PMI VECTORLINK LIBERIA PROJECT

PMI VECTORLINK LIBERIA ENTOMOLOGICAL MONITORING FINAL REPORT

OCTOBER 1, 2020–SEPTEMBER 30, 2021

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ACRONYMS

CDC	Centers for Disease Control and Prevention
CHW	Community health worker
ELISA	Enzyme-linked immunosorbent assay
HLC	Human landing catch
IRS	Indoor Residual Spray
ITNs	Insecticide-Treated Nets
LIBR	Liberia Institute for Biomedical Research
LISGIS	Liberia Institute of Statistics and Geo-Information Services
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
SBC	Social and behavior change
SOP	Standard Operation Procedure
UL	University of Liberia

EXECUTIVE SUMMARY

VectorLink Liberia and the National Malaria Control Program (NMCP) performed malaria vector monitoring and insecticide resistance testing in selected sites from October 2020 to September 2021. During that period, monthly longitudinal entomological monitoring (vector bionomics) data were collected from eight sites: Fissebu (Lofa County), Koryah (Bong County), Madina (Grand Cape Mount County), Saint John (Grand Bassa County), Gbedin Camp 3 (Nimba County), Jackson Farm (Margibi County), Zeansue (Bong County) and Suehn Town (Bomi County). The main aim of these collections is to generate entomological data that can be used in the planning and impact monitoring of malaria prevention interventions, such as the Interceptor® G2 ITNs distributed countrywide in June – July 2021.

In longitudinal monitoring sites, three different methods of collection were used: pyrethrum spray catch (PSC) applied in 25 houses per site, human landing catch (HLC) with indoor and outdoor collections, and Centers for Disease Control and Prevention light traps (CDC-LTs) installed indoors only. To assess the susceptibility of *Anopheles gambiae* s.l. populations to insecticide, the CDC bottle bioassay method was utilized and tests were conducted in ten sentinel sites across the country (refer to map – Figure 1). The role of the synergist, piperonyl butoxide (PBO) in combination with alpha-cypermethrin on susceptibility of the vector was also tested using CDC bottle assays. In the eleventh site which is Zeansue, the number of larvae and subsequent adults available were insufficient to perform the susceptibility test.

From October 2020 to September 2021, using the three methods of collections (PSC, HLC, and CDC LTs), 11,572 *An. gambiae* s.l. (78.0%) and 3,203 *Anopheles funestus* s.l. (21.6%) and 64 other *Anopheles* mosquito species (0.4%) were collected in the eight sentinel sites. *Anopheles gambiae* s.l. was the predominant vector in six out of eight sites: Koryah (57.1%), Madina (98.9%), Saint John (96.2%), Jackson Farm (87.3%), and Gbedin Camp 3 (97.9%). The abundance of *An. funestus* s.l. was higher in Zeansue (82.2%) and Fissebu (66.7%).

Using PSC, the highest indoor resting density (IRD) of *An. gambiae* s.l. was recorded in: Gbedin Camp 3 (12 mosquitoes per house per day in November 2020), Suehn Town (10.4 mosquitoes per house per day in November 2020), and Jackson Farm (7.6 mosquitoes per house per day in May and June 2021). For *An. funestus* s.l., the highest IRD was observed in Zeansue (5.5 mosquitoes per house per day) in March 2021.

With CDC-LT collections, the highest number of *An. gambiae* s.l. was recorded in Gbedin Camp 3 (33.6 mosquitoes per trap per night) in August 2021. The period of June – August 2021 was a peak of abundance since the mean numbers per trap were in the interval 28.1 – 33.6 mosquitoes per trap per night. In Jackson Farm, the number of mosquitoes was 22.5 mosquitoes per trap per night in June 2021. In Fissebu, 10.3 *An. funestus* s.l. mosquitoes per trap per night were recorded in May 2021, while in Zeansue, 7.9 *An funestus* s.l. mosquitoes per trap per night were observed in April 2021.

Anopheles gambiae s.l. collected using HLCs showed that the highest mean indoor human biting rate (HBR) was 29.8 bites per person per night in Gbedin Camp 3 and 27 bites per person per night in Jackson Farm for both sites in June 2021. For outdoor collections, 32.2 bites per person per night was recorded in Gbedin Camp 3 and 29.3 bites per person per night in Jackson Farm, both in June 2021. From the HLCs collections, the highest HBR for *An. funestus* s.l. was in Zeansue in March 2021, 16.3 bites per person per night indoors and 17.5 bites per person per night outdoors.

Anopheles gambiae s.l. larva collected from various sites and reared to adults were tested for susceptibility to different insecticides. *Anopheles. gambiae* s.l. were resistant to alpha-cypermethrin in all nine test sites. However, pre-exposure of *An. gambiae* s.l. mosquitoes to the synergist PBO restored full susceptibility to alpha-cypermethrin in five of the nine sites. With the tests performed on chlorfenapyr using *An. gambiae* s.l. mosquitoes, the post-exposure mortalities were recorded after 24 hours, 48 hours and 72 hours. Overall, after 72 hours holding period, the mortality was 100% in nine sites with the tenth site, Harper, have a mortality of 98.8%. Based on these findings, it is expected that the Interceptor® G2 ITNs distributed in 2021 should have a better impact on *An. gambiae* s.l. resistant to pyrethroids.

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I. INTRODUCTION

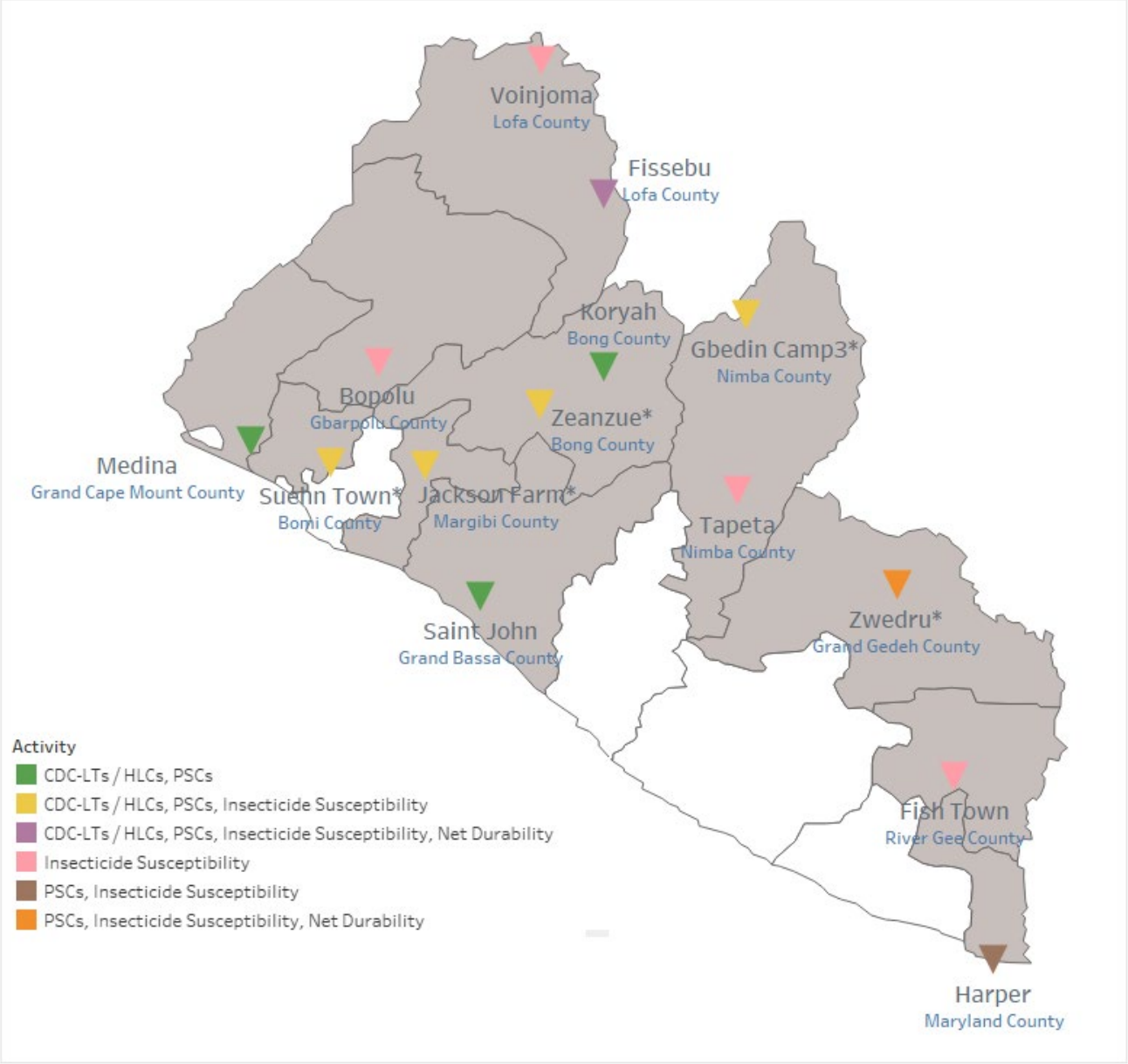
From 2009 to 2013, indoor residual spraying (IRS) was implemented in Liberia by the U.S. President's Malaria Initiative (PMI) in collaboration with the National Malaria Control Program (NMCP) and Ministry of Health (MOH). In 2012, IRS was implemented in 14 districts selected from five counties (Grand Bassa, Montserrado, Margibi, Bong and Bong). Due to an increase of insecticide and operation cost, PMI decided to shift support to insecticide resistance surveillance and vector monitoring to generate data which would guide decision making. After presenting field data on vector resistance to pyrethroids, PMI, NMCP, and the Ministry of Health agreed to switch from standard pyrethroid nets to Interceptor® G2 nets for mass distribution in 2021 to mitigate the resistance of the major malaria vector *An. gambiae* s.l. to pyrethroids.

In January 2020, the team started collecting vector bionomics data in eight sites: Fissebu (Lofa County), Gbedin Camp 3 (Nimba County), Jackson Farm (Margibi County), Koryah (Bong County), Zeansue (Bong County), Madina (Grand Cape Mount County), Saint John (Grand Bassa County), Suehn Town (Bomi County), (Figure 1). Due to COVID-19 pandemic, the data collection was disrupted from March 2020 to July 2020 when Liberia health authorities restricted travel to counties. In Year 4 (October 2020 to September 2021), the field activities were performed monthly in the eight sites listed above. The methods of collection were kept the same in order to obtain two consecutive years of data for an Interceptor® G2 ITN impact assessment. Pyrethrum Spray Catches (PSCs), Human Landing Catches (HLCs), and Centers for Disease Control light traps (CDC-LTs) were used to collect mosquitoes and allow for monitoring seasonal vector density, species composition, and behavior.

In Year 4, insecticide resistance testing was conducted in ten sites including counties from South – East regions where the road access is challenging during the rainy season. VectorLink and NMCP assessed *An. gambiae* s.l. susceptibility status to alpha-cypermethrin with or without piperonyl butoxide (PBO) as synergist and chlorfenapyr.

In addition to these activities, the team performed cone bioassays on samples of Duranet® nets, a pyrethroid only treated standard insecticide treated nets (ITNs), from two counties Lofa and Grand Gedeh for the 36-month durability monitoring of the ITNs distributed in the 2018 ITN mass distribution in Liberia.

FIGURE 1: VECTORLINK LIBERIA ENTOMOLOGICAL MONITORING, INSECTICIDE RESISTANCE AND DURABILITY MONITORING SITES, OCTOBER 2020 – SEPTEMBER 2021[1]



In Year 4, the main objectives of VectorLink Liberia entomological monitoring activities were:

- Assess *Anopheles* vector bionomics, including species composition, density, and behavior, in eight 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.

[1] Insecticide susceptibility testing was planned in 11 sites, but only conducted in 10 sites due to low number of larvae found in Zeansue.

- 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 11 sites.
- Maintain and support a functional insectary for bioassays tests in country.
- Conduct bioassays on ITNs in support of ITN 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 2020 to September 2021, VectorLink working with the NMCP and the University of Liberia (UL), was able to conduct monthly longitudinal entomological monitoring activities in eight sites with the support of community health workers (CHWs) in Liberia.

Insecticide resistance tests (on pyrethroid and chlorfenapyr) were performed in ten sites out of the eleven sites planned for the year (Table 1) by VectorLink and NMCP staff with the involvement of CHWs trained by our joint team. For cost savings, vector monitoring trips and larval collections for insecticide resistance testing were conducted during a single trip. Larva were transported to Monrovia for rearing and tests on adult mosquitoes were performed at the VectorLink insectary facility.

Due to limited accessibility in three sites located in the Southeast of the country (Zwedru, Fish Town and Harper), all larval collection were done by local CHWs teams supervised by one experienced CHW from Zwedru, who was trained on mosquito larval collections and rearing by VectorLink. The teams were able to pack and ship live larva and adult mosquitoes from these three sites by domestic flight without any incident.

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

Province/ Region	County	Site	PSC / CDC-LT / HLCs	Insecticide Susceptibility	ITN Durability Monitoring
North Central	Lofa	Fissebu	X	X	X
		Voinjoma		X	
	Bong	Koryah	X		
		Zeansue	X	X*	
	Bomi	Suehn Town	X	X	
	Nimba	Gbedin Camp 3	X	X	
		Tappita		X	
South Central	Grand Bassa	Saint John	X		
	Margibi	Jackson Farm	X	X	
Northwestern	Grand Cape Mount	Medina	X		
	Gbarpolu	Bopolu		X	
Southeastern A	Grand Gedeh	Zwedru		X	X
Southeastern B	Maryland	Harper		X	
	River Gee	Fish Town		X	

**: In Zeansue, few larvae were collected, so the number of adults was not sufficient to perform a test.*

2.1.2 Routine Monitoring Site Collection methods

Three methods were used to collect adult mosquitoes in the longitudinal entomological monitoring sites: 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.

2.1.2.1 Pyrethrum Spray Catches

PSCs were performed in 25 houses for two consecutive days per site. A commercial pyrethroid insecticide spray including PBO named Kwik,[2] was used according to VectorLink SOP (SOP03/01). Following PMI guideline for COVID-19 mitigation measures, and for precaution, a set of white bedsheets was used one time per house in all the eight sites.

2.1.2.2 CDC Light Trap Collection

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

2.1.2.3 Human Landing Collection

HLCs were conducted both indoors and outdoors using glass tubes instead of mouth aspirator for COVID-19 mitigation.

Morphological identification of all mosquitoes collected according to Coetzee et al. (2020) was completed at each site by VectorLink, NMCP, and UL staff under a dissection microscope. *An. gambiae* s.l. mosquitoes were counted based on their blood-digestion stage while they are fresh. *Anopheles* samples were preserved in silica gel for further processing of the head-thorax portions in the laboratory at Liberia Institute for Biomedical Research (LIBR) using enzyme-linked immunosorbent assay (ELISA) for sporozoite rate assessment.

2.1.3 Insecticide Resistance Monitoring

The sites where VectorLink, the NMCP, and CHWs conducted insecticide resistance monitoring from October 2020 to September 2021 are shown in Table 3. Susceptibility tests for alpha-cypermethrin were performed in

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

nine sites and vector susceptibility to chlorfenapyr was tested in ten sites. Larvae collected in sites closest to Monrovia and those sites in the south-east which had to be transported via air were transported to the main insectary in Monrovia for rearing and testing of adult females according to SOP14/01. Testing at the other sites were done in the field. To prevent cross contamination, wildtype and susceptible lab colony larva were reared in two different rooms in the insectary.

TABLE 3: SUMMARY OF INSECTICIDE SUSCEPTIBILITY AND SYNERGIST ASSAYS PERFORMED FROM OCTOBER 2020 – SEPTEMBER 2021

Province/ Region	County	Site	Insecticides Tested	
			Alpha-cypermethrin ±PBO	Chlorfenapyr
North Central	Lofa	Fissebu	X	X
		Voinjoma	X	X
	Bong	Zeansue*		
	Bomi	Suehn Town	X	X
	Nimba	Gbedin Camp 3	X	X
		Tappita	X	X
	Margibi	Jackson Farm	X	X
Northwestern	Gbarpolu	Bopolu		X
Southeastern A	Grand Gedeh	Zwedru	X	X
Southeastern B	Maryland	Harper	X	X
	River Gee	Fish Town	X	X

** In Zeansue, few larvae were collected, so the number of adults was not sufficient to perform a test.*

A subsample of dead and surviving mosquitoes were 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

In Year 4, 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 according to SOP04/01. It was tested alone as well as with a synergist (PBO).

2.1.3.2 INSECTICIDE SUSCEPTIBILITY TEST FOR CHLORFENAPYR – CDC BOTTLE ASSAY METHOD

An. gambiae s.l. was tested for susceptibility to chlorfenapyr using the CDC bottle assay method as per the VectorLink SOP (SOP04/01). Chlorfenapyr was tested in 11 sites from five provinces of Liberia. Insecticide grade chlorfenapyr in vials were provided free of charge to VectorLink by CDC Atlanta Entomology Branch. Each pre-weighed technical grade of chlorfenapyr was dissolved in 50 ml acetone. 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 kept covered overnight to dry before using 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. Due to the slow acting characteristics of the insecticide, 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

Synergist assays were performed using the CDC bottle assay method as per the VectorLink SOP (SOP04/01). Pre-exposure to a synergist, PBO, was done before introducing mosquitoes to the diagnostic dose of alpha-cypermethrin to assess whether susceptibility could be improved or fully restored. 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. These were left to dry overnight. 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.

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 a 36-month standard durability monitoring study of ITNs, which concluded in Year 4. In addition, VectorLink initiated a streamlined durability monitoring study of Interceptor® G2 ITNs.

2.2.1 STANDARD DURABILITY MONITORING BIOASSAYS

Cone bioassays were conducted on ITNs (Duranet) collected as part of the 36-month durability monitoring study. A total of 60 ITNs were collected in the two sites (30 ITNs in Lofa County and 30 ITNs in Grand Gedeh County). On each ITN, two sets of five pieces were cut, labeled, and packed. One set of the ITN pieces were used for in-country cone bioassays testing using lab colony *An. coluzzii* strain susceptible to insecticide. In total, 100 female mosquitoes were tested per ITN. The second set of ITN samples was sent to CDC Atlanta for chemical content tests. The results of bioassays and chemical content tests were shared in the ITN durability monitoring report.

2.2.2 STREAMLINED DURABILITY MONITORING BIOASSAYS AND TUNNEL TESTS

In June – July 2021, Interceptor® G2 ITNs were distributed countrywide in Liberia for ITN mass campaign. The VectorLink team conducted pre-distribution bioassays (cone and tunnel tests) on 10 Interceptor® G2 ITNs. From each ITN, 5 pieces were tested using cone bioassay method with susceptible *An. coluzzii* lab colony strain from the VectorLink Liberia insectary. For tunnel bioassays, ten samples (one piece per ITN) were cut, labeled and shipped to PAMVERC/Benin for tunnel testing. The tunnel test results are expected from the subcontractor by the end of December 2021. Nets for chemical residue analysis will be sent to CDC by the end of December 2021 or January 2022. A combined report of cone bioassay, tunnel test and chemical residue analysis for the Interceptor® G2 ITN pre-distribution time point will be submitted as soon as all results become available.

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.

3.1.1 Vector Abundance and Species Composition

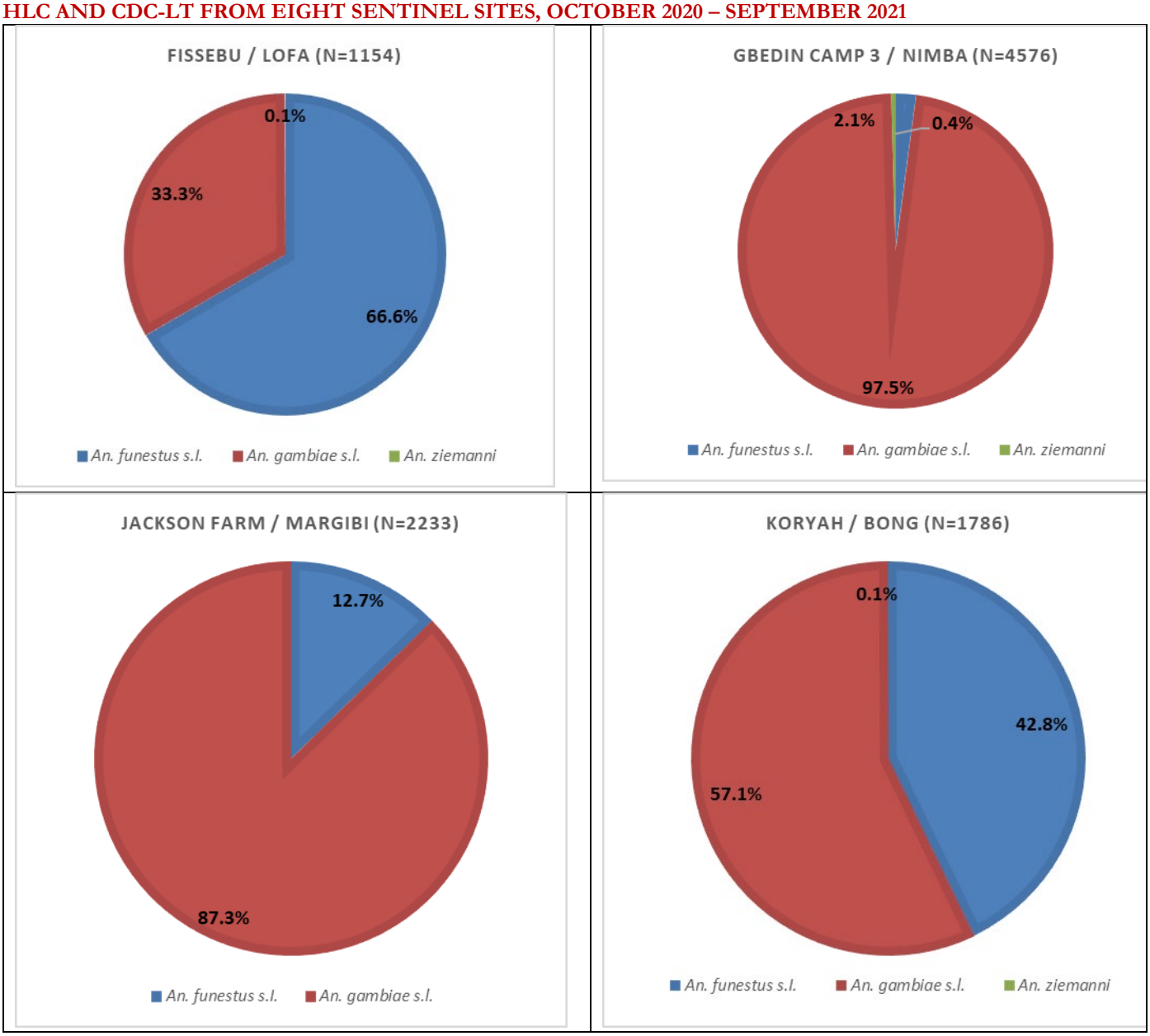
Using the three methods of collection (PSC, CDC-LTs and HLC), 14,839*Anopheles* mosquitoes were collected in eight sites. The proportions per species are: 78.0% *An. gambiae* s.l., 21.6% *An. funestus* s.l. and 0.4% other *Anopheles* species (*An. ziemanni* and *An. rufipes*) (Table 4). *Anopheles* mosquitoes were most abundant in Gbedin Camp 3 (30.8%), Jackson Farm (15%) and Suehn Town (14.1%). All these three sites are rice irrigated zones with higher potential breeding sites for *Anopheles* mosquitoes.

Table 4: Total Mosquitoes Collected from Eight Sentinel Sites, October 2020– September 2021 (All Collection Methods)

Site	*An. gambiae s.l.*		*An. funestus s.l.*		*An. rufipes*		*An. ziemanni*		Total *Anopheles*	
	n	%	n	%	n	%	n	%	n	%
Fissebu / Lofa	384	33.3	769	66.6	0	0	1	0.1	1,154	7.8
Gbedin Camp 3 / Nimba	4,460	97.5	96	2.1	0	0	20	0.4	4,576	30.8
Jackson Farm / Margibi	1,950	87.3	283	12.7	0	0	0	0	2,233	15.0
Koryah / Bong	1,020	57.1	765	42.8	0	0	1	0.1	1,786	12.0
Madina	538	98.0	6	1.1	0	0	5	0.9	549	3.7
Saint John / Grand Bassa	920	93.6	36	3.7	1	0.1	26	2.6	983	6.6
Suehn Town / Bomi	2,039	97.5	44	2.1	0	0	9	0.4	2,092	14.1
Zeansue / Bong	261	17.8	1,204	82.1	0	0	1	0.1	1,466	9.9
Total	11,572	78.0	3,203	21.6	1	0	63	0.4	14,839	100.0

In six out of the eight sentinel sites, *An. gambiae* s.l. is the most abundant malaria vector while *An. funestus* s.l. was predominant in two sites (Zeansue and Fissebu), (Table 4 and Figure 2). These two sites have suitable breeding sites for *An. funestus* s.l. which is known to be adapted to breeding in permanent and semi-permanent swamps with aquatic vegetation.

FIGURE 2: SPECIES COMPOSITION OF *ANOPHELES* MOSQUITOES COLLECTED BY PSC, HLC AND CDC-LT FROM EIGHT SENTINEL SITES, OCTOBER 2020 – SEPTEMBER 2021



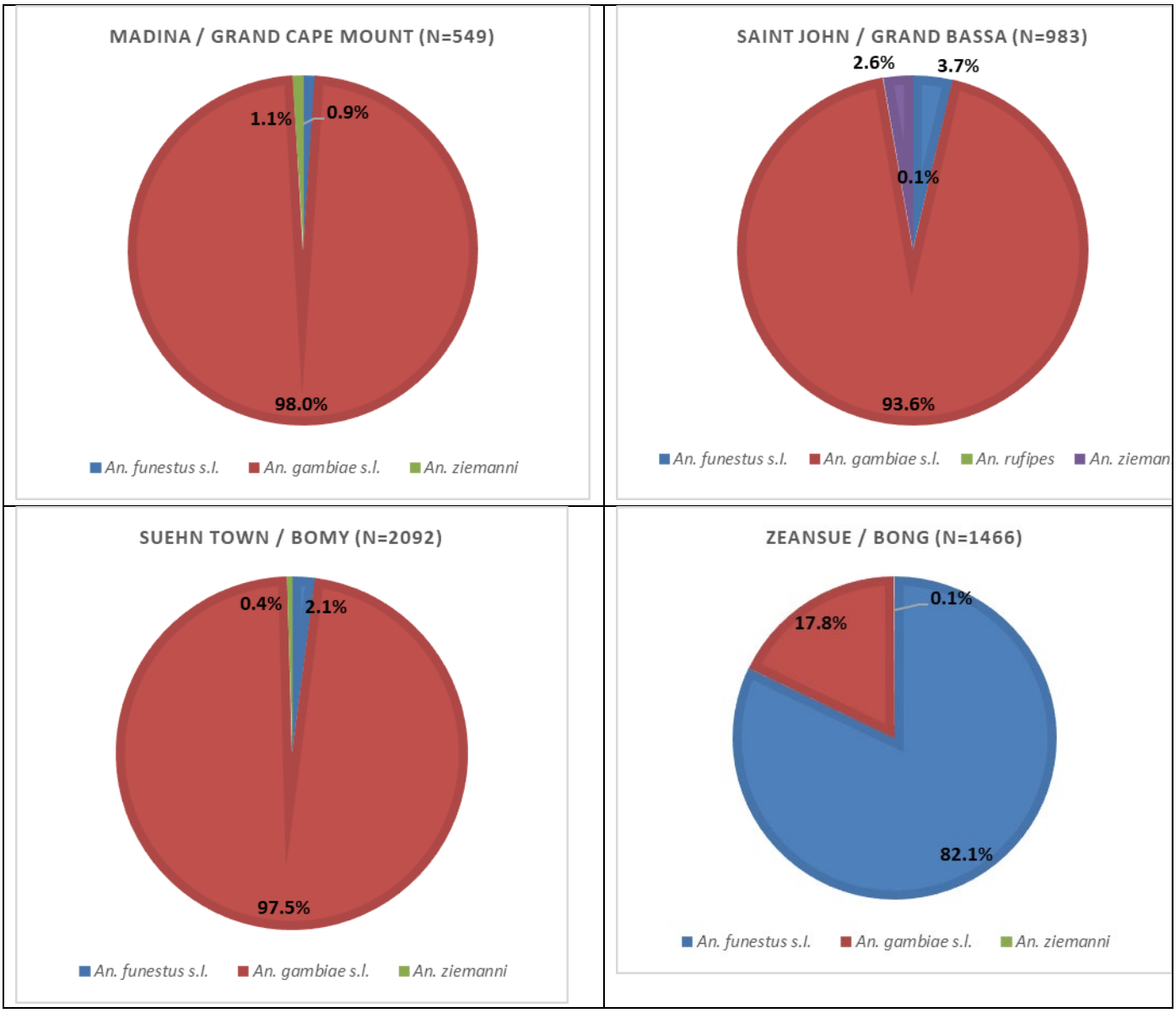


Table 5 summarizes the total number of mosquitoes collected per site using the three methods. The HLC collections were performed for eight person-nights per month per site and similarly the CDC-LT collections represent eight trap-night per month per site. However, overall, the HLC (38.7%) seems to collect more mosquitoes than CDC-LT (23.5%). The HLC collections were also higher (38.7%) than the PSC (37.8%) even though the PSC collections represent 25 house-days per site per month. For *An. gambiae* s.l., 39.9% mosquitoes were collected by HLC, 36.5 % by PSC and 23.5% for CDC-LT. The proportion of *An. funestus* s.l. mosquitoes collected resting indoor by PSC (42.6%) was higher than HLC and CDC-LT, 33.8% and 23.7%, respectively. The higher proportion of *An. funestus* s.l. mosquitoes collected resting indoors by PSC could associated to their more endophilic behavior than *An. gambiae* s.l. More *An. ziemanni* were collected by HLC than the other methods possibly due to their preference to outdoor biting and resting.

TABLE 5: *ANOPHELES* MOSQUITOES SPECIES COLLECTED IN EIGHT SENTINEL SITES, OCTOBER 2020 –– SEPTEMBER 2021.

Site	Method	*An. gambiae* s.l.		*An. funestus* s.l.		*An. rufipes*		*An. ziemanni*		Total	
		n	%	*n*	%	*n*	%	*n*			
Fissebu	PSC	76	19.8%	237	30.8%	0	-	0	0.0%	313	27.1%
	CDC-LT	76	19.8%	276	35.9%	0	-	0	0.0%	352	30.5%
	HLC	232	60.4%	256	33.3%	0	-	1	100.0%	489	42.4%
	Sub Total	**384**		**769**		**0**		**1**			
Koryah	PSC	521	51%	400	52.3%	0	-	0	0.0%	921	51.6%
	CDC-LT	148	15%	153	20.0%	0	-	0	0.0%	301	16.9%
	HLC	351	34%	212	27.7%	0	-	1	100.0%	564	31.6%
	Sub Total	**1020**		**765**		**0**		**1**			
Madina	PSC	391	72.7%	4	66.7%	0	-	0	0.0%	395	71.9%
	CDC-LT	22	4.1%	0	0.0%	0	-	1	20.0%	23	4.2%
	HLC	125	23.2%	2	33.3%	0	-	4	80.0%	131	23.9%
	Sub Total	**538**		**6**		**0**		**5**			
Saint John	PSC	476	51.7%	23	63.9%	0	0%	3	11.5%	502	51.1%
	CDC-LT	97	10.5%	1	2.8%	0	0%	2	7.7%	100	10.2%
	HLC	347	37.7%	12	33.3%	1	100%	21	80.8%	381	38.8%
	Sub Total	**920**		**36**		1		**26**			
Gbedin Camp 3	PSC	1039	23.3%	24	25.0%	0	-	11	55.0%	1,074	23.5%
	CDC-LT	1815	40.7%	49	51.0%	0	-	8	40.0%	1,872	40.9%
	HLC	1606	36.0%	23	24.0%	0	-	1	5.0%	1,630	35.6%
	Sub Total	**4,460**		**96**		**0**		**20**			
Suehn Town	PSC	805	39.5%	13	29.5%	0	-	0	0.0%	818	39.1%
	CDC-LT	135	6.6%	6	13.6%	0	-	0	0.0%	141	6.7%
	HLC	1099	53.9%	25	56.8%	0	-	9	100.0%	1,133	54.2%
	Sub Total	**2,039**		**44**		**0**		**9**			
Jackson Farm	PSC	798	40.9%	178	62.9%	0	-	0	-	976	43.7%
	CDC-LT	397	20.4%	47	16.6%	0	-	0	-	444	19.9%
	HLC	755	38.7%	58	20.5%	0	-	0	-	813	36.4%
	Sub Total	**1950**		**283**		**0**		**0**			
Zeansue	PSC	120	46.0%	484	40.2%	0	-	0	0.0%	604	41.2%
	CDC-LT	33	12.6%	226	18.8%	0	-	1	100.0%	260	17.7%
	HLC	108	41.4%	494	41.0%	0	-	0	0.0%	602	41.1%
	Sub Total	**261**		**1,204**		0		**1**			
Total	PSC	**4,226**	36.5%	**1363**	42.6%	**0**	0%	**14**	22.2%	5,603	37.8%
	CDC-LT	**2,723**	23.5%	**758**	23.7%	**0**	0%	**12**	19.0%	3,493	23.5%
	HLC	**4,623**	39.9%	**1082**	33.8%	**1**	100%	**37**	58.7%	5,743	38.7%
	Total	**11,572**	19.8%	**3203**		**1**		**63**		**11,572**	**100%**

3.1.2 INDOOR RESTING DENSITY

The highest indoor resting densities were observed in Gbedin Camp 3 (12 mosquitoes per house per day) and in Suehn Town (10.4 mosquitoes per house per day) in November 2020 , followed by Jackson Farm (7.6 mosquitoes per house per day) in May and June 2021, (Figure 3).

In Zeansue, a peak of *An. funestus* s.l. indoor resting density (5.5 mosquitoes per house per day) was noted in March 2021 (Figure 4), followed by Jackson Farm (3.2 mosquitoes per house per day in October 2020) and Koryah (2.8 and 2.3 mosquitoes per house per day in April 2021 and December 2020, respectively). In Year 3, these sites also had higher densities of *An. funestus* s.l. comparatively to the other sites.

FIGURE 3: INDOOR DENSITIES OF *AN. GAMBIAE* S.L. COLLECTED BY PSC, OCTOBER 2020-SEPTEMBER 2021.

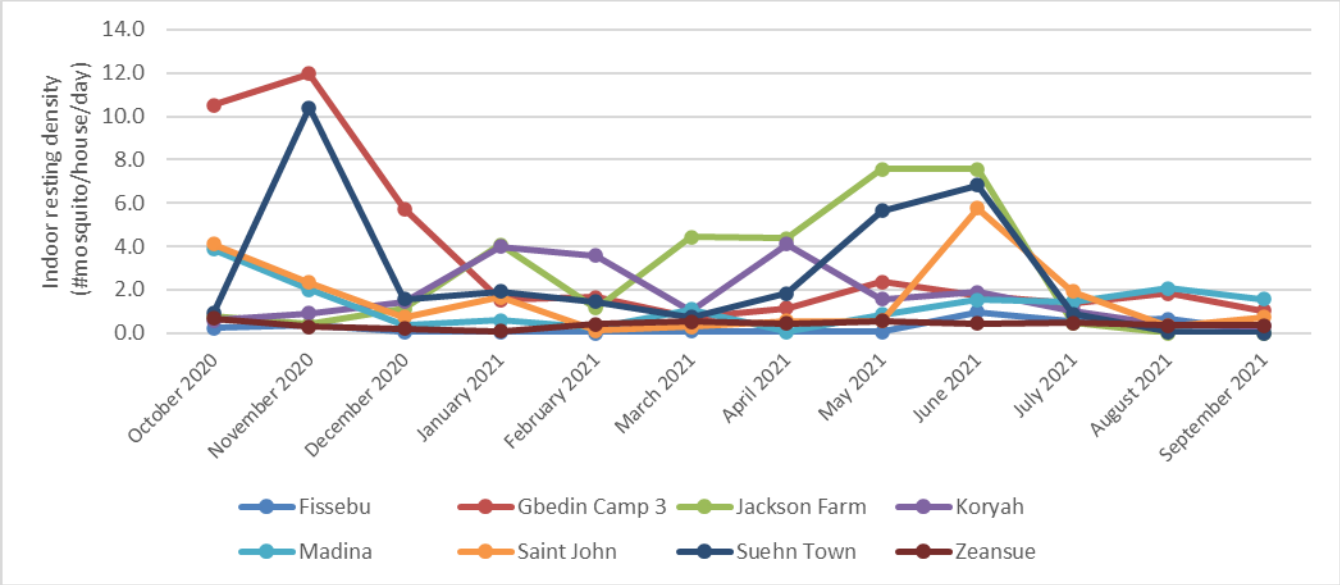
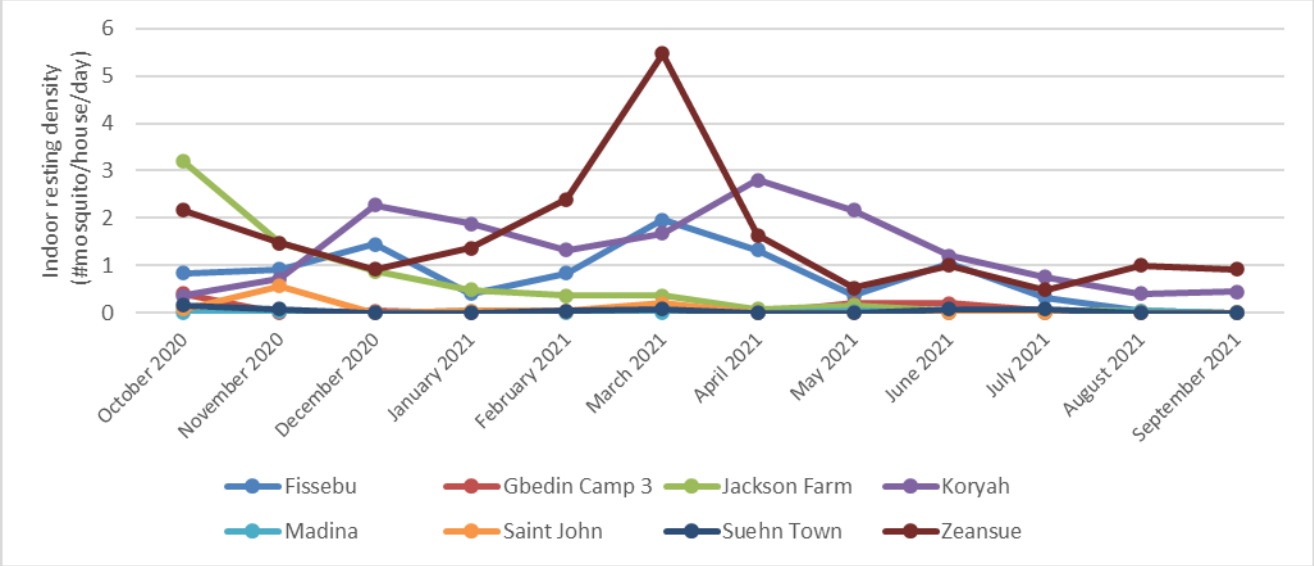


FIGURE 4: INDOOR DENSITIES OF *AN. FUNESTUS* S.L. COLLECTED BY PSC, OCTOBER 2020-SEPTEMBER 2021.



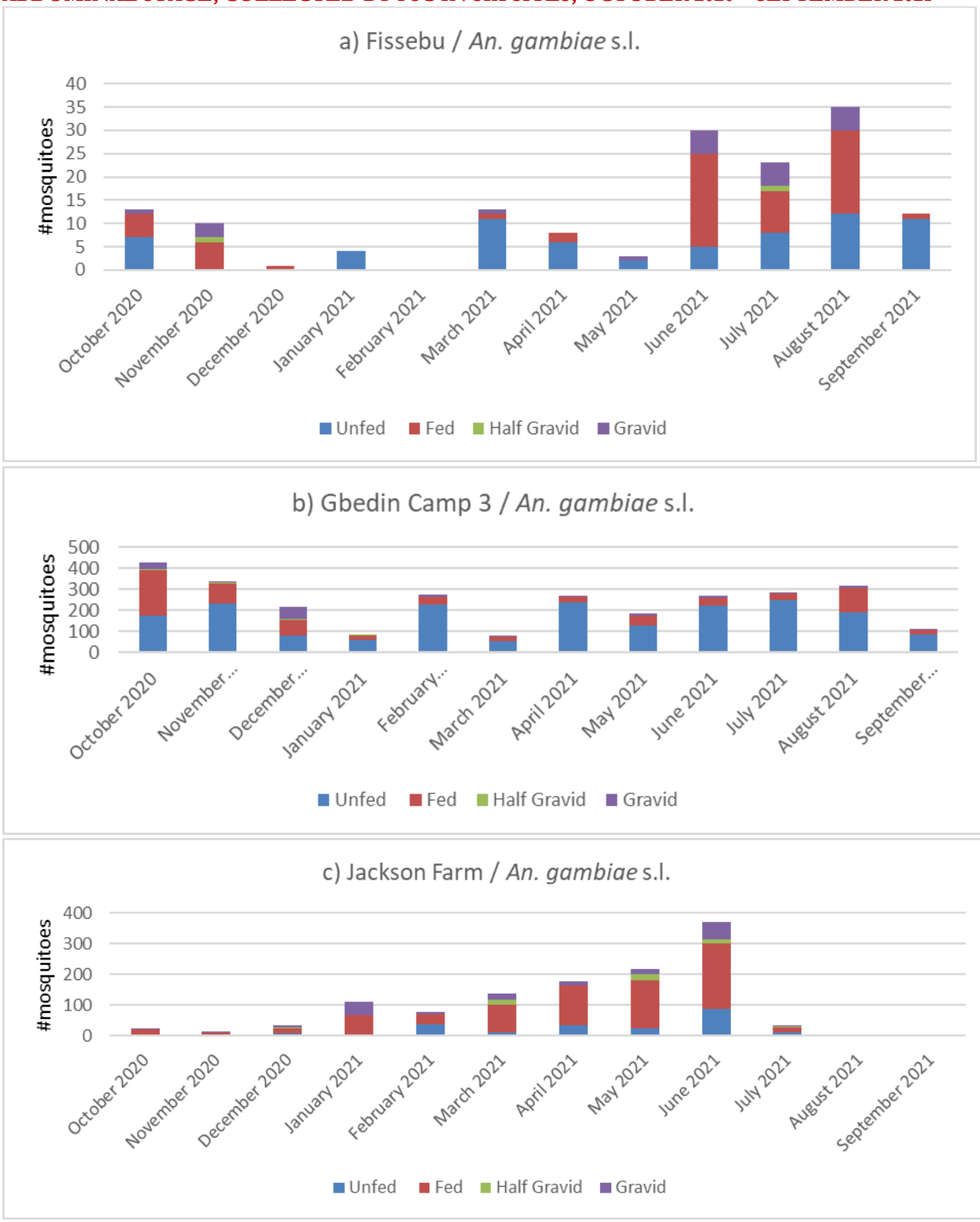
3.1.3 ABDOMINAL STAGE

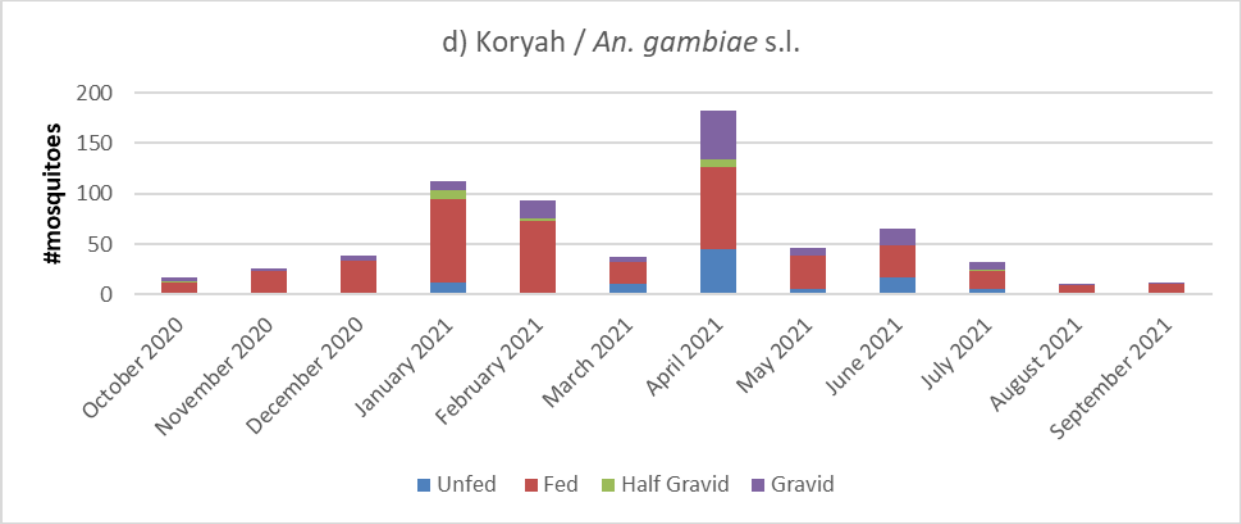
Figure 5 shows the pattern of blood digestion stages per month for each site. The proportions of fresh blood-fed *An. gambiae* s.l. were high in all the sentinel sites except in Gbedin Camp 3, where the overall number of blood-fed was less than the number unfed mosquitoes probably indicating differences in net use among communities.

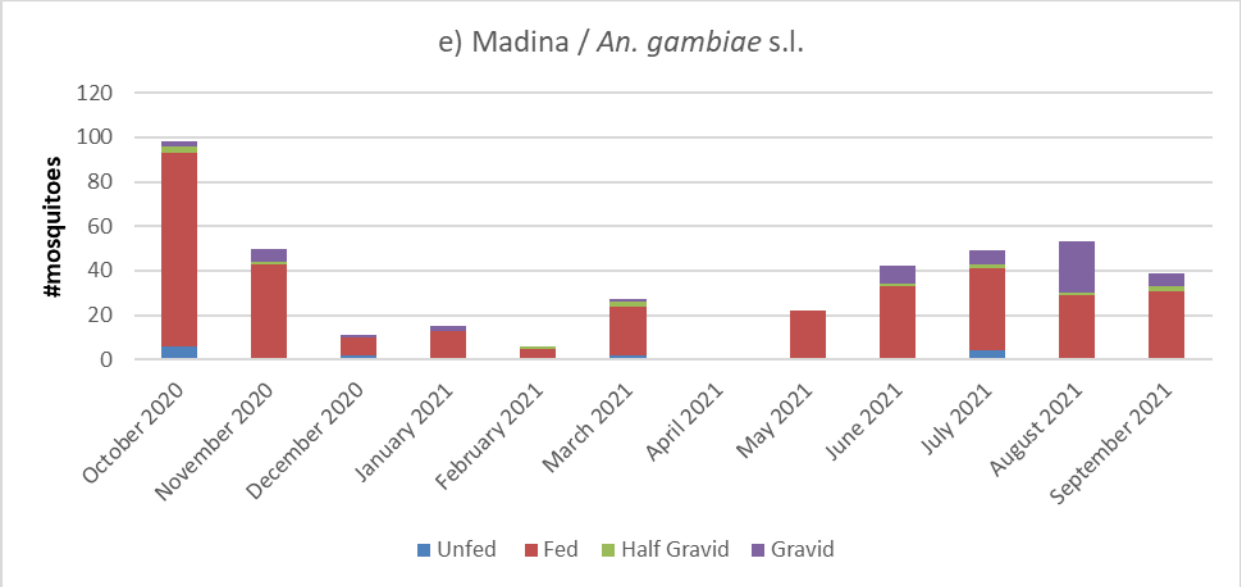
The proportions of females fed on humans or animals will be assessed using molecular techniques, which is more sensitive than standard ELISA blood meal method. Social and behavior change communication (SBCC)

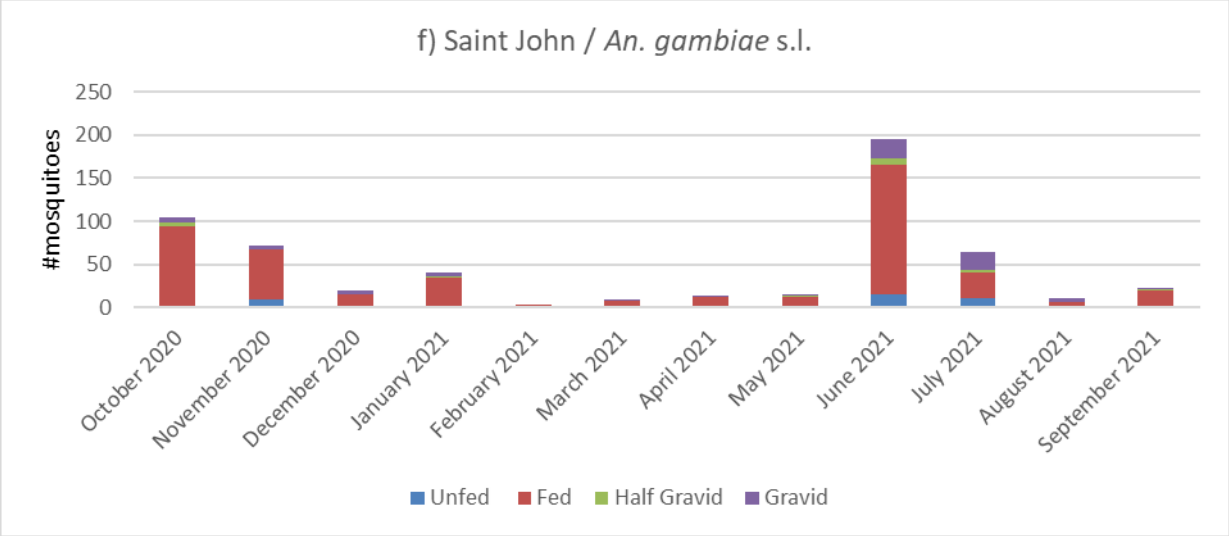
messages could be developed using the blood feeding results to improve the use of ITNs to prevent mosquitoes bite and exposure to malaria infection.

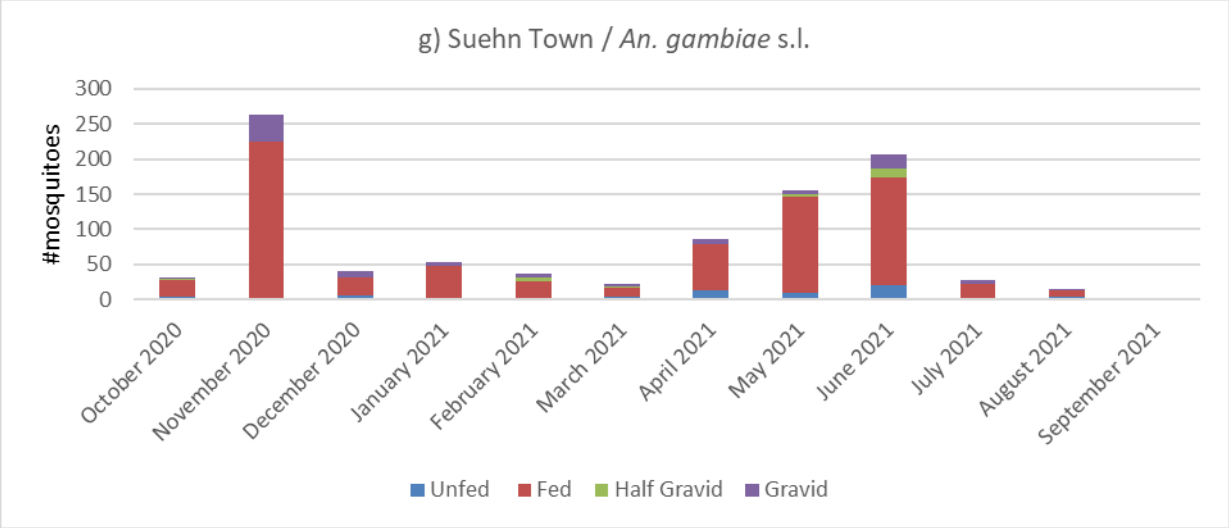
FIGURE 5 DISTRIBUTION OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSC IN SIX SITES, OCTOBER 2020 – SEPTEMBER 2021

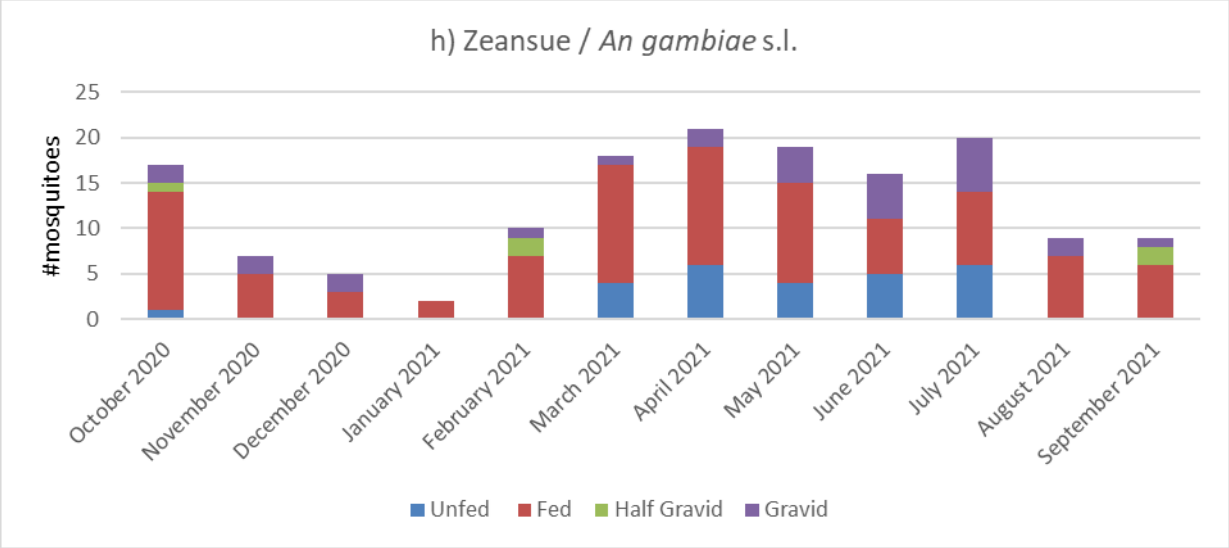


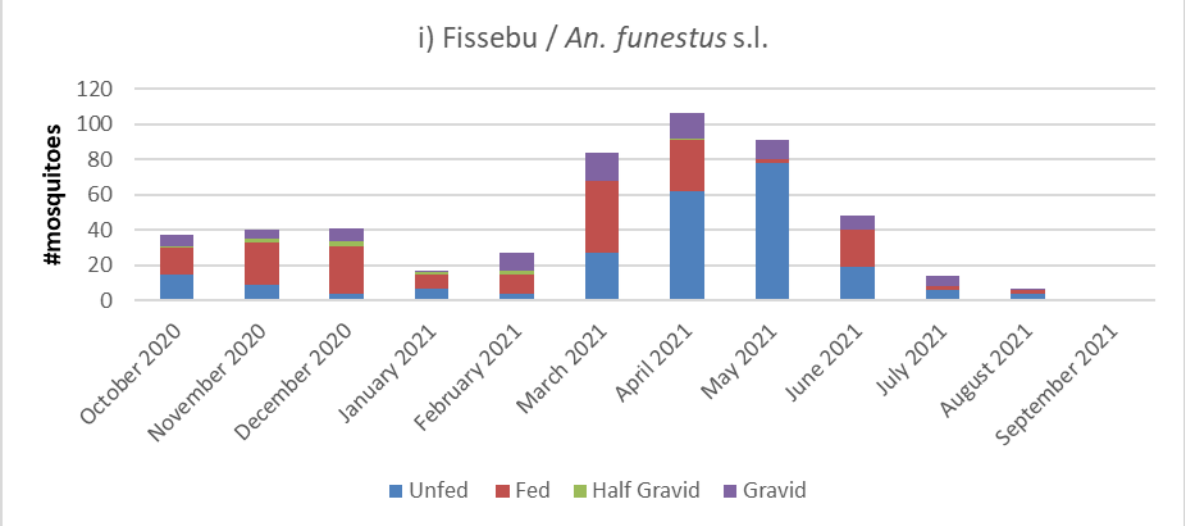

d) Koryah / An. gambiae s.l.
#mosquitoes
200
150
100
50
0
October 2020
November 2020
December 2020
January 2021
February 2021
March 2021
April 2021
May 2021
June 2021
July 2021
August 2021
September 2021
Unfed
Fed
Half Gravid
Gravid

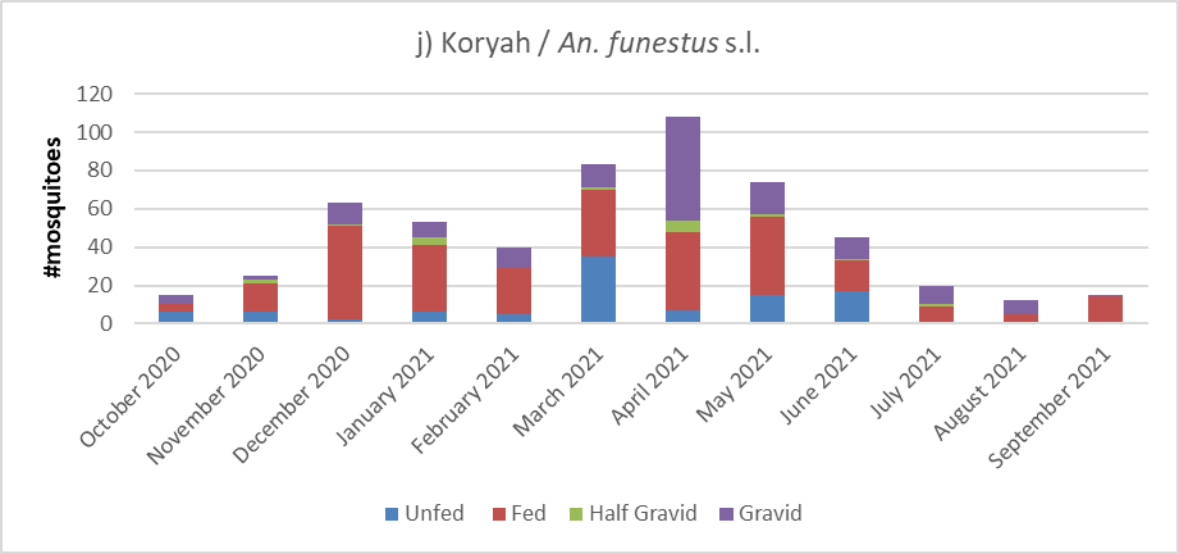

e) Madina / An. gambiae s.l.
#mosquitoes
120
100
80
60
40
20
0
October 2020
November 2020
December 2020
January 2021
February 2021
March 2021
April 2021
May 2021
June 2021
July 2021
August 2021
September 2021
Unfed
Fed
Half Gravid
Gravid

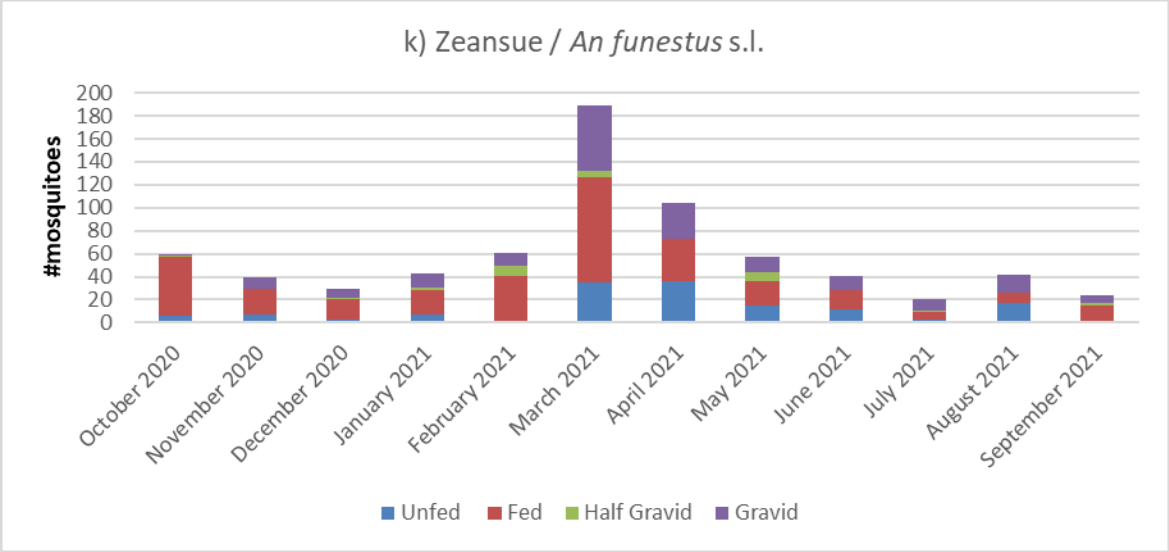

f) Saint John / An. gambiae s.l.
#mosquitoes
250
200
150
100
50
0
October 2020
November 2020
December 2020
January 2021
February 2021
March 2021
April 2021
May 2021
June 2021
July 2021
August 2021
September 2021
Unfed
Fed
Half Gravid
Gravid


g) Suehn Town / An. gambiae s.l.
#mosquitoes
300
250
200
150
100
50
0
October 2020
November 2020
December 2020
January 2021
February 2021
March 2021
April 2021
May 2021
June 2021
July 2021
August 2021
September 2021
Unfed
Fed
Half Gravid
Gravid


h) Zeansue / An gambiae s.l.
#mosquitoes
25
20
15
10
5
0
October 2020
November 2020
December 2020
January 2021
February 2021
March 2021
April 2021
May 2021
June 2021
July 2021
August 2021
September 2021
Unfed
Fed
Half Gravid
Gravid


i) Fissebu / An. funestus s.l.
#mosquitoes
120
100
80
60
40
20
0
October 2020
November 2020
December 2020
January 2021
February 2021
March 2021
April 2021
May 2021
June 2021
July 2021
August 2021
September 2021
Unfed
Fed
Half Gravid
Gravid





3.1.4 CDC Light Trap Collections

Using the CDC LTs collections, 2,723 *An. gambiae* s.l. mosquitoes were collected indoors from October 2020 to September 2021 (Table 6). In the eight sites, using CDC-LTs, 66.7% of *An. gambiae* s.l. mosquitoes were collected in Gbedin Camp 3. Overall, the numbers were higher between the months of April and August.

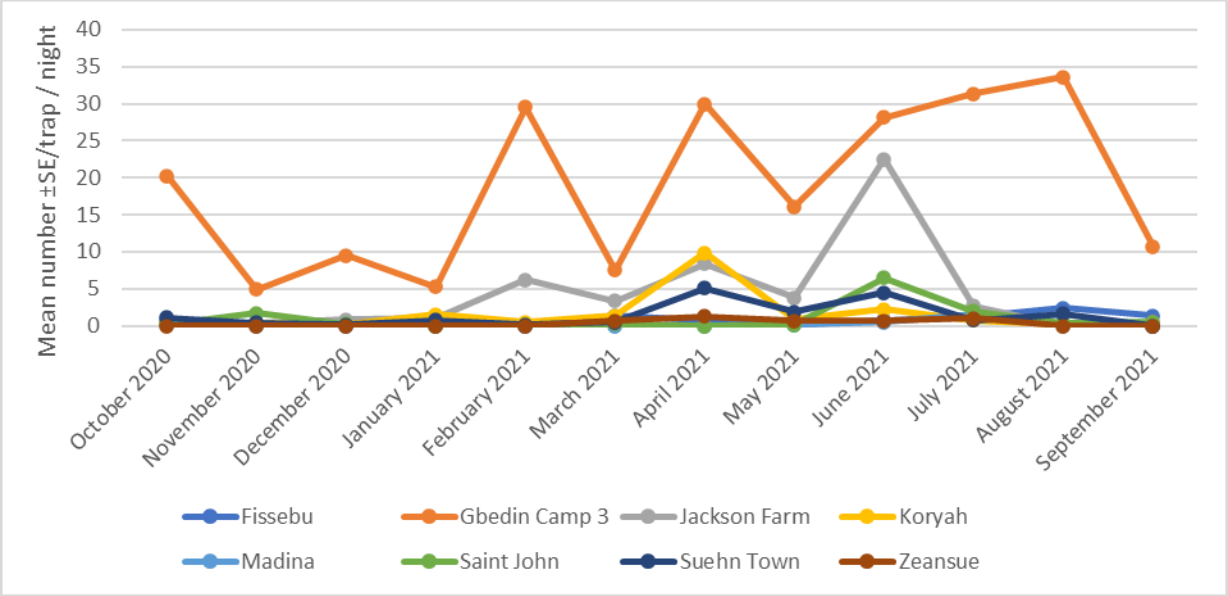
TABLE 6: *AN. GAMBIAE* S.L. COLLECTED BY CDC LT, INDOOR, OCTOBER 2020- SEPTEMBER 2021

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2020	7	162	3	2	1	2	9	0	186
November 2020	1	39	3	3	0	14	3	0	63
December 2020	0	76	7	2	2	2	1	0	90
January 2021	3	42	9	12	0	0	6	0	72
February 2021	0	236	50	4	0	1	1	0	292
March 2021	11	60	27	11	0	2	4	5	120
April 2021	6	240	67	79	0	0	41	10	443
May 2021	2	129	30	7	1	1	15	5	190

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
June 2021	6	225	180	18	4	52	36	5	526
July 2021	10	251	21	6	13	16	6	8	331
August 2021	19	269	0	1	1	3	13	0	306
September 2021	11	86	0	3	0	4	0	0	104
Total	76	1815	397	148	22	97	135	33	2723
%	2.8	66.7	14.6	5.4	0.8	3.6	5.0	1.2	100

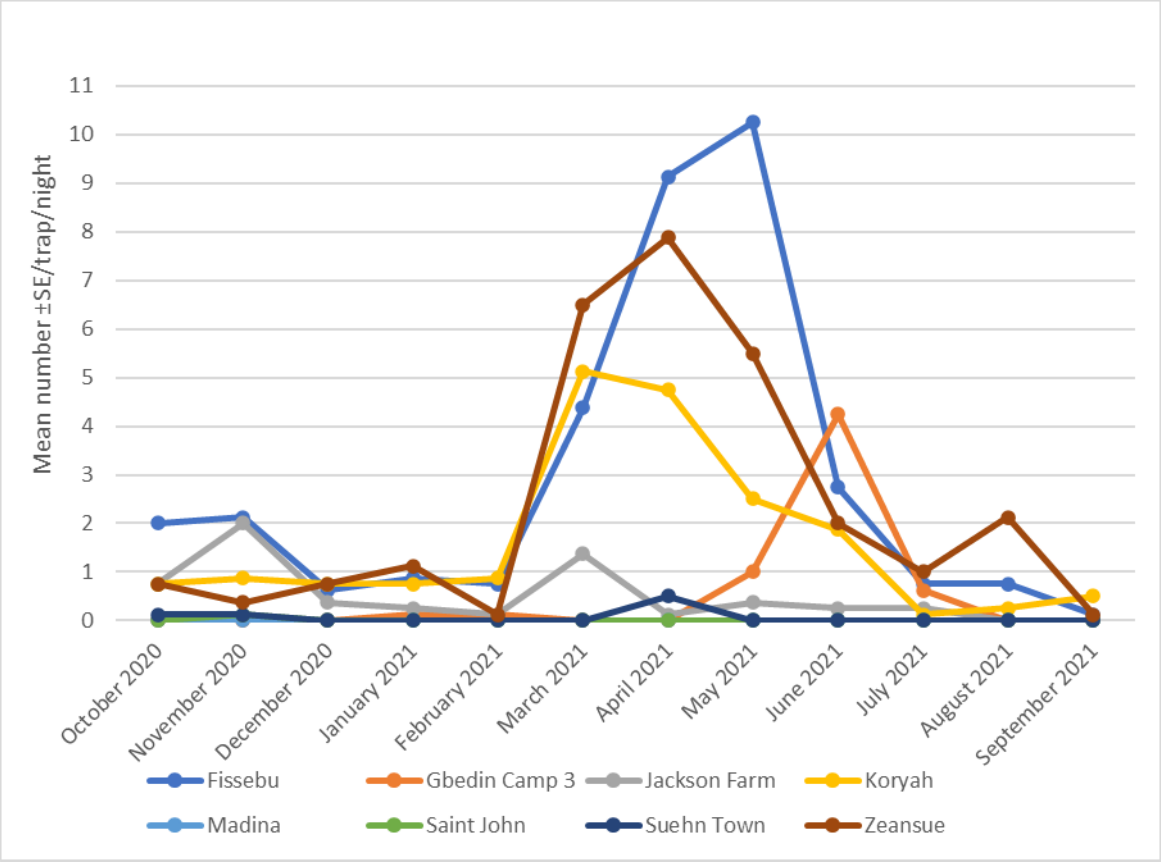
In Gbedin Camp 3, for *An. gambiae* s.l., the density per trap per night was highest in August 2021 (33.6 mosquitoes per trap per night). For Jackson Farm the highest density (22.5 per trap per night) was recorded in June 2021 (Figure 6). These two sites have rice fields which may provide more permanent breeding environment. Though there were some peaks in February and April for some sites, the main peak season for *An. gambiae* s.l. was June-August.

FIGURE 6: MEAN DENSITIES PER TRAP PER NIGHT OF *AN. GAMBIAE* S.L. SAMPLES COLLECTED BY CDC-LT, INDOOR, OCTOBER 2020- SEPTEMBER 2021.



Using CDC LTs, the highest number of *An. funestus* s.l. mosquitoes were collected from Fissebu in May 2021 (10.3 mosquitoes per trap per night), (Figure 7). *An. funestus* s.l. was collected from all sites though it was more abundant in Fissebu (10. 3 mosquito per trap per night in May 2021) than, Zeansue (7.9 mosquito per trap per night in April 2021), Koryah (5.1 mosquito per trap per night in March 2021) and Gbedin Camp 3 (4.3 mosquito per trap per night in June 2021). They were present throughout the year though the peak season for *An. funestus* s.l. was March to June.

FIGURE 7: MEAN DENSITIES PER TRAP PER NIGHT OF *AN. FUNESTUS S.L.* SAMPLES COLLECTED BY CDC-LT, INDOOR, OCTOBER 2020- SEPTEMBER 2021.



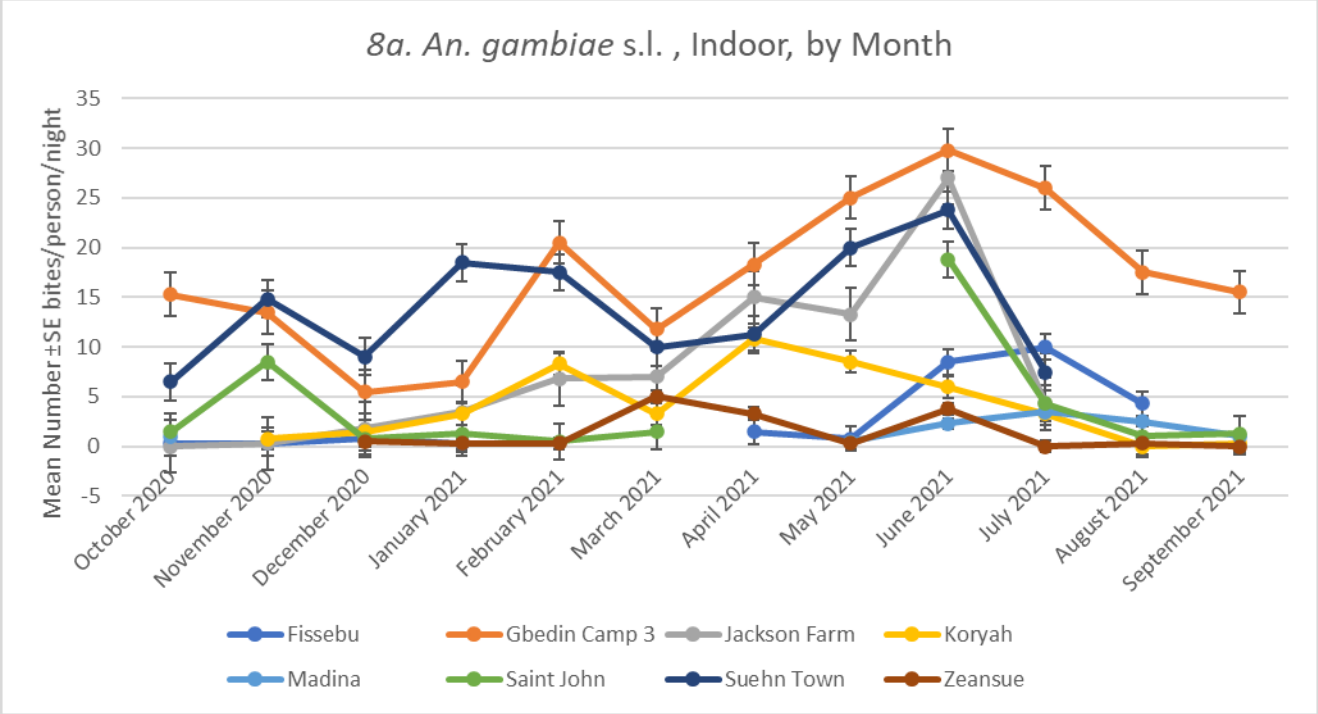
3.1.5 HUMAN BITING RATE AND PATTERN

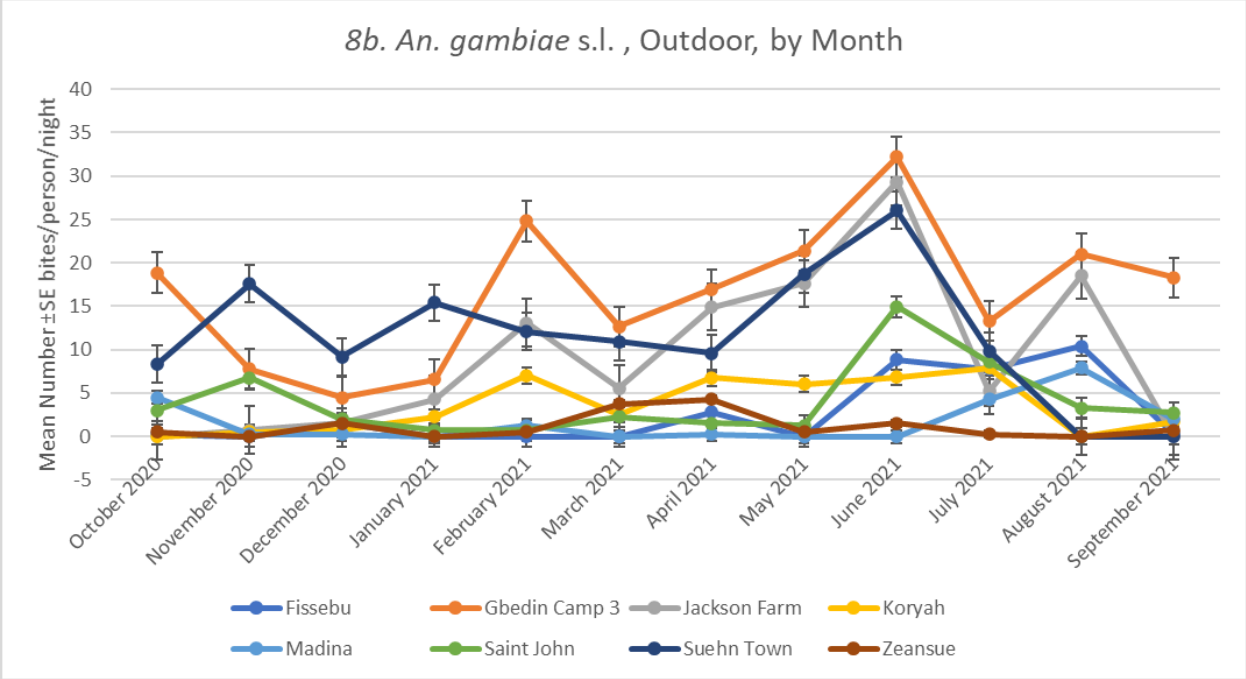
The highest indoor biting rate of *An. gambiae* s.l., as measure by HLCs, was recorded in Gbedin Camp 3 in June 2021 (29.8 bites per person per night), (Figure 8a and 8b). The second site with highest indoor biting rate was Jackson Farm (27 bites per person per night).

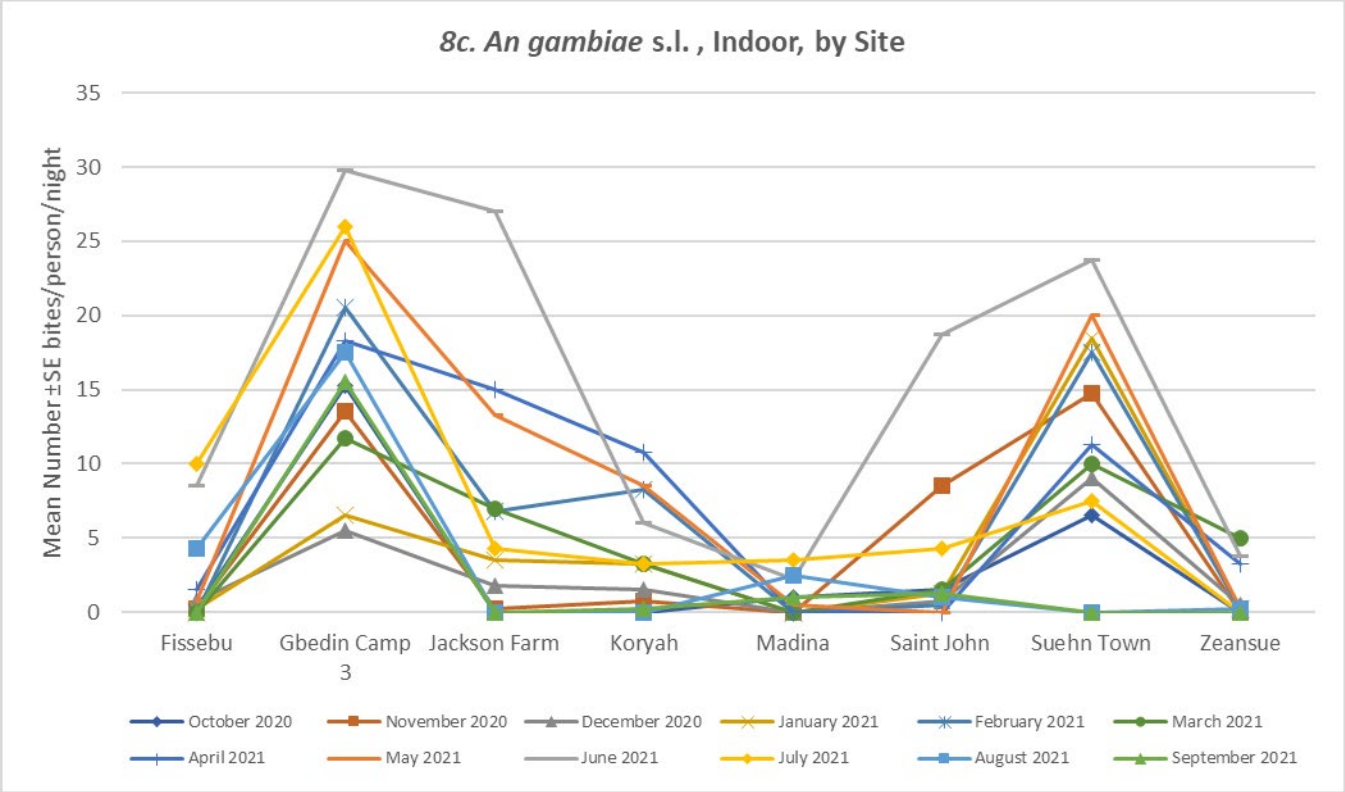
The highest outdoor biting rate, as measured by HLCs, was observed in Gbedin Camp 3 in June 2021 (32.2 bites per person per night). In the same month, a second peak of biting was noted in Jackson Farm (29.3 bites per person per night).

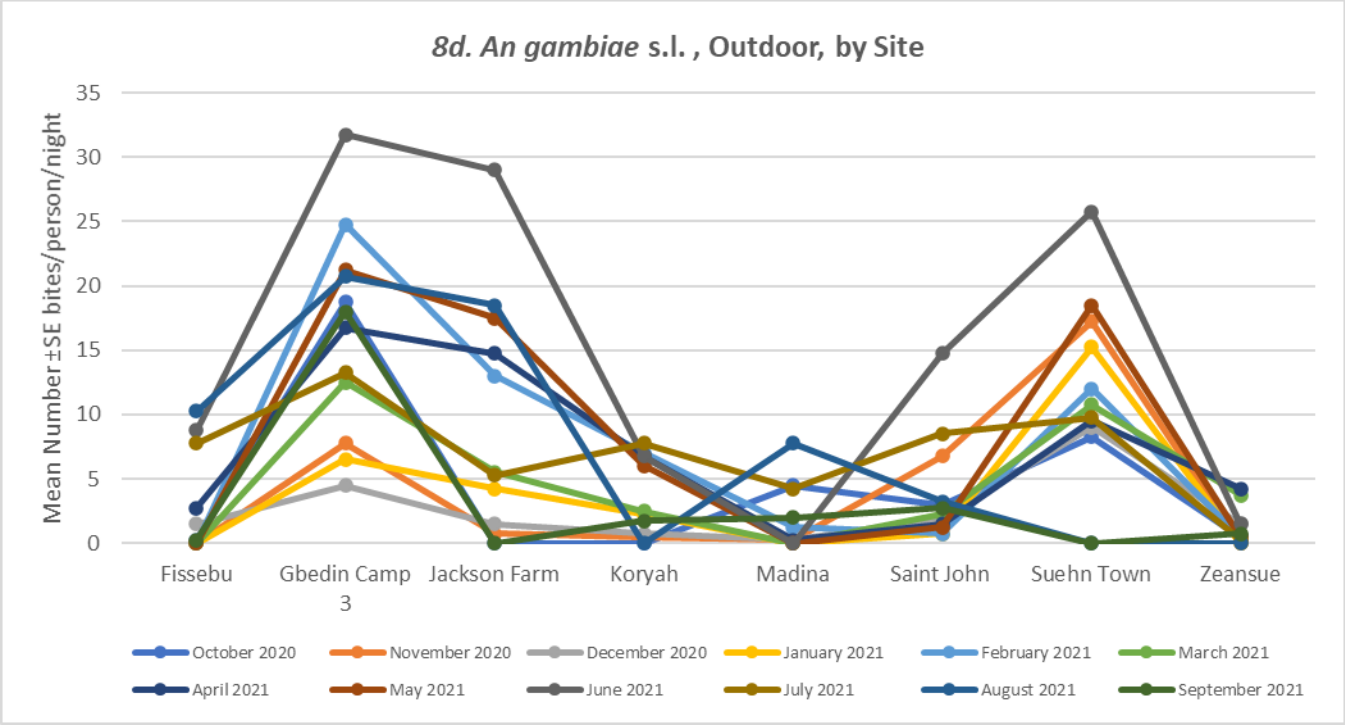
Figure 8c and 8d show biting numbers by site with the highest biting overall occuring in two sites: Gbedin Camp 3and Suehn Town.

FIGURE 8 NIGHTLY HUMAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED FROM INDOOR AND OUTDOOR HLC, OCTOBER 2020 – SEPTEMBER 2021.






8c. An gambiae s.l. , Indoor, by Site
Mean Number ±SE bites/person/night
35
30
25
20
15
10
5
0
Fissebu
Gbedin Camp 3
Jackson Farm
Koryah
Madina
Saint John
Suehn Town
Zeansue
October 2020
November 2020
December 2020
January 2021
February 2021
March 2021
April 2021
May 2021
June 2021
July 2021
August 2021
September 2021

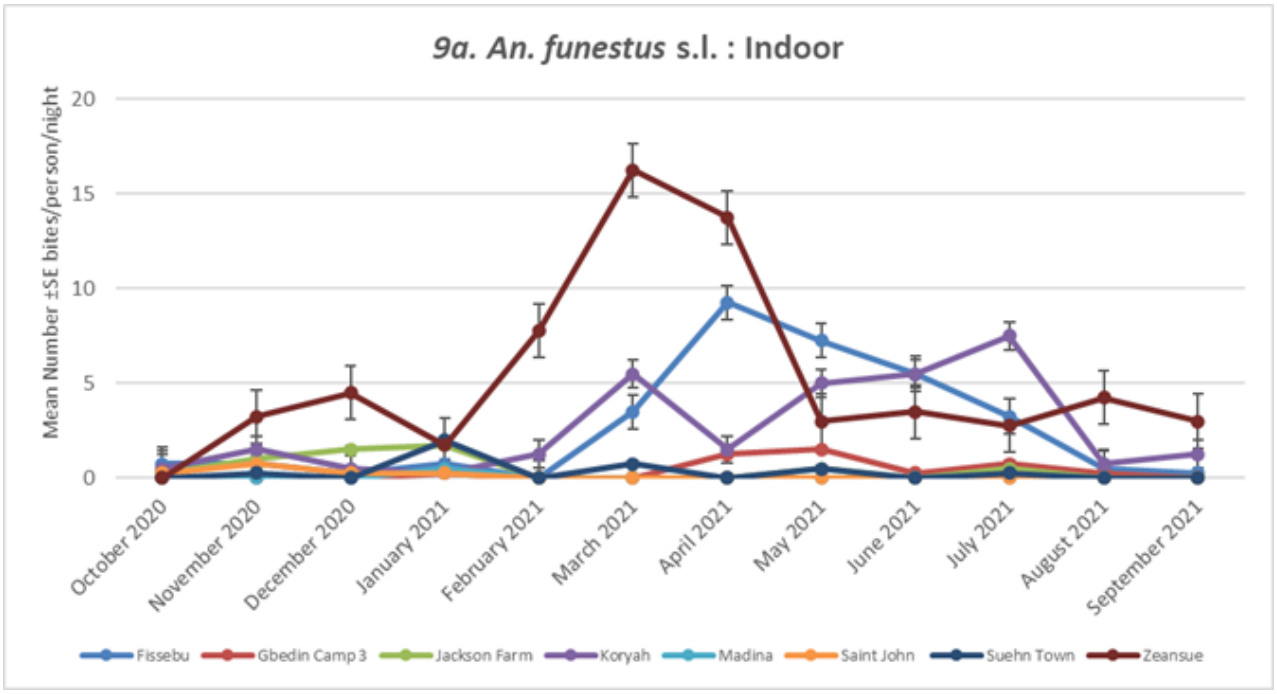

8d. An gambiae s.l. , Outdoor, by Site
Mean Number ±SE bites/person/night
35
30
25
20
15
10
5
0
Fissebu
Gbedin Camp 3
Jackson Farm
Koryah
Madina
Saint John
Suehn Town
Zeansue
October 2020
November 2020
December 2020
January 2021
February 2021
March 2021
April 2021
May 2021
June 2021
July 2021
August 2021
September 2021

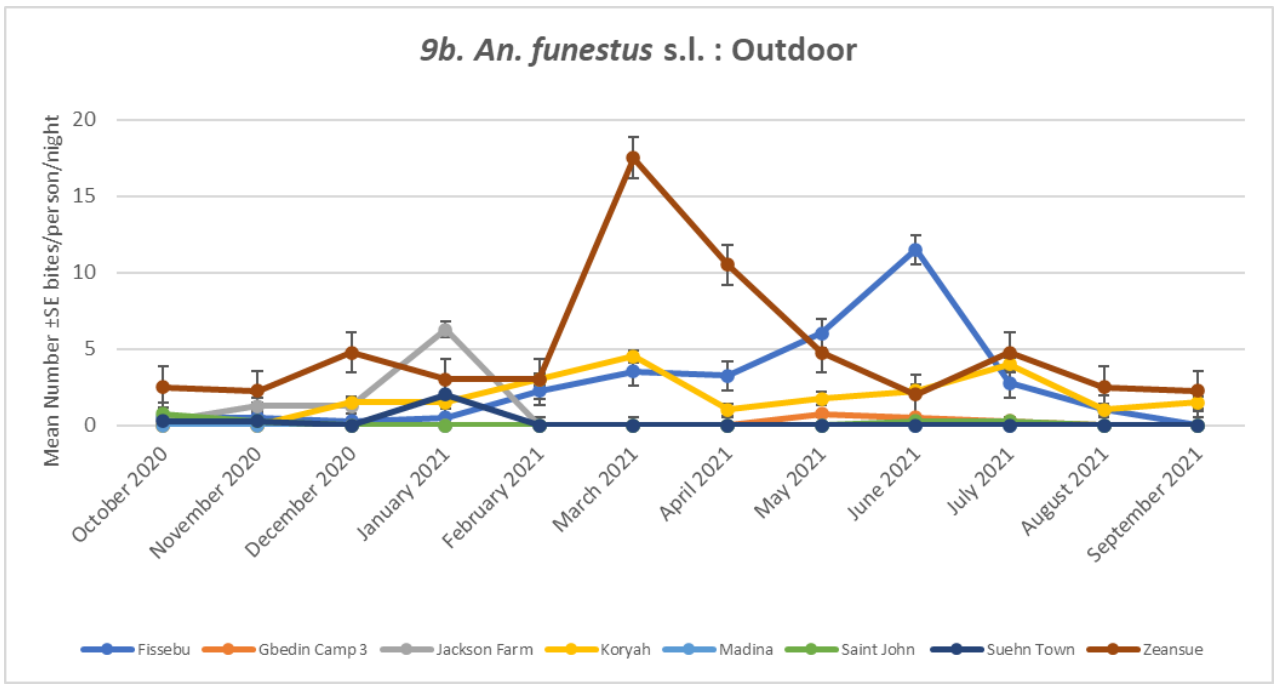
The indoor biting rate by *An. funestus* s.l. was overall lower than *An. gambiae* s.l. The highest indoor biting rate occurred at Zeansue with 16.3 bites per person per night in March 2021. Fissebu was the second site with highest indoor biting rate (9.3 bites per person per night) in April 2021.

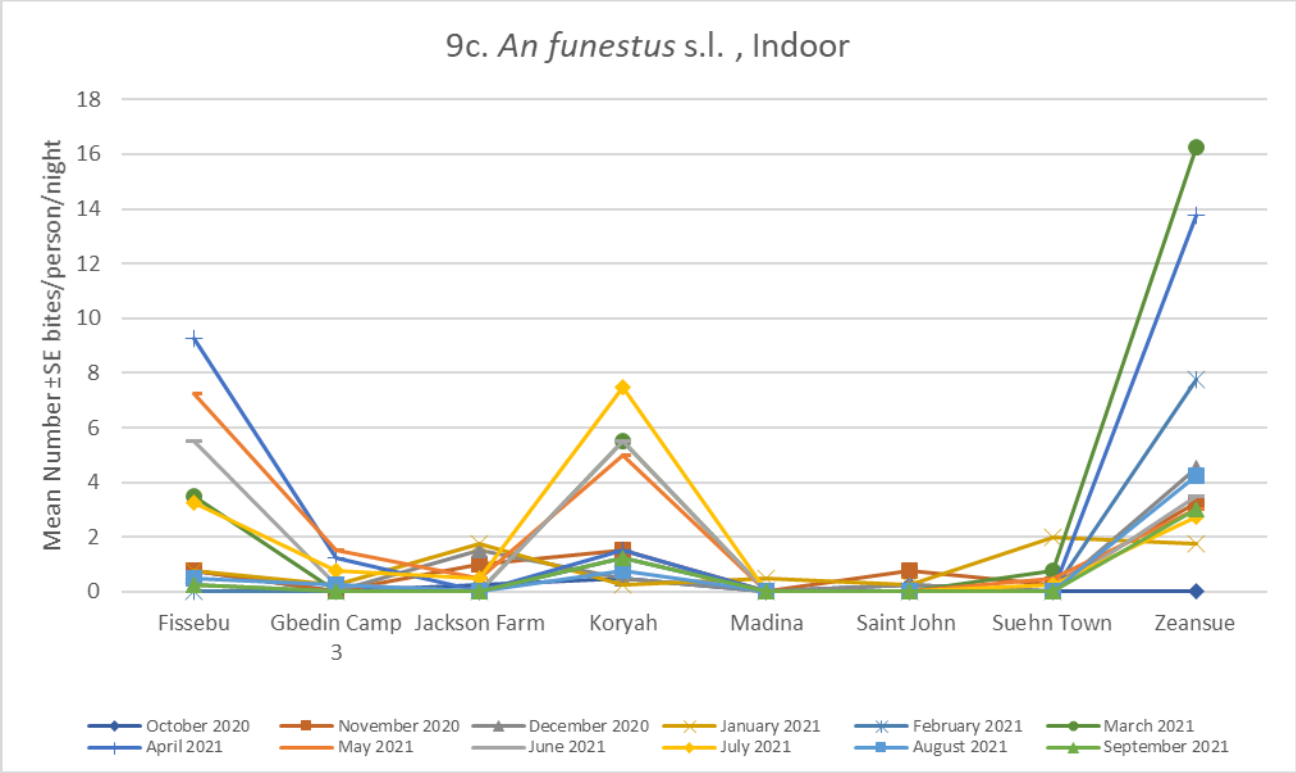
For the outdoor collection using HLC, the peak of *An. funestus* s.l. outdoor biting was observed in Zeansue in March 2021 (17.5 bites per person per night) followed by Fissebu with 11.5 bites per person per night in June 2021 (Figure 9a and 9b). Unlike the *An. gambiae* s.l. biting rates, *An. funestus* s.l. seem to peak earlier around March-April before the start of the rainy season.

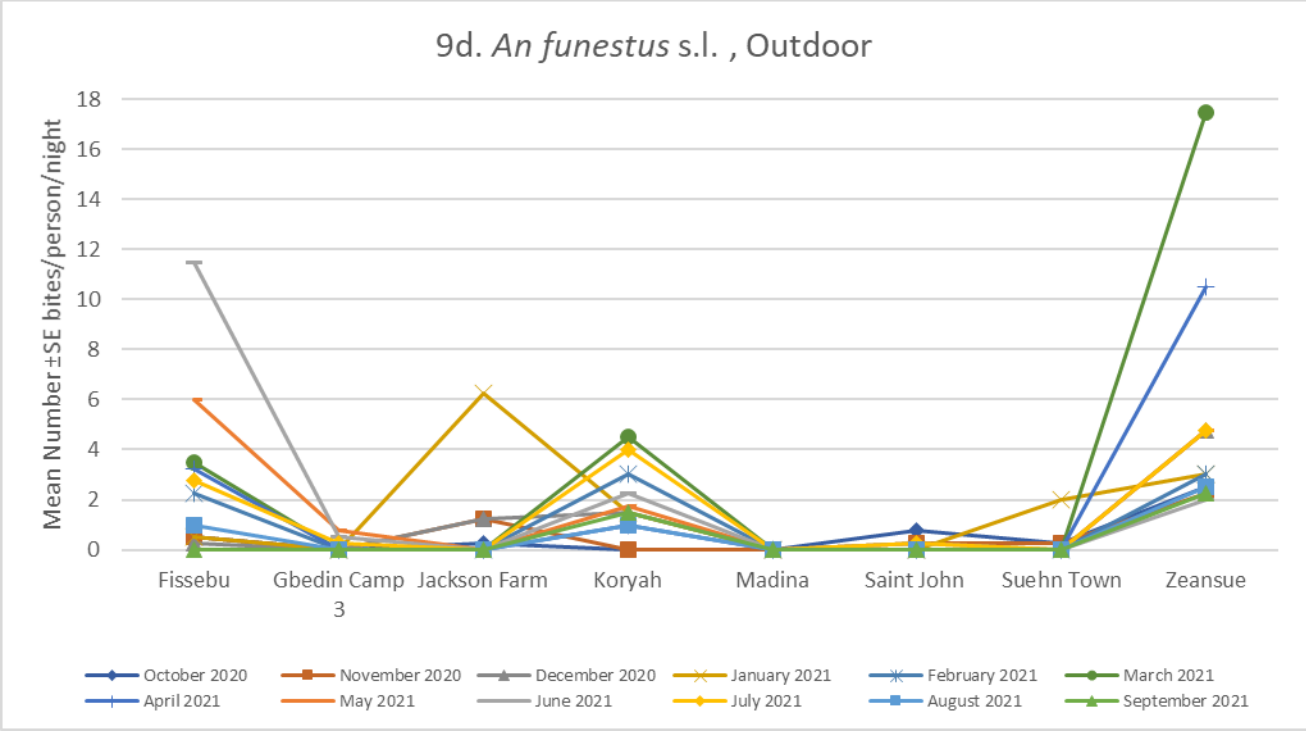
Figure 9c and 9d show biting numbers of *An. funestus* s.l. by site and the highest biting numbers from HLC occur in three sites: Zeansue, Fissebu and Koryah. There was unexpected peak in Jackson Farm in January 2021 outdoor collection, which quickly dropped down after January.

FIGURE 9: NIGHTLY HUMAN BITING RATE OF *AN. FUNESTUS* S.L. COLLECTED FROM INDOOR AND OUTDOOR HLC, OCTOBER 2020–SEPTEMBER 2021.



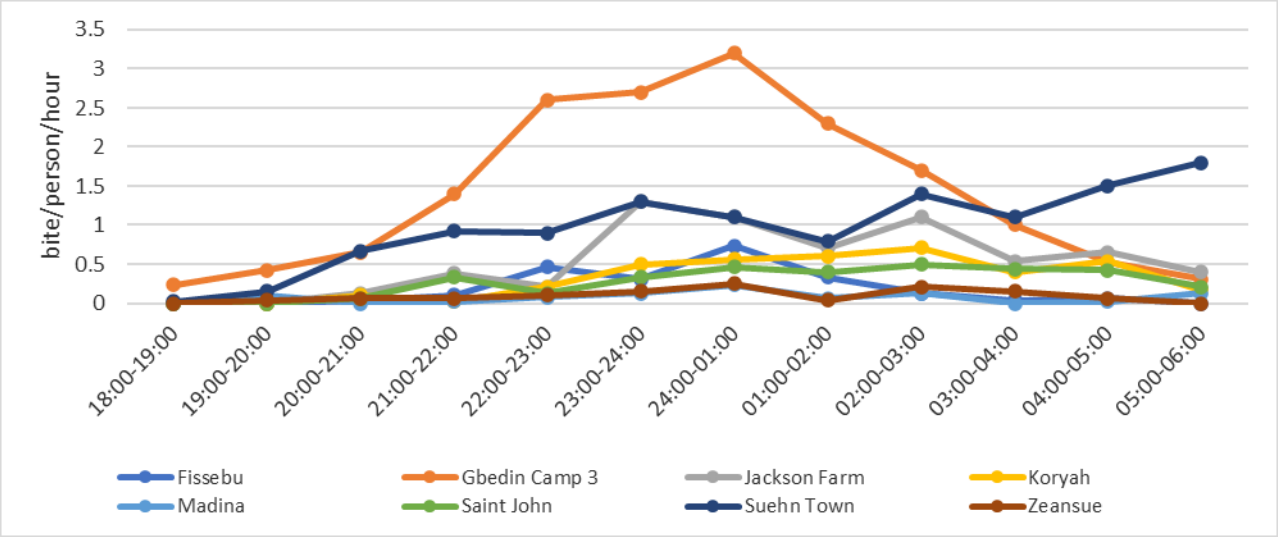






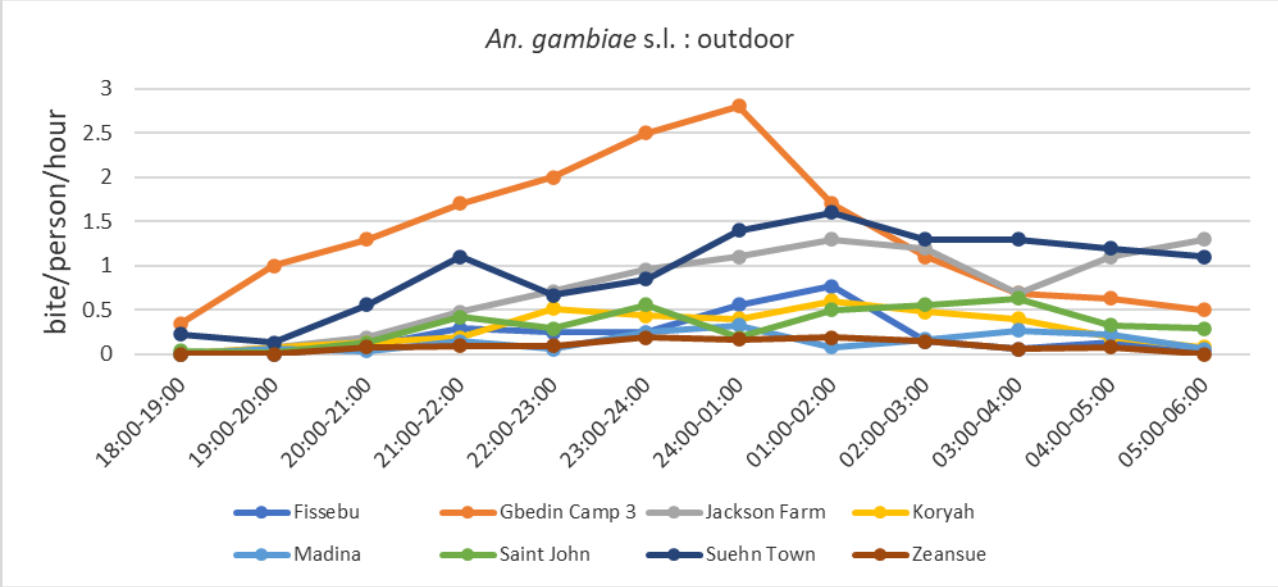
Overall, there is an increased trend in hourly biting activity after 21:00 and up to 03:00. Gbedin Camp 3 has the highest hourly indoor biting rates. Though the hourly rates are low, the increasing trend in biting after 4:00 in Suehn Town, may require additional human behavior observations and probably also extending the HLC collection into the early hours of the morning.

FIGURE 10: HOURLY HUMAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED FROM INDOOR HLC IN EIGHT SITES, OCTOBER 2020 – SEPTEMBER 2021.



Outdoor biting hourly rates across the eight sites is presented in Figure 11. The highest hourly indoor biting hourly are also recorded from Gbedin Camp 3. Overall, outdoor biting activity seems to peak around mid-night.

FIGURE 11: HOURLY HUMAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED FROM OUTDOOR HLC IN EIGHT SITES, OCTOBER 2020 - SEPTEMBER 2021.



Indoor and outdoor humane biting rates of *An. gambiae* s.l. and *An. funestus* s.l. showed that both species did not show any distinct preferent for indoor or outdoor biting, provided the host is available at both locations. Overall, all indoor HBR for *An. gambiae* s.l. was not significantly different from outdoor HBR except in Jackson Farm and Madina (Table 7). For *An. funestus* s.l. indoor HBRs was not significantly different from outdoor HBRs in all sites (Table 8).

TABLE 7: *ANOPHELES GAMBIAE* S.L. BITING PLACE PREFERENCE, INDOOR AND OUTDOOR, OCTOBER 2020 - SEPTEMBER 2021

An. gambiae s.l.	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
Indoor Biting Rate (b/p/n)	2.21	17.08	6.56	3.81	0.90	3.27	11.56	1.13	46.52
Outdoor Biting Rate (b/p/n)	2.63	16.38	9.17	3.50	1.71	3.96	11.33	1.13	49.79
Total	4.83	33.46	15.73	7.31	2.60	7.23	22.90	2.25	96.31
Endophagic Index (a)	0.46	0.51	0.42	0.52	0.34	0.45	0.51	0.50	0.48
Exophagic Index (b)	0.54	0.49	0.58	0.48	0.66	0.55	0.49	0.50	0.52
Chi^2 (p-value)	0.19	0.40	<0.001	0.42	<0.001	0.08	0.74	1.00	0.02

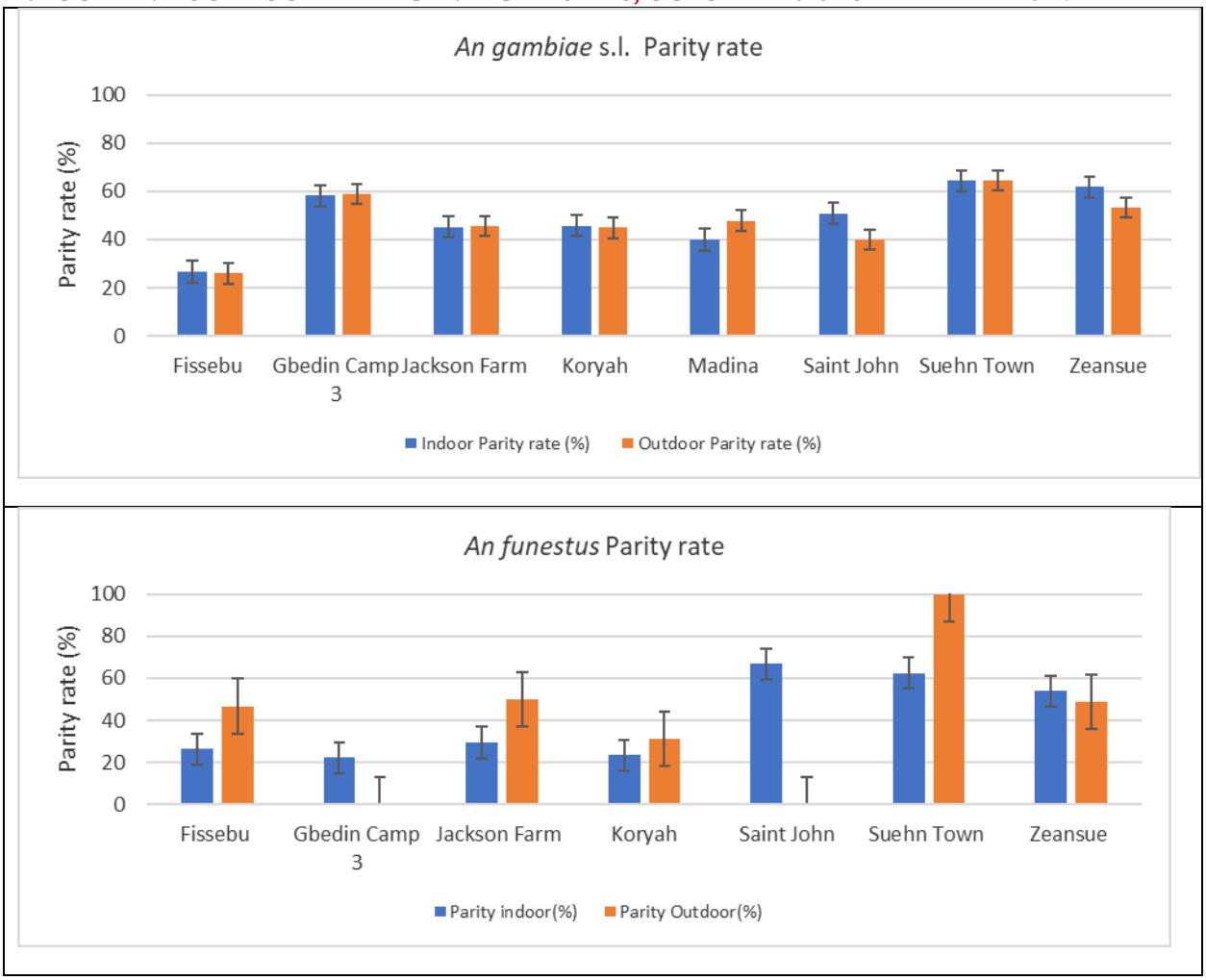
TABLE 8: *ANOPHELES FUNESTUS* S.L. BITING PLACE PREFERENCE, INDOOR AND OUTDOOR, OCTOBER 2020 - SEPTEMBER 2021

An funestus s.l.	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
Indoor Biting Rate (b/p/n)	2.65	0.31	0.46	2.40	0.04	0.13	0.21	5.13	11.31
Outdoor Biting Rate (b/p/n)	2.69	0.17	0.75	2.02	0.00	0.13	0.31	5.17	11.23
Total	5.33	0.48	1.21	4.42	0.04	0.25	0.52	10.29	22.54
Endophagic Index (a)	0.50	0.65	0.38	0.54	1.00	0.50	0.40	0.50	0.50
Exophagic Index (b)	0.50	0.35	0.62	0.46	0.00	0.50	0.60	0.50	0.50
Chi^2 (p-value)	0.90	0.14	0.07	0.22	0.16	1.00	0.32	0.93	0.90

3.1.6 PARITY RATE

The parity was assessed by extracting the ovaries of unfed mosquitoes into a slide mounted on dissecting microscope and reading the slides under a compound microscope. Figure 12 shows the percentage parous mosquitoes (mosquitoes that has laid eggs at least once in their lifetime). Overall, for *An. gambiae* s.l., the parity rates are comparable between indoor and outdoor in the eight sites (Figure 12) except in Madina where parity was slightly higher in outdoor collected mosquitoes and in Saint John parity was higher indoors. For *An. funestus* s.l., parity seems to be higher in outdoor collected mosquitoes than indoors for all the sites with indoor and outdoor parity data, except in Zeansue.

FIGURE 12: PARITY RATE OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED FROM INDOOR AND OUTDOOR BY HLC IN EIGHT SITES, OCTOBER 2020 - SEPTEMBER 2021.

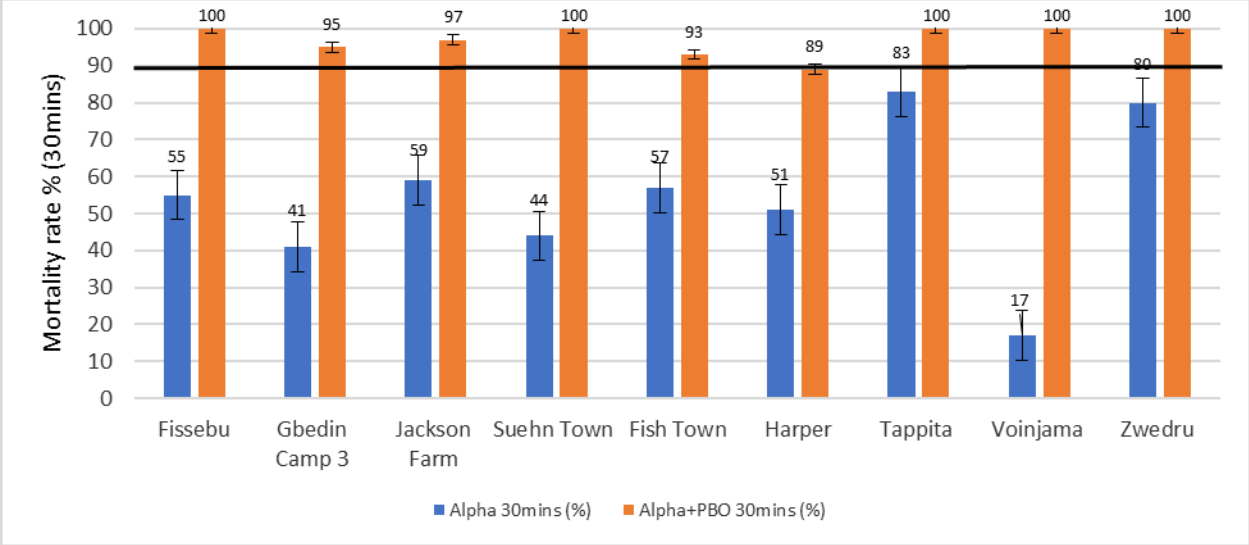


3.2 INSECTICIDE RESISTANCE MONITORING

3.2.1 INSECTICIDE SUSCEPTIBILITY TEST FOR PYRETHROIDS

In Year 3, tests were performed using the diagnostic dose (1x) of deltamethrin and permethrin. The results showed that *An. gambiae* s.l. populations were resistant to these two insecticides. In Year 4, focus was put on the active ingredients of the Interceptor® G2 ITNs. As such, alpha-cypermethrin with and without PBO was tested in nine sites (Figure 13). Alpha-cypermethrin resistance was observed in all nine test sites. In five out of the nine sites 100% mortality rate was observed among mosquitoes pre-exposed to PBO before being tested with alpha-cypermethrin. PBO did not restore full susceptibility to pyrethroids at the 30 minutes diagnostic time in four sites. Molecular analysis of resistance mechanisms to insecticide will be assessed in Year 5 after PCR training for LIBR staff is completed.

FIGURE 13: 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, FEBRUARY– SEPTEMBER 2020

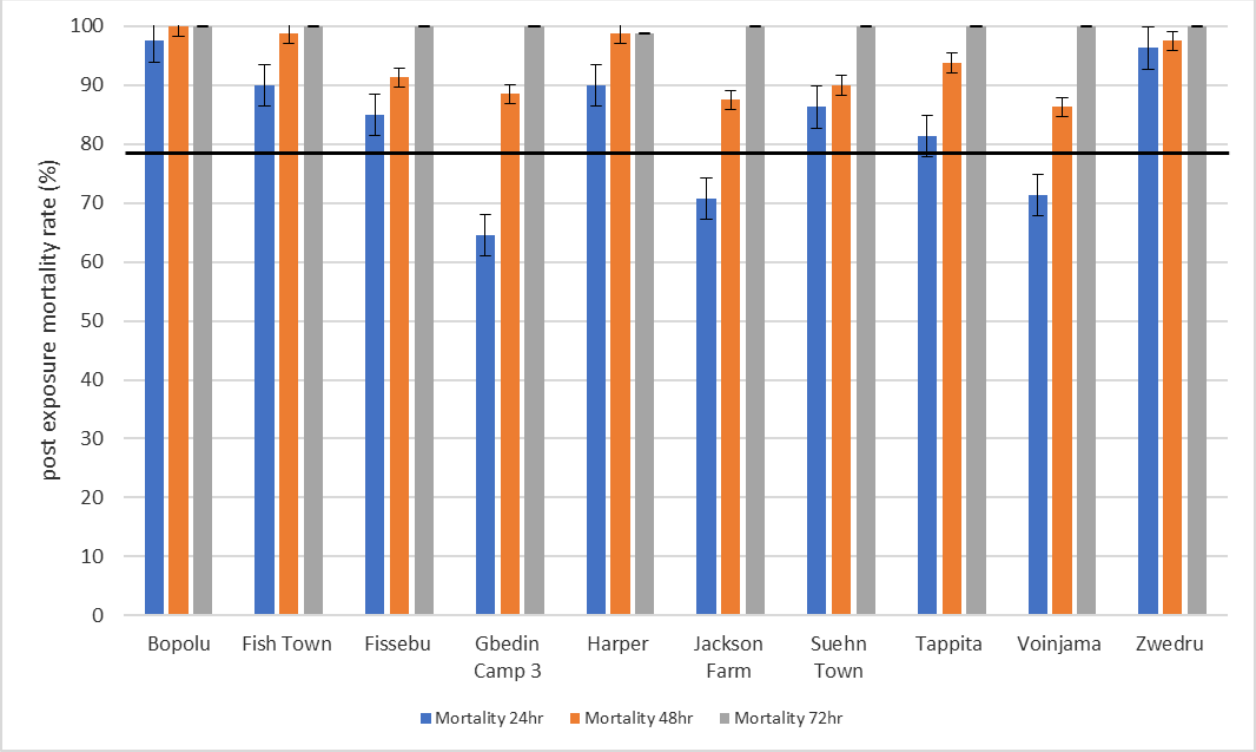


Note: Black horizontal line indicates WHO cut-off point for susceptibility

3.2.2 INSECTICIDE SUSCEPTIBILITY TESTING OF CHLORFENAPYR

In Year 4, priority was given to the testing of alpha-cypermethrin and chlorfenapyr, the two main ingredients of the Interceptor® G2 ITNs distributed, countrywide, in Liberia in 2021. *An. gambiae* s.l. adult females reared from larvae collected in ten sites were tested for susceptibility to chlorfenapyr (Figure 14). The vector is fully susceptible to this insecticide in all ten sites. Mortality at the 72 hours post-exposure holding period was 100% in all sites, except in Harper, where the mortality was 98.8%.

FIGURE 14: MORTALITY RATE OF *AN. GAMBIAE* S.L. PER EXPOSURE TO CHLORFENAPYR USING CDC BOTTLE ASSAY



Note: Black horizontal line indicates WHO cut-off point for susceptibility

3.3 CONE BIOASSAYS OF STANDARD AND IG2 ITNS

The cone bioassay results of the standard (Duranet) nets were reported as part of the 36 months durability monitoring report. A separate report for the cone assay, tunnel test and chemical residue analysis results for the Interceptor® G2 ITNs sampled at pre-distribution will be submitted as soon as all results become available.

3.4 NEW INSECTARY CONSTRUCTION PROCESS

In Year 4, VectorLink negotiated with NPHIL to identify space where containers could be installed for a new insectary to be completed in Year 5 to rear a susceptible and resistant strain of *Anopheles* mosquitoes. VectorLink worked with a local architect to render an insectary design and presented this design to PMI during a monthly update meeting. A risk management document is under review and will be submitted to PMI for approval in Year 5.

3.5 ITN MONITORING DASHBOARD

In Year 4, VectorLink completed the baseline ITN monitoring dashboard for the period of October 2020 to March 2021 which included entomological, epidemiological and climate data. This dashboard was presented to PMI and the NMCP.

3.6 LABORATORY ANALYSES

LIBR staff are processing project mosquito samples to assess the sporozoite rate using ELISA-CSP. As soon as the entire results of these tests are available, they will be included in this report or as an addendum. Other

molecular analysis activities that include PCR species identification, molecular resistance mechanism and blood meal analysis will be performed by LIBR in Year 5. All consumables and reagents are procured and on-site and remote training of LIBR staff by CDC/VectorLink is scheduled for January 2022.

3.7 COVID-19 PREVENTION MEASURES

PMI's guidance for COVID mitigation was followed by VectorLink and NMCP staff and CHWs supporting the field activities; mask wearing, disinfectant, social distance, and body temperature recording. The bedsheets for PSC were used one time per house to avoid using the same set of bedsheets in different houses. The CHWs were provided with 5ml glass tubes to catch mosquitoes landing on their legs instead of using mouth aspirators.

3.8 CAPACITY BUILDING ACTIVITIES

Over the past years, VectorLink worked to build the capacity of the NMCP, CHWs, and UL in entomological monitoring (Table 9). In the project monthly longitudinal entomological monitoring and insecticide resistance testing sites, VectorLink, and the NMCP staff providing on-site, practical training to CHWs on basic morphology of adult and larval mosquitoes. During periodic supervision visits to the field, the VectorLink Chief of Party assesses issues related to activity implementation including skills of the technical team, filling of data recording forms, logistic challenges like working space and the team's relation with local communities. VectorLink was able to get access to insecticide resistance data from hard-to-reach areas through capacity building of a CHW from Zwedru, who led larval collections and shipped samples via domestic flight following all safety measure for live mosquitoes.

While VectorLink has successfully built laboratory capacity at LIBR in ELISA methods, there remains a gap in PCR methods. VectorLink coordinated with LIBR and CDC staff to prepare for a molecular training for LIBR staff on PCR methods for molecular identification of samples and diagnostic of mutations involved in insecticide resistance. As part of this preparation, VectorLink worked with the CDC to procure needed supplies and equipment for a training on PCR methods while working with LIBR to prepare a laboratory space. The virtual training is tentatively planned for January 2022.

TABLE 9: SUMMARY OF VECTORLINK CAPACITY BUILDING ACTIVITIES IN LIBERIA IN YEAR 4

	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	1	51
Field morphological ID	4	32	16	1	51
ITN durability monitoring bioassay training	2				2
Mosquito midgut dissection				1	1

Due to COVID-19-, all international training sessions and local in-country training with involvement of counties representatives were canceled. The in-country training will be held in Year 4 for participants vaccinated.

4. OBSERVATIONS AND CONCLUSIONS

Overall, a full year of vector bionomics data were collected in the eight sites without any disruption despite COVID context. Data on vector abundance, species composition, temporal, and spatial trends, biting rate, biting location preference were collected. *Anopheles gambiae* s.l. was the main vector at six out of the eight sentinel sites where the vector monitoring was conducted. Gbedin Camp 3, Jackson Farm and Suehn Town have higher vector densities of *An. gambiae* s.l. as compared to other sites.

Though *An. funestus* s.l. was also collected from all sites, it was more dominant in Fissebu and Zeansue than the others which is likely due to the presence of breeding habitats preferred by this vector. In Year 5, with more ELISA tests and if possible, also insecticide susceptibility tests, the project will work to understand the contribution of this vector in malaria transmission in Liberia.

An. gambiae s.l. populations tested in nine sites showed that this vector was highly resistant to pyrethroids (alpha-cypermethrin). The pre-exposure of *An. gambiae* s.l. to PBO has restored 100% susceptibility to alpha-cypermethrin in five out of the nine sites.

Bottle assays performed with adult mosquitoes reared from wild larva collected in ten sites cross Liberia showed that *An. gambiae* s.l. was fully susceptible to chlorfenapyr at 72 hours post-exposure. This finding is encouraging since Interceptor® G2 ITNs (treated with both alpha-cypermethrin and chlorfenapyr) have been distributed in the mass campaign held in June – July 2021.

Biting pattern of both *An. gambiae* s.l. and *An. funestus* s.l. showed an increase of activity after 20:00 hours and peaking around midnight suggesting that correct and sustained use of ITNs during bedtime and sleeping hours could provide protection again mosquitoes bites and malaria. Outdoor and indoor biting patterns were similar in sentinel sites.

There was a high proportion of blood fed mosquitoes found during Year 4 collections, which means that there is more human to vector contact and higher chance for transmission of malaria. Liberia does do mass distribution of ITNs, but the latest mass distribution campaign occurred in June / July 2021, so much of the year, communities had ITNs from the previous campaign in 2018, so the increase in blood fed mosquitoes could be due to aging and attrition of ITNs. VectorLink will continue to monitor this trend in Year 5.

In addition to field work skills, the NMCP and VectorLink staff gained experience on mosquito lab colony maintenance and ITN bioassays which are relevant to ITN durability monitoring activities.

Continuous efforts were made on building the VectorLink Collect database for Liberia through data entry and dashboard setup. The updated database will allow project partners access to timely entomological data for use in decision making.

5. REFERENCES

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6. ANNEXES

ANNEX 1: *ANOPHELES GAMBIAE* S.L. COLLECTED BY PSC, OCTOBER 2020 –-SEPTEMBER 2021

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2020	6	263	19	15	97	103	23	17	543
November 2020	9	299	10	23	50	58	260	7	716
December 2020	1	143	28	36	9	18	39	5	279
January 2021	1	38	102	100	15	41	48	2	347
February 2021	0	41	29	89	6	3	36	10	214
March 2021	2	18	111	26	27	7	18	13	222
April 2021	2	29	109	103	1	14	46	11	315
May 2021	1	59	189	39	21	14	141	14	478
June 2021	24	43	189	47	38	144	171	11	667
July 2021	13	34	12	26	36	48	22	12	203
August 2021	16	46	0	9	52	8	1	9	141
September 2021	1	26	0	8	39	18	0	9	101
Total	76	1039	798	521	391	476	805	120	4226

ANNEX 2: *ANOPHELES FUNESTUS* S.L. COLLECTED BY PSC, OCTOBER 2020–SEPTEMBER 2021

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2020	21	10	80	9	0	2	4	54	180
November 2020	23	0	37	18	1	14	2	37	132
December 2020	36	1	22	57	0	0	0	23	139
January 2021	10	0	12	47	1	1	0	34	105
February 2021	21	1	9	33	0	1	1	60	126
March 2021	49	1	9	42	0	5	2	137	245
April 2021	33	0	2	70	0	0	0	41	146
May 2021	9	5	4	54	1	0	0	13	86
June 2021	26	5	1	30	0	0	2	25	89
July 2021	8	1	1	19	0	0	2	12	43
August 2021	1	0	1	10	1	0	0	25	38
September 2021	0	0	0	11	0	0	0	23	34
Total	237	24	178	400	4	23	13	484	1363

ANNEX 3: *ANOPHELES GAMBIAE* S.L. COLLECTED BY HLC INDOOR, OCTOBER 2020 –-SEPTEMBER 2021

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2020	1	61	0	0	4	6	26	0	98
November 2020	1	54	1	3	0	34	59	0	152
December 2020	3	22	7	6	0	3	36	2	79
January 2021	1	26	14	13	0	5	74	1	134
February 2021	0	82	27	33	0	2	70	1	215
March 2021	0	47	28	13	0	6	40	20	154
April 2021	6	73	60	43	0	0	45	13	240
May 2021	3	100	53	34	2	0	80	1	273
June 2021	34	119	108	24	9	75	95	15	479
July 2021	40	104	17	13	14	17	30	0	235
August 2021	17	70	0	0	10	4	0	1	102
September 2021	0	62		1	4	5	0	0	72
Total	106	820	315	183	43	157	555	54	2233

ANNEX 4: *ANOPHELES GAMBIAE* S.L. COLLECTED BY HLC OUTDOOR, OCTOBER 2020 –-SEPTEMBER 2021

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2020	1	75	0	0	18	12	33	2	141
November 2020	0	31	3	2	1	27	69	0	133
December 2020	6	18	6	3	1	8	36	6	84
January 2021	0	26	17	9	0	3	61	0	116
February 2021	0	99	52	28	5	3	48	2	237
March 2021	0	50	22	10	0	9	43	15	149
April 2021	11	67	59	27	1	6	38	17	226
May 2021	0	85	70	24	0	5	74	2	260
June 2021	35	127	116	27	0	59	103	6	473
July 2021	31	53	21	31	17	34	39	1	227
August 2021	41	83	74	0	31	13	0	0	242
September 2021	1	72		7	8	11	0	3	102
Total	126	786	440	168	82	190	544	54	2390

ANNEX 5: *ANOPHELES FUNESTUS* S.L. COLLECTED BY HLC INDOOR, OCTOBER 2020 –-SEPTEMBER 2021

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2020	3	0	1	2	0	1	0	0	7
November 2020	3	0	4	6	0	3	1	13	30
December 2020	1	0	6	2	0	1	0	18	28
January 2021	3	1	7	1	2	1	8	7	30
February 2021	0	0	0	5	0	0	0	31	36
March 2021	14	0	0	22	0	0	3	65	104
April 2021	37	5	0	6	0	0	0	55	103
May 2021	29	6	2	20	0	0	2	12	71
June 2021	22	1	0	22	0	0	0	14	59
July 2021	13	3	2	30	0	0	1	11	60
August 2021	2	1	0	3	0	0	0	17	23
September 2021	1	0	0	5	0	0	0	12	18
Total	128	17	22	124	2	6	15	255	569

ANNEX 6: *ANOPHELES FUNESTUS* S.L. COLLECTED BY HLC OUTDOOR, OCTOBER 2020 –-SEPTEMBER 2021

Month	Fissebu	Gbedin Camp 3	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeansue	Total
October 2020	2	0	1	0	0	3	1	10	17
November 2020	2	0	5	0	0	1	1	9	18
December 2020	1	0	5	6	0	0	0	19	31
January 2021	2	0	25	6	0	0	8	12	53
February 2021	9	0	0	12	0	0	0	12	33
March 2021	14	0	0	18	0	0	0	70	102
April 2021	13	0	0	4	0	0	0	42	59
May 2021	24	3	0	7	0	0	0	19	53
June 2021	46	2	0	9	0	1	0	8	66
July 2021	11	1	0	16	0	1	0	19	48
August 2021	4	0	0	4	0	0	0	10	18
September 2021	0	0	0	6	0	0	0	9	15
Total	128	6	36	88	0	6	10	239	513