Aggressive mosquito fauna and malaria transmission in a forest area targeted for the creation of an agro-industrial complex in the south of Cameroon

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Abstract

Baseline entomological information should be collected before the implementation of industrial projects in malaria endemic areas. This allows for subsequent monitoring and evaluation of the project impact on malaria vectors. This study aimed at assessing the vectorial system and malaria transmission in two ecologically different villages of the South-Cameroon forest bloc targeted for the creation of an agro-industrial complex.

For four consecutive seasons in 2013, adult mosquitoes were captured using Human Landing Catch in NDELLE village (located along a main road in a degraded forest with many fish ponds) and KOMBO village (located 5km far from the main road in a darker forest and crossed by the Mvobo River). Morpho-taxonomic techniques were used alongside molecular techniques for the identification of mosquito species. ELISA test was used for the detection of circumsporozoite protein antigen of *Plasmodium falciparum*.

Mosquito biting rate was higher in NDELLE than in KOMBO (28.18 *versus* 17.34 bites per person per night). Mosquitoes had a strong tendency to endophagy both in NDELLE (73.57%) and KOMBO (70.21%). Three anophelines species were identified; *An. gambiae, An. funestus* s.s and *An. moucheti* s.s.. *An. gambiae* and *An. funestus* s.s. represented the bulk of aggressive mosquitoes in NDELLE (n=10,891; 96.62%). *An. gambiae* was responsible for 62.6% and 77.72% of malaria transmission in KOMBO and NDELLE respectively. Mean entomological inoculation rate recorded in KOMBO and NDELLE were 4.82 and 2.02 infective bites per person per night respectively. Vector control was mainly based on the use of long-lasting insecticidal nets and indoor residual spraying.

The degraded forest environment added to the presence of fishponds resulted in the increase of aggressive mosquito density but not of malaria transmission. The managers should use these data for monitoring and evaluation of the impact of their project; malaria control strategies should be included in their project in order to mitigate the risk of increased malaria transmission as a result of the implementation of their projects.

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Introduction

Vector control remains an important component in the fight against malaria in endemic countries. According to many authors, vector control is one of the first prevention methods (Ross, 1911; WHO, 1994). However, the choice of an appropriate vector control strategy is based on sound knowledge of systematics and bio-ecology of vectors as well as their susceptibility to the various insecticides recommended by the World Health Organisation (WHO). Many studies have shown that the distribution of malaria is closely related to eco-climatic and hydrographic conditions (Dossou-Yovo *et al.,* 1998; Carnevale & Robert, 2009). For decades, the epidemiological map of malaria in Africa as well as in Cameroon is summarized into two main distribution areas; the equatorial zone where transmission is perennial, driven by *An.*

gambiae s.s. and the tropical zone with seasonal transmission essentially driven by *An. arabiensis* (Same-Ekobo, 1997). Beside the characteristics of these two main areas that determine the density and distribution of the Anopheles fauna responsible for malaria transmission, it is necessary to consider the particular situations encountered in large cities, villages, areas with dams and agricultural areas. Indeed, the increasing population observed in African countries in recent decades has triggered various development projects including agro-pastoral activities to ensure social and dietary well-being. Changes to the environment even at small scale can affect mosquito population as well as malaria transmission (Nzeyimana *et al.,* 2002). However, an objective evaluation of the impact of these projects requires baseline entomological data collected before their implementation.

KOMBO and NDELLE are two villages of the AYOS health district (Cameroon). NDELLE is a village located along a main road, in a degraded forest with many fishponds meanwhile. KOMBO village is located 5 km far from the main road, in a darker forest, crossed by the Mvobo river. Both villages are targeted by the AYOS city council for the creation of an agro-industrial complex covering up to 25 hectares in KOMBO and 35 hectares in NDELLE. The implementation of this project as from February 2013 would obviously result in important ecological changes with possible consequences on the health of local populations. The project may have mild impact on anopheline proliferation and malaria transmission in NDELLE, given the anthropic face of the village, very favourable to the proliferation of Anopheles. In KOMBO however, the deforestation and anthropization that would result from the implementation of this project could lead to the proliferation of *An. gambiae* s.l. (heliophilic species) following the increase in sunshine of mosquito breeding sites. This study assesses agressive mosquito diversity and malaria transmission levels in KOMBO and NDELLE.

Materials and methods

Description of study sites

The study was conducted in the Ayos health district (03° 54'N, 12° 31'E), an area of degraded forest of South Cameroon (Figure 1). Ayos is in the equatorial area with a Guinean type climate including two rainy seasons (September to November and March to June) alternating with two dry seasons (December to February and July-August). Meteorological data recorded during the past 5 years show an average annual rainfall of 1980.5 mm/year, average annual temperatures of 26°C and an average humidity rate of 80% (National Meteorology Service of Cameroon, 2014). Winds are frequent and humid, blowing from south-west to west and from north to west for stronger winds (Wété *et al*., 2003). The hydrographic network is dense, consisting of swampy low lands and many small streams that flow into the river Nyong.

The survey was conducted in two villages of the Ayos health district, precisely NDELLE and KOMBO. NDELLE is a village of about 1500 inhabitants located along a road whose recent construction has led to significant deforestation and the creation of numerous shoals on either side of the road. The village is highly anthropized as confirmed by the presence of cultivated lands and the destruction of the forest. It is also characterized by the presence of fishponds covering a surface of about 5 hectares. *Oreochromis niloticus* Linnaeus, 1758 (Tilapia) and *Clarias gariepinus* Burchell, 1822 (catfish) are the main fish species farmed in these ponds (MINEPIA 2009). KOMBO is a village of about 700 inhabitants located 5 km far from the road, in a darker forest with very little cultivated lands. The village is little anthropized with the presence of numerous trees over 10 m height and a canopy that often shelters from sun beams. It is crossed by the Mvobo stream, which flows, into the river Nyong. This

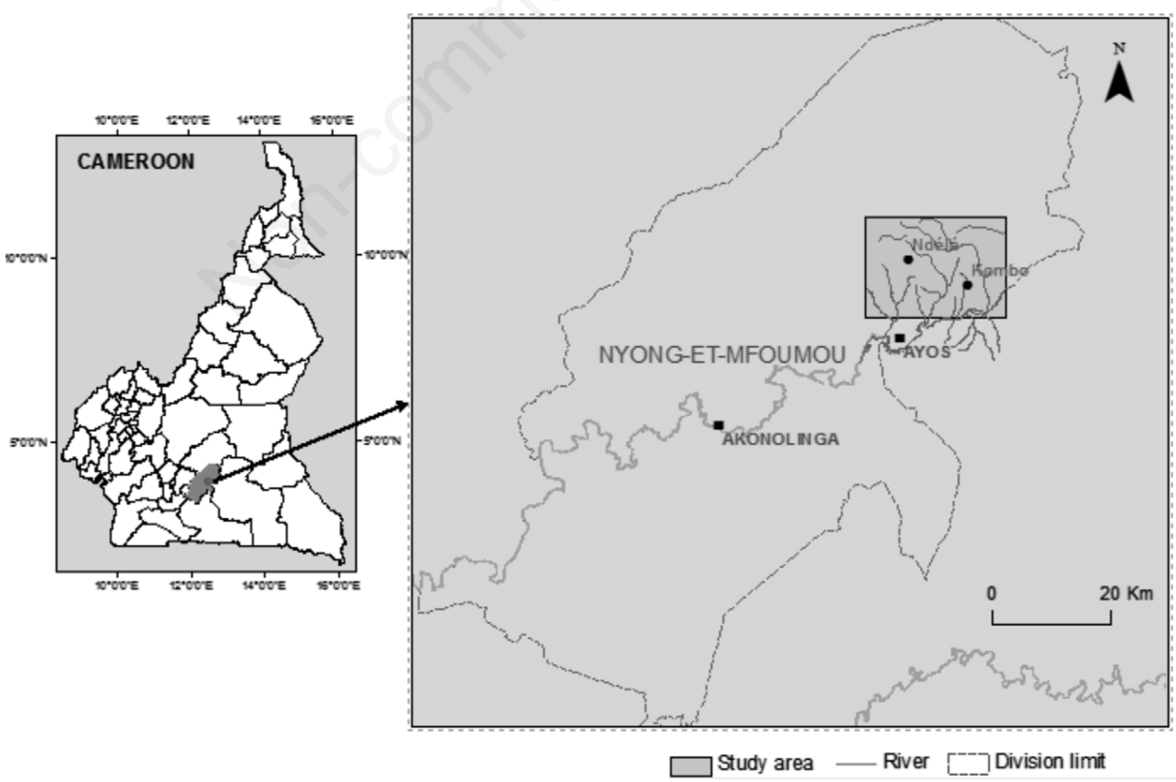

Figure 1. Map of the study area.

river hosts *Gambusia affinis* Baird and Girard, 1853; a species known for its predatory skills against Anopheles larvae.

Populations in the two villages are mainly indigenous, belonging to the Mvog-Abada and Yetenga ethnics. They live on agriculture, hunting and fishing. There is no large-scale livestock breeding, though some dogs, cats, pigs and chickens wander through the villages. Malaria cases are usually cared for in the Ayos District Hospital. Prevention is ensured by the use of insecticidal sprays and long lasting insecticidal nets distributed in 2011. Many of these mosquito nets however present with holes.

Capture of adult mosquitoes

Ten residential houses located nearby major mosquito breeding sites were selected in each of the villages, upon the approval of residents. The sampling technique used for capturing female adult mosquitoes was nocturnal human landing catch on volunteers, indoors and outdoors. Catches were performed each season in 2013, for 5 consecutive days per season, in both study sites simultaneously. In each of the residences, 2 capturers were working in turn in two shifts; one from 6 p.m. to midnight and the other from midnight to 6 a.m. A total of 100 volunteers were used per season in each of the study site. During the capture period, a window or door was left slightly opened. Mosquitoes were morphologically identified to species following the taxonomic keys then stored in silicagel containing Eppendorf tubes at –20°C, for subsequent laboratory analysis (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987; Jupp, 1996).

Laboratory analysis

Heads and thoraces of some specimens of adult female Anopheles mosquitoes were analysed for the presence of sporozoite of malaria parasites through the detection of the circumsporozoite protein (CSP), by the ELISA technique (Burkot *et al,.* 1984; Wirtz *et al.,* 1987). All positive samples were re-tested to confirm the results. DNA extracted from legs and wings was used for biomolecular identification of mosquitoes. Species and molecular forms of *An. gambiae* complex were identified by a PCR-based method (Fanello *et al.,* 2002). Species of *An. funestus*, *An. nili* and *An. moucheti* groups were identified by the use of PCR-based methods (Cohuet *et al.,* 2003; Kengne *et al.,* 2003; Kengne *et al.,* 2007).

Physico-chemical analysis of water from breeding sites

Orion™ 5-star portable multi-parameter (Thermo-Scientific) was used to determine physico-chemical parameters including pH, conductivity (µS/cm), salinity, TDS (mg/L), turbidity (NTU) and dissolved oxygen (%). A thermometer was used to measure the temperatures of water. Aquatic macro-invertebrates were sampled with a kick-net with a mesh of 2.5 mm in diameter.

Statistical analysis of data

Biting rate (number of bites per person per night or b/p/n) was calculated as the number of mosquitoes caught in one night divided by the number of capturers. The infection rate (%) was calculated as the number of infected Anopheles female mosquitoes divided by the total number of Anopheles analyzed times 100. The entomological inoculation rate (EIR, number of infected bites per person per night, ib/p/n) was calculated as the cross product of infection rate and biting rate. Statistical analyses were performed with SPSS software (version 19.0 for Windows, SPSS Inc., Chicago, IL, USA). The Wilcoxon test was used to compare the aggressive densities and average entomological inoculation rates between species, months and study sites.

Informed consent and ethical clearance

This study received administrative clearance from local authorities. Volunteers aged 20 to 30 years were recruited in the local community and trained on the anthropophilic mosquitoes capture technique. Informed consent was obtained from each volunteer involved in the study. Prior to the capture, selected volunteers were immunized against yellow fever; they also received antimalarial chemoprophylaxis consisting in 3 tablets of sulphadoxine-pyrimethamine (500/25 mg).

Results

Mosquito diversity in the study sites

A total of 18 208 female adult mosquitoes of 11 species were caught in the two study sites (Table 1). The Anophelinae (n=17,553; 96.40%)

Table 1. Female adult mosquitoes captured on volunteer in NDELLE and KOMBO.

	Number of female adult mosquitoes captured								
	NDELLE			KOMBO			Both sites		
Species	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total n. (%)*
An. gambiae	7194	2499	9693	3340	1495	4835	10,534	3994	14,528 (79.78)
An. funestus s.s	845	353	1198	620	248	868	1465	601	2066 (11.34)
An. moucheti s.s	-	-	-	728	231	959	728	231	959 (5.26)
Cx. pipiens	146	79	225	17	11	28	163	90	253 (1.38)
Cx. duttoni	46	26	72	33	15	48	79	41	120 (0.65)
Cx. zombaensis	-	-	-	11	2	13	11	2	13 (0.06)
Cx. univittatus	5	0	5	-	-	-	5	0	5 (0.03)
Ae. albopictus	-	-	-	121	64	185	121	64	185 (1.06)
Ae. simpsoni	10	6	16	-	-	-	10	6	16 (0.09)
Ae. furcifer	3	2	5	-	-	-	3	2	5 (0.03)
M. uniformis	44	14	58	-	-	-	44	14	58 (0.32)
Total	8293	2979	11,272	4870	2066	6936	13,163	5045	18,208 (100)
		S1= 8			S2=7			S=11	

%, percentage; S, number of species.

occupy most of the general mosquito wildlife. The Culicinae (n=655; 3.60%) were less represented.

Degraded forest site (NDELLE)

A total of 11,272 female adult mosquitoes of 8 species were captured in NDELLE (Table 1). *An. gambiae* (lone species of the Gambiae complex out of 3000 specimens successfully identified by the PCR technique) and *An. funestus* s.s. (lone species of the Funestus group out of 500 specimens successfully identified by PCR technique) are the only aggressive species of anopheles in this locality. The Anopheles species represents the bulk of the local mosquito wildlife (n=10,891; %=96.62). The Culicinae are represented by *Culex duttoni*, *Cx. pipiens*, *Cx. univittatus*, *Aedes simpsoni*, *Ae. furcifer* and M*ansonia uniformis* (n=381; %=3.38) (Table 1).

Non-degraded forest site (KOMBO)

A total of 6936 female adult mosquitoes belonging to 7 species were captured in KOMBO (Table 1). *An. gambiae* (lone species of the Gambiae complex out of 3000 specimens successfully identified by the PCR technique), *An. funestus* s.s. (lone species of the Funestus group out of 500 specimens successfully identified by PCR technique) and *An. moucheti* s.s (lone species of the Moucheti group out of 300 specimens successfully identified by the PCR technique) are the only aggressive species of anopheles in the locality. The Anopheles species represent the bulk of the local mosquito wildlife (n=6662; %=96.05). The Culicinae are represented by *Culex pipiens*, *Cx. zombaensis*, *Cx. duttoni* and *Aedes albopictus* (n=274; %=3.95) (Table 1).

Comparison of diversity in both sites

The degraded forest site (NDELLE; n=11,272; %=61.9) recorded a significantly larger number of aggressive mosquitoes than non-degraded forest site (KOMBO; n=6936; %=38.1) (P=0.01). However, the difference between the number of species recorded in both sites was not significant (P=0.8). In both sites, the number of captured mosquitoes was higher indoors than outdoors (P<0.0001).

Biting rate and their variations

Degraded forest site (NDELLE)

A total of 11,272 female mosquitoes were captured in 400 men night catches. The overall average biting rate recorded is 28.18 b/p/n, that is 41.46 b/p/n indoors and 14.89 b/p/n outdoors. Anopheles mosquitoes contribute 96.61% of these biting rates, the remaining 3.39% being insured by Culicinae. *An. gambiae* appears *to* be the most aggressive species (24, 23 b/p/n), followed by *An. funestus* s.s. (2.99 b/p/n). Compared to August, biting rates were significantly higher in January (P=0.043), April (P=0.04) and October (P=0.04) (Table 2).

Non-degraded forest site (KOMBO)

A total of 6,936 female mosquitoes were captured in 400 men night catches. The overall average biting rate recorded is 17.34 b/p/n that is 24.35 b/p/n indoors and 10.33 b/p/n outdoors (Table 2). Anopheles mosquitoes ensure 96.06% of the aggressive activity, the remaining 3.94% being insured by Culicinae. *An. gambiae* appears to be the most aggressive species (12.08 b/p/n), followed by *An. moucheti* s.s. (2.39 b/p/n) and *An. funestus* s.s. (2.17 b/p/n). Biting rates were higher in April and August (small dry and small rainy seasons) and are lower in January and October (main dry and main rainy seasons) (Table 2).

Comparison of biting rates

The overall average daily biting rate recorded is 17.34 b/p/n in the non-degraded forest site and 28.18 b/p/n in the degraded forest site. There is a significant difference between the overall average biting rate recorded in both sites (P=0.01). Moreover, endophilic mosquitoes were significantly more abundant than exophilic mosquitoes in both study sites (P<0.0001) (Table 3).

Infection rate and malaria vectors

Non-degraded forest site (KOMBO)

In the non-degraded forest site, a total of 1333 female Anopheles mosquitoes belonging to 3 species were tested with the ELISA CSP assay. Mean annual infection rates were 5.3% (n=51); 10.3% (n=18) and 6.2% (n=12) for *An. gambiae, An. funestus* s.s. and *An. moucheti* s.s. respectively (Table 4). The number of infected endophilic females was significantly higher than that of infected exophilic females (P<0.0001). Infected females were captured in all seasons. *Plasmodium falciparum* was the only plasmodium species identified in infected female mosquitoes.

Table 2. Number of mosquitoes captured on volunteers and variation of biting rates (b/p/n) in NDELLE and KOMBO.

Species	NDELLE								KOMBO							
	January		April		August		October		January		April		August		October	
	Capt.*	BR°	Capt.	BR	Capt.	BR	Capt.	BR	Capt.	BR	Capt.	BR	Capt.	BR	Capt.	BR
An. gambiae	2761	27.61	4035	40.35	532	5.32	2365	23.65	800	8	1750	17.5	1635	16.35	650	6.5
An. funestus s.s	363	3.63	502	5.02	131	1.31	202	2.02	58	0.58	362	3.62	400	4	48	0.48
An. moucheti s.s	-	-	-	-	-	-	-	-	435	4.35	103	1.03	115	1.15	306	3.06
Cx. pipiens	45	0.45	138	1.38	25	0.25	17	0.17	15	0.15	7	0.07	0	0	6	0.06
Cx. duttoni	15	0.15	36	0.36	21	0.21	0	0	15	0.15	6	0.06	7	0.07	20	0.2
Cx. zombaensis	-	-	-	-	-	-	-	-	5	0.05	3	0.03	1	0.01	4	0.04
Cx. univittatus	0	0	5	0.05	0	0	0	0	-	-	-	-	-	-	-	-
Ae. albopictus	-	-	-	-	-	-	-	-	45	0.45	28	0.28	67	0.67	45	0.45
Ae. simpsoni	2	0.02	3	0.03	5	0.05	6	0.06	-	-	-	-	-	-	-	-
Ae. furcifer	0	0	4	0.04	1	0.01	0	0	-	-	-	-	-	-	-	-
M. uniformis	15	0.15	32	0.32	5	0.05	6	0.06	-	-	-	-	-	-	-	-
Total	3201	32.01	4755	47.55	720	7.2	2596	25.96	1373	13.73	2259	22.59	2225	22.25	1079	10.79

*Capt., number of mosquitoes captured; °BR, biting rate.

Degraded forest site (NDELLE)

A total of 2,179/10,891 female mosquitoes were tested with the ELISA CSP assay; 35 females were found to be infected with *Plasmodium falciparum*. Annual average infection rates were 1.4% (n=27) and 3.3% (n=8) for *An. gambiae* and *An. funestus* s.s. respectively (Table 4). The number of infected females was significantly higher inside and outside homes (P<0.0001). Infected females were captured at each capture season.

Entomological inoculation rates and malaria transmission

Non-degraded forest site (KOMBO)

Malaria transmission is ensured by the three anopheles species identified in this site. The average annual EIR recorded is 4.82 ib/p/n. *An. gambiae* (3.02 ib/p/n) appears to be the major malaria vector in the locality. *An. funestus* s.s. (1085 ib/p/n) and *An. moucheti* s.s. (0.72 ib/p/n) are considered secondary vectors (Table 5). The average EIR was significantly higher for *An. gambiae* than *An. funestus* s.s. (P<0.0001) and *An. moucheti* s.s. (P<0.0001).

An. gambiae recorded high EIR in the months of January, August and October (Table 5). EIR were significantly higher indoors than outdoors (P=0.04).

Degraded forest site (NDELLE)

Malaria transmission is ensured by the 2 anopheles identified in the site. The average annual EIR recorded is 2.02 ib/p/n. *An. gambiae* with an EIR of 1.57 ib/p/n, appears to be major malaria vector in this locality. *An. funestus* s.s. (0.45 ib/p/n) is regarded as a secondary vector (Table 5). The average EIR are significantly different between *An. gambiae* and *An. funestus* s.s. (P=0.03). *An. gambiae* EIR was at its peak in January (Table 5).

Comparison of entomological inoculation rate

The overall average EIR recorded was 4.82 ib/p/n and 2.02 ib/p/n, in the non-degraded forest site and in the degraded forest site respectively. The difference between the overall average daily EIR in both sites is significant (P=0.002). EIR were significantly higher indoors and outdoors in the two study sites (Table 3).

Physico-chemical parameters of breeding sites

Fish ponds and river bank breeding sites are acidic (4.5≤pH≤.7,3) and adequately oxygenated (dissolved oxygen from 6.9 to 11.2 mg/L). The solubilization ratio is low (95.3 mg/L≤TDS≤170.4 mg/L). These parameters are favourable for mosquito larvae survival and growth. However, the riverbank site (non-degraded forest site) hosts a larvivorous fish species (*Gambusia affinis*) and is richer in aquatic macro-invertebrates than the fish-farming site (degraded forest site) (Table 6).

Discussion

This study compared aggressive mosquito diversity and the transmission of human malaria in a non-degraded forest and degraded forest sites in a rural area of the South of Cameroon. It appears that the mosquito fauna in both sites is abundant and diverse. It consists of the genus *Culex, Aedes, Anopheles* and *Mansonia.* However, Anophelinae are more abundant than Culicinae (Table 1). This is consistent with findings by many authors who have carried out similar studies in tropical African villages (Robert *et al.,* 1998). This may be bound to the nature of the breeding sites that are less polluted and have low content of dissolved organic matter. Molecular techniques coupled with conventional taxonomy methods have shown the presence of three species of

Table 3. Comparison of biting rates and entomological inoculation rates in the study sites.

		Study site		
		NDELLE	KOMBO	P
Biting rate (b/p/n)	Indoor	41.46	24.35	0.001
	Outdoor	14.89	10.33	0.13
	P	<0.0001	<0.0001	-
	Total	28.18	17.34	0.01
EIR (ib/p/n)	Indoor	1.41	3.49	0.12
	Outdoor	0.61	1.33	<0.0001
	P	0.001	<0.0001	-
	Total	2.02	4.82	0.002

EIR, entomological inoculation rates.

Table 4. Number of infected mosquitoes and variations of infection rates in NDELLE and KOMBO.

Month of survey	NDELLE				KOMBO					
	An. gambiae		*An. funestus* s.s.		*An. gambiae*		*An. funestus* s.s.		*An. moucheti* s.s.	
	Tested (infected)	Infection rate (95% CI)	Tested (infected)	Infection rate (95% CI)	Tested (infected)	Infection rate (95% CI)	Tested (infected)	Infection rate (95% CI)	Tested (infected)	Infection rate (95% CI)
January	553 (11)	2.0 (1.6-2.3)	73 (3)	4.1 (3.8-4.4)	160 (15)	9.4 (8.7-10.2)	12 (1)	8.3 (7.3-9.2)	87 (4)	4.6 (3.9-5.3)
April	807 (5)	0.6 (0.5-0.7)	100 (2)	2.0 (1.6-2.3)	350 (8)	2.3 (1.9-2.7)	73 (7)	9.6 (9.1-10.1)	21 (2)	9.5 (8.2-10.7)
August	107 (8)	7.4 (6.9-7.9)	26 (2)	7.7 (7.1-8.2)	327 (9)	2.7 (1.9-3.6)	80 (8)	10 (8.7-11.2)	23 (4)	17.4 (13.9-20.9)
October	473 (3)	0.6 (0.5-0.7)	40 (1)	2.5 (1.9-3.1)	130 (19)	14.6 (13.1-16.2)	10 (2)	20 (17.3-22.5)	62 (2)	3.2 (2.4-4.1)
Overall	1939 (27)	1.4 (1.1-1.7)	240 (8)	3.3 (3.0-3.5)	967 (51)	5.3 (4.3-6.2)	174 (18)	10.3 (8.6-11.9)	192 (12)	6.2 (5.7-6.9)

CI, confidence interval.

anopheles out of the forty Anopheles species identified in Cameroon (Hervy *et al.,* 1998). These are *An. gambiae*, *An. funestus* s.s. and *An. moucheti* s.s. Table 1 shows that *An. gambiae* is twice as frequent in NDELLE than in KOMBO. This species has been reported by many authors as the major malaria vector in degraded forest area in tropical Africa (Languillon *et al.,* 1956; Akono *et al.,* 2014). The adaptation of this species could be explained by the presence of sunlit breeding sites. In fact, the degraded environment in NDELLE favour better exposure of breeding sites to sunlight *An. gambiae* being known as an heliophilic species. On the other hand, these breeding sites are less polluted, and made of freshwater collections of various dimensions. Females *An. moucheti* s.s. were only captured in the non-degraded forest site. The presence of this species appears to depend on the Mvobo river. In fact, *An. moucheti* s.s. larvae live preferentially in permanent slow-flowing waters more or less rich in plant species like *Pistia sp* (Njan Nloga *et al.,* 1993). In addition, *Anopheles funestus* s.s. larvae are heliophobic though having same environmental requirements as those of *An. gambiae* (Hamon *et al.,* 1956). The presence of some shaded breeding sites with an upright vegetation and low dissolved organic matter rate in the study sites may explain the presence of this species in houses.

Biting rates were significantly higher in the degraded forest sites. In addition to the density of the forest that limits the exposure of breading sites to sunlight and thus the abundance of *An. gambiae* larvae in KOMBO, the difference of mosquito abundance between the sites could be related to differences in density of macro-invertebrates and aquatic vertebrates, which may regulate the populations of aquatic mosquito larvae by exerting predation on them or depriving them from essential nutrient found in the site (Mereta *et al.,* 2012). Lepidoptera, beetles and frogs larvae are long known for their impact on aquatic mosquito fauna (Humberto & Ariadna, 2007). A comparison between the two sites showed a higher abundance of macro-invertebrates in the Mvobo river; this may explaining the weak biting rate recorded in non-degraded forest site compared with the degraded forest site. Furthermore, *Gambusia affinis*, a larvivorous fish species originating from the south of the United States of America lives in the Mvobo river. This aquatic vertebrate has often been captured by fishermen in the area. It has been used successfully in some countries for the biological control of mosquitoes. In India for instance, its introduction in rice paddies has resulted in an 88% reduction of mosquito larvae population. This fish species, capable of consuming over 165 Anopheles larvae in two hours (Lemasson, 1957), could well be regarded as one of the regulatory factors of mosquito biting rates in this site. Captured mosquitoes were more endophilic than exophilic. The feeding behavior of vectors appears to be subservient to the behavior of the inhabitants of the study sites. In fact, inhabitants of the study sites carry out agricultural activity during day time; they go to bed early enough. Moreover, in the non-degraded forest site, the fishermen go to bed early, because they start fishing activities before sunrise. This habit of early sleeping forces mosquitoes to mostly seek blood meal indoors than outdoors.

Seasonal fluctuations of biting rates for *An. gambiae* and *An. funestus* s.s. follow the same trend and are associated with the operations in the fish ponds of degraded forest site. The results showed low biting rates in August. This result could be explained by the fact that August is the period during which in NDELLE, fishponds are drained. This maintenance of fish ponds resulted in the interruption of the mosquito larvae development cycle, thereby contributing to lower biting rate. On the reverse, in the non-degraded forest site, the seasonal variations of biting rates of *An. gambiae* and *An. funestus* s.s. are influenced by the

Table 5. Variation of entomological inoculation rate (ib/p/n) in NDELLE and KOMBO.

Month of survey	NDELLE *An. gambiae*	KOMBO *An. funestus s.s.*	*An. gambiae*	*An. funestus s.s.*	*An. moucheti s.s.*
January	2.71	0.73	3.6	0.03	1.09
April	2.02	0.5	1.75	1.81	0.46
August	1.86	0.52	2.45	2	0.98
October	0.71	0.3	4.87	0.48	0.46
Overall	1.57	0.45	3.02	1.085	0.72

ib/p/n, infective bites per person per night.

Table 6. Physicochemical parameters of mosquito breeding sites.

Parametres	Breeding sites			
	NDELLE		KOMBO	
	Mean± SE	Range	Mean± SE	Range
Dissolvedoxygen	7.2±1.2	6.9-8.3	8.9±0.8	8.6-11.2
pH	6.03±0.2	5.3-6.7	6.9±0.7	4.5-7.3
Salinity (%)	0.07±0.03	0.05-0.1	0.08±0.02	0.06-0.1
Conductivity (µS/cm)	280.2±5.6	255.7-290.5	273.5±6.2	250.3-300.7
Water temperature (°C)	26.9±2.7	23.2-31.5	24.1±3.5	22.3-27.6
Total dissolved solids (mg/L)	150.6±2.6	140.4-170.4	106.3±1.2	95.3-114.9
Turbidity (NTU)	30.2±1.7	26.0 - 37.0	28.4±3.7	23-32
Aquatic plants	(+)	-	(+++)	-
Gambusia affinis	(-)	-	(+++)	-
Aquaticinvertebrates	(-)	-	(+++)	-

(+++), very abundant; (+), scarce; (-), absent - no data available.

rainfall. January is the long dry season, characterized by the drying up of most of the existing temporary water collections in the site; which explains the low biting rates recorded in this period. The spacing of rainfalls observed in April and August allows for the reconstitution of previously drained breeding sites, explaining the high biting rates in this time of year. The abundance and regularity of rainfalls in October favored the leaching of breeding sites and the larvae they contain, resulting in lower biting rates observed at this time of the year. The survival of *An. moucheti* s.s. is especially related to aquatic plant species such as *Pistia sp.* (Njan Nloga *et al.,* 1993). For some years, the inhabitants carry out weeding of the river Mvobo to get the bed rid of invasive plant species. This weeding activity by local residents carried out in April and August each year result in the disruption of the ecology of *An. moucheti* s.s. larvae of resulting in a lower biting rate of *An. moucheti* s.s. in the study site at these periods. Similar patterns of seasonal fluctuations in aggressive Anopheles mosquito densities have been reported in other villages crossed by rivers, noting influence of these rivers in malaria transmission (Akono *et al.,* 2014).

The CSP ELISA tests revealed that malaria transmission is maintained throughout the year in the study sites, driven by *An. gambiae*, *An. funestus s.s* and *An. moucheti* s.s.. In both sites, *An. gambiae* appears to be the major malaria vector. It is followed in this role by *An. funestus* s.s. and *An. moucheti* s.s. in the non-degraded forest site and *An. funestus* s.s. only in the degraded forest site. This is consistent with findings in most villages of forest areas in tropical Africa where *An. gambiae* is the most adapted species and the major malaria (Languillon *et al.,* 1956). *An. gambiae* is the only species of the Gambiae complex that was identified and found to be infected in the study sites. This result is all the more reliable as other studies have reported that *An. gambiae* flourishes better and transmits malaria in sunlit forest context (Akono *et al.,* 2014), in contrast to *An. coluzzii* which tends to fit better in polluted towns of tropical Africa as well as in brackish water collections (Antonio-Nkondjio *et al.,* 2012; Overgaard *et al.,* 2012). *An. moucheti* s.s. and *An. funestus* s.s. are secondary malaria vectors in the study area. Studies on their roles in the transmission have been conducted by many authors (Njan Nloga *et al*., 1993; Antonio-Nkondjio *et al*., 2008; Akono, 2011). In some localities, *An. moucheti* s.s. has shown to be a locally important vector (Njan Nloga *et al*., 1993). While in others, *An. funestus* s.s. was reported as subsequent in time to *An. gambiae* in its role as major vector malaria (Dossou-Yovo, 1998; Akono, 2011). The results also show that the entomological inoculation rate (EIR) vary with sites and months. A comparative analysis shows that in October, the non-degraded forest site record higher EIR than the degraded forest site. This could be justified by differences in aggressive mosquito densities observed in the two sites in this period. In fact, the leaching of Anopheles larvae that occurs with heavy rainfall results in a very low biting rate and subsequent increase in the proportion of infected female anopheles in the general mosquito community. Ultimately, the overall average EIR recorded in the non-degraded forest and in the degraded forest sites were respectively 4.82 ib/p/n and 2.02 ib/p/n. Thus, the degraded forest environment and the presence of many fishponds in NDELLE have led to a proliferation of Anopheles mosquitoes; however, it has not resulted in a significant increase of malaria transmission.

These results should be used for monitoring and evaluation of the impact of the future agro-industrial complex on aggressive mosquito diversity and malaria transmission in these villages. An increase in entomological values of malaria parasite transmission in the course of the implementation of the project should trigger the reinforcing of malaria control strategies by project managers and local authorities, mainly mass distribution of new LLINs and elimination of anopheline breeding sites set up during the earthmoving of the site alongside health education on malaria. The managers should thus include malaria prevention strategies in their project in order to mitigate the risk of increased malaria transmission as a result of the implementation of their projects.

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