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Full Length Research

Exploratory studies of *Anopheles* species abundance in five selected communities of Emohua LGA, Rivers State

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ABSTRACT: Malaria is one of the public health problems facing people in many parts of Nigeria. To effectively implement malaria control program, baseline studies of species abundance and their susceptibility status to insecticides is required. The aim of the study was to carry out baseline studies on malaria vectors, species abundance and susceptibility status of local malaria vectors in five communities in Emohua Local Government Area of Rivers State. Mosquito larvae were collected from different breeding sites, reared in the insectary and identified morphologically. Data collected were analyzed using SAS software and statistics software GraphPad Software. Spearman's rank correlation coefficient (RHO) was used in comparing Indoor Resting Density (IRD) and Man Biting Rates (MBR). A Total of 1,415 adult indoor mosquitoes were caught comprising of two (2) genera. Of these, 87.21% were *Anopheles gambiae* s.l. and 12.79% were *Culex* spp*.* There was a statistical difference (p<0.0001) in the distribution of *Anopheles* and *Culex* mosquitoes caught. The highest percentage of *Anopheles* mosquitoes were caught at Emohua 26.74%, followed by Rumuji 22.77%, Egbeda 21.96%, Akpabu 17.10% and the least was Elele 11.43%. Examination of their abdominal conditions to determine feeding frequency indicated that the average number of fed mosquitoes ranged from 0.17-15.13 with a peak of 15.13 in the month of September in Elele. There was a significant difference (p<0.0001) in Indoor Resting Density (IRD) and Man Biting Rate (MBR) in the area. This study has contributed to the understanding of the distribution, composition and indoor resting behaviour of mosquito vectors in the study areas.

Keywords: *Anopheles gambiae* s.l, *Culex* spp, Emohua, insecticides, Rivers State.

INTRODUCTION

Malaria is the most important parasitic disease of public health importance caused by five species of *Plasmodium* parasites namely: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. According to World Health organization (2013), malaria is presently considered endemic in a total of 104 countries and territories. Globally, an estimated 3.4 billion people are at risk of malaria. Most malaria morbidity cases (80%) and deaths (90%) occurred in Africa, and most deaths (77%) were in children under 5 years of age. Malaria remains a major public health problem in many countries of the world despite the progress in reducing cases and deaths. The global burden of mortality is dominated by countries in sub-Saharan Africa, with the Democratic Republic of Congo and Nigeria together accounting for more than 35% of the global total of estimated malaria deaths.

In Nigeria, malaria remains a public health problem and is a risk for 97% of the population. It is known to contribute to over 300,000 deaths per annum and 11% of maternal mortality (Oduola *et al*., 2013). Nigeria has the largest burden of malaria in Africa, yet not much is known about the vectors, how the species interact, overlap or differ across the country (Okorie *et al.,* 2011).

Malaria is transmitted through the bite of an infected female mosquito of the genus *Anopheles*. There are about 400 different species of *Anopheles* mosquitoes, but only 30 are vectors of major importance for malaria. Each species of *Anopheles* mosquito has its own preferred aquatic habitat; for example, some prefer small, shallow collections of fresh water, such as puddles and hoof prints, which are abundant during the rainy season in tropical countries. It is an established fact that mosquitoes are the most important insects affecting human health (Woodbridge and Edward, 2006) chiefly in the spread of malaria the most common lethal disease second only to HIV/AIDS (Rowton, 2005; Hay *et al*., 2009). Malaria transmission is variable from one area to another and this has an impact on its epidemiology and control (CDC, 2004). In another study in Badagry by Okwa *et al*. (2007b), a coastal area of southern Nigeria, several species of *Anopheles* occur in sympatry. These species all contribute to the transmission of malaria as potential vectors. The study is aimed at determining the abundance and distribution of *Anopheles* species in the selected communities of Emohua LGA, Rivers State.

MATERIALS AND METHODS

Study area

This study was conducted in Emohua Local Government Area in Rivers State, south-south Nigeria. It is one of the 23 Local Government Areas of the State. The major ethnic groups include the Kalabaris, Ikwerres, Ogonis, Ekpeyes, Ogbas, Engenes, Ibanis and Okrikas. Emohua has an estimated population of 201,901 at the 2006 census (NPC, 2006). The geographical coordinates of Emohua LGA are 4°52'44"N latitude and 6˚51'40"E longitude (Figure 1). It rains always with annual rain fall averaging 1500 mm, and relative humidity over 80%. The climate is characterized with two distinct seasons, the wet and dry seasons the former taking place from April to October and dry season between November and March. Although Rivers State experience rain fall no matter how little every month of the year, this does not fall on every part of the state at the same time. The expanse of freshwater swamps, dense rain forest and intricate network of creeks and coastal ridges promote breeding of malaria vectors (Tobin-West and Babatunde, 2011). Over 70% of the inhabitants reside in rural areas and are engaged in subsistence fishing and farming, with the involvement of some in petty trading.

Selection of communities and households

Communities and households sampled were selected based on areas where IRS (Indoor Residual Spraying) intervention program had not taken place.

Collection of mosquito vectors

Collection of mosquito larvae

Collection, processing, rearing and identification of mosquito larvae were as described by Awolola *et al*. (2002). Larvae were collected from puddles, shallow wells, gutters and farms. The larvae were collected using the dipping technique in which the dipper was lowered gently about 10 times at an angle of 45° just below the surface so that water flows in together with any larvae that were present. All larvae collected were transported in a container to the insectary at the Rivers State University (RSU) and reared for a period of one week in bowls containing water which were covered with mesh and fed with a mixture of yeast and low-fat biscuits at ratio 1:9. The emerged adults were transferred into cages using mechanical aspirator.

Collection of indoor mosquitoes

Indoor adult mosquito collections were carried out from September 2014 to February 2015 in all the five communities in Emohua using Pyrethrum Sheet Collection (PSC) method as described by the WHO (1975) for sampling indoor-resting mosquitoes. The houses were sampled using an aerosol insecticide (Raid) containing the active ingredients of 0.250% Allethrin, 0.150% Tetramethrin, 0.015% Deltamethrin and 99.585 % inert ingredients. This was carried out by spraying inside the room as well as the eaves outside of the house. The doors were closed for 15 minutes, and opened for collection. Mosquitoes that were knocked down were taken outside by four people, each holding one edge of the sheet for illumination. The mosquitoes were collected using forceps and placed in petri dishes containing damp filter paper. Anopheline mosquitoes were kept on damp absorbent paper in a cool box and later identified to the species level by morphological criteria (Gillies and Coetzee, 1987). Monthly Indoor Resting Density (IRD) was calculated by collecting indoor resting mosquitoes in a number of selected houses (two houses were selected per community) by PSC method. Mosquitoes collected were separated morphologically and counted by species and gender. The IRD of female *Anopheles* mosquitoes was computed using WHO (2003) criteria, viz: IRD=Number of females mosquitoes collected/number of houses sampled x number of nights (Umar *et al*., 2015).

Morphological identification and preservation of mosquitoes

After each indoor pyrethrum spray, mosquitoes were sorted by genus as anopheline and culicine and were

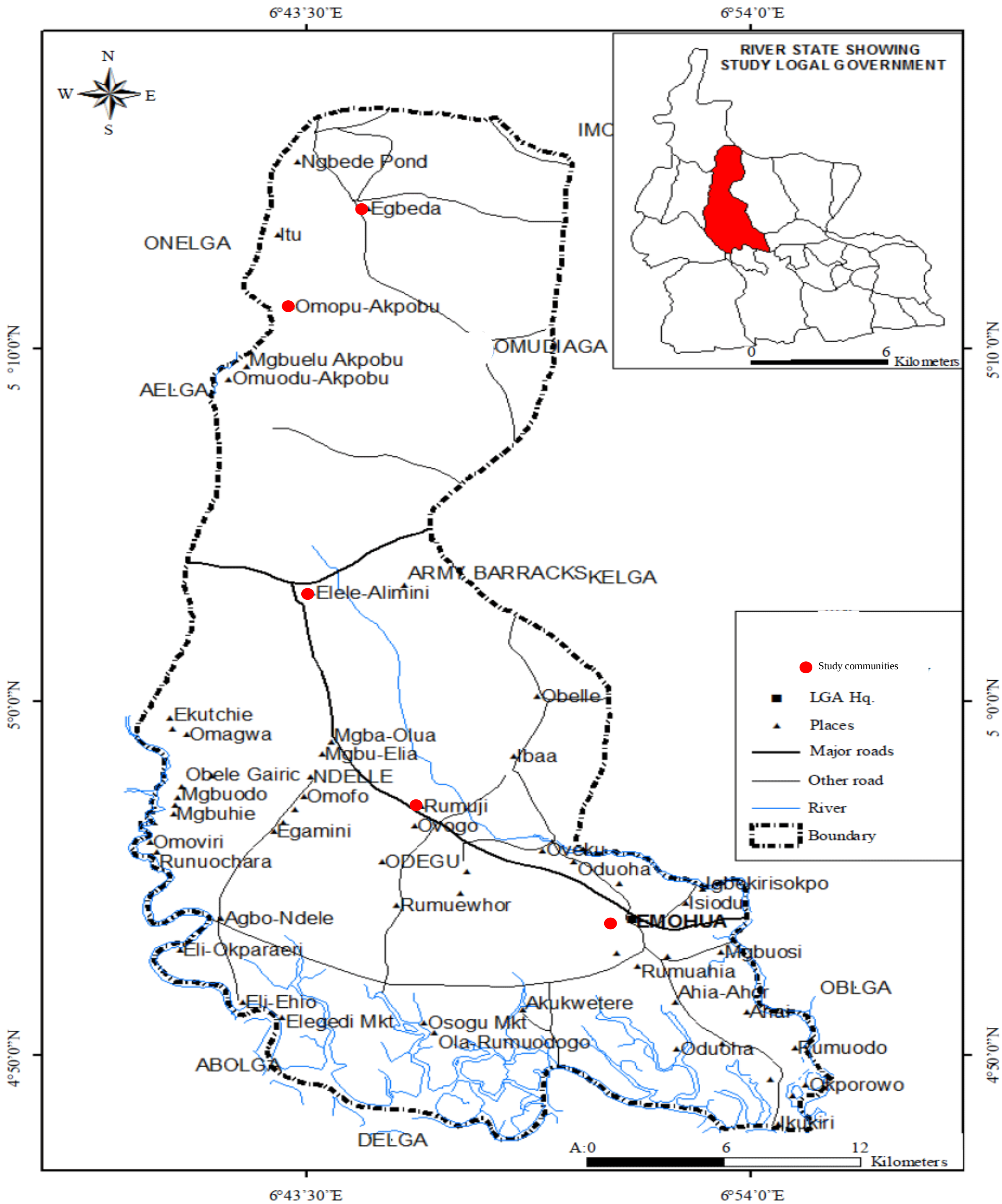

Figure 1. Map of Emohua (Study area) showing study sites (Source: Edu et al., 2015).

counted. Anophelines were morphologically identified using keys of Gillies and DeMeillon (1968) and Gillies and Coetzee (1987). Each of the mosquito was preserved in a well labelled 1 ml Eppendorf tubes loaded with silica gel and cotton wool for further analysis.

Determination of Man Biting Rate (MBR)

The various degrees of blood meal digestion of the mosquito species caught at the various sites (Akpabu, Egbeda, Elele, Emohua and Rumuji) was used to

Table 1. Species of mosquitoes caught in communities in Emohua LGA in Rivers State.

Communities	Total number of mosquitoes	Total number of Anopheles (%)	95% confidence Interval	Total number of culicines (%)	95% Confidence interval
Rumuji	322	281 (22.8)	85.91-88.69	41 (12.7)	3.16-22.24
Elele	157	141(11.4)	88.2-91.4	16 (10.2)	-4.9-24.3
Emohua	404	330 (26.7)	79.91-83.49	74 (18.3)	10.33-26.27
Egbeda	298	271 (22.0)	89.87-91.93	27 (9.1)	-2.26-19.46
Akpabu	234	211 (17.1)	88.94-91.46	23 (9.8)	-2.76-21.36
Total	1,415	1,234 (87.2)		181 (12.8)	

(χ^2= 783.61; df = 1; P= 0.001).

determine the man biting rates. This was done from September to February according to the recommended WHO standard (WHO, 2002). The Man-Biting Rate (MBR) described as the number of bites a person receives from a specific vector species per night was indirectly estimated from Pyrethrum Spray Collections (PSC) by separating all freshly blood fed (F) female mosquitoes collected by species and counted. The total number of females of the species was then divided by the total number of occupants (W) who spent the night in the rooms that were used for the collection (Sinka *et al*., 2012).

Determination of the stages of blood meal digestion

Blood digestion stage refers to the appearance of the abdomen of the female *Anopheles* as the result of the blood digestion and ovarian development. In anophelines, ovary maturation (egg development) occurs at the same time as blood digestion based on their blood digestion stage or abdominal condition, anopheline are grouped as unfed, freshly fed, half-gravid, and gravid. With the aid of a hand lens, the stages of blood meal digestion of the mosquitoes caught were determined and recorded.

Statistical analysis

Data collected were analyzed using SAS software (Statistics SAS Institute Inc., Cary, NC 27513, USA) and statistics software GraphPad Software, Inc. (7825 Fay Avenue, Suite 230 La Jolla, CA 92037 USA). Spearman's rank correlation coefficient (RHO) calculator was used in comparing Indoor Resting Density (IRD) and Man Biting Rates (MBR).

RESULTS

Mosquito genera encountered and their relative abundance

A total of 1,415 adult indoor mosquitoes comprising two genera namely *Anopheles* and *Culex* were caught in all the sampled communities. Of these, 87.2% were *Anopheles gambiae* s.l. and 12.8% were *Culex* spp*.* Out of the 1,234 *Anopheles* mosquitoes caught, 26.7%, 22.8%, 22.0%, 17.1% and 11.4% were caught at Emohua, Rumuji, Egbeda, Akpabu and Elele, respectively as shown in Table 1.

Man Biting Rates (MBR) and Indoor Resting Density (IRD) in five communities of Rivers State

Across the five communities significantly higher collections of *Anopheles* mosquitoes were recorded in the months of September and October of the rainy season ranging between an average of 6.88 to 20.25 (Tables 2 and 3) mosquitoes compared to the dry season months of November, December, January, and February (Tables 4 to 7) ranging between 0.13 to 6.13 mosquitoes (Table 2). Examination of their abdominal conditions to determine feeding frequency indicated that the average number of fed mosquitoes ranged from 0.17 to 15.13 with a peak of 15.13 in the month of September in Elele. Higher numbers of half gravid and gravid mosquitoes were also collected in Elele with 1.38 and 2.38 respectively (Table 2). There was a significant difference ($F_{4,45}$ = 3.77; P<0.01) between Indoor Resting Density (IRD) and Man Biting Rate (MBR) observed in the area. Indoor Resting Density (IRD) ranged from 0.33 to 0.52 with a peak IRD of 0.52 at Rumuji in the month of February. Man Biting Rates in the months of September and December was observed to range from 0.04 to 5.89, the peak of which was 5.89 recorded at Elele community. The peak Man Biting Rates of 5.89 was recorded at Elele in the month of September (Table 2).

DISCUSSION

This study investigated the principal vectors of the malaria parasite in five communities in Emohua LGA of Rivers State. Although the findings of Gillies and De Mellion (1968) and Sinka *et al. (*2010) suggested that members of *An. gambiae* and *An. funestus* complexes being Africa's most important malaria vectors because of their widespread distribution, can be found in sympatry and present across all geographical zones in the country, only

Table 2. Man biting rates (MBR) and indoor resting density (IRD) in five communities of Rivers State in the month of September 2014.

Communities	Average No. of occupants	Average No. of mosquitoes sampled	Average of *Anopheles*	Average of unfed	Average of fed	Average of half gravid	Average of gravid	Average of IRD	Average of MBR
Akpabu	3	19	17	2	13	1	1	0.3	5.7
Egbeda	3	9	8	1	6	1	1	0.3	2.3
Elele	3	26	20	1	15	1	2	0.4	5.9
Emohua	4	11	10	1	8	1	2	0.5	3.1
Rumuji	3	15	13	1	10	1	2	0.3	3.8
Total	**3**	**16**	**14**	**1**	**10**	**1**	**2**	**0.4**	**4.2**

R-value = 0.7. Between 0.5 and 1 strong positive. Correlation between IRD and MBR recorded.

Table 3. Man biting rates (MBR) and indoor resting density (IRD) in five communities of Rivers State in the month of October 2014.

Communities	Average No. of occupants	Average No. of mosquitoes sampled	Average of *Anopheles*	Average of Unfed	Average of Fed	Average of Half gravid	Average of Gravid	Average of IRD	Average of MBR
Akpabu	3	13	12	1	10	1	1	0.4	3.9
Egbeda	3	7	7	0	6	1	1	0.3	2.6
Elele	2	19	17	1	12	1	3	0.3	5.2
Emohua	3	16	14	1	11	1	1	0.4	4.9
Rumuji	3	9	8	1	6	1	1	0.4	2.3
Total	3	13	12	1	9	1	1	0.4	3.8

R-value = 0.175.

Table 4. Man biting rates (MBR) and indoor resting density (IRD) in five communities of Rivers State in the month of November 2014.

Communities	Average No. of occupants	Average No. of mosquitoes sampled	Average of *Anopheles*	Average of unfed	Average of fed	Average of half gravid	Average of gravid	Average of IRD	Average of MBR
Akpabu	3	8	6	1	5	0	1	0.4	1.5
Egbeda	3	3	2	0	2	0	1	0.3	0.7
Elele	3	4	4	0	3	0	1	0.4	1.2
Emohua	3	9	8	0	6	1	1	0.4	2.0
Rumuji	4	5	5	0	4	0	1	0.4	1.0
Total	3	6	5	0	4	0	1	0.4	1.3

R-value = 0.075.

An. gambiae was found in this study. The finding that the *An. gambiae* complex is the most abundant is consistent with the findings of Owusu-Ofori *et al.* (2013). They concluded that *An. gambiae* s.s. is omnipresent in Nigeria because of its indiscriminate breeding habits. Morphological identification revealed that *An. gambiae* s.l. is predominant in this study area compared to other *Anopheles* groups.

Table 5. Man biting rates (MBR) and indoor resting density (IRD) in five communities of Rivers State in the month of December 2014.

Communities	Average No. of occupants	Average No. of mosquitoes sampled	Average of *Anopheles*	Average of unfed	Average of fed	Average of half gravid	Average of gravid	Average of IRD	Average of MBR
Akpabu	3	1	0	0	0	0	0	0.3	0.2
Egbeda	3	0	0	0	0	0	0	0.4	0.1
Elele	3	0	0	0	0	0	0	0.3	0.1
Emohua	4	1	1	0	1	0	0	0.4	0.4
Rumuji	3	0	0	0	0	0	0	0.4	0.0
Total	3	0	0	0	0	0	0	0.4	0.1

R-Value = 0.5.

Table 6. Man biting rates (MBR) and indoor resting density (IRD) in five communities of Rivers state in the month of January 2015.

Communities	Average No. of occupants	Average No. of mosquitoes sampled	Average of *Anopheles*	Average of unfed	Average of fed	Average of half gravid	Average of gravid	Average of IRD	Average of MBR
Akpabu	3	0	0	0	0	0	0	0.4	0.0
Egbeda	3	0	0	0	0	0	0	0.3	0.0
Elele	3	0	0	0	0	0	0	0.3	0.0
Emohua	3	0	0	0	0	0	0	0.4	0.0
Rumuji	4	0	0	0	0	0	0	0.5	0.0
Total	3	0	0	0	0	0	0	0.4	0.0

R-value=0.55.

Table 7. Man biting rates (MBR) and indoor resting density (IRD) in five communities of Rivers State in the month of February 2015.

Communities	Average No. of occupants	Average No. of mosquitoes sampled	Average of *Anopheles*	Average of unfed	Average of fed	Average of half gravid	Average of gravid	Average of IRD	Average of MBR
Akpabu	2	0	0	0	0	0	0	0.3	0.0
Egbeda	3	0	0	0	0	0	0	0.3	0.0
Elele	2	1	1	0	1	0	0	0.3	0.4
Emohua	4	0	0	0	0	0	0	0.4	0.0
Rumuji	4	0	0	0	0	0	0	0.5	0.1
Total	3	0	0	0	0	0	0	0.4	0.1

R-value =0.175.

Both *Anopheles gambiae* s.l. and *Culex* mosquitoes were encountered during the course of this study. The species of mosquitoes reported in this study, and probably their susceptibility and resistance status have been reported by different studies in other parts of Nigeria (Ossè *et al*., 2012, Awolola *et al*., 2014; Okorie *et al*., 2015; Nwankwo *et al*., 2017). The higher proportion of *Anopheles mosquitoes* (87%) when compared to *Culex* mosquitoes (13%) in this study is in accordance with the findings of Ndams *et al*. (2006). Relative abundance of Anopheline mosquitoes is as a result of suitable environmental and climatic breeding conditions which favour the breeding of *Anopheles* larvae as also reported by Aribodor *et al*. (2013). The higher density of the anopheline mosquitoes in this study is perhaps an outcome of favourable ecological conditions, rich forest cover of congenial breeding environment. The average indoor resting density recorded in these communities seems to be fairly high which is in accordance with the work done by Jubilate *et al.* (2012).

Across the months, the average indoor resting densities were relatively high in all the communities which confirm that malaria vectors are endophilic and endophagic in the study area. Total average indoor resting density recorded ranged from 0.30 to 0.52 with a peak IRD of 0.52 at Rumuji in the month of February. Man-biting rates in the months of September and December was observed to be 0.04 and 5.89 respectively, with the peak of 5.89 which was recorded at Elele community. This finding on Indoor Resting Density (IRD) and Man Biting Rates (MBR) according to community is critical to the stratification of malaria by risk and vector resting behavior. This variability can be studied in relation to the potential effectiveness and sustainability of available interventions and to identify which vector control measures might work best under which circumstances (WHO, 2013). Stratification unites areas, populations or situations that exhibit a relative resemblance of a set of specified relevant characteristics, known as variables, thereby distinguishing them from other areas, populations or situations dissimilar by the same set of characteristics (WHO, 2013). The high proportion of *Anopheles gambiae* s.l. in the months of September and October during the study period could be attributable to availability of more breeding sites during this season; the low number of *Anopheles* mosquitoes recorded in other months in the study community could be due to the dry season. This is in consonance with the findings of Oduola *et al*. (2012 and 2013) who reported a significantly higher proportion of *An. gambiae* s.s. during the rains. The presence of other non-malaria vector i.e. *Culex* is suggestive of the level of nuisance experienced by the inhabitants of these areas, and the density of *Culex* species is a bio-indication of filariasis in this locality which also is of public concern. The preponderance of the *An. gambiae* s.l group in this study is attributable to their anthropophilic behaviour. This, as above explains the variations observed in different communities during the course of this work.

In some cases, very low number of indoor mosquitoes was recorded. This may be due largely to the residual effects of IRS intervention carried out in the locality, according to the findings of Masaninga *et al*. (2012) in Lusaka Zambia where a consistent decline in the anopheline vectors was observed in the Nation from 2004 to 2010 due to an effective implementation of malaria vectors control mechanism.

With respect to the feeding status of mosquitoes, it was observed that 81% of the mosquitoes caught had taken blood meal from the occupants and were resting in the rooms in the morning, 6.6% of the mosquitoes were unfed, 5.2 and 7.2% were half gravid and gravid respectively, which explains the host preference of local indoor resting mosquitoes in the study communities. This is in accordance with the findings of Adeleke *et al*. (2010) where the fed mosquitoes dominate over unfed mosquitoes. But somehow in contrast with the findings of Okwa *et al*. (2007a) where most of the communities sampled had a higher number of unfed mosquitoes. Elele community recorded the highest number of average fed mosquitoes with 15.11% in the month of September which is the peak of rainy season compared to other communities. This is an indication of the fact that the inhabitants of Elele are more prone to malaria infection since *Anopheles* mosquitoes have been incriminated as an efficient vector of malaria (Adeleke *et al*, 2010).

The abundance of mosquitoes in Elele, Emohua, Akpabu and Rumuji are all higher than that of Egbeda community. This observation could be due to microecological differences in these communities. The malaria vector abundance status in relation to stomach stages revealed more mosquitoes in the month of September. This is in accordance with the findings of Emidi *et al.* (2015) who reported the highest abundance of mosquitoes to be in September and October in Kintampo Ghana. September being the peak of rainy season and most efficient period for malaria vector distribution and abundance recorded the highest number of half-gravid and gravid mosquitoes 32 and 61 respectively, this is an indication of more mosquitoes and if allowed to oviposit without control, the vector abundance will be very high. From the tables, Elele still recorded the highest number of gravid mosquitoes (2.50) in September indicating more malaria abundance in the area. Abundance of *anophelines* is as a result of suitable environmental and climatic breeding conditions which favour the survival of larvae. This was also reported in the findings of Minakawa *et al.* (2002).

Conclusion

In conclusion, this study has contributed to the understanding of the distribution, composition and indoor resting behaviour of mosquito vectors in the study areas.

Recommendations

It is recommended that the findings are of immense value for working out vector intervention strategies in the study area, taking into consideration the fact that malaria vector abundance in the area is due to favourable breeding conditions found in the area, since from the findings of this study *An. gambiae* s.l is the most important malaria vector found in these communities.

CONFLICT OF INTEREST

The authors declare that they have not conflict of interest.

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