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Intense malarial transmission during the dry season in irrigated rice-growing areas: a case study in Sakassou, Côte d'Ivoire

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ABSTRACT

The health district of Sakassou is one of the 83 health districts in Côte d'Ivoire, located in a zone with very high malarial transmission rates, with an incidence rate of ≥40% Therefore, to guide vector control methods more effectively, it was crucial to have a good understanding of the vectors in the area. This study aimed to determine the level of malarial transmission during the dry season in Sakassou, Côte d'Ivoire. Female *Anopheles* mosquitoes were sampled using human landing catches (HLCs) and pyrethrum spraying catches (PSCs). The larvae were collected using the 'dipping' method. A total of 10,875 adult female mosquitoes of *Anopheles gambiae* were collected. The PCR analysis revealed that all individuals were *Anopheles coluzzii*. The geographical distribution of potential breeding sites of *Anopheles* showed the presence of *An. coluzzii* in all the wetlands of the city of Sakassou. During the dry season, the human-biting rate of *An. coluzzii* was 139.1 bites/person/night. An exophagic trend was displayed by an adult female of *An. coluzzii*. The entomological inoculation rate during the dry season was 1.49 infectious bites/person/night. This study demonstrated that *An. coluzzii* was the main vector of malarial transmission in Sakassou, and the intensity of transmission remains high throughout the dry season.

Key words: *Anopheles coluzzii*, dry season, irrigated rice-growing, malarial transmission, Sakassou

HIGHLIGHTS

- Effect of rice-growing regions on malarial transmission during the dry season in Sakassou was studied.
- Provide novel insights into the dynamics of transmission during this period.
- Areas used for irrigation of rice are potential breeding sites of *Anopheles coluzzii*.
- This geographical focus provides valuable information for targeted vector control interventions in specific areas.
- Offering critical information for malaria control programs.

BACKGROUND

Malaria remains one of the world's most serious diseases. In 2021, the World Health Organization (WHO) reported 247 million malarial cases, with 95% of infection and 96% of deaths occurring in Africa. The diversity of malarial epidemiological profiles is related to climatic, hydrographic, and ecological conditions. At the ecological level, some agricultural practices, such as growing rice in shallow irrigated areas, lead to proliferation of mosquito-breeding sites and often increase malaria incidence.

In sub-Saharan Africa, the proliferation of *Anopheles gambiae*, the main vector of malaria, is associated with rice-growing practices (Reimer *et al.* 2005). In these rice-growing areas, the implementation of hydraulic structures raises significant public health concerns (Klinkenberg *et al.* 2005). Several studies conducted in sub-Saharan Africa indicated that the expansion of irrigated areas leads to an increase in the number of vectors and their long-term reproduction, thereby intensifying human–vector contact (Sissoko *et al.* 2004; Diuk-Wasser *et al.* 2005; Walker & Lynch 2007). Consequently, an upsurge in the prevalence and incidence rates of various vector-borne diseases, including malaria, has been reported in some regions (Plaen & Geneau 2002).

In Côte d'Ivoire, numerous previous studies showed that *An. gambiae* s.l., *An. funestus* s.l., and *An. nili* s.l. are responsible for the transmission of three *Plasmodium* species to humans in the country, namely *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium ovale* (Zoh *et al.* 2020). The transformation of wetland areas to grow rice in several localities in the country led to a significant increase in *An. gambiae* densities, which fortunately is not always followed by a rise in malarial transmission (Dossou-Yovo *et al.* 1998a, 1998b). Studies conducted in the savanna areas indicated that an increased density of *Anopheles* mosquitoes does not necessarily influence malarial transmission. However, in the forest area in western Côte d'Ivoire, the high density of *An. funestus* led to a malarial transmission upsurge in villages practicing annual rice growth.

Several studies carried out in central of Côte d'Ivoire, in the Gbêkê region (in the savanna areas), revealed a high malaria endemicity caused by the three *Plasmodium* species identified in Côte d'Ivoire, with a peak during the rainy season (Dossou-Yovo *et al.* 1998a, 1998b; Zoh *et al.* 2020). Studies in this region also showed the agricultural practices impact on malarial vector proliferation (Dossou-Yovo *et al.* 1998a, 1998b).

Sakassou City, located in the Gbêkê region, is characterized by the presence of irrigated wetlands extending across urban and rural areas. Over 900 ha of these areas are dedicated to year-round irrigation of rice growing, resulting in the availability of permanent breeding sites for malarial vectors, even during the dry season. In general, malarial transmission is observed in the Gbêkê region during the rainy season, with a significant decrease during the dry season. However, with the presence of irrigated wetlands and their year-round exploitation, what would be the malarial transmission situation in Sakassou during the dry season?

This study aimed to determine the malarial transmission level during the dry season in Sakassou. This study could provide the National Malaria Control Program (NMCP) precise information on when vector control should be implemented in this type of area.

MATERIALS AND METHODS

Study sites

Sakassou (7°27′16″ N; 5°17′33″ W) is located in the north-central region, 43 km from Bouaké. The city has a humid tropical climate, with annual precipitation ranging from 1,500 to 2,200 mm. A Guinean forest–savanna mosaic belt characterizes the vegetation. There are two main seasons in this locality: a dry season from December to February and a rainy season from March to November. The study was conducted in a village (Kpétébonou) and an urban district (Habitat-Texas) of Sakassou.

The village of Kpétébonou, is approximately 5 km from Sakassou northwest. The village is surrounded by wetlands. The majority of families practice livestock farming with oxen, sheep, and goats. There are also rice farming and vegetable crops such as tomatoes and okra in the surrounding areas. According to the latest census conducted in 2014, Kpétébonou has a population of 505 inhabitants.

The urban area, Habitat-Texas, is located in the downtown area of Sakassou. This study site is surrounded by wetlands used for growing rice (Figure 1).

Georeferencing of potential breeding sites of mosquitoes

Surveys were conducted in March 2019 in both study sites. All water points that could potentially serve as permanent or temporary breeding sites for *Anopheles* mosquitoes were studied. The larvae were collected using the 'dipping' method. All sampling points were georeferenced with a GPS version Garmin eTrex 20x and all mosquito-breeding sites were described to determine their distribution in each study site. The studied site was considered positive with all breeding sites containing at least one mosquito larva or pupa.

The *Anopheles* larvae were collected at different breeding sites and transported to the insectarium where the collected larvae were counted and reared in the water from the original breeding site until they emerged at room temperature. The adult stage was determined by the Anophelinae of Gillies and Meillon (Gillies & De Meillon 1968) key determination. The breeding sites were labeled according to their location and origin.

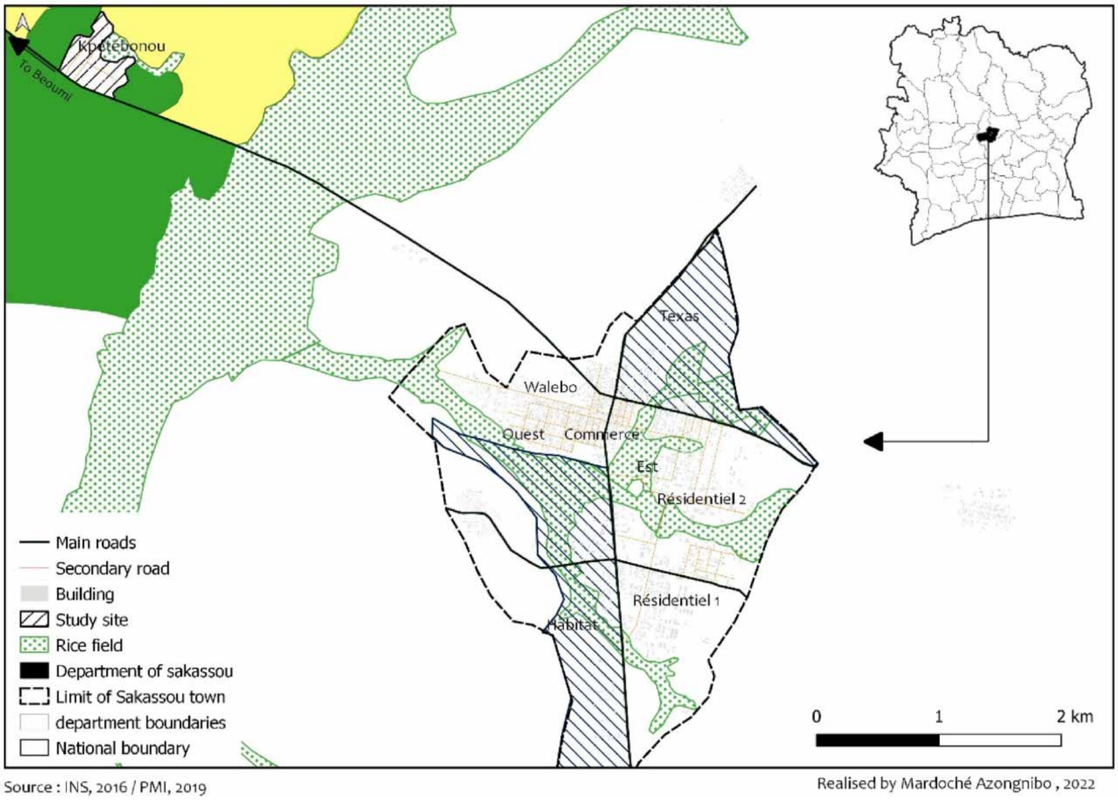

Figure 1 | Location of the study sites.

Adult mosquito sampling

Adult female mosquitoes were collected using human landing catches (HLCs) from November 2018 to March 2019 in 10 capture sessions, totaling 80 person-nights. These captures took place both inside and outside dwellings from 6 p.m. to 6 a.m. The captures were carried out in two teams, with the first team working from 6 p.m. to 12 a.m. and the second from 12 a.m. to 6 a.m. This method involved a catcher seated on a chair or stool with bare legs up to the knees, capturing all mosquitoes that landed on them before biting. The captured mosquitoes were carefully stored in closed hemolysis tubes with cotton and kept in pockets labeled by the time and location of the capture. A circular permutation of the catchers was necessary to limit the margin of error. The determination keys of the Anophelinae of Gillies and DeMeillon (Gillies & De Meillon 1968) were used for morphological identification of mosquitoes. Only female mosquitoes of the malarial vector species collected by HLCs were kept for ovary dissection according to Detinova (Detinova 1962).

Laboratory processing

The head and thorax of each mosquito were carefully separated from the rest of the body and placed in new coded tubes for the detection of *P. falciparum* circumsporozoite protein (CSP) according to Burkot *et al.* (1984); Wirtz *et al.* (1987). The rest of the mosquito body (abdomen and legs) was preserved for molecular identification of species. DNA from the abdomen and legs of each *Anopheles* was extracted. Species molecular identification was performed using PCR SINE 200 Amplification.

Entomological parameters in the dry season

Several entomological indicators were used, including:

(i) The human-biting rate (HBR): the number of *Anopheles* bites per person per night (b/p/n).
(ii) The infection rate (IR): the proportion of female mosquitoes infected by *P. falciparum*.
(iii) The parity rate (PR): the proportion of parous female mosquitoes.

(iv) The Entomological Inoculation Rate (EIR), which is the number of infective bites per person per night by *anopheline* mosquitoes, was calculated as the product of the HBR and the IR of mosquitoes collected on humans. The overall EIR for a given period was calculated as the sum of the EIR of each mosquito species.

Statistical analysis

For statistical analysis, the data were entered into Microsoft Office Excel 2007 software. The HBR, PR, and IR were calculated using the STATA version 8.0 software (College Station, TX: StataCorp LP). A 95% confidence interval was used, and statistical significance was determined at $P < 0.05$ using Mann–Whitney and χ^2 tests to compare the HBR and different rates between both study sites.

RESULTS

Anopheles breeding sites and *Anopheles coluzzii* larval distribution in study sites

In total, 47 potential breeding sites were characterized in both study sites. Among these 47 potential breeding sites, 38 (80.85%) were found positive for anopheline larvae. Molecular identification at the adult stage of anopheline larvae showed that all larvae were *An. coluzzii*. These larvae were found in six (6) types of breeding sites, namely rice fields, ponds, mangroves, puddles, swamps, and abandoned containers. The majority of these breeding sites were permanent except puddles (Table 1).

In Kpétébonou, five of six potential breeding types identified (except abandoned containers) were found with 65.96% (31/47) of breeding sites described in both study sites. Rice fields were predominant with 77.42% (24/31) of all breeding sites identified in the village. Out of 31 breeding sites described, 87.10% (27/31) were found positive except for puddles. *An. coluzzii* larvae were found in breeding sites located on the outskirts of the village (Figure 2).

In Habitat-Texas, five of the six types of potential breeding sites described were identified (except Mangrove), predominated by rice fields with 62.5% (10/16) of all breeding sites identified in the Sakassou urban area. On a total of 16 breeding sites identified, 75% (12/16) were found to be positive. As observed in Kpétébonou, no larva was found in the puddle. *An. coluzzii* larvae were more concentrated in breeding sites located inside the neighborhood (Figure 3).

Anopheline fauna collected by HLCs in sakassou study sites

A total of 11,142 anopheline species was collected in both study sites of Sakassou, with 8,094 (72.64%) in Kpétébonou village and 3,048 (27.36%) in the Habitat-Texas district. Three species were morphologically identified in this study, namely *An. gambiae* complex, *An. Funestus*, and *An. pharoensis*. However, molecular identification showed that all *An. gambiae* complex species belonged to *An. coluzzii*.

In Kpétébonou village, two species of *Anopheles* were identified with *An. coluzzii* as the predominate *Anopheles* species (97%) and *An. pharoensis* (2%).

In the Habitat-Texas district, all three *Anopheles* species were collected and mainly dominated by *An. coluzzii* (97%), followed by *An. pharoensis* (2%) and *An. funestus* (less than 1%) (Table 2).

Table 1 | Characterization of breeding sites of *An. coluzzii* during March 2019 in Sakassou

	Kpétébonou		Habitat-Texas		
Breeding sites	***N*** **(+)**	**Periodicity**	***N*** **(+)**	**Periodicity**	**Total**
Rice field	24 (24)	Permanent	10 (8)	Permanent	**34 (32)**
Pond	1 (1)	Permanent	2 (2)	Permanent	**3 (3)**
Mangrove	1 (1)	Permanent	0	–	**1 (1)**
Puddle	4 (0)	Temporal	1 (0)	Permanent	**5 (0)**
Swamp	1 (1)	Permanent	1 (1)	Permanent	**2 (2)**
Abandoned container	0	–	2 (0)	Permanent	**2 (0)**
Total	**31 (27)**		**16 (11)**		**47 (38)**

N indicates the number of potential breeding sites; (+) indicates the number of positive breeding sites.

Figure 2 | Distribution of breeding habitat of *Anopheles* in Kpétébonou.

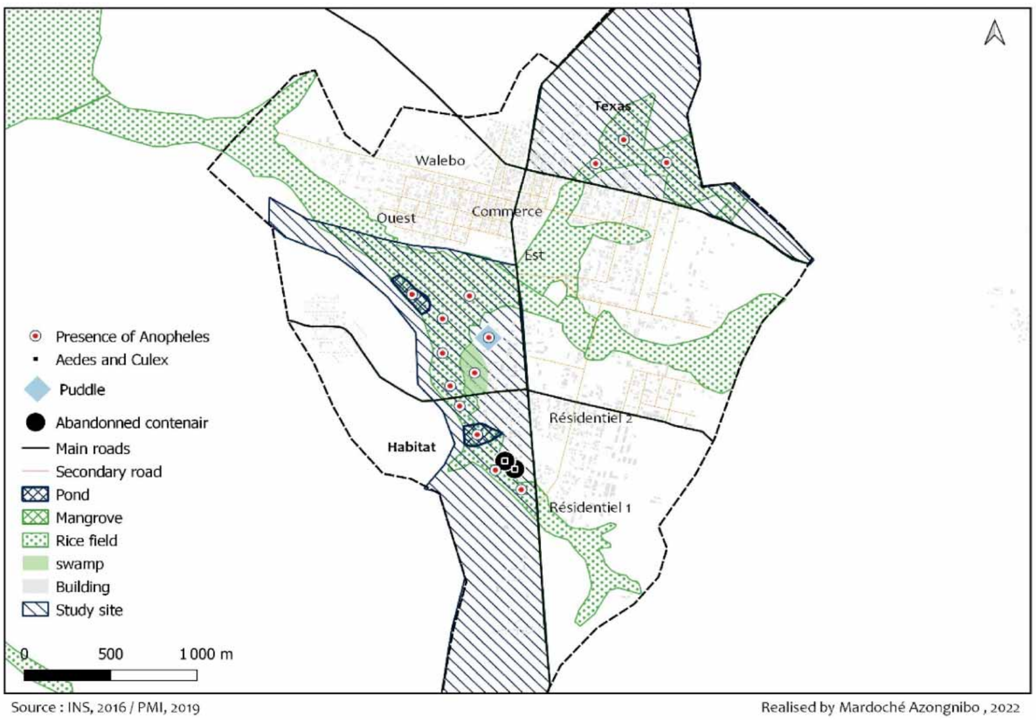

Figure 3 | Distribution of breeding habitat of *Anopheles* in Habitat-Texas.

Table 2 | Composition of mosquito fauna collected by HLCs during the dry season in Sakassou

Species	Kpétébonou			Habitat-Texas		
	Indoor	Outdoor (%)	Total (%)	Indoor	Outdoor (%)	Total (%)
***An. gambiae* s.l.**	3,707	4,190	7,897 (97.57)	1,361	1,617	2,978 (97.70)
***An. funestus* s.l.**	0	0	0	2	1	3 (0.10)
An. pharoensis	89	108	197 (2.43)	17	50	67 (2.20)
Total	***3,796***	***4,298***	***8,094***	***1,380***	***1,668***	***3,048***

In this study, 10,875 female mosquitoes of *An. coluzzii* were identified in both study sites as main vectors. In total, three (03) female mosquitoes of *An. funestus* were collected only in Habitat-Texas with a low proportion. So, only *An. coluzzii* has been considered for the rest of the study.

Biting behavior of *An. coluzzii*

Conducting catches both inside and outside dwellings facilitated the study of the biting behavior of *An. coluzzii* female mosquitoes.

In Kpétébonou and Habitat-Texas, female mosquitoes of *An. coluzzii* have presented an exophagic trend (53.06%), indicating a preference for outdoor biting.

Malarial transmission during the dry season in Kpétébonou and Habitat-Texas

Biting rate of *An. coluzzii*

The average HBR of *An. coluzzii* female mosquitoes recorded in Sakassou during this study was 139.10 bites per person per night (b/p/n). The average biting rate of *An. coluzzii* in Kpétébonou village was 197.43 b/p/n (CI = 178.01–216.83). This rate was significantly higher ($P < 0.0001$) than that recorded in the Habitat-Texas district, with 80.78 b/p/n (CI = 64.66–96.89).

In the dry season (December, January, and February), the biting rate recorded in study sites was 110.56 b/p/n. In Kpétébonou, the average biting rate recorded during this season was 168.70 b/p/n (CI = 149.30–188.10). This rate was comparable ($P = 0.0938$) to that recorded at the rainy season in November (209.88 b/p/n, CI = 160–259.76) but, lower ($P = 0.0001$) than that recorded at the start of the rainy season in March (271.13 b/p/n, CI = 247.14–295.11). In Habitat-Texas, the average biting rate of *An. coluzzii* was 52.42 b/p/n (CI = 39.88–64.96) in the dry season and that was significantly lower than those recorded at the start and the end of the rainy season (121.13 b/p/n, CI = 92.39–149.86 and 125.5, CI = 78.50–172.50, respectively).

In both study sites, the average biting rate of *An. coluzzii* recorded during this season was also higher ($P < 0.0001$) in Kpétébonou than in Habitat district (Table 3).

PR of *An. coluzzii*

During the study, of 2,179 female *An. coluzzii* mosquitoes dissected in two study sites, 61.59% were found to be parous. The PR of *An. coluzzii* female mosquitoes were 60.80% ($n = 1,176$, CI = 58–63.59) in Kpétébonou and 62.51% ($n = 1,003$, CI = 59.51–65.51) in Habitat, and did not vary significantly ($P = 0.4382$) between the two environments. In the dry season, the PR was estimated at 57.44% in Kpétébonou and 61.59% in Habitat-Texas.

Plasmodium IR and EIR

Head and thorax of 1,126 *Anopheles* analyzed by ELISA-CSP revealed a CSP rate of 1.07%. In the village of Kpétébonou, the IR of *P. falciparum* was 0.68% ($n = 587$, CI = 0.01–1.35). *P. falciparum* infections were observed throughout the study except in March at the start of the rainy season. The rates recorded ranged from 0.59 to 1.20%. In Habitat-Texas, the IR was 1.49% ($n = 539$, CI = 0.46–2.51). *P. falciparum* was detected in the months of November, February, and March, with rates between 1.79% and 3.77%. The rate of *P. falciparum* infection did not vary between both study sites ($P = 0.2487$). In Kpétébonou, *P. falciparum* was detected each month in the dry season with an average rate of 1% ($n = 299$, CI = 0–2.14), while in Habitat-Texas, *P. falciparum* was only detected in the month of February (1.90%), with an infectivity rate of 0.62% ($n = 321$, CI = 0–1).

Table 3 | Biting rate of *An. coluzzii* from November 2018 to March 2019 at study sites

	Period	m.a. (b/p/n)	CI	Dry season average
Kpétébonou				
End of rainy season	**Nov-18**	209.88	*160–259.76*	
Dry season	**Dec-18**	159.88	*106.22–213.53*	**168.7 (CI = 149.30–188.10)**
	Jan-19	173.13	*137.40–208.85*	
	Feb-19	173.13	*148.77–197.48*	
Start of rainy season	**March-19**	271.13	*247.14–295.11*	
Kpétébonou average	***197.43***	***178.01–216.83***		
Habitat-Texas				
End of rainy season	**Nov-18**	121.13	*92.39–149.86*	
Dry season	**Dec-18**	67.86	*34.88–100.87*	**52,42 (CI = 39.48–104.96)**
	Jan-19	50.36	*31.85–68.90*	
	Feb-19	39	*23–55*	
Start of rainy season	**March-19**	125.5	*78.50–172.50*	
Habitat-Texas average		***80.78***	***64.66–96.89***	
Average		**139.10**		**110.56**

m.a., biting rate; **CI**, confidence interval; **b/p/n**, bites per person per night.

This rate was significantly lower ($P = 0.0356$) than that recorded at the end of the rainy season (3.77%), but comparable ($P = 0.2760$) to the start of the rainy season (1.79%). However, the infectivity rates recorded between these sites were comparable ($P = 0.6761$) in the dry season.

The EIR of *An. coluzzii* recorded in Sakassou was 1.49 infectious bites/person/night (ib/p/n), or 44.7 ib/p/month. In Kpétébonou, malarial transmission was permanent during the dry season, with *P. falciparum* inoculation rates ranging from 1.49 to 2.08 ib/p/n, with an average rate of 1.69 ib/p/n, i.e. around 51 infectious bites received per person per month. In this village, transmission begins at the end of the rainy season, remains higher during the dry season, and ceases at the start of the rainy season. However, in Habitat-Texas, *P. falciparum* was only transmitted in February, with a rate of 0.74 ib/p/n. The average inoculation rate during the dry season was estimated at 0.33 ib/p/n or 10 ib/p/month. Transmission remains higher at the end and at the start of the rainy season, with rates of 4.57 and 2.25 ib/p/n, respectively. During the dry season in Sakassou, a person living in a rural area receives five times more *P. falciparum*-infected bites than a person living in an urban area (Table 4).

DISCUSSION

This study contributes valuable insights into the dynamics of malarial transmission during the dry season in Sakassou, a region with a notorious prevalence of malaria. The predominance of *An. coluzzii* and its identification as the only malarial vector were due to the availability of specific breeding sites for this species. These breeding sites such as rice fields, ponds, swamps, and mangroves, identified in both study sites are mainly permanent and constitute preferential breeding sites for *An. coluzzii* (Lehmann & Diabate 2008; Simard *et al.* 2009; Kudom 2015).

The exophagic behavior of *An. coluzzii* observed in both study sites would suggest that malarial transmission occurs mainly outdoors. This behavior would be influenced by vector control measures such as insecticide-impregnated bed nets, which can have a repellent effect on vectors. However, comparative analyses between malarial transmission levels inside and outside the home could confirm or refute this suggestion. In addition, knowledge of vector behavior can contribute to the design and implementation of effective control strategies specific to biting behaviors of each vector.

In the dry season, the biting rate of *An. coluzzii*, although higher in Kpétébonou village, remains important (52.42 b/p/n) in Habitat-Texas, in urban areas. This rate remains higher than that recorded in Bouaké urban areas (4.2 b/p/n).

Despite the *P. falciparum* detection throughout the dry season in Kpétébonou, and only in February in Habitat-Texas, the IR remains comparable between the two study sites. This suggests that the risk of malarial transmission is the same in both Sakassou's rural and urban areas in the dry season. *P. falciparum* transmission recorded in the dry season in Kpétébonou was

Table 4 | IR (%) and EIR (ib/p/n) to *Plasmodium* of *An. coluzzii* from November 2018 to March 2019 at study sites

	Period	N	S (95% CI)	SDS average	EIR (ib/p/n)	EIR DS average
Kpétébonou						
End of rainy season	**Nov-18**	170	0.59 *(0–1.75)*		1.24	
Dry season	**Dec-18**	108	0.93 *(0–2.76)*	**1 (*0–2.14*)**	1.49	**1.69**
	Jan-19	83	1.2 *(0–3.60)*		2.08	
	Feb-19	108	0.93 *(0–2.76)*		1.61	
Start of rainy season	**March-19**	118	0		0	
Kpétébonou average		***587***	***0.68 (0.01–1.35)***		***1.34***	
Habitat-Texas						
End of the rainy season	**Nov-18**	106	3.77 *(0.09–7.46)*		4.57	
Dry season	**Dec-18**	101	0	**0.62 *(0–1)***	0	**0.33**
	Jan-19	115	0		0	
	Feb-19	105	1.90 *(0–4.56)*		0.74	
Start of rainy season	**March-19**	112	1.79 *(0–4.28)*		2.24	
Habitat-Texas average		***539***	***1.49 (0.46–2.51)***		***0.54***	
Average		**1,126**	**1.07**	**0.8**	**1.49**	**0.88**

N, number of *An. coluzzii* female tested; *S*, infectivity rate; SDS, infectivity rate in dry season ; EIR, entomological inoculation rate.; ib/p/n, infectious bite received per person per night; DS, dry season.

five times higher than that recorded in Habitat-Texas. This difference observed in *Plasmodium* transmission level between study sites was related to vector density, as the IR was similar in both sites. In Kpétébonou, *Plasmodium* transmission was observed throughout the dry season and remained very high during this period. The same trend was observed in Petessou village in Bouaké where high *Plasmodium* transmission was observed during the dry season (Zoh *et al.* 2020). However, in Habitat-Texas, transmission was less high and only observed in February, but was three times higher than that recorded in Kennedy neighborhood in Bouaké. A study in the rainy season would be necessary to determine the vectorial transmission dynamic throughout the year.

CONCLUSIONS

This study revealed a diversity of breeding sites for *An. coluzzii*, the only malarial vector identified in the Sakassou area. These female mosquitoes showed a general tendency to exophagy and a high PR. Although the IR was similar between both study sites, malarial transmission in Kpétébonou, a rural area, was five times higher than in Habitat-Texas, an urban area. In the Sakassou area, malarial transmission was high during the dry season and was observed throughout the season in the rural area. For better vector control in Sakassou, the vector control must be carried out throughout the dry season. This should include the treatment of breeding sites.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the National Ethics Committee. All the mosquito collectors were adult volunteers from the study village and the neighborhood of Habitat-Texas. Each of these volunteers gave his or her informed consent, were protected with appropriate antimalarial prophylaxis, and were immunized against yellow fever.

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DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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