

Original Research Article

Assessing the Entomological Parameters of Malaria Vectors in Anambra State, Southeast Nigeria

Abstract

Adequate knowledge of entomological parameters within a defined geographic endemic area is basic for effective planning of malaria vector control for malaria elimination. The study investigated indoor resting density (IRD), human biting rate (HBR), blood meal source (BMS), human blood index (HBI), sporozoite rate (SR) and entomological inoculation rate (EIR) of malaria vectors in Awka North, Awka South and Njikoka Local Government Areas in Anambra State, southeast Nigeria. Pyrethrum spray collection (PSC), Centre for Disease Control (CDC) light trap, and human landing catch (HLC) techniques were used for collection of indoor and outdoor malaria vectors. Mosquitoes collected were sorted according to species and sex; and identified using standard morphological and molecular techniques. Chi-square test was used for data analysis. A total of 2,870 *Anopheles* mosquitoes were collected, male 1949 (67.9%) and female 921 (32.1%). All female species identified morphologically belong to the *Anopheles gambiae* s. l. complex. From the molecular and siblings species separation, *Anopheles gambiae* recorded the highest abundance of 54.2% and *Anopheles coluzzii* the least abundance of 45.8%. The IRD was found to be 1.40 per man per night with an average HBR of 5.05. The blood meal source showed that human blood source was the highest number 46.2%, followed by goat blood source 32.7%, and combination of human and goat blood was the least 21.2%. The result also showed a HBI of 0.51. The test for the presence of circumsporozoite protein (CSP) of *Plasmodium falciparum* shows that none of the malaria vectors was positive for sporozoite; thus, the EIR could not be determined and compared among the study population. The allopatric breeding of the two sibling species of *Anopheles gambiae* and *Anopheles coluzzii* reported in this study is probably the second of such report in Nigeria and therefore has huge implications for malaria vector control and malaria control in Anambra State, Nigeria.

Keywords: Blood meal source, entomological parameters, malaria vectors, Anambra State

Introduction:

Anopheles gambiae remains the dominant and most efficient vector of human malaria in the tropics including Nigeria (CDC, 2015) based on its high abundance, longevity, high propensity for humans feeding and high vectorial capacity (Autino *et al.*, 2012). The correct analysis of the distribution of specific malaria vectors and its subsequent entomological parameters remains the prerequisites for meaningful epidemiological studies and for planning and monitoring of successful malaria control or eradication program. In the past, large areas of Nigeria had no reliable data on presence and absence of vectors, yet it has been established that there are diversities of malaria vectors in Nigeria and they have different bionomics and vector competences. Several studies conducted in the past observed that *Anopheles arabiensis* has a low Human Blood Index (HBI) and shows a marked preference for cattle and other warm-

blooded animals (Gillies *et al.*, 1987). For instance, it has a high degree of zoophily in Madagascar as demonstrated by HBI reported from various environmental settings (Scott *et al.*, 1993 and Kirby *et al.*, 2018). Lower proportion of human blood meals (26%) were also recorded in *An. arabiensis* collected from sites where cattle were kept closer to human housing than in those collected from sites where cattle were kept some distance from humans (Smith *et al.*, 2012; Lindsay *et al.*, 2018 and Onoriet *et al.*, 2021). The entomological parameters allows a better understanding of the transmission dynamics and the design of more effective malaria vector control strategies. The information collected in this study will both complement and support other studies that describe the entomological parameters of malaria vectors which include but not limited to the indoor resting density (IRD), human biting rate (HBR), blood meal source, human blood index (HBI), sporozoite rate (SR) and entomological inoculation rate (EIR) of the study sites. The collection and integration of these information will characterize malaria in the LGAs and establish a robust framework for developing future interventions against malaria.

Materials and methods:

Study area: The research was carried out in Awka South, Awka North and Njikoka LGAs of Anambra State Nigeria from September 2021 to August, 2022 covering the two major seasons of the year. The State has twenty one Local Government Areas with a total land kilometer of 4,844km² (1,870sqm) (Microsoft Encarta, 2009). The 2020 projected population of Anambra State is 6,182,924 (NBS, 2016). The official language of the people of Anambra State is Igbo although English language is widely spoken throughout the state as a secondary language (Microsoft Encarta, 2009). The State is located on Latitude 6° 12' 45.68" North of Equator and Longitude 7° 04' 19.16" East of Greenwich and its average daily temperature is 26.8 °C / 80.2 °F (Microsoft Encarta, 2009). Anambra State has seasonal climatic conditions, the rainy season (which falls between April and October) and the dry season (which falls within November and March) with a short spell of harmattan between November and January which is a period of cold weather when the atmosphere is generally misty (Enete, 2008). The total annual rainfall of the State is above 11,450mm for the six to seven months of the rainy season whereas the mean annual temperature range is 30.0–36.0 °C. (Enete, 2004). Awka South Local Government Area (LGA) is made up of nine (9) communities namely; Awka, Amawbia, Ezinato, Isiagu, Mbaukwu, Nibo, Nise, Okpuno and Umuawulu (Microsoft Encarta, 2009). Awka South LGA has one ethnic group and the LGA is located on Latitude 6° 10' North of equator and Longitude 7° 04' East of Greenwich. Awka North is also a Local Government Area in Anambra State with coordinates of 6° 15' North of equator and 7° 10' East of Greenwich. Communities that make up the local government are ten (10) in number which includes: AwbaOfemili, Amansea, Ugbene, Ebenebe, Achalla, Urum, Amanuke, IsuAniocha, Mgbakwu and Ugbenu with its local government headquarters at Achalla. (Microsoft Encarta, 2009). Njikoka is a Local Government Area in Anambra State, South-Central Nigeria lying between latitude 06° 20' 58" N to 06° 21' 00" N and longitude 06° 52' 55" E. The area is characterized by a temperature range of 27° - 30°C and has a double maxima rainfall peaks in July and September. Six (6) towns make up the local government which include; Abagana, Enugwu-Ukwu, Enugwu-Agidi, Nawfia, Nimo and Abba.

The area has a land mass of 84.40 km² area with an annual population change of 2.8% and 2020 projected population of 219,571(NPC, 2016).

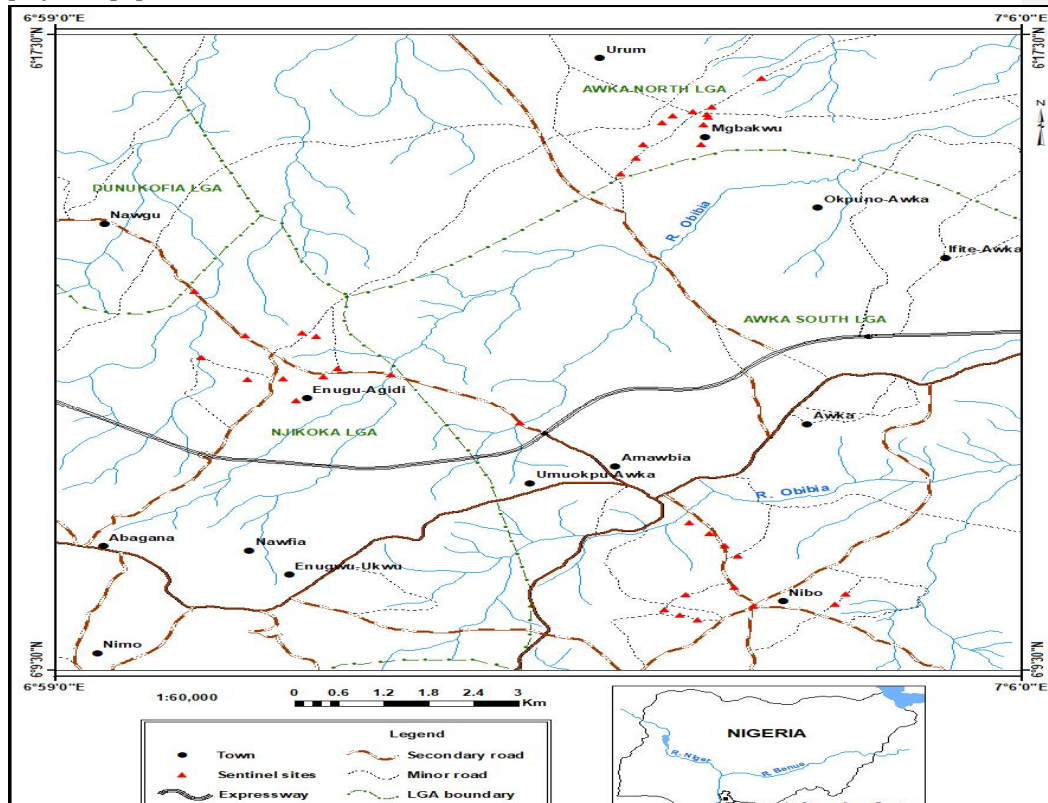


Fig. 1: GIS map of the study areas showing Nibo in Awka South, Mgbakwu in Awka North and EnugwuAgidi in Njikoka Local Government Areas. Source: Modified from Google map on 20th December, 2022

Selection of communities and households: One community was randomly selected from each LGA to represent all the communities in the local government area. A village was randomly selected from a pool of villages with equal representation from the different sites with each village having an equal chance of being selected. According to the method described by WHO (1992), households were randomly selected from a pool of village with equal representation from the different sites with each household having an equal chance of being selected. Rooms were randomly selected from the different collection sites at the three study areas from a pool of houses with equal representation with each houses having an equal chance of being selected. Mosquitoes were captured between September 2021 and August 2022, covering the rainy and dry seasons of the year.

Study design:

Descriptive and comparative study design was used in the study and simple random sampling as described by WHO (1992) was used to select households for the study.

Advocacy and community mobilization:

Advocacy visit to the communities was accompanied with a letter of introduction obtained from the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka Anambra State. Three levels of advocacy were carried out; first to the heads in charge of Health Department through the Local Government Chairman of the selected LGAs, followed by advocacy visit to the community leaders/president general of the selected LGAs and finally to the households/individuals of the selected communities that will participate in the study. Sensitization was done through churches, markets and kindred enclaves. Mobilization for the study involved youths, women, and men in the study community. The participants were mobilized by a sensitization rally during which the objective of the study, explanation of the project intent and its methodology was made. Approval and informed consent of the participants for the study was obtained before the commencement of the study and the identity of the participants were anonymous during and after the survey. Participation was also voluntary and participants had the liberty to withdraw from the study without giving any reason.

Sampling procedures for entomological investigations: The following sample procedures were engaged: pyrethrum spray trap collections for indoor mosquito vectors, CDC light trap collections for outdoor and indoor mosquito vectors, human landing catches for outdoor and indoor mosquito vectors.

Collection of adult mosquito vector: Outdoor and Indoor resting mosquitoes were collected from the study locations using pyrethrum spray collection techniques, CDC light trap collection and human landing catches methods. The collections were performed monthly during three consecutive nights/days, and each collection period lasted for twelve hours, from 6:00pm to 6:00am for the Human Landing Catch and CDC Light Trap, from 6:00am to 9:00am for the Pyrethrum Spray Collections. Under these conditions, the mosquitoes were captured concomitantly in the intra (indoor) and peridomestic (outdoor) environments. In all the collections, mosquitoes collected were transferred into moist filter papers in Petri dishes as described by WHO (2015) properly labeled with location, date, hour, collection method.

Pyrethrum spray collections for indoor mosquito vectors: Indoor mosquito collection was done monthly using pyrethrum spray catches (PSC) method as described by Abdoon and Alshahrani (2003). The rationale behind selection of this method is that it is considered most successful in capturing anthropophilic (human biting) and endophilic (indoor resting) mosquitoes. According to Curtis (2003), it is also considered far less ethically objectionable in areas of multidrug resistance. In each site, households were randomly selected for the spray collections and sampled monthly. Pyrethrum spray collections took place between 06.00am and 09.00am using BMC branded pyrethroid mosquito spray which constitutes the following active ingredients (0.26% Esbiothrin, 0.28% Permethrin, 0.1% Beta-Cypermethrin and 0.31% Lemon). Food and water were removed from the house and white sheets spread out on the floor and over the furniture in the house. Two field workers were engaged, one inside the house and the other

outside who sprayed around the eaves with this non-residual pyrethroid. The field worker inside the house then sprayed the roof and walls. The house was closed for 20 minutes after which the white sheets were brought outside (where there is sufficient light to see the dead and dying mosquitoes).

CDC light trap collections for indoor and outdoor malaria vectors: Indoor and Outdoor mosquito collection was also conducted monthly using modified miniature light traps as described by the Centers for Disease Control (CDC) (John W. Hock Company, model 512). The (CDC) was used to collect mosquitoes during night time for three consecutive nights. The CDC light traps were placed approximately 1.2m above the floor in and outside the room. Traps were switched on from 6:00 pm and removed by 6:00 am the next day. Each night, 6 traps (one trap indoor and one trap outdoor at three different locations) were set for three nights for each site each month and they were rotated in the same order each month.

Human landing catches for outdoor and indoor malaria vectors: The human landing catch (HLC) method which is the standard reference method for measuring human exposure to mosquito bite was used to assess the human biting rate (HBR) of mosquito in the study areas as described by WHO (2015). HLC was performed for 50 minutes each hour with 10 minutes break for the collectors. Human-landing catches was conducted indoors and outdoors in and around the randomly selected houses at each site each month. At each site, different houses were selected each night, at least 300 m apart, for three consecutive nights. Thus, all households were sampled in the same week each month. Catches were designed to replicate normal human subject behaviour, assuming many residents were outdoors in the early evening, and that most will retire to bed before 22.00. At each house two adults were stationed outdoors 10 m from the house, and two were stationed indoors. Outdoor collections were conducted from 6.00pm to 9.50pm, after which time few people were outdoors, and indoor collections from 6.00pm to 05.50am. Collectors sat on chairs with their legs exposed. Using flashlights, collectors caught landing mosquitoes with a hand – held mouth aspirator and improvised test tubes and each hour's collection were kept separately and the collectors were rotated between sites on different nights.

Preservation of adult malaria vector: All adult malaria vectors collected were temporary preserved on moist filter papers in petri dishes as described by WHO (2015) and transferred to the Vector Research Department at the National Arbovirus and Vectors Research Centre (NAVRC), Enugu, Nigeria for identification and further analysis.

Morphological and molecular identification of malaria vectors: All the mosquito vectors collected were identified and sorted out under a Stereomicroscope Leica model NSW series IMNS 210 entomological microscope. The mosquitoes were identified to species level using morphological keys of Gillies and De Meillon (1968), Gillies and Coetzee (1987). Adult female mosquitoes were stored dry on silica gel for species identification and molecular assays (PCR and ELISA) subsequently of the *Anopheles gambiae* complex. Each mosquito sample was preserved individually in an eppendorf tube and were transferred to the Molecular Entomology and Vector Control Research Laboratory of the Department of Public Health and Epidemiology

at the Nigerian Institute of Medical Research (NIMR) Yaba Lagos, Lagos State Nigeria for further molecular identification and analyses.

Analysis of Data: Descriptive statistics (mean and standard deviation) was used for sample characterization. One-way analysis of variance (ANOVA). Data were subjected to Chi-square using the SPSS Software package (Version 20) to assess possible correlation whereas *P-values* < 0.05 was considered statistically significant.

Results:

Table 1: Vector composition and abundance of *Anopheles* species collected from Awka South, Awka North and Njikoka Local Government Areas in Anambra State

Sex of <i>Anopheles</i> species	Awka South (%)	Awka North (%)	Njikoka (%)	Total (%)
Male	412 (21.1)	540 (27.7)	997 (51.2)	1949 (67.9)
Female	329 (35.7)	311 (33.8)	281 (30.5)	921 (32.1)
Grand total	741 (25.8)	851 (29.7)	1278 (44.5)	2870 (100)

$\chi^2=119.125$, $df=2$, $Pv=0.000$

Table 2: Determination of Indoor Resting Density (IRD) of Malaria Vectors in Awka South, Awka North and Njikoka LGA, Anambra State

Number of Indoor Anopheline Mosquitoes Collected

LGA/	Average No. of Rooms sprayed per month	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	Total number of mosquitoes collected	Total number of rooms sprayed	IRD
AWKA SOUTH	36	72	123	15	13	52	41	76	31	20	24	37	85	589	432	1.36
		(12.2)	(20.9)	(2.5)	(2.2)	(8.8)	(7.0)	(12.9)	(5.3)	(3.4)	(4.1)	(6.3)	(14.4)	(100)		
AWKA NORTH	36	17	30	11	27	100	35	43	51	21	120	85	81	621	432	1.44
		(2.7)	(4.8)	(1.8)	(4.3)	(16.1)	(5.6)	(6.9)	(8.2)	(3.4)	(19.3)	(13.7)	(13.0)	(100)		
NJIKOKA	36	55	46	14	12	18	58	51	19	25	49	85	81	513	432	1.39
		(10.0)	(9.0)	(2.7)	(2.2)	(3.5)	(11.1)	(9.9)	(3.3)	(4.9)	(9.6)	(16.1)	(15.0)	(100)		

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	144	199	40	52	170	134	170	101	66	193	207	247	1723	1296	1.40
TOTAL	(8.4)	(11.5)	(2.3)	(3.0)	(9.9)	(7.8)	(9.9)	(5.9)	(3.8)	(11.2)	(12.0)	(14.3)	(100)		

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$\chi^2=290.370$, $df=22$, $Pv=0.000$

Table 3: Determination of Human Biting Rate (HBR) of Malaria Vectors in Awka South, Awka North and Njikoka Local Government Areas, Anambra State

Months	Awka South LGA				Awka North LGA				Njikoka LGA				Average HBR
	Average No. of Occupants	Total No. of Mosquitoes Sampled	Average No. of freshly fed <i>Anopheles spp</i>	HBR	Average No. of Occupants	Total No. of Mosquitoes Sampled	Average No. of freshly fed <i>Anopheles spp</i>	HBR	Average No. of Occupants	Total No. of Mosquitoes Sampled	Average No. of freshly fed <i>Anopheles spp</i>	HBR	
Sept	3	72	24	8	3	17	0	0	3	55	19	6.33	
Oct	2	123	0	0	4	30	0	0	4	46	45	11.25	
Nov	3	15	3	1	4	11	0	0	3	14	0	0	
Dec	4	13	2	0.5	4	27	1	0.25	4	12	4	1	
Jan	4	52	31	7.75	2	100	87	43.5	3	18	16	5.33	
Feb	3	41	9	3	4	35	12	3	2	58	12	6	
Mar	3	76	33	11	3	43	29	9.67	2	51	39	19.5	
Apr	3	31	5	1.67	3	51	7	2.33	4	19	3	0.75	
May	3	20	3	1	3	21	2	0.67	3	25	3	1	
Jun	2	24	4	2	3	120	15	5	3	49	9	3	
Jul	3	37	6	3	4	85	9	2.25	4	85	12	3	
Aug	3	85	12	4	2	81	19	9.5	3	81	16	5.33	
Total	36	589	132	3.58	39	621	181	6.35	38	513	178	5.21	5.0

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R-value = -99.23 (negative correlated at 0.7). Between 0.5 and 1 = strong positive correlation between IRD and HBR recorded.

Table 4: Identification of Blood Meal Source in *Anopheles gambiae* and *Anopheles coluzzii* collected indoors in Awka South, Awka North and Njikoka LGA, Anambra State

LGA	T. Collected	Blood Meal Source in <i>An. gambiae</i> . l.				
		N. Human (%)	N. Goat (%)	N. Bovine (%)	N. Human (%) + N. Goat (%)	T. Blood fed (%)

AWKA SOUTH	60	15 (36.6)	14 (34.2)	0 (0)	12 (29.3)	41 (39.4)
AWKA NORTH	60	24 (63.2)	9 (23.7)	0 (0)	5 (13.2)	38 (36.5)
NJIKOKA	60	9 (36.0)	11 (44.0)	0 (0)	5 (20.0)	25 (24.0)
TOTAL	180	48 (46.2)	34 (32.7)	0 (0)	22 (21.2)	104 (57.8)

$$\chi^2=18.408, df=6, Pv=0.005$$

T= total of mosquitoes; N = number of mosquitoes having taken their blood meal on: Human

(H), Goat (G), and Bovine (B).

Table 5: Determination of Human Blood Index (HBI) of Malaria Vectors in Awka South, Awka North and Njikoka Local Government Areas, Anambra State

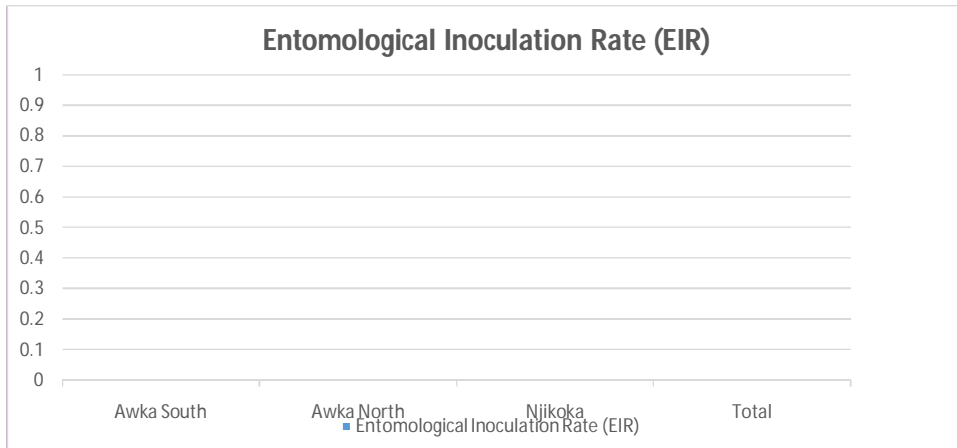
LGA	T. Collected	T. Blood fed (%)	N. Human (%)	Human Blood Index (HBI)
Awka South	60	41 (68.3)	15 (36.6)	0.37
Awka North	60	38 (63.3)	24 (63.2)	0.63
Njikoka	60	25 (41.7)	9 (36.0)	0.36
Total	180	104 (57.8)	53 (51.0)	0.51

$$\chi^2=69.35768, df=1, Pv=0.0992$$

Table 6: Determination of malaria sporozoite / infection rate in Awka South, Awka North and Njikoka Local Government Areas, Anambra State

LGA	T. Collected	<i>Anopheles gambiae</i> (%)	<i>Anopheles coluzzii</i> (%)	T. Blood fed / Processed (%)	Positive for Sporozoite (%)	Sporozoite Rate (%)
Awka South	80	45 (56.3)	35 (43.8)	41 (51.25)	0 (0)	0 (0)
Awka North	80	61 (76.3)	19 (23.8)	38 (47.50)	0 (0)	0 (0)
Njikoka	80	24 (30.0)	56 (70)	25 (31.25)	0 (0)	0 (0)
Total	240	130 (54.2)	110 (45.8)	104 (43.33)	0 (0)	0 (0)

Sporozoite rate = number positive/ number processed (WHO, 2013).



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Fig 2: Determination of EIR of malaria vectors in Awka South, Awka North and Njikoka Local Government Areas, Anambra State

Discussion:

A total number of 2,870 *Anopheles* mosquitoes were collected in the study. Of this number, 67.9% (1949/2870) were males and 32.1% (921/2870) were females. All the female species identified morphologically belonged to the *Anopheles gambiaes. l.* complex. The highest number of female malaria vectors was 35.7% (329/921) collected from Awka South LGA, followed by 33.8% (311/921) in Awka North LGA and then 30.5% (281/921) in Njikoka LGA in Anambra. The female mosquitoes 32.1% caught in the present study is relatively low compared to that caught by Kevanet *et al.*, (2019) where a total of 7146 female *Anopheles* mosquitoes of seven different species (*An. bancroftii*, *An. farautis.s*, *An. farautino. 4*, *An. hinesorum*, *An. koliensis*, *An. longirostris* and *An. punctulatus.s*) were collected, including 2611 (36.5%) blood-fed and 4535 (63.5%) unfed; gravid females were very low in numbers (< 0.1%, and not considered further) to investigate species composition, abundance, and nocturnal activity of *Anopheles* populations in the study villages. The number was very high because mosquitoes were sampled from 6 pm to 6 am in five villages from 2012 to 2016 (a four years survey) whereas the present study was conducted under a year.

The IRD in Awka South LGA was found to be 1.36 per man per night whereas Awka North and Njikoka LGAs recorded an IRD of 1.44 per man per night and 1.39 per man per night respectively. The average IRD in the overall study was found to be 1.40 per man per night. This is very high when compared with the overall indoor resting density (IRD) of 0.02 bites per man per night required for maintaining transmission and this might also account for high malaria prevalence in the area. Earlier, WHO (2020) observed that *A. gambiae* has high indoor resting density and typically long lived mosquitoes connected with stable malaria in equatorial Africa. The higher density of the anopheline mosquitoes in the present study is perhaps an outcome of favorable ecological conditions, good breeding environment (presence of conducive habitats) and is attributed to high malaria incidence in this part of the state. Nevertheless, the result was lower than the total average indoor resting density recorded by Dash *et al.* (2000) which was (46.32/man hr) in the region seems to be fairly high. Separately, the average density for *Anopheles* and *Culex* was 30.80 and 12.95, respectively Kulkarni (1990). Indoor resting density

of anopheline species was 66.79. Sharma (1998) observed high density (11.3–125.1) of *A. culicifacies* in their study areas where the average density was high in August–September and February–March 2003, declined gradually and was lowest in summer season. Kulkarni (1990) reported two peaks, one in July–August and another in February–March. Several of the climatic factors viz. rainfall, humidity and temperature and biological activities like housing style, pattern of villages, larval breeding grounds, socioeconomic conditions and adopted control measures are found to be the regulatory factors of indoor mosquito density in this study (Kulkarni, 2019; Mahesh and Jauhari 2004).

Awka North LGA recorded the highest HBR of 6.35 followed by Njikoka LGA which had the HBR of 5.21 whereas Awka South recorded the least HBR of 3.58. But the average HBR in the 3 LGAs was found to be 5.05. The result is lower than the result study done by Ojira *et al.*, (2016) who reported that the overall (indoor and outdoor combined) mean human-biting rate (HBR) of *Anopheles* mosquitoes was 85.2 mosquitoes/person/night (m/p/n). The total (indoor and outdoor combined) mean HBRs for *An. zeimanni* was 56.7, *An. arabiensis* 21.1. The mean hourly human-biting density of *An. arabiensis* was greater outdoors than indoors and peaked between the months of January and March. However, *An. arabiensis* parous population showed high indoor man biting activities during February and also during bedtimes (22:00 to 05:00 h) when the local people were indoor and potentially protected by IRS and LLINs. The finding in the present study is consistent with previous reports by (Aboseet *et al.*, 1998 and Krafur, 2017). Because *Anopheles gambiae* was more exophagic than *Anopheles coluzzi*, it appears to have less chance to come in contact with LLINs and IRS. This might have favored *Anopheles gambiae*, while *Anopheles coluzzi* population was affected by IRS and LLINs interventions. Results show that mosquito human-biting activities occurs both outdoors and indoors and during early parts of the night, implying higher outdoor malaria transmission potential in the area. However, high bedtime (22:00 to 05:00 h) indoor biting activities of parous *Anopheles gambiae* and *Anopheles coluzzi* suggest high potential intervention impact of IRS and LLINs on indoor malaria transmission. The scale up of IRS and LLINs during the last decade has substantially reduced malaria incidence in many parts of the study areas (D'Acremont *et al.*, 2010 and Mharakurwa *et al.*, 2013). These interventions reduce the density, feeding frequency and longevity of malaria vectors by killing the vectors with insecticides or blocking their contact with humans (Mutuku *et al.*, 2011 and Gimnig *et al.*, 2013) and primarily target malaria vectors that feed indoors and at night on sleeping humans (WHO, 2014).

Since female mosquitoes require blood meals for their egg maturation, it is very crucial that the blood meal source of *Anopheles gambiae* and *Anopheles coluzzi* in the current study be identified. The blood meal source of malaria vectors in the study were found to be goat, human, combination of human and goat, but no malaria vector had bovine blood meal source. Human blood source recorded the highest number with 46.2% (48/104) followed by goat blood source 32.7% (34/104), whereas (human and goat) recorded the least number of 21.2% (22/104). Awka South LGA had the highest number of blood fed malaria vectors 39.4% (41/104) followed by Awka North 36.5% (38/104) and Njikoka respectively 24.0% (25/104). In a study done by Mwanga *et al.*, 2019 who used the logistic regression (LR) model to achieve the highest accuracy, correctly predicting true vertebrate bloodmeal sources with overall accuracy of 98.4%. It was reported that the model correctly identified 96% goat blood meals, 97% of bovine blood meals, 100% of chicken blood meals and 100% of human blood in *Anopheles arabiensis*. The study was also in line with that of Kent and Norris, 2005 who noted in their study that a total of 194 blood-fed *An. arabiensis* (identified by PCR) that were collected by indoor resting

collections were tested for vertebrate host blood source (human, bovine, goat, dog), the overall proportion of *An. arabiensis* that fed on humans (including mixed blood meals on both human and animal) was 59.3% (115/194), with cattle blood being the most common non-human source. An estimated 32.5% (63/194) of *An. arabiensis* fed on both human and animals, demonstrating opportunistic feeding behaviour, while only 26.8% fed only on humans (Chow *et al.*, 1993). The figure for blood meal source in this study is very high and this probably accounts for high malaria prevalence in the area. Earlier, (WHO, 2020) observed that *Anopheles gambiae* has high human blood index, and are typically long-lived mosquitoes connected with stable malaria in equatorial Africa.

The Human Blood Index (HBI) which represents the proportion of blood meals derived from humans by mosquito vectors and may be used to estimate the human biting habit, an important component of vectorial capacity, as a proxy measure of malaria transmission. It was observed that the HBI of malaria vectors in Awka South LGA was 0.37 (15/41), 0.63 (24/38) in Awka North and 0.36 (9/25) in Njikoka LGAs shown in Table 5. The overall HBI in the study was 0.51 which was very high. Kent and Norris, 2005 noted that the overall human blood index in their study was 0.62 whereas (Burkotet *et al.*, 1989) noted the HBI of 0.55 in their study. Marco Pombiet *et al.*, 2018 in their study noted that low values of human blood index (0.12) observed in the major malaria vectors in the area (*Anopheles coluzzii*: N = 263, 20.1%; *An. arabiensis*: 5.8%, N = 103) are consistent with the hypothesis that LLINs reduced the availability of human hosts to mosquitoes. The result is also in line with the study done by Vasilikiet *et al.*, 2011, who reported that the majority of blood meals were derived from humans. Four mosquitoes took mixed blood meals (human and dog), and one took a blood meal derived from dog and the crude Human Blood Index (HBIs) among the different mosquito species ranged from 0.56 to 1, whereas all adjusted HBIs were equal to 1. *Anopheles gambiae* is widely regarded as a highly anthropophilic and domestic mosquito vector (Muirhead, 1957). However, some studies report *An. gambiae* preferentially feeding on dogs or cattle over human hosts (Sousa *et al.*, 2001; Teklehaimanotet *et al.*, 2009; Ducheminet *et al.*, 2001). The zoophily of these vectors could be explained as a response to indoor residual spraying (IRS) interventions combined with the use of long-lasting insecticide-treated nets (LLINs) (Teklehaimanotet *et al.*, 2009). The insecticides used in such interventions have excito-repellent effects that drive host-seeking mosquitoes outdoors, where they may come into contact with non-human hosts. It may be that, as a consequence of indoor-based antivector interventions, host-seeking mosquitoes are diverted to seek blood meals in outdoor venues, where a greater diversity of non-human hosts may be encountered and fed on. The high degree of anthropophily observed in the present study is consistent with findings reported in other studies (Githekoet *et al.*, 1994; Okwaet *et al.*, 2009; Wanjiat *et al.*, 2003; Tangaet *et al.*, 2010; Magbityet *et al.*, 1997). Although some studies report zoophily in Afro-tropical vector populations (Silver, (2018), although such behaviors were not detected. In these situations, ongoing antimalarial interventions may not be enough to divert malaria vectors to non-human hosts. Different results may also be attributed to behavioral differences in local vector population or differences in collection methods. In this study, a high degree of anthropophily in the vector population was observed in this study and this suggests that ongoing IRS activities may not be sufficient to reduce the HBI because of a variety of factors, including diminished residual insecticidal activity and household coverage and lack of available alternative vertebrate hosts. And this call into question the appropriateness of current methods of assessing host preferences among disease vectors and have important implications for strategizing vector control.

Since the epidemiological significance of *Anopheles* as malaria vectors is defined by a range of factors, the most important among them including: the rate of *Anopheles* species susceptibility to infection by human plasmodia, the duration of sporozoites' preservation in salivary glands of the mosquitoes, the species numbers, and the degree of relationship with man, the individual life span of the mosquito. In this study, a total of 130 *Anopheles gambiae* and 110 *Anopheles coluzzii* from Awka South, Awka North and Njikoka LGAs were tested for the presence of CSP of *P. falciparum*. However, none was found positive for sporozoite. The study was in line with another study done by Ledayane *et al.*, 2016 who also noted that using the PCR technique, no *Anopheline* was found infected with the malaria parasite. But in contrast with the study done by Burkot *et al.*, 1989 who reported that between 2016 and 2017 in the study area, the overall *P. falciparum* sporozoite rate across all sites for all *Anopheles* (*An. funestus*, *An. arabiensis*, *An. gambiae*, and *An. parensis*) combined was estimated as 1.72% (286/16,670). The overall sporozoite infection rate was higher in unsprayed sites, estimated as 2.02% (115/5,686) than in sprayed sites at 1.56% (171/10,984) and the mean sporozoite rates were generally less than 2% for all sprayed sites (from 2016 to 2017) in the same study. Marco Pombiet *et al.*, (2018) in their study noted that a regression meta-analysis of data from a systematic review of published studies reporting HBI and sporozoite rates (SR) for *An. gambiae* complex revealed that the observed SR values (*An. coluzzii*: 7.6%, N = 503; *An. arabiensis*: 5.3%, N = 225) are out of the ranges expected based on the low HBI observed. Sporozoite rates of *An. coluzzii* females were higher for the human-fed sub-sample (22.9%; N = 35) than for animal-fed (8.4%; N = 143) or unfed-gravid subsamples (5.5%; N = 200), as well as for the sub-sample of fed-females with unidentified blood-meals (5.6%; N = 125). This suggests that females that have already taken a blood-meal on a (infected) human host and/or that are ready to transmit the malaria parasite, have a higher tendency to bite human again (Koella *et al.*, 1998; Nyasembe *et al.*, 2014 and Smallegange, *et al.*, 2013). It is believed that *Plasmodium* can potentially affect the complex mosquito host-seeking behaviour (in the experimental model *P. yoelii*-*An. stephensi*) due to neurophysiological mechanism, based on changes in the responsiveness of mosquito odorant receptors (Cator *et al.*, 2013). Thus, the higher SR observed in human-fed *A. coluzzii* could be due to a significant increase in infected mosquito attraction to human odours triggered by the parasite itself in order to enhance its chances of transmission to the appropriate host (Koella *et al.*, 1998 and Nyasembe *et al.*, 2014).

Entomological inoculation rate (EIR) which is the product of man biting rate and sporozoite rate, but none of the malaria vectors were found to be positive for CSP of *P. falciparum* (sporozoite). For this reason, the EIR could not be determined and compared among the study populations. The allopatric breeding of the two sibling species of *Anopheles gambiae* and *Anopheles coluzzii* reported in this study is probably the second of such report in Nigeria and therefore has huge implications for malaria vector control and malaria control in Anambra State, Nigeria.

Data availability: The data used to support the findings of this study are available upon request.

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