
Temporal Ecologic Adaptability of the Principal Vector of Malaria, *Anopheles gambiae* s.l. (Diptera: Culicidae), in North-central Nigeria

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ABSTRACT: The need for a better understanding of vectorial epidemiology of malaria transmission in North-central Nigeria, a pre-requisite for effective control, informed this study. The study elucidated seasonal dynamics of bio-behavioural ecology of the principal vector of malaria in the area, i.e., *Anopheles gambiae* s.l., in relation to the species potentials for disease transmission. Adult female mosquitoes were collected bi-weekly, from locations typical of the ecotypes of North-central Nigeria, using all-night human landing catches. Mosquito collections were made both indoors and outdoors during the seasonal periods identified namely, dry, transition and rainy seasons. The wing length of the mosquitoes was measured as a proxy for adult body size. Immature Developmental rates and insecticide susceptibility of the mosquito species were evaluated during the peak of each seasonal period, following standard WHO procedures. Significant ($P < 0.05$) seasonal variations in vectorial attributes were exhibited by *An. gambiae* in the area. The species was most abundant in the rainy season and least encountered during the dry period. While the mosquitoes were significantly exophagic during dry and transition seasons (56.22 and 63.50% respectively), they showed no such locational feeding preference during the rainy season. Analysis of hourly biting density of the mosquitoes indicated that they were not active during the first half of the night (i.e., 1700 – 2400hrs), both indoors and outdoors, during the transition season. However, while outdoor human biting activity was most intense during the early part of the second half of the night (i.e., 2400 - 0300hrs) in the rainy season, indoor hourly biting density varied within narrow limits in the dry season. While, immature survival rates were generally high (>80%) and insignificantly different ($P > 0.05$) in all seasons, duration of development ranged significantly from 9.40 ± 2.45 days in the dry season to 11.20 ± 2.67 days in rainy. Also, significantly smaller mosquitoes (mean wing length = 2.94 ± 0.36 mm) were encountered in the dry season. The adult mosquitoes were susceptible to permethrin insecticide in all seasons, unlike the larval stage that indicated potential resistance during transition and rainy seasons. The findings of this study revealed that seasonal influence plays significant roles in vectorial dynamics of malaria transmission in North-central Nigeria, thus, providing requisite information for fine-tuning malaria vector control strategy for year-round optimum efficacy.

Keywords: Feeding Location, Human Biting Rates, Immature Development, Insecticide Susceptibility, Seasons and Wing Length.

INTRODUCTION

Anopheles gambiae is the principal vector of malaria in sub-Saharan Africa (Gillies and Coetzee, 1987; Sinka, 2010), where more than 90% of the World's clinical cases are recorded (Sachs and Malaney, 2002; Breman, 2001refs). According to recent World Health Organisation's statistics, malaria threatens the health of about two-thirds of the world's human population; resulting in as much as 600 million clinical attacks and an estimated one million deaths annually. The disproportionately high

intensity of malaria transmission in sub-Saharan Africa is due to the widespread distribution and high vectorial capacity of the primary vector, i.e., *An. gambiae* in the region (Hodges, 2013). Studies have established this anopheline species as one of the most efficient transmitter of *Plasmodium* parasites in the world. The epidemiological success of *An. gambiae* is largely dependent on its highly dynamic ecological behavior (White, 2011), that have evolved over a long time to take advantage of certain tropical clement weather conditions that promote mosquito proliferation and human/vector contact.

Though, *An. gambiae* is widely distributed in sub-Saharan Africa, its behavior and ecological adaptability vary considerably from one locality to another, partly dictated by spatio-temporal differences in seasonal weather conditions (Gimonneau, 2010; Olayemi, 2011). Such temporal variations in anopheline vector behavior, in response to seasonal changes in weather conditions in an area, are responsible for enormous heterogeneity in the intensity of malaria transmission and efficacy of control measures (Paajimans, 2011). Studies have shown that in the rainy season, anopheline mosquitoes tend to be more endophagic, endophilic and anthropophagic, in order to avoid the harsh environmental conditions outdoor (Jaeson, 1994; Paison, 2004; Loaiza, 2008; Olayemi and Ande, 2008a;). Also, these mosquitoes breed more in natural larval habitats, such as temporary sunlit ground-pools, in the rainy season due to high proliferation of such sites during the period, as well as, guaranteeing faster developmental and higher survival rates (Olayemi and Ande, 2008b; Imbahale, 2011). The local interactions of combinations of these important entomological drivers of malaria transmission, occasioned by behavioural responses of anopheline mosquitoes to prevailing weather conditions, will go a long way in determining vectorial efficiency and hence, the patterns of malaria transmission, as well as, the efficacy of implemented vector-control measures. Anopheline mosquitoes pose less threat to human health during periods when they are compelled to be zoophilic or breed in less productive and hazardous sites. Also, residual indoor-spraying with insecticides such as pyrethroids and the use of insecticide-treated bed nets are more effective at controlling malaria vectors, when such vectors prefer to feed and rest indoors (Roland, 2000).

Nigeria, with its distinct tropical annual rainy and dry seasons, coupled with local heterogeneity in the intensity and distribution of climatic factors such as rainfall, relative humidity and temperature, in its different eco-geographical zones, is bound to elicit behavioural changes in anopheline mosquitoes at different times of the year, that may influence the epidemiology of malaria and efficacies of vector control measures. Though, the patterns of population dynamics of anopheline mosquitoes in almost all the geo-ecological zones of Nigeria have been elucidated (Awolola, 2002; Oyewole, 2005; Olayemi and Ande, 2005c&d; White, 2011), similar variations in behavioural traits in relation to the seasons of the year remain poorly understood. This study was, therefore, carried out to fill this information gap by determining and comparing certain aspects of the behavior of *An. gambiae* including, peak time and locational biting preferences, duration of development and survivorship of immature stage, susceptibility to insecticides, etc, during the different seasonal periods in North-central Nigeria.

MATERIALS AND METHODS

Description of the Study Area

The study was carried out in North Central Nigeria, which serves as a gate-way between the northern and southern parts of the country. Two State Capital cities namely, Ilorin and Minna, generally representative of the eco-type of the area, were selected for data collection. While Ilorin is located within longitudes 4°30' and 4°45' E, and latitudes 8°25' and 8°40' N, covering an estimated land area of 75km² with about 1.4 million human population; Minna, on the other hand, lies within longitude 6°33' E and 9°37' N on a land area of 88km², and having an estimated 1.2 million inhabitants (The Nigerian Congress, 2007 and The World Gazetteer, 2007). The climate in North Central Nigeria is that of a tropical continental region (Iloje, 1972), which is characterized by relatively wide annual temperature range and a restricted rainfall. The mean annual temperature range from 27.00°C to 30.20°C in Ilorin and Minna, respectively, and mean annual relative humidity is higher in the former (76.00%) than the latter (61.00%) while, mean annual rainfall range from 1,334.00mm in Minna to 1,800mm in Ilorin.

The climate of the area is marked by two distinct weather seasons, i.e., rainy and dry. The rainy season starts in the month of May and lasts till October, with June and August as the months of peak rainfall. The dry season, extending from December to March, is completely devoid of rains and characterized by harmattan with dust-laden cold winds informed by the Northeast Trade wind. The departure of harmattan, for the rains is informed by the arrival of moist tropical maritime air mass of the Southwest Trade wind. This point is usually marked by hot sunny days with temperature range of 34 – 40°C, the highest of which occur in March. The months of April and November, being transition periods at the beginning and end of the rainy season, respectively, usually record little amount of rainfall. For the purpose of this study, the seasons in study area were divided into three, on the basis of the amount of recorded rainfall, the principal climatic factor determining seasons in the Tropics. The three seasons are: Rainy (May – October), Dry (December – March) and Transitional (April and November). The vegetation in North Central Nigeria reflects that of the Guinea Savanna zone, characterized by a predominance of tall grass species with scattered trees. This vegetation is frequently removed by bush burning in the dry season.

Mosquito Collection, Preservation and Identification

Anopheline mosquitoes were collected monthly from the study area between 2004 and 2009. The larvae were sampled from randomly selected conventional mosquito breeding habitats, using standard 250ml capacity 'Dippers' (Service, 1993), between the hours of 0700 and 1200. Twenty 'Dipper' samples were taken randomly from each breeding habitat type, and the average of these samples was calculated. On the other hand, the adult female mosquitoes were collected from randomly selected sites using the Spread-sheet Pyrethrum Spray Catches (SPSC) and all-night Human Bait Catches (HBC) (World Health Organization, 1975). HBC mosquito collections were carried out both indoors and outdoors simultaneously, between the hours of 1700 and 0700 the following day. Collected mosquito specimens were preserved using 4% formaldehyde solution and identification was done using the keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987).

Determination of Species Composition and Relative Abundance

Species composition and relative abundance of the anopheline mosquitoes were determined by noting the total number of species, at both larval and adult stages, and percentage distribution of such species in the total specimen collections, respectively.

Determination of Biting Behaviour

Adult mosquitoes collected using all-night Human Landing Catches, were sorted according to the hour of collection. Mosquito density was calculated as number of specimens caught per man per hour.

Determination of Adult Body Size and Wing Symmetry

Insecticide-treated Test Papers

The susceptibility of the anopheline mosquitoes to Permethrin were characteristically conducted during the months of August, February and April, being periods typical of the three seasons, i.e., Rainy, Dry and Transitional seasons, respectively. Permethrin was selected as the insecticide of choice, as a result of the on-going World Health Organization's Roll-Back-Malaria campaign in Nigeria, emphasizing the use of pyrethroid insecticide treated bed nets. The larval and adult anopheline insecticide bioassays were carried out according to WHO recommended procedures (WHO, 1981; WHO, 1998).

Collection of Meteorological Data

Meteorological data of rainfall, temperature and relative humidity during the study period, for Ilorin, were obtained from the Records Department of the weather station in Ilorin International Airport while, similar data for Minna were obtained from Minna Airport.

Data Analysis

Mosquito Larval Density (LD) was determined as the average number of specimens collected per 'Dip'. Adult Mosquito Density, expressed as Human Biting Rate (HBR), was calculated as the total number of specimens collected from a room divided by the number of people that slept in the room the previous night (World Health Organization, 1975).

RESULTS AND DISCUSSION

RESULTS

Seasonal influence on abundance of *Anopheles gambiae* feeding both indoors and outdoors was apparent during the study period (Figure 1 & 2). On the whole, significantly ($P < 0.05$) higher densities of the mosquito species were collected during the rainy season, and least encountered in the dry season. Outdoor nocturnal biting density of the mosquitoes was distinctly uni-modal during the rainy season but multi-modal during the transition and dry seasons, though, those of the former were not very distinct (Figure 1). While intense outdoor human biting activities of the mosquitoes occurred during the first half of the night (i.e. 1700-2400 hours) in the transition and dry season; it extended into the early part (i.e. 2400-0300 hours) of the second half when peak biting rate was recorded, during the rainy season.

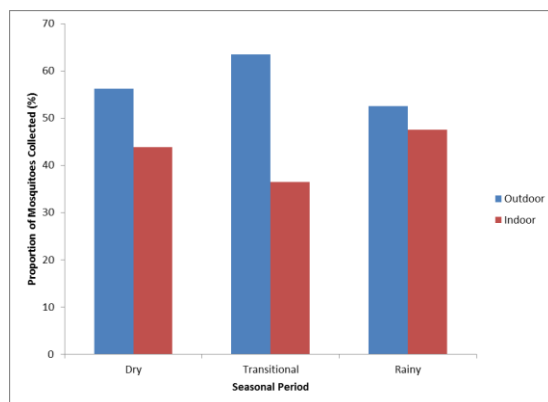


Figure 1. Feeding location preferences of *An. gambiae* mosquito populations in North-central Nigeria, during the seasonal periods

The pattern of distribution of indoor human biting rates of the mosquitoes differed from that of those biting outdoors. While outdoor mosquitoes human biting rates were bi-modal and multi-modal in the rainy and dry season respectively, those of the dry season showed no clear peaks, though, intense biting activities were recorded late in the night (2300-0400 hours) during this season (Figure 2). Also, while outdoor intense biting rates of mosquitoes were recorded in the 2nd half of the night during the rainy season, the mosquitoes were more active during the 1st half in the transition season.

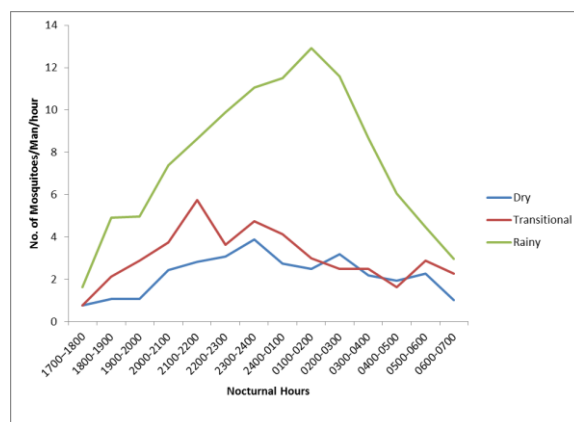


Figure 2. Hourly distribution of outdoor biting density of *An. gambiae* mosquito populations in North-central Nigeria, during the seasonal periods

The locational feeding preferences of the mosquitoes are presented in figure 3. The mosquitoes were significantly ($P < 0.05$) exophagic during the dry and rainy seasons (56.22 and 63.50% respectively), but showed no such significant ($P < 0.05$) preference for biting either outdoor or indoor during the rainy season. The effects of seasons on developmental indices and insecticide susceptibility of *Anopheles gambiae* in northcentral Nigeria are highlighted in Table 1. While survival rates of the immature stage were generally high ($>80\%$) and varied insignificantly across the seasons, the duration of development differed significantly (range= 9.40 ± 2.45 days in the dry season, to 11.20 ± 2.67 days in the rainy season). Wing length (proxy for adult body size) varied within narrow limits between the transition and rainy seasons (mean= 3.20mm) but however, the *Anopheles gambiae* mosquitoes collected in the dry season were significantly smaller (mean wing length= $2.94 \pm 0.36\text{mm}$). While the adult stage of the mosquito species was entirely (i.e. 100%) susceptible to pyrethroid insecticide in all the seasons, indications of resistance were recorded among the larval populations during transition and rainy seasons.

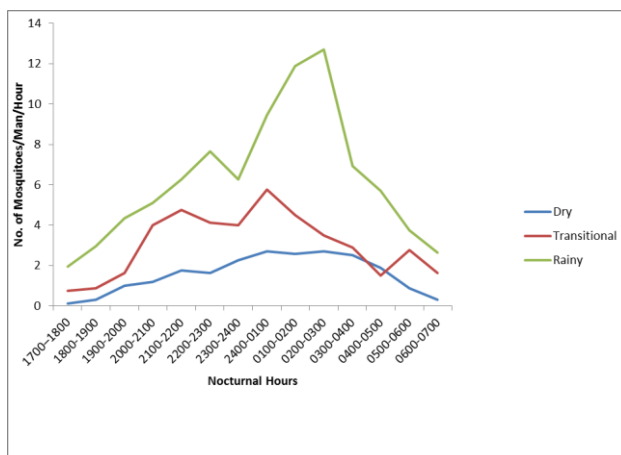


Figure 3. Hourly distribution of indoor biting density of *An. gambiae* mosquito populations in North-central Nigeria, during the seasonal periods

Table 1. Mean climatic factors and entomologic attributes of *An. gambiae* mosquito populations in North-central Nigeria, during the seasonal periods

Parameters	Seasonal Period		
	Dry (Dec. – Mar.)	Transition (Apr. and Nov.)	Rainy (May – Oct.)
Climatic Factors			
Temperature (°C)	28.91±0.66 ^{ab}	28.84±0.74 ^b	26.18±0.33 ^a
Rainfall (mm)	7.06±6.33 ^a	37.36±14.32 ^b	194.39±7.56 ^c
Relative Humidity (%)	45.47±17.53 ^a	58.69±16.06 ^b	77.06±6.53 ^c
Entomologic Attributes			
Duration of Immature Development (days)	9.40±2.45 ^a	10.65±1.11 ^{ab}	11.20±2.67 ^b
Survivorship of Immature Stage (%)	84.14±6.90 ^a	83.90±2.57 ^a	82.45±4.24 ^a
Wing Length (mm)	2.94±0.36 ^a	3.20±0.30 ^b	3.22±0.12 ^b
Larval Insecticide Susceptibility (%)	100.00±0.00 ^b	96.93±2.04 ^a	98.24±0.95 ^{ab}
Adult Insecticide Susceptibility	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a

DISCUSSION

The ecology and behavior of *An. gambiae* mosquito populations investigated in this study were greatly influenced by the prevailing seasons in North-central Nigeria. Significantly ($P < 0.05$), higher mosquito densities were encountered in the rainy than other seasons, i.e., transition and dry. Marked seasonality in spatial and temporal distributions of anopheline mosquitoes are typical of the Tropics (Shililu, 2003; Munga, 2006); and the preponderance of anopheline mosquitoes in the rainy season have been attributed to the proliferation of rain pools, the preferred breeding sites of anophelines, as well as the resultant improved humidity for adult- mosquito survival and dispersal (Fillinger, 2004; Koendraat, 2004). The significantly higher densities of anopheline mosquitoes collected during the rainy season in this study, to a large extent, explains the equally seasonal pattern of clinical cases of malaria, with peak transmission shortly after maximum annual rainfall, in Nigeria as a whole (Leighton and Foster, 1993) and North-central part of the country in particular (Olayemi, 2009).

The pattern of modal distribution of hourly biting density of the mosquitoes, both indoors and outdoors, varied considerably with seasons. This finding indicate that variable weather conditions that characterize different seasons in the Tropics influence anopheline blood feeding activities and, hence, malaria transmission. For example, relative humidity affects mosquito flight activities and blood meal seeking behavior (Vandyk, 2006). Also, the results of this study showed significant seasonal influence on the preferences of anopheline mosquitoes for feeding indoors and outdoors. Therefore, with malaria vector control in Nigeria, as a whole, relying heavily on mass distribution of insecticide-treated bed nets, a tool whose effectiveness demands that anopheline populations be principally endophagic and bite late in the night (Lengeler and Snow, 1996; Curtis, 2006), there is need to incorporate other vector control strategies, especially during seasonal periods when and/or where significant anopheline biting activities are at variance with functional requirements of bed nets.

The immature survival rates of the anopheline mosquitoes were generally high and insignificantly different in all seasons. These results suggest that the anopheline species have evolved to adapt optimally to the diverse tropical conditions prevailing in North-central Nigeria. This may mean equally high anopheline production and, hence, malaria transmission even in the dry season, if larval breeding habitats are available. This is more so, as the duration of immature development was significantly reduced in the dry season. The faster rate of immature development in the dry season is, probably, due to the relatively higher temperatures that characterize the season.

Significantly, smaller anopheline mosquitoes were collected in the dry than other seasons. This may be due to ecologic stress on the mosquitoes during immature development, and occasioned by inclement dry season weather conditions (Olayemi, 2008; Yoshioka, 2012). Ecological studies have established positive proportional relationships between anopheline body size and vectorial capacity for malaria transmission (Menge, 2005). This finding could contribute a quota to the significantly higher intensities of malaria transmission in North-central Nigeria during the rainy season (Leighton and Foster, 1993; Olayemi, 2009), when significantly bigger mosquitoes are produced judging from the results of this study.

To a large extent, the *An. gambiae* population in North-central Nigeria, is still susceptible to pyrethroid insecticide, despite decades of the use of the insecticidal compound in the country. This finding while, it justifies the continued retention of pyrethroids as the insecticidal compound of choice in the production of treated bed nets, for malaria vector control in the study area, calls for pro-active management of suspected resistance among the larval populations, as significant seasonal influence on insecticide susceptibility was apparent. According to World Health Organisation's guidelines for assessing the susceptibility of mosquitoes to insecticides (WHO, 2005), 24-hours post-exposure mortality of <98% indicates susceptibility; 80 – 98% suggests probable resistance that calls for close monitoring; and <80% means that the mosquito population is resistant. The significant lower insecticide susceptibility of the larval population in the rainy than dry season, for example, may be due to the selective pressure occasioned by exposure of the larvae to agricultural insecticides of the same or related Class as pyrethroids, that are washed from farmlands to surrounding mosquito breeding habitats. North-central Nigeria is known for widespread intensive crop production, characterized by heavy insecticide usage, to the extent that the region has acquired the status of 'Food basket of the Nation'.

CONCLUSION

Seasonal influence plays significant roles in the dynamics of behavior and ecology of *An. gambiae*, the primary vector of malaria in North-central Nigeria. Such influences favour increased transmission of malaria in the rainy season, as well as, the bridged-sustenance of transmission during the less conducive dry season. The seasonal variability in feeding behavior namely, peak biting period and preferred biting location, of this important vector threatens the success of the on-going WHO's RMB-supported malaria vector control in North-central Nigeria. The main-thrust, i.e., the use of insecticide-treated bed nets, is more effective against endophagic mosquitoes biting late in the night; vector behavior that are at variance with those demonstrated by *An. gambiae* in this study, especially during the dry and transition seasons, when significant proportions of the mosquitoes were exophagic and early night biters. There are indications that the immature population development of the mosquito species has adapted optimally to the different seasons in the area, as well as, seasonal tendency for the development of resistance to pyrethroids (the first-line insecticide for mosquito vector control in the area), during the rainy season when malaria transmission is significantly at its peak. The findings of this study have provided stake-holders with information for fine-tuning malaria vector control strategies in North-central Nigeria, in a way that the program will, in addition, be season-sensitive, for optimum year-round success.

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