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Cartography of the lists of mosquitoes in the department of the littoral, a tool for fight against urban malaria in Benin

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Abstract
The study was carried out from January to December 2013 during the period of alternation between the long dry season and the small rainy season in the city of Cotonou, Southern Benin. The aim of the present study was to provide a mapping of breeding sites that highlights the boroughs of Cotonou with strong development of *Anopheles*, *Culex* and other mosquitoes. For this purpose, breeding sites were identified in each borough during both dry and rainy seasons. Then, the geographical coordinates of each site were noted. Mosquito larvae were collected and their density evaluated.
A total of 997 breeding sites were enumerated. The most prolific areas in both rainy and dry seasons were identified: 1st, 6th, 12th and 13th borough. In these boroughs, a multitude of breeding sites were found throughout the year. This situation is probably due to the lack of adequate road network, predominance of flood areas, inadequate and poorly managed sanitation and uncontrolled territorial occupation.

Keywords: Cartography, breeding site, *Anopheles*, *Culex*, Cotonou

1. Introduction
Humanity undergoes important nuisances and diseases with disastrous human and economic consequences because of mosquitoes [1, 2]. On the one hand, their bites can cause severe irritations for human being or animals [2] and on the other hand, mosquitoes are responsible for the transmission of many disease-causing agents of medical and veterinary importance (virus, bacteria, protozoa or nematodes) [2]. Mosquito vector-borne diseases have recently experienced a strong upsurge [3] and account for 14% of infectious diseases and 28% of emerging diseases that affect public health and the global economy [3-5]. Environment is a major determinant of the epidemiology of malaria because of the vectorial nature of its transmission and the bioecological preferences of vectors. Diseases whose pathogens are transmitted by mosquitoes are present in all regions worldwide. The vector species belong mainly to three genera: *Anopheles*, *Culex* and *Aedes*. Thus, half of the world population is now exposed to the risk of malaria which is transmitted only by mosquitoes of the *Anopheles* genus [6]. Despite the efforts made to reduce the transmission of this disease, it remains a major public health problem in the world, particularly in Benin. It remains a permanent threat due to its high morbidity (212 million worldwide) and mortality (429,000) rates with Africa being the most affected continent (92%) far ahead of the regions of South-East Asia (6%) and Eastern Mediterranean (2%) [7]. Improving the health of populations is a concern at both international and national levels. In Benin, improvement of health care and hospital technical platform observed over past decades has favored a significant demographic growth with a population which has increased from 2082511 in 1961 to 6769914 in 2002 and 9364619 in 2012 [7]. This demographic explosion over a limited space has led to major consequences including the anarchic occupation of the land and the degradation of the living environment of the population (Bio Bangana, personal communication). In Cotonou, many mosquito breeding sites are produced in neighborhoods due to poor environmental management and inadequate drainage system [8]. Density of the *Anopheles* and *Culex* mosquitoes varies according to the degree of urbanization of neighborhoods. One of the factors which influences the distribution and availability of breeding sites in the Littoral department is flooding. Classic periods of flood and recession modify the distribution of mosquito larval habitats.

Indeed, ends of third quarter and of last quarter of the year correspond respectively to the period of flood and recession [9]. In addition, the littoral department presents a very favorable environment for the development of mosquitoes due to the presence of numerous shallows and the shallow water table. In addition, the existence of two rainy seasons in southern Benin is an important factor in the long presence of mosquito breeding sites in Cotonou [10]. The purpose of this study was to identify the boroughs of high larval development of the *Anopheles* and *Culex* mosquitoes to allow the Ministry of Health to better refine its strategies to protect populations from mosquito biting.

2. Materials and methods

2.1 Study area

The present study was carried out from January to December 2013 during the period of alternation between the long dry season and the small rainy season in the city of Cotonou, Southern Benin. The city of Cotonou is located on a low and sandy coastal plain between 6 ° 20' and 6 ° 23 N latitude and 2 ° 22 and 2 ° 30' E longitude (Fig 1). It is limited to the East by the district of Sèmè-kpodji, to the West by the district of Abomey-Calavi, to the North by the districts of Sô-Ava and Aguégués and to the South by the Atlantic Ocean. It extends on both sides of a channel that connects Lake Nokoué and the Atlantic Ocean on an area of 7006 hectares of which 70% are located west of the channel. 33% of the area of Cotonou consists of swampy areas and 67% of urbanized areas [11]. The relief; climatic, edaphic and hydrological conditions and populations' density as well as behaviors and practices of individuals are factors which allow the proliferation of mosquitoes in the Littoral department.

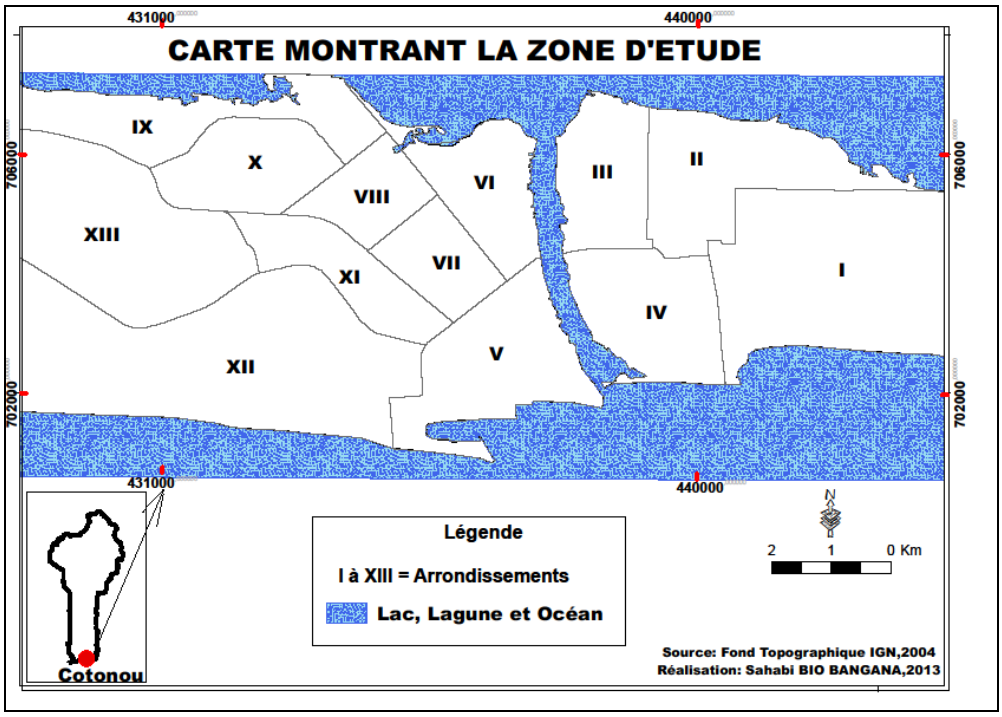

Fig 1: Map showing the study area

2.2 Field data collection

It was carried out through identification and taking of geographical coordinates of mosquito breeding sites. The ladle method was used to assess mosquito larvae density [12]. Indeed, the transition period between the dry and the rainy seasons was chosen as a favorable period for taking geographic coordinates of mosquito breeding sites. All the boroughs of Cotonou were investigated in a scrutiny way for the identification of the mosquito breeding sites. The global position system (GPS) was used to determine the geographic coordinates of the identified breeding sites. Two neighborhoods were chosen per day and all breeding sites were enumerated through all streets.

Mosquito larvae density was assessed using two methods. A visual method relied on counting the number of larvae present in a given setting was used for the determination of larval density. To this has been coupled the traditional method of ladle [13]. Depending on the extent of the larval site, this method is based on two techniques.

When the breeding site is extended and/or deep (gutter, pond, sump, well... etc), larvae density measurement was made in a specific way because it is impossible to collect all the water so as to count the number of larvae. For this, the breeding site was divided into 4 and with a ladle, the bed water was sampled ten times in each of the four portions. Then, the number of *Anopheles* and / or *Culex* larvae observed in each of the portions was counted using a pipette. Finally, the density of *Anopheles* and / or *Culex* larvae was calculated using the Bruce-Chwatt technique [13].

By cons, when the breeding site is very small (small water collections, footprints, flowerpot, abandoned shoes, etc...), larvae density measurement was made by taking all the water in a plastic cup using a ladle of fine capacity. The water was left to decant and then using a ladle, it was taken ten times and then introduced into a second plastic cup. The *Anopheles* and *Culex* larvae were then identified, separated and counted. The total number of mosquito larvae in the breeding site was determined by the method of Bruce-Chwatt [13, 14].

2.3 Data processing

Geographical coordinates of the identified breeding sites in the city of Cotonou were recorded thanks to a computer equipped with maps processing softwares (Arc-view 3.2 and Arc-Gis 9.3). Then, in order to establish homogeneous classes of Cotonou's boroughs according to the duration of impoundment of the mosquito breeding sites, a numerical

classification was carried out with SAS software. The description of the different classes was made from a Principal Component Analysis (PCA) on the established classes. The log-linear analysis applied to the absolute frequencies of the larval breeding sites of the different types of mosquito according to the duration of impoundment was also carried out within the various boroughs.

3. Results

3.1 Availability and characterization of breeding sites in the districts of Cotonou

Of a total of 997 identified breeding sites in the study area, 44% were positive. The distribution of positive and potential breeding sites varied from one borough to another. Overall, the frequency of breeding sites was low in the 2nd, 3rd, 4th, 7th, 8th, 9th, 10th and 11th boroughs while it was high in the 1st, 5th, 6th, 12th and 13th boroughs. The largest number of potential breeding sites was recorded in the 13th borough (n = 156) followed by the 1st borough (n = 117) (Fig 2).

Of the 443 identified positive breeding sites, mixed breeding sites with Anopheles and Culex larvae accounted for 42%, compared with 35% for Culex breeding sites and 23% for Anopheles breeding sites. The Aedes, Mansonia, Erecmapodites and Uranotenia breeding sites were rare in the study area.

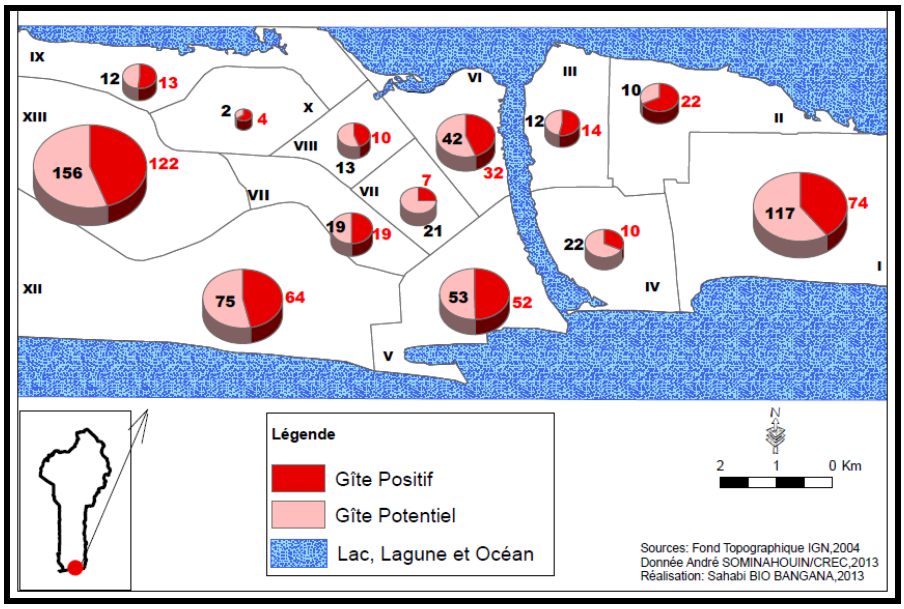

Fig 2: Spatial distribution of positive and potential breeding sites

3.2 Distribution of mosquito breeding sites according to the duration of impoundment

135 (31%) of the 443 positive breeding sites were temporary, 34% were permanent, and 35% were semi- permanent.

In addition, the numerical classification performed with the SAS software grouped the 13 boroughs into 3 classes with a value of R^2 (coefficient of determination) equal to 0.75 (Fig 3). The 13th borough of Cotonou was the place where permanent, semi-permanent and temporary mosquito breeding sites were all found in large numbers, whereas in the 1st, 6th and 12th borough, *Anopheles* permanent breeding sites predominated. The other boroughs have relatively low frequencies of breeding sites (Fig 3). Photos 1, 2, 3 and 4 show the different types of breeding sites encountered in the various boroughs of Cotonou.

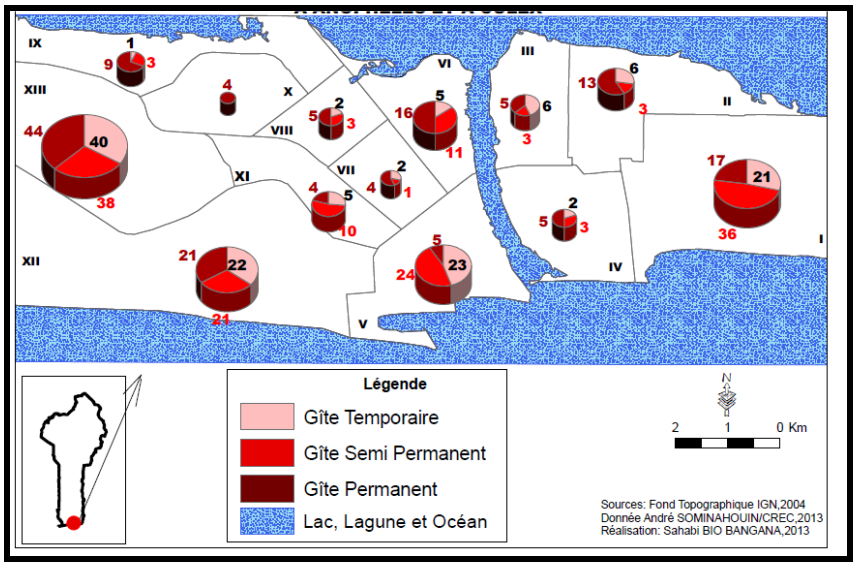

Fig 3: Map showing the impoundment of the *Anopheles* and *Culex* breeding sites

Photo 1: Culex Permanent breeding site at Donatin, 1st borough

Photo 2: Anopheles Permanent breeding site at Houéyiho, 11th borough

Photo 3: Mixed temporary breeding site at Mènontin, 9th borough

Photo 4: Anopheles and Culex mixed semi- permanent breeding site at Vossa, 6th borough

3.3 Frequency and spatial distribution of *Anopheles* and *Culex* breeding sites by season

Table 1 presents the results of analysis of the relationship between the absolute frequencies of breeding sites of different genera of mosquitoes and the duration of impoundment. The frequency of the mosquito genus was highly dependent on the duration of the impoundment of the breeding sites (p<0.05) in the various boroughs of Cotonou. The same result was obtained regarding the interaction between these two factors, which implies that the effect of the genus on the frequency depends on the duration of impoundment of mosquitoes and vice versa.

Table 1: Results of log-linear analysis on breeding sites and the duration of impoundment.

Source	DDL	Khi-2	Prob. > Khi-2
Larval gîtes	2	1872.87	<0,0001
Duration of impoundment	2	431.18	<0,0001
Kind*Duration	4	82.28	<0,0001

DDL= Degree of freedom Prob= Probability

- ***Anopheles* breeding sites**

Larvae collections allowed identification of 101 breeding sites of *Anopheles*, of which 43 temporary (42%), 35 semi-permanent (35%) and 23 permanent (23%). This distribution of the *Anopheles* breeding sites according to the duration of their impoundment revealed that the *Anopheles* larvae prefer more the temporary breeding sites than those semi-permanent and permanent. In addition, the spatial distribution of the *Anopheles* breeding sites showed that Anopheles' temporary and semi-permanent breeding sites predominated in the 1st, 2nd, 5th, 12th and 13th boroughs. The permanent breeding sites of Anopheles were in the majority in the 12th, 1st, 5th and 8th boroughs (fig 4).

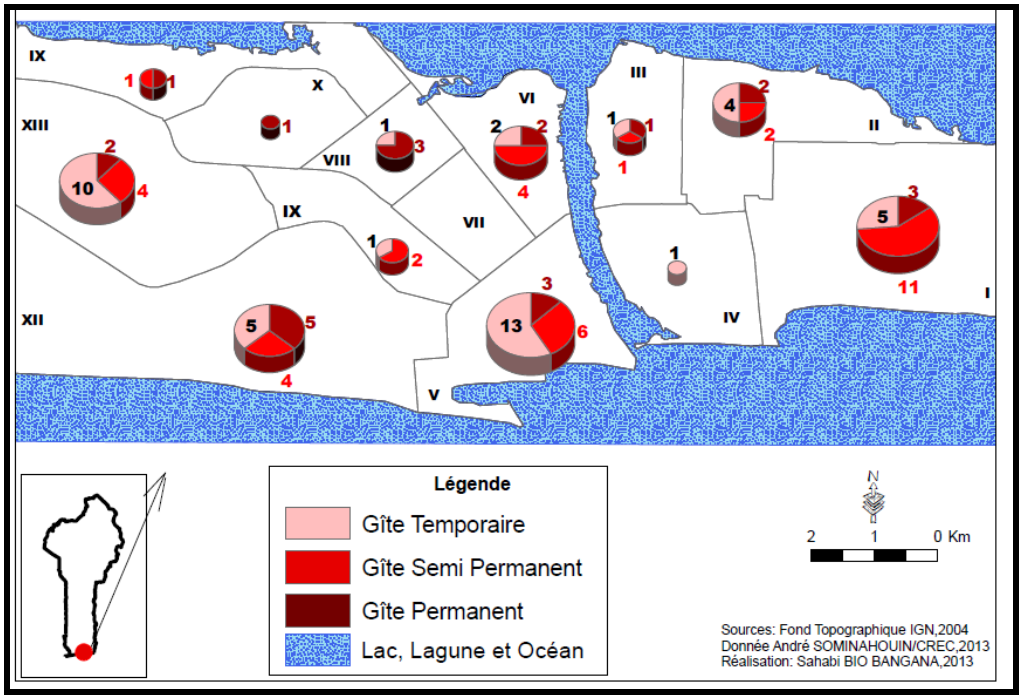

Fig 4: Spatial distribution of *Anopheles* breeding sites in the districts of Cotonou

- ***Culex* breeding sites**

Of a total of 154 breeding sites of *Culex* encountered, 40% were permanent, 33% semi-permanent and 27% temporary. So, *Culex* mosquito larvae preferred permanent breeding sites rather than those semi-permanent and temporary. Analysis of Fig 5 showed that a high frequency of permanent breeding sites of *Culex* was recorded in the 13th borough compared to the 1st, 2nd, 6th and 12th boroughs. By cons, the temporary and semi-permanent breeding sites of *Culex* were present in the 1st, 5th, 12th and 13th boroughs of Cotonou.

In addition, mixed *Anopheles* and *Culex* breeding sites were registered. Thus, out of a total of 188 mixed breeding sites identified, 37% were semi-permanent, 36% permanent and 27% temporary. These mixed breeding sites are unequally distributed in the boroughs. In the 1st, 12th and 13th boroughs, a high frequency of permanent, semi-permanent and temporary breeding sites was recorded (Fig 6). Moreover, in the 2nd, 9th and 10th boroughs, almost all (93%) of the mixed breeding sites were permanent.

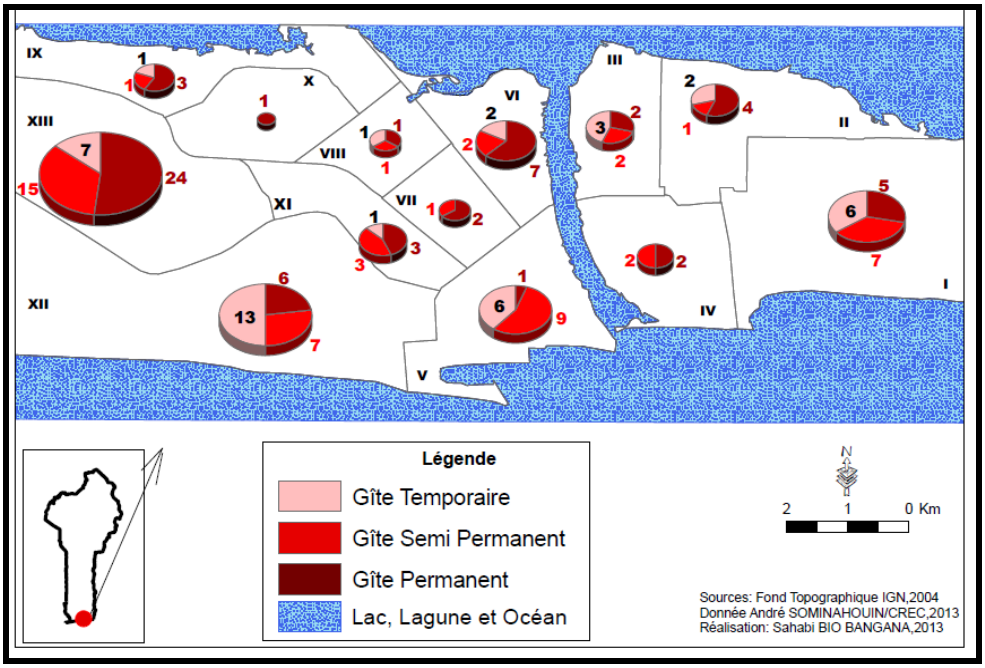

Fig 5: Spatial distribution of *Culex* breeding sites in the boroughs of Cotonou

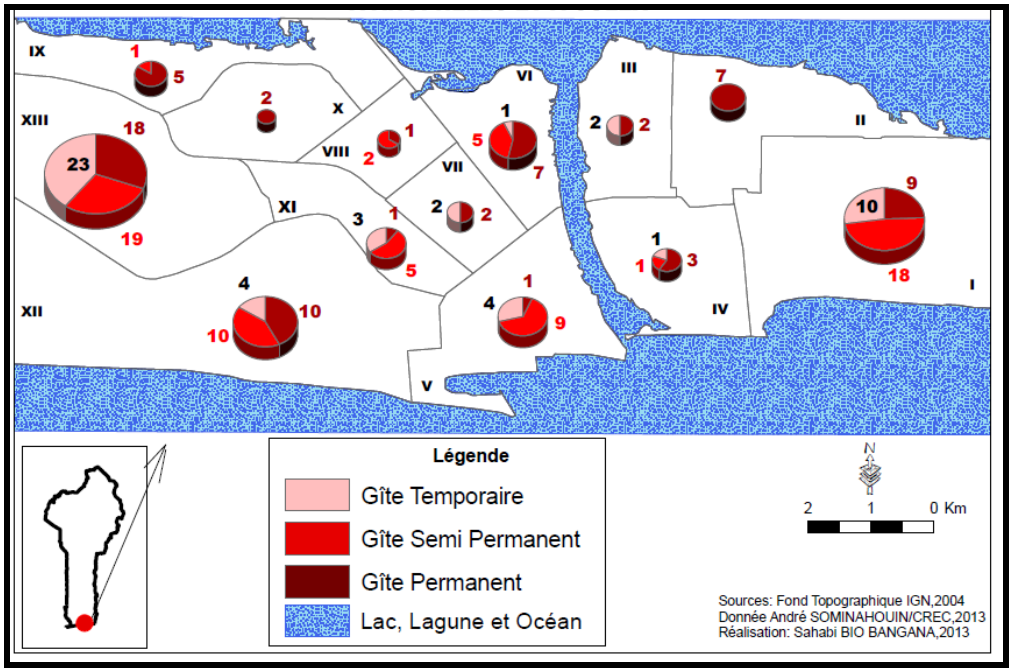

Fig 6: Spatial distribution of mixed (*Anopheles* and *Culex)* breeding sites in the Cotonou boroughs

3.4 Typology of positive breeding sites

The typology of the *Anopheles* breeding sites has shown that the larvae of this genus of mosquito proliferate much more in the shallows in the 9th and 10th boroughs (100%) and also in the puddles created by the imprints of vehicles in the 1st (58%), 5th (41%), 6th (63%) and 13th (63%) boroughs. In these same boroughs, the frequency of *Anopheles* larvae was also as follows in the wells (16% in the 1st, 23% in the 5th and 19% in the 13th), in the gutters (16% in the 1st and 23% in the 5th) and in the sumps (25% in the 6th) (Fig 7). In the Culex breeding sites, a high frequency of larvae was recorded in sumps (28%, 71%, 100%, 25%, 31% and 26% respectively in the 1st, 3rd, 4th, 5th, 12th and 13rd boroughs), in the puddles (33% in the 1st and 45% in the 6th borough), in the gutters (44% in the 5th and 38% in the 12th borough) and in the shallows (40 % in the 9th and 22% in the 13th borough) (Fig 8). Within mixed breeding sites (Anopheles and Culex), a high rate of larvae of these two genera of mosquitoes was recorded in the sumps (21% in the 12th and 23% in the 13th borough), the shallows (29% in the 12th borough) and 35% in the 13th borough), gutters (50% in the 4th and 50% in the 7th borough), puddles (67% in the 11th borough), wells (59% in the 13th borough) and flooded houses (12% in the 13th borough) (Fig 9).

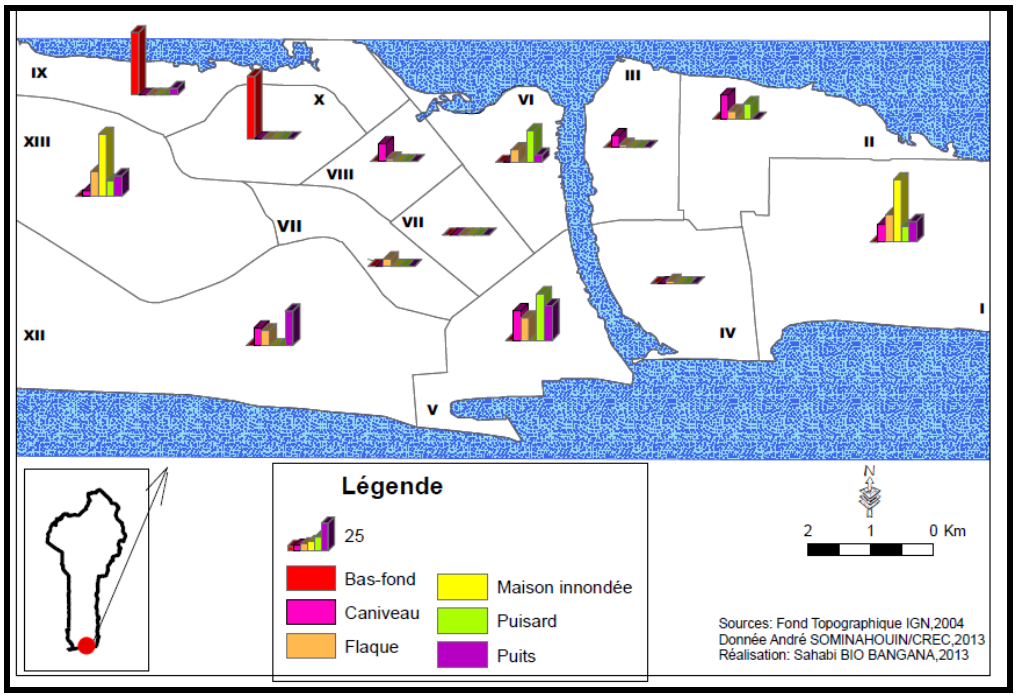

Fig 7: Typology of *Anopheles* breeding sites at Cotonou

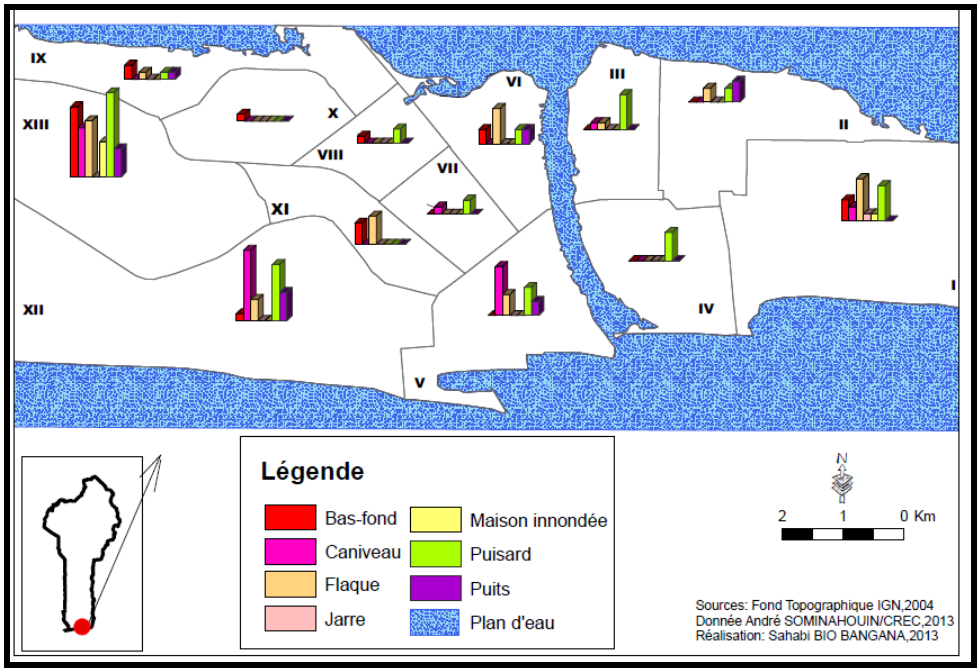

Fig 8: Typology of *Culex* breeding sites at Cotonou

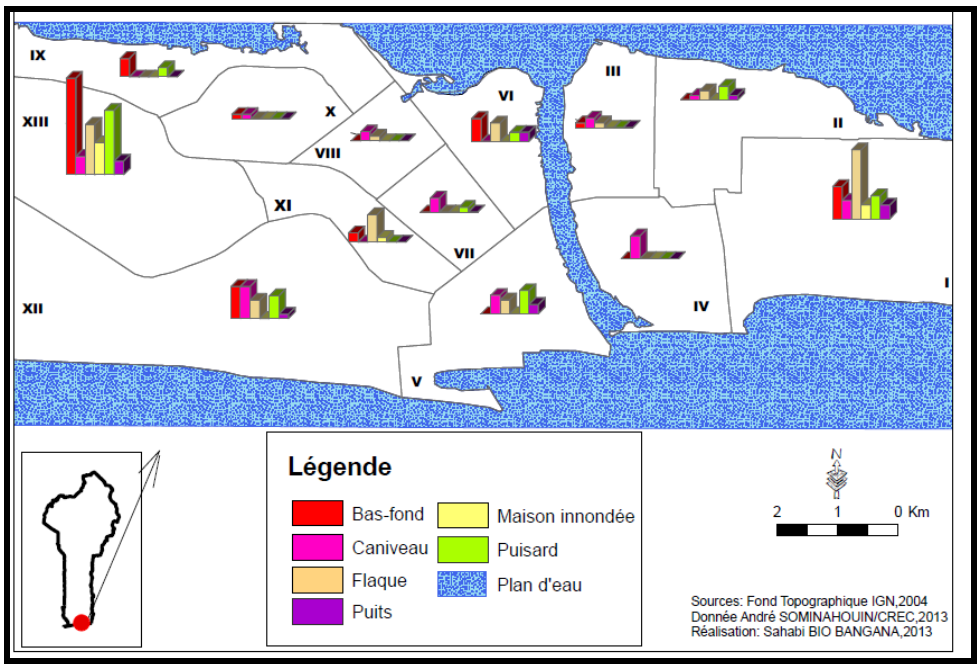

Fig 9: Typology of Anopheles and Culex breeding sites

3.5 Larval density according to the boroughs

A total of 1, 8307 mosquito larvae were counted. The spatial distribution of the cumulative larval density of mosquito breeding sites showed a high density of mosquito larvae in the 1st, 2nd, 6th, 12th and 13th boroughs. In addition, the cumulative density of *Anopheles* and *Culex* larvae (mixed breeding sites) was high in the 1*st*, 6th, 12th and 13th boroughs of Cotonou while that of Culex larvae was high in the 2nd, 3rd, 12th and 13th boroughs. As for the density of *Anopheles* larvae, it was high in the 1st, 5th, 8th, 12th and 13th districts (Fig 10).

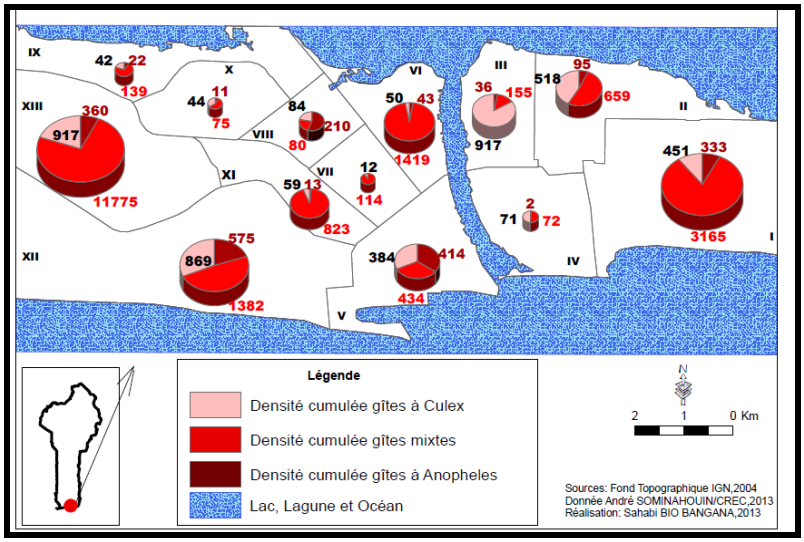

Fig 10: Spatial distribution of cumulative density of mosquito breeding sites

4. Discussion

The current study showed heterogeneity of the distribution of permanent, semi-permanent and temporary breeding sites in the thirteen boroughs of Cotonou according to the seasons. These three impoundments are characterized by different biotic and abiotic factors and have impacts on the spatial distribution of mosquito larvae in the Littoral Department. The 1st, 12th and 13th boroughs were characterized by a multiplicity of mosquito breeding sites. This is mainly due to a water drainage system which is almost non-existent. As a result, water stays in the same place in houses during a good period of the dry season. The sumps that exist there are not protected. In addition, domestic wastewater is either dumped on the streets or thrown into the few existing gutters. This results in an abundant proliferation of *Culex* breeding sites. From January to December 2013, we noted the presence of two types of Culicidae breeding sites in the 13 boroughs. Of a total of 997 listed breeding sites, 443 were positive. The mixed (Anopheles and Culex) breeding sites accounted for 42%, followed by the Culex breeding sites (35%) and the Anopheles breeding sites (23%). The rarity of Mansonia breeding sites could be due to the "dipping" method used to collect mosquito larvae. This method is not suitable for collecting larvae of this genus, which live most on aquatic plants [15].

Of the 443 identified positive breeding sites, 35% were semi-permanent, 34% permanent, and 31% temporary. Differences noted between the frequency of temporary, semi-permanent and permanent breeding sites are significant (p<0.05). In the district of Cotonou, most breeding sites are semi-permanent. This result is contrary to the works of Bio Bangana *et al.* [16] who have shown that temporary breeding sites were the most commonly found in the Ouidah Kpomassè Tori bossito region, an area located a few kilometers from Cotonou. This observed difference could be due to the different nature of the soil of the two zones. In the first and sixth boroughs, the number of permanent and semi-permanent breeding sites is high. This is explained by the fact that these boroughs are flooded during the rainy season and at the advent of flood. Also, the high concentration of administrative real estate infrastructures in these boroughs is still not favorable to the reduction of the breeding sites. So, there is stagnation, that is to say a very difficult flow of runoff in these boroughs.

The Anopheles breeding sites were temporary (42%) and represented by puddles, while those of Culex were permanent (40%) and represented by sumps, gutters and shallows. The differences noted between the two genera would be related to differences in biotic and abiotic conditions of the environment [12]. Mosquito breeding sites proliferate more in the rainy season than in the dry season. These results confirm with those of Patrick [15] and Bio Bangana *et al.* [16]. According to the latter, seasonal variations of Culicidae larvae are strongly correlated with rainfall. The high density of mosquitoes observed in the first, sixth, twelfth and thirteenth districts during the rainy season (October) compared to the dry season (December and January) would be explained by several biotic and abiotic factors specific to each borough and each middle. In fact, during the month of October, repeated rains help maintain a waterlogged soil. Such soil is very favorable to the production of larval breeding sites, since without water intake, mosquitoes cannot develop. This situation, added to the very low groundwater level and the sandy soil texture of the study area, would limit the infiltration of rainwater in places and, maintain temporary mosquito breeding sites during a good period of the rainy season. It should also be noted that during this period, the sunshine is moderate, which would limit the evaporation of the water.

Our works are similar to those Mouchet *et al* which showed the different species of anopheles exploiting a large variety of water collections as lodgings, including residual pools of sunny stagnant surfaces, upright ponds, brackish water etc. *Anopheles gambiae* preferentially uses residual pools of sunny stagnant surfaces in several parts of Africa [17].

The conductivity and turbidity of larval breeding sites has been elevated in Bandundu-ville and as such has a negative influence on density. Malaria transmission is sustainable in Bandundu-ville because of the permanence of the breeding sites both during the rainy season and during the dry season and the presence of the New Year. gambiae sl, as well as its infectivity to plasmodia [18-20].

Moreover, the high density of *Anopheles* larvae recorded in the 1st, 5th, 8th, 12th and 13th boroughs could be explained by the presence of temporary and semi-permanent breeding sites of *Anopheles* gambiae constituted by the water table, practices and behaviors of individuals. The low density of mosquito breeding sites found in the fifth and seventh districts confirms the works of Bio Bangana in 2004 (Bio Bangana, personnal communication). This situation can be due to the quite good state of the drainage system in these boroughs and the protection of the majority of sumps.

Larvae of *Anopheles gambiae* are never found in brackish water. This explains the considerable reduction in the density of *Anopheles* larvae in the second, third, fourth, ninth and tenth districts of the city of Cotonou, which are boroughs bordering the Nokoué lake, the Cotonou Lagoon and the Atlantic Ocean where a relatively high salinity rate has been shown by surveys of the National Institute of Statistics and Economic Analysis [21].

5. Conclusion

The study showed that the frequency of breeding sites was low in the 2nd, 3rd, 4th, 7th, 8th, 9th, 10th and 11th boroughs while it was high in the 1st, 5th, 6th, 12th and 13th boroughs. The largest number of potential breeding sites was recorded in the 13th borough followed by the 1st borough. Furthermore, the 13th borough of Cotonou was the place where permanent, semi-permanent and temporary mosquito breeding sites were all found in large numbers, whereas in the 1st, 6th and 12th borough, *Anopheles* permanent breeding sites predominated.

Overall, there are three types of mosquito breeding sites in the city of Cotonou, namely temporary, semi-permanent and permanent breeding sites. Finally, raising awareness of hygiene and environmental sanitation remains a challenge for authorities in charge of vector control, who should consider this strategy as an important activity in the vector control.

6. Competing interests

The authors declare that they have no competing interests.

7. Acknowledgment

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