Mosquito species diversity and malaria transmission in Ayos, an area of degraded forest targeted for universal long-lasting insecticidal net distribution in southern Cameroon

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This study was conducted from January to December 2010 to evaluate the anopheline diversity and transmission of malaria in Ayos, a degraded forest area in the south of Cameroon, targeted for the distribution of long-lasting insecticidal nets (LLINs). Mosquito larvae were collected by the dipping method and endophilic female adult mosquitoes were captured on volunteers. Molecular techniques were used alongside morphological techniques for mosquito identification; ELISA was used for the detection of plasmodium circumsporozoite antigens. Ten mosquito species, including four *Anopheles* species (*Anopheles gambiae s.s.*, *An. funestus s.s.*, *An. moucheti s.s.* and *An. hancocki*), were identified. The mean biting rate of these *Anopheles* species was 12.7 bites per person per night (b/p/n). *An. gambiae s.s.* (6.9 b/p/n) appeared to be the most aggressive species. Malaria transmission is mainly ensured by *An. gambiae s.s.*, *An. funestus ss.* and *An. moucheti s.s. Plasmodium falciparum* was the only malaria parasite transmitted. The mean entomological inoculation rate (EIR) for these vectors was 0.7 infecting b/p/n. *An. gambiae s.s.* (65.6 %) is the major vector, with an annual EIR of 167.9 infectious b/p/n/year. The utilization of LLINs alongside other methods would highly contribute to effective malaria control in Ayos.

Key words: *Anopheles*, transmission, malaria, LLINs, Cameroon.

INTRODUCTION

More than a century after the discovery of its causal agent and the role of the mosquito vector, malaria remains a scourge (Carnevale & Robert 2009). About 154 to 289 million persons are infected each year with subsequent 490 000 to 836 000 deaths, mostly in children under five years of age. About 90 % of this burden is recorded in Africa. This disease is one of the most serious obstacles to socio-economic development in Africa (WHO 2012). For several decades, malaria control has been a priority for endemic African countries. Nevertheless, these efforts have long been hampered by new strains of the malaria parasite showing resistance to common antimalarial drugs (Ridley *et al.* 2002) and vector resistance to insecticides (Chouaibou *et al.* 2006; Etang *et al.* 2003, 2006, 2007). It is in this context that Cameroon, in the framework of the adjustment of its national malaria control policy in 2007, has adopted an integrated approach including long-lasting insecticidal nets (LLINs) distribution and Indoor Residual Spraying (IRS) as main interventions. There is need for baseline entomological data for the selection as well as the evaluation of control interventions. Entomological surveys have been conducted in many eco-climatic areas of Cameroon (Bigoga *et al.* 2007; Manga *et al.* 1995; Wanji *et al.* 2003). The present study was conducted in Ayos in the southern part of Cameroon, an area of degraded forest. The locality is crossed by the Nyong River, a perennial, slow-flowing river. Ayos has experienced profound environmental changes due to the tarring of the Yaounde–Bonis road, linking the Centre to the East Region of the country. This study reports on mosquito species diversity, abundance as well as malaria transmission in Ayos with the aim of complementing existing baseline entomological data. This will enable follow-up and the evaluation of the impact of the distribution of LLINs in this area.

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MATERIAL AND METHODS

Description of the study area

The study was conducted in Ayos (03°54′N 12°31′E), a locality situated 160 km east of Yaoundé, the capital city of Cameroon (Fig. 1). The total pop-

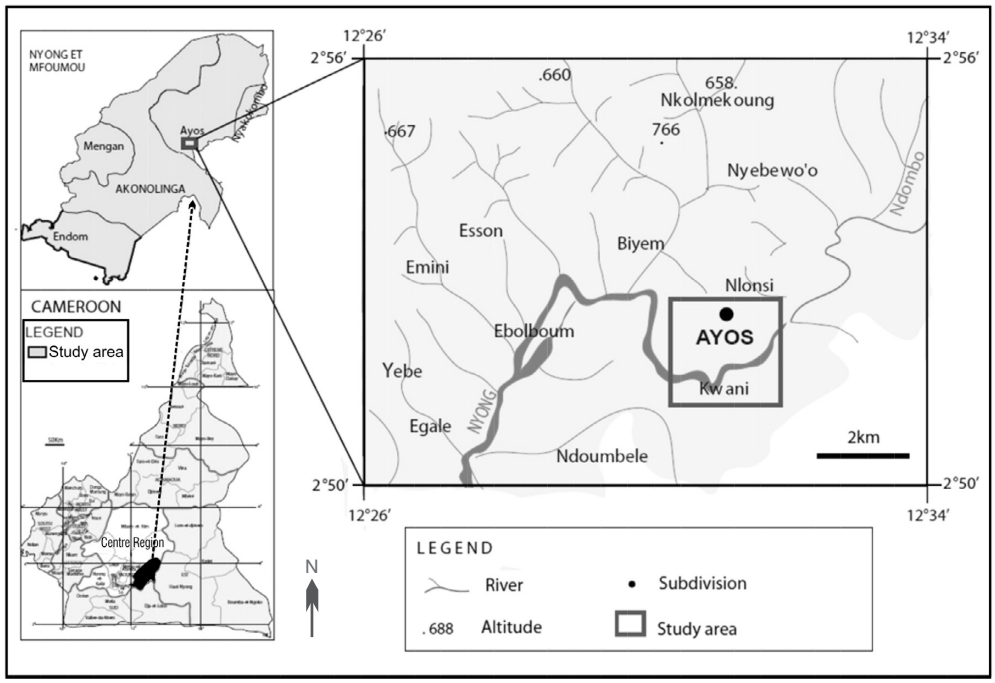

Fig. 1. Map of the study area.

ulation was estimated at 14 950 inhabitants. They live mainly on the production of cash crops (cocoa, coffee), food crops (groundnuts, maize and cocoyam) and fishing. Animal husbandry is also practiced. Most of the houses have mud walls and corrugated iron roofs. The hydrographic network is dense and consist of many brooks and streams that flow into the Nyong River, a slow-flowing river invaded by two plant species (*Pistia* sp. and *Eichhornia crassipes*), which crosses the town east to west. It is an area of semi-deciduous forest, made up of plants of the Ulmaceae, Piperaceae and Graminae families. The vegetation has suffered severe degradation as a result of urbanization and the tarring of the Yaounde–Bonis road, connecting the Centre and the East Regions. The climate is equatorial (Guinean type) with four seasons, two rainy (September to November and March to June) and two dry ones (December to February and July to August). Annual rainfall was 1971.2 mm in 2010; mean temperature was 25.53 °C and relative humidity was 80 % (Fig. 2).

Study design and ethical consideration

This longitudinal study was carried out for 12 months, from January to December 2010, with the permission of local authorities. Ethical clearance was obtained from the National Ethical Review Committee of Cameroon. Participation in the study was voluntary. All participants gave informed written consent; prior to field work, they were immunized against yellow fever. Antimalarial chemoprophylaxis was administered up to one month after the end of the study.

Sampling and processing of larvae and adult mosquitoes

Sampling was done in the months of January, April, August and October (corresponding to the four seasons of the year), for five consecutive days each time. Mosquito larvae were collected from breeding sites along the Nyong River. Female endophilic adult mosquitoes were captured indoors by human volunteers.

All permanent and temporary water collections on the banks of the Nyong River were examined for larvae and pupae. Larvae and pupae were collected, from 10:00 to 14:00 using the standard dipping method that consists in performing 20 to 100 dips, depending on the size of the breeding site (Services 1993). Larvae and pupae were then transported to the laboratory, in water collected from their respective breeding sites and fed on

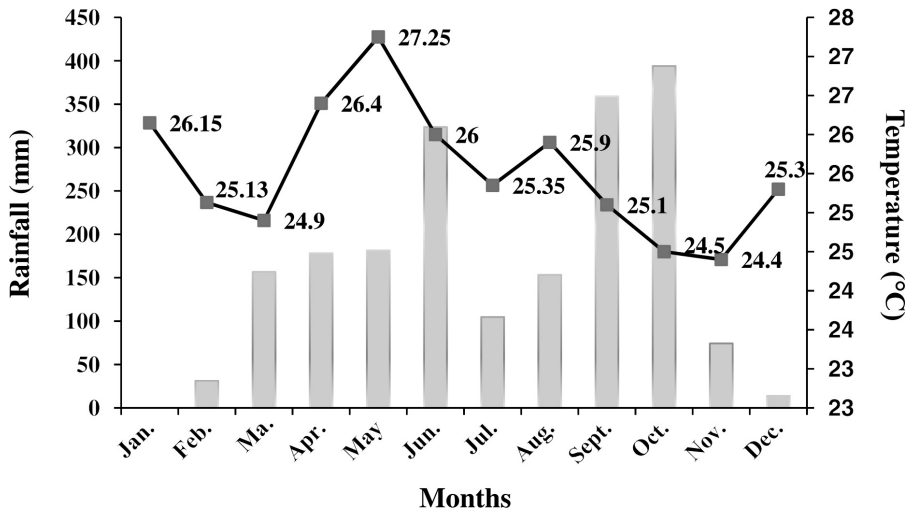

Fig. 2. Rainfall and temperatures for 2010 in Ayos (National Meteorological Service).

Tetrababy fish food, to adult stage. The larval density was 100 larvae per litre of water. Emerging adults were morphologically identified and stored in silica gel containing Eppendorf tubes at –20 °C, for subsequent laboratory analysis.

Adult mosquito sampling was carried out by human landing catch. Female adult mosquitoes were captured indoors, from 18:00 to 06:00 on human volunteers who prior to the catch were immunized against yellow fever and administered antimalarial chemoprophylaxis. Catches were performed in five residences selected along the river, with the approval of residents. In each of the residences, two catchers were working in turn in two shifts; one from 18:00 to midnight and the other from midnight to 06:00. During the capture period, a window or door was left slightly open. Mosquitoes were morphologically identified to species following the taxonomic keys of Gillies & De Meillon (1968), Gillies & Coetzee (1987) and Edwards (1941) then stored in silica gel containing Eppendorf tubes at –20 °C, for subsequent laboratory analysis.

Laboratory analysis

Legs and wings were used for molecular identification of mosquitoes belonging to the *An. gambiae* Giles complex as well as *An. funestus* Giles and *An. moucheti* Evans groups. Species and molecular forms of *An. gambiae* complex were identified by a PCR-based method described by Fanello *et al.* (2002). Species of the *An. funestus* and *An. moucheti* groups were identified by PCR-based methods described by Cohuet *et al.* (2003) and Kengne *et al.* (2007), respectively. Debris of the head and thorax of adult female *Anopheles* mosquitoes were analysed for the presence of sporozoites of malaria parasites through the detection of the circumsporozoite protein (CSP), by the ELISA technique described by Burkot *et al.* (1984), and amended by Wirtz *et al.* (1987). All positive samples were retested to confirm the result.

Data analysis

The biting rate was expressed as the average number of bites received per person per night of collection. The sporozoite index was expressed as the proportion of mosquitoes found to contain circumsporozoite antigens by ELISA. The entomological inoculation rate (EIR) was expressed as the number of infective bites per person per night or year (ib/p/n or ib/p/y) and calculated as the product of the sporozoite index and the biting rate. All statistical analyses were performed using SPSS software (Version 19.0 for Windows, SPSS Inc., Chicago, IL, U.S.A.). The Pearson correlation test was used to study the linear relationship between the biting rates of various species while ANOVA test was used to compare biting rates and EIR between species.

RESULTS

Diversity of the mosquito fauna in Ayos

Overall 14 128 adult mosquitoes were obtained both by human landing catch and rearing of larvae in the laboratory. Ten species of mosquitoes were

Table 1. Diversity of mosquitoes in Ayos*.

Mosquito species	Adults from landing catch	Adults that emerged from larvae	Total number of adults obtained
Anopheles gambiae s.s.	6993 (53.9 %)	182 (15.9 %)	7175 (50.8 %)
Anopheles funestus s.s.	3442 (26.5 %)	138 (12.0 %)	3580 (25.3 %)
Anopheles moucheti s.s.	2388 (18.4 %)	246 (21.5 %)	2634 (18.6 %)
Anopheles hancocki	25 (0.2 %)	0 (0 %)	25 (0.2 %)
Mansonia uniformis	0 (0 %)	20 (1.7 %)	20 (0.1 %)
Mansonia africana	0 (0 %)	32 (2.8 %)	32 (0.2 %)
Culex duttoni	9 (0.1 %)	116 (10.1 %)	125 (0.9 %)
Culex pipiens	23 (0.2 %)	76 (6.6 %)	99 (0.7 %)
Aedes albopictus	17 (0.1 %)	35 (3.1 %)	52 (0.4 %)
Aedes aegypti	85 (0.7 %)	301 (26.3 %)	386 (2.8 %)
Total	12982 S1 = 8	1146* S2 = 9	14128 (100 %) S = 10

*Adult mosquitoes obtained from 1301 larvae and pupae reared in the laboratory; S1 = number of biting species; S2 = number of species found in breeding site; S = total number of species; *s.s.* = *sensu stricto.*

identified: *An. gambiae s.s.* (the only species of the *An. gambiae* complex); *An. funestus s.s.* (the only species identified in the *An. funestus* group); *An. moucheti s.s.* (the only species identified in the *An. moucheti* group); *An. hancocki* Edwards; *Mansonia uniformis* Blanchard; *Mansonia africana* Theobald; *Culex duttoni* Theobald; *Culex pipiens* Linnaeus; *Aedes albopictus* Skuse and *Aedes aegypti* Linnaeus (Table 1).

Of the 10 identified species, those of the genus *Mansonia* were captured in their larval stages only, while no larva or pupa of *An. hancocki* were collected. The seven other species were captured both in their larval and adult stages (Table 1).

Mosquitoes of the subfamily Anophelinae were 94.9 % (n = 13 414) of the total number of mosquitoes. *An. gambiae s.s.* (50.8 %, n = 7175) was the most abundant species, followed by *An. funestus s.s.* (25.3%, n = 3580) and *An. moucheti s.s.* (18.6 %, n = 2634). The Culicinae were poorly represented (5.1 %, n = 714).

Biting activity, sporozoite index and entomological inoculation rate of *Anopheles* mosquitoes

A total of 12 982 female mosquitoes captured in 200 man-nights were found biting humans, of which 99 % were *Anopheles* mosquitoes (Table 1). The average biting rate of these *Anopheles* mosquitoes was 12.7 b/p/n. *An. gambiae s.s.* was the most aggressive species with 6.9 b/p/n, followed by *An. funestus s.s.* (3.4 b/p/n) and *An. moucheti s.s.* (2.4 b/p/n). There were statistically significant differences in the biting rates of *An. gambiae s.s.* and *An. funestus s.s.* (F = 5.089, d.f. = 38, P = 0.03) as well as between *An. gambiae s.s.* and *An moucheti s.s.* (F = 8.48, d.f. = 38, P = 0.006).

A total of 3700 females of the four identified *Anopheles* species were analysed with ELISA circumsporozoite antigen test. The annual average sporozoite index was 1.7 % (n = 1731), 1.3 % (n = 1150), 1.7 % (n = 797) and 4.5 % (n = 22) for *An. gambiae s.s.*, *An. funestus s.s.*, *An. moucheti s.s.* and *An. hancocki*, respectively (Table 2). Infected females of *An. gambiae s.s.* and *An. funestus s.s.* were captured in all seasons while those of *An. moucheti s.s.* were absent in April during the short rainy season. The single infected *An. hancocki* specimen was found in January (Table 2). *Plasmodium falciparum* was the only malaria parasite species found in infected mosquitoes.

In Ayos, *An. gambiae s.s.* alone contributes to 65.62 % of the transmission, with an average EIR of 0.46 ib/p/n. *Anopheles funestus s.s.* and *An. moucheti s.s.* appear as secondary vectors with an EIR of 0.14 ib/p/n and 0.1 ib/p/n, respectively (Table 3). *Anopheles hancocki* could be considered an occasional vector with 0.001 ib/p/n. Average EIRs were significantly different between *An. gambiae s.s.* and *An. funestus s.s.* (F = 30.9, d.f. = 1, P = 0.0001) as well as between *An. gambiae s.s.* and *An. moucheti s.s.* (F = 36.4, d.f. = 1, P = 0.0001). Overall transmission due to the four vectors is estimated at 255.86 ib/p/y. This transmission occurred

Table 2. Average sporozoitic index (SI) for the different *Anopheles* species.

Anopheles species (%)	Period	Tested	Infected	Sporozoitic index
Anopheles gambiae s.s.	January	83	9	10.8
	April	1100	3	0.3
	August	314	14	4.5
	October	234	4	1.7
	Over the year	1731	30	1.7
Anopheles funestus s.s.	January	18	3	16.6
	April	787	2	0.25
	August	235	7	2.9
	October	110	3	2.7
	Over the year	1150	15	1.3
Anopheles moucheti s.s.	January	109	5	4.5
	April	6	0	0
	August	15	1	6.6
	October	667	8	1.2
	Over the year	797	14	1.8
Anopheles hancocki	January	10	1	10
	April	0	0	0
	August	7	0	0
	October	5	0	0
	Over the year	22	1	4.5

s.s. = s*ensu stricto*.

throughout the entire period of study with some variations in time.

Variation in mosquito activity

The biting rates varied in a similar manner for *An. gambiae s.s. and An. funestus s.s.* (R^2 = 0.993, P = 0.034); they were lower in January (long dry season), higher in April (short rainy season), then decreased steadily from August (short dry season) to October (long rainy season). Biting rates of *An. moucheti s.s.* decreased from January to April, then increased to reach its maximum in October. The number of specimens of *An. hancocki* captured was not enough to assess variations in biting rates in this species (Fig. 3). Sporozoite index was at the highest for the four *Anopheles* species during the long dry season then decreased to the lowest during the short rainy season; it rose again towards the short dry season before decreasing steadily with the rains of the long rainy season (Fig. 4). The EIR followed a similar trend for *An. gambiae s.s., An. hancocki* and *An. funestus s.s.* For *An. moucheti s.s.*, however, it decreased to the lowest during the short rainy season then kept increasing throughout the short dry season and the long rainy season (Fig. 5).

Table 3. Average entomological inoculation rate (EIR) for different anopheles species.

Period	*Anopheles* species			
	Anopheles gambiae s.s.	*Anopheles funestus s.s.*	*Anopheles moucheti s.s.*	*Anopheles hancocki*
January (ib/p/n)	0.53	0.11	0.15	0.01
April (ib/p/n)	0.2	0.07	0.0	0.0
August (ib/p/n)	0.8	0.25	0.04	0.0
October (ib/p/n)	0.3	0.12	0.24	0.0
Annual EIR (ib/p/y)	167.9	51.1	36.5	0.36

ib/p/n = infectve bites per person per night; ib/p/y = infective bites per person per year; *s.s. = sensu stricto*.

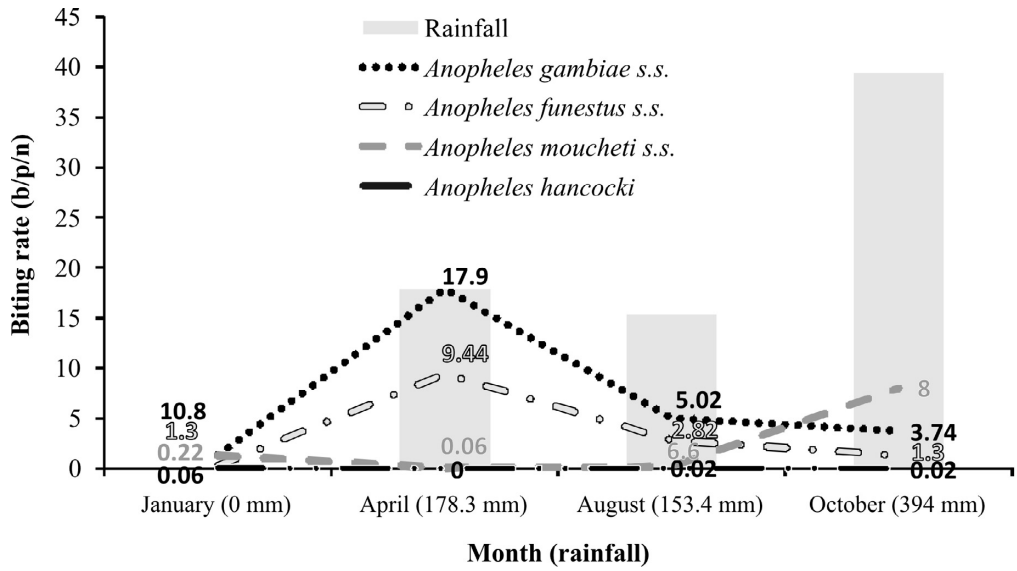

Fig. 3. Variation in biting rates with season/rainfall.

DISCUSSION

This study investigated the diversity of mosquito species and malaria transmission in Ayos. Our findings showed that there is an abundant and diversified mosquito fauna, ensuring perennial malaria transmission, with variations related to rainfall. Mosquitoes of the genus *Anopheles* were the most abundant (94.9 %). Most of the breeding sites found in the study area were less polluted and had low quantities of dissolved organic matter making them suitable for anopheline breeding. The physico-chemical analyses of water samples collected from the study site showed that the river and most of the breeding sites contained weakly basic water (pH 7.9–8.1). In addition, they showed a very low organic matter content (essentially consists of vegetable matter, TDS between 62 and

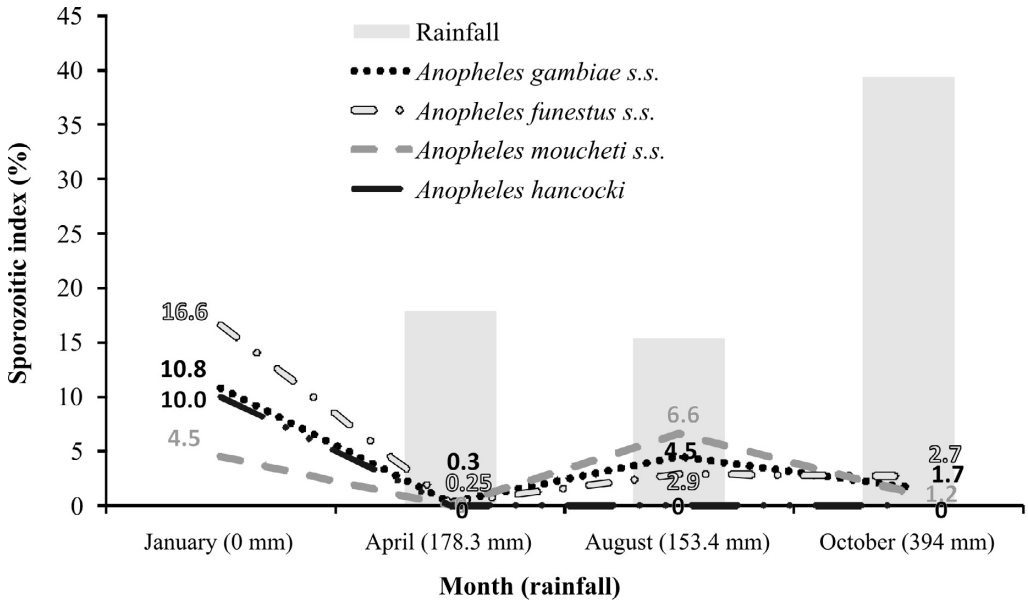

Fig. 4. Variation in the sporozoitic index with season/rainfall.

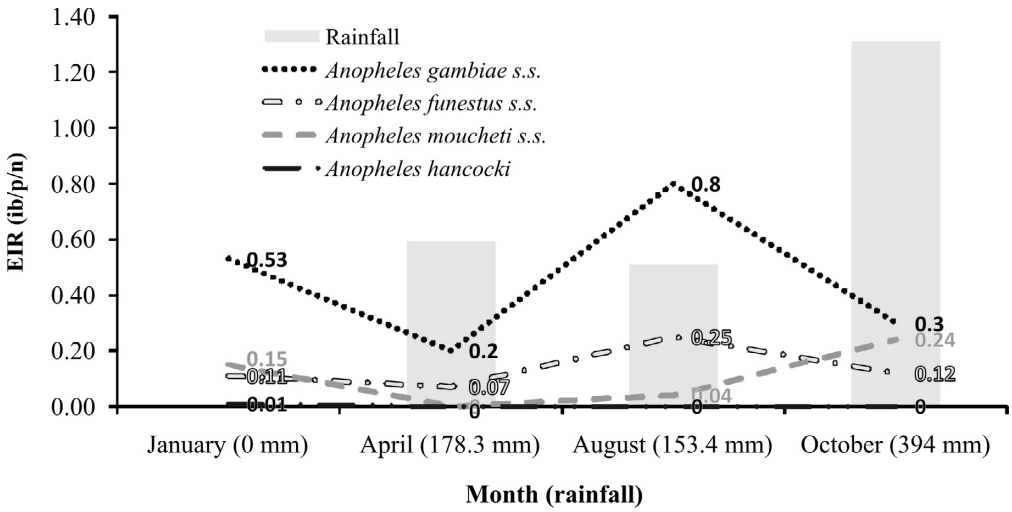

Fig. 5. Variation in the entomological inoculation rate (EIR) with season/rainfall.

75 mg/l) and a low amount of nitrate, chlorides, phosphates and potassium ions. These ecological conditions, added to deforestation that has reduced shade in the area, may explain the proliferation of *Anopheles* to the detriment of species of the family Culicinae. *Anopheles gambiae s.s.*, *An. funestus s.s.*, *An. coluzzii* Coetzee & Wilkerson, *An. arabiensis*, *An. nili s.s.* and *An. moucheti s.s.* are considered the most important vectors in sub-Saharan Africa (Coz 1973; De Meillon 1956; Fontenille *et al.* 2003; Le Goff *et al*. 1993). In our study, *An. gambiae s.s.* was the most abundant malaria vector, differing from previous findings by Manga *et al.* (1993) and Manga (1999), who found *An. gambiae s.s.* not to be the major species in rural areas of southern-forested Cameroon. *An. gambiae s.s.* was the only member of the Gambiae complex identified through molecular assays. Wanji (pers. comm.) found that in the southwest of Cameroon, *An. melas* and probably *An. coluzzii* adapt better in the brackish surface water while *An. gambiae s.s.* prefers sunny freshwater bodies. The tarring of the Yaounde–Bonis road led to the destruction of the forest, thereby contributing to the formation of freshwater bodies exposed to sunlight. This might explain the proliferation of *An. gambiae s.s.* and its shift to become the major malaria vector in this area of the country. *An. funestus s.s.* larvae prefer permanent, vegetated water although their other ecological requirements are similar to those of *An. gambiae s.s.* (Hamon *et al.* 1956). Most of the larvae of *An. funestus s.s.* were collected in shaded water bodies. The Nyong River offers adequate conditions for the development of larvae of *An. moucheti s.s.* which prefer permanent, slow-moving water bodies, with plant species such as *Pistia* sp. (Njan Nloga *et al.* 1993). *An. hancocki* had long been reported in this area by Adam *et al.* (1956). This species would have resisted the profound ecological changes that took place in Ayos. Only adult stages of this species were captured. We thus cannot describe the larval ecology of this anopheline species.

The biting rates of *An. gambiae s.s.* and *An. funestus s.s.* showed similar trends that depended on rainfall. They decreased in January during the long dry season, as a result of the drying up of temporary water collections that serve as breeding sites for mosquitoes. The months of April and August correspond to the short dry season and short rainy season, respectively. These two seasons are generally characterized by the spacing of the rhythm of rains which are scarce. Rainwater collects in previously dry breeding sites but does not flow because of its reduced quantity; this results in the increase in the number of breeding sites, resulting in the proliferation of larvae and adults of *An. gambiae s.s.* and *An. funestus s.s.*. The heavy precipitation recorded in the long rainy season favours the drainage of breeding sites, washing away the larvae, hence the lower biting rates recorded for the two species during this period. Biting rates of *An. moucheti s.s.* are particularly low in April and August (Fig. 3). This might have resulted from the maintenance of the river

undertaken by riparians during that period to rid the waterway of invasive plants such as *Pistia* sp. and *Eichhornia crassipes*. This maintenance activity would have disrupted the ecology of larvae of *An. moucheti s.s.,* causing the lower biting rates observed during this period.

Night-landing catches on volunteers showed that *An. gambiae s.s.* and *An. funestus s.s.* transmitted *Plasmodium falciparum* in all seasons. *An. gambiae s.s.* was the most effective malaria vector at this study site, with seasonal EIR ranging between 0.2 and 0.8 ib/p/n. These observations are in agreement with findings in most of the degraded forest areas of southern Cameroon, where *An. gambiae s.s.* is recognized as the major malaria vector *(*Languillon *et al.* 1956). They, however, differ from other studies in various ecological zones of southern Cameroon crossed by major rivers. Njan Nloga *et al.* (1993) found that *An. moucheti s.l.* was the major vector of malaria in Ebogo; Carnevale *et al.* (1992) and Le Goff *et al.* (1997) also reported *An. nili s.l.* as the major malaria vector along the Sanaga River. The development of *An. gambiae s.s.* as the major malaria vector in Ayos has probably been favoured by the presence of many breeding sites suitable for the development of *An. gambiae s.s.* larvae, as a result of urbanization and the tarring of the Yaounde–Bonis road. The average EIR of vectors identified in the study site was 0.7 ib/p/n. This somewhat low malaria transmission rate nevertheless has an impact on the population's health in Ayos. Correct use of the LLINs given by the government to households is highly recommended as this would lead to the reduction of malaria prevalence to an acceptable level in this locality.

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