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Impact of extensive rice cultivation on the culicid fauna and the transmission of malaria in Tonga, West Cameroon

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Key words: Rice field, culicid fauna, malaria transmission, Tonga

1 SUMMARY

Anopheles mosquitoes cause malaria in man. The aim of this study was to assess the impact of a rice field on the Anopheles fauna and on the transmission of malaria in a village located in a degraded forest at high altitude (820 m). The analysis took place from January to December 2006 in Tonga subdivision of Nde Division, Cameroon. Larva were collected by dipping technique while female Anopheles were captured on humans. A total of 7 species of Anopheles were identified in the two sites assessed. *Anopheles gambiae* and *A. nili* were the only aggressive anophelienne species for humans in Nde neighborhood. Their daily average aggressivity rate (m.a) is 6 bites/man/night (b/m/n) against 83.5 bites/man/night registered at Bandounga rice field for the species of *A. gambiae*, *A. funestus,* and *A. wellcomei.* At each site, the transmission of malaria was low and was spread throughout the whole year with a higher rate from December to March. The global yearly entomological rate is estimated at 16.5 infected bites /man in 10 months studies in the Nde neighborhood. The rate is 27 infected bites/man/year at Bandounga. *A. gambiae* accounted for more than 80% of the transmission at the two sites and appears to be the main malaria vector in Tonga. *A. nili, A. wellcomei* and *A. funestus* play a secondary role in the transmission. The creation of a rice farm in Bandounga has led to a qualitative and quantitative increase in the Anopheles fauna in the area.

2 INTRODUCTION

The transformation of swampy areas into hydro-agricultural zones in African villages and towns is increasing due to the high food shortage experienced in every day (Dossou-Yovo *et al*., 1998). In most cases, the placement of an agricultural project in a given locality is not always accompanied by the necessary assessment of environmental impact and the consequent sanitary risks on the population. According to data obtained over 20 years, it has been unanimously agreed that the exploitation of hydro-agricultural ecosystems has an impact in the short, middle and long term on the incidence of vectorial diseases (Manga et al.,

1992; Robert et al., 1992).
In Cameroun, research work carried out in the forest and Sudano-sahelian zone has showed that agricultural installations increase the density of aggressive anopheles mosquitoes, which may be accompanied by modifications in the dynamics of transmission and epidemiology of malaria (Blancheteau *et* Picot, 1983; Fondjo *et al*., 1999). However, very few entomological studies on the impact of agricultural projects have been carried out in the mountainous area of west Cameroon. The belief that the climatic characteristics of mountainous areas do not favor the proliferation of Anopheles vectors (Cot *et al*., 1992) could be responsible for the scarcity of entomological studies in this region of the country.
A few years ago, this region was exposed to numerous anthropogenic actions, with the aim of resolving the problems of food insecurity and housing as a result of the creation of universities in Bangangte, Dschang and Bandjoun in the 1990s. Tomatoes, vegetables, maize, potatoes, are among the main food crops grown by the population of this locality. Due to the closure of the Rice Expansion Company of the Mbo plain in Santchou subdivision in the West of Cameroon, rice cultivation is only practiced in the localities of Bandounga, Baloua and Babitchoua. Irrigated *rice* growing areas have been known as excellent breeding grounds for culicidians. Their incidence on the transmission of malaria in the rural and urban areas is known (Coosemans et al., 1984; Robert *et al*., 1986; Robert *et al*., 1992; Faye *et al*., 1993; Dossou-yovo *et al*., 1998).
The impact of rice cultivation at high altitudes on the health of the population is not well known. Due to their altitudinal position, rice cultivation in this region opposes two ecological concepts, i.e. the climatic factors which create unfavorable conditions for the survival of germs and mosquitoes (Cot *et al*., 1992) and the presence of breeding grounds which are favorable for their proliferation (Dossou *et al*., 1998).
The objectives of this study were to determine the diversity, aggressivity and physiological age of the Anopheles mosquitoes in Nde division; and characterize the infectivity of malaria vectors and the rate of entomological inoculation (E.I.R) and transmission of malaria in the area.

3 MATERIALS AND METHODS

3.1 Study site: This study was carried out in the Tonga area from January to December 2006 (4°58'N; 10°41'E; 820m of altitude). It is located in the degraded forestry zone, at a distance of 300 km from Yaoundé, the Capital of Cameroon. The primary forestry zone was changed to a secondary forest as a result of human activities. Apart from the sacred trees which constitute the relics of the forest, the vegetation is made up of fruit trees and rice. This gives a prairie dimension to the environment (Feukou, 1994). There is a lowly dense hydrographic network, made up of some streams, low lands and marshy areas. The population of Tonga (about 25,000 inhabitants), is made up mainly of Baloua, Babitchoua and Ngodikam, who are adapted to the local climate that is characterized by a relatively short dry season (mid- November to February), followed by a long rainy season (mid- March to mid- November) (Fig. 1). Precipitation is high, from 1278.7 mm/year, annual average temperature is 21.6°C, with values ranging from 20.3 - 23.2°C. Average humidity is 68%, and varies between 53 and 78%.
The entomological inquiries were carried out in two areas of Tonga Bandounga and Nde. These two quarters distant by 33km and linked by an untarred road with "pot holes" holding temporal pools of water during the rainy season. These pools can represent breeding grounds for mosquito larvae. In Bandounga more than 80% of the cultivable land is transformed into rice fields. The seasonal presence of water, exclusively during the rainy season is favorable for rice cultivation which is essentially practiced during the months of August and November. The Nde area is rich in many fish species, with the carpe being the most consumed by the population. The habitat has remained traditional in the two localities. The huts are rectangular, made

from earth, the roof is thatched, husbandry is very low with some pigs, goats and chicken loitering around the compounds. Fishing and agriculture are the main traditional economic activities of Bandounga.

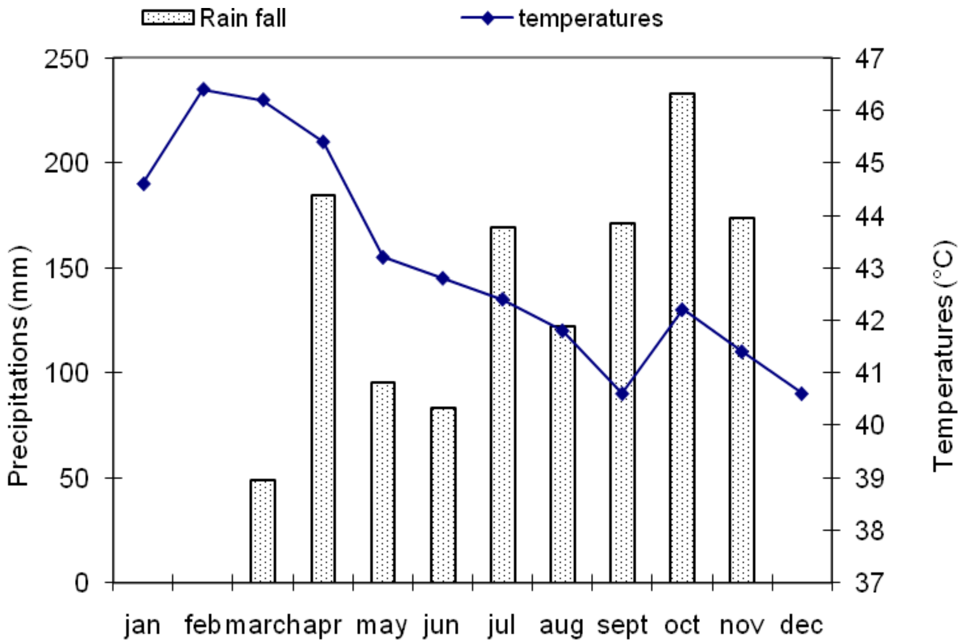

Figure 1: Rainfall and temperature variations at Tonga, west Cameroon.

3.2 Capture of larval forms of mosquitoes: The larval prospection's took place in the rice breeding fields, in the breeding ground of the Nde river and in all permanent and temporal water points in the two sites. The larvae sampled were conserved in polystyrene bottles of 1.5 liters each containing breeding water and they were transported to the Zoology laboratory of the University of Yaoundé 1 for breeding. After regrouping the larva of the same genera, they were classified based on their developmental stages, then bred in cylindrical plastic containers of 10cl each. The larvae were made to develop in their breeding water in which fish food was added (Tetrabady fish food L) (Desfontaines *et al.,* 1991). The water was changed every 3 days in order to avoid the death of larva by asphyxiation due to the decomposition of food nutrients (Ravaonjanahary, 1978). The larval density applied was 10 larvae for 8 cl of water. The nymphs obtained were transversed into aluminum recipients, then placed inside the cubic cages of 4 liters each and then covered with mosquito net. The emerging adults were sacrificed by an insecticide spray and then identified in accordance to the specialized keys proposed by Edwards (1941), Harry and Chester (1963), Gillies and De Meillon (1968) and Peter Jupp (1996).

3.3 Capture of female adult mosquitoes: The night capture of anthropophilic Culicidian populations was carried out in each area using human subjects (protected from malaria by a preventive dosage of artesunate and amodiaquine, yellow fever vaccines). After an identification phase of the houses of inhabitants enrolled in the study, three capture points were selected during the entire period of sampling in both quarters. The organisms were captured inside the houses from 6 pm to 12pm at a rhythm of 3 consecutive days per month. In each compound, 2 human subjects relayed during the night with one working from 6pm to 12pm and the other from 12pm to 6am. The mosquitoes were captured with a tube immediately they landed on the bare limbs of a human subject. The mosquitoes that were captured in each time frame were killed by an insecticide and then identified with the help of specialized identification

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keys. The physiological age of each female was determined by the nature of its ovarian tracheoles (OMS, 1975).The sporozoïtes were identified with the help of an optical microscope at the objective 40x in the salivary glands by wet mounts, using a drop of physiological liquid (solution NaCl, 8%).

3.4 Data analysis: The specific richness of the communities was calculated. The degree of similarity was expressed by the Sorensen index (Is= (2c x 100) / (a+b) [a=number of species in site 1; b= number of species in site 2; c=number of species common to the two sites (Barbault, 1992). The SAS software, version 9.1 was applied in Pearson correlation test to study the variations of the aggressivity densities as a function of pluviometry, infection rate and the fluctuations in parturition.

4 RESULTS

Specific richness: The sampling of mosquito larva in the breeding ground of Bandounga and the Nde quarter revealed the presence of 7 species of Anopheles belonging to the genera (Anopheles) and subfamily Anophelinae. The rice fields breeding ground had a lower number of species (S=3) than the breeding at Nde (S=6) (table 1). *Anopheles gambiae*, *A. funestus* and *A. wellcomei* are species that were sampled in the rice breeding area. Apart from the species *A. gambiae* and *A. wellcomei* considered as ubiquitous in the two zones prospected, *A. paludis, A. nili*, *A. ziemanni* and *A. hancocki* were sampled in the Nde area. The Sorensen indices (IS=44%) attest to the fact that the species identified in the two sites do not belong to the same community.

Abundance: A total of 12, 257 mosquito larvae were collected from the two sites during 36 capture sessions. The breeding of these larva up to the adult stage yielded 93 % imago (11, 442 imagoes) (Table 2).The Culicid fauna of Tonga is more abundant than the rice zone and it is dominated by *A. gambiae* group (51.47%; n=5,889).

Of the 4,722 mosquito larva in Nde bred in insectariums, only 4,573 emerged to the adult stage, giving an emergence rate of 96.84%. A. nili was the main specie (70. 68%, n=3,232) followed by *A. gambiae* (28. 87%; n=1,320). The larva of *A. nili* were however absent in the rice field. The dominant species were *A. gambiae* (66. 5%, n=4,569) and *A. funestus* (n=27. 9%; n= 1,917).

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Table 1: Specific richness of larval forms in Tonga, Cameroon.

Sites / Species	Bandounga rice field breeding ground	Rivers breeding ground in the Nde quarter
Anopheles gambiae	+	+
Anopheles funestus	+	-
Anopheles wellcomei	+	+
Anopheles paludis	-	+
Anopheles nili	-	+
Anopheles ziemanni	-	+
Anopheles hancocki	-	+
Total	**S1= 3**	**S2= 6**

(+) = Present; (-) = absent; S = Number of species

Table 2: Abundance of mosquitoes in the prospected sites.

Sites / Species	Rice field breeding site		River breeding		The two sites	
	Effective	**Percentage**	**Effective**	**Percentage**	**Effective**	**Percentage**
Anopheles gambiae	4,569	66.5%	1,320	28.87%	5,889	51.47%
Anopheles funestus	1,917	27.9%	_	_	1,917	16.75%
Anopheles wellcomei	383	5.6%	2	0.04%	385	3.37%
Anopheles paludis	_	_	9	0.2%	9	0.08%
Anopheles ziemanni	_	_	8	0.17%	8	0.07%
Anopheles hancocki	_	_	2	0.04%	2	0.02%
Anopheles nili	_	_	3,232	70.68%	3,232	28.24%
Total	**6869**	**100%**	**4,573**	**100%**	**11,442**	**100%**

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%centage = Percentage

4.1 Anopheles aggressivity and seasonal variations in the Nde quarter: *A. gambiae and A. nili* were the main species captured (fig. 2a and 2b). During the study period, 3,924 adult females mosquitoes were captured inside the houses in the Nde area in 648-men nights. The species captured were *Anopheles gambiae and A. nili.*

The average aggressivity rate of the two species of anopheles was 6 bites per man per night. We can estimate at 2,190 the number of bites of the 2 species received by an individual on a yearly basis. Of the two species, *A. gambiae* appeared to be the most aggressive on man with 4.2 bites/man/night. This rate remains lower at 1 bites/man/night during the dry season which runs from December to April. There was a rapid increase in this rate (7 bites/man/night) in the month of April. With 9.4 bites/man/night of average aggressivity rate, the month of August has the highest possibility of nuisance of the population of the Nde area due to *A. gambiae.* The aggressivity densities of these species remained higher at 2 bites/man/night in September and November (Fig. 2a).

The daily aggressivity rate due to *A. nili* was 1.8 bites/man/night with a maximum of 3.4 bites/man/night in the month of May and a minimum of 0.1 bites/man/night in the month of January and February. This aggressivity remained low during the dry season with an average rate of 0.2 bites/man/night (Fig. 2b).The monthly variations of the aggressivity density of *Anopheles gambiae* and *A. nili* show a positive correlation with rainfall (r= 0.6110 ; p= 0.035 for *A. gambiae*) and (r= 0.6458, p= 0.023 for *A. nili*).

4.2 Anopheles aggressivity and seasonal variations in Bandounga: A total of 54,103 female mosquitoes were collected at Bandounga in 648 men-nights of capture. Anopheles represented 99.63% of this fauna. Apart from *A. gambiae* which represents 90.87% of Anopheles, *A. funestus* and *A. wellcomei* were equally captured.The daily average value of bites was 83.5 bites/man/night, an inhabitant of this quarter will then receive about 30,370 bites of mosquitoes annually, thus 14 times more mosquitoes than in the Nde area.

The daily average rate of biting due uniquely to *A. gambiae* was 75.6 bites/man/night (27,594bites/man/year) against 5 bites/man/night (1,825 bites/man/year) due to *A. funestus* and 2.6 bites/man/night (949 bites/man/year) due to *A. wellcomei.* The monthly variation of the aggressivity densities of *A. gambiae* shows a positive correlation with rainfall (r=0.6565; p=0.028). These densities remained very low from November to March, followed by a sharp increase from April to October when there is an average aggressivity rate of 122.8p/h/n (Fig. 3a). However, the high aggressivity rate of *A. funestus* was only registered in the months of September to November which corresponds to the period of heavy downpour (Fig. 3b)

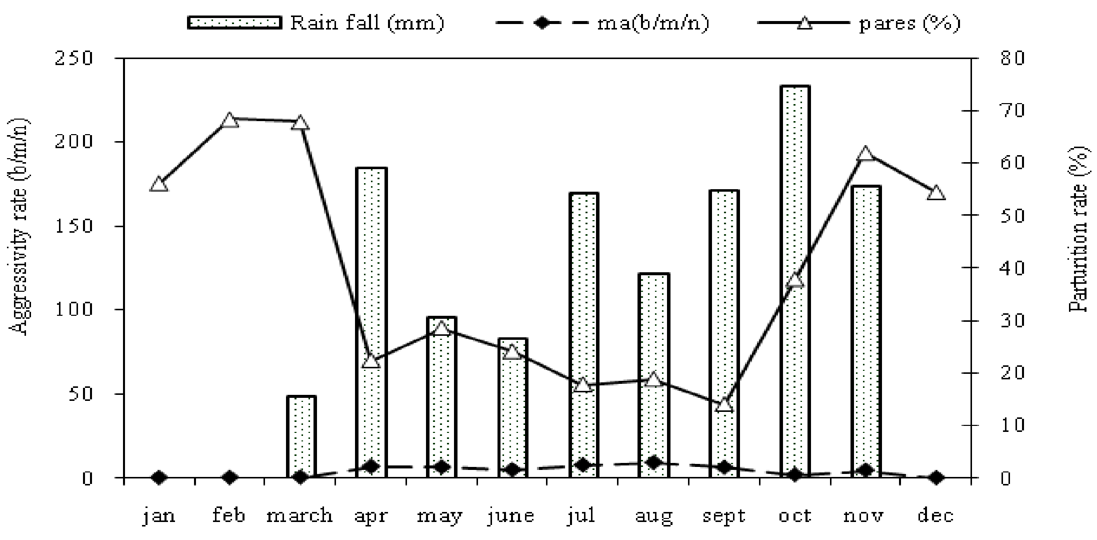

Figure 2a: Evolution of the rate of parturition and aggressivity of *A. gambiae* as a function of precipitation in

the Nde quarter

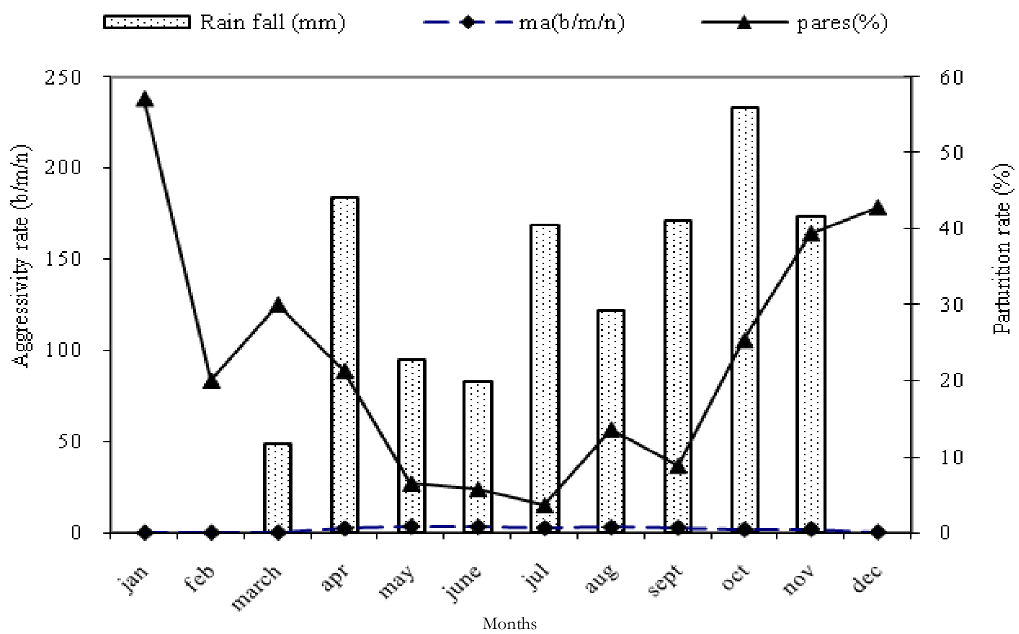

Figure 2b: Evolution of the rate of parturition and aggressivity of *A. nili* as a function of precipitation in the Nde quarter

4.3 Physiological age of females of *A. gambiae*, *A. funestus* and *A. nili*: The rates of parturition of A. *gambiae* and *A. nili* registered in the Nde area was 26.4% (n=696) and 14.9% (n=171), respectively. These rates are 12.4% (n=6,028) for *A. gambiae* and 18.24% (n=578) for *A. funestus* at Bandounga in the rice cultivating area. The rates of pare females in the two quarters and for the 3 species are high during the dry season and low during the rainy season (Fig. 2a, 2b, 3a, 3b). The monthly variability of the aggressivity densities show a negative correlation with the fluctuations of the rate of parturition of *A. gambiae* in the two sites (r= -0.8373; p= 0.001 for the Ndé area and r= -0.9189; p= 0.00001 for the Bandounga village).

4.4 Infectivity of the malaria vectors

4.4.1 Fluvial zone: The average sporozoitic index of A. *gambiae* registered in the Nde quarter was 1.2% (n=34) and 0.2% for *A. nili*. The females of infected *A. gambiae* were found during the whole year of study, except in the months of May and June. The rate of infection varied as a function of pluviometry. The maximal period of infection with *A. gambiae* spread from December to March (higher than 26%).There was a negative correlation between the monthly aggressivity and the infection rate (r= -0. 6626; p= 0.026).

4.4.2 Rice cultivating area: The annual sporozoitic index for *A. gambiae* registered in Bandounga is 0. 08% (n=38), 0.3% (n=11) for *A. funestus* and 1.4% (n=2) for *A. wellcomei*. The females of infected *A. gambiae* were encountered during the 12 months of the year. The maximum study period spread from December to March.

4.4.3 Rate of entomological inoculation (E.I.R) and the transmission of Malaria at Bandounga: At Bandounga, the transmission of malaria is assured by 3 anopheles species: *A. gambiae, A. funestus* and *A. wellcomei*. This transmission runs throughout the entire year. *A. gambiae* alone was observed to be responsible for 82.19% of transmission, with an average rate of 0.06 infected bites/man/night (bi/m/n) giving 21.9 infected bites/man/year. Globally, the transmission due to the 3 anopheles species is estimated at 0.073 infected bites/man/night giving 27 infected bites/man/year. There is a low level of transmission throughout the year, with a slight increase during the period from December to March (Fig.5).

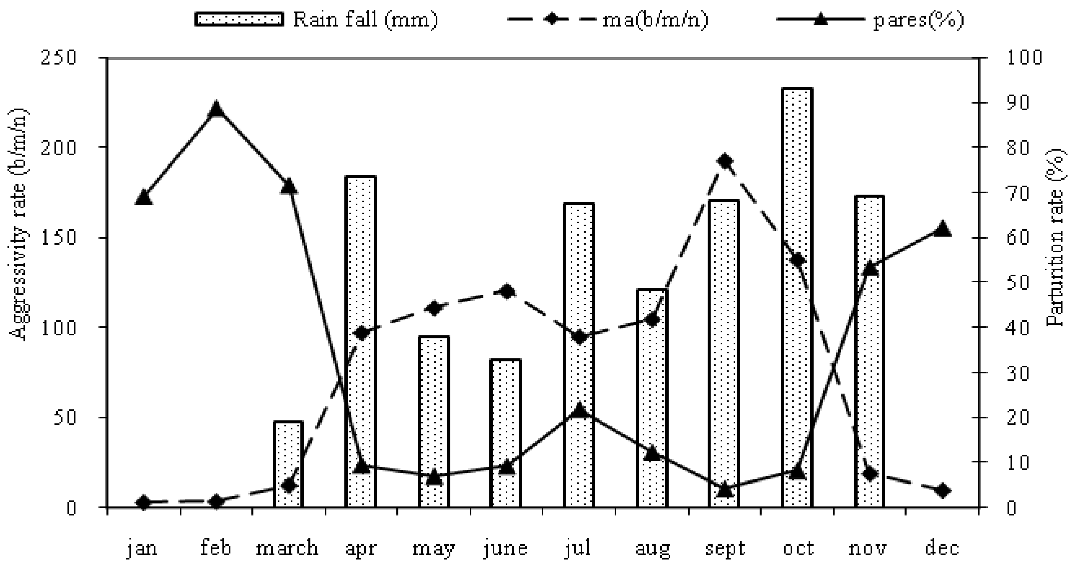

Figure 3a: Evolution of the rate of parturition and aggressivity of *A. gambiae* as a function of precipitation in the Bandounga quarter

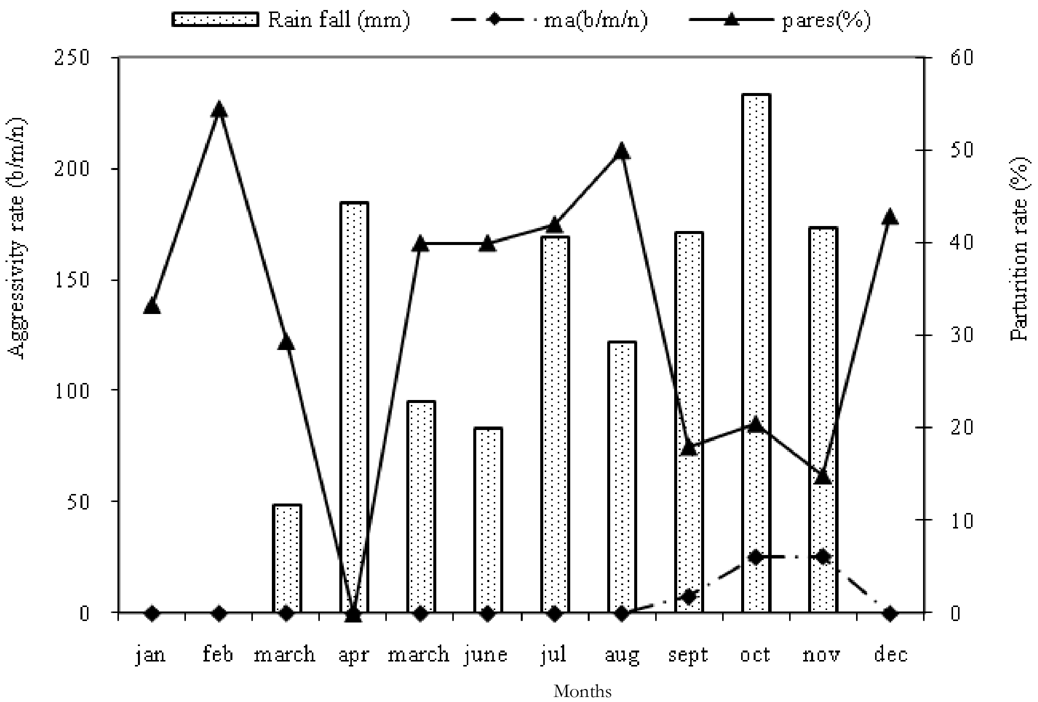

Figure 3b: Evolution of the rate of parturition and aggressivity of *A. funestus* as a function of precipitation in the Bandounga quarter

4.5 Fluvial Zone: The transmission of malaria is assured by *A. gambiae* and *A. nili* in the Nde quarter. *A. gambiae* is responsible for 90.9% of the transmission. Each person in the area receives an average of 0.05 infected bites/night, thus 15 infectious bites that were spread for ten months. Three salivary glands of the *A. nili* females contained sporozoites, one in January, and the 2 others in July and December. The transmission of malaria as a result of the 2 Anopheles mosquitoes is estimated at 0.055 infected bites/man/night thus 16.5 infectious bites in 10 months. This transmission is significant in the months of December to March (Fig. 4).

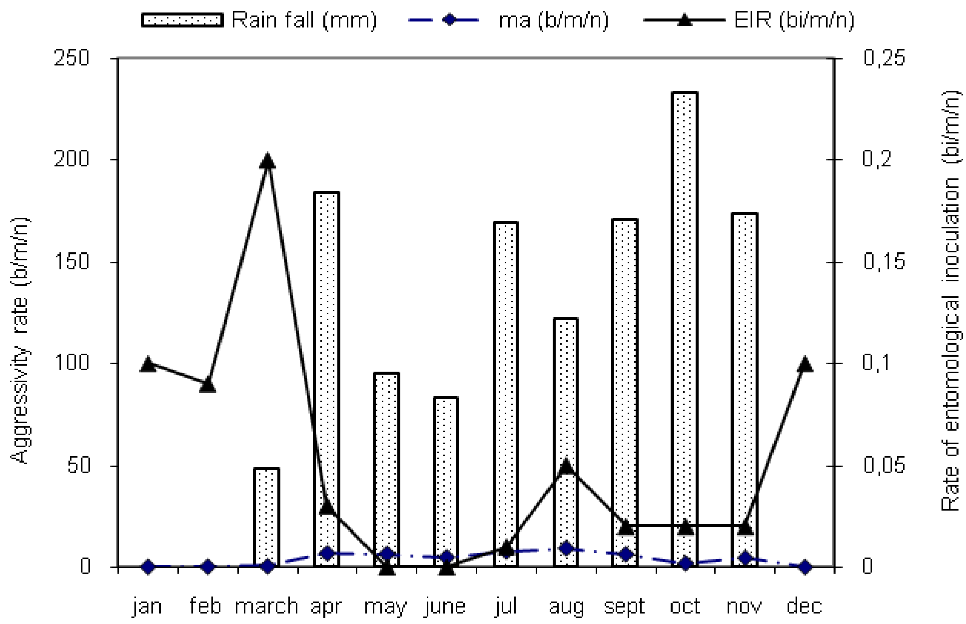

Figure 4: Evolution of the rate of entomological inoculation and aggressivity of *A. gambiae* as a function of precipitation in the Nde quarter

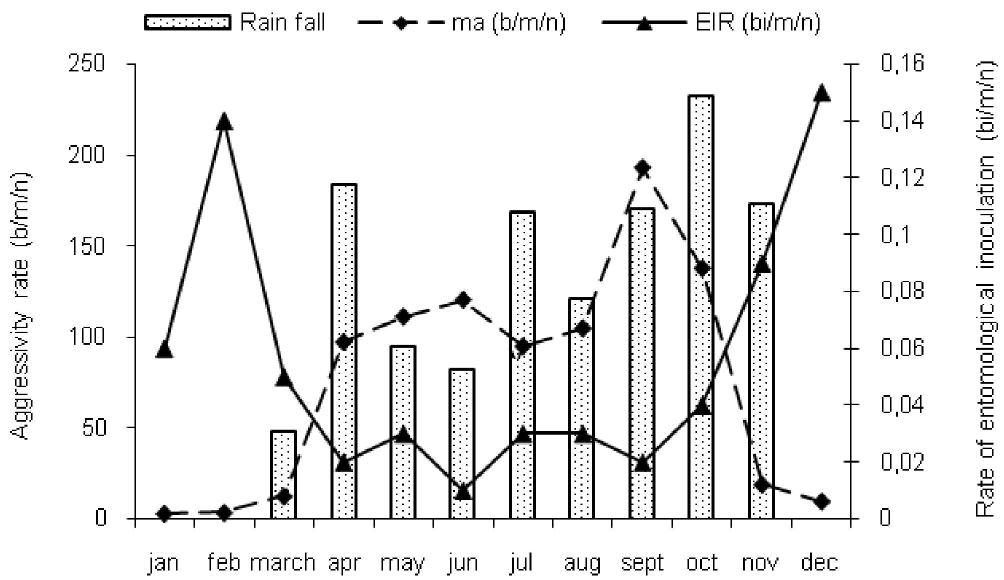

Figure 5: Evolution of the rate of entomological inoculation and aggressivity of *A. gambiae* as a function of precipitation in the Bandounga quarter

5 DISCUSSION

This study that was carried out in Tonga reveals the rich and diversified culicid fauna in the Western region of Cameroon. The transmission of malaria is low but permanent and it is mostly transmitted by *Anopheles gambiae*, *A. nili* and *A. funestus*. The presence of these 3 species, which are considered as the most important vectors of malaria in tropical Africa, (Adja *et al.,* 2006) prove that the Tonga climate constitutes a barrier to the transmission of malaria species or the proliferation of mosquitoes. Similar findings were recorded by Tchuinkam *et al.* (2003) in a town located at 1,400m altitude in West Cameroon. He described 8 anopheles species in Dschang, in which 3 were responsible for the transmission of the illness. The existence of transmission in Tonga could be explained by the presence of climatic conditions that are favourable for the survival of the vector and the parasite. During the study period, the average yearly temperature was 21.6°C. This value is higher than 19°C which is considered as the temperature below which the parasites have low chances of survival and propagation (Philippe *et al.*, 1995).

Our entomological inquiry also showed that the specific richness of the collected mosquitoes, their abundance and the monthly fluctuations of their larval and adult densities are dependent on the environmental and ecological characteristics that are inherent to each site assessed (Dossou-yovo *et al.*, 1998).

Culicid fauna in the Nde quarter originates mostly from the Anopheles genera (Fig. 2a, 2b and Table 1). In areas where the swampy rice fields are nonexistent, *Anopheles gambiae* recorded in our results come mainly from temporal water spots which are lowly charged in organic matter. These breeding grounds of *A. gambiae* are similar to those described by Mouchet *et al.,* (1961). This makes us believe that the adults of these species captured on volunteers arise from the Nde river. The seasonal fluctuations of the population of these two species of Anopheles are in line with the functional dynamics of their respective larval breeding grounds (Brengues *et al.*, 1973).

The densities of *Anopheles gambiae* remain rare from the dry season which runs from December to March and increase significantly from the beginning of the rainy season (April) (Fig. 2a, 3a). This is explained by the fact that the breeding ground of the *A. gambiae* considerably increases at the beginning of the rainy season. This is due to input of water into the dry pools. The pot holes leads to the creation of water ponds which receive direct solar radiation and thus constitute very suitable breeding grounds for this species. The spacing of rainfall can also constitute a favourable factor for the breeding of larval forms during the rainy season and enables favourable multiplication of mosquitoes during the months of July and August (fig. 2a).

The tendency for zoophily and exophagy of some species of the group of *Nili* such as *Anopheles somalicus* (Fontenille *et al.*, 2003) enabled a biased appreciation of their seasonal fluctuations, the sampling being carried out mostly on human subjects and inside houses. Despite this, the period of high proliferation of *A. nili* which spreads from April to September (Fig. 2b) depends mostly on the depth of the Nde river as postulated by Adja *et al.*, (2006) in Gansé, Côte d'Ivoire. The imago and larval densities of *A. nili* increase with respect to the rainfall and the storm water running into the river. The biocenology of *A. wellcomei*, A. *hancocki, A. ziemanni and A. paludis* remains unclear but they play important roles in transmission. However, due to the fact that their larva was collected from the Nde River, we can conclude that their biocenology is similar to that of *A. nili.*

In the Bandounga rice field, six species bite men, among which are Anopheles species. It stands out clearly from these studies that there are about 13.5 times more mosquitoes in Bandounga than in the Nde area. These results are in line with those of Dossou –Yovo (1998) in Bouake and confirm the rice field as the ideal medium for the proliferation of Anopheles. The annual fluctuation of Anopheles is not correlated to the rhythms of rainfall as it is to the evolution of rice field ecosystem. In effect, due to the cultural cycle, the rice field is a medium in which there is a succession of many biotopes (Speelman et al., 1986); each type of biotope created enabling ecological conditions for the multiplication of a particular type of species. This is why the densities of *A. gambiae* present a higher density in the month of September (Fig. 3a) which corresponds to the germination of the young rice plant. The constituted biotope leads to the proliferation of *A. gambiae* (heliophilic specie), at the detriment of the larva of *A. funestus* which is more susceptible to shady breeding grounds (Hamon *et al.,* 1956; Brengues and Coz, 1973). Meanwhile the growth of rice plants which follows will progressively put in place the shady breeding which is favorable for the larva of *A. funestus*.

The transmission of malaria even though it is low, remains permanently assured and in a permanent manner by *A. gambiae* in the two areas studied. *A. nili* and *A. funestus* are equally implicated in the transmission in a residual manner. These observations are in line with the findings of most of the rice fields of tropical Africa in which *A. gambiae* is known as the major vector of malaria (Dossou-yovo, 1998). The transmission pathway for malaria in the two areas of this subdivision is almost identical and it follows a seasonal pattern. It commences from the beginning of the dry season to the end of November and remains very high towards the second half of the season with a very high infectivity of the population of *A. gambiae* and lower levels of mosquitoes bites (Fig. 4 and 5). It drastically reduces when the aggressivity of *A. gambiae* increases (Fig. 4 and 5).

This can be explained by the fact that during the rainy season, the renewal of the population is very important; this reduces the rate of Anopheles infected in the general Anopheles community. Meanwhile this renewal is lower during the dry season; this increases the average age of the anopheles population and the possibility of encountering infested anopheles (Fig. 4 and 5). The average global rate of entomological inoculation (taking the entire anopheles population into consideration) obtained at Tonga is 0.12 infected bites/man/night. This rate expresses the hypo endemic level of transmission and reveals that the establishment of a rice field in Bandounga, at high altitude leads to an increase in the Anopheles densities, without a corresponding modification of the transmission of malaria. This situation is compatible with the analysis of Robert *et al.* (1986) in the Kou valley of Burkina Faso.

The people of Tonga remain exposed to a permanent pressure of malaria. The data obtained through this study is very useful and should therefore be taken into consideration so as to ensure an efficient anti-vectorial campaign of malaria in the study site.

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