is one month before spraying, T+1 is one month after spraying) instead of calendar months, enabling comparison between districts and summing across districts. Rainfall data presented in the graphs is based on the Level 3 Global Precipitation Measurement (GPM) mission's Integrated Multi-satellite Retrievals of GPM data obtained from the Giovanni online data system, developed and maintained

by the U.S. National Aeronautics and Space Administration Goddard Earth Sciences Data and Information Services Center[13].

*An. funestus* s.l. indoor densities reduced after IRS at three of the seven IRS sites (Lunga, Milenge district- Figure 5K, Nkana, Lufwanyama district Figure 5M and Kawama, Chililabombwe district Figure 5O). In Mambwe (Figure 5G), density reduced in the first month post-IRS, but increased in the second month, though densities in both months were extremely low. In Chiloba, Katete district (Figure 5I) the low densities before IRS were maintained after IRS. No reductions in *An. gambiae* s.l. indoor densities were observed after IRS at any of the IRS sites. IRS in Serenje was conducted by the government in December and therefore, we currently do not have enough data to adequately describe the post-spray trend in this district. Figures 5C to 5P display the indoor densities for both *An. funestus* s.l. and *An. gambiae* s.l. vectors at sprayed and unsprayed sites in all seven districts between June 2019 and February 2020 with 95% confidence intervals.

There were significantly less *An. funestus* s.l. indoor resting mosquitoes in the sprayed sites as compared to the unsprayed sites (36.6% less, p=0.02), while more *An. gambiae* s.l. was observed at the sprayed sites relative to the control sites (42.1% more, p=0.03). When all sprayed sites were combined, there was no decrease in the indoor resting density of either *An. funestus* s.l. or *An. gambiae* s.l. during the post-IRS period relative to the pre-IRS period. However, we observed a large and significant increase in *An. gambiae* s.l. over the period (93.9% increase, p<0.001). Detailed output of statistical analyses of the impact of IRS on indoor resting density are presented in Annex 4A.

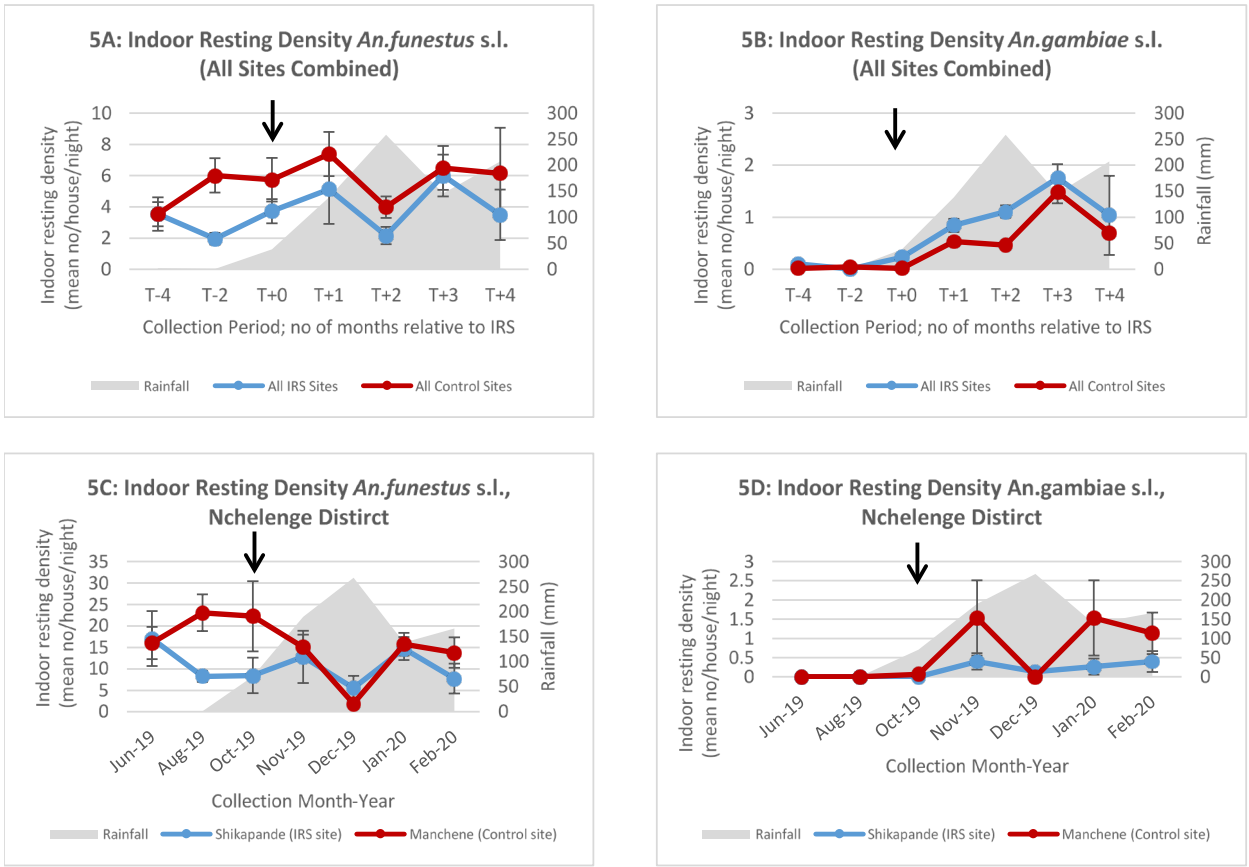
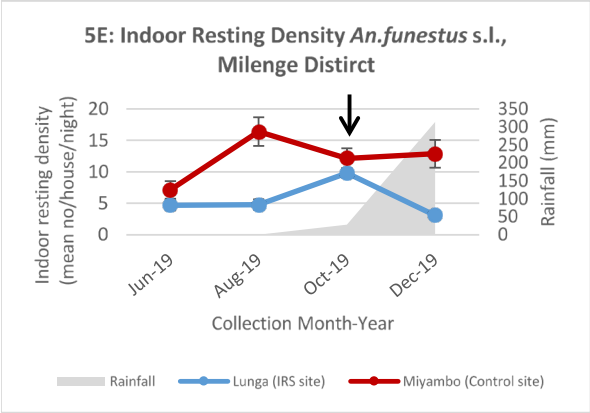
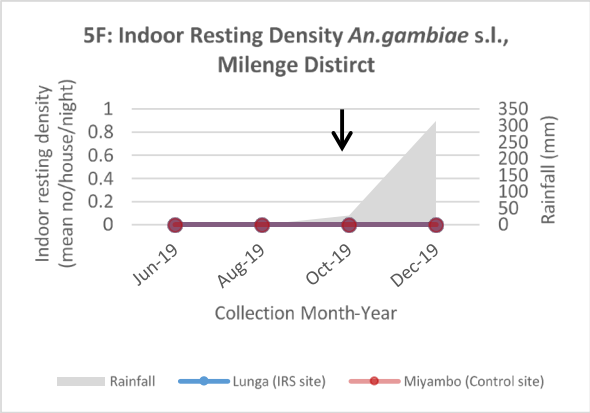


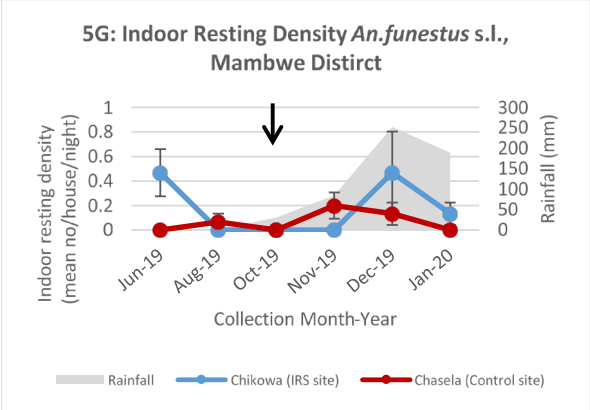

**Figure 5: Indoor Resting Density of *An. funestus* s.l. and *An. gambiae* s.l. Across Sites (June 2019-February 2020)**

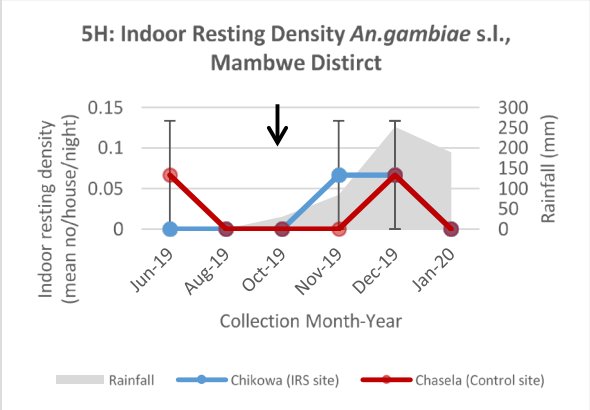
[Arrow indicates when IRS was implemented.]

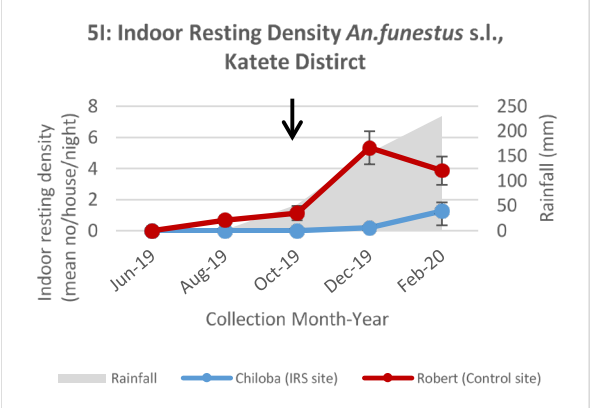
[13] https://giovanni.gsfc.nasa.gov



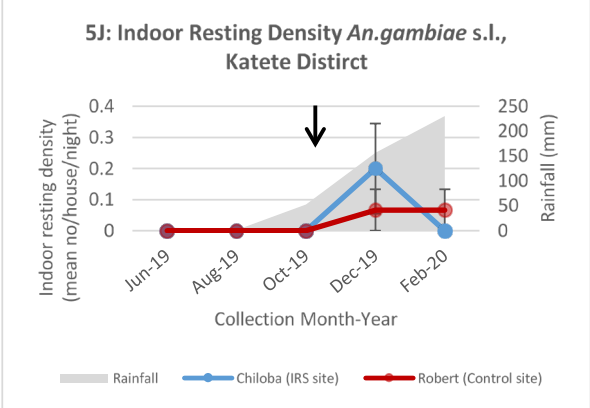
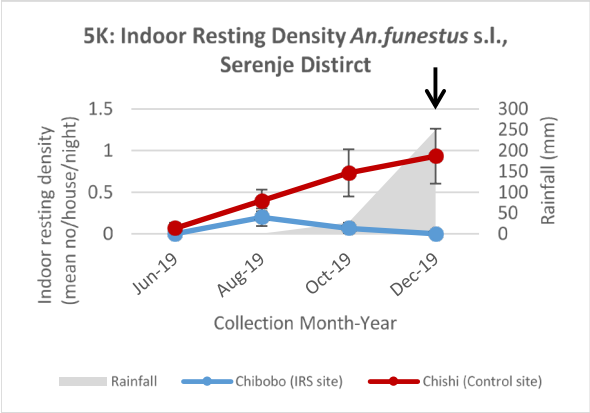
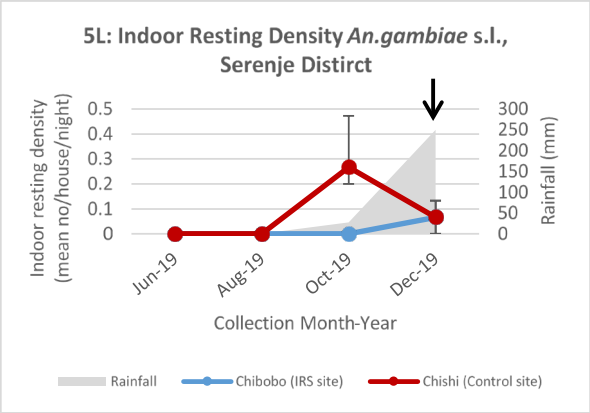
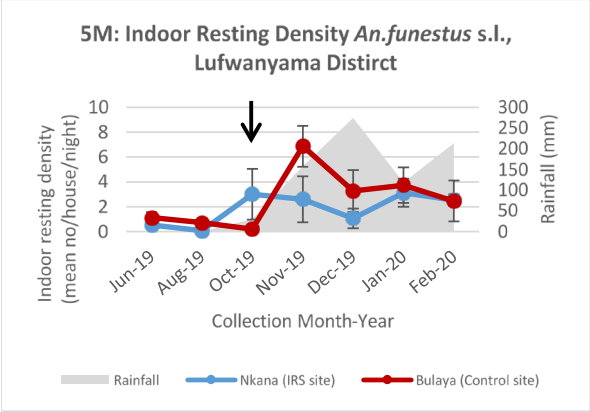



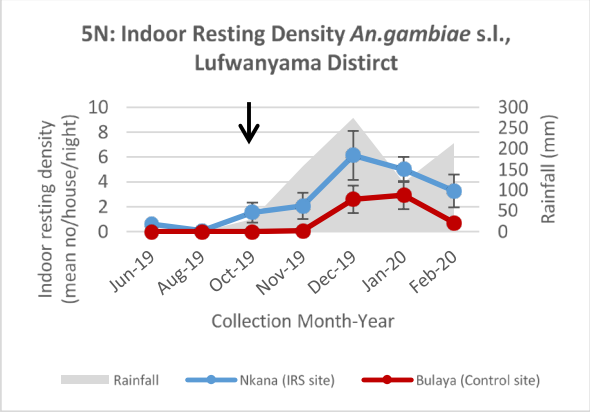

**Figure 5: Indoor Resting Density of *An. funestus* s.l. and *An. gambiae* s.l. Across Sites (June 2019-February 2020) (cont.)**

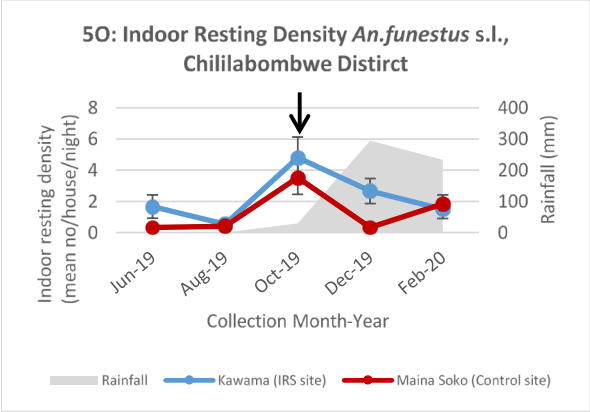
[Arrow indicates when IRS was implemented.]



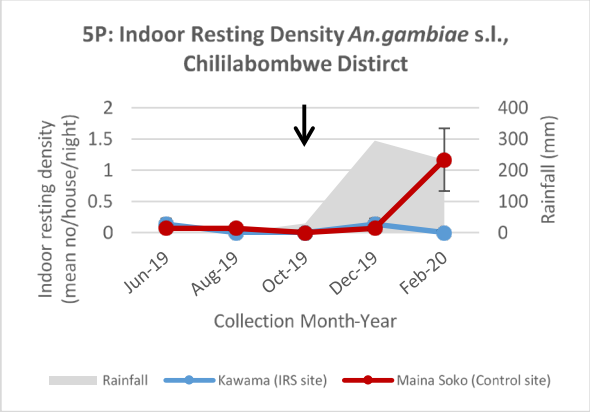



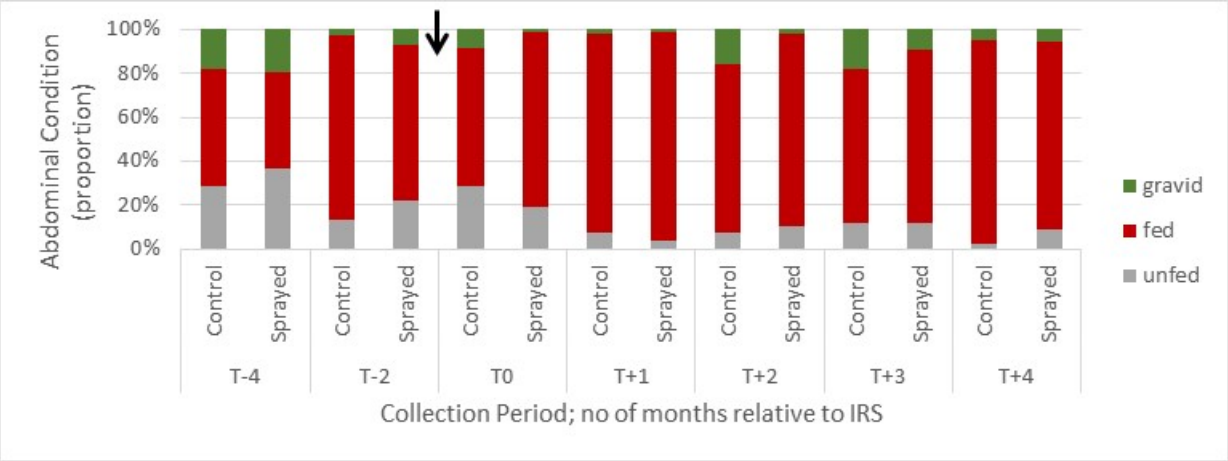

**Figure 5: Indoor Resting Density of *An. funestus* s.l. and *An. gambiae* s.l. Across Sites (June 2019-February 2020) (cont.)**

[Arrow indicates when IRS was implemented.]

### 3.2.2 Abdominal Condition of *An. funestus* s.l. Collected by PSCs

Figures 6A and 6B show the abdominal status of *An. funestus* s.l. and *An. gambiae* s.l. collected indoors by PSCs from sprayed and control sites before and after IRS. Abdominal condition, namely the proportions of unfed, fed, and gravid *An. funestus* s.l. and *An. gambiae* s.l. mosquitoes, was determined for a total of 4,705 *An. funestus* s.l. (1,841 from sprayed sites and 2,864 from control sites) and 465 *An. gambiae* s.l. (328 from sprayed sites and 137 from control sites). Overall, the proportions of fed and gravid *An. funestus* s.l. mosquitoes were 74.6% and 7.3% in the sprayed sites and 74.7% and 9.5% in the control sites, respectively, while the proportions of fed and gravid *An. gambiae* s.l. were 77.1% and 3.4% in the sprayed sites and 81.8% and 3.6% in the control sites, respectively. After IRS, there were consistently fewer gravid mosquitoes at the sprayed sites compared to the control sites for *An. funestus* s.l. This trend was not consistent for *An. gambiae* s.l. Overall, there was a significant reduction (76.3%) in gravid *An. funestus* s.l. vectors in the sprayed sites after IRS compared to the period before IRS (IRR 0.23, p=0.002). There was also an overall 62% reduction in gravid *An. gambiae* s.l. at the sprayed sites after IRS, but the decrease was not statistically significant (IRR 0.27, p=0.24). See detailed statistical output in Annex 4B.

**6A: *Anopheles funestus* s.l.**




**6B: *Anopheles gambiae* s.l.**

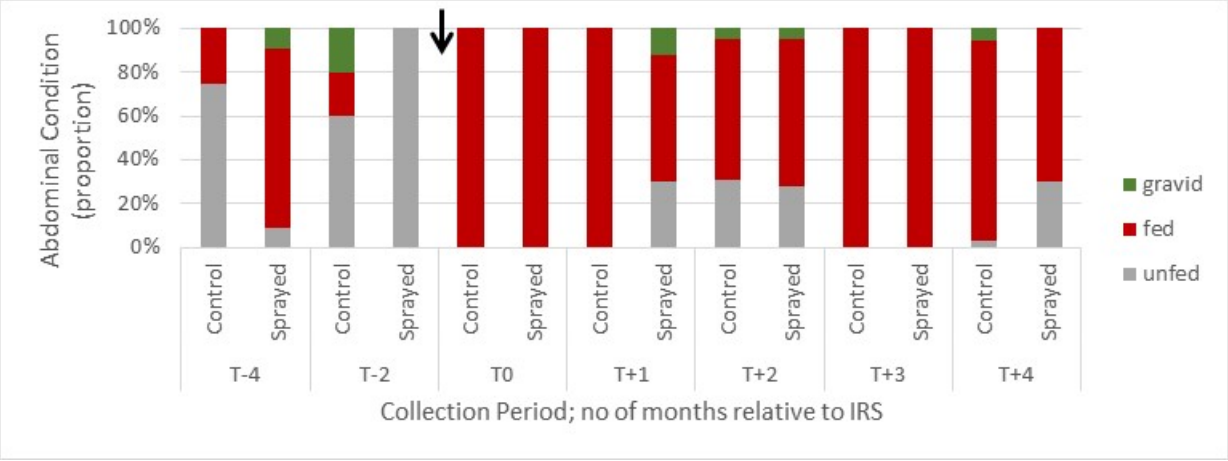



**Figure 6: Abdominal Condition of *An. funestus* s.l. (A) and *An. gambiae* s.l. (B) Collected by PSCs in Sprayed and Control Sites (June 2019-February 2020)**

[Arrow indicates the time IRS was implemented]

### 3.2.3 BITING RATE OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY HLC

The human biting rates (HBRs) indoors and outdoors of *An. funestus* s.l. and *An. gambiae* s.l. in the IRS and control sites are presented in Figure 7. There were overall fewer bites from *An. funestus* s.l. and more bites from *An. gambiae* s.l. at the IRS sites compared to the control sites, though neither was statistically significant. The highest impact of IRS on HBR was observed for *An. funestus* s.l. two months post-IRS at the IRS sites: 19.3 to 9.7 bites per person per night indoors and 15.6 to 4.8 bites per person per night outdoors (Fig. 7A). At the control sites, there was an increase in the number of *An. funestus* s.l. bites per person two months after IRS from 15.1 to 31.5 indoors and from 7.3 to 19.7 outdoors. Conversely, overall biting rates for *An. gambiae* s.l. increased after IRS from 0.2 to 6.6 bites per person per night indoors and 0.3 to 4.2 outdoors at the IRS sites and from 0.1 to 3.1 bites per person per night indoors and 0.1 to 2.6 outdoors (Fig. 7B). At the site level, *An. funestus* s.l. human biting rates reduced after IRS at two of the seven IRS sites (Shikapande-Fig. 7C and Fig. Lunga-7E) and for *An. gambiae* s.l. at Shikapande-7D. Low biting rates were maintained after IRS in Chiloba (Katete district) for *An. funestus* s.l. and in Lunga (Milenge district) for *An. gambiae* s.l. Pre-IRS human biting rates were higher at the IRS sites in Nchelenge (Fig. 7C), Mambwe (Fig. 7G), and Chililabombwe (Fig. 7O) and at the control sites in Milenge (Fig. 7E), Katete (Fig. 7I), Serenje (Fig. 6K), and Lufwanyama (Fig. 7M). In Chililabombwe, there was a slight increase in biting rate at the IRS site; however, at baseline, the rates at the IRS sites were higher than the non-IRS sites. Given Serenje was sprayed in December, we do not have enough data to describe the impact of IRS. There was more indoor biting than outdoor biting for both *An. funestus* s.l. and *An. gambiae* s.l. See detailed statistical output in Annex 4C. We observed less indoor and outdoor biting by *An. funestus* s.l. at the sprayed sites compared to the control sites but the differences were not statistically significant (39.4% less indoor biting, p=0.49 and 38.2% less outdoor biting, p=0.23, respectively). For both *An. funestus* s.l. and *An. gambiae* s.l., indoor and outdoor bites per person per night significantly increased after IRS at both the IRS sites and the control sites. See Annex 4D and 4E for details.

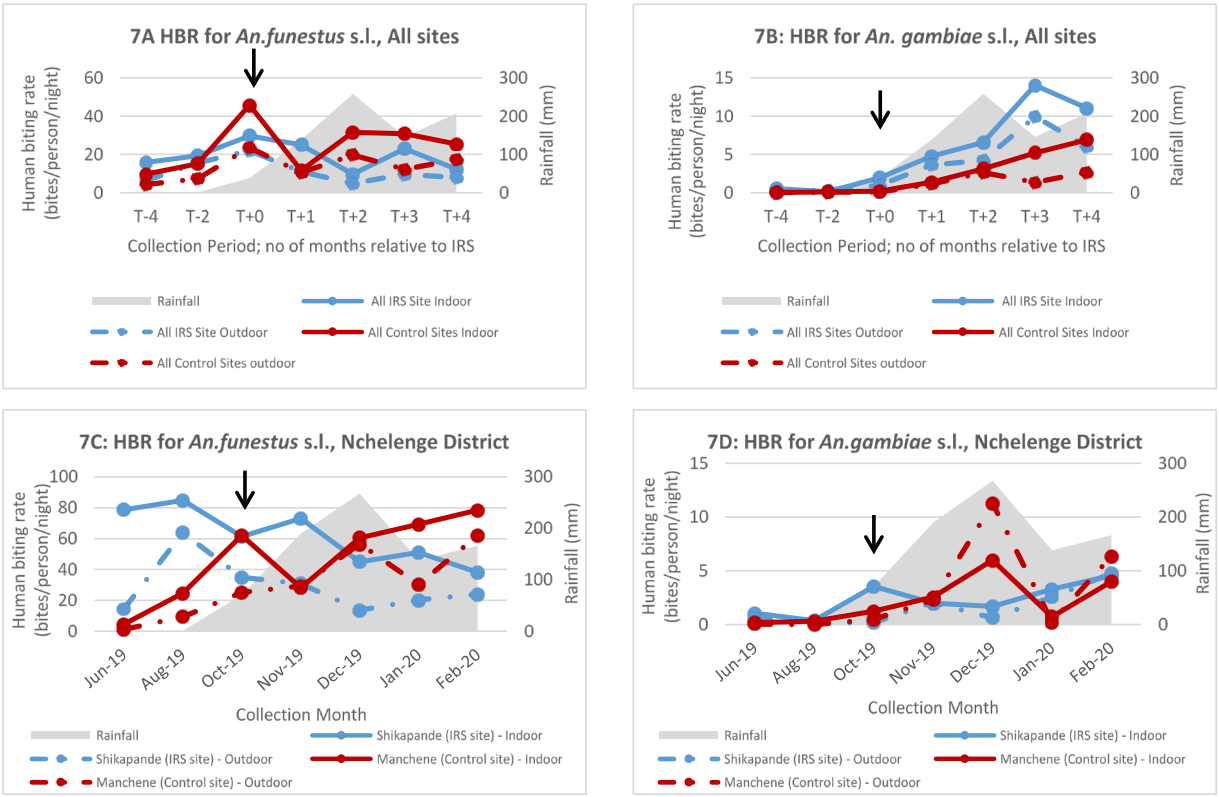
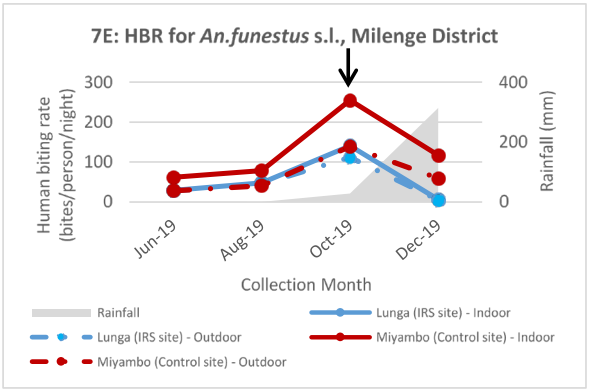
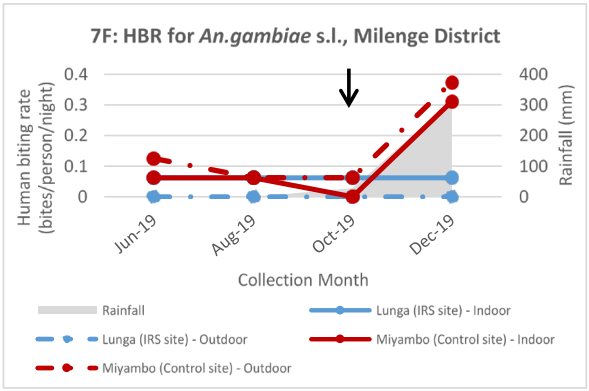
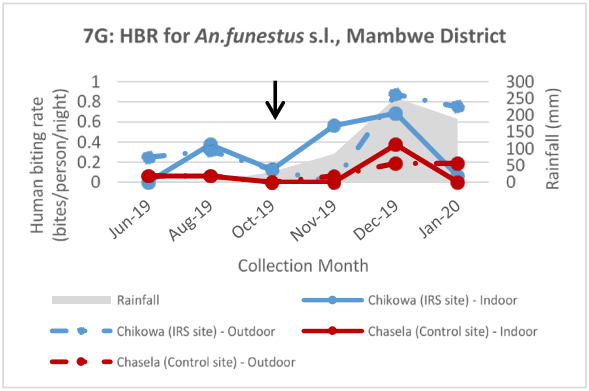


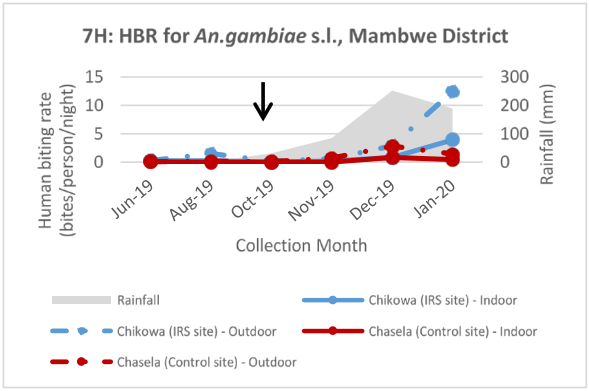

**Figure 7: Human Biting Rate of *An. funestus* s.l. and *An. gambiae* s.l. Across Sites (June 2019-February 2020)**

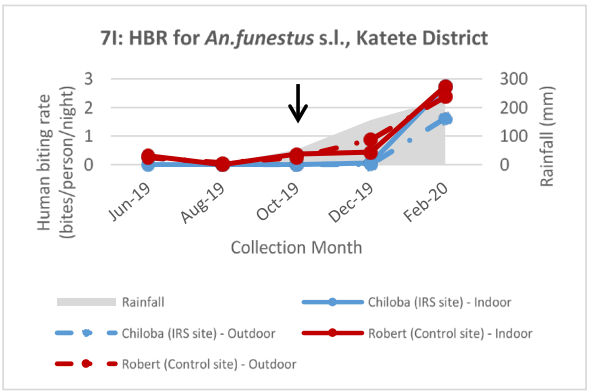
[Arrow indicates when IRS was implemented.]

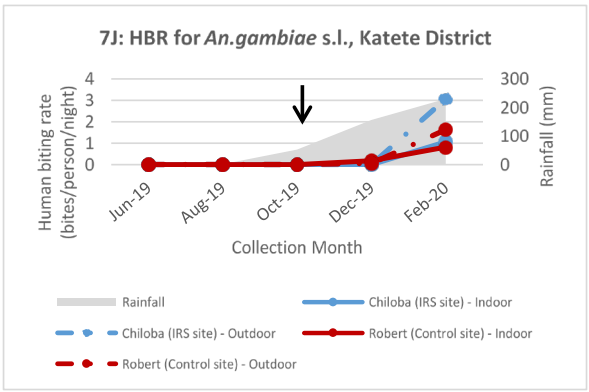


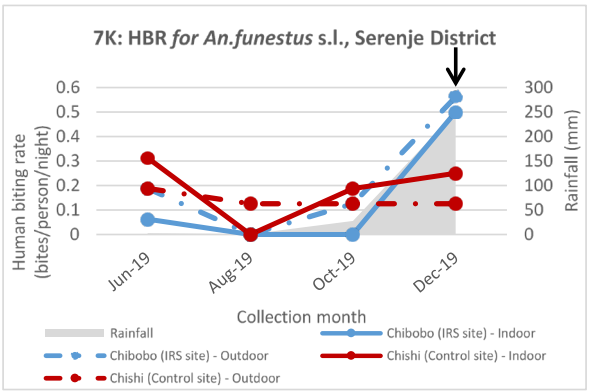



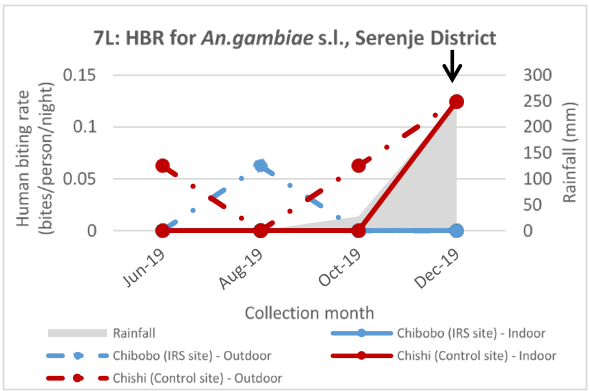




**Figure 7: Human Biting Rate of *An. funestus* s.l. and *An. gambiae* s.l. Across Sites (June 2019-February 2020) (cont.)**

[Arrow indicates when IRS was implemented.]

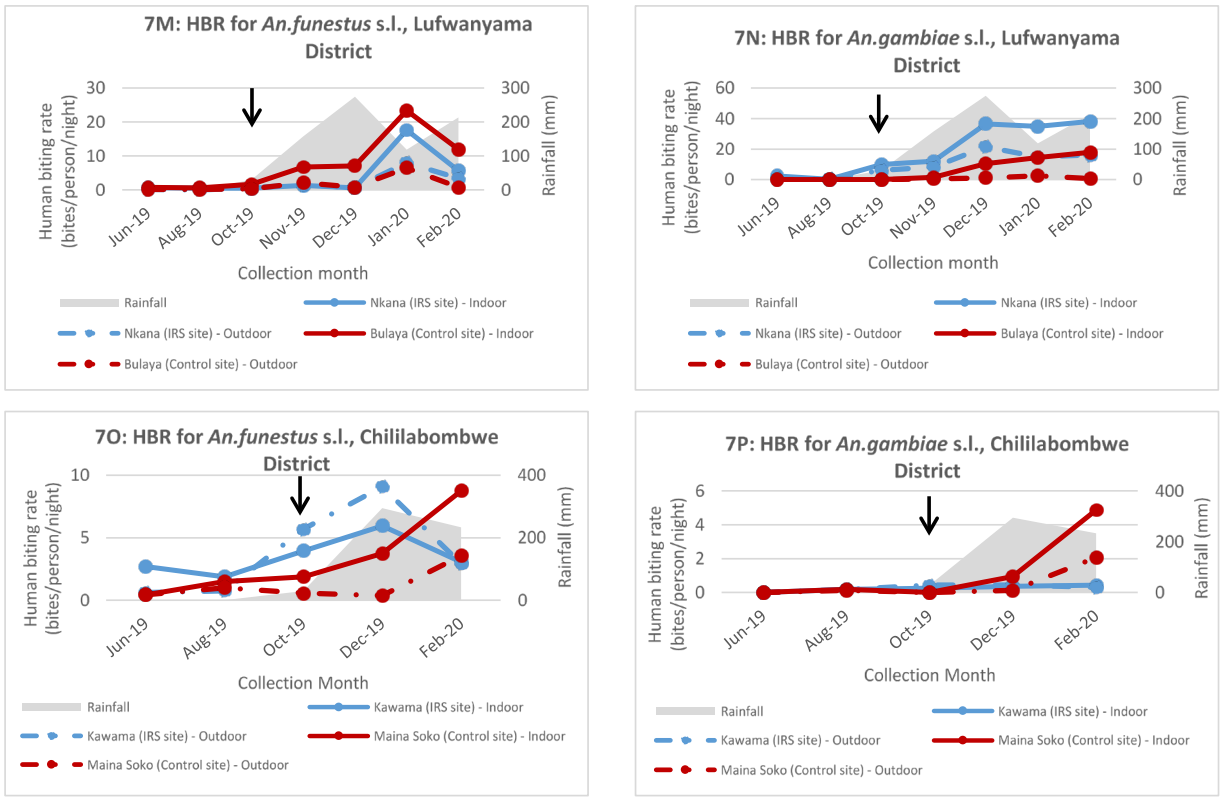



**Figure 7: Human Biting Rate of *An. funestus* s.l. and *An. gambiae* s.l. Across Sites (June 2019-February 2020) (cont.)**

[Arrow indicates when IRS was implemented.]

### 3.2.4 *An. funestus* s.l. and *An. gambiae* s.l. Feeding Location and Time

The feeding location (indoor or outdoor) and biting times for *An. funestus* s.l. and *An. gambiae* s.l. mosquitoes for all sentinel sites are presented in Figure 8. *An. funestus* s.l. exhibited predominantly indoor feeding behavior (Shikapande-Fig. 6A and Manchene-Fig. 8B in Nchelenge, Miyambo-Fig. 6D in Milenge, Nkana-Fig. 6K and Bulaya-6L in Lufwanyama, and Maina Soko-Fig. 8N in Chililabombwe) while *An. gambiae* s.l. exhibited both indoor feeding behavior (Nkana-Fig. 8K, Bulaya-Fig. 8L, and Maina Soko-Fig. 8N) and outdoor feeding behavior (Chikowa-Fig. 8E and Chasela-Fig. 8F in Mambwe). The biting trend was mainly unimodal at sites with high vector numbers (more than five bites/person/hour), peaking generally between 12 a.m. and 4 a.m. (Fig. 8A-D). For areas with low vector numbers, we observed multiple peaks throughout the night.

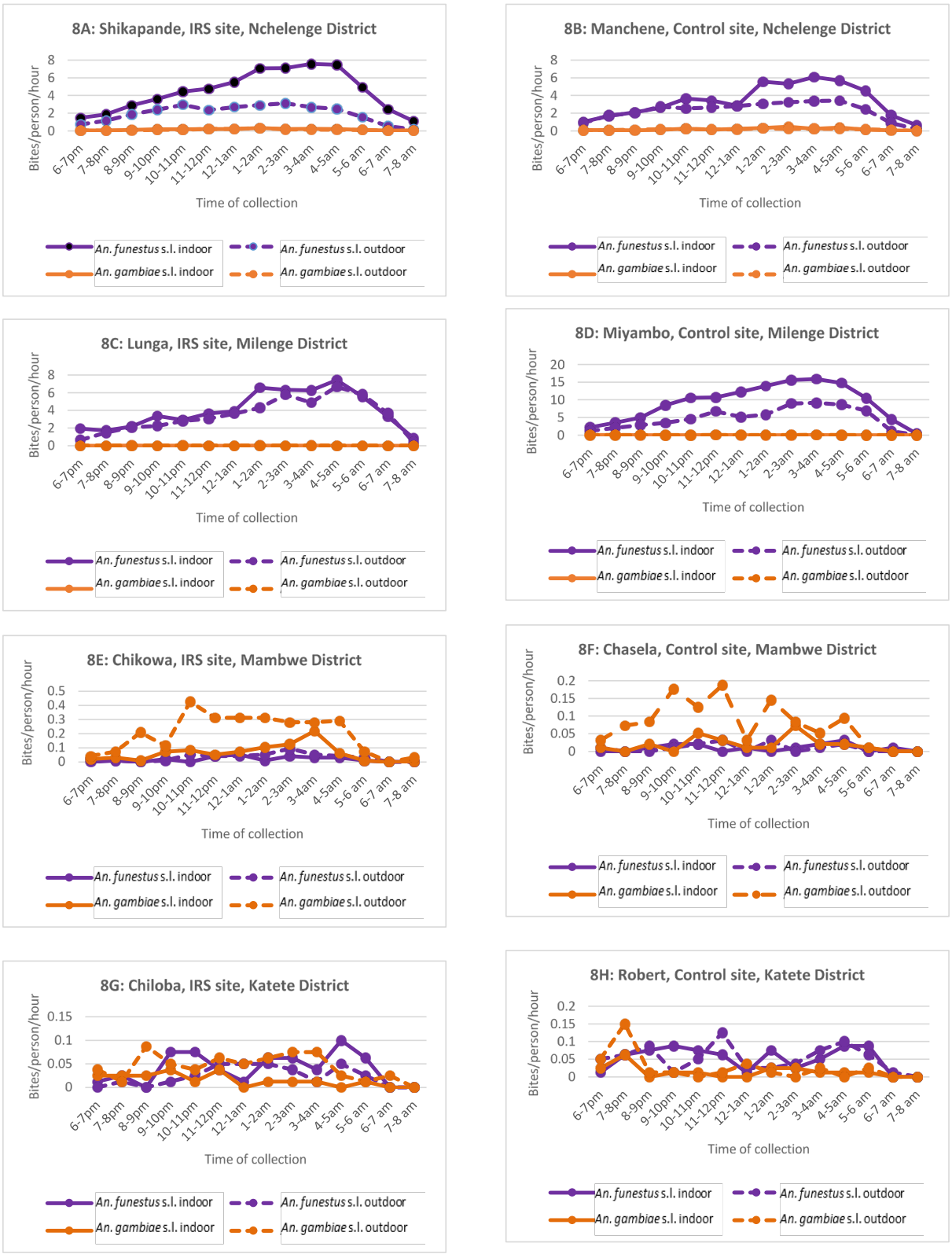



**Figure 8: Hourly Biting Rates of *An. funestus* s.l. and *An. gambiae* s.l. by Site (June 2019-February 2020)**

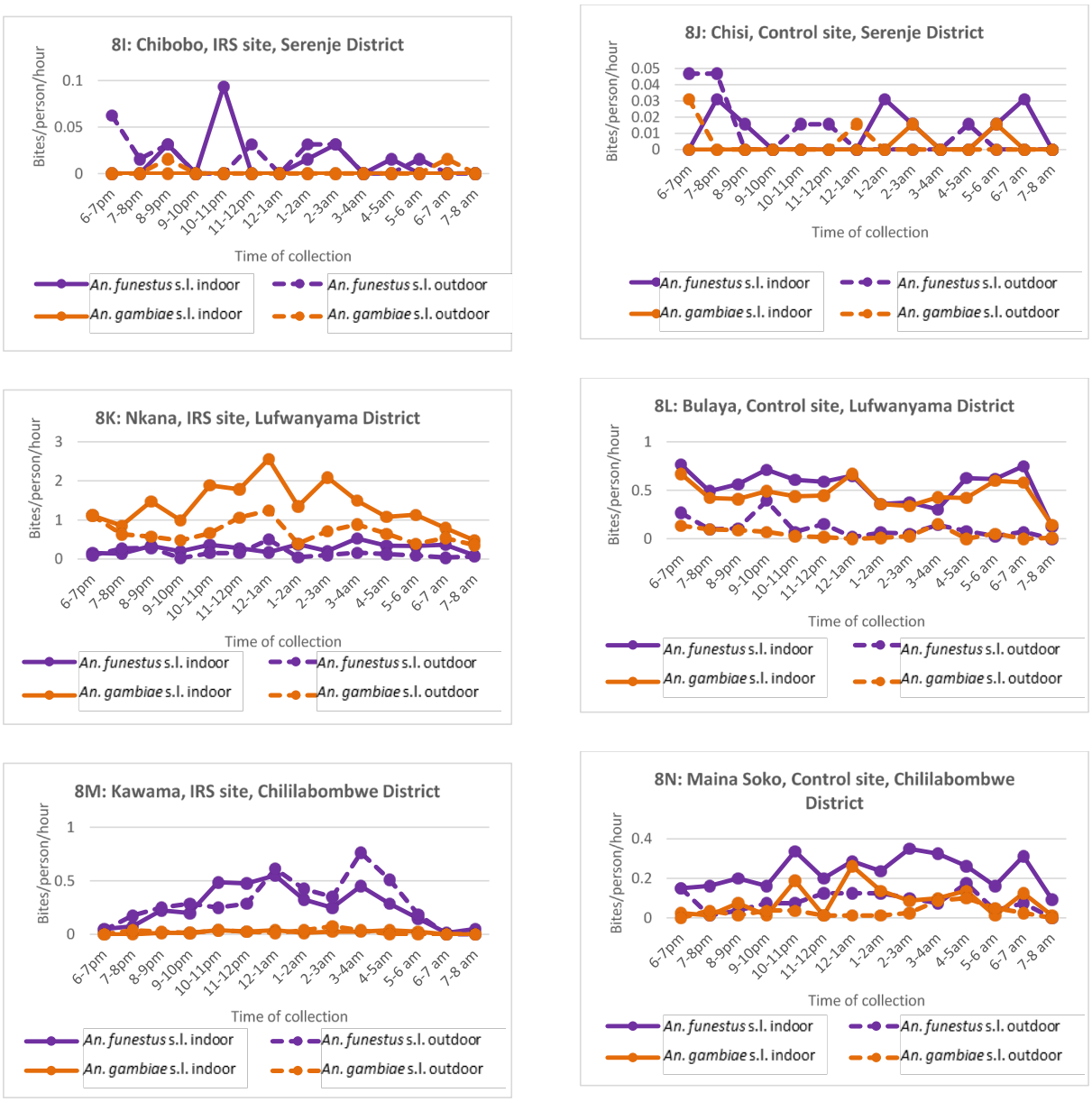



**Figure 8: Hourly Biting Rates of *An. funestus* s.l. and *An. gambiae* s.l. by Site (June 2019-February 2020) (cont.)**

## 3.3 PARITY RATE

A total of 5,802 unfed female *An. funestus* s.l. and 2,996 unfed female *An. gambiae* s.l. collected by HLCs were examined for parity status (SOP 10/01)[14] during the reporting period. Parity rates for *An. funestus* s.l. before and after IRS at the sprayed sites were 57.7% versus 36.8% and at the control sites were 54.3% versus 54.7% while parity rates for *An. gambiae* s.l. were 49.5% versus 38.7% at the sprayed sites and 40.7% versus 52.4% at the control sites respectively. Figure 9 shows monthly parity rates for *An. funestus* s.l. and *An. gambiae* s.l. to displaying rates between sprayed and control sites for each of the months before and after IRS. There were significantly fewer numbers of parous *An. funestus* s.l. and *An. gambiae* s.l. vectors (27.3% fewer, p<0.001, and

[14] SOP 10/01. Standard Operating Procedures for Ovary dissection, January 2020.

28.8% fewer, p<0.001 respectively) at the sprayed sites compared to the control sites. Parity rates at four months post-IRS (T+4) were comparable between sprayed and control sites for both species. When data from the pre-spray period were combined and compared to combined post-spray period data, we found a significantly lower number of parous vectors during the post-spray period relative to the pre-spray period for *An. funestus* s.l. (35% reduction, IRR=0.65, p<0.001). However for *An. gambiae* s.l., the overall decrease during the post spray period relative to the pre-spray period was not statistically significant (18.5% reduction, IRR=0.81, P=0.171). Since no data was collected after T+4, it cannot be determined whether or not the post-IRS reductions at sprayed sites relative to control sites seen up to T+3 had truly ceased. See Annex 4F for statistical output of comparisons of vector parity between sprayed and control sites as well as pre-IRS and post-IRS periods.

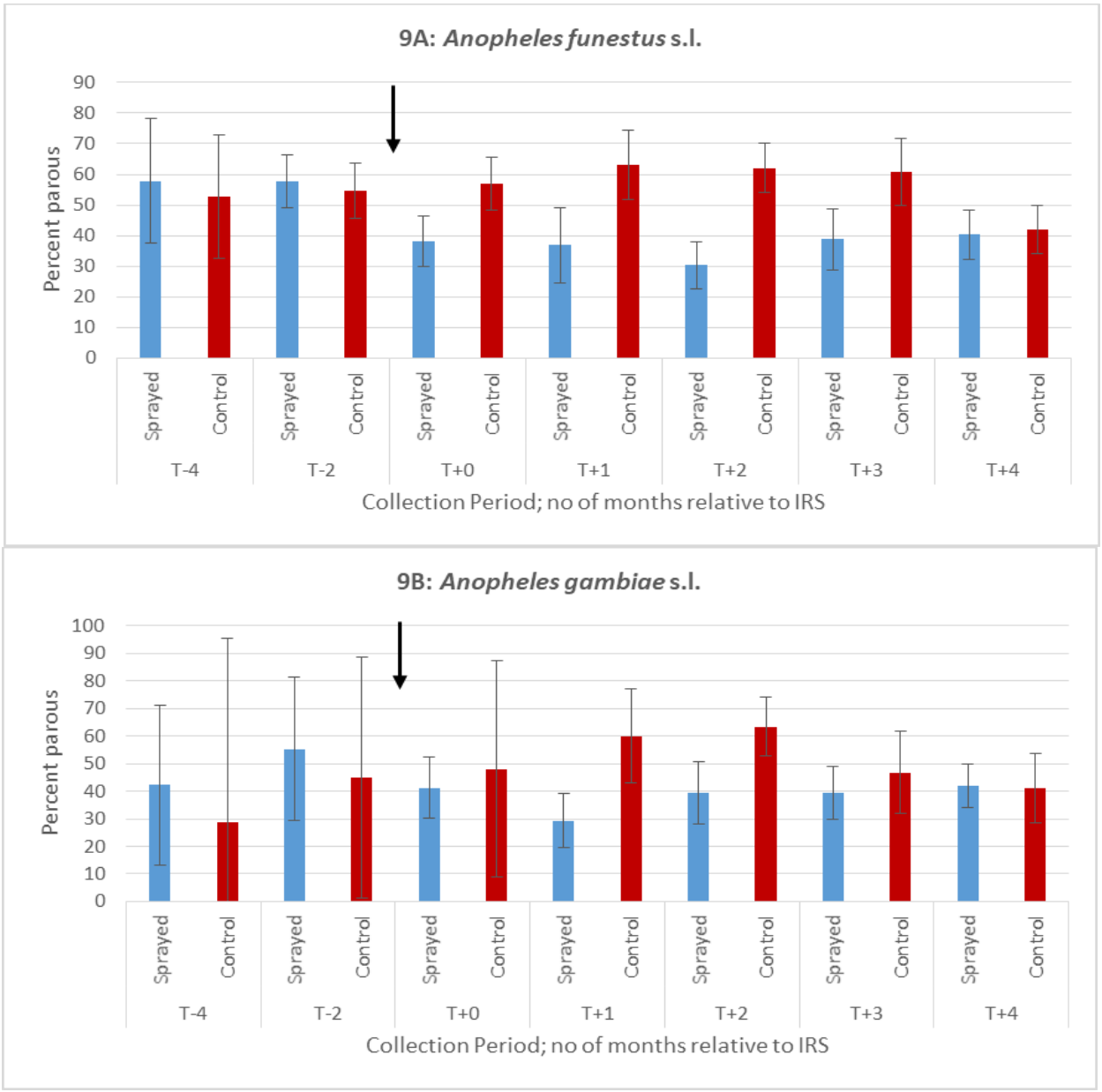



**Figure 9: Parity Rate of *An. funestus* s.l. (A) and *An. gambiae* s.l. (B) in Intervention and Control Sites Before and After IRS (June 2019-February 2020)**

[Arrow indicates the time IRS was implemented; 95% confidence interval shown with bracket]

## 3.4 LABORATORY RESULTS: ADVANCED INDICATORS

Zambia has experienced perennial challenges conducting timely laboratory analysis to measure advanced entomological indicators such as molecular species composition, sporozoite infectivity rates, and source of mosquito blood meal. In 2018, PMI authorized the procurement of equipment and reagents to bolster the molecular laboratory capacity at the NMEC and afford VectorLink Zambia the opportunity to conduct laboratory analysis in a timely manner. A backlog of the 2018/2019 samples were analyzed up to the first quarter of 2020 and the results were presented as an addendum to the 2018/2019 annual report. The plan has been to clear the 2019/2020 backlog of samples ahead of the commencement of the 2020/2021 vector surveillance activities so that the future samples can be analyzed promptly with not more than 1-2 months lag-time. This has not been achieved due to the COVID-19 restrictions at the NMEC laboratory that came into effect by the end of the first quarter where a strict schedule was enforced on number of staff that can be permitted in the laboratories at any given time. We have not been able to clear the backlog of samples before the commencement of the collection of the 2020/2021 samples. The data presented here is based on the samples analyzed to date; 37.8% of the 1,554 samples targeted for PCR analysis, 93% of the 2,125 samples targeted for ELISA analysis, and 29% of the 560 samples targeted for blood meal source determination (2019 work plan targets).

### 3.4.1 PCR IDENTIFICATION OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L.

Of the 632 *An. gambiae* s.l. and 1,249 *An. funestus* s.l. tested by PCR, 203 and 384 successfully amplified, respectively [15]. Among the *An. gambiae* s.l. that amplified, 70.9% were *An. gambiae* s.s. and 29.1% were *An. arabiensis*. Lufwanyama district (Copperbelt Province) accounted for more than 94% of *An. gambiae* s.s. detected. Of the 384 *An. funestus* s.l. that successfully amplified, 31.3% were found to be *An. funestus* s.s., while the majority (62.2%) were *An. rivulorum*. Other species included *An. rivoluruom*-like (5.9%), *An. vaneedeni* (0.3%), and *An. parensis* (0.3%). *An. rivulorum* and *An. rivulorum*–like[16] species were observed occurring together in Milenge district. Table 5 below shows the distribution of the different molecular species of *An. gambiae* s.l. and *An. funestus* s.l. vectors during the 2019/2020 surveillance period by district.

**Table 5: Molecular Identification of *An. gambiae* s.l. and *An. funestus* s.l. Collected from Sentinel Districts (June 2019-February 2020)**

| District | A: *An. gambiae* s.l. | | | |
|---|---|---|---|---|
| | Total Tested | Total Amplified | *An. gambiae* s.s. | *An. arabiensis* |
| Mambwe | 214 | 57 | 7 | 50 |
| Katete | 137 | 7 | 0 | 7 |
| Lufwanyama | 276 | 138 | 136 | 2 |
| Chililabombwe | 5 | 1 | 1 | 0 |
| **Total** | **632** | **203** | 144 | 59 |
| **% of total amplified** | | | **70.94** | **29.06** |

[15] We are currently optimizing the DNA extraction process in response to the low amplification observed. We are changing the extraction process in the current standard operating procedure from use to water to use of an extraction reagent.

[16] Cohuet A, Simard F, Toto JC, Kengne P, Coetzee M, Fontenille D. Species identification within the Anopheles funestus group of malaria vectors in Cameroon and evidence for a new species. Am J Trop Med Hyg. 2003;69:200–5.

| District | B: *An. funestus* s.l. | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total tested | Total amplified | *An. funestus* s.s. | *An. rivulorum* | *An. rivulorum*-like | *An. vaneedeni* | *An. parensis* |
| Nchelenge | 240 | 59 | 43 | 16 | 0 | 0 | 0 |
| Milenge | 539 | 168 | 1 | 146 | 21 | 0 | 0 |
| Mambwe | 84 | 23 | 22 | 0 | 1 | 0 | 0 |
| Katete | 132 | 8 | 7 | 0 | 0 | 0 | 1 |
| Serenje | 31 | 20 | 4 | 15 | 0 | 1 | 0 |
| Lufwanyama | 136 | 67 | 7 | 59 | 1 | 0 | 0 |
| Chililabombwe | 87 | 39 | 36 | 3 | 0 | 0 | 0 |
| **Total** | **1249** | **384** | **120** | **239** | **23** | **1** | **1** |
| **% of total amplified** | | | **31.25** | **62.24** | **5.99** | **0.26** | **0.26** |

### 3.4.2 SPOROZOITE RATES AND EIRS

A total of 515 *An. gambiae* s.l. and 1,526 *An. funestus* s.l. collected by HLCs from both sprayed and control sites were tested for *Plasmodium* circumsporozoite proteins. The sporozoite rate for the two species were 0.8% and 3.3%, respectively. In the *An. funestus* s.l. group, *An. rivulorum*-like had the highest sporozoite positive rate (13.0%), followed by *An. rivulorum* (5.0%) and *An. funestus* s.s. (2.6%).

Sporozoite infection rates by collection month are shown in Figure 10. January was the peak sporozoite infection month for *An. funestus* s.l. vectors (with fewer sporozoite infected vectors at the sprayed sites compared to the control sites) while November was the peak for *An. gambiae* s.l. vectors (all sporozoite infected vectors were collected from the sprayed sites).

**Table 6: Sporozoite Rates by Molecular Species (June 2019-February 2020)**

| Species Complex | Molecular Species | # Tested | # Sporozoite Positive | Sporozoite Rate (% Positive) |
|---|---|---|---|---|
| *An. gambiae* s.l. | *An. gambiae* s.s. | 40 | 0 | 0.0 |
| | *An. arabiensis* | 144 | 2 | 1.4 |
| | Undetermined* | 331 | 2 | 0.6 |
| | **TOTAL** | **515** | **4** | **0.8** |

| Species Complex | Molecular Species | # Tested | # Sporozoite Positive | Sporozoite Rate (% Positive) |
|---|---|---|---|---|
| *An. funestus* s.l. | *An. funestus* s.s. | 117 | 3 | 2.6 |
| | *An. rivulorum* | 239 | 12 | 5.0 |
| | *An. rivulorum*-like | 23 | 3 | 13.0 |
| | *An. vaneedeni* | 1 | 0 | 0.0 |
| | *An. parensis* | 1 | 0 | 0.0 |
| | Undetermined* | 1,145 | 33 | 2.9 |
| | **TOTAL** | **1,526** | **51** | **3.3** |

*Undetermined refers to *An. funestus* s.l. or *An. gambiae* s.l. samples on which sporozoite ELISAs were performed to determine infectivity that were subjected to PCR and the PCR products did not show any bands to determine the species as well as samples that were not subjected to PCR to determine the molecular species.

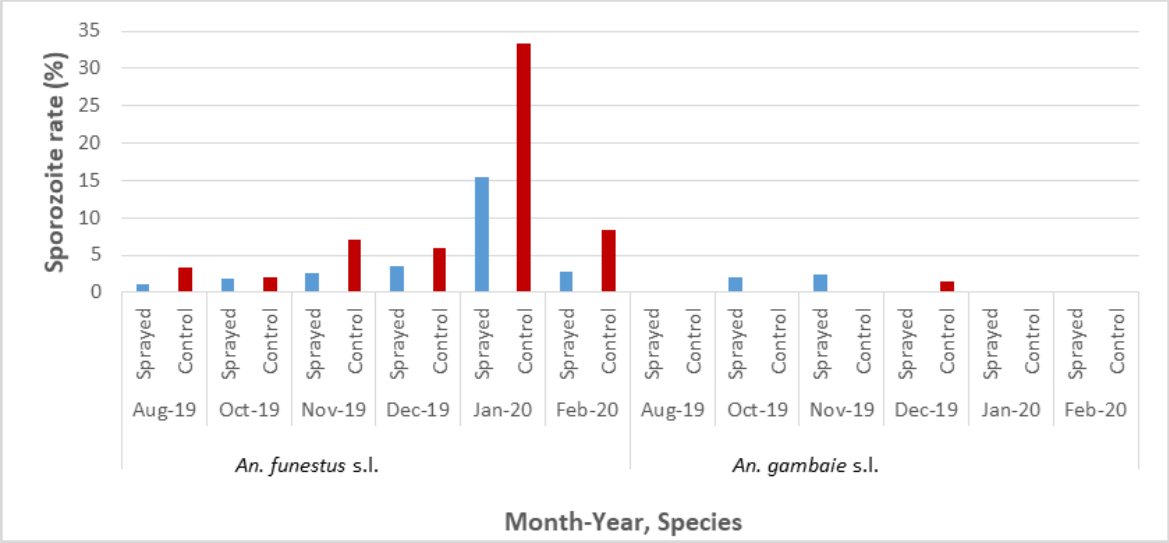
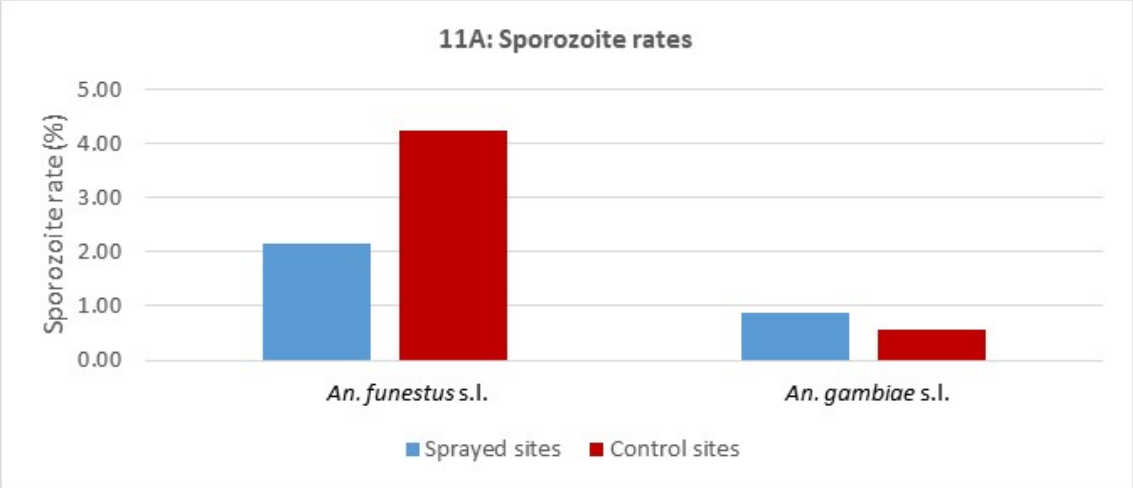



**Figure 10: *An. funestus* s.l. and *An. gambiae* s.l. Sporozoite Infection Rates By Month of Collection (June 2019-February 2020)**

*An. funestus* s.l. and *An. gambiae* s.l. sporozoite infection rates and entomological inoculation rates at combined sprayed sites and combined control sites are shown in Figures 11A and 11B. Sporozoite rates for *An. funestus* s.l. were higher at the control sites (4.2%) compared to the sprayed sites (2.2%) while for *An. gambiae* s.l. sporozoite rates were lower at the control sites (0.6%) compared to the sprayed sites (0.9%). The average EIR for *An. funestus* s.l. at the combined sprayed sites (0.33 infective bites per person per night - ib/p/n) compared to EIR at the control sites (0.80 ib/p/n) while for *An. gambiae* s.l., the average EIR at the combined sprayed sites was higher (0.04 ib/p/n) compared to the control sites (0.01 ib/p/n).



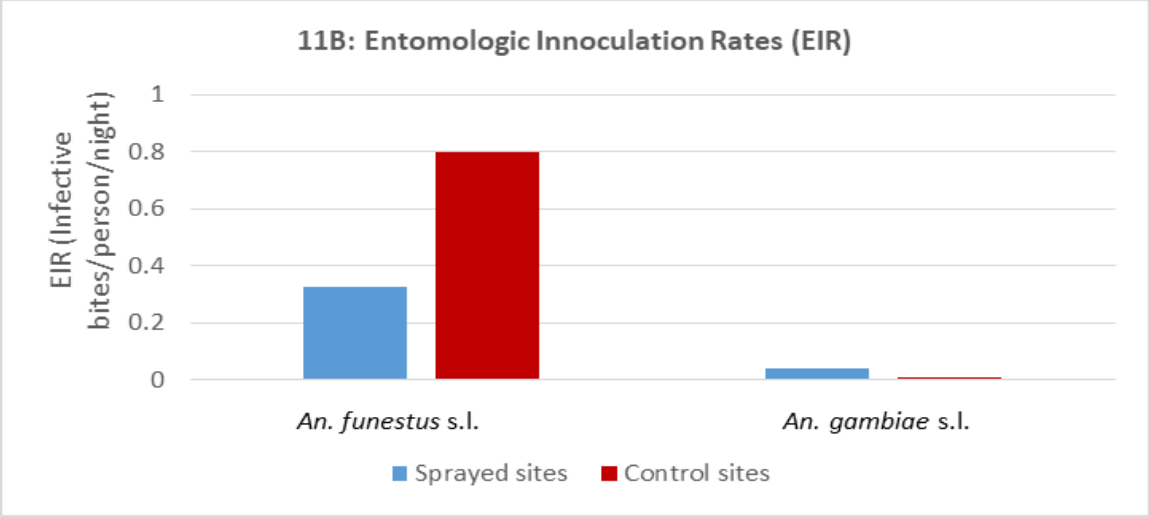




**Figure 11: *An. funestus* s.l. and *An. gambiae* s.l. Sporozoite Infection Rates (A) and Entomologic Innoculation Rates (B) At Sprayed and Control Sites (June 2019-February 2020)**

Due to the low sporozoite positivity rates, we could not estimate EIR to compare indoors and outdoors or pre- and post-IRS at the sprayed and control sites. The number of molecular species tested and number positive, along with a breakdown of numbers tested and numbers positive for indoor and outdoor *An. funestus* s.l. and *An. gambiae* s.l. before and after IRS, are provided in Annex 5.

### 3.4.3 Blood Meal Sources

Out of the 119 blood meals identified from 119 fed *An. funestus* s.l. vectors, 93.3% were from humans followed by 4.2% from cows. Of the 46 blood meals identified from 45 fed *An. gambiae* s.l. (one sample had a mixed human and dog blood meals), 93.5% were from humans and 4.3% were from cows. The human blood index was similar for both species at about 93%. When blood meal sources were grouped into control and intervention sites, the human blood index for *An. funestus* s.l. was slightly higher in the combined control sites (94.9) compared to the combined sprayed sites (85.0), while the human blood index for *An. gambiae* s.l. was about one point higher in the combined sprayed sites (93.8) compared to the control sites (92.9) (Figure 12). This finding suggests that, in the entire region, the majority of vectors resting indoors obtain their blood meals from humans.

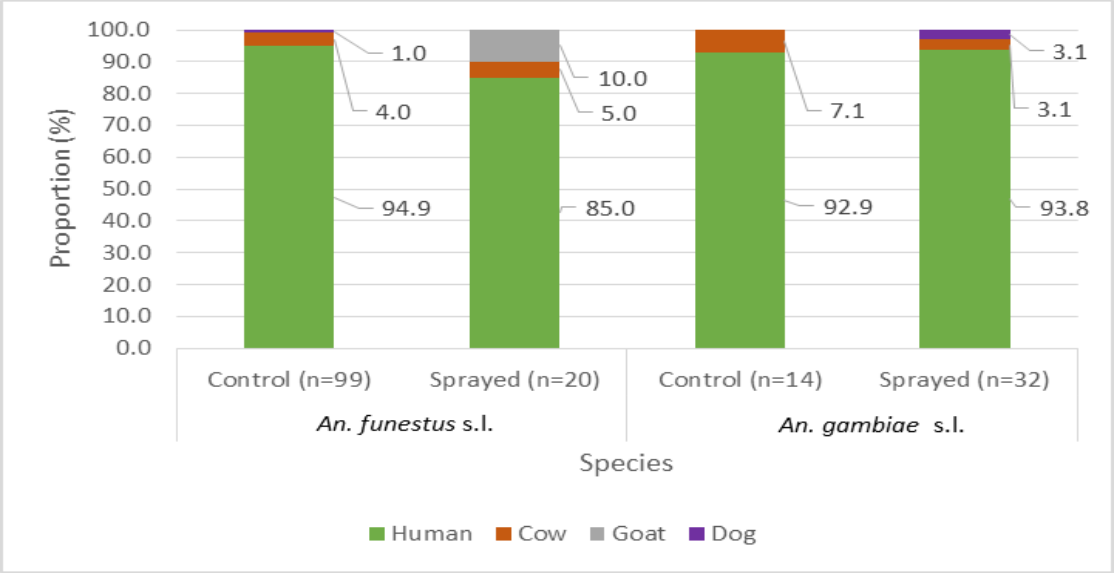



**Figure 12: Sources of Blood Meal for *An. funestus* s.l. and *An. gambiae* s.l. Vectors from Indoor Resting Collections (June 2019-February 2020)**

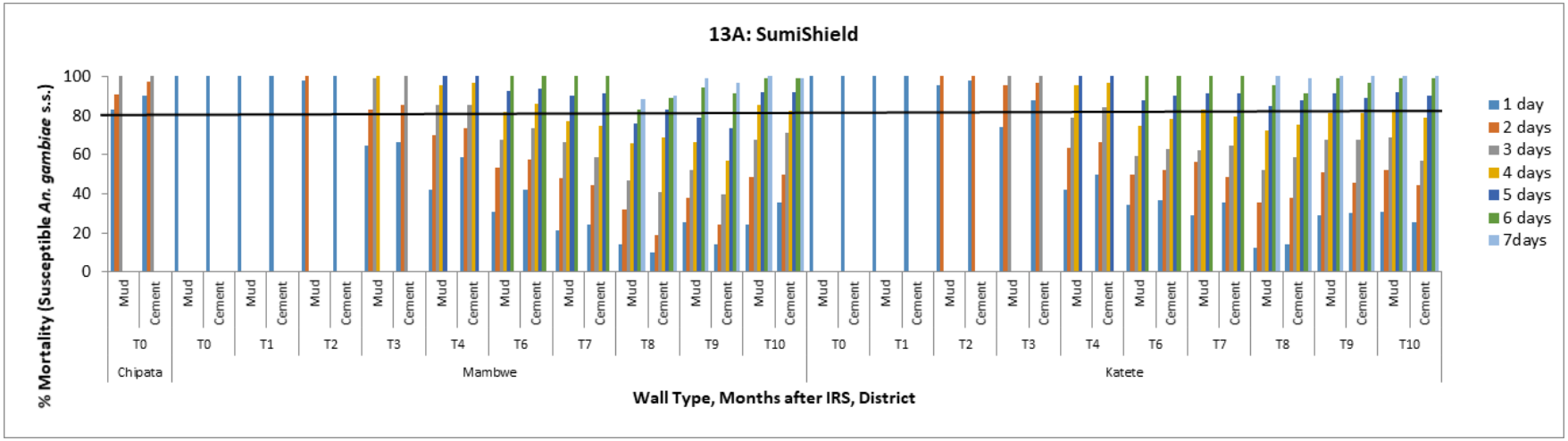
## 3.5 Quality Assurance of IRS and Insecticide Decay Rate

### 3.5.1 Quality Assurance

Wall cone bioassays were conducted in a total of 42 treated houses (21 mud and 21 cement houses) and 14 control (unsprayed) houses (seven mud and seven cement) in seven districts where VectorLink conducted IRS at T0 (during the quality of spray determination at the start of the 2019 IRS campaign). The assays were performed 24 hours after the house was sprayed. All mosquitoes exposed to the sprayed walls were dead after the 24-hour holding period in the districts sprayed with SumiShield with the exception of Chipata where 100% mortality occurred at 72 hours on both mud and cement walls (Figure 13A). In the districts sprayed with Fludora Fusion, all exposed mosquitoes died at 24 hours period except in Nchelenge where 100% mortality on mud walls occurred at 48 hours (Figure 13B). There was no variation in mortality rates of mosquitoes exposed at the different wall heights in 41 out of the 42 houses sampled. The data suggests a high quality of spray during the 2019 IRS campaign. In addition, all *An. gambiae* s.s. exposed 10 cm away from treated walls (to assess the fumigant effect of SumiShield) were dead after the 24-hour holding period (n=120), while in the districts being sprayed with Fludora Fusion, 100% mortality was achieved after 48 hours in Chililabombwe (n=60) and after 120 hours in Lufwanyama (n=60) and Nchelenge (n=60). Mortality in all control tests during the quality assurance bioassays was below the 5% threshold, thus there was no correction of exposed mortality required.

### 3.5.2 Insecticide Decay Rate

The residual efficacy represented by the mosquito mortality from T1 (one-month post-IRS) to T10 (ten months post-IRS) show mosquito mortalities above the 80% threshold for both insecticides. Corrected mortality was calculated for exposures in which control mortality was between 5-20%. No control mortalities were in excess of 20%. Monitoring of residual efficacy is planned to continue until the mortality is below the 80% cut-off for two consecutive months or the commencement of the new cycle of IRS, whichever is earlier.



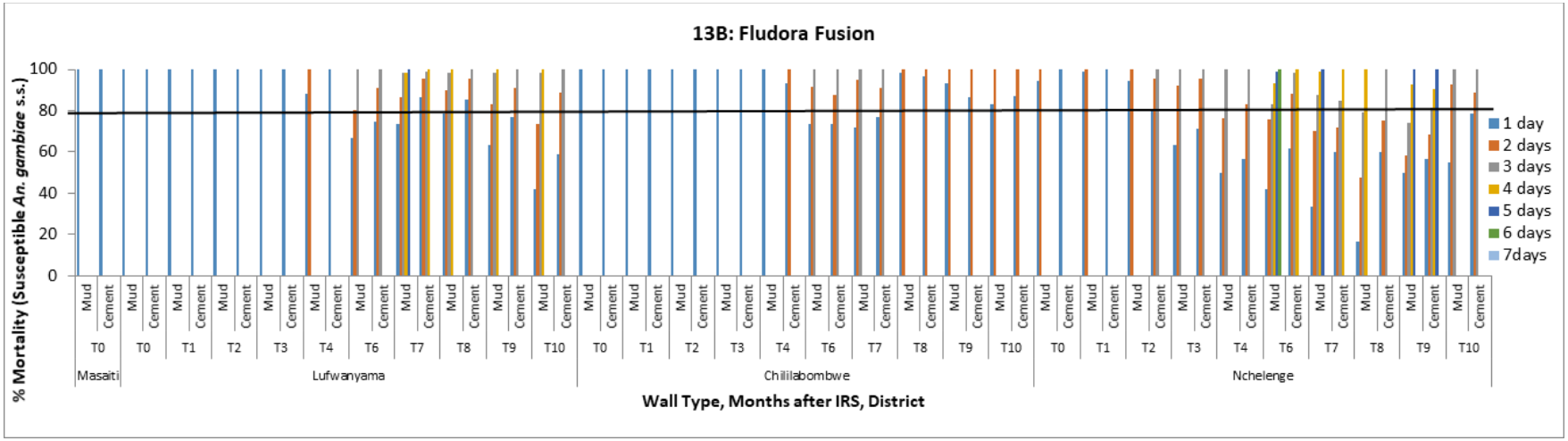




**Figure 13: Residual Efficacy - Mortality of *An. gambiae* s.l. Kisumu Strain on Surfaces Sprayed with SumiShield (A) and Fludora Fusion (B) (Oct 2019-Jul 2020)**

Note: In Figures 13A and 13B, 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.

## 3.6 INSECTICIDE SUSCEPTIBILITY TESTS

*An. funestus* s.l. and *An. gambiae* s.l. were fully susceptible to clothianidin 2%, chlorfenapyr (100 µg/bottle), and pirimiphos methyl 0.25% at all sites tested. Susceptibility to chlorfenapyr (98% post exposure mortality) among *An. funestus* s.l. populations was determined at 24hrs at one site and at 72hrs at the second site investigated, while among *An. gambiae* s.l. populations susceptibility was determined at 24hrs at eight sites and 48hrs at the ninth site investigated. Probable or confirmed resistance to DDT was observed for both *An. funestus* s.l. and *An. gambiae* s.l. at some of the sites tested. *An. gambiae* s.l. was susceptible to deltamethrin at all sites tested while probable or confirmed resistance was observed for *An. funestus* s.l. Susceptibility tests using adult mosquitoes collected from inside houses was suspended in March 2020 due to the COVID-19 pandemic. As a result, all tests conducted after this time used samples reared from larval/immature stages collected from larval habitats within the communities. More tests were done for *An. gambiae* s.l. during this period (as it was easier to rear this species from their larval stages) and all priority insecticides were covered for this species. Mortality in all control tests (non-insecticide-treated papers or untreated bottles) was below the 5% threshold, thus no correction of exposed mortality was needed. Figures 14 and 15 show susceptibility data for *An. funestus* s.l. and *An. gambiae* s.l. for all insecticides and monitoring sites. Exposed mosquito mortality of 98% (shown by the top dotted line) or above indicates susceptibility, while mortality below 90% (shown by the bottom line) indicates confirmed resistance. Mortality between the two is indicative of probable resistance. Annex 6 contains a table of the insecticide susceptibility test results conducted from December 2019 to April 2020 for both species.

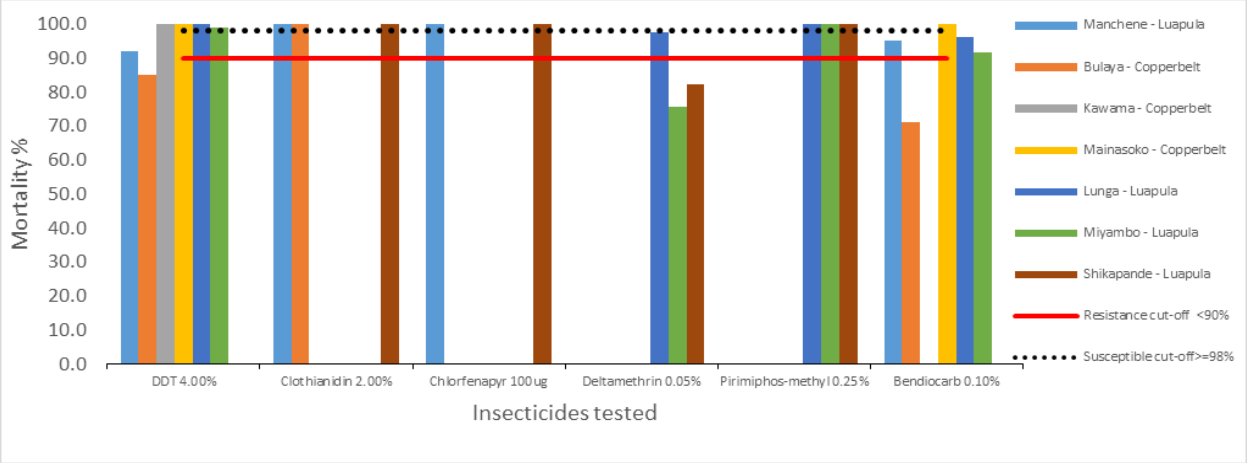



**Figure 14: Insecticide Susceptibility Status of *An. funestus* s.l. (December 2019-April 2020)**

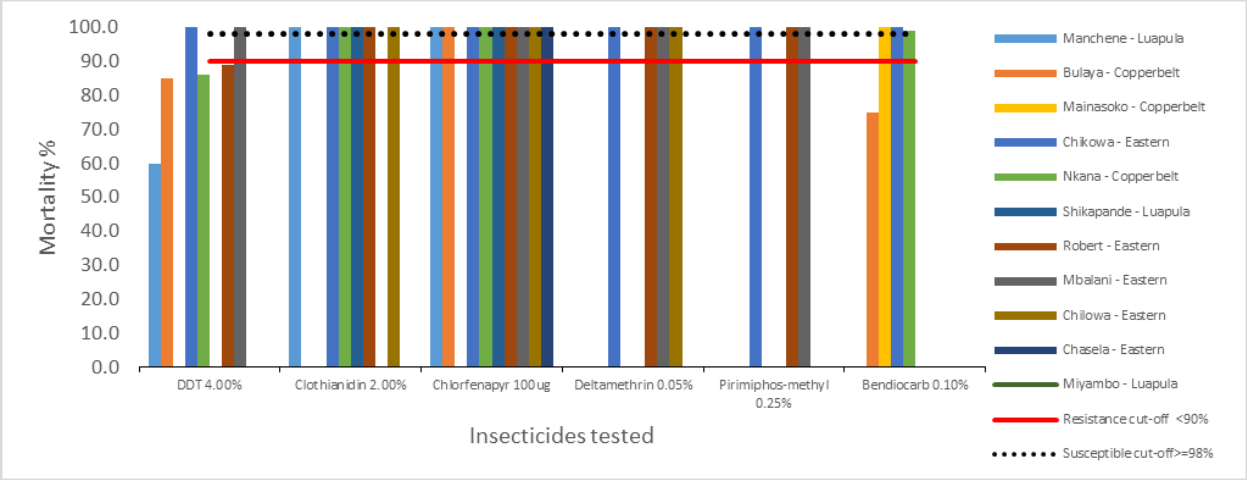



**Figure 15: Insecticide Susceptibility Status of *An. gambiae* s.l. (December 2019-April 2020)**

# 4. Discussion and Conclusions

## 4.1 Species Composition and Vector Density

*An. funestus* s.l. remains the predominant *Anopheles* species and predominant malaria vector at most surveillance sites. *Anopheles* species diversity observed during this surveillance period was similar to previous years with a significant presence of *An. ziemanni namibiensis* s.l. and *An. tchekedii*. 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 87.9% similar to what was observed in 2018-2019 period (87.6%). In this reporting year, we observed an upsurge of *An. funestus* s.l. (61.5%) among all *Anopheles* species collected at the sprayed sites compared to 46% in 2018-2019, and 27% in the 2017-2018 reporting period. Caution is required in interpreting this result as more than 40% of the sites in the current reporting period were new sites. *An. funestus* s.l. vector numbers were highest in the two districts in Luapula Province. This trend in *An. funestus* s.l. vector numbers has been reported for Luapula previously and attributed to the formation of marshes and other water bodies from the Luapula River in many parts of the province which creates permanent *An. funestus* s.l. breeding sites. *An. gambiae* s.l. vector numbers were highest in Mambwe and Nchelenge districts and lowest in Serenje and Milenge districts.

Indoor densities of *An. funestus* s.l. vectors were reduced after IRS at only two of the six sites for which data was available. The situation was similar at the control sites where *An. funestus* s.l. indoor densities increased at four of the six sites. Among the four districts where post-IRS vector numbers increased at both IRS and control sites, the difference-in-differences was positive in two districts: 1) Katete, where the increase at the IRS site was only 15% of the increase at the control site, and 2) Lufwanyama, where the increase at the two sites were almost the same at 91%. Differences in the landscape and ecological characteristics between the IRS and control sites may explain some of the observed trends. Most notably, the IRS sites are located closer to disproportionately more potential vector habitats than the control sites.

At both IRS and control sites, vector indoor densities and human biting rates increased after IRS at most sites relative to the period before IRS. These increases post-IRS may reflect the seasonal increase following the rainy season in these areas: total monthly rainfall in all seven districts increased from 6 mm before IRS in August to 308 mm immediately after IRS in October, 1,105 mm in November, and 2,067 mm in December.

Vector numbers were aggregated across districts to compare sprayed versus control sites over time. The variation in vector numbers observed among districts necessitates that all districts contribute equally to all collection periods that need to be compared, that is, collection of data in all districts should be done at equal intervals either monthly or bimonthly. A monthly regime is preferable to be able to capture expected month to month variations in mosquito numbers.

Longitudinal monitoring activities were suspended in March 2020 due to the COVID-19 pandemic and no vector surveillance data was collected in the months from March to June as planned. A mitigation plan for conducting HLCs and PSCs safely in the context of COVID-19 was developed and, after four months of consultations with PMI, the NMEP, and the WHO-Zambia office, vector surveillance activities resumed in August 2020. The data obtained will be reported in subsequent entomological monitoring reports as the first pre-IRS data for the 2020 IRS campaign.

## 4.2 Vector Biting Behavior

A discernable unimodal peak in human biting was observed at sites in Luapula Province, while at most of the other sites, there were several small peaks throughout the night. Most of the human biting in Luapula Province by both *An. funestus* s.l. and *An. gambiae* s.l. occurred late at night, when people were likely asleep and

ITNs are a very suitable intervention against this type of biting pattern. There was more biting indoors than outdoors for both *An. funestus* s.l. and *An. gambiae* s.l., as reported in previous years, making indoor vector control strategies that require vectors to enter dwellings (such as IRS and ITNs) still effective in these areas.

## 4.3 Vector Abdominal Status, Parity Rates, and Advanced Indicators

Fewer parous *An. funestus* s.l. and *An. gambiae* s.l. vectors were caught after IRS at the intervention sites compared to the period before IRS. Vector parity was lower at all IRS sites compared to control sites after IRS implementation for up to three months. Data collected from a few sites in February at four months post-IRS (that is, T+4) did not show any difference in parity between sprayed and control sites. Also, there was no data past T+4 due to the suspension of activities as a result of the COVID-19 outbreak. As a result, it is difficult to determine the duration of the effect of IRS on parity; that is, whether or not the post-IRS reductions at sprayed sites relative to control sites truly ceased at four months after IRS. Parity rates are monitored to determine the age structure of a vector population. Parity rates indicative of an older vector population increase the likelihood of malaria transmission because the vectors have survived long enough for the parasite to complete the sporogonic cycle and develop into the infective stage within the mosquito. A decrease in parity rates implies a reduction in the average longevity of the vectors which reduces the ability of the vector to transmit malaria and is the desired outcome for vector control interventions such as IRS and ITNs.

The proportion of gravid *An. funestus* s.l. and *An. gambiae* s.l. vectors were reduced at the sprayed sites after IRS compared to the period before IRS. The reductions were statistically significant for *An. funestus* s.l. but not for *An. gambiae* s.l. While fewer gravid mosquitoes is a crude indication of younger vector populations, it is still a desired outcome of vector control interventions.

*An. rivulorum* has now been observed by the project in six different provinces: Northern, Muchinga, Eastern, and Luapula Provinces (2018/2019, and Central and Copperbelt Provinces (2019/2020). *An. rivulorum* and *An. vaneedeni* are primarily zoophilic but are capable of *Plasmodium* transmission and therefore considered secondary vectors. Both vectors have been involved in malaria transmission; *An. rivulorum* in Tanzania and Kenya and *An. vaneedeni* in South Africa[17]. *An. rivulorum* and *An. rivulorum*–like were found to occur together in Milenge district. This confirms reports that the two species are sympatric in parts of Zambia[18]. *An. rivulorum* (63.1%) remains the predominant member of the *An. funestus* complex group; in the 2018/2019 laboratory report, *An. rivulorum* made up 68.9% of the *An. funestus* s.l. tested. If this trend continues, we may be observing a process in which *An. rivulorum* is replacing *An. funestus* s.s. as the predominant malaria vector due to the impact of IRS on the latter[19]. These results suggest a sustained positive impact on the more endophilic and anthropophilic *An. funestus* s.s. vector in sprayed areas. The proportion of *An. funestus* s.s. reduced from 31.1% in 2018/2019 to 15.2% in 2019/2020. The reduction in a predominantly indoor-biting species suggests IRS has had an impact over time.

Among the *An. funestus* s.l. samples, sporozoite positivity was detected in the *An. rivulorum* and *An. rivulorum*-like samples. This is an indication that both species may be important secondary vectors of *Plasmodium* in the area. Among the *An. gambiae* s.l. samples tested, sporozoite positivity was detected in *An. gambiae* s.s. but not in *An. arabiensis*. After aggregating data from all IRS sites and that from all control sites, the number of *An. funestus* s.l. infective bites received per night was lower at the IRS sites compared to the control sites, while that for *An. gambiae* s.l. was higher at the intervention sites compared to the control sites. The reduction in the

[17] Kyalo, D., Amratia, P., Mundia, C. W., Mbogo, C. M., Coetzee, M., & Snow, R. W. (2017). A geo-coded inventory of anophelines in the Afrotropical Region south of the Sahara: 1898-2016. *Wellcome open research*, *2*, 57. https://doi.org/10.12688/wellcomeopenres.12187.1

[18] Norris LC, Norris DE. Phylogeny of anopheline (Diptera: Culicidae) species in southern Africa, based on nuclear and mitochondrial genes. J Vector Ecol. 2015;40:16–27.

[19] Gillies MT, Smith A (1960): The effect of a residual house spraying campaign in East Africa on species balance in the *Anopheles funestus* group: the replacement of *Anopheles funestus* Giles by *Anopheles rivulorum* Leeson. Bull Entomol Res., 51: 243-252.

number of infective bites observed for *An. funestus* s.l. is an indication of a positive outcome of IRS in the area.

The human blood index was more than 90% for both primary species complexes, 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 been finalized. Optimization of all laboratory protocols (PCR and ELISA) with technical assistance from an established molecular laboratory at NMEC is currently underway. When the situation normalizes, we are confident that measurement of all advanced PMI indicators, including human blood index and *kdr* frequency will be done, and any future backlog of samples will be significantly minimized.

## 4.4 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 at the time of compiling this report. 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. However, Zambia may be faced with a crucial decision as to whether to continue using clothianidin based products for IRS or rotate to another active ingredient as this product would have approached two years of deployment in many districts by the 2021 IRS campaign. Currently, the only viable active ingredient to rotate to is pirimiphos methyl, which has been out of use for at least two consecutive years in most districts. In addition, 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. The hope is that a new product (Sylando® 240SC) with a new active ingredient chlorfenapyr obtains WHO pre-qualification listing and may be registered and available for use at that time. This product has been reported to show 7-10 months of residual efficacy on cement walls in experimental hut trials[20]. If a new product is not available, Zambia may have to continue the use of clothianidin-based products in some districts for the third year in most districts and for the fourth year in about three districts, raising concerns of the onset of insecticide resistance.

## 4.5 Insecticide Susceptibility

*An. funestus* s.l. and *An. gambiae* s.l. were both fully susceptible to clothianidin 2% and chlorfenapyr 100 µg/bottle at all sites tested. We found a mix of full susceptibility, possible resistance and confirmed resistance to DDT among populations of both species, a mix of possible and confirmed resistance to deltamethrin among *An. funestus* s.l. but full susceptibility among *An. gambiae* s.l. populations. There was full susceptibility to pirimiphos-methyl and a mix of full susceptibility and possible resistance to bendiocarb for both vector species. Some of the target insecticides, including chlorfenapyr and clothianidin, were not fully covered for *An. funestus* s.l. during the reporting period due to change from deploying both indoor collections of live adult mosquitoes and collection of immatures from larval habitats to collection from larval habitats only, which limited the number of mosquitoes that could be collected and tested. This was a result of the mitigation measures employed during the wake of the COVID-19 pandemic. More *An. gambiae* s.l. than *An. funestus* s.l. vectors were obtained during the intervening period and as a result more tests were performed on *An. gambiae* s.l. vectors.

[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

## 4.6 CONCLUSIONS

- The desirable outcome of 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 compared to before IRS, 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. This reduction was observed for up to three months after IRS; data collected four months after IRS indicated comparable parity rates at both sprayed and control sites. We discontinued data collection at four months and we cannot make valid conclusions on the duration of the effect of IRS on parity.
- 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.
- After IRS, there were consistently fewer gravid *An. funestus* s.l. mosquitoes at the sprayed sites compared to the control sites, an indication of a reduction in older mosquitoes.
- On mud and cement walls, 100% mortality was observed at all houses tested immediately after spraying. These findings signify high-quality wall coverage by spray operators in the 2019 spray campaign.
- Residual efficacy is at least 11 months for both SumiShield and Fludora Fusion.
- *An. funestus* s.l. and *An. gambiae* s.l. were fully susceptible to clothianidin and chlorfenapyr in all the three provinces tested: Luapula, Eastern, and Copperbelt.
- DDT resistance was confirmed among *An. gambiae* s.l. populations in Luapula, Copperbelt, and Eastern Provinces and among *An. funestus* s.l. vectors in Copperbelt Province while *An. funestus* s.l. vectors in Luapula Province were either susceptible or possible resistant.
- Deltamethrin resistance was confirmed among *An. funestus* in Luapula Province, while *An. gambiae* s.l. populations in Eastern Province were fully susceptible. No data is available Copperbelt Province.
- *An. funestus* s.l. was the predominant primary malaria vector in Luapula and Central Provinces while *An. gambiae* s.l. was the predominant vector in Eastern and Copperbelt Provinces. The entomological features of the predominant species (e.g. insecticide susceptibility) can be broadly used to make decisions for each of the provinces.
- *An. funestus* s.l., the predominant vector species, was highly endophilic, thus IRS remains an appropriate malaria intervention strategy for this part of Zambia.
- *An. arabiensis* was the predominant member of the *An. gambiae* s.l. group in Eastern Province while *An. gambiae* s.s. was the predominant species in Copperbelt Province. *An. arabiensis* is known to show variable feeding and resting habits, and will readily feed and rest outdoors. Due to the substantial outdoor biting by *An. gambiae* s.l. in Eastern Province, additional vector control interventions targeting outdoor vector populations (e.g. larval source management) can be considered for deployment.
- *An. rivulorum* was found to be positive with *Plasmodium* sporozoites in Luapula and Copperbelt Provinces. This increases the likelihood that this species is a potentially major malaria vector in this area.
- There was little or no impact on the numbers of *An. gambiae* s.l. indoor resting or human biting rates. This may be related to proliferation of the vectors during the rainy season that coincides with the post-intervention period. However, parity was low in the first three months of post-IRS, and the population is likely dominated by freshly emerging vector mosquitoes during the rainy season. Also, *An. arabiensis*, a predominant member of this complex at some of the sites, is known to exhibit exophagic behavior and may not be subject to the full effect of the sprayed walls.

- The marginal impact on vector density at sprayed sites has been observed since 2017, indicating a stagnation of vector numbers in the region. Complementary vector control interventions should be explored to further reduce vector numbers below the current levels. For instance, universal coverage with ITNs in all endemic districts and larval source management in suitable districts in Eastern Province should be considered.
- ITNs can be an effective intervention in Luapula Province where most of the human biting by both *An. funestus* s.l. and *An. gambiae* s.l. occurred late at night, when people were likely asleep.
- Both primary vectors rely heavily on the human host for blood feeding and thus, interventions targeting human dwellings remain valid.

# 5. RECOMMENDATIONS

- As outdoor biting occurred at many sites in Eastern Province, identify areas where community-based larval source management is feasible and consider its implementation as a complementary intervention.
- If faced with the decision of either rotating to a susceptible shorter duration insecticide such as pirimiphos-methyl or continuing the deployment of clothianidin-based insecticides, consider the use of the clothianidin based insecticide for another year (third year in most districts already deploying this active ingredient and fourth year in three districts in Eastern).
- Due to the continued resistance of local vectors to pyrethroid insecticides in some areas, consider introducing PBO nets (that is, nets with the synergist piperonyl butoxide) or nets with dual active ingredients (that is pyrethroid, plus a pyrrole) in select areas, especially where ITNs are the major vector control intervention.
- If feasible, conduct monthly vector surveillance in all sentinel districts during the 2021 work plan period to capture the expected month-to-month variations in mosquito numbers and ensure sites contribute equally to data aggregated by time period.
- Conduct additional susceptibility assays for insecticides not fully represented in terms of sites and number of samples tested ahead of the 2021 Technical Advisory Committee meeting on insecticide choice. These include deltamethrin tests among *An. funestus* in Copperbelt and Eastern Provinces and among *An. gambiae* s.l. populations in Luapula and Copperbelt Provinces, and also PBO synergist tests in all provinces showing deltamethrin resistance.
- Consider relocating the surveillance sites in Serenje district to sites in health facility catchment areas reporting higher malaria cases. This is due to the consistently low numbers of vector species collected at the current sites.
- Continue to maintain the collaboration with NMEP laboratory in Lusaka, which is receiving support from the Malaria Control and Elimination Partnership in Africa project (MACEPA) to supplement molecular analyses of mosquito samples to solve the recurrent issue of delays in obtaining vital data from mosquito samples collected in the field. Work closely with PATH laboratory in Zambia to address the issue of low PCR amplification rates, which usually necessitates repeating analysis on the same samples and slows down sample turnover in the laboratory. Consider having PATH commence work on the 2020/2021 samples and put into effect prompt and regular analysis of samples (as opposed to long term storage) to significantly help resolve backlog.

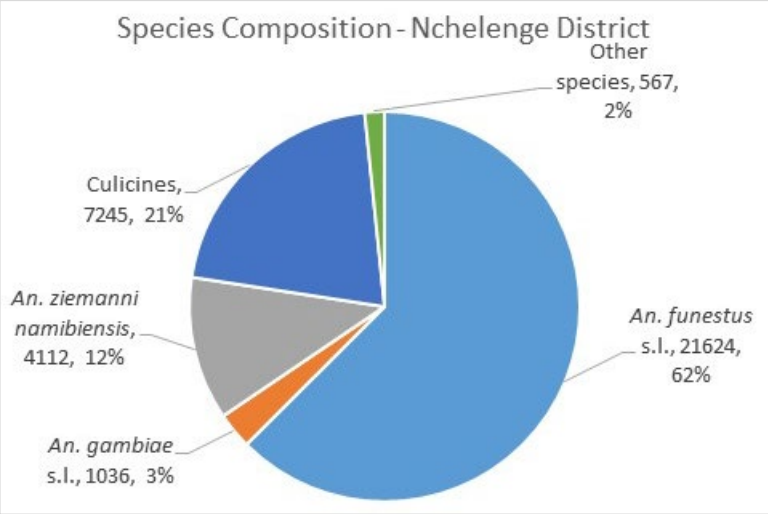
# ANNEX 1: CULICIDAE COLLECTED IN SPRAYED AND CONTROL SITES BY COLLECTION METHOD (JUNE 2019-FEBRUARY 2020)

| District | Village | Status | *An. funestus* s.l. | *An. gambiae* s.l. | *An. ziemanni namibiensis* | *An. maculipalpis* | *An. coustani* | *An. tenebrosus* | *An. gibbinsi* | *An. rufipes* | *An. pretoriensis* | *An. squamosus* | *An. argentio-lobatus* | *An. tchekedii* | *Culicines* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HLC Indoor** | | | | | | | | | | | | | | | |
| Nchelenge | Shikapande | Sprayed | 6,978 | 276 | 385 | 13 | 56 | 3 | 0 | 5 | 3 | 3 | 0 | 0 | 462 |
| | Mancehe | Control | 5,245 | 248 | 1,272 | 51 | 57 | 4 | 3 | 4 | 13 | 6 | 0 | 0 | 1,613 |
| Milenge | Lunga | Sprayed | 3,579 | 4 | 468 | 0 | 20 | 0 | 0 | 0 | 0 | 29 | 0 | 388 | 225 |
| | Miyambo | Control | 8,189 | 7 | 457 | 0 | 3 | 0 | 0 | 0 | 0 | 102 | 21 | 215 | 182 |
| Mambwe | Chikowa | Sprayed | 29 | 87 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 58 |
| | Chasela | Control | 2 | 21 | 0 | 1 | 1 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 314 |
| Katete | Chilowa | Sprayed | 45 | 17 | 0 | 3 | 43 | 0 | 0 | 7 | 0 | 1 | 0 | 0 | 71 |
| | Robert | Control | 62 | 16 | 2 | 5 | 8 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 91 |
| Serenje | Chibobo | Sprayed | 6 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 4 |
| | Chish | Control | 16 | 0 | 0 | 0 | 0 | 4 | 2 | 6 | 0 | 0 | 0 | 0 | 13 |
| Lufwanyama | Nkana | Sprayed | 433 | 2,144 | 10 | 13 | 3 | 0 | 13 | 20 | 0 | 0 | 0 | 0 | 453 |
| | Bulaya | Control | 838 | 720 | 49 | 30 | 20 | 0 | 5 | 12 | 0 | 5 | 0 | 1 | 235 |
| Chililabombwe | Kawama | Sprayed | 287 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89 |
| | Maina Soko | Control | 236 | 77 | 63 | 159 | 0 | 0 | 1 | 0 | 0 | 24 | 2 | 0 | 89 |
| **Total** | | | **25,945** | **3,639** | **2,706** | **275** | **213** | **11** | **24** | **74** | **16** | **171** | **23** | **604** | **3,899** |

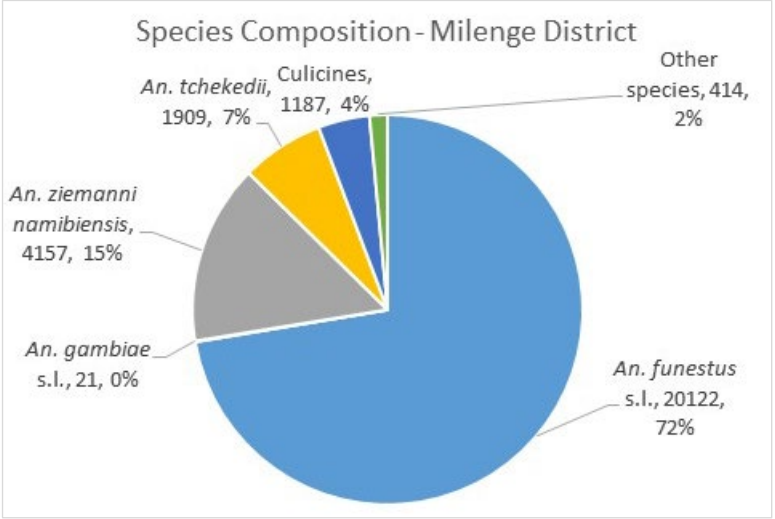
| District | Village | Status | *An. funestus* s.l. | *An. gambiae* s.l. | *An. ziemanni namibiensis* | *An. maculipalpis* | *An. coustani* | *An. tenebrosus* | *An. gibbinsi* | *An. rufipes* | *An. pretoriensis* | *An. squamosus* | *An. argentio-lobatus* | *An. tchekedii* | *Culicines* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HLC Outdoor** | | | | | | | | | | | | | | | |
| Nchelenge | Shikapande | Sprayed | 3,242 | 182 | 484 | 6 | 77 | 3 | 0 | 1 | 2 | 5 | 0 | 0 | 1,710 |
| | Mancehe | Control | 3,422 | 248 | 1,785 | 114 | 76 | 0 | 5 | 9 | 22 | 25 | 0 | 0 | 3,428 |
| Milenge | Lunga | Sprayed | 3,032 | 0 | 635 | 0 | 31 | 0 | 0 | 0 | 0 | 52 | 0 | 646 | 298 |
| | Miyambo | Control | 4,259 | 10 | 2,597 | 0 | 35 | 0 | 0 | 2 | 0 | 71 | 48 | 659 | 471 |
| Mambwe | Chikowa | Sprayed | 47 | 286 | 2 | 0 | 20 | 5 | 0 | 3 | 0 | 0 | 0 | 0 | 308 |
| | Chasela | Control | 9 | 84 | 0 | 2 | 1 | 0 | 0 | 28 | 0 | 1 | 0 | 0 | 585 |
| Katete | Chilowa | Sprayed | 26 | 49 | 0 | 1 | 103 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 128 |
| | Robert | Control | 61 | 27 | 1 | 3 | 8 | 0 | 0 | 10 | 0 | 2 | 0 | 0 | 114 |
| Serenje | Chibobo | Sprayed | 8 | 3 | 0 | 0 | 2 | 1 | 0 | 10 | 0 | 0 | 0 | 0 | 7 |
| | Chish | Control | 16 | 2 | 0 | 0 | 0 | 6 | 1 | 4 | 0 | 0 | 0 | 0 | 23 |
| Lufwanyama | Nkana | Sprayed | 234 | 1,084 | 13 | 25 | 2 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 323 |
| | Bulaya | Control | 173 | 77 | 240 | 62 | 55 | 1 | 2 | 1 | 0 | 17 | 0 | 0 | 156 |
| Chililabombwe | Kawama | Sprayed | 331 | 25 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 96 |
| | Maina Soko | Control | 81 | 34 | 108 | 123 | 0 | 1 | 0 | 0 | 0 | 23 | 0 | 0 | 50 |
| **Total** | | | **14,941** | **2,111** | **5,875** | **336** | **410** | **17** | **11** | **76** | **24** | **200** | **48** | **1,305** | **7,697** |

| District | Village | Status | *An. funestus* s.l. | *An. gambiae* s.l. | *An. ziemanni namibiensis* | *An. maculipalpis* | *An. coustani* | *An. tenebrosus* | *An. gibbinsi* | *An. rufipes* | *An. pretoriensis* | *An. squamosus* | *An. argentio-lobatus* | *An. tchekedii* | *Culicines* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PSC** | | | | | | | | | | | | | | | |
| Nchelenge | Shikapande | Sprayed | 1,153 | 18 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| | Mancehe | Control | 1,584 | 64 | 186 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 |
| Milenge | Lunga | Sprayed | 335 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | Miyambo | Control | 728 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 |
| Mambwe | Chikowa | Sprayed | 16 | 2 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 16 |
| | Chasela | Control | 12 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 82 |
| Katete | Chilowa | Sprayed | 21 | 5 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Robert | Control | 165 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Serenje | Chibobo | Sprayed | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Chish | Control | 30 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lufwanyama | Nkana | Sprayed | 193 | 280 | 0 | 0 | 0 | 0 | 10 | 15 | 0 | 0 | 0 | 0 | 6 |
| | Bulaya | Control | 276 | 95 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chililabombwe | Kawama | Sprayed | 153 | 4 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 144 |
| | Maina Soko | Control | 81 | 10 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 |
| **Total** | | | **4,751** | **488** | **224** | **0** | **4** | **1** | **10** | **19** | **0** | **0** | **0** | **1** | **312** |

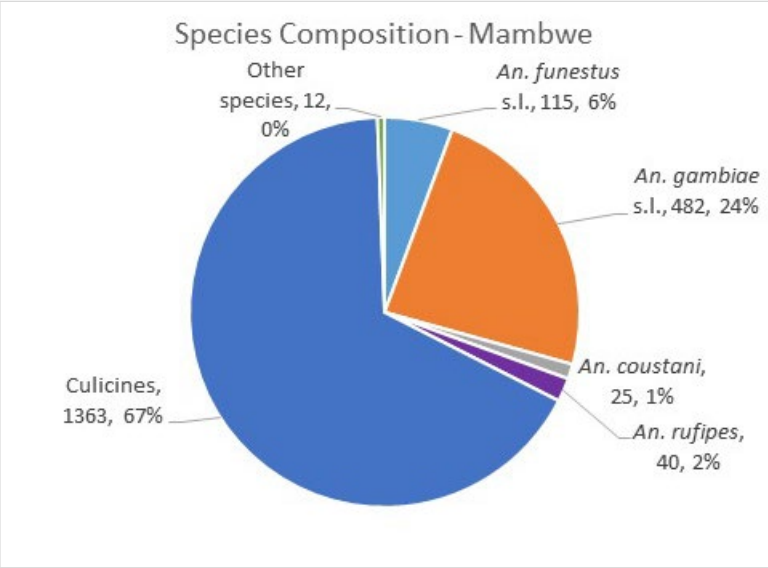
# ANNEX 2: SPECIES COMPOSITION BY DISTRICT



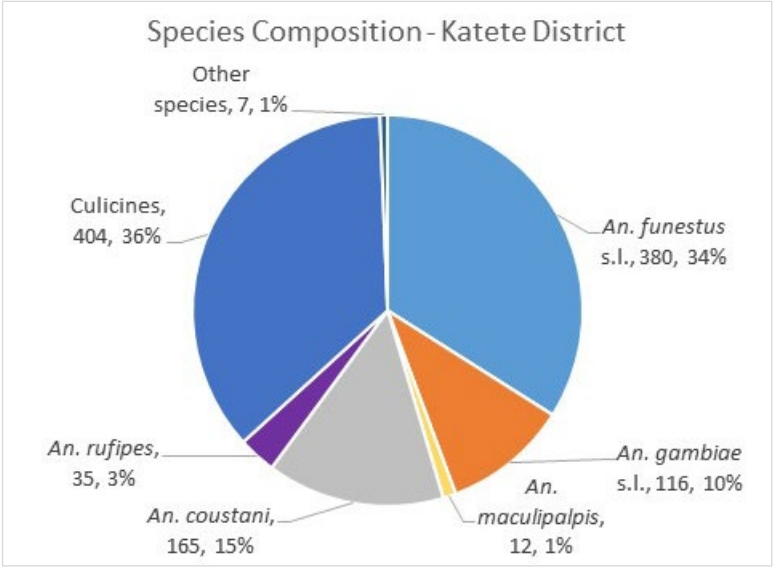

Other species include *An. coustani* (266, 0.77%), *An. maculipalpis* (184, 0.53%), *An. pretoriensis* (40, 0.12%), *An. squamosus* (39, 0.11%), *An. rufipes* (20, 0.06%), *An. tenebrosus* (10, 0.03), and *An. gibbinsi* (8, 0.02%).



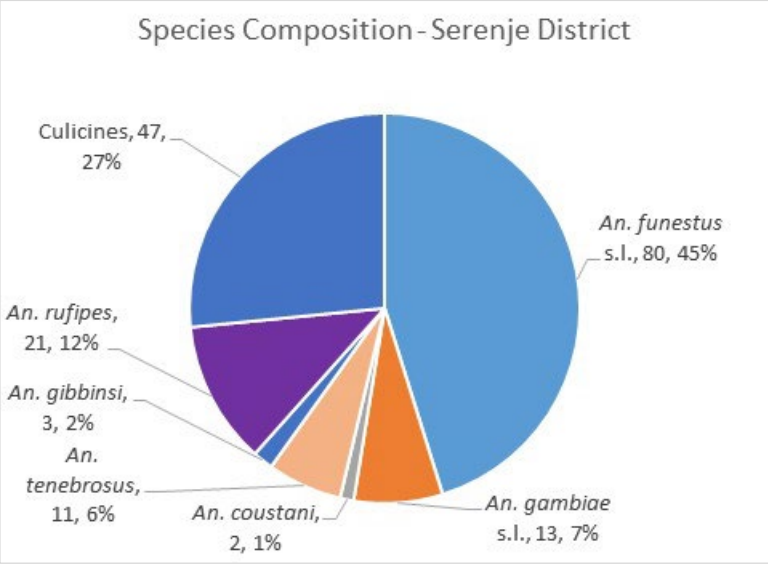

Other species include *An. squamosus* (254, 0.91%), *An. coustani* (89, 0.32%), *An. argentiolobatus* (69, 0.25%), and *An. rufipes* (2, 0.01%).

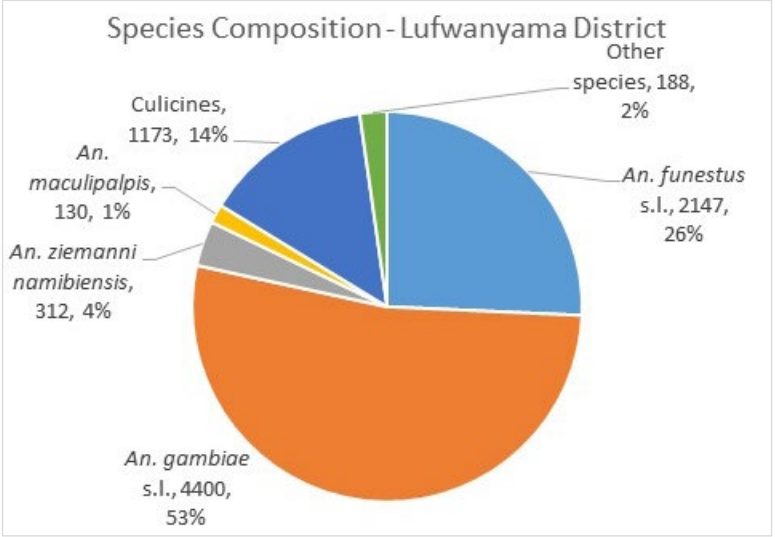



Other species include *An. tenebrosus* (5, 0.25%), *An. maculipalpis* (3, 0.15%), *An. ziemanni namibiensis* (2, 0.1%), and *An. squamosus* (2, 0.1%)

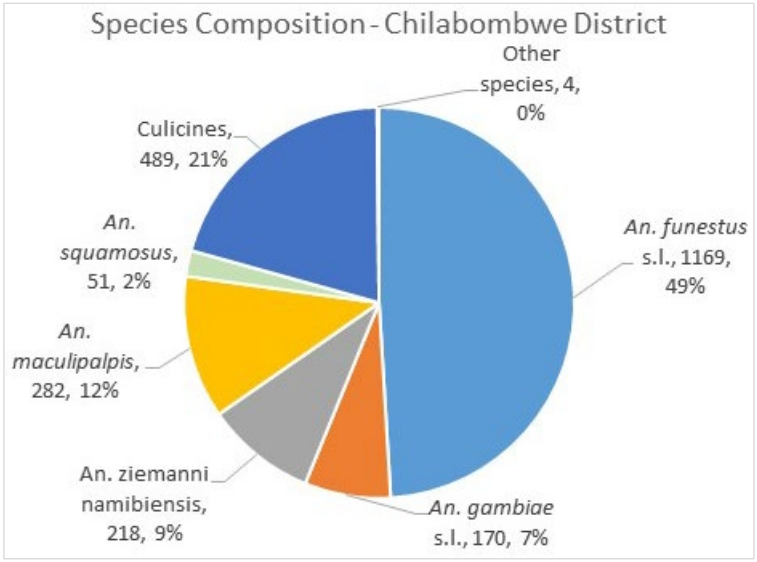



Other species include *An. ziemanni namibiensis* (4, 0.36%), *An. squamosus* (3, 0.27%), and *An. tenebrosus* (1, 0.09%).




Other species include *An. coustani* (80, 0.96%), *An. rufipes* (51, 0.61%), *An. gibbinsi* (33, 0.46%), An. squamosus (22, 0.26%), *An. tenebrosus* (1, 0.01%), and *An. tchekedii* (1, 0.01%).




Other species include *An. argentiolobatus* (2, 0.08%), *An. tenebrosus* (1, 0.04), and *An. gibbinsi* (1, 0.04%).

# ANNEX 3: *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. BY MONTH, SITE, AND COLLECTION METHOD (JUNE 2019-FEBRUARY 2020)

| Month, Year | District | Site | Status | *An. funestus* s.l. | | | | *An. gambiae* s.l. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Number Collected by Indoor HLC | Number Collected by Outdoor HLC | Number Collected by PSC | Monthly Total Collected | Number Collected by Indoor HLC | Number Collected by Outdoor HLC | Number Collected by PSC | Monthly Total Collected |
| June, 2019 | Nchelenge | Shikapande | Spayed | 1,258 | 232 | 256 | 4,798 | 17 | 9 | 0 | 105 |
| | | Manchene | Control | 74 | 19 | 240 | | 3 | 1 | 0 | |
| | Milenge | Lunga | Sprayed | 469 | 452 | 70 | | 1 | 0 | 0 | |
| | | Miyambo | Control | 987 | 449 | 107 | | 1 | 2 | 0 | |
| | Mambwe | Chikowa | Sprayed | 0 | 4 | 7 | | 4 | 4 | 0 | |
| | | Chasela | Control | 1 | 1 | 0 | | 1 | 1 | 1 | |
| | Katete | Mbalani | Sprayed | 0 | 0 | 0 | | 0 | 0 | 0 | |
| | | Robert | Control | 5 | 4 | 0 | | 0 | 0 | 0 | |
| | Serenje | Chibobo | Sprayed | 1 | 3 | 0 | | 0 | 0 | 0 | |
| | | Chishi | Control | 5 | 3 | 1 | | 0 | 1 | 0 | |
| | Lufwanyama | Nkana | Sprayed | 11 | 3 | 7 | | 38 | 9 | 9 | |
| | | Bulaya | Control | 13 | 2 | 17 | | 0 | 0 | 0 | |
| | Chililabombwe | Kawama | Sprayed | 43 | 10 | 25 | | 0 | 0 | 2 | |
| | | Maina Soko | Control | 7 | 6 | 6 | | 0 | 0 | 1 | |

| Month, Year | District | Site | Status | *An. funestus* s.l. | | | | *An. gambiae* s.l. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Number Collected by Indoor HLC | Number Collected by Outdoor HLC | Number Collected by PSC | Monthly Total Collected | Number Collected by Indoor HLC | Number Collected by Outdoor HLC | Number Collected by PSC | Monthly Total Collected |
| August, 2019 | Nchelenge | Shikapande | Sprayed | 1,357 | 1,020 | 124 | 7,251 | 6 | 3 | 0 | 78 |
| | | Manchene | Control | 395 | 150 | 346 | | 5 | 0 | 0 | |
| | Milenge | Lunga | Spayed | 763 | 716 | 72 | | 1 | 0 | 0 | |
| | | Miyambo | Control | 1,255 | 643 | 246 | | 1 | 1 | 0 | |
| | Mambwe | Chikowa | Sprayed | 6 | 5 | 0 | | 4 | 24 | 0 | |
| | | Chasela | Control | 1 | 1 | 1 | | 0 | 3 | 0 | |
| | Katete | Chiloba | Sprayed | 0 | 0 | 0 | | 0 | 0 | 0 | |
| | | Robert | Control | 0 | 1 | 10 | | 0 | 0 | 0 | |
| | Serenje | Chibobo | Sprayed | 0 | 0 | 3 | | 0 | 1 | 0 | |
| | | Chishi | Control | 0 | 2 | 6 | | 0 | 0 | 0 | |
| | Lufwanyama | Nkana | Sprayed | 8 | 0 | 1 | | 4 | 8 | 1 | |
| | | Bulaya | Control | 10 | 2 | 11 | | 2 | 2 | 0 | |
| | Chililabombwe | Kawama | Sprayed | 30 | 12 | 8 | | 3 | 3 | 0 | |
| | | Maina Soko | Control | 24 | 16 | 6 | | 3 | 2 | 1 | |

| Month, Year | District | Site | Status | *An. funestus* s.l. | | | | *An. gambiae* s.l. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Number Collected by Indoor HLC | Number Collected by Outdoor HLC | Number Collected by PSC | Monthly Total Collected | Number Collected by Indoor HLC | Number Collected by Outdoor HLC | Number Collected by PSC | Monthly Total Collected |
| October, 2019 | Nchelenge | Shikapande | Sprayed | 981 | 559 | 127 | 14,527 | 57 | 3 | 0 | 390 |
| | | Manchene | Control | 994 | 403 | 334 | | 20 | 8 | 1 | |
| | Milenge | Lunga | Spayed | 2,253 | 1,789 | 147 | | 1 | 0 | 0 | |
| | | Miyambo | Control | 4,076 | 2,215 | 182 | | 0 | 1 | 0 | |
| | Mambwe | Chikowa | Sprayed | 2 | 2 | 0 | | 0 | 3 | 0 | |
| | | Chasela | Control | 0 | 0 | 0 | | 0 | 1 | 0 | |
| | Katete | Chiloba | Sprayed | 0 | 0 | 0 | | 0 | 0 | 0 | |
| | | Robert | Control | 6 | 4 | 17 | | 0 | 0 | 0 | |
| | Serenje | Chibobo | Sprayed | 3 | 2 | 1 | | 0 | 0 | 0 | |
| | | Chishi | Control | 3 | 2 | 9 | | 0 | 1 | 4 | |
| | Lufwanyama | Nkana | Sprayed | 8 | 10 | 45 | | 158 | 97 | 23 | |
| | | Bulaya | Control | 27 | 5 | 3 | | 1 | 0 | 0 | |
| | Chililabombwe | Kawama | Sprayed | 63 | 91 | 72 | | 4 | 7 | 0 | |
| | | Maina Soko | Control | 30 | 9 | 53 | | 0 | 0 | 0 | |
| November, 2019 | Nchelenge | Shikapande | Sprayed | 1,169 | 493 | 226 | 3,355 | 32 | 33 | 6 | 585 |
| | | Manchene | Control | 451 | 469 | 192 | | 41 | 37 | 23 | |
| | Mambwe | Chikowa | Spayed | 9 | 10 | 0 | | 3 | 10 | 1 | |
| | | Chasela | Control | 0 | 1 | 3 | | 0 | 11 | 0 | |
| | Lufwanyama | Nkana | Sprayed | 22 | 26 | 39 | | 193 | 131 | 31 | |
| | | Bulaya | Control | 108 | 34 | 103 | | 25 | 7 | 1 | |

| Month, Year | District | Site | Status | *An. funestus* s.l. | | | | *An. gambiae* s.l. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Number Collected by Indoor HLC | Number Collected by Outdoor HLC | Number Collected by PSC | Monthly Total Collected | Number Collected by Indoor HLC | Number Collected by Outdoor HLC | Number Collected by PSC | Monthly Total Collected |
| December, 2019 | Nchelenge | Shikapande | Sprayed | 720 | 220 | 83 | 6,901 | 27 | 10 | 2 | 1,661 |
| | | Manchene | Control | 967 | 904 | 29 | | 103 | 96 | 0 | |
| | Milenge | Lunga | Sprayed | 94 | 75 | 46 | | 1 | 0 | 0 | |
| | | Miyambo | Control | 1,871 | 952 | 193 | | 5 | 6 | 0 | |
| | Mambwe | Chikowa | Sprayed | 11 | 14 | 7 | | 13 | 46 | 1 | |
| | | Chasela | Control | 0 | 3 | 8 | | 13 | 45 | 1 | |
| | Katete | Chiloba | Sprayed | 1 | 0 | 3 | | 0 | 0 | 3 | |
| | | Robert | Control | 7 | 14 | 80 | | 3 | 1 | 1 | |
| | Serenje | Chibobo | Sprayed | 2 | 3 | 0 | | 2 | 2 | 1 | |
| | | Chishi | Control | 8 | 9 | 14 | | 0 | 0 | 1 | |
| | Lufwanyama | Nkana | Spayed | 10 | 11 | 16 | | 584 | 344 | 92 | |
| | | Bulaya | Control | 113 | 13 | 49 | | 168 | 18 | 39 | |
| | Chililabombwe | Kawama | Sprayed | 95 | 145 | 40 | | 6 | 7 | 2 | |
| | | Maina Soko | Control | 60 | 6 | 5 | | 15 | 2 | 1 | |
| January, 2020 | Nchelenge | Shikapande | Sprayed | 817 | 325 | 221 | 4,202 | 53 | 41 | 4 | 1,612 |
| | | Manchene | Control | 1,108 | 484 | 236 | | 12 | 4 | 23 | |
| | Mambwe | Chikowa | Sprayed | 1 | 12 | 2 | | 63 | 199 | 0 | |
| | | Chasela | Control | 0 | 3 | 0 | | 7 | 23 | 0 | |
| | Lufwanyama | Nkana | Sprayed | 283 | 129 | 47 | | 557 | 237 | 75 | |
| | | Bulaya | Control | 374 | 104 | 56 | | 231 | 39 | 44 | |

| Month, Year | District | Site | Status | *An. funestus* s.l. | | | | *An. gambiae* s.l. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Number Collected by Indoor HLC | Number Collected by Outdoor HLC | Number Collected by PSC | Monthly Total Collected | Number Collected by Indoor HLC | Number Collected by Outdoor HLC | Number Collected by PSC | Monthly Total Collected |
| February, 2020 | Nchelenge | Shikapande | Sprayed | 676 | 393 | 116 | 4,603 | 84 | 83 | 6 | 1,897 |
| | | Manchene | Control | 1,256 | 993 | 207 | | 64 | 102 | 17 | |
| | Katete | Chilowa | Sprayed | 44 | 26 | 18 | | 17 | 49 | 2 | |
| | | Robert | Control | 44 | 38 | 58 | | 13 | 26 | 1 | |
| | Lufwanyama | Nkana | Sprayed | 91 | 55 | 38 | | 610 | 258 | 49 | |
| | | Bulaya | Control | 193 | 13 | 37 | | 293 | 11 | 11 | |
| | Chililabombwe | Kawama | Sprayed | 56 | 73 | 8 | | 7 | 8 | 0 | |
| | | Maina Soko | Control | 115 | 44 | 11 | | 59 | 30 | 7 | |
| **TOTAL** | | | | **25,945** | **14,941** | **4,751** | **45,637** | **3,639** | **2,111** | **488** | **6328** |

# ANNEX 4: 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 (Jun 2019-Feb 2020)**

**A. 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 | 5.18 | 3.29 | 0.74 | 0.02 | 0.32 | 0.55 | 1.69 | 0.03 |
| ALL-Sprayed | Pre-IRS v Post-IRS | 2.57 | 3.76 | 1.16 | 0.26 | 0.05 | 0.88 | 3.98 | <0.0001 |
| ALL-Control | Pre-IRS v Post-IRS | 4.48 | 5.65 | 1.37 | 0.01 | 0.03 | 0.51 | 6.27 | 0.0001 |
| Nchelenge | Control v Sprayed | 15.41 | 10.66 | 0.69 | 0.05 | 0.61 | 0.17 | 0.28 | 0.07 |
| Milenge | Control v Sprayed | 12.13 | 5.58 | 0.46 | 0.0000 | 0.00 | 0.00 | N/A | N/A |
| Mambwe | Control v Sprayed | 0.07 | 0.18 | 2.67 | 0.14 | 0.02 | 0.02 | 1.00 | 1.00 |
| Katete | Control v Sprayed | 2.20 | 0.30 | 0.14 | <0.0001 | 0.03 | 0.04 | 1.52 | 0.6871 |
| Serenje | Control v Sprayed | 0.53 | 0.07 | 0.13 | 0.0001 | 0.08 | 0.02 | 0.20 | 0.2020 |
| Lufwanyama | Control v Sprayed | 2.63 | 1.85 | 0.70 | 0.18 | 0.90 | 2.67 | 2.95 | <0.0001 |
| Chililabombwe | Control v Sprayed | 1.21 | 2.31 | 1.92 | 0.02 | 0.15 | 0.06 | 0.41 | 0.21 |

| 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 |
| Shikapande-Sprayed | Pre-IRS v Post-IRS | 12.67 | 9.85 | 0.78 | <0.0001 | 0.00 | 0.24 | N/A | N/A |
| Manchene-Control | Pre-IRS v Post-IRS | 19.53 | 13.76 | 0.70 | <0.0001 | 0.00 | 0.85 | N/A | N/A |
| Lunga-Sprayed | Pre-IRS v Post-IRS | 4.73 | 6.43 | 1.36 | 0.006 | 0.00 | 0.00 | N/A | N/A |
| Miyambo-Control | Pre-IRS v Post-IRS | 11.77 | 12.50 | 1.06 | 0.41 | 0.00 | 0.00 | N/A | N/A |
| Chikowa-Sprayed | Pre-IRS v Post-IRS | 0.23 | 0.15 | 0.64 | 0.38 | 0.00 | 0.03 | N/A | N/A |
| Chasela-Control | Pre-IRS v Post-IRS | 0.03 | 0.08 | 2.50 | 0.40 | 0.03 | 0.02 | 0.50 | 0.62 |
| Chiloba-Sprayed | Pre-IRS v Post-IRS | 0.00 | 0.49 | N/A | N/A | 0.00 | 0.07 | N/A | N/A |
| Robert-Control | Pre-IRS v Post-IRS | 0.33 | 3.44 | 10.33 | <0.0001 | 0.00 | 0.04 | N/A | N/A |
| Chibobo-Sprayed | Pre-IRS v Post-IRS | 0.09 | 0.00 | N/A | N/A | 0.00 | 0.07 | N/A | N/A |
| Chishi-Control | Pre-IRS v Post-IRS | 0.40 | 0.93 | 2.33 | 0.02 | 0.09 | 0.07 | 0.75 | 0.797 |
| Nkana-Sprayed | Pre-IRS v Post-IRS | 0.30 | 2.47 | 8.22 | <0.0001 | 0.33 | 3.60 | 10.80 | <0.0001 |
| Bulaya-Control | Pre-IRS v Post-IRS | 0.93 | 3.31 | 3.54 | <0.0001 | 0.00 | 1.27 | N/A | N/A |
| Kawama-Sprayed | Pre-IRS v Post-IRS | 1.10 | 3.26 | 2.78 | <0.0001 | 0.07 | 0.05 | 0.82 | 0.84 |
| Maina Soko-Control | Pre-IRS v Post-IRS | 0.37 | 1.92 | 4.78 | <0.0001 | 0.07 | 0.22 | 2.79 | 0.20 |

*For IRR, the reference group is “control" or "pre-intervention period”.
Shaded cells significant at 0.05%. N/A means no p-values obtained because two sites had the same value or one site had two zero values

## B. Abdominal Condition - Vectors Collected by PSC

| Site | Comparison | *An. funestus* s.l. | | | | *An. gambiae* s.l. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean Proportion Gravid [First group]: | Mean Proportion Gravid [Second group]: | Random Effects IRR | p-value | Mean Proportion Gravid [First group]: | Mean Proportion Gravid [Second group]: | Random Effects IRR | p-value |
| All | Control v Sprayed | 9% | 7% | 0.87 | 0.678 | 4% | 3% | 0.83 | 0.76 |
| ALL-Sprayed | Pre-IRS v Post-IRS | 15% | 4% | 0.23 | 0.002 | 8% | 3% | 0.27 | 0.24 |
| ALL-Control | Pre-IRS v Post-IRS | 9% | 10% | 0.59 | 0.19 | 11% | 3% | 0.27 | 0.25 |
| Nchelenge | Control v Sprayed | 6% | 8% | 1.33 | 0.67 | - | - | N/A | N/A |
| Milenge | Control v Sprayed | 8% | 5% | 2.01 | 0.48 | - | - | N/A | N/A |
| Mambwe | Control v Sprayed | 57% | 14% | 0.25 | 0.11 | 0% | 25% | N/A | N/A |
| Katete | Control v Sprayed | 46% | 48% | 1.33 | 0.74 | 0% | 40% | N/A | N/A |
| Serenje | Control v Sprayed | 42% | 0% | 0.00 | 1.00 | 25% | | N/A | N/A |
| Lufwanyama | Control v Sprayed | 6% | 5% | 1.46 | 0.60 | 3% | 3% | 0.83 | 0.82 |
| Chililabombwe | Control v Sprayed | 6% | 2% | 0.22 | 0.20 | 10% | 0% | N/A | N/A |

| Site | Comparison | *An. funestus* s.l. | | | | *An. gambiae* s.l. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean Proportion Gravid [First group]: | Mean Proportion Gravid [Second group]: | Random Effects IRR | p-value | Mean Proportion Gravid [First group]: | Mean Proportion Gravid [Second group]: | Random Effects IRR | p-value |
| Shikapande-Sprayed | Pre-IRS v Post-IRS | 17% | 4% | 0.12 | 0.02 | - | - | N/A | N/A |
| Manchene-Control | Pre-IRS v Post-IRS | 2% | 8% | 0.60 | 0.60 | - | - | N/A | N/A |
| Lunga-Sprayed | Pre-IRS v Post-IRS | 11% | 2% | 0.62 | 0.73 | - | - | N/A | N/A |
| Miyambo-Control | Pre-IRS v Post-IRS | 12% | 4% | 0.99 | 0.99 | - | - | N/A | N/A |
| Chikowa-Sprayed | Pre-IRS v Post-IRS | 0% | 29% | N/A | N/A | 25% | - | N/A | N/A |
| Chasela-Control | Pre-IRS v Post-IRS | 100% | 50% | 0.50 | 0.55 | 0% | 0% | N/A | N/A |
| Chiloba-Sprayed | Pre-IRS v Post-IRS | 48% | | N/A | N/A | 40% | - | N/A | N/A |
| Robert-Control | Pre-IRS v Post-IRS | 40% | 46% | 0.36 | 0.37 | 0% | - | N/A | N/A |
| Chibobo-Sprayed | Pre-IRS v Post-IRS | 0% | 0% | N/A | N/A | - | - | N/A | N/A |
| Chishi-Control | Pre-IRS v Post-IRS | 56% | 27% | 0.48 | 0.22 | 25% | - | N/A | N/A |
| Nkana-Sprayed | Pre-IRS v Post-IRS | 56% | 3% | 0.05 | <0.0001 | 10% | 2% | 0.12 | 0.08 |
| Bulaya-Control | Pre-IRS v Post-IRS | 43% | 2% | 0.03 | 0.002 | 3% | - | N/A | N/A |
| Kawama-Sprayed | Pre-IRS v Post-IRS | 9% | 0% | N/A | N/A | 0% | 0% | N/A | N/A |
| Maina Soko-Control | Pre-IRS v Post-IRS | 27% | 3% | 0.15 | 0.15 | 0% | 13% | N/A | N/A |

*For IRR, the reference group is “control" or "pre-intervention period”.
Shaded cells significant 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 (-)

## C. 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 | 43.6 | 26.6 | 0.67 | 0.24 | 1.6 | 6.9 | 1.19 | 0.57 |
| ALL-Sprayed | Pre-IRS v Post-IRS | 23.7 | 28.4 | 2.01 | 0.02 | 0.5 | 10.7 | 2.83 | 0.003 |
| ALL-Control | Pre-IRS v Post-IRS | 19.5 | 61.0 | 2.76 | <0.001 | 0.1 | 2.7 | 2.49 | 0.03 |
| Nchelenge | Control v Sprayed | 77.4 | 90.6 | N/A | N/A | 4.4 | 3.9 | 1.30 | 0.54 |
| Milenge | Control v Sprayed | 194.5 | 103.3 | N/A | N/A | 0.3 | 0.1 | 0.34 | 0.11 |
| Mambwe | Control v Sprayed | 0.1 | 0.8 | 4.94 | <0.001 | 1.1 | 3.9 | 1.71 | 0.30 |
| Katete | Control v Sprayed | 1.5 | 0.89 | 0.22 | 0.066 | 0.5 | 0.8 | 0.47 | 0.54 |
| Serenje | Control v Sprayed | 0.5 | 0.2 | 0.49 | 0.17 | 0.0 | 0.1 | 1.80 | 0.56 |
| Lufwanyama | Control v Sprayed | 0.0 | 7.5 | N/A | N/A | 0.0 | 17.9 | N/A | N/A |
| Chililabombwe | Control v Sprayed | 4.4 | 7.3 | N/A | N/A | 1.7 | 0.5 | 1.32 | 0.70 |

| 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 |
| Shikapande-Sprayed | Pre-IRS v Post-IRS | 120.8 | 78.5 | 0.66 | 0.03 | 1.09 | 5.06 | 3.60 | 0.02 |
| Manchene-Control | Pre-IRS v Post-IRS | 19.9 | 100.4 | 5.25 | 0.001 | 0.28 | 6.09 | 3.85 | 0.09 |
| Lunga-Sprayed | Pre-IRS v Post-IRS | 75.0 | 131.6 | 0.82 | 0.81 | 0.06 | 0.06 | 1.00 | 1.00 |
| Miyambo-Control | Pre-IRS v Post-IRS | 104.2 | 284.8 | 2.36 | 0.04 | 0.16 | 0.38 | 1.43 | 0.68 |
| Chikowa-Sprayed | Pre-IRS v Post-IRS | 0.5 | 1.0 | 1.75 | 0.31 | 1.13 | 5.27 | 1.30 | 0.74 |
| Chasela-Control | Pre-IRS v Post-IRS | 0.1 | 0.1 | 0.88 | 0.83 | 0.16 | 1.56 | 2.17 | 0.35 |
| Chiloba-Sprayed | Pre-IRS v Post-IRS | 0.0 | 1.5 | N/A | N/A | 0.00 | 1.38 | N/A | N/A |
| Robert-Control | Pre-IRS v Post-IRS | 0.3 | 2.4 | 3.30 | 0.15 | 0.00 | 0.90 | N/A | N/A |
| Chibobo-Sprayed | Pre-IRS v Post-IRS | 0.2 | 0.3 | 1.94 | 0.43 | 0.02 | 0.25 | 12.00 | 0.03 |
| Chishi-Control | Pre-IRS v Post-IRS | 0.3 | 1.1 | 3.42 | 0.002 | 0.04 | 0.00 | N/A | N/A |
| Nkana-Sprayed | Pre-IRS v Post-IRS | 0.7 | 8.1 | 2.25 | 0.30 | 1.84 | 39.61 | 8.01 | 0.004 |
| Bulaya-Control | Pre-IRS v Post-IRS | 0.8 | 12.3 | 3.77 | 0.08 | 0.13 | 9.84 | 5.33 | 0.13 |
| Kawama-Sprayed | Pre-IRS v Post-IRS | 3.0 | 10.2 | 2.93 | 0.009 | 0.19 | 0.75 | 5.15 | 0.03 |
| Maina Soko-Control | Pre-IRS v Post-IRS | 1.7 | 6.3 | 2.49 | 0.17 | 0.16 | 2.67 | 2.09 | 0.55 |

*For IRR, the reference group is “control" or "pre-intervention period”.
Shaded cells significant at 0.05%.
N/A = no estimated computed either because two sites had the same value or one site had two zero values.

## D. Indoor 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 | 27.8 | 16.8 | 0.77 | 0.49 | 0.8 | 4.5 | 1.56 | 0.22 |
| ALL-Sprayed | Pre-IRS v Post-IRS | 14.6 | 18.2 | 1.90 | 0.03 | 0.3 | 7.1 | 3.41 | 0.001 |
| ALL-Control | Pre-IRS v Post-IRS | 13.3 | 38.3 | 2.80 | 0.001 | 0.1 | 1.3 | 2.91 | 0.03 |
| Nchelenge | Control v Sprayed | 46.8 | 61.7 | 1.58 | 0.08 | 2.2 | 2.4 | 1.33 | 0.46 |
| Milenge | Control v Sprayed | 128.0 | 55.9 | 0.40 | 0.05 | 0.1 | 0.1 | 0.60 | 0.49 |
| Mambwe | Control v Sprayed | 0.0 | 0.3 | 6.48 | 0.02 | 0.2 | 0.9 | 2.63 | 0.17 |
| Katete | Control v Sprayed | 0.8 | 0.6 | N/A | N/A | 0.2 | 0.2 | 0.47 | 0.53 |
| Serenje | Control v Sprayed | 0.3 | 0.1 | 0.44 | 0.19 | 0.0 | 0.0 | N/A | N/A |
| Lufwanyama | Control v Sprayed | 0.0 | 5.7 | N/A | N/A | 0.0 | 12.8 | N/A | N/A |
| Chililabombwe | Control v Sprayed | 3.3 | 3.5 | 1.40 | 0.38 | 1.2 | 0.3 | 1.10 | 0.90 |

| 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 |
| Shikapande-Sprayed | Pre-IRS v Post-IRS | 81.7 | 53.7 | 0.65 | 0.004 | 0.7 | 3.0 | 3.78 | 0.006 |
| Manchene-Control | Pre-IRS v Post-IRS | 14.7 | 59.7 | 4.16 | 0.004 | 0.3 | 3.0 | 4.05 | 0.06 |
| Lunga-Sprayed | Pre-IRS v Post-IRS | 38.5 | 73.3 | 0.86 | 0.855 | 0.1 | 0.1 | 1.00 | 1.00 |
| Miyambo-Control | Pre-IRS v Post-IRS | 70.1 | 185.8 | 2.31 | 0.03 | 0.1 | 0.2 | 1.13 | 0.92 |
| Chikowa-Sprayed | Pre-IRS v Post-IRS | 0.2 | 0.4 | 2.36 | 0.39 | 0.3 | 1.2 | 0.99 | 0.99 |
| Chasela-Control | Pre-IRS v Post-IRS | 0.1 | 0.0 | N/A | N/A | 0.0 | 0.3 | 1.61 | 0.70 |
| Chiloba-Sprayed | Pre-IRS v Post-IRS | 0.0 | 0.9 | N/A | N/A | 0.0 | 0.4 | N/A | N/A |
| Robert-Control | Pre-IRS v Post-IRS | 0.2 | 1.2 | 4.64 | 0.16 | 0.0 | 0.3 | N/A | N/A |
| Chibobo-Sprayed | Pre-IRS v Post-IRS | 0.1 | 0.1 | 1.60 | 0.63 | 0.0 | 0.1 | N/A | N/A |
| Chishi-Control | Pre-IRS v Post-IRS | 0.2 | 0.5 | 3.26 | 0.11 | 0.0 | 0.0 | N/A | N/A |
| Nkana-Sprayed | Pre-IRS v Post-IRS | 0.6 | 5.2 | 1.74 | 0.47 | 1.3 | 26.3 | 7.88 | 0.01 |
| Bulaya-Control | Pre-IRS v Post-IRS | 0.7 | 10.2 | 3.85 | 0.07 | 0.1 | 8.9 | 5.61 | 0.12 |
| Kawama-Sprayed | Pre-IRS v Post-IRS | 2.3 | 4.3 | 1.82 | 0.03 | 0.1 | 0.4 | 3.78 | 0.03 |
| Maina Soko-Control | Pre-IRS v Post-IRS | 1.0 | 4.8 | 3.21 | 0.09 | 0.1 | 1.9 | 2.23 | 0.52 |

*For IRR, the reference group is “control" or "pre-intervention period”.
Shaded cells significant at 0.05%.
N/A = no estimated computed either because two sites had the same value or one site had two zero values.

## E. Outdoor Human Biting Rates - Vectors Collected by HLC (Human Landing Catches)

| 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 | 15.8 | 9.8 | 0.66 | 0.23 | 0.8 | 2.4 | 1.18 | 0.62 |
| ALL-Sprayed | Pre-IRS v Post-IRS | 9.1 | 10.3 | 2.30 | 0.01 | 0.2 | 3.6 | 3.19 | 0.002 |
| ALL-Control | Pre-IRS v Post-IRS | 6.2 | 22.8 | 2.67 | 0.001 | 0.1 | 1.4 | 2.72 | 0.02 |
| Nchelenge | Control v Sprayed | 30.6 | 28.9 | N/A | N/A | 2.2 | 1.6 | 1.29 | 0.61 |
| Milenge | Control v Sprayed | 66.5 | 47.4 | N/A | N/A | 0.2 | 0.0 | N/A | N/A |
| Mambwe | Control v Sprayed | 0.1 | 0.5 | 4.53 | 0.001 | 0.9 | 3.0 | 1.67 | 0.33 |
| Katete | Control v Sprayed | 0.8 | 0.3 | 0.12 | 0.05 | 0.3 | 0.6 | 0.52 | 0.59 |
| Serenje | Control v Sprayed | 0.3 | 0.1 | 0.53 | 0.22 | 0.0 | 0.0 | 1.50 | 0.66 |
| Lufwanyama | Control v Sprayed | 0.0 | 1.8 | N/A | N/A | 0.0 | 5.2 | N/A | N/A |
| Chililabombwe | Control v Sprayed | 1.2 | 3.8 | 2.04 | 0.15 | 0.5 | 0.3 | 1.57 | 0.53 |

| 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 |
| Shikapande-Sprayed | Pre-IRS v Post-IRS | 39.1 | 24.8 | 0.80 | 0.57 | 0.38 | 2.04 | 2.19 | 0.29 |
| Manchene-Control | Pre-IRS v Post-IRS | 5.3 | 40.7 | 6.78 | 0.002 | 0.03 | 3.09 | 7.00 | 0.08 |
| Lunga-Sprayed | Pre-IRS v Post-IRS | 36.5 | 58.3 | 0.77 | 0.75 | 0.00 | 0.00 | N/A | N/A |
| Miyambo-Control | Pre-IRS v Post-IRS | 34.1 | 99.0 | 2.46 | 0.04 | 0.09 | 0.22 | 2.02 | 0.40 |
| Chikowa-Sprayed | Pre-IRS v Post-IRS | 0.3 | 0.6 | 1.82 | 0.26 | 0.88 | 4.03 | 1.43 | 0.65 |
| Chasela-Control | Pre-IRS v Post-IRS | 0.1 | 0.1 | 1.75 | 0.49 | 0.13 | 1.25 | 2.52 | 0.27 |
| Chiloba-Sprayed | Pre-IRS v Post-IRS | 0.0 | 0.5 | N/A | N/A | 0.00 | 1.02 | N/A | N/A |
| Robert-Control | Pre-IRS v Post-IRS | 0.2 | 1.2 | 3.15 | 0.17 | 0.00 | 0.56 | N/A | N/A |
| Chibobo-Sprayed | Pre-IRS v Post-IRS | 0.1 | 0.2 | 1.80 | 0.42 | 0.02 | 0.13 | 6.00 | 0.14 |
| Chishi-Control | Pre-IRS v Post-IRS | 0.1 | 0.6 | N/A | N/A | 0.04 | 0.00 | N/A | N/A |
| Nkana-Sprayed | Pre-IRS v Post-IRS | 0.1 | 2.9 | 7.05 | 0.07 | 0.53 | 13.34 | 9.37 | 0.002 |
| Bulaya-Control | Pre-IRS v Post-IRS | 0.1 | 2.1 | 3.03 | 0.17 | 0.06 | 0.94 | 3.84 | 0.23 |
| Kawama-Sprayed | Pre-IRS v Post-IRS | 0.7 | 5.9 | 5.44 | 0.006 | 0.09 | 0.40 | 4.22 | 0.02 |
| Maina Soko-Control | Pre-IRS v Post-IRS | 0.7 | 1.5 | 1.31 | 0.71 | 0.06 | 0.73 | 1.87 | 0.61 |

*For IRR, the reference group is “control" or "pre-intervention period”.
Shaded cells significant at 0.05%.
N/A = no estimated computed either because two sites had the same value or one site had two zero values.

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

| Site | Comparison | *An. funestus* s.l. | | | | *An. gambiae* s.l. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean Proportion Parous [First group] | Mean Proportion Parous [Second group] | Random Effects IRR* | p-value | Mean Proportion Parous [First group] | Mean Proportion Parous [Second group] | Random Effects IRR* | p-value |
| All | Control v Sprayed | 57% | 41% | 0.70 | <0.0001 | 55% | 39% | 0.71 | <0.0001 |
| ALL-Sprayed | Pre-IRS v Post-IRS | 57% | 37% | 0.65 | <0.0001 | 48% | 39% | 0.81 | 0.171 |
| ALL-Control | Pre-IRS v Post-IRS | 53% | 57% | 1.09 | 0.18 | 35% | 55% | 1.57 | 0.270 |
| Nchelenge | Control v Sprayed | 54% | 45% | 0.82 | 0.003 | 57% | 40% | 0.71 | 0.002 |
| Milenge | Control v Sprayed | 60% | 47% | 0.76 | 0.03 | 46% | | N/A | N/A |
| Mambwe | Control v Sprayed | 62% | 40% | 0.65 | 0.2867 | 56% | 46% | 0.82 | 0.2402 |
| Katete | Control v Sprayed | 57% | 31% | 0.54 | 0.04 | 75% | 46% | 0.61 | 0.0980 |
| Serenje | Control v Sprayed | 0% | 0% | N/A | N/A | 25% | | N/A | N/A |
| Lufwanyama | Control v Sprayed | 60% | 34% | 0.56 | <0.0001 | 54% | 37% | 0.68 | 0.001 |
| Chililabombwe | Control v Sprayed | 55% | 32% | 0.56 | 0.001 | 47% | 40% | 0.85 | 0.50 |

| Site | Comparison | *An. funestus* s.l. | | | | *An. gambiae* s.l. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean Proportion Parous [First group] | Mean Proportion Parous [Second group] | Random Effects IRR* | p-value | Mean Proportion Parous [First group] | Mean Proportion Parous [Second group] | Random Effects IRR* | p-value |
| Shikapande-Sprayed | Pre-IRS v Post-IRS | 54% | 42% | 0.78 | 0.01 | 30% | 41% | 1.70 | 0.304 |
| Manchene-Control | Pre-IRS v Post-IRS | 51% | 55% | 1.07 | 0.6327 | 0% | 57% | N/A | N/A |
| Lunga-Sprayed | Pre-IRS v Post-IRS | 60% | 37% | 0.61 | 0.002 | - | - | N/A | N/A |
| Miyambo-Control | Pre-IRS v Post-IRS | 58% | 61% | 1.06 | 0.59 | 50% | 45% | 0.91 | 0.93 |
| Chikowa-Sprayed | Pre-IRS v Post-IRS | 55% | 38% | 0.69 | 0.42 | 56% | 44% | 0.79 | 0.34 |
| Chasela-Control | Pre-IRS v Post-IRS | 50% | 67% | 1.33 | 0.72 | 50% | 56% | 1.11 | 0.92 |
| Chiloba-Sprayed | Pre-IRS v Post-IRS | 31% | - | N/A | N/A | 46% | - | N/A | N/A |
| Robert-Control | Pre-IRS v Post-IRS | 56% | 58% | 1.04 | 0.9373 | 75% | - | N/A | N/A |
| Chibobo-Sprayed | Pre-IRS v Post-IRS | 0% | - | N/A | N/A | 25% | - | N/A | N/A |
| Chishi-Control | Pre-IRS v Post-IRS | 0% | - | N/A | N/A | - | - | N/A | N/A |
| Nkana-Sprayed | Pre-IRS v Post-IRS | 70% | 33% | 0.39 | 0.16 | 57% | 37% | 0.58 | 0.18 |
| Bulaya-Control | Pre-IRS v Post-IRS | 59% | 60% | 1.01 | 0.9694 | 75% | 54% | 0.72 | 0.5755 |
| Kawama-Sprayed | Pre-IRS v Post-IRS | 59% | 28% | 0.45 | 0.008 | 50% | 39% | 0.78 | 0.69 |
| Maina Soko-Control | Pre-IRS v Post-IRS | 54% | 55% | 1.03 | 0.8841 | 20% | 49% | 2.44 | 0.38 |

*For IRR, the reference group is “control" or "pre-intervention period”.
Shaded cells significant 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 5: SPOROZOITE RATES (JUNE 2019-FEBRUARY 2020)

**A: *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 | # Tested | # Positive | Sporozoite Rate |
| *An. funestus* s.l. | Indoor | Pre-IRS | 164 | 5 | 3.05 | 217 | 2 | 0.92 |
| | | Post-IRS | 402 | 16 | 3.98 | 239 | 7 | 2.93 |
| | Outdoor | Pre-IRS | 77 | 2 | 2.60 | 82 | 1 | 1.22 |
| | | Post-IRS | 232 | 14 | 6.03 | 110 | 4 | 3.64 |
| | All Locations | Pre-IRS | 241 | 7 | 2.90 | 299 | 3 | 1.00 |
| | All Locations | Post-IRS | 634 | 30 | 4.73 | 349 | 11 | 3.15 |
| | **TOTAL** | | **875** | **37** | **4.23** | **648** | **14** | **2.16** |

| Species | Location | Time | Intervention sites | | | Control sites | | |
|---|---|---|---|---|---|---|---|---|
| | | | # Tested | # Positive | Sporozoite Rate | # Tested | # Positive | Sporozoite Rate |
| *An. gambiae* s.l. | Indoor | Pre-IRS | 5 | 0 | 0.00 | - | - | -* |
| | | Post-IRS | 182 | 2 | 1.10 | 84 | 0 | 0.00 |
| | Outdoor | Pre-IRS | 24 | 0 | 0.00 | 3 | 0 | 0.00 |
| | | Post-IRS | 127 | 1 | 0.79 | 93 | 1 | 1.08 |
| | All Locations | Pre-IRS | 29 | 0 | 0.00 | 3 | 0 | 0.00 |
| | All Locations | Post-IRS | 309 | 3 | 0.97 | 177 | 1 | 0.56 |
| | **TOTAL** | | **180** | **1** | **0.56** | **338** | **3** | **0.89** |

*No pre-IRS indoor biting *An. gambiae* s.l. tested

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

| District | Molecular Species | Total Tested | Number Positive |
|---|---|---|---|
| Nchelenge | *An. funestus* | 41 | 0 |
| | *An. rivulorum* | 16 | 1 |
| Milenge | *An. funestus* | 1 | 0 |
| | *An. rivulorum* | 146 | 9 |
| | *An. rivulorum*- like | 21 | 3 |
| Mambwe | *An. arabiensis* | 37 | 0 |
| | *An. funestus* | 22 | 2 |
| | *An. gambiae* | 7 | 0 |
| | *An. rivulorum*- like | 1 | 0 |
| Katete | *An. arabiensis* | 1 | 0 |
| | *An. funestus* | 6 | 0 |
| | *An. parensis* | 1 | 0 |
| Serenje | *An. funestus* | 4 | 0 |
| | *An. rivulorum* | 15 | 0 |
| | *An. vanedeni* | 1 | 0 |

| District | Molecular Species | Total Tested | Number Positive |
|---|---|---|---|
| Lufwanyama | *An. arabiensis* | 2 | 0 |
| | *An. funestus* | 7 | 0 |
| | *An. gambiae* | 136 | 2 |
| | *An. rivulorum* | 59 | 2 |
| | *An. rivulorum*- like | 1 | 0 |
| Chililabombwe | *An. funestus* | 36 | 1 |
| | *An. gambiae* | 1 | 0 |
| | *An. rivulorum* | 3 | 0 |

# ANNEX 6: INSECTICIDE SUSCEPTIBILITY TEST RESULTS

**A: *An. funestus* s.l. Insect Susceptibility Test Results (December 2019-February 2020)**

| Chemical | District, Sentinel Site | Intervention Status | # Exposed | # Dead after 24 Hours | % Mortality after 24 Hours | % Mortality after 48 Hours | % Mortality after 72 Hours |
|---|---|---|---|---|---|---|---|
| DDT (4%) | Lufwanyama, Bulaya | Control | 61 | 52 | 85 | N/A | N/A |
| | Chililabombwe, Kawama | Sprayed | 31 | 31 | 100 | N/A | N/A |
| | Milenge, Lunga | Sprayed | 56 | 56 | 100 | N/A | N/A |
| | Chililabombwe, Maina Soko | Control | 20 | 20 | 100 | N/A | N/A |
| | Nchelenge, Manchene | Control | 125 | 115 | 92 | N/A | N/A |
| | Milenge, Miyambo | Control | 131 | 130 | 99 | N/A | N/A |
| Clothianidin (2%) | Lufwanyama, Bulaya | Control | 50 | 50 | 100 | - | - |
| | Nchelenge, Manchene | Control | 90 | 90 | 100 | - | - |
| | Nchelenge, Shikapande | Sprayed | 89 | 84 | 94.4 | 100 | - |

| Chemical | District, Sentinel Site | Intervention Status | # Exposed | # Dead after 24 Hours | % Mortality after 24 Hours | % Mortality after 48 Hours | % Mortality after 72 Hours |
|---|---|---|---|---|---|---|---|
| Chlorfenapyr (100ug) | Nchelenge, Manchene | Control | 78 | 78 | 74.4 | 91.0 | 100 |
| | Nchelenge, Shikapande | Sprayed | 82 | 82 | 100 | - | - |
| Deltamethrin (0.05%) | Milenge, Lunga | Sprayed | 137 | 134 | 97.5 | N/A | N/A |
| | Milenge, Miyambo | Control | 174 | 131 | 75.5 | N/A | N/A |
| | Nchelenge, Shikapande | Sprayed | 140 | 115 | 82.3 | N/A | N/A |
| Pirimiphos-methyl (0.25%) | Milenge, Lunga | Sprayed | 130 | 130 | 100 | N/A | N/A |
| | Milenge, Miyambo | Control | 162 | 162 | 100 | N/A | N/A |
| | Nchelenge, Shikapande | Sprayed | 38 | 38 | 100 | N/A | N/A |
| Bendiocarb (0.1%) | Lufwanyama, Bulaya | Control | 24 | 17 | 71 | N/A | N/A |
| | Milenge, Lunga | Sprayed | 75 | 72 | 96 | N/A | N/A |
| | Chililabombwe, Maina Soko | Control | 33 | 33 | 100 | N/A | N/A |
| | Nchelenge, Manchene | Control | 110 | 105 | 95 | N/A | N/A |
| | Milenge, Miyambo | Control | 108 | 99 | 91.5 | N/A | N/A |

**B: *An. gambiae* s.l. Insecticide Susceptibility Test Results (December 2019 - April 2020)**

| Chemical | District/ Sentinel Site | Intervention Status | # Exposed | # Dead after 24 Hours | % Mortality after 24 Hours | % Mortality after 48 Hours | % Mortality after 72 hours |
|---|---|---|---|---|---|---|---|
| DDT (4%) | Lufwanyama, Bulaya | Control | 84 | 71 | 85 | N/A | N/A |
| | Mambwe, Chikowa | Sprayed | 80 | 80 | 100 | N/A | N/A |
| | Nchelenge, Manchene | Control | 10 | 6 | 60 | - | - |
| | Katete, Mbalani | Sprayed | 71 | 71 | 100 | N/A | N/A |
| | Lufwanyama, Nkana | Sprayed | 129 | 110 | 85.5 | N/A | N/A |
| | Katete, Robert | Control | 29 | 26 | 89 | N/A | N/A |
| Clothianidin (2%) | Mambwe, Chikowa | Sprayed | 80 | 80 | 100 | - | - |
| | Katete, Chilowa | Sprayed | 80 | 80 | 100 | - | - |
| | Nchelenge, Manchene | Control | 25 | 23 | 92.0 | 100 | |
| | Milenge, Miyambo | Control | 7 | 7 | 100 | - | - |
| | Lufwanyama, Nkana | Sprayed | 44 | 44 | 100 | - | - |
| | Katete, Robert | Control | 100 | 100 | 100 | - | - |
| | Nchelenge, Shikapande | Sprayed | 11 | 11 | 100 | - | - |

| Chemical | District/ Sentinel Site | Intervention Status | # Exposed | # Dead after 24 Hours | % Mortality after 24 Hours | % Mortality after 48 Hours | % Mortality after 72 hours |
|---|---|---|---|---|---|---|---|
| Chlorfenapyr (100ug) | Lufwanyama, Bulaya | Control | 100 | 100 | 100 | - | - |
| | Mambwe, Chasela | Control | 20 | 20 | 100 | - | - |
| | Mambwe, Chikowa | Sprayed | 80 | 80 | 100 | - | - |
| | Katete, Chilowa | Sprayed | 80 | 80 | 100 | - | - |
| | Nchelenge, Manchene | Control | 22 | 22 | 90.9 | 100 | - |
| | Katete, Mbalani | Sprayed | 80 | 80 | 100 | - | - |
| | Lufwanyama, Nkana | Sprayed | 75 | 75 | 100 | - | - |
| | Katete, Robert | Control | 100 | 100 | 100 | - | - |
| | Nchelenge, Shikapande | Sprayed | 18 | 18 | 100 | - | - |
| Deltamethrin (0.05%) | Mambwe, Chikowa | Sprayed | 80 | 80 | 100 | N/A | N/A |
| | Katete, Chilowa | Sprayed | 80 | 80 | 100 | N/A | N/A |
| | Katete, Mbalani | Sprayed | 136 | 136 | 100 | N/A | N/A |
| | Katete, Robert | Control | 133 | 133 | 100 | N/A | N/A |

| Chemical | District/ Sentinel Site | Intervention Status | # Exposed | # Dead after 24 Hours | % Mortality after 24 Hours | % Mortality after 48 Hours | % Mortality after 72 hours |
|---|---|---|---|---|---|---|---|
| Pirimiphos-methyl (0.25%) | Mambwe, Chikowa | Sprayed | 80 | 80 | 100 | N/A | N/A |
| | Katete, Mbalani | Sprayed | 100 | 100 | 100 | N/A | N/A |
| | Katete, Robert | Control | 100 | 100 | 100 | N/A | N/A |
| Bendiocarb (0.1%) | Lufwanyama, Bulaya | Control | 20 | 15 | 75 | N/A | N/A |
| | Mambwe, Chikowa | Sprayed | 80 | 80 | 100 | N/A | N/A |
| | Chililabombwe, Maina Soko | Control | 17 | 17 | 100 | N/A | N/A |
| | Lufwanyama, Nkana | Sprayed | 95 | 94 | 99 | N/A | N/A |

Key: Red shading indicates <90% mortality (confirmed resistance), yellow indicates 90-97% mortality (probable resistance), and green indicates ≥98% mortality (susceptible). N/A = Not applicable.
Control mortalities were below 5% in all of the tests conducted therefore no corrected mortality was calculated for any of the tests.