U.S. PRESIDENT'S MALARIA INITIATIVE

THE PMI VECTORLINK SIERRA LEONE 2022 ANNUAL ENTOMOLOGICAL MONITORING REPORT

MARCH 1, 2022 – FEBRUARY 28, 2023

Recommended Citation: The PMI VectorLink Project. June 2023. *Sierra Leone 2022 Annual Entomological Monitoring Report, March 1, 2022 - February 28, 2023*. Rockville, MD. The PMI VectorLink Project, Abt Associates Inc.

Contract: AID-OAA-I-17-00008

Task Order: AID-OAA-TO-17-00027

Submitted to: United States Agency for International Development/PMI

Submitted on: June 01, 2023

Approved on: July 17, 2023

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THE PMI VECTORLINK
SIERRA LEONE
2022 ANNUAL ENTOMOLOGICAL
MONITORING REPORT

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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
gDNA	Genomic DNA
HBR	Human Biting Rate
HLC	Human Landing Collection
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 Collection
USAID	United States Agency for International Development
VBDIL	Vector Borne Disease Insectary and Laboratory
VCNA	Vector Control Needs Assessment
VLC	VectorLink Collect Database
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 piperonyl butoxide (PBO) ITNs (Olyset Plus and PermaNet 3.0) countrywide. In May 2021, the NMCP with PMI support, introduced and implemented IRS with the clothianidin insecticide SumiShield™ in Bo and Bombali districts. After three consecutive years of spraying SumiShield™ in the same districts in Sierra Leone, the project is currently considering an insecticide rotation according to the Insecticide Resistance Monitoring and Management Plan (IRMMP) to minimize development and spread of insecticide resistance. To inform the decision on which insecticide to be selected for the 2024 IRS campaign, NMCP through VectorLink is piloting the pirimiphos-methyl insecticide Actellic in two sites (one in Bo and one in Bombali) to generate residual efficacy data.

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 2022 and February 2023, 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 was tested. Between July 2020 and April 2022, the country investigated the impact of co-deployment of PBO ITNs and IRS on malaria incidence and entomological indicators of transmission. This investigation was done in two intervention districts (Bo and Bombali) where IRS was implemented, as well as in 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 residual efficacy of SumiShield™, which was sprayed in Bombali and Bo districts during the 2022 IRS campaign.

Vector Bionomics: *An. gambiae* s.l. was the predominant vector representing 81.8% (13,630) of all mosquitoes collected, followed by *An. funestus* group 17.6% (2,941), *An. coustani* 0.6% (94), and others 0.0% (5). The proportion of *An. funestus* s.l. collected using pyrethrum spray collection (PSC) (47.3%) was higher than the proportions collected from human landing collection (HLC) (44.3%) and Centers for Disease Control and Prevention-light trap (CDC-LT) (8.5%), indicating a more endophilic behavior of this mosquito. Mosquito density, both for *An. gambiae* s.l. and *An. funestus* s.l. was highest in the IRS districts of Bo and Bombali compared to other districts consistent with previous years' reports. In Bo District, the mean indoor resting density (IRD) was highest in June in Largor rural site with 10.4/house/day, a reduction from previous year with 14.9/house/day, which was also the highest IRD recorded. In Bombali District, the highest IRD was recorded in June in Kamaranka rural site with 5.6/house/day, representing an increase from the previous year with 4.3/house/day.

Human Biting Rate (HBR) and Location: The mean biting density varied by site, district, and month of collection. The HBR in IRS sites continued to decline compared to previous years, although the average HBR still remained high in IRS districts compared to non-IRS districts. The highest indoor HBR was recorded in June in Largor, Bo district with 49 bites/person/night compared to the previous year where the highest HBR was recorded in Masongbo, Bombali district with 80.6 bites/person/night. In Bombali, the highest HBR was recorded in June in Masongbo (29.3 bites/person/night). In all districts, the HBR followed the rainfall pattern with a peak observed between June and September and declining into the dry season. There was no clear outdoor/indoor biting pattern in both *An. gambiae* s.l. and *An. funestus* s.l. in all sites except Masongbo in Bombali district and New Maforki in Port Loko district where more outdoor biting was observed. 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 CDC-LT, also varied by site, district, and month, and followed the rainfall patterns in Sierra Leone. The highest average density of *An. gambiae* s.l./trap/night was recorded in June in the non-IRS site of Bakolo, Port Loko district (12.4/trap/night). In the IRS districts, the highest density was recorded in Kamaranka rural site, Bombali district with 8.4 *An. gambiae* s.l./trap/night, a reduction from the 2021-2022 period (21.4/trap/night) and 2020-2021 period (34.4/trap/night). Both IRS districts report great reduction in average density per trap with density averaging less than 3/trap/night. For *An. funestus* s.l., the highest average density was recorded in December in Masongbo peri-urban site, Bombali district with 2.9/trap/night followed by Largor (Bo district) in May with 2.6/trap/night.

Molecular Identification: Species identification using polymerase chain reaction (PCR) was successfully conducted on a total of 2,552 out of 2,603 (98%) *An. gambiae* s.l. sent for molecular species identification; a total of 51 (2%) failed to amplify for *An. gambiae* species ID PCR and will be screened using *An. stephensi* PCR ID; *An. gambiae* s.s. were predominant at 60.07% (1,533/2,552), followed by *An. coluzzii* at 39.85% (1,017/2,552), two hybrid of *An. gambiae* s.s./*An. coluzzii* at 0.08% (2/2,552). This distribution is consistent with the species distribution in the previous year where *An. gambiae* s.s. was predominant. Molecular identification was also successfully conducted on 262 out of 266 (98.5%) *An. funestus* group sent to the laboratory for molecular identification; 98.5% (258/262) were identified as *An. funestus* s.s. and 1.5% (4/262) as *An. leesoni*.

Parity Rate: The majority of malaria vectors collected during the sampling period had laid eggs at least once (parous) with 85.8% (1,296/1,503), while 14.2% (214/1,503) 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 the rainy season. In all the districts, apart from New Maforki in Port Loko, there was no clear pattern in the proportion of parous mosquitoes following the rainfall season.

Abdominal Condition of Indoor Resting Collections: Over 80% of both *An. gambiae* s.l. and *An. funestus* group in Sierra Leone collected using PSC had fed on a host. This high bloodmeal access has remained high for the past four years. Given they were collected inside sleeping and living rooms, it is expected that these were fed on humans. Additional analysis to identify the source of bloodmeal is ongoing and will be reported in the addendum report.

Insecticide Resistance: *An. gambiae* s.l. are resistant to permethrin, deltamethrin and alpha-cypermethrin with very low mortalities ranging from 2% in deltamethrin-only to 36% in alpha-cypermethrin-only exposures. However, PBO was able to restore partial susceptibility by 38-72% to alpha-cypermethrin, by 49-72% to deltamethrin and by 2-41% to permethrin, indicating that mono-oxygenases are partially involved in conferring pyrethroid resistance in Sierra Leone. There was susceptibility to pirimiphos-methyl apart from Port Loko and Western Area Rural; however, the vectors were fully susceptible to chlorfenapyr at all sites. The team also observed an emerging resistance to clothianidin with an average mortality of 89.4% ranging from 81% in Port Loko district to 100% in Kono district. Molecular markers of insecticide resistance were observed at various frequencies; *kdr-w* mutation was fixed or approaching fixation (>80%), *kdr-e* (0.4) *Ace-1*(G119S) 2.2% and N1575Y 1.1%.

Decay Rate of IRS Insecticide: SumiShield™ remained effective against laboratory strain (*An. gambiae* s.s. Kisumu) mosquitoes, 10 months after the second spray campaign in May 2022, indicating long residual effect of the insecticide.

Conclusion: Malaria vectors in Sierra Leone 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. Greater reduction in entomological indicators have been observed in IRS sites, highlighting the contribution of IRS in vector control 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 the *An. funestus* group and partially maintained by dry season irrigation farming, was observed in December in IRS sites. The full susceptibility of mosquitoes to chlorfenapyr indicates that deploying interceptor G2 (IG2) nets in the 2023 mass ITN distribution could also be effective in controlling the pyrethroid resistant mosquitoes. SumiShield™ is effective for IRS in Sierra Leone and has been used for the third time during the April 2023 campaign; therefore there is need for an insecticide rotation in line with the IRMMP. Malaria vectors still have access to bloodmeal, and NMCP could benefit from innovative behavior change activities and communication such as those used during the pilot of school-based ITN distribution in Kono whereby the highest response rate to radio messaging by the community was recorded. The numbers of collected mosquitoes by the three trapping methods 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. There was a reduction in frequency of *Ace-1* mutation compared to 2021 and robust larval sampling should be carried out to represent true frequency of resistance alleles to better guide vector control decision making.

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. The most recent ITN mass campaign distributed the synergist piperonyl butoxide (PBO)-permethrin nets (Olyset Plus) and PBO-deltamethrin nets (PermaNet 3.0) in May -June 2020. In May 2021, the Sierra Leone NMCP, in collaboration with PMI, introduced indoor residual spraying (IRS) with SumiShield™ (clothianidin) in the districts of Bo and Bombali after the initial pilot led by NMCP that took place in 2011-2012 in selected chiefdoms in four districts (Bo, Bombali, Kono and Western Rural Area). In May 2022, the second round of IRS with SumiShield™ was carried out in the same districts while the third round was implemented earlier in April 2023. The April spray date does not impact the effectiveness of the IRS intervention because the rainy season in Sierra Leone begins in May and residual efficacy data of SumiShield™ indicates that it remains effective on walls up to 10 months (Annual Sierra Leone Entomology Report 2021) post-IRS. In 2021, malaria prevalence in Bo had reduced from 38% to 25.4% and from 40% to 12.8% in Bombali, indicating progress in malaria reduction because of PBO ITN and IRS use in those districts (*MIS 2016, MIS 2021*). Evaluation of the impact of PBO-ITN and IRS with clothianidin in Bo and Bombali is still underway and further analysis will shed more light on the effect of each intervention in malaria control in Sierra Leone.

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 and has supported the establishment and maintenance of two insectaries (one in Freetown and another in Makeni -Vector Borne Disease Insectary and Laboratory (VBDIL) in Bombali District).

Between March 2022 and February 2023, 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 in the North West(to take into account another 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 project also monitored residual efficacy of SumiShield™ insecticide used in IRS.

The current report covers monthly entomological surveillance in the five districts (Bo, Bombali, Port Loko, Kono and Western Area Rural; Figure 1). Insecticide resistance testing with pyrethroids with and without the synergist PBO, pirimiphos-methyl, clothianidin and chlorfenapyr was also conducted in selected sites. Capacity strengthening activities to improve entomological capacity in Sierra Leone was also undertaken.

The objectives of the 2022/2023 entomological monitoring plan were to:

- Understand malaria vector bionomics, densities, and behavior in all districts.
- Assess the impact of vector control interventions.

- 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 in Bombali and one in 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 IRMMP.

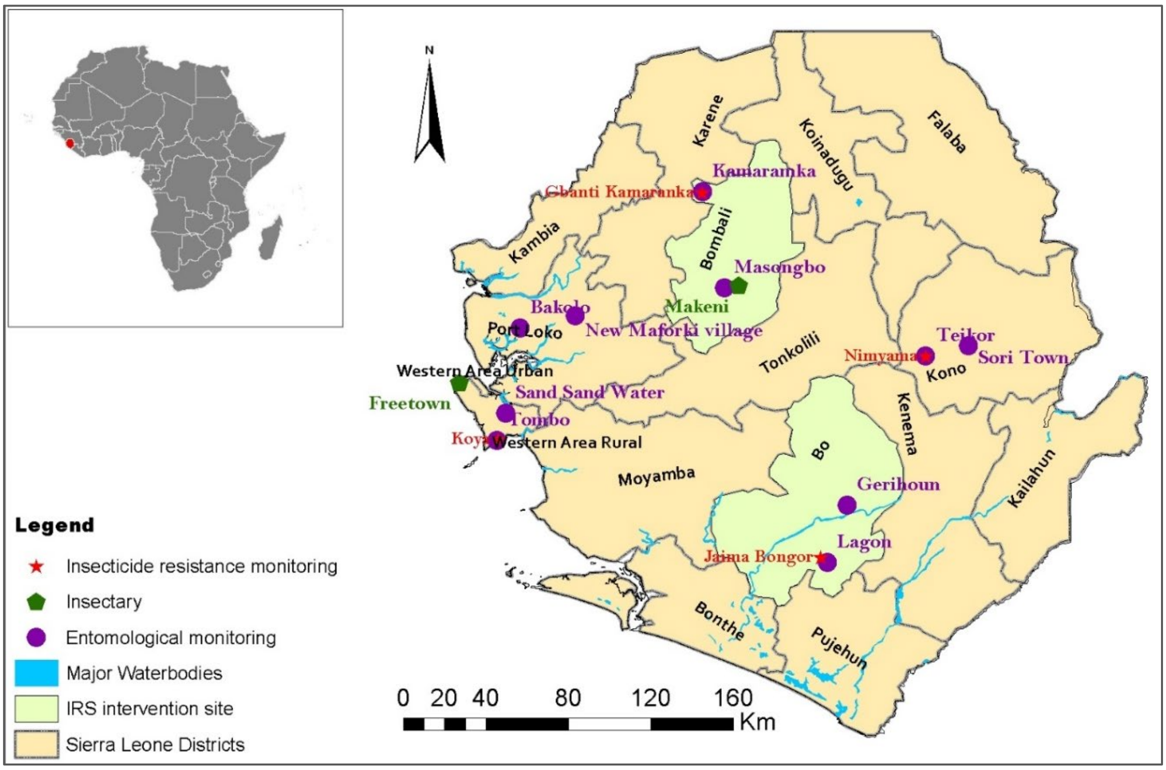
2. METHODOLOGY

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 2022 to February 2023 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 was conducted with pyrethrum spray collections (PSCs), human landing collections (HLCs), and U.S. Centers for Disease Control and Prevention (CDC) light traps, and insecticide resistance testing, and monitoring of the decay rate of SumiShield™ was performed using VectorLink standard operating procedures (SOPs[1]).

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		IRS Site
					Vector Bionomic Monitoring (PSC, CDC LT, and HLC)	Vector Insecticide Resistance	
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): PSC, CDC-LT and HLC. Outdoor resting collections were piloted in Bombali and Port Loko districts between September and November.

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 data collected from the rural sites were compared to data from peri-urban sites and the difference in IRD between IRS and non-IRS sites assessed.

HLC Collections: Monthly HLCs were also performed according to VL SOP02/01. Mosquito collections were conducted in two houses per site in the five routine districts (Bo, Bombali, Kono, Western Rural Area and Port Loko). 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.

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. 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 for extraction of ovaries, which were 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 beginning in May 2021.

2.3.1 WHO Tube Tests

The project team performed insecticide susceptibility tests using the WHO tube method (SOP06/01), in Bo, Bombali, Port Loko, Kono, 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 alpha-cypermethrin 0.05%, deltamethrin 0.05% and permethrin 0.75% in all the districts.

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

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).

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.

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 second 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 2022 to February 2023 using wall cone assays according to VL SOP009/01. The assessment was done on twenty houses of different wall types, six mud and four cement, selected based on the most common wall type available in the intervention areas. The twenty houses sprayed with SumiShield™ were tested every month. 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, 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 cone assays in houses 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.

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). 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.

2.6 ANALYSIS AND MOLECULAR EVALUATIONS

In December 2022, the NMCP and Njala University signed an MoU to allow molecular processing of mosquitoes in country. Njala University was then tasked to process samples collected in the second half of the workplan year while CRID was to process those collected in the first half. A Subset of collected samples between March 2022 and August 2022 were shipped to CRID in Cameroon while a second subset of samples collected between September 2022 - February 2023 were shipped to Njala University in Sierra Leone 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* group 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 (VLC), 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 16,670 *Anopheles* mosquitoes were collected during the monthly collections conducted from March 2022 to February 2023 using PSC, HLC, and CDC light traps in both rural and peri-urban sites in five districts. The HLC sampled more mosquitoes, 66% (11,026/16,670), compared to PSC, 24% (3,989/16,670) and CDC-LT ,10% (1,655/16,670) (Annex A-D). Of the *Anopheles* mosquitoes collected, *An. gambiae* s.l. was the predominant vector with 81.8% (13,630), followed by *An. funestus* group 17.6% (2,941), *An. coustani* 0.6% (94), and others 0.0% (5) (Annex A). The proportion of *An. funestus* s.l. collected using PSC (47.3%) was higher than the proportions collected from HLC (44.3%) and CDC-LT (8.5%), indicating the more endophilic behavior of this mosquito compared to *An. gambiae* s.l. (Annex A-D). The HLC sampled more *An. gambiae* s.l. compared to PSC and CDC-LT. Total number of mosquitoes collected, mosquito density, both for *An. gambiae* s.l. and *An. funestus* s.l. was highest in the IRS districts of Bo and Bombali compared to other districts (Annex A-D) consistent with previous years reports.

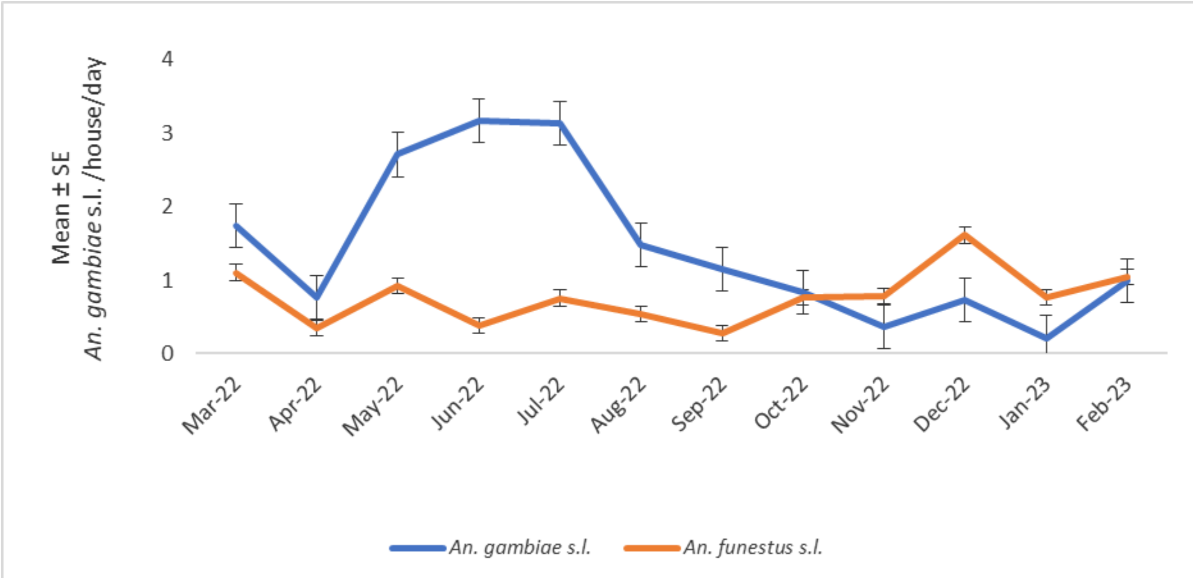
Detailed numbers collected by each sampling tool are presented in Annexes A-D.

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* s.l., a smaller peak was observed in December (Figure 2). In April 2022, at the tail end of the dry season, there was a reduction in mean resting density in all sites, which rose again from May with the onset of the rainy season (Figure 2-4)

Figure 2: Mean IRD of *An. gambiae* s.l. and *An. funestus* s.l. Across All Sentinel Sites, March 2022–February 2023



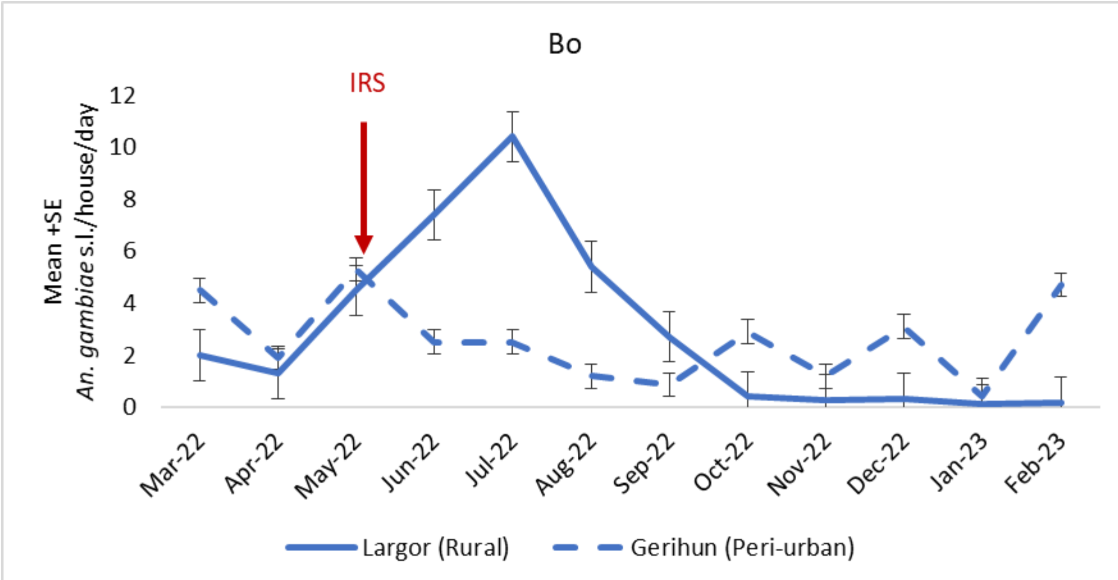
The IRD of *An. gambiae* s.l. per house per day between March 2022 and February 2023 varied across village, district, and month (Figures 3 and 4). The mean IRD in all sites peaked between June and September except in Sorie Town, Kono district where peak IRD was recorded in March 2022.

In Bo District, the mean IRD was highest in July in Largor rural site with 10.4/house/day, a reduction from the previous year with (14.9/house/day) (Figure 8), which was also the highest IRD recorded. In Bombali district, the highest IRD was recorded in June in Kamaranka rural site (5.6/house/day), an increase from 4.3/house/day the previous year. During the rainy season, the mean resting density in rural site in Bo was highest compared to peri-urban site but was higher in peri-urban site during the dry season (Figure 3). In Bombali, there was no clear difference in mean resting density between peri-urban and rural sites (Figure 3). Density peaked in June following the rains and the timing of IRS was timed in May before the high mosquito season.

In Port Loko, highest IRD was recorded in peri-urban site of New Maforki, 4.1/house/day in June (Figure 9) and this followed the rainfall pattern. There was no clear pattern in Bakolo, and the mean IRD did not follow the rainfall pattern (Figure 4).

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 March (5.1/house/day). The mean IRD declined in April before rising in May and June then declining through the rainy season (Figure 4) albeit non-uniformly. Similar to previous years reports, dips in July and August in the middle of the rainy season have been observed and could be due to the rains affecting the mosquito breeding sites. The mean IRD in Western Rural also followed the rainfall pattern with two peaks in May and July and a smaller one in December with highest IRD recorded in the rural site of Sand Sand Water (4.6/house/day) (Figure 4).

Figure 3: Mean IRDs of *An. gambiae* s.l. in Bo and Bombali Districts, Comparing Rural Versus Peri-Urban sites, March 2022–February 2023



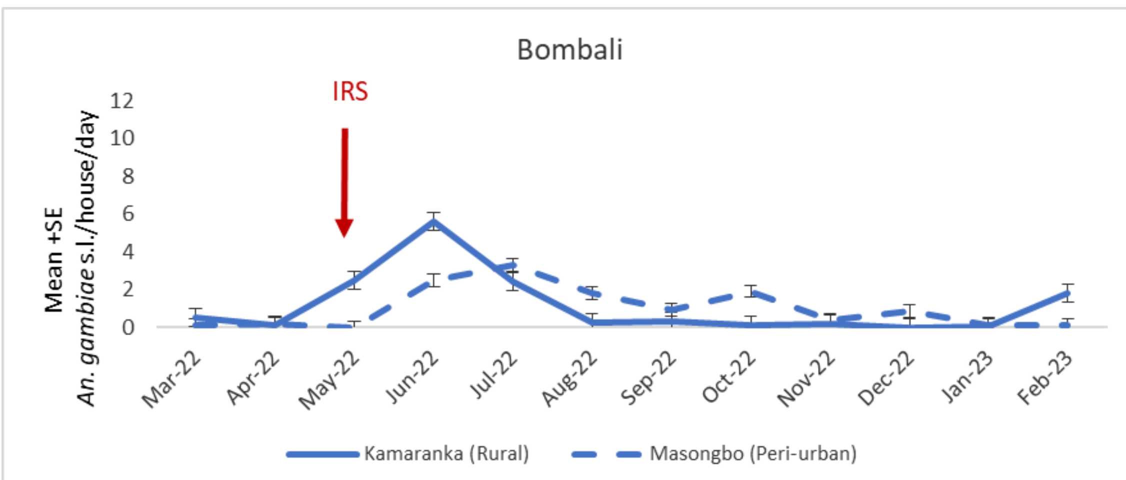
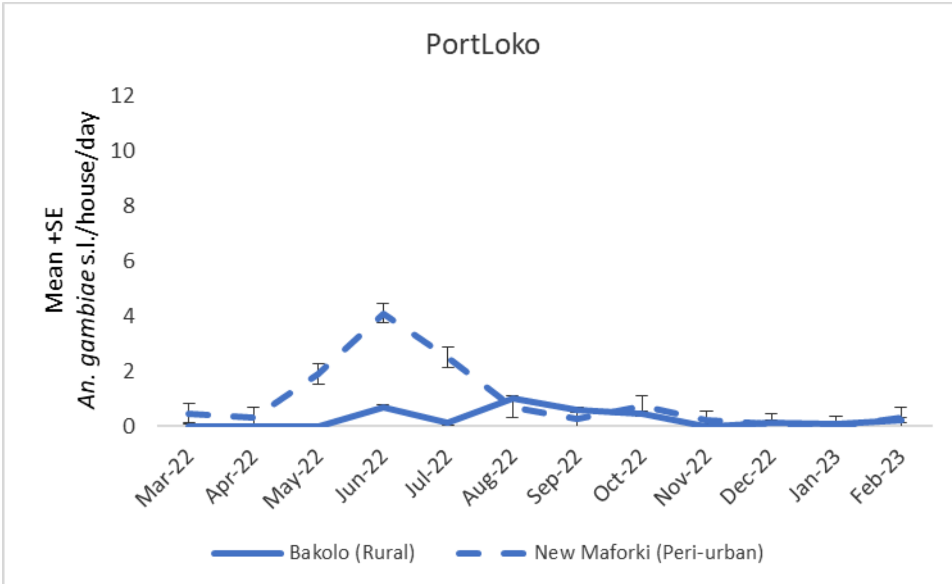
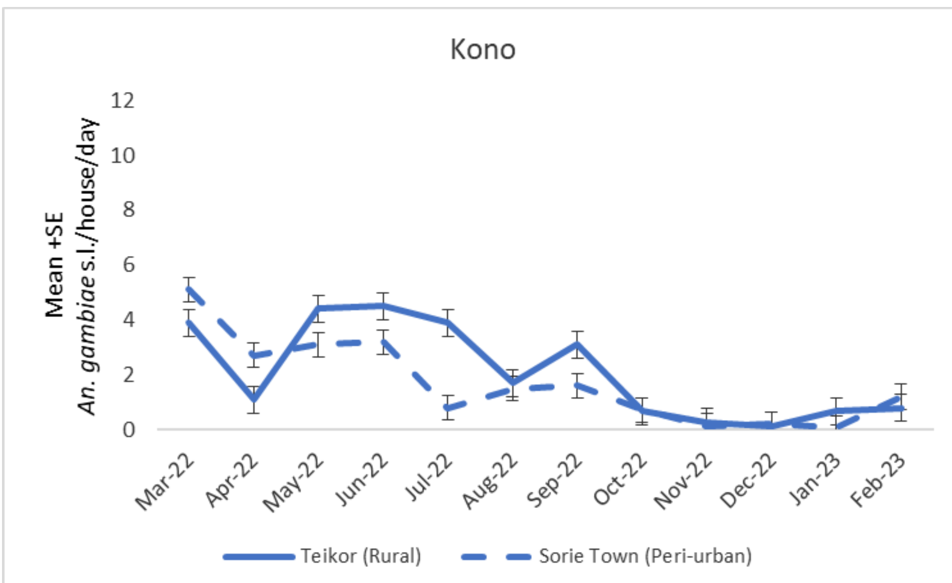
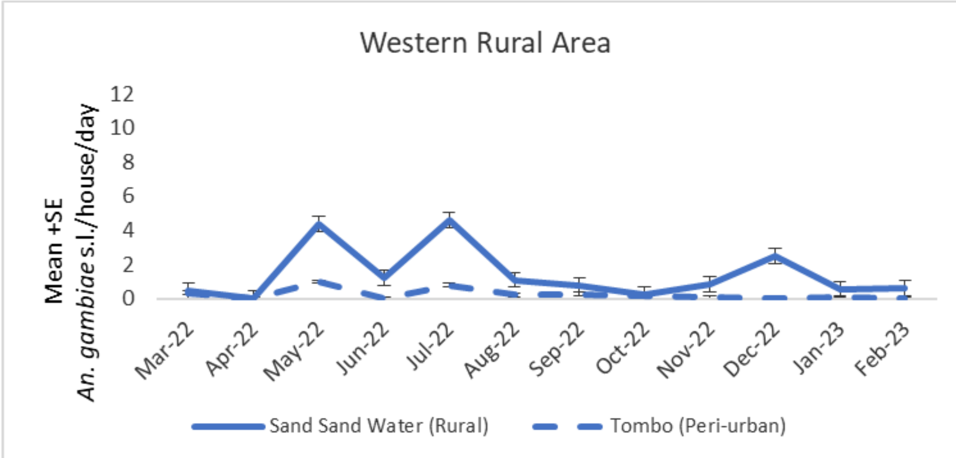


Figure 4: Mean IRDs of *An. gambiae* s.l. in Port Loko, Kono and Western Area Rural Districts, Comparing Rural Vs Peri-Urban sites, March 2022–February 2023



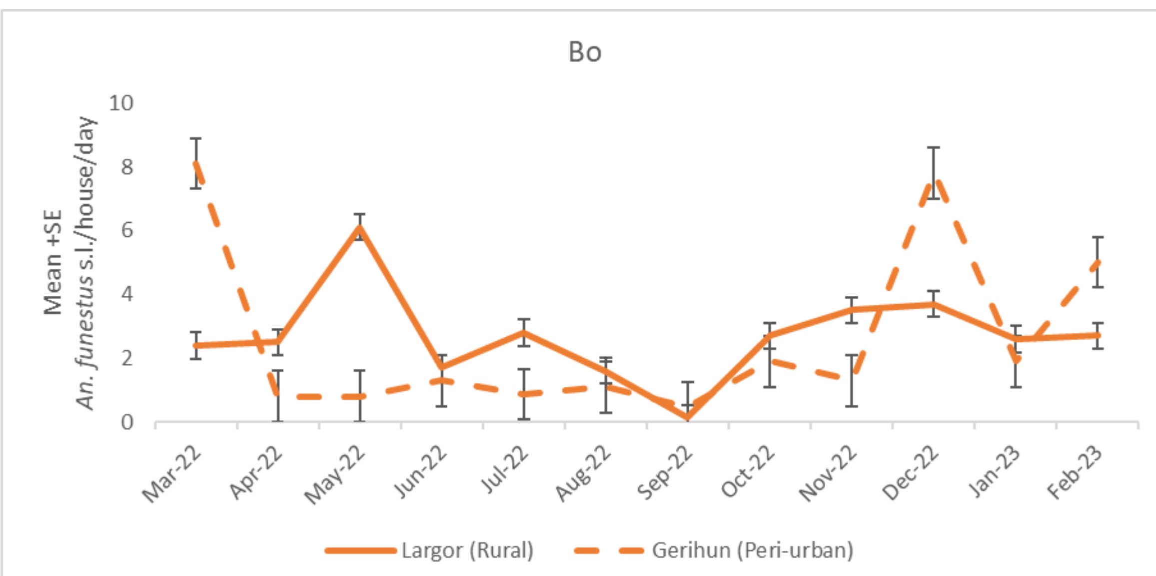




Indoor Resting Density of *Anopheles funestus* s.l. Collected by PSC

Anopheles. funestus s.l. was sampled in all districts but appreciable numbers were collected in Bo, followed by Bombali, Kono and Port Loko, which contributed more to overall IRD trends (Figures 5 and 6). In all sites, the highest IRD was always recorded in the dry seasons indicating differences in ecology of this vector in Sierra Leone. In Bo, there were two peaks of *An. funestus*, both recorded in Gerihun peri-urban site, in March 8.1/house/day and in December 7.8/house/day. In Largor rural site in Bo, the highest IRD was recorded in May 6.1/house/day (Figure 5). Similarly, in Bombali district, the highest IRD was recorded in December, during the dry season with 4.1/house/day in the peri-urban site of Masongbo. In Kono district, two peaks were recorded in Teikor rural site, one in July (1.5/house/day) and another in February 91.8/house/day) (Figure 6).

Figure 5: Mean IRDs of *An. funestus* s.l. in Bo and Bombali Districts, Comparing Rural Versus Peri-Urban Sites, March 2022–February 2023



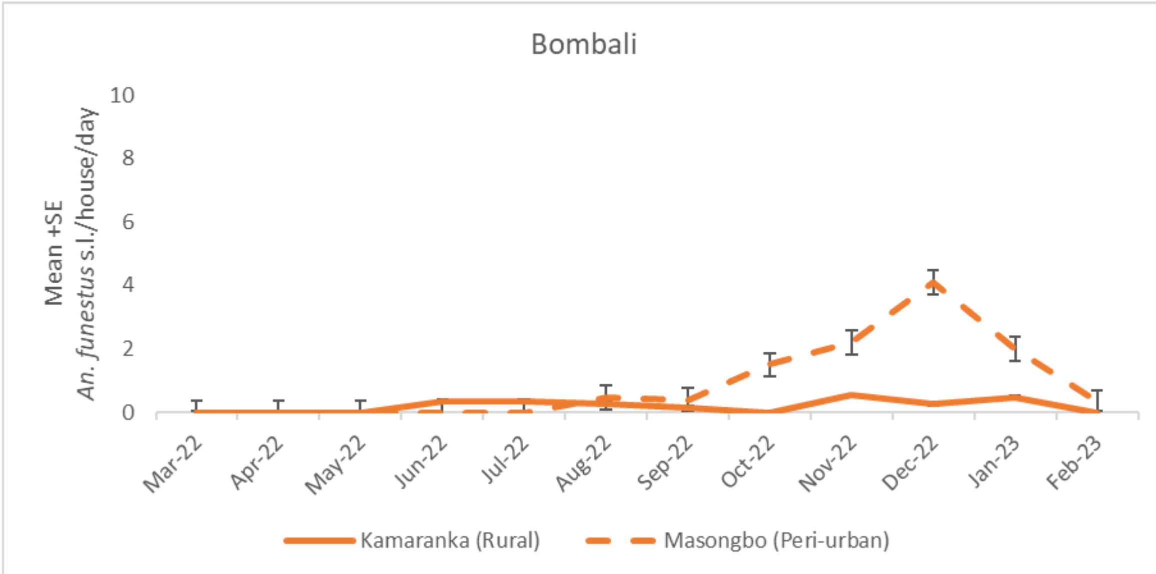
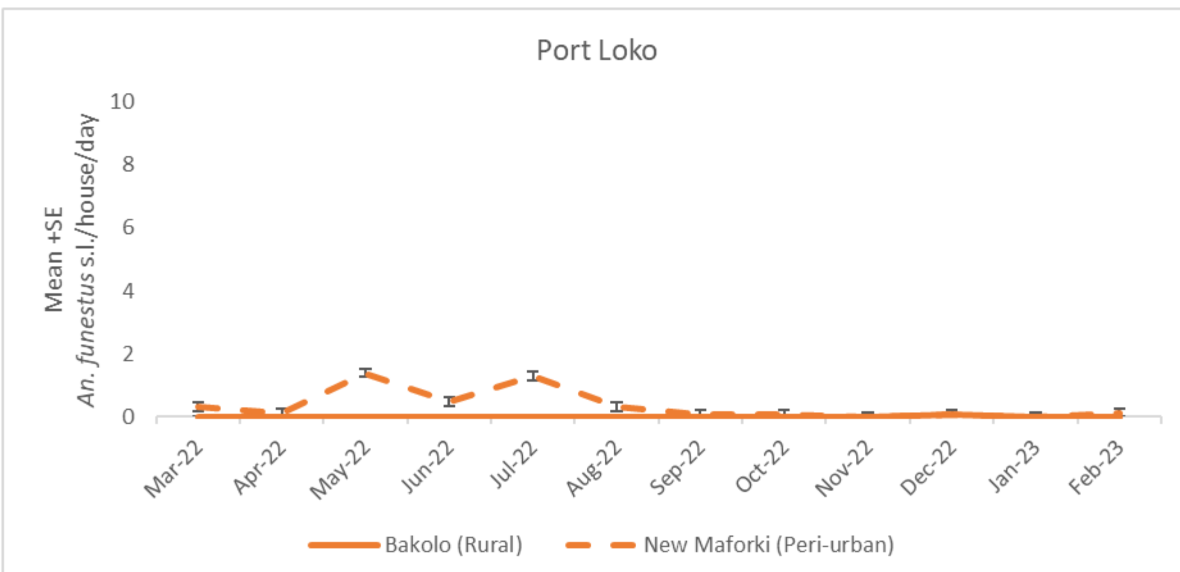
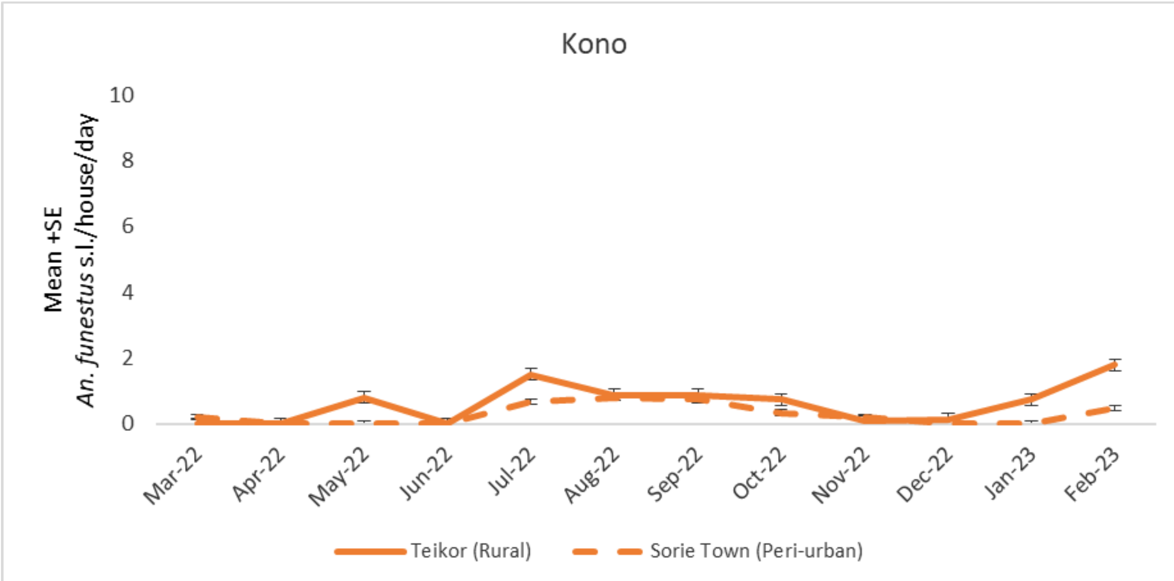
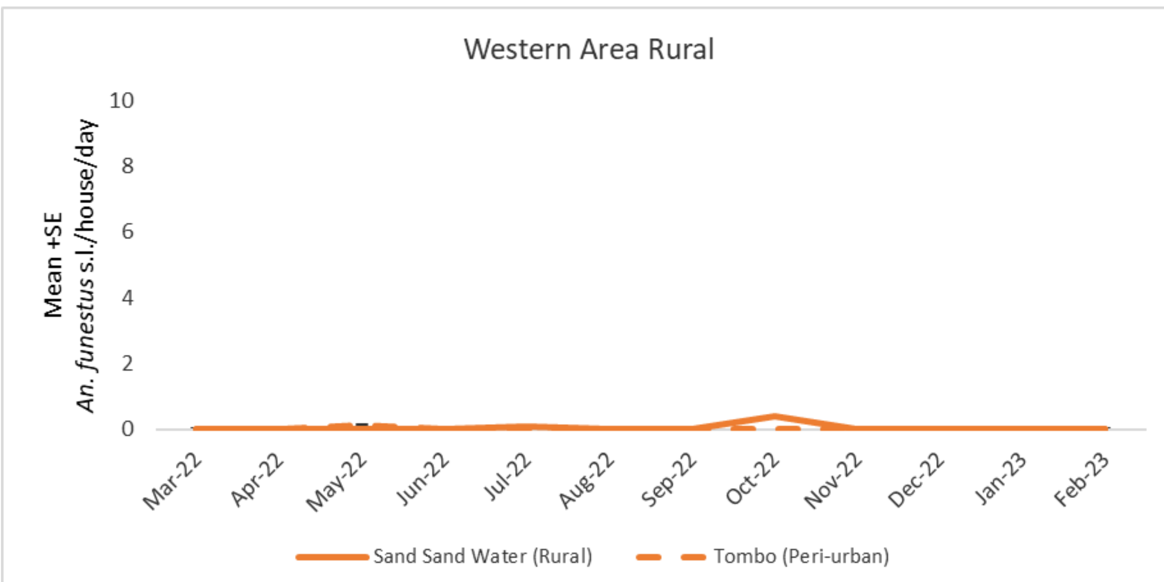


Figure 6: Mean IRDS of *An. funestus* s.l. in Port Loko, Kono and Western Area Rural Districts, Comparing Rural Versus Peri-Urban Sites March 2022–February 2023



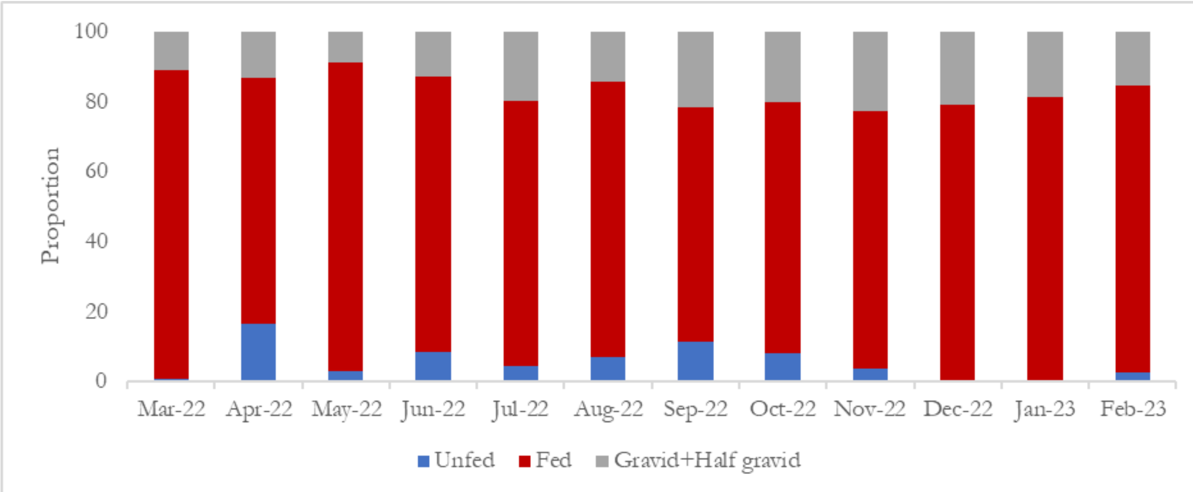




3.1.3 Abdominal Blood Digestions Stages of *An. gambiae* s.l. Collected by PSC

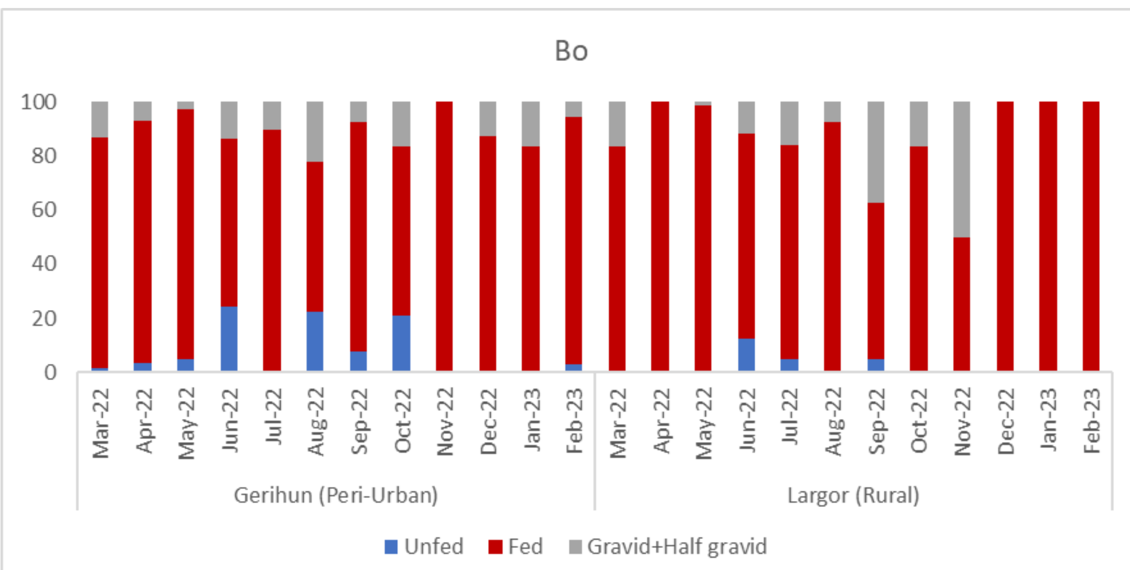
A higher number of fed compared to unfed/gravid *An. gambiae* s.l. mosquitoes were sampled by PSC all through the sampling period (Figure 7). This has remained so for the past four years during routine monitoring. There was no relationship/trend between proportion fed and season (Figures 8 and 9). The proportion of fed mosquitoes was higher throughout the year. The highest proportion of gravid/half gravid mosquitoes were sampled in September toward the end of the rainy season (Figure 7). This raises the question whether people are sleeping under the net or whether most of the biting is occurring during the hours when people are out of bed.

Figure 7: Abdominal Blood Digestions Stages of *An. gambiae* s.l. Collected by PSC Across All Sentinel Sites, March 2022–February 2023



When disaggregated by district, there were still more blood fed *An. gambiae* s.l. compared to gravid and unfed (Figures 7 - 9). Empty bars indicate months where PSC had no mosquito catches. In Bombali, there were relatively less blood fed mosquitoes than were found in Bo; however, since the proportion of gravid were high, it implies high access to blood meal source in both districts.

Figure 8: Abdominal Blood Digestions Stages of *An. gambiae* s.l. in Bo and Bombali, Collected by PSC, March 2022–February 2023



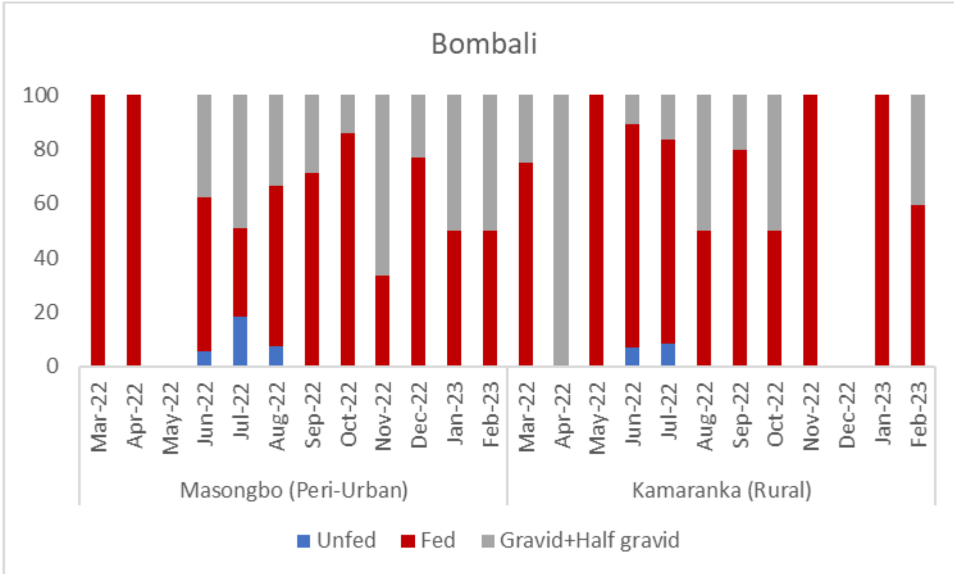
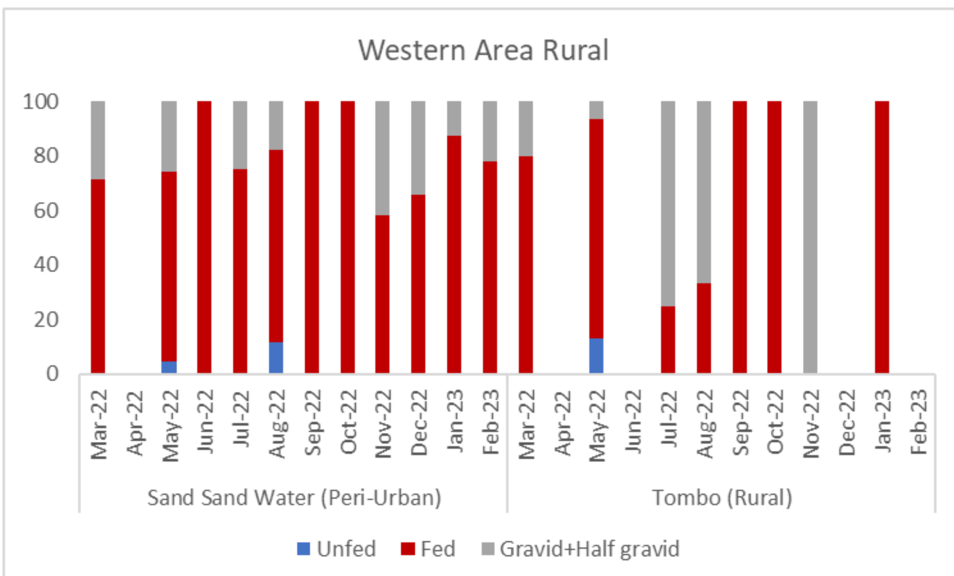
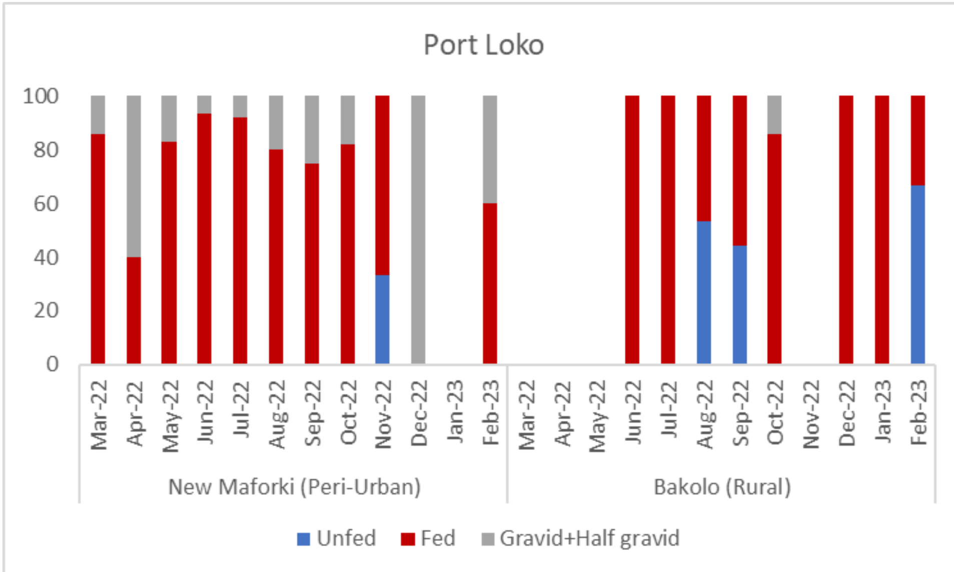
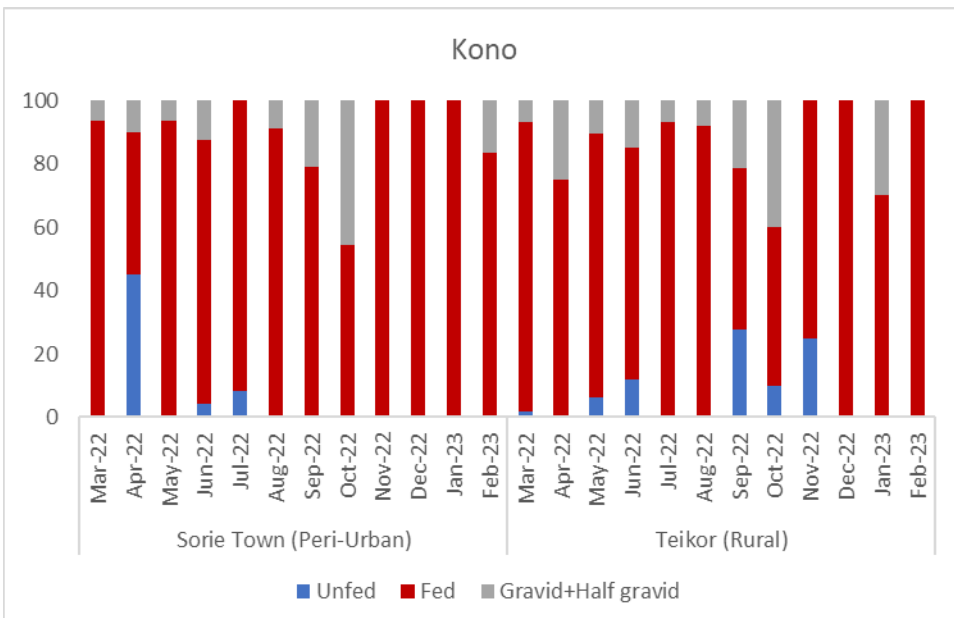


Figure 9: Abdominal Blood Digestions Stages of *An. gambiae* s.l. in Western Rural, Port Loko and Kono, Collected by PSC, March 2022–February 2023







3.1.4 Abdominal Blood Digestions Stages of *An. funestus* Group Collected by PSC

Overall, there was more blood fed mosquitoes than unfed/gravid (Figure 10). In Bo, where the majority of *An. funestus* s.l. were sampled, there appeared to be an increase in the number of blood fed mosquitoes at the end of the rainy season from September/October into the dry season peaking in either December (Largor) or February (Gerihun) (Figure 11). A rise in proportion of blood fed mosquitoes from October to February was also observed in Masongbo peri-urban site (Figure 11).

Figure 10: Abdominal Blood Digestions Stages of *An. funestus* s.l. Collected by PSC Across All Sentinel Sites, March 2021- February 2022

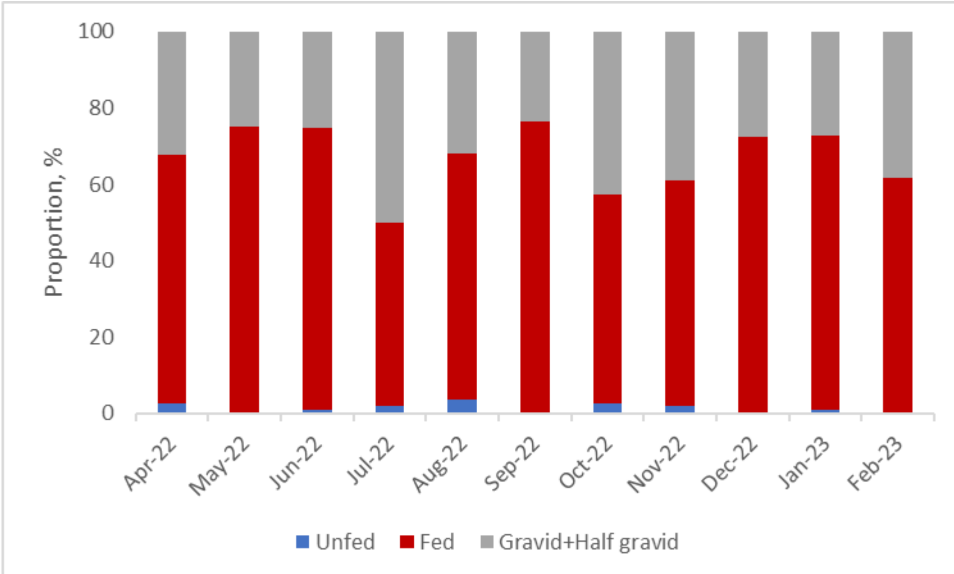
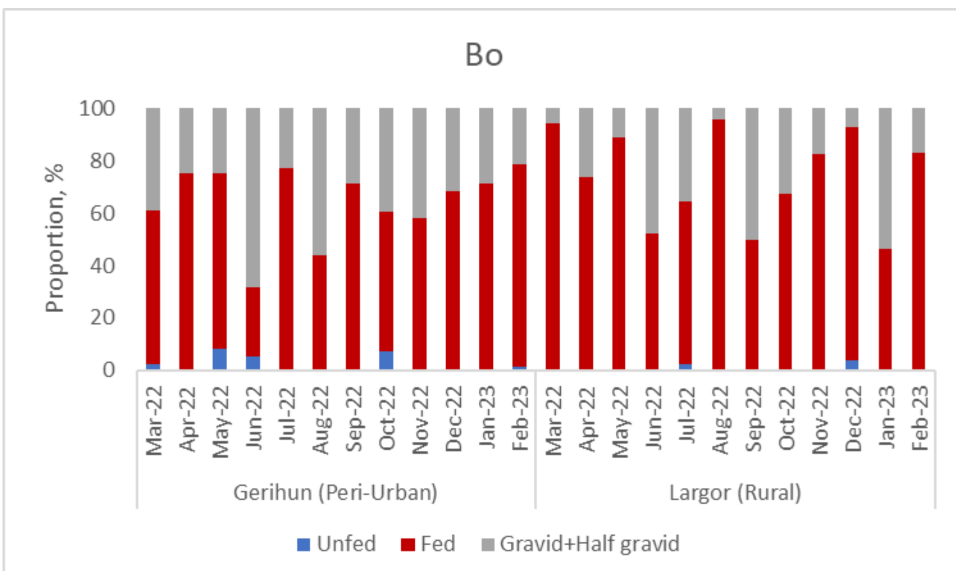
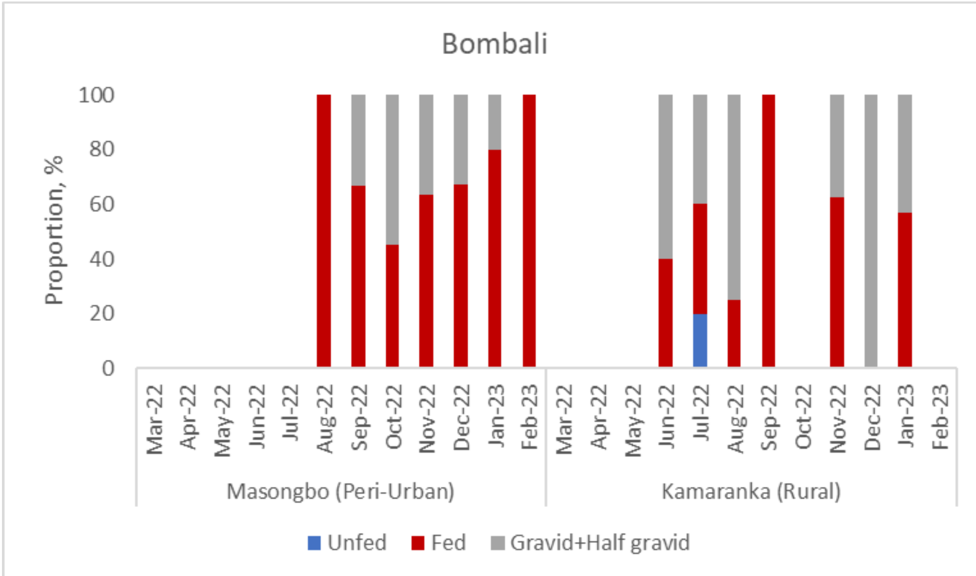
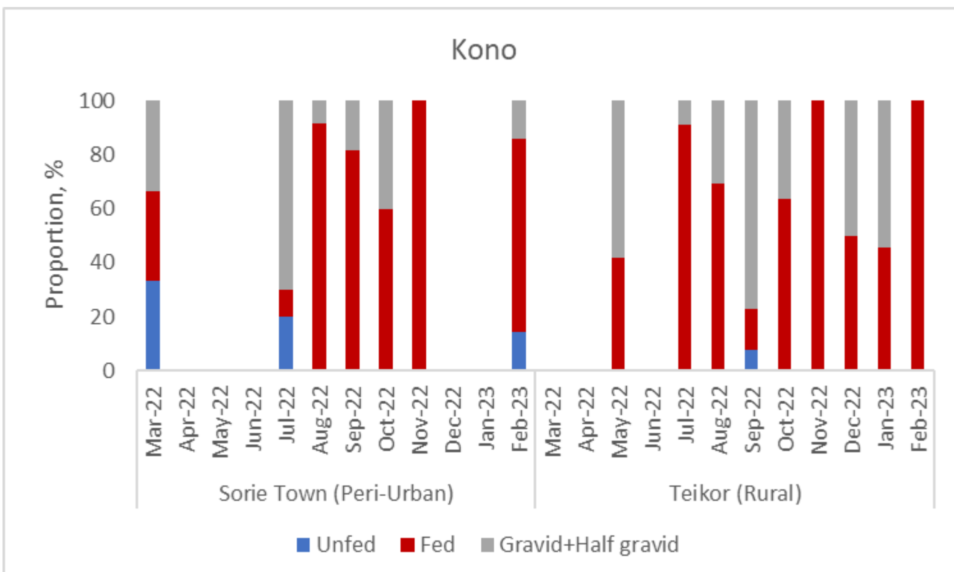
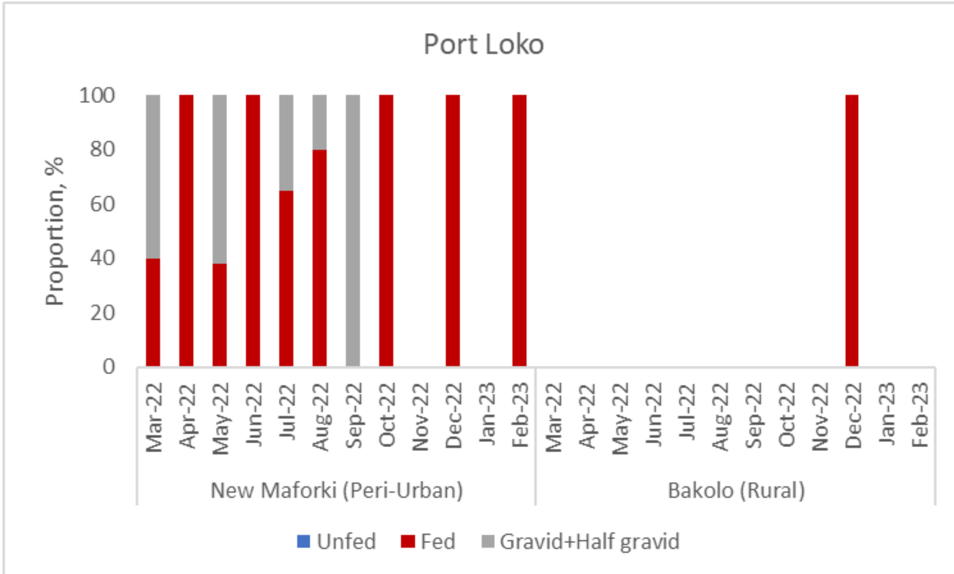


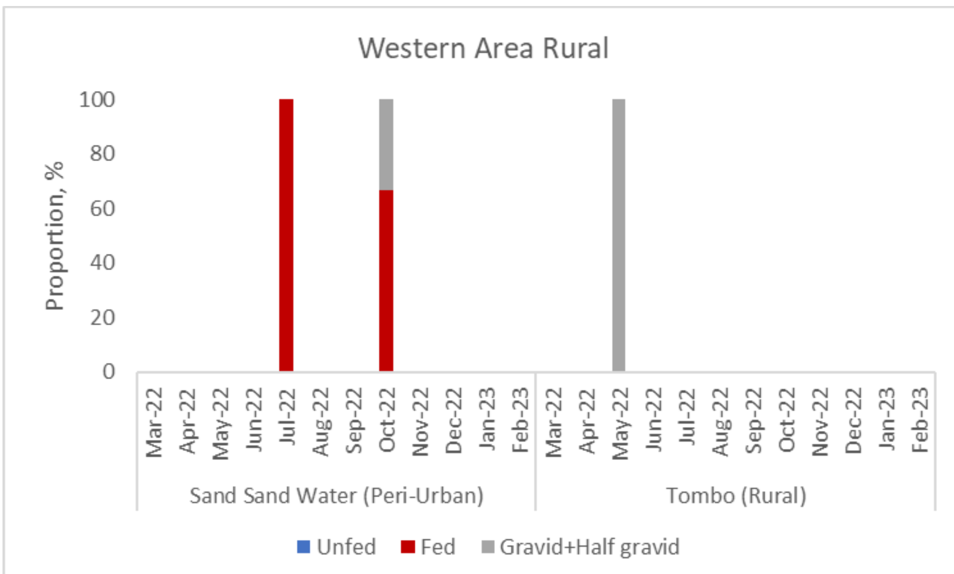
Figure 11: Abdominal Blood Digestions Stages of *An. funestus* s.l. in Bo, Bombali, Kono, Port Loko and Western Area Rural, Collected by PSC, March 2022–February 2023




Bombali
Proportion, %
100
80
60
40
20
0
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Masongbo (Peri-Urban)
Kamaranka (Rural)
Unfed
Fed
Gravid+Half gravid


Kono
Proportion, %
100
80
60
40
20
0
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Sorie Town (Peri-Urban)
Teikor (Rural)
Unfed
Fed
Gravid+Half gravid



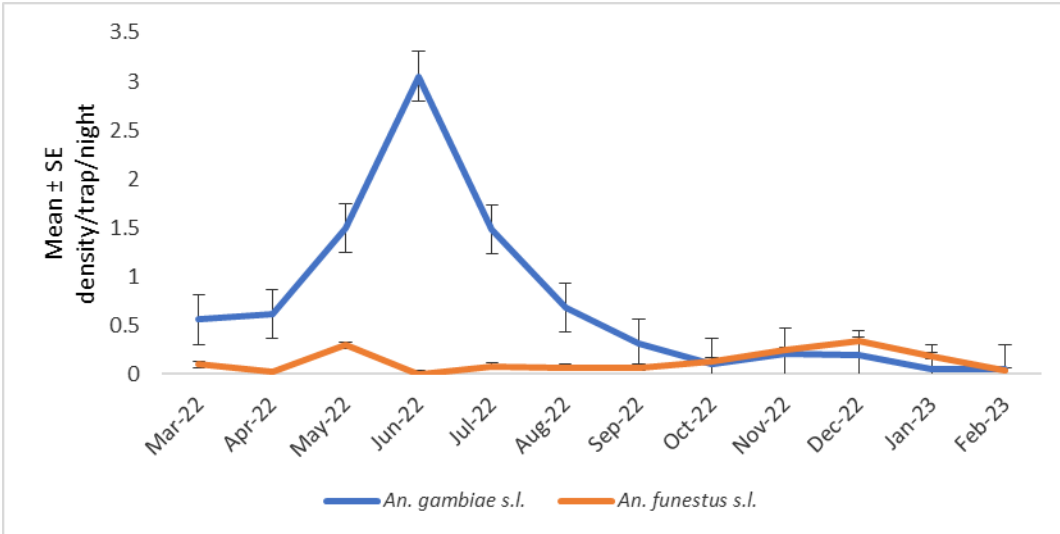


3.1.5 CDC Light Trap Collection

Anopheles gambiae s.l. was also the most predominant species by CDC-LT. Few numbers of *An. funestus* s.l. were collected. The overall density per trap followed the rainfall pattern with peak density in June (Figure 12). The density of *An. funestus* was low; therefore, no peak was observed (Figure 12).

The average density of *An. gambiae* s.l. per trap per night varied by district, site, and month during the sampling period (Figures 13 and 14).

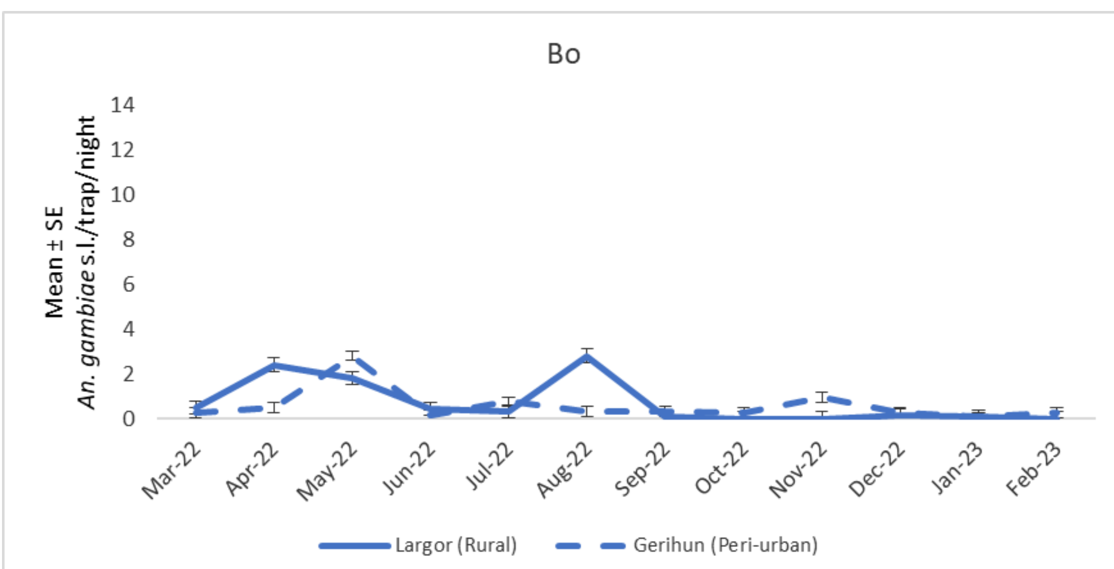
Figure 12: Density of *An. gambiae* s.l. and *An. funestus* s.l. from CDC Light Trap Collections Across All Sentinel Sites, March 2022–February 2023.



The highest average density of *An. gambiae* s.l./trap/night was recorded in June in non-IRS site of Bakolo, Port Loko district (12.4/trap/night) (Figure 14). In the IRS districts, the highest density was recorded in Kamaranka rural site, Bombali district with 8.4 *An. gambiae* s.l./trap/night, a reduction from the 2021-2022 period (21.4/trap/night) and 2020-2021 period (34.4/trap/night). Both IRS districts report great reduction in average density per trap with density averaging less than 3/trap/night (Figure 13). In all sites, peak density was recorded during the rainy season (Figures 13-14).

For *An. funestus* s.l., the highest average density was recorded in December in Masongbo peri-urban site, Bombali district with 2.9/trap/night followed by Largor (Bo district) in May with 2.6/trap/night. In Kono and Port Loko, the mean density per trap was always below 1 and therefore no clear pattern could be observed (Figure 15).

Figure 13: Density of *An. gambiae* s.l. from CDC Light Trap Collections in Bo and Bombali Districts, Comparing Rural Vs Peri-Urban Sites, March 2022–February 2023



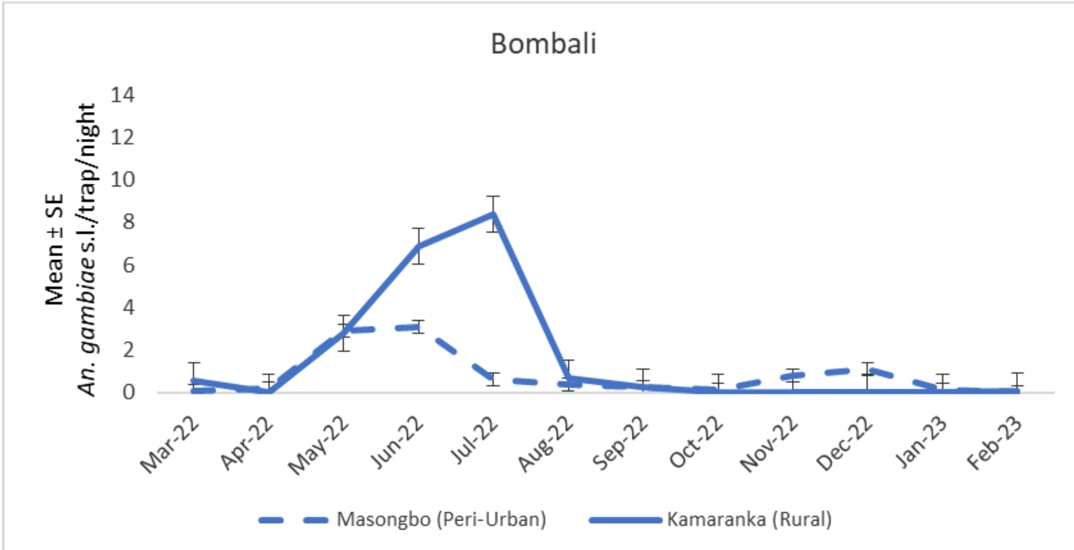
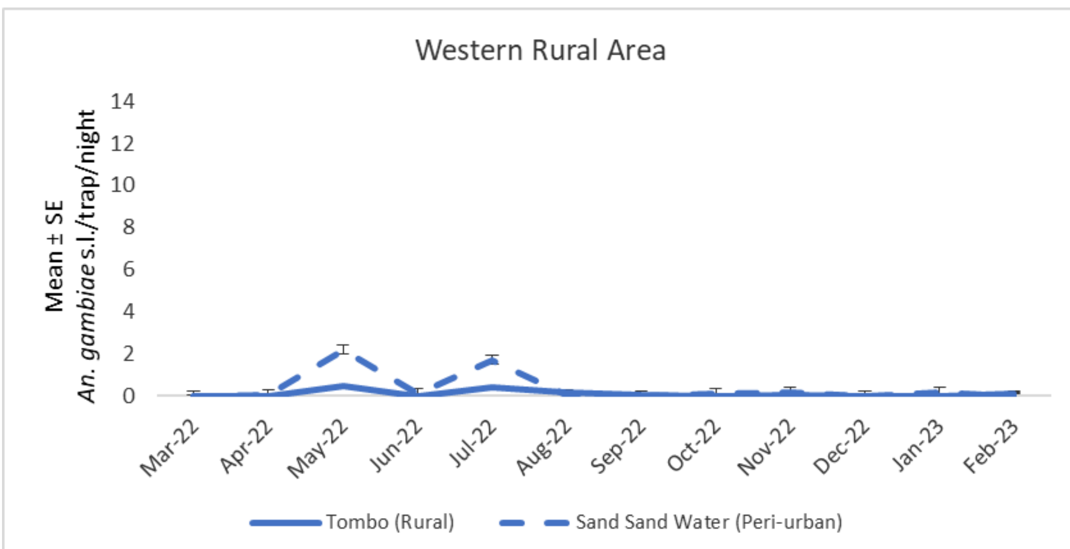
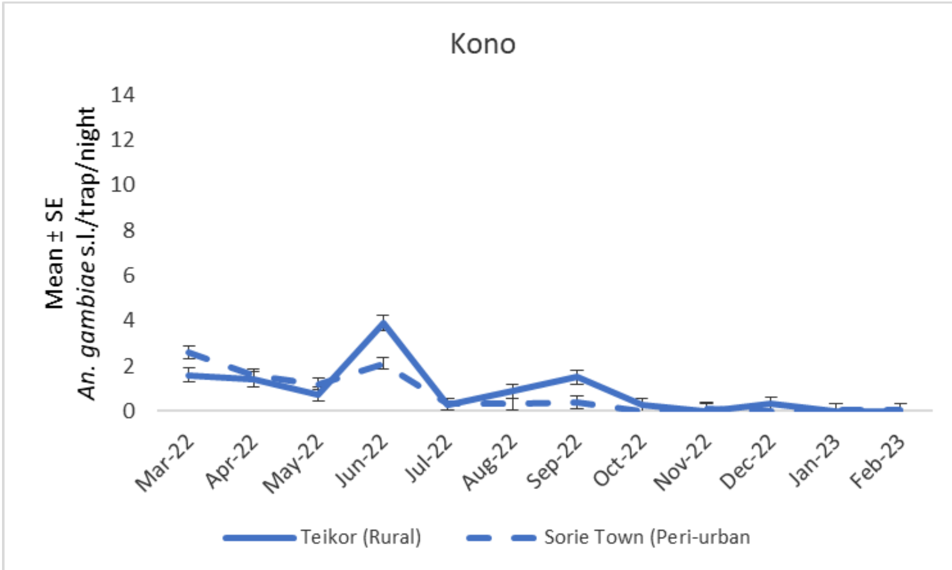


Figure 14: Density of *An. gambiae* s.l. from CDC Light Trap Collections in Western Rural, Kono and Port Loko Districts, Comparing Rural Versus Peri-Urban Sites, March 2022–February 2023




Kono
Mean ± SE
An. gambiae s.l./trap/night
14
12
10
8
6
4
2
0
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Teikor (Rural)
Sorie Town (Peri-urban

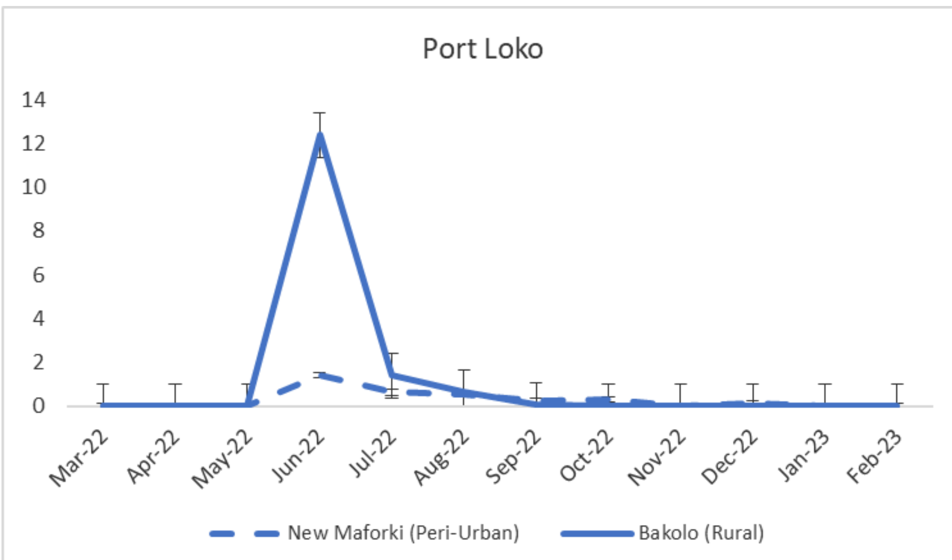
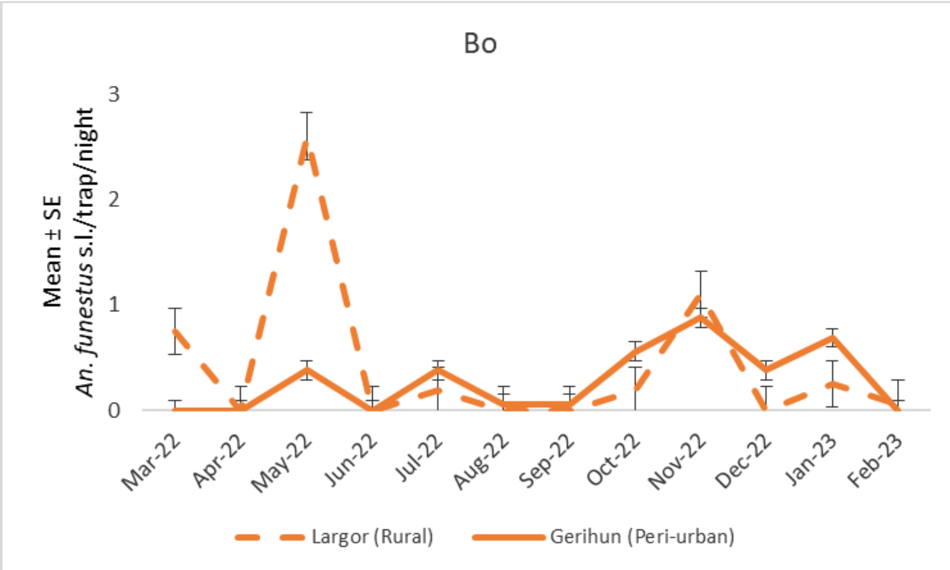
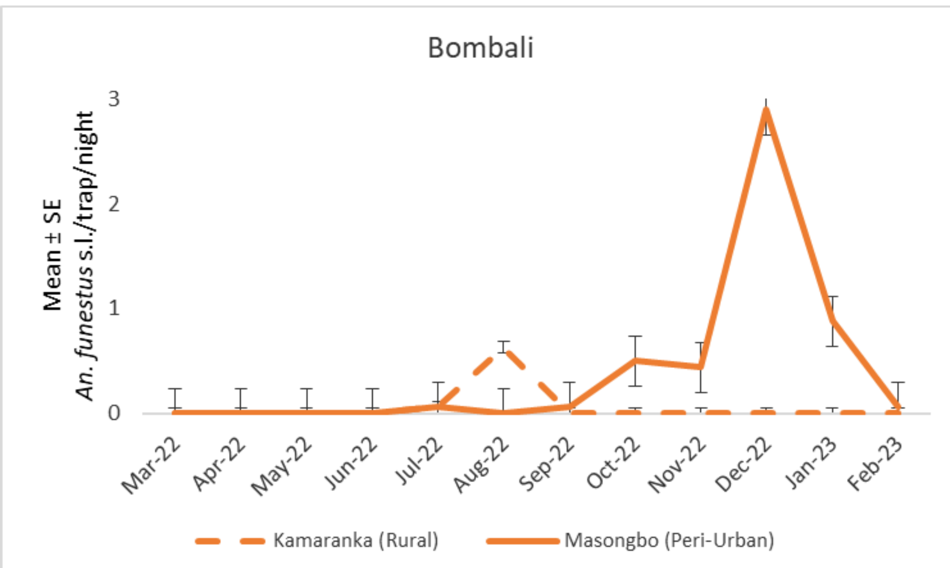
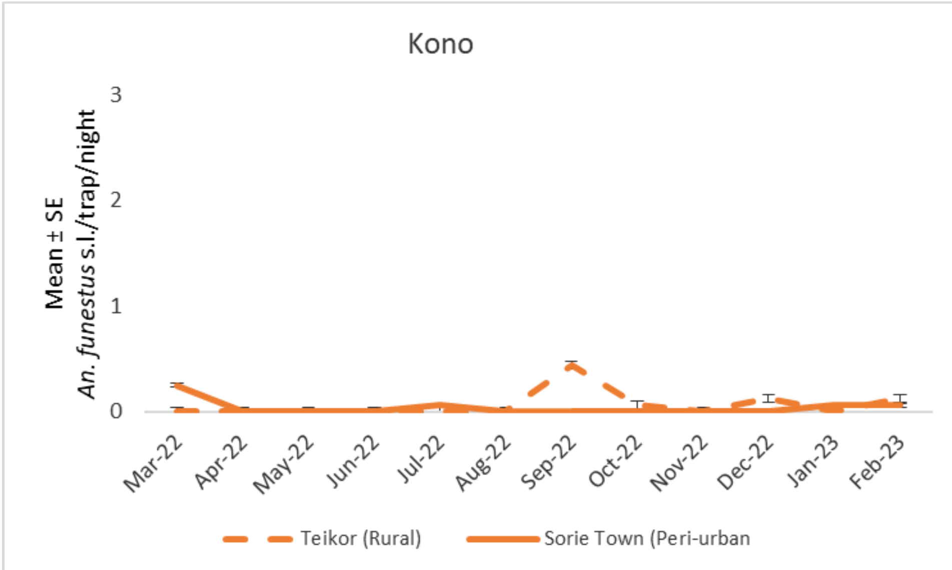
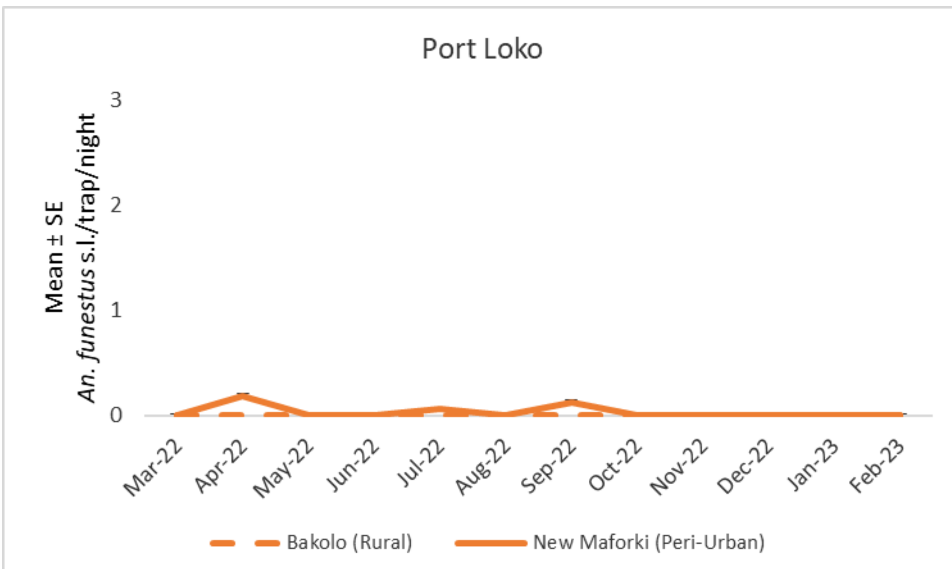

Port Loko
14
12
10
8
6
4
2
0
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
New Maforki (Peri-Urban)
Bakolo (Rural)

Figure 15: Density of *An. funestus* s.l. from CDC Light Trap Collections in Bo, Bombali and Kono Districts, Comparing Rural Versus Peri-Urban Sites, March 2022–February 2023





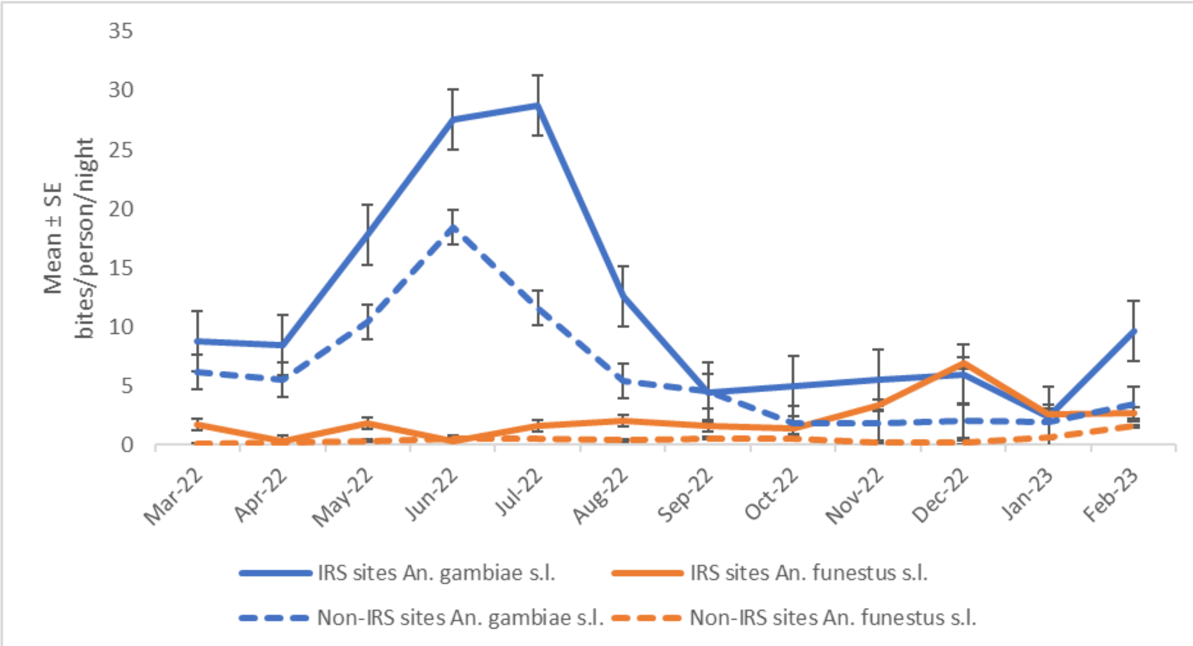




3.1.6 HUMAN LANDING COLLECTIONS

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 16). For *An. funestus* s.l., there was no observable pattern apart from a small peak observed in December 2022 in IRS sites (Figure 16). The mean HBR was highest in IRS sites compared to non-IRS sites for both species.

Figure 16: Mean HBR of *An. gambiae* s.l. and *An. funestus* s.l. Across all Sentinel Sites, March 2022–February 2023.



The outdoor and indoor biting patterns seem to be similar throughout the collection period although in the IRS sites, outdoor biting was more consistent during the rainy season (Figure 17). When data was aggregated across all sites, the HBR followed the rainfall pattern, peaking in June and declining through February 2022 (Figure 17).

The HBR in IRS sites continued to decline compared to previous years although the average HBR still remained high in IRS districts (Figure 17). The highest indoor HBR was recorded in June in Largor, Bo district with 49 bites/person/night compared to previous year where the highest was recorded in Masongbo, Bombali district with 80.6 bites/person/night. In Bombali, the highest HBR was recorded in June in Masongbo (29.3 bites/person/night) (Figure 18). In all districts, the HBR followed the rainfall pattern with a peak observed between May and September and declining into the dry season (Figures 17 - 19). There was no clear outdoor/indoor biting pattern difference in all sites except in Masongbo, Bombali district and New Maforki, Port Loko district where more outdoor biting was observed (Figures 17 - 19).

The mean HBR of *An funestus* s.l. did not follow the rainfall pattern with highest peak observed during the dry season in December in Gerihun, Bo district with 13 bites/person/night (Figure 20 and 21). The majority of the *An. funestus* s.l. was collected in Bo followed by Bombali, Kono and Port Loko. These three districts contributed most to the overall trends in HBR for *An. funestus* s.l.. The highest HBR was recorded outdoors in December in Gerihun, Bo District with 13 bites/person/night followed by Masongbo in Bo district with 10 bites/person/night also outdoor (Figure 21). This is an interesting trend to monitor given *An. funestus* is predominantly an indoor feeder. In Kono district, the highest HBR was in Sorie town with 7.3 bites/person/night outdoors in February. Western rural district peak was recorded indoors in Sand Sand Water with 2.8 bites/person/night (Figure 21).

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

Figure 17: *An. gambiae* s.l. Mean HBR Across All Sentinel Sites, March 2022–February 2023

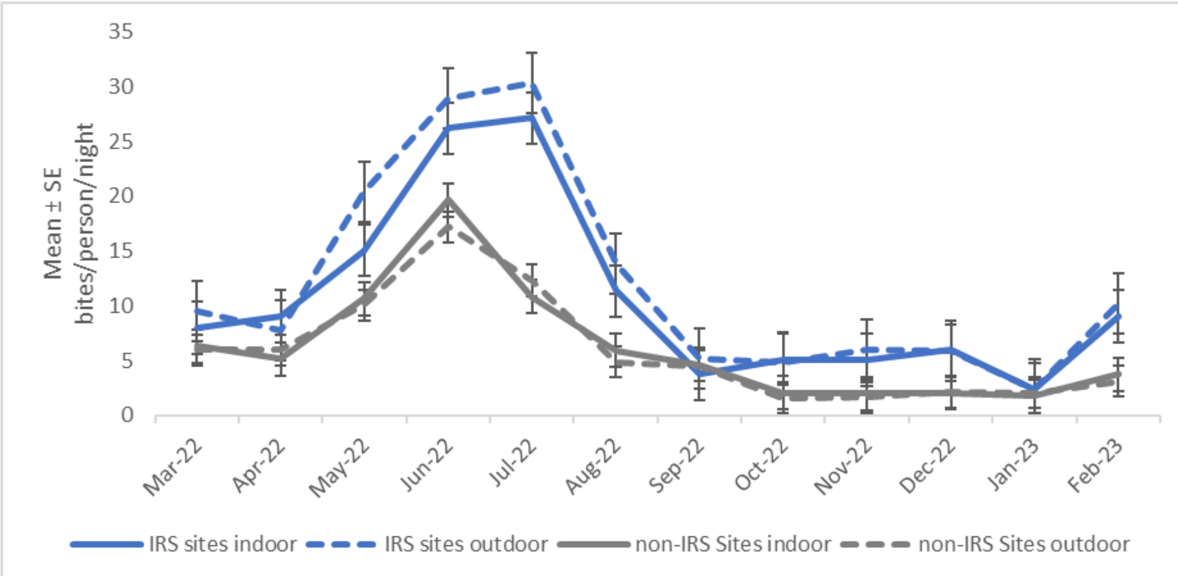
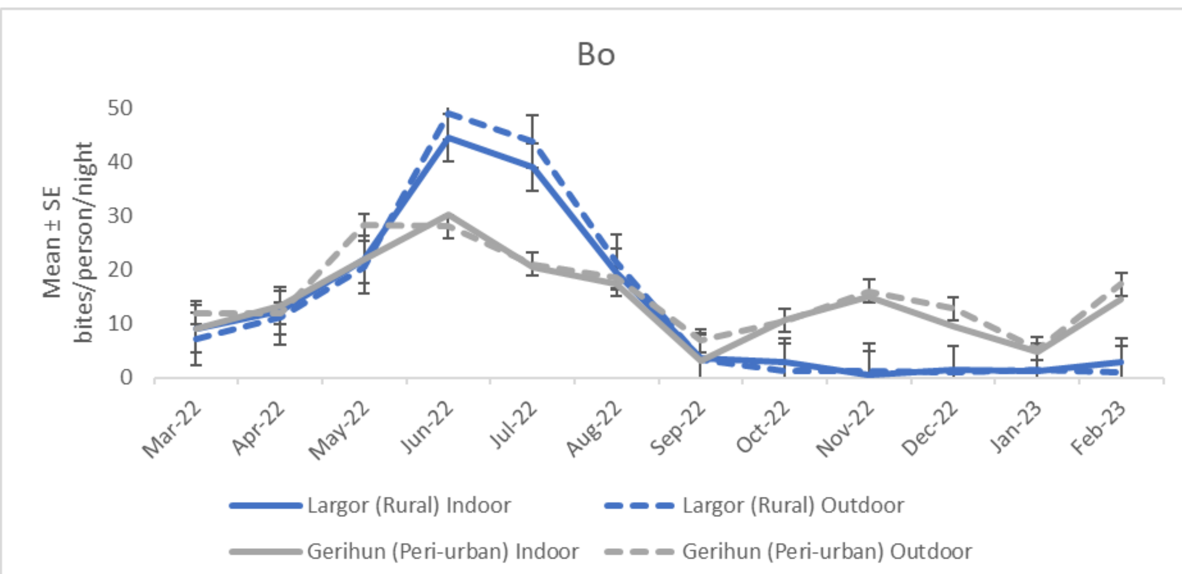


Figure 18: *An. gambiae* s.l. Mean HBR in Bo and Bombali Districts, March 2022–February 2023



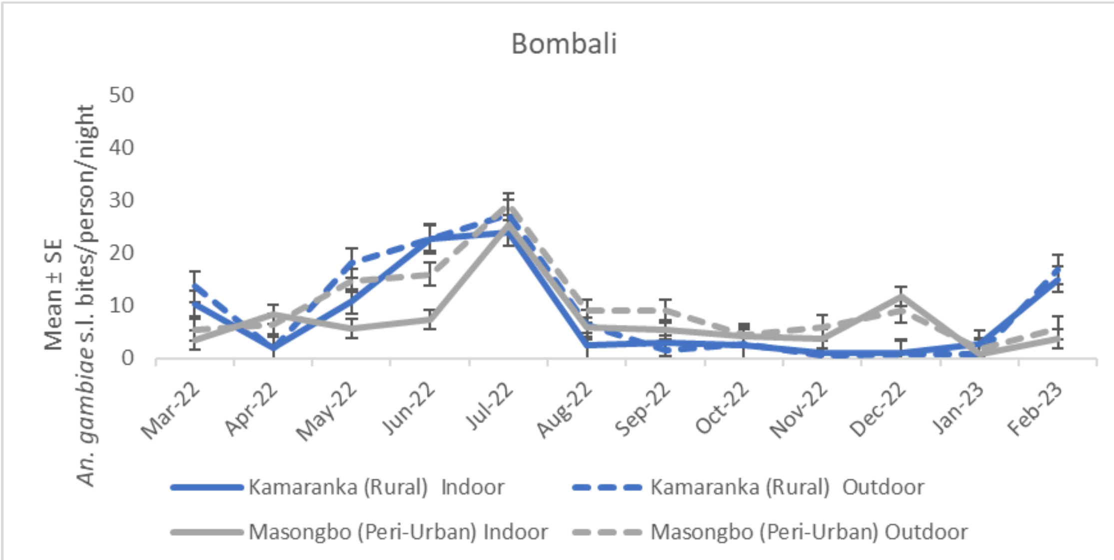
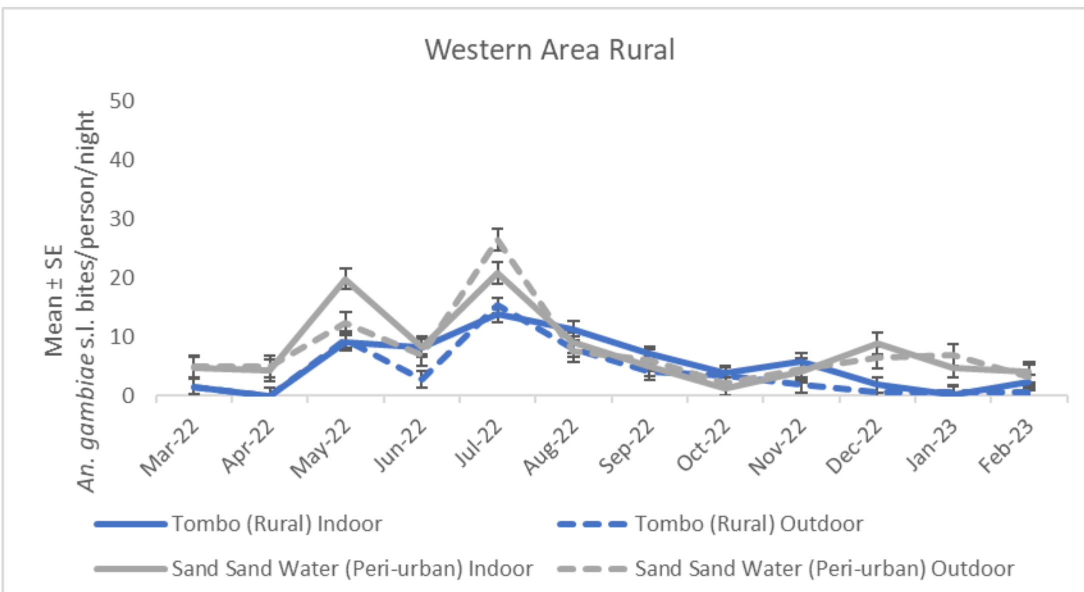
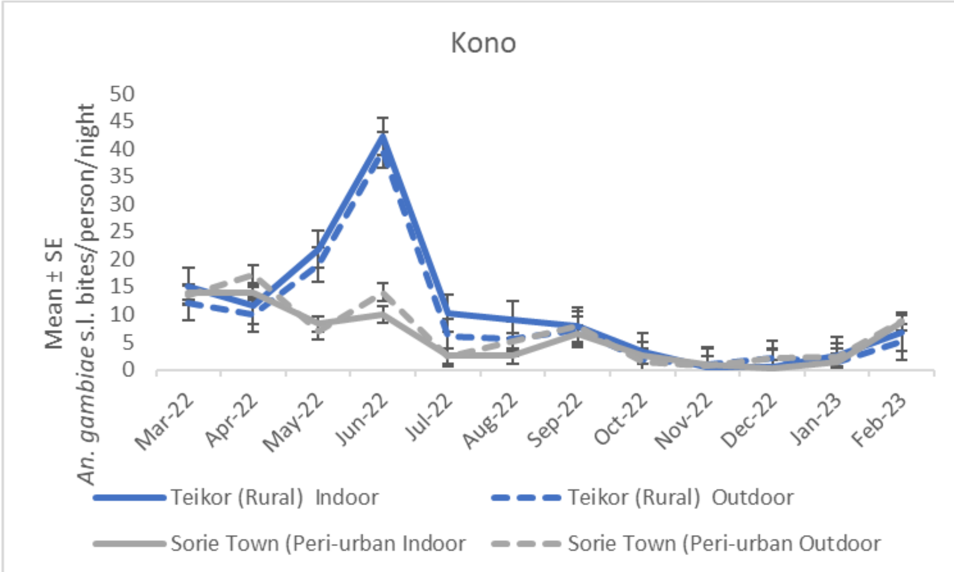


Figure 19: *An. gambiae* s.l. Mean HBR in Western Rural, Kono and Port Loko Districts, March 2022–February 2023




Kono
Mean ± SE
An. gambiae s.l. bites/person/night
50
45
40
35
30
25
20
15
10
5
0
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Teikor (Rural) Indoor
Teikor (Rural) Outdoor
Sorie Town (Peri-urban Indoor
Sorie Town (Peri-urban Outdoor

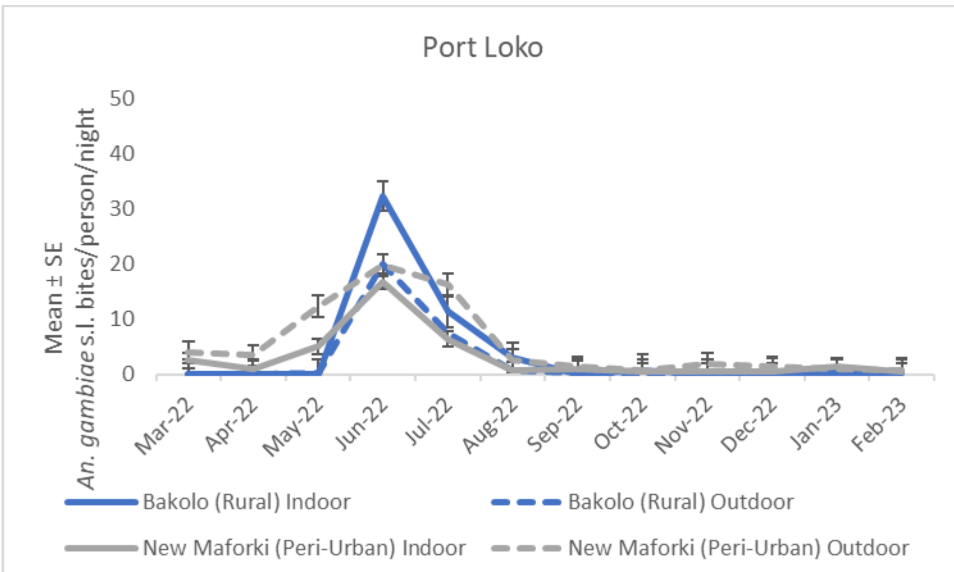

Port Loko
Mean ± SE
An. gambiae s.l. bites/person/night
50
40
30
20
10
0
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Bakolo (Rural) Indoor
Bakolo (Rural) Outdoor
New Maforki (Peri-Urban) Indoor
New Maforki (Peri-Urban) Outdoor

Figure 20: ***An. funestus*** **s.l. Mean HBR across Bo, Bombali, Kono, Port Loko and Western Rural Sentinel Sites, March 2022–February 2023**

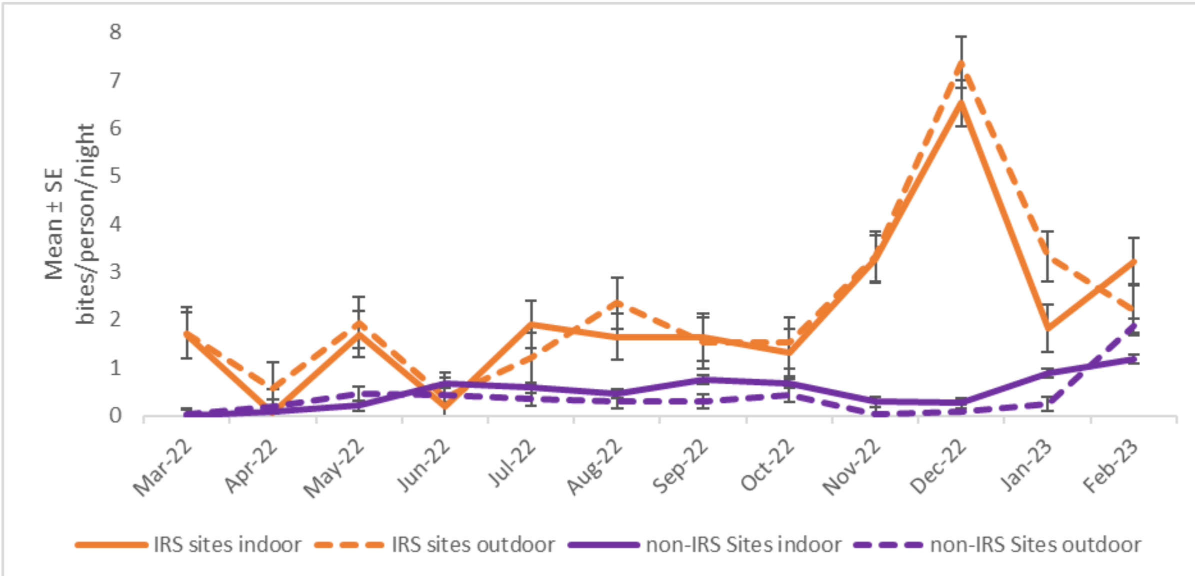
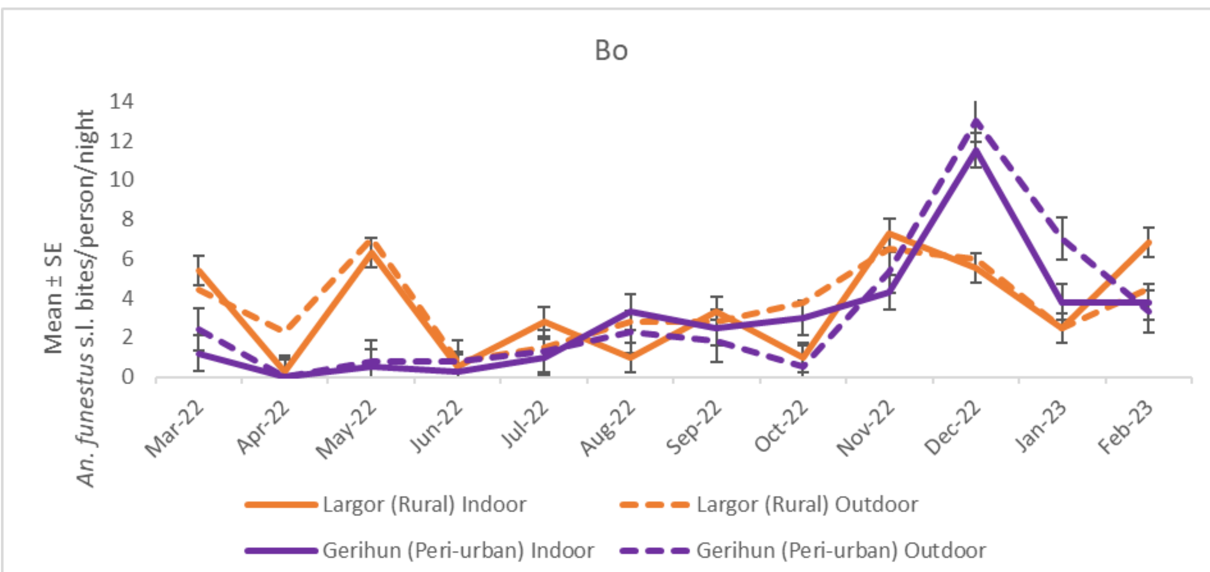
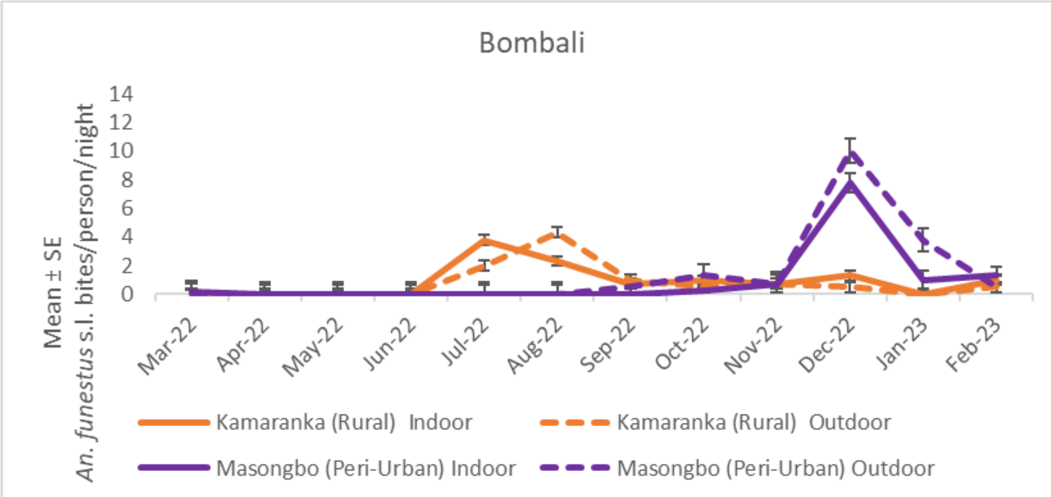
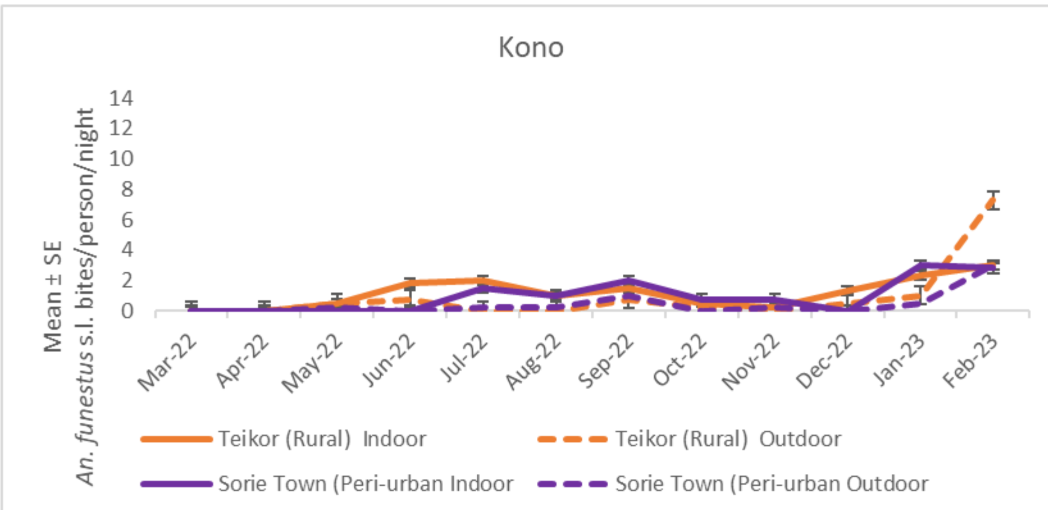
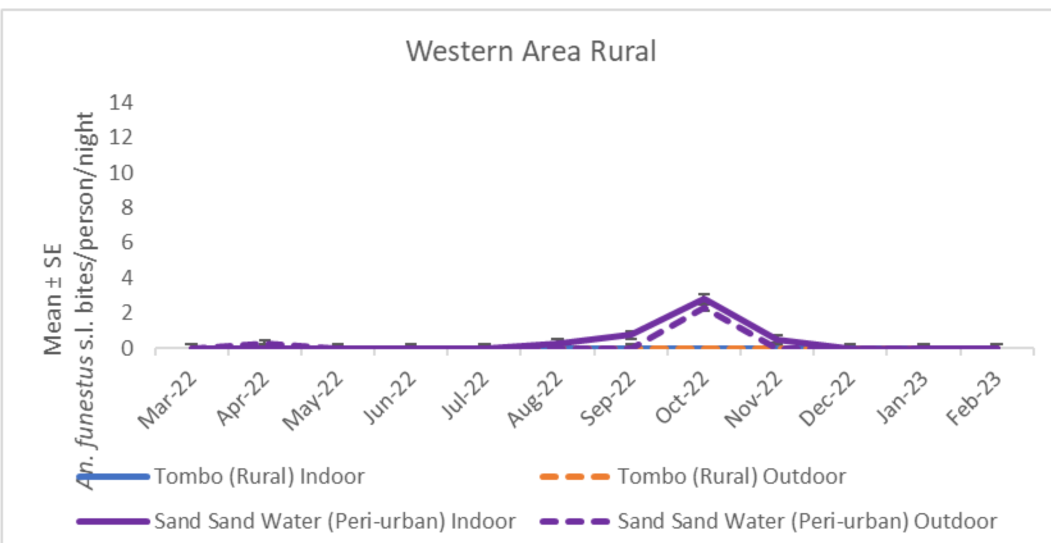


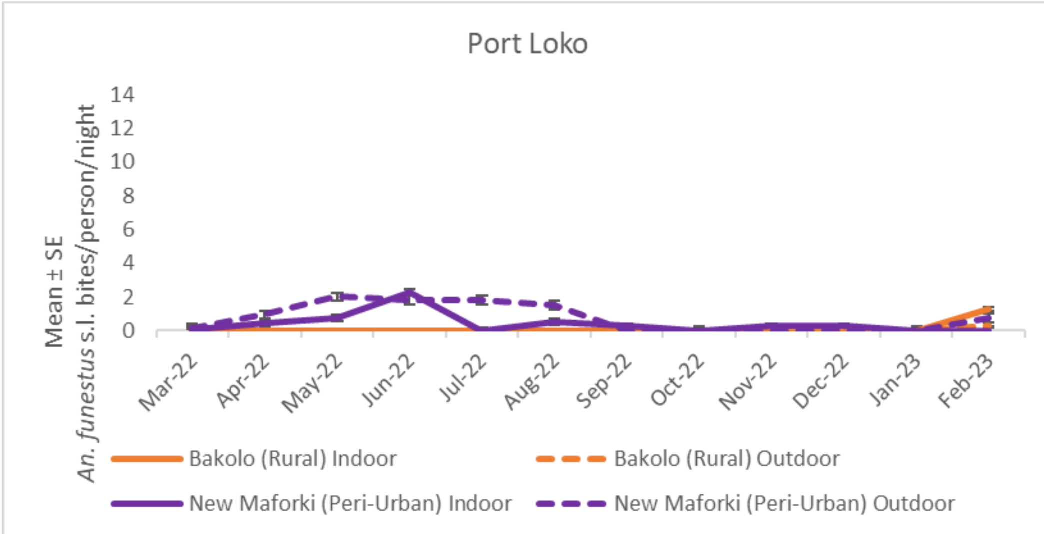
Figure 21: ***An. funestus*** **s.l. Mean HBR in Bo, Bombali, Kono, Western Rural and Port Loko Districts, March 2022–February 2023**




Bombali
Mean ± SE
An. funestus s.l. bites/person/night
14
12
10
8
6
4
2
0
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Kamaranka (Rural) Indoor
Kamaranka (Rural) Outdoor
Masongbo (Peri-Urban) Indoor
Masongbo (Peri-Urban) Outdoor


Kono
Mean ± SE
An. funestus s.l. bites/person/night
14
12
10
8
6
4
2
0
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Teikor (Rural) Indoor
Teikor (Rural) Outdoor
Sorie Town (Peri-urban Indoor
Sorie Town (Peri-urban Outdoor

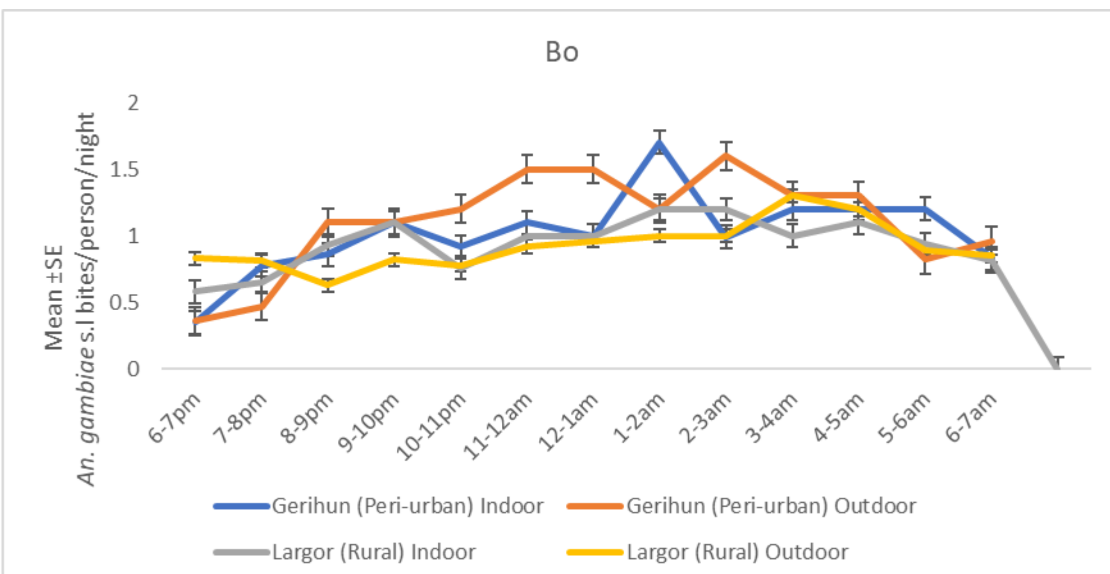

Western Area Rural
Mean ± SE
An. funestus s.l. bites/person/night
14
12
10
8
6
4
2
0
Mar-22
Apr-22
May-22
Jun-22
Jul-22
Aug-22
Sep-22
Oct-22
Nov-22
Dec-22
Jan-23
Feb-23
Tombo (Rural) Indoor
Tombo (Rural) Outdoor
Sand Sand Water (Peri-urban) Indoor
Sand Sand Water (Peri-urban) Outdoor



Biting Time: Overall, biting activity of *An. gambiae* s.l. peaked between 10:00pm and 5:00am although peaks varied by district (Figures 22 and 23). In almost all sites, biting activity was highest between 10:00 pm-5:00am, peaking at 1:00-2:00am for Bo and Bombali, 2:00-3:00am in Port Loko, 3:00-5:00am in Kono and 2:00-4:00am in Western Rural (Figures 22 and 23).

For *An. funestus* s.l., the mean HBR per hour was always below one; therefore, no clear biting time could be inferred (Figure 24).

Figure 22: *An. gambiae* s.l. Biting Time in Bo and Bombali Districts, Comparing Indoor Versus Outdoor, March 2022–February 2023



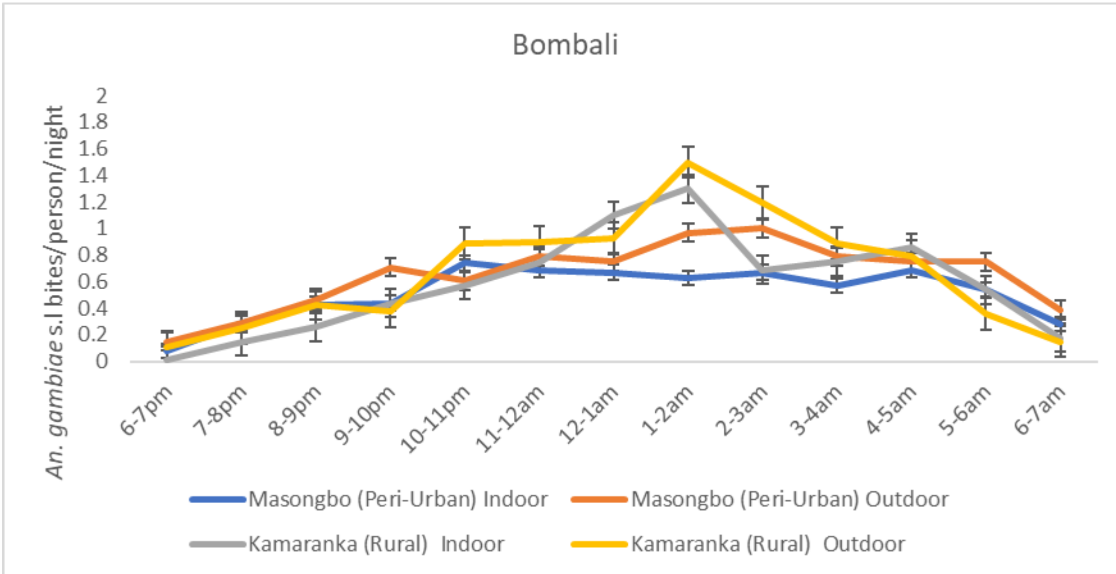
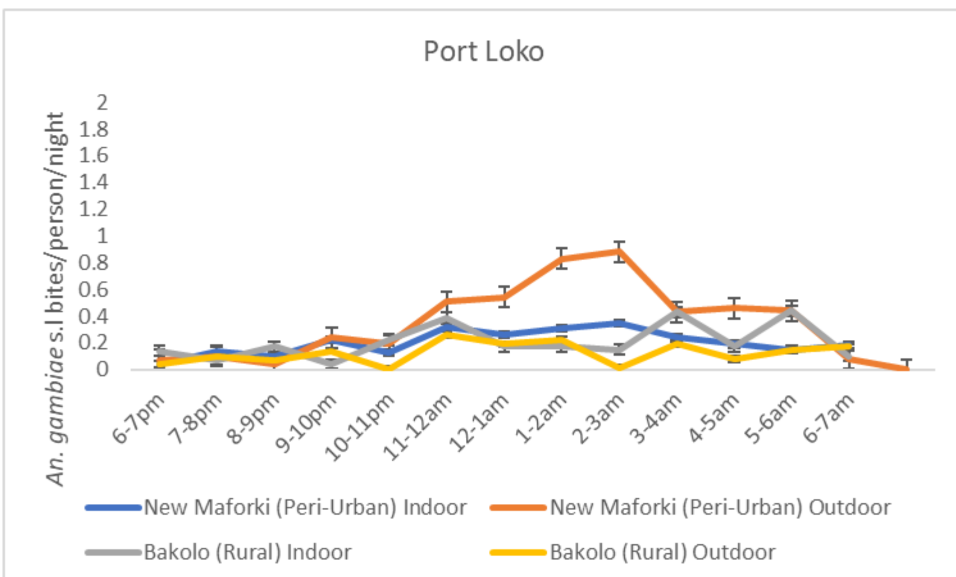
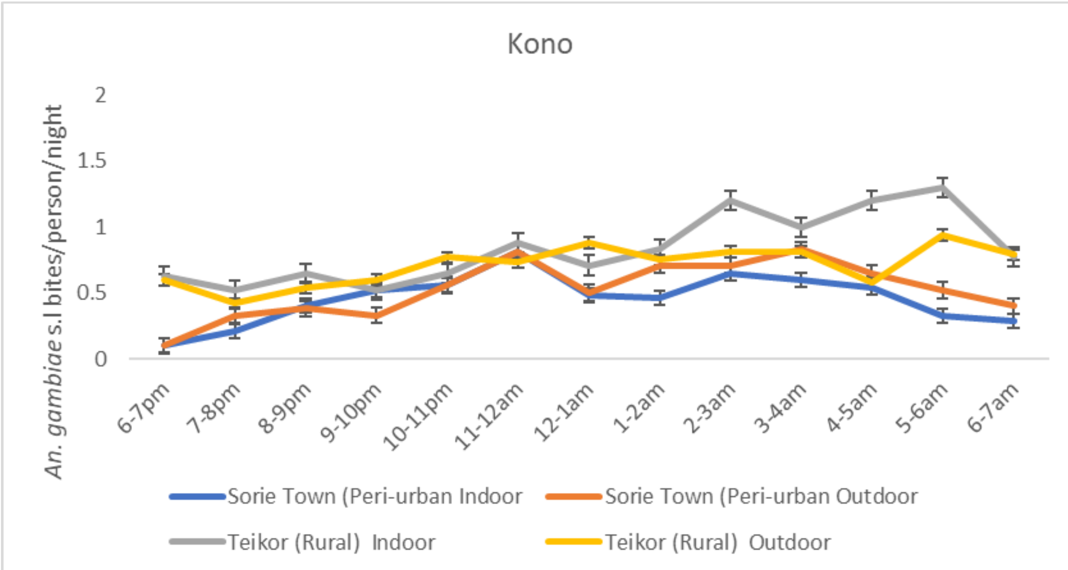


Figure 23: *An. gambiae* s.l. Biting Time in Port Loko, Kono and Western Area Rural Districts, Comparing Indoor Versus Outdoor, March 2022–February 2023




Kono
An. gambiae s.l bites/person/night
2
1.5
1
0.5
0
6-7pm
7-8pm
8-9pm
9-10pm
10-11pm
11-12am
12-1am
1-2am
2-3am
3-4am
4-5am
5-6am
6-7am
Sorie Town (Peri-urban Indoor
Sorie Town (Peri-urban Outdoor
Teikor (Rural) Indoor
Teikor (Rural) Outdoor

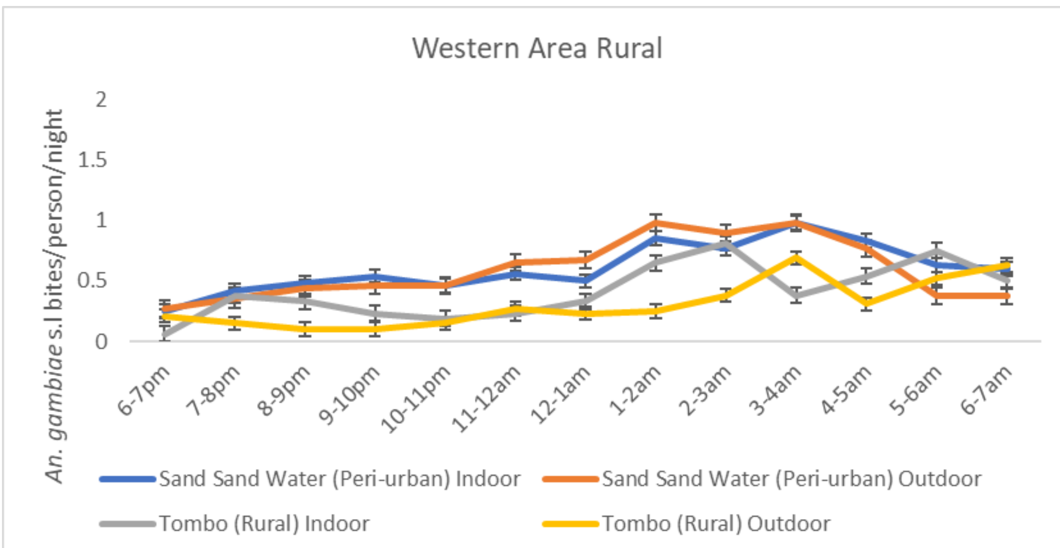
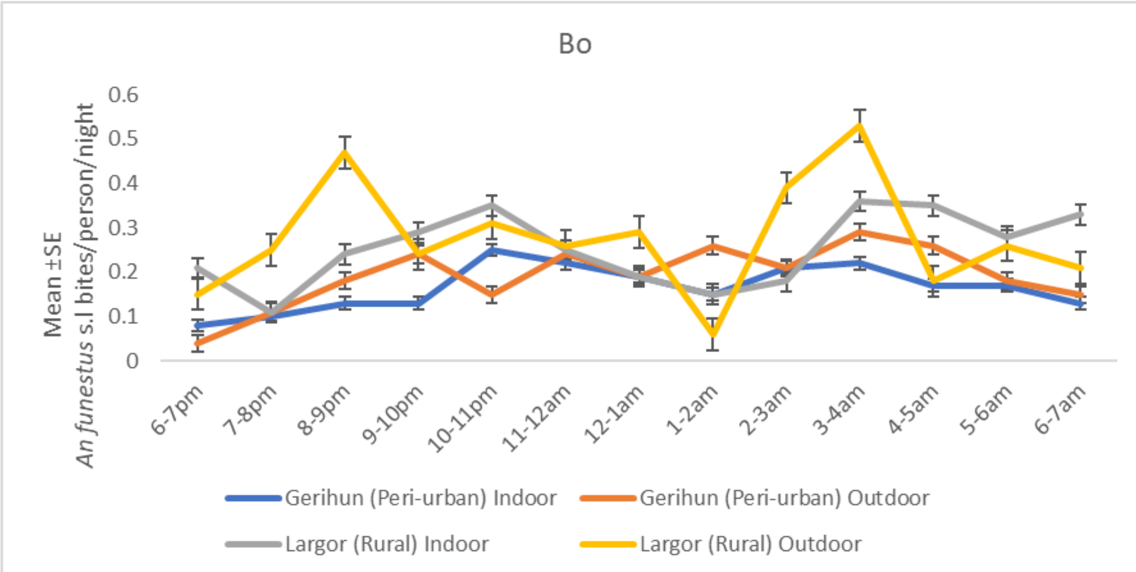
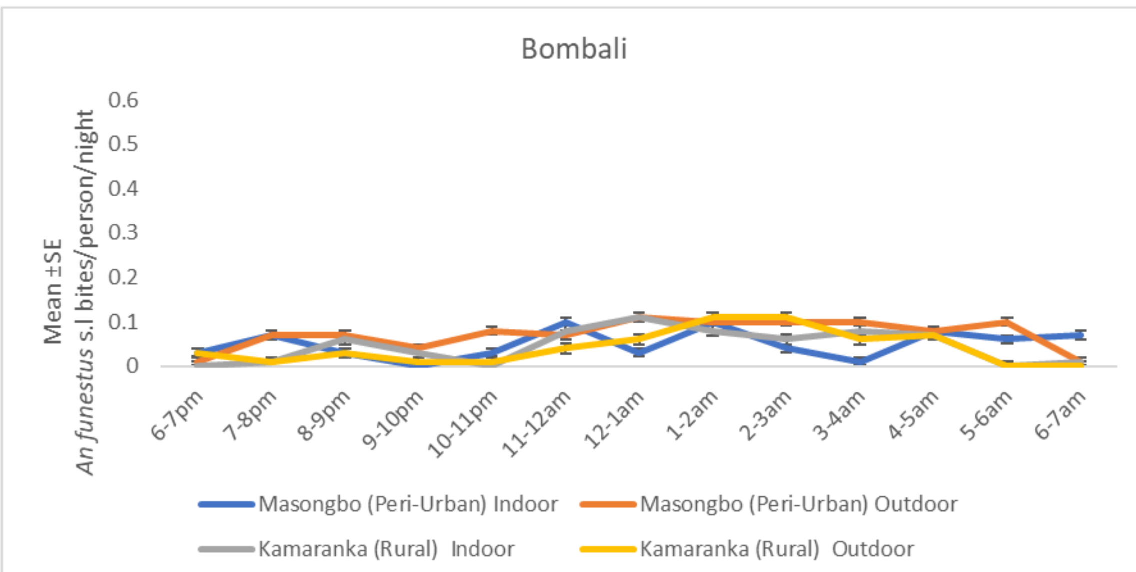
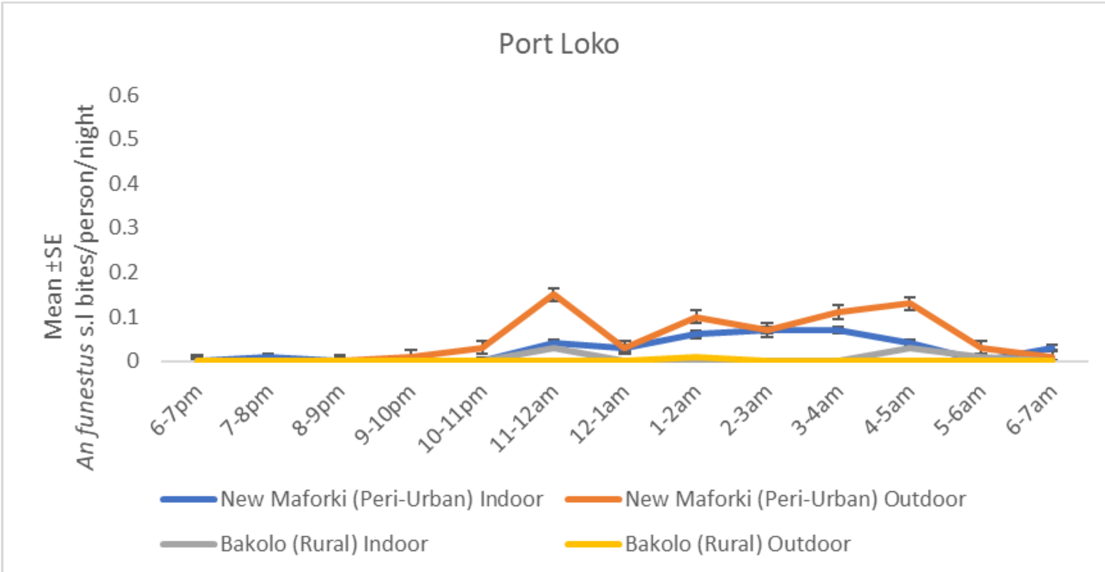
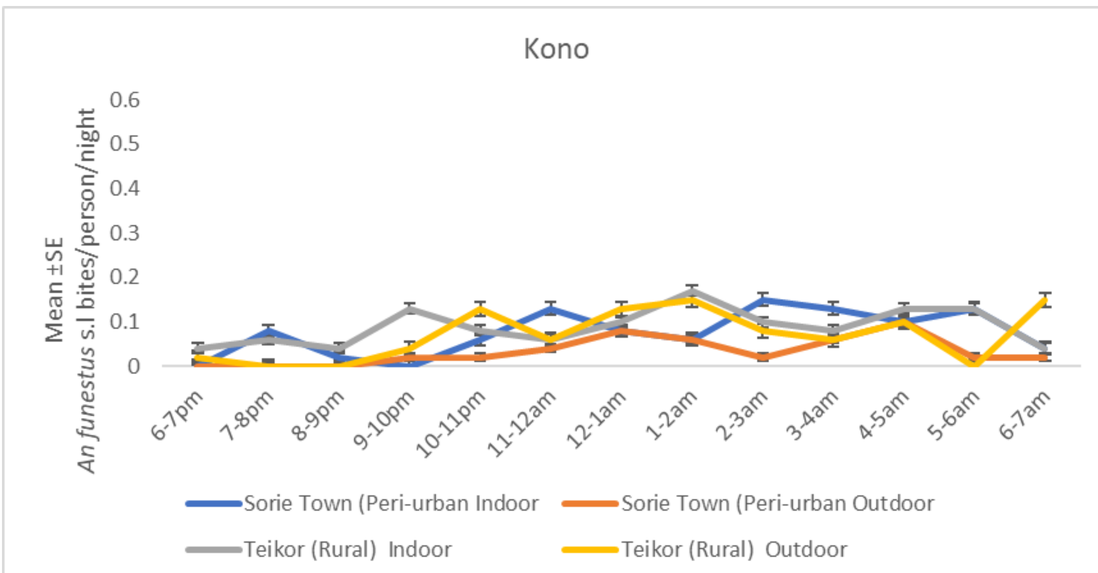

Western Area Rural
An. gambiae s.l bites/person/night
2
1.5
1
0.5
0
6-7pm
7-8pm
8-9pm
9-10pm
10-11pm
11-12am
12-1am
1-2am
2-3am
3-4am
4-5am
5-6am
6-7am
Sand Sand Water (Peri-urban) Indoor
Sand Sand Water (Peri-urban) Outdoor
Tombo (Rural) Indoor
Tombo (Rural) Outdoor

Figure 24: ***An. funestus*** **s.l. Biting Time in Bo, Bombali, Port Loko and Kono Districts, Comparing Indoor and Outdoor, March 2022–February 2023**









3.1.7 PARITY STATUS OF MALARIA VECTORS COLLECTED BY HLC AND PSC

A total of 1,592 malaria vectors collected using HLC were dissected to determine the parity status, whether they had laid eggs at least once or not (Annex E). *Anopheles gambiae* s.l. was the species most dissected (95.4 %; 1,592) followed by *An. funestus* group (4.6%; 73). From the 1,592 dissected mosquitoes, 81.4% (1,296) were parous, 13.5% (215) nulliparous, while 5.1% (81) were undetermined due to incorrect dissection that destroyed the ovary. The number of undetermined or destroyed ovaries in 2022 was lower compared to 2021 where about 7.7% were unreadable, indicating improved ovary dissections on the team. For those successfully identified, 85.8% (1,296) were parous while 14.2% (215) were nulliparous.

Parity status was examined by sentinel site and district by month. In all the districts, apart from New Maforki in Port Loko, there was no clear pattern in proportion of parous mosquitoes following the rainfall season. Parity rate remained high in many sites across the months with only few months having lower proportion of parous mosquitoes (Figures 25 - 27).

Figure 25: *An. gambiae* s.l. Parity Status Across Sentinel Sites, Comparing IRS and Non-IRS sites, March 2022–February 2023

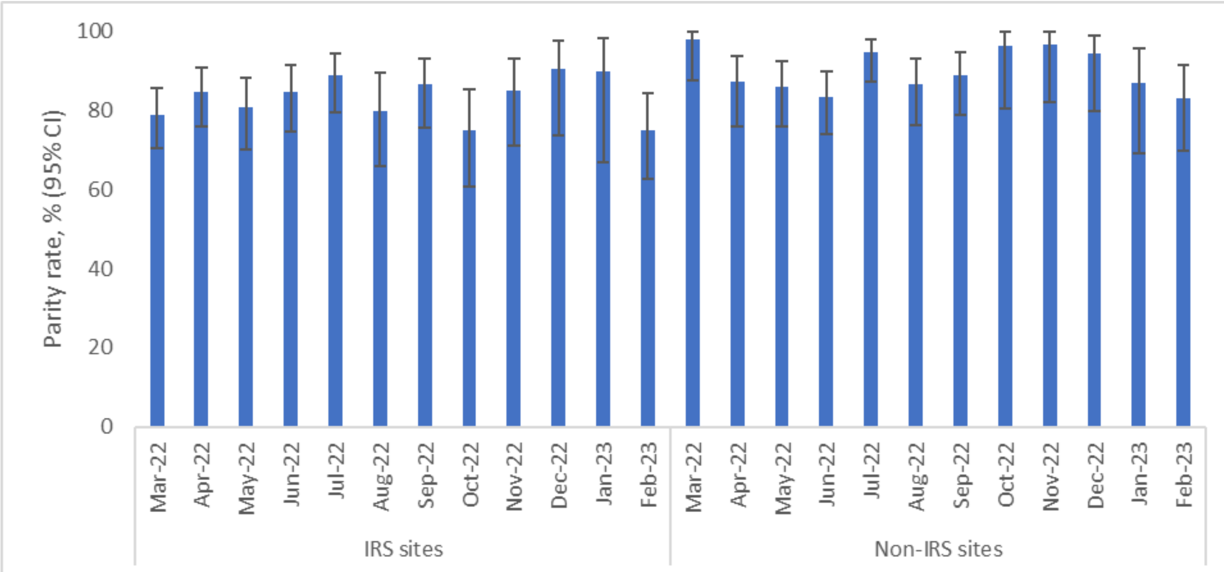
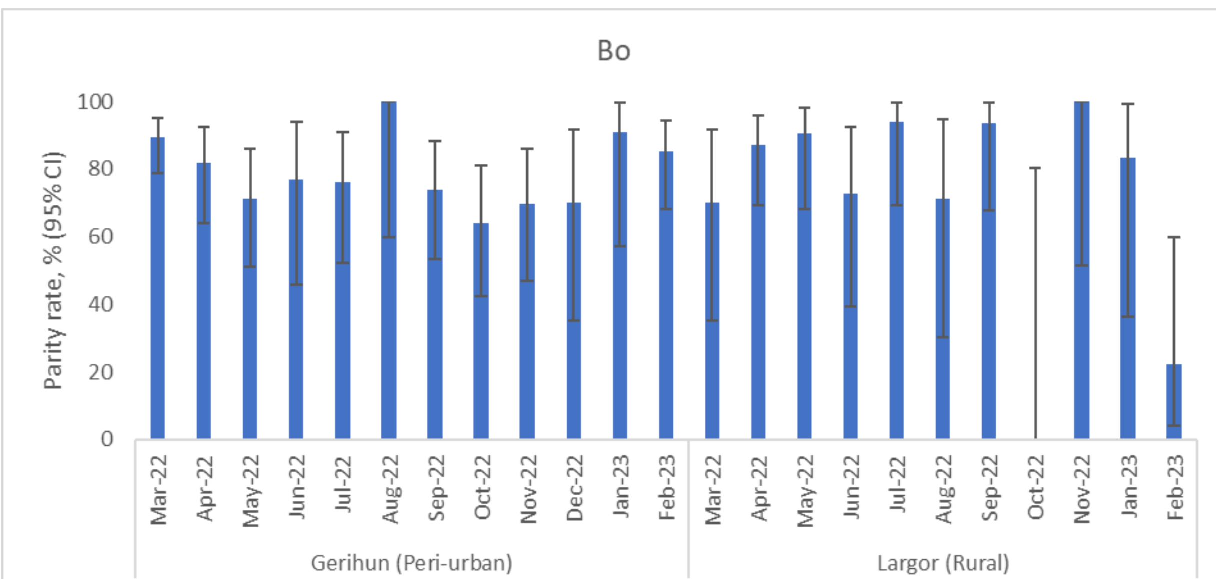


Figure 26: *An. gambiae* s.l. Parity Status in Bo and Bombali Districts, Comparing Peri-urban and Rural Sites, March 2022–February 2023



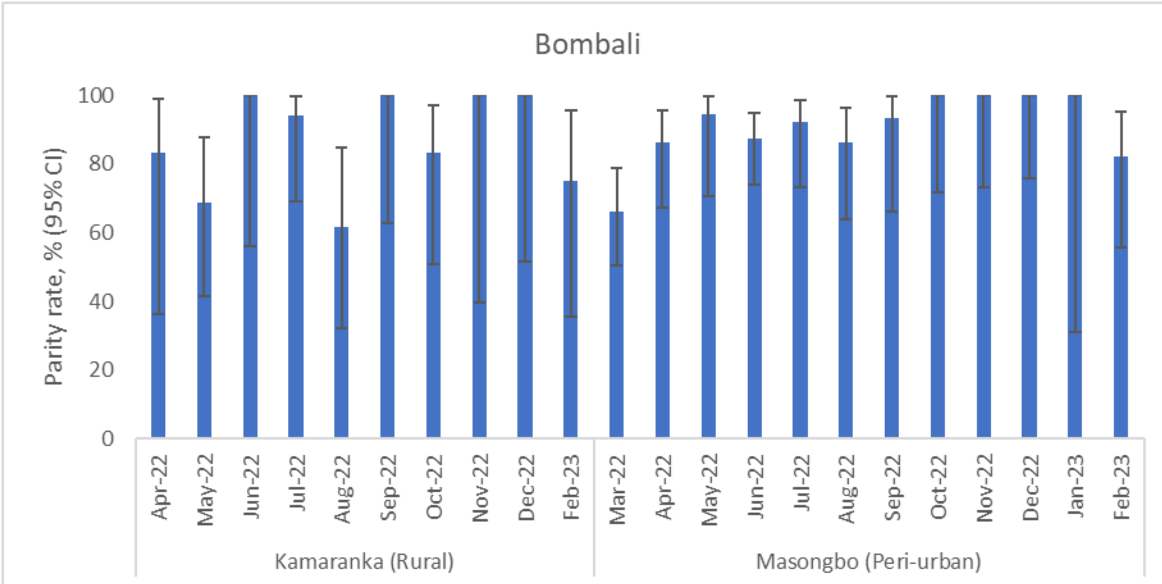
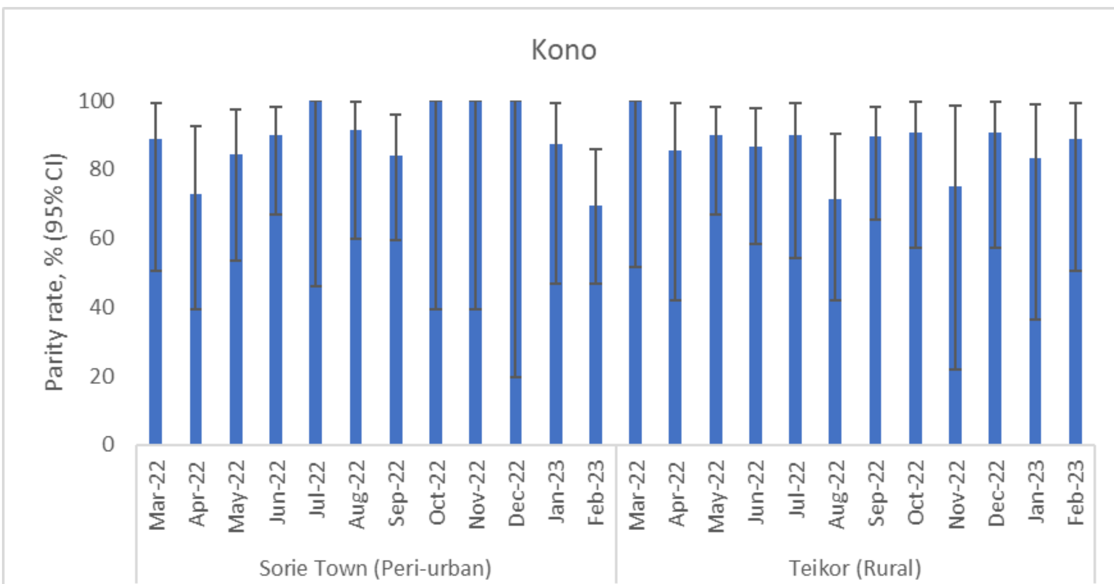
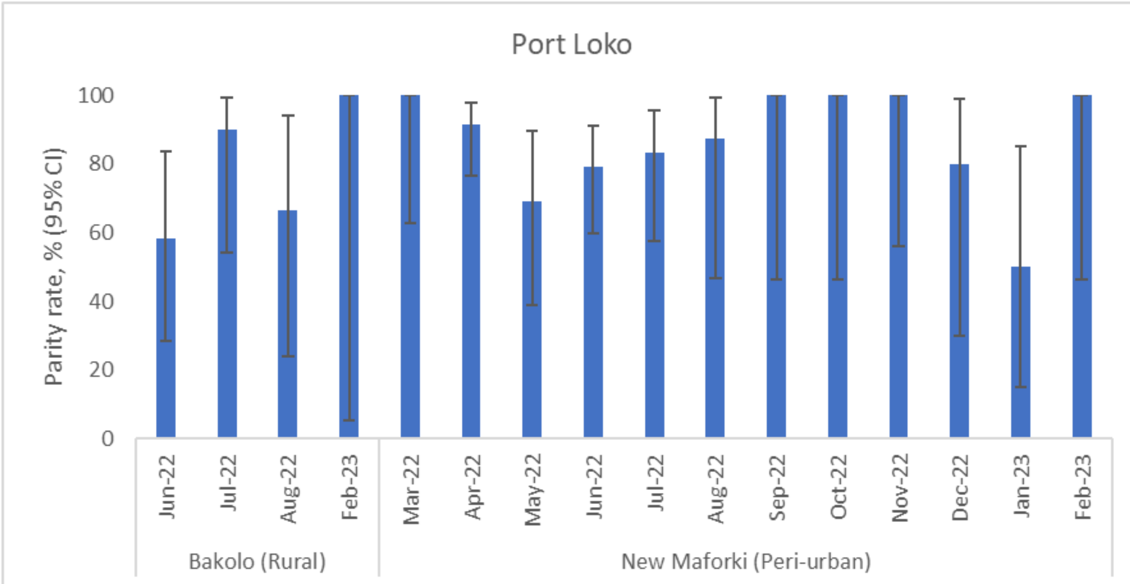
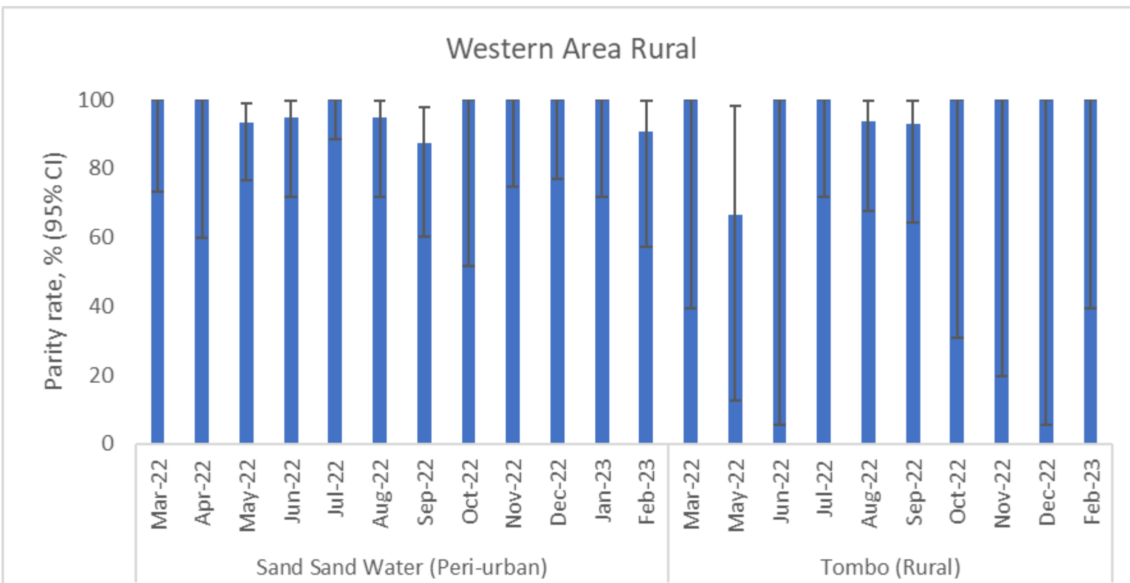


Figure 27: *An. gambiae* s.l. Parity Status in Kono, Port Loko and Western Rural Area Districts, Comparing Peri-Urban and Rural Sites, March 2022–February 2023







3.2 DECAY RATE OF SUMISHIELD (CLOTHIANIDIN) IN 2022

In May 2022, the quality of spray was assessed using WHO wall cone bioassays (VL SOP6) in Gerihun, Bo district and Masongbo, Bombali district within one week of spraying with SumiShield™. This formed the T0 of residual effectiveness monitoring (Figures 28 and 29). The assessment of decay rate thus began in June for SumiShield™. Within one week of IRS, over 95% mortality was observed in all sites on both mud and cement walls after day 3 (Figure 28 and 29). Mortality remained above 80%, the WHO minimum mortality threshold for IRS insecticide (Figure 30). 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 cone assays in houses 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 had painted mud or cement walls for the cone wall assays.

Figure 28: 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 (1-5 days post spray).

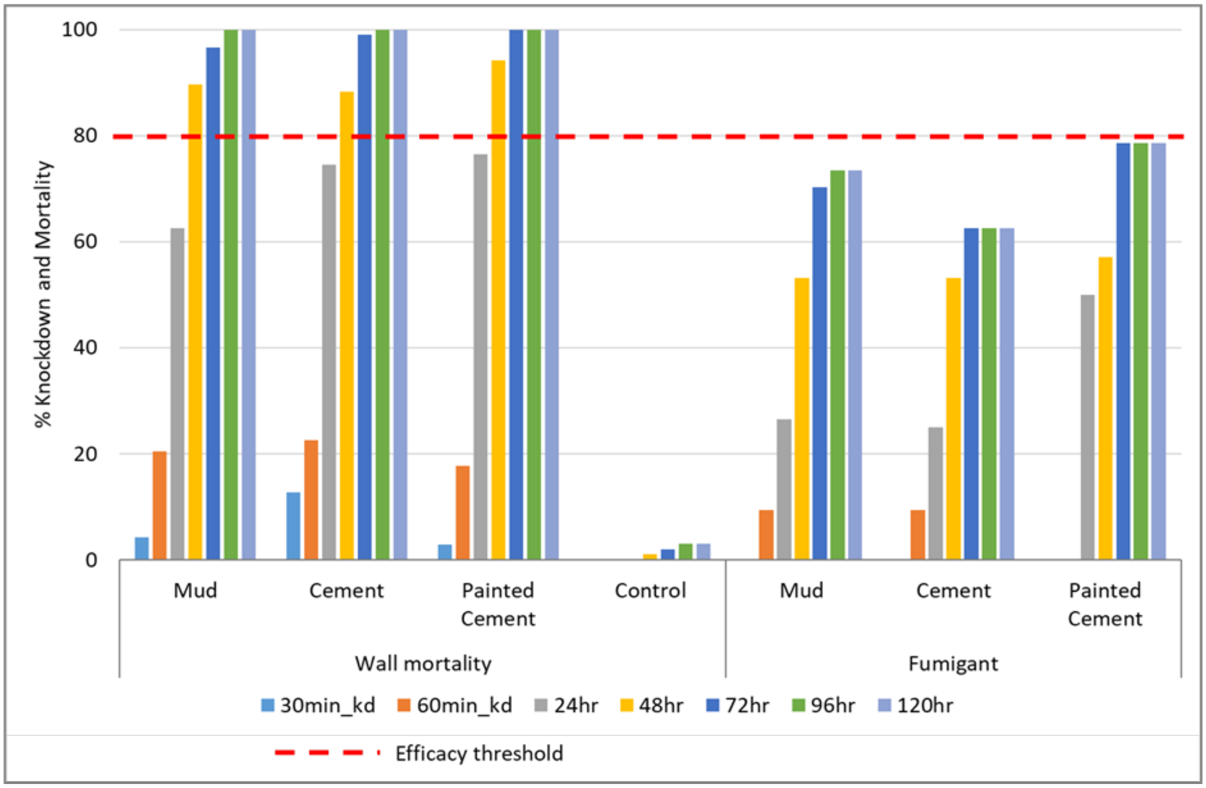


Figure 29: Knock-Down (30 and 60 mins) and Mortality of *An. gambiae* s.s. Kisumu Strain Exposed in WHO Cone Bioassay to SumiShield™ 50WG Sprayed Mud and Cement Walls in Gerihun in Bo District, Sierra Leone (1-5 Days Post Spray).

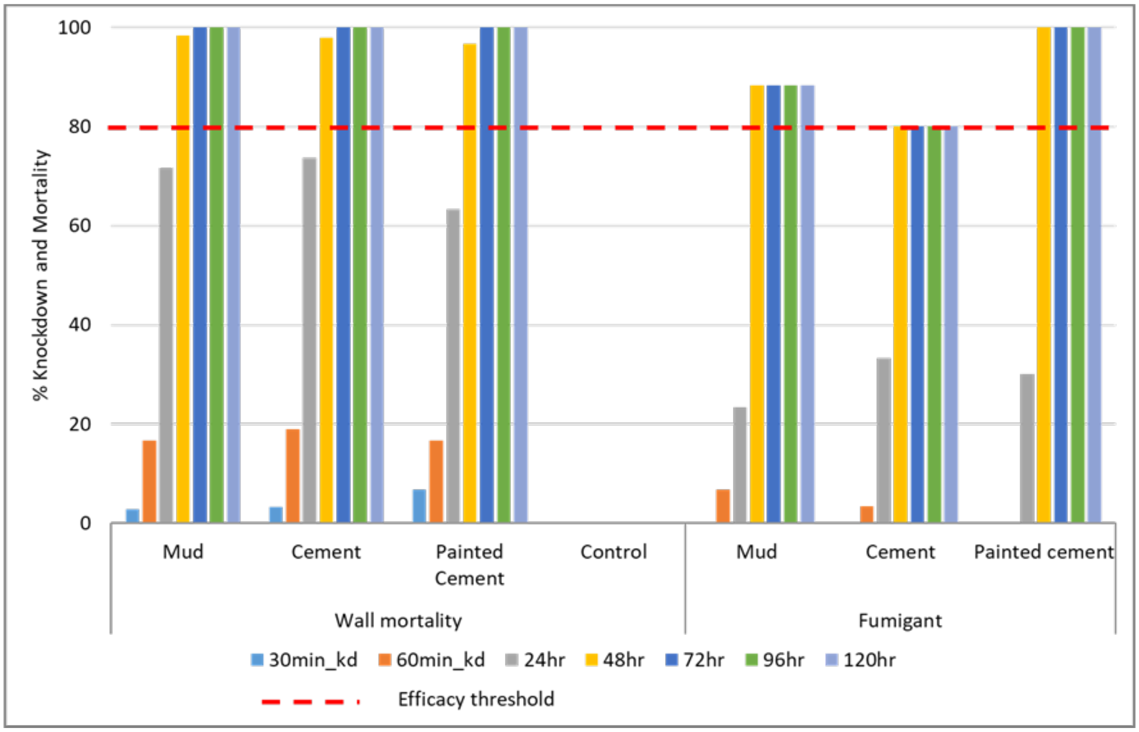
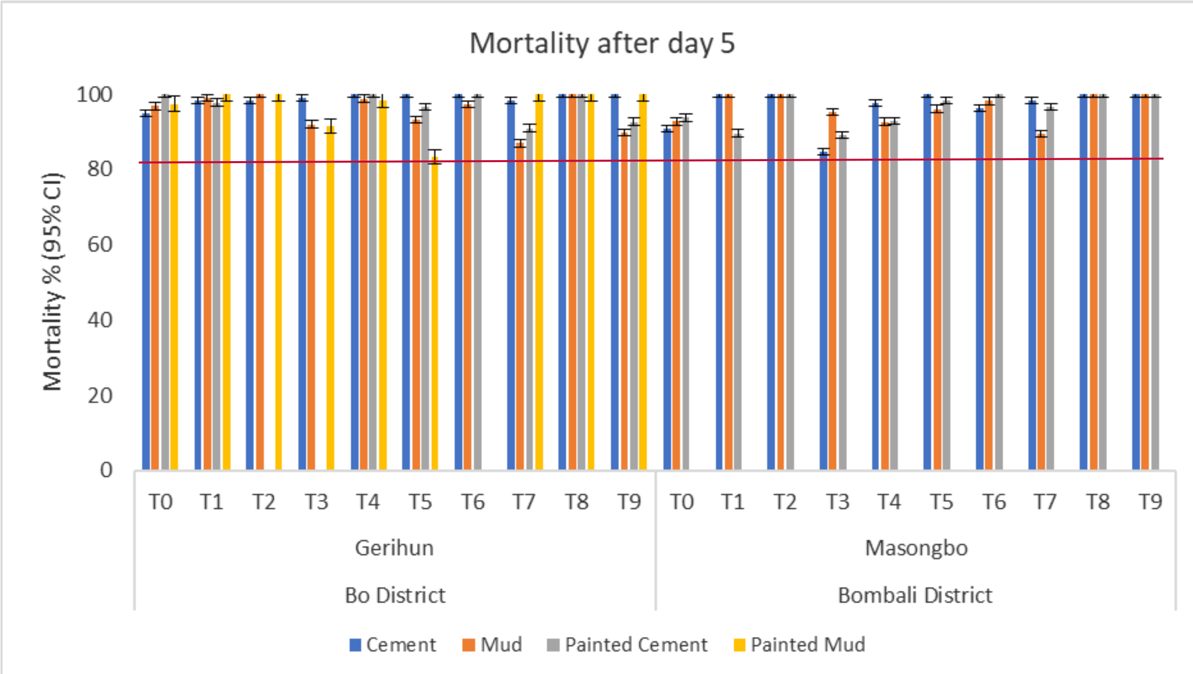


Figure 30: The WHO Cone Bioassay Mortality by Wall Type by Month Showing Decay Rate of SumiShield™ (Clothianidin) in Gerihun, Bo District and Masongbo, Bombali District Between May (T0) 2022-February (T9) 2023.



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 between March – August 2022 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, reared to adults and tested for phenotypic resistance were also sent to CRID to screen for molecular markers of insecticide resistance and identify sibling species. Another subset of samples collected between September 2022 and February 2023 were sent to Njala University, also for molecular analysis. This section reports on results of the molecular analysis done at CRID and Njala University. CRID completed the processing of mosquito samples collected between March and August 2022 while Njala University is still processing samples collected between September 2022 and February 2023. Only molecular species ID has been completed by Njala University; thus, only molecular species identification and insecticide resistance data is reported in this report; the full molecular analysis will be presented in an addendum report.

3.3.1 Species Identification

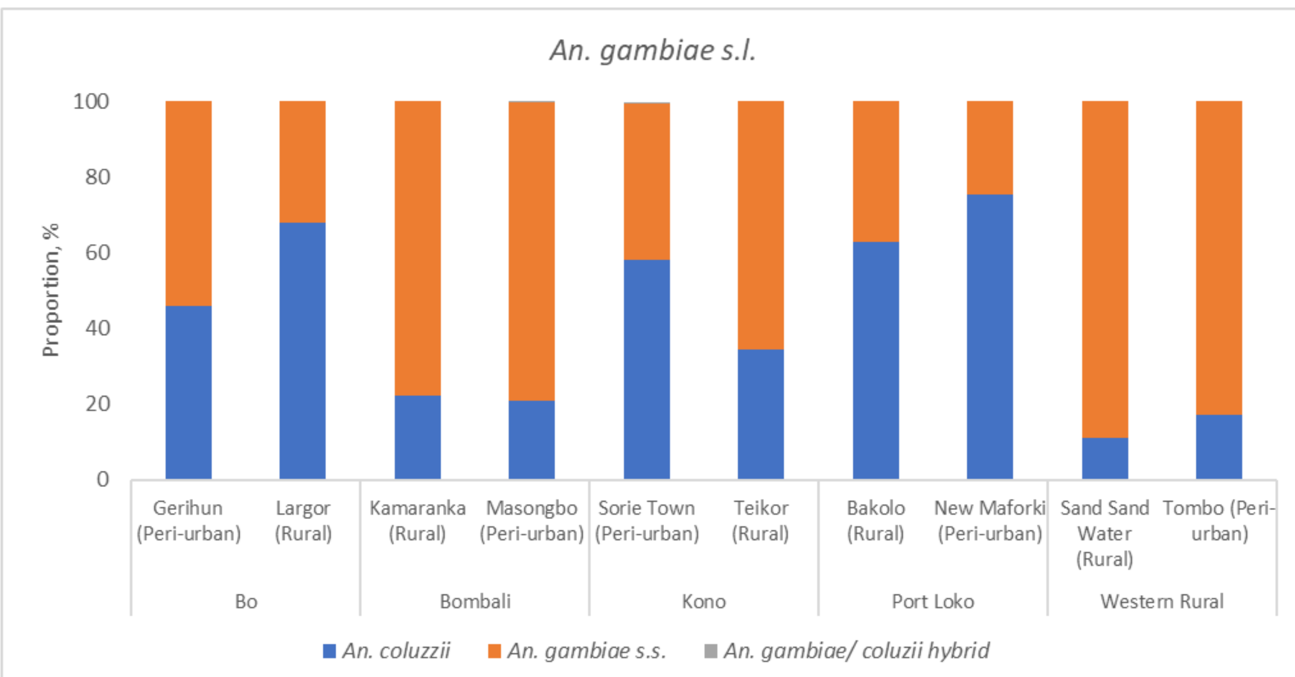
A total of 2,869 *Anopheles* mosquitoes sampled between March 2022 and February 2023 were analyzed for molecular species identification. Among these, 1,363 came from HLC collection, 993 from PSC & CDC-LT collection and 458 from phenotypic WHO susceptibility bioassays (Table 3). Out of the 2,869, a total of 2,814 (2,552 *An. gambiae* s.l. and 262 *An funestus* s.l.) were successfully identified to sibling species, translating to 98.1% (2,814/2,869) while 1.9% (55) were not identified.

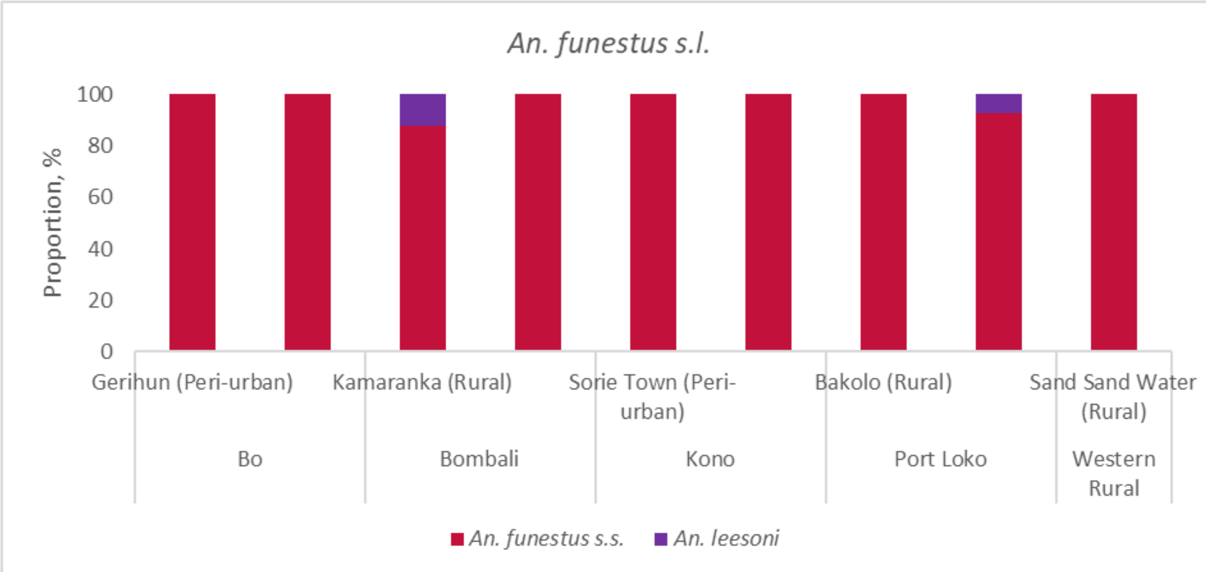
Table 3: Number of Samples Analyzed for Molecular Species Identification, March 2022-February 2023.

Districts	HLC	PSC and CDC	Resistance	Total
Bo	323	294	120	**737**
Bombali	278	210	122	**610**
Kono	268	221	60	**549**
Port Loko	276	108	99	**483**
Western Rural	218	160	57	**435**
Total	**1363**	**993**	**458**	**2814**

Within the *An. gambiae* complex, the majority (60.07%; 1,533/2,552) of the samples screened were *An. gambiae* s.s. followed by *An. coluzzii* 39.85% (1,017/2,552) and hybrid between *An. coluzzii/An. gambiae*, 0.08% (2/2,552). For *An. funestus* group, majority 98.5% (258/262) were *An. funestus* s.s. followed by *An. leesoni* 1.5% (4/262). There is no observable species change across the districts and the distribution followed the pattern in 2020-2021. The *An. gambiae* s.s. was predominant in Bombali, Kono and Port Loko districts while *An. coluzzii* was common in Bo and Western Area Rural. For *An. funestus* group, there was no change in composition as majority were *An. funestus* s.s. (Figure 31).

Figure 31: Molecular Species Distribution of *An. gambiae* s.l. and *An. funestus* s.l. Across Sampling Methods and Sites, March 2022–February 2023

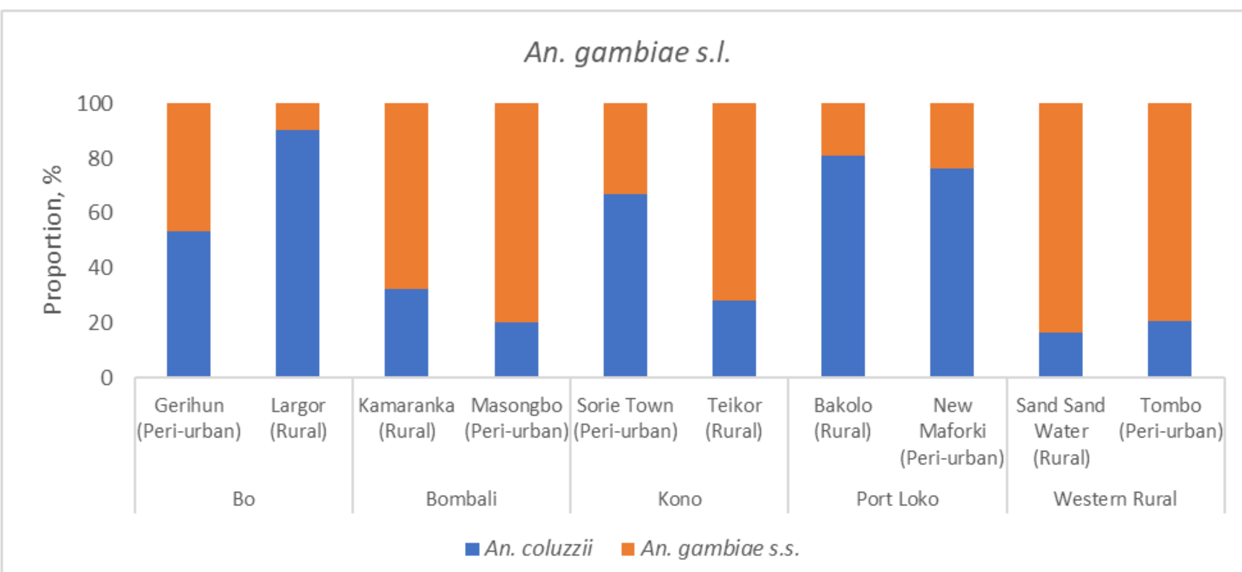


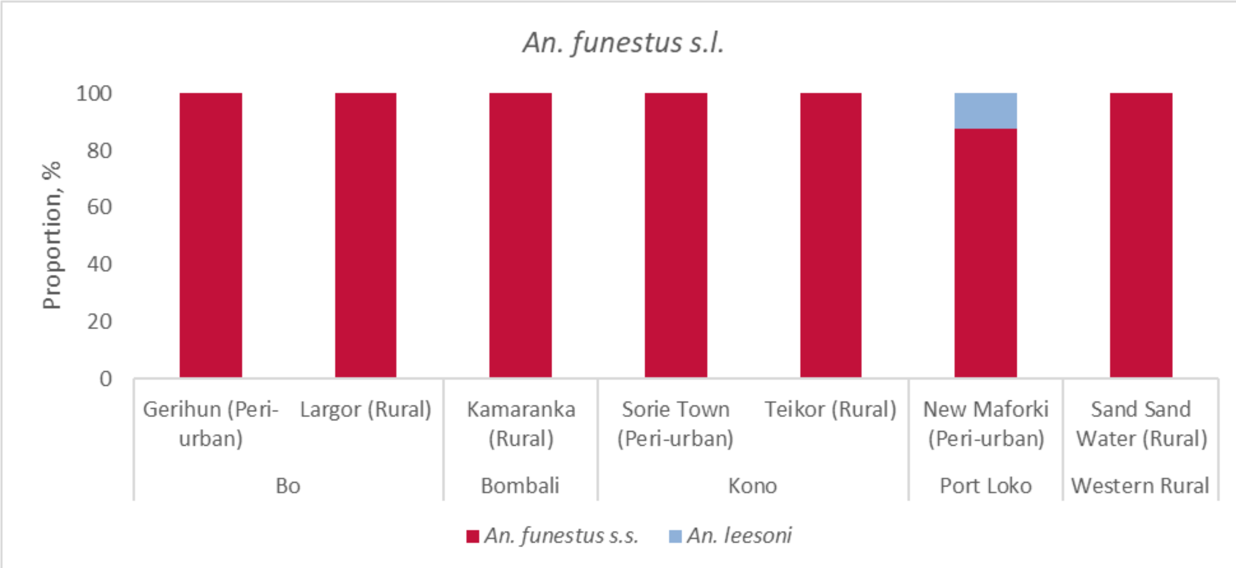


3.3.2 Species Distribution by District

HLC collections: All HLC samples that tested positive for *Plasmodium* infection and additional 50 random samples per site were identified to sibling species. The molecular species composition followed similar pattern observed in the previous year across all districts. In Largor and Gerihun (Bo district), Sorie Town (Kono district) and Bakolo and New Marforki (Port Loko district), *An. coluzzii* was the predominant species (Figure 32). 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 32). Within the *An. funestus* group, all were *An. funestus* s.s. apart from New Maforki (Port Loko) that had *An. Leesoni* (Figure 32).

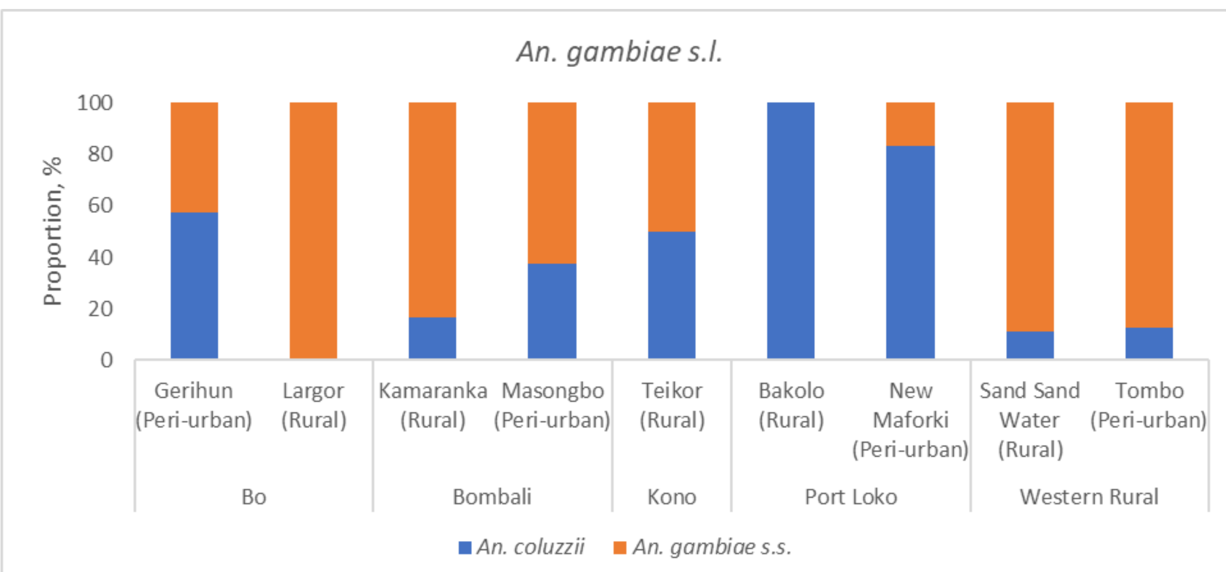
Figure 32: Molecular Species Distribution of *Anopheles gambiae* s.l. and *An. funestus* s.l. Samples Collected by HLC by District, March 2022–February 2023





PSC and CDC-LT Collections: Species distribution varied by district and site. Overall, *An. gambiae* s.s. was the most common in CDC-LT collections apart from Port Loko where majority were *An. coluzzii* (Figure 33). Similar pattern was observed in PSC collections, apart from Largor (Bo) which had more *An. coluzzii* (Figure 34). *An. funestus* s.s. was only sampled in three sites using CDC-LT; however, in PSC collections, the species was sampled in nearly all sites with the majority being *An. funestus* s.s. (Figure 33).

Figure 33: Molecular Species Distribution of *Anopheles* Samples Collected by CDC-LT Across Districts, March 2022–February 2023



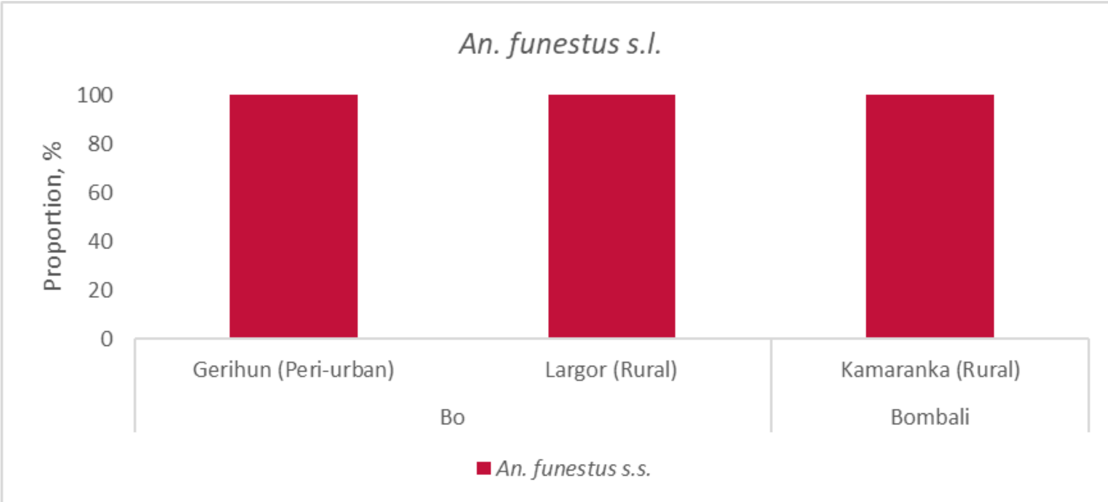
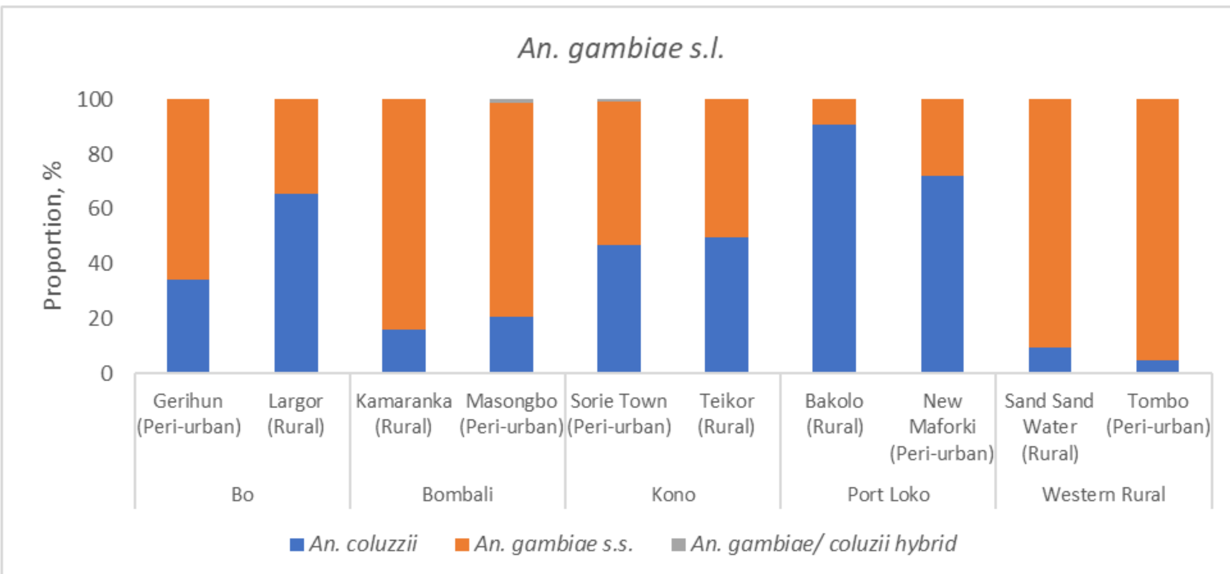
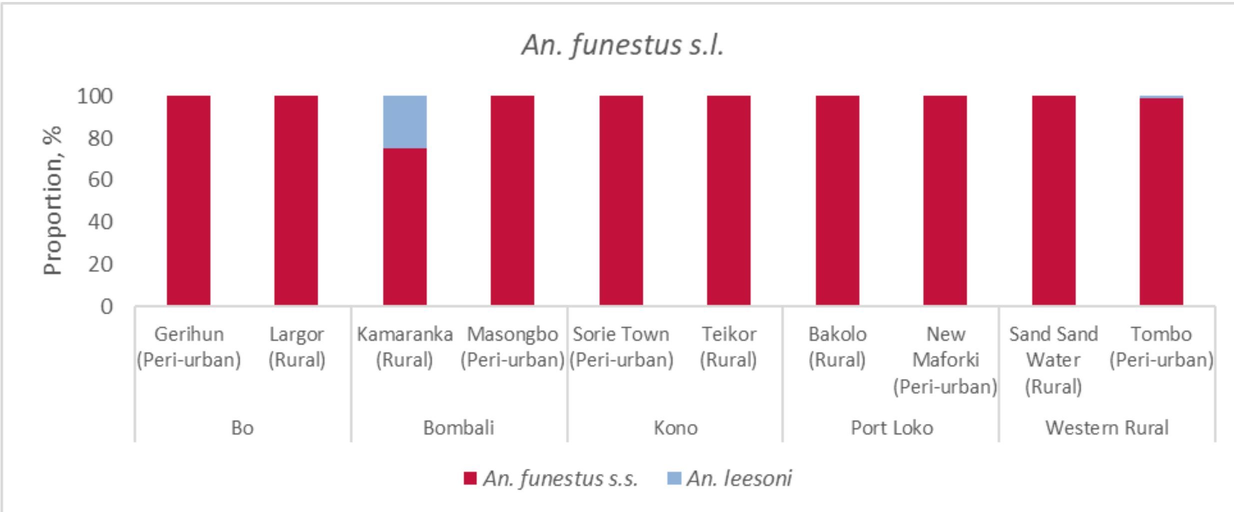


Figure 34: Molecular Species Distribution of *Anopheles* Samples Collected by PSC Across Districts, March 2022–February 2023





3.3.3 *Plasmodium* Infection

Due to delays in obtaining ELISA results from Njala University, this will be reported in addendum report.

3.3.4 Entomological Inoculation Rate

Due to delays in obtaining ELISA results from Njala University, this will be reported in addendum report.

3.3.5 Blood Meal Origin

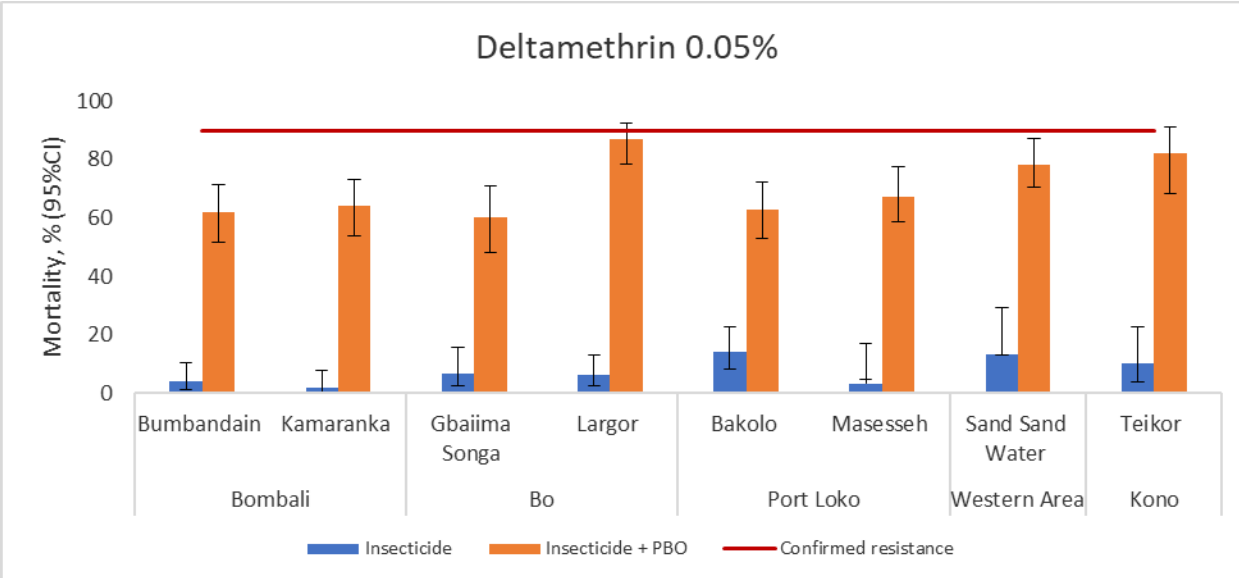
Due to delays in obtaining ELISA results from Njala University, this will be reported in addendum report.

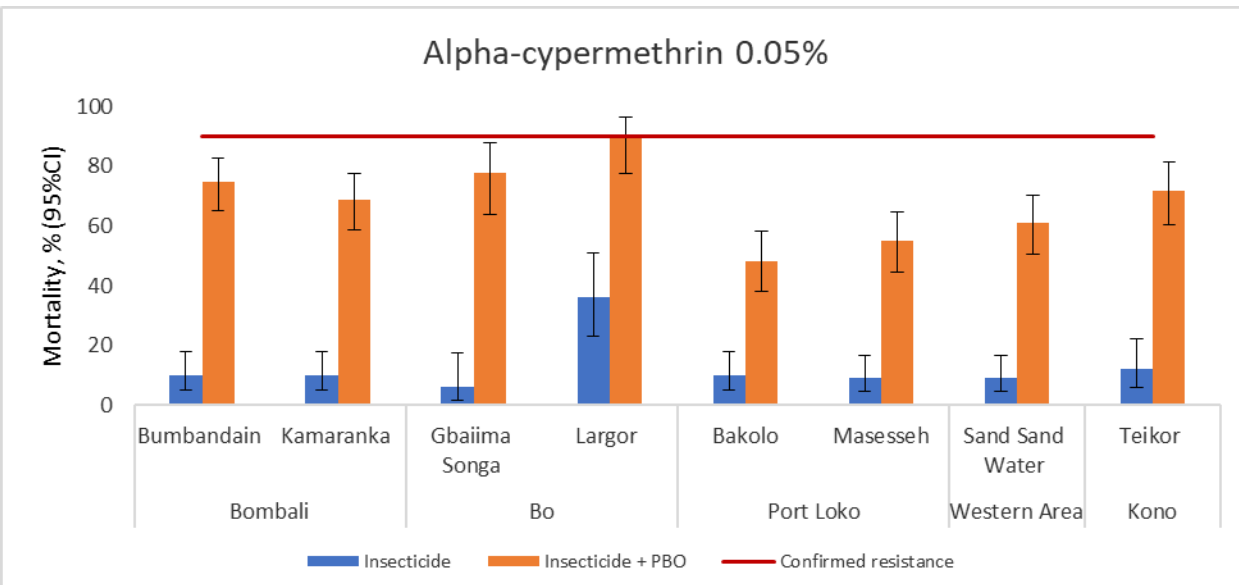
3.4 Insecticide Resistance Monitoring

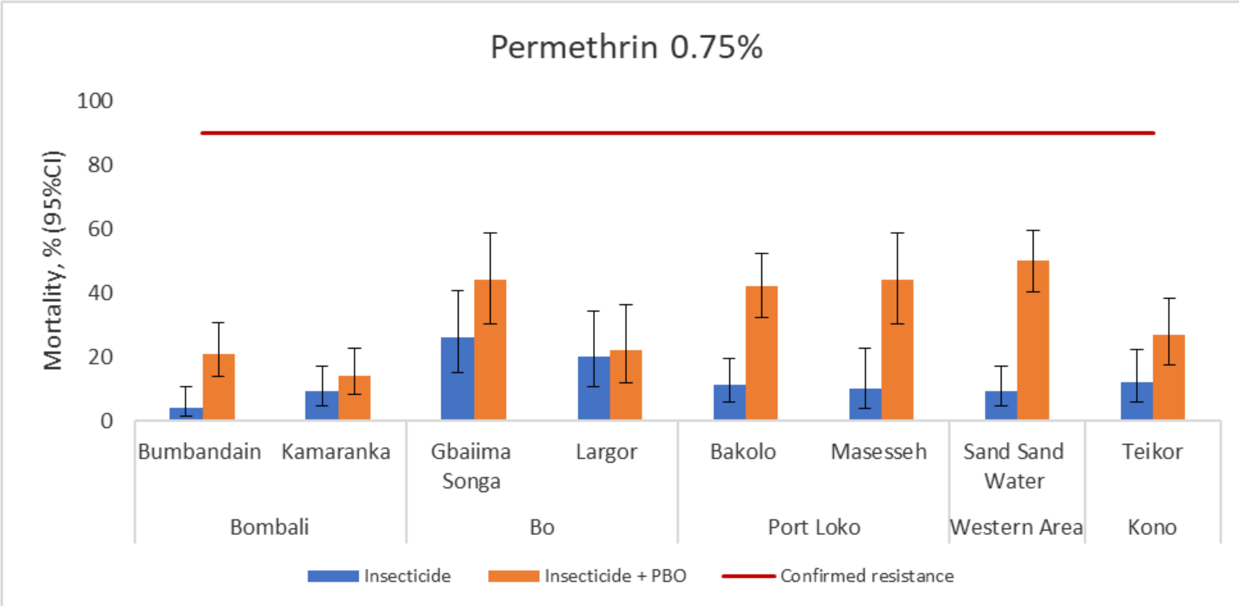
3.4.1 Synergist Assays

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 35). Resistance was high in 2022 and mortality ranged from 2% in Kamaranka (Bombali District) to 10% in Teikor (Kono District) for deltamethrin; 4% in Bumbadain (Bombali District) to 26% in Gbaima Songa (Bo District) for permethrin, and 6% in Gbaima Songa (Bo) to 36% in Largor (Bo) for alpha-cypermethrin (Figure 35; Annex F). The PBO partially restored susceptibility to all the pyrethroids, suggesting the partial involvement of monooxygenase-based resistance mechanism in Sierra Leone (Figure 35). The minimum increase in mortality after PBO also varied by insecticide and site (Figure 35). For Permethrin, PBO increased mortality by a minimum of 2%, 49% for deltamethrin, and 38% for alpha-cypermethrin. The lowest restoration of susceptibility was for permethrin-PBO, where its mortality was restored by a range of 2-41%. Mortality after pre-exposure to PBO in all the three pyrethroids was less than 90% in all but only one site (Largor) when tested against alpha-cypermethrin. Mortality after pre-exposure to PBO and then permethrin ranged between 14 and 50%.

Figure 35: Susceptibility of *An. gambiae* s.l. to Deltamethrin 0.05%, Permethrin 0.75% and Alpha-cypermethrin 0.05% With or Without PBO in 2022



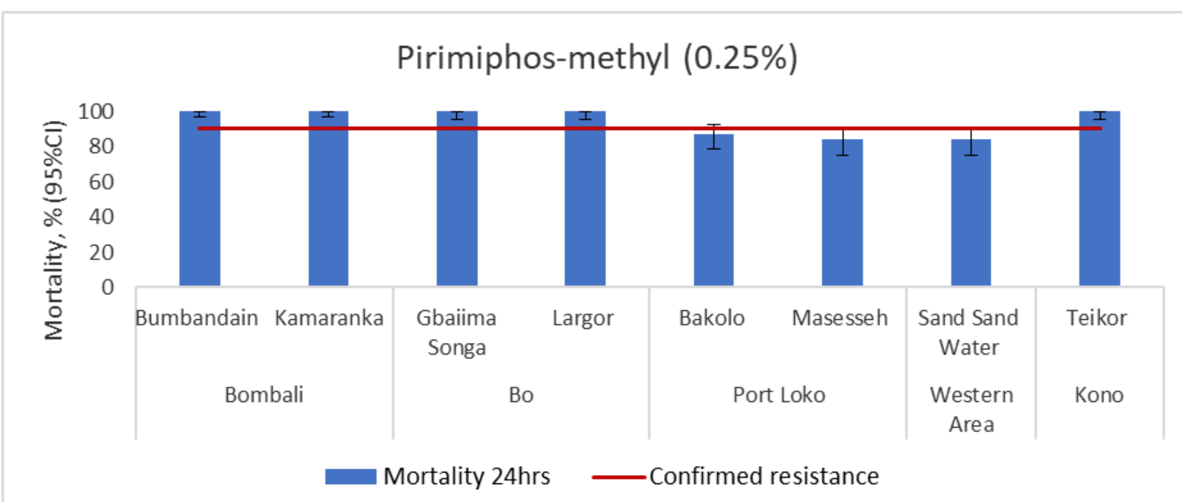




3.4.2 *An. gambiae* s.l. Susceptibility to Organophosphates

An. gambiae s.l. were exposed to pirimiphos-methyl (0.25%) in Bo, Bombali, Western Rural, Kono and Port Loko (Figure 36). There was resistance to pirimiphos-methyl in Port Loko and Western Area districts only. This varies from previous year where there was reduced susceptibility to pirimiphos-methyl in all sites tested. Pirimiphos-methyl susceptibility has varied in the previous three years, and it is not clear whether it is due to impregnation of the papers supplied or different cohorts of vectors sampled each year. Since different mosquito populations may be sampled, variability in susceptibility is always observed especially if the majority of larvae was collected from one habitat with few family lines. The frequency of *Ace-1* mutation that confers resistance to organophosphates and carbamates was 2.2% in 2022 compared to the previous year of 14.6%. This indicates that the vector population sampled were susceptible in 2022 and is a major reason for variability in resistance pattern in Sierra Leone. The project will aim to intensify and collect larvae from as many sources as possible to have robust vector composition. (Table 3). To confirm that the WHO test papers used were effective; the team exposed susceptible *An. gambiae* Kisumu to the test papers, which resulted in 100% mortality.

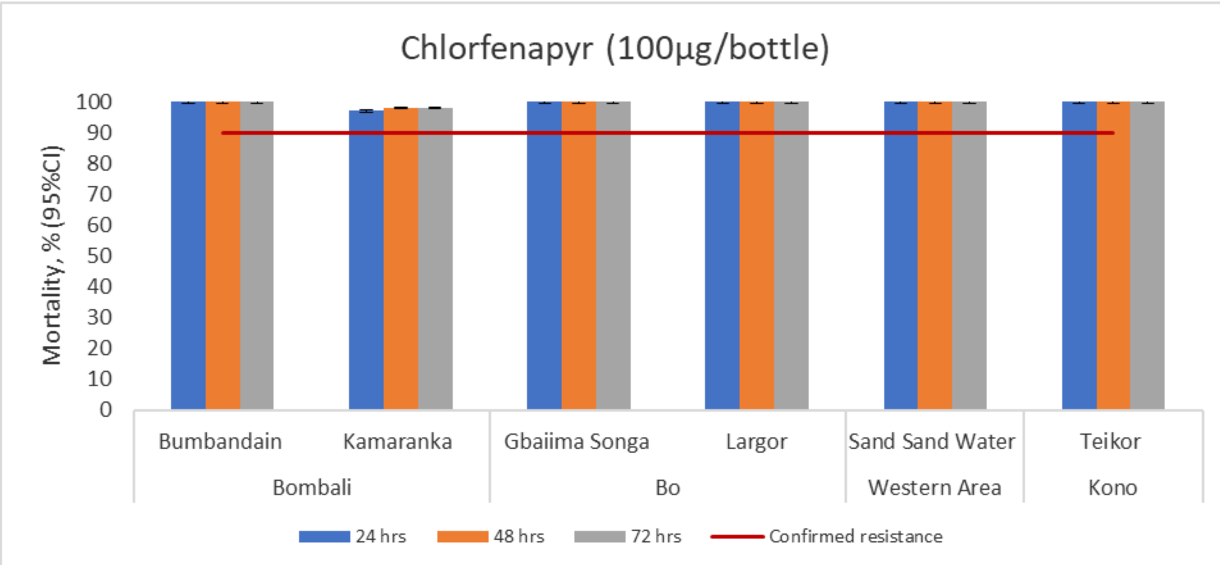
Figure 36: Susceptibility Status of *An. gambiae* s.l. to Pirimiphos-methyl (0.25%) in 2022-2023



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

The *An. gambiae* s.l. mosquitoes from Bo, Bombali, Kono and Western Rural that were exposed to chlorfenapyr were fully susceptible with all mosquitoes dying after day 2 of exposure (Figure 37).

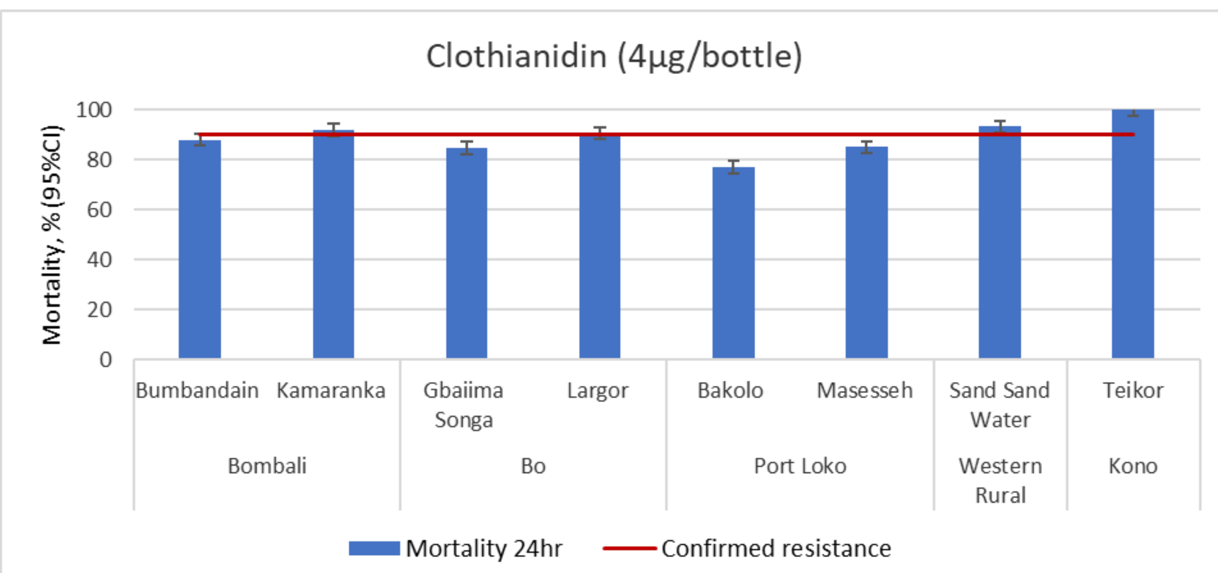
Figure 37: Susceptibility of *An. gambiae* s.l. to Chlorfenapyr (100 µg/Bottle) in 2022



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

There is emerging resistance to clothianidin in all districts where malaria vectors were tested against clothianidin (Figure 38) apart from Kono where there was full susceptibility. The project is aiming to do additional tests this year in 2023 using mosquitoes derived from as many larval breeding habitats in order to monitor this resistance whose molecular mechanism is not yet fully understood.

Figure 38: Susceptibility of *An. gambiae* s.l. to Clothianidin (4µg/bottle) in 2022



3.5 PCR Analysis for Mechanism of Resistance

In 2022, 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 499 mosquitoes out of 7,100 were assayed for molecular species identification and screened for resistance mutations. Of these, 41 samples did not amplify and were not identified to species level. Of the remaining 458 that were successfully identified, 87.12% were *An. gambiae* s.s. while 12.66% were *An. coluzzii* with one hybrid (0.22%) of *An. gambiae* s.s. and *An. coluzzii*. This distribution is consistent with the species identification on insecticide resistance samples collected in 2021.

In all sites, *kdr-w* mutation was fixed or approaching fixation in the sampled *An. gambiae* s.s. mosquitoes indicating high level of pyrethroid resistance in Sierra Leone in this species (Table 4). The *kdr-w* mutation in *An. coluzzii* is also approaching fixation, and the frequency in all sites was above 50% (Table 4). The *kdr-e* mutation was also found in few samples in very low frequencies in both species indicating that it is not a common mutation typical of West Africa (Table 3). In 2018, the frequency of *kdr-e* was about 2%; however, in 2021, there was no sample carrying *kdr-e* mutation (Table 4); therefore, there is a need for further monitoring of the trend and its implication. The *Ace-1*mutation that confers resistance to carbamates/organophosphates is present in Sierra Leone and the frequency dropped down from 21.9% in 2022 to 2.2% in 2022. This probably explains the lower mortality against pirimiphos-methyl observed in 2022 across all districts, except Western Area and Kono (Table 4). Since these classes of insecticide (carbamates/organophosphates) are not used for malaria vector control, it will be crucial to work with the agriculture department to monitor the trend in agricultural pesticides use 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 1.1% (Table 5).

Table 4: 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 December 2022 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. gambiae*	87	7	2	0.94	90.6	0	2	97	0.01	0.0	0	0	97	0.00	0.0
	An. coluzzii	7	9	2	0.64	38.9	0	10	8	0.28	0.0	0	0	18	0.00	0.0
Bombali	*An. gambiae*	104	4	3	0.95	93.7	1	7	101	0.04	0.9	0	11	99	0.05	0.0
	An. coluzzii	6	4		0.80	60.0	0	1	9	0.05	0.0	0	0	10	0.00	0.0
	An. gam + An. coluzzii (Hybrid)	1	0	0	1.00	100.0	0		1	0.00	0.0	0	0	1	0.00	0.0
Kono	*An. gambiae*	31	1	12	0.72	70.5	1	11	32	0.15	2.3	0	3	41	0.03	0.0
	An. coluzzii	11	3	2	0.78	68.8	0	3	13	0.09	0.0	0	1	15	0.03	0.0
Port Loko	*An. gambiae*	88	0	0	1.00	100.0	0		88	0.00	0.0	3	28	56	0.20	3.4
	An. coluzzii	6	3	2	0.68	54.5	0	5	6	0.23	0.0	0	0	11	0.00	0.0
Western rural area	*An. gambiae*	52	0	0	1.00	100.0	0	0	56	0.00	0.0	7	12	35	0.24	13.0
	An. coluzzii	87	0	1	0.00	0.0	0	1	0	0.50	0.0	0	0	1	0.00	0.0
Grand Total		393	31	24	0.91	87.7	2	40	411	0.05	0.4	10	55	384	0.08	2.2

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 5: Distribution of Insecticide Resistance Mutation to Pyrethroids (N1575Y), in *An. gambiae* s.l., Sampled in the Rainy Season between May and December 2022 in Sierra Leone.

District	PCR M/S	YY	YN	NN	f (Y)	%YY
Bo	*An. gambiae*		12	86	0.06	0.0
	An. coluzzii		1	17	0.03	0.0
Bombali	*An. gambiae*	3	9	98	0.07	2.7
	An. coluzzii			10	0.00	0.0
	An. gam + An. col (Hybrid)			1	0.00	0.0
Kono	*An. gambiae*		6	38	0.07	0.0
	An. coluzzii			16	0.00	0.0
Port Loko	*An. gambiae*	1	7	77	0.05	1.2
	An. coluzzii		1	10	0.05	0.0
Western rural area	*An. gambiae*	1	3	45	0.05	2.0
	An. coluzzii			1	0.00	0.0
Total		5	39	399	0.06	1.1

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 6). There was no *Kdr-w* and *Kdr-e* mutations in the samples tested. These were F21, F22 and F23 generations reared from eggs received from MR4 in December 2021. Two samples could not be established because of amplification challenges.

Table 6: 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	f(W)	EE	ES	SS	f (E)
Freetown	*An. gambiae* s.s.	0	0	50	50	0	0	50	0.0
Makeni	*An. gambiae* s.s.	0	0	50	50	0	0	50	0.0
Total		**0**	**0**	**100**	**100**	**0**	**0**	**100**	**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

In April 2022, VectorLink Sierra Leone conducted entomology training to field supervisors, technicians and selected vector collectors on entomology and entomological surveillance techniques. This training was composed of 24 field supervisors within the district health management teams (DHMT) in Bo, Bombali, Karene, Port Loko, Kono, Western area Rural, as well as six seasonal entomology technicians. The training was led by the VectorLink Technical Manager and supported by the VectorLink field entomology technicians. The participation of VectorLink field technicians as facilitators allowed them to strengthen their entomology skills while learning how to share knowledge with others. The trainees were taught about the different malaria vectors, their ecology and behaviors and how to sample them using various tools like PSC, HLC, CDC-LT and also using larval dippers to sample juvenile stages of mosquitoes. The training was an avenue to listen to challenges and experiences of supervisors during routine monthly monitoring in our sentinel sites.

In August 2022, the VectorLink project successfully launched the electronic data collection in Western rural Area district following a successful training of the VectorLink entomology team on how to use mobile devices to enter data. This training was led by the Entomology Monitoring and Evaluation Specialist from VectorLink home office supported by the in-country entomology Database Manager and Technical Manager. The VectorLink project has now rolled the electronic data capture in all districts, and the last district to fully migrate will be Kono during the May 2023 routine entomological surveillance. During the rollout in the districts, all sites entered data both on paper-based forms and on VLC mobile Application to verify and scrutinize data quality. By July 2023, the VL Sierra Leone project shall be fully transitioned to electronic data entry.

Between November and December, the VectorLink Sierra Leone project, led by the VectorLink Regional Molecular Entomologist and supported by the Technical Manager undertook a molecular training to Njala University laboratory technicians on how to process mosquitoes using molecular entomological assays. A total of six Njala university technicians and two field entomology technicians benefited from the highly skilled training on molecular entomology. The team of eight learned how to receive mosquito samples from the field, bisect the mosquitoes to prepare them for assays, conduct DNA extraction, use molecular assays to identify sibling species and screen for insecticide resistance mutations. The participants were also trained on theory of ELISA and how it is used to detect mosquitoes infected with *Plasmodium* parasites and identify source of blood meal. A second phase of training on how to perform ELISA will be conducted when the current 2022 samples are set to be tested by ELISA.

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 and implemented with the NMCP an assessment tool (standard operating procedure) that gauged standard laboratory practices within each laboratory. The laboratories were assessed on three broad areas: i) Laboratory structure and management (including equipment), ii) Good laboratory practices and availability of competent staff, and iii) Collaboration structures and existing collaboration including student programs.

While Njala university has begun processing the samples, it is still slow due to operational and logistical plans, which the VectorLink project is planning to help improve as we fully transition to use Njala University to process molecular samples. In order to ensure data quality, CRID in Cameroon will still be able to run quality control checks to ensure accurate and quality data for Sierra Leone. In addition, VectorLink Sierra Leone is at an advanced stage of establishing a container laboratory " to support sample storage and reception at the Bo Campus, paramedical school that is hosting the laboratory. This will be in place by the end of June 2023 as the project prepares to transition into PMI Evolve. The project has conducted a Vector Control Needs Assessment (VCNA) to document and highlight key gaps and strengths in vector control program in Sierra Leone to develop an integrated vector management strategy. The final VCNA document is expected to be finalized by end of July 2023. The project will work in close collaboration with Malaria Consortium to undertake this activity and present the document to aide in planning activities as the project transitions to PMI Evolve.

5. Discussion, Lessons Learned / Challenges, and Recommendations

5.1 Discussion

In Sierra Leone, *An. gambiae* s.l. is still the principal malaria vector with 81.8% (13,630), followed by *An. funestus* s.l. with 17.6% (2,941). This distribution pattern was consistent both in IRS and non-IRS sites. Since 2021, there is a general decline in mosquito density and parameters in IRS sites, although higher density of mosquitoes was identified through collections in IRS sites compared to non-IRS sites and affirms the choice of Bo and Bombali as the IRS districts due to high malaria risk as measured by entomological indicators-of malaria risk. Within *An. gambiae* s.l. complex, *An. gambiae* s.s. is still the major vector (60.07%) followed by *An. coluzzii* (39.85%) and hybrid of *An. gambiae/An. coluzzii* at 0.08%. This composition is similar to the 2021 period, but varies from the 2020 period when *An. colluzzii* was the predominant vector identified at 64%. Within the *An. funestus* group, the majority of 98.3% (469) were identified as *An. funestus* s.s. and 1.7% (8) as *An. leesoni*. From 499 samples collected as larvae and reared to adults during insecticide susceptibility exposure assays, *An. gambiae* s.s. was the predominant sibling species followed by *An. coluzzii*. This follows previous years' data indicating that *An. gambiae* s.s. is the major vector followed by *An. coluzzii* within the *An. gambiae* s.l. complex. There is no major species change following two rounds of IRS with *An. gambiae* s.l. being the major vector in both IRS and non-IRS sites.

The presence of *An. funestus* s.l. in IRS sites with a peak at the beginning of dry season in December poses challenges for IRS implementation because of the need to have an insecticide with long residual life on the walls. Indeed SumiShield™ has remained effective at killing mosquitoes up 10 months but because the country has sprayed SumiShield™ for a third year in a row in 2023 and there is emerging resistance, the NMCP is seeking recommendations for a switch to a differnet class of insecticide. The emerging insecticide resistance to clothianidin was confirmed in December 2022 after the procurement process had been completed for 2023 IRS campaign.The high *An. funestus* s.l. density in Bo and Bombali could be as a result of irrigation farming during that period and controlling this vector willl require coordinated efforts from other departments like ministries of agriculture, environment and planning.

Similar to previous reports, 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 HBR in IRS sites continued to decline compared to previous years although the average HBR still remained high in IRS districts. The biting pattern, however, varied by district, but major biting activity happened from 10:00pm to 5:00am overall. The highest indoor HBR was recorded in June in Largor, Bo district with 49 bites/person/night compared to previous year 2021 where the highest was recorded in Masongbo, Bombali district with 80.6 bites/person/night. In Bombali, in 2022, the highest HBR was recorded in June in Masongbo (29.3 bites/person/night), representing over 50% progressive reduction in IRS sites (2020 and 2021 Sierra Leone Annual Entomological reports). In a separate analysis, the evaluation of impact of PBO ITN and IRS with clothianidin indicated that HBR reduced significantly in IRS sites. Further analysis is ongoing to investigate this further and will be reported on separately.

In all districts, the HBR followed the rainfall pattern with a peak observed between June to September and declining into the dry season. There was no clear outdoor/indoor biting pattern difference in all sites except Masongbo in Bombali and New Maforki in Port Loko district where more outdoor biting was observed. The mean HBR of *An funestus* s.l. did not follow the rainfall pattern with highest peak observed outdoor during the dry season in December in Gerihun, Bo district with 13 bites/person/night. In Masongbo

(Bombali) and Sorie town (Kono), the highest HBR was also outdoor and presents an interesting trend to monitor given *An. funestus* is predominantly indoor feeder.

Malaria vectors in Sierra Leone still have high access to blood meal as indicated by the high number of blood fed mosquitoes collected resting indoors. Previous reports indicate that over 90% of all blood meals are from humans and therefore stronger communication should be carried out by NMCP to encourage ITN use. The country is set to distribute PBO ITN and Interceptor G2 (IG2) during the mass net campaign in October 2023. This will present an opportunity to accompany the distribution with stronger innovative social behavior change to encourage proper ITN use. In late April of 2023, NMCP piloted distribution of ITNs through primary schools to promote ITN-use within households. Indeed, the radio communication message they carried out received the highest response rate ever for any ITN-based radio messaging, highlighting the role that distribution at schools could play in increasing ITN use in Sierra Leone. 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. The third round of IRS commenced on April 11 in Bo and Bombali, a month earlier than the previous two rounds, to limit large operational activities during the period close to national elections scheduled for June 24th. The April start date is also recommended by the Integrated Vector Management Technical Working Group (IVM-TWG) that reviews entomology and rainfall data to decide on best IRS campaign approaches. The heavy rainfall accompanied by strong winds between July-September make it challenging to do outdoor HLC and contributes to observed trends in vector density even to other sampling tools.

There was high parity rate in *An gambiae* s.l. across all sites including IRS and non-IRS sites with no clear relationship with rainfall patterns. This makes it challenging to evaluate the impact of IRS using this parameter.

There was high insecticide resistance to pyrethroids recorded for *An. gambiae* s.l. in Sierra Leone in 2022 with lowest mortality of 2% for deltamethrin. In some instances, PBO only had a modest effect, increasing mortality by only 2% for permethrin in Largor. In other sites, PBO was able to restore susceptibility by more than 40% but not close to the mortality threshold for susceptibility. For pirimiphos-methyl, there was full susceptibility to mosquitoes collected in Bo, Bombali, and Kono but not in Western Area Rural and Port Loko. This increased susceptibility varies from the previous year where there was recorded resistance to pirimiphos-methyl. This finding could be due to a difference in the vector population as compared to the previous year, and indeed, *Ace-1* molecular marker of resistance was also low (2.2%) this year compared to the previous year, where *Ace-1* prevalence was 21.9%. There was also recorded emerging resistance to clothianidin, active ingredient used in IRS since 2021 in Sierra Leone, but the fact that even resistant populations were found in districts where IRS is not implemented indicates that there could be a secondary source of insecticide pressure either in agriculture or other domestic uses for pest control. Malaria vectors were still fully susceptible to chlorfenapyr, and this finding supports the decision by the NMCP to procure IG2 nets that has chlorfenapyr as one of the active ingredients.

SumiShield™ has been used in a district wide IRS campaign in Bo and Bombali for three years beginning in May 2021, and the team monitored its effectiveness from May 2022 to February 2023. 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, Kono 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 continue to distribute remaining stocks of PBO-ITNs during 2023. Malaria vectors are fully susceptible to chlorfenapyr, justifying the decision to procure over 2 million IG2 nets for mass net campaign in 2023 and to begin procuring IG2 nets for routine distribution this year. Malaria vectors were susceptible to pirimiphos-methyl, except in Kono and Port Loko (non-IRS districts) and could be considered as one of the insecticide options for IRS in Sierra Leone. The project is monitoring the residual efficacy of Actellic (pirimiphos-methyl) in Bo and Bombali to generate data for decision making on IRS insecticide for future IRS campaigns. SumiShield™ remained effective in killing mosquitoes, nine months after the May 2022 spray campaign. Malaria vectors still prefer to bite in the middle of the night and NMCP could benefit from using innovative SBC strategies to target households. This report includes all vector bionomics data and insecticide resistance data collected for the entire reporting year of March 2022 – February 2023 although biochemical and molecular analysis is currently incomplete for this entire time period. This is because of delayed procurement process and completion of CSP ELISA and bloodmeal identification at Njala University. An addendum report with the remaining laboratory analysis findings and molecular data for Sierra Leone will be submitted later.

5.2 LESSONS LEARNED AND CHALLENGES

- Malaria vectors in Sierra Leone are highly resistant to deltamethrin alpha-cypermethrin and permethrin insecticides; however, PBO was able to restore partial susceptibility. The restoration of susceptibility in the permethrin assay was much lower compared to other pyrethroids and it seems PBO and permethrin combination is less effective than deltamethrin/alphacypermethrin PBO combinations. Resistance to pirimiphos-methyl was only detected in Port Loko and Western Area District. It is necessary for the government to continue monitoring insecticide resistance to all classes of insecticides to guide vector control.
- Insecticide resistance to clothianidin and pirimiphos-methyl was recorded in districts where they are not used for public health. The country does not also have data that indicates the classes and types of pesticides used in agriculture limiting decision-making in selecting insecticide for malaria 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.
- A great majority of collected mosquitoes were still found to have fed on humans, indicating a challenge with ITN use in Sierra Leone. The VectorLink project is supporting NMCP in collaboration with Breakthrough Action project to learn how school based ITN distribution messaging can elicit and enhance ITN use awareness.
- The team successfully launched electronic data entry in five districts where routine entomological surveillance is conducted. Only one or two supervisors had challenges using the VLC mobile App to enter entomology data; thus, mutual agreements were reached between DHMT to seek replacements as to who can handle electronic data entry exercises. This was successfully done for Kono and Port Loko.
- Feld technicians and entomology field supervisors are all comfortable using the electronic data entry system. It will be crucial to monitor and care for the mobile devices to ensure they are always ready while in the field. Provision is also being made to provide power banks to charge the devices while in the field.
- The project has begun processing mosquito samples locally at Njala University using the university technicians. However, the process is slow because they are yet to abide by the set timelines, deliverables and the project's expectations to communicate all results within a specific period of time. Discussions are underway to discuss future engagement with Njala University under PMI evolve to ensure timely completion of activities.
- In 2022, the entomology team further improved in parity dissections, and only 5% of ovaries could not be read.
- Involvement of national government staff at NMCP and regional MOHS officers in the routine monitoring helps foster confidence of Government officials 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:

- More mosquitoes were still found to have fed on humans, indicating a challenge with ITN use in Sierra Leone. To estimate the risk of biting exposure and recommend corrective measures, the team plans to conduct a direct observation-based HBO in Year 1 of the Evolve project.
- Maintain the new colony of *An gambiae* s.s. Kisumu strain, from MR4, in Freetown and Makeni.
- Continue with the assessment of residual effectiveness of SumiShield™ and Actellic in Bo and Bombali districts where IRS is being implemented.
- Discuss the assessment of Vectron 500 insecticide in Sierra Leone with NMCP and IVM-TWG as another alternative insecticide for IRS.
- 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.
- Develop an MoU with Njala University to mitigate and fix the logistical and operational challenges limiting molecular processing of samples at Njala University.
- Install, equip and refurbish the container laboratory at Njala University, Bo campus for sample storage and for project use during residual efficacy monitoring in Bo district.
- Conduct regular molecular entomology training/supervision to technicians, hire project staff to be seconded to Njala university to increase capacity in the laboratory by fostering prompt analysis and reporting of samples.

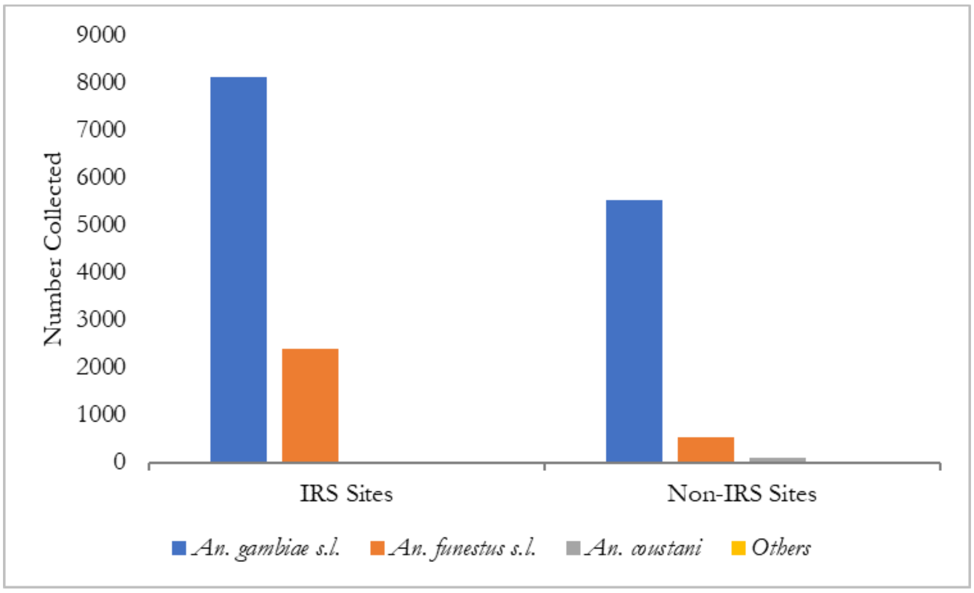
For the NMCP and VectorLink Sierra Leone:

- Implement community sensitization on the use of ITNs and benefits of IRS to reduce human-vector contact.
- Agree on the next IRS insecticide to be used for future IRS campaigns.
- Conduct IRS in 2024 between April-May just before the onset of the rainy season.
- Assess the residual life of SumiShield™ and Actellic in Sierra Leone after the 2022 IRS campaign
- Liaise with the Ministry of Agriculture to undertake a research study to document classes and types of pesticides used in Agriculture.

ANNEX A: NUMBER OF *ANOPHELES* COLLECTED BY PSC, CDC-LT AND HLC, MARCH 2022–FEBRUARY 2023

District	Site	*An. funestus* s.l.	*An. gambiae* s.l.	*An. coustani*	Other	Total
Bo	Gerihun (Peri-urban)	856	2,561	0	0	3,417
	Largor (Rural)	1,063	2,417	0	0	3,480
Bombali	Kamaranka (Rural)	128	1,701	0	1	1,830
	Masongbo (Peri-Urban)	356	1,430	3	0	1,789
Kono	Sorie Town (Peri-Urban)	127	1,058	2	1	1,188
	Teikor (Rural)	224	1,517	1	0	1,742
Port Loko	Bakolo (Rural)	7	589	82	0	6,78
	New Maforki (Peri-Urban)	144	762	5	3	9,14
Western Rural	Sand Sand Water (Rural)	34	1,081	1	0	1,116
	Tombo (Peri-Urban)	2	514	0	0	516
Total		2,941	13,630	94	5	**16,670**

Total *Anopheles* Species Collected (PSC, HLC, and CDC-LT) in IRS Versus Non-IRS Sites, Between March 2022 and February 2023

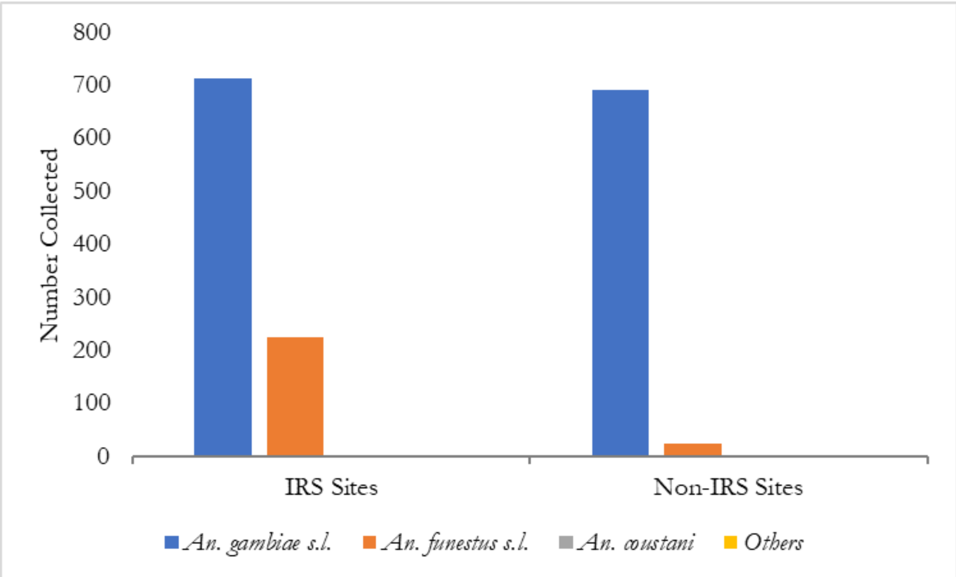


***Anopheles* Species Collected (PSC, HLC, and CDC-LT) by District/Site, Between March 2022 and February 2023.**

ANNEX B: NUMBER OF *ANOPHELES* COLLECTED BY CDC LIGHT TRAPS, MARCH 2022–FEBRUARY 2023

District	Site	*An. gambiae* s.l.	*An. funestus* s.l.	*An. coustani*	Other	Total
Bo	Gerihun (Rural)	54	108	0	0	162
	Largor (Rural)	81	136	0	0	217
Bombali	Kamaranka (Rural)	11	314	0	0	325
	Masongbo (Peri-Urban)	78	155	0	0	233
Kono	Sorie Town (Peri-Urban)	7	139	0	1	147
	Teikor (Rural)	12	173	0	0	185
Port Loko	Bakolo (Rural)	0	231	0	0	231
	New Maforki (Peri-Urban)	6	52	1	0	59
Western Rural	Sand Sand Water (Rural)	0	74	0	0	74
	Tombo (Peri-Urban)	0	22	0	0	22
Total		**249**	**1,404**	**1**	**1**	**1,655**

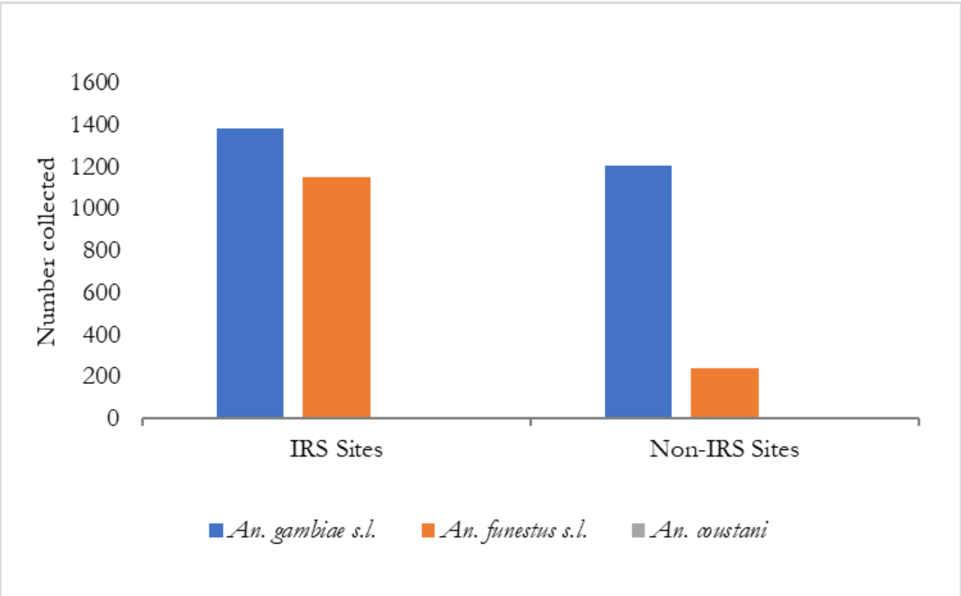
Total *Anopheles* Species Collected by CDC-LT in IRS Versus Non-IRS Sites, Between March 2022 and February 2023



ANNEX C: NUMBER OF *ANOPHELES* COLLECTED BY PSC, MARCH 2022-FEBRUARY 2023

District	Site	*An. gambiae* s.l.	*An. funestus* s.l.	*An. coustani*	Other	Total
Bo	Gerihun (Rural)	467	467	0	0	934
	Largor (Rural)	486	525	0	0	1,011
Bombali	Kamaranka (Rural)	35	210	0	0	245
	Masongbo (Peri-Urban)	164	183	0	0	347
Kono	Sorie Town (Peri-Urban)	51	305	0	0	356
	Teikor (Rural)	112	376	0	0	488
Port Loko	Bakolo (Rural)	1	49	6	0	56
	New Maforki (Peri-Urban)	65	174	0	0	239
Western Rural	Sand Sand Water (Rural)	7	261	0	0	268
	Tombo (Peri-Urban)	2	43	0	0	45
Total		**1,390**	**2,593**	**6**	**0**	**3,989**

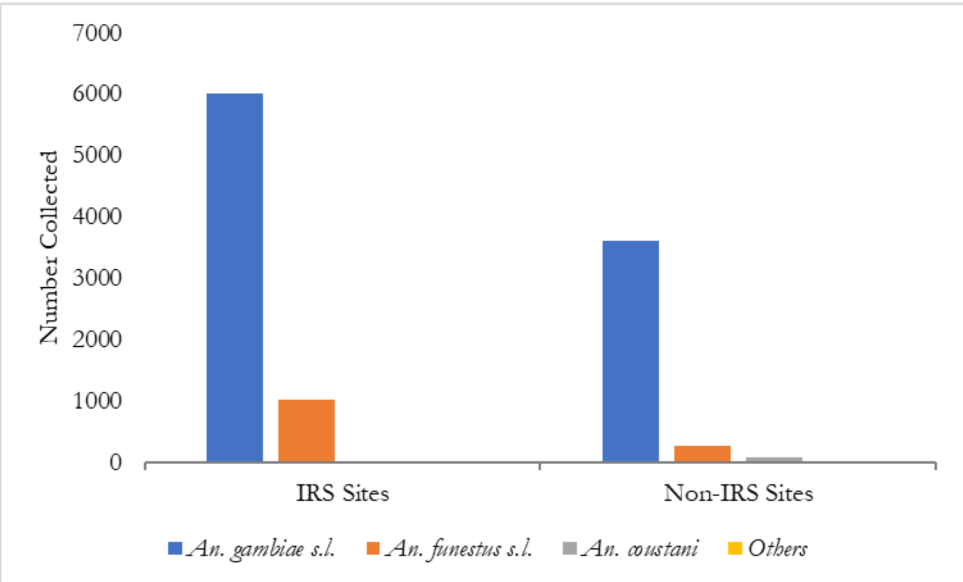
Total *Anopheles* Species Collected by PSC in IRS Versus Non-IRS sites, Between March 2022 and February 2023



ANNEX D: NUMBER OF *ANOPHELES* COLLECTED BY HLC, MARCH 2022-FEBRUARY 2023

District	Site	*An. gambiae* s.l.	*An. funestus* s.l.	*An. coustani*	Other	Total
Bo	Gerihun (Rural)	335	1986	0	0	2,321
	Largor (Rural)	496	1756	0	0	2,252
Bombali	Kamaranka (Rural)	82	1177	0	1	1,260
	Masongbo (Peri-Urban)	114	1092	3	0	1,209
Kono	Sorie Town (Peri-Urban)	69	614	2	0	685
	Teikor (Rural)	100	968	1	0	1,069
Port Loko	Bakolo (Rural)	6	309	76	0	391
	New Maforki (Peri-Urban)	73	536	4	3	616
Western Rural	Sand Sand Water (Rural)	27	746	1	0	774
	Tombo (Peri-Urban)	0	449	0	0	449
Total		**1,302**	**9,633**	**87**	**4**	**11,026**

Total *Anopheles* Species Collected by HLC in IRS Versus Non-IRS Sites, Between March 2022 and February 2023



Annex E: Number of *Anopheles* Dissected for Parity Rate; *An gambiae* s.l. and *An funestus* s.l., March 2022-February 2023

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-22	7	59	3	66	4.5	66	89.4	78.8	95.3
		Apr-22	6	27	1	33	3.0	33	81.8	63.9	92.4
		May-22	8	20		28	0.0	28	71.4	51.1	86.0
		Jun-22	3	10	8	13	61.5	13	76.9	46.0	93.8
		Jul-22	5	16		21	0.0	21	76.2	52.5	90.9
		Aug-22		8		8	0.0	8	100.0	59.8	100.0
		Sep-22	7	20	3	27	11.1	27	74.1	53.4	88.1
		Oct-22	9	16		25	0.0	25	64.0	42.6	81.3
		Nov-22	7	16	1	23	4.3	23	69.6	47.0	85.9
		Dec-22	3	7		10	0.0	10	70.0	35.4	91.9
		Jan-23	1	10		11	0.0	11	90.9	57.1	99.5
		Feb-23	5	29		34	0.0	34	85.3	68.2	94.5

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
	Largor	Mar-22	3	7	1	10	10.0	10	70.0	35.4	91.9
		Apr-22	4	27		31	0.0	31	87.1	69.2	95.8
		May-22	2	19	2	21	9.5	21	90.5	68.2	98.3
		Jun-22	3	8	1	11	9.1	11	72.7	39.3	92.7
		Jul-22	1	16		17	0.0	17	94.1	69.2	99.7
		Aug-22	2	5		7	0.0	7	71.4	30.3	94.9
		Sep-22	1	15	4	16	25.0	16	93.8	67.7	99.7
		Oct-22	2			2	0.0	2	0.0	0.0	80.2
		Nov-22		6		6	0.0	6	100.0	51.7	100.0
		Jan-23	1	5		6	0.0	6	83.3	36.5	99.1
		Feb-23	7	2	1	9	11.1	9	22.2	3.9	59.8
Bombali	**Kamaranka**	Apr-22	1	5		6	0.0	6	83.3	36.5	99.1
		May-22	5	11		16	0.0	16	68.8	41.5	87.9
		Jun-22		7	1	7	14.3	7	100.0	56.1	100.0
		Jul-22	1	16		17	0.0	17	94.1	69.2	99.7
		Aug-22	5	8	3	13	23.1	13	61.5	32.3	84.9
		Sep-22		9	6	9	66.7	9	100.0	62.9	100.0

District	Village	Month	Nulliparous (N)	Parous (P)	Undetermined (U)	Grand Total	Proportion Undetermined	Total (P+N)	Parity Rate (P/(P+N) *100	95 % Confidence Intervals	
										Lower	Upper
		Oct-22	2	10		12	0.0	12	83.3	50.9	97.1
		Nov-22		4		4	0.0	4	100.0	39.6	100.0
		Dec-22		6		6	0.0	6	100.0	51.7	100.0
		Feb-23	2	6		8	0.0	8	75.0	35.6	95.5
	Masongbo	Mar-22	16	31		47	0.0	47	66.0	50.6	78.7
		Apr-22	4	25		29	0.0	29	86.2	67.4	95.5
		May-22	1	17		18	0.0	18	94.4	70.6	99.7
		Jun-22	6	42		48	0.0	48	87.5	74.1	94.8
		Jul-22	2	24		26	0.0	26	92.3	73.4	98.7
		Aug-22	3	19	11	22	50.0	22	86.4	64.0	96.4
		Sep-22	1	14	8	15	53.3	15	93.3	66.0	99.7
		Oct-22		13	2	13	15.4	13	100.0	71.7	100.0
		Nov-22		14	2	14	14.3	14	100.0	73.2	100.0
		Dec-22		16		16	0.0	16	100.0	75.9	100.0
		Jan-23		3		3	0.0	3	100.0	31.0	100.0
		Feb-23	3	14		17	0.0	17	82.4	55.8	95.3

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
Kono	**Sorie Town**	Mar-22	1	8		9	0.0	9	88.9	50.7	99.4
		Apr-22	3	8		11	0.0	11	72.7	39.3	92.7
		May-22	2	11		13	0.0	13	84.6	53.7	97.3
		Jun-22	2	18		20	0.0	20	90.0	66.9	98.2
		Jul-22		5		5	0.0	5	100.0	46.3	100.0
		Aug-22	1	11		12	0.0	12	91.7	59.8	99.6
		Sep-22	3	16		19	0.0	19	84.2	59.5	95.8
		Oct-22		4		4	0.0	4	100.0	39.6	100.0
		Nov-22		4		4	0.0	4	100.0	39.6	100.0
		Dec-22		2		2	0.0	2	100.0	19.8	100.0
		Jan-23	1	7		8	0.0	8	87.5	46.7	99.3
		Feb-23	7	16	1	23	4.3	23	69.6	47.0	85.9
	Teikor	Mar-22		6		6	0.0	6	100.0	51.7	100.0
		Apr-22	1	6		7	0.0	7	85.7	42.0	99.2
		May-22	2	18		20	0.0	20	90.0	66.9	98.2
		Jun-22	2	13		15	0.0	15	86.7	58.4	97.7
		Jul-22	1	9		10	0.0	10	90.0	54.1	99.5

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
		Aug-22	4	10		14	0.0	14	71.4	42.0	90.4
		Sep-22	2	17		19	0.0	19	89.5	65.5	98.2
		Oct-22	1	10	1	11	9.1	11	90.9	57.1	99.5
		Nov-22	1	3		4	0.0	4	75.0	21.9	98.7
		Dec-22	1	10		11	0.0	11	90.9	57.1	99.5
		Jan-23	1	5		6	0.0	6	83.3	36.5	99.1
		Feb-23	1	8	1	9	11.1	9	88.9	50.7	99.4
Port Loko	**Bakolo**	Jun-22	5	7		12	0.0	12	58.3	28.6	83.5
		Jul-22	1	9	1	10	10.0	10	90.0	54.1	99.5
		Aug-22	2	4		6	0.0	6	66.7	24.1	94.0
		Feb-23		1		1	0.0	1	100.0	5.5	100.0
	New Maforki	Mar-22		9		9	0.0	9	100.0	62.9	100.0
		Apr-22	3	33	1	36	2.8	36	91.7	76.4	97.8
		May-22	4	9		13	0.0	13	69.2	38.9	89.6
		Jun-22	6	23		29	0.0	29	79.3	59.7	91.3
		Jul-22	3	15	3	18	16.7	18	83.3	57.7	95.6
		Aug-22	1	7		8	0.0	8	87.5	46.7	99.3

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
		Sep-22		5	1	5	20.0	5	100.0	46.3	100.0
		Oct-22		5	1	5	20.0	5	100.0	46.3	100.0
		Nov-22		7		7	0.0	7	100.0	56.1	100.0
		Dec-22	1	4		5	0.0	5	80.0	29.9	98.9
		Jan-23	2	2		4	0.0	4	50.0	15.0	85.0
		Feb-23		5		5	0.0	5	100.0	46.3	100.0
Western Rural	**Sand Sand Water**	Mar-22		14		14	0.0	14	100.0	73.2	100.0
		Apr-22		8		8	0.0	8	100.0	59.8	100.0
		May-22	2	28		30	0.0	30	93.3	76.5	98.8
		Jun-22	1	18	1	19	5.3	19	94.7	71.9	99.7
		Jul-22		38	1	38	2.6	38	100.0	88.6	100.0
		Aug-22	1	18		19	0.0	19	94.7	71.9	99.7
		Sep-22	2	14		16	0.0	16	87.5	60.4	97.8
		Oct-22		6		6	0.0	6	100.0	51.7	100.0
		Nov-22		15		15	0.0	15	100.0	74.7	100.0
		Dec-22		17	3	17	17.6	17	100.0	77.1	100.0
		Jan-23		13		13	0.0	13	100.0	71.7	100.0

District	Village	Month	Nulliparous (N)	Parous (P)	Undetermined (U)	Grand Total	Proportion Undetermined	Total (P+N)	Parity Rate (P/(P+N) *100	95 % Confidence Intervals Lower	Upper
		Feb-23	1	10		11	0.0	11	90.9	57.1	99.5
	Tombo	Mar-22		4		4	0.0	4	100.0	39.6	100.0
		May-22	1	2	1	3	33.3	3	66.7	12.5	98.2
		Jun-22		1	2	1	200.0	1	100.0	5.5	100.0
		Jul-22		13		13	0.0	13	100.0	71.7	100.0
		Aug-22	1	15	4	16	25.0	16	93.8	67.7	99.7
		Sep-22	1	13	1	14	7.1	14	92.9	64.2	99.6
		Oct-22		3		3	0.0	3	100.0	31.0	100.0
		Nov-22		2		2	0.0	2	100.0	19.8	100.0
		Dec-22		1		1	0.0	1	100.0	5.5	100.0
		Feb-23		4		4	0.0	4	100.0	39.6	100.0

ANNEX F: WHO SUSCEPTIBILITY TEST AND CDC BOTTLE ASSAYS RESULTS, MAY-NOVEMBER 2022

A. **Pyrethroids**

District	Village	Alphacypermethrin 0.05%				Deltamethrin 0.05%				Permethrin 0.75%			
		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	10	50	78	75	6.7	75	60	50	26	50	44
	Largor	50	36	50	90	100	6	100	87	50	20	50	22
Bombali	Bumbadain	100	10	100	75	100	4	100	62	100	4	100	21
	Kamaranka	100	10	100	69	100	2	100	64	100	9	100	14
Port Loko	Bakolo	100	10	100	48	100	14	100	63	100	11	100	42
	Masesseh	100	9	100	55	100	3.2	100	67	50	10	50	44
Western Area	Sand Sand Water	100	9	75	61	100	13	100	78.3	100	9	100	50
Kono	Teikor	75	12	75	72	50	10	50	82	75	12	75	26.7
Key	Susceptible	Resistant											

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	250	84.8	100	100
	Largor	100	100	300	90.7	100	100
Bombali	Bumbadain	100	100	250	88	150	100
	Kamaranka	100	98	250	92	150	100
Port Loko	Bakolo	100	100	100	77	100	87
	Masesseh	100	100	100	85	100	84
Western Area	Sand Sand Water	100	100	150	93.3	100	84
Kono	Teikor	100	100	150	100	100	100
Key	Susceptible	Resistant					

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