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Anopheles Species Prevalence, Diversity, Behaviour and Their Implications to Tourist Activities in Uyo, South-South Nigeria

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Authors' contributions

The work was carried out in collaboration between all authors. Author IAA designed the study, wrote the protocol and the first draft of the manuscript. Author MEA managed the literature searches, analyses of the study, performed the field and laboratory data collection and analyses. Author NIU managed the experimental process and joined in the writing of the first draft of the manuscript. All the authors read and approved the final manuscript.

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ABSTRACT

Aims: Malaria poses a medical and public health challenge in Nigeria. The burden of the disease has been a major source of concern to tourists in Uyo. Knowledge on the biting behaviour and the Human Biting Rates (HBR) are needed to assess the epidemiology of the disease and in estimating the vector - human contact.
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

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households 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 Chain Reaction (PCR).

Results: Two anophelines species were collected by the sampling methods consisting of 21(23.3%) *Anopheles nili* and 69(76.7%) *Anophele 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 11 pm (indoor) and 7 pm (outdoor) in July whereas that of *An. nili* occurred at 10 pm (indoor) and 6 pm (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: Better understanding of the behaviour of the sibling species within the complex is important to help identify their roles in disease transmission and to facilitate vector control.

Keywords: An. gambiae; An. nili; PCR; Nigeria.

1. INTRODUCTION

Malaria is a vector-borne disease and Anopheles mosquitoes are implicated agents of malaria parasite transmission [1]. Female anopheles mosquitoes take blood meals to carry out egg production and such blood meals are the link between human and mosquitoes in the parasite life cycle [1]. Malaria is caused by five different *Plasmodium* species and these are; *Plasmodium ovale, Plasmodium vivax, Plasmodium malariae, Plasmodium falciparum* and *Plasmodium knowlesi. Plasmodium falciparum* is the most dangerous of the five [2]. *Plasmodium falciparum* is the most common species specific malaria parasite. Most of the infected cases with this parasite showed anaemia, elevated Erythrocyte Sedimentation Rate (ESR) than patient with lower risk species [3].

Nigerian, health statistics show that an estimated 300,000 children die due to malaria each year. Thirty percent of children under-five and 11 percent of pregnant women die from this disease [4]. The development of the malaria parasites inside the mosquito (sporogonic cycle) is more rapid as the temperature rises and increased rainfall, stagnant pools of water or surface water provide hospitable breeding grounds for the anopheles mosquitoes [5]. Temperature and rainfall have been associated with the dynamics of malaria vector population. Daily survival of malaria vector is dependent on temperature [6]. Rainfall provides breeding sites for mosquitoes to lay their eggs. The development of the parasites within the mosquito (sporogonic cycle) is dependent on temperature [7]. The sporogonic cycle takes about 9 to 10 days at temperature of 28℃ but stops at temperature below 16℃. The survival of mosquito drops rapidly at temperature above 36℃ [7].

The burden of malaria remains high in many African countries despite increasing effort to control the vector, and this has been a source of concern to tourism [8].

In Nigeria, one single mosquito bite could lead an individual to frequent clinical visits, a large out of pocket spending on drugs and many days of absence from work [9]. The effect of mosquito bites could be very devastating on the human body and particularly on the health of vulnerable group which are pregnant women and children under-five [9].

In sub-Sahara Africa, anopheles mosquitoes comprise of diverse species whose distribution and composition vary from one geographical location to another being influenced by the prevailing climatic conditions [1].

Based on collections of indoor-resting females, Okwa et al. [5] reported that the distribution of *An. gambiae* and *An. arabiensis* across the ecological zones in Nigeria is “puzzling”. They found that *An. gambiae* was prevalent in the forest zones but also abundant in several localities in the savanna. *An. Arabiensis* was prevalent in localities in the Southern Guinea Savanna. Onyebe and Conn [10] reported the distribution of two major malaria vectors, *An. gambiae* and *An. arabiensis* in the Southern Nigeria and Awolola et al. [9] focused mainly on the *An. funestus* group. Coetzee et al. [1,5] reported that *An. arabiensis* tends to predominate in arid savannas, whereas *An. gambiae* is the predominant species in humid

forest zones. Nigeria has little information on the biting behaviour in Southern Nigeria [10]. Correct analysis of the distribution of specific malaria vectors is one of the prerequisites for meaningful epidemiological studies and for planning and monitoring of successful malaria vector control [11].

Malaria vector control activities in Nigeria focus mainly on the use of insecticide treated bed nets. Whereas effective vector control strategies requires information on the vector behaviour, their distribution and efficiency in malaria transmission [12].

Uyo is an ecological area that has undergone serious environmental modifications over the years owing to rapid growth in population and urbanization. The city is gradually developing into a potential tourist centre and people are coming in because of the ongoing transformation. The sanitation standard in Uyo is poor which can enhance the proliferation of malaria vectors in the city. Therefore this study sought to describe the behavioural patterns of anopheles mosquitoes in Uyo.

2. METHODOLOGY

2.1 Study Area

The study was conducted in Ewet Offot in Uyo Local Government Area which is a peri-urban settlement. Uyo lies between latitude 5.05° 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℃ - 31℃ 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 Street which are 2 kilometers apart from each other. These areas are usually characterized by stagnant pools in the rainy season and Urua Udofia Street is surrounded by streams, valleys and borrow pits. Most houses were built in clusters in Urua Udofia Street and some compounds consist of a group of houses separated from each other by gardens in Orok Close. Many inhabitants are traders and farmers. During the rainy season, when mosquito densities are high, mosquito nets and fumigating coils are frequently used for personal protection. In Urua Udofia Street, five households used impregnated bed nets while two households used coils. In Orok Close, only two households used impregnated bed nets.

2.2 Anopheles Species Breeding Habitat Identification

Mosquitoes are capable of colonizing every conceivable type of water except fast running waters in rivers. Systematic ground surveys were conducted at one month intervals in the months of May to October representing the rainy season to determine the possible anopheles aquatic breeding habitats. This excluded fast running water because mosquitoes breed in calm, slow, moving water and in containers in houses [8]. Aquatic habitats found present in the study sites were drains, ditches stagnant pools, ponds and streams. A dipper (13 cm in diameter and 6.5 cm deep with handle)\ sieve\ pipette was gently lowered at an angle of 45℃ just below the water surface. This was to ensure that water flowed into it or sucked (by pipette) together with any larvae that might be present. During dipping, care was taken not to disturb the water too much and make larvae swim downwards. The filled dipper\ sieve\ pipette was carefully lifted, taking care not to spill the water containing the larvae. The drawn water containing the larvae was poured into white rectangular trays and checked visually, and larval samples when present were collected by pipette. Three dippers\ pipette\ sieve collections were made per each aquatic habitat. If none had anopheline larvae, then the site was declared anopheline negative. The larval samples from each habitat were transferred into container/ vials labeled appropriately indicating date, collection site and type of habitat and to transport to Nigeria Institute of Medical Research (NIMR) for identification. The identified anopheline positive breeding sites were noted, and the location recorded.

2.3 Sampling Methods

Adult mosquitoes were collected in two areas in Ewet Offot Community (Orok Close and Urua Udofia Street). Nine households 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 method was used for the collection of indoor mosquitoes. Total indoor resting mosquitoes were collected from randomly selected households by Pyrethrum Spray Collection (PSC) [13] three times a week. White calico clothes were spread on the floor and the windows, doors, and all other exit points closed. Knockdown insecticide consisting of Transfluthrin 0.25% and Permethrin 0.5% was sprayed on all doors, windows and the entire space of all rooms in the house according to manufacturer's instruction. The house was closed for 15 minutes. After 15 minutes, all knocked down anopheles species were collected carefully with forceps and placed in petri dishes lined with moist filter paper. The collections were transported to Nigeria Institute of Medical Research (NIMR) laboratories. The samples were preserved on silica gel in a labeled 1.5 ml eppendorf tubes prior to species identification. Three households were used for collection of mosquitoes using this method.

2.3.2 Human Landing Catches (HLC)

Human Landing Catches (HLC) was carried out according to World Health Organization standard procedure [13] which adopts the standard human bait collector method. In this procedure, a volunteer will sit outdoor from 6 pm to 10 pm and collect the mosquitoes that come to bite. This technique was also performed from 11 pm – 5 am indoor in three compounds different from the one that was used for knockdown technique. Six households were used for the collection of mosquitoes using this technique throughout the study period. Malaria prophylaxis with Aretemeter and Lumenfanthrin (the recommended chemoprophylaxis in Nigeria) was provided for the volunteers once a month throughout the study period. Samples collected were kept in a labeled 1.5 ml eppendorf tubes containing silica gel desiccant. The samples were stored in the laboratory at room temperature (35℃ - 37℃).

2.4 Identification of Samples

2.4.1 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 [14] in a WHO Reference Laboratory. The anopheles mosquitoes were sorted out to separate the females from the males. The females were identified to species level using morphological features with the aid of identification manuals [14].

2.4.2 Molecular identification of anopheles mosquitoes

The females of all morphologically identified female *Anopheles gambiae* mosquitoes collected were identified to sibling species using Polymerase Chain Reaction (PCR) as described by Scot et al. [15]. The PCR assay involved the following key steps: mosquito DNA extraction using the potassium acetate precipitation technique making of PCR master mix (mixture of buffer, ions, primers and enzymes in water); electrophoresis; gel visualization and photography. The distribution of bands in gel after electrophoresis was used to identify and determine *An. gambiae* sibling species as *Anopheles gambiae sensu stricto (s.s).* The PCR DNA 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).

2.5 Statistical Analysis

The entomological parameters that were determined were the Human-Biting Rates (HBR) [16]. Human Biting Rates was calculated by dividing the total number of *An. gambiae* caught by PSC by the total number of occupants who slept in the houses the night before collection.

The rate of endophagy was calculated as;

[Mosquitoes caught indoors by HLC/
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 AND DISCUSSION

3.1 Results

Most of the larval habitats were man-made that arise from human related activities and confined to valley bottoms. The drainage channels formed water collection points in streams, breeding was common on edges where water flow from slow and calm. The larval species found in Urua Udofia were; *An. gambiae* (20%), *An. nili* (15%),

Culex quinquefasciatus (45%), *Aedes aegypti* (20%). In Orok Close, the larval species found were; *An. gambiae* (15%), *An. nili* (25%), *Culex quinquefasciatus* (43%), *Aedes aegypti* (17%)

3.2 Morphological Identification of Mosquitoes

A total of 700 mosquitoes were collected from the study area. They were identified by their morphological features. The malaria vectors were *An. gambiae* and *An. nili*. *An. gambiae* was the most predominant malaria vector species. The diversity of anopheles mosquitoes in the study area is shown on Table 1. PCR analysis was not used to identify the specific sibling species within the *An. nili* complex. Other mosquito species collected from the study area were *Aedes aegypti* and *Culex quinquefasciatus.* These mosquito species were excluded from the study because they are not malaria vectors.

3.3 Molecular Characterization of Anopheles Mosquitoes

The 69 An. gambiae sample specimens collected from the study sites were analyzed by PCR [15] were found to belong to one sibling species. *An. gambiae sensu stricto (s.s)* indicating that it was possibly the only sibling species from the *An. gambiae* complex circulation in the study area. The result is presented in form of photographed gel under UV light (Fig. 1).

The highest number of *An. gambiae s.s* was collected and recorded in the month of July after two months of onset of rain. The highest number of *An. nili* complex was recorded in the month of June (Fig. 3).

An. 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).

Table 1. Species composition of female mosquitoes collected in both Orok Close and Urua Udofia Street

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

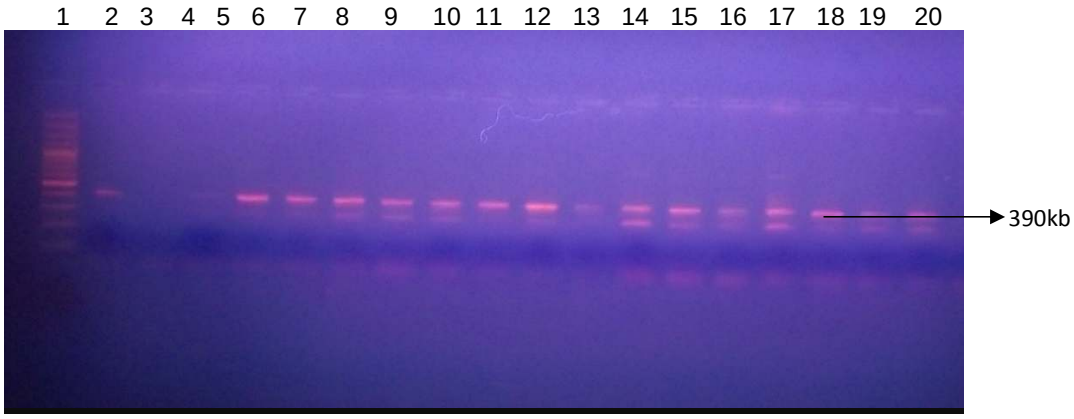

Fig. 1. Molecular characterization of sibling species of *An. gambiae* complex
Lane 1 = 100kb DNA ladder, Lane 2 = Standard strain of An. gambiae s. s
Lanes 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20 = An. gambiae s. s
The molecular weight of An. gambiae s. s is 390kb

An. gambiae and *An. nili* displayed similar biting activities. Biting generally commences at 6 pm (60% of samples were collected outdoors). *An. gambiae* indoors biting activities peaked at 11 pm and 3 am (Table 3 and Table 4).

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 5. The rate of endophagy of *An. gambiae s.s* in Orok close was 0.14 and *An. nili* was 0. In Urua Udofia Street the rate of endophagy of *An. gambiae* was 0.3 and *An. nili* was 0.2.

Some of the most important health considerations of travel to the study area are those who are visiting friends and relatives. These travelers often do not seek pre-travel health advice since they are returning to their land of origin. Also some tourist visit the area and stay outdoors without protecting themselves and thus are at risk of travel-related illnesses such as malaria.

4. DISCUSSION

The study area is characterized by hill-valley topography. These topographical features determine the formation of aquatic habitats. Hills-valley topography facilities run-off down-hill to settle in the valley bottoms forming aquatic habitats such as streams and swamps. The malaria vectors present in the area were *An. gambiae* and *An. nili.*

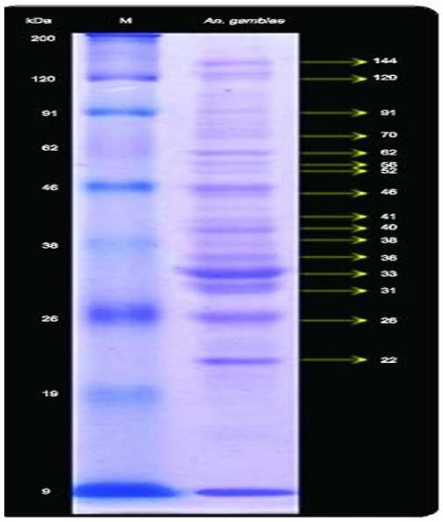

Fig. 2. Salivary gland proteins from female *Anopheles gambiae* mosquitoes, separated using 12%

SDS-PAGE (right lane) and stained with Coomassie Blue. Molecular weight markers are shown in the left lane. kDa: Kilodalton; M: Marker; An.: Anopheles; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis

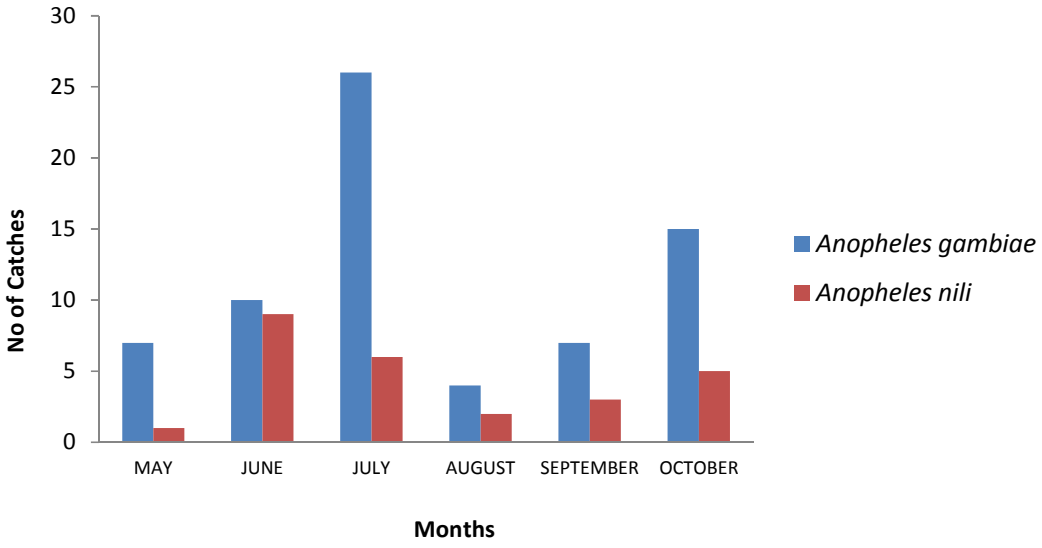

Fig. 3. Variation in monthly collection of anopheles mosquitoes

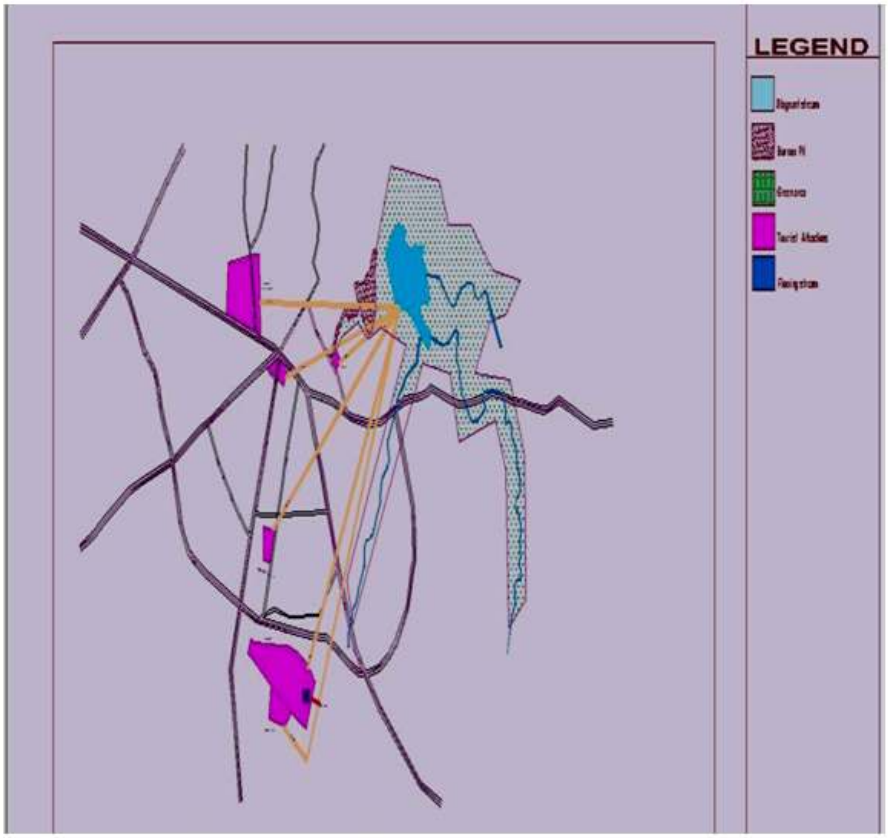

Fig. 4. Breeding sites of anopheles mosquitoes and tourist attractions in Uyo

Distance from the breeding sites of anopheles mosquitoes to tourist centers in Uyo, Akwa Ibom State,

Tourist centers	**Distance from anopheles mosquitoes breeding sites**
Tropicana	3.73 km
Ibom Plaza	1.98 km
Uyo sport ground	2.62 km
Playground	400 metres
University of Uyo	2.56 km

(Google earth image 2014 digital globe)

Table 2. 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

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

Reports from previous studies by Awolola et al. [9], Pates and Curtis, [17] reported the biting behavior of mosquitoes in Southern Nigeria showed that *An. gambiae s.I* 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. Member of *An. gambiae* complex identify in this study was *An. gambiae s.s*. This findings was consistence with studies by Awolola

et al. [9] and Oyewole et al. [12] which reported the distribution of malaria vectors in Southern Nigeria.

Table 3. Indoor host seeking behaviour of *An. gambiae* and *An. nili*

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

Table 4. Outdoor host seeking behaviour of *An. gambiae* and *An. nili*

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

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 [16,18]. 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.

Anopheles mosquitoes collected in Urua Udofia Street could be due to the location of the study area and its proximity to slow flowing streams. This finding agrees with previous studies by Sungvornyothin [19], Trung et al. [20] and Spitzeen et al. [21] 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.

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 [5,12] even though endophagy has been shown to be a variable trait [22,23]. 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 6 pm (outdoor) with the peak biting activity at 11 pm (indoor) in the July. However studies conducted by Dandalo [24] reported that the biting activity of *An. gambiae* complex species commenced at 7 pm and ceased at 5 am with peak biting activity in March [5,18] 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.

On the other hand *An. nili* was more exophagic with 60% of biting activity occurring outdoor. Biting activity of *An. nili* commenced at 6 pm and peak at 10 pm and decreased thereafter at 3am in June. In Cameroon the peak biting activity of *An. nili* occurred between midnight and 1 am [25]. A study in Italy showed host seeking to be highest during early hours of sunset but activity continued through the night [22,25,26]. Similar reports were made in other studies conducted in Indonesia, India and other countries [6,7,3]. In endemic areas, this species is perceived as a serious biting nuisance [1,17]. Takken [22] 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 6 pm shows that the possibility of outdoor transmission may be high because at this period the inhabitants of the areas are still active outdoors. The risk of contracting malaria is a deterrent for some tourist, particularly during high transmission periods of the year in certain places [27]. In South Africa, a group of researchers surveyed tourist facilities in Kwa-Zulu-Natal and found that risk of contracting malaria was the major cause behind lack of bed occupancy [25]. In India incidence of some illnesses, such as those transmitting by mosquitoes increase during the monsoon season (May – October) with high temperatures, heavy rains and the risk of flooding [21,23].

Table 5. 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

Previous studies in Southern Nigeria by Awono-Ambene et al. [4] and Awolola et al. [9], indicate 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 [4,9]. 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 [17] 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, so wealthy tourists may put off visiting areas where they are likely to get disease. In order to re assure tourists, hotels must spend money on Insecticide Treated Nets (ITNs) also based on the exophilic behaviour of *An. nil*i 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 endophilic behaviour of *An.gambiae* would be a prerequisite for significant contact between the vector species and man.

The highest number of *An. gambiae* was collected in July whereas the highest number of *An. nili* collected was recorded in June. The Human Biting Rates (HBR) recorded for *An. gambiae* was higher than *An. nili* (76.7%). This agrees with previous findings by Awolola [9] which reported similar HBR of *An. gambiae.* Also better understanding of each sibling species within the complex is quite important to help identify their respective roles in disease transmission and the Human Biting Rates (HBR) can be an important factor in the epidemiology of the disease and in estimating the vector-human contact.

5. CONCLUSIONS

The results from this study show that *An. gambiae* and *An. nili* co-exist in Ewet Offot, Uyo, Akwa Ibom State, Nigeria. *Anopheles gambiae* is the predominant species in this area. The differences in the Human Biting Rates (HBR) for A*n. 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 11 pm indoor and 7 pm outdoor in July whereas the peak of biting activity for *An. nili* occurred at 10 pm indoor and 6 pm 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. Improving tourists awareness of how malaria infection is transmitted and ways of preventing the infection such as proper environmental sanitation will go a long way to reducing the negative impact of the disease on activities of tourists in the area. The findings from this study contribute to the basic understanding of the behaviour of anopheles mosquitoes with respect to species composition and heterogeneities that could serve as base - line data for further entomological studies in the area.

ETHICAL CONSIDERATIONS

Ethical clearance for the work was obtained from Ewet Offot Village Council. The objectives and potential benefits of the study were made known to all the participants. Oral consent was obtained from all participants that were involved in both Knockdown Technique and Human Landing Catches (HLC).The study and methodology of the study were explained, and arrangements made with participants from the two study sites on when to collect the samples.

Malaria Prophylaxis with Aretemether and Lumefantrine (the recommended chemoprophylaxis in Nigeria) was provided for the HLC volunteers once a month throughout the study period.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Coetzee M, Craig M, Lesueur D. Distribution of the African malaria mosquitoes belonging to the *An. gambiae* complex. Parasitol Today. 2000;6(2):74-8.
2. FMH. National Anti-malarial treatment policy. Abuja: Federal Ministry of Health, National Malaria and Vector Control Division. 2008;1183-88.

3. Das LK, Pan SP Clinical manifestation of severe form of *P. falciparum* malaria in Koraput District of Orissa State. India. J Vector Borne Disease. 2006;43:104-143.
4. Awono-Ambene H, Kergne P, Simard F Antonio, Nkondjio Fontenille D. Description and bionomics of anopheles (Dipteria; Culicidae) a new malaria vector species of the *Anopheles nili* group from South Cameroon. J Med Entomol. 2004;41(4): 561-68.
5. Coetzee M. Distribution of the African malaria vectors of the *An. gambiae* complex. American Society Trop Med Hyg. 2004;70(4):103-4.
6. Elyazar IR, Sinka ME, Gething PW, Tarnidzi SN, Sunya A, Kusriastuti R, et al. The distribution and bionomic of anopheles malaria vector mosquitoes in Indonesia. Advanced Parasitol. 2015;83:173-266.
7. World Malaria Report. Geneva, Switzerland: Malaria report; 2015.
   Available:http://www.who.int/malaria/publications/world
   (Accessed December 9th, 2015)
8. Boccolini D, Toma L, DiLuca M, Severini F, Cocchi M, Bella, et al. Impact of environment changes and human related factors on the potential malaria vector, *An labranchiae* (Dipteria Culcidae) in Maremma. Central Italy. J. Med. Entomol. 2012;49(4):833–842.
9. Awolola TS, Ibrahim K, Okorie T, Koekomoer LL, Hunt RH, Coetzee M. Species composition and biting activities and their role in malaria transmission in a holoendemic area of South Western Nigeria. Africa Entomol. 2003;11(2):227-32.
10. Onyabe DS, Conn JC. The distribution of two major malaria vectors *An. gambiae* and *An. arabiensis* in Nigeria. J Med Entomol. 2001;96(26):1081-1084.
11. CDC. Chikungunya fever fact sheet. Division for Vector-borne Infectious Diseases Centers for Disease Control. 2007;12:6.
    Available:http//:wwwedegov/ncidadldubid/chikungunya
    (Accessed May 2013)
12. Oyewole IO, Ibidapo CA, Oduola AO, Awolola TS, Obansa JA. Molecular identification and population dynamics of the major malaria vectors in a rainforest zone of Nigeria. Biochem. 2005;17(20): 171-78.
13. WHO. Manual on practical entomology in malaria, part II methods and techniques. Geneva; division of malaria and other parasitic diseases; World Health Organization. 1975;39-55.
14. Gilles MT, Coetzee M. A supplement to the anophelinae of Africa South of the Sahara (Afro-tropical region). Johannesburg: South African Institute for Medical Research. 1987;55.
15. Scott JA, Brogdon WG, Collins FH. Identification of single specimen of *An. gambiae* complex by the polymerase chain reaction. J Trop Hyg. 1993;49(4):520-29.
16. Lines JD, Curtis CF, Wilkes IL, Njunwa WJL. Monitoring human-biting mosquitoes (Dipteria: Culicidae) in Tanzania with light-traps hung beside mosquito nets. J Med Entomol. 1991;81(3):77-84.
17. Pates H, Curtis C. Mosquito behaviour and vector control. Annual Review Entomol. 2005;50(2):53-70.
18. Hay SI, Okiro EA, Gething PW, Patil AP, Tatem AJ, Gueria CA, et al. Estimating the global clinical burden of *Plasmodium falciparum*. Med Parasitol. 2010;7(6):624-40.
19. Sungvornyothin S, Muenvorn V, Grarros C, Prabaripai A, Bangs MJ, Manguin S, et al. Tropic behavior and biting activity of two sibling species of anopheles minimus complex in western Thailand. J Vector Ecol. 2006;1:252–261.
20. Trung HD, VanBortel W, Sochantho J, Koekenchanh K, Breet OJT, Coosemans M. Behavioural heterogeneity of anopheles species in ecological different localities in Southern Asia: A challenge for vector control. J Trop Hyg. 2005;10(3):222-51.
21. Spitzen J, Cornelis W, Spoor FG, Cajioter B, Jacob B, Sjaak P, et al. A 30 analysis of flight behaviour of *Anopheles sensu stricto* malaria mosquitoes in response to human odour and heat. 2013;13:2.
    Available:http://edis,ifas.ufl.eduin6299
    (Accessed 3 May 2014)
22. Takken W, Knols B. Malaria vector control: Current and future strategies trends. Parasitol. 2009;25(2):101-4.
23. Service MW, Townson H. Malaria vector species distribution and culicidae mosquitoes. J Med. Entomol. 2012;2(1):428-432.
24. Dandolo LC. The abundance and biting behaviour of *An. gambiae* (Donitz) in Gokwe, South District Zimbabwe. Thesis, University of Zimbabwe. 2007;23-29.

25. DiLuca M, Boccolini D, Severini F, Toma L, Barbieri FM, Massa A, et al. A 2 year entomogical study of potential malaria vectors in central Italy. Vector Borne Zoonotic Disease. 2009;9(6):703–711.
26. Sharp BL. *Anopheles merus* (Donitz) its biting cycle in relation to environmental parameters. J Entomol Society in Southern Africa. 1983;46(2):367-74.
27. WHO. World Malaria Report Switzerland. 2013;10:2. Available:http://www.who.int/malaria/worldmalariareport/en/index/-html/Geneva (Accessed 12 February 2014)

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