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Abundance and diversity of man-biting mosquito species in the tropical rainforest belt of Southeastern Nigeria

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Abstract
Abundance and diversity of man-biting mosquito species in the tropical rainforest belt of Southeastern Nigeria was undertaken in three selected communities. The study lasted 12 months cutting across wet and dry seasons. Adult mosquitoes were collected, both indoors and outdoors, using pyrethrum spreadsheet method (PSC) and human landing catch technique (HLC). The mosquitoes were morphologically identified using identification guides, later confirmed by expert taxonomists. Data were analyzed using statistical package for Social Sciences (SPSS version 21.0) Percentages were used to determine relative abundance of the mosquitoes. Species richness was determined using Shannon-Weiner Indices. A total of 4,434 adult mosquitoes consisting of 936 (21.11%) indoors and 3,498 (78.89%) outdoors were collected. The mosquito collections were made of 15 species belonging to six genera. *Aedes* mosquitoes 3,072 (69.28%) significantly topped the list of the mosquito population ($P < 0.05$) and was the most abundant species caught outdoors. *Anopheles* mosquitoes 627 (14.14%) were significantly higher indoors ($P < 0.05$) than all the mosquitoes collected. Monthly abundance of the mosquitoes showed a bi-modal peak with a minor peak occurring in June and a major peak in October. In the dry season, mosquito abundance in the three communities was similar, but differed significantly ($P < 0.05$) during the wet season. Shannon-Weiner Indices ranked Okija community as the most diverse in species richness. The preponderance of *Anopheles*, *Culex* and *Aedes* species in the study area was viewed as a danger signal against mosquito-borne diseases transmission as the collected species are proven vectors of public health diseases. Health education is advocated for protection and improvement of the people's health.

Keywords: Mosquitoes, abundance, diversity, public health

1. Introduction
Mosquitoes are blood-sucking insects with a cosmopolitan distribution [1]. Though widely distributed, they are more abundant in the tropical countries of the world [2, 3]. Over 3500 species of mosquitoes exist and have been classified into three subfamilies; Anophelinae, Culicinae and Toxorhynchitinae.

Mosquitoes are found in almost all types of aquatic habitats for breeding [4, 5]. Most of them breed in both natural and artificial habitats, which include drains, pools of water, plastic cans and tins, tree holes, leaf axils and soak-away pits [6].

The most important human biting mosquitoes belong to the genera *Anopheles, Culex, Aedes, Mansonia, Haemagogus, Sabethes* and *Psorophora.* Certain dreadful diseases which cause serious health problems to humans are transmitted by mosquitoes [7]. Since female mosquitoes must have a blood meal as a requirement for oviposition, this gives them a great opportunity to bite man and transmit diseases such as malaria, arborviruses such as zika, yellow fever, dengue, chikungunya, filariasis among others [4, 8]. The affected persons suffer immensely and development is hindered [9].

They breed in almost all types of aquatic habitats. The knowledge of mosquito breeding ecology, including their resting habits and biting patterns are needed for any vector control measure to be effective and successful. This study was therefore designed to investigate the abundance and diversity of the existing mosquito fauna and its possible public health implication on the residents of Okija, Ukpor and Orsumoghu communities in Anambra State, Nigeria.

2. Materials and Methods

2.1 Study Area

The study was carried out in Anambra State, in the Southeastern 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 [10]. These communities lie within the tropical rainforest zone of Nigeria with well-marked wet and dry seasons. They have about 8 months of wet season (April to November) and about 4 months of dry season (December to March). There is also a short harmattan period from December to January within the dry season. The relative humidity of these communities is about 85.8% in the dry season and 91.1% in the wet season. The annual rainfall for the communities was 97.3 mm in the dry season and 2064 mm in the wet season. The average temperature range of the areas are 24.1 °C minimum and 35 °C maximum during the dry season and 23.5 °C minimum and 31.5 °C maximum during the wet season [11].

2.2 Informed Consent and Permission to carry out study

Prior to the study, advocacy visits to the traditional rulers and opinion leaders of the communities and proper explanation of the project intent were used to obtain permission to carry out the study in these communities. Oral consent was obtained from all participants involved in sample collection. Yellow fever vaccines and malaria prophylaxis were administered to the volunteers from the community health facility.

2.3 Study Design

The study adopted a cross-sectional and longitudinal study design for the aspects of ecology of human biting mosquito population in Okija, Ukpor and Orsumoghu communities. The study spanned over a period of one year cutting across two seasons (the dry and the wet seasons).

2.4 Selection of houses for entomological surveillance

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 indoor resting mosquitoes and out-door biting mosquitoes. Collection was done bi-weekly for a period of one year.

2.5 Adult Mosquito Collection

Adult mosquitoes were collected using two methods: The Pyrethrum Spreadsheet Collection (PSC) and the Human Landing Catches (HLC) methods [12]. The PSC was done bi-weekly between the hours of 06:00 and 09:00, while the HLC was equally done bi-weekly between the hours of 16:00 and 19:00.

2.5.1 Pyrethrum spreadsheet collection: This study employed the insecticidal knockdown technique of mosquitoes [13]. On the morning of the sampling, care was taken to make sure that any resting mosquitoes were not disturbed. All persons were requested to leave the room, all food items and water in the room were properly covered. As much as possible, any removable item (furniture) was carried out. The white sheet (4m x 4m) was then spread so that it completely covered the floor and every other furniture that remained, the doors and windows were closed. Cracks and crevices on the wall were covered with wrung papers. The outside of the room was inspected and in cases of unceiled roofs, pyrethroid-based insecticide aerosol (Baygon) was sprayed in the, the openings and cracks in the walls and roof before spraying the inside of the rooms. Inside the room, the doors, windows and vents were shut and in a clockwise direction, the insecticide was quickly sprayed towards the ceiling and corners of the room until the room was filled with insecticide. The room was left undisturbed for about 15 minutes. After the time elapsed, starting from the doorway the sheet was picked from their corners and brought outside. The sheet was carefully spread out and using entomological forceps, the knocked down mosquitoes were picked up and placed in a labeled petri dish lined with damp cotton wool and filter paper.

2.5.2 The human landing catches: This involved using humans as bait for trapping mosquitoes. It was carried out bi-weekly in each study community between the hours of 16:00 to 19:00. Collectors wore long-sleeve clothes with scarves or caps on the heads, scarves around the necks while exposing their lower legs for mosquitoes to land on. With the aid of torch-light the landing mosquitoes were collected using test tube vials or aspirators, covered with cotton balls and time of collection placed on the container. The collected mosquitoes were collated at quarter-hourly intervals and placed in separate polyethylene bags for transportation to the laboratory.

2.5.3 Identification of Adult Mosquitoes: Morphological identification of the mosquitoes was carried out using a stereomicroscope model no ST-30-2L S/ST Series Pec Medicals USA, with the aid of published keys by [14, 15] The identification was based on gross morphological features, appearances of palps, proboscis, antennae, thorax, wings, terminal abdominal segment and colour of the hind tarsi.

2.6 Data Analysis

The abundance of the individual mosquitoes was determined as percentage of the total mosquitoes collected at each study station. Faunal diversity index for species richness (D) was analyzed using Margalef's diversity index for species (taxa) richness, while Shannon Wiener index (H) was used for general diversity and equitability of evenness (E) of distribution [16, 17].

SPSS version 21.0 employed the use of ANOVA, Duncan's New Multiple Range Test, Student's t-test and Chi square test to analyze this data.

3. Results

A total of 4,434 adult mosquitoes belonging to 6 genera namely: *Anopheles, Culex, Aedes, Mansonia, Eretmapodites* and *Coquillettidia* and comprised of 15 species were collected during the study. Of the six genera *Aedes* 3072 (69.28%) was the most abundant and *Coquillettidia* 6 (0.14%), was the least. Anopheles vectors of malaria parasites, were also collected in considerable numbers 702 (15.83%). Of the 15 species *Ae. africanus* 1587 (33.79%) was the most abundant while *Er. quinquevittatus* 3 (0.07%) was the least (Table 1).

Monthly abundance of mosquitoes in the study area showed a bi-modal peak in October and June. January and February had the lowest population. In January, the mosquito population was in all-time low of 1.08%, from where it gradually rose to the first peak of 12.95% of the total mosquito collection in June. The mosquito population dropped again in July to 6.75%, remained more or less steady in August to 6.28%. In September the mosquito population started increasing again and reached the highest peak of 17.62% in October, dropped

rapidly to 4.8% in December (Fig.1).

Mosquito abundance in the three communities studied was similar in dry season. Ukpor community 34.72% had slightly but non-significant higher mosquito population than Okija 32.83% and Orsumoghu 32.45%. In the wet season Ukpor (41.15%) had significantly higher mosquito abundance ($P < 0.05$) than Orsumoghu (34.39%) and Okija (24.46%) (Fig.2).

Relative abundance of mosquito species in the studied communities showed Orsumoghu with the highest percentage abundance of 45.47%, while Okija recorded the least 20.84%. The distribution of the 15 species of mosquitoes were as follows; Okija 13 species, Ukpor 8 species and Orsumoghu 12 species (Table 2).

Of the 15 species of mosquitoes collected from the study sites, six species namely *Anopheles gambiae*, *An. mouchetti*, *Culex quinquefasciatus*, *Mansonia africana* and *Coquillettidia aurites* were collected indoors. Anopheles species 927 (66.99%) significantly topped the list in abundance ($P < 0.05$), followed by *Culex quiquefasciatus* 276 (29.49%) *Mansonia* and *Coquillettidia* 6 (0.64%) each (Table 3).

Eleven mosquito species composed of six species of *Aedes*, *Culex quinquefasciatus*, *Cx. annulioris*, *Eretmapodites chrysogaster*, *Er. quinquevittatus* and *Mansonia africana* were collected outdoors. *Aedes* group of mosquitoes accounted for 3498 (78.89%) of the outdoor biting mosquitoes and were significantly higher in abundance ($P < 0.05$) than other outdoor biting mosquitoes collected (Table 3). No *Anopheles* species was collected outdoors. *Culex quinquefasciatus* were collected both indoors and outdoors in significant numbers. *Mansonia africana* was collected both indoors and outdoors but in relatively very small numbers.

Margalef index gave the highest species richness to Okija community while Ukpor ranked least. Shannon –Weiner diversity index ranked Okija also as the most diverse community, Ukpor also ranked least. However the eveness index was highest in Okija (Table 4).

Table 1: Man-biting mosquito species collected in the study communities

Mosquito species	Number collected	Percentage
Anopheles gambiae	600	13.53
Anopheles funestus	21	0.47
Anopheles mouchetti	6	0.14
Culex quinquefasciatus	696	15.69
Culex annulioris	6	0.14
Culex chrysogaster	12	0.27
Culex quinquevittatus	3	0.07
Aedes albopictus	1059	23.88
Aedes aegypti	279	6.29
Aedes africanus	1587	35.79
Aedes luteocephalus	123	2.77
Aedes circumluteolus	12	0.27
Aedes simpsoni	12	0.27
Mansonia africana	12	0.27
Coquillettidia aurites	6	0.14
Total	4434	100.00

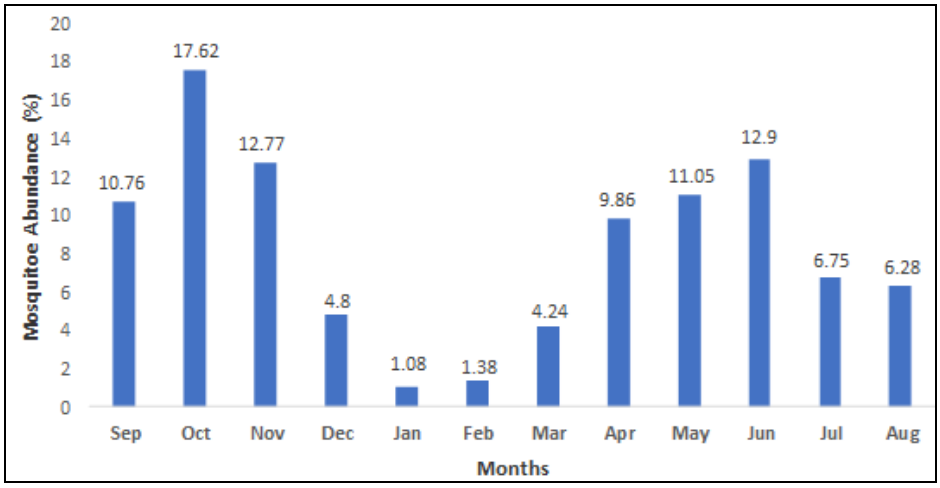

Fig 1: Gross monthly abundance of mosquitoes in the study area

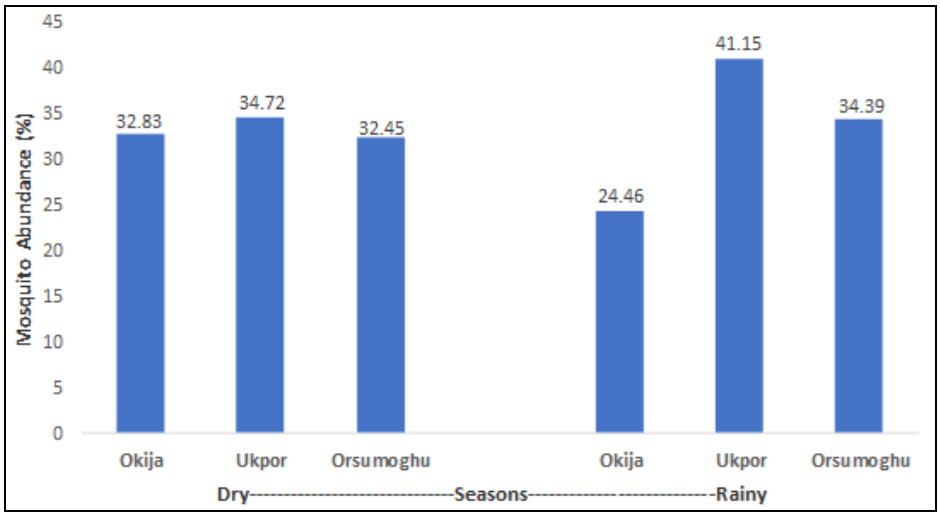

Fig 2: Seasonal abundance of the mosquitoes in the three communities studied

Table 2: Relative abundance of man-biting mosquito species in the studied communities

Mosquito species	Communities			Total (%)
	Okija	Ukpor	Orsumoghu	
Aedes aegypti	150	69	51	279 (6.29)
Aedes albopictus	186	705	168	1059 (23.88)
Aedes africanus	60	288	1239	1587 (35.79)
Aedes luteocephalus	30	60	33	123 (2.77)
Aedes circumluteolus	9	0	3	12 (0.27)
Aedes simpsoni	9	0	3	12 (0.27)
Anopheles gambiae	240	183	177	600(13.53)
Anopheles funestus	12	0	9	21(0.47)
Anopheles mouchetti	6	0	0	6(0.14)
Culex quinquefasciatus	201	174	321	696 (15.70)
Culex annulioris	0	3	3	6 (0.14)
Coquillettidia aurites	6	0	0	6(0.14)
Eretmapodite chrysogaster	6	0	6	12 (0.27)
Eretmapodite quinquevittatus	0	0	3	3 (0.07)
Mansonia africana	9	3	0	12 (0.27)
	924(20.84)	1494(33.69)	2016(45.47)	4434

Table 3: Indoor and outdoor abundance of man-biting mosquito species in the studied communities

Mosquito species	Indoor collection	Outdoor collection	Total (%)
Aedes albopictus	6	1053	1,059(23.88)
Aedes aegypti	15	264	279(6.29)
Aedes africanus	0	1587	1,587(35.79)
Aedes luteocephalus	0	123	123(2.77)
Aedes circumluteolus	0	12	12(0.27)
Aedes simpsoni	0	12	12(0.27)
Anopheles gambiae	600	0	600 (13.53)
Anopheles funestus	21	0	21 (0.47)
Anopheles mouchetti	6	0	6 (0.14)
Culex quinquefasciatus	276	420	696 (15.70)
Culex annulioris	0	6	6 (0.14)
Coquelittidia aurites	6	0	6 (0.14)
Eretmapodites chrysogaster	0	12	12(0.27)
Eretmapodites quinquevittatus	0	3	3(0.07)
Mansonia africana	6	6	12 (0.27)
Total	936(21.11)	3498(78.89)	4434(100.00)

Table 4: Species richness, diversity, dominance and evenness indices calculated for mosquito species in the studied communities

Indices	Communities		
	Okija	Ukpor	Orsumoghu
Margalef's index (d)	1.751	1.144	1.558
Menhinick's	0.3418	0.1959	0.2763
Dominance (D)	0.2588	0.2777	0.3551
Simpson (1 - D)	0.7412	0.7223	0.6449
Reciprocal Simpson (1/D)	3.8640	3.6010	1.5506
Shannon Wiener index (H)	1.7224	1.5519	1.4421
Highest species diversity (H_{max})	2.6389	1.0717	2.5651
Berger-Parker	0.4493	0.4267	0.5596
Fisher_alpha	2.093	1.317	1.831
Evenness _ e^H/S	0.3999	0.4721	0.3254
Equitability (J)	0.6527	0.6740	0.5622
Brillouin	1.703	1.542	1.429
No. of Species	14	9	13
No. of individuals	1678	2606	2214

4. Discussion and Recommendation

Six genera of mosquitoes *Aedes, Anopheles, Culex, Mansonia, Eretmapodites* and *Coquillettidia* composed of fifteen species were observed in this study. Similar findings were recorded by some previous works on mosquito distribution and abundance in Nigeria [1, 8, 3, 9]. This wide variety of mosquito species observed in this study could be as a result of the land use and human activities of the inhabitants of these communities [4]. Rivers traverse these communities and the rich forested vegetation and swampy banks of these rivers could be excellent breeding and resting grounds for these mosquitoes.

Mosquito abundance in this study revealed the most abundant genus as *Aedes*, followed by *Culex*, next was *Anopheles*, *Eretmapodites*, *Mansonia* with *Coquillettidia* being the least.The most abundant species was *Aedes africanus*, followed by *Ae. albopictus*, then *Cx. quinquefasciatus*, the lowest percentage abundance was recorded by *Er. quinquevittatus.* The composition of mosquito species recorded in this study was similar to that observed in a study carried out in the Nnamdi Azikiwe University Awka community [18]. Six genera of culicines yielded thirteen species of mosquitoes. Similar to what was obtained in this present work, *Culex* species and *Aedes* species were found in all study locations [18]. However this study differed from the work [7] done in a rice growing community of Kwara State which recorded four genera of *Culicidae* with only four species of mosquitoes. A high preponderance of *An. gambiae* complex was recorded with it having the highest percentage abundance in the community during the study period. This could easily be attributed to the swampy nature of the rice growing environment with pools of water that could serve as breeding sites for *Anopheles* species.

Seasonal variations in the distribution of mosquitoes were observed in this study. The abundance of mosquitoes increased during the wet seasons. Bimodal peak abundance of mosquito species in the study communities were recorded in October and June, while the months of January recorded the least abundance. This agreed with the work of Ajao and Adeleke [7], showing a similar trend with a peak in the month of October and the least abundance being recorded in the month of January. The agricultural activities of the inhabitants provided breeding grounds for these mosquitoes especially within the peri-domestic environments. The practice of having farms within the immediate environments of the living areas gave rise to a number of plantain and banana plants, also cocoyam and pineapples plants whose leaf axils served as good breeding grounds for culicine mosquitoes especially *Aedes* species [4]. In addition, the practice of cultivating ridges and mounds helps to create water pools within the farms for the breeding of *Anopheles* mosquitoes [5]. The indiscriminate

disposal of cans, tins and plastic containers within the environment served as good breeding grounds for mosquito species [19]. Similar reports had been given [20, 21], showing that the preponderance of *Ae. aegypti* over other species could be as a result of the presence of artificial containers in and around homes and provided them with suitable breeding sites.

Mosquito species richness and diversity as determined using Shannon-Weiner and Margalef indices ranked Okija highest, followed closely by Orsumoghu with Ukpor being the least. However Ukpor ranked highest in eveness index.

It should be noted that the four species of mosquitoes *Aedes africanus, Aedes albopictus, Culex quinquefasciatus* and *Anopheles gambiae* which recorded higher densities than others are known vectors of life threatening diseases such as yellow fever, dengue fever, malaria and bancroftian filariasis in Africa [1]. Their dominance in the study area signifies that the inhabitants of the communities are at risk of these diseases if neglected and can in turn affect and impede the productivity and life span of the residents in general. Efforts therefore should be made by all stakeholders of the communities to stem the man-mosquito contact in the area.

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