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THE PMI VECTORLINK SIERRA LEONE ANNUAL ENTOMOLOGICAL MONITORING REPORT

MARCH 1, 2020 – FEBRUARY 28, 2021

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ACRONYMS

Ace-1	Acetylcholinesterase 1
CDC	U.S. Centers for Disease Control and Prevention
CSRS	Centre Suisse de Recherches Scientifiques / Swiss Center for Scientific Research
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
EPI	Expanded Program on Immunization
gDNA	Genomic DNA
HBR	Human Biting Rate
HLC	Human Landing Catch
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated Net
IRMMP	Insecticide Resistance Monitoring and Management plan
kdr	Knockdown Resistance
LLIN	Long-Lasting Insecticidal Net
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
VBDIL	Vector Borne Disease Insectary and Laboratory
VCTWG	Vector Control Technical Working Group
VL-C	VectorLink-Collect
WHO	World Health Organization

EXECUTIVE SUMMARY

Malaria remains one of the biggest public health challenges in Sierra Leone with year-round transmission and peaks during the rainy season between May and October. To combat malaria, the US President's Malaria Initiative (PMI) in collaboration with the Sierra Leone National Malaria Control Program (NMCP) invests in the distribution of insecticide-treated nets (ITNs) and more recently, indoor residual spraying (IRS) in selected high disease burden districts. In a mass campaign in 2020, the NMCP distributed Olyset Plus and PermaNet 3.0 piperonyl butoxide (PBO)-based nets countrywide. In May 2021, the NMCP with President's Malaria Initiative (PMI) support, introduced and implemented Indoor Residual Spraying (IRS) with SumiShield in Bo and Bombali districts.

Beginning March 2018, the PMI VectorLink Project in Sierra Leone has supported the NMCP in conducting longitudinal entomological monitoring to guide vector control decisions. Between March 2020 and February 2021, mosquitoes were collected in five districts namely Bo, Bombali, Kono, Western Rural and Port Loko. The susceptibility of the main malaria vector in Sierra Leone, *Anopheles gambiae* s.l., to pyrethroids with and without the synergist PBO, clothianidin, chlorfenapyr and pirimiphos-methyl were tested. In July 2020, the country began a study to investigate the impact of co-de-deployment of PBO-ITNs and IRS on malaria incidence and entomological indicators of transmission. This was done in two intervention districts (Bo and Bombali) where IRS was implemented and two control districts (Karene and Port Loko) that received only PBO-ITNs. The results of the co-deployment study will be reported on separately.

Vector Bionomics: *An. gambiae* s.l. was the predominant (91.8%) mosquito species collected, followed by *An. funestus* group (6.1%), *An. coustani* (1.3%) and others (0.4%). *Anopheles funestus* s.l. were collected mainly in Bo and Bombali. Molecular species identification using polymerase chain reaction (PCR) was done on a total of 5,054 *An. gambiae* s.l. collected in 2020–2021; *An. coluzzii* were predominant at 64.5% (3,258), followed by *An. gambiae* s.s. at 34.3% (1,732), *An. melas* at 1.0 % (51), hybrid of *An. gambiae* s.s. and *An. coluzzii* at 0.2% (11), two *An. arabiensis* (0.03%). Molecular identification was also done on 186 *An. funestus* group; 92.5% (172) were identified as *An. funestus* s.s., 7.0% (13) *An. leesoni* and *An. parensis* 0.5 % (1).

Human Biting Rate (HBR) and Location: The mean biting density varied by site, district, and month of collection. The highest indoor HBR/person/night was recorded in June in Masongbo, Bombali district, 175.5 bites/person/night. In all districts, the HBR followed the rainfall pattern (May-October) with a peak observed between June and September and declining into the dry season apart from Kono that had peaks in March and September. The highest HBR for *An funestus* s.l. was recorded in Gerihun, Bo district with 11.7 bites/person/night. The density of *An. funestus* s.l. was low and comparisons could only be made in Bo, Kono and Port Loko districts due to the sample size. Both *An. gambiae* s.l. and *An. funestus* s.l. did not show any clear preference to feeding indoors or outdoors. The peak biting time for both species occurred in the middle of the night and towards the early morning.

Vector density: Vector density varied by site, district, and month, but followed the rainfall patterns in Sierra Leone. The highest average density of *An. gambiae* s.l./trap/house was recorded in June in Masongbo, Bombali district (34.4/trap/house), followed by Gerihun (rural) in Bo in March (6.6./trap/house). Mean density was relatively higher in rural compared to peri-urban sites apart from Bombali and Western Area districts.

Blood Meal Source: Both *An. gambiae* s.l. and *An. funestus* group in Sierra Leone prefer to feed on human blood rather than any other source of blood. The minimum Human Blood Index (HBI) was 75% and was recorded in Moyamba district.

Parity Rate: Majority of malaria vectors collected during the sampling period had laid eggs at least once (parous) 69.1% (849) while 30.9% (379) had not laid eggs (nulliparous). In all the districts, there was no clear trend in parity status by month of the mosquitoes that were dissected apart from in Western Rural Area district.

Insecticide Resistance: *An. gambiae* s.l. are resistant to permethrin, deltamethrin and alpha-cypermethrin with high resistance intensity in 2020. However, PBO was able to restore partial susceptibility, indicating that mono-oxygenases are involved in conferring resistance in Sierra Leone. These mosquitoes are, however, fully susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. Molecular analysis is ongoing to determine the molecular markers of insecticide resistance in *An. gambiae* s.l.

Conclusion: Malaria vectors prefer to bite late at night and show no preference to either indoor or outdoor biting. This implies that the proper use of PBO-ITNs at night when in bed, in addition to IRS coverage of over 80% should significantly reduce malaria burden in Sierra Leone from indoor biting, but other forms of outdoor malaria prevention should be considered and continued robust investment in malaria treatment and control should be maintained. The PBO-permethrin/deltamethrin nets distributed in the national 2020 mass campaign should be able to kill pyrethroid resistant mosquitoes. There was a high proportion of collected mosquitoes that had a blood meal, indicating that it is crucial for the NMCP to intensify behavior change activities to promote correct use of ITNs. The biting density followed the rainfall pattern and started to peak from June. This supports the decision to implement IRS in April-May before the onset of rain. The VectorLink Sierra Leone project is also collecting entomological monitoring data as part of the IRS/PBO-net co-deployment assessment. Data is being analyzed and an update report on findings for the same entomological indicators will be made available in November 2021.

1. INTRODUCTION

Malaria is endemic in Sierra Leone with stable and perennial transmission. Malaria prevalence was 40% in 2016 among children aged 6–59 months (NMCP 2016). It is currently the main cause of morbidity and mortality among children under 5 years. Malaria prevalence is two times higher in rural areas (49%) than in urban areas (25%) (NMCP 2016).

The main malaria vector control intervention deployed in Sierra-Leone are insecticide-treated nets (ITNs). The ITNs are normally distributed through mass campaigns, antenatal care clinics and the expanded immunization program (EPI). The most recent ITN mass campaign distributed piperonyl butoxide (PBO)-permethrin nets (Olyset Plus) and PBO-deltamethrin nets (PermaNet 3.0) in May -June 2020. In May 2021, the Sierra Leone National Malaria Control Program (NMCP) introduced indoor residual spraying (IRS) with SumiShield (clothianidin) in the districts of Bo and Bombali after the initial pilot that took place in 2011-2012 in selected chiefdoms in four districts (Bo, Bombali, Kono and Western Rural Area). The two 2021 IRS districts were selected from the four districts where NMCP was conducting insecticide resistance monitoring and piloted IRS in 2011-2012. The selection was made based on the criteria of having malaria prevalence between 38% and 40% and on the findings from the longitudinal entomological monitoring, which indicated Bo and Bombali as the districts with the highest entomological inoculation rate (EIR) compared to Kono and Western Rural Area. However, Port Loko, Tonkolili and Koinadougu have higher malaria prevalence, but these sites lacked entomological monitoring data. The President's Malaria Initiative (PMI) VectorLink project in Sierra Leone worked with the NMCP to add Port Loko as an additional site for longitudinal entomological monitoring and the findings will guide country decision making for future IRS campaign district selection.

The continued use of insecticide-based vector control interventions is threatened by the development of insecticide resistance. In collaboration with the NMCP, the project supported the revitalization of the Vector Control Technical Working Group that formulated the Insecticide Resistance Monitoring and Management Pan (IRMMP) and selected the insecticide for use in IRS in 2021.

To manage insecticide resistance and formulate vector control policies, the NMCP, through the project, has been monitoring malaria vectors since March 2018. The project has collected data on vector bionomics and insecticide resistance. The project has also supported the establishment of an insectary in Freetown, and the maintenance of the Makeni Vector Borne Disease Insectary and Laboratory (VBDIL) in Bombali District, which was established in 2018.

Between March 2020 and February 2021, monthly routine entomological monitoring activities were conducted in five districts previously selected in consultation with the NMCP: Bo, Sierra Leone's second-largest district (South), Bombali), Kono, where there are large-scale mining activities (East), Western Rural Area Coastal area (Westand Port Loko (North) to take into account a district with high malaria burden. In each district, vector bionomics monitoring was conducted in two chiefdoms with one community/site per chiefdom for a total of ten sentinel sites. In April and May 2020, no monitoring was undertaken because of the Covid-19 pandemic. Insecticide susceptibility tests were performed in one rural chiefdom per district, where more *Anopheles gambiae* s.l. breeding sites were available

The current report covers monthly entomological surveillance in the five districts (Bo, Bombali, Port Loko, Kono and Western Area Rural.). Insecticide resistance testing with pyrethroids with and without the synergist PBO pirimiphos-methyl, clothianidin and chlorfenapyr was also conducted in selected sites. Capacity building activities to improve entomological capacity in Sierra Leone was also undertaken.

The objectives of the 2020/2021 entomological monitoring plan were to:

- Monitor malaria vector bionomics, densities, and behavior in all districts.
- Determine levels and mechanisms of insecticide resistance of local malaria vector populations. Insecticide resistance monitoring was also conducted to inform decision making for IRS and ITNs.

Results from this monitoring are intended to guide decision making by the NMCP and other development partners in the fight against malaria and will be used to update the Insecticide Resistance Monitoring and Management plan (IRMMP).

2. METHODOLOGY

2.1 ENTOMOLOGICAL MONITORING SITES

Sierra Leone has a tropical climate with wet and dry seasons. The wet season lasts from May - October while the dry season lasts from November -April. Port Loko and Moyamba districts border the Atlantic Ocean and have a coastal ecosystem where brackish water larval breeding grounds are common. Adult mosquitoes were collected monthly from March 2020 to February 2021 in the routine monitoring sites. However, the number of sites where collections were done varied in particular months. There was no collection in April and May in all sites due to the Covid-19 pandemic.

In each district, mosquitoes were collected in one peri-urban and two rural sentinel sites (Table 1). The peri-urban sites are close to a metropolitan municipality representing transition zones between the urban and the rural areas, with a mix of traditional rural dwellings and relatively modern houses, the presence of agricultural lands, and a highly concentrated population. The rural sites are in communities away from the urban centers with traditional houses made of mud and cement, with the agricultural sector being the main source of jobs in these sites.

Figure 1. PMI VectorLink Sierra Leone Entomological Monitoring Sites

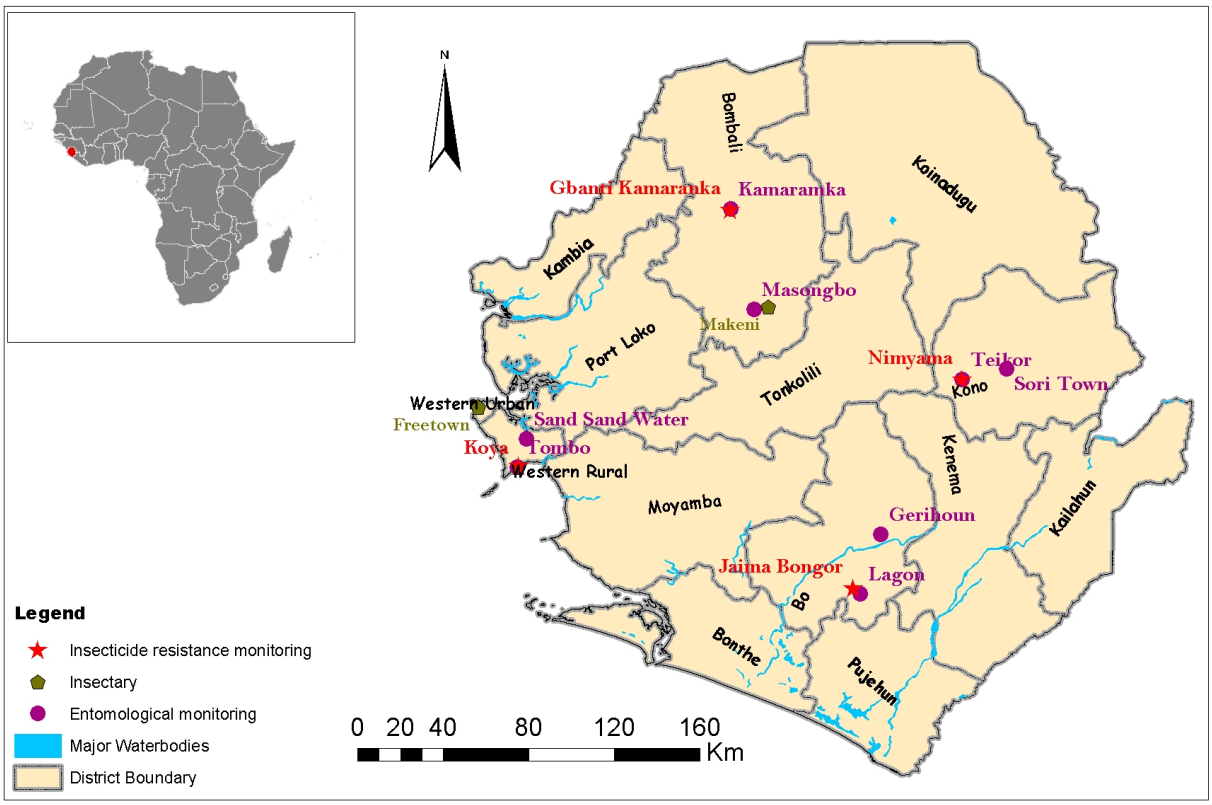


Table 1: Sentinel Sites for Entomological Monitoring

Province	District	Chiefdom	Sentinel Site	Location	Activity		IRS Site
					Vector Bionomic Monitoring (PSC, CDC LT, and HLC)	Vector Susceptibility	
Southern	Bo	Jaima Bongor	Largor	Rural	✓	✓	✓
		Boama	Gerihun	Peri-urban	✓		✓
Northern	Bombali	Gbanti Kamaranka	Kamaranka	Rural	✓	✓	✓
		Makari Gbanti	Mansongbo	Peri-urban	✓		✓
	Port Loko	Lokomasama	Bakolo	Rural	✓	✓	
		Maforki	New Maforki Village	Peri-Urban	✓		
Eastern	Kono	Nimikoro	Sori Town	Peri-urban	✓		
		Nimyama	Teikor	Rural	✓	✓	
Western	Western Area Rural	York Rural	Tombo	Peri urban	✓		
		Koya Rural	Sand Sand Water	Rural	✓	✓	

2.2 LONGITUDINAL ENTOMOLOGICAL MONITORING

In all sites, three sampling methods were used to assess the vectors bionomics (Table 2): Pyrethrum spray catches (PSCs), human landing catches (HLCs), and U.S. Centers for Disease Control and Prevention (CDC) light traps. Due to the Covid-19 pandemic, collections were incomplete or suspended between March – June 2020.

Table 2: Longitudinal Monitoring Adult Mosquito Collection Methods

Collection Method	Time	Frequency	Sample
PSC	5:00 am to 9:00 am	3 days per site per month	15 houses per site (5 houses each day for 3 consecutive days)
HLC	6:00 p.m. to 7:00 am	2 nights per month	2 houses for 2 consecutive nights (both inside and outside) per site
CDC light trap	6:00 p.m. to 7:00 am	4 nights every month	4 houses for 4 consecutive nights (inside) per site

PSC Collections: The project entomology team conducted PSCs based on the VL protocol (SOP03/01) in five houses per day for three consecutive days each month in each sentinel site. The PSCs were performed in the morning hours (5:00 am to 9:00 am) to determine indoor resting mosquito species and densities. Collections took place in rooms where people had slept the preceding night. The number and different gonotrophic stages (unfed, blood fed, half-gravid and gravid) of female *Anopheles* mosquitoes collected were recorded, and the samples were placed in moist petri dishes and transported to the hotel or laboratory (VBDIL) for morphological identification. Vector species collected were preserved in labeled 1.5 ml screw-top tubes with silica gel. The same sentinel sites and houses were visited for the monthly collections throughout the study period. The mosquitoes collected were used to assess vector species composition, abundance, and indoor resting density. The data collected from the rural sites were compared to data from peri-urban sites.

HLC Collections: Monthly HLCs were also performed according to VL SOP SOP02/01. Mosquito collections were done in two houses per site in the five routine districts (Bo, Bombali, Kono, Western Rural Area and Port Loko). In each house, hourly collections were done simultaneously indoor and outdoor by two trained collectors, one inside and another outside the house from 6:00 pm to 12:00 am. The two were replaced by another team of two collectors from 12:00 am to 7:00 am. The proportion of mud and cement

houses varied by site depending on the most common building material in the site. The houses were randomly located either close to or further away from breeding sites to account for spatial variation in distribution of malaria vectors. The same sentinel sites and houses were visited for the monthly collections throughout the study period.

On each night, collectors (one inside and another one outside the house) sat on chairs with their legs exposed up to the knee (a total of 16 person-nights/district). Each hour, mosquitoes attempting to feed on the collectors were caught either using mouth aspirators and flashlights and transferred into labelled paper cups or tubes and placed in labelled Ziplock bags. A different paper cup/Ziplock bag was used for indoor and outdoor collections and for each hourly catch. The collectors worked overnight and exchanged positions indoors and outdoors every hour to reduce possible collection bias due to differences in their attractiveness to mosquitoes.

The data was used to determine the human biting rate (HBR) (mean number of mosquito bites per person per unit of time), as well as feeding locations and peak biting times. Subsamples of the *An. gambiae* s.l. and *An. funestus* s.l. collected were further analyzed by polymerase chain reaction (PCR). A subsample of unfed *An. gambiae* s.l. collected were dissected to determine parity rate.

CDC-LT Collections: CDC light traps (SOP01/01) were set up indoors in four randomly selected houses where people slept under an untreated mosquito net for four consecutive nights each month. The same sentinel sites and houses were visited for the monthly collections throughout the study period. The consent of the head of each household was acquired beforehand. The traps were placed approximately 150 cm above the sleeper's legs and about 50 cm from the human sleeping under an ITN. The traps operated from 6:00 pm to 7:00 am for four nights in each house (4 houses x 2 sites x 4 nights = 32 house-nights per district). Mosquitoes were collected from the traps once in the morning. Mosquitoes captured were transported to the VBDIL or hotel for morphological identification.

A taxonomic key (Coetzee 2020) was used to morphologically identify all *Anopheles* mosquitoes collected by each method. All mosquitoes collected were preserved in 1.5 ml Eppendorf tubes with desiccant for further analysis at the Center for Research in Infectious Diseases (CRID) laboratory in Cameroon.

Determination of Parity Rate: Ovarian dissection of unfed mosquitoes collected by HLC, and CDC LT was done to determine the proportion of mosquitoes that had laid eggs at least once. Parity rate was determined in the sites each month. Thus, *An. gambiae* s.l. and *An. funestus* s.l. specimens were dissected under a dissecting microscope and examined under a compound microscope to determine parity rate as described by Gillies and Wilkes (1963) and WHO (2013b).

2.3 Insecticide Susceptibility Tests

World Health Organization (WHO) tube tests (SOP06/01) and CDC bottle assays (SOP04/01) were performed to assess the susceptibility of local vector populations to the insecticides used for IRS and for ITNs. All the sentinel sites have a long history of ITN coverage, gained through mass distribution campaigns and maintained through routine distribution at antenatal care and expanded program on immunization visits, as well as during Maternal and Child Health Week. PBO ITNs were distributed in May 2020 in all districts, and the country implemented IRS with clothianidin in Bo and Bombali districts in May 2021.

2.3.1 WHO Tube Tests

The project team performed insecticide susceptibility tests using the WHO tube method in Bo, Bombali, Port Loko, Kono, Karene and Western Rural districts. Larvae and pupae of *Anopheles* mosquitoes for susceptibility tests were collected from breeding sites in and around the sentinel sites and reared to adults at the Makeni insectary or in Freetown. Mosquitoes were morphologically identified at the adult stage and only *An. gambiae* s.l. were used for the susceptibility tests. Synergist assays with PBO using WHO tube tests were conducted with deltamethrin 0.05% and permethrin 0.75% in all the districts and with alpha-cypermethrin 0.05% in the four districts (Bombali, Bo, Western Rural and Kono).

The team also conducted susceptibility tests of *An. gambiae* s.l. to pirimiphos-methyl 0.25% using the WHO tube method.

After the 24-hour holding period, the number of dead and alive mosquitoes in both the exposure and the control tubes was recorded. For all tests, mortalities were corrected using Abbott's formula if the control mortalities were between 5% and 20%, but tests were discarded and repeated if control mortalities were ≥20% (Abbott 1925).

Additionally, the team determined the susceptibility status of wild *An. gambiae* s.l. from selected sites to 2% clothianidin, using papers that were impregnated in country. Knockdown was recorded after a 60-minute exposure and mortalities recorded every 24 hours for seven days post-exposure.

The team evaluated susceptibility levels of *An. gambiae* s.l. on the basis of the WHO criteria of test mortality (WHO 2013): 98–100% mortality after the holding period indicates susceptibility. Mortality of <98% suggests the existence of resistance and the need for further investigation. If the observed mortality (corrected if necessary) is >90% but <98%, the presence of resistance in the vector population must be confirmed with a repeat test. Mortality of <90% confirms the presence of resistant individuals in the vector population.

2.3.2 CDC Bottle Assays

The project team conducted the CDC bottle assay with chlorfenapyr, using a method described by Brogdon and Chan (2010) with some modifications (60 minutes of exposure time). *An. gambiae* s.l. reared from larvae were exposed to 250ml Wheaton bottles treated with a diagnostic concentration of 100µg/bottle. Tests with *An. gambiae* Kisumu were run in parallel as positive controls. Mosquitoes were introduced in batches of 20–25 in one replicate of each concentration. After the exposure period, mosquitoes were released into clean cages and then gently aspirated into labeled paper cups covered with untreated netting and provided with 10% sugar solution. Knockdown was recorded 60 minutes after the start of the test, while mosquitoes were still in the bottle. Mortality was recorded one, two, and three days after the end of exposure. A negative control was tested at the same time and mortality recorded at one, two, and three days so that corrected mortality could be calculated.

2.4 Resistance Intensity Assays

Intensity assays were carried out only in three sites, Largor (Bo), Kamaranka (Bombali) and Sand Sand Water (Western Area Rural) for deltamethrin and in two sites, Kamaranka (Bombali) and Largor (Bo) for permethrin. Not enough mosquitoes were collected in other sites to allow for the resistance intensity assays to be carried out. Additional tests have been scheduled for the 2021-2022 period. The project team used the CDC bottle bioassay resistance Intensity Rapid Diagnostic Test (I-RDT) (Brogdon and Chan 2010) to assess the intensity of resistance (5x and 10x) observed using discriminating concentrations of deltamethrin and permethrin. Four replicates of 500µl of acetone were added to each insecticide vial and washed off into a 50ml graduated falcon tube. The falcon tube was topped up to the 50ml mark. The prepared insecticide solutions were stored in a refrigerator at 4°C until use. The control bottle was prepared by adding 1ml of acetone into a 250ml Wheaton bottle and coated as described by Brogdon and Chan (2010). Four test bottles were then coated with 1ml of different concentrations of the prepared insecticide solutions to get one bottle each of the required insecticide concentration. Between 20 and 25 mosquitoes were introduced into each of the four replicates. A control bottle (coated with acetone only) was run alongside the tests. The knockdown rate was recorded at 15-minute intervals in each bottle.

2.5 Synergist Assays

Synergist assays with PBO using the WHO procedure were conducted with deltamethrin 0.05%, permethrin 0.75%, and alpha-cypermethrin 0.05% in the six districts.

2.6 Analysis and Molecular Evaluations

Subset of collected samples were shipped to CRID, Cameroon for molecular analysis to:

- Determine sporozoite rates and calculate entomological inoculation rates (EIRs)
- Identify members of the *An. gambiae* s.l. and the *An. funestus* s.l. complex to species (Scott et al. 1993)

- Determine the source of blood meal
- Determine the mechanism of target site resistance and the frequency of gene mutations related insecticide resistance.

This report covers only the molecular species identification and bloodmeal source. Results on EIR and insecticide resistance mechanisms will be reported in an addendum report.

2.6.1 Mosquitoes Species Identification

DNA Extraction

Prior to species identification and genotyping experiments, genomic DNA (gDNA) was extracted from samples of mosquitoes according to the Livak method (Livak 1984). using a LIVAK grind buffer (1.6 ml 5M NaCl, 5.48 g sucrose, 1.57g Tris, 10.16 ml 0.5M EDTA, 2.5 ml 20% SDS). Each individual mosquito was ground in 100µl of preheated LIVAK grind buffer and incubated at 65°C for 30 minutes. 14 µl 8M K-acetate was added and gently mixed. After 30 minutes of incubation on ice, the thicker mixture was centrifuged at 13,000 rpm for 20 minutes (4°C). 200 µl 100% EtOH was added to the supernatant (transferred in new Eppendorf tube 1.5 ml) and the mixture was subjected to a new centrifugation at 13,000 rpm for 15 minutes (4°C). After discarding the supernatant, the pellet was rinsed with 100 µl ice cold 70% EtOH. Dried pellets were then re-suspended in 100 µl TAE buffer. The DNA was used for species identification and target site genotyping.

Polymerase Chain Reaction

Members of *An. gambiae* s.l. and *An. funestus* s.l. groups were identified to species by PCR, following the protocols developed in Scott et al. (1993) and Santolamazza et al. (2008) for *An. gambiae* s.l. and *An. funestus* s.l. (Koekemoer et al. 2002). A total volume of 24.75 µl was used per reaction for the master mix containing 18.83 µl DNase free water, 2.5 µl Buffer 10X, 0.75 µl MgCl2 (25mM), 1 µl dNTP (10mM), 1 µl Sine 6.1a (10 µM) 5'-CGCTTCAAGAATTCGAG ATAC-3', 1 µl Sine 6.1b (10 µM) 5'-TCGCCTTAGA CCTTGCGTTA-3' and 0.17 µl Kappa Taq. Each PCR product had 23 µl of mix and 2 µl of genomic DNA. The PCR product was amplified at 94°C for three minutes followed by 35 cycles of 94°C, 62°C, and 72°C for 30 minutes respectively, and a last cycle of five minutes at 72°C. Products were then run on 1.5% agarose gels.

2.6.2 Molecular Characterization of Insecticide Resistance Mechanisms

KDR West and KDR East Genotyping

This will be reported in an addendum report.

Ace-1 genotyping

This will be reported in an addendum report.

2.6.3 Determination of Infection Rate

This will be reported in an addendum report.

2.6.4 Determination of Blood Meal Origin

The host preference was assessed by analyzing the blood meal among the potential hosts in the study areas, using ELISA blood meal (Beier et al. 1988). Each mosquito sample (abdomen) was analyzed for the possible host blood: human, bovine, porcine, and chicken. A plate was sensitized with 50 µl of anti-human diluted to 1/2,000 in PBS (pH 7.2), the second plate was sensitized with anti-bovine 1/1,000 in PBS (pH 7.2), the third plate with anti-porcine (1/300 in PBS, pH 7.2), and the fourth with anti-chicken (1/2,000 in PBS, pH 7.2). All these plates were stored for 24 hours (at least one night) at +4°C. Plates were washed three times after 24 hours with 0.05% PBS-Tween. Plate wells were then filled with 200 µl of 0.1% PBS-Tween and stored for 30 minutes at 37°C. After one hour, the wells were filled with 50 µl of human serum, 0.1% PBS-

Tween, or samples to be tested. The same sample was tested in all plates. Then wells were incubated for two hours at room temperature (20–25 °C) and washed three times with 0.05% PBS-Tween. Then wells were filled with 50 µl of anti-human HRPO diluted to 1/30,000 in PBS-Tween, 50 µl HRPO anti-bovine 1/5,000, and finally 50 µl HRPO anti-chicken to 1/20,000. The plates were incubated for one hour at 37°C. After the incubation, they were washed three times with 0.05% PBS-Tween and then once with distilled water. They were immediately filled with 50 µl of substrate per well. Four to six minutes later, the reaction was stopped with 25 µl of H_2SO_48N per well. The plates were read in a spectrophotometer at 492 nm. The optical density of the positive samples ranges between 900 and 1,200 nm. Each plate contained positive controls and negative controls.

2.6.5 Analysis of Data

Data was entered into VectorLink collect, the DHSI2 database platform for VectorLink project. Summaries were generated using the analysis App in the VL-collect software. Additional analysis was done in Microsoft Excel. R (R core team, 2020, Version 4.0.2) statistical software was used to compare proportions and trend analysis.

The following parameters were estimated:

- Human Biting Rate (HBR) per night = the total number of vectors collected/number of collectors/number of nights of capture (reported as bites per person per night).
- Human Blood Index (HBI) per district = the proportion of mosquitoes that fed on human blood.
- Mean Biting Density = the mean number of mosquitoes sampled using CDC-LT per house per night.
- Indoor Resting Density = the mean number of indoor resting mosquitoes per house per day sampled using PSC.
- Parity rate = the proportion of parous mosquitoes among those successfully dissected.

The following will be reported in the addendum report.

- Sporozoite rate = the proportion of *Anopheles* found positive for *Plasmodium* infection
- Monthly EIR = daily EIR x number of days in the month
- Annual EIR = Σ Monthly EIRs

3. RESULTS

3.1 LONGITUDINAL MONITORING

3.1.1 SPECIES COMPOSITION

A total of 24,424 *Anopheles* mosquitoes were collected during the monthly collections conducted from March 2020 to February 2021 using PSC, HLC, and CDC light traps in both rural and peri-urban sites in five districts. The HLC sampled more mosquitoes, (80.3%), compared to CDC-LT (9.3%) and PSC (10.4%). Of the anophelines collected, *An. gambiae* s.l. was the predominant vector with 91.8% (22,431), followed by *An. funestus* group, 6.1% (1,482), *An. coustani* 1.3% (322), *An. squamosus* 0.4% (87) and others 0.4% (102) (Figure 2).

Detailed numbers collected by each sampling tool are presented in Annexes A-D.

Figure 2: Total *Anopheles* Species Collected (PSC, HLC, and CDC-LT) Between March 2020 and February 2021

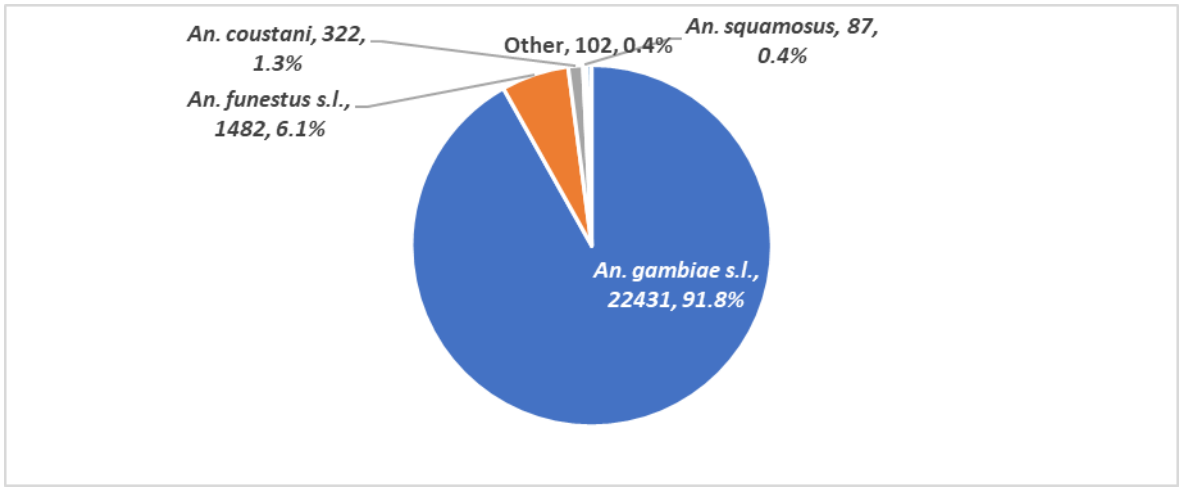


Figure 3: Total *Anopheles* Species Collected by HLC between March 2020 and February 2021

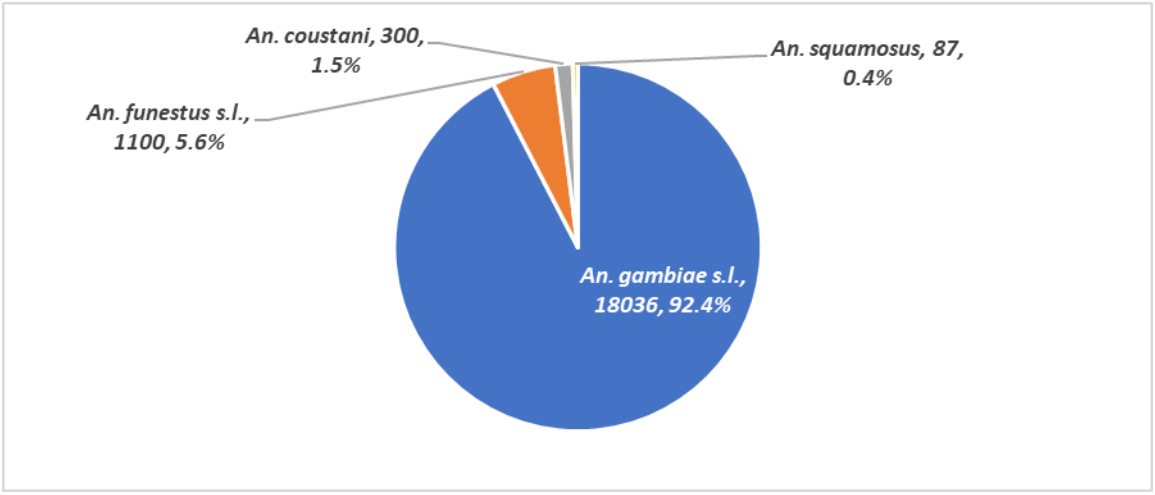


Figure 4: Total *Anopheles* Species Collected by CDC-LT between March 2020 and February 2021

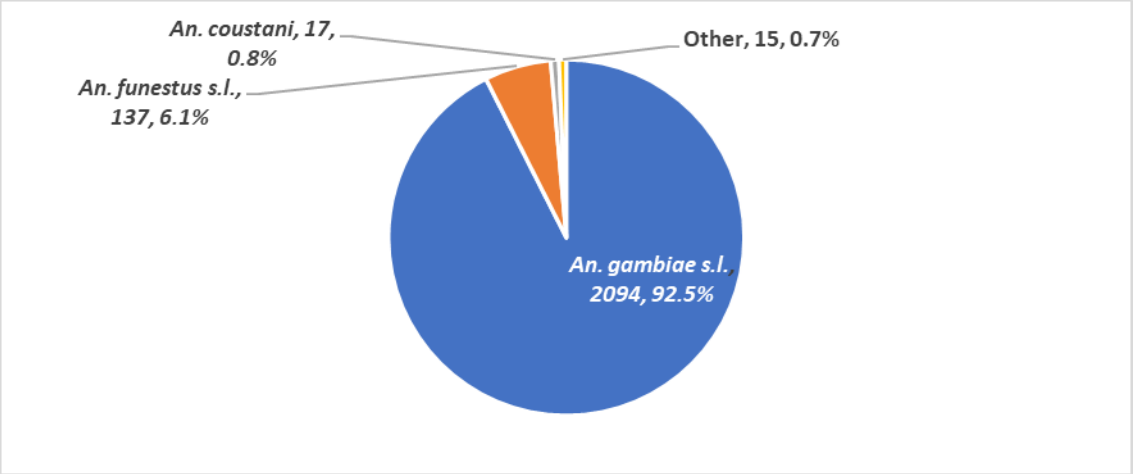
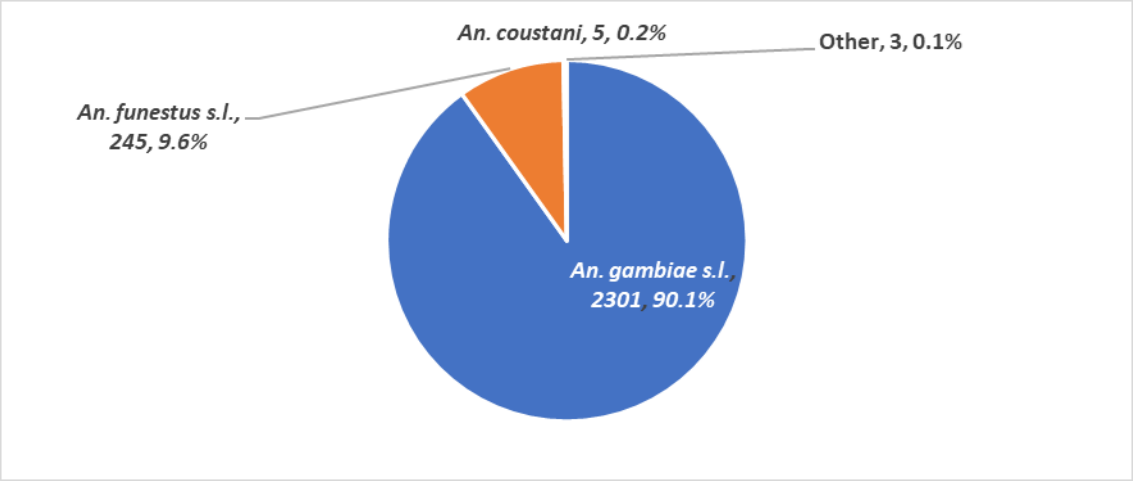


Figure 5: Total *Anopheles* Species Collected by PSC Between March 2020 and February 2021



3.1.2 PYRETHRUM SPRAY COLLECTIONS

INDOOR RESTING DENSITY (PSC COLLECTION)

The indoor density of malaria vectors in Sierra Leone followed the rainfall pattern with peaks observed between May and September.

The Indoor Resting Density (IRD) of *An. gambiae* s.l. per house per day between March 2020 and February 2021 varied across village, district, and month. The mean IRD in all sites peaked between June and August except in Western rural districts where another peak was observed between January-February 2021. Due to the Covid-19 pandemic, there were only few collections in March and June, while no collection was done in April and May 2020.

In Bombali district, the mean IRD was highest in March in Kamaranka rural site (10.3/house/day) (Figure 6). In the rainy season, the highest IRD was in Masongbo peri-urban village (4.7/house/day). In Bo District, the mean IRD remained relatively high but with a declining trend in Largor rural site all through the sampling period with the highest IRD in July (10.4/house/day). The mean resting density was higher in rural compared to peri-urban sites (Figure 6).

In Kono district, the highest mean IRD was in September in Sorie town (peri-urban) with 4.6/house/day and Teikor (rural) with 4.1/house/day. Mean density appeared not to follow the rainfall pattern in Kono.

Figure 6: Mean IRDS of *An. gambiae* s.l. in Bo and Bombali Districts, March 2020–February 2021

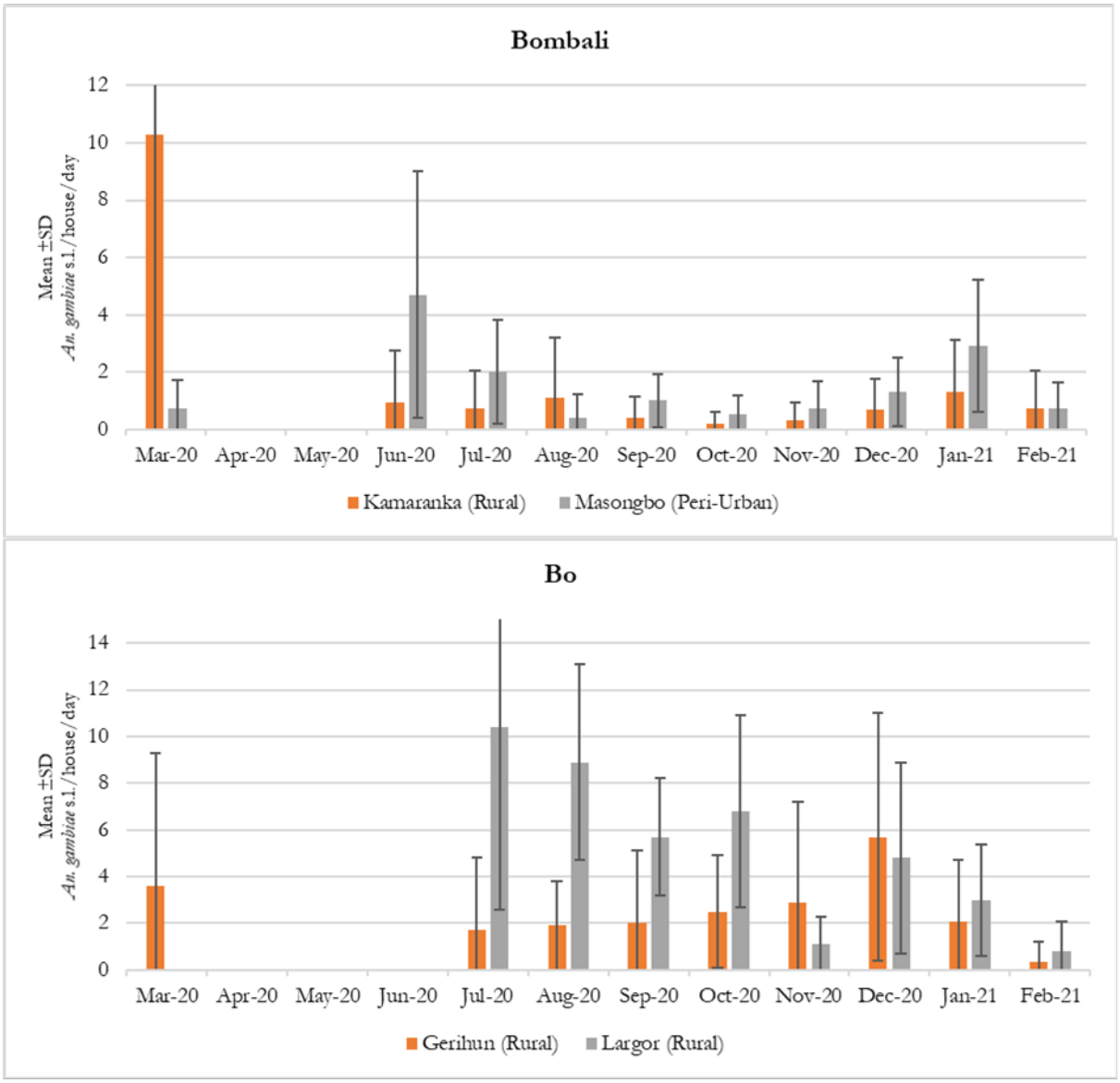
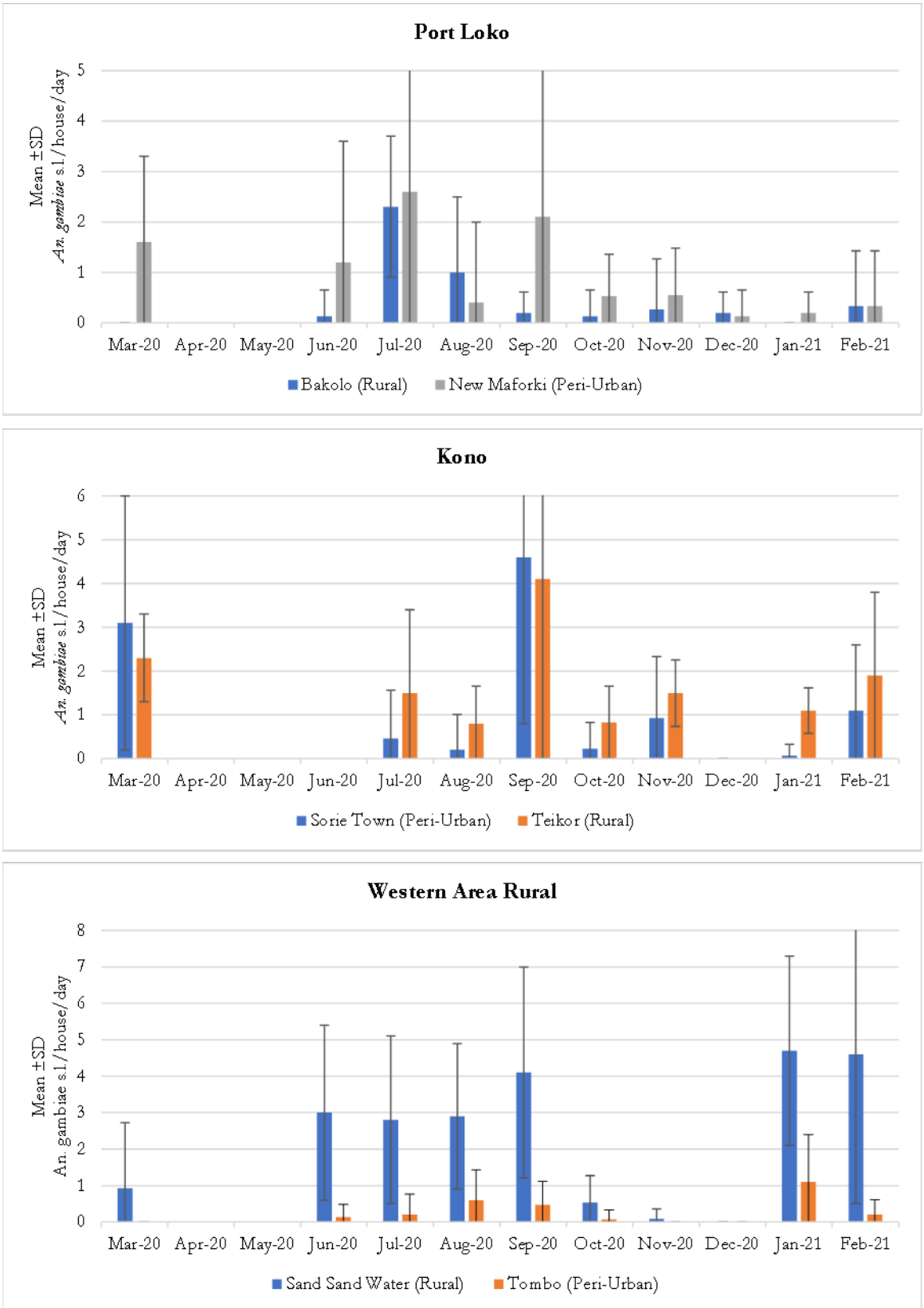


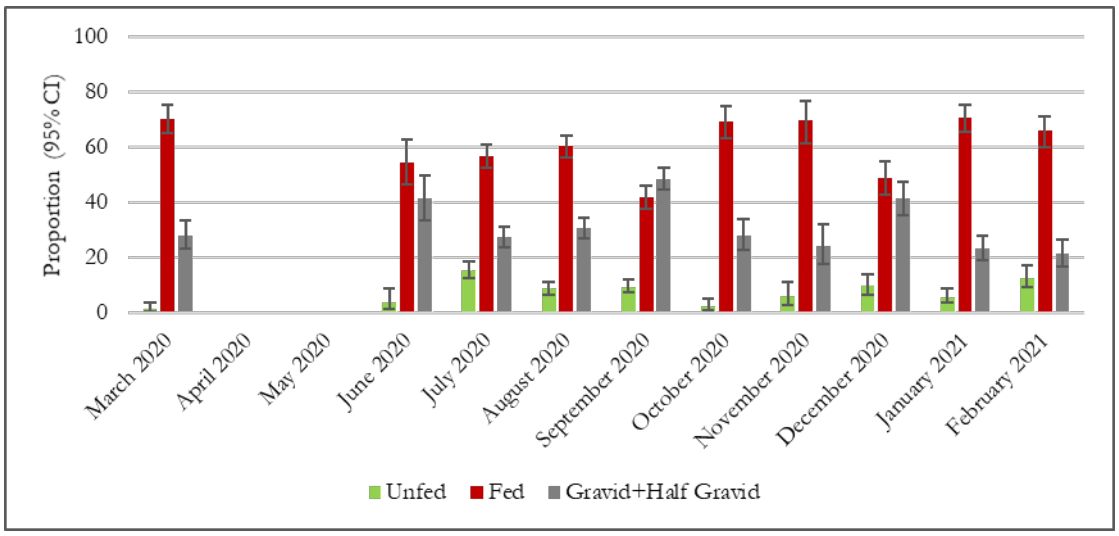
Figure 7: Mean IRDS of *An. gambiae* s.l. in Port Loko, Kono and Western Area Rural Districts, March 2020–February 2021



Abdominal Condition of *An. gambiae* s.l. Collected by PSC

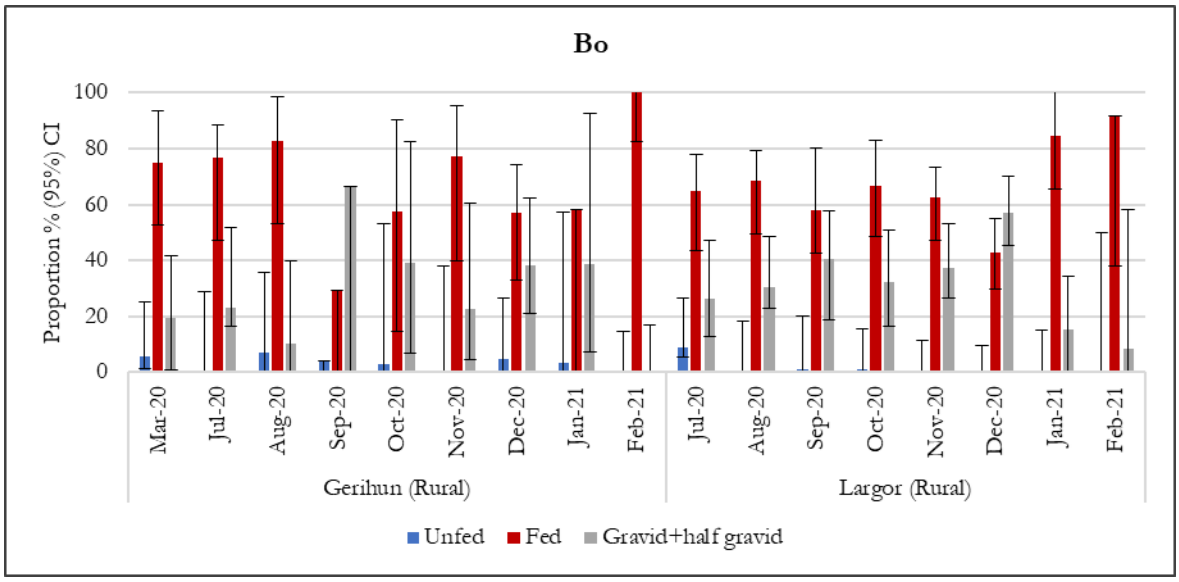
The PSC collected more *An. gambiae* s.l. mosquitoes that were fed compared to unfed/gravid mosquitoes all through the sampling period (Figure 8). There was no relationship/trend between proportion fed with rainy season. The highest number of gravid/half gravid mosquitoes were sampled in September, coinciding with the middle of the rainy season (Figure 6).

Figure 8: Abdominal Condition of *An. gambiae* S.L. Collected by PSC Across all Sentinel Sites, March 2020–February 2021



When disaggregated by district, there were still more blood fed *An. gambiae* s.l. compared to gravid and unfed (Figures 9-10). The proportion of unfed mosquitoes did not follow the rainfall pattern where more unfed mosquitoes would be expected.

Figure 9: Abdominal Condition of *An. gambiae* s.l. in Bo and Bombali, Collected by PSC, March 2020–February 2021



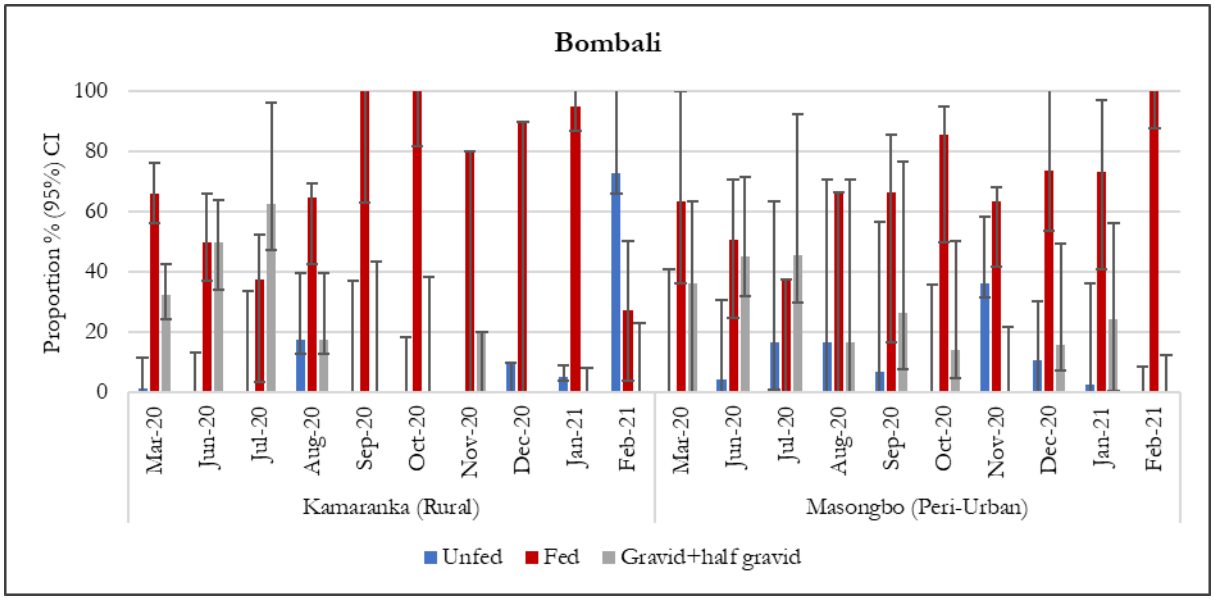
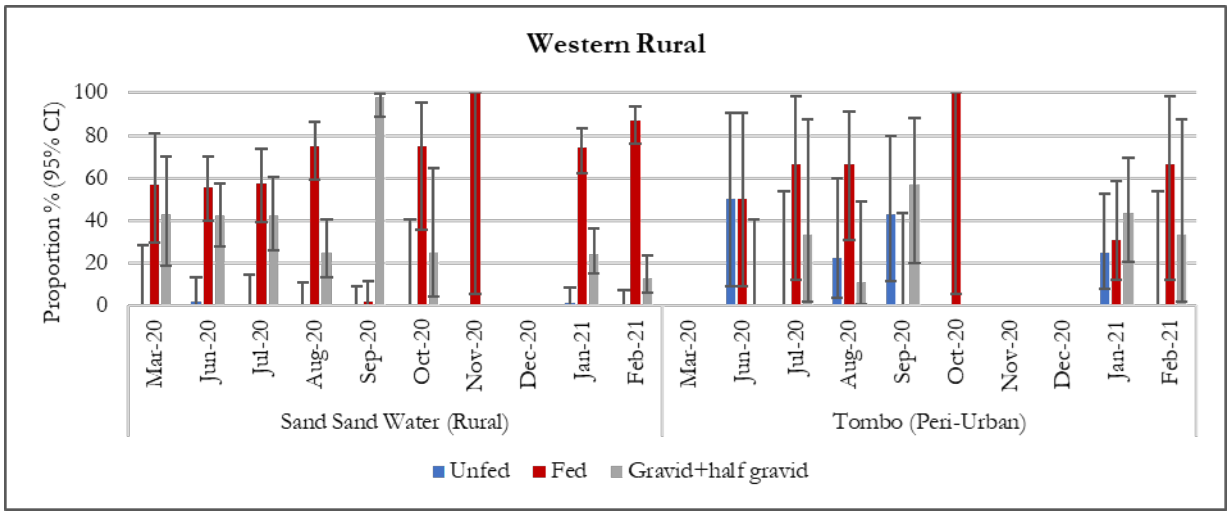
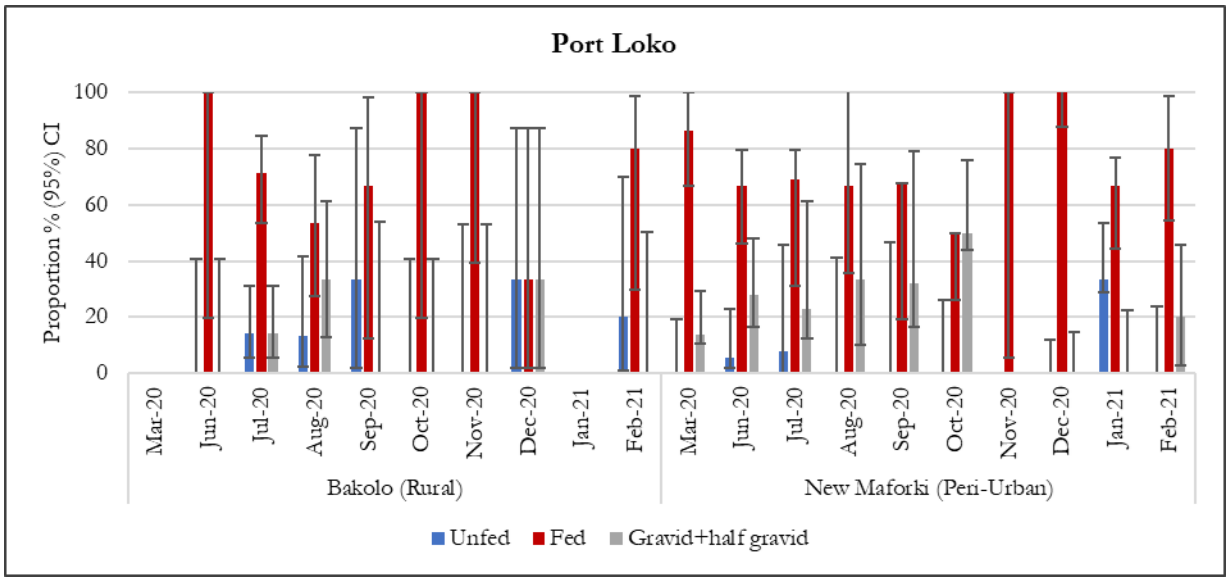
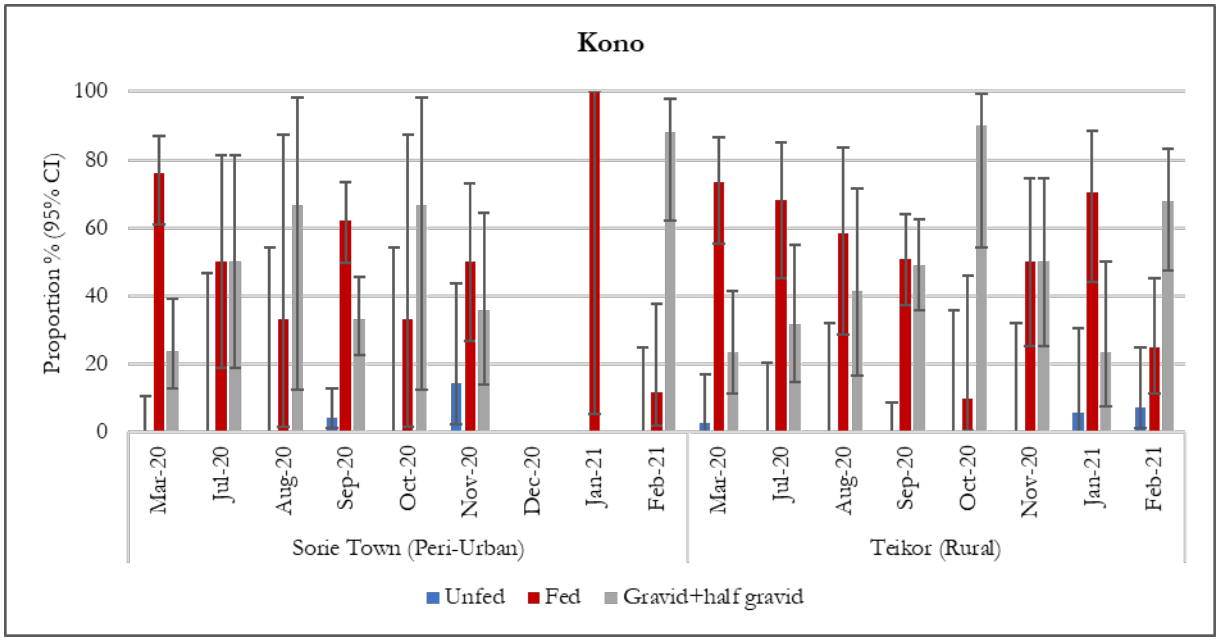


Figure 10: Abdominal Condition of *An. gambiae* s.l. in Western Rural, Port Loko and Kono, Collected by PSC, March 2020–February 2021







3.1.3 ABDOMINAL CONDITION OF *AN. FUNESTUS* GROUP COLLECTED BY PSC

There was no difference between proportion of fed *An. funestus* compared to proportion gravid/half gravid all through the sampling period (Figure 11). The proportion of unfed mosquitoes was always very low apart from July, August, December, and January (Figure 11).

For *An funestus* s.l., the density was very low in all districts apart from Bo and Kono to allow for an estimation of the blood feeding index. Meaningful comparison could only be made in Bo and Kono districts. (Figure 12). In Bo, both fed and gravid were collected more compared to unfed mosquitoes, while in Kono, more mosquitoes that were fed were sampled (Figure 12).

Figure 11: Abdominal Condition of *An. funestus* Group Collected by PSC Across all Sentinel Sites, March 2020- February 2021

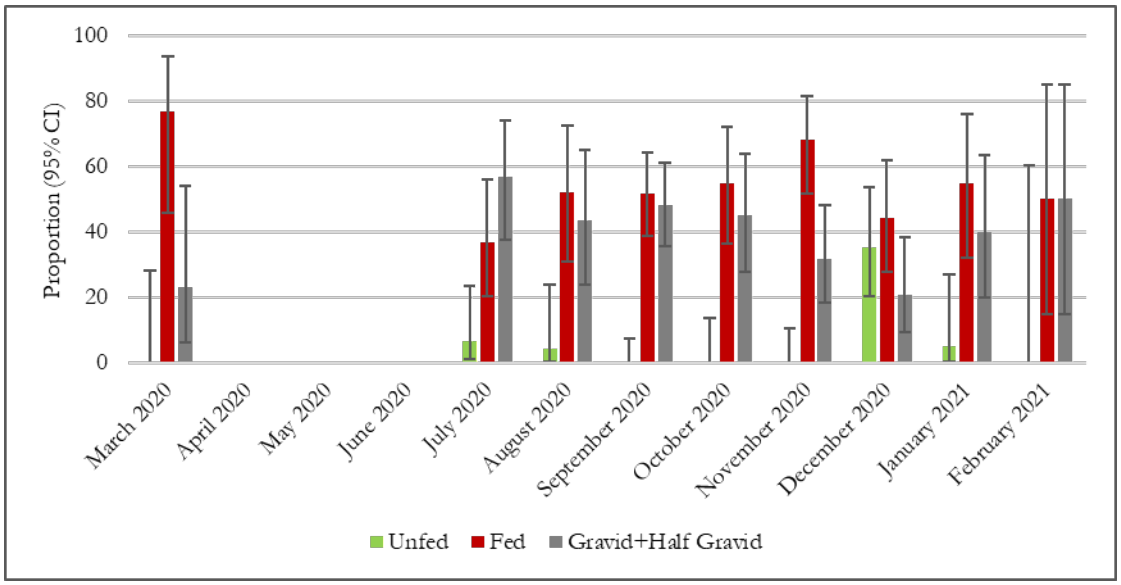
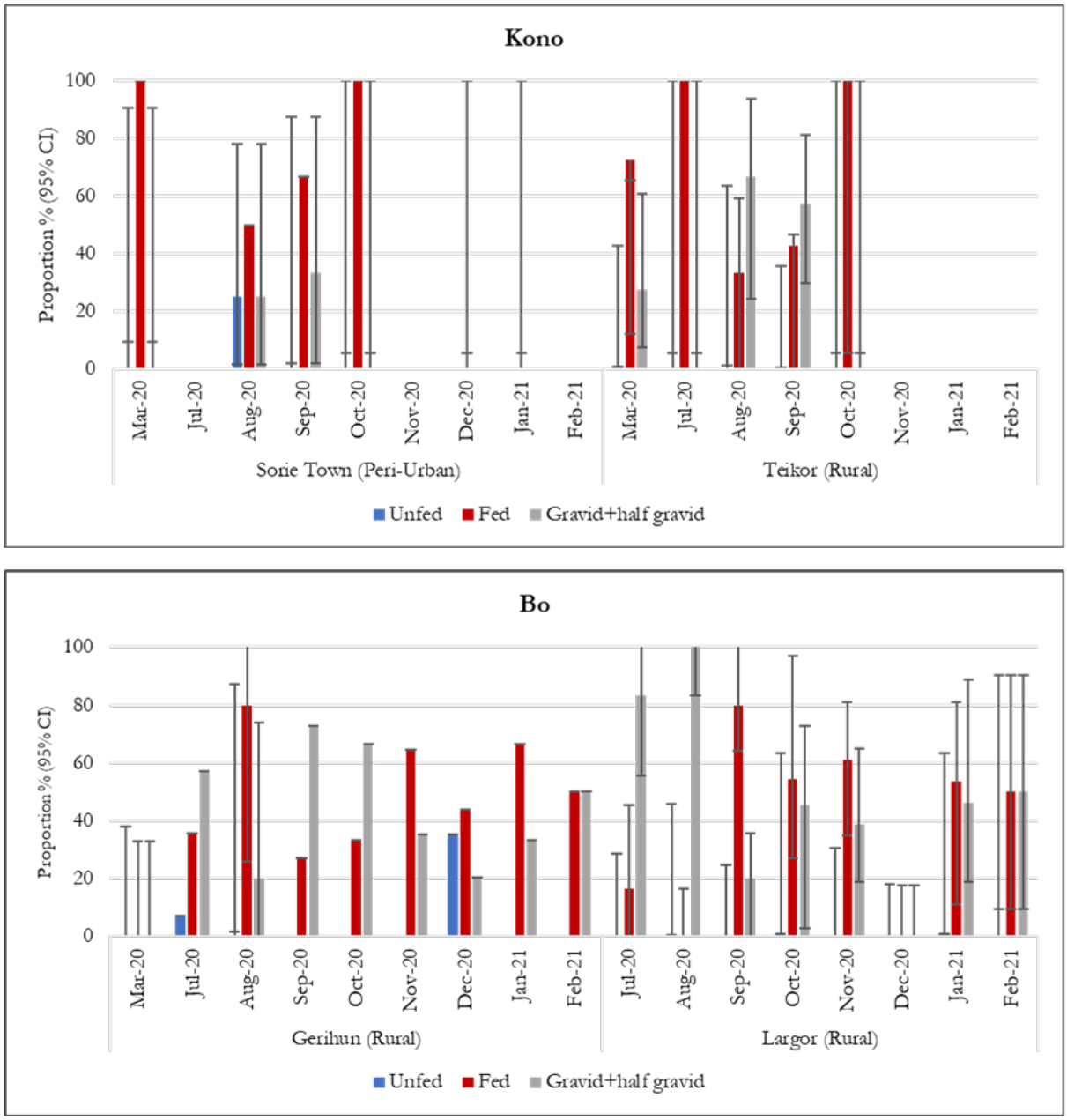


Figure 12: Abdominal Condition of *An. funestus* Group in Bo and Kono, Collected by PSC, March 2020–February 2021



3.1.4 CDC Light Trap Collection

The average density of *An. gambiae* s.l. per trap per night varied by district, site, and month during the sampling period. The highest average density of *An. gambiae* s.l./trap/house was recorded in June in Masongbo, Bombali district (34.4/trap/night) followed by Gerihun (rural) in Bo (6.6./trap/house) in March (Figure 13). Mean density was relatively higher in rural sites compared to peri-urban sites apart from Bombali and Western Area districts (Figure 13).

In Bo district, mean density was highest in March and decreased through September when density rose again, peaking in December (5.4/trap/house). In Bombali, the density remained relatively low averaging 2-3/trap/house (Figure13).

In Western Rural district, the mean density followed the rainfall pattern, with highest density recorded in July (4.7/trap/house). The pattern was similar in Port Loko and Kono districts. (Figure 14).

For *An. funestus* s.l., the few numbers sampled could not allow meaningful comparisons to be made.

Figure 13: Density of *An. gambiae* s.l. from CDC Light Trap Collections in Bo and Bombali Districts, Comparing Rural Vs Peri-Urban Sites, March 2020–February 2021

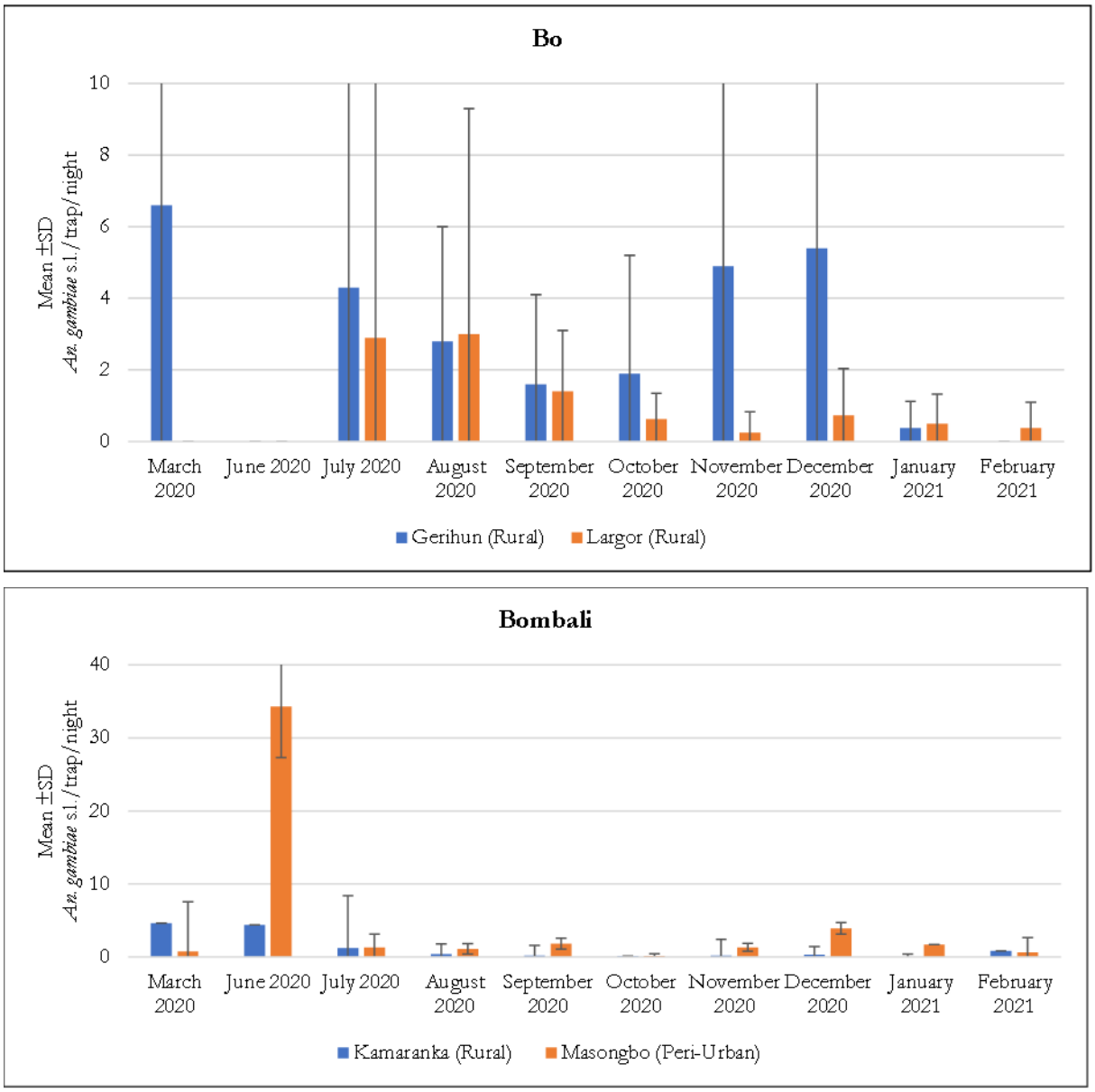
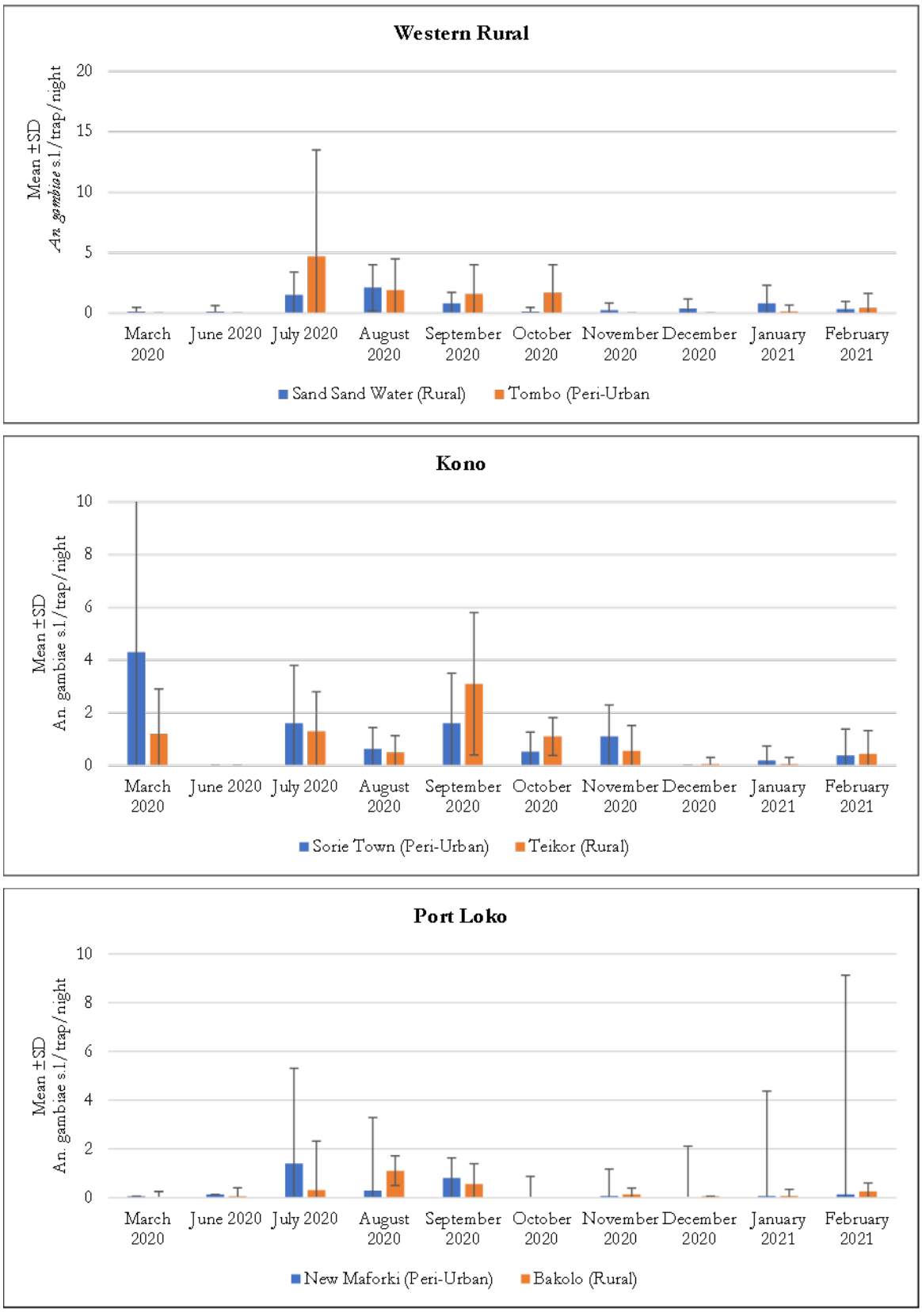


Figure 14: Density of *An. gambiae* s.l. from CDC Light Trap Collections in Western Rural, Kono and Port Loko Districts, Comparing Rural Vs Peri-Urban Sites, March 2020–February 2021



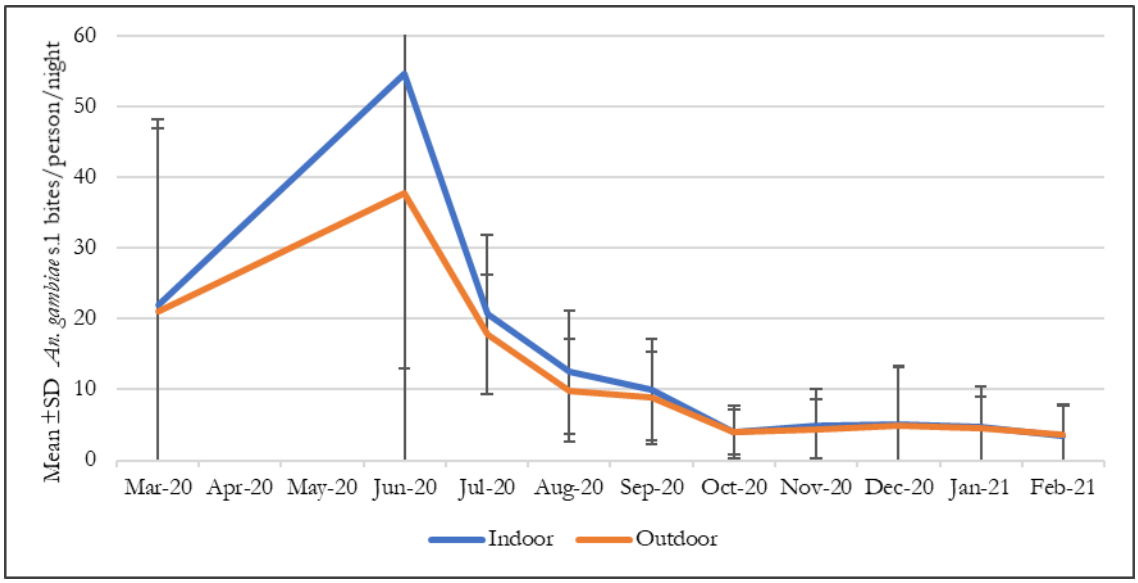
Human Landing Collections

Human Biting Rate (HBR): The HBR of *An. gambiae* s.l. varied by village, district, and month through the sampling period. There was no significant difference between outdoor and indoor biting patterns (Figure 15). When data was aggregated across all sites, the HBR followed the rainfall pattern, peaking in June and declining through February 2021 (Figure 15).

The highest indoor HBR/person/night was recorded in June in Masongbo, Bombali district with 175.5 bites/person/night (Figure 16). In all districts, the HBR followed the rainfall pattern with a peak observed between June to September and declining into the dry season, apart from Kono that had peaks in March and September (Figures 16 and 17). In Bo, more mosquitoes were collected in rural site while in Bombali, peri-urban site (Figure 16).

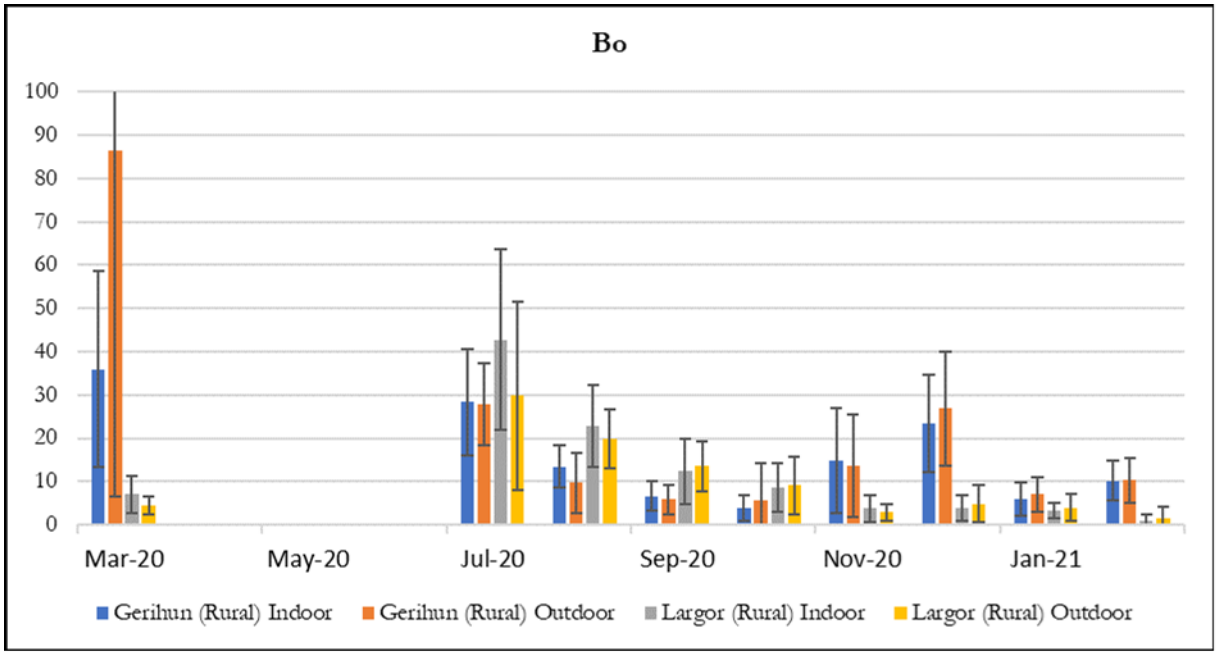
The HBR of *An funestus* s.l. was low and could only be compared in Bo, Kono and Port Loko districts(Figure 18). The highest HBR recorded was indoor in Teikor, Kono district, 9.1 bites/person/night (Figure 18). The highest were recorded in July in Bo, August in Kono and June in Port Loko districts (Figure 18).

Figure 15: *An. gambiae* s.l. Mean HBR across all Sentinel Sites, March 2019–February 2020



Biting Location: There was no difference between indoor and outdoor biting for *An. gambiae* s.l. For *An. funestus* s.l., in Gerihun in Bo District, HBR was higher indoors compared to outdoors, but not significantly (Figure 18).

Figure 16: *An. gambiae* s.l. Mean HBR in Bo and Bombali Districts, March 2020–February 2021



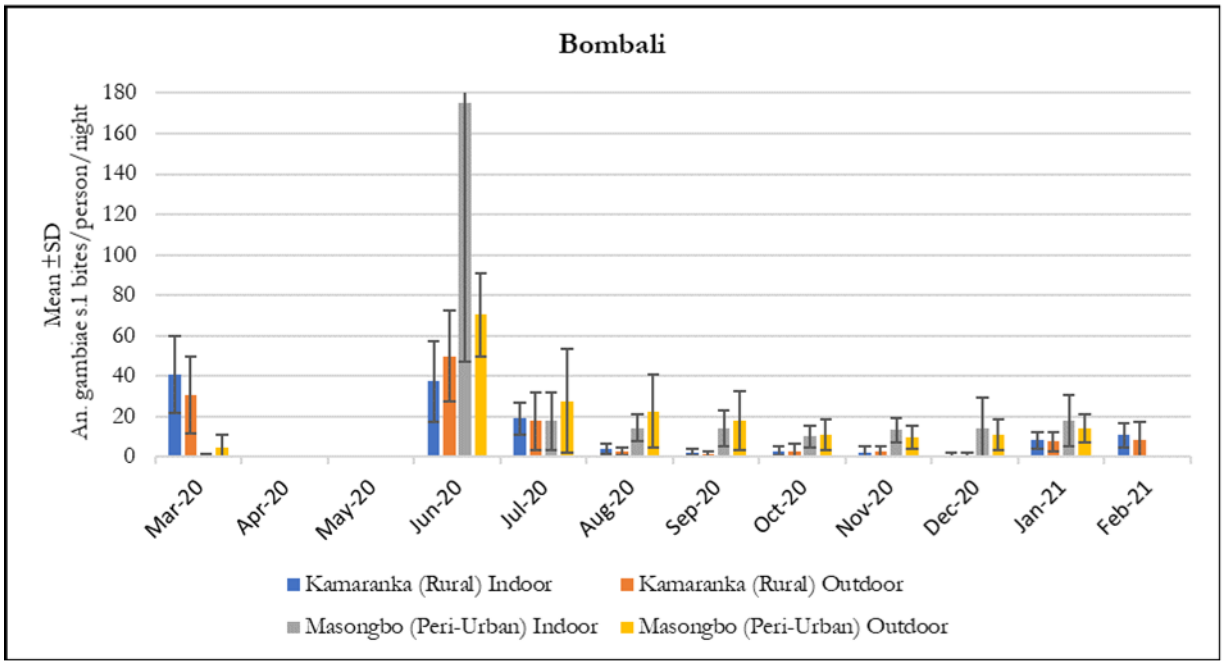


Figure 17: *An. gambiae* s.l. Mean HBR in Western Rural, Kono and Port Loko Districts, March 2020–February 2021

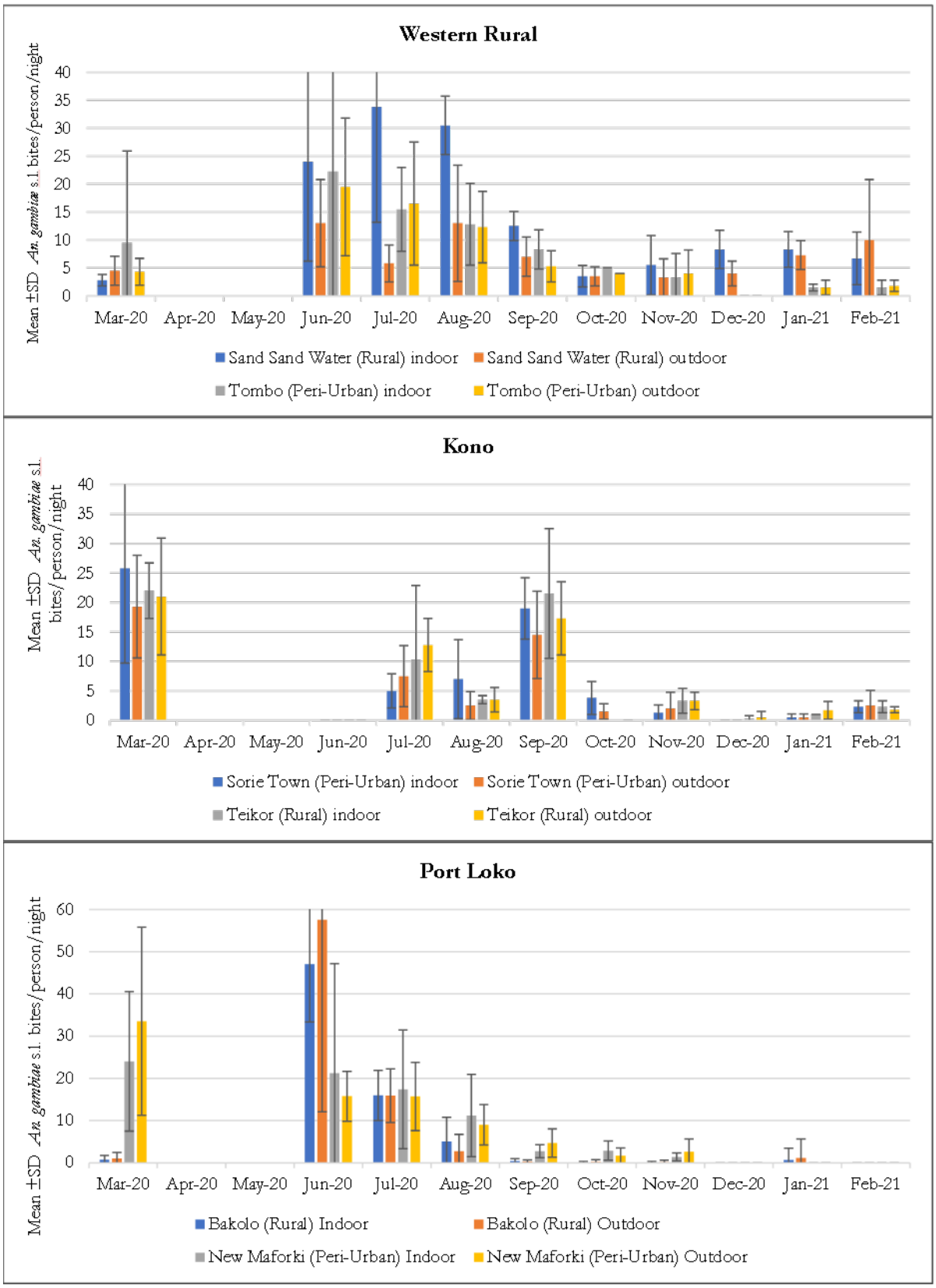
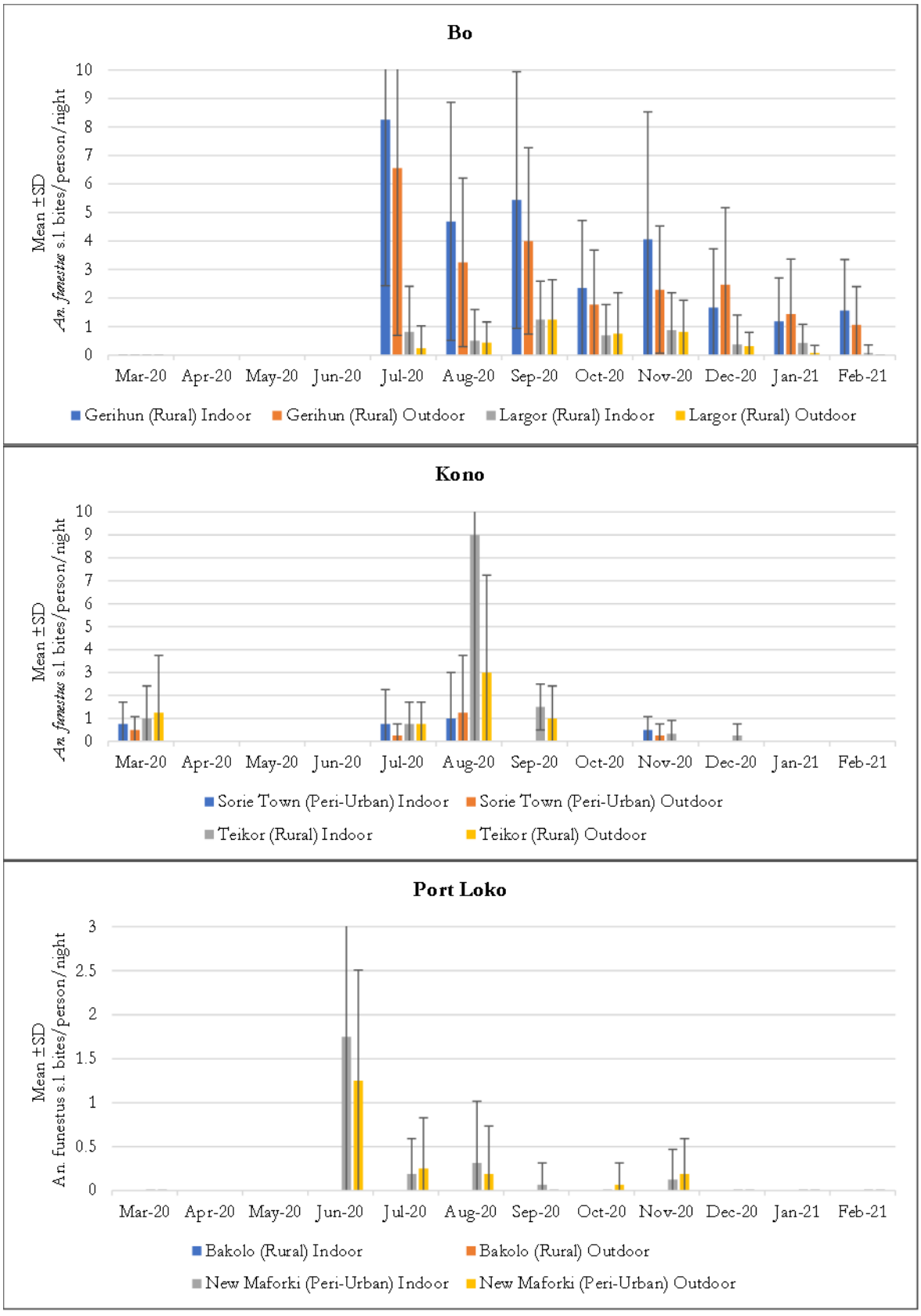


Figure 18: *An. funestus* Group Mean HBR in Bo and Kono and Port Loko Districts, March 2020–February 2021



Biting Time: Biting activity of *An. gambiae* s.l. peaked at different times across the sites and districts. In Bo and Bombali, biting activity was highest between 11:00 pm-5:00 am, peaking at 3:00-4:00 am for Bo and 2:00-3:00 am for Bombali (Figure 19). The majority of households are expected to be in bed and using ITNs at that time and given that peak biting activity falls during this time, it is crucial that people living in these areas continue using ITNs while in bed.

In Port Loko and Kono, bites/person/night was around one with no clear pattern of biting activity (Figure 20). In Western Rural, biting activity started late in the night between 11pm and midnight with peaks between 2am -3am, and 5am-6am (Figure 20).

For *An. funestus* s.l., the density was too low to allow comparisons between sites and month (Figure 21).

Figure 19: *An. gambiae* s.l. Biting Time in Bo and Bombali Districts, Comparing Indoor Vs Outdoor, March 2020–February 2021

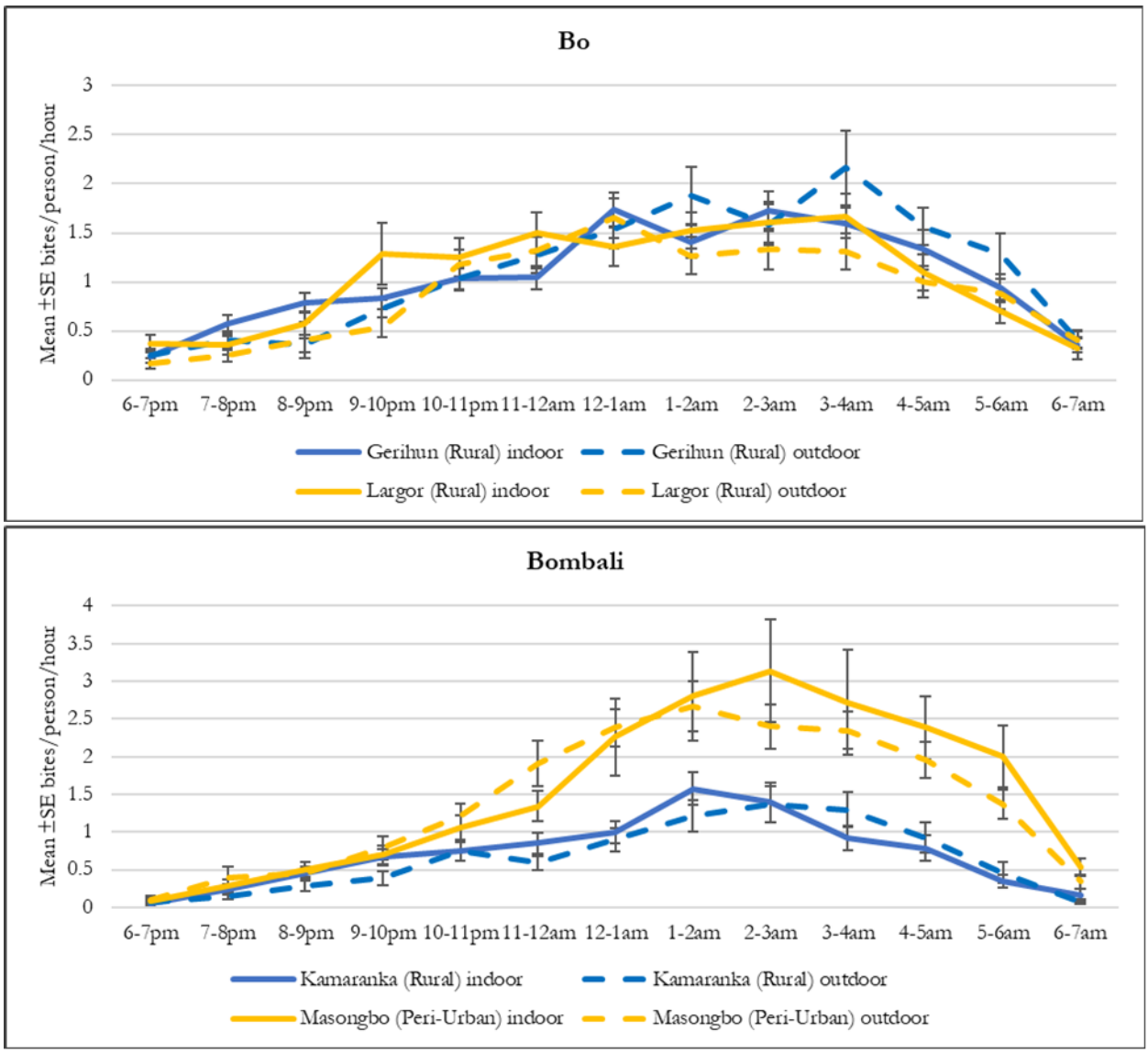
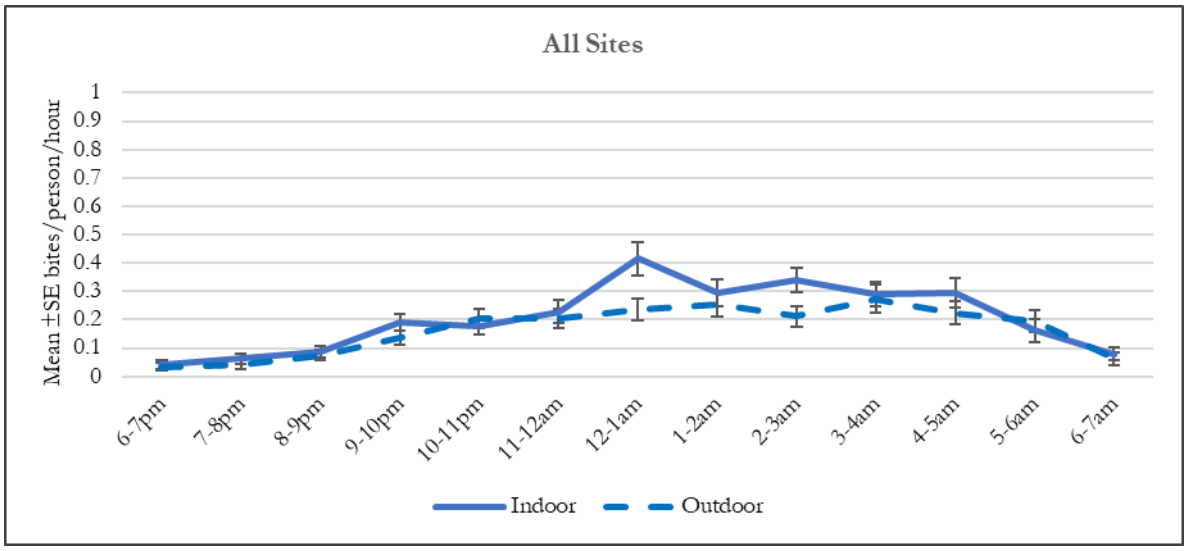


Figure 20: *An. gambiae* s.l. Biting Time in Port Loko, Kono and Western Area Rural Districts, Comparing Indoor Vs Outdoor, March 2020–February 2021

Figure 21: *An. funestus* Group Biting Time Across all Sentinel Sites, Comparing Indoor and Outdoor, March 2020–February 2021

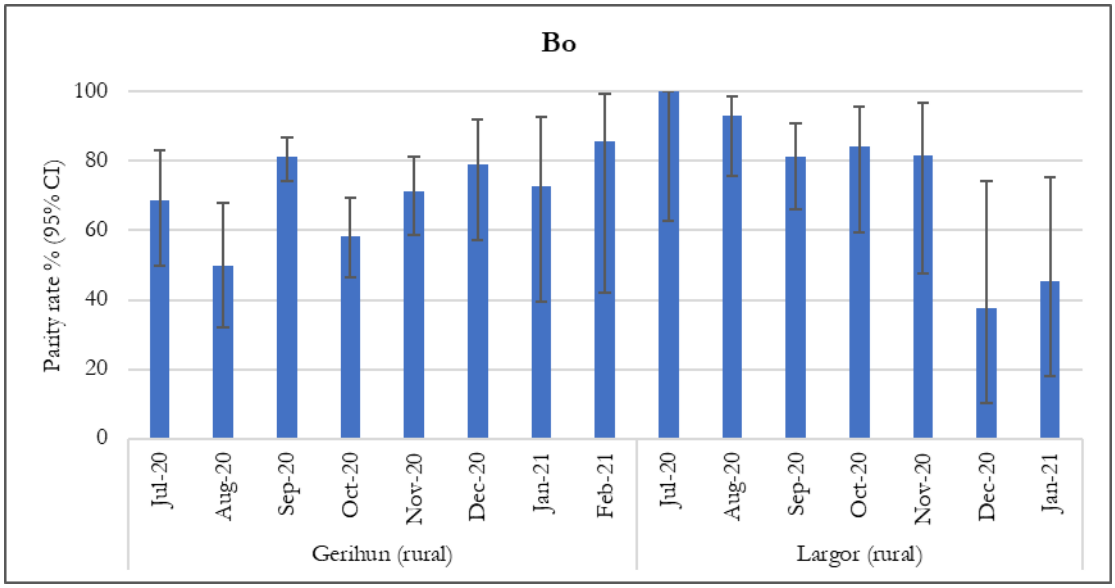


3.1.5 PARITY STATUS OF MALARIA VECTORS COLLECTED BY HLC AND CDC-LT

A total of 1,557 malaria vectors were dissected to determine the parity status, whether they had laid eggs or not (Annex E). Of these, 97.4 % (1,557) came from HLC while 2.6% (40) came from CDC-LT. *Anopheles gambiae* s.l. were the majority of species (98.8 %; 1,538) followed by *An. funestus* group (1.2 %; 19). From the 1,557 dissected mosquitoes, 54.5% (849) were parous, 24.4% (379) nulliparous while 21.1 % (329) were undetermined due to incorrect dissection that destroyed the ovary. For those successfully identified, 69.1% (849) were parous while 30.9% (379) were nulliparous.

Parity status was examined by sentinel site and district by month. In all the districts, there was no clear trend in parity status of the mosquitoes that were dissected apart from Western Rural Area District where, parity rate appeared to follow the expected pattern where parity rate increases as rainfall reduces (Figures 22 and 23).

Figure 22: *An. gambiae* s.l. Parity status in Bo and Bombali Districts, comparing Peri-urban and Rural Sites, June 2020–February 2021



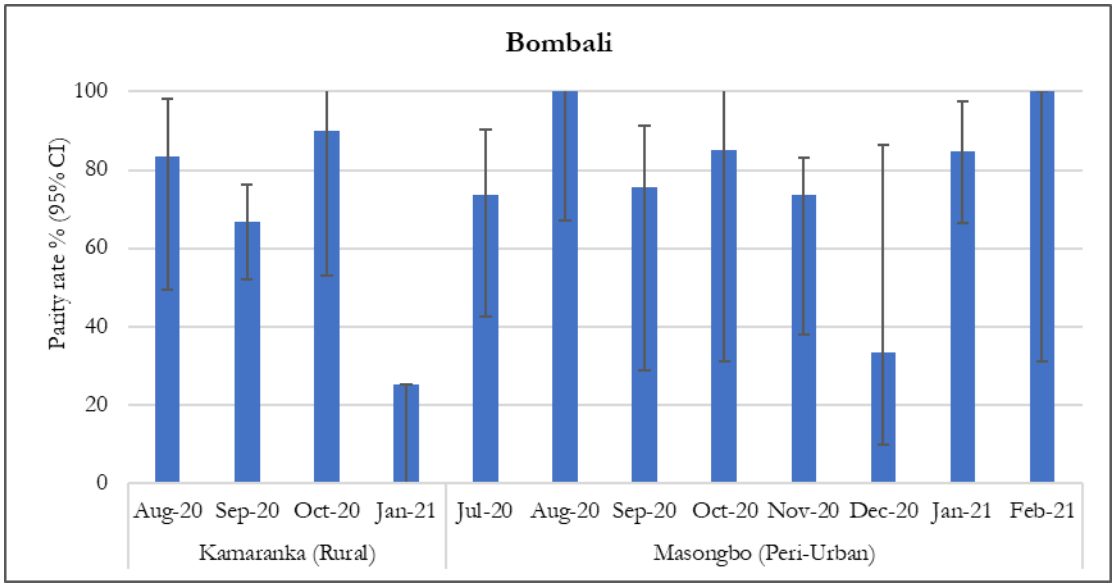
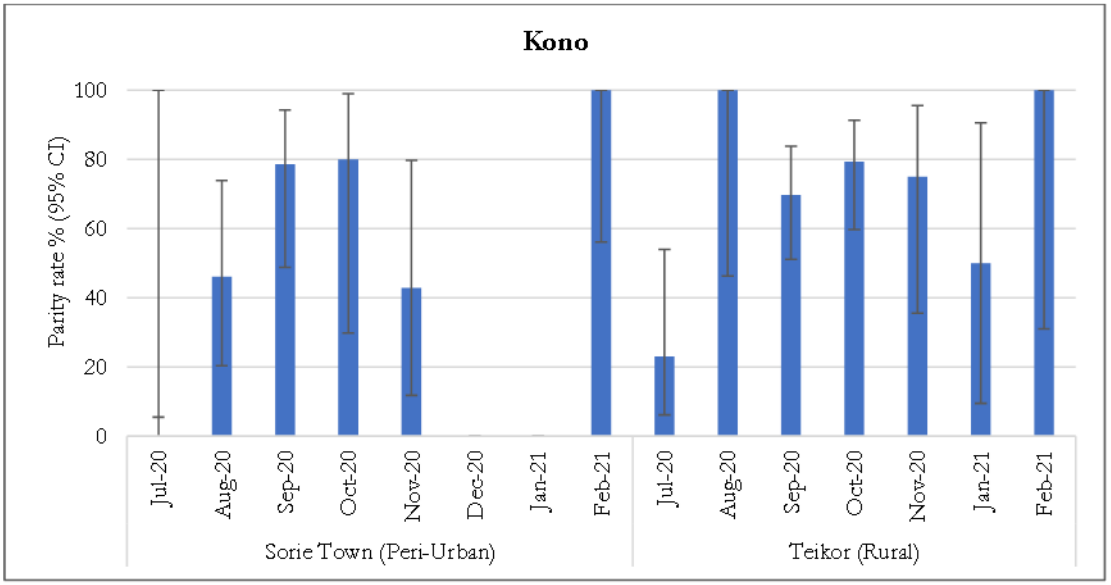
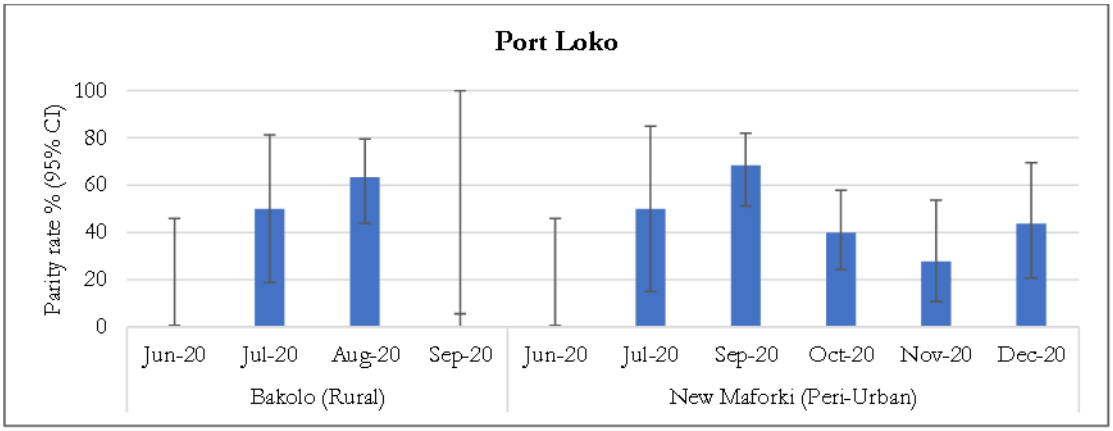
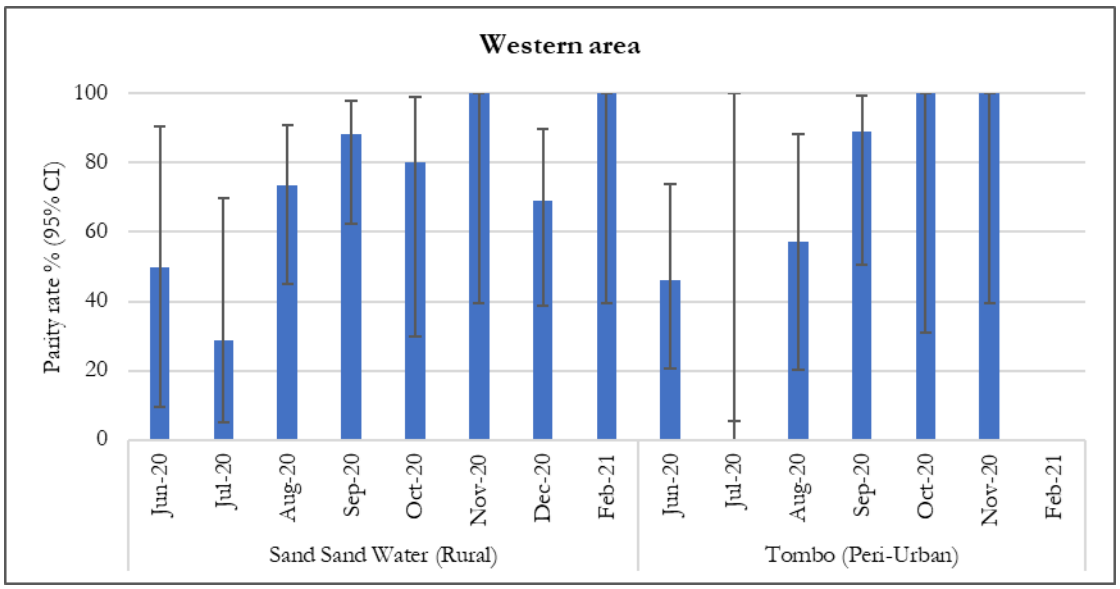


Figure 23: ***An. gambiae*** **s.l. Parity Status in Kono, Port Loko and Western Rural Area Districts, Comparing Peri-Urban and Rural Sites, June 2020–February 2021**







3.2 Laboratory Analysis for Species Identification, Screening for Sporozoite Infection, Molecular Markers of Insecticide Resistance and Blood Meal Source.

A subset of samples collected using PSC, CDC-LT, HLC were sent to CRID in Cameroon for molecular analysis to identify sibling species, screen for sporozoite infection and identify source of blood meal for the fed mosquitoes. Another subset of those collected through larvae and tested for phenotypic resistance were also sent to CRID to screen for molecular markers of insecticide resistance and identify sibling species. This section reports on results from samples already processed thus far. A complete analysis with all samples screened shall be added in the next draft submission of this report or as separate addendum report.

3.2.1 Species Identification

Thus far, for routine sentinel sites, a total of 5,638 *Anopheles* mosquitoes sampled between March 2020 and February 2021 have been analyzed for molecular species identification. Among these, 4,604 came from HLC collection, 852 from PSC & CDC-LT collection and 168 from phenotypic WHO susceptibility bioassays (Table 3).

Table 3: Number of Samples Analyzed for Molecular Species Identification, March 2020-February 2021

Districts	HLC	PSC and CDC	Resistance	Total
Bo	1253	317	59	1639
Bombali	680	276	109	1065
Kono	341	69	0	410
Port Loko	1340	130	0	1474
Western Rural	990	60	0	1050
Total	**4,604**	**852**	**168**	**5,638**

Within the *An. gambiae* s.l. complex, majority of the samples screened were *An. coluzzii* (64.5%) followed by *An. gambiae* (34.3%), *An. melas* (1.0%) and hybrid between *An. coluzzii* and *An. gambiae* ,0.2%. (Figure 24). For *An. funestus* group, majority were *An. funestus* s.s. (92.5%), followed by *An. leesoni* (7.0%) and *An. parensis* (0.5%) (Figure 25).

Figure 24: Molecular Species Distribution of *An. gambiae* s.l. Across Sampling Methods and Sites, March 2020–February 2021

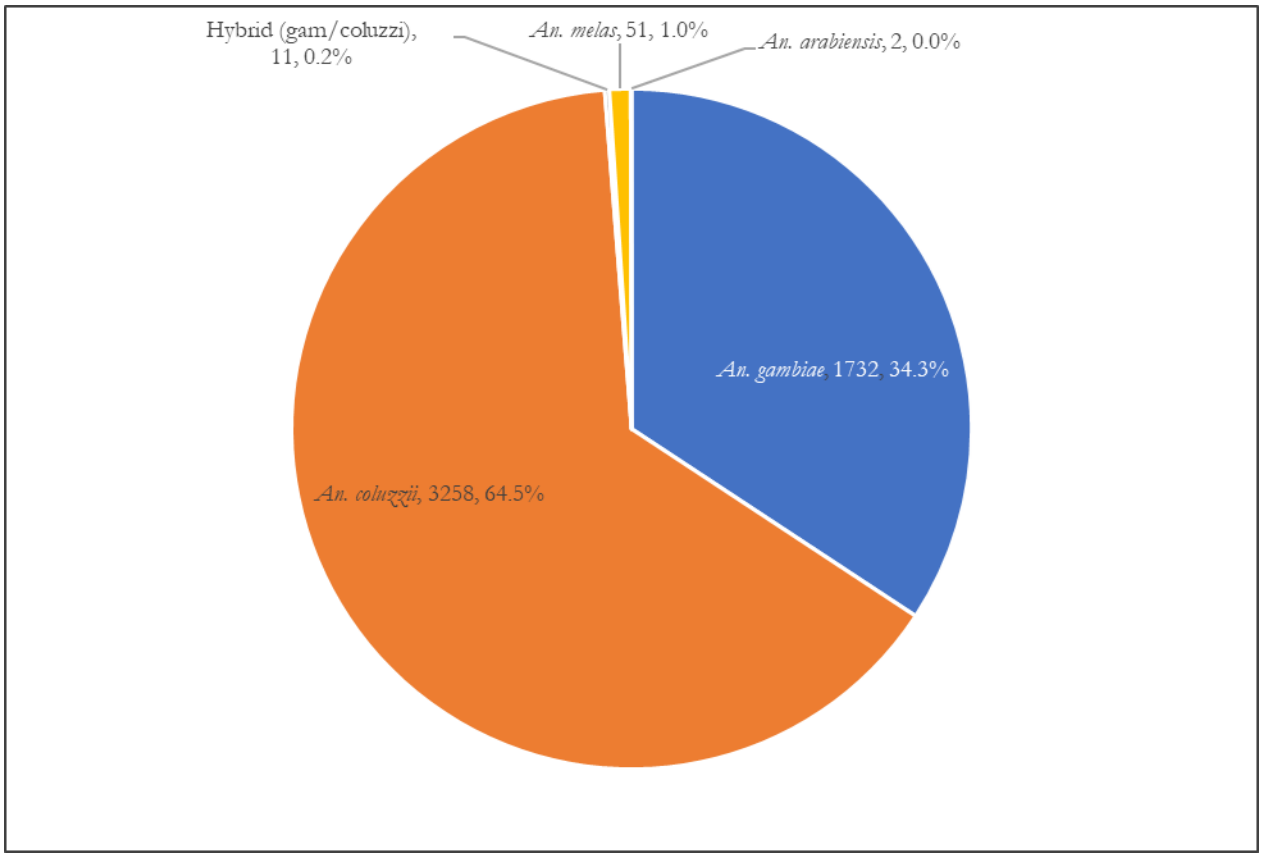
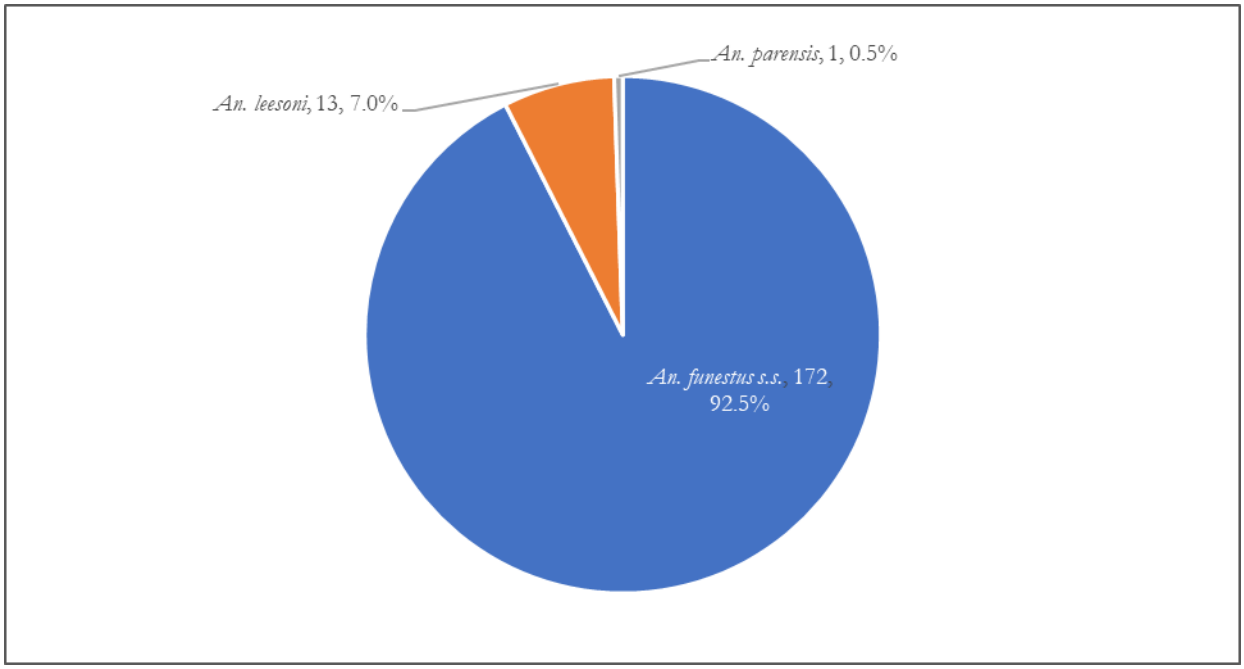


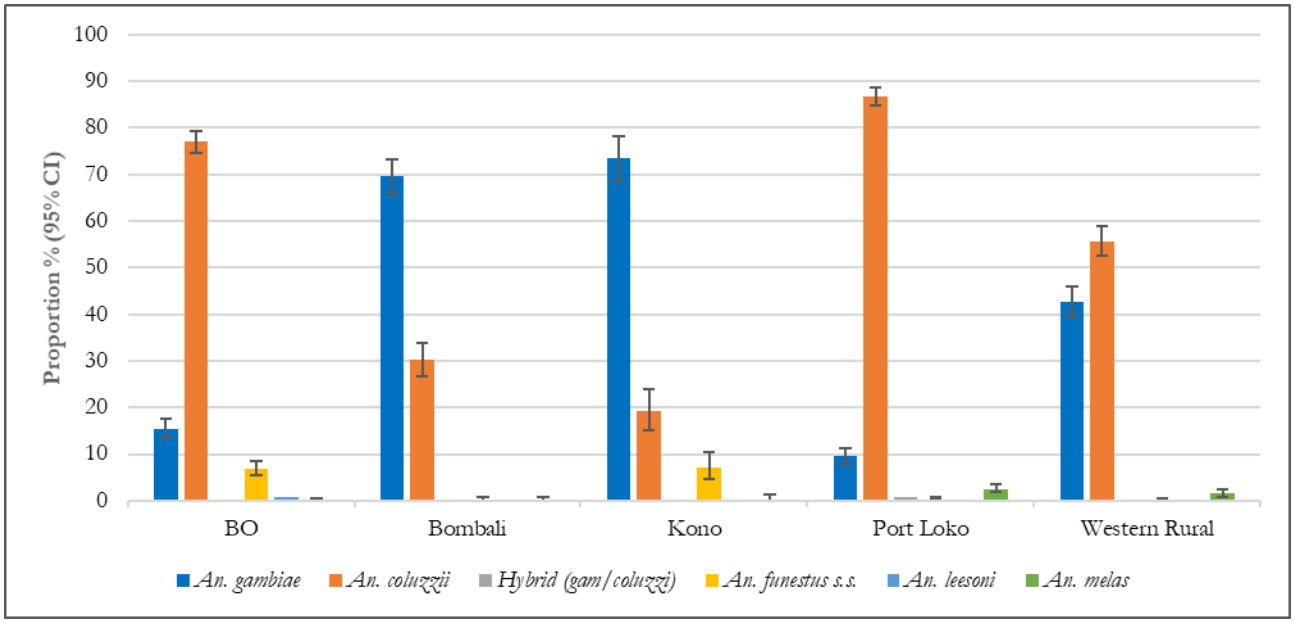
Figure 25: Molecular Species Distribution of *An. funestus* group Across Sampling Methods and Sites, March 2020–February 2021



3.2.2 SPECIES DISTRIBUTION BY DISTRICT

HLC collections: In Bombali and Kono districts, *An. gambiae* was the predominant species while *An. coluzzii* was predominant in Bo, Port Loko and Western Rural (Figure 26). *Anopheles melas* was sampled in Port Loko district that is adjacent to the coastal ecosystem with brackish waters breeding grounds (Figure 26). A few hybrids of *An. gambiae* and *An. coluzzii* were detected in Bombali and Port Loko and this is consistent with documented levels of hybridization in the *An. gambiae* complex of the Far West distribution scale. The *An. funestus* s.s. and *An. leesoni* were detected in Bo and Kono districts (Figure 26).

Figure 26: Molecular Species Distribution of Samples Collected by HLC by District, March 2020–February 2021



PSC and CDC-LT Collections: Out of the 996 samples analyzed, 204 samples failed to amplify. Of those that successfully amplified, 726 were from *An. gambiae* complex, 66 from the *An. funestus* group. Within the *An. gambiae* complex (Figure 27), *An. coluzzii* was the most abundant (56.89%; 413), followed by *An. gambiae* (42.84%; 311), whereas only two samples were identified as *An. arabiensis* (0.28%; 2) (Figure 27). Within the *An. funestus* group, majority were *An. funestus* s.s. (92.4%) followed by *An. leesoni* (6.1%) and *An. parensis* (1.5%), (Figure 28). Further distribution per district will be presented in the addendum report.

Figure 27: Molecular Species Distribution of *An. gambiae* s.l. Samples Collected by PSC & CDC-LT Across Districts, March 2020–February 2021

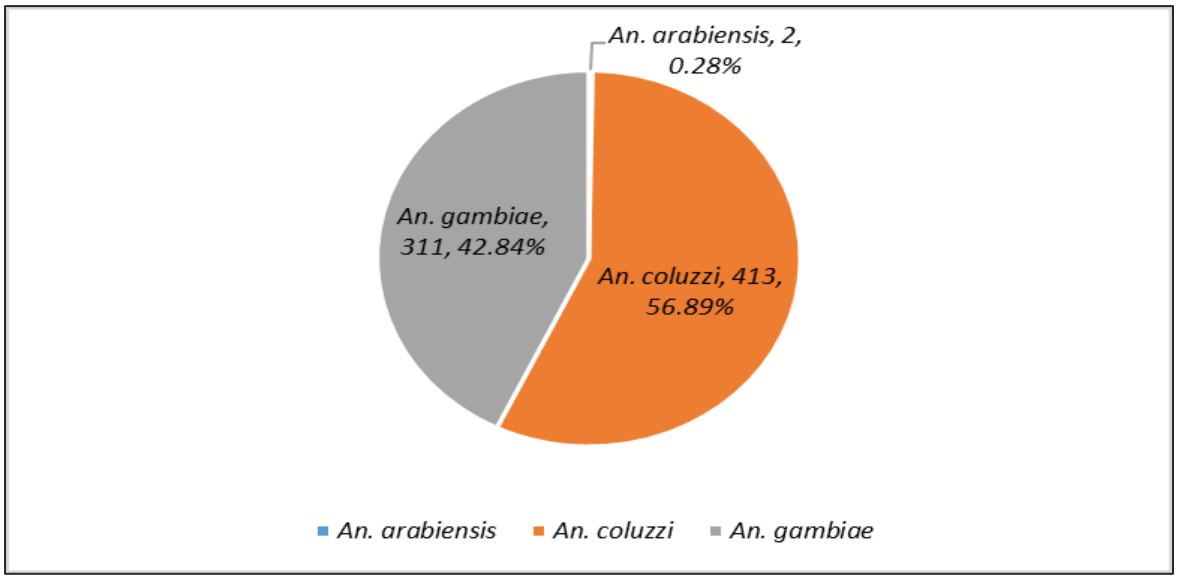
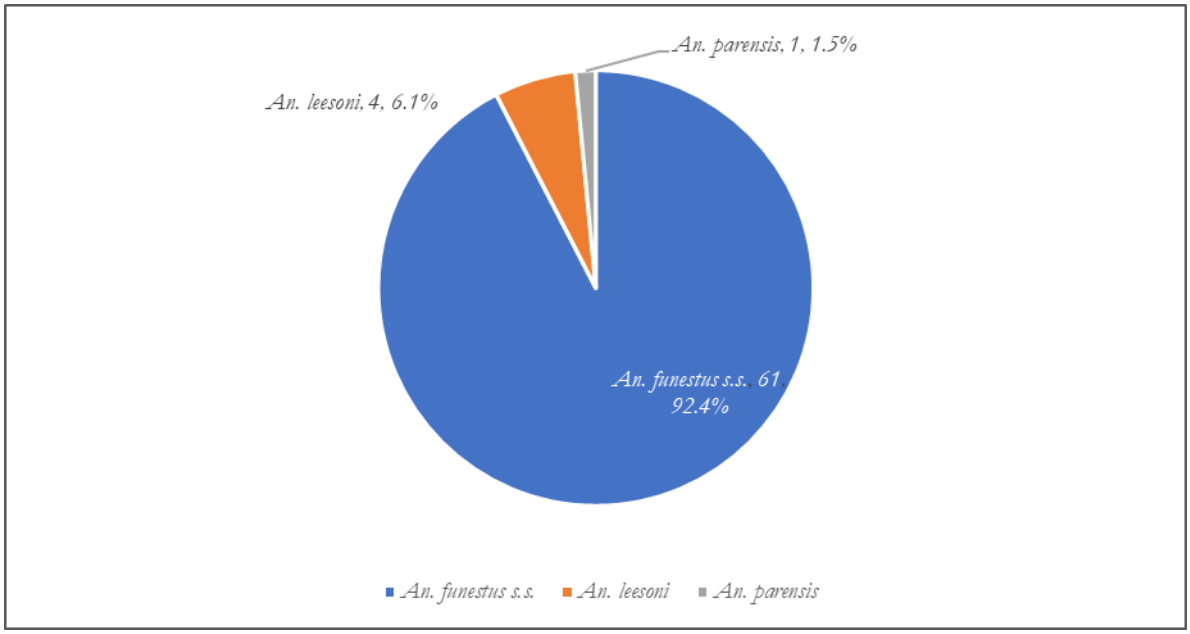


Figure 28: Molecular Species Distribution of *An. funestus* group Samples Collected by PSC & CDC-LT across Districts, March 2020–February 2021



3.2.3 *Plasmodium* Infection

Screening of vectors for plasmodium infection is ongoing and will be reported as an addendum.

3.2.4 Entomological Inoculation Rate

Screening of mosquitoes for infection with *Plasmodium* is ongoing and will be reported as an addendum.

3.2.5 Blood Meal Origin

A total of 996 blood fed mosquito samples from CDC-LT and PSC collections were screened with ELISA for source of blood meal. Of these, blood meal source was successfully identified for 895 blood fed

mosquitoes. The remaining 101 could not be identified (Table 4). Of the 895 mosquitoes with known blood meal source, 736 were identified to the species level using PCR. The remaining 159 were morphologically identified to the species complex level: *An. gambiae* s.l. (151) and *An. funestus* s.l. (8). Overall, 840 (93.9%) fed on human blood, 46 (5.1%) on mixed human and animal blood and 9 (1.0%) on animal blood. The Human Blood index (HBI) was high in all members of *An. gambiae* and *An. funestus* groups identified with values ranging from 84 in *An. gambiae* s.l. in Bo to 100 in several districts (Table 5). The data indicates that the malaria vectors in Sierra Leone are highly anthropophagic with 99.0% of them fed either fully or partly on human blood (Table 4 and 5).

Table 4: Host Preference for *Anopheles* Mosquitoes Across Districts, March 2020-February 2021

Species	Blood meal sources				
	Animal	Human	Mixed (Animal/ Human)	No Source	Total
An. arabiensis		2			2
An. coluzzii	3	373	14	23	413
An. funestus s.s.		53	4	4	61
An. gambiae	3	267	14	27	311
An. leesoni		3		1	4
An. parensis				1	1
An. gambiae s.l.	2	137	12	40	191
An. funestus s.l.	1	5	2	5	13
Total	**9**	**840**	**46**	**101**	**996**

Table 5: Human Blood Index by District from PSC & Light Trap Collections, March 2020 – February 2021

District	Species	Total	Animal	Mixed (Animal/ Human)	Human	Human Blood Index (% Human)
Bo	*An. gambiae*	50	1	1	48	96.0
	An. coluzzii	151	0	3	148	98.0
	An. funestus s.s.	41	0	4	37	90.2
	An. leesoni	3	0	0	3	100.0
	An. gambiae s.l.	50	0	8	42	84.0
	An. funestus s.l.	3	0	0	3	100.0
Bombali	*An. arabiensis*	1	0	0	1	100.0
	An. coluzzii	59	0	5	54	91.5
	An. gambiae	145	1	9	135	93.1
	An. funestus s.s.	2	0	0	2	100.0
	An. gambiae s.l.	34	0	1	33	97.1
	An. funestus s.l.	2	1	1	0	0.0
Kono	*An. arabiensis*	1	0	0	1	100.0
	An. coluzzii	14	0	0	14	100.0
	An. gambiae	18	0	0	18	100.0
	An. funestus s.s.	14	0	0	14	100.0
	An. gambiae s.l.	10	0	0	10	100.0
	An. funestus s.l.	1	0	0	1	100.0

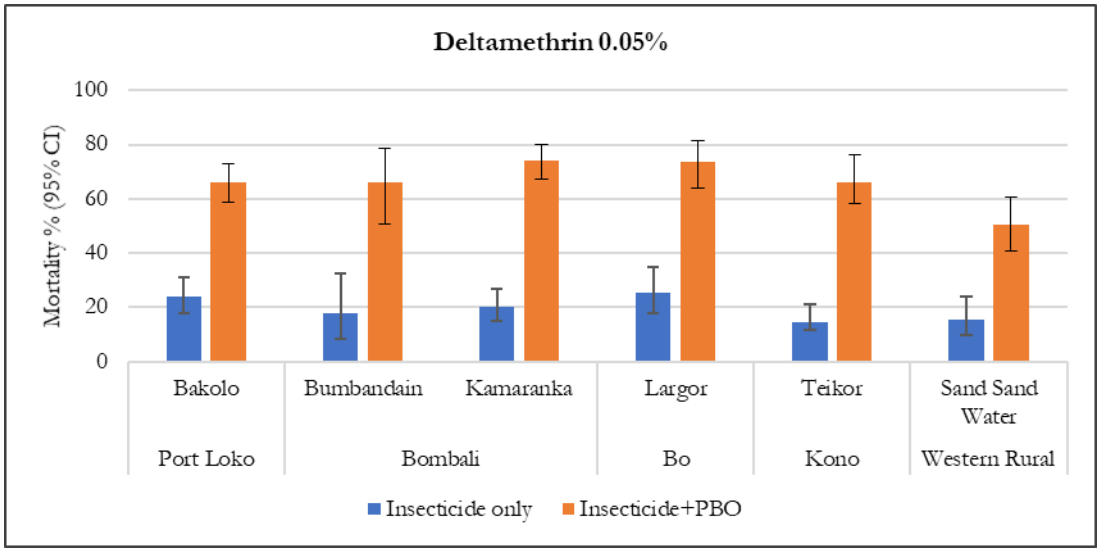
District	Species	Total	Animal	Mixed (Animal/ Human)	Human	Human Blood Index (% Human)
Port Loko	*An. coluzzii*	86	2	2	82	95.3
	An. gambiae	10	0	0	10	100.0
	An. gambiae s.l.	20	1	2	17	85.0
	An. funestus s.l.	0	0	0	0	0
Western Rural	*An. coluzzii*	14	0	0	14	100.0
	An. gambiae	34	1	1	32	94.1
	An. gambiae s.l.	6	0	0	6	100.0
	An. funestus s.l.	1	0	0	1	100.0
Total		**895**	**9**	**46**	**840**	**93.9**

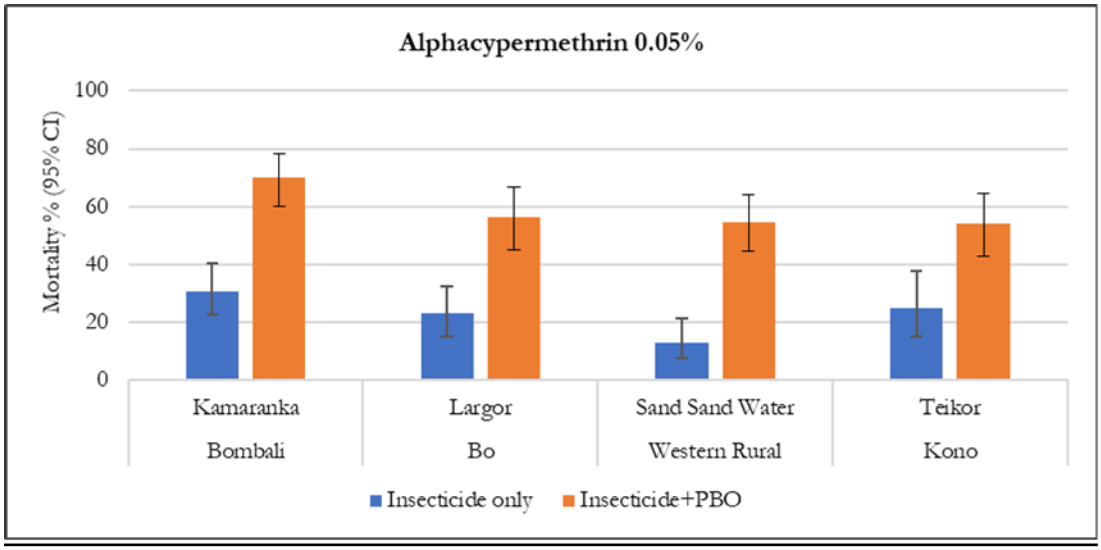
3.3 INSECTICIDE RESISTANCE MONITORING

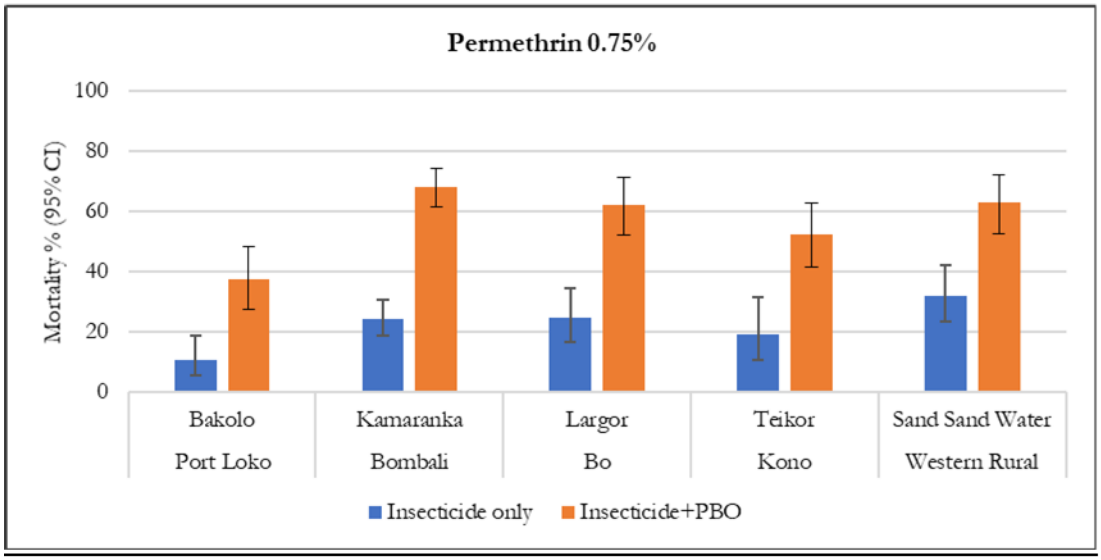
3.3.1 SYNERGIST ASSAYS

Anopheles gambiae s.l. sampled in Bombali, Port Loko, Kono, Bo and Western Rural districts were exposed to the pyrethroid insecticides deltamethrin, permethrin, and alpha-cypermethrin in PBO synergist assays. *Anopheles gambiae* s.l. was resistant to all the pyrethroids tested in all the districts (Figure 29). Mortality ranged from 4.2% to 25.5% for deltamethrin; 8.7% to 32% for permethrin, and 12.5% to 30.9% for alpha-cypermethrin (Figure 29; Annex F). The PBO partially restored susceptibility to all the pyrethroids, suggesting the partial involvement of monooxygenase-based resistance mechanism in Sierra Leone (Figure 29).

Figure 29: Susceptibility of *An. gambiae* s.l. to Deltamethrin 0.05%, Permethrin 0.75% and Alpha-Cypermethrin 0.05% with or without PBO in 2020



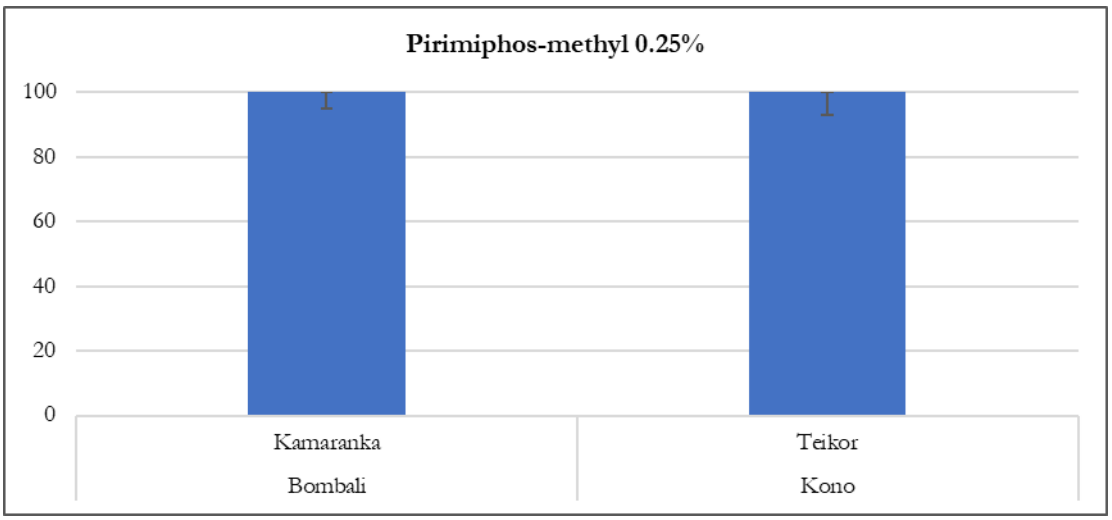




3.3.2 *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO ORGANOPHOSPHATES

An. gambiae s.l. exposed to pirimiphos-methyl (0.245%) in Kamaranka, Bombali district and Teikor in Kono district were fully susceptible (Figure 30).

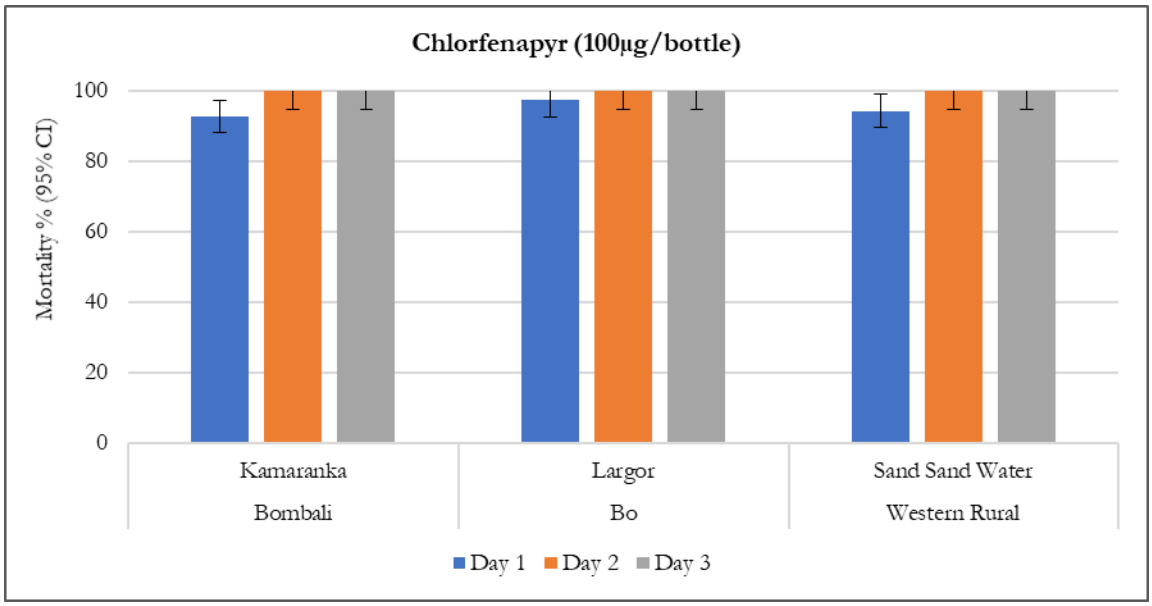
Figure 30: Susceptibility Status of *An. gambiae* s.l. to Pirimiphos-Methyl (0.25%) in 2020



3.3.3 *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO CHLORFENAPYR

The *An. gambiae* s.l. mosquitoes from three districts, Bombali, Bo and Western Rural that were exposed to chlorfenapyr were fully susceptible with all mosquitoes dying after day 2 of exposure (Figure 31).

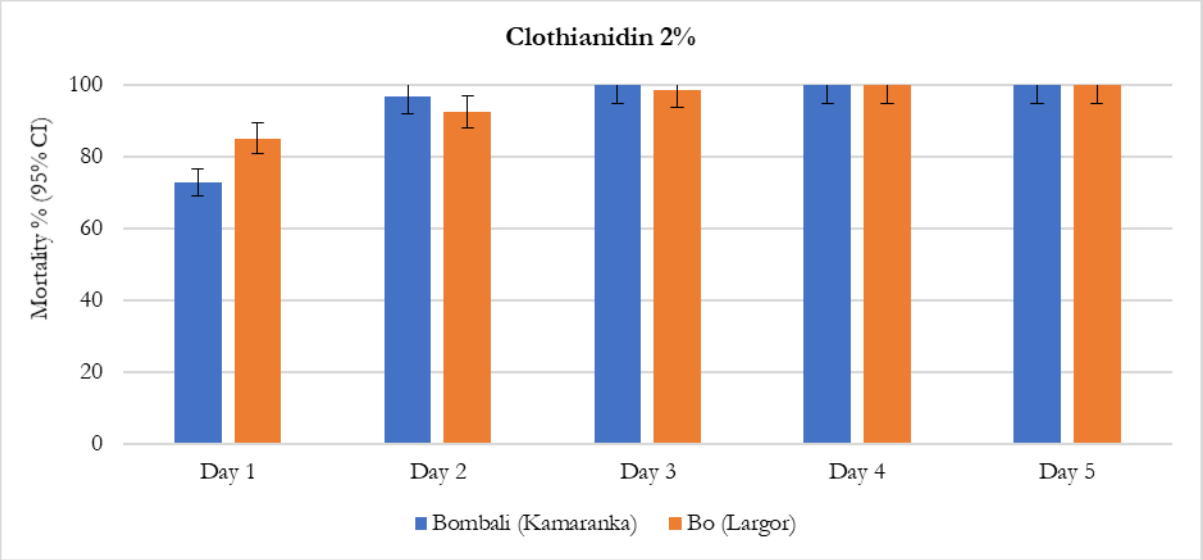
Figure 31: Susceptibility of *An. gambiae* s.l. to Chlorfenapyr (100 µg/Bottle) in 2020



3.3.4 *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO CLOTHIANIDIN

Malaria vectors in Bo and Bombali are still susceptible to clothianidin (2%). In Bombali, 100% mortality was achieved after day 3, while in Bo, 100% mortality was observed on day 4 (Figure 32).

Figure 32: Susceptibility of *An. gambiae* s.l. to Clothianidin (13.2 mg/Paper), 2020



3.3.5 DETERMINATION OF THE INTENSITY OF RESISTANCE

The intensity of pyrethroid resistance in *An. gambiae* s.l. was determined in three sites in three districts for deltamethrin (Figure 33) and two sites for permethrin (Figure 34). There is high intensity resistance to both deltamethrin and permethrin in the sites tested. Additional collections and testing have been scheduled for 2021-2022 work plan period.

Figure 33: Intensity of Insecticide Resistance of *An. Gambiae* s.l. to Deltamethrin in Sierra Leone in 2020 using the CDC Bottle Bioassay for Intensity.

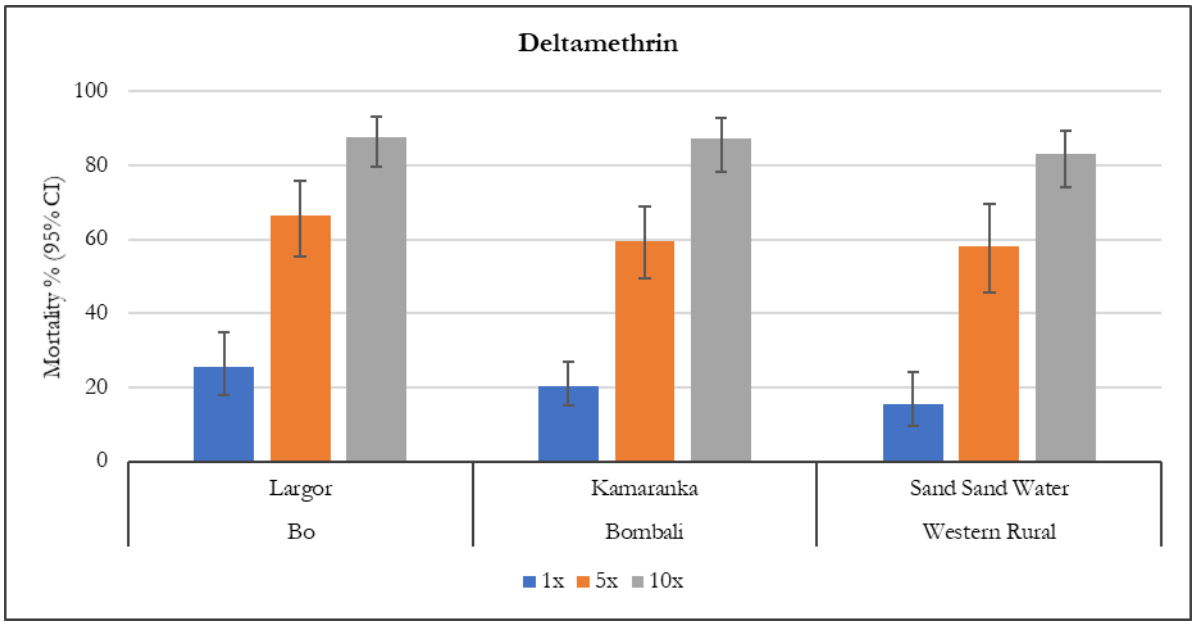
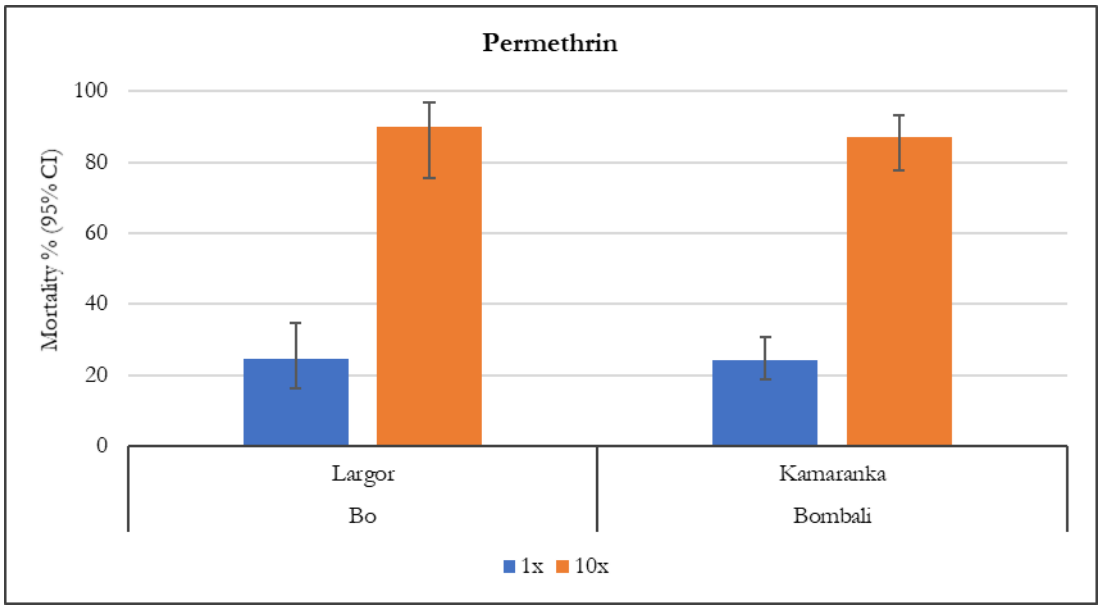


Figure 34: Intensity of Insecticide Resistance of *An. gambiae* s.l. to Permethrin in Sierra Leone in 2020 using the CDC Bottle Bioassay for Intensity.



3.4 PCR Analysis for Mechanism of Resistance

Laboratory analysis is ongoing and will be reported on as an addendum report.

4. CAPACITY BUILDING

- In July 2020, VectorLink Sierra Leone conducted two sessions of four-day training for government supervisors and seasonal technicians supporting the entomological monitoring in the new sites in Bo, Bombali, Port Loko, Karene and Moyamba. All trainings were undertaken while observing the Covid-19 mitigation measures; participants maintained a minimum distance of two meters; face masks were worn at all times; a hand washing station was placed at the entrance of the training venue; hand sanitizers were placed on each table and where microscopes were located. There was 70% alcohol for cleaning forceps and microscopes after each use.
- A total of 22 environmental health officers from each of the sentinel districts and four seasonal field technicians were trained in Freetown on entomological monitoring, including morphological identification of *Anopheles*, susceptibility tests and cone bioassays. The in-class sessions of the training were conducted in Family Kingdom conference hall in Freetown, and the microscopes from PMI, CDC and NTD allowed the participants to have more time for practice on mosquito's identification and dissection.
- The practical session was conducted in class, and the backup insectary located in the VectorLink office provided mosquitoes for the cone bioassay and susceptibility tests.
- A practical session was also conducted in Sand Sand Water in Western Rural Area where participants had the opportunity to practice CDC light trap setting and also human landing catches.

5. Discussion, Lessons Learned / Challenges, and Recommendations

5.1 Discussion

In Sierra Leone, *An. gambiae* s.l. is still the principal malaria vector. Within this complex, *An. coluzzii* is the major vector (64.5%) followed by *An. gambiae* (34.3%). *Anopheles melas* was sampled in Port Loko, and Western Rural districts, which is consistent with the expected distribution of this species along the coastal areas. Two *Anopheles arabiensis* and eleven hybrids between *An. gambiae* and *An. coluzzii* were also identified from the 5,638 samples tested by PCR. *An. funestus* group was the second main vector but only in Bo, Kono and Port Loko. Very low numbers were sampled in other districts. Of those sampled, *An. funestus* and *An. leesoni* were the major sibling species.

The *An. gambiae* s.l. vectors in Sierra Leone bite both indoors and outdoors, with no significant difference between indoor and outdoor biting behavior, even when comparing peri-urban to rural sites. The biting pattern however, varied by district, but major biting activity happened from 10:00 pm until 3:00 am overall. The highest indoor *An. gambiae* s.l. HBR was recorded in the peri-urban site of Masongbo in Bombali District (175.5 bites/person/night). This HBR of 175.5 was very high relative to other sites but in 2019, HBR of 111.8 bites/person/night was recorded in Gerihun in Bo district (2020 Sierra Leone Annual Entomological report). Malaria vectors in Sierra Leone prefer to feed on human rather than animal blood. Throughout the sampling period, a higher proportion of *An. gambiae* s.l. collected were fed compared to unfed and gravid mosquitoes. The HBI was very high with the lowest HBI of 75% in Port Loko district. The HBI was also high for *An. funestus*, with the lowest HBI of 90.2% recorded in Bo district. This is probably indicative of high human-vector contact despite the recent mass distribution of ITNs.

The vector abundance and biting rates followed the rainfall pattern with peaks observed between June-September and another smaller peak observed between December-February, consistent with previous years' reports. This supports the implementation of IRS in April-May before the start of the rainy season in May. Most human vector contact occurred during the second part of the night (after midnight). Since most bites still happen indoors at night, it is expected that a combination of IRS and PBO ITNs may result in a reduction of malaria in Bo and Bombali districts if both interventions are correctly utilized. The VectorLink Sierra Leone project is implementing a study to investigate the additional benefits of co-deployment of IRS with clothianidin (SumiShield) in addition to PBO-ITN in Bombali and Bo districts. However, scale up of SBC on net use would be necessary given the high proportion of fed mosquitoes collected inside houses.

During each month, there was a higher number of mosquitoes that had laid eggs at least once throughout the sampling period, supporting the observation of high proportion of mosquitoes that had successfully acquired a blood meal. There was no clear trend in parity rate and the monthly patterns did not follow the rainfall pattern where it is expected that parity rate increases as rainfall subsides.

The *An. gambiae* s.l. in Sierra Leone are resistant to deltamethrin, permethrin and alpha-cypermethrin with average mortality below 40%. The Intensity of deltamethrin/pyrethroid resistance is very high in Sierra Leone. However, PBO partially restored susceptibility to all the pyrethroids but this varied by insecticide and site. On average, PBO increased susceptibility by 46% for those exposed to deltamethrin, 35.6% to those exposed to alphacypermethrin and 34.54% to those exposed to permethrin. This suggests that monooxygenase-based resistance mechanism is partially involved in the resistance to pyrethroids. The distribution of PBO nets in May 2020 is expected to kill even the resistant mosquitoes resulting in reduced malaria transmission. *An. gambiae* s.l. is fully susceptible to pirimiphos-methyl, chlorfenapyr and clothianidin and this supports the justification for the use of SumiShield (clothianidin) for IRS in Sierra Leone.

In conclusion, *An. gambiae* s.l. and *An. funestus* s.l. are the major vectors of malaria in Sierra Leone. The PBO synergist was able to partially restore susceptibility of pyrethroid resistant *An. gambiae* s.l., supporting the decision by NMCP to distribute PBO-ITNs in 2020. Malaria vectors are still susceptible to pirimiphos-methyl and clothianidin and this supports the use of SumiShield (clothianidin) insecticide for IRS in Bo and Bombali districts. Malaria vectors still prefer to bite in the middle of the night. There was a high number of blood fed mosquitoes and therefore NMCP should undertake SBC on correct ITN use. This report includes all the entomological data collected for the reporting year of March 20 – February 2021. Data collection for the PBO nets-IRS co-deployment started in July 2020 and it will be one year at the end of June 2021. The team plans to compile and analyze the entomological monitoring data of 12 months for the co-deployment assessment only and submit a separate progress report in November 2021.

5.2 Lessons Learned and Challenges

- Malaria vectors in Sierra Leone are highly resistant to deltamethrin and permethrin insecticides; however, PBO was able to restore partial but significant susceptibility. It is necessary for the government to continue monitoring insecticide resistance to all classes of insecticides to guide vector control.
- There is heavy rainfall from around July-September and this limits sampling of malaria vectors, especially larvae used in testing for insecticide resistance. For future collections, the team is developing a framework to sample malaria vectors for insecticide resistance in May/June just before the middle of rainy season and in October/November after the rains.
- More mosquitoes were fed on humans indicating a challenge with ITN use in Sierra Leone. The NMCP should therefore carry out social behavior change communication activities to increase bed net use.
- At the start of the Covid-19 pandemic, the community was hesitant to allow monitoring to continue because they assumed the project team was there to spread the virus. This hesitancy can partly be explained by residual suspicions from the communities' experiences during the past Ebola virus disease outbreak. The PMI-supported entomological surveillance project presently involves community stake holders, regional government representatives and ministry of health officers to improve community support for monitoring activities.
- The project has adapted to undertaking entomology activities while observing Covid-19 prevention guidelines.
- The project suspended entomological field activities from March to June. The project was also not able to send participants to attend regional trainings organized by VectorLink senior entomologists because these events were cancelled due to the Covid-19 pandemic.
- Shipping samples out of the country to other laboratories for molecular analysis has potential drawbacks in that project technical staff are not present to observe and conduct quality assessment of the laboratory assays.
- In 2020, the entomology team had challenges with parity dissections and reading of the ovary dissection scores. This was addressed with a refresher training undertaken in December 2020. Further guidance and monitoring of parity dissections will be needed to ensure the competence is maintained.
- The team was not able to establish a new colony of *An. gambiae* s.s. Kisumu from MR4 because the eggs arrived in Freetown four days after they were shipped from CDC-Atlanta. This delay might have affected the egg hatch and viable larvae numbers. We have placed another order for more eggs, and we are working with CDC to speed up shipment and improve larval propagation.
- Involvement of national government staff at NMCP and regional ministry of health officers in the routine monitoring helps improve confidence of the ministry in the data that VectorLink collects for Sierra Leone. It also creates a buy-in rapport that helps engender a cordial working relationship. VectorLink will continue to operate with this principle in mind.

5.3 RECOMMENDATIONS

For VectorLink Sierra Leone:

- Establish a new colony of *An gambiae* s.s. Kisumu strain, using certified eggs from MR4, in Freetown and replace the Makeni colony with this new colony to be established.
- Continue with assessment of residual effectiveness of SumiShield in Bo and Bombali districts where IRS is being implemented.
- Conduct refresher training for the laboratory technicians on laboratory protocol and emphasize good practices to ensure colony purity.
- Carry out a refresher training to field technicians and supervisors, emphazise on quality data collection and data entry, and strenthen quality assurance systems.
- The team is in the process of verifying the parity data for reporting purposes.

For the NMCP and VectorLink Sierra Leone:

- Implement community sensitization on the use of ITNs and benefits of IRS to reduce human-vector contact.
- Conduct IRS in 2022 between April-May just before onset of rainy season.
- Ensure high-quality application of insecticide during the 2022 IRS campaign to ensure longer residual effect of IRS insecticide.
- Assess the residual life of SumiShield (clothianidin) in Sierra Leone after the 2021 IRS campaign.

ANNEX A: NUMBER OF *ANOPHELES* COLLECTED BY PSC, CDC-LT AND HLC, MARCH 2020–FEBRUARY 2021

District	Site	*An. funestus* s.l.	*An. gambiae* s.l.	*An. coustani*	*An. squamosus*	Other	Total
Bo	Gerihun (Rural)	1,046	4,308	18	0	2	5,374
	Largor (Rural)	237	3,716	7	0	0	3,960
Bombali	Kamaranka (Rural)	0	2,585	9	0	3	2,597
	Masongbo (Peri-Urban)	14	5,331	210	0	5	5,560
Kono	Sorie Town (Peri-Urban)	36	784	1	0	2	823
	Teikor (Rural)	94	806	10	0	1	911
Port Loko	Bakolo (Rural)	1	1,215	22	87	188	1,513
	New Maforki (Peri-Urban)	44	1,672	42	0	39	1,797
Western Rural	Sand Sand Water (Rural)	8	1,251	3	0	0	1,262
	Tombo (Peri-Urban)	2	763	0	0	1	766
Total		**1,482**	**22,431**	**322**	**87**	**102**	**24,424**

ANNEX B: NUMBER OF *ANOPHELES* COLLECTED BY CDC LIGHT TRAPS, MARCH 2020–FEBRUARY 2021

District	Site	*An. gambiae* s.l.	*An. funestus* s.l.	*An. coustani*	Other	Total
Bo	Gerihun (Rural)	370	96	0	0	466
	Largor (Rural)	156	12	1	0	169
Bombali	Kamaranka (Rural)	184	0	1	0	185
	Masongbo (Peri-Urban)	739	2	4	0	745
Kono	Sorie Town (Peri-Urban)	166	5	1	0	172
	Teikor (Rural)	134	10	5	1	150
Port Loko	Bakolo (Rural)	41	1	3	2	47
	New Maforki (Peri-Urban)	46	10	2	12	70
Western Rural	Sand Sand Water (Rural)	103	0	0	0	103
	Tombo (Peri-Urban)	155	1	0	0	156
Total		**2,094**	**137**	**17**	**15**	**2,263**

ANNEX C: NUMBER OF *ANOPHELES* COLLECTED BY PSC, MARCH 2020-FEBRUARY 2021

District	Site	*An. gambiae* s.l.	*An. funestus* s.l.	*An. coustani*	Other	Total
Bo	Gerihun (Rural)	291	111	1	0	403
	Largor (Rural)	613	84		0	697
Bombali	Kamaranka (Rural)	248	0	1	0	249
	Masongbo (Peri-Urban)	211	0	1	0	212
Kono	Sorie Town (Peri-Urban)	159	10		0	169
	Teikor (Rural)	192	33		0	225
Port Loko	Bakolo (Rural)	69	0	2	1	72
	New Maforki (Peri-Urban)	140	0	0	1	141
Western Rural	Sand Sand Water (Rural)	337	7		0	344
	Tombo (Peri-Urban)	41	0	0	1	42
Total		**2,301**	**245**	**5**	**3**	**2,554**

ANNEX D: NUMBER OF *ANOPHELES* COLLECTED BY HLC, MARCH 2020-FEBRUARY 2021

District	Site	*An. funestus* s.l.	*An. gambiae* s.l.	*An. coustani*	*An. squamosus*	Other	Total
Bo	Gerihun (Rural)	839	3,647	17	0	2	4,505
	Largor (Rural)	141	2,947	6	0	0	3,094
Bombali	Kamaranka (Rural)	0	2,153	7	0	3	2,163
	Masongbo (Peri-Urban)	12	4,381	205	0	5	4,603
Kono	Sorie Town (Peri-Urban)	21	459	0	0	2	482
	Teikor (Rural)	51	480	5	0	0	536
Port Loko	Bakolo (Rural)	0	1,105	17	87	185	1,394
	New Maforki (Peri-Urban)	34	1,486	40	0	26	1,586
Western Rural	Sand Sand Water (Rural)	1	811	3	0	0	815
	Tombo (Peri-Urban)	1	567	0	0	0	568
Total		**1,100**	**18,036**	**300**	**87**	**223**	**19,746**

ANNEX E: NUMBER OF *ANOPHELES* DISSECTED FOR PARITY RATE; *AN GAMBIAE* S.L. AND *AN FUNESTUS* S.L., MARCH 2020-FEBRUARY 2021

A. *An. gambiae* s.l.

District	Village	Month	Nulliparous (N)	Parous (P)	Undetermined (U)	Grand Total	Proportion undetermined	Total (P+N)	Parity Rate (P/(P+N) *100	95 % Confidence intervals	
										Lower	Upper
Bo	Gerihun	Jul-20	10	22	2	34	5.88	32	68.75	49.9	83.3
		Aug-20	13	13	4	30	13.33	26	50	32.1	67.9
		Sep-20	29	126	6	161	3.72	155	81.29	74.1	86.9
		Oct-20	32	45	2	79	2.53	77	58.44	46.6	69.4
		Nov-20	19	47	4	70	5.71	66	71.21	58.6	81.4
		Dec-20	5	19	24	48	50	24	79.16	57.3	92.1
		Jan-21	3	8	14	25	56	11	72.72	39.3	92.7
		Feb-21	1	6	2	9	22.22	7	85.71	42.0	99.2
	Largor	Jul-20		9	4	13	30.76	9	100	62.9	100.0
		Aug-20	2	27	9	38	23.68	29	93.10	75.8	98.8
		Sep-20	8	35	1	44	2.27	43	81.39	66.1	91.1
		Oct-20	3	16		19	0	19	84.21	59.5	95.8
		Nov-20	2	9	2	13	15.38	11	81.81	47.8	96.8
		Dec-20	5	3	7	15	46.66	8	37.5	10.2	74.1
		Jan-21	6	5	16	27	59.25	11	45.45	18.1	75.4
Bombali	Kamaranka	Aug-20	1	5	1	7	14.28	6	83.33	36.5	99.1
		Sep-20	1	2		3	0	3	66.66	12.5	98.2
		Oct-20	1	9		10	0	10	90	54.1	99.5
		Jan-21	3	1	1	5	20	4	25	1.3	78.1
	Masongbo	Jul-20	9	25	2	36	5.55	34	73.52	55.3	86.5
		Aug-20		3	19	22	86.36	3	100	31.0	100.0
		Sep-20	21	65	4	90	4.44	86	75.58	64.9	83.9
		Oct-20	6	34		40	0	40	85	69.5	93.8
		Nov-20	10	28	2	40	5	38	73.68	56.6	86.0
		Dec-20	14	7	11	32	34.37	21	33.33	15.5	56.9

District	Village	Month	Nulliparous (N)	Parous (P)	Undetermined (U)	Grand Total	Proportion undetermined	Total (P+N)	Parity Rate (P/(P+N) *100	95 % Confidence intervals	
										Lower	Upper
		Jan-21	4	22	20	46	43.47	26	84.61	64.3	95.0
		Feb-21		7	9	16	56.25	7	100	56.1	100.0
Kono	Sorie Town	Jul-20	1		1	2	50	1	0	5.5	100.0
		Aug-20	7	6	5	18	27.77	13	46.15	20.4	73.9
		Sep-20	3	11	3	17	17.64	14	78.57	48.8	94.3
		Oct-20	1	4		5	0	5	80	29.9	98.9
		Nov-20	4	3		7	0	7	42.85	11.8	79.8
		Dec-20			1	1	100	0	0	0.0	0.0
		Jan-21			2	2	100	0	0	0.0	0.0
		Feb-21		7	6	13	46.15	7	100	56.1	100.0
	Teikor	Jul-20	10	3	6	19	31.57	13	23.07	6.2	54.0
		Aug-20		5	3	8	37.5	5	100	46.3	100.0
		Sep-20	10	23	4	37	10.81	33	69.69	51.1	83.8
		Oct-20	6	23		29	0	29	79.31	59.7	91.3
		Nov-20	2	6	1	9	11.11	8	75	35.6	95.5
		Jan-21	1	1	2	4	50	2	50	9.5	90.5
		Feb-21		3		3	0	3	100	31.0	100.0
Port Loko	Bakolo	Jun-20	10		11	21	52.38	10	0	0.5	45.9
		Jul-20	3	3	6	12	50	6	50	18.8	81.2
		Aug-20	11	19	6	36	16.66	30	63.33	43.9	79.5
		Sep-20	1			1	0	1	0	5.5	100.0
	New Maforki	Jun-20	10		11	21	52.38	10	0	0.5	45.9
		Jul-20	2	2	3	7	42.85	4	50	15.0	85.0
		Sep-20	12	26		38	0	38	68.42	51.2	82.0
		Oct-20	21	14		35	0	35	40	24.4	57.8
		Nov-20	13	5	2	20	10	18	27.77	10.7	53.6
		Dec-20	9	7	3	19	15.78	16	43.75	20.8	69.4
Western Rural	Sand Sand Water	Jun-20	1	1	7	9	77.77	2	50	9.5	90.5
		Jul-20	5	2	17	24	70.83	7	28.57	5.1	69.7
		Aug-20	4	11	8	23	34.78	15	73.33	44.8	91.1
		Sep-20	2	15	2	19	10.52	17	88.23	62.3	97.9
		Oct-20	1	4	3	8	37.5	5	80	29.9	98.9
		Nov-20		4	6	10	60	4	100	39.6	100.0
		Dec-20	4	9	3	16	18.75	13	69.23	38.9	89.6
		Feb-21		4	6	10	60	4	100	39.6	100.0

District	Village	Month	Nulliparous (N)	Parous (P)	Undetermined (U)	Grand Total	Proportion undetermined	Total (P+N)	Parity Rate (P/(P+N) *100	95 % Confidence intervals	
										Lower	Upper
	Tombo	Jun-20	7	6	18	31	58.06	13	46.15	20.4	73.9
		Jul-20	1		3	4	75	1	0	5.5	100.0
		Aug-20	3	4	3	10	30	7	57.14	20.2	88.2
		Sep-20	1	8		9	0	9	88.88	50.7	99.4
		Oct-20		3		3	0	3	100	31.0	100.0
		Nov-20		4	1	5	20	4	100	39.6	100.0
		Feb-21			1	1	100	0	0	0.0	0.0
Grand Total			**374**	**844**	**320**	**1538**	**29.59**	**1218**	**61.95**		

B. *An. funestus* s.l.

District	Village	Month	Nulliparous (N)	Parous (P)	Undetermined (U)	Grand Total	Proportion undetermined	Total (P+N)	Parity Rate (P/(P+N) *100	95% Confidence Interval	
										Lower	Upper
Bo	Gerihun	Dec-20	4	3	9	16	56.25	7	42.85	11.8	79.8
		Jan-21	1			1	0	1	0	0	94.5
Kono	Teikor	Oct-20		2		2	0	2	100	19.8	100

ANNEX F: WHO SUSCEPTIBILITY TEST AND CDC BOTTLE ASSAYS RESULTS

District (Site)	Bo (Largor)		Bombali (Kamaranka)		Kono (Teikor)		Port Loko (Bakolo)		Western Rural (Sand Sand Water)	
	#No Exposed	% Mortality	# Exposed	% Mortality	# Exposed	% Mortality	# Exposed	% Mortality	# Exposed	% Mortality
Deltamethrin 0.05%	106	25.5	196	20.4	76	14.5	176	23.9	109	15.6
PBO+Deltamethrin 0.05%	102	73.5	200	74	77	66.2	186	66.1	103	50.5
Permethrin 0.75%	94	24.5	206	24.3	63	19	96	10.4	100	32
PBO+Permethrin 0.75%	103	62.1	208	68.3	92	52.2	91	37.4	97	62.9
Alphacypermethrin 0.05%	100	23	110	30.9	64	25	ND	ND	106	13.2
PBO+Alpha-cypermethrin 0.05%	89	56.2	103	69.9	87	54	ND	ND	110	54.5
Pirimiphos-methyl	ND	ND	94	100	61	100	ND	ND	ND	ND
Clothianidin 2%	81	100	100	100	ND	ND	ND	ND	99	100
Chlorfenapyr (100 µg)	85	100	98	100	ND	ND	ND	ND	108	100
Key	Susceptible	Resistant								

ND= Not Done

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