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Distribution and Characterization of Culicids (Diptera: Culicidae) in Two Urban Communes of Estuaire Province of Gabon

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ABSTRACT

Background and Objective: The increasing urbanization in Libreville, the capital city of Gabon favors the creation and expansion of mosquito breeding sites that represent a high risk for an increased population of vectors of dangerous parasitic diseases such as malaria and arboviruses (chikungunya, dengue, yellow fever and Zika). Despite this growing risk, the mosquito population in Libreville and Akanda has not been thoroughly studied. The present study was designed to fill these knowledge gaps and provide mosquito breeding and distribution maps to ease their control. The purpose of this study is to improve understanding of the mosquito population in these areas by identifying different types of mosquito breeding grounds and adult mosquito species and mapping their distribution. **Materials and Methods:** An entomological prospection was conducted in Akanda and Libreville communities for 30 days during the rainy season from February 9th to April 6th. The physical characteristics of mosquito breeding spots were analyzed and mosquito larvae were raised in the Transmissible Diseases Ecology Laboratory (TDEL) under standard conditions. The adult mosquitoes were identified using standard taxonomic keys. Geospatial analysis was conducted using the Geographic Information System (GIS). **Results:** Of the 250 potential mosquito breeding microhabitats prospected, 122 of them were positive. The rate of mosquito breeding habitats was higher in Libreville (51%) than in Akanda (46%). The most common and positive breeding site was plastic containers, while the most densely populated was an abandoned frying pan. The genera *Culex* and *Aedes* were found in both communes, but *Anopheles* was only found in Libreville. **Conclusion:** It is important to dispose of household items like plastic containers and frying pans in Akanda and Libreville to prevent the invasion and spread of mosquito vectors. Mosquito control authorities can use distribution map to help plan and implement their mitigation system.

KEYWORDS

Ecology, mosquito control, microhabitat, Akanda, Libreville

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INTRODUCTION

Mosquitoes are extensively studied due to their role in spreading viral and parasitic diseases of medical and zoonotic significance[1]. Over 3000 species of mosquitoes spread diseases like malaria, lymphatic filariasis and arboviruses[2,3]. The capital cities of Africa have been reported to be one of the hotspot areas for the spread of dangerous vector-borne diseases (VBDs) such as malaria[4] and arboviruses (chikungunya, dengue, Zika and yellow fever)[5]. The growing economic and infrastructural developments and climate change witnessed in Libreville in the past decades could favor the invasion and proliferation of certain mosquito species and the spread of diseases they are capable of transmitting. A report by Mourou *et al.*[6] and Sevidzem *et al.*[7] revealed a high frequency of mosquitoes in urban areas of Libreville. Other studies report outbreaks of some mosquito-borne arboviruses such as chikungunya and dengue in Libreville[8]. Despite its malaria endemic and arboviruses hotspot status, only three entomological studies have been conducted in Libreville with one of them being a transmission study using human landing catch as the collection method[9], while the other two utilized the larval collection approach to evaluate some ecological indices of mosquitoes in Akanda by Sevidzem *et al.*[7] and in Libreville by Mourou *et al.*[6]. The urban communes of Akanda and Libreville were the focus of a recent study. So far, there has been no comprehensive and comparative analysis to identify mosquito breeding sites and map their distribution by studying adult mosquitoes. To address this, a thorough search for potential breeding grounds is required in the neighborhoods of these two communes. To contribute to the existing knowledge on mosquito ecology in Libreville and provide entomological data to aid its control, this study aims to identify mosquito breeding sites and adult mosquito species and map the distribution of larval microhabitats and adult mosquito species in two communes (Libreville and peripheral Akanda City) in the Estuaire Province.

MATERIALS AND METHODS

Study area: Libreville is a city in Gabon located at Latitude 0°23'24"North and Longitude 9°27'15"East. It is the capital and has a population of over 703,940 people, which is half of the country's population. The city is situated on the coast of the Atlantic Ocean and is drained by the Komo River to the South and North. The area is home to mangroves and patches of dense forests and several rivers run through the city. The climate in Libreville is tropical, with an average temperature of 26.3°C and an annual average rainfall of 1970.6 mm. The humidity level averages at 79% per year. Due to heavy rainfall year-round, many neighborhoods in the city experience severe flooding[7].

Akanda is situated at Latitude 0°30'00"North and Longitude 09°30'00"East. It has been the capital of the Estuaire Province since, 2022 and boasts a thriving economy with rapid urbanization. The tropical climate of Akanda experiences a humidity level that ranges between 85 and 100% during the rainy season. Heavy rainfall averaging between 2000 to 3800 mm per year is common. The average annual temperature is around 26°C. Akanda is predominantly flat and characterized by plains[7].

Larval prospection, laboratory rearing and identification of adults: This study was conducted from February, 9 to April, 9, 2023, during the heavy rainy season to identify different mosquito larval microhabitats in the neighborhoods of the commune of Libreville and the peri-urban commune of Akanda. Purposive random sampling and an observational study were used. The entomological survey was carried out synchronously from 8:30 am to 2:30 pm for two to three consecutive days per week in both communes. Potential mosquito breeding sites were systematically checked and larvae and nymphs were collected from each breeding site using a standard 350 mL long-handled dipper. Larval microhabitats were classified as positive if they harbored larvae or nymphs, but their negative counterparts were those without larvae or nymphs. The GPS points were recorded for each potential positive microhabitat, along with the type of breeding site, distance to the nearest human dwelling using a measuring tape and larval density. The collected larvae were transported to TDEL for the rearing of larvae and nymphs and the identification of adults upon emergence. The immature stages were raised to the adult stage in the TDEL insectary under standard conditions before identification. Adult mosquitoes obtained from the nymphs were killed using an insecticide spray for their morphological identification. Adult mosquitoes were identified using the identification keys of Edwards[10] and Huang[11].

Determination of some ecological indices: The density of mosquito larvae and their distribution proportions in Akanda and Libreville were determined using formula (1):

$$\text{Larval density} = \frac{\text{Total number of larvae}}{\text{Total number of dips}} \quad (1)$$

Distribution rate of positive larval microhabitats: The distribution rate of positive larval microhabitats for mosquitoes was determined using formula (2) from Bashar *et al.*[12]:

$$\text{Distribution rate} = \frac{\text{Total number of positive microhabitats}}{\text{Total number of potential microhabitats}} \quad (2)$$

Statistical analysis: The information gathered from the field collection sheets was entered into MS Excel, 2013. The data was then analysed to determine the frequency and proportions of the various parameters studied. To compare the proportions of larvae at both study sites, the Chi^2 test was utilized. Additionally, the mean density of larvae collected from different microhabitats was compared using the Student's t-test. The significant level for both tests was determined to be $p<0.05$.

Creation of distribution maps: Geographic coordinates collected at all sampling sites were verified and then compiled into Access, 2013 databases (Microsoft), then attached to locality shapefiles produced from GPS handset gpx files in ArcMap™ version 10.1, using geographic information system software (Environmental Systems Research Institute, United States of America).

RESULTS

Positivity rate of breeding sites per commune: In the present study, 250 potential breeding sites were systematically examined, including 90 in Akanda and 160 in Libreville. The overall positivity rate of breeding sites for the two communes was 48.8%. At the communal level, in Akanda, 41% of the 90 potential breeding sites were positive while 51% of the 160 surveyed in Libreville were positive. However, there was no statistically significant difference (X^2 = 2.00; df = 1 and p = 0.157) in the positivity rate with study communes.

Mosquito larval microhabitats: For both communes, the most frequently encountered microhabitats in order of magnitude were: Plastic containers, tires and gutters. A high positivity rate was recorded for plastic containers, followed by tires and then by surface water (Fig. 1).

Average mosquito larval density by microhabitat: Of the different microhabitats prospected, that with the highest average density was the frying pan (138 larvae/five dips), followed by plastic containers (9.73 larvae/five dips) and the least dense was the drum (0.56 larvae/five dips). However, there was no statistically significant difference in the average mosquito larval density of the microhabitats prospected (t = 1.302; df = 6 and p = 0.241) (Fig. 2).

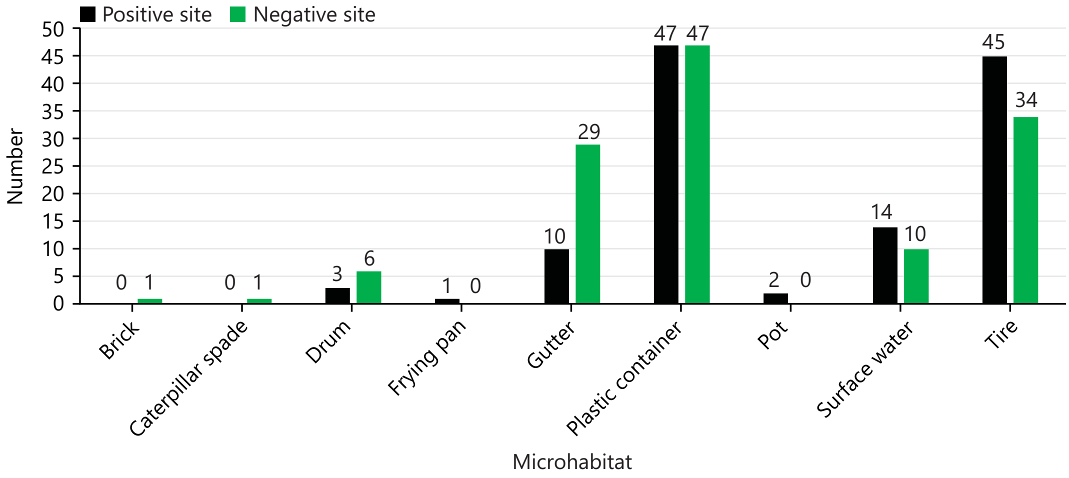

Fig. 1: Frequency of mosquito larval habitats encountered in the prospected communes

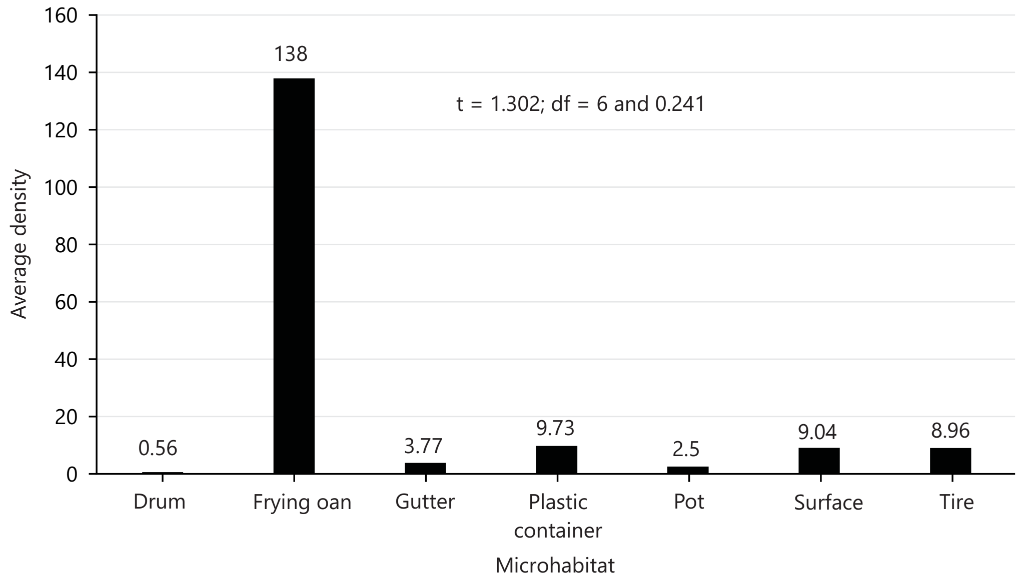

Fig. 2: Average mosquito larval density of the prospected microhabitats

Number of positive larval microhabitats and distance from human dwellings: A high number of positive mosquito larval microhabitats were close to human dwellings (0 to 10 m). The least number of positive microhabitats were found at a distance range of 51 to 60 m from human dwellings. Details on the frequency of positive microhabitats and distance from households can be seen in Fig. 3.

Mosquito species: In the two communes of Grand Libreville, 122 mosquito larval microhabitats were observed and immature stages were collected, resulting in the emergence of 519 adult mosquitoes of these, 61.5% were female and 38.5% were male. More females emerged than males in both communes, as shown in Table 1. The identified mosquito species belonged to three genera: *Culex*, *Aedes* and *Anopheles*. *Culex* was the most frequently identified genus overall. In Akanda, two genera were found - *Culex* and *Aedes*. Two species of Aedes, *Ae. albopictus* and *Ae. aegypti*, as well as two species of *Culex* (*Cx. decens* and *Cx. quinquefasciatus*) were identified. In Libreville, three genera were identified -*Aedes*, *Anopheles* and *Culex*. Two species of *Aedes* (*Ae. aegypti* and *Ae. albopictus*), one species of Anopheles (*An. gambiae*) and three species of *Culex* (*Cx. naevei*, *Cx. pipiens* and *Cx. quinquefasciatus*) were identified, as shown in Table 1.

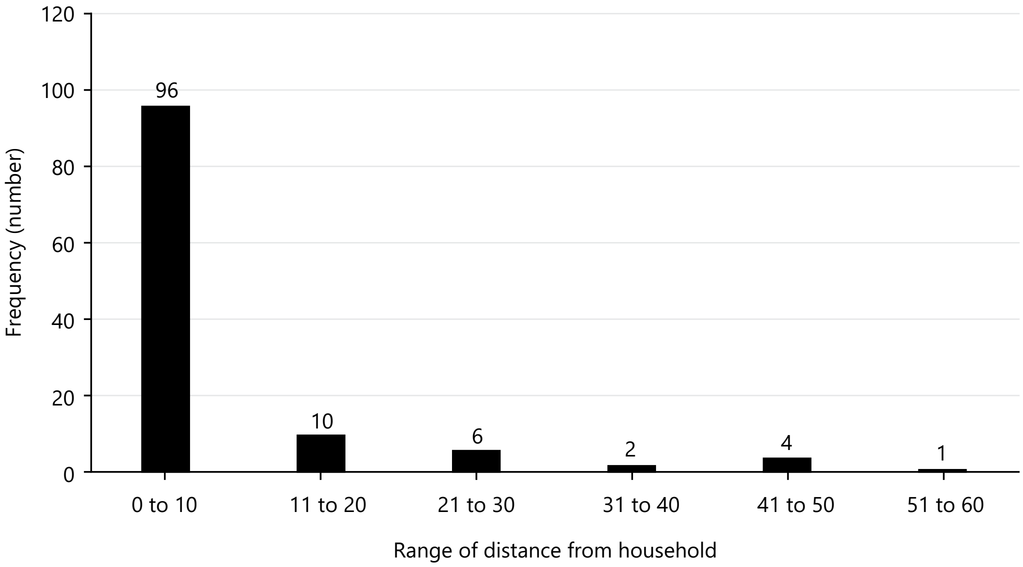

Fig. 3: Number of positive mosquito larval microhabitats and distance from households

Table 1: Mosquito species in Akanda and Libreville

			Sex		
Commune	Genus	Species	Female	Male	Total
Akanda		*Aedes aegypti*	1 (100)	/	1 (100)
	Aedes	*Aedes albopictus*	5 (55.6)	4 (44.4)	9 (100)
	Culex	*Culex decens*	3 (50)	3 (50)	6 (100)
		Culex quinquefasciatus	1 (50)	1 (50)	2 (100)
		Culex spp.	57 (57.6)	42 (42.4)	99 (100)
	Total		67 (57.3)	50 (42.7)	117 (100)
Libreville	*Aedes*	*Aedes aegypti*	62 (70.5)	26 (29.5)	88 (100)
		Aedes albopictus	19 (79.2)	5 (20.8)	24 (100)
		Aedes spp.	/	2 (100)	2 (100)
	Anopheles	*Anopheles gambiae* s.l.	3 (75)	1 (25)	4 (100)
	Culex	*Culex naevei*	1 (100)	/	1 (100)
		Culex pipiens s.l.	6 (100)	/	6 (100)
		Culex quinquefasciatus	5 (100)	/	5 (100)
		Culex spp.	156 (57.4)	116 (42.6)	272 (100)
	Total		252 (62.7)	150 (37.3)	402 (100)
Total général			319 (61.5)	200 (38.5)	519 (100)

Mosquito larval micohabitat distribution map: The distribution rate of mosquito breeding habitats was higher in Libreville commune (51%) than in Akanda (46%), as shown in Fig. 4.

Mosquito larval density: High larval density values were frequently recorded in the commune of Libreville than in Akanda. The details of the larval density in the two communes were shown in Fig. 5.

Distribution map of the genus *Culex*: Both communes frequently collected *Culex* mosquitoes. In Akanda, *Cx. quinquefasciatus* and other *Culex* spp. were found in close proximity in areas such as Malibé 2 and Gigi. In Libreville, *Cx. pipiens* coexisted with other *Culex* spp. In Haut Gué-Gué, *Cx. naevei* shared breeding habitats with other Culex spp. and Camp de Gaulle also had *Culex* mosquitoes present (Fig. 6).

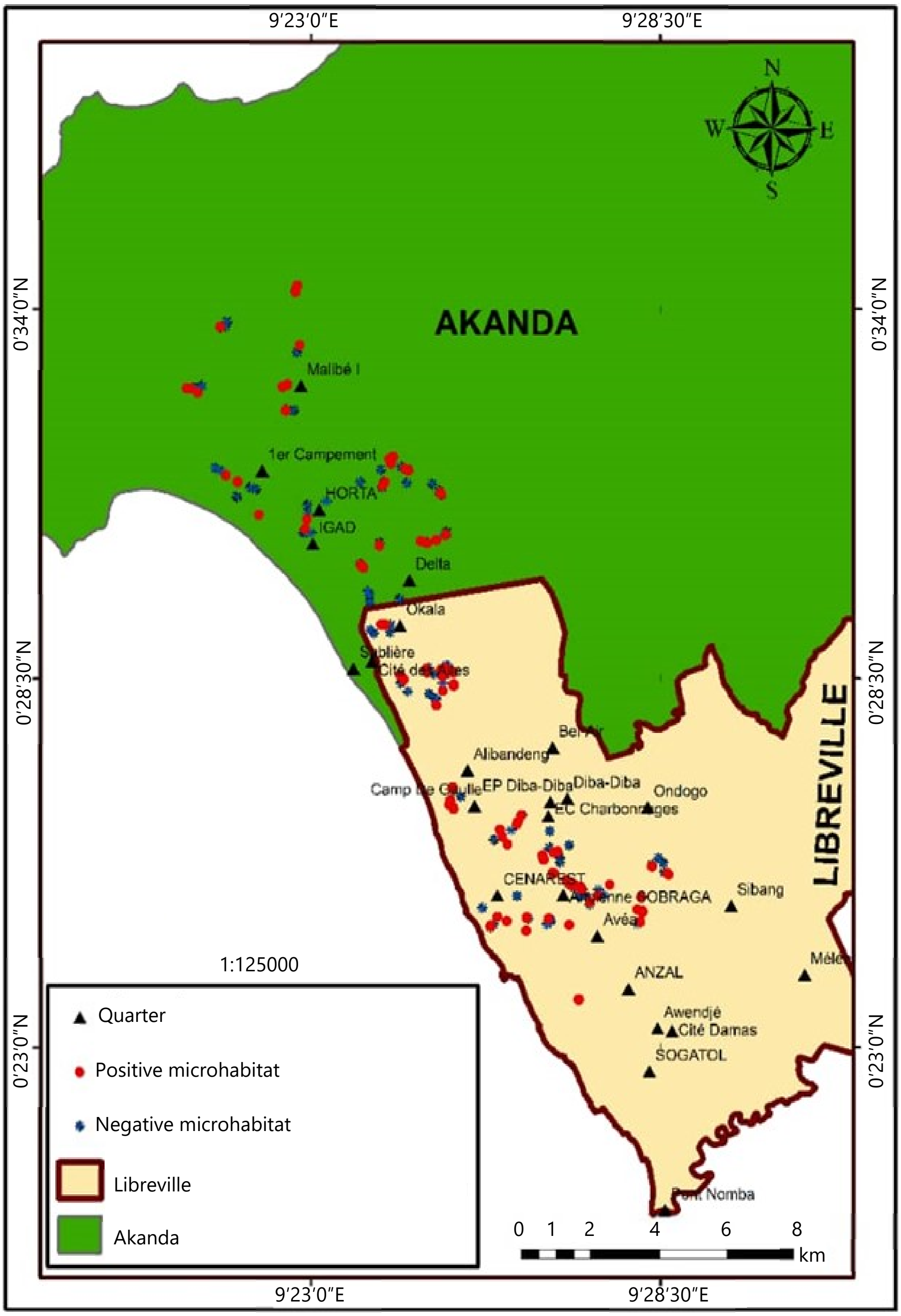

Fig. 4: Mosquito larval microhabitat distribution map for Akanda and Libreville

Distribution of the genus *Anopheles*: The only identified species of *Anopheles* was *An. gambiae*, found only in the Okala Rivier quarter of the Libreville commune. This microhabitat was not mixed and was a puddle.

Distribution of the genus *Aedes*: *Aedes* spp. was found in both prospected communes of grand Libreville. In Akanda, *Ae. aegypti* and *Ae. albopictus* sympatrically coexisted in the Angondje quarter. In Libreville, this sympatric occurrence of *Aedes* spp. was noted in two quarters (Plaine Oréty and Ancienne Sobraga). Interestingly, both species were able to colonize the same breeding habitat with other *Aedes* spp. as in the case of a positive microhabitat identified in Ancienne Sobraga (Fig. 7).

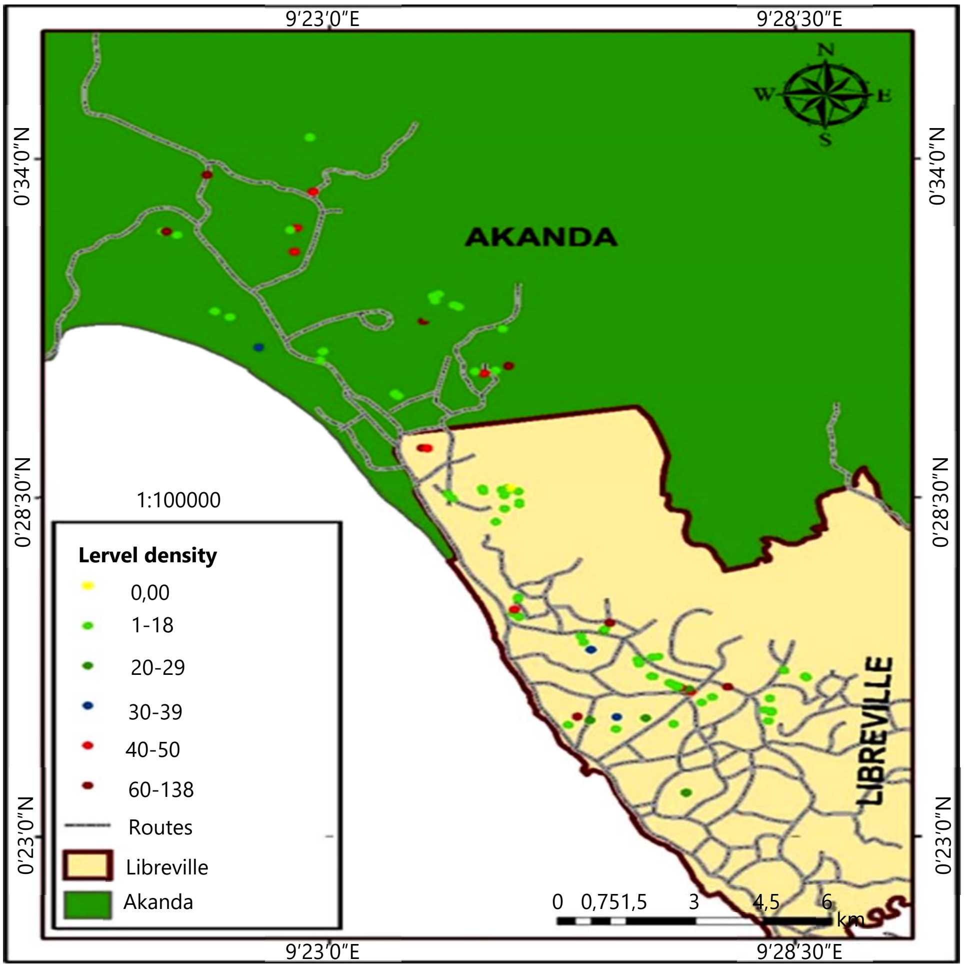

Fig. 5: Larval density in prospected breeding sites in the two communes (Akanda and Libreville)

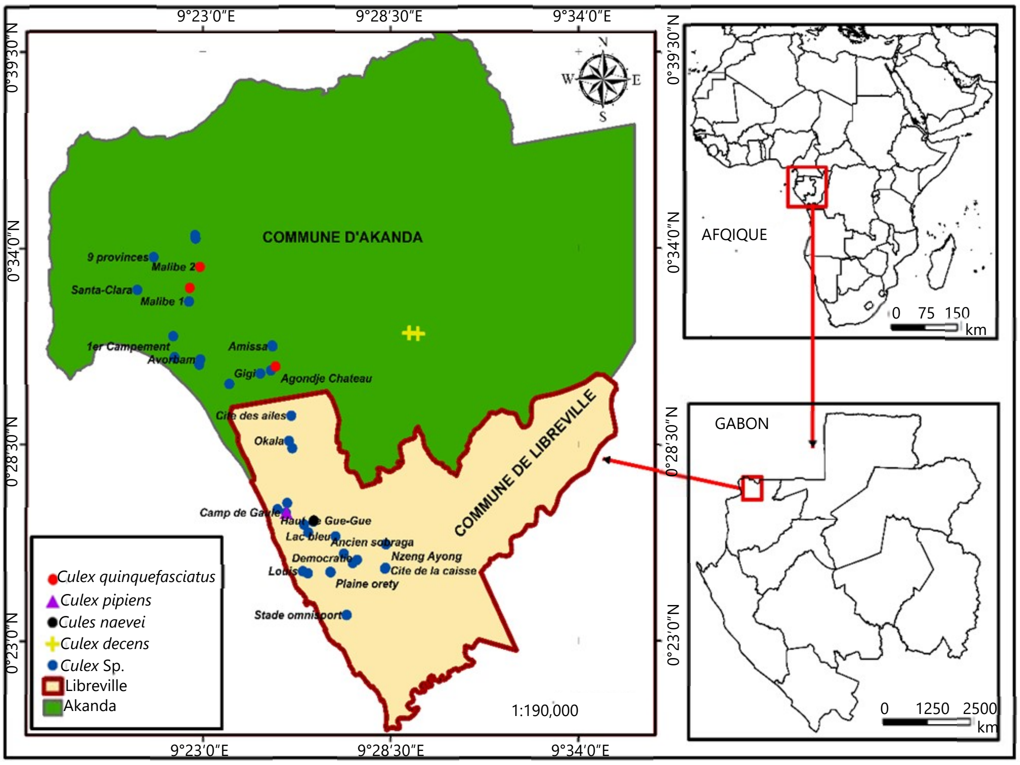

Fig. 6: Distribution of *Culex* spp. in Akanda and Libreville

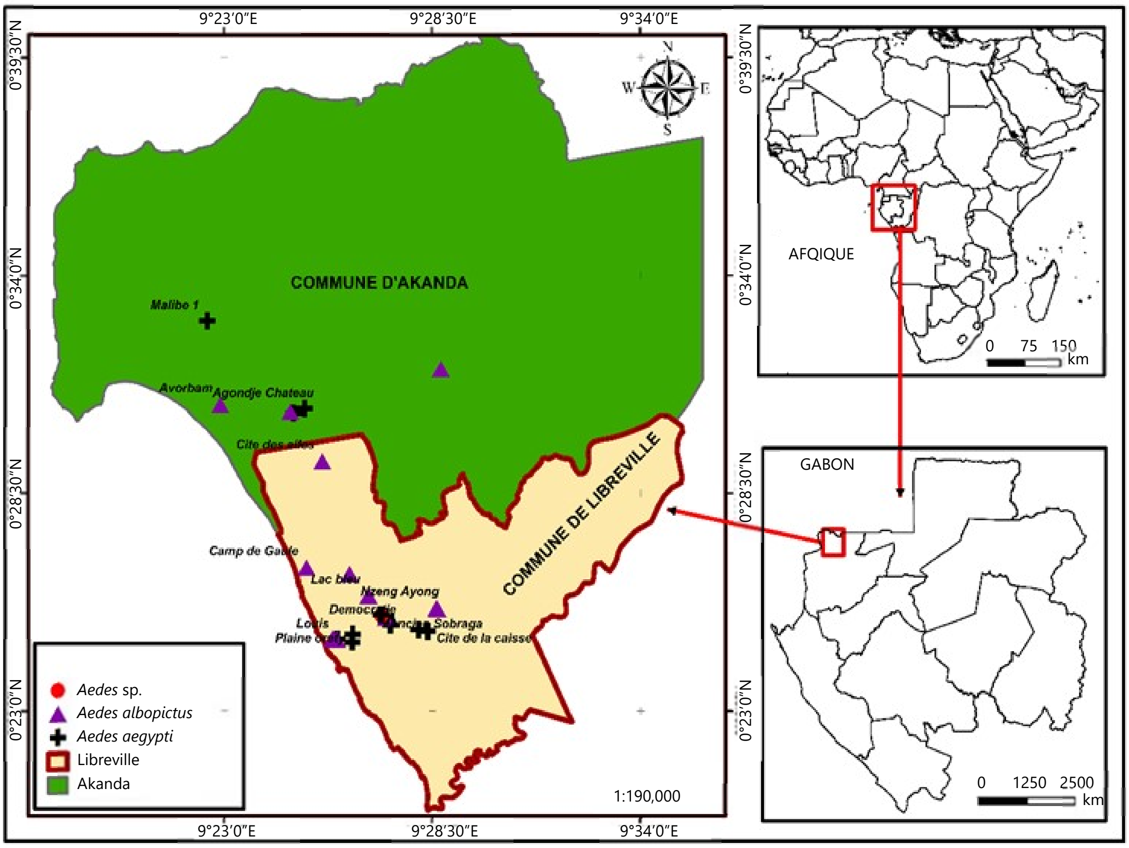

Fig. 7: Distribution of *Aedes* spp. in Libreville and Akanda

DISCUSSION

This investigation aims to improve the knowledge about the Culcidian fauna in two communes, Akanda and Libreville, located in the Estuaire Province of Gabon. The findings indicate that Libreville had a greater number of mosquito larval microhabitats (n = 160) compared to Akanda (n = 90). The most commonly observed and favorable microhabitat in both communes was plastic containers. This may be due to the recent water shortage in Libreville, which has resulted in residents purchasing plastic water collection containers for household and construction purposes. Consequently, these containers have become optimal breeding sites for mosquito larvae. A study in Gabon reported that urbanization and community actions (conservation of water due to shortages) are the major factors leading to the creation of artificial mosquito larval breeding grounds[13]. Furthermore, plastic containers were the most preferred larval breeding grounds in the prospected quarters. This finding was similar to the reports of Mourou *et al.*[6] and Sevidzem *et al.*[7] in Akanda and Libreville where containers were the most frequent and positive mosquito larval breeding ground. However, the report of Kutomy *et al.*[14] reported puddles as the most positive larval microhabitat for *Anopheles* in the Oyem area. This observation of Kutomy *et al.*[14] after the collection of larvae of *Anopheles* and raising them to identify adults was the reason why they mostly prospected puddles which have already been reported to be favorable breeding grounds for the larvae of this genus[15]. The majority of the larval breeding microhabitats were typical of that of Culicinae as already described for *Culex pipiens* with the following characteristics such as minimally polluted water (gutters, containers and puddles) by Shaman *et al.*[16].

Of the 250 larval microhabitats prospected and 122 positive, a total of 519 mosquito adults emerged and 61.5% were females and 38.5% males. Three genera (*Aedes*, *Anopheles* and *Culex*) were identified. The species *Ae. aegypti* and *Ae. albopictus* were found in both communes. The genus *Anopheles* was only found in Libreville and *Culex* was present in both communes. The predominance of the genus *Culex* could be explained by the capacity of its larvae to adapt and develop in diverse microhabitats. The Culcidian fauna of this current study was similar to that reported by Kutomy *et al.*[14] in Sevidzem *et al.*[7] in the Akanda area, where they mentioned the presence of all three genera. Furthermore, of the 250 potential microhabitats surveyed and with a high larval density recorded, the number of adults that emerged under standard laboratory-rearing conditions was very low and this could probably be due to the following reasons: (i) The existence of intraspecies and interspecies competition[17] and with the possibility of cannibalism[18] and (ii) The switch from natural microhabitat to laboratory could have led to their stress and more so when the physicochemical parameters of the laboratory rearing container are not all met (temperature, light, humidity)[19].

The distribution rate of mosquito larval microhabitats was higher in Libreville (51%) compared to Akanda (46%). This difference could be explained by the fact that the study was carried out during the rainy season when the capital city (Libreville) was characterized by flooding, leading to the creation and expansion of several artificial microhabitats (containers, gutters, puddles, tires, etc). The higher and more widespread average larval density intervals in Libreville than in Akanda were also found. This could be due to the recent upsurge in anthropic activities that could enhance the development of mosquito breeding sites[20]. A study was conducted in the two communes of Grand Libreville to map the distribution of *Anopheles* species. The results showed that only one species, *An. gambiae*, was present in Okala Rivière of the Akanda commune and the species was found in a non-mixed microhabitat consisting of a puddle. While some studies have reported mixed species mosquito larval microhabitats for *Anopheles*, this study found that they were in non-mixed microhabitats based on the physical and chemical characteristics of the breeding substrates. In contrast, *Aedes* spp. larvae were frequently found in mixed microhabitats (*Ae. aegypti*+*Ae. albopictus*) in both communes. This is not surprising as the breeding substrates observed in the field were typical of an *Aedes* larval microhabitat, which allows females of different species to lay eggs and promote interspecific coexistence. The coexistence of *Ae. aegypti* and *Ae. albopictus* larvae have been reported in other studies as well. The coexistence of the larvae of *Ae. aegypti* and *Ae. albopictus* has already been reported elsewhere[21]. The coexistence of these two taxa in the same microhabitat is conditioned by food regime, temperature and multifactorial interactions[22]. The species of the genus *Culex* were widely distributed and occurred in sympatry with other species in the two study communes. The *Cx. quinquefasciatus* mixed larval microhabitats occurred in Akanda while in Libreville such breeding grounds were observed for *Cx. pipiens* and *Cx. naevei*. The coexistence of the larvae of the species of the genus *Culex* and *Aedes* in the same microhabitat has already been reported[22]. Generally, it is not uncommon for several species of adults to colonize the same microhabitat, especially when such breeding substrates are not available. Artificial water recipients such as containers, are usually colonized by several species (*Ae. aegypti* and *Ae. albopictus*) inside or near human dwellings. Others, like *Cx. quinquefasciatus* and *Cx. pipiens*, tend to settle in waters relatively or heavily polluted by organic matter, such as latrines, septic tanks, or wastewater treatment plants[19]. Future studies will include a physicochemical analysis of the breeding sites of mosquitoes to establish the physical and chemical conditions preferred by these dipterids for their development. In addition, seasonal studies will be conducted to understand the dynamics of the local population of mosquitoes to better plan control interventions.

CONCLUSION

To summarize, a total of 122 mosquito breeding sites were discovered in Akanda and Libreville, with plastic containers being the most commonly used and frying pans being the most densely populated. These breeding spots were predominantly located within 0 to 10 m from homesteads. Compared to Akanda, Libreville exhibited a higher rate of mosquito breeding and distribution. The emergence of 519 adult mosquitoes revealed three different genera -*Anopheles*, *Aedes* and *Culex*- with *Culex* being the most species-rich. Factors such as urbanization and poor drainage systems in Akanda and Libreville may have contributed to the development and spread of mosquito breeding sites.

SIGNIFICANCE STATEMENT

The objective of this study was to characterize the mosquito larval microhabitats and provide distribution maps to aid control. A high density of mosquito larvae was encountered in frying pans, but plastic containers represented the most preferred breeding microhabitat. Most positive microhabitats were in close proximity to human dwellings. *Anopheles gambiae*, the main vector of malaria raised in the laboratory was from collections made in Libreville. The widespread of *Culex* spp. and *Aedes* spp. represents a risk for the spillover of arboviruses.

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