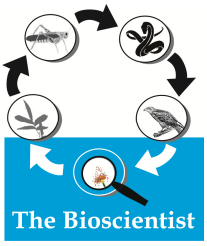
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COMPOSITION OF MOSQUITO SPECIES AND PHYSIOLOGICAL STATES OF INDOOR MAN-BITING MOSQUITOES AT NTEJE, SOUTH-EASTERN NIGERIA

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ABSTRACT

Mosquitoes are vectors of human and animal diseases. A survey ofman-biting mosquito species was conducted in Nteje, Oyi Local Government Area, Anambra State, from November, 2021 - January, 2022. The aimwas to determine the composition of mosquito species, their biting time and the physiological states of indoor man-biting mosquitoes in the area. Larvalmosquitoes were collected by dipper method,indoor biting mosquitoes were collected usingpyrethriod-based insecticide knockdown (PKD)while outdoor biting adults were collected using human bait collection (HBC) method. The physiological states of mosquitoes were determinedthrough observation of their abdomen. A total of 1,262 mosquitoes were collected from the study. Of this number, 528(41.83%) were larvae, 185(14.65%) were indoor biting adults while 549(43.50%) were outdoor bitters. Six mosquito species; *Culex quinquefasciatus* 201(38.06%), *Aedes cumminsi* 6(1.13%), *Culex tigripes*27(5.11%), *Aedes aegypti* 180(34.09%), *Aedes albopictus* 105(19.88%) and *Culex nigripalpus* 9(1.70%) were collected during larval sampling. There was no significant difference in the numbers of mosquito species collected as larvae (P>0.05). Three mosquito species; *C. quinquefasciatus* 109(58.9%), *Anopheles gambiae* 46(24.9%) and*Ae. aegypti* 30(16.2%) were collected indoors. There was no significant difference in the numbers of mosquito species collected indoors (P>0.05). Of the 185(100%) adult mosquitoes collected indoors, 53(28.64%) were unfed, 79(42.70%) were freshly fed, 32(17.29%) were half gravid while 21(11.35%) were gravid. There was also no significant difference in the physiological state of the mosquitoes (P>0.05). *Aedes aegypti* 292(53.2%) and *Aedes albopictus* 257(46.8%) were collected outdoors. The abundance of different species of mosquitoes including *An. Gambiae* complex which is a major vector of the malaria disease in the tropics demands that mosquito control interventions should be intensified in the study area to save the people from mosquito-borne infections.

Keywords: Mosquitoes, Vectors, Breeding sites, *Anopheles gambiae*, *Culex quinquefasciatus*

INTRODUCTION

Mosquitoes are the most common blood sucking arthropods vectors of human and animal diseases. Mosquito-borne diseases have emerged as a major public health concern globally, but mostly in developing countries of the world (Nnamonu*et al*., 2019).Several mosquito species belonging to genus *Anopheles*, *Culex* and *Aedes* has been incriminated with the transmission of some serious parasitic and viral diseases (Umeanaeto*et al*., 2017;Irikannu*et al*., 2020; Ikpo*et al*., 2021). The most common diseases they transmit includes malaria, dengue fever, filariasis and yellow fever (Irikannu *et al*., 2019; Egbuche *et al*., 2020).Mosquitoes also cause serious biting annoyance, noise nuisance, sleeplessness and allergic reactions through their bites (Onyido *et al*., 2009).

Mosquitoes are widely distributed throughout the world, with difference species having preference for peculiar breeding sites (Adeleke *et al*., 2008; Moussa *et al*., 2013). Many mosquito species breed in both natural and man-made containers such as ground pools, gutters, coconut shells, tree holes, bamboo stumps, leaf axils, concrete mixers and septic tanks amongst others (Onyido *et al*., 2009; Oyewole *et al*., 2009; Onyido *et al*., 2016;Irikannu*et al*., 2021). Mosquito species distribution across geographical areas is also influenced by climatic and environmental factors such as temperature, relative humidity and rainfall (Kudom*et al*., 2011).

One of the major ways to prevent or reduce mosquito-borne diseases isto ensure reduction in vector population and human-vector contact. Investigating the mosquito species composition in an area before initiating a control program is necessary to avoid waste of resources in combating non-vector species (WHO, 2013). This study was conducted to investigate mosquito species composition and the physiological state of indoor man-biting adult mosquitoes in Nteje, Oyi Local Government Area, Anambra State, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted in Nteje, the headquarters of Oyi local government Area(L.G.A) of Anambra State, Nigeria. The town is situated about 25 kilometers North-East of Onitsha between longitude 6.45^{o}E and the latitude 6.14^{o}N.The study area is about 500 feet above the sea level (Akwue, 1980). The area has an average annual rainfall of about 1700mm to 2100mm and average daily atmospheric temperature of 26.8^{o}C. There are two distinct seasons, the wet (March – October) and dry season (November – February). The inhabitants of the L.G.A are predominantly Igbos with an estimated population of 168,201 people (NPC, 2006). The inhabitants are mainly farmers, others engage in trading, while a few are in civil service.

Study Design

The study is a field survey of larval, outdoor and indoor biting adult mosquitoes, conducted from November, 2021 - January, 2022.

Advocacy Visit and Sensitization

An advocacy visit to the community was made using an introductory letter obtained from the head of the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka. The study intent and their significance were explained to the community leaders and other inhabitants. Their oral consent was obtained for the use of their environment for the study.

Ethical Approval

Ethical approval for the study was obtained from the Health Research Ethics Committee

of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku Awka (Ref No. COOUTH/CMAC/ETH.C/Vol.1/FN:04/146)

Larval Collection and Rearing to Adults

Larval collection was done using 350ml standard dipper (WHO, 2013). Collection was made in all forms of breeding sites including ground pools, discarded containers and discarded used tyres, amongst others. Sampling was carried out between 10:00am to 2:00pm (local time) for about 30 minutes in each larval habitat for 3 days. All larvae collected were transported in containers labeled according to the site of collection to the laboratory of the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka were they were reared to adults before identification. During rearing, the larvae were fed with fish feed (Tropical Flakes, Samyu Pets Corp and Shulin City) in bowls measuring 500ml, while the adult mosquitoes were fed with a 10% sugar solution in cotton wool on emergence.

Collection of Indoor Man-Biting and Resting Mosquitoes Species

Indoor man-biting and resting mosquitoes were collected from the community using pyrethroid-based insecticide knock down (PKD) (Onyido *et al*., 2016). The adult mosquitoes were collected from rooms where people slept the previous night from 6:00am – 8:00am (local time). In the rooms, the doors and windows were shut and white spread sheets were laid from wall to wall, covering furniture and other non-movable items in the rooms. A pyrethroid-based insecticide aerosol (Raid®manufactured by Johnson Wax Nigeria Ltd, 13/14 Abimbola Street, Isolo Industrial Estate, Lagos Nigeria. Expiry Date: 20/07/2023) was sprayed in the rooms and allowed to remain for 20 minutes before collection. At the end of the 20 minutes interval after spraying, the white spread sheet was folded. It was taken outside the room and spread out again to collect the knocked down mosquitoes into damp-petri dishes using a pair of entomological forceps.

Physiological State of the Indoor Man-Biting Mosquitoes Species

The physiological states of female mosquitoes collected indoors was determined by observing their abdomen to determine those that had bloodmeal using the method earlier described by Service (1985). The mosquitoes were grouped into four categories; unfed, freshly fed, half gravid and gravid.

Collection of Outdoor Man-Biting Mosquitoes Species

Outdoor man-biting mosquitoes were collected using human-bait collection (HBC) (Onyido *et al*., 2016). The collections were done quarter-hourly for three nights from 4.00pm-8.00pm (local time). The volunteer collectors were humans who were already vaccinated for yellow fever. They exposed their legs and hands for mosquito landing. Mosquitoes alighting on them were collected using test tube vials. Each vial was quickly covered with a ball of cotton wool to avoid escape of the mosquito. The time of collection of each mosquito were properly recorded.

Morphological Identification of the Mosquitoes

All mosquitoes collected were properly labeled and sent to the Laboratory Unit of the Department of Parasitology and Entomology, Nnamdi Azikiwe University for identification. The mosquitoes were identified using the gross morphological features of the species earlier described (Gillies and De Meillon, 1968; Gillet, 1972 and Gillies and Coetzee, 1987).

Data Analysis

Analysis of variance (ANOVA) was used for test of significant difference in mosquito species composition at 95% confidence level.

RESULTS

A total of 528 larvae were collected from three different breeding sites and six species were identified after rearing to adult. *Culex quinquefasciatus* (Plate 1), 201(38.06%) was the most abundant while *Ae. cumminsi*, 6(1.13%) was the least (Table 1). Other mosquito species collected were*C.tigripes*,27(5.11%), *Ae. aegypti*, 180(34.09%), *Ae. albopictus*, 105(19.88%) and *C. nigripalpus*, 9(1.70%).There was no significant difference in the numbers of larval mosquito species collected (P>0.05, P=0.212). The highest number of mosquito larvae was collected from domestic containers, 213(40.34%) while the least,114(21.59%)was from ground pool. Also, 201 (38.06%) was collected from discarded tyres. There was also no significant difference in larval abundance across breeding sites sampled (P>0.05, P=0.786).

A total of 185 indoor biting mosquitoes were collected. *Culex quinquefasciatus*, 109(58.9%) was the most prevalent indoor while*An. gambiae* had 46(24.9%) and *Ae. aegypti*, 30(16.2%) was the least (Fig 1). There was no significant difference in the numbers of indoor mosquito species collected (P>0.05, P=0.181).

Of the 185 adult mosquitoes collected indoors, 53(28.64%) were unfed, 79(42.70%) were freshly fed, 32(17.29%) were half gravid while 21(11.35%) were gravid (Table 2). There was also no significant difference in the physiological state of the mosquitoes collected (P>0.05, P=0.515).

Table 1: Mosquito larval sampling in Nteje, Oyi Local Government Area, Anambra State, Nigeria.

	Breeding sites			
Mosquito Species	Domestic containers (%)	Discarded tyres (%)	Ground pool (%)	Total (%)
Culex quinquefasciatus	102 (50.74)	0 (0.00)	99 (49.25)	201 (38.06)
Culex tigripes	12 (44.44)	0 (0.00)	15 (55.55)	27 (5.11)
Culex nigripalpus	9 (100)	0 (0.00)	0 (0.00)	9 (1.70)
Aedes aegypti	81 (45)	99 (55)	0 (0.00)	180 (34.09)
Aedes albopictus	9 (8.57)	96 (91.42)	0 (0.00)	105 (19.88)
Aedes cumminsi	0 (0.00)	6 (100)	0 (0.00)	6 (1.13)
Total	213 (40.34)	201 (38.06)	114 (21.59)	528 (100)

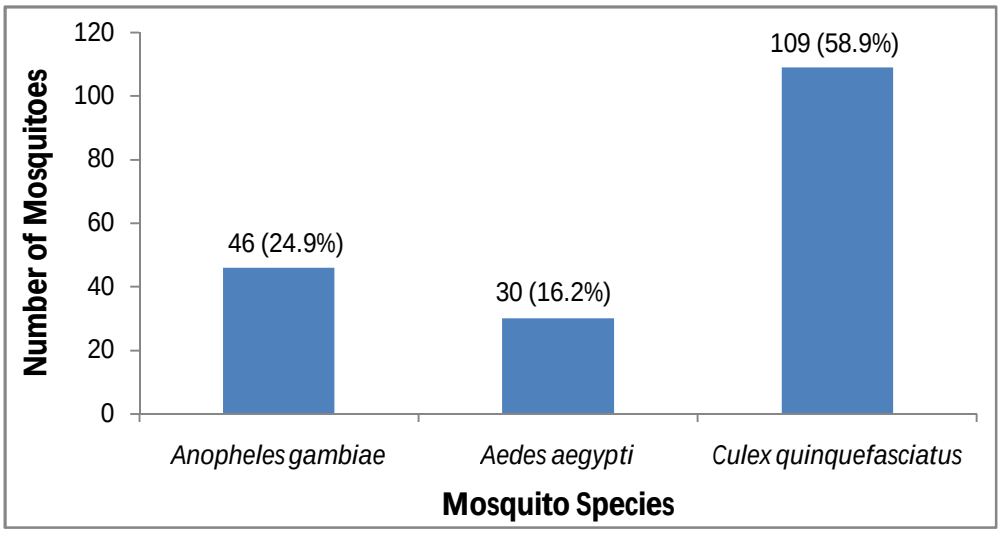


Fig. 1: Collection of indoor man-biting and resting mosquito species in Nteje, Oyi L.G.A, Anambra State.

Table 2: Abdominal grading of indoor-biting adult mosquitoes collected to determine their physiological state.

	Physiological state				
Mosquito Species	Unfed (%)	Freshly fed (%)	Half gravid (%)	Gravid (%)	Total (%)
Anopheles gambiae	8 (17.39)	34 (73.91)	4 (8.69)	0 (0.00)	46 (24.9)
Aedes aegypti	0 (0.00)	30 (100)	0 (0.00)	0 (0.00)	30 (16.2)
Culex quinquefasciatus	45 (41.28)	15 (13.76)	28 (25.68)	21 (19.26)	109 (58.9)
Total	53 (28.64)	79 (42.70)	32 (17.29)	21 (11.35)	185(100)

A total of 549(100%) outdoor man-biting mosquitoes were collected from Nteje of which *Ae. aegypti*, 292(53.2%) was more prevalent while *Ae. albopictus*, 257(46.8%) was the least (Fig. 2).

Two dominant mosquito species namely; *Ae. aegypti*(Plate 2) and *Ae. albopictus*(Plate 3) were collected outdoors during quarter-hourly collection (Fig 3). *Ae. aegypti* and *Ae. albopictus* had their peaks between 6.00-6.14pm followed by a gradual decline in population by 7.00pm.

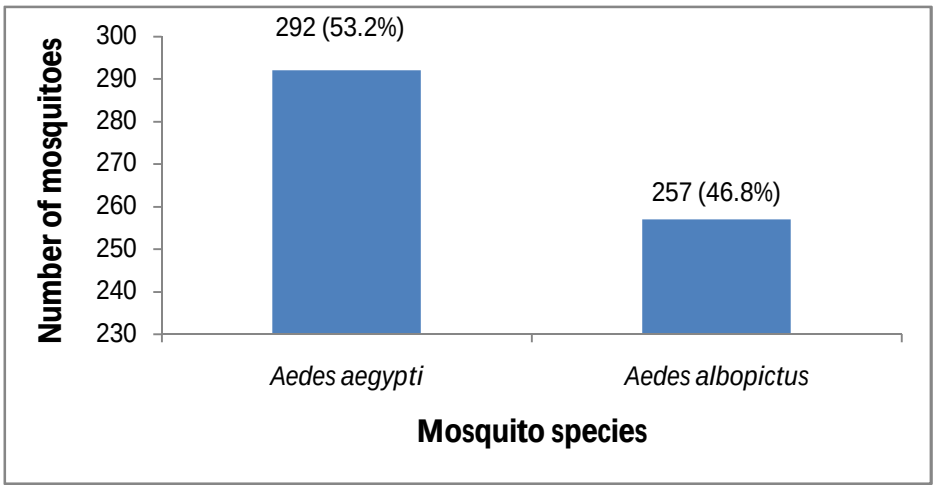


Fig. 2: Collection of outdoor man-biting mosquito species in Nteje, Oyi L.G.A, Anambra State.

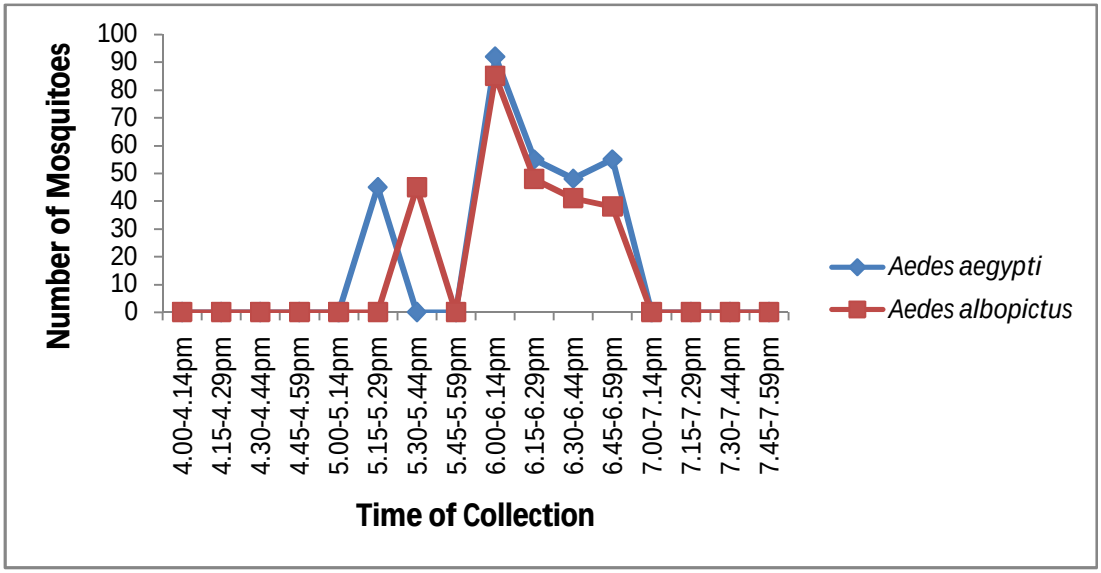


Fig. 3: Quarter-hourly collection of outdoor biting mosquito species in Nteje, Oyi L.G.A, Anambra State.



Plate 1. Microscopic image of adult *Culex quinquefasciatus*



Plate 2. Microscopic image of adult *Aedes agypti*



Plate 3. Microscopic image of adult *Aedes albopictus*

DISCUSSION

A total of seven mosquito species; *An. gambiae*, *Ae. aegypti*, *Ae. albopictus*,*Ae. cumminsi*, *C. quinquefasciatus*,*C. tigripes*, and *C. nigripalpus* were collected either as larvae, indoor or outdoor biting adult mosquitoes in the study area. The availability of these species in these communities was due to the presence of suitable breeding sites which include ground water pools, domestic containers, and discarded used vehicle tyres around homes where they breed (Mbanugo and Okpalaononuju, 2003). All the adult mosquitoes collected in this study have also been reported in different places in Nigeria. Onyido*et al.* (2016) reported *An. gambiae*, *C. quinquefasciatus*, *Ae. aegypti* and *Ae. albopictus* in Nibo community in Anambra State. Same speciesamongst others have been reported in several studies near the study area (Umeanaeto *et al.*, 2017; Egbuche*et al.*, 2020; Irikannu *et al.*, 2020; Ikpo *et al.*, 2021; Irikannu *et al.*, 2021; Onwuzulike *et al.*, 2021).

In this study,*C. quinquefasciatus*was largely responsible for indoor biting activities in the study area. The observation is in tandem with Umeanaeto*et al.* (2017) were*C. quinquefasciatus* (61.43%) constituted the most abundant speciesbiting indoors in the female hostel of Nnamdi Azikiwe University, Awka. Also *C. quinquefasciatus* was themost abundant species collected by Onwuzulike *et al.* (2021) and Irikannu*et al.* (2020) in other parts of Anambra State.*Culex quinquefasciatus* has been observed to breed near homes and bite humans indoor even during the dry season due to the availability of water and their continuous breeding in septic tanks throughout the year (Irikannu*et al.*, 2021).Most mosquitoes collected indoors (42.70%) were freshly fed, exhibiting endophilic behavior. This observation shows that infected mosquitoes may transmit pathogens during bloodmeal indoors.

The most abundant outdoor man-biting mosquito in the study area was *Ae. aegypti*. This corroborate the findings of Onyido *et al.* (2009) in Nnamdi Azikiwe University hostels, Awka, Anambra State. The culicines, *Ae. aegypti* and *Ae. albopictus* had their peaks between 6:00-6:14pm and gradually stopped biting between 7:00-7:14pm. This observation shows that these species are twilight biters and can have contact with humans during outdoor activities, which increases the chances of mosquito-borne diseases transmission.

CONCLUSION

The availability of mosquito species proven to be vectors of several parasitic and viral diseases in their substantial number in the study area raises concern. These mosquitoes feeding on human blood constitutes risk of disease transmission to humans in the area. Integrated interventions with mosquito control programs in the area and health education is strongly recommended.

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