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Breeding substrates and diversity of aedes species in Periurban areas of Côte d'Ivoire

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Abstract

As part of the arboviral disease vectors surveillance in Côte d'Ivoire, a longitudinal entomological prospection was undertaken in five stations. Mosquito larvae collections were carried out monthly from March 2018 to September 2019 in breeding sites and using the WHO-type oviposition trap. These larvae were reared till adult stage at the insectarium of the Institut National d'Hygiène Publique (INHP) in Abidjan. Adult mosquitoes were morphologically identified using standard keys. In total, 896 potential mosquito larval breeding substrates were identified. Aedes larvae were collected from 225 sites. The ideal breeding substrates for Aedes consisted of abandoned containers/objects (39.6%), natural breeding sites (31.6%), and storage containers (22.2%). It was noticed that used tires (5.3%) and other breeding substrates (1.3%) were poorly represented. There was a statistically significant difference ($\chi^2 = 48.748$; $ddl = 16$; $P = 0.000$) in the positive proportions of Aedes with breeding substrates. The ideal height for Aedes breeding is 1.5m. In terms of species diversity, 16 Aedes species were identified and the most frequent was *Aedes aegypti* (80.8%). From the Shannon index (H'), Aedes species are not very diversified ($H' = 0.42$ in the south, $H' = 1.08$ in the west, $H' = 0.38$ in the north-west, $H' = 0.69$ in the north-east and $H' = 1.09$ in the center). The baseline data obtained from this study could instruct decision makers involved in antivectorial fight to better design and implement control measures against arboviruses vectors.

Keywords: Aedes, species composition, breeding sites, arbovirus, Côte d'Ivoire.

1. Introduction

Within the insect class, Culicidae occupy an important space ^[1] with more than 3,500 species already described ^[2]. These insects, represented by two subfamilies (Culicinae and Anophelinae) are considered to be the largest group of arthropods of medical importance ^[3, 4, 5]. They are involved in the transmission of several parasites and viruses that cause diseases such as malaria and arboviruses ^[5, 6, 7].

Arboviruses such as dengue fever, Japanese encephalitis, chikungunya, yellow fever and Zika fever threaten nearly 831 million people worldwide, including 70% of the African population ^[8]. Today, these diseases constitute a major threat to the health of human populations ^[9].

In Côte d'Ivoire, dengue fever and yellow fever are growing concern for health authorities ^[10, 11, 12, 13, 14, 15]. Indeed, in recent decades, the country has experienced an increase in cases associated with these vector-borne diseases in both urban and rural areas. In urban areas, the only vector suspected to be implicated in causing arbovirus epidemics is *Aedes aegypti*. *Aedes aegypti* breed in different breeding sites that are mostly encountered around households ^[15, 16, 17, 18]. In rural and/or Sylvatic areas the following *Aedes* spp. : *Ae. aegypti*, *Ae. africanus*, *Ae. luteocephalus*, and *Ae. usambara* are encountered ^[19, 20, 21, 22, 23]. However, in arbovirus monitoring stations of Côte d'Ivoire, very few studies on Aedes have been conducted. This study seeks to improve on the knowledge of the ecology and species diversity of genus *Aedes* in five stations in Côte d'Ivoire.

2. Materials and Methods

2.1 Study zone

This study was conducted from March 2018 to September 2019 in five different stations in Côte d'Ivoire, located in the south (Banco National Park (5 ° 23'40"N and 4 ° 03'07"W)), West (Vapleu village (6 ° 49'N and 8 ° 16'W)), northwest (Tron Touba village (9 ° 43'N and 7 °

23W)), north east (Toupé village (9 ° 06'53"N and 3 ° 43'49"W)), and center (Sokala Sobara village (8 ° 27 ' N and 4 ° 31'W)) (Fig. 1). The climate of these stations is generally hot and humid ranging from 20 ° C to 33 ° C. The area has three climates i.e. hot (November to February), hot and dry

(March to April), and very hot and dry (June to October). The choice of the different study stations was based on their differences in relief, vegetation, rainfall and temperature patterns.

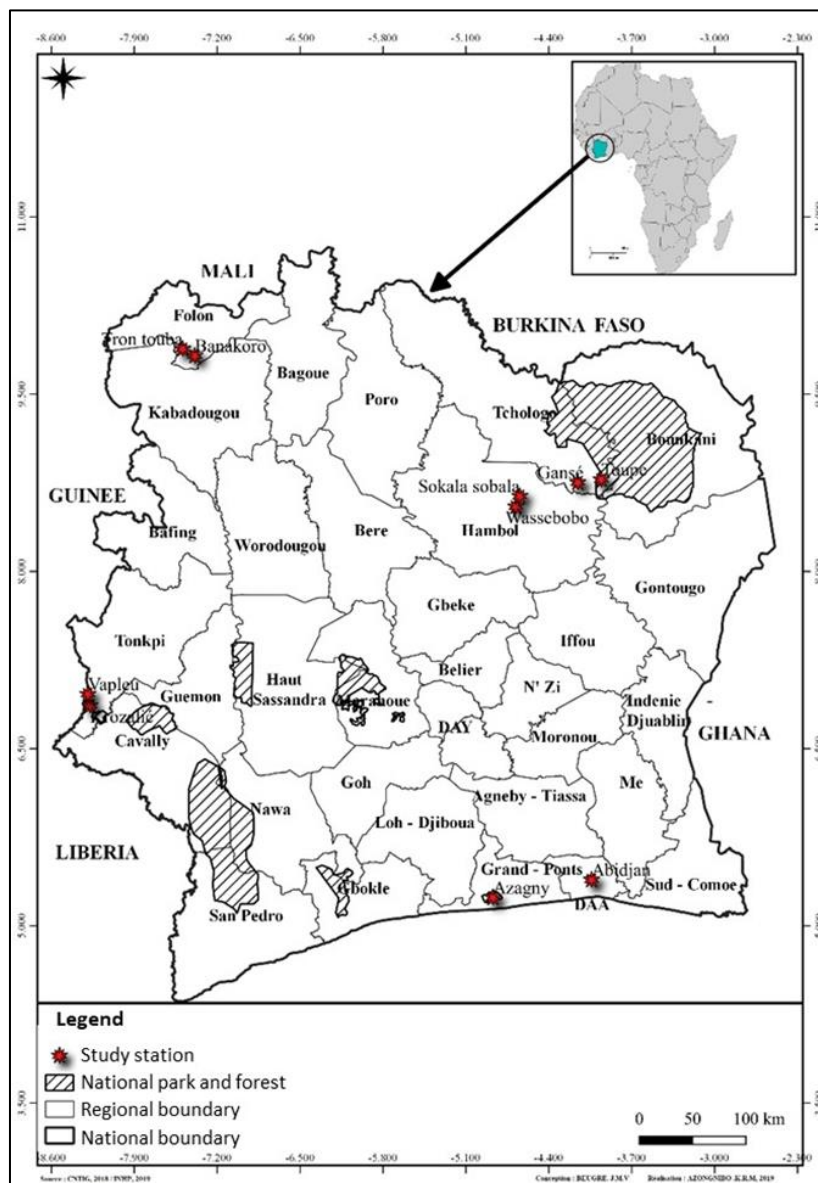


Fig 1: map of Côte d'Ivoire showing the study areas and sites

2.2 Installation of oviposition traps in study stations

In order to establish the species composition of mosquitoes of the genus *Aedes*, oviposition traps (WHO model) [24, 25, 26] were set in the field. Ninety (90) oviposition traps were set in each of the five stations. The contents of these traps were removed four days after installation. They were transferred to the insectarium of the Institut National d'Hygiène Publique in Abidjan. The larvae from hatched eggs were raised to the adult stage for identification at the species level.

2.3 Identification of *Aedes* breeding sites

This study was done through systematic search and identification of potential *Aedes* breeding substrates around dwellings, forests, and villages. Water points where *Aedes* larvae were visually confirmed were considered to be breeding substrates (identified in the forest) [18, 27]. All

collected larvae were transferred into labeled containers using pipettes [28, 29]. These different containers were sent to the station laboratory or to the insectarium of the Institut National d'Hygiène Publique in Abidjan for rearing. All the larvae collected were raised to the adult stage for identification at the species level.

2.4 Morphological identification of *Aedes* spp.

All adult mosquitoes were identified under a binocular microscope using morphological identification keys [30, 31, 32].

2.5 Data analysis

The Shannon index was used to establish the heterogeneity in the spatial distribution of *Aedes* spp. as follows:

$$H' = -\sum ((N_i / N) \times \ln (N_i / N))$$

Where

Ni= number of individuals of a given species

N= total number of individuals.

The interpretation of the Shannon index was carried out following Grall and Coïc [33].

The different statistical tests were carried out using the SPSS 25 statistical software. The Fisher's test was used to compare the percentages of larval substrates. The degree of significance was set at 5% ($p < 0.05$).

3. Results

3.1 Types of *Aedes* breeding substrates in study stations

Of the 896 potential mosquito breeding substrates identified, 317 were positive (35.37%) and 579 were negative (64.63%). The breeding substrates were grouped into 5 types: storage containers, abandoned containers / objects, used tires, natural and others. Among the positive substrates, nearly 225 harbored *Aedes* larvae while 92 were substrates consisting of *Eretmapodites*, *Anopheles* or *Culex* (Table 1). However, these different *Aedes* breeding substrates recorded different proportions ranging from 1.3% to 39.6%. The positive proportion test recorded a statistically significant difference between breeding substrates ($P = 0.000$; Fisher test).

3.2 Breeding substrate predilection of *Aedes* in prospected stations

In Banco station, the preferred breeding substrate included abandoned containers / objects (35.6%) followed by storage containers (30.6%), natural substrates (16.1%), and used tires (14.5%). Other breeding substrates represented only 3.2% of the total. Furthermore, there was a statistically significant

difference ($P = 0.000$; Fisher test) between the positive proportions of breeding substrates in the area (Table 1).

At Vapleu station, 3 types of substrates harbored *Aedes* larvae and included storage containers, abandoned containers / objects and natural deposits. Among these different types of breeding substrates, the natural deposits represented the most frequent (52.8%) *Aedes* breeding substrate. In contrast, storage containers were rare (5.6%). The results of the Fisher test indicated that there was a statistically significant difference ($P = 0.001$; Fisher test) between the different proportions of identified breeding substrates (Table 1).

In Tron Touba station, most *Aedes* larvae were encountered in abandoned containers / objects ($\% = 52.8$). Storage containers (22.6%), natural deposits/used tires (1.9%) were rare *Aedes* breeding substrates (Table 1). There was a statistically significant difference ($P = 0.000$; Fisher test) in the frequency of *Aedes* breeding substrates at Tron Touba.

At Toupé station, abandoned containers / objects, storage containers and natural deposits recorded superior *Aedes* numbers. Among these types of deposits, the most frequent (44.4%) was abandoned containers / objects (Table 1). The results of the Fisher test did not show any statistically significant difference ($P = 0.429$; Fisher test) between the proportions of different *Aedes* larval substrates encountered at Toupé.

Finally, at the Sokala Sobara station, natural deposits were the most frequent (52.6%) *Aedes* breeding substrate. However, the other breeding substrates were rare (2.6%) (Table 1). Statistically, a significant difference ($P = 0.000$; Fisher test) was noted between the proportions of *Aedes* breeding substrates in Sokala Sobara.

Table 1: Proportion of *Aedes* breeding substrates in sampled stations

Study stations	Potential breeding substrates	Positive breeding substrates	<i>Aedes</i> breeding substrates					Total
			Storage containers	Abandoned storage containers/objects	Abandoned tires	Natural	Others	
Banco National Park	159	77 (48,42%)	19 (30,6%)	22 (35,5%)	9 (14,5%)	10 (16,1%)	2(3,2%)	62(100%)
Vapleu village	105	52 (49,53%)	2 (5,6%)	15 (41,7%)	0 (0%)	19 (52,8%)	0 (0%)	36 (100%)
Tron Touba village	322	77 (23,92%)	12 (22,6%)	28 (52,8%)	1 (1,9%)	12 (22,6%)	0(0%)	53 (100%)
Toupé village	208	64 (30,76%)	10 (27,8%)	16 (44,4%)	0 (0%)	10 (27,8%)	0 (0%)	36 (100%)
Sokala Sobara village	102	47 (46,07%)	7 (18,4%)	8 (21,1%)	2 (5,3%)	20 (52,6%)	1(2,6%)	38 (100%)
Total	896	317 (35,37%)	50 (22,2%)	89 (39,6%)	12 (5,3%)	71 (31,6%)	3(1,3%)	225 (100%)

Storage containers (kettles, barrels, barrels, canaries, cans, basins, bowls, buckets), **Abandoned storage containers/objects**: disposable cups, abandoned plates, boxes, abandoned calabashes, broken canaries, children's toys, cut cans, broken basins; **Natural** (tree hollows, sheathing plants, tree leaves, rock hollows); **Others** (concrete tanks, hollow bricks).

3.3 *Aedes* species identified at different oviposition trap heights in the stations

During this study, 16 species of the genus *Aedes* were identified including: *Aedes aegypti*, *Aedes africanus*, *Aedes apicoargenteus*, *Aedes denderensis*, *Aedes dendrophilus*, *Aedes fraseri*, *Aedes haworthi*, *Aedes lilii*, *Aedes longipalpalis*, *Aedes luteocephalus*, *Aedes metallicus*, *Aedes opok*, *Aedes palpalisi*, *Aedes schwetzi*, and *Aedes vittatus*. It was noticed that *Aedes aegypti* was most frequent

(Table 2). The oviposition traps set in the stations at different levels above the ground, enabled the identification of four species at heights ≥ 5 m: *Aedes apicoargenteus* (at 5, 10, 15 and 20 meters from the ground), *Aedes aegypti* (at 5 and 20 meters from the ground), *Aedes haworthi* (at 5, 10, 15 and 20 meters from the ground) and *Aedes lilii* (at 5 meters from the ground). It was noticed that the ideal height for *Aedes* breeding was 1.5m (Table 2).

Table 2 : *Aedes* spp. identified from oviposition traps at different heights in the sampled stations

Species	Height of oviposition trap				
	1.5 m	5 m	10 m	15 m	20 m
<i>Ae. aegypti</i>	452 (55.7%)	12 (21.8%)	0 (0%)	0 (0%)	5 (83.3%)
<i>Ae. africanus</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. apicoargenteus</i>	126 (15.5%)	36 (65.5%)	8 (100%)	7 (87.5%)	1 (16.7%)
<i>Ae. denderensis</i>	62 (7.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. dendrophilus</i>	20 (2.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. fraseri</i>	79 (9.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. haworthi</i>	17 (2.1%)	1 (1.8%)	0 (0%)	1 (12.5%)	0 (0%)
<i>Ae. lillii</i>	37 (4.6%)	6 (10.9%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. longipalpalis</i>	1 (0.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. luteocephalus</i>	16 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. metallicus</i>	1 (0.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. opok</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. palpalis</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. schwetzi</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. unilineatus</i>	1 (0.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Ae. vittatus</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Species number	11	4	1	2	2
Total	812 (100%)	55 (100%)	8 (100%)	8 (100%)	6 (100%)

3.4 *Aedes* species diversity in prospected stations

The Shannon diversity index (H') calculated for each station indicated that for the south station (Banco national park), 10 *Aedes* species were collected after oviposition trapping and larval surveys. Here, the H' was 0.42 with a maximum diversity (H' max) of 2.3 (Table 3). In the western station (Vapleu village), 8 species were

identified and Shannon's index and H' max were 1.09 and 2.07 respectively (Table 3). In the northwest (Tron Touba village) and northeast (Toupé) stations, 11 species were collected with H' max of 2.39 (Table 3). At the Sokala Sobara station (center), 9 species were identified and the Shannon index and maximum diversity values were 1.09 and 2.19, respectively (Table 3).

Table 3 : Species diversity of *Aedes* in sampled stations

Species	Study stations				
	PNB	VAP	TRON	TOUP	SOKA
<i>Aedes aegypti</i>	318	502	1076	1104	356
<i>Aedes africanus</i>	1	-	-	38	-
<i>Aedes apicoargenteus</i>	9	189	28	-	54
<i>Aedes denderensis</i>	2	38	12	45	7
<i>Aedes dendrophilus</i>	2	-	3	19	6
<i>Aedes fraseri</i>	6	63	6	50	4
<i>Aedes haworthi</i>	-	-	-	17	2
<i>Aedes lillii</i>	-	6	3	11	68
<i>Aedes longipalpalis</i>	-	-	1	-	-
<i>Aedes luteocephalus</i>	2	5	10	29	-
<i>Aedes metallicus</i>	-	8	5	3	-
<i>Aedes opok</i>	2	-	-	-	-
<i>Aedes palpalis</i>	1	-	-	1	-
<i>Aedes schwetzi</i>	2	2	-	-	1
<i>Aedes unilineatus</i>	-	-	1	-	-
<i>Aedes vittatus</i>	-	-	11	3	26
Total	345	811	1156	1320	524
Species number	10	8	11	11	9
Shannon index (H')	0,42	1,08	0,38	0,69	1,09
Maximum diversity (H' max)	2,3	2,7	2,29	2,39	2,19

PNB: Banco National Park; **VAP:** Vapleu village; **TRON:** Tron Touba village; **TOUP** : Toupé village; **SOKA** : Sokala Sobara.

4. Discussion

Aedes colonize a wide range of breeding places. In this study, *Aedes* deposits were mainly storage containers, abandoned containers / objects, abandoned tires, natural deposits and other types of

deposits. In most prospected sites, the presence of larval stages of *Aedes* in water collections has been strongly influenced by human activities such as tourist hikes (in Banco National Park), management of water storage materials, construction of houses, and

installation of garages. Moreover, majority of these deposits have been encountered around human dwellings [34]. This observation is similar to that made by Tia *et al.* [35] (2016) in Oussou-yaokro and Korhogo (Côte d'Ivoire) and by Koumba *et al.* [28] in Mouila (Gabon). The proximity of individuals to *Aedes* sp. breeding places could pose a health risk to them.

Five *Aedes* larval breeding substrates were identified in the sampled stations including: storage containers, abandoned containers / objects, abandoned tires, natural deposits and others. These *Aedes* breeding substrates have already been reported in urban areas of Côte d'Ivoire [16, 15, 17, 18]. Similarly, in the forest environment of Senegal and Côte d'Ivoire *Aedes* females preferred laying eggs in wide variety of substrates [22, 36].

Sixteen species of *Aedes* were identified in this study and included *Aedes aegypti*, *Aedes africanus* and *Aedes luteocephalus*, vectors of several arboviruses [37, 38]. *Aedes aegypti* was most frequent in the study area and several studies have established its role in the transmission of yellow fever and dengue viruses in urban and rural areas of Côte d'Ivoire [10, 15, 16, 17, 18, 26]. However, *Aedes africanus* and *Aedes luteocephalus* are vectors of yellow fever virus [23, 37, 38]. The species number in this study is superior to that obtained by Zahouli [8], but inferior to that of Zahouli *et al.* [39] in the south-east of Côte d'Ivoire. This difference in species numbers obtained in the different studies could be related to the differences in sampling methods, the duration of sampling and number of study stations.

All the species of the genus *Aedes* identified in this study occurred in sympatry or allopatry in the different environments. This distribution could be due to the anthropophilic and / or zoophilic traits of these mosquito populations as well as presence of nutritional sources, landscape, and breeding sites. The aforementioned factors, associated with weather variables could be the drivers of the distribution of mosquitoes in an environment [40, 41, 42, 43, 44]. Oviposition traps placed at different altitudinal levels revealed that females of the following: *Aedes aegypti*, *Aedes apicoargenteus*, *Aedes haworthi*, *Aedes lilii* bred at 20 meters above the ground. This could be due to the high plasticity of these species that enables them to adapt to such breed at such a height.

The highest frequency of *Aedes aegypti* in the sampled stations resulted in a relatively low Shannon indices compared to maximum diversity values. The Shannon diversity index of 0.42 was obtained for the Southern station, 1.08 for the western station, 0.38 for the northwest station, 0.69 for the northeast station, and 1.09 for the center station. These low Shannon's diversity indices reflects the poor species diversity in the study area.

5. Conclusion

The main breeding substrates of *Aedes* spp. in the prospected stations consisted of storage containers, abandoned storage containers/objects, abandoned tires, and natural. Sixteen species of *Aedes* spp. were identified in the study area with *Aedes aegypti* recording the highest frequency. The ideal *Aedes* breeding height in the prospected stations is 1.5 m. Highest *Aedes* species diversity in the study area was recorded at Sokala Sobara and Wasségbôgbô stations as well as in Vapleu and Krozialé stations. The occurrence of several species of *Aedes* indicates the risk of arboviruses spillover in the study area.

6. Declaration of interest

The authors declare that they have no competing interest in relation to this article. All the authors read and approved the final version.

7. Acknowledgements

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