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THE DEMOCRATIC REPUBLIC OF THE CONGO ENTOMOLOGICAL MONITORING

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ACRONYMS

AIRS	Africa Indoor Residual Spraying Project
CDC	Centers for Disease Control and Prevention
DRC	Democratic Republic of Congo
ELISA	Enzyme-linked Immunosorbent Assay
HBR	Human Biting Rate
HLCs	Human Landing Catches
INRB	National Institute of Bio-medical Research/Institut National de Recherche Bio-médicale
IRS	Indoor Residual Spraying
Kdr	Knockdown Resistance
LLIN	Long Lasting Insecticide-treated nets
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Collection
USAID	United States Agency for International Development
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Schemes

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1. EXECUTIVE SUMMARY

An. gambiae s.l. continued to be the predominant malaria vector caught during 2016 through human landing catch (HLC) and pyrethrum spray catch (PSC) in most sentinel sites. Molecular species identification showed that all An. gambiae s.l. from 2016 were either An. gambiae s.s. or An. coluzzii. No An. arabiensis or An. melas were captured. An. gambiae s.s. constituted 63% (95% CI; 57-69) of An. gambiae s.l. analyzed, with 5% (95% CI; 3-8) An. coluzzii and 32% (95% CI; 26-37) hybrids of the two species. The proportion of hybrids was far greater than expected and most likely due to non-specific amplification during Polymerase Chain Reaction (PCR) resulting in false positives. Troubleshooting is ongoing in collaboration with the University of Notre Dame in the USA. The exceptions were in Katana (Sud Kivu) and Mikalayi (Kasaï) where more An. funestus s.l. were captured than An. gambiae s.l. The proportion of An. funestus s.l. captured increased substantially in Kapolowe (Haut Katanga), Katana, Kingasani (Kinshasa) and Mikalayi compared to 2015. The mean *Plasmodium falciparum* sporozoite rate in the Democratic Republic of Congo (DRC) was 5.1% (95% CI; 3.5-6.7) in An. gambiae s.l. and 3.3% (95% CI; 1.3-5.3) in An. funestus s.l.

World Health Organization (WHO) cylinder tests showed *An. gambiae* s.l. was fully susceptible to bendiocarb (0.1%) at six sites, with possible resistance in Kabondo (Tshopo). A high frequency of resistance to permethrin (0.75%) was recorded in five of the seven sites. Despite a high frequency of permethrin resistance, full susceptibility was recorded to deltamethrin (0.05%) in

Kingasani, Kalemie (Tanganyika) and Lodja (Sankuru). A sub-sample of specimens was tested for the presence of the voltage-gated sodium channel (*V*gsc) 1014F allele; however 41% of samples failed to amplify. *An. funestus* s.l. is likely to be an important malaria vector in DRC, and testing in 2017 should be conducted to determine resistance frequencies and mechanisms.

The mean *An. gambiae* s.l. indoor human biting rate in 2016 varied between six bites per person per night in Katana and Kalemie, and up to 30 bites per person per night in Kabondo. Comparison of biting rates could not be made between regions due to the relatively small number of houses sampled. There was significant outdoor biting potential with outdoor biting rates similar to indoor biting rates for both *An. gambiae* s.l. and *An. funestus* s.l. The highest outdoor biting rates were in Kabondo for *An. gambiae* s.l. with 20 bites per person per night. In general, indoor biting by *An. gambiae* s.l. and *An. funestus* s.l. and *An. funestus* s.l. was primarily late at night between 22:00 and 05:00, and mirrored outdoor biting started between 18:00-19.00 and continued throughout the night both indoors and outdoors until 06.00. As documented in 2015, *An. paludis* biting in Lodja was focused outdoors with an intense peak between 19:00 and 20:00, followed by a gradual decline until midnight.

An. gambiae s.l was the dominant species collected both indoors and outdoors through PSC and HLC in Kalemie, Kingasani, and Kabondo. In Katana and Kapolowe, *An. funestus* s.l. was the most common species collected resting indoors by PSC, although *An. gambiae* s.l. was the predominant species caught indoors through HLC. This may indicate either that *An. gambiae* s.l. exit houses earlier than *An. funestus* s.l., or that *An. funestus* s.l. are entering houses to rest after feeding elsewhere. Few *An. paludis* in Kapolowe and Lodja were collected by PSC, confirming its exophilic tendencies. *An. paludis* in Lodja was primarily exophagic, with the majority of human biting occurring outdoors during the early evening. This is in contrast to Kapolowe, where a similar

proportion of *An. paludis* were captured by HLC both indoors and outdoors. Sequencing data collected in partnership with University of Notre Dame from 2015 indicates the probable presence of at least one sub-species of *An. paludis*.

As the majority of malaria vectors at sentinel sites were biting indoors and late at night, use of long-lasting insecticide-treated nets (LLINs) should provide some protection in all locations. There was a significant amount of outdoor biting, but this was mostly late at night and the level of importance will depend on local nighttime behaviors.

2. INTRODUCTION

Malaria is a major health challenge in the Democratic Republic of Congo (DRC). According to the 2013/14 Demographic and Health Survey (DHS), from a total of 7,250 dried blood spots tested from children sampled across all 26 Division Provinciales de la Santé (DPS) 30.9% were positive for P. falciparum using rapid diagnostic tests (RDT) and 34.1% by PCR. The distribution of long-lasting insecticide-treated nets (LLINs) is the main method of malaria vector control in the DRC. LLIN mass distribution campaigns are organized with the support of donors, including the United States Agency for International Development (USAID), and occur throughout many DRC regions. The NMCP has planned LLIN distributions every three years since 2007. Distribution is done on a rolling basis with mass distribution campaigns taking place annually in different provinces. In 2016, mass distribution campaigns were conducted with Dawaplus 2.0 in Mongala, Tshuapa, Haut Katanga and Kinshasa Provinces, while Duranet was distributed in Sud Ubangi. In addition to mass distributions, LLINs are distributed to pregnant women during antenatal care visits and to children under one year of age at health clinics. Abt Associates conducts entomological monitoring and surveillance through the President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) Project in the DRC to help evaluate the impact of LLIN use on malaria vectors (vector density, seasonal distribution, behavior, and species composition). This report covers the entomological activities undertaken in seven sites (Kingasani, Kalemie, Katana, Mikalayi, Lodja, Kapolowe and Kabondo) during year two of the TO6 contract.

3. PROJECT OBJECTIVES

The aim for entomological activities in DRC is to build capacity in generating data on malaria vectors and develop specific tools to fight and eliminate malaria vectors. The objectives are to:

- 1. Continue to conduct high-quality entomological monitoring activities with minimum technical assistance from outside the DRC;
- Continue support for monitoring species composition, seasonality, behavior, and infectivity of malaria vectors in seven sentinel sites within seven provinces (Kinshasa, Kasai, Sankuru, Tshopo, Sud Kivu, Haut Katanga and Tanganyika);
- 3. Determine the susceptibility level of the main malaria vector, *Anopheles gambiae* s.l. to three classes of insecticides approved by the World Health Organization Pesticide Evaluation Scheme (WHOPES) in seven sentinel sites. DDT is not currently being considered for use in the DRC, and was replaced in this year's plan with alpha-cypermethrin from the pyrethroid class; the intensity of resistance to pyrethroid insecticides using the Centers for Disease Control (CDC) bottle assays was also determined;
- Continue to support the evaluation of *Anopheles paludis* as a malaria vector in the Sankuru-Western Kasai region and in Kapolowe site in Katanga Province in close collaboration with CDC, National Institute of Biomedical Research (INRB), and NMCP;
- 5. Provide technical assistance to NMCP in the development of its indoor residual spraying (IRS) strategy and national resistance monitoring plan;
- 6. Continue to support small-scale entomological studies related to the ongoing sentinel site work, including evaluation of the Suna Trap for sampling hostseeking malaria vectors in selected areas (Kingasani neighborhood of Kinshasa), as well as the impact of LLINs mass distribution on the intensity of insecticide resistance in Kinshasa.

4. PROJECT ACTIVITIES AND METHODOLOGY

This final report covers entomological activities undertaken in seven sentinel sites (Kingasani, Kalemie, Katana, Mikalayi, Lodja, Kabondo and Kapolowe) in the DRC based on the 2015/16 work plan.



Figure 1: 2015 Sentinel Sites for Entomological Activity

Table 1 contains the schedule and status for each activity.

Table 1: Schedule for entomological activities conducted between Januaryand October 2016

Activity	Timeline	Frequency	Curent Status
Vector density and seasonal change	Ð	7 sites, 3 times per site	Complete
	June		

	January-June	7 sites, 3 times per	
Vector behavior	2016	site	Complete
Molecular assays	January-October 2016	7 sites, once per site	INRB analyzed many samples and identified problems with the results for species ID and Vgsc-1014F frequency. Dr Neil Lobo of Notre Dame is assisting with troubleshooting.
ELISA work	January-October 2016	7 sites, once per site	Complete
Evaluation of new mosquito trapping techniques (Suna Trap)	January-October 2016	1 site, once or twice per year	Complete
Evaluation of Anopheles paludis as malaria vector	January 2016- January 2017	2 sites, 12 times a year	In progress for both Lodja and Kapolowe. The final report will be submitted in February 2017.
Impact of mass distribution of LLINs on the intensity of insecticide resistance in Kinshasa	January 2016- December 2017 (LLIN distribution scheduled for December 2016)	2 times before LLIN distribution, (March/April, June) 2016 & scheduled for 3 times after in 2017	2 rounds of bottle bioassays were conducted before LLIN distribution (December 2016) in 5 sites (4 in Kinshasa area and 1 in Kongo central (Kasangulu)

Scientific support -	An STTA trip was conducted in August 2016. The technical
short term	team visited field sites in Kapolowe and Fungurume via
technical assitance	Lubumbashi, including a village in Fungurume where malaria
(STTA) in DRC	vector control was conducted using non-pyrethroid
from	impregnated wall liner. The financial team explored the
Seth Irish (CDC),	financial network. Neil Lobo, Seth Irish, Richard Oxborough
Richard	and Djenam Jacob visited the Biomolecular Unit of
Oxborough (AIRS),	INRB, where Neil provided guidance as to how to properly
and Neil Lobo	conduct a DNA extraction with the AIRS technician (Mr. Joel
(Notre Dame).	Imponge).
Technical,	
Administrative and	
financial support	
from Djenam	
Jacob (AIRS).	
	The entomological training in Benin supported by NMCP and
	INRB, with financial support from Abt through PMI AIRS, is
Training	complete. The team has gained considerable experience from
	their time spent in Benin and will be part of the upcoming
	training sessions as trainers for other NMCP and INRB
	entomologists.

INRB staff traveled to each site to provide technical support during three sampling periods: January-February 2016, March-April 2016, and May-June 2016. The exceptions were Kapolowe and Lodja where vector monitoring was conducted monthly to determine dynamics and vector status of *An. paludis*.

The methods used are described below.

Human Biting Rate

Human Landing Catch

The Human Landing Catch (HLC) method was used to assess mosquito biting times and feeding behavior, as well as to monitor species composition and sporozoite rates. Two teams collected adult mosquitoes during four consecutive nights (in two houses each night), with one person indoor and the other outdoor in each selected house. The two collectors in each location were assigned shifts (one person from 18:00 to midnight and the other from midnight to 06:00). The human biting rate (HBR) was calculated for each sampling period based on eight person nights of collection.

Indoor resting densities

Pyrethrum Spray Catch

Pyrethrum Spray Catch (PSC) was used to estimate the indoor resting density of mosquito species. Indoor PSC was used in ten houses/bedrooms in each sentinel site. The PSCs were carried out between 06:00 and 10:00. All occupants were asked to move water, food, or anything that could be harmed by the insecticide out of the house prior to spraying. A commercial aerosol containing several pyrethroid insecticides was sprayed in each room, and white sheets were lined on floors and other surfaces to collect mosquitoes. All mosquitoes were collected from the white sheets 20 minutes after spraying. Female *Anopheles* mosquitoes were classified according to abdominal status (unfed, fed, half-gravid, or gravid). Each mosquito was labeled for subsequent analysis at INRB for sibling species identification using PCR and other labbased analysis following standard procedures.

Molecular analysis of mosquito samples

In total, 280 samples of *Anopheles gambiae* s.l. were analyzed for species identification according to the protocol of Wilkins et al. 2006.. DNA was extracted from the wings and legs using the Chelex method. Amplification and migration were undertaken on agarose gel (1.5%) using ethidium bromide. Pictures of gels were obtained after running the migration for 40 minutes. According to protocol, *Anopheles melas* bands were at 464/466 bp, *Anopheles gambiae* at 390 bp, and *An. arabiensis* at 315 bp.

Identification of Vgsc-1014F

In total, 105 samples of *An. gambiae* s.l. were analyzed for *Vgsc*-1014F; 15 samples per site, according to the protocol or Martinez-Torres et al. (1998) and Basilua et al. (2013). The following primers were used to identify the *V*gsc-L1014F:

- AgD1 (ATAGATTCCCCGACCATG)
- AgD2 (AGACAAGGATGATGAACC)
- AgD3 (AATTTGCATTACTTACGACA)
- AGD4 (CTGTAGTGATAGGAAATTTA).

After the amplification and 40 minutes migration on agarose gel (Figure 3), the primers produced DNA bands at 293 bp for internal control, 195 bp for presence of Vgsc-1014F, or 137bp for absence of Vgsc-1014F.

Evaluation of mosquito trapping techniques (Suna Trap and CDC Light Trap)

HLC has been used in DRC to monitor human biting rates both indoors and outdoors. However, this method is labor intensive and results in a small sample size. Therefore, in May 2016, AIRS compared HLC, with two alternative methods using CDC Light Traps and the Suna Trap both indoors and outdoors at Kingasani sentinel site (Kinshasa).

CDC light traps have been used as an alternative for human landing catches in several countries. However, the CDC-LT does not always produce accurate measures of human biting in some settings. The Suna trap was developed recently by Biogents AG (Germany) working with Wageningen University (Netherlands) as a tool for mosquito monitoring. The trap is baited with a synthetic attractant lure which mimics the odor of a human, but has the advantage of being standardized so that multiple traps exhibit the same level of attraction to mosquitoes. Six houses were used for trapping each night with each house receiving 1 trapping method either indoors or outdoors.

House 1- CDC-LT indoors

House 2- CDC-LT outdoors

House 3- Suna trap indoors

House 4- Suna trap outdoors

House 5- HLC indoors

House 6- HLC outdoors

Trapping was conducted for a total of 18 nights according to a Latin Square design, with each trapping method being rotated between houses nightly.

Impact of mass distribution of LLINs on the intensity of insecticide resistance in Kinshasa

The objective of this study was to determine if selection pressure due to mass distribution of LLINs affects insecticide resistance intensity in Kinshasa. Insecticide resistance intensity in *Anopheles gambiae* s.l. was monitored in four sites within Kinshasa before the start of a mass LLIN distribution campaign. Resistance was monitored using the intensity assay (bottle bioassays using 1, 2, 5, and 10 times the diagnostic dose of permethrin and deltamethrin). Insecticide resistance intensity was also monitored in Kasungulu, a control area 40km from Kinshasa where there is no plan for mass distribution of LLINs (the last distribution in Kasungulu was in 2014). Intensity assays will be conducted in 2017 at one, three and six months following LLIN distribution.

Evaluation of Anopheles paludis as malaria vector

Further evaluation was conducted to determine whether *Anopheles paludis* is a malaria vector in the region of Sankuru and Kapolowe (Haut Katanga). Sporozoite infection rates of *Anopheles paludis* were determined using ELISA.

5. Species Composition For PSC And HLC Collection

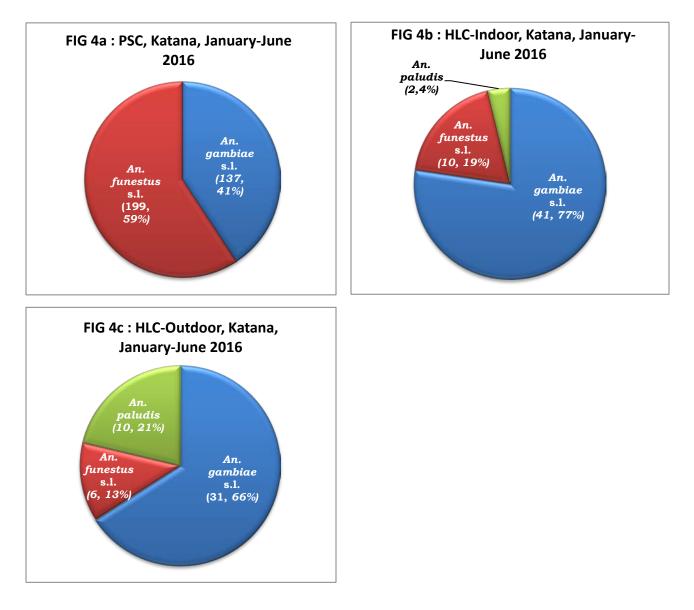
5.1. SUD KIVU PROVINCE, KATANA SITE (JANUARY – JUNE 2016)

Table 2: Distribution and abundance of mosquitoes collected in Katana by species and method (PSC and HLC) of collection

Site	KATANA			
Species / Method	PSC	HLC indoors	HLC outdoors	
	January – Feb	ruary 2016	I	
An. gambiae s.1.	94 (57 %)	15 (63%)	13 (48%)	
An. funestus s.l.	70 (43 %)	7 (29%)	4 (15%)	
An. paludis	0 (0 %)	2 (8%)	10 (37%)	
Total 1	164 (100 %)	24 (100%)	27 (100%)	
	March – Apı	il 2016		
An. gambiae s.1.	29 (21 %)	12 (80%)	13 (80%)	
An. funestus s.l.	110 (79 %)	3 (20%)	2 (20%)	
Total 2	139 (100 %)	15 (100%)	15 (100%)	
	May – Jun	e 2016		
An. gambiae s.1.	14 (42 %)	14 (100%)	5 (100%)	
An. funestus s.l.	19 (58 %)	0	0	
Total 3	33 (100 %)	14 (100%)	5 (100%)	
All Thr	ee Periods: Ja	nuary – June 2	2016	
An. gambiae s.1.	137 (41 %)	41 (77%)	31 (66%)	
An. funestus s.l.	199 (59 %)	10 (19%)	6 (13%)	
An. paludis	0 (0 %)	2 (4%)	10 (21%)	

TOTAL	336 (100 %)	53 (100%)	47 (100%)
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Figure 2: Distribution of Anopheles captured by PSC and HLC indoors and outdoors in Katana



In Katana, despite *An. gambiae* s.l. being the predominant species caught hostseeking indoors by HLC, *An. funestus* s.l. was the most abundant in PSC resting collections. This may indicate that *An. gambiae* s.l. exit earlier than *An. funestus* s.l. or that *An. funestus* s.l. are entering houses to rest after feeding elsewhere.

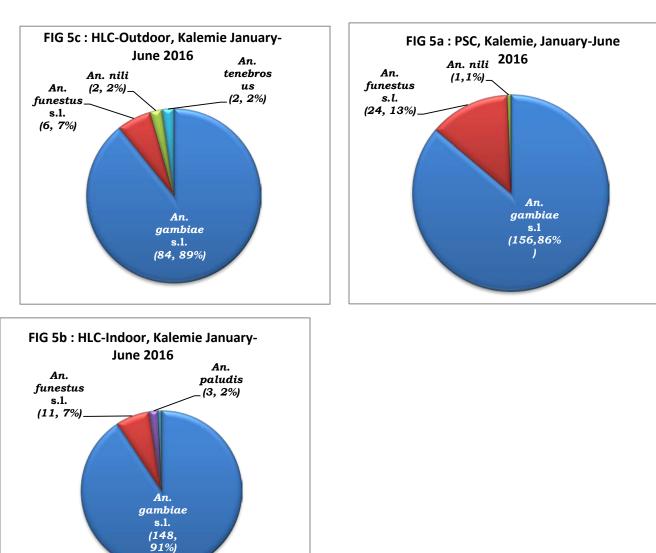
5.2. TANGANYIKA PROVINCE, KALEMIE SITE (JANUARY - JUNE 2016)

Site	KALEMIE					
Species /Method	PSC	HLC indoors	HLC outdoors			
	January – Fe	bruary 2016				
An. gambiae s.l.	81 (89 %)	42 (98%)	23 (100%)			
An. funestus s.l.	9 (10 %)	1 (2%)	0			
An. nili	1 (1 %)	0	0			
Total 1	91 (100 %)	43 (100%)	23 (100%)			
	March – A	pril 2016				
An. gambiae s.l.	64 (85 %)	54 (89%)	25 (86%)			
An. funestus s.1.	11 (15 %)	4 (7%)	2 (7%)			
An. paludis	0 (0 %)	3 (5%)	0			
An. nili	0 (0 %)	0	2 (7%)			
Total 2	75 (100 %)	61 (100%)	29 (100%)			
	May – Ju	ne 2016				
An. gambiae s.l.	11 (73 %)	52 (88%)	36 (86%)			
An. funestus s.1.	4 (27 %)	6 (10%)	4 (10%)			
An. tenebrosus	0 (0 %)	1 (2%)	2 (5%)			

Table 3: Distribution and abundance of Anopheles collected by species and method (PSC & HLC) of collection in Kalemie

Total 3	15 (100 %)	59 (100%)	42 (100%)
All Th	ree Periods: J	anuary – June	2016
An. gambiae s.l.	156 (86 %)	148 (91%)	84 (89%)
An. funestus s.l.	24 (13 %)	11 (7%)	6 (6%)
An. nili	1 (1 %)	0	2 (2%)
An. paludis	0 (0 %)	3 (2%)	0
An. tenebrosus	0 (0 %)	1 (1%)	2 (2%)
TOTAL	181 (100 %)	163 (100%)	94 (100%)

FIGURE 3: DISTRIBUTION OF ANOPHELES CAPTURED BY INDOORS AND OUTDOORS USING HLC IN KALEMIE SITE



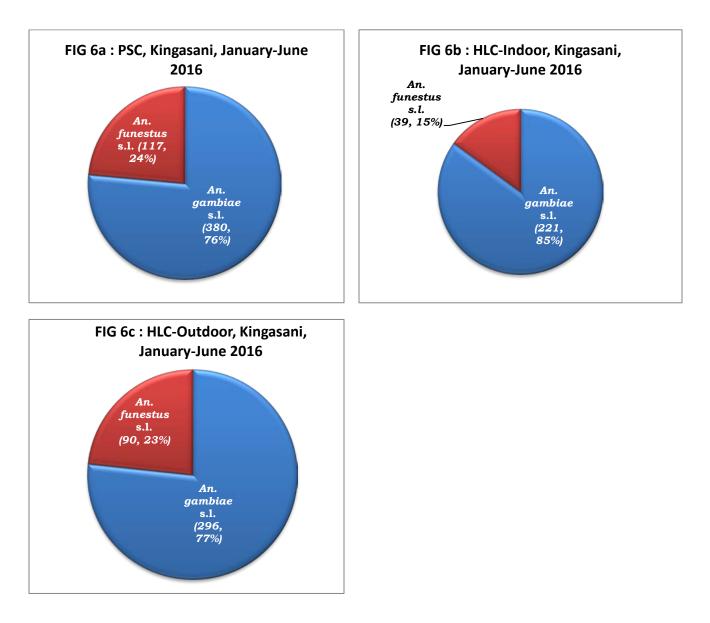
In Kalemie, *An. gambiae* s.l. was the predominant species collected by PSC and HLC both indoors and outdoors.

5.3. KINSHASA PROVINCE, KINGASANI SITE (JANUARY- JUNE 2016)

Table 4: Distribution and abundance of mosquitoes collected in Kingasani by species and method (PSC and HLC) of collection

Site	KINGASANI		
Species	PSC	HLC indoors	HLC outdoors
	January	– February 2016	
An. gambiae s.l.	11 (100 %)	18 (75%)	55 (92%)
An. funestus s.l.	0 (0 %)	6 (25%)	5 (8%)
Total 1	11 (100 %)	24 (100%)	60 (100%)
	March	1 - April 2016	
An. gambiae s.l.	90 (68 %)	92 (84%)	91 (75%)
An. funestus s.l.	43 (32 %)	17 (16%)	31 (25%)
Total 2	133 (100 %)	109 (100%)	122 (100%)
	Мау	- June 2016	
An. gambiae s.l.	279 (79 %)	111 (87%)	150 (74%)
An. funestus s.l.	74 (21 %)	16 (13%)	54 (26%)
Total 3	353 (100 %)	127 (100%)	204 (100%)
A1 1	Three Period	ls: January – June	2016
An. gambiae s.l.	380 (76 %)	221 (85%)	296 (77%)
An. funestus s.1.	117 (24 %)	39 (15%)	90 (23%)
TOTAL	497 (100 %)	260 (100%)	386 (100%)

FIGURE 4: DISTRIBUTION OF ANOPHELES CAPTURED BY INDOORS AND OUTDOORS BY HLC IN KINGASANI SITE



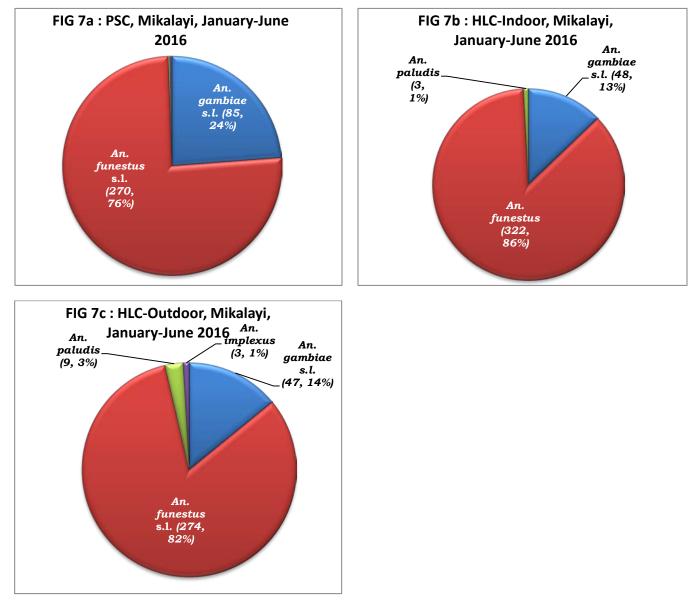
In Kingasani, *An. gambiae* s.l. was the predominant species collected by PSC and HLC both indoors and outdoors.

5.4. KASAÏ CENTRAL PROVINCE, MIKALAYI SITE (JANUARY - JUNE 2016)

Table 5: Distribution of mosquitoes collected by species and method (PSC and HLC) of capture in Mikalayi

Site	MIKALAYI				
Period	January-June	, 2016			
Species/Method	PSC	HLC indoors	HLC outdoors		
	January – Feb	ruary 2016	<u> </u>		
An. gambiae s.l.	45 (23 %)	3 (2%)	8 (15%)		
An. funestus s.l.	148 (77 %)	124 (97%)	44 (80%)		
An. paludis	0 (0 %)	1 (1%)	2 (4%)		
An. implexus	0 (0 %)	0	1 (2%)		
Total 1	193 (100 %)	128 (100%)	55 (100%)		
	March-Ap	ril 2016	1		
An. gambiae s.1.	35 (38 %)	36 (27%)	31 (21%)		
An. funestus s.l.	56 (60 %)	98 (73%)	112 (76%)		
An. paludis	1 (1 %)	0	4 (3%)		
An. implexus	1 (1 %)	0	0		
Table 2	93 (100 %)	134 (100%)	147 (100%)		
	May-Jun	e 2016			
An. gambiae s.1.	5 (7 %)	9 (8%)	8 (6%)		
An. funestus s.l.	66 (93 %)	100 (90%)	118 (90%)		
An. paludis	(0 %)	2 (2%)	3 (2%)		
An. implexus	(0 %)	0	2 (2%)		
Total 3	71 (100 %)	111 (100%)	131 (100%)		
All Th	ree Periods : J	anuary-June 2	016		
An. gambiae s.l.	85 (24 %)	48 (13%)	47 (14%)		
An. funestus s.l.	270 (76 %)	322 (86%)	274 (82%)		
An. paludis	1 (<1 %)	3 (1%)	9 (3%)		
An. implexus	1 (<1 %)	0	3 (1%)		

FIGURE 5: DISTRIBUTION OF ANOPHELES CAPTURED BY HLC INDOORS AND OUTDOORS IN MIKALAYI SITE



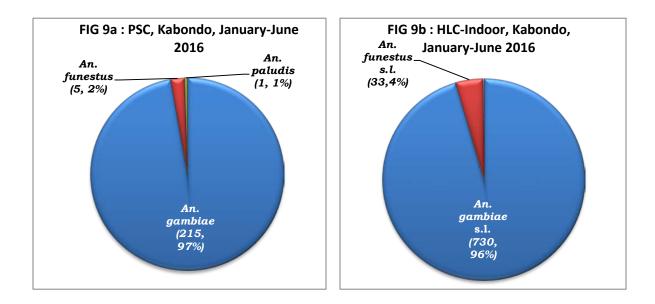
In Mikalayi, *An. funestus* s.l. was the predominant species collected by PSC and HLC both indoors and outdoors.

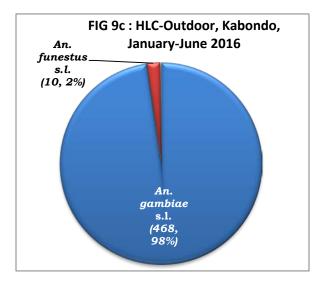
5.5. TSHOPO PROVINCE, KABONDO SITE (JANUARY- JUNE 2016)

Table 6: Distribution and abundance of mosquitoes collected in Kabondo by species and method (PSC and HLC) of collection

Site	KABONDO				
Period	J	anuary – June	2016		
Species	PSC	HLC indoors	HLC outdoors		
	January – February 2016				
An. gambiae s.l.	78 (99%)	140 (97%)	111 (100%)		
An. funestus s.l.	0	4 (3%)	0		
An. paludis	1 (1%)	0	0		
Total 1	79 (100%)	144 (100%)	111 (100%)		
	1	March – April 2	016		
An. gambiae s.l.	63 (95%)	545 (97%)	309 (98%)		
An. funestus s.l.	3 (5%)	18 (3%)	5 (2%)		
Total 2	66 (100%)	563 (100%)	314 (100%)		
		May – June 20	016		
An. gambiae s.l.	74 (97%)	45 (79%)	48 (89%)		
An. funestus s.l.	2 (3%)	11 (19%)	5 (9%)		
An. paludis	0	1 (2%)	1 (2%)		
Total 3	76 (100%)	57 (100%)	54 (100%)		
	All three p	periods : Janua	ry-June 2016		
An. gambiae s.l.	215 (97%)	730 (96%)	468 (98%)		
An. funestus s.l.	5 (2%)	33 (4%)	10 (2%)		
An. paludis	1 (<1%)	1 (<1%)	1 (<1%)		
TOTAL	221 (100%)	764 (100%)	479 (100%)		

FIGURE 6: DISTRIBUTION OF ANOPHELES CAPTURED BY SPECIES, METHOD (HLC) AND LOCATION IN KABONDO SITE





In Kabondo, *An. gambiae* s.l. was the predominant species collected by PSC and HLC both indoors and outdoors.

6. HUMAN BITING RATE OF MALARIA VECTORS INDOORS AND OUTDOORS COLLECTED BY HLC

6.1. SUD KIVU PROVINCE, KATANA SITE

Table7: HBR of malaria vectors indoors and outdoors in the Katana site (January-February, March-April and May-June 2016)

Site		KATANA						
Period	January – June							
Method	HLC							
Species	Area	Variable	Jan-Feb	Marc-	May-			
Species	Area	Variable	Jan-red	April	June			
		Total mosquitoes	15	12	14			
An. gambiae	Indoor	Nbr person-nights	8	8	8			
s.l.		HBR/night	2	2	2			
5.1.	Outdoor	Total mosquitoes	13	13	5			
		Nbr person-nights	8	8	8			
		HBR/night	2	2	1			
		Total mosquitoes	77	3	10			
An. funestus	Indoor	Nbr person-nights	8	8	8			
s.l.		HBR/night	10	0	1			
	Outdoor	Total mosquitoes	4	2	0			
		Nbr person-nights	8	8	8			
		HBR/night	1	0	0			

6.2. TANGANYIKA PROVINCE, KALEMIE SITE

Table 8: HBR of malaria vectors indoors and outdoors in Kalemie site (January-February, March-April and May-June 2016).

Site	KALEMIE							
Period	January – June 2016							
Method		HLC	;					
Species	Area	Variable	Jan-Feb	March- April	May-June			
		Total mosquitoes	42	54	52			
	Indoors	Nbr person-nights	8	8	8			
An.		HBR/night	5	7	7			
gambiae s.l.		Total mosquitoes	23	25	36			
	Outdoors	Nbr person-nights	8	8	8			
		HBR/night	3	3	5			
	Indoors	Total mosquitoes	1	4	6			
		Nbr person-nights	8	8	8			
An.		HBR/night	0	0	0			
funestus s.l.		Total mosquitoes	0	2	4			
	Outdoors	Nbr person-nights	8	8	8			
		HBR/night	0	0	1			
		Total mosquitoes	0	3	0			
	Indoors	Nbr person-nights	8	8	8			
An naludia		HBR/night	0	0	0			
An. paludis		Total mosquitoes	0	0	0			
	Outdoors	Nbr person-nights	8	8	8			
		HBR/night	0	0	0			
		Total mosquitoes	0	0	0			
An. nili	Indoors	Nbr person-nights	8	8	8			
		HBR/night	0	0	0			

		Total mosquitoes	0	2	0
	Outdoors	Nbr person-nights	8	8	8
		HBR/night	0	0	0
		Total mosquitoes	0	0	1
	Indoor	Nbr person-nights	8	8	8
An.		HBR/night	0	0	0
tenebrosus		Total mosquitoes	0	0	2
	Outdoors	Nbr person-nights	8	8	8
		HBR/night	0	0	0

6.3. KINSHASA PROVINCE, KINGASANI SITE

Table 9: HBR of malaria vectors Indoors and Outdoors in the Kingasani site (January-February, March-April and May-June 2016)

Site	KINGASANI							
Period	January - June 2016							
Method	HLC							
Sites	Area	Variable	Jan/Feb	Marc/Apr	May/June			
		Total mosquitoes	18	92	111			
An.	Indoor	Nbr person- nights	8	8	8			
gambiae s.l		HBR/night	2	12	14			
5.1		Total mosquitoes	55	91	150			
Outdoor	Outdoor	Nbr person- nights	8	8	8			
		HBR/night	7	11	19			
		Total mosquitoes	6	60	16			
An.	Indoor	Nbr person- nights	8	8	8			
funestus		HBR/night	1	8	2			
s.l.		Total mosquitoes	5	31	54			
	Outdoor	Nbr person- nights	8	8	8			
		HBR/night	1	4	7			

6.4. KASAI CENTRAL PROVINCE, MIKALAYI SITE

Table 10: HBR of malaria vectors Indoors and Outdoors in Mikalayi (January-February, March-April and May-June 2016).

Site	MIKALAYI						
Period	January – June 2016						
Method		1	HLC				
Species	Area	Variable	Jan/Feb	Mar/Apr	May/June		
		Total mosquitoes	48	36	9		
An.	Indoor	Nbr person- nights	8	8	8		
gambiae s.1		HBR/night	0	5	1		
5.1		Total mosquitoes	8	31	8		
	Outdoor	Nbr person- nights	8	8	8		
		HBR/night	1	4	1		
	Indoor	Total mosquitoes	124	98	100		
An.		Nbr person- nights	8	8	8		
funestus s.l.		HBR/night	16	12	13		
5.1.		Total mosquitoes	44	112	118		
	Outdoor	Nbr person- nights	8	8	8		
		HBR/night	6	14	15		
		Total mosquitoes	1	0	2		
	Indoor	Nbr person- nights	8	8	8		
An.		HBR/night	0	0	0		
paludis	0	Total mosquitoes	2	4	3		
	Outdoor	Nbr persons	8	8	8		
		HBR/night	0	1	0		
An.	Indoor	Total mosquitoes	0	0	0		
implexus		Nbr person-	8	8	8		

	1	nights			
	1	HBR/night	0	0	0
		Total mosquitoes	1	0	2
Outdoor		Nbr person- nights	8	8	8
	J	HBR/night	0	0	0

6.5. TSHOPO PROVINCE, KABONDO SITE

Table 11: HBR of malaria vectors indoors and outdoors in the Kabondo site (January-February, March-April and May-June 2016)

Site	KABONI	00								
Period	January – June 2016									
Method	HLC									
Species	Area	Variable	Jan/Feb	March/April	May/June					
		Total mosquitos	140	545	45					
	Indoor	Nbr persons	8	8	8					
An.		HBR / night	18	68	6					
gambiae s.l.	Outdoor	Total mosquitos	111	309	48					
		Nbr night	8	8	8					
		HBR / night	14	39	6					
An.	Indoor	Total mosquitos	251	18	11					
		Nbr person-nights	8	8	8					
		HBR /night	31	2	1					
funestus s.l.	Outdoor	Total mosquitos	4	5	5					
		Nbr person-nights	8	8	8					
		HBR / night	1	1	1					
		Total mosquitos	0	0	1					
	Indoor	Nbr person-nights	8	8	8					
An.		HBR / night	0	0	0					
paludis		Total mosquitos	0	0	1					
	Outdoor	Nbr person-nights	8	8	8					
		HBR / night	0	0	0					

a	INDOORS			OUTDOORS			
Sentinel site and species	Nbr mosquitoe s	Nbr person s	HBR/nigh t	Nbr mosquitoe s	Nbr person s	HBR/nigh t	
Katana							
An. gambiae s.1.	41	24	2	31	24	1	
Kalemie							
An. gambiae s.1.	148	24	6	84	24	4	
Kingasani							
An. gambiae s.1.	221	24	9	296	24	12	
An. funestus s.l.	82	24	3	90	24	4	
Mikalayi							
An. gambiae s.l.	93	24	4	47	24	2	
An. funestus s.l.	322	24	13	274	24	11	
Kabondo							
An. gambiae s.l.	730	24	30	468	24	20	
An. funestus s.l.	280	24	12	14	24	1	

Table 12: Indoor HBR of malaria vectors (January-June 2016)

The mean indoor human biting rate of *An. gambiae* s.l. in 2016 varied between two bites per person per night in Katana and up to 30 bites per person per night in Kabondo. The biting rate of *An. funestus* s.l. was substantial in four sites, with biting rates varying between three bites per person per night in Kingasani and up to 13 in Mikalayi. Comparison of biting rates could not be made between regions due to the relatively small number of houses sampled. There was significant outdoor biting potential, with outdoor biting rates similar to indoor biting rates for both *An. gambiae* s.l. and *An. funestus* s.l. The highest outdoor biting rates were in Kabondo for *An. gambiae* s.l. with 20 bites per person per night.

7. BITING TIMES OF MALARIA VECTORS INDOORS AND OUTDOORS

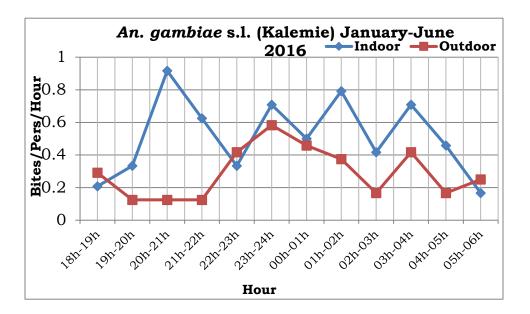
Biting trends are presented only for locations where the total caught per species was >200 between January and June 2016. In general, indoor biting by *An. gambiae* s.l. and *An. funestus* s.l. was primarily late at night between 22:00 and 05:00 and mirrored outdoor biting trends.

7.1. SUD-KIVU PROVINCE, KATANA SITE

The number of An. gambiae s.l. was too few to present clear biting trends.

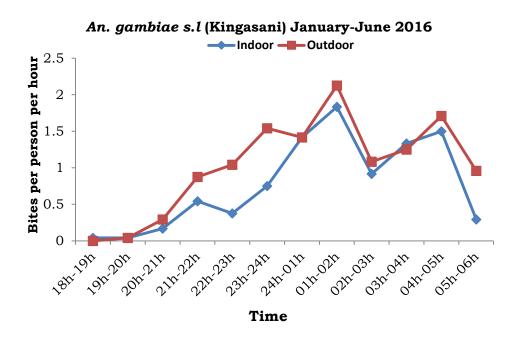
7.2. TANGANYIKA PROVINCE, KALEMIE SITE

FIGURE 7: BITING ACTIVITY OF AN. GAMBIAE S.L. AT KALEMIE SITE (JANUARY – JUNE 2016)



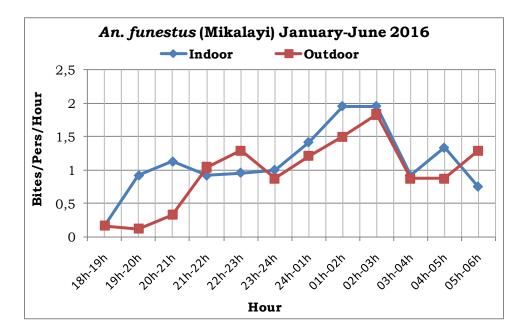
7.3. KINSHASA PROVINCE, KINGASANI SITE

FIGURE 8: BITING ACTIVITY OF AN. GAMBIAE S.L. AT KINGASANI SITE (JANUARY – JUNE 2016)



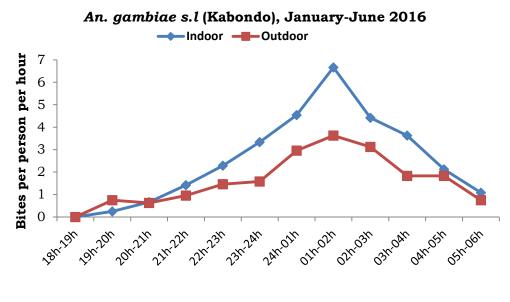
7.4. KASAI PROVINCE, MIKALAYI SITE

FIGURE 9: BITING ACTIVITY OF AN. FUNESTUS S.L. AT MIKALAYI SITE (JANUARY – JUNE 2016)



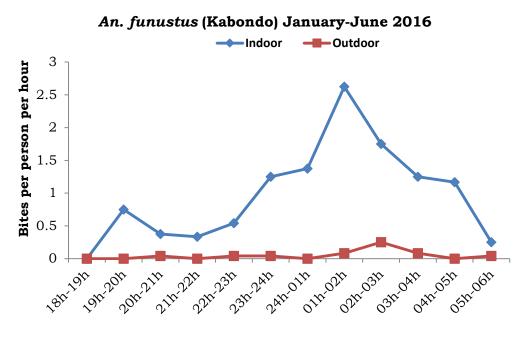
7.5. TSHOPO PROVINCE, KABONDO SITE

FIGURE 10: BITING ACTIVITY OF AN. GAMBIAE S.L. AT KABONDO SITE (JANUARY – JUNE 2016)



Time

FIGURE 11: BITING ACTIVITY OF AN. GAMBIAE S.L. AT KABONDO SITE (JANUARY – JUNE 2016)



Time

8. BLOOD DIGESTION STAGE OF MALARIA VECTORS COLLECTED USING PSC

A summary of abdominal status for malaria vectors collected by PSC between January and June 2016 is presented in Table 17. See Annex A for more detailed data broken down by collection period. In all sites, the majority of *An. gambiae* s.l. and *An. funestus* s.l. collected by PSC were blood-fed (60-92%), with <10% being either half-gravid or gravid.

Table 13: Abdominal status of malaria vectors collected resting indoorsthrough PSC (January-June 2016)

Sentinel site &			status		
species	Unfed	Fed	Half gravid	Gravid	TOTAL
Katana					
An. gambiae s.l.	13 (7%) 15	177 (89%) 133	7 (4%)	2 (1%)	199
An. funestus s.l.	(10%)	(85%)	4 (3%)	4 (3%)	156
Kalemie		. ,	· · /	· · ·	
An. gambiae s.l.	16 (10%) 4	130 (83%)	2 (1%)	8 (5%)	156
An. funestus s.l.	(17%)	18 (75%)	0	2 (8%)	24
Kingasani		, ,		()	
An. gambiae s.1.	12 (3%)	350 (92%) 106	1 <1%)	17 (5%)	380
An. funestus s.l.	6 (5%)	(91%)	2 (2%)	3 (3%)	117
Mikalayi	. ,				
An. gambiae s.l.	8 (9%) 56	76 (89%) 211	0	1 (1%)	85
An. funestus s.l.	(21%)	(78%)	0	3 (1%)	270
Kabondo					
An. gambiae s.l.	74 (34%)	128 (60%)	13 (6%)	0	215

9. MONTHLY MONITORING OF MALARIA VECTORS IN LODJA AND KAPOLOWE (JANUARY – JUNE 2016)

9.1 BITING RATE JANUARY-JUNE 2016

Samples were collected monthly between January to December in Kapolowe and Lodja, but so far only samples from January – June 2016 were checked for species identification and analyzed. The rest of the samples will be reported in a supplement in February 2017.

Indoor and outdoor biting rates were extremely high in Lodja and Kapolowe throughout the period of January to June. In Lodja, *An. gambiae* s.l. was the primary malaria vector, with a biting peak in April indoors at 27 bites per person per night and outdoors at 39 bites per person per night (Figures 12 & 13). *An. paludis* biting was predominantly outdoors and peaked in January with 70 bites per person per night (Figure 13). In Kapolowe the peak biting rates for *An. gambiae* s.l. were in February, followed by a later peak for *An. funestus* s.l. in May (Figures 15 & 16). The period of peak *An. funestus* s.l. biting coincided with a typically dry period of the year (Figure 17).

FIGURE 12: MONTHLY INDOOR BITING RATE OF ANOPHELES SPECIES IN LODJA (SANKURU) FROM HLC COLLECTIONS (8 HOUSES PER MONTH).

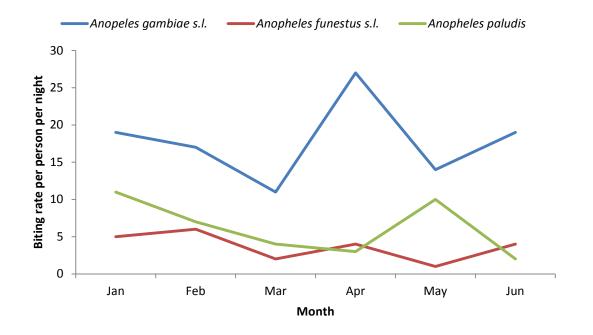
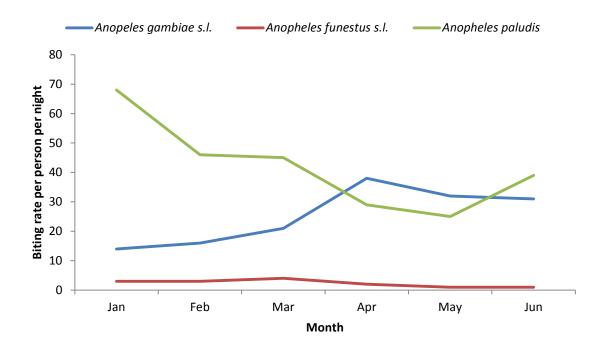


FIGURE 13: MONTHLY OUTDOOR BITING RATE OF ANOPHELES SPECIES IN LODJA (SANKURU) FROM HLC COLLECTIONS (8 HOUSES PER MONTH).



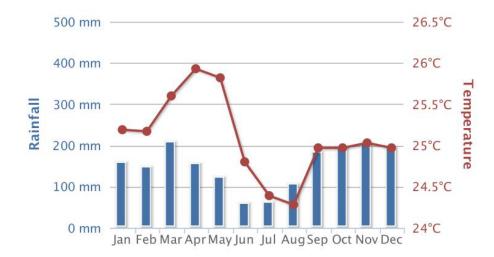


FIGURE 14: MEAN CLIMATE DATA BETWEEN 2000-2012 FOR LODJA (SANKURU)

*Data taken from the Climatic Research Unit (CRU) of University of East Anglia (UEA).

FIGURE 15: MONTHLY INDOOR BITING RATE OF *ANOPHELES* SPECIES IN KAPOLOWE (HAUT KATANGA) FROM HLC COLLECTIONS (8 HOUSES PER MONTH).

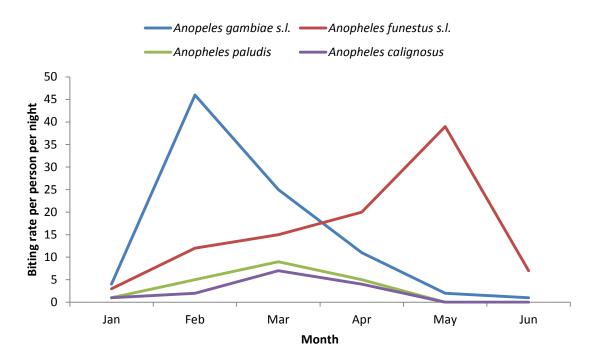


FIGURE 16: MONTHLY OUTDOOR BITING RATE OF ANOPHELES SPECIES IN KAPOLOWE (HAUT KATANGA) FROM HLC COLLECTIONS (8 HOUSES PER MONTH).

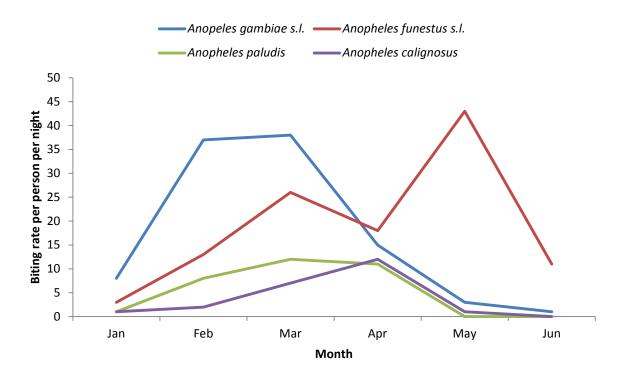
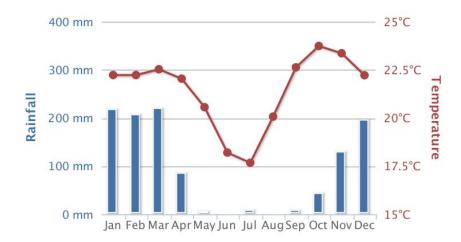


FIGURE 17: MEAN CLIMATE DATA BETWEEN 2000-2012 FOR KAPOLOWE (HAUT KATANGA)



*Data taken from the Climatic Research Unit (CRU) of University of East Anglia (UEA).

9.2 BITING TIMES OF ANOPHELES SPECIES INDOORS AND OUTDOORS

In Lodja, *An. gambiae* s.l. biting trends were mirrored indoors and outdoors, with a gradual increase in biting rate until the peak was reached at 1am, followed by a gradual decrease until dawn (Figure 18). Biting of *An. funestus* s.l. was generally late at night with a prolonged peak indoors between 1am and 5am, followed by a sharp decrease in biting rate at dawn (Figure 19). *An. paludis* was recorded biting predominantly outdoors, with a clear early evening peak at 19-20:00 followed by a gradual decrease up to 23:00 which reached a plateau at 1-2 bites p/person/hr until 05:00 (Figure 20).

In Kapolowe, the biting trends were less clear for all species, with similar biting rates throughout the night. *An. paludis* in Kapolowe was captured biting indoors and outdoors at similar frequency and did not display the early evening outdoor biting peak (Figure 23).

FIGURE 18: NOCTURNAL BITING TIMES OF AN. GAMBIAE S.L. INDOORS AND OUTDOORS IN LODJA SITE (SANKURU).

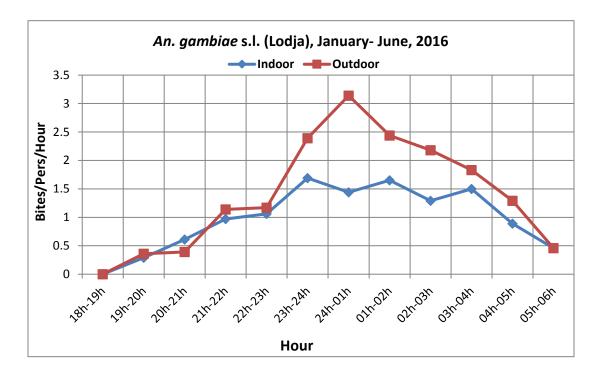


FIGURE 19: NOCTURNAL BITING TIMES OF AN. FUNESTUS S.L. INDOORS AND OUTDOORS IN LODJA SITE (SANKURU)

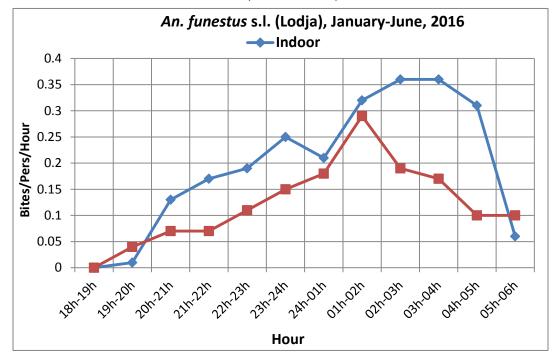


FIGURE 20: NOCTURNAL BITING TIMES OF AN. PALUDIS INDOORS AND OUTDOORS IN LODJA SITE (SANKURU)

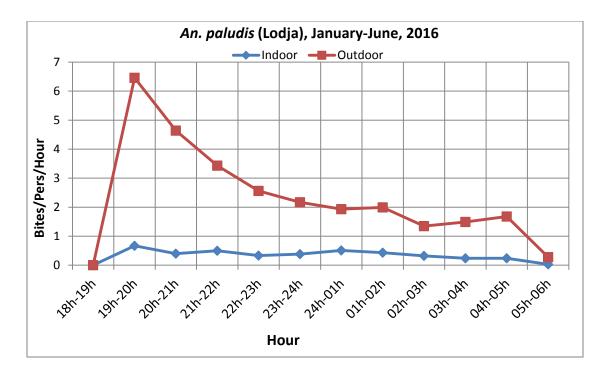


Figure 21: Nocturnal biting times of *An. gambiae* s.l. indoors and outdoors in Kapolowe (Haut Katanga).

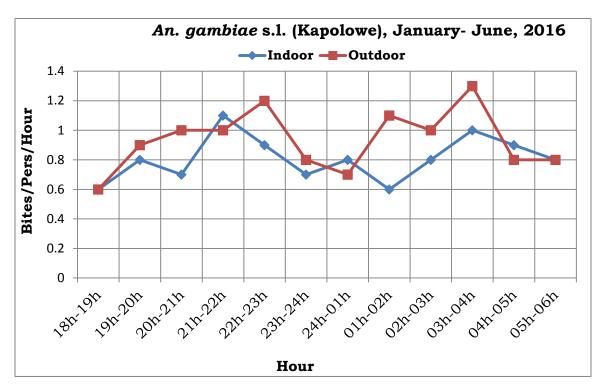


Figure 22: Nocturnal biting times of *An. funestus* s.l. indoors and outdoors in Kapolowe (Haut Katanga).

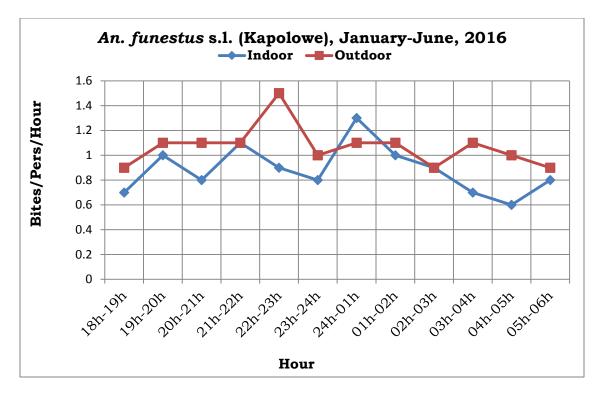
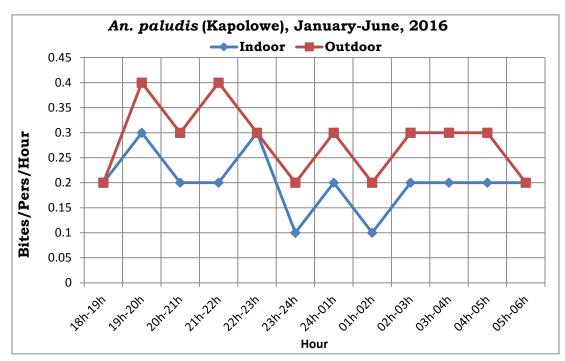


Figure 23: Nocturnal biting times of *An. paludis* indoors and outdoors in Kapolowe (Haut Katanga).



9.3 MONTHLY MONITORING OF ABDOMINAL STATUS FOR MALARIA VECTORS CAUGHT RESTING INDOORS BY PSC

In Lodja, the majority of *An. gambiae* s.l. and *An. funestus* s.l. captured by PSC indoors between January and June were either unfed (37%) or blood-fed (54%), with very few half-gravid (9%) or gravid (1%) females. In Kapolowe, the majority of captured *An. gambiae* s.l. and *An. funestus* s.l.were blood-fed (89%).

		January	2016		
Species	Unfed	Blood-fed	Half-gravid	Gravid	Total PSC
An. gambiae s.l.	9	23	1	0	33
An. funestus s.l.	1	21	1	0	23
An. paludis	2	4	0	0	6
Total Jan	12	48	2	0	62
		February	2016		
An. gambiae s.l.	13	27	5	1	46
An. <i>funestus</i> s.l.	8	8	4	2	22
An. paludis	4	7	0	0	11
Total Feb	25	42	9	3	79
		March 2	2016		
An. gambiae s.l.	11	12	4	0	27
An. <i>funestus</i> s.l.	1	6	1	0	8
An. paludis	0	1	0	0	1
Total March	12	19	5	0	36
		April 2	016		
An. gambiae s.l.	9	16	2	0	27
An. funestus s.1.	0	5	4	0	9
An. paludis	4	2	0	0	6
Total April	13	23	6	0	42
		May 20	016		
An. gambiae s.l.	12	10	0	0	22
An. funestus s.l.	3	0	0	0	3
An. paludis	5	3	1	0	9
Total May	20	13	1	0	34
		June 2	016		1
An. gambiae s.l.	15	4	1	0	20
An. funestus s.1.	1	1	0	0	2
An. paludis	6	1	0	0	7
			1		1

Table 14: Monthly Indoor Resting Density of *Anopheles* species in Lodja (Sankuru) from PSC collections (8 houses per month).

Total June	22	6	1	0	29				
TOTAL Jan-June 2016									
An. gambiae s.l.	69 (39%)	92 (53%)	13 (7%)	1 (1%)	175				
An. funestus s.l.	14 (21%)	41 (61%)	10 (15%)	2 (3%)	67				
An. paludis	21 (53%)	18 (45%)	1 (3%)	0	40				
All Anopheles	104 (37%)	151 (54%)	24 (9%)	3 (1%)	282				

Table 15: Monthly Indoor Resting Density of *Anopheles* species in Kapolowe (Haut Katanga) from PSC collections (8 houses per month).

		Janua	ary 2016		
Species	Unfed	Blood-fed	Half-Gravid	Gravid	Total PSC
An. gambiae s.l.	2	60	0	0	62
An. funestus s.l.	1	49	0	0	50
Total January	3	109	0	0	112
		Febru	ary 2016		
An. gambiae s.l.	1	77	0	0	78
An. funestus s.l.	0	36	0	0	36
Total February	1	113	0	0	114
		Mare	ch 2016		·
An. gambiae s.l.	7	12	0	0	19
An. funestus s.l.	3	43	0	0	46
Total Mach	10	55	0	0	65
		Apr	il 2016		·
An. gambiae s.l.	1	4	1	0	6
An. funestus s.l.	17	100	3	1	121
Total April	18	104	4	1	127
		Mag	y 2016		
An. gambiae s.l.	1	1	0	0	2
An. funestus s.l.	10	38	0	0	48
Total May	11	39	0	0	50
	-	Jun	e 2016		
An. gambiae s.l.	0	1	0	0	1
An. funestus s.l.	4	21	2	1	28
Total June	4	22	2	1	29
		TOTAL Ja	an-June 2016	-	
An. gambiae s.l.	12 (7%)	155 (92%)	1 (1%)	0	168
An. funestus s.l.	35 (11%)	287 (87%)	5 (2%)	2 (1%)	329
Total	47 (9%)	442 (89%)	6 (1%)	2 (<1%)	497

10. CIRCUMSPOROZOITE INDEX

Table 16: *Plasmodium falciparum* circumsporozoite index by site and *Anopheles* species (collected by PSC)

SITES	SPECIES	NBR ANOPHELES TESTED	NBR ANOPHELES POSITIVE	% positive (95% CI)
KATANA	An. gambiae s.l.	137	7	5.1 (1.4-8.8)
KATANA	An. funestus s.l.	70	2	2.9 (0.1-6.8)
	An gambiae s.l.	145	7	4.8 (1.3-8.3)
KALEMIE	An. funestus s.l.	9	1	11.1 (0.1-31.6)
	An. nili	1	1	100
KINGASANI	An gambiae s.l.	101	4	4.0 (0.2-7.8)
	An. gambiae s.l.	84	4	4.8 (0.2-9.3)
MIKALAYI	An. funestus s.l.	149	3	2.0 (0.1-4.3)

KABONDO	An gambiae s.l.	142	5	3.5 (0.5-6.6)
	An. gambiae s.l.	62	7	11.3 (3.4-19.2)
KAPOLOWE	An. funestus s.l.	50	4	8.0 (0.5-15.5)
	An. paludis	165	1	0.6 (0.1-1.8)
	An gambiae s.l.	33	2	6.1 (0.1-14.2)
LODJA	An. funestus s.l.	24	0	0
	An. paludis	200	0	0
	An gambiae s.l.	704	36	5.1 (3.5-6.7)
OVERALL	An. funestus s.l.	302	10	3.3 (1.3-5.3)
	An. paludis	365	1	0.3 (0.1-0.8)

All *Anopheles* analyzed for the presence of circumsporozoites were captured by PSC. The mean *Plasmodium falciparum* sporozoite rate in DRC was 5.1% (95% CI; 3.5-6.7) in *An. gambiae* s.1. and 3.3% (95% CI; 1.3-5.3) in *An. funestus* s.1. Of 365 *An. paludis* analyzed, one from Kapolowe was positive, but this needs to be confirmed. This is the first evidence of *An. paludis* being a malaria vector from this study. In 2015, there were no positive samples from the 1,366 *An. paludis* that were tested.

$11. Insecticide \ susceptibility$

Table 17: Distribution in the sites of the status of An. gambiae s.l.
exposed to different insecticides in 2016.

Sentinel Sites	Insecticides	2016 Nbr Exposed (test)	2016 24 hrs % Mortality (95% CI)	2016 Status	2015 24 hrs % Mortality (95% CI)	2015 Status
	Bendiocarb 0,1	100	100	S	100	S
	Deltamethrin 0,05	100	100	S	98 (95-99)	S
Katana	Permethrin 0,75	100	100	s	92 (87-97)	PR
	Bendiocarb 0,1	100	100	S	100	S
	Deltamethrin 0,05	100	100	S	100	S
Kalemie	Permethrin 0,75	100	40 (30-50)	R	55 (45-65)	R
	Bendiocarb 0,1	100	100	S	100	S
	Deltamethrin 0,05	100	100	S	97 (94-98)	PR
Kingasani	Permethrin 0,75	100	21 (13-29)	R	91 (85-97)	PR
	Bendiocarb 0,1	100	100	S	100	S
Mikalayi	Deltamethrin 0,05	100	88 (82-94)	R	100	S

			36		30	R
	Permethrin 0,75	100	(27-45)	R	(21-39)	
	Bendiocarb 0,1	100	96	PR	100	S
Kabondo	Deltamethrin 0,05	100	76 (68-84)	R	85 (78-92)	R
	Permethrin 0,75	100	12 (6-18)	R	52 (42-62)	R
	Bendiocarb 0,1	100	100	S	100	S
	Deltamethrin 0,05	100	100	s	100	S
Kapolowe	Permethrin 0,75	100	100	S	53 (42-63)	R
	Bendiocarb 0,1	100	100	S	100	S
	Deltamethrin 0,05	100	100	S	100	S
Lodja	Permethrin 0,75	100	69 (60-78)	R	68 (59-77)	R

Notes: S = susceptibility R = resistance PR = possible resistance

There was no mortality in untreated negative control bioassays conducted in 2016. Bendiocarb 0.1% killed 100% of *An gambiae* s.l. in all sites except Kabondo where possible resistance was detected. *An. gambiae* s.l. resistance to permethrin was recorded in five sites (Kingasani, Kabondo, Mikalayi, Kalemie, and Lodja). Despite resistance to permethrin at high frequency, deltamethrin resistance was only recorded in Kabondo and Mikalayi. Permethrin resistance frequency remained stable between 2015 and 2016 in Kalemie, Kabondo, Mikalayi, and Lodja. In Kingasani (Kinshasa), the frequency of permethrin resistance increased from 91% (95% CI: 85-97) in 2015 to 21% (95% CI: 13-29) in 2016. Conversely in Kapolowe, resistance to permethrin was recorded in 2015, but there was full susceptibility in 2016. Results for pirimiphos-methyl are not presented as the dose tested (0.01%) was 25 times lower than the WHO recommended diagnostic dose.

12. INTENSITY OF PYRETHROID RESISTANCE OF ANOPHELES GAMBIAE S.L. IN KINSHASA

Table 18: Resistance intensity of *An. gambiae* s.l. in Kinshasa and Kasangulu to insecticides (deltamethrin and permethrin)

		Permethrin dose % Mortality (95% CI)					
Village	Nbr tested	1x	2x	5x	10x		
Control 40			44	O A	IOA		
Kasungulu	50	6 (1-13)	14 (4-24)	30 (17-43)	50 (36-64)		
Kinshasa a	rea						
Bu Village	50	2 (1-6)	4 (1-9)	18 (7-29)	48 (34-62)		
Kimpoko	50	6 (1-13)	12 (3-21)	26 (14-38)	66 (53-79)		
Kingasani	50	10 (2-18)	20 (9-31)	40 (26-54)	52 (38-66)		
Kinkole	50	4(1-9)	4 (1-9)	8 (1-16)	50 (36-64)		
Overall	200	4 (2-7)	10 (6-14)	23 (17-29)	54 (47-61)		

		Deltamethrin dose % Mortality (95% CI)					
Village	Nbr tested	1x	2 x	5x	10x		
Control 40			48	JX	10x		
Kasungulu	50	20 (9-31)	40 (26-54)	74 (62-86)	98 (94-99)		
Kinshasa a	rea						
Bu Village	50	30 (17-43)	46 (32-60)	54 (40-68)	56 (42-70)		
Kimpoko	50	12 (3-21)	34 (21-47)	46 (32-60)	64 (51-77)		
Kingasani	50	62 (49-76)	80 (69-91)	88 (79-97)	98 (94-99)		
Kinkole	50	4 (1-9)	22 (11-34)	42 (28-56)	74 (62-86)		
			46 (39-	·			
Overall	200	27 (21-33)	52)	58 (51-64)	73 (67-79)		

*Note-In each test, 25 were tested in an untreated control. Mortality was zero in all.

Mortality to permethrin was extremely low (<20%) in all sites for 1x and 2x times the diagnostic dose of permethrin. Although mortality increased for 5x and 10x times the diagnostic dose, approximately half the *An. gambiae* s.l. tested survived the 10x dose. However, there was large variation in mortality between replicates for all sites (See Annex B; confidence intervals did not take into account replicate variation). Although WHO susceptibility tests indicated full susceptibility to deltamethrin in Kingasani, there were survivors at 1x, 2x, 5x and 10x times the diagnostic dose in bottle bioassays. Distribution of LLINs is ongoing in December 2016. The quality of the baseline data needs to be scrutinized to determine whether the work plan for 2017 needs to be amended.

Species	Sites of capture							Total
species	Mikalayi	Kabondo	Katana	Lodja	Kalemie	Kingasani	Kapolowe	Total
An.	34	37	37	36	39	38	36	257
gambiae	34	57	57	50	39	50	50	231
An. melas	0	0	0	0	0	0	0	0
An.	0	0	0	0	0	0	0	0
arabiensis	0	0	0	0	0	0	0	0
Did not	6	3	3	4	1	2	4	23
amplify	0	5	5	4	T	4	+	20
Total	40	40	40	40	40	40	40	280

Table 19: Species identification using the method of Wilkins et al. (2006)

DNA was extracted from the wings and legs of 280 *An. gambiae* s.l. from all seven sites. Of these, 257 samples were *An. gambiae* and 23 failed to amplify. No *An. arabiensis* or *An. melas* were detected.

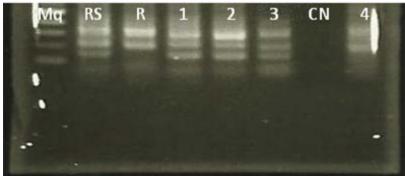
TABLE 20: Species identification to molecular forms using the method in Wilkins et al. (2006)

Molecular	Sites of capture								
Forms	Mikalayi	Kabondo	Katana	Lodja	Kalemie	Kingasani	Kapolowe	Total	
An.	2	0	1	0	6	3	2	14	
coluzzii	4	0	Ĩ	0	0	5	Ч	11	
An.									
gambiae	18	29	34	27	16	15	23	162	
s.s.									
Hybrids	14	8	2	9	17	20	11	81	
Total	34	37	37	36	39	38	36	257	

Out of the 257 DNA samples that were extracted, 63% (95% CI: 57-69) were confirmed as *An. gambiae* s.s., 5% *An. coluzzii* and 32% (95% CI: 26-37) hybrids of *An. gambiae* s.s. and *coluzzii*. The proportion of hybrids is unexpectedly high and most likely indicates that there are problems with the methodology. A similar result was also recorded in Mali in 2015 and was later

confirmed by collaborators at UC-Davis, USA to be a false result. University of Notre Dame, USA are currently testing samples from DRC using more recent techniques of Santolamazza et al. to determine whether this is an improved method.





Mq: marker RS: hybrid; R:resistant Samples hybrids (RS): 1; 2; 3. Resistant: 4 CN: negative control

Table 21: Frequency of Vgsc-1014F in An. gambiae s.l.

Anopheles	Sites of capture								
gambiae	Mikalayi	Kabondo	Katana	Lodja	Kalemie	Kingasani	Kapolowe	Total	
s.1.									
RR	13	12	3	0	1	4	2	35	
RS	0	0	7	1	6	4	1	19	
SS	0	0	3	3	1	0	1	8	
Did not	2	3	2	11	7	7	11	43	
amplify									
Total	15	15	15	15	15	15	15	105	

Out of the 105 samples analyzed for the presence of Vgsc-1014F, 41% (95% CI; 32-50) failed to amplify. This may be due to sample degradation due to inadequate storage (not all Eppendorf tubes contained silica gel). In the work plan for 2017, laboratory quality assurance training will be conducted by the

University of Notre Dame, which will include detailed procedures for sample storage.

14. Comparison of HLC, CDC-LT and Suna Trap for Determining Human Biting Rates in Kinshasa

Table 22: Comparison of An. gambiae s.l. and An. funestus s.l. catch size by method and location (indoors or outdoors).

Species	Method/Location	Nbr collected (% compared with HLC)	Total/Method
	HLC indoors	81	165
An. gambiae s.l.	HLC outdoors	84	165
	CDC-LT indoors	3 (4%)	2 (09/)
	CDC-LT outdoors	0	3 (2%)
	Suna Trap indoors	18 (22%)	00 (1997)
	Suna Trap outdoors	11 (13%)	29 (18%)
	HLC indoors	3	7
An. funestus	HLC outdoors	4	1
s.1.	CDC-LT indoors	0	1 (149/)
	CDC-LT outdoors	1 (25%)	1 (14%)

In May 2016 a total of 1,053 mosquitoes were captured using three methods: Suna Trap, CDC-LT and HLC in Kingasani (Kinshasa). HLC is considered the gold standard for measuring human biting rate. Two hundred and five specimens were identified as Anophelinae, and of these 197 were *An. gambiae* s.1. (96%; 197/205). HLC caught a much greater number of *Anopheles* both indoors and outdoors than the Suna Trap and CDC-LT combined. The number of *An. gambiae* s.1. and *An. funestus* s.1. captured by the CDC-LT was remarkably low. Based on these results, neither the CDC-LT nor the Suna Trap provided accurate measures of human biting exposure. HLC will therefore continue to be the method of choice in 2017 to determine human biting rates.

15.CONCLUSION

Species	Katana	Kalemie	Mikalayi	Kingasani	Kabondo	Kapolowe	Lodja
An.							
gambiae	+	+	+	+	+	+	+
s.1.							
An.	+	+	+	+	+	+	+
funestus	•			•	•	•	
An.	_		+	-	+	+	+
paludis						•	
An. nili	-	+	-	-	-	-	-
An.	-	-	+	-	_	_	_
implexus			•				

Table 23: Distribution and abundance of Anophelinae in the sites.

Five species of *Anophelinae* were caught in the sites: *An. gambiae* s.l., *An. funestus, An. paludis, An. nili and An. implexus* (see Table 27). *An. gambiae* s.l. and *An. funestus* s.l. were captured in all sites.

Out of all species captured, three are known malaria vectors: *An. gambiae* s.l., *An. funestus and An. nili.* In addition, *An. paludis* has been previously found to be a malaria vector in Kwango Province (Karch, 1992). *An. gambiae* s.l. and *An. funestus* were present in all the sites. *An. paludis* was captured in three sites (Mikalayi, Kabondo, Kapolowe, and Lodja). *An. nili* is recognized as a malaria vector in certain areas, including Kinshasa. *An. gambiae* s.l. continued to be the predominant malaria vector caught during 2016 through human landing catch (HLC) and pyrethrum spray catch (PSC) in most sentinel sites. The exceptions were in Katana (Sud Kivu) and Mikalayi (Kasaï) where more *An. funestus* s.l. were captured than *An. gambiae* s.l. The proportion of *An.*

funestus s.l. captured increased substantially in Kapolowe (Haut Katanga), Katana, Kingasani (Kinshasa) and Mikalayi compared to 2015.

The mean *Plasmodium falciparum* sporozoite rate in the Democratic Republic of Congo (DRC) was 5.1% (95% CI; 3.5-6.7) in *An. gambiae* s.l. and 3.3% (95% CI; 1.3-5.3) in *An. funestus* s.l.

World Health Organization (WHO) cylinder tests showed *An. gambiae* s.l. was fully susceptible to bendiocarb (0.1%) at six sites, with possible resistance in Kabondo (Tshopo). A high frequency of resistance to permethrin (0.75%) was recorded in five of the seven sites. Despite a high frequency of permethrin resistance, full susceptibility was recorded to deltamethrin (0.05%) in Kingasani, Kalemie (Tanganyika) and Lodja (Sankuru). A sub-sample of specimens was tested for the presence of the voltage-gated sodium channel (*V*gsc) 1014F allele; however 41% of samples failed to amplify. *An. funestus* s.l. is likely to be an important malaria vector in DRC, and testing in 2017 should be conducted to determine resistance frequencies and mechanisms.

The mean *An. gambiae* s.l. indoor human biting rate in 2016 varied between two bites per person per night in Katana up to 30 bites per person per night in Kabondo. Comparison of biting rates could not be made between regions due to the relatively small number of houses sampled. There was significant outdoor biting potential with outdoor biting rates similar to indoor biting rates for both *An. gambiae* s.l. and *An. funestus* s.l. The highest outdoor biting rates were in Kabondo for *An. gambiae* s.l. with 20 bites per person per night. In general, indoor biting by *An. gambiae* s.l. and *An. funestus* s.l. was primarily late at night between 22:00 and 05:00, and mirrored outdoor biting tends. As documented in 2015, *An. paludis* biting in Lodja was focused outdoors with an intense peak between 19:00 and 20:00, followed by a gradual decline until midnight. *An. gambiae* s.l was the dominant species collected both indoors and outdoors through PSC and HLC in Kalemie, Kingasani, and Kabondo. In Katana and Kapolowe, *An. funestus* s.l. was the most common species collected resting indoors by PSC, although *An. gambiae* s.l. was the predominant species caught indoors through HLC. This may indicate either that *An. gambiae* s.l. exit houses earlier than *An. funestus* s.l., or that *An. funestus* s.l. are entering houses to rest after feeding elsewhere. Few *An. paludis* in Kapolowe and Lodja were collected by PSC, confirming its exophilic tendencies. *An. paludis* in Lodja was primarily exophagic, with the majority of human biting occurring outdoors during the early evening. This is in contrast to Kapolowe, where a similar proportion of *An. paludis* were captured by HLC both indoors and outdoors. Sequencing data collected in partnership with University of Notre Dame from 2015 indicates the probable presence of at least one sub-species of *An. paludis*.

As the majority of malaria vectors at sentinel sites were biting indoors and late at night, use of long-lasting insecticide-treated nets (LLINs) should provide some protection in all locations. There was a significant amount of outdoor biting, but this was mostly late at night and the level of importance will depend on local nighttime behaviors.

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ANNEX A: ABDOMINAL STATUS OF INDOOR RESTING MOSQUITOES CAUGHT BY PSC

A1.1: SUD KIVU PROVINCE, KATANA SITE

Table 24: Blood digestion stage of malaria vectors collected in Kalemie during January - June 2016 using PSC.

Site	Katana							
Period	January – June 2016							
Method	PSC 1							
Species	Fed	Unfed	Half- gravid	PSC				
	January	– Febru	ary 2016	,				
An. gambiae s.l.	81	9	3	1	94			
An. funestus s.l.	63	4	0	3	70			
Total	144	13	3	4	164			
	Marcl	h – April	2016					
An. gambiae s.l.	24	2	0	3	29			
An. funestus s.l.	101	3	2	4	110			
Total	125	5	2	7	139			
	Мау	– June	2016					
An. gambiae s.l.	9	4	1	0	14			
An. funestus s.l.	13	6	0	0	19			
Total	22	10	1	0	33			
All three	e period	s : Janu	ary – Ju	ne 2016				
An. gambiae s.l.	114	15	4	4	137			
An. funestus s.l.	177	13	2	7	199			
TOTAL	291	28	6	11	336			

A1.2: TANGANYIKA PROVINCE, KALEMIE SITE

Table 25: Blood digestion stage of malaria vectors collected in Kalemieduring January – June 2016 using Anopheles captured by PSC

Site		KALEMIE					
Period		January-February 2016					
Method			PSC		Madal DSO		
Species	Fed	Unfed	Gravid	Half-Gravid	Total PSC		
		Ja	anuary – Fel	oruary 2016			
An. gambiae s.l.	79	2	0	0	81		
An. funestus s.l.	7	2	0	0	9		
An. nili	1	0	0	0	1		
Total 1	87	4	0	0	91		
			March – A	pril 2016			
An. gambiae s.l.	43	13	7	1	64		
An. funestus s.l.	7	2	2	0	11		
Total 2	50	15	9	1	75		
			May – Ju	ne 2016			
An. gambiae s.l.	8	1	1	1	11		
An. funestus	4	0	0	0	4		
Total	12	1	1	1	15		
		All per	riods : Janu	ary – June 2010	б		
An. gambiae s.l.	130	16	8	2	156		
An. funestus s.l.	18	4	2	0	24		
An. nili	1	0	0	0	1		
GENERAL TOTAL	149	20	10	2	181		

A1.3: KASAI CENTRAL PROVINCE, MIKALAYI SITE

Table 26: Blood digestion stage of malaria vectors collected in Kalemieduring January – June using Anopheles captured by PSC.

Site			MIKAL	AYI					
Period	January – June 2016								
Method			PSC						
January – February 2016									
Species	Fed	Unfed	Half-gravid	Gravid	Total				
An. gambiae s.l.	38	6	0	1	45				
An. funestus s.l.	98	47	0	3	148				
Total 1	136	53	0	4	193				
	N	Iarch –	April 2016						
An. gambiae s.l.	33	2	0	0	35				
An. funestus s.l.	55	1	0	0	56				
An. paludis	0	1	0	0	1				
An. implexus	1	0	0	0	1				
Total 2	89	4	0	0	93				
		May – J	une 2016						
An. gambiae s.1.	5	0	0	0	5				
An. funestus s.l.	58	8	0	0	66				
Total 3	63	8	0	0	71				
All tl	hree Pe	eriods : .	January – Ju	ne 2016					
An. gambiae s.l.	76	8	0	1	85				
An. funestus s.l.	211	56	0	3	270				
An. paludis	0	1	0	0	1				
An. implexus	1	0	0	0	1				
GENERAL TOTAL	288	65	0	4	357				

A1.4: KINSHASA PROVINCE, KINGASANI SITE

Table 27: Blood digestion stage of malaria vectors collected in Kingasani
during January – June 2016 using Anopheles captured by PSC.

Site	KINGASANI						
Period	January – June 2016						
Method			PSC				
Species	Fed	Unfed	Half-Gravid	Gravid	Total PSC		
	Ja	nuary –	February 201	6	·		
An. gambiae s.l.	11	0	0	0	11		
Total	11	0	0	0	11		
			March – Ap	ril 2016			
An. gambiae s.l.	85	1	3	1	90		
An. funestus s.l.	39	2	0	2	43		
Total	124	3	3	3	133		
		May -	June 2016		·		
An. gambiae s.l.	254	11	14	0	279		
An. funestus s.l.	67	4	3	0	74		
Total	321	15	17	0	353		
	All thr	ee perio	ds : January –	June	·		
An. gambiae s.l.	350	12	17	1	380		
An. funestus s.l.	106	6	3	2	117		
GENERAL TOTAL	456	18	20	3	497		

A1.5: TSHOPOPROVINCE, KABONDO SITE

Table 28: Blood digestion stage of malaria vectors collected in Kabondoduring January - June 2016 using Anopheles captured by PSC.

Site	KABONDO							
Period		January – June 2016						
Method	PSC	PSC						
Species	Fed	Unfed Half gravid G		Gravid	Total			
		January	- February	7				
<i>An. gambiae</i> s.1.	45	33	0	0	78			
An. paludis	0	1	0	0	1			

Total 1	45	34	0	0	79				
March – April 2016									
An. gambiae s.l.	31	25	7	0	63				
An. funestus s.l.	2	1	0	0	3				
Total 2	33	26	7	0	66				
		May – J	une 2016						
An. gambiae s.l.	52	16	6	0	74				
<i>An. funestus</i> s.1.	2	0	0	0	2				
Total 3	54	16	6	0	76				
A11	three pe	eriods :	January –	June 2016					
An. gambiae s.l.	128	74	13	0	215				
An ; funestus s.l.	2	0	0	0	2				
An. paludis	2	2	0	0	4				
GENERAL TOTAL	132	76	13	0	221				

A1.6: HAUT KATANGA PROVINCE, KAPOLOWE SITE

Table 29: Blood digestion stage of malaria vectors collected in Kapoloweduring January - June 2016 using Anopheles captured by PSC.

Site		Kapolowe								
Period	January 2016									
Method		PSC								
Species	Fed	Fed Unfed Half gravid Gravid Total								
	Januar	ry – Februa	ry 2016							
An. gambiae s.l.	60	2	0	0	62					
An. funestus s.l.	49	1	0	0	50					
General Total	109	109 3 0 0 112								
	Mar	ch – April	2016							
An. gambiae s.l.	12	7	0	0	19					
An. funestus s.l.	43	3	0	0	46					
Total 2	55	10	0	0	65					
May – June 2016										
An. gambiae s.l.	4	1	1	0	6					
An. funestus s.l.	100	17	3	1	121					

Total 3	104	18	8 4		127			
All three periods : January – June 2016								
An. gambiae s.l.	76	10	1	0	87			
An. funestus s.l.	192	21	3	1	217			
Total 3	268	31	4	1	304			

A1.7: SANKURU PROVINCE, LODJA SITE

Table 30: Blood digestion stage of malaria vectors collected in Lodja during January - June 2016 using Anopheles captured by PSC.

Site	LODJA							
Period	January 2016							
Method	PSC							
Species	feld	Unfeld	Half gravid	Gravid	Total			
January – February 2016								
An. gambiae s.l.	23	9	0	1	33			
An. funestus s.l.	21	1	0	1	23			
An. paludis	4	2	0	0	6			
Total	48	12	0	2	62			
	Ma	rch – April	2016					
An. gambiae s.l.	12	11	0	4	27			
An. funestus s.l.	6	1	0	1	8			
An. paludis	1	0	0	0	1			
Total	19	12	0	5	36			
	Ma	ay – June 2	2016		•			
An. gambiae s.l.	16	9	2	0	27			
An. funestus s.l.	5	0	4	0	9			
An. paludis	2	4	0	0	6			
General Total	23	13	6	0	42			
All th	All three periods : January – June 2016							
An. gambiae s.l.	51	29	2	5	87			
An. funestus s.l.	32	2	4	2	40			
An. paludis	7	6	0	0	13			
GENERAL TOTAL	90	37	6	7	140			

ANNEX B: INTENSITY OF PYRETHROID RESISTANCE OF ANOPHELES GAMBIAE S.L. IN KINSHASA

Table 31: Results of resistance intensity bottle bioassays for An. gambiaes.l. in Kinshasa and Kasangulu to deltamethrin and permethrin

Sites	Insecticide	Concentration	Control Exposed	Control Died	Nbr Exposed (test)	Observed 30 min Mortality
Bu	Permethrin	1X	25	0	25	1%
Bu	Permethrin	2X	25	0	25	1%
Bu	Permethrin	5X	25	0	25	1%
Bu	Permethrin	10X	25	0	25	1%
Bu	Deltamethrin	1X	25	0	25	8%
Bu	Deltamethrin	2X	25	0	25	10%
Bu	Deltamethrin	5X	25	0	25	10%
Bu	Deltamethrin	10X	25	0	25	12%
Bu	Permethrin	1X	25	0	25	3%
Bu	Permethrin	2X	25	0	25	7%
Bu	Permethrin	5X	25	0	25	33%
Bu	Permethrin	10X	25	0	25	93%
Bu	Deltamethrin	1X	25	0	25	52%
Bu	Deltamethrin	2X	25	0	25	82%
Bu	Deltamethrin	5X	25	0	25	96%
Bu	Deltamethrin	10X	25	0	25	100%
Kasangulu	Permethrin	1X	25	0	25	0%
Kasangulu	Permethrin	2X	25	0	25	0%
Kasangulu	Permethrin	5X	25	0	25	0%
Kasangulu	Permethrin	10X	25	0	25	0%
Kasangulu	Deltamethrin	1X	25	0	25	16%
Kasangulu	Deltamethrin	2X	25	0	25	23%
Kasangulu	Deltamethrin	5X	25	0	25	48%
	Deltamethrin	10X	25	0	25	97%
	Permethrin	1X	25	0	25	13%
Kasangulu	Permethrin	2X	25	0	25	28%

Kasangulu	Permethrin	5X	25	0	25	60%
	Permethrin	10X	25	0	25	98%
Kasangulu	Deltamethrin	1X	25	0	25	24%
	Deltamethrin	2X	25	0	25	58%
Kasangulu	Deltamethrin	5X	25	0	25	99%
Kasangulu	Deltamethrin	10X	25	0	25	100%
Kinshasa	Permethrin	1X	25	0	25	0%
Kinshasa	Permethrin	2X	25	0	25	0%
Kinshasa	Permethrin	5X	25	0	25	0%
Kinshasa	Permethrin	10X	25	0	25	4%
Kinshasa	Deltamethrin	1X	25	0	25	42%
Kinshasa	Deltamethrin	2X	25	0	25	69%
Kinshasa	Deltamethrin	5X	25	0	25	78%
Kinshasa	Deltamethrin	10X	25	0	25	96%
Kinshasa	Permethrin	1X	25	0	25	21%
Kinshasa	Permethrin	2X	25	0	25	40
Kinshasa	Permethrin	5X	25	0	25	80
Kinshasa	Permethrin	10X	25	0	25	100%
Kinshasa	Deltamethrin	1X	25	0	25	80%
Kinshasa	Deltamethrin	2X	25	0	25	90%
Kinshasa	Deltamethrin	5X	25	0	25	98%
Kinshasa	Deltamethrin	10X	25	0	25	100%
Kimpoko	Permethrin	1X	25	0	25	0%
Kimpoko	Permethrin	2X	25	0	25	0%
Kimpoko	Permethrin	5X	25	0	25	0%
Kimpoko	Permethrin	10X	25	0	25	55%
Kimpoko	Deltamethrin	1X	25	0	25	0%
Kimpoko	Deltamethrin	2X	25	0	25	13%
Kimpoko	Deltamethrin	5X	25	0	25	23%
Kimpoko	Deltamethrin	10X	25	0	25	29%
Kimpoko	Permethrin	1X	25	0	25	13%
Kimpoko	Permethrin	2X	25	0	25	23%
Kimpoko	Permethrin	5X	25	0	25	50%
Kimpoko	Permethrin	10X	25	0	25	78%
Kimpoko	Deltamethrin	1X	25	0	25	23%
Kimpoko	Deltamethrin	2X	25	0	25	53%
Kimpoko	Deltamethrin	5X	25	0	25	70%
Kimpoko	Deltamethrin	10X	25	0	25	100%
Kinkole	Permethrin	1X	25	0	25	2%
Kinkole	Permethrin	2X	25	0	25	6%
Kinkole	Permethrin	5X	25	0	25	8%

Kinkole	Permethrin	10X	25	0	25	82%
Kinkole	Deltamethrin	1X	25		25	3%
Kinkole	Deltamethrin	2X	25		25	24%
Kinkole	Deltamethrin	5X	25		25	48%
Kinkole	Deltamethrin	10X	25		25	78%
Kinkole	Permethrin	1X	25	0	25	4%
Kinkole	Permethrin	2X	25	0	25	2%
Kinkole	Permethrin	5X	25	0	25	7%
Kinkole	Permethrin	10X	25	0	25	18%
Kinkole	Deltamethrin	1X	25	0	25	3%
Kinkole	Deltamethrin	2X	25	0	25	19%
Kinkole	Deltamethrin	5X	25	0	25	35%
Kinkole	Deltamethrin	10X	25	0	25	71%