

## Research Article

# Filariasis Control in Coastal Nigeria: Predictive Significance of Baseline Entomological Indices of *Anopheles gambiae* s.l. (Diptera: Culicidae)

Emmanuel C. Uttah,<sup>1,2</sup> Dominic Ibe,<sup>3</sup> and Gloria N. Wokem<sup>4</sup>

<sup>1</sup> Department of Animal and Environmental Biology, University of Port Harcourt, Port Harcourt, Nigeria

<sup>2</sup> Department of Biological Sciences, Cross River University of Technology, Calabar, Nigeria

<sup>3</sup> Public Health Technology Programme, Federal University of Technology, Owerri, Nigeria

<sup>4</sup> Department of Medical Laboratory Sciences, Rivers State University of Science and Technology, Port Harcourt, Nigeria

Correspondence should be addressed to Emmanuel C. Uttah; drecuttah@yahoo.com

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This work aimed at collecting filariasis transmission data of *Anopheles gambiae* to be used in predicting future trends in filariasis transmission and control programme outcomes. Collection of the mosquitoes was made by human landing catch and light trap methods. In all, 5,813 females were caught from September 2005 to August 2006. Mosquito population started to expand at the onset of the rains. The highest density was found after peak temperature. The *A. gambiae* s.l. biting peaked around midnight; 39.7% were parous and 0.3% were infective. The highest percentage of parous females caught was near midnight, ranging between 42.0% and 47.5% from 22.00 to 03.00 hours. Biting rate in the rainy season was 2.6 times higher than it in the dry season. Transmission potential was 3.6 times higher during the rains than during the dry season. The percentage infectivity was relatively high (13.2%) in June, corresponding to 8.8 infective bites per person per month. All infective *A. gambiae*, were caught between 22.00 and 03.00 hours. The average load of L3 larvae per infective *A. gambiae* was 1.4 L3/mosquito. The monthly transmission potential calculated for each month indicated that transmission was ongoing for most of the months of the year, especially in the rainy season.

## 1. Introduction

The World Health Organization did estimate that over 1.25 billion people (18 percent of the world's population) are at risk of lymphatic filariasis in 83 countries and territories, with approximately 120 million already infected and over 40 million seriously incapacitated and disfigured by the disease [1]. The overall global burden of disease (GBD) estimates suggest a global filariasis case prevalence of 3.39% (for both *Wuchereria bancrofti* (order Spirurida, family Onchocercidae) and *Brugia malayi* (order Spirurida, family Onchocercidae) infections) in exposed populations, with the highest regional bancroftian filariasis prevalence of (9.0%) in sub-Saharan Africa [2].

To deal decisively with this global menace, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was

launched to massively administer drugs for 4 to 6 years, with the goal of achieving worldwide elimination of this mosquito-borne parasitic disease by the year 2020 [3, 4]. To achieve this, the need for close monitoring of infection trends to ascertain the right time to declare cessation of transmission cannot be overemphasized. Although the prevalence of microfilaraemia is an indicator of transmission levels that are monitored at sentinel sites [1], verification of quotients of microfilaraemia in the human population is not enough for one to be quantitatively certain of the dynamics of incidence of new infections [5]. To this end, monitoring the corresponding changes and levels of infection indicative of transmission endpoints in the vector population becomes extremely necessary; therefore, parameters such as annual biting rates (ABRs) and annual transmission potential

(ATP) from dissected mosquitoes collected from human landing catch and light trap methods would be central in this regard [4, 6]. Additionally, knowledge of the biology and ecology of the mosquito is essential for predicting outbreaks of disease and control of mosquitoes [7]. Furthermore, there exist possible tangents between microfilaria (Mf) density in human blood and the number of third stage (L3) larvae developing in some mosquito vector species after blood-feeding [8]. This observation deserves further exploration to establish correlates to enable the estimation of prevalence of microfilaraemia in the human population using the infective and infection rates among the vectors in an endemic area. This would be desirable as it would deemphasize the use of protocols that involve the collection of human blood. With the well-known barriers posed by sociocultural beliefs and superstitions against collection of human blood during filariasis surveys and with the HIV/AIDS pandemic posing serious threat around the world, there is increasing apathy by subjects towards presenting themselves for blood collection during surveys, and therefore compliance rates at filariasis surveys have fallen over the years. Furthermore, verification of success or otherwise of control programmes will not have to rely solely on the use of expensive and cumbersome human blood protocols but on the relatively convenient vector studies protocols.

As the filariasis elimination programmes get underway around the world, the need for control predictive signatures from entomological indices as efficient monitoring tools in filariasis elimination becomes a crucial priority [4]. This work is a preliminary longitudinal study and it is aimed at collecting entomological indices on *Anopheles gambiae* (Diptera: Culicidae) as baseline data to be used in monitoring future trends and control programmes outcomes in the area. The entomological indices to be ascertained include ABR, ATP, monthly biting rates (MBR), monthly transmission potential (MTP), infective and infectious rates.

## 2. Materials and Methods

**2.1. Description of the Study Area.** The study was carried out in Ogbakiri, a filariasis endemic coastal area in Rivers State, Nigeria, and a suburb of Port Harcourt, by the Atlantic Ocean. Ogbakiri is drained by the New Calabar Creek and lies in the rainforest belt, with a mean annual rainfall of 2,403 mm. The temperature range is between 19°C and 33.2°C, while the mean relative humidity is 80%.

**2.2. Method of Collection of *A. gambiae*.** Every week, during 12 months from September 2005 to August 2006, two nighttime human landing catch (HLC) collections were carried out. A four-man team of collectors alternated pair-wise between collecting and resting from 18.00 to 06.00 hours (18.00 hours to 22.00 hours outdoors, 22.00 to 06.00 hours indoors), following the indoor and outdoor most common behavioral pattern of the local endemic community. The pairing of collectors and working hours were shifted systematically on each catching day to eliminate any possible bias that might arise from differences in each individual's

attraction and catching prowess. Hourly collections were kept separately in labeled cups, which were covered with fine nylon nets held with rubber bands. After each hourly collection, the cups with the mosquitoes were provided with a pad of wet cotton wool on the top and kept cool in an insulated box with cooling elements until dissected (those alive), after being sexed and identified based on external morphology using Gillies and Coetzee [9], Gillies and de Meillon [10] and Edwards [11].

During dissection, ovaries were extracted and quickly transferred to a drop of distilled water on a slide, left to dry before being examined under high magnification for tracheal skeins and classified as parous or nulliparous [12]. The head, thorax, and the remaining parts of the abdomen were then separated and put in different drops of saline. These parts were teased and examined under the microscope at  $\times 40$  magnification for filarial larvae. Filarial larvae were identified as L<sub>1</sub>, L<sub>2</sub>, or L<sub>3</sub> larvae after World Health Organization [13] and counted. All *A. gambiae* females were dissected fully whether parous or nulliparous.

**2.3. Data Analysis.** The SPSS for Windows (1995 version) was used for both entering of data and for data analysis. The annual biting rate (ABR) and the annual transmission potential (ATP) were calculated after Walsh and coworkers [14]. ABR is an estimate of the number of a particular vector coming to bite one person who is exposed to biting during all biting hours of the vector every day for one year. It was calculated as the annual total of the monthly biting rate (MBR) as follows:

$$\begin{aligned} \text{MBR} = & (\text{number of females collected person per month} \\ & \times \text{number of days in the month}) \\ & \times (\text{number of catching days in the month})^{-1}. \end{aligned} \quad (1)$$

ATP is an estimate of the total number of infective larvae (L3) transmitted to one person exposed to biting during all biting hours in a year and was calculated as an annual total of the individual monthly transmission potential obtained from the following formula:

$$\text{MTP} = \frac{(\text{total number of L3 larvae per month} \times \text{MBR})}{(\text{number of female vector dissected per month})}. \quad (2)$$

## 3. Results

**3.1. Abundance, Seasonality, and Circadian Biting Pattern.** A total of 5,813 *A. gambiae* females were caught from September 2005 to August 2006 by human landing catch (see Table 1). Abundance followed the seasons. The population started to expand at the onset of the rains. The circadian biting activities of *A. gambiae* showed a pronounced peak around midnight but maintained a relatively high level throughout (see Table 2). The indoor catch exceeded almost three times the outdoor catch.

TABLE 1: Monthly entomological indices for *Anopheles gambiae* in the costal Nigeria based on human landing catch.

Month	Number of days	Collection dissected (%)	Number of <i>A. gambiae</i> females parous (%)	infective (%)	(L3/inf) <sup>a</sup>	Number of L3s	MBR <sup>b</sup>	MTP <sup>c</sup>
September 2005	4	468	328 (70.1)	120 (36.6)	0 (0)	0 (0.0)	1755	0.0
October 2005	4	416	290 (69.7)	112 (38.6)	3 (1.0)	5 (1.7)	1612	27.8
November 2005	5	293	193 (63.9)	132 (68.4)	1 (0.5)	2 (0.5)	879	9.1
December 2005	5	211	169 (80.1)	73 (43.2)	0 (0.0)	0 (0.0)	654	0.0
January 2006	4	238	191 (80.3)	110 (57.6)	1 (0.5)	2 (0.5)	922	9.7
February 2006	4	235	203 (86.4)	88 (43.3)	0 (0.0)	0 (0.0)	852	0.0
March 2006	4	288	217 (75.3)	89 (41.0)	0 (0.0)	0 (0.0)	1116	0.0
April 2006	4	367	330 (89.9)	114 (35.5)	2 (0.6)	2 (1.0)	1376	8.3
May 2006	6	566	412 (72.8)	141 (34.2)	0 (0.0)	0 (0.0)	1462	0.0
June 2006	4	763	652 (85.5)	271 (41.6)	2 (0.3)	3 (1.5)	2861	13.2
July 2006	4	838	733 (87.5)	287 (39.2)	2 (0.3)	2 (1.0)	3247	8.9
August 2006	4	1130	975 (86.3)	325 (33.3)	2 (0.2)	2 (1.0)	4379	9.0
Total	52	5813	4693 (80.7)	1862 (39.7)	13 (0.3)	18 (1.4)	21115 <sup>d</sup>	86.0 <sup>e</sup>

<sup>a</sup>L3/inf: number of L3 per infective *A. gambiae*.<sup>b</sup>MBR: monthly biting rate.<sup>c</sup>MTP: monthly transmission potential.<sup>d</sup>ABR: annual biting rate.<sup>e</sup>ATP: annual transmission potential.

TABLE 2: Circadian variation in parity and infectivity of human landing *A. gambiae* s.l. in coastal Nigeria.

Time	Dissected	Number of <i>A. gambiae</i> females		Number of L <sub>3</sub>
		Parous (%)	Infective	
18.00–19.00	286	84 (29.4)	0	0
19.00–20.00	297	85 (28.4)	0	0
20.00–21.00	309	95 (30.7)	0	0
21.00–22.00	307	118 (38.4)	0	0
22.00–23.00	518	246 (47.5)	1	1
23.00–00.00	620	292 (47.1)	5	7
00.00–01.00	582	272 (46.7)	5	6
01.00–02.00	526	239 (45.4)	0	0
02.00–03.00	324	136 (42.0)	2	4
03.00–04.00	219	73 (33.3)	0	0
04.00–05.00	276	84 (30.4)	0	0
05.00–06.00	429	138 (32.2)	0	0
Total	4693	1862 (39.7)	13	18

TABLE 3: Seasonality of entomological indices of *A. gambiae* s.l. in coastal Nigeria.

Description	Dry season	Rainy season
Total number of days	152	214
Number of catch days	22	30
Number of females collected	1265	4548
Number of females dissected (%)	973 (76.9)	3720 (81.8)
Number parous (%)	492 (50.6)	1370 (38.8)
Number infective (%)	2 (0.2)	11 (0.3)
Number of L3s (L3/inf)	4 (2.0)	14 (1.3)
Seasonal biting rate	4423	16692
Seasonal transmission potential	18.8	67.2

**3.2. Parity and Infectivity.** A high percentage (80.7%) of the mosquitoes collected by human landing catch remained alive until dissected within thirty hours of collection. Out of these, 39.7% were parous and 0.3% were infective. An average of 1.4 L3 larvae per infective mosquito was obtained, making *A. gambiae* s.l. the proven vector.

The percentage of females that were parous was significantly higher in the dry season (50.6%; November to March) than in the rainy season (36.8%; April to October) ( $\chi^2$ -test;  $P < 0.001$ ).

The monthly and circadian variation in parity of man-landing night biting females (see Table 3) showed that the highest percentage of parous females caught was near midnight. Parity was in the range of 42.0%–47.5% from 22.00 hours to 03.00 hours. The lowest percentage of parous females (28.4%) was recorded from 19.00 to 20.00 hours. The difference in parity between the peak from 22.00 to 2.00 hours (56.7% of total parous) and the rest of the night (33.2% of total parous) was statistically significant ( $\chi^2$ -test;  $P < 0.001$ ).

The monthly transmission potential was the highest in October (27.8), corresponding to 16.7 infective bites per person per month. It was also relatively high in June (13.2), corresponding to 8.8 infective bites per person per month. It

was lowest in September, December, February, March, and April when no infective larvae were found. Generally, the MTP did not follow any definite pattern in relation either to parity or relative abundance. The parity tended towards an inverse relationship to relative abundance.

All the infective *A. gambiae* were caught between the hours of 22.00 and 03.00, with the peak from 23.00 to 01.00 hours. The average load of L3 larvae per infective *A. gambiae* was 1.4 L3/mosquito.

In terms of seasonality of entomological indices (see Table 3), more mosquitoes survived until dissected during the rainy season than during the dry season ( $\chi^2_{M-H} = 15.13$ ;  $P < 0.001$ ). The biting rate in the rainy season was 2.6 times higher than that in the dry season. The transmission potential was also 3.6 times higher in the rainy season than in the dry season. The percentage of infectivity was higher in the dry season, but this was not statistically significant ( $\chi^2_{M-H} = 0.23$ ;  $P > 0.05$ ).

#### 4. Discussion

Bancroftian filariasis is endemic in coastal Nigeria [15], where *A. gambiae* is the predominant vector species. Understanding the factors that regulate the size of mosquito populations is fundamental to the ability to predict transmission rates and for vector population control [16]. Effective control of vector-borne parasitic infections through vector management requires information on the abundance of vectors in the targeted areas [17]. In this study, there were marked seasonal variations in the mosquito abundance, with more abundance recorded during the rainy season than during the dry season. This was expected as mosquito population density variations are closely linked to rainfall and temperature [18, 19]. The pattern observed in this study was similar to those reported elsewhere [17, 20]. The rains make more vector breeding sites available, and therefore areas of rain-dependent agriculture precursor ideal aquatic habitats that support high density of diverse mosquito species [21]. The shallow edges of the

New Calabar River, which is the predominant water body in the study area, provide suitable all-year-round breeding sites for *A. gambiae*. The other breeding sites such as sunlit pools and forest ponds, as well as numerous temporary ponds which are the most numerous during the rainy season add to ensure sustenance of higher population density during the rains. A similar seasonal pattern of biting preponderance during the rainy season has been reported from many regions of the world [22–28]. Observations from this study indicate that whereas rainfall had a positive relationship with the relative mosquito abundance, temperature tended to have an inverse relationship with the relative mosquito abundance. This agrees with the observations reported elsewhere [18, 19, 29].

The annual biting rate (ABR) recorded in this study was higher than that reported for this species in the forest area of Liberia, but comparable to both of that reported from the savannah area [30], and that reported in the Teressa Island [31]. The circadian biting showed pronounced peak biting between 23.00 and 02.00 hours indoors, coinciding with the time when the microfilariae of the nocturnally periodic *W. bancrofti* are abundant in the peripheral blood circulation. This is the acknowledged peak biting time for the vectors of filariasis in many endemic areas [32]. Our study was adapted after the routine times of the local people, which meant that the mosquito sampling was done outdoors during the time when people usually stayed outdoors (up till 22.00 hours) and inside (22.00–06.00 hours) when they were normally indoors. The peak biting reported in the Gambia [33] occurred later, between 03.00 and 04.00 hours.

An individual in coastal Nigeria would receive 27.2% of bites from *A. gambiae* while outdoors and 72.8% while indoors. All the infective *A. gambiae* were caught during the indoor hours, specifically between 22.00 hours and 03.00 hours, indicating that transmission of lymphatic filariasis was mostly indoors. Similarly, majority of parous females fed within this peak period, supporting the observation elsewhere that physiological age may influence the circadian biting time [34]. The risk of infection can be determined from the density of parous females collected per hour and the mean number of infective larvae per parous mosquitoes, since this has been found to correlate significantly with the prevalence of microfilaraemia [35].

Survival of *A. gambiae* between capture and dissection showed differences seasonally, being higher during the rainy season, perhaps due to higher humidity. A total of 39.7% of all collections were parous, and 0.3% were infective. Although lower than the 3.3% infective rate observed for *A. gambiae* in Malumfsshi, a savannah area of northern Nigeria about two and a half decades earlier [36], it is comparable with the 0.4% infected rate reported in Tanzanian study. This indicates that *A. gambiae* is probably a better vector in a Tanzanian area than in the Imo River Basin. The average number of infective larvae per female *A. gambiae* was 1.4 L3s per infective female. This may reflect a lower intensity of microfilariae in humans in coastal Nigeria, since the number of microfilariae in the mosquito is directly proportional to the number of microfilariae in the peripheral blood of the human being at the time of feeding [37].

The relatively lower parity rates in coastal Nigeria may be due to the fact that favourable environmental conditions ensured recruitment of abundant nullipars that led to the lower proportion of parous females caught. On the other hand, this could be related to the level of anthropophily of *A. gambiae s.l.* in the area and to their low chances of finding the human host, taking into account the working habits of the local people [38]. Parity was inversely proportional to relative abundance in coastal Nigeria.

The relatively low L3 burden in vectors in our study area may be indicative of relatively low microfilariae intensity in the study populations [8]. Microfilariae uptake by mosquitoes depends on the carrier's Mf density, but as mf densities decrease, the concentration capacity of the mosquito increases [39]. This means that the low microfilarial intensities constitute a significant pool for the infection of mosquitoes. The infectivity of the vectors biting in human populations untreated for filariasis, such as the study population, is higher than those of vectors biting in populations treated for filariasis [39].

Genetic and environmental reasons may also explain the low number of L3 larvae in *A. gambiae*, as the yield of infective *W. bancrofti* larvae may vary among different strains of the same mosquito species. The probability that an infective vector transmits infective parasites is closely related to its physiological status [39]. Secondly, the infection rate and survival rate are partly dependent on environmental conditions experienced during larval development [39]. The quality of larval diet has been reported to affect the eventual adult size of mosquitoes [40], and the adult size affects the volume of blood meal, blood-feeding behavior, duration of gonotrophic cycles, and longevity [41–43].

The annual transmission potential (ATP) was useful in assessing intensity of transmission during different months [44]. ATP was extensively used in the evaluation of Onchocerciasis Control Programme (OCP) in West Africa. The MTPs from this study indicate that transmission was ongoing for most of the months of the year, especially in the rainy season.

## Ethical Approval

Ethical approval was received from the Ministry of Health, Emohua Local Government Area, Rivers State, Nigeria and from the Department of Animal and Environmental Biology, University of Port Harcourt Ethical Committee, Nigeria.

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