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Species Composition and Role of *Anopheles* Mosquitoes in Malaria Transmission Along Badagry Axis of Lagos Lagoon, Lagos, Nigeria

I.O. Oyewole[1], C.A. Ibidapo[2], O.O. Okwa[2], A.O. Oduola[3,5], G.O. Adeoye[3], H.I. Okoh[4] and T.S. Awolola[5]

[1]Department of Biosciences and Biotechnology, Babcock University, Ilisan-Remo, Ogun State, Nigeria. [2]Zoology Department, Lagos State University, Ojo, Lagos, Nigeria. [3]University of Lagos, [4]Biochemistry Department, [4,5]Public Health Division, Nigerian Institute of Medical Research, Yaba, Lagos. Corresponding author email: oyewoleio@gmail.com

Abstract: Three communities along Badagry axis of the Lagos lagoon were sampled for indoor resting *Anopheles* mosquitoes in order to determine their species composition, relative abundance, density and contribution to malaria transmission in the coastal ecosystem. A total of 1938 adult female *Anopheles* mosquitoes collected from 2005 to 2007 constituted three species viz *Anopheles gambiae, An melas and An. nili*. The Polymerase Chain Reaction (PCR)—based tests indicated that more than three-fourth of the *An. gambiae* s.l (75.8%) population belongs to *An. gambiae* s.s the remaining were *An. melas*. Further analysis showed that all the *An. gambiae* s.s was the M form. ELISA-based analyses indicated that *An. gambiae* s.s and *An. melas* were the main vectors of malaria in this area with an overall *P. falciparum* sporozoite infection rate of 4.8% and 6.5% respectively. Both species also maintained relatively high EIR indicating their prominent roles in malaria transmission in the study area. All the *An. nili* tested were negative for *P. falciparum* sporozoite infection. This study provides baseline information for planning vector control programme relevant to reduction of malaria transmission in the coastal areas of Nigeria.

Keywords: *Anopheles gambiae, An. melas, An. nili,* PCR, ELISA, sporozoite rates, coastal lagoon, Nigeria

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Introduction

Insect-transmitted disease remains a major source of illness and death worldwide. Mosquitoes alone transmit diseases to more than 700 million people annually.[1] Malaria remains one of the major endemic diseases in the tropics due to high frequency of transmission of *Plasmodium* species by a large number of *Anopheles* mosquito.[2,3] Despite the major attempts over the past century to control malaria, vector resistance to insecticides and malaria parasite resistance to multiple drugs have stood in the way of malaria control.[4,5] The World Health Organization has recommended vector control as an important component of the global strategy for preventing malaria, while vector identification forms an essential component of the strategy.[6,7] The existence of species complexes containing morphologically cryptic sibling or isomorphic forms presents a major challenge to malaria control programmes as these require vector identification using molecular techniques.[8] *Anopheles gambiae* s.l Giles (Diptera: Culicidae) is one of the most important malaria vectors in Africa, where 90% of the world malaria cases occur.[9] The complex consists of at least seven sibling species, five of which are vectors of human malaria parasites with varying degree of efficiencies.[10–12]

Accurate identification of the several malaria transmitting genetic species within a complex, a knowledge of behavior and involvement of each in malaria transmission in the different ecological settings are desirable due to variation.[8] This work attempts to report for the first time the *Anopheles* species composition and respective role of each species in malaria transmission along Badagry axis of Lagos lagoon.

Materials and Methods

Study site

Three communities, Iworo (06°24′54^{s} N3°1′15^{s}E), Epe (06°24′52^{s} N3°2′53^{s}E) and Moba (06°24′18^{s}N 3°3′45^{s}E) along the Badagry axis of the Lagos lagoon were sampled for *Anopheles* mosquitoes. The rainy season usually lasts from April to October with scanty occurrence between January and March. There are usually two peaks of rains from April to June (338.9 mm) and September to November (94.3 mm) when heavy rainfall causes serious flash floods with small creeks becoming choked with water lettuce (*Pistia stratiotes)*. In the dry season (November to April) temperatures are high and the streams and creeks dry up. Vegetation here is characterized as a mangrove swampy forest type.

Houses are diverse in design, structure and constructing materials (mud, mat, stone, thatch, wooden, cement block, thatched or corrugated iron roofing).

Mosquito collections

Mosquitoes resting indoors were collected in the study villages twice a month between May 2005 and June 2007 using indoor pyrethrum spray catches (PSC) and hand collection methods with the aid of aspirator and searchlight. Collections were made early in the morning between 0600 and 0900 hrs in houses occupied by humans and those cohabiting by humans and other domestic animals. Specimens were collected in 10 randomly selected dwellings in each village from rooms where people slept in the previous night but different from those rooms used for human bait catches. Samples were preserved dry over silica gel in eppendorf tubes prior to identification and ELISA tests.

Night biting catches (NBC)

Adult mosquitoes were collected each night (1800–2400 hrs) on volunteer human baits following WHO[13] procedures to establish feeding habits and biting activities of the vectors. Collection was made both indoor and outdoor as soon as the mosquito landed on the host to bite or while in the process of biting. Mosquitoes were also collected occasionally where possible while biting the sleeping occupants in the selected houses.

Species identification

Identification of mosquito was done using morphological keys of Gillies and De Meillon,[14] Gillies and Coetzee.[11] Molecular assay was carried out using the species-specific PCR[15] with minor modifications as detailed in Van Rensburg et al[16] for the confirmatory identification of the members within *An. gambiae* complex. Abdominal conditions of the identified species were simultaneously analyzed.

PCR-RFLP assay was used to identify the molecular M and S forms of *An. gambiae* s.s. following the method described by Favia et al.[17]

ELISA tests

The circumsporozoite proteins of *Plasmodium* species present on the head and thorax of 209 *Anopheles* mosquitoes were tested following the method of Wirtz et al.[18] Sporozoite rates were determined photometrically as described by Beier et al.[19]

Blood meal sources of blood-fed *Anopheles* mosquitoes were identified by direct Enzyme-linked Immunosorbent assay (ELISA) for human, bovine, ovine (sheep and goat), equine (horse and donkey), pig, or chicken hosts.[20]

Ethical approval

Ethical approval for the study was granted by the Ethical Committee of the Nigerian Institute of Medical Research, Lagos, Nigeria.

Statistical analysis

Data collected were analyzed using SAS software (Statistics SAS Institute Inc., Cary, NC 27513, USA), while ANOVA was used as test statistics. The entomological inoculation rate (EIR) was calculated for each species as the product of the sporozoite and human biting rates (using data from HLC or NBC).[21] Indoor resting density was calculated as the total *Anopheles* sampled per community divided by number of sampled houses.

Results

Species composition and relative abundance

In all 1938 adult female *Anopheles* mosquitoes were collected, a significantly higher proportion 64.1% (n = 1242) ($P < 0.05$) of these were caught using PSC. Table 1 shows the species composition and their relative abundance in each location. This was expressed as the percentage of the total number of *Anopheles* collected.

Table 1. Percentage composition of *Anopheles* caught in the sampled communities.

	Total no of the *Anopheles* caught	Communities sampled		
		Iworo	Epe	Moba
An. gambiae	1461 (75.6)	637	453	371
An. melas	470 (24.0)	0	0	470
An. nili	7 (0.4)	7	0	0
Total	**1938**	**644**	**453**	**841**

The products of the species-specific PCR assay showed that species collected fall into two major groups of *Anopheles* mosquitoes: *An. gambiae* s.l. constituting 99.6% (n = 1931), and *An. nili* 0.4% (n = 7) of the total collection. *An. gambiae* s.l. constituted 75.6% (n = 1461) *An. gambiae* s.s. and 24.0% (n = 470) *An. melas*. The molecular analysis of the M and S forms of *An. gambiae* s.s indicated that the entire sample belongs to M form. There was no significant difference in the indoor resting density of *Anopheles* collected in all the communities for the period of study, 2005 ($F_{2, 33} = 1.39$, $P = 0.26455$), 2006 ($F_{2, 33} = 1.53$, $P = 0.2318$), 2007 ($F_{2, 15} = 0.73$, $P = 0.4981$). A large number of mosquitoes were sampled in Moba and Epe with a daily indoor resting density of 40.1 and 15.1 per household respectively (Table 2).

Biting activities

Large numbers of biting species of anopheline were collected in June and this coincided with the peak of the rain. Precipitation was recorded in all the months of study except in October to December 2006. Biting population followed the trend of precipitation in each year, however, the biting activity of female *An. gambiae* was more pronounced during the wet season (10.2 bite/person/night) in June and (4.3 bite/person/night) in September. The biting

Table 2. Indoor resting densities of *Anopheles* mosquitoes collected in the study communities.

Communities surveyed	Total sample	*Anopheles* density	# Houses sample	Mean	Standard deviation	95%	CI
Iworo	644 (33.2)	10.7	60	26.8	18.66	–1.6	15.5
Epe	453 (23.4)	15.1	30	18.9	12.62	–10.2	19.6
Moba	841 (43.4)	40.1	21	35.0	23.51	–6.8	10.7

Notes: *Anopheles* density, total sampled per number of household; Standard deviation, measure of the tendency of individual values to vary from the mean; 95% CI, 95% of the values within the standard deviation of the mean.

activity showed statistical difference in the level of anthropophilic rates in the three species ($F_{2, 33} = 11.1$, $P = 0.002$). However, more species of *An. gambiae* and *An. melas* were caught on human bait than was *An. nili*. A few numbers of *An. gambiae* and *An. melas* were still caught persistently biting during the drier months (Nov-Feb) (Fig. 1). Indoor biting activity in *An. gambiae* commenced around 1900 h with active biting between 2200 h and 2300 h. Biting continued throughout the night and reached the highest biting peak between 0200 h and 0300 h when most villagers have retired indoors. For *An. melas* indoor biting commenced later around 2000 h reaching the peak between 2200 h and 2300 h and decline drastically thereafter. Active biting was resumed again between 0300 h and 0500 h. *Anopheles nili* was not caught biting indoors throughout the night (Fig. 2).

Blood meal sources

ELISA results for the sources of the blood meal indicated that most (66.9%) of the blood meals were from humans. This was similar to that of the blood—fed mosquitoes caught by PSC. The percentages that had only bovine blood and those with mixed human and bovine blood meals were (1.9%) and (0.5%) respectively (Table 3).

Plasmodium falciparum sporozoite 'Rates'

Table 4 shows that 6.0% *An. gambiae* s.s (n = 84) were found positive for *P. falciparum* circumsporozoite antigen at Iworo compared to 3.0% and 5.2% at Epe (n = 67) and Moba (n = 58) respectively. Of the total 209 *Anopheles* mosquitoes tested for circumsporozoite proteins, only 4.8% (n = 10) *An. gambiae* s.s and 6.5% (n = 5) *An. melas* were positive. None of the *An. nili* was positive for *P. falciparum* circumsporozoite antigen. However, there was no comparative significant difference in the sporozoite rates for *An. gambiae* s.s and *An. melas* in the study area ($F_{1, 22} = 0.12$, $P = 0.7353$).

Entomological inoculation rates (EIR)

The EIR for *An. gambiae* s.s and *An. melas* were 0.031 and 0.007 infective bites/person/night respectively. Overall, the EIR for the period of the study indicates

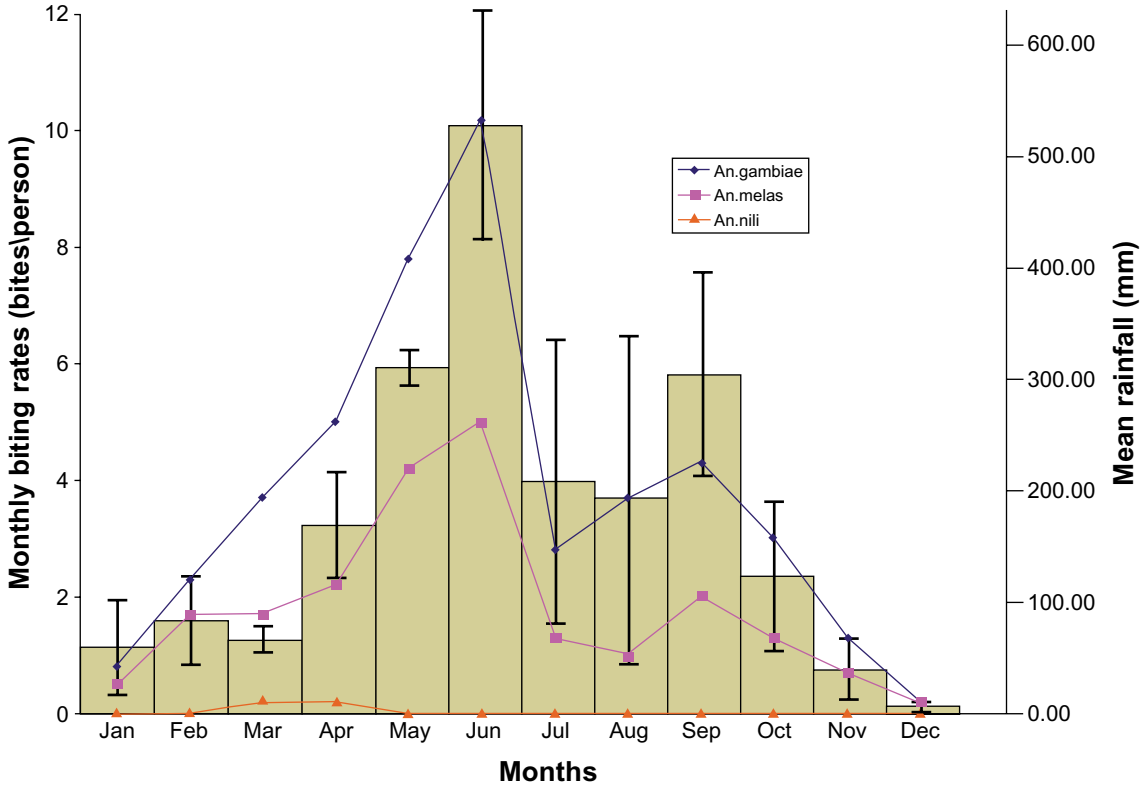

Figure 1. Mean monthly biting rates for *Anopheles* mosquitoes and monthly rainfall for the study period (2005–2007) in the coastal area of Lagos.

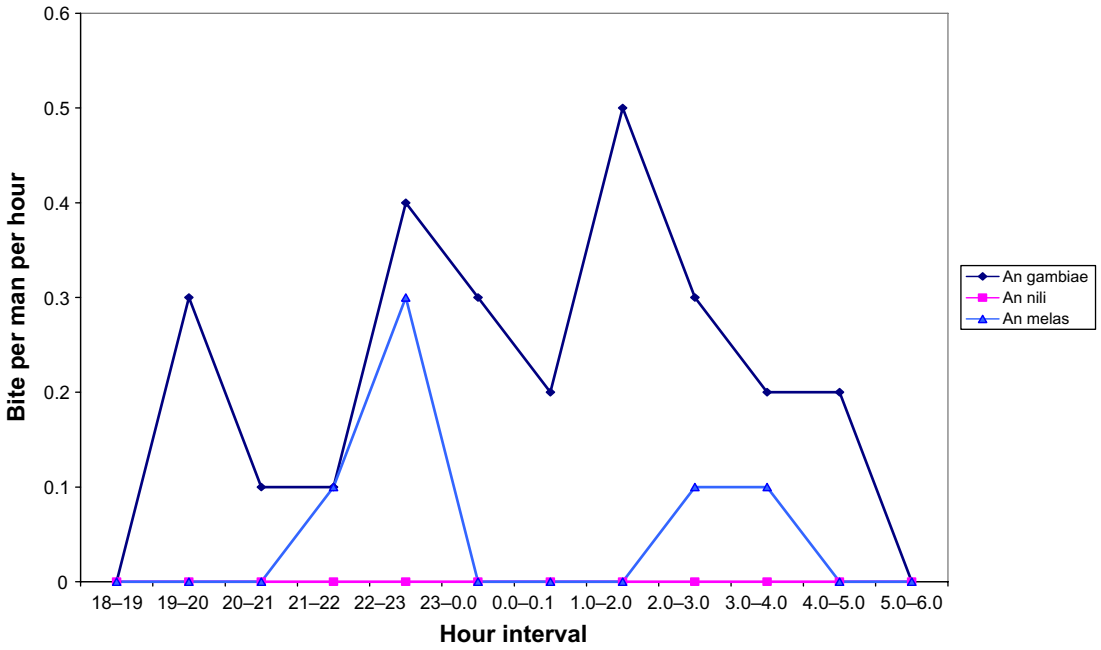

Figure 2. Biting cycle of *Anopheles* species in the study communities.

that each person in the study area is exposed to a mean of 13.9 infective bites/year, that is, 11.3 from *An. gambiae* s.s, 2.5 from *An. melas* and 0.00 from *An. nili* (Table 2). This showed that *An. gambiae* s.s contributed >80% of the overall EIR in the study area.

Discussion

Previous reports have shown that malaria is a common occurrence throughout the year and this was attributed to the year-round presence of the adult *Anopheles* mosquitoes in the coastal communities of Lagos.[3] However, in the present study transmission appeared to be seasonal whereby the population density of the anopheline species increased tremendously between May and June and this corresponds to the peak of rains. During this period, higher sporozoite rates and EIR were also recorded compared to the other months. Seasonal variability in malaria transmission has also been reported elsewhere in Nigeria and other parts of Africa.[3,22,23] The presence of *An. gambiae* s.s cut across the study communities and it was found to be the predominant species in the area and could as well be largely responsible for malaria transmission in the coastal area. However, *An. melas* was the predominant species in Moba indicating that this species breed mainly in the salt water environment since this community is located close to the Atlantic Ocean. Both species of *An. gambiae* s.s and *An. melas* were found to be endophilic and endophagic in contrast to *An. nili* with low indoor density. The present study showed that the molecular 'M' form was predominant in this area and the larger population was recorded

Table 3. The numbers and percentages of *Anopheles* mosquitoes found with humans and bovine blood.

Species	No	Examined		Blood	Sources		
	No tested	PSC N (%)	NBC N (%)	Human only N (%)	Bovine only N (%)	Human+ Bovine N (%)	Negative for Human+ Bovine N (%)
An. gambiae	90	52 (57.8)	38 (42.2)	57 (63.3)	1 (1.1)	–	32 (35.6)
An. nili	6	4 (66.7)	2 (33.3)	–	2 (33.3)	1 (16.7)	3 (50)
An. melas	107	79 (73.8)	28 (26.2)	79 (73.8)	1 (0.9)	–	27 (25.2)
Total	**203**	**135**	**68**	**136**	**4**	**1**	**62**

Table 4. Number of *Anopheles* mosquitoes tested and number positive for *Plasmodium falciparum* circumsporozoite antigen and corresponding SPR and EIR.

Study area	*An. gambiae* s.s				*An. melas*				*An. nili*			
	N	No +ve	SPR	EIR	N	No +ve	SPR	EIR	N	No +ve	SPR	EIR
Iworo	84	5	6.0	16.5	0	0	0	0	3	0	0	0
Epe	67	2	3.0	6.0	0	0	0	0	0	0	0	0
Moba	58	3	5.2	12.1	77	5	6.5	7.1	0	0	0	0
Total	**209**	**10**	**4.8**	**34.6**	**77**	**5**	**6.5**	**7.1**	**3**	**0**	**0**	**0**

Abbreviations: N, number of mosquito tested; No +ve, number positive for *P. falciparum* circumsporozoite antigen; SPR, percentage sporozoite 'rates'; EIR, entomological inoculation rate.

in the wet season. However, this may be in conflict with the previous reports elsewhere in Nigeria where the molecular 'S' form was found to be prominent species.[24,25] Meanwhile, the distribution of the molecular M and S forms of *An. gambiae* s.s is still being determined for much of the West African regions.

In this study the sporozoite rates (SPR) for *An. gambiae* s.s ranged from 3.0%–6.0% and this conforms to the sporozoite rates reported by the previous authors in some parts of Nigeria. For instance, Hanney[26] reported SPR of 5.3% in Zaria province while Molineaux and Gramiccia[31] gave SPR of 7.6% for *An. gambiae* s.s in Garki (Kano State). Another reports elsewhere in West Africa showed a range of 3.5%–7.5% for *An. gambiae* s.s.[27,28] Meanwhile, *An. melas* provided the highest SPR of 7.1%, which may be an indication of high vectorial capacity for this species within the locality.

The EIR usually serves as an indicator for the level of parasite transmission in a given area. In the present study, the overall EIR in the study area was lower than that reported for coastal areas elsewhere in West Africa.[28,29] The presence of *An. melas* contributed immensely to the infectivity rates in the area, although, the species has been reported to be more zoophilic and exophilic than *An. gambiae* s.s. in other parts of West Africa.[30] In addition, relative EIR maintained by *An. gambiae* s.s in the present study also indicates the role played by this species in malaria transmission in this region. These results suggest the need to plan control programmes that will target the implicated species in this study.

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Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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