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Population Dynamics of *Anopheles gambiae* s.l. and *Culex quinquefasciatus* in Rural and Urban Settings Before an Indoor Residual Spraying Campaign in Northern Benin

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Abstract

Background: The purpose of this report is to provide information on Culicidae diversity; biting behavior and spatio-seasonal variation of abundance of *Anopheles gambiae* s.l. and *Culex quinquefasciatus* in rural and urban settings of the Alibori and Donga regions, Northern Benin, where an indoor residual spraying (IRS) campaign to control malaria is planned.
Methods: Both human landing catches, associated with pyrethrum spray catches were used to monitor the mosquito populations in 12 sites with 1 urban and 1 rural located in each of the 6 districts randomly selected in the two targeted regions. After morphological identification of all mosquito specimens, biting behavior and density of *An. gambiae* s.l. and *Cx quinquefasciatus* were studied. PCR was also performed on *An. gambiae* s.l., to identify sibling species and its seasonal variation.
Results: A total of 10,367 mosquitoes were captured, related to 14 species of the genera, *Anopheles*, *Aedes*, *Culex* and *Mansonia*. Of the total species collection, 40.39% were *An. gambiae* s.l. and 56.85% were *Cx. quinquefasciatus. An. gambiae* s.l. was more abundant in Donga (2521 specimens) compared with Alibori (1666 specimens). The opposite trend was observed with *Cx. quinquefasciatus* (2162 specimens in Donga against 4028 in Alibori). *An. gambiae* s.l. was predominant and displayed a higher blood feeding rate in rural areas, whereas *Cx. quinquefasciatus* was in majority in urban areas. *An. gambiae* s.l. was more endophagic, whereas *Cx. quinquefasciatus* showed similar indoor and outdoor biting behavior. *An. gambiae* s.l. was composed of *An. coluzzii* found in majority in the drought, and *An. gambiae*, which was predominant in the rainy season.
Conclusion: The predominance of the malaria vector, *An. gambiae* s.l. and their higher blood feeding rate and their significantly high endophagy in rural areas indicate that these areas should be primarily targeted with the IRS operations to have a substantial impact on malaria transmission. Endophagy, characteristic of *An. gambiae* s.l. in our study area, suggests that IRS will have a positive impact on vector control if implemented 1 week before June that is the onset of the rainy season.

Keywords: *Anopheles gambiae* s.l., *Culex quinquefasciatus*, biting behavior, seasonal variation, Alibori, Donga, Benin

Background

In public health, mosquitoes are considered as one of the most important groups of arthropods (Schaffner et al. 2001, Becker et al. 2010). Those belonging to the *Anopheles*, *Culex*, and *Aedes* genera, are the most studied because of their role in the transmission of a variety of human and animal diseases. According to the World Health Organization (WHO 2014), vector-borne diseases account for 17% of the estimated global burden of all infectious diseases; the main

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vector-borne disease in West Africa being malaria. In Benin, *Anopheles gambiae* s.l. is the major vector of malaria (Akogbeto 1992, Akogbeto and Di Deco 1995), which is the leading cause of morbidity and mortality in children under 5 years old (Ministère de la Santé/DPP 2013).

Long-lasting insecticidal nets (LLINs) massively distributed in the country in 2014 and indoor residual spraying (IRS) remain the strategic pillars in the prevention of malaria in Benin. In 2008, IRS was introduced in the Oueme department, Southern Benin with a carbamate insecticide (Bendiocarb) as the high pyrethroid resistance observed in malaria vectors (Akogbéto and Yacoubou 1999) has reduced the efficacy of standard LLINs (N'guessan et al. 2007). The strategy which displayed a great success (Akogbeto et al. 2011, 2015, Osse et al. 2012) was then relocated in the Atacora department, northern Benin from 2011 to 2015.

The technical rationale for this relocation was the short transmission period that can be easily covered by a single spray round in the new targeted department, allowing a better cost effectiveness. IRS success has also been recorded in other countries, namely Madagascar (Randriantsimaniry 1995), Equatorial Guinea (Bioko Island) (Sharp et al. 2007) and Southern Africa (Sharp et al. 2000, Mabaso et al. 2004). Despite these successes, major challenges remain, including the fact that some countries implement vector control interventions without reliable baseline on entomological data. Indeed, those data are necessary as they allow national malaria control program to know the exact places where vector biting rates or malaria transmission are high and also, the right moment for the implementation of a control intervention such as IRS to have the maximum impact on the peak in biting of malaria vectors.

For that, the availability of sufficient entomological baseline data should be a priority to guide the development of vector control policies (Marsh 2010). Success in the planning, implementation, and evaluation of vector control interventions depends on the knowledge of spatial and temporal variation of disease vectors (Killeen et al. 2011).

In Benin, since the advent of IRS, the monitoring of its impact has focused solely on the behavioral change of *Anopheles*, vectors of malaria (Akogbeto et al. 2011, 2015, Osse et al. 2012). However, *Culex quinquefasciatus*, which is a vector of lymphatic filariasis (*Wuchereria bancrofti*) (Maxwell 1990), have been neglected in entomological evaluations. Although high resistance to insecticides (Yadouleton et al. 2015) has been detected in *Cx. quinquefasciatus*, some authors have reported that some strategies could have a positive impact on the control of this mosquito (Hougard et al. 1993). So far, lymphatic filariasis has not been a public health problem in Benin, but the nuisance and even the daily stress caused by *Culex* bites are important factors that should not be neglected (Viniaker and Lavaud 2005).

In 2017, an IRS campaign with Actellic 300CS was planned to target the Alibori and Donga regions. As a prelude to this control campaign, this study was initiated in some urban and rural sites of the targeted regions to obtain information on the distribution, density, biting behavior, and seasonal variations of *An. gambiae* s.l., main malaria vector (Akogbeto 1992, Akogbeto and Di Deco 1995) and *Cx. quinquefasciatus*, mosquito of high nuisance (Agbanrin et al. 2015) in Benin.

Materials and Methods

Study area

Data collection took place in six districts located in two regions: the Kandi, Gogounou and Segbana (KGS) districts in the Alibori region, then the Djougou, Copargo and Ouake (DCO) districts in the Donga region. The Alibori region of is characterized by a Sudanese climate and the Donga region by a Sudano–Guinean climate. Both regions have a single dry season (December to May) and a single rainy season (June to November). Overall, the average monthly temperature varies between 23°C and 40°C. The region of Donga has more rivers than the region of Alibori. The DCO and KGS health zones cover an area of 5465 km^2 and 12,943 km^2 with a population of 424,425 and 325,522 inhabitants, respectively, mostly farmers. The incidence of malaria was 21.4% in the DCO health zone and 19.4% in the KGS health zone (SGSI/DPP/Zs-DCO/MS 2016) (SGSI/DPP/Zs-KGS/MS 2014).

In the current study, one urban and one rural sites were selected in each district for the monthly collections of mosquitoes. Urban sites have several development infrastructures, such as gutters, sewers, and roads, unlike rural sites that do not have. Urban/Rural villages were Bantansoue/Gounarou, Kossarou/Sonsoro, Segbana-Centre/Liboussou, Parakounan/Kataban, Zountori/Barienou, and Aboulaoude/Komde, respectively, in the districts of Gogounou, Kandi, Segbana, Copargo, Djougou, and Ouake (Fig. 1).

Mosquito sampling

Human landing catches (HLCs) were conducted in two houses per village from 9 PM to 5 AM for two nights per month over 7 months (May 2016 to February 2017, with no data collected in September, November, and December 2016) to monitor the mosquito populations. At each house, one indoor mosquito collector and one outdoor mosquito collector collected all mosquitoes that landed on their feet (World Health Organization 1993). The number of collectors and the number of sessions were the same for all surveyed districts in each month. In each district, recorded data were used to evaluate the mosquito species composition and to determine the human biting rate (HBR) of *Anopheles* and *Cx. quinquefasciatus*.

In addition, pyrethrum spray catches (PSCs) were performed, as described by WHO 1993 for 20 rooms (10 in the urban site and 10 in the rural site) per month over the same 7 months to assess the resting behavior and the vectors' blood feeding rate in each of the six districts. The number of sessions and of investigated rooms were the same for all surveyed districts in each month. PSCs consisted of covering the floor with white sheets, spraying the rooms and collecting all fallen mosquito specimens. After collection, mosquitoes were counted and morphologically identified.

Processing methodology

After each collection, collected mosquitoes were counted and separated into Culicinae and Anophelinae using a stereomicroscope. Mosquitoes were identified using the taxonomic keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987). Specimens of *An. gambiae* s.l. were separated for further molecular analyses.

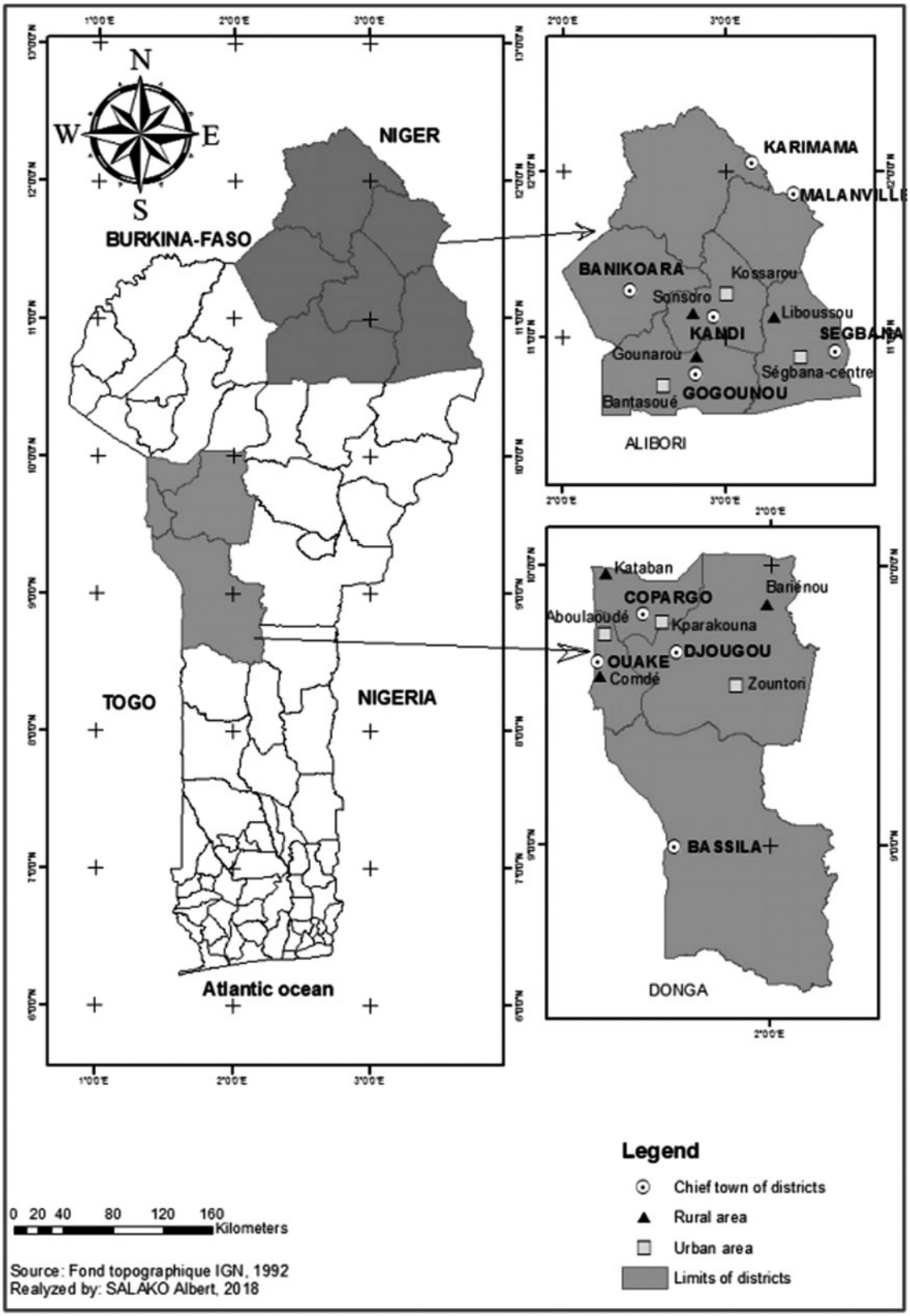


FIG. 1. Map showing the study area.

A sample of 8–68 female specimens of *An. gambiae* s.l., randomly sampled per district per month, bringing to a total number of 111–350 over the study period according to the district, was analyzed by PCR according to the protocol of Santolamazza et al. (2008) for the determination of the molecular species of the *An. gambiae* complex. Thus, specific primers (F6: TCG CCT TAG ACC TTG CGT TA and R6: CGC TTC AAG AAT TCG AGA) were used. The reactions were carried out in a total volume of 25 μL containing 10 pmol of each primer, 0.2 mM of each dNTP, 1.5 mM of $MgCl_2$, 1 U of Taq polymerase and 0.5 μL of the template DNA.

The thermocycler conditions were 94°C for 5 min (step 1), followed by 35 cycles (94°C for 30 s [step 2], 54°C for 30 s [step 3], 72°C for 1 min [step 4]), and 72°C for 7 min (step 5). The products resulting from the amplification were analyzed on a 1.5% gel stained with ethidium bromide.

Data analysis

Species Richness (S), which corresponds to the number of collected species, and their relative abundance (Pi) were assessed (Pi = ni/*N*, where ni = number of species i; *N* = total number of species encountered, $i = 1$) by district.

The Shannon–Wiener index (H) shows that the species diversity was determined by district using the formula $H = -\sum Pi \times \log (Pi)$ (Shannon 1948).

The equitability index was also calculated. It equals to the ratio of the Shannon index to the maximum value that this index could attain if all species in the district were represented in similar proportions.

The Simpson index (D), which is a dominance index was determined according to the formula: $D = 1/\sum Pi$ (Simpson 1949).

The HBR for *An. gambiae* or *Cx. quinquefasciatus* was calculated as the number of collected mosquito species divided by the number of collectors and of nights of sampling effort. The mean indoor resting density of *An. gambiae* s.l. was determined by the following way: The total number of vectors collected by PSC/Total number of rooms surveyed. The Poisson method (Rothman 2012) was used to estimate confidence intervals for biting rates and compare them between districts, urbanization level (rural and urban), and location (indoors and outdoors). The same method was also used to compare the average indoor resting densities between rural and urban areas. Densities of *An. gambiae* (s.l.) with different superscripts (a, b) are significantly different ($p < 0.05$).

The abundance of a given species of mosquito was obtained by cumulating its total number obtained through both HLC and PSC. To assess the spatiotemporal variation of this indicator, it was blot versus monthly rainfall data obtained from Agence pour la Sécurité de la Navigation aérienne en Afrique et à Madagascar (ASECNA) using an excel spreadsheet.

The blood feeding rate of *An. gambiae* s.l. was obtained by dividing the number of fed and half gravid mosquitoes by the total number of collected mosquitoes. The chi-squared test of comparison of proportions was used to compare the proportions of sibling species of the *An. gambiae* complex on one hand, and the blood feeding rates of *An. gambiae* (s.l.) between the rural and urban areas on the other hand, using the R software, version 3.3.2.

Ethics approval

This study was approved by the Institutional Ethics Committee of CREC (IECC) (N°338/MS/DC/SGM/DRFMT/CREC/CEI-CREC/SA).

Mosquito collectors were regularly monitored. In case of confirmed malaria, they were immediately taken care of by the medical doctor of the team according to protocol.

Results

Mosquito species composition

A total of 10,367 mosquitoes were captured during the study period in the six districts using both HLC and PSC. The mosquitoes belonged to 4 genera *(Anopheles*, *Aedes*, *Culex*, *Mansonia*) and 14 species. Overall, seven *Anopheles* species were harvested, including the two main malaria vectors: *An. gambiae* s.l. (40.39%) and *An. funestus*: (0.98%) (Table 1). As shown by the Table, the other species of *Anopheles* captured included *An. nili* (0.01%), *An. pharoensis* (0.03%), *An. ziemanni* (0.02%), *An. paludis* (0.01%) and *An. coustani* (0.09%) (Table 1).

Cx. quinquefasciatus was collected at high densities (56.85%) unlike other *Culex* species, such as *Cx. nebulosus* and *Cx. decens*. *Cx. quinquefasciatus* was the most captured mosquito species in Segbana (81.15%), Gogounou (75.71%), Kandi (56.76%), Ouake (55.72%), and Djougou (49.53%). *Mansonia africana* was also collected in most districts but at very low frequency (0.22% on overall) (Table 1).

The species richness observed at Copargo (11 species) is similar to that of Kandi (10 species), Gogounou (9 species), Djougou (8 species), Segbana (7 species), and Ouake (6 species) ($p > 0.05$).

The Shannon–Wiener index was 0.89 [0.87–0.92] in the DCO health zone against 0.69 [0.68–0.72] in the KGS health zone ($p < 0.05$). The Simpson indices were 0.56 [0.53–0.58] and 0.43 [0.42–0.44], respectively, in the DCO health zone and the KGS health zone.

Monthly variation of abundance of An. gambiae *s.l. and* Cx. quinquefasciatus *in Alibori and Donga*

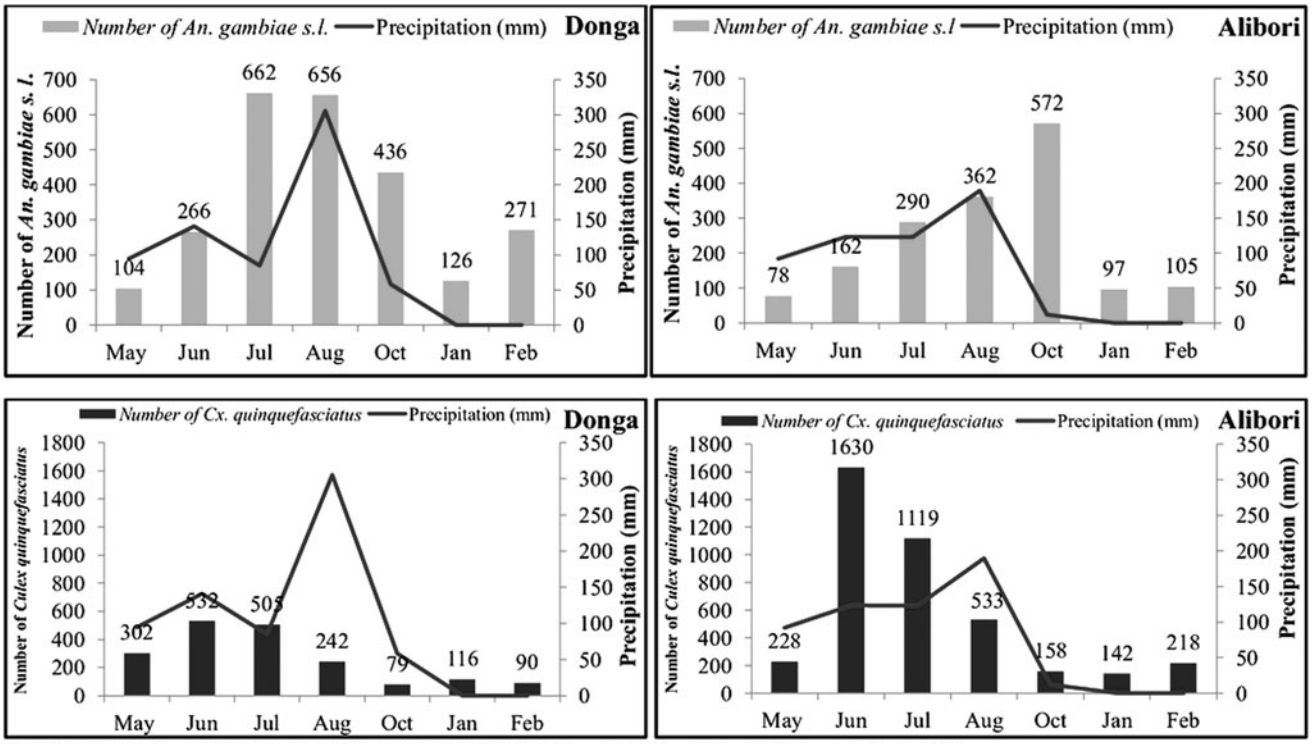
An. gambiae s.l. and *Cx. quinquefasciatus* were present permanently throughout the study period in Alibori and Donga regions, but the abundance of both species varied seasonally. The density of *An. gambiae* s.l. was more abundant during the rainy months in the two regions with a single peak in Alibori (October) compared with two peaks in Donga (July and August). Conversely, the density of *An. gambiae* s.l. was relatively low from January to May during the drought and the Harmattan period (Fig. 2). For *Cx. quinquefasciatus*, the period of highest density coincided with the driest and warmest months, whereas the period of lowest density corresponded with the rainy months.

Spatiotemporal distribution of species of the An. gambiae *s.l. complex*

Out of the 1497 specimens of *An. gambiae* s.l. analyzed by PCR, two sibling species were observed: *An. coluzzii* (56%, $n = 835$) and *An. gambiae* (44%, $n = 662$) ($p = 0.0000324$). The distribution of these species varied in time and space (Fig. 3). Overall, in Alibori (KGS combined), 69% (452/658)

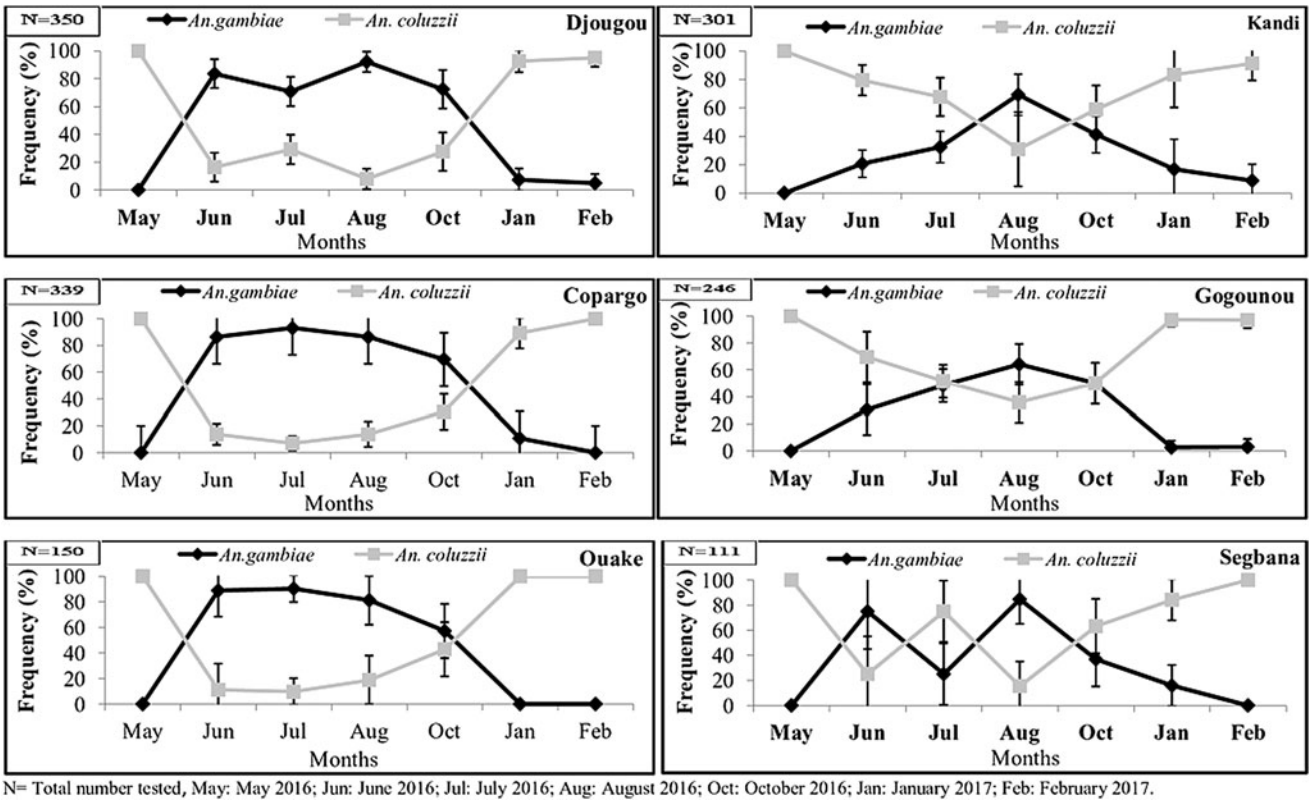
Table 1. Mosquito Species Composition and Density in the Study Sites with the Two Collection Methods (Human Landing Catch and Pyrethrum Spray Catch Methods)

	Donga region								*Alibori region*									
	Djougou		*Copargo*		*Ouake*		*Total Donga*		*Kandi*		*Gogounou*		*Segbana*		*Total Alibori*		*Total*	
Species	*ni*	*Pi (%)*	*ni*	*Pi (%)*	*ni*	*Pi (%)*	*ni*	*Pi (%)*	*ni*	*Pi (%)*	*ni*	*Pi (%)*	*ni*	*Pi (%)*	*ni*	*Pi (%)*	*ni*	*Pi (%)*
Anopheles gambiae s.l.	1087	46.14	1188	76.74	246	36.07	2521	54.97	869	41.80	649	22.62	148	17.77	1666	28.82	4187	40.39
Anopheles funestus	60	2.55	8	0.52	27	3.96	95	2.07	4	0.19	1	0.03	2	0.24	7	0.12	102	0.98
Anopheles nili	0	0	0	0	1	0.15	1	0.02	0	0	0	0	0	0	0	0.00	1	0.01
Anopheles pharoensis	0	0	2	0.13	0	0	2	0.04	0	0	1	0.03	0	0	1	0.02	3	0.03
Anopheles ziemanni	0	0	1	0.06	0	0	1	0.02	1	0.05	0	0	0	0	1	0.02	2	0.02
Anopheles coustani	1	0.04	8	0.52	0	0	9	0.20	0	0	0	0	0	0	0	0	9	0.09
Anopheles paludis	0	0	1	0.06	0	0	1	0.02	0	0	0	0	0	0	0	0	1	0.01
Aedes aegypti	14	0.59	8	0.52	5	0.73	27	0.59	11	0.53	15	0.52	2	0.24	28	0.48	55	0.53
Aedes vittatus	0	0	0	0	0	0	0	0	1	0.05	0	0	0	0	1	0.02	1	0.01
Aedes luteocephalus	0	0	0	0	0	0	0	0	1	0.05	1	0.03	0	0	2	0.03	2	0.02
Culex quinquefasciatus	1167	49.53	319	20.61	380	55.72	1866	40.69	1180	56.76	2172	75.71	676	81.15	4028	69.68	5893	56.85
Culex gr *decens*	3	0.13	1	0.06	0	0	4	0.09	1	0.05	3	0.10	1	0.12	5	0.09	9	0.09
Culex nebulosus	17	0.72	9	0.58	23	3.37	49	1.07	8	0.38	18	0.63	3	0.36	29	0.50	78	0.75
Mansonia Africana	7	0.3	3	0.19	0	0	10	0.22	3	0.14	9	0.31	1	0.12	13	0.22	23	0.22
Total	2356	100	1548	100	682	100	4586	100	2079	100	2869	100	833	100	5781	100	10367	100
Taxas_S	8		11		6		12		10		9		7		11		14	
Shannon diversity index	0.89 [0.86–0.92]		0.67 [0.63–0.72]		0.98 [0.92–1.04]		0.89 [0.87–0.92]		0.77 [0.75–0.80]		0.64 [0.61–0.67]		0.54 [0.49–0.59]		0.69 [0.68–0.72]			
Simpson's dominance	0.54 [0.53–0.54]		0.37 [0.34–0.39]		0.56 [0.53–0.58]		0.53 [0.52–0.54]		0.5 [0.49–0.51]		0.38 [0.36–0.39]		0.31 [0.28–0.34]		0.43 [0.42–0.44]			
Equitability Index	0.43 [0.42–0.44]		0.28 [0.26–0.30]		0.55 [0.52–0.58]		0.36 [0.35–0.37]		0.34 [0.33–0.35]		0.29 [0.28–0.31]		0.28 [0.26–0.32]		0.29 [0.28–0.3]			



May: May 2016; Jun: June 2016; Jul: July 2016; Aug: August 2016; Oct: October 2016; Jan: January 2017; Feb: February 2017.

FIG. 2. Spatiotemporal distribution of abundance of *Anopheles gambiae* s.l. and *Culex quinquefasciatus* according to the rainfall in Alibori and Donga



N= Total number tested, May: May 2016; Jun: June 2016; Jul: July 2016; Aug: August 2016; Oct: October 2016; Jan: January 2017; Feb: February 2017.

FIG. 3. Spatiotemporal distribution of species of the *Anopheles gambiae* s.l. *complex* in Alibori and Donga.

TABLE 2. VARIABILITY OF *ANOPHELES GAMBIAE* S.L. BITING RATE

Variables	*Modality*	*Total of* Anopheles gambiae *s.l. collected*	*Nb of collectors*	*HBR*	*HBR/month*	*95% CI [HBR]*
Donga	Djougou	682	104	6.56	196.73	[6.07–7.07]
	Copargo	782	104	7.52	225.57	[7.00–8.07]
Alibori	Kandi	498	104	4.78	143.65	[4.37–5.22]
	Gogounou	458	104	4.4	132.11	[4.00–4.83]
Urbanization level	Urban area	459	208	2.20	66.2	[1.99–2.39]
	Rural area	1961	208	9.42	282.83	[9.01–9.85]
Location	Indoor	1326	208	6.37	191.25	[6.03–6.72]
	Outdoor	1094	208	5.25	157.78	[4.95–5.58]

Nb, number; HBR, human biting rate; CI, confidence intervals.

of specimens were *An. coluzzii* and 31% ($n=206/658$) were *An. gambiae* ($p=0.00000022$). In Donga (DCO cumulated), 54% ($n=456/839$) of specimens were *An. gambiae* and 46% ($n=383/839$) were *An. coluzzii* ($p=0.00043$). Both species were present throughout the study period, except between January and February when *An. gambiae* was not found in most of the surveyed districts (Fig. 3). *An. coluzzii* predominated during the dry season (Periods May to June and February to January), whereas *An. gambiae* predominated from June to October (rainy season) (Fig. 3).

HBR of An. gambiae *s.l.*

The HBR of *An. gambiae* s.l. varied significantly from one region to another ($p<0.001$) with 132–143 bites/man/month in Alibori and 196–225 bites/man/month in Donga (Table 2). Overall, the HBR was four times higher in rural areas than in urban areas (rate ratio = 4.27; $p<0.001$) and was higher indoors (191.25 bites/man/month) than outdoors (157.78 bites/man/month) ($p=0.0000002$) (Table 2).

HBR of Cx. quinquefasciatus

The HBR of *Cx. quinquefasciatus* was higher in Gogounou, Kandi (Alibori region), and Djougou (Donga region) (Table 3). Contrary to *An. gambiae* s.l., the HBR of *Cx. quinquefasciatus* was significantly higher in urban areas than in rural areas ($p<0.001$) with 291 bites/man/month in urban areas compared with 128 bites/man/month in rural areas. Moreover, this species exhibits similar biting behavior indoors (201.63 bites/man/month) and outdoors (217.78 bites/man/month) with an endophagic index of 0.48 [(1398)/(1398 + 1510)] against an exophagic index of 0.52 [(1510)/(1398 + 1510)] (Table 3).

Blood feeding rate, indoor resting density of An. gambiae *s.l.*

Table 4 shows the blood feeding rate and the indoor resting density of *An. gambiae* s.l. in rural and urban areas of the Alibori and Donga regions.

Overall, cumulated data show that vector density was higher in rural than in urban areas in each of the two regions (2.3 *An. gambiae* s.l./room vs. 0.56 in Donga [$p=0.000$] and 1.35 *An. gambiae s.l.*/room vs. 0.49 in Alibori [$p=0.0001$]).

The blood feeding rate was high and similar in urban and rural areas of Donga, 93.27% and 96.11% ($p=0.111$), respectively. By contrast, a significant higher blood feeding rate was observed in rural (93.64%) than in urban (86.17%) areas ($p=0.002$) of Alibori. Similarly, combined data of both regions show a higher blood feeding rate in rural areas (95.17%) as compared with urban areas (89.9%) ($p=0.00016$).

Discussion

This study provides some entomological baseline data on mosquito population dynamics in Alibori and Donga regions, two areas in northern Benin, where IRS was planned to be applied. A total of 14 different species of mosquitoes were collected during this study, compared with 13 and 15 species collected in southeastern Benin by Huttel (1950) and Hamon (1954), respectively. Our data are similar to those of Gnanguenon et al. (2014) who reported 12 species of mosquitoes in sentinel sites, including the Borgou region, which is not far from Alibori and Donga.

The Shannon–Wiener and Simpson indices, which are higher in the DCO health zone compared with the KGS health zone show that mosquito population is more diverse in the DCO health zone than that of KGS.

TABLE 3. VARIABILITY OF *CULEX QUINQUEFASCIATUS* BITING RATE

Variables	*Modality*	*Total of* Culex quinquefasciatus *collected*	*Nb of collectors*	*HBR*	*HBR/month*	*95% CI [HBR]*
Donga	Djougou	732	104	7.03	211.15	[6.53–7.56]
	Copargo	167	104	1.6	48.17	[1.37–1.86]
Alibori	Kandi	698	104	6.71	201.34	[6.22–7.22]
	Gogounou	1311	104	12.6	378.17	[11.93–13.30]
Urbanization level	Urban area	2017	208	9.69	290.91	[9.27–10.13]
	Rural area	891	208	4.28	128.50	[4.00–4.57]
Location	Indoor	1398	208	6.72	201.63	[6.37–7.08]
	Outdoor	1510	208	7.26	217.78	[6.89–7.63]

TABLE 4. BLOOD FEEDING RATE AND INDOOR RESTING DENSITY OF *ANOPHELES GAMBIAE* S.L. COLLECTED BY PYRETHRUM SPRAY CATCH METHOD IN RURAL AND URBAN AREAS OF ALIBORI AND DONGA

Variables	*Modality*	*Zone*	*Total of* Anopheles gambiae *collected*	*Unfed*	*Fed*	*Gravid*	*Half-gravid*	*Nb of rooms*	*Room density*	*Blood feeding rate (%)*	p-*Value*
Donga	Djougou	Rural	352	7	319	5	21	120	2.93[a]	96.59	0.0871
		Urban	52	5	45	0	2	119	0.44[b]	90.38	
	Copargo	Rural	366	4	331	13	18	129	2.84[a]	95.36	0.683
		Urban	40	1	36	2	1	130	0.31[b]	92.5	
	Ouake	Rural	130	2	115	2	11	119	1.09[a]	96.92	0.611
		Urban	116	4	104	2	6	120	0.97[a]	94.83	
	Total (DCO)	Rural	848	13	765	20	50	368	2.3[a]	96.11	
		Urban	208	10	185	4	9	369	0.56[b]	93.27	0.111
Alibori	Kandi	Rural	336	9	277	12	38	125	2.69[a]	93.75	0.864
		Urban	35	3	29	0	3	129	0.27[b]	91.43	
	Gogounou	Rural	96	3	88	4	1	130	0.74[a]	92.71	0.090
		Urban	92	2	75	13	2	128	0.72[a]	83.7	
	Segbana	Rural	87	1	73	4	9	130	0.67[a]	94.25	0.206
		Urban	61	3	48	5	5	130	0.47[a]	86.89	
	Total (KGS)	Rural	519	13	438	20	48	385	1.35[a]	93.64	
		Urban	188	8	152	18	10	387	0.49[b]	86.17	0.002
Donga and Alibori	Grand Total	Rural	1367	26	1203	40	98	753	1.82[a]	95.17	0.00016
		Urban	396	18	337	22	19	756	0.52[b]	89.9	

p-Value: *p*-Value of comparison of the blood feeding rate of *Anopheles gambiae* s.l. between rural and urban areas; densities of *Anopheles gambiae* s.l. with different superscript (a, b) are significantly different ($p < 0.05$).
KGS, Kandi, Gogounou and Segbana; DCO, Djougou, Copargo, and Ouake.

The abundance of *An. gambiae* s.l. in both regions confirms earlier findings of studies carried out in northern Benin (Aïkpon et al. 2013, Gnanguenon et al. 2014). The relative abundance of *Cx. quinquefasciatus* in urban sites would be due to the presence of several larval habitats (sewers, abandoned wells, and cisterns) containing organic matters, and which were favorable to the development of *Cx. quinquefasciatus*, around houses.

In rural areas, where there is a lack of development infrastructures, such as gutters, drainage channel, and roads, much less polluted breeding sites that are conducive to the development of *Anopheles*, were formed in large numbers after rains. Such larval habitats were very close to the rural agglomerations made of several houses, which facilitated the blood meal intake and the oviposition of *Anopheles gambiae* s.l. resulting thus in their proliferation as observed by Govoetchan et al. (2014). Similarly, Fontenille et al. (1997), Gila et al. (2003), and Hay et al. (2005) have noticed a higher risk of malaria transmission in rural areas than in urban areas. This suggests that, vector control interventions, such as IRS operations must focus more on rural areas so as to have a substantial impact on malaria transmission.

Indoor and outdoor HBR of the two species differs by study site. Contrary to *Cx. quinquefasciatus,* which had similar indoor and outdoor trophic activity, the proportion of *An. gambiae* s.l. inside dwellings was relatively high throughout the study areas. The indoor biting behavior observed in *An. gambiae* s.l. is justified by its anthropophilic characteristics and by the fact that people do not typically rest outside their dwellings in the study area during the rainy season, which is also the period with the highest vector abundance. These observations support the plans to implement IRS, which preferentially targets endophagic and/or endophilic vectors.

Moreover, the data collected simultaneously on the levels of resistance to different classes of insecticide showed resistance to pyrethroids (permethrin and deltamethrin), emerging resistance to bendiocarb and full susceptibility to pirimiphos-methyl in *An. gambiae* (s.l.) populations in both targeted regions (Salako et al. 2018). However, the short residual life (3 months) of pirimiphos-methyl observed by Aïkpon et al. (2014) in the neighboring region of Atacora suggests the use of another effective insecticide that has at least 6 months of remanence. For that, Fludora® Fusion, a mixture of clothianidin and deltamethrin that displayed a residual life of 10 months (Agossa et al. 2018) in a small-scale field evaluation carried out in Benin could be a good candidate.

Given the relationship between increasing rainfall and increasing density of *An. gambiae* s.l., it is likely that rainfall is a key factor in determining the existence and abundance of *Anopheles* species and the duration of seasonal malaria transmission (Lindsay and Birley 1996, Besancenot et al. 2004). In the Sahel in Senegal, Fontenille et al. (2003) noticed a decrease in *Anopheles* density following a decrease in precipitation. That should be taken into account in setting the spraying schedule. Indeed, for a more efficient insecticide treatment, the spraying campaign should start 1 week before June, the onset of the rainy season to protect people against malaria when the bites of *Anopheles* are likely to be high.

Only two species of the *An. gambiae* s.l. complex, *An. gambiae* and *An. coluzzii*, were involved in malaria transmission in both regions (Akogbéto et al. 2018) with predominance of *An. coluzzii* further north in Alibori probably

due to higher aridity as reported in some studies in Nigeria (Coluzzi et al. 1979, 1985), Cameroon (Onyabe and Conn 2001, Simard et al. 2009), and Burkina Faso (Diabate et al. 2005). In the Alibori, a region in the extreme north of Benin, where the degree of aridity is high, *An. coluzzii* was predominant (69%) during most of the study period. On the other hand, in the more southern Donga region, *An. gambiae* was predominant. These results confirm the works of Toure et al. (1994) who reported a gradual decrease in the frequency of *An. coluzzii* when moving from a Sahelian environment in the north to a forest environment in the south.

The abundance of *An. gambiae* during the rainy season is due to the creation of numerous temporary larval breeding sites during raining season (Diabate et al. 2008, Lehmann and Diabate 2008). No specimen of *An. arabiensis*, a malaria vector of dry savanna areas, was found in our samples, whereas Akogbeto (1992), Akogbeto and Di Deco (1995), and Djogbenou et al. (2010) previously reported their presence in sympatry with *An. gambiae* s.s. in northern Benin.

Our results confirm works by Aïkpon et al. (2014), which also reported the only presence of *An. gambiae* and *An. coluzzii* in Kouande, Tanguieta, and Copargo districts, which are quite close to our study area. The absence of *An. arabiensis* could be due to an accelerated urbanization that destroyed the natural habitats of this species, which are mostly dry savannas (Dukeen and Omer 1986). This species is also known as being zoophilic (Duchemin et al. 2001), but the longer droughts observed in recent years in northern Benin have created a lack of grazing leading to the displacement from the north to the center of the country of most animals on which *An. arabiensis* used to feed blood, hence its absence.

The blood feeding rate, which is a proxy indicator of the man–vector contact frequency and of malaria transmission risk (Garrett-Jones and Shidrawi 1969) was overall higher in rural than in urban areas ($p = 0.00016$). This could be due to a stronger man–vector contact facilitated by the proximity, between numerous breeding sites of *Anopheles* and the houses in rural areas. Another contributing factor could also be, the fact that most rural populations might have spent more time outdoors before sleeping under their nets or preferred to spend most nights outside their nets, because of their low level of education that does not allow them to know the importance of the use of LLINs to avoid infected bites of mosquitoes as compared with urban populations. This suggests that people from rural areas need more sensitization.

The cross-sectional nature (only 7 months) of the current study stresses the need for further elaborate studies to complete the picture of population dynamics of vectors for all seasons.

Conclusion

This study revealed that *An. gambiae* s.l. and *Cx. quinquefasciatus* were the most abundant mosquito species in our study area, but with different levels according to the region. The endophagy character of *An. gambiae* s.l. noted in the study is an asset for IRS implementation 1 week before June, which is the start of the rainy season. *Cx. quinquefasciatus* had a similar indoor and outdoor trophic habit and predominated during the dry season, during which time IRS has little residual efficacy. The high abundance of *An. gambiae* s.l. and its higher blood feeding rate in rural areas stress the need for those areas to be primarily targeted by the IRS operations to have a substantial impact on malaria transmission. A longitudinal study covering at least all 12 months of a year is required to have sufficient data on the vector population dynamics for all seasons.

Ethics Approval and Consent to Participate

The protocol of this study was reviewed and approved by the Institutional Ethics Committee of CREC (IECC). Before mosquito collectors were involved in this study, they gave their consent to participate. They were vaccinated against yellow fever, regularly checked up by a medical doctor, and taken care in case of confirmed malaria case.

Availability of Data and Materials

The data used and/or analyzed in this study are available from the corresponding author on reasonable request.

Author's Contributions

A.S.S., R.O., R.A., G.G.P., and M.C.A. conceived the study. A.S.S., R.A., C.K., H.S., and M.C.A. have participated in the design of the study. A.S.S., G.G.P., R.O., C.K., H.S., and R.A. carried out the field activities and the laboratory analysis. A.S. and M.C.A. drafted the article. A.S.S., A.S., F.D., M.S., and M.C.A. critically revised the article for intellectual content. All authors read and approved the final article.

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Author Disclosure Statement

No conflicting financial interests exist.

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