U.S. PRESIDENT'S MALARIA INITIATIVE

PMI VECTORLINK LIBERIA PROJECT

PMI VECTORLINK LIBERIA ENTOMOLOGICAL MONITORING YEAR 5 FINAL REPORT

OCTOBER 1, 2021–SEPTEMBER 30, 2022

Recommended Citation: *The PMI VectorLink Project Year 5 Annual Report: October 1, 2021 - September 30, 2022.* Rockville, Maryland. 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: January 3, 2023

Abt Associates | 6130 Executive Blvd |
| Rockville, MD 20852 | T. 301.347.5000 | F. 301.913.9061
| www.abtassociates.com

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS

CDC	Centers for Disease Control and Prevention
CHW	Community health worker
ELISA	Enzyme-linked immunosorbent assay
HBR	Human biting rate
HLC	Human landing catch
IRD	Indoor resting density
IRS	Indoor Residual Spray
ITNs	Insecticide-Treated Nets
LIBR	Liberia Institute for Biomedical Research
LT	Light trap
MOH	Ministry of Health
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase chain reaction
PMI	U.S. President's Malaria Initiative
PSC	Pyrethrum spray catch
SBCC	Social and behavior change communication
SOP	Standard Operation Procedure

EXECUTIVE SUMMARY

The US President's Malaria Initiative (PMI) has been supporting routine malaria vector monitoring and insecticide resistance testing in selected sites in Liberia for more than 10 years. The PMI VectorLink Liberia Project and the National Malaria Control Program (NMCP) perform these activities based on approved annual workplans. In this report, the data collected from October 2021 to September 2022 are presented with analysis for trends by season, collection techniques and sites. The data collection for entomological monitoring (vector bionomics) was done monthly in eight sites. To obtain two consecutive years' data, the following sites were visited during the past two years: Fissebu (Lofa County), Gbedin Camp 3 (Nimba County), Jackson Farm (Margibi County), Koryah (Bong County), Madina (Grand Cape Mount County), Saint John (Grand Bassa County), Suehn Town (Bomi County) and Zeansue (Bong County). Three different methods of collection were used for the longitudinal monitoring of malaria vectors in these sentinel sites: pyrethrum spray catch (PSC) performed in 25 houses per site, human landing catch (HLC) including indoor and outdoor collections in two houses, and Centers for Disease Control and Prevention light traps (CDC-LTs) setup indoor in eight houses.

In addition to understanding vector bionomics across the country, the benefit of these collections taking place before and after the mass distribution of Interceptor® G2 ITNs countrywide (June – July 2021) allows for vector data to be used in an integrated analysis, including epidemiological and climatic data, to assess the impact of Interceptor® G2 ITNs distribution on malaria transmission.

In selected sites across the country, insecticide resistance tests were conducted annually to assess the susceptibility of *Anopheles gambiae* s.l. populations to alpha-cypermethrin (pyrethroid) and chlorfenapyr (pyrrole), the two active ingredients on Interceptor® G2 ITNs. Larva were collected in the field and sent to Monrovia for rearing to adult, then batches of females *An. gambiae* s.l. were tested in the insectary where relative humidity (80%±10) and temperature (27±2 degrees Celsius) were under control.

Alpha-cypermethrin was tested alone and with a synergist, piperonyl butoxide (PBO), which can inhibit the activity of metabolic enzymes of the cytochrome P450 family known to detoxify insecticides including pyrethroids. The inhibition of these enzymes is supposed to increase the susceptibility of *An. gambiae* s.l. mosquitoes to alpha-cypermethrin. The outcome data allowed to assess if the combination of pyrethroid and synergist could reduce the resistance level or restore full susceptibility.

Across the study period, for three methods of collections (PSC, HLC, and CDC-LTs), in total, 7,748 *An. gambiae* s.l. (76.1%) and 2,417 *Anopheles funestus* s.l. (23.7%) and 21 other *Anopheles* mosquito species (0.2%) were collected in the eight sentinel sites. Comparing the abundance of *An. gambiae* s.l. and *An. funestus* s.l. by site, the proportion of *An. gambiae* s.l. was higher Jackson Farm (99.6%; n=1,409), Madina (99.3%; n=1,011), Saint John (99.6%; n=1,346) and Gbedin Camp 3 (98.5%; n=1,733). The abundance of *An. funestus* s.l. was highest in Zeansue (89.1%; n=1,906). *Anopheles funestus* s.l. was also collected from Koryah (30.6%; n=406) and Fissebu (20.8%; n=63).

For indoor resting density (IRD) of *An. gambiae* s.l. collected by PSC method, the highest values were reported in: Madina (6.5 mosquitoes per house per day in July 2022), Jackson Farm (5.7 mosquitoes per house per day in June 2022), and Koryah (3.7 mosquitoes per house per day in May 2022). In Zeansue, the highest IRD of *An. funestus* s.l. (9.7 mosquitoes per house per day) was reported in May 2022.

The highest number of *An. gambiae* s.l. with sampling using CDC-LT was reported in Gbedin Camp 3 (19.5 mosquitoes per trap per night) in October 2021, November 2021, and April 2022 with 19.5, 18.5 and 15.4 mosquitoes collected per trap per night. In Zeansue, the numbers of *An. funestus* s.l. collected increased from January 2022 (3.0 mosquitoes per trap per night) to April 2022 (23.8 mosquitoes per trap per night) then it decreased to less than two mosquitoes per trap per night from June to September 2022.

With HLC indoor collections, the highest mean indoor human biting rate (HBR) of *Anopheles gambiae* s.l. was recorded in Saint John (38.0 bites per person per night) in June 2022, followed by Gbedin Camp 3 (21.8 bites per person per night) in November 2021 and Jackson Farm (19.8 bites per person per night) in May 2022. The values for HLC outdoor collections were higher than for HLC indoor collections. The highest mean outdoor HBR of *Anopheles gambiae* s.l. was recorded in June with 41.5 bites per person per night in Jackson Farm, and 37.3 bites per person per night in Saint John and in July with 34.8 bites per person per night for Madina. For *An. funestus* s.l., the highest HBR was reported in Zeansue in February 2022 with 12.0 bites per person per night indoors and 17.5 bites per person per night outdoors in March 2022.

In all the sites where larvae were collected for resistance testing, the *An. gambiae* s.l., vector populations were resistant to alpha-cypermethrin. With the pre-exposure of the mosquitoes to PBO, full susceptibility was observed one in one site, Gbedin Camp 3. In the other sites, the susceptibility increased after pre-exposure to PBO, but full susceptibility was not restored. A series of tests were performed for susceptibility of the vector to chlorfenapyr, and mortality was >98% at the 72h holding period, for all sites except the Barclayville in Grand Kru County, where mortality was 94%.

1. INTRODUCTION

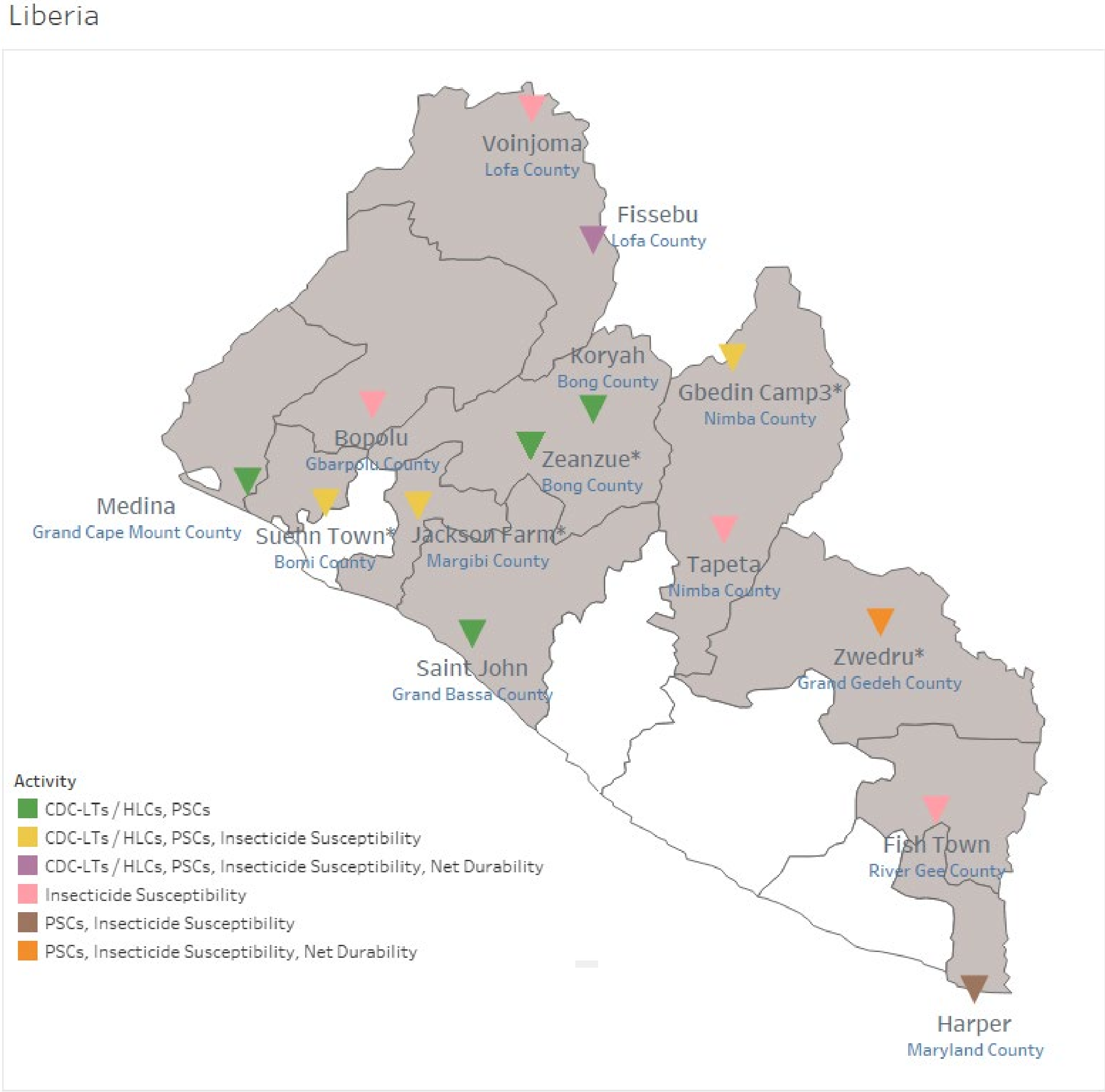
From 2009 to 2013, indoor residual spraying (IRS) was implemented in Liberia by the U.S. President's Malaria Initiative (PMI) in collaboration with the National Malaria Control Program (NMCP) and Ministry of Health (MOH). In 2012, IRS was implemented in 14 districts selected from five counties (Grand Bassa, Montserrado, Margibi, Bong and Bong). Due to insecticide and operation cost, PMI decided to shift support to insecticide resistance surveillance and vector monitoring to generate data which would guide decision making on vector control options other than IRS, primarily insecticide-treated nets (ITNs). Based on the resistance monitoring data collected up to 2020 that showed the malaria vectors were resistant to pyrethroids, PMI, NMCP, and the MOH decided to switch from standard pyrethroid ITNs to dual active ingredient Interceptor® G2 ITNs (chlorfenapyr and alpha-cypermethrin) for mass distribution in 2021.

From October 2020 to September 2021 (Year 4), entomological monitoring was performed monthly in eight sites **(Figure 1)**: Fissebu (Lofa County), Gbedin Camp 3 (Nimba County), Jackson Farm (Margibi County), Koryah (Bong County), Madina (Grand Cape Mount County), Saint John (Grand Bassa County), and Suehn Town (Bomi County). In Year 5 (October 2021 to September 2022), the same sites were visited monthly for vector bionomics data collection using the same protocol of data collection including Pyrethrum Spray Catches (PSCs), Human Landing Catches (HLCs), and Centers for Disease Control light traps (CDC-LTs). The main indicators assessed were density, species composition, and behavior.

In addition to these activities, insecticide resistance testing was performed in nine of the ten planned sites. In the tenth site – Zeansue – collection of larvae was done in September 2022, but the larvae died unexpectedly. Another collection was done in October 2022 and insecticide resistance testing was conducted successfully. However, given this is outside the reporting period, it will be reported in the next annual report. In seven sites, both insecticides (alpha-cypermethrin with or without pre-exposure of mosquitoes to piperonyl butoxide (PBO) and chlorfenapyr) were tested. In the other two sites at least one insecticide was tested but both were unable to be tested due to insufficient mosquitoes.

Bioassays were conducted on samples (n=20) of Interceptor® G2 ITNs collected from warehouse before the mass campaign and on a subsample of 60 ITNs collected 12 months post-distribution in two districts (Suakoko in Bong County and Suehn Mecca in Bomi County), as part of the Streamlined Durability Monitoring Study led by VectorLink. For bioassay tests, ITN samples were tested locally using the cone test method while a sample (one piece / ITN) was sent abroad to the Pan African Malaria Vector Research Consortium (PAMVERC) laboratory in Benin to conduct tunnel tests with resistant strains (VKPER) of *An. gambiae* s.l. For chemical content analysis, samples of ITNs were sent to CDC Atlanta. The data for these activities are reported in Streamlined Durability Monitoring reports which also include household survey data.

FIGURE 1: VECTORLINK LIBERIA ENTOMOLOGICAL MONITORING, INSECTICIDE RESISTANCE AND DURABILITY MONITORING SITES, OCTOBER 2021 – SEPTEMBER 2022



In Year 5 (October 1, 2021 – September 30, 2022), the main objectives of VectorLink Liberia entomological monitoring activities were:

- Assess *Anopheles* vector bionomics, including species composition, density, and behavior, in eight sentinel sites across seven counties.
- Conduct molecular analyses to determine the species, sporozoite infection rates, and blood-meal sources of mosquitoes collected during routine collection, as well as identify potential mutations contributing to insecticide resistance.
- Determine insecticide susceptibility of *An. gambiae* s.l., the primary local malaria vector, to pyrethroids (with or without pre-exposure to synergist piperonyl butoxide (PBO)) and to chlorfenapyr in ten sites.
- Maintain and support a functional insectary for bioassays tests in country.

- Conduct bioassays on ITNs in support of ITN streamlined durability monitoring.
- Build local capacity in entomological surveillance methods and techniques through formal and informal training.

2. MATERIALS AND METHODS

2.1 SAMPLING SITES AND COLLECTION METHODS

2.1.1 SAMPLING SITES

From October 2021 to September 2022, all field activities related to monthly longitudinal entomological monitoring in the eight sites were performed by VectorLink and NMCP staff who were supported in the field by the community health workers (CHWs) from the sentinel sites in Liberia. VectorLink and NMCP staffs also worked together to conduct insecticide resistance tests in the selected sites Insecticide resistance tests were performed in nine sites. In two sites – Barclayille and Greenville – insufficient mosquitoes were available to perform testing with both insecticides. CHWs trained over the years by VectorLink and NMCP supported larvae collection and rearing in the field before samples were transported by road or flight to Monrovia. Once in Monrovia, VectorLink conducted additional rearing and insecticide resistance tests on adult mosquitoes at the insectary facility. VectorLink communicates directly CHWs when planning field visits for larvae collections. VectorLink calls CHWs to inquire about water levels in main breeding sites and presence of larvae. If CHWs confirm positive conditions, they work with VectorLink to decide on the number of days needed for collection based on CHW intel. This approach has reduced the number of days that the teams spend in the field, along with the associated costs – two years ago the team was spending 12-14 days in the field for collection, rearing and testing while this has now been reduced to 5-6 days in Year 5. Durability monitoring of ITNS will be conducted in 2 sites **(Table 1).**

TABLE 1: SUMMARY OF SITES PLANNED FOR ENTOMOLOGICAL SURVEILLANCE AND INSECTICIDE RESISTANCE MONITORING ACTIVITIES

Province/Regio	County	Site	CDC-LTs / HLCs / PSCs	Insecticide Susceptibility	Streamlined Durability Monitoring^
North Central	Lofa	Fissebu	X		
	Bong	Koryah	X		
		Zeansue*	X	X**	X
	Bomi	Suehn Town*	X	X	X
	Nimba	Gbedin Camp3*	X	X	
South Central	Grand Bassa	Saint John	X		
	Grand Bassa	Compound 3		X	
	Margibi	Jackson Farm*	X	X	
Northwestern	Montserrado	Bentol		X	
	Grand Cape Mount	Madina	X		
		Robert Sport		X	
Southeastern A	Sinoe	Greenville		X	
Southeastern B	Grand Kru	Barclayville		X	
	River Cess	Cestos City		X	

**Site selected for Interceptor® G2 ITN Monitoring*

***Insecticide resistance testing for Zeansue conducted outside the reporting period in October 2022.*

^ SDM is occurring in Suakoko District/Bong County and Suehn Mecca District/Bomi County

Adult mosquitoes were collected using three methods: PSC, indoor CDC-LTs, and HLC (**Table 2**).

TABLE 2: ADULT MOSQUITO COLLECTION METHODS USED FOR LONGITUDINAL ENTOMOLOGICAL MONITORING

Method	Time	Frequency	Sample*	Indicator
PSC	5:00 am to 8:00 am	Monthly	25 houses per site over two consecutive days	Indoor resting density: # mosquitoes collected per room
CDC-LT (Indoors only)	6:00 pm to 6:00 am	Monthly	Eight houses per site (four houses per night), using four baited, indoor CDC-LTs	Indoor trap density: # mosquitoes collected per trap per night
HLC	6:00 pm to 6:00 am	Monthly	Two houses per site for two consecutive nights using two persons - one indoor and one outdoor - per house	Indoor and outdoor human biting rate: # bites per person per hour and per night

**All houses were randomly selected, and the same houses were visited each month, to the greatest extent possible.*

2.1.2 Routine Monitoring Site Collection methods

The adult mosquitoes were collected using three methods: PSC, indoor CDC-LTs, and HLC.

2.1.2.1 Pyrethrum Spray Catches

PSCs were performed in 25 houses per site, for two consecutive days using a commercial insecticide spray named Kwik[1] (pyrethroid plus PBO) following VectorLink SOP (SOP03/01)[2].

2.1.2.2 CDC Light Trap Collection

CDC-LTs were installed indoors by the feet side of a person sleeping under an ITN at selected houses according to VectorLink protocols (SOP01/01)[3].

2.1.2.3 Human Landing Collection

All HLCs were performed (indoors and outdoors) with glass tubes packed hourly in plastic bags.

2.1.2.4 Sorting and Identification of Mosquitoes

Collected mosquitoes were identified morphologically using a dissecting microscope according to Coetzee et al. (2020) in the field by VectorLink and NMCP staff. Both *An. gambiae* s.l. and *An. funestus* s.l. mosquitoes collected from PSC were sorted based on their blood-digestion stages. Mosquito samples were preserved in individual tubes with silica gel for further processing at Liberia Institute for Biomedical Research (LIBR) using enzyme-linked immunosorbent assay (ELISA) for sporozoite rate assessment and bloodmeal analysis and polymerase chain reaction (PCR) for molecular identification.

2.1.3 Insecticide Resistance Monitoring

VectorLink and NMCP conducted susceptibility tests for alpha-cypermethrin (with and without PBO synergist) and chlorfenapyr as per the VectorLink SOP (SOP04/01)[4] using the CDC bottle assay (Brogdon WG and, Chan A., 2010) method, in the sites listed in **Table 3**. Since temperature and relative humidity are under control

[1]Kwik ingredients: Transfluthrin 0.05%; Tetramethrin 0.20%; B-Cyfluthrin 0.20%; PBO 0.50%; Solvent & Propellant 99.55%, ASI Al Sharhan Industries, Kuwait.

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

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

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

in the testing room at VectorLink office, the preference was to perform all the tests in Monrovia to prevent any variability related to field site testing conditions.

TABLE 3: SUMMARY OF INSECTICIDE SUSCEPTIBILITY TESTS AND SYNERGIST ASSAYS PERFORMED FROM OCTOBER 2021 – SEPTEMBER 2022

Province/Region	County	Site	Alpha-cypermethrin ±PBO	Chlorfenapyr
North Central	Lofa	Fissebu		
	Bong	Koryah		
		Zeansue*		
	Bomi	Suehn Town	X	X
	Nimba	Gbedin Camp3	X	X
South Central	Grand Bassa	Saint John		
		Compound 3	X	X
	Margibi	Jackson Farm	X	X
Northwestern	Montserrado	Bentol	X	X
	Grand Cape Mount	Madina		
		Robert Sport	X	X
Southeastern A	Sinoe	Greenville**	X	
Southeastern B	Grand Kru	Barclayville*		X
	River Cess	Cestos City	X	X

** In Zeansue and Barclayville, larvae were collected in Sep. 2022, due to unexpected death of mosquitoes, testing could not be done with either insecticide on samples from Zeansue and with alpha-cypermethrin on samples from Barclayville. A second collection was performed in October 2022 which was followed by successful insecticide resistance testing.*

***Not enough larvae were collected in Greenville to conduct chlorfenapyr testing.*

A subsample of dead and surviving mosquitoes was stored in labeled individual Eppendorf tubes for further molecular laboratory analysis to assess the presence of *kdr* or *Ace-1* mutations using PCR.

2.1.3.1 INSECTICIDE SUSCEPTIBILITY TEST FOR PYRETHROIDS– CDC BOTTLE ASSAY METHOD

The susceptibility assays of *An. gambiae* s.l. to the pyrethroids (alpha-cypermethrin 12.5µg per bottle) were performed using technical grade insecticide concentrations provided by CDC Atlanta.

2.1.3.2 INSECTICIDE SUSCEPTIBILITY TEST FOR CHLORFENAPYR – CDC BOTTLE ASSAY METHOD

Anopheles gambiae s.l. was tested for susceptibility to chlorfenapyr as per the VectorLink SOP (SOP04/01) using CDC bottle assay. Insecticide grade chlorfenapyr in vials were provided to VectorLink by CDC Atlanta Entomology Branch. Each pre-weighed technical grade of chlorfenapyr was dissolved in 50 ml acetone to coat the bottles. Four bottle replicates were coated with one ml of the prepared insecticide solutions containing 100µg/ml. One ml acetone only was used to coat the control bottles. All the bottles were left to dry overnight before using them the next day. Twenty-five female two to five days old *An. gambiae* s.l. were transferred into each of the bottles.

Knockdown was recorded at 60 minutes post exposure while mosquitoes were still in the bottle. Then, mosquitoes were transferred into cups and fed with a 10% sugar solution for holding up to 72 hours. Mortality was recorded every 24 hours for up to 72 hours or until 100% mortality was recorded, whichever came first.

2.1.3.3 SYNERGIST ASSAYS - CDC BOTTLE ASSAY METHOD

Using the CDC bottle assay method as per the VectorLink SOP (SOP04/01), pre-exposure to PBO as a synergist was performed before mosquitoes were exposed to the diagnostic dose of alpha-cypermethrin. A concentration (100µg per bottle) of PBO was diluted with 50 ml acetone solution. One bottle was coated with one ml of PBO solution to be used as the synergist-exposure bottle. A second bottle was coated with one ml of acetone to serve as a synergist-control bottle. The bottles were left to dry overnight before being used for testing. The next day, a subsample of 125 female *An. gambiae* s.l. were introduced into the synergist-exposure bottle for one hour. Another 125 female *An. gambiae* s.l. were introduced for one hour into the synergist-control bottle coated with acetone only.

After one hour, the mosquitoes were transferred to two holding cages-one for the synergist-control mosquitoes and another for the synergist-exposure mosquitoes. Four replicate tests were done for PBO and non-PBO, based on the CDC bioassays method, using eight bottles (four bottles for PBO and four bottles for non-PBO) with one bottle for each set coated with acetone only as a control. In each insecticide-coated and control bottle, 25 females were introduced using a mouth aspirator, and mortality was recorded every 15 minutes for up to two hours. For all the tests performed, controls tests were run to validate the tests.

CDC bioassay tests were discarded when control mortality was > 10%. Abbott's formula was used to correct results if the mortality in the control bottle was between 3% and 10%.

2.2 ITN DURABILITY MONITORING

VectorLink supported the Streamlined Durability Monitoring study of Interceptor® G2 ITNs. This study includes two main activities: a household survey, which is conducted by subcontractor UL-PIRE and the bioassays (cone, tunnel, and chemical) performed partially in-country as explained above.

2.2.1 STREAMLINED DURABILITY MONITORING BIOASSAYS AND TUNNEL TESTS

For bioassays performed in-country, VectorLink tested five pieces from each ITN using the cone bioassay method with susceptible *An. coluzzii* lab colony strain from the VectorLink Liberia insectary. For tunnel testing bioassays, ten samples (one piece per ITN) were cut, labeled, and shipped to PAMVERC/Benin. The results of tunnel tests and chemical residue analysis were analyzed and shared in a report with PMI in Year 5. The results of tunnel test and chemical residue for 12 month Streamlined Durability Monitoring are expected from PAMVERC and CDC Atlanta Entomology Branch, respectively, and will be reported as part of the Streamlined Durability Monitoring reporting process.

3. RESULTS AND DISCUSSION

3.1 VECTOR BIONOMICS

Below are the results from PSC, CDC-LT and HLC collections of *Anopheles* mosquitoes for the reporting period October 2021 to September 2022.

3.1.1 VECTOR ABUNDANCE AND SPECIES COMPOSITION

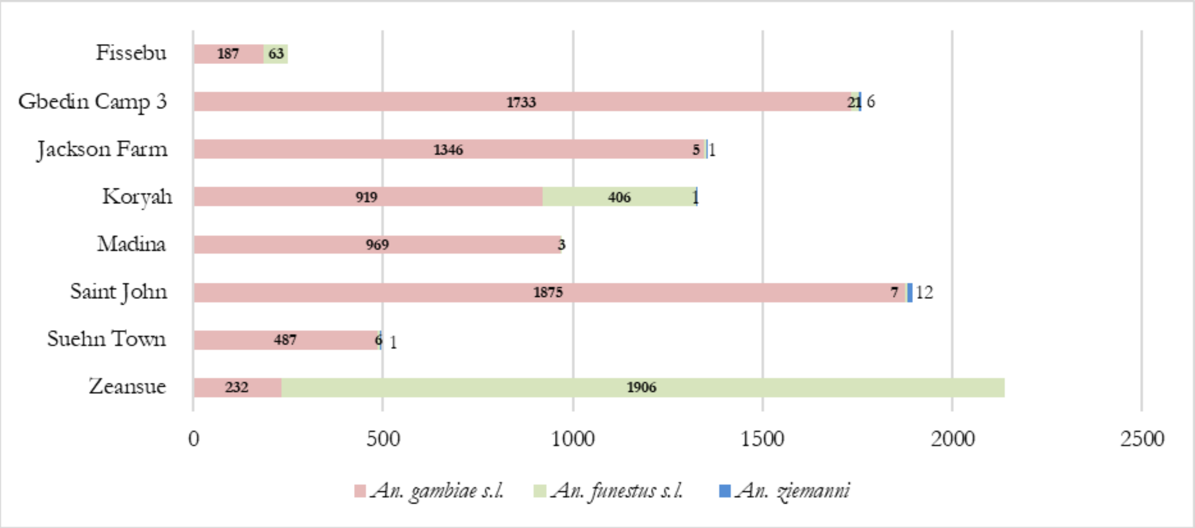
Overall, 10,186 *Anopheles* mosquitoes were collected in eight sites using the three methods of collection (PSC, CDC-LTs and HLC). Of these, 76.1% were *An. gambiae* s.l., 23.7% *An. funestus* s.l. and 0.2% other *Anopheles* species (**Table 4**). Of the total 7,748 *An. gambiae* s.l. collected in all sites, the highest proportions of *An. gambiae* s.l. mosquitoes were caught in in Saint John (24.2%; n=1875), Gbedin Camp 3 (22.4%; n=1733) and Jackson Farm (17.4%; n=1346). *Anopheles funestus* s.l. was mainly collected from Zeansue (78.9%; 1,906/2417) and Koryah (16.8%; 406/2417). Zeansue site from Bong County also had the highest proportion of the total collected mosquitoes (21.0%; 2,138/10,186).

TABLE 4: TOTAL MOSQUITOES COLLECTED FROM EIGHT SENTINEL SITES, OCTOBER 2021–SEPTEMBER 2022 (ALL COLLECTION METHODS)

County	Site	*An. gambiae s.l.*		*An. funestus s.l.*		*An. ziemanni*		*Total Anopheles*		% *Anopheles* species		
		n	%	n	%	n	%	n	% Total *by site*	% *An. gambiae* s.l.	% *An. funestus* s.l.	% *An. ziemanni*
Lofa	Fissebu	187	2.4%	63	2.6%	0	0.0%	250	2.5%	74.8%	25.2%	0.0%
Nimba	Gbedin Camp 3	1733	22.4%	21	0.9%	6	28.6%	1760	17.3%	98.5%	1.2%	0.3%
Margibi	Jackson Farm	1346	17.4%	5	0.2%	1	4.8%	1352	13.3%	99.6%	0.4%	0.1%
Bong	Koryah	919	11.9%	406	16.8%	1	4.8%	1326	13.0%	69.3%	30.6%	0.1%
Grand Cape Mount	Madina	969	12.5%	3	0.1%	0	0.0%	972	9.5%	99.7%	0.3%	0.0%
Grand Bassa	Saint John	1875	24.2%	7	0.3%	12	57.1%	1894	18.6%	99.0%	0.4%	0.6%
Bomi	Suehn Town	487	6.3%	6	0.2%	1	4.8%	494	4.8%	98.6%	1.2%	0.2%
Bong	Zeansue	232	3.0%	1906	78.9%	0	0.0%	2138	21.0%	10.9%	89.1%	0.0%
	Total	**7748**	**100.0%**	**2417**	**100.0%**	**21**	**100.0%**	**10,186**	**100.0%**	**76.1%**	**23.7%**	**0.2%**

Figure 2 shows *An. gambiae* s.l. as the most abundant vector in all sites, except in Zeansue.

FIGURE 2: SPECIES COMPOSITION OF *ANOPHELES* MOSQUITOES COLLECTED FROM EIGHT SENTINEL SITES, OCTOBER 2021 – SEPTEMBER 2022



Overall, more *An. gambiae* s.l., were caught with HLC (57.0%) than with PSC (27.0%) and CDC-LT (16.0%). PSC collection was performed in 25 houses (25 house-days) while for HLC, only two houses were sampled using two collectors per house for two consecutive nights (eight person-nights) and one trap was set up in eight houses for one night each per month for CDC-LT (**Table 5**). The higher proportion *An. gambiae* s.l. of from HLC collections indicates that the HLC method was more efficient in the collection of this vector, and this is despite it was performed in only two houses for two consecutive nights.

TABLE 5: *ANOPHELES* MOSQUITOES SPECIES COLLECTED IN EIGHT SENTINEL SITES, OCTOBER 2021–– SEPTEMBER 2022.

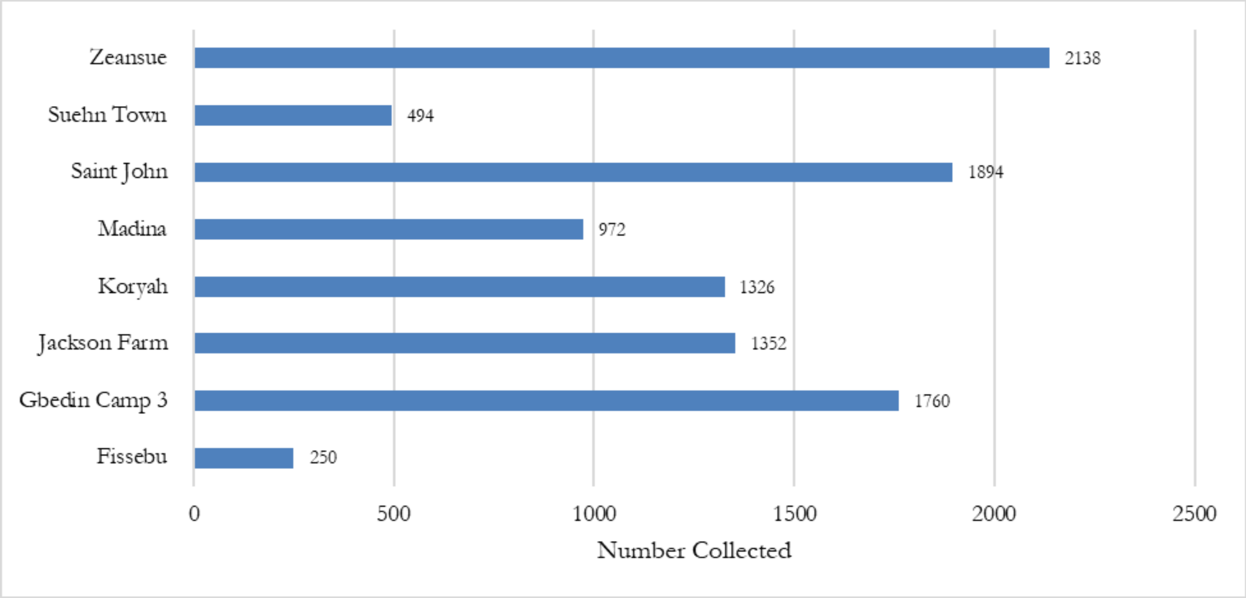
	An. gambiae s.l.					*An. funestus* s.l.					*Total An. gambiae s.l. + An. funestus s.l.*
Site		PSC	CDC-LT	HLC	Total		PSC	CDC-LT	HLC	Total	% of the total
Fissebu	n	38	63	86	187	n	18	12	33	63	
	%	20.3	33.7	46.0	100	%	28.6	19.0	52.4	100	2.46
Gbedin Camp 3	n	251	743	739	1733	n	4	13	4	21	
	%	14.5	42.9	42.6	100	%	19.0	61.9	19.0	100	17.26
Jackson Farm	n	347	123	876	1346	n	2	1	2	5	
	%	25.8	9.1	65.1	100	%	40.0	20.0	40.0	100	13.29
Koryah	n	354	91	474	919	n	150	99	157	406	
	%	38.5	9.9	51.6	100	%	36.9	24.4	38.7	100	13.03
Madina	n	480	26	463	969	n	1	0	2	3	
	%	49.5	2.7	47.8	100	%	33.3	0.0	66.7	100	9.56
Saint John	n	385	118	1372	1875	n	1	2	4	7	
	%	20.5	6.3	73.2	100	%	14.3	28.6	57.1	100	18.51
Suehn Town	n	102	31	354	487	n	1	2	3	6	
	%	20.9	6.4	72.7	100	%	16.7	33.3	50.0	100	4.85
Zeansue	n	132	45	55	232	n	914	499	493	1906	
	%	56.9	19.4	23.7	100	%	48.0	26.2	25.9	100	21.03
Total	**n**	**2089**	**1240**	**4419**	**7748**	**n**	**1091**	**628**	**698**	**2417**	
	%	**27.0**	**16.0**	**57.0**	**100**	**%**	**45.1**	**26.0**	**28.9**	**100**	

Unlike *An. gambiae* s.l., for *An. funestus* s.l. higher proportions were caught from PSC (45.1%) than from HLC (26.0%) and CDC-LT (28.9%). The higher numbers of *An. funestus* s.l. in the PSC collection than the two collection methods can be partially attributed to the higher number of houses sampled for PSC than HLC or CDC-LT. However, this trend is different for the *An. gambiae* s.l. where the proportion in HLC collections was higher than in the PSC method even though the same houses were sampled, and the same frequencies of collection used for both species (**Figure 3 and Table 5**). The proportion of *An. funestus* s.l. collected from PSC was significantly higher than the proportion of *An. gambiae* s.l. collected by the same method (45.1% vs 27.0%; χ=280.6; p<0.0001). This mostly likely suggests more *An. funestus* s.l. mosquitoes are staying resting indoors compared to *An. gambiae* s.l. indicating that *An. funestus* s.l. is more endophilic than *An. gambiae* s.l. in the Liberian context.

FIGURE 3: SPECIES COMPOSITION OF *ANOPHELES* MOSQUITOES BY COLLECTION METHOD (PSC, HLC AND CDC-LT) FROM EIGHT SENTINEL SITES, OCTOBER 2021 – SEPTEMBER 2022

When the numbers collected by the three methods are combined for the two important vector of malaria, *An. gambiae* s.l. and *An. funestus* s.l., the aggregate numbers are highest in Zeansue making 21.3% (n=2,138) of the total collections from the eight sites **(Figure 4)**. This frequency is significantly higher than the site with the next higher proportion for both species combined, Saint John [χ=16.3; (n=1,894); p=0.001]. Given both species are equally efficient vectors of malaria and the same collection methods, and frequency were used in all sites, the higher numbers likely indicate higher risk of malaria transmission in Zeansue.

FIGURE 4: TOTAL *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED BY ALL COLLECTION METHODS (PSC, HLC AND CDC-LT) AND BY SITE FROM EIGHT SENTINEL SITES, OCTOBER 2021 – SEPTEMBER 2022



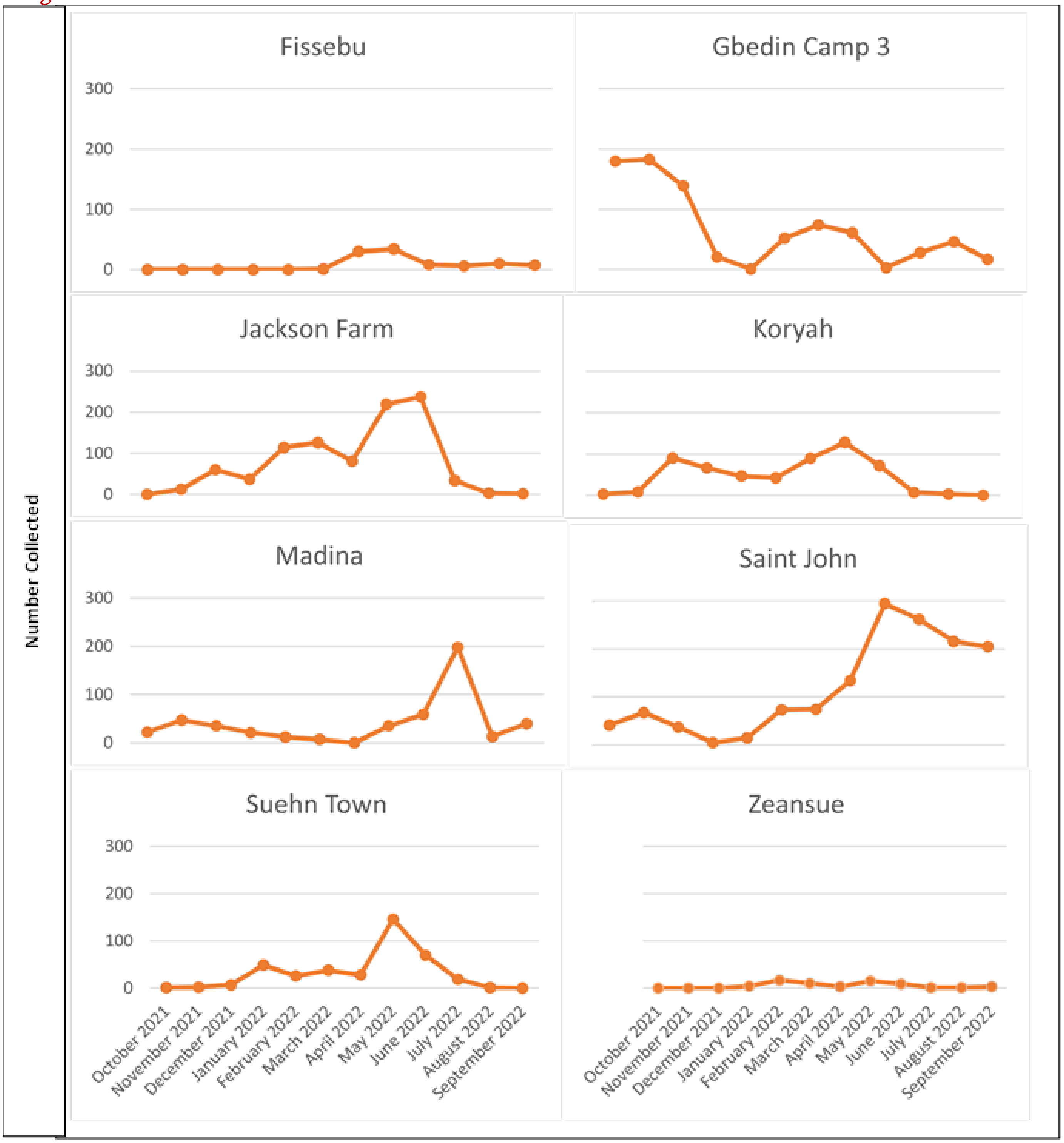
3.1.2 Seasonal Trends of the *An. gambiae* s.l. and *An. funestus* s.l. population from HLC collections

Figure 5 shows seasonal trends in the vector population plotted from HLC collection. In general, the data indicates seasonal malaria risk could be relatively uniform across all the sampling sites, with a peak between April and June. For *An. gambiae* s.l. the seasonality of the vector is similar across all the sentinel sites except for Gbedin Camp 3, where the population seems stable throughout the year with peaks during the dry months October to December. The *An. gambiae* s.l. population in Zeansue is relatively low throughout the year with minor peaks in February-March and May-June. In almost all the other six sites, population starts to build up in April and starts to drop down in July.

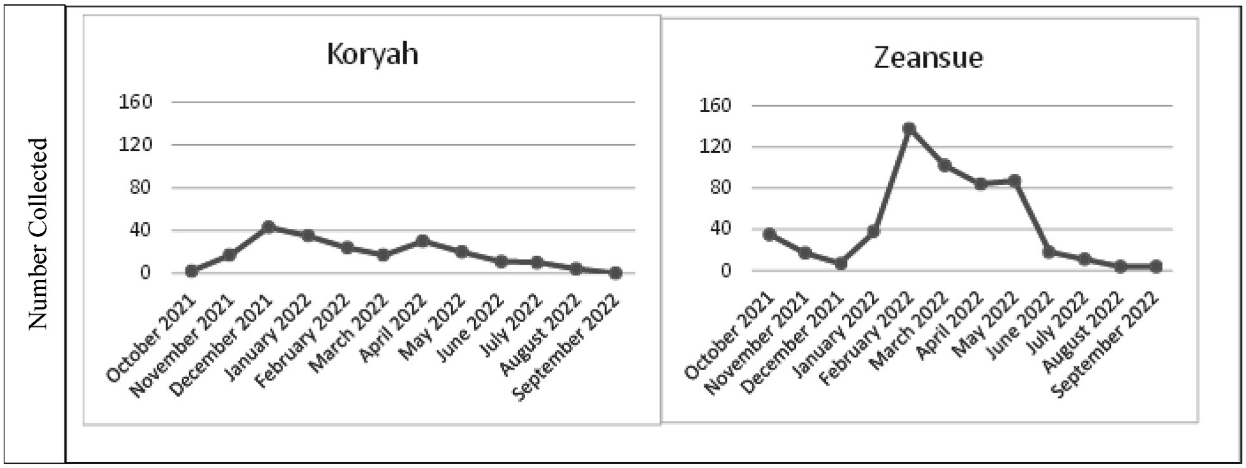
The seasonal trend in *An. funestus* s.l. can be more clearly observed from the Zeansue collections, where this vector is more abundant. The trend is similar to that of *An. gambiae* s.l. except that the peak starts earlier in January and drops in June.

FIGURE 5: SEASONAL TRENDS IN THE AN. GAMBIAE S.L. AND AN. FUNESTUS S.L. POPULATION BY SITE (FROM HLC COLLECTIONS)

An. gambiae s.l.



An. funestus s.l.



3.1.3 Indoor Resting Density

In general, *An. gambiae* s.l. IRDs increased from May to July 2022, then decreased due the frequent rains in the July and August period where larva breeding sites are washed out. The highest indoor resting density (IRD) for *An. gambiae* s.l. was recorded in Madina (6.5 mosquitoes per house per day) in July 2022 and 5.7 mosquitoes per house per day in Jackson Farm in June 2022 **(Figure 6)**. Smaller peaks were also observed in Madina in November 2021 and Koryah in December 2021.

In Zeansue, IRDs for *An. funestus* s.l. increased from January to May 2022, with a peak in May (9.7 mosquitoes per house per day) (**Figure 7**). In Koryah, *An. funestus* s.l. IRDs were less than two mosquitoes per house per day throughout the year. In the other sites, the *An. funestus* s.l. IRDs were very low or zero.

FIGURE 6: INDOOR DENSITIES OF *AN. GAMBIAE* S.L. COLLECTED BY PSC, OCTOBER 2021-SEPTEMBER 2022.

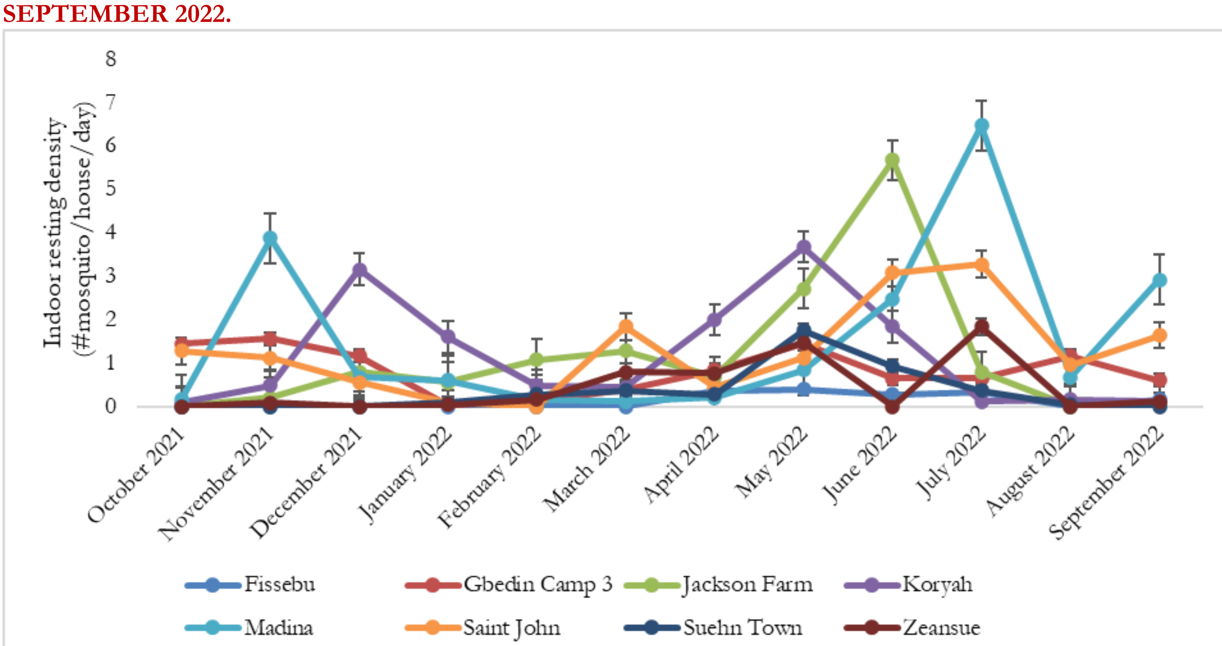
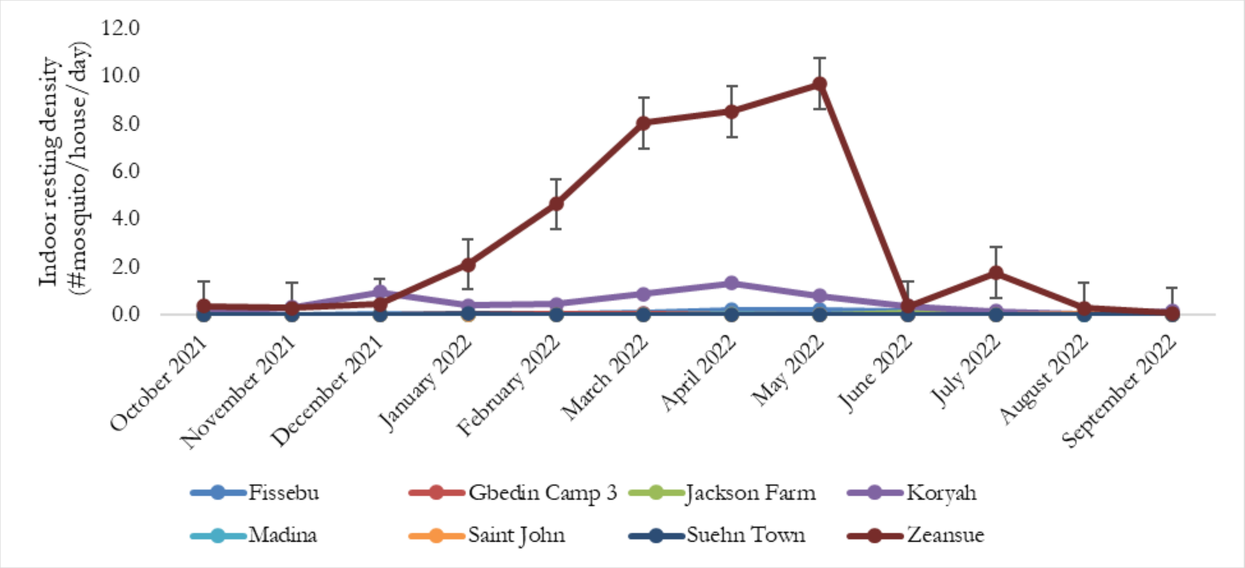


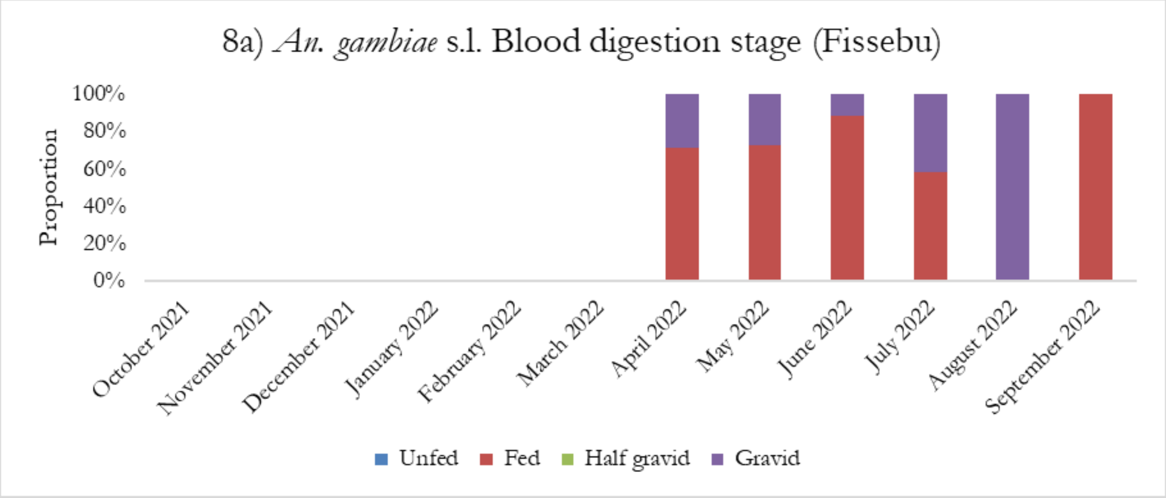
FIGURE 7: INDOOR DENSITIES OF *AN. FUNESTUS* S.L. COLLECTED BY PSC, OCTOBER 2021-SEPTEMBER 2022.



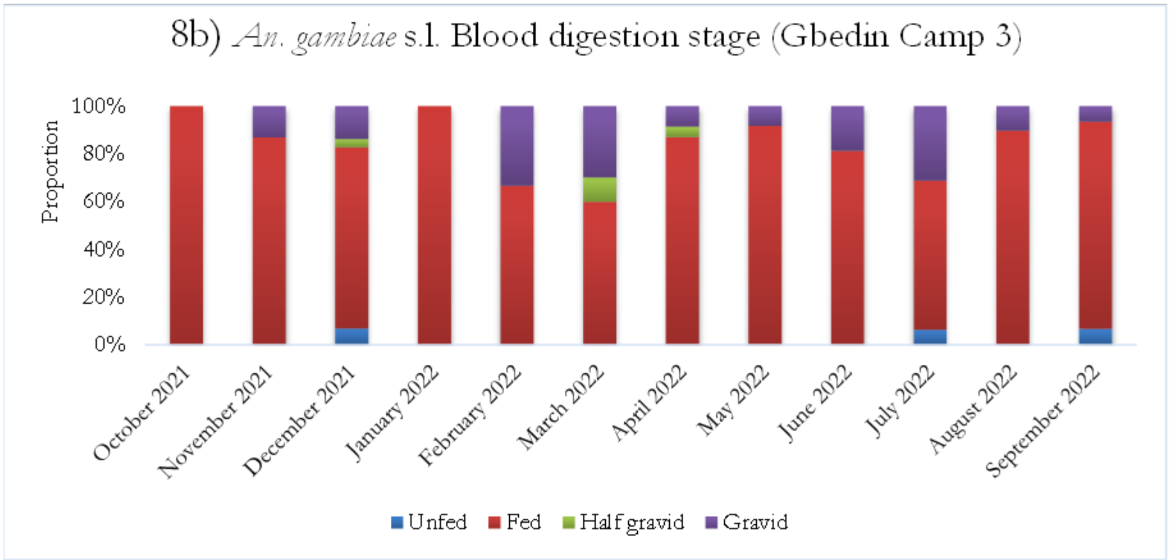
3.1.4 ABDOMINAL STAGE

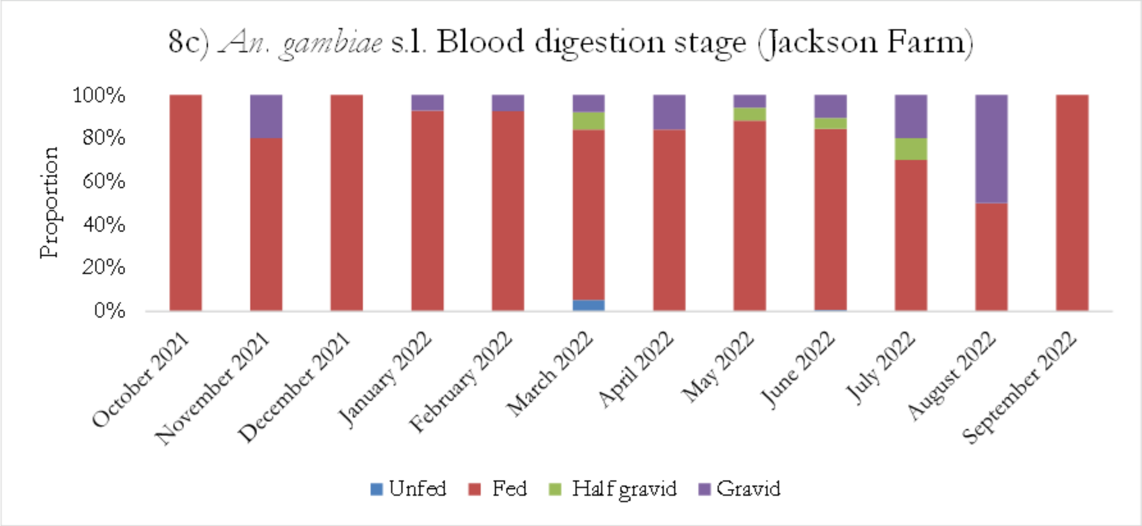
For *An. gambiae* s.l. mosquitoes collected by PSCs and sorted in the field according to the blood-digestion stage are displayed in **Figure 8**, by site and by month. The proportions of fresh blood-fed *An. gambiae* s.l. were high in all sentinel sites. This same pattern was observed in Year 4 (October 1, 2020-September 30, 2021) except in Gbedin Camp 3. The type of houses in Liberia with eaves allow access to host seeking mosquitoes and the high proportion of freshly fed mosquitoes may indicate people are not sleeping under ITNs for part of or the whole night while indoors. The identification of the proportions of females fed on humans or animals is pending until the LIBR staff will be trained on appropriate PCR techniques, which is planned for Year 6.

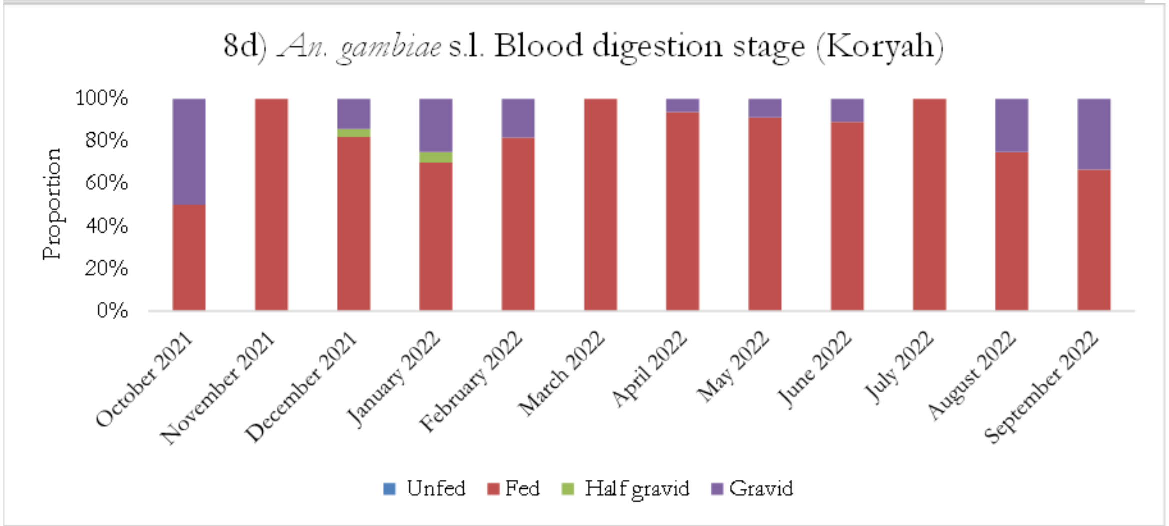
FIGURE 8: DISTRIBUTION OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSC IN EIGHT SITES, OCTOBER 2021 – SEPTEMBER 2022

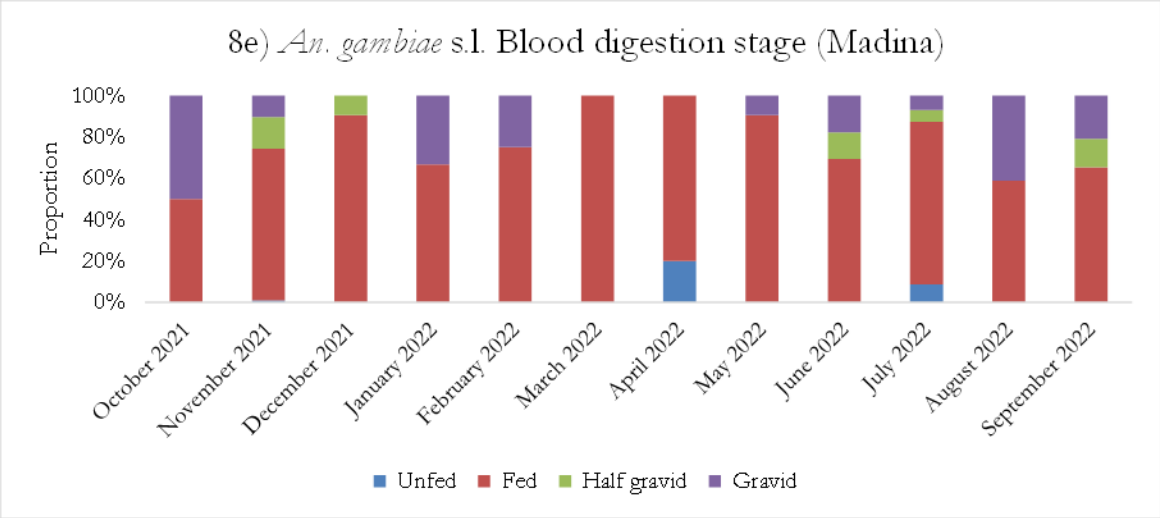


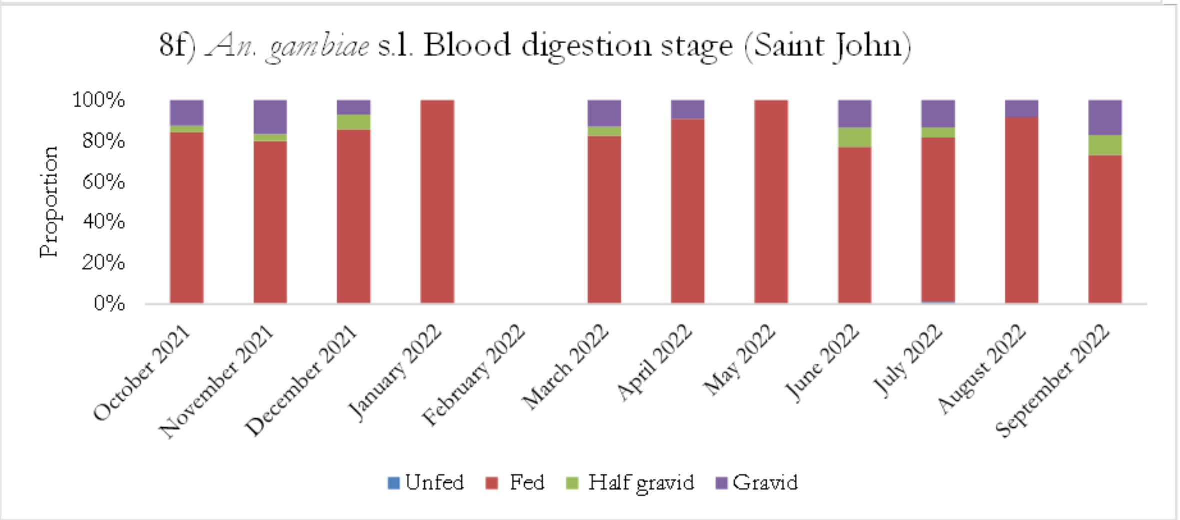
**No or very few indoor resting mosquitoes were collected in Fissebu, Oct 2021 to March 2022 due to the dry season.*


8b) An. gambiae s.l. Blood digestion stage (Gbedin Camp 3)
Proportion
100%
80%
60%
40%
20%
0%
October 2021
November 2021
December 2021
January 2022
February 2022
March 2022
April 2022
May 2022
June 2022
July 2022
August 2022
September 2022
Unfed
Fed
Half gravid
Gravid

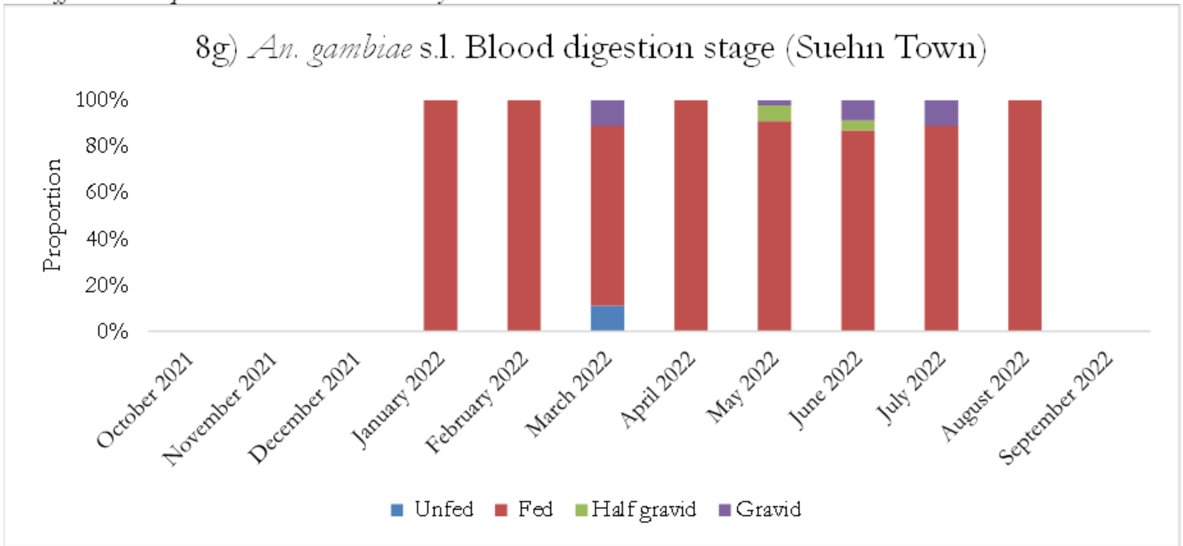

8c) An. gambiae s.l. Blood digestion stage (Jackson Farm)
Proportion
100%
80%
60%
40%
20%
0%
October 2021
November 2021
December 2021
January 2022
February 2022
March 2022
April 2022
May 2022
June 2022
July 2022
August 2022
September 2022
Unfed
Fed
Half gravid
Gravid


8d) An. gambiae s.l. Blood digestion stage (Koryah)
Proportion
100%
80%
60%
40%
20%
0%
October 2021
November 2021
December 2021
January 2022
February 2022
March 2022
April 2022
May 2022
June 2022
July 2022
August 2022
September 2022
Unfed
Fed
Half gravid
Gravid

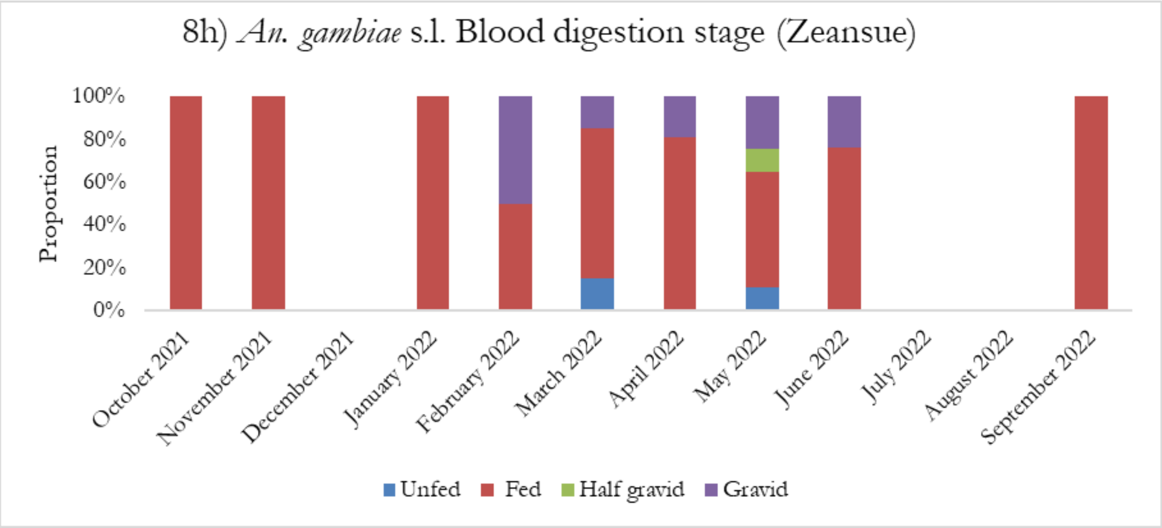




**Insufficient mosquitoes collected in February 2022.*



**No or very few indoor resting mosquitoes were collected in Suehn Town Oct-December 2021 and September 2022.*

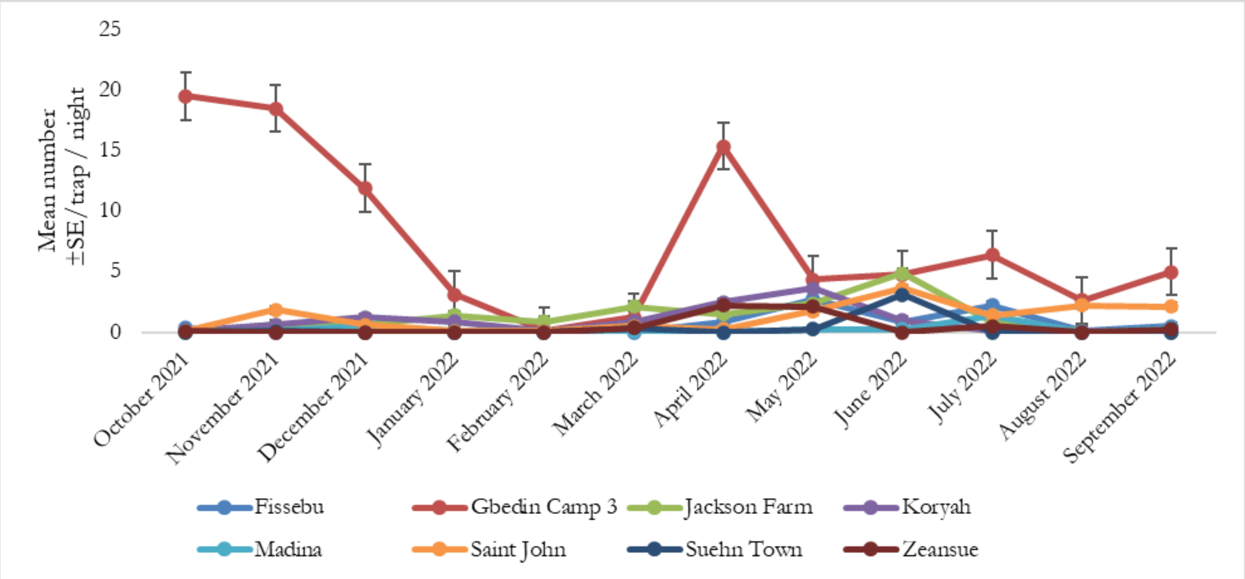


**No or very few indoor resting mosquitoes were collected in Zeansue in December 2021, and July/August 2022.*

3.1.5 CDC Light Trap Collections

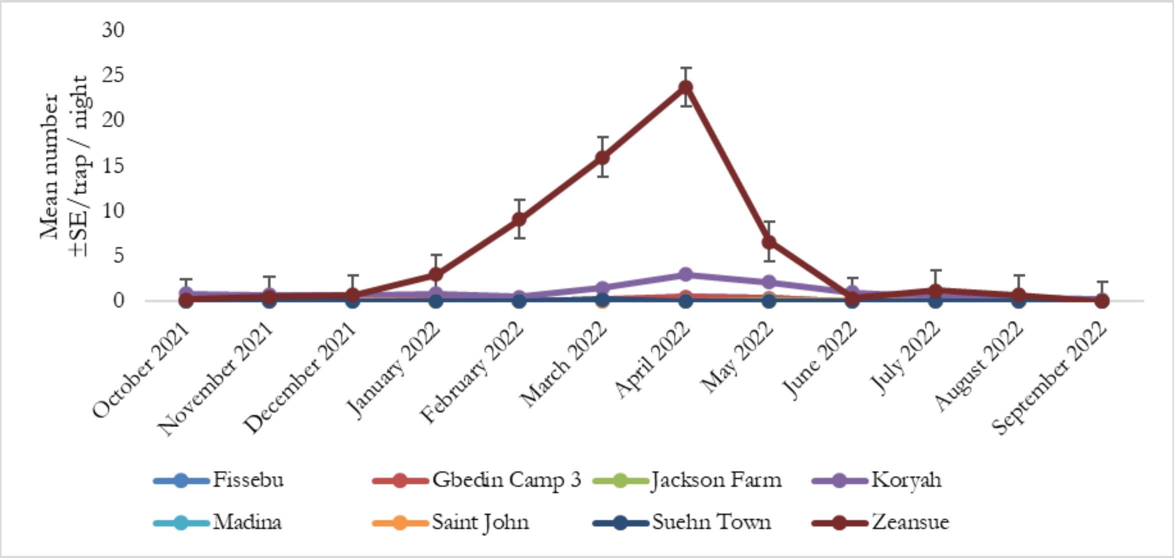
Using the CDC-LTs collections, 1,240 *An. gambiae* s.l. mosquitoes were collected from traps mounted indoors from October 2021 to September 2022 in the eight sites. The density per trap per night was the highest in Gbedin Camp 3 in October 2021 (19.5 mosquitoes per trap per night) for *An. gambiae* s.l. **(Figure 9)**.

FIGURE 9: MEAN DENSITIES PER TRAP PER NIGHT OF *AN. GAMBIAE* S.L. SAMPLES COLLECTED BY CDC-LT, INDOOR, OCTOBER 2021- SEPTEMBER 2022.



Using CDC-LTs collections, the highest number of *An. funestus* s.l. mosquitoes were collected from Zeansue **(Figure 10)**. In Zeansue, the *An. funestus* s.l. population started to increase in January and peaked in April 2022 (23.8 mosquitoes per trap per night), with a sharp fall in May and June 2022. The numbers remained low between June and September 2022.

FIGURE 10: MEAN DENSITIES PER TRAP PER NIGHT OF *AN. FUNESTUS S.L.* SAMPLES COLLECTED BY CDC-LT, INDOOR, OCTOBER 2021- SEPTEMBER 2022.



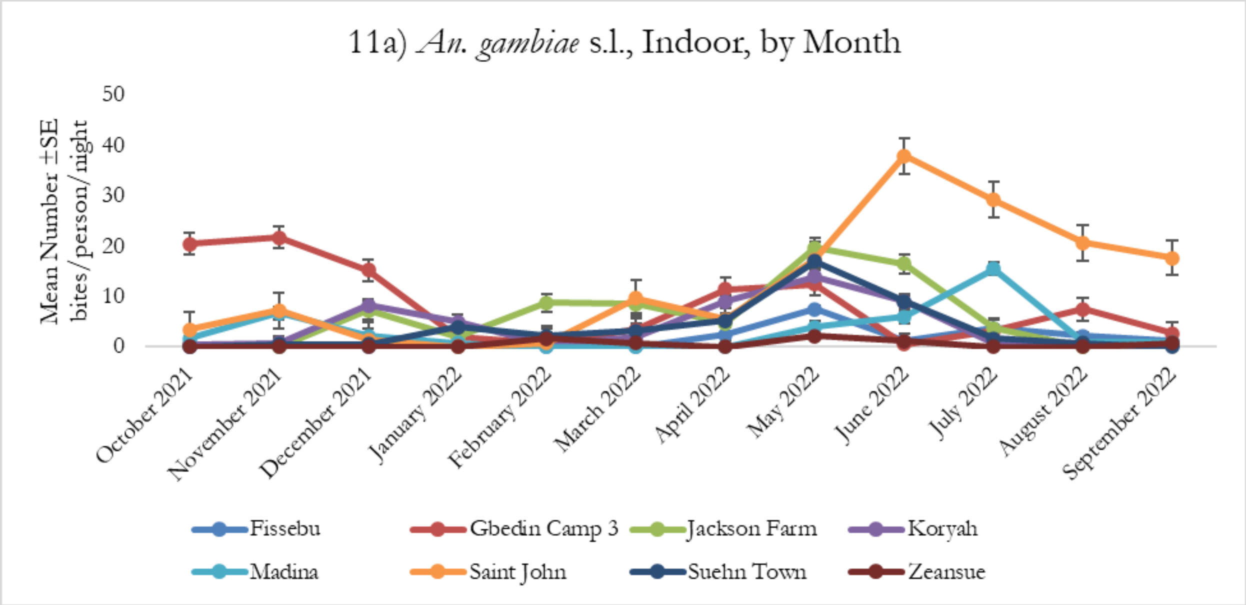
3.1.6 HUMAN BITING RATE AND PATTERN

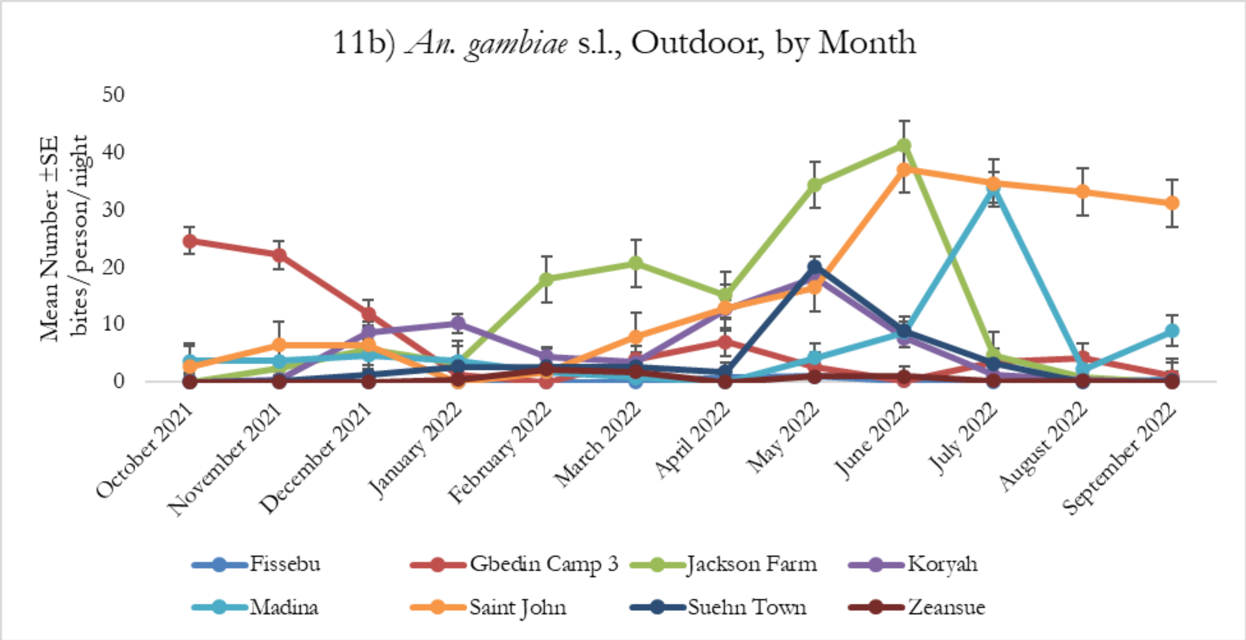
The highest indoor biting rate of *An. gambiae* s.l. (38.0 bites per person per night), reported from HLC was in Saint John, in June 2022 **(Figure 11a)**. In Gbedin Camp 3, the biting rate was highest in November 2021 (21.8 bites per person per night). For most sites, biting rate was lower between January and March 2022 and peaked from March to July 2022, followed by a drop in August due to the frequent rains.

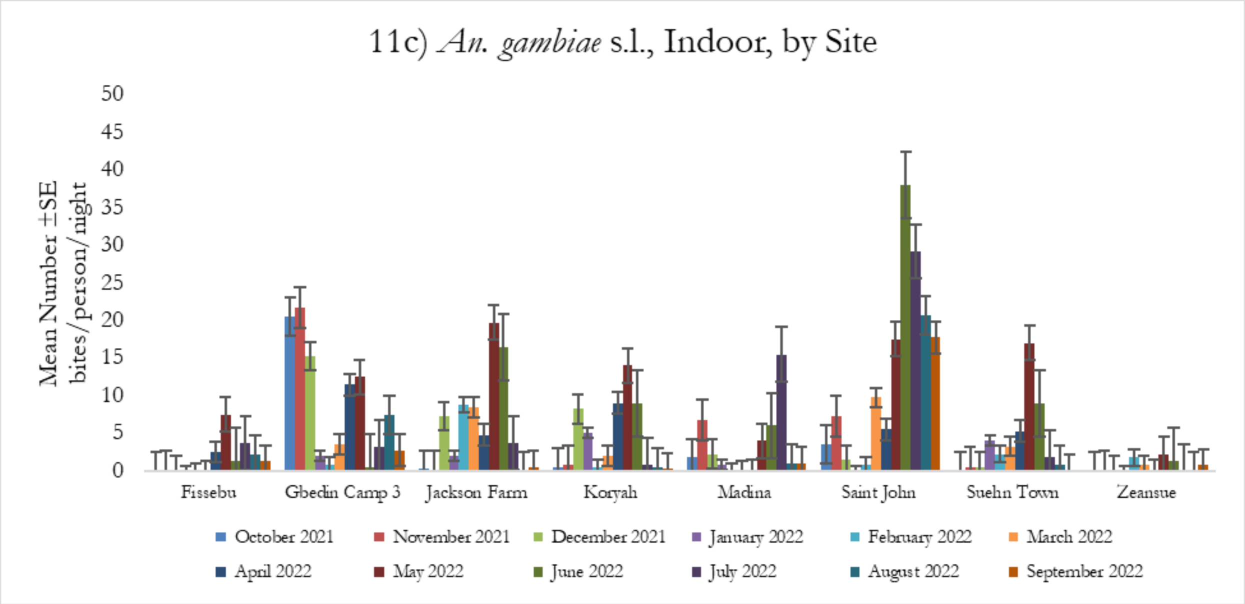
For the outdoor HLC, the highest biting rate was reported in Jackson Farm with 41.5 bites per person per night in June 2022 **(Figure 11b)**. High outdoor biting rates were also observed in Saint John for four consecutive months between June and September 2022 with the highest in June (37.3 bites per person per night). Rates also peaked for Madina in July with 34.0 bites per person per night. Overall, the biting rates were highest from April to July 2022 for most sites with a decline starting August 2022 due to the heavy rains which affect the mosquito breeding sites.

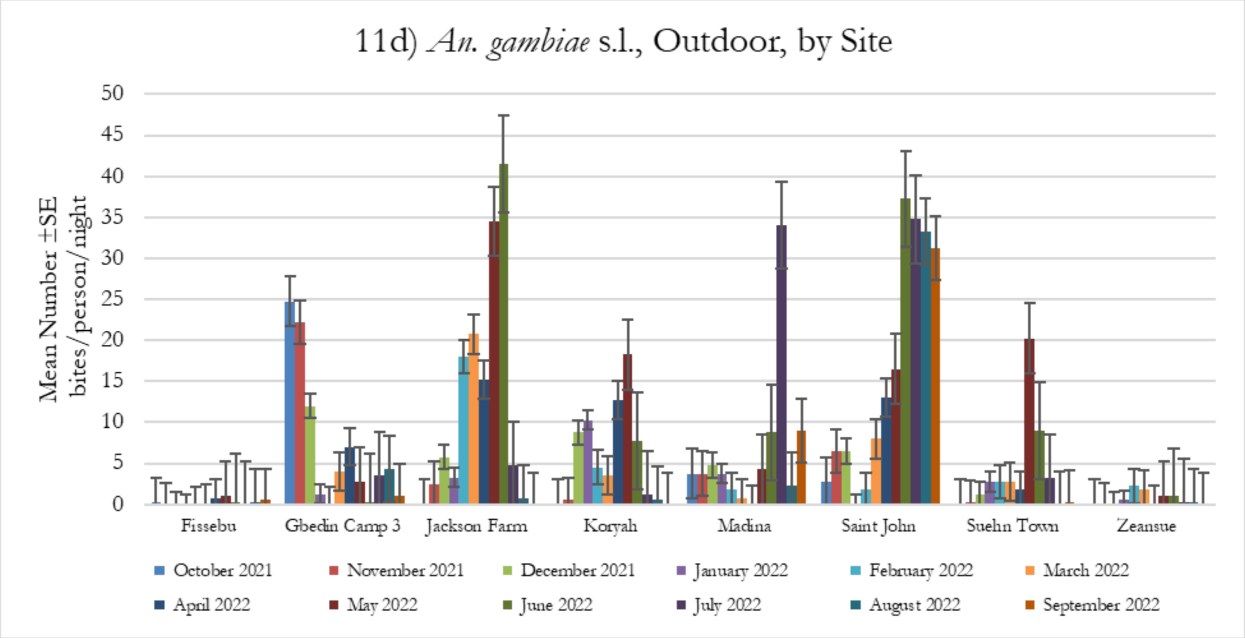
Figures 11c and 11d show that overall, Gbedin Camp 3, Saint John and Jackson Farm had the highest biting rates compared to the other sites. Both Gbedin Camp 3 and Jackson Farm are rice growing areas. The high biting rate in Saint John, which is not a rice growing area, could be due to the intensive local mining activities around this site which creates additional mosquito breeding grounds.

FIGURE 11: NIGHTLY HUMAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED FROM INDOOR AND OUTDOOR HLC, OCTOBER 2021 – SEPTEMBER 2022.







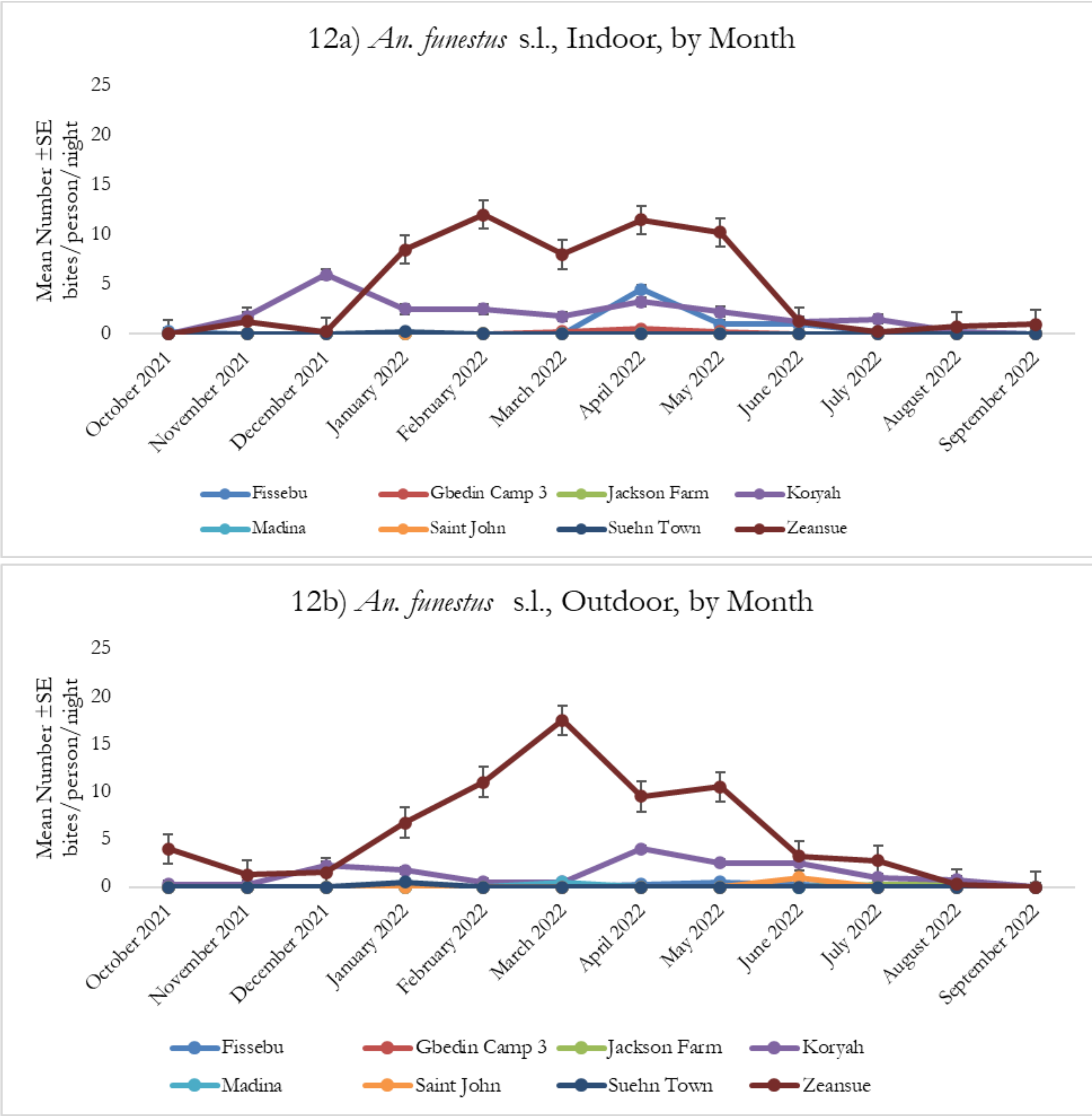


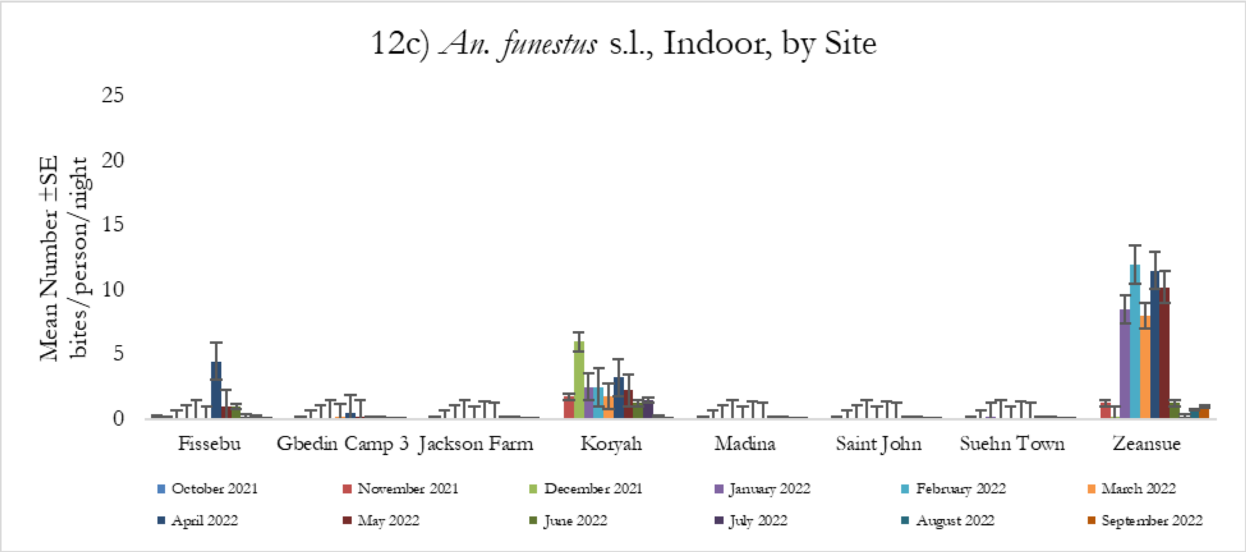
The indoor and outdoor biting rates of *An. funestus* s.l. by site is shown in **Figures 12a and 12d**. Zeansue and Koryah are the two sites where most *An. funestus* s.l. activity occurs. The highest indoor biting rate for *An. funestus* s.l. was recorded in Zeansue with 12.0 bites per person per night in February 2022 with biting rate starting to increase after December 2021 and starting to decline after May 2022 **(Figure 12a)**. The peak for Koryah (6.0 bites per person per night) was observed in December 2021.

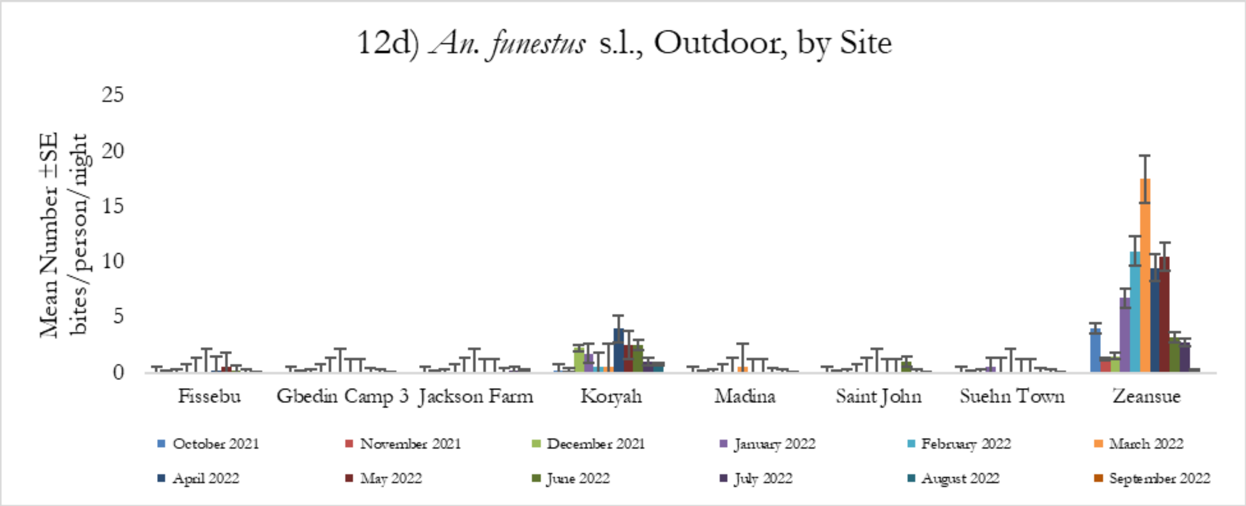
The peak *An. funestus* s.l. outdoor HLC biting was recorded in Zeansue in March 2022 (17.5 bites per person per night) and biting rate was relatively higher between December 2021 and June 2022, **(Figure 12b)**.

Figures 12c and 12d show that Zeansue and Koryah had the highest biting rates compared to the other sites both indoors and outdoors.

FIGURE 12: NIGHTLY HUMAN BITING RATE OF *AN. FUNESTUS* S.L. COLLECTED FROM INDOOR AND OUTDOOR HLC, OCTOBER 2021–SEPTEMBER 2022.

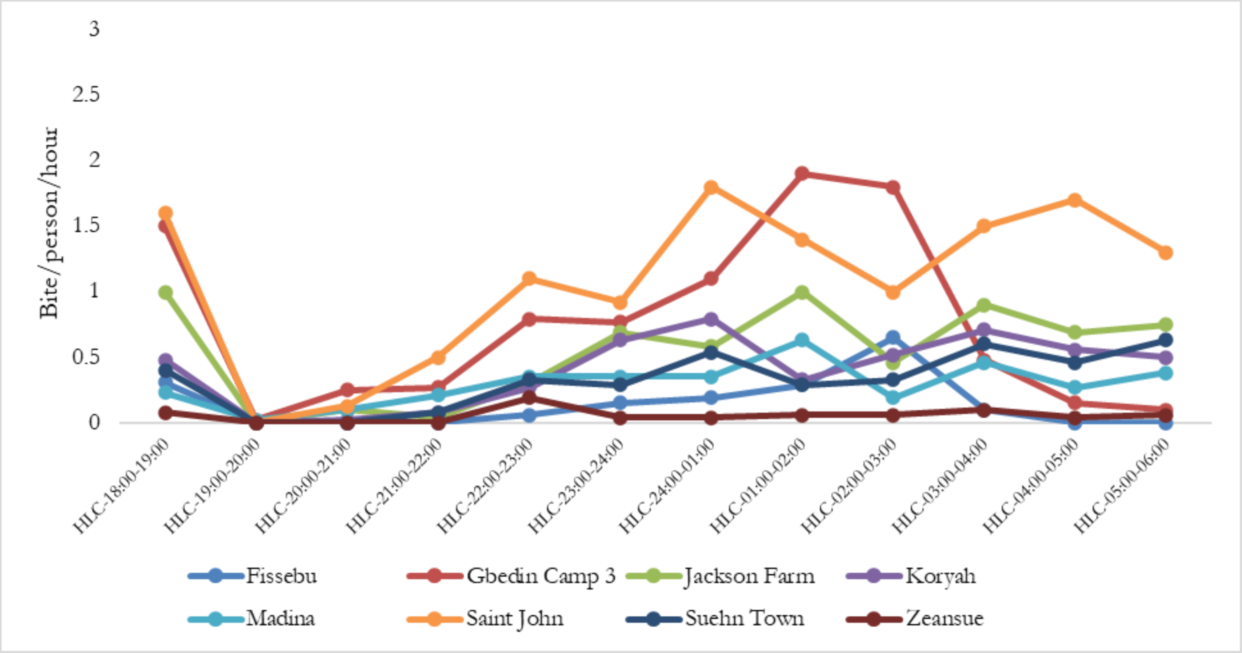






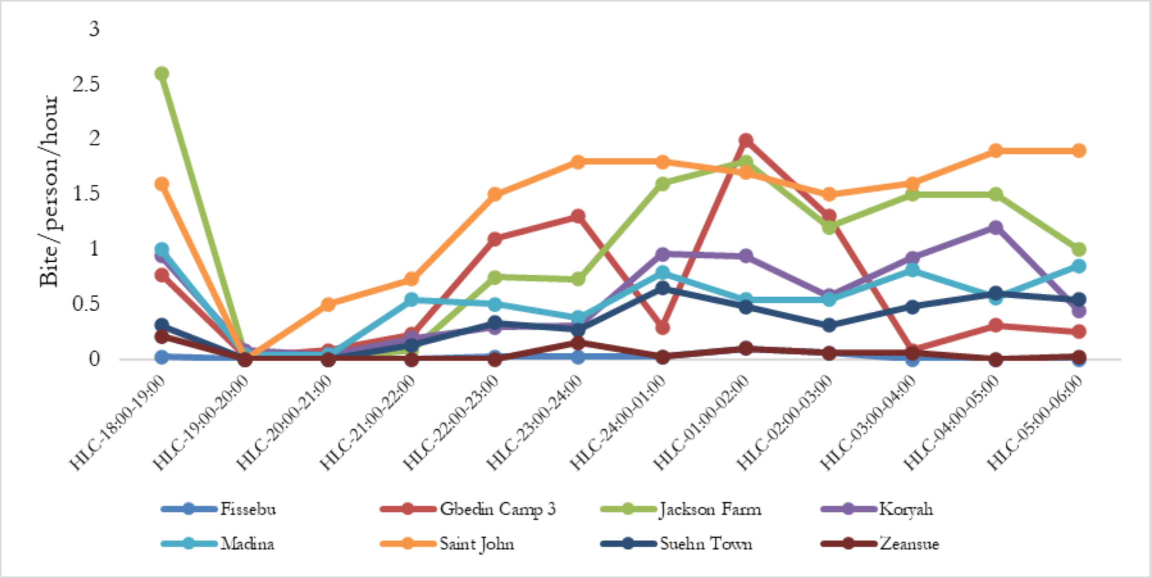
For *An. gambiae* s.l., the hourly indoor biting activity increased after 22:00 in most of the sites **(Figure 13 and 14)**. The biting pattern indicates that the high biting period corresponds to the period when people are more likely to be indoors and protected if they are using ITNs properly. The hourly biting rates are generally higher in Gbedin Camp 3 and Saint John sites.

FIGURE 13: HOURLY HUMAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED FROM INDOOR HLC IN EIGHT SITES, OCTOBER 2021 – SEPTEMBER 2022.



Overall, the outdoor hourly biting rates in the sites shows an increasing trend after 22:00, which is similar to the trend with indoor biting times.

FIGURE 14: HOURLY HUMAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED FROM OUTDOOR HLC IN EIGHT SITES, OCTOBER 2021 - SEPTEMBER 2022.



Biting Place Preference

Indoor and outdoor human biting rates of *An. gambiae* s.l. did show significant difference in biting locations, depending on the sites. For *An. gambiae* s.l., the indoor human biting rate (HBR) was significantly higher than the outdoor HBR in Fissebu and Gbedin Camp 3 sites **(Table 6)**. In Jackson Farm, Koryah, Madina and Saint John, the outdoor biting was significantly higher than indoor biting. For the two other sites, Suehn Town and Zeansue, there was no significant difference between indoor and outdoor biting; p-values: 1.000 and 0.8927, respectively.

For *An. funestus* s.l., the number collected indoor and outdoor were low in most sites except in Zeansue and Koryah **(Table 7)**. In Koryah, there was more indoor biting than outdoor biting with a significant difference (p-value = 0.0312); while in Zeansue, the outdoor biting was higher than indoor, and the difference was also significant (p-value = 0.017). After aggregating data from the eight sites, there was no significant difference between indoor and outdoor biting by *An. funestus* s.l.

TABLE 6: *ANOPHELES GAMBIAE* S.L. BITING PLACE PREFERENCE, INDOOR AND OUTDOOR, OCTOBER 2021 - SEPTEMBER 2022

Anopheles gambiae s.l.	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Grand Total
Indoor HLC (collected)	74	407	288	202	156	606	177	27	1937
Outdoor HLC (collected)	12	332	588	272	307	766	177	28	2482
Total	86	739	876	474	463	1372	354	55	4419
Endophagic Index (A)	0.86	0.55	0.33	0.43	0.34	0.44	0.50	0.49	0.44
Exophagic Index (B)	0.14	0.45	0.67	0.57	0.66	0.56	0.50	0.51	0.56
Ratio (A/B)	6.17	1.23	0.49	0.74	0.51	0.79	1.00	0.96	0.78
Chi^2 (p-value)	2.30E-11*	0.0058*	3.8E-24*	0.00134*	2.26E-12*	1.563E-05*	1.00	0.8927	2.40E-16*

*= difference significant (<0.05)

TABLE 7: *ANOPHELES FUNESTUS* S.L. BITING PLACE PREFERENCE, INDOOR AND OUTDOOR, OCTOBER 2021 - SEPTEMBER 2022

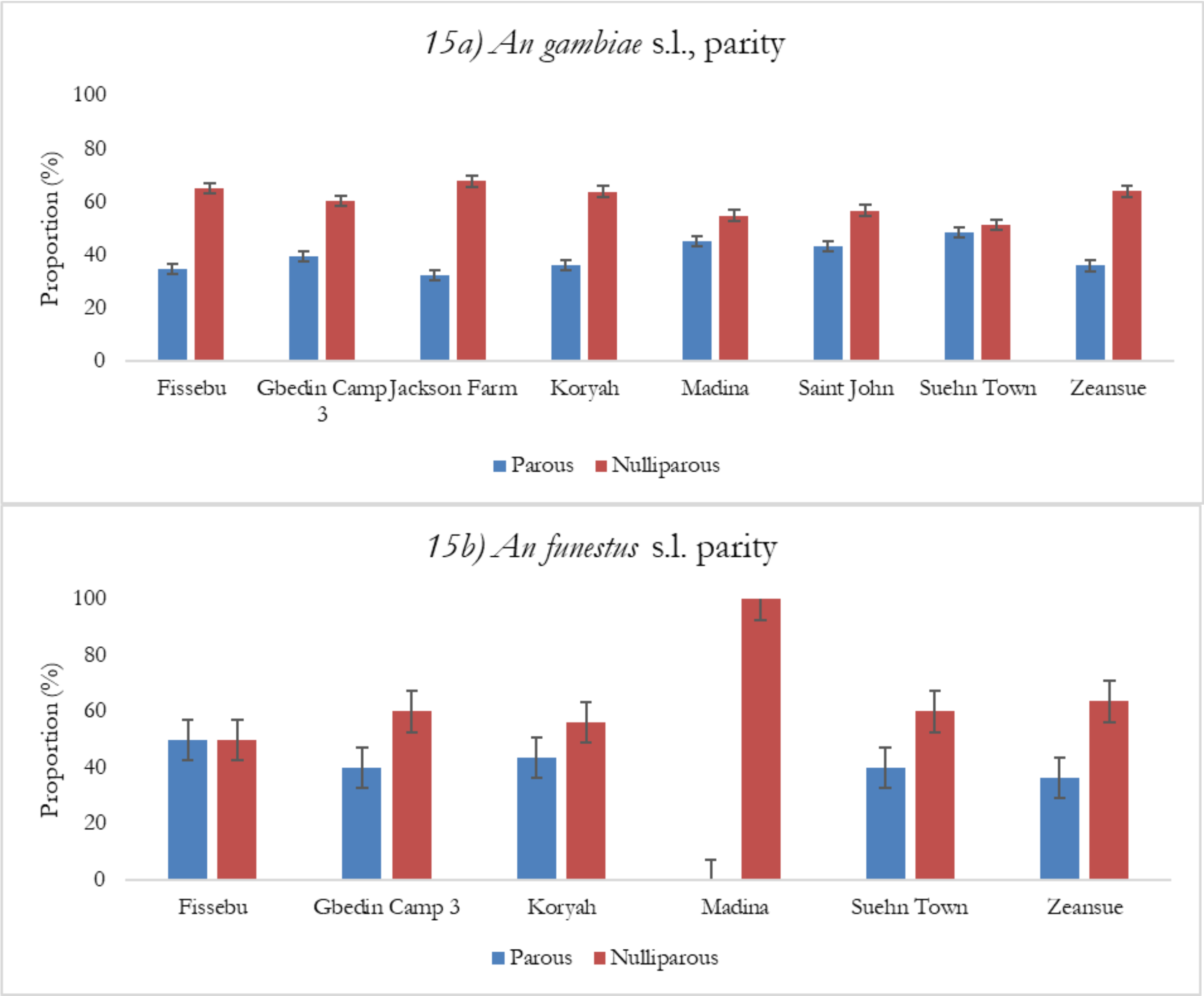
Anopheles funestus s.l.	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Grand Total
Indoor HLC (collected)	29	4	0	92	0	0	1	220	346
Outdoor HLC (collected)	4	0	2	65	2	4	2	273	352
Total	**33**	**4**	**2**	**157**	**2**	**4**	**3**	**493**	**698**
Endophagic Index (A)	0.88	1.00	0.00	0.59	0.00	0.00	0.33	0.45	0.50
Exophagic Index (B)	0.12	0.00	1.00	0.41	1.00	1.00	0.67	0.55	0.50
Ratio (A/B)	7.25	-	-	1.42	-	-	0.50	0.81	0.98
Chi^2 (p-value)	1.35E-05*	-	-	0.0312*	-	-	0.480	0.017*	0.820

*= difference significant (<0.05)

3.1.7 PARITY RATE

The ovaries of unfed *An. gambiae* s.l. and *An. funestus* s.l. were dissected in the field and read under a compound microscope. **Figure 15a and 15b** show the percentages of parous mosquitoes (mosquito that has laid eggs at least once in their lifetime) and nulliparous (mosquito that has not laid) for *An. gambiae* s.l. and *An. funestus* s.l. In general, the proportions of parous females were less than 50%. The highest parity rate for *An. gambiae* s.l. was observed in Suehn Town (48.5%). In Zeansue and Koryah, where most of the *An. funestus* s.l. mosquitoes were collected and dissected, the proportion of parous mosquitoes was lower than the nulliparous.

FIGURE 15: PARITY RATE OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED FROM INDOOR AND OUTDOOR BY HLC IN EIGHT SITES, OCTOBER 2021 - SEPTEMBER 2022.

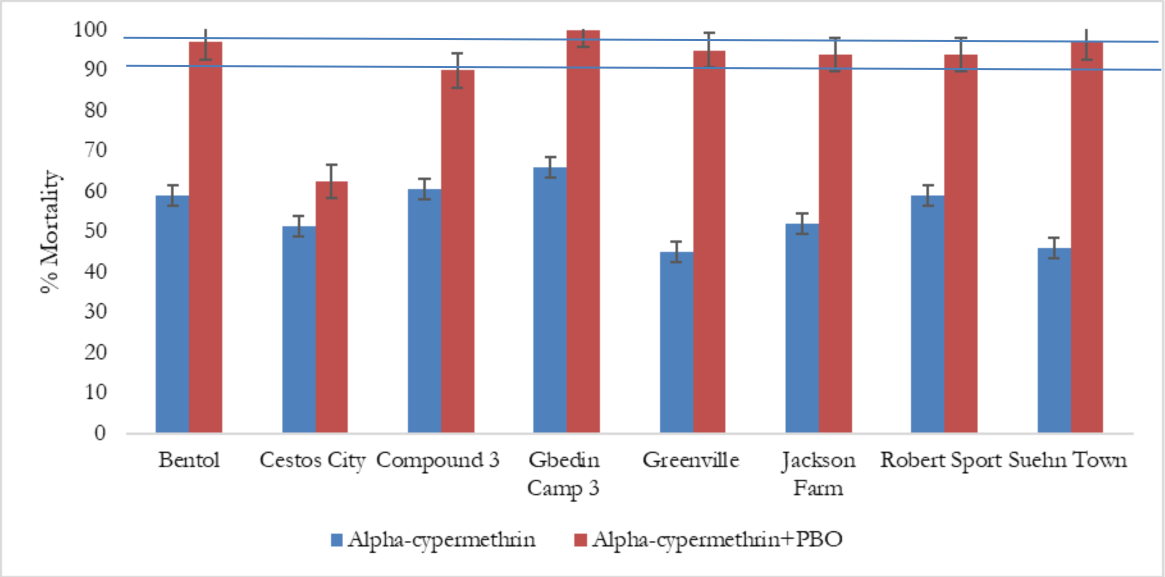


3.2 INSECTICIDE RESISTANCE MONITORING

3.2.1 INSECTICIDE SUSCEPTIBILITY TEST FOR PYRETHROIDS AND SYNERGIST ASSAYS

During the reporting year, *An. gambiae* s.l. tested against the diagnostic dose (1x) of alpha-cypermethrin have showed resistance to this insecticide in all sites. Pre-exposure to PBO did not restore full susceptibility to alpha-cypermethrin at the 30 minutes diagnostic time in most the sites, except in Gbedin Camp 3 where the mortality rate increased from 66.0% with alpha-cypermethrin only to 100% with alpha-cypermethrin + PBO **(Figure 16)**. This trend was similar to last year's resistance reports. Molecular analysis of insecticide resistance mechanisms will help to complete a comprehensive insecticide resistance profile in Liberia. The team is preserving mosquito samples after each test so that these analyses can be conducted in Year 6.

FIGURE 16: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. FROM SITES IN LIBERIA EXPOSED TO DIAGNOSTIC DOSE (1X) OF ALPHA-CYPERMETHRIN WITH AND WITHOUT PRE-EXPOSURE TO PBO: CDC BOTTLE ASSAYS, NOVEMBER 2021– SEPTEMBER 2022

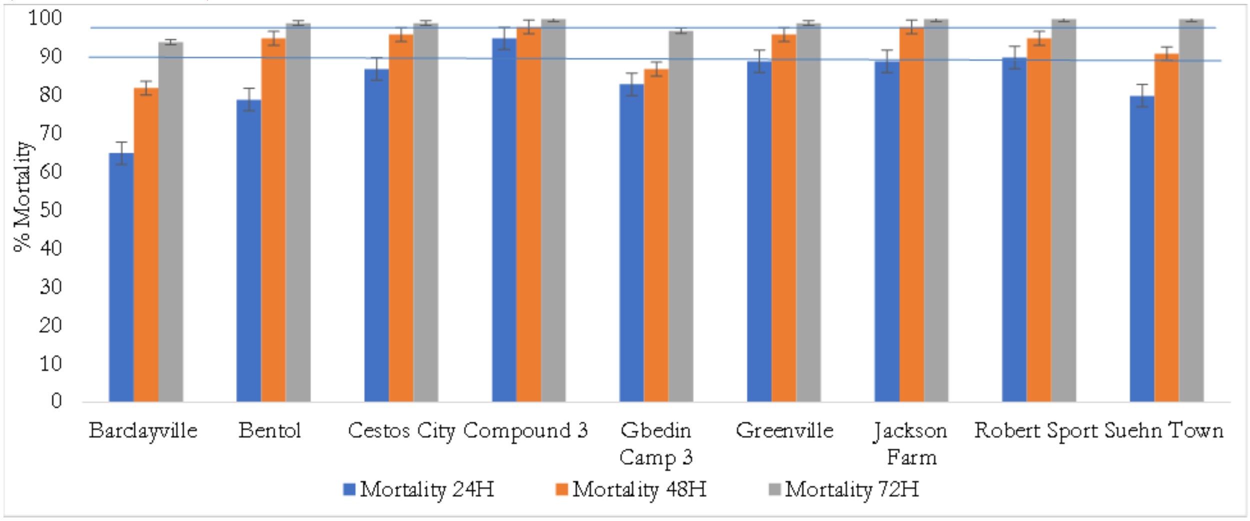


3.2.2 INSECTICIDE SUSCEPTIBILITY TESTING OF CHLORFENAPYR

Alpha-cypermethrin and chlorfenapyr are the two main active ingredients of the Interceptor® G2 ITNs distributed in 2021. During this reporting year, susceptibility of the vector to chlorfenapyr was tested in nine sites using adult *An. gambiae* s.l. from reared larvae. Based on the data after 72 hours, full susceptibility (100% mortality) was recorded in all sites except in two - Barclayville (94%) and Gbedin Camp 3 (97%) **(Figure 17)**. According to the new SOP[5] for tests against chlorfenapyr, mortality >90% is considered as susceptible given it is performed under controlled temperature and humidity. All tests in Liberia are performed at the insectary under a controlled environment. The new SOP for chlorfenapyr testing which includes parallel testing of susceptible colony, will be implemented in Year 6.

[5]Determining susceptibility status of *Anopheles* mosquitoes to a discriminating concentration of chlorfenapyr (100µg/bottle) in bottle bioassays

FIGURE 17: MORTALITY RATE OF *AN. GAMBIAE* S.L. TESTED AGAINST CHLORFENAPYR (100µG/BOTTLE) USING CDC BOTTLE ASSAY, NOVEMBER 2021– SEPTEMBER 2022



BIOASSAYS OF INTERCEPTOR® G2 ITNS

Cone assays, tunnel tests and chemical content analysis for 12 months post-distribution of Interceptor® G2 ITNs are underway. Results will be included in the 12-month Streamlined Durability Monitoring Report.

3.3 New Insectary Relocation Process

VectorLink is working with the NMCP to relocate the insectary to host both the resistance and susceptible mosquito colonies. Site visits conducted by PMI, NMCP and VectorLink concluded that the most suitable location is the National Public Health Institute of Liberia (NPHIL)/LIBR. VectorLink Home Office is working to procure a local vendor for the containers.

3.4 ITN Monitoring Dashboard

Entomological data available in VectorLink Collect will be used for an ITN monitoring dashboard created by PATH and presented to NMCP in Year 6. The main aim of this tool is to perform integrated data analysis including entomological, epidemiological and climate data to assess the impact of the Interceptor® G2 ITNs distributed in Liberia at the national level in 2021.

3.5 Laboratory Analyses

At the time of this report, five months of ELISA-CSP data are available and there is an ongoing effort by VectorLink to support LIBR on the sample preparation process including head-thorax cutting which is time consuming for the small team at LIBR. The ELISA results will be analyzed with the HBR and sporozoite rate and will be an addendum to this report once available.

In Year 5, PMI through CDC Atlanta Entomology Branch provided remote training on PCR for molecular identification to the LIBR team. Despite some challenges, the LIBR lab staff made initial progress on this technique. However, troubleshooting is ongoing regarding the PCR product volumes for accurate gel analysis. An in-person training by the CDC team is planned for the LIBR team in Year 6 (January 2023) to address PCR issues as well as provide training on molecular resistance mechanism protocols and blood meal analysis using PCR.

3.6 COVID-19 Prevention Measures

In the field, guidance for COVID mitigation was followed by VectorLink and NMCP staff and CHWs. The team used bedsheets for PSC one time per house and CHWs were provided with 5ml glass tubes (instead of aspirators) to catch mosquitoes during HLC.

3.7 Capacity Strengthening Activities

Over the past five years, the PMI VectorLink Liberia project has worked to strengthen capacity of the NMCP and local staff. VectorLink has included NMCP staff in field data collection and supported NMCP staff to participate in meetings, conferences, and trainings. VectorLink has also worked closely with the University of Liberia entomologist by including him in field activities. His participation in entomological monitoring activities has improved his skills and motivated him to take a research position where he is working now to setup a laboratory to support research on fruit flies. The CHWs and NMCP staff who have been trained in the field by VectorLink are now able to perform field collection of larvae independently.

After setting up a laboratory room at LIBR and training LIBR staff on ELISA-CSP testing in Year 4, LIBR has been successfully and efficiently conducting ELISA analyses. VectorLink is focusing on building local capacity in PCR techniques in the coming Year 6.

Table 8 shows the types of trainings and number of people trained during the reporting year.

TABLE 8: SUMMARY OF VECTORLINK CAPACITY STRENGTHENING ACTIVITIES IN LIBERIA IN YEAR 5

	Numbers Trained				
Activity	**NMCP**	**CHWs**	**Field Supervisors**	**University of Liberia or NPHIL**	**Total**
Insecticide resistance testing	4	28			32
Adult and larval mosquito collection methods	2	32	16	2	52
Field morphological ID	4	32	16	2	54
ITN durability monitoring bioassay training	2				2
Mosquito rearing				2	2

**One NMCP staff attended PMI VectorLink regional entomology training held in Senegal.*

4. OBSERVATIONS AND CONCLUSIONS

From October 2021 to September 2022 (Year 5), VectorLink and NMCP were able to successfully collect monthly data on vector bionomics and annual insecticide resistance data of the main malaria vectors at eight sites across Liberia. The bionomics data includes data on vector abundance, species composition, temporal and spatial trends, biting rate, and biting location preference. Conclusions are summarized below:

- The population dynamics of *An. gambiae* s.l. as a major vector is better understood in the sentinel sites and its densities are higher during the period of April to July, with a decrease in August following the heavy rain season. Messages or interventions to protect the community should be timed immediately before the mosquito peak month of April.

- *Anopheles funestus* s.l. was most predominant in Zeansue, with a moderate abundance in Koryah followed by Fissebu. The ongoing ELISA tests included *An. funestus* s.l. samples to assess its contribution in malaria transmission in the study sites. The presence of *An. funestus* s.l. in these sites is due to the type of breeding (permanent swamps with vegetation) which are more suitable for this species.

- The results of this year have confirmed the trend observed last year showing that *An. gambiae* s.l. populations were resistant to the tested pyrethroid (alpha-cypermethrin) in all sites. Despite an increase of susceptibility after a pre-exposure to PBO as synergist, full susceptibility was observed in one site only (Gbedin Camp 3). While PBO also improved the susceptibility of the vector in the other sites, the final absolute mortalities were still less than the 98% threshold.

- *Anopheles gambiae* s.l. is susceptible to chlorfenapyr in all sites. The tests performed using chlorfenapyr showed an increase of mortality rates from 24hours to 48hours and full susceptibility occurred at 72 hours post-exposure.

- For *An. gambiae* s.l. and *An. funestus* s.l., an increase of biting pattern after 22:00 hours was reported in the sentinel sites. This is the time where most household members should be sleeping, so proper use of ITNs should be reinforced.

- Though both vectors (*An. gambiae* s.l. and *An. funestus* s.l.) slightly prefer biting outdoors than indoors, further human behavior observation assessment would be required to determine the human-vector contact throughout the night.

- Given a very high proportion of the mosquitoes collected by PSC were blood fed (future PCR tests will confirm blood meal host), SBC activities for sustained and appropriate use of ITNs should be strengthened. These finding would be discussed during the Technical Working Group meeting and recommendations forwarded to strengthen the SBCC messages promoting sustained use of ITNs.

- HLC seems to be the more efficient method of mosquito collection compared to the PSC and CDC-LT methods.

- The setup of the insectary for rearing of susceptible and resistant strains of *An. gambiae* s.l. and the training of lab techniques will help to promote entomological research and decision-making in Liberia.

5. REFERENCES

1. Coetzee, M. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). *Malar J* 19, 70 (2020). https://doi.org/10.1186/s12936-020-3144-9 National Malaria Control Program (NMCP) [Liberia], Ministry of Health (MOH), Liberia Institute of Statistics and Geo-Information Services (LISGIS), and ICF. 2017. Liberia Malaria Indicator Survey 2016. Monrovia, Liberia: MOH, LISGIS, and ICF.

2. Guidelines for evaluating insecticide resistance in vectors using the CDC bottle bioassay/methods in Anopheles research. CDC Atlanta USA: CDC Technical Report; p. 28.

6. ANNEXES

Annex 1: *Anopheles gambiae* s.l. Collected by PSC, October 2021 – September 2022

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2021	0	36	0	2	4	32	0	0	74
November 2021	0	39	5	12	97	28	0	2	183
December 2021	0	29	20	79	17	14	0	0	159
January 2022	0	1	14	40	15	2	2	1	75
February 2022	0	3	27	12	4	0	7	4	57
March 2022	0	10	32	11	3	46	9	20	131
April 2022	9	21	17	50	5	11	7	19	139
May 2022	10	36	68	92	21	28	44	37	336
June 2022	7	16	142	46	62	77	23	0	373
July 2022	8	16	20	3	162	82	9	46	346
August 2022	0	29	0	4	17	24	1	0	75
September 2022	4	15	2	3	73	41	0	3	141
Total	38	251	347	354	480	385	102	132	2089

Annex 2: *Anopheles funestus* s.l. Collected by PSC, October 2021–September 2022

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2021	0	0	0	4	0	0	0	9	13
November 2021	0	0	0	8	0	0	0	7	15
December 2021	1	0	0	24	0	0	0	11	36
January 2022	0	1	0	10	0	0	1	53	65
February 2022	1	1	0	11	0	0	0	116	129
March 2022	2	1	0	22	0	0	0	201	226
April 2022	5	1	0	33	1	0	0	213	253
May 2022	5	0	0	20	0	0	0	242	267
June 2022	3	0	2	9	0	0	0	9	23
July 2022	1	0	0	4	0	0	0	44	49
August 2022	0	0	0	1	0	1	0	7	9
September 2022	0	0	0	4	0	0	0	2	6
Total	18	4	2	150	1	1	1	914	1091

Annex 3: *Anopheles gambiae* s.l. Collected by HLC Indoor, October 2021 –September 2022

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2021	0	82	1	2	7	14	0	0	106
November 2021	0	87	0	3	27	29	2	0	148
December 2021	0	61	29	33	9	6	2	0	140
January 2022	0	8	8	20	3	0	16	0	55
February 2022	0	3	35	2	0	3	9	7	59
March 2022	0	14	34	8	0	39	13	3	111
April 2022	10	46	19	36	0	22	21	0	154
May 2022	30	50	79	56	16	70	68	9	378
June 2022	5	2	66	36	24	152	36	5	326
July 2022	15	13	15	3	62	117	7	0	232
August 2022	9	30	0	2	4	83	3	0	131
September 2022	5	11	2	1	4	71	0	3	97
Total	74	407	288	202	156	606	177	27	1937

Annex 4: *Anopheles gambiae* s.l. Collected by HLC Outdoor, October 2021 –September 2022

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2021	1	99	0	0	15	11	0	0	126
November 2021	0	89	10	2	15	26	1	0	143
December 2021	0	48	23	35	19	26	5	0	156
January 2022	0	5	13	41	15	0	11	2	87
February 2022	0	0	72	18	7	7	11	9	124
March 2022	0	16	83	14	3	32	11	7	166
April 2022	3	28	61	51	0	52	7	0	202
May 2022	4	11	138	73	17	66	81	4	394
June 2022	1	1	166	31	35	149	36	4	423
July 2022	0	14	19	5	136	139	13	1	327
August 2022	1	17	3	2	9	133	0	1	166
September 2022	2	4	0	0	36	125	1	0	168
Total	12	332	588	272	307	766	177	28	2482

Annex 5: *Anopheles funestus* s.l. Collected by HLC Indoor, October 2021 –September 2022

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2021	1	0	0	0	0	0	0	0	1
November 2021	0	0	0	7	0	0	0	5	12
December 2021	0	0	0	24	0	0	0	1	25
January 2022	0	0	0	10	0	0	1	34	45
February 2022	0	0	0	10	0	0	0	48	58
March 2022	0	1	0	7	0	0	0	32	40
April 2022	18	2	0	13	0	0	0	46	79
May 2022	4	1	0	9	0	0	0	41	55
June 2022	4	0	0	5	0	0	0	5	14
July 2022	1	0	0	6	0	0	0	1	8
August 2022	1	0	0	1	0	0	0	3	5
September 2022	0	0	0	0	0	0	0	4	4
Total	29	4	0	92	0	0	1	220	346

Annex 6: *Anopheles funestus* s.l. Collected by HLC Outdoor, October 2021 –September 2022

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2021	0	0	0	1	0	0	0	16	17
November 2021	0	0	0	1	0	0	0	5	6
December 2021	0	0	0	9	0	0	0	6	15
January 2022	0	0	0	7	0	0	2	27	36
February 2022	0	0	0	2	0	0	0	44	46
March 2022	0	0	0	2	2	0	0	70	74
April 2022	1	0	0	16	0	0	0	38	55
May 2022	2	0	0	10	0	0	0	42	54
June 2022	1	0	0	10	0	4	0	13	28
July 2022	0	0	1	4	0	0	0	11	16
August 2022	0	0	1	3	0	0	0	1	5
September 2022	0	0	0	0	0	0	0	0	0
Total	4	0	2	65	2	4	2	273	352

Annex 7: *Anopheles funestus* s.l. Collected by CDC-LT indoor, October 2021 –-September 2022

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2021	1	0	0	7	0	0	0	2	10
November 2021	0	0	0	6	0	0	0	4	10
December 2021	0	1	0	5	0	1	0	6	13
January 2022	1	1	0	7	0	0	0	24	33
February 2022	1	0	0	4	0	0	0	73	78
March 2022	1	2	0	12	0	0	2	128	145
April 2022	4	4	0	24	0	0	0	190	222
May 2022	3	3	1	17	0	0	0	53	77
June 2022	0	0	0	8	0	1	0	3	12
July 2022	1	2	0	4	0	0	0	10	17
August 2022	0	0	0	3	0	0	0	6	9
September 2022	0	0	0	2	0	0	0	0	2
Total	12	13	1	99	0	2	2	499	628

Annex 8: Proportions of *Anopheles gambiae s.l.* parous and nulliparous in the eight sites, October 2021 –-September 2022.

Site	Parous	Nulliparous	Total	p-values
Fissebu	23	43	66	0.014
Gbedin Camp 3	113	173	286	0.000
Jackson Farm	50	105	155	0.000
Koryah	79	140	219	0.000
Madina	56	68	124	0.281*
Saint John	92	121	213	0.047
Suehn Town	66	70	136	0.732*
Zeansue	9	16	25	0.162*
Total	488	736	1224	

Annex 9: Proportions of *Anopheles funestus s.l.* parous and nulliparous in six of the eight sites, October 2021 –-September 2022. *No data from Jackson Farm or Saint John due to low numbers.

Site	Parous	Nulliparous	Total
Fissebu	15	15	30
Gbedin Camp 3	2	3	5
Koryah	38	49	87
Madina	0	1	1
Suehn Town	2	3	5
Zeansue	75	131	206
Total	132	202	334