MOLECULAR CHARACTERIZATION AND POTENTIALITY OF *ANOPHELES COLUZZII* IN DISEASE TRANSMISSION IN DIFFERENT COMMUNITIES IN UGHELLI NORTH LGA, DELTA STATE, NIGERIA

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ABSTRACT

A thorough understanding of the malaria vector's dynamics is a crucial strategy for preventing mosquito bites and vector control. This study assessed the species composition, wing length and biting behavior with respect to seasons in Ughelli North LGA, Delta State, Nigeria. CDC light traps were used to capture mosquitoes hourly, indoors and outdoors in four settlements (Ugono Orogun, Orhomuru Orogun, Emonu Orogun and Ovara Orogun) for 10 months (May 2022 – February 2023). Mosquitoes collected outdoor (187) and indoor (138) were significantly different (p<0.05), with most abundant indoor recorded in June and July (5.25 ± 0.48 respectively), and outdoor in June (6.75 ± 0.48). Monthly variation in mosquito species was not significant (F = 0.71, df = 9, p = 0.695). The biting peaks of mosquitoes in the outdoor and indoor were observed between the hours of 8 – 9 pm respectively, also 3 – 4 am for indoor and 4 – 5 am for outdoor mosquitoes. Furthermore, all Anopheles mosquitoes collected were Anopheles gambiae sensu lato and confirmed molecularly to species level as Anopheles coluzzii. Mosquitoes outdoor had wing length significantly longer (p<0.05) than the mosquitoes obtained indoor and this may justify the reason for more outdoor mosquitoes. More mosquitoes were recorded in the wet months (F = 9.70, p = 0.0002) than in dry months. In conclusion, in order to lessen the prevalence of malaria in the study area, focused control actions of An. coluzzii, a key vector that transmits malaria parasites, should be directed outdoors before the month of June.

Keywords: *Anopheles coluzzii*, Delta State, Disease transmission, Entomological indices, Molecular studies

INTRODUCTION

Mosquitoes as vectors, the significant and deadly diseases they cause such as malaria, lymphatic filariasis, dengue and yellow fever are the main aspects of public health needing urgent global attention and intervention (WHO, 2022). Amongst these diseases, malaria stands out as a major disease causing severe problems especially in the WHO African region. Global malaria report in 2021 revealed an estimated significant decline in malaria deaths from 896,000 in 2000 to 619,000 malaria deaths in 2023, also, malaria cases reduced to 247 million

cases from the year 2000 (WHO, 2022). The WHO African region especially Nigeria has suffered the most burden of this disease. As a result, malaria continues to be a significant public health burden in Nigeria, with new emerging case reports in the face of current interventions. Nigeria alone accounted for 27% and 23% respectively, of all cases and fatalities worldwide which relate to 61 million cases and 96,000 fatalities in 2019 (WHO, 2020). This represents a 17% increase in cases but a 46% decrease in deaths since 2000. Despite significant variations by state and location, the national frequency decreased from 42% in 2010 (NMIS, 2010) to 23% in 2018 (NDHS, 2019). However, between the year 2000 and today, the fight against malaria has produced notable outcomes related to intervening such as vaccine and drug discovery, insecticide actions and many technological advances in mosquito research (Mahmoudi and Keshavarz, 2018; Ojianwuna *et al.,* 2021a; Ojianwuna and Enwemiwe, 2022; El-Moamly and El-Sweify, 2023).

To reduce the diseases caused by mosquitoes, WHO has recommended the use of insecticides in four classes; pyrethroid, carbamates, organochlorine and organophosphates. It is well known that current vector control tactics adopting insecticides focus on the use of Long Lasting Insecticidal Nets (LLINs), Indoor Residual Spraying (IRS) and Larval Source Management (LSM). Even with these interventions, malaria is still one of the leading causes of death in Africa. However, long-term vector control initiatives based on IRS and LLINs have helped to significantly reduce malaria related mortality over the past two decades (WHO, 2020). The Federal Ministry of Health target for net is complete ownership and at least 80% population usage which would realize the national goal in the malaria strategic plan, and the nets have been widely deployed to households through mass net campaigns and regular routes (Masaninga *et al.,* 2018). There is no holistic study to establish the quantity of nets deployed in Nigeria. However, Solanke *et al.* (2023) has reported net ownership and usage in Northern area of Nigeria. Studies also exist for Southwestern and Southeastern Nigeria (Adebayo *et al*., 2014; Okeke *et al*., 2016; Orji *et al*., 2018; Alawode *et al*., 2019). Adding up the net ownership reported in these studies among others, over 10 million nets have been deployed in Nigeria. In the African region alone over 100 million LLINs have been distributed (Njatosoa *et al.,* 2021; Ng'ang'a *et al.,* 2021). So far, UNICEF (2022) reported that over 275 million insecticide treated nets have been distributed in the fight against malaria. Similar report has been made by malaria consortium in 2022 that over seven million insecticide treated nets were distributed in Nigeria (Malaria Consortium, 2023). Despite the efforts in assuring availability and coverage of LLINs, relatively few numbers of states in Nigeria have adopted and put into practice other vector control measures like IRS and LSM as key elements of the malaria control program (NMEP, 2020). In one way, collection or over collection of adult mosquitoes and even the destruction of breeding sites are control strategies. The CDC light trap was designed to passively collect *Anopheles* mosquitoes, while diverting mosquito attention on humans. Unfortunately, its effectiveness is restricted in terms of collecting blood fed mosquitoes and number of catches.

Research-wise, a lot has been done and a lot is still needed to unravel the mysteries behind malaria in Nigeria (Oduola *et al.,* 2012; Evans and Adenomon, 2014; Ndenga *et al.*, 2016; Adepoju and Akpan, 2017). Assessments of the spatial pattern of malaria infection in Nigeria indicated that seasonal variations play significant roles in malaria infection in Nigeria (Ugwu and Zewotir, 2020). It also showed high prevalence of malaria infections in some few states (Ayanlade *et al*., 2013; Onwuemele, 2014). A study by Onyiri (2015) geospatially modelled malaria burden in Nigeria and observed that the two main environmental covariates correlating with malaria infection were land surface temperature for day and rainfall just as closeness to water bodies. Geospatial mapping of malaria vectors is important in the control of malaria and monitoring of malaria prevalence especially in the African region. This is because the species composition, spatial distribution and other biological parameters of the mosquitoes are

poorly known in different ecological zones of Nigeria. More so, in most of the malaria endemic areas, financial constraints in the molecular identification of certain species complexes and the right knowledge of interpretation of results have presented difficulty in the design of vector control programme to tackle the prevalence of the disease in endemic areas. The Federal Ministry of Health reported that approximately 50% of Nigerians suffered from one form of malaria or the other making it the most significant health problem (FMoH, 2014). This was acknowledged by officially commemorating April 25 every year, the date in 2000 when leaders of 44 African nations met in Abuja, Nigeria, and committed their countries to reducing the number of malaria-related deaths (CDC, 2017). This is part of the efforts made to eradicate malaria.

One notable setback to the success in vector control is the emergence of resistance to pyrethroid, the only class of insecticide-treated bed nets licensed for the management of malaria in West and East Africa. In Nigeria, mosquito resistance to pyrethroid insecticide and other common insecticides have been reported (Djouaka *et al.*, 2016; Chukwuekezie *et al.*, 2020; Ojianwuna *et al.,* 2021b). All efforts aimed at controlling the vectors and preventing malaria are still constrained by these predisposing factors. The information on insecticide resistance available in Nigeria is insufficient to support an evidence-based choice about national malaria control, despite the fact that some studies points to insecticide resistance as a key obstacle to the global effort to eliminate malaria (Awolola *et al.,* 2018; NMEP, 2020). Delta State of Nigeria is endemic to malaria with reported prevalence of above 30.0% (Odoko *et al.*, 2020). Baseline entomological studies are important in mapping out malaria endemic areas to enhance the control of malaria parasite vectors in Nigeria. Studies on species composition and abundance, biting dynamics, spatial distribution and other biological parameters of the mosquitoes are well documented in Southern Nigeria especially most malaria endemic areas of Delta State. Given the high prevalence of malaria in endemic areas, not much attention has been given to the vector causing the disease in terms of molecular identification. More importantly, the unavailability of correct information on vector dynamics presents difficulty in designing vector control program and disease eradication. Furthermore, a control strategy involving indoor-based methods is not sufficient to eliminate malaria transmission in most endemic areas due to changes in anthropogenic activities and temperature gradients. This is because most of residents tend to remain outdoors for longer periods of time and are often bitten by mosquitoes before sheltering indoors. Considering the need of information on species abundance and sporozoite load in *Anopheles* mosquitoes, this study was designed to determine the molecular characterization of *Anopheles coluzzii* and potentiality of disease transmission in different communities in Ughelli North LGA, Delta State, Nigeria. This was with the specific objective of determining the species of mosquitoes, seasonal abundance, biting time of the mosquitoes both indoor and outdoor, and wing variations.

MATERIALS AND METHODS

Study Area: This study was conducted at four communities in Orogun clan in Ughelli North Local Government Area of Delta State. The studied communities comprised of Ugono (5.4029^{0}N, 6.0646^{0}E), Orhomuru (5.3935^{0}N, 6.0953^{0}E), Emonu (5.3901^{0}N, 6.1719^{0}E), and Ovara-Umusu (5.3948^{0}N, 6.0847^{0}E) which are the four selected villages in Orogun Clan (Figure 1).

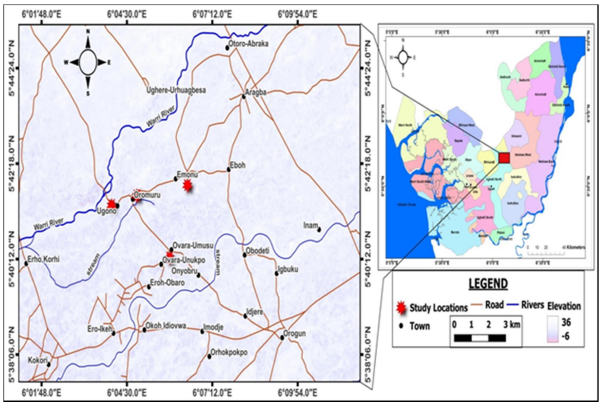

Figure 1: Map of Orogun, Ughelli North Local Government Area showing the sampled villages

The communities have a scattered settlement surrounded by bushes, forest trees, swamps and rivers. The residents' main occupations are farming and fishing. Most houses are built with low thatched roof, the eaves of most houses are open and the windows are without net which facilitate the entrance and exit of mosquitoes. It is a practice by the residents to store water in containers for various domestic activities which sometimes become breeding sites for mosquitoes. Stagnant water in gutters also serves as breeding habitat in the study area.

Study Design: Stratified sampling was used in community selection, while simple random sampling techniques was applied in house selection based on house condition whether they were netted or not, with eaves or not and other characteristics such as presence of breeding sites. The study spanned for 10 months between May 2022 and February 2023. The study spanned six months during the wet season (May 2022 – October 2022) and four months during the dry season (November 2022 – February 2023). Environmental parameters such as temperature, rainfall and humidity were recorded throughout the months of the study.

Mosquito Sampling Techniques: Mosquitoes were collected on once a week for each location over the night with strict adherence to these time ranges; 6 – 7 pm, 7 – 8 pm, 8 – 9 pm, 9 – 10 pm, 10 – 11 pm, 11 pm – 12 am, 12 – 1 am, 1 – 2 am, 2 – 3 am, 3 – 4 am, 4 – 5 am and 5 – 6 am. The collection was done using the CDC light trap, one positioned indoor and the other outdoor. The devices were set up at a height of about 1.5 metres above the human bait under a non-treated bed net. The light trap was thoughtfully positioned at the end were the human bait's head were. These light traps were set up 6 pm after sunset and were retrieved 6 am before sunrise the following day. The light trap cups were emptied at the end of each hour; environmental variables (temperature, relative humidity and rainfall) were recorded. Collected mosquitoes were kept in well labeled Petri dishes half-filled with self-indicating silica gel, according to where and when they were collected. Finally, collected mosquitoes were taken to the laboratory for counting and further morphological identification.

Mosquito Sorting, Morphological Identification and Preservation: Mosquitoes sampled from the various communities were taken to the Laboratory of the Department of Animal and Environmental Biology, Delta State University, Abraka, Nigeria for sorting. Male mosquitoes were sorted from females, before they were further identified morphologically using dissecting microscope following the taxonomic keys of Gillies and Coetzee (1987) and Coetzee (2020). The morphological identification of different species of female *Anopheles* mosquitoes was done with reference to the bands on the palps at the head region, the presence or absence of spots on the wings, and the legs. Individual mosquitoes were preserved in Eppendorf tubes (1.5 ml) stuffed with silica gel for molecular identification.

Environmental Variables: Thermo hygrometer was used to take the record of temperature and relative humidity in both indoor and outdoor where traps were set. Readings were taken immediately after five minutes of acclimatization with the environment. Rainfall data was gotten from Nigerian Meteorological Agency for Orogun communities.

Biting Time, Peak and Seasonality: This was determined in line with the time of sampling and determination of the abundance of mosquitoes encountered for outdoor and indoor. The biting peak was determined using the formula:

$$\text{Biting peak} = \frac{Abundance\ of\ mosquitoes}{Number\ of\ months\ for\ sampling}$$

Seasonality of mosquito was determined using the method of Roca-Feltrer *et al.* (2009).

Wing Length: The wings of the sampled mosquitoes were measured in the course of the study. Wing length was taken using meter rule positioned under the electrified light microscope.

Feeding Condition, Gravidity and Sporozoite Load: The feeding condition of morphologically

identified mosquitoes; fed, half-fed, or unfed as well as their gravidity of whether they were gravid or half gravid was determined using the appearance of the abdomen as reference point. Fully fed mosquitoes have their abdomen fully expanded antero-posteriorly, half-fed will have only their anterior part of abdomen slightly expanded. Gravid mosquitoes are characterized by milky appearance of the abdomen while half gravid has the posterior to mid-abdomen milky. Sporozoite Load was determined using a modified method of Ponnudurai *et al.* (1989).

Molecular Identification

DNA extraction: Mosquito DNA extraction was performed according to Genomic DNA extraction protocol (Nieman *et al.*, 2015). Prior to the extraction, abdomens of preserved mosquitoes were dissected to avoid blood contamination. The individual dissected mosquitoes were placed in a well labeled microcentrifuge tubes (UN1-50) and 500 µl of Lysis Buffer were added to each tube. The mosquitoes were crushed using mortar and pestle. The contents of each tube were mixed using Vortex and incubated at temperature of 56^0C for ten minutes. Thereafter, it was centrifuged at 10,000 rpm for a few seconds to spin down the content without compacting it. After spinning, 200 µl of absolute ethanol were added to each tube. The mixture was transferred into a spin column, mixed well by inversion and centrifuged at 10,000 rpm for 30 seconds. After spinning, the flow-through was discarded and the collection tubes were blotted neatly on a tissue paper. A further 500 µl of wash buffer 1 was added to the spin column and again centrifuged at 10,000 rpm for 30 seconds and after which the flow-through were discarded and collection tubes were blotted on a tissue paper. 500 µl of wash buffer 2 was added to the spin column, and then centrifuged at 10,000 rpm for 1 minute. Flow-through was discarded and collection tubes were blotted neatly on a tissue paper. Spin column as further centrifuged at 13,000 rpm for 3 minutes to remove all traces of ethanol. The spin column was placed into another microcentrifuge tube and 50 µl Elution Buffer was added to the centre of the column. The content was allowed to incubate at room temperature for 2 minutes then centrifuge at 10,000 rpm for 1 minute to elude the DNA (Figure 2). The resulted DNA is stored at -20^0C for Polymerase Chain Reaction (PCR) amplification.

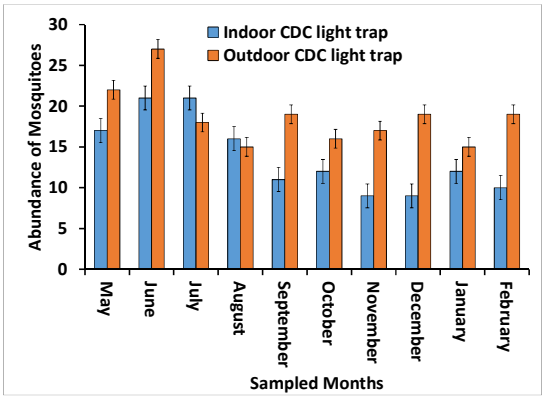

Figure 2: Monthly variation in mosquitoes sampled indoor and outdoor in Ughelli North LGA, Delta State. ***Key:*** *(F (Traps) = 13.10, df = 1, p = 0.0056; F (months) = 2.78, df = 9, p = 0.072)*

PCR amplification: Protocol provided by Wilkins *et al.* (2006) was used during the amplification of extracted DNA for PCR. The DNA cocktail used for species identification contain specific species primers for *Anopheles merus* (5-CAACCCACTCCCTTGACGATG-3), *Anopheles gambiae* (5-GCTTACTGGTTTGGT CGGCATTG-3), *Anopheles arabiensis* (5-GTGTTAAGTGTCCTTCTCCGTC-3) and *An. quadriannulatus* (5-GCATGTCCAAGATGGTTCG CTG3), *An. coluzzii* (M form; 5TAGCCAGCTCT TGTCCACTAGTTTT-3) *Anopheles sensu stricto* (S form; 5-CCAGACCAAGATGGTTCGCTG-3). The prepared ready to load master mix contained 2.5 µl pre-mix, 0.5 µl of IMP-UN, AR-3T, QD-3T GA-3T, ME-3T, IMP-S1 and IMP-M1 specific primers, 5.5 µl ddH^0CO for both forward and reverse reaction respectively. Prepared PCR master mix of 12.5 µl was added into each 200 µl tube thereby individual extracted DNA template (1 µl) was added to each tube. The amplicons of the PCR products undergo initial denaturation at 95^0C for 5 minutes (1 cycle), denaturation 95^0C for 30 seconds, annealing at 59.2^0C for 30 seconds followed by extension at 72^0C for 30 seconds (30 cycles), final extension at 72^0C for 5 minutes (1 cycle) and final hold at 4^0C.

Sporozoite PCR amplification: The DNA cocktail used for sporozoites identification contain specific *Plasmodium* primers for *P. falciparum* (5-CAAATGTAGCATAAAAATCCAAG-3) and *P. vivax* (5-CTGATTTTCCGCGTAACAATG-3) (Tham *et al.*, 1999). The DNA amplification was done by adding; 7.5 µl of ddH_2O, 2.5 pre-mix with BSA, 25 pmol/l of Pvr47, 25 pmol/l of pvr47R, 25 pmol/l of pfr364F, 25 pmol/l of pfr364R was added to the PCR tubes respectively. 1.0 µl of the extracted DNA was then added to each of the tubes and was properly vortex and allowed to cool before transferring to the PCR machine (Themocycler). The tubes were arranged carefully on the machine and were set to undergo initial denaturation at 95^0C for 5 minutes. At the end of the initial denaturation, it was programmed again for main denaturation for 95^0C for 2 minutes then allowed to cool. After cooling, the content was subjected to annealing temperature at 56^0C for 30 seconds to enable the primers bind to the flank region of the DNA. All samples in the PCR tubes were further subjected to extension at 72^0C for 5 minutes and then hold for 4^0C (Demas *et al.*, 2011).

Gel electrophoresis: 1.5 g of agarose gel with 100 ml of ethylenediaminetetraacetic acid (EDTA) was used in electrophoresed of the PCR product. The agarose was melted in a microwave for about 2 minutes until it is completely dissolved and allowed to cool sufficiently. The gel was stained with 5 µl ethidium bromide (visualizing dye) for the visualization and detection of amplified DNA fragment. After cooling to the point of not too hot to touch, the gel was poured into a clean well casting chamber and clean electrophoresis comb had been inserted as appropriate to create wells into which amplicons were loaded. The cast was placed in the electrophoresis tank containing 1X TAE (Tris base, acetic acid and EDTA) buffer to cover the gel and wells follows by the gentle removal of the comb from the well in a way to avoid cracking of wells or gel. Molecular ladder (5 µ) was carefully dispensed into the first well of the gel or any designated well for ladder. Follow by 7.5 µl of each amplicon (DNA) were appropriately loaded into corresponding wells. The electrophoresis tank covered with its lid and the tank cables were appropriately connected to the electric source and set to run at 80v with 150 mA for an hour. Afterward, the gel was viewed to check for the bands and taken under UV transilluminator for documentation.

Data Analysis: Data obtained for the study were entered in Microsoft Excel Spread Sheet (2013 version). Two-way Analysis of Variance (ANOVA) test was done to ascertain the level of significance within and between the environmental variables, mosquitoes collected indoor and outdoor of houses with month. Canonical correlation analysis (CCoA) was done to check foe correspondence between environmental variables and mosquito abundance. Spatio-temporal variation of mosquito abundance and environmental variables were presented using line graphs, bar charts, mean and standard error in tables.

RESULTS

Abundance of Mosquitoes: All the mosquitoes sampled from this study were morphologically identified as *An. gambiae* and were molecularly confirmed as *An. coluzzii*. A total catch of 329 mosquitoes was recorded in Ughelli North LGA, Delta State (May 2022 – February 2023). The majority of which was recorded in June as shown in Table 1. More mosquitoes were collected in the wet months. The total catch was lowest in November. In this study, mosquitoes were collected more outdoor (187) than indoor (138). Majority of the mosquitoes indoor was collected in June and July (5.25 ± 0.48 respectively), and outdoor in June (6.75 ± 0.48). The mean abundance of mosquitoes indoor and outdoor were not significantly different within the sampled months ($p>0.05$) (Table 1). However, the difference between mosquitoes sampled with months was significant ($F_{(months)} = 2.78$, df = 9, $p = 0.072$). The lowest mosquito abundance indoor was collected in November and December, while mosquito abundance outdoor was recorded in August and January (Figure 2). The difference between the abundance of mosquitoes indoor and outdoor was significant ($F_{(Traps)} = 13.10$, df = 1, $p = 0.0056$).

Table 1: Seasonal and monthly abundance of anopheline mosquitoes in Ughelli North LGA, Delta State (May 2022 – February 2023)

Seasons	Months	Total catch	Mean catch	
			Indoor	Outdoor
Wet	May	40	4.25 ± 0.25^{bcd} (0 – 5)	5.50 ± 0.65^{bc} (0 – 7)
	June	48	5.25 ± 0.48^{cd} (0 – 6)	6.75 ± 0.48^{c} (0 – 8)
	July	38	5.25 ± 0.48^{cd} (0 – 6)	4.50 ± 0.29^{ab} (0 – 5)
	August	31	4.00 ± 0.58^{abc} (0 – 5)	3.75 ± 0.48^{a} (0 – 5)
	September	29	2.75 ± 0.25^{a} (0 – 3)	4.75 ± 0.48^{ab} (0 – 6)
Subtotal		186	4.30 ± 0.41 (0 – 6)	5.05 ± 0.48* (0 – 7)
Dry	October	28	3.00 ± 0.41^{ab} (0 – 4)	4.00 ± 0.58^{a} (0 – 5)
	November	26	2.25 ± 0.25^{a} (0 – 3)	4.25 ± 0.48^{ab} (0 – 5)
	December	28	2.25 ± 0.25^{a} (0 – 3)	4.75 ± 0.25^{ab} (0 – 5)
	January	27	3.00 ± 0.41^{ab} (0 – 4)	3.75 ± 0.48^{a} (0 – 5)
	February	34	2.50 ± 0.29^{a} (0 – 3)	4.75 ± 0.25^{ab} (0 – 5)
Subtotal		143	2.60 ± 0.32 (0 – 4)	4.30 ± 0.41* (0 – 5)
Total		329	3.45 ± 0.37	4.68 ± 0.45

Note: *values separated with ± are means and standard error of mosquito collections. Values in parentheses are ranges of mosquito. Asterisk (*) on the highest subtotal indicates the significant difference in the seasonal abundance. Mean values with different superscript letters along the columns differ significantly*

Morphological and Molecular Identity of Mosquito: The Anopheles mosquitoes sampled from Ughelli North LGA were identified morphologically as *An. gambiae*. Molecular identification using PCR assured *An. gambiae* s.l. at 463 bp; however, PCR to species level confirmed it as *An. coluzzii* with bands at 333 bp as shown in Plate 1 and this was higher in June compared to other months (Figure 2). Although, the difference between the mosquito species across months was not significant ($F_{(months)}$ = 0.71, df = 9, p = 0.695). Also, sporozoite PCR Amplification indicated that there were no sporozoites found in the sampled mosquitoes as shown in Plate 2.

Biting Time, Peak and Seasonality: Mosquito abundance was highest in 8 – 9 pm collection time. This is closely followed by collections between 3 and 4 am, and 4 and 5 am. There was no mosquito collected in this study between 6 and 7 pm. This trend was observed in the biting peak of mosquitoes shown in Figure 3.

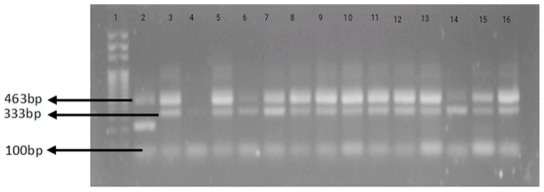

Plate 1: Showing a molecular result of the sampled mosquitoes. ***Key:*** *Lane 1 = 100bp. DNA ladder lane 2 = positive control. Lane 3 – 16 = DNA of pooled samples positive for An. coluzzii (M form)*

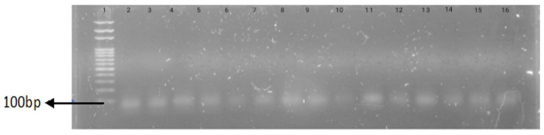

Plate 2: Showing a sporozoite result of the sampled mosquitoes

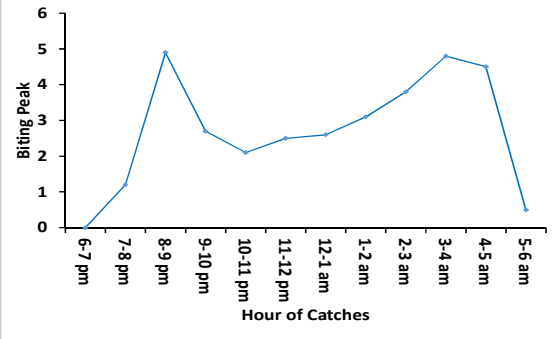

Figure 3: Biting peak of *An. coluzzii* mosquitoes in Ughelli North LGA, Delta State, Nigeria

More mosquitoes were collected outdoor in this study and was observed from 8 – 9 pm to 4 – 5 am (Figure 4).

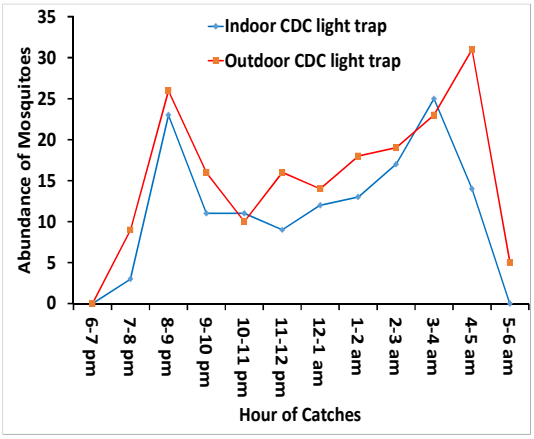

Figure 4: Abundance of *An. coluzzii mosquitoes* indoor and outdoor in relation to biting time in Ughelli North LGA, Delta State, Nigeria. *Key:* *($F_{(biting\ time)}$ = 10.43, p = 0.0003; $F_{(trap)}$ =8.12, p = 0.0016)*

Two peaks were observed in the outdoor and indoor collection, it was recorded at 8 – 9 pm respectively, 3 – 4 am for indoor and 4 – 5 am for outdoor mosquitoes. This followed a regular M- pattern throughout the night. The differences between the abundance of mosquitoes with biting time and CDC light traps were significant ($F_{(biting\ time)}$ = 10.43, p = 0.0003; $F_{(trap)}$ = 8.12, p = 0.0016).

Seasonal abundance of mosquitoes in Ughelli North LGA, Delta State is shown in Figure 5.

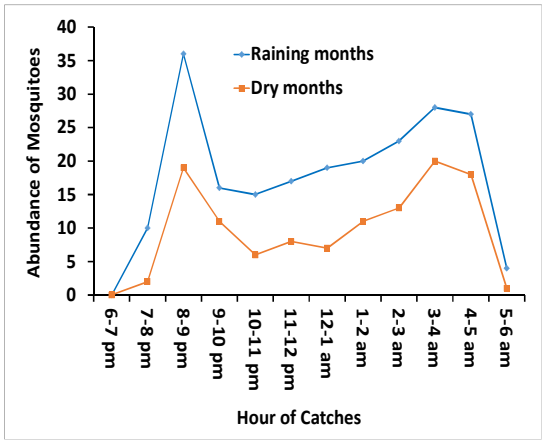

Figure 5: Abundance of *An. coluzzii mosquitoes* in relation to seasons in Ughelli North LGA, Delta State, Nigeria. *Key:* *($F_{(seasons)}$= 9.70, p = 0.0002; $F_{(biting\ time)}$=8.30, p = 0.0007)*

There were more mosquitoes recorded in the wet season than in dry season with time of collection. The difference was significant ($F_{(seasons)}$ = 9.70, p = 0.0002).

Monthly Variation in Environmental Condition: Mean monthly variations in temperature and relative humidity indoor and outdoor in Ughelli North, Delta State, Nigeria are shown in Table 2. Temperature and relative humidity recorded in this study are within the ranges for mosquito survival. The highest temperature indoor and outdoor of sampled houses was recorded in January. The lowest temperature indoor and outdoor was recorded in August and July respectively. The highest relative humidity indoor and outdoor of houses was recorded in August and June respectively. Furthermore, relative humidity indoor and outdoor was lowest in December and February respectively. The highest and lowest rainfall in the sampled location was recorded in September and January respectively. The differences in the environmental variables were significant across months ($p<0.05$).

Correlation Analysis: Canonical correlation analysis of *An. coluzzii mosquito* abundance with sampled months and environmental variables in Ughelli North LGA, Delta State is shown in Figure 6. The environmental variables are correlated except for relative humidity indoor.

Table 2: Mean monthly variations in temperature and relative humidity indoor and outdoor in Ughelli North, Delta State, Nigeria

Months	Temperature (^{0}C)		Relative humidity (%)		Rainfall (mm)
	Indoor	Outdoor	Indoor	Outdoor	
May	24.65 ± 0.45^{ab} (23.0-27.5)	29.43 ± 0.27^{bc} (28.7-30.0)	79.27 ± 5.83^{c} (48.0-95.0)	84.50 ± 0.29^{de} (82.0-89.0)	14.97 ± 0.13^{b} (14.6-15.24)
June	24.98 ± 0.44^{ab} (22.8-27.3)	27.27 ± 0.69^{ab} (26.6-27.8)	82.29 ± 2.82^{d} (66.3-95.0)	87.25 ± 0.63^{ef} (86.0-89.0)	21.24 ± 0.19^{c} (20.7-21.6)
July	25.02 ± 0.42^{ab} (23.0-27.3)	25.96 ± 0.23^{a} (25.3-26.4)	75.46 ± 4.48^{ab} (47.5-95.0)	86.75 ± 0.75^{ef} (85.0-88.0)	26.79 ± 0.24^{d} (26.1-27.3)
August	23.48 ± 0.20^{a} (22.5-24.8)	26.68 ± 0.24^{a} (26.0- 27.2)	93.5 ± 1.01^{f} (86.8-97.3)	80.00 ± 3.08^{d} (75.0-89.0)	1.51 ± 0.02^{a} (1.47-1.54)
September	25.00 ± 0.29^{ab} (23.5 – 26.3)	26.79 ± 2.24^{a} (26.1-27.3)	85.02 ± 3.14^{de} (62.0-95.5)	85.75 ± 1.49^{de} (82.0-89.0)	37.71 ± 0.34^{f} (36.8-38.4)
October	25.00 ± 0.26^{ab} (23.3 – 26.0)	27.38 ± 0.55^{ab} (26.2-28.9)	84.46 ± 3.45^{de} (63.5-96.0)	86.00 ± 1.87^{de} (81.0-90.0)	30.85 ± 0.28^{de} (30.1- 31.4)
November	24.88 ± 0.56^{ab} (23.0-29.5)	29.56 ± 0.27^{bc} (28.8-30.1)	76.60 ± 5.55^{bc} (39.8-94.5)	81.75 ± 2.14^{d} (76.0-85.0)	14.08 ± 0.13^{b} (13.7-14.3)
December	25.23 ± 0.68^{ab} (23.3-31.3)	31.75 ± 0.28^{cd} (31.0-32.3)	74.23 ± 6.32^{a} (33.5-94.5)	74.25 ± 2.66^{c} (68.0-80.0)	2.51 ± 0.12^{a} (2.16-2.68)
January	25.56 ± 0.35^{ab} (23.8-27.3)	32.45 ± 0.29^{cd} (31.6-33.0)	75.21 ± 5.01^{ab} (45.0-93.8)	69.50 ± 3.23^{ab} (64.0-78.0)	0.27 ± 0.00^{a} (0.26-0.28)
February	25.15 ± 0.64^{ab} (23.3-31.0)	32.24 ± 0.29^{cd} (31.4-32.8)	74.48 ± 6.16^{a} (33.5-94.3)	67.75 ± 1.89^{a} (63.0-72.0)	2.06 ± 0.02^{a} (2.01-2.10)

Note: *Mean values with different superscript letters along the columns differ significantly*

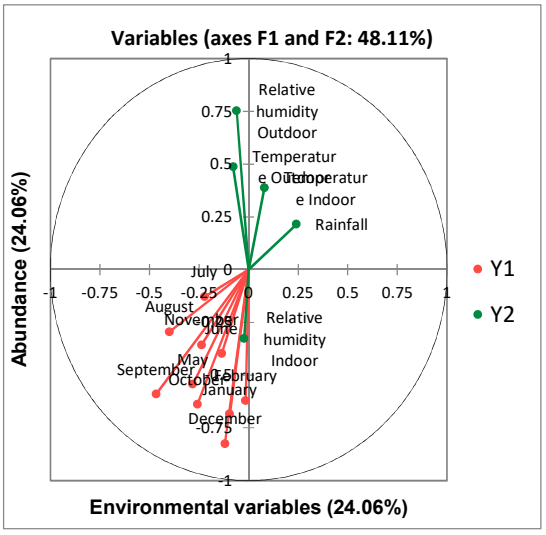

Figure 6: Canonical correlation analysis of *An. coluzzii mosquito* abundance with sampled months and environmental variables in Ughelli North LGA, Delta State, Nigeria. ***Key:*** *Y1 = Sampled months, Y2 = Environmental variables*

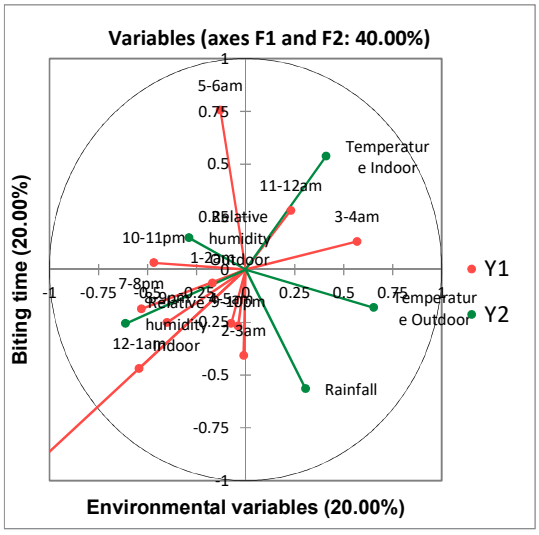

Figure 7: Canonical correlation analysis of *An. coluzzii mosquito* abundance with sampled time and environmental variables in Ughelli North LGA, Delta State, Nigeria. ***Key:*** *Y1 = Sampled time, Y2 = Environmental variable*

It appears that relative humidity indoor influences the monthly abundance of *Anopheles* mosquitoes in the study. The Eigen values show that temperature and relative humidity indoor and outdoor of houses contributed 24% respectively of the variability to the abundance of mosquitoes while rainfall contributed only 3.8%. Mosquito abundance in the sampled months was positively correlated with relative humidity indoor.

Canonical correlation analysis of *An. coluzzii mosquito* abundance with sampled time and environmental variables in Ughelli North LGA, Delta State is shown in Figure 7. The environmental variables were correlated. The Eigen values show that the environmental variables contributed 20% respectively of the variability to the abundance of mosquitoes. Mosquito abundance in 9 pm – 12 am, and 2 – 6 am was positively correlated with temperature indoor while 7 – 9 pm and 12 – 2 am was negatively correlated. Temperature outdoor was negatively correlated to mosquito abundance in 7 – 9 pm, 10 pm – 2 am and 4 – 6 am.

It was positively correlated with mosquito abundance in 9 – 10 pm, and 2 – 4 am. Relative humidity indoor was positively correlated with mosquito abundance in 7 – 9 pm, 12 – 2 am, and 5 – 6 am. Similarly, relative humidity outdoor was positively correlated with mosquito abundance in 7 – 9 pm, 10 – 2 am and 4 – 6 am. The mosquito abundance recorded in other hours was negatively correlated with relative humidity indoor and outdoor. Rainfall was positively correlated with mosquito abundance except in 8 – 9 pm and 5 – 6 am.

Feeding Condition and Sporozoite Load in Mosquitoes: The feeding characteristics of mosquitoes collected from Ughelli North, Delta State is shown in Table 3. Unfed mosquitoes were more in this study (≈80%) than fed mosquitoes. No blood fed mosquitoes were recorded (0%). The 100 ELISA tested mosquitoes showed no sporozoite in them.

Wing Length: It was observed that the mean wing length of *An. coluzzii* obtained outdoor in this present study were significantly ($p<0.05$) longer than the mosquitoes obtained indoor (Figure 8).

DISCUSSION

Adequate entomological surveillance is required to understanding the vector biting dynamics of the malaria vector, their composition and behavior which will inform the design of proper intervention and management policies against the diseases caused by these vectors. In this regard, this study evaluated some entomological indices of malaria vectors in Ughelli North, Delta State, Nigeria.

Table 3: Feeding conditions and gravidity of *An. coluzzii* mosquitoes from Ughelli North, Delta State, Nigeria

Mosquito Species	Seasons	Feeding conditions (%)	
		Fed	Unfed
Anopheles	Wet	0 (0.00)	187 (56.84)
	Dry	0 (0.00)	93 (28.27)
	Total	0 (0.00)	264 (80.24)
		Gravidity (%)	
		Gravid	Half Gravid
	Wet	16 (4.86)	10 (3.04)
	Dry	9 (2.74)	10 (3.04)
	Total	25 (7.60)	20 (6.08)

F-ANOVA = 116.9, p<0.0001

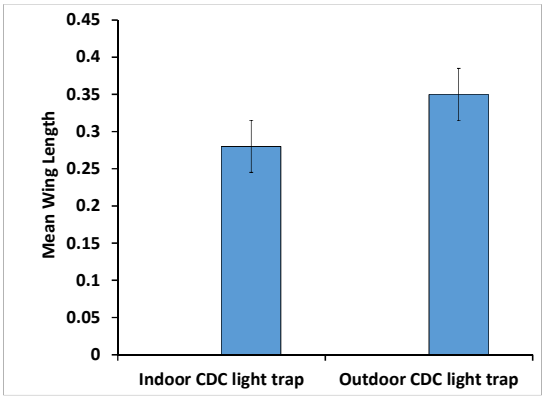

Figure 8: Mean of wing length of *An. coluzzii mosquito*es collected indoor and outdoor of Ughelli North LGA, Delta State, Nigeria

Considering the information on literature, some studies have reported the abundance and distribution of mosquitoes in Delta State, Nigeria (Onodua *et al.*, 2020; Ojianwuna *et al.*, 2021a, b; Ojianwuna and Enwemiwe, 2022). Hence, this study wishes to provide more in-depth information on indoor resting species. In this study, only *An. gambiae,* subspecies *An. coluzzii* was the anopheline mosquito recorded. The abundance of *An. gambiae* complex across the study areas agrees with previous studies in Taraba, Kwara and Cross River States (Oduola

et al., 2012; Lamidi *et al.,* 2017; Akpan *et al.,* 2018). Another interesting study by Lamidi *et al.* (2017) recorded more species of *Anopheles* including *An. coluzzii*, *An. gambiae*, hybrid species and *An. arabiensis* in molecular determined species. In characterizing the genus *Anopheles,* twenty-eight complexes are known including *An. albitarsis, An. benarrochi, An. crucians, An. konderi, An. cruzii, An. oswaldoi* and *An. nuneztovari* complexes endemic in American countries, *An. annularis*, *An. barbirostris*, *An. culicifacies, An. dirus, An. fluviatilis, An. gigas, An. leucosphyrus, An. lindesayi, An. minimus, An. nivipes, An. subpictus, An. sundaicus, An. superpictus* and *An. stenphensi* endemic in Asian countries, *An. annulipes, An. farauti* and *An. lungae* complexes are endemic in Asian-Pacific countries and *An. claviger* is endemic in Europe, while *An. nili*, *An. funestus* and *An. gambiae* are endemic in Africa (Dev and Manguin, 2020). More species were reported in most study including a Bangladesh study by Bashar and Tuno (2014) that reported over 2400 individual mosquitoes belonging to twenty-two mosquito species. Other unidentified species collected and irrelevant to this present study were excluded. Sometimes, *Culex* and other insects collected by CDC light trap may outnumber *Anopheles* mosquitoes in same collection as observed by Degefa *et al.* (2017) and a study in Southwest Nigeria by Okorie *et al.* (2014) where *Culex* species was much more abundant than *Anopheles* species. Similar trend was observed by Simon-Oke and Olofintoye (2015) in Ekiti State, Nigeria. In this present study, 329 mosquitoes were recorded using the carbon (IV) oxide-baited CDC light trap. This was considered low and it may be linked to the low number of traps used in mosquito trapping, personal mosquito control approaches adopted by resident or the coverage of the trap. The total catches recorded in this study were lower than those reported in several studies such as Bashar and Tuno (2014), Lamidi *et al.* (2017) and Ojianwuna *et al.* (2021b). These differences in the catches may be due to the trapping method applied, for this study CDC light trapping was used and in most other studies pyrethrium spray catches (PSC), human landing catches and larval collection and rearing to adult were adopted. The number of species recorded in this study did not compare favourably to the study of Bedasso *et al.* (2022) as more species were collected and this could be linked to the differences in geographical setting, and human activities can affect the species abundance and composition. The chances of getting *An. gambiae s.l.* in Nigeria as one of the primary vector have been established in literature (Lamidi, 2009; Lamidi *et al.,* 2017).

In this present study, more *An. gambiae s.l.* mosquitoes were encountered in May and June, and this coincides with the wet months. It was confirmed by Lamidi (2009) that seasonality can influence the appearance or disappearance of species in a location. For instance, *An. gambiae* were observed to appear at the onset of rain, *An. funestus* at the termination of rain and *An. arabiensis* in dry period. This was not the case for this present study as only one species was encountered in the ten-month survey. Lamidi (2009) confirmed that complementation effect of these vectors in sustaining malaria spread is possible. Lowest catches of mosquitoes in this study was made in November, the onset of dry period. This contradicts the study of Bashar and Tuno (2014) that observed a decline in mosquito abundance between the onset of the wet season and August break and an upsurge in September. With regards to month, majority of the mosquitoes indoor were collected in June and July and outdoor in June.

The highest abundance of mosquito in this study was collected between 8 and 9 pm, when the activities of locating and entering the houses were high. The mosquito abundance was equally high between 3 and 4 am, and 4 and 5 am. There was no mosquito collected in this study between 6 and 7 pm (both indoor and outdoor). This may be because as the night fall commenced around this time and day was still bright, mosquito species may not have come out of their hide outs. The observation in this study showed that mosquitoes collection peaked twice both for indoor and outdoor at 8 – 9 pm and this may be because residents were retiring to their bed as well as more social activities happening outdoor. Again, mosquito biting peaked at 4 – 5 am and this may be because

most residents wake up early to start their day. Hence, the mosquitoes may have adapted behaviourally to suite this period. This was in agreement with the findings of Reddy *et al.* (2011) in Equatorial Guinea, Cooke *et al.* (2015) in Kenya and Abong'o *et al.* (2021) in Western Kenya where CDC light-traps were used as sampling tool for replacement of human landing catches in outdoor host-seeking preferences and densities.

Considering seasonality, there were more mosquitoes in the wet season than in dry season, this may be as a result of gutters, puddles and pot holes are covered with water serving as breeding sites for the mosquitoes. This finding was not in agreement with the findings of Animut and Negash (2018) in West Gojjham zone, Ethiopia that reported high proportion of *Anopheles* mosquitoes during the dry season due to availability of streams, ponds and swamps, and high population of indoor resting ones. The environment has a key impact in defining the *Anopheles* species in the region. Therefore, the temperature, relative humidity and rainfall recorded in this study were within the ranges for mosquito survival and abundance. The finding of this study corresponded to the observation made by Ojianwuna *et al.* (2021a). The evening temperatures in the locations of this study were usually cool outdoor and warm indoor. This observation may explain why mosquitoes in this study seek warmth indoor as well as blood meal from their host. The highest temperatures indoor and outdoor of sampled houses were recorded in January. It was expected because of the low rainfall during this period. The lowest temperature indoor and outdoor was recorded in August and July respectively. The highest relative humidity indoor and outdoor of houses was recorded in August and June respectively. Furthermore, relative humidity indoor and outdoor was lowest in December and February respectively. The highest and lowest rainfall in the sampled location was recorded in September and January respectively. Rainfall was a significant environmental factor in determining species abundance in this present study. In this present study, rainfall was positively correlated with mosquito abundance except in 8 – 9 pm and 5 – 6 am. This predicts that the onset of rain at the early hours of the night and the terminating time of collection may influence abundance of species. However, water source for mosquito breeding comes from rainfall and the bulk of species come from outdoor. This observation is in consonance with the study of Bashar and Tuno (2014) that ascribed the decline in rainfall to the reason that threatens the natural breeding habitat. In this study, mosquito abundance increased as rainfall increased and this may be linked to increase in malaria incidence which is not in accordance with the findings of Briët *et al.* (2008) which noted that malaria incidence would increase as rainfall decreases.

The environmental condition in this study was different within the month. There was a strong correlation between the environmental variables recorded indoor and outdoor in this study except for relative humidity indoor. It appears that relative humidity indoor influences the monthly abundance of Anopheles mosquitoes in the study. The Eigen values of the correspondence analysis reveal that temperature and relative humidity indoor and outdoor of houses contributed 24% respectively of the variability to the abundance of mosquitoes while rainfall contributed only 3.8%. Relative humidity indoor was a positively factor that correlated with mosquito abundance in the sampled months. This observation corroborated the study of Bashar and Tuno (2014) that revealed that relative humidity was a significant determinant of species density. Monitoring the influence of environmental condition on mosquito abundance within a short time is quite difficult. It is important to know the possible time mosquito abundance will be high within the nights. This will help in the control of mosquito activities. The environmental variables in this present study were correlated. The Eigen values of the correspondence analysis show that the environmental variables contributed 20% respectively to the variability of mosquito abundance. This study showed that the temperature indoor of houses sampled was positively correlated with the mosquito abundance throughout the night except for the cold hours of the morning from 12 – 2 am and

cold evenings 7 – 9 pm. It was observed in the cool evenings that mosquitoes try to gain access into the houses through cracks, crevices, eaves and torn windows nets and open doors.

Outdoor temperature was negatively correlated to mosquito abundance between 7 – 9 pm, 10 – 2 am and 4 – 6 am. It was positively correlated with mosquito abundance between 9 – 10 pm and 2 – 4 am. Indoor relative humidity was positively correlated with mosquito abundance between 7 – 9 pm, 12 – 2 am and 5 – 6 am. Similarly, outdoor relative humidity was positively correlated with mosquito abundance between 7 – 9 pm, 10 – 2 am and 4 – 6 am. The mosquito abundance recorded in other hours was negatively correlated with indoor and outdoor relative humidity. Rainfall was positively correlated with mosquito abundance except in 8 – 9 pm and 5 – 6 am. The feeding characteristics of *Anopheles* mosquitoes collected from this study showed that the unfed mosquitoes were more (≈80%) than the fed mosquitoes. No blood fed mosquitoes were recorded (0%). This finding was consistent with the findings of Fornadel *et al.* (2010) in Zambia, Bashar *et al.* (2012) in Bangladesh, Getachew *et al.* (2019) and Adugna *et al.* (2021) in Ethiopia, using CDC light trap. The reason for these females being unfed is not clear. However, this observation suggests a very low flight activity of fed mosquitoes. These unfed mosquitoes must have been parous females attracted and caught by light traps while searching for blood meals, or whether they are nulliparous (newly-emerged). A study in Tanzania reported that mosquito biting time was not influenced by parity status (Milali *et al.,* 2017). In this present study, mosquito activities were observed in all hours of the night and in early morning. This implies that protection against mosquitoes at all times is a relevant measure to preventing mosquito-borne disease transmission. These findings was in agreement with a recent epidemiological study in urban Dar es Salaam, Tanzania, where individuals who sleep in houses with complete window screening and under bed nets enjoyed a reduction in risk of contracting malaria if only their evenings and mornings were spent indoors (Msellemu *et al.,* 2016). The CDC-Light trapped mosquitoes in this present study were more unfed, gravid and half-gravid. None were blood fed. This could be because the light trap was designed to divert the attention of mosquitoes from humans towards the light trap due to the similar carbon dioxide gas and light they produce in dark rooms (Sriwichai *et al.*, 2015). The finding of this study is not in line with the study of Abdelwhab *et al.* (2021). This is probably due to the difference in the collection techniques adopted, in their study, pyrethrum spray catch was adopted which best explains why blood fed mosquitoes would be easily captured from resting wall surfaces. There was no record of sporozoite in the mosquitoes tested by ELISA in this study and this observation corroborates the finding of Elmahdi *et al.* (2012). In the present study, longer wing length were encountered in *An. coluzzii* mosquitoes outdoor compared to those collected indoors. This may be the reason why there were more species outdoor as they could be able to withstand wind pressure through the nights and colonize wider range of habitats outdoor compared to those obtained indoors. The study of Foley *et al.* (2020) observed that short-winged *Anopheles* mosquitoes were accustomed to warmer and humid environment, which may equally be the reason why longer-winged mosquitoes colonized cooler outdoor. Another study by Charlwood *et al.* (2003) opined that increased wing size benefits the search for mates than for feeding. This predicts that small-sized wing of mosquitoes in this study is an adaptation of indoor species to blood feed.

Conclusion: Adequate entomological surveillance is required to understanding the vector biting dynamics of the malaria vector, their composition, and behavior which could inform the design of proper intervention and management policies against the diseases caused by these vectors. In this study, only *An. coluzzii* was the *Anopheles* mosquito recorded. It was also observed that the biting peak was highest between 8 – 9 pm and equally between 3 – 5 am. Generally, most mosquitoes were collected within wet months than the dry months in order to lessen the prevalence of malaria in the study area, targeted control measures of *An. coluzzii*, a key vector that

transmits malaria parasites, should be directed outdoors before the onset of the wet season.

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