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Diversity of Culicidae, determination of entomological parameters of the transmission of *Plasmodium* spp. In Maga, Far North Cameroon

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Abstract
From March to October 2015, an entomological study based on the diversity of culicidae and the determination of entomological parameters of the transmission of *Plasmodium* parasite was carried out in three quarters of Maga Subdivision. A total of 6469 mosquitoes were collected at the moment of a blood meal, the Anophelinae (3393/6469) presented a high number of 2431 *Anopheles gambiae* (37.58%) and 962 *Anopheles funestus* (14.87%). The anthropophilic culicinae (3076/6469) consisted of 2332 *Culex quinquefasciatus* (36.04%) and 744 *Aedes aegypti* (11.5%). During the period of study, the general rate of aggressiveness in Maga was 67.38 bite/man/night with 35.34 bite/man/night for Anophelinae and 32.04 bite/man/night for Culicidae. On the 3227 ovaries obtained from dissected female Anopheles, 2027 presented parous ovaries with an annual medium rate of parturity of PR = 62.81%. More than half of the adult of the population of *Anopheles* being parous, makes it possible to declare the locality of Maga as a risky malaria transmission area.

Keywords: Culicidae diversity, entomological parameters, transmission, *Plasmodium*, Maga, Cameroon

1. Introduction
In the World, the number of malaria cases in 2015 is estimated at 214 million, resulting in the death of 438 000 people, among them a high percentage of young children (78%) living in sub-Saharan Africa (OMS, 2015) [25]. In Cameroon, the mortality rate due to malaria remains high with an average of 4000 deaths in 2012 (OMS, 2014) [26]. Far North and North regions are the most affected with 40% of deaths (Minsanté, 2014) [22]. In Maga, the construction of the dike is the cause of the permanent existence of breeding sites and the multiplication of mosquitoes, contributing to the outbreak of malaria in the area (Koudou *et al*., 2005) [16]. If the rice-growing component is very important, the control of the vector is unavoidable in a global fight against malaria (WHO, 2013) [32]. In fact, vector control is based on a better knowledge of the vector (Fournet *et al*., 2010) [9]. In the locality of Maga, the lack of data on the entomological study is a handicap in the elaboration of strategies for an efficient fight. The overall objective of this work is to contribute to the fight against malaria by an evaluation of the main entomological parameters directly involved in the transmission of the *Plasmodium* parasite to man. The specific objectives consisted in:

- making an inventory of the anthropophilic Culicidae fauna of Maga;
- dissecting the abdomens to determine the physiological age of female *Anopheles*;
- Calculate entomological evidence from the dissected organs to assess the magnitude of its transmission in Maga town.

2. Materials and Methods
2.1 Presentation of the study site
Maga sub-division is located in the Far North Region Cameroon, Mayo – Danay Division, between 10 ° 32'57 " - 11 ° 58'00 " North latitude and 14 ° 34 '- 15 ° 10'13' East longitude (Leumbe *et al*., 2015) [18]. Its climate is sudano-sahelian, characterized by a long dry season that begins in October and ends in April (Leumbe *et al*., 2015) [18]. Rainfall varies between 530 to 630 mm/year with maxima in August (250 mm) and Septembe

The average temperature is 28 °C; the warmest months are March, April and May with a maximum value of 32 ° C. The floristic groupings are made up of a shrubby savanna (weakly wooded), herbaceous steppes with very marked Grass (Poaceae) in floodplains and sparse vegetation in degraded areas (Milleville & Serpantie, 1994) [21]. On a geological scale, recent and old alluvial deposits of varied nature occupy the entire area and on a pedological level there are poorly developed soils, vertisols, and tropical ferruginous soils, hydromorphic soils and halomorphous soils (Seignobos & Moukouri, 2000) [31]. On the human level, the population is made up of the Massa, Toupouri, Musgum, Mousey, Peulh, Kanuri, Kéra, etc. The main activities are agriculture, livestock and fisheries (Seignobos & Moukouri, 2000) [31].

2.2 Methodology

2.2.1 Inventory of the Culicidae fauna

From March to October 2015, mosquitoes were captured on human subjects according to the method of Le Goff *et al*. (1992) [17]. Mosquitoes catchers were put under drug prophylaxis by taking Coartem® before they were introduced into the capture houses. The catching was carried out in 8 months, four months in the dry season and four months in the rainy season. During the survey, three out of five quarters were selected to perform nightly catching. These were Madalam (Abattoir), Gamah and Sirata (Maga Nord II) quarters. The choice of quarters obeyed a number of criteria, namely the proximity of permanent breeding sites, the number of people living in the houses, the populous quarter character and the willingness of household heads to accept catchers in their homes. In each of the three quarters, 4 capture houses were selected; corresponding to 4 men-nights or capturers. This makes a total of 12 capture houses. The capture rate was one passage per month, or 8 outputs in total. It makes up 4houses x 3quarters (=12catchers) x 8months x 1outputs = 96men-nights. Capture houses remained fixed and unchanged until the end of the work. Catchings were carried out from 7 pm to 5 am.

2.2.2 Method of capture on human subjects

The catchings were made according to the method of Le Goff *et al*., 1992[17]. The catchers seated in breeches inside the house of capture, lights his lamp on his legs to see if a mosquito has landed to feed itself on blood. If a mosquito is detected, it is captured using a glass test tube closed with a cotton wool once inside.

2.2.3 Morphological identification of mosquitoes

The morphological identification of the mosquitoes was done in the Laboratory of Life and Earth Sciences of the Higher Teachers' Training College of the University of Maroua using binocular loupes and electric microscopes with X10 and X40 objectives. Identification keys by Gillies and De Meillon (1968) [12] and Gillies and Coetzee (1987) [11] were also used to separate the female *Anopheles* mosquitoes from the others. Identification keys by Edwards (1941) [7] and Jupp (1996) [14] were used for Culicinae. The illustrated key of Holstein, (1952) [13] to determine the morphological characters of adult *Anopheles gambiae* was used.

2.2.4 Dissection of female anopheles caught on human subjects

After identification, unopened female living anophelinae were separated from each other for dissection at the level of their abdomen. Dissections were carried out in the early hours of the morning (between 6 am and 10 am) according to the method of Russel *et al*. (1963) [28]. The *Anopheles* is knocked down inside the test tube, then placed back side down on the slide with the lap portion placed at the edge of a drop of distilled water, a section was made between segments 6 and 7; the *Anopheles* is held at the trunk with the left hand, and the terminal part of the abdomen is pulled down by the right hand.; released ovaries were placed on a microscope slide in a drop of distilled water and the whole was left to dry for at least 6 hours. The ovaries, completely dried, are examined in the electronic microscope with either X10 or X40 objective. The observation in the microscope easily distinguish ovarian structures nulliparous and parous. A nulliparous female presents tracheoles ovarian curled and those of female ovary barrier consist of scalp tracheoles distended network.

2.2.5 Entomological calculated indexes

- The biting rate (BR) is the average number of mosquito bites received by an individual per unit of time. It is calculated by the formula of (MacDonald, 1957) [19]: BR = number of mosquitoes stinging a man / total number of catchers.
- The Parturity rate (PR), which is the ratio of parous throughout parous and nulliparous; it is calculated by the formulas of Denitova (1963) [5]; it is expressed in percentage.
- $\mathrm{PR} = \mathrm{P}/\mathrm{NP}\ \mathrm{X}100$, with P = number of parous and NP = number of nulliparous females.
- The Survival rate (P): this is the probability of daily survival female *Anopheles*. It is obtained by the formula of Davidson (1954) [4]: $\mathrm{P} = \sqrt[\ell]{\mathrm{P}/(\mathrm{P}+\mathrm{NP})}$ ℓ = being the duration of the gonotrophic cycle (3 days).
- Life expectancy (LE): this corresponds to the average length of life of individuals. It is calculated using MacDonald's formula (1957): $\mathrm{LE} = 1/{-\log_{e}}\mathrm{P}$, where p is the daily survival rate.
- Vectorial Capacity (VC): The vectorial capacity represents the rate of potential infectious contacts (or daily spread of malaria) between individuals through the *Anopheles* vector (Garret - Jones & Schidrawi, 1969) [10]. The vectorial capacity is also defined as "the daily rate of a future inoculations initiated by each infected case."
- It is calculated using the formula: $\mathrm{VC} = (\mathrm{BR}^2).\mathrm{P}^n/{-\log_{e}}\mathrm{P}$
- Stability index (Si): this is a parameter that allows the assessment and the classification of malaria endemicity in a given area. It is calculated using the following formula (Macdonald, 1957) [19]. $\mathrm{Si} = \mathrm{B}/{-\log_{e}}\mathrm{P}$ B= number of persons bitten per day; P = survival rate.

Areas are classified according to the following criteria:

- stable malaria zone: Si> 2.5;
- unstable malaria zone: Si <0.5;
- intermediate zone: 0.5 ≤ Si ≥ 2.5.

2.2.6 Data analysis

- descriptive statistics to calculate averages, standard deviations and percentages
- Z-test and Student's t-test to compare two averages
- Chi - square ($\chi 2$) to compare percentages
- Pearson correlation coefficient (r) to evaluate the degree of relationship between two parameters

Excel 2007 software to make graphics For all analyzes, $P < 0.05$

3. Results and Discussion

3.1 Abundance of adult mosquitoes caught in Maga

At the end of the investigation, 6469 adult mosquitoes of all species were captured on human subjects. These mosquitoes belong to two subfamilies: Anophelinae (3393, 52.45%) and Culicinae (3076; 47.55%). A non significant difference (X^2= 0.2626, ddl = 1) was recorded between the proportions of the individuals of the two subfamilies. These results are different from those recorded by Akono (2005) [2], who collected at Nkolbisson in Yaoundé 62.48% of Anophelinae versus 37.57% of Culicinae. The considerable proportion of individuals in the subfamily of Anophelinae in Maga is explained by the presence of suitable deposits for the development of larvae (collection of clear water, calm, sunny and less rich in organic matter). The Anophelinae are made up of *Anopheles gambiae* (2431, 37.58%) and *Anopheles funestus* (962; 4.87%); While the Culicinae are made up of 2332 *Culex quinquefasciatus* (36.04%) and 744 *Aedes aegypti* (11.5%). A non-significant difference (X^2= 0.5628, ddl = 3, p> 0.05) was observed between the four species.

The predominance of the genus *Anopheles*, in particular *A. gambiae*, can be explained by the influence of rice traps which offer shallow water points (a few centimeters deep), calm, sunny and clear. The same observation was made by Konan *et al*. (2008) [15] in Tiassalekro, an irrigated rice growing village in the southern forest zone of Côte d'Ivoire. The high numbers of *Anopheles gambiae* individuals can be justified by the proximity of the dwelling houses selected for captures. Robert *et al*. (2003) [27] showed that *Anopheles* have a small radius of activity in areas with a high human population density considering the availability of the blood meal after their emergence in their immediate environment. The presence of *Culex* is linked to the level of environmental pollution and to the absence of a drainage system of wastewater. Similar observations were made by Diallo *et al*. (2000) [6] in Dakar, Senegal. Because of its mainly diurnal activity, the mosquito of the *Aedes* genus is weakly represented (11.26%). Adult mosquitoes are caught both in the dry season (3367, 52.04%) and in the rainy season (3102, 48%). A non-significant difference was observed between the number of individuals in both seasons (t = 0.3805, ddl = 1, p> 0.05). This could be explained by the presence of water in all seasons in Maga due to the existence of the lake.

3.2 Cucicidae biting rate (BR)

The Culicidae aggressiveness average rate in Maga was BR = 67.38bite/man/night with 35.34bite/man/night for Anophelinae and 32.04bite/man/night for Culicidae. A non-significant difference (t = 0.59; ddl = 1; p> 0.05) is noted between the two rates of culicidae aggressiveness. However, the Anophelian aggressiveness is higher than that of the Culicinae. This is due to irrigation, which changes the dynamics of anopheline populations. This situation confirms the remark made by Faye *et al*. (1993) [8] who conducted a study on the entomological aspects of malaria transmission in Senegal and showed that the aggressive density of *Anopheles gambiae* is 7 to 16 times higher in the rice-growing area than in other areas of rainwater cultivation. The Spermann test revealed a weak negative correlation (r = -0.12) between aggressive densities and rainfall. This means that the aggressiveness in Maga is not related to the precipitation regime, but rather to the presence of the lake that irrigates the rice fields which are close to the dwelling houses. In Cotonou-Benin, Akogbeto *et al.* (1992) [1] found a strong positive correlation between precipitation and the aggressive density of mosquitoes. On the other hand, the importance of the swamps and the clay structure of the soil of Maga can prolong the duration of some temporary shelters. During the dry season (March, April, May and October), the average aggressive rate is 70.14bite/man/night with 36.81bite / man / night for Anophelinae and 33.34 bite / man / night for the Culicinae. Whereas, during the rainy season (June, July, August and September), the average aggressive rate is 64.62bite/man/night divided into 33.87 bite / man / night for Anophelinae and 30, 74bite/man/night for Culicinae. A non-significant difference (t = 0.98, ddl = 6, p> 0.05) was recorded between seasons. Whereas, the dry season showed a high rate of aggressiveness compared to the rainy season. The low rate of aggression in the rainy season is due to the leaching phenomenon as reported by Njan Nlôga (1994) [24] in Ebogo in Southern Cameroon.

3.3 Parturity rate (PR)

Out of 3227 ovaries of female of *Anopheles* dissected, 2027 had parous ovaries, an average parturity of PR = 62.81%. More than half of the adult of the population of *Anopheles* being parous, makes it possible to declare the locality of Maga as a risky malaria transmission area. In the rainy season, the average rate of parturity is 31.42% and in the dry season it is 31.39%. A non-significant difference (t = 1.44; ddl = 6; p> 0.05) was observed between the two seasons. However, there was a slight predominance of the parturity rate in the rainy season. The stability of larvae breeding sites and the absence of leaching phenomenon at the beginning and at the end of the rainy season are necessary factors for the regular development of mosquito larvae (Saotoing *et al*., 2011) [30]. A non-significant correlation (r = 0.47) was recorded between the parturity rate and rainfall. Whereas, between the number of *Anopheles* caught and the parous ovaries, the correlation is very highly significant (r = 0.98). This means that female of *Anopheles* are aggressive and possibly capable of transmitting plasmodium. A similar fluctuation was observed in the forest zone of South Cameroon by Njan Nl6ga (1994) [24] who found parturity rates oscillating between 45.3% at the beginning of the small rainy season and 72.8% at the end of the rainy seasons. Faye *et al*. (1993) [8] revealed very high parturity rates in Senegal of about 70% in *Anopheles gambiae.*

3.4 Survival rate (P)

In general, the daily survival rate of the adult population of *Anopheles* in Maga calculated using the Davidson formula (1954) are P = 0.84 in the dry season and P = 0.86 in the rainy season. A non-significant difference (χ^2 = 0.00023; ddl = 6; p> 0.05) was noted between daily survival rates for both seasons. *Anopheles funestus* recorded a survival rate of 0.83 and 0.87 in the dry and rainy seasons, respectively. A non-significant difference ($\chi2$ = 0.00094, ddl = 6, p> 0.05) was observed between daily survival rates of the two seasons. Carnevale *et al*. (1992) [3] have carried out a study in the forest zone of Cameroon and obtained a daily survival rate of *Anopheles gambiae* of P = 0.85. The average daily survival rate for *Anopheles gambiae* and *Anopheles funestus* in the town of Maga is 0.85. This rate approaching 1 is then high enough to maintain the anopheles alive to meet the preferred host who is the Man.

3.5 Life expectancy

Overall, the life expectancy in Maga was 18 days, which is high compared to that found by Metelo *et al*. (2015) [20] in the Democratic Republic of Congo (16.4 days). This difference is due to climatic and ecological conditions very favorable to the development of *Anopheles* in Maga. As concerning the *Anopheles gambiae* the average annual life expectancy is 18.46 days during the study period. Distributed according to the seasons, it is 17.65 days in the dry season and 20 days in the rainy season. A non-significant difference (χ^2 = 0.15, ddl = 6, p> 0.05) was observed between the life expectancies of both seasons. In Maga, the female *Anopheles* have the tendency to live longer during the rainy season than during the dry season.

3.6 Vectorial Capacity

In Maga, the daily vectorial capacity is 14. This means that *Anopheles* could theoretically be responsible for 14 new infections every day in the absence of protection against the bites of female *Anopheles*. In Maroua, Saotoing (2017) [29] obtained a daily vectorial capacity of 0.15 at *Anopheles gambiae*, which is by far less than that found at Maga. The difference in numbers of cases of malaria infection could may be linked to the higher biting rate in Maga than in Maroua. It is by far superior to that obtained by Njan Nlôga (1994) [24] in the forest region of southern Cameroon where 7 new infections every day due to *Anopheles moucheti* have been recorded. Overall, the potential of infection is quite high, placing Maga in a situation of high risk of malaria transmission.

3.7 Stability Index

During the study period (March to October 2015), the average stability index of malaria in Maga calculated according to the Macdonald formula (1957) was Si = 1.84 with 1.76 and 2.15 in the dry season and in the rainy season. These values are used to classify the city of Maga town as intermediate transmission zone. The stability index is higher in the rainy season than in the dry season. Mouchet *et al*., (1961) [23] have shown that malaria transmission is high during the rainy season and low or zero during the dry season in the Sahel regions.

4. Conclusion

The adult and anthropophilic culicidae fauna of the locality of Maga consists mainly of Culicinae and Anophelinae. The Culicinae are mainly represented by *Culex quinquefasciatus* and *Aedes aegypti*. The Anophelinae are mainly made up of *Anopheles gambia* and also *Anopheles funestus*. The average rate of Anophenine aggressiveness was 67.38bite/man/night with 35.34bite/man/night for Anophelinae and 32.04bite/man/night for Culicidae. The variation in aggressive densities was inversely proportional to rainfall. The average annual rate of parturity is PR = 62.81%. More than half of the imaginative population of *Anopheles* being a barrier, this makes it possible to declare the locality of Maga a risky area of malaria transmission. The average daily vectorial capacity in Maga is 14 new cases of malaria infection per day. The average annual malaria stability index in Maga is Si = 1.84 classifying the Maga locality as an intermediate transmission zone. In the coming days, a biomolecular study of the Anophelian populations in the city of Maga should be conducted in order to know their biodiversity and to determine the mechanisms involved in resistance to insecticides.

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