Typology of Breeding Sites and Species Diversity of Culicids (Diptera: *Culicidae*) in Akanda and its Environs (North West, Gabon)

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ABSTRACT

The typology of larval habitats and the species diversity of culicids were conducted in the Akanda and its environs by collecting mosquito larvae and nymphs present in water collections, from May to June 2019. A total of 334 breeding sites of which 158 positive (47.31%) and 176 negative (52.69%) were identified. Water storage media such as: barrels (n = 95; 28.44%), puddles (n = 73; 21.86%), ponds (n = 47; 14.07%), tires (n = 31; 9.28%) and drains (n = 22; 6.59%) were the most frequent types of breeding sites. In addition, 1 549 mosquito larvae were collected, of which 59.39% belong to the sub-family Culicinae and 40.61% belong to the sub family Anophelinae. After rearing, 790 adults were obtained and divided into 8 species, including *Aedes albopictus*, *Aedes aegypti*, *Anopheles gambiae* s.l., *Anopheles pharaensis*, *Culex tigripes*, *Culex decens*, *Culex quinquefasciatus*, and *Mansonia uniformis*. Although preliminary, this information on the culicid fauna could be useful for the implementation of appropriate control strategies in the study area. Likewise, the presence of *Anopheles gambiae* s.l. in this area requires that additional studies on its resistance to insecticides be conducted as recommended by the World Health Organization.

Keywords: Typology, breeding sites, species diversity, mosquitoes, Akanda, Gabon

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I. INTRODUCTION

Malaria remains by far the most widespread parasitic disease in the intertropical world, and particularly in sub-Saharan Africa [1,2]. According to the World Health Organization (WHO), malaria affected nearly 228 million people worldwide in 2019 compared to 219 million in 2017 with 405,000 human losses [3]. Among the deaths attributable to this parasitosis, 67% (272,000) involved children under the age of 5 (WHO, 2019). In addition, Africa remains the continent most affected by this disease with 93% (213 millions) of malaria episodes and 94% of deaths [3].

Besides this parasitosis, arboviruses such as dengue, chikungunya, yellow fever and Zika fever, threaten 831 millions people worldwide, including 70% of the African

population [4]. These diseases represent a major scourge to the health of human populations [5], [6], [7].

In Gabon, vector-borne diseases represent public health problems [8, 9]. Referring to the National Health Information System (NHIS), malaria was the cause of 153,666 cases of consultation and 160 deaths recorded in health facilities in 2017 [10]. According to Leroy *et al.* [11], the 2007 Chikungunya epidemic caused nearly 20,000 cases in the country.

Also, these diseases, notably malaria, appear as one of the main obstacles on economic development in Gabon with relatively high cases of death and associated school and professional absenteeism [12]. The fight against these vector-borne diseases is essentially based on the control of vector populations. Indeed, vector control is one of the effective

approaches to reduce the incidence of a disease in endemic areas. However, the effectiveness of this method requires an up-to-date entomological database. Indeed, the work conducted by Akono *et al.* [13] has shown that the establishment of an effective vector control method requires, for a given eco-climatic aspect, a good knowledge of the ecology of the vectors, in particular the identity and the bio-ecology of the vector mosquitoes and the characterization of their breeding sites. However, such knowledge is not available in the majority of localities in Gabon.

It is in this context that a preliminary entomological survey was conducted in Akanda and its environs, located in a degraded forest area, at 30 km from the city of Libreville, capital of Gabon. This town, created in 2013, has been subject to a significant urban dynamics associated with an increase in population. The installation of infrastructures and the construction of new human dwellings have strongly modified the natural landscape of this locality thus causing important disturbances among the populations of vectors.

The purpose of this study was to identify the different types of mosquito breeding sites, to determine the species diversity of Culicidae and to characterize the larval breeding sites of these insects in Akanda and its surroundings.

II. MATERIALS AND METHODS

A. Study Area

This study was conducted from May to June 2019 (rainy season) in the city of Akanda (0° 30'00" N; 09° 30'00" E), a sub-urban area located in the northern periphery of the city of Libreville, capital of Gabon (Fig. 1). This area was declared as a municipality in 2013. Akanda covers an area of 45,482 hectares and is part of the département du Komo-Mondah, in the province de l'Estuaire. It includes two boroughs divided into 15 districts [14]. There are several socio-economic activities including trading, sports, health, education, and tourism.

Fig. 1. Map of the study area.

From a climatic point of view, Akanda has an equatorial climate, with humidity between 85 % and 100 % in the rainy season [15]. It is marked by an alternation of four seasons, namely: a short dry season (from December to January), a long rainy season (from February to May) with high rainfalls, a long dry season (from June to September) and a short rainy season (October to November). This area experiences heavy rainfall with nearly 2,000 to 3,800 mm on average per year [15]. The average annual temperature is 26 °C [15].

At the topographic level, Akanda is characterized, as a whole, by a flat relief with a predominance of plains. It lays on an uneven oro-hydrographic complex, endowed with multiple hills and valleys crossed by numerous rivers in the estuaire du Komo river [15]. In addition, the Akanda area has diversified flora and fauna colonizing the coastal forests and mangroves of Pongara National Park, Akanda National Park, and the Raponda Walker Arboretum. The vegetation in this area is deeply threatened by the current urban extension. According to the 2013 General Population and Housing Census (GPHC), this city has about 34,548 inhabitants distributed unevenly [16].

B. Identification of Breeding Sites in the Study Area

Mosquito breeding sites were surveyed in several places in Akanda, including: Marseille Mveng-Ayong, Premier Campement Makwengue, Cap-Estérias, Sablière, Avorbam, Angondjé Centre Amissa, Cap Caravane, Entraco, and Okala. The choice of these sites was guided by their ecological profile and the structuring of their landscape. These surveys were conducted during the rainy season, from May to June 2019. The survey consisted of direct observation of the presence or not of the pre-imaginal stages of mosquitoes [17]. All breeding sites likely to contain immature stages of mosquitoes were geo-located using the Garmin GPSMAP 62st device [18]. Other information concerning the identified breeding sites (type of water collection, origin of the water, presence of larvae or nymphs of mosquitoes) was noted in order to better characterize them.

C. Sampling of Immature Stages of Mosquitoes

Mosquito larvae and nymphs were collected during the study period from the different sites chosen, using the "dipping" method [17], [19], [20]. Each sample was transferred to labeled trays, using 300 ml entomological pipettes. Subsequently, all the trays were put in coolers and transported to the insectarium of the Vector Ecology Laboratory (LEV) of the Research Institute in Tropical Ecology (IRET-CENAREST) for the rearing.

D. Rearing Of Mosquito Larvae and Pupae

In the laboratory, the different larvae collected were sorted and counted by sub-family (*Anophelinae* or *Culicinae*). They were then raised in plastic trays placed on the palliase and covered with a piece of mosquito net to avoid possible egg-laying of other mosquitoes and the escape of adult mosquitoes in case of emergence. These larvae were fed every day with fish food (*Sera Vipagran*) [21]. The water in each tray (the water from the original breeding sites) was changed once every three days to avoid death by asphyxiation of the larvae further to the decomposition of food.

In addition, all the nymphs obtained were sampled daily and placed in 0.25 l plastic cups containing water from the breeding sites. These cylindrical cups were placed in emergence cages covered with a mosquito net. The adult mosquitoes obtained from these nymphs were killed using an insecticide spray (Baygon) for their morphological identification.

E. Morphological Identification of Adult Mosquitoes

The adult mosquitoes that emerged from the nymphs were identified on the basis of the morphological criteria using a dissecting microscope and the taxonomic key of Central African mosquitoes by Baldacchino and Paupy [22], and the ORSTOM software for *Culicidae* identification.

F. Data Analysis

Data on breeding sites, larvae and adult mosquitoes were entered and organized in the Excel 2013 spreadsheet. This spreadsheet allowed us to calculate the numbers and percentages of adults and immature stages of mosquitoes, and to produce tables and graphs. Moreover, the GPS points of the study sites were integrated into ArcGIS 10.2 for the elaboration of the map of the study area.

III. RESULTS

A. Types of Breeding Sites in the Study Area

A total of 334 mosquito breeding sites divided into 24 types were identified during this study. Among the types of breeding sites encountered, we identified : barrels, puddles, ponds, tires, gutters, pits, tanks, buckets, pools, wells, tree holes, abandoned canoes, car carcasses, water-trays, boxes, abandoned lids, cans, bowls, pots, bath-tubes, cement bricks, jars, and car-prints (Fig. 2).

Fig. 2. Types of mosquito breeding sites in Akanda and its environs.

Among the identified breeding sites, barrels (n = 95; 28.44 %), puddles (n = 73; 21.86 %), ponds (n = 47; 14.07 %), tires (n = 31; 9.28 %), and channels (n = 22; 6.59 %) were the most numerous types of larval habitats (Fig. 2). The other types represented approximately 19.76% (n = 66) of the breeding sites listed in the study area. Most of these mosquito breeding places were temporary (n = 244), unclear (n = 205), and natural (n = 204) (Table 2).

TABLE 2 : CHARACTERISTICS OF MOSQUITO BREEDING SITES IN AKANDA AND ITS ENVIRONS

	Seasonality		Water aspect		Degree of disturbance	
	Temporary	Permanent	Clear	Unclear	Natural	Anthropic
Number of mosquito breeding sites	244	90	129	205	204	130

B. Positivity Of Mosquito Breeding Sites

Among the positive breeding sites recorded, 117 harbored *Culicinae* larvae (74.05%), 23 harbored *Anophelinae* (14.56%), and 18 were *Culicinae+Anophelinae* (11.39%) (Fig. 3).

Fig. 3. Proportion of breeding sites in Akanda and its environs.

C. Spatial Distribution Of Positive Breeding Sites

Of the 334 larval habitats identified, 158 (47.31%) were positive, while 176 (52.69%) were negative (Table 3). The maximum number of positive breeding sites identified were recorded at Ecole Nationale des Eaux et Forêts (ENEF) (n = 44) and Premier Campement (n = 36). The other districts registered less than 20 positive breeding sites and included Okala Carrière (n = 19), Mikoloungou (n = 18), Entraco-Stade de l'Amitié (n = 15), Sablière (n = 11), Cap Caravane (n = 8), Cités Angondjé (n = 5), and Angondjé Village (n = 2) (Table 3).

TABLE 3: DISTRIBUTION OF BREEDING SITES IN THE STUDY AREA

Study sites	Number of negative	Number of positive
Okala Carrière	0	19
Entraco-Stade de l'Amitié	0	15
Sablière	13	11
Cap Caravane	22	8
ENEF	70	44
Premier Campement	48	36
Cités Angondjé	9	5
Angondjé Village	0	2
Mikoloungou	14	18
Total	176	158

D. Abundance And Distribution Of Mosquito Larvae

During this study, 2,288 mosquito larvae were collected, including 1,659 individuals from *Culicinae* (72.51%) and 629 specimens of *Anophelinae* (27.49%) (Table 4). The Premier Campement was the study site that presented the highest number of larvae (n = 543), followed by the ENEF (n = 399) and that of Entraco-Stade de l'Amitié (n = 300). The rest of the study sites recorded less than 200 larvae (Table 4).

TABLE 4: ABUNDANCE OF LARVAL POPULATIONS ACCORDING TO THE STUDY SITES

Study sites	Total number of larvae collected
<i>Okala Carrière</i>	380
<i>Entraco-Stade de l'Amitié</i>	300
<i>Sablière</i>	163
<i>Cap Caravane</i>	150
<i>ENEF</i>	399
<i>Premier Campement</i>	543
<i>Cités Angondjé</i>	47
<i>Angondjé village</i>	198
<i>Mikoloungou</i>	108
Total	2 288

E. Species Richness Of Culicid Fauna

During this study, eight (8) mosquito species including *Aedes albopictus*, *Aedes aegypti*, *Anopheles gambiae s.l.*, *Anopheles pharaensis*, *Culex tigripes*, *Culex decens*, *Culex quinquefasciatus*, and *Mansonia uniformis* were identified (Table 5). All these species belonged to 2 subfamilies (*Anophelinae* and *Culicinae*) and to 4 genera (*Aedes*, *Anopheles*, *Culex* and *Mansonia*). The *Anophelinae* subfamily was represented by two species while the *Culicinae* subfamily was represented by 6 species (Table 5). The genera *Anopheles* and *Culex* were the most species-rich taxa (3 species). The other genera were represented by two species (*Aedes*) and one species (*Mansonia*) (Table 5).

TABLE 5 : SPECIES RICHNESS OF THE CULICIDIAN FAUNA IN THE STUDY AREA

Sub-family	Genus	Species
<i>Anophelinae</i>	<i>Anopheles</i>	<i>Anopheles gambiae s.l</i>
	<i>Anopheles</i>	<i>Anopheles pharaensis</i>
	S1	2
<i>Culicinae</i>	<i>Aedes</i>	<i>Aedes aegypti</i>
	<i>Aedes</i>	<i>Aedes albopictus</i>
	S2	2
	<i>Culex</i>	<i>Culex tigripes</i>
	<i>Culex</i>	<i>Culex decens</i>
	<i>Culex</i>	<i>Culex quinquefasciatus</i>
	S3	3
	<i>Mansonia</i>	<i>Mansonia uniformis</i>
	S4	1

F. Abundance And Distribution Of Adult Mosquitoes

After the rearing, nearly 1,182 adults were obtained including 444 belonging to the genus *Culex* (37.56%), 405 *Aedes* (34.26%), 322 *Anopheles* (27.24%) and 11 *Mansonia* (0.94%). Among these adult mosquitoes, 343 (29.02%) were collected from Okala Carrière, 277 (23.43%) from Entraco-Stade de l'Amitié, 200 (16.92%) from ENEF, and 161 (13.62%) from the Sablière. In contrast, all other study sites recorded fewer than 61 adults (<5.16%) (Table 6).

TABLE 6: ABUNDANCE OF ADULTS MOSQUITOE ACCORDING TO THE STUDY SITES

Study sites	<i>Anopheles</i>	<i>Culex</i>	<i>Aedes</i>	<i>Mansonia</i>
<i>Okala Carrière</i>	105	73	161	4
<i>Entraco-Stade de l'Amitié</i>	53	74	144	6
<i>Sablière</i>	33	42	85	1
<i>Cap Caravane</i>	20	30	0	0
<i>ENEF</i>	70	115	15	0
<i>Premier Campement</i>	20	40	0	0
<i>Cités Angondjé</i>	10	20	0	0
<i>Angondjé Village</i>	6	30	0	0
<i>Mikoloungou</i>	5	20	0	0
Total	322	444	405	11

IV. DISCUSSION

This study identified the different breeding sites of mosquitoes and species diversity in Akanda and its environs. In fact, nearly 334 breeding sites grouped into 24 types have been identified. This multiplicity of mosquito breeding sites could be linked to the uncontrolled urban extension, the non-application of adequate hygiene measures and the lack of sanitation in the immediate environment of human habitations in the Akanda area. Our results corroborate with those obtained by numerous authors who recorded in various rural and urban localities of Central Africa several mosquito breeding sites [13, 17]. Furthermore, these results are similar to those observed by several authors in certain villages and cities in Africa [23,24,25,26]. Moreover, the studies conducted by Koumba *et al.* [17] on mosquito larval habitats in the Mouila area, (southern Gabon) showed a large multiplicity of mosquito breeding sites.

The breeding sites of *Culicinae* were the most abundant compared to the other types of positive larval habitats. Indeed, in recent years, the Akanda area, like all other African cities, has undergone various demographic as well as infrastructural changes, marked by anarchic urbanization and high rainfall. All these factors have an impact on the bioecology of *Culicinae*, thereby favorising the proliferation of the pre-imaginal stages of certain species of the genus *Culex* and *Aedes* [13]. Also, these results are similar to those obtained by Koumba *et al.* [26] in Mouila, in Gabon, and they highlighted that the occurrence of diversified breeding sites could be related to certain factors including demography and human activities.

The abundance of mosquitoes of the genera *Culex* and *Aedes* could be due to environmental conditions and anthropic activities, favorable for the proliferation of these taxa. These results corroborate those obtained by several authors who have shown that this sub-family adapts better to the environmental conditions offered by most localities [26, 27]. Furthermore, the abundance of species of the *Culicinae* sub-family (6 species) is due to the high plasticity of this sub-family in colonizing diversified ecosystems. Excessive household waste, watering activities, and vehicle prints create favourable conditions for the development and maintenance of *Culex* and *Aedes* [28, 29]. However, it should be noted that the low presence of mosquitoes of the genera *Anopheles* and *Mansonia* is due to the scarcity of their preferred breeding sites. According to Teklu *et al.* [30], *Anopheles* spp. prefers sunny natural breeding sites, without vegetation and unpolluted. In contrast, *Mansonia* larvae are found in water

collections with aquatic plants such as *Pistia* spp. and polluted waters [31]. The insufficiency of these two types of larval habitats in the study area could therefore explain the low abundance of these taxa. On the other hand, the presence of certain species including *Anopheles gambiae* s.l. could constitute a major risk of malaria occurrence in Akanda. Also, preventive measures to control malaria vectors must be developed in this study area.

V. CONCLUSION

Although preliminary, the results of this study contribute to the knowledge of the bio-ecology of the culicids fauna of the Akanda area. Also, it appears that the study sites are hosts for the vectors of *Plasmodium*, filaria, and arboviruses. The breeding sites for these blood-sucking insects are maintained by rain and human activities. Awareness and sanitation campaigns should be conducted in order to educate the local populations on the good practices of the prevention and control of these vectors that cause malaria and other diseases.

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