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Gonotrophic stages of *Anopheles gambiae* and *Culex quinquefasciatus* and their relationships with climatic conditions in selected school dormitories in Benin City, Nigeria

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Abstract

Mosquito-borne pathogens are transmitted mainly during blood feeding by infective female mosquito vectors. Blood meals' digestion stages reveal necessary entomological information for retrospective assessment and future plan for vector control. This study was done to assess the blood digestion stages of *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes and their relationships with climatic conditions in school dormitories in Egor, Oredo and Ikpoba Okha Local Government Areas (LGAs) of Benin City, Edo State. Indoor mosquitoes were collected between 08:00 and 11:00 hours from randomly selected dormitories bi-monthly for 6 months, using sweep net and mechanical aspirator. Female mosquitoes were identified and grouped into various blood digestion stages using relevant keys. A total of 439 adult female mosquitoes identified as *An. gambiae* (n=28) and *Cx. quinquefasciatus* (n=411) were examined for gonotrophic status. Total gonotrophic stages of both vector species in the collective LGAs were highly significant ($p<0.01$). Only *Cx. quinquefasciatus* revealed significant variation ($p<0.01$) in monthly mean percentage abundance of the gonotrophic stages across the study months in the combined LGA data. Regarding each LGA, there was significant variation in monthly mean percentage abundance of *Cx. quinquefasciatus* gonotrophic stages across the study months ($p<0.05$) in Egor and Ikpoba Okha except Oredo. As for *An. gambiae*, no significant difference ($p>0.05$) was observed per LGA. Correlations between gravid *Cx. quinquefasciatus* vs relative humidity, unfed *Cx. quinquefasciatus* vs rainfall and *An. gambiae* vs rainfall were the only associations that correlated significantly ($p<0.05$). The results indicate the need for deployment of thorough and effective vector management strategy.

Keywords: Gonotrophic or Blood digestion stage, Anopheles gambiae, Culex quinquefasciatus, mosquito vector control, boarding school dormitories

Introduction

After emergence from their pupal stage of development, adult female mosquitoes seek out suitable hosts either animal or human to obtain blood meals necessary for their energy requirement and most importantly the requisite nutrient to aid production of their eggs (Service, 2012; Fereda, 2022). Blood in the abdominal region of blood fed female mosquitoes begin to digest immediately after ingestion; a process that occurs concurrently as their ovaries' development (Charlwood *et al.*, 2000). During the process of digestion, blood is actively used to produce eggs. This is shown in the gradual change in the appearance of a completely reddish abdomen of a freshly fed female mosquito compared to an entirely whitish abdomen of a gravid female mosquito with fully developed eggs (Service, 2012; Fereda, 2022). Mosquito-borne diseases are transmitted essentially during blood feeding by the infective mosquito vectors (Lehane, 2005; Scott and Takken, 2012; Service, 2012). Of the list of mosquito-borne infections, malaria and lymphatic filariasis stand out in having debilitating effects and ability to cause serious illnesses and death to their hosts. Malaria, caused by the *Plasmodium* parasites is transmitted to humans by *Anopheles* mosquito vectors and African infections of Lymphatic filariasis infections in Africa is caused by the parasitic *Wuchereria bancrofti* nematodes and transmitted by the *Anopheles* and *Culex* mosquito vector species (Service, 2012; WHO, 2013).

Lymphatic filariasis is classed as one of the world's major Neglected Tropical Diseases based on prevalence (Hotez *et al.*, 2007). It's global prevalence is about 120 million; being most prevalent in sub-Saharan Africa, India, South Asia, East Asia and Pacific Islands and about 1.3 billion persons are at risk of the disease (Ottesen, 2006; WHO, 2006). Nigeria has had or still holds the highest rank for the country with the most Lymphatic filariasis cases in Africa (Njepuome *et al.*, 2009). According to WHO (2021), there were about 228 million estimated malaria cases and 602,000 deaths reported in the WHO African Region including Nigeria in the year 2020; accounting for about 95% of cases and 96% of deaths globally. With a total record of 64 million cases, Nigeria ranked as the country amongst other countries of the world with the most number of malaria cases (WHO, 2021).

The success of a method chosen to control a certain mosquito population is greatly reliant on the accuracy and compliance level of the vectors to the ascertained indices that informed the choice of the method in the first instance (Pates and Curtis, 2005). Blood digestion status assessments provide information on the unfed, blood fed, half gravid and gravid abdominal status of resting mosquitoes in the population (WHO, 2003). The presence of each stage has some relevance with respect to providing information on existing vector control efficacy. For instance, having blood fed endophagic and endophilic mosquitoes in a population could be evident of successful vector – host contacts. Also, the presence and number of unfed mosquitoes in the population could be indicative of the existence of nearby viable larval breeding sites and lastly, the occurrence of all the stages could well imply a dearth of effective mosquito vector control tool that targets the freshly fed, half gravid and gravid as they rest and the unfed as they seek suitable host to obtain blood meals..

Weather conditions such as temperature (Maiga *et al*., 2012), rainfall (Fereda, 2022) and humidity (WHO, 1975) do affect the progression and duration of blood digestion in mosquitoes. This synchronously impacts on their disease transmission risk. For instance, the faster rate of digestion of blood meal by malaria mosquito vectors during warmer temperature periods will consequently result in increased frequency of blood feeding and ultimately increased risk of malaria transmission (Fereda, 2022).

Previous reports have mainly centred on description of the gonotrophic or blood digestion stages of mosquito species in study localities or areas with respect to total count or percentage abundance for the entire study period (Adeleke *et al*., 2010; Ebenezer *et al*., 2014; Degefa *et al*., 2017; Umeanaeto *et al*., 2017; Getachew *et al*., 2019; Njila *et al.,* 2019; Adugna *et al.,* 2021; Irikannu *et al*., 2022). The reason for this may not be unconnected with the fact that determination of certain aspects of gonotrophic status of mosquito vectors especially the unfed and the blood fed stages are pre-requirements for conducting other linking studies such as on parity and blood meal origin (WHO, 2013). Rarely is there data on monthly gonotrophic status of mosquito vector species and when available, are mostly reported in monthly total count and/or percentage abundance (Okorie *et al*., 2014; Lelisa *et al*., 2017; Hassan *et al*., 2019). Furthermore, there is no comprehensive data on gonotrophic status of mosquito vector species in boarding school dormitories and also on their relationships with weather conditions in Benin City, Edo State. Although, Omoregie and Aigbodion (2022) reported total count of freshly fed and unfed gonotrophic stages of *Anopheles gambiae* and *Culex quinquefasciatus*, records of their monthly average percentage abundance and of other stages were absent.

This study therefore evaluated the abundance (mean monthly and total) of the gonotrophic stages such as unfed (uf), freshly fed (ff), half gravid (hg) and gravid (g) of *An. gambiae* and *Cx. quinquefasciatus* mosquito vectors collected from boarding school dormitories in 3 LGAs in Edo State and relationships that existed between them and the prevailing weather conditions. Data obtained will serve as a guide in determining impact of vector control and aid proper planning for malaria control programmes.

Materials and Methods

The study was conducted in Egor, Oredo and Ikpoba-Okha Local Government Areas (LGA) in Benin City, Edo State, Nigeria. Randomly selected school dormitories of study were located in 06°20.899'N, 005°36.677'E; 06°19.937'N, 005°38.808'E and 06°18.018'N, 005°36.358E in Egor, Oredo and Ikpoba Okha LGAs respectively which have been extensively described by Omoregie *et al.* (2019) and Omoregie and Aigbodion (2022).

Resting adult mosquitoes were sampled from 08:00 – 11:00 hours in five dormitories randomly selected from each of the Local Government Areas bimonthly from January to June 2012. Techniques used for the adult mosquito collection and handling in the laboratory were also reported by Omoregie *et al.* (2019) and Omoregie and Aigbodion (2022). Mosquitoes brought from the field in specimen bottles were first anaesthetized or killed before identification. Few drops of ethyl acetate were introduced into the cotton wool laden specimen bottle and left for about 10 mins till the mosquitoes were knocked down or dead. Each mosquito was then placed on a glass slide and examined under a stereomicroscope at the X10 magnification and identified morphologically using taxonomic keys as described by Gillett and Smith (1972), Coetzee (2020) and WHO (2020). Female mosquitoes identified as *An. gambiae* and *Cx. quinquefasciatus* were examined for gonotrophic status on a glass slide under a stereomicroscope at X10 using the quantity and quality of the blood in the mosquito midgut as described by WHO (2003). Blood digestion stages were categorized as unfed (uf), freshly fed (ff), half gravid (hg) and gravid (g). Weather data on mean relative humidity (%), monthly mean temperature (%) and total rainfall (mm) for the period of study was collected from the Nigeria Meteorological Agency (NIMET) Benin Office at the Benin Airport, Benin City. The data have been reported elsewhere (Omoregie *et al*., 2019).

Data collected were analysed using the Statistical Package for Social Scientists (SPSS 16.0) and Microsoft Excel, 2010 packages. Data presented in tables were expressed as mean percentages and standard deviations. The Chi-square test and Analysis of Variance (ANOVA) were used to determine variation in the blood feeding stages of mosquito vectors. Tests for significant difference in relationships between the blood feeding stages of the mosquitoes and weather condition was done using the simple linear correlation. Significance in comparisons was set at $p<0.05$

Results

A total of 439 adult female mosquitoes identified as *An. gambiae* ($n = 28$) and *Cx. quinquefasciatus* ($n = 411$) were examined for gonotrophic (physiological) status (Table 1 and 2). There was significant difference in the total gonotrophic stages of both vector species in the collective LGAs; *An. gambiae* ($\chi^2 = 61.760$; df = 3; $p < 0.01$) and *Cx. quinquefasciatus* ($\chi^2 = 68.560$; df = 3; $p < 0.01$). The freshly fed digestion stage was highest in abundance in both vector species while the lowest were recorded by the unfed (7.1%) and half gravid (10.7%) stages in *An. gambiae* and only the unfed stage (5.8%) in the *Cx. quinquefasciatus* samples.

Table 1: Blood digestion stages of *Cx. quinquefasciatus* in school dormitories in the combined LGAs (Egor, Oredo and Ikpoba Okha):

Months	Unfed (%) ($\bar{x}$±SD)	Freshly fed (%) ($\bar{x}$±SD)	Half gravid (%) ($\bar{x}$±SD)	Gravid (%) ($\bar{x}$±SD)
January	18.3±5.6	54.6±1.4	12.7±2.2	14.5±4.8
February	7.1±10.1	56.9±6.4	26.3±0.1	9.7±3.6
March	4.7±1.3	54.7±2.7	27.5±4.3	13.1±2.8
April	4.0±5.7	34.0±48.1	50.9±43.7	11.1±10.0
May	2.9±0.1	53.5±13.3	33.4±10.9	10.2±2.3
June	4.1±5.8	76.3±6.9	9.3±4.5	10.3±5.7
Total	**5.8 (24)**	**58.6 (241)**	**24.1 (99)**	**11.4 (47)**

Table 2: Blood digestion stages of *An. gambiae* in school dormitories in the combined LGAs (Oredo and Ikpoba Okha):

Months	Unfed (%) ($\bar{x}$±SD)	Freshly fed (%) ($\bar{x}$±SD)	Half gravid (%) ($\bar{x}$±SD)	Gravid (%) ($\bar{x}$±SD)
January	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
February	0.0±0.0	50.0±70.7	50.0±70.7	0.0±0.0
March	0.0±0.0	33.3±47.1	16.7±23.6	0.0±0.0
April	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
May	0.0±0.0	83.3±23.6	0.0±0.0	16.7±23.6
June	16.3±12.2	30.8±43.5	12.5±17.7	40.4±13.6
Total	**7.1 (2.0)**	**57.1 (16.0)**	**10.7 (3.0)**	**25.0 (7.0)**

Result of statistical analysis on the difference in mean percentage abundance of the gonotrophic stages of both mosquitoes across the study months in the combined LGA data revealed significant variation only for *Cx. quinquefasciatus* ($\chi^2_{(3)} = 17.540$; $p < 0.01$). There were records of freshly fed vectors in all the study months where mosquitoes occurred. As for *Cx. quinquefasciatus*, the freshly fed mosquitoes were highest in monthly mean percentage abundance in all the months across all dormitories, except in April when those that were half gravid were the most abundant (50.9 ± 43.7 %). Conversely, their unfeds were the least abundant in all the months except January where the position was shared among the unfed (18.3 ± 5.6 %), half gravid (12.7 ± 2.2 %) and gravid (14.5 ± 4.8 %) mosquitoes (Table 1).The freshly fed *An. gambiae* mosquitoes were also highest in monthly mean percentage abundance across the study months where they occurred; although, the position was jointly shared with the half gravids in February. Nevertheless, the least abundant were the mosquitoes that were unfed and gravid in February and March (unfed = 0.0 ± 0.0 %; gravid = 0.0 ± 0.0 %), unfed and half gravid in May (unfed = 0.0 ± 0.0 %; half gravid = 0.0 ± 0.0 %) and in June (unfed = 16.3 ± 12.2 %; half gravid = 12.5 ± 17.7 %) (Table 2).

Concerning each LGA, blood digestion stages of *Cx. quinquefasciatus* varied significantly ($p<0.05$) in monthly mean percentage abundance in Egor and Ikpoba Okha LGAs except Oredo ($\chi^2_{(3)} = 2.649$, $p>0.05$) (Figures 1, 2 and 3). There was also no significant difference ($p>0.05$) in the blood digestion stages of the *An. gambiae* vectors evaluated each in Oredo and Ikpoba Okha LGAs (Figures 4 and 5). In Egor, the freshly fed *Cx. quinquefasciatus* mosquitoes were the most abundant in all the months but shared the position with the half gravid mosquitoes in March and April. The unfed mosquitoes were the least abundant (Figure 1). In Oredo LGA, *Cx. quinquefasciatus* mosquitoes that were unfed in January and February, freshly fed in March and June and gravid in April were the most abundant. Also, the freshly fed having similar monthly mean percentage abundance with half gravid mosquitoes, were the most abundant in May (Figure 3). As for *An. gambiae*, the freshly fed blood digestion stage was the most abundant in March, May and June (Figure 4). In Ikpoba Okha, freshly fed *Cx. quinquefasciatus* mosquitoes had the highest monthly mean in all the months of its occurrence in March, May and June. In February, the half gravid mosquitoes having similar mean percentage abundance with the freshly fed were the most abundant (Figure 2). Freshly fed *An. gambiae* having tied with those that were half gravid in their monthly mean in February, the half gravids in March, the freshly feds in May and the gravids in June all had the highest monthly mean percentage abundance in their respective months in Ikpoba Okha LGA (Figure 5).

Significant correlations between the different gonotrophic stages and the prevailing weather conditions were revealed only between gravid *Cx. quinquefasciatus* vs relative humidity, unfed *Cx. quinquefasciatus* vs rainfall and *An. gambiae* vs rainfall ($p < 0.05$) (Table 3).

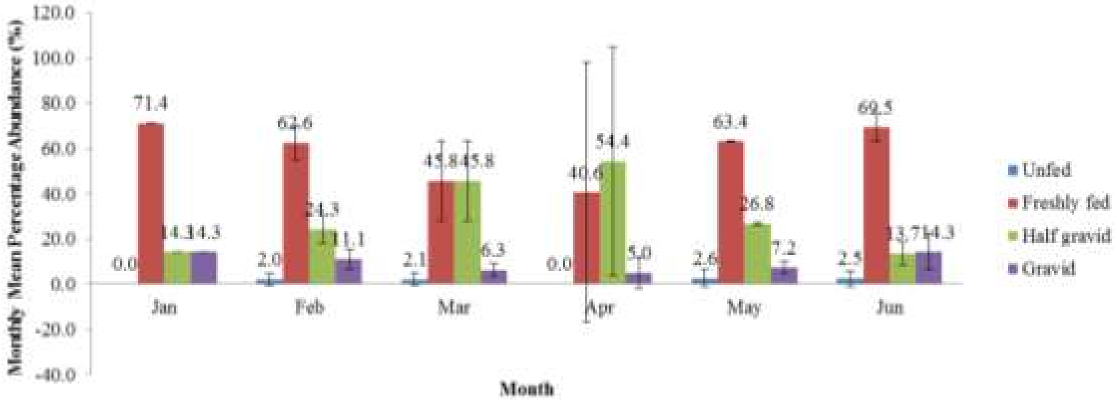

Figure 1: Blood digestion stages of *Cx. quinquefasciatus* in school dormitories in Egor.

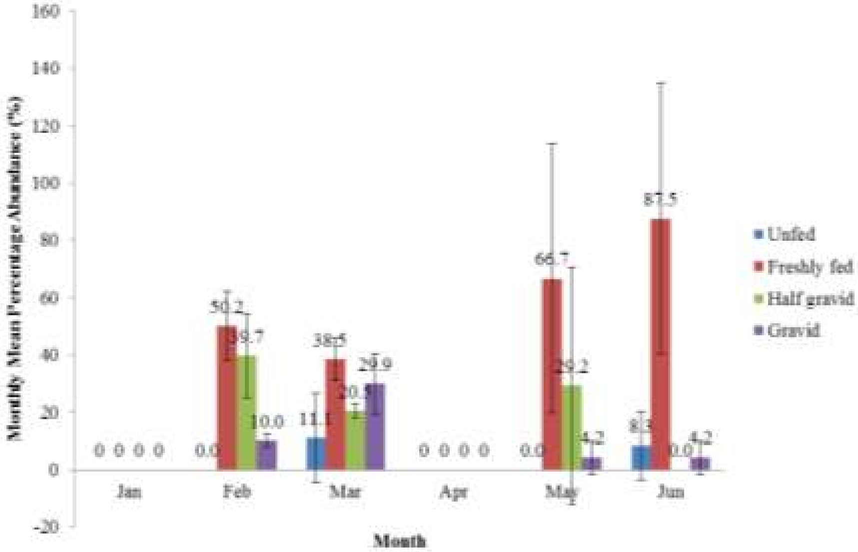

Figure 2: Blood digestion stages of *Cx. quinquefasciatus* in school dormitories in Ikpoba Okha.

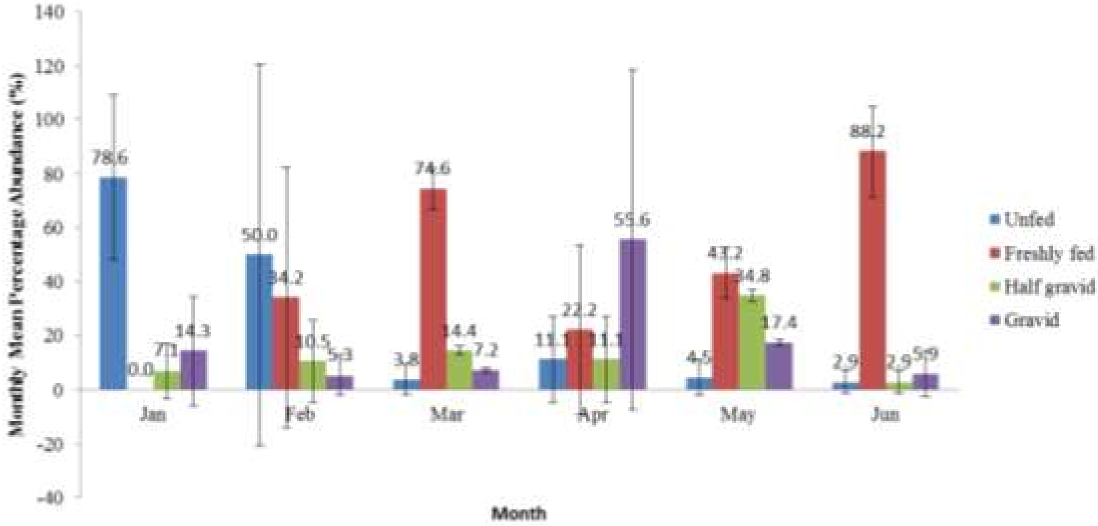

Figure 3: Blood digestion stages of *Cx. quinquefasciatus* in school dormitories in Oredo.

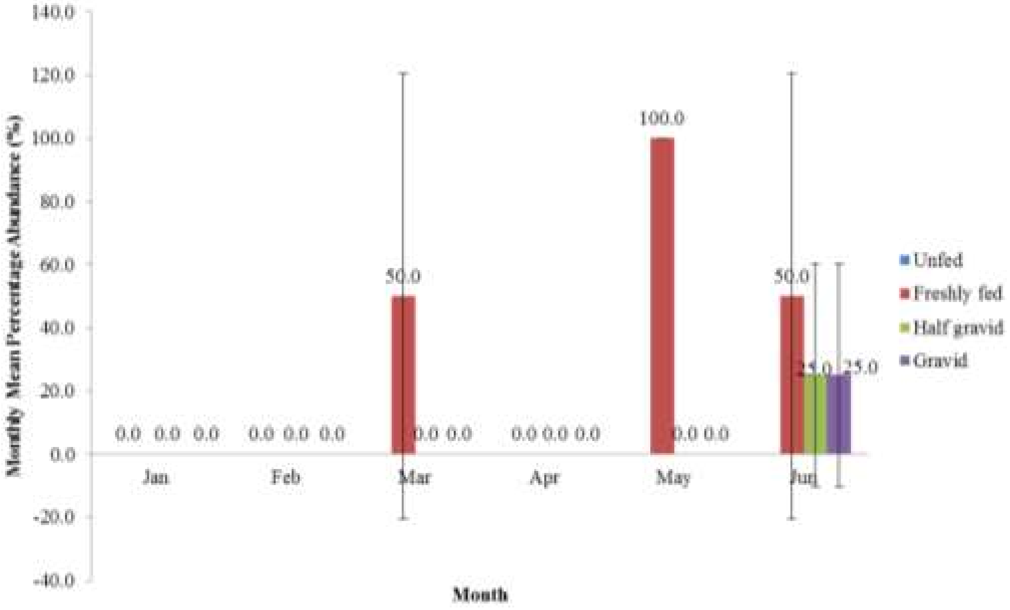

Figure 4: Blood digestion stages of *An. gambiae* in school dormitories in Oredo.

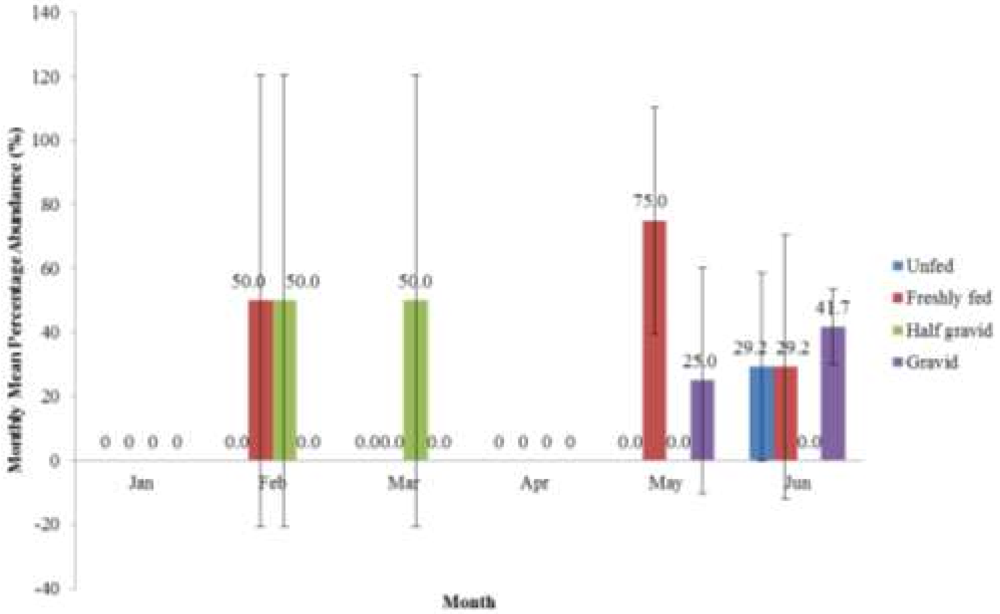

Figure 5: Blood digestion stages of *An. gambiae* in school dormitories in Ikpoba Okha.

Table 3: Correlation between blood digestion stages and weather condition in school dormitories in Benin City

Gonotrophic Stage Vs Climatic Condition	Correlation coefficient
Unfed *Cx. quinquefasciatus* vs Temperature	-0.3
Unfed *Cx. quinquefasciatus* vs Relative Humidity	-0.3
Unfed *Cx. quinquefasciatus* vs Rainfall	-0.8*
Freshly fed *Cx. quinquefasciatus* vs Temperature	-0.7
Freshly fed *Cx. quinquefasciatus* vs Relative Humidity	0.4
Freshly fed *Cx. quinquefasciatus* vs Rainfall	0.5
Half gravid *Cx. quinquefasciatus* vs Temperature	0.7
Half gravid *Cx. quinquefasciatus* vs Relative Humidity	0.0
Half gravid *Cx. quinquefasciatus* vs Rainfall	-0.2
Gravid *Cx. quinquefasciatus* vs Temperature	0.2
Gravid *Cx. quinquefasciatus* vs Relative Humidity	-0.9*
Gravid *Cx. quinquefasciatus* vs Rainfall	-0.5
Unfed *An. gambiae* vs Temperature	-0.7
Unfed *An. gambiae* vs Relative Humidity	0.7
Unfed *An. gambiae* vs Rainfall	0.7
Freshly fed *An. gambiae* vs Temperature	0.0
Freshly fed *An. gambiae* vs Relative Humidity	0.6
Freshly fed *An. gambiae* vs Rainfall	0.4
Half gravid *An. gambiae* vs Temperature	0.0
Half gravid *An. gambiae* vs Relative Humidity	0.5
Half gravid *An. gambiae* vs Rainfall	-0.2
Gravid *An. gambiae* vs Temperature	-0.5
Gravid *An. gambiae* vs Relative Humidity	0.7
Gravid *An. gambiae* vs Rainfall	0.8*

* Correlation is significant at the 0.05 level (2-tailed)

Discussion

There was higher total abundance of the proportion of freshly fed mosquito vectors (58.6% and 57.1%) compared to other blood digestion stages in the collective data from the LGAs for *Cx. quinquefasciatus* and *An. gambiae* respectively. These findings agree with that of Adeleke *et al.* (2010) that reported higher abundance of blood fed *An. gambiae* (61.64%) in Abeokuta, Ogun State. Blood fed mosquitoes were also reported to be more in abundance (42.05%) compared to other stages for the total indoor resting mosquitoes (*An. gambiae*, *An. funestus*, *Cx. quinquefasciatus* and *Cx. Annulioris*) which were collected in Nnamdi Azikiwe University Female Hostel in Awka (Umeanaeto *et al.*, (2017). Similarly, blood fed *An. gambiae* were reported to have higher abundance values of 196 individuals from southern Ethiopia (Lelisa *et al.*, 2017), 63.2% in Russau village in Jos-North LGA of Plateau State (Njila *et al.*, 2019) and 34.3% in 7 towns/villages in 7 LGAs in 3 eco-vegetational zones in Bayelsa State (Ebenezer *et al.*, 2014) compared to other gonotrophic stages The comparatively higher overall proportion of freshly fed blood digestion stage and lower unfeds as reported in the findings of Njila *et al.* (2019) was attributed to none compliance of sleeping under the mosquito nets by inhabitants of the dwellings and the dearth of an efficient vector control system in place to prevent mosquito bites (WHO, 2015).

Records of higher proportion of freshly fed blood digestion stage in each study LGA and also as per the result of the collective data of all the LGAs combined is indicative that efforts made by the dormitories' dwellers in controlling the vector populations such as having either their windows or doors covered with net screens, use of insecticide treated bed nets and use of chemical insecticides were all inadequate. The purpose of control is unachieved when the tools deployed are not formidable and in good condition such as expired, weak and/or torn Long Lasting Insecticidal Nets (LLINs), screens with tears, and the likes. These were the case with most dormitories in the study.

The unfed *Cx. quinquefasciatus* were relatively higher in number in in the months of January (78.6±30.3 %) and February (50.0±70.7 %) in Oredo LGA. This is contrary to the findings of Okorie *et al.* (2014) where highest unfed blood digestion stages were reported in most of the study months in *An. gambiae* in December 2012, January, April and June 2013 while for *Cx. quinquefasciatus,* in all the study months (December 2012 to June 2013) except June 2013. Hassan *et al* (2019) also reported higher abundance of unfed mosquitoes in April, May and June study periods in Gitata and Panda communities of Karu LGA of Nasarawa State. On the other hand, Lelisa *et al* (2017) had no record of higher abundance of the unfed blood digestion stage from June to December 2014 in southwestern Ethiopia. The presence and upsurge in the number of unfed mosquitoes in some of the months in this study is linked with ineffective control of nearby breeding sites larval and indoor adult mosquito vector populations in the areas. Higher mean percentage abundance of gravid *An. gambiae* s.l. was recorded in Ikpoba Okha LGA in the rainy season month of June (41.7±11.8 %). Relating this finding with the number of eggs a female mosquito is able to lay during oviposition which is up to 200 (Service, 2012; Fereda, 2022) is something to be concerned with - as this could translate to geometric increase in the vector population and disease burden thereafter. Months that had no record of blood digestion stage may have been periods when the dormitory dwellers' were on vacation or the mosquitoes caught were all males that are not used for blood digestion studies.

Gravid and unfed *Cx. quinquefasciatus* mosquitoes had significant negative correlations with relative humidity and rainfall respectively. This result was seen in January; being the least humid month, it had the highest mean percentage abundant gravid *Cx. quinquefasciatus* (14.5±4.8 %) in comparison to other months. Furthermore, the month of January where the monthly mean percentage abundance of unfed *Cx. quinquefasciatus* was highest (18.3±5.6 %) also had the lowest monthly total rainfall compared to other months. *An. gambiae* being positively correlated significantly with rainfall recorded its highest monthly mean percentage abundant gravid population in the study in June (40.4±13.6 %) also with the highest monthly rainfall. The variance in weather condition of lower relative humidity and higher rainfall may have helped to produce conducive microclimates within the dwellings that favoured the digestion of blood meals by the respective resting *Cx. quinquefasciatus* and *An. gambiae* mosquitoes till the gravid stage. Rainfall is needed for the successful existence and maintenance of the larval habitats of most mosquito species (Paaijmans *et al.,* 2007). However, flooding of these habitats as a result of excessive rainfall reduces the number of larvae that eventually emerge as adults; thereby causing a decrease in the number of the unfed and host seeking newly emerged adult population. This may have been the case with the negative relationship that existed between unfed *Cx. quinquefasciatus* and rainfall. Similar to our findings, Okorie *et al.* (2014) reported the collection of highest monthly unfed and gravid *Cx. quinquefasciatus* mosquito samples in the dryer months in December 2012 (n = 398) and January 2013 (n = 19) respectively. In that study, December had the least monthly rainfall and January had the third least monthly relative humidity readings. Moreover, Lelisa *et al.* (2917) reported relatively higher monthly data of half gravid and gravid *An. gambiae* s.l. mosquitoes from southwestern Ethiopia in the long rainy season months of July (n = 24; second highest monthly data) and August (n = 69; highest monthly data). The fewer outcomes of number of significant correlations gotten from the assessment of the relationship between the different blood digestion stages of the mosquito vectors and the various weather parameters may have been as a result of the suggestive considerably smaller number of mosquitoes and months that were computed. The total number of unfed and freshly fed *An. gambiae* and *Cx. quinquefasciatus* mosquito vectors collected in this study has been previously reported by Omoregie and Aigbodion (2022).

Conclusion

The result of higher mean percentage abundance of the different blood digestion stages at different times especially for the freshly fed stage has to be considered and proper measures taken in preventing further human – mosquito contact and by extension, exacerbation of the risk of malaria and lymphatic filariasis transmission. A holistic and pro-active approach that would involve good sanitation measure and deploying other effective vector control tools that targets both the egg, immature and adult stages of the mosquitoes should be adopted in a strategic form.

Conclusion

We appreciate the leadership of the respective schools whose dormitories were used in the study and also for appropriate support they provided. The technologists and laboratory staff members of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin are acknowledged for their assistance in the laboratory aspect as well.

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