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Indoor resting densities and feeding behavior of malaria vectors in a malaria sentinel site in Nigeria

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Abstract

A study was conducted to determine the mosquito vector responsible for malaria transmission in a rural malaria vector sentinel site and to determine their behavioral pattern. The study was carried out in Oduoha-Emohua, in Emohua Local Government Area of Rivers State which represents a typical tropical rainforest. The present report represents the findings of the 12 months of the survey carried out between the month of March 2014 and February 2015. The survey involved sampling of adult mosquitoes using pyrethrum spray collection (PSC) and CDC light trap stationed both indoors and outdoors. Various relevant parameters, including G.P.S. locations of the sampling points were recorded. A total of 32 structures were sampled per month using PSC, while 2 structures were sampled for indoor and outdoor collections for 3 consecutive nights per month using CDC light traps. A total of 887 mosquitoes were sampled from these structures, out of these number, 822 (92.67%) were morphologically identified as female Anopheles mosquitoes while 65(7.33%) were culicine. Further identification of the Anopheline species using the morphological key revealed that the entire Anopheles belonged to the *Anopheles gambiae* complex (s.l.). *Anopheles gambiae* s.s. was found to account for over 85% of Anopheles species collected in the study area of which 80.29% was blood fed. The total number of the *Anopheles gambiae* collected across the months varied significantly (P<005), the highest was obtained in October, followed by May at the onset of the rains while the dry season months witnessed very low collections with zero collections in January and February. The indoor resting density followed similar trend and exceeded the critical value of 0.05 mosquitoes per household. The range obtained varied between 1.0 and 6.7 mosquitoes per household. The value was considered high and could be associated with epidermic situation and high level of malaria endemicity in the zone. These mosquitoes displayed several behavioural patterns and feeding habits. The number of indoor bitters was significantly higher than the outdoor bitters within the period under survey. The vectors displayed high level of endophilly and considerable level of exophilly. However, the endophilic species had their peak activities in the night and early morning, increasing the vector-human contact. While the outdoor bitters (exophilic) commenced their activities earlier and had their peak biting periods before midnight in some cases and early morning.

Keywords: Abundance, temperature, relative humidity, light trap, blood digestion

Introduction

Malaria as a sickness has become a house hold name in tropical African countries and accounts for much of the disease burden in the continent. In Nigeria, malaria is highly endemic and has claimed thousands of lives and caused massive economic losses. There were an estimated 263 million cases and 597 000 malaria deaths worldwide in 2023 (WHO, 2024) [30, 31]. Many control efforts have been formulated by various international agencies involved in the elimination of the disease but the problem still stare us on our faces.

To effectively control malaria, the study of the vectors remains paramount. Malaria remains one of the most important global health challenges, the greatest burden of malaria, by far, remains in the heartland of Africa, characterized by large contiguous areas of high transmission, low coverage of proven control interventions, and limited infrastructure to monitor trends in malaria burden and measure the impact of interventions (WHO, 2024) [30].

Females are able to locate hosts by following their carbon dioxide and odour gradients. When in close proximity to the host, visual cues, temperature and relative humidity are also used. Mosquitoes of the *Anopheles gambiae* complex are one of the major vectors of malaria in sub-Saharan Africa. Their ability to transmit this disease of major public health importance is dependent on their abundance, biting behaviour, susceptibility and their ability to survive long enough to transmit malaria parasites. Most blood-feeding occurs at night, indoors. A deeper understanding of this behaviour can be exploited for improving vector surveillance and malaria control (Takken *et al.,* 2024) [30]. The knowledge of the biting cycle of a species is essential in ascertaining whether peak biting coincides with the outdoor activities of local human population or whether house entry by a mosquito would be necessary to establish significant contact (Sharp, 1983) [26]. This will help in determining the appropriate prevention and control measures against the vectors.

The epidemiological importance of the time of the day mosquito bite and how much this contributes to residual transmission is unclear. The scale of the problem must be understood to demonstrate the need for outdoor vector control tools. Mosquito species and behavior data, together with people's resting and sleeping patterns are needed to fully measure indoor intervention efficacy and accurately quantify residual transmission. (Sherrard-Smith *et al.,* 2019) [27]

Solid knowledge of the vector's biting behavior is an important prerequisite to better understand and reduce malaria transmission. It is known that anopheline species differ regarding their preferred biting time and their favored biting and resting places (Githeko *et al.,* 1996, *Sinka et al.,* 2010) [12, 28]. The biting behavior of anophelines is an important determinant of malaria transmission. Understanding the local vector host-seeking behavior, its outdoor/ indoor biting preference, and nocturnal biting periods is essential for effectively applying and improving vector control methods, such as Long-Lasting Insecticidal Nets (LLINs) and personal protective measures (Dambach *et al.,* 2018) [5].

However, the sustainability of this achievement is threatened by the shift in biting and resting behaviors and emergence of insecticide resistance by the primary malaria vector (Bedasso *et al.,* 2022) [3]. Successful female An. gambiae complex mosquitoes mating is accomplished by host seeking, blood feeding and digestion, egg development and oviposition (Fereda, 2022) [10]. Egg development takes two or three days depending on temperature (Takken *et al.,* 2024) [30].

The sequential process of host finding and blood feeding followed by blood digestion and simultaneously egg maturation and accomplished by searching of oviposition site and oviposition is referred to as gonotrophic cycle. The time period between two successive blood feedings or two successive ovipositions is said to be gonotrophic period. The length of gonotrophic period depends on temperature, number of previous gonotrophic cycle, host and breeding site availability. In addition to population net reproduction of the colony, gonotrophic cycle is also used to estimate female mosquitoes' age by determining the parity (Fereda, 2022) [10].

Seasonal patterns of malaria cases in many parts of Africa are generally associated with rainfall, yet in the dry seasons, malaria transmission declines but does not always cease. It is important to understand what conditions support these periodic cases. Aerial moisture is thought to be important for mosquito survival and ability to forage (Duque *et al.,* 2022) [6].

Understanding ectotherm responses to environmental change is central to coping with many of humanity's current and future challenges in public health, biodiversity conservation, and food security. Complex relationships between abiotic and biotic factors can influence ectotherm abundance and distribution patterns by introducing stage–specific variation in fitness trait responses. Variation in temperature, rainfall, competition, and habitat have all been considered in previous attempts to understand how environmental factors can interact and vary in their relative influence on species' maximal population growth rates, r_m. However, the combined effects of temperature and humidity on this fundamental metric are poorly understood. We show that variation in relative humidity can influence juvenile trait responses and r_m's temperature dependence in *Anopheles stephensi*, an important malaria vector. Our climate suitability maps show that the interactive effects of temperature × humidity on juvenile traits have important implications for predicting how environmental change will influence arthropod–mediated systems (Huxley *et al.,* 2025) [14]. The relevance of mosquito sampling and surveillance cannot be over – emphasized, regarding the menace caused by this organism "mosquito' to the human population.

Methodology

1. The Study Site

The study was conducted in Oduoha-Emuoha community of Rivers State, which is one of the malaria endemic zones in the Niger Delta region of Nigeria. It lies between: 04°52'44"N and 06°51'40"E. It is a rural community characterized by tropical rain forest and is bounded by the New Calabar River which empties into the Bonny Estuaries.

The locals depend mainly on farming as their income. Other economic activities include fishing and trading. The topography is flat with pockets of forest stream, which also serve as source of drinking water. Due to the flat topography of the area and constant rainfall there are pockets of temporary pools of water within the community which further encourage mosquitoe breeding.

Their dwellings are mostly modern houses (cemented and partially cemented, painted and unpainted) with iron roofing sheets, only a few dwell in traditional houses (mud or wooden) with thatch or Zink roof. The dwelling houses are surrounded by a variety of homestead vegetation types including swamps, crop fields and plantain plantation. The climate is characterized with two distinct seasons, the wet and dry seasons, the former taking place from April to October and dry season between November and March. They live in clusters and are mostly Christians; a few are traditional worshippers.

2. Study Strategy

2.1. Advocacy And Sensitization

Prior to the commencement of the studies, advocacy and sensitization visits were carried out in the community to alert and keep the community fully informed of the proposed malaria vector surveillance studies and to seek for their support, ethical consent and permission to carry out the project in their domain.

2.2. Reconnaissance Visit/Mapping

Reconnaissance visit was carried out to identify suitable site for the studies. This was followed by mapping and numbering of the houses. The houses were mapped with hand held GPS. The house owners of the Identified structures were instructed to be around and make their houses available for the study. They were also instructed not to apply any insecticide two days to the sampling date. Anopheles breeding sites were also identified and geo referenced with a GPS. Suitable sites and structures for CDC light trap were also mapped.

2.3. Adult Female Collections

The study was conducted in 32 randomly selected dwelling houses in the community. Standard collection protocols recommended by the Federal Ministry of Health and World Health Organization (WHO, 1995) [35], was used in the collection of adult anopheline mosquitoes. The mosquitoes were collected using spray sheet (room collection or pyrethrum knock down collection). At each house selected for pyrethrum spray catch (PSC), latitude and longitude coordinates and certain other identifiable data were gathered and recorded accordingly.

After taking the GPS, the number of persons who slept in each of the room, the previous night was noted. White sheets were then spread to cover the entire floor surface of all the rooms to be sprayed, all exit from the house were covered. The rooms were then sprayed from both the inside and outside with pyrethrum. The rooms were closed for 10 minutes during which everyone waited outside.

The sheets were taken outside after the 10 minutes and examined for adult *Anopheles* mosquitoes. Magnifying hand lens was used to differentiate between female anopheles and other mosquitoes using morphological characteristics. The female anophelines were preserved individually in a well labeled Eppendorf plastic vial half filled with silica gel and stuffed with white papers.

The preserved mosquitoes were identified using the morphological keys of Gillies and Coetzee (1987) [11]. Data about the type of house, the number of people per sampled room of the selected houses, the nature of the environment, climatic conditions, inspectors name, date of inspection, etc. were recorded.

2.4. CDC Light Trap

CDC light trapping was carried out in two selected houses per night according to the standard methods. Human-baited light traps were set up both indoor and outdoor in both structures. The CDC light trap collecting bags were removed hourly and replaced by an empty one to determine feeding time of the vectors. The light traps collections were carried out from 6pm to 6am for three nights. The mosquitoes caught in each of the structures per hour were placed in labeled ventilated paper cups for later laboratory analysis. GPS locations of the sampling points are as follows. Structure A, N04 ^{0} 55. 452' E0060 49.969, Structure B, N04 ^{0} 54.979' E006 ^{0} 50. 127'

Environmental parameters such as temperature and humidity were taken for both indoors and outdoors and recorded hourly. In the laboratory, the mosquitoe species were preliminarily identified into species and species complex using methods described by Gillies and Coetzee (1987) [11].

2.5. Determination Of Man-Biting Rate

Man-biting rate (MBR) described as the number of bites a person receives from a specific vector species per night was indirectly estimated from pyrethrum spray catch (PSC) by separating all freshly blood fed (F) female anopheles mosquitoes collected by species and counted. The total number of female species was then divided by the total number of occupants who spent the night in the rooms that were used for the collections.

Man-biting rate (MBR) = F/W

Where F=total number of freshly fed mosquitoes of the particular species and

W= total number of occupants in the rooms used for collection

The assumptions for this indirect estimation are that:

1. All freshly fed mosquitoes obtained the blood from the occupants of the house that night
2. No freshly fed mosquito exited the house that night (Williams and Pinto, 2012)

2.6. Statistical Analysis

Microsoft excel statistical package was used to summarize the data, Minitop software was used for data analysis. The statistical variations between the mean numbers of mosquitoes sampled in the 32 structures using PSC across the months were determined using one way analysis of variance (ANOVA). T-test was used to separate the means. Comparison between the indoor and outdoor behaviours was carried out using two ways ANOVA. TUKEY method was also used to separate the means: Sigma plot was used to draw the graphs of the variation of the number of mosquitoes sampled indoors and outdoors across the month and sampling hours. The same methods were applicable for the analysis of variations of the temperature and relative humidity over the periods. Correlation between the density of mosquitoes sampled indoor and outdoor with the mean temperature and relative humidity over the study period was analyzed using regression test.

Results

1. Species Composition

A total of 887 mosquitoes were sampled indoors using PSC in 384 structures with a total of 1935 occupants, of which, 822 (92.67 %) were female *Anopheles* mosquitoes (Table 1) while 65 (7.32 %) were culicines. The *Anopheles* mosquitoes were morphologically identified to belong to *Anopheles gambiae complex.*

Table 1: Density of Anopheles Mosquito Collected at Rivers State Sentinel Site from March 2014 – February, 2015

Months of sampling	No of human occupants	No. of Structures	No. of mosquitoes sampled		Total No of mosquitoes sampled
			Culicine	Anopheline	
March,2014	237	32	27	35	62
April, 2014	186	32	3	45	48
May, 2014	162	32	2	181	183
June, 2014	157	32	19	80	99
July, 2014	175	32	4	49	53
August, 2014	165	32	2	90	92
Sept, 2014	158	32	4	77	81
Oct, 2014	172	32	2	215	217
November, 2014	126	32	0	31	31
December,2014	138	32	2	19	21
January,2015	139	32	0	0	0
February,2015	120	32	0	0	0
Total	1935	384	65	822	887

2. Vector Density

The density of the malaria vectors peaked significantly in May and October (Fig. 1), the lowest densities occurred in March and April while no mosquito was caught in January and February. The indoor resting densities also followed similar trend with a range of 0 - 6.8 mosquitoes/room with an overall mean of 2.1 (Fig. 1).

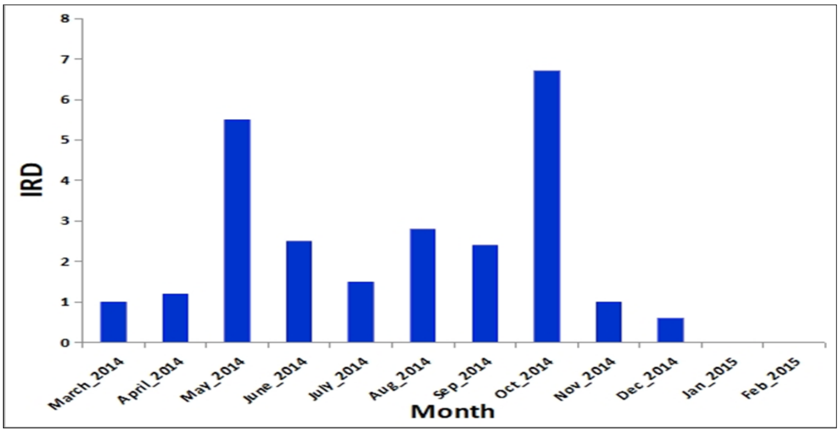

Fig 1: Indoor resting densities across the months

3. Gonotrophic Analysis

The gonotrophic analysis (Fig. 2) also revealed that the month of April recorded the highest number of blood fed malaria vectors, (90%), this was followed by the month of November (> 80%). The least blood fed month was in June (50%). The highest half-gravid vectors were in the months of March and June (25%) respectively and the lowest proportion of half-gravid was seen in the month of May (< 5%). September recorded the highest proportion of gravid vectors (15%) while July recorded the least proportion of gravid vectors (< 5%). There were no gravid vectors in the months of May, December and February. The month with the highest unfed proportion of the vectors was in February (50%) while the least unfed proportion of (< 5%) was observed in the month of November. There was no record taken at all for the month of January.

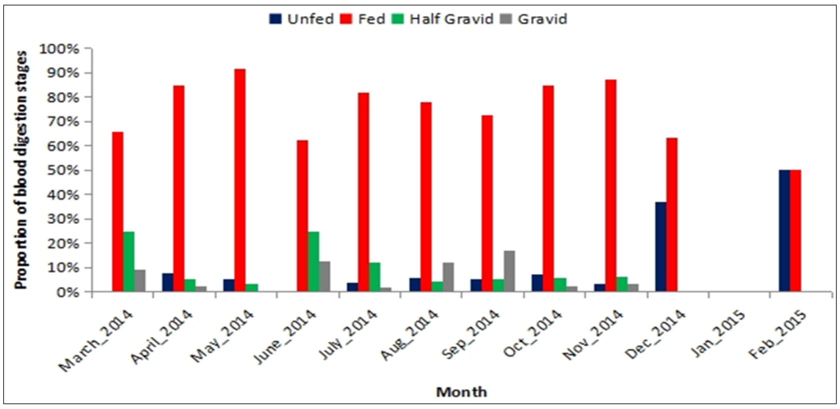

Fig 2: Gonotrophic analysis

4. Blood Digestion Stages

Fig. 3.0 shows the proportion of blood digestion stages across the months which also shows, that the percentage of mosquitoes that were blood fed (80.84%) was quite high in each of the months sampled. Unfed mosquitoes across the months were the next highest stage (11.74%), followed by half-gravid (7.66%) while the gravid stage was the lowest in proportion (5.56%) within the 12 months.

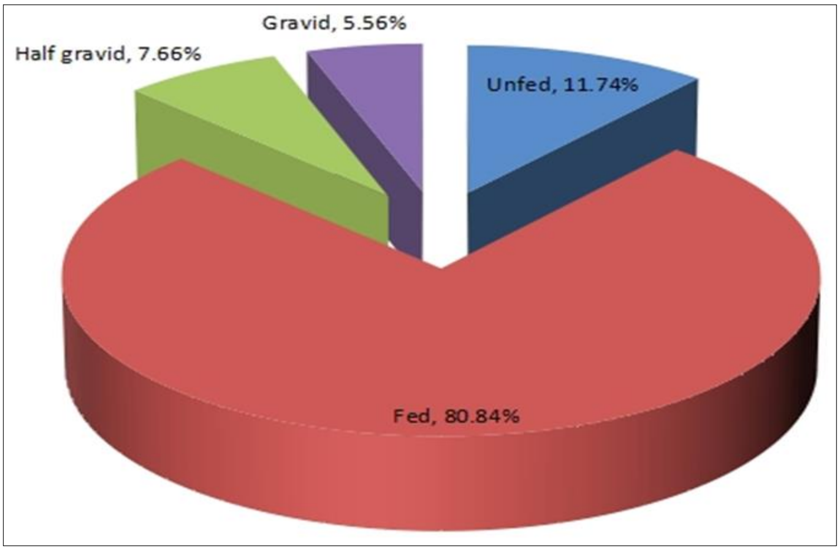

Fig 3: Percentage blood digestion stages

5. Vector Behaviour And Feeding Time

5.1. Proportion Of Mosquitoes Sampled Indoors And Outdoors

A total of 503 *Anopheles gambiae s.l* were caught with the CDC light trap. Out of this, 56.86% (N = 286) were collected indoors, while 43.14% (N = 217) were caught outdoors (Fig. 4).

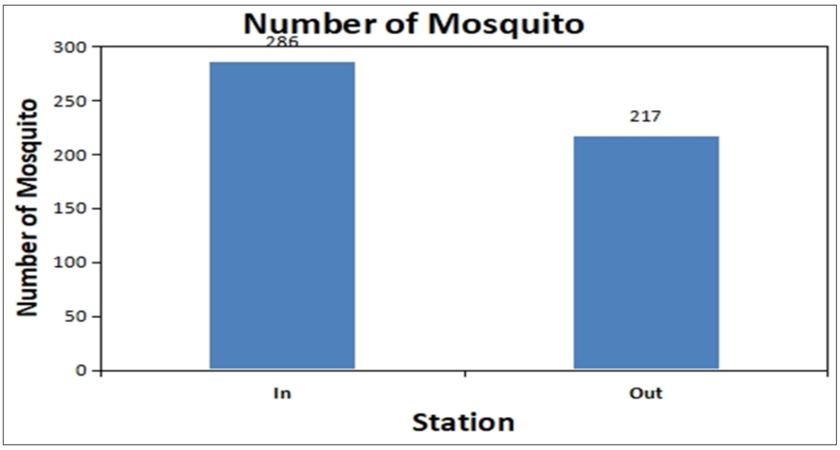

Fig 4: Proportion of Mosquitoes sampled Indoors and Outdoors

5.2. Relative Abundance of *Anopheles Gambiae s.l.* Sampled With CDC Light Trap

The highest collections were obtained in May (> 130), followed by August (< 90). The lowest collections were made in March (< 10) and February (< 10). No mosquito was collected in January (0) (Fig. 5).

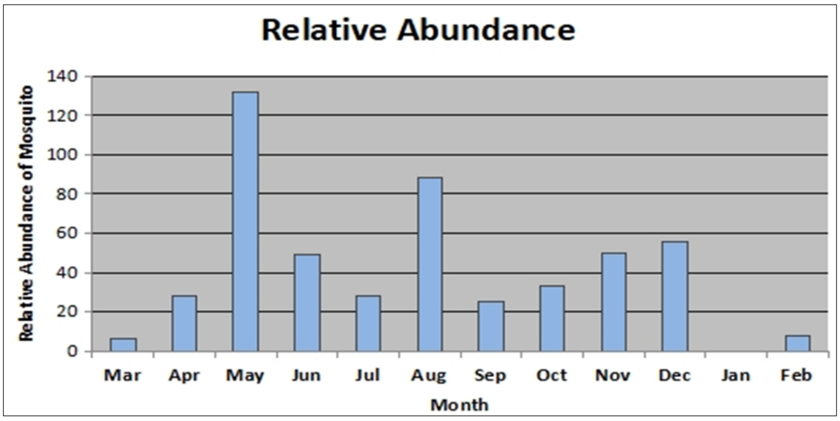

Fig 5: Summary of the relative Abundance of *Anopheles gambiae s.l.* sampled with CDC Light Trap in Rivers State sentinel site

5.3. Mean Monthly Feeding Pattern Of *Anopheles gambiae s.l.*

The outdoor biters (Fig. 6) showed three peaked activities in May (2.5), August (>2.5) and December (2.5) and sharply reduced in January (0), February (1) and March (0). The indoor biters showed a sharp peak in May (>7.5) and gradually reduced with a slight increase in August (>2.5) and later reduced steadily from September (3) to December (>3) with virtually no biting activities in March (0), January (0) and February (0) corresponding to the main months of the dry season.

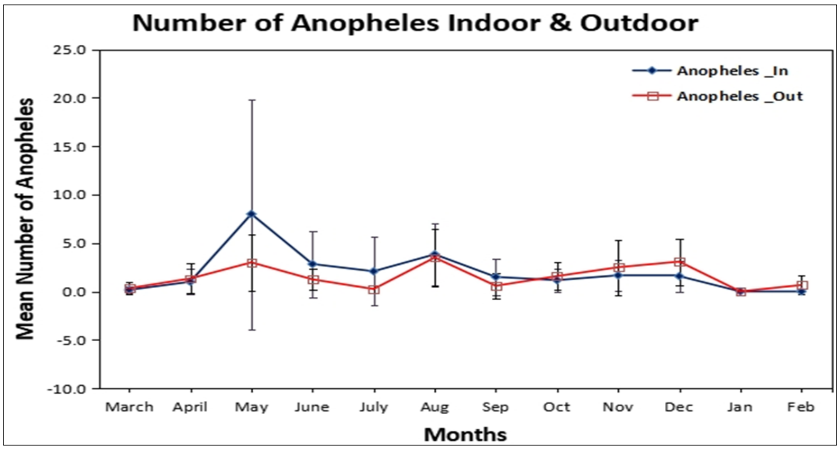

Fig 6: Mean Monthly feeding pattern of *Anopheles gambiae s.l.* in Emohua sentinel site

5.4. Mean Hourly Biting and Feeding Pattern Of *Anopheles gambiae s.l.*

The indoor biters (Fig. 7) made their appearance from 6pm and maintained a low appearance (n < 2) till 2am before peaking between 2am - 5am (n = 2 to 5). Their activities gradually reduced from 5am till 6am (n = 0). The outdoor biters also appeared from 6pm, had two smaller peaks between 7am - 8pm (n < 2) and 10pm - 11pm (n > 2). It maintained a low but steady appearance before peaking between 4am - 5am (n = 2) and later decreased till 6am (n < 1).

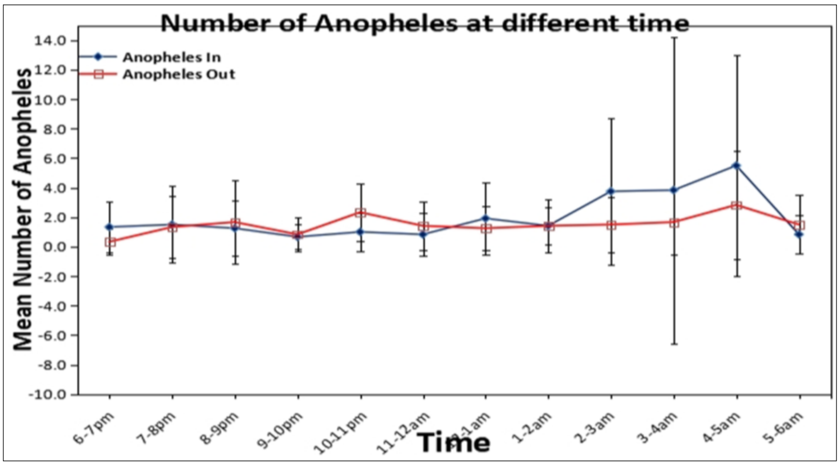

Fig 7: Mean hourly biting and feeding pattern of *Anopheles gambiae* sampled in Emohua Community

6. Temperature And Relative Humidity Variation

The mean temperature ranged from 24.67 ^{0}c (August) to 27.67 ^{0}c (March). While the mean relative humidity ranged from 81.28% (March) to 90.55% (September) (Fig. 8).

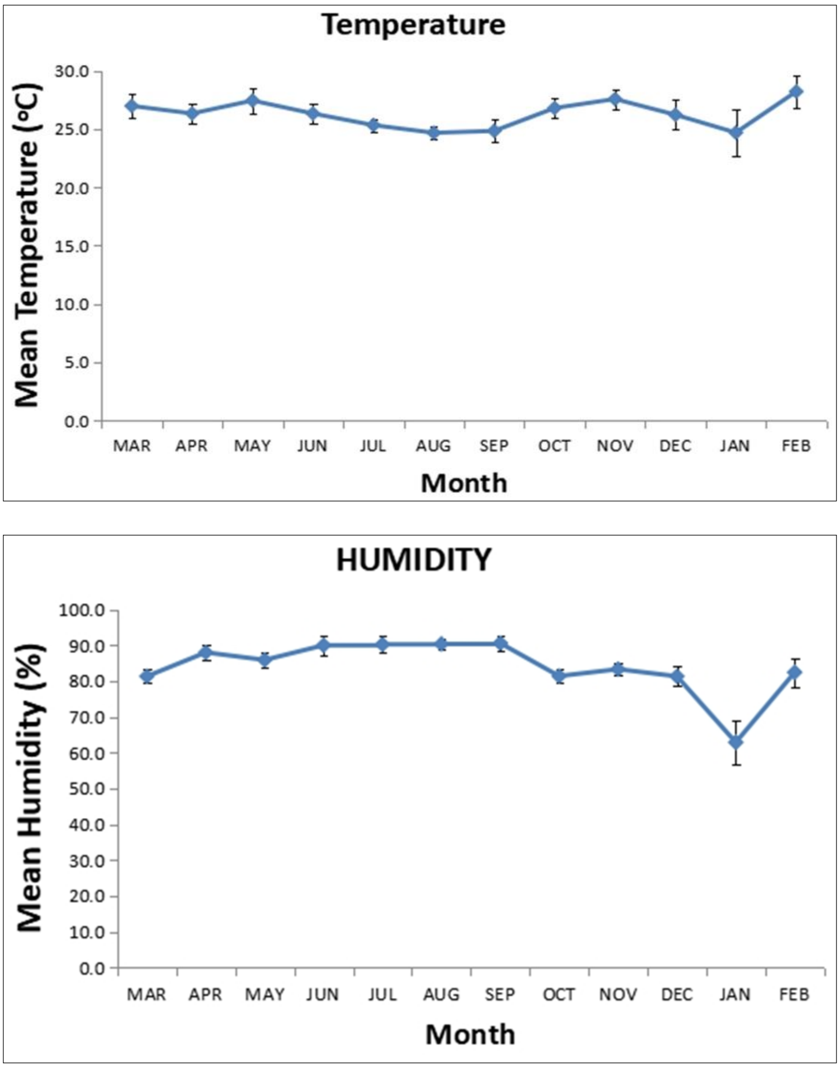

Fig 8: Mean Temperature and Mean Humidity

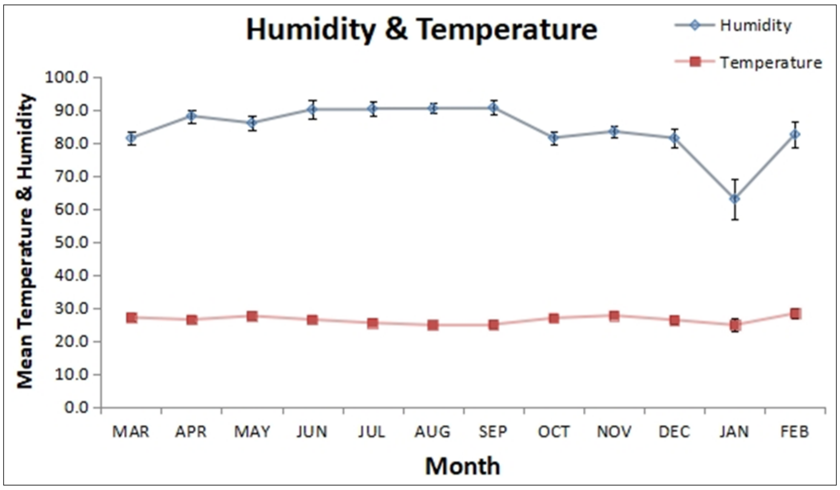

Fig 9: Combination of the Mean Temperature and Mean Humidity

7. Relationship Between Environmental Parameters And Vector Abundance

Regression analysis between the mean temperature and vector abundance (Fig. 10) over the sampling period showed a non-significant negative correlation ($r = 0.41$, $p < 0.05$). Similarly, that of relative humidity and vector abundance (Fig. 11) showed a non-significant positive correlation ($r = 0.42$, $p > 0.05$).

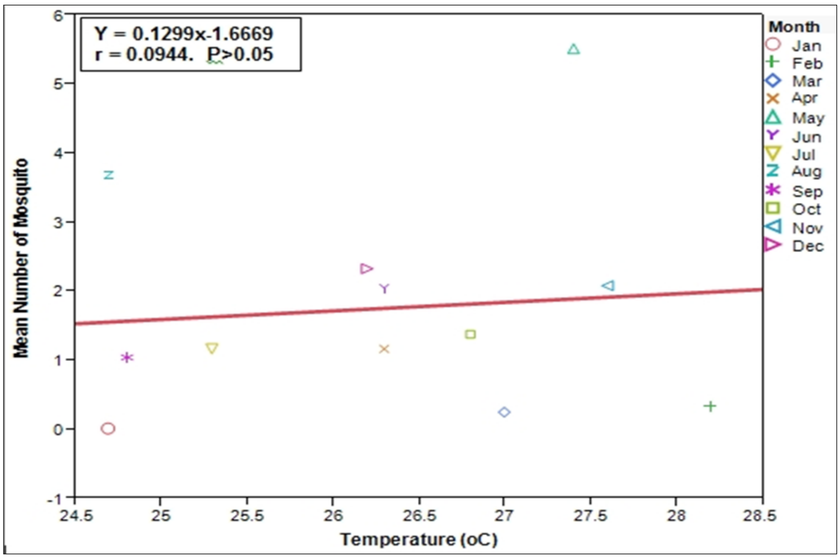

Fig 10: Relationship between temperature and vector population

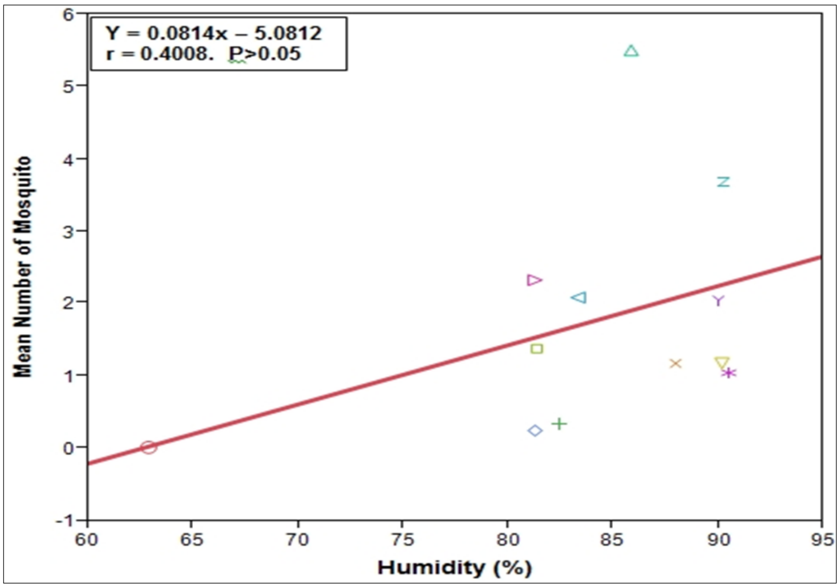

Fig 11: Relationship between Relative Humidity and Vector Population

Discussions

Anopheles gambiae s.s. was the predominant (> 80%) of the members of the complex found at all the sites. *Anopheles arabiensis* (< 5%) was the other member of the complex identified. Another study showed that *An. gambiae s.l.* was composed of two sibling species, namely *An. coluzzii*, *An. gambiae s.s.* and the hybrids. Among the two sibling species *An. coluzzii* was the predominant (88.24%) species (Kouamé *et al.,* 2024) [15]. Results from the present study revealed that *Anopheles gambiae s.l.* is the main malaria vector in the geographical zone. This finding is also in agreement with the earlier baseline survey in the region (Ebere, 2011;2013) [7, 8] and the report of Maaji *et al.* (2024) [18], in which a total of 755 Anopheles mosquitoes collected by PSC were all found to be *Anopheles gambiae s.l.* Out of this, 568 (75%) *An. gambiae s.l.* were collected from mud houses while 189 *An. gambiae s.l.* were collected from brick houses. *An. gambiae s.l.* was the most abundant mosquitoe over other species. According to White (1974) [33], mosquitoes of the *An. gambiae* complex have been reported as one of the most efficient vectors of malaria in the Afro tropical region. The complex consists of many species. The abundance of the species in this area could be associated to the favourable climatic condition characteristics of the zone and abundance of breeding habitats.

Comparing the two collection methods used, a greater number of *An. gambiae s.l.* were collected using PSC (822) than with CDC LT (503).This is in contrast with a study in which Anopheles mosquitoes were collected in May than in April following the onset of rainfall which tends to create more breeding sites which in turn increase mosquito population, longevity and hence increase malaria transmission. A greater number of *An. gambiae s.l.* was collected using CDC LT (4565) than with PSC (757) (Maaji *et al*., 2024) [18].

The vector densities were not uniformly distributed across the months (ANOVA, F = 10.75, df = 7, P < 0.05). The higher density of the anopheline mosquitoes in the region, in the present study is perhaps an outcome of favorable ecological conditions, rich forest cover and congenial breeding environment (presence of large number of ponds and river tributaries) and is attributed to high malaria incidence in this part of the State. Spatial determinants of malaria risk within communities are associated with heterogeneity of exposure to vector mosquitoes. The abundance of adult malaria vectors inside people's houses, where most transmission takes place, should be associated with several factors: proximity of houses to larval habitats, structural characteristics of houses, indoor use of vector control tools containing insecticides, and human behavioural and environmental factors in and near houses (Robert *et al.,* 2017). The high density of *An. gambiae* highlighted the high number of the female that emerged when the environmental condition is favorable following the wide distribution of breeding site among the communities. *An. gambiae* are known vectors of malaria (Abdullahi *et al.,* 2024) [1].

In the current study, fully fed mosquitoes were far higher than unfed mosquitoes, indicating a high transmission rate. This agrees with Ajayi *et al.* (2025) [2] in which a total of 298 *An. gambiae* complex mosquitoes were examined. Fully fed mosquitoes accounted for 77.2%, while the half gravid in the current study was higher than the gravid, this contrasts with the report in which 12.4% were gravid, and 5.4% were half-gravid. Blood digestion analysis also agreed with a previous report which shows that *Anopheles gambiae s.l.* is highly anthropophagic, endophilic and endophagic (Oylewole *et al.,* 2007, Echodu *et al.,* 2010) [9].

There were variations in the number of vectors collected both indoors and outdoors across the months with CDC light trap. However, over the period, the indoor biters were significantly higher than the outdoor biters (ANOVA, F = 405, df = 1, P < 0.05). Comparatively, in a report, a total of 5322 Anopheles belonging to five different species were identified. 4565 Anopheles mosquito species were collected by CDC LT method. Out of this, 4411 (96.6%) were *Anopheles gambiae s.l.* 143 (3.1%) were *An. pharoensis,* 7 (0.2%) were *An. coustani*, 3 (0.06%) were *An. funestus* and 2 (0.04%) were *An. rufipes* (Maaji *et al.,* 2024) [18]. In another study, a total of (n=1005) mosquitoes belonging to 5 genera and 21 species were caught from the sites during the study. Eight hundred and forty-eight (848) were caught indoors while one hundred and fifty-seven (157) were caught outdoors (Abdullahi *et al.,* 2024) [1]. Comparison of the indoor and outdoor collections over the study period clearly indicated significant activities of indoor biters over outdoor biters. However, in some months of sampling the differences were not significant (Reddy *et al.,* 2011) [25]. In another study, a large proportion of Anopheles females exiting the houses during the night were not blood-fed, suggesting that bed-net use prevented them to find an available host indoors and led them to move outdoors to find one. However, a large proportion of females collected indoors were blood-fed, suggesting that prevention of bites was incomplete even when all persons in a house were declared to have slept under bed-net (Tondossam *et al.,* 2023). This trend implied that probably at one time, the outdoor human biting rates equaled the indoor biting rates as observed in the month of May. The effectiveness of the vector control intervention such as ITN/LLINs, local herbs and insecticide under use in this zone could also influence the indoor collections. Other prevailing factors like late sleeping due to excessive heat can also contribute to the observation.

Hourly studies of the behavior of the vectors in different months showed variations in the feeding pattern. However, comparison of the mean abundance and biting pattern of the vectors over the sampling period (Fig. 7) clearly revealed that the mosquitoes exhibited both endophillic and exophillic pattern of behavior as their presence were visible throughout the period of study. Previous studies have also reported an increase in the outdoor biting rates of the *Anopheline* species over the indoor biters (Reddy *et al.,* 2011) [25] high levels of exophagy and opportunistic feeding on animals have been observed in *An. gambaie s.l.* mosquitoes in specific ecological context throughout sub-Sahara Africa (Wanji *et al.,* 2003; Bryan *et al.,* 1987; Sousa *et al.,* 2001; Govella, 2010) [4, 13, 29, 32].

According to Reddy *et al.* (2011) [25], though *Anopheles gambiae* is typically considered as endophagic mosquito, this tract appears to vary between locations, seasons or both. *Anopheles gambiae* will feed on cows, dogs in the absence of available human hosts (Bryan *et al.,* 1987, Sousa *et al.,* 2001 [4, 29], Lefivre *et al.,* 2009). This result implies that *Anopheles gambiae s.l* mosquitoes seek hosts outdoor as well as indoors in this zone. This contrasts with an earlier position of exclusive endophagy in this region.

Mosquito biting times may also change due to environmental cues with the productivity of different breeding sites varying according to local weather patterns. The arrival of electric lighting in the last few decades may also have changed behaviors over time as people may stay up, or out of bed, for longer into the evening (Sherrard-Smith *et al.,* 2019) [27].

The *An. gambiae s.l.* activities indoors generally showed peak biting activities after midnight a time when the local human population is asleep in their houses. The endophillic behavior would be a prerequisite for significant contact between the vector species and man. The occurrence of most of the peak biting after midnight is consistent with the already known time of peak biting by the majority of human-biting sporozoite positive *An. gambiae* mosquitoes which is between 23.00 and 5.00 hours, a period when most people are in bed and covered with nets (Maxwell *et al.,* 1998; Ministry of Health Uganda, 1999) [19, 21].

The outdoor biters (exophillic species) showed regular appearance throughout the night with intensified biting activities before midnight and later peaked between 4am - 5am. This corresponds with the time the rural dwellers wake up to prepare for their farming activities. The increase in the biting activities of the outdoor biters before midnight is quite understandable as the periods in question is a period where a larger proportion of the rural population is still active outdoors especially during the months in dry season due to excessive heat.

The circadian activity rhythm, distance and abundance of aquatic habitats and human population, topography, vegetation, housing quality and weather can affect the shape of the biting cycles of *An. gambiae.* This probably explains why biting patterns can vary markedly from site to site. Yet, summarizing 92 recent studies from sub-Saharan Africa showed that there was a clear tendency for *An. gambiae* biting activity to increase from dusk to a maximum at 01.00 h before slowly declining until 05.00 h, before dropping precipitously to low levels at 07.00 h (Fig. 6) (Takken *et al.*, 2024) [30].

The temperature and relative humidity varied significantly across the months of sampling (F = 13.5, df = 7, P < 0.05, F = 33.02, df = 7, p < 0.05 respectively). The mean humidity recorded in the months of March (> 80%), October (80%) and January (> 60%) are significantly lower than the rest months by Tukey mean separation analysis. Combination of the mean temperature and mean humidity (Fig. 9) shows that the lower the temperature, the higher the humidity from the months of March to December, this is due to the moist air that prevails from constant rainfall, which as well results in drop in the temperature. In January it was observed that the lower temperature resulted to low humidity because of the harmattan season which is responsible for decrease moisture in the air as the dry wind blows with its coolness which makes the temperature to drop.

Meteorological factors are important drivers of malaria transmission. Temperature, rainfall and humidity have been associated with the dynamics of malaria vector population and, therefore; with spread of the disease. This is attested by the range of temperature (24.67 ^{0}C - 27.67 ^{0}C) and relative humidity (81.28% - 90.55%) observed in this survey, which are quite favourable for breeding the mosquitoe. Rainfall provides breeding sites for mosquitoes to lay their eggs, and ensures a suitable relative humidity of at least 50% to 60% to prolong mosquito survival. Relative humidity below 60% shortens the life span of the mosquitoes. *Anopheles gambiae* complex breed in temporary man-made water bodies e.g. pools, puddles or brick pits, field construction sites, hoof prints and even tire tracts. These micro habitats are common site in the study area coupled with some home stead crops like plantain which can favour the breeding of mosquitoes in their leaf axils. Significantly higher catches of the Anopheles species were realized at the onsets of rain (May and October), this being attributed to the fact that the rainy season provides enough temporary pool of water for mosquitoes to breed (Echodu *et al.,* 2010) [9] and increases humidity of mosquito survival (Nicholas, 1998) [22]. The positive correlation obtained between the mosquitoe density and relative humidity over the study period clearly attest to this. The indoor resting density of the mosquitoes per household varied between 1.0 and 6.7. The observed densities exceeded the critical range of 0.05 mosquitoes per household and could be associated to epidermic transmission. The high densities observed in both the onsets of the rain and the dry season months (April and March) are a pointer that, the mosquitoes could transmit malaria in the region all year round.

In another study correlations between gravid *Cx. quinquefasciatus* versus relative humidity, unfed *Cx. quinquefasciatus* versus rainfall and *An. gambiae* versus rainfall were the only associations that correlated significantly (p<0.05) (Omoregie and Aigbodion, 2022) [23].

Temperature had an interactive effect on malaria with relative humidity and rainfall. High temperature together with high relative humidity and high rainfall could accelerate the transmission of malaria. Meteorological factors may affect malaria transmission interactively (Liu *et al.,* 2021) [17]. Higher temperatures within suitable ranges were generally associated with increased transmission risks, while excessively high or low temperatures had adverse effects. Humidity exhibited complex nonlinear relationships, facilitating transmission in certain temperature zones but inhibiting it in others. Heavy rainfall and high humidity were linked to vector-borne disease outbreaks such as malaria by enabling vector breeding. However, reduced incidence of some diseases like dengue fever was observed with high rainfall (Zhang *et al.,* 2024) [36].

Conclusion

An. gambaie s.l. is the predominant vector of malaria in the region. The presence of this species throughout the study period is due to favourable environmental condition in the area. The indoor resting density of the vectors was high which indicate that the great proportion of the mosquitoes are endophillic and anthropophagic. Blood digestion stages reflected a sequence in which fully fed mosquitoes were far higher than unfed mosquitoes and the half gravid was higher than the gravid. The predominance of this species of mosquitoes is a warning signal that the rural dwellers are predisposed to malaria attack. The vectors showed high level of endophily and considerable level of exophily suggesting that the long-term indoor application of residual insecticides in combination with LLINs is needed for control of the malaria vectors. Generally, the mosquitoes were more active in the night and increased their feeding and biting activities early in the morning.

Authors' Contributions

Nwabueze Ebere was involved in all study processes including design, data collection, analysis, and interpretation of results, as well as drafting of the manuscript. Idorenyin

Bassey Ekerette was involved in study design supervision and manuscript drafting. Nioking Amadi participated in study design, data analysis and manuscript revision. All authors read and approved the final manuscript.

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