AGE STRUCTURE OF MOSQUITO VECTORS FROM BOARDING SCHOOL DORMITORIES IN BENIN CITY, EDO STATE, NIGERIA

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ABSTRACT

Anopheles and Culex species, notorious vectors of malaria and lymphatic filariasis diseases respectively are known to thrive in poor third world countries. Regular monitoring of entomological indices are necessary pre-requisites for instituting effective control against the vectors. An assessment of the parity of Anopheles gambiae and Culex quinquefasciatus mosquitoes and their relationships with temperature, relative humidity, rainfall and malaria cases was carried out in school dormitories in Egor, Oredo and Ikpoba Okha Local Government Areas (LGAs) of Benin City, Edo State. Resting adult mosquitoes were collected from randomly selected school dormitories using sweep net and mechanical aspirator between 08:00 and 11:00 hours, twice every month for 6 months. Collected mosquitoes were identified and classified as being freshly fed and unfed using relevant published keys. A total of 283 female Cx. quinquefasciatus (n = 265; 93.6 %) and An. gambiae (n = 18; 6.4 %) were dissected and examined for parity. There was significant difference (p<0.05) between total parous and nulliparous rates of An. gambiae in all the LGAs it occurred and also for Cx. quinquefasciatus, except in Ikpoba Okha (p>0.05). Variation in the monthly parous rates of the study mosquitoes in all their LGAs of incidence were not significant (p>0.05). Although, no significant association existed between parous rate of study mosquitoes and weather conditions (p>0.05), parous An. gambiae and treated malaria cases related highly significantly (p<0.01). Results from this study emphasize the need for establishment of effective control against mosquito vectors within and around human dwellings.

Keywords: *Culex quinquefasciatus*, *Anopheles gambiae* s.l., Parous rate, Weather condition, Treated malaria cases

INTRODUCTION

In most mosquito species, eggs can only be produced and deposited when mating and insemination has occurred (OECD, 2018). As a rule, female mosquitoes mate before taking a first blood meal, but in several anophelines a large proportion of virgins may blood-feed prior to mating. Such a blood meal is essential for the development of a metabolic energy reservoir and the necessary nutrients it requires for her egg development (anautogenous development) with the exception of a few (autogenous development) (Service, 2012).

The parity status of mosquito vectors which depends on the proportion of mosquitoes that have ever blood fed, is among the most important factors in vector-borne diseases' epidemiology (Uttah *et al.*, 2013). It is a key indicator in assessing vector competence, the length of gonotrophic cycle, recruitment rate and longevity of adult vectors (Goindin *et al*., 2015). This means that parous mosquitoes (mosquitoes that have previously laid at least a batch of eggs successfully) are older mosquitoes with the possibility of being infected or infective. Malaria and lymphatic filariasis whose main vectors are *Anopheles gambiae* s.l. and *Culex quinquefasciatus,* are examples of such diseases transmitted through this means. These vectors have been previously reported as common mosquitoes that breed and also rest indoors in Benin City, Edo State (Aigbodion and Uyi, 2013; Omoregie *et al*., 2019).

As of present, only a few reports on parous rate of mosquito vectors abound in different parts of Nigeria and Africa and those that are, are mostly on anopheline mosquitoes alone (Uttah *et al*., 2013; Okorie *et al*., 2014; Amaechi *et al*., 2018; Njila *et al*., 2019; Adugna *et al*., 2022). Apart from the aforementioned, there is also paucity of such information from dedicated school dwelling for students including hostels or boarding school dormitories generally and certainly none from Edo State. This study therefore evaluated the parous rates of indoor resting *An. gambiae* and *Cx. quinquefasciatus*, their relationships (parous mosquito vectors with weather conditions and parous *An. gambiae* with malaria case data) in dormitories of school children in randomly selected day/boarding secondary schools within three Local Government Areas were Egor, Oredo and Ikpoba Okha in Benin City, Edo State.

MATERIALS AND METHODS

Study Area: This study was carried out in randomly selected dormitories of schools located in Egor, Oredo and Ikpoba Okha Local Government Areas (LGAs) respectively in Benin City, Edo State, South-South, Nigeria. The selected schools using GPS 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. Around these dormitories were stagnant water of different sources and kinds including waters in abandoned containers, collection points of waste waters, fish pond and moats. They all had capacity of supporting the breeding habits of mosquitoes. The dormitories lacked effective coordinated mosquito control program. Where nets were hanged over beds or used as protection on doors and/or windows, they were hardly in proper condition. It was either the bednets were overused and too old or were torn at some points. There were some other instances wherein screens were made only on windows without a corresponding door nets to keep the mosquito vector completely away from gaining entry into the dormitories.

Sampling of Adult Mosquito: Adult mosquitoes were collected in five randomly selected dormitories in each of the Local Government Areas twice every month using a combination of aerial net and mouth aspirator from January to June 2012. Resting mosquitoes were collected from 08:00 – 11:00 hours; during which time, the inhabitants (students) of the dormitories had all left for their classroom. Approximately, 30 minutes was spent in each dormitory. Prior to sampling, hidden places such as underneath sleeping beds, within clothes in wardrobe, around shoes, within cupboards were shook and dusted to dislodge any resting mosquito around there. Flying mosquitoes including those now dislodged were collected using the aerial net, from where they were transferred into specimen bottles with mouth aspirators. This method was best suiting for this sampling owing to the sizes of the dormitories that were visited which were mostly large. It would be difficult to use the popular pyrethrum spray catch method as adult sampling method here owed partly to the longer time it would take to clear out and re-arrange the dormitories pre spray and post spray and the large volume of insecticide it would require to create an insecticide saturated air, enough to knock down the mosquitoes. Collected mosquitoes were thereafter introduced into appropriately labelled specimen bottles laden with cotton wool and then taken to the Postgraduate Laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria for further study.

Taxonomic Identification of Mosquitoes: In the laboratory, few drops of ethyl acetate were introduced into each specimen bottle and left for about 10 minutes until live wild caught mosquitoes were totally immobilized or dead. Mosquitoes were placed individually on a glass slide and examined at x10 under a stereomicroscope and identified to the genus and species levels using gross external morphological keys as described by Coetzee (2020) and WHO (2020).

Determination of Unfed and Freshly Fed Gonotrophic Stages: Female mosquitoes were examined 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). Gonotrophic stages of the mosquito classified as unfed and freshly fed were recorded accordingly and separated for studies to determine parity status.

Determination of Parity: Ovaries of unfed and the fed female *An. gambiae* and *Cx. quinquefasciatus* were dissected to determine parity by observing the degree of coiling of tracheoles (WHO, 2003; Hugo *et al*., 2014). Anesthetized adult females were placed in a drop of physiological saline or soapy water solution on a glass slide under the stereomicroscope using forceps and thereafter dissected to extract the ovaries. The ovaries were further viewed under the compound microscope to determine their parity status as either nulliparous or parous. Females whose ovaries have tracheolar skeins that are coiled were regarded as nulliparous while ovaries with stretched out tracheoles are parous. The parous rate was calculated using the formula: Parous Rate = Number of parous females / Number of females dissected.

Collection of Malaria Data: The number of cases of students treated for malaria infection for the period of the study was obtained from the dispensary unit of each school.

Collection of Meteorological Data: Meteorological data of mean monthly temperature (%), mean relative humidity (%) and total rainfall (mm) covering the study period was collected from the Nigeria Meteorological Agency (NIMET) Benin Office at the Benin Airport, Benin City. The data on weather condition used for this study have been reported elsewhere (Omoregie *et al*., 2019).

Statistical Analysis: Data collected were analysed using a number of statistical tools including Statistical Package for Social Scientists (SPSS 16.0) and Microsoft Excel, 2010. Data presented in tables were expressed as percentages, means and standard deviations. The Chi-square test, Analysis of Variance (ANOVA) and unpaired t-test were used to determine variation in the parous rates of study mosquitoes. Tests for significant difference in relationships between the parous rate of mosquitoes and weather condition and, parous rate of *An. gambiae* and treated malaria cases in the study LGAs were done using the simple linear correlation. Significance in comparisons was set at $p<0.05$.

RESULTS AND DISCUSSION

Variation in Parous Rates of the Mosquito Species: A total of 283 female mosquitoes identified as *Cx. quinquefasciatus* (n = 265; 93.64 %) and *An. gambiae* (n = 18; 6.36%) were dissected and examined for parity. The *Cx. quinquefasciatus* mosquitoes being greater in number than *An. gambiae* is not unconnected with the presence of stagnant water of different sources around the vicinities of the dormitories that support the breeding of mosquito species as earlier described. Previous reports (Okogun *et al*., 2005; Adeleke *et al*., 2008; Egwu *et al*., 2018) have shown the proliferation of mosquito vector species around suitable habitats. Our observation supports the findings of Aigbodion and Uyi (2013) who reported *Cx. quinquefasciatus* to be more abundant of all the mosquito species encountered in pools, containers, domestic run-offs and gutters in Benin City, Edo State. Egwu *et al*. (2018) also reported higher abundance of *Cx. quinquefasciatus* (40 %) than *An. gambiae* (14 %) in Ohafia, Abia State, Nigeria.

There was significant difference between the total parous and nulliparous rates of *Cx. quinquefasciatus* in Egor (χ^2 = 4.55, df = 1, p<0.05); Oredo (χ^2 = 12.63, df =1, p<0.05) except in Ikpoba Okha where the difference was not statistically significant (χ^2 = 1.44, df = 1, p>0.05). Also for *An. gambiae*, the variation in parous and nulliparous rates were significant in Oredo (χ^2 = 36.00, df = 1, p<0.05) and Ikpoba Okha LGAs (χ^2 = 29.16, df = 1, p<0.05). The differences between the total parous and nulliparous rates may have been as a consequence of the dynamics in the presence and proximity of active vector breeding sites to human habitats/dwellings, suitable weather conditions, impact of control measures (if any and how effective) against the vector population and other factors that influence mosquito biology and ecology (WHO, 2003; Service, 2012). The finding of a non-significant variation between the total parous and nulliparous rates observed for *Cx. quinquefasciatus* in Ikpoba Okha LGA was in agreement with the report of Njila *et al.* (2019) of a similar non-significant difference (p>0.05) between the parous and nulliparous mosquito vectors studied in Russau village of Jos-North LGA of Plateau State. Adugna *et al.* (2022) also had corresponding report on variation between the overall parous and nulliparous *Anopheles* mosquitoes in Bure district of north-western Ethiopia.

The monthly mean parous rate of *Cx. quinquefasciatus* across the study months were observed to be higher in February (65.3 ± 13.7 %), March (77.5 ± 3.5 %), May (70.3 ± 1.6 %) and June (59.9 ± 26.4 %) in Egor; January (62.5 ± 17.7 %), March (59.2 ± 1.2 %), May (71.7 ± 16.5 %) and June (79.8 ± 18.5 %) in Oredo and March (55.7 ± 22.8 %), May (75.0 ± 0.0 %) and June (52.3 ± 3.2 %) in Ikpoba Okha LGA. Rates in *An. gambiae* were also observed to be highest in the month of May in both Oredo (100.0 ± 0.0 %) and Ikpoba Okha (75.0 ± 35.4 %) LGA which may have been as a result of build-up of the vector population owing to the well favoured environmental condition for their proliferation at this time of the year; added to a likely case of an overwhelmed uncoordinated system of mosquito vector control in the study localities permitting them access to blood meal and subsequent laying of eggs (Table 1). In the study by Adugna *et al.* (2022) highest parous rate for *An. funestus* was recorded in March (83.0 %) and it was also assumed to be as a result of large collections of the vector at that time and also the presence of extended marshland.

Variation in the overall parous rate of *Cx. quinquefasciatus* and *An. gambiae* in the study was not statistically significant (χ^2 = 2.08, df = 1, p>0.05) (Table 2). Njila *et al.* (2019) also reported a non-significant difference in parity between vector types and it was attributed to likelihood factors such as constitution of the vector population by older members, continuous replenishment of the population from a nearby breeding site with high fecundity and favourable weather condition in the area including temperature, rainfall and high relative humidity (WHO, 2003; Adeleke *et al.*, 2010).

The overall parous rates of *An. gambiae* in this study differed and shared similarities with other reports including those of *An. arabiensis* (also a malaria vector) from Nigeria and other parts of the Africa: in having values like 77.8 % for A*n. gambiae* in Russau village of Jos-North LGA of Plateau State, Nigeria (Njila *et al.*, 2019); 57.1 % for *An. gambiae* s.s. in Beere, Ibadan North/East LGA, Oyo State, Nigeria (Okorie *et al.*, 2014); 69.4 % for *An. gambiae* s.l. in Metema-Armachiho lowland, Northwest Ethiopia (Aschale *et al.*, 2020); 63.0 % for *An. arabiensis* in Lanre district, Ethiopia (Taye *et al.*, 2017); 49.3 % for *An. arabiensis* in South-eastern Tanzania (Kaindoa *et al.*, 2017) and 45.0 % for *An. arabiensis* during the long rainy season in the two irrigated agro-ecosystems of western Ethiopia (Jaleta *et al.*, 2013). Also for *Culex* species, Okorie *et al.* (2014) reported parous rate of 64.9 % in Ibadan, South-western Nigeria and Njila *et al.* (2019) 51.6 % for *Cx. quinquefasciatus* in a peri-urban area of Plateau State, Nigeria.

Variation in Parous Rates in all the Study LGAs: In comparing parity rates in all the study LGAs, there was no significant difference (p>0.05) in the monthly parous rates of *Cx. quinquefasciatus* in Egor, Oredo and Ikpoba Okha LGAs and likewise *An. gambiae* in Oredo and Ikpoba LGAs.

Table 1: Parous rate of *Culex quinquefasciatus* and *Anopheles gambiae* in school dormitories in Benin City, Edo State, Nigeria

Month	*Cx. Quinquefasciatus*			*An. gambiae*	
	Egor	Oredo	Ikpoba Okha	Oredo	Ikpoba Okha
January	36.7 ± 33.0^{1b}	62.5 ± 17.7^{2c}	0.0 ± 0.0^{1a}	0.0 ± 0.0^{1}	0.0 ± 0.0^{1}
February	65.3 ± 13.7^{2b}	46.9 ± 9.8^{1a}	40.0 ± 28.3^{2a}	0.0 ± 0.0^{1}	$50.0 \pm 70.7^{2*}$
March	77.5 ± 3.5^{2}	59.2 ± 1.2^{2}	55.7 ± 22.2^{3}	$50.0 \pm 70.7^{2*}$	0.0 ± 0.0^{1}
April	30.8 ± 43.5^{1b}	33.3 ± 47.1^{1b}	0.0 ± 0.0^{1a}	0.0 ± 0.0^{1}	0.0 ± 0.0^{1}
May	70.3 ± 1.6^{2}	71.7 ± 16.5^{2}	75.0 ± 0.0^{3}	100.0 ± 0.0^{3}	75.0 ± 35.4^{3}
June	59.9 ± 26.4^{2a}	79.8 ± 18.5^{2b}	52.3 ± 3.2^{3a}	0.0 ± 0.0^{1}	$43.8 \pm 61.9^{2*}$
Total	59.4	66.3	55.8	80.0	76.9

Means on the same row with different letter superscript are significantly different (p<0.05). Mean same row with asterisk is significantly different (p<0.05). Means on the same column with different number superscript are significantly different (p<0.05)

Table 2: Overall Parous rate of study mosquito species in school dormitories in Benin City, Edo State, Nigeria

Month	*Cx. quinquefasciatus*	*An. gambiae*
January	$41.7 \pm 21.8^{1*}$	0.0 ± 0.0^{1}
February	54.6 ± 11.6^{2}	50.0 ± 70.7^{3}
March	64.5 ± 10.4^{2}	50.0 ± 70.7^{3}
April	$31.6 \pm 44.7^{1*}$	0.0 ± 0.0^{1}
May	71.3 ± 3.7^{2}	83.3 ± 23.6^{4}
June	$61.5 \pm 21.8^{2*}$	38.9 ± 55.0^{2}
Total	60.8	77.8

Mean on a row with asterisk is significantly different (p<0.05). Means on the same column with different number superscript are significantly different (p<0.05)

Variation in the study localities and LGA had no influence on the parous rate of vector types, *Cx. quinquefasciatus* and *An. gambiae*. This may have been as a result of a level of similarity in the environmental condition of the studied localities and LGAs with respect to presence of mosquito breeding sites in the form of pools of stagnant water, moat and unused container litters around the dormitories and an ineffective and uncoordinated control strategy in handling the mosquito vector problem. These factors presented a situation in all the LGAs in having a consequent similar chance of intensity of mosquito infestation and access to obtaining blood meal from human hosts; thus affecting the parity status.

Unlike the findings of this study, Adugna *et al.* (2022) reported a significant difference (p<0.05) in parous rates in the study villages in Bure district in north-western Ethiopia, wherein *An. arabiensis* recorded its highest rates (52.0 %) in Shnebekuma village than other villages and *An. cinerus* in Bukta (63.0 %) than other villages.

Relationship between Parous Rate of the Mosquitoes and Weather Condition: Test of relationship between the monthly mean parous rate of the studied mosquito species and the concurrent weather conditions showed a non-significant relationship (p>0.05) (Table 3).

Table 3: Correlation between parous mosquito species population and climatic condition in school dormitories in Benin City, Edo State, Nigeria

Parous mosquito species vs. Climatic condition	Correlation value
An. gambiae vs. Mean temperature	0.7
Cx. quinquefasciatus vs. Mean temperature	-0.1
An. gambiae vs. Relative humidity	0.6
Cx. quinquefasciatus vs. Relative humidity	0.6
An. gambiae vs. Total rainfall	0.4
Cx. quinquefasciatus vs. Total rainfall	0.5

Mosquitoes tend to increase their quest for blood meals during times of higher environmental temperature due to increased sweat production by the human hosts which attract them. The time it takes to digest the blood meal taken up also depends on temperature (Service, 2012). Although this has a great probability of ultimately increasing parity rates of the vector population, result of their relationships between the mosquito vectors and the weather conditions clearly indicated the presence of other factors that influenced the parity rates in this study which include proliferation of mosquito breeding sites around the dwellings and the ease of their access to blood meal. Okorie *et al.* (2014) also reported the variation in monthly parity rates of mosquito vectors to have been possibly connected with the seasonal abundance of mosquito breeding sites. This emphasizes the importance of nearby breeding sites to determining parity rates. In North Central Nigeria, Amaechi *et al.* (2018) recorded more parous female anopheline mosquitoes in the dry season (48.1 %) than in the rainy season (31.4 %). Uttah *et al.* (2013) also reported highest parity rate of the anopheline mosquitoes in the dryer month of December (91.3 %) in Ekorinim area of Calabar, Cross River State, Nigeria.

Relationship between Parous Rate of *An. gambiae* and Treated Malaria Cases: Relationship between the sum total monthly mean parous *An. gambiae* and total record of treated malaria cases in the LGAs where they occurred were significantly different (p<0.05) (Table 4).

Table 4: Relationship between parous *Anopheles gambiae* mosquito population and treated malaria cases in school dormitories in Benin City, Edo State, Nigeria

Parous Mosquito Vs. Malaria cases	Correlation value
***Anopheles gambiae* vs. Malaria cases**	1.0**

*** Correlation is significant at the 0.01 level*

Generally, there were more parous *An. gambiae* and *Cx. quinquefasciatus* in this study compared to nulliparous ones. Records of higher parous rates are indications that the vector population comprises older adult females and have a high rate of survival (WHO, 2003). This also implied higher chances of carrying and transmitting their related disease pathogen to suitable hosts successfully. Therefore, it is highly likely that the months of February, March and May had comparably increased transmission of malaria parasite and February, March, May and June of filarial worm parasites; if present in the population in the school dormitories in the combined LGAs in Benin City. However, this will further be enhanced by complementary environmental and weather conditions (Mohammadkhani *et al*., 2016; Laneri *et al*., 2019). The possibility of that of higher malaria transmission in the above said months is further supported by the highly significant positive relationship that existed between the parous female malaria vector and treated malaria cases in this study.

The records of zero parity gotten from some of the studied LGAs may have been times when the period of sampling fell into the time of students' holiday or all the mosquitoes collected during that time were all nulliparous or didn't meet the requirement for those that could be used for parous rate determination. Parity rates of a given mosquito vector species in any locality at a given time by the ovary tracheation method used in this study can be gotten only when the female mosquitoes collected meet the requirement for being used to determine parity (WHO, 2003; Hugo *et al*., 2014). Hence, results of parity rates are likely to vary as seen in this study and in other reports (Njila *et al*., 2019; Adugna *et al*., 2022).

Conclusion: Results from this study accentuate the need for reduction of man-adult mosquito contact irrespective of the predisposed season or weather condition and especially in places that recorded higher parous rates. This could be done through proper planning and execution of effective control measure against the mosquito vectors such as the use of bednets, doors and windows screens and, the use of insecticides. Reductions of larval sources in the form of breeding sites around dwellings should also support the vector control drive. This will not only reduce the parous rate of the indoor resting mosquito vectors alone but also reduce their populations in the first instance and thereby ensuring reduced risk of disease transmission altogether.

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