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RESEARCH ARTICLE

THE DISTRIBUTION OF MOSQUITO LARVAE AND PUPAE ON A UNIVERSITY CAMPUS IN AWKA, ANAMBRA STATE, NIGERIA; A COMPARATIVE INVESTIGATION

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ABSTRACT

Background: The World Malaria Report has estimated that 3.4 billion people are still at risk of infection via malarial parasites irrespective of global efforts to control infection and elimination. Over 80% of these cases are diagnosed in Africa.
Aims: The primary aim of this study was to investigate the distribution of mosquitoes across a residential university campus (Nnamdi Azikiwe) via the collection of eggs, larvae and pupae. The secondary aim was then to compare the relative incidence and prevalence rates of malaria diagnosis with the relative distribution of these malarial vectors amongst students living in the study area.
Methods: A cross sectional survey was executed with 384 participants living in residential accommodation at Nnamdi Azikiwe University via a questionnaire. Mosquito eggs, larvae and pupae were manually collected using ovitraps and ladles. The mosquitoes were taken to the National Arbovirus and Vectors Research Division Enugu, Nigeria for positive identification.
Results: The study showed that an increase in malaria vector had a significant impact in the prevalence rates of malaria diagnosed amongst students living in residential accommodation at Nnamdi Azikiwe University. The comparator study recorded 58 (39%) prevalence in 2012 while the research study indicated a prevalence of 116 (43%) which was calculated to be double the prevalence of the students recorded as being exposed in 2012). Malaria vector abundance also increased by a ratio of 1:5 between 2012 with 38 (19%) and 195 (61%) in 2016.
Conclusion: Ensuring adequate interventions to maintain vector density low as well as continuous vector surveillance to prevent increasing incidence and prevalence rates of malaria are required.

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INTRODUCTION

Malaria elimination has long been a challenge in both public and global health. The sixth Millenium Development Goal (MDG) aimed to combat HIV/AIDS, malaria and infectious diseases (WHO, 2016). According to Aribodor *et al.* (2016) some of the notable challenges to achieving malaria elimination in Nigeria revolve around treatment failure attributable to drug resistance; insecticide resistance; climate change due to global warming; conflicts, terrorism or insurgency; attitudinal and behavioural change; the political will to implement policies; insufficient funding and a lack of evidence based research data for implementation in continuous health improvement strategies.

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Entomological surveillance in public health (Govella *et al.* 2013; WHO 2015) remain key mechanisms of generating comprehensive information which can inform the most appropriate mechanisms of mosquito (vector) control. They are most significant in generating comprehensive information on vector abundance (Oduola *et al.*, 2013). Routine and continuous surveys are used to monitor those specific species of mosquito known for their ongoing contribution to malarial transmission. Other strategies for health protection in mosquito endemic areas include environmental sanitation; use of long-lasting insecticide nets (LLINs); indoor residual spraying (IRS); larval source management; elimination of breeding sites; insecticide resistance policies and insect repellents (WHO, 2015 and CDC, 2016).

Vector Transmission

Mosquitoes are vectors of public health importance. The bite of the bloodsucking females who depend on blood meal for egg development can transmit deadly diseases to man and other animals (Deng *et al*., 2012). The feeding process involves salivary contact from the mosquito to its host (Hopp and Sinnis, 2015). Thus, transmission may occur if the mosquito is infected. Man is a host for many mosquito diseases. Some of which are Malaria, Dengue Fever, Yellow Fever, Chikungunya virus, Zika virus and La Crosse Encephalitis virus (Medlock *et al*., 2015). These infections are transmitted by the female Aedes species except for malaria transmitted by female Anopheles species. Those transmitted by the female Culex species include West Nile virus, Saint Louis encephalitis virus, Japanese encephalitis virus, Western equine encephalitis virus, Eastern equine encephalitis virus, and Venezuelan equine encephalitis virus (CDC 2016 and Reiss 2016). Of all these, malaria is the most common in Africa with 1.2 billion people living in high-risk areas (WHO, 2015) and several challenges to its elimination.

Epidemiology and Transmission

The Malaria parasite was first discovered in the erythrocytes of patients by Alphonse Laveran in 1880 (Taki and Smooker, 2014). Further research by Ronald Ross in 1897 described the transmission pattern more specifically (Ahorlu, Kumordzi, and Koram, 2014). Since then, several studies have been undertaken to understand the scope of malarial infection and how control might be achieved. The World Malaria Report provided an estimate of 3.4 billion people still at risk of infection with malarial parasites irrespective of global efforts in controlling and eliminating disease vectors (WHO, 2016). Murray *et al*. (2012) in a systematic analysis of global malaria mortality highlighted that, whereas African nations observed a consistent reduction in malaria deaths from 502,000 in 1980 to 104,000 in 2010, malarial mortality in Africa increased from 493,000 to 1.6 million between 1980 to 2004 and dropped by 480,000 in 2010 with to just over 1 million deaths. Across Nigeria, approximately 51 million people have malaria with over 207 thousand reported deaths per annum (Noland *et al*., 2014). Vector control is still a fundamental weapon in the fight against vector borne diseases (WHO 2016; Guest, 2014). Hence, entomological surveys in public health predominantly aim to measure the relative abundance of arthropod vectors transmitting human diseases so that the initiation of control strategies can begin.

Research Aims and Objectives

The study was designed to compare the relative abundance of the malaria vector and the relative rate of malaria diagnosis amongst students, living in residential accommodation, with a previous data set from the area. The design aimed to facilitate:

- Exploration and description of the most prevalent human mosquito vectors present.
- Identification of egg, larvae and pupae breeding sites of malarial vectors within the geographical area.
- Determination of the frequency of malarial diagnosis among the students residing within the area relative to the prevalence of malarial parasites.

Research Question

'Is there a comparison between the relative abundance of malaria vector and the rate of malaria diagnosis among the residential students in Nnamdi Azikiwe University Awka, Anambra State, Nigeria?'

Hypothesis

H0: There is no comparison between the relative abundance of malaria vector and the rate of malaria diagnosis among the students in Nnamdi Azikiwe University Awka, Anambra State, Nigeria

HA: There is a comparison between the relative abundance of malaria vector and the rate of malaria diagnosis among the students in Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

Research Design and Methodology

Study Area

The study was conducted in Nnamdi Azikiwe University (latitude 6.2445° N and longitude 7.1214° E) between 15th and 30th August 2016. The area is located along Enugu-Onitsha Expressway, Ifite Awka, Anambra state, Nigeria. The University and its environs are considered high transmission areas with more than one case per 1000 population (Nworah, 2013). The estimated number of students living inside the university is approximately 5,000 persons per session. Student accommodation within the university is of two main types particularly the male, and then the female hostels. The female hostels are Dora Akunyuli and Stella Okoli hostels (Block A and Block B respectively) while the male hostels are Block D and Block E. There is also the postgraduate hostel (Block C). However, the university accommodation has equal number of rooms designated for each group such that the ratio of male to female residents is 1:1. Two dominant seasonal conditions are experienced in the area. One, is the dry season which comes up between November and March, and the other is the rainy season from April to October. The dry season is accompanied by harmattan from December to January. With an altitude of 81m, the annual precipitation is 1808mm. However, the difference in precipitation between the driest and wettest months (December and September) is 297mm. Average precipitation for the month of August is 202mm. Average annual temperature is 27.1°C but the variation in temperatures all year round is 3.7°C. The maximum and minimum temperatures for the month of August are 28.3°C and 22.5°C respectively whereas on average it is 25.4°C. Climate is tropical and classified by the Köppen-Geiger climate as Aw.

MATERIALS

The materials used include; oviposition cups, filter paper, ladle, sieve, white plastic bowl, transparent plastic container, and questionnaires.

Materials used for Mosquito egg, larvae and pupae sampling

- Oviposition cups: Fourteen plastic cups of 13cm length and 22cm diameter were used as ovitraps in suspected breeding sites within the study area. The observed suspected breeding sites were majorly fast flowing waters in open drainage systems close to the students' residence.

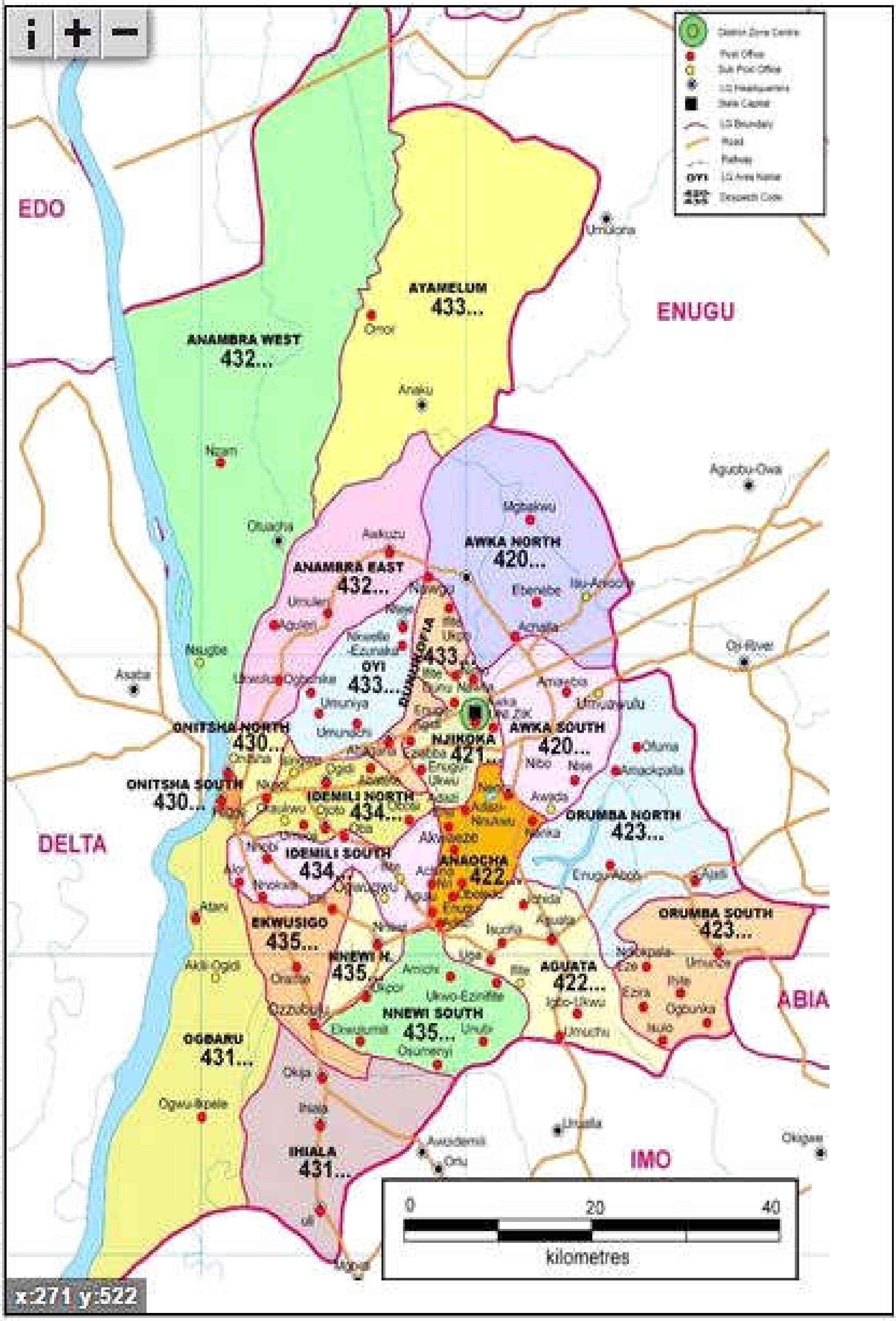

Figure 1. Map of Geographical Location of Anambra State

- Cream coloured fabric: This an inexpensive fabric was laid inside each oviposition cup to cover all its sides from the top side to the bottom side leaving the two ends uncovered.
- Ladle: A ladle was used to scoop for larvae and pupae of mosquitoes from open drainage systems close to the students' residence using the method applied by Le Goff *et al*., (2014).
- Sieve: The content of the ladle was poured into the sieve to drain off debris and dirt scooped along with the larvae and pupae.
- White plastic bowls: The sieve with its open end facing downwards was washed into the white plastic bowl using a 60cl of visibly clean water to release the aquatic larvae and pupae.
- Transparent plastic containers: These were used to temporarily store the larvae and pupae of mosquitoes collected on site.

Materials used for Survey Questionnaire

The WHO household Malaria Indicator Survey (MIS) was used as a guideline to extract the survey questions. In Specifics, questions from section 1 (demographics) were extracted from the column 2 of MIS; section 2 (trips/movement) from questions 103, 2B, 2C and column 2 of MIS; section 3 (symptoms/diagnosis) from questions 218 and 221 of MIS while section 4 (prevention/control) was extracted from questions 112-121 of MIS.

Study Design

A cross-sectional survey design was used to examine the exposure of students to malarial parasites who live within university residential accommodation. Practically, the study involved sampling students to collect prevalence data on malaria status and sampling mosquitoes to determine the population of the malaria vector in the study area between 15th and 30th August 2016. Cross-sectional studies were used to examine the relationship between a health related state and other variables existing in a defined population at a particular point in time (Lobo *et al*., 2016). They aided in describing the prevalence of disease occurrence and required data on all variables to be collected at once (ibid., 2016). This was a purposive approach adopted as a means of obtaining health related characteristics or disease frequencies, such as exposure variables, at a given point in time within a population.The descriptive cross-sectional survey was used to estimate disease burden in a given population, while the analytical surveys were used to investigate relationships between hypothetical risk factors and defined health outcomes (Hair, 2015).

Sample Size

A survey system sample size calculator was used to calculate the sample size for the survey population. The sample size was determined to be 384 with a confidence interval of 5 at 95% confidence level. In statistics, confidence intervals represent the measure of uncertainty associated with the estimated sample of a population parameter (Moore and Langley, 2011). Alternatively, confidence levels could be used to describe the percentage of all possible samples expected to determine the actual population parameter (ibid., 2011). This meant that at 95% confidence level, 95% of the confidence interval determined the actual population parameter.

Sampling

Sampling aided the collection of data from a target population. However, certain considerations are involved in specifying how each target population would be fully represented in any given sample. Some of which include sample size, sample error, and sample bias. All of these were considered in the course of sampling the target populations involved in the study (mosquito population and students of Nnamdi Azikiwe University residential accommodation). Sampling of the student population was undertaken using a simple random method whilst mosquito-sampling techniques were used in sampling the mosquito population. The simple random method was used in preference to a stratified random sampling technique as the confounding variables of age and gender involved in the research have not formally been attributed to the spread or control of malaria. However early researchers recorded some gender attribution to mosquito bite preference (Logan, 2008; Pérez-Guerra, 2009). They suggested that men were less likely to be bitten by mosquitoes because the body odour produced by their sweat glands repelled mosquitoes. The research was geared towards collecting data at the conclusion of the examinations period in order to be able to access a higher number of students, with the intention of increasing the response rate. Using a stratified sampling technique would have increased the number of participants and this may not be have been achievable within the time frame of the research.

Core Considerations

In Nnamdi Azikiwe University, does the abundance of the malaria vector, compared to low abundance, decrease malaria prevalence?

P Population	Residential Students of Nnamdi Azikiwe University
I Intervention	Vector abundance control measures
C Comparator	No measures taken
O Outcome	Reduction in vector abundance

- **Mosquito collection:** Three developmental stages of mosquitoes were collected in the study area. These include eggs, larvae and pupae of mosquitoes, which were collected in their breeding sites as well as small water pools around the student residence with the aid of the materials earlier mentioned.
- **For Survey Questionnaire:** The researcher self-administered the questionnaires to students in the visiting halls of each hostel. The PICO criterial was used to choose the participants as well.

P Population	Residential Students of Nnamdi Azikiwe University
I Intervention	Malaria preventive measures (LLIN and IRS)
C Comparator	No preventive measures
O Outcome	Reduction in malaria prevalence

Data Collection Procedure: This involves detailed records of sampling, identification and collation of questionnaires.

- **Egg collection:** Fourteen ovipositon containers of 300ml capacity each were placed in 7 different sampling sites between 22nd and 26th August 2016. The Blocks A and B are the female hostels labelled as female hostel bus stop (FH), female hostel 1 entrance (FH 1), female hostel 2 entrance (FH 2), and female hostel 3 entrance (FH 3). In like manner, the male hostels (block D and E) alongside the postgraduate hostel (block C), were labelled as male hostel 1 entrance (MH1), male hostel 2 entrance

(MH 2) and male hostel bus stop (MH) respectively. These 7 location sites were points within 1-Km radius of the hostels as such, a single ovitrap was used for sampling each site. All ovitraps were layered with cream coloured fabric as a substrate on the inside, according to the method described by Service (2012). The traps were labelled with code numbers representing each location after which, three-quarter of the ovitrap containers were filled with water. All the ovitraps were placed in suspected mosquito breeding sites for 24hours in contrast with weekly collections observed in many studies due to time constraint. However, it was considered that the results observed in a daily collection and that observed in a weekly can be equitable since

Figure 2. Oviposition container with substrate

- **Larvae and Pupae Collection**: A dipping method was adopted to collect the mosquito larvae and pupae between 26th and 30th August 2016. Sample collections were carried out within the 7 different location sites in the study area. Each location was sampled once every day for five days covering all potential larval habitats within 1-km radius of the student accommodation. Practically, this involved immersing a 35cm ladle into an open drainage with an estimated $13m^2$ surface area and a capacity of 1260L. The ladle which could hold a 175ml was allowed only three-quarter of water at each dip to prevent the larvae from escaping as a result of the sudden distortion of water. After each dip, contents were emptied into transparent containers of 340ml as described by Okeke *et al*. (2016) if the water was clean. However, some researchers transfer the larvae directly to tiny hole sieves where they were washed in to white plastic containers with clean water as described by Onyiudo *et al*. (2012) when the water is cloudy. In general, both techniques were used as the open drainage in the female hostel had visibly clear water while that of the male hostel required a clean water wash. A 165cm sieve was used to drain the larvae and clean sachet water of approximately 0.6L was used to wash the larvae into a 2.5L white bowl.

- **Comparator study:** The comparator study was performed by Onyiudo *et al*. (2012). The study objective was to determine the abundance of mosquitoes and investigate malaria prevalence among students living in Nnamdi Azikiwe University residential accommodation. To achieve this, 149 volunteer students were examined for malaria using rapid diagnostic tests. Mosquito collection was conducted using two sampling techniques. The first was the larval collection within the hostel environs and the second was the collection of adult mosquitoes from the postgraduate block as well as hostels A, B and D. The target breeding sites were septic tanks, littered containers, construction sites, stagnant water pools and tyres around the student hostels. Larvae collection was by the use of a ladle while aerosol-based pyrethroid (Raid) was used for adult collection. The rooms of the participants were sampled by convenience. The larvae and adult samples of mosquitoes collected were taken to the National Arbovirus and Vectors Research Institute, Enugu for identification.

Data Analysis: Oneway ANOVA was used to test for relationships between the locations and the mosquito species collected. The analysis was carried out using SPSS 22.0.0.0 to interpret and determine the relationship between the variables (location and mosquitoes) involved in the research. Specifically, the Excel was used to analyse the malaria survey.

- **Mosquito Survey:** The collected mosquito eggs, larvae and pupae were taken to the National Arbovirus and Vectors Research Division of the Federal Ministry of Health, Enugu state for identification.
- **Survey Questionnaire:** Students who completed the questionnaires made contact with the researcher through flash calls (in which case, the researcher called back), free short message services (SMS), mails and other social network groups such as 'Whats App' to discuss the time for collection in the visiting halls of each hostel primarily at their convenience. This was achieved using a mobile number specifically assigned for the project which was made available on the consent form and the participant information sheet as well as the questionnaire.

Using ovitraps, 219 mosquito eggs were collected and immersed in water till they metamorphosed to larvae. Of the 219 eggs, 101 hatched to larvae. Table 1 shows the eggs collected with ovitraps, number of days collection was made, number of ovitraps set, number of positive ovitraps, number of negative ovitraps, and those hatched in to larvae during the study period, from 15th to 30th August 2016. Of the 101 larvae hatched out, 3 mosquito genera were identified. The identification process at the National Arbovirus and Vectors Research Division was based on the morphological features of the larvae. Table 2 shows the distribution of the hatched larvae by location and genus. The numbers in parenthesis indicate the calculated percentage of mosquitoes collected. Three Mosquito genera were identified in the 7 different locations of Nnamdi Azikiwe University hostel where FH represents female hostel and MH stands for male hostel. The identification was done at the larval stage using its morphological features and behaviour in water. The genera include the Anopheles 60 (59.41%), Culex 12 (11.88%), and Aedes 29 (28.71%). The Anopheles genus was identified in all 7 locations followed by the genus Aedes identified in 6, of the 7 locations. The results indicate the abundance of the malaria vector present in the area unlike the genus Culex which appeared not so abundant. Although the Anopheles genera was identified in all the 7 locations, it appears less abundant in the Male hostels but more abundant in the female hostels.

Table 1. Showing Eggs Collected with Ovitraps between 22nd and 26th of August 2016

Days	Number of Ovitraps	Number of positive Ovitraps	Number of negative Ovitraps	Number of eggs	Number of larvae Hatched Out
DAY1	14	7	7	30	10
DAY2	14	7	7	27	14
DAY3	14	12	2	15	7
DAY4	14	10	4	12	5
DAY 5	14	14	0	135	65
TOTAL				219	101

Table 2. Distribution of Mosquito larvae (collected as eggs) According to Location and Genus between 22nd and 26th of August 2016

LOCATION	Anopheles (%)	Culex (%)	Aedes (%)	TOTAL (%)
FH 1 bus stop	10(66.67)	3(20.00)	2(13.33)	15(14.85)
FH 2 entrance	11(64.71)	0(0)	6(35.29)	17(16.83)
MH 1 entrance	7(38.89)	3(16.67)	8(44.44)	18(17.82)
MH 1 bus stop	4(36.36)	4(36.36)	3(27.27)	11(10.89)
MH 2 entrance	6(46.15)	0(0)	7(53.85)	13(12.87)
FH 3 entrance	12(92.3)	1(7.69)	0(0)	13(12.87)
FH 1 entrance	10(71.43)	1(7.14)	3(21.43)	14(13.86)
TOTAL	60(59.41)	12(11.88)	29(28.71)	101

Table 3. Shows the Larvae and Pupae collected (with Ladle) between 26th and 30th August 2016

Days	Number Of larvae Collected	Number of pupae Hatched Out	Number Of pupae Collected	Number of adult Hatched Out
DAY1	10	6	22	20
DAY2	7	3	32	28
DAY3	1	0	16	12
DAY4	12	5	12	6
DAY 5	15	7	15	9
TOTAL	45	21	97	75

Table 4. showing the Genus of larval mosquitoes collected with ladle between 26th and 30th August 2016

DAYS	Anopheles	Culex	Aedes	Number of unhatched Larvae collected with ladle
1	3	0	1	4
2	2	1	1	4
3	1	0	0	1
4	4	1	2	7
5	5	1	2	8
Total %	15(62.50)	3(12.50)	6(25.00)	24

Table 5. shows the distribution of pupae (by genera) collected between 26th and 30th August 2016

DAYS	Anopheles	Culex	Aedes	Total Number of Pupae (ladle collection + hatched
1	20	4	4	28
2	26	5	4	35
3	12	1	3	16
4	10	2	5	17
5	12	4	6	22
Total %	80(67.80)	16(13.56)	22(18.64)	118

Table 6. Distribution of Adult Mosquitoes (collected as pupae) According to Location and Specie from 26th to 30th August

LOCATION	*Anopheles funestus* (%)	*Culex Quinquefasciatus* (%)	*Aedes eagypti* (%)	TOTAL (%)
FH 1 bus stop	6(46.15)	3(23.07)	4(30.77)	13(17.33)
FH 2 entrance	7(50.00)	3(21.43)	4(28.57)	14(18.67)
MH 1 entrance	4(57.14)	3(42.86)	0(0)	7(9.33)
MH 1 bus stop	4(50.00)	2(25.00)	2(25.00)	8(10.67)
MH 2 entrance	4(66.67)	0(0)	2(33.33)	6(8.00)
FH 3 entrance	5(55.56)	4(44.44)	0(0)	9(12.00)
FH 1 entrance	10(55.56)	6(33.33)	2(11.11)	18(24.00)
TOTAL	40(53.33)	21(28.00)	14(18.67)	75

Table 7. Socio-demographics of Respondents

	Category	Frequency
Age range		
	16-20	111(39.50%)
	21-25	155(55.16%)
	26-30	14(4.98%)
	Above 30	1(0.36%)
Gender		
	Male	143(49.31%)
	Female	147(50.69%)
Hostel Resident		
	Yes	292(95.74%)
	No	13(4.26%)

Table 8. Student Movement During Campus Teaching Sessions

Variables	Responses	Frequency
Travels		
	Yes	182(62.12%)
	No	111(37.88%)
Over 1 week travel		
	Yes	66(23.40%)
	No	216(76.60%)
Visit friends in school		
	Yes	192(66.21%)
	No	98(33.79%)
Over 3 days visiting friends		
	Yes	112(39.72%)
	No	170(60.28%)

Statistical analyses however, indicate that there is no relationship (P>0.05) between the location and the abundance of mosquito genus identified (see appendix 2). This means that the location does not determine the kind of mosquito larvae collected.

Using a ladle, 45 mosquito larvae were collected alongside 97 mosquito pupae. Of the 45 mosquito larvae collected, 21 hatched in to pupae between the 26th and 30th of August 2016, while 75 adult mosquito species hatched out of the 97 mosquitoes collected as pupae within the same period. Altogether, the larvae, pupae and adult mosquitoes were identified to belong to three mosquito genera. This includes the Anopheles, Culex and Aedes. Both the larvae and pupae where identified to genus level only using their morphological features and breeding habits. However, the adults were identified to specie level indicating the abundance of *Anopheles funestus*, *Culex quinquefasciatus*, *and Aedes eagypti*. Thus, table 3 (above) represents the number of larvae collected with ladle, number of pupae hatched out of the larvae, the number of pupae collected with ladle and the number of adults hatched from the pupae collected. Table 4 and 5 (below) show the distribution of the three mosquito genera across the number of larvae collected with ladle and the total number of pupae (consisting of the ladle collected pupae and the hatched ones) respectively. Of the 45 larvae collected with ladle, 24 mosquito larvae did not hatch in to pupae. Thus they were identified to genus level at the larval stage and three genera of economic importance were observed. These are the Anopheles 15(62.50%), the Culex 3(12.50%) and Aedes 6 (25.00%). The Anopheles species appear to be more abundant followed by the genus Aedes. One way analysis of variance was used to test for relationships between the days and the larvae collected. The statistical analysis showed no difference (P>0.05) between the day each larvae was collected and the genus that was identified (see appendix 3).

This means that the number of pupae collected per day is not determined by the actual day of collection. All the pupae collected by ladle (97) and those hatched out (21) between the 26th and 30th of August were identified to genus level. Three main genera were observed in the study area. They include the Anopheles 80(67.80%), Culex 16(13.56%) and Aedes 22(18.64%). Analysis of variance was used to test for an association between the days of collection and the pupae collected. However, statistical Analysis show there is no relationship (P>0.05) between the day each Pupae was collected and the abundance of the genus identified (see appendix 4). This practically means that the 20 Anopheles pupae collected on day 1 (26th August, 2016) was not collected particularly because it was day 1. In other words, the days do not determine the number of pupae collected.

Of the 97 pupae collected with ladle, 75 metamorphosed to adult. These adult mosquitoes were identified to specie level. They consist of 3 mosquito species important to man and they belong to three different mosquito genera. They include *Anopheles funestus* 40(53.33%), *Culex quinquefasciatus* 21(28.00%), and *Aedes eagypti* 14(8.67%). They all belong to the genus Anopheles, Culex and Aedes respectively. The location symbols FH and MH represent the female hostels and the male hostels in the study area. From the calculated percentages in parenthesis, *Anopheles funestus* and *Culex quinquefasciatus* appear to be most abundant in the female hostel 1 entrance. However, statistical analysis show there is no difference (P>0.05) between the location where each mosquito was collected and the mosquito specie identified (see appendix 5).

Results for Survey questionnaire on malaria

384 questionnaires were administered during the study to both male and female residents in the study area.

Table 9. Symptoms and Malaria status of Respondents in the Study Area

Variables	Responses	Frequency
Extreme weakness	Never	52(17.33%)
	Sometimes	180(60.00%)
	Most times	41(13.67%)
	Always	27(9.00%)
Cold and Shivering	Never	63(21.07%)
	Sometimes	146(48.83%)
	Most times	48(16.05%)
	Always	42(14.05%)
Headache	Never	28(9.30%)
	Sometimes	156(51.83%)
	Most times	64(21.26%)
	Always	53(17.61%)
Vomiting	Never	154(52.03%)
	Sometimes	107(36.15%)
	Most times	23(7.77%)
	Always	12(4.05%)
Feverish	Never	63(25.00%)
	Sometimes	63(25.00%)
	Most times	63(25.00%)
	Always	63(25.00%)
Tired/Sweaty yet normal temp	Never	76(26.03%)
	Sometimes	152(52.05%)
	Most times	37(12.67%)
	Always	27(9.25%)
Body pains	Never	84(28.38%)
	Sometimes	149(50.34%)
	Most times	34(11.49%)
	Always	29(9.80%)
Mild Jaundice/Yellow skin	Never	261(88.78%)
	Sometimes	22(7.48%)
	Most times	6(2.04%)
	Always	5(1.70%)
Breathing difficulty	Never	222(75.51%)
	Sometimes	59(20.07%)
	Most times	6(2.04%)
	Always	7(2.38%)
Dark Urine	Never	222(74.50%)
	Sometimes	50(16.78%)
	Most times	18(6.04%)
	Always	9(2.68%)
Abnormal Bleeding	Never	272(91.28%)
	Sometimes	21(7.05%)
	Most times	4(1.34%)
	Always	1(0.34%)
Diagnosed of malaria during exams	Never	100(34.13%)
	Sometimes	139(47.44)
	Most times	39(13.31%)
	Always	15(5.12)
drugs for malaria in past 2 weeks	Never	154(57.04%)
	Sometimes	73(27.04%)
	Most times	20(7.41%)
	Always	23(8.52%)

Of the 384, 305 questionnaires were returned giving a calculative response rate over 79%. The completes from the males were 143 questionnaires consisting of 49% of the total filled and returned questionnaires. Likewise, the female completes were 147 questionnaires consisting of over 50% of the total filled and returned questionnaires. Table 7 shows the socio-demographics of the respondents. Of the 305 returned questionnaires, 13 (4%) respondents do not live in the university accommodation while 292 (95%) respondents admitted to living within the area.

Respondents between the ages of 21 and 25 appeared the largest age group of 155 students (55%) in the study area followed by respondents in the age range of 16-20 111(39.5%). This suggests that majority of the students living in the university accommodation are undergraduates in their first year or “freshers” as they are locally called. Apparently, it could be argued that many of the post graduate students do not live in the school accommodation as the 26-30 and above 30 age brackets are less than 10%. However, it may be that the cost effectiveness of living in the university accommodation is

Table 10. Prevention and Control measures used in the study area

Variables	Responses	Frequency
Use mosquito net		
	Never	105(34.77%)
	Sometimes	65(21.52%)
	Most times	45(14.90%)
	Always	87(28.81%)
Have more than one net		
	Never	247(83.16%)
	Sometimes	23(7.74%)
	Most times	9(3.03%)
	Always	18(6.06%)
LLIN more than a month old		
	Never	128(44.14%)
	Sometimes	27(9.31%)
	Most times	24(8.28%)
	Always	111(38.28%)
Soaked LLIN in liquid to repel mosquito		
	Never	262(88.23%)
	Sometimes	23(7.74%)
	Most times	8(2.69%)
	Always	4(1.35%)
Soaked LLIN in liquid to repel mosquito less than a month		
	Never	267(89.90%)
	Sometimes	15(5.05%)
	Most times	3(1.01%)
	Always	12(4.04%)
Slept under LLIN last night		
	Never	163(54.88%)
	Sometimes	22(7.41%)
	Most times	11(3.70%)
	Always	101(34.00%)
IRS sprayed within 6 months		
	Never	225(75.50%)
	Sometimes	46(15.44%)
	Most times	13(4.36%)
	Always	14(4.70%)
Personally spray mosquito repellent		
	Never	191(66.09%)
	Sometimes	61(21.11%)
	Most times	15(5.19%)
	Always	22(7.61%)

quite adaptive and could serve as a driving force for incoming students compared to post graduate students who possibly have a steady source of income. Hence, they could live in the university and make regular trips home. Table 8 shows the pattern of student movement during campus teaching sessions; which could effectively increase or decrease their relative chances of malaria infection. Trips outside the university appear to be consistent. The respondents who make trips outside the university are approximately two times more than those who do not. However, the trips appear not to last longer than 3 days. Research has shown that host movement within perimeters of high vector densities can increase chances of exposure even in endemic zones (Idris *et al*., 2014; CDC, 2016). This implies that within endemic zones, some places could have higher endemicity than others which further increases the chances of exposure. WHO (2015) has categorized this as high transmission, low transmission or malaria free zones based on the level of cases per 1000 population. In Nigeria, 76% of the population live in high transmission areas with over 135,000,000 at risk (WHO, 2015). Thus CDC (2016) advice travellers to conduct individual risk assessment which considers not just the destination country profile but also, specific cities, accommodation types, seasons and means of transportation in the area. Table 9 shows the signs and symptoms observed by the respondents as well as their malaria status since they have been in the school accommodation which is usually a one-year period. In practice, signs and symptoms generally observed by individuals are not enough for diagnostic inference. This is to say that the signs and symptoms represented by the respondents cannot be used to make conclusions on their malaria health status. However, the respondents were asked if they had ever taken anti-malaria drugs prescribed by a doctor for a 2-week period since their stay in the university accommodation. To this, 154 (57%) students responded never, another 73 (27%) appeared to have sometimes received treatment while 20 (7%) others have received such treatments most times. However, 23 (9%) students indicated they had always received a 2-week prescribed treatment for malaria in the study area. This could be interpreted to mean that within an academic session, a period of 8 to 9 months, 57% of students living in the university accommodation have not at onetime, been given anti-malaria medication while 34% intermittently receive treatment. In addition, some of the students 139 (47%) admitted to have sometimes been treated during exams while 100 (34%) students responded never when asked if they had

Table 11. Comparator Study Results

Variables	Comparator Study	Research under study
Objective	to determine the abundance of mosquitoes and investigate malaria prevalence among students living in Nnamdi Azikiwe University accommodation	to compare the relationship between the relative abundance of malaria vector and the rate of malaria diagnosis among the students in Nnamdi Azikiwe University accomodation
Materials	Ladle, aerosol-based pyrethroid (Raid) and RDT kits	oviposition cups, filter paper, ladle, sieve, white plastic bowl, transparent plastic container, and questionnaires
Study design	Cross-sectional Survey	Cross-sectional Survey
Sample size	149 participants	384 participants
Method of Sampling	Convenience sampling	Simple random sampling
Data collection procedure	• Collection sites include septic tanks, littered containers, construction sites, stagnant water pools and tyres around the student hostels. • Use of ladle for larvae collection • Use of aerosol based pyrethroid (Raid) for adult collection • Use of RDT for malaria tests	• Collection site was majorly open drainage (gutters) with an approximate capacity of 1260L • Use of oviposition trap for egg collection • Use of ladle for larvae collection • Use of questionnaire for malaria prevalence determination
Data Analysis	• Identification of mosquitoes done at National Arbovirus and Vectors Research Institute, Enugu • RDT analysed in Nnamdi Azikiwe University Parasitology laboratory	• Identification of mosquitoes done at National Arbovirus and Vectors Research Institute, Enugu • Malaria questionnaire analysed using microsoft Excel
Result on mosquito Abundance	• 720 mosquito larvae collected • 201 adult mosquitoes collected • Mosquito Genera collected • Anopheles 38(18.91%) • Culex 87(43.28%) • Aedes 123(61.19%) • Species of adult mosquitoes collected • Anopheles gambiae 38(18.91%) • Culex quinquefasciatus 87(43.28%) • Aedes albopictus 87(43.28%) • Aedes eagypti 36(17.91%)	• 219 eggs collected • 125 larvae (non-metamorphosed larvae from ladle collection + larvae hatched from egg) • 43 pupae (ladle collected pupae + larvae that developed to pupae) • 75 adult mosquitoes (developed adults from pupae) • Mosquito Genera collected • Anopheles 195(61.32%) • Culex 52(16.35%) • Aedes 71(22.35%) • Species of adult mosquitoes collected • Anopheles funestus 40(53.33%) • Culex quinquefasciatus 21(28.00%) • Aedes eagypti 14(18.67%)
Results on malaria Prevalence	• Of 149 participants, 58(38.93%) infected	• Of 384 participants, 116(42.97%) probably infected

ever been diagnosed of malaria during exams. The respondents were also asked for observed signs or symptoms. 180(60%) students indicated they sometimes feel extreme weakness, 189 (75%) feel feverish, while 152 (52%) feel tired and sweaty even on a standard body temperature. 146 (49%) students sometimes have cold and shivers, 156 (52%) sometimes have a headache while 149 (50%) sometimes experience body pains. Majority of respondents indicated they never experienced mild jaundice 261 (89%), breathing difficulty 222 (76%), or abnormal bleeding 272 (91%). Similarly, an approximated 76% (222) indicated they never had dark urine.

All in all, general conclusions on malaria status were deduced based on the respondents who sometimes, most times or always taken anti-malaria drugs for a 2-week period since they had been living in the school accommodation. Treatments were confirmed by asking respondents to include the name of the anti-malaria drug used. Figure 11 and figure 12 are graphs showing students who treat malaria during exams and the common anti-malaria drugs used in the study area as a means of controlling the parasite. Table 10 however shows the prevention and control measures used by students to control the vectors. Of the 305 respondents, 87(28%) always use mosquito net however, 105 (35%) never use mosquito net. It could be argued that 35% of the respondents do not have mosquito net since 65 (22%) and 45 (15%) admitted to sometimes or most times use their mosquito net. Again, 247 (83%) respondents indicated not having more than one mosquito net leaving only 18(6%) students who admitted to having more than one.

This is to say that students who do not use mosquito net have a mere 6% chance of borrowing one hence, it could be assumed that the students who do not use mosquito nets do not have one. Long lasting insecticidal nets have been widely used by the world health organization to control mosquito bite. Mosquito nets require treatment using insecticide kits to ensure their efficacy (Hunter el., 2014). However, only 4 (1.35%) respondents admitted to have always soaked their mosquito net in a liquid to repel mosquitoes since their stay in the university accommodation while 12(4%) respondents indicated they had soaked their nets less than once a month.

In reality, this means that majority of the students do not treat their mosquito nets. Irrespective of that, 101(34%) respondents always slept under their mosquito net the night preceding the questionnaire distribution whilst 22(7%) and 11(4%) of the respondents reported sleeping under their mosquito net the majority of the time. This means that 12% of the respondents removed their mosquito net at one time during their sleep. The factors that guided the removal of mosquito nets while asleep, might also be responsible for the 163(55%) who did not sleep under a mosquito net at all the night before. Another alternative method of protection against the malaria vector widely used by WHO member countries is the indoor residual spraying. However, mosquito repellents or insecticides are locally used. Of the 305 respondents, 225(76%) never had anyone come to spray against mosquitoes in the last six months although 22(8%) respondents indicated they always spray mosquito repellents personally. On the other hand, 191(66%) students attested to never using mosquito repellent.

It could therefore be asserted that LLINs are more popular for malaria control in the area as the ratio of LLINs to IRS application is 6:1. Figure 14 and figure 15 shows the frequency of use of LLINs and IRS respectively, while figure 16 show the most common types of mosquito repellent found in the study area. The two studies showed the presence of the malaria vector in the study area. The comparator study indicated an abundance of 38(18.91%) while the research study recorded an abundance of 195(61.32%). The Abundance of the anopheles vector found in the research study was 5 times more than that of the comparative study as the ratio of abundance in the comparator study to that of the research study is 1:5. This suggests that between 2012 and 2016, malaria vector density has multiplied by 5. This might have explained why the malaria prevalence rates doubled as the ratio of malaria prevalence in the comparator study area to that of the research study is 1:2. Furthermore, the two studies recorded the presence of *Anopheles gambiae* and *Anopheles funestus*. According to WHO (2015) they are competent vectors of malaria parasite in Nigeria. As a result, it could be argued that the malaria prevalence rate which doubled between 2012 and 2016 is justifiable. Alternatively, two different mosquito species (*Culex quinquefasciatus* and *Aedes eagypti*) where recorded in both studies. This might be an indicator of a favourable condition for breeding present in the study area. Categorically, it could be asserted that the larvae breeding sites of the *Culex quinquefasciatus* and *Aedes eagypti* were present in the study area.

DISCUSSION

With respect to the research question, it was found that as malaria vector abundance increased by 5, malaria prevalence doubled in a space of 4 years. From a public health perspective, the expected vector capacity and malaria prevalence in 12 years would experience an increase by 15 and 6 respectively. This implies that by 2028, it is expected that malaria prevalence would increase by 30%. One of the interesting findings was that 105(35%) of the students do not use and probably may not have long lasting insecticidal mosquito net. Another important finding like the latter is that 225(76%) of the students indicated they never saw anyone come in for indoor residual spraying and only 22(8%) indicated they personally use mosquito repellent all the time as a means to control the malaria vector. Malaria vector abundance is the most common entomological measure used to describe the relationship between vectors and malaria (Gatton *et al*., 2013). Thus, the study suggests that there is a frequency of 2.5 increase between the malaria vector in the study area and the malaria prevalence observed. However, this does not really indicate a coherence or consistency as there is need for future and continuous research to be able to make this inference. Okeke, Magbo and Nwakwusi (2013) compared the malaria vector abundance of Nnamdi Azikiwe University and that of Ifite. In his comparison, he discovered 79(46.47%) species of mosquitoes of which the genus Anopheles was 17(10%). However, his sampling studies were carried out at the onset of rainfall unlike the research study carried out at the peak of the rains. Rainfall, temperature and altitudes are conditions which determine the species of mosquito likely to be found in an environment (MacLeod and Morse, 2014). Such climatic conditions could increase vector abundance at some time of the year or decrease them. For instance, mosquitoes are not found in the arctic regions due to its cold climate (gomes *et al*., 2013).

A study conducted by Aribodor *et al*. (2016) asserted that climatic changes resulting from global warming has contributed to the fight against malaria in Nigeria. They further argued that the changes in the climatic condition of Nigeria has increased the population of people who do not use LLINs as well as those who use them but remove them at some point during their sleep due to heat. Surprisingly, 225(76%) of the respondents admitted never to having seen anyone come in to spray any insecticide to repel malaria in the last six months. By implication, it means that since they had been in the university accommodation (a 9 month period), no form of residual spraying ha, to their knowledge, been undertaken. However, some students (8%) personally use mosquito repellents to control malaria all the time. It was somewhat interesting to note that the repellent found to be most common among the students (Raid) was also used by Onyiudo *et al* (2012) in the comparator study. This result is in line with those of previous studies done in Nigeria where the aerosol based pyrethroid was used for IRS (Mgbemena *et al*., 2012; Nnanna *et al*., 2016). Researchers have admitted that insecticide resistance by malaria vectors are increasingly become a global concern (Thomas and Read 2016; Aribodor *et al*. 2016).

The consistent use of a particular type of insecticide compared to an integrated approach could bring about resistance. Thus dominance of a certain brand of insecticide would increase future tendencies of resistance in the study area. However, many individuals of the middle and lower strata in the community might continue this pattern of behaviour. According to Aribodor *et al*. (2016) insecticide vector resistance to pyrethroid and DDT has been reported in Nigeria. By implication, this means that future malaria vector control plans would be intensive and financial burden would increase for the Nigerian state. Nigeria is a country with a GDP of 521.8 billion USD and a population of 173.6 million people (WHO, 2016). Major funding for public health policies might not be implemented if intense burden arises due to insecticide resistance. As a matter of fact, most WHO policies for member states are yet to be implemented. The inability to implement and keep up policies have resulted to gross loss of evidence data which affect future research. However, the Nigerian state has successfully implemented the use of LLINs in most areas (ibid., 2016).

Long lasting insecticidal nets have been used by the public health unit to control malaria. It has been recorded that many member states have successfully controlled malaria with the use of LLINs (WHO, 2016). However, records also indicate that many individuals use LLINs without treatment (Anshebo *et al*., 2014). Results from the study area also support this evidence as 262(88%) indicated they have never soaked their mosquito net in a liquid to control malaria. The efficacy of the LLINs is dependent on the insecticide on the net and frequency of use by the individual. Thus, if nets are continuously used without treatment, its ability to control the malaria vector would be reduced. A case study was analysed in Aguleri local government where homes produced worn out nets as evidence of use (Aribodor, 2016). Continuous use of worn out nets could lead to recurrent infection with malaria. Malaria parasite infection was observed to be 116(43%) amongst students in the study area. However, in comparison to places like Abuja where the prevalence is 61% among students it is fairly low (Onyiudo *et al*., 2012). A possible explanation to this could be the knowledge, attitude and practice of individuals. Onyiudo *et al*. (2012) argued that studies conducted at the peak of the raining season would have increased abundance of vectors that would possibly reflect on the malaria prevalence.

Conclusion

A typical necessary control approach required in the study area is the covering of all currently open drainage systems across the University campus since the major site of collection of mosquitoes within the university hostels is the open drainage systems. These drainage systems have an approximate capacity of 1260L each. This may be seen to be a small contribution to prevention but at the peak of the raining season, they can harbour over 142 (45%) mosquitoes, if estimates are made from the number of larval and pupae collections in this study. Covering the drainage could thus reduce the exposure of students to malarial vectors alongside IRS to reduce vector density in their residential accommodation. Construction sites could serve as location sites for containing and studying container breeding mosquitoes such as the Aedes species which had an abundance of 123(61.19%) in the comparator study. Mosquito-borne diseases such as malaria, have become a global health concern of ever increasing proportion however the role of mosquitoes in malarial transmission can be contained. Nigeria remains an endemic state thus integrated mosquito control approaches ought to be continually integrated to reduce malarial vector density. The study demonstrated that the increase in malarial vectors had a significant impact in the prevalence of malaria among students living in Nnamdi Azikiwe University, thus, taking adequate measures to keep malarial vector density low as well as malaria prevalence rates, must become a key priority in any continuous surveillance approach.

Ethical Approval

Formal ethical approval was obtained from the University of Sunderland Ethics Committee, UK and the Nnamdi Azikiwe University Ethics Committee, Awka, Anambra State, Nigeria.

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