

Original Research Article
Species Composition and Biting Activities of Mosquitoes in a Selected Community in Awka South Local Government Area, Anambra State Nigeria

ABSTRACT

Mosquitoes are common biting and sucking arthropods whose females bite man and animals for their blood meal which leads to itching and causes diseases. The study investigated the species composition and biting activities of mosquitoes at Ifite-Awka, Awka South Local Government Area, Anambra State. The study was carried out between November and December, 2021. The mosquitoes were collected using the Human Landing Catch (HLC) and Pyrethrum Spray Collection Method (PSC), whereas Larval Sampling (LS) method was used to collect the immature stages of mosquito. The Human Landing Catch was carried out from 5:00pm to 8:00pm and the biting peak was observed from 5:45pm to 7:00pm. A total of 158 adult mosquitoes were collected which comprised of 3 species namely *Culex quinquefasciatus*, *Anopheles gambiae* and *Mansonia africana*. *Culex quinquefasciatus* was the most abundant species irrespective of the method of collection which was attributed to their anthropophilic, endophagic and endophilic nature. Pyrethrum Spray Collection method (PKC) produced the highest species of 120 mosquitoes which constitute of 91(75.83%) *Culex quinquefasciatus*, 27 (22.5%) *Anopheles gambiae* and 2(1.66%) *Mansonia africana*. The Human Landing Catch Method caught 28 mosquitoes of two species of mosquitoes collected which was *Culex quinquefasciatus* 25(89.29%) and *Mansonia africana* 3(10.71%). The Larval Sampling Method caught 10 *Culex quinquefasciatus*. Although there was no significant difference among the methods of collection ($p>0.05$), but in the distribution of the mosquitoes species, there was a significant difference ($p<0.05$) using the seven (7) lodges sampled. The study observed high exposure to mosquitoes and the possibility of epidemics of mosquito -borne diseases among Ifite-Awka residents. Need of proper health education, immediate and effective control measures of the mosquito vectors, which includes environmental sanitation.

Comment [MOU1]: In summary, the abstract provides a good overview of the study's objectives, methods, and findings, but could be improved by providing more detailed information, including a statement of the significance of the findings, and being more concise.

Keywords: Mosquitoes; Biting Activities; Specie Composition; Ifite; Anambra State

1. INTRODUCTION

Mosquitoes are common biting and sucking arthropods that belong to the class Insecta, Order Diptera and Culicidae. Mosquitoes are the world's deadliest animals (CDC, 2021) and are distributed worldwide where they are found in both aquatic and terrestrial habitats (Egbuche *et al.*, 2021). Mosquitoes undergo complete metamorphosis consisting of egg, larva, pupa and adults. The female mosquitoes bite people and animals for their blood meal (Nikbakhtzadeh *et al.*,

Comment [MOU2]: Is the writing of this introduction in accordance with the journal guidelines? it would be better if the writing is given a main idea in the form of several paragraphs that represent the idea of the discussion and write down the justification for the significance of this research to be carried out.

2016). These blood meals are necessary to produce eggs. When people or animals are bitten by mosquitoes, it commonly leads to itching and swelling (CDC, 2020). Mosquitoes have a slender, elongated body covered with scales. The body is divided into three (3) parts namely, Head, thorax and abdomen. The head contains an elongated piercing mouthparts with which they bite and feed from their hosts (Gurera *et al.*, 2018). Mosquitoes are distinguished from other flies by their long proboscis, characteristic wing venation and scales on the wing. The male mosquitoes can be differentiated from the female mosquitoes by their form of antennae. The male antennae are very plumose while the female antennae have few hairs. There are over 3500 species of mosquitoes (Nicoletti, 2020) of 112 genera. The most important man-biting mosquitoes of these species belong to the genera; *Mansonia*, *Haemagogus*, *Sabethes* and *Psorophora* (Aribodor *et al.*, 2016). Studies from Nigeria have revealed various species of mosquitoes from three (3) genera (Egwu *et al.*, 2018 and Ifeoluwa *et al.*, 2020). These genera are *Anopheles*, *Culex* and *Aedes*. *Anopheles* mosquitoes are distributed worldwide and can be found in both tropical and temperate regions. *Anopheles* mosquitoes go through the four stages of their life cycle: egg, larva, pupa and adult. The first 3 stages are aquatic and last 7-14 days (CDC, 2020). They lay their eggs singly and float on water surface with the aid of air filled floats (Service, 2008). The larva lie parallel to the water surface due to the absence of siphon. Most *Anopheles* mosquitoes have spotted wings. Malaria and lymphatic filariasis (LF) are primarily transmitted by *Anopheles* mosquitoes (WHO, 2010). Recent studies have identified *An. gambiae* and *Anopheles funestus* as the main vectors of these diseases (Sinka *et al.*, 2010). *Culex* mosquitoes are distributed worldwide except in the extreme northern parts of the temperate regions. They undergo the four stages of their life cycle: egg, larva, pupa and adult. The life cycle takes about 7-10 days. The adult mosquito measures from 4- 10 mm (ECDC, 2020). The eggs are laid one at a time and stick together to form of raft of 100 to 300 eggs (CDC, 2020). The rafts floats in water. The larvae live in water and are very active shedding their skin severally at this stage (Low *et al.*, 2012). *Culex* mosquitoes are the most widespread mosquito species and contain about 1000 species (Mohammed, *et al.*, 2021). These species serve as vectors of important diseases of birds, humans, and other animals (Ferraguti *et al.*, 2021). *Aedes* mosquitoes originated in Africa (Powell *et al.*, 2018) but is now found in tropical, sub-tropical and temperate regions throughout the world. *Aedes* mosquitoes have distinctive white markings on the leg and on the thorax (Ferraguti *et al.*, 2021). There are approximately 190 species of *Aedes* mosquitoes (Ferraguti *et al.*, 2021). *Aedes* mosquitoes undergo the four stages of their life cycle: egg, larva, pupa and adult. They only need a small amount of water to lay eggs. The eggs look like black dirt and stick to the container walls like glue. They can stay up to 8 months depending on environmental conditions without drying out (McGregor and Connelly, 2021). The larvae have four instar stages. The adults emerge from seven days to several weeks after egg hatching and has a narrow black body (Rogers, 2019). *Aedes* mosquitoes feed during the day (Kamgang *et al.*, 2012) with peak feeding activities between dawn and dusk (Rogers, 2019). Many of the species prefer to feed outdoor though some still prefer to live close to humans and lay their eggs indoors (Rogers, 2019). Mosquitoes serve as vector of diseases. Three (3) main genera of mosquitoes which includes *Aedes*, *Culex* and *Anopheles* transmit the causative agents of various diseases to man and animals (Achee, *et al.*, 2019). Malaria is the most important characteristic disease of man currently endemic in about 80 countries and transmitted by anopheline mosquitoes (Dahmana and Mediannikov, 2020). Malaria is caused by the parasite, *Plasmodium*. There are about 430 species of *Anopheles species* of which only 30-40 commonly serve as vectors of malaria to humans (Elbers *et al.*, 2015). Mosquitoes also serves as vectors of Lymphatic filariasis. Lymphatic filariasis is endemic in 50 countries and is caused by filarial parasites *Wuchereria bancrofti*, *Brugia malayi* or *Brugia timori* (WHO, 2021). The genera of mosquito that transmits the parasites depends on the geographically location (CDC, 2018). The most common vector in Africa is *Anopheles* (CDC, 2018) though *Culex* has been reported as a vector of Mosquito in Africa (Ughasi *et al.*, 2021). In

America, the parasites are transmitted by *Culex* mosquitoes (CDC, 2018) while in the Pacific and Asia, it is transmitted by *Aedes* and *Mansonia* (CDC, 2018). The breeding sites of mosquitoes are increased by abundant rainfall and human activities such poor sanitation, and indiscriminate rate of waste disposal. For effective reduction of mosquito breeding sites and control strategies, the knowledge of the bionomics of mosquitoes is essential (Korgaonkar *et al.*, 2012). For instance, knowledge of a biting activity of mosquito at night will lead to the use of repellent or protective collecting. These control methods resulting from bionomics knowledge are grouped into chemical, biological, mechanical, and environmental control methods. Various Mosquito control interventions have been implemented in various regions though with varying successes (Huang *et al.*, 2019, Filho, *et al.*, 2019). There is also need for health education, sensitization, and adequate policies to improve sanitation for effective control of mosquitoes.

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2. MATERIAL AND METHODS

2.1 Study area

The study was carried out in Ifite Awka, Awka South Local Government Area. Awka being the capital city of Anambra state, Nigeria is located at 199.1km by road. Ifite Awka is located geographically in Awka-South, Anambra state Nigeria. The geographical coordinates of the area are 6°15N, 7°5E and 150m above sea level. Ifite Awka lies below 300m above sea level in a valley on the plains of the Manu River. Ifite Awka is sited in a fertile tropical valley and being in the tropical rainforest zone of Nigeria they experience both the rainy and dry seasons. The temperature is generally 27°C-30°C between June and December but rises to 32°C-34°C between January and April, with the last few months of the dry season marked by intense heat. The area is density populated by staff and students of Nnamdi Azikiwe University (NAU) as well as public servants who live in and around commissioner's quarter's axis due to its proximity to the Anambra State Government. The villages that made up of Ifite-Awka is Enu-Ifite, Ezinato-Ifite and Agbada-Ifite (Wikipedia, 2021).

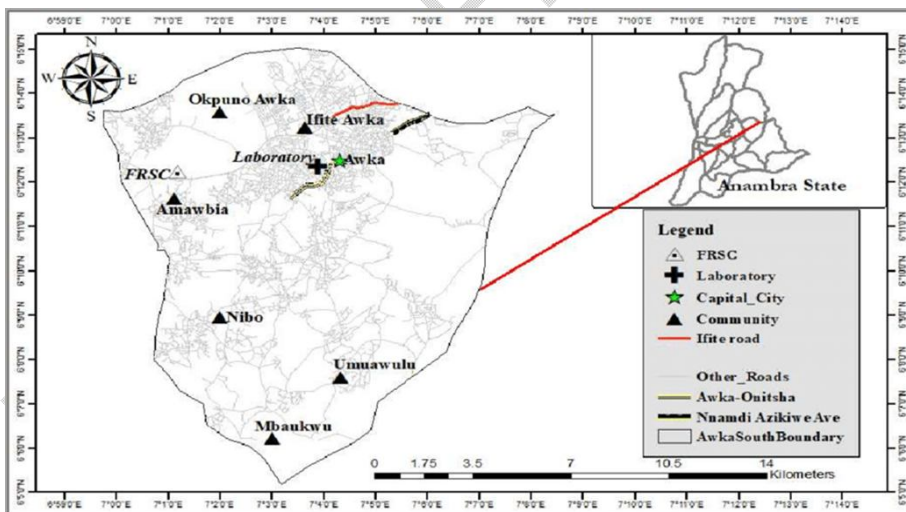


Figure 1: Map of Ifite Awka, Awka South Local Government Area, Anambra State, Nigeria. Source: Norris D.E. (2004)

2.2 Study design

Simple Random Sampling design was used in the study. Each of the lodges sampled were randomly selected to represent the lodges in Ifite Awka, Awka South L.G.A., Anambra State.

Comment [MOU4]: Why suddenly designated lodging as a place to catch mosquitoes without any previous scientific/rationale explanation

Comment [MOU5]: Mention the reasons for choosing lodging as a location for catching mosquitoes rather than other places of residence such as local settlements or local households

2.3 Sampling techniques

The sampling techniques used was Human landing catch method (used to collect the outdoor biting mosquitoes). Pyrethrum Spray Collection method (used to collect the indoor resting mosquitoes) and larval sampling (used to collect the immature stages of mosquitoes and subsequently reared to Adults)

2.3.1 Indoor collection of adult mosquitoes

This was achieved using pyrethrum Spray Collection Method (using BNC mosquito insecticides, the active ingredients include; Esbiothrin: 0.26%, Permethrin: 0.28%, Beta-Cypermethrin: 0.1%, LEMON: 0.31% which is less toxic to humans). The collections were made in the early hours between 6:00am-8:00am rooms where at least one person slept the previous night, was chosen randomly for the exercise as described by Okorie *et al.*, (2014). When entering the rooms; the windows and doors were properly closed to avoid the escape of the insecticides, spaces were made in the room to be able to cover all the edges of rooms and a white cloth sheets measuring 12m×12m were used to cover the floor of each room. The white cloth sheets were covered properly to avoid escape of mosquitoes. The rooms were left for 15 to 20mins to allow the insecticides sufficient time to knock down the mosquitoes. After 15-20mins the doors and windows were opened; carefully entered the room. The white sheets were then folded starting from the edges and with gentle jerking to ensure that all fallen mosquitoes were collected with an entomological forceps into a damp Petri dish prepared with cotton wool soaked in ethyl acetate and covered with filter paper as described by Onyido *et al.* (2016).

2.3.2 Outdoor collection of adult mosquitoes

These were collected on human volunteers and research participants who gave informed consent to be part of the study. Human Landing Catch (HLC) was used in this study. All catches were recorded between 5:00pm to 8:00pm. Mosquitoes are attracted to odor and carbon dioxide emitted from human body by the help of their palms. All the human volunteers were short skirts and knickers with sleeves shirts and singlet to expose the hands and legs to bites from mosquitoes. The volunteers sat on a low stool with 10m apart from each other and carefully, gently and consistently with patience searches for alighting mosquitoes all over their bodies when the mosquitoes would have come for a blood meal. The alighting mosquitoes on their bodies were allowed to settle before being collected with a test tube and sealed with a ball of damped cotton wool soaked with 70% Ethanol. The time and place of each collection was recorded subsequently.

Comment [MOU6]: Is this the standard method? because I think the overnight capture method will better describe the bionomics and behavior of the mosquito. Provide a standard reference about this method.

2.3.3 Collection of immature stages of mosquitoes

Mosquito larvae and pupae were collected from different mosquito breeding sites such as ground water pools, stills and standing water, discarded tins, polluted water in gutters around homes, puddles made from vehicles. The eggs of mosquitoes were seen together in form of rafts. The immature stages of mosquitos' collections were done between 10:00am-12pm as described by Onyido *et al.*, (2009).

2.3.4 Rearing of immature stages of mosquitoes

The mosquito larvae and pupae collected were reared into adult mosquitoes. The collected larvae were placed in white small bowl covered with nets and reared in the insect rearing cage which served as the insectary. The larvae were fed with a mixture of yeast and biscuits. The adults that emerged from the larvae were fed with glucose solution as described by Ekwebene *et al.*, 2020.

2.3.5 Identification of the collected mosquitoes

All the adult mosquitoes reared from the larvae or collected through indoor and outdoor collections methods were transported to the Laboratory Unit of the Department of Parasitology and Entomology, Nnamdi Azikwe University Awka for proper Identification. The mosquitoes were identified to the specie level using morphological keys as described by Service (2008), Gillett (1972) and Hopkins (1952).

2.4 Analysis of data

Data obtained from the study was summarized using Tables. Test of statistical significance at 5% levels was done. Analysis of variance (ANOVA) and Chi Square were used to compare the species composition of different mosquito genera and species in the study area.

3. RESULTS AND DISCUSSION

3.1 RESULTS

A total of 158 mosquitoes were collected from the study area, 10 of them were collected at the immature stage whereas 148 adults mosquitoes were collected. These adult mosquitoes were collected through human Landing catch and pyrethrum Spray Collection Method (for indoor resting collection). A total of 28 were collected through human Landing catch which consists of 2 species, *Culex quinquefasciatus* and *Mansonia africana*. Whereas 120 mosquitoes were collected from Pyrethrum Spray Collection Method, and it is made up *Culex quinquefasciatus*, *Anopheles gambiae* and *Mansonia africana*.

The table below Shows the composition of mosquitoes species collected from various methods of sample collection, However, analysis of variance shows no significant difference in the three methods of mosquitoes collection (ANOVA $P>0.05$).

Table 1: Identification of Mosquitoes Species in Ifite-Awka, Awka South L.G.A, Anambra State

Mosquito species	Human Landing catch	Larva sampling	Pyrethrum Spray Collection	Total (%)	Mean±S.E
<i>Culex quinquefasciatus</i>	25(19.84%)	10(7.94%)	91(72.22%)	126(79.75%)	42.0±24.8
<i>Anopheles gambiae</i>	0	0	27(100.0%)	27(17.09%)	9.0±9.0
<i>Mansonia africana</i>	3(60.0%)	0	2(40.0%)	5(3.16%)	1.6±0.9
Total	28(17.72%)	10(6.33%)	120(75.95%)	158(100%)	17.5±9.8
Mean±S.E	9.3±7.8	3.3±3.3	40.0±26.5	17.5±9.8	

The figure below shows the quarterly biting time of mosquitoes in outdoor locations using human bait collection method.

The peak biting of *Culex quinquefasciatus* which is the most predominant is between 6:00pm to 7:00pm.

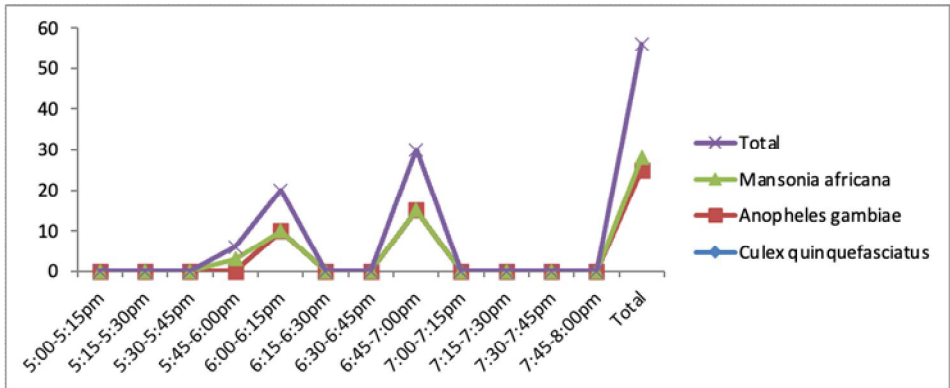
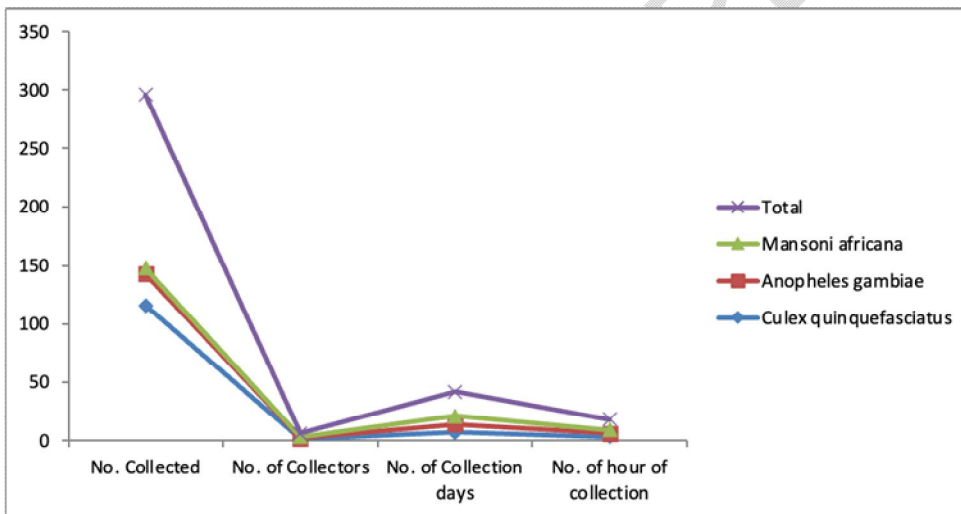


Figure 2: Collection of Mosquitoes Using the Human Landing Catch Method at Ifite-Awka, Awka South L.G.A, Anambra State

The figure below shows the quarterly biting time of all mosquitoes collected in the biting area. Mosquitoes were between 5:00pm to 8:00pm. The quarterly peak biting time is 5:45pm-7:00pm (figure 3).

Comment [MOU7]: Narrate the resulting data so that the reader better understands the intent of the data presented

Comment [MOU8]: Indeed, these data reflect the peak of biting in the observed period, but would this represent the biting behavior of mosquitoes over the overnight period? Please be careful in drawing conclusions about this fact



No. = Number

Figure 3: Effects of the Biting Time of Mosquitoes Species in Ifite-Awka, Awka-South L.G.A, Anambra State

This table shows the number of mosquitoes species collected indoor both the male and female. *Culex quinquefasciatus* is the most predominant with 91(75.83%) whereas *Mansonia africana* was the least the least prominent at (1.66%).

Comment [MOU9]: Describe again clearly how to read the graph above, and what is the significance of the data for the findings?

Table 2: Mosquito Species Collected Indoors at Ifite-Awka, Awka South L.G.A, Anambra State

Mosquito species	Number of females	Number of males	Total	% collected
<i>Culex quinquefasciatus</i>	20	7	27	22.5
<i>Anopheles gambiae</i>	78	13	91	75.83

Mansoni africana	2	0	2	1.66
Total	102(85%)	18(15%)	120	100

It was observed that *Culex quinquefasciatus* was the most predominant with 116 (78.38%). However, statistical analysis of variance (ANOVA $P < 0.05$) showed a significant difference in the distribution of mosquitoes species within the lodges.

Table 3: Distribution of Mosquitoes Species within Seven lodges representing in Ifite Awka, Awka South L.G.A, Anambra State

Mosquito species	Favour lodge	Justice court	Emeka Akaigwe	El glory	Our Lady's hotel 3	Thank God	Next Level	Total	Mean \pm S.E
<i>Culex quinquefasciatus</i>	25 (21.55)	11 (9.48)	17 (14.66)	7 (6.03)	22 (18.97)	21 (18.10)	13 (11.21)	116 (78.38)	16.5 \pm 2.4
<i>Anopheles gambiae</i>	10 (37.04)	0	10 (37.04)	0	7 (25.93)	0	0	27 (18.24)	3.8 \pm 1.8
<i>Mansoni africana</i>	2 (40.0)	0	3(60.0)		0	0	0	5 (3.38)	0.7 \pm 0.4
Total	37 (25.0)	11 (7.4)	30 (20.3)	7 (4.7)	29 (19.6)	21 (14.2)	13 (8.7)	148 (100)	7.0 \pm 1.8
Mean \pm S.E	12.3 \pm 6.7	3.6 \pm 3.6	10.0 \pm 4.0	2.3 \pm 2.3	9.6 \pm 6.4	7.0 \pm 7.0	4.3 \pm 4.3	7.0 \pm 1.8	

Comment [MOU10]: the location of the seven lodges has not been clearly identified in the method, it is better to state clearly the determination of study locations in the method and what is the purpose of determining the location

3.2 DISCUSSION

One hundred and fifty-eight (158) mosquitoes were collected in the study area. Three (3) species of mosquito which includes *Culex quinquefasciatus*, *Anopheles gambiae* and *Mansonia africana*. These are the most dominant species of mosquito in Nigeria as they have been reported in the researches of Okorie *et al.*, (2014), Joseph *et al.*, (2013), Omoregie *et al.*, (2019) and Umeanaeto *et al.*, (2017). They are also vector borne diseases of economic importance (Okorie *et al.*, 2014 and Umaru *et al.*, 2014). All Ten (10) mosquitoes collected through larval samplings were that of *Culex quinquefasciatus* and were from polluted water in gutters. This is similar to the research of Okoye *et al.*, 2020 were *Culex* species were found to breed in polluted groundwater pools. *Culex quinquefasciatus* was the most abundant specie accounting for 126(79.75%) of the total mosquitoes collection. This corresponds the research of Yayock *et al.*, (2021) and Ajao *et al.*, (2014) which reports a high abundance of *Culex* species.

The study indicates a high survival and fecundity rate *Culex quinquefasciatus* due to its high indoor resting density 91(75.83%) collaborating with the research of Irikannu *et al.*, 2021. The predominance of *Culex quinquefasciatus* over *Anopheles gambiae* and *Mansonia africana* mosquitoes at the area may be associated to the dry seasons of collections. This was explained by Umeanato *et al.*, (2017) as a result of presence of large blocked drainage with very dirty stagnant water, ground collections of dirty water, and soak-away pits among others which serve as a breeding grounds. The identified species of mosquito are vector borne diseases of economic importance (Okorie *et al.*, (2014) and Umaru *et al.*,

(2014). *Culex*, *Anopheles* and *Mansonia*, are known vectors of malaria and lymphatic filariasis. *Culex quinquefasciatus* is responsible for the transmission of filariasis, especially Bancroftian filariasis caused by *Wuchereria bancrofti*. This is highly prevalent in Nigeria with about 106 million people at risk in Nigeria (Hotez *et al.*, 2012). The result obtained from this study showed composition in mosquitoes species present at the study area. In Africa, the prevalence of lymphatic filariasis is on the increase (Agibodion *et al.*, 2008)

Adult female mosquitoes generally differ in their behavior especially biting activities including host blood meal preferences, time, and place of biting and resting sites, which are very important in the epidemiology of diseases transmission (Onyido *et al.*, 2016). *Culex quinquefasciatus* predominating in the collections. This is so because adults of *Culex quinquefasciatus* are anthropophilic (biting humans only), endophagic and endophilic (biting and resting indoors) (Onyido *et al.*, 2016). The more important vectors of human diseases are those that show close association with Man and prefer man to animals as source or food. Most *Culex* and *Anopheles* are night feeders they attack their victims when they are at rest indoor. The presence of mosquito vector habitat is directly associated with diseases transmission corresponding with the study of Mbanugo and Okpalanonuju (2003). In the pyrethrum spray collection method, *Culex quinquefasciatus* was the dominant species followed by *Anopheles gambiae* and *Mansonia africana*. This study agrees with that of Gbaye *et al.*, (2017) which reported that *Culex quinquefasciatus* was the most predominant mosquito species recorded in a south-western state in Nigeria and that of Dalhatu *et al.*, (2016) in Bauchi state, Nigeria.

4. CONCLUSION

There is a fast rate of urbanization going on in Ifite-Awka which is indicated by the presence of *Culex* mosquitoes since they have a predilection for urban environments. Urbanization implies that there is an increasing human population in the areas which is increasing the number of people that will be susceptible to lymphatic filariasis and malaria. It was observed that most of the lodges have no window and door nets and residents do not sleep with Long Lasting Insecticidal nets (LLINs) and the environment is litter with organic Matter and polluted waters, so the residents should be enlightened about the dangers of dumping refuse and sewage into drainage systems. Cleaning of already existing drainage and good construction practices will help to make the water unsuitable as a mosquito breeding habitats. Above all, massive larvae control interventions in the gutters and canals should be carried out especially in the dry season.

Comment [MOU11]: Make conclusions according to the result data and elaborate sentences precisely and carefully according to the findings obtained instead of making assumptions.

Comment [MOU12]: this content is more suitable if included in the results or detailed discussion.

REFERENCES

- Achee, N. L., Grieco, J. P., Vatandoost, H. Seixas, G. Pinto, J. Ching-Ng, L. and Martins AJ, Juntarajumnonng W, Corbel V, Gouagna C, David JP, Logan JG, Orsborne J, Marois E, Devine GJ, Vontas J. (2019). Alternative strategies for mosquito-borne arbovirus control. *PLoS Neglected Tropical Disease*. 3:13
- Aigbodion, F. I. and Emiebor, G. O. (2008). Biting cycle of *Culex quinquefasciatus* (Diptera: Culicidae) in Benin City, Nigeria. *Bioscience Research Communications* 20(5): 243-248
- Ajao, A. M., & Adeleke, M. A. (2014). Species Composition And Seasonal Abundance Of Mosquito Vectors In A Rice Growing Community In Kwara State, North Central, Nigeria. *Municipal Entomological Zoology*, 9, 838-841.
- Aribodor, N. D., Nwabueze, O. G., Kosisochukwu, U. I., Benedicta, A. O., and Amauche, I. E. (2016). Species composition and infection rate of mosquito vectors following the indoor residual spraying exercise in three communities in Awka north L.G.A of Anambra state, Nigeria. *Annual Research and Review in Biology*, 10(2).
- Centre for Disease Prevention and control, CDC, (2021). Dengue. Accessed on 31st November, 2021.
- Centre for Disease Prevention and control, CDC, (2020). Parasites. Accessed on 28th November, 2021
- Dahmana H, and Mediannikov O. (2020) Mosquito-Borne Diseases Emergence/Resurgence and How to Effectively Control It Biologically. *Pathogens*. 9(4):310.
- Dalhatu, A., Omar, A. A., & Bagari, H. (2016). Surveillance of mosquito species abundance and composition in Azare, Katagum local government of Bauchi State, Nigeria. *IOSR Journal of Pharmaceutical and Biological Sciences*, 11(6), 105-9.
- ECDC, 2020 *Culex pipiens* - Factsheet for experts <https://www.ecdc.europa.eu/en/all-topics-z/disease-vectors/facts/mosquito-factsheets/Culex-pipiens-factsheet-experts> accessed on 15th November, 2021.

- Egbuche, Ephraim Ezaka, Chiemelie Okwubanego, Ifeanyi Omah, Victor Odoh, Chidera Obi, Tangkat Dashar, Johnpaul Egbobe, Sandra Olisakwe, Tochukwu Okafor, Ifunanya Nwankwo (2021). Mosquito fauna of Anambra East LGA, Anambra state, Nigeria. *Journal of Entomology and Zoology Students*. 9 (4):32-39.
- Egwu, O., Ohaeri, C.C., Amaechi, E.C., and Ehisianyana, C.N. (2018). Distribution and abundance of mosquito larvae in Ohafia, Abia State, Nigeria. *Cuadernos de Investigación* 10.2
- Ekwebene, O. C., Ogbuagu, C. N., Ononye, B. U., & Orji, A. E. (2020). Ecological Survey of Man-Biting Mosquitoes in Nnewi Metropolis South-East Nigeria. *European Journal of Medical and Health Sciences*, 2(5).
- Elbers A. R. W., Koenraadt, C. J. M., and Meiswinkel, R. (2015) Mosquitoes and Culicoides biting midges: vector range and the influence of climate change. *Revue Scientifique Technique* 34: 123–137.
- Ferraguti, M., Heesterbeek, H., Martínez-de la Puente, J. (2021). The role of different *Culex* mosquito species in the transmission of West Nile virus and avian malaria parasites in Mediterranean areas. *Transbound Emerging Diseases*. 68: 920– 930.
- Filho, W.L., Scheday, S., Boonecke, J., Gogoi, A., Maharaj, A., Korovou, S. (2019). Climate Change, Health and Mosquito-Borne Diseases: Trends and Implications to the Pacific Region. *International Journal of Environmental Research and Public Health* 16:14.
- Gbaye, O. A., Afolabi, O. J., Simon-Oke, I. A., & Lasisi, A. O. (2017). Abundance and spatial distribution of mosquitoes across three ecological zones of Ondo State Nigeria. *International Journal of Mosquito Research*. 4(5), 23-27.
- Gillet, J. D. (1972). Common Africa mosquitoes and their medical importance: William Heinmann Medical book. Limited London, 106.
- Gurera Dev, Bharat Bhushan, Navin Kumar. (2018) Lessons from mosquitoesâ painless piercing. *Journal of the Mechanical Behavior of Biomedical Materials*, 84, 178-187
- Hopkins, G. H. E. (1952). Mosquitoes of the Ethiopian Region. I. Larval Bionomics of Mosquitoes and Taxonomy of Culicine Larvae. *Mosquitoes of the Ethiopian Region. I. Larval Bionomics of Mosquitoes and Taxonomy of Culicine Larvae.* (Edn 2).
- Hotez, P.J., Asojo, O.A. and Adesina, A.M. (2012). Nigeria: "Ground Zero" for high prevalence of neglected tropical diseases. *PLOS Neglected Tropical Diseases*. 6, e1600.
- Huang, Y. S., Higgs, S., Vanlandingham, D. L. (2019). Emergence, and re-emergence of mosquito-borne arboviruses. *Current Opinion in Virology*, 34:104–109.
- Ifeoluwa K. F., Emmanuel T.I., Taiwo S.A., Olubunmi A.O. (2020). Seasonal abundance and larval habitats characterization of mosquito species in Lagos State, Nigeria. *Scientific African*, 10, 6-11
- Irikannu, K. C., Onyido, A. E., Umeanaeto, P. U., Onyebueke, A. C., Nzeukwu, C. I., Ogbonna, C. U., & Asogwa, K. K. (2021). Breeding ecology and physicochemical properties of mosquito breeding sites in Awka South Local Government Area, Anambra State, Nigeria. *The Bioscientist Journal*, 10(1), 113-122.
- Joseph, A. O., Adepeju, S. O. I., & Omosalewa, O. B. (2013). Distribution, abundance and diversity of mosquitoes in Akure, Ondo State, Nigeria. *Journal of Parasitology and Vector Biology*, 5(10), 132-136.
- Kamgang, B., Nchoutpouen, E., Simard, F. (2012). Notes on the blood-feeding behavior of *Aedes albopictus* (Diptera: Culicidae) in Cameroon. *Parasites Vectors* 5, 57.
- Korgaonkar NS, Kumar A, Yadav RS, Kabadi D, Dash AP. (2021). Mosquito biting activity on humans and detection of Plasmodium falciparum infection in *Anopheles stephensi* in Goa, India. *Indian Journal of Medical Research*. 135(1):120-6.
- Low, V. L., Chen, C. D., Lee, H. L., Lim, P. E., Leong, C. S., Sofian-Azirun, M. (2012) Nation-wide distribution of *Culex* mosquitoes and associated habitat characteristics at residential areas in Malaysia. *Journal of American Mosquito Control Association*. 28(3):160-9.
- Mbanugo, J. I., & Okpalaonuju, C. N. (2003). Surveillance of mosquito vectors in some habitats of Awka metropolis, Anambra, Nigeria. *Nigerian Journal of Parasitology*, 24(1), 185-190.
- McGregor, L. M. and Connelly, C. X. (2021). A Review of the Control of *Aedes aegypti* (Diptera: Culicidae) in the Continental United States, *Journal of Medical Entomology*, Volume 58, Issue 1, Pages 10–25
- Mohammed, B.R. A.M. Yayo, O.J. Ajanusi, I.A. Lawal, (2021) Relative abundance and molecular identification of *Culex pipiens* complex (Diptera: Culicidae), in Kura Local Government Area, North-western Nigeria, *Parasite Epidemiology and Control*, 14
- Nicoletti, M. (2020). Chapter Five - Three scenarios in insect-borne diseases. In *Insect-Borne Diseases in the 21st Century*. Academic Press, 99-251,
- Nikbakhtzadeh, M., Buss, G., & Leal, W. (2016). Toxic Effect of Blood Feeding in Male Mosquitoes. *Frontiers in physiology*, 7(JAN), 4. Report #: ARTN 4.
- Okorie, P.N., Popoola, K. O.K., Awobifa, O. M., Ibrahim, K. T. and Ademowo, G. O. (2014). Species composition and temporal distribution of mosquito populations in Ibadan, Southwest Nigeria. *Journal of Entomology and Zoology Studies*, 2(4): 164-169.
- Okoye, N. T., Iheukwumere, S. O., Nkwocha, K. F., & Igboanugo, I. R. (2020). Trend Analysis of Road Transport Infrastructure Development in Awka, Anambra State, Nigeria. *The International Journal of Science & Technology*, 8(6).
- Omoriegbe, A. O., Omoriegbe, M. E., Adetimehin, A. D., & Aigbodion, F. I. (2019). Species Composition of Mosquitoes from Boarding School Dormitories in Benin City, Edo State, Nigeria. *Nigerian Annals Of Pure And Applied Sciences*, 2, 25-34.
- Onyido, A. E., Ezeani, A. C., Irikannu, K. C., Umeanaeto, P. U., Egbuche, C. M., Chikezie, F. M., & Ugha, C. N. (2016). Anthropophilic Mosquito Species Prevalence in Nibo Community, Awka South Local Government Area, Anambra State, Southeastern Nigeria. *Ewemen Journal of Epidemiology and Clinical Medicine*. 2(1), 14-20.
- Onyido, A., Ezike V., Ozumba, N., Nwosu, E., Ikpeze, O., Obiukwu, M. and Amadi E. (2009) Crepuscular Man-Biting Mosquitoes of a Tropical Zoological Garden in Enugu, South-Eastern Nigeria. *The Internet Journal of Parasitic Diseases*. 4(2)
- Powell, J. R., Gloria-Soria, A., & Kotsakiozi, P. (2018). Recent History of *Aedes aegypti*: Vector Genomics and Epidemiology Records. *Bioscience*, 68(11), 854–860.
- Rogers, K. I. and Kara, M. J. (2019) "Aedes". Encyclopedia Britannica, <https://www.britannica.com/animal/Aedes>. Accessed 15 November 2021.
- Service, M. (2008). Anopheline mosquitoes (Anophelinae). In *Medical Entomology for Students* (pp. 33-51). Cambridge: Cambridge University Press.
- Sinka, M. E., Bangs, M. J., Manguin, S. (2010). The dominant *Anopheles* vectors of human malaria in Africa, Europe, and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasites Vectors* 3, 117. p40.
- Umaru, N. F., Akogun, O. B. and Owuama, C. I. (2014). Biological and chemical factors associated with survival of *Anopheles* and *Culex* mosquitoes in captivity in Yola, Nigeria. *International Journal of Applied Microbiology and Biotechnology Research*, 2: 43-51.

Comment [MOU13]: Too old reference

Comment [MOU14]: May be there is the more recent study in the site.

Umeanaeto, P. U., Asogwa, A. N., Onyido, A. E., Irikannu, K. C., & Ifeanyichukwu, M. O. (2017). The parity rate of indoor-resting adult female *Anopheles* and *Culex* mosquitoes and their implication in disease transmission in Nnamdi Azikiwe University female hostels Awka, south eastern Nigeria. *International Journal of Environment, Agriculture and Biotechnology*, 2(4), 238825.

World Health Organization (2010). World malaria report 2010. WHO Global Malaria Programme. http://whqlibdoc.who.int/publications/2010/9789241564106_eng.pdf. Accessed on 15th November, 2021.

Yayock, H. C., Osagiede, N. O., Bitrus, S., Gimba, H. S., & Shehu, R. (2021). Survey of Mosquito Species, Composition and Breeding Habitats in Four Communities of Kaduna Metropolis, Nigeria. *Fudma Journal of Sciences*, 5(3), 122-129.

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