PMI VECTORLINK PROJECT

THE DEMOCRATIC REPUBLIC OF CONGO
ANNUAL ENTOMOLOGICAL MONITORING REPORT

JANUARY–DECEMBER 2022

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Abt Associates | 6130 Executive Boulevard | Rockville, MD 20852
T. 301.347.5000
abtassociates.com

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ACRONYMS

CDC	Centers for Disease Control and Prevention
CI	confidence interval
CTAB	cetyltrimethylammonium bromide
DRC	Democratic Republic of Congo
EIR	entomological inoculation rate
ELISA	enzyme-linked immunosorbent assay
HBR	human biting rate
HLC	human landing catch
INRB	*Institut National de Recherche Biomédicale* / National Institute of Biomedical Research
ITN	insecticide-treated net
kdr	knockdown resistance
nbr	number
NMCP	National Malaria Control Program
PBO	piperonyl butoxide
PCR	polymerase chain reaction
PMI	President's Malaria Initiative
PSC	pyrethrum spray catch
SOP	standard operating procedure
SR	sporozoite rate
VGSC	voltage gated sodium channel
WHO	World Health Organization

EXECUTIVE SUMMARY

The President's Malaria Initiative (PMI) VectorLink Project conducted entomological monitoring in the Democratic Republic of Congo (DRC) from January to December 2022. Activities took place in 14 sentinel sites distributed nationwide, including monthly sites. Sites were chosen strategically to ensure data was collected to inform ITN procurement for upcoming mass distribution campaigns. NMCP through Vector Control Working Group (VCWG) did coordination for site selection by avoiding overlapping of activities between partners. The project also conducted monthly longitudinal monitoring of malaria vector biting rates, resting densities, and entomological inoculation rates (EIRs) in three sites (Kenge, Lodja, and Karawa-part of the 14 sentinel sites). Indoor resting densities were determined using pyrethrum spray catch (PSC) collections; human landing catch (HLC) was undertaken indoors and outdoors to determine malaria vector biting rates; and human observation took place indoors in the HLC houses to determine the adjusted biting rates.

To inform the National Malaria Control Program's (NMCP's) choice of insecticides for future insecticide-treated net (ITN) distribution campaigns, insecticide susceptibility tests were conducted in all 14 sites. Resistance intensity bioassays on *Anopheles gambiae* s.l. were conducted with permethrin, deltamethrin, and alpha-cypermethrin at five, and 10 times the diagnostic concentration, according to World Health Organization protocols. The project conducted synergist bioassays using piperonyl butoxide (PBO) and pyrethroids and determined susceptibility status to chlorfenapyr using the Centers for Disease Control and Prevention bottle bioassay. The planned molecular analyses were performed at the National Institute of Biomedical Research / Institut National de Recherche Biomédicale (INRB) between April 2022 and January 2023. All entomological data collected in 2022 was entered into VectorLink Collect and exported for data analysis. An online data visualization platform within VectorLink Collect was developed. Stakeholders, including INRB, NMCP, and PMI, have access to data summaries and raw data.

Transmission

Anopheles gambiae s.l. was the predominant malaria vector throughout the year in Kenge, Lodja, and Karawa. The abundance in all sites was greater from HLC (89%: 5,894/6,641) than from PSC (11%: 747/6,641). This could indicate that *An. gambiae* s.l. and *An. funestus* s.l. are less likely to enter houses to rest indoor or may exit houses early (before PSC sampling, which started at 06:00) perhaps due to the presence of ITNs which may have induced excito-repellency. Biting rates of *An. gambiae* s.l. were particularly high in Karawa and Lodja throughout the year and high in the rainy season in Kenge. The high mean indoor resting density per house per day of *An. gambiae* s.l. was 4.3 in May in Kenge, 3.1 in March in Lodja, and 6.9 in July in Karawa over the reporting period. The majority of *Anopheles* collected by PSC were blood-fed in Kenge, Lodja, and Karawa.

In Kenge, the mean *An. gambiae* s.l. biting rate was 3.3 bites per person per night indoors and 3.6 outdoors, with a malaria sporozoite rate (SR) of 13% (54/432), giving an annual EIR of 105 infectious bites per person. At the same site (Kenge), the mean *An. funestus* s.l. biting rate was 0.1 bites per person per night indoors and 0.2 bites outdoors, with a malaria SR of 4.3% (1/23), giving an annual EIR of 1.5 infectious bites per person. The combined annual EIR for the three vector species was 106.5 in Kenge.

In Lodja, the mean *An. gambiae* s.l. biting rate was 6.8 bites per person per night indoors and 13.9 outdoors, with a malaria SR of 1.8% (36/1,974) and an annual EIR of 73.1 infectious bites per person.

In Karawa, the mean *An. gambiae* s.l. biting rate was 16.7 bites per person per night indoors and 13.5 outdoors, with a malaria SR of 11% (169/1,593), giving an annual EIR of 387.3 infectious bites per person. At the same site (Karawa), the mean *An. funestus* s.l. biting rate was 2.1 bites per person per night indoors and 2.5 bites outdoors, with a malaria SR of 7.4% (11/148), giving an annual EIR of 43 infectious bites per person. The mean *An. moucheti* biting rate was 1.8 bites per person per night indoors and 2 bites outdoors, with a malaria SR of 2.7% (5/182), giving an annual EIR of 9.2 infectious bites per person. The combined annual EIR for the three vector species was 439.5 in Karawa.

The results highlight high year-round malaria transmission risk in all sites. When considering the sleeping habits of people in Kenge, Lodja, and Karawa, the majority (60% to 80%) of people were asleep indoors under ITNs during the peak mosquito host-seeking periods between 20:00 and 5:00 hours. Therefore, the adjusted indoor and outdoor mean biting risk in all sites remained low (<0.6 bites per person per hour indoors and outdoors in Kenge and Lodja, and around 0.6 bites per person per hour indoors and 3.2 outdoors in Karawa).

The biting risk for unprotected people indoors is high in all sites, pointing to the importance for all householders to sleep under ITN each night. In addition, the data also show that there is heterogeneity across the country, with Karawa having a high transmission risk. Hence, the results showed the implication of *An. moucheti* in malaria transmission in Karawa, northern DRC.

Insecticide Susceptibility

Insecticide susceptibility tests showed that pyrethroid resistance is widespread. In all sites, *An. gambiae* s.l. were resistant to permethrin, deltamethrin, and alpha-cypermethrin. Resistance intensity varied by site and by insecticide but was usually moderate or high, where *low* was >98% mortality at ×5 dose, *moderate* was <98% mortality at ×5 and >98% at ×10 dose; and *high* was <98% mortality at ×10 dose. The intensity of the three most common pyrethroids used on ITNs was as follows: low in three sites, moderate in five sites, and high in six sites for permethrin; low in five sites, moderate in five sites, and high in four sites for deltamethrin; and low in two sites, moderate in three sites, and high in nine sites for alpha-cypermethrin.

Resistance intensity	Insecticides/sites		
	Permethrin	Deltamethrin	Alpha-cypermethrin
Low	Kapolowe, Mikalayi and Mweka)	Lodja, Pawa, Mikalayi, Mweka and Nyankunde	Mikalayi and Mweka
Moderate	Kenge, Kabondo, Karawa, Kalemie and Nyankunde	Kenge, Kingasani, Kapolowe, Karawa and Kamina	Kingasani, Karawa and Nyankunde
High	Lodja, Buta, Pawa, Kingasani, Kamina and Rutshuru	Buta, Kabondo, Kalemie and Rutshuru	Kenge, Lodja, Buta, Pawa, Kabondo, Kapolowe, Kamina, Kalemie and Rutshuru

The widespread pyrethroid resistance observed suggests that pyrethroid ITNs may no longer provide optimal protection against malaria. The high intensity of pyrethroid resistance reinforces the NMCP's decision to prioritize distribution of new types of nets, as funding allows. In all sites, bioassays with pyrethroids following pre-exposure to PBO showed an increase in mortality compared with tested pyrethroid alone, though mortality for permethrin was still <90% in seven sites (Lodja, Pawa, Kingasani, Kabondo, Karawa, Kamina, and

Rutshuru); for deltamethrin, in two sites (Lodja and Karawa); and for alpha-cypermethrin in six sites (Kenge, Lodja, Pawa, Kingasani, Mikalayi, and Rutshuru).

The general increase in mortality when PBO synergist was used indicates that ITNs containing PBO may provide greater control compared to pyrethroid-only ITNs, although susceptibility was not fully restored at some sites. A better option may be dual active ingredient chlorfenapyr (AI) ITNs, as susceptibility to chlorfenapyr 100ug/bottle was recorded at all sites in 2022 compared to 2021[1] where susceptibility was recorded in seven sites (Boende, Inongo, Kimpese, Kingasani, Lisala, Mbandaka, and Mweneditu) and resistance in two sites (Karawa and Pawa) where bioassays were performed.

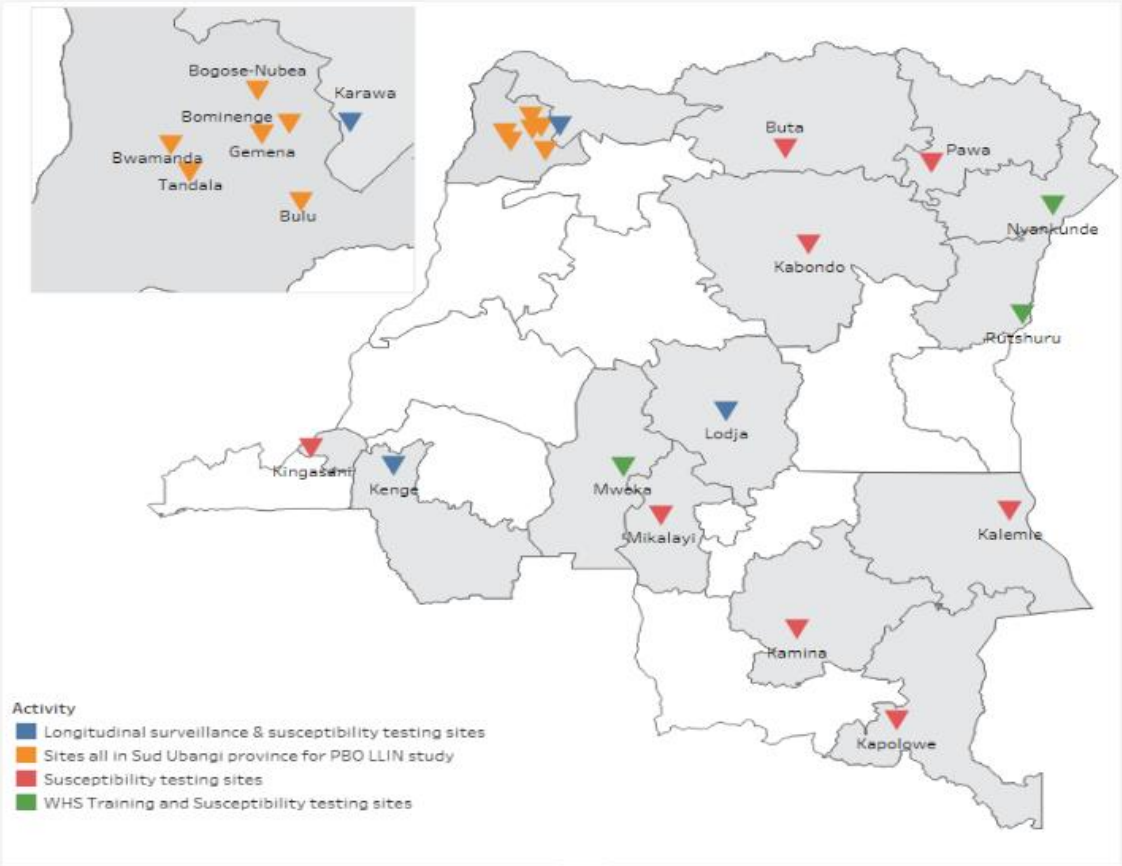
[1] The PMI VectorLink Project, *The Democratic Republic of Congo Annual Entomological Monitoring Report, January–December, 2021*. Rockville, MD: The PMI VectorLink Project, Abt Associates Inc.

1. METHODOLOGY

1.1 STUDY AREA

The President's Malaria Initiative (PMI) VectorLink Project conducted entomological monitoring in the Democratic Republic of Congo (DRC) in 14 sites in 2022 (Figure 1). Results of a piperonyl butoxide (PBO) insecticide treated net (ITN) study conducted in Sud Ubangi, a PBO durability study in Tanganyika, and pre-distribution testing of Interceptor G2 in Nord Ubangi are presented in separate reports.

Figure 1: Location of Sentinel Sites in DRC for Entomological Monitoring in 2022.



The activities were conducted according to the PMI VectorLink DRC Year 5 work plan (Table 1).

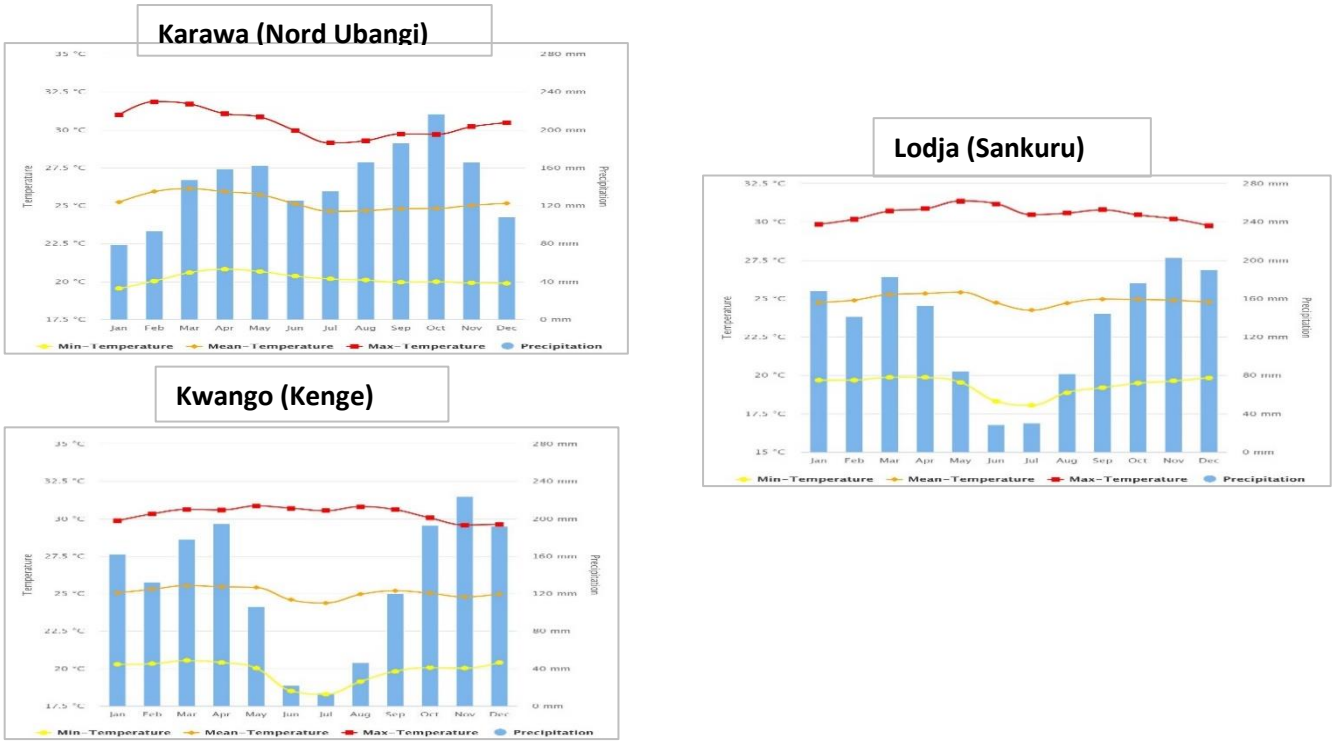
Table 1: Summary PMI VectorLink DRC 2022 Entomological Activities.

Activity	Purpose	Sites	Timeline	Frequency	Status
Vector susceptibility and intensity of resistance	• To determine vector susceptibility to three pyrethroid insecticides with/without the synergist PBO. • To determine pyrethroid resistance intensity. • To determine susceptibility to chlorfenapyr.	Kenge*, Lodja, Karawa, Kamina, Buta, Pawa, Kingasani, Kabondo, Kapolowe, Mikalayi, Kalemie, Nyankunde*, Rutshuru*, and Mweka*	May–Dec 2022	14 sites, once per site	Completed
Monthly species composition, biting rate, biting times, and indoor resting densities	• To gather more detailed longitudinal information on malaria vector dynamics and behavior.	Kenge, Karawa, and Lodja	Jan–Dec 2022 in Lodja and May–Dec 2022 in Kenge and Karawa	3 sites, every month	Completed
Tanganyika 12-month ITN bio-efficacy and durability	• To assess the physical and biological durability of the two ITN brands: Veeralin and SafeNet.	Kalemie and Manono	Feb 2022	2 sites	Completed with report approved
Pre-distribution testing of IG2 in Nord Ubangi	• To determine the bio-efficacy of Interceptor G2.	Gbadolite (Nord Ubangi)	Nov–Dec 2022	1 site	Completed with reports approved
Molecular assays	• To identify mosquito species of the *An. gambiae* s.l. species complex, mechanisms of pyrethroid resistance (*kdr*), and sporozoite rates.	Kenge*, Lodja, Karawa, Kamina, Buta, Pawa, Kingasani, Kabondo, Kapolowe, Mikalayi, Kalemie, Nyankunde*, Rutshuru*, and Mweka*	Jan–Dec 2022	12 sites	Completed
Sud Ubangi ITN monitoring	• To collect data on the density of vectors and the susceptibility of *An. gambiae* s.l. to deltamethrin with and without PBO. • To conduct ITN bioassay testing of PBO and pyrethroid nets using locally collected *An. gambiae* s.l. from Gemena.	Sud Ubangi in 6 health zones	May–June and Oct–Nov 2022	6 sites, once per site	Completed for 24 and 28 months with reports approved

Key: ITN=insecticide-treated net. *kdr*=knockdown resistance. PBO=piperonyl butoxide. PCR=polymerase chain reaction.
*New site for 2022.

Figure 2 shows the mean rainfall and temperature for the three longitudinal monitoring sites (Kenge, Lodja, and Karawa). Lodja and Kenge have a dry season in the middle of the year, which lasts approximately four months in Lodja and five months in Kenge. Karawa has considerable rainfall for most of the year, with peaks in April/May and October. The mean temperature in all sites is quite stable year-round, with a mean of 77–79°F (25–26°C), providing perfect conditions for malaria vector survival.

Figure 2: Average Monthly Temperature and Rainfall (1991–2016) in Longitudinal Entomological Monitoring Sites.



Source: Climatic Research Unit of University of East Anglia

Mosquitoes were collected monthly using human landing catch (HLC) and pyrethrum spray catch (PSC) in Kenge, Lodja, and Karawa (Table 2). Houses were sampled monthly in the same village from May to December 2022 in the health zone of Kenge (Mupepe: 4.82660, 17.03111) and in the health zone of Karawa (Bayasekado: 3.33116, 20.31728), and from January to December in the health zone of Lodja (-3.539530, 23.583070).

Table 2: Summary of Collection Methods.

Collection Method	Time	Frequency	Sample
Human landing catch	18:00 to 06:00	4 days per site per month	8 houses per site per month (2 houses per night)
Pyrethrum spray catch	06:00 to 09:00	2 days per site per month	10 houses per site per month (5 houses per day)

1.2 Human Landing Catch

HLCs were performed to assess mosquito biting time, feeding behavior, and biting rates and to monitor species composition and sporozoite infection rates. Trained residents collected adult mosquitoes over four consecutive

nights in two different houses each night (total of eight houses per month), with one person located indoors and another outdoors in each selected house. Collectors rotated indoors and outdoors every hour. The same houses were used every month in Kenge, Karawa, and Lodja. All *Anopheles* mosquitoes collected by HLC per the standard operating procedure (SOP) 02/01[2] were identified to species morphologically in the field and cross-checked by National Institute of Biomedical Research / Institut National de Recherche Biomédicale (INRB) entomologists either in the field or in Kinshasa (depending on the supervision schedule). All *Anopheles* were preserved in 1.5 ml Eppendorf tubes on silica gel for further molecular analysis in Kinshasa by INRB.

1.3 Pyrethrum Spray Catch

PSCs were conducted following the SOP 03/01[3] from 06:00 to 09:00 in the same areas as for HLC (but in different houses) to estimate the indoor resting density of mosquito species. Before the PSCs were performed, all occupants were asked to move out of the house. The rooms were sprayed following SOP 03/01[3]. Each mosquito collected was labeled properly, stored in an Eppendorf tube with silica gel, identified to species morphologically in the field, and cross-checked by an INRB entomologist once back at the central lab.

1.4 Human Behavior in Household

The monitoring of human behavior occurred each month in the eight HLC households from August to December in Lodja and from July to December in Kenge and Karawa. The observation of human behavior in households was conducted by family members, mostly heads of households or main caregivers who were literate. These observers were paid for their services. Individuals selected to monitor the behavior of household members were trained to fill out the standardized observation worksheet (Table A4 in Annex). This worksheet allowed the observers to monitor the behavior of family members (not including the observer) every 30 minutes. Rather than attempting to monitor behavior over the entire 30 minutes, the behaviors of each member of the household were measured at each half hour. The observers were loaned a timer that sounded every 30 minutes.

In addition to the observation in the household, there were supervisors in the area who were available by phone (particularly early in the evening) in case observers had any questions.

Observations started at 18:00 and finished at 06:00. Observations occurred each night over four consecutive nights each month.

1.5 Insecticide Susceptibility, PBO Synergy, and Resistance Intensity Testing

Insecticide susceptibility and resistance intensity testing were conducted per the SOP 06/01[4] in 14 sentinel sites. As part of decentralization of entomological surveillance, insecticide susceptibility testing was conducted by the

[2] 2-SOP "Indoor and Outdoor Human Landing Catch." PMI VectorLink Entomology Standard Operating Procedures: https://docs.google.com/document/d/1NKDTyKUeXDActWsCKoMYRVugAvFbmUFw/edit?usp=share_link&ouid=105827530796748925129&rtpof=true&sd=true

[3] 3-SOP "Pyrethrum Spray Catch." PMI VectorLink Entomology Standard Operating Procedures: https://docs.google.com/document/d/1E7a6USMaJG7BSFPCZwrKssFiWtkafi5v/edit?usp=share_link&ouid=105827530796748925129&rtpof=true&sd=true

[4] 6-SOP "Insecticide Susceptibility Test, Intensity and Synergist Assay Using WHO Test Kits," PMI VectorLink Entomology Standard Operating Procedures: https://docs.google.com/document/d/128jamIR3sjnNXqc98t78LlPX8RgjHl9R/edit?usp=share_link&ouid=105827530796748925129&rtpof=true&sd=true

staff from five of the sentinel sites, with no supervision from INRB. The five sites were Kabondo, Kapolowe, Kalemie, Lodja, and Mikalayi, where local staff are relatively well trained and had performed testing using wild *An. gambiae* s.l. collected as larvae, following SOPs. Insecticide papers and chlorfenapyr were sent from Kinshasa to the remote sites; after testing, mosquito samples and data sheets were returned to INRB.

Susceptibility testing was conducted in another four sites (Kamina, Kingasani, Kenge, and Karawa) with in-person supervision from INRB and in two sites (Buta and Pawa) through the Kabondo-based National Malaria Control Program (NMCP) supervisor as he is located in a neighbor province and well trained. In another three, unsecured/inaccessible sites (Nyankunde, Mweka, and Rutshuru), where the Water Hygiene and Sanitation supervisors were trained, resistance monitoring was done through in-person supervision from the Public Health School of Kinshasa.

In addition to tests at the diagnostic dose, World Health Organization (WHO) intensity bioassays were also conducted by testing with pyrethroid papers treated with five and 10 times the diagnostic dose. The insecticides tested in 2022 were:

- Deltamethrin ×1, ×5, ×10 (0.05%, 0.25%, 0.5%)
- Permethrin ×1, ×5, ×10 (0.75%, 3.75%, 7.5%)
- Alpha-cypermethrin ×1, ×5, ×10 (0.05%, 0.25%, 0.5%)
- Deltamethrin 0.05% with pre-exposure to PBO 4%
- Permethrin 0.75% with pre-exposure to PBO 4%
- Alpha-cypermethrin 0.05% with pre-exposure to PBO 4%
- Chlorfenapyr 100µg/bottle[5]

In all sites, susceptibility testing was conducted with adult *An. gambiae* s.l. following the WHO method (with the exception of chlorfenapyr in Lodja, where the field team was unable to conduct testing due to lack of CDC bottles which were broken during shipment of materials from Kinshasa). WHO susceptibility tests were conducted on deltamethrin, permethrin, and alpha-cypermethrin, with pre-exposure to PBO for 60 minutes[3] to determine the change in mortality rates with PBO exposure.

CDC bottle bioassays were completed following the SOP 04/01[6] in the 13 sites (Kabondo, Kapolowe, Kalemie, Mikalayi, Kamina, Kingasani, Kenge, Karawa, Buta, Pawa, Nyakunde, Mweka, and Rutshuru) to determine the susceptibility status of *An. gambiae* s.l. populations to chlorfenapyr using a diagnostic dose of 100µg/bottle. Four replicates of 20–25 *An. gambiae* s.l. were exposed for 60 minutes to chlorfenapyr 100ug/bottle. To ensure the insecticide's quality before it was used in the sites, the susceptible *An. coluzzii*, being raised in the INRB insectary was tested following the SOP in the laboratory.

[5] Chlorfenapyr was tested following the Centers for Disease Control and Prevention bottle bioassays.

[6] 4-SOP "Susceptibility Testing, Resistance Intensity and Synergist Assays Using the CDC Bottle Bioassay," PMI VectorLink Entomology Standard Operating Procedures: https://docs.google.com/document/d/1kgOs50Bi79IHnycfFE7aCm_AsTOoq6s7/edit?usp=share_link&ouid=105827530796748925129&rtpof=true&sd=true

1.6 MOLECULAR ANALYSIS

Molecular analyses were conducted in a molecular laboratory at INRB, which was equipped by the project and dedicated to entomology. The mosquito samples collected from sentinel sites were transported to INRB for processing and analysis. Technicians conducted laboratory analyses under the supervision of the INRB focal point entomologist and PMI VectorLink entomologist, following the protocols described in Table 3.

Table 3: Protocols Used for Laboratory Analysis of Malaria Vectors.

Molecular Analysis	Protocol	Output
ELISA	Malaria Research and Reference Reagent Resource Centre, "*Anopheles* in Research" (based on Wirtz et al. [1987])[7]	Sporozoite identification: Identified mosquito samples that were positive for *Plasmodium falciparum* sporozoites.
PCR	Santolamazza et al. (2008)[8]	Species identification: Identified *An. gambiae* complex sibling species including *An. coluzzii*, *An. gambiae,* and *An. arabiensis.*
	Huynh et al. (2007)[9]	Vgsc-L1014F allele frequency: Monitored pyrethroid target site resistance mechanism frequency.
qPCR	Messenger L (unpublished)[10]	G119-ACE1 allele frequency: Identified the presence/absence of carbamate/organophosphate target site resistance mechanism frequency in *An. coluzzii* and *An. gambiae.*
	Messenger L (unpublished)[11]	L43F-CYP4J5 allele frequency: Monitored molecular resistance mechanism frequency in *An. coluzzii* and *An. gambiae.*
	Messenger L (unpublished)[10]	N1575Y allele frequency: Monitored molecular resistance mechanism frequency in *An. coluzzii* and *An. gambiae.*

Key: ELISA=enzyme-linked immunosorbent assay. VGSC=voltage gated sodium channel. PCR=polymerase chain reaction.

Sporozoite infection rate testing was completed for 3,999 *An. gambiae* s.l. collected from Kenge, Lodja, and Karawa in 2022 using an enzyme-linked immunosorbent assay (ELISA), 2,800 *An. gambiae* s.l. using polymerase chain reaction (PCR) for species identification and resistance mechanism detection, and 257 *An. gambiae* s.l. using quantitative PCR (qPCR) for the molecular monitoring of G119-ACE1, L43F-CYP4J5, and N1575Y. The results are reported in Sections 2.6 and 2.9.

ELISA tests were conducted on a subsample of *Anopheles* collected through HLCs in Kenge, Lodja, and Karawa (targeted at a sample of 200 *An. gambiae* s.l. per month, or 2,400 total; and 20 *An. funestus* s.l. per month, or 240 total). PCR analysis was performed on a subsample of *An. gambiae* s.l. collected through HLCs and susceptibility

[7] Wirtz R, Zavala F, Charoenvit Y, Campbell G, Burkot T, Schneider I, et al. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. *Bull World Health Organ.* 1987; **65**:39.

[8] Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, Torre DA. Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. *Malar J.* 2008; **7**:163.

[9] Huynh L, Sandve S, Hannan L, Van Ert M, Gimnig J. *Fitness costs of pyrethroid insecticide resistance in Anopheles gambiae.* Christchurch: New Zealand; 2007.

[10] Messenger L et al. Identification the presence/absence of carbamate/organophosphate target site resistance mechanism frequency in *An. coluzzii* and *An. gambiae.* (unpublished).

[11] Messenger L et al. Monitoring molecular resistance mechanism frequency in *An. coluzzii* and *An. gambiae* (Unpublished).

tests in 14 sites. The number of *An. gambiae* s.l. analyzed by each method in each sentinel site is shown in Table 4.

Table 4: Number of *An. gambiae* s.l. Samples Analyzed at INRB, Kinshasa, Compared with Number Planned.

Sentinel Site	Total *An. gambiae* s.l. Collected in 2022 by HLC	HLC Sporozoite ELISA Monthly Monitoring**	HLC Species ID Monthly Monitoring**	WHO Pyrethroid Susceptibility Test, Species ID**	WHO Pyrethroid Susceptibility Test, VGSC L1014F Frequency**
Kenge*	439	432/1,600	400/400	100/100	50/50
Lodja*	1,986	1,974/2,400	600/600	100/100	50/50
Karawa*	1,927	1,593/1,600	400/400	100/100	50/50
Kabondo	-	-	-	100/100	50/50
Kapolowe	-	-	-	100/100	50/50
Kalemie	-	-	-	100/100	50/50
Mikalayi	-	-	-	100/100	50/50
Kamina	-	-	-	100/100	50/50
Kingasani	-	-	-	100/100	50/50
Buta	-	-	-	100/100	50/50
Pawa	-	-	-	100/100	50/50
Nyankunde	-	-	-	100/100	50/50
Mweka				100/100	50/50
Rutshuru				100/100	50/50
Total	**4,352**	**3,999/5,600**	**1,400/1,400**	**1,400/1,400**	**700/700**

*Only 439 *An. gambiae* s.l. were collected in Kenge (May–December 2022), 1,986 in Lodja (January–December 2022).
**Numbers represent the total number tested in the reporting period/the total number targeted. For those where the targets were not met, not enough mosquitoes could be found in those sites.

1.7 Data Management and Analysis

The District Health Information Software (DHIS2)-based VectorLink Collect programs for entomological data management were used. The Home Office staff remotely supported INRB and Abt entomologist technicians and database managers on updated data workflows – including field paper collections, technical reviews, data entry, data cleaning, and analytics – to support the generation and use of high-quality entomological data. All entomological data collected in DRC in 2022 was managed within VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results. Stakeholders, including INRB, NMCP, and PMI, have access to these results dashboards to support timely decision-making.

The following formulas were used to calculate entomological indicators:

- Sporozoite rate= (total ELISA positive/total number tested) × 100
- Human biting rate (HBR)=total # of each *Anopheles* species collected by HLCs during a specific period/total number of trap nights

- Human behavior adjusted biting rate indoor (HBABRi)=HBR indoor * Proportion of people indoors during a specific period
- Human behavior adjusted biting rate outdoor (HBABRo)=HBR outdoor * Proportion of people outdoors during a specific period
- Nightly entomological inoculation rate (EIR)=nightly HBR × sporozoite rate
- Monthly EIR=nightly mean EIR × number of nights in the month
- Yearly EIR=sum of monthly EIRs

The WHO recommendations below are used for interpretation of results from susceptibility testing and the intensity bioassays:

- *Susceptible* when a mortality in the range of 98–100% is recorded at the diagnostic dose.
- *Resistance* when a mortality <90% is recorded at the diagnostic dose.
- *Possible resistance* when a mortality of 90–97% is recorded at the diagnostic dose.
- Low resistance intensity when a mortality of 98–100% is recorded at the 5× concentration.
- Moderate resistance intensity when a mortality <98% is recorded at the 5× concentration.
- High resistance intensity when a mortality of <98% is recorded at the 10× concentration.

2. RESULTS

2.1 MALARIA VECTOR SPECIES COMPOSITION

Over the study period (January to December 2022 for Lodja and May to December for Karawa and Kenge), a total of 6,641 *Anopheles* mosquitoes were collected from the three longitudinal monitoring sites through monthly HLC and PSC. Seven *Anopheles* species (*An. gambiae* s.l., *An. funestus* s.l., *An. paludis, An. coustani, An. tenebrosus, An. ziemanni,* and *An. moucheti*) were collected, with *An. gambiae* s.l. being the most common (*n*=4,920), followed by *An. paludis* (*n*=893), *An. funestus* s.l. (*n*=546), *An. moucheti* (*n*=245), *An. tenebrosus* (*n*=19), *An. coustani* (*n*=16), and *An. zieamanni* (*n*=2).

In all sites, the abundance of *Anopheles* species was greater using HLC (89%: 5,894/6,641) than PSC (11%: 747/6,641). *Anopheles moucheti* was only found in Karawa. The species composition is presented by site and collection method in Figures 3, 4, and 5. Details about the indoor resting densities and biting rates are presented in Sections 2.2 and 2.3. Molecular species composition is presented in Section 2.9.

Figure 3: Species Composition of *Anopheles* Captured by PSC and HLC (Indoors and Outdoors) in Kenge from Monthly Collections.

Fig. 3a: Kenge, PSC-Indoor, May–Dec 2022 (*n*=95)

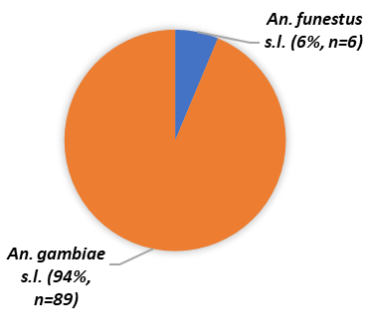


Fig. 3b: Kenge, HLC-Indoor, May–Dec 2022 (*n*=225)

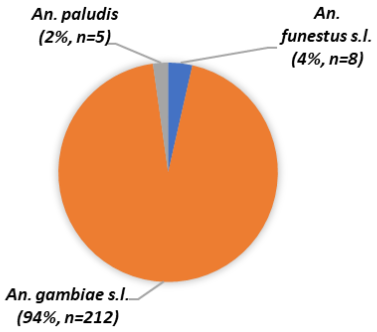


Fig. 3c: Kenge, HLC-outdoor, May–Dec 2022 (*n*=250)

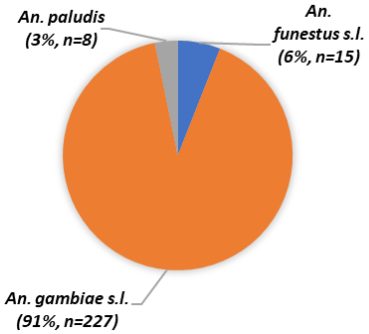


Figure 4: Species Composition of *Anopheles* Captured by PSC and HLC (Indoors and Outdoors) in Lodja from Monthly Collections.

Fig 4a: Lodja, PSC-Indoor, Jan–Dec 2022 (*n*=232)

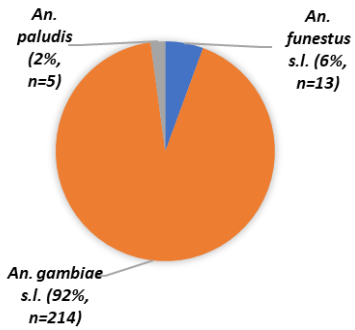


Fig. 4b: Lodja, HLC-Indoor, Jan–Dec 2022 (*n*=842)

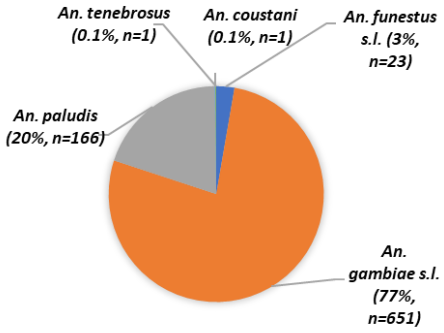


Fig. 4c: Lodja, HLC-outdoor, Jan–Dec 2022 (*n*=1,831)

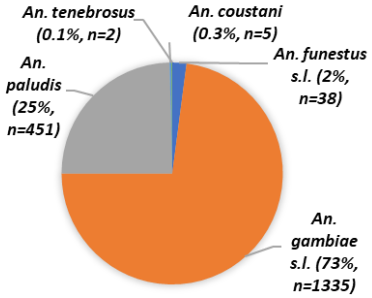


Figure 5: Species Composition of *Anopheles* Captured by PSC and HLC (Indoors and Outdoors) in Karawa from Monthly Collections.

Fig.5a: Karawa, PSC-Indoor, May–Dec 2022 (*n*=420)

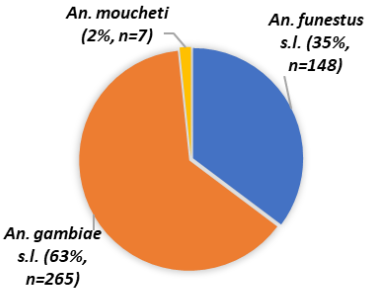


Fig. 5b: Karawa, HLC-Indoor, May–Dec 2022 (*n*=1,474)

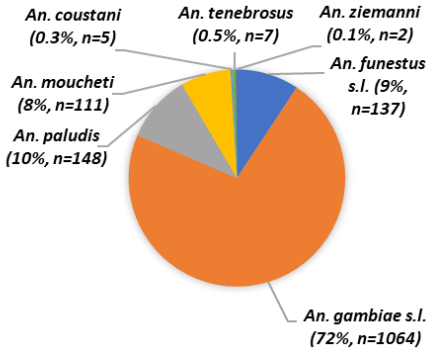
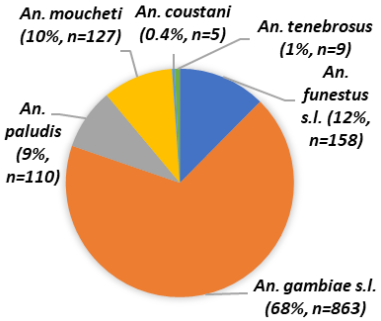


Fig. 5c: Karawa, HLC-outdoor, May–Dec 2022 (*n*=1,272)



2.2 Malaria Indoor Vector Resting Density (by PSC)

Figures 6, 7, and 8 show the mean indoor resting density of *An. gambiae* s.l., *An. funestus* s.l., *An. paludis*, and *An. moucheti* per house per day collected by PSC. In Lodja, the indoor resting density was fairly stable throughout the year, with three peaks of 3.1, 1.8, and 2.5 of *An. gambiae* s.l. per house per day in March, July, and October. The high mean indoor resting density per house per day of *An. gambiae* s.l. was 4.3 in May in Kenge, 3.1 in March in Lodja, and 6.9 in July in Karawa. The density of *An. gambiae* s.l. was higher than that of other species, except in Karawa where the density of *An. funestus* s.l. was higher than that of *An. gambiae* s.l. from September to December.

The majority of *Anopheles* mosquitoes collected were blood-fed (45% overall) in Kenge, Lodja, and Karawa.

Figure 6: Mean Indoor Resting Density per House per Day of *Anopheles species (An. gambiae s.l.: n=89 and An. funestus s.l.: n=6)* Captured by PSC in Kenge from Monthly Collections.

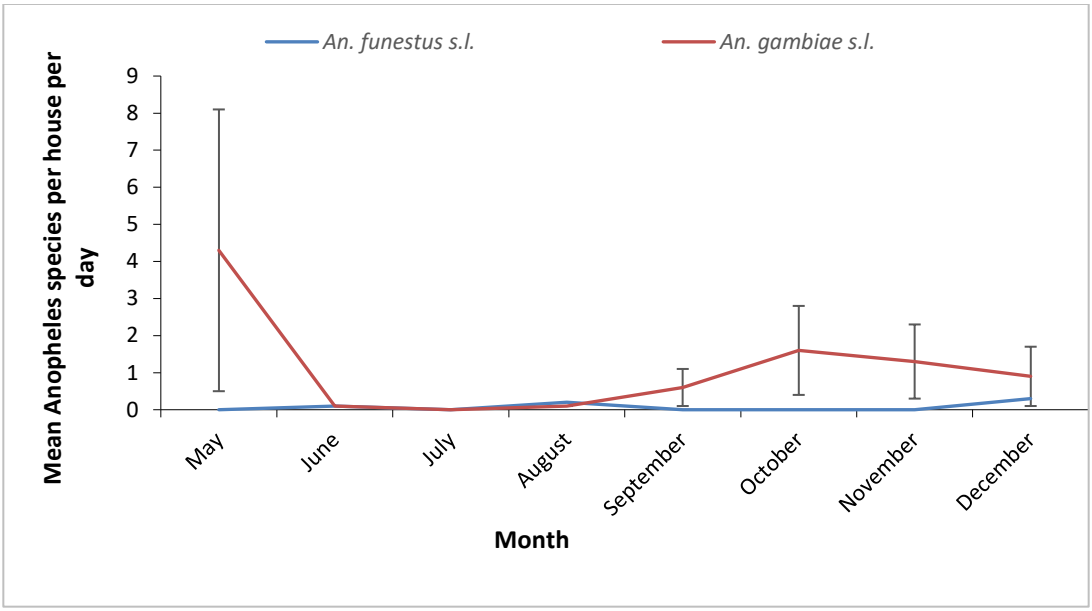


Figure 7: Mean Indoor Resting Density per House per Day of *Anopheles species (An. gambiae s.l.: n=214; An. funestus s.l.: n=13; An. paludis: n=5)* Captured by PSC in Lodja from Monthly Collections.

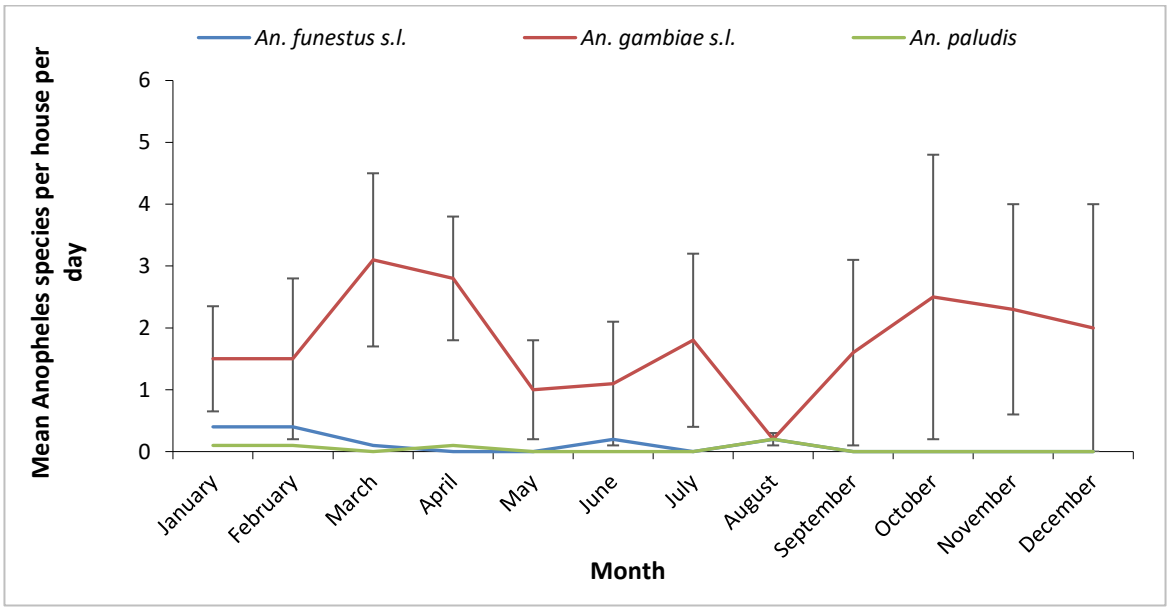
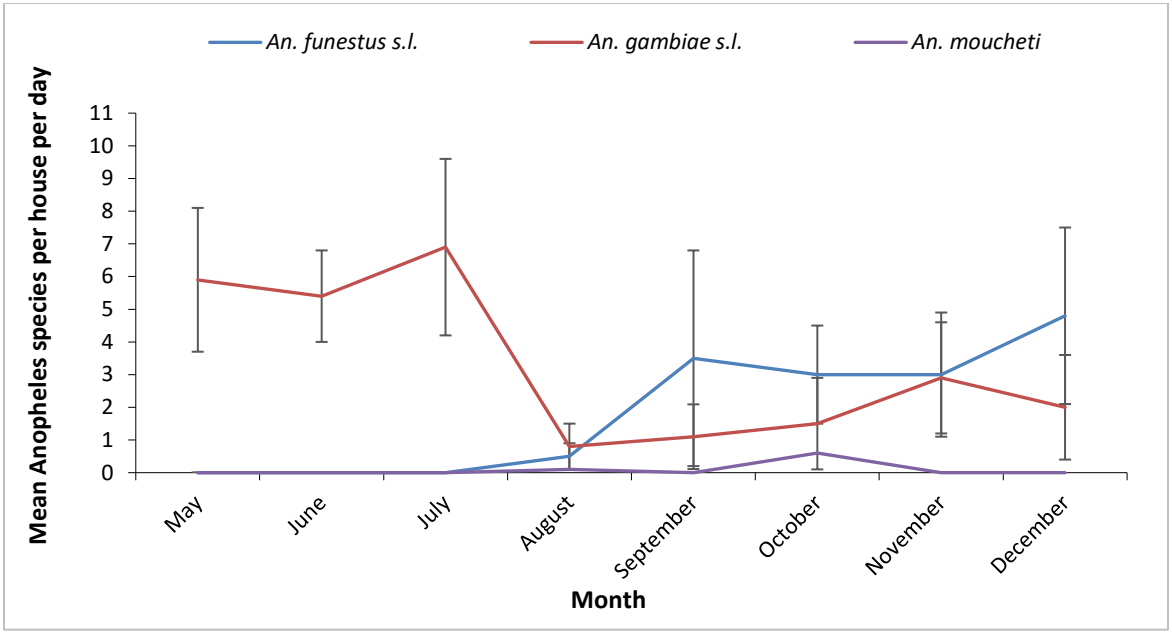


Figure 8: Mean Indoor Resting Density per House per Day of *Anopheles species (An. gambiae s.l.: n=265; An. funestus s.l.: n=148; An. moucheti: n=7)* Captured by PSC in Karawa from Monthly Collections.



2.3 Monthly Malaria Vector Human Biting Rates (by HLC)

Figures 9 to 14 show the mean monthly biting rates per person per night in Kenge, Lodja, and Karawa by vector species (details in Annex Tables A1, A2, and A3).

Anopheles gambiae s.l. biting rates were particularly high in the rainy season, with a mean of 44 bites per person per night indoors in May and 28 outdoors in July in Karawa (Figures 13 and 14), a mean of 15 indoors in October and 27 outdoors in April in Lodja (Figures 11 and 12), and a mean of seven bites per person indoors in October and 10 outdoors in October in Kenge (Figures 9 and 10). The mean higher biting rates of *An. paludis* of six bites per person per night indoors in August and 12 outdoors in February were observed in Lodja (Figures 11 and 12). The biting rates were erratic in Lodja, with high rates observed in the rainy season between January to May and October to December. The biting rate of *An. funestus* s.l. was low year-round in all sites, with a mean biting rate ranging between zero and five bites per person per night in both indoor and outdoor collections.

Figure 9: Mean Monthly Indoor *An. gambiae* s.l. (*n*=212), *An. funestus* s.l. (*n*=8), and *An. paludis* (*n*=5) Biting Rate in Kenge

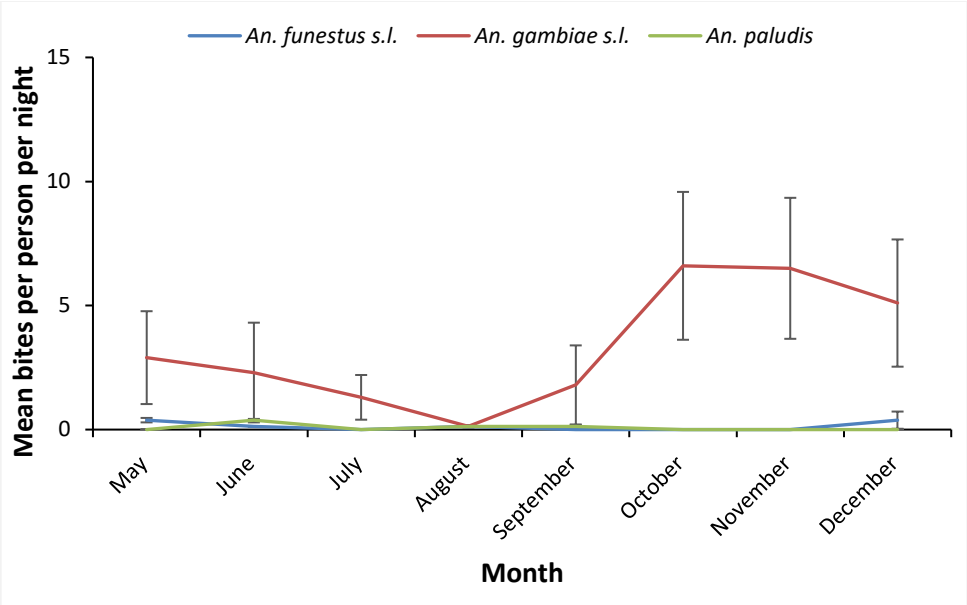


Figure 10: Mean Monthly Outdoor *An. gambiae* s.l. (*n*=227), *An. funestus* s.l. (*n*=15), and *An. paludis* (*n*=8) Biting Rate in Kenge.

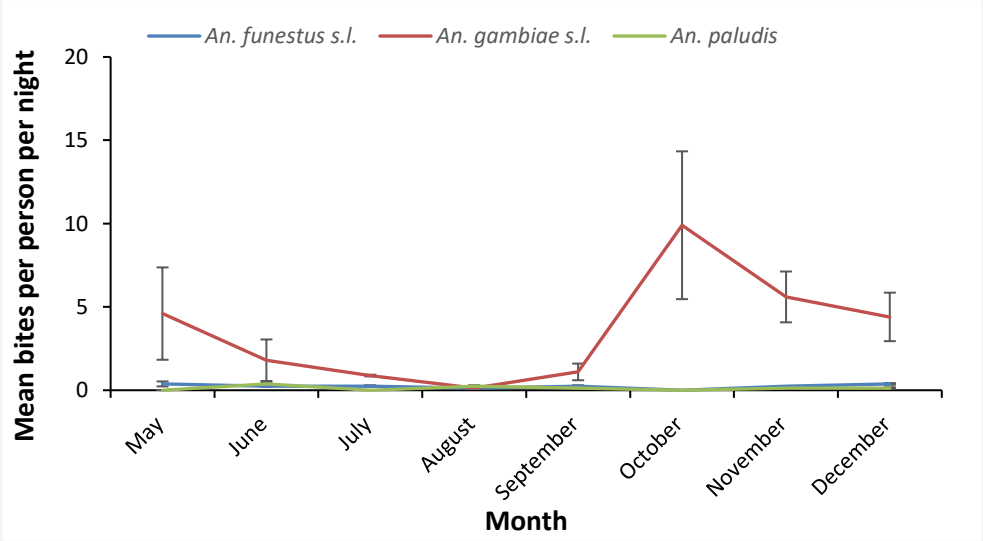


Figure 11: Mean Monthly Indoor *An. gambiae* s.l. (*n*=651), *An. funestus* s.l. (*n*=23), and *An. paludis* (*n*=166) Biting Rate in Lodja.

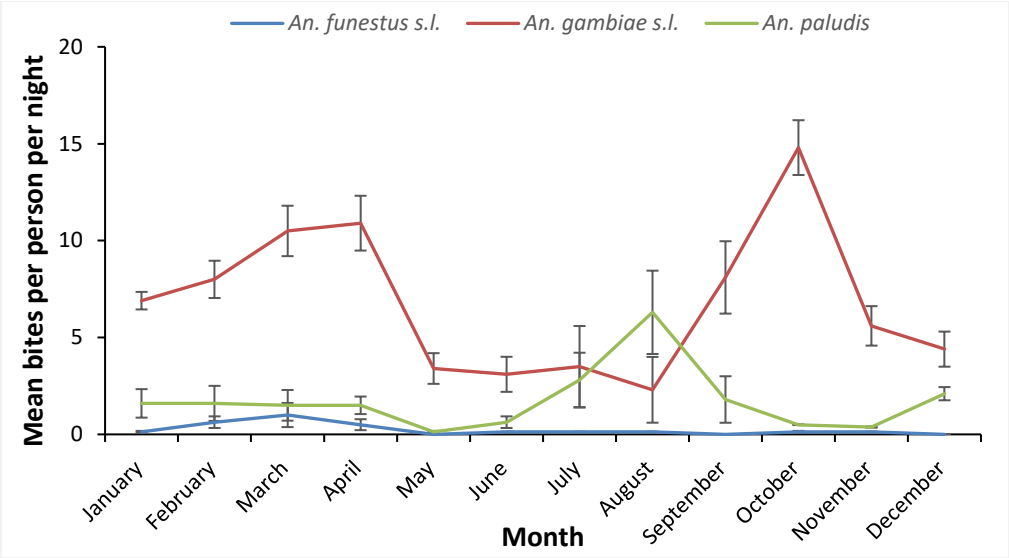


Figure 12: Mean Monthly Outdoor *An. gambiae* s.l. (*n*=1,335), *An. funestus* s.l. (*n*=38), and *An. paludis* (*n*=451) Biting Rate in Lodja.

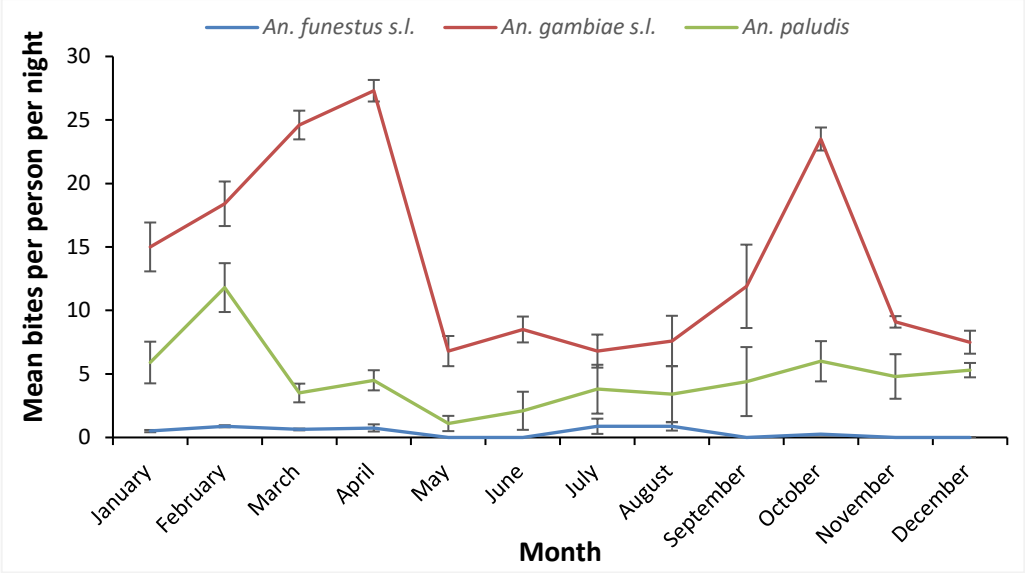


Figure 13: Mean Monthly Indoor *An. gambiae* s.l. (*n*=1,064), *An. funestus* s.l. (*n*=137), *An. paludis* (*n*=148), and *An. moucheti* (*n*=111) Biting Rate in Karawa.

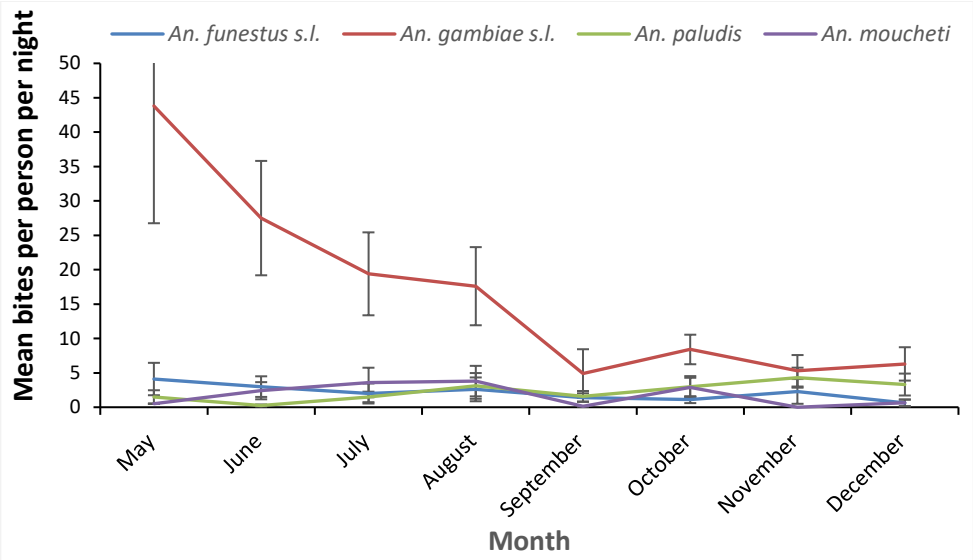
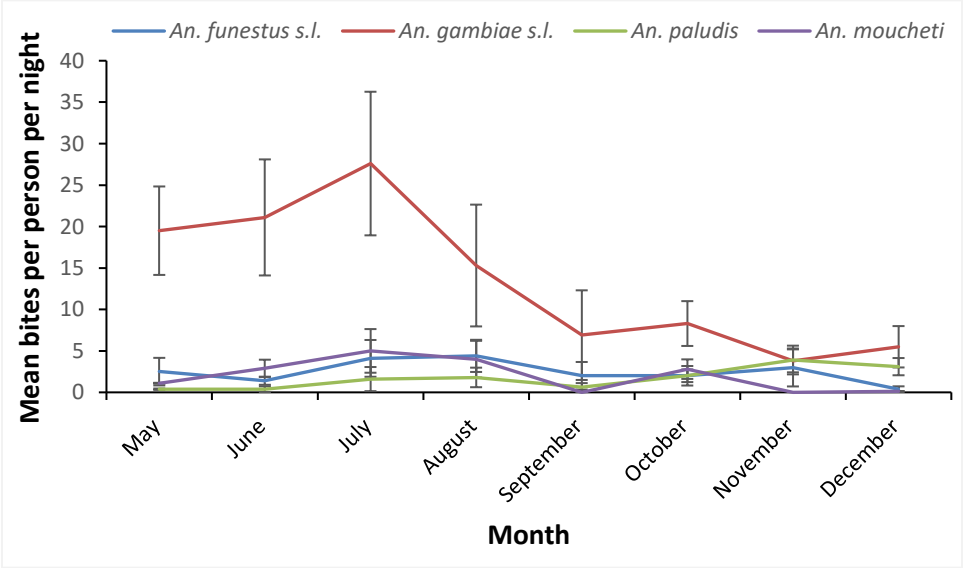


Figure 14: Mean Monthly Outdoor *An. gambiae* s.l. (*n*=863), *An. funestus* s.l. (*n*=158), *An. paludis* (*n*=110), and *An. moucheti* (*n*=127) Biting Rate in Karawa.



2.4 Biting Times of Malaria Vectors Collected Indoors and Outdoors by HLC

In general, the peak period of *An. gambiae* s.l. and *An. funestus* s.l. indoor biting was late at night, between 22:00 and 05:00, which mirrored outdoor biting trends in all sites (Figures 15 to 18). Biting rates of *An. gambiae* s.l. were substantially greater indoors than outdoors, particularly between midnight and 04:00 in Karawa, and greater outdoors than indoors, particularly between 20:00 and 06:00. *An. funestus* s.l. biting activities were low (<0.1 bite per person per hour) indoors and outdoors in Kenge and Lodja, whereas biting rates in Karawa were high indoors and outdoors (Figures 17 and 18). The peak period of *An. paludis* indoor biting was earlier, between

18:00 and 23:00, which mirrored outdoor biting trends in Lodja and Karawa (Figures 19 and 20). The peak of *An. moucheti* indoor biting was around 23:00 indoors and outdoors in Karawa (Figures 21 and 22).

Figure 15: Mean Hourly Indoor *An. gambiae* s.l. Biting Rates in Kenge (*n*=212), Lodja (*n*=651), and Karawa (*n*=1,064).

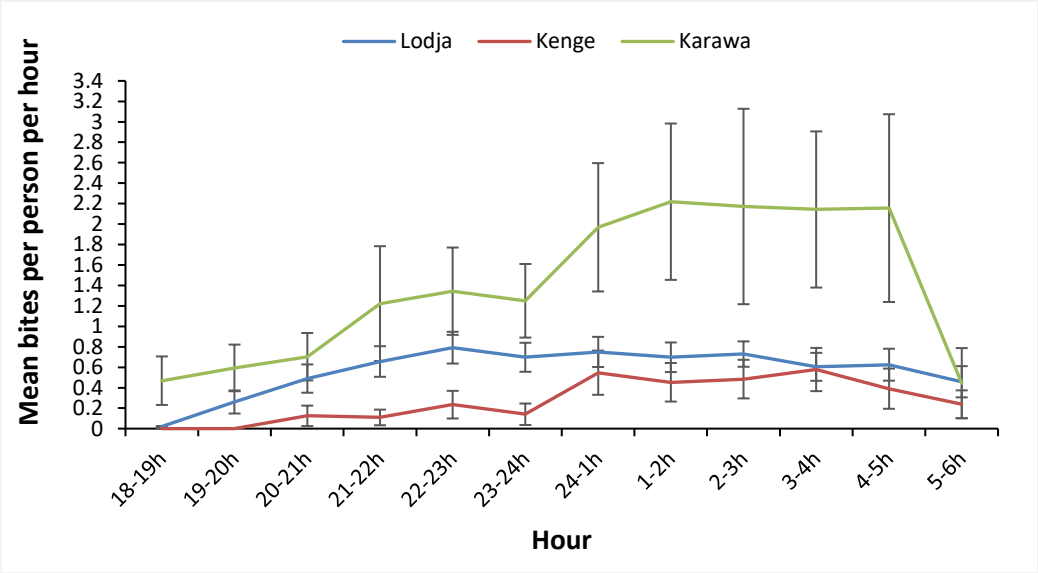


Figure 16: Mean Hourly Outdoor *An. gambiae* s.l. Biting Rates in Kenge (*n*=227), Lodja (*n*=1,335), and Karawa (*n*=863).

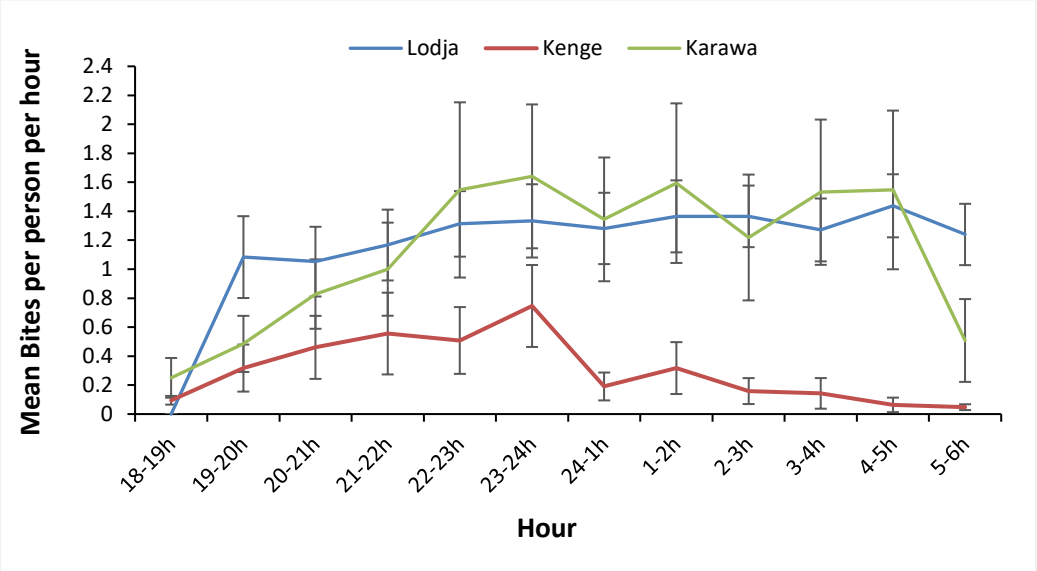


Figure 17: Mean Hourly Indoor *An. funestus* s.l. Biting Rates in Kenge (*n*=8), Lodja (*n*=23), and Karawa (*n*=137).

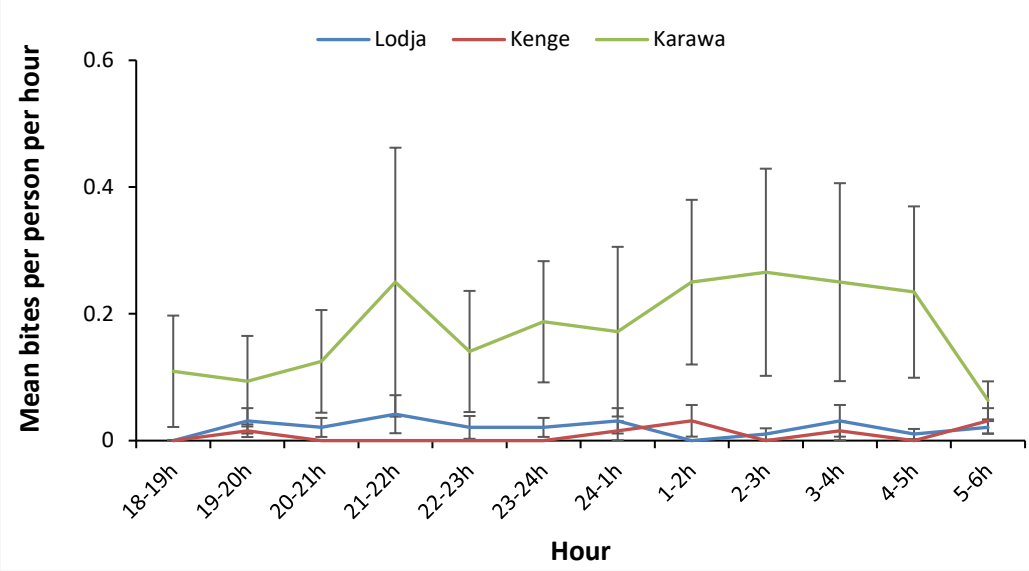


Figure 18: Mean Hourly Outdoor *An. funestus* s.l. Biting Rates in Kenge (*n*=15), Lodja (*n*=38), and Karawa (*n*=158).

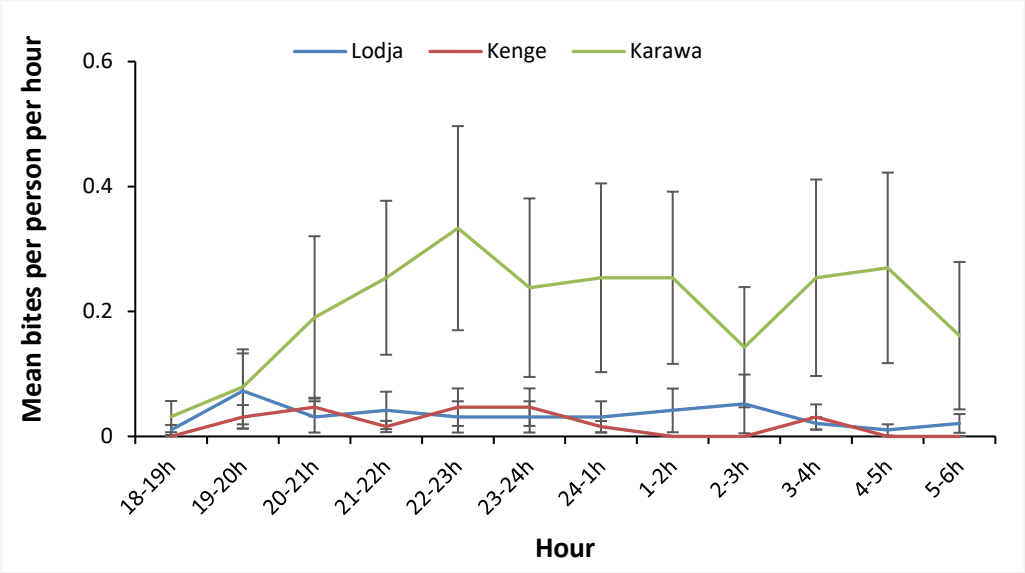


Figure 19: Mean Hourly Indoor *An. paludis* Biting Rates in Kenge (*n*=5), Lodja (*n*=166), and Karawa (*n*=148).

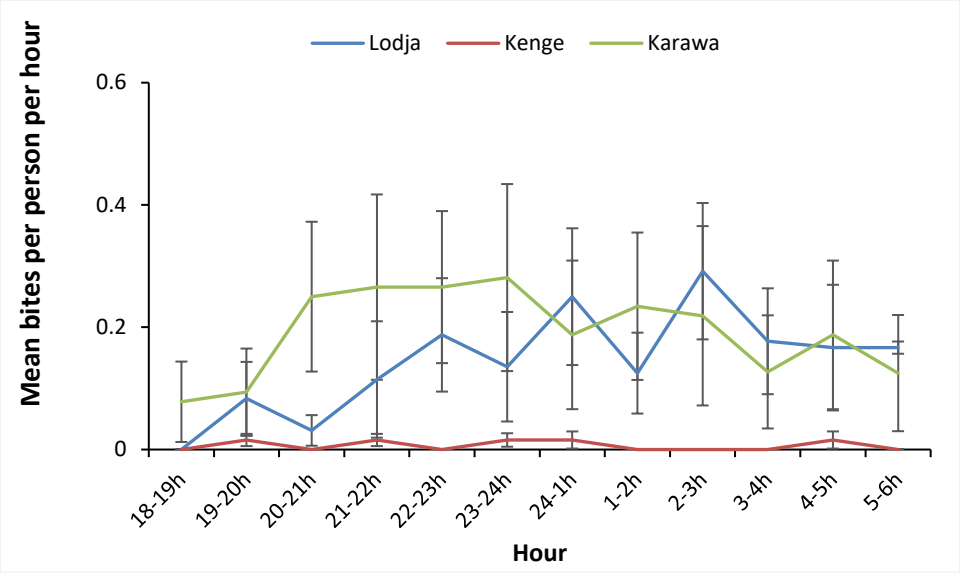


Figure 20: Mean Hourly Outdoor *An. paludis* Biting Rates in Kenge (*n*=8), Lodja (*n*=451), and Karawa (*n*=110).

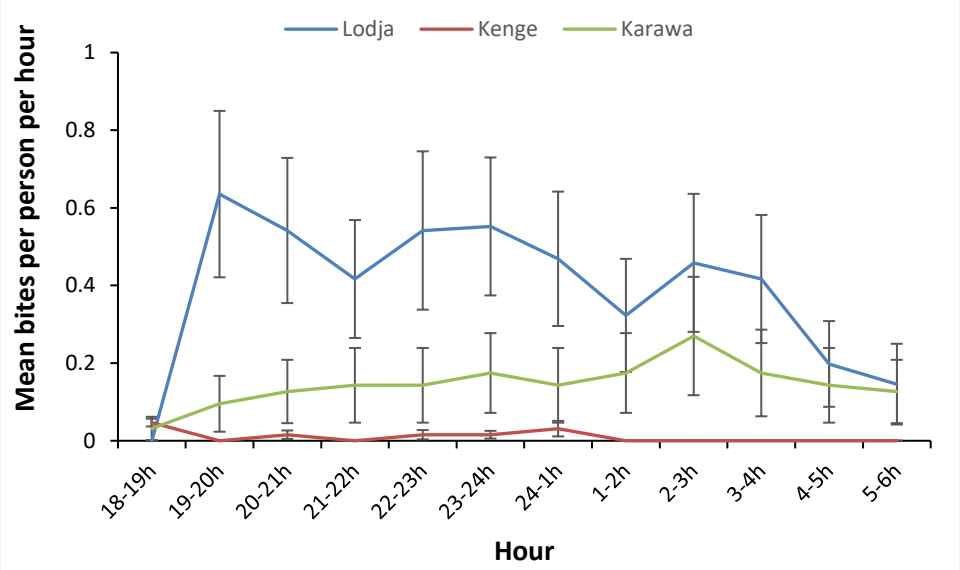


Figure 21: Mean Hourly Indoor *An. moucheti* Biting Rates in Kenge (*n*=0), Lodja (*n*=0), and Karawa (*n*=111).

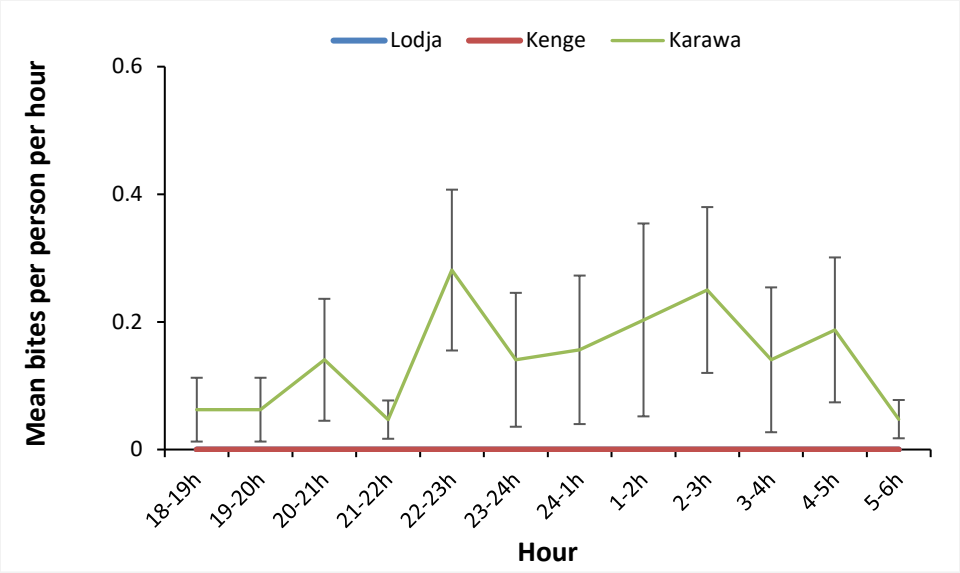
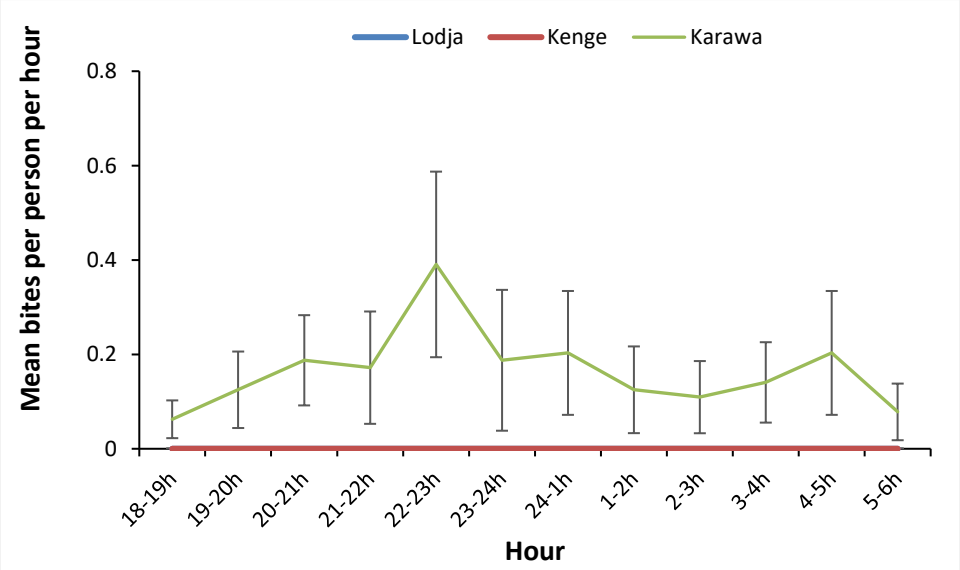


Figure 22: Mean Hourly Outdoor *An. moucheti* Biting Rates in Kenge (*n*=0), Lodja (*n*=0), and Karawa (*n*=127).



2.5 Observation of ITN Use in Households

Observations of household members were made mostly by the heads of households themselves. The activities performed by householders in the night are summarized in Annex in Figures A1 to A9. The activities are divided into groups according to household compartments where they took place: (1) *outdoor* activities, including talking, cooking, eating, resting, or working (Annex Figures A1–A3); (2) *sitting room* activities, including watching TV, sleeping under ITN, or any other activities performed there (Annex Figures A4–A6); (3) *bedroom* activities, mainly sleeping under ITN but any other activity performed there (Annex Figures A7–A9). More details on the activities are provided in the figure keys and in Annex Table A4.

Generally, activities outside the houses gradually decreased from 19:00 onwards. The proportion of people in the sitting rooms and bedrooms increased between 21:00 and 05:30. (Annex Figures A1–A3). In the sitting room, overall, almost 23% of householders slept under ITNs from 22:00 to 03:00 in Kenge. However, household members moved out of the sitting rooms to sleep in other rooms between 22:00 and 4:00 in Lodja or between 22:00 and 06:00 in Karawa (Annex Figures A4–A6).

Overall, almost 80% of householders used an ITN between 21:00 and 05:00 in Kenge, almost 70% between 21:00 and 04:00 in Lodja, and almost 68% between 21:00 and 04:00 in Karawa (Annex Figures A10–A12). Incorrect sleeping under ITNs (almost 8%) was observed only in Kenge, which happened between 20:00 p.m. and 23:00 and between 02:00 and 05:00. Mostly children, who slept normally under an ITN, were regularly observed to be partially outside the ITN overnight, putting this proportion of the population at a higher risk for malaria transmission.

Figures 23 and 24 show directly measured and adjusted biting risk of the major vectors *An. gambiae* s.l and *An. funestus* s.l. in Kenge. The peaks of the unadjusted biting rates were 0.3 bites per person per hour indoors and 0.4 bites outdoors (Figure 23). When considering the sleeping habits of people in Kenge, the majority of people were asleep indoors under ITNs during the peak mosquito host-seeking period between 20:00 and 04:00. Therefore, the adjusted indoor and outdoor mean biting risk in Kenge remained very low (around 0.2 bites per person per hour indoors and around 0.1 outdoors) (Figure 24). In Kenge, the biting risk for unprotected people indoors is very low (about 0.03 bites per hour per person) between 22:00 and 05:00 (Figure 24).

Figure 23: Directly Measured Biting Rate (*An. gambiae* s.l. and *An. funestus* s.l. Combined) and Human Behavior and Location in Kenge.

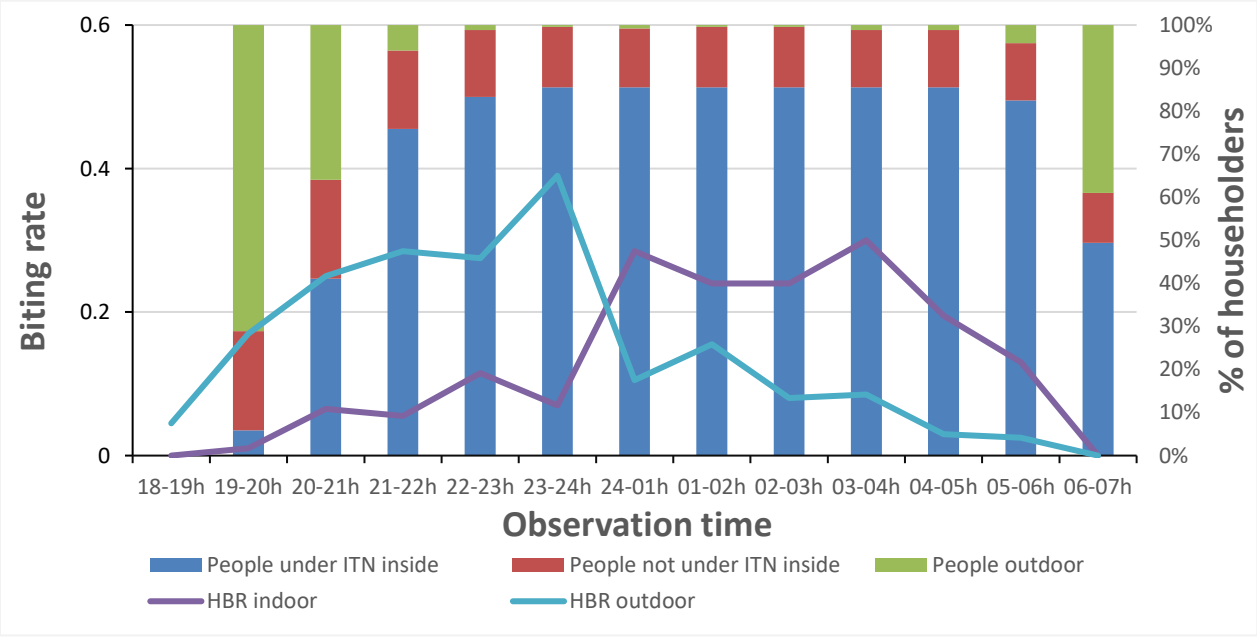
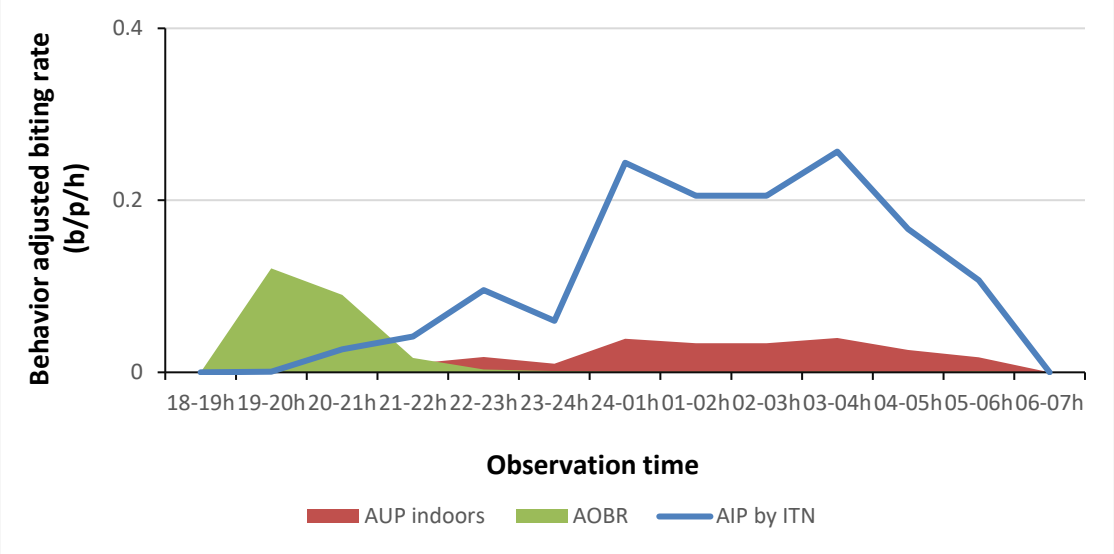


Figure 24: Behavior Adjusted Biting Rate Showing Malaria Vector Biting Risk (*An. gambiae* s.l. and *An. funestus* s.l. Combined) Indoor and Outdoor for Unprotected Individuals and Predicted Bites Prevented by ITNs in Kenge.



Key: AIP=adjusted indoor protected by insecticide-treated tent. AUP=adjusted unprotected indoors. AOBR=adjusted outdoor biting risk. b/p/h=bites per person per hour.

Figures 25 and 26 show directly and adjusted biting risk of the major vectors *An. gambiae* s.l and *An. funestus* s.l. in Lodja. The peaks of the unadjusted biting rates were 0.4 bites per person per hour indoors and 0.7 bites outdoors (Figure 25). When considering the sleeping habits of people in Lodja, the majority of people were asleep indoors under ITNs during the peak mosquito host-seeking period between 20:00 and 05:00. Therefore, the adjusted indoor and outdoor mean biting risk in Lodja remained low (around 0.3 bites per person per hour indoors and around 0.5 outdoors) (Figure 26). In Lodja, the biting risk for unprotected people indoors is high (around 0.1) between 22:00 and 05:00. (Figure 26).

Figure 25: Directly Measured Biting Rate (*An. gambiae* s.l. and *An. funestus* s.l. Combined) and Human Behavior and Location in Lodja.

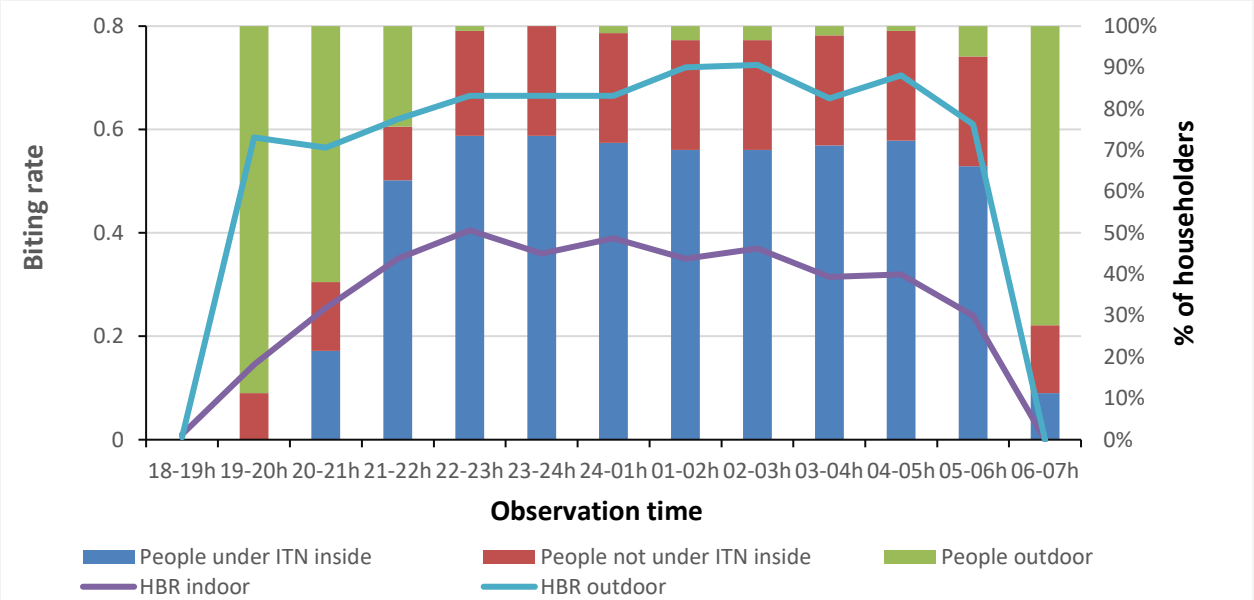
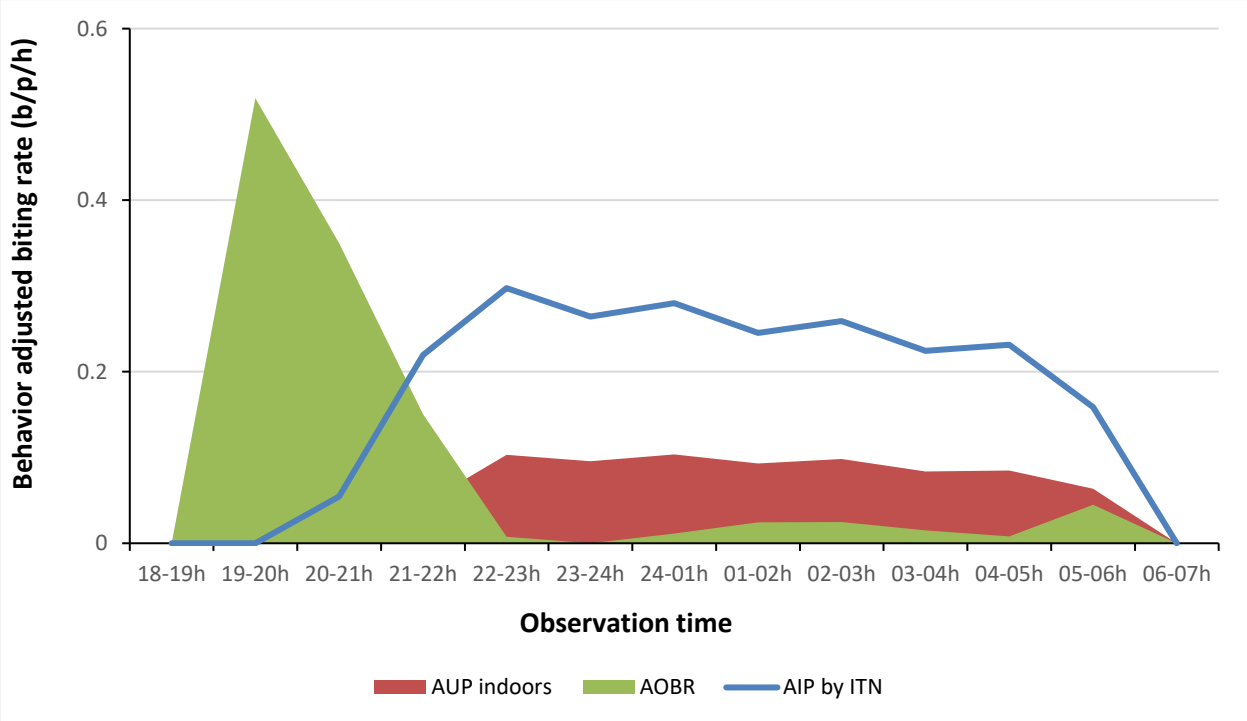


Figure 26: Behavior Adjusted Biting Rate Showing Malaria Vector Biting Risk (*An. gambiae* s.l. and *An. funestus* s.l. Combined) Indoor and Outdoor for Unprotected Individuals and Predicted Bites Prevented by ITNs in Lodja.



Key: AIP=adjusted indoor protected by insecticide-treated tent. AUP=adjusted unprotected indoors. AOBR=adjusted outdoor biting risk. b/p/h=bites per person per hour.

Figures 27 and 28 show directly and adjusted biting risk of the major vectors *An. gambiae* s.l., *An. funestus* s.l., and *An. moucheti* in Karawa. The peaks of the unadjusted biting rates were 0.9 bites per person per hour indoors and 0.7 bites outdoors (Figure 27). When considering the sleeping habits of people in Karawa, the majority (at least 65%) of people were asleep indoors under ITNs during the peak mosquito host-seeking period between 20:00 and 06:00. Therefore, the adjusted indoor and outdoor mean biting risk in Karawa remained low (around 0.6 bites per person per hour indoors and around 3.2 outdoors) (Figure 28). In Karawa, the biting risk for unprotected people indoors is high (around 0.2 bites per person per hour) between 22:00 and 05:00 (Figure 28).

Figure 27: Directly Measured Biting Rate (*An. gambiae* s.l., *An. funestus* s.l., and *An. moucheti* Combined) and Human Behavior and Location in Karawa.

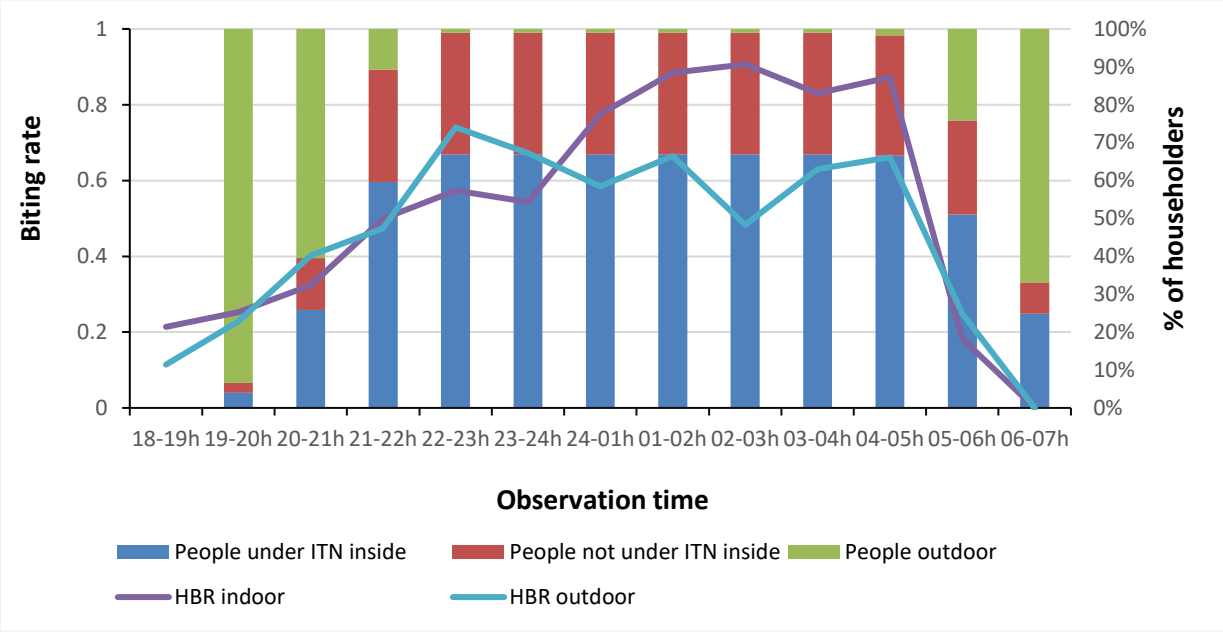
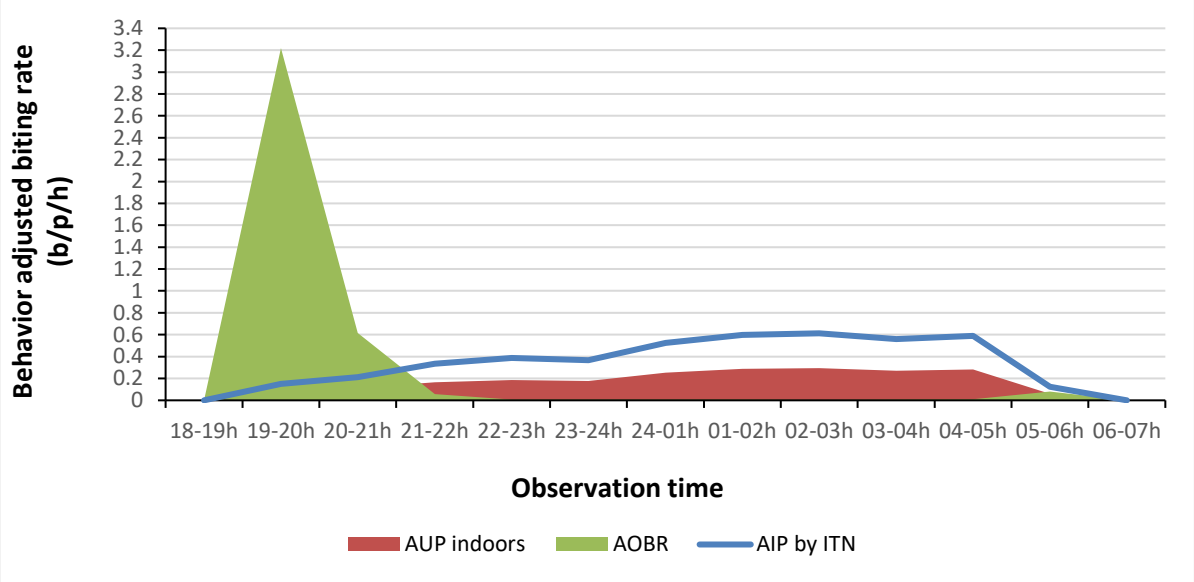


Figure 28: Behavior Adjusted Biting Rate Showing Malaria Vector Biting Risk (*An. gambiae* s.l., *An. funestus* s.l., and *An. moucheti* Combined) Indoor and Outdoor for Unprotected Individuals and Predicted Bites Prevented by ITNs in Karawa.



Key: AIP=adjusted indoor protected by insecticide-treated tent. AUP=adjusted unprotected indoors. AOBR=adjusted outdoor biting risk. b/p/h=bites per person per hour.

2.6 PLASMODIUM FALCIPARUM SPOROZOITE RATE

The number of *An. gambiae* s.l. and *An. funestus* s.l. analyzed for presence of sporozoites from each site is shown in Table 5. The total number of *An. gambiae* s.l. collected did not meet work plan targets of 1,600 for Kenge, 2,600 for Lodja, and 1,600 for Karawa due to insufficient mosquitoes collected. Specimens processed were 432, 1,974, and 1,593 for Kenge, Lodja, and Karawa, respectively. In addition, a subsample of 238 *An. moucheti* was processed for their potential implication in malaria transmission in Karawa.

Table 5: Number of *An. gambiae* s.l., *An. funestus* s.l., and *An. moucheti* Samples Collected by HLC and Tested for Presence of *P. falciparum* Sporozoites.

Sentinel site	Total *An. gambiae* s.l Collected by HLC	Total *An. gambiae* s.l. Tested (tested/expected)	% Mean Sporozoite Count (positive/tested)	Total. *An. funestus* s.l. Collected by HLC	Total *An. funestus* s.l. (tested/expected)	% Sporozoite Rate (positive/tested)	Total *An. moucheti* Collected by HLC	Total *An. moucheti* Tested	% Sporozoite Rate (positive/tested)
Kenge	439 **	432/1,600	13% (54/432)	23 *	23/160 (all collected)	4.3% (1/23)	0	0	0
Lodja	1,986 **	1,974/2,400	1.8% (36/1,974)	61 *	52/240	0% (0/52)	0	0	0
Karawa	1,927	1,593 /1,600	11% (169/1,593)	295	148/160 (20 per month)	7.4% (11/148)	238 ***	182	2.7% (5/182)
Total	**4,352**	**3,999**	**6.5%**	**379**	**223**	**5.4%**	**238**	**182**	**2.7%**

			(259/3,999)			(12/223)			(5/182)

*Insufficient *An. funestus* s.l. captured in Kenge and Lodja for testing.
**Insufficient *An. gambiae* s.l. captured in Kenge and Lodja for testing.
***Subsample of *An. moucheti* were processed.

The mean *An. gambiae* s.l. infection rate over 12 months in Lodja was 1.8% (95% confidence interval [CI], 1.3–2.5). It was 13% (95% CI, 9.7–16) and 11% (95% CI, 9.2–12.2) respectively in Kenge and Karawa over eight months (May-December). The mean *An. funestus* s.l. sporozoite rate over eight months was 4.3% (95% CI, 0.8–21) in Kenge and 7.4% (95% CI, 4.2–12.8) in Karawa. In Lodja and Inongo, no *An. funestus* tested positive (Table 5). Some *An. moucheti* tested positive (5/182) in Karawa, and the mean sporozoite rate over eight months was 2.7% (95% CI, 1.2–6.3). The monthly *An. gambiae* s.l., *An. funestus* s.l., and *An. moucheti* sporozoite rates from HLCs are presented in Figures 29–33.

Although it appeared that confidence intervals were quite large, as only 200 mosquitoes were tested per month, *An. gambiae* s.l. infections occurred mostly in the rainy season (mid-September to mid-May) in all sites (Figures 29, 30, and 31); *An. funestus* s.l. infections were observed in May to September and December in Karawa; and *An. moucheti* infections were observed in June–August in Karawa, where it rains all year, but less over June–July and December–February (Figures 32 and 33).

Figure 29: Monthly *P. falciparum* Sporozoite Rate of *An. gambiae* s.l. Collected by HLC in Kenge.

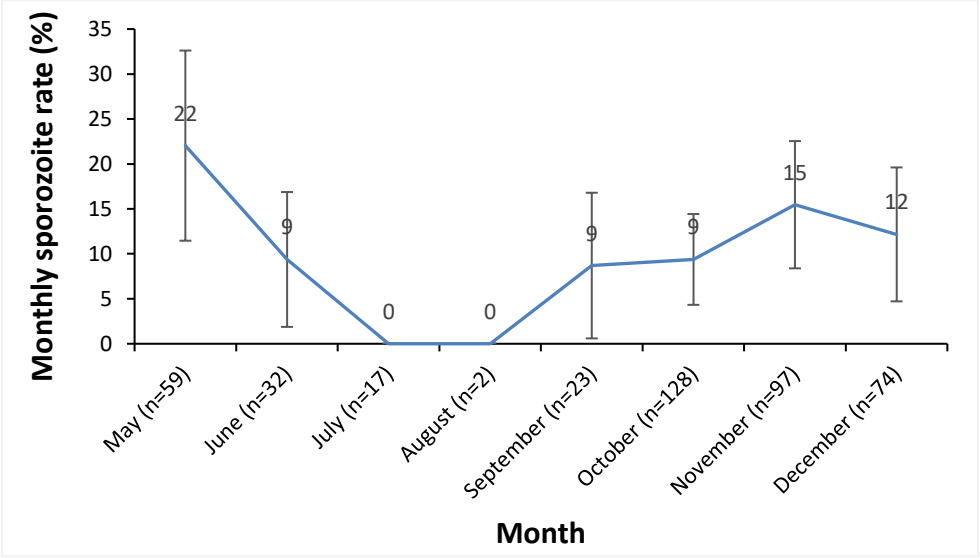


Figure 30: Monthly *P. falciparum* Sporozoite Rate of *An. gambiae* s.l. Collected by HLC in Lodja.

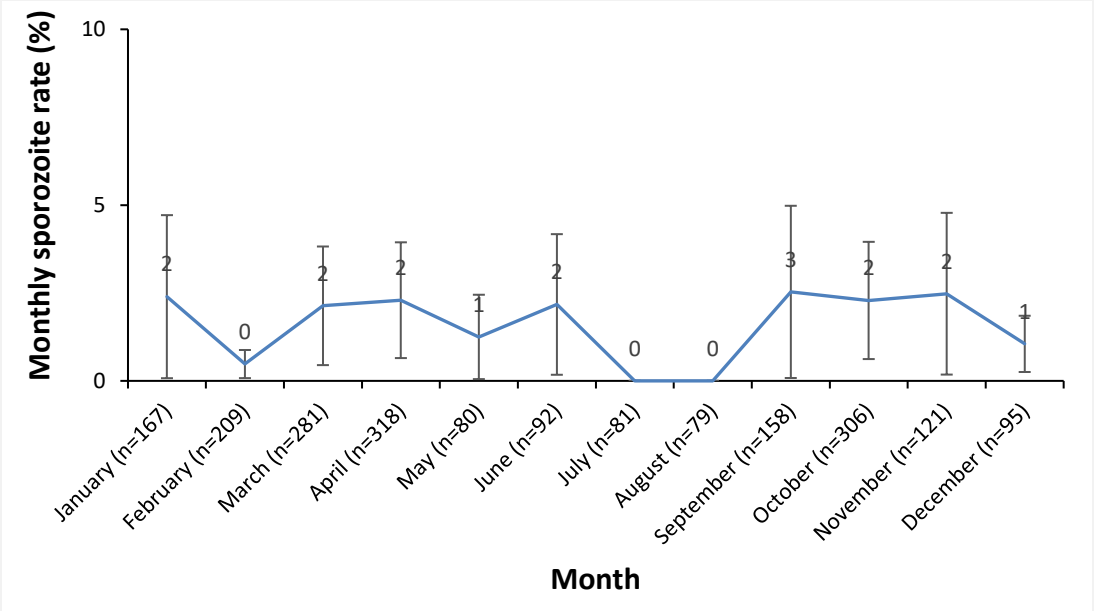


Figure 31: Monthly *P. falciparum* Sporozoite Rate of *An. gambiae* s.l. Collected by HLC in Karawa.

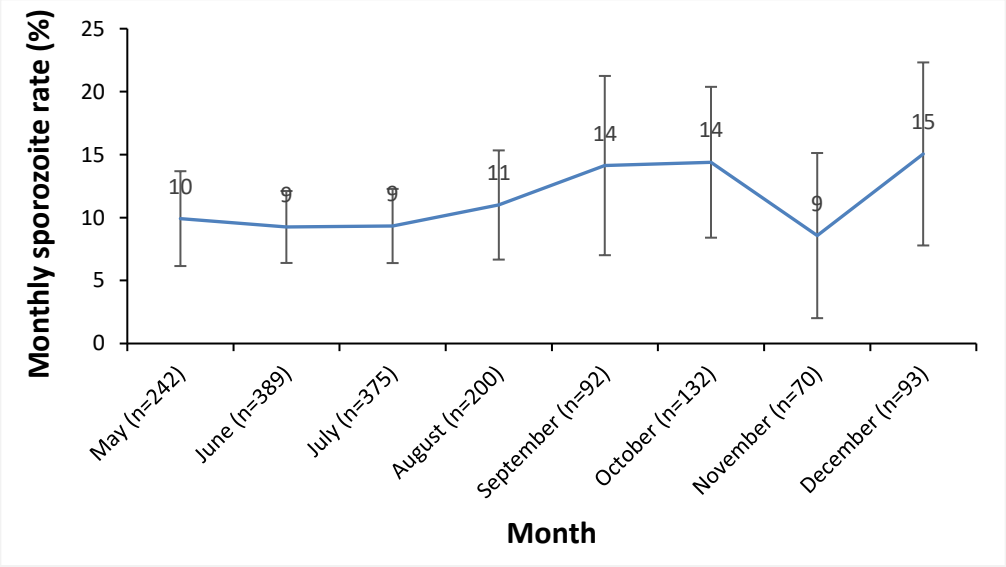


Figure 32: Monthly *P. falciparum* Sporozoite Rate of *An. funestus* s.l. Collected by HLC in Karawa.

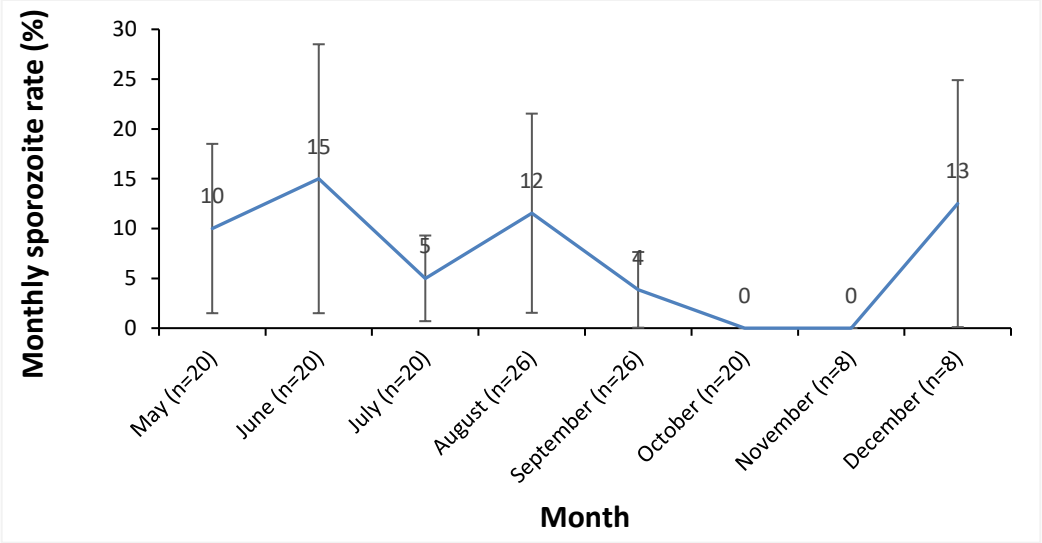
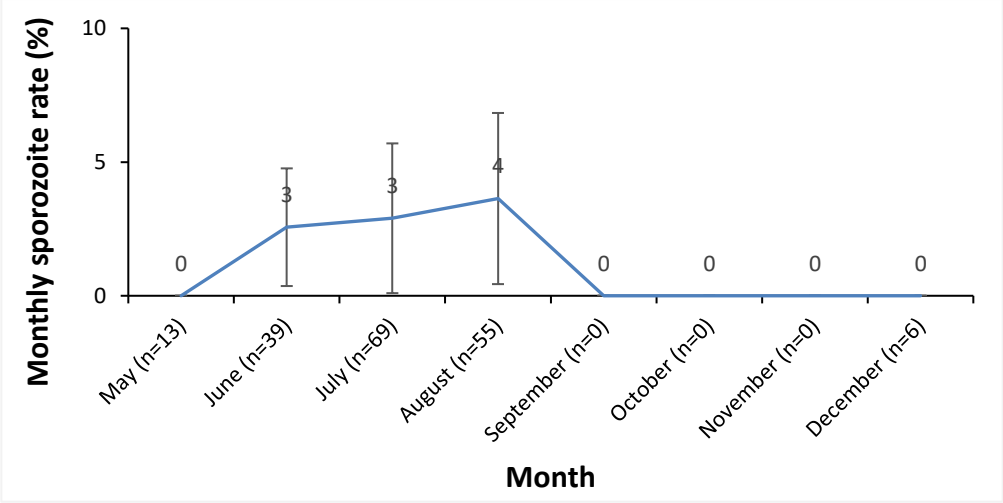


Figure 33: Monthly *P. falciparum* Sporozoite Rate of *An. moucheti* Collected by HLC in Karawa.



2.7 Entomological Inoculation Rate

Tables 6 to 9 summarize the combined indoor and outdoor monthly EIRs for *An. gambiae* s.l. in Kenge, Lodja, and Karawa and for *An. funestus* s.l. in Karawa for 2022. Summing the monthly EIRs gave an annual *An. gambiae* s.l. EIR of 105 infectious bites per person per year for Kenge (Table 6), 73.1 for Lodja (Table 7), and 387.3 for Karawa (Table 8). The annual *An. funestus* s.l. EIR was 43 infectious bites per person per year for Karawa (Table 9). Sporozoite-positive *An. funestus* s.l. were detected in Kenge in November, giving an estimated annual EIR of 1.5 infectious bites per person.

Sporozoite-positive *An. moucheti* were detected in Karawa only in June, July, and August, giving an estimated annual EIR of 9.2 infectious bites per person.

The combined annual EIR (*An. funestus* s.l. and *An. gambiae* s.l.) in Kenge was 106.5 infectious bites per person per year. The combined annual EIR (*An. gambiae* s.l., *An. funestus* s.l., and *An. moucheti*) in Karawa was 439.5 infectious bites per person per year.

Overall, these results demonstrate the high malaria transmission risk year-round in Kenge and Karawa, despite the use of pyrethroid ITNs. However, the malaria transmission risk was low in Lodja in 2022 compared to 2021,[12] when the annual EIR (*An. gambiae* s.l.) was 242.6 infectious bites per person per year.

[12] PMI VectorLink Project. *The Democratic Republic of Congo Annual Entomological Monitoring Report, January–December, 2021.* Rockville, MD: PMI VectorLink Project, Abt Associates, Inc.

Table 6: Monthly *An. gambiae* s.l. EIR in Kenge.

	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	2022 Total
Total *An. gambiae* s.l. (HLC) collected	60	32	17	2	23	132	97	76	**439**
HLC trap nights (indoors + outdoors)	16	16	16	16	16	16	16	16	**128**
HBR per night	3.8	2.0	1.1	0.1	1.4	8.3	6.1	4.8	**3.4 (mean)**
Total *An. gambiae* s.l. tested by ELISA	59	32	17	2	23	128	97	74	**432**
Sporozoite rate (%)	22.0	9.4	0	0	8.7	9.4	15.5	12.2	**9.6 (mean)**
EIR per night	0.8	0.2	0.0	0.0	0.1	0.8	0.9	0.6	**0.4 (mean)**
EIR per month*	25	6	0	0	3	25	27	19	**13.1 (mean)**
Kenge 8-month EIR May–Dec 2022=105 infectious bites per person (by *An. gambiae* s.l.)									

Key: EIR=entomological inoculation rate. ELISA=enzyme-linked immunosorbent assay. HBR=human biting rate. HLC=human landing catch.
*Nightly EIR is multiplied by number of nights in that month.

Table 7: Monthly *An. gambiae* s.l. EIR in Lodja.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	2022 Total
Total *An. gambiae* s.l. (HLC) collected	175	211	281	305	81	93	82	79	160	306	118	95	**1,986**
HLC trap nights (indoors + outdoors)	16	16	16	16	16	16	16	16	16	16	16	16	**192**
HBR per night	10.9	13.2	17.6	19.1	5.1	5.8	5.1	4.9	10.0	19.1	7.4	5.9	**10.3 (mean)**
Total *An. gambiae* s.l. tested by ELISA	167	209	281	305	80	92	81	79	158	306	121	95	**1,974**
Sporozoite rate (%)	2.4	0.5	2.1	2.2	1.3	2.2	0.0	0.0	2.5	2.3	2.5	1.1	**1.6 (mean)**
EIR per night	0.3	0.1	0.4	0.4	0.1	0.1	0.0	0.0	0.3	0.4	0.2	0.1	**0.2 (mean)**
EIR per month*	9.3	2.8	12.4	12	3.1	3	0.0	0.0	9	12.4	6	3.1	**6.1 (mean)**
Lodja 12-month EIR Jan–Dec 2022=73.1 infectious bites per person (by *An. gambiae* s.l.)													

Key: EIR=entomological inoculation rate. ELISA=enzyme-linked immunosorbent assay. HBR=human biting rate. HLC=human landing catch.
*****Nightly EIR is multiplied by number of nights in that month.

Table 8: Monthly *An. gambiae* s.l. EIR in Karawa.

	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	2022 Total
Total *An. gambiae* s.l. (HLC) collected	506	389	376	263	94	133	72	94	**1,927**
HLC trap nights (indoors + outdoors)	16	16	16	16	16	16	16	16	**128**
HBR per night	31.6	24.3	23.5	16.4	5.9	8.3	4.5	5.9	**15.1 (mean)**
Total *An. gambiae* s.l. tested by ELISA	242	389	375	200	92	132	70	93	**1,593**
Sporozoite rate (%)	9.9	9.3	9.3	11.0	14.4	14.4	8.6	15.1	**11.5 (mean)**
EIR per night	3.1	2.2	2.2	1.8	0.8	1.2	0.4	0.9	**1.6 (mean)**
EIR per month*	96.1	66	68.2	55.8	24	37.2	12	28	**48.4 (mean)**
Kenge 8-month EIR May–Dec 2022=387.3 infectious bites per person (by *An. gambiae* s.l.)									

Key: EIR=entomological inoculation rate. ELISA=enzyme-linked immunosorbent assay. HBR=human biting rate. HLC=human landing catch.
*Nightly EIR is multiplied by number of nights in that month.

Table 9: Monthly *An. funestus* s.l. EIR in Karawa.

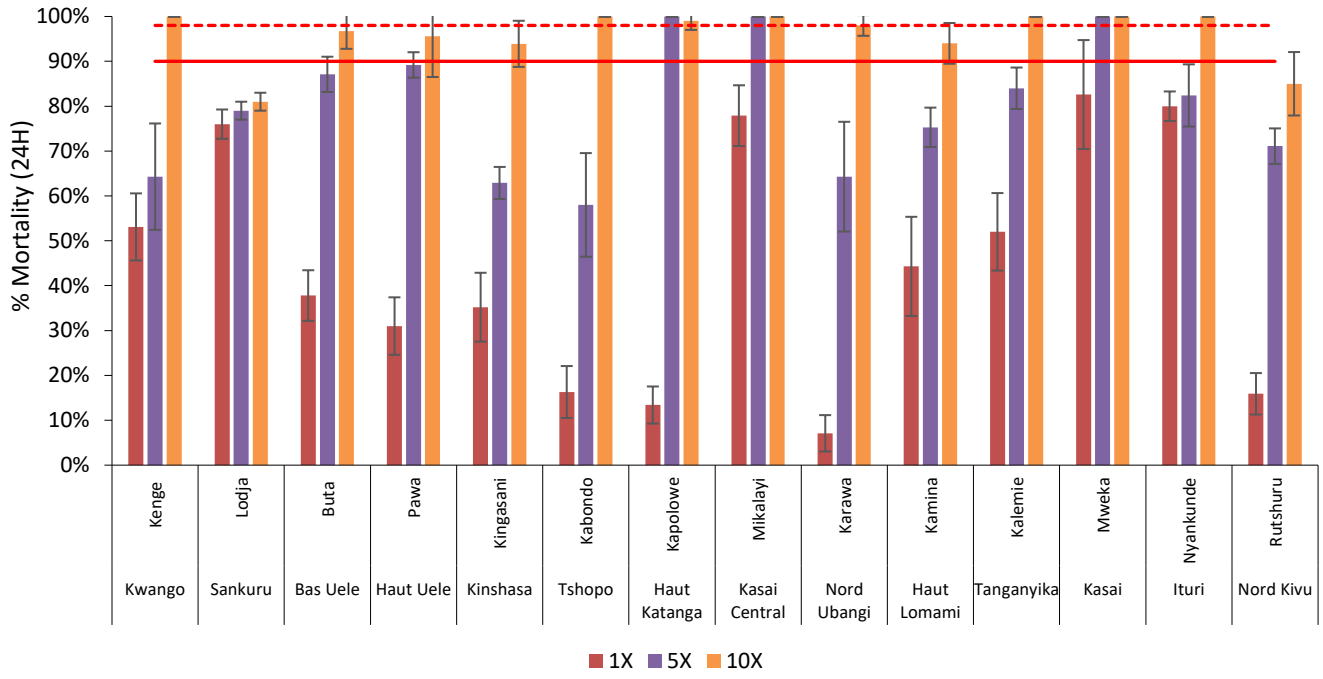
	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	2022 Total
Total *An. gambiae* s.l. (HLC) collected	53	35	49	56	27	25	42	8	**295**
HLC trap nights (indoors + outdoors)	16	16	16	16	16	16	16	16	**128**
HBR per night	3.3	2.2	3.1	3.5	1.7	1.6	2.6	0.5	**2.3 (mean)**
Total *An. gambiae* s.l. tested by ELISA	20	20	20	26	26	20	8	8	**148**
Sporozoite rate (%)	10	15	5	11.5	3.8	0	0	12.5	**7.2 (mean)**
EIR per night	0.3	0.3	0.2	0.4	0.1	0.0	0.0	0.1	**0.2 (mean)**
EIR per month*	9.3	9	6.2	12.4	3	0.0	0.0	3.1	**5.4 (mean)**
Kenge 8-month EIR May–Dec 2022=43 infectious bites per person (by *An. funestus* s.l.)									

Key: EIR=entomological inoculation rate. ELISA=enzyme-linked immunosorbent assay. HBR=human biting rate. HLC=human landing catch.
*Nightly EIR is multiplied by number of nights in that month.

2.8 Insecticide Susceptibility, PBO Synergist, and Pyrethroid Resistance Intensity

WHO insecticide susceptibility and resistance intensity tests were completed with *An. gambiae* s.l. populations that were collected as larvae in all 14 sites. Figure 34 shows the percentage mortality of *An. gambiae* s.l. in ×1, ×5, and ×10 doses permethrin. Resistance to permethrin (×1 dose) observed in all sites. Resistance intensity to permethrin was low (>98% mortality at ×5) in three sites (Kapolowe, Mikalayi and Mweka); moderate (<98% mortality at ×5 and >98% at ×10) in five sites (Kenge, Kabondo, Karawa, Kalemie, and Nyankunde); and high (<98% mortality at ×10 dose) in six sites (Lodja, Buta, Pawa, Kingasani, Kamina, and Rutshuru).

Figure 34: Percentage Mortality of *An. gambiae* s.l. after Exposure to Permethrin at ×1, ×5, and ×10 the Diagnostic Concentration in WHO Tube Tests in 14 Sites.



As shown in Figure 35 below, resistance to deltamethrin was recorded in all sites. The intensity of deltamethrin resistance was low in five sites (Lodja, Pawa, Mikalayi, Mweka, and Nyankunde), moderate in five sites (Kenge, Kingasani, Kapolowe, Karawa, and Kamina), and high in four sites (Buta, Kabondo, Kalemie, and Rutshuru).

Resistance to alpha-cypermethrin was also observed in all sites (Figure 36). The intensity of alpha-cypermethrin resistance was low in two sites (Mikalayi and Mweka), moderate in three sites (Kingasani, Karawa, and Nyankunde), and high in nine sites (Kenge, Lodja, Buta, Pawa, Kabondo, Kapolowe, Kamina, Kalemie, and Rutshuru).

Figure 35: Percentage Mortality of *An. gambiae* s.l. after Exposure to Deltamethrin at ×1, ×5, and ×10 the Diagnostic Concentration in WHO Tube Tests in 14 Sites.

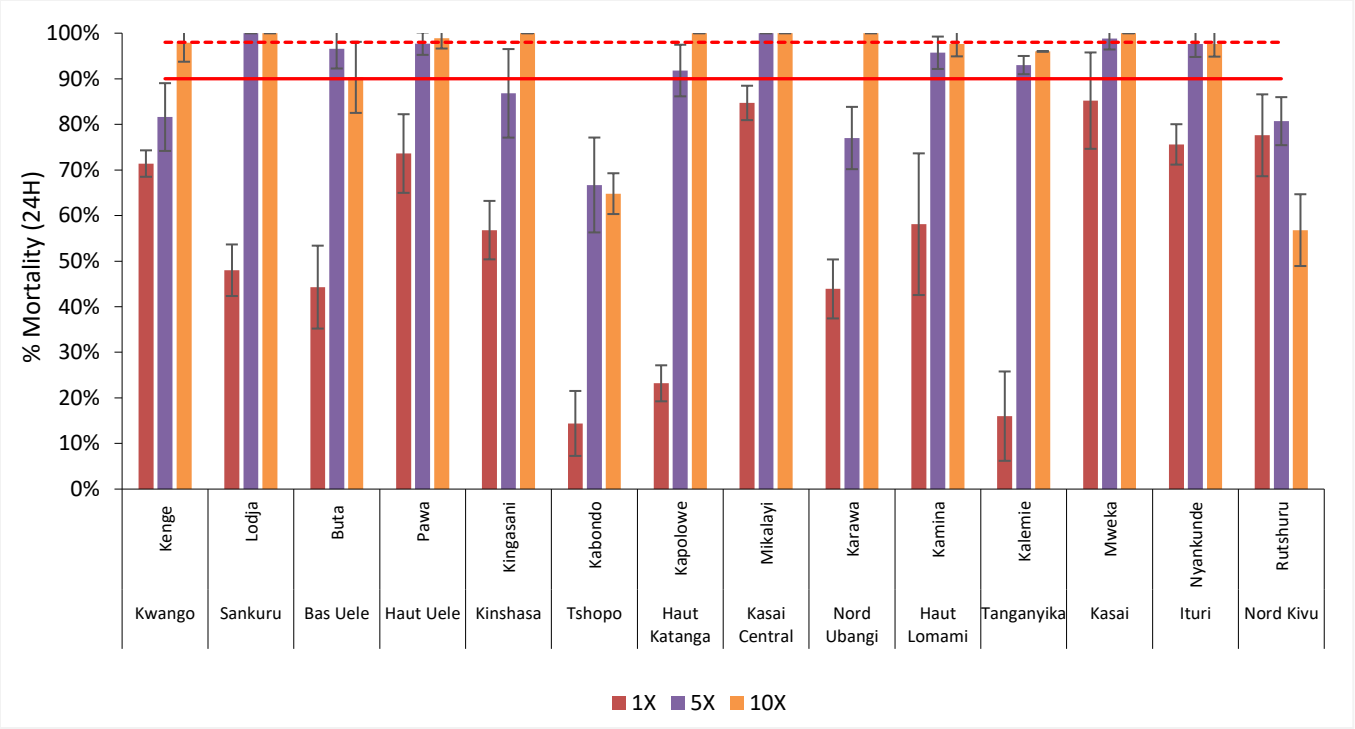
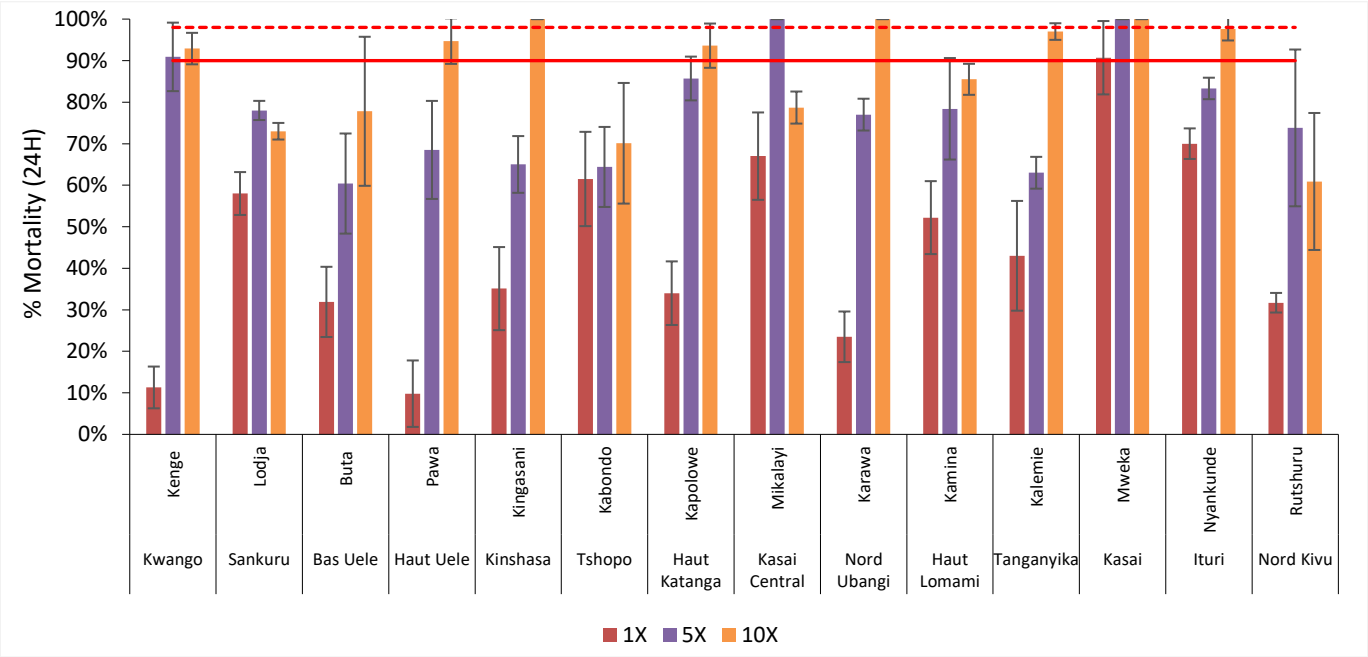
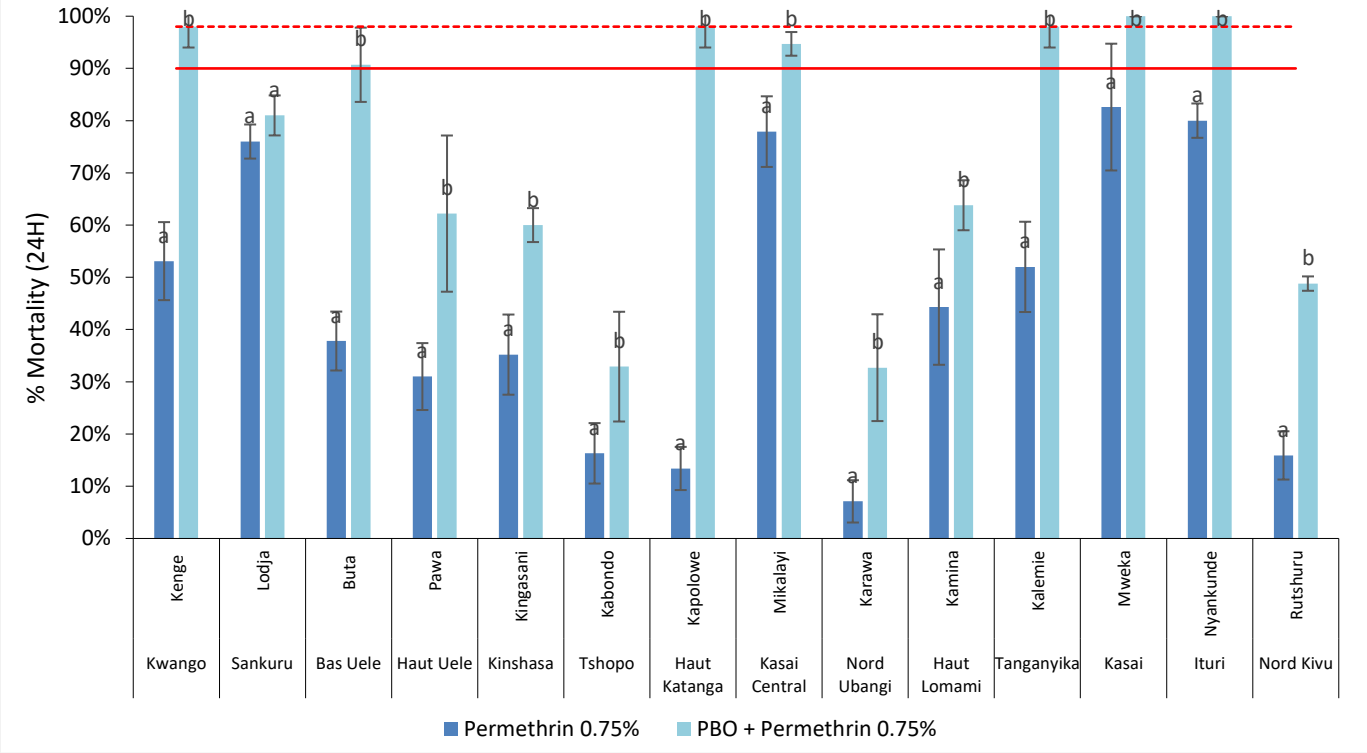


Figure 36: Percentage Mortality of *An. gambiae* s.l. after Exposure to Alpha-Cypermethrin at ×1, ×5, and ×10 the Diagnostic Concentration in WHO Tube Tests in 14 Sites.



Bioassays with permethrin (×1 dose) following pre-exposure to PBO 4% in WHO tube tests showed an increase in mortality ranging from 33% to 100% compared with permethrin alone in all sites (Figure 37). There was no significant increase in mortality in Lodja. Despite an increase in mortality after pre-exposure to PBO, mortality was still <90% in seven sites (Lodja, Pawa, Kingasani, Kabondo, Karawa, Kamina, and Rutshuru).

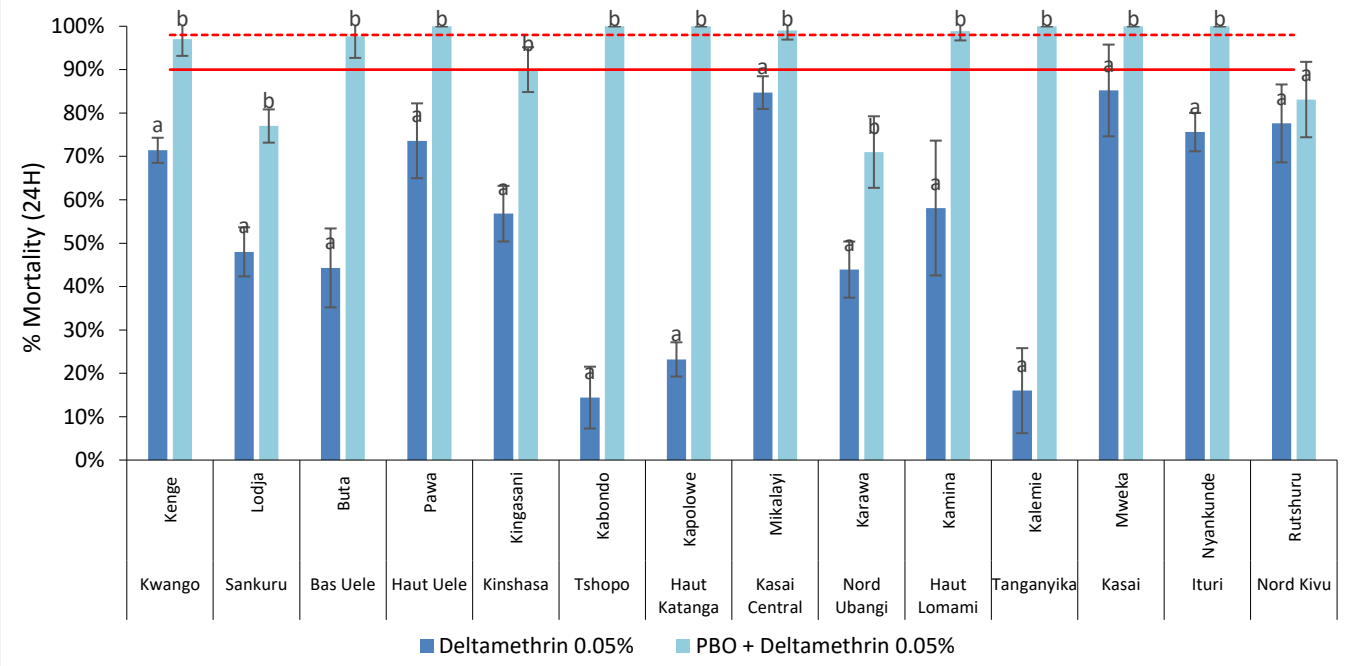
Figure 37: Percentage Mortality of *An. gambiae* s.l. after Pre-Exposure to PBO Followed by Permethrin at the Diagnostic Concentration in WHO Tube Tests in 14 Sites.



Note: Superscripts The letters at the top of the columns (a or b) indicate whether % mortality for permethrin is significantly different from % mortality for permethrin + PBO.
a, b=significant difference, $p<.05$.
a, a=no significant difference, $p>.05$.

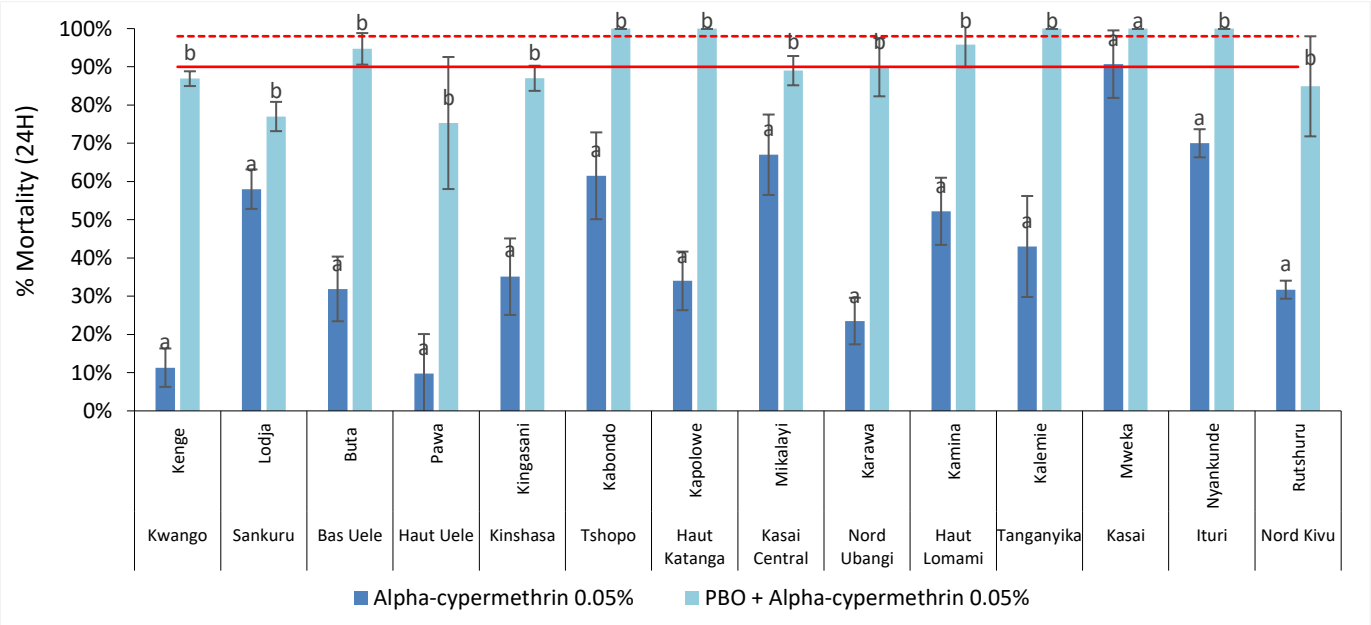
There was a significant increase in mortality with deltamethrin and alpha-cypermethrin (×1 dose) following pre-exposure to PBO 4% in WHO tube tests in 13 sites (Figures 38 and 39), except Rutshuru for PBO + deltamethrin and Mweka for PBO + alpha-cypermethrin. For deltamethrin, there were significant increases in mortality (≥90% mortality, $p < 0.05\%$) in Kenge, Buta, Pawa, Kingasani, Kabondo, Kapolowe, Mikalayi, Kamina, Kalemie, Mweka, and Nyankunde (Figure 38). Despite an increase in mortality after pre-exposure to PBO, mortality was still <90% in Lodja and Karawa for deltamethrin and in Kenge, Lodja, Pawa, Kingasani, Mikalayi, and Rutshuru for alpha-cypermethrin (Figures 38 and 39).

Figure 38: Percentage Mortality of *An. gambiae* s.l. after Pre-Exposure to PBO Followed by Deltamethrin at the Diagnostic Concentration in WHO Tube Tests in 14 Sites.



Note: Superscripts indicate whether % mortality for deltamethrin is significantly different to % mortality for deltamethrin + PBO.
a, b=significant difference, $p<.05$.
a, a=no significant difference, $p>.05$.

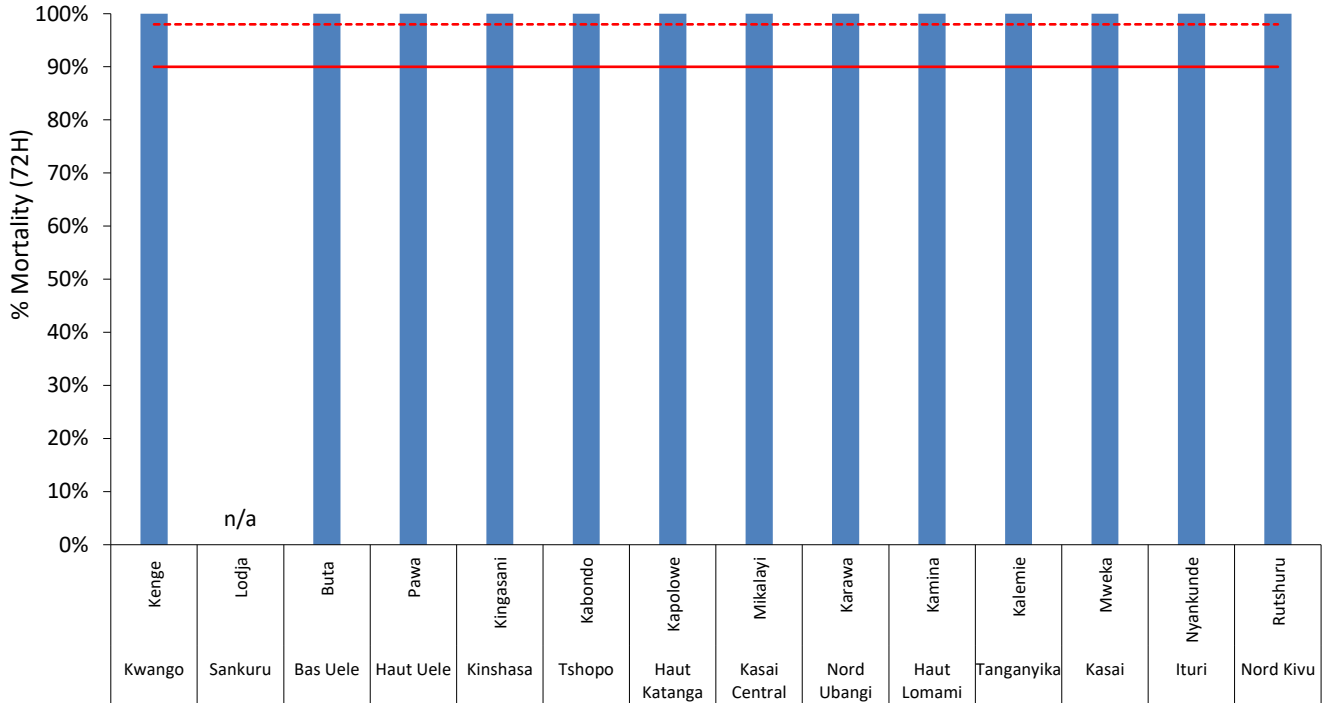
Figure 39: Percentage Mortality of *An. gambiae* s.l. after Pre-Exposure to PBO Followed by Alpha-cypermethrin at the Diagnostic Concentration in WHO Tube Tests in 14 Sites.



Note: Superscripts indicate whether % mortality for alpha-cypermethrin is significantly different from % mortality for alpha-cypermethrin + PBO.
a, b=significant difference, $p<.05$.
a, a=no significant difference, $p>.05$.

CDC bottle bioassays using 100µg/bottle as the diagnostic dose for chlorfenapyr produced 100% mortality after 72 hours exposure in all sites where testing was performed (Figure 40). Testing with susceptible *An. coluzzii* resulted in 100% mortality rates after 24 hours exposure. The field team was unable to conduct testing in Lodja due to lack of CDC bottles, which were broken during shipment of materials from Kinshasa.

Figure 40: Percentage Mortality of *An. gambiae* s.l. after Exposure to Chlorfenapyr 100ug/bottle in CDC Bottle Tests in 13 Sites.



2.9 Molecular Species Identification of the *An. gambiae* Species Complex

Out of 2,800 *An. gambiae* s.l. analyzed, 80% were identified as *An. gambiae* (*n*=2,242) and 13% as *An. coluzzii* (*n*=370). Seven percent (*n*=188) did not amplify. No hybrid (*An. gambiae* s.s./*An. coluzzii*) was detected (Figure 41).

Figure 41: Molecular Species Composition of *An. gambiae* across 14 Sites.

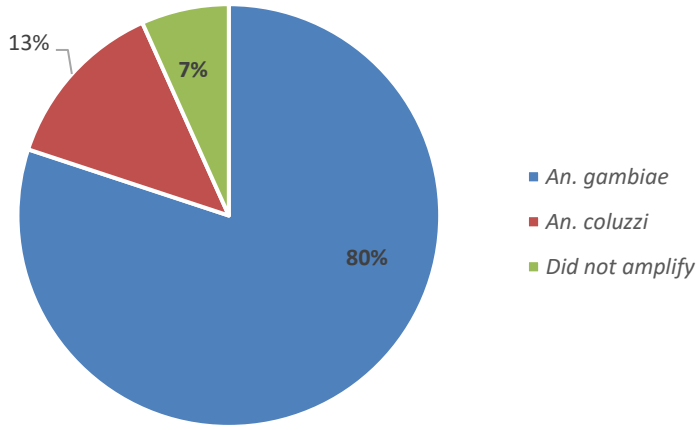


Table 10 shows the proportion of each species per site (collected by HLC), and Figure 42 below shows molecular species composition of those mosquitoes used for insecticides susceptibility tests. In all sites, there were sympatric populations of *An. gambiae* and *An. coluzzii*, with *An. gambiae* as the most common species collected in Kenge and Karawa (90% and 93% of amplified samples, respectively). Conversely to 2021, the proportion of *An. coluzzii* of 57% was superior to that of *An. gambiae* at 40%. Samples from monthly HLC collections in Kenge, Lodja, and Karawa showed no clear seasonal variation in species composition. The sympatric populations of *An. gambiae* and *An. coluzzii* were detected in five sites (Kabondo, Kenge, Kingasani, Lodja, and Mikalayi) from *An. gambiae* s.l. collected as larvae for insecticides susceptibility tests (Figure 42).

Table 10: Molecular Species Composition of *An. gambiae* s.l. Collected by HLC.

Site	*An. gambiae*	*An. coluzzii*	Hybrid	*An. arabiensis*	Did Not Amplify	2022 Total
Kenge	360 (90%)	4 (1%)	0	0	36 (9%)	**400**
Lodja	237 (40%)	343 (57%)	0	0	20 (3%)	**600**
Karawa	371 (93%)	4 (1%)	0	0	25 (6%)	**400**

Figure 42: Molecular Species Composition of *An. gambiae* s.l. Collected as Larvae for WHO Susceptibility Tests.

An. gambiae was the predominant species used in WHO susceptibility tests (collected as larvae) at all 14 sites. *An. coluzzii* were detected in Kabondo (1%), Kenge (6%), Kingasani (8%), Lodja (2%), and Mikalayi (2%) (Figure 42).

2.10 Frequency of the Voltage Gated Sodium Channel Mutation L1014F

Of the total 700 *An. gambiae* and *An. coluzzii* specimens analyzed, 583 (83.1%) were homozygous for Vgsc-L1014F, nine (1.3%) were wild type susceptible, and 61 (8.7%) heterozygous resistant. The remaining 47 (6.7%) did not amplify (Table 11). The high proportion of homozygous resistant samples is in support of previous results of *An. gambiae* s.l. collected from 11 sites in 2018 that were tested by the CDC and found to be 85% (883/1,039) homozygous resistant, compared to only 3% wild type susceptible or heterozygous resistant (28/1,039), with the remainder not amplified.[13]

Table 11: Frequency of the Vgsc L1014F Mutation (Formerly *kdr*-west) in *An. gambiae* s.s. and *An. coluzzii.*

Site	Number Tested	Homozygous Resistant	Heterozygous Resistant	Wild Type Susceptible	Did Not Amplify	Frequency of 1014F
Buta	50	37	8	4	1	0.84
Kabondo	50	43	2	1	4	0.96
Kalemie	50	40	3	0	7	0.97

[13] Wat'senga et al. (2020). Intensity of pyrethroid resistance in *Anopheles gambiae* before and after a mass distribution of insecticide-treated nets in Kinshasa and in 11 provinces of the Democratic Republic of Congo. *Malaria Journal, 19*, 169.

Site	Number Tested	Homozygous Resistant	Heterozygous Resistant	Wild Type Susceptible	Did Not Amplify	Frequency of 1014F
Kamina	50	44	1	0	5	0.99
Kapolowe	50	44	5	0	1	0.95
Karawa	50	42	4	0	4	0.96
Kenge	50	45	1	1	3	0.97
Kingasani	50	46	2	0	2	0.98
Lodja	50	39	1	1	9	0.96
Mikalyi	50	43	2	0	5	0.98
Pawa	50	39	7	2	2	0.89
Mweka	50	39	9	0	2	0.91
Nyankunde	50	40	9	0	1	0.91
Rutshuru	50	42	7	0	1	0.93

2.11 Frequency of the G119-ACE 1, N1575Y and L43F-CYP4J5 Mutations

Following London School Tropical Health and Medicine training on qPCR in the INRB laboratory in October 2022, the team successfully performed analysis on sub samples of 257 *An. gambiae* and *An. coluzzii* specimens from Karawa. Out of the total number tested for each mutation, the frequency of the resistance allele was 0% for G119-Ace 1, 12% for L43F-CYP4J5, and 7% for 1575Y. The remaining 157 (61%) did not amplify (Table 12). These results showed that metabolic markers occurred concurrently in addition with the knockdown resistance (Vgsc-1014F) in *An. gambiae* population from Karawa. The identification of new candidate markers in vector populations highlighted the evidence of ongoing selection pressure and warrant larger-scale monitoring in the DRC to inform vector control decisions by the NMCP. Beyond results, INRB has the facility to perform qPCR and the project should continue capacity building for the resolution of remaining troubleshooting and technical issues such as the non-amplify samples.

Table 12: Frequency of the G119-ACE 1, L43F-CYP4J5, and N1575Y Mutations in *An. gambiae* s.s. and *An. coluzzii.*

Site	Number Tested	Homozygous Resistant	Heterozygous Resistant	Wild Type Susceptible	Did Not Amplify	Frequency of Resistance Allele
G119-ACE 1	93	0	0	24	69	0.00
L43F-CYP4J5	93	1	4	20	68	0.12
N1575Y	71	0	7	44	20	0.07

3. CAPACITY BUILDING

In 2022, VectorLink continued to strengthen capacity at the central and provincial levels. Dr. Rodrigue Agossa, the in-country entomologist, supported project activities by assisting with data entry, cleaning and analysis in VectorLink Collect, updating and translating SOPs for susceptibility and intensity tests, WHO cone bioassays, Prokopack aspiration, molecular species identification, and knockdown resistance (*kdr*) mutation detection into French. Dr. Agossa worked with INRB and the University of Kinshasa to implement activities. He also provided technical assistance to NMCP to support Vector Control Working Group (Groupe de Travail Lutte Anti-Vectorielle) activities and the dissemination of results in meetings. Other efforts are summarized below.

Training	• VectorLink and INRB participated to the regional entomology M&E DHIS2 training in Yaounde, Cameroon, on June 11–17, 2022, on the functionality of VectorLink Collect. All 2022 entomological data (HLC, PSC, and susceptibility data) were successfully entered and cleaned in VectorLink Collect. • NMCP staff Mr. Narcisse Basosila participated in the VectorLink regional entomology training on August 21–29, 2022. • VectorLink and INRB trained the technicians assigned to the new sites (Kamina and Kenge). • The VectorLink Chief of Party facilitated sessions on resistance and bio-ecology of vector modules for Water, Hygiene, and Sanitation supervisors from Ituri, Kasai, and Nord Kivu provinces. The one-month training was held August 22 to September 21, 2022, at Public Health School of Kinshasa (PHSK). • A five-day laboratory practical training was held October 17–21, 2022, at INRB, Kinshasa, on molecular insecticide resistance monitoring techniques for *Anopheles* malaria vector species. The training was led by Dr. Louisa Messenger and Ms. Bethanie Pelloquin, both of London School of Hygiene and Tropical Medicine (LSHTM), with significant support from Dr. Agossa.
Meetings	• VectorLink DRC financially and technically supported NMCP to plan and hold two national-level meetings of the Vector Control Working Group (Groupe de Travail Lutte Anti-Vectorielle) at Carita Congo Hotel on May 27, 2022, and November 15, 2022. This multi-sectoral group is composed of participants from NMCP; the ministries of Health, Agriculture, Environment and Hygiene, and Plant Protection; the directorates of Disease and Pharmacy; and universities, research institutions, civil society organizations, mining companies, and technical partners.

4. DISCUSSION

The climate of Karawa province in northern DRC is particularly favorable for the proliferation of malaria vectors, with year-round high temperatures and no dry season. Several vectors including *An. gambiae* s.l., *An. funestus* s.l., and *An. moucheti* are involved in malaria transmission in Karawa. The combined annual EIRs of 439.5 infectious bites per person per year in Karawa and 106.5 infectious bites per person per year in Kenge highlight the extremely high year-round malaria transmission risk in these provinces. However, in Lodja the annual EIR (*An. gambiae* s.l.) was reduced to 73.1 infectious bites per person per year compared to 218 and 242.6 infectious bites per person per year in 2020 and 2021, respectively.

The observed decrease of malaria transmission risk in Lodja may be justified by the reported rapid urbanization of the sampling village. The high rates of ITN use by householders and the good sleeping behaviors observed in all sites (>60%) reduced the exposure risks to infectious bites. However, NMCP should increase communications on ITNs care and prevent their early damaging, which should exacerbate malaria risk transmission, particularly in Karawa. In high transmission areas such as Karawa, multiple interventions (e.g., indoor residual spraying, bait-trap), not just ITNs, can help reduce malaria transmission.

Insecticide susceptibility tests showed a similar pattern as previous years with widespread pyrethroid resistance. In all sites, except Lodja, *An. gambiae* s.l. were resistant to permethrin, deltamethrin, and alpha-cypermethrin. Resistance intensity varied by site and by insecticide but was usually moderate or high. In regard to the high proportion of *An. coluzzii* observed in Lodja and its presence observed in southern DRC, future investigation may be focused on the distribution, resistance pattern, and behavior of this species in the country.

Despite uncertainty regarding the impact of pyrethroid resistance, WHO states that, "When resistance is confirmed at the 5× and especially at the 10× concentrations, operational failure is likely[14]." Throughout the DRC monitoring sites, resistance to the three most common pyrethroids used on ITNs was observed at the 5× and 10× concentrations, therefore making it highly likely that pyrethroid ITNs are no longer providing optimal protection against malaria (though this needs to be confirmed through epidemiological data).

The high intensity of pyrethroid resistance reinforces the NMCP's decision to prioritize distribution of new types of nets, as funding allows. In all 14 sites, bioassays with pyrethroids following pre-exposure to PBO showed an increase in mortality over pyrethroid alone, though mortality was still <90% in seven sites (Lodja, Pawa, Kingasani, Kabondo, Karawa, Kamina, and Rutshuru); in two sites (Lodja and Karawa) for deltamethrin; and in six sites (Kenge, Lodja, Pawa, Kingasani, Mikalayi, and Rutshuru) for alpha-cypermethrin.

The general increase in mortality when a PBO synergist was used indicates that ITNs containing PBO may provide greater control compared to pyrethroid only ITNs, although full susceptibility was not restored. A better option may be Dual AI ITNs, as susceptibility to chlorfenapyr was recorded in all 13 sites where bioassays were performed. The results of PBO and Interceptor G2 ITN durability studies being conducted by VectorLink over three years in Sud Ubangi and Tanganyika (PBO) and in Nord Ubangi (Interceptor G2) provinces will provide additional data to inform the NMCP and stakeholders.

[14] WHO 2016. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, second edition.

To continue decentralized insecticide resistance testing, refresher training should be organized every two years in Kinshasa to bring all field teams together for standardized training. Training would also be needed if additional new sites are added and new bioassay methods were to be implemented, such as pyriproxyfen and chlorfenapyr susceptibility testing using CDC bottle bioassays. Trainees from unsecured (Ituri and Nord Kivu) and inaccessible (Kasai) provinces successfully conducted insecticide testing in 2022, after a one-month training in Kinshasa.

5. ANNEX

Table A1: Monthly HBR of Malaria Vectors Collected Indoors and Outdoors by HLC in Kenge.

Species	Location	Variables	May	Jun	July	Aug	Sep	Oct	Nov	Dec	2022 Overall
An. gambiae s.l.	Indoor	Total collected	23	18	10	1	14	53	52	41	212
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	**3**	**2**	**1**	**0**	**2**	**7**	**7**	**5**	**3**
	Outdoor	Total collected	37	14	7	1	9	79	45	35	227
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	5	2	1	0	1	10	6	4	4
An. funestus s.l.	Indoor	Total collected	3	1	0	1	0	0	0	3	8
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Outdoor	Total collected	**3**	**2**	**2**	**1**	**2**	**0**	**2**	**3**	15
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	0	0	0	0	0	0	0	0	0
An. paludis	Indoor	Total collected	0	3	0	1	1	0	0	0	5
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	0	0	0	0	0	0	0	0	0
	Outdoor	Total collected	**0**	**3**	**0**	**2**	**1**	**0**	**1**	**1**	8
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Note: HBR=human biting rate. *nbr=number.*

Table A2: Monthly HBR of Malaria Vectors Collected Indoors and Outdoors by HLC in Lodja.

Species	Location	Variables	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	2022 Overall
An. gambiae s.l.	Indoor	Total collected	55	64	84	87	27	25	28	18	65	118	45	35	651
		***nbr* person-nights**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**96**
		HBR/night	**6.9**	**8.0**	**10.5**	**10.9**	**3.4**	**3.1**	**3.5**	**2.3**	**8.1**	**14.8**	**5.6**	**4.4**	**7**
	Outdoor	Total collected	120	147	197	218	54	68	54	61	95	188	73	60	1335
		***nbr* person-nights**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**96**
		HBR/night	15.0	18.4	24.6	27.3	6.8	8.5	6.8	7.6	11.9	23.5	9.1	7.5	14
An. funestus s.l.	Indoor	Total collected	**1**	**5**	**8**	**4**	**0**	**1**	**1**	**1**	**0**	**1**	**1**	**0**	23
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**96**
		HBR/night	0.1	0.6	1.0	0.5	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.0	**0**
	Outdoor	Total collected	4	7	5	6	0	0	7	7	0	2	0	0	38
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**96**
		HBR/night	**0.5**	**0.9**	**0.6**	**0.8**	**0.0**	**0.0**	**0.9**	**0.9**	**0.0**	**0.3**	**0.0**	**0.0**	**0**
An. paludis	Indoor	Total collected	13	13	12	12	1	5	22	50	14	4	3	17	166
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**96**
		HBR/night	**1.6**	**1.6**	**1.5**	**1.5**	**0.1**	**0.6**	**2.8**	**6.3**	**1.8**	**0.5**	**0.4**	**2.1**	**2**
	Outdoor	Total collected	47	94	28	36	9	17	30	27	35	48	38	42	451
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**96**
		HBR/night	**5.9**	**11.8**	**3.5**	**4.5**	**1.1**	**2.1**	**3.8**	**3.4**	**4.4**	**6.0**	**4.8**	**5.3**	**5**

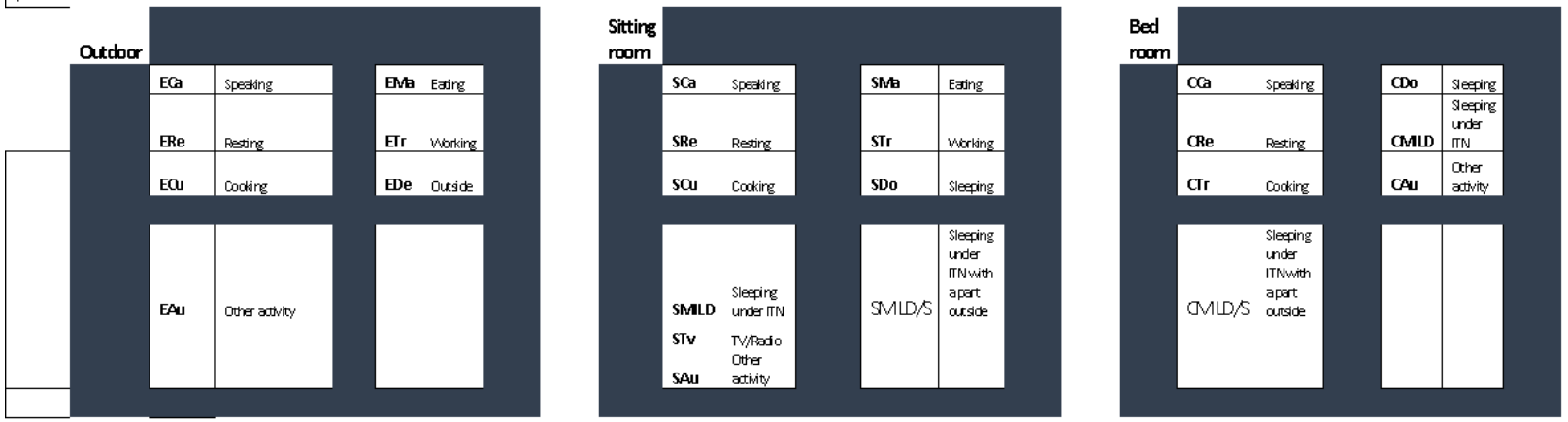
Note: HBR=human biting rate. *nbr=number.*

Table A3: Monthly HBR of Malaria Vectors Collected Indoors and Outdoors by HLC in Karawa.

Species	Location	Variables	May	Jun	July	Aug	Sep	Oct	Nov	Dec	2022 Overall
An. gambiae s.l.	Indoor	Total collected	**350**	**220**	**155**	**141**	**39**	**67**	**42**	**50**	1064
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	**44**	**28**	**19**	**18**	**5**	**8**	**5**	**6**	**17**
	Outdoor	Total collected	**156**	**169**	**221**	**122**	**55**	**66**	**30**	**44**	863
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	**20**	**21**	**28**	**15**	**7**	**8**	**4**	**6**	**13**
An. funestus s.l.	Indoor	Total collected	**33**	**24**	**16**	**21**	**11**	**9**	**18**	**5**	137
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	**4**	**3**	**2**	**3**	**1**	**1**	**2**	**1**	**2**
	Outdoor	Total collected	**20**	**11**	**33**	**35**	**16**	**16**	**24**	**3**	158
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	**3**	**1**	**4**	**4**	**2**	**2**	**3**	**0**	**2**
An. paludis	Indoor	Total collected	**12**	**2**	**12**	**25**	**13**	**24**	**34**	**26**	148
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	**2**	**0**	**2**	**3**	**2**	**3**	**4**	**3**	**2**
	Outdoor	Total collected	**3**	**3**	**13**	**14**	**5**	**16**	**31**	**25**	110
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	**0**	**0**	**2**	**2**	**1**	**2**	**4**	**3**	**2**
An. moucheti	Indoor	Total collected	**4**	**19**	**29**	**30**	**1**	**23**	**0**	**5**	111
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	**1**	**2**	**4**	**4**	**0**	**3**	**0**	**1**	**2**
	Outdoor	Total collected	**9**	**23**	**40**	**32**	**0**	**22**	**0**	**1**	127
		nbr person-nights	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	64
		HBR/night	**1**	**3**	**5**	**4**	**0**	**3**	**0**	**0**	**2**

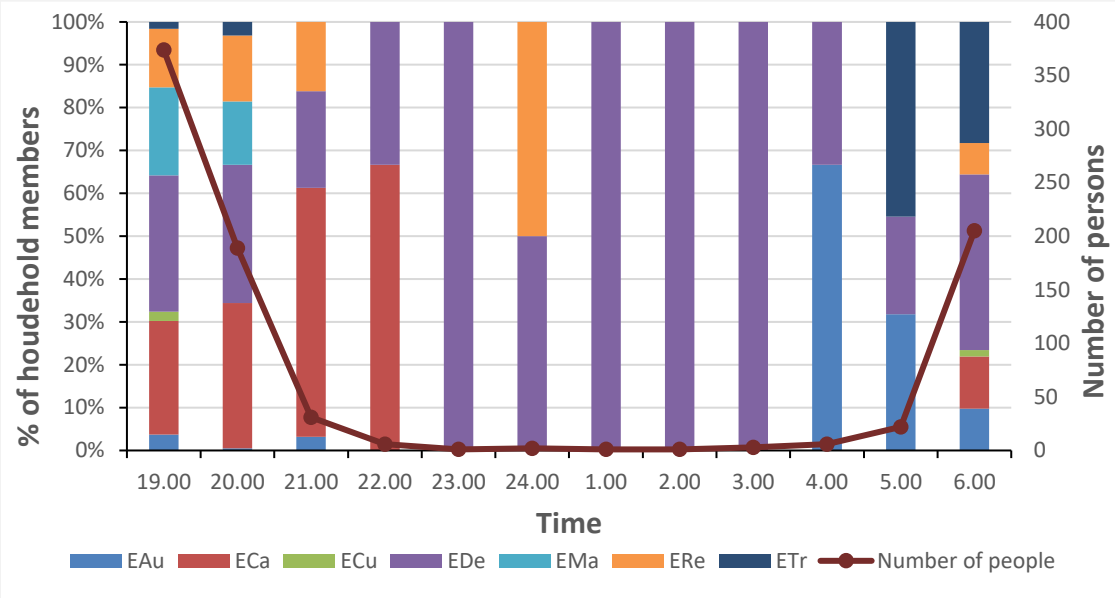
Table A4: Observation Worksheet.

Village :



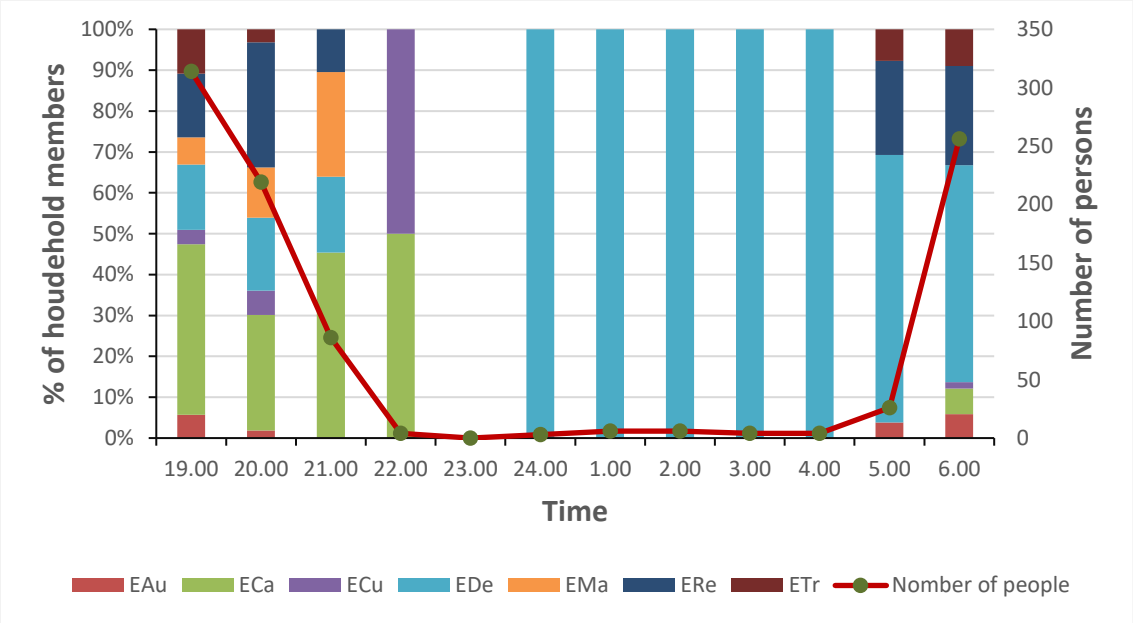
Date J 1			Time																			
N°	Householders	Sex	Age	19:00	19:30	20:00	20:30	21:00	21:30	22:00	22:30	23:00	24:00	01:00	02:00	03:00	04:30	05:00	05:30	06:00	06:30	07:00	ITN type	ITN code
1																								
2 3 4																								
5																								
6																								
7																								
8																								

Figure A1: Mean Outdoor Activities Performed by Household Members at Night in Kenge.



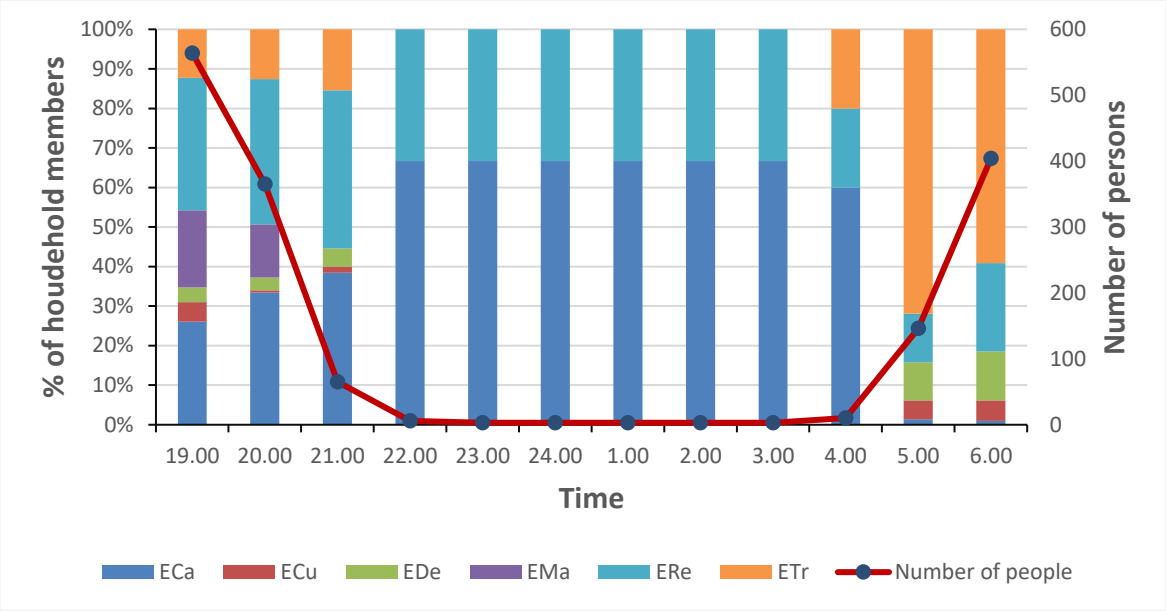
Key:
Location: E=outdoors.
Activity: Ca=speaking. Cu=cooking. Re=resting. Ma=eating. Tr=working. De=outside. Au=other.

Figure A2: Mean Outdoor Activities Performed by Household Members at Night in Lodja.



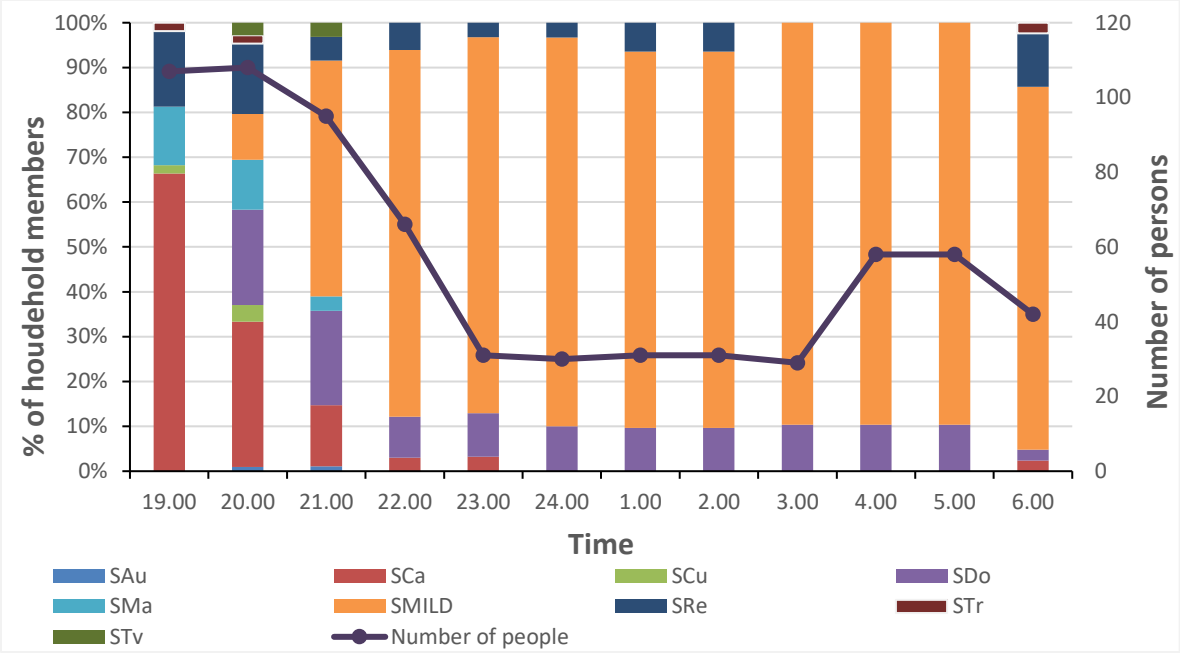
Key:
Location: E=outdoors.
Activity: Ca=speaking. Cu=cooking. Re=resting. Ma=eating. Tr=working. De=outside. Au=other.

Figure A3: Mean Outdoor Activities Performed by Household Members at Night in Karawa.



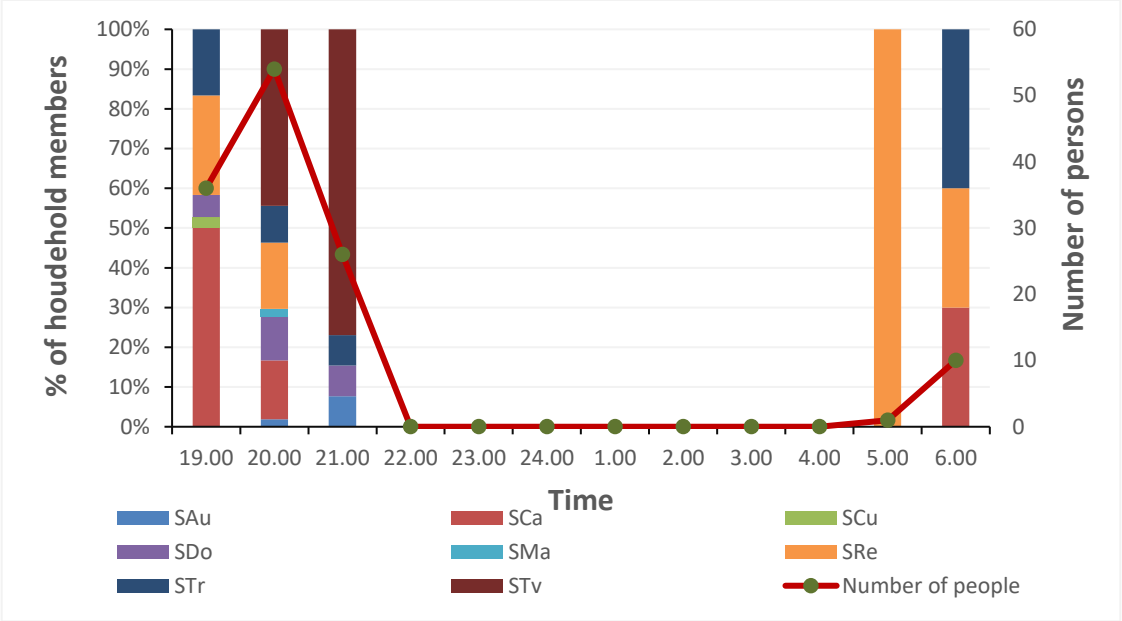
Key:
Location: E=outdoors.
Activity: Ca=speaking. Cu=cooking. Re=resting. Ma=eating. Tr=working. De=outside. Au=other.

Figure A4: Mean Sitting Room Activities Performed by Household Members at Night in Kenge.



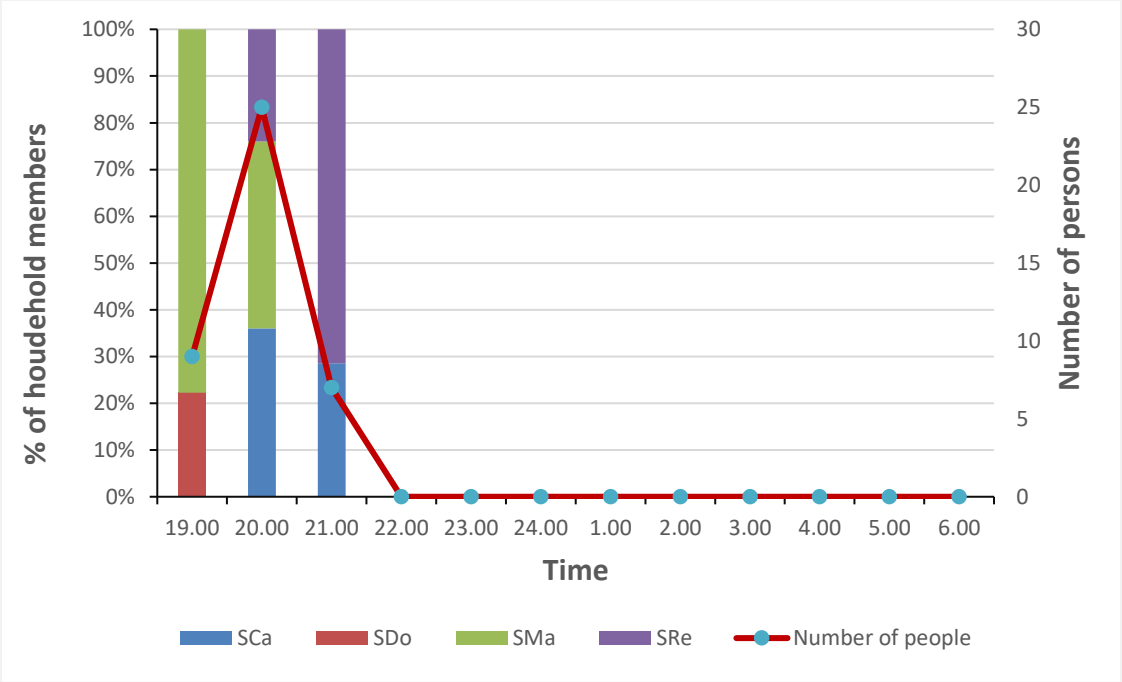
Key:
Location: S=sitting room.
Activity: Ca=speaking. Cu=cooking. Re=resting. Ma=eating. Tr=working. TV=watching TV/listening to radio. Do=sleeping. MILD=sleeping under a net. MILD/S=sleeping under a net with a body part out the net. Au=other.

Figure A5: Mean Sitting Room Activities Performed by Household Members at Night in Lodja.



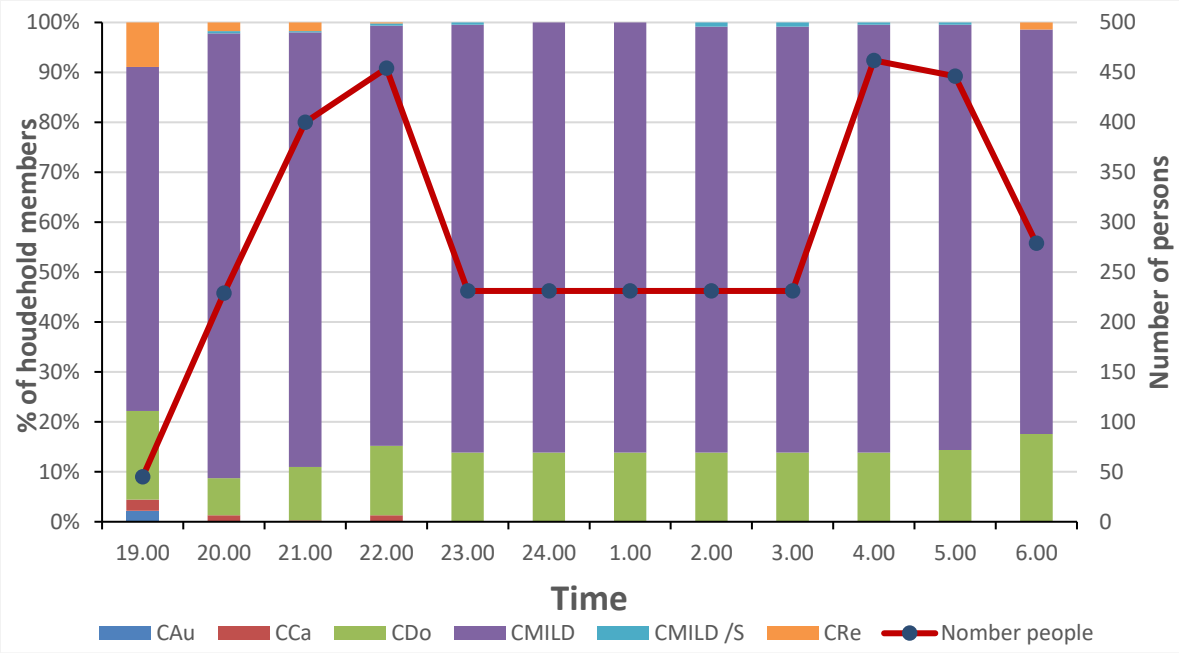
Key:
Location: S=sitting room.
Activity: Ca=speaking. Cu=cooking. Re=resting. Ma=eating. Tr=working. TV=watching TV/listening to radio. Do=sleeping. MILD=sleeping under a net. MILD/S=sleeping under a net with a body part out the net. Au=other.

Figure A6: Mean Sitting Room Activities Performed by Household Members at Night in Karawa.



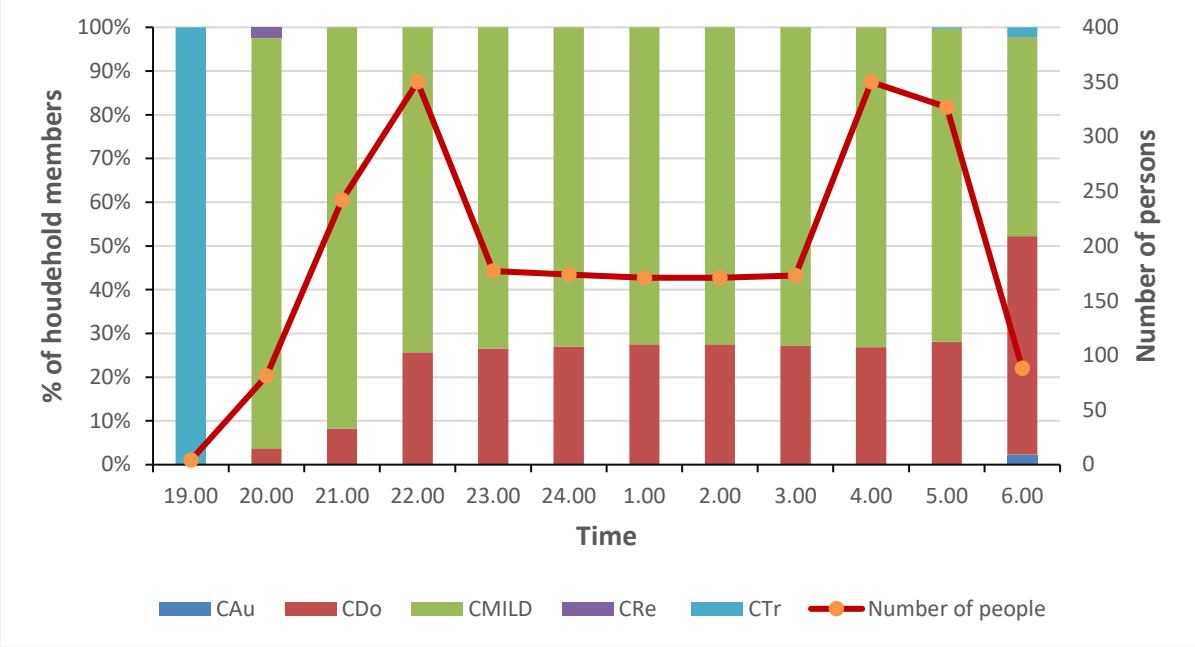
Key:
Location: S=sitting room.
Activity: Ca=speaking. Cu=cooking. Re=resting. Ma=eating. Tr=working. TV=watching TV/listening to radio. Do=sleeping. MILD=sleeping under a net. MILD/S=sleeping under a net with a body part out the net. Au=other.

Figure A7: Mean Bedroom Activities Performed by Household Members at Night in Kenge.



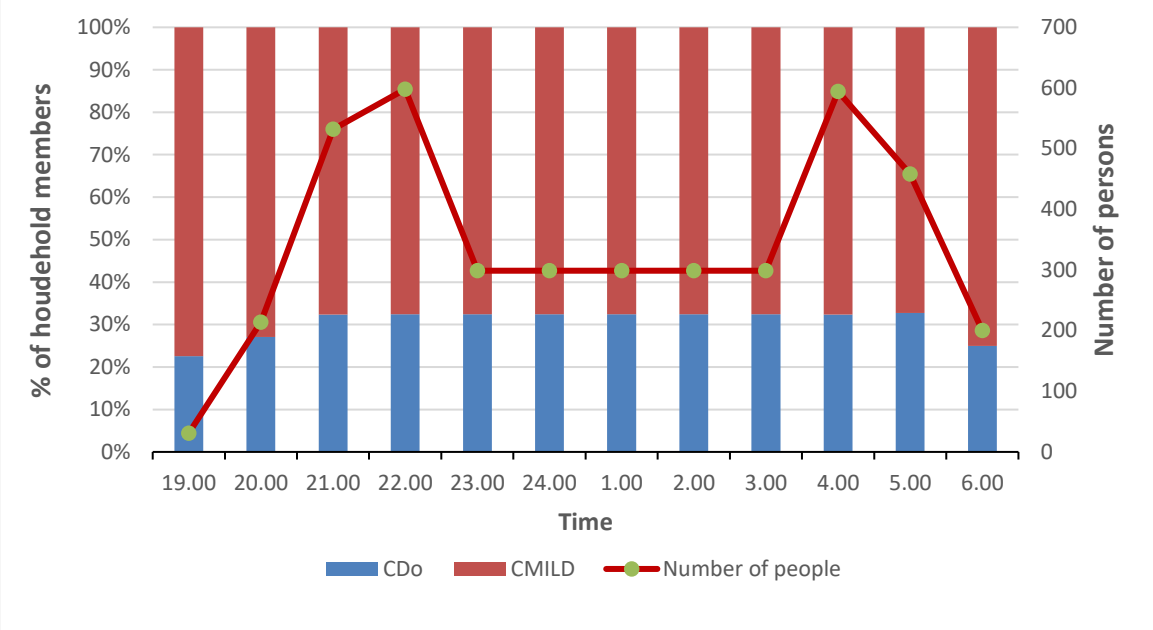
Key:
Location: C=bedroom.
Activity: Ca=speaking. Re=resting. Tr=working. Do=sleeping. MILD=sleeping correctly under a net. MILD/S=sleeping under a net with a body part out the net. Au=other.

Figure A8: Mean Bedroom Activities Performed by Household Members at Night in Lodja.



Key:
Location: C=bedroom.
Activity: Ca=speaking. Re=resting. Tr=working. Do=sleeping. MILD=sleeping correctly under a net. MILD/S=sleeping under a net with a body part out the net. Au=other.

Figure A9: Mean Bedroom Activities Performed by Household Members at Night in Karawa.



Key:
Location: C=bedroom.
Activity: Ca=speaking. Re=resting. Tr=working. Do=sleeping. MILD=sleeping correctly under a net. MILD/S=sleeping under a net with a body part out the net. Au=other.

Figure A10: Overall Household ITN Use in Kenge.

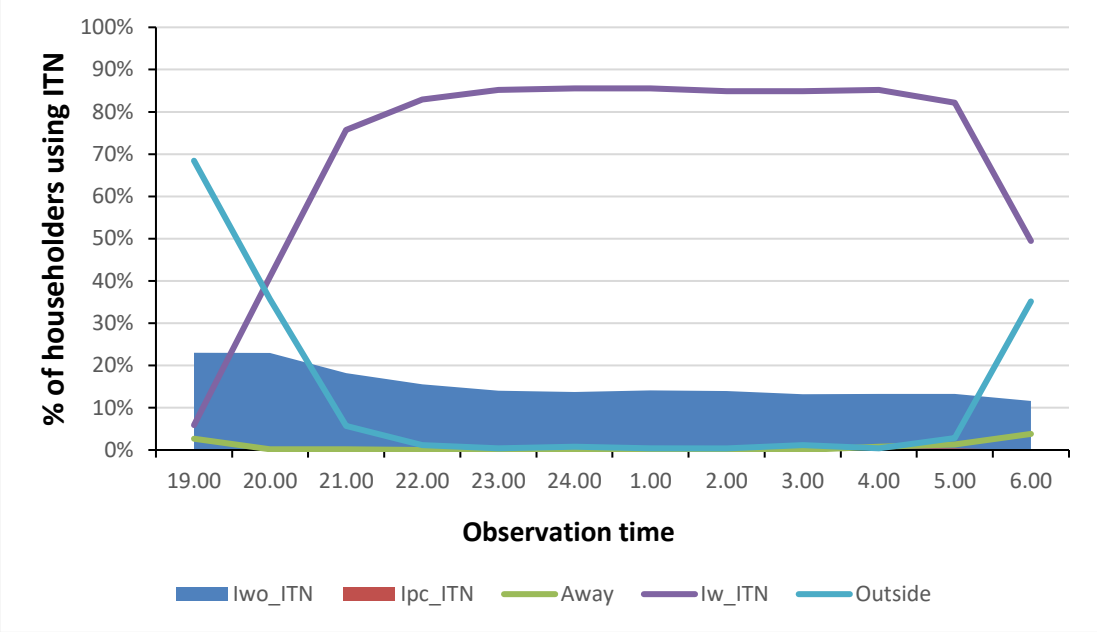


Figure A11: Overall Household ITN Use in Lodja.

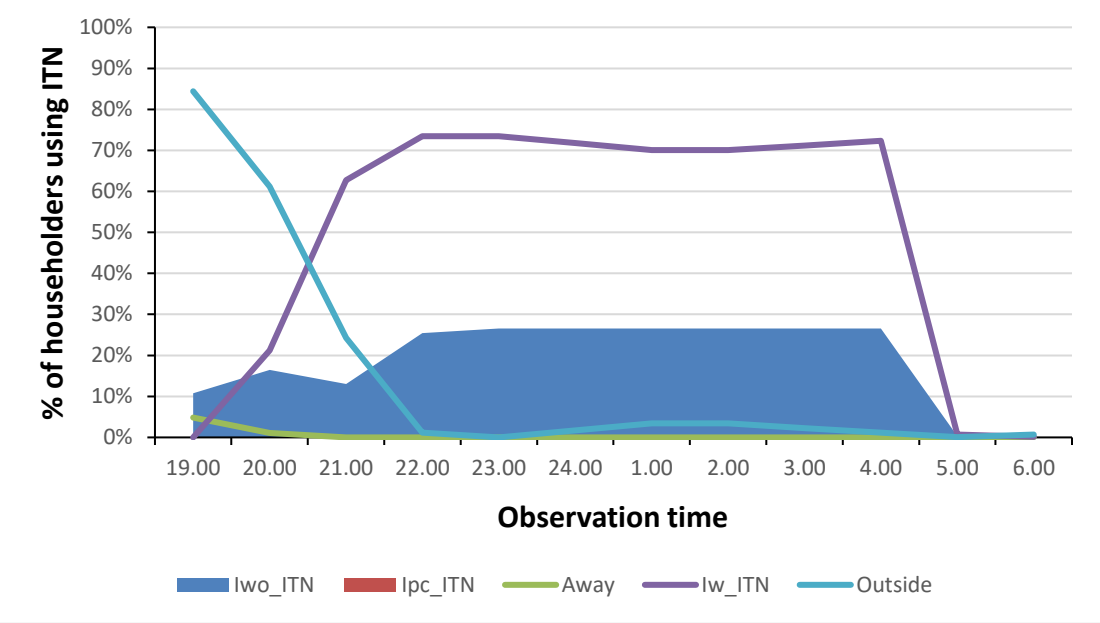


Figure A12: Overall Household ITN use in Karawa.