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BREEDING POPULATIONS OF CULICINES WITHIN THE PERI-DOMESTIC ENVIRONMENT OF SOME COMMUNITIES IN ANAMBRA STATE, SOUTHEAST, NIGERIA

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Abstract

A longitudinal study was carried out from September 2017 to August 2018 to investigate the breeding populations of culicines in peri-domestic environments at Okija, Ukpor and Orsumoghu communities of Anambra State. A bi-weekly mosquito larval collection was done. All water storage and discarded water-holding containers were sampled. For standardization, ten dips were made on each large container while smaller containers were pooled together to get representative samples. Disposable plastic pipettes of 1.5ml capacity were used to aspirate immature stages of mosquitoes from plant axils. Data was analyzed using SPSS version 21.0. A total of 3,225 mosquito larvae/pupae were collected during the study. *Aedes albopictus* 573 (17.77%), *Aedes aegypti* 372 (11.53 %), *Aedes simpsoni* 798 (24.74 %), *Culex quinquefasciatus* 1,374 (42.6 %), *Culex tigripes*, 99 (3.07 %) and *Eretmapodites quinquevittatus* 9 (0.28 %), emerged from the collections. A significantly higher number of larvae, 2,421 (75.07%), were collected from artificial containers than in the plant axils, 804 (24.93%) ($P < 0.05$). *Aedes simpsoni* was only recovered from the plant axils. The abundance of Culicine mosquitoes was significantly more in the rainy season than in the dry season ($P < 0.05$). Keeping the surroundings free from discarded water-holding containers, properly covering water storage containers and practicising subsistence agriculture for the implicated plants well away from our immediate living environment are sure ways of controlling these mosquitoes, as several of the mosquitoes collected are vectors of major public health diseases.

Keywords: Mosquito, larvae, breeding site, plant axils

Introduction

Mosquitoes are cosmopolitan in distribution and have been found to inhabit both aquatic and terrestrial habitats (Egbuche *et al*., 2021). Their ability to exhibit great ecological flexibility makes them able to adapt to various habitats such as highly polluted or clean bodies of water; Swampy woodlands, floodplains, tree-holes, leaf axils, small accumulations of water in buckets, flower vases, discarded tyres, hoof prints (Becker, *et al*., 2010). Natural factors such as rainfall, humidity and temperature have an effect on the overall spatial and temporal distribution and abundance of mosquitoes. Several activities of man especially those that bring about environmental changes also affect the distribution and survival of mosquito species (Egbuche, *et al*., 2021). At present there are more than 2000 species of mosquitoes which have been identified world-wide as vectors of human diseases (Onudua *et al*., 2020). Globally mosquitoes are one of the most important vectors of diseases chief of which is malaria infection in the tropics and sub-tropics, and some other arboviral infections such as dengue fever, chikungunya fever, West Nile virus, yellow fever virus among others.

Larvae of mosquitoes have been found to thrive in different aquatic environments (Ajayi *et al*., 2010; *Odo et al*., 2015). These include fresh or salt water marshes, grassy ditches, edges of streams, small temporary rain pools, artificial containers and even plastic sachets of water. Few species prefer leaf axils and tree holes (Anosike *et al*., 2007). Studies have shown that while certain aquatic habitats might be convenient for some species of mosquitoes it is inadequate for some other species (Afolabi *et al.*, 2013). The principal vectors of malaria in sub-Saharan Africa are *An. gambiae, An. funestus* and *An. Arabiensis* (Coetzee, 2004). They breed predominantly in environments where there is high humidity and rainfall (Irikannu *et al*., 2021). *An. nili* and *An. mouchetti* have also been included as part of the principal vectors. About 8 other species belong to what have been grouped as secondary or local vectors (Onyido *et al*., 2009; Koffi *et al*., 2023).

The recent increase in environmental and ecological modifications due to agricultural activities and urbanization has contributed to the breeding of various mosquito species (Amusan *et al*., 2005). The latest trend of production of most edibles in sachets and plastic containers have also contributed to the increase of breeding sites for mosquitoes coupled with the fact that our people have not learnt the discipline of proper disposal of waste. Reemergence of some diseases thought to have been controlled in the past such as the recent outbreak of yellow fever in Kwara State, Nigeria, the emergence of Zika virus infection and Dengue viral infections which have been grossly misdiagnosed and under reported in Nigeria, maybe due to lack of awareness (Ayukekbong, 2014). Additional vector control interventions that will complement existing adulticiding measures, such as larval control measures are therefore required to build integrated mosquito vector control programmes.

Successful larval control requires a good knowledge of the breeding ecology of mosquitoes, types of and preferences for larval habitats, spatial and temporal distribution of breeding sites. This information is lacking in some communities in Anambra State. It therefore becomes pertinent that continous updating of information on the hematophagous insect species composition and their abundance in some of these communities be made to

complement efforts geared towards effective and integrated vector control strategies.

The aim of this study was to determine the breeding population of Culicine mosquitoes within the peridomestic environment of Okija, Ukpor and Orsumogho communities.

Materials and Method

Study Area

The research was carried out in Anambra State, in the south eastern region of Nigeria. The three study sites were Ukpor community (Nnewi South L.G.A.) situated between latitude 5°54"34"N and latitude 6° 55" 58", Okija community (Ihiala L.G.A.) lies between latitude 5° 54" 0" N and longitude 6° 50" 0" E and Orsumoghu community (Ihiala L.G.A.) located between latitude 5° 51" 14" N and Longitude 6° 51" 36" E (Google imagery, 2016). These communities lie within the tropical rainforest zone of Nigeria with well marked wet and dry seasons. Ihiala L.G.A. has a population of 302,277 inhabitants, while Nnewi South L.G.A. has a population of 233,362 inhabitants (NPC, 2006). These three communities are Igbo speaking, though other tribes live there peacefully with them.

Ethical Consideration and Informed Consent

Prior to the study, advocacy visits to the traditional rulers and opinion leaders of the communities were made. Proper explanation of the project intent was made and used to obtain written permission to carry out the study in these communities. Oral consent was obtained from all volunteers involved in sample collection.

Study Design

The study adopted a cross-sectional and longitudinal study design. The study spanned over a period of one year cutting across two the dry and the wet seasons, from September 2017 to August 2018.

Selection of houses for entomological surveillance

Stratified random sampling was used in selecting the houses that were used for the surveillance. A total of ninety houses (30 per community) were randomly selected in the three communities for the collection of larval mosquitoes. Collection was done bi-weekly for a period of one year from September 2017 to August 2018. Immature stages of mosquitoes were also collected from the compounds involved in the study.

Collection of Mosquito Larvae

Mosquito larvae were collected from their different breeding sites such as artificial containers (discarded tyres, old pots, plastic water containers, cups, tins and cans) and leaf axils. This was done bi-weekly within the hours of 09:00 to 11:00. Small containers were emptied into white collecting bowls while ladles were used to scoop out water containing larvae from larger containers into the bowls. A sieve of about 0.55mm mesh size was used to separate larvae from debris. For standardization purposes, 10 dips were made at each large container while smaller containers were pooled together to get a representative sample (Yayock *et al.*, 2014). Plastic pipettes of 3.0 ml capacity were used to aspirate larval samples from the leaf axils of plants such as banana, plantain, cocoyam and pineapples and emptied into clean plastic jars and appropriately labeled. They were later sent to National Arbovirus and Vectors Research Centre (NAVRC) Enugu for rearing to adult stage in the laboratory and identification to species level.

Protocols for the Rearing of Mosquitoes

Plastic bowls were half filled with clean water. Larval samples were aspirated into the bowls. The larvae were fed with 3 or 4

cubes of poultry feed as food. Cabin biscuits were also used for a small colony. The larvae were washed every other day as they grew older, using sieve. The 1st or 2nd instar stage larvae were not washed to avoid losing much through sieving. The pupae were picked using pipette when they started pupating and introduced into a cage. Ten percent sugar solution were prepared, cotton wool were damped in it and rolled like a ball and put inside the cage on top of a small plastic container. The emerged adults fed on it and were subsequently collected for identification adapted from (Maiga *et al*., 2017).

Identification of Adult Mosquitoes

This was carried out microscopically using a stereomicroscope model no ST-30-2L S/ST Series Pec Medicals USA, with the aid of published keys by Gillet (1972) and Gilles and Coetzee (1987). The identification was based on gross morphological features, appearances of palps, proboscis, antennae, thorax, wings, terminal abdominal segment and colour on the hind tarsals.

Data Analysis

The total number of mosquitoes were determined as percentage composition at each study location. Data was analysed using SPSS version 21.0. Data was compared using analysis of variance (ANOVA). Duncan's New Multiple Range Test (DNMRT) was used to separate monthly, and location means. Student t-test was used to compare two different data. Level of significance was kept at ($P<0.05$).

Result

Table 1 shows the composition, distribution and abundance of mosquito larvae and variations in relation to communities and breeding sites. A total of three thousand two hundred and twenty five (3225) larvae were collected. Six mosquito species (*Ae. albopictus, Ae. simpsoni, Ae. aegypti, Cx. quinquefasciatus, Cx. tigripes* and *Er. quinquevittatus*) belonging to 3 genera (*Aedes, Culex* and *Eretmapodites*) were identified from the larvae. The most abundant species was *Cx. quinquefasciatus* (1374, 56.75 %), followed by *Ae. simpsoni* (798, 24.74 %). The least was *Er. quinquevittatus* (9, 0.28 %). There was a significant difference in abundance between the species observed during the study ($P < 0.05$). The larval breeding sites observed were artificial containers, comprising of all plastic containers and jars, metal or aluminium cans, discarded bottles of drinks, broken or whole earthenware vessels and discarded used tyres; and the leaf axils of bananas, plantains, pineapples and cocoyam plants. A significantly higher number of larvae, 2421 (75.07%), were collected from artificial containers than in the leaf axils 804 (24.93%) ($P < 0.05$). Five (5) different mosquito species were harvested from the artificial containers while only two (2) species were recorded in the leaf axils. *Ae. albopictus* was recorded in both sites (Table 1).

Of the 3225 larvae collected, the highest number 1611 (49.95%) was collected at Okija and the least 567 (17.58%) was from Orsumoghu, At Ukpor, 1042 (32.47%) mosquito larvae were collected.

High predominance of mosquito larvae occurred in November 822 (22.29%) while low mosquito larvae abundance occurred in December 66 (1.79%). Mosquito larvae were not encountered in February and March. The results of the monthly variations in mosquito larvae samples from Okija, Ukpor and Orsumoghu communities of Anambra State are presented in Table 2. *Cx. quinquefasciatus* was the most dominant species in June through December and January with percentage contribution of 384 (20.91%), then 36 (1.96%) and 78 (4.24%) respectively. However, this species was not recorded from February to May. *Ae.*

simpsoni was recorded most in April and May, with respective percentage abundances of 111 (13.91 %) and 126 (15.79%). *Ae. aegypti* and *Ae. albopictus* were the most abundant species in July and August with percentage contributions of 72 (19.40 %) and 69 (12.04 %) respectively. *Ae. aegypti* was not recorded from November to March. Similarly, *Ae. simpsoni* was not recorded from December to March. *Cx. tigripes* was not encountered in September, and from December to April. *Er. quinquevittatus* was only ecountered in July. *Cx. quinquefasciatus* was recorded as the most abundant species 1836 (49.77 %), followed by *Ae. simpsoni* 798 (21.64 %), while *Er. quinquevittattus* recorded the lowest species relative abundance of 9 (0.24 %).

Table 3 showed that the mean values of *Ae. aegypti* (109.33 ± 4.91), *Er. quinquevittatus* (4.33 ± 0.88) and *Cx. quinquefasciatus* (444.00 ± 16.17) were recorded highest in Okija when compared with their corresponding mean values recorded in Ukpor and Orsumoghu ($p < 0.05$). Ukpor had the highest mean values of *Ae. albopictus* (114 ± 7.50), *Ae. simpsoni* (187.33 ± 1.45) and *Cx. tigripes* (24.00 ± 1.73). *Cx. quinquefasciatus* recorded the highest mean value among all the larval species collected. Its value was highest in Okija community and differed significantly ($p < 0.05$) from the values obtained in Ukpor and Orsumoghu. Orsumoghu recorded the least mean values of mosquito larvae in all the species recorded. However, *Cx. quinquefasciatus* had the highest overall mean value of 229.00 ± 54.75, followed by *Ae. simpsoni* (133.00 ± 14.62).

The mean values of mosquito larvae varied greatly between the breeding sites in all the sampled communities (Table 4). Artificial containers recorded the highest mean values of 681.00 ± 16.16, 334.33 ± 12.99 and 195.00 ± 2.31 in Okija, Ukpor and Orsumoghu respectively. There was a significant difference between the breeding sites in all the communities ($p < 0.01$). The overall mean values of 403.44 ± 72.49 and 134.11 ± 15.71 were recorded in artificial containers and Leaf axil respectively. Comparing the mean values of the mosquito larvae across the communities, the highest significant mean values of mosquito larvae in artificial containers was recorded at Okija, while Leaf axil was recorded at Ukpor community ($p < 0.05$).

Table 1: Distribution and abundance of mosquito larvae from different breeding sites in the studied communities

Mosquito Species	Total (%)	Communities			Breeding sites	
		Okija	Ukpor	Orsumoghu	Artificial containers	Leaf axil
	1,743(54.05)	678(38.90)	705(40.44)	360(20.70)	939(53.90)	804(46.10)
Ae. albopictus	573(17.77)	213(37.20)	228(39.80)	132(23.04)	567(99.00)	6(1.00)
Ae. simpsoni	798(24.74)	246(30.83)	375(46.99)	177(22.18)	0(0.00)	798(100.00)
Ae. aegypti	372(11.53)	219(58.90)	102(27.42)	51(13.71)	372(100.00)	0(0.00)
	1,473(45.67)	924(62.73)	342(23.22)	207(14.10)	1,473(100.00)	0(0.00)
Cx. quinquefasciatus	1374(42.60)	888(64.63)	294(21.40)	192(13.97)	1374(100.00)	0(0.00)
Cx. tigripes	99(3.07)	36(36.36)	48(48.48)	15(15.15)	99(100.00)	0(0.00)
Er. quinquevittatus	9(0.28)	9(100.00)	0(0.00)	0(0.00)	9(100.00)	0(0.00)
Total	**3225(100.00)**	**1,611(49.95)**	**1,047(32.47)**	**567(17.58)**	**2,421(75.07)**	**804(24.93)**

The figures in parenthesis show percentage relative abundance of the species.

Table 2: Monthly distribution of mosquito larvae collected from the studied communities

Species	Total (%)	Months											
		Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Ae. albopictus	573 (15.54)	108 (18.84)	114 (19.90)	123 (21.50)	30 (5.24)	42 (7.33)	0 (0.00)	0 (0.00)	30 (5.24)	6 (1.05)	0 (0.00)	51 (8.90)	69 (12.04)
Ae. aegypti	372 (10.09)	9 (2.42)	42 (11.30)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	78 (21.00)	81 (21.80)	24 (6.50)	72 (19.40)	66 (17.74)
Ae. simpsoni	798 (21.64)	159 (19.92)	81 (10.15)	243 (30.45)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	111 (13.91)	126 (15.79)	27 (3.38)	15 (1.88)	36 (4.51)
Cx. Quinquefasciatus	1836 (49.77)	177 (9.64)	225 (12.25)	396 (19.38)	36 (1.96)	78 (4.24)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	384 (20.91)	18 (0.98)	60 (3.27)
Cx. tigripes	99 (2.69)	0 (0.00)	9 (9.10)	60 (60.61)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	15 (15.20)	0 (0.00)	9 (9.10)	6 (6.10)
Er. Quinquevittatus	(0.24)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	9 (100.00)	0 (0.00)
Total	**3687 (100.00)**	**453 (12.29)**	**471 (12.77)**	**822 (22.29)**	**66 (1.79)**	**120 (3.25)**	**0 (0.00)**	**0 (0.00)**	**219 (5.94)**	**228 (6.18)**	**435 (11.80)**	**174 (4.71)**	**237 (6.43)**

The figures in parenthesis show percentage relative abundance of the species

Table 3: Mean variations of mosquito larvae species in the studied communities

Species	Communities			Pooled mean/species
	Okija	Ukpor	Orsumoghu	
Ae. albopictus	106.33 ± 6.06^{b}	114.00 ± 7.50^{b}	66.00 ± 0.58^{a}	95.44 ± 7.95
Ae. simpsoni	123.00 ± 6.93^{b}	187.33 ± 1.45^{c}	88.67 ± 2.60^{a}	133.00 ± 14.62
Ae. aegypti	109.33 ± 4.91^{c}	51.00 ± 2.31^{b}	25.67 ± 1.45^{a}	62.00 ± 12.49
Cx. quinquefasciatus	444.00 ± 16.17^{b}	147.00 ± 19.63^{a}	96.00 ± 3.46^{a}	229.00 ± 54.75
Cx. tigripes	18.00 ± 0.58^{b}	24.00 ± 1.73^{c}	7.67 ± 0.88^{a}	16.56 ± 2.46
Er. quinquevittatus	4.33 ± 0.88^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.44 ± 0.77

The figures in parenthesis show range at station samples of the species. Mean (±S. E) values with the same superscript on the same row are not significantly different from each other at $p > 0.05$

Table 4: Mean Variations of mosquito larvae from different breeding sites in the studied communities

Breeding sites	Communities			Pooled mean/species
	Okija	Ukpor	Orsumoghu	
Artificial containers	681.00 ± 16.16^{c}	334.33 ± 12.99^{b}	195.00 ± 2.31^{a}	403.44 ± 72.49
Leaf axil	124.67 ± 3.76^{a}	189.00 ± 19.05^{b}	88.67 ± 1.45^{a}	134.11 ± 15.71
T-test	27.929	23.965	120.571	
p-value	0.001	0.002	0.0001	

Mean values with different alphabets as superscripts in a row differ significantly ($p < 0.05$). Level of significance along column: * = $p < 0.05$; ** = $p < 0.001$

Discussion

Larval sampling in this study revealed three genera of culicidae (*Aedes, Culex and Eretmapodites*). *Cx. quinquefasciatus* was the most abundant species followed by *Ae. simpsoni, Ae. albopictus, Ae. aegypti, Cx. tigripes and Er. quinquevittatus* which recorded the least abundance. This agreed with the findings of Olayemi *et al*. (2014) and Okonkwo *et al*. (2014) who observed that *Culex spp* was the most abundant species in their studies.

Okogun *et al*. (2003) in a larval study at Ekpoma recorded three genera of Culicidae (*Anopheles, Aedes* and *Culex*). *Anopheles* species were the most abundant, being recovered from both artificial and natural receptacles. Adeleke *et al*. (2008), in a larval sampling at Abeokuta recorded five genera of Culicidae (*Anopheles, Aedes, Culex, Mansonia* and *Eretmapodites*), with *Anopheles* having the second highest abundance on record and was equally recovered from both ground pools and artificial containers. These reports contradicted the present study which recorded absence of *Anopheles* in larval sampling. This could be as a result of the fact that no ground pools were available in the peridomestic environment of the study area. Such collections of water have been documented as the usual breeding ground of *Anopheles* (Okogun *et al*., 2003). In agreement with this study *Ae. simpsoni* was recovered only in leaf axils in Ekpoma as recorded in this present study. Some other researchers recorded high abundance of *Cx. quinquefasciatus* in their larval sampling (Dalhatu *et al*., 2016). This is in agreement with the records obtained in this current study.

A close look at the distribution and abundance of larval mosquitoes in this surveillance revealed that though a diverse number of mosquito species were harvested from this study generally using different methods, larval searches were limited to the peri-domestic environment hence no samples from the natural bodies of water were collected. There were no organized drainage systems in the study areas. Collections were mainly from artificial containers discarded carelessly in the environment, household water containers, and leaf axils of plants such as banana, plantain, cocoyam and pineapples.

The absence of *Ae. africanus* from the larval collections goes to confirm that this species of mosquito is a sylvatic vector breeding mainly in tree holes and bamboo stumps which are mainly situated in the forest areas (Anosike *et al*., 2007). The high abundance of *Cx. quinquefasciatus* could be attributed to the fact that part of the larval collections were from domestic waste water poured into old earthen-ware pots and left standing in the environment. Some of the liquid waste were water used in washing plates and cassava peels because they were not promptly disposed they served as breeding ground for *Culex* mosquitoes. *Culex* mosquitoes are known to breed in contaminated and polluted waters (Mafiana, 1989; Dalhatu *et al*., 2016) Among the communities Okija recorded the highest abundance of larval mosquitoes, followed by Ukpor community, while Orsumoghu community recorded the lowest. There was significant variation ($p < 0.01$) in the distribution of the different larval mosquitoes among the communities. While *Cx. quinquefasciatus* and *Ae. simpsoni* were encountered in all the communities, the rest species varied in their distribution from community to community. *Ae. simpsoni* was encountered only in leaf axils, they were not recovered from any artificial containers in this study. This could be as a result of microhabitat preferences. Walter *et al.* (2014) documented that *Ae. simpsoni* complex mosquitoes are known to be associated with cultivated microhabitat and prefers to breed in phytotelma.

Highest abundance of larval mosquitoes were recorded at the peak of wet season

suggesting that sufficient rain supports the breeding of mosquito species. Zero abundance were recorded in the months of February and March which corresponded with the peak of dry season, when the environment is dry and all the containers were virtually dried up. Okogun *et al.* (2005) noted that extremes of temperature are lethal to aquatic stages of mosquitoes especially the larvae. When categorized into two, the larval breeding sites came under two distinct categories – the artificial containers and the leaf axils. Artificial containers yielded six species of mosquitoes while leaf axil yielded only two species. There is a significant difference in the mean number of larvae collected between the artificial containers and leaf axils ($P < 0.01$). A related work by Anosike *et al*., (2007) recovered up to eleven species of mosquitoes in banana/ pineapple leaf axils in Imo state. The increased number of species of mosquitoes could be as a result of the fact that the study was carried out both in the peridomestic environment and the forest reserve areas. Adebote (2006) was able to collect five different species of container breeding mosquitoes when he investigated some settlements around Zaria, Nigeria, for yellow fever risk potentials. They were *Ae. aegypti, Cx. horridus, Cx. nebulosus, Cx. quinquefasciatus* and *Cx. tigripes. Ae. aegypti* and *Cx. tigripes* ranking highest and least in abundance respectively.

Conclusion

Keeping our surroundings free from discarded water-holding containers, properly covering water storage containers are advocated. The practice of subsistence agriculture is very important in our present economy; however, this should be done from a safe distance to our immediate living environment. By doing this we are putting in place control measures for these mosquitoes as several of the mosquitoes collected are vectors of major public health diseases.

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