Djoufounna *et al.*
The Journal of Basic and Applied Zoology (2022) 83:30
https://doi.org/10.1186/s41936-022-00290-x

The Journal of Basic and Applied Zoology

RESEARCH

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Larval habitats characterization and population dynamics of *Culex* mosquitoes in two localities of the Menoua Division, Dschang and Santchou, West Cameroon

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Abstract

Background: Choosing an appropriate strategy for mosquito vector control in an eco-climatic facies requires knowledge on vector ecology including population dynamics of species and productivity of the breeding sites. The aim of this study was to characterize *Culex* mosquitoes breeding places and to determine their population dynamics in two localities (Dschang and Santchou) separated by a forest cliff in the West Region of Cameroon. Field surveys were conducted from November 2019 to June 2020 in Dschang and Santchou. Mosquito breeding sites were georeferenced, and for each breeding site, physical parameters were measured and immature stages were collected. The collected immatures were reared in the laboratory until adult stage. Adult mosquitoes were identified using a stereomicroscope and morphological identification keys.

Results: A total of 44 breeding sites were identified: 24 in Dschang and 20 in Santchou. They were grouped into seven types and were mostly shallow, close to human dwellings, sunny, with organic matters and of anthropogenic nature. A total of 2706 mosquitoes belonging to four genera were identified. *Culex* genus was the most represented (90.4%) and was made up of *Culex pipiens* s.l. (61.79%), *Culex duttoni* (23.17%) and *Culex (Culiciomyia)* sp. (05.46%). High abundance of *Culex* species was observed in Santchou (52.71%) compared to Dschang (47.28%), while Dschang ($S=3$; $H=0.87$; $D=0.54$) recorded the greatest diversity compared to Santchou ($S=2$; $H=0.23$; $D=0.11$).

Conclusions: The proliferation and persistence of *Culex* mosquitoes independently of the breeding sites and localities might be attributable to the poor environmental management which favor the creation mosquito breeding sites. This study highlights the fact that prompt sanitation measures could be undertaken in these two localities to reduce mosquito abundance and the risk of vector-borne diseases.

Keywords: Mosquito diversity, *Culex*, Breeding sites, Dschang, Santchou, Cameroon

Background

Mosquitoes threaten the live and livelihood of millions of people worldwide (Boussès et al., 2018; Braaks et al., 2018; Carnevale & Robert, 2009; WHO, 2014) because they can transmit diseases such as malaria, dengue, chikungunya, filariasis, encephalitis, Rift Valley fever, yellow fever and Zika fever (Ikram et al., 2016). According

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to WHO, mosquito-borne diseases account for about 17% of the total burden of all infectious diseases across the world (WHO, 2014). In Cameroon, several studies have been carried out on *Anopheles* mosquitoes (Antonio-Nkondjio et al., 2005; Antonio-Nkondjio et al., 2019; Bamou et al., 2018; Djamouko-Djonkam et al., 2019; Mbida et al., 2018; Tchuinkam et al., 2011; 2010), the vectors of human malaria, but little is known about the systematics and ecology of other mosquitoes species including those of the genus *Culex*. *Culex* mosquitoes are responsible of high nuisance and painful bites (Nchoutpouen et al., 2019) and are known to transmit several diseases such as lymphatic filariasis, Rift Valley fever, West Nile fever and avian malaria (Al-Ashry et al., 2018; Ikram et al., 2016; Ochieng et al., 2013; Schmid et al., 2017; Viniaker & Lavaud, 2005). These mosquitoes are largely distributed in urban settings of Cameroon (Nchoutpouen et al., 2019; Ngadvou et al., 2020) where previous studies indicated circulation of arboviruses in *Culex* mosquitoes species (Brottes et al., 1996; Salaun et al., 1969).

Like most of the large agglomerations in the intertropical zone, the cities of Dschang and Santchou are undergoing a rapid demographic growth due to population displacement resulting from social crisis. This results in a proliferation of wastewater collections which constitute potential breeding sites for *Culex* mosquitoes, which preferentially breed and adapt in permanent and/or semipermanent, organically polluted water collections (Nchoutpouen et al., 2019).

Currently, integrated vectors management is the most recommended strategy for the control of mosquito vectors. Larval source management (LSM) which requires better understanding of the ecology and composition of the local mosquito fauna is an important component of vector control (Mo'awia et al., 2020). Hence, characterization of mosquito larval habitats is a prerequisite to designing any efficient control strategy. To date, little work has been conducted in western Cameroon on *Culex* species and their larval habitats.

The current study was carried out to identify and characterize *Culex* mosquitoes breeding places and to determine their population dynamics in the western highlands (Dschang) and in the "Mbô" plain (Santchou). The cliff that separates Dschang and Santchou would be an example of an isolation barrier that would make it possible to assess the evolution of the specific richness of mosquitoes within the same region at different altitudes.

Methods

Study sites

This study was carried out from November 2019 to June 2020 (except in May) in two localities of the Menoua Division in the West Region of Cameroon, namely Dschang (N 05° 26.522, E 010° 03.153) in the western highlands (located at 1400 m of altitude) and Santchou (N 05° 16.945, E 009° 58.647) in the Mbô plain (located at 700 m of altitude) (Fig. 1), both separated by a forest cliff. Additional differences between the two sites are depicted in Table 1.

Water collections, large or small, artificial or natural, favorable to the development of immature stages of mosquitoes (i.e., eggs, larvae, pupae) were surveyed monthly. Only water collections with *Culex* larvae were considered. *Culex* breeding sites were georeferenced using a Garmin eTrex 10 Handheld GPS (Global Position System) and revisited monthly for larval collection during both rainy and dry seasons to assess the effect of seasons on mosquito presence and distribution (Fig. 1).

Physical characterization of breeding sites and collection of *Culex* immature stages

Mosquito breeding sites were characterized according to seven criteria: (a) type of breeding sites (ponds, tires, swamps, streams, etc.), (b) vegetation (absent/present), (c) distance to the nearest human habitation, (d) water quality (clear or trouble), (e) depth of breeding site, (f) water movement (current/stagnant), (g) presence/absence of organic matter, and (h) presence/absence of other Culicidae larvae. Eggs, larvae and nymphs of *Culex* mosquitoes were collected in each breeding site of each locality using the "Dipping" technique (Service, 1993). The number of dips varied according to the size of the breeding site, the quantity of water and the presence of larvae. The collected immature stages were stored in several plastic bottles (of at least 1 L of volume) labeled and then transported to the VBID-RUBAE (Vector Borne Diseases Laboratory of the Research Unit of Biology and Applied Ecology) of the University of Dschang. They were reared to adult stage in the insectary under standard laboratory conditions (27–28°C temperature; 70–80% hygrometry) before identification.

Rearing of immature stages and identification of adult mosquitoes

Immature stages collected at the different sampling sites were transferred to labeled rearing trays. Larvae were fed with MosquiFood® as described by Tchuinkam et al. (2011), and nymphs were introduced into cardboard boxes covered with mosquito nets until adult emergence. Adult mosquitoes were later knocked down in the refrigerator (for about 15 min) and identified under a binocular magnifying glass using identification keys of Jupp (1996) and Edwards (1941).

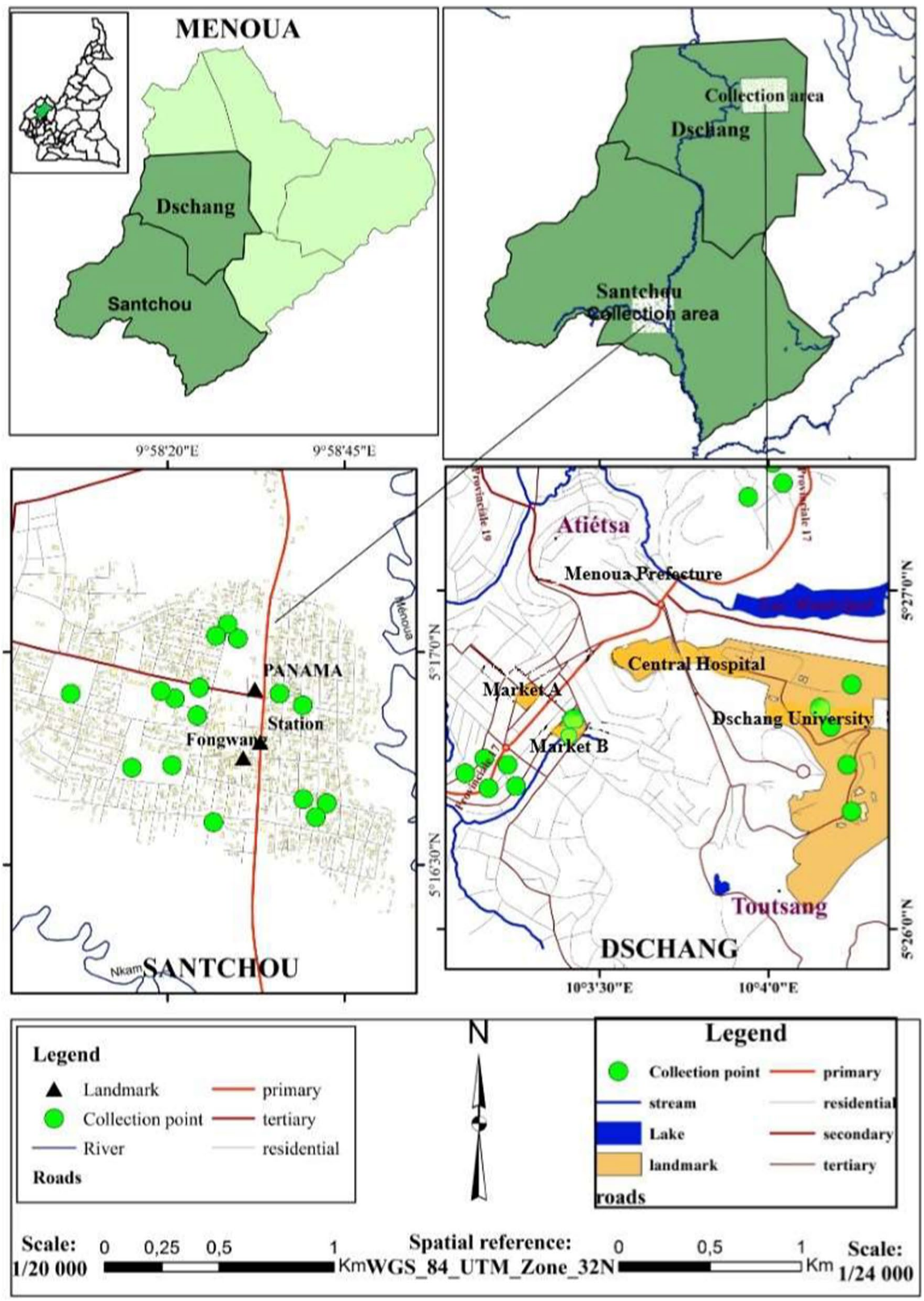

Fig. 1 Map showing the study sites Dschang and Santchou with the surveyed breeding sites

Table 1 Main characteristics of the study sites (PDCD, 2011; PDCS, 2015)

Characteristics	Study sites	
	Dschang	**Santchou**
Climate	Equatorial monsoon climate at mountain facies	Equatorial climate of Guinean type
Altitude	1400 m	700 m
Seasons	Rainy season: March–October	Rainy season: March–October
	Dry season: November–February	Dry season: November–February
Soils type	Ferralitic/basic soil	Hydromorphic soils
Temperature	18.9–21.1 °C	20.83–23.35 °C
Surface area	262 Km^2	335 Km^2
Relief	Tray	Plain
Precipitation	1654.2 mm/year	1363.3 mm/year

Data analysis

Statistical analyses were performed using R software version 4.0.3 (R version 4.0.3 2020). A Mann–Whitney test was used to compare the abundance and richness of *Culex* mosquitoes across localities and seasons. *Culex* abundance according to water quality, presence/absence of vegetation, water movement, presence/absence of organic matter and presence/absence of other Culicidae larvae were assessed using a Mann–Whitney test. A Kruskal–Wallis test was used to assess *Culex* abundance according to sunshine (sunny, semi-shaded, shady). Spearman correlation was used to compare the abundance of *Culex* according to the depth of breeding site and the distance of the breeding site from human dwellings. For all analyses, the differences were considered significant at $p < 0.05$.

Results

Typology and distribution of breeding sites in Dschang and Santchou

A total of 44 breeding sites were identified and characterized during the field survey, with 24 sites found in Dschang and 20 in Santchou. They were grouped into seven categories: gutters (A), pools (B), tires (C), metallic containers (D), plastic containers (E and G), swamps (F) and wells (H) (Fig. 2). Overall, gutters were the most frequent breeding habitats (43.2%) followed by tires (27.3%). More specifically, in Dschang, tires and gutters were the most prevalent breeding sites (37.5% and 33.3%, respectively), while in Santchou the most encountered breeding sites were gutters and pools (55% and 20%, respectively). Wells were less prevalent in both sites (4.2% in Dschang and 5% in Santchou) (Table 2).

Monthly distribution of breeding sites in Dschang and Santchou

Among the seven categories of breeding sites identified, the greatest number (six breeding sites) was recorded in November (at the end of the rainy season) in Dschang (Fig. 3). The months of January and February (long dry season) recorded only one type of breeding habitats (gutters), while the months of December, March and June (rainy season) recorded two types of breeding habitats (Fig. 3). Gutters were the most encountered and colonized by *Culex* mosquitoes and were found in six months out of the seven months of collection, especially in June.

An irregularity of the breeding sites according to months was observed in Santchou (Fig. 3). With the exception of the months of January and March (marked by the presence of only one type of habitats (gutters)), all other months recorded two types of breeding habitats, all different from 1 month to the next. Pools and gutters were the most colonized, especially in May and December, respectively.

Physical characteristics of *Culex* breeding habitats in Dschang and Santchou

Of the 24 breeding sites found in Dschang, 43.97% had only *Culex* larvae, while 56.02% were considered as mixed (presence of other larvae of Culicidae) (Table 3). On the other hand, sunny habitats contained a much greater abundance of mosquitoes (51.68%) compared to the semi-shaded and shaded habitats. In addition, mosquitoes preferred stagnant water (97.76%), without vegetation (75.28%) with a depth greater than 10 cm (72.08%). However, no significant difference of the abundance according to the different physical characteristics was observed ($p > 0.05$).

Of the 20 breeding sites found in Santchou, 46.21% consisted solely of *Culex* larvae, while 53.78% contained larvae of other Culicidae. Sunny breeding sites (66.04%) represented the most suitable places for *Culex* larvae development and appeared trouble (69.37%) with vegetation (60.23%) (Table 4). However, no significant difference of the abundance according to the different physical characteristics was observed ($p > 0.05$), except for the

Fig. 2 Breeding sites found in Dschang and Santchou

Table 2 Distribution of breeding sites in Dschang and Santchou

Types of breeding sites	Study sites					
	Dschang		Santchou		Overall	
	N	%	*N*	%	*N*	%
Swamps	0	0	1	5	1	2.3
Tires	9	37.5	3	15	12	27.3
Gutters	8	33.3	11	55	19	43.2
Wells	1	4.2	1	5	2	4.5
Plastic containers	3	12.5	0	0	3	6.8
Metallic containers	1	4.2	0	0	1	2.3
Pools	2	8.3	4	20	6	13.6
Total	24	100	20	100	44	100

N Total breeding sites in Dschang and Santchou

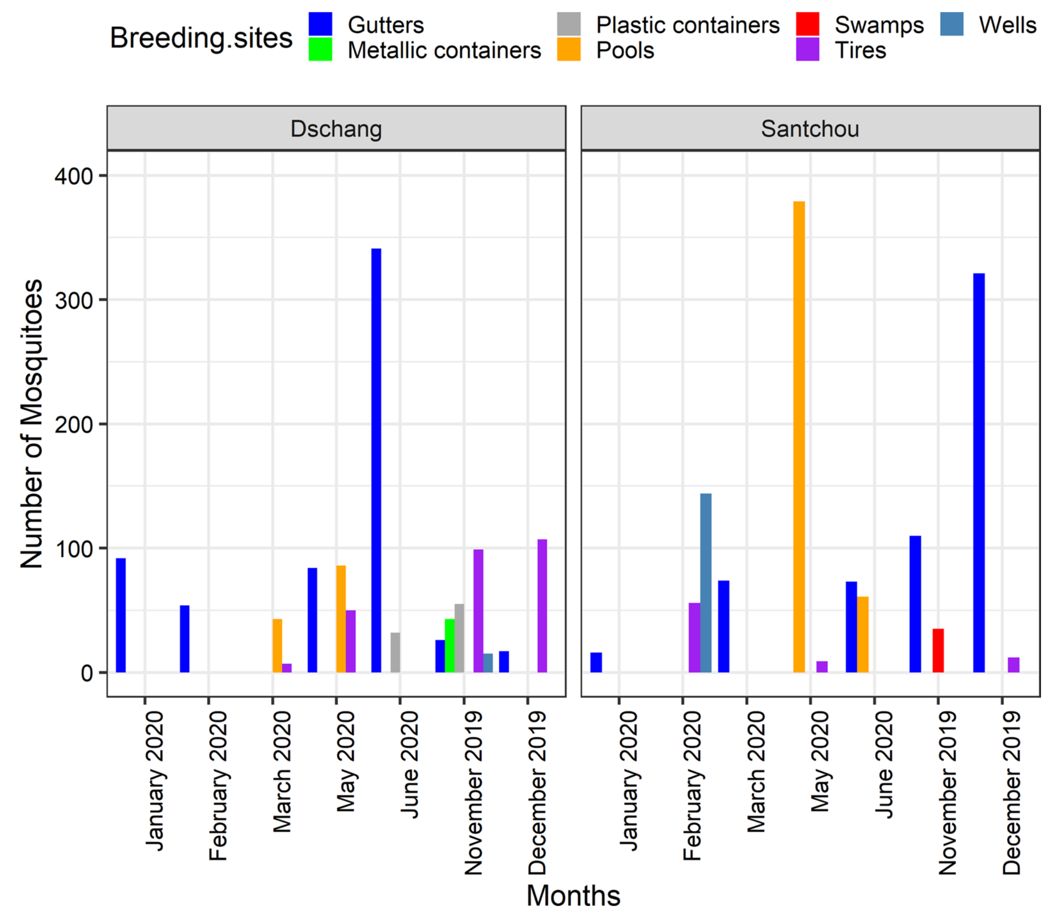

Fig. 3 Monthly variation of *Culex* breeding sites in Dschang and Santchou

Table 3 Variation of *Culex* mosquito abundance according to the physical characteristics of breeding habitats in Dschang

Characteristics	Variables	Number of breeding sites	Mosquito abundance (%)	*p* value
Sunshine	Sunny	8	598 (51.68%)	0.62
	Semi-shaded	6	260 (22.47%)	
	Shady	8	299 (25. 84%)	
Depth	≤ 10	8	323 (27.91%)	0.14
	> 10	14	834 (72.08)	
Vegetation	Present	7	286 (24.71)	0.2
	Absent	15	871 (75.28)	
Organic matters	Present	16	945 (81.67)	0.1
	Absent	6	212 (18.32)	
Water aspect	Clear	3	76 (6.56)	0.1
	Trouble	19	1081 (93.43)	
Water movement	Current	1	26 (2.24%)	0.08
	Stagnant	21	1131 (97.76%)	
Distance to human dwellings	≤ 10	14	603 (52.11%)	0.65
	> 10	8	554 (47.88)	
Other larvae of Culicidae	Present	17	739 (56.02)	0.4
	Absent	7	580 (43.97%)	

Table 4 Variation of *Culex* mosquito abundance according to the physical characteristics of breeding habitats in Santchou

Characteristics	Variables	Number of breeding sites	Mosquito abundance (%)	*p* value
Sunshine	Sunny	11	852 (66.04%)	0.56
	Semi-shaded	6	361 (27.98%)	
	Shady	3	77 (5.96%)	
Depth	≤ 10	7	234 (18.13%)	0.04
	> 10	13	1056 (81.86%)	
Vegetation	Present	12	777 (60.23%)	1
	Absent	8	513 (39.76%)	
Organic matters	Present	10	756 (58.60%)	1
	Absent	10	534 (41.39%)	
Water aspect	Clear	5	395 (30.62%)	1
	Trouble	15	895 (69.37%)	
Water movement	Current	3	78 (6.04%)	1
	Stagnant	17	1212 (93.95%)	
Distance to human dwellings	≤ 10	12	582 (45.11%)	0.36
	> 10	8	708 (54.88%)	
Other larvae of Culicidae	Present	10	746 (53.78%)	0.7
	Absent	10	641 (46.21%)	

depth which significantly influenced mosquito abundance ($p = 0.04575$) in Santchou.

Abundance and richness of *Culex* mosquitoes in Dschang and Santchou

A total of 2706 mosquitoes belonging to four genera (*Aedes*, *Anopheles*, *Culex* and *Lutzia*) and two subfamilies (Anophelinae and Culicinae) were collected in the two localities. *Culex* genus was the most abundant and represented 90.4% of the total mosquitoes collected, while *Aedes* spp., *Anopheles* spp. and *Lutzia tigripes* accounted for less than 10% of the mosquitoes collected. Species of the *Culex pipiens* s.l. were the most prevalent, representing 61.79% of the total samples followed by *Culex duttoni* (23.17%) and *Culex (Culiciomyia)* sp. (05.46%). *Culex* mosquito abundance was higher in Santchou ($n = 1290$–52.72%) compared to Dschang ($n = 1157$–47.28%), while species richness and diversity were higher in Dschang ($S = 3$; $H = 0.87$, $D = 0.54$) compared to Santchou ($S = 2$; $H = 0.23$, $D = 0.11$) (Table 5). However, no significant difference in the abundance according to the sites was observed ($p = 0.8048$). This was not the case with species richness which significantly varied across sites ($p = 0.0027$).

Figure 4 allows to appreciate species richness in both localities. It appears that the city of Dschang was richer in *Culex* mosquito species compared to Santchou.

Mosquito abundance in Dschang was higher during the rainy season ($n = 643$) compared to the dry season ($n = 514$), while the same species richness ($S = 3$) was observed in both seasons (Fig. 5). However, no significant difference in the abundance ($p = 1$) and species richness ($p = 0.4232$) across seasons was observed in Dschang.

In Santchou, the abundance of *Culex* was higher in the dry ($n = 694$) than in the rainy season ($n = 596$). Species richness was also greater during the dry season ($S = 2$) compared to the rainy season ($S = 1$) (Fig. 5). However, no

Table 5 Abundance and diversity of *Culex* mosquitoes in Dschang and Santchou

	Study sites	
	Dschang	Santchou
Abundance	1157	1290
Species richness (*S*)	3	2
Shannon index (*H*)	0.87	0.23
Simpson index (*D*)	0.54	0.11

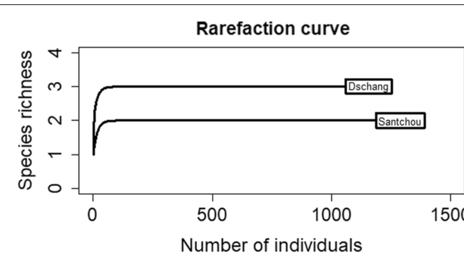

Fig. 4 *Culex* rarefaction curve in Dschang and Santchou

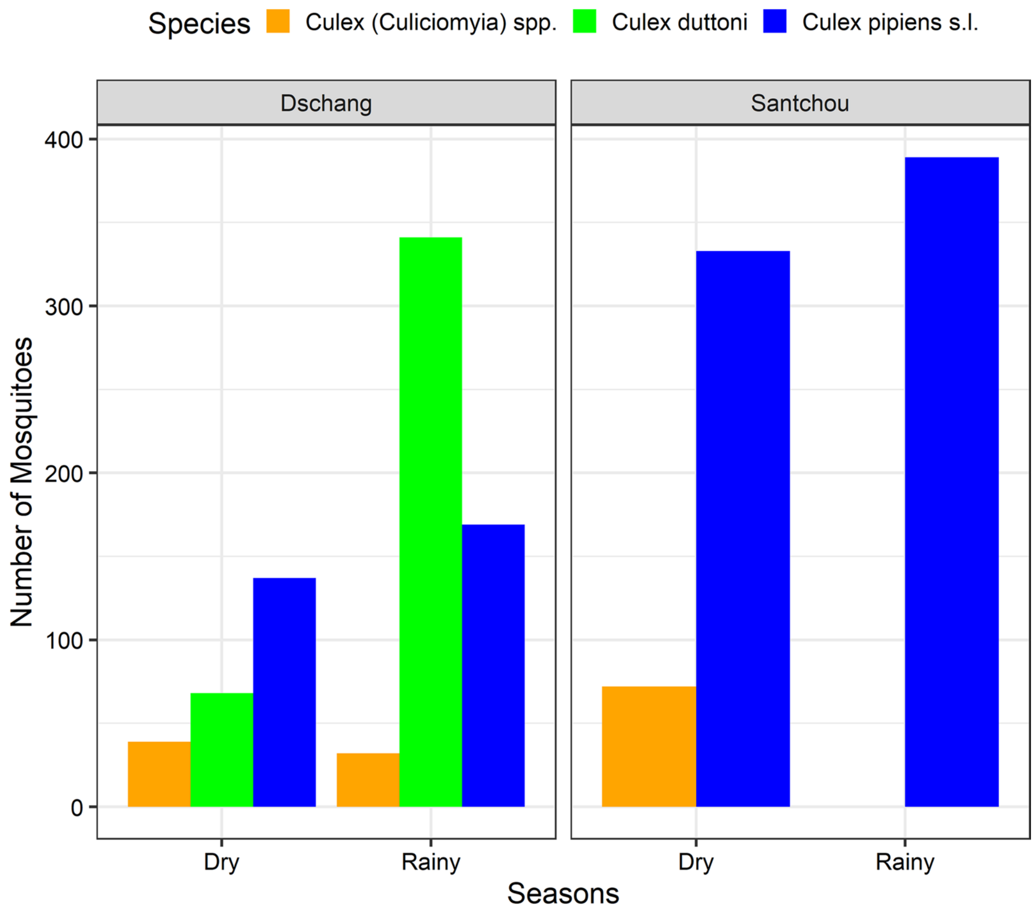

Fig. 5 Abundance and species richness of *Culex* per season in Dschang and Santchou

significant difference in the abundance ($p = 1$) and species richness ($p = 0.4232$) across seasons was observed.

Discussion

The findings of this study revealed that artificial (man-made) breeding sites such as gutters and tires were the most abundant and frequent in both localities. The abundance of these two types of larval habitats could be explained by intensive human activities (economic and industrial), a poorly controlled urbanization and lack of sanitation around habitations in these localities. These results are in accordance with those obtained by Koumba et al. (2018) in agricultural areas in Gabon, Mbida et al. (2018) in the surroundings of Wouri in the Cameroon coast, and Benhissen et al. (2017) in the region of Biskra in Algeria who found that human activities and poor environmental management impact the proliferation of mosquito breeding sites. However, in a different ecosystem (the case of the forest for example), tree holes, bamboo hollows and stagnant water created by public work machines (caterpillars) represent development sites of *Culex* mosquitoes (Mayi et al., 2019). Additional research on the physicochemical characteristics of the different *Culex* breeding sites encountered should be carried out.

High densities of *Culex* spp. were found in turbid, stagnant and sunny water bodies, rich in organic matters in Dschang and Santchou. These results are similar to those of Berchi et al. (2012) in Algeria who indicated that *Culex* larvae were more frequently encountered in turbid water containing organic matter because this provides a substantial nutrient supply for their development and reduces competition rates (Souza et al., 2019). Previous studies conducted in Cameroon by Nchoutpouen et al. (2019) in Yaoundé, Ngadvou et al. (2020) in Ngaoundéré, Mbida et al. (2018) in Douala and Saotoing et al. (2014) in Maroua also revealed that mosquitoes of this genus prefer breeding sites with turbid and more or less polluted water. According to Hassaine (2002), the characters clear/turbid, sunny/shaded nature of the water in most breeding sites, added to the fact that they are poor or rich in dissolved organic matter seem to contribute largely to the proliferation of certain species. However, no significant difference in abundance based on different characteristics of immature stages was observed in Dschang and Santchou except for the depth, which significantly influenced mosquito abundance in Santchou.

Although considered as a secondary ecological factor, depth can provide an important indication on the field. It is assessed from two categories of larval habitat: deep larval habitat, in this case the depth is greater than 50 cm, and shallow larval habitat (less than 50 cm) (Hassaine, 2002). In general, the majority of Culicidae rarely frequent deep breeding sites. High densities of *Culex* spp. were found in shallow and sunny water bodies in Dschang and Santchou. This could be explained by the fact that, in these habitats, water temperature rises rapidly due to its exposure to sun, which accelerates the speed of larval development. In fact, eggs hatch faster when they are subjected to higher temperatures (Hassaine, 2002). Similar results on the variation in abundance according to depth were reported in *An. labranchiae* by Tabbabi et al. (2015).

It is known that the density of mosquito larvae in breeding sites is highly influenced by other factors such as vegetation and the surrounding habitats. In Dschang, the highest density (75%) of *Culex* larvae was found in breeding sites without vegetation and this is because the presence of such vegetation in water body may cause barriers for the oviposition of gravid females, shadowing the water surface as well as microbial growth. It is also very important to mention that 52% of mosquitoes in Dschang were found at a distance less than 10 m from human dwellings. The occurrence of larval habitats with a high density of mosquito larvae around houses might represent a risk of future transmission of *Culex*-borne diseases among the population of Dschang especially.

The knowledge of the seasonal abundance of mosquitoes is of importance in predicting the period of maximum risk of disease transmission and for carrying out an effective control program (Ashry et al., 2018). The high abundance recorded during the rainy season in Dschang shows that monthly mosquito productivity was strongly influenced by rainfall (this was not the case in Santchou). Indeed, rain offers more possibilities in the choice of breeding sites for different Culicidian species in terms of quality and quantity. These results corroborate those by Nchoutpouen et al. (2019) in Yaoundé where the abundance of *Culex* increased with rains. They are also similar to those of Saotoing et al. (2014) in Maroua. According to these authors, mosquitoes are present throughout the year with a greater abundance at the start and the end of the rainy season. However, in order to better appreciate mosquito dynamics across seasons, an extension of the collection period throughout the year is important.

A greater abundance of mosquitoes was observed in Santchou compared to Dschang. It is known that areas of low altitude are generally subjected to higher temperatures than those of high altitudes. However, temperature and precipitation have a direct influence on the biology of vectors and the pathogens they transmit (Carnevale & Robert, 2009). In fact, when temperature rises, female mosquitoes digest the blood meal more quickly and therefore lay eggs more frequently, which increases the chances of encountering a high density of larvae in the breeding sites. In addition, this can increase water temperature which favors larval development (Hassaine, 2002). The town of Santchou, located at a low altitude, would therefore offer more possibilities for egg-laying by female mosquitoes compared to the town of Dschang. On the other hand, this result could also be explained by the fact that in some breeding sites, the amount of water collected was greater than in others, and that egg rafts were also collected in addition to the larvae.

Overall, species richness was low because only three *Culex* species were identified, namely *Culex pipiens s.l*, *Culex duttoni* and *Culex (Culiciomyia)* spp. This could be explained by the use of only one collection method (the "dipping" or ladle stroke). Indeed, several collection methods are important to increase the chances of having greater diversity. Carlson et al. (2015) showed that at least five collection methods are necessary to have a great diversity of mosquitoes in different habitats. Dschang was more diverse than Santchou in terms of mosquito richness. *Culex pipiens* s.l. and *Culex (Culiciomyia)* sp. were found in both localities, while *Cx. duttoni* was only found in Dschang. The absence of *Culex duttoni* in Santchou could be explained by the fact that the breeding sites of Santchou did not present all the conditions required and favorable for their development. Based on studies by Mayi et al. (2019, 2020) and Nchoutpouen et al. (2019), the presence of Culicinae species and their abundance could also vary depending on the type of landscape, altitude and/or the presence/absence of vegetation cover. Indeed, areas found at high altitude generally have a greater species richness than those found at low altitude (Stevens, 1992). These results are contrary with the work by Tchuinkam et al. (2010) on the bionomic of *Anopheles* following an altitudinal transect in the region of West Cameroon, where the diversity of *Anopheles* species decreased from areas of low altitudes to those of high altitudes. A similar study at a much higher altitude and on the forest cliff itself should be carried out in order to assess the evolution of *Culex* mosquitoes following an altitudinal transect in the West Region of Cameroon.

Conclusions

A great diversity of *Culex* breeding sites were encountered in both localities of which mostly were shallow, close to human dwellings, sunny, trouble, loaded with organic matter and of an anthropogenic nature. While Santchou recorded the greatest abundance of *Culex*, Dschang was found to be more diverse in terms of species richness, suggesting a possible effect of the altitudinal differences of the two localities on *Culex* mosquitoes. The proliferation of *Culex* mosquitoes in the two localities would be attributable to the unsanitary conditions and to human activities which create and ensure the maintenance of breeding sites for mosquitoes; this could increase the risk of vector-borne diseases to the human population of Dschang and Santchou. A sustainable public health campaign on vector management and control should therefore be intensified in these cities.

Abbreviations

Cx: *Culex*; H: Shannon index; D: Simpson index; GPS: Global Positioning System; S: Species richness; Sp: Species.

Acknowledgements

The authors thank the members of VBID-RUBAE from the University of Dschang for their assistance during field work and the populations of Dschang and Santchou for their collaborations.

Author contributions

DJ, MMPA, NLG, MFO and DTB carried out the field and laboratory studies. DJ, MMPA, BR, ANC and TT designed and supervised the study. MMPA carried out the data analysis. DJ wrote the first manuscript draft. ANC, TT, BR, DTB, DDL and MMPA critically reviewed the manuscript. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

Data generated during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 4 March 2021 Accepted: 5 June 2022
Published online: 17 June 2022

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