

## A Cross Sectional Survey of Yellow Fever and Dengue Virus Vectors in Four Communities of Ayamelum Local Government Area (LGA), Anambra State, Southeast Nigeria

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**Abstract:** Several *Aedes* species have been incriminated in the transmission of yellow fever. Also, *Aedes aegypti* and *Aedes albopictus* are established transmitters of various serotypes of dengue. Both diseases have been recorded in Nigeria, as recent reports show that seroprevalence of dengue virus in the country is high. This study sought to establish the presence of yellow fever and dengue vectors in the study area. Baseline study to establish presence of the vectors was done in 4 communities (Ifite Ogwari, Anaku, Omor and Igbakwu) of Ayamelum LGA, Anambra state. To ascertain the local vector biting and breeding behaviour, major mosquito sampling methods (ovitrapping, larval sampling, human bait collection, Pyrethrum spray collection (PSC) and light trapping) were employed. Day and night collections were made. Adult collections were identified fresh in the field, while immature stages were reared to adults for proper identification. Results show that 1,531 mosquitoes of 16 species in 8 genera were collected. Igbakwu accounted for most of the collections, 670 (43.8%), while Anaku recorded the least, 190 (12.4%). A total of 422 (27.6%) mosquitoes from all collections were yellow fever vectors. Of these, 375 (88.9%) were also vectors of dengue. Yellow fever and dengue vectors were collected from all the communities, as well as all but one (PSC) of the sampling methods. This work establishes the presence and abundance of yellow fever and dengue vectors in the study area. Hence, there is need for simultaneous entomological and epidemiological monitoring of the viruses and their vectors throughout the country.

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### 1. Introduction

Mosquitoes are involved in transmission of arboviruses, filarial worms and protozoa (Service, 2008). Four communities of Ayamelum LGA in Anambra State, Nigeria were surveyed in research of vectors of yellow fever and dengue viruses. The potential yellow fever vectors in Nigeria are *Aedes aegypti*, *Aedes africanus*, *Aedes luteocephalus*, *Aedes albopictus*, *Aedes simpsoni* complex and *Aedes vittatus*. *Aedes aegypti* is commonly involved in transmission of all four serotypes of dengue and *Aedes albopictus* has been incriminated as a maintenance vector of dengue in rural areas of dengue-endemic countries. It is also competent vector of several other viruses in human and veterinary diseases (Gratz, 1999; Foster and Walker, 2002).

Initially, *Aedes simpsoni* complex was reported not to bite man in Nigeria (Iwuala and Ezike, 1979), but this trend seems to have changed as recent reports including this study shows otherwise. *Aedes africanus* is the most important in the transmission of the jungle yellow fever. *Aedes africanus* is found from the rain forest south up to the northern part of Kaduna while,

*Aedes aegypti*, *Aedes luteocephalus* and *Aedes vittatus* are found all over Nigeria (Iwuala and Ezike, 1979; Chukwuekezie, unpublished). *Aedes albopictus* is originally indigenous to South-east Asia, islands of the Western Pacific and Indian Ocean. This mosquito has spread to the mid-east, Europe, the North America, South America and Africa. The presence of *Aedes albopictus* was established in Nigeria in 1991 by National Arbovirus and Vectors Research Centre (NAVRC).

*Aedes* eggs are laid singly on damp substrates just beyond the water line (damp mud and leaf litter of pools, on damp walls of clay pots, rock-pools and tree-holes). The eggs can withstand desiccation. When flooded, some eggs may hatch within a few minutes, while others of the same batch may require longer immersion in water, so hatching may be spread over days or weeks. There is a resting period (state of diapause). The eggs can hatch in installments and the ability to withstand desiccation can create problem in controlling the immature stages.

High vector population densities precede human and animal diseases, so that estimates of these can

provide an early warning of the outbreaks and thus permit timely intervention to avoid or abort such outbreaks. Environmental factors such as weather pattern may influence vector density. Factors like presence of virus, temperature patterns, precipitation, flood control measures and herd immunity also influence the possibility of outbreaks (Foster and Walker, 2002).

The incidence of dengue has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings (WHO, 2009). An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries (WHO, 2008). Dengue is endemic in Nigeria, with seroprevalence of about 73% in some areas (Ayukekbong, 2014). Data recording and reporting are poor. Outbreak reports exist, although they are not complete, and there is evidence that dengue outbreaks are increasing in size and frequency (Nathan and Dayal-Drager, 2007). In Nigeria most cases of dengue are undiagnosed, misdiagnosed as malaria or referred to as fever of unknown cause (Ayukekbong, 2014).

The yellow fever epidemic in Nigeria in 1969-70 emphasized the lack of data concerning the possible importance of *Aedes aegypti* and other *Stegomyia* mosquitos as vectors. It was concluded that the only potential yellow fever vectors in North West were *Aedes aegypti* and *Aedes vittatus* (Service, 1974). Nigeria recorded outbreaks of yellow fever in former Benue-Plateau, South eastern, South south, and South western States in different periods. The National Primary Health Care Development Agency (NPHCDA) asserted that over 100 million Nigerians are at risk of yellow fever. Three hundred and seventy-seven Local Government Areas in 25 States are risk areas. Recently, there were reports of yellow fever outbreaks in six districts of Cameroun bordering Cross River State (Tomori, 2015). Hence, this study strives to establish the presence and abundance of yellow fever and dengue vectors in the study area.

## 2. Materials and Methods

### 2.1 Study Area

Ayamelum is an LGA in Anambra State. Its headquarters is at Anaku. The LGA has an area of 200 km<sup>2</sup> and a population of 197,573 (projected from 2006 population census). It lies between Latitude 06°30'17.54"N and Longitude 06°58'09.53"E at an altitude of 213 meters above sea. The climate is tropical with two seasons: the wet and dry. It has five months of dry season (November to March) and seven months of wet season (April to October). The LGA has a substantial annual average temperature of 20°C to 28°C and 18°C in the coldest month (Iheke and Nwaru, 2009). The mean annual rainfall varies

between 1500mm to 2250mm (Iloeje,1980). Eight communities (Anaku, Ifite Ogwari, Igbakwu, Omasi Omor, Umueje, Umerum and Umumbo) from which 11 political wards were carved, make up the LGA.

All the 8 communities in the LGA are agrarian, as they are noted for farming of rice, plantain and okra in very large quantities. The LGA has a mixture of mangrove/forest, and guinea-savanna vegetation. It has 2 major rivers (Omambala and Ezu). The name "Anambra" was derived from River Omambara.

### 2.2 Site Selection

Four of the 8 communities in the LGA were selected for the study. This was based on vegetation, population and proximity to the Omambala River. As a result, Anaku, Ifite Ogwari, Omor and Igbakwu were selected for the study.

### 2.3 Sampling methods

#### 2.3.1 Ovitrap Setting/Collection

Twenty ovitraps were set at strategic positions in each of the 4 communities (Ifite Ogwari, Anaku, Omor and Igbakwu). The traps were set in all the communities between the first and second day of arrival in the field. Care was taken to ensure that the traps and their ribbons were retrieved after 48 hours. On collection, the ribbons were air-dried, labeled and stored appropriately for further works in the laboratory.

#### 2.3.2 Larval Sampling

Twenty houses were sampled in each of the 4 communities, once in the course of the surveillance. Man-made containers (both discarded and those rarely used) around human dwellings were sampled for larvae. Collections were stored in well-labeled transparent larval containers for transportation back to our insectary.

#### 2.3.3 Human Bait Collection (HBC)

HBC was done once in each of the selected communities between 4:30pm in the evening and 7:45pm at night. Two members each of four different groups sat about hundred meters apart to do the collection in each locality. Collections were done with well labeled test tubes for accuracy of data. Mosquitoes collected at intervals of 15 minutes were stored separately, for further analysis.

#### 2.3.4 Light trapping

Two CDC Light traps and 1 WHO Light trap were set once in each of the communities during the study. One CDC Light trap was set outdoors and the other indoors, while the WHO Light trap was set only outdoors. Collections were labeled accordingly in the collection cups.

#### 2.3.5 Pyrethrum Spray sheet Collection

This type of collection was done uniformly in 1 room each from 5 houses per community. Care was taken to ensure that occupants of the room understood and gave their consent for the activity. The activity

also started as early as 6a.m so as not to allow escape of exophilic mosquitoes once doors and windows are opened by occupants of the room. By means of forceps, collections were transferred to well-labeled Petri dishes stuffed with wet cotton wool and filter papers for temporary preservation.

### 2.3.6 Identification of samples

In the course of the work, samples were identified in the field using standard identification/taxonomic keys. This is because identification is particularly done better when the vectors are fresh. Hence, a suitable site in the field was always chosen with the support of community stakeholders, for identification. Adult mosquitoes collected from each community were identified the next day (there in the community). Larval emergence was continually identified as soon as they emerged both in the field and later in the laboratory. Eggs collected using the ovitrap were carefully counted under the microscope, recorded and packed accordingly. They were eventually soaked in the laboratory and the adults that eventually emerged, identified. After identification, all collections were preserved in well-labeled Eppendorf tubes stuffed with Silica Gel.

### 3.0 Results

Of the 80 ovitraps set across the 4 communities, 27 (33.75%) were positive for eggs. Omor recorded the highest number of positive ribbons, 11 (55%), while Ifite Ogwari and Anaku recorded the least, 5(25%). From the ribbons, a total of 333 eggs were recovered. Igbakwu recorded the highest number, 97 (29.1%), while Anaku recorded the least, 55 (16.5%).

Only 239 (71.8%) of the eggs eventually hatched. Again, Igbakwu had the most 70 (29.3%), while Anaku recorded the least (15.9%). All these are shown in Tables 1 and 2.

Tables 3 and 4 represent larval sampling activities. Of the 397 containers in 80 households checked for larvae, only 45 (11.3%) were positive with larvae. A total of 243 mosquitoes in 4 genera and 7 species were collected from the sampling. Ifite Ogwari recorded the most 174 (71.6%), while Igbakwu had the least, 2 (0.8%).

Human bait collection is shown in Table 5. Across the 4 communities, 607 mosquitoes were collected. These were found to be 12 different species in 5 genera. Once again, the mosquitoes were by far most abundant in Igbakwu, 280 (46.1%) and least in Anaku 68 (11.2%). Remarkably, the dengue vectors though present, were very few. *Mansonia uniformis*, 465 (76.6%) was overwhelmingly predominant in the entire study area.

A total of 359 mosquitoes were collected using both the CDC and the WHO light traps, indoors and outdoors. These mosquitoes were spread into 9 species in 6 genera. Of these, only 1 (0.3%) dengue vector, *Aedes albopictus* was collected. This is shown in Table 6.

In Table 7, PSC was shown. Of the 20 houses sampled across the 4 communities, 92 mosquitoes were collected. These were in 3 mosquito species from 3 genera. *Anopheles gambiae*, 84 (91.3%) was the predominant species, while *Culex quinquefasciatus* 1 (1.1%) was the least collected. No dengue vector was collected from the pyrethrum spraysheet collection.

**Table 1: Egg Collections from Ovitrap**

S/N	Community	No. of Ribbons Set	No. of Positive Ribbons	% of positive Ribbons	Total No. of Eggs
1.	Ifite Ogwari	20	5	25	87
2.	Anaku	20	5	25	55
3	Omor	20	11	55	94
4.	Igbakwu	20	6	30	97
<b>Total</b>		<b>80</b>	<b>27</b>	<b>33.75</b>	<b>333</b>

**Table 2: Hatches from the eggs**

S/N	Community	Total No. Of Eggs Collected	Emergence	Mosquito Species
1.	Ifite Ogwari	87	71	<i>Aedes aegypti</i> 47, <i>Aedes albopictus</i> 24
2.	Anaku	55	38	<i>Aedes albopictus</i> 18, <i>Aedes aegypti</i> 20
3.	Omor	94	60	<i>Aedes albopictus</i> 45 <i>Aedes aegypti</i> 1, <i>Aedes simpsoni complex</i> 14,
4.	Igbakwu	97	70	<i>Aedes albopictus</i> 45, <i>Aedes aegypti</i> 24, <i>Aedes simpsoni complex</i> 1
<b>Total</b>		<b>333</b>	<b>239</b>	<b><i>Aedes aegypti</i> 92, <i>Aedes albopictus</i> 132, <i>Aedes simpsoni complex</i> 15( 71.8% )</b>

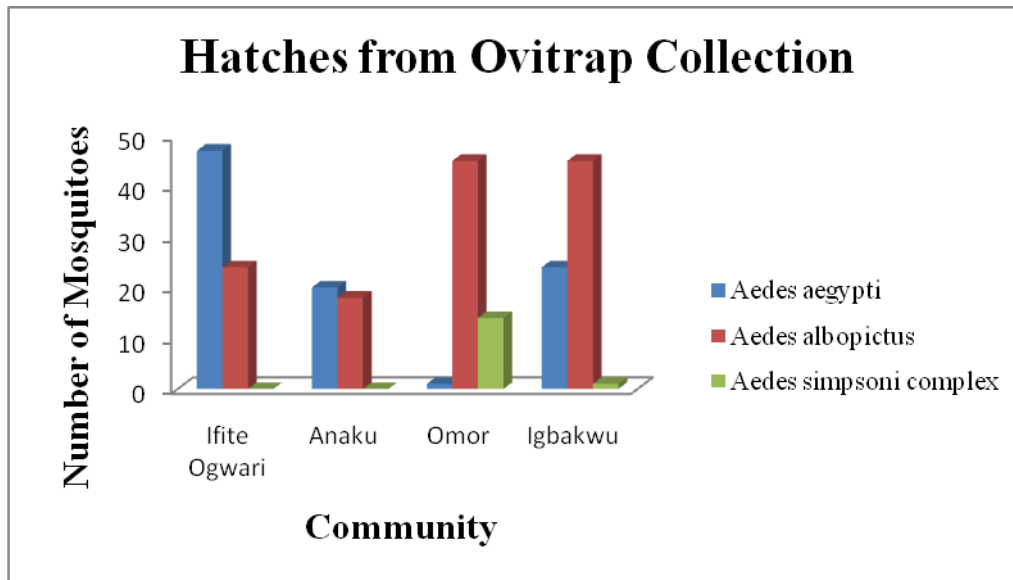


Figure 1: Number of larvae that hatched from eggs collected per community

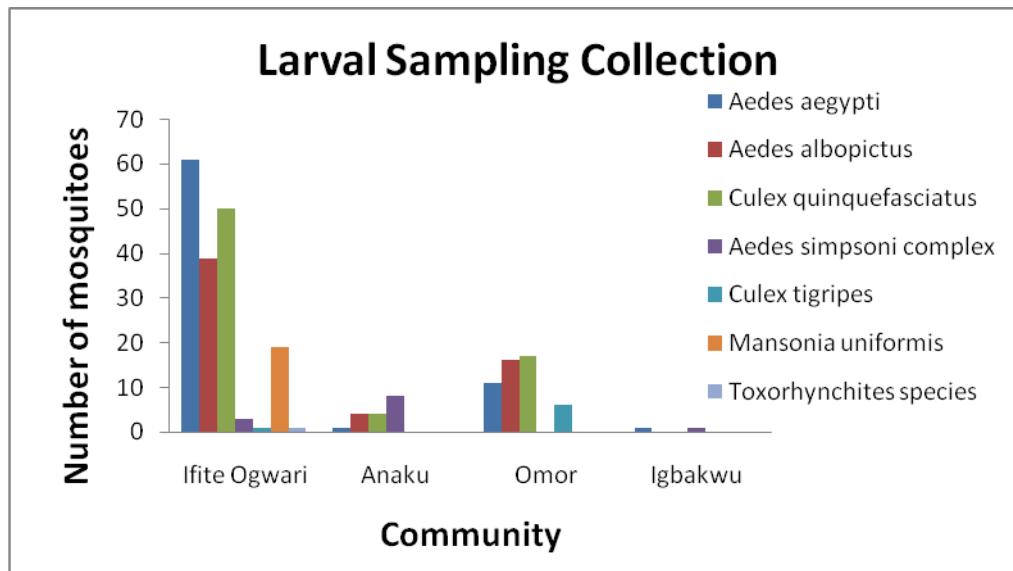


Figure 2: Collections from Larval sampling

Table 3: Larval Sampling

S/N	Community	No. of Households	No. of Containers With Water	No. of Containers Without Water	No. of Containers Positive With Larvae
1.	Ifite Ogwari	20	68	28	13
2.	Anaku	20	70	48	17
3.	Omor	20	107	25	8
4.	Igbakwu	20	27	24	7
<b>Total</b>		<b>80</b>	<b>272</b>	<b>125</b>	<b>45</b>

**Table 4: Larval Sampling Emergence**

S/N	Mosquito Species	Emergence per community				Total
		Ifite Ogwari	Anaku	Omor	Igbakwu	
1.	<i>Aedes aegypti</i>	61	1	11	1	74
2.	<i>Aedes albopictus</i>	39	4	16	0	59
3.	<i>Culex quinquefasciatus</i>	50	4	17	0	71
4.	<i>Aedes simpsoni complex</i>	3	8	0	1	12
5.	<i>Culex tigripes</i>	1	0	6	0	7
6.	<i>Mansonia uniformis</i>	19	0	0	0	19
7.	<i>Toxorhynchites species</i>	1	0	0	0	1
<b>Total</b>		<b>174</b>	<b>17</b>	<b>50</b>	<b>2</b>	<b>243</b>

**Table 5: Summary of Mosquitoes Collected From Human Bait Collection (HBC)**

S/N	Mosquito Species	Community				Total
		Ifite Ogwari	Anaku	Omor	Igbakwu	
1.	<i>Aedes aegypti</i>	3	0	2	0	5
2.	<i>Aedes albopictus</i>	4	0	2	6	12
3.	<i>Aedes africanus</i>	8	0	0	4	12
4.	<i>Aedes vittatus</i>	1	0	0	0	1
5.	<i>Anopheles gambiae</i>	1	0	1	0	2
6.	<i>Culex quinquefasciatus</i>	0	0	5	18	23
7.	<i>Eretmapodites chrysogaster</i>	2	0	0	1	3
8.	<i>Mansonia africana</i>	13	3	15	13	44
9.	<i>Mansonia uniformis</i>	105	62	77	221	465
10.	<i>Anopheles coustani</i>	2	2	5	14	23
11.	<i>Aedes simpsoni complex</i>	3	0	3	1	7
12.	<i>Culex poicilipes</i>	3	1	4	2	10
<b>Total</b>		<b>145</b>	<b>68</b>	<b>114</b>	<b>280</b>	<b>607</b>

**Table 6: Light Trap Collection**

S/N	Community	Type Of Light Trap	Number Set	Indoor Catch	Mosquito Species	Outdoor Catch	Mosquito Species
1.	Ifite Ogwari	CDC	2	0	Nil	0	Nil
		WHO	1	N/A	N/A	0	Nil
2.	Anaku	CDC	2	0	Nil	5	<i>Anopheles coustani</i> 2 <i>Mansonia uniformis</i> 3
		WHO	1	N/A	N/A	0	Nil
3.	Omor	CDC	2	33	<i>Anopheles coustani</i> 11, <i>Anopheles gambiae</i> 2, <i>Culex quinquefasciatus</i> 13, <i>Culex poicilipes</i> 2, <i>Mansonia uniformis</i> 2, <i>Eretmapodites chrysogaster</i> 2, <i>Aedes albopictus</i> 1	14	<i>Culex quinquefasciatus</i> 14
		WHO	1	N/A	N/A	3	<i>Coquillettidia</i> species 2 and an unidentified species

4.	Igbakwu	CDC	2	287	<i>Mansonia uniformis</i> 169, <i>Mansonia africana</i> 94, <i>Culex quinquefasciatus</i> 18, <i>Anopheles gambiae</i> 3, <i>Anopheles coustani</i> 3	17	<i>Culex quinquefasciatus</i> 1, <i>Mansonia africana</i> 3, <i>Mansonia uniformis</i> 12, <i>Coquillettidia</i> species 1
		WHO	1	N/A	N/A	0	Nil
<b>Total</b>				<b>CDC 320</b>	<i>Anopheles coustani</i> 14, <i>Anopheles gambiae</i> 5, <i>Culex quinquefasciatus</i> 31, <i>Culex poicilipes</i> 2, <i>Mansonia uniformis</i> 171, <i>Eretmapodites chrysogaster</i> 2, <i>Aedes albopictus</i> 1, <i>Mansonia africana</i> 94	<b>CDC 36</b> <b>WHO 3</b>	<i>Anopheles coustani</i> 2, <i>Mansonia uniformis</i> 15, <i>Culex quinquefasciatus</i> 15, <i>Mansonia africana</i> 3, <i>Coquillettidia</i> species 1 <i>Coquillettidia</i> species 2, and 1 unidentified species

**Table 6: Pyrethrum Spraysheet Collection**

S/N	Community	House No.	No. collected (Total)	Mosquito species Identified	Abdominal Grading
1.	Ifite Ogwari	1. 2. 3. 4. 5.	2 0 0 0 1(3)	<i>Anopheles gambiae</i> 1, <i>Mansonia uniformis</i> 1 Nil Nil Nil <i>Anopheles gambiae</i> 1	FF2 Nil Nil Nil FF1
2.	Anaku	1. 2. 3. 4. 5.	11 0 0 38 17(66)	<i>Anopheles gambiae</i> 10, <i>Mansonia uniformis</i> 1 Nil Nil <i>Anopheles gambiae</i> 35, <i>Mansonia uniformis</i> 3 <i>Anopheles gambiae</i> 17	FF 9, LF 2 Nil Nil FF 33, HG 5 FF15, Gravid 2
3.	Omor	1. 2. 3. 4. 5.	3 2 3 1 0(9)	<i>Anopheles gambiae</i> 3 <i>Anopheles gambiae</i> 1, <i>Mansonia uniformis</i> 1 <i>Anopheles gambiae</i> 2, <i>Culex quinquefasciatus</i> 1 <i>Anopheles gambiae</i> 1 Nil	FF 3 FF1, HG 1 FF3 FF 1 Nil
4.	Igbakwu	1. 2. 3. 4. 5.	6 7 0 0 1(14)	<i>Anopheles gambiae</i> 5, <i>Mansonia uniformis</i> 1 <i>Anopheles gambiae</i> 7 Nil Nil <i>Anopheles gambiae</i> 1	FF 5, HG 1 FF 7 Nil Nil FF 1
<b>Total</b>		20 Houses	92 Mosquitoes	<i>Anopheles gambiae</i> 84 <i>Mansonia uniformis</i> 7 <i>Culex quinquefasciatus</i> 1	FF 73, HG 9, GRAVID 2 FF 6, HG 1 FF 1

**KEY**

FF = Freshly Fed; HG = Half Gravid

**Table 7: Summary of all collections per community**

	Collection Per Community (Total)				Grand Total
	Ifite Ogwari	Anaku	Omor	Igbakwu	
Mosquito species	<i>Aedes aegypti</i> 111, <i>Aedes albopictus</i> 67, <i>Aedes africanus</i> 8, <i>Aedes vittatus</i> 1, <i>Anopheles gambiae</i> 1, <i>Eretmapodites chrysogaster</i> 2, <i>Mansonia africana</i> 13, <i>Mansonia uniformis</i> 125, <i>Anopheles coustani</i> 2, <i>Aedes simpsoni</i> complex 6, <i>Culex quinquefasciatus</i> 50, <i>Culex tigripes</i> 1, <i>Culex poicilipes</i> 3, <i>Toxorhynchites species</i> 1 <b>(391)</b>	<i>Aedes aegypti</i> 21, <i>Aedes albopictus</i> 22, <i>Aedes simpsoni</i> complex 8, <i>Anopheles gambiae</i> 62, <i>Mansonia africana</i> 3, <i>Mansonia uniformis</i> 69, <i>Anopheles coustani</i> 4, <i>Culex poicilipes</i> 1 <b>(190)</b>	<i>Aedes aegypti</i> 14, <i>Aedes albopictus</i> 64, <i>Anopheles gambiae</i> 10, <i>Culex quinquefasciatus</i> 50, <i>Mansonia africana</i> 15, <i>Mansonia uniformis</i> 80, <i>Anopheles coustani</i> 16, <i>Aedes simpsoni</i> complex 17, <i>Culex tigripes</i> 6, <i>Culex poicilipes</i> 6, <i>Coquillettidia</i> sp. 2, Unidentified sp. 1 <b>(281)</b>	<i>Aedes aegypti</i> 25, <i>Aedes albopictus</i> 51, <i>Aedes africanus</i> 4, <i>Culex quinquefasciatus</i> 37, <i>Eretmapodites chrysogaster</i> 1, <i>Mansonia africana</i> 110, <i>Mansonia uniformis</i> 403, <i>Anopheles gambiae</i> 16, <i>Anopheles coustani</i> 17, <i>Aedes simpsoni</i> complex 3, <i>Culex poicilipes</i> 2 <i>Coquillettidia</i> sp. 1 <b>(670)</b>	<b>1,532</b>

#### 4. Discussions

Emergence from Ovitrap collections showed that *Aedes albopictus* is the predominant species in all four communities, followed by *Aedes aegypti*. *Aedes albopictus* was also more in number in all sampling methods across board, except for Ifite Ogwari. This is not surprising or conflicting with most findings, as it is often described as an invasive *Aedes* species. Bonizzoni *et al.*, (2013) stated that one of the most dynamic events in public health is being mediated by the global spread of the invasive mosquito, *Aedes albopictus*. Also, many researchers have reported that *Aedes albopictus* has spread rapidly in Nigeria since its presence was announced in 1991 by the National Arbovirus and Vectors Research Centre, Enugu. This is corroborated by Adeleke *et al.*, (2015) who reported that the mosquito has spread rapidly in the Southern part of Nigeria.

The usual man-made container breeders were collected from larval sampling, of which *Aedes aegypti* predominated while *Toxorhynchites* species (not too often a man-made container breeder) was the least. This is in line with a study carried out in Enugu, where Onyido *et al.*, (2009) also collected more of *Aedes aegypti*. *Aedes albopictus* was also well represented in the collections. In Ifite Ogwari community, there was an uncommon finding as *Mansonia uniformis* was collected from household containers along with the dengue vectors. It is well documented that this mosquito species utilizes root parts of water plants for survival during the larval stages (Service, 2008). It is difficult to determine the exact type of container from

where they were collected or whether there were aquatic plants in the containers. Nevertheless, further studies need to be done on this.

In HBC, 12 different mosquito species including the 2 dengue vectors, were collected. This simply shows the abundance of mosquitoes in the study areas. Three of the 4 community had uniform peak biting period of 6:30 – 6:45pm (except Omor which had 7:30 – 7:45pm). This is an indication that mosquito biting activity may have some form of uniformity across communities in the LGA. The man-biting rate is alarming. It was found to be 2.83, 4.75, 6.04 and 11.67 mosquito/man/hour in Anaku, Omor, Ifite Ogwari and Igbakwu communities, respectively. Also, worthy of note, is the fact that *Aedes simpsoni* complex was collected from 3 (Ifite Ogwari, Omor and Igbakwu) of the 4 communities. This is suggestive that what was collected is *Aedes bromeliae*, which is the widely distributed anthropophilic member of the complex in Africa (Huang, 1979).

PSC activity from all 4 communities showed that the usual indoor biters were collected – *Anopheles gambiae*, *Culex quinquefasciatus* and *Mansonia uniformis*. Also as expected, *Anopheles gambiae* accounted for most (91.3%) of the collections. This is due to its anthropophilic, endophilic and endophilic nature, unlike *Mansonia uniformis* which is endophilic but exophilic. In line with well established facts, none of the dengue vectors were collected by this method in all 4 communities. In contrast to the PSC, one dengue vector, was collected from light trapping. This may be due to chance.

## 5. Conclusion

This study shows that Yellow fever vectors and the dengue vectors (*Aedes aegypti* and *Aedes albopictus*) are well established in the LGA, even in a community (Igbakwu) where outdoor water storage in containers was almost non-existent. The fact that in such a short period, 16 mosquito species, in 8 genera were collected, also points to the fact that the study area has abundance of mosquitoes. The data presented in this work along with previous works, suggests the urgent need for continuous entomological and epidemiological monitoring of the diseases and their vectors, particularly with the increasing reports of dengue fever in Nigeria and *Aedes* transmitted disease globally.

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