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THE PMI VECTORLINK CAMBODIA ENTOMOLOGICAL MONITORING ANNUAL REPORT

OCTOBER 1, 2020–NOVEMBER 30, 2021

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

CDN	Cow-baited double-net trap
CDC	U.S. Centers for Disease Control and Prevention
CNM	National Center for Parasitology, Entomology and Malaria Control
CSP	Circum-sporozoite protein
ELISA	Enzyme-linked immunosorbent assay
FTT	Furvela tent trap
HDN	Human-baited double-net trap
LLIN	Long-lasting insecticidal net
P.	*Plasmodium*
PCR	Polymerase Chain Reaction
PHD	Provincial Health Department
PMI	President's Malaria Initiative
SOP	Standard Operating Procedure
WHO	World Health Organization

EXECUTIVE SUMMARY

Entomological longitudinal monitoring and insecticide resistance monitoring of malaria vectors were the main activities VectorLink Cambodia performed in collaboration with the National Center for Parasitology, Entomology and Malaria Control (CNM) from October 2020 to November 2021. Initial training on morphological identification and field trapping methods for the VectorLink Cambodia team, CNM, and Cambodia Provincial Health Department (PHD) staff was completed in 2020 before the start of the entomological monitoring. Monthly longitudinal entomological monitoring was completed for 12 months during the reporting period (except in March and April 2021, due to COVID-19 travel restrictions) in four sites comprising an annex village site and a forest fringe site in each of Mondulkiri and Stung Treng provinces. The reporting period therefore represents 12 months of collections, but not 12 consecutive calendar months.

Four different trapping methods were used for collections done in the annex village sites: outdoor cow-baited double-net traps (CDNs), outdoor human-baited double-net traps (HDNs), outdoor Furvela tent traps (FTTs), and indoor U.S. Centers for Disease Control and Prevention (CDC) light traps. Two of these trapping methods were also used for collections in the forest fringe sites: outdoor HDN collections and outdoor FTT collections. Insecticide resistance monitoring was started in the four sites in September 2021, after the start of the rainy season.

Anopheles dirus, An. minimus s.l., and *An. maculatus* s.l. are the primary malaria vectors in Cambodia. In Mondulkiri and Stung Treng, all three of these vector species were proportionately more prevalent in the forest fringe sites than the annex village sites, and *An. dirus* was especially predominant in the forest. In the two annex village sites from all trapping methods combined, only 851 *An. dirus* (4.0% of all mosquitoes collected), 781 *An. maculatus* s.l. (3.6%), and 1,034 *An. minimus* s.l. (4.8%) were collected, whereas in the two forest fringe sites 1,303 *An. dirus* (47.4%), 225 *An. maculatus* s.l. (8.2%), and 165 *An. minimus* s.l. (6.0%) were caught. Of the four trapping methods deployed in the two annex village sites, CDNs collected the largest number of species and the highest numbers of *Anopheles* compared with the three other trapping techniques. In Mondulkiri annex village, CDNs also caught higher numbers of the three primary vector species than the other methods. This was also true for *An. minimus* s.l. and *An. maculatus* s.l. in the Stung Treng annex village, but not for *An. dirus*, which was caught in greater numbers using HDNs and CDC light traps. In all sites (village and forest), FTTs collected the lowest numbers of *Anopheles* and smallest numbers of species.

Because of the interruption of field collections due to COVID-19, it was not possible to record a complete picture of seasonal trends in vector abundance in relation to the rainy and dry seasons. Nevertheless, in both provinces, an increase of *An. dirus* was observed from August with the arrival of the rainy season in both forest and annex village sites. For *An. maculatus* s.l., the increase was 1–2 months later and only apparent in Mondulkiri as this species was not common in Stung Treng. For *An. minimus* s.l., peak densities were recorded in February, notably different from the other two primary vector species.

Among the three primary vectors, the highest human biting rates at all sites (estimated from HDNs) were observed in *An. dirus* in September 2021, ranging from 43.3 to 64.0 bites per human per night. The highest cow biting rate was observed in *An. minimus* s.l. in February (60 bites per cow per night in Mondulkiri Province). There was a clear spatial difference between the two provinces – *An. minimus* s.l. and *An. maculatus* s.l. cow and human biting rates were much higher in Mondulkiri compared to Stung Treng.

A clear pattern in the preferred human biting times for *An. dirus* was observed: peak biting was from 19:00 to 22:00 in Mondulkiri (annex village and forest sites) and from 19:00 to 20:00 in the forest fringe site of Stung Treng. A peak in cow biting activity for *An. minimus* s.l. was observed from 19:00 to 22:00 in the annex village

site of Mondulkiri Province. A clear biting pattern was not established for *An. maculatus* s.l. in any of the sites for either human or cow hosts.

All three primary vectors from the annex village site of Mondulkiri were susceptible to deltamethrin (0.05%). *An. dirus* from the forest fringe site of Mondulkiri was susceptible to the diagnostic doses of alpha-cypermethrin (0.05%), deltamethrin (0.05%), and permethrin (0.75%). In the forest fringe site of Stung Treng, *An. dirus* was shown to be susceptible to alpha-cypermethrin (0.05%). Testing of the other pyrethroids against other primary vectors from Stung Treng has not yet been completed. The only species tested that was found to be resistant to pyrethroids was the secondary vector, *An. peditaeniatus*, from the annex village site of Stung Treng. This might be related to their preference for breeding in nearby rice fields that have been treated with insecticides.

Molecular laboratory analyses of the collected *Anopheles* mosquitoes were delayed by requirements for export permits and documentation of compliance with the Nagoya Protocol. As a result of this delay, the molecular laboratory analyses will be included in the future as an addendum to this report. Future molecular analyses will be performed in Cambodia after establishment of in-country molecular laboratory capacity in Year 4.

I. INTRODUCTION

The U.S. President's Malaria Initiative (PMI) VectorLink Project began entomological monitoring activities in Cambodia in October 2019. The project's primary objective is to support entomological surveillance focused on higher-burden geographical areas, with an emphasis on improved insecticide resistance monitoring and routine vector surveillance using a range of trapping techniques.

Cambodia has experienced a significant reduction in malaria morbidity and mortality over the last 10 years. Nevertheless, an estimated 58% of the population, or approximately 9.3 million people, in 55 operational districts remain at risk, with the highest transmission risk in forested or forest-fringe areas in the northeastern part of the country. Malaria cases in Cambodia are caused by *Plasmodium falciparum, P. vivax*, or a mix of both species, and the main malaria vectors are *An. dirus, An. minimus* s.l., and *An. maculatus* s.l. Malaria transmission occurs primarily in the hot and rainy season, between July and November. *An. dirus* is found in forested mountains and foothills, cultivated forests, and rubber plantations, whereas *An. minimus* s.l. is found outside the forests or in areas that have been cleared of forest. *An. maculatus* s.l. is found in hilly or mountainous areas, and breeds in or near permanent or semi-permanent bodies of clean water such as streams or rivers.[1]

The specific objectives of VectorLink Cambodia are as follows:

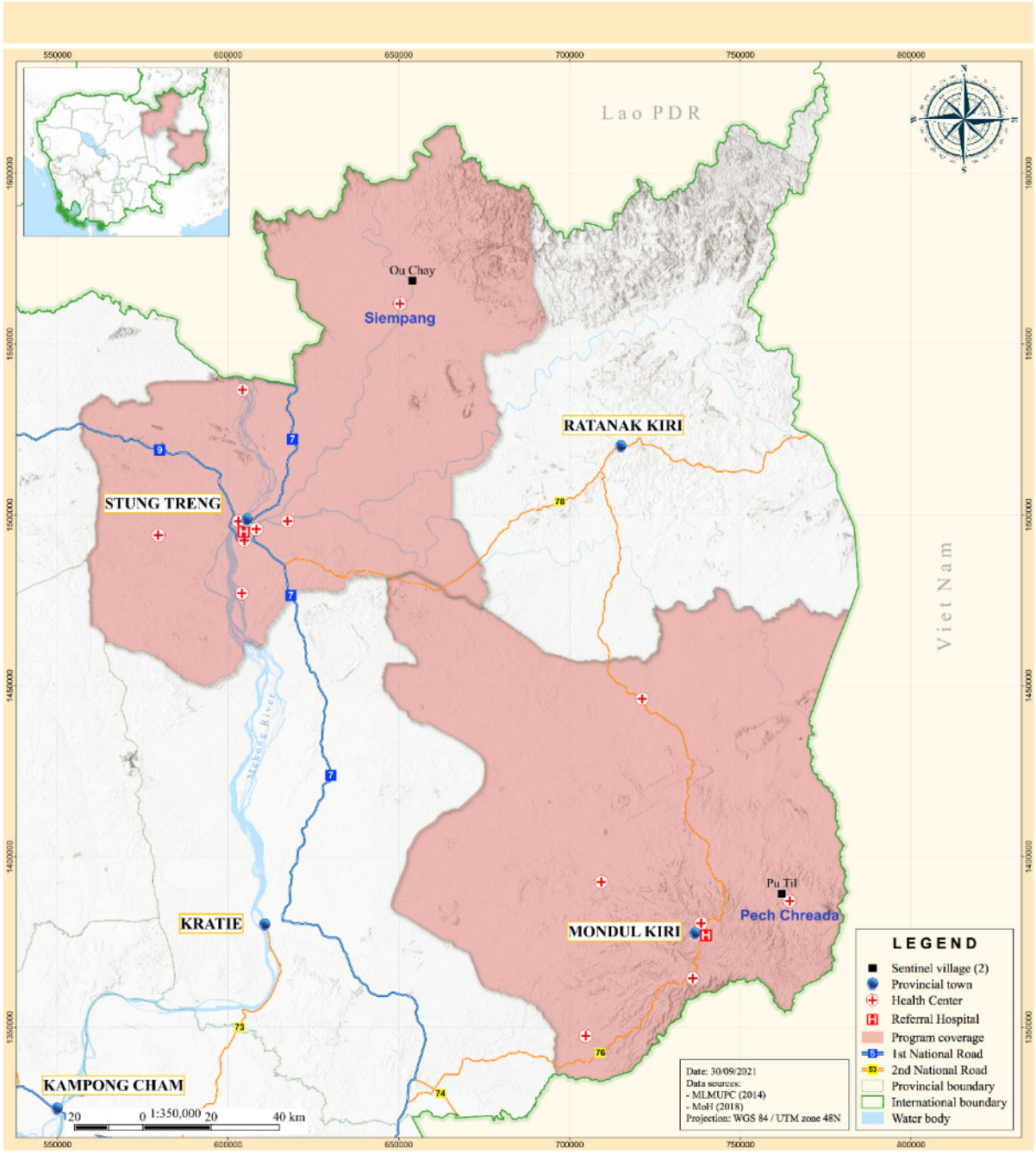
- To characterize *Anopheles* species composition, density, seasonality, and biting behavior
- To determine the insecticide susceptibility of the main malaria vectors to pyrethroid insecticides
- Molecular analysis of *Anopheles* mosquito samples, generating sporozoite rates using circumsporozoite protein (CSP) enzyme-linked immunosorbent assay (ELISA) for *P. falciparum* and *P. vivax,* and determining species identification by polymerase chain reaction (PCR).

Initial training on mosquito species identification was conducted in Phnom Penh for CNM, PHD, and PMI VectorLink Cambodia staff in August 2020, followed by a field training program for trapping techniques conducted in Stung Treng Province in September 2020. On-the-job training for CNM, PHD, and health center staff was carried out when monthly collections were done in the provinces.

Entomological monitoring was conducted from October 2020 to November 2021 in two provinces of relatively high malaria transmission, Stung Treng and Mondulkiri (Figure 1), which have an Annual Parasite Index of 20–50 and >50, respectively. VectorLink Cambodia, in partnership with CNM and the two PHDs and their operational districts, conducted monthly longitudinal entomological monitoring in an annex village and a forest site in the each of the provinces. Between September 2021 and November 2021, insecticide susceptibility testing of the three primary malaria vector species and one secondary vector species, against pyrethroid insecticides, was conducted in the two sites (annex village and forest) in each province.

[1] Sinka, ME, Bangs, MJ, Manguin, S. et al. 2011. The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. *Parasites & Vectors* 4, No. 89.

FIGURE 1: ENTOMOLOGICAL MONITORING SITES



2. METHODOLOGY

2.1 SAMPLING SITES AND COLLECTION METHODS

Two sites in each province were selected for monthly monitoring: one in the forest fringe and one in an annex village close to the forest (Figures 2 and 3). Annex villages are informal settlements, often geographically isolated, established because of population movement. The annex village of Pu Til in Pech Chreada District of Mondulkiri Province and the annex village of Ou Chay in Siem Pang District of Stung Treng Province were each paired with a nearby forest fringe site approximately 1.5 to 2 kilometers away. Villagers enter the forest fringe sites for farming, cutting trees, hunting, and other activities.

Monthly entomological monitoring was conducted using outdoor CDNs, HDNs, and FTTs, and indoor CDC light traps (Table 1). One CDN, one HDN, and one FTT were set up outdoors within the annex villages for three consecutive nights each month. CDC light traps were set indoors in four houses in each annex village for three consecutive nights on a monthly basis. In each forest site, three consecutive nights of trapping using one HDN and one FTT were conducted monthly.

FIGURE 2. MAP OF TRAPPING LOCATIONS FOR MONTHLY COLLECTIONS, PU TIL VILLAGE AND THE FOREST FRINGE, MONDULKIRI.

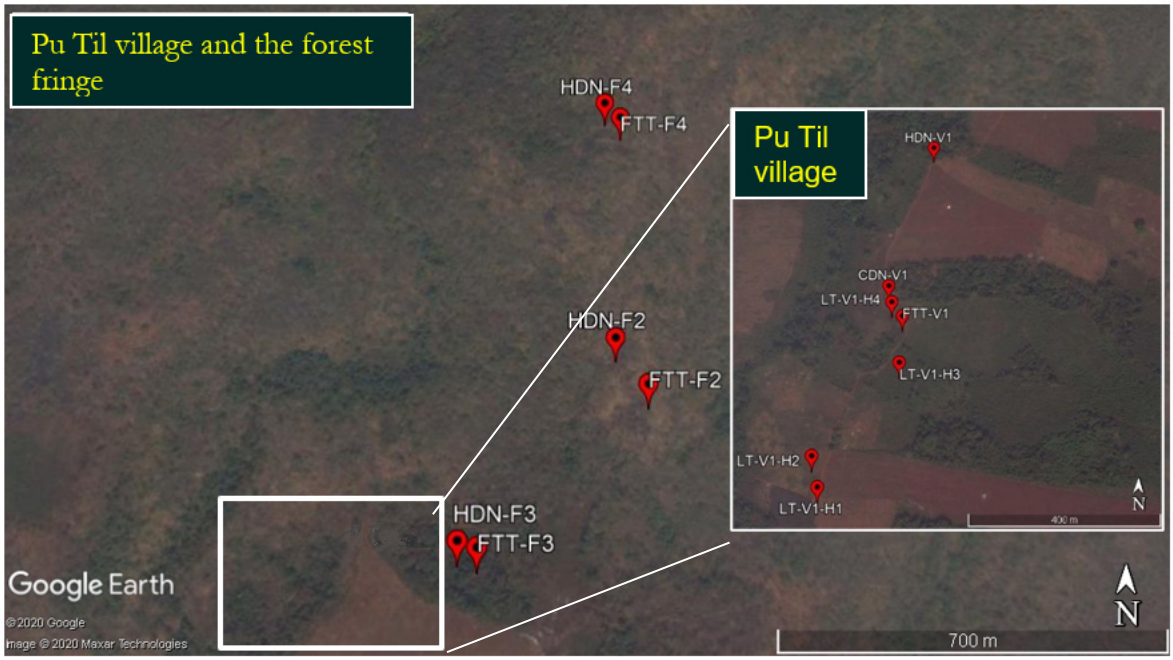


FIGURE 3. MAP OF TRAPPING LOCATIONS FOR MONTHLY COLLECTIONS, IN THE OU CHAY ANNEX VILLAGE AND THE FOREST FRINGE, STUNG TRENG

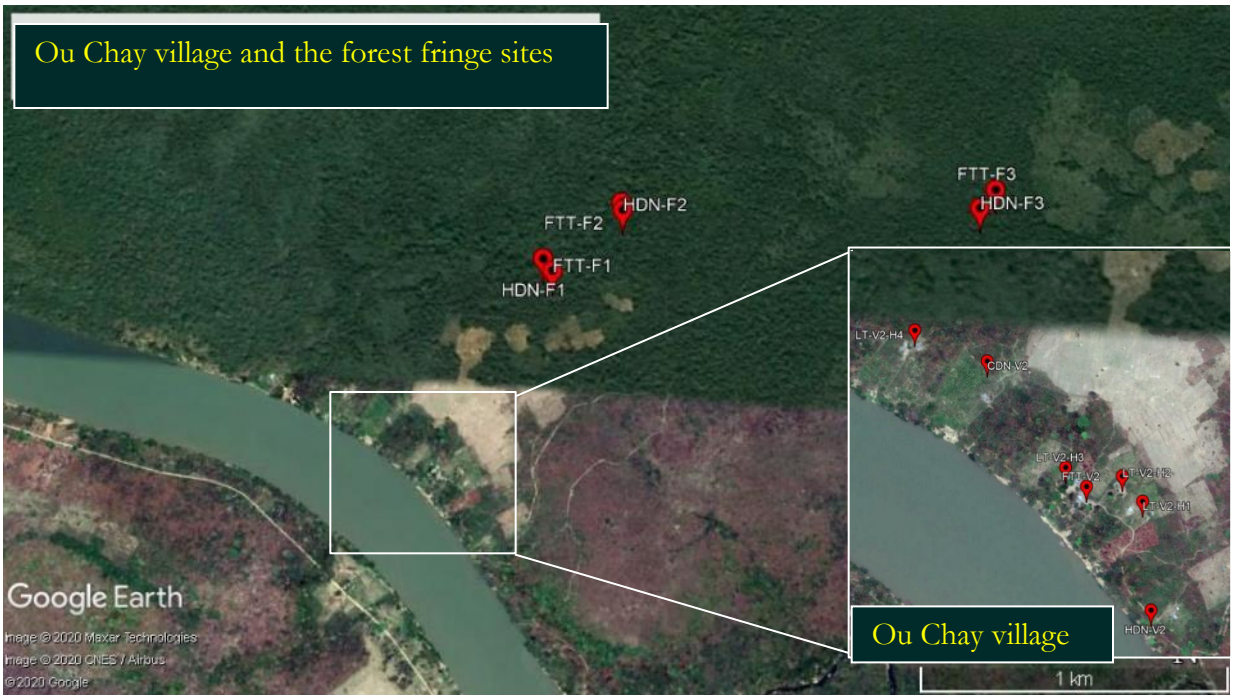


TABLE 1: ADULT MOSQUITO COLLECTION METHODS USED FOR LONGITUDINAL ENTOMOLOGICAL MONITORING

Collection method	Time	Frequency and Site	Sample	Indicators
CDN	Hourly collections 6:00 p.m. to 6:00 a.m.	Monthly for annex village sites only	Three traps/month/site	Outdoor cattle biting rate: number per trap per hour
HDN	Hourly collections 6:00 p.m. to 6:00 a.m.	Monthly for all sites	Three traps/month/site	Outdoor human biting rate: number per trap per hour
FTT	Hourly collections 6:00 p.m. to 6:00 a.m.	Monthly for all sites	Three traps/month/site	Outdoor human biting rate: number per trap per hour
Indoor CDC Light trap	6:00 p.m. to 6:00 a.m.	Monthly for annex village sites only	Twelve traps/month/site	Indoor human biting rate: number per trap

2.1.1 CDNs

CDNs were used outdoors from October 2020. They were set up in the annex village sites only, because it was difficult to obtain cows to use in the forest sites. The cow was kept inside the double net from sunset to dawn. Collections were carried out using glass tubes to catch the mosquitoes trapped inside the outer net. Hourly collections were made according to PMI VectorLink Standard Operating Procedure (SOP) 01/01. From June 2021, after each hour of manual collection of mosquitoes, a Prokopack aspirator was used to catch any remaining mosquitoes inside the trap, to check the efficiency of the collectors using the glass tubes.

2.1.2 HDNs

HDN collections were conducted outdoors from October 2020. They were set up in the annex village sites and in the forest sites. A human volunteer slept inside the double net from sunset to dawn. Collections were carried out using glass tubes to catch the mosquitoes trapped inside the outer net. Hourly collections were made according to PMI VectorLink SOP 02/01. From June 2021, after each hour of manual collection of mosquitoes, a Prokopack aspirator was used to catch the remaining mosquitoes inside the trap.

2.1.3 FTTs

FTT collections were conducted outdoors from October 2020 in the annex village sites and forest sites. A human host was protected from mosquito bites inside the tent. Mosquitoes were attracted to the odor and exhaled gases of the sleeper inside the trap, which emanate from a gap the diameter of a CDC trap, in the zipped-up door of the tent. The mosquitoes trying to enter the tent are sucked into a CDC trap conical collection bag (without the light, lid, or grid) positioned horizontally outside the tent, between 2 and 3 centimeters from the opening in the door. Hourly collections were made using different the collection bag using mouth aspirators in accordance with PMI VectorLink SOP 04/01.

2.1.4 CDC Light Traps

Indoor CDC light trap collections were conducted from October 2020. These traps were set up in four annex-village houses' bedrooms next to a volunteer who would sleep under an existing long-lasting insecticide-treated net (LLIN). Mosquitoes trapped inside the collection cup were collected the following morning, in accordance with PMI VectorLink SOP 03/01.

2.1.5 Morphological Identification of *Anopheles* Mosquitoes and Storage

All *Anopheles* mosquitoes collected using the four methods were identified morphologically and cross-checked by the VectorLink Cambodia entomology supervisory team in the field. Female *Anopheles* mosquitoes collected by CDC light traps were classified according to the four abdominal stages (unfed, fed, gravid, and half-gravid). All *Anopheles* were initially preserved in 1.5 mL Eppendorf tubes with silica gel and transferred later to a freezer for preservation for future molecular laboratory analysis.

2.2 Insecticide Resistance Monitoring

The VectorLink Cambodia entomology team and CNM conducted insecticide susceptibility testing from September 2021 through November 2021 following PMI VectorLink SOP 06/01, based on the World Health Organization (WHO) susceptibility tube test procedure. Adult females of *An. dirus, An. maculatus* s.l., and *An. minimus* s.l. collected by the HDN and CDN methods were used.

The tests prioritized pyrethroids of the LLINs used in the annex villages in both provinces. In Mondulkiri Province, the LLINs used in the annex village houses are PermaNet 2.0 (deltamethrin), SafeNet (alpha-cypermethrin), and Yahe (deltamethrin). In Stung Treng Province, the LLINs used in the annex village houses are SafeNet and PermaNet 2.0, with a majority being SafeNet. Thus, in Mondulkiri, testing priority was given to deltamethrin then alpha-cypermethrin, whereas in Stung Treng, priority was given to alpha-cypermethrin. The exposure to diagnostic dose test papers was 60 minutes, and mortality was recorded 24 hours after exposure.

Interpretation of the results followed WHO guidance, i.e., 98–100% mortality is defined as susceptibility, 90–97% mortality as possible resistance (further investigations needed), and less than 90% mortality as confirmed resistance. To increase the mosquito numbers collected for susceptibility tests, VectorLink Cambodia doubled the number of HDNs in the forest sites from September 2021 through November 2021 and in both annex village and forest fringe sites in November 2021 in the two provinces.

3. RESULTS

3.1 VECTOR BIONOMICS

Outdoor CDNs, HDNs, and FTTs, and indoor CDC light traps were used to collect mosquitoes every month from October 2020 through November 2021, except in March and April 2021, when collections were interrupted by COVID-19 pandemic restrictions. The reporting period therefore represents 12 months of collections, but not 12 consecutive calendar months.

3.1.1 *ANOPHELES* FEMALE MOSQUITOES COLLECTED FROM THE ANNEX VILLAGE AND THE FOREST FRINGE IN TWO PROVINCES

Anopheles females were caught from a total of 36 CDN collections, 72 HDN collections (36 in village sites and 36 in forest fringe sites), 72 FTT collections (36 in village sites and 36 in forest fringe sites), and 144 CDC light-trap collections in each province (Tables 2 and 3). A total of 24,291 *Anopheles* were collected (both provinces combined) during the reporting period. CDNs were by far the most productive trapping method (18,237), followed by HDNs (4,940). CDC light traps (947) and FTTs (167) caught relatively few *Anopheles*. In both provinces, FTTs in the forest caught a higher density of *Anopheles* females than did FTTs in the annex village sites, whereas the densities and species richness caught by the HDNs in the forest were more similar to those of the annex villages. The highest number of different species (species richness) were found in CDNs in both provinces.

TABLE 2. TOTAL NUMBER, DENSITY, AND SPECIES RICHNESS OF *ANOPHELES* FEMALES IN VILLAGE AND FOREST FRINGE IN MONDULKIRI

Trapping method	Village Site				Forest Fringe Site			
	Number of traps	Anopheles (females)	Anopheles (females) per trap	Number of species	Number of traps	Anopheles (females)	Anopheles (females) per trap	Number of species
CDN	36	10,199	283.3	28	NA			
HDN	36	1,381	38.4	25	36	1,399	38.8	23
CDC light trap	144	343	2.4	21	NA			
FTT	36	46	1.3	16	36	91	2.5	10

TABLE 3. TOTAL NUMBER, DENSITY, AND SPECIES RICHNESS OF *ANOPHELES* FEMALES IN VILLAGE AND FOREST FRINGE IN STUNG TRENG

Trapping method	Village Site				Forest Fringe Site			
	Number of traps	*Anopheles* (females)	*Anopheles* (females) per trap	Number of species	Number of traps	*Anopheles* (females)	*Anopheles* (females) per trap	Number of species
CDN	36	8,038	223.3	27	NA			
HDN	36	915	25.4	19	36	1,245	34.6	16
CDC light trap	144	604	4.2	15	NA			
FTT	36	14	0.4	4	36	16	2.5	6

3.1.2 Species Composition and Vector Abundance

Total Number and Percentage of Anopheles Females in Annex Village and Forest Fringe Sites in Mondulkiri and Stung Treng Provinces

Thirty-one *Anopheles* species were found in Mondulkiri Province (all trapping methods combined) during the 12 months of collections (Table 4). *An. philippinensis* (20.4%) was the most abundant at the annex village site. *An. dirus* (52.3%) was the most abundant at the forest fringe site, followed by *An. maculatus* s.l. (14.4%) and *An. minimus* s.l. (10.8%).

Table 4. Total Number and Percentage of *Anopheles* Females in Village and Forest Fringe Sites in Mondulkiri (Primary Vectors Highlighted in Gray Color)

Species Name	Village		Forest		Total	
	n	%	n	%	n	%
An. philippinensis	2,442	20.4%	55	3.7%	2,497	18.6%
An. aconitus	1,755	14.7%	17	1.1%	1,772	13.2%
An. jamesii	1,458	12.2%	2	0.1%	1,460	10.9%
An. minimus s.l.	1,005	8.4%	161	10.8%	1,166	8.7%
An. peditaeniatus	782	6.5%	3	0.2%	785	5.8%
An. maculatus s.l.	683	5.7%	214	14.4%	897	6.7%
An. campestris	468	3.9%	7	0.5%	475	3.5%
An. splendidus	447	3.7%	25	1.7%	472	3.5%
An. vagus	409	3.4%	2	0.1%	411	3.1%
An. nigerrimus	391	3.3%	56	3.8%	447	3.3%
An. dirus	367	3.1%	780	52.3%	1,147	8.5%
An. sawadwongporni s.l.	323	2.7%	83	5.6%	405	3.0%
An. nivipes	258	2.2%	4	0.3%	262	2.0%
Unidentified	236	2.0%	22	1.5%	258	1.9%
An. indefinitus	213	1.8%	0	0.0%	213	1.6%
An. crawfordi	154	1.3%	19	1.3%	173	1.3%
An. barbirostris (a, b, and c)	130	1.1%	7	0.5%	137	1.0%
An. nitidus	123	1.0%	3	0.2%	126	0.9%
An. varuna	88	0.7%	5	0.3%	93	0.7%
An. kochi	74	0.6%	2	0.1%	76	0. 6%
An. sinensis	38	0.3%	1	0.1%	39	0.3%
An. tessellatus	37	0.3%	11	0.7%	48	0.4%
An. karwari	32	0.3%	0	0.0%	32	0.2%
An. subpictus	27	0.2%	0	0.0%	27	0.2%
An. pseudojamesi	17	0.1%	2	0.1%	19	0.1%
An. argyropus	3	-<0.1%	0	0.0%	3	<0.1%
An. baimaii	2	<0.1%	7	0.5%	9	<0.1%
An. notanandai	2	<0.1%	0	0.0%	2	<0.1%
An. pampanai	1	<0.1%	0	0.0%	1	<0.1%
An. pursati	1	<0.1%	0	0.0%	1	<0.1%
An. willmori	1	<0.1%	2	0.1%	3	<0.1%
An. interruptus	1	<0.1%	0	0.0%	1	<0.1%
Total	11,969	100.0%	1,490	100.0%	13,459	100.0%

A total of 27 different *Anopheles* species were found in Stung Treng Province (all trapping methods combined) during the 12 months of collections (Table 5). *An. peditaeniatus* (35.5%) was the most abundant in the annex village site. Forest site collections were dominated by *An. dirus* (41.5%) and *An. tessellatus* (37.4%).

TABLE 5. TOTAL NUMBER OF *ANOPHELES* FEMALES IN VILLAGE AND FOREST FRINGE IN STUNG TRENG (PRIMARY VECTORS HIGHLIGHTED IN GRAY COLOR)

Species Name	Village		Forest Fringe		Total	
	n	%	n	%	n	%
An. peditaeniatus	3,392	35.5%	17	1.4%	3,409	31.5%
An. tessellatus	1,448	15.1%	472	37.4%	1,920	17.7%
An. kochi	908	9.5%	41	3.3%	949	8.8%
An. philippinensis	791	8.3%	45	3.6%	836	7.7%
An. dirus	484	5.1%	523	41.5%	1,007	9.3%
An. nivipes	375	3.9%	22	1.7%	397	3.7%
An. barbirostris (a, b, and c)	359	3.8%	54	4.3%	413	3.8%
Unidentified	366	3.8%	8	0.6%	374	3.5%
An. sawadwongporni s.l.	343	3.6%	16	1.3%	359	3.3%
An. vagus	254	2.7%	0	0.0%	254	2.3%
An. campestris	224	2.3%	37	2.9%	261	2.4%
An. nigerrimus	189	2.0%	3	0.2%	192	1.8%
An. indefinitus	124	1.3%	0	0.0%	124	1.1%
An. maculatus s.l.	98	1.0%	11	0. 9%	109	1.0%
An. argyropus	89	0.9%	0	0.0%	89	0.8%
An. aconitus	32	0.3%	2	0.2%	34	0.3%
An. minimus s.l.	29	0.3%	4	0.3%	33	0.3%
An. crawfordi	21	0.2%	0	0.0%	21	0.2%
An. karwari	11	0.1%	0	0.0%	11	0.1%
An. subpictus	9	0.1%	0	0.0%	9	0.1%
An. jamesii	6	0.1%	0	0.0%	6	0.1%
An. nitidus	5	0.1%	0	0.0%	5	0.1%
An. baimaii	4	<0.1%	4	0.3%	8	0.1%
An. notanandai	3	<0.1%	1	0.1%	4	<0.1%
An. pampanai	2	<0.1%	1	0.1%	3	<0.1%
An. sinensis	2	<0.1%	0	0.0%	2	<0.1%
An. varuna	2	<0.1%	0	0.0%	2	<0.1%
An. willmori	1	<0.1%	0	0.0%	1	<0.1%
Total	9,571	100.0%	1,261	100.0%	10,832	100.0%

Species Composition by Trapping Method in the Mondulkiri Annex Village Site

CDNs Set Outdoors (36 traps total, n=10,199)

Compared to the other trapping methods, CDNs in Mondulkiri caught a greater number of *Anopheles* mosquitoes, and a higher species richness was observed: 28 *Anopheles* species were collected from CDNs. *An. philippinensis* was the most abundant species (n=2,257, 22.1%), followed by *An. aconitus* (n=1,588, 15.6%) (Figure 4A). The primary malaria vectors found in CDNs were as follows: *An. dirus* (n=22, 0.2%), *An. maculatus* (n=524, 5.1%), and *An. minimus* s.l. (n=789, 7.7%). Species that were less than 2% of the total caught are grouped together and represented in Figure 4A as "others." These were *An. argyropus, An. barbirostris, An. crawfordi, An. dirus, An. kochi, An. nitidus, An. notanandai, An. pseudojamesii, An. pursati, An. sinensis, An. subpictus, An. tessellatus, An. varuna, An. wiullmori,* and unidentified *Anopheles.*

HDNs Set Outdoors (36 traps total, n=1,381)

Twenty-five *Anopheles* species were caught in HDN collections in the Mondulkiri annex village site (Figure 4B). The primary malaria vector *An. dirus* was the most abundant species (n=295, 21.4%). The second most abundant species was *An. philippinensis* (n=171, 12.4%). Of the other primary malaria vectors, *An. maculatus* was the third most common species (n=148, 10.7%) and *An. minimus* s.l. was found in moderate numbers (n=133, 9.6%). Species that were less than 5% of the total caught are grouped together and represented in Figure 4B as "others." These include *An. baimai, An. barbirostris, An. crawfordi, An. indefinitus, An. jamesii, An. kawari, An. kochi, An. nitidus, An. nivipes, An. notanandai, An. pampani, An. peditaeniatus, An. pseudojamesii, An. splendidus, An. tessellatus, An. vagus, An. varuna, An. willmori,* and unidentified *Anopheles.*

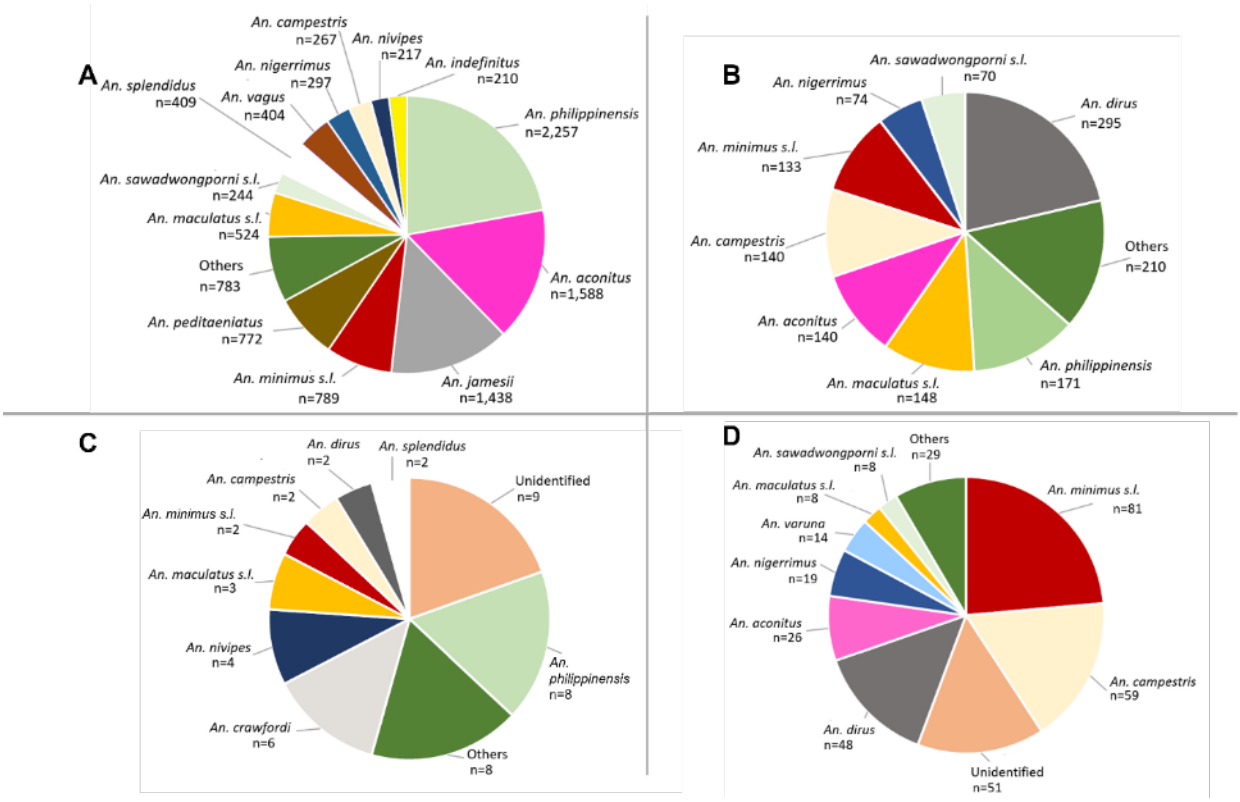
FTTs Set Outdoors (36 traps total, n=46)

Sixteen *Anopheles* mosquitoes were caught in FTT collections in the Mondulkiri annex village site (Figure 4C). The majority of them were unidentifiable to species level because morphological features were damaged by the traps (n=8, 19.57%). Of the primary malaria vectors, only two *An. dirus* (4.35%), three *An. maculatus* (6.52%), and two *An. minimus* s.l. (4.35%) were collected. Species that were less than 4% of the total caught are grouped together and represented in Figure 4C as "others." They are *An. aconitus*, *An. jamesii, An. nigerrimus, An. nitidus, An. pseudojamesii, An. sawadwongporni, An. tessellatus*, and *An. crawfordi.*

CDC Light Traps Set Inside Village Houses (144 traps total, n=343)

A large proportion of the mosquitoes collected from the CDC light trap were damaged and therefore unidentifiable to species level (n=51, 14.9%). Of the identified mosquitoes, a total of 21 species were found (Figure 4D). The primary malaria vector *An. minimus* s.l. was the most abundant (n=81, 23.6%). Other primary vectors found from CDC light trap collection were *An. dirus* (n=48, 14.0%) and *An. maculatus* s.l. (n=8, 2.3%). Species that were less than 2% of the total caught are grouped together and represented in Figure 4D as "others." These were *An. barbirostris, An. baimai, An. crawfordi, An. interruptus, An. kochi, An. jamesii, An. philippinensis, An. peditaeniatus, An. nivipes, An. pseudojamesii, An. sinensis, An. splendidus,* and *An. vagus.* The majority of mosquitoes caught using this method were unfed (91.0%).

FIGURE 4 A–D. *ANOPHELES* SPECIES COMPOSITION FROM COLLECTIONS IN PU TIL ANNEX VILLAGE, USING A. CDNS SET OUTDOORS; B. HDNS SET OUTDOORS; C. FTTS SET OUTDOORS; D. CDC LIGHT TRAPS SET INDOORS



SPECIES COMPOSITION BY TRAPPING METHOD IN MONDULKIRI FOREST FRINGE SITE

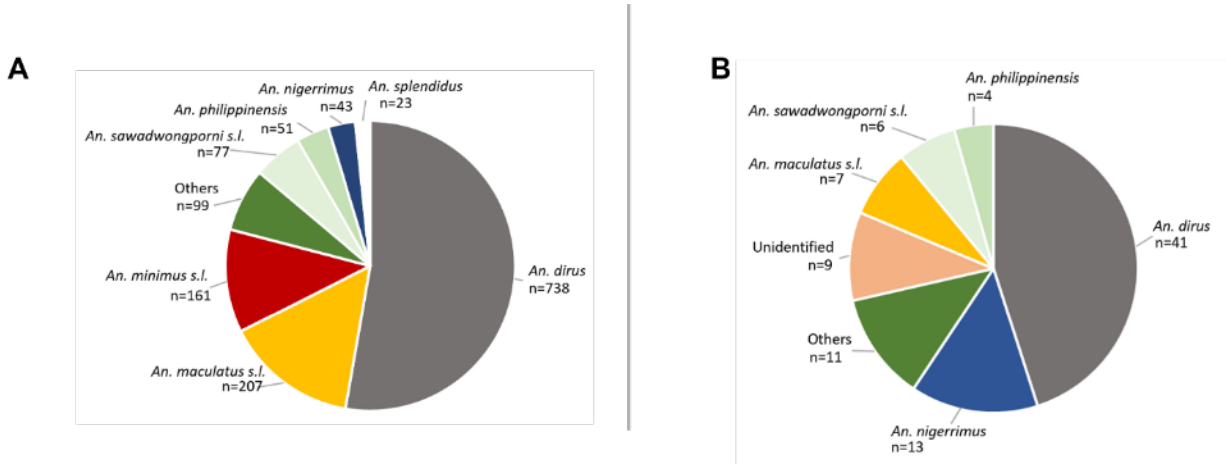
HDNS SET OUTDOORS (36 TRAPS TOTAL, N=1,399)

Twenty-three *Anopheles* species were caught in HDN collections in the Mondulkiri forest fringe site (Figure 5A). The primary malaria vector *An. dirus* was the most abundant species found in HDNs (n=738, 52.8%). The next most abundant species were also primary vectors: *An. maculatus* s.l. (n=207, 14.8%) and *An. minimus* s.l. (n=161, 11.5%). Species that individually made up less than 2% of the total caught are grouped together and represented as "others" in Figure 5A. They were *An. aconitus, An. baimai, An. barbirostris, An. campestris, An. crawfordi, An. jamesii, An. kochi, An. nitidus, An. nivipes, An. peditaeniatus, An. pseudojamesii, An. sinensis, An. tessellatus, An. varuna, An. vagus, An. willmori* and unidentified *Anopheles*.

FTTS SET OUTDOORS (36 TRAPS TOTAL, N=91)

Ten *Anopheles* species were caught in FTT collections in the Mondulkiri forest fringe site (Figure 5B). The primary malaria vector *An. dirus* was the most abundant (n=41, 45.1%). Of the other primary vectors, *An. maculatus* s.l. was found in low numbers (n=7, 7.7%), and no *An. minimus* s.l. were collected. Species that made up less than 4% of the total are grouped together and represented in Figure 5B as "others." They are *An. crawfordi, An. aconitus, An. campesitris, An. splendidus,* and *An. tessellatus.*

FIGURE 5. *ANOPHELES* SPECIES COMPOSITION FROM A. HDNS SET OUTDOORS IN THE FOREST FRINGE; B. FTTS SET OUTDOORS IN THE FOREST FRINGE IN MONDULKIRI PROVINCE



SPECIES COMPOSITION BY TRAPPING METHOD IN STUNG TRENG ANNEX VILLAGE SITE

CDNS SET OUTDOORS (36 TRAPS TOTAL, N=8,038)

Twenty-seven *Anopheles* species were collected from CDNs in Stung Treng annex village site (Figure 6A). *An. peditaeniatus* was the most abundant species in CDNs (n=3,221, 40.1%), followed by *An. tessellatus* (n=1,149, 14.3%). The primary malaria vectors found in CDNs were as follows: *An. dirus* (n=120, 1.5%); *An. maculatus* (n=87, 1.1%); and *An. minimus* (n=25, 0.3%). Species that made up less than 2% of the total are grouped together as "others" and represented in Figure 6A. They are *An. aconitus, An. argyropus, An. baimai, An. crawfordi, An. dirus, An. maculatus* s.l., *An. minimus* s.l., An. *kochi, An. nitidus, An. notanandai, An. pseudojamesii, An. pursati, An. sinensis, An. subpictus, An. varuna, An. willmori,* and unidentified *Anopheles*.

HDNS SET OUTDOORS (36 TRAPS TOTAL, N=915)

Twenty *Anopheles* species were found from the HDN collections in Stung Treng annex village site (Figure 6B). *An. tessellatus* was the most abundant species in HDNs (n=268, 29.3%). Primary malaria vector *An. dirus* was the second most abundant species in HDN (n=241, 26.3%). Of the other primary malaria vectors, *An. maculatus* s.l.(n=4, 0.4%) and *An. minimus* s.l. (n=2, 0.2%) were caught in very low numbers. Species that made up less than 2% of the total are grouped and represented as "others" in Figure 6B. They are *An. aconitus, An. argyropus, An. baimai, An. campestris, An. indefinitus, An. kawari, An. kochi, An. maculatus* s.l., *An. minimus* s.l., *An. nitidus, An. nigerrimus, An. sawadwongporni, and An. vagus.*

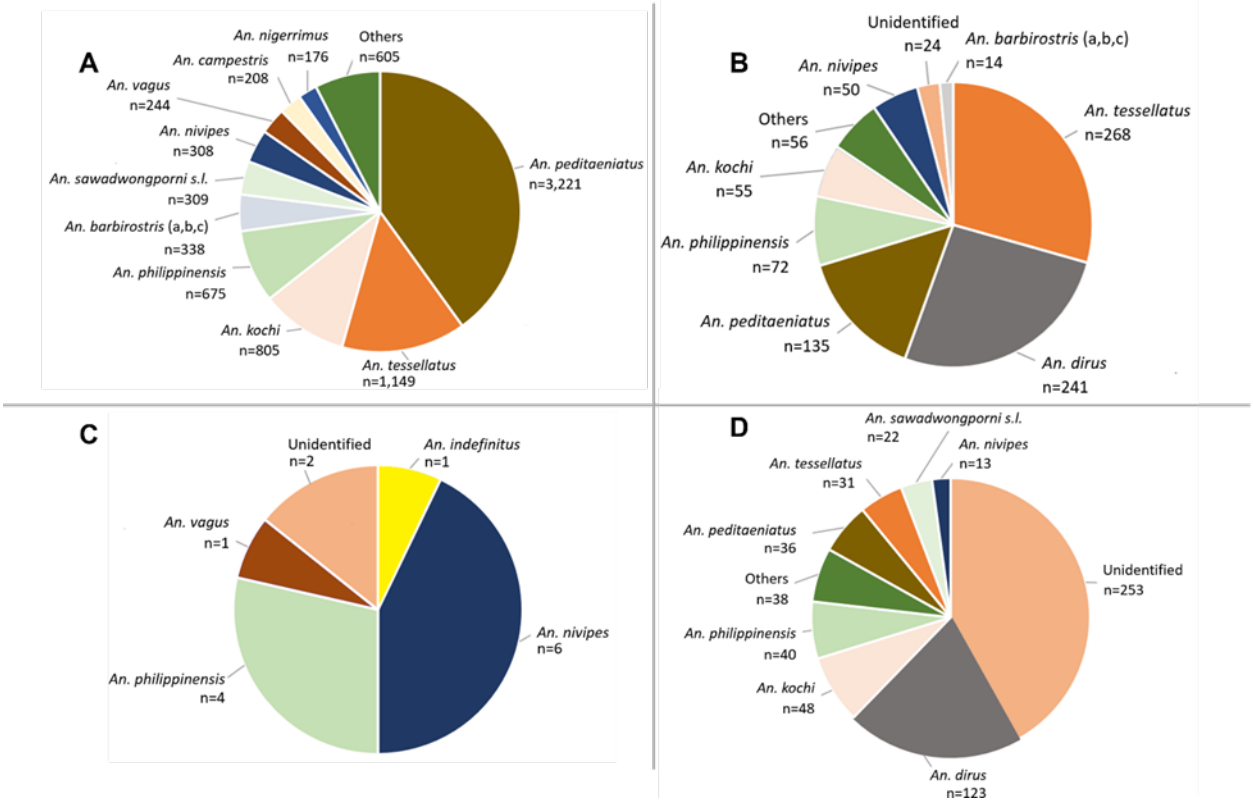
FTTS SET OUTDOORS (36 TRAPS TOTAL, N=14)

Only four species of *Anopheles* mosquitoes were found from the 36 FTT collections in the Stung Treng annex village site (Figure 6C). The most abundant species was *An. nivipes* (n=6, 42.9%). The other three species collected were *An. philippinensis, An. indefinitus*, and *An. vagus.* Two *Anopheles* were unidentifiable to species (Figure 6C). No primary malaria vector species were collected from the FTTs.

CDC LIGHT TRAP SET INSIDE VILLAGE HOUSES (144 TRAPS TOTAL, N=604)

A large proportion of the mosquitoes collected from the CDC light traps were damaged and unidentifiable to species (n=253,41.9%). Of the identified *Anopheles*, a total of 16 species were found, and the primary malaria vector *An. dirus* was the most abundant (n=123, 20.4%) (Figure 6D). Other primary vectors found from CDC light traps are *An. maculatus* s.l. (n=7, 1.7%) and *An. minimus* s.l. (n=2, 0.3%). Species that made up less than 2% of the total caught are grouped together as "others" in Figure 6D. They are *An. aconitus, An. argyropus, An. barbirostris, An. campestris, An. interruptus, An. maculatus* s.l., *An. minimus* s.l., *An. nigerrimus,* and *An. vagus.* Almost all mosquitoes caught using this method were unfed (95.7%).

FIGURE 6. *ANOPHELES* SPECIES COMPOSITION FROM COLLECTIONS IN OU CHAY ANNEX VILLAGE USING A. CDNS SET OUTDOORS; B. HDNS; C. FTTS SET OUTDOORS; D. CDC LIGHT TRAPS SET INDOORS



SPECIES COMPOSITION BY TRAPPING METHOD IN STUNG TRENG FOREST FRINGE SITE

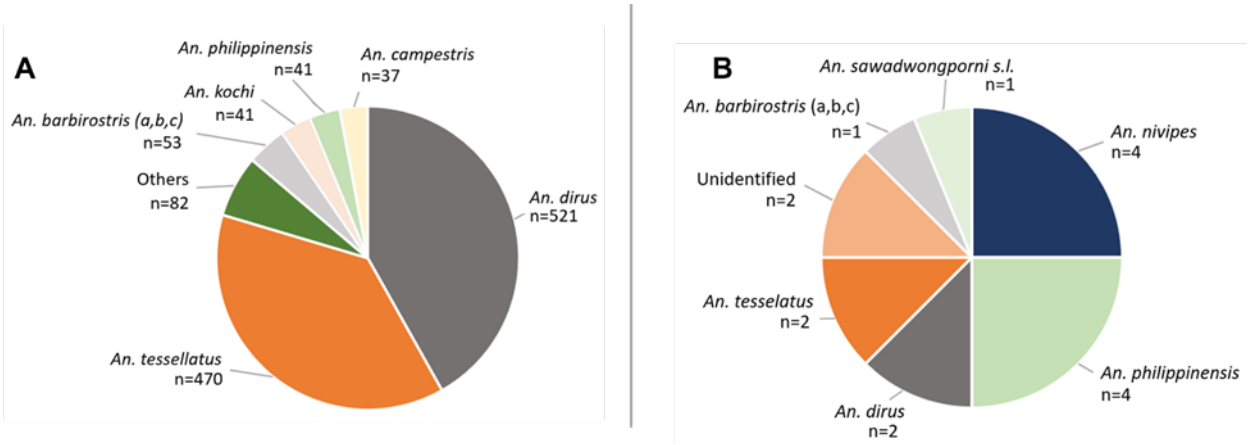
HDNS IN THE FOREST FRINGE (36 TRAPS TOTAL, N=1,245)

Sixteen *Anopheles* species were caught using HDNs in the Stung Treng forest fringe site (Figure 7A). The primary malaria vector *An. dirus* was the most abundant species found in HDNs (n=521, 41.9%). The second most abundant species was *An. tessellatus* (n=470, 37.8%). Other primary malaria vectors, *An. maculatus* s.l. (n=11, 0.9%) and *An. minimus* s.l. (n=4, 0.3%), were found in very low numbers. Species that amounted to less than 2% are grouped together and represented as "others" in Figure 7A. They are *An. aconitus, An. baimai, An. maculatus* s.l., *An. minimus* s.l., *An. nigerrimus, An. nivipes, An. notanandai, An. pampani, An. peditaeniatus, An. sawadwongporni,* and unidentified *Anopheles*.

FTTS IN THE FOREST FRINGE (36 TRAPS TOTAL, N=16)

Only six *Anopheles* species were caught in FTTs in the Stung Treng forest fringe site (Figure 7B). The highest numbers collected were *An. nivipes* and *An. philippinensis* (n=4, 25.0%). The other species were An. *dirus* (n=2, 12.5%), *An. tessellatus* (n=2, 12.5%), *An. barbirostris* (n=1, 6.3%), and *An. sawadwongporni* (n=1, 6.3%). Two *Anopheles* were unidentifiable to species (Figure 7B).

FIGURE 7. *ANOPHELES* SPECIES COMPOSITION FROM A. HDNS SET OUTDOORS IN THE FOREST FRINGE; B. FTTS SET OUTDOORS IN THE FOREST FRINGE IN STUNG TRENG PROVINCE



3.1.3 SEASONAL BITING RATES OF THE PRIMARY MALARIA VECTORS

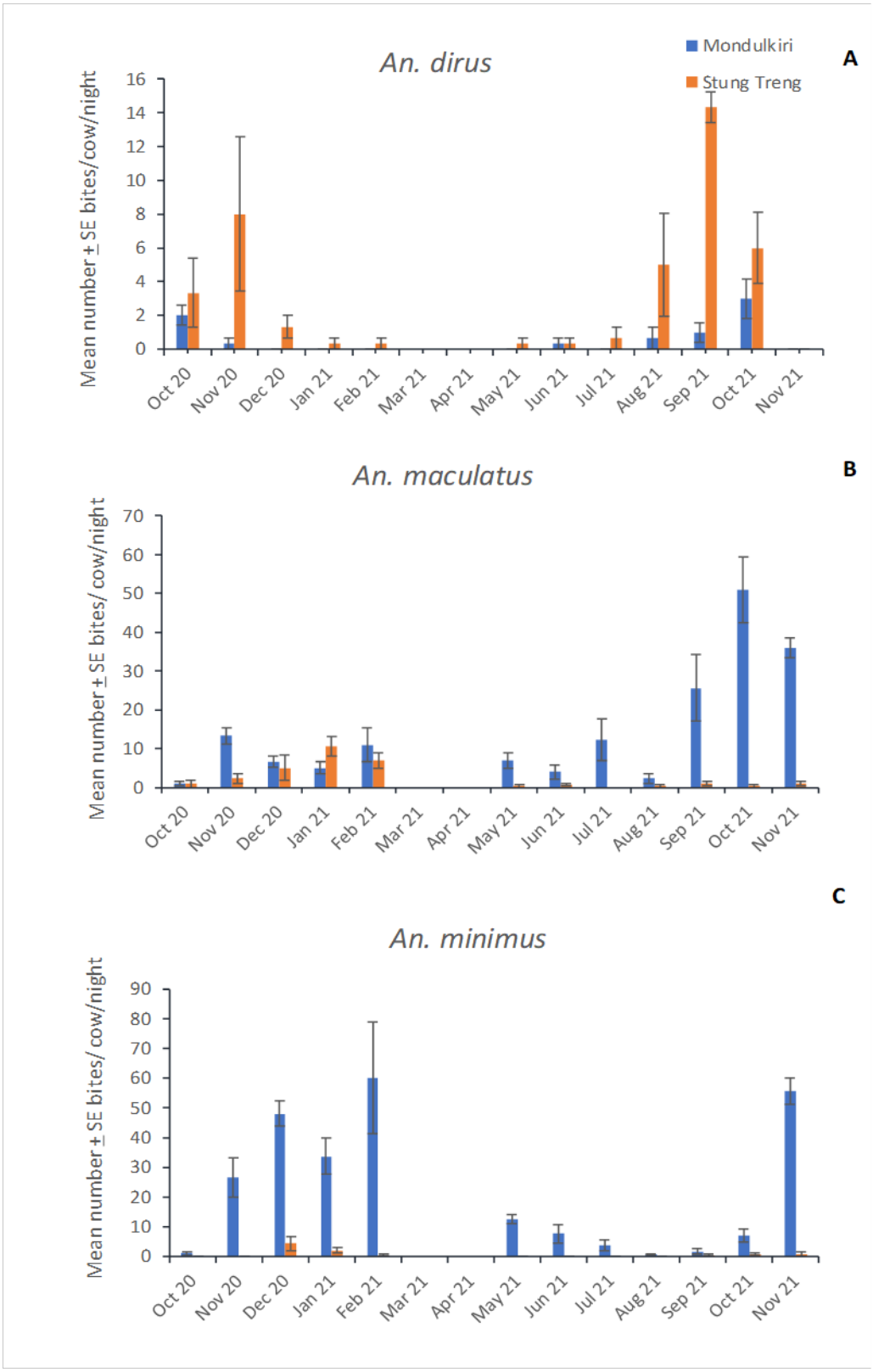
There were clear spatial (between the provinces) and temporal trends in cow and human biting rates of the three primary malaria vectors *An. dirus, An. maculatus* s.l., and *An. minimus* s.l.

OUTDOOR COW BITING RATES

The cow biting rates of *An. dirus* were consistently higher in Stung Treng than in Mondulkiri. The highest rate was observed in September 2021 in Stung Treng (14.3 bites per cow per night), and in October 2021 in Mondulkiri (3.0 bites per cow per night) (Figure 8A).

In contrast, cow biting rates of both *An. maculatus* s.l. and *An. minimus* s.l. were consistently higher in Mondulkiri Province. There, the highest cow biting rate of *An. maculatus* s.l. – 51 bites per cow per night – was observed in October 2021, whereas the highest rate in Stung Treng was just 10.7 bites per cow per night, observed in January 2021 (Figure 8B). The highest cow biting rate of *An. minimus* s.l. – 60 bites per cow per night – was observed in February 2021 in Mondulkiri Province; in Stung Treng, the highest rate, only 2.3 bites per cow per night, was observed in December 2020 (Figure 8C).

FIGURE 8. MEAN COW BITING RATE OF A. *AN. DIRUS;* B. *AN. MACULATUS* S.L.; C. *AN. MINIMUS* S.L. IN MONDULKIRI AND STUNG TRENG PROVINCES FROM OCTOBER 2020 THROUGH NOVEMBER 2021



Outdoor Human Biting Rates

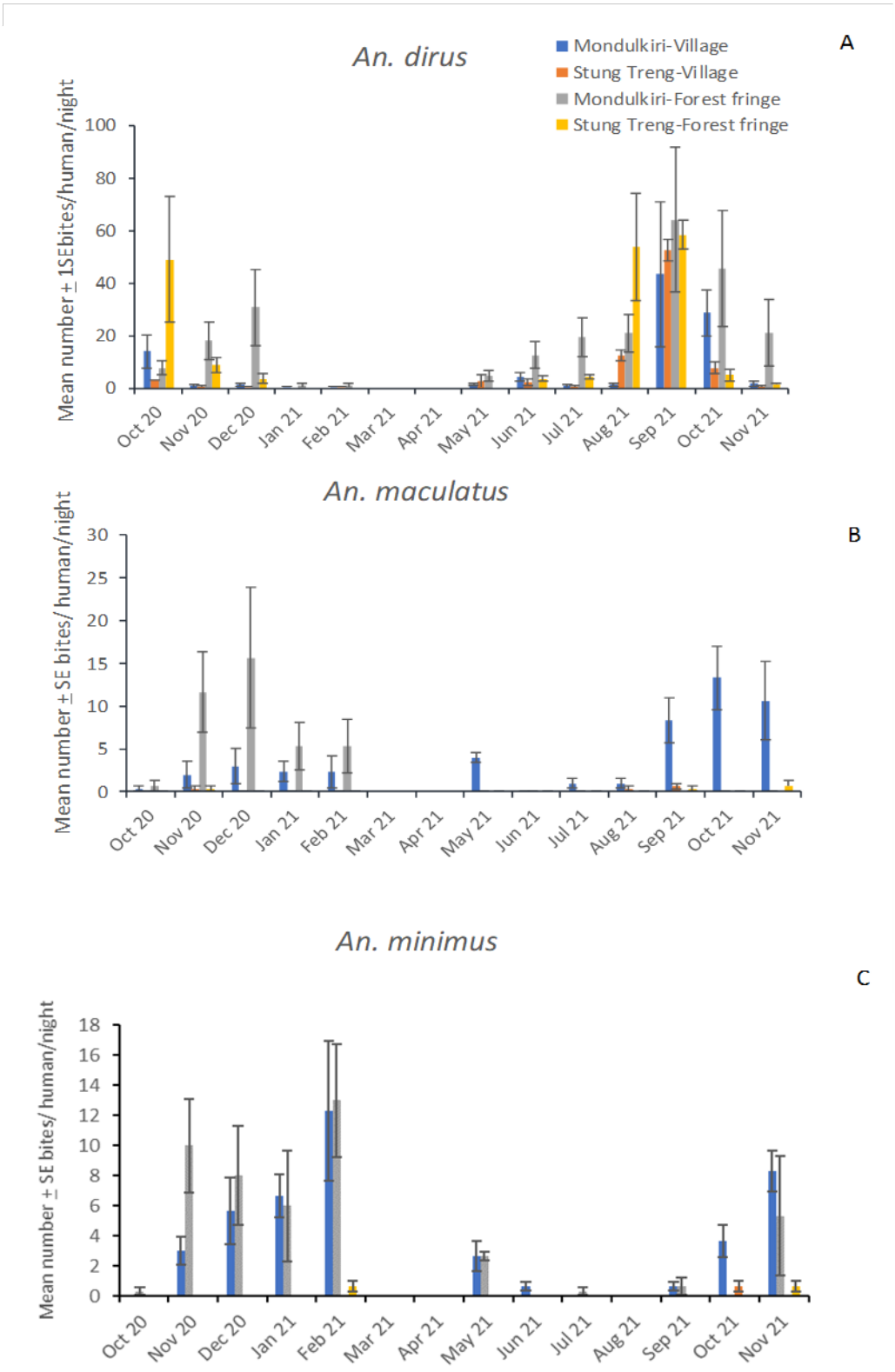
HDNs in Annex Village and Forest Fringe

The three primary vectors had different seasonal peaks in the two provinces during the sampling period. The highest human biting rate of *An. dirus* estimated from HDN catches was observed in September 2021 in the annex village and forest sites of both provinces (Figure 9A). In the Mondulkiri annex village site, the human biting rate peaked at 43.3 bites per human per night; in the forest site, the peak was 64.0 bites per human per night. Similar biting rates were observed in Stung Treng: there were 52.7 bites per human per night in the annex village site and 58.3 bites per human per night in the forest site.

The human biting rates for *An. maculatus* s.l. differed between the two provinces. Mondulkiri Province had higher biting rates than did Stung Treng (Figure 9B). In Mondulkiri, the highest human biting rates were observed in October 2021 (13.3 bites per human per night in the annex village site) and December 2020 (15.7 bites per human per night in the forest site). In Stung Treng, the highest biting rate happened at a similar time of the year, in September 2021 (0.67 bites per human per night in the annex village site) and November 2021 (0.67 bites per human per night in the forest site), but biting rates were much lower than in Mondulkiri.

An. minimus s.l. differed in its peak seasonal human biting from the other two species (Figure 9C): peak human biting rates occurred in February 2021 in the annex village (12.3 bites per human per night) and forest fringe sites (13.0 bites per human per night) of Mondulkiri Province and in the forest site of the Stung Treng Province (0.67 bites per human per night). The annex village site of Stung Treng Province recorded human biting of *An. minimus* s.l. in October 2021 only (0.67 bites per human per night).

FIGURE 9. MEAN HUMAN BITING RATE OF A. *AN. DIRUS;* B. *AN. MACULATUS* S.L.; C. *AN. MINIMUS* S.L. FROM HDN IN MONDULKIRI AND STUNG TRENG PROVINCES FROM OCTOBER 2020 TO NOVEMBER 2021



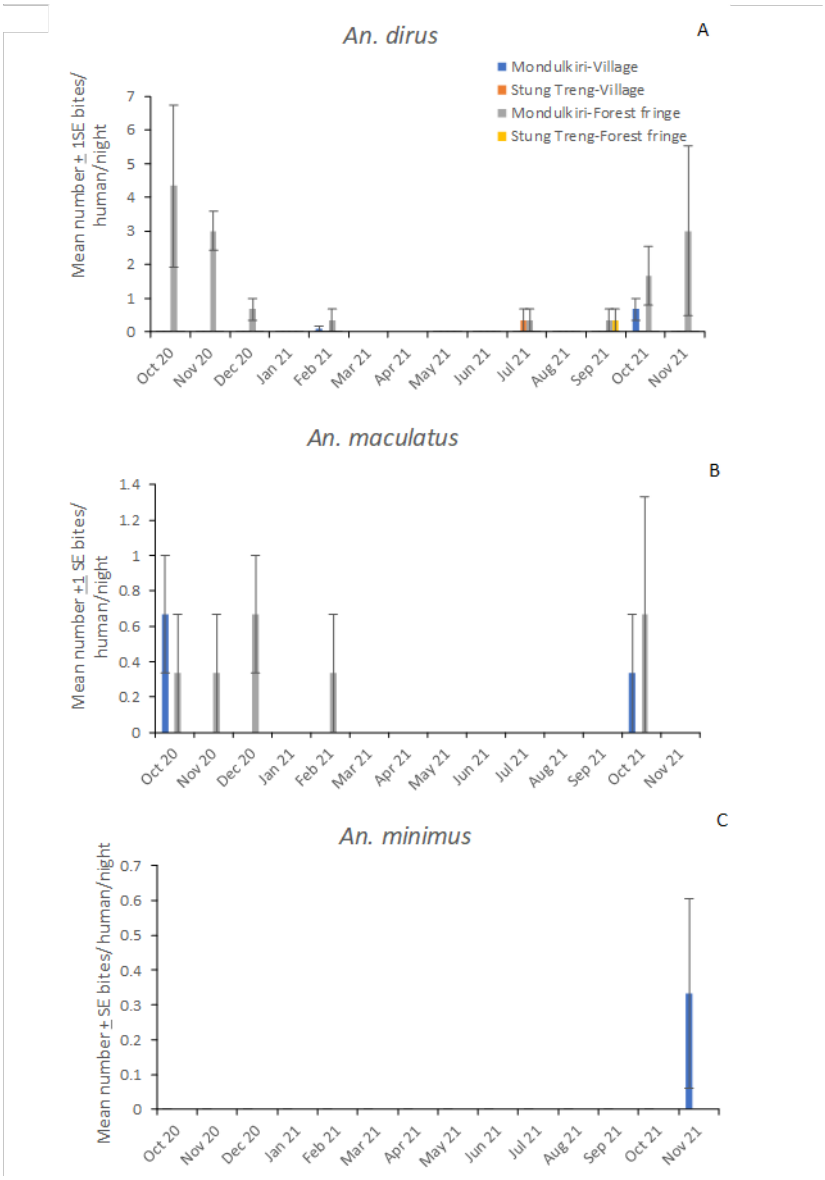
FTTs in Annex Village and Forest Fringe

Human biting rates estimated from FTTs were considerably lower than those from HDNs in both provinces. Human biting of *An. dirus* from FTTs in the Mondulkiri annex village site was recorded only in two months: February 2021 (0.08 bites per human per night) and October 2021 (0.03 bites per human per night). The highest human biting rate (4.3 bites per human per night) from FTTs in the Mondulkiri forest fringe site was recorded in October 2020. Human biting of *An. dirus* from FTTs in Stung Treng was recorded only in one month in the annex village (July 2021) and the forest site (September 2021) (Figure 10A).

Human biting from FTTs of *An. maculatus* s.l. was observed only in Mondulkiri Province. The highest human biting rate was observed in October 2020 (0.7 bites per human per night) in the annex village site and in December 2020 and October 2021 (0.7 bites per human per night) in the forest site (Figure 10B).

Human biting of *An. minimus* s.l. from FTTs was observed only in one instance, in November 2021 in the Mondulkiri annex village site (0.33 bites per human per night) (Figure 10C).

Figure 10. Mean Human Biting Rate of A. *An. dirus;* B. *An. maculatus* s.l.; C. *An. minimus* s.l. from FTT in Mondulkiri and Stung Treng Provinces from October 2020 to November 2021

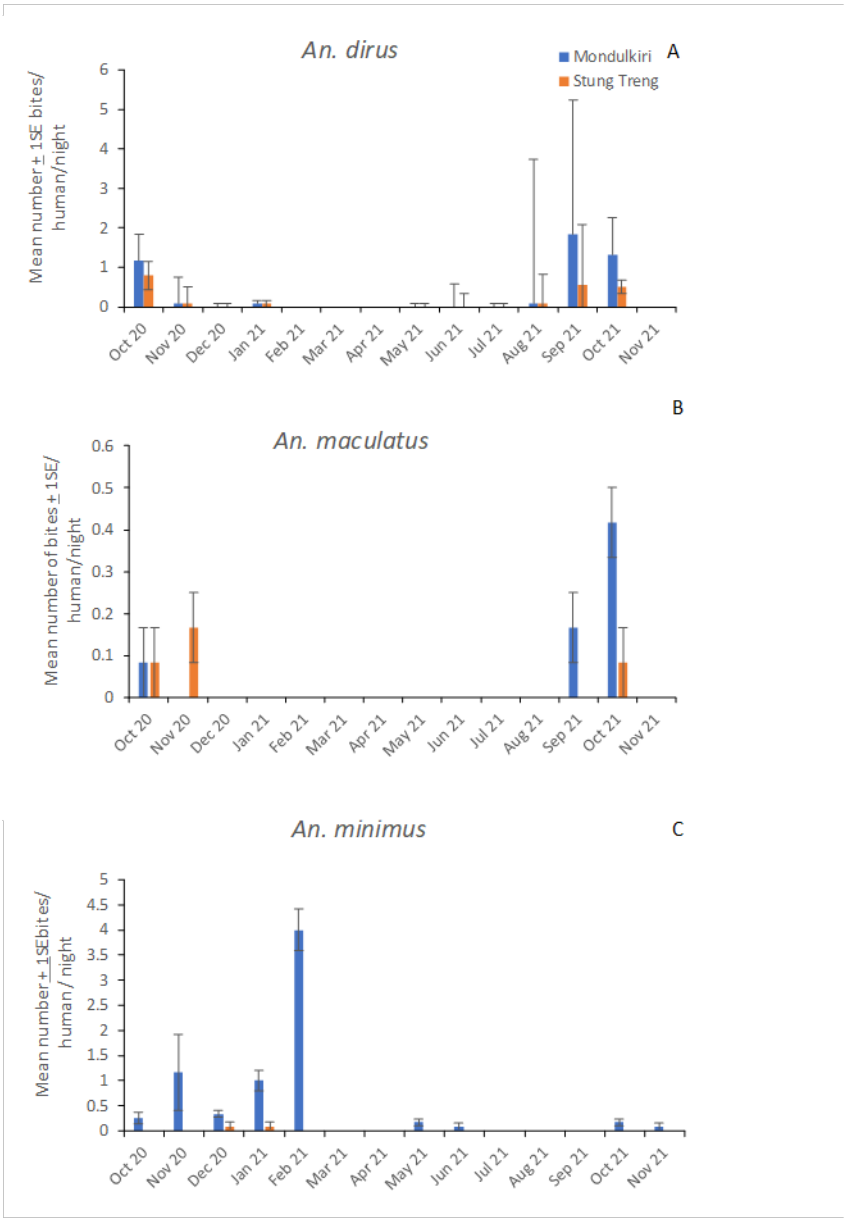


INDOOR HUMAN BITING RATES FROM CDC LIGHT TRAPS

Indoor human biting rates of the primary malaria vectors as estimated by indoor CDC light trap catches are presented in Figure 11. The highest indoor biting rates of *An. dirus* were observed in October 2020 (Stung Treng) and September 2021 (Mondulkiri) (Figure 11A).

The highest indoor biting rates of *An. maculatus* s.l. were observed in October 2021 in Mondulkiri (0.41 bites per human per night) and in November 2020 in Stung Treng (0.16 bites per human per night) (Figure 11B). For *An. minimus* s.l., the highest indoor biting rate was recorded in February 2021 (4 bites per human per night) in Mondulkiri Province. In Stung Treng province, *An. minimus* s.l. human biting was observed from indoor CDC light traps on only two occasions: in December 2020 and January 2021 (0.08 bites per human per night, Figure 11C).

FIGURE 11. MEAN HUMAN BITING RATE OF A. *AN. DIRUS;* B. *AN. MACULATUS*; C. *AN. MINIMUS* FROM CDC LIGHT TRAPS IN MONDULKIRI AND STUNG TRENG PROVINCES FROM OCTOBER 2020 TO NOVEMBER 2021

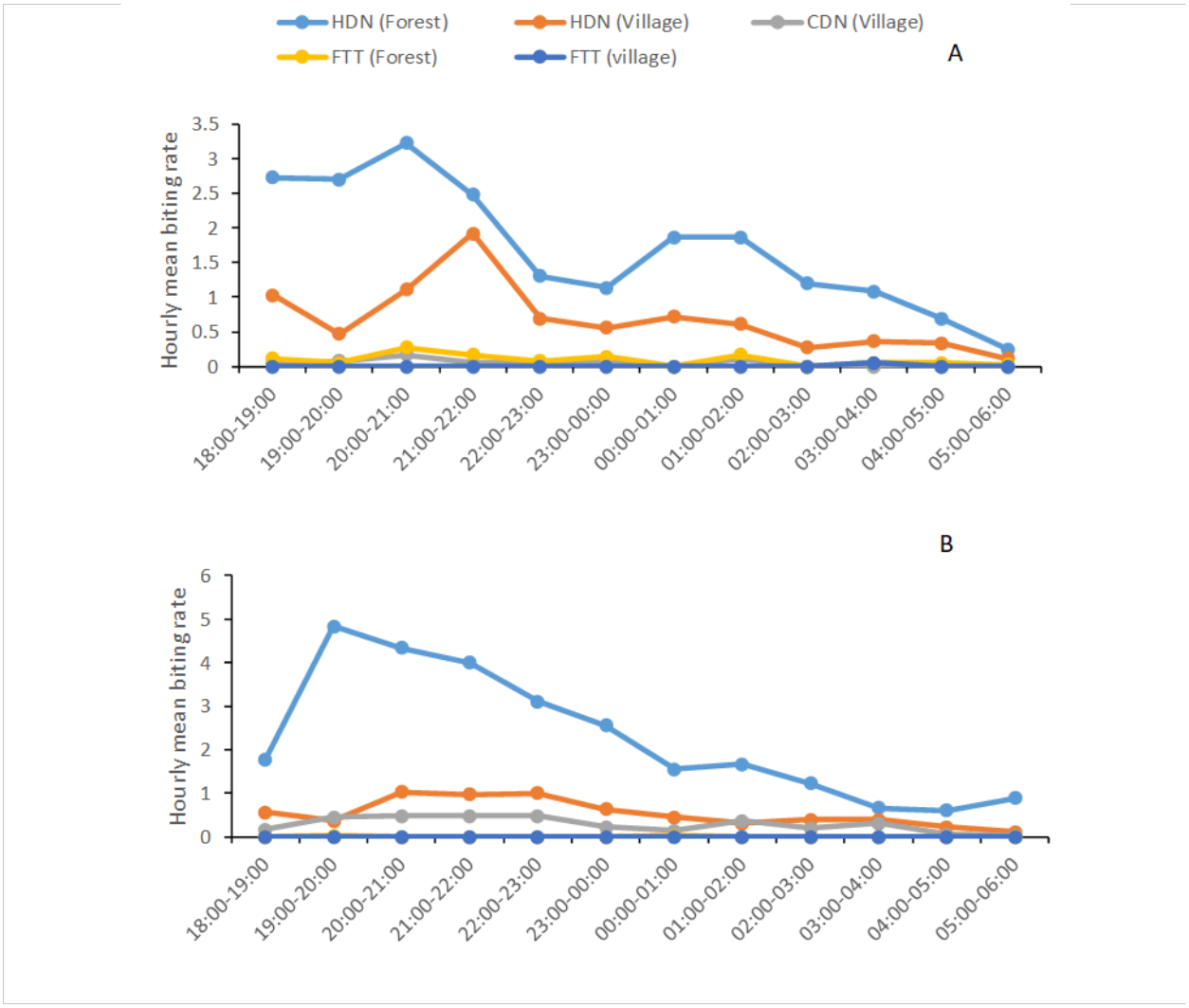


3.1.4 Hourly Biting Rates of Primary Malaria Vectors

Hourly Biting Rates of An. dirus

A clear pattern in the hourly biting rates of *An. dirus* was observed in Mondulkiri Province from HDNs, both in the annex village site and in the forest site. There are two peaks: a primary peak from 18:00 to 22:00 and a secondary peak from 23:00 to 02:00. The hourly biting rates from the FTT and CDN collections are so low that trends are not obviously apparent (Figure 12A). In Stung Treng Province, early biting was also apparent from the HDNs at the forest site with a peak at 19:00 to 20:00 (Figure 12B). Trends in biting times were less identifiable from any of the other trapping techniques.

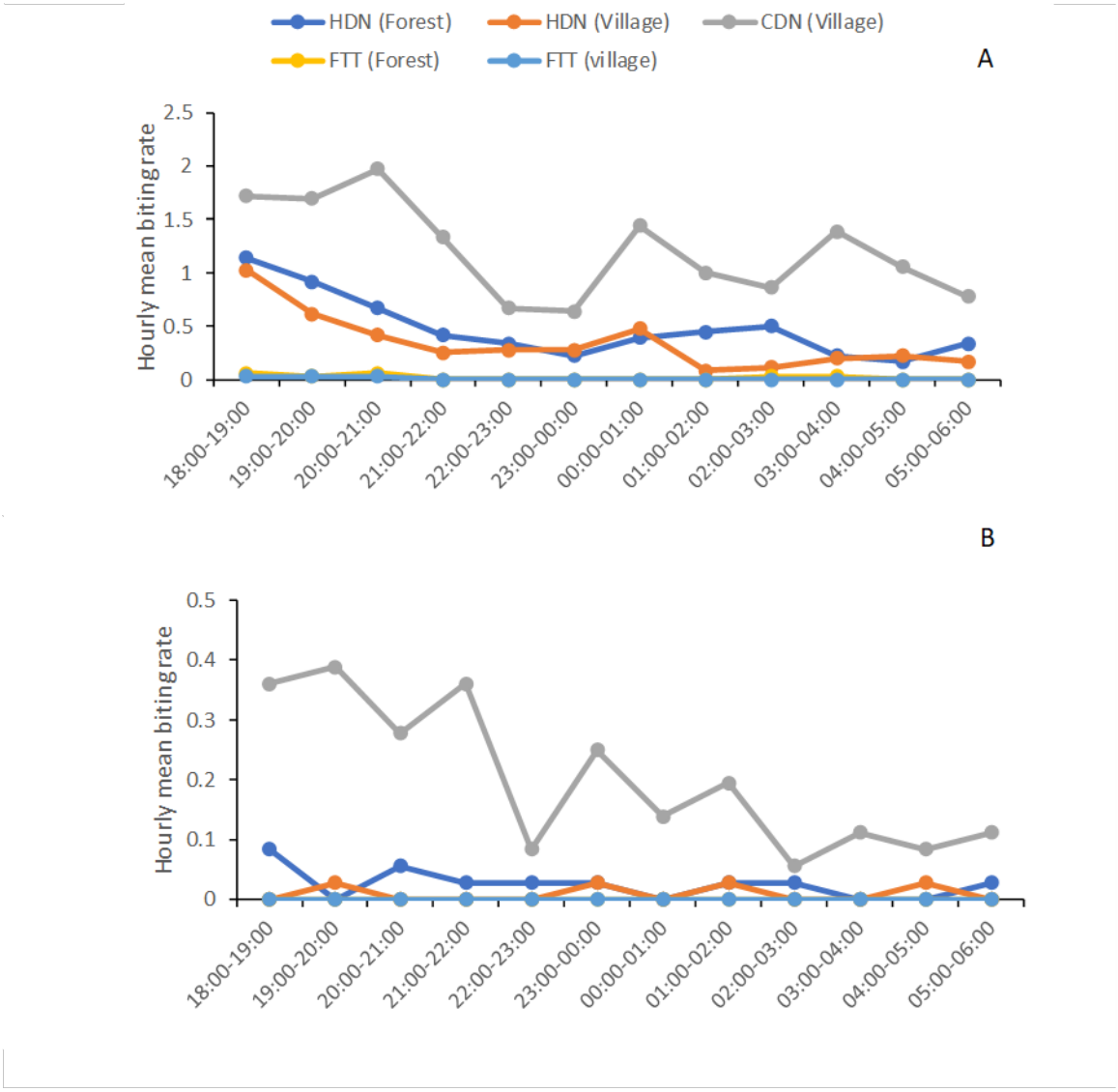
Figure 12. Mean Human Biting Rates of *An. dirus* in A. Mondulkiri and B. Stung Treng Provinces from October 2020 to November 2021



HOURLY BITING RATES OF *AN. MACULATUS* S.L.

The hourly biting rates of *An. maculatus* s.l. were higher in Mondulkiri Province than in Stung Treng Province, but a consistent biting pattern is not apparent in either province (Figure 13A and 13B).

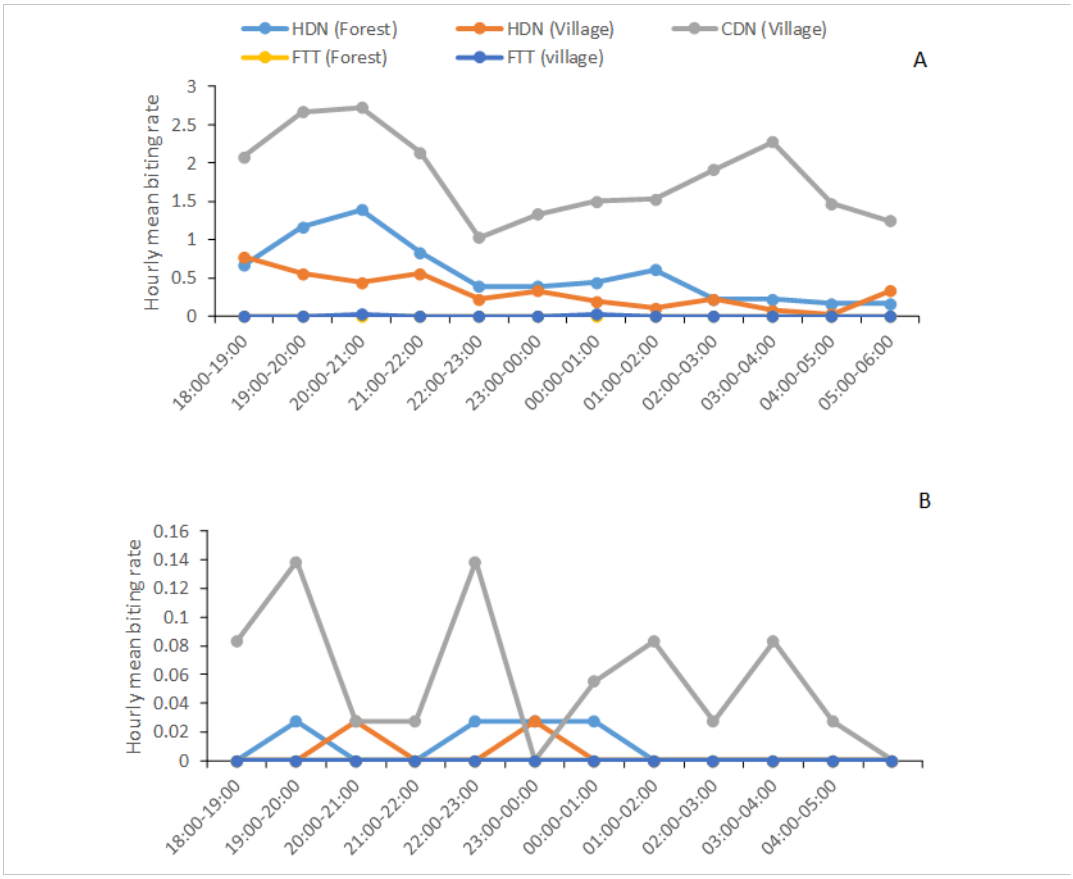
FIGURE 13. MEAN HUMAN BITING RATES OF *AN. MACULATUS* S.L. IN A. MONDULKIRI AND B. STUNG TRENG PROVINCES FROM OCTOBER 2020 TO NOVEMBER 2021



HOURLY BITING RATES OF *AN. MINIMUS* S.L.

The hourly biting rates of *An. minimus* s.l. were higher in Mondulkiri Province than in Stung Treng Province. A pattern of early biting could be seen for CDN and HDN forest site collections. The CDN data show two peaks: a major peak from 18:00 to 22:00, and a minor peak from 01:00 to 04:00. Early biting from 18:00 to 22:00 is also apparent in the HDN forest site data. No clear biting patterns were observed for *An. minimus* s.l. caught using other trapping methods in Mondulkiri Province (Figure 14A). In Stung Treng Province there is no clear biting pattern observed for CDNs. Two minor peaks at 19:00 to 20:00 and 00:00 to 01:00 are observed for HDN in the annex village site but biting rates are extremely low (Figure 14B).

FIGURE 14. MEAN HUMAN BITING RATES OF *AN. MINIMUS* S.L. IN A. MONDULKIRI AND B. STUNG TRENG PROVINCES FROM OCTOBER 2020 TO NOVEMBER 2021



PROKOPACK ASPIRATOR COLLECTIONS IN CDN AND HDN COLLECTIONS

This additional Prokopack aspiration activity started after Prokopack aspirators were received in Cambodia. From June 2021 through November 2021, Prokopacks were used in both provinces to catch any *Anopheles* mosquitoes that remained after hourly manual collections from the CDN and HDN collections. Prokopack collections caught 2,438 *Anopheles* females. As a percentage of the total collected, the Prokopack collections represented 10% or less in all instances except for CDN collections in Mondulkiri (Table 6). This suggests the collectors were efficient at collecting the mosquitoes using the manual technique in most cases. The large number of mosquitoes missed with the manual collections in the CDN traps in Mondulkiri means that data should be interpreted with some caution.

TABLE 6. TOTAL NUMBER OF *ANOPHELES* FEMALES COLLECTED FROM PROKOPACK COLLECTIONS IN ANNEX VILLAGE AND FOREST FRINGE IN MONDULKIRI AND STUNG TRENG PROVINCES

Trapping method	Mondulkiri				Stung Treng			
	Manual collections	Prokopack collections	Total collected	Percentage of prokopack collections	Manual collections	Prokopack collections	Total collected	Percentage of prokopack collections
CDN	5882	1727	7609	23%	4846	551	5397	10%
HDN-Village	876	19	895	2%	808	36	844	4%
HDN-Forest	812	82	894	9%	933	23	956	2%
Total	**7570**	**1828**	**9398**	**19%**	**6587**	**610**	**7197**	**8%**

3.2 INSECTICIDE RESISTANCE MONITORING

3.2.1 INSECTICIDE SUSCEPTIBILITY TESTING FOR PYRETHROID INSECTICIDES

Insecticide susceptibility testing for the three primary vectors (*An. dirus, An. maculatus* s.l., and *An. minimus* s.l.), and one secondary vector (*An. peditaeniatus*), using the WHO tube method, started in September 2021 during the rainy season. The insecticides tested were alpha-cypermethrin 0.05%, deltamethrin 0.05%, and permethrin 0.75%.

An. dirus collected from the Mondulkiri forest site was susceptible to all three pyrethroids. In Stung Treng, fewer tests were run on *An. dirus* from the forest site, as transportation from the distant site to the field laboratory had a negative impact on the numbers available for testing. The low densities of *An. dirus* collected in the annex village sites in both provinces also limited the number of tests completed. The assays that were run demonstrated full susceptibility to alpha-cypermethrin 0.05% (Ou Chay annex village, Stung Treng) and deltamethrin 0.05% (Pu Til annex village, Mondulkiri). In the absence of primary vectors in adequate numbers in Stung Treng after October, testing of the secondary vector species *An. peditaeniatus* was conducted in November 2021. *An. peditaeniatus* was resistant to alpha-cypermethrin 0.05% and deltamethrin 0.05%.

In Mondulkiri, insecticide susceptibility tests of a limited number of *An. maculatus* s.l. and *An. minimus* s.l. collected from CDNs in Pu Til annex village were run in November 2021. Those tested were susceptible to deltamethrin 0.05% (Table 7).

TABLE 7. RESULTS OF INSECTICIDE SUSCEPTIBILITY TESTS ON PRIMARY AND SECONDARY MALARIA VECTORS IN SENTINEL SITES OF MONDULKIRI AND STUNG TRENG PROVINCES

Species tested	Province	Sentinel Site	Month/s of Test	Trapping Technique	Insecticide and Concentration	Number of Mosquitoes Tested	Resistance Status (Corrected Mortality %)
An. dirus	Mondulkiri	Pu Til Village	Sep 2021	HDN	Deltamethrin 0.05%	48	S (100%)
		Forest Fringe	Sep 2021	HDN	Deltamethrin 0.05%	100	S (100%)
			Sep, Oct, and Nov 2021	HDN	Alpha-cypermethrin 0.05%	150	S (100%)
			Oct and Nov 2021	HDN	Permethrin 0.75%	116	S (100%)
	Stung Treng	Ou Chay Village	Sep 2021	HDN	Alpha-cypermethrin 0.05%	25	S (100%)
		Forest Fringe	Sep 2021	HDN	Alpha-cypermethrin 0.05%	50	S (100%)
An. maculatus s.l.	Mondulkiri	Pu Til Village	Nov 2021	CDN	Deltamethrin 0.05%	31	S (100%)
An. minimus s.l.	Mondulkiri		Nov 2021	CDN	Deltamethrin 0.05%	52	S (100%)
An. peditaeniatus	Stung Treng	Ou Chay Village	Nov 2021	CDN	Alpha-cypermethrin 0.05%	100	R (37%)
			Nov 2021	CDN	Deltamethrin 0.05%	50	R (50%)

3.3 LABORATORY ANALYSES

Mosquito samples collected from sentinel sites were stored individually in Eppendorf tubes for molecular testing. CSP ELISA will be used to detect *P. falciparum* and *P. vivax* sporozoites. Training on the CSP ELISA is planned for the VectorLink Cambodia entomology team in the coming months with the guidance of the PMI VectorLink molecular biologist and a local consultant once the reagents and supplies required are received in Cambodia.

A subsample of 1,053 mosquito samples from *An. dirus*, *An. minimus,* and *An. maculatus* complexes have been selected to be sent to CDC Atlanta for species confirmation by PCR pending approval from the CNM. A laboratory capacity assessment was completed in August 2021 for the CNM and Royal University of Phnom Penh with the aim of building in-country capacity for future laboratory analyses of mosquito samples.

3.4 CAPACITY-BUILDING ACTIVITIES

On-the-job training was provided for CNM, PHD, and health center staff during monthly collections in the provinces for trap setting, collections of mosquitoes, and morphological identification of mosquitoes. On-the-job training for insecticide resistance monitoring was provided for CNM technicians.

4. OBSERVATIONS AND CONCLUSIONS

The species compositions, densities, and species richness of four trapping methods for the *Anopheles* species, along with the seasonal abundance and hourly biting rates of the primary malaria vectors (*An. dirus*, *An. maculatus* s.l., and *An. minimus* s.l.) were explored during Year 3 using monthly longitudinal entomological monitoring in two high-burden provinces, Stung Treng and Mondulkiri, in Cambodia.

The three primary malaria vectors were found in different densities among the sentinel sites monitored, and their biting behavior was demonstrably different. *An. dirus* was found in all four sites but at higher densities in the forest sites than in the annex village sites in both provinces. Of the three primary vectors, *An. dirus* was also predominant in the forest sites, where *An. minimus* s.l. and *An. maculatus* s.l. were rarely found. This is consistent with the notion that the preferred habitat of *An. dirus* is forested mountains and foothills. *An. maculatus* s.l. and *An. minimus* s.l. were found in higher densities in the two sites of Mondulkiri Province than in the two sites in Stung Treng Province, where these species were not common. *An. maculatus* s.l. and *An. minimus* s.l. were more prevalent than *An. dirus* in the village site of Mondulkiri, but not in the village site of Stung Treng. High densities of the primary vector species *An. dirus* in the forests suggest that it may be the species most responsible for forest malaria transmission, but this can only be determined by completion of the ELISA testing for sporozoites. The densities also suggest a possible role of *An. minimus* s.l. and *An. maculatus* (to a lesser extent) in malaria transmission in and around human dwellings in Mondulkiri. Conclusions based on densities alone should be treated with caution until they can be corroborated with sporozoite rate and entomological inoculation rate calculations.

Seasonal trends in the densities of the primary vectors were observed. *An. dirus* densities increased with the start of the continuous rains from July through September in all sites in both provinces, at which time this species was caught by all trapping methods. Because the peak malaria transmission season is July–November, this would also suggest *An. dirus* is an important vector. In contrast, *An. maculatus* s.l. and *An. minimus* s.l. densities started to increase from October, toward the end of the rainy season. *An. minimus* s.l. in particular reached highest densities during the dry season in February.

Of the four trapping methods that were used during the reporting period, CDNs caught the highest numbers, densities, and species richness of *Anopheles*, but because this method uses cows and not humans it may be less relevant for monitoring malaria vectors compared to HDNs and CDC light traps. Of the three primary vectors, *An. dirus* was caught at the highest densities from HDNs in the annex village and forest sites of both provinces, while *An. maculatus* s.l. and *An. minimus* s.l. were caught in highest densities from CDNs. CDC light traps caught *An. dirus* in higher densities during the rainy season, indicating this species may also act as a vector in annex village sites.

FTT was not an efficient method compared with the other three techniques and it was not useful for estimating human biting rates – FTT caught so few mosquitoes that the project does not recommend continuing with this method. HDNs and CDC light traps that target anthropophilic vectors may be more appropriate for use in longitudinal monitoring of vectors and in foci investigations than the other trapping methods. The seasonality in human biting rates as estimated by HDN and CDC light traps is similar, but the biting rate estimated by HDNs outdoors is considerably higher than the biting rate indoors as estimated by CDC light traps. This observation likely reflects the exophagic nature of the primary malaria vectors rather than any difference in trapping efficiency between the two methods.

The primary vector *An. dirus* showed a tendency to bite early in the evening in the forest sites of both provinces and in the annex village site of Stung Treng Province; in the latter site, it also had a second biting peak at around midnight, a bimodal pattern of biting. Because the biting rate was already high during the first hour (18:00–19:00) of recording, VectorLink Cambodia proposes to start the collections earlier, at 16:00, in order to detect the onset of early biting. CDN collections at the annex village site of Mondulkiri found two biting peaks for *An. minimus* s.l.*,* one early and one around midnight. No clear hourly biting pattern was established for *An. maculatus* s.l.

Insecticide susceptibility was demonstrated for *An. dirus* in the forest site of Mondulkiri Province for all three pyrethroids (alpha-cypermethrin, deltamethrin, and permethrin) used in the LLINs distributed in Cambodia. No resistance was detected either in *An. maculatus* s.l. or *An. minimus* s.l.*,* although lower numbers were tested, so these data should be treated with some caution. Nonetheless, these data provide some confidence that LLINs would kill these mosquitoes if they came into contact with them.

In light of observations of early biting behavior of primary malaria vectors, further studies on use of LLINs and observations of human behavior would help in identifying the gaps in the vector control in Cambodia. A previous study in Cambodia concluded that heterogeneity of human behavior and the variations of the vector densities and biting behaviors might lead to a considerable proportion of exposure occurring during times that people not protected by LLINs.[2] Moreover, future work needs to explore the vectorial status of other species of mosquito beyond the three primary malaria vectors already identified, to understand the future threats to malaria elimination in Cambodia. This is particularly relevant for secondary vectors like *An. peditaeniatus*, for which resistance to deltamethrin and alpha-cypermethrin was demonstrated in Stung Treng Province and is the most abundant species in the Stung Treng annex village. Insecticide resistance in this species has been recorded in the region including in neighboring countries. It is likely that resistance has emerged in this species because of a preference to breed in rice fields, which are often treated with pesticides, thereby exposing larval stages to insecticide selection pressure.[3]

The absence of laboratory analyses in Year 3 limits our ability to interpret the vector bionomic data in terms of vectorial status. Once the laboratory assays for sporozoite ELISA are completed for the backlog of Year 3 samples, a clearer picture of the vectorial status of *Anopheles* in the two high-burden provinces will emerge. PCR species confirmation followed by the sporozoite ELISA would be helpful in correctly identifying and implicating the primary vectors that are members of species complexes.

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ANNEX 1. ANOPHELES SPECIES COMPOSITION FROM CDN TRAPS SET OUTDOORS IN THE ANNEX VILLAGE IN MONDULKIRI (36 TRAPS TOTAL) (N=10,199)

Species	Number	%
An. philippinensis	2,257	22.1%
An. aconitus	1,588	15.6%
An. jamesii	1,438	14.1%
An. minimus s.l.	789	7.7%
An. peditaeniatus	772	7.6%
An. maculatus s.l.	524	5.1%
An. sawadwongporni s.l.	244	2.4%
An. splendidus	409	4.0%
An. vagus	404	3.7%
An. nigerrimus	297	2.9%
An. campestris	267	2.6%
An. nivipes	217	2.1%
An. indefinitus	210	2.1%
Unidentified	159	1.6%
An. nitidus	115	1.1%
An. barbirostris (a, b, and c)	110	1.1%
An. crawfordi	108	1.1%
An. kochi	68	0.7%
An. varuna	62	0.6%
An. sinensis	37	0.4%
An. karwari	28	0.3%
An. subpictus	27	0.3%
An. tessellatus	27	0.3%
An. dirus	22	0.2%
An. pseudojamesi	14	0.1%
An. argyropus	3	<0.1%
An. willmori	1	<0.1%
An. notanandai	1	<0.1%
An. pursati	1	<0.1%
Total	10,199	100.0%

ANNEX 2. ANOPHELES SPECIES COMPOSITION FROM HDNS SET OUTDOORS IN THE ANNEX VILLAGE IN MONDULKIRI (36 TRAPS TOTAL) (N=1,381)

Species	Number	%
An. dirus	295	21.4%
An. philippinensis	171	12.4%
An. maculatus s.l.	148	10.7%
An. aconitus	140	10.1%
An. campestris	140	10.1%
An. minimus s.l.	133	9.6%
An. nigerrimus	74	5.4%
An. sawadwongporni s.l.	70	5.1%
An. crawfordi	38	2.8%
An. nivipes	35	2.5%
An. splendidus	35	2.5%
An. barbirostris (a, b and c)	18	1.3%
An. jamesii	13	0.9%
An. varuna	13	0.9%
An. tessellatus	9	0.7%
An. nitidus	7	0.5%
An. kochi	5	0.4%
An. peditaeniatus	5	0.4%
An. karwari	4	0.3%
An. vagus	4	0.3%
An. indefinitus	3	0.2%
An. baimaii	1	0.1%
An. notanandai	1	0.1%
An. pampanai	1	0.1%
An. pseudojamesi	1	0.1%
Unidentified	17	1.2%
Total	1,381	100.0%

ANNEX 3. ANOPHELES SPECIES COMPOSITION FROM FTTS SET OUTDOORS IN THE ANNEX VILLAGE IN MONDULKIRI (36 TRAPS TOTAL) (N=46)

Species	Number	%
An. philippinensis	8	17.4%
An. crawfordi	6	13.0%
An. nivipes	4	8.7%
An. maculatus s.l.	3	6.5%
An. minimus s.l.	2	4.4%
An. campestris	2	4.4%
An. dirus	2	4.4%
An. splendidus	2	4.4%
An. aconitus	1	2.2%
An. jamesii	1	2.2%
An. nigerrimus	1	2.2%
An. nitidus	1	2.2%
An. pseudojamesi	1	2.2%
An. sawadwongporni s.l.	1	2.2%
An. tessellatus	1	2.2%
An. crawfordi	1	2.2%
Unidentified	9	19.6%
Total	46	100.0%

ANNEX 4. ANOPHELES SPECIES COMPOSITION FROM CDC LIGHT TRAPS SET INDOORS IN THE ANNEX VILLAGE IN MONDULKIRI (144 TRAPS TOTAL) (N=343)

Species	Number	%
An. minimus s.l.	81	23.6%
An. campestris	59	17.2%
An. dirus	48	14.0%
An. aconitus	26	7.6%
An. nigerrimus	19	5.5%
An. varuna	14	4.1%
An. maculatus s.l.	8	2.3%
An. sawadwongporni s.l.	8	2.3%
An. jamesii	6	1.8%
An. philippinensis	6	1.8%
An. peditaeniatus	5	1.5%
An. barbirostris (a,b,c)	2	0.6%
An. nivipes	2	0.6%
An. baimaii	1	0.3%
An. crawfordi	1	0.3%
An. interruptus	1	0.3%
An. kochi	1	0.3%
An. pseudojamesi	1	0.3%
An. sinensis	1	0.3%
An. splendidus	1	0.3%
An. vagus	1	0.3%
Unidentified	51	14.9%
Total	343	100.0%

ANNEX 5. ANOPHELES SPECIES COMPOSITION FROM HDNS SET OUTDOORS IN THE FOREST FRINGE IN MONDULKIRI (36 TRAPS TOTAL) (N=1,399)

Species	Number	%
An. dirus	738	52.8%
An. maculatus s.l.	207	14.8%
An. minimus s.l.	161	11.5%
An. sawadwongporni s.l.	77	5.5%
An. philippinensis	51	3.7%
An. nigerrimus	43	3.1%
An. splendidus	23	1.6%
An. crawfordi	16	1.1%
An. aconitus	15	1.1%
Unidentified	13	0.9%
An. tessellatus	10	0.7%
An. barbirostris (a, b and c)	7	0.5%
An. baimaii	7	0.5%
An. campestris	5	0.4%
An. varuna	5	0.4%
An. nivipes	4	0.3%
An. nitidus	3	0.2%
An. peditaeniatus	3	0.2%
An. jamesii	2	0.1%
An. kochi	2	0.1%
An. pseudojamesi	2	0.1%
An. vagus	2	0.1%
An. willmori	2	0.1%
An. sinensis	1	0.1%
Total	1,399	100.0%

ANNEX 6. ANOPHELES SPECIES COMPOSITION FROM FTTS SET OUTDOORS IN THE FOREST FRINGE IN MONDULKIRI (36 TRAPS TOTAL) (N=91)

Species	Number	%
An. dirus	41	45.0%
An. nigerrimus	13	14.3%
An. maculatus s.l.	7	7.7%
An. sawadwongporni s.l.	6	6.6%
An. philippinensis	4	4.4%
An. crawfordi	3	3.3%
An. aconitus	2	2.2%
An. campestris	2	2.2%
An. splendidus	2	2.2%
An. tessellatus	1	1.1%
An. baimaii	1	1.1%
Unidentified	9	9.9%
Total	91	100.0%

ANNEX 7. *ANOPHELES* SPECIES COMPOSITION FROM CDN TRAPS SET OUTDOORS IN THE ANNEX VILLAGE IN STUNG TRENG (36 TRAPS TOTAL) (N=8,038)

Species	Number	%
An. peditaeniatus	3,221	40.1%
An. tessellatus	1,149	14.3%
An. kochi	805	10.0%
An. philippinensis	675	8.4%
An. barbirostris (a, b and c)	338	4.2%
An. sawadwongporni s.l.	309	3.8%
An. nivipes	308	3.8%
An. vagus	244	3.0%
An. campestris	208	2.6%
An. nigerrimus	176	2.2%
An. indefinitus	121	1.5%
An. dirus	120	1.5%
An. maculatus s.l.	87	1.1%
An. argyropus	79	0.9%
An. minimus s.l.	25	0.3%
An. aconitus	23	0.3%
An. crawfordi	21	0.3%
An. karwari	10	0.1%
An. subpictus	9	0.1%
An. jamesii	6	0.1%
An. nitidus	4	<0.1%
An. baimaii	3	<0.1%
An. notanandai	3	<0.1%
An. pampanai	2	<0.1%
An. sinensis	2	<0.1%
An. varuna	2	<0.1%
An. willmori	1	<0.1%
Unidentified	87	1.1%
Total	8,038	100.0%

ANNEX 8. *ANOPHELES* SPECIES COMPOSITION FROM HDNS SET OUTDOORS IN THE ANNEX VILLAGE IN STUNG TRENG (36 TRAPS TOTAL) (N=915)

Species	Number	%
An. tessellatus	268	29.3%
An. dirus	241	26.3%
An. peditaeniatus	135	14.8%
An. philippinensis	72	7.9%
An. kochi	55	6.0%
An. nivipes	48	5.3%
Unidentified	24	2.6%
An. barbirostris (a, b, and c)	14	1.5%
An. sawadwongporni s.l.	12	1.3%
An. campestris	12	1.3%
An. aconitus	8	0.9%
An. argyropus	9	1.0%
An. maculatus s.l.	4	0.4%
An. nigerrimus	4	0.4%
An. indefinitus	2	0.2%
An. minimus s.l.	2	0.2%
An. vagus	2	0.2%
An. baimaii	1	0.1%
An. karwari	1	0.1%
An. nitidus	1	0.1%
Total	915	100.0%

ANNEX 9. *ANOPHELES* SPECIES COMPOSITION FROM FTTS SET OUTDOORS IN THE ANNEX VILLAGE IN STUNG TRENG (36 TRAPS TOTAL) (N=14)

Species	Number	%
An. indefinitus	1	7.1%
An. nivipes	6	42.9%
An. philippinensis	4	28.6%
An. vagus	1	7.1%
Unidentified	2	14.3%
Total	14	100.0%

ANNEX 10. *ANOPHELES* SPECIES COMPOSITION FROM CDC LIGHT TRAPS SET INDOORS IN THE ANNEX VILLAGE IN STUNG TRENG (144 TRAPS TOTAL) (N=604)

Species	Number	%
Unidentified	253	41.9%
An. dirus	123	20.4%
An. kochi	48	8.0%
An. philippinensis	40	6.6%
An. peditaeniatus	36	6.0%
An. tessellatus	31	5.1%
An. sawadwongporni	22	3.6%
An. nivipes	13	2.2%
An. nigerrimus	9	1.5%
An. barbirostris (a,b, and c)	7	1.2%
An. maculatus s.l.	7	1.2%
An. vagus	7	1.2%
An. campestris	4	0.7%
An. minimus s.l.	2	0.3%
An. aconitus	1	0.2%
An. argyropus	1	0.2%
Total	604	100.0%

ANNEX 11. *ANOPHELES* SPECIES COMPOSITION FROM HDNS SET OUTDOORS IN THE FOREST FRINGE IN STUNG TRENG (36 TRAPS TOTAL) (N=312)

Species	Number	%
An. dirus	521	41.9%
An. tessellatus	470	37.8%
An. barbirostris (a,b, and c)	53	4.3%
An. kochi	41	3.3%
An. philippinensis	41	3.3%
An. campestris	37	3.0%
An. peditaeniatus	17	1.4%
An. nivipes	18	1.5%
An. sawadwongporni s.l.	15	1.2%
An. maculatus	11	0.9%
An. baimaii	4	0.3%
An. minimus s.l.	4	0.3%
An. nigerrimus	3	0.2%
Unidentified	6	0.5%
An. aconitus	2	0.2%
An. notanandai	1	0.1%
An. pampanai	1	0.1%
Total	1,245	100.0%

ANNEX 12. *ANOPHELES* SPECIES COMPOSITION FROM FTT TRAPS SET OUTDOORS IN THE FOREST FRINGE IN STUNG TRENG (36 TRAPS TOTAL) (N=16)

Species	Number	%
An. nivipes	4	25.0%
An. philippinensis	4	25.0%
An. dirus	2	12.5%
An. tessellatus	2	12.5%
Unidentified	2	12.5%
An. barbirostris (a,b and c)	1	6.3%
An. sawadwongporni	1	6.3%
Total	16	100.0%

ANNEX 13. MEAN COW BITING RATES OF PRIMARY MALARIA VECTORS (*AN. DIRUS, AN. MACULATUS, AND AN. MINIMUS)* OF MONDULKIRI AND STUNG TRENG PROVINCES FROM OCTOBER 2020 TO NOVEMBER 2021

Month	*An. dirus*		*An. maculatus*		*An. minimus*	
	Mondulkiri	Stung Treng	Mondulkiri	Stung Treng	Mondulkiri	Stung Treng
Oct-20	2.0	3.3	1.0	1.0	1.0	0.0
Nov-20	0.3	8.0	13.3	2.3	26.7	0.0
Dec-20	0.0	1.3	6.7	5.0	48.0	4.3
Jan-21	0.0	0.3	5.0	10.7	33.7	2.0
Feb-21	0.0	0.3	11.0	7.0	60.0	0.3
Mar-21	NA	NA	NA	NA	NA	NA
Apr-21	NA	NA	NA	NA	NA	NA
May-21	0.0	0.3	7.0	0.3	12.7	0.0
Jun-21	0.3	0.3	4.0	0.7	7.7	0.0
Jul-21	0.0	0.7	12.3	0.0	3.7	0.0
Aug-21	0.7	5.0	2.3	0.3	0.7	0.0
Sep-21	1.0	14.3	25.7	1.0	1.7	0.3
Oct-21	3.0	6.0	51.0	0.3	7.0	0.7
Nov-21	0.0	0.0	36.0	1.0	55.7	0.7

ANNEX 14. MEAN HUMAN BITING RATES OF PRIMARY MALARIA VECTORS (*AN. DIRUS, AN. MACULATUS, AND AN. MINIMUS)* FROM HDN IN MONDULKIRI AND STUNG TRENG PROVINCES FROM OCTOBER 2020 TO NOVEMBER 2021

Month	*An. dirus*				*An. maculatus*				*An. minimus*			
	Mondulkiri		Stung Treng		Mondulkiri		Stung Treng		Mondulkiri		Stung Treng	
	Village	Forest fringe	Village	Forest fringe	Village	Forest fringe	Village	Forest fringe	Village	Forest fringe	Village	Forest fringe
Oct-20	14.0	7.7	3.0	49.0	0.3	0.7	0.0	0.0	0.0	0.3	0.0	0.0
Nov-20	1.0	18.0	0.7	8.7	2.0	11.7	0.3	0.3	3.0	10.0	0.0	0.0
Dec-20	1.3	30.7	0.3	3.7	3.0	15.7	0.0	0.0	5.7	8.0	0.0	0.0
Jan-21	0.3	1.0	0.0	0.0	2.3	5.3	0.0	0.0	6.7	6.0	0.0	0.0
Feb-21	0.3	1.0	0.3	0.0	2.3	5.3	0.0	0.0	12.3	13.0	0.0	0.7
Mar-21	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Apr-21	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
May-21	1.3	4.7	2.7	0.0	4.0	1.3	0.0	0.0	2.7	2.7	0.0	0.0
Jun-21	4.3	12.7	2.3	3.7	0.0	0.7	0.0	0.0	0.7	0.0	0.0	0.0
Jul-21	1.0	19.3	0.7	4.3	1.0	3.7	0.0	0.0	0.0	0.3	0.0	0.0
Aug-21	1.3	21.0	12.3	53.7	1.0	2.7	0.3	0.0	0.0	0.0	0.0	0.0
Sep-21	43.3	64.0	52.7	58.3	8.3	2.7	0.7	0.3	0.7	0.7	0.0	0.0
Oct-21	28.7	45.7	7.7	5.0	13.3	8.3	0.0	0.0	3.7	0.0	0.7	0.0
Nov-21	1.7	21.0	0.7	1.3	10.7	11.0	0.0	0.7	8.3	5.3	0.0	0.7

ANNEX 15. MEAN HUMAN BITING RATES OF PRIMARY MALARIA VECTORS (*AN. DIRUS, AN. MACULATUS, AND AN. MINIMUS*) FROM FTT IN MONDULKIRI AND STUNG TRENG PROVINCES FROM OCTOBER 2020 TO NOVEMBER 2021

Month	*An. dirus*				*An. maculatus*				*An. minimus*			
	Mondulkiri		Stung Treng		Mondulkiri		Stung Treng		Mondulkiri		Stung Treng	
	Village	Forest fringe	Village	Forest fringe	Village	Forest fringe	Village	Forest fringe	Village	Forest fringe	Village	Forest fringe
Oct-20	0.0	4.3	0.0	0.0	0.7	0.0	0.3	0.0	0.0	0.0	0.0	0.0
Nov-20	0.0	3.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
Dec-20	0.0	0.7	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Jan-21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Feb-21	0.1	0.3	0.0	0.0	0.0	0.0	<0.1	0.0	0.0	0.0	0.0	0.0
Mar-21	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Apr-21	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
May-21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Jun-21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Jul-21	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aug-21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sep-21	0.0	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oct-21	0.7	1.7	0.0	0.0	0.3	0.0	0.7	0.0	0.0	0.0	0.0	0.0
Nov-21	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0

ANNEX 16. MEAN HUMAN BITING RATES OF PRIMARY MALARIA VECTORS (*AN. DIRUS, AN. MACULATUS, AND AN. MINIMUS)* FROM CDC LIGHT TRAPS IN MONDULKIRI AND STUNG TRENG PROVINCES FROM OCTOBER 2020 TO NOVEMBER 2021

Month	*An. dirus*		*An. maculatus*		*An. minimus*	
	Mondulkiri	Stung Treng	Mondulkiri	Stung Treng	Mondulkiri	Stung Treng
Oct-20	1.2	0.8	0.1	0.1	0.3	0.0
Nov-20	0.1	0.1	0.0	0.2	1.2	0.0
Dec-20	0.0	0.0	0.0	0.0	0.3	0.1
Jan-21	0.1	0.1	0.0	0.0	1.0	0.1
Feb-21	0.0	0.0	0.0	0.0	4.0	0.0
Mar-21	NA	NA	NA	NA	NA	NA
Apr-21	NA	NA	NA	NA	NA	NA
May-21	0.0	0.0	0.0	0.0	0.2	0.0
Jun-21	0.0	0.0	0.0	0.0	0.1	0.0
Jul-21	0.0	0.0	0.0	0.0	0.0	0.0
Aug-21	0.1	0.1	0.0	0.0	0.0	0.0
Sep-21	1.8	0.6	0.2	0.0	0.0	0.0
Oct-21	1.3	0.5	0.4	0.1	0.2	0.0
Nov-21	0.0	0.0	0.0	0.0	0.1	0.0

ANNEX 17. MEAN HOURLY BITING RATE OF *AN. DIRUS* FROM CDN, HDN, AND FTT IN VILLAGE AND FOREST FRINGE SITES IN MONDULKIRI PROVINCE FROM OCTOBER 2020 TO NOVEMBER 2021

Hour	CDN		HDN				FTT			
	Number caught (annex village) (36 nights)	Biting rate (Village)	Number caught (annex village) (36 nights)	Biting rate (Village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)	Number caught (annex village)	Biting rate (village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)
18:00–19:00	2	0.1	37	1.0	98	2.7	0	0.0	4	0.1
19:00–20:00	3	0.1	17	0.5	97	2.7	0	0.0	2	0.1
20:00–21:00	6	0.2	40	1.1	116	3.2	0	0.0	10	0.3
21:00–22:00	2	0.1	69	1.9	89	2.5	0	0.0	6	0.2
22:00–23:00	2	0.1	25	0.7	47	1.3	0	0.0	3	0.1
23:00–00:00	3	0.1	20	0.6	41	1.1	0	0.0	5	0.1
00:00–01:00	0	0.0	26	0.7	67	1.9	0	0.0	0	0.0
01:00–02:00	4	0.1	22	0.6	67	1.9	0	0.0	6	0.2
02:00–03:00	0	0.0	10	0.3	43	1.2	0	0.0	0	0.0
03:00–04:00	0	0.0	13	0.4	39	1.1	2	0.1	2	0.1
04:00–05:00	0	0.0	12	0.3	25	0.7	0	0.0	2	0.1
05:00–06:00	0	0.0	4	0.1	9	0.3	0	0.0	1	<0.1

ANNEX 18. MEAN HOURLY BITING RATE OF *AN. MACULATUS* FROM CDN, HDN, AND FTT IN VILLAGE AND FOREST FRINGE SITES IN MONDULKIRI PROVINCE FROM OCTOBER 2020 TO NOVEMBER 2021

Hour	CDN		HDN				FTT			
	Number caught (annex village) (36 nights)	Biting rate (village)	Number caught (annex village) (36 nights)	(Biting rate (Village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)	Number caught (annex village)	Biting rate (village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)
18:00–19:00	62	1.7	37	1.0	41	1.1	1	<0.1	2	0.1
19:00–20:00	61	1.7	22	0.6	33	0.9	1	<0.1	1	<0.1
20:00–21:00	71	2.0	15	0.4	24	0.7	1	<0.1	2	0.1
21:00–22:00	48	1.3	9	0.3	15	0.4	0	0.0	0	0.0
22:00–23:00	24	0.7	10	0.3	12	0.3	0	0.0	0	0.0
23:00–00:00	23	0.6	10	0.3	8	0.2	0	0.0	0	0.0
00:00–01:00	52	1.4	17	0.5	14	0.4	0	0.0	0	0.0
01:00–02:00	36	1.0	3	0.1	16	0.4	0	0.0	0	0.0
02:00–03:00	31	0.9	4	0.1	18	0.5	0	0.0	1	<0.1
03:00–04:00	50	1.4	7	0.2	8	0.2	0	0.0	1	<0.1
04:00–05:00	38	1.1	8	0.2	6	0.2	0	0.0	0	0.0
05:00–06:00	28	0.8	6	0.2	12	0.3	0	0.0	0	0.0

ANNEX 19. MEAN HOURLY BITING RATE OF *AN. MINIMUS* FROM CDN, HDN, AND FTT IN VILLAGE AND FOREST FRINGE SITES IN MONDULKIRI PROVINCE FROM OCTOBER 2020 TO NOVEMBER 2021

Hour	CDN		HDN				FTT			
	Number caught (annex village) (36 nights)	Biting rate (Village)	Number caught (annex village) (36 nights)	(Biting rate (village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)	Number caught (annex village)	Biting rate (village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)
18:00–19:00	75	2.1	28	0.8	15	0.4	0	0.0	0	0.0
19:00–20:00	96	2.7	20	0.6	24	0.7	0	0.0	0	0.0
20:00–21:00	98	2.7	16	0.4	29	0.8	1	<0.1	0	0.0
21:00–22:00	77	2.1	20	0.6	17	0.5	0	0.0	0	0.0
22:00–23:00	37	1.0	8	0.2	9	0.3	0	0.0	0	0.0
23:00–00:00	48	1.3	12	0.3	9	0.3	0	0.0	0	0.0
00:00–01:00	54	1.5	7	0.2	10	0.3	1	<0.1	0	0.0
01:00–02:00	55	1.5	4	0.1	14	0.4	0	0.0	0	0.0
02:00–03:00	69	1.9	8	0.2	10	0.3	0	0.0	0	0.0
03:00–04:00	82	2.3	3	0.1	5	0.1	0	0.0	0	0.0
04:00–05:00	53	1.5	1	<0.1	8	0.2	0	0.0	0	0.0
05:00–06:00	45	1.3	6	0.2	11	0.3	0	0.0	0	0.0

ANNEX 20. MEAN HOURLY BITING RATE OF *AN. DIRUS* FROM CDN, HDN, AND FTT IN VILLAGE AND FOREST FRINGE SITES IN STUNG TRENG PROVINCE FROM OCTOBER 2020 TO NOVEMBER 2021

Hour	CDN		HDN				FTT			
	Number caught (annex village) (36 nights)	Biting rate (village)	Number caught (village) (36 nights)	Biting rate (village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)	Number caught (annex village)	Biting rate (village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)
18:00–19:00	6	0.2	21	0.6	64	1.8	0	0.0	0	0.0
19:00–20:00	16	0.4	15	0.4	86	2.4	0	0.0	1	<0.1
20:00–21:00	17	0.5	38	1.1	78	2.2	0	0.0	0	0.0
21:00–22:00	17	0.5	35	1.0	72	2.0	0	0.0	0	0.0
22:00–23:00	17	0.5	37	1.0	56	1.6	0	0.0	0	0.0
23:00–00:00	8	0.2	27	1.0	46	1.3	0	0.0	0	0.0
00:00–01:00	5	0.1	17	0.8	28	0.8	0	0.0	1	<0.1
01:00–02:00	13	0.4	10	0.3	30	0.8	0	0.0	0	0.0
02:00–03:00	7	0.2	14	0.4	22	0.6	0	0.0	0	0.0
03:00–04:00	11	0.3	14	0.4	12	0.3	0	0.0	0	0.0
04:00–05:00	2	0.1	9	0.3	11	0.3	0	0.0	0	0.0
05:00–06:00	1	<0.1	4	0.1	16	0.4	0	0.0	0	0.0

ANNEX 21. MEAN HOURLY BITING RATE OF *AN. MACULATUS* FROM CDN, HDN, AND FTT IN VILLAGE AND FOREST FRINGE SITES IN STUNG TRENG PROVINCE FROM OCTOBER 2020 TO NOVEMBER 2021

Hour	CDN		HDN				FTT			
	Number caught (annex village) (36 nights)	(Biting rate (village)	Number caught (annex village)	Biting rate (village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)	Number caught (annex village)	Biting rate (village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)
18:00–19:00	13	0.4	0	0.0	3	0.1	0	0.0	0	0.0
19:00–20:00	14	0.4	1	<0.1	0	0.0	0	0.0	0	0.0
20:00–21:00	10	0.3	0	0.0	2	0.1	0	0.0	0	0.0
21:00–22:00	13	0.4	0	0.0	1	<0.1	0	0.0	0	0.0
22:00–23:00	3	0.1	0	0.0	1	<0.1	0	0.0	0	0.0
23:00–00:00	9	0.3	1	<0.1	1	<0.1	0	0.0	0	0.0
00:00–01:00	5	0.1	0	0.0	0	0.0	0	0.0	0	0.0
01:00–02:00	7	0.2	1	<0.1	1	<0.1	0	0.0	0	0.0
02:00–03:00	2	0.1	0	0.0	1	<0.1	0	0.0	0	0.0
03:00–04:00	4	0.1	0	0.0	0	0.0	0	0.0	0	0.0
04:00–05:00	3	0.1	1	0.1	0	0.0	0	0.0	0	0.0
05:00–06:00	4	0.1	0	0.0	1	<0.1	0	0.0	0	0.0

ANNEX 22. MEAN HOURLY BITING RATE OF *AN. MINIMUS* FROM CDN, HDN, AND FTT IN VILLAGE AND FOREST FRINGE SITES IN STUNG TRENG PROVINCE FROM OCTOBER 2020 TO NOVEMBER 2021

Hour	CDN		HDN				FTT			
	Number caught (annex village) (36 nights)	Biting rate (village)	Number caught (annex village)	Biting rate (village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)	Number caught (annex village)	Biting rate (village)	Number caught (forest fringe) (36 nights)	Biting rate (forest fringe)
18:00–19:00	3	0.1	0	0.0	0	0.0	0	0.0	0	0.0
19:00–20:00	5	0.1	0	0.0	1	<0.1	0	0.0	0	0.0
20:00–21:00	1	<0.1	1	<0.1	0	0.0	0	0.0	0	0.0
21:00–22:00	1	<0.1	0	0.0	0	0.0	0	0.0	0	0.0
22:00–23:00	5	0.1	0	0.0	1	<0.1	0	0.0	0	0.0
23:00–00:00	0	<0.1	1	<0.1	1	<0.1	0	0.0	0	0.0
00:00–01:00	2	0.1	0	0.0	1	<0.1	0	0.0	0	0.0
01:00–02:00	3	0.1	0	0.0	0	0.0	0	0.0	0	0.0
02:00–03:00	1	<0.1	0	0.0	0	0.0	0	0.0	0	0.0
03:00–04:00	3	0.1	0	0.0	0	0.0	0	0.0	0	0.0
04:00–05:00	1	<0.1	0	0.0	0	0.0	0	0.0	0	0.0
05:00–06:00	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0