Feeding Behaviour of Mosquito Species in Mararraba-Akunza, Lafia Local Government Area, Nasarawa State, Nigeria

Ombugadu A1*, Ekawu RA2, Odey SA1, Igboanugo SI1, Luka J1, Ajah LJ1, Adejoh VA1, Micah EM1, Echor BO3, Samuel MD1, Dogo KS1, Ahmed HO3, Ayim JO1, Ewa PE1, Aimankhu OP1, Uzoigwe NR1, Mafuyai MJ4, Yina GI5, Pam DD5, Lapang MP5, Aliyu AA1, Ayuba SO1, Nkup CD6, Angbalaga GA7 and Mwansat GS5

1Department of Zoology, Faculty of Science, Federal University of Lafia, Nasarawa State, Nigeria.
2Department of Zoology, Faculty of Natural Sciences, Nasarawa State University, Keffi, Nigeria.
3Department of Science Laboratory Technology, Faculty of Natural Sciences, University of Jos, Plateau State, Nigeria.
4Department of Pest Management Technology, Forestry Research Institute of Nigeria, Federal College of Forestry, Jos, Plateau State, Nigeria.
5Department of Zoology, Faculty of Natural Sciences, University of Jos, Plateau State, Nigeria.
6Department of Biology, College of Arts, Science and Technology, Kurgwi, Qua’an Pan LGA, Plateau State, Nigeria.
7Department of Microbiology, Faculty of Science, Federal University of Lafia, Nasarawa State, Nigeria.

*Corresponding author: Ombugadu A, Department of Zoology, Faculty of Science, Federal University of Lafia, Nasarawa State, Nigeria, E-mail: akwash24@gmail.com

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Abbreviations: CDC: Centers for Disease Control; HLC: Human Landing Collection; CDC LT: CDC Light Trap

ABSTRACT

The knowledge on human-vectors contact is of great importance in order to tackle the point of transmission of mosquito-borne diseases through relevant control measures and interventions. To this end, a study on the feeding behaviour of mosquito species in Mararraba-Akunza, Lafia Local Government Area, Nasarawa State, Nigeria was carried out for 8 nights in May 2018 using modified CDC Light Traps set in indoor and outdoor points. The traps were suspended by the foot side of the bed having human bait laying under an untreated mosquito bed net. Mosquitoes were collected hourly between 6:00pm and 6:00am and transferred into well labelled paper cups. Hourly temperature and relative humidity were recorded using digital thermo-hygrometer. A total of 95 mosquitoes belonging to An. gambiae 49 (51.6%) > Culex quinquefasciatus 40 (42.1%) > Aedes aegypti 4 (4.2%) > Mansonia uniformis 2 (2.1%) were collected. The abundance of mosquitoes in relation to feeding points showed no significant difference (P > 0.5). Outdoor point had higher abundance of 52 (54.7%). The feeding behaviour in both points showed that mosquitoes feed most from 1:00 to 2:00am and decreased around 5:00 – 6:00am. The indoor and outdoor mosquitoes feeding time peaked at 9:00 – 10:00pm and 3:00 – 4:00am respectively. Temperature influenced indoor mosquitoes abundance while outdoor mosquitoes abundance was influenced by relative humidity. The indoors and outdoors man biting rate of mosquitoes per person per night was 5.0 and 5.9 respectively. Therefore, inhabitants of the area are encouraged to sleep under Long Lasting Insecticide Treated Nets in both points from dusk to dawn so as to prevent them from mosquitoes bites and consequently mosquito-borne diseases.
Introduction

Mosquitoes are considered as serious problem everywhere because they proliferate in large number WHO [1] and are resistant to insecticides (Badolo et al. [2]; Oyewole et al. [3]; Awolola et al. [4]; Mwansat et al. [5]; Oduola et al. [6]; Lynd et al. [7]; Sherrard-Smith et al. [8]) leading to successful transmissions of malaria which is the leading cause of morbidity and mortality in Nigeria (WHO [9]). The mosquitogenic conditions are promoted by the anthropogenic activities and the creation and existence of several habitats throughout the year in the urban areas. The problem is more severe in rural areas especially because these areas are associated with well irrigated agroecosystems (Emidi et al. [10]). In these areas, poor sanitation facilities, usual agricultural practices and lack of sanitation facilities enhance the diversity and density of the population of mosquitoes and vector borne diseases. To control these remarkably adapted mosquitoes is a serious problem and therefore the mosquitoes peacefully co-exist with man. Many researchers have attempted to study the impacts of microhabitats and the differences in the behavioural patterns of vector and non-vector species of mosquitoes (WHO [1]).

The resting and feeding behaviour of mosquito is of great importance with respect to the efficacy of control measures. Indoor-based methods of control, such as the use of insecticide-impregnated bed nets and house-spraying with residual insecticides, are highly effective against mosquito species which mainly feeds indoors on humans and rests there once fed. Residual transmission is encouraged through mosquitoes bites outdoor which is a determinant of the maximum effectiveness on current prevention of mosquito-borne diseases (Sherrard-Smith et al. [8]). The degree to which other species feeds on non-human hosts and rests outdoors reduces the efficacy of these control methods, but also offers opportunities for other approaches (Reisen et al. [11]). An annual estimation of 10.6 million malaria cases across Africa is predicted to result due to higher outdoor transmission if universal coverage of LLIN and IRS is achieved (Sherrard-Smith et al. [8]).

Sampling vector populations in relation to surveys and compilation of checklist of species present in an area; identification of those species which bite man and determination of those that are important or potential vectors of disease is very essential Service [12]. The different aspects of mosquitoes are studied by many workers under different situations. Traps are effective surveillance tools for monitoring prevalence and the species composition of mosquitoes, along with reducing mosquito numbers nearby Moore, et al. [13].

Consequently, Centers for Disease Control (CDC) light traps hung beside occupied beds protected by bed nets remain a preferred alternative to an indoor human landing collection (HLC) for collecting host-seeking vectors over a wide range of mosquito densities Becker, et al. [14]. The CDC light traps are affordable, easy to use, and have relatively high sampling efficiency, although the relationship between CDC trap and indoor HLC collections needs to be verified locally for each study area because vector species composition and intraspecific variation in feeding and resting behaviour can have a significant impact on the quantitative association between the two methods Moore, et al. [13]. Thus, this study investigated the concurrent feeding behaviour of mosquito species in Mararraba-Akunza, Lafia Local Government Area, Nasarawa State, Nigeria.

Materials And Methods

Study Area

This study was carried out in Mararraba-Akunza area (Figure 1) of Lafia Local Government Area of Nasarawa State, Nigeria. Mararraba-Akunza area is a peri-urban settlement where various agricultural to anthropogenic activities takes place. Lafia is a town in central Nigeria (Latitude 8°24’N, 9°1’E and Longitude 8°13’E, 9°8’N). It has a total landmass of about 2797.53km² Lafia L.G.A shares boundary with Plateau State in the north east, Obi and Doma L.G.A in the South, Nasarawa Eggon in the West and Wamba LGA in the North. Nasarawa State is located in the North Central region of Nigeria with a land area of 27,137.8square kilometers. The main economic activities of the State is agriculture: cash crops such as yam, cassava and melon are grown Agidi, et al. [15]. Lafia L. G. A. has a tropical sub-humid climate, with two distinct seasons which are wet season and dry season. The wet season lasts for seven months which is between April and October, while the dry season is between November and March. Rainfall is moderately high in Lafia, ranging from 1200mm to 1600mm. Average maximum and minimum daily temperatures are 35 °C and 21 °C in rainy season and 37 °C and 16 °C in dry season respectively Agidi, et al. [13].

Sample Collection

Mosquito samples were collected in accordance to the standard by AIRS Nigeria [17]. The night catch of mosquitoes was carried out between the hours of 6:00pm to 6:00am for eight nights in May 2018 using the modified CDC light trap (CDC LT) concurrently in both indoor and outdoor points. One trap was set up at each point. The trap was suspended 1.5 meters above the floor. The trap was hung by the foot side of the bed where human bait was lying down under an untreated mosquito bed net. The temperature and relative humidity was taken hourly for each point using digital thermometer and hygrometer. Mosquitoes trapped in the collection bag were transferred hourly into well labelled paper cups indicating the hour and point of catch. A mesh was placed on top of the cups and held by a rubber band/ring so as to prevent mosquitoes from escaping. The samples were later transported to the insectary for sorting and morphological identification with the aid of a dissecting microscope.
Morphological Identification and Preservation of Samples

Mosquitoes were identified using morphological keys by Gillies, and De Meillon [18], Gillies and Coetzee [19] and Kent [20]. Samples were sorted according to species, sex, abdominal conditions, time, catch point, and then counted. All mosquitoes collected were then preserved in 1.5ml eppendorf tubes containing silica gel desiccant beneath a layer of cottonwool.

Statistical Analysis

Man Biting Rate (MBR): this is expressed as the number of bites a person receives from a specific vector species per night. This parameter can indirectly be estimated from mosquito day or night catches (e.g. PS/C, HLC, CDC LT) using the formula by Williams and Pinto [21] below:

\[
MBR = \frac{\text{Number of mosquitoes collected}}{\text{Number of people that slept in the previous night}}
\]

Results and Discussion

Composition of Mosquitoes in Mararraba-Akunza Area, Lafia LGA, Nasarawa State, Nigeria

Results obtained showed that the study area is populated by anopheline (Anopheles gambiae as shown on Plate 1) and culicine mosquitoes (Culex quinquefasciatus and Aedes aegypti on Plate 2 and plate 3) as shown in Table 1. This possibly suggests the likelihood that the inhabitants of Mararaba-Akunza community may be infected with various mosquitoes-borne diseases if bitten by mosquitoes carrying infective stage of any of the parasite gotten from an infected person while feeding on blood meal host. The four diverse mosquito species recorded in this study showed that the human-baited CDC light trap is an efficient mosquito sampling tool. This is in agreement with the finding by Ezeigwe, et al. [22] who trapped five Anopheles species via the CDC light trap ina study on Anopheles species diversity, behaviour, and sporozoite rates in six states of Nigeria. Also, this in an agreement with the finding of Sriwichai, et al. [23] who reported the effectiveness of CDC light trap on mosquito surveillance in a malaria endemic area on the Thai-Myanmar border in Thailand.
Table 1: Mosquitoes trapped in Relation to Feeding Points in Mararraban-Akunza Area, Lafia LGA, Nasarawa State, Nigeria Using CDC Light Trap, May 2018.

| Sex      | An. gambiae Indoor | An. gambiae Outdoor | Culicine Indoor | Culicine Outdoor | Sub-Total (%) Indoor | Sub-Total (%) Outdoor | Total (%) Indoor | Total (%) Outdoor |
|----------|--------------------|---------------------|----------------|-----------------|----------------------|----------------------|-----------------|-----------------|
| Male     | 1                  | 0                   | 2              | 5               | 3 (37.5)             | 5 (62.5)             | 8 (8.4)         |
| Female   | 21                 | 27                  | 19             | 20              | 40 (46.0)            | 47 (54.0)            | 87 (91.6)       |
| Sub-Total (%) | 22 (44.9)   | 27 (55.1)       | 21 (45.7)     | 25 (54.3)      | 43 (45.3)            | 52 (54.7)            |                 |
| Total (%)                      | 49 (51.6)     | 46 (48.4)       | 95             | 95              |                      |                      |                 |

Note: Abundance between anopheline and culicine mosquitoes: \( x^2 = 0.1024, \) df = 1, \( P = 0.749 \)
Abundance between female and male mosquitoes: \( x^2 = 69.222, \) df = 1, \( P < 0.0001^* \)

*: Not significant
**: Significant

Mosquitoes Abundance

Of the 95 individuals collected, *Anopheles gambiae* was more dominant 49 (51.6%) than the culicine mosquitoes 46 (48.4%) as shown in Table 1. There was however no significant difference between the abundance of anopheline and culicine mosquitoes \( (x^2 = 0.1024, \) df = 1, \( P = 0.749) \). This is accordance with the finding by Sriwichai, et al. [23] who reported no significant variation in abundance between the two mosquitoes groups. *Anopheles gambiae* was the most abundant mosquito collected from both indoor and outdoor points (Table 1). This possibly suggests that the area is a good breeding ground (based on the presence of farmlands such as rice paddy fields and animal hoofs) for malaria vector which invariably will increase the risk of malaria transmission in the area. This agrees with the findings of Ezeigwe, et al. [22] who reported high number of *Anopheles* species over culicine group using same trap. This stands in contrast to study by Sriwichai, et al. [23] who used the CO\(_2\) baited CDC light traps and found *Culex quinquesfasciatus* as the predominant mosquito species in both indoor and outdoor points. Also, the finding of Norma, et al. [24] showed that *Culex quinquefasciatus* had the highest number of individuals in feeding points with more mosquitoes trapped at a municipality of Niter\’i, a coastal residential district along a 700m beach in Brazil. Consequently, Sherrard-Smith, et al. [8] reported some evidence for more outdoor biting for mosquito species that were not *An. gambiae* s.l. or *An. funestus*.

*Aedes aegypti* was one of the least predominant mosquito in the area (Table 1). This may probably be due to the fact that they are diurnal mosquitoes and the CDC light trap was set up to collect nocturnal mosquitoes from dusk to dawn. This is in line with previous study by Sriwichai, et al. [23] who set up same time and collected very few *Aedes aegypti* in comparison to other mosquito species. Most of the collected mosquitoes were female 87 (91.6%) than males 8 (8.4%) (Table 1) with significant difference between the abundance of female to male mosquitoes \( (x^2 = 69.222, \) df = 1, \( P < 0.0001) \). This clearly shows that there will be a very high human-vector contact as females go in search of blood meal host. This is in accordance with the report by Sriwichai, et al. [23] who
showed that more females (94%) were collected over male mosquitoes in Thai-Myanmar border of Thailand.

Table 2 showed that *Cx. quinquefasciatus* was the most dominant 40 (87%) amongst the culicine mosquitoes followed by *Ae. aegypti* 4 (8.7%) and the least 2 (4.3%) was *Mn. uniformis*. Therefore, there was a very high significant difference ($\chi^2 = 129.9, df = 2, P < 0.0001$) in abundance between culicine species. The dominance of *Cx. quinquefasciatus* over other members in the culicine group may be due to the nature of the settlement of the study area which is peri-urban with a lot of stagnant water bodies in the neighborhood which serves as a good breeding ground for such species. Similarly, Sriwichai, et al. [23] showed that *Culex* species was the most predominant within the culicine group.

**Table 2**: Checklist of Culicine Mosquitoes trapped in Relation to Feeding Points in Mararraban-Akunza Area, Lafia LGA, Nasarawa State, Nigeria Using CDC Light Trap, May 2018.

| Sex   | *Ae. Aegypti* | *Cx. quinquefasciatus* | *Mn. uniformis* | Total (%) |
|-------|---------------|------------------------|-----------------|-----------|
|       | Indoor        | Outdoor                | Indoor          | Outdoor   |
| Male  | 0             | 0                      | 2               | 6         | 8 (17.4) |
| Female| 2             | 2                      | 16              | 16        | 38 (82.6) |
| Sub-Total| 2          | 2                      | 18              | 22        | 46       |
| Total (%)| 4 (8.7)       | 40 (87.0)              | 2 (4.3)         | 46        |

Note: Abundance between culicine species: $\chi^2 = 129.9, df = 2, P < 0.0001^*$

*M: Significant  

Mosquitoes Abundance In Relation to Feeding Points

The outdoor point had higher abundance of mosquitoes caught 52 (54.7%) as compared to the indoor point 43 (45.3%). Nevertheless, the abundance of mosquitoes in relation to feeding points showed no significant difference ($t = -0.43308, df = 12.516, P = 0.6723$) as shown in Figure 2. The lack of variation in the abundance of mosquitoes in relation to feeding points possibly suggests no preferred feeding point and the amount of CO2 being exhaled by the human baits laying down under the untreated bed nets may be equal alongside microclimatic conditions. On the contrary, Sherrard-Smith, et al. [8] reported that Burkina Faso, Eritrea, Ethiopia, Gabon, and Tanzania had relatively low proportions of mosquitoes feeding when people were indoors and in bed. Also, studies by Saavedra et al. [25] in riverine communities in the Peruvian Amazon showed that there were more anophelinae collected consistenly in the peridomestic area outdoors compared with inside houses. Likewise, Sriwichai, et al. [23] collected more mosquitoes outdoor than indoor. Furthermore, the proportion of mosquito bites taken outside is nearly 10% higher in 2018 compared with 2003 Sherrard-Smith, et al. [8]. Furthermore, there is considerable variability in the level of outdoor mosquito biting across Africa, which is likely to result in substantial differences in residual transmission and the effectiveness of current malaria prevention activities. Mathematical models suggest that even relatively modest changes in outdoor biting can have a substantial public health impact Sherrard-Smith, et al. [8]. Findings from Tirados et al. [26] and Keana et al. [27] showed that more mosquitoes were trapped outdoors than indoors. On the other hand, Ezeigwe, et al. [22] recorded more *Anopheles* indoors than outdoors.

Although, results obtained in this study, showed that on the average more mosquitoes were trapped outdoor than indoor which may possibly be due to the fact that collection commenced at a transition period from late dry season (hot period) into early wet season and as well as various indoor activities like cooking with stoves, spraying of aerosols, and use of mosquito repellants by occupants in the area which might kill or drive out indoor resting mosquitoes out.

Pooled mosquitoes abundance in both indoor and outdoor points in relation to hourly time of collection was highest with 15 individuals at 1-2am followed by 14 individuals at 9-10pm then 12 individuals at 3-4am, 11 caught at 12-1am while 8-9am had with zero catch at both points as shown in Table 3. However, there was no significant difference ($F_{179} = 1.456, Adjusted R^2 = 0.02786, P = 0.1447$) in the abundance of pooled mosquitoes from both points in relation to feeding time. This shows that mosquitoes do not have a preferred feeding hour rather the feed all through from early dusk hour to dawn. Correspondingly, Keven, et al. [28] in a study on female *Anopheles* in malaria-endemic area of Papua New Guinea showed no significant variation in the abundance of hourly collections.
Pattern of Mosquitoes Peak Feeding Time in Relation to Points

Concurrent mosquitoes feeding time peaked in indoor and outdoor points at 9-10pm and 3-4am respectively with 9 individuals each as shown in Table 3. This is probably due to variations in microclimatic conditions (temperature and relative humidity) at each point per collection time. Accordingly, Ngowo, et al. [25] recorded that variations in household microclimate affect outdoor-biting behaviour of malaria vectors in four villages in south-eastern Tanzania. Anopheles gambiae feeding peaked at 9-10pm and 10-11pm/3-4am in indoor and outdoor points respectively with 9 individuals each (Figure 3). This result agrees with the finding of AIRS Nigeria [14] which reported that An. gambiae had high feeding peak between 2am and 4am. *Cx. quinquefasciatus* feeding peaked at 1-2am and 3-4am in indoor and outdoor points respectively with 5 individuals each (Figure 4). *Aedes aegypti* feeding peaked at 6-7pm and 8-9pm/11pm-12am in indoor and outdoor with an individual each (Figure 5). *Mansonia uniformis* feeding peaked at 9-10pm and 2-3am in indoor and outdoor points with 1 individual (Figure 6). Similarly, AIRS Nigeria [17] reported that the feeding peak time for culicine species was between 1-2am at the indoor and outdoor respectively.

Table 3: Mosquito Abundance in Relation to Feeding Time and Points.

| Time  | No. of Mosquitoes in Points | Total |
|-------|-----------------------------|-------|
|       | Indoor                     | Outdoor |       |
| 6-7pm | 2                          | 1       | 3     |
| 7-8   | 3                          | 3       | 6     |
| 8-9   | 2                          | 4       | 6     |
| 9-10  | 9                          | 5       | 14    |
| 10-11 | 3                          | 6       | 9     |
| 11-12 | 3                          | 5       | 8     |
| 12-1am| 6                          | 5       | 11    |
| 1-2   | 8                          | 7       | 15    |
| 2-3   | 3                          | 3       | 6     |
| 3-4   | 3                          | 9       | 12    |
| 4-5   | 1                          | 4       | 5     |
| 5-6am | 0                          | 0       | 0     |
| Total | 42                         | 52      | 94    |

Note: Abundance between time and feeding points: $F_{179} = 1.456$, Adjusted $R^2 = 0.02786$, $P = 0.1447^{ns}$

Figure 3: Feeding behavior of *Anopheles gambiae* in relation to hourly collection time.
Association between Mosquito Abundance and Physicochemical Parameters within Feeding Points

**Indoor:** Mosquitoes abundance indoor showed a weak positive association with temperature ($t = 0.57654, df = 94, P = 0.5656, r = 0.05936096, Figure 7a$) but negatively associated with relative humidity ($t = -0.65621, df = 94, P = 0.5133, r = -0.06752828, Figure 7b$). The weak positive influence of indoor temperature on mosquitoes abundance may probably be due to the fact that temperature did not reach the maximum threshold (Figure 7a) as described by Mordecai, et al. [30]. This is inconsistent with the observation by Ngowo, et al. [29] in south-eastern Tanzania that a 1°C rise in the relative difference between indoor and outdoor temperature will prominently tend to drive out mosquitoes.
outdoor thereby increasing exophily An. funestus abundance by ~66% and a marginal nightly increase in An. arabiensis abundance by ~11%. Also, previous studies have shown that An. arabiensis and An. gambiae s.s. are capable of detecting and responding to an increase in temperature of a few degrees through the use of thermohygroreceptor cells which makes them assess microclimatic conditions and modify their behaviour (Kirby and Lindsay [31]; Kessler and Guerin [32]; Paaijmans et al. [33]).

**Outdoor:** The abundance of mosquitoes outdoor negatively associated with outdoor temperature \( t = -0.61791, \text{df} = 94, P = 0.5381, r = -0.06360321, \) Figure 7c) while relative humidity positively associated with mosquitoes abundance at outdoor point \( t = 0.93477, \text{df} = 94, p\text{-value} = 0.3523, r = 0.09596935, \) Figure 7d). The influence of relative humidity on mosquitoes abundance at outdoor point may be due to consistent rise in outdoor relative humidity. This is consistent with the findings of Kessler, et al. [28,25] who recorded that mosquitoes move towards a more humid location. Also, a rise in indoor relative humidity will result to a decrease in exophily mosquitoes abundance Ngowo, et al. [25].

Figure 7a-d: Association between mosquito abundance and physico-chemical parameters in concurrent feeding points.

**Association of An. gambiae Average Abundance to Temperature and Relative Humidity**

Figure 8: The simultaneous association of An. gambiae average abundance to temperature and relative humidity at both points.
The average abundance of An. gambiae relatively increased with increase in relative humidity while temperature decreased as shown in Figure 8. This stands in agreement with the finding by Ngowo, et al. [29] who demonstrated that the malaria vector An. arabiensis shifts the location of its biting from indoors to outdoors in association with relative differences in microclimatic conditions, they prefer to bite in relatively cooler and humid places. Also, this matches with laboratory observations by Kessler and Guerin [32] where An. gambiae s.l., An. stephensi and Cx. pipiens moved toward the more humid and cooler parts of a cage such as the roof, in comparison to other parts.

**Man biting rate (MBR) of Mosquitoes in Relation to Indoor and Outdoor Points**

The MBR of An. gambiae per person per night was 2.6 and 3.4 An. gambiae in indoor and outdoor points respectively whereas, culicine MBR in indoor and outdoor points was 2.4 and 2.5 respectively. This is in line with work by Keana, et al. [27].

**Table 4:** Abdominal Condition of Mosquitoes Caught in May 2018.

| Species             | Unfed | Fed | Half gravid | Gravid | Total |
|---------------------|-------|-----|-------------|--------|-------|
|                     | In    | Out | In          | Out    | In    | Out    | In | Out |
| Ae. aegypti         | 1     | 2   | 0           | 0      | 1     | 0      | 0  | 2   |
| An. gambiae         | 17    | 21  | 3           | 6      | 0     | 0      | 1  | 27  |
| Cx. quinquefasciatus| 13    | 17  | 0           | 1      | 0     | 0      | 2  | 20  |
| Mn. uniformis       | 1     | 1   | 0           | 0      | 0     | 0      | 0  | 1   |
| Sub-Total           | 32    | 41  | 3           | 7      | 1     | 0      | 1  | 50  |
| Total(%)            | 73(83.9) | 10 (11.6) | 1(1.1) | 3(3.4) | 87    |

**Conclusion**

This study shows that CDC light trap is very effective and has high efficiency in trapping of mosquito species. A total of 95 mosquitoes were recorded in both indoor and outdoor points in which the anopheline group had only An. gambiae while, Cx. quinquefasciatus was the most abundant among the culicines caught. Temperature and relative humidity influences the abundance of the mosquitoes in Mararaba-Akunza Lafia, Nasarawa State. More mosquitoes were caught outdoors than indoors due to the transition period in the month of May between late dry and early wet seasons. Mosquito-vectors population increased as temperature decreased with an increase in relative humidity while their population decreased as temperature increased with a decrease in relative humidity. Mosquito feeding habit in relation to time also shows that the vectors are specific in their feeding time, recording high peak feeding time at 9-10pm and 1-2am respectively. Since most of the mosquitoes were unfed there is a need in future studies for the ratio of nulliparous to parous female mosquitoes be determined through dissection. The findings from this study shows that there is need for the people to be properly and effectively educated on sanitation, environmental health, and the use of treated mosquito nets both indoors and outdoors so as to reduce human-vectors contact in the study area.

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