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SURVEY OF ADULT *Anopheles* MOSQUITOES IN ORUMBA NORTH LOCAL GOVERNMENT AREA, ANAMBRA STATE, NIGERIA

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Abstract

Insect-transmitted diseases remain a major source of illness and death worldwide. Malaria parasites affecting humans are transmitted through the bites of infected female *Anopheles* mosquitoes. However, the vector distribution varies in locations. This study aimed to survey adult *Anopheles* mosquitoes in Orumba North Local Government Area, Anambra State, Nigeria from August 2022 to July 2023. Adult *Anopheles* mosquitoes were collected from indoor and outdoor locations using Pyrethrum Knockdown Collection (PKC) and Human Bait Collection (HBC) methods. Morphological identification of *Anopheles* species was carried out using standard identification keys. Molecular identification of sibling species of *Anopheles gambiae* complex was also done using Polymerase Chain Reaction (PCR). A total of 2,252 adult *Anopheles* mosquitoes were collected from the study area. Of the total number, 764(33.9%) were collected outdoors while 1488(66.1%) were collected indoors. The total collections comprised of two *Anopheles* mosquito species namely; *An. gambiae* 1618 (71.8%) and *An. funestus* 634(28.2%). Molecular identification of sibling species of *Anopheles gambiae* complex was also done using Polymerase Chain Reaction (PCR). Out of 300 *An. gambiae* mosquitoes that were subjected to PCR, 272(90.67%) were amplified and identified as *An. gambiae s.s*. while 28(9.33%) were unamplified and could not be identified. Of the 1488 adult mosquitoes collected indoors, the highest number 822(55.2%) were freshly fed, while the least 131(8.8%) were gravid. The cumulative Indoor Resting Density of the two *Anopheles* species was 2.9 mosquitoes/room/night, while Man Biting Rate was 0.8 bite/man/night. Of the 2,252 mosquitoes, 2,229(99.0%) were collected in the rainy season while 23(1.0%) were collected in the dry season. The presence of *An. gambiae* and *An. funestus* in the area shows that the inhabitants were exposed to their bites. It is recommended that integrated vector control is used against the vectors in order to reduce malaria transmission to levels below public health importance.

Keywords: Malaria, Mosquitoes, *Anopheles gambiae, Anopheles funestus.*

INTRODUCTION

Many of the most important significant infectious diseases of humans are transmitted by arthropods and in particular by mosquitoes (Culicidae). Mosquitoes are the greatest enemies of man because of widespread suffering and death caused by the diseases transmitted. Mosquitoes belong to the family Culicidae, the insect order known as Diptera (True flies). There are three subfamilies of the family Culicidae; Anophelinae, Culicinae and Toxorhynchitinae (Snow, 1990). It is only subfamilies of Anophelinae and Culicinae that contain medically important man-biting mosquitoes (Walker and Lynch, 2007). Mosquitoes are important vectors of human diseases and the most common blood sucking arthropods. They are ubiquitous in distribution and are found in both tropical and temperate regions of the world (Onyido *et al.*, 2010).

The most important is malaria vectored by *Anopheles* mosquitoes (Gilles *et al.,* 1993). In spite of the global effort to eliminate malaria, it remains the most significant vector- borne disease of humans. *Anopheles* mosquitoes are responsible for the transmission of a number of diseases in the world including malaria, lymphatic filariasis (*Wuchereria bancrofti* and *Brugia malayi)* and viruses such as one that causes O'nyong' nyong fever among others (Yaw *et al.,* 2012).

Anopheles mosquitoes have plagued the world with malaria for decades and centuries now. Malaria is caused by protozoan parasites of the genus *Plasmodium* and transmitted by female *Anopheles* mosquitoes. There are five different human malaria species; *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale* and *Plasmodium knowlesi* (Gontie *et al.*, 2020). Malaria is transmitted through the bite of infected female *Anopheles* mosquitoes. *Anopheles gambiae* is the dominant and most efficient vector of human malaria in Africa based on its high abundance, longevity, high propensity for human feeding and high vectorial capacity (Autino *et al.,* 2012). It plays a prominent role in the transmission of the most dangerous malaria parasite species- *P. falciparum* (Yaw *et al.,* 2012) and this account for about 80% morbidity and 90% mortality.

There are approximately 460 recognized *Anopheles* mosquito species worldwide and over hundred of them are capable of transmitting human or animal diseases (Yaw *et al.,* 2012). In sub-Saharan Africa, there are 140 *Anopheles* species (Foley *et al.,* 2010). *Anopheles gambiae* complex referred to as *An. gambiae sensu lato* is made up of eight (8) reproductively isolated species that are almost indistinguishable morphologically; *An. arabiensis, An. gambiae sensu stricto, An. bwambae, An. melas, An. merus, An. quadriannulatus, An. coluzzi,* and *An. amharicus* (Akpan *et al.,* 2018). Of the 37 *Anopheles* species documented vectors of malaria in Nigeria, studies have identified mosquitoes of the *An. gambiae* (principally *An. gambiae sensu stricto, An. arabiensis funestus* complexes as the main vectors of malaria (Oyewole *et al.,* 2010; Sinka *et al.*, 2010). *Anopheles melas* is found in the coastal areas and is involved in malaria transmission (Oyewole *et al.*, 2010).

The aim of this study was to conduct a survey on adult *Anopheles* species in the study area. The specific objectives were to determine the abundance of *Anopheles* species in the study area through morphological and molecular identification, distribution of both the outdoor biting and indoor biting *Anopheles* mosquitoes, physiological state of indoor biting *Anopheles* mosquitoes collected, indoor resting density and man biting rate of *Anopheles* mosquitoes and monthly distribution of *Anopheles* mosquito species in the study area.

MATERIALS AND METHODS

Description of the Study Area

The study was carried out in Orumba North Local Government Area of Anambra State Southeast Nigeria in the following communities; Amaokpala, Awgbu, Awa, Omogho, Ndikelionwu, Ndiokpalaeze and Ufuma. The area lies between latitudes $5^0$58'N -$5^0$60'N and longitudes $6^0$47'E-6^0.57'E. The study area has a rainforest climate with 7-8 months of wet season (April to November) and 4-5 months of dry season (December to March) with a short period of harmattan (December to January). The area has distinct rainy and dry seasons.

Study Design

The study was a field survey of adult *Anopheles* mosquitoes and laboratory based molecular characterization of the identified mosquitoes in the study area.

Advocacy Visits and Community Mobilization

Advocacy visits were made with an introductory letter from the Head of Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli to Orumba North Local Government Area Chairman. Visits were also made to the traditional leaders of the selected communities to obtain permission to work with their people. Furthermore, consent was also sought from head of households of selected houses. Sensitization was done house to house in selected communities before mosquito collection. Volunteers involved in the collection of mosquitoes were educated on the nature of the field work and the implications. They were trained on how to collect landing mosquitoes before blood feeding to minimize the risk of malaria transmission. They were given prophylactic treatment for malaria 10 days before commencement of the study according to Onyido *et al.* (2008).

Selection of Houses for Mosquito Collection

Purposive sampling was used in selecting houses with each village forming a stratum. House structures were selected based on the number of pregnant women in the study area.

Collection of Adult Mosquitoes

Outdoor biting mosquitoes and indoor biting and resting adult mosquitoes were collected. Mosquitoes were collected through the assistance of volunteers.

Collection of Indoor Biting Mosquitoes

Indoor biting mosquitoes were collected using Pyrethrum Knock-down Collection (PKC) method as described by Gillett (1972). This was done in the early hours of the day between 6.00am and 8.00am from rooms slept in by at least one person the previous night. Head count of each selected household was done and the number of persons that slept in each room was noted. In each room, the doors and windows were shut and white spread sheets laid from wall to wall covering furniture and other non-movable items in the rooms. A pyrethroid-based insecticide aerosol (Raid$^{@}$) was sprayed in the rooms and allowed to stay for 20 minutes before collection. Cracks or any observed escape routes from the walls, doors and windows were closed with old newspaper to prevent escape of mosquitoes through them. At the end of the time interval after spraying, the white spread sheets were folded and mosquitoes were collected using a pair of entomological forceps into a well-labelled petri dish lined with damp cotton wool.

Collection of Outdoor Biting Mosquitoes

Outdoor biting mosquitoes were collected using Human Bait Collection (HBC) method as described by Gillett (1972). In this method of collection, volunteers were used as baits. Collection was done all night from 6.00pm to 6.00am (Local time). Torchlight, cotton wool, test tube vials, wrist watches for keeping time, pen and paper for recording the time of collection, cellophane bags for collection and collation of catches and low stools or benches were the materials that were used. The volunteers sat on low stools and benches at a little distance away from each other, exposed their legs and hands for mosquito bites by rolling up their trousers and shirt sleeves to knee and elbow levels respectively and searched for mosquitoes all over their bodies (with torchlight).

Mosquitoes alighting on their bodies to suck blood were collected with the aid of test tube vials and torchlight. On collection of each mosquito, the vial was quickly covered with a ball of cotton wool to avoid escape of mosquitoes. The time of collection of each mosquito was properly recorded. Mosquito collections was collated at 15minutes interval and placed in separate bags.

Morphological Identification of the Mosquitoes Collected

At the end of each collection period, all the mosquitoes collected were properly labelled and sent to the Entomology Laboratory of the Department of Parasitology and Entomology, Nnamdi Azikiwe University Awka for identification. The mosquitoes were identified using the gross morphology of the species especially the body colour, patches of scales on the palps, antennae, proboscis, patches of pale and black scales on the wings and legs and the terminal abdominal segments using standard key as described by Gillett (1972).

Preservation of Mosquitoes for Molecular Identification

After morphological identification, all the outdoor and indoor biting adult *Anopheles* mosquitoes belonging to *An. gambiae* complex were preserved in Eppendorf tubes for molecular studies. An Eppendorf tube was used to preserve a single adult mosquito. Each Eppendorf tube was 70% filled with silica gel in its solid form. The silica gel served as a preservative to prevent the mosquito from decaying during transportation. A ball of cotton wool was placed in the tube to separate the preservative from the adult mosquito. Each adult mosquito was placed on the cotton ball and the Eppendorf tube was covered. All the tubes containing the preserved mosquitoes were transported to the Laboratory of Nigerian Institute of Medical Research, Yaba, Lagos State, for Polymerase Chain Reaction (PCR) studies.

Molecular Identification of Sibling Species of *Anopheles gambiae* Complex Using Polymerase Chain Reaction (PCR)

- **Deoxyribonucleic acid (DNA) Extraction**

PCR amplification of DNA sibling species of *An. gambiae* complex was performed as described by Scott *et al.* (1993). The wings and legs of each mosquito were severed using a scalpel and were put into centrifuge tubes for DNA extraction. The DNA was extracted following the manufacturer's instruction was used. Blood-Animal-Plant DNA preparation kit manufactured by Jena Bioscience, Germany. The extraction was done by adding the severed specimens to a ZR Bashing Bead lysis tube. Then 750µl lysis solution was added to the tube. The set-up was secured in a bead beater fitted with a 2ml tube holder assembly and was processed at maximum speed for 10 minutes. The ZR Bashing Bead lysis tube was centrifuged at ≥10,000rpm for 1 minute and 400µl of the supernatant was transferred to Zymo-Spin IV Spin Filter (orange top) in a collection tube, centrifuged at 7,000rpm for 1 minute; 1200µl of Genome Lysis Buffer was added to the filtrate in the collection tube and mixed. Eight hundred microlitres (800µl) of the mixture was transferred to a Zymo- Spin IIC column in a collection tube and centrifuged at 10,000rpm for 1 minute. The flow through the collection tube was discarded and the previous process was repeated. Two hundred microlitres (200µl) of DNA Pre-Wash Buffer was added to the Zymo-Spin IIC column in a new collection tube and was centrifuged at 10,000rpm for 1 minute. Five hundred microlitres (500µl) g-DNA Wash Buffer was added to the Zymo-Spin IIC column and centrifuged at 10,000rpm for 1 minute. The Zymo-Spin IIC column was transferred to a clean 1.5ml microcentrifuge tube and 50µl DNA Elution Buffer was added directly to the column matrix. It was then centrifuged at 10,000rpm for 30 seconds and the DNA was eluted.

- **Preparation of Master Mix**

A master mix for *An. gambiae* complex mosquitoes was prepared by mixing the primers and other reagents in the following proportion (Pre-mix 4.0 µl, ddH2O 5.25 µl, ME 0.5 µl, AR 0.5 µl, GA 0.5 µl, UN 0.5 µl, OD 0.25 µl, DNA 1.0 µl. The total mixture obtained is called the master mix which was used for PCR in step 3.

- **PCR Procedure for *Anopheles gambiae* Complex**

Twelve and half microlitres (12.5 µl) of PCR master mix of each adult mosquito was added into each of the two hundred microlitres (200µl) tube. One microlitre (1µl) of DNA was added into each tube. Each of the tube was loaded in the PCR machine and an appropriate programme and PCR condition was chosen on the machine. The PCR conditions for *An. gambiae* complex chosen were; Initial Denaturation @ 95^{o}C – 2 mins, Denaturation @ 95^{o}C – 30sec, Annealing @ 55^{o}C – 30sec, Extension @ 72^{o}C – 40sec, Final extension @ 72^{o}C – 7mins. All the conditions were set to run for 30 cycles. After subjecting to polymerase chain reaction, 1.5% agarose gel was prepared by weighing 1.5g of agarose powder in 100ml of Trisborate ethylene-di-amino tetraacetic acid (TBE) buffer and boiled in microwave until the solution was clear. This was brought out and allowed to cool for few minutes until no steam was observed and 10µl of ethidium bromide was added. The gel was poured into a trough and allowed to solidify. Ten (10µl) of the microlitres µl of DNA ladder,

negative control and PCR product was then added into each well for electrophoresis and gel was viewed using gel documentation machine.

Determination of Physiological State of the Mosquitoes Collected

The physiological state of female mosquitoes collected indoors was determined in order to observe mosquitoes that had blood meal and those that had not fed. The mosquitoes were grouped into four categories; Unfed, freshly fed, half gravid and gravid according to Service (1985).

Determination of Indoor Resting Density of the Mosquitoes

The indoor resting density of the mosquitoes collected was calculated as described by Ezihe *et al.,* (2017). The room density was determined by the total number of *Anopheles* mosquitoes collected divided by the total number of rooms sampled and the total number of nights the mosquitoes were collected. It was calculated as thus;

Indoor Resting Density (D) = (Number of *Anopheles* females ÷ Number of rooms) ÷ Number of nights

The result was expressed as number of mosquitoes/room/night.

Determination of Man Biting Rate of the Mosquitoes Collected

Man biting rate as described by Irikannu *et al.,* (2019) was expressed as the number of bites a person receives from a specific vector per night. This was determined from PKC as the total number of freshly fed *Anopheles* females of a species collected divided by the total number of occupants who spent the night in the rooms and then the total number of nights that was used for the collection. It was calculated as thus;

Man Biting Rate= (Number of freshly fed females ÷ Total number of occupants) ÷ Total number of nights

The results were expressed as mosquito bites/man/night.

Data Summary and Statistical Analysis

Data collected were summarised using tables, graphs and charts. Test of statistical significance was conducted using Chi square and one way Analysis of Variance (ANOVA) at $p < 0.05$. The statistical package employed was SPSS version 25.0.

RESULTS

Abundance of *Anopheles* Mosquito Species in the Study Area

A total of 2,252 adult *Anopheles* mosquitoes were collected from the study area throughout the study period (Table 1). Of the total number, 764(33.9%) were collected outdoors while 1488(66.1%) were collected indoors. The total collections comprised of two *Anopheles* mosquito species in varying proportions namely; *An. gambiae* 1618 (71.8%) and *An. funestus* 634 (28.2%).

Table 1: Abundance of *Anopheles* mosquito species collected from the study area

Mosquito Species	Outdoor biting population (%)	Indoor biting population (%)	Total (%)
Anopheles gambiae	546	1072	1618 (71.8)
Anopheles funestus	218	416	634 (28.2)
Total	764 (33.9%)	1488 (66.1%)	2,252 (100)

Molecular Identification of Sibling Species of *An. gambiae* Complex by Polymerase Chain Reaction

A total of 300 *An. gambiae* complex mosquitoes were subjected to PCR, 272(90.67%) were amplified and identified while 28(9.33%) were unamplified and could not be identified. All the amplified mosquitoes were identified as *An. gambiae* sensu stricto. (Fig. I).

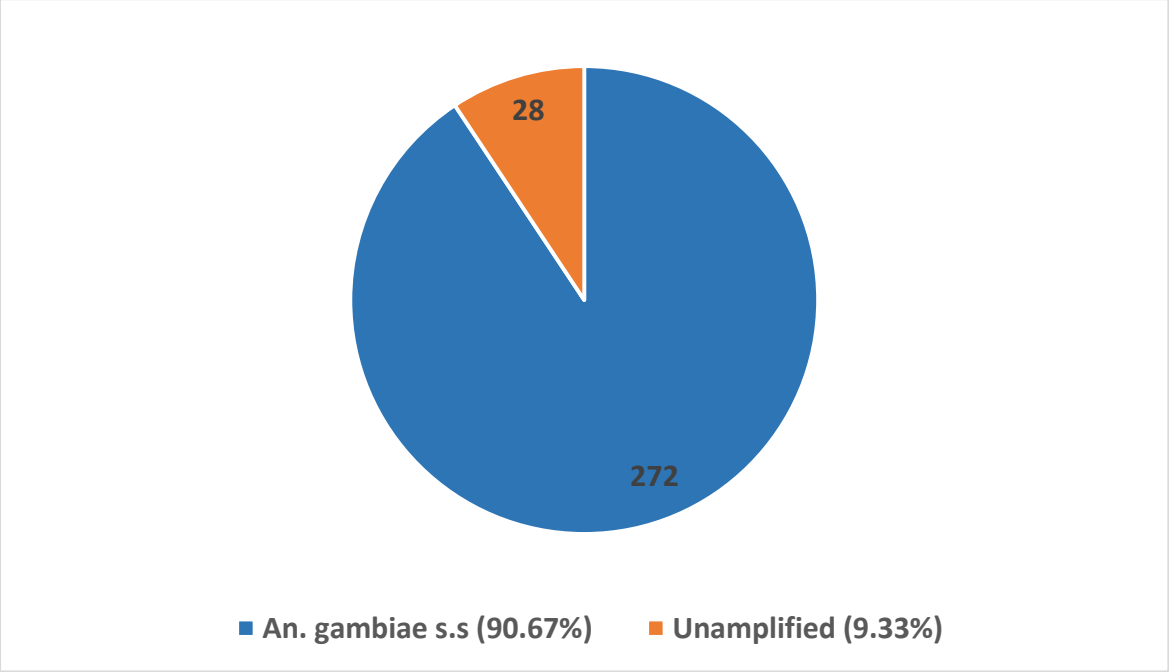


Fig I. Chart showing the percentage of *Anopheles gambiae* complex amplified and unamplified bands

A total of 764 adult *Anopheles* mosquitoes were collected outdoors from different communities in the study area (Table 2). The outdoor collections comprised of two *Anopheles* species. They

were *An. gambiae* 546(71.5%) and *An. funestus* 218(28.5%). The number of *Anopheles* mosquitoes collected outdoors varied across communities. The highest number 234(30.6%) were collected in Ufuma while the least number 46(6.0%) were collected in Amaokpala. Total number of *Anopheles* mosquitoes collected from other communities was Awgbu 51(6.7%), Awa 63(8.2%), Omogho 79(10.3%), Ndikelionwu 104(13.6%), and Ndiokpalaeze 187(24.5%). There was significant difference in abundance of outdoor biting *Anopheles* mosquito species across communities ($P<0.05$)

Table 2: Distribution of outdoor biting *Anopheles* mosquito species in communities

Communities	*Anopheles gambiae*	*Anopheles funestus*	Total	Percentage (%)
Amaokpala	27	19	46	6.0
Awgbu	31	20	51	6.7
Awa	41	22	63	8.2
Omogho	50	29	79	10.3
Ndikelionwu	79	25	104	13.6

Ndiokpalaeze	101	86	187	24.5
Ufuma	167	67	234	30.6
Total	546 (71.5)	218 (28.5)	764	100

X^2 =20.870, df=6, P=0.002 (P<0.05)

All adult *An. gambiae* and *An. funestus* mosquitoes collected outdoors were recorded according to their time of collection (Fig I1). *An. gambiae* were collected between 10.00pm to 6.00am while *An. funestus* were collected between 11.00pm to 5.00am. Both *An. gambiae* and *An. funestus* recorded their biting peak between 1.00am-2.00am.

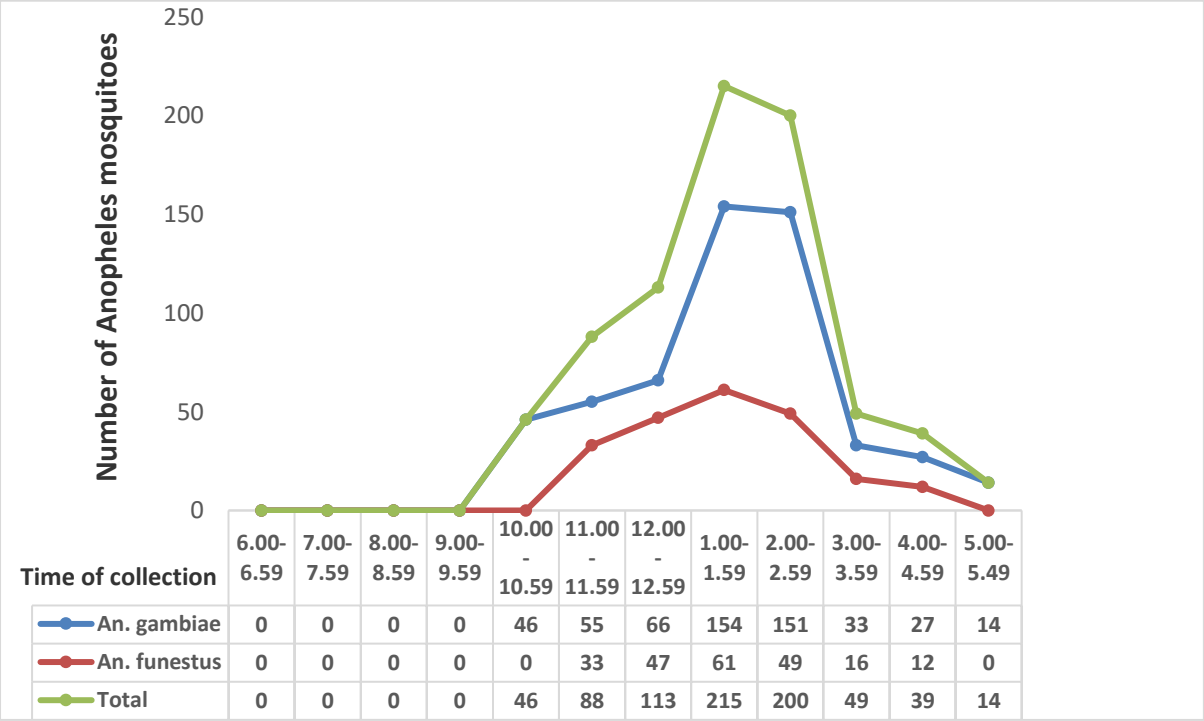


Fig 1I: All-night hourly biting pattern of *Anopheles gambiae* and *Anopheles funestus* mosquitoes in the study area

A total of 1488 adult *Anopheles* mosquitoes were collected indoors from different communities in the study area (Table 3). The indoor collections also comprised of two *Anopheles* species. They were *An. gambiae* 1072(72.0%) and *An. funestus* 416(28.0%). The number of *Anopheles* mosquitoes collected indoors varied across communities. The highest number 462(31.0%) were collected in Ufuma while the least number 86(5.8%) were collected in Amaokpala. Total number of *Anopheles* mosquitoes collected from other communities were; Awgbu 96(6.5%),

Awa 122(8.2%), Omogho 152(10.2%), Ndikelionwu 202(13.6%), and Ndiokpalaeze 368(24.7%). There was significant difference in abundance of indoor biting *Anopheles* mosquito species across communities (P<0.05).

Table 3: Distribution of indoor biting *Anopheles* mosquito species in communities

Communities	*Anopheles gambiae*	*Anopheles funestus*	Total	Percentage (%)
Amaokpala	52	34	86	5.8
Awgbu	63	33	96	6.5
Awa	79	43	122	8.2
Omogho	98	54	152	10.2
Ndikelionwu	153	49	202	13.6
Ndiokpalaeze	299	69	368	24.7
Ufuma	316	146	462	31.0
Total	1072 (72.0)	416 (28.0)	1488	100

X^2 =34.066, df=6, P=0.000 (P<0.05)

Malaria Transmission Potentials of *Anopheles* Mosquitoes Collected

The abdominal status of the *An. gambiae* and *An. funestus* mosquitoes collected indoors were observed and recorded (Fig III). Of the 1488 adult mosquitoes collected indoors, the highest number 822(55.2%) were freshly fed, while the least 131(8.8%) were gravid. Others were 312(21.0%) unfed while 223(15.0%) were half gravid.

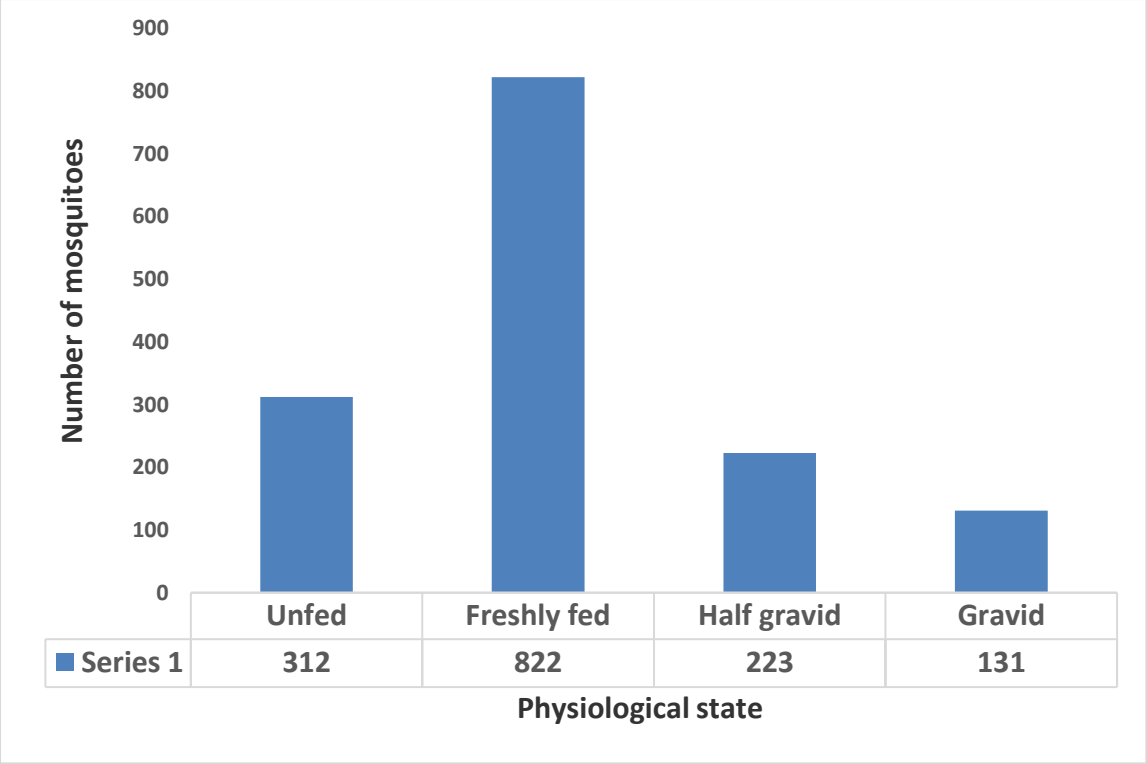


Fig III: Physiological state of indoor biting *Anopheles* mosquitoes collected in the study area

The Indoor Resting Density (IRD) and Man Biting Rate (MBR) of *Anopheles* mosquito species collected in the study area was calculated (Table 4). The cumulative IRD of the two *Anopheles* mosquitoes in the area was 2.9 mosquitoes/room/night while the cumulative MBR was 0.8 bite/man/night. By species, *An. gambiae* had the highest IRD of 2.1 mosquitoes/room/night and MBR of 0.6 bite/man/night. In contrast, *An. funestus* has the least IRD of 0.8 mosquitoes/room/night and MBR of 0.2 bite/man/night.

Table 4: Indoor resting density and man biting rate of *Anopheles* mosquito species in the study area

Anopheles mosquito collected indoors	Number of *Anopheles* mosquitoes collected	Number of *Anopheles* mosquitoes freshly fed	Indoor Resting Density (IRD) No. of mosquito/room/night	Man Biting Rate (MBR) No. of bite/man/night
Anopheles gambiae	1072	624	2.1	0.6

Anopheles funestus	416	198	0.8	0.2
Total	1488	822	2.9	0.8

Monthly Distribution of *Anopheles* Mosquito Species

All the adult *An. gambiae* mosquitoes collected were recorded according to month of collection (Fig IV). Of the 1618 collected both indoors and outdoors in the study, the highest (n=316) was collected in the month of August (rainy season) while none (n=0) was collected between November and February (dry season). Total collections in other months were; March (n=23), April (n=126), May (n=206), June (n=245), July (n=286), September (n=214) and October (n=202).

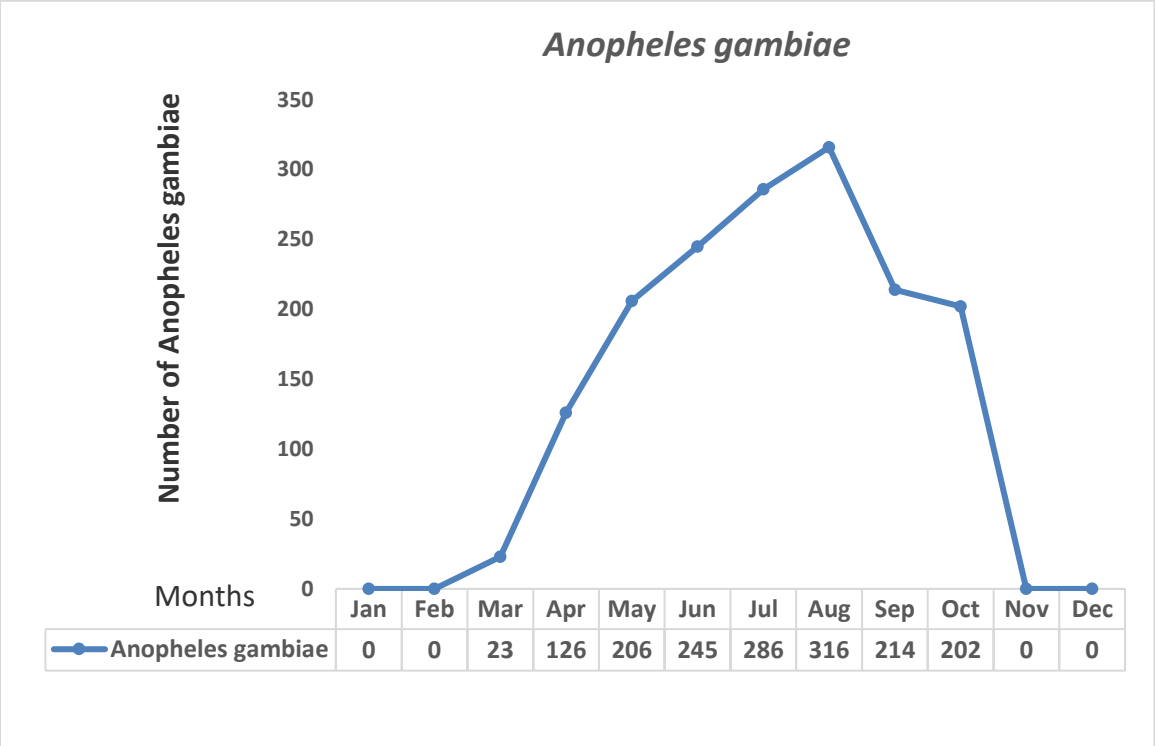
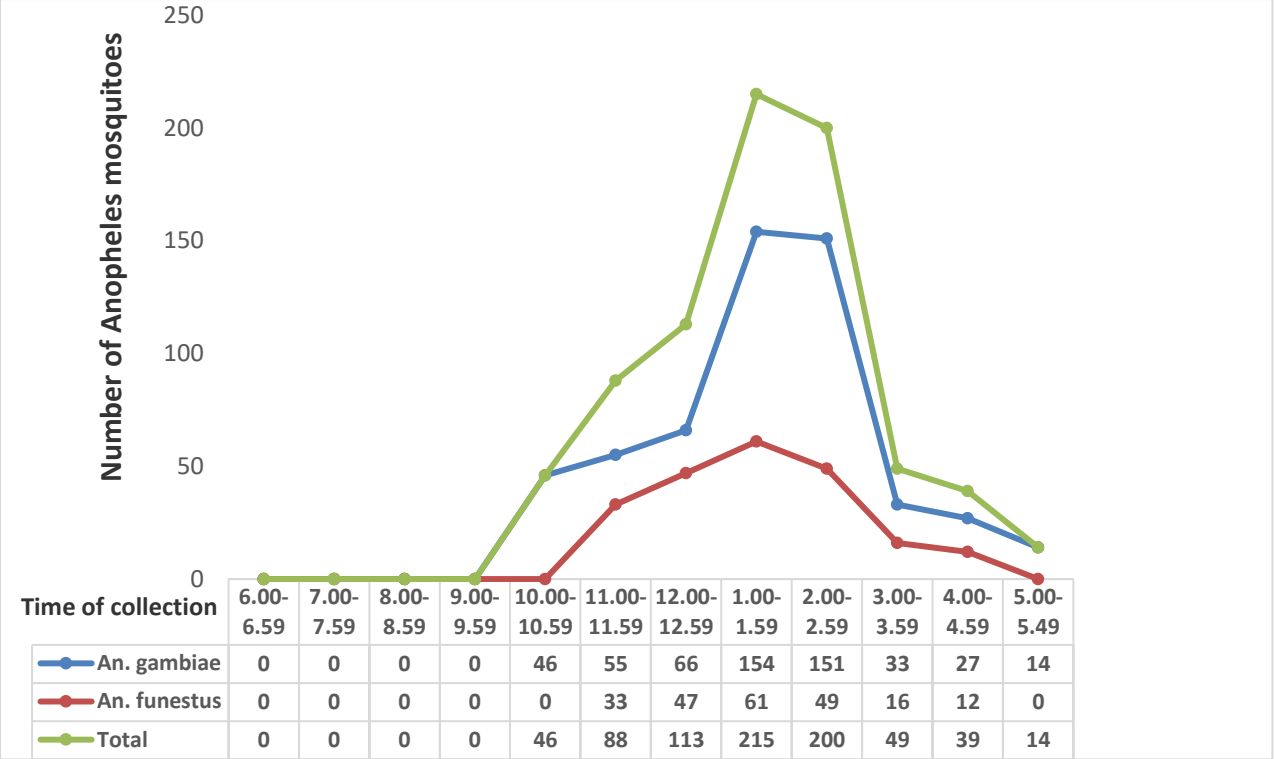


Fig IV: Monthly distribution of *Anopheles gambiae* mosquitoes collected in the study area



Time of collection	6.00-6.59	7.00-7.59	8.00-8.59	9.00-9.59	10.00-10.59	11.00-11.59	12.00-12.59	1.00-1.59	2.00-2.59	3.00-3.59	4.00-4.59	5.00-5.49
An. gambiae	0	0	0	0	46	55	66	154	151	33	27	14
An. funestus	0	0	0	0	0	33	47	61	49	16	12	0
Total	0	0	0	0	46	88	113	215	200	49	39	14

Fig V: All-night hourly biting pattern of *Anopheles gambiae* and *Anopheles funestus* mosquitoes in the study area

A total of 1488 adult *Anopheles* mosquitoes were collected indoors from different communities in the study area (Table 3). The indoor collections also comprised of two *Anopheles* species. They were *An. gambiae* 1072(72.0%) and *An. funestus* 416(28.0%). The number of *Anopheles* mosquitoes collected indoors varied across communities. The highest number 462(31.0%) were collected in Ufuma while the least number 86(5.8%) were collected in Amaokpala. Total number of *Anopheles* mosquitoes collected from other communities were; Awgbu 96(6.5%), Awa 122(8.2%), Omogho 152(10.2%), Ndikelionwu 202(13.6%), and Ndiokpalaeze 368(24.7%). There was significant difference in abundance of indoor biting *Anopheles* mosquito species across communities ($P<0.05$).

Table 3: Distribution of indoor biting *Anopheles* mosquito species in communities

Communities	*Anopheles gambiae*	*Anopheles funestus*	Total	Percentage (%)
Amaokpala	52	34	86	5.8

Awgbu	63	33	96	6.5
Awa	79	43	122	8.2
Omogho	98	54	152	10.2
Ndikelionwu	153	49	202	13.6
Ndiokpalaeze	299	69	368	24.7
Ufuma	316	146	462	31.0
Total	1072 (72.0)	416 (28.0)	1488	100

X^2 =34.066, df=6, P=0.000 (P<0.05)

DISCUSSION

A total of 2,252 adult *Anopheles* mosquitoes were collected from the study area throughout the study period. The total collection is much lower when compared to Egbuche *et al*. (2020) who collected 8181 *Anopheles* mosquitoes in Anambra East, a riverine Local Government Area in Anambra State and higher than Irikannu *et al*. (2019) who collected a total of 211 *Anopheles* mosquitoes in Awka Metropolis, Anambra State. Lamidi *et al*. (2017) collected 1073 *Anopheles* mosquitoes in three selected areas in Taraba State, North-eastern Nigeria. Several other studies have reported the collection of varying population of *Anopheles* mosquitoes in different locations in Anambra State, Nigeria (Onwuzuike *et al*., 2021a; Onwuzuike *et al*. 2021b; Irikannu *et al*. 2021). It has been noted that the availability of breeding sites in an area determines the population of mosquitoes in that area (Irikannu *et al*. 2021; Egbuche *et al*. 2020). Of the total number of *Anopheles* mosquitoes collected, 33.9% were collected outdoors while 66.1% were collected indoors. This shows that the malaria vectors biting activities takes place indoors than outdoors. This is an important epidemiological factor of the malaria disease in the study area.

The outdoor and indoor collections comprised of two *Anopheles* species. They were *An. gambiae* and *An. funestus*. These two species of mosquitoes have been reported to be the major malaria transmitters in Africa by various studies (Lamidi *et al*., 2017; Irikannu *et al*. 2021; Egbuche *et al*. 2020; Onwuzuike *et al*., 2021a; Onwuzuike *et al*. 2021b). Their availability in the study area and their indiscriminate biting activity suggest that the malaria disease is

effectively transmitted from human to human in the study area and more especially among pregnant women, one of the venerable groups.

In both outdoor and indoor collections, the highest were collected in Ufuma community while the least number were collected in Amaokpala community. Irikannu *et al*. (2020) observed in their study that the uneven distribution of mosquitoes in the communities is dependent on the availability of human hosts since more mosquitoes were collected from highly populated communities where human hosts were readily available. Their observation agrees with this study findings as more mosquitoes were collected in high density population communities.

In this study, the only sibling species of *An. gambiae* reported was *An. gambiae s.s.* The observation is in agreement with some other studies conducted in Awka South L.G.A and Anambra East L.G.A respectively, both in Anambra State, where *An. gambiae s.s* was the only sibling species reported (Irikannu *et al*., 2019; Egbuche *et al.,* 2020). *An. gambiae s.s* has also been reported in the 3 senatorial districts in Enugu State (Ikpo *et al*., 2021b). The observation shows that *An. gambiae s.s* is either the only or the most active member of the *An. gambiae* complex transmitting malaria in the study area.

An. gambiae were collected between 10.00pm to 6.00am while *An. funestus* were collected between 11.00pm to 5.00am. Both *An. gambiae* and *An. funestus* recorded their biting peak between 1.00am-2.00am. These observation shows that *Anopheles* mosquitoes are midnight biters in the study area. The findings on the peak biting hour of *Anopheles* mosquito species which are important malaria vectors is in agreement with Aribodor (2012) who reported that *Anopheles* species are midnight biters, as such, the mosquitoes could transmit malaria parasites to humans while they are fast asleep.

The highest number of indoor biting mosquitoes 55.2% was freshly fed, while the least 8.8% was gravid. The percentage value of freshly fed *Anopheles* mosquitoes indicates that a large number of the mosquitoes have had contact with human host and as such, there could be chances of the infected mosquitoes transmitting the malaria parasite in the area (Irikannu, 2019; Aribodor 2012). Is also demonstrates high vectoral capacity of the *Anopheles* mosquitoes in the area.

The Indoor Resting Density of the *Anopheles* mosquitoes in the study area was 2.9 mosquitoes/room/night while Man Biting Rate was 0.8 bite/man/night. These observations were higher that Irikannu *et al.* (2019) where *An. gambiae* had a room density of 0.30 mosquitoes/room/night and man biting rate of 0.017 bites/man/night. They were also higher than the findings in Enugu State, where *An. gambiae* had a room density of 0.66 mosquitoes/room/night and but with a higher man biting rate of 3.9 mosquitoes/man/night (Ezihe *et al*. 2017). From this study, the findings were far below the reports in Bayelsa State, where *An. gambiae* had a man-biting rate of 8.7 bites/man/night and room density of 20.5 mosquitoes/room/night (Ebenezer *et al*., 2013).

Of the 2252 mosquitoes, 99.0% were collected in the rainy season while 1.0% was collected in the dry season. Irikannu *et al*. (2021) made similar collections with highest been in the rainy season and the least in the dry season. This is because rainy season provide water breeding sites

prompting mosquito breeding. Also, the study area is located in the tropical rainforest area of Nigeria and thus experience several months of rainfall in a year giving the *Anopheles* mosquitoes opportunity to breed maximally. Thus, malaria transmission follows same cycle and pattern of rainfall in the communities as earlier mentioned.

CONCLUSION

The knowledge of vector characteristics of malaria parasites is important in order to make selective, targeted, site specific, ecologically sound and cost-effective vector control strategy. The study showed that *An. gambiae* and *An. funestus* were the vectors of malaria parasite identified in the study area. All the *Anopheles* species identified were found in indoor and outdoor locations throughout the study period. The number of species collected indoors was higher than the number collected outdoors. This is an indication that biting activities takes place indoors than outdoors. Biting peak for both species was revealed to be between 1.00am to 2.00am. Therefore, indicating that they are midnight bitters. Mosquito collections were greater during the rainy season than dry season. This is because rainy season is favourable to support their continual breeding and survival.

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