Original Research Articles

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF ANOPHELES MOSQUITOES AND THEIR BEHAVIOURAL PATTERNS IN UYO, NIGERIA

ABSTRACT

Aims: Malaria is a public health problem in Nigeria. Knowledge of some baseline data such as Human Biting Rates (HBR) is needed to assess the epidemiology of the disease and in estimating the vector-human contact. This study provides information on the behavioural patterns of anopheles mosquitoes in Uyo.

Study design: A six months study was carried out in Uyo, Nigeria where no information exists on the major malaria vectors associated with human malaria. Sample collection was carried out between May and October 2013 using Knockdown and Human Landing Catches (HLC) techniques.

Methodology: Adults mosquitoes were collected in two areas in Ewet Offot Community. Nine houses were randomly selected in each location using Simple Random Sampling Method. Mosquitoes belonging to the *Anopheles gambiae* complex were further characterized and identified by a molecular method using Polymerase Chaiin Reaction (PCR).

Results: Two anophelines species were collected by the sampling methods consisting of 21(23.3%) *Anopheles nili* and 69(76.7%) *Anopheles gambiae* complex. A Polymerase Chain Reaction (PCR) based test on the *An. gambiae* complex identified 66(96.0%) as *An. gambiae sensu stricto*. The study also revealed that the resting behaviour of *An. gambiae* complex species in this area is endophilic whereas the resting behaviour of *An. nili i*s exophagic/exophilic. The peak biting activity of *An. gambiae* complex species occurred at 11pm (indoor) and 7pm (outdoor) in July whereas that of *An. nili* occurred at 10pm (indoor) and 6pm (outdoor) in June. The Human Biting Rates (HBR) recorded for *An. gambiae* was higher than *An. nili* and the total number *An. gambiae* collected was more than *An. nili.*

Conclusion: There is a need for a comprehensive knowledge on the behaviour and heterogeneities that exist within and among malaria vector species in this area if the goal of malaria elimination is to be achieved.

Keywords: *An. gambiae, An.nili,* PCR, Nigeria

1. INTRODUCTION

Malaria is a vector-borne disease caused by single celled parasites of the genus Plasmodium. It is transmitted by infected female anopheles mosquitoes [1,20]. It is the major public health problem in Nigeria contributing a quarter of the malaria burden in Africa [25]. The disease is the most important cause of human morbidity and mortality and with enormous medical, emotional and economic impact in the world [4,7]. The Federal Ministry of Health [10] estimates that approximately 30% of the Nigerian population lives in areas of high to very high malaria transmission intensity while 67% live in moderate transmission zone. Also, the disease is responsible for 60% outpatient visits to health facilities, 30% childhood mortality and 11% maternal mortality thereby imposing great burden on the country in terms of pains and trauma suffered by its victims [10].

Malaria is caused by four different protozoa in the Plasmodium genus and these are: *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium falciparum*, the most dangerous of the four. The zoonotic species, *Plasmodium knowlesi* can also cause severe infections in humans [17]. Over 90% of the population in Nigeria is at risk of acquiring malaria infection because of drug resistance in the parasite strain, insecticide resistance in the vectors and the presence of very efficient mosquito vectors of *Plasmodium falciparum* [18,19]. Vectorial capacity takes into account environment, behavioural, cellular and biochemical factors that influence the association between vector, pathogen transmitted by the vector and the vertebrate host to which the pathogen is transmitted [3].

The burden of malaria remains high in many African countries, despite increasing effort to control the disease due to a persistent high level of transmission [14]. Malaria transmission is variable from one area to another and this has an impact on its epidemiology and control [4]. The development of the malaria parasites inside the mosquito (sporogonic cycle) is more rapid as the temperature rises and increased rainfall and stagnant pools of water or surface water provide hospitable breeding grounds for the mosquitoes [4, 18].

The role of anophelines in transmitting the disease depends on their occurrence and preference to feed on humans [14] which in turn is affected by socio-economic as well as environmental factors [14]. Thus, preventing humans from the bite of vectors can reduce malaria transmission. However, implementation of preventing tools requires knowledge on occurrence, feeding behaviour of the vectors [23].

In Nigeria, there is little baseline information on vector populations and variation in the intensity of malaria transmission but the use of vector control methods via Indoor Residual Sprays (IRS) and Insecticide Treated Nets (ITNs) are applied without regard to the local epidemiology of the disease especially the relationship to vector behaviour and intensity of transmission [19].

Uyo is the capital of Akwa Ibom State and it is an ecological area that has undergone serious environmental modifications over the years owing to rapid growth in population and urbanization. Such modifications can lead to ecological changes that can affect vector population structure, distribution and density, all of which would impact on the efficiency in transmitting malaria. Therefore, this study seeks to describe the behavioural patterns of anopheline mosquitoes in Uyo.

2. METHODOLOGY

2.1 Study Area

The study was conducted in Ewet Offot in Uyo Local Government area. Uyo lies between latitude 5.05^{o} North and Longitude 80° East. It is within the equatorial rainforest belt, which is a tropical zone that houses vegetation of green foliage of trees, shrubs and oil palm trees. Local climate is tropical, with dry season between November and March and wet season between April and October.

The average temperature ranges from 23°C -31°C providing an ideal climate for malaria transmission throughout the year. Ewet Offot is located along Nwaniba road and beside Oron road in Uyo Local Government Area. Based on their dense population 2 areas were selected for the study. These areas were Orok Close and Urua Udofia Streets which are 2 kilometers apart from each other. These areas are usually characterized by stagnant pools in the rainy season and Urua Udofia Streets are surrounded by streams, valleys and borrow pits. Most houses were built in clusters in Urua Udofia Streets and some compounds consist of a group of houses separated from each other by gardens in Orok Close.

2.3 Sampling Methods

Adult mosquitoes were collected in two areas in Ewet Offot Community (Orok Close and Urua Udofia Streets). Nine houses were randomly selected in each location using Simple Random Sampling Method. Sample collection was carried out for six months (May- October, 2013) using the following techniques:

2.3.1 Knockdown Technique

This technique involved the laying of Calico cloth on the floor and on the surfaces of immovable household furniture. The houses were sprayed using knockdown Insecticide consisting of Transfluthrin 0.25% and Permethrin 0.5% according to the manufacturer's instruction. After 10 minutes the Calico cloth was carefully taken out of the house and knocked-down mosquitoes were collected using forceps. Three houses with nets and three houses without nets were used for the collection of samples using this technique.

2.3.2 Human Landing Catches (HLC)

Human Landing Catches (HLC) was carried out according to World Health Organization standard procedure [15] which adopts the standard human bait collector method. In this procedure, a volunteer will sit outdoor from 6pm to 10pm and collect the mosquitoes that come to bite him. This technique was also performed from 11pm- 5am indoor in three compounds different from the one that was used for knockdown technique. These compounds were used for the collection of mosquitoes using this technique throughout the study period.

2.4 Sample Preservation

All the adult mosquitoes that were collected were kept in a labeled 1.5ml eppendorf tubes containing silica gel desiccant. Samples were stored in the laboratory at room temperature (35^{0}C-37^{0}C).

2.5 Identification of Samples

2.5.1 Morphological Identification

Anopheles mosquitoes were morphologically identified and differentiated from *Culex quinquefasciatus* and *Aedes aegypt*i using Binocular microscope with the aid of identification Manual [12,5] in a WHO Reference Laboratory.

2.5.2 Molecular Identification of Anopheles Mosquitoes

Polymerase Chain Reaction (PCR) technique of Scott, Brogon and Collins [24,2] was used to identify species of *Anopheles gambiae.*

The steps used in carrying out PCR analysis were:

i. Extraction of DNA: Preserved legs and wings of mosquitoes were washed in phosphate buffer saline (PBS) to remove excess alcohol. The mosquitoes were ground in 1.5ml eppendorf tubes with micropistle in 50-100µl buffer along with 100mM sucrose. 1 x Sodium Acetate(STE) buffer was added to a total volume of 300 - 500µl for a single mosquito. Then 1% Sodium lauryl sulfate (SDS),1% Triton –X, 10µl/ml Rnase A (20mg/ml) and 20µl/1ml Proteinase K(20mg/ml) were added and were mixed then lysed for 1hour 30minutes at 37^0C. The tube was gently mixed by inverting every 15minutes and was centrifuged at 12,000g per 10minutes at 4^0C. The supernatant was transferred to a fresh tube and equal volume of phenol; Chloroform (1:1) was added to it. The tube was shake for 5minutes and centrifuged at 12,000g for 10minutes at 4^0C. Phenol: Chloroform (1:1) was added again to the tube alongside with Chloroform: Isoamyl alcohol (24:1), centrifuged at 12,000g for 10minutes at 4^0C. The clear supernatant was transferred to a fresh tube and two fold volumes cold Isopropanol was added to it and was kept for 1hour at -20^0C. It was centrifuged at 12,000g for 30minutes at 4^0C and then the supernatant was removed and the pellet was washed with 70% ethanol and was kept at 37^0C for 10minutes. The dried pellet was dissolved in TE buffer (ph 8.0) and DNA was stored at -20^0C.

ii. Amplification of DNA: Polymerase Chain Reaction (PCR) is the most common DNA amplification method in molecular biology [22].The method used for the PCR analysis was positive control method. Anopheles gambiae primers used were as follows:
GA (Gambiae)
AA (Arabiensis)
ME (Melas)
QD (Quadriannulatus)
UN (Universal)
These primers bind to specific anopheles mosquito species. The primers were stored in a frozen state and were removed from the refrigerator and placed on an icepack. The buffer, deoxynucleotidetriphosphate (dNTPs), primers,Magnesium Chloride (MgCl2), water and RTag (an enzyme) were measured according to their proportion using microtitrepipette into eppendorf tubes and then place inside a vortex machine for it to mix. 23 PCR tubes were arranged into tubes stand and the PCR master mix were measured in the PCR tubes. The model of PCR machine was 25.2 automated PCR machine.
The three processes involved in the PCR analysis were;
Denaturation – DNA strands was separated and it occurred at 94^0C.
Annealation – binds the primers to the specific DNA and it occurred at 50^0C -54^0C.
Enlongation or Extension – binds the primers to the dNTPs and it occurred at 72^0C.
These three processes involved in PCR analysis were programmed inside a 25.2 automated PCR machine.
To check whether the PCR generated the anticipated DNA fragment (amplicon), agarose gel electrophoresis was employed for size separation of the PCR products. The size of PCR product was determined by comparison with a DNA ladder (a molecular weight marker), which contained DNA fragments of known size run on the gel alongside the PCR products.

iii Gel Electrophoresis: 1.5% agarose powder was washed and dissolved in 100ml TBE buffer (containing 0.089MTris,0.089Mboric acid, 0.0025M Ethylenedinitriolo tetra-acetic acid) was

placed inside a microwave oven for 3minutes until the medium was clear. Then it was allowed to cool to about 50^0C.

3.0µl of Ethidium bromide was added to it and was poured into the trough. A comb was placed at the edge and in the middle of the trough. The comb was removed after it solidified and the TBE buffer was poured into the electrophoretic machine. 2µl loading dye was dropped on a foil paper and the PCR amplicons were picked and mixed with the loading dye. The mixture was picked and dropped in their respective wells one after the other.

1st well on the upper and down roll was the DNA ladder
2nd well on the upper and down roll was positive control
3rd well on the upper and down roll was negative control

The trough was covered with a lid and was plugged according to how it was charged from negative to positive because DNA is negatively charged. Migration was from negative to positive poles. After 45minutes, the gel was removed and was placed on a UV light machine and the DNA bands were visualized under short wavelength UV light.

2.6 Statistical Analysis

The entomological parameters that were determined was the Human-Biting Rates (HBR), calculated as the number of mosquito bites received per person per night of collection using the formula [16].

The rate of endophagy, defined as the proportion of mosquitoes caught indoors by HLC over the total numbers of mosquitoes collected by HLC (indoors and outdoors).
Chi-square test was used to compare the results from the two locations.

3. RESULTS

3.2 Morphological Identification of Mosquitoes

A total of 1300 mosquitoes were collected from two areas in Ewet Offot within the period of 6 months but only 700 were used for morphological identification and 90 (12.8%)of these were identified as female anopheles mosquitoes consisting of *An. nili* 21 (23.3%) and *An. gambiae* complex 69 (76.7%) (Table 3). *Culex quinquefasciatus* were 300 (42.9%) in Orok Close and 214 (30.6%) in Urua Udofia Streets. *Aedes aegypti* collected in Orok Close were 46 (6.6%) and 50 (7.1%) in Urua Udofia Streets (Table 2).

Aedes aegypti and *Culex quinquefasciatus* collected were excluded from the study because they are not malaria vectors. PCR analysis was not used to identify the specific sibling species within the *Anopheles nili* complex. During the rainy season however, when mosquito densities are high, mosquito nets and fumigating coils was frequently used for personal protection. Household ownership of mosquito nets was around 36% in Orok Close and 25% in Urua Udofia Streets.

3.3 Molecular Characterization of Anopheles Mosquitoes

The *Anopheles gambiae* species specific PCR was employed to distinguish the species present within the *An. gambiae* of the total *An. gambiae* collected 66 (96%) were identified as *An. gambiae s.s* and 3 (4%) were negative (Figure 1). DNA bands were visualized under short wave length UV light. The PCR DNA (Figure 1) enabled the identification of sibling species of *An. gambiae* complex as *An. gambiae s. s* by comparison with a DNA ladder (a molecular weight marker).

Table 1: Classification into species based on morphological characteristics

Features	***An. gambiae***	***An. nili***
Wings	dark spots on the wing veins	pale wings
Proboscis/ Palps	black spot on the palps	white spots on the palps
Legs	spotted or speckled legs	dark legs
Colour	yellowish brown to brown	bright yellowish red

Table 2: **Species composition of female mosquitoes collected in both Orok Close and Urua Udofia Streets.**

Areas	Mosquito species	No. identified	%
Orok close	*An. gamibiae* complex	20	2.9
	An. nili complex	7	1.0
	Culex quiu-quefasciatus	300	42.9
	Aedes aegypti	46	6.6
Urua Udofia Streets	*An. gamibiae complex*	49	7.0
	An. nili complex	14	2.0
	Culex quiu-quefasciatus	214	30.6
	Aedes aegypti	50	7.1

Table 3: **Sibling species composition of the two anopheles vector groups identified in Orok Close and Urua Udofia Streets.**

Location	Group	Sibling species	No. identified	%
Orok Close	*An. Gambiae*	*An. gambiae* s.s	20	22.2
	An. Nili	-	7	7.7
Urua Udofia Streets	*An. Gambiae*	*An. gambiae* s.s	49	54.4
	An. Nili	-	14	15.6
Total			90	100

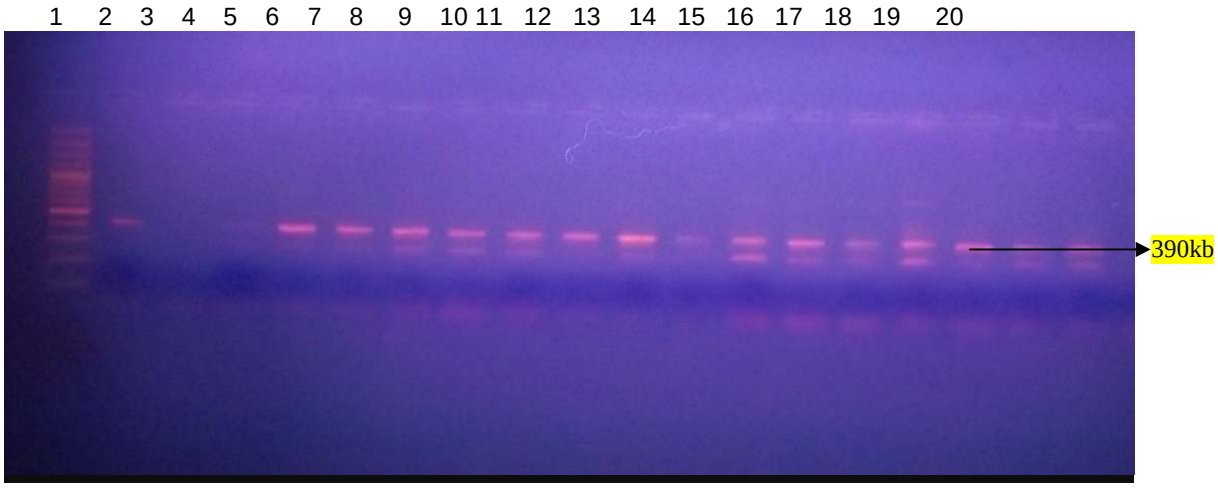

Fig I: **Molecular Characterization of sibling species of *An. gambiae* complex**

Lane 1 = 100kb DNA ladder, Lane 2 = Standard strain of An. gambiae s. s
Lanes 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20 = An. gambiae s. s
The molecular weight of An. gambiae s. s is 390kb.

Anopheles gambiae and *Anopheles nili* displayed similar biting activities. The observed differences in the proportion of *Anopheles gambiae* collected showed that the total number of *Anopheles gambiae sensu stricto* collected outdoor and in houses without nets was more than those collected indoor and in houses with nets (Table 4 and Table 5). Biting generally commences at 6pm (60% of samples were collected outdoors). *Anopheles gambiae* indoors peaked at 11pm and 3am (Table 6 and Table 7).

Anopheles nili were collected resting outdoors and few were found resting indoor after feeding. No male sample of any anopheles mosquito was collected by Human Landing Catches (HLC). However, there was statistical significant difference between indoor and outdoor host seeking behaviour of *An. gambiae* and *An. nili* in the area.

The population of the species collected in the two areas shown on Tables 4 and 5 showed that the proportion of *An. gambiae* and *An. nili* complex collected using HLC outdoor was more than *An. nili* in houses without nets in both Orok Close and Urua Udofia Street. The highest number of *An. gambiae s.s* was collected and recorded in the July after two months onset of rain. The highest number of *An. nili* complex was recorded in the June Figure 2.

However, the total number of *An. gambiae* collected across the two areas was more than the number of *An. nili* caught within the same period. The Human Biting Rates (HBR) recorded for *An. gambiae s.s* was higher than *An. nili* complex although increase in man biting rate in each species coincides with the level of rainfall reaching maximum at different months throughout the study period Table 8. The rate of endophagy of *An. gambiae s.s* in Orok close was 0.14 and *An. nili* was 0. In Urua Udofia the rate of endophagy of *An. gambiae* was 0.3 and *An. nili* was 0.2.

Table 4: Species composition of indoor and outdoor biting *An. gambiae* and *An. nili* mosquitoes in the two different areas in Ewet Offot using human landing catches (HLC) method.

	Indoor		Outdoor	
Areas	***An. gambiae* No. (%)**	***An. nili* No. (%)**	***An. gambiae* No. (%)**	***An. nili* No. (%)**
Orok Close	1 (20)	0	6 (33.3)	2 (20)
Urua Udofia Streets	4 (80)	2 (100)	12 (66.7)	8 (80)
Total	5 (100)	2 (100)	18 (100)	10 (100)

Table 5: Total number of *An. gambiae* and *An. nili* mosquitoes collected in houses with nets and without nets using knock-down technique in two different areas in Ewet Offot Community.

	Houses with nets		Houses without nets	
Areas	***An. gambiae* No. (%)**	***An. nili* No. (%)**	***An.gambiae* No. (%)**	***An. nili* No. (%)**
Orok Close	2 (25)	1 (50)	10 (26.4)	2 (28.6)
Urua Udofia Streets	6 (75)	1 (50)	28 (73.6)	5 (71.4)
Total	8 (100)	2 (100)	38 (100)	7 (100)

Table 6: Indoor host seeking behaviour of *An. gambiae* and *An. nili*

Months	*An.gambiae* HLC Indoor No. of Catches	*An. nili* HLC indoor No. of catches	Time
May	1	0	10pm
June	0	3	10pm
July	3	0	11pm
August	0	0	11pm
Sept.	0	0	11pm
Oct.	1	1	3am

Table 7: Outdoor host seeking behaviour of *An. gambiae* and *An. nili*

Months	*An. gambiae* HLC outdoor No. of Catches	*An. nili* HLC outdoor No. of catches	Time
May	4	2	6pm
June	2	3	6pm
July	9	1	7pm
August	1	0	6pm
Sept.	2	1	8pm
Oct.	5	1	6pm

Anopheles gambiae

Anopheles nili

Figure 2: Variation in monthly collection of anopheles mosquitoes

Table 8: Human biting rates of *An. gambiae* sensu stricto and *An. nili* complex

Months	Species	No. of Nights of Catch	No. of baits		Total catches indoors		Total catches outdoors	Human biting rate		Total
			Indoors	Outdoors	6pm – 10pm	10pm – 6am	6 – 10 pm	Indoors (3+8hrs)	Outdoors (1hr)	
May	*An. gambiae*	2	1	1	0	0	3	0.5	0.4	0.9
June		2	1	1	1	0	1	0.4	0.1	0.5
July		2	1	1	1	2	9	1.3	1.1	2.4
August		2	1	1	0	0	1	0.5	0.1	0.6
Sept		2	1	1	0	0	2	0	0.3	0.3
Oct		2	1	1	0	1	5	0.5	0.6	1.1
May	*An. nili*	2	1	1	0	0	2	0	0.3	0.3
June		2	1	1	0	2	3	1	0.4	1.4
July		2	1	1	0	0	1	0	0.1	0.1
August		2	1	1	0	0	0	0	0	0
Sept		2	1	1	0	0	1	0	0	0
Oct		2	1	1	0	0	1	0	0	0

4. DISCUSSION

The rainforest zone of Nigeria has been described as a malaria endemic area because of the high level of human- vector contact, which has been attributed to high density of anopheles mosquito vectors in the area [21]. Reports from previous studies by [17] showed that *An. gambiae s.l* and *An. funestus* group constitute the largest proportion of malaria vectors in Nigeria. In this study, An. gambiae and An. nili were the malaria vectors found in the study area. *An. nili* was an important malaria vector in the study area according to [7,17]. Member of *An. gambiae* complex identify in this study was *An. gambiae s.s*.

Vector abundance increased with increasing rainfall, resulting in the proliferation of anopheles mosquito populations. The 12.8% of anopheles mosquitoes collected in the study area showed that anopheles mosquitoes were not predominant in the areas. This could be because anopheles mosquitoes are not predominant in the urban areas [8,19]. Collection of samples were most abundant in the month of June which coincided with the peak of rainy season, however collection of *An. gambiae* was most abundant in July after two months of onset of rain and collection of *An. nili* was abundant in the month of June.

Large number of anopheles mosquitoes collected in Urua Udofia Street could be due to the location of the area and its proximity to slow moving streams. This finding agrees with previous studies by [4,10,18] which reported that anopheles mosquitoes were predominant in villages with proximity to slow moving streams. *An. nili* was found to be one of the main malaria vectors in the study area.

This study indicates that *An. gambiae s.s* seeks host in outdoor venues in Ewet Offot, Uyo. The greater proportion of *An. gambiae s.s* in this area leave houses in the morning and rest outdoors. This species was predominant and the most aggressive vector in the area. The abundance of *An. gambiae* had been reported in the Southwest Nigeria by [7,13]. This has been found to be connected with certain factors most especially the annual precipitation which appears to influence the range and relative abundance of this species. *Anopheles nili* complex were highly exophagic/exophilic in the study areas. This finding also agrees with previous findings by [4,19] which reported that *An. nili* complex are highly exophagic and exophilic and can bite man as well as other vertebrates in remote areas. However, in this study more than 70% host seeking mosquitoes were collected outdoors in the early evenings.

Biting activities of both *An. gambiae s.s* and *An. nili* commenced effectively at the early part of the night before the inhabitants retire to bed. *An. gambiae s.s* are considered to exhibit highly endophagic and endophilic behaviour and to feed primarily on human hosts [5,8,13] even though endophagy has been shown to be a variable trait [7]. In this study, *An. gambiae s.s* was responsible for both indoor and outdoor biting activities and was found to be highly endophilous compared to *An. nili*. This indicates that this species preferred to rest indoor after feeding. It was shown that the biting activity of *An. gambiae* complex species commenced at 6pm (outdoor) with the peak biting activity at 11pm (indoor) in the July. However studies conducted by Dandalo [11] reported that the biting activity of *An. gambiae* complex species commenced at 7pm and ceased at 5am with peak biting activity in March [13,19] reported that the biting activity of *An. gambiae* complex gradually increased throughout the night with a peak biting attained three hours before dawn in Kenya.

The biting activity of *An. gambiae* complex is dynamic and varies in response to changes in environmental factors such as wind and rainfall [6]. On the other hand *An. nili* was more exophagic with 60% of biting activity occurring outdoor. Biting activity of *An. nili* commenced at 6pm and peak at 10pm and decreased thereafter at 3am in June. In Cameroon the peak biting activity of *An. nili* occurred between midnight and 1am [23]. Takken [23] reported that the biting activity of *An.nili* can be disrupted by changes in environmental factors during the night, example rain and wind. The peak biting activity of anopheles mosquitoes in the early hours of the evening 6pm shows that the possibility of outdoor transmission may be high because at this period the inhabitants of the areas are still active outdoors.

The endophilic behaviour of *An.gambiae* would be a prerequisite for significant contact between the vector species and man. The resting behaviour of *An. nili* species in Ewet Offot, Uyo is mainly exophilic /endophagic. The highest number of *An. gambiae* was collected in July whereas the highest number of *An. nili* collected was recorded in June. Based on the exophilic behaviour of *An. nili* complex at Ewet Offot, Uyo, Residual Insecticidal Treatment of houses would not be ideal for vector control since it is mostly designed to kill indoor resting fraction of malaria vector population. The Human Biting Rates (HBR) recorded for *An. gambiae* was higher than *An. nili* (76.7%). This agrees with previous findings by Awolola [3] which reported similar HBR of *An. gambiae.* The Human Biting Rates (HBR) can be an important factor in the epidemiology of the disease and in estimating the vector-human contact.

5. CONCLUSION

The results from this study show that *An. gambiae* and *An. nili* co-exist in Ewet Offot, Uyo, Akwa Ibom State. *An. gambiae* is the predominant species in this area. The biting patterns of *An. gambiae* and *An. nil*i vary in response to changes in environmental factors such as wind and rainfall. The differences in the Human Biting Rates (HBR) for *An. gambiae s.s* and *An. nili* complex during the six months of sampling exhibit variations in biting activity of these species at Ewet Offot. The peak of biting activity of *An. gambiae s.s* occurred before mid - night at 11pm indoor and 7pm outdoor in July whereas the peak of biting activity for *An. nili* occurred at 10pm indoor and 6pm outdoor in June. The resting behaviour of *An. gambiae s.s* at Ewet Offot is endophilic. Low rate of endophagy (0.2) of *An. nili* indicates that a greater proportion of this species in the area is exophilic. The findings from this study contributes to the basic understanding of the distribution of anopheles mosquitoes with respect to species composition and heterogeneities that could serve as a base- line data for further entomological studies in the area.

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Competing Interest
"Authors have declared that no competing interests exist."

Author's Contributions

Topic, research design, input, literature review, methodology, result and discussion were done by the first author. The second author did the data collection, data analysis and produced the draft on the paper.

All authors read and approved the final manuscript.

Ethical Consideration:

Oral consent was obtained from all participants that were involved in both knockdown technique and Human Landing Catches (HLC).

Malaria prophylaxis with Artemether and Lumefantrine (the recommended Chemoprophylaxis in Nigeria) was provided for the HLC volunteers once a month throughout the study period.

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