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Involvement of both *Anopheles gambiae* and *Anopheles funestus* (Diptera: Culicidae) in the perennial malaria transmission through a seasonal abondance in savannah area in Benin

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Abstract
The involvement of *Anopheles gambiae s.l.* and *Anopheles funestus* in malaria transmission was investigated in Savannah area in Benin. This study shows the trends in malaria vectors dynamics and their part of contribution in malaria transmission.
Female mosquitoes were collected, using human landing catches. All the anopheline mosquitoes were assessed for species identify and sporozoite infection status.
Most of the anopheline mosquitoes collected were members of the *An. gambiae* complex (80.90%) and *An. funestus* group (14.36%). *An. gambiae* and *An. coluzzii* were found in sympatry. All of the females of the *An. funestus* group investigated were identified as *An. funestus* s.s. In spite of being the major malaria vector as far as abundance is concerned, sporozoite prevalence was three times higher with *An. funestus* than *An. gambiae*.
This study provides useful informations on the relative contribution of malaria vectors to the perennial malaria transmission in the study area.

Keywords: Perennial malaria transmission, *Anopheles gambiae*, *Anopheles funestus*, contribution

Introduction
Malaria transmission is sustained through vector interaction [1] and the current effective vector control tools include the use of Long Lasting Insecticide Nets (LLIN) and Indoor Residual Spraying (IRS) [2]. Although malaria can be severely limited, if not eliminated, by effective vector, the implementation of any successful vector-control measures requires knowledge of the biology of the anopheline species present in the area to be targetd.
The malaria vectorial system in tropical Africa is dominated by four species of major importance, *Anopheles gambiae*, *Anopheles coluzzii*, *Anopheles arabiensis* and *Anopheles funestus*, which are broadly codistributed across much of tropical Africa in close association with humans [3, 4]. *Anopheles gambiae*, *Anopheles coluzzii* and *Anopheles arabiensis* belong to the same cryptic species complex (the *An. gambiae* complex) whose members cannot be distinguished morphologically at any developmental stage, although they differ in aquatic larval ecology and adult behaviours relevant to malaria transmission and control (e.g., degree of anthropophily and tendency to blood-feed or rest indoors) [5,6]. *Anopheles funestus* and its presently recognized closest relatives are classified into a group and subgroup [7,8] rather than a species complex, owing to slight morphological distinctions mainly at immature stages.
Although *Anopheles gambiae* Giles complex and *Anopheles funestus* Giles are the primary malaria vectors in sub-saharan Africa, *Anopheles gambiae* s.l is often regarded as the most important vector species across Africa [9-11] and, because of its almost entirely anthropophagic and endophilic behaviour, it is the species that has been targeted most effectively by LLINs. Nevertheless, *Anopheles funestus* is also a very anthropophic and endophilic mosquito and it too can be a highly efficient malaria vector [4, 11, 12]. However, despite its obvious importance as vector, *An funestus* s.l has been sadly neglected compared to *An. gambiae* s.l. Undoubtedly, this has been due to the adaptability of the *An. gambiae* complex to laboratory conditions and the ease with which species in the group be colonized.

The present study aims to investigate the contribution of *Anopheles gambiae* and *An. funestus* mosquitoes to malaria transmission in Copargo district in a savanah area in Benin.

Methods

Study area

The study was carried out in two villages (Kantaban and Kparakounan) of Copargo district in Donga department (9° 50'15" N, 1°32'53" E) located in North-west of Benin (Figure 1). Copargo district covered 876 km2 and had an estimated population of 70,820 in 2012. Donga department has a sub-equatorial type climate with one dry season (December-May) and only one rainy season (June to November). The annual mean rainfall is 1,300 mm and the mean monthly temperature ranges between 22 and 33 °C. The department is irrigated by three major rivers: the Ouémé, the Yan, and the Gbangbaré. The major economic activity is agriculture and it is characterized by the production of cotton and yam where various classes of pesticides are used for pest control.

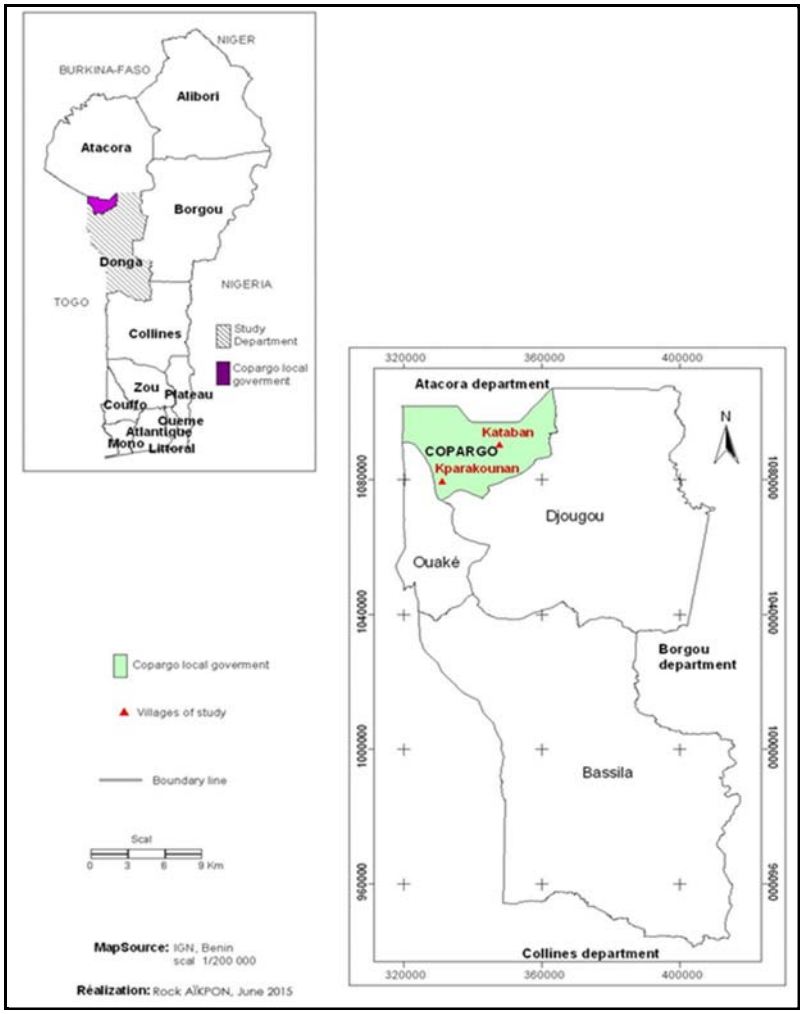

Fig 1: Map of study area

Mosquito sampling

In Copargo district, two villages were selected, and ten houses were chosen per village for mosquito collection to monitor malaria transmission. Monthly, mosquito collections were carried out from 6 p.m. to 6 a.m. inside and outside human dwellings using a mouth aspirator, by human volunteers who had previously given consent. Two nights of mosquito collections a month were carried out from January to December 2015. A total of 24 night catches were conducted in each village. In each village, twenty collectors were selected for the collection of mosquitoes. The recorded data were used to assess the aggressiveness (HBR), and the entomological inoculation rate vectors (EIR).

Laboratory processing

After each night catch, Anophelines were morphologically identified to species using taxonomic keys of Gillies & De Meillon [4] and Gillies & Coetzie [11]. Mosquito infectivity rates were determined from head and thorax of all female anopheline specimens by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies against *Plasmodium falciparum* circum sporozoite protein (CSP) as described by Wirtz *et al.* [13]. The carcass of these females (abdomens, wing and legs) were stored in individual tubes with silicagel and preserved at −20 °C in the laboratory for individual molecular species identification using polymerase chain reaction (PCR) assay for the *An. gambiae* complex [14] and *An. funestus* group [15].

Data analysis

The human biting rate [number of bites/man/night] (HBR), the sporozoïte rate (Is) and the entomological inoculation rate (EIR) were calculated monthly and the seasonal (dry/rainy) EIR was estimated for each species. Comparisons of these seasonal EIR were made by the Chi-square test. Is= Number of positive sporozoite mosquitoes/total tested; HBR= Total collected/ number of human cathes; EIR= HBR x Is.

Results

Composition and abundance of mosquito fauna

A total of 1,257 mosquitoes belonging to 14 different species were caught in the lading catches during the study period. Most (63.72%) of these were anopheline, and most the anopheline mosquitoes collected were members of the *An. gambiae* complex (80.90%) and *An. funestus* group (14.36%) (Table 1).

Among the 648 female *An. gambiae* s.l identified in species level in PCR-based assay,

An. gambiae and *An. coluzzii* were found in sympatry in the study area. However, *An. gambiae* was predominant, representing 85.96% (n=557) against 14.04% for *An. coluzzii* (n=91). Besides, All of the females of the *An. funestus* group investigated were identified as *An. funestus* s.s.

Table 1: The numbers of adult female mosquitoes collected on human bait in study area from January to December 2013.

Species	Number	Percentage (%)
Anopheles gambiae	648	51,55
Anopheles funestus	115	9,15
Anopheles pharoensis	5	0,40
Anopheles ziemanni	33	2,63
Total Anopheles	801	63,72
Aedes aegypti	16	1,27
Aedes vittatus	17	1,35
Aedes longipalpis	4	0,32
Aedes gr. palpalis	3	0,24
Aedes gr. tarsalis	2	0,16
Culex quinquefasciatus	249	19,81
Culex gr decens	9	0,72
Culex nebulosus	8	0,64
Culex fatigans	104	8,27
Mansonia africana	44	3,50
Total	1257	

Seasonal variation in vector abundance

The period from June to November was characterized as the rainy season, and December-May as the dry season. *An. gambiae* s.l was the most important species in both seasons with 4.74 bites per man per night (b/m/n) in the rainy season and 2.01 b/m/n in the dry season, against 0.35 b/m/n and 0.84 b/m/n for *An. funestus* respectively in the rainy season and the dry season (table 2).

Table 2. Seasonal variation in the human-biting rates of *Anopheles gambiae* s.l and *An. funestus* in Copargo district, from January to December 2013.

Seasons	Species	Total caught	nb human cathes	HBR/night	Thorax +	IS	EIR/night	EIR/season
Rainy season	*An. gambiae*	455	96	4.74	40	0.088	0.4167^{a}	75^{a}
	An. funestus	34	96	0.354	10	0.294	0.1042^{b}	18.75^{b}
Dry season	*An. gambiae*	193	96	2.01	15	0.078	0.1563^{a}	28.13^{a}
	An. funestus	81	96	0.844	20	0.247	0.2083^{b}	37.5^{b}

For each season, numbers in the same column with the different superscripts differ significantly by Fisher's exact test ($p < 0.0001$).

Malaria transmission

A total of 763 individual mosquitoes (648 *An. gambiae* s.l and 115 *An. funestus*) were screened for *Plasmodium falciparum* sporozoites of wich 85 were positive (11.14% sporozoite prevalence). In spite of being the major malaria vector as far as abundance is concerned, sporozoite prevalence was three times higher with *An. funestus* than *An. gambiae* (8,49% for *An. gambiae* s.l against 26,08% for *An. funestus*).

The Table 3 and figure 2 show the dynamic of the malaria vector composition, sporozoite indice, biting rate and entomological inoculation rate (EIR) for *Anopheles gambiae* s.l and *Anopheles funestus* and their overall estimated contribution to malaria transmission from January to December 2013 in the study area.

The first observation of the dynamic of malaria transmission in the study area is the perennial aspect of this transmission with is permanent the year long. During the period study, the average EIR for *P. falciparum* was estimated at 26.56 infective bites/person-month, with *An. gambiae* s.l and *An. funestus* responsible for 17.19 and 9.37 of those infective bites respectively. The lowest EIR was recorded in February (3.75 infective bites/person-month) and the highest was recorded in November (33.8 infective bites/person-month).

Besides, although *An. gambiae* s.*l* and *An. funestus* are both involved in malaria, their relative importance varied significatively with season (rainy or dry). In fact, during the rainy season, *An. gambiae* s.l insure 80% of malaria transmission (75/93.75 infective bites). Conversely, during the dry season, *An. funestus* insure 57.1% of malaria transmission (37.5/65.6 infective bites) (figure 3).

Table 3. Dynamic of the malaria vector composition, sporozoite indice (IS), biting rate (HBR) and entomological inoculation rate (EIR) for *Anopheles gambiae* s.l and *Anopheles funestus* and their overall estimated contribution to malaria transmission from January to December 2013 in the study area.

Vectors	Indicators	January	February	March	April	May	June	July	August	September	October	November	December
An. gambiae	Total caught	1	5	30	30	124	70	118	46	130	59	32	3
	nb human cathes	16	16	16	16	16	16	16	16	16	16	16	16
	HBR/night	0,0625	0,313	1,875	1,875	7,75	4,375	7,375	2,875	8,13	3,688	2	0,19
	Thorax +	0	1	3	2	9	6	7	2	3	13	9	0
	IS	0	0,20	0,10	0,07	0,07	0,09	0,06	0,04	0,02	0,22	0,28	0,00
	EIR/night	0,000	0,063	0,188	0,125	0,563	0,375	0,438	0,125	0,188	0,813	0,563	0,000
An. funesus	Total caught	11	5	27	18	3	1	0	0	0	3	30	17
	nb human cathes	16	16	16	16	16	16	16	16	16	16	16	16
	HBR/night	0,6875	0,313	1,688	1,125	0,188	0,0625	0	0	0	0,188	1,88	1,06
	Thorax +	3	1	3	4	1	0	0	0	0	1	9	8
	IS	0,27273	0,2	0,111	0,222	0,333	0	0	0	0	0,333	0,3	0,47
	EIR/night	0,1875	0,063	0,188	0,25	0,063	0	0	0	0	0,063	0,56	0,5
Total vectors (*An. gambiae* + *An. funestus*)	Total caught	12	10	57	48	127	71	118	46	130	62	62	20
	nb human cathes	16	16	16	16	16	16	16	16	16	16	16	16
	HBR/night	0,75	0,625	3,563	3	7,938	4,4375	7,375	2,875	8,13	3,875	3,88	1,25
	Thorax +	3	2	6	6	10	6	7	2	3	14	18	8
	IS	0,25	0,2	0,105	0,125	0,079	0,0845	0,059	0,043	0,02	0,226	0,29	0,4
	EIR/night	0,1875	0,125	0,375	0,375	0,625	0,375	0,438	0,125	0,19	0,875	1,13	0,5

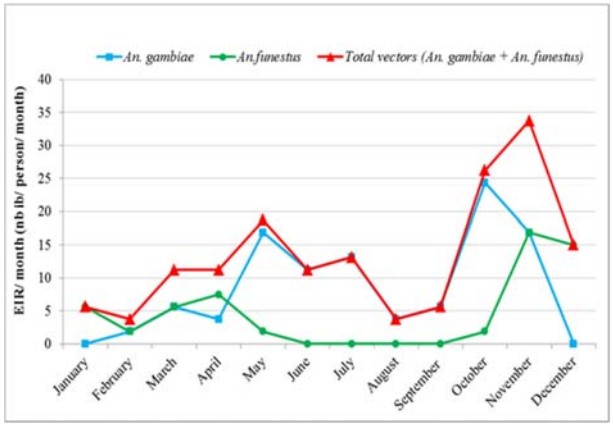

Fig 2: Monthly entomological inoculation (EIR) of *An. gambiae s.l* and *An. funestus* from January from December 2013 in the study area

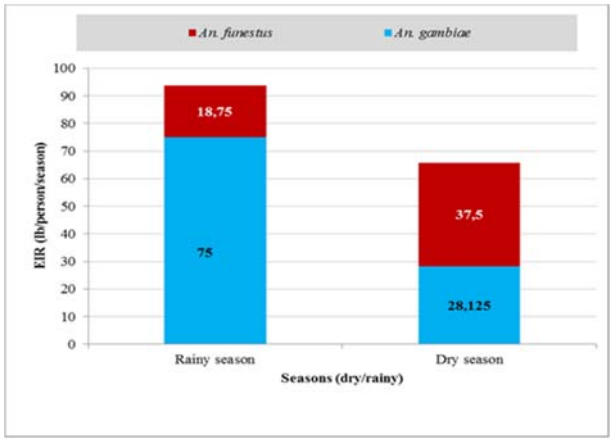

Fig 3: Seasonal variation of vectors contribution of malaria transmission in study area

Discussion

The results of the present study provide substantial information on malaria vector dynamics and their contribution to malaria transmission in Copargo district in Donga department in savannah area in Benin.

In this study, both *An. funestus* and *An. gambiae* s.l. were found in sympatric and were shown to be the main vectors in the study area and their abundance varied with season, confirming previous study in West and East Africa [16-19]. The relative abundance of *An. gambiae* s.l. densities is facilitated by a wide range of ephemeral, sunlit, breeding habitats, such as hoof prints, rice puddles and ground depressions created during the rainy season [4, 20]. The temporary nature of these habitats tends to reduce predation rate but also allows quick development of the juvenile stage, which results in *An. gambiae s.l.* domination during the rainy season [4]. On the contrary, *An. funestus* prefers vegetated semi-permanent and permanent breeding habitats, such as swamps and large ponds [4]. *An. funestus* remained at a detectable density across the rainy and dry seasons in the study area even if its appear more abundant in the dry season, probably due to their breeding habitat stability against desiccation [21]. Despite high abundance of *An. gambiae s.l.*, *An. funestus* has displayed high sporozoite prevalence, similar to that observed in a recent study in Tanzania [19]. This trend of high sporozoite prevalence of *An. funestus* has been also observed in western Kenya [22] and so appears to represent a trend across several regions of East Africa. A previous study has shown that despite high coverage and use of LLINs, a high proportion of mosquitoes still enter houses [23]. Therefore, the increase in *An. funestus* in the dry season, is likely to exacerbate the problem.

The present results demonstrate very high levels of *P. falciparum* transmission in Copargo district in north of Benin. Although the relative abundance and biting rate of each vector species varied between the seasons, it was the presence of two species, exploiting different ecological niches that allowed transmission to occur year-round. Malaria control by indoor residual spraying should be implemented independently the rainy season around the months of February and September before the peaks of malaria transmission. In addition to IRS and LLINs, larval source management strategy (which includes larviciding and source reduction) presents another potential intervention that may be promoted in this part of the country in the context of integrated vector management strategy [24-27].

Conclusion

In conclusion, the findings in the present study provide useful information on the seasonal abundances of *Anopheles gambiae* and *An. funestus* mosquito species and their contribution to the perennial malaria transmission in Copargo district in north of Benin. This may be a basis for formulating appropriate malaria control interventions in this area.

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Competing interests

The authors declare that they have no competing interests.

Ethical consideration and consent to participate

Ethical approval for this study was granted by the Ethical Committee of the Ministry of Health in Benin. The mosquito collectors gave prior verbal consent and they were vaccinated against yellow fever. They were also subjected to regular medical check-ups with preventive treatments of malaria.

Authors' contributions

MA, RA, RO, GP, AS, ID designed the study. RA and MA carried out the field activities. RA drafted the manuscript and analyzed the data. MA critically revised the manuscript. RA, and MA conceived and designed the study and MA revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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