U.S. PRESIDENT'S MALARIA INITIATIVE

THE PMI VECTORLINK SIERRA LEONE 2021 ANNUAL ENTOMOLOGICAL MONITORING REPORT

MARCH 1, 2021 – FEBRUARY 28, 2022

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Abt Associates Inc. | 6130 Executive Blvd | Rockville, MD 20852

T. 301.347.5000 | F. 301.913.9061 | www.abtassociates.com

THE PMI VECTORLINK
SIERRA LEONE
2021 ANNUAL ENTOMOLOGICAL
MONITORING REPORT

MARCH 1, 2021 – FEBRUARY 28, 2022

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ACRONYMS

Ace-1	Acetylcholinesterase 1
CDC	U.S. Centers for Disease Control and Prevention
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
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
WHO	World Health Organization

Executive Summary

Sierra Leone has made significant gains in the fight against malaria with the recent 2021 malaria indicator survey showing malaria prevalence at the national level has halved to 22% in children below five years of age. The National Malaria Control Program (NMCP) has employed various tools since 2020 to reduce malaria burden. The U.S. President's Malaria Initiative (PMI), in collaboration with the Sierra Leone 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 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 2021 and February 2022, mosquitoes were collected from 10 sentinel sites 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-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 separately. The project also monitored the decay rate of SumiShield™ (clothianidin), which was sprayed in Bombali and Bo districts during the 2021 IRS campaign.

Vector Bionomics: *An. gambiae* s.l. was the predominant vector collected with 87.2% (30,795), followed by *An. funestus* group, 11.8% (4,168), *An. coustani* 0.7% (244), and others 0.3% (105). *Anopheles funestus* s.l. was sampled in all districts but appreciable numbers were collected in Bo and Kono districts. In Bo, the highest *An. funestus* s.l. indoor resting density (IRD) was recorded in August in Largor rural site (6.6/house/day). The highest mean IRD for *An. gambiae* s.l. was recorded in June in Largor rural site (14.9/house/day). There was no clear difference in mean resting density between peri-urban and rural sites.

Human Biting Rate (HBR) and Location: The mean biting density varied by site, district, and month of collection. The highest indoor human biting rate (HBR)/ person/night for *An. gambiae* s.l. was recorded in June in Masongbo, Bombali district with 80.6 bites/person/night. In all districts, the HBR followed the rainfall pattern with a peak observed in the wet season between June and September and declining into the dry season. The highest HBR for *An funestus* s.l. was recorded in Gerihun, Bo district with 14.3 bites/person/night. The majority of the *An. funestus* s.l. were collected in Bo and contributed most to the overall trends in HBR. 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 as measured by the Centers for Disease Control and Prevention light traps (CDC-LT) varied by site, district, and month, but followed the rainfall patterns in Sierra Leone. The highest average density of *An. gambiae* s.l./trap/night was recorded in June in Masongbo, Bombali District (21.4/trap/night), a 38% reduction from the 2020-2021 period. For *An. funestus* s.l., the mean density per trap per night was always below 1, and therefore no clear pattern could be observed in the three districts (Bo, Bombali and Kono) where *An. funestus* s.l. were sampled.

Molecular identification: Species identification using polymerase chain reaction (PCR) was done on a total of 3,016 *An. gambiae* s.l. collected in 2021–2022; *An. gambiae* s.s. were predominant at 56.1% (1,694), followed by *An. coluzzii* at 43.8% (1,320), hybrid of *An. gambiae* s.s./*An. coluzzii* at 0.1% (2). Molecular identification was also done on 477 *An. funestus* group; 98.3% (469) were identified as *An. funestus* s.s. and 1.7% (8) as *An. leesoni.*

Entomological Inoculation Rate (EIR): The EIR, expressed as the number of infective bites per person per unit time (either month (ib/p/m) or year (ib/p/y)) was estimated for each site during the sampling period of March 2021 - February 2022. The highest monthly EIR (mEIR) was recorded indoor in June in Teikor rural site in Kono District (142.2 ib/p/m) followed by Sand Sand Water rural site in Western Area district, outdoor mEIR of 75.8 ib/p/m in January 2022. This represents a 50% drop in EIR from the previous year. The highest aEIR was in Sand Sand Water (213.1 ib/p/y). The mEIR for all sites combined peaked in June and decreased progressively into the dry season with some peaks observed in September and December. Indoor and outdoor mEIR were comparable when data was aggregated but varied when data was disaggregated by site. Between the rainy season of June and September, non-IRS sites had higher EIR than IRS sites.

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 54% in Bo District.

Parity Rate: The majority of malaria vectors collected during the sampling period had laid eggs at least once (parous) with 73.5% (778), while 26.5% (280) had not laid eggs (nulliparous). In all the districts, apart from Western Rural, before the rains, parity rate was relatively high as expected and declined either in May/June with the onset of rains and later rose toward the end of rainy season.

Insecticide Resistance: *An. gambiae* s.l. are resistant to permethrin, deltamethrin and alpha-cypermethrin. However, PBO was able to restore partial susceptibility by 48-65% to alphacypermethrin, 20-55% to deltamethrin and 17-61% to permethrin, indicating that mono-oxygenases are involved in conferring pyrethroid resistance in Sierra Leone. The effect of PBO in 2021 is similar to its activity in 2020 where mortality was restored by about 27-53.6%. Resistance to pirimiphos-methyl was also reported in all districts but were fully susceptible to chlorfenapyr and clothianidin. Molecular markers of insecticide resistance were observed at various frequencies; *kdr-w* mutation was fixed or approaching fixation (>80%), *ace-1*(G119S) 21.9% and N1575Y 0.5%.

Decay rate of IRS-Insecticide: SumiShield™ (clothianidin) remained effective against both field collected and laboratory strain (*An. gambiae* s.s. Kisumu) mosquitoes, 10 months after the first spray campaign in May 2021 indicating long residual effect of the insecticide.

Conclusion: Malaria vectors prefer to bite late at night and show no preference to either indoor or outdoor biting. The longitudinal data indicates that entomological indicators of malaria transmission has gone down in the sentinel areas including the IRS sites. This implies that the proper use of PBO-ITNs at night when in bed should contribute to reduction of malaria burden in Sierra Leone from indoor biting, but other forms of outdoor malaria prevention tools should be considered and continued robust investment in malaria treatment and control should be maintained. Another peak of malaria transmission mediated by *An. funestus* and partially maintained by dry season irrigation farming, was observed in December in IRS sites. This poses a challenge for IRS which targets the first peak in June. The full susceptibility of mosquitoes to chlorfenapyr indicates that deploying interceptor G2 (IG2) nets could also be effective at controlling the pyrethroid resistant mosquitoes. SumiShield™ (clothianidin) is effective for IRS in Sierra Leone since there is no reported resistance to this insecticide and the residual life is long. 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 during the wet month of June. This supports the decision to implement IRS in April-May before the onset of rains. The VectorLink Sierra Leone project is also collecting entomological monitoring data as part of the IRS/PBO-net co-deployment assessment (which will be presented in a separate report). There was an increase in frequency in *Ace-1* mutation, and NMCP should continue monitoring this to inform selection of appropriate insecticides for vector control.

I. INTRODUCTION

Malaria is endemic in Sierra Leone with stable and perennial transmission. In 2021, malaria prevalence by microscopy was 22% among children aged 6–59 months and 25% in children aged 5-9 years old (*MIS 2021, under review*). This represents a 45% drop from the malaria indicator survey of 2016. Malaria is still the main cause of morbidity and mortality among children under five years of age. Malaria prevalence is two times higher in rural areas (26%) compared to urban areas (14%) (*MIS 2021, under review*). The reduction in malaria prevalence is attributed to the use of a combination of interventions targeting the mosquito vector and parasite including human behavior change interventions.

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) in collaboration with the President's Malaria Initiative (PMI) 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). NMCP, in coordination with PMI, has also planned another IRS mass campaign in May 2022 in the same districts. The two 2021 IRS districts were selected from the four districts where NMCP/PMI 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% (*MIS 2016*) 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. In 2021, malaria prevalence in Bo was 25.4%, and 12.8% in Bombali (*MIS 2021, under review*).

The continued use of insecticide-based vector control interventions is threatened by the development of insecticide resistance. To manage insecticide resistance and formulate vector control policies, the NMCP, through the project, has been monitoring malaria vectors since March 2018, in line with the strategy document of Insecticide Resistance Monitoring and Management Pan (IRMMP). 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 2021 and February 2022, monthly routine entomological monitoring activities were conducted in five districts previously selected in consultation with the NMCP: Bo (Sierra Leone's second-largest district in the South), Bombali (representing the Northern part of the country), Kono (where there are large-scale mining activities in the East), Western Rural Area (in the Coastal area), and Port Loko 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. 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 2021/2022 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.

- Monitor the decay rate of SumiShield (clothianidin) insecticide in two sites, one each in Bombali and Bo where IRS was implemented.

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).

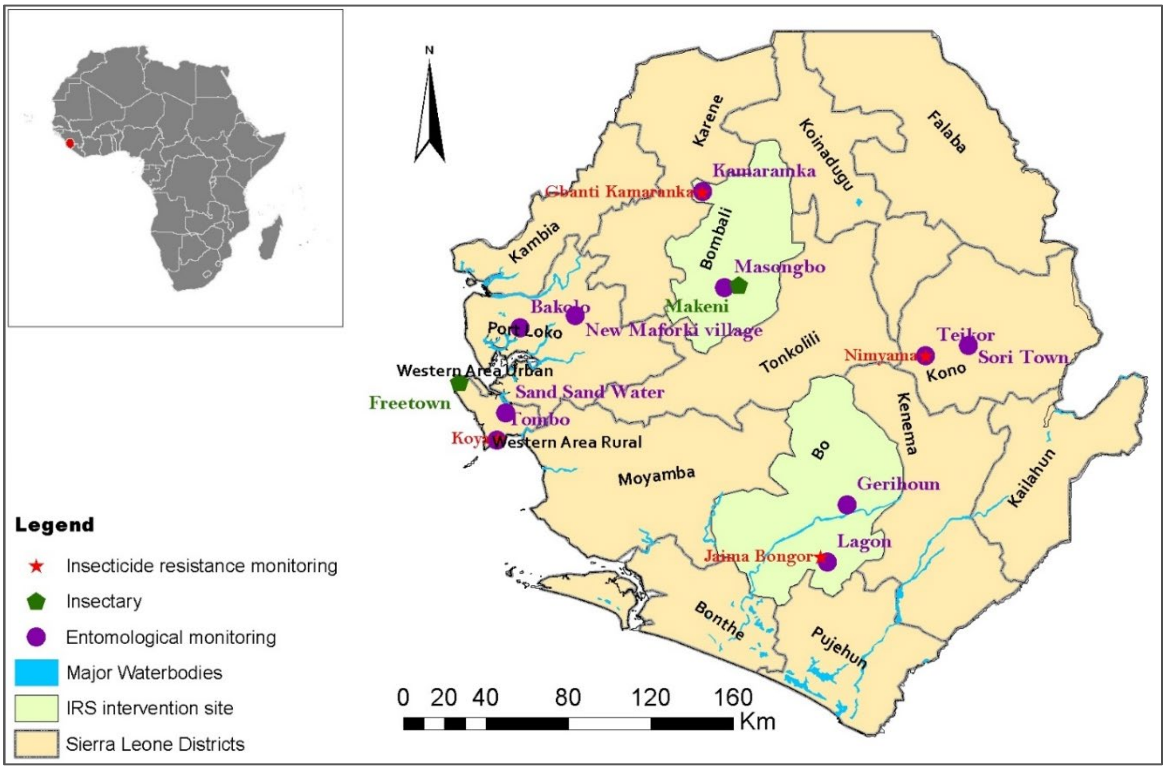
2. METHODOLOGY

2.1 ENTOMOLOGICAL MONITORING SITES

Sierra Leone has a tropical climate with wet and dry seasons. The wet season lasts from May to October, while the dry season is from November to April. Port Loko and Western rural Area 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 2021 to February 2022 in the routine monitoring sites.

In each district, mosquitoes were collected in one peri-urban site and one rural sentinel site (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, and with the agricultural sector being the main source of jobs. Monthly entomological surveillance with PSC, CDC-LT and HLC, insecticide resistance testing, and monitoring of the decay rate of SumiShield (clothianidin) was performed using VL standard operating procedures (SOPs[1]) protocols.

Figure 1. PMI VectorLink Sierra Leone Entomological Monitoring Sites



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

Table 1: Sentinel Sites for Entomological Monitoring

Province	District	Chiefdom	Sentinel Site	Location	Activity		
					Vector Bionomic Monitoring (PSC, CDC LT, and HLC)	Vector Susceptibility	IRS Site
Southern	Bo	Jaima Bongor	Largor	Rural	✓	✓	✓
		Boama	Gerihun	Peri-urban	✓		✓
Northern	Bombali	Gbanti Kamaranka	Kamaranka	Rural	✓	✓	✓
		Makari Gbanti	Mansongbo	Peri-urban	✓		✓
Northwestern	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.

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 transported to the field sorting site 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 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.

During each night, collectors (one inside and another 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) for species identification (Table 3). A subsample of unfed *An. gambiae* s.l. collected were kept in a moist petri dish and dissected to determine parity rate (Annex E).

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 at 07:00am. Mosquitoes captured were transported to the VBDIL or field sorting site 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. based on 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 and clothianidin, 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 for chlorfenapyr and 4µg/bottle for clothianidin. 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 24 hours post-exposure for clothianidin and one, two, and three days after the end of exposure for chlorfenapyr. A negative control was tested at the same time and used to calculate corrected mortality where applicable.

2.4 Synergist Assays

Synergist assays with PBO using the WHO tube tests and SOP06/01 procedure were conducted with deltamethrin 0.05%, permethrin 0.75%, and alpha-cypermethrin 0.05% in the five districts.

2.5 Quality of Spray and Decay Rate of Insecticide on the Wall

Sierra Leone implemented the first IRS campaign using SumiShield™ in Gerihun, Bo District and Masongbo, Bombali District. The quality of spray was assessed seven days after the start of the spray campaign and residual efficacy monitored from May 2021 to February 2022 using wall cone assay according to VL SOP009/01.

To determine the quality of spray and residual duration of insecticide on the walls, wall bioassays were conducted within two weeks of IRS and monthly using an insectary-reared susceptible colony of *An. gambiae* s.s. Kisumu. The results of the spray quality assessment in May were taken as T0 (baseline) for the consecutive monthly assays. Bioassays were conducted using the WHO cone bioassay in Bo and Bombali districts in 10 houses (for a total of 20 houses per month per district). In each site, 10 houses of different wall types were selected based on the most common house type in Sierra Leone. At the start of T0 (May), six mud houses and four cemented houses (one painted cement in Bo) were selected for the wall bioassays. Cone bioassays were conducted early in the morning according to VL SOP6. The exposure time of 30 minutes with mosquitoes was monitored for mortality every 24 hours post exposure to SumiShield-sprayed walls for five days. A parallel control exposure was run on an unsprayed surface (block board) close to each sprayed house. When mortality decreased, the team investigated whether houses were re-plastered/re-smeared after IRS and if so, additional houses were selected for wall bioassays. Further, if a household owner wished to discontinue, the team would search for a replacement of similar wall type house, otherwise any wall type would be selected.

To check the air-borne fumigant effect of the insecticide, a small cage (20 cm x 20 cm x 20 cm) containing 10 insectary-reared *An. gambiae* s.s. or field collected *An. gambiae* s.l. was placed one meter away from the sprayed wall. The mosquitoes were exposed for 30 minutes then transferred into paper cups and taken to an untreated holding room. Mortality was recorded every 24 hours post exposure. Fumigant tests were conducted monthly in parallel with cone bioassays.

The assessment of decay rate thus began in June for SumiShield™. In 2020-2021, the insectary had *Kdr-East* contamination and a population crash due to use of cold water fish flakes in place of tropical fish flakes as larval food; therefore both susceptible insectary strain *An. gambiae* s.s. Kisumu and field collected pyrethroid resistant mosquitoes were used at different time points. In May, June, January and February, the susceptible insectary strain *An. gambiae* s.s. Kisumu was exposed to sprayed walls at different heights in both villages. Between July and December, field collected pyrethroid resistant *An. gambiae* s.l. mosquitoes were exposed to sprayed walls in both villages. Twenty houses sprayed with SumiShield were tested every month (six mud and four cement houses per site). At different months, some houses were changed to houses with the same type of surface (unless similar type could not be consented) because household owners did not want to continue, while in some instances, houses were brought down. Therefore, two houses had different wall types after they consented to be part of the evaluation of decay rate of SumiShield™.

2.6 Analysis and Molecular Evaluations

Subset of collected samples were shipped to CRID in 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.

2.6.1 Mosquitoes Species Identification

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 by Scott et al. (1993) and Santolamazza et al. (2008) for *An. gambiae* s.l. and *An. funestus* s.l. (Koekemoer et al. 2002).

2.6.2 Molecular Characterization of Insecticide Resistance Mechanisms

KDR West and KDR East Genotyping

A TaqMan assay with two labeled Fluorochrome probes, FAM and HEX, was used to screen for the L1014F and L1014S *kdr* mutations (Bass et al. 2007).

Ace-1 Genotyping

A TaqMan assay with two labeled Fluorochrome probes, FAM and HEX, was used to screen for the *Ace-1* mutations (Bass et al. 2007).

2.6.3 Determination of Infection Rate

Enzyme-linked immunosorbent assay (ELISA) (Wirtz et al, 2010) was used to determine the sporozoite infection rate.

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).

2.6.5 ANALYSIS OF DATA

Data was entered into VectorLink Collect, the DHSI2 database platform for the VectorLink project. Summaries were generated using the analysis App in the VL Collect software. Additional analysis was conducted 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 hour or per night (reported as bites per person per hour or per night)
    - = the total number of vectors collected/number of collectors per hour or per night /number of nights of capture
- Sporozoite rate = the proportion of *Anopheles* found positive for *Plasmodium* infection
- Daily EIR = sporozoite rate x HBR
- Monthly EIR = daily EIR x number of days in the month
- Seasonal EIR = Σ monthly EIR for months of either wet (May-October) or dry (November to February and March 2020) seasons.
- Annual EIR = Σ Monthly EIRs
- Human Blood Index (HBI) per district = the proportion of mosquitoes that fed on human blood.
- Mean Numbers per Trap = 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.

3. Results

3.1 Longitudinal Monitoring

3.1.1 Species Composition

A total of 35,312 *Anopheles* mosquitoes were collected during the monthly collections conducted from March 2021 to February 2022 using PSC, HLC, and CDC light traps in both rural and peri-urban sites in five districts. The HLC sampled more mosquitoes (82%), compared to PSC (12%) and CDC-LT (6%) (Annex A). Of the *Anopheles* mosquitoes collected, *An. gambiae* s.l. was the predominant vector with 87.2% (30,795), followed by *An. funestus* group 11.8% (4,168), *An. coustani* 0.7% (244), and others 0.3% (105) (Figure 2, Annex A). The proportion of *An. funestus* s.l. collected using PSC (22.8%) was higher than the proportions collected from HLC (10.5%) and CDC-LT (8.2%), indicating the more endophilic behavior of this mosquito compared to *An. gambiae* s.l. (Figures 3-5, Annex A-D). Mosquito density was highest in Bo and Bombali compared to other districts (Figure 6).

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 2021 and February 2022

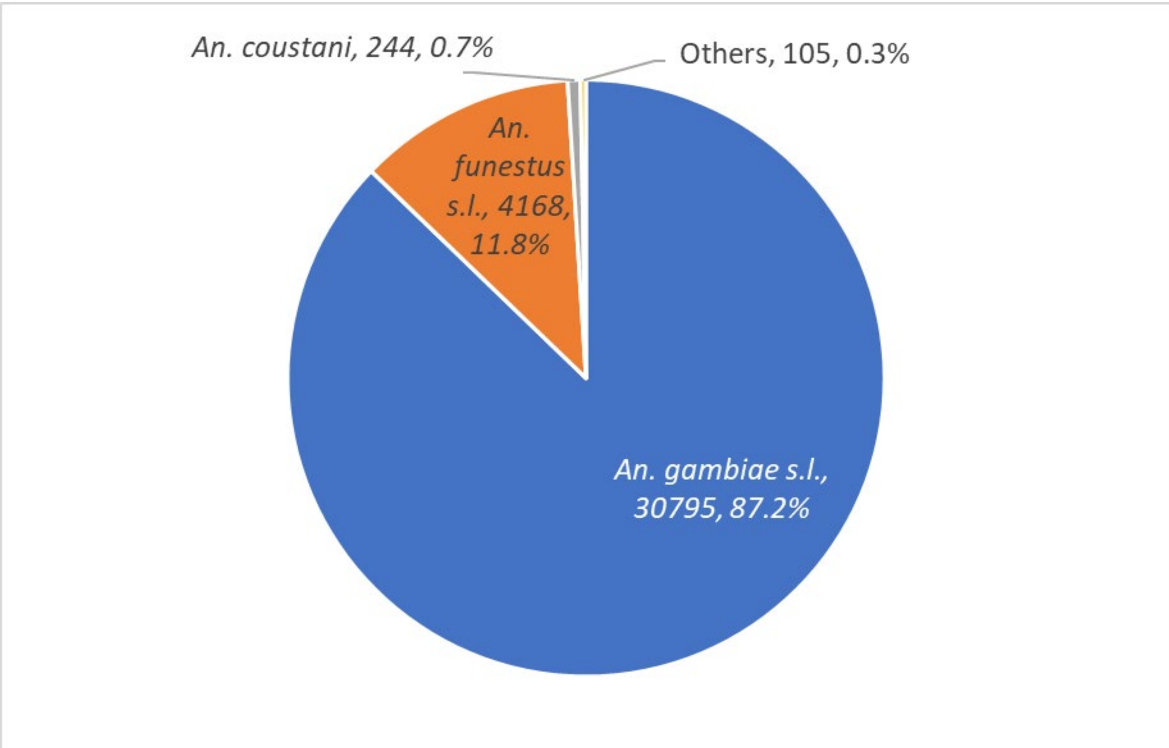


Figure 3: Total *Anopheles* Species Collected by HLC Between March 2021 and February 2022

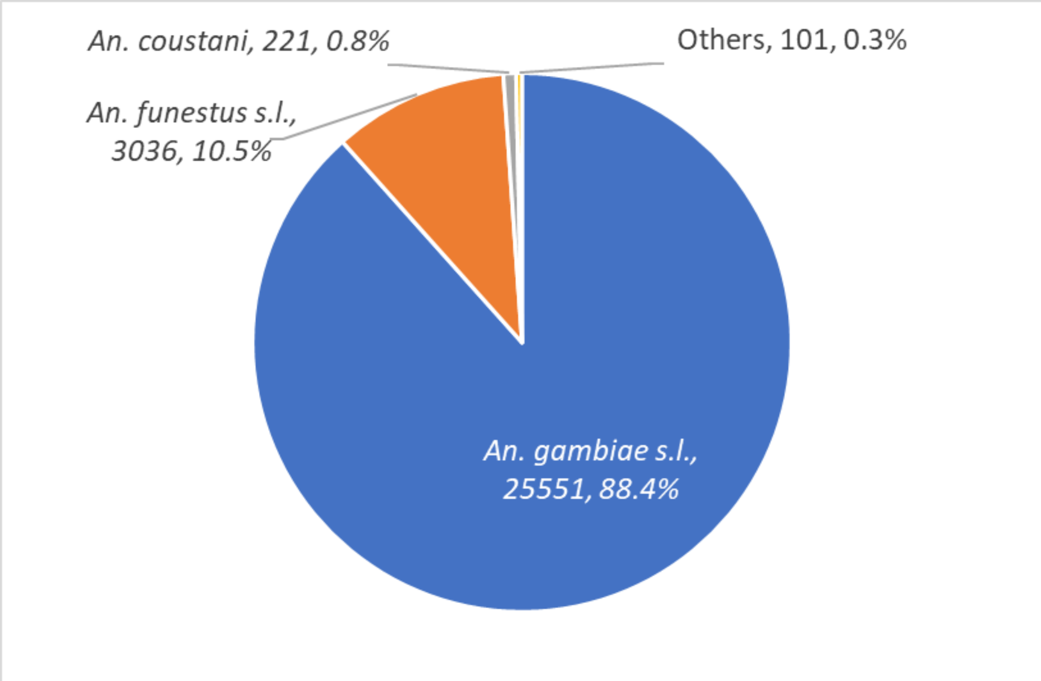


Figure 4: Total *Anopheles* Species Collected by CDC-LT Between March 2021 and February 2022

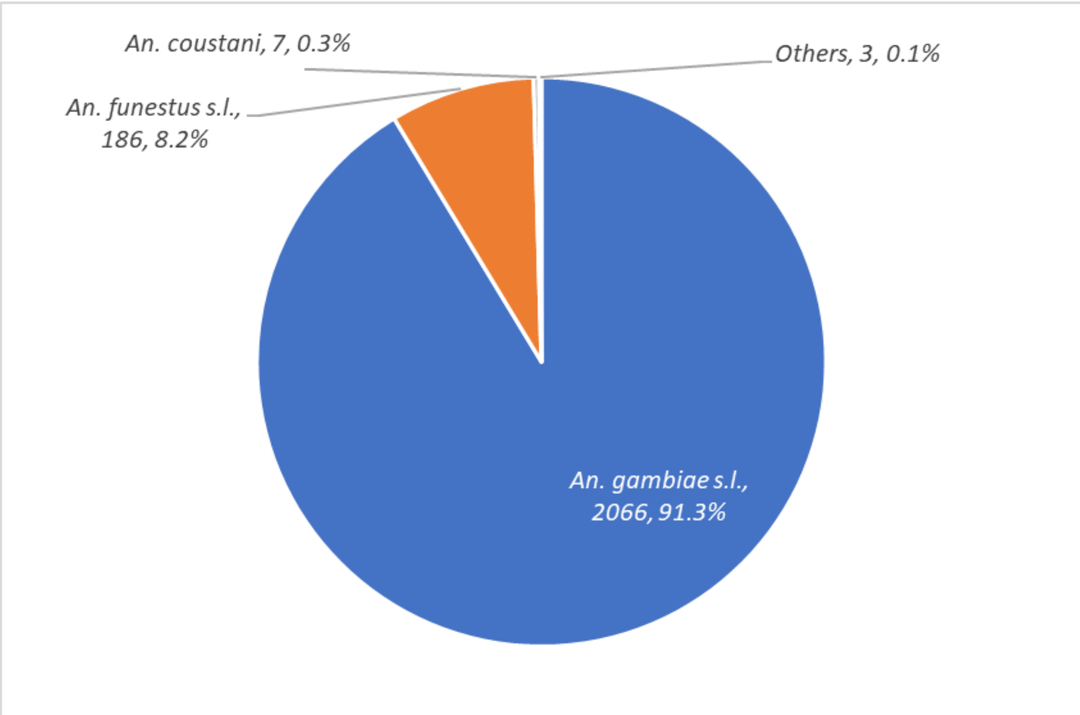


Figure 5: Total *Anopheles* Species Collected by PSC Between March 2021 and February 2022

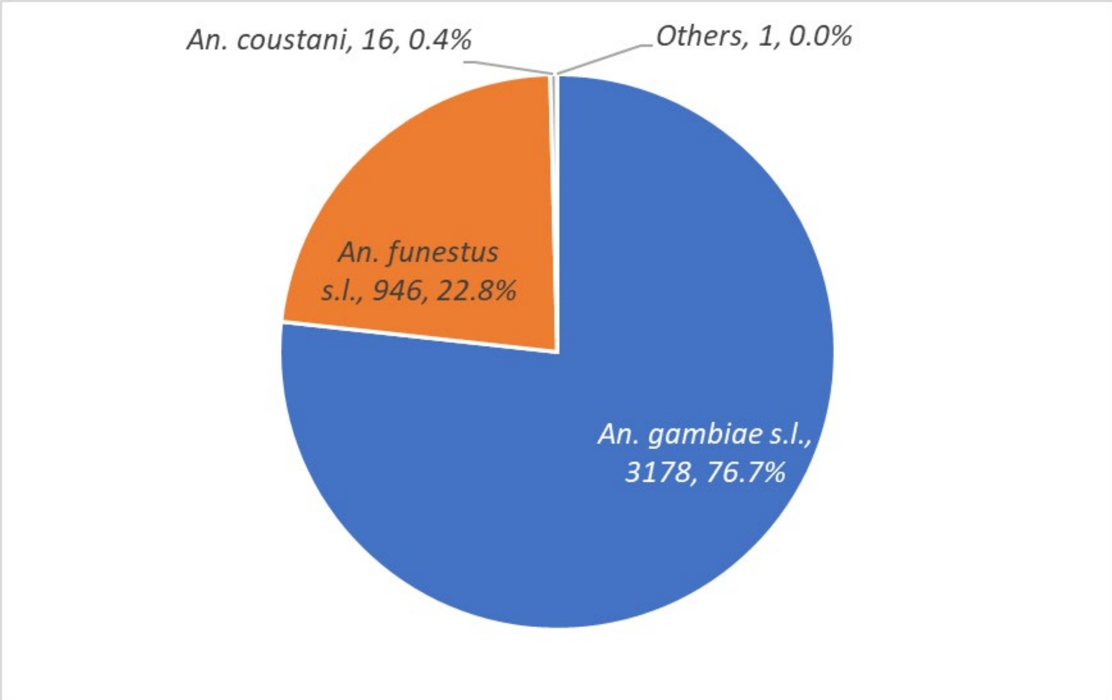
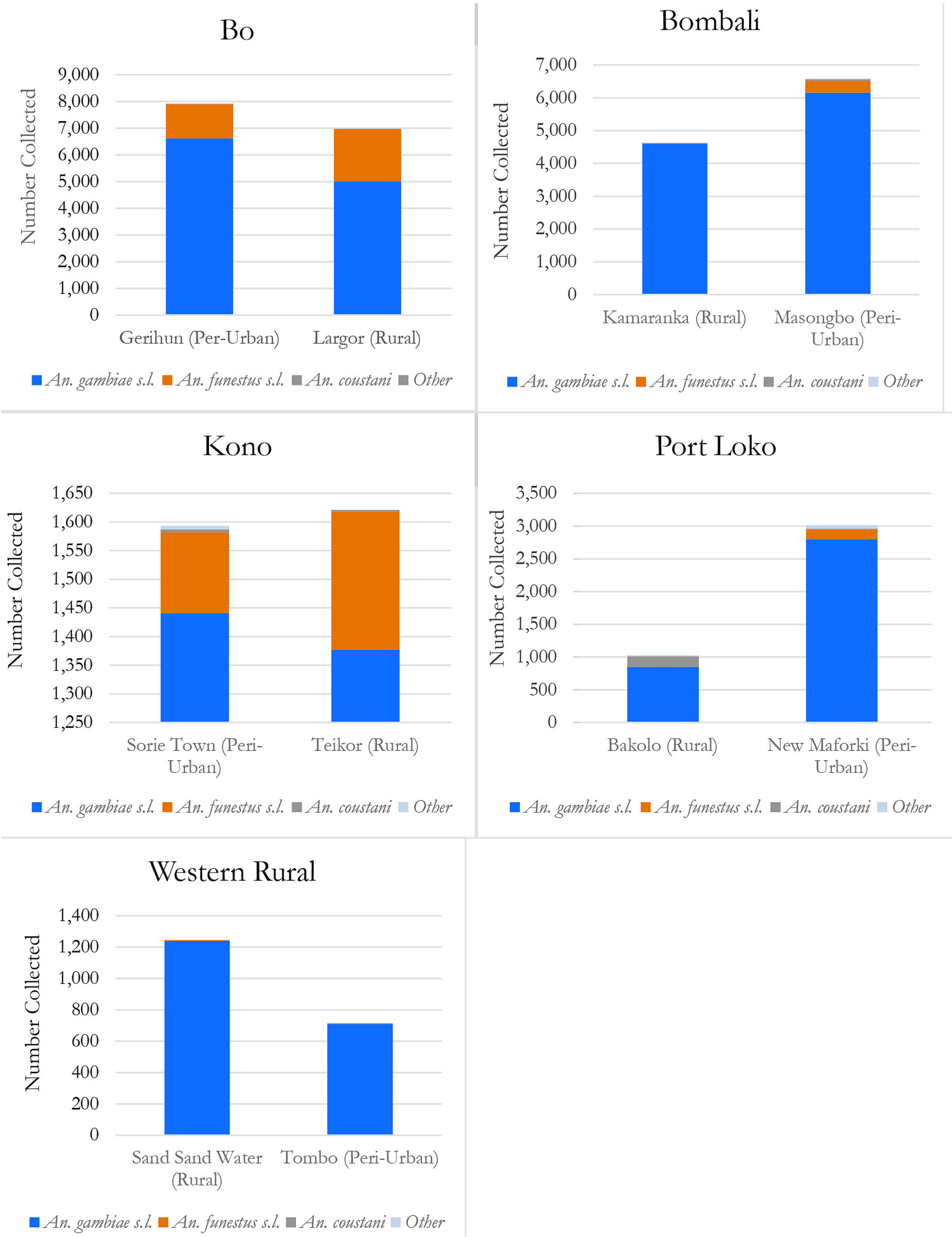


Figure 6: *Anopheles* Species Collected (PSC, HLC, and CDC-LT) by District/Site, Between March 2021 and February 2022.

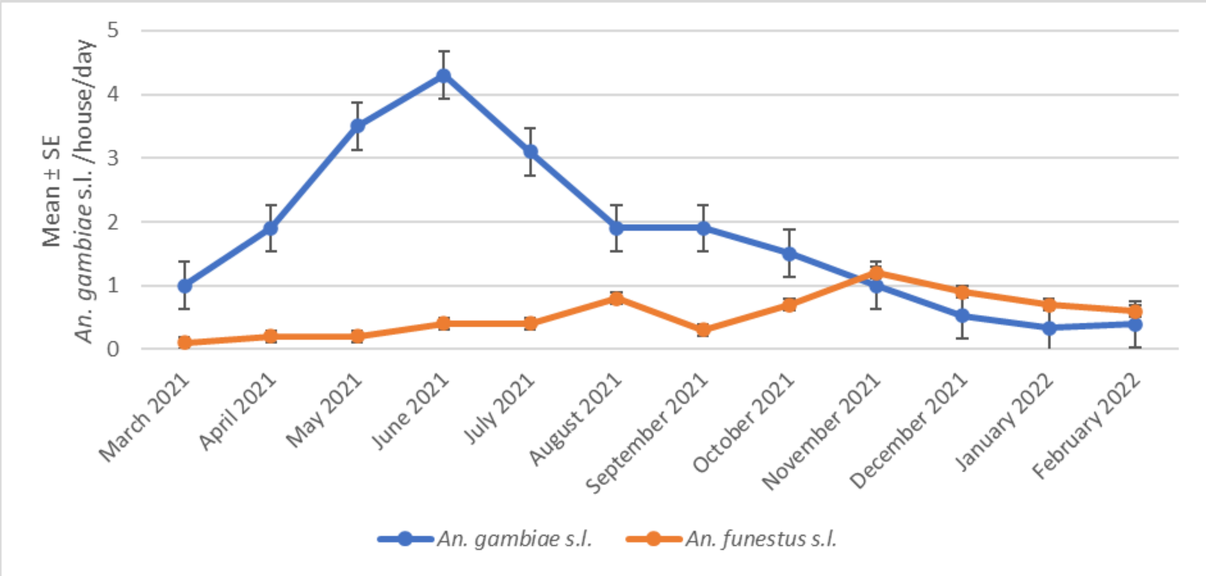


3.1.2 Pyrethrum Spray Collections

Indoor Resting Density (PSC Collection)

The indoor resting density (IRD) of malaria vectors in Sierra Leone followed the rainfall pattern with peaks observed between May and September. Overall, the highest indoor resting density peak was observed in June for *An. gambiae* s.l. while for *An. funestus*, smaller peak was observed in November (Figure 7).

Figure 7: Mean IRD of *An. gambiae* s.l. and *An. funestus* s.l. Across All Sentinel Sites, March 2021–February 2022



The IRD of *An. gambiae* s.l. per house per day between March 2021 and February 2022 varied across village, district, and month (Figures 8 and 9). The mean IRD in all sites peaked between June and September except in Kono district where peak IRD was recorded in April 2021.

In Bo District, the mean IRD was highest in June in Largor rural site (14.9/house/day) (Figure 8) which was also the highest IRD recorded. In Bombali District, the highest IRD was recorded in Kamaranka rural site (4.3/house/day), also in June. A similar value (4.7/house/day) was recorded the previous year in 2020 before IRS. There was no clear difference in mean resting density between peri-urban and rural sites (Figure 8). Density peaked in June following the rains and the timing of IRS was timed in May before the high mosquito season.

In Port Loko, there was no clear pattern in New Maforki in the rainy season with the highest IRD being in September in the peri-urban site of New Maforki (2.7/house/day) (Figure 9). Mean IRD in Bakolo followed rainfall pattern, rising from June, and peaking in August (2/house/day) (Figure 9).

In Kono district, the mean IRD did not follow the rainfall pattern. The highest IRD was recorded in peri-urban site of Sorie town in April (6.7/house/day) and it declined through the rainy season (Figure 9). The dip in density observed in July could be due to the rains affecting the mosquito breeding sites. The mean IRD in Western Rural also followed the rainfall pattern with highest IRD recorded in the rural site of Sand Sand Water (8.7/house/day) (Figure 9).

Figure 8: Mean IRDs of *An. gambiae* s.l. in Bo and Bombali Districts, Comparing Rural Vs Peri-Urban sites, March 2021–February 2022

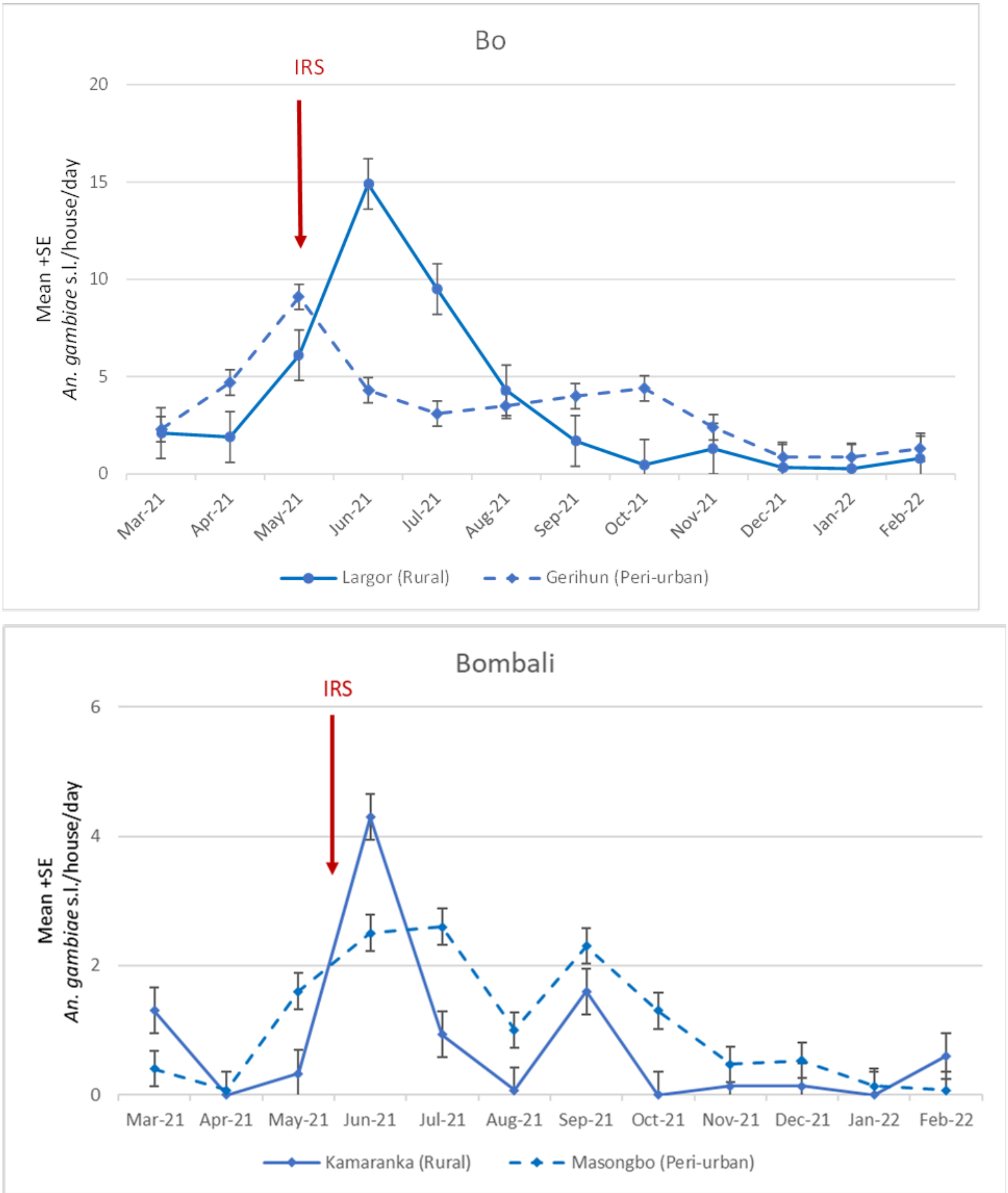
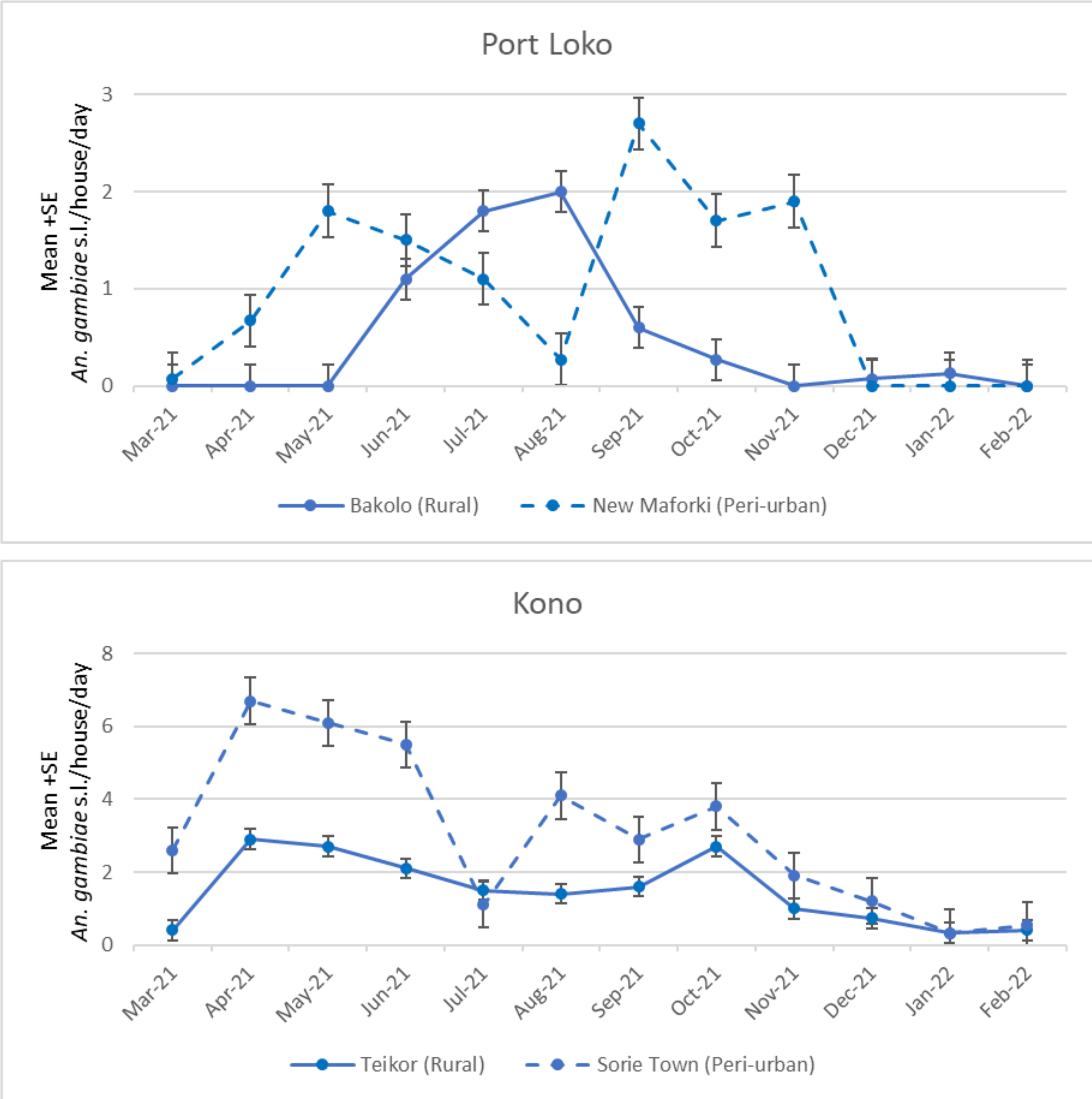
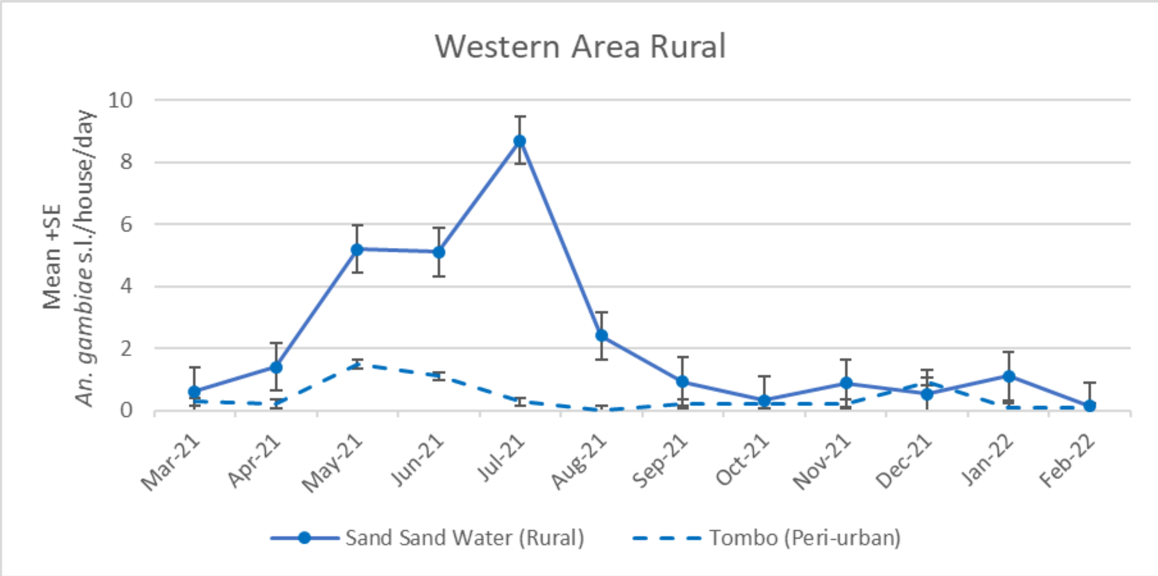


Figure 9: Mean IRDs of *An. gambiae* s.l. in Port Loko, Kono and Western Area Rural Districts, Comparing Rural Vs Peri-Urban sites, March 2021–February 2022

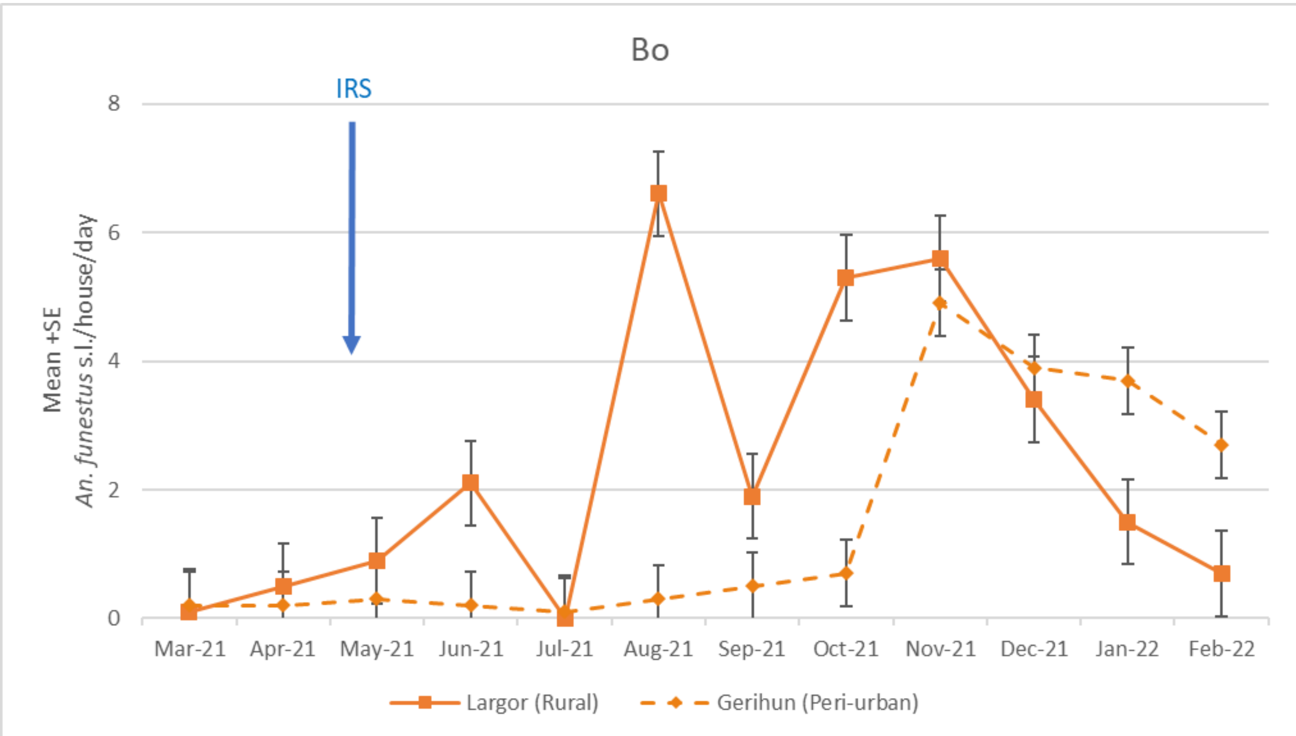




Indoor Resting Density of *Anopheles funestus* s.l. Collected by PSC

Anopheles. funestus s.l. was sampled in all districts but appreciable numbers were collected in Bo and Kono districts, which contributed more to overall IRD trends (Figures 10 and 11). In Bo, the highest IRD was recorded in August in Largor rural site (6.6/house/day) (Figure 10). The mean densities were also higher between October and November and started declining in December with the lowest number collected in March and July. In Kono district, two peaks were recorded, one in July in both Sorie Town and Teikor (1.7/house/day) and another in February in Teikor rural site (2.2/house/day) (Figure 11).

Figure 10: Mean IRDs of *An. funestus* s.l. in Bo and Bombali Districts, Comparing Rural Vs Peri-Urban sites, March 2021–February 2022



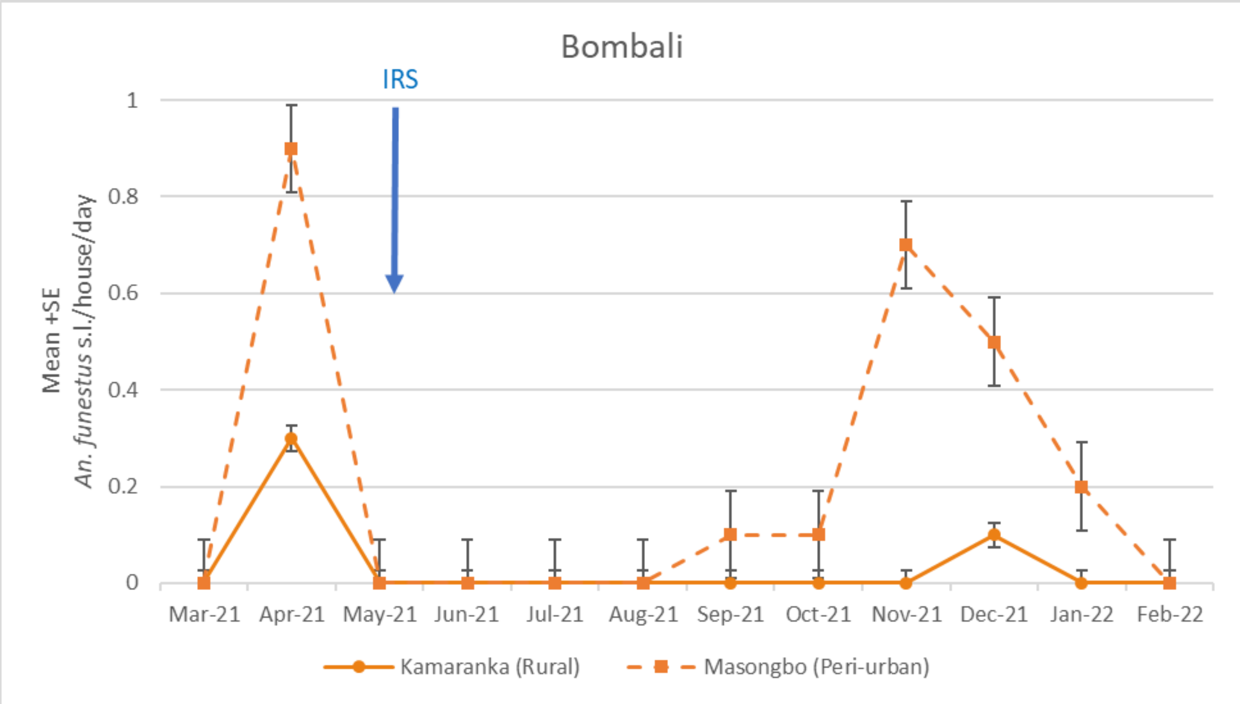
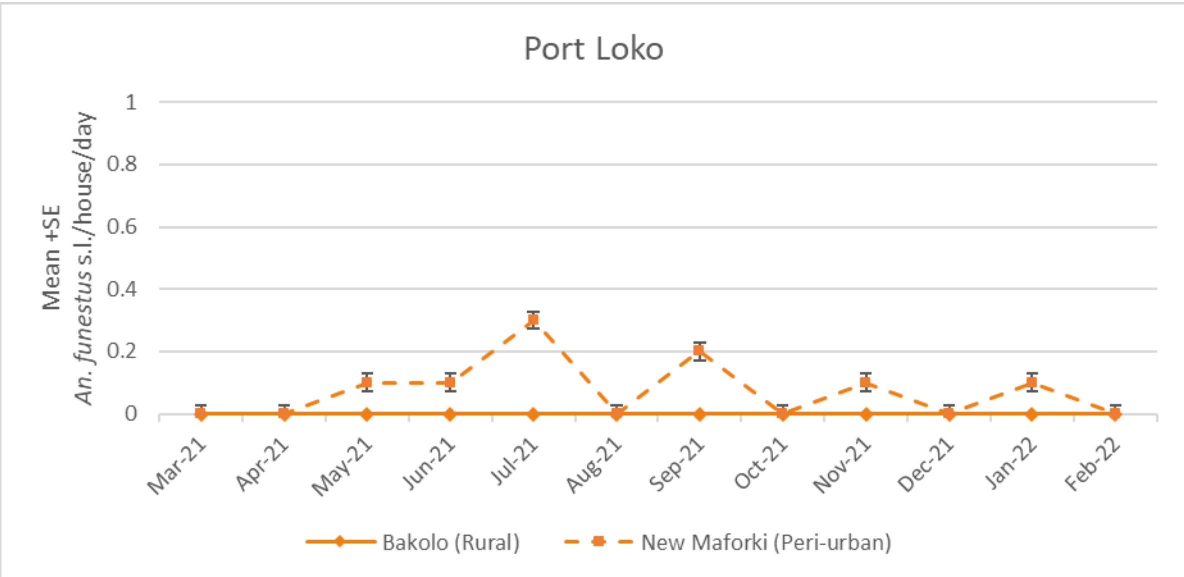
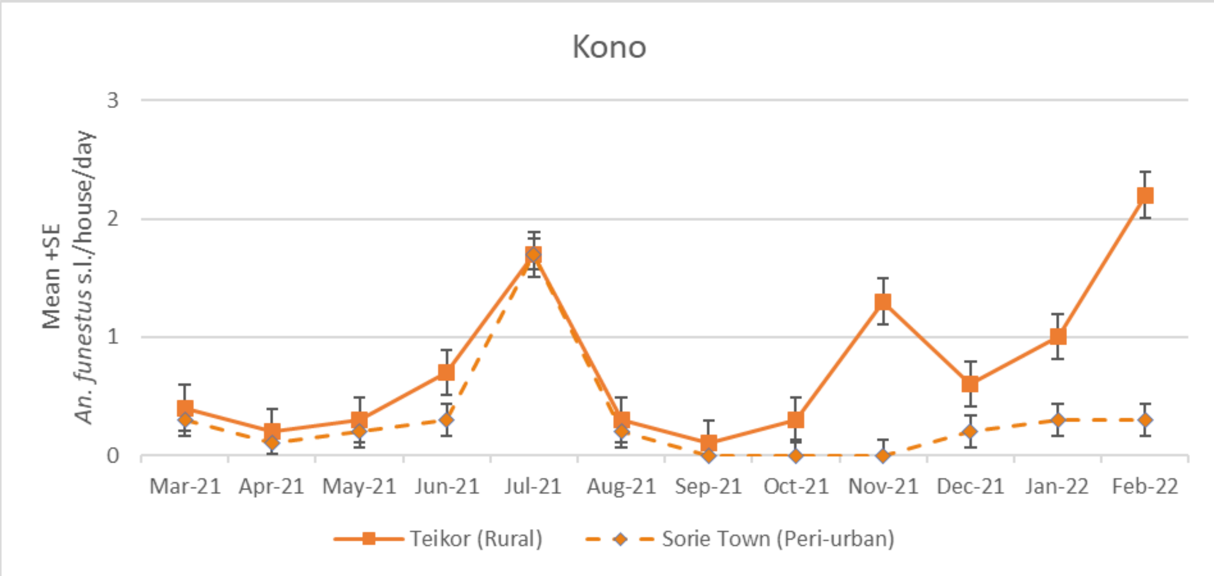
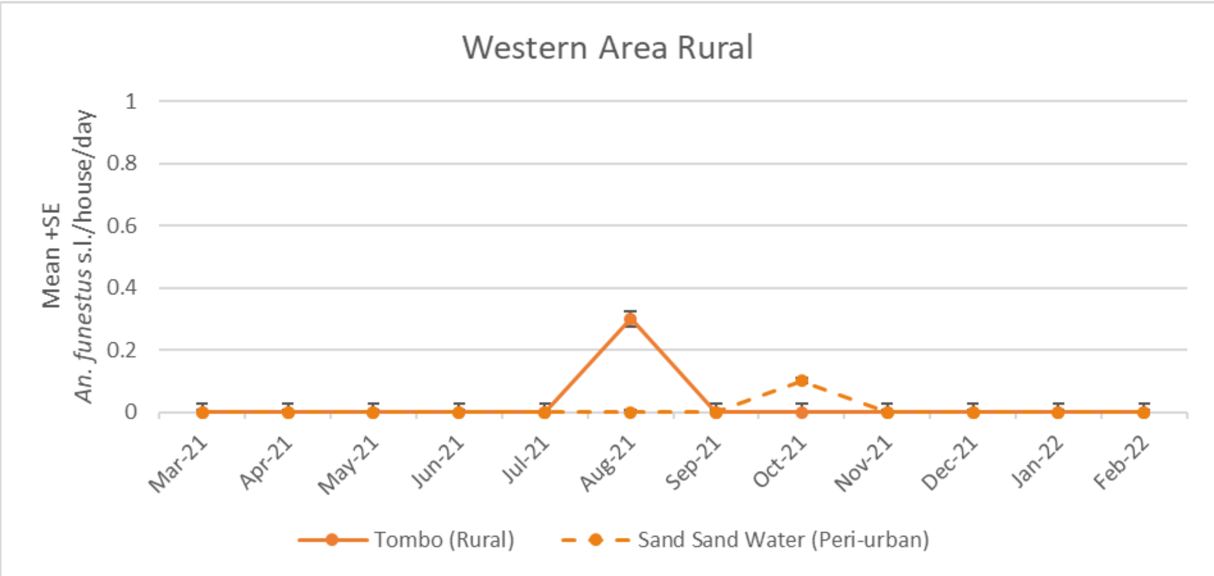


Figure 11: Mean IRDS of *An. funestus* s.l. in Port Loko, Kono and Western Area Rural Districts, Comparing Rural Vs Peri-Urban sites March 2021–February 2022



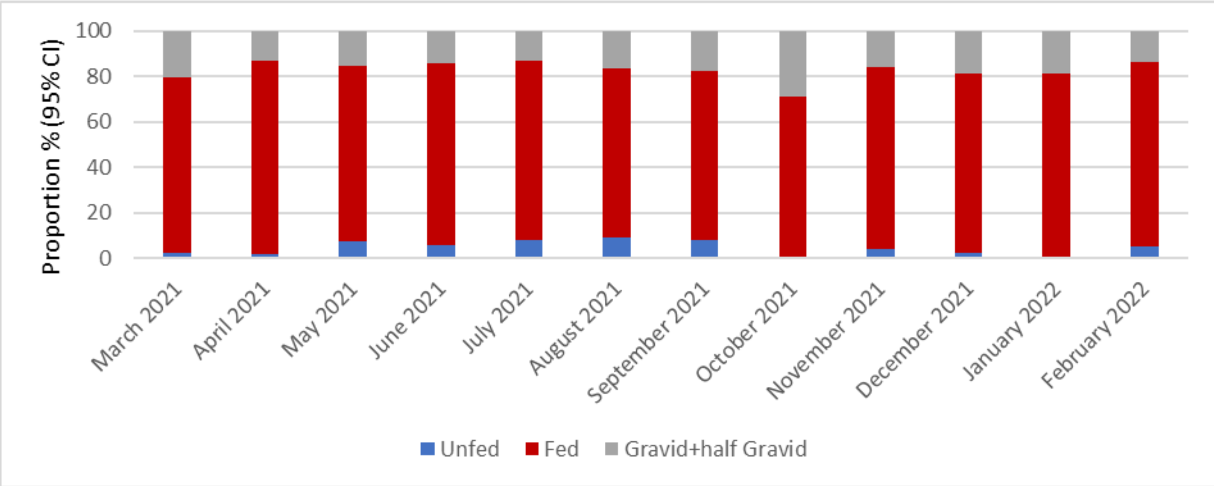




3.1.3 Abdominal Blood Digestions Stages of *An. gambiae* s.l. Collected by PSC

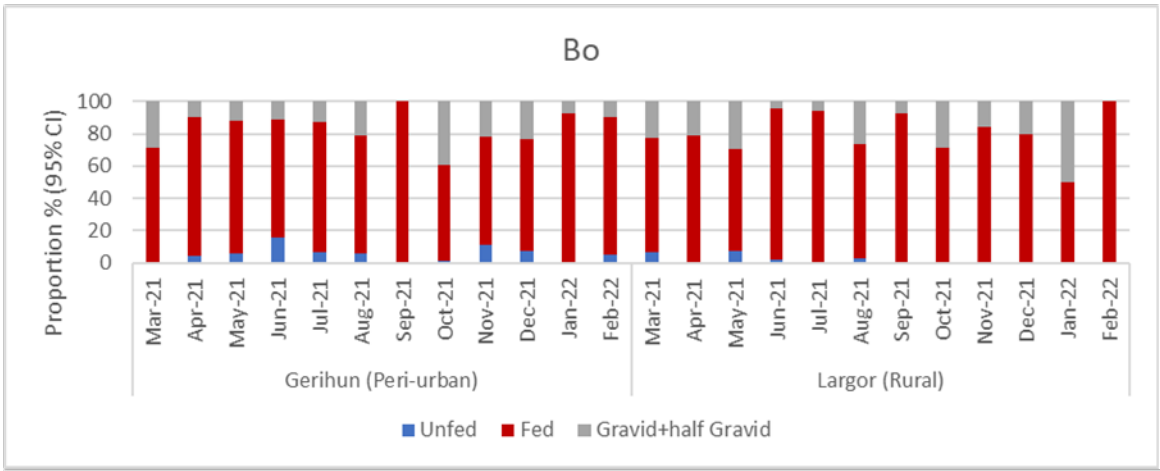
A higher number of fed compared to unfed/gravid *An. gambiae* s.l. mosquitoes were sampled by PSC all through the sampling period (Figure 12). This has remained so for the past three years during routine monitoring. There was no relationship/trend between proportion fed and season (Figures 13 and 14). The proportion of fed mosquitoes was higher throughout the year. The highest proportion of gravid/half gravid mosquitoes were sampled in October toward the end of the rainy season (Figure 12).

Figure 12: Abdominal Blood Digestions Stages of *An. gambiae* s.l. Collected by PSC Across All Sentinel Sites, March 2021–February 2022



When disaggregated by district, there were still more blood fed *An. gambiae* s.l. compared to gravid and unfed (Figures 12-13). Empty bars indicate months where no mosquitoes were sampled by PSC.

Figure 13: Abdominal Blood Digestions Stages of *An. gambiae* s.l. in Bo and Bombali, Collected by PSC, March 2021–February 2022



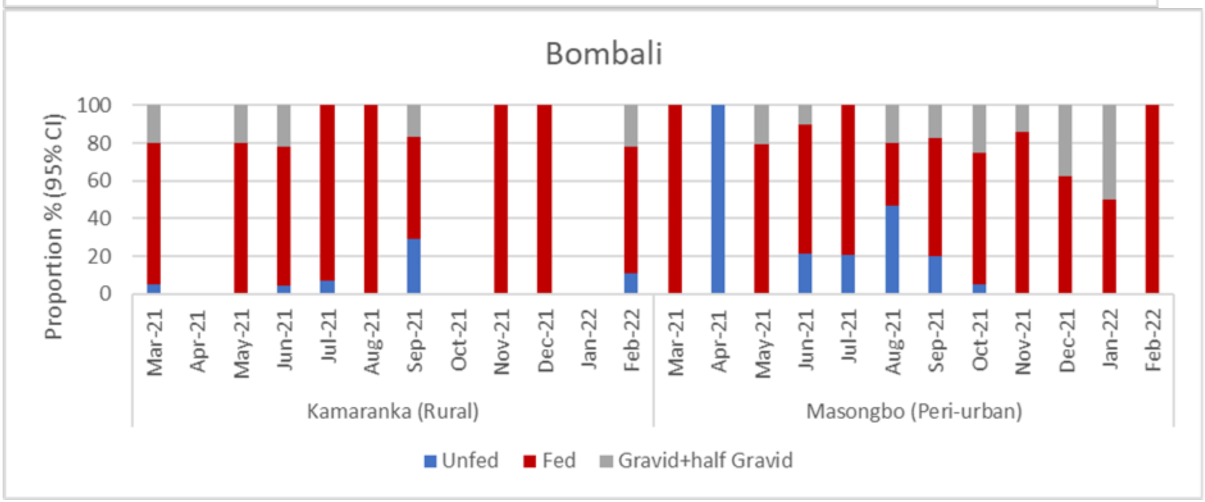
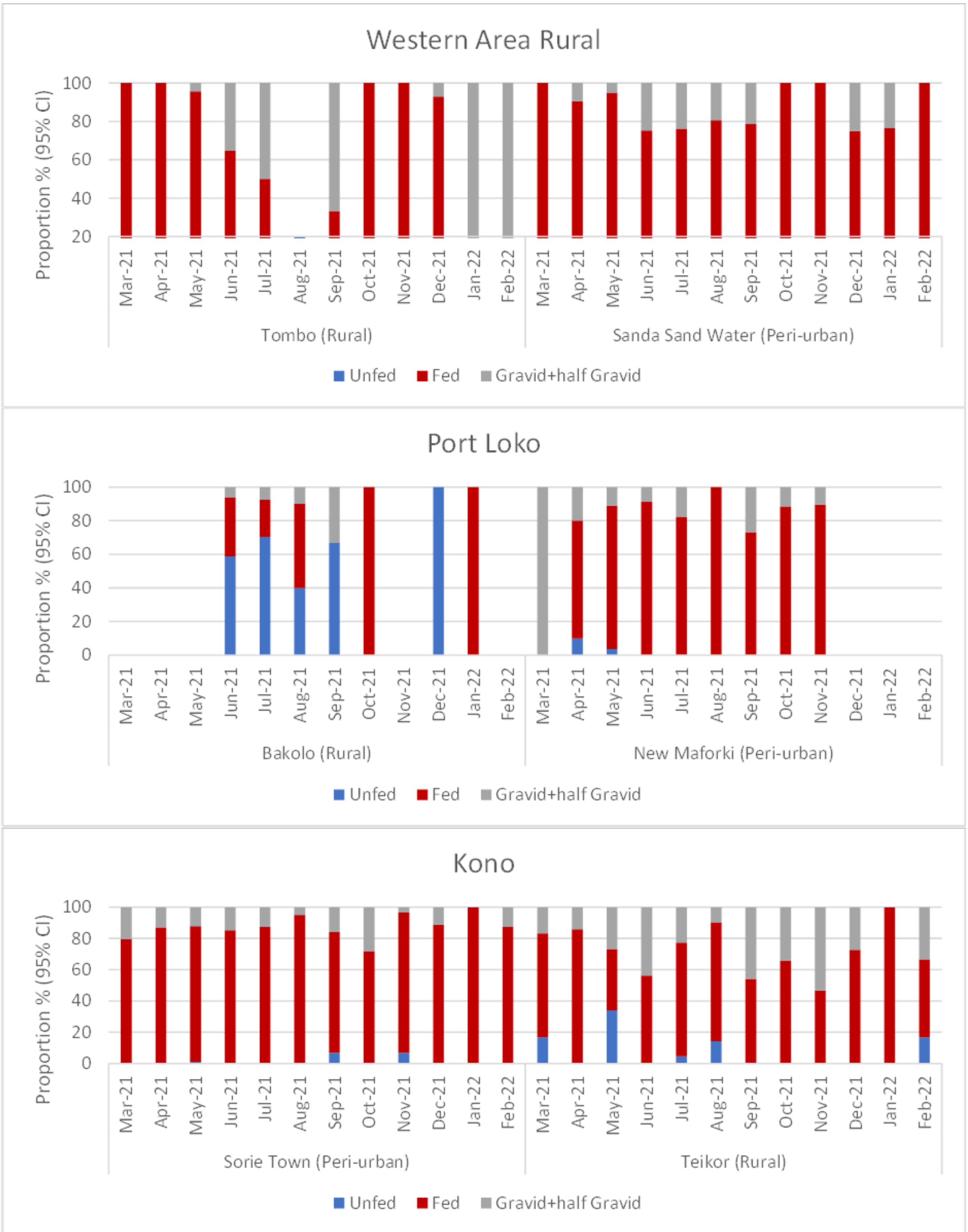


Figure 14: Abdominal Blood Digestions Stages of *An. gambiae* s.l. in Western Rural, Port Loko and Kono, Collected by PSC, March 2021–February 2022



3.1.4 ABDOMINAL BLOOD DIGESTIONS STAGES OF *AN. FUNESTUS* GROUP COLLECTED BY PSC

There was no difference between the proportion of fed *An. funestus* group compared to the proportion of gravid/half gravid all through the sampling period (Figures 15 and 16). The proportion of unfed mosquitoes was always low apart from July and October (Figure 15). 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 (Figure 16).

Figure 15: Abdominal Blood Digestions Stages of *An. funestus* Group Collected by PSC Across All Sentinel Sites, March 2021- February 2022

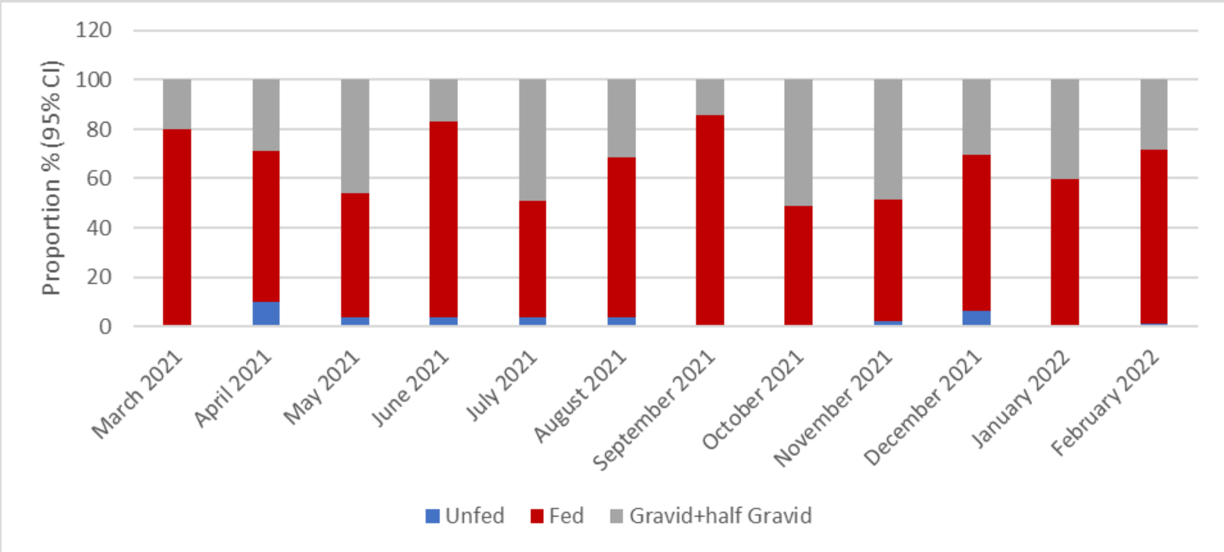
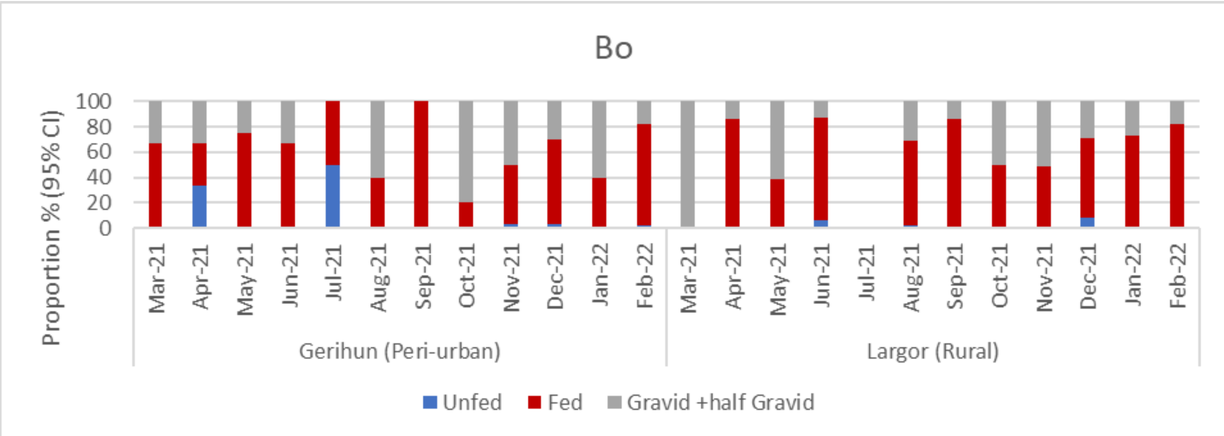
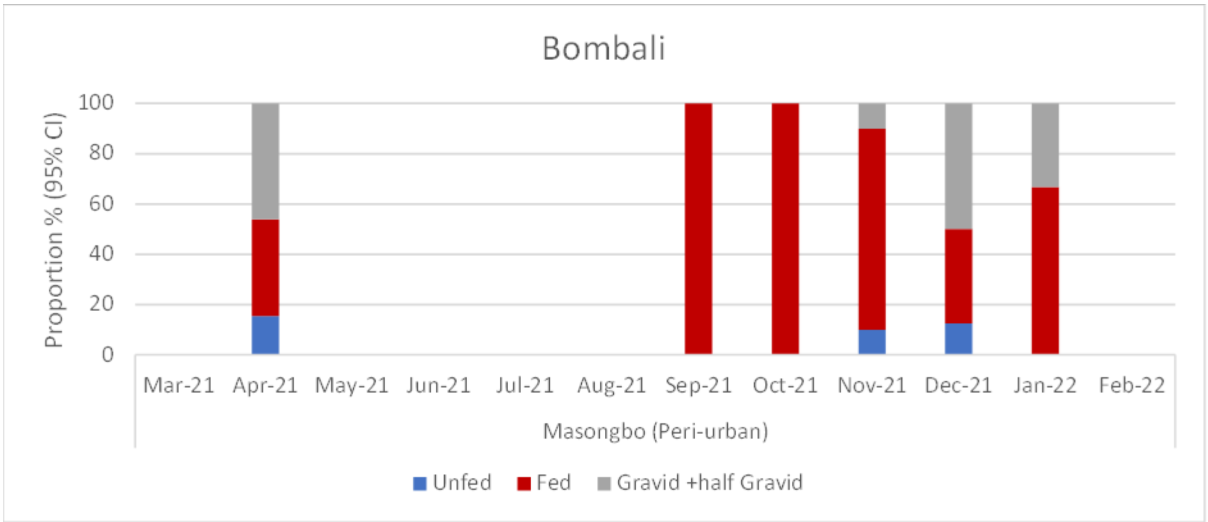
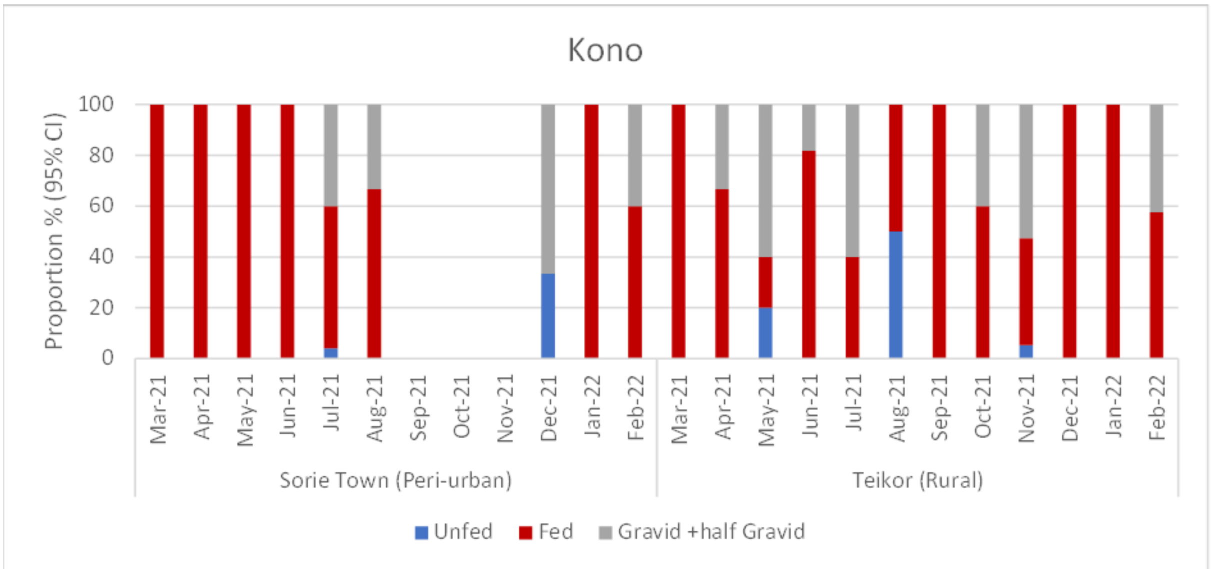
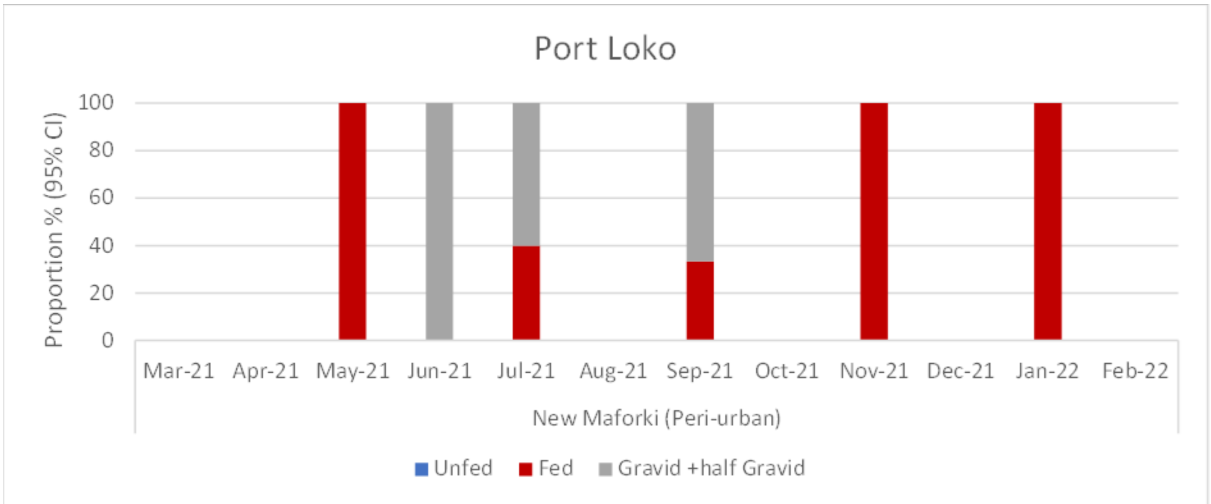


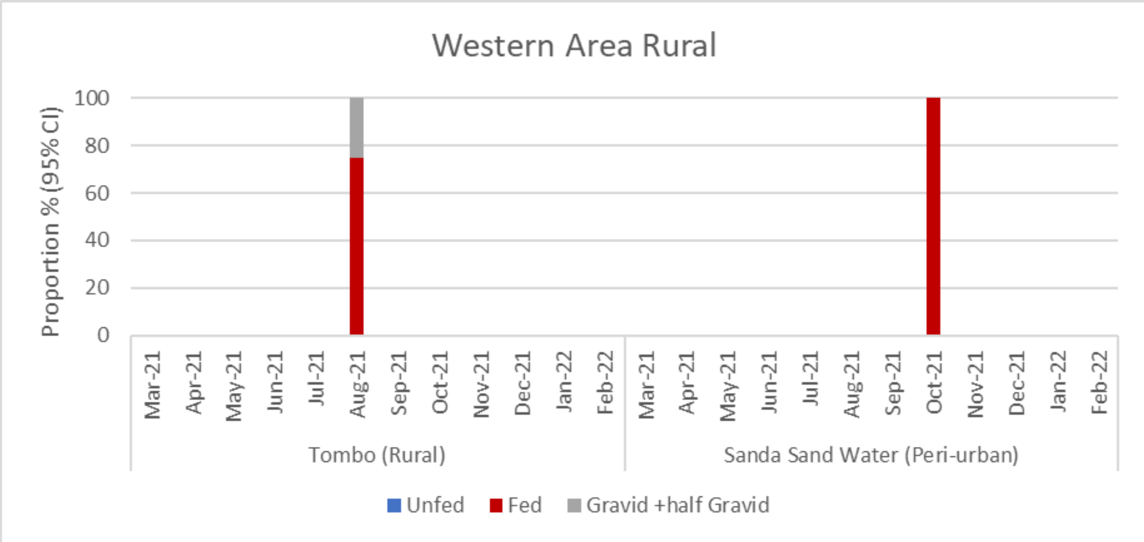
Figure 16: Abdominal Blood Digestions Stages of *An. funestus* Group in Bo, Bombali, Kono, Port Loko and Western Area Rural, Collected by PSC, March 2021–February 2022




Bombali
Proportion % (95% CI)
100
80
60
40
20
0
Mar-21
Apr-21
May-21
Jun-21
Jul-21
Aug-21
Sep-21
Oct-21
Nov-21
Dec-21
Jan-22
Feb-22
Masongbo (Peri-urban)
Unfed
Fed
Gravid +half Gravid


Kono
Proportion % (95% CI)
100
80
60
40
20
0
Mar-21
Apr-21
May-21
Jun-21
Jul-21
Aug-21
Sep-21
Oct-21
Nov-21
Dec-21
Jan-22
Feb-22
Sorie Town (Peri-urban)
Teikor (Rural)
Unfed
Fed
Gravid +half Gravid


Port Loko
Proportion % (95% CI)
100
80
60
40
20
0
Mar-21
Apr-21
May-21
Jun-21
Jul-21
Aug-21
Sep-21
Oct-21
Nov-21
Dec-21
Jan-22
Feb-22
New Maforki (Peri-urban)
Unfed
Fed
Gravid +half Gravid

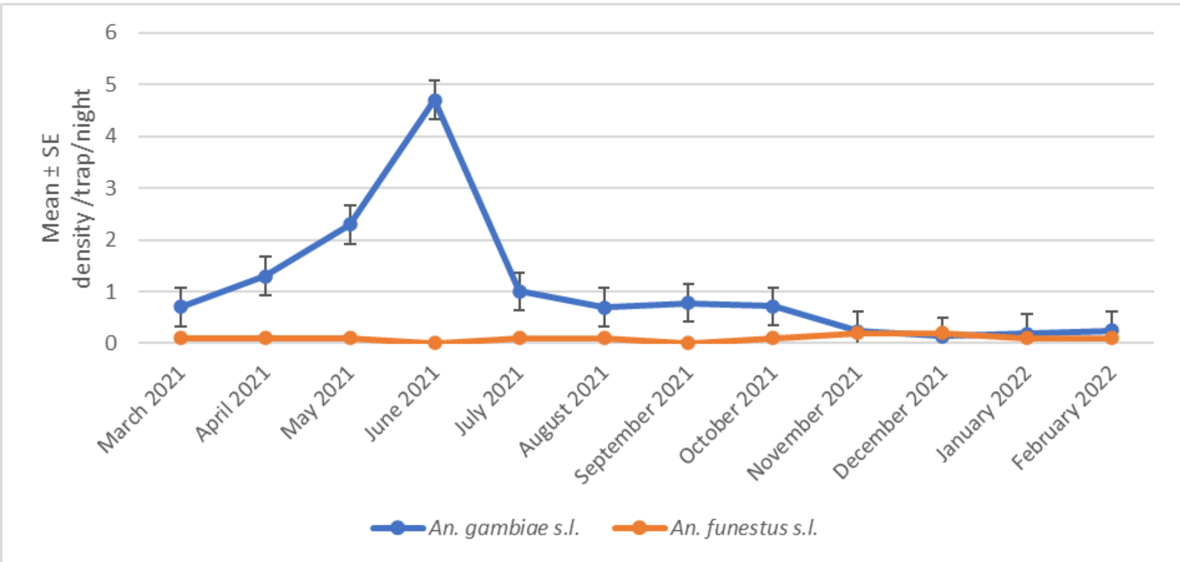


3.1.5 CDC Light Trap Collection

Anopheles gambiae s.l. was also the most predominant species by CDC-LT. Few numbers of *An. funestus* s.l. were also collected. The overall density per trap followed the rainfall pattern with peak density in June 2021 (Figure 17). The density of *An. funestus* was low; therefore no peak was observed (Figure 17).

The average density of *An. gambiae* s.l. per trap per night varied by district, site, and month during the sampling period (Figures 18 and 19).

Figure 17: Density of *An. gambiae* s.l. and *An. funestus* s.l. from CDC Light Trap Collections Across All Sentinel Sites, March 2021–February 2022.



The highest average density of *An. gambiae* s.l./trap/night was recorded in June in Masongbo, Bombali district (21.4/trap/night), a reduction from the 2020-2021 period (34.4/trap/night). Second highest density was in Gerihun (rural) in Bo (7.1/trap/night) in May (Figure 18). There was no clear difference in mean density between rural and peri-urban sites except in Bo where density seems to be higher in the peri-urban sites (Figures 18-19).

In Bo, Kono and Western Rural, the density per trap was highest at the beginning of the rainy season and declined through February (Figures 18-19). There was no clear pattern in Bombali and Port Loko districts (Figures 18-19).

For *An. funestus* s.l., the mean density per trap was always below 1 and therefore no clear pattern could be observed in the three districts (Bo, Bombali and Kono) where they were sampled (Figure 20).

Figure 18: Density of *An. gambiae* s.l. from CDC Light Trap Collections in Bo and Bombali Districts, Comparing Rural Vs Peri-Urban Sites, March 2021–February 2022

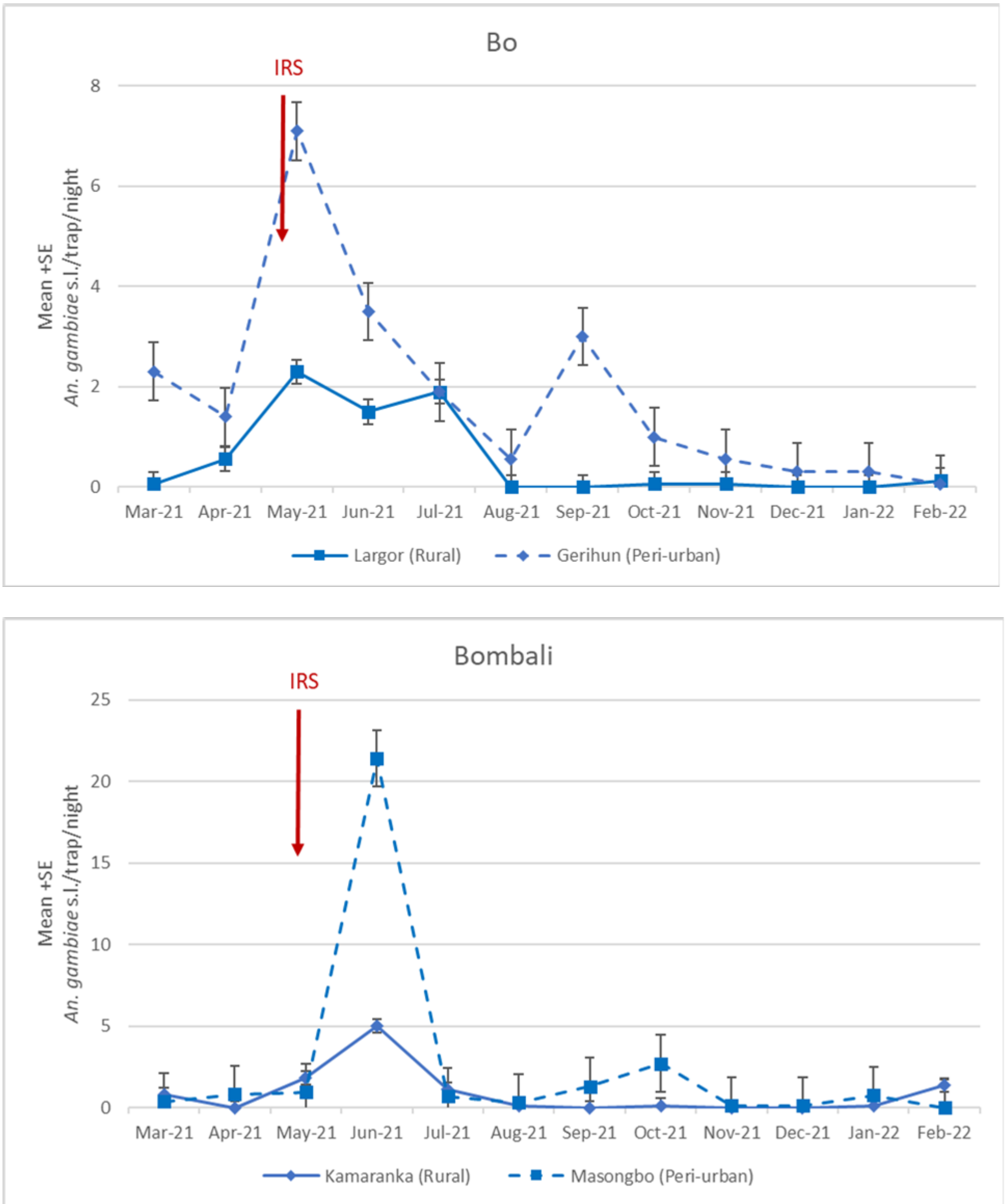
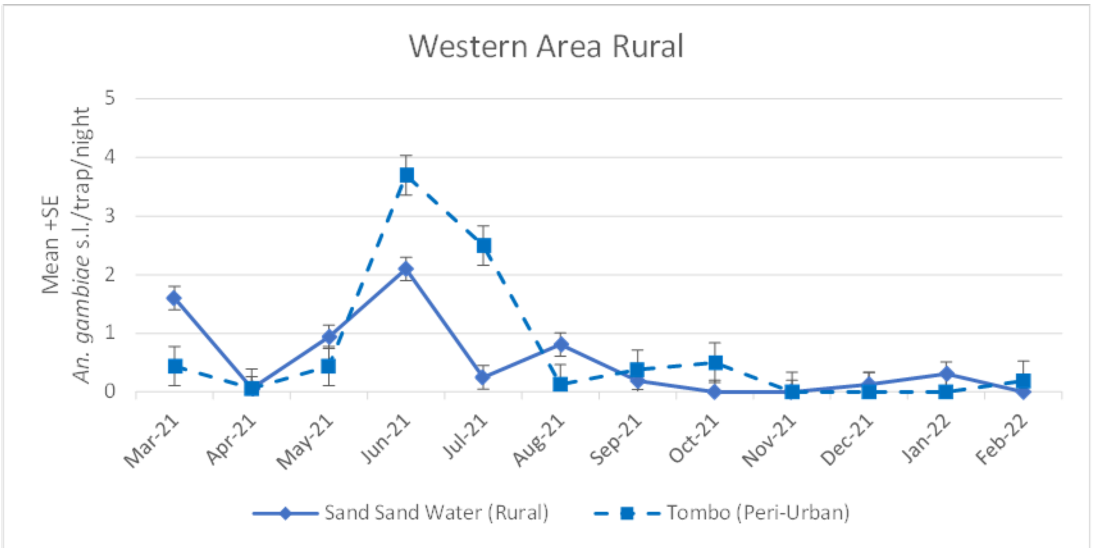
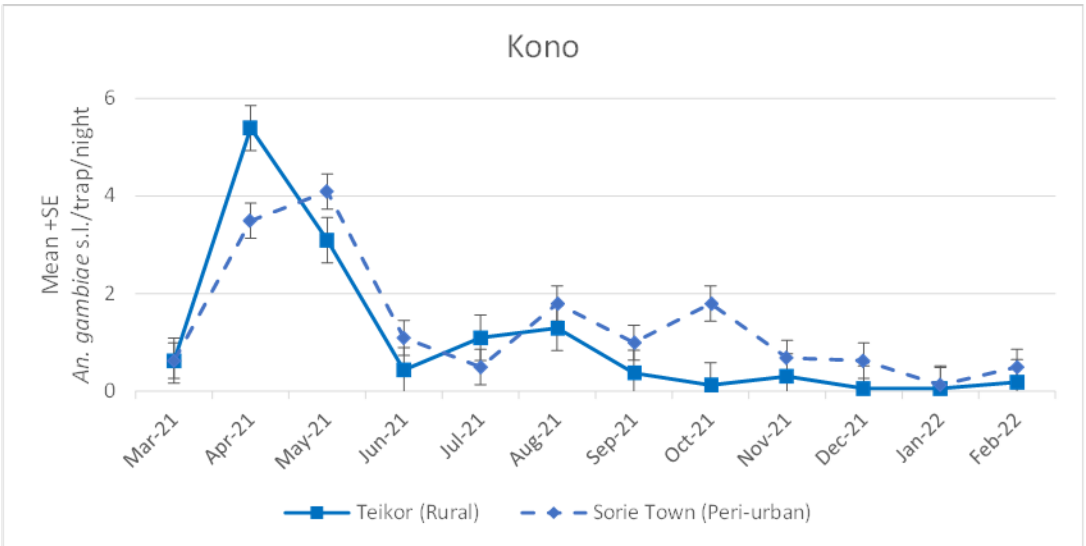


Figure 19: Density of *An. gambiae* s.l. from CDC Light Trap Collections in Western Rural, Kono and Port Loko Districts, Comparing Rural Vs Peri-Urban Sites, March 2021–February 2022





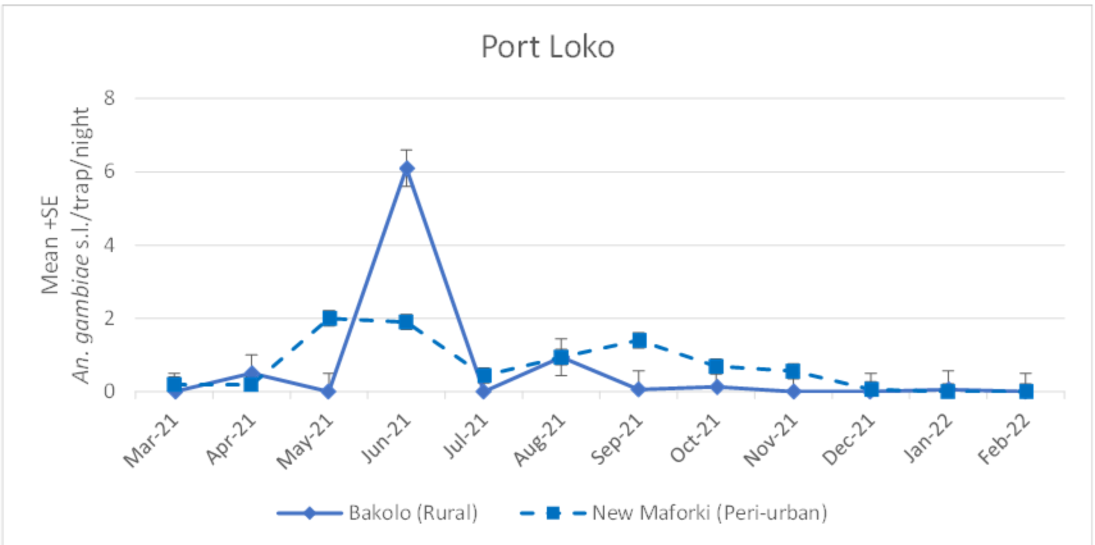
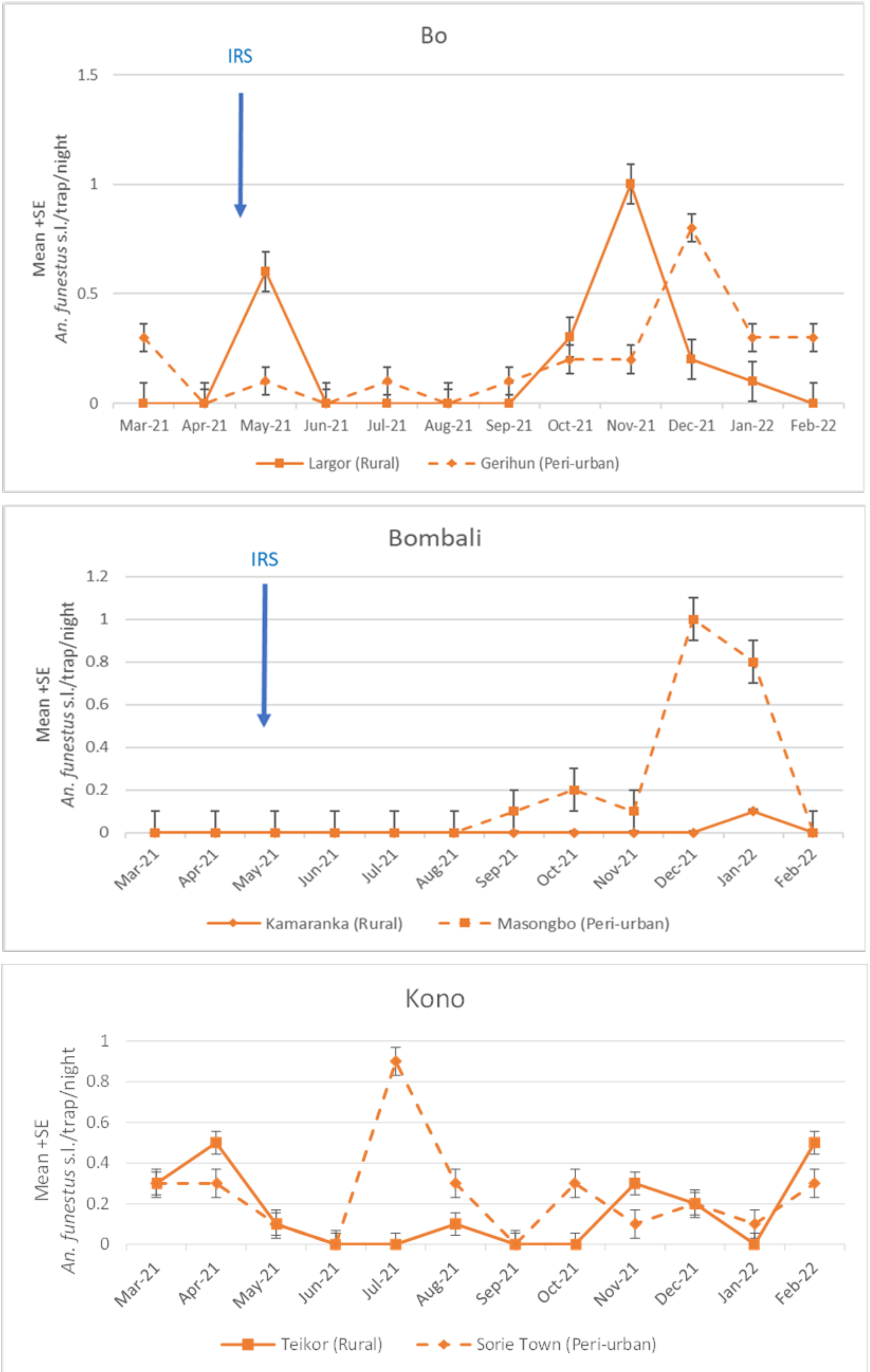


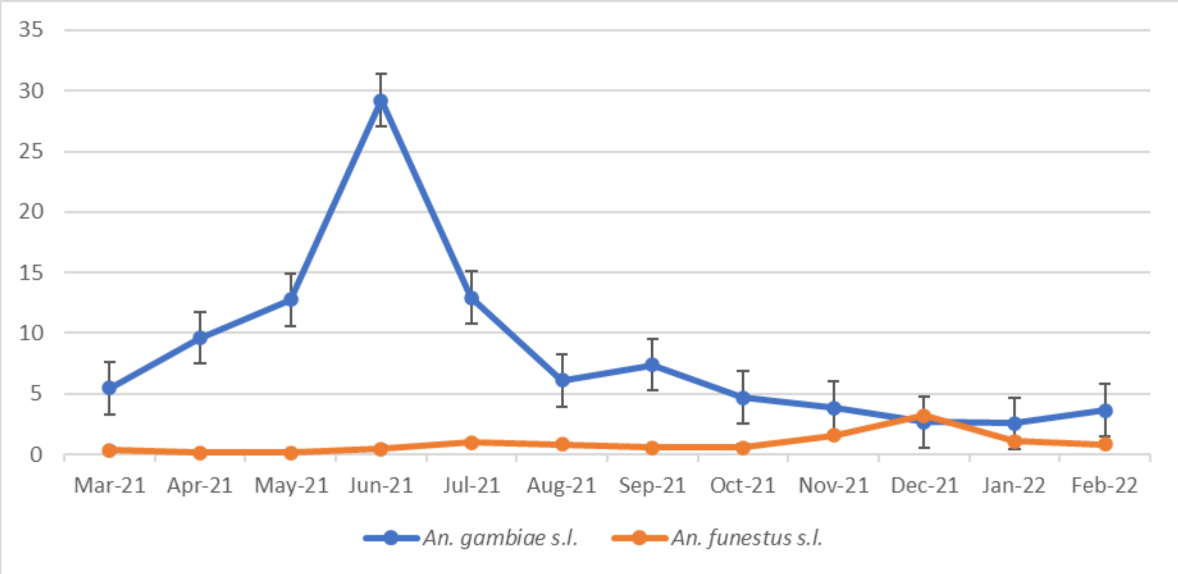
Figure 20: Density of *An. funestus* s.l. from CDC Light Trap Collections in Bo, Bombali and Kono Districts, Comparing Rural Vs Peri-Urban Sites, March 2021–February 2022



3.1.6 HUMAN LANDING COLLECTIONS

Human Biting Rate (HBR): The HBR (bites/person/night) of *An. gambiae* s.l. varied by district, site, and month through the sampling period. The mean HBR for *An. gambiae* s.l. followed the rainfall pattern, peaking in June and declining through February (Figure 21). For *An. funestus*, there was no observable pattern apart from a small peak observed in December 2021 (Figure 21).

Figure 21: Mean HBR of *An. gambiae* s.l. and *An. funestus* s.l. across all Sentinel Sites, March 2021–February 2022.



The outdoor and indoor biting patterns seem to be similar throughout the collection period (Figure 22). When data was aggregated across all sites, the HBR followed the rainfall pattern, peaking in June and declining through February 2022 (Figure 22).

The highest indoor HBR was recorded in June in Masongbo, Bombali district with 80.6 bites/person/night (Figure 23) representing a 50% reduction from the previous year (2020). In all districts, the HBR followed the rainfall pattern with a peak observed between June to September and declining into the dry season (Figures 22 and 23). There was no clear outdoor/indoor biting pattern difference in all sites (Figures 22 and 23).

The mean HBR of *An funestus* s.l. did not follow the rainfall pattern with highest peak observed during the dry season in December (Figure 25). The majority of the *An. funestus* s.l. was collected in Bo and contributed most to the overall trends in HBR. The highest HBR was recorded indoor in December in Gerihun, Bo District, 14.3 bites/person/night (Figure 26). In Bombali, the highest HBR was in December in Masongbo, with 4.4 bites/person/night, while in Kono District the highest was in Sorie town with 2.8 bites/person/night. Western rural was less than 1 bite/person/night (Figure 26).

Biting Location: There was no difference between indoor and outdoor biting for both *An. gambiae* s.l. and *An. funestus* s.l. in all sites.

Figure 22: *An. gambiae* s.l. Mean HBR Across All Sentinel Sites, March 2021–February 2022

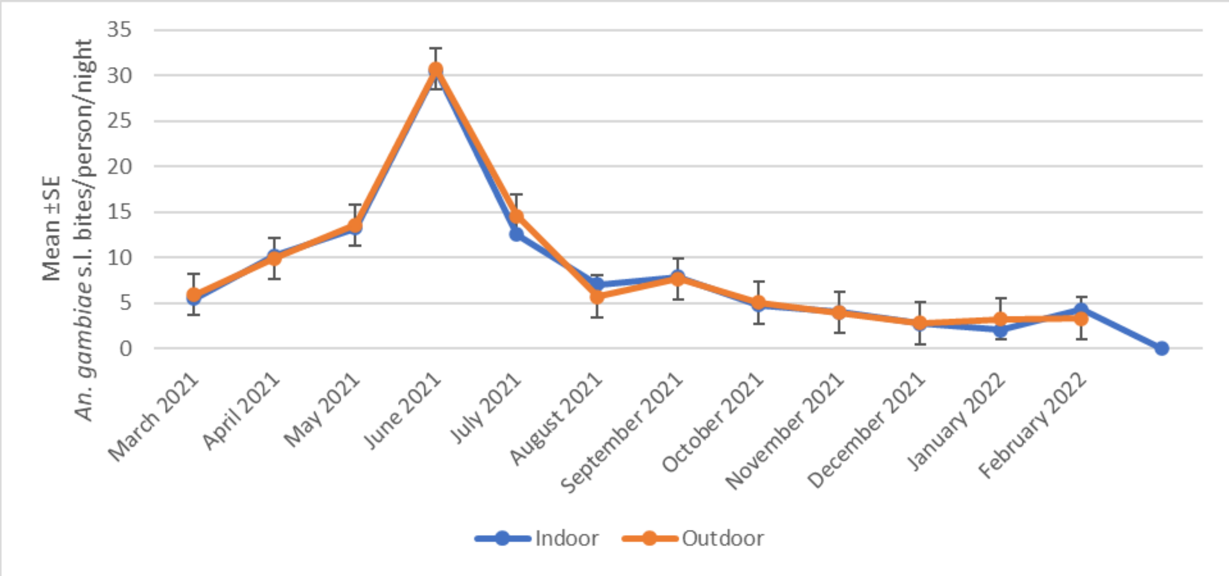
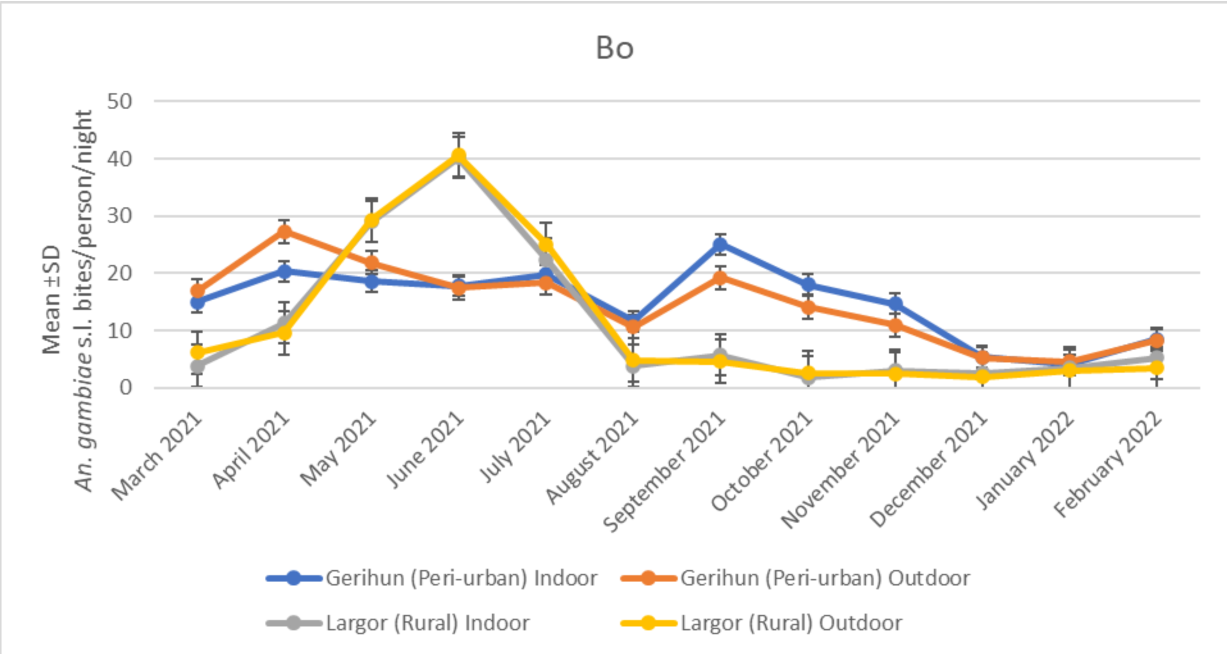


Figure 23: *An. gambiae* s.l. Mean HBR in Bo and Bombali Districts, March 2021–February 2022



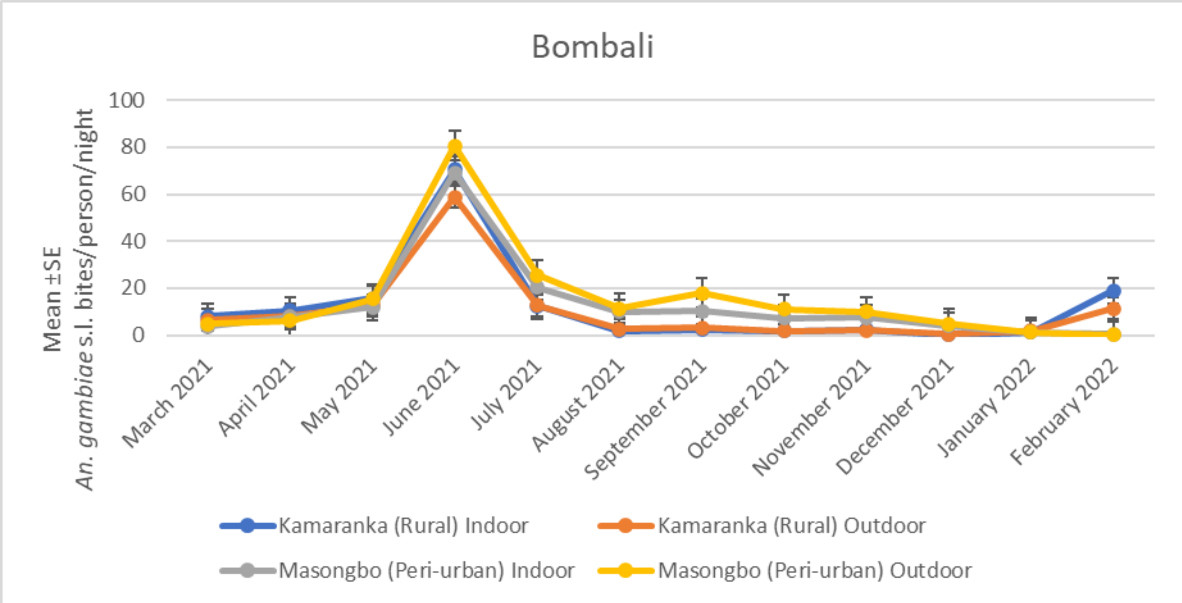
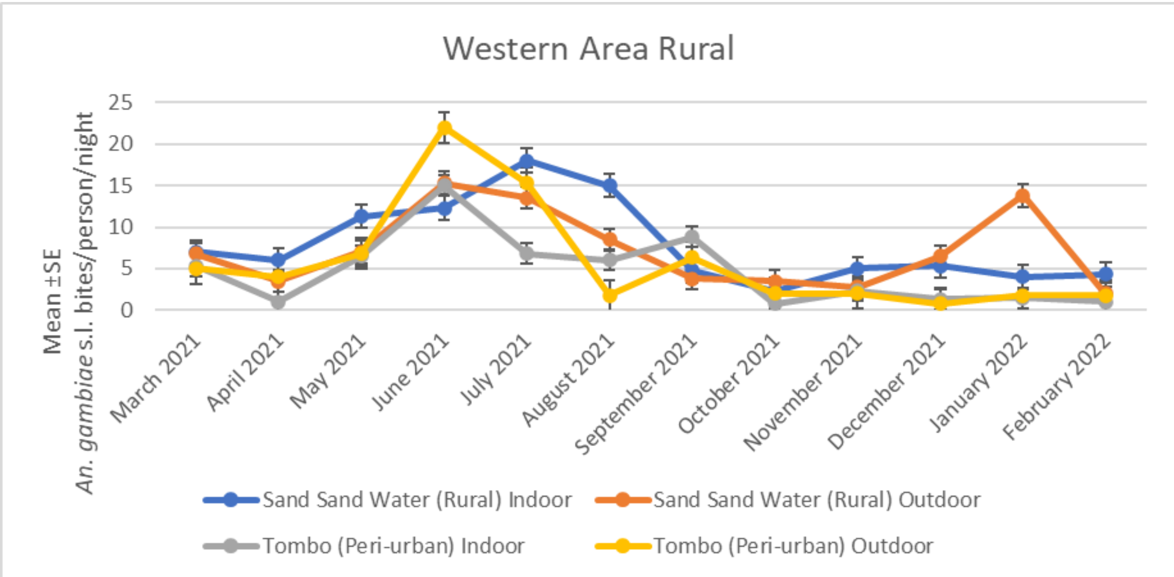
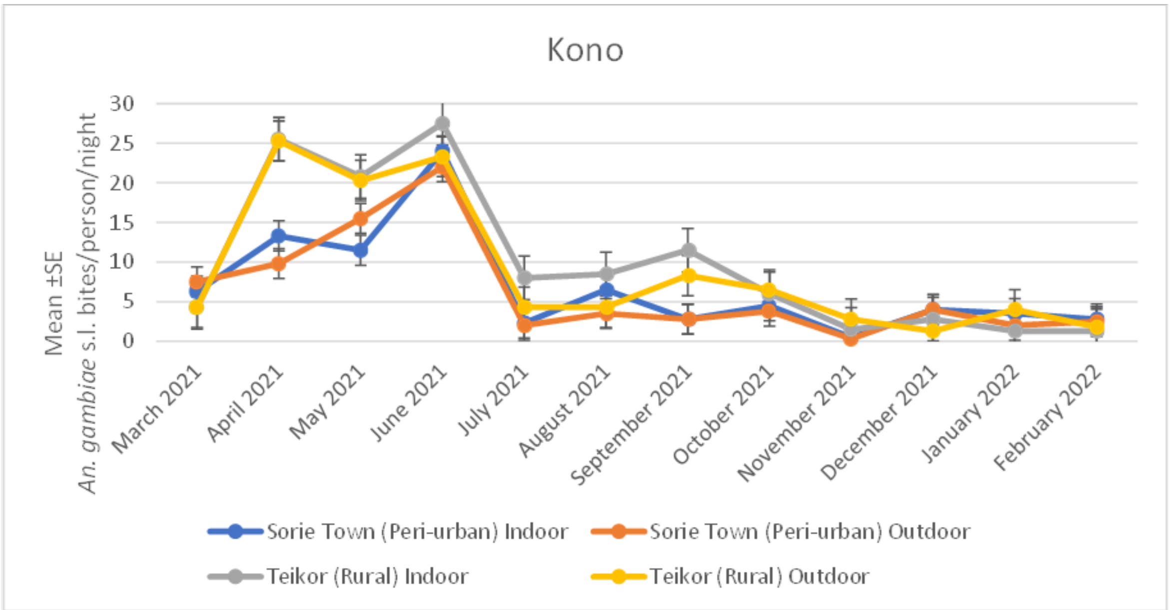


Figure 24: *An. gambiae* s.l. Mean HBR in Western Rural, Kono and Port Loko Districts, March 2021–February 2022




Kono
Mean ±SE
An. gambiae s.l. bites/person/night
30
25
20
15
10
5
0
March 2021
April 2021
May 2021
June 2021
July 2021
August 2021
September 2021
October 2021
November 2021
December 2021
January 2022
February 2022
Sorie Town (Peri-urban) Indoor
Sorie Town (Peri-urban) Outdoor
Teikor (Rural) Indoor
Teikor (Rural) Outdoor

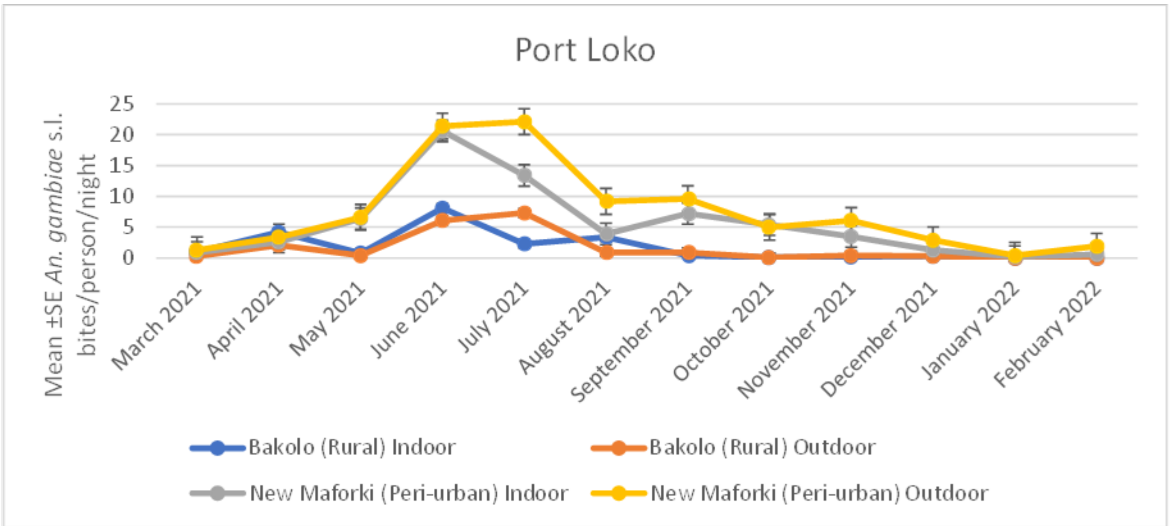

Port Loko
Mean ±SE An. gambiae s.l.
bites/person/night
25
20
15
10
5
0
March 2021
April 2021
May 2021
June 2021
July 2021
August 2021
September 2021
October 2021
November 2021
December 2021
January 2022
February 2022
Bakolo (Rural) Indoor
Bakolo (Rural) Outdoor
New Maforki (Peri-urban) Indoor
New Maforki (Peri-urban) Outdoor

Figure 25: *An. funestus* s.l. Mean HBR across Bo, Bombali, Kono and Western Rural Sentinel Sites, March 2021–February 2022

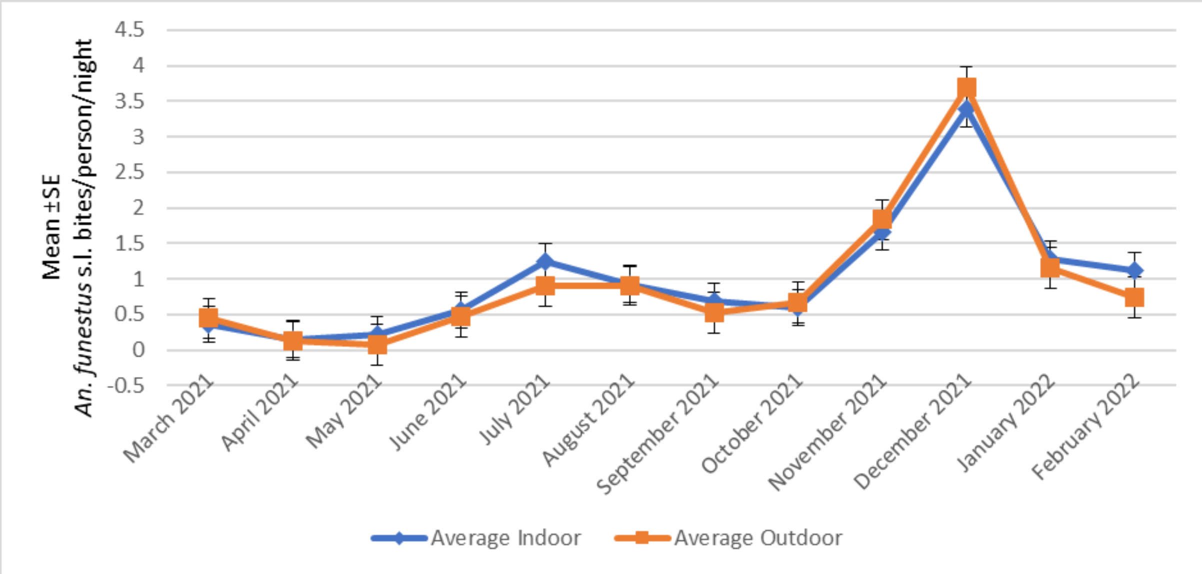
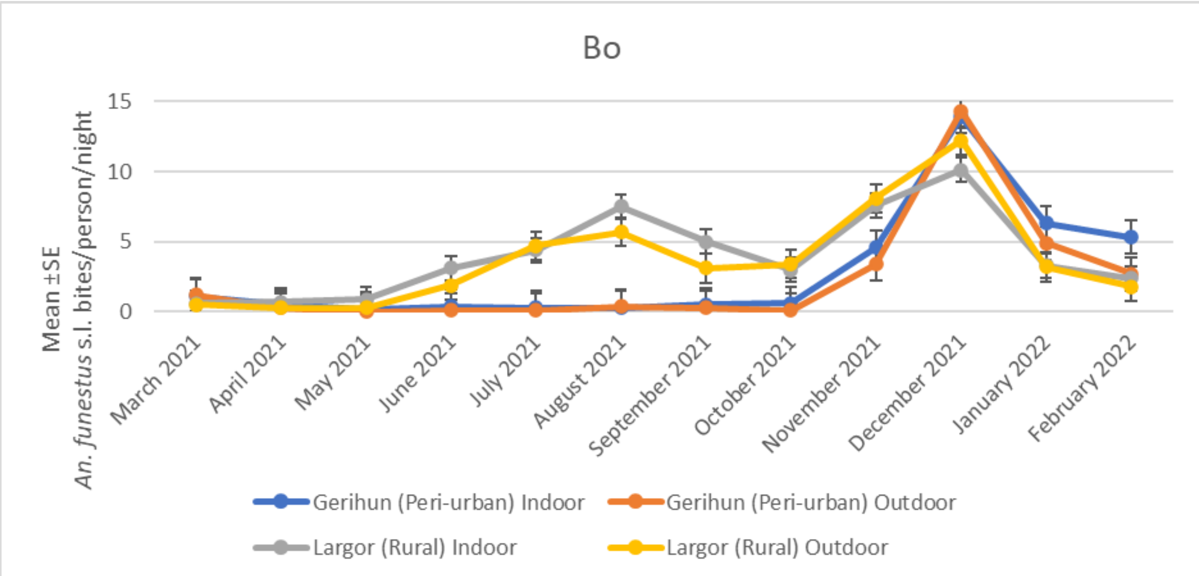
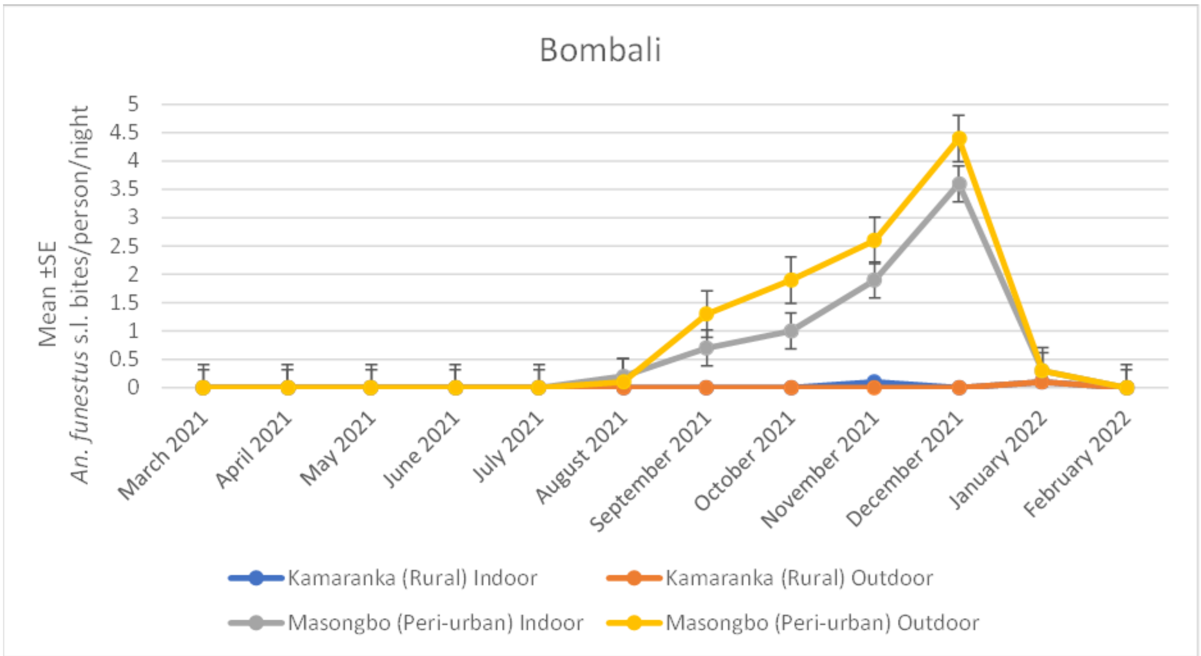
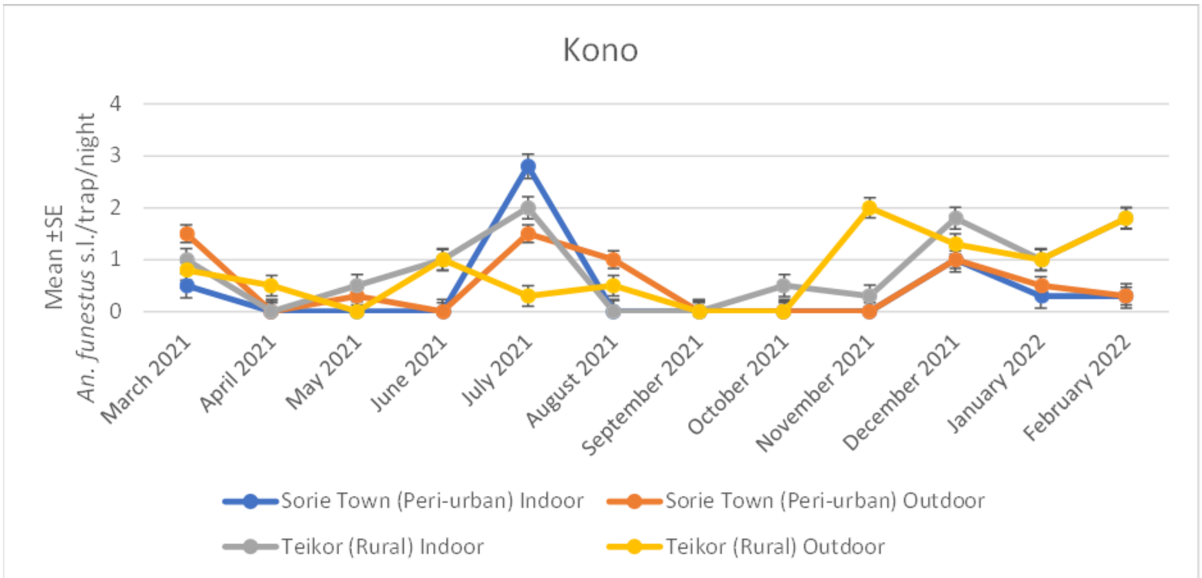


Figure 26: *An. funestus* s.l. Mean HBR in Bo, Bombali, Kono and Western Rural Districts, March 2021–February 2022



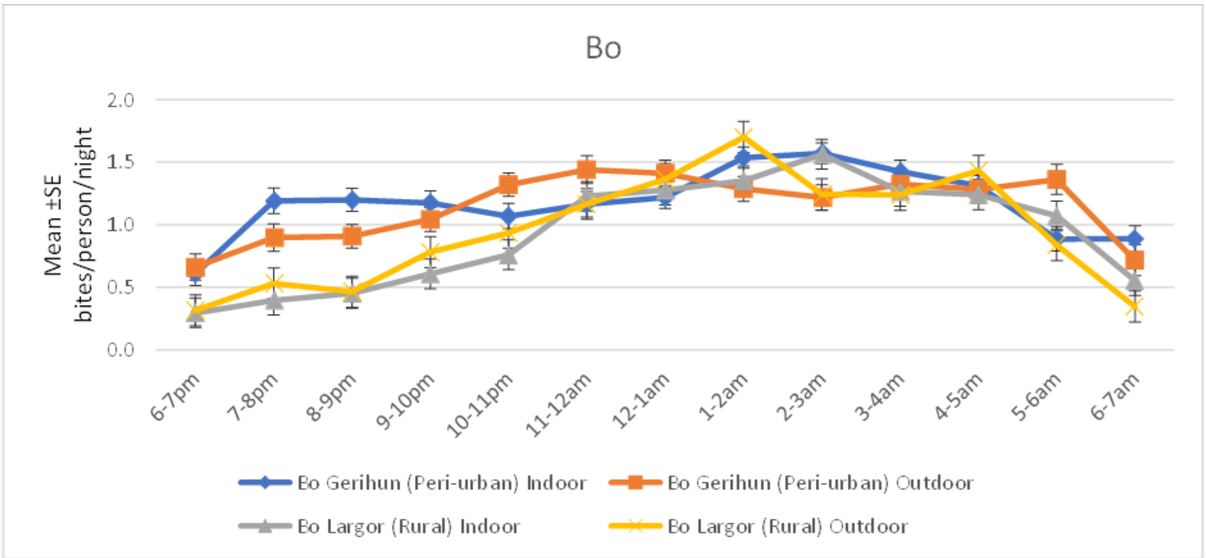




Biting Time: Biting activity of *An. gambiae* s.l. peaked at different times across the sites and districts. In almost all the sites, biting activity was highest between 11:00 pm-5:00 am, peaking at 1:00-2:00 am for Bo, 12:00-4:00 am in Bombali, 2:00-3:00am in Port Loko, 1:00-2:00am in Kono and 2:00-3:00am in Western Rural (Figures 27 and 28).

For *An. funestus* s.l., the mean HBR per hour was always below one; therefore no clear biting time could be inferred (Figure 29).

Figure 27: *An. gambiae* s.l. Biting Time in Bo and Bombali Districts, Comparing Indoor Vs Outdoor, March 2021–February 2022



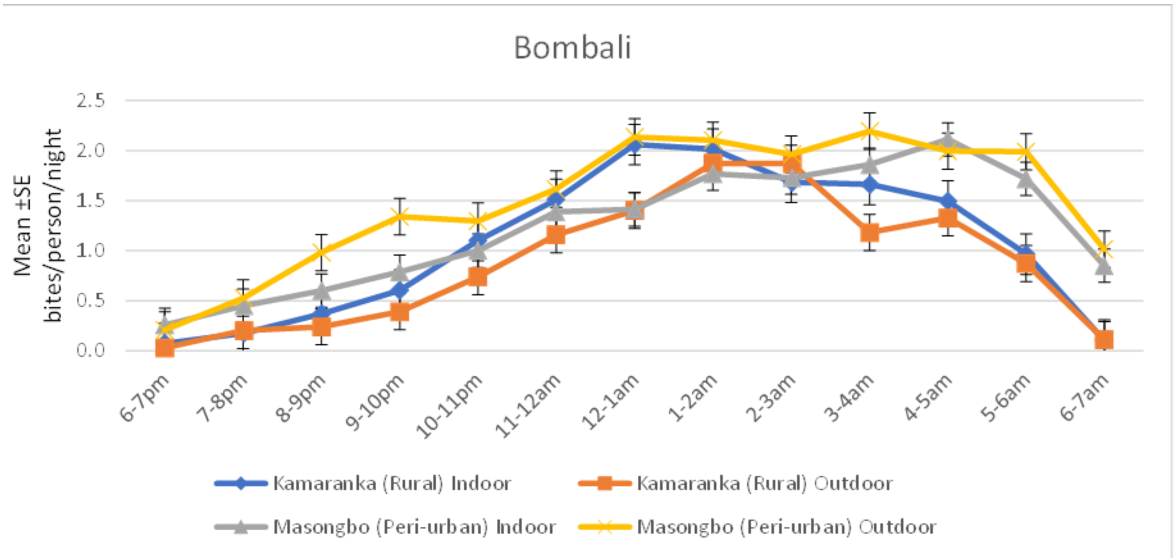


Figure 28: *An. gambiae* s.l. Biting Time in Port Loko, Kono and Western Area Rural Districts, Comparing Indoor Vs Outdoor, March 2021–February 2022

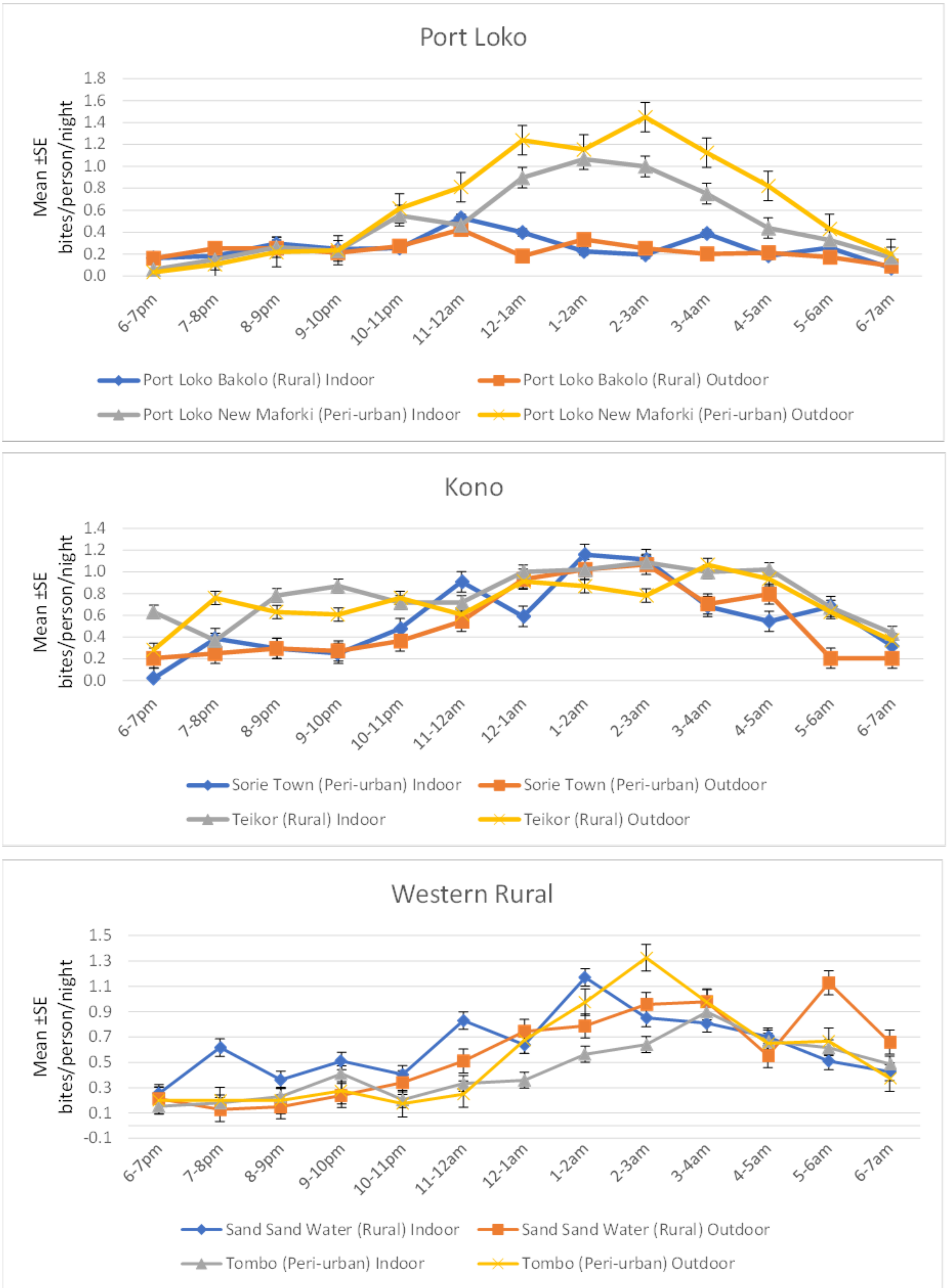
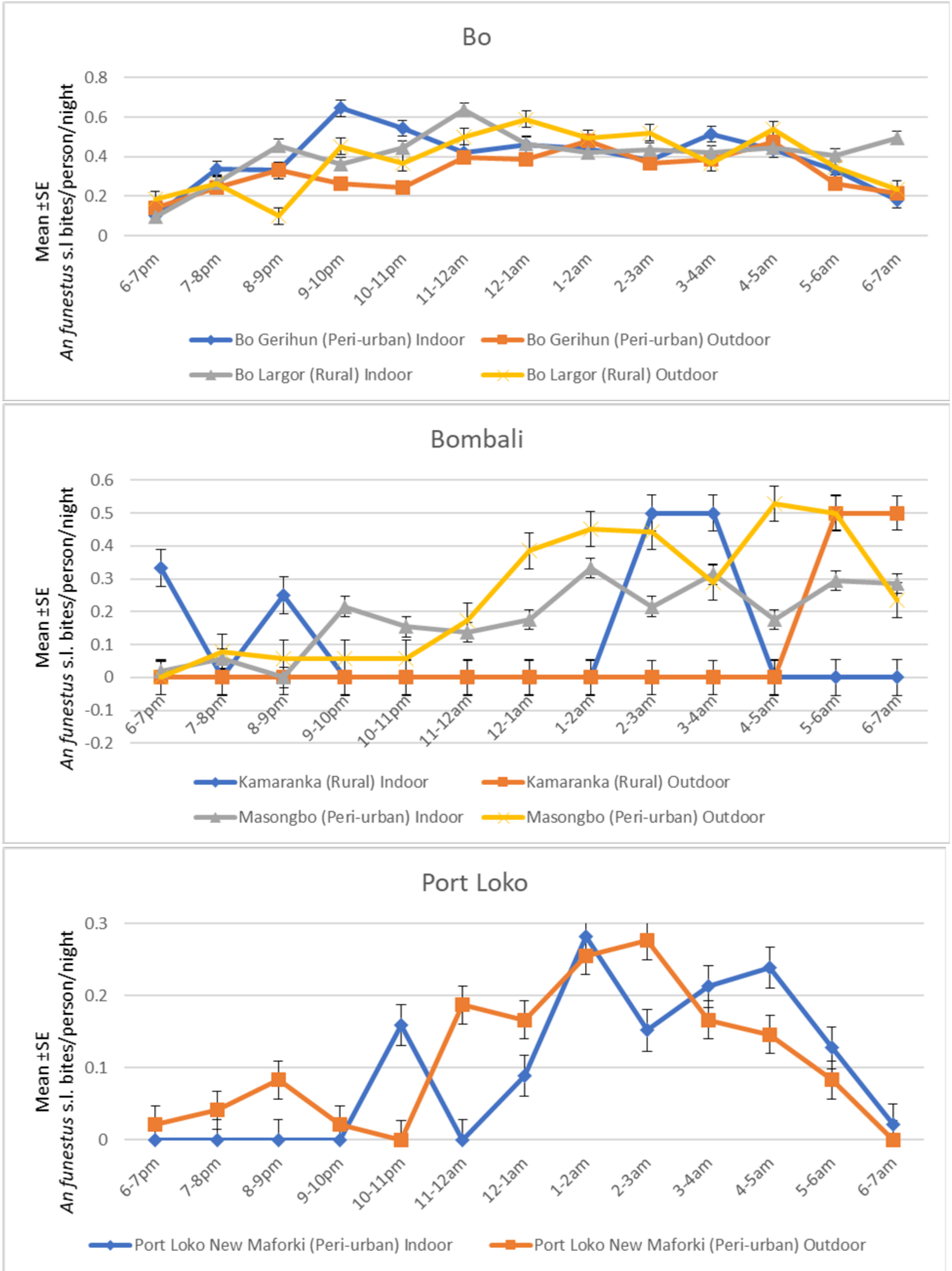
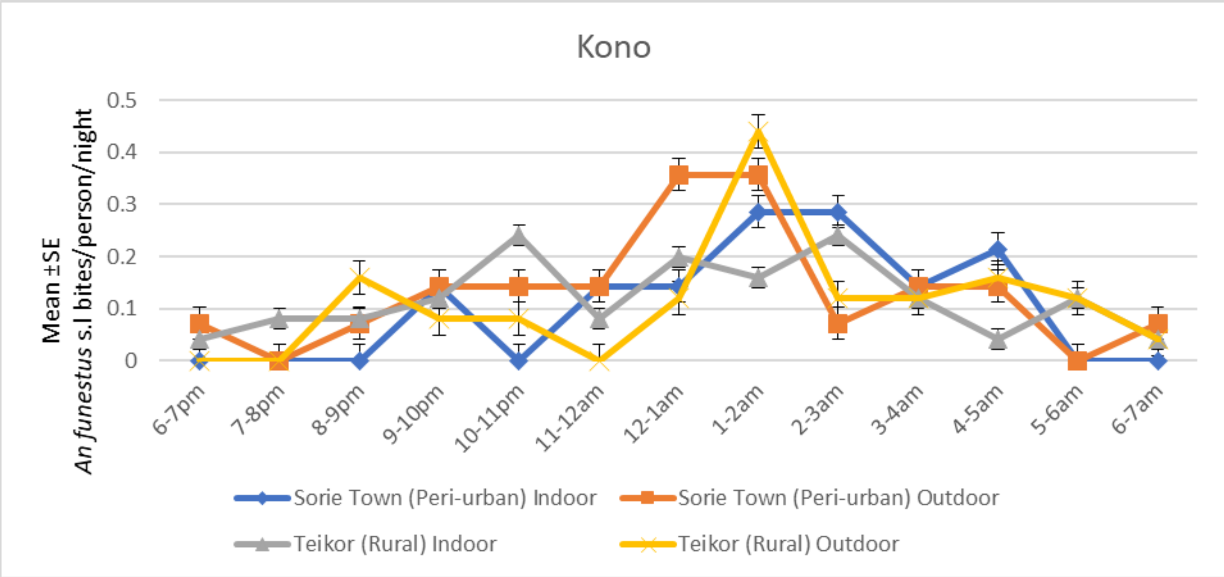


Figure 29: *An. funestus* s.l. Biting Time in Bo, Bombali, Port Loko and Kono Districts, Comparing Indoor and Outdoor, March 2021–February 2022



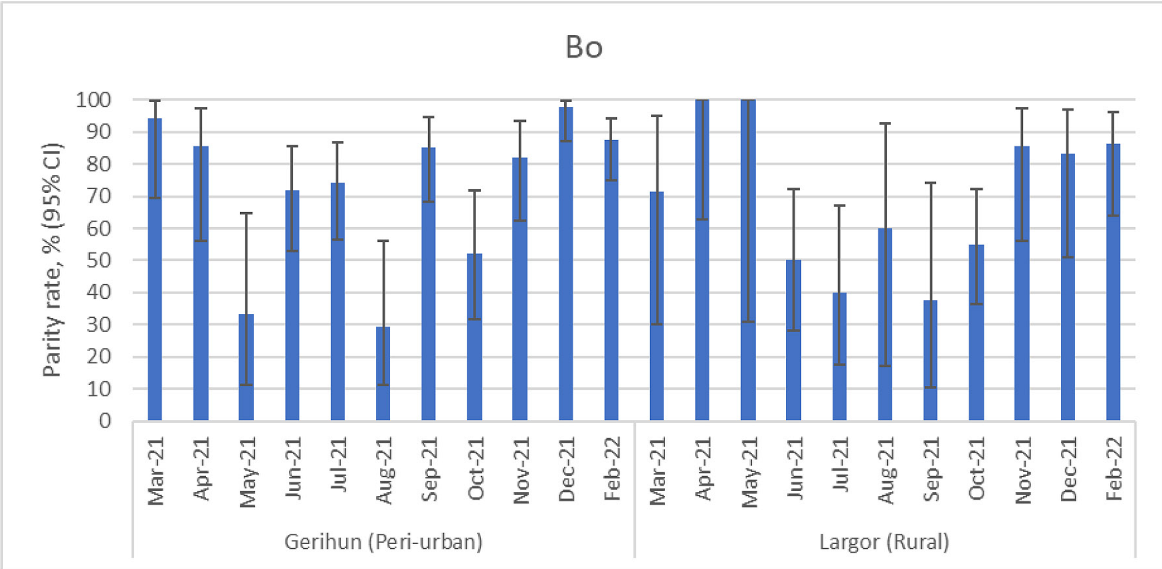


3.1.7 Parity Status of Malaria Vectors Collected by HLC and PSC

A total of 1,412 malaria vectors were dissected to determine the parity status, whether they had laid eggs at least once or not (Annex E). Of these, 99.8 % (1,409) came from HLC while 0.2% (3) came from PSC. *Anopheles gambiae* s.l. were most of the species dissected (98.4 %; 1,390) followed by *An. funestus* group (1.6 %; 18). From the 1,412 dissected mosquitoes, 70.7% (998) were parous, 21.6% (303) nulliparous while 7.7% (109) were undetermined due to incorrect dissection that destroyed the ovary. The number of undetermined or destroyed ovaries in 2021 was lower compared to 2020 where about 21% were unreadable, indicating improved ovary dissections in the team. For those successfully identified, 76.6% (998) were parous while 23.4% (305) were nulliparous.

Parity status was examined by sentinel site and district by month. In all the districts apart from Western Rural, before the rains, parity rate was high as expected and declined either in May/June with onset of rains and later rose toward the end of rainy season in November (Figures 29 and 30).

Figure 30: *An. gambiae* s.l. Parity status in Bo and Bombali Districts, Comparing Peri-urban and Rural Sites, March 2021–February 2022



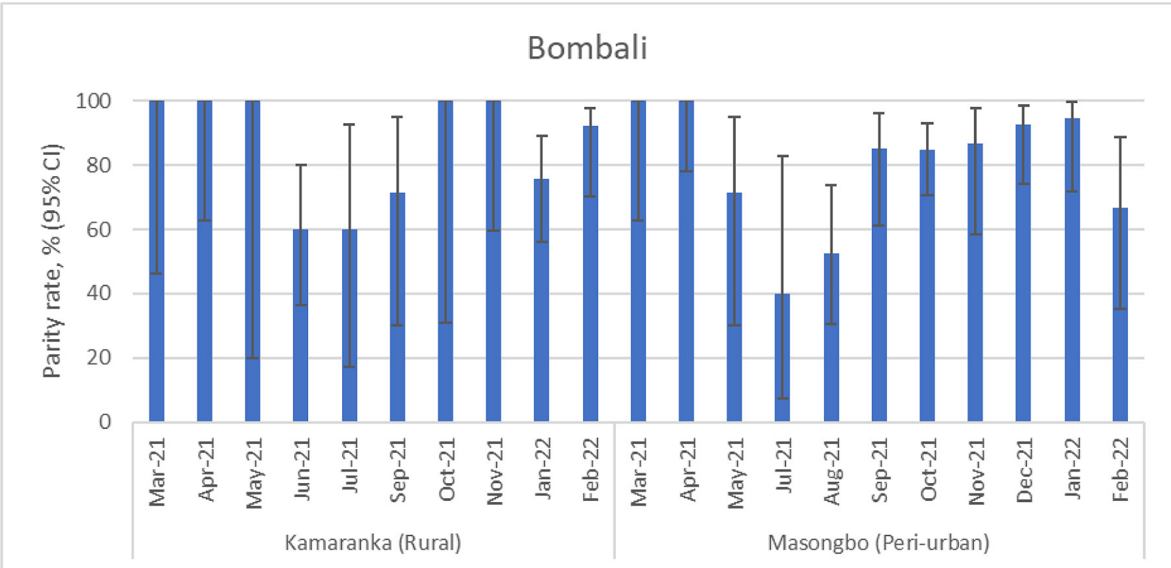
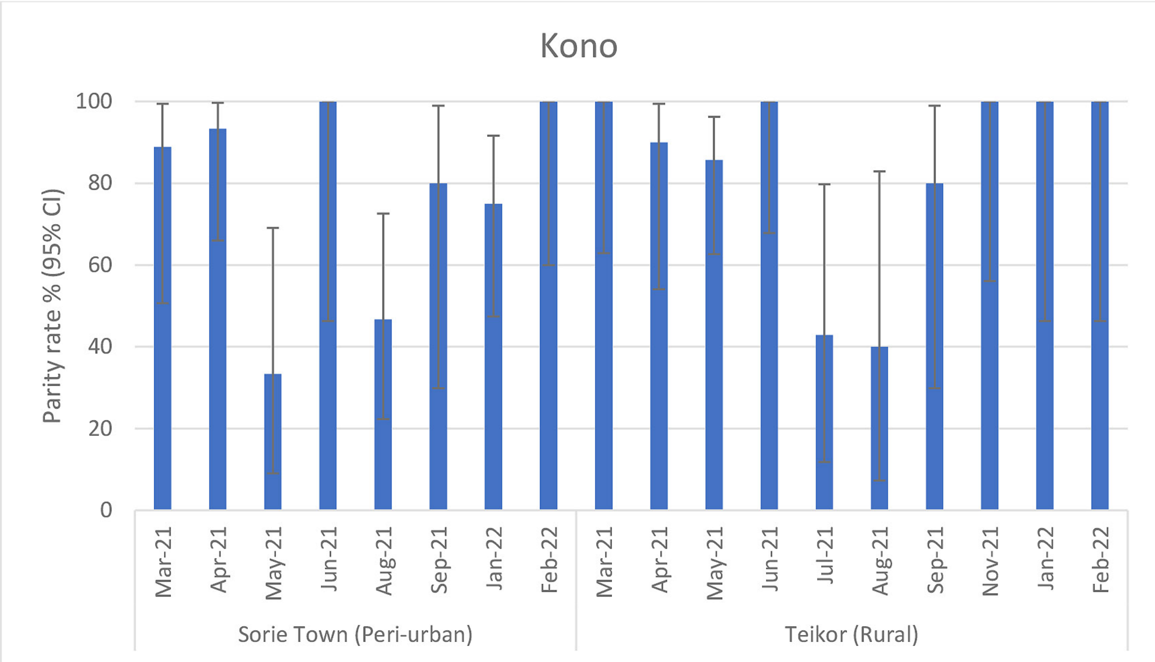
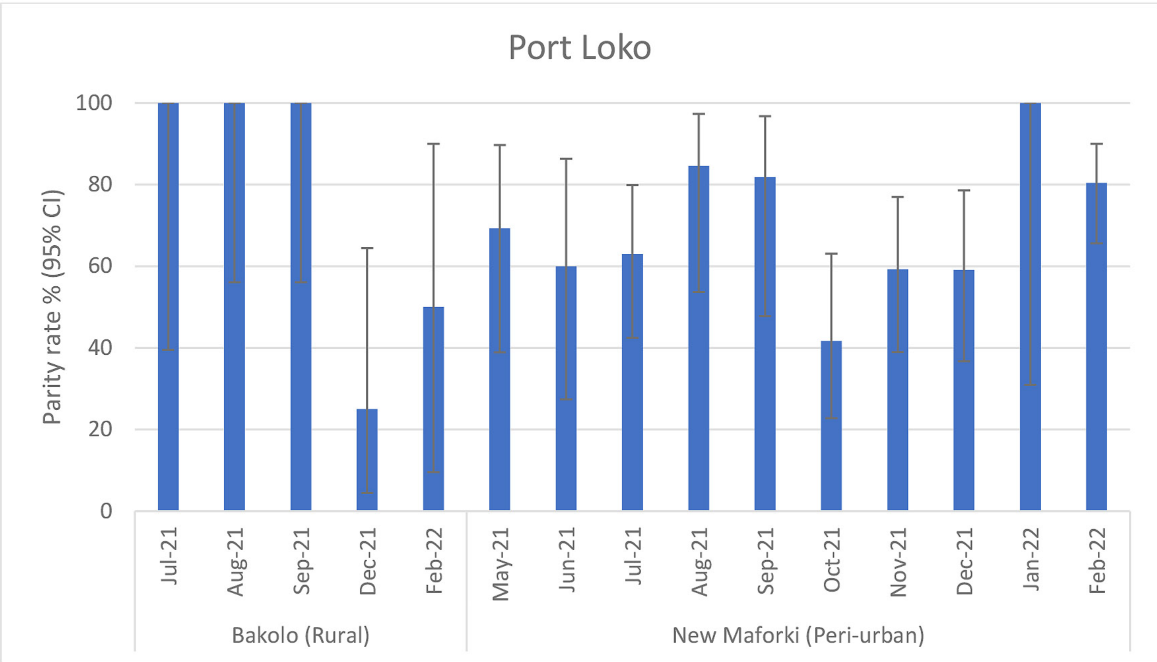
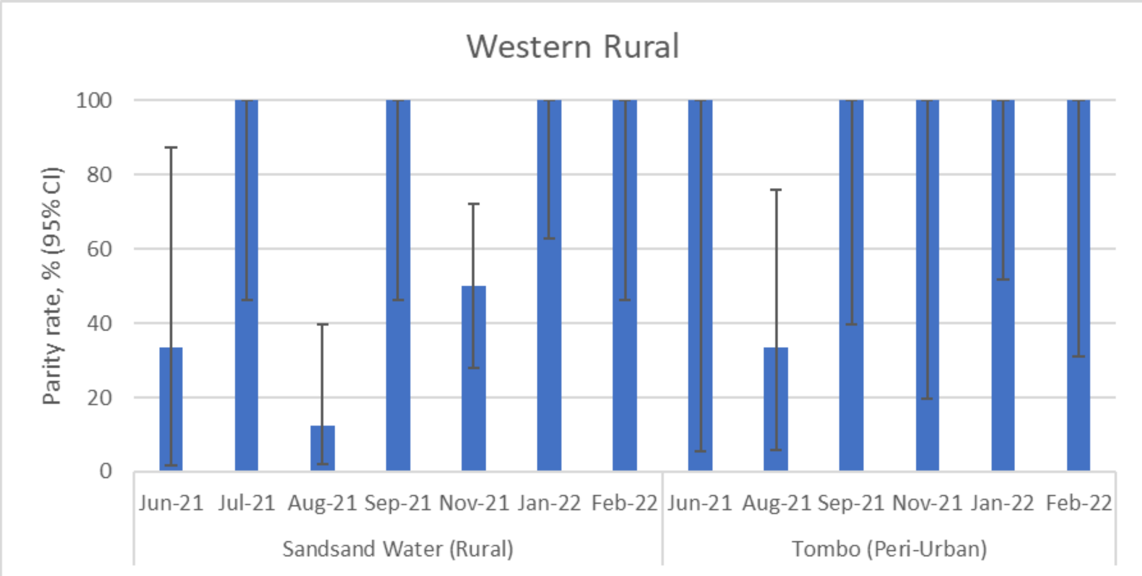


Figure 31: *An. gambiae* s.l. Parity Status in Kono, Port Loko and Western Rural Area Districts, Comparing Peri-Urban and Rural Sites, March 2021–February 2022







3.2 Decay Rate of SumiShield (Clothianidin) in 2021

In May 2021, one week after IRS spray campaign with SumiShield™ had started, the quality of spray was assessed using WHO wall cone bioassays (VL SOP6) in Gerihun, Bo District and Masongbo, Bombali District. This formed the T0 of residual effectiveness monitoring (Figures 32 and 33). The assessment of decay rate thus began in June for SumiShield™. In each site, 10 houses of different wall types (mud, painted mud, cemented, and painted-cement) were selected based on the most common house type in Sierra Leone. At the start of T0 (May), six mud houses and four cemented houses (one painted cement in Bo) were selected for the wall bioassays. In 2020-2021, the insectary had contamination and a crash in population; therefore both susceptible insectary strains, *An. gambiae* s.s. Kisumu and field collected pyrethroid resistant mosquitoes, were used at different time points. However, the temporary crash in the Kisumu colony and test with wild mosquitoes had no effect in interpretation of the wall assay results, as the wild was also equally susceptible to clothianidin and post exposure mortality was 100%. In May, June, January and February, the susceptible insectary strain *An. gambiae* s.s. Kisumu was exposed to sprayed walls at different heights in both villages. Between July to December, field collected pyrethroid resistant mosquitoes were exposed to sprayed walls in both villages. Twenty houses sprayed with SumiShield™ were tested every month (six mud and four cement houses per site). Within one week of IRS, over 95% mortality was observed in all sites on both mud and cement walls after day 5 (Figure 32 and 33). Mortality to both mosquito populations remained above 80%, the WHO minimum mortality threshold for IRS insecticide (Figure 34). At different months, some houses were changed because household owners did not want to continue while in some instances, houses were locked down. Therefore, one or two houses had different wall types after they consented to be part of the evaluation of decay rate of SumiShield™. The team started the cone assays in house with mud, cement and painted cement walls in Bo and in houses with mud and cement walls in Bombali. However, when some houses were not available, they were replaced with other adjacent houses that have painted mud or cement walls for the cone wall assays.

Figure 32: Knock-down (30 and 60 mins) and mortality of *An. gambiae* s.s. Kisumu strain exposed in WHO cone bioassay to SumiShield™ 50WG sprayed mud and cement walls in Masongbo in Bombali district, Sierra Leone.

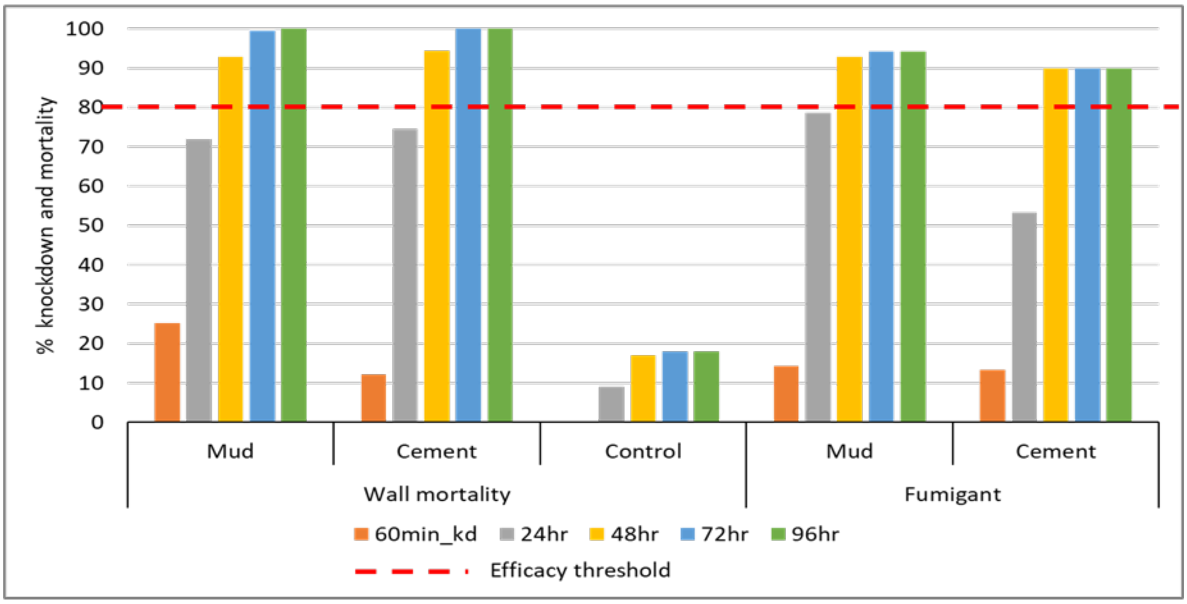


Figure 33: Knock-down (30 and 60 mins) and mortality of *An. gambiae* s.s. Kisumu strain exposed in WHO Cone Bioassay to SumiShield™ 50WG Sprayed Mud and Cement Walls in Gerihun in Bo district, Sierra Leone.

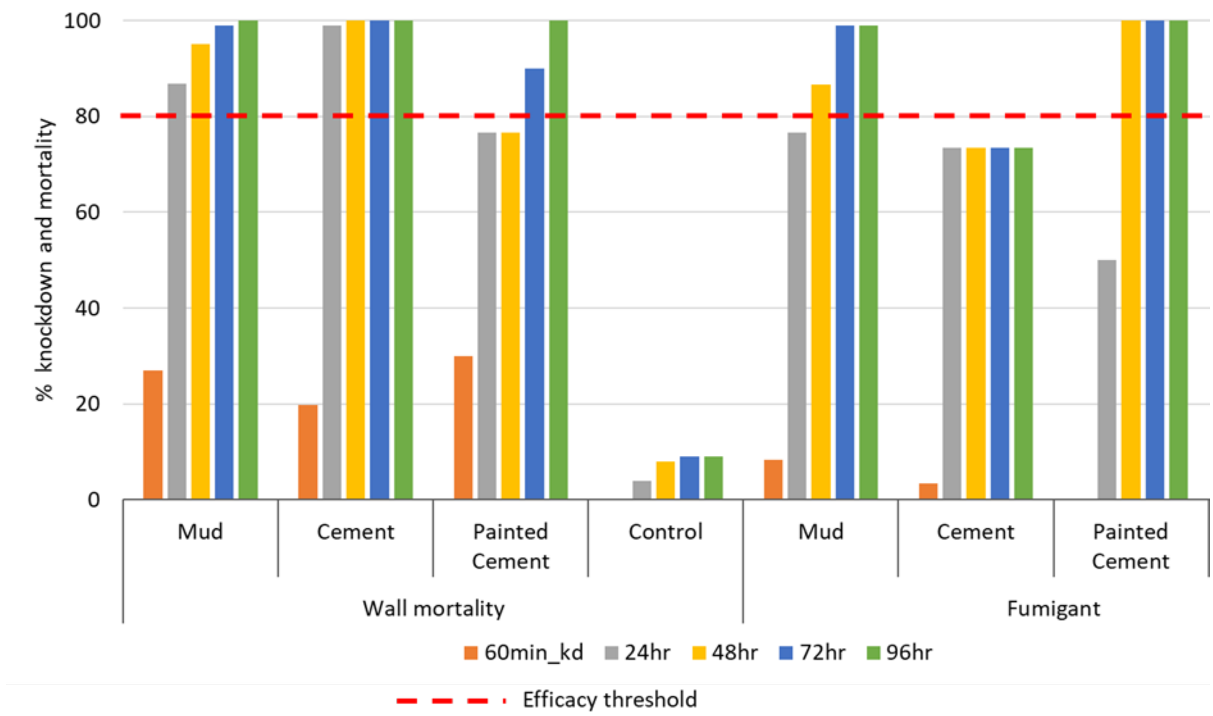
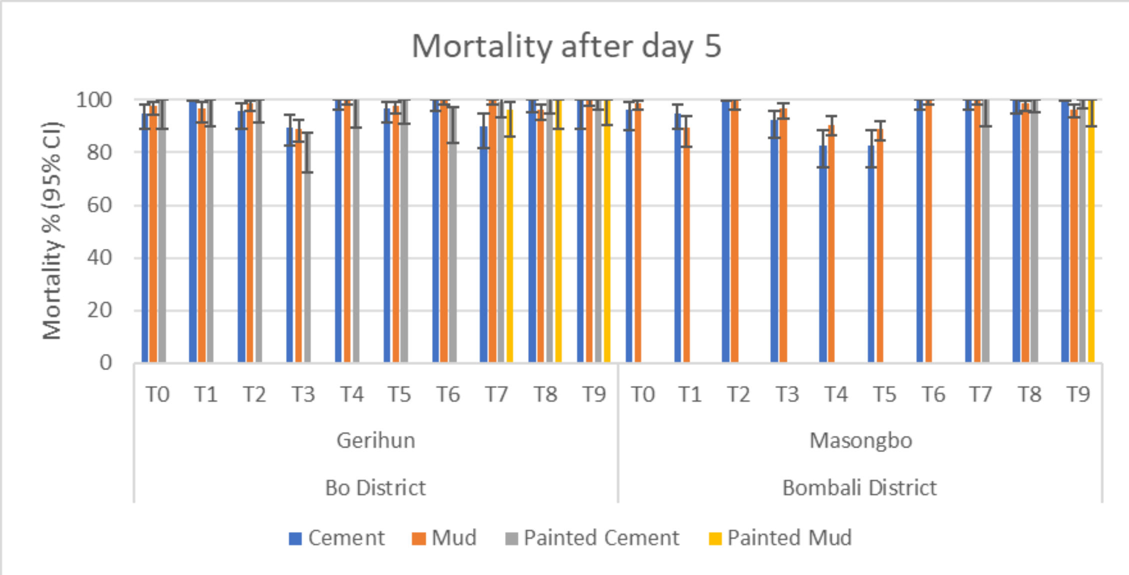


Figure 34: The WHO Cone Bioassay Mortality by Wall Type by Month Showing Decay Rate of SumiShield (Clothianidin) in Gerihun, Bo District and Masongbo, Bombali District Between May (T0) 2021-February (T9) 2022.



3.3 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 of the molecular analysis done at CRID.

3.3.1 Species Identification

A total of 3,946 *Anopheles* mosquitoes sampled between March 2021 and February 2022 were analyzed for molecular species identification. Among these, 232 came from HLC collection, 3,319 from PSC & CDC-LT collection and 395 from phenotypic WHO susceptibility bioassays (Table 3). Out of the 3,946, a total of 3,493 (3,016 *An. gambiae* s.l. and 477 *An funestus* s.l.) were successfully identified to sibling species translating to 88.52% (3,493/3,94s6) while 3.2% (128) were not identified.

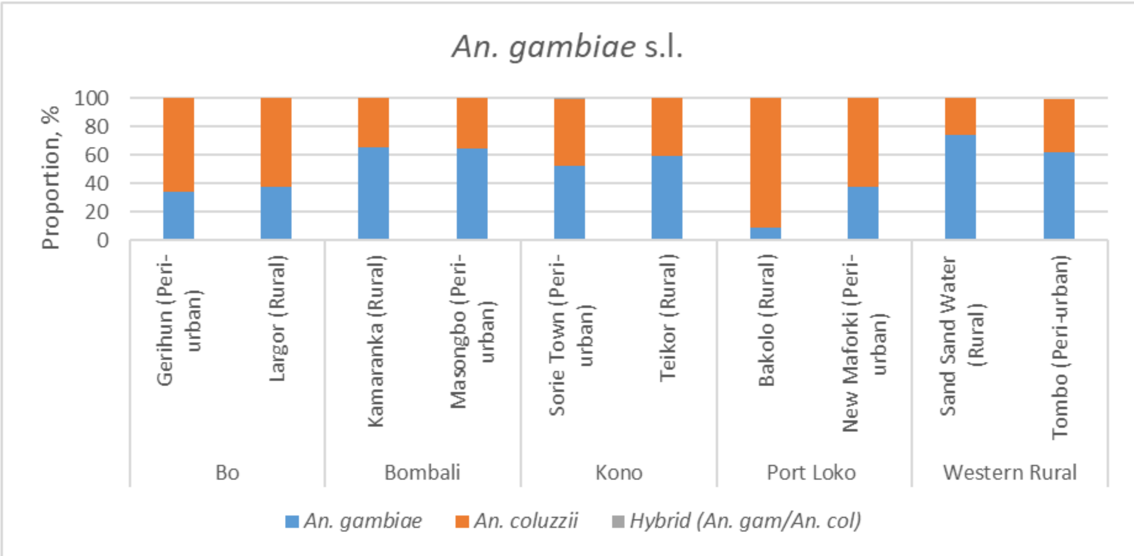
Table 3: Number of Samples Analyzed for Molecular Species Identification, March 2021-February 2022.

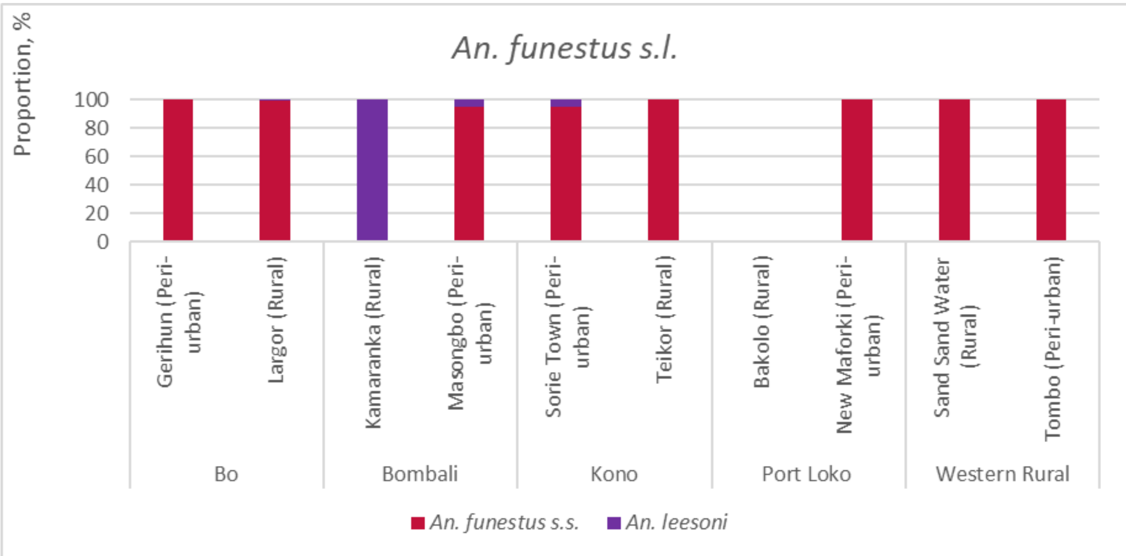
Districts	HLC	PSC and CDC	Resistance	Total
Bo	60	962	98	1120
Bombali	68	554	104	726
Kono	17	897	30	944
Port Loko	35	364	111	510
Western Rural	52	542	52	646

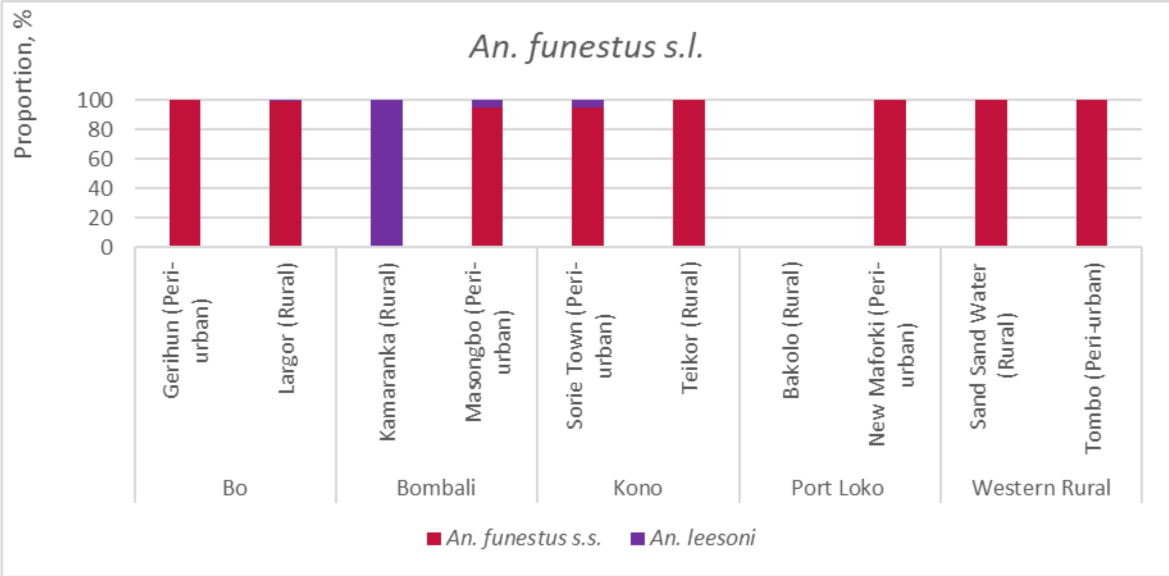
Districts	HLC	PSC and CDC	Resistance	Total
Total	**232**	**3,319**	**395**	**3,946**

Within the *An. gambiae* complex, majority 56.1% (1,694/3,016) of the samples screened were *An. gambiae* s.s. followed by *An. coluzzii* 43.8% (1,320/3016) and hybrid between *An. coluzzii/An. gambiae*, 0.1% (2/3,016). For *An. funestus* group, majority 98.3% (469/477) were *An. funestus* s.s. followed by *An. leesoni* 1.7% (8/477). *An. gambiae* s.s. was predominant in Bombali, Kono, Western Rural and Bakolo (Port Loko District) while *An. coluzzii* was prevalent in Bo District (Figure 35).

Figure 35: Molecular Species Distribution of *An. gambiae* s.l. and *An. funestus* s.l. Across Sampling Methods and Sites, March 2021–February 2022.



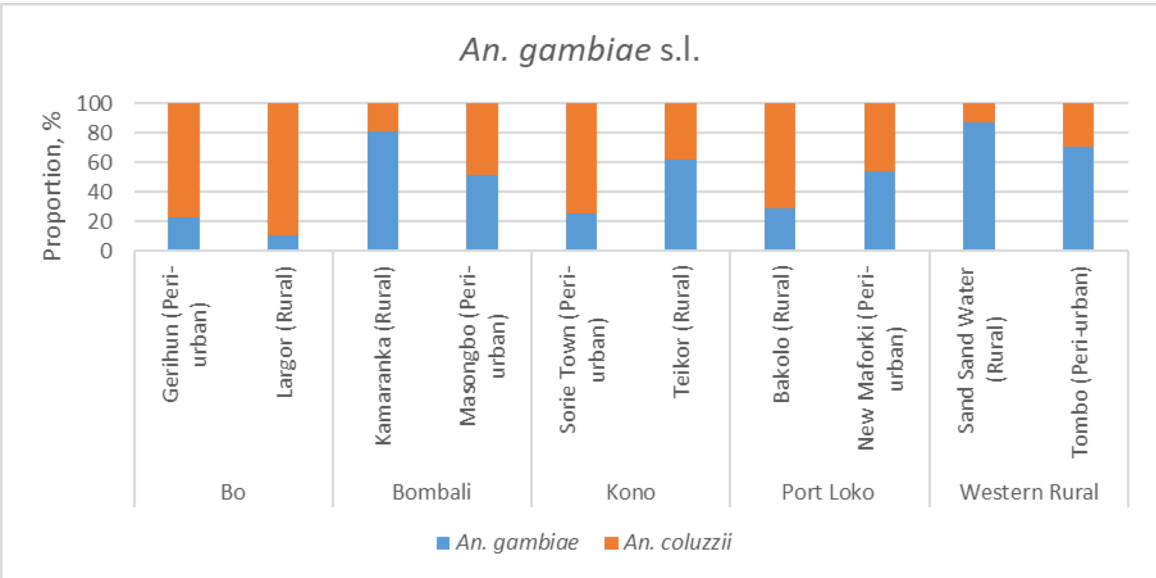


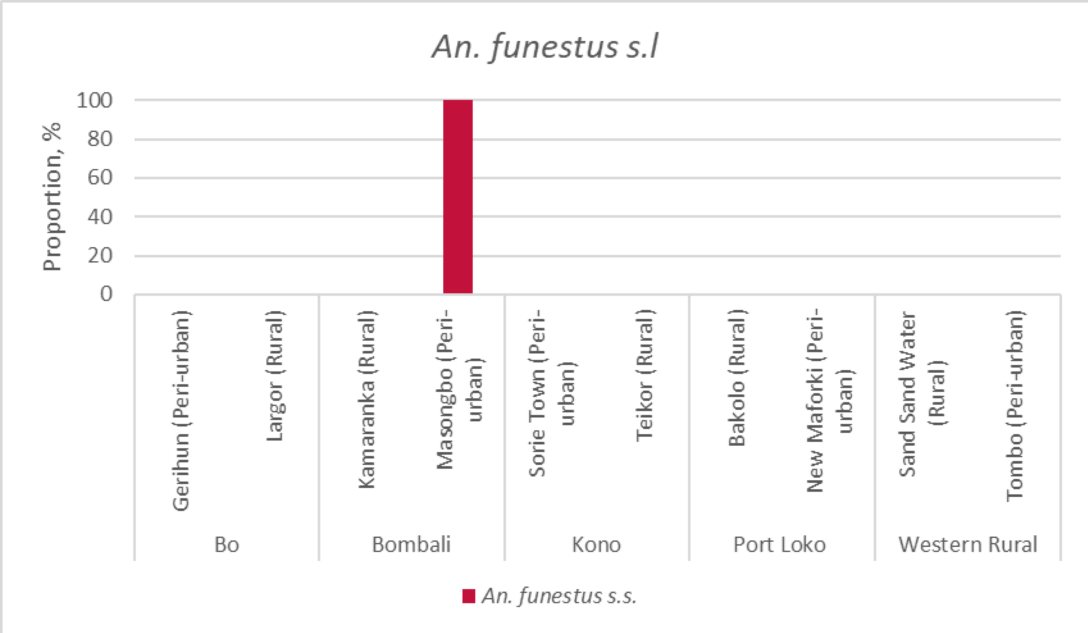


3.3.2 Species Distribution by District

HLC collections: Only HLC samples that tested positive for *Plasmodium* infection were identified to sibling species. In Largor and Gerihun (Bo District), Sorie Town (Kono District) and Bakolo (Port Loko District), *An. coluzzii* was the predominant species (Figure 36). In Bombali (Kamaranka and Masongbo), Western Rural (Sand Sand Water and Tombo) and Teikor (Kono District), *An. gambiae* s.s. was the predominant species (Figure 36).

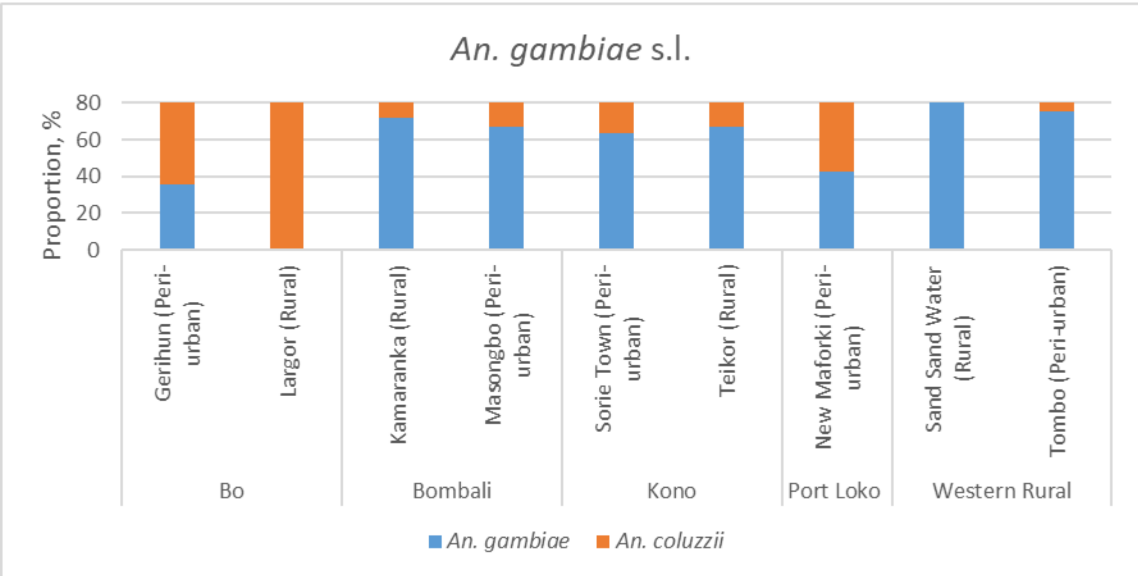
Figure 36: Molecular Species Distribution of *Anopheles gambiae* s.l. and *An. funestus* s.l. Samples Collected by HLC by District, March 2021–February 2022





PSC and CDC-LT Collections: Out of the 3,319 samples analyzed, 92 samples failed to amplify compared to 204 in 2020. Species distribution varied by district and site. Overall, *An. gambiae* s.s. was the most common in CDC-LT collections (Figure 37) while there was no clear difference in species distribution for those sampled via PSC (Figure 38). *An. funestus* was absent from Kamaranka (Bombali District, New Maforki (Port Loko District) and Sand Sand Water (Western Rural Area District) (Figure 37).

Figure 37: Molecular Species Distribution of *Anopheles* Samples Collected by CDC-LT Across Districts, March 2021–February 2022



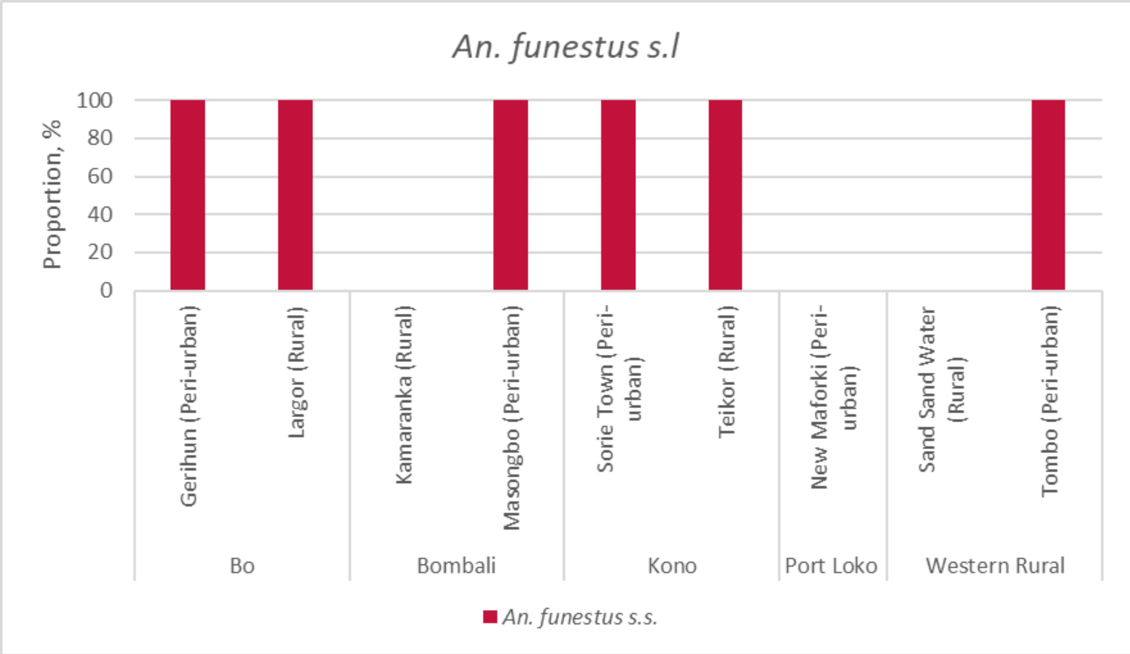
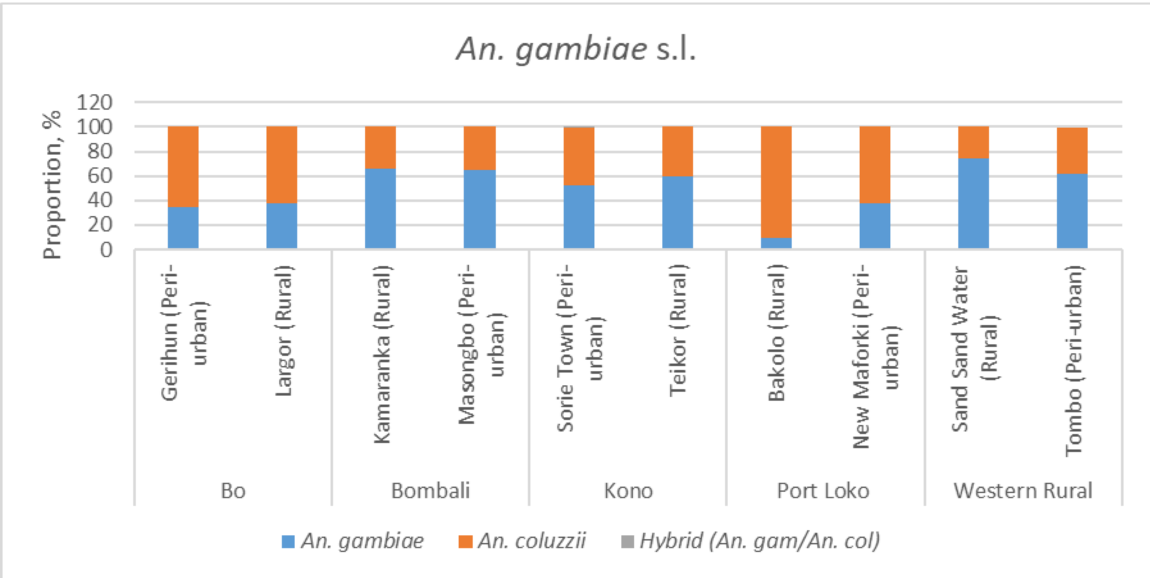
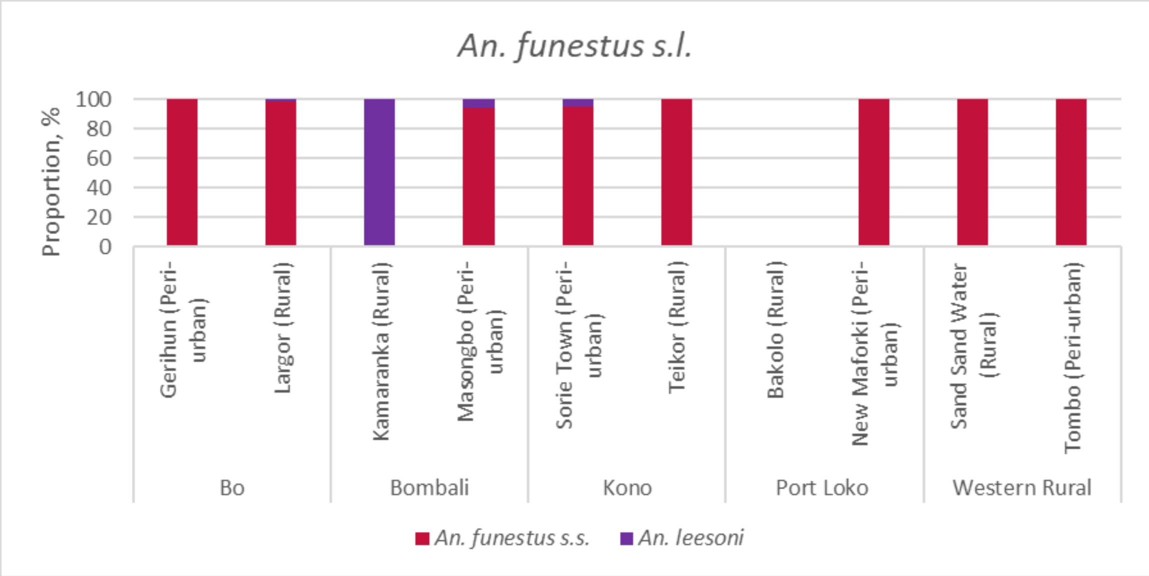


Figure 38: Molecular Species Distribution of *Anopheles* Samples Collected by PSC Across Districts, March 2021–February 2022

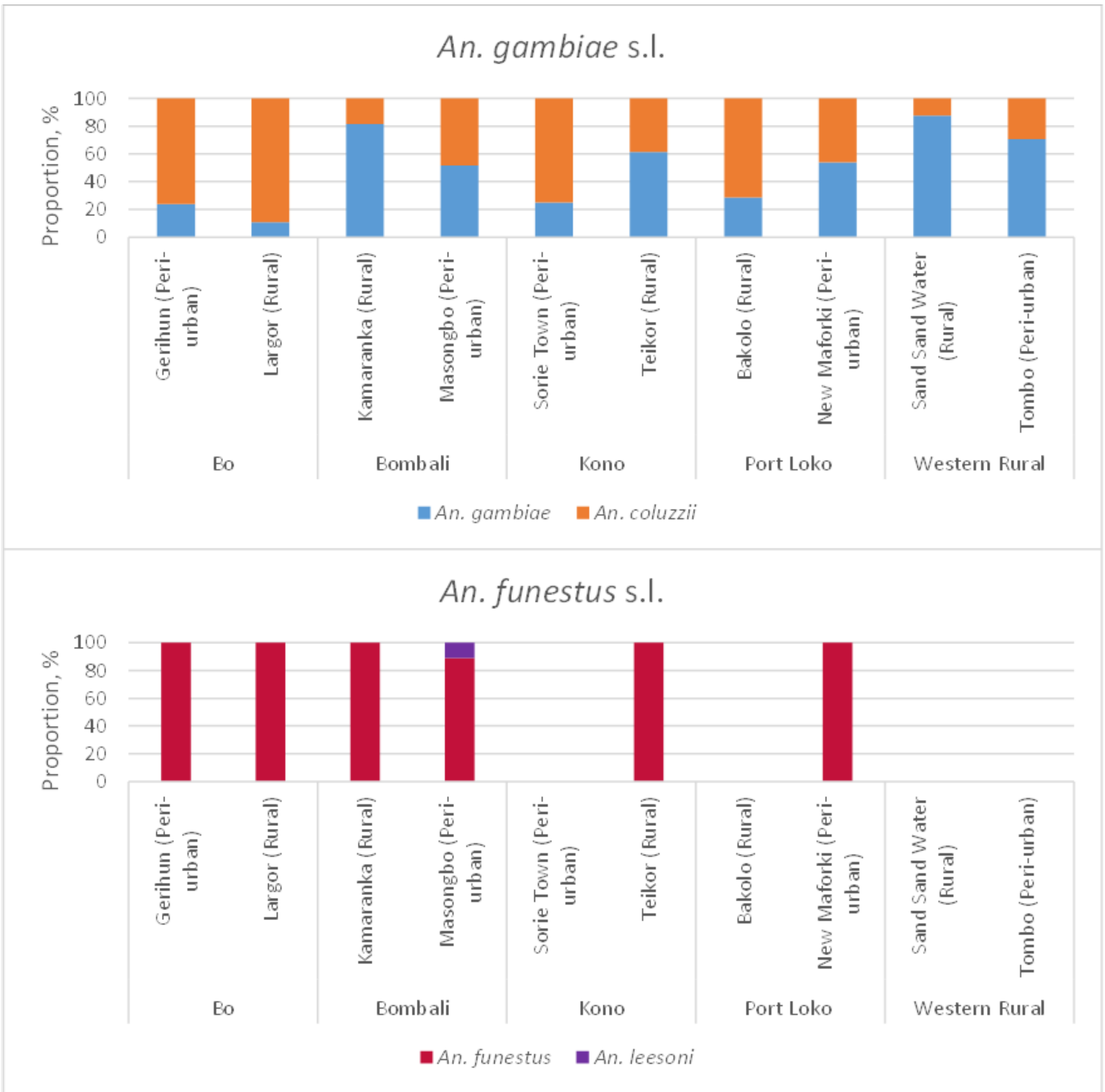




3.3.3 *Plasmodium* Infection

A total of 7,909 *An. gambiae* s.l. and 1,134 *An. funestus* s.l. were tested for *Plasmodium* infection, and 270/9,043 (2.99%) were found to be positive (Annex G, H and I). *Plasmodium* infection was detected in 3.02% (239/7,909) of *An. gambiae* s.l. and 2.73% (31/1,134) of *An. funestus* s.l. All 270 (239 *An. gambiae* s.l. and 31 *An. funestus* s.l.) mosquitoes that tested positive for *Plasmodium* were identified to sibling species using molecular method and a total of 236 were successfully identified to species level. Of the *An. gambiae* s.l. that tested positive, the majority of 53.9% (110/204) were *An. gambiae* and 46.1 % (94/204) were *An. coluzzii*. For *An. funestus* s.l., 96.77% (30/31) were *An. funestus* s.s. with one identified as *An. leesoni* at 3.22% (1/31)/. This species distribution indicates that *Plasmodium* infection was highest in *An. gambiae* s.s. followed by *An. coluzzii* and is consistent with the previous year, where *Plasmodium* infection was highest in *An. gambiae*. Sporozoite infected *An. funestus* s.l. was sampled in all districts apart from Western Rural (Figure 39, Annex G-I).

Figure 39: Distribution of Species Among *An. gambiae* s.l. and *An. funestus* s.l. Samples that Tested Positive for *Plasmodium* Circumsporozoite Protein (CSP), All Sites, March 2021 – February 2022.



The sporozoite rate, defined as number of mosquitoes that tested positive divided by the total mosquitoes screened for *Plasmodium* sporozoites, varied between sites, species, and months (Figures 40, Tables 4-9, Annex G-I). In Bombali, the sampled *An. funestus* s.l. had higher sporozoite compared to *An. gambiae* s.l. when data was aggregated by district (Figure 40). Sand Sand Water rural site in Western Area had the highest sporozoite rate in *An. gambiae* s.l. (Figure 40). The data presented in this report covers two months before and nine months after the first IRS spray campaign in Bo and Bombali in May 2021. Overall, sporozoite rate was lower in 2021 (2.99%) across the country compared to the 4.77% in 2020 (Figure 40, Tables 4-9)). In the IRS sites, the highest *An. gambiae* s.l. sporozoite rate of 6% was recorded in Bo in March 2021 (Table 4). Sporozoite rates remained below 1% in May - July after IRS, then rose to peak at 4.63% in January 2022 (Table 4). In Non-IRS sites, the highest sporozoite rate was 12.5% in Western Area in January 2022 (Table 4). For *An. funestus*, the highest sporozoite rate was observed in March 2021, Kono District, 28% (2/7) (Table 7) with variability between months and sites (Table 8 and 9).

Figure 40: Sporozoite Rates of *An. gambiae* s.l. and *An. funestus* s.l. Collected Indoor and Outdoor by HLC by Site, March 2021-February 2022.

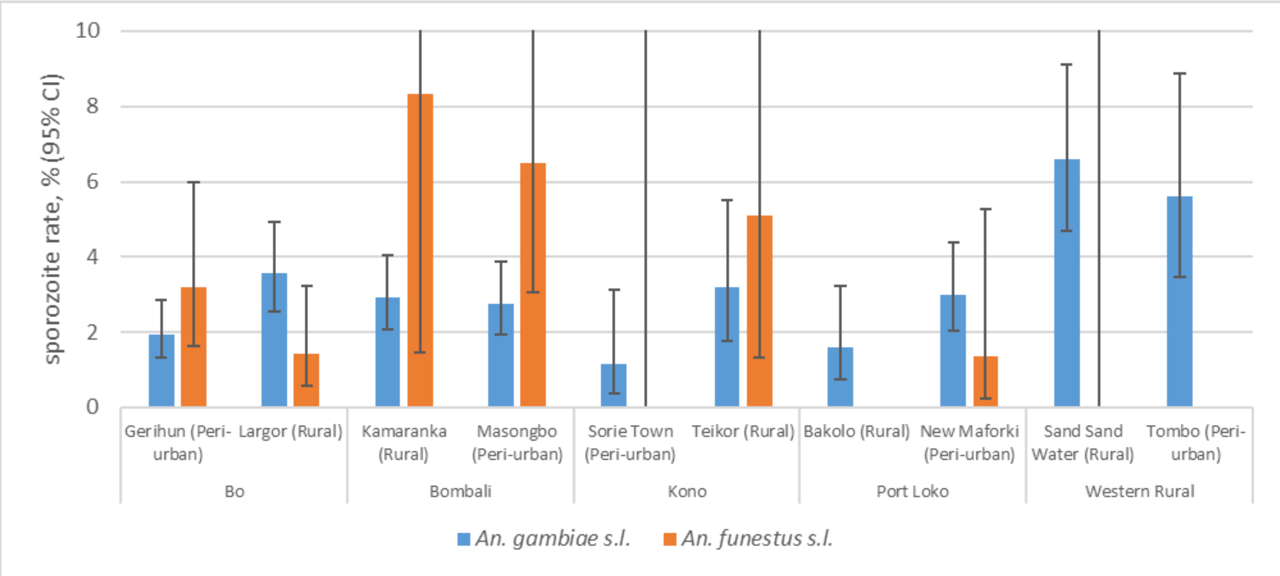


Table 4: Sporozoite Rates in *An. gambiae* s.l. Collected by HLC in IRS Intervention Sites and Non-IRS Sites Between March 2021 and February 2022

Months	IRS Sites						Non-IRS Sites								
	Bo			Bombali			Kono			Port Loko			Western Rural		
	# Tested	# +VE	% +VE	# Tested	# +VE	% +VE	# Tested	# +VE	% +VE	# Tested	# +VE	% +VE	# Tested	# +VE	% +VE
21-Mar	509	31	6.09	354	6	1.69	13	0	0.00	16	0	0.00	72	8	11.11
21-Apr	492	10	2.03	343	12	3.50	88	1	1.14	216	1	0.46	42	0	0.00
21-May	209	2	0.96	245	10	4.08	102	2	1.96	153	1	0.65	68	0	0.00
21-Jun	172	1	0.58	232	0	0.00	104	1	0.96	184	4	2.17	111	7	6.31
21-Jul	129	0	0.00	216	3	1.39	60	6	10.00	174	6	3.45	137	8	5.84
21-Aug	215	7	3.26	247	7	2.83	88	0	0.00	145	0	0.00	138	4	2.90
21-Sep	211	0	0.00	171	7	4.09	75	3	4.00	180	7	3.89	81	9	11.11
21-Oct	101	2	1.98	159	7	4.40	77	3	3.90	89	9	10.11	43	4	9.30
21-Nov	104	3	2.88	140	5	3.57	21	0	0.00	89	4	4.49	50	5	10.00
21-Dec	75	3	4.00	94	9	9.57	49	0	0.00	96	4	4.17	60	4	6.67
22-Jan	108	5	4.63	128	3	2.34	45	1	2.22	28	0	0.00	71	9	12.68
22-Feb	105	0	0.00	108	0	0.00	33	0	0.00	179	1	0.56	36	1	2.78

Table 5: Sporozoite Rates in *An. gambiae* s.l. Collected By HLC in IRS Intervention Sites Between March 2021 and February 2022

Months	Bo				Bombali			
	Gerihun (Peri-urban)		Largor (Rural)		Kamaranka (Rural)		Masongbo (Peri-urban)	
	# Tested	% +Ve	# Tested	% +Ve	# Tested	% +Ve	# Tested	% +Ve
21-Mar	381	4.2	128	11.72	188	2.13	166	1.20
21-Apr	235	0.85	257	3.11	169	5.33	174	1.72
21-May	103	0.97	106	0.94	122	5.74	123	2.44
21-Jun	85	0.00	87	1.15	117	0.00	115	0.00
21-Jul	67	0.00	62	0.00	112	1.79	104	0.96
21-Aug	114	1.75	101	4.95	94	1.06	153	3.92
21-Sep	124	0	87	0.00	95	4.21	76	3.95
21-Oct	71	2.82	30	0.00	79	5.06	80	3.75
21-Nov	58	3.45	46	2.17	84	4.76	56	1.79
21-Dec	38	0.00	37	8.11	30	3.33	64	12.5
22-Jan	62	3.23	46	6.52	60	0.00	68	4.41
22-Feb	55	0.00	50	0.00	87	0.00	21	0.00

Table 6: Sporozoite Rates in *An. gambiae* s.l. Collected By HLC in Non-IRS Intervention Sites Between March 2021 and February 2022

Months	Kono				Port Loko				Western Rural			
	Sorie Town		Teikor		Bakolo		New Maforki		Sand Sand Water		Tombo	
	# Tested	% +Ve	# Tested	% +Ve	# Tested	% +Ve	# Tested	% +Ve	# Tested	% +Ve	# Tested	% +Ve
21-Mar	13	0.00	0	0.00	6	0.00	10	0	46	13	17	0.00
21-Apr	38	0.00	50	2.00	111	0.00	105	0.95	32	0.00	18	0.00
21-May	52	3.85	50	0.00	21	0.00	132	0.76	55	0.00	43	2.33
21-Jun	53	0.00	51	1.96	116	2.6	68	1.47	53	7.55	51	1.96
21-Jul	17	0.00	43	14.00	84	3.6	90	3.33	48	6.25	52	9.62
21-Aug	39	0.00	49	0.00	86	0.00	59	0	78	3.85	30	3.33
21-Sep	22	4.55	53	3.77	58	3.5	122	4.1	34	8.82	53	13.21
21-Oct	31	0.00	46	6.52	2	0.00	87	10.3	23	8.70	11	9.09
21-Nov	3	0.00	18	0.00	10	0.00	79	5.06	30	6.67	14	14.29
21-Dec	33	0.00	16	0.00	8	0.00	88	4.55	47	6.38	8	0.00
22-Jan	24	4.17	21	0.00	-	-	18	0.00	59	15.3	13	0.00
22-Feb	21	0.00	12	0.00	-	-	74	0.00	27	0.00	11	0.00

Table 7: Sporozoite Rates in *An. funestus* s.l. Collected by Human Landing Catch in IRS Intervention Sites and Non-IRS Sites between March 2021 and February 2022

Months	IRS Sites						Non-IRS Sites								
	Bo			Bombali			Kono			Port Loko			Western Rural		
	# Tested	# +VE	% +VE	# Tested	# +VE	% +VE	# Tested	# +VE	% +VE	# Tested	# +VE	% +VE	# Tested	# +VE	% +VE
21-Mar	31	1	3.23	0	0	0.00	7	2	28.57	16	0	0.00	0	0	0.00
21-Apr	0	0	0.00	0	0	0.00	0	0	0.00	12	0	0.00	0	0	0.00
21-May	33	0	0.00	0	0	0.00	3	0	0.00	4	0	0.00	0	0	0.00
21-Jun	60	0	0.00	0	0	0.00	1	0	0.00	48	1	2.08	0	0	0.00
21-Jul	112	0	0.00	0	0	0.00	26	1	3.85	31	0	0.00	0	0	0.00
21-Aug	95	2	2.11	1	0	0.00	9	0	0.00	28	0	0.00	0	0	0.00
21-Sep	36	0	0.00	22	1	4.55	0	0	0.00	2	0	0.00	0	0	0.00
21-Oct	70	0	0.00	34	0	0.00	0	0	0.00	0	0	0.00	3	0	0.00
21-Nov	66	4	6.06	32	2	6.25	8	0	0.00	8	1	12.50	0	0	0.00
21-Dec	98	5	5.10	27	4	14.81	21	0	0.00	3	0	0.00	0	0	0.00
22-Jan	65	4	6.15	31	3	9.68	9	0	0.00	16	0	0.00	0	0	0.00
22-Feb	69	0	0.00	0	0	0.00	16	0	0.00	12	0	0.00	0	0	0.00

Table 8: Sporozoite Rates in *An. funestus* s.l. Collected By HLC in IRS Intervention Sites Between March 2021 and February 2022

Months	Bo				Bombali			
	Gerihun		Largor		Kamaranka		Masongbo	
	# Tested	% +ve	# Tested	% +ve	# Tested	% +ve	# Tested	% +ve
21-Mar	30	3.33	1	0.00	0	0.00	0	0.00
21-Apr	0	0.00	0	0.00	0	0.00	0	0.00
21-May	20	0.00	13	0.00	0	0.00	0	0.00
21-Jun	31	0.00	29	0.00	0	0.00	0	0.00
21-Jul	54	0.00	58	0.00	0	0.00	0	0.00
21-Aug	32	3.13	63	1.60	0	0.00	1	0.00
21-Sep	0	0.00	36	0.00	0	0.00	22	4.60
21-Oct	15	0.00	55	0.00	0	0.00	34	0.00
21-Nov	26	7.69	40	5.00	2	0.00	30	6.70
21-Dec	48	8.33	50	2.00	7	0.00	20	20.0
22-Jan	25	8.00	40	5.00	15	13.33	16	6.30
22-Feb	32	0.00	37	0.00	0	0.00	0	0.00

Table 9: Sporozoite Rates in *An. funestus* s.l. Collected By HLC in Non-IRS Intervention Sites Between March 2021 and February 2022sd

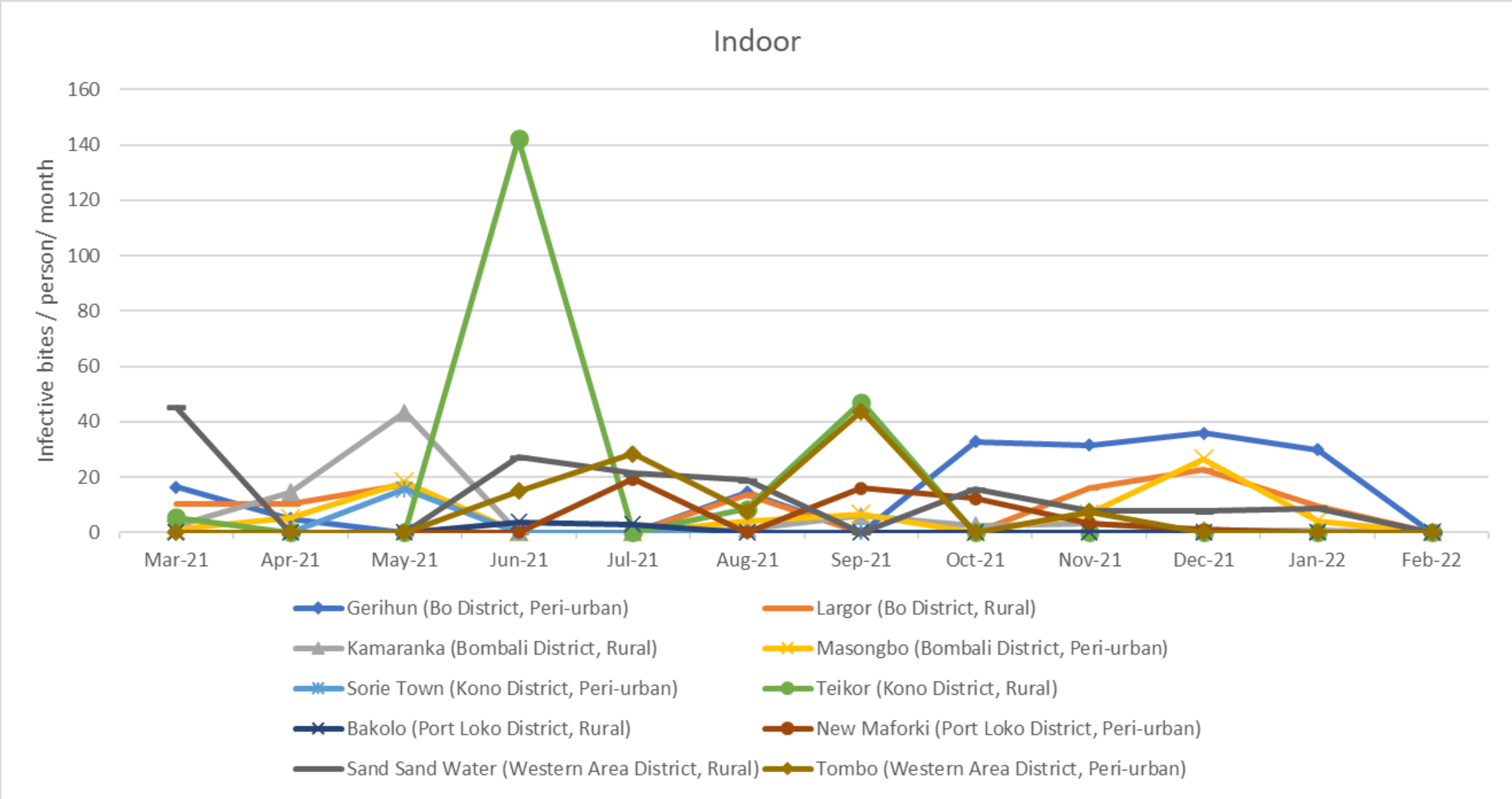
Months	Kono				Port Loko				Western Rural			
	Sorie Town		Teikor		Bakolo		New Maforki		Sand Sand Water		Tombo	
	# Tested	% +ve	# Tested	% +ve	# Tested	% +ve	# Tested	% +ve	# Tested	% +ve	# Tested	% +ve
21-Mar	5	0.00	2	100.0	0	0.00	16	0.00	0	0.00	0	0.00
21-Apr	0	0.00	0	0.00	0	0.00	12	0.00	0	0.00	0	0.00
21-May	1	0.00	2	0.00	4	0.00	0	0.00	0	0.00	0	0.00
21-Jun	0	0.00	1	0.00	48	2.08	0	0.00	0	0.00	0	0.00
21-Jul	16	0.00	10	10.0	31	0.00	0	0.00	0	0.00	0	0.00
21-Aug	7	0.00	2	0.00	28	0.00	0	0.00	0	0.00	0	0.00
21-Sep	0	0.00	0	0.00	2	0.00	0	0.00	0	0.00	0	0.00
21-Oct	0	0.00	0	0.00	0	0.00	0	0.00	3	0.00	0	0.00
21-Nov	0	0.00	8	0.00	8	12.50	0	0.00	0	0.00	0	0.00
21-Dec	9	0.00	12	0.00	0	0.00	3	0.00	0	0.00	0	0.00
22-Jan	1	0.00	8	0.00	0	0.00	0	0.00	0	0.00	0	0.00
22-Feb	2	0.00	14	0.00	0	0.00	0	0.00	0	0.00	0	0.00

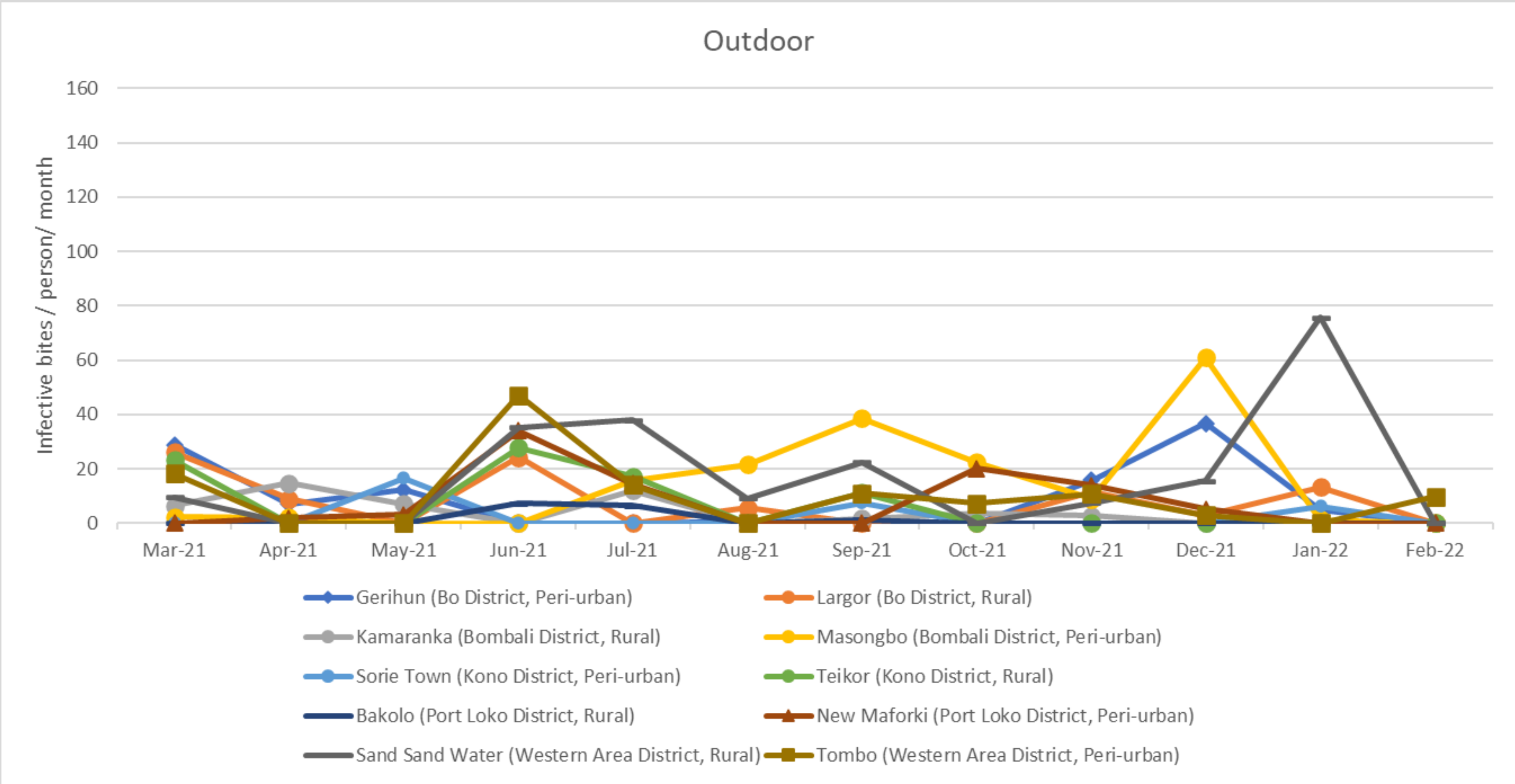
3.3.4 Entomological Inoculation Rate

The Entomological Inoculation Rate (EIR), expressed as the number of infective bites per person per unit time, either month (ib/p/m), or year (ib/p/y) was estimated for each site during the sampling period of March 2021 - February 2022 (Annex G, H and I). Seasonal EIR was estimated with wet season EIR covering between June-October 2021, while dry season covering from November 2021 to February 2022 and March 2022. The annual EIR was estimated from all the 12 sampling months (Annex G-I).

The highest monthly EIR (mEIR) was recorded indoor in June in Teikor rural site in Kono District (142.2 ib/p/m) followed by Sand Sand Water rural site in Western Area district, outdoor mEIR of 75.8 ib/p/m in January 2022 (Figure 41 and 42). Sand Sand Water is a rural site in the West Coast region that has persistent mosquito breeding habitats. The previous year, the highest EIR was in the IRS District of Bombali (Masongbo peri-urban site).

Figure 41: Monthly Entomological Inoculation Rates (mEIR) by Site, March 2021-February 2022




Outdoor
Infective bites / person/ month
160
140
120
100
80
60
40
20
0
Mar-21
Apr-21
May-21
Jun-21
Jul-21
Aug-21
Sep-21
Oct-21
Nov-21
Dec-21
Jan-22
Feb-22
Gerihun (Bo District, Peri-urban)
Largor (Bo District, Rural)
Kamaranka (Bombali District, Rural)
Masongbo (Bombali District, Peri-urban)
Sorie Town (Kono District, Peri-urban)
Teikor (Kono District, Rural)
Bakolo (Port Loko District, Rural)
New Maforki (Port Loko District, Peri-urban)
Sand Sand Water (Western Area District, Rural)
Tombo (Western Area District, Peri-urban)

The monthly EIR (mEIR) for all sites combined peaked in June (for those sampled) and decreased progressively into the dry season with some peaks observed in September and December (Figure 42). Indoor and outdoor mEIR were comparable when data was aggregated but varied when data was disaggregated by site (Figure 43-45). When data was aggregated by IRS intervention and non-IRS sites, the highest mEIR was recorded in June in non-IRS sites (Figure 43). Between the rainy season of June and September, non-IRS sites had higher EIR than IRS sites but reversed in December with IRS sites having higher EIR than Non-IRS sites possibly due to high HBR of *An. funestus* in December (Figure 43). There was no clear relationship between mEIR estimates within sites and rainfall pattern in Sierra Leone but when aggregated together, estimates followed the rainfall patterns (Figure 42-45).

Figure 42: Monthly Mean Indoor and Outdoor Entomological Inoculation Rate (mEIR) in All Sites, March 2021–February 2022

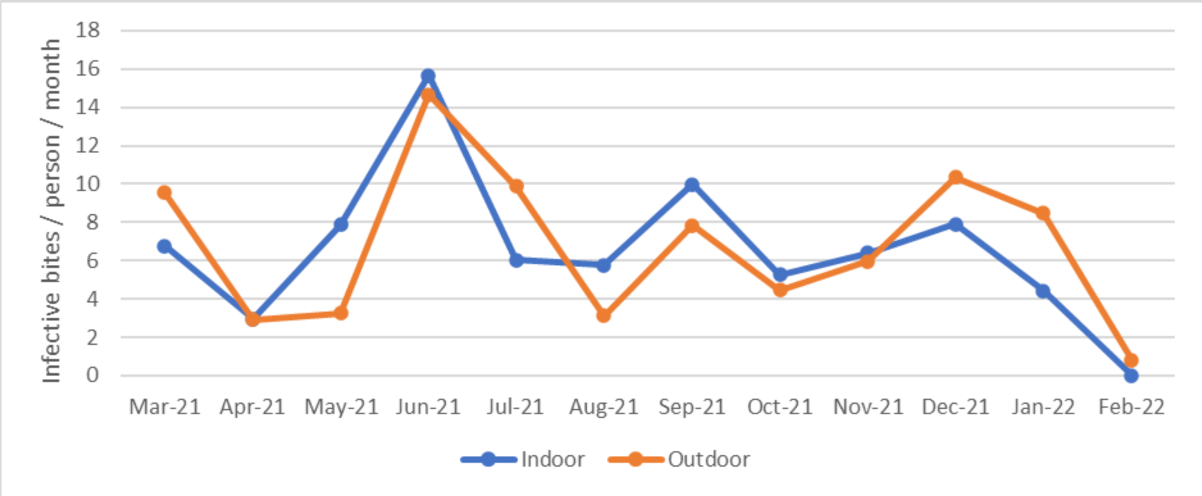


Figure 43: Monthly Indoor and Outdoor Entomological Inoculation Rate (mEIR) in Indoor Residual Spraying (IRS) and Non-IRS Districts by Month, March 2021–February 2022.

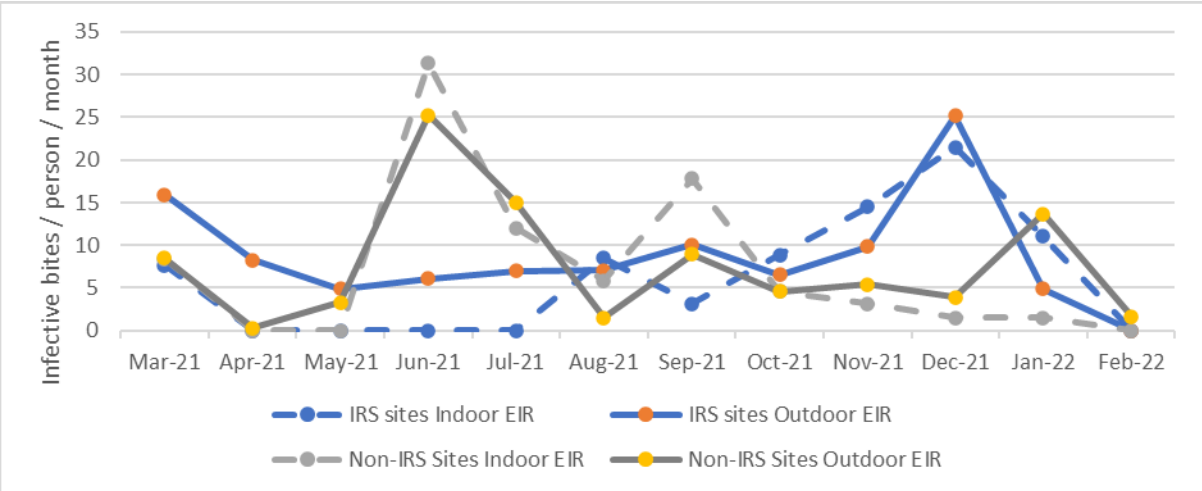
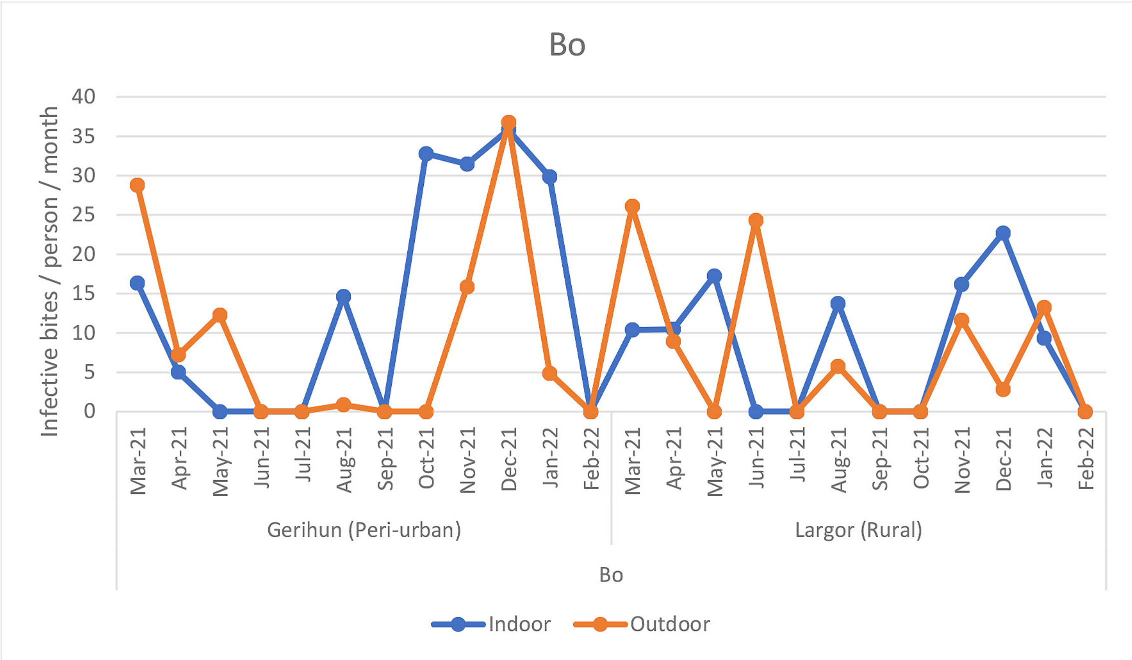


Figure 44: Monthly Indoor and Outdoor Entomological Inoculation Rate (mEIR) in Indoor Residual Spraying (IRS) Districts by Month and Site, March 2021–February 2022.



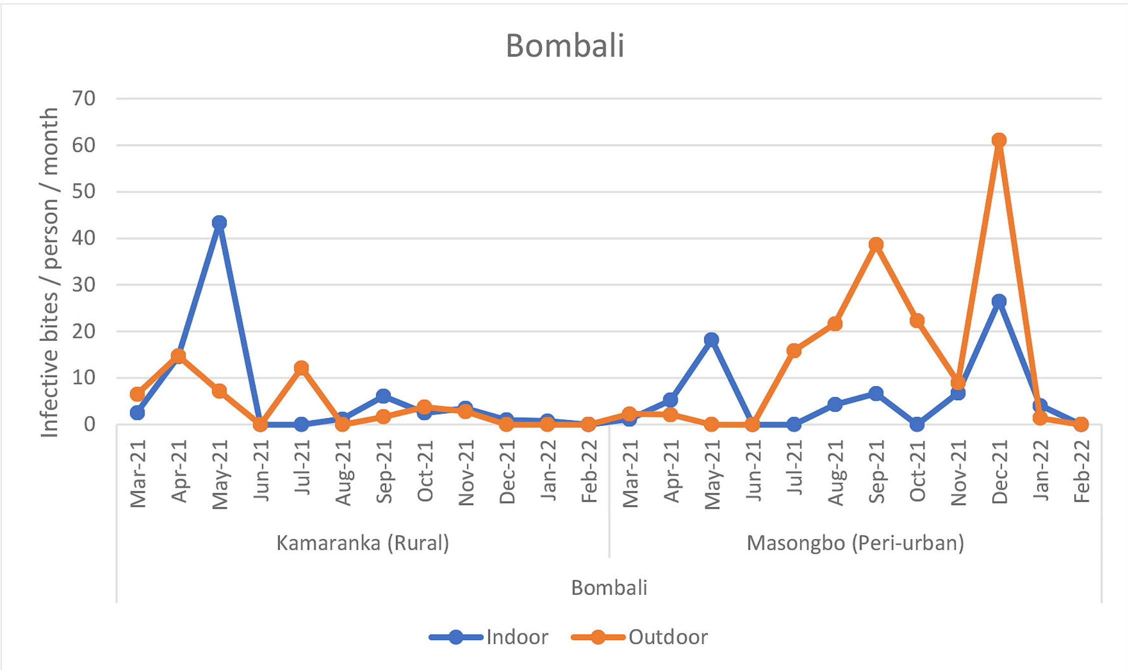
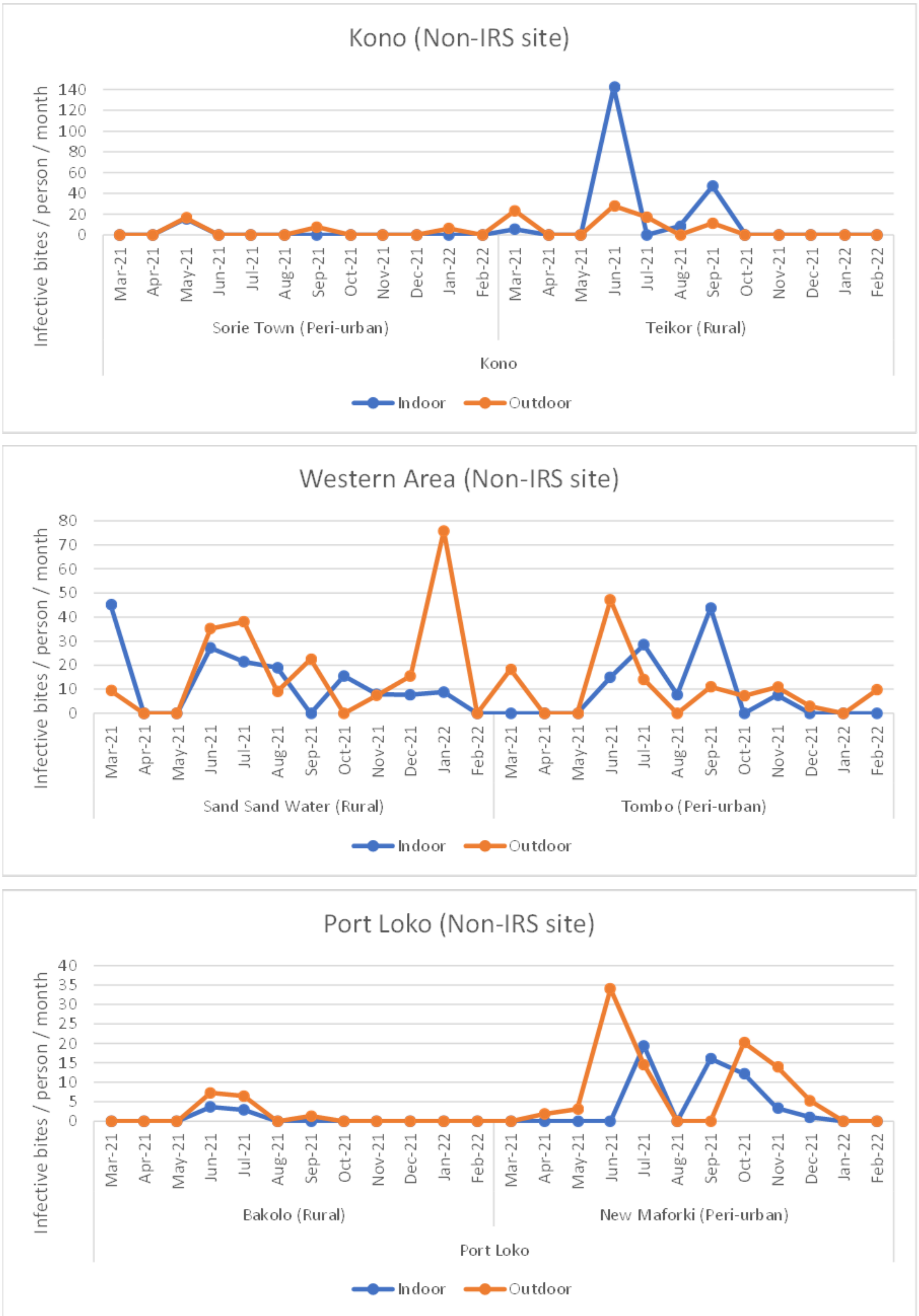


Figure 45: Monthly Indoor and Outdoor Entomological Inoculation Rate (mEIR) in Non-Indoor Residual Spraying (Non-IRS) Districts by Month and Site, March 2021–February 2022.



3.3.5 BLOOD MEAL ORIGIN

A total of 3,107 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 2,988 blood fed mosquitoes. The remaining 121 could not be identified (Table 10). Of the 2,988 (96.1%) mosquitoes with known blood meal source, 2,985 were identified to the species level using PCR. The remaining 124 were morphologically identified to the species complex level as *An. gambiae* s.l. Overall, 2,530 (81.4%) fed on human blood, 451 (14.5%) on mixed human and animal blood and 7 (0.23%) on animal blood. The Human Blood Index (HBI) in all members of *An. gambiae* and *An. funestus* groups identified was over 70% apart from Bo District where the HBI was between 54% to 96% (Table 11). The data indicates that the malaria vectors in Sierra Leone are highly anthropophagic with 80% of them fed either fully or partly on human blood (Table 10 and 11).

Table 10: Host Preference for *Anopheles* Mosquitoes Across Districts, March 2021-February 2022

Species	Blood meal sources				
	Animal	**Human**	**Mixed (Animal/ Human)**	**No Source**	**Total**
An. coluzzii	0.2 % (3)	86.6% (1,048)	11.5% (139)	1.7% (20)	1210
An. gambiae	0.1% (2)	78.6% (1162)	16.8% (248)	4.5% (67)	1479
An. gambiae s.l.	1.6% (2)	79.8% (99)	5.6% (7)	12.9% (16)	124
An. funestus s.s.	0%	74.0% (213)	19.5% (18)	6.3 (18)	288
An. leesoni	0%	100% (6)	0%	0%	6
Total	**7 (0.23%)**	**2528 (81.4%)**	**451 (14.5%)**	**121 (3.9%)**	**3,107**

Table 11: Human Blood Index by District from PSC & Light Trap Collections, March 2021 – February 2022

District	Village	Species	Total	Animal	Mixed	Human	Human Blood Index (HBI)
Bo	Gerihun	*An. coluzzii*	130		25	105	80.8
		An. gambiae s.s.	121		28	93	76.9
		An. gambiae s.l.	25		1	24	96.0
		An. funestus s.s.	44		9	35	79.5
	Largor	*An. coluzzii*	74		25	49	66.2
		An. gambiae s.s.	48		22	26	54.2
		An. funestus s.s.	65		8	57	87.7
Bombali	Kamaranka	*An. coluzzii*	23		5	18	78.3
		An. gambiae s.s.	48		18	30	62.5
	Masongbo	*An. coluzzii*	50		3	47	94.0
		An. gambiae s.s.	84		20	64	76.2

		An. gambiae. s.l.	2		0	2	100.0
		An. funestus s.s.	26		8	18	69.2
Kono	Sorie Town	*An. coluzzii*	141	2	14	125	88.7
		An. gambiae s.s.	147		23	124	84.4
		An. coluzzii/ *An. gambiae* s.s.	2		0	2	100.0
		An. funestus s.s.	36		10	26	72.2
		NA	8		2	6	75.0
	Teikor	*An. coluzzii*	86		15	71	82.6
		An. gambiae s.s.	155		25	130	83.9
		An. funestus s.s.	69		17	50	72.5
		NA	23		0	23	100.0
Port Loko	Bakolo	*An. coluzzii*	20		0	20	100.0
		An. gambiae s.s.	2		0	2	100.0
	New Maforki	*An. coluzzii*	125		6	119	95.2
		An. gambiae s.s.	54		8	46	85.2
		An. gambiae s.l.	5		0	5	100.0
		An. funestus s.s.	2		0	2	100.0
Western Rural	Sand Sand Water	*An. coluzzii*	70		4	66	94.3
		An. gambiae s.s.	162		12	150	92.6
		An. gambiae s.l.	5		0	5	100.0
	Tombo	*An. coluzzii*	67	1	1	65	97.0
		An. gambiae s.s.	86		10	76	88.4
		An. gambiae s.l.	10		0	10	100.0
		An. funestus s.s.	6		1	5	83.3

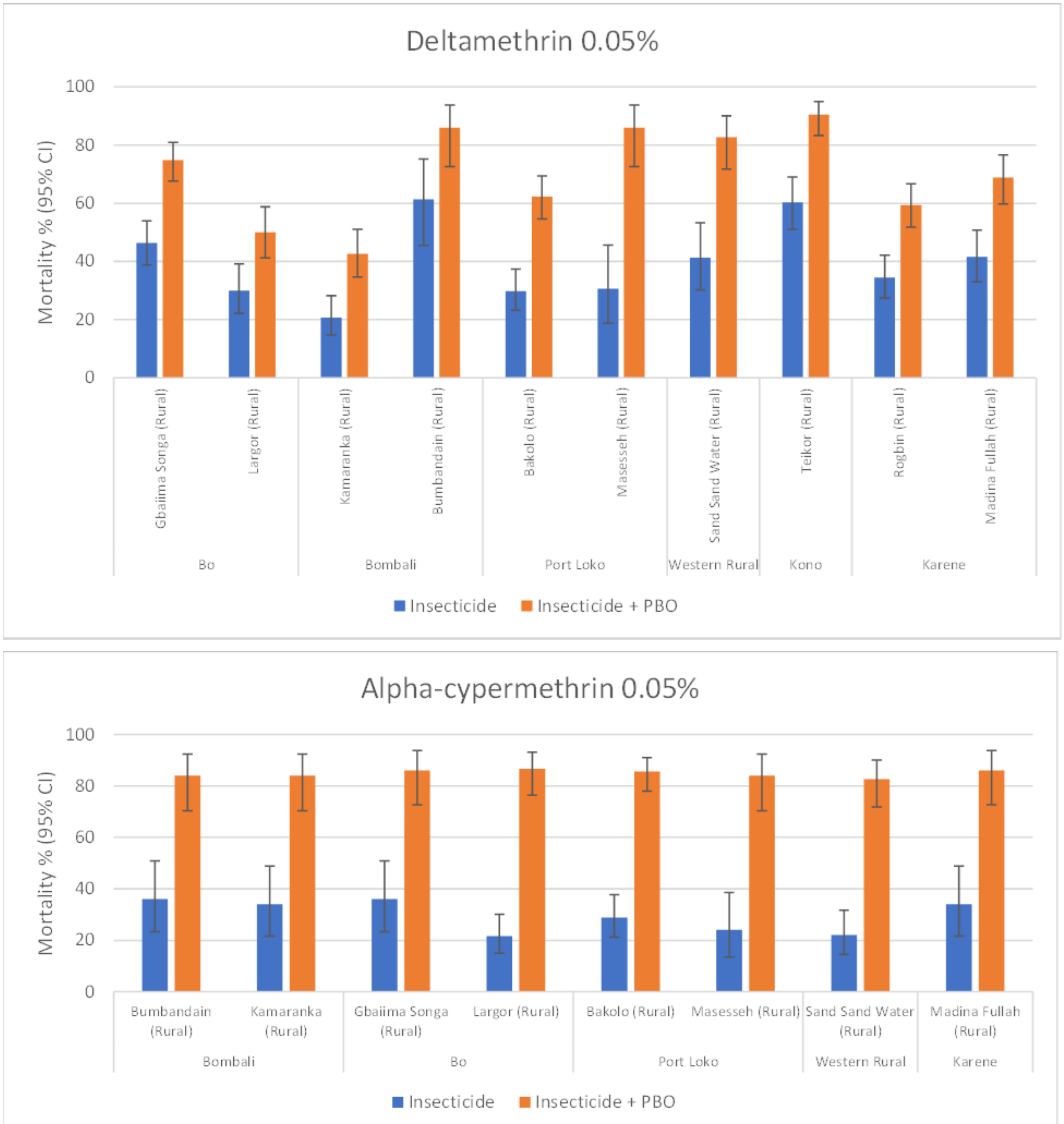
3.4 Insecticide Resistance Monitoring

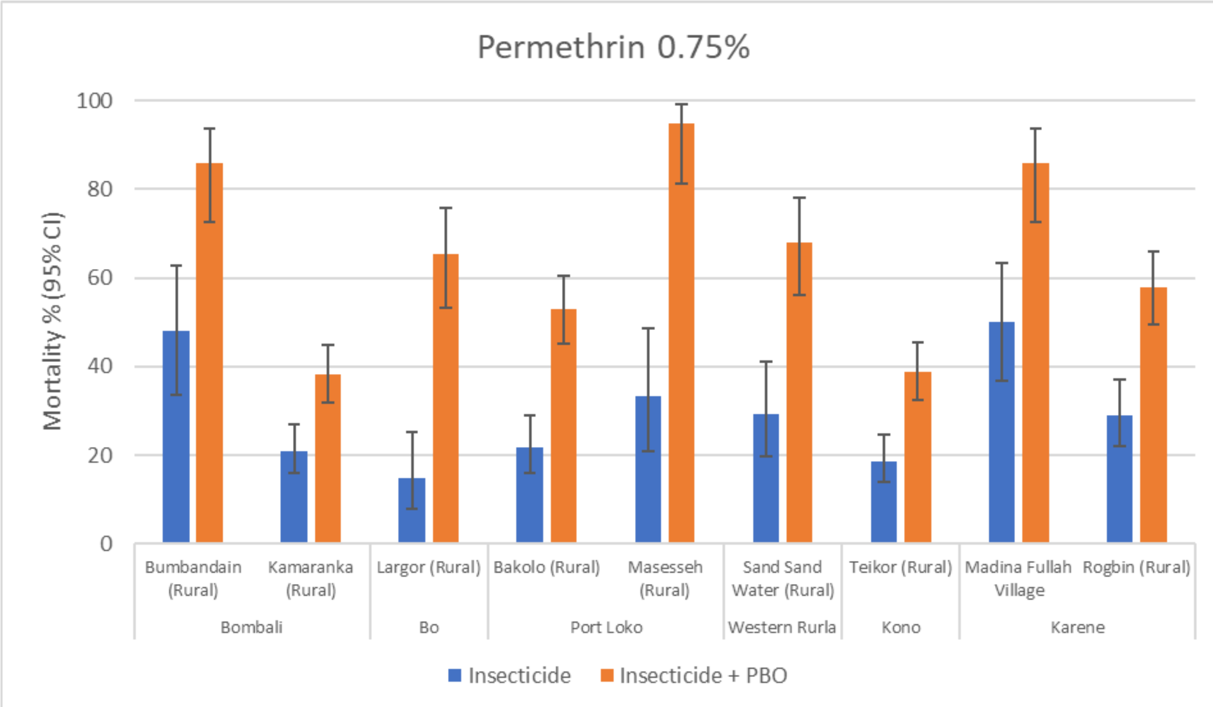
3.4.1 Synergist Assays

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 46). Mortality ranged from 20.7% in Kamaranka (Bombali District) to 61.4% in Bumbadain (Bombali District) for deltamethrin; 14.7% in Largor (Bo District) to 50% in Madina Fulah (Karene District) for permethrin, and 21.6% in Largor (Bo) to 36% in Bumbadain (Bombali) for alpha-cypermethrin (Figure 46; Annex F). The PBO partially restored susceptibility to all the pyrethroids, suggesting the partial involvement of monooxygenase-based resistance mechanism in Sierra Leone (Figure 46). The minimum increase in mortality after PBO also varied by insecticide and site (Figure 46). For Permethrin, PBO increased mortality by a minimum of 17.3%, 20% for deltamethrin, and 48%

for alpha-cypermethrin. The effect of PBO in 2021 is similar to its activity in 2020 where mortality was restored by about 27-53.6%. This implies PBO-ITNs are still effective against pyrethroid resistant mosquitoes in Sierra Leone.

Figure 46: Susceptibility of *An. gambiae* s.l. to Deltamethrin 0.05%, Permethrin 0.75% and Alpha-cypermethrin 0.05% With or Without PBO in 2021

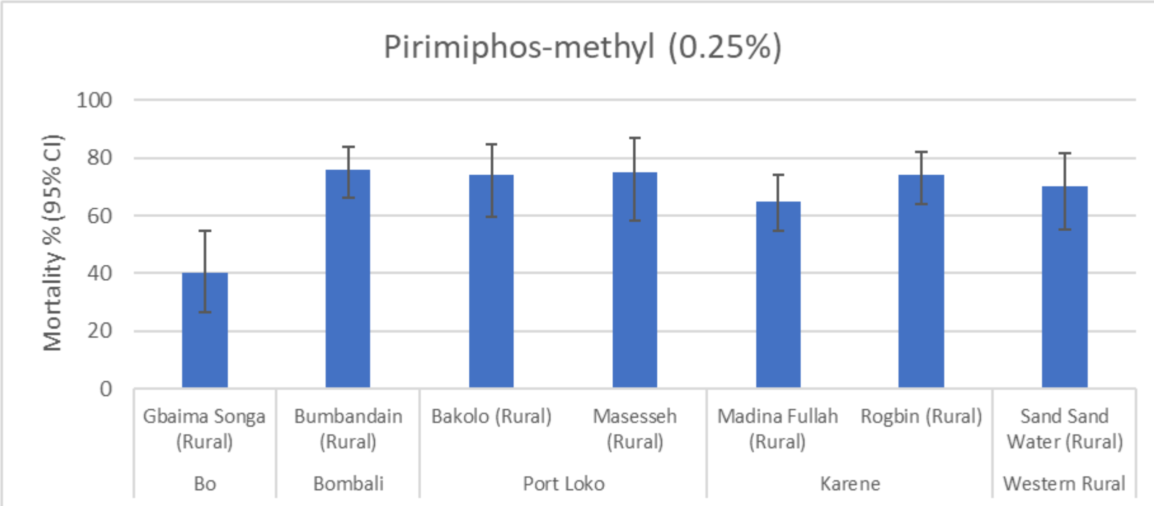




3.4.2 *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO ORGANOPHOSPHATES

An. gambiae s.l. were exposed to pirimiphos-methyl (0.25%) in Bo, Bombali, Western Rural, Kono and Port Loko (Figure 47). There was resistance to pirimiphos-methyl in all 7 sites tested. In 2020, pirimiphos-methyl susceptibility was investigated in only two sites, (Kamaranka (Bombali District) and Teikor (Kono District) and there was full susceptibility. Since different mosquito populations may be sampled, variability in susceptibility is always observed especially if majority of larvae was collected from one habitat with few family lines. In the previous year, the frequency of *Ace-1* mutation that confers resistance to organophosphates and carbamates was 14.6%, indicating that the mutation is present in Sierra Leone. The frequency of *Ace-1* for this period is 21.9% (Table 12). This probably comes from the use of pesticides in agriculture or gene flow sweeping across west Africa. To confirm that the WHO test papers used were effective, the team exposed susceptible *An. gambiae* Kisumu to the test papers and there was 100% mortality.

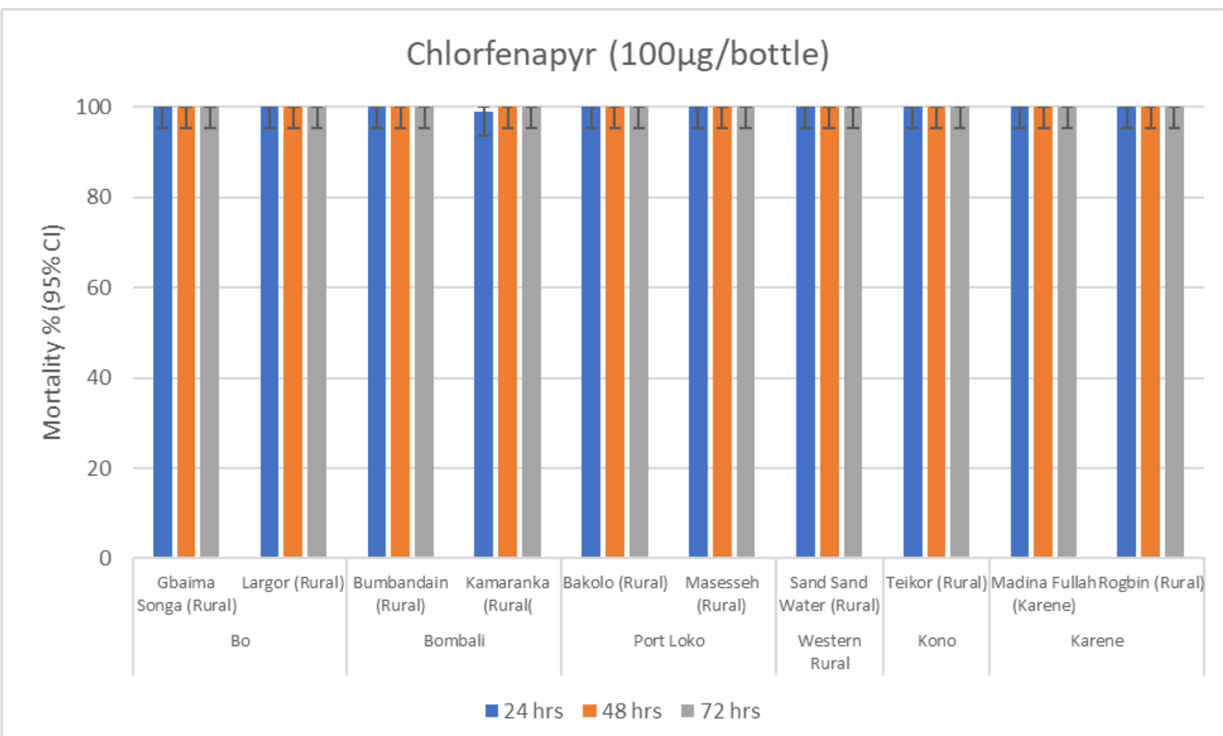
Figure 47: Susceptibility Status of *An. gambiae* s.l. to Pirimiphos-methyl (0.25%) in 2021-2022



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

The *An. gambiae* s.l. mosquitoes from Bo, Bombali, Port Loko, Kono, Western Rural and Karene that were exposed to chlorfenapyr were fully susceptible with all mosquitoes dying after day 2 of exposure (Figure 48).

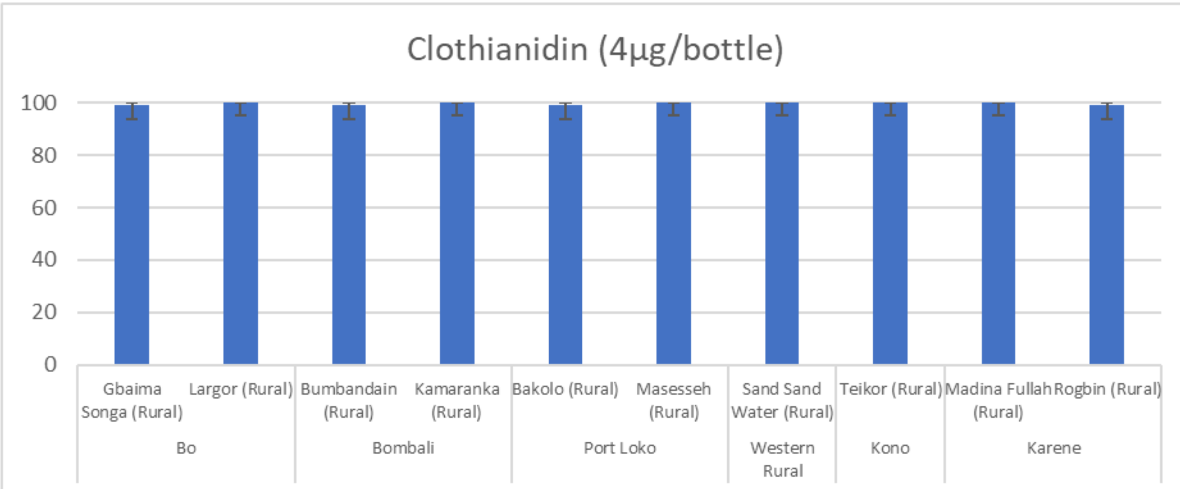
Figure 48: Susceptibility of *An. gambiae* s.l. to Chlorfenapyr (100 µg/Bottle) in 2021



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

Malaria vectors in Bo, Bombali, Kono, Port Loko, Western Rural and Karene districts were still susceptible to clothianidin (4µg/bottle) (Figure 49).

Figure 49: Susceptibility of *An. gambiae* s.l. to Clothianidin (4µg/bottle) in 2021



3.4.5 DETERMINATION OF THE INTENSITY OF RESISTANCE

The intensity of pyrethroid resistance in *An. gambiae* s.l. was not determined in any of the sites due to the unavailability of mosquitoes and the order of priority of insecticide resistance testing for Sierra Leone NMCP.

3.5 PCR ANALYSIS FOR MECHANISM OF RESISTANCE

In 2021, a sub sample of mosquitoes from the susceptibility tests were screened for the presence of *kdr-w* (L1014F), *kdr-e* (L1014S), *ace-1*(G119S) and N1575Y. A total of 395 mosquitoes out of 8,137 were assayed for molecular species identification and screened for resistance mutations. Of these, 13 were not identified to species level. Of the remaining 382 that were successfully identified, 87.4% were *An. gambiae* s.s. while 12.6% were *An. coluzzii*. This distribution is consistent with the species identification on adults collected via HLC/PSC/CDC-LT.

In all sites, in both species, *kdr-w* mutation was fixed or approaching fixation in the sampled mosquitoes indicating high level of pyrethroid resistance in Sierra Leone (Table 12). There was no sample carrying *kdr-e* mutation (Table 12). The *ace-1*mutation that confers resistance to carbamates/organophosphates is present in Sierra Leone and the frequency increased from 14.6% in 2020 to 21.9% in 2021. This probably explains the lower mortality against pirimiphos-methyl observed in 2021 (Table 12). Since these classes of insecticide (carbamates/organophosphates) are not used for malaria vector control, it will be crucial to monitor agricultural pesticides in Sierra Leone. Regular monitoring of the *ace-1* distribution would be useful in the selection of insecticides for vector control. The presence of N1575Y mutation was detected at low frequency of 0.5% (Table 13).

Table 12: Distribution of Insecticide Resistance Mutation to Pyrethroids (*Kdr-w/e*) and Carbamates/Organophosphates (*Ace-1*), in *An. gambiae* s.l. Sampled During the Rainy Season Between May and October 2021 in Sierra Leone.

		Kdr-w						*Kdr*-e				*Ace-1*				
District	Species	WW	WS	SS	f (W)	% WW	EE	ES	SS	f (E)	%EE	AG	AG	GG	f (A)	%AA
Bo	*An. coluzzii*	16	7	0	0.85	69.6	0	0	23	0.0	0.0	0	0	22	0.0	0.0
	An. gambiae	70	0	0	1.00	100.0	0	0	71	0.0	0.0	21	0	51	0.3	29.2
Bombali	*An. coluzzii*	12	0	0	1.00	100.0	0	0	12	0.0	0.0	0	0	12	0.0	0.0
	An. gambiae	90	0	0	1.00	100.0	0	0	90	0.0	0.0	9	0	80	0.1	10.1
Kono	*An. coluzzii*	2	1	0	0.83	66.7	0	0	3	0.0	0.0	0	0	3	0.0	0.0
	An. gambiae	26	0	0	1.00	100.0	0	0	27	0.0	0.0	1	0	25	0.0	3.8
Port Loko	*An. coluzzii*	6	0	0	1.00	100.0	0	0	6	0.0	0.0	1	0	5	0.2	16.7
	An. gambiae	100	0	1	0.99	99.0	0	0	101	0.0	0.0	40	0	62	0.4	39.2
Western rural area	*An. coluzzii*	2	2	0	0.75	50.0	0	0	4	0.0	0.0	2	0	2	0.5	50.0
	An. gambiae	42	0	0	1.00	100.0	0	0	43	0.0	0.0	9	0	34	0.2	20.9
Grand Total		366	10	1	0.98	97.1	0	0	380	0.0	0.0	83	0	296	0.2	21.9

Frequency (f) of the resistance allele [(2WW + WS)/2(WW+WS+SS)]
W=west mutation (Phenylalanine); E = east mutation (Serine); A =*Ace-1* mutation (Serine); S=susceptible (Leucine); G=susceptible (Glycine)

Table 13: Distribution of Insecticide Resistance Mutation to Pyrethroids (N1575Y), in *An. gambiae* s.l., Sampled in the Rainy Season between May and October 2021 in Sierra Leone.

District	PCR M/S	YY	YN	NN	f (Y)	%YY
Bo	*An. coluzzii*	0	0	23	0.0	0.0
	An. gambiae	0	14	57	0.1	0.0
Bombali	*An. coluzzii*	0	2	10	0.1	0.0
	An. gambiae	0	15	75	0.1	0.0
Kono	*An. coluzzii*	0	0	3	0.0	0.0
	An. gambiae	0	1	26	0.0	0.0
Port Loko	*An. coluzzii*	0	0	5	0.0	0.0
	An. gambiae	1	13	80	0.1	1.1
Western rural area	*An. coluzzii*	0	0	4	0.0	0.0
	An. gambiae	1	2	40	0.0	2.3
Total		2	47	323	0.1	0.5

Frequency (f) of the resistance allele [(2YY + YN)/2(YY+YN+NN)]; Y=mutation (Tyrosine)

3.5.1 VERIFICATION OF *AN. GAMBIAE* S.S. KISUMU COLONY

In 2022, a subsample of 100 laboratory colony strains from Freetown and Makeni was sent to CRID for molecular analysis. All mosquitoes were identified as *An. gambiae* s.s. (Table 14). There was no *Kdr-w* and *Kdr-e* mutations in the samples tested. These were F2 and F3 generations reared from eggs received from MR4 in December 2021. Eight samples could not be established because of amplification challenges.

Table 14: Distribution of Insecticide Resistance Mutation to Pyrethroids (*Kdr-w/e*) in Susceptible Laboratory Colony in Makeni and Freetown, Sierra Leone, 2022

		kdr-w				*kdr-e*				
Strain source	species	WW	WS	SS	Total	EE	ES	SS	f (E)	% EE
Freetown	*An. gambiae* s.s.	0	0	48	50	0	0	49	0.0	0.0
Makeni	*An. gambiae* s.s.	0	0	44	50	0	0	48	0.0	0.0
Total		**0**	**0**	**92**	**100**	**0**	**0**	**97**	**0.0**	**0.0**

Frequency (f) of the resistance allele [(2EE + ES)/2(EE+ES+SS)]

W=west mutation (Phenylalanine); E = east mutation (Serine); S=susceptible (Leucine)

4. CAPACITY BUILDING

Between July and December 2021, VectorLink Sierra Leone conducted assessments of 18 local laboratories consisting of public and private institutions, including universities and public health laboratories in hospitals. The aim was to support NMCP in identifying and selecting a local laboratory to undertake molecular analysis of mosquito samples collected during routine monitoring. The VectorLink team developed a guide (standard operating procedure) that contained standard laboratory practices that was used to assess each laboratory. The laboratories were assessed on three broad areas: i) Laboratory structure and management (including equipment), ii) Good laboratory practices (GCLP) and availability of competent staff and iii). Collaboration structures and existing collaboration including student programs.

The Njala laboratory in Bo was selected as the most suitable out of top three institutions, Makeni university laboratory in Makeni, Bombali district and the Tekko Veterinary laboratory in Tekko (Makeni outskirts), Bombali district. Njala university demonstrated their experience in molecular research of other pathogens including malaria and other vector borne disease screening. Another strong point was their MSs-Public Health and entomology student program that would ensure continuous training of new technicians and students for Sierra Leone.

It is expected that Njala University will begin processing mosquito samples in September of 2022 after they sign the Memorandum of understanding with NMCP. VectorLink is also expected to lead training of existing technicians in August 2022 at Njala laboratory to be able to carry out mosquito molecular analysis. VectorLink will be responsible for initial analysis and will work together with the Technical Manager to ensure that all protocols are understood, including interpreting results.

5. Discussion, Lessons Learned / Challenges, and Recommendations

5.1 Discussion

In Sierra Leone, *An. gambiae* s.l. is still the principal malaria vector with 87.2% (30,795), followed by *An. funestus* s.l. with 11.8% (4,168). Within *An. gambiae* s.l. complex, *An. gambiae* s.s. is the major vector (56.1%) followed by *An. coluzzii* (43.8%) and hybrid of *An. gambiae*/*An. coluzzii* at 0.1%. This composition differs from the 2020 period when *An. colluzzii* was the predominant vector identified at 64%. However, considering that both sibling species were infected with malaria sporozoites, they are both involved in malaria transmission in Sierra Leone. Within *An. funestus* group, the majority of 98.3% (469) were identified as *An. funestus* s.s. and 1.7% (8) as *An. leesoni.* The number of mosquitoes infected with malaria sporozoites reduced from 4.77% in 2020 to 2.99% in 2021. Peak malaria transmission still occurs in June with the highest HBR and EIR. The EIRs recorded across the sites indicate a great reduction in EIR and agrees with the recent malaria indicator survey (MIS 2021) showing national malaria prevalence dropped from 40 to 25% in children aged below 5 years. The aggregated data shows that EIR (outdoor and indoor) in IRS sites remained relatively low even after IRS in May 2021 compared to non-IRS sites. However, in December, EIR was higher in IRS-sites possibly because of the high *An. funestus* density and HBR in Bo and Bombali, maintained by irrigation farming during that period. This presents a challenge for IRS which is implemented in May and targets peak malaria season of June.

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 10:00pm to 3:00am overall. The highest indoor *An. gambiae* s.l. HBR was recorded in the peri-urban site of Masongbo in Bombali District (80.6 bites/person/night), and this reduced by 54% from previous year which had 175.5 bites/person/night (2020 Sierra Leone Annual Entomological report). The VectorLink project will investigate whether the reduced HBR is because of co-intervention of IRS with clothianidin and PBO-ITNs. This will be reported on separately.

Malaria vectors in Sierra Leone prefer to feed on humans rather than animal blood. The minimum Human Blood Index (HBI) was 54% in Bo District. 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* s.l., 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. In the absence of humans, malaria vectors will feed on other available hosts but *An. funestus* is expected to prefer humans (higher HBI) compared to *An. gambiae.* s.l. (Killeen et al 2001). Although national malaria prevalence has declined from 44% in 2016 to 22% in 2021, the high HBI has remained the same since 2019 and indicates that ITN use is not optimum in Sierra Leone, translating to the observed high malaria transmission (MIS 2016 and 2021). The high proportion of fed mosquitoes over half gravid/gravid could also indicate an exophilic tendency of the vector (mosquitoes leaving the house after feeding before they become gravid) or the impact of IRS and ITNs, killing the mosquitoes before they start digesting their bloodmeal and get the chance to be half or fully gravid.

The vector abundance and biting rates followed the rainfall pattern with peaks observed between June-September, 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. However, scale up of SBC on net use could be beneficial given the high proportion of fed mosquitoes collected inside houses.

During each month, there was a high proportion of mosquitoes that had laid eggs (parous) at least once throughout the sampling period (13-100%), supporting the observation of high proportion of mosquitoes that had successfully acquired a blood meal. The parity rate was high in the dry months of March and April, and this rate declined with the onset of rains in May/June with random variations and later increasing toward the end of rainy season in October/November through February.

The *An. gambiae* s.l. in Sierra Leone are resistant to pyrethroids with average mortality varying per insecticide, deltamethrin (40%), permethrin (30%) and alpha-cypermethrin (30%). Previous reports indicate intensity of deltamethrin/pyrethroid resistance is very high in Sierra Leone when read together with average mortality per insecticide. However, PBO partially restored susceptibility to all the pyrethroids, but this varied by insecticide and site. On average, PBO increased susceptibility by 30% for those exposed to deltamethrin, 55% to those exposed to alphacypermethrin and 36% to those exposed to permethrin. This suggests that monooxygenase-based resistance mechanism is partially involved in the resistance to pyrethroids. There was resistance to pirimiphos-methyl in all sites despite this insecticide not being used for vector control in Sierra Leone. The frequency of *ace*-1 mutation that confers resistance to pirimiphos-methyl increased to 21.9%, indicating that resistance to organophosphates/carbamates could be existing. It is possible that organophosphates/carbamate pesticides used in agriculture could have contributed to this finding. Additionally, there is strong phylogenetic selection wave sweeping across West Africa and the presence of this mutation could be because of gene flow (Lynd et al 2010, Edi, C. V 2012). This mutation presents opportunities for capacity building for Sierra Leone to study evolution of insecticide resistance through post-graduate studies. *An. gambiae* s.l. is fully susceptible to chlorfenapyr and clothianidin and this supports the justification for the use of SumiShield (clothianidin) for IRS in Sierra Leone. Additionally, the NMCP also procured dual active nets Interceptor G2 that has alpha-cypermethrin and chlorfenapyr as active ingredients.

SumiShield™ (clothianidin) has been used in a district wide IRS campaign in Bo and Bombali for two years beginning in May 2021 and the team monitored the effectiveness from May 2021 to February 2022. In all, during the 10 months post spray, the insecticide killed over 80% of mosquitoes indicating longer duration of effectiveness of clothianidin compared to pyrethroids, consistent with reports from other countries.

In conclusion, *An. gambiae* s.l. and *An. funestus* s.l. are the major vectors of malaria in Sierra Leone with *An. gambiae* s.l. driving transmission in all sites during the rainy season while *An. funestus* s.l. is implicated during the dry season especially in Bo, Bombali and Western Area rural districts. 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. There is resistance to pirimiphos methyl. Malaria vectors are still susceptible to chlorfenapyr and clothianidin and this supports the use of SumiShield (clothianidin) insecticide for IRS in Bo and Bombali districts and procurement of IG2 nets for 2023 mass net campaign and for all continuous distribution channels beginning in 2023. SumiShield (clothianidin) remained effective in killing mosquitoes, nine months after spray campaign in May 2021. 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 expand effective SBC on improved ITN use. This report includes all the entomological data collected for the reporting year of March 21 – February 2022. Entomological data collection for the PBO nets-IRS co-deployment started in July 2020 and ended successfully in April 2022. The team plans to compile and analyze the entomological monitoring data before and after the co-deployment and submit a separate report in October 2022.

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. Resistance to pirimiphos-methyl was also detected in all five districts. 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. The team developed 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, with the team achieving over 85% target in monitoring insecticide resistance in Sierra Leone.

- More mosquitoes were still found to have fed on humans, indicating a challenge with ITN use in Sierra Leone. The VectorLink project is supporting NMCP and the Breakthrough Action project to evaluate the reasons for low net use resulting in high blood fed mosquitoes.
- The project successfully closed entomological surveillance sites used during the co-deployment study, and the VectorLink project met with District Health Management teams in Bo, Bombali, Port Loko and Karene including the supervisors and vector collectors.
- The project has adapted to undertaking entomology activities while observing Covid-19 prevention guidelines.
- 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 2021, the entomology team greatly improved in parity dissections after refresher training and guidance during routine mosquito collections.
- The team was able to establish a new colony of *An. gambiae* s.s. Kisumu from MR4, CDC Atlanta, US. The colony is vibrant and has supplied susceptible mosquitoes for the monitoring of decay rate of SumiShield (clothianidin) in Bo and Bombali IRS districts.
- 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:

- Maintain the new colony of *An gambiae* s.s. Kisumu strain, from MR4, in Freetown and Makeni.
- 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 strengthen quality assurance systems.
- Coordinate with NMCP and Breakthrough action to understand reasons for low net use in Sierra Leone

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 the onset of the 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 2022 IRS campaign.

ANNEX A: NUMBER OF *ANOPHELES* COLLECTED BY PSC, CDC-LT AND HLC, MARCH 2021–FEBRUARY 2022

District	Site	*An. funestus* s.l.	*An. gambiae* s.l.	*An. coustani*	Other	Total
Bo	Gerihun (Peri-urban)	6,614	1,289	10	1	7,914
	Largor (Rural)	5,007	1,966	1	0	6,974
Bombali	Kamaranka (Rural)	4,603	12	2	3	4,620
	Masongbo (Peri-Urban)	6,155	361	47	24	6,587
Kono	Sorie Town (Peri-Urban)	1,441	141	5	6	1,593
	Teikor (Rural)	1,377	241	3	0	1,621
Port Loko	Bakolo (Rural)	846	2	164	16	1,028
	New Maforki (Peri-Urban)	2,802	147	12	55	3,016
Western Rural	Sand Sand Water (Rural)	1,240	5	0	0	1,245
	Tombo (Peri-Urban)	710	4	0	0	714
Total		**30,795**	**4,168**	**244**	**105**	**35,312**

ANNEX B: NUMBER OF *ANOPHELES* COLLECTED BY CDC LIGHT TRAPS, MARCH 2021–FEBRUARY 2022

District	Site	*An. gambiae* s.l.	*An. funestus* s.l.	*An. coustani*	Other	Total
Bo	Gerihun (Rural)	353	36	0	0	389
	Largor (Rural)	105	34	0	0	139
Bombali	Kamaranka (Rural)	168	1	0	1	170
	Masongbo (Peri-Urban)	472	34	2	1	509
Kono	Sorie Town (Peri-Urban)	261	44	1	0	306
	Teikor (Rural)	211	30	1	0	242
Port Loko	Bakolo (Rural)	125	2	0	0	127
	New Maforki (Peri-Urban)	135	5	3	1	144
Western Rural	Sand Sand Water (Rural)	103	0	0	0	103
	Tombo (Peri-Urban)	133	0	0	0	133
Total		**2,066**	**186**	**7**	**3**	**2,262**

ANNEX C: NUMBER OF *ANOPHELES* COLLECTED BY PSC, MARCH 2021-FEBRUARY 2022

District	Site	*An. gambiae* s.l.	*An. funestus* s.l.	*An. coustani*	Other	Total
Bo	Gerihun (Rural)	613	265	0	0	878
	Largor (Rural)	656	430	0	0	1086
Bombali	Kamaranka (Rural)	141	5	0	0	146
	Masongbo (Peri-Urban)	196	38	0	0	234
Kono	Sorie Town (Peri-Urban)	551	54	0	0	605
	Teikor (Rural)	267	136	0	0	403
Port Loko	Bakolo (Rural)	90	0	16	1	107
	New Maforki (Peri-Urban)	178	13	0	0	191
Western Rural	Sand Sand Water (Rural)	410	1	0	0	411
	Tombo (Peri-Urban)	76	4	0	0	80
Total		3178	946	16	1	**4,141**

ANNEX D: NUMBER OF *ANOPHELES* COLLECTED BY HLC, MARCH 2021-FEBRUARY 2022

District	Site	*An. gambiae* s.l.	*An. funestus* s.l.	*An. coustani*	Other	Total
Bo	Gerihun (Rural)	5648	988	10	1	6647
	Largor (Rural)	4246	1502	1	0	5749
Bombali	Kamaranka (Rural)	4294	6	2	2	4304
	Masongbo (Peri-Urban)	5487	289	45	23	5850
Kono	Sorie Town (Peri-Urban)	629	43	4	6	682
	Teikor (Rural)	899	75	2	0	976
Port Loko	Bakolo (Rural)	631	0	148	15	794
	New Maforki (Peri-Urban)	2489	129	9	54	2686
Western Rural	Sand Sand Water (Rural)	727	4	0	0	731
	Tombo (Peri-Urban)	501	0	0	0	501
Total		**25,551**	**3,036**	**221**	**101**	**28,920**

ANNEX E: NUMBER OF *ANOPHELES* DISSECTED FOR PARITY RATE; *AN GAMBIAE* S.L. AND *AN FUNESTUS* S.L., MARCH 2021-FEBRUARY 2022

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**	Mar-21	1	16	11	28	39.3	17	94.1	69.2	99.7
		Apr-21	2	12	1	15	6.7	14	85.7	56.2	97.5
		Jun-21	8	4		12	0.0	12	33.3	11.3	64.6
		Jul-21	9	23		32	0.0	32	71.9	53.0	85.6
		Aug-21	9	26		35	0.0	35	74.3	56.4	86.9
		Sep-21	12	5	9	26	34.6	17	29.4	11.4	56.0
		Oct-21	5	29		34	0.0	34	85.3	68.2	94.5
		Nov-21	12	13	1	26	3.8	25	52.0	31.8	71.7
		Dec-21	5	23		28	0.0	28	82.1	62.4	93.2
		Jan-22	1	45	1	47	2.1	46	97.8	87.0	99.9
		Feb-22	7	49		56	0.0	56	87.5	75.3	94.4
	Largor	Mar-21	2	5	6	13	46.2	7	71.4	30.3	94.9
		Apr-21		9		9	0.0	9	100.0	62.9	100.0
		May-21		3		3	0.0	3	100.0	31.0	100.0
		Jun-21	8	8		16	0.0	16	50.0	28.0	72.0
		Jul-21	9	6		15	0.0	15	40.0	17.5	67.1
		Aug-21	2	3	1	6	16.7	5	60.0	17.0	92.7
		Sep-21	5	3	3	11	27.3	8	37.5	10.2	74.1
		Oct-21	3	13		16	0.0	16	81.3	53.7	95.0
		Nov-21	11	4		15	0.0	15	26.7	8.9	55.2
		Dec-21	2	12		14	0.0	14	85.7	56.2	97.5
		Jan-22	2	10		12	0.0	12	83.3	50.9	97.1
		Feb-22	3	19		22	0.0	22	86.4	64.0	96.4

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
Bombali	**Kamaranka**	Mar-21		5	8	13	61.5	5	100.0	46.3	100.0
		Apr-21		9	2	11	18.2	9	100.0	62.9	100.0
		May-21		2	3	5	60.0	2	100.0	19.8	100.0
		Jun-21	8	12	5	25	20.0	20	60.0	36.4	80.0
		Jul-21	2	3	2	7	28.6	5	60.0	17.0	92.7
		Sep-21	2	5		7	0.0	7	71.4	30.3	94.9
		Oct-21		3	1	4	25.0	3	100.0	31.0	100.0
		Nov-21		8		8	0.0	8	100.0	59.8	100.0
		Jan-22	7	22		29	0.0	29	75.9	56.1	89.0
		Feb-22		35	3	38	7.9	35	100.0	87.7	100.0
	Masongbo	Mar-21		9	3	12	25.0	9	100.0	62.9	100.0
		Apr-21		18		18	0.0	18	100.0	78.1	100.0
		May-21	2	5	7	14	50.0	7	71.4	30.3	94.9
		Jul-21	3	2	1	6	16.7	5	40.0	7.3	83.0
		Aug-21	10	11	1	22	4.5	21	52.4	30.3	73.6
		Sep-21	3	17		20	0.0	20	85.0	61.1	96.0
		Oct-21	7	39	2	48	4.2	46	84.8	70.5	93.2
		Nov-21	2	13		15	0.0	15	86.7	58.4	97.7
		Dec-21	2	25		27	0.0	27	92.6	74.2	98.7
		Jan-22	1	18	1	20	5.0	19	94.7	71.9	99.7
		Feb-22	3	8	1	12	8.3	11	72.7	39.3	92.7
Kono	**Sorie Town**	Mar-21	1	8		9	0.0	9	88.9	50.7	99.4
		Apr-21	1	14		15	0.0	15	93.3	66.0	99.7
		May-21	6	3		9	0.0	9	33.3	9.0	69.1
		Jun-21		5		5	0.0	5	100.0	46.3	100.0
		Aug-21	8	7		15	0.0	15	46.7	22.3	72.6
		Sep-21	1	4		5	0.0	5	80.0	29.9	98.9
		Oct-21		2		2	0.0	2	100.0	19.8	100.0
		Dec-21	3	15	1	19	5.3	18	83.3	57.7	95.6
		Jan-22	4	12		16	0.0	16	75.0	47.4	91.7
		Feb-22		8		8	0.0	8	100.0	59.8	100.0
	Teikor	Mar-21		9		9	0.0	9	100.0	62.9	100.0
		Apr-21	1	9		10	0.0	10	90.0	54.1	99.5
		May-21	3	18		21	0.0	21	85.7	62.6	96.2
		Jun-21		11		11	0.0	11	100.0	67.9	100.0
		Jul-21	4	3	1	8	12.5	7	42.9	11.8	79.8
		Aug-21	3	2		5	0.0	5	40.0	7.3	83.0
		Sep-21	1	4		5	0.0	5	80.0	29.9	98.9

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
		Oct-21		6		6	0.0	6	100.0	51.7	100.0
		Nov-21		7		7	0.0	7	100.0	56.1	100.0
		Dec-21	1	3		4	0.0	4	75.0	21.9	98.7
		Jan-22		5		5	0.0	5	100.0	46.3	100.0
		Feb-22		5		5	0.0	5	100.0	46.3	100.0
Port Loko	**Bakolo**	Jul-21		4		4	0.0	4	100.0	39.6	100.0
		Aug-21		7		7	0.0	7	100.0	56.1	100.0
		Sep-21		7		7	0.0	7	100.0	56.1	100.0
		Dec-21	6	2		8	0.0	8	25.0	4.5	64.4
		Feb-22	1	1		2	0.0	2	50.0	9.5	90.5
	New Maforki	May-21	4	9	1	14	7.1	13	69.2	38.9	89.6
		Jun-21	4	6	3	13	23.1	10	60.0	27.4	86.3
		Jul-21	10	17	2	29	6.9	27	63.0	42.5	79.9
		Aug-21	2	11	3	16	18.8	13	84.6	53.7	97.3
		Sep-21	2	9		11	0.0	11	81.8	47.8	96.8
		Oct-21	14	10	1	25	4.0	24	41.7	22.8	63.1
		Nov-21	11	16	1	28	3.6	27	59.3	39.0	77.0
		Dec-21	9	13		22	0.0	22	59.1	36.7	78.5
		Jan-22		3		3	0.0	3	100.0	31.0	100.0
		Feb-22	5	38	4	47	8.5	43	88.4	74.1	95.6
Western Rural	**Sand Sand Water**	Jun-21	2	1	2	5	40.0	3	33.3	1.8	87.5
		Jul-21		5	9	14	64.3	5	100.0	46.3	100.0
		Aug-21	14	2	1	17	5.9	16	12.5	2.2	39.6
		Sep-21		5		5	0.0	5	100.0	46.3	100.0
		Oct-21		13		13	0.0	13	100.0	71.7	100.0
		Nov-21	8	8	1	17	5.9	16	50.0	28.0	72.0
		Dec-21	2	8		10	0.0	10	80.0	44.2	96.5
		Jan-22		9	1	10	10.0	9	100.0	62.9	100.0
		Feb-22		5		5	0.0	5	100.0	46.3	100.0
	Tombo	Jun-21		1	2	3	66.7	1	100.0	5.5	100.0
		Aug-21	4	2		6	0.0	6	33.3	6.0	75.9
		Sep-21		4		4	0.0	4	100.0	39.6	100.0
		Nov-21		2	1	3	33.3	2	100.0	19.8	100.0
		Dec-21		2		2	0.0	2	100.0	19.8	100.0
		Jan-22		6	2	8	25.0	6	100.0	51.7	100.0
		Feb-22		3		3	0.0	3	100.0	31.0	100.0
Total			**305**	**998**	**109**	**1,412**	**7.7**	**1,303**			

ANNEX F: WHO SUSCEPTIBILITY TEST AND CDC BOTTLE ASSAYS RESULTS, MAY-NOVEMBER 2021

A. Pyrethroids

		Alphacypermethrin 0.05%				Deltamethrin 0.05%				Permethrin 0.75%			
District	Village	Insecticide only		Insecticide + PBO 4%		Insecticide only		Insecticide + PBO 4%		Insecticide only		Insecticide + PBO 4%	
		Exposed	Mortality	Exposed	Mortality	Exposed	Mortality	Exposed	Mortality	Exposed	Mortality	Exposed	Mortality
Bo	Gbaima Songa	50	36	50	86	175	45.4	175	74.3	ND	ND	ND	ND
	Largor	125	21.6	75	86.7	120	30	120	50	75	14.7	75	65.3
Bombali	Bumbadain	50	36	50	84	44	53	50	84	48	46	50	85.4
	Kamaranka	50	34	50	84	150	20.7	150	42.7	225	20.9	225	38.2
Port Loko	Bakolo	125	28.8	125	85.6	171	29.8	175	62.3	165	20.5	170	52.6
	Masesseh	50	24	50	84	49	30.6	50	86	48	27.5	39	94.4
Karene	Madina Fullah	50	34	50	86	125	39.8	125	68	50	50	50	86
	Rogbin	ND	ND	ND	ND	171	34	175	59.3	145	29	145	57.9
Western Area	Sand Sand Water	100	22	75	82.7	75	41.3	75	82.7	75	29.3	75	68
Kono	Teikor	ND	ND	ND	ND	121	55.7	116	89.1	225	18.7	225	38.7
Key	Susceptible	Resistant											

ND= Not Done

B. Pyrroles (Chlorfenapyr), Neonicotinoids (Clothianidin) and Organophosphate (Pirimiphos-Methyl)

District	Village	Chlorfenapyr (100 µg)		Clothianidin 4µg/bottle		Pirimiphos-methyl	
		Exposed	Mortality	Exposed	Mortality	Exposed	Mortality
Bo	Gbaima Songa	100	100	100	99	50	40
	Largor	100	100	100	100	ND	ND
Bombali	Bumbadain	100	100	100	99	100	76
	Kamaranka	100	100	100	100	ND	ND
Port Loko	Bakolo	100	100	100	99	50	74
	Masesseh	100	100	100	100	40	75
Karene	Madina Fullah	100	100	100	100	100	65
	Rogbin	100	100	100	99	100	74
Western Area	Sand Sand Water	100	100	100	100	50	70
Kono	Teikor	100	100	100	100	ND	ND
Key	Susceptible	Resistant					

ND= Not Done

ANNEX G: *PLASMODIUM* INFECTION RATES, ALL SITES, MARCH 2021-FEBRUARY 2022

		An. gambiae		*An. coluzzii*		*An. funestus*		*An. leesoni*		
District	Village	*Pf* Positive	Proportion	*Pf* Positive	Proportion	*Pf* Positive	Proportion	*Pf* Positive	Proportion	**Grand Total**
Bo	Gerihun (Peri-urban)	5	16.7	16	53.3	9	30.0	0	0.0	30
	Largor (Rural)	3	8.8	25	73.5	6	17.6	0	0.0	34
Bombali	Kamaranka (Rural)	22	75.9	5	17.2	2	6.9	0	0.0	29
	Masongbo (Peri-urban)	15	39.5	14	36.8	8	21.1	1	2.6	38
Kono	Sorie Town (Peri-urban)	1	25.0	3	75.0	0	0.0	0	0.0	4
	Teikor (Rural)	8	50.0	5	31.3	3	18.8	0	0.0	16
Port Loko	Bakolo (Rural)	2	28.6	5	71.4	0	0.0	0	0.0	7
	New Maforki (Peri-urban)	14	50.0	12	42.9	2	7.1	0	0.0	28
Western Rural	Sand Sand Water (Rural)	28	87.5	4	12.5	0	0.0	0	0.0	32
	Tombo (Peri-urban)	12	70.6	5	29.4	0	0.0	0	0.0	17
Total		**110**	**46.8**	**94**	**40.0**	**30**	**12.8**	**1**	**0.4**	**235**

ANNEX H: *PLASMODIUM* INFECTION RATES, BY SITE AND MONTH, MARCH 2021-FEBRUARY 2022

Species	District	Village	Month	Negative	Sporozoite positive	Total	% Positive
***An. gambiae* s.l.**	**Bo**	**Gerihun**	Mar-21	365	16	381	4.20
			Apr-21	233	2	235	0.85
			May-21	102	1	103	0.97
			Jun-21	85	0	85	0.00
			Jul-21	67	0	67	0.00
			Aug-21	112	2	114	1.75
			Sep-21	124	0	124	0.00
			Oct-21	69	2	71	2.82
			Nov-21	56	2	58	3.45
			Dec-21	38	0	38	0.00
			Jan-22	60	2	62	3.23
			Feb-22	55	0	55	0.00
		Largor	Mar-21	113	15	128	11.72
			Apr-21	249	8	257	3.11
			May-21	105	1	106	0.94
			Jun-21	86	1	87	1.15
			Jul-21	62	0	62	0.00
			Aug-21	96	5	101	4.95
			Sep-21	87	0	87	0.00
			Oct-21	30	0	30	0.00
			Nov-21	45	1	46	2.17
			Dec-21	34	3	37	8.11
			Jan-22	43	3	46	6.52
			Feb-22	50	0	50	0.00

Species	District	Village	Month	Negative	Sporozoite positive	Total	% Positive
	Bombali	Kamarank	Mar-21	184	4	188	2.13
			Apr-21	160	9	169	5.33
			May-21	115	7	122	5.74
			Jun-21	117	0	117	0.00
			Jul-21	110	2	112	1.79
			Aug-21	93	1	94	1.06
			Sep-21	91	4	95	4.21
			Oct-21	75	4	79	5.06
			Nov-21	80	4	84	4.76
			Dec-21	29	1	30	3.33
			Jan-22	60	0	60	0.00
			Feb-22	87	0	87	0.00
		Masongbo	Mar-21	164	2	166	1.20
			Apr-21	171	3	174	1.72
			May-21	120	3	123	2.44
			Jun-21	115	0	115	0.00
			Jul-21	103	1	104	0.96
			Aug-21	147	6	153	3.92
			Sep-21	73	3	76	3.95
			Oct-21	77	3	80	3.75
			Nov-21	55	1	56	1.79
			Dec-21	56	8	64	12.50
			Jan-22	65	3	68	4.41
			Feb-22	21	0	21	0.00
	Kono	Sorie Town	Mar-21	13	0	13	0.00
			Apr-21	38	0	38	0.00
			May-21	50	2	52	3.85
			Jun-21	53	0	53	0.00
			Jul-21	17	0	17	0.00
			Aug-21	39	0	39	0.00
			Sep-21	21	1	22	4.55

Species	District	Village	Month	Negative	Sporozoite positive	Total	% Positive
			Oct-21	31	0	31	0.00
			Nov-21	3	0	3	0.00
			Dec-21	33	0	33	0.00
			Jan-22	23	1	24	4.17
			Feb-22	21	0	21	0.00
		Teikor	Mar-21	23	1	24	4.17
			Apr-21	51	0	51	0.00
			May-21	51	0	51	0.00
			Jun-21	48	6	54	11.11
			Jul-21	46	1	47	2.13
			Aug-21	46	1	47	2.13
			Sep-21	40	4	44	9.09
			Oct-21	30	0	30	0.00
			Nov-21	23	0	23	0.00
			Dec-21	10	0	10	0.00
			Jan-22	21	0	21	0.00
			Feb-22	16	0	16	0.00
	Port Loko	Bakolo	Mar-21	6	0	6	0.00
			Apr-21	111	0	111	0.00
			May-21	21	0	21	0.00
			Jun-21	113	3	116	2.59
			Jul-21	81	3	84	3.57
			Aug-21	86	0	86	0.00
			Sep-21	56	2	58	3.45
			Oct-21	2	0	2	0.00
			Nov-21	10	0	10	0.00
			Dec-21	8	0	8	0.00
		New Maforki	Mar-21	10	0	10	0.00
			Apr-21	104	1	105	0.95
			May-21	131	1	132	0.76
			Jun-21	67	1	68	1.47

Species	District	Village	Month	Negative	Sporozoite positive	Total	% Positive
			Jul-21	87	3	90	3.33
			Aug-21	59	0	59	0.00
			Sep-21	117	5	122	4.10
			Oct-21	78	9	87	10.34
			Nov-21	75	4	79	5.06
			Dec-21	84	4	88	4.55
			Jan-22	18	0	18	0.00
			Feb-22	74	0	74	0.00
	Western Rural	Sand Sand Water	Mar-21	40	6	46	13.04
			Apr-21	32	0	32	0.00
			May-21	55	0	55	0.00
			Jun-21	49	4	53	7.55
			Jul-21	45	3	48	6.25
			Aug-21	75	3	78	3.85
			Sep-21	31	3	34	8.82
			Oct-21	21	2	23	8.70
			Nov-21	28	2	30	6.67
			Dec-21	44	3	47	6.38
			Jan-22	50	9	59	15.25
			Feb-22	27	0	27	0.00
		Tombo	Mar-21	17	0	17	0.00
			Apr-21	18	0	18	0.00
			May-21	42	1	43	2.33
			Jun-21	50	1	51	1.96
			Jul-21	47	5	52	9.62
			Aug-21	29	1	30	3.33
			Sep-21	46	7	53	13.21
			Oct-21	10	1	11	9.09
			Nov-21	12	2	14	14.29
			Dec-21	8	0	8	0.00
			Jan-22	13	0	13	0.00

Species	District	Village	Month	Negative	Sporozoite positive	Total	% Positive
			Feb-22	11	0	11	0.00
***An. gambiae* s.l. Total**				**7679**	**239**	**7918**	3.02
An. funestus s.l.	**Bo**	Gerihun	Mar-21	29	1	30	3.33
			May-21	20	0	20	0.00
			Jun-21	31	0	31	0.00
			Jul-21	54	0	54	0.00
			Aug-21	31	1	32	3.13
			Oct-21	15	0	15	0.00
			Nov-21	24	2	26	7.69
			Dec-21	44	4	48	8.33
			Jan-22	23	2	25	8.00
			Feb-22	32	0	32	0.00
		Largor	Mar-21	1	0	1	0.00
			May-21	13	0	13	0.00
			Jun-21	29	0	29	0.00
			Jul-21	58	0	58	0.00
			Aug-21	62	1	63	1.59
			Sep-21	36	0	36	0.00
			Oct-21	55	0	55	0.00
			Nov-21	38	2	40	5.00
			Dec-21	49	1	50	2.00
			Jan-22	38	2	40	5.00
			Feb-22	37	0	37	0.00
	Bombali	Kamaranka	Nov-21	2	0	2	0.00
			Dec-21	7	0	7	0.00
			Jan-22	13	2	15	13.33
		Masongbo	Aug-21	1	0	1	0.00
			Sep-21	21	1	22	4.55
			Oct-21	34	0	34	0.00
			Nov-21	28	2	30	6.67

Species	District	Village	Month	Negative	Sporozoite positive	Total	% Positive
			Dec-21	16	4	20	20.00
			Jan-22	15	1	16	6.25
	Kono	Sorie Town	Mar-21	5	0	5	0.00
			May-21	1	0	1	0.00
			Jul-21	16	0	16	0.00
			Aug-21	7	0	7	0.00
			Dec-21	9	0	9	0.00
			Jan-22	1	0	1	0.00
			Feb-22	2	0	2	0.00
		Teikor	Mar-21	4	2	2	50.00
			Jun-21	1	0	1	0.00
			Jul-21	9	1	10	10.00
			Aug-21	2	0	2	0.00
			Nov-21	8	0	8	0.00
			Dec-21	12	0	12	0.00
			Jan-22	8	0	8	0.00
			Feb-22	14	0	14	0.00
	Port Loko	New Maforki	May-21	4	0	4	0.00
			Jun-21	47	1	48	2.08
			Jul-21	31	0	31	0.00
			Aug-21	28	0	28	0.00
			Sep-21	2	0	2	0.00
			Nov-21	7	1	8	12.50
			Jan-22	16	0	16	0.00
			Feb-22	12	0	12	0.00
	Western Rural	Sand Sand Water	Oct-21	3	0	3	0.00
		Sand Sand Water Total		3	0	3	0.00
***An. funestus* s.l. Total**				**1108**	**31**	**1135**	2.73
Total all Species				**8787**	**270**	**9053**	2.98

ANNEX I: SPOROZOITE RATE AND EIR OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. IN SIERRA LEONE, MARCH 2021 – FEBRUARY 2022

A. Bo District

		Gerihun											
Indoor	Month	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	240	324	297	284	316	185	400	288	235	86	64	136
	HBR	15	20.25	18.56	17.75	19.75	11.56	25	18	14.69	5.38	4	8.5
	Total tested	199	121	48	35	30	49	64	34	28	11	33	29
	Spz +ve	7	1	0	0	0	2	0	2	2	0	1	0
	Sporozoite rate	0.04	0.01	0.00	0.00	0.00	0.04	0.00	0.06	0.07	0.00	0.03	0.00
	Nightly Indoor EIR	0.53	0.17	0.00	0.00	0.00	0.47	0.00	1.06	1.05	0.00	0.12	0.00
	Monthly EIR	**16.36**	**5.02**	**0.00**	**0.00**	**0.00**	**14.63**	**0.00**	**32.82**	**31.47**	**0.00**	**3.76**	**0.00**
An. funestus s.l.	Total collected	18	8	3	6	5	4	8	10	74	222	101	84
	HBR	1.135	0.5	0.19	0.38	0.31	0.25	0.5	0.63	4.63	13.99	6.31	5.25
	Total tested	17		11	23	33	16		11	13	12	15	16
	Spz +ve	0		0	0	0			0	0	1	2	0
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.13	0.00
	Nightly Indoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.16	0.84	0.00
	Monthly EIR	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**35.8**	**26.1**	**0.0**
	Total monthly Indoor EIR	**16.36**	**5.02**	**0.00**	**0.00**	**0.00**	**14.63**	**0.00**	**32.82**	**31.47**	**35.84**	**29.85**	**0.00**

Outdoor	Month	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	271	437	348	279	293	169	307	226	175	83	73	132
	HBR	16.94	27.31	21.75	17.44	18.31	10.56	19.19	14.12	10.94	5.19	4.56	8.25
	Total tested	182	114	55	50	37	65	60	37	30	27	29	26
	Spz +ve	9	1	1	0	0	0	0	0	0	0	1	0
	Sporozoite rate	0.05	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
	Nightly Outdoor EIR	0.84	0.24	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00
	Month EIR	**25.96**	**7.19**	**12.26**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**4.88**	**0.00**
An. funestus s.l.	Total collected	19	5	0	1	2	7	5	2	55	228	78	43
	HBR	1.19	0.31	0	0.06	0.125	0.4375	0.3125	0.125	3.44	14.25	4.88	2.69
	Total tested	13		9	8	21	16		4	13	36	10	16
	Spz +ve	1		0	0	0	1		0	2	3	0	0
	Sporozoite rate	0.08	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.15	0.08	0.00	0.00

	Nightly Outdoor EIR	0.09	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.53	1.19	0.00	0.00
	Monthly EIR	**2.83**	**0.00**	**0.00**	**0.00**	**0.00**	**0.85**	**0.00**	**0.00**	**15.87**	**36.81**	**0.00**	**0.00**
	Total monthly Outdoor EIR	**28.80**	**7.19**	**12.26**	**0.00**	**0.00**	**0.85**	**0.00**	**0.00**	**15.87**	**36.81**	**4.88**	**0.00**

Largor

Indoor	Month	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
	Total collected	60	180	464	643	359	60	91	28	48	42	54	83
	HBR	3.75	11.25	29	40.19	22.44	3.75	5.69	1.75	3	2.635	3.385	5.19
	Total tested	56	129	52	37	37	49	38	16	21	16	23	29
An. gambiae s.l.	Spz +ve	5	4	1	0	0	3	0	0	1	2	1	0
	Sporozoite rate	0.09	0.03	0.02	0.00	0.00	0.06	0.00	0.00	0.05	0.13	0.04	0.00
	Nightly Indoor EIR	0.33	0.35	0.56	0.00	0.00	0.23	0.00	0.00	0.14	0.33	0.15	0.00
	Monthly EIR	**10.38**	**10.47**	**17.29**	**0.00**	**0.00**	**7.12**	**0.00**	**0.00**	**4.29**	**10.17**	**4.55**	**0.00**
	Total collected	10	11	15	49	71	120	80	48	121	162	52	39
	HBR	0.63	0.69	0.945	3.06	4.44	7.5	5	3	7.56	10.13	3.25	2.44
	Total tested	1		7	17	27	35	22	32	19	25	21	22
An funestus s.l.	Spz +ve	0		0	0	0	1	0	0	1	1	1	0
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.05	0.04	0.05	0.00
	Nightly Indoor EIR	0.00	0.00	0.00	0.00	0.00	0.21	0.00	0.00	0.40	0.41	0.15	0.00
	Monthly EIR	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**6.64**	**0.00**	**0.00**	**11.94**	**12.56**	**4.80**	**0.00**
	Total monthly Indoor EIR	**10.38**	**10.47**	**17.29**	**0.00**	**0.00**	**13.76**	**0.00**	**0.00**	**16.23**	**22.73**	**9.35**	**0.00**

Outdoor	Month	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
	Total collected	97	153	469	649	402	77	73	41	40	30	48	55
	HBR	6.06	9.56	29.31	40.56	25.13	4.81	4.56	2.56	2.5	1.99	3	3.44
	Total tested	72	128	54	50	25	52	49	14	25	21	23	21
An. gambiae s.l.	Spz +ve	10	4	0	1	0	2	0	0	0	1	2	0
	Sporozoite rate	0.14	0.03	0.00	0.02	0.00	0.04	0.00	0.00	0.00	0.05	0.09	0.00
	Nightly Outdoor EIR	0.84	0.30	0.00	0.81	0.00	0.19	0.00	0.00	0.00	0.09	0.26	0.00
	Monthly EIR	**26.10**	**8.96**	**0.00**	**24.34**	**0.00**	**5.74**	**0.00**	**0.00**	**0.00**	**2.77**	**8.09**	**0.00**

Indoor	Month	Largor											
		Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. funestus s.l.	Total collected	8	5	4	31	75	91	50	55	130	195	51	29
	HBR	0.5	0.31	0.25	1.94	4.69	5.69	3.13	3.44	8.13	12.19	3.19	1.81
	Total tested			6	12	31	28	14	23	21	25	19	15
	Spz +ve			0	0	0	0	0	0	1	0	1	0
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.05	0.00
	Nightly Outdoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.00	0.17	0.00
	Monthly EIR	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**11.61**	**0.00**	**5.20**	**0.00**
	Total monthly Outdoor EIR	**26.10**	**8.96**	**0.00**	**24.34**	**0.00**	**5.74**	**0.00**	**0.00**	**11.61**	**2.77**	**13.29**	**0.00**

B. Bombali District

Indoor	**month**	**Kamaranka**											
		Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	128	169	250	1130	198	27	39	31	38	5	22	303
	HBR	8	10.56	15.63	70.63	12.38	1.69	2.44	1.94	2.38	0.31	1.38	18.94
	Total tested	95	87	67	66	48	43	36	48	41	10	24	33
	Spz +ve	1	4	6	0	0	1	3	2	2	1	0	0
	Sporozoite rate	0.01	0.05	0.09	0.00	0.00	0.02	0.08	0.04	0.05	0.10	0.00	0.00
	Nightly Indoor EIR	0.08	0.49	1.40	0.00	0.00	0.04	0.20	0.08	0.12	0.03	0.00	0.00
	Monthly EIR	**2.61**	**14.57**	**43.38**	**0.00**	**0.00**	**1.22**	**6.09**	**2.50**	**3.48**	**0.97**	**0.00**	**0.00**
An. funestus s.l.	Total collected	0	0	0	0	0	0	0	0	2	4	2	0
	HBR	0	0	0	0	0	0	0	0	0.125	0.25	0.125	0
	Total tested									2	4	10	23
	Spz +ve									0	0	2	0
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00
	Nightly Indoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
	Monthly EIR	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.775**	**0**
	Total Monthly Indoor EIR	**2.61**	**14.57**	**43.38**	**0.00**	**0.00**	**1.22**	**6.09**	**2.50**	**3.48**	**0.97**	**0.78**	**0.00**
Outdoor	**month**	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	104	129	203	943	201	40	52	30	32	8	28	184
	HBR	6.5	8.06	12.69	58.94	12.56	2.5	3.25	1.88	2	0.5	1.75	11.5
	Total tested	93	82	55	51	64	51	59	31	43	20	36	54
	Spz +ve	3	5	1	0	2	0	1	2	2	0	0	0

		Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
	Kamaranka												
	Sporozoite rate	0.03	0.06	0.02	0.00	0.03	0.00	0.02	0.06	0.05	0.00	0.00	0.00
	Nightly Outdoor EIR	0.21	0.49	0.23	0.00	0.39	0.00	0.06	0.12	0.09	0.00	0.00	0.00
	Monthly EIR	**6.5**	**14.75**	**7.15**	**0**	**12.17**	**0**	**1.65**	**3.75**	**2.79**	**0**	**0**	**0**
An. funestus s.l.	Total collected	0	0	0	0	0	0	0	0	0	3	5	
	HBR	0			0	0	0.00	0.00	0.00	0.00	0.00	0.13	0.00
	Total tested										3	5	
	Spz +ve										0	0	
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Nightly Outdoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Monthly EIR	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Total monthly Outdoor EIR	**6.50**	**14.75**	**7.15**	**0.00**	**12.17**	**0.00**	**1.65**	**3.75**	**2.79**	**0.00**	**0.00**	**0.00**

Indoor	**Masongbo**												
	Month	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	61	124	188	1109	326	159	163	119	120	65	21	8
	HBR	3.81	7.75	11.75	69.31	20.38	9.94	10.19	7.44	7.50	4.06	1.31	0.50
	Total tested	100	89	60	67	54	72	46	34	33	31	37	16
	Spz +ve	1	2	3	0	0	1	1	0	1	2	2	0
	Sporozoite rate	0.01	0.02	0.05	0.00	0.00	0.01	0.02	0.00	0.03	0.06	0.05	0.00
	Nightly Indoor EIR	0.04	0.17	0.59	0.00	0.00	0.14	0.22	0.00	0.23	0.26	0.07	0.00
	Monthly EIR	**1.18**	**5.22**	**18.21**	**0.00**	**0.00**	**4.28**	**6.64**	**0.00**	**6.82**	**8.13**	**2.20**	**0.00**
An. funestus s.l.	Total collected	0	0	0	0	0	3	11	26	30	57	4	0
	HBR	0			0	0.00	0.19	0.69	1.63	1.88	3.56	0.25	0.00
	Total tested						1	10	26	13	12	4	
	Spz +ve						0	0	0	0	2	1	
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.25	0.00
	Nightly Indoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.59	0.06	0.00
	Month EIR	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**18.41**	**1.94**	**0.00**
	Total Monthly Indoor EIR	**1.18**	**5.22**	**18.21**	**0.00**	**0.00**	**4.28**	**6.64**	**0.00**	**6.82**	**26.53**	**4.14**	**0.00**

Indoor		Masongbo											
	Month	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
		Masongbo											
Outdoor	Month	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	79	97	246	1289	410	181	284	177	160	77	22	2
	HBR	4.94	6.06	15.38	80.56	25.63	11.31	17.75	11.06	10.00	4.81	1.38	0.13
	Total tested	66	85	63	48	50	81	30	46	23	33	31	5
	Spz +ve	1	1	0	0	1	5	2	3	0	6	1	0
	Sporozoite rate	0.02	0.01	0.00	0.00	0.02	0.06	0.07	0.07	0.00	0.18	0.03	0.00
	Nightly Outdoor EIR	0.07	0.07	0.00	0.00	0.51	0.70	1.18	0.72	0.00	0.88	0.04	0.00
	Monthly EIR	**2.32**	**2.14**	**0.00**	**0.00**	**15.89**	**21.65**	**35.50**	**22.37**	**0.00**	**27.13**	**1.38**	**0.00**
An. funestus s.l.	Total collected	0	0	0	0	0	2	20	31	41	70	12	0
	HBR	0			0	0.00	0.13	1.25	1.94	2.56	4.38	0.75	0.00
	Total tested							12	8	17	8	12	
	Spz +ve							1	0	2	2	0	
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.12	0.25	0.00	0.00
	Nightly Outdoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.30	1.09	0.00	0.00
	Month EIR	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**3.13**	**0.00**	**9.04**	**33.91**	**0.00**	**0.00**
	Total Monthly Outdoor EIR	**2.32**	**2.14**	**0.00**	**0.00**	**15.89**	**21.65**	**38.63**	**22.37**	**9.04**	**61.03**	**1.38**	**0.00**

C. Kono District

		Sorie Town											
Indoor	**Month**	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	25	53	46	96	9	26	11	18	2	16	14	11
	HBR	6.25	13.25	11.5	24	2.25	6.5	2.75	4.5	0.5	4	3.5	2.75
	Total tested	9	19	23	29	11	25	11	15	2	17	14	11
	Spz +ve	0	0	1	0	0	0	0	0	0	0	0	0
	Sporozoite rate	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Nightly indoor EIR	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Monthly EIR	**0.00**	**0.00**	**15.50**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
An. funestus s.l.	Total collected	2	0	0	0	11	0	0	0	0	6	1	1
	HBR	0.5	0	0	0	2.75	0	0	0	0	1	0.25	0.25

	Total tested	2				9	1				6	1	1
	Spz +ve	0				0	0				0	0	0
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Nightly Indoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Monthly Indoor EIR	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Total Monthly Indoor EIR	**0.00**	**0.00**	**15.50**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

							Sorie Town						
Outdoor	**Month**	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	30	39	62	88	8	14	11	15	1	16	8	10
	HBR	7.5	9.75	15.5	22	2	3.5	2.75	3.75	0.25	4	2	2.5
	Total tested	4	19	29	24	6	14	11	16	1	16	10	10
	Spz +ve	0	0	1	0	0	0	1	0	0	0	1	0
	Sporozoite rate	0.00	0.00	0.03	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.10	0.00
	Nightly Outdoor EIR	0.00	0.00	0.53	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.20	0.00
	Monthly EIR	**0.00**	**0.00**	**16.57**	**0.00**	**0.00**	**0.00**	**7.50**	**0.00**	**0.00**	**0.00**	**6.20**	**0.00**
An. funestus s.l.	Total collected	6	0	1	0	7	6	0	0	0	4	2	1
	HBR	1.5	0	0.25	0	1.5	1	0	0	0	1	0.5	0.25
	Total tested	3		1		7	6				3		1
	Spz +ve	0		0		0	0				0		0
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Nightly Outdoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Monthly Outdoor EIR	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Total Monthly Outdoor EIR	**0.00**	**0.00**	**16.57**	**0.00**	**0.00**	**0.00**	**7.50**	**0.00**	**0.00**	**0.00**	**6.20**	**0.00**

	Site						**Teikor**						
Indoor	**Month**	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	17	102	83	110	32	34	46	24	6	11	5	5
	HBR	4.25	25.5	20.75	27.5	8	8.5	11.5	6	1.5	2.75	1.25	1.25
	Total tested	24	25	26	29	33	31	22	6	11	5	5	9
	Spz +ve	1	0	0	5	0	1	3	0	0	0	0	0
	Sporozoite rate	0.04	0.00	0.00	0.17	0.00	0.03	0.14	0.00	0.00	0.00	0.00	0.00
	Nightly Indoor EIR	0.18	0.00	0.00	4.74	0.00	0.27	1.57	0.00	0.00	0.00	0.00	0.00

	Monthly EIR	**5.49**	**0.00**	**0.00**	**142.24**	**0.00**	**8.50**	**47.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
An. funestus s.l.	Total collected	4	0	2	4	9	0	0	2	1	7	4	8
	HBR	1	0	0.5	1	2.25	0	0	0.5	0.25	1.75	1	2
	Total tested	2			1	9				1	7	4	8
	Spz +ve	0			0	0				0	0	0	0
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Nightly indoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Month EIR	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Total Monthly Indoor EIR	**5.49**	**0.00**	**0.00**	**142.24**	**0.00**	**8.50**	**47.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

	Site	**Teikor**											
Outdoor	**Month**	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	17	101	81	93	17	17	33	26	11	5	16	7
	HBR	4.25	25.25	20.25	23.25	4.25	4.25	8.25	6.5	2.75	1.25	4	1.75
	Total tested		26	25	25	14	16	22	24	12	5	16	7
	Spz +ve		0	0	1	1	0	1	0	0	0	0	0
	Sporozoite rate	0.00	0.00	0.00	0.04	0.07	0.00	0.05	0.00	0.00	0.00	0.00	0.00
	Nightly outdoor EIR	0.00	0.00	0.00	0.93	0.30	0.00	0.38	0.00	0.00	0.00	0.00	0.00
	Monthly EIR	**0.00**	**0.00**	**0.00**	**27.90**	**9.41**	**0.00**	**11.25**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
An. funestus s.l.	Total collected	3	2	0	4	1	2	0	0	8	5	4	7
	HBR	0.75	0.5	0	1	0.25	0.5	0	0	2	1.25	1	1.75
	Total tested	2				1	2			7	5	4	6
	Spz +ve	2				1	0			0	0	0	0
	Sporozoite rate	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Nightly Outdoor EIR	0.75	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Month EIR	**23.25**	**0**	**0**	**0**	**7.75**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Total Monthly Outdoor EIR	**23.25**	**0.00**	**0.00**	**27.90**	**17.16**	**0.00**	**11.25**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

D. Port Loko

	Site	**Bakolo**											
	Month	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
Indoor	Total collected	16	67	13	130	37	55	6	1	3	4	0	0

	HBR	1	4.1875	0.8125	8.125	2.3125	3.4375	0.375	0.0625	0.1875	0.25	0	0
	Total tested	3	55	9	66	49	36	19		3	5		
	Spz +ve	0	0	0	1	2	0	0		0	0		
	Sporozoite rate	0.00	0.00	0.00	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Nightly Indoor EIR	0.00	0.00	0.00	0.12	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Monthly Indoor EIR	**0.00**	**0.00**	**0.00**	**3.69**	**2.93**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Outdoor	Total collected	4	34	6	97	117	15	14	1	7	4	0	0
	HBR	0.25	2.125	0.375	6.0625	7.3125	0.9375	0.875	0.0625	0.4375	0.25	0	0
	Total tested	3	56	12	50	35	50	39	2	7	3	0	0
	Spz +ve	0	0	0	2	1	0	2	0	0	0	0	0
	Sporozoite rate	0.00	0.00	0.00	0.04	0.03	0.00	0.05	0.00	0.00	0.00	0.00	0.00
	Nightly Outdoor EIR	0.00	0.00	0.00	0.24	0.21	0.00	0.04	0.00	0.00	0.00	0.00	0.00
	Monthly EIR	**0.00**	**0.00**	**0.00**	**7.28**	**6.48**	**0.00**	**1.35**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

	Site	**New Maforki**											
Indoor	**Month**	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	14	42	102	329	215	62	115	86	56	20	3	10
	HBR	0.875	2.625	6.375	20.5625	13.4375	3.875	7.1875	5.375	3.5	1.25	0.1875	0.625
	Total tested	7	51	67	48	43	30	67	41	31	37	8	22
	Spz +ve	0	0	0	0	2	0	5	3	1	1	0	0
	Sporozoite rate	0.00	0.00	0.00	0.00	0.05	0.00	0.07	0.07	0.03	0.03	0.00	0.00
	Nightly Indoor EIR	0.00	0.00	0.00	0.00	0.63	0.00	0.54	0.39	0.11	0.03	0.00	0.00
	Monthly EIR	**0.00**	**0.00**	**0.00**	**0.00**	**19.38**	**0.00**	**16.09**	**12.19**	**3.39**	**1.05**	**0.00**	**0.00**
An. funestus s.l.	Total collected	0	1	5	20	27	8	0	0	3	2	3	6
	HBR	0	0.0625	0.3125	1.25	1.6875	0.5	0	0	0.1875	0.125	0.1875	0.375
	Total tested			3	20	10	8	2		3		2	6
	Spz +ve			0	0	0	0	0		0		0	0
	Sporozoite rate	0	0	0	0	0	0	0	0	0	0	0	0
	Nightly Indoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Monthly EIR	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Total Monthly Indoor EIR	**0.00**	**0.00**	**0.00**	**0.00**	**19.38**	**0.00**	**16.09**	**12.19**	**3.39**	**1.05**	**0.00**	**0.00**

	Site	**New Maforki**											
Outdoor	**Month**	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	20	54	105	343	353	147	154	80	97	46	6	30

	HBR	1.25	3.38	6.56	21.44	22.06	9.19	9.63	5.00	6.06	2.88	0.38	1.88
	Total tested	3	54	65	20	47	29	55	46	48	51	10	52
	Spz +ve	0	1	1	1	1	0	0	6	3	3	0	0
	Sporozoite rate	0.00	0.02	0.02	0.05	0.02	0.00	0.00	0.13	0.06	0.06	0.00	0.00
	Nightly Outdoor EIR	0.00	0.06	0.10	1.07	0.47	0.00	0.00	0.65	0.38	0.17	0.00	0.00
	Monthly EIR	**0.00**	**1.88**	**3.13**	**32.16**	**14.55**	**0.00**	**0.00**	**20.22**	**11.37**	**5.24**	**0.00**	**0.00**
An. funestus s.l.	Total collected	0	1	1	28	24	20	0	1	7	1	14	6
	HBR	0.00	0.06	0.06	1.75	1.50	1.25	0.00	0.06	0.44	0.06	0.88	0.38
	Total tested			1	28	21	20			5		14	6
	Spz +ve			0	1	0	0			1		0	0
	Sporozoite rate	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00
	Nightly Outdoor EIR	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00
	Monthly EIR	**0.00**	**0.00**	**0.00**	**1.88**	**0.00**	**0.00**	**0.00**	**0.00**	**2.63**	**0.00**	**0.00**	**0.00**
	Total Monthly Outdoor EIR	**0.00**	**1.88**	**3.13**	**34.03**	**14.55**	**0.00**	**0.00**	**20.22**	**13.99**	**5.24**	**0.00**	**0.00**

E. Western Area District

	Site						**Sand Sand Water**						
Indoor	**Month**	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	28	24	45	49	72	60	19	9	20	21	16	17
	HBR	7	6	11.25	12.25	18	15	4.75	2.25	5	5.25	4	4.25
	total tested	24	20	30	27	26	49	19	9	19	21	14	19
	Spz +ve	5	0	0	2	1	2	0	2	1	1	1	0
	Sporozoite rate	0.21	0.00	0.00	0.07	0.04	0.04	0.00	0.22	0.05	0.05	0.07	0.00
	Nightly Indoor EIR	1.46	0.00	0.00	0.91	0.69	0.61	0.00	0.50	0.26	0.25	0.29	0.00
	Monthly EIR	**45.21**	**0.00**	**0.00**	**27.22**	**21.46**	**18.98**	**0.00**	**15.50**	**7.89**	**7.75**	**8.86**	**0.00**
An. funestus s.l.	Total collected	0	0	0	0	0	0	0	1	1	0	0	0
	HBR	0			0	0	0	0	0.25	0.25	0	0	0
	Total tested								1				
	Spz +ve								0				
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Nightly Indoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Monthly EIR	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

	Total Monthly Indoor EIR	**45.21**	**0.00**	**0.00**	**27.22**	**21.46**	**18.98**	**0.00**	**15.50**	**7.89**	**7.75**	**8.86**	**0.00**

	Site						**Sand Sand Water**						
Outdoor	**Month**	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
An. gambiae s.l.	Total collected	27	14	28	61	54	34	15	14	11	26	55	8
	HBR	6.75	3.5	7	15.25	13.5	8.5	3.75	3.5	2.75	6.5	13.75	2
	total tested	22	12	25	26	22	29	15	14	11	26	45	8
	Spz +ve	1	0	0	2	2	1	3	0	1	2	8	0
	Sporozoite rate	0.05	0.00	0.00	0.08	0.09	0.03	0.20	0.00	0.09	0.08	0.18	0.00
	Nightly Outdoor EIR	0.31	0.00	0.00	1.17	1.23	0.29	0.75	0.00	0.25	0.50	2.44	0.00
	Monthly EIR	**9.51**	**0.00**	**0.00**	**35.19**	**38.05**	**9.09**	**22.50**	**0.00**	**7.50**	**15.50**	**75.78**	**0.00**
An. funestus s.l.	Total collected	0	0	0	0	0	0	0	2	0	0	0	0
	HBR	0	0	0	0	0	0	0	0.5	0	0	0	0
	Total tested								2				
	Spz +ve								0				
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Nightly Outdoor EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Monthly EIR	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Total Monthly Outdoor EIR	**9.51**	**0.00**	**0.00**	**35.19**	**38.05**	**9.09**	**22.50**	**0.00**	**7.50**	**15.50**	**75.78**	**0.00**

	Site	**Tombo**											
	Month	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22
Indoor	Total collected	21	4	26	60	27	24	35	3	9	5	6	4
	HBR	5.25	1	6.5	15	6.75	6	8.75	0.75	2.25	1.25	1.5	1
	Total tested	9	10	13	30	22	24	30	3	9	5	6	4
	Spz +ve	0	0	0	1	3	1	5	0	1	0	0	0
	sporozoite rate	0.00	0.00	0.00	0.03	0.14	0.04	0.17	0.00	0.11	0.00	0.00	0.00
	Nightly Indoor EIR	0.00	0.00	0.00	0.50	0.92	0.25	1.46	0.00	0.25	0.00	0.00	0.00
	Monthly EIR	**0.00**	**0.00**	**0.00**	**15.00**	**28.53**	**7.75**	**43.75**	**0.00**	**7.50**	**0.00**	**0.00**	**0.00**
Outdoor	Total collected	20	16	27	88	61	7	25	8	8	3	7	7
	HBR	5	4	6.75	22	15.25	1.75	6.25	2	2	0.75	1.75	1.75
	Total tested	17			28	67	36	17	17	11	8	6	5
	Spz +ve	2			2	2	0	1	2	2	1	0	1
	Sporozoite rate	0.12	0.00	0.00	0.07	0.03	0.00	0.06	0.12	0.18	0.13	0.00	0.20

	EIR	Nightly Outdoor	0.59	0.00	0.00	1.57	0.46	0.00	0.37	0.24	0.36	0.09	0.00	0.35
		Monthly EIR	**18.24**	**0.00**	**0.00**	**47.14**	**14.11**	**0.00**	**11.03**	**7.29**	**10.91**	**2.91**	**0.00**	**9.80**

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