



U.S. PRESIDENT'S MALARIA INITIATIVE



# THE PMI VECTORLINK PROJECT MOZAMBIQUE ENTOMOLOGICAL MONITORING

## ANNUAL REPORT JULY 2018 – JUNE 2019

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# ACRONYMS

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<b>AIRS</b>	Africa Indoor Residual Spraying
<b>b/p/h</b>	bites per person per hour
<b>b/p/n</b>	bites per person per night
<b>CDC</b>	Centers for Disease Control and Prevention
<b>ELISA</b>	Enzyme-linked Immunosorbent Assay
<b>HLC</b>	Human Landing Catch
<b>INS</b>	<i>Instituto Nacional de Saúde</i>
<b>IRS</b>	Indoor Residual Spraying
<b>ITNs</b>	Insecticide treated bednet
<b>KD</b>	Knock Down
<b><i>kdr</i></b>	knockdown resistance gene
<b>LLIN</b>	Long-lasting Insecticide-treated Bednet
<b>m/t/n</b>	mosquitoes per trap per night
<b>NMCP</b>	National Malaria Control Program
<b>PBO</b>	Piperonyl butoxide
<b>PCR</b>	Polymerase Chain Reaction
<b>PMI</b>	President's Malaria Initiative
<b>PSC</b>	Pyrethrum Spray Catch
<b>USAID</b>	United States Agency for International Development
<b>WG</b>	Wettable Granules
<b>WHO</b>	World Health Organization

# EXECUTIVE SUMMARY

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Indoor residual spraying (IRS) and insecticide-treated nets (ITNs) remain the primary mosquito vector control interventions in many parts of world, including sub-Saharan Africa, where the disease continues to be a major public health concern.

In Mozambique, Abt Associates implemented the U.S. President's Malaria Initiative (PMI) VectorLink Mozambique Project from July 1, 2018, to June 30, 2019. In the 2018 spray campaign from October to November 2018, VectorLink Mozambique conducted IRS with pirimiphos-methyl (Actellic® 300CS) in the four target districts of Derre, Maganja da Costa, Milange, and Molumbo and with SumiShield® 50WG in the two districts of Morrumbala and Mopeia. To guide proper targeting of IRS, monthly entomological monitoring was performed in a set of intervention districts and one control district of Lugela that had not received IRS. Surveillance employed techniques such as CDC light traps, human landing catches (HLCs), pyrethrum spray catches (PSCs), and cone wall bioassays (used only in sprayed areas). Annual insecticide susceptibility tests were carried out in the six sprayed and one control districts.

In Nampula, the Government of Mozambique conducted IRS using pirimiphos-methyl (Actellic® 300CS) in eight districts: Nampula City, Meconta, Monapo, Nacala, Murrupula, Ribaue, Angoche, and Rapali. VectorLink Mozambique performed monthly entomological monitoring using CDC light traps, HLCs, and PSCs in two intervention districts, Nampula City and Monapo. Erati was included as the control district.

Mopeia District collections contribute to the epidemiological study entitled “A cluster randomized trial to measure the impact of indoor residual spraying with a third-generation indoor residual spray (3GIRS) product in combination with long-lasting insecticidal nets in Zambezia, Mozambique.” The study worked in five sentinel villages in Mopeia's intervention areas and five in control areas up to October 2018, when spraying with SumiShield® 50WG was extended into the previously unsprayed control areas. The same 10 sentinel villages were maintained thereafter in the extension of the study period. CDC light trap and HLCs collections were used to sample mosquitoes in the study areas.

Mosquito collections using the methods described above demonstrated the presence of highly diverse community composition of anophelines, which included both the main vectors *Anopheles funestus* s.l. and *An. gambiae* s.l., and other potential vectors and non-vectors such as *An. coustani*, *An. pharoensis*, *An. pretoriensis*, *An. squamosus*, *An. tenebrosus*, *An. ziemanni*, *An. brucei*, *An. caliginosus*, *An. dancallicus*, *An. rufipes*, *An. natalensis*, and *An. maculipalpis*. The role of these potential vectors in malaria transmission remains to be investigated in the surveyed districts. Our findings highlight high levels of heterogeneity and diversity in mosquito vector species composition and behavior in the monitored areas.

Following IRS, in general, *An. funestus* s.l. densities were suppressed in comparison with pre-IRS (July to October) densities. *An. gambiae* s.l. densities appear to have increased in January and February, most likely because of the rapid build-up of breeding habitats due to the high level of precipitation during that period.

Malaria vectors *An. gambiae* s.l. and *An. funestus* s.l. were collected both indoors and outdoors. In some districts, the densities were higher outdoors and in others they were higher indoors. *An. funestus* s.l. tended to be found predominantly indoors, even after IRS in most districts. Biting activity seemed to follow human sleeping patterns, with peak indoor biting activity occurring at around midnight from 10:00-11:00 pm extending towards morning hours of 1:00-2:00 am, and 3:00-4:00 am.

Quality of IRS assessed by cone wall bioassays showed that spray teams were able to achieve optimal insecticide application in all districts. The insecticide decay rate assessment show that Actellic® 300CS had variable decay periods, from four to six months. SumiShield® 50WG was found to last up to 10 months. This is the longest period that an insecticide has been reported to remain effective on a sprayed wall surface in Mozambique.

Results of insecticide susceptibility tests show that local vectors are fully susceptible to pirimiphos-methyl, clorfenapyr, clothianidin, bendiocarb, and dichlorodiphenyltrichloroethane (DDT). Assays for pyrethroids once again revealed occurrence of widespread vector resistance to pyrethroids. Further assays to assess the strength of resistance in *An. gambiae* s.l. show presence of moderate to high intensity resistance to pyrethroids. This finding demonstrates that the current situation poses a major threat of potential intervention failure for tools dependent on pyrethroid insecticides. However, synergist assays with piperonyl butoxide (PBO) demonstrated recovery of mortality, indicating involvement of oxidase-mediated resistance mechanisms. This strongly suggests the potential for PBO nets to effectively overcome the observed pyrethroid resistance threat.

# I. INTRODUCTION

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Through support of the U.S. President's Malaria Initiative (PMI), the Africa Indoor Residual Spraying (AIRS) project implemented five rounds of indoor residual spraying (IRS) in Zambezia Province of Mozambique. During the 2018 spray campaign, successor project PMI VectorLink Mozambique conducted IRS in six target districts from October to November 2018: Derre, Maganja da Costa, Milange, Molumbo, Morrumbala, and Mopeia.

PMI VectorLink is implemented by Abt Associates in close collaboration with Mozambique's National Malaria Control Program (NMCP), the Provincial Directorates of Health in Zambézia and Nampula provinces, the District Services for Health, Women and Social Welfare at the district level, and the ministries of Agriculture and Food Security, and of Land, Environment and Rural Development at the provincial and district levels.

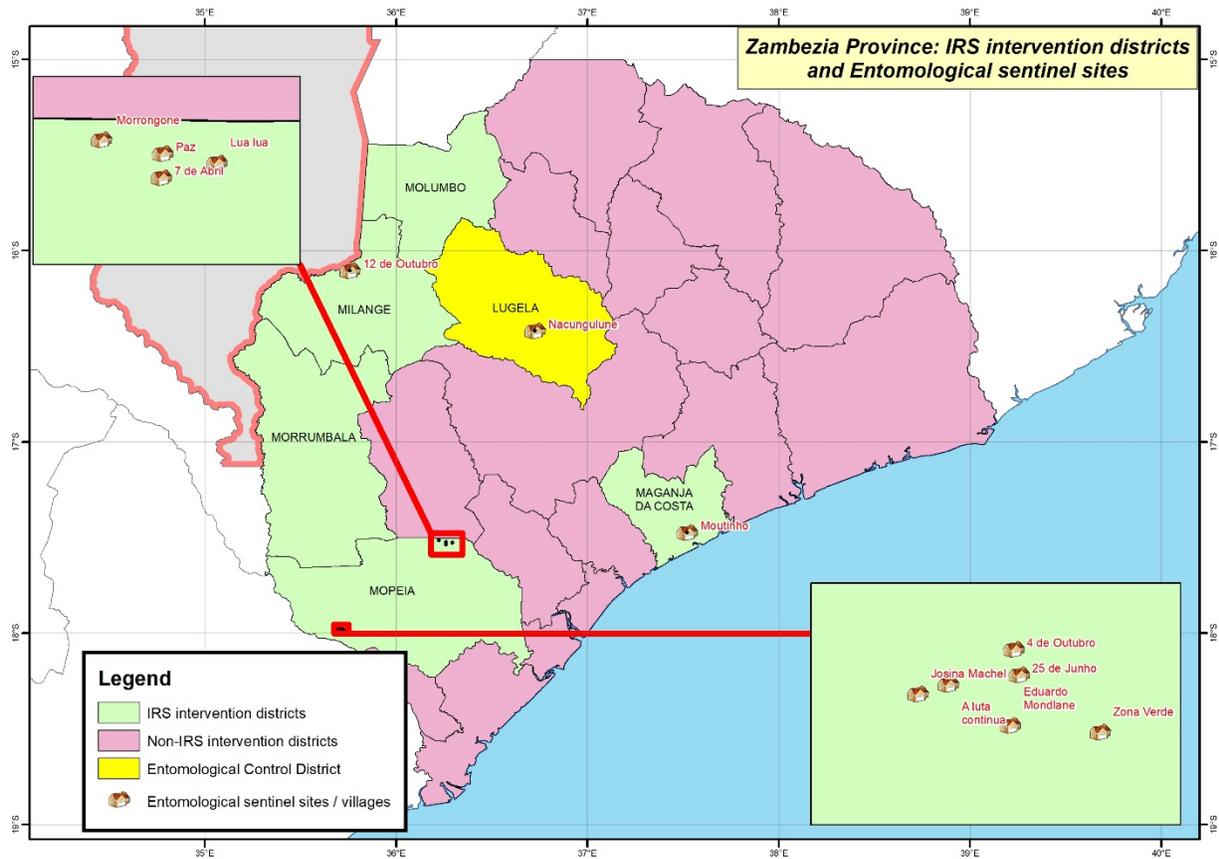
Also in 2018, VectorLink Mozambique carried out entomological monitoring activities in Zambezia and Nampula provinces, in collaboration with the NMCP. In addition to producing entomological data, this collaboration has been enhancing in-country capacity for entomological monitoring. Having these data to supplement epidemiological data is essential to properly target IRS; evaluate the susceptibility level of the local vectors to different insecticides and determine the underlying mechanisms; inform selection of insecticides; ensure the quality of spraying; monitor the impact of IRS on vector density, vector behavior, and composition; and monitor the residual life of different insecticides on different types of wall surfaces.

This entomological monitoring annual report covers the period from July 1, 2018, through June 30, 2019. Over this year, entomological monitoring was conducted in the six Zambezia IRS intervention districts listed above. Unsprayed Lugela District was used as a control. Longitudinal surveillance running throughout the year was maintained in three intervention districts (Milange, Maganja da Costa, and Mopeia) and the control district (Lugela). The three districts were selected among the six sprayed, after reviewing epidemiological data of all the six districts and considering the three as representative of the rest. Among the three, Mopeia was selected for the cluster randomized trial (CRT) due its relatively high level of transmission than the rest.

Surveillance activities in Mopeia District contributed data to the extended year of the epidemiological study on "A cluster randomized trial to measure the impact of indoor residual spraying with a third-generation indoor residual spray (3GIRS) product in combination with long-lasting insecticidal nets in Zambezia, Mozambique." This study was implemented in five villages in Mopeia's intervention areas and five in control areas until October 2018, at which time IRS was expanded throughout the district and thus covered the previously unsprayed control areas. The same 10 sentinel villages were maintained during the extension of epidemiological study, which is scheduled to end October 31, 2019.

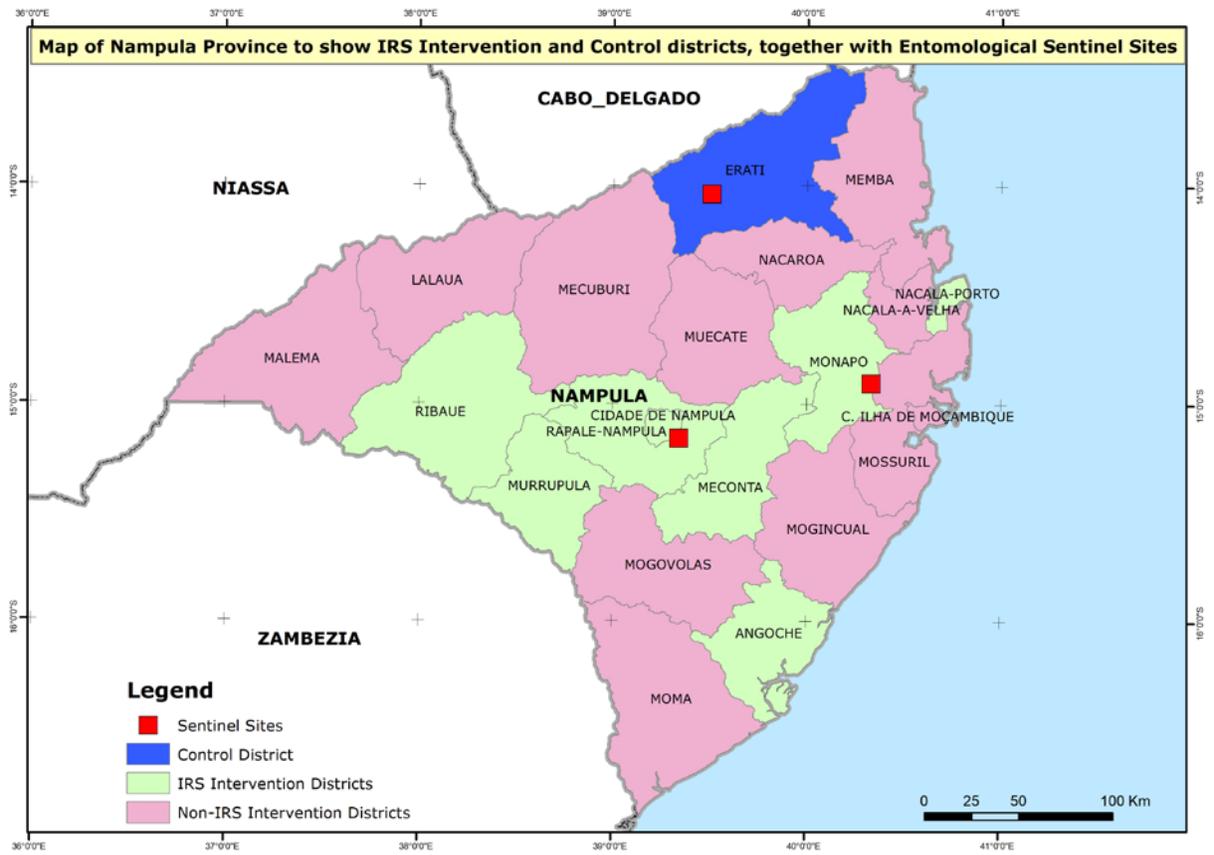
In Mopeia, entomological monitoring data were collected using the Centers for Disease Control and Prevention (CDC) light trap and human landing catch (HLC) collection methods. In the other districts in Zambezia and Nampula, entomological monitoring data were collected using pyrethrum spray catch (PSC), CDC light trap, and HLC methods. For susceptibility tests, Prokopack collections were used in all districts to collect adult *An. funestus* s.l.; larval collections were conducted to collect *An. gambiae* s.l. The map of Zambezia Province in Figure 1A shows IRS intervention and control districts, and the sentinel sites where the entomological activities took place.

**FIGURE 1A. ZAMBEZIA PROVINCE IRS INTERVENTION AND CONTROL DISTRICTS, AND ENTOMOLOGICAL SENTINEL SITES**



The government of Mozambique, through the NMCP, conducts IRS in Nampula Province, in eight districts (Nampula City, Meconta, Monapo, Nacala, Murrupula, Ribaué, Angoche, and Rapali) with insecticide procured through the Global Fund to Fight AIDS, Tuberculosis and Malaria. VectorLink Mozambique provided technical support to Nampula to implement entomological surveillance in two intervention districts, Nampula City and Monapo as well as in the control district of Erati. This support includes carrying out entomological monitoring activities in Nampula province, in collaboration with the NMCP and Provincial Department of Health. In addition to producing entomological data, this collaboration is meant to enhance provincial capacity for entomological monitoring. Having entomological data to supplement epidemiological data is essential to properly target IRS; evaluate the susceptibility level of the local vectors to different insecticides and determine the underlying mechanisms; inform selection of insecticides; ensure the quality of spraying; monitor the impact of IRS on vector density, vector behavior, and composition; and monitor the residual life of different insecticides sprayed on different types of wall surfaces. The map of in Figure 1B shows Nampula Province’s IRS intervention and control districts, together with the entomological sentinel sites.

**FIGURE 1B: NAMPULA PROVINCE IRS INTERVENTION AND CONTROL DISTRICTS, AND ENTOMOLOGICAL SENTINEL SITES<sup>1</sup>**



<sup>1</sup> NMCP conducts IRS and entomological activities in Nampula with VL support

## 2. METHODOLOGY

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### 2.1 BEHAVIOR AND DENSITY

#### 2.1.1 PYRETHRUM SPRAY CATCH

The PSC method of mosquito collection was conducted in sentinel sites in selected IRS intervention and control districts in Zambezia and Nampula provinces. In Zambezia, the intervention districts were Maganja da Costa and Milange, and control district was Lugela. In Nampula, intervention districts were Nampula City and Monapo and the control district was Erati. Two villages per district, and 10 houses per village were selected to participate, for a total of 20 houses per district. The same houses were visited each month. PSC was conducted from 5:00 am to 8:00 am, over four consecutive days in each district. Data were collected in five houses per day per district. The first collection was conducted three months prior to the IRS campaign and collection continued after the campaign. In each house, one sleeping room was selected and sprayed with Baygon (commercial nomenclature), an aerosol containing the pyrethroids deltamethrin 0.5 g/kg and imiprothrin 1.0 g/kg, to knock down mosquitoes. The room was closed for 10 minutes after spraying, and then the knocked-down mosquitoes were collected using forceps and placed in a labeled petri dish. The samples were identified morphologically and preserved in 1.5 ml eppendorf tubes containing silica gel for further identification using the Polymerase Chain Reaction (PCR) technique. The National Institute of Health (Instituto Nacional de Saúde, INS) laboratory in Maputo received samples for the PCR analysis.

#### 2.1.2 HUMAN LANDING CATCH

In Nampula Province, HLCs were conducted in the intervention districts of Nampula City and Monapo and in the control district of Erati. In Zambezia Province, HLCs were conducted in the intervention districts of Maganja da Costa, Milange, and Mopeia, and the control district of Lugela. With the exception of Mopeia, two houses were sampled in a selected village on three consecutive nights to obtain six person-nights of collection per district per month (2 houses x 3 collection nights = 6 person-nights). In Mopeia, one house in each village in the intervention and control areas was selected for a total of eight houses (four in intervention and four in control districts). Collections were conducted on three consecutive nights to obtain 12 person-nights per area per month (4 houses x 3 collection nights = 12 person-nights). In all districts, two human volunteers were positioned, one inside the house and the other outside, to collect mosquitoes. Collections were conducted from 6:00 pm to 6:00 am. Over each hour of collection, collectors collected mosquitoes for 50 minutes and rested for 10 minutes, during which they exchanged positions and recorded humidity and temperature. During the time of collection, the collectors sat quietly on a small chair and exposed part of their legs (up to the knees); when they felt a landing mosquito, they turned on a torch and collected the mosquito using a mouth aspirator. Collected mosquitoes were transferred into labeled paper cups assigned for each hourly collection. Collected mosquitoes were subsequently killed using cotton soaked in chloroform; identified; counted by species, location, and hour of collection; and preserved in 1.5 ml eppendorf tubes with silica gel.

#### 2.1.3 CDC LIGHT TRAP

In Nampula Province, CDC light traps were installed in four houses in the two intervention districts of Monapo and Nampula City and the control district of Erati. Likewise, in Zambezia Province, CDC light traps were installed in four houses in two of the intervention districts, Maganja da Costa and Milange, as well as in the control district of Lugela. Each month, the traps were set over three consecutive nights, from 6:00 pm to 6:00 am, for a total of 12 trap nights per month for each district.

In Mopeia, 10 villages were selected (five in intervention areas and five in the previous control areas) as sentinel sites for CDC light trapping. Eight houses were selected in each village. Data were collected on three consecutive nights, from 6:00 pm to 6:00 am. This resulted in 240 traps nights per month, with an equal number in intervention and control areas.

The traps were set up inside the house in the bedroom beside the bed, at the bed’s footrest, with humans sleeping under untreated bed nets, about 1.5 m from the floor. After each night of collection, chloroform was used to kill the mosquitoes in the paper cups, and the mosquitoes were identified and preserved in 1.5 ml eppendorf tubes for future species identification using PCR. The same houses were used each month.

Data were collected from July 2018 to June 2019.

Some of the samples from the collections in Mopeia were sent to the Walter Reed Biosystematics Unit for PCR analyses; the rest of the Mopeia samples, as well as the samples from other districts were sent to the INS for PCR analysis. Samples were sent in May and July 2019.

Table 1 summarizes the monthly schedule for the longitudinal monitoring conducted using the three collection methods described above

**TABLE 1. MONTHLY SCHEDULE FOR LONGITUDINAL MONITORING OF ADULT MOSQUITO COLLECTIONS IN EACH SENTINEL DISTRICT IN ZAMBEZIA AND NAMPULA PROVINCES**

Province / District	Sampling Time	Number of Villages in Each District	Method, Frequency, and Intensity of Collection per Month		
			HLC	CDC LT	PSC
Zambezia Mopeia*	6:00pm – 6:00am	8 for HLC; 10 for CDC LTs	1 house × 3 nights × 8 villages = 24 person-nights	8 CDC LTs × 3 nights × 10 villages = 240 trap nights	Not used
Zambezia Milange, Maganja da Costa, and Lugela#	6:00pm – 6:00am for HLC and CDC LT; 5:00am –8:00am for PSC	1 each for HLC and CDC LT, 2 for PSC	2 houses × 3 nights = 6 nights	4 CDC LTs × 3 nights = 12 trap nights	10 houses/ village × 2 villages = 20 houses
Nampula City, Monapo and Erati#	6:00pm – 6:00am for HLC and CDC LT; 5:00am – 8:00am for PSC	1 each for HLC and CDC LT, 2 for PSC	2 houses × 3 nights = 6 nights	4 CDC LTs × 3 nights = 12 trap nights	10 houses/ village × 2 villages = 20 houses

Note: LT=light trap

\*Mopeia collections form part of an epidemiological study (“A cluster randomized trial to measure the impact of indoor residual spraying with a third-generation indoor residual spray (3GIRS) product in combination with long-lasting insecticidal nets in Zambezia, Mozambique”) that had 5 villages in control areas and 5 in intervention areas up to October 2018, when spraying with SumiShield® 50WG was extended to cover the unsprayed control areas.

#Lugela and Erati are unsprayed control districts, in Zambezia and Nampula provinces, respectively.

As noted above, until October 2018, only parts of Mopeia District were sprayed; vector control in the other areas consisted of insecticide-treated nets. Following the expansion of IRS throughout Mopeia, the newly sprayed areas became known as the “previous control” area; the areas where IRS began two years earlier are still referred to as the “intervention area” in this report.

## 2.1.4 VECTOR SUSCEPTIBILITY TESTING

In Maganja da Costa, Milange, and Lugela (Zambezia), the project attempted from July to December 2018 to collect adult *An. funestus* s.l. mosquitoes using Prokopack aspirators and to test them for insecticide susceptibility. Immature malaria vectors were collected from different larval habitats in all seven districts (six intervention, one control) from January to May 2019. In Nampula Province, immature *An. gambiae* s.l. malaria vectors were collected from different larval habitats in Nampula City, Monapo, and Erati districts.

Field-collected larvae of *An. gambiae* s.l. were reared in the insectary to adult stage. Batches of 25 females, sugar-fed and from three to five days old, were subjected to World Health Organization (WHO) tube tests following the standard WHO 2016 protocol. These females were exposed to pirimiphos-methyl 0.25%, alpha-cypermethrin 0.05%, permethrin 0.75%, dichlorodiphenyltrichloroethane (DDT) 4%, bendiocarb 0.1%, and deltamethrin 0.05% on WHO impregnated filter papers for 60 minutes. Knockdown was scored at 60 minutes, immediately after the exposure period, at which time all mosquitoes were gently transferred to holding tubes. Mortality was recorded at 24 hours after exposure for all insecticides except for chlorfenapyr and clothianidin, for which the holding period was longer, up to seven days (Oxborough et al. 2019). Where control mortality scored higher than 5% but less than 20%, Abbott's correction was applied to test mortalities (Abbott 1925). Those above 20% led to tests being discarded. Susceptibility levels of *An. gambiae* s.l. and *An. funestus* s.l. were evaluated based on WHO criteria, which classifies test mortality rates higher than 98% as susceptible, between 90% and 97% as suggestive of resistance and requiring further investigation, and below 90% as resistant (WHO 2016).

Intensity assays were conducted by exposing wild-caught vector mosquitoes to insecticide dosages of 5× and 10× the diagnostic concentrations of permethrin and alpha-cypermethrin, according to the standard WHO bioassay method. All exposures were for one hour, and final mortality was scored after a 24-hour holding period during which a 10% sugar solution was made available to surviving mosquitoes.

Synergist assays were conducted using mosquitoes reared from field-collected larvae. Four bioassay exposures were done as follows: In the first group of replicates, the mosquitoes were exposed to the insecticide only (alpha-cypermethrin), the second group was exposed to 4% piperonyl-butoxide (PBO) only, the third group to 4% PBO followed by insecticide, and the last group was exposed to the solvent (control). All replicates were exposed for 60 minutes and mortality was recorded 24 hours after exposure, according to the WHO (2016) protocol. This process was repeated three times based on the standard procedure.

For clothianidin susceptibility tests, freshly treated filter papers<sup>2</sup> were inserted into plastic cylinders and tested according to standard WHO susceptibility test protocols. The exposure time was 60 minutes. Afterward, mosquitoes were transferred into holding cylinders with filter paper treated only with distilled water and provided with lightly moistened cotton wool containing 10% sugar solution that was changed daily. Knockdown was recorded halfway through the test at 30 minutes and at the end of the test at 60 minutes. Mortality was recorded on days 1, 2, 3, 4, 5, and 6, and final mortality on day 7 after exposure. A negative control was tested at the same time and mortality recorded on days 1 through 7. The test was conducted with *An. gambiae* s.l. collected from several breeding sites in villages in two different districts (Mopeia and Morrumbala). For each village, four replicates of 25 mosquitoes were tested (for a total of 100 sugar-fed females) with clothianidin papers, and two replicates were used at the same time with the negative control papers (impregnated only with distilled water). In addition to the negative control described above, a positive control was done by similarly exposing a laboratory-reared susceptible *An. arabiensis* KGB strain.

Chlorfenapyr susceptibility tests were conducted using the CDC bottle assay technique in the field as described in the CDC Guideline for Evaluating Insecticide Resistance in Vectors Using the CDC Bottle Bioassay ([https://www.cdc.gov/malaria/resources/pdf/fsp/ir\\_manual/ir\\_cdc\\_bioassay\\_en.pdf](https://www.cdc.gov/malaria/resources/pdf/fsp/ir_manual/ir_cdc_bioassay_en.pdf)).

All the above susceptibility tests were conducted to the extent possible under the recommended optimal conditions, at temperatures around 27°C ±2°C and 70–80% relative humidity. Similar to other collections, a portion of Mopeia samples from these tests were sent to the Walter Reed Biosystematics Unit and the rest to Mozambique's *Instituto Nacional de Saúde* (INS), for PCR assays to identify sibling species and detect presence of mutations on the knockdown resistance (*kdr*) and acetylcholinesterase-1 (*Ace-1*) genes.

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<sup>2</sup> Treated based on the AIRS protocol

## 2.1.5 MONITORING IRS QUALITY, INSECTICIDE DECAY RATE AND AIRBORNE FUMIGANT EFFECT

In Zambezia, standard WHO cone bioassay tests were performed in Maganja da Costa, Milange, and Mopeia (in the village of 24 de Julho) districts, from October/ November 2018 through July 2019 to evaluate the spray quality and residual efficacy of the insecticide used in the 2018 spray campaign. The same activity was performed in Nampula City and Monapo districts of Nampula Province. Wall bioassays were conducted 24 hours after spraying and subsequently monitored monthly up to July 2018 or until mortality dropped below 80% in two consecutive months. Quality assurance tests were carried out in October 2018.

In each village, five houses were randomly selected. The same houses were used each month. Cones were placed at heights of 0.5 m, 1.0 m, and 1.5 m from the floor, arranged diagonally across a sprayed wall surface. The cones were lined with self-adhesive tape. The control cone was affixed on a wall lined with a paperboard with adhesive in an unsprayed house or in the shade of a tree in the yard away from the sprayed house to avoid any potential airborne effect. Two-to-five day-old female mosquitoes were used for the tests. Susceptible *An. arabiensis* KGB strain mosquitoes were introduced into the plastic cones in batches of 10 and left exposed on the sprayed surface for 30 minutes at different heights. For houses sprayed with pirimiphos-methyl (Actellic® 300CS), numbers of mosquitoes knocked down at the 30<sup>th</sup> minute were recorded. At that time, the mosquitoes were carefully collected and transferred to paper cups and provided with 10% sugar solution soaked on cotton wool pads placed on top of the paper cups covered with net. Knockdown was also recorded in SumiShield® sprayed houses. Mortality was scored after 24 hours holding for Actellic® and every 24 hours up to seven days for SumiShield®.

Tests for the airborne fumigant effect of pirimiphos-methyl (Actellic® 300CS) and SumiShield® 50WG were conducted with laboratory reared *An. arabiensis* KGB strain mosquitoes placed inside a netting cage and hung 10 cm away from the sprayed wall surfaces at a height of 1.5 m above the floor. The mosquitoes were transferred into clean paper cups that were kept for a 24-hours (for Actellic) or up to 72 hours (for SumiShield) holding period. Dead and live mosquitoes were counted after 24 or 72 hours, and the percentage mortality was calculated in the replicates for each house and recorded according to WHO protocol.

## 2.2 STATISTICAL TESTS

The average number of mosquitoes collected by the HLC method was calculated. To compare mean indoor and outdoor biting rates, Chi-square tests were used, and *P* values less than 0.05 were considered significant.

## 3. RESULTS: ZAMBEZIA

VectorLink Mozambique conducted IRS with pirimiphos-methyl (Actellic® 300CS) in the four target districts of Derre, Maganja da Costa, Milange, and Molumbo and with SumiShield® 50WG in the two districts of Morrumbala and Mopeia.

### 3.1 ANOPHELINE SPECIES COLLECTED BY DIFFERENT METHODS

During the reporting period, July 2018 through June 2019, in the intervention districts of Maganja da Costa and Milange, the intervention and previous control arms of Mopeia, and the control district of Lugela, a total of 18,916 anophelines belonging to 14 different species and species complexes were collected using the three collection methods (PSC, CDC light trap, and HLC) and morphologically identified (Gillies & Coetzee. 1987). The anophelines collected included *An. funestus* s.l., *An. gambiae* s.l., *An. coustani*, *An. pharoensis*, *An. pretoriensis*, *An. squamosus*, *An. tenebrosus*, *An. ziemanni*, *An. brucei*, *An. caliginosus*, *An. dancallicus*, *An. rufipes*, *An. natalensis*, and *An. maculipalpis*. Table 2 enumerates the number of mosquitoes collected, by district and species during the period.

**TABLE 2. NUMBER OF MOSQUITOES COLLECTED IN EACH DISTRICT BY ALL THREE COLLECTION METHODS**

Species Collected	Maganja da Costa	Milange	Mopeia	Lugela	Total per Species
<i>An. funestus</i> s.l.	369	608	11,683	2,967	15,627
<i>An. gambiae</i> s.l.	192	343	1,441	331	2,307
<i>An. coustani</i>	12	29	17	3	61
<i>An. pharoensis</i>	0	0	26	0	26
<i>An. pretoriensis</i>	0	3	2	7	12
<i>An. squamosus</i>	0	1	157	0	158
<i>An. tenebrosus</i>	124	1	235	6	366
<i>An. ziemanni</i>	84	60	169	0	313
<i>An. brucei</i>	0	0	1	0	1
<i>An. caliginosus</i>	0	0	1	0	1
<i>An. dancallicus</i>	0	1	1	0	2
<i>An. rufipes</i>	0	8	2	0	10
<i>An. natalensis</i>	1	0	0	0	1
<i>An. maculipalpis</i>	1	10	0	20	31
<b>Total</b>	<b>783</b>	<b>1,064</b>	<b>13,735</b>	<b>3,334</b>	<b>18,916</b>

#### 3.1.1 PYRETHRUM SPRAY CATCH

PSC collections yielded a total of 1,215 *Anopheles* mosquitos (Table 3). Morphological identification found that 1,121 of these belonged to *An. funestus* s.l. (92.3%), 93 to *An. gambiae* s.l., and one to *An. coustani* (0.1%) groups of species.

**TABLE 3. NUMBER OF MOSQUITOES BY SPECIES COLLECTED USING PSC IN TWO INTERVENTION DISTRICTS AND THE CONTROL DISTRICT**

Mosquito Species/District	Maganja da Costa	Milange	Lugela	Total
<i>An. funestus</i> s.l.	95	251	775	1,121
<i>An. gambiae</i> s.l.	14	25	54	93
<i>An. coustani</i>	1	0	0	1
<b>Total</b>	110	276	829	1,215

The indoor resting density of *An. funestus* s.l. and *An. gambiae* s.l. established by PSC sampling was very low in both intervention and control sites. The mean vector density was estimated at less than seven and one for *An. funestus* s.l. and *An. gambiae* s.l. per room per day, respectively. These low indoor resting densities were found at most of the collection sites before and after IRS intervention during the monitoring period (Figures 2A and 2B).

**FIGURE 2. INDOOR RESTING DENSITIES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN THREE DISTRICTS BEFORE AND AFTER IRS INTERVENTION**

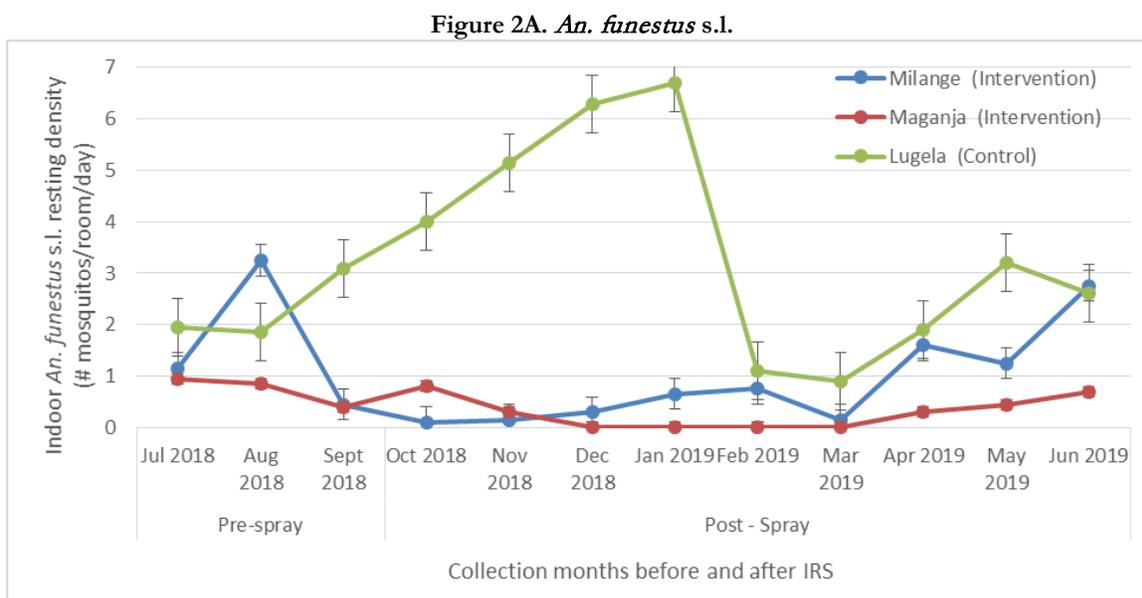
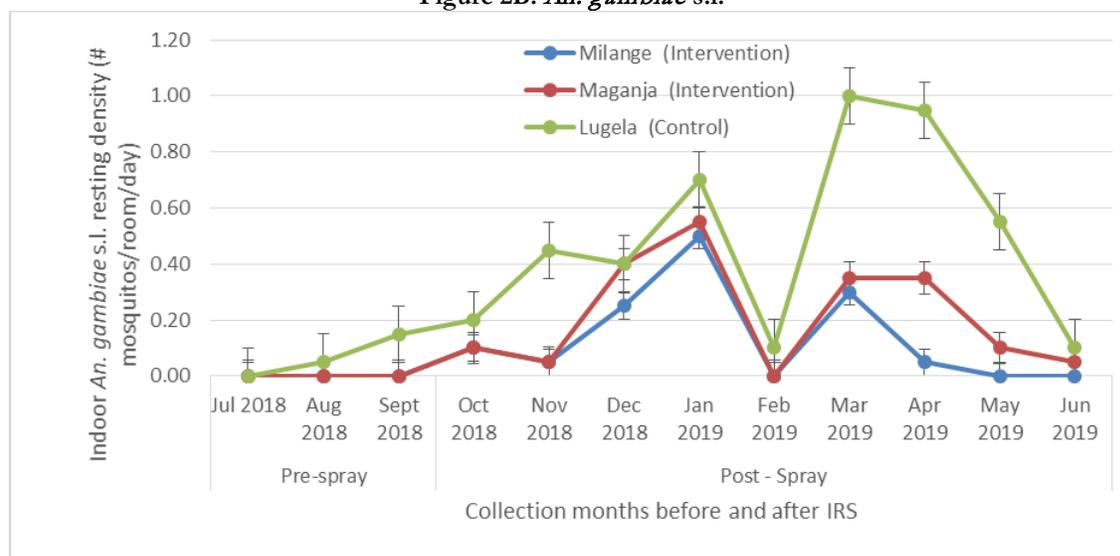


Figure 2B. *An. gambiae* s.l.



### 3.1.2 HUMAN LANDING CATCHES

A total of 2,277 Anopheles mosquitoes were collected using the HLC technique. Morphological identification showed that 1,370 were *An. funestus* s.l.; 544 *An. gambiae* s.l.; 39 *An. coustani*; 130 *An. tenebrosus*; 10 *An. pretoriensis*; 1 *An. squamosus*; 8 *An. rufipes*; 142 *An. ziemanni*; 1 *An. natalensis*; 31 *An. maculipalpis*; and 1 *An. dancallicus*. Milange had highest anopheline diversity, followed by Maganja da Costa and Lugela.

Table 4 shows that significant differences were observed between total numbers of *An. funestus* s.l. samples collected indoors and outdoors (with  $p < 0.05$ ) in Maganja da Costa, Milange, and Lugela (control). More *An. funestus* s.l. mosquitoes were collected indoors than outdoors in Maganja da Costa and Lugela.

Significant differences were observed between total numbers of *An. gambiae* s.l. samples collected indoors and outdoors (with  $p < 0.05$ ) in Milange. More *An. gambiae* s.l. mosquitoes were collected outdoors than indoors in both districts.

TABLE 4. COMPARISON OF TOTAL NUMBER OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY HLC INDOORS AND OUTDOORS IN THREE DISTRICTS

District	<i>An. funestus</i> s.l.				<i>An. gambiae</i> s.l.			
	# Collected indoors	# Collected outdoors	X <sup>2</sup>	p-value	# Collected indoors	# Collected outdoors	X <sup>2</sup>	p-value
Maganja da Costa	127	56	27.55	<0.00001*	68	74	0.25	0.614607
Milange	86	127	7.89	0.004965*	72	199	59.52	<0.00001*
Lugela (Control)	659	315	121.49	<0.00001*	60	71	0.92	0.336515

\*p-value significant

Table 5 summarizes the combined outdoor and indoor collections by species from intervention and control districts, showing mean biting rates per night (b/p/n) for each. Overall combined data for all vectors shows that an unprotected person in control areas experienced about twice (0.72 b/p/n) as many bites as one in the intervention areas (0.36 b/p/n). For *An. funestus* s.l., alone, an unprotected person in the control areas experienced about five times (6.76 b/p/n) more bites than one in the intervention areas (1.38 b/p/n).

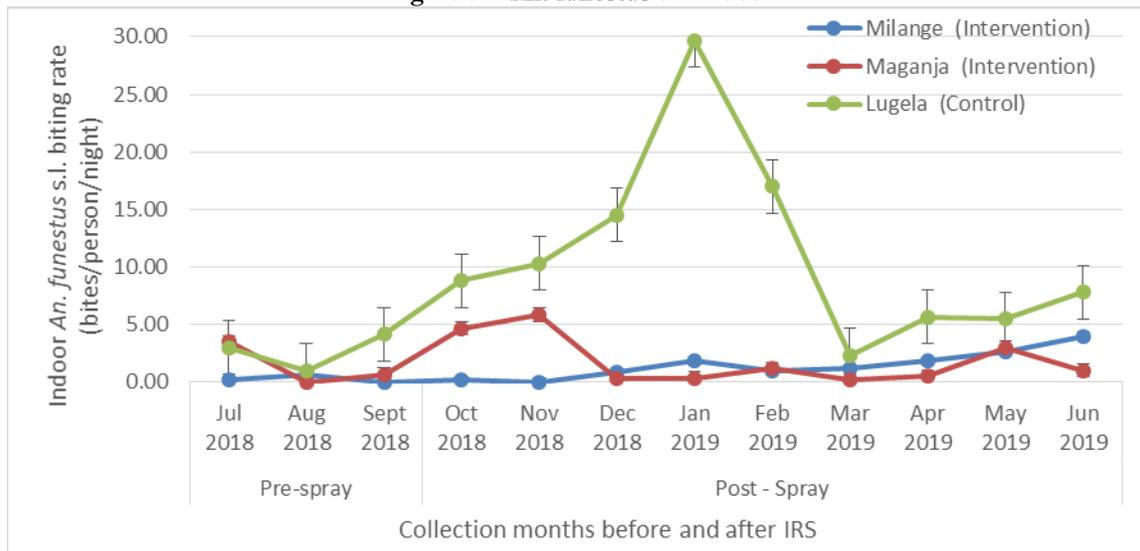
**TABLE 5. MOSQUITO SPECIES COLLECTED BY HLC AND THEIR COMBINED OUTDOOR AND INDOOR MEAN BITING RATES IN INTERVENTION DISTRICTS OF MAGANJA DA COSTA AND MILANGE AND CONTROL AREA OF LUGELA**

Species Collected	Intervention Area			Control Area		
	Total numbers collected	Total person nights	b/p/n	Total numbers collected	Total person nights	b/p/n
<i>An. funestus</i> s.l.	396	288	1.38	974	144	6.76
<i>An. gambiae</i> s.l.	413	288	1.43	131	144	0.91
<i>An. coustani</i>	37	288	0.13	2	144	0.01
<i>An. natalensis</i>	1	288	0.00	0	144	0.00
<i>An. pretoriensis</i>	3	288	0.01	7	144	0.05
<i>An. squamosus</i>	1	288	0.00	0	144	0.00
<i>An. tenebrosus</i>	124	288	0.43	6	144	0.04
<i>An. ziemanni</i>	142	288	0.49	0	144	0.00
<i>An. dancallicus</i>	1	288	0.00	0	144	0.00
<i>An. maculipalpis</i>	11	288	0.04	20	144	0.14
<i>An. rufipes</i>	8	288	0.03	0	144	0.00
Total	1,137	3,168	0.36	1,140	1,584	0.72

Figures 3A and 3B show that *An. funestus* s.l. demonstrated a similar biting pattern across the year both indoors and outdoors, albeit with the biting intensity being almost two times higher indoors than outdoors. The rate was found to be low before IRS,  $\leq 4.1$  b/p/n indoors and  $\leq 1.67$  b/p/n outdoors in all districts. In the control district, where the biting rate was consistently higher than in the intervention districts, biting rates were observed to go up, reaching a peak in January 2019, both indoors (at 29.7 b/p/n) and outdoors (at 15.5 b/p/n). This was followed by a dramatic decrease that bottomed out in March 2019 at 2.33 b/p/n indoors and 1.5 b/p/n outdoors. In the intervention districts of Maganja da Costa and Milange, indoor bites remained relatively low after IRS, at below 5.83 b/p/n for the rest of the year. A similar pattern was observed outdoors, where bites remained below 2.3 b/p/n until April 2019, when there was a slight increase in Milange, to 6.33 b/p/n in June 2019.

**FIGURE 3. INDOOR AND OUTDOOR BITING RATES FOR *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN TWO INTERVENTION DISTRICTS AND ONE CONTROL DISTRICT, BEFORE AND AFTER IRS**

**Figure 3A. *An. funestus* s.l. Indoor**



**Figure 3B. *An. funestus* s.l. Outdoor**

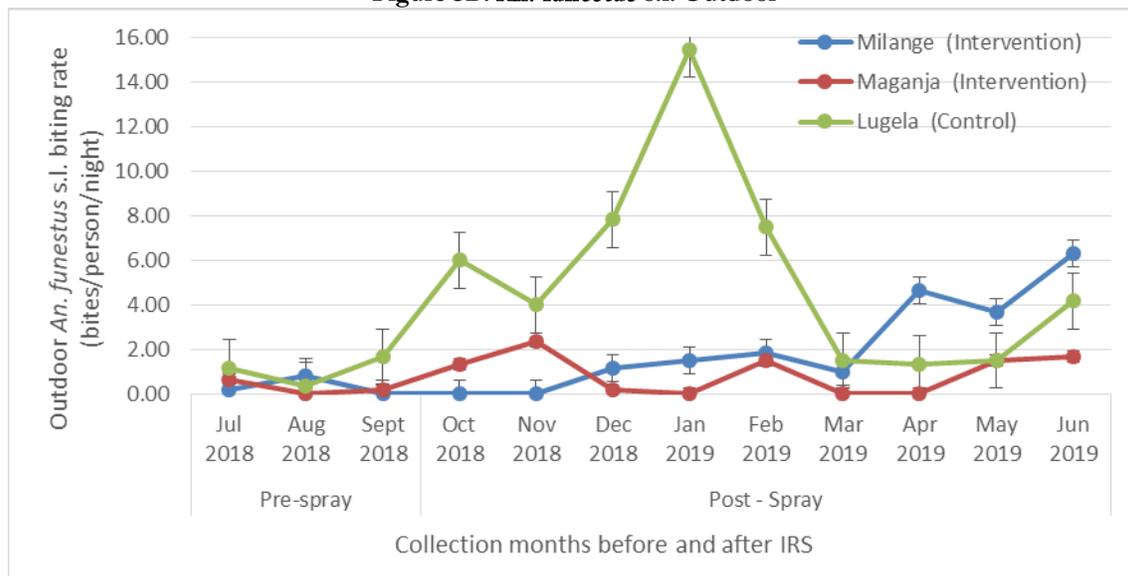
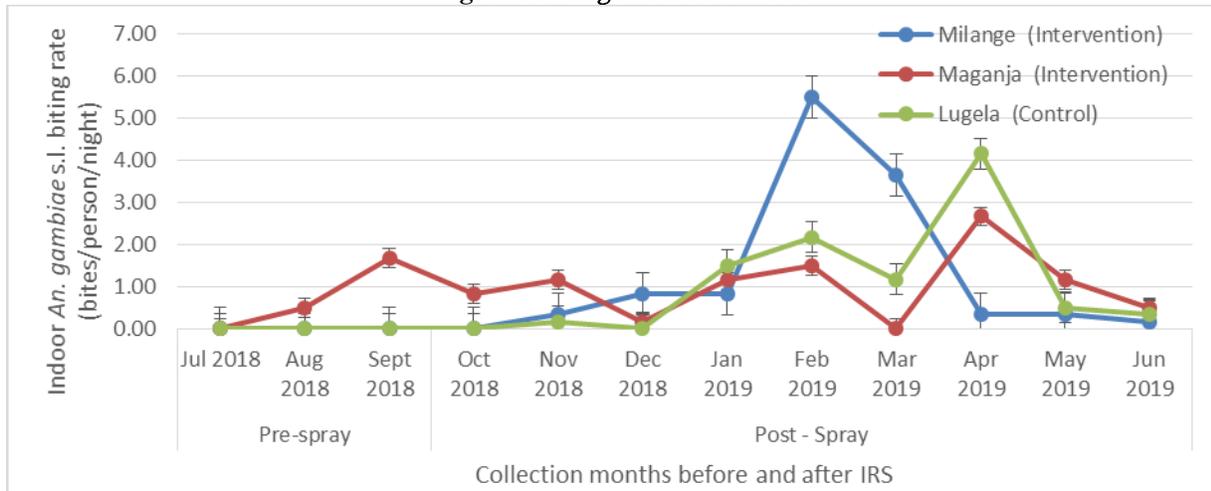


Figure 3C shows that *An. gambiae* s.l. biting rate before IRS was observed to be low both indoors ( $\leq 1.67$  b/p/n) and outdoors ( $\leq 0.67$  b/p/n). The indoor biting rates remained low after IRS for several months, up to February 2019 when indoor biting in Milange spiked to 5.5 b/p/n; possibly due to temporal increase in breeding sites. It thereafter dropped dramatically to less than 0.33 b/p/n. The other districts demonstrated a gradual and small increase that peaked in April 2019 at 4.1 in Lugela and 2.7 b/p/n Maganja da Costa. Thereafter, there was a sharp drop in both districts, to 0.33 b/p/n, by June 2019.

Figure 3C *An. gambiae* s.l. Indoor



The *An. gambiae* s.l. outdoor pattern is shown in Figure 3D. Outdoor biting rates remained low after IRS for a shorter period than was observed indoors. In Milange, rates increased steadily beginning in November 2018, and peaked in March 2019 at 10.5 b/p/n; thereafter they dropped dramatically to 1.1 b/p/n in April 2019. The biting rates in Maganja da Costa and Lugela increased later, beginning in December and January 2019, respectively, and reaching a peak in February 2019 at 3.3 b/p/n and 5.0 b/p/n and thereafter dropping dramatically to 0.00 and 0.33 b/p/n by June.

Figure 3D. *An. gambiae* s.l. Outdoor

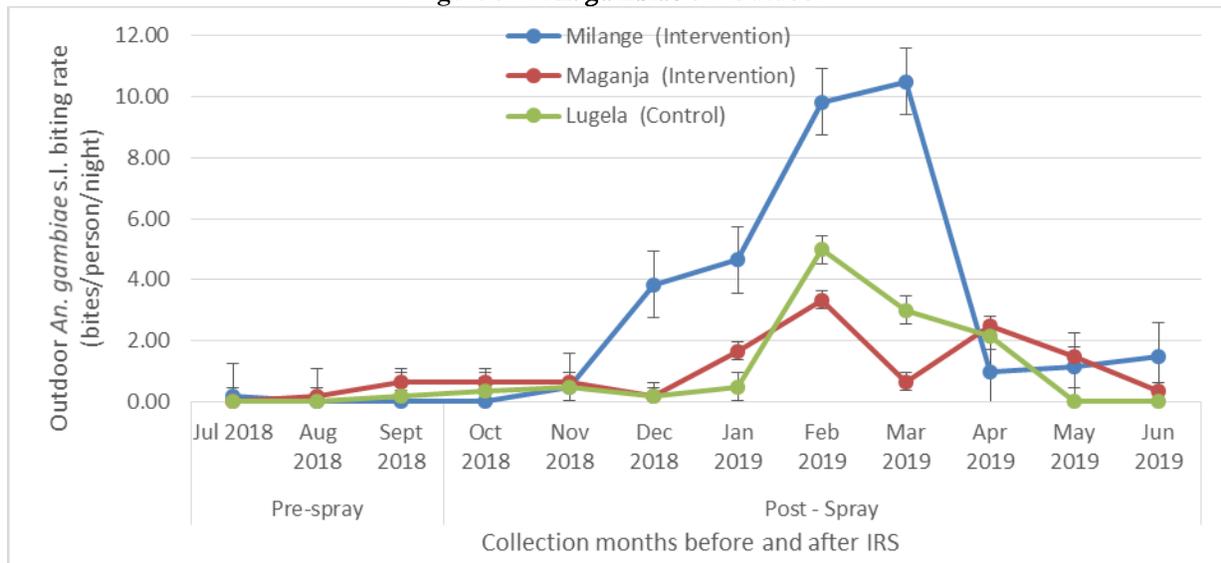


Table 6 shows the mean indoor and outdoor vector biting rates for *An. funestus* s.l. and *An. gambiae* s.l. before and after spraying. The biting rates were observed to have risen for both species indoors and outdoors in all districts. Lugela, the control district, demonstrated the highest pre-spray biting rates for both species indoors and outdoors except for *An. gambiae* s.l. in Maganja da Costa. The two highest biting rates were recorded with *An. funestus* s.l. both indoors (11.3 b/p/n) and outdoors (5.4 b/p/n) in Lugela.

**TABLE 6. INDOOR AND OUTDOOR MEAN BITING RATE FOR *FUNESTUS S.L* AND *AN. GAMBIAE S.L.*, ESTIMATED USING HLC FROM ALL COLLECTION ROUNDS, BY DISTRICT, BEFORE AND AFTER SPRAYING**

District	<i>An. funestus s.l.</i>				<i>An. gambiae s.l.</i>			
	(b/p/n)				(b/p/n)			
	Indoors		Outdoors		Indoors		Outdoors	
	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray
Maganja	1.39	1.89	0.28	0.94	0.72	1.02	0.28	1.28
Milange	0.28	1.50	0.33	2.24	0.00	1.33	0.06	3.67
Lugela*	2.72	11.30	1.06	5.48	0.00	1.11	0.06	1.30

\*Unsprayed control District.

The pre- and post-spray estimates are based on the period before spraying was done in intervention districts (July–September 2018): pre-spray comprised the three months before spraying and post-spray, nine months after spraying, to June 2019.

The overnight indoor and outdoor biting pattern of *An. funestus s.l.* is displayed in Figures 4A and 4B respectively. The indoor biting activity in Maganja da Costa and Milange remained consistently below 2.0 bites per hour (b/p/h) up to midnight indoors and 1:00 am outdoors. An indoor biting peak was observed in Maganja da Costa (3.44 b/p/h) and in Milange (3.83 b/p/h) between 1:00 am and 2:00 am. The control district of Lugela demonstrated the highest biting activity from 9:00 pm to 6:00 am, both indoors and outdoors. Both indoor and outdoor biting activity there increased steadily starting at 7:00 pm; activity reached a peak indoors (15.8 b/p/h) between 1:00 am and 2:00 am and outdoors (9.3 b/p/h) between 2:00 am and 3:00 am.

**FIGURE 4. HOURLY BITING RATES OF *AN. FUNESTUS S.L.* AND *AN. GAMBIAE S.L.* IN MAGANJA DA COSTA, MILANGE, AND LUGELA AS DETERMINED THROUGH HLCs**

Figure 4A. *An. funestus s.l.* Indoor

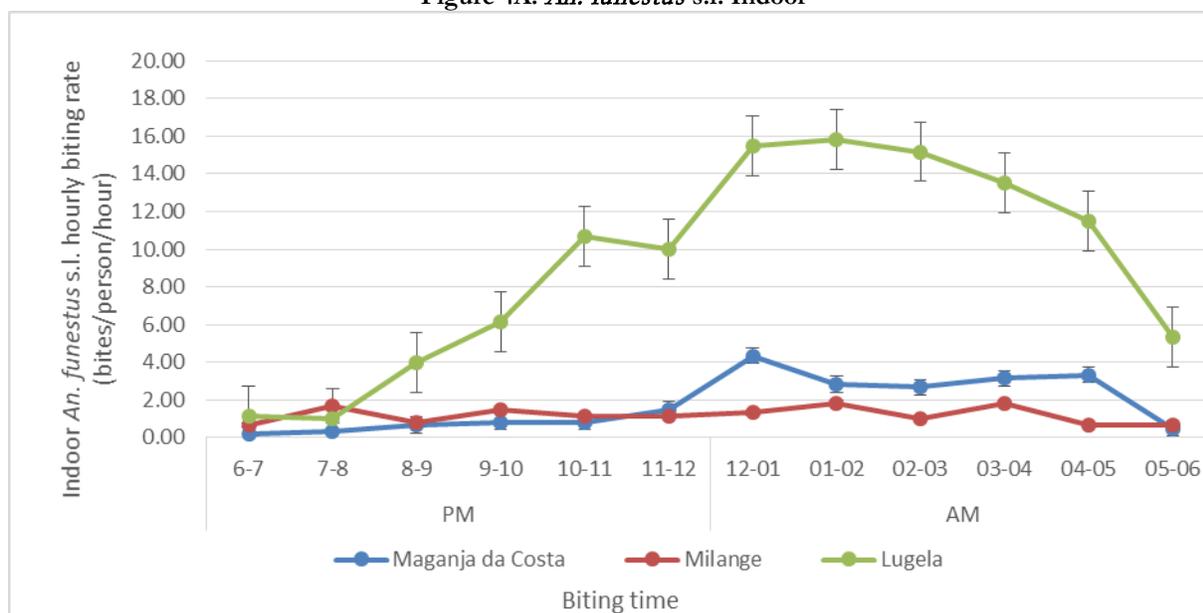
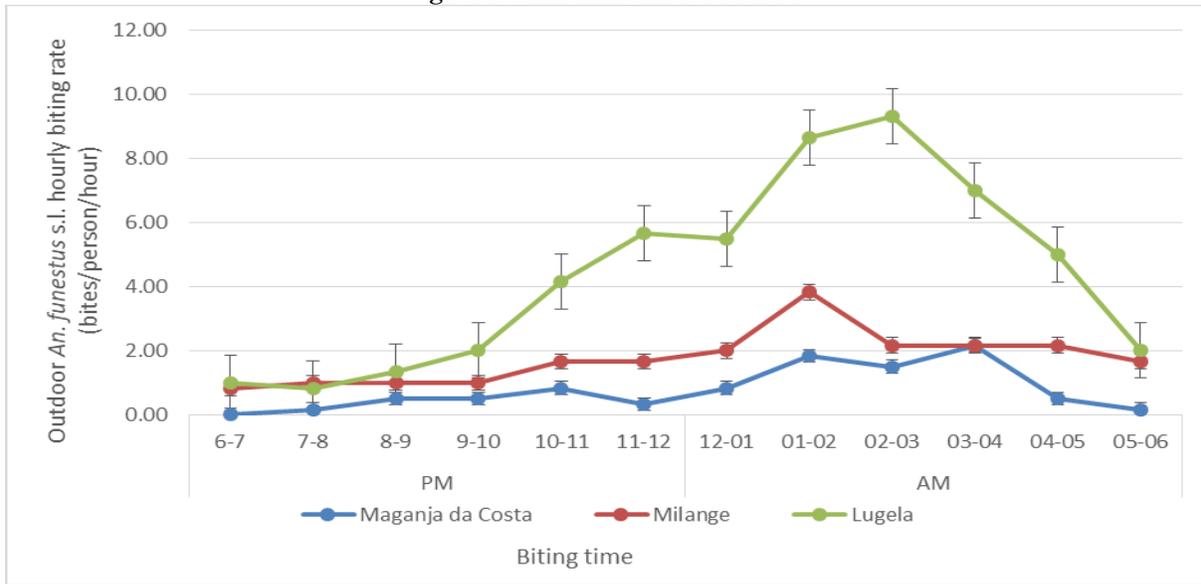


Figure 4B. *An. funestus* s.l. Outdoor



The indoor and outdoor hourly biting activity for *An. gambiae* s.l. is shown in Figures 4C and 4D. The indoor biting activity remained below 1.0 b/p/h in all districts from 6:00 pm to 10:00 pm and then increased steadily, reaching a peak between 12:00 pm and 1:00 am in Maganja da Costa (2.67 b/p/h) and Milange (2.5 b/p/h) and between 02:00 pm and 03:00 pm in Lugela (2.33 b/p/h). Most biting appears to have taken place between 11:00 pm and 4:00 am but especially around midnight in the two intervention districts and between 2:00 am and 3:00 am in the control. Unlike the *An. funestus* s.l. biting pattern shown above, where the biting activity in Lugela control district was well above activity in the intervention districts, for *An. gambiae* s.l., the biting activity varied little among the three districts.

The outdoor *An. gambiae* s.l. biting activity remained below and around 1.0 b/p/h in Maganja da Costa and Lugela during the early evening hours, from 6:00 pm to 11:00 pm, and thereafter slightly increased to reach a peak in Lugela (1.83 b/p/h) around midnight and Maganja da Costa (2.33 b/p/h) between 2:00 am and 3:00 am. Surprisingly, the biting activity in Milange intervention district was found to be consistently higher than the rest of the districts throughout the night, reaching a peak of 5.0 b/p/h after midnight, between 1:00 am and 2:00 am. Similar to the other districts, overall biting activity was highest between 11:00 pm and 3:00 am. Similar pattern of highest outdoor biting activity was observed in Milange in 2017, but was not the case in the 2018 reporting period. This might be due to variability of abundance of mosquito breeding habitats and microclimatic conditions in and around the collection sites.

Figure 4C. *An. gambiae* s.l. Indoor

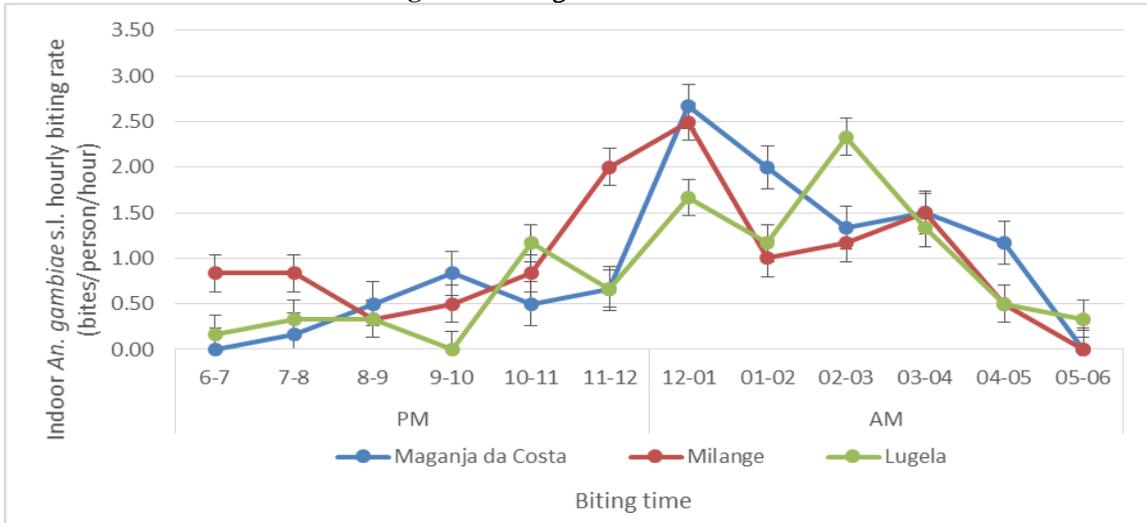
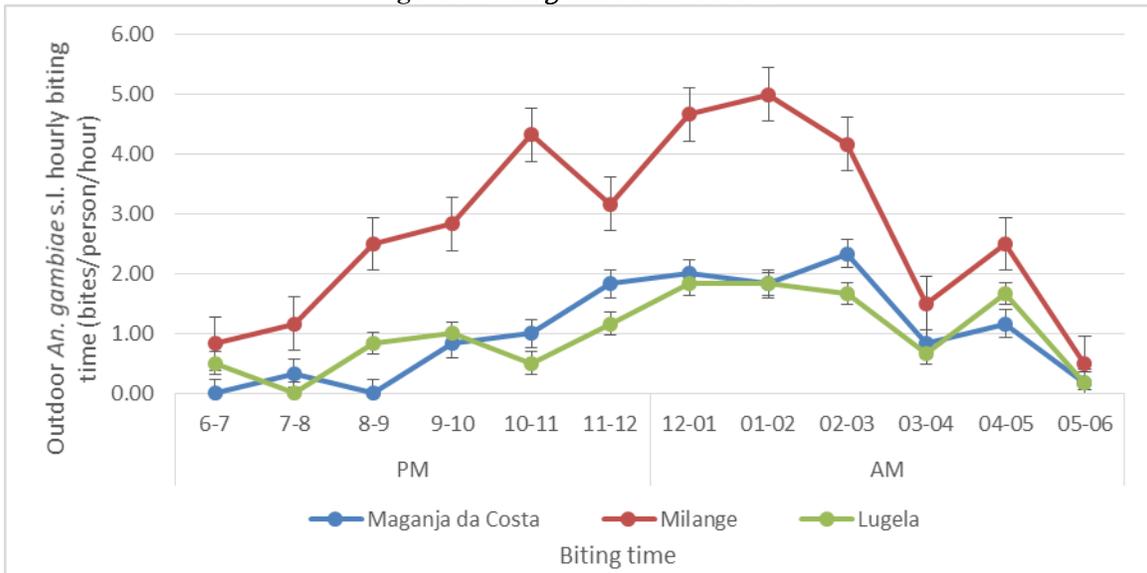


Figure 4D. *An. gambiae* s.l. Outdoor



### 3.1.3 MOPEIA STUDY

In 2018, Mopeia was one of two districts in Zambezia province that was sprayed with SumiShield 50WG. Preliminary analysis of HLC data from Mopeia clearly shows that there was a significant difference in collections in the intervention area that had received IRS intervention for the past two years and the previous control area, which was sprayed for the first time in October 2018.

Surveillance including HLC was performed there in the three months prior to IRS intervention, and then afterward, through June 2019. In both the intervention and previous control areas, the most abundant mosquito species collected HLCs were *An. funestus* s.l. (1,479) and *An. gambiae* s.l. (268). An additional 520 mosquitoes were morphologically identified as *An. coustani* (14), *An. tenebrosus* (195), *An. squamosus* (134), *An. ziemanni* (152), *An. pretoriensis* (1), *An. pharoensis* (22), *An. brucei* (1), and *An. caliginosus* (1). Table 7 shows numbers of each mosquito species collected in intervention and previous control areas, indicating for each the total person-nights and subsequent biting rate. The

major vectors *An. funestus* s.l. and *An. gambiae* s.l. were observed to contribute a combined proportion to the tune of 60.0% and 84.5% of the bites in the intervention and previous control areas, respectively. Other avid biters included *An. tenebrosus* (16%) and *An. ziemanni* (15.7%) in the intervention and *An. squamosus* (5.9%) and *An. tenebrosus* (5.2%) in the previous control areas. Overall, the mean bites experienced in the intervention and previous control areas were estimated as 0.48 and 1.1 b/p/n, respectively. This indicates that a person in the previous control area would experience more than twice (2.3 times) the bites experienced in the intervention area.

**TABLE 7. MOSQUITO SPECIES COLLECTED BY HLC AND THEIR MEAN BITING RATES IN MOPEIA INTERVENTION AND PREVIOUS CONTROL AREAS**

Species Collected	Intervention Area			Previous Control Area		
	Total numbers collected	Total person nights	b/p/n	Total numbers collected	Total person nights	b/p/n
<i>An. funestus</i> s.l.	287	144	1.99	1,192	144	8.28
<i>An. gambiae</i> s.l.	133	144	0.92	135	144	0.94
<i>An. coustani</i>	6	144	0.04	8	144	0.06
<i>An. pharoensis</i>	10	144	0.07	12	144	0.08
<i>An. pretoriensis</i>	1	144	0.01	0	144	0.00
<i>An. squamosus</i>	40	144	0.28	94	144	0.65
<i>An. tenebrosus</i>	112	144	0.78	83	144	0.58
<i>An. ziemanni</i>	110	144	0.76	42	144	0.29
<i>An. bucei</i>	0	144	0.00	1	144	0.01
<i>An. caliginosus</i>	1	144	0.01	0	144	0.00
Total	<b>700</b>	1,440	0.48	<b>1,567</b>	1,440	1.1

Table 8 shows that in Mopeia, the mean *An. funestus* s.l. biting rate in the intervention areas was low both indoors and outdoors throughout the collection period, scoring less than 1.23 b/p/n indoors and 1.21 b/p/n outdoors throughout the period. In the previous control areas, the mean indoor and outdoor biting rates were much higher, estimated as 5.20 and 4.74 b/p/n, respectively. A mixed observation was noted for *An. gambiae* s.l.; its mean biting rate was higher indoors in the previous control areas (0.64 against 0.48 b/p/n) whereas its outdoor rate was higher in the intervention (0.75 against 0.59 b/p/n).

**TABLE 8. INDOOR AND OUTDOOR MEAN BITING RATE FOR *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. IN MOPEIA DISTRICT, ESTIMATED USING HLC, BEFORE AND AFTER SPRAYING**

District	<i>An. funestus</i> s.l. (b/p/n)				<i>An. gambiae</i> s.l. (b/p/n)			
	Indoors		Outdoors		Indoors		Outdoors	
	Pre-Spray	Post-Spray	Pre-Spray	Post-Spray	Pre-Spray	Post-Spray	Pre-Spray	Post-Spray
Mopeia - Intervention	0.33	1.23	0.31	1.21	0.00	0.48	0.00	0.75
Mopeia - Previous control	1.75	5.20	0.53	4.74	0.03	0.64	0.03	0.59

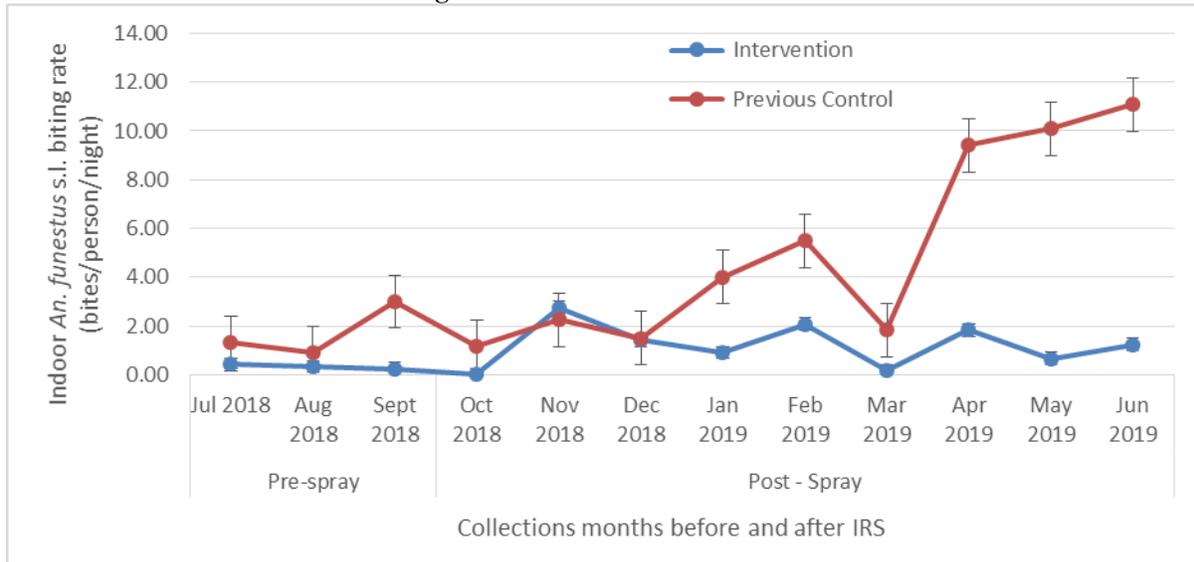
The pre- and post-spray estimates are based on the period before spraying was done in intervention districts (July-September 2018): Pre-spray comprised three months before spraying and post-spray comprised the subsequent nine months after spraying, October 2018-June 2019.

The monthly profile of indoor and outdoor biting pattern of *An. funestus* s.l. is presented in Figures 5A and 5B. The indoor biting activity remained below 3.0 b/p/n throughout the pre-spray period and into the post-spray period in both intervention and previous control areas until December 2018, when a generally upward trend started in the previous control areas. No increase was recorded in the intervention areas where the rates

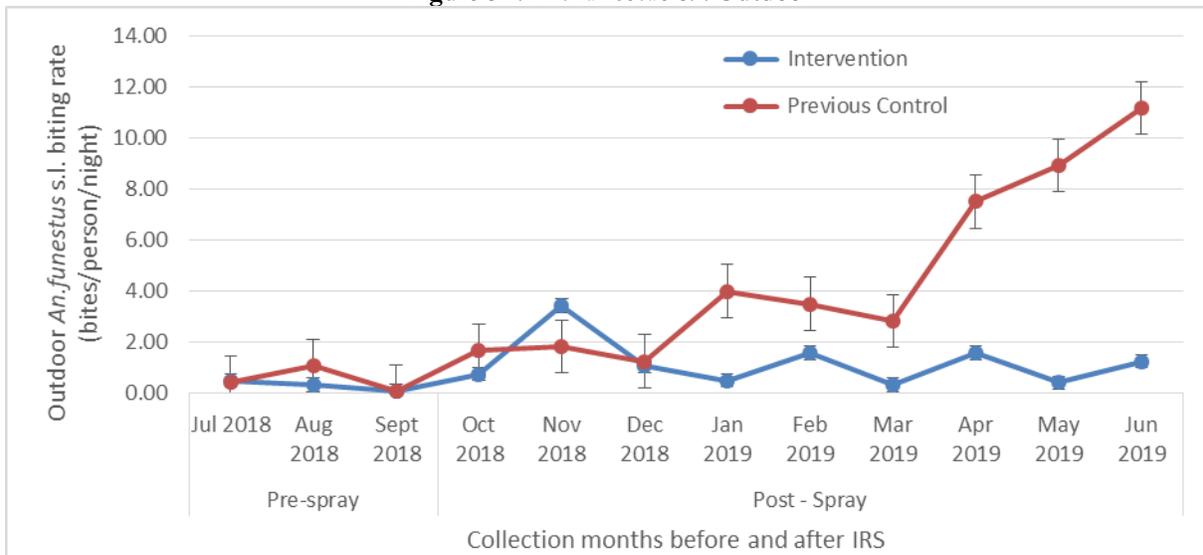
remained low, below 2.1 b/p/n, through the end of June 2019. Indoor biting activity in the previous control areas increased steadily from January 2019 (except for a dip observed in March 2019) to a peak of 11.8 b/p/n recorded in June 2019. Outdoor biting rates in previous control areas was similar to the indoor pattern, and peaked at 11.1 b/p/n in June 2019.

**FIGURE 5. INDOOR AND OUTDOOR HUMAN BITING RATES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN MOPEIA INTERVENTION AND PREVIOUS CONTROL AREAS**

**Figure 5A. *An. funestus* s.l. Indoor**



**Figure 5B. *An. funestus* s.l. Outdoor**



The monthly indoor and outdoor biting pattern of *An. gambiae* s.l. is presented in Figures 5C and 5D. Indoor biting in the intervention areas started off at a low level, never exceeding 0.08 b/p/n, then spiked to 1.08 b/p/n in November 2018 and again to 1.5 b/p/n in April 2019 before dropping to 0.33 b/p/n in May 2019 and to 0.08 in June 2019. The previous control demonstrated only one peak, in April 2019, scoring 1.75 b/p/n and then dropping to 0.33 b/p/n in May 2019 and to 0.08 b/p/n in June 2019. The different patterns in the two sites might also be associated with temporal and spatial abundance of mosquito breeding habitat in different months and sites. But, the biting rates are generally low to provide definitive explanations for variability between the two sites.

The outdoor biting activity remained very low, around 0.08 b/p/n, until a first spike was recorded in the intervention areas (2.58 b/p/n) in November 2018 and in the previous control area (1.0 b/p/n) in February 2019. A second spike in the previous control areas was recorded as 1.83 b/p/n in April 2019, and then a drop to 0.17 b/p/n in June 2019. Intervention areas demonstrated further peaks in February and April 2019 (both at 1.42 b/p/n), then dropping to 0.08 b/p/n in May and June 2019.

Figure 5C. *An. gambiae* s.l. Indoor

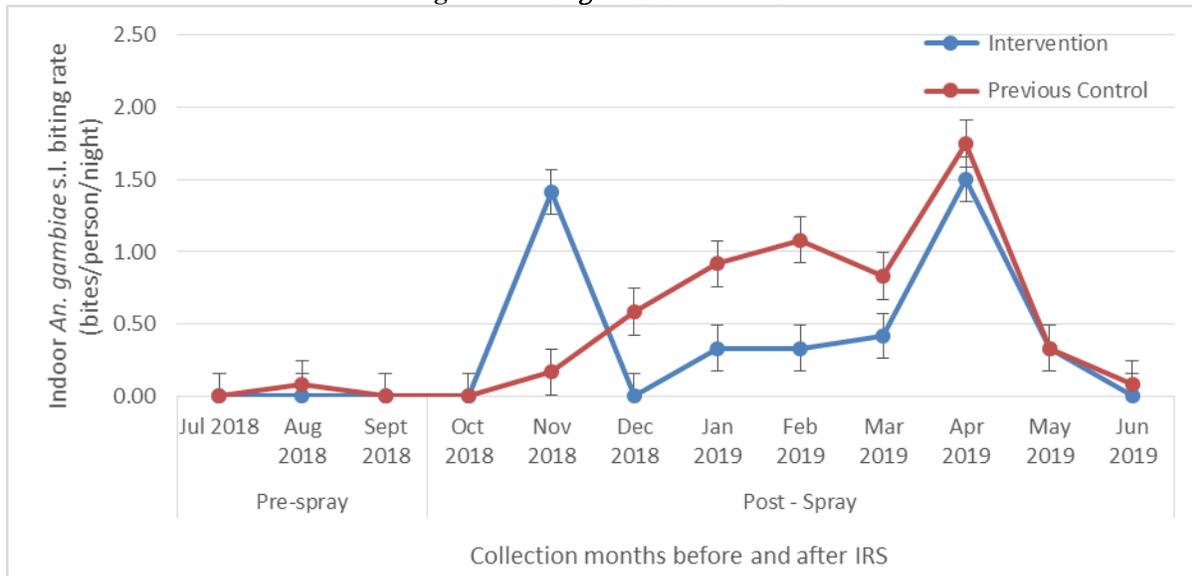
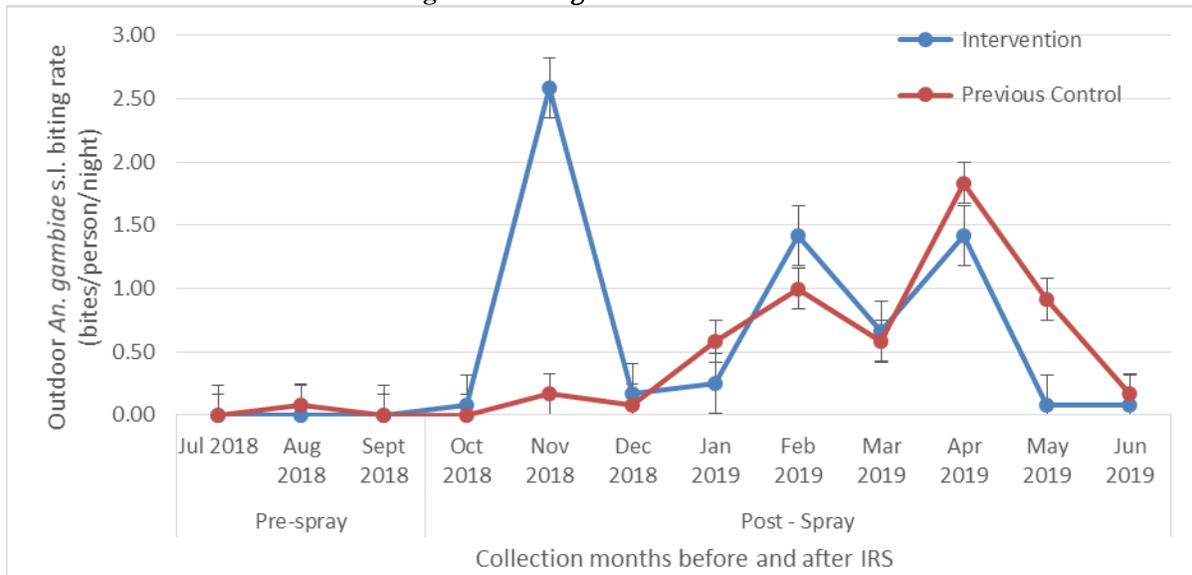


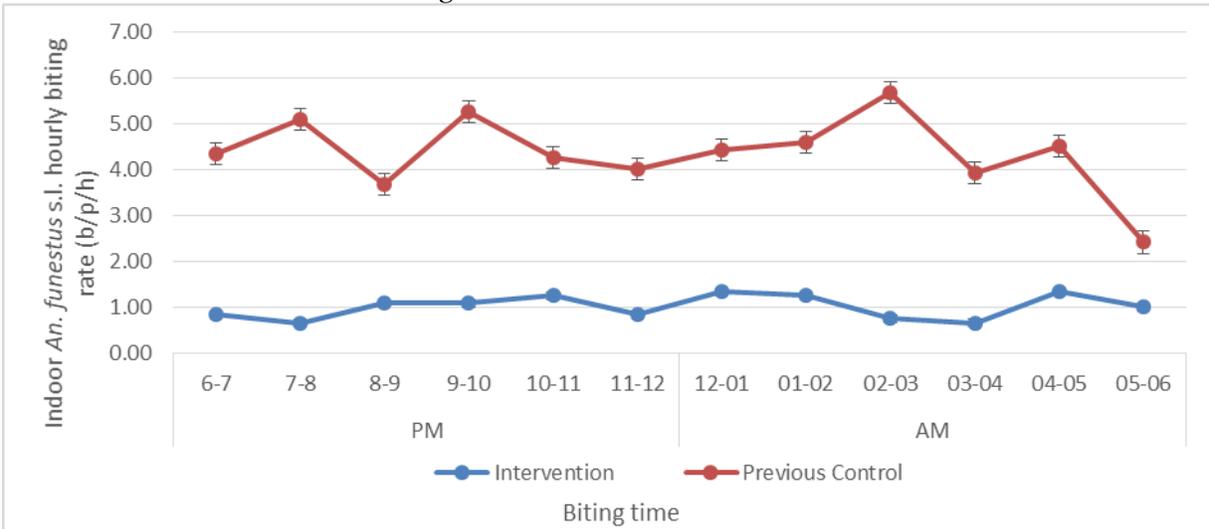
Figure 5D. *An. gambiae* s.l. Outdoor



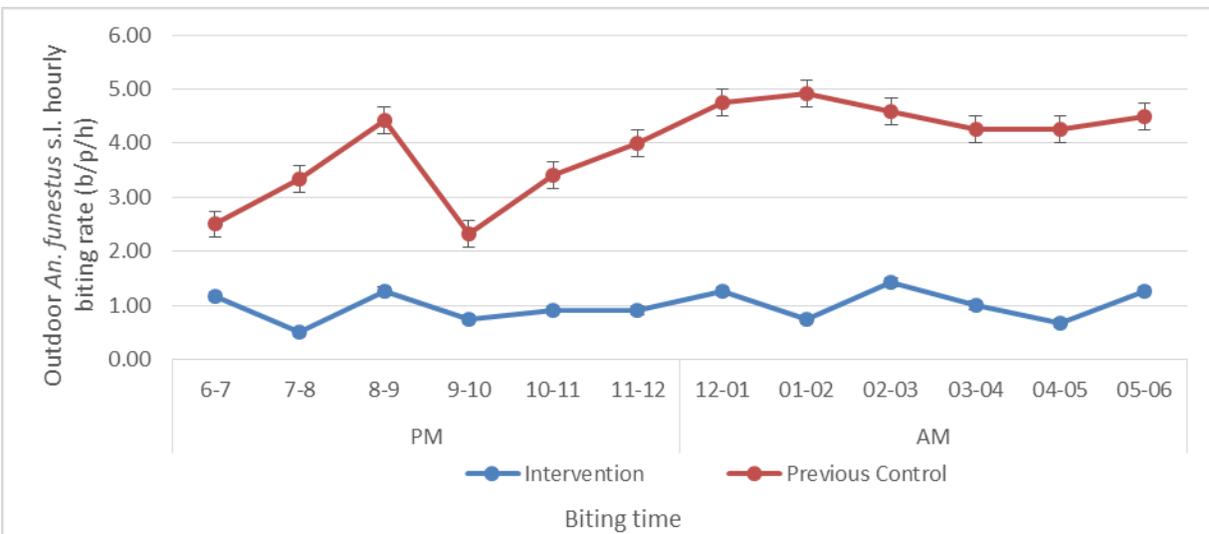
Results of the overnight *An. funestus* s.l. hourly biting pattern indoors and outdoors in Mopeia are plotted in Figures 6A and 6B. The biting rates both indoors and outdoors in the two areas appears to be at two levels of activity. In the intervention areas, it remained consistently below 1.33 b/p/h indoors and 1.42 b/p/h outdoors. In the previous control areas indoors, it was found to be consistently above 4.33 b/p/h over most of the night, with a slight peak (at 5.67 b/p/h) observed at 2:00–3:00 am; it then dropped to 2.41 b/p/h by 5:00 am–6:00 am. The biting activity pattern outdoors in the previous control areas showed a steady increase in biting activity from 2.5 b/p/h at 6:00 pm–7:00 pm, reaching a peak of 4.92 b/p/h by 2:00 am–3:00 am and thereafter dropping gently to 4.5 b/p/h by 05:00am–06:00am.

**FIGURE 6. HOURLY BITING RATES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN MOPEIA DISTRICT, INTERVENTION AND PREVIOUS CONTROL AS DETERMINED THROUGH HLCs**

**Figure 6A. *An. funestus* s.l. Indoor**



**Figure 6B. *An. funestus* s.l. Outdoor**



Results of the overnight *An. gambiae* s.l. hourly biting pattern indoors and outdoors in Mopeia are plotted in Figures 6C and 6D. In the intervention areas, the biting activity remained low (not exceeding 0.33 b/p/h) for much of the night, until it increased to 0.58 b/p/h at 2:00 am–3:00 am; it then dropped to 0.42 b/p/h by 5:00 am–6:00 am. Indoor bites in the previous control areas rose after 8:00–9:00 pm to an estimated peak of 0.83 b/p/h at 11:00 pm–12:00 am; thereafter activity dropped steadily to 0.08 b/p/h recorded at 6:00 am. The fairly low hourly biting rates were ostensibly due to the low numbers of *An. gambiae* s.l. caught in Mopeia.

The outdoor biting pattern in both areas show that activity started at a low level not exceeding 0.42 b/p/h until at 10:00 pm–11:00 pm, when it was observed to increase in the intervention areas to a peak of 1.08 b/p/h. Biting activity in the previous control areas peaked at 0.92 b/p/h around midnight (11:00 pm–1:00 am) and then fluctuated, ending as 0.5 b/p/h by 5:00 am–6:00 am.

Figure 6C. *An. gambiae* s.l. Indoor

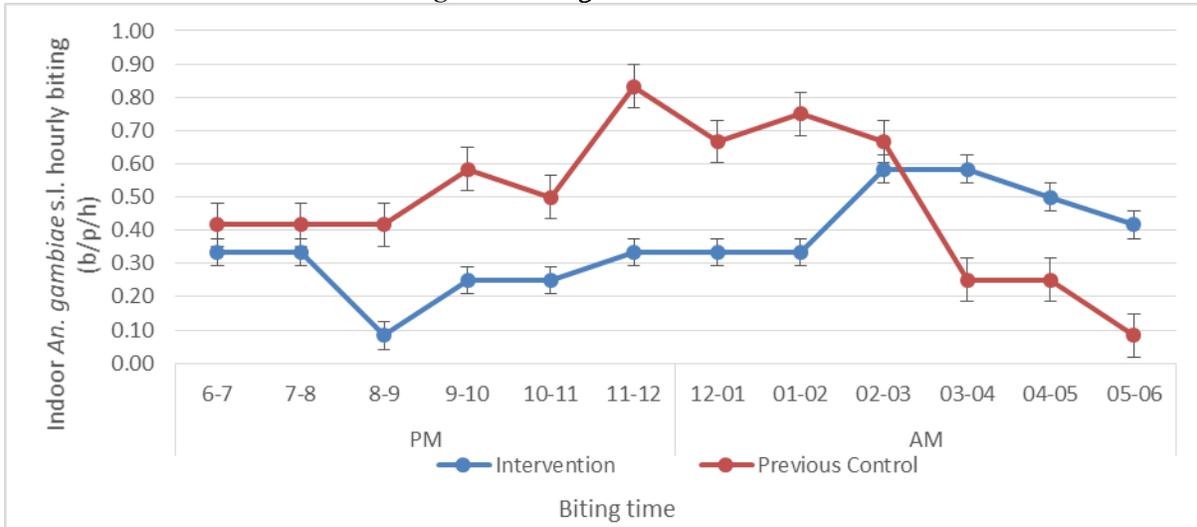
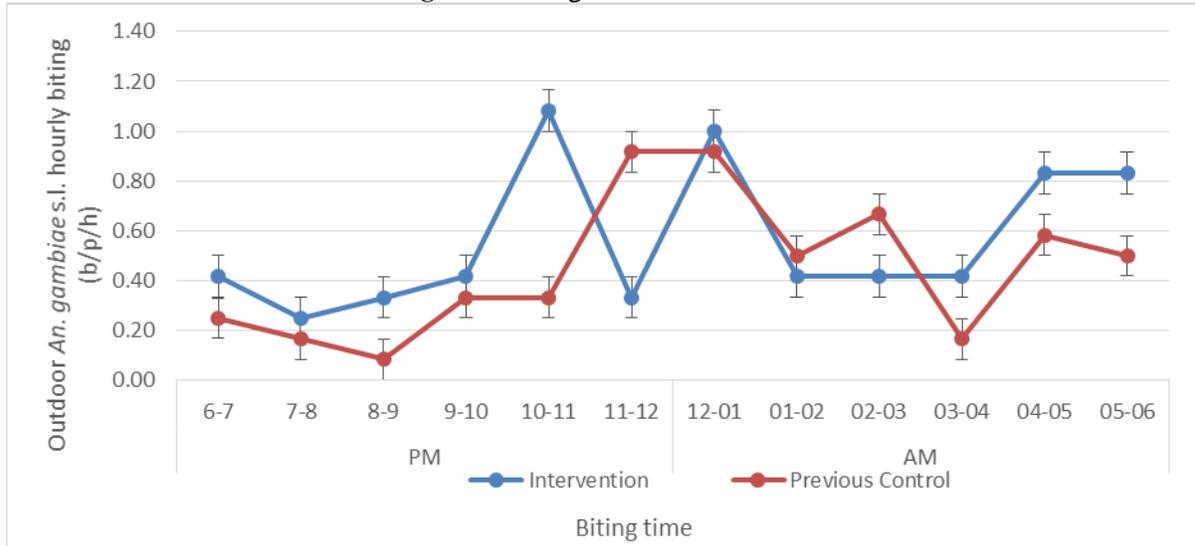


Figure 6D. *An. gambiae* s.l. Outdoor



### 3.1.4 CDC LIGHT TRAP

The CDC light trap collections yielded a total of 1,689 *Anopheles* mosquitoes from two intervention districts (Milange and Maganja da Costa, excluding Mopeia) and the control District. Morphological identification of the mosquitoes revealed that 1,453 (86.03%) were *An. funestus* s.l., 229 (13.56%) were *An. gambiae* s.l., 4 (0.24%) were *An. coustani*, 2 (0.12%) were *An. ziemanni*, and 1 (0.06%) were *An. tenebrosus*. Lugela (control) had the highest percentage of all *An. funestus* s.l. collected, 72.11%, followed by Milange at 8.53%.

Table 9 provides a summary of CDC light trap data from monthly collection in the three districts. Comparing mosquito densities between control and intervention districts, the data demonstrate a significant difference in *An. funestus* s.l. between vector densities recorded in control (Lugela) and intervention areas ( $p < 0.05$  was obtained with a  $X^2$  of 5.88, and 6.74 for Milange and Maganja da Costa, respectively), while for *An. gambiae* s.l. the data showed no significant difference on the mean densities ( $p > 0.05$  and  $X^2$  of 0.35 and 0.46 for Milange and Maganja da Costa).

**TABLE 9. SUMMARY OF CDC LIGHT TRAP DATA FROM MONTHLY COLLECTION IN THREE DISTRICTS, ZAMBEZIA**

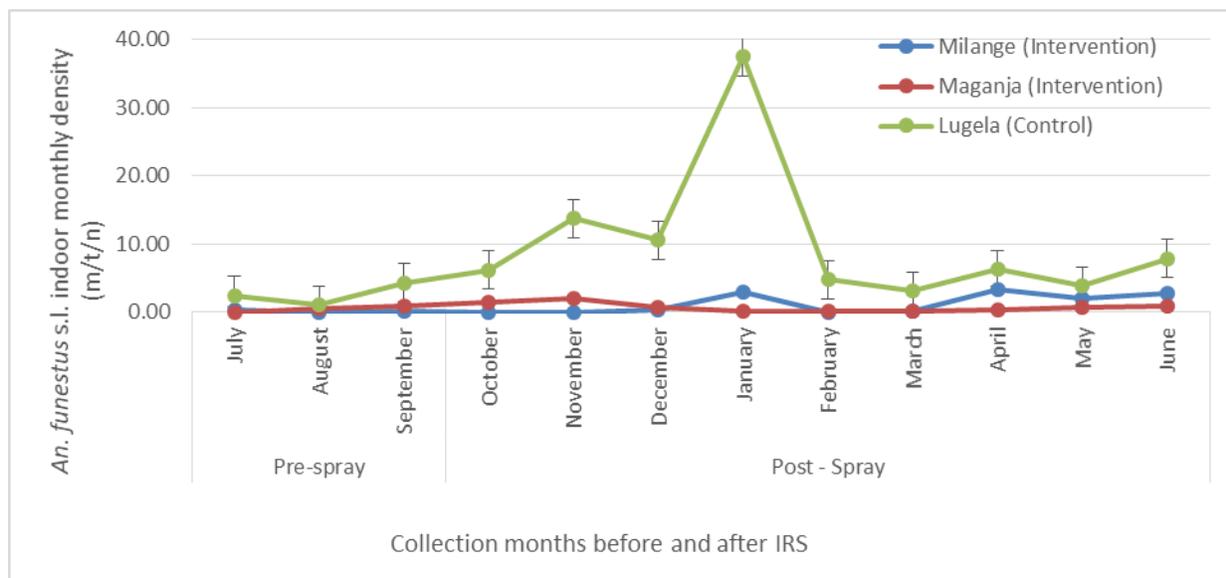
Districts	Species	2018						2019						Total	
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June		
Milange	<i>An. funestus</i> s.l.	4	0	1	0	0	4	36	0	1	40	24	34	144	191
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.33	0.00	0.08	0.00	0.00	0.33	3.00	0.00	0.08	3.33	2.00	2.83	1.00	
	<i>An. gambiae</i> s.l.	0	0	0	0	0	6	18	0	0	12	10	1	47	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.00	0.00	0.00	0.00	0.00	0.50	1.50	0.00	0.00	1.00	0.83	0.08	0.33	
Maganja	<i>An. funestus</i> s.l.	0	6	11	17	24	8	1	1	1	3	8	11	91	127
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.00	0.50	0.92	1.42	2.00	0.67	0.08	0.08	0.08	0.25	0.67	0.92	0.63	
	<i>An. gambiae</i> s.l.	0	3	4	0	3	3	4	0	1	9	6	3	36	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.00	0.25	0.33	0.00	0.25	0.25	0.33	0.00	0.08	0.75	0.50	0.25	0.25	
Lugela	<i>An. funestus</i> s.l.	29	12	52	74	165	127	450	57	37	75	46	94	1218	1364
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	2.42	1.00	4.33	6.17	13.75	10.58	37.50	4.75	3.08	6.25	3.83	7.83	8.46	
	<i>An. gambiae</i> s.l.	1	0	1	1	2	3	23	7	20	77	5	6	146	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.08	0.00	0.08	0.08	0.17	0.25	1.92	0.58	1.67	6.42	0.42	0.50	1.01	
<b>Total</b>		<b>34</b>	<b>21</b>	<b>69</b>	<b>92</b>	<b>194</b>	<b>151</b>	<b>532</b>	<b>65</b>	<b>60</b>	<b>216</b>	<b>99</b>	<b>149</b>	<b>1682</b>	

Table 9 also shows that, in terms of mean collections, *An. funestus* s.l., at 8.4 mosquitoes per trap per night (m/t/n) over the 12 collection months, was most abundant in Lugela control district, followed by 1.0 m/t/n in Milange.

Table 9, as well as Figures 7A and 7B, show clearly that before IRS, CDC light traps recorded low *An. gambiae* s.l. densities estimated at less than 0.5 m/t/n in both intervention and control districts. *An. funestus* s.l. were more than two to eight-fold higher, estimated at 4.33 m/t/n in Lugela and 0.92 m/t/n in Maganja da Costa and Milange. Following IRS, densities of *An. funestus* s.l. remained low (<3.4 m/t/n) in intervention districts through the rest of the year. As expected, there was a steady increase in *An. funestus* s.l. densities in Lugela, in January 2019 reaching a peak estimated at 37.5 m/t/n and thereafter dropping sharply to less than 8.0 m/t/n from February to June 2019. Likewise, *An. gambiae* s.l. densities remained low in intervention districts with a slight increase to 1.50 m/t/n observed in January 2019, and thereafter a decrease to 0.25 m/t/n in June 2019.

**FIGURE 7. INDOOR CDC LIGHT TRAP DENSITY PER TRAP PER NIGHT FOR *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN MILANGE, MAGANJA DA COSTA, AND LUGELA DISTRICTS**

**Figure 7A. *An. funestus* s.l.**



**Figure 7B. *An. gambiae* s.l.**

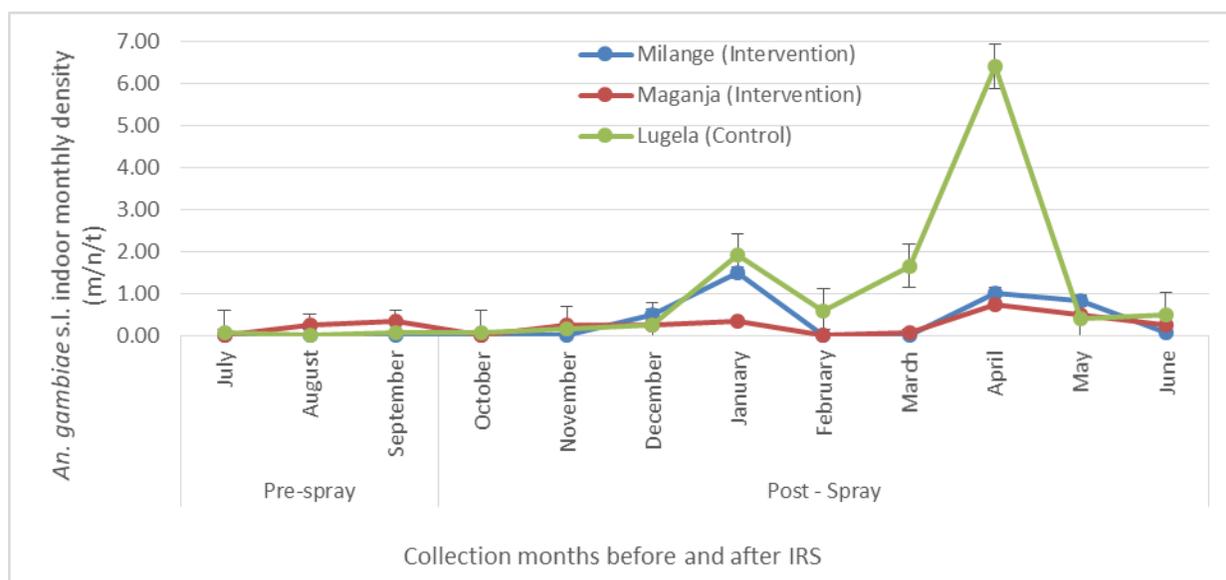


Table 10 shows that in Mopeia District, CDC light traps caught a total of 11,468 *Anopheles* mosquitoes, of which 7,097 (61.88%) were collected in previous control areas and 4,371 (38.11%) in intervention areas. *An. funestus* s.l. was the most abundant mosquito species, accounting for 10,204 (88.98%) mosquitoes collected, followed by *An. gambiae* s.l. accounting for 1,173 (10.23%) mosquitoes collected. Other species caught, in low numbers, were *An. tenebrosus* 40 (0.35%), *An. squamosus* 23 (0.20%), *An. ziemanni* 17 (0.15%), *An. pharoensis* 4 (0.03%), *An. coustani*, 3 (0.03%), *An. rufipes* 2 (0.02%), *An. pretoriensis* 1 (0.01%), and *An. dancallicus* 1 (0.01%). Peak densities for *An. funestus* s.l. were observed from April to June 2019 in both intervention (12.9 m/t/n) and previous control areas (9.4 m/t/n).

Table 10 also shows that the Mopeia intervention area yielded fewer anopheline mosquitoes than the previous control area did. A statistical test for observed differences in densities of the two major vectors shows the

estimates for *An. funestus* s.l. ( $X^2 = 760.66$ ;  $p = 1.9 \times 10^{-167}$ ) and *An. gambiae* s.l. ( $X^2 = 4.30$ ;  $p = 0.038168$ ) as statistically significant.

**TABLE 10. ANOPHELINE SPECIES COLLECTED BY CDC LIGHT TRAPS IN MOPEIA DISTRICT**

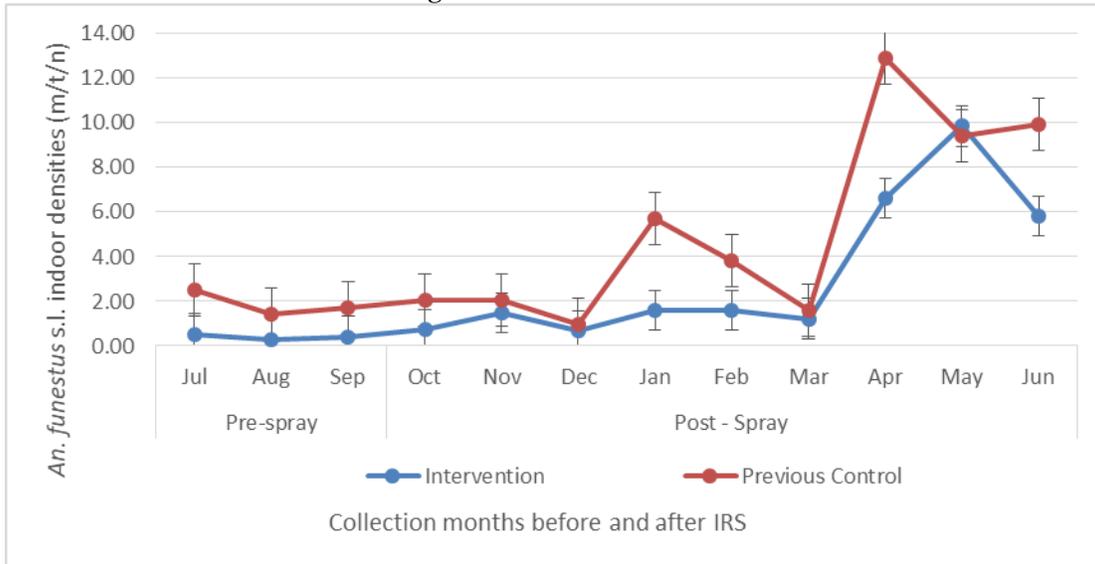
District	Species	Total Collection per Month												Total	Proportion (%)	
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun			
Mopeia (Previous control)	<i>An. funestus</i> s.l.	302	174	207	245	246	115	687	458	191	1549	1,129	1,192	6,495	7,097	61.89
	<i>An. gambiae</i> s.l.	4	0	0	36	34	37	44	41	19	275	36	25	551		
	<i>An. tenebrosus</i>	3	3	0	0	0	0	0	0	2	5	3	0	16		
	<i>An. squamosus</i>	0	0	0	0	0	0	0	0	13	4	0	1	18		
	<i>An. ziemanni</i>	0	0	0	0	0	0	0	0	0	7	0	5	12		
	<i>An. pharoensis</i>	0	0	0	0	0	0	0	0	1	1	0	0	2		
	<i>An. coustani</i>	0	0	0	0	0	0	0	0	0	0	0	0	0		
	<i>An. rufipes</i>	0	0	0	0	0	0	0	0	0	2	0	0	2		
	<i>An. pretoriensis</i>	0	0	0	0	0	0	0	0	0	0	0	1	1		
	<i>An. danallicus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0		
Mopeia (Intervention)	<i>An. funestus</i> s.l.	64	37	51	90	180	80	191	192	147	793	1183	701	3,709	4,371	38.11
	<i>An. gambiae</i> s.l.	3	4	1	22	49	5	38	61	30	325	54	30	622		
	<i>An. tenebrosus</i>	2	11	0	0	0	0	1	0	7	2	1	0	24		
	<i>An. squamosus</i>	0	0	0	0	0	0	0	0	3	2	0	0	5		
	<i>An. ziemanni</i>	0	0	0	0	0	0	0	0	0	5	0	0	5		
	<i>An. pharoensis</i>	0	0	0	0	0	0	0	0	1	1	0	0	2		
	<i>An. coustani</i>	0	0	0	0	0	0	0	0	0	2	0	1	3		
	<i>An. rufipes</i>	0	0	0	0	0	0	0	0	0	0	0	0	0		
	<i>An. pretoriensis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0		
	<i>An. danallicus</i>	0	0	0	0	0	0	0	0	1	0	0	0	1		
Total	378	229	259	393	509	237	961	752	415	2973	2406	1956	11,468			

The *An. gambiae* s.l. population demonstrated consistently low densities (below 0.51 m/t/n) in both intervention and control areas, except in April 2019, when its density increased slightly to around 2.7 m/t/n both in intervention and previous control areas (Figures 8A and 8B).

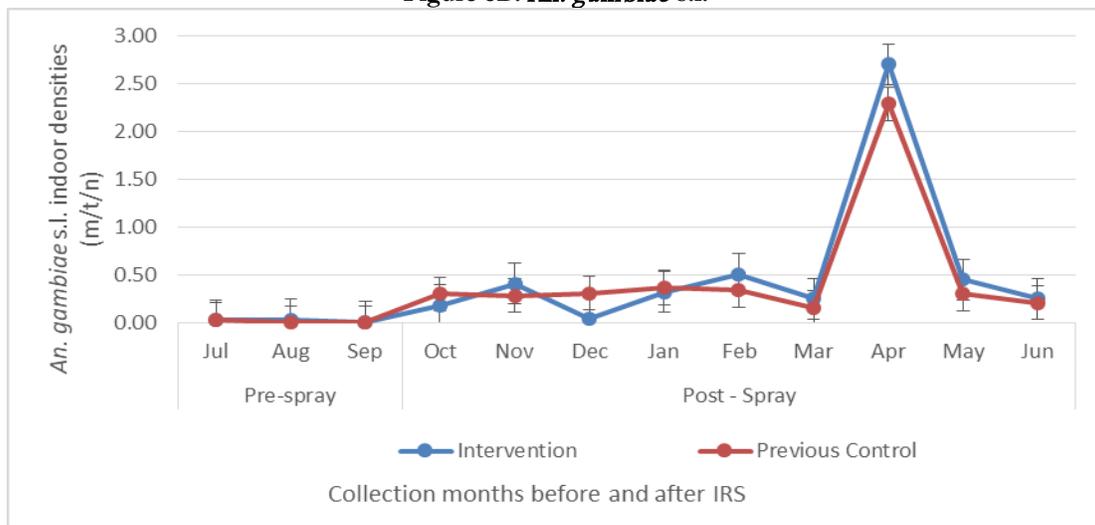
On the other hand *An. funestus* s.l. was collected in much higher densities up to three to four times the densities of *An. gambiae* s.l. in intervention and previous control areas, respectively. *An. funestus* s.l. densities in intervention areas remained below 1.6 m/t/n over most of the period from July 2018 to March 2019, increasing steadily to a peak of 9.6 m/t/n in May 2019. In previous control areas, it remained below 2.52 m/t/n from July to December 2018, rising to small peak at 5.73 m/t/n in January 2019, thereafter decreasing to 1.59 m/t/n in March 2019. This was followed by a sharp rise to a peak April 2019, estimated at 12.92 m/t/n.

**FIGURE 8. INDOOR CDC LIGHT TRAP DENSITY OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. PER TRAP PER NIGHT IN MOPEIA**

**Figure 8A. *An. funestus* s.l.**



**Figure 8B. *An. gambiae* s.l.**



## 3.2 CONE WALL BIOASSAY TESTS

During spray operations in October 2018, cone wall bioassays were conducted to measure the quality of the spray starting 24 hours after spray. Thereafter, monthly assays were performed to monitor the insecticide decay rate on various sprayed wall surfaces. Results of the quality assurance and decay rate monitoring of SumiShield® 50WG (clothianidin 500G) in Mopeia and Actellic® 300CS in Maganja da Costa and Milange, districts are shown below in Section 3.2.2 (Figures 9 and 10, respectively).

### 3.2.1 QUALITY OF SPRAYING

For SumiShield® 50WG mortality scored at T<sub>0</sub> was 100% in all houses tested with cone wall bioassays one day after spraying.

For Actellic® 300CS, in all sites tested in all five intervention districts, 24-hour mortality was scored at 99% to 100%. (PMI-VectorLink Mozambique 2018 Spray Quality Assessment Report, October 2018)

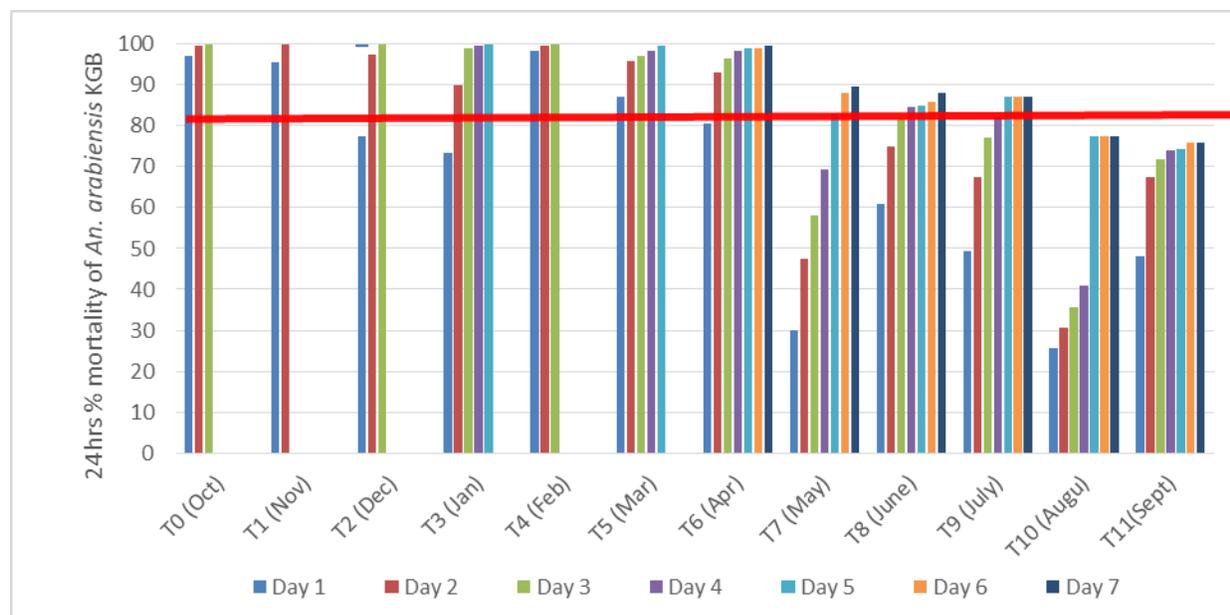
Bioassay results for assessing the quality of spraying exhibited high mortalities ranging from 99% to 100% of female *An. arabiensis* KGB strain upon exposure to all three types of sprayed surfaces. As expected for Actellic® 300CS (pirimiphos-methyl), low levels of knockdown were observed 60 minutes post-exposure to almost all sprayed substrates, whereas SumiShield® (clothianidin) elicited high knockdowns at 60 minutes post-exposure. SumiShield® demonstrated its typical slow-acting characteristic where mosquitoes were observed to survive up to 72 hours after exposure, at which 100% mortality was recorded. The results obtained from these wall assays strongly suggest that the spray teams were skilled in applying the insecticide uniformly, resulting in high 24- and 72-hour mortalities for Actellic® and SumiShield®, respectively.

### 3.2.2 INSECTICIDE DECAY RATE

#### SUMISHIELD® 50WG DECAY RATE

Baseline cone wall bioassays for assessing SumiShield® 50WG IRS quality and subsequent monitoring of its decay rate was conducted in 24 de Julho village in Mopeia. Baseline denoted as T<sub>0</sub> was conducted in October 2019, eliciting a 100% mortality by day 3 post exposure (Figure 9). Subsequent monthly cone bioassays resulted in more than 80% mortality up to month 10 post spray. Overall mortality below 80% was first observed in month 10. It was also noted that scores of 100% were observed up to six months post IRS. However, there was a notable increase in the number of days when 100% mortality was achieved, from three days during the first three months to five days by the fourth month and up to seven days by the seventh month. This is presumably due to decreasing efficacy of the insecticide deposits on the sprayed surface with time. These results show that SumiShield® 50WG remained efficacious up to nine months post spray.

**FIGURE 9: SPRAY QUALITY ASSESSMENT AND RESIDUAL BIOEFFICACY OF SUMISHIELD® 50WG (CLOTHIANIDIN 50WG)**



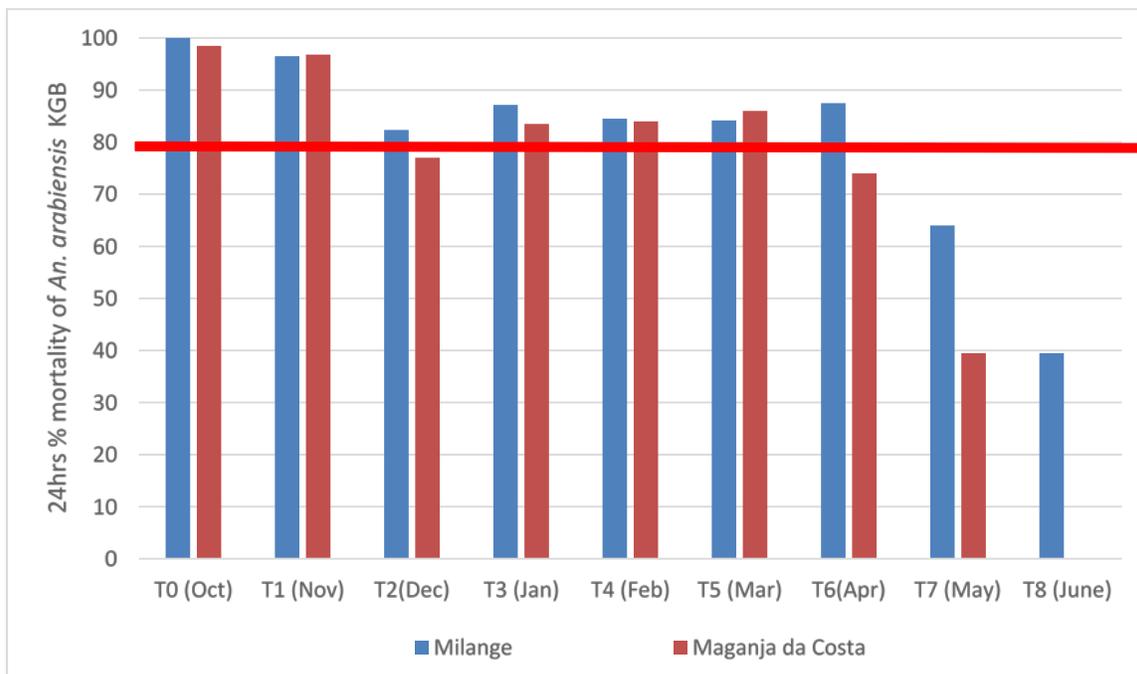
Red line indicates the 80% mortality cut-off point.

#### ACTELIC® 300CS DECAY RATE

Baseline cone wall bioassays for assessing Actellic® 300CS IRS quality and subsequent monitoring of its decay rate were conducted in Milange and Maganja da Costa. Baseline (T<sub>0</sub>) was conducted in October 2019, eliciting 100% mortality in Milange and 98.5% mortality in Maganja da Costa (Figure 10). Subsequent cone

bioassays observed a first drop in mortality, to 77%, in Maganja da Costa two months post spray ( $T_2$ ), followed immediately by a recovery in month three ( $T_3$ ) to 83% mortality. A subsequent drop below the cut-off point to 74% was observed six months ( $T_6$ ) post spray, persisting during month seven and therefore calling for termination on monitoring. In Milange, a first drop in mortality below the cut-off point to 68% was observed seven months post spray and persisted in month eight, calling for termination of monitoring. These results show that Actellic® 300CS remained efficacious up to five and six months post spray in Maganja da Costa and Milange, respectively.

**FIGURE 10: SPRAY QUALITY ASSESSMENT AND RESIDUAL BIOEFFICACY OF ACTELLIC® 300CS**



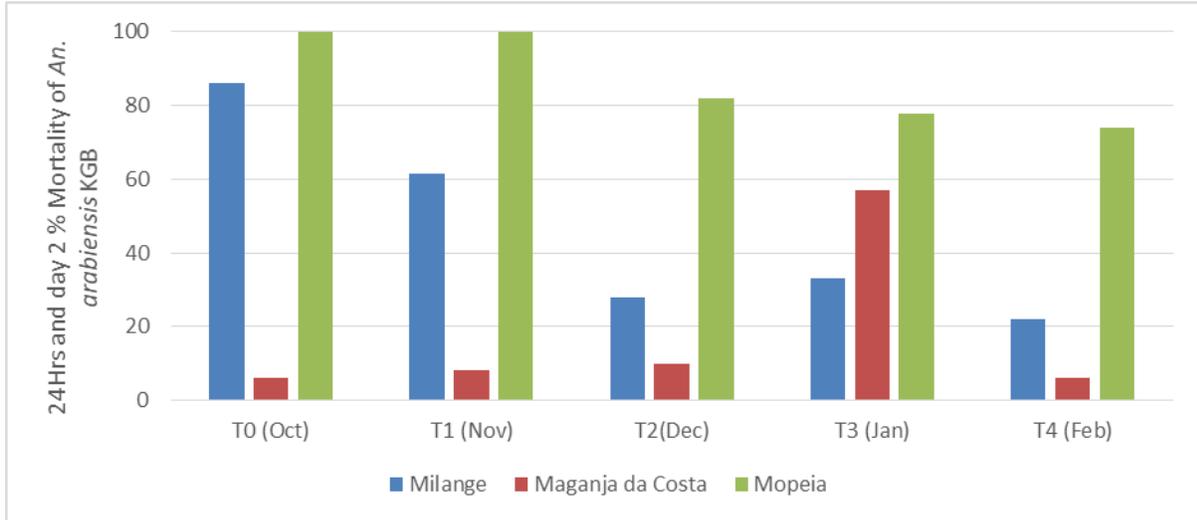
Red line indicates the 80% mortality cutoff point.

### 3.2.3 THE AIRBORNE FUMIGANT EFFECT

Figure 11 uses bioassay data to illustrate the airborne effect of the insecticides. The airborne fumigant effect of Actellic® 300CS was found to be low in Maganja da Costa producing only 6% mortality 24 hours post exposure, while a much higher score, 86%, was recorded in Milange at  $T_0$ . In contrast, SumiShield® 50WG demonstrated an extremely high effect, scored at 96% mortality at 24 hours post-exposure and 100% at 48 hours.

The low mortalities observed with airborne fumigant effect assays with Actellic® 300CS suggest low levels of fumigant effect in the sprayed houses, ostensibly due to low vapor pressure of the insecticide microencapsulated formulation. The reasons for the wide margin between the observations in Maganja and Milange is yet to be established. The different findings in houses sprayed with SumiShield® 50WG suggest a high airborne effect of the formulation.

**FIGURE 11. PERCENT MORTALITY OF AN. ARABIENSIS KGB SUSCEPTIBLE STRAIN ON AIRBORNE FUMIGANT EFFECT TEST AGAINST ACTELIC® 300CS IN MILANGE AND MAGANJA DA COSTA OR SUMISHIELD® 50WG IN MOPEIA**

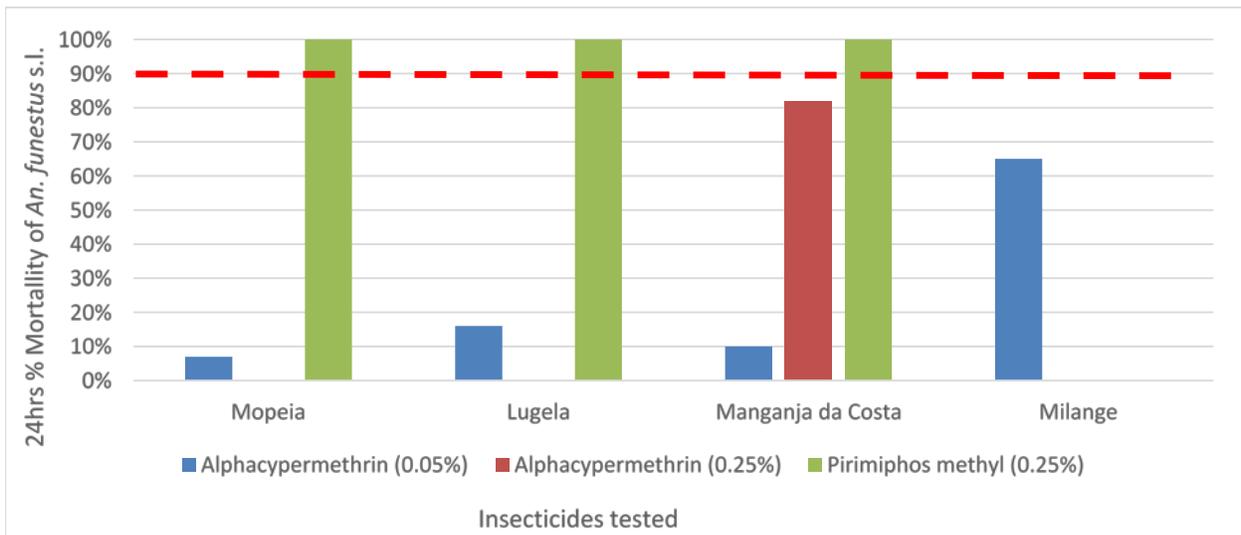


### 3.3 WHO SUSCEPTIBILITY TESTING

Susceptibility testing was conducted in August to September 2018 in Derre, Maganja da Costa, Milange, Molumbo, Morrumbala, Mopeia, and Lugela districts. Tests conducted in Derre and Lugela could not cover all planned target insecticides due to a shortage of mosquitoes in the field.

Adult *An. funestus* s.l. were collected indoors using Prokopac aspirators and immediately tested in Mopeia, Lugela, Maganja da Costa, and Milage. These were exposed to diagnostic dosages of pirimiphos-methyl in Maganja da Costa, Mopeia, and Lugela and found to be fully susceptible to the product. Resistance was detected against alpha-cypermethrin in Maganja da Costa, Lugela, and Milange. In Maganja da Costa, the strength of resistance was explored against *An. funestus* s.l. with a 5× discriminating concentration and found to be of moderate to high resistance intensity (Figure 12).

**FIGURE 12: 24-HOUR MORTALITY FROM THE WHO TUBE TESTS OF ADULT AN. FUNESTUS S.L. COLLECTED BY PROKOPACK COLLECTIONS**

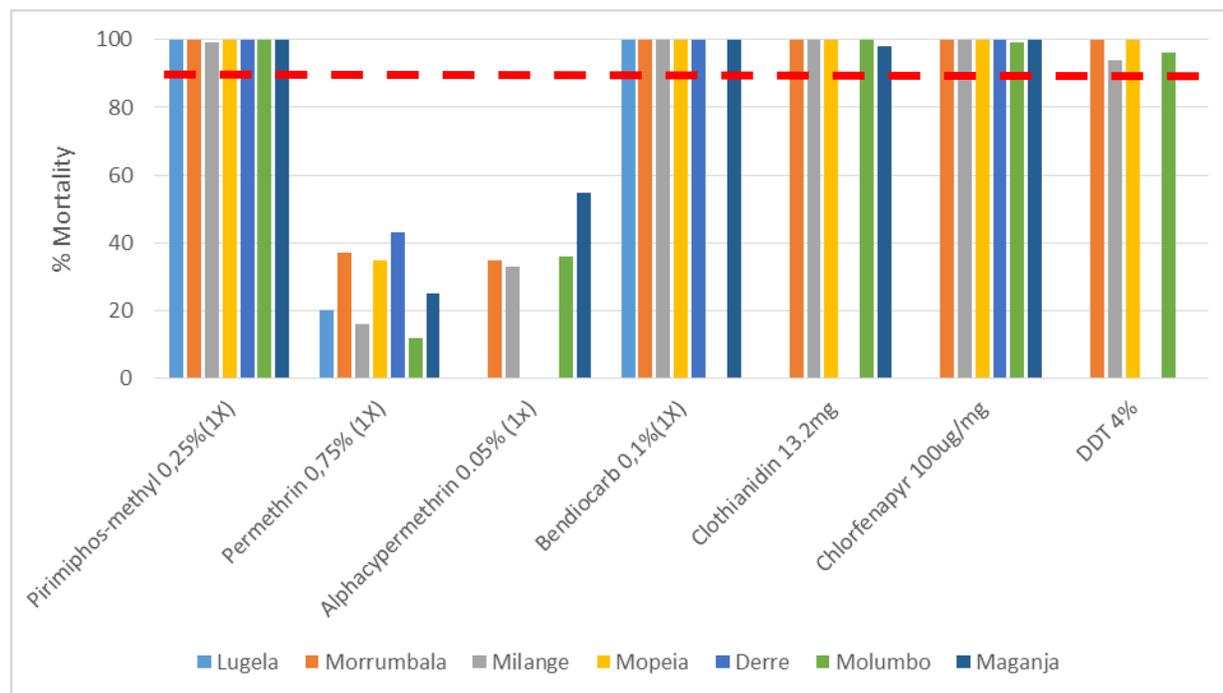


Red line indicates cut-off mortality (90%) below which a vector population is considered resistant.

Susceptibility tests of *An. gambiae* s.l. were conducted from January through to April 2019 in Lugela, Morrumbala, Milange, Mopeia, Derre, Molumbo, and Maganja da Costa, by exposing them to diagnostic dosages of pirimiphos-methyl, DDT, bendiocarb, clothianidin, chlorfenapyr, permethrin, and alpha-cypermethrin,

The mortality results presented in Figure 13 show that *An. gambiae* s.l. in all districts tested were fully susceptible to pirimiphos-methyl. *An. gambiae* s.l. exposed to DDT were susceptible in Morrumbala and Mopeia. Possible resistance to DDT was detected among the vectors in Milange and Molumbo. Exposure to bendiocarb revealed full susceptibility in Lugela, Morrumbala, Milange, Mopeia, Derre, and Maganja. Likewise, full susceptibility to clothianidin was demonstrated in Morrumbala, Milange, Mopeia, and Molumbo. Vectors in Morrumbala, Milange, Mopeia, Derre, Molumbo, and Maganja da Costa demonstrated full susceptibility to chlorfenapyr. Resistance to permethrin was detected in all seven districts and to alpha-cypermethrin in Morrumbala, Milange, Molumbo, and Maganja da Costa.

**FIGURE 13: 24-HOUR MORTALITY OR 48–72 HOUR MORTALITY OF ADULT *AN. GAMBIAE* S.L. RAISED FROM LARVAL COLLECTIONS EXPOSED TO A RANGE OF INSECTICIDES AT RESPECTIVE DIAGNOSTIC CONCENTRATIONS**



— — Red line indicates mortality below 90% are resistance mosquito

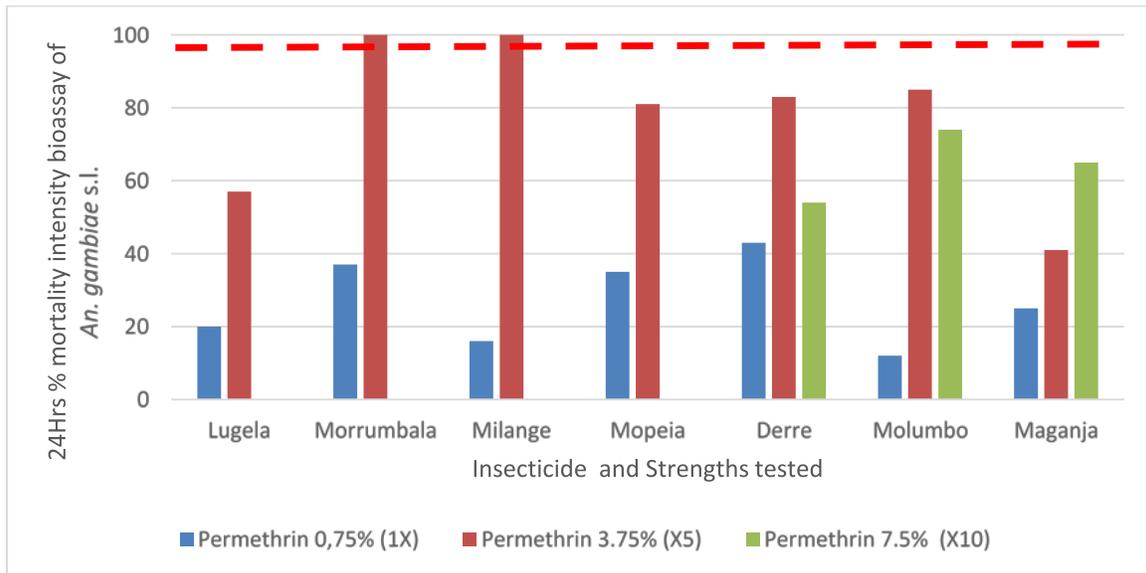
24 hour Mortality for Pirimiphos-methyl, Permethrin, Alphacypermethrin, Bendiocarb, and DDT  
48 – 72 hours Mortality for Clothianidin and Clorfenapyr

### 3.3.1 DETERMINING THE INTENSITY OF RESISTANCE AND SYNERGIST ASSAYS USING WHO TUBE TESTS

Bioassays for intensity of resistance were conducted where *An. gambiae* s.l. resistance was detected in discriminating concentrations (24 hrs mortality <90%) of the respective insecticides. Resistance intensity is considered to be of low intensity if < 98% mortality at 1×diagnostic dose; moderate intensity if <98% mortality at 5× diagnostic dose and high intensity if <98% mortality at 10× dose (WHO, 2016)

Figure 14 shows results of exposure to 5× permethrin indicating the presence of moderate intensity resistance in Lugela and Mopeia districts, where mortality was scored at less than 98%. Further exposure to 10× permethrin revealed the presence of high-intensity resistance in Derre, Molumbo, and Maganja da Costa, where mortalities were scored as less than 98%. Presence of medium to high intensity resistance suggests the need or importance of next generation IRS or insecticide-treated bednets for malaria vector control in the areas.

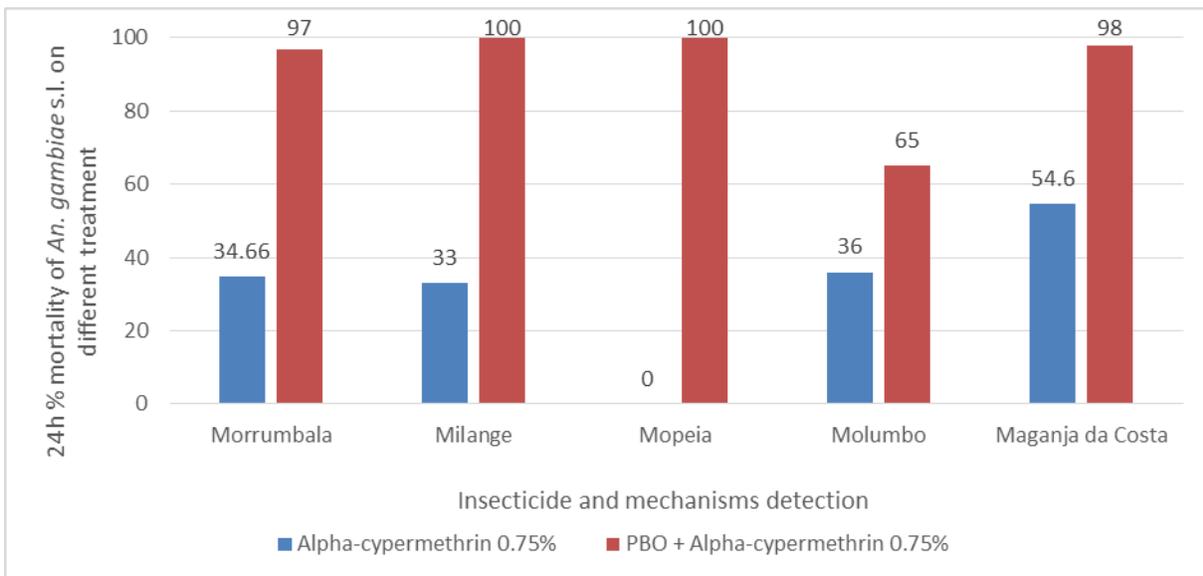
**FIGURE 14. TO SHOW 24-HOUR MORTALITY OF ADULT *AN. GAMBIAE* S.L. FOLLOWING EXPOSURE TO INCREASING MULTIPLES OF RESPECTIVE DIAGNOSTIC CONCENTRATIONS UP TO  $\times 10$**



Red line indicates mortality cut-off of 98% for intensity of resistance.

Figure 15 summarizes the results of synergist assays on *An. gambiae* s.l. from Morrumbala, Milange, Mopeia, Molumbo, and Maganja da Costa. The synergist PBO restored full susceptibility to alpha-cypermethrin, estimated as  $\geq 98\%$  mortality, up from 33% to 100% in Milange, from 0% to 100% in Mopeia, and from 54.6% to 98% in Maganja da Costa. Partial restoration, from 34.6% to 97%, was observed in Morrumbala and from 36% to 65% in Molumbo. This observation suggests that monooxygenases are the only form of metabolic resistance mechanisms prevailing in Milange, Mopeia, and Maganja da Costa and possibly a combination of monooxygenases and other mechanisms in Morrumbala and Molumbo.

**FIGURE 15: SYNERGIST ASSAY MORTALITY RESULTS IN *AN. GAMBIAE* S.L. FROM FIVE INTERVENTION DISTRICTS**



# 4. RESULTS: NAMPULA

## 4.1 ANOPHELINE SPECIES COLLECTED BY DIFFERENT METHODS

In the three participating districts of Nampula Province (intervention districts of Nampula city and Monapo and control district of Erati), a total of 3,778 anophelines belonging to five species and species complexes were collected using the three collection methods (PSC, CDC light traps, and HLC) and morphologically identified. Table 11 below provides a summary of the number of mosquitoes collected, by district and species, during the reporting period.

**TABLE 11. NUMBER OF MOSQUITOES COLLECTED IN EACH DISTRICT BY ALL THREE COLLECTION METHODS**

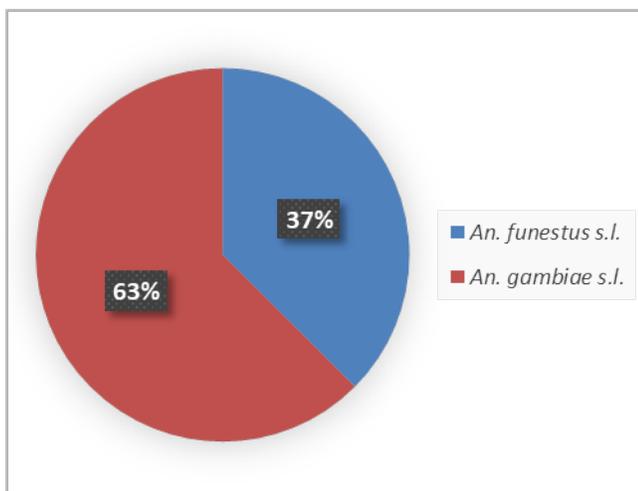
Species Collected	Erati	Nampula City	Monapo	Total per Species
<i>An. funestus</i> s.l.	341	891	181	1413
<i>An. gambiae</i> s.l.	988	926	445	2359
<i>An. coustani</i>	0	2	0	2
<i>An. pretoriensis</i>	0	0	1	1
<i>An. rufipes</i>	0	3	0	3
<b>Total</b>	<b>1329</b>	<b>1822</b>	<b>627</b>	<b>3,778</b>

Figure 16 illustrates the proportions of the two major species collected, *An. gambiae* s.l. (63%) and *An. funestus* s.l. (37%). As Table 11 shows, other species (*An. coustani*, *An. rufipes*, and *An. pretoriensis*) were collected in numbers too small to include in the figure.

### 4.1.1 PYRETHRUM SPRAY COLLECTION

PSC conducted in the three districts yielded mainly *An. gambiae* s.l. and *An. funestus* s.l. as determined by morphological identification. The total collection of 1,035 *Anopheles* mosquitos broke down to 536 (51.79%) belonging to the *An. funestus* s.l. group and 499 (48.21%) to *An. gambiae* s.l. Table 12 shows the numbers of each species collected by PSC.

**FIGURE 16: PROPORTION OF MAJOR SPECIES OF ANOPHELES MOSQUITOES COLLECTED BY ALL COLLECTION METHODS**



**TABLE 12. NUMBER OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING PSC, BY DISTRICT**

Species	District			Total
	Erati	Monapo	Nampula	
<i>An. funestus</i> s.l.	65	63	408	536
<i>An. gambiae</i> s.l.	143	183	173	499
<b>Total</b>	<b>208</b>	<b>246</b>	<b>581</b>	<b>1,035</b>

As Figures 17A and 17B show, indoor resting density established by PSC sampling was very low in both intervention and control sites for both *An. funestus* s.l. (<4.8 m/h/d) and *An. gambiae* s.l. (<2.5 m/h/d). These low indoor resting densities were found at most of the collection sites before and after IRS intervention. Surprisingly, the densities in the control site were found to be lower than in the intervention sites for both species, before and after spraying.

**FIGURE 17. INDOOR RESTING DENSITIES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. ESTIMATED USING PSC, BY DISTRICT**

Figure 17A: *An. funestus* s.l.

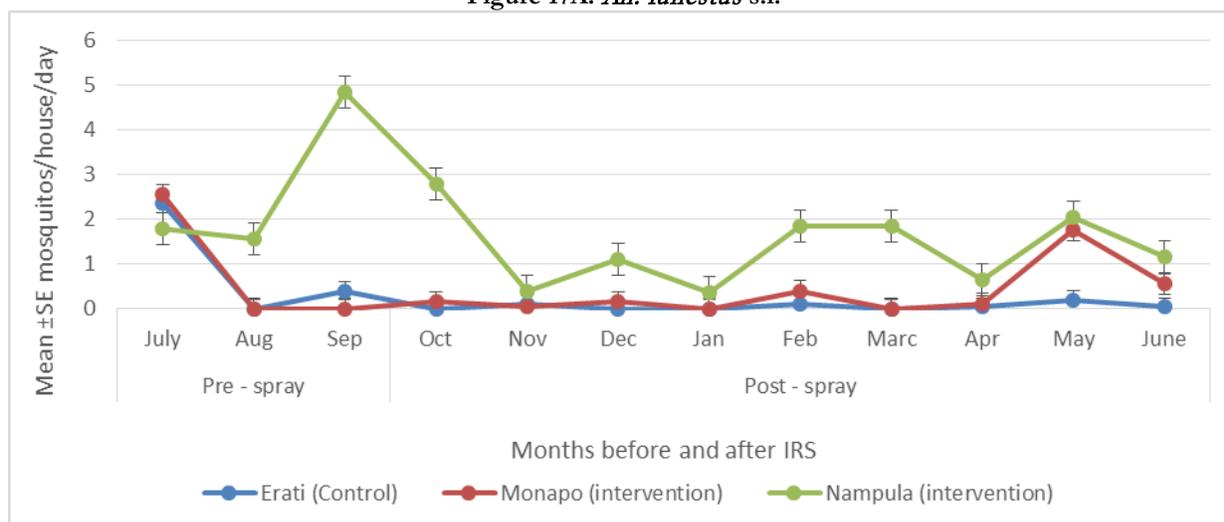
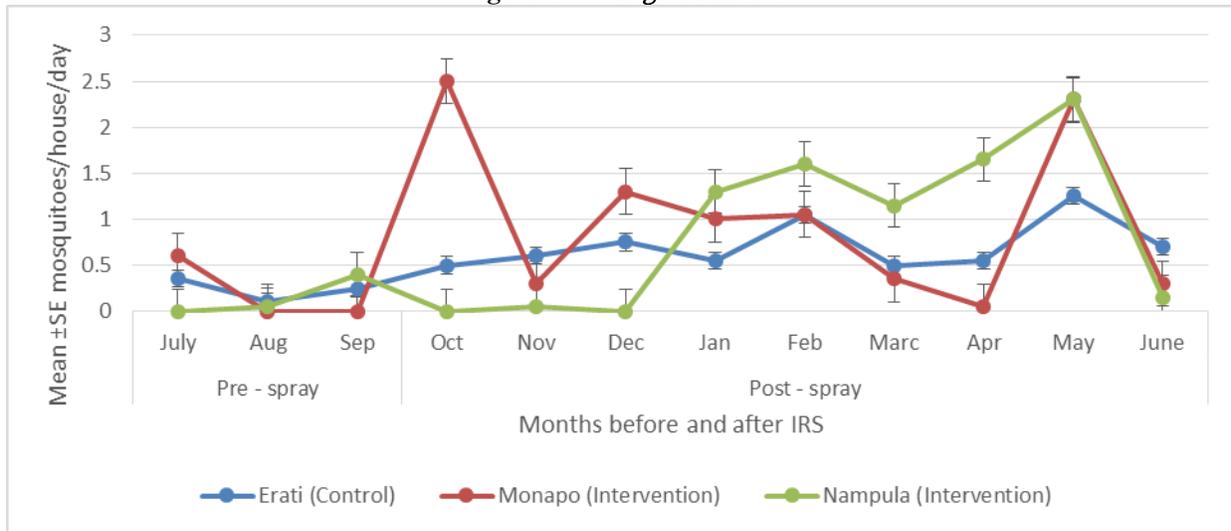


Figure 17B: *An. gambiae* s.l.



#### 4.1.2 HUMAN LANDING CATCHES

A total of 1,432 *Anopheles* mosquitoes were collected using the HLC technique from July 2018 to June 2019. The species identified morphologically from this collection were found to belong to *An. gambiae* s.l., (1,041), *An. funestus* s.l., (386), *An. coustani* (2), *An. rufipes* (2), and *An. pretoriensis* (1). Nampula City and Monapo were the districts with highest diversity of *Anopheline* mosquitos collected.

Table 13 shows that after IRS, there was a notable drop in *An. funestus* s.l. biting rate both indoors and outdoors in control and intervention districts. In contrast, *An. gambiae* s.l. biting rates were found to have increased both indoors and outdoors after spraying except for Monapo, where outdoor biting decreased. These observations were as expected because they reflect natural fluctuations in vector population densities: the *An. funestus* s.l. population tends to drop for seasonal reasons and *An. gambiae* s.l. population increased due to rains that came after spraying.

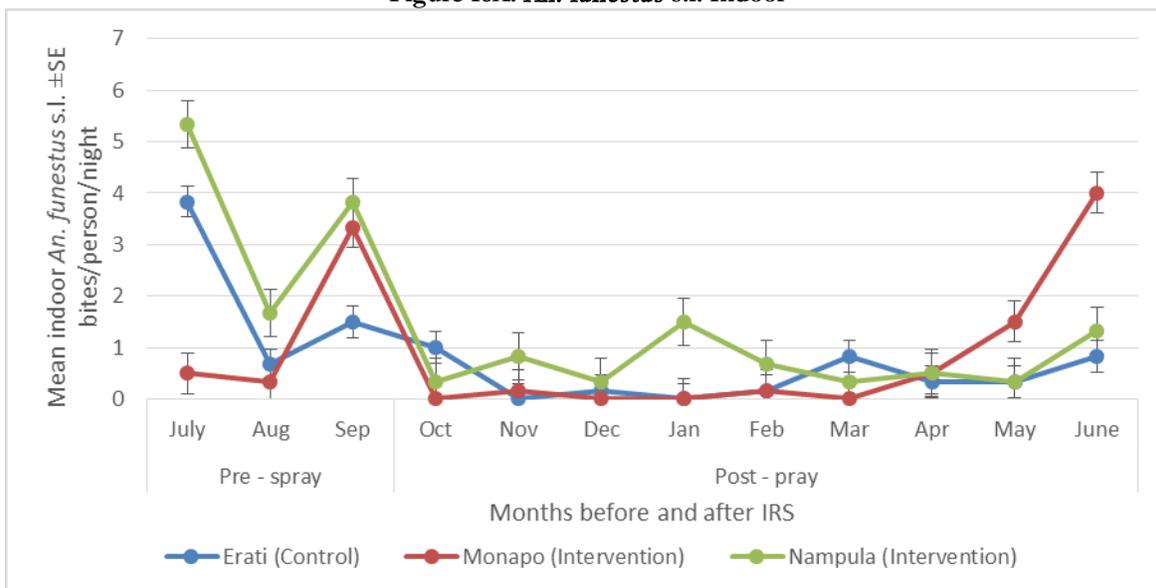
TABLE 13. INDOOR AND OUTDOOR MEAN BITING RATE FOR *FUNESTUS* S.L AND *AN. GAMBIAE* S.L., ESTIMATED USING HLC, BY DISTRICT, BEFORE AND AFTER SPRAYING

District	<i>An. funestus</i> s.l. (b/p/n)				<i>An. gambiae</i> s.l. (b/p/n)			
	Indoor		Outdoor		Indoor		Outdoor	
	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray
Erati	2	0.41	1.83	0.31	0.94	4.87	0.78	3.78
Nampula	3.61	0.98	2.56	0.78	0.33	3.44	0.11	3.94
Monapo	1.39	0.41	0.5	0.3	0.22	1.26	1.17	0.8

Figures 18A and 18B show that the *An. funestus* s.l. biting activity declined immediately after spraying both indoors and outdoors and remained well below 2.0 bites per person per night in intervention and control districts over several months, picking up in June 2019 indoors in Monapo and outdoors in Nampula City intervention districts, but with very little change in Erati control district.

**FIGURE 18. INDOOR AND OUTDOOR BITING RATES FOR *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN INTERVENTION AND CONTROL DISTRICTS, BEFORE AND AFTER IRS**

**Figure 18A: *An. funestus* s.l. Indoor**



**Figure 18B: *An. funestus* s.l. Outdoor**

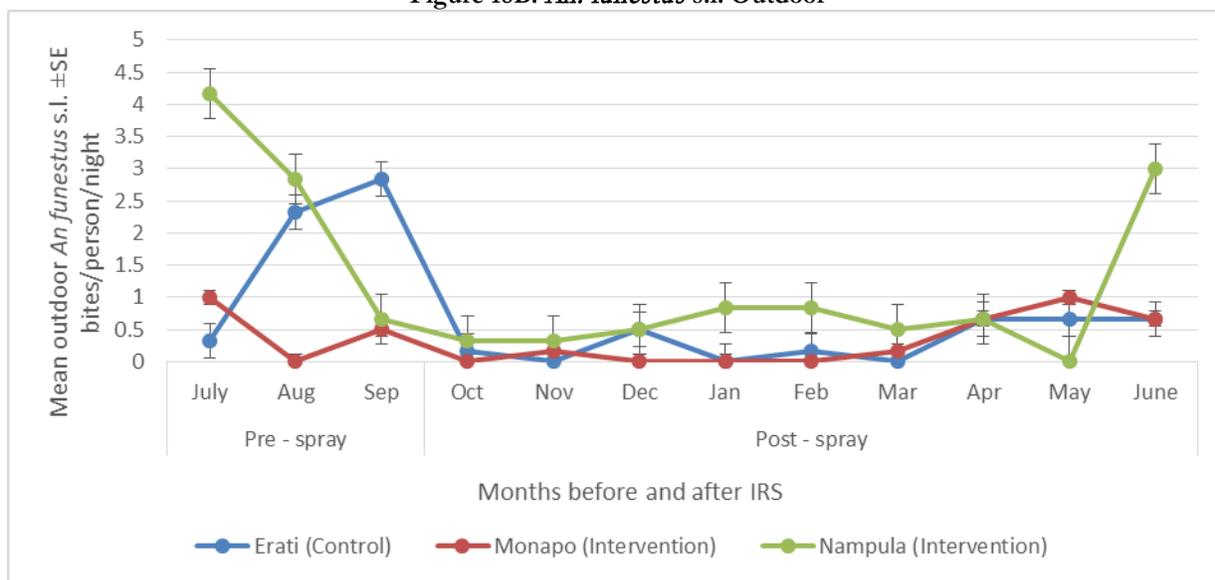


Figure 18C and 18D show an increase in *An. gambiae* s.l. indoor biting activity in all districts indoors immediately after the IRS intervention. The upsurge was sharp in Erati control district, where it reached 9.33 b/p/n in October 2018. In Nampula City, the increase was gradual, until it peaked at 8.0 b/p/n in January 2019. The increase was much less in Monapo, where the highest point was 3.5 b/p/n in January 2019, followed by a drop to less than 0.66 bites per person per night from March 2019 and beyond.

Figure 18C: *An. gambiae* s.l. Indoor

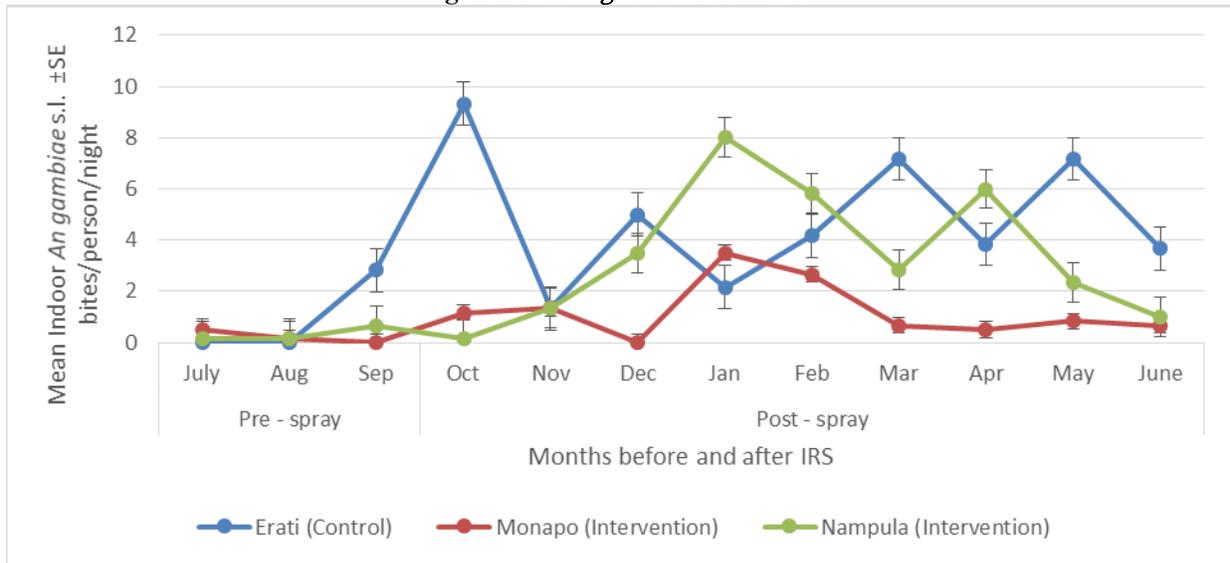


Figure 18D: *An. gambiae* s.l. Outdoor

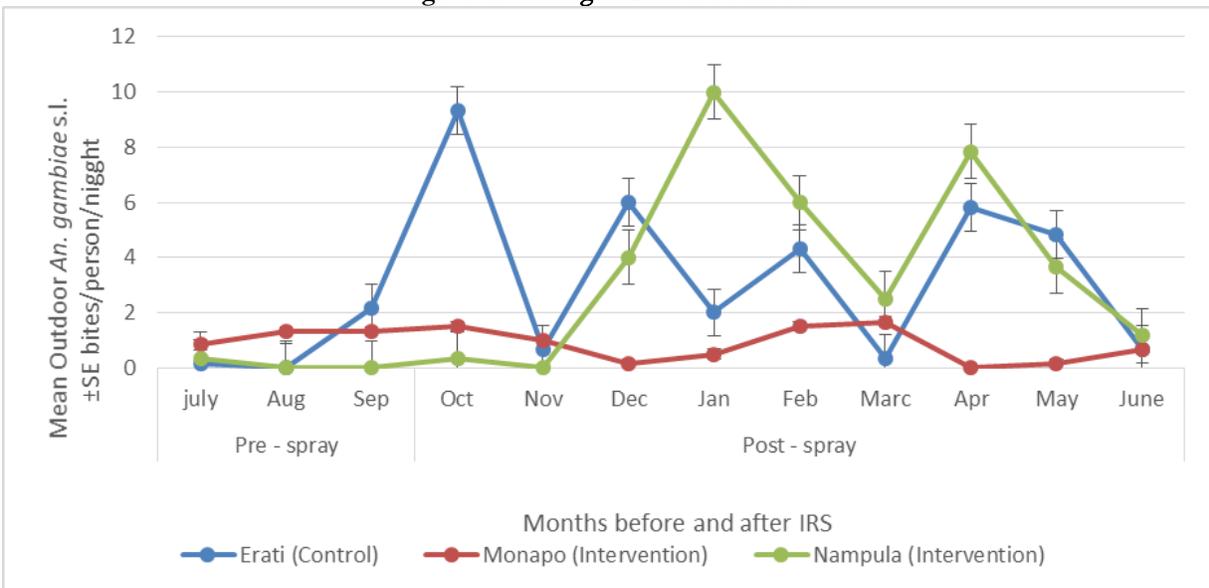


Table 14 compares the biting preferences of the two vectors in the intervention and control districts. *An. funestus* s.l. showed a significantly higher preference for indoor biting in Nampula City ( $p=0.0366$ ) and Monapo ( $p=0.0095$ ), despite the districts having been sprayed. No such preference could be observed in the unsprayed control district of Erati ( $p=0.4414$ ). *An. gambiae* s.l. demonstrated a different pattern, showing a higher preference for indoor biting in Erati ( $p=0.0055$ ). This preference was not found in Nampula City ( $p=0.2543$ ) or Monapo ( $p=0.4927$ ).

**TABLE 14. COMPARISON OF TOTAL NUMBER OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY HLC INDOORS AND OUTDOORS IN THREE DISTRICTS**

District	<i>An. funestus</i> s.l.				<i>An. gambiae</i> s.l.			
	# Collected indoors	# Collected outdoors	X <sup>2</sup>	p-value	# Collected indoors	# Collected outdoors	X <sup>2</sup>	p-value
Nampula	118	88	4.37	0.0366*	192	215	1.30	0.2543
Monapo	47	25	6.7	0.0095*	72	64	0.47	0.4927
Erati (Control)	58	50	0.5	0.4414	280	218	7.72	0.0055*

\*p – value significant

Figures 19A and 19B show the overnight biting pattern of *An. funestus* s.l. indoors and outdoors. Both indoors and outdoors, biting activity was at its lowest during the early evening hours, 6:00–8:00 pm, after which there was a steady increase in activity, especially indoors. Most indoor bites took place from 10:00 pm to 2:00am, peaking at 3.0 b/p/h in Nampula City at 10:00pm–11:00pm, at 2.67 b/p/h in Erati at 12:00 pm–1:00 am, and at 1.83 b/p/h in Monapo at 11:00pm–12:00pm. Outdoor biting in Monapo remained low (<0.5 b/p/h) throughout the night except for a small spike, estimated at 0.8 b/p/h, recorded at 10:00 pm–11:00 pm.

**FIGURE 19. HOURLY BITING RATES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. INDOOR AND OUTDOOR DETERMINED THROUGH HLCs**

Figure 19A. *An. funestus* s.l. Indoor

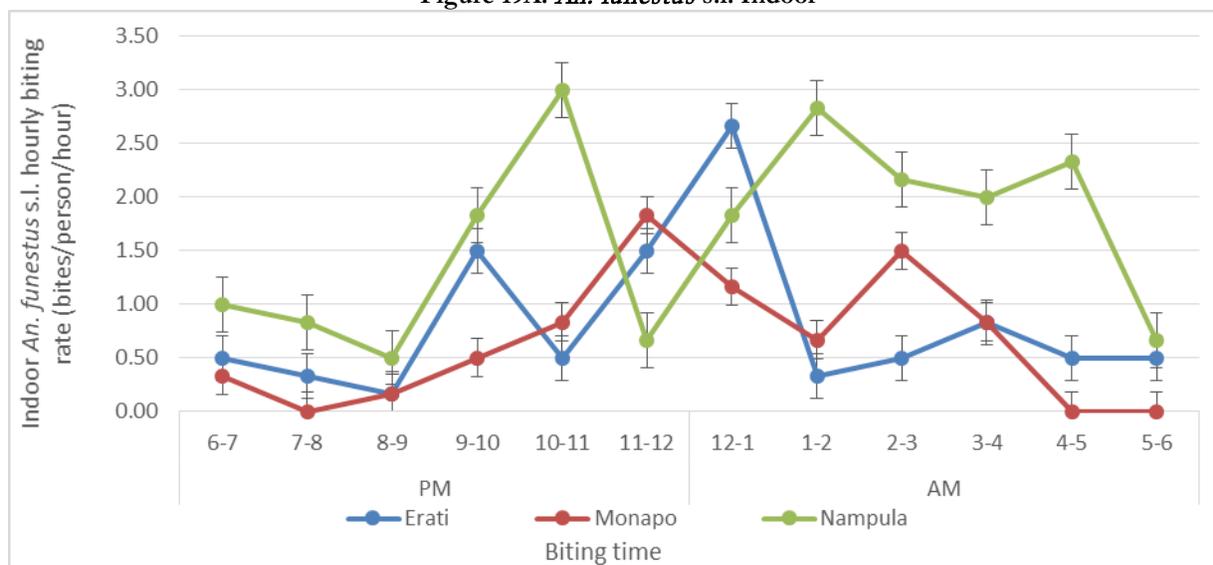
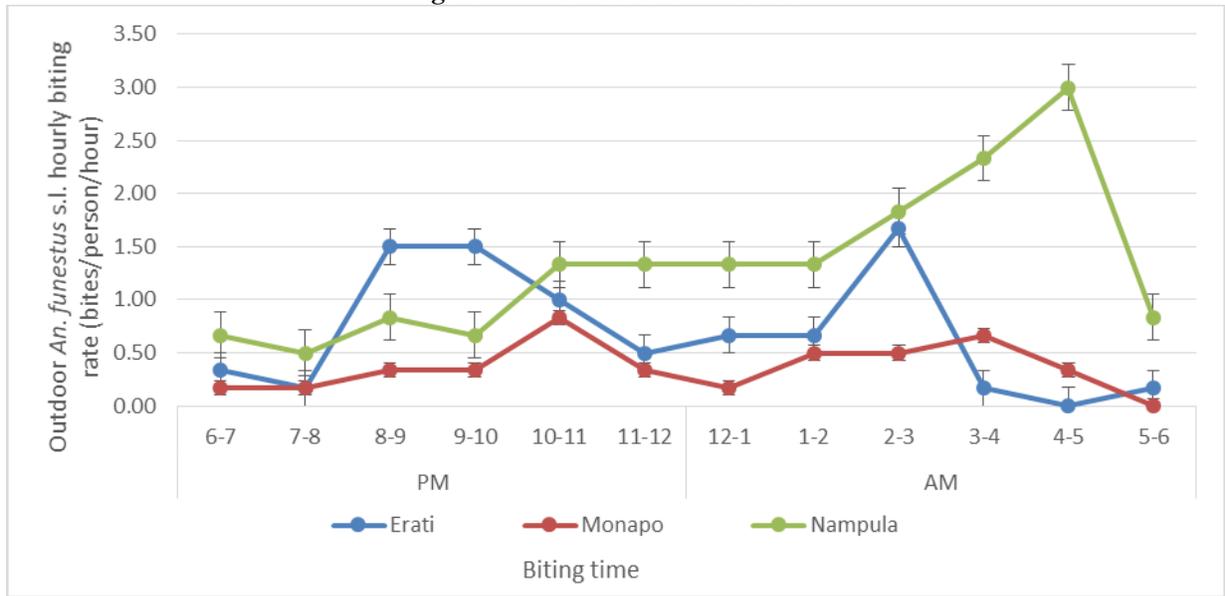


Figure 19B. *An. funestus* s.l. Outdoor



Figures 19C and 19D show the overnight biting pattern of *An. gambiae* s.l. indoors and outdoors. Both indoor and outdoor biting activity in Erati and Nampula City (2.0–2.5 b/p/h) start at a higher level than in Monapo (0.17–0.33 b/p/h). Most *An. gambiae* s.l. bites were observed to take place between 9:00 pm and 2:00am both indoors and outdoors. The peak biting, an estimated 6.83 b/p/h, was recorded indoors in Erati. Indoor and outdoor biting activity in Monapo continued at a low level through the night, at less than 1.0 b/p/h with occasional, and slight spikes not exceeding 2.0 b/p/h occurring mainly indoors at 10:00 pm–11:00 pm.

Figure 19C. *An. gambiae* s.l. Indoor

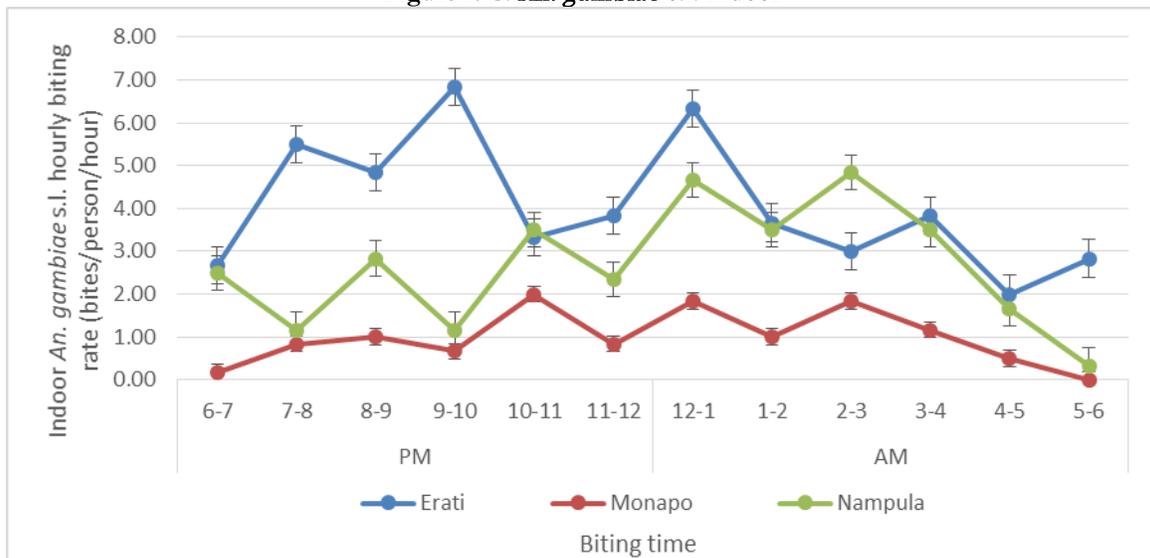
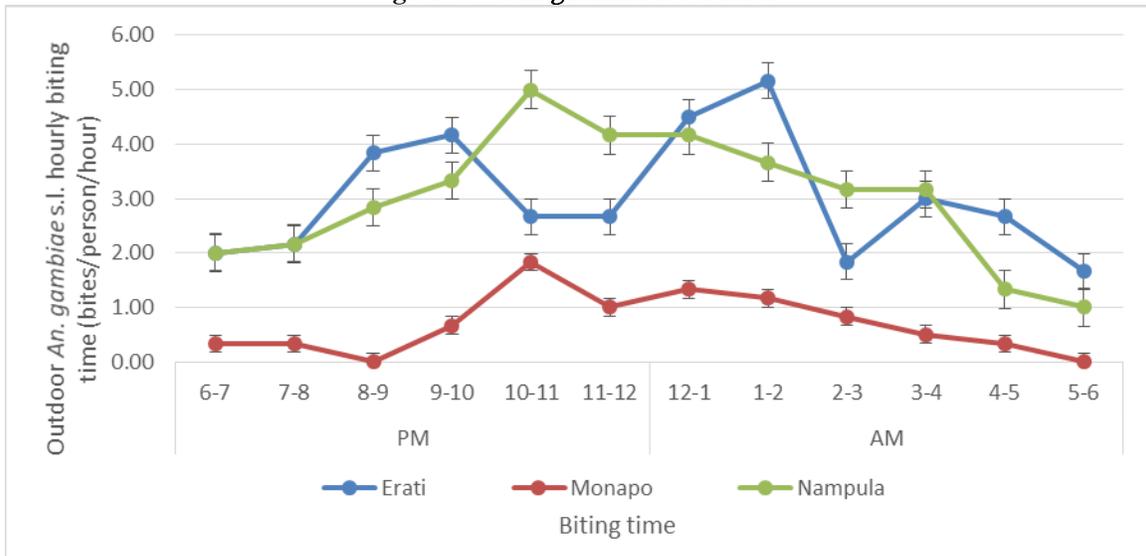


Figure 19D. *An. gambiae* s.l. Outdoor



#### 4.1.3 CDC LIGHT TRAP COLLECTIONS

The CDC light traps collected a total of 1,320 vector mosquitoes from the three districts. Table 15 below shows the major vector species identified morphologically from these collections: 819 (62.6%) were *An. gambiae* s.l., and 491 (37.4%) were *An. funestus* s.l. Nampula and Erati were the districts where the most anopheline mosquitos were collected, 623 (45.56%) and 515 (39.31%), respectively. In Monapo, 172 (13.13%) were collected.

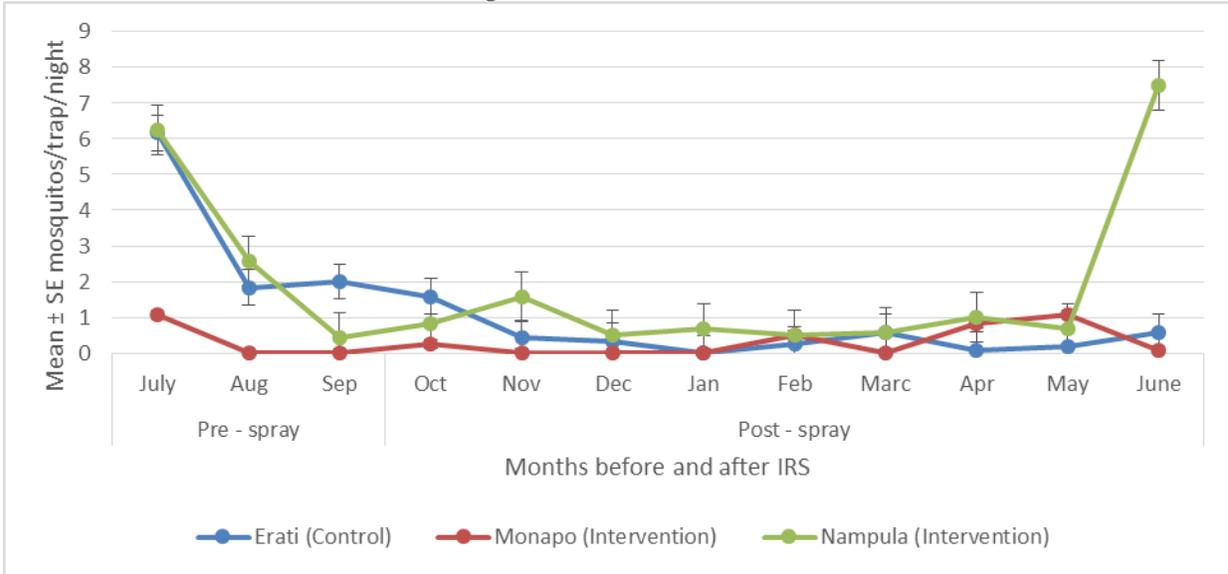
**TABLE 15. CDC LIGHT TRAP DATA FOR MONTHLY COLLECTION OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED IN NAMPULA PROVINCE**

Districts	Species	2018						2019						Total & Average Densities/Month /Night	
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June		
Erati	<i>An. funestus</i> s.l.	74	22	24	19	5	4	0	3	7	1	2	7	168	515
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	6.17	1.83	2.0	1.58	0.42	0.33	0	0.30	0.58	0.08	0.17	0.58	1.17	
	<i>An. gambiae</i> s.l.	40	0	19	30	24	25	26	41	74	11	26	31	347	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	3.33	0.0	1.58	2.5	2.0	2.08	2.17	3.4	6.17	0.92	2.17	2.58	2.41	
Monapo	<i>An. funestus</i> s.l.	13	0	0	3	0	0	0	6	0	10	13	1	46	172
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	1.08	0.0	0.0	0.25	0.0	0.0	0.0	0.5	0.0	0.83	1.08	0.08	0.32	
	<i>An. gambiae</i> s.l.	2	11	17	14	3	1	26	25	2	16	9	0	126	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.17	0.92	1.42	1.17	0.25	0.08	2.17	2.10	0.17	1.33	0.75	0	0.88	
Nampula City	<i>An. funestus</i> s.l.	75	31	5	10	19	6	8	6	7	12	8	90	277	623
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	6.25	2.58	0.42	0.83	1.58	0.50	0.67	0.50	0.58	1.0	0.67	7.5	1.92	
	<i>An. gambiae</i> s.l.	1	4	0	0	3	28	69	44	34	117	28	18	346	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.08	0.33	0	0	0.25	2.33	5.75	3.70	2.83	9.75	2.33	1.5	2.4	
<b>Total</b>		<b>205</b>	<b>68</b>	<b>65</b>	<b>76</b>	<b>64</b>	<b>64</b>	<b>129</b>	<b>125</b>	<b>124</b>	<b>167</b>	<b>86</b>	<b>147</b>	<b>1,320</b>	

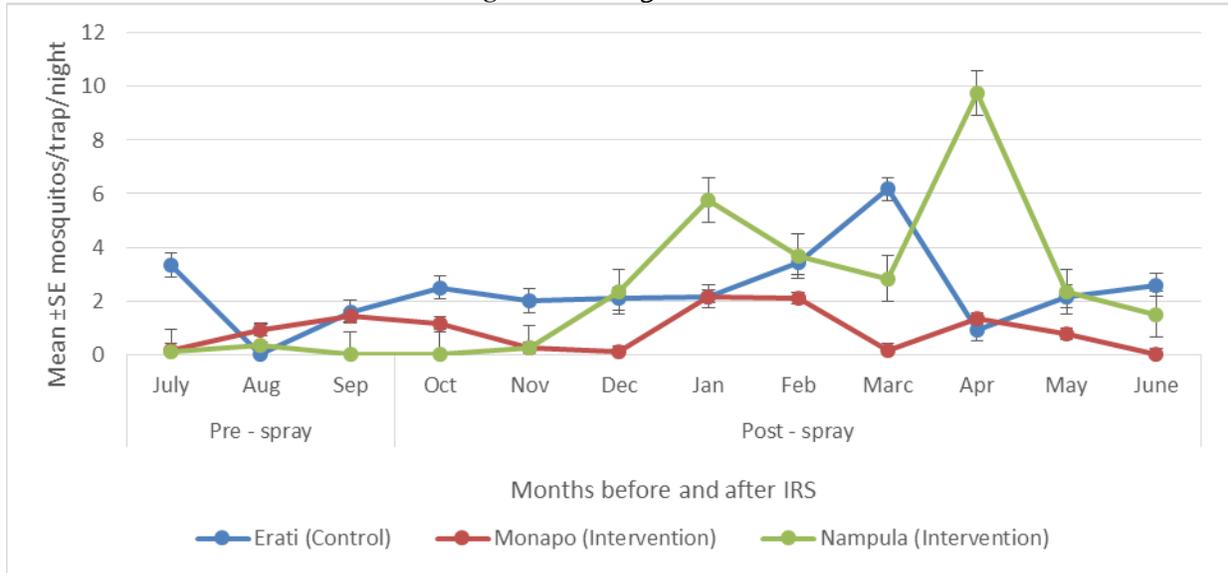
As shown in Figures 20A and 20B, over the year, *An. funestus* s.l. was most abundant in Nampula City (mean collection of 1.92 m/t/n), followed closely by Erati control district (mean collection of 1.17 m/t/n). *An. gambiae* s.l. was the most abundant species in Erati (2.41 m/t/n), followed by Nampula City (2.40 m/t/n). Monapo had the lowest mean collection for both *An. funestus* s.l. (0.32 m/t/n) and *An. gambiae* s.l. (0.88 m/t/n).

**FIGURE 20. AN. FUNESTUS S.L. AND AN. GAMBIAE S.L. DENSITIES ESTIMATED FROM CDC LIGHT TRAP COLLECTIONS**

**Figure 20A: *An. funestus* s.l.**



**Figure 20B: *An. gambiae* s.l.**



## 4.2 CONE WALL BIOASSAYS

In September 2018, cone wall bioassays were conducted in the two intervention districts, to measure spray quality in the period from 24 hours to 14 days after spray. Thereafter, monthly assays were performed to monitor the insecticide decay rate on various wall surfaces sprayed with Actellic® 300CS. Figure 21 summarizes the results.

### 4.2.1 QUALITY OF SPRAY

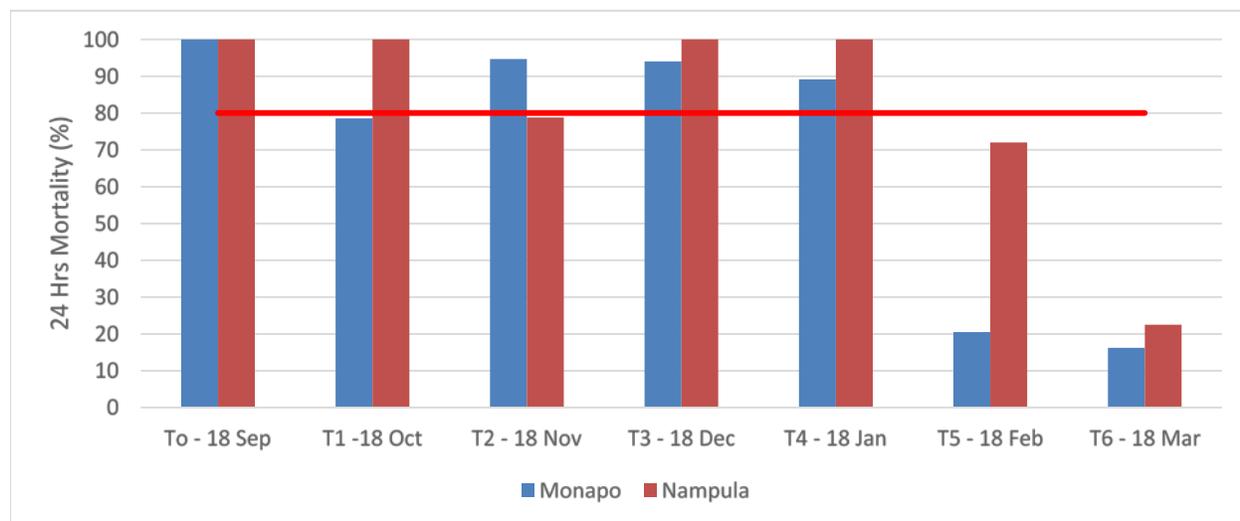
In Monapo and Nampula City, 24-hour mortality from cone wall bioassays were scored at 100%. One month later (at T<sub>1</sub>), mortality at 78.56% and 100%; reverting to 94.7% and 78.79% at T<sub>2</sub>; then up to 94.2% and 100% at T<sub>3</sub> as observed in the respective districts, showing satisfactory level of spray quality.

### 4.2.2 INSECTICIDE DECAY RATE

Figure 21 shows the results of the monthly cone wall bioassays done in Monapo and Nampula City to measure quality assurance (see above) for two months and decay rate monitoring through March 2019.

In Monapo, mortality dropped below 80% by T<sub>1</sub> (October 2018), then recovered to above 90% during T<sub>2</sub> (November 2018) and held there through T<sub>4</sub> (January 2019). Thereafter, mortality dropped dramatically, to less than 20% in T<sub>5</sub> and T<sub>6</sub> (February and March 2019), leading to suspension of monitoring. In Nampula City, mortality dropped below 80% by T<sub>2</sub> (November 2018), then recovering to 100% in T<sub>3</sub> (December 2018). It held there for one month then dropped appreciably to 72% in T<sub>5</sub> (February 2019) and even lower in T<sub>6</sub> leading to monitoring being suspended. IRS with Actellic® 300CS thus remained efficacious on sprayed walls up to four months post-spray in the Nampula intervention districts.

**FIGURE 21. RESULTS OF CONE WALL BIOASSAYS ON WALLS SPRAYED WITH ACTELLIC® 300CS IN MONAPO AND NAMPULA CITY**



Red line indicates the 80% mortality cutoff point.

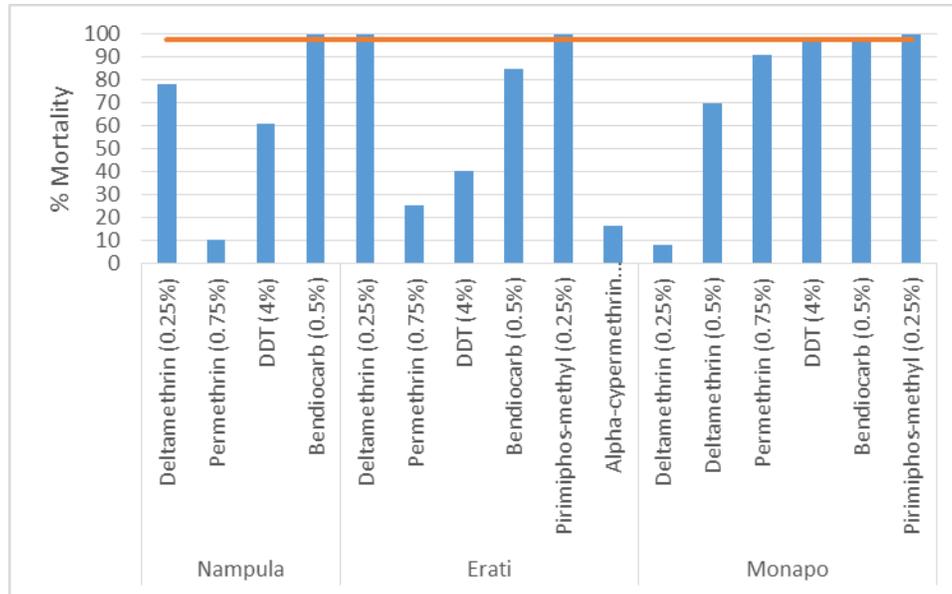
## 4.3 WHO SUSCEPTIBILITY TESTS

Testing for insecticide resistance in Nampula Province was limited to WHO susceptibility tests with pyrethroids (permethrin 0.75%, deltamethrin 0.25%, and alpha-cypermethrin 0.5%), carbamates (bendiocarb 0.5%), organophosphates (pirimiphos-methyl 0.25%), and organochlorine (DDT 4%).

Figure 22 shows the results of WHO susceptibility tests performed with wild-caught *An. gambiae* s.l. in the three districts. The vectors demonstrated high levels of resistance to the three pyrethroids tested with the

exception of deltamethrin in Erati. Further resistance was recorded with DDT in Nampula City and Erati, as well as bendiocarb in Erati. However, full susceptibility was recorded with bendiocarb in Nampula City and Monapo. Tests with pirimiphos-methyl show that the vector is fully susceptible to the insecticide.

**FIGURE 22: RESULTS OF WHO INSECTICIDE SUSCEPTIBILITY TESTS EXPRESSED AS 24-HOUR MORTALITY AGAINST WILD *AN. GAMBIAE* S.L. COLLECTED FROM NAMPULA, MONAPO AND ERATI DISTRICTS**



# 5. DISCUSSION, LESSONS LEARNED, AND CHALLENGES

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## 5.1 ZAMBEZIA PROVINCE

The entomological surveillance conducted in Zambezia employed three main collection methods, namely PSC, HLC, and CDC light traps. Anophelines collected by these methods were identified using the morphological identification key, revealing the presence of 14 anopheline species, with *An. funestus* s.l. being the most abundant, followed by *An. gambiae* s.l. Together, the two vectors constituted over 85% of the anopheline population collected. Coincidentally, the two species are known to be the most efficient malaria vectors in Africa. HLC was the method that collected the greatest diversity of anopheline species, 11 species out of the total 14 collected.

Low levels of indoor resting mosquitoes were recorded in both intervention and control districts for the two main vector species, though it was higher among *An. gambiae* s.l. Monthly indoor resting patterns show *An. funestus* s.l. to be more abundant during the dry season between May and January with a peak in January. It is succeeded by *An. gambiae* s.l. during the rainy season with a peak between March and April. This observation is considered to show how species succession reflects the seasonal variations driven by the annual climatic cycle.

Our findings show that our IRS intervention reduced overall combined bites from all anophelines by half (0.72 b/p/n in intervention areas against 0.36 b/p/n in the control districts). When considering bites by the leading malaria vector, *An. funestus* s.l. only, people in the control districts are apt to receive about five times (6.76 b/p/n) more bites than those in the intervention areas (1.38 b/p/n). Mosquito abundance data collected from 2016 to present show a progressive decline in mosquito densities estimated from year to year through the three main vector sampling techniques including light traps (CDC-LT), pyrethrum spray collection (PSC) and human landing catches (HLC).

Comparison of Mopeia vector biting rates between intervention areas and previous control areas shows that people in the latter areas do experience more than twice ( $\times 2.3$ ) the bites experienced in the former areas. This may demonstrate a higher impact against vectors of the cumulative IRS intervention with Actellic® 300CS and SumiShield® 50WG over the last two years (2016 and 2017 + 2018) in the intervention areas against the single year (2018 only) IRS impact in the previous control areas.

In most districts, biting activity was found to be higher indoor than outdoors, even in intervention districts. This difference was statistically significant in Maganja da Costa. Outdoor biting was significantly higher in Milange for both *An. funestus* s.l. and *An. gambiae* s.l. These observations suggest a strong endophagic behavior of the two vectors – they preferred entering houses to feed even though the houses had been sprayed.

The monthly biting pattern across the 12 months shows a similar pattern indoors and outdoors within the same species. As with indoor resting, the annual pattern depicts the seasonal abundance of each species across the year.

Overnight biting patterns for both *An. funestus* s.l. and *An. gambiae* s.l. show that most occur around midnight and in the early morning hours, when most people are expected to be sleeping in their houses under a treated net. This finding shows the potential for sprayed houses and treated nets in protecting communities against infective bites from the two major vectors.

The quality of IRS assessed by cone wall bioassays showed that spray teams were able to achieve optimal insecticide application in all districts, demonstrating appreciable skills in consistent uniform application of insecticides across districts. Subsequent monthly cone wall bioassays to monitor insecticide decay rates found that Actellic® 300CS had variable decay periods ranging from four to six months. The effective period was estimated as five to six months. Interestingly, SumiShield® 50WG sprayed in Mopeia was found to remain effective on sprayed walls up to 10 months. This is the longest period that an insecticide has been reported to remain effective on a sprayed wall surface in Mozambique. With such encouraging observations, it is time to rotate from Actellic 300CS which has been in use for the past four years to the newer IRS molecules, including SumiShield and Fludora Fusion. The airborne fumigant effect of Actellic® 300CS was found to be low, while that of SumiShield® 50WG was found to be high. This observation suggests a comparatively low vapor pressure of the insecticide's microencapsulated formulation. Insecticide susceptibility tests results show that local vectors are fully susceptible to pirimiphos-methyl, chlorfenapyr, clothianidin, bendiocarb, and DDT. Assays for pyrethroids again revealed widespread vector resistance to pyrethroids among *An. funestus* s.l. and *An. gambiae* s.l. Further assays to assess the strength of the observed resistance in *An. gambiae* s.l. show the presence of moderate to high intensity resistance to pyrethroids in all the five districts tested in Zambezia. Confirmed resistance of moderate to high intensity indicates that operational failure of pyrethroid only LLINs is likely. The results suggest the need or importance of next generation IRS or insecticide-treated nets such as PBO LLINs or Interceptor G2 may be needed for effective malaria vector control in the areas. Synergist assays with PBO demonstrated recovery of mortality indicating involvement of oxidase-mediated resistance mechanisms. This would be good news for the country as it further shows the potential for PBO nets to effectively overcome the observed pyrethroid resistance threat.

## 5.2 NAMPULA PROVINCE

A total of 3,778 anophelines were collected in surveyed districts of Nampula Province, using PSC, CDC light trap, and HLC techniques. The anopheline mosquitoes were found to belong to five species and species complexes: *An. gambiae* s.l., *An. funestus* s.l., *An. coustani*, *An. pretoriensis*, and *An. rufipes*. *An. gambiae* s.l. and *An. funestus* s.l. were the major vectors, making up 63% and 37%, respectively, of the mosquitoes caught.

Low indoor resting densities were observed in the two intervention districts before and after IRS. Surprisingly, the densities recorded in the control site were found to be much lower than in the intervention sites for both *An. funestus* s.l. and *An. gambiae* s.l. before and after spraying, regardless of presence of insecticide indoors in intervention areas which was expected to deter mosquitoes from resting indoors. Available information do not suggest any obvious reason for such an observation. However, this could possibly result from presence of higher vector densities in intervention areas with more inclination towards indoor resting. Other collections using CDC-LT show that indoor densities for Erati was almost similar to those in Nampula City, but much higher than in Monapo. HLC data for *An. funestus* s.l. biting activity shows that while there was an increase in activity in Erati, the control district, a drop was recorded in both intervention districts. *An. gambiae* s.l. activity demonstrated an increase in both control and intervention districts. These findings could potentially be an outcome of interaction between variation in vector behavior and seasonal changes in vector species abundance in the two areas.

The HLC data show that after IRS, there was a notable drop in the *An. funestus* s.l. biting rate both indoors and outdoors in both control and intervention districts. In contrast, *An. gambiae* s.l. biting rates were found to increase both indoors and outdoors after spraying except in Monapo, where there was a drop in outdoor biting following spraying. These observations were expected: they simply reflect natural, seasonal fluctuations in vector population densities in which the *An. funestus* s.l. population tends to drop and the *An. gambiae* s.l. population to rise with the rains that came after spraying. Mosquito density estimated using CDC light traps shows that *An. funestus* s.l. was most abundant in Nampula City followed closely by Erati control district. The reverse was found for *An. gambiae* s.l.; it was most abundant in Erati, followed by Nampula City. Monapo had the lowest mean collection for both *An. funestus* s.l. and *An. gambiae* s.l. This observation could reflect the differences in the natural mosquito productivities of the three districts, which are influenced by other

ecological factors that affect the two species in different ways. As a result, densities of *An. funestus* s.l. in Nampula City could remain higher than in Erati control district even with IRS.

The quality of spray established with cone bioassays immediately after spraying with Actellic® 300CS showed that spray teams were able to achieve optimal insecticide application in all districts, demonstrating appreciable skills in consistent uniform application of insecticides across districts. Subsequent monthly cone wall bioassays to monitor insecticide decay rates found that Actellic® 300CS could last up to four months on sprayed walls in Nampula.

Wild *An. gambiae* s.l. vectors collected in Nampula demonstrated high levels of resistance to pyrethroids tested, including permethrin, deltamethrin, and alpha-cypermethrin, with the exception of deltamethrin in Erati. Further resistance was recorded with DDT in Nampula City and Erati, as well as bendiocarb in Erati. However, full susceptibility was recorded with bendiocarb in Nampula City and Monapo. Tests with pirimiphos-methyl in sprayed districts of Nampula Province show that the vector is fully susceptible to the insecticide.

Molecular assays of vector samples this study collected in Mopeia District are underway at the Walter Reed Biosystematics Unit in the United States, and at the INS in Maputo for the rest of the samples from districts in Zambezia and Nampula. The assays include identification of sibling species, detecting presence of human Plasmodium parasites, detecting mutations on the knockdown resistance (*knr*) and Acetylcholinesterase-1 (*Ace-1*) genes and identifying sources of mosquito bloodmeals.

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