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The Circadian and seasonal biting patterns of *Anopheles gambiae* sl in Bayelsa State, Nigeria

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Accurate knowledge on the biting pattern of *Anopheles gambiae* sl is a prerequisites for mounting long lasting control intervention in malaria endemic areas. A descriptive study was undertaken to determine the hourly biting cycle of *A. gambiae* in some randomly selected communities in Bayelsa State, Nigeria during January, 2014 and December, 2015. The two methods used for mosquito collection were human baits (indoor and outdoor). Hourly mosquito collections were undertaken twice quarterly from 1900 to 0400 h. Mosquitoes collected were identified morphologically following a standard key. The hourly and seasonal biting rates of *A. gambiae* were calculated. Two thousand and seven female *A. gambiae* were identified in 16 man-night. The total bites were 62.72 bites/person/night. The mosquito biting rates were higher outdoor than indoor collections. Similar results were recorded for seasonal and ecovegetation collections. Wet seasons had higher mosquito biting rates (71.58 bites/person/night) than that of the dry seasons collection (36.358 bites/person/night). The biting rates of *A. gambiae* sl were 2-fold higher in fresh water than in brackish water swamp forest and mangrove coastal water forest. The hourly biting rates of *A. gambiae* peaked at 2300 h, while the seasonal biting rates peaked at 2300 h and 4 am. This result has demonstrated the inefficiency of the indoor residual spray (IRS) and long lasting insecticidal nets (LLNs) as the only malaria control measure in this area. Outdoor protective control measure is recommended alongside indoor IRS and LLNs.

Key words: Circadian, Seasons, biting patterns, *Anopheles gambiae*, Bayelsa State.

INTRODUCTION

Anopheles gambiae is the principal malaria vector confined exclusively in sub-Saharan Africa (Gillet, 1972; Coetzee, 2004). Their vectorial competence is controlled by several factors, such as ability to locate their host and initiate infective blood meal bite (Mboera et al., 1997), availability of breeding sites (Okiwelu and Noutcha, 2012), poor sanitation habit through urban expansion (Nwoke and Eboh, 1991) and climatic conditions that enhances the parasite development (Theresa et al., 2006).

Malaria accounts for over 1.09 million deaths mostly in children under the age of 5 years around the globes (WHO, 2006) and 90% of total deaths in Africa (NIH, 2001). In Nigeria alone, malaria account for over 60%

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of the total outpatient visits to health facilities, 30%childhood death and 11% of maternal death (NDHS, 2011). Every year, the nation loses over N132 billion for cost of treatment and absenteeism from work, schools and farms (FMH, 2005). In Bayelsa State, malaria infection account for 102 deaths out of 210 recorded from 35 different diseases in the year 2011 (Ambah, 2012).

The menace of mosquito borne diseases has been controlled with different strategies around the globe (WHO, 1995; Najera, 2000). A strategy targeted at reducing the mosquito-man-contact has proven to be most promising (Lwetoijera et al., 2013; Trig and Wemndorfe, 1995). Insecticide treated bed nets and long lasting insecticidal nets are the most widely used intervention in Nigeria to break vector human contact and reduce incidences of malaria (WHO, 2005). Despite the enormous effort to control mosquito bites in Nigeria, the incidences of malaria still remain unchanged.

Insecticide treated bed nets, long lasting insecticidal nets and residual spray have demonstrated a positive potent in reducing man mosquito contact in many malaria endemic areas (WHO, 2007). However, these interventions have also changed the behavior of *Anopheles* mosquitoes from biting indoor to biting outdoor (Moiroux et al., 2012). In Bayelsa State, a personal interaction with most people showed that the use of the non-treated bed net are more predominant than the treated net especially among the rural dwellers. This may have accounted for the high incidence rates of malaria recorded in previous studies in the same area (Ebenezer et al., 2014). Good knowledge on the biting pattern of *A. gambiae* is a pre-requisite for monitoring control measures of malaria infection in endemic areas (Kabbale et al., 2013). Studies have demonstrated the biting behavior of *A. gambiae* in some parts of Nigeria (Awolola et al., 2002; Awolola et al., 2003; Oyewole et al., 2007; Okwa et al., 2009). This information is scarce in Bayelsa State. This pioneering study shall therefore provide base line information on the hourly biting cycle of *Anopheles gambiae* in Bayelsa State.

MATERIALS AND METHODS

Study area

Bayelsa State (5°22′- 6°45′E and 4° 10′- 5° 23′N) copies the lower deltaic plan of Nigeria (Alagoa, 1999). The study was conducted in two randomly selected communities across the 3 eco-vegetation. Details about the study locations have been extensively described (Ebenezer et al., 2012).

Mosquito collections

Two collection methods were adopted: indoor and outdoor human catch. A modified bed net trap in Kabbale et al. (2013) was employed. The bed net trap was constructed to the shape of a pyramid with a diameter of 6 × 4 × 5 ft. The bed net trap was constructed with non-treated nets. Each side of the pyramid was made up of 3 layers of the non-treated net. The second layer was perforated into 8-10 inches holes. The third layer was permanently fixed while the first layer was designed in the form of flip over on the second layer. The first layer serves as door, which are closed against the second layer after an hourly interval. This is in a bid to reduce mosquito escape at the time of collection. The third layer that is permanently fixed gives protection to the human bait. The whole pyramid was set up over a wooden bed with human sleeping on it as bait.

Two sets of human bait volunteers were involved; one serving as indoor catchers and one serves as outdoor catchers. A total of 16 volunteers were involved in the study; two collectors per night per station. They were made to sleep on the bed net trap at some distance apart. Mosquito collection was made from 1900-0400 h for two consecutive nights at each quarter during January, 2014 and December, 2015. In each night, the catchers were made to sleep on the constructed net, leaving the first layer of the trap totally flipped open. Mosquitoes attracted to the human bait were trapped in the second layer. At each hour, the first layer of the net construction was flipped down and the mosquitoes trapped at the second layer were collected using mouth aspirator. The hourly collection represents the mosquito that actively sought for blood meal bite from the host in that particular hour.

Mosquitoes collected were placed in different Petri dishes duly labeled against the hour of collection. Morphological identification of the mosquitoes was undertaken at the Entomology Laboratory of the Department of Animal and Environmental Biology, University of Port-Harcourt. The identification followed standard key in Gillies and Coetzee (1987). *Anopheles gambiae* were preserved dried in silica gel in a labeled 1.5 ml Eppendorf tube for further studies.

Ethical consideration

Verbal consents were obtained from the community heads and house hold heads. Consents were also obtained from human bait volunteers after properly informing them on the purpose of the study. Each volunteer was treated with anti-malaria drugs on and after each collection night.

Data analysis

Data were analyzed in SPSS version 20.3 software. Chi-square and ANOVA were the statistical tools used.

RESULTS

A total of two thousand and seven female *Anopheles gambiae* were identified in 16 man – night of an indoor and outdoor collections. Details are shown in Table 1. The biting rate of *A. gambiae* was 62.72 bites/person/night. The outdoor mosquito abundance (70.5%) was higher than that of the indoor mosquito abundance (29.5%) with statistically significant difference (F=50.241 df= 1; p<0.05). The biting rates of *A. gambiae* of outdoor collection (88.44 bites/person/night) was higher than that of the biting rates of indoor collections (37.00 bites/person/night) with significant difference (t=12.083 df=1 p<0.05).

A. gambiae biting rates vary across seasons. In wet seasons, *A. gambiae* biting rates was 71.58 bites/person/night. During dry seasons, the biting rate was 36.13

Table 1. Biting rates of *A. gambiae* in Bayelsa State, January, 2014 and December, 2015.

Variables	No. of catchers	No. of night	Man- night	No(%) of Anopheles	Biting rates
Collection methods					
Indoor	8	8	16	592 (29.98)	37.00
Outdoor	8	8	16	1415(70.50)	88.44
Total	16	16	32	2007	62.72
Seasons					
Wet					
Indoor	6	6	12	543 (31.60)	45.25
Outdoor	6	6	12	1175(68.40)	97.93
Total	12	12	24	1718(85.60)	71.58
Dry					
indoor	2	2	4	75 (26.00)	18.75
outdoor	2	2	4	214 (74.40)	53.50
Total	4	4	8	289 (14.40)	36.13
Ecovegetation					
FWSF					
Indoor	8	8	16	290(28.88)	18.13
Outdoor	8	8	16	714(71.12)	44.63
Total	16	16	32	1004(50.02)	62.75
BWSF					
Indoor	8	8	16	181(33.39)	11.31
Outdoor	8	8	16	361(66.61)	22.26
TOTAL	16	16	32	542(27.01)	16.94
MCWF					
Indoor	8	8	16	144(31.24)	9.00
Outdoor	8	8	16	317(68.72)	19.81
Total	16	16	32	461(22.97)	14.41

FWSF = Fresh water swamp forest; BWSF = brackish water swamp forest; MCWF = mangrove coastal water forest.

bites/person/night. The differences were significant (F=9.685 df=1 $p<0.05$). The biting rates of *A. gambiae* in fresh water swamp forest were 2-fold higher than those in brackish water swamp forest and mangrove coastal water forest, respectively. The differences were significant (X^2=9.488 df=4 $p<0.05$).

The hourly biting patterns of *A. gambiae* vary across seasons (Figure 1). The biting pattern was said to be biphasic peaking at 2300hrs and 4am.

DISCUSSION

The biting rates of *A. gambiae* in this present studies was 62.72 bites/person/night. This value was 17-fold higher than the 3.51 reported by Bradley et al. (2015) in Bioko, Equatorial Guinea. The significantly higher outdoor biting rates than indoor biting rates correspond with the report of Kenea et al. (2016). The observed higher outdoor biting rate is an indication that the indoor control measure could be rendered ineffective (Bradley et al., 2015). Although, in this study, speciation of the *A. gambiae* were not undertaken, the higher outdoor catches may be unrelated to the already existing plasticity of most vectors to host preferences (Noutcha and Anumudu, 2009; Sane et al., 2016). It is possible that most of the *A. gambiae* caught were rather host seeking than resting. *A. gambiae* showed higher propensity to biting outdoor.

Studies have demonstrated that the biting behavior of A. *gambiae* was altered when IRS or LLNs were used as indoor control interventions (Moiroux et al., 2012). Surprisingly, such intervention is not feasibly monitored in these study locations. A personal interaction with most rural dwellers showed that people relied mostly on the use of the untreated bed net than the treated net for mosquito control. Both outdoor and indoor mosquito density reported in this study highlighted the adaptive indoor and outdoor biting behavior of *A. gambiae* (Taye et al., 2016).

The hourly seasonal biting rates of *A. gambiae* in this

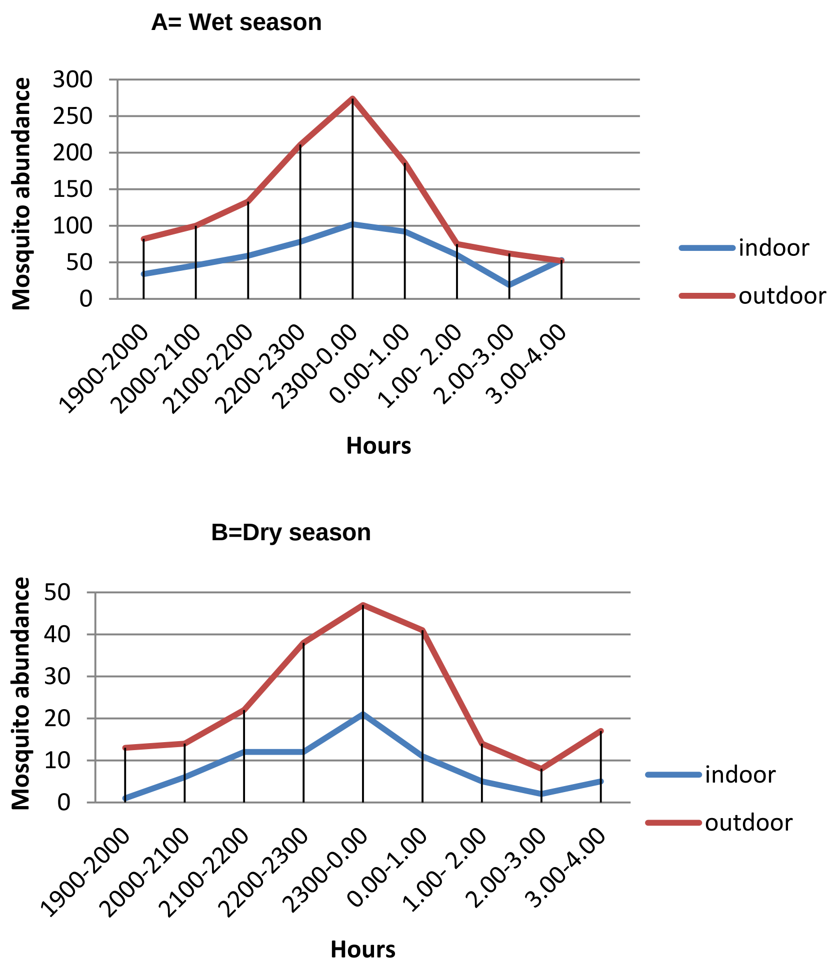

Figure 1. Seasonal circadian *A. gambiae* biting rates.

present study highlighted the importance of suitable timing for mosquito control intervention (Kabbale et al., 2013). The high biting rates during wet seasons is an indication that availability of mosquito breeding sites can increase the propagation of larva population that transits to adulthood. High humidity during the raining seasons is responsible for the high density of adults (Sande et al., 2016).

Mosquitoes in the study locations bite all-night. The biting pattern was said to be biphasic peaking at 23:00 h and 3am. This biting pattern has been reported elsewhere (Amerasinghe and Indrajith, 1995; Sande et al., 2016; Kenea et al., 2016). However the report contrasted the findings of Taye et al. (2016) who reported a biphasic cycle that peaked at 8 pm and 3 am. The peak biting period of *A. gambiae* in this present study corresponds with the exposure time of the inhabitants to fishing activities. The double peak period correspond exactly with late fish trapping period and the early fish harvesting period at the study locations. This timing has been reported by Cooke et al. (2016).

Hourly biting rates was higher outdoor. This highlights the possibilities of acquiring vector-borne infections even before and after bed time; Fishing and trading activities are the two major occupations in the area (Alagoa, 1999). These activities exposes people to outdoor biting of *A. gambiae,* which could also be one of the determinant of higher incidence rates of malaria recorded in previous studies (Ebenezer et al., 2014*).*

CONCLUSION

A. gambiae in this study have shown the tendency of biting all night. This is a concern for public health intervention. However, outdoor biting rates were higher than those of the indoor biting rates. This behavioral plasticity may undermine the conventional indoor control intervention with IRS and LLNS. More so, since the peak biting rates of the mosquitoes synchronize with fishing and trading activities of inhabitants in the study locations, it is recommended however that an outdoor protective coat impregnated with pyrethroid could be the best control measure for outdoor activities when the IRS and LLNs are used indoor.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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