CHAPTER ONE
INTRODUCTION

Background of the study

Mosquitoes are the most important dipteran pests and are probably the most serious blood-sucking arthropod worldwide (Clements, 2012). Mosquitoes are of economic importance because of their biting nuisance and disease transmission to man and his livestock (Harbach, 2007). They thrive in both urban and rural areas, in close association with humans thus making them exceptionally successful disease vectors. Transmission of diseases by mosquitoes is as a result of their very high avidity for feeding on man, high reproductive potential and remarkable longevity (Onyido *et al.*, 2010).

The most important man-biting mosquitoes belong to the genera, *Anopheles*, *Culex, Mansoni, Aedes, Psorphora, Haemagogus and Sabethes*. The last three genera are found in Central and South America (Service, 1980). In Nigeria, species of mosquitoes include; *Anopheles gambiae* complex, *An. funestus* complex, *An. moucheti, Aedes aegypti*, *Ae. simpsoni*, *Ae. albopictus, Ae. vittatus*, *Ae. unilineatus*, *Ae. luteocephalus*, *Ae. africanus*, *Culex quinquefasciatus*, *Cx. nebulosus*, *Cx. tigripes*, *Cx. decens*, *Cx. pipiens*, *Cx. moucheti* and *Cx. cinereus* (Oguoma *et al.,* 2010; Aigbodion and Osariyekemwen, 2013).

Mosquitoes are distributed in both tropical and temperate regions of the world (Akunne *et al.,* 2015). The only areas from which they are absent are the Antarctica and a few islands (Mullen and Durden, 2009). Some oceanic islands appear to have been free from mosquitoes before the advent of man and modern travel and some still are (Erling, 2014). A wingless species has been reported from Antarctica (Goto *et al.*, 2015). Over 3,500 species of mosquitoes have been described from various parts of the world (Harbach and Howard, 2007). Mosquitoes are found at altitudes of over 4700m. Besides a worldwide distribution, they are predominantly tropical insects.

Originating in Africa, *Ae. aegypti* is now present globally in tropical and sub-tropical regions (Cutwa-Francis and O'Meara, 2007). Nowadays, developmental activities, urban development associated with rapid growth of townships have accentuated the problem of mosquito bite and mosquito-borne diseases. Humans are responsible for the creation of mosquitogenic environment (Chen *et al.,* 2009). According to Opoku *et al.* (2005), rapid rates of urbanization with the attendant sanitation and public health problems such as inadequate waste disposal facilities, poor drainage system and poor water supply among many others have led to the creation of a congenial environment for the breeding of water related insect vectors including mosquitoes. A recent study in a rural village in Western Kenya showed that burrow pits alone accounted for 60-78% of the total mosquito pupa productivity (Mutuku *et al.,* 2006).

Onyido *et al.* (2006a,b) reported in a study at Government Reservation Area of Enugu metropolis in South-Eastern Nigeria, that mosquitoes have many breeding sites which include stagnant water, swamps, broken bamboo stems, tin cans, plastic containers, water-holding cisterns and tanks, coconut shells, foot prints of man and animals, sand excavation ditches, stone quarry sites, motor vehicle tyre prints, sunlit or shaded quiescent water, scoops in concrete slabs used in feeding animals and cassava fermentation pots. Developmental projects, such as civil engineering constructions, mining and agricultural projects including deforestation, intended to improve the quality of life of the people often bring with them favourable conditions for the breeding of large populations of disease vectors including mosquitoes and associated disease burden (Mbanugo and Okpalaononuju, 2003).

In a mosquito larval habitat at Abeokuta, Ogun State Nigeria, Adeleke *et al.* (2008) encountered ten species of mosquito in ground pools/ponds, gutters/open drains, tyres, domestic containers and tree-holes/axils. *Ae. aegypti* bred in all these habitats while *Cx. quinquefasciatus* bred in all

habitats tree holes/leaf axils. *An. gambiae* and *Ae. albopictus* occurred in three of the habitats while other species bred only in one or two habitats. However, ground pools and domestic containers recorded the highest number of species followed by gutter/open drains. While tree holes/leaf axils were the least preferred habitat with the lowest number of species occurrence. Okogun *et al.* (2005), reported that more mosquito larvae were collected in artificial than natural sources. Plastics and metal cans were the predominant artificial sources of larvae, indicating the importance of human activities, environmental modification, attitudes and practices on mosquito-borne disease transmission.

The distribution of mosquitoes is influenced both directly and indirectly by climatic and environmental factors (Akram *et al.,* 2009). Differences in abiotic and biotic factors could have a major influence in the distribution of mosquito species (Teng and Apperson, 2000). The biotic and abiotic factors affect the growth, development and survival of larvae of mosquitoes. Biotic factors are intraspecific competition, cannibalism, interspecific competition, predation by sibling species, predators, parasites and/or pathogens and nutrition (Paaijmans, 2008). Abiotic factors are physical and chemical factors (such as water temperature, light, water movement, wave action, vegetation, hydrogen, ion concentration, soil type, salinity, calcium, magnesium sulphate, nitrate, phosphate) (Muturi *et al.*, 2008). Optimum temperature (27.7^0C - 30.1^0C), pH (4.74 - 7.54) and dissolved oxygen (9.20mg/L - 9.94mg/L) might have provided conducive environment for survival and breeding activity of the *Anopheline* species (Oyewole *et al.*, 2009). The climatic factors, for example, mean monthly precipitation accumulation of at least 80mm, temperatures between 18^0C and 32^0C, and relative humidity of at least 60% are considered suitable for the sustenance of significant mosquito population density and vectorial capacity (McMichael and Martens, 1995).

Mosquitoes prefer an environment with certain resources (food, shelter, breeding sites, favourable temperature and suitable humidity) in sufficient amount and at appropriate time for survival and development (Akram *et al.,* 2009). Increase in ecological and environmental modification due to agricultural activities and urbanization has been observed to contribute to the breeding of various mosquito species (Amusan *et al.,* 2005).

Amaechi *et al.* (2014) noted in their study in Asa-Obingwu, a rural community in Abia State South-Eastern Nigeria, that abundance of *Anopheline* is as a result of suitable environmental and climatic breeding conditions which favour the breeding of larva of the vectors. The high indices of *Anopheles gambiae* in Asa-Obingwu was due to the proximity of residential areas to farm lands which were most often water logged (Amaechi *et al.,* 2014).

Through mosquitoes' blood sucking habit, they act as vectors of a variety of human pathogens like viruses, bacteria, protozoa and parasitic helminths. Mosquitoes transmit to man such deadly diseases as malaria, yellow fever, filariasis, dengue and various forms of viral encephalitis (West Nile Virus, St. Louis Encephalitis Virus, LaCrosse Encephalitis Virus, Eastern Equine Encephalitis Virus, and Western Equine Encephalitis Virus) (Ukpai and Ajoku, 2001). The *Anopheles* mosquitoes especially, *Anopheles gambiae,* transmit malaria and filariasis. The *Aedes* mosquitoes particularly *Aedes aegypti, Ae. albopictus, Ae. africanus, Ae. luteocephalus* and *Ae. simpsoni*, transmit yellow fever, dengue haemorrhagic fever and various forms of viral encephalitis. The *Culex* and *Mansonia* mosquitoes particularly *Culex quinquefasciatus* and *Mansonia uniformis* are very important transmitters of filarial worms especially *Wuchereria bancrofti* which causes elephantiasis, as well as transmit various forms of viral encephalitis (Onyido *et al.,* 2009b). *Mansonia* transmit Rift valley disease in some parts of East Africa (Sang *et al.,* 2010). Mosquitoes are also viscious biters and constitute biting nuisance, cause allergic reactions, skin irritations, scratching, restlessness

and sleepless nights (Amusan *et al,* 2003, Onyido *et al.,* 2008, Onyido *et al.,* 2009a).

In Nigeria, there have been reports on mosquito borne disease outbreaks. Nigeria Centre for Disease Control (2019) reported outbreaks of yellow fever in the 36 States and Federal Capital Territory (FCT). Baud *et al.* (2017) reported Nigeria as one of the African countries that encountered zikka virus outbreak while Ayukekbong (2014) reported that dengue fever is endemic in Nigeria with sero-prevalence of about 73% in some areas. WHO (2011) reported that the burden of lymphatic filariasis is heaviest in Nigeria, Democratic Republic of Congo, India, Indonesia and Bangladesh.

Statement of the problem

Rapid increase in infrastructural development and human population influx into Oraifite in Anambra State, Nigeria has resulted to environmental modifications with multiple mosquito breeding habitats in the area. Also, despite all mosquito control measures in Oraifite, mosquito-borne diseases have continued to be threats to health and socioeconomic development of the people in the area. Although some level of mosquito control has been achieved by spraying Pyrethroids (Mortein, Mobil, Raid) as contact insecticides and use of Insecticidal Treated Nets, mosquitoes still bite indiscriminately in Oraifite, hence the need for the study.

Aim

To study the bionomics and distribution of man-biting mosquitoes in Oraifite, Ekwusigo Local Government Area, Anambra State, Nigeria.

Specific objectives

1) To identify mosquito species at both morphological and molecular level and their composition in various locations at Oraifite.

2) To determine the breeding sites of the mosquito vectors.

3) To determine the ecological factors that influences the survival of mosquitoes in their breeding sites.

4) To study the temporal distribution of the mosquito vectors in relation to the climatic factors.

Significance of the study

There is dearth of information in the literature about mosquito bionomics and distribution in Oraifite, Anambra State, Nigeria. Therefore, studies on the bionomics and distribution of man-biting mosquitoes shall contribute to the baseline information and help in evidence-based policy decision for the control of mosquitoes and mosquito-borne diseases in the area. The results of this study provided the much needed literature and empirical data on mosquito distribution and their breeding sites in Oraifite, Anambra State for comparisons with other areas within and outside the State. Thus, this study has provided some significant reference points for further research work.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF MOSQUITOES

Many studies have been carried out in different regions of Nigeria to identify mosquito species which include; *Anopheles gambiae* complex, *An. funestus* complex, *An. moucheti, Aedes aegypti*, *Ae. simpsoni*, *Ae. albopictus, Ae. vittatus*, *Ae. unilineatus*, *Ae. luteocephalus*, *Ae. africanus*, *Culex quinquefasciatus*, *Cx. nebulosus*, *Cx. tigripes*, *Cx. decens*, *Cx. pipiens*, *Mansonia africana, Mansonia uniformis, Eretmapodites chrysogaster, Coquillettidia maculipennis, Cx. moucheti* and *Cx. cinereus* (Abdullahi *et al*, 2010; Akunne *et al.*,2015; Aribodor *et al.*, 2016; Egbuche *et al.,* 2016; Ezihe *et al.,* 2017; Odo *et al.*, 2015; Oduola *et al.*, 2013; Oguoma and Ikpeze, 2010; Okonkwo *et al.*, 2014; Olayemi *et al.*, 2014; Onyido *et al.*, 2016; Onyido *et al.*, 2014; Onyido *et al.*, 2013; Onyido *et al.*, 2011; Onyido *et al.*, 2009; Oyewole *et al.*, 2009; Umar *et al.*, 2015; Umeanaeto *et al.*, 2016 and Yayock *et al.,* 2014).

Mosquitoes are classified under the family: Culicidae; subfamilies: Anophelinae, Culicinae, Toxorhynchitinae and genera: *Anopheles, Aedes, Culex, Toxorhynchites, Mansonia* (Harbach, 2007). The subfamily Anophelinae contains three genera; *Anopheles* (which are cosmopolitan), *Bironella* and *Chagasia. Anopheles* species is proven to be of medical importance (Yayock *et al.,* 2014). The Culicine mosquitoes of medical importance belong to the genera; *Culex, Aedes, Mansonia, Sabethes, Haemagogus and Psorophora* (Service, 1993). The subfamily Toxorhynchitinae contains only one genus; *Toxorhynchites* and are mainly found in Africa, Asia, Central and South America and in coastal areas of Japan and Russia. The commonest in Nigeria is *Toxorhynchites brevipalpis* (Theo) (Yayock *et al.,* 2014).

2.2 GLOBAL DISTRIBUTION OF MOSQUITOES

Mosquitoes have worldwide distribution in both tropical and temperate regions (Akunne, 2015). The only areas where they are absent are the Antarctica and a few islands (Mullen and Durden, 2009) but a wingless species has recently been reported from Antarctica (Goto *et al.*, 2015), though iceland appear to have been free from mosquitoes (Erling, 2014). Over 3,500 species of mosquitoes have been described from different parts of the world (Harbach and Howard, 2007). Mansonia transmit Rift valley disease in some part of East Africa (Sang *et al.,* 2010). Mosquitoes are found at altitudes of over 4700m. Besides being worldwide in distribution, mosquitoes are predominantly tropical insects. Originating in Africa, *Ae. aegypti* is now present in tropical and sub-tropical regions (Cutwa-Francis and O'Meara, 2007).

2.3 MOSQUITO SPECIES COMPOSITION IN NIGERIA

Mosquitoes are widely distributed in Nigeria; the most common species belong to the genera *Anopheles, Culex* and *Aedes*. The mosquito species which have been incriminated in the transmission of malaria (*Anopheles gambiae*, *An. arabiensis*), yellow fever (*Aedes aegypti*) and lymphatic filariasis (*Anopheles gambiae* s.l and *Culex quinquefasciatus*), have also been identified in Nigeria (Richards *et al.*, 2005). In Nigeria, *An. gambiae* is the main vector of malaria. It breeds in exposed, often muddy sunlit, ground pools of water of various sizes, brick pits, animal footprints and vehicle tyre prints. It is occasionally found in man-made containers such as wheel barrows, mortar pans, open tanks, canoes and abandoned concrete mixers (Onyido *et al.*, 2009b).

In Awka Metropolis, Anambra State, South-Eastern Nigeria, Mbanugo and Okpalaononuju (2003) identified *Ae. albopictus*, *Ae. aegypti*, *An. gambiae*, *An. funestus*. Similarly Onyido *et al.* (2009a) reported five species of Culicine mosquitoes (*Ae. aegypti, Ae. africanus, Ae. albopictus Ae. luteocephalus* and *Mansonia africana*) in Enugu Municipality, South-Eastern Nigeria. Anosike

et al. (2007) reported sixteen mosquito species (*Ae. aegypti, Ae. africanus, Ae. simpsoni, Ae. albopictus, Ae. stokesi, Ae. taylori Ae. apicoargenteus, Cx. quinquefasciatus, Cx. nebulosus, Cx. trigipes, Cx. decens An. gambiae, An. funestus, An. coustani* and *T. viridibasis*) from tree-holes in Imo State, South-Eastern Nigeria.

An. gambiae s. s, An. funestus s. s., *An. arabiensis* and *An. melas* have been reported as the most important malaria transmitting species in Nigeria (Alaribe *et al.*, 2002). In Anambra State, Nigeria, *An. gambiae* s. l. has been reported as the main malaria vector (Onyido *et al.*, 2011b; 2014) and also the predominating species among the indoor biting mosquitoes (Onyido *et al.*, 2016). *Anopheles gambiae*, *An. funestus, T. viridibasis, Ae. aegypti, Ae. albopictus, Ae. africanus, Cx. quinquefasciatus* and *Cx. tigripes* were also present in the sample collections at Umumpamma-Aborji and Isu-Umuabo Village, Oba in Anambra State (Okonkwo *et al.*, 2014). He also reported that Umumpamma-Aborji village had the highest Shannon-Wiener index of diversity (0.703) while Isu-Umuabu village had the highest Simpson's dominant index (0.265) at Oba, Anambra State, Nigeria. Lamidi *et al.* (2017) reported that Bali had the highest Shannon-Wiener index of diversity (0.8753) while Ardo-Kola had the highest Simpson's dominant index (0.4587557) in Taraba State, Nigeria. In Abagana, three mosquito species, *An. gambiae* 95(62.5%), *Cx. quinquefasciatus* 33(21.71%) and *Ae. aegypti* 24(15.79%) were collected by Onyido *et al.* (2014) while Aribodor (2012) observed that *Anopheles* species were most abundant indoors in Nimo, Anambra State, Nigeria. Okwa *et al.* (2007) reported *An. gambiae* as the most abundance mosquito vector in Badagry, Lagos State. Simon-Oke *et al.* (2012) observed that mosquito distribution and abundance are related to population, land use and human activities in Ekiti State, Nigeria. In Awka North L.G.A. of Anambra State, Aribodor *et al.* (2016) reported that the availability of indoor mosquito species was due to the presence of ground water pools, domestic containers, plant axils and bushes around the houses where they breed and readily fly into houses to rest and feed on humans.

while Ezihe *et al.* (2017b) reported that areas with increased social and human activities such as markets, hospitals, churches tend to have increased relative abundance of mosquito species at Enugu Municipal, Enugu State.

In Mid-Western Nigeria, Okogun *et al.* (2005) reported seventeen mosquito species which included eight *Aedes* species (*Ae. albopictus, Ae. luteocephalus, Ae. simpsoni Ae. africanus, Ae. palpalis, Ae. aegypti, Ae. unlingeatus and Ae. vittatus*), six *Culex* species (Cx. *fatigans, Cx. pipiens, Cx. albovitrolus, Cx. perfuscus Cx. decens and Cx. quinquefasciatus) and Anopheles* species (*An. gambiae, An. pseudopunctipennis* and *An. funestus*). In Abeokuta, Ogun State, ten species of mosquitoes were encountered including; *M. africana, M. uniformis, Cx. quinquefasciatus, Ae. aegypti, Ae. albopictus, Ae. vittatus, Cx. tigripes, An. gambiae s.l., An. funestus and E. chrysogaster* (Adeleke *et al.*, 2008). Oguoma and Ikpeze, (2008) reported *An. gambaie* as the most diverse (0.1415) and dominant (0.427) mosquito species in a study at Gewaza, Agro-ecological zone of North-Central Nigeria.

Onyido *et al.* (2008) reported nine mosquito species namely, *Ae. aegypti, Ae. africanus, Ae. vittatus, Ae. luteocephalus, Cx. quinquefasciatus, Coquilletidia metallica, Eretmapodites quinquevittatus, E. inormatus* and *E. chrysogaster* in Jos North-Central, Nigeria while Oguoma and Ikpeze (2008) encountered eighteen mosquito species in an irrigation system in North-Central Nigeria which include *An gambiae* complex, *An. funestus* complex, *An. pharoensis*, *An. coustani*, *An. rhodesiensis*, *Cx. quinquefasciatus*, *Cx. pipiens fatigans*, *Cx. pipiens pipiens*, *Cx. tigripes*, *Ae. aegypti*, *Ae. albopictus*, *Ae. africanus*, *Ae. taylori*, *Ae. luteocephalus*, *Ae.vittatus*, *Ae. simpsoni*, *Mansonia* species and *Psorophora* species. Also Oguoma *et al.* (2010) identified man-biting mosquitoes, in three villages of Uratta in Imo State which were *Anopheles gambiae* complex, *Anopheles funestus* complex, *Anopheles coustani* and *Anopheles moucheti*. In Yola, Northern Nigeria, Umaru *et al.* (2006) reported *An. gambiae* complex, *An. funestus* complex, *An. pharoensis*, *An.*

rhodesiensis. *Cx. quinquefasciatus*, *Cx. pipiens fatigans* and *Cx. tigripes* while Bunza *et al.* (2010) recorded four mosquito species; *An. gambiae*, *Cx. pipiens*, *An. arabiensis* and *An. funestus* in Kastina. Immature stages of *An. arabiensis* and other mosquito species in a rice agro-ecosystem in Kenya recorded by Mwangangi *et al.* (2007) were *An. arabiensis, An. funestus*. *An. pharoensis, An. maculipalpis*, *An. coustani*, Cx. *quinquefasciatus, Cx. poicilipes, Cx. annulioris, Cx. tigripes* and *Cx. duttoni.* Umeanaeto *et al.* (2016) reported *An. gambiae, Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus* and *Cx. tigripes* in Okpatu, Enugu State.

In Ado-Ekiti, Nigeria, Olorunniyi (2016) reported three genera of mosquitoes which included *Anopheles, Aedes* and *Culex.* He further stated that *Anopheles* mosquitoes were the most abundant mosquito genera, thus making malaria prevalent in the area.

2.4 THE BREEDING HABITATS OF MOSQUITO VECTORS

Mosquitoes utilize different water bodies for their breeding throughout the world (Akram *et al.*, 2009). Many species breed in both natural and artificial containers such as pools, gutters, coconut shells, tree holes, bamboo stumps, leaf axils, and septic tank (Aigbodion and Osariyekemwen, 2013). Mosquitoes prefer an environment with certain resources (food, shelter, breeding sites, favourable temperature and suitable humidity) in sufficient amount and at appropriate time for survival and development (Sattler *et al.,* 2005). The recent increase in ecological and environmental modification due to agricultural activities and urbanization has been observed to contribute to the breeding of various mosquito species (Amusan *et al.,* 2005).

Mosquito species, groups, subgenus and genus have preferred habitats based on locations and conditions of the water body, pH, habitat size and temperature which are determinants of mosquito species abundance and distribution (Opoku *et al.,* 2005). Eni *et al.* (2014) observed that edges of

streams and swamps are good breeding sites for mosquitoes. The larval stages of antropophilic species such as *An. gambiae* larvae are found in habitats closer to human dwellings (Minakawa *et al.*, 2002). Also *An. arabiensis,* a sibling species of *An. gambiae,* is found in the same habitat as *An. gambiae* s.s.

Some mosquito species (e.g. *An. gambiae*) breed in clean fresh water whereas others (e.g. *Ae. sundaicus*) are adapted to breeding in brackish water while highly polluted water with organic matter serve as breeding habitats for *Cx. quinquefasciatus* and *Ae. stephensi* in some areas. Some species are restricted to a single type of breeding habitat while others possess a larger adaptability. The presence of mosquito larvae in a given habitat is due to the oviposition habits of the female, which influences the presence of larvae in different types of habitats (Pates and Curtis, 2005). The selection of the larval habitat by the adult female and the preference for one breeding habitat over another is more or less genetically fixed by natural selection, and same breeding place might attract one species and deter another. The example of *Cx. quinquefaciatus* breeding in highly polluted water and in general absence of anopheline larvae is explained by the positive and negative attraction exercised by the same breeding place. *Anopheles* siphon would prefer clean-oxygenated water body while siphon of *Culex* larvae (surface breathing) enables it to feed in polluted water body. Gu *et al.* (2008) opined that the spatiotemporal patterns of mosquito production are driven by two mechanisms namely; variations in intrinsic properties of breeding habitats, which affect growth and survival of larval populations and secondly spatial locations of the focal habitat in relation to blood-meal sources.

Oviposition habitat selection is influenced by a diversity of chemical, physical and physiological factors (Oyewole *et al.,* 2009). In many species, the selection of particular oviposition sites is determined by certain qualities of the aqueous, organic and mineral content of such sites that attract adult

female mosquitoes. The ability of gravid females to distinguish among potential oviposition sites that will or will not support the growth, development and survival of their offspring is critical to the maintenance of the mosquito population (Chen *et al.,* 2007). Once attracted to the oviposition site, gravid females use visual (colour, texture and brightness) and olfactory clues (semi-chemicals) to decide the suitability of a potential habitat for egg laying (Attardo *et al.,* 2005). However the choice or suitability of breeding place is not determined entirely by the marked selective power of the female mosquitoes, because after the egg has been laid, subsequent alterations in the nature of the breeding site caused for example by flood, pollution or drying out may render the habitat unsuitable for development of larvae (Attardo *et al.,* 2005).

Larval developmental sites vary considerably and can include leaf axils, pitcher plants, and even crab-holes but typical sites, however, are temporary or permanent shallow bodies of freshwater with little water movement (Clements, 1992, Harbach, 2007). Though *Anopheles* species prefer freshwater, *Aedes* and *Culex* species are adapted to brackish or saline waters in salt marshes or inland saline pools, roadside ditches, catch basins and backyard habitats, such as clogged rain gutters, discarded tyres or un-maintained bird baths (Clements, 1992). Nearly all mosquito larvae must obtain atmospheric oxygen to breathe. Egbuche *et al.* (2016) noted the preference of *Anopheles* species being selective in their oviposition sites as they prefer to breed in open drains and ground pools in Agulueri, Nigeria.

Mwangangi *et al.* (2007) demonstrated that the type and spatial distribution of habitat characteristics for *Anopheles* larvae along the Kenya coast were important in determining the abundance and diversity of the species composition. They further showed that there was no habitat found to be having only a single species of mosquitoes. Thus co-existence of mosquito larvae ensures that there is adult mosquito productivity of different species throughout the year, as these species use the same habitats. Agro-

ecosystems, especially where there is extensive irrigation as found in rice farming, are known to bring about breeding sites for both *Anopheles* and *Culex* mosquito species.

Mosquitoes of different species breed in certain light conditions which include shades, sunlit, surface debris, algae and emergent plants, turbidity and brightness. There are species occurring mostly in sun-exposed environments such as *An. gambiae* s.s., *An. albimanus, An. pseudopunctipennis*, members of the *An. sundaicus* complex*, An. sinensis, An. aconitus* while others seem to prefer shaded water bodies (*An. funestus*, *An. vestitipennis*) (Bugoro *et al.*, 2011). The *Anopheles* species occur in a wide range of habitats, but with relatively low nutrient status and high oxygen levels (Opoku *et al.,* 2005). The relative abundance of *An. gambiae* larvae is significantly associated with the distance between a larval habitat and the nearest human dwellings (Maciel De Freitas *et al.*, 2007). Caillouet *et al.* (2008) reported that larvae of *An. albimanus* were found in permanent and semi-permanent groundwater habitats including (in order of greatest abundance) hoof and footprints, ditches, rice fields, and ground pools. *Anopheles* species largely breed in temporary pools such as hoof prints, footprints, roadside ditches, natural depressions and holes created by man (Opoku *et al.,* 2005). The preponderance of *An. gambiae* in such small, open, temporal pools can be explained by the fact that the females preferentially select them for oviposition because they are highly oxygenated at the time of oviposition (Opoku *et al.,* 2005). Onyido *et al.* (2011b) reported highest collection of *An. gambiae* in ground pools in Umudioka, Anambra State while Umeanaeto *et al.* (2016) noted that *An. gambiae* was collected only in ground pools at Ikeghe Okpatu, Enugu State.

The primary habitats of *Aedes* mosquito larvae are artificial containers such as flowerpots, cans, buckets, ornamental ponds, birdbaths, old tyres, cemetery vases, and clogged rain gutters. Their ability to breed in artificial containers facilitated their passive spread in the last decades through main

transportation routes (Mbanugo and Okpalaononuju, 2003). Asian tiger mosquitoes (*Ae. albopictus*) still utilize natural containers such as tree-holes, bamboo stumps, and leaf axils. As long as water remains in these containers long enough to complete their larval and pupae stages, *Ae albopictus* will use these items to successfully reproduce (Eritja *et al.*, 2005).

Cx. quinquefasciatus has been observed to breed in stagnant and polluted water with high organic content because they are surface breeders. Similarly, Muturi *et al.* (2007) reported that the species prefer habitats with turbid water where turbidity was caused by organic matter, and observed that poor drainage and sanitation, discharge and scattering of empty cans, fermentation of cassava in vessels in homes and the increased building of houses create wells in almost every home. They also noted that ventilated septic tanks associated with these houses were also responsible for increasing the number of breeding sites in public and residential habitats for *Cx. quinquefasciatus*. Preechaporn *et al.* (2006) reported that *Cx. quinquefasciatus* breeds only in plastic containers, whereas Hriber *et al.* (2001) and Preechaporn *et al.* (2007) reported that this species breeds in several types of water containers including ceramic vessels, metal vessels, plastic and metal water barrels and concrete water tanks.

Mansonia species breed mainly in permanent collections of water that has floating vegetation. Larvae of this species only detach themselves from plants but rise to the surface of the water if disturbed. Since they are attached almost permanently to plants in their habitats, *Mansonia* larvae are frequently missed during larval survey (Opoku *et al.,* 2005). Of the three major mosquito breeding sites at Abagana, ground water pools and discarded tyres yielded very large populations of mosquito larvae while domestic water containers yielded relatively few (Onyido *et al.,* 2014) while Ezihe *et al.* (2017b) reported that seven species of mosquitoes including; *Ae. aegypti*, *Ae. luteocephalus*, *Ae. albopictus*, *Cx. quinquefasciatus*, *An.*

gambiae, *Ae. simpsoni,* and *Cx. tigripes* were collected from different breeding sites in Enugu Municipal, Enugu State.

2.5 ECOLOGICAL FACTORS AFFECTING SURVIVAL AND DISTRIBUTION OF MOSQUITOES

A good knowledge of the abiotic and biotic conditions of the mosquito larval breeding sites could benefit future mosquito management programs (Rao *et al.*, 2011). The abiotic factors include physical and chemical properties of water. The biotic factors include presence of food substances (organic), presence of predacious mosquito larvae, fishes, other insects, crustaceans and arachnids (Okogun, 2005). Physical and chemical factors (such as water temperature, light, water movement, wave action, vegetation, hydrogen, ion concentration, soil type, salinity, calcium, magnesium sulphate, nitrate, phosphate) and biotic interactions (such as predation) are known to influence mosquito species abundance (Muturi *et al.*, 2008). Thus, the water chemistry of aquatic habitats plays a critical role in determining the survival rate of mosquitoes (Chen *et al.*, 2007).

2.5.1 Abiotic factors (Physicochemical properties of water) in mosquito breeding habitats.

The physicochemical compositions of the water bodies that influence oviposition, survival, and the spatio-temporal distribution of important disease vector species include salts, dissolved organic and inorganic matter, degree of eutrophication, turbidity, presence of suspended mud, presence or absence of plants, temperature, light and shade, and hydrogen ion concentration (Grillet, 2000). However, high water current and flooding have been reported to lead to *Anopheles* species larval deaths due to reduction in oxygen tension causing physical harm to the larvae (Okogun, 2005). Water of a near neutral pH 6.8 - 7.2 was found most optimal for the weakening of the egg shells for the first instar larvae stage to emerge (Okogun *et al.*, 2003). In a study at the Obuasi municipality in Ghana, mosquito breeding habitat of pH below 4.5 or above 10 leads to mosquito larvae mortality (Kwasi *et al.,* 2012). Other factors such as optimum temperature (27.7^{0}C -

30.1^{0}C), pH (4.74 - 7.54) and dissolved oxygen (9.20mg/L -9.94mg/L) might have provided conducive environment for survival and breeding activity of the *Anopheline* species (Oyewole *et al.*, 2009). He further stated that physicochemical parameters such as temperature, salinity, conductivity, total dissolved solids and pH have significant influence on mosquito larval abundance.

Clark *et al.* (2004) reported that pH value of 4.0 has no significant effect on the growth and development of *Ae. aegypti*. According to Dario and Nicolas (2002), pre-imaginal stages of mosquito develop in artificial containers of small volume, such as flask, bottles and flower vases. Odo *et al.* (2015) noted that volume of water had no effect on most of the mosquito species except *Ae. albopictus* in Nsukka ecological zone. He further stressed that the fact that only a few *species* abundance was affected by some physicochemical parameters is in line with the report of Adebote *et al.* (2008) in which no physicochemical parameter correlated with larvae abundance in two of the sites he sampled in Zaria, Northern Nigeria and while other species abundance correlated significantly with the physicochemical parameters in the third site. He further stressed that abundance of *Cx. nebulosus* in phytotelmata correlates positively and significantly with total dissolved solids ($p<0.05$; $r = 0.302$), *Culex horridus* population correlates positively and highly significantly with temperature ($p<0.05$; $r = 0.323$) and the concentration of cadmium ($p<0.0001$; $r = 0.661$) of water in phytotelmata.

Musonda and Sichilima (2019) in a study at Kapiri Mposhi District of Zambia reported that a positive significant Pearson correlation between salinity($r=0.240$, $p = 0.000$), electrical conductivity($r=0.120$ $p=0.003$) and larvae abundance existed while a correlation between total dissolved solids and larvae abundance ($r=0.018$ $p=0.663$) was not significant. They further stressed that the physicochemical parameters (conductivity and salinity) have significant relationship with the abundance of *Anopheles* mosquito

while total dissolved solids have insignificant positive linear relationship with the abundance of *Anopheles* mosquito in various breeding sites of Kapiri Mposhi districts in Zaria. Ezihe *et al.* (2017b) reported that mosquito larvae abundance had strong positive and significant correlation with temperature ($P<0.05$, $r=0.5$) in a study at Enugu Municipal, Enugu State. Ukonze *et al.* (2017) reported in a study at Omor that mosquito abundance is negatively associated with pH, Sulphate and TSS ($r= -0.071$; $p= 0.72$). Correlation analysis revealed that abundance of mosquitoes decreases with increase in physicochemical parameters (Ukonze *et al.*, 2017).

An. gambiae seems to prefer edges of river and ocean as breeding habitat since these water bodies contain physicochemical properties such as calcium, magnesium sulphate, nitrate, phosphate and dissolved solids in high proportion as nutrient composition (Oyewole *et al.*, 2009). According to Tyagi *et al.* (2013), Spearman correlation analysis between the vector abundance and 12 parameters of larval water showed that *An. culicifacies* was strongly and positively associated with dissolved oxygen (DO; $r = 0.618$, $p < 0.05$) and the maximum occurrence of larvae was also associated with pH range 6-7 (pH; $r = -0.49$, $p < 0.05$). *An. culicifacies s.l.* naturally breeds in clear water and when given an opportunity to select waters with different amounts of free ammonia and ammonium carbonate, lays eggs indiscriminately, even in water containing 6.6 ppm of saline ammonia (Barik *et al.*, 2009).

Coconut shells were observed to be chosen more often than other containers, by mosquitoes for breeding sites, especially by *Ae. albopictus* (Rao *et al.*, 2011). Coconut shells rich in calcium, potassium, sodium, sulphur and magnesium form ideal breeding grounds for *Ae. albopictus.* A pH variation outside the range of 7-8 could be used as a tool for management of *Ae. albopictus* (Rao *et al.*, 2011).

Tiimub *et al.* (2012) analysed the physicochemical assessment of mosquito breeding sites from selected mining communities at the Obuasi Municipality in Ghana, mean temperature of water from various mosquito breeding sites sampled varied between 17.03 ± 0.18^0C and 24.06 ± 0.18^0C; total suspended solids (TSS) ranged from 17.03 ± 4.04mg/l - 96.67 ± 4.04mg/l; mean total dissolved solids (TDS) varied between 1.09±3.23mg/l and 35.67±3.23mg/l; dissolved oxygen (DO) ranged from 3.97 ± 0.13mg/l - 7.43 ± 0.13mg/l and pH levels varied between 7.77 ± 0.01 and10.70 ± 0.01.
In turbid breeding sites, Culicine larvae would more likely be present, while *Anopheles sp.* larvae would be absent (Chavasse *et al.* (1995) but Gimnig *et al.* (2001) found increasing *A. gambiae s.l.* larvae densities with increasing turbidity. The preference of Culicine mosquitoes for turbid water is coherent with their known breeding site preferences, as they breed successfully in rather polluted environment such as blocked drains and septic tanks (Chavasse *et al.*, 1995).

Thangamathi *et al.* (2014), analysed the physicochemical characteristics of water in the mosquito breeding sites and observed that the pH ranged from 6.38 ± 0.38 in waste bucket to 6.73 ± 0.67 in coconut shell. Conductivity (mho/cm) ranged from 115.3 ± 0.38 in waste bucket to 989.9 ± 0.73 in tree holes. Total dissolved solids (mg/l) was least in waste bucket (79.83 ± 1.75) and highest in tree holes (693.00 ± 0.5). Total hardness (mg/l) was highest in tree holes (83.83 ± 0.76) and least in waste bucket (22.06 ± 0.60). Turbidity of breeding water ranged from 20.66 ± 2.08 in waste bucket to 29.66 ± 1.52 in coconut shell. Calcium (mg/l) ranged from 55.93 ± 1.10 in coconut shell to 20.16 ± 1.25 in tires. Mutero *et al.* (2004) reported that various chemical properties and physicochemical characteristics of the larval habitat observed in gutters, peri-domestic runoff and domestic containers ranging from pH, optimum temperature, total suspended solids, total dissolved solids and electrical conductivity have been found to affect larval development and survival. Garba and Olayemi (2015) reported that temperature was highest in ground and riverbed pools that were usually

fully exposed to the sun compared to streambed pools and marshes in Minna, Nigeria. Emidi *et al.* (2017) noted that Pond water reservoirs had the highest mean temperature (41.9 ± 0.8), Tds (990.0 ± 550.7), salinity (8.3 ± 0.0) and conductivity (809.2 ± 453.9). They further noted that none of the physicochemical parameters studied (pH, salinity, temperature, conductivity and total dissolved solids) was found to be significantly associated with the presence of *Culex* mosquito abundance.

Apart from the other factors like concentration of various nutrients and minerals, temperature is the main factor responsible during larval growth and development (Bayoh and Lindsay, 2004). Higher temperature of the water may result in rapid development of the mosquito immatures but causes decrease in their size, while the same rise in temperature produces fewer adults (Bayoh and Lindsay, 2004). Water temperature is influenced by various parameters, such as local climate, water depth and movement, habitat size and geometry, land cover type or canopy overgrowth, presence of vegetation and or algae, soil properties and turbidity (Paaijmans *et al.*, 2008).

A study examined the relationship between habitat characteristics and larval abundance (Miller *et al.*, 2007). Others have reported significant relationships. For example, in Eritrea, *An. arabiensis* was associated with shallow, clean water and sunlit habitats (Shililu *et al.*, 2003) as were those reported for *An. gambiae s.s* in Western Kenya (Munga *et al.*, 2005). Fluctuations in physicochemical factors of the rice field environment during the course of the rice growing cycle also impacted significantly on temporal distribution and abundance of *An. arabiensis* (Muturi *et al.,* 2007).

A major challenge faced by all mosquito larvae is the tendency for larval habitats to fluctuate widely in salinity due to changes in rainfall and evaporation (Smith *et al.*, 2008). Organisms living in brackish and saline environments have evolved various mechanisms of coping with increased

salinity, and in order to survive in these conditions, they have to be able to regulate their osmotic potential. Larvae of salinity tolerant mosquito possess cuticles that are less permeable to water than freshwater forms, and their pupae have thickened and sclerotized cuticles that are impermeable to water and ions. Larval survival depends upon the ability to regulate hemolymph osmolarity by absorbing and excreting ions (Smith *et al.*, 2008). Osmoregulatory mechanisms vary among various mosquito genera, for example, *An. albimanus* larvae osmoregulate through rectal ion excretion and the larvae undergo a dramatic shift in rectal Na+/K+-ATPase (an enzyme important for ion regulation) localization when reared in freshwater *vs.* saline water (Smith *et al.*, 2008).

2.5.2 Biotic factors affecting the breeding sites of mosquitoes

The nutrient value of the breeding habitat provides favourable conditions for the breeding of bacteria, algae, yeast, fungal spores and protozoa which are the types of food that majority of mosquito larvae ingest. In the absence of predators, the highest survival of larvae had been observed at 66% algal cover. Greater quantities of algae reduced survival possibly by presenting a physical barrier to larval respiration or feeding activity close to the air-water interface. Lesser quantities of algae may possibly reduce mosquito survival by exposing larvae to harmful level of ultra violet light (Burma *et al.,* 2003).

Various species of plants are indicators for a given type of water, the plants reflecting not only the physical characteristics of a breeding habitat but also the chemical content. Emergent plants and floating debris have also been described as one of the best predictors of larval abundance in aquatic habitats (Mwangangi *et al.,* 2007).

In addition to providing food, vegetation as part of larval habitat acts in a number of other ways. For instance, it directly affects the temperature, evaporation, surface characteristics and chemical composition of the water. It also influences the amount of light reaching the water surface, as well as

provides harbourage (e.g. tree holes and leaf axils) and provides sites of attachment and source of oxygen for *Mansonia* immature stages. Mwangangi *et al.* (2007) observed that in Kenya habitat type, floating debris and emergent plants are key factors determining the presence of *Anopheles* larvae in the habitats. In Nigeria, composition of mosquito fauna of a pool is also influenced by the temporary or permanent nature of the pools (Okogun *et al.*, 2005). Small containers are often inhabited by a relatively consistent fauna. Included in this groups are tree holes (cut or bored bamboos), artificial containers, including discarded tins, bottles, motor vehicle tyres and tubes, barrels, tanks, earthenware of varying sizes and rain water drains. Also included are water collections in depressions of fallen leaves, leaf axils, crab holes in banks of streams and sea shores which are not saline (Okogun *et al.,* 2003). Small habitats have been shown to be more productive for mosquito larvae compared to large habitats during the rainy season (Mwangangi *et al.,* 2007).

2.6 TEMPORAL DISTRIBUTION OF MOSQUITOES

In Nigeria, developmental activities, urban development associated with rapid growth of townships have accentuated the problem of mosquito bite and mosquito borne diseases. Human ecology is responsible for the creation of mosquitogenic environment (Chen *et al.,* 2009). According to Opoku *et al.* (2005), rapid rates of urbanization with the attendant sanitation and public health problems such as inadequate waste disposal facilities, poor drainage system and poor water supply among many others lead to the creation of a congenial environment for the breeding of water bleeding insect vectors such as mosquitoes. For example, in a rural village in western Kenya, burrow pits alone accounted for 60-78% of the total mosquito pupa productivity (Mutuku *et al.,* 2006).

Oduola and Awe (2006) reported that the spatial and temporal distribution of mosquitoes will contribute to the design of malarial vector control which is a major component of the global malarial strategies and still remains the

most general effective measure to prevent malarial transmission. Anopheline larvae were predominantly found in habitats covered by relatively short vegetation such as early stage of rice, grass, and sedge (Fillinger *et al.,* 2008) which allow water bodies to be exposed to sunlight, a situation preferred by ovipositing mosquitoes, unlike tall and thick vegetation.

Human activities can greatly affect the distribution of mosquito populations. The movement of large segments of population into suburban and rural areas increases human and domestic animal exposure to mosquitoes such as *Ochlerotatus triseriatus* and *O. canadensis*. The construction of man-made lakes and the use of irrigation agriculture have increased the numbers of many mosquitoes such as malaria associated mosquitoes (*Anopheles*) and rice field mosquitoes (Onyido *et al.,* 2009a). The increased use of non-degradable plastic, glass, and metal containers and discarded tires has increased population of container breeding mosquitoes such as the yellow fever mosquito (*Aedes aegypti*) (Onyido *et al.,* 2006a,b). Expansion in international trade has increased the chances of introducing new mosquito species into new areas, as has recently happened with the Asian tiger mosquito (*Aedes albopictus*) (Mbanugo and Okpalaononuju, 2003) introduced from Asia to Nigeria. Environmental changes due to human activities greatly influence the distribution and survival of many mosquito species (Adeleke *et al.*, 2008). Amusan *et al.* (2005) opined that increase in agricultural activities and urbanization contributed to the breeding of different mosquito species in South-Western Nigeria. Over population in cities and indiscriminate disposal of waste materials (including metallic and cans and discarded household materials) due to improper town planning and lack of adequate sanitary education increase the potential for breeding disease vector mosquito species (Okogun *et al.*, 2005).

Poor economy, low literacy, poor sanitation, indiscriminate disposal of wastes, abundant numbers of abandoned construction sites, uncontrolled domestic run-offs and poor maintenance of gutters and drainages, were

attributed to be responsible for the increase in mosquitoes breeding sites in Nigeria. The consequence of one or a combination of these factors is the increase in mosquito vector abundance and vectorial capacity (Adeleke *et al.*, 2008; Aigbodion and Odiachi, 2003; Akram *et al.*, 2009; Ezike *et al.*, 2001 and Okogun *et al.*, 2005).

2.6.1 Seasonal distribution of mosquitoes

The seasonal distribution of mosquitoes depends on climatic conditions which reflect individual survival strategies in response to climatic variation (Hugo, 2008). Nearly all species of mosquitoes have definite patterns of seasonality. These patterns vary depending on the geographical region inhabited by individual populations (Eldridge *et al.,* 2008). Some species produce only a single population in a year (univoltine). Some species, such as *Aedes tahoensis*, the larvae of which develop in melted snow, have life cycles that are not capable of additional generations, even under highly favourable weather conditions. Other species, especially those having very large geographical distributions, may have many generations in warmer parts of their range (multivoltine), but have only a single generation in colder regions.

In Nigeria, many species are active almost all through the year, going into short periods of inactivity only during the dry season. The population of *Aedes aegypti* fluctuates with temperature, rainfall and humidity. Dengue infectios was generally encountered during or after rain along with the rise in the vector *Aedes aegypti* population (Ananya and Andrew, 2012). In extremes of weather in winter and summer, *Aedes aegypti* larvae die because of low and high temperature. The amount and distribution of rainfall as well as temperatures of the North-Central, Nigeria, are such that, respectively, promote rapid mosquito population development and parasite maturation; the relative humidity appears limiting to mosquito survival (Olayemi *et al.*, 2014).

2.7 CLIMATIC FACTORS AFFECTING SURVIVAL AND DISTRIBUTION OF MOSQUITOES

The effects of weather factors on both mosquito population and their distribution as well as on the parasites they transmit have been documented (Okogun *et al.*, 2003; Service, 2012). Mosquitoes show limited geographical range because, each vector species can survive only under certain optimal environmental conditions. Temperature, rainfall, relative humidity and altitude are the four major abiotic factors affecting the presence and abundance of *Anopheles* mosquitoes in a given area (Kim *et al.*, 2010). The abundance, behaviour, spatiotemporal distribution and population dynamics of mosquito species are known to be influenced by factors such as climate, seasonality, availability of micro-habitats for breeding, physicochemical parameters of breeding sites and anthropogenic related factors (Aigbodion and Odiachi, 2003; Akram *et al.*, 2009; Impoinvil *et al.*, 2008; Kim *et al.*, 2010; Koenraadt *et al.*, 2004; Midega *et al.*, 2010; Muturi *et al.*, 2008 and Stoops *et al.*, 2007). It is a general consensus among several researchers (Akram *et al.*, 2009; Bradshaw *et al.*, 2000; Koenraadt *et al.*, 2004 and Stoops *et al.*, 2007) that abiotic factors of temperature, relative humidity, altitude and rainfall play a vital role in mosquito development which in turn influences their population density. For example, mean monthly rainfall of at least 80mm, temperatures between 18 and 32^{o}C, and relative humidity of at least 60% are considered suitable for the sustenance of significant mosquito population density and vectorial capacity (McMichael and Martens, 1995).

Tropical areas, including Nigeria, have the best combination of adequate rainfall, temperature and humidity allowing for breeding and survival of mosquitoes (Ayanda, 2009). Reports from the National Institute of Allergy and Infectious Diseases (NIAID, 2007) pointed out that climate affects both parasites and mosquito vectors. Mosquitoes cannot survive in low humidity; rainfall expands breeding grounds, and in many tropical areas, malaria and other mosquito vector disease cases increase during the rainy season (NIAID, 2007). Masaninga *et al.* (2012) reported that these factors

(temperature, rainfall and relative humidity) played insignificant roles in the mosquito distribution in their study area in an urban setting in Zambia. Rainfall provides breeding sites for mosquito to lay their eggs and ensures a suitable relative humidity of at least 50-60% to prolong mosquito survival. Relative humidity below 60% shortens the life span of the mosquito vectors (Rogers *et al.*, 2006).

2.7.1 Temperature

Temperature affects all the important processes such as the rate of larval development and survivorship, pupation rates, larval-to-adult survivorship and larval-to adult development time (Ndenga *et al.*, 2011). Service (2012) stated that the higher the temperature, the faster the gonotrophic cycle of mosquitoes and vice versa. Mosquitoes can survive low temperatures but their metabolic processes are slowed down or even arrested when temperature falls below the threshold. At temperatures higher than 32^0C - 35^0C, the metabolism is also modified in the sense of slowing the physiological process. The average optimum temperature for the development of most mosquito species is around 25^0C - 27^0C and development can be completely arrested at 10^0C or over 40^0C when high mortality may occur (Adeleke *et al.*, 2010).

At very low temperature, mosquito development is slow, while at high temperature, mosquito development is rapid but producing sterile adults. Death also occurs at temperature above 35^0C (Adeleke *et al.*, 2010). Temperature has strong influence on longevity. The daily survival of mosquito vector is dependent on temperature between 16^0C and 36^0C, the daily survival drops rapidly at temperature above 36^0C.

Tropical species do not withstand temperature near freezing point, while permanent high temperatures over 27^0C - 30^0C will reduce the average life of a mosquito population. For instance, *An. maculipennis* larvae (a temperate zone species) will become completely inactive, remain at the surface and will

be killed during freezing of the superficial layer of the water, whereas *An. claviger* and *An. plumbeus* larvae will move actively at temperatures around 0^{0C} and survive within pockets of water enclosed in ice. In North-Central Nigeria, Olayemi and Ande (2008) observed that a $4^{0}C$ increase in atmospheric temperature resulted in a shortening of *Plasmodium* duration of sporogony in mosquitoes by about two days.

Manyi *et al.* (2015) reported that the highest and lowest monthly mean temperature values ($29.5^{0}C$ and $38.2^{0}C$) during the study period in Makurdi imply that temperature had no adverse effect on the mosquito population in the study area for both dry and wet seasons. This is because the mean temperature values were within the optimum temperature range for insects, particularly mosquitoes as reported by Githeko *et al.* (2000).

2.7.2 Relative humidity

Mosquitoes abound in an environment with high humidity and moisture which does not only provide suitable condition for the survival and increase in fecundity of females but also makes available the presence and abundance of breeding places as well as ensures the survival of the immature stages. For instance, the greater dependency of *An. gambiae* s.l. on humid conditions has been described by Charlwood *et al.* (2000). Sutherst (2004) observed that climate is an important determining factor in the distribution of mosquito vectors. While investigating the survivorship of *Anopheles gambiae* population in relation to indoor micro-climatic conditions in North-Central Nigeria, Olayemi (2011) recorded much higher relative humidity of almost 60% indoors, and such relative humidities correlated strongly with indoor mosquito resting density (i.e., endophily), daily survival and adult longevity. Opayele *et al.* (2017) reported that fluctuations in relative humidity were not significantly correlated with mosquito abundance except for *Mansonia* species whose population increased with increased relative humidity in Ibadan, Nigeria. However, such endophagic and endophilic behaviours of adult mosquitoes will no doubts

have serious implications for the selection and efficacy of mosquito adulticiding interventions in the area.

Olayemi *et al.* (2014) noted that the mean relative humidity established for North-Central Nigeria (i.e., slightly >50%), is relatively low and may not be conducive for adult mosquito dispersal and foraging activities for blood meal and oviposition. Low relative humidity is known to cause death of mosquitoes through desiccation (Olayemi, 2011). Because of the tracheal system of respiration, insects in general are particularly susceptible to desiccation. Thus, humidity can act as a limiting factor in species distribution and longevity. Forest species are more susceptible to humidity changes than those living in areas with a dry climate. However, Okogun *et al.* (2003) reported that extreme relative humidity retards the activity of mosquitoes, thereby making them stationary in their breeding, biting and resting places. Manyi *et al.* (2015) noted in a study on the relationship between weather parameters and female mosquitoes' abundance and distribution in Makurdi, that relative humidity ranged from 44% - 86% have a positive correlation with the mosquito populations during the 12 months study period. The strength of this relationship was determined to be strong by regression analysis. The relative humidity of at least 50- 55% prolong mosquito survival (Simon-Oke and Olofintoye, 2015).

2.7.3 Rainfall

Mosquitoes often dominate in wetland ecosystems where suitable breeding sites are abundant and other physical factors are optimal for adult survival (Okorie *et al.*, 2014). The immature stages of *An. gambiae* require an aquatic environment to develop and are often found in transient, sunlit and generally small pools (Onyido *et al.,* 2009b).The availability of these aquatic habitats depends on precipitation (Fillinger *et al.*, 2004). Precipitation creates new breeding sites and adds water to existing ones. The availability, persistence and dimensions of mosquito larval habitats depend to a large extent on the frequency, duration and intensity of precipitation (Gimnig *et*

al., 2001). It has been suggested that precipitation could affect larval population dynamics by flooding the habitats and consequently flushing out larvae (Impoinvil *et al.*, 2008).

The increased near–surface humidity associated with rainfall enhances mosquito flight activity and host seeking behaviours (Shaman and Day, 2005). There are lower numbers of mosquitoes during the dry seasons (Alaribe *et al.,* 2002; Suleiman, 2012; Oduola *et al.*, 2013; Amaechi *et al.,* 2014; Onyido *et al.*, 2014). The low populations of female *Anopheles* mosquitoes could be attributed to fewer breeding habitats existing in the sentinel community during the dry season (Umar *et al.*, 2015). Adebote *et al.* (2008) also stressed on aquatic microhabitat drying out due to cessation of rainfall. Odo *et al.* (2015) reported that the number of mosquitoes encountered in the study decreased from rainy to dry season (that is from October to December). Reudal *et al.* (2010) observed the increase in mosquito population across all seasons of the year with peak in rainy season and least population in the dry season in Republic of Korea. Opayele *et al.* (2017) reported that local mosquito population decreases due to high rainfall that results to flood, thereby washing away mosquito larvae from their breeding sites in Ibadan, Nigeria.

Olayemi *et al.* (2014) recorded that annual rainfall amount of 1247.52±166.68 in North-Central Nigeria is such that will create and sustain highly productive mosquito breeding habitats, thereby resulting in the production of many high-density generations of mosquitoes particularly during the rainy season. Yet, Olayemi (2012) reported high prevalence of malaria, and intense mosquito breeding activities in the study area during the dry season. These findings, therefore, suggest that mosquito population development and intensity of disease transmission in North-Central Nigeria may not be so heavily dependent on rainfall, but perhaps equally influenced by non-climatic drivers, such as human behaviour (Olayemi *et al.*, 2014).

Tuno *et al.* (2007) observed a high larval mortality in open habitats during rainy season in the Western Kenya highlands and suggested a damaging effect of raindrops on larvae. The possible effect of mortality by the direct hit of a raindrop was studied by Impoinvil *et al.* (2008), who exposed larvae to rain showers. However, in the study, no damaging effect was observed. Tuno *et al.* (2005) proposed that the direct damage to anopheline larvae by precipitation may depend on raindrop size.

An increase in rainfall will increase the availability, persistence and dimensions of larval habitats, although this will depend on parameters such as local evaporation rates, soil percolation and slope of the terrain (Shaman and Day, 2007). Rainfall provides breeding sites for mosquito to lay their eggs and ensures a suitable relative humidity of at least 50-60% to prolong mosquito survival (Simon-Oke and Olofintoye, 2015).

In a study on the relationship between weather parameters and female mosquitoes' abundance and distribution in Makurdi, Manyi *et al.* (2015) observed that the mean monthly rainfall range during the study period was between 5 mm and 293.4 mm, distributed over the 12 month study period. He further noted that there was a strong positive correlation between the mosquito populations and rainfall throughout the study period.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA

The study was conducted in Oraifite, Ekwusigo Local Government Area of Anambra State. Oraifite (5·56^{0}N to 6·03^{0}N and longitude 006·49^{0}E to 006·86^{0}E) has a population of 42,346 (NPC/FRN, 2006). Oraifite is bounded by Oba to the north, Ozubulu to the south, Atani to the west and Nnewi to the east in the rain forest belt of Nigeria. Oraifite enjoys equatorial tropical climate, characterised by eight months of rainy season (April to November) and four months dry season (December to March). The relative humidity of the area is about 80% reaching 92% during wet season and an annual rainfall of about 2200m. Temperature is high throughout the year with day time range of 23^{0}C to 35^{0}C. The main language of the people is Igbo. The land is drained by Ekulu River and river Eze. Oraifite people are mostly farmers, fishermen and palm wine tappers and few civil servants. It has schools, hospitals, markets, road networks, etc. (Oraifite Welfare Association, 2014).

Oraifite has four major quarters; Unodu, Ezumeri, Ifite and Irefi and a total of 14 communities. Unodu comprises Ibolo, Umuezopi and Isingwu. Ezumeri comprises Ogbe, Umuonyeagolu, Umuafa and Umuezikem. Ifite comprises Awor, Amakom, Urudunu and Umunakwa. Irefi is comprised of Umudisi, Nkalafia and Umueshi. Oraifite is a predominantly Christian community, with many traditionalists. Rapid infrastructural development and population influx into Oraifite have given rise to environmental changes that have created artificial breeding habitats for mosquito larvae, in addition to natural body of water that are suitable for mosquito breeding.

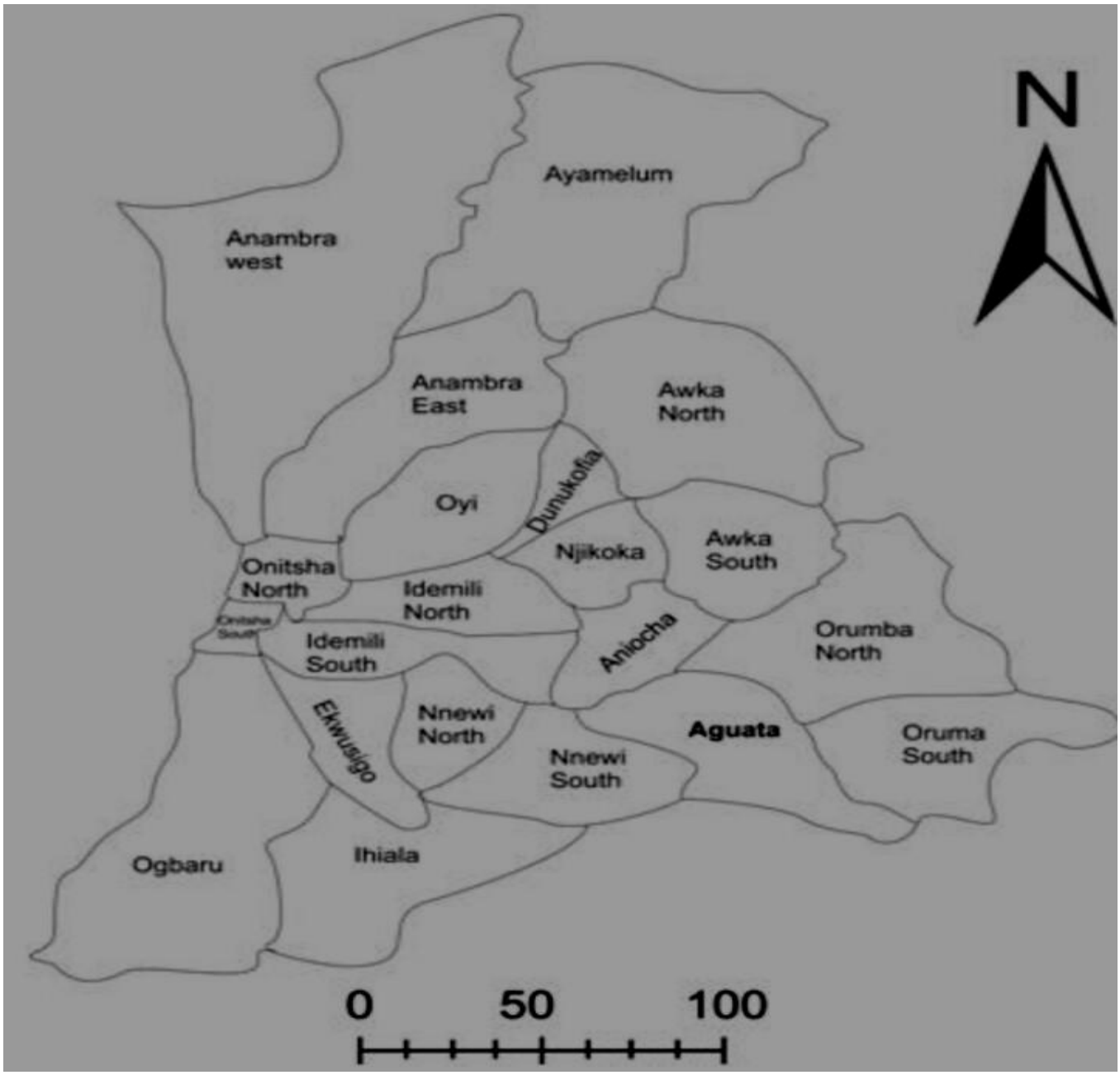

Fig1. Map of Anambra State showing the different L.G.A (**source:** https://www.researchgate.net/publication/259802565_Spatial_Patterns_of_Residential_water_Supply_Accessibility_Levels_in_Anambra_State_Nigeria/figures?lo=1&utm_-source=google&utm_medium=organic.

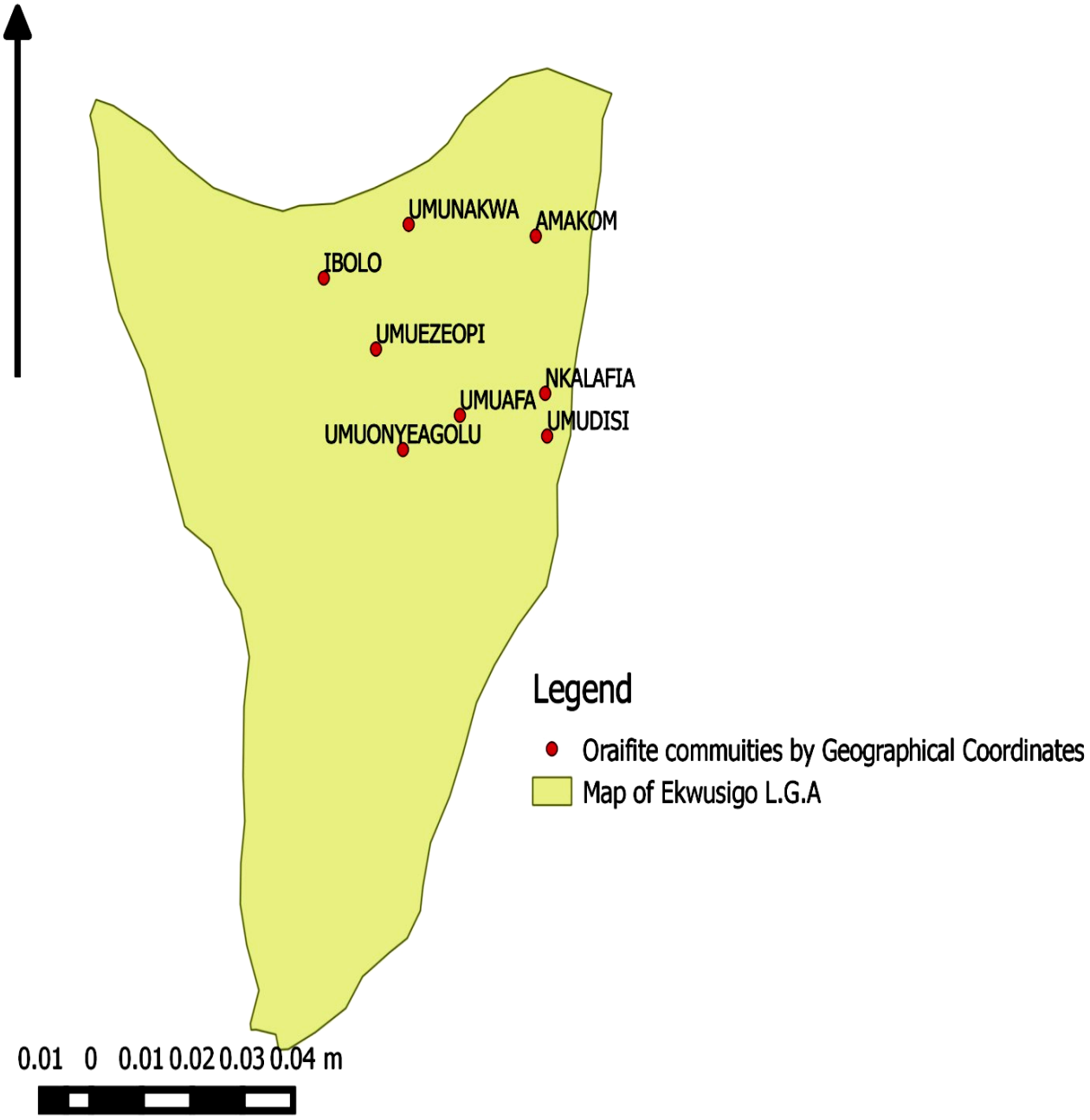

Fig. 2: Map of Ekwusigo L.G.A showing the eight communities of Oraifite studied (Made with GIS Software Version 2.10.1).

3.2 STUDY DESIGN

The study was a field longitudinal survey of mosquitoes and their breeding sites in the community. The study was carried out over a period of one year, cutting across two seasons (dry and wet seasons). The study was done for a period of nineteen months (January, 2017 to July, 2018). House to house visit was used in mosquito collection.

3.3 ETHICAL CONSIDERATION

Ethical clearance was obtained from Chukwuemeka Odumegwu Ojukwu Teaching Hospital (COOTH) Amaku Awka, Anambra State. After this, advocacy visits were made to the traditional rulers and cabinets of Oraifite community, to obtain their permission to work in their community. Thereafter, announcements were made publicly in the community through the help of the Town Criers and Church Leaders, informing them about the study. This facilitated both access and co-operation of the people. House to house sensitization was done prior to sample collection, where explanation of what was done in the study was given to them.

3.4 SELECTION OF HOUSES

Stratified random sampling was used in selecting the houses. Oraifite community is made up of four quarters with a total of 14 main communities; Unodu (Ibolo, Umuezopi and Isingwu), Ezumeri (Ogbe, Umuonyeagolu, Umuafa and Umuezikem), Ifite (Awor, Amakom, Urudunu and Umunakwa) and Irefi (Umudisi, Nkalafia and Umueshi). Two communities were selected from each quarter. A total of eight (8) villages were selected. The selected communities include Amakom, Ibolo, Nkalafia, Umuafa, Umudisi, Umuezeopi, Umunakwa, and Umuonyeagolu. Equal number of communities was selected from each quarter using simple random sampling/balloting. Each community formed a stratum. An average of three (3) houses (Onyido *et al.*, 2014) was randomly selected from each community using their household list. This gave a total of 24 houses in each month. Each community contributed equal number of households.

Sampling of mosquitoes was carried out in two (2) consecutive days in a month for a period of 12months. One room was used in each household for collection of indoor biting and resting mosquitoes using Pyrethroid Knockdown Collection (PKC) method. PKC was done in 3 rooms each for community twice every month. Outdoor biting adult mosquito collection was done using Human Bait Collection (HBC) method. Puddles, ponds, stagnant water, tree-holes, plant axils, clay pots, used or discarded vehicle tyres and domestic containers were sampled for larvae.

IDENTIFICATION OF MOSQUITO SPECIES COMPOSITION IN VARIOUS LOCATIONS OF ORAIFITE AT MOLECULAR LEVEL.

3.5 COLLECTION OF MOSQUITOES

3.5.1 Collection of indoor biting and resting adult mosquitoes using Pyrethrum Knockdown Collection (PKC) method.

Adult mosquitoes that bite and rest indoors were sampled in the eight selected communities (Amakom, Umunakwa, Nkalafia, Umudisi, Umuezopi, Ibolo, Umuafa and Umuonyeagolu) in Oraifite from January to December, 2017. It was done using Pyrethrum Knockdown Collection method between 6.00hrs and 8.00hrs local time according to World Health Organisation (WHO) standard procedure (WHO, 1995). The rooms in which the occupants slept previous night were used. Food items were properly secured. Windows and doors were shut to prevent escape of mosquitoes. White sheets were spread from wall to wall with sufficient overlaps at their joints to avoid escape of fallen mosquitoes. The floor surfaces as well as the beds and any other area were completely covered. No space was left between the walls and all the surfaces were covered to prevent loss of any mosquito. The Pyrethroid-based insecticide (Mortein®) with active ingredients Allethrin (2.09g/kg) and Resmethrin (0.39g/kg) was sprayed in the rooms. After 20 minutes of fleeting each room, the doors and windows were opened and the spread sheets were carefully removed by lifting them at the four edges and

jerking them gently so that incapacitated and dead mosquitoes would aggregate in the middle of the sheets. The sheets were taken out and examined in daylight. The numbers of mosquitoes collected were recorded.

The mosquitoes were picked up using a pair of forceps into a damp petri-dish. The petri-dishes were prepared with cotton wool moistened with water and layered with filter papers. The mosquitoes were preserved and identified morphologically after two days at Department of Parasitology and Entomology laboratory, Awka. *Anopheles gambiae* complex were preserved from Jan, 2017 to July, 2018 and were further identified into sibling species by DNA-PCR technique at the Nigerian Institute of Medical Research (NIMR), Lagos.

3.5.1.1 Determination of the gonotrophic stages of adult mosquitoes collected indoors using Pyrethroid Knockdown Collection (PKC) method.

The gonotrophic stages of female mosquitoes collected indoors was determined in order to detect mosquitoes that fed on blood and those that did not feed. The gonotrophic stages were grouped as fed, unfed, half gravid and gravid (GMEM, 1994).

Unfed: mosquitoes that have not had bloodmeal. The abdomen was flat.

Blood fed (fleshly fed): mosquitoes that have just taken bloodmeal. The abdomen was reddish.

Half gravid: mosquitoes in which the bloodmeal was not completely digested. The abdomen becomes whitish posteriorly and dark reddish anteriorly.

Gravid: mosquitoes in which all the bloodmeal was digested and the abdomen becomes fully dilated and whitish due to the formation of fully developed eggs.

3.5.1.2 Determination of Indoor resting density of mosquitoes

The indoor resting density (IRD) of mosquitoes collected from the eight villages in Oraifite was determined using the result of PKC method. The IRD of mosquitoes collected indoors was computed using WHO (2003) criteria, viz: IRD=Number of mosquitoes collected/number of houses sampled/ number of nights

3.5.1.3 Determination of man-biting rate (indoor biting rate) of mosquitoes

Man-biting rate of mosquitoes collected indoors implies the number of bites each person receives from a mosquito species per night. It was calculated using mosquitoes collected by PKC method as the total number of freshly fed mosquito of a species divided by the total number of occupants that spent the night in the room and then the total number of night collection was made (Ezihe *et al.*, 2017a). It is expressed as; Man-biting rate (MBR) = (number of freshly fed female mosquitoes/total number of occupants)/ total number of nights.

3.5.2 Collection of outdoor biting mosquitoes using Human Bait Collection (HBC) method.

Outdoor biting mosquitoes were collected from eight communities (Amakom, Umunakwa, Nkalafia, Umudisi, Umuezopi, Ibolo, Umuafa and Umuonyeagolu) in Oraifite from January to December, 2017 using Human Bait Collection (HBC) method. Human landing catches were carried out using World Health Organisation (WHO) standard procedure (WHO, 1995), which adopts the stationed human bait collection method. This was done in the evening hours between 16.30hrs and 19.30hrs local time. This method was used to collect mosquitoes when they land to bite or in the process of biting a human. At each point, three to four volunteers sat on low chairs (small stools) and exposed their legs and hands by rolling up their trousers and sleeves of their shirts to knees and elbows respectively. Mosquitoes coming to feed on the exposed parts of the body were collected with test tube

vials and torchlight. The test tubes containing mosquitoes were covered with cotton wool to avoid escaping of the mosquitoes and the time of collection labelled on the vial. Data recordings were taken at 15minutes interval starting from 16.30 to 19.30hrs local time. Mosquitoes collected were preserved in an eppendorf tube containing silica-gel with the collection date, village name and household number clearly labelled on the tube. The silica gel was covered with paper before placing the mosquito so as to prevent direct contact of silica gel with the mosquito. Mosquitoes were put in the tube in order to prevent the delicate body parts of the insect such as palps, antenna, wings and legs, which are of significant importance in identification from damaging. The preserved mosquitoes were identified morphologically at Department of Parasitology and Entomology laboratory, Awka.

DETERMINATION OF THE BREEDING SITES OF THE MOSQUITO VECTORS.

3.6 BREEDING SITES OF MOSQUITOES

Mosquito breeding sites were determined by sampling larval and pupal stages of mosquitoes.

3.6.1 Sampling of larval and pupal stages of mosquitoes

Mosquito larvae were sampled in the eight selected communities (Amakom, Umunakwa, Nkalafia, Umudisi, Umuezopi, Ibolo, Umuafa and Umuonyeagolu) in Oraifite from January to December, 2017. The sampling methods were according to those of (Onyido *et al.,* 2011). During the sampling of mosquito larvae and pupae, specimen were collected from man-made containers (discarded tyres, water containers for domestic use, including metal basins, discarded cans, clay pots, plastic buckets, metal and plastic drums), ground pools (puddles, ponds) and plant axils (cocoyam axil, pineapple axil). The water in the puddles as well as those in large man-made containers such as metals was collected with the aid of ladles into the

bowls. They were carefully observed for the presence of mosquito larvae. Mosquito larvae collected were concentrated in a sieve and carefully picked with Pasteur pipette into labelled specimen containers. The larvae and pupae in discarded tyres were collected with the aid of suction pipettes. The contents of smaller containers of the same type/group in a compound or area were carefully pooled together into a white bowl. A 0.55mm mesh sieve was used to separate the larvae from debris. The larvae and pupae collected were transferred into clean jam containers half-filled with water from the breeding places. The bottles were properly labelled, bearing the time, date and place of collection for ease of identification.

The sampled immature stages of mosquitoes were separated according to their breeding sites and counted to know the number of pupae and larvae collected from each breeding site. The immature stages of mosquitoes were also reared to adult stage before identification and classification into genera and species composition.

3.6.2 Rearing of larval and pupal stages of mosquitoes to adult stage.

Mosquito larvae collected from different breeding sites were separately reared to adult stage for proper identification to species level. In the laboratory, mosquito larvae collected from different breeding sites and locations were put into separate bowls and labelled appropriately. The larvae were reared at room temperature and fed yeast. Water in larval bowls were regularly changed due to excess feed to prevent larval mortality. Then, Pasteur pipette was used to transfer emerged pupae into well-labelled plastic cups covered with nets so as to prevent the mosquitoes from flying out when emerged to adults. An opening, from where the emerged adults were aspirated was created on the net and covered with cotton wool. They were closely monitored and the ones that emerged to adults were aspirated and identified to species level (Hashmat *et al.*, 2014). Both male and female mosquitoes were identified but male mosquitoes were discarded because they are not man-biting mosquitoes.

3.7 IDENTIFICATION OF MOSQUITOES

All the mosquitoes collected in the eight villages from January to December, 2017 were identified morphologically and by molecular identification.

3.7.1 Morphological identification of mosquitoes

The morphological identification of mosquitoes was done microscopically in the Parasitology and Entomology laboratory, on the indoor and outdoor biting mosquitoes collected and adult mosquitoes that emerged from larvae with the aid of published keys by Gillies and Coetzee (1987) and Gillett (1972). The collected samples were identified to genera and species levels. The identification was based on the gross external morphology, appearance of the head, thorax, colour of the wings and hind leg tarsal segments. The process was carried out by placing the mosquito specimen in a petri-dish and viewing under stereo-microscope.

3.7.2 Molecular Identification of Sibling species of *Anopheles gambiae* complex.

The molecular identification of Sibling species of *Anopheles gambiae* complex was carried out in July, 2018, using the preserved mosquitoes. The PCR amplification of Deoxyribonucleic Acid (DNA) from legs and wings of the *Anopheles gambiae* sl complex collected in the study was done in order to identify the sibling species of *Anopheles gambiae* complex. It was done at NIMR, Lagos using the method described by Scott *et al.* (1993).

3.7.2.1 Procedure for extraction of mosquito DNA

The wings and legs of each mosquito were severed using a scalpel and were put into centrifuge tubes for Deoxyribonucleic Acid (DNA) extraction. The DNA was extracted using Blood-Animal-Plant DNA preparation Kit produced by Jena Bioscience. The extraction was done by adding the severed specimens to a ZR BashingBead lysis tube and 750µl lysis solution was added to the tube. The set-up was secured in a bead beater fitted with a 2ml tube holder assembly and was processed at maximum speed for 10 minutes. The ZR Bashing Bead lysis tube was centrifuged at ≥10,000rpm for 1minute.

Four hundred microliter (400µl) of the supernatant was transferred to Zymo-Spin IV Spin Filter (orange top) in a collection tube and centrifuged at 7,000rpm for 1minute. 1,200µl of Genome Lysis Buffer was added to the filtrate in the collection tube and was mixed. 800µl of the mixture was transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000rpm for 1minute. The flow through the collection tube was discarded and the previous process was repeated. Four hundred microliter (400µl)µl of DNA Pre-Wash Buffer was added to the Zymo-Spin IIC column in a new collection tube and was centrifuged at 10,000rpm for 1minute. Five hundred microliter (500µl) g-DNA Wash Buffer was added to the Zymo-Spin IIC column and centrifuged at 10,000rpm for 1minute. The Zymo-Spin IIC column was transferred to a clean 1.5ml micro-centrifuge tube and 50µl DNA Elution Buffer was added directly to the column matrix. Then it was centrifuged at 10,000rpm for 30 seconds and the DNA was eluted.

Compositions of pre mix to obtain Master Mix for the PCR of *An. gambiae* complex.

A master mix for the PCR of *An. gambiae* was obtained by mixing the pre mix and other reagents in the proportion shown in Table 1.

Table 1: *Anopheles gambiae* complex master mix.

Reagent	**X_1 (µl)**
Pre-mix	3.0
ddH_2O	8.0
81F	0.5
691R	0.5
DNA	1.0
TOTAL	12.5

3.7.2.2 PCR procedure for *An. gambiae* complex after DNA extraction and obtaining the Master Mix

PCR master mix (12.5µl) was added into each of the 200µl tube. One microlitre (1µl) of DNA was added into each tube. Each of the tubes was loaded in the PCR machine and appropriate program and PCR conditions were selected on the machine. The PCR conditions for *An. gambiae* complex selected were; Initial Denaturation @ 95^{o}C – 2mins, Denaturation @ 95^{o}C – 30secs, Annealing @ 55^{o}C – 30secs, Extension @ 72^{o}C – 40sec, Final extension @ 72^{o}C –7mins. All the conditions selected were set to run for 30cycles. Agarose gel of 1.5% was prepared with Tris-borate ethylene-di-amino tetra-acetic acid (TBE) buffer; such as 1.5g of agarose gel in 100ml of TBE buffer. It was mixed and boiled in microwave until solution was clear. It was cooled down for 5mins and the skin on top was removed and 100µl of ethidium bromide was added. The gel was poured into the trough. Ten microlitres (10µl) of the PCR product was loaded with 1µl of loading buffer into each well. Ten microlitres (10µl) of standard marker per gel was loaded in the electrophoresis machine (Figure 3). PCR product was run at 100volts and not more than 120-150mA in the electrophoresis machine. The gel picture was taken under UV light using gel documentation machine (Figure 19). It was read using the molecular weights of the *An. gambiae* sibling species as follows: *Anopheles gambiae s.s* - 390 base pair, *Anopheles arabiensis*- 315 base pair, *Anopheles merus* – 464 base pair and *Anopheles quadriannulatus* – 153 base pair (Figure 20).

Figure 3: Agarose gel electrophoresis loaded with applicorn (mosquito PCR product) prior to migration of the mosquito band.

DETERMINATION OF THE ECOLOGICAL FACTORS THAT INFLUENCE THE SURVIVAL OF MOSQUITOES IN THEIR BREEDING SITES.

3.8 ECOLOGICAL FACTORS THAT INFLUENCE MOSQUITOES

The ecological factors necessary for mosquito survival in their breeding sites include; physicochemical characteristics (e.g. temperature, pH, salinity, sulphate, total suspended solids, total dissolved solids, dissolved oxygen and turbidity) and biological characteristics (vertebrates and invertebrate) (Tadesse *et al.,* 2011). The breeding sites of mosquito larvae were grouped into three (3) which include; man-made containers, ground pools and plant axils. Water sample collected from the breeding sites were analysed monthly except for plant axils due to insufficient water for analysis.

3.8.1 Determination of physicochemical characteristics of the various breeding sites (Abiotic factors).

The physicochemical characteristics of the various breeding sites were determined using the method described by Patil *et al.* (2012). Water sample used were obtained from two breeding sites; man-made containers and ground pools. Each water sample was analysed for physicochemical constituents in Applied Biochemistry laboratory of NAU Awka from March to December, 2017. Each parameter was analysed three times for accuracy, after which the average was taken. Salinimetric method was used to determine salinity. Sulphate was measured using nephelometric method. The dissolved oxygen (DO) demand was measured using Winkler's titration method. Water temperature was determined at sites during sampling using mercury in glass thermometer; pH was measured using potentiometric method or pH meter. Total dissolved solids and total suspended solids were also determined by conventional method.

3.8.1.1 Determination of pH

The pH meter was switched on and the pH electrode was placed into the pH buffer solutions one after the other. 250ml Beaker filled with water was kept standby for rinsing before changing over from one buffer solution to another. The pH meter was calibrated with buffer 4.01, 7 and 9.21 by pressing CAL button. After calibration, the samples were read by pressing READ button. The blinking values were steady with one, indicating the pH value of the sample being read.

3.8.1.2 Determination of total dissolved solids (TDS)

An empty beaker was weighed, 50ml of the water sample was measured and the water sample was filtered into a weighed beaker. It was heat to dryness. The beaker was cooled and weighed after cooling

Calculation:

$$\text{Dissolved Solid (mg/l)} = \frac{\text{DS residue x 1000}}{\text{Volume of sample used}}$$

Dissolved residue = (Weight of sample + beaker after dryness) – Weight of empty beaker.

3.8.1.3 Determination of total suspended solids (TSS)

1000ml of the water sample was measured and was filtered through an already dried and weighed filter paper. When the filtration was completed, the filter paper was carefully transferred into the oven and was allowed to dry at 103^0C for at least one hour in the first instance. The filter was cooled and weighed. The weight was noted and the filter paper was put back inside the oven to dry more. It was weighed again and the process continued until a constant weight was obtained.

Calculation:

$TSS = 1000 \times T_1 - T_2$ ppm

Where T_1 = Weight of filter paper + suspended solid

T_2 = Weight of filter paper.

3.8.1.4 Determination of dissolved oxygen (DO)

The water sample was put in 300ml bottle, 2ml of $MnSO_4$ solution and 2ml alkali-iodide-azide were added well below the surface of the liquid. It was stoppered with care to exclude air bubbles and mixed by inverting the bottle a number of times until clear supernatant water was obtained. It was allowed to settle for 2minutes. 2ml Conc. H_2SO_4 was added by allowing the acid to run down the neck of the bottle, stoppered and mixed until gentle dissolution was completed. Iodine was distributed uniformly through the solution before decanting the quantity needed for titration. The mixture (203ml) was titrated with 0.0125M $Na_2S_2O_3$.$5H_2O$ solution to a pale straw colour. 1-2ml starch solution was added and the colour became blue, titration was continued by adding the thiosulphate solution drop-wise until the blue colour disappeared.

Calculation:

$$mg/l\ DO = \frac{16{,}000 \times M \times V}{(V_1 - 2.0)}$$

Where M = Molarity of the thiosulphate solution

V_1 = Volume of the bottle with stopper in place

3.8.1.5 Determination of sulphate

10ml of water sample was pipette into a conical flask and 5ml of 2M HCl was added. 2ml of 0.05M BaCl was also added. The mixture was boiled for 5minutes and allowed to cool. 2ml of ammonia and 5ml of 0.01N EDTA were added to the mixture and boiled for 5minutes. 5ml of buffer 10 and 3 drops of Eriot (solochrome Black T) indicator were also added. The mixture was titrated with 0.01M $Mgcl_2$

Note: Colour changes from deep blue to light purple

Calculation:

Sulphate = [10- (Tv x 0.93)] x 96.01464mg/l

Where Tv = titre value of sample

96.01464 = molecular weight of sulphate

3.8.1.6 Determination of salinity

50ml of water sample was measured and 3 drops of potassium chromate indicator was added into it. The mixture was then titrated against 0.02M $AgNO_3$ solution with a colour change from yellow to red.

Calculation:

$$\text{Salinity (mg/l)} = \frac{\text{Volume of } AgNO_3 \times \text{Molarity} \times 70{,}900}{\text{Volume of sample}}$$

3.8.1.7 Determination of surface water temperature

The temperature of each water sample was determined immediately on the field using water thermometer. It was measured in ^0C.

TEMPORAL DISTRIBUTION OF THE MOSQUITO VECTORS IN RELATION TO THE CLIMATIC FACTORS.

3.9 MOSQUITO MONTHLY ABUNDANCE AND CLIMATIC FACTORS

The abundance of the indoor and outdoor biting mosquitoes collected from the households in relation to the climatic factors in Oraifite was done by counting the number of indoor and outdoor mosquitoes collected from Jan to Dec, 2017 and observing their response to the data on monthly climatic conditions in Oraifite. The data on climatic conditions of Oraifite such as rainfall, temperature and relative humidity were collected from Weather Atlas (2017).

3.10 DATA ANALYSIS

The data collected were subjected to statistical analysis using SPSS (20.0) version and Microsoft Excel 2010 version. Objectives 1 and 2 were analysed using tables, graphs and charts. Objectives 3 and 4 were analysed using correlation coefficient to test for relationship between mosquito larval abundance and individual physicochemical parameters of the breeding sites and to establish the association between adult mosquito population and climatic factors. Correlation coefficient used to test relationship between two variables was interpreted according to Onuoha *et al*. (2011). Analysis of Variance (ANOVA) and T-test were used to compare different mosquito populations at 5% significant level. Simpson's dominance index was used to determine the prevalence of different mosquito species while Shannon-Weiner Index was used to determine species diversity in the study area (Ogbeibu, 2005).

CHAPTER FOUR

RESULTS

Indoor biting mosquitoes

A total of 1418 indoor biting adult mosquitoes were collected from the eight communitties for a period of twelve months using Pyrethrum Knockdown Collection (PKC) method (Table 2). Of the total number collected, the highest 239(16.85%) was collected in Umuafa while the least 109(7.68%) was from Amakom. The other collections were from Nkalafia 201(14.17%), Ibolo 189(13.33%), Umudisi 183(12.90%), Umuezopi 177(12.48%), Umunakwa and Umuonyeagolu 160(11.28%) respectively.

A total of twelve species formed the 1418 mosquitoes collected indoors. The highest collected indoors was *Anopheles gambiae* 294(20.73%) while the least was *Coquillettidia maculipennis* 1(0.07%). Others included *Culex quinquefasciatus* 271(19.11%), *Anopheles funestus* 228(16.08%), *Aedes albopictus* 202(14.25%), *Aedes aegypti* 180(12.70%), *Culex decens* 95(6.70%), *Mansonia africana* 49(3.46%), *Culex poicilipes* 45(3.17%), *Mansonia uniformis* 22(1.55%), *Anopheles moucheti* 19(3.46%) and *Culex nebulosus* 12(0.85%). There was significant difference in mosquito species collected indoors from Oraifite ($p<0.05$). The significant difference in number occurred when *An. gambiae* was compared with all other species except *Ae. aegypti, Ae. albopictus, An. funestus* and *Cx. quinquefasciatus* (See appendix 4).

Table 2: Indoor biting adult mosquitoes collected using Pyrethroid Knockdown Collection Method (PKC) in Oraifite.

Adult mosquito species	Communities								Total (%)
	Amakom	Umunakwa	Umudisi	Nkalafia	Umuafa	Umuezopi	Ibolo	Umuonyeagolu	
Ae. aegypti	12	22	19	17	28	31	29	22	180(12.70)
Ae. albopictus	9	17	34	39	22	41	13	27	202(14.25)
An. gambiae	29	36	47	31	49	38	41	23	294(20.73)
An. funestus	27	44	39	41	29	17	13	18	228(16.08)
Cx. decens	0	0	0	10	34	0	20	31	95(6.70)
Cx. nebulosus	0	0	12	0	0	0	0	0	12(0.85)
Cx. poicilipes	0	0	0	0	26	0	19	0	45(3.17)
Cx. quinquefasciatus	32	29	21	37	41	27	46	38	271(19.1)
Cq. maculipennis	0	0	0	0	0	0	0	1	1(0.07)
An. moucheti	0	12	0	4	3	0	0	0	19(1.34)
M. africana	0	0	11	9	7	14	8	0	49(3.46)
M. uniformis	0	0	0	13	0	9	0	0	22(1.55)
Total(%)	**109 (7.68)**	**160 (11.28)**	**183 (12.90)**	**201 (14.17)**	**239 (16.85)**	**177 (12.48)**	**189 (13.33)**	**160 (11.28)**	**1418(100)**

Significance level: ($P<0.05$; $P=3.38*10^{-19}$).

Percentage distributions of indoor-biting and resting mosquitoes sampled using PKC method in Oraifite communities

Figure 4 shows that out of eight communities sampled in Oraifite, Umuafa had the highest percentage distribution (16.85%) while Amakom had the least (7.68%) (See appendix 2a). From the error bars, there was significant difference ($P<0.05$) between the indoor biting and resting mosquitoes collected from Umuafa and other communities. There was no significant difference ($P>0.05$) between the indoor biting and resting mosquitoes collected from Umuonyeagolu, Umuezopi, Umudisi and Umunakwa. Also, there was no significant difference ($P>0.05$) between the indoor biting and resting mosquitoes collected from Ibolo and Nkalafia while there was significant difference ($P<0.05$) between the indoor biting and resting mosquitoes collected from Amakom and the other communities.

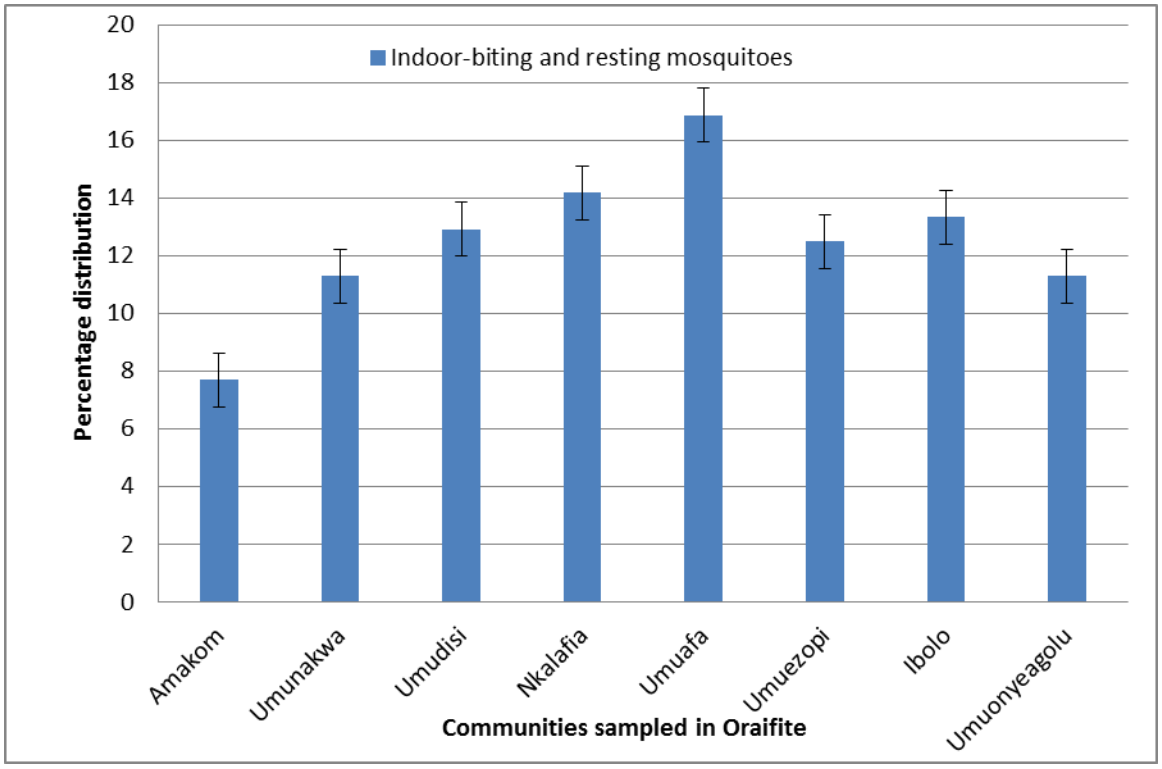

Figure 4: Percentage distributions of indoor-biting and resting mosquitoes sampled in Oraifite communities.

Percentage of species composition of Indoor-biting and resting mosquito sampled in Oraifite communities

In figure 5, out of 12 mosquito species collected indoors in Oraifite communities using PKC method, *An. gambiae* had the highest distribution (20.73%) while *Coquillettidia maculipennis* had the least (0.07%) (See appendix 2b). From the error bars, there was significant difference ($P<0.05$) between *An. gambiae* and other mosquito species collected indoors in Oraifite communities while *Cx. quinquefasciatus* and *An. funestus* had no significance difference ($P>0.05$). There was no significant difference ($P>0.05$) between *M. africana* and *Cx. poicilipes*. Also, there was no significant difference ($P>0.05$) between *Ae. albopictus and Ae. aegypti*.

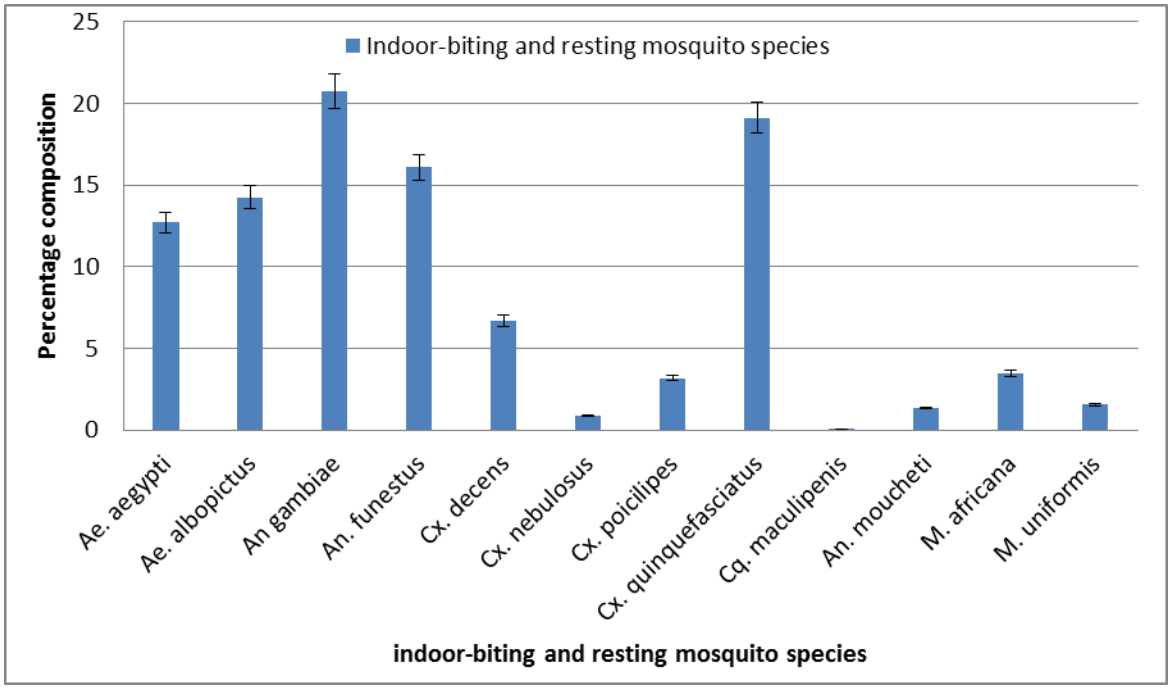

Figure 5: Percentage of species composition of Indoor-biting and resting mosquito sampled in Oraifite communities.

Percentage composition of Gonotrophic stages of adult mosquitoes collected by PKC method

Figure 6 shows that freshly fed mosquitoes had the highest percentage (79.63%) while the gravid mosquitoes had the least percentage (6.06%) (See appendix 3b). There was significant difference ($p<0.05$, $P = 2.08*10^{-5}$) between freshly fed stage and other gonotrophic stages of mosquitoes collected indoors in Oraifite communities (Appendix 6). The error bar showed that there was no significant difference ($P>0.05$) between the unfed, half-gravid and gravid stages of mosquitoes collected indoors.

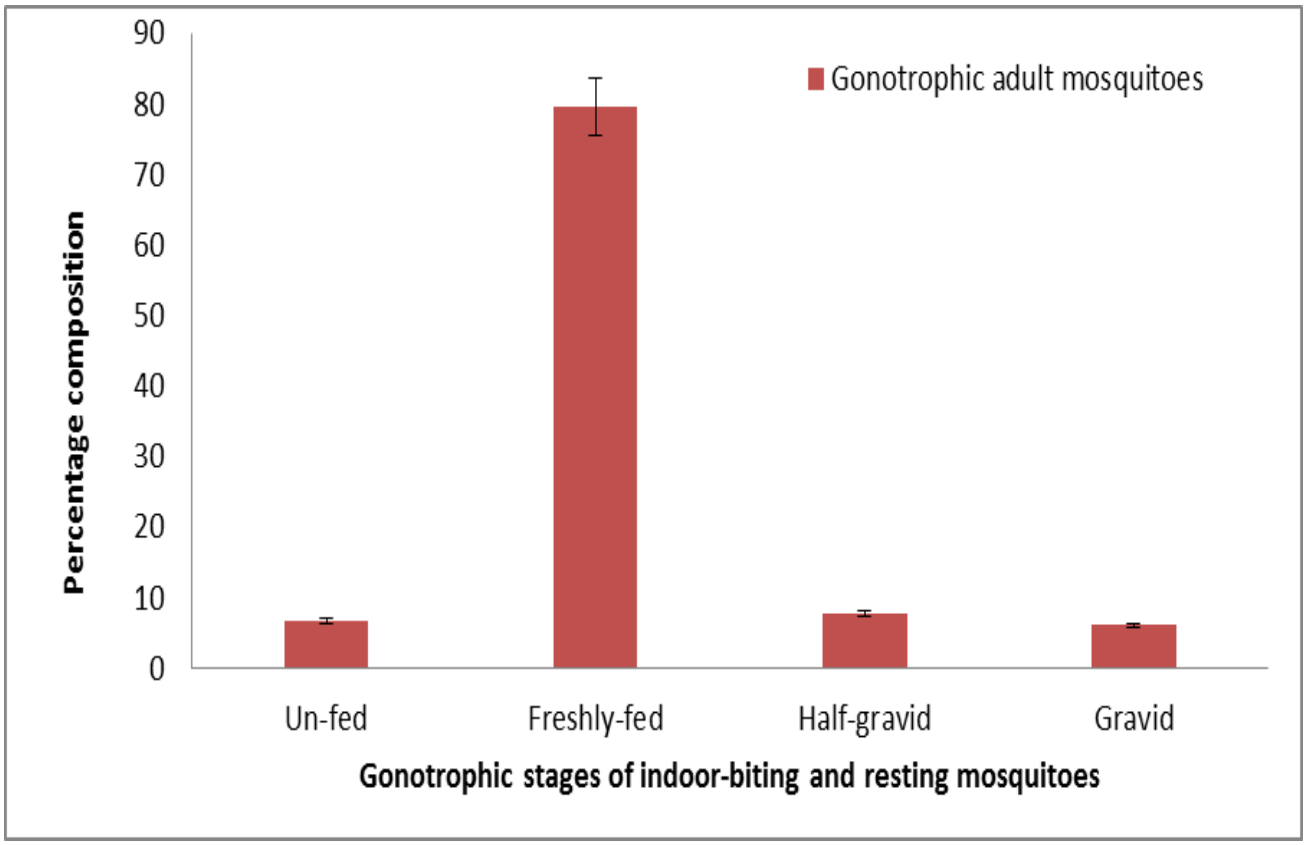

Figure 6: Percentage composition of gonotrophic stages of adult mosquitoes collected by PKC method.

Percentage composition of gonotrophic stages of indoor-biting and resting mosquito species

In figure 7, *An. gambiae* had the highest percentage of freshly fed mosquito species (17.07%) while *Cq. maculipennis* had the least (0.07%) (See appendix 3b). There was significant difference (P<0.05) between freshly fed *An. gambiae* and other freshly fed mosquitoes species collected indoors in Oraifite communities while there was no significant difference (P>0.05) between the freshly fed *An. gambiae* and freshly fed *Cx. quinquefasciatus.* The percentages of freshly fed mosquito species were significantly higher (P<0.05) than the percentages of the unfed, half gravid and gravid mosquitoes. The error bar showed that there was no significant difference (P>0.05) between the unfed, half-gravid and gravid stages of all mosquitoes species collected indoors.

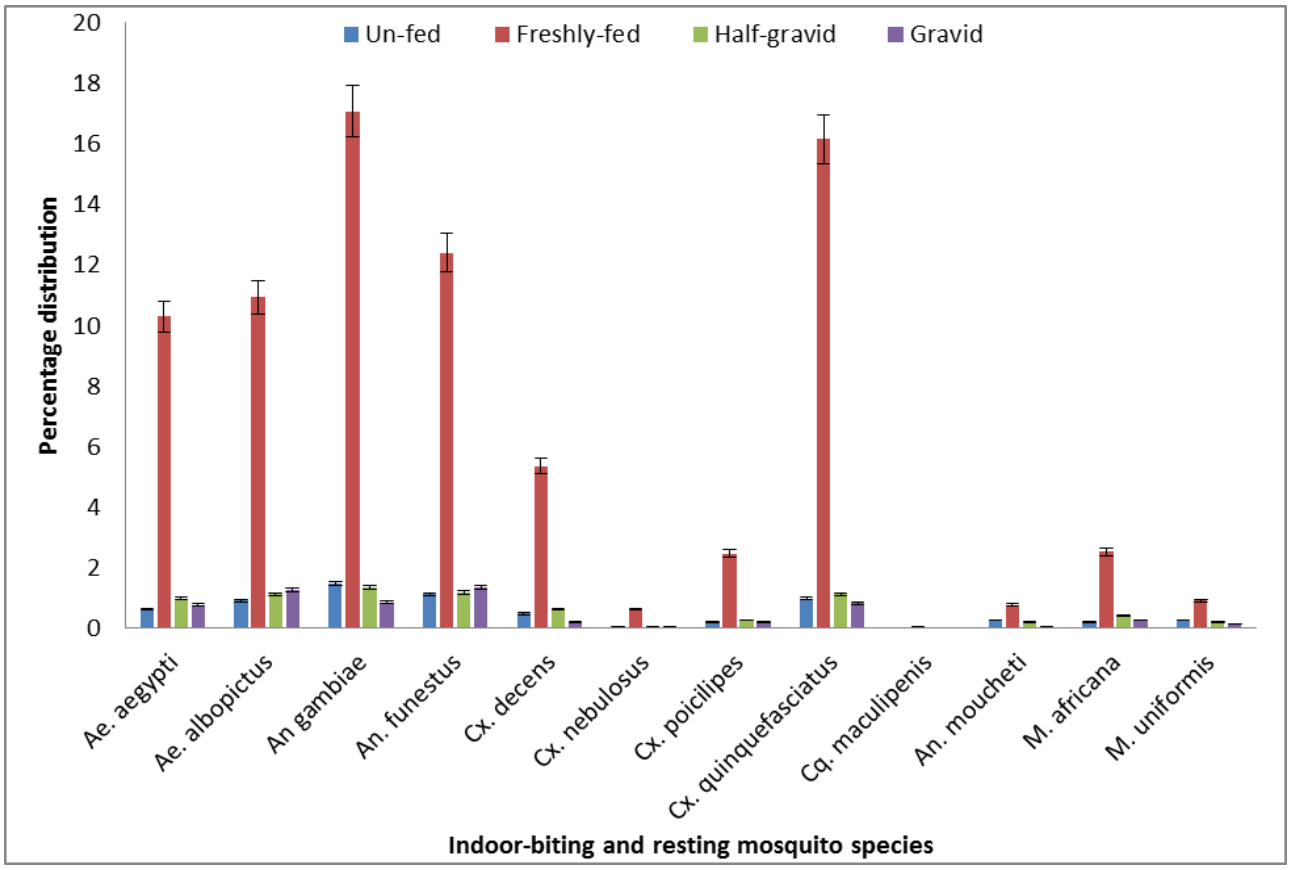

Figure 7: Percentage composition of gonotrophic stages of indoor-biting and resting mosquito species.

Relationship between Indoor resting density (IRD) and man-biting rate (MBR) of mosquitoes collected indoors.

The indoor resting density and man-biting rate of the different mosquito species in the study area were calculated and shown in appendix 4b. *An. gambiae* had the highest indoor resting density and man biting rate of 0.51 and 0.20 while *Cq. maculipennis* had the least of 0.002 and 0.0008 respectively.

In Fig 8, data transformed to Log_{10} (IRD + 10) and Log_{10} (MBR + 10) were used to determine the relationship between indoor resting density (IRD) and man-biting rate (MBR) of mosquitoes. Log_{10} (IRD + 10) and Log_{10} (MBR + 10) had a linear relationship because, as Log_{10} (IRD + 10) of mosquito species increases, Log_{10} (MBR + 10) of mosquito species also increases.

Indoor resting density (IRD) had a significant effect on man-biting rate (MBR) because, as IRD increases MBR increases. The higher the number of mosquito species resting indoors, the higher the biting rate of the mosquitoes. The effect of IRD on MBR was significant ($p<0.05$) at 95% confidence interval because, from our simple regression/ANOVA table (See appendix 4c).

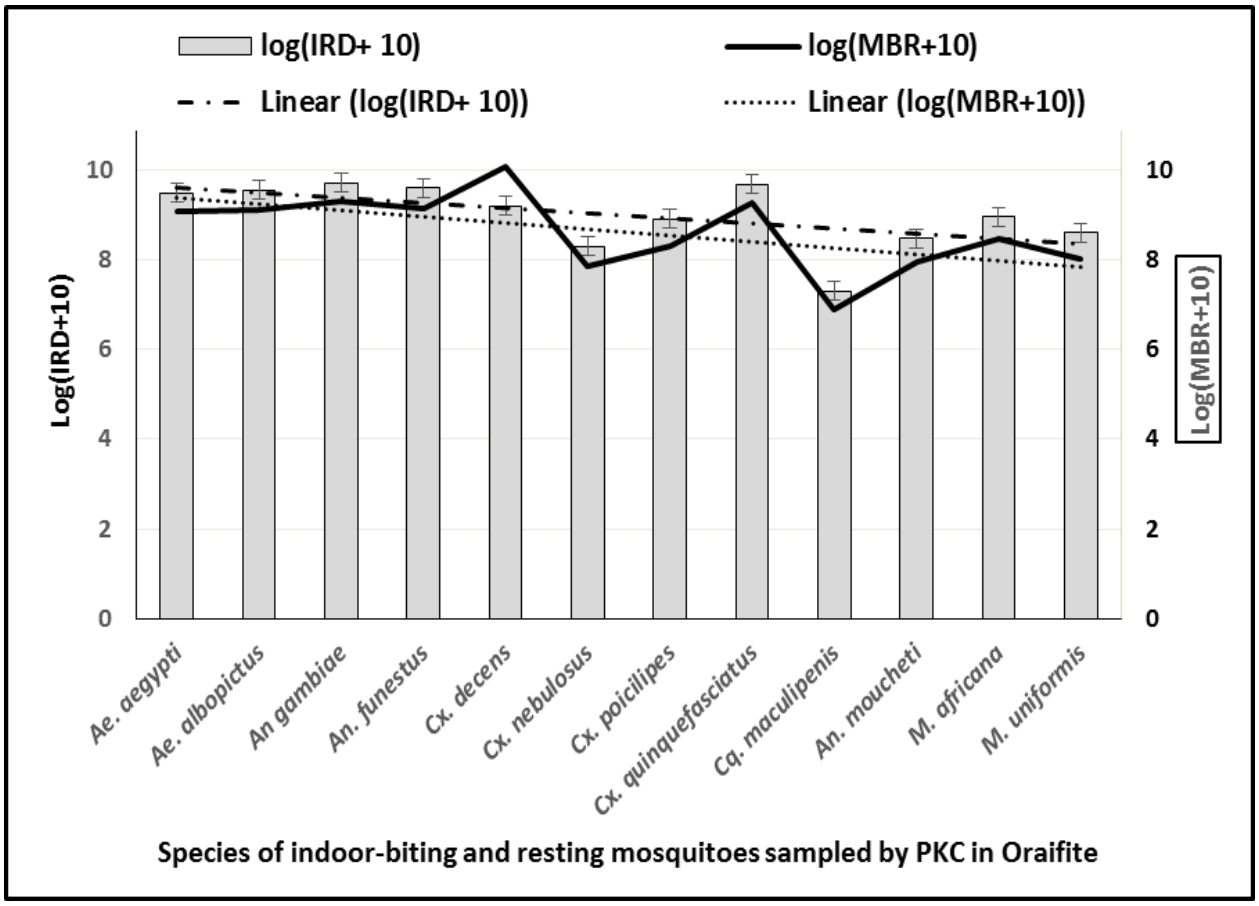

Figure 8: Relationship between Indoor resting density (IRD) and man-biting rate (MBR).

Percentage distribution of outdoor biting mosquitoes collected in Oraifite Communities.

Fig. 9 shows that out of 952 mosquitoes collected outdoors from the eight communities for a period of twelve months using Human Bait Collection method (HBC), Nkalafia had the highest percentage distribution (17.12%), followed by Umuafa (13.76%), Ibolo (12.92%), Umuezopi (12.71%), Umudisi (12.39%), Umuonyeagolu (11.55%), Amakom (10.29%) and Umunakwa (9.24%) (See appendix 5). The error bars indicated that there was significant difference ($P<0.05$) between the outdoor mosquitoes collected in Nkalafia and other communities while mosquitoes collected from Umuonyeagolu, Amakom and Umunakwa were not significantly different ($P>0.05$). Also, there was no significant difference ($P>0.05$) between outdoor mosquitoes collected in Umudisi, Ibolo and Umuezopi.

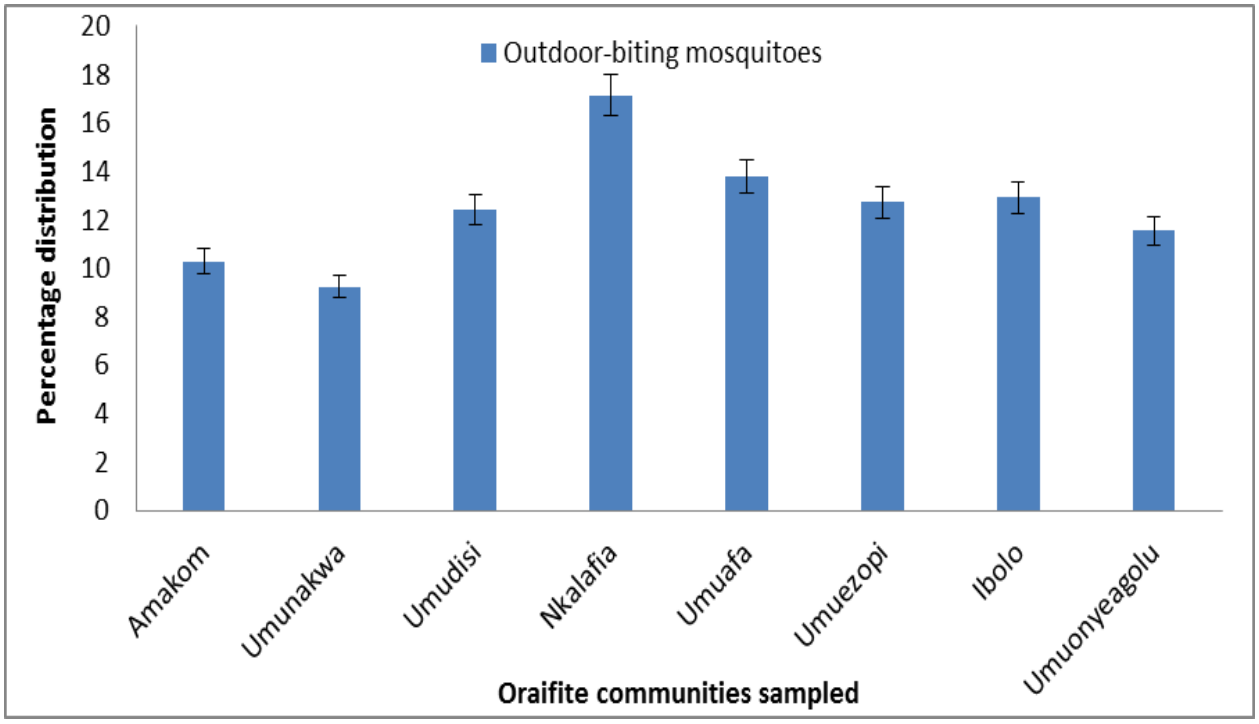

Figure 9: Percentage distribution of outdoor biting mosquitoes collected in Oraifite Communities.

Percentage composition of outdoor biting species of mosquitoes collected by human bait catch (HBC) method.

Figure 10 shows that out of 14 species of mosquitoes collected outdoors by HBC method, *Ae. albopictus* had the highest percentage composition (25.00%) while *Cx. nigripalpus* had the least (0.21%) (See appendix 5). There was significant difference ($P<0.05$), P-value = $1.3*10^{-28}$ in mosquito species collected outdoors in Oraifite. The significant difference in number occurred when *Ae. albopictus* was compared with all other species except *Ae. aegypti* and *Cx. quinquefasciatus* (Appendix 5b). The error bar showed that there was no significant difference ($P>0.05$) between *Ae. africanus, Ae. circumluteolus, Cx. nebulosus, Cx. poicilipes, Cx. annulioris, Cx. nigripalpus, Er. chrysogaster, M. africana* and *M. uniformis.*

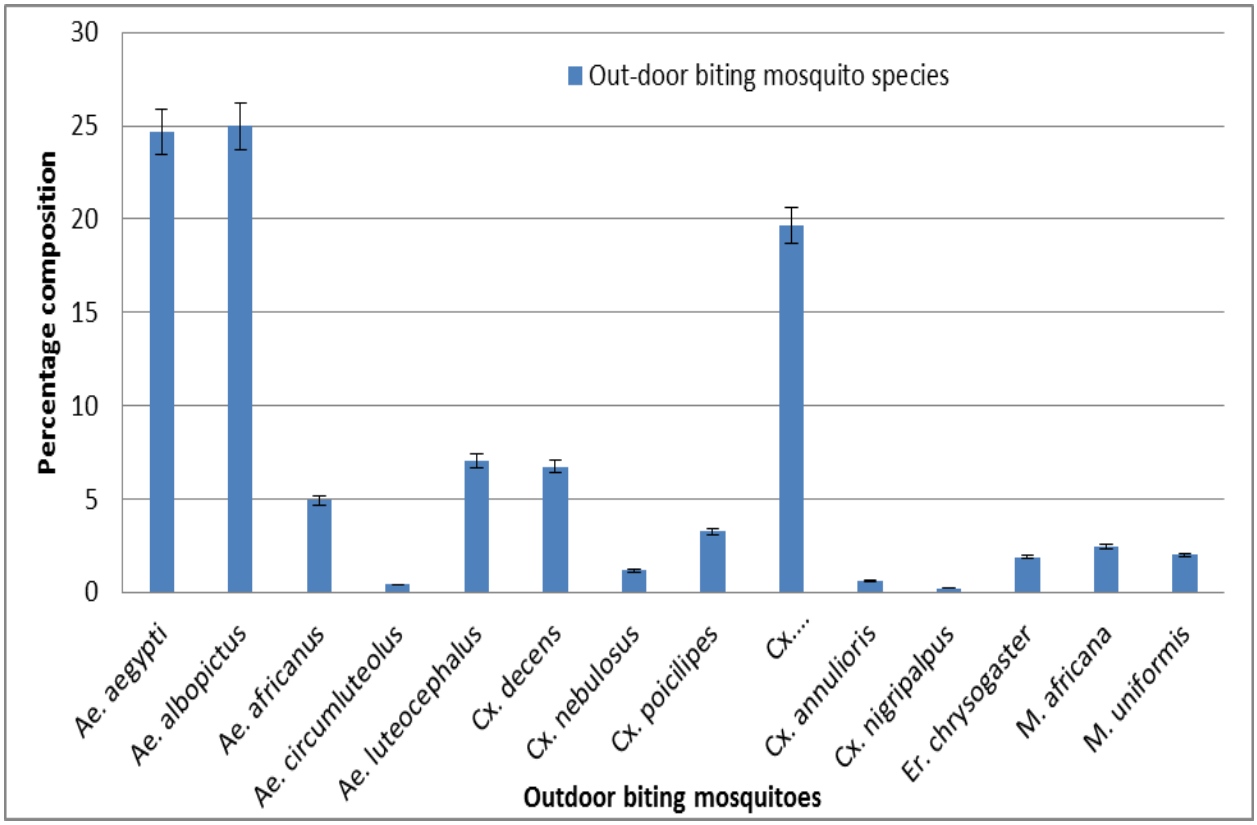

Figure 10: Percentage composition of Out-door biting species of mosquitoes collected by human bait catch (HBC) method.

Percentage distribution of outdoor biting mosquito species with respect to time of the day

Figure 11a shows that outdoor biting mosquito collections using Human Bait Collection method was done from 4.30pm – 7.30pm. The highest percentage of mosquitoes was collected at interval of 7.15 - 7.30pm (12.89%) while the least was at 4.30-4.45pm (2.3%) (See appendix 6). The error bars indicated that the percentage of mosquitoes collected between 6.45 - 7.00pm, 7.00 - 7.15pm and 7.15 - 7.30pm were significantly difference ($P<0.05$) from the percentage of mosquitoes collected between 4.30 – 4.45pm, 4.45 - 5.00pm, 5.00 – 5.15pm, 5.15 - 5.30pm, 5.30 – 5.45pm and 5.45 – 6.00pm.

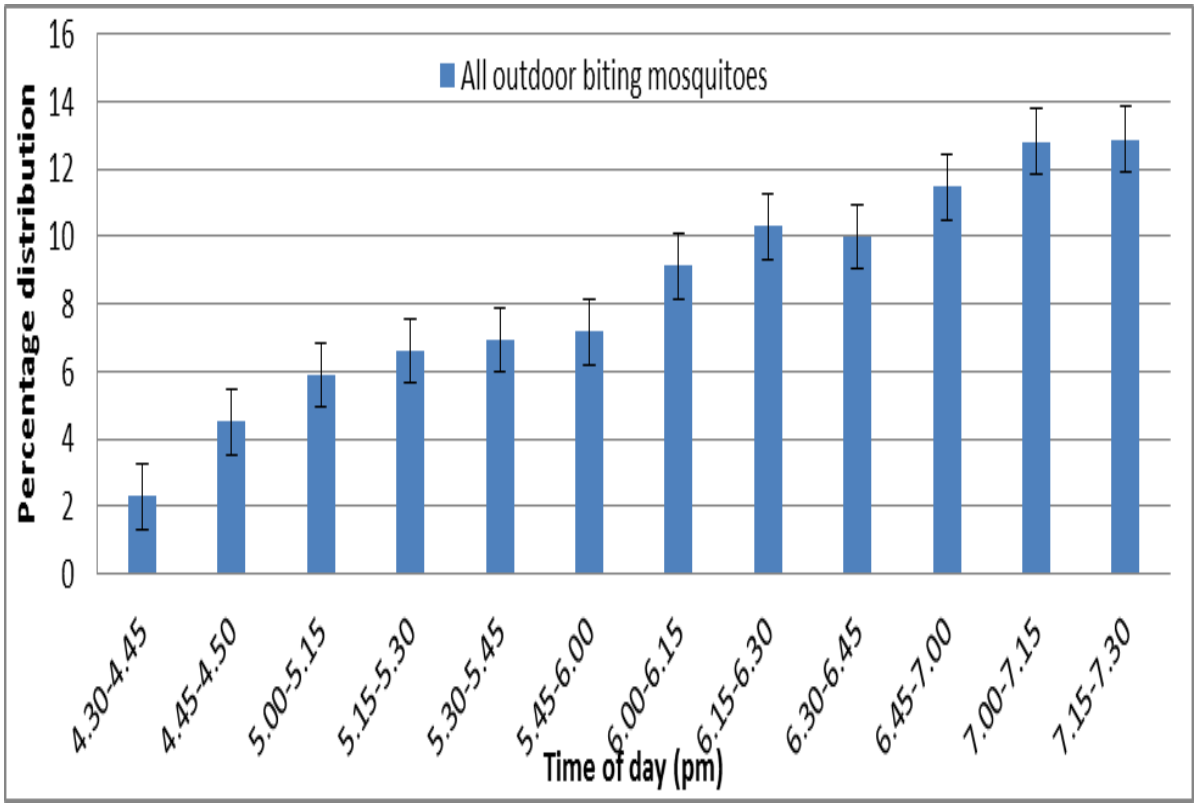

Fig. 11a: Percentage distribution of outdoor biting mosquito species with respect to time of day.

Percentage distribution of *Aedes* mosquitoes collected outdoors with respect to time of the day.

Fig. 11b shows that *Ae. aegypti* and *Ae. albopictus* were the only mosquito species collected from 4.30 – 7.30pm. *Ae. albopictus* had the highest Percentage distribution and its peak of collection was at the interval of 7.00 – 7.15pm. The highest percentage of *Ae. aegypti* was collected from 7.15 – 7.30pm while the least was collected at the interval of 4.30 – 4.45pm. High Percentage of *Ae. africanus* was collected from 7.00 – 7.15pm while the least was collected from 5.30 – 5.45pm and 5.45 – 6.00pm, respectively and none was collected from 4.30 – 5.30pm. *Ae. circumluteolus* had its peak at the interval of 6.15 – 6.30pm and was least collected at 5.15 – 5.30 and 5.45 – 6.00pm respectively. *Ae. luteocephalus* had its biting peak at 6.45 – 7.00pm and 5.30 – 5.45pm, respectively while the least collection was at 5.45 – 6.00pm and 6.00 – 6.15pm, respectively. The error bar shows that the percentage of *Ae. albopictus* collected from 7.00 – 7.15pm was significantly different ($P<0.05$) from other *Aedes* mosquitoes collected at different time intervals

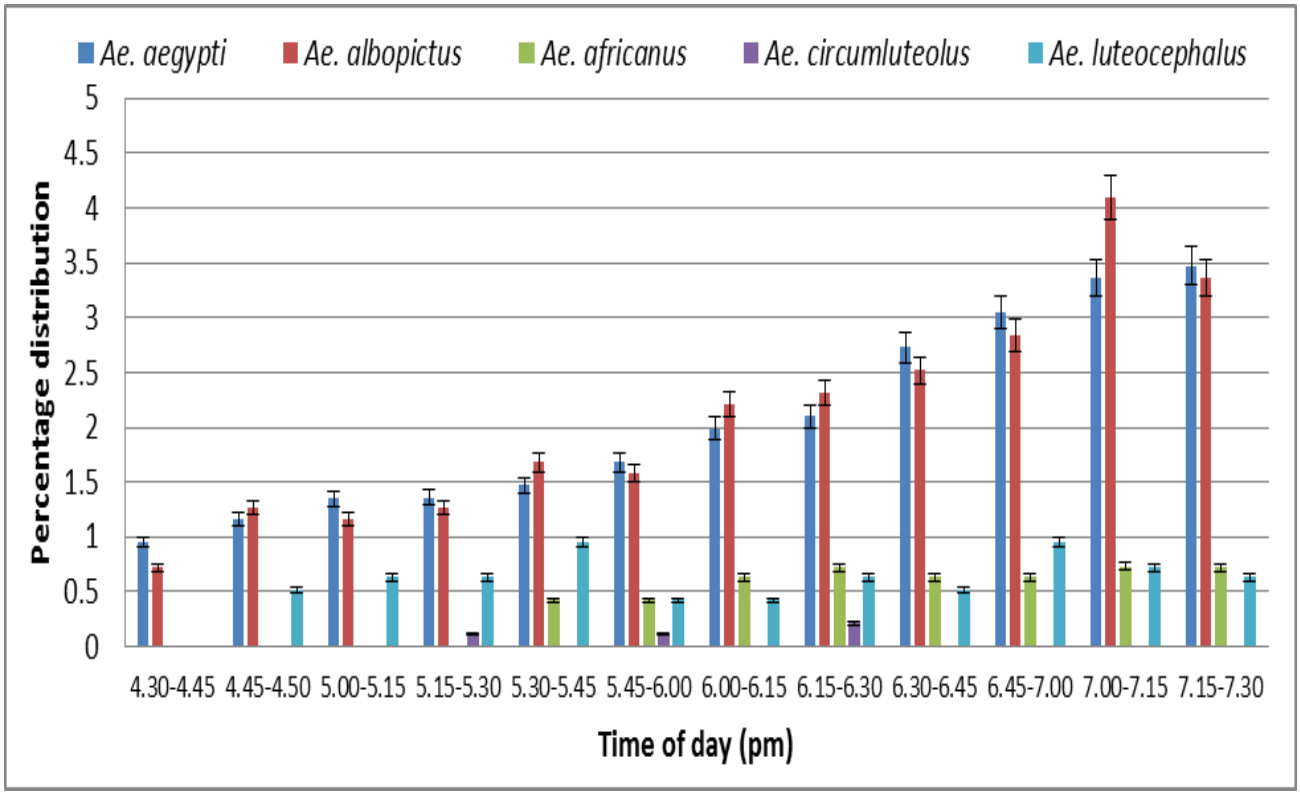

Fig. 11b: Percentage distribution of *Aedes* mosquitoes with respect to time of day.

Percentage distribution of *Culex* species collected outdoors with respect to time of day.

Fig. 11c shows that *Cx. quinquefasciatus* was the only *Culex* species that was collected from 4.30 – 7.30pm. *Cx. quinquefasciatus* had the highest Percentage distribution and its peak of collection was at the interval of 7.15 – 7.30pm while it was least collected from 4.30 – 4.45pm. *Cx. decens* had its biting peak at the interval of 5.45 – 6.00pm, it was collected at all intervals except at 4.30 – 4.45pm. *Cx. nebulosus* had its biting peak at the interval of 6.45 – 7.00pm while *Cx. poicilipes* had its highest Percentage distribution from 6.15 – 6.30pm. *Cx. annulioris* had its biting peak at the interval of 6.00 – 6.15pm while *Cx. nigripalpus* had its highest Percentage distribution from 5.45 – 6.00pm and 6.00 – 6.15pm, respectively. There was significant difference ($P<0.05$) between *Cx. quinquefasciatus* collected at all intervals and the other *Culex* species. At the interval of 5.45 – 6.00pm, all the six species of *Culex* mosquitoes were collected but not the same in the other time intervals.

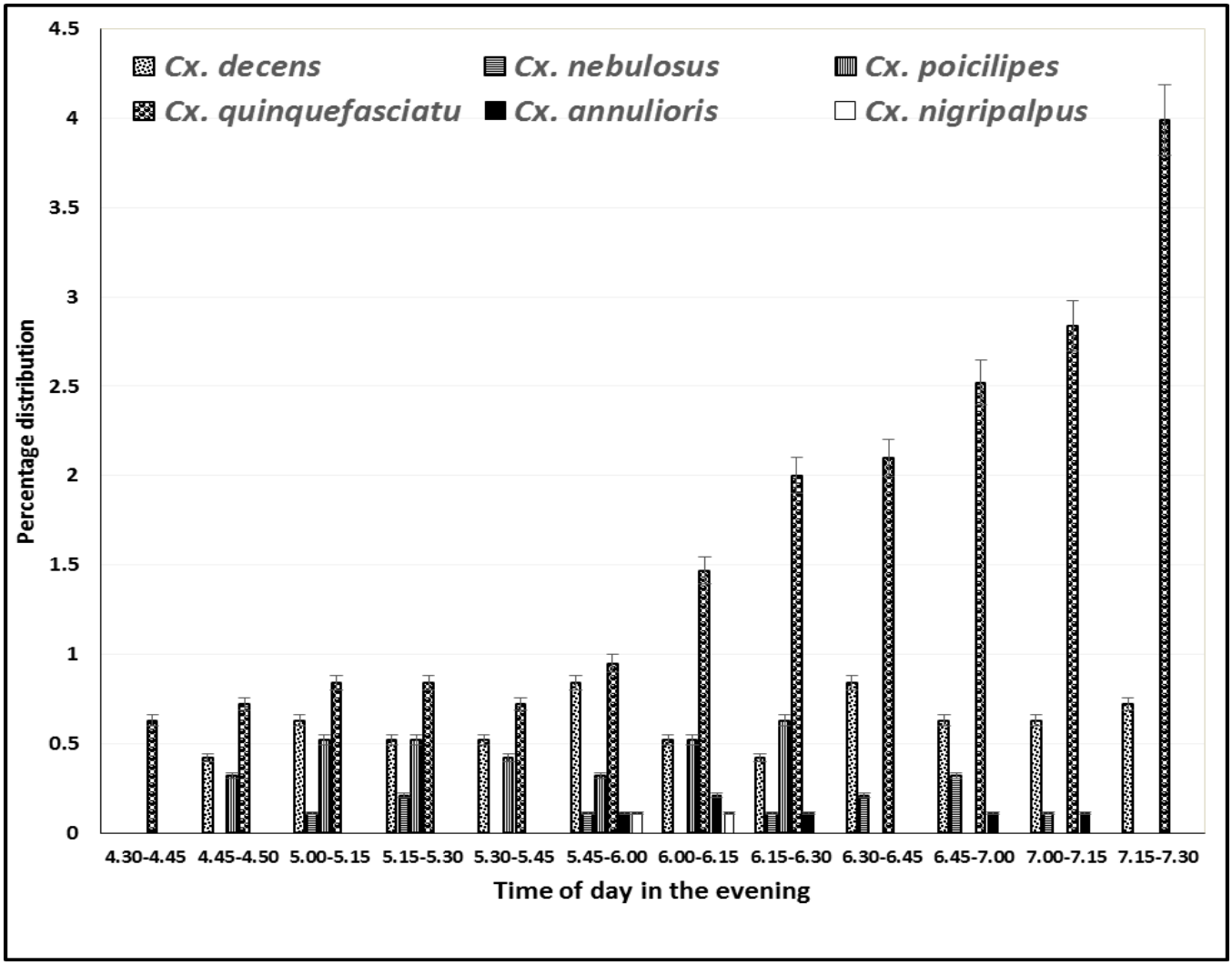

Fig. 11c: *Culex* species percentage distribution with respect to time of day

Percentage distribution of *Eretmapodites* and *Mansonia* species with respect to time of day.

In Fig. 11d, *Er. chrysogaster* had its biting peak at the intervals of 5.15 – 5.30pm and 6.00 – 6.15pm respectively. It was only collected between the intervals of 5.00 – 6.45pm. *M. africana* had its biting peak at the interval of 6.15 – 6.30pm while *M. uniformis* had its biting peak at the interval of 5.15 – 5.30pm. There was significant difference ($P<0.05$) between *M. africana* collected at the interval of 6.15 – 6.30pm and the other species collected at all time intervals. No species of mosquites was collected at the time intervals of 4.30 – 4.45pm and 7.15 – 7.30pm.

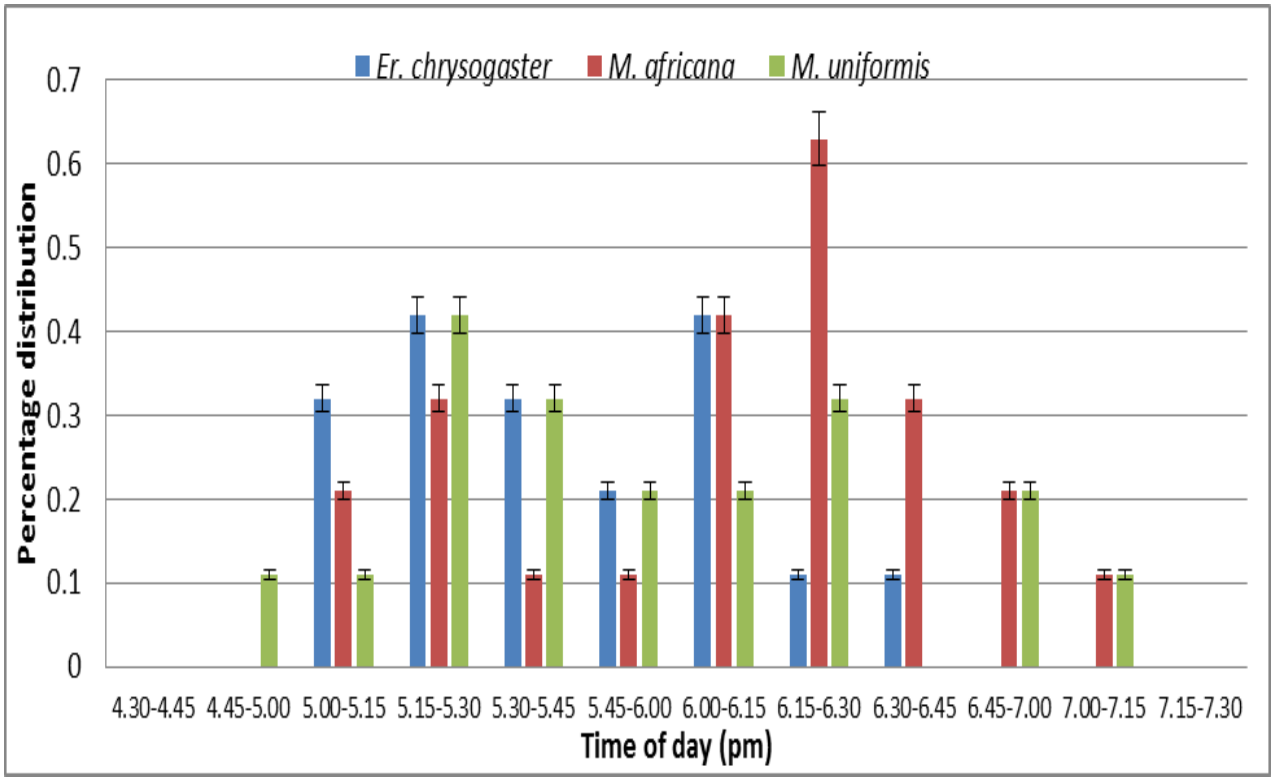

Fig. 11d: Percentage distribution of *Eretmapodites* and *Mansonia* species with respect to time of day.

Monthly percentage distribution of all mosquito genera collected in the study

Table 3 shows that *Aedes* mosquitoes had the highest percentage distribution in the month of September (9.11%) and lowest percentage distribution in the month of December (1.26%) while there was no distribution in the months of January, February and March.

Anopheles mosquitoes had the highest percentage distribution in the month of September (5.15%); the least percentage distribution was collected in the month of December (0.89%) while no *Anopheles* mosquitoes were collected in the months of January, February and March.

Culex mosquitoes was the only genera collected throughout the months of the year. *Culex* mosquitoes had the highest percentage distribution in the month of July (9.58%) and least percentage distribution in the month of February (0.38%).

Coquillettidia and *Eretmapodites* were only collected in the months of June (0.04%) and August (0.76%). *Mansonia* mosquitoes had the highest percentage distribution in the month of August (2.49%) and least collected in the month of July (0.17%) while no *Mansonia* mosquitoes were collected in the months of December to June except in March.

Table 3: Monthly distribution (%) of all mosquito genera collected in the study.

Mosquito Genera	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Total (%)
Aedes	1.26	0	0	0	3.21	4.34	4.55	4.94	4.85	9.11	4.72	4.05	**41.03**
Anopheles	0.89	0	0	0	2.15	1.9	2.49	3.03	3.37	5.15	2.23	1.61	**22.82**
Culex	1.86	1.1	0.38	1.05	1.73	1.86	1.69	9.58	2.82	2.91	3.12	2.44	**30.54**
Coquillettidia	0	0	0	0	0	0	0.04	0	0	0	0	0	**0.04**
Eretmapodites	0	0	0	0	0	0	0	0	0.76	0	0	0	**0.76**
Mansonia	0	0	0	0.38	0	0	0	0.17	2.49	0.29	0.84	0.59	**4.76**
Total (%)	**4.01**	**1.1**	**0.38**	**1.43**	**7.09**	**8.1**	**8.77**	**17.72**	**14.29**	**17.46**	**10.91**	**8.69**	**99.95**

Seasonal percentage distributions of all mosquito genera

Fig. 12 shows that among the six mosquito genera collected in both wet and dry seasons, *Culex* mosquitoes had the highest percentage distribution in dry season (4.39%) while *Mansonia* mosquitoes had the least percentage distribution (0.38%). *Coquillettidia* and *Eretmapodites* were not collected in dry season but they were collected in wet season. In wet season, *Aedes* mosquitoes had the highest percentage distribution (39.77%) while *Coquillettidia* had the least percentage distribution (0.04%) (See appendix 8). The percentage distribution of mosquito genera was very high in wet season and low in dry season. Mosquito genera collected in wet season were significantly different ($P<0.05$) while Mosquito genera collected in dry season were not significantly different ($P>0.05$).

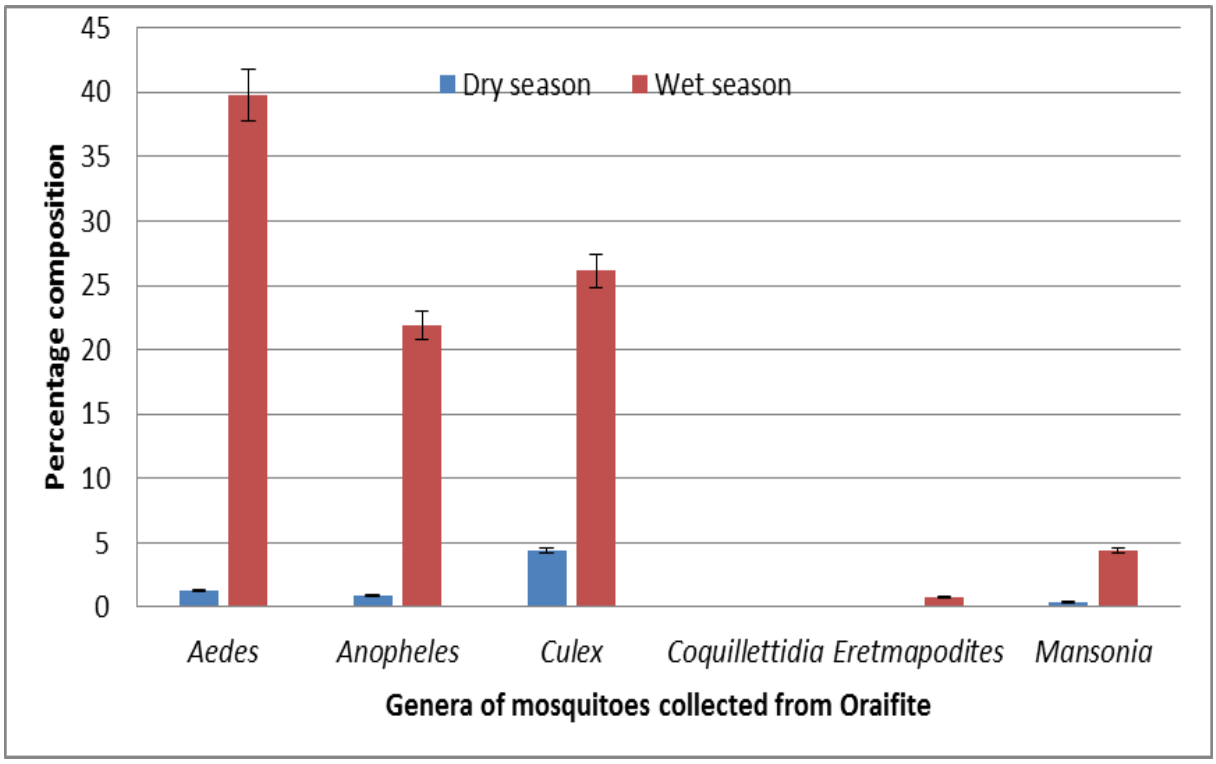

Fig. 12: Seasonal distributions (%) of all mosquito genera.

Seasonal distributions (%) of all adult mosquito species collected in the study area.

In Fig. 13, *Aedes albopictus* had the highest distribution in wet season (17.89%) while *Cx. quinquefasciatus* had the highest distribution in dry season (4.01%) (See appendix 9). There was significant difference between *Cx. quinquefasciatus* and other mosquito species collected in dry season ($P<0.05$). *Cx. quinquefasciatus, Ae. aegypti* and *Ae. albopictus* were significantly different ($P<0.05$) from other adult mosquito species collected in wet season.

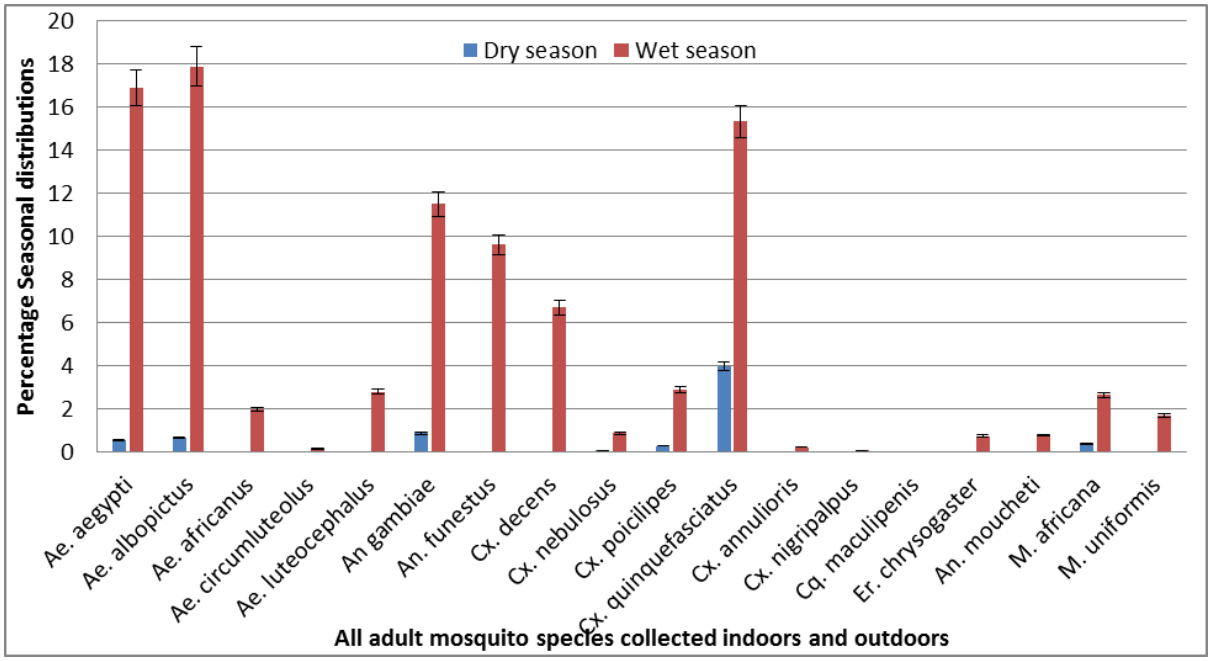

Fig. 13: Seasonal distributions (%) of all mosquito species collected indoors and outdoors.

Seasonal percentage distribution of indoor and outdoor adult biting mosquitoes

Fig. 14 shows that in dry and wet seasons, indoor biting and resting mosquitoes collected by PKC method had the percentage distributions of 5.2% and 54.6% respectively while the outdoor biting mosquitoes collected by HBC method had the percentage distributions of 1.8% and 38.4% (See appendix 10). There was no significant difference ($P>0.05$) between the adult mosquito population collected indoors and outdoors during dry season while there was significant difference ($P<0.05$) between the adult mosquito species collected indoors and outdoors in wet season. In addition, there was significant difference ($p<0.05$) in the wet and dry season of outdoor mosquito population. Indoor mosquito population collected in the wet and dry season were significantly different ($p<0.05$) (See appendix 10b & 10c). All adult mosquito population was high in wet and very low in dry season.

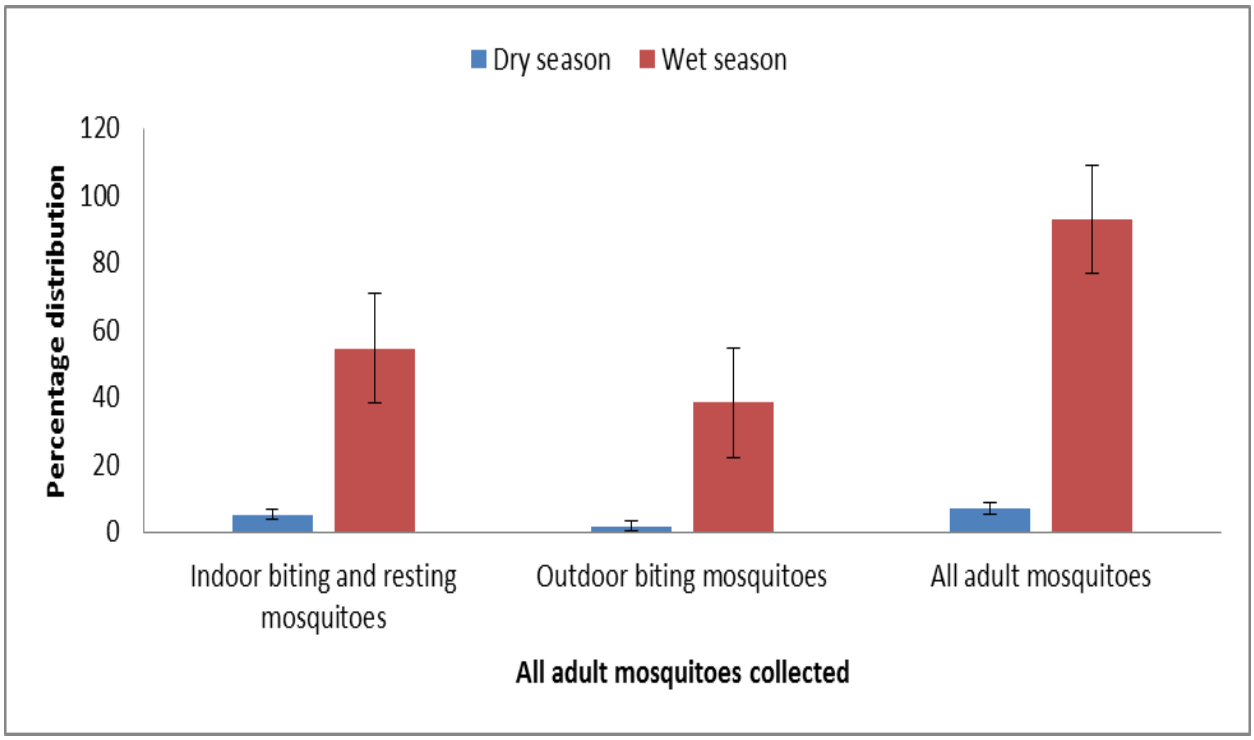

Fig. 14: Seasonal percentage distribution of indoor and outdoor adult biting mosquitoes.

Monthly percentage distribution of indoor and outdoor adult mosquitoes collected in the study area.

In Fig. 15, indoor biting and resting mosquitoes were collected from January to December while outdoor biting resting mosquitoes were collected in all months except in February. There was also a significant difference ($p<0.05$, $p=2.3*10^{-34}$) in mosquito population collected in different months in the study area (See appendix 11b).

The error bars indicate that there was no significant difference ($P>0.05$) between the outdoor mosquitoes collected from Dec to March ($P>0.05$). Indoor biting and resting mosquitoes had highest percentage distribution in the month of July (11.05%) and the least in February (0.38%) (See appendix 11). Outdoor biting mosquitoes had highest percentage distribution in the month of September (7.64%) and the least in the months of January (0.51%) and March (0.51%) respectively. The error bar shows that there was no significant difference ($P>0.05$) in all adult mosquitoes collected in the month of July and September.

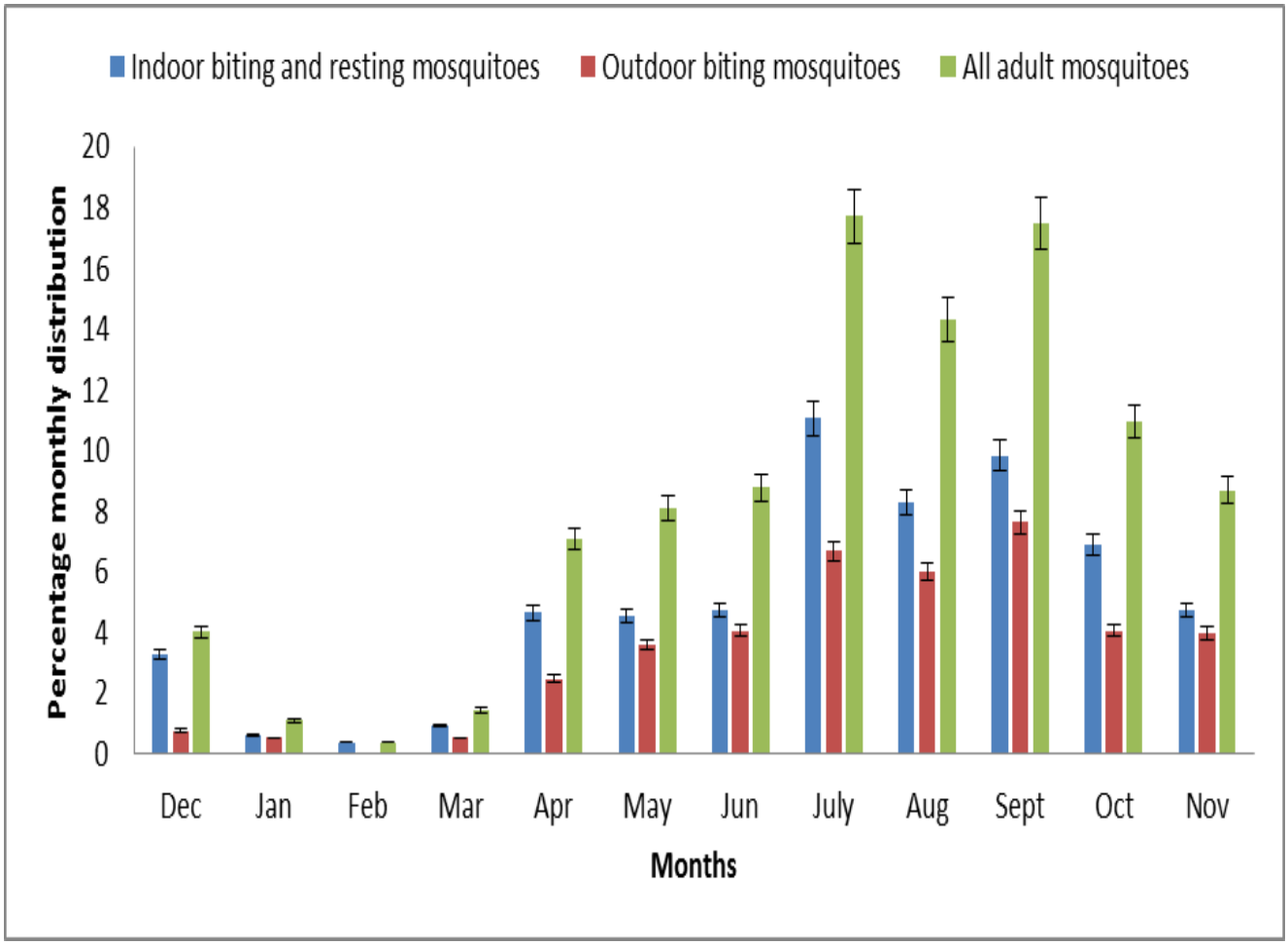

Fig. 15: Monthly percentage distribution of all adult mosquitoes

Determination of the breeding sites of mosquito vectors

All the 1,156 mosquito species emerged from larvae were collected from three different breeding sites sampled (Figure 16). They included; man-made containers, ground pools and plant axils. Man-made containers had the highest percentage of mosquito species (45.16%), followed by ground pools (43.25%) and plant axils (11.60%) (See appendix 12). *An. gambiae* was collected only in ground pools. The mosquito species collected from man-made container were *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus, An. gambiae*, *Ae. simpsoni, Cx. tigripes* and *Ae. luteocephalus.* The ground pool breeders were *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus, An. gambiae* and *Cx. tigripes* while the species collected from plant axils were *Ae. aegypti*, *Ae. albopictus* and *Ae. simpsoni*. The error bars indicated that there was no significant difference ($p>0.05$) between *Cx. tigripes* collected from ground pools and man-made containers. Also, there was no significant difference ($p>0.05$) between *Ae. aegypti* collected from ground pools and *Cx. quinquefasciatus* collected from man-made containers while percentage of *Ae. simpsoni* collected from man-made container and plant axils were significantly different ($p<0.05$).

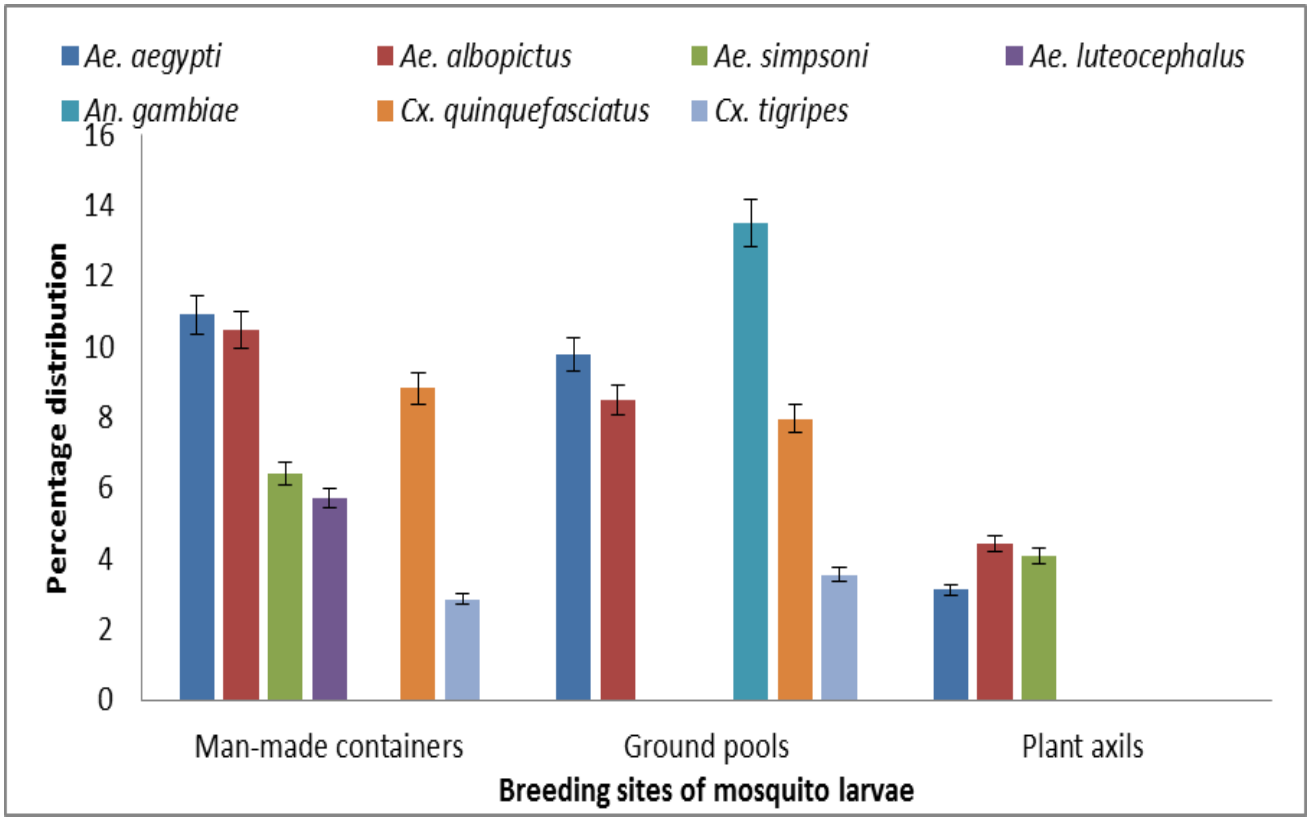

Figure 16: Percentage distribution of mosquito larvae collected from different breeding sites sampled.

Percentage distribution of adult mosquitoes emerged from larvae collected from breeding sites sampled.

Figure 17 shows the percentage distribution of seven mosquito species that were collected in the study. Out of seven species of mosquitoes collected as larvae, the mosquito species with highest percentage distribution was *Ae. aegypti* 23.70% while the least was *Ae. luteocephalus* 5.71%. Others were *Ae. albopictus* 23.36%, *Cx. quinquefasciatus* 16.78%, *An. gambiae* 13.50%, *Ae. simpsoni* 10.47% and *Cx. tigripes* 6.40% (See appendix 12). The error bars indicated that there was no significant difference ($p>0.05$) between the percentage distributions of *Ae. aegypti* and *Ae. albopictus* while there was significant difference ($p<0.05$) when the percentage distributions of *Ae. aegypti* and *Ae. albopictus* were compared with that of other mosquito species emerged from larvae.

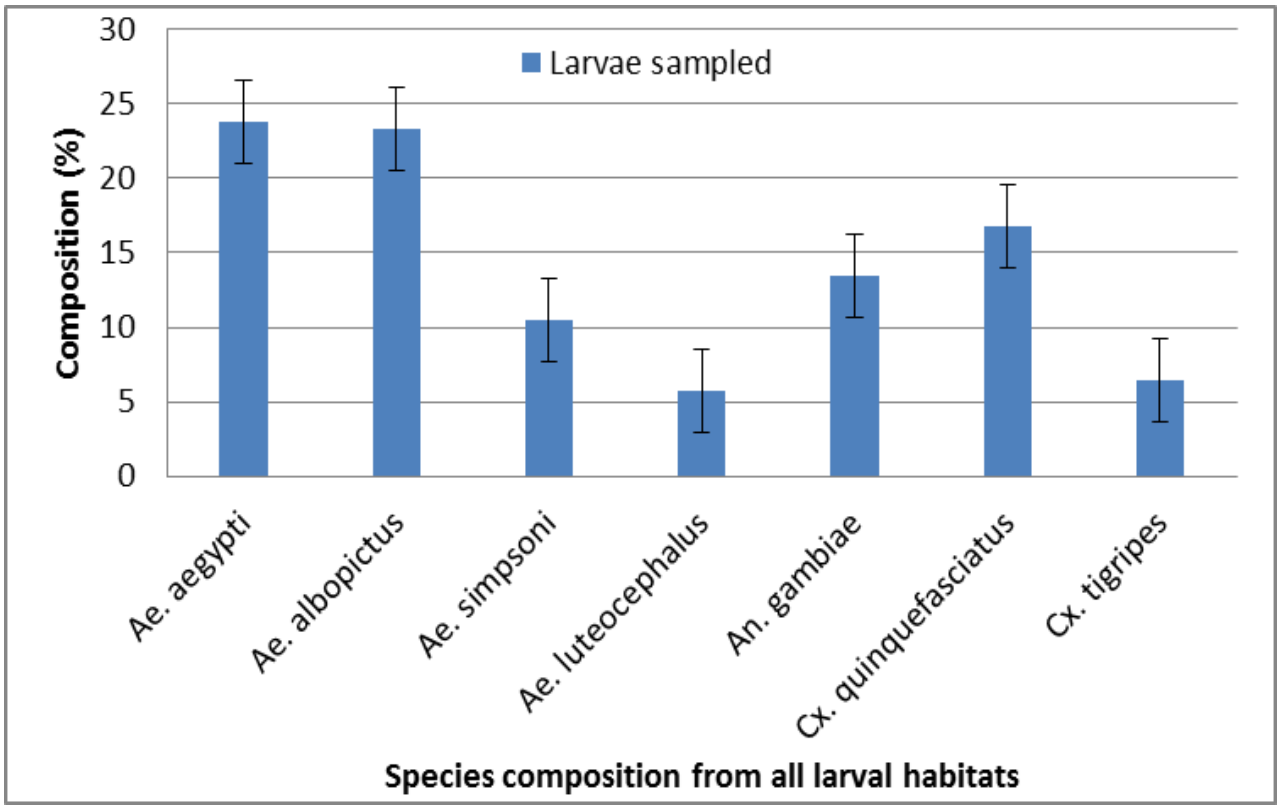

Figure 17: Percentage distribution of adult mosquitoes emerged from larvae collected from breeding sites sampled.

Percentage distribution of mosquito larvae collected from breeding sites in communities sampled

Figure 18 shows that out of seven different mosquito species emerged from larvae in different breeding sites sampled in eight communities, the highest collections 21.54% were made in Umudisi while the least collections 6.92% were made in Umuezopi. Others were Umuafa 20.59%, Umunakwa 11.76%, Umuonyeagolu 10.81%, Nkalafia 10.55%, Amakom and Ibolo 10.81% respectively (Appendix 13). *Ae. simpsoni* was only collected in Umudisi while *Ae. luteocephalus* and *Cx. tigripes* were collected only in Umuafa community. The error bars indicated significant difference at 5% level of probability, thus, there was no significant difference (P>0.05) between *An. gambiae* collected in the eight communities.

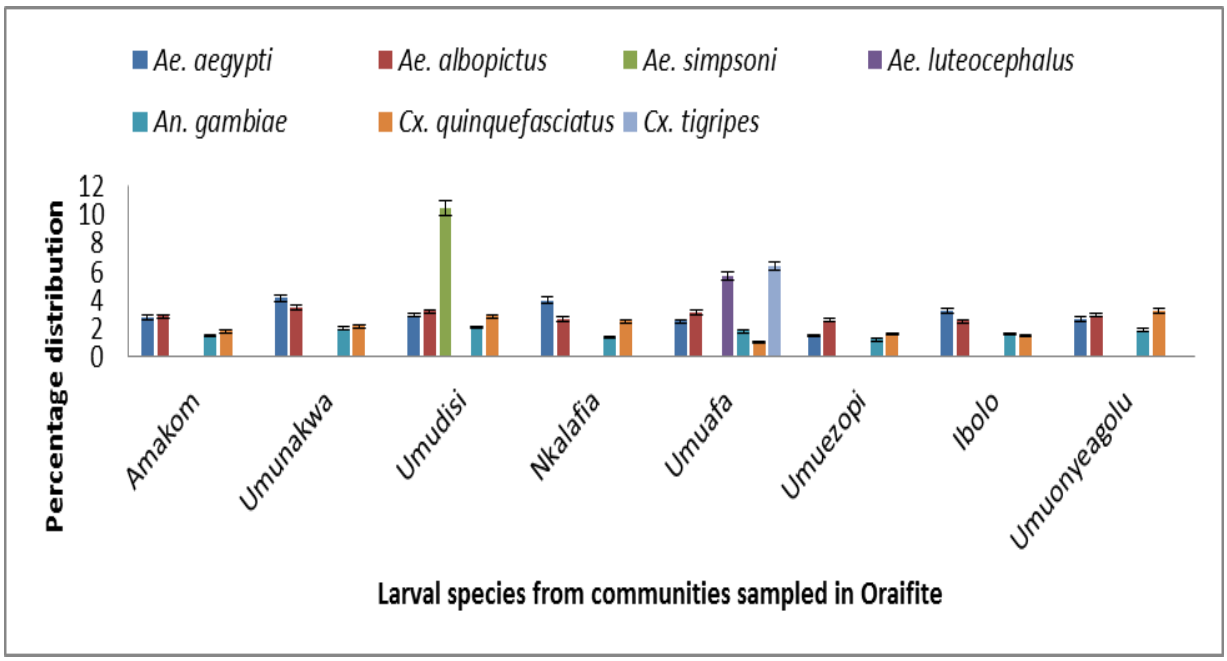

Figure 18: Percentage distribution of mosquito larvae collected from breeding sites in communities sampled.

Molecular Identification of Siblings species of *An. gambiae* complex by Polymerase Chain Reaction (PCR).

Figure 19 shows that out of 160 *An. gambiae* complex mosquitoes that was run on PCR for molecular identification, 125(78.1%) were amplified while 35(21.9%) were unamplified. All the amplified 125(78.1%) *An. gambiae* complex were identified as *An. gambiae* sensu stricto. There was significant difference ($P<0.05$) between amplified and unamplified *An. gambiae* complex, $P = 6.04*10^{-6}$ (See appendix 14).

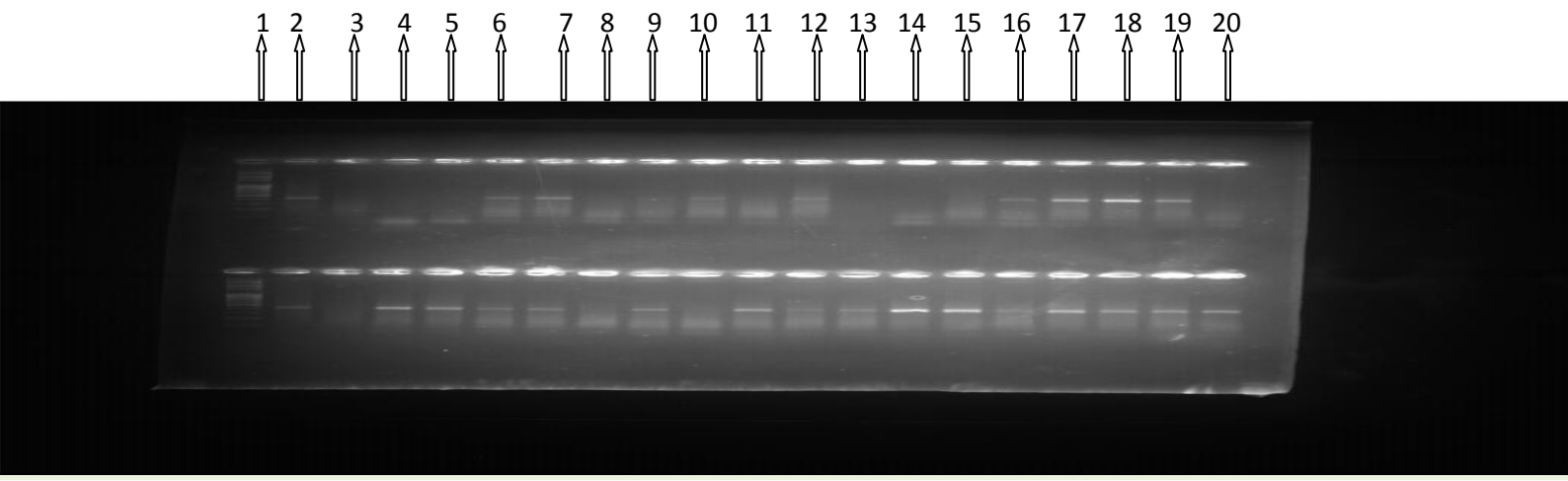

Figure 19: PCR product of Agarose gel electrophoresis for *An. gambiae* complex. Lane 1 is DNA ladder, lane 2 is positive control, lane 3 is negative control, lanes 4,5,6,7,9,11,12,13,14,15,16,17,18,19 and 20 were products of *An. gambiae* s.s while lanes 8 and 10 were unamplified (unidentified).

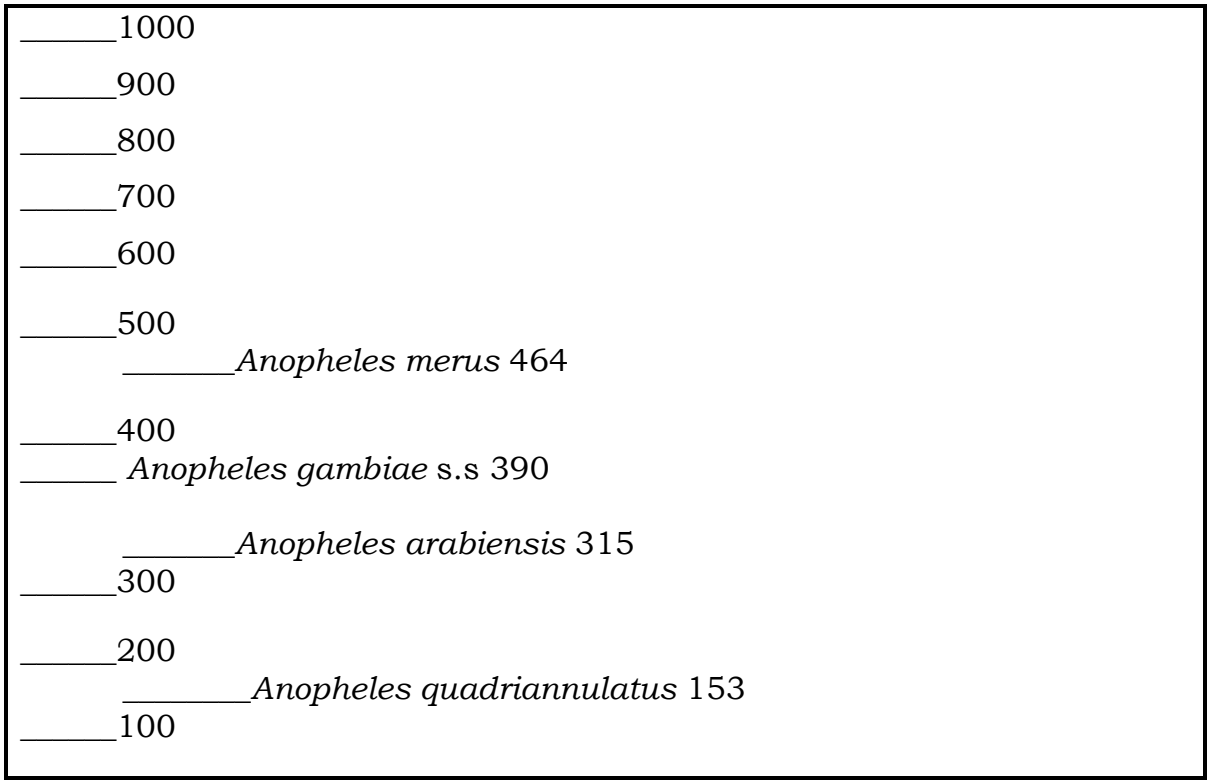

Figure 20: DNA ladder containing molecular weight of mosquitoes.
(Number 100 to 1000 represents the molecular weight of different mosquito species)

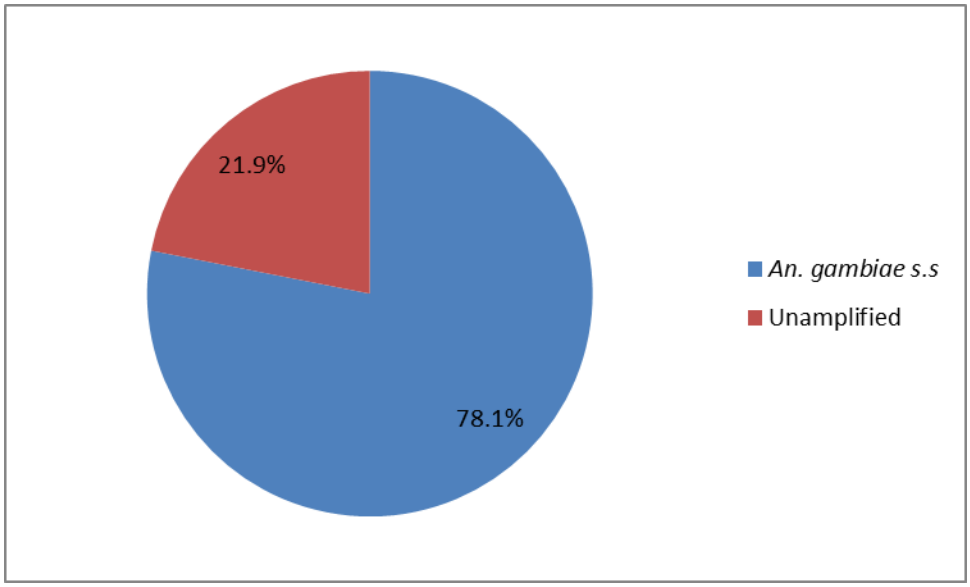

Figure 21: *Anopheles gambiae* s.s. mosquitoes as identified using Polymerase Chain Reaction (PCR) technique.

Amplified and unamplified *An. gambiae* complex in Oraifite Communities.

Figure 22 shows that out of 160 *An. gambiae* complex mosquitoes that was run on PCR for molecular identification, Umudisi had the highest number of amplified *An. gambaie* s.s 19(11.9%), followed by Umuonyeagolu and Nkalafia of number 18(11.3%) respectively. Umuafa had 17(10.6%), Ibolo had 16(10.0%), Umuezopi had 14(8.8%) and Amakom had 13 (8.1%) while the least was Umunakwa 9(5.6%).

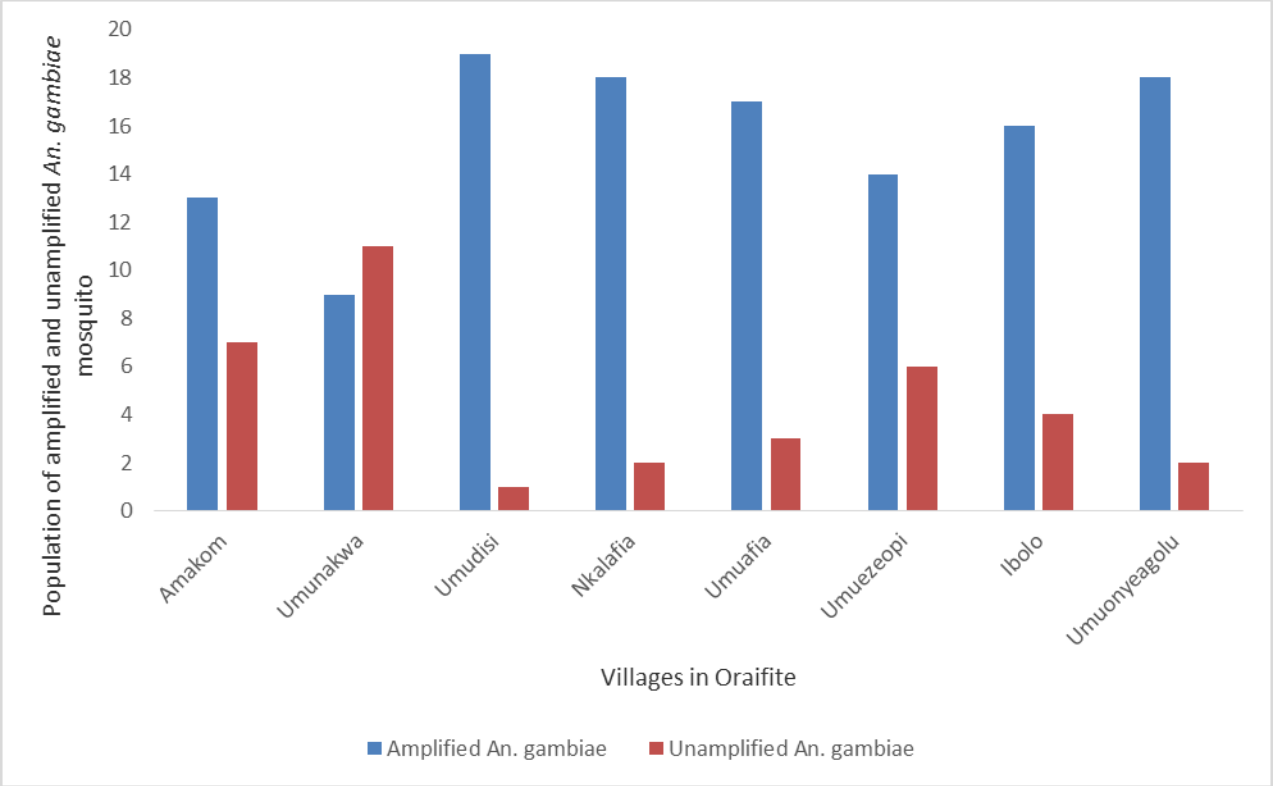

Figure 22: Chart showing amplified and unamplified *An. gambiae* complex in Oraifite Communities.

Index of species diversity and dominance index for all mosquito species

Of all the twenty species of mosquitoes collected in this study, *Ae. albopictus* had the highest diversity index of 0.140 while *Cq. maculipennis* had the least 0.0001 (Table 4). Others were *Ae. aegypti* 0.139, *Cx. quinquefasciatus* 0.136, *An. gambiae* 0.114, *An. funestus* 0.077, *Cx. decens* 0.061, *Ae. luteocephalus* 0.053, *Ae. simpsoni* 0.050, *Cx. poicilipes* 0.036, *Cx. tigripes* 0.035, *M. africana* 0.034, *Ae. africanus* 0.025, *M. uniformis* 0.023, *Cx. nebulosus* 0.015, *An. moucheti* 0.012, *Cx. annulioris* 0.005, *Ae. circumluteolus* 0.004, *Cx. nigripalpus* 0.002 and *Er. chrysogaster* 0.0012.

Ae. albopictus had the highest dominance index of 0.041 while *Cq. maculipennis* had the least 0.000000080. Others were *Ae. aegypti* 0.038, *Cx. quinquefasciatus* 0.034, *An. gambiae* 0.0163, *An. funestus* 0.0042, *Cx. decens* 0.020, *Ae. luteocephalus* 0.0014, *Ae. simpsoni* 0.0012, *Cx. poicilipes* 0.00046, *Cx. tigripes* 0.00044, *M. africana* 0.00042, *Ae. africanus* 0.00017, *M. uniformis* 0.00014, *Cx. nebulosus* 0.000043, *An. moucheti* 0.000029, *Cx. annulioris* 0.0000029, *Ae. circumluteolus* 0.0000012, *Cx. nigripalpus* 0.00000032 and *Er. chrysogaster* 0.000026.

There was significant difference ($P<0.05$, $P=1.89*10^{-07}$) between mosquito species collected from eight communities in Oraifite. The significant difference in number occurred when the *Ae. aegypti* was compared with all other species except *Ae. albopictus* and *Cx. quinquefasciatus* (See appendix 15e).

Table 4: Species diversity and dominance indices for all mosquitoes collected in Oraifite, Ekwusigo Local Government Area.

Mosquito species	Shannon-Wiener diversity index (H)	Simpson's dominance index (C)
Ae. aegypti	0.139	0.038
Ae. albopictus	0.140	0.041
Ae. africanus	0.025	0.00017
Ae. circumluteolus	0.004	0.0000012
Ae. luteocephalus	0.053	0.0014
Ae. simpsoni	0.050	0.0012
An. gambiae	0.114	0.0163
An. funestus	0.077	0.0042
Cx. decens	0.061	0.0020
Cx. nebulosus	0.015	0.000043
Cx. poicilipes	0.036	0.00046
Cx. quinquefasciatus	0.136	0.034
Cx. annulioris	0.005	0.0000029
Cx. nigripalpus	0.002	0.00000032
Cx. tigripes	0.035	0.00044
Cq. maculipennis	0.0001	0.000000080
Er. chrysogaster	0.0012	0.000026
M africana	0.034	0.00042
M. uniformis	0.023	0.00014
An. moucheti	0.012	0.000029
Total	**H = 0.973**	**C = 0.287**

Significance level: ($P<0.05$), P = 1.89E-07.

Comparison between diversity and dominance indices of mosquito species sampled in Oraifite.

Transformed data was used to compare between diversity and dominance indices of mosquito species sampled in Oraifite. In Fig. 23, Umuafa had the highest mosquitoes diversity index of 1.0411 while Umuezopi had the least mosquito diversity index of 1.0287 (See appendix 15d). There was significant difference ($P<0.05$) between the diversity index of Umuafa and other communities sampled. Umuonyeagolu had the highest dominance index of mosquito species of 1.0086 while Umudisi had the least dominance index of 1.0054. There was significant difference ($P<0.05$) in diversity and dominance indices of mosquito species sampled in Oraifite.

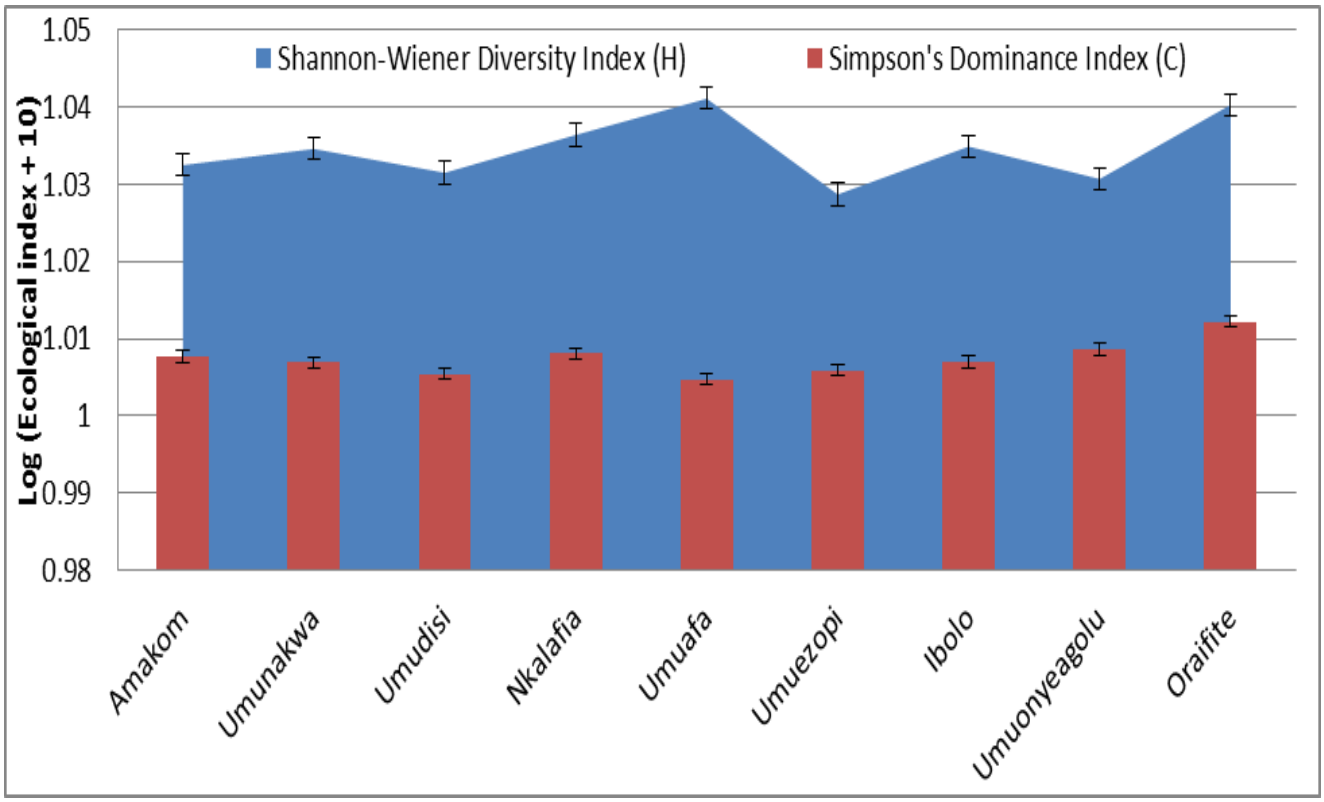

Figure 23: Index of species diversity and dominance index for all mosquitoes collected.

Determination of the ecological factors that influence the survival of mosquitoes in their breeding sites.

Table 5 shows the correlation coefficients (r) of *Ae aegypti, Ae. albopictus, Cx. quinquefasciatus, Cx. tigripes* abundance and the physicochemical conditions of man- made container breeding sites (See appendix 16 - 22). *Ae. aegypti* larvae abundance correlated positively and highly significant with sulphate ($p<0.05$; $r = 0.628$), dissolved oxygen ($p<0.01$; $r = 0.806$) and surface water temperature ($p<0.05$; $r = 0.634$) in man-made containers. *Ae. aegypti* abundance larvae had weak positive relationship and not significant with salinity and total suspended solids ($p>0.05$; $r = 0.268$ and 0.253 respectively). *Ae. aegypti* population correlated positively with pH and negatively with total dissolved solids without significance ($p>0.05$; $r = 0.533$ and -0.068).

Ae. albopictus larvae abundance correlated positively and not significant with pH, sulphate and surface water temperature ($p>0.05$; $r = 0.461$, 0.555 and 0.554) in man-made containers. *Ae. albopictus* larvae abundance correlated positively and highly significant with dissolved oxygen ($p<0.01$; $r = 0.781$). It had a weak positive relationship with salinity and total suspended solids ($p>0.05$; $r = 0.188$ and 0.188 respectively) and weak negative relationship with total dissolved solids ($r = -0.059$).

Cx. quinquefasciatus larvae abundance correlated positively and highly significant with pH ($P<0.01$; $r = 0.763$), salinity ($P<0.05$; $r = 0.600$), total suspended solids ($p<0.05$; $r = 0.596$), dissolved oxygen ($p<0.01$; $r = 0.763$), sulphate ($P<0.01$; $r = 0.773$) and surface water temperature ($p<0.01$; $r = 0.795$) in man-made containers. It had a weak negative relationship with total dissolved solids ($r = -0.177$).

Abundance of *Cx. tigripes* larvae correlated positively and not significant with pH, sulphate and surface water temperature ($p>0.05$; $r = 0.403$, 0.483 and 0.501) in ground pools. *Cx. tigripes* larvae abundance correlated

positively and highly significant with dissolved oxygen ($p<0.01$; $r = 0.765$). It had a weak positive relationship with salinity and total suspended solids ($p>0.05$; $r = 0.096$ and 0.098) and weak negative relationship with total dissolved solids ($r = -0.298$).

Table 5: Correlation coefficients (r) on relationship between mosquito larvae and physicochemical parameters in man-made containers breeding sites in Oraifite.

Mosquito species	pH	Salinity	Total suspended solid	Total dissolved solid	Dissolved oxygen	Sulphate	Surface water temperature
Aedes aegypti	0.533	0.268	0.253	-0.068	0.806**	0.628*	0.634*
Aedes albopictus	0.461	0.188	0.188	-0.059	0.781**	0.555	0.554
Culex quinquefasciatus	0.763**	0.600*	0.596*	-0.177	0.763**	0.773**	0.795**
Culex tigripes	0.403	0.096	0.098	-0.298	0.765**	0.483	0.501

****** Correlation is significant at the 0.01 level (2-tailed).

***** Correlation is significant at the 0.05 level (2-tailed) (See appendix 16 to 22).

Correlation coefficients (r) on relationship between mosquito larvae and physicochemical parameters in ground pools breeding sites in Oraifite.

In table 6, the correlation coefficients (r) of *Ae. aegypti, Ae. albopictus, An. gambiae, Cx. quinquefasciatus*, *Cx. tigripes* and the physicochemical conditions of the ground pools breeding sites were shown (See appendix 22 - 29). *Ae. aegypti* larvae abundance correlated positively and highly significant with pH ($p<0.05$; $r = 0.658$), dissolved oxygen ($p<0.01$; $r = 0.784$) and surface water temperature ($p<0.05$; $r = 0.583$) in ground pools. *Ae. aegypti* larvae abundance had weak positive relationship and not significant with sulphate and total dissolved solids ($p>0.05$; $r = 0.167$ and 0.083 respectively). *Ae. aegypti* larvae population correlated positively with salinity and total suspended solids without significant effect ($p>0.05$; $r = 0.430$ and 0.419).

Ae. albopictus larvae abundance correlated positively and not significant with salinity and total suspended solids ($p>0.05$; $r = 0.445$ and 0.429) in ground pools. *Ae. albopictus* larvae abundance correlated positively and highly significant with pH ($p<0.05$; $r = 0.658$), dissolved oxygen ($p<0.01$; $r = 0.741$) and surface water temperature ($p<0.05$; $r = 0.589$). It had a weak positive relationship with sulphate and total dissolved solids without significance ($p>0.05$; $r = 0.221$ and 0.109 respectively).

An. gambiae larvae abundance correlates positively and not significantly with pH and surface water temperature ($p>0.05$; $r = 0.549$ and 0.450) in ground pools. *An. gambiae* larvae abundance correlates positively and highly significantly with dissolved oxygen ($p<0.01$; $r = 0.771$). It had a weak positive relationship with salinity, total suspended solids and sulphate without significance ($p>0.05$; $r = 0.284$, 0.331 and 0.082) while it correlates weakly and negatively with total dissolved solids (-0.075).

Cx. quinquefasciatus larvae population correlated positively and highly significant with pH ($P<0.01$; $r = 0.812$), salinity ($P<0.05$; $r = 0.624$), total suspended solids ($p<0.05$; $r = 0.606$), dissolved oxygen ($p<0.01$; $r = 0.833$)

and surface water temperature ($p<0.01$; $r = 0.754$) in ground pools. It had a weak positive relationship with total dissolved solids and sulphate ($r = 0.268$ and 0.348) in ground pools.

Abundance of *Cx. tigripes* larvae correlated positively and not significant with pH ($p>0.05$; $r = 0.489$) ground pools. *Cx. tigripes* larvae population correlated positively and highly significant with dissolved oxygen ($p<0.01$; $r = 0.741$). It had a weak positive relationship with salinity ($r = 0.234$), total suspended solids ($r = 0.281$), total dissolved solids ($r = 0.096$), sulphate ($r = 0.040$) and surface water temperature ($r = 0.096$).

Table 6: Correlation coefficients (r) on relationship between mosquito larvae and physicochemical parameters in ground pools breeding sites in Oraifite.

Mosquito larvae	pH	Salinity	Total suspended solid	Total dissolved solid	Dissolved oxygen	Sulphate	Surface water temperature
Aedes aegypti	0.658*	0.430	0. 419	0.083	0.784**	0.167	0.583*
Aedes albopictus	0.658*	0.445	0. 429	0.109	0.741**	0.221	0.589*
Anopheles gambiae	0.549	0.284	0.331	-0.075	0.771**	0.082	0.450
Culex quinquefasciatus	0.812**	0. 624*	0. 606*	0.268	0.833**	0.348	0.754**
Culex tigripes	0.489	0.234	0.281	0.096	0.741**	0.040	0.395

****** Correlation is significant at the 0.01 level (2-tailed).

***** Correlation is significant at the 0.05 level (2-tailed) (See appendix 22 to 29).

Mean physicochemical parameters of mosquito larvae breeding sites in Oraifite communities.

The physicochemical parameters of mosquito larvae breeding sites in Oraifite ranged from pH 4.70-6.37, salinity 58.33-98.31(mg/l), total suspended solid 10.0-30.0(mg/l), total dissolved solid 200-1870(mg/l), dissolved oxygen 2.26-7.83(mg/l), sulphate 281.51-811.53(mg/l) and surface water temperature 25.5-29.5(^{o}C) (See appendix 30).

From table 7 below, the mean±se of pH, salinity, total suspended solids, total dissolved solids, dissolved oxygen, sulphate and surface water temperature of water collected from man-made containers and ground pools were determined. The pH levels of the mosquito larvae breeding sites varied from 5.343±0.108 to 5.47±0.139. The highest value was collected from man-made containers and the lowest from ground pools. The salinity content of the mosquito breeding sites ranged from 62.695±5.1989mg/l - 75.361±3.4915mg/l. The mean total suspended solids (TSS) concentration in the mosquito breeding waters ranged from 23.329±1.5736mg/l - 23.336±2.1075mg/l. In addition, the mean total dissolved solids (TDS) concentration of the water varied from 600±154.0mg/l 683±132.5mg/l from the various sites. The dissolved oxygen (DO) content of the mosquito breeding sites ranged from 4.609±0.556mg/l - 5±0.445mg/l. The sulphate content of the mosquito breeding sites ranged from 603.88±63.896mg/l - 771.441±18.047mg/l. The mean temperature of surface water from various mosquito breeding sites in Oraifite varied from 26.9±0.21^{0}C to 28.19±0.31^{0}C respectively. The lowest value was collected from man-made containers and the highest from ground pools. The physicochemical parameters collected from man-made containers and ground pools breeding sites had no significant difference (P>0.05), P=0.952852.

Table 7: Mean physicochemical parameters of mosquito larvae breeding sites in Oraifite

PARAMETERS	BREEDING SITES	
	MAN-MADE CONTAINERS MEAN±SE	GROUND POOLS MEAN±SE
pH	5.47±0.139	5.343±0.108
Salinity (mg/l)	62.695±5.1989	75.361±3.4915
TSS(mg/l)	23.336±2.1075	23.329±1.5736
TDS(mg/l)	600±154.0	683±132.5
DO(mg/l)	5±0.45	4.609±0.556
Sulphate (mg/l)	771.441±18.047	603.879±63.896
Surface water temperature (^{0}C)	26.9±0.207	28.19±0.314

Relationship between mosquito abundance and temperature, relative humidity and rainfall.

The response of indoor biting adult mosquito collections to temperature**,** relative humidity and rainfall in the study area was observed (Figures 24-26). Correlation (relationship) between indoor biting mosquitoes and temperature was strongly negative (r = -0.87643) (See appendix 31). The R^2 value (0.7681) still showed the extent of dependency of indoor mosquito population on temperature. The equation showed linear model relationship between temperature and indoor mosquito population.

Correlation between indoor biting mosquito population and relative humidity was strongly positive (r = 0.883495) (See appendix 31). The R^2 value (0.7806) still showed the extent of dependency of indoor mosquito population on relative humidity. The equation showed linear model relationship between relative humidity and indoor mosquito population.

Correlation between indoor biting population and rainfall was strongly positive (r = 0.911659) (See appendix 31). The R^2 value (0.8311) still showed the extent of dependency of indoor mosquito population on rainfall. The equation showed linear model relationship between rainfall and indoor mosquito population.

In appendix 31, the ANOVA table showed that these climatic factors have significant effects ($p<0.05$) on indoor mosquito population.

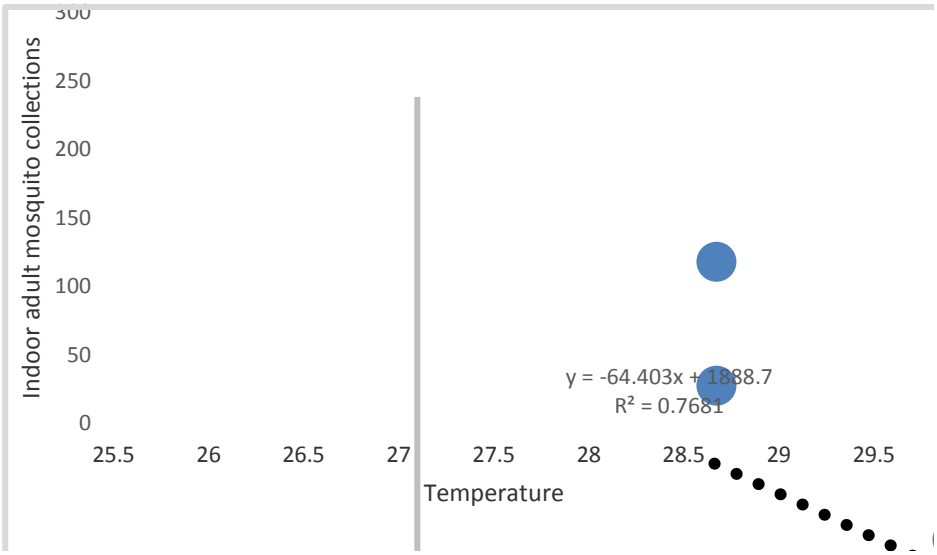

Figure 24: Scatter plot showing level of correlation between indoor biting mosquito population and temperature.

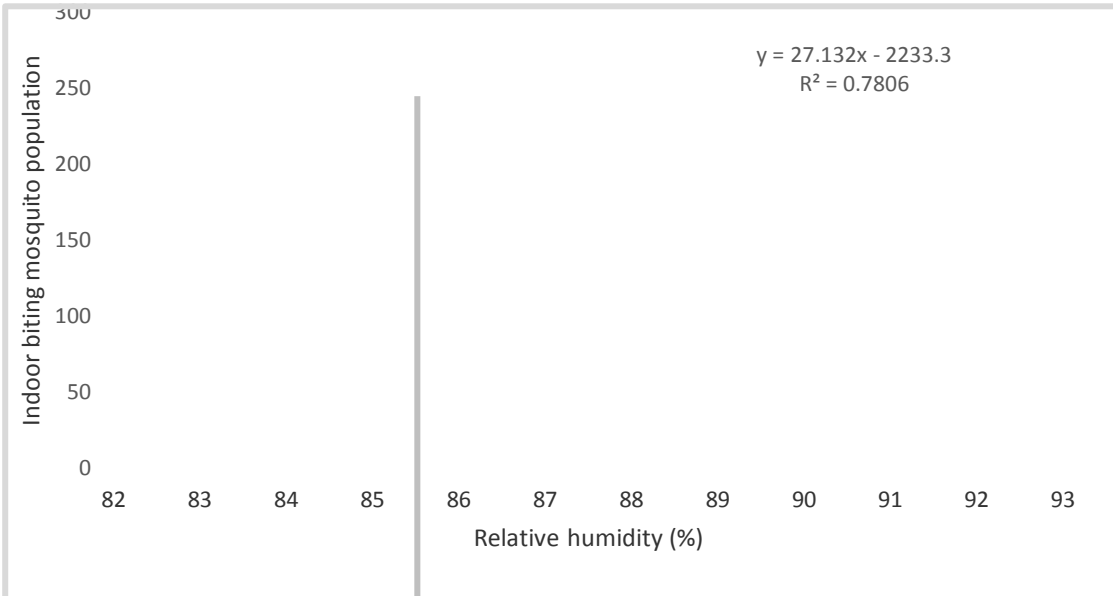

Figure 25: Scatter plot showing level of correlation between indoor biting mosquito population and relative humidity.

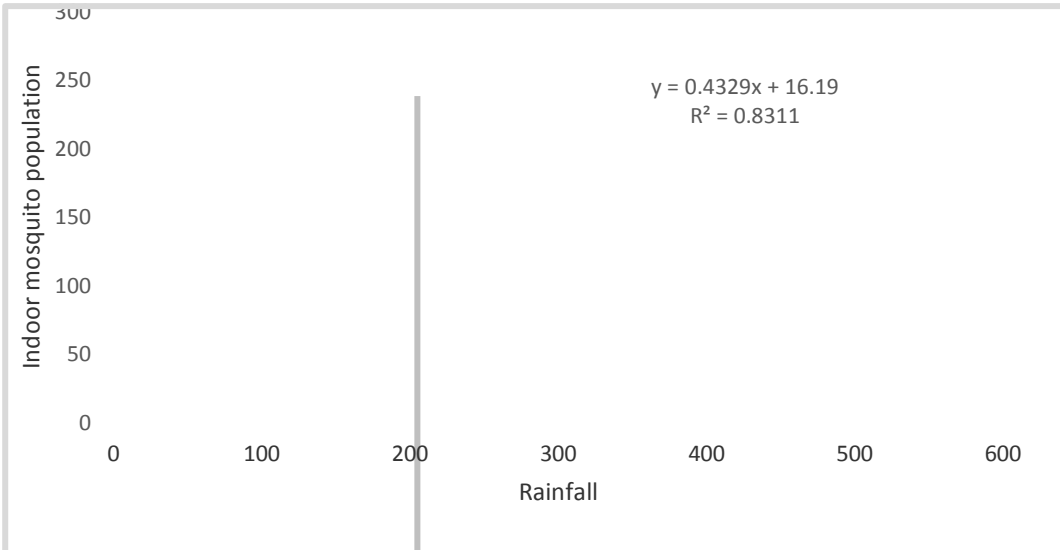

Figure 26: Scatter plot showing level of correlation between indoor biting mosquito population and rainfall.

The response of outdoor biting adult mosquito collections to temperature, relative humidity and rainfall in the study.

The response of indoor biting adult mosquito collections to temperature**,** relative humidity and rainfall in the study area was observed (Figures 27-29). Correlation (relationship) between indoor biting mosquitoes and temperature was strongly negative (r = -0.8978) (See appendix 32). The R^2 value (0.806) still showed the extent of dependency of outdoor mosquito population on temperature. The equation showed linear model relationship between temperature and outdoor mosquito population.

Correlation between indoor biting mosquito population and relative humidity was strongly positive (r = 0.885085) (See appendix 32). The R^2 value (0.7834) still showed the extent of dependency of outdoor mosquito population on relative humidity. The equation showed a linear model relationship between relative humidity and outdoor mosquito population.

Correlation between indoor biting mosquito population and rainfall was strongly positive (r = 0.936896) (See appendix 32). The R^2 value (0.8778) still showed the extent of dependency of outdoor mosquito population on rainfall. The equation showed a linear model relationship between rainfall and outdoor mosquito population.

In appendix 32, the ANOVA table shows that these climatic factors have significant effects ($p<0.05$) on outdoor mosquito population.

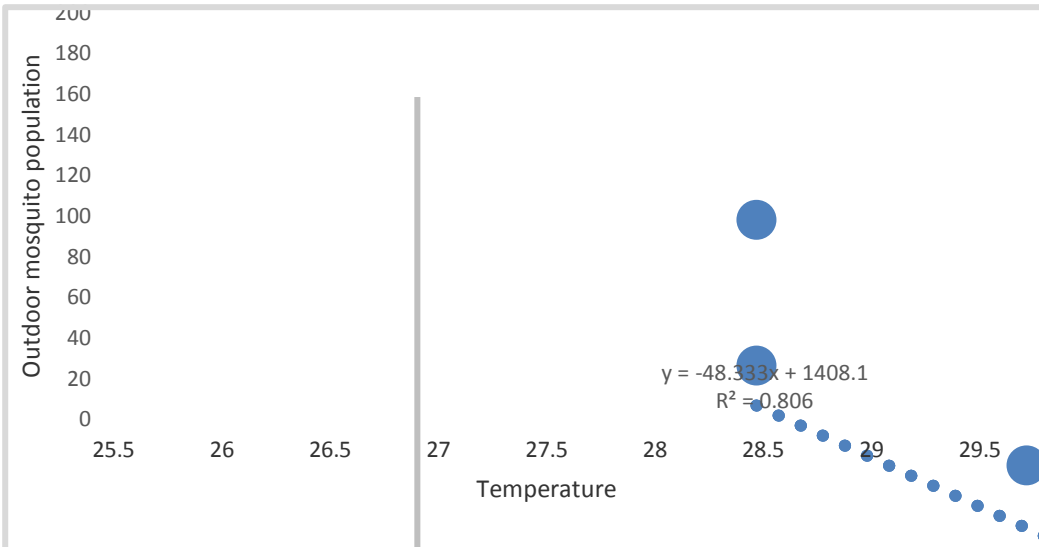

Figure 27: Scatter plot showing level of correlation between outdoor biting mosquito population and temperature.

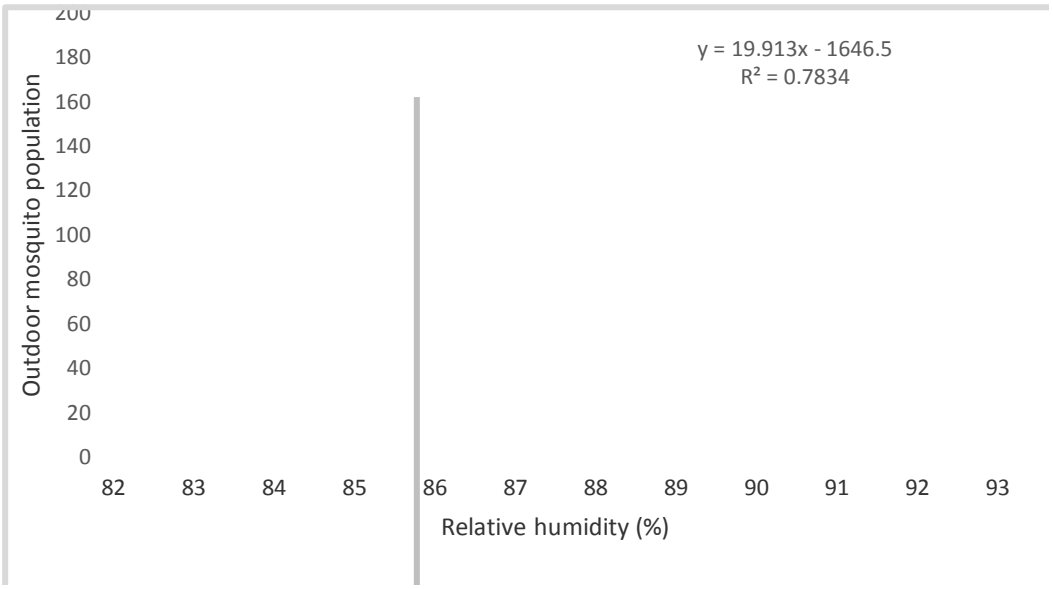

Figure 28: Scatter plot showing level of correlation between outdoor biting mosquito population and relative humidity.

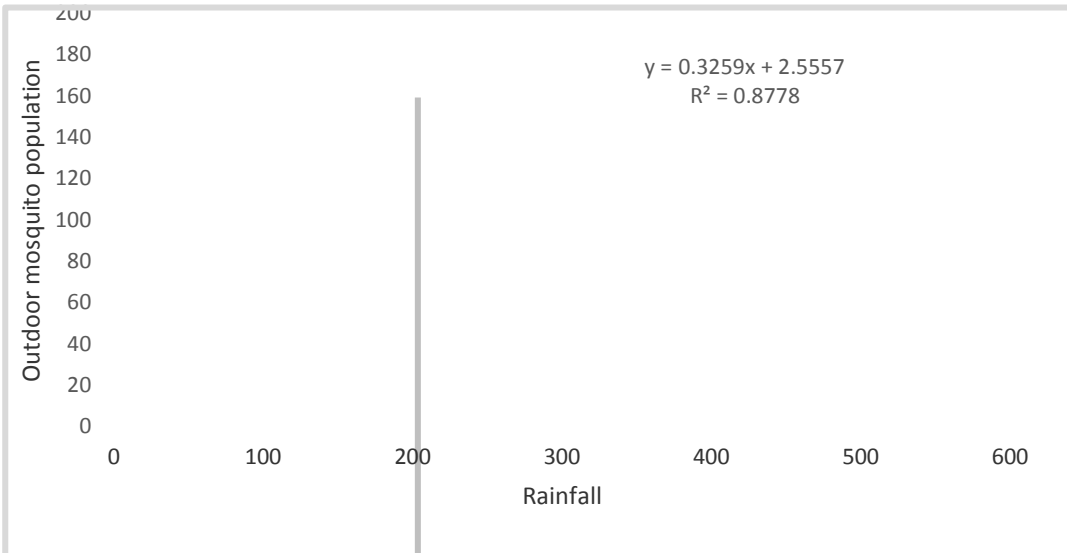

Figure 29: Scatter plot showing level of correlation between outdoor biting mosquito population and rainfall.

Seasonal variations in climatic factors during indoor and outdoor mosquito collection.

Table 8 shows the seasonal variations in temperature, relative humidity and rainfall. During dry season, the average indoor and outdoor collections were 30.5 and 10.5, respectively. During wet season, the average indoor and outdoor collections were 162 and 113.75, respectively. The average temperature was higher during dry season (28.53^{0}C) than in wet season (26.98^{0}C). Thus, as mosquito population increases, temperature decreases. The average relative humidity and rainfall was higher during wet season (87.88% and 325.9mm) than in dry season (84.25% and 55mm). Therefore, mosquito population inceases as relative humidity and rainfall increase. There was significant difference ($P<0.05$, 1.24E-06) between indoor and outdoor mosquito population collected in the communities (Appendix 11c)

Table 8: Seasonal variations in climatic factors during indoor and outdoor collections.

Seasons		Indoor collection	Outdoor collection	Temperature °C	Relative humidity %	Rainfall Mm
Dry season	Dec	77	18	28.2	86	32
	Jan	14	12	28.2	84	21
	Feb	9	0	29.1	83	34
	Mar	22	12	28.6	84	133
	Total	**122**	**42**	**114.1**	**337**	**220**
	Average	**30.5**	**10.5**	**28.53**	**84.25**	**55**
Wet season	Apr	110	58	28.6	85	208
	May	107	85	28.1	85	221
	June	112	96	26.4	87	300
	July	262	158	26.0	89	438
	Aug	197	142	26.4	92	506
	Sep	233	181	26.0	90	491
	Oct	163	96	26.6	88	305
	Nov	112	94	27.7	87	138
	Total	**1296**	**910**	**215.8**	**703**	**2607**
	average	**162**	**113.75**	**26.98**	**87.88**	**325.9**

CHAPTER FIVE

DISCUSSION

A total of 1418 indoor biting adult mosquitoes were collected using pyrethrium knock-down collection (PKC) method (Table 2). Of the total number, the highest 16.85% were collected in Umuafa while the least 7.68% were from Amakom. This could be due to differences in the management of the environment by the inhabiting population of the areas. Umuafa community had many mosquito breeding sites like ground pools (ponds, stagnant water) and man-made containers (discarded tins, clay pots, drums, plastic buckets) compared to other communities. These breeding sites were not much in Amakom especially man-made containers due to proper disposal by the inhabitants. This is in line with the findings of Simon-Oke *et al*. (2012) that mosquito distribution and abundance are related to population, land-use and human activities in Ekiti State, Nigeria.

A total of twelve species formed the 1418 mosquitoes collected indoors. The mosquito species included *Anopheles gambiae*, *Coquillettidia maculipennis*, *Culex quinquefasciatus*, *Anopheles funestus*, *Aedes albopictus*, *Aedes aegypti*, *Culex decens Mansonia africana*, *Culex poicilipes*, *Mansonia uniformis*, *Anopheles moucheti* and *Culex nebulosus*. The occurrence of these species was due to the presence of ground pools, man-made containers and plant axils near the houses where they breed before entering the rooms to feed on human blood. This agrees with the observations in a study at Awka North L.G.A. of Anambra State, where Aribodor *et al.* (2016) reported that the availability of indoor mosquito species was due to the presence of ground water pools, domestic containers, plant axils and bushes around the houses where they breed and readily fly into houses to rest and feed on humans. *An. gambiae* was the highest collected indoor mosquito species. The observation here corroborates with the findings of Aribodor (2012) in Nimo, Anambra State, Nigeria that *Anopheles* species were the most abundant indoor mosquitoes. It also agrees with Onyido *et al.* (2016) in

Anambra State, Nigeria, that *An. gambiae* s. l. has been reported as the main malaria vector (Onyido *et al.*, 2011; 2014) and the predominating species among the indoor biting mosquitoes. The abundance of *An. gambiae* in Oraifite was as a result of suitable environmental and climatic breeding conditions which favour the breeding of larvae of the vectors. These breeding conditions include suitable levels of physicochemical parameters that increased *An. gambiae* abundance in their breeding sites. This observation confirms the findings of Amaechi *et al.* (2014) in a study at Asa-Obingwu, a rural community in Abia State South-Eastern Nigeria, that high indices of *Anopheles gambiae* in Asa-Obingwu was due to the close proximity of residential areas to their farm lands which was most often water logged area. Therefore, the high abundance of indoor man-biting mosquitoes observed in this study could pose a potential danger for epidemics if a mosquito-borne disease is introduced in the area. Presently, malaria is the only disease prevalent in Oraifite but immigrants from disease endemic areas can introduce other mosquito-borne diseases like yellow fever and filariasis in the study area.

The gonotrophic stages of adult mosquitoes collected indoors (Figure 6) showed that greater number of the mosquitoes was blood fed, indicating that a good number of the mosquitoes have had contact with human host. This is in line with the findings of Savage *et al.* (1992) in Continental Africa who reported high number of bloodfed mosquitoes and that chances exist that the infected ones have transmitted parasites and viruses to susceptible human victims during bites. Of the mosquito species collected indoors, *An. gambiae* had the highest percentage of freshly fed mosquito species (17.07%). This observation showed that *An. gambiae* are highly anthropophilic and endophilic in nature and its high quest for bloodmeal could lead to high malaria transmission in an endemic area.

The study on indoor resting density and man-biting rate of different mosquito species in the study area showed that *An. gambiae* had the highest indoor resting density and man-biting rate of 0.51 and 0.20 while

Cq. maculipennis had the least of 0.002 and 0.0008 respectively. This observation conforms with the report of Ezihe *et al.* (2017a) who demonstrated in a study at Ahani-Achi East, Enugu State that mosquito density per room suggests that one *Anopheles* species might have fed on each occupant of the house per night. Ezihe *et al.* (2017a) further suggested that presence of *An. gambiae* sl and *An. funestus* complex in a room serves as a threat to public health because they are very efficient malaria transmitters. *An. gambiae* which was the most dominant indoor adult mosquitoes in the study area agrees with the report of Okwa *et al.* (2007) who observed that *An. gambiae* as the most abundant mosquito vector in Badagry, Lagos State. This study showed that IRD had significant effect ($p<0.05$) on MBR because, increase in the number of IRD of *An. gambiae* resulted in increase in the number of MBR of *An. gambiae*. The biting rates of *An. gambiae* are enough to intensify the spread of malaria in Oraifite and can jeopardize the malaria elimination efforts of the Ministry of health which includes provision of long lasting Insecticide Treated Net (ITN).

A total of 952 outdoor biting mosquitoes were collected outdoors using human bait collection method (HBC) from the eight communities (Fig. 11). Nkalafia had the highest percentage distribution (17.12%) and Umunakwa had the least collection (9.24%). In this study, communities with increased rapid infrastructural development, social and human activities have increased relative abundance of mosquito species. The present study showed that *Anopheles* species was not collected outdoors in the evening hours. This observation agrees with the report of Aribodor (2012) that *Anopheles* species bite at midnight and not mainly in the twilight hours because they are nocturnal feeders.

Fourteen mosquito species made up the 952 mosquitoes collected outdoors in Oraifite. The mosquito species included; *Ae. albopictus*, *Ae. aegypti*, *Cx. quinquefasciatus*, *Ae. luteocephalus*, *Cx. decens*, *Ae. africanus*, *Cx. poicilipes*, *M. africana*, *M. uniformis*, *Erytmapodite chrysogaster*, *Cx. nebulosus*, *Cx.*

annulioris, Culex nigripalpus and *Ae. circumluteolus.* Some of these mosquito species were reported by Onyido *et al.* (2008) in Jos North-central, Nigeria; Oguoma and Ikpeze (2008) in Gewaza, North-Central Nigeria; Okogun *et al.* (2005) in mid-Western Nigeria; Onyido *et al.* (2009a) in Enugu Municipality, South-Eastern Nigeria and Anosike *et al.* (2007) in Imo state, South-Eastern Nigeria. The outdoor mosquito species collected in Oraifite are of public health importance and vectors of mosquito-borne diseases. *Ae. albopictus* which was most abundant outdoor biting species in the study area has been reported as a vector of yellow fever, dengue fever, zikka virus and other arboviruses (Service, 1980). This suggests that a trace of yellow fever, dengue fever, zikka virus and other mosquito-borne diseases vectored by *Ae. albopictus* would result in an outbreak in Oraifite community. Baud *et al.* (2017) reported Nigeria as one of the African countries that encountered zikka virus outbreak while Nigeria Centre for Disease Control (2019) reported outbreaks of yellow fever in the 36 States and Federal Capital Territory (FCT). Ayukekbong (2014) reported that dengue fever is endemic in Nigeria with sero-prevalence of about 73% in some areas.

The mosquito species collected outdoors had their different biting peaks. The outdoor biting mosquito collections using Human Bait Collection method was done from 4.30pm – 7.30pm. There was a distinct difference in the peak periods of activity of individual mosquito species in Oraifite. The highest percentage of mosquitoes was collected between the intervals of 7.15 - 7.30pm while the least was at 4.30-4.45pm. This observation disagrees with the findings of Umeanaeto *et al.* (2016) at Okpatu, Enugu State that the highest percentage of mosquitoes was collected between the hours of 4.45pm to 5.00pm and between 6.00pm to 6.15pm using Human Bait Catch (HBC) method. *Ae. albopictus* which was the most abundant *Aedes* species had its biting peak from 7.00pm – 7.15pm. The high percentage of *Ae. albopictus* indicates that Oraifite communities are at risk of yellow fever, dengue fever and other mosquito-borne diseases *Ae. albopictus* transmits. *Cx. quinquefasciatus* was the most abundant and the only *Culex* mosquitoes

collected outdoors at dusk from 4.30pm – 7.30pm. It had its biting peak from 7.15pm – 7.30pm. *Mansonia* and *Eretmapodites* were collected between 5.00pm to 7.15pm. The implication of this finding is that the occupants of Oraifite could be at risk of lymphatic filariasis which *Cx. quinquefasciatus* transmits. The *Culex* and *Mansonia* mosquitoes particularly *Culex quinquefasciatus* and *Mansonia uniformis* are very important transmitters of filarial worms especially *Wuchereria bancrofti* which causes lymphatic filariasis, elephantiasis, as well as transmit various forms of viral encephalitis in Africa (Onyido *et al.,* 2009b). WHO (2011) reported that the burden of lymphatic filariasis is heaviest in Nigeriria, Democratic Republic of Congo, India, Indonesia and Bangladesh.

The mosquito genera collected in Oraifite included *Aedes, Anophele, Culex, Coquillettidia, Mansonia* and *Eretmapodites.* The implication of the presence of these vectors is that Oraifite is at risk of mosquito-borne diseases. Ae*des* are vectors of yellow fever and other arboviral diseases; *Culex* mosquitoes are vectors of bancroftian filariasis while *Anopheles mosquitoes* transmit malaria and filariasis in Nigeria (Onyido *et al.,* 2009b). *Mansonia* transmit Rift valley virus in some parts of East Africa (Sang *et al.,* 2010). *Coquillettidia* and *Eretmapodites* mosquitoes were not known to transmit any disease in Nigeria though they transmit eastern equine encephalitis virus (EEEV) in North America (Onyido *et al.,* 2008). *Mansonia* transmit Rift valley disease in some parts of East Africa (Sang *et al.,* 2010). Monthly percentage distribution of all mosquito genera collected in the study showed that *Aedes* mosquitoes which had the highest percentage distribution in the month of September (9.11%) was the most abundance in Oraifite (Table 3). This explains why Oraifite inhabitants are at great potential risk of yellow fever since female *Aedes* mosquitoes are known to transmit yellow fever and other mosquito-borne diseases. Unlike the present observations, *Anopheles* mosquitoes were the most abundant genera in a study at Ilokun and Irasa communities in Ado-Ekiti, Nigeria (Olorunniyi, 2016). This could be as a result of favourable environmental conditions for *Anopheles* mosquito

oviposition and survival in their breeding sites which led to increase in adult mosquito population in Ilokun and Irasa communities.

Seasonal percentage distribution of all mosquito genera shows that *Culex* mosquitoes had the highest percentage distribution in dry season while *Aedes* mosquitoes had the highest percentage distribution in wet season (Figure 14). *Aedes* and *Culex* mosquito were the most abundant genera collected in Oraifite. This is in agreement with observation made in Enugu metropolis (Onyido *et al.,* 2010), Awka metropolis (Mbanugo and Okpalaononuju, 2003) and in Midwestern Nigeria (Okogun *et al.*, 2005) where *Aedes* and *Culex* mosquitoes were reported as the most abundant mosquito species. However, it contrasted with the findings from Katstina state Nigeria (Bunza *et al.*, 2010) and North-central Nigeria (Oguoma and Ikpeze, 2008) where *Anopheles* species were reported as the most abundant mosquito species. The abundance of *Aedes* and *Culex* mosquito in the eight communities in Oraifite shows that they are very versatile and highly adapted to different types of environment in the study area. The percentage distribution of mosquito genera was very high in wet season and low in dry season, thus disease transmission by the observed mosquito genera would be at peak in wet season in Oraifite**.**

The seasonal percentage distribution of all adult mosquito species collected both indoors and outdoors in this study showed that adult mosquito population was high in wet season (93%) and very low during dry season (7%). In dry and wet seasons, indoor biting and resting mosquitoes collected by PKC method had the percentage distribution of 5.2% and 54.6% respectively while the outdoor biting mosquitoes collected by HBC method had the percentage distribution of 1.8% and 38.4% (Figure 16). Indoor biting and resting mosquito population was higher than outdoor biting mosquito population in both wet and dry seasons. At the beginning of wet season, mosquito breeding sites were few and dried up easily but in mid wet season, the breeding sites increased, providing more sites for mosquito oviposition

and thereby increasing the larval abundance in the breeding sites. At the beginning of dry season, the condition of mosquito breeding sites were the same with early wet season, few breeding sites were seen while in mid dry season, temporal breeding sites of mosquitoes became dry due to either no or little rainfall but natural breeding sites still remain. It was observed that wet season favours mosquito distribution more than dry season because, high percentage of mosquito species were distributed in wet season than in dry season due to increased mosquito breeding sites. The present observation agrees with the studies on seasonal distributions of mosquitoes in Nigeria (Alaribe *et al.,* Amaechi *et al.,* 2014; 2002; Oduola *et al.*, 2013; Onyido *et al.*, 2014; Suleiman, 2012) noted lower numbers of mosquitoes during the dry seasons. The findings here agrees with Reudal *et al.* (2010) who observed the increase in *Anopheles* mosquito population across all seasons of the year with peak in rainy season and least population in the dry season in Korea Republic. It disagrees with the findings of Olayemi (2012) who reported high prevalence of malaria, and intense mosquito breeding activities in Minna during the dry season because of non-climatic drivers such as human behaviour.

Monthly percentage distribution of indoor and outdoor adult mosquitoes collected in the study area showed that indoor biting and resting mosquitoes were collected from January (2017) to December (2017) while outdoor biting mosquitoes were collected in all months except in February, the peak of dry season, when temporal mosquito breeding sites were eliminated due to harsh weather, caused by low rainfall, relative humidity and high temperature in the study area. Indoor biting and resting mosquitoes had highest percentage distribution in July (11.05%) and the least in February (0.38%). Outdoor biting mosquitoes had highest percentage distribution in September (7.64%) and the least in January (0.51%) and March (0.51%) respectively. This implies that the indoor environment seems to encourage mosquito survival all through the months, unlike outdoor environment where high temperature and low rainfall disrupt mosquito breeding sites.

Mosquito species use different natural and artificial water collection for oviposition and larval breeding. These breeding sites were numerous in Oraifite. This was due to various human activities, increased number of construction sites, poor sanitation level and indiscriminate disposal of discarded household materials. It resulted to abundance of man-made containers of varying sizes and ground pools. This observation is in line with the findings of Hriber *et al.* (2001) and Preechaporn *et al.* (2007) that reported that mosquito species use ceramic vessels, metal vessels, plastic and metal water barrels and concrete water tanks for oviposition and breeding. Oraifite also lies in the tropical rainforest area of Nigeria, an area that has been described by Onyido *et al.* (2010) as conducive for the breeding and survival of mosquitoes of different species due to its high annual rainfall and biodiversity.

Seven species of mosquitoes which included; *Ae. aegypti*, *Ae. luteocephalus*, *Ae. albopictus*, *Cx. quinquefasciatus*, *An. gambiae*, *Ae. simpsoni,* and *Cx. tigripes* consisted the 1156 emerged mosquitoes collected as larvae from different breeding sites in Oraifite. The present observation agrees with Ezihe *et al.* (2017b) that reported seven species of mosquitoes in a study at Enugu Municipal, Enugu State. *Ae. aegypti* and *Ae. albopictus* were collected from man-made containers, ground pools and plant axils. *Ae. luteocephalus* was collected only in man-made containers. *An. gambiae* was collected only in ground pools. *Cx. quinquefasciatus* and *Cx. tigripes* were both collected in man-made containers and ground pools while *Ae. simpsoni* was collected in man-made containers and plant axils. The collection of mosquitoes from different breeding sites suggests that the physicochemical properties of the bleeding sites were favourable for larval development. This corroborates with the report of Onyido *et al.* (2009a) that mosquitoes can take advantage of any little collection of water within their reach to establish a habitat. *An. gambiae* was collected only in ground pools. This finding conforms to the report of Egbuche *et al.* (2016) of the preference of *Anopheles* species being selective in their oviposition sites as they prefer to

breed in highly oxygenated open drains and ground pools in Agulueri, Nigeria. However, *Ae. aegypti* was the highest adult mosquito emerged from larvae in Oraifite. This was due to its ability to breed in any available water collection especially man made containers.

All the 1,156 mosquitoes collected as larvae were from eight communities in Oraifite. The high mosquito number may be as a result of the large-scaled infrastructural development and other anthropogenic activities in the study area which resulted in proliferation of different conducive mosquito breeding sites. Umeanaeto *et al.* (2016) had observed that the intensive breeding of mosquitoes at Okpatu, Enugu State was as a result of human activities that led to the littering of the environment with all sorts of containers serving as mosquito breeding sites. The observed differences in the number of mosquito populations collected in the different communities shows that mosquito larvae were not evenly distributed in all the communities, similar to the findings of Mbanugo and Okpalaononuju (2003) that mosquito larvae are likely to be more in communities with many breeding sites such as water in discarded plastics and tin cans, bottles, disposable cups, vehicle tyres and ground water pools.

Out of 160 *An. gambiae* complex mosquitoes that were run on PCR for molecular identification, 125(78.1%) were amplified while 35(22.0%) were not. All amplified *An. gambiae* complex were identified as *An. gambiae sensu stricto*. This is an indication that *An. gambiae* s. s. would be the primary vector of malaria parasite in Oraifite.

Simpson's dominant index [*C*] and Shannon-Wiener index of diversity [*H*] revealed that *Ae. albopictus* was the dominant species (*C* = 0.041) with the highest index of diversity (*H* = 0.140) (Table 4). *Ae. albopictus* were collected in large numbers, biting during the evening hours and were observed to breed in any available container in the study area. Comparison between diversity and dominance indices of mosquito species sampled in Oraifite

shows that Umuafa had the highest mosquitoes diversity index of 1.0411 and Umuonyeagolu had the highest dominance index of mosquitoes species of 1.0086 (Figure 23). At a nearby community of Oba, it was observed (Okonkwo *et al.*, 2014) that Umumpamma-Aborji village had Shannon-Wiener index of diversity of 0.703 while Isu-Umuabu village had Simpson's dominant index of 0.265; but in far-a-away Taraba State Nigeria, Lamidi *et al.* (2017) noted that Bali had Shannon-Wiener index of diversity of 0.8753 while Ardo-Kola had Simpson's dominant index of 0.4587.

There were observed relationships between mosquito larvae abundance and the physicochemical parameters of water in man–made containers and ground pools in Oraifite (Tables 5 and 6). In man-made containers, water pH correlates positively with *Ae. aegypti, Ae. albopictus, Cx. quinquefasciatus* and *Cx. tigripes* but pH only had significant relationship with *Cx. quinquefasciatus* ($P<0.01$; $r = 0.763$). Thus, *Cx. quinquefasciatus* larvae abundance increases with increase in pH. In ground pools, water pH correlates positively with *Ae. aegypti, Ae. albopictus, An, gambiae, Cx. quinquefasciatus* and *Cx. tigripes* but it only had significant relationship with *Ae. aegypti* ($p<0.05$; $r = 0.658$), *Ae. albopictus* ($p<0.05$; $r = 0.658$) and *Cx. quinquefasciatus* ($P<0.01$; $r = 0.763$). However, larvae abundance of *Ae. aegypti, Ae. albopictus* and *Cx. quinquefasciatus* increases in water of pH 4.70-5.94. The pH levels of the mosquito larvae breeding sites in Oraifite varied from 5.343±0.108 and 5.47±0.139. Unlike this present study, Tiimub *et al.* (2012) reported mean pH levels varied between 7.77 ± 0.01 and10.70 ± 0.01 in breeding sites sampled at the Obuasi Municipality in Ghana. According to Kwasi *et al.* (2012) in a study at *the* Obuasi municipality in Ghana, pH of mosquito breeding sites below 4.5 or above 10 results to mortalities of mosquito larvae. However, the pH of mosquito breeding sites in Oraifite was favourable for mosquito and they were within the range stated by Kwasi *et al.* (2012). The present study observed that pH 4.70-5.94 had significant effect on *Aedes aegypti* abundance. This observation is in line with the report of Clark *et al.* (2004) that pH value of 4.0 has no

significant effect on the growth and development of *Ae. aegypti* but disagrees with the findings of Umar and Don-pedro (2008) that the survival of *Ae. aegypti* occurred mainly between pH 6.5 and 8.0.

Salinity and total suspended solids had weak positive relationship with larvae abundance but significantly influence *Cx. quinquefasciatus* larvae abundance in both breeding sites. A study at Omor on effect of physicochemical parameters on mosquito abundance in rice fields reported that mosquito abundance is negatively associated with pH, sulphate and TSS (r= -0.071; p= 0.72), thus correlation analysis revealed that abundance of mosquitoes decreases with pH, sulphate and TSS (Ukonze *et al.*, 2017). The present study which observed that pH, sulphate and total suspended solids have positive relationship with larvae abundance in man–made containers and ground pools is not in agreement with the findings of Ukonze *et al.* (2017) in rice fields at Omor, Anambra State. This could be attributed to differences in breeding sites sampled for larvae. In this study, the mean salinity content of the mosquito breeding sites ranged from 62.695±5.199mg/l - 75.361±3.492mg/l while the mean total suspended solids (TSS) ranged from 23.329±1.574mg/l - 23.336±2.108mg/l. In line with the present study, Tiimub *et al.* (2012) reported that mean total suspended solids (TSS) ranged from 17.03 ± 4.04mg/l - 96.67 ± 4.04mg/l.

Total dissolved solids had a weak positive and negative not significant relationship with larvae abundance in man-made container and ground pools breeding sites. Thus, increase or decrease in total dissolved solids had no influence on larvae abundance in Oraifite. It is in agreement with the findings of Musonda and Sichilima (2019) in a study at Kapiri Mposhi District of Zambia that a correlation between total dissolved solids and larvae abundance (r=0.018 p=0.663) was not significant, thus, total dissolved solids have insignificant relationship with the abundance of mosquito larvae in various breeding sites. The mean total dissolved solids (TDS) varied from 600±154.0mg/l and 683±132.5mg/l. Unlike this present

study, Tiimub *et al.* (2012) reported mean total dissolved solids (TDS) varied between 1.09±3.23mg/l - 35.67±3.23mg/l in breeding sites sampled at the Obuasi Municipality in Ghana

Dissolved oxygen correlated positively and highly significant (P<0.01) with all larvae abundance in both breeding sites. Dissolved oxygen had great influence on mosquito larvae abundance thus, larvae abundance increases with increase in dissolved oxygen. In this study, dissolved oxygen was the main parameter responsible for larval growth and development because it correlated positively with high significant effect (P<0.01) on larvae abundance. Dissolved oxygen (DO) ranged from 4.609±0.556mg/l - 5±0.445mg/l, however, the DO requirement of the mosquito breeding sites were generally less than 10mg/l at the breeding sites studied in oraifite. This trend revealed that the habitats were generally favourable sites for prolific breeding of the mosquitoes. In line with the present study, Tiimub *et al.* (2012) reported that mean dissolved oxygen (DO) ranged from 3.97 ± 0.13mg/l - 7.43 ± 0.13mg/l increases mosquito larvae abundance.

In man-made containers breeding sites**,** sulphate and surface water temperature correlated positively with all larvae abundance but they only have significant effects only on *Ae aegypti* and *Cx. quinquefasciatus.* In ground pools breeding sites**,** sulphate had a positive no significant relationship with larvae abundance while surface water temperature correlated positively and significantly with all larvae abundance except *An. gambiae* and *Cx. tigripes.* This observation disagrees with the report of Ezihe *et al.* (2017b) that mosquito larvae abundance had strong positive and significant correlation with temperature (P<0.05, r=0.5) in a study area in Enugu Municipal, Enugu State.

In this study, abundance *of Ae. aegypti*, *Ae. albopictus, An. gambiae, Cx. quinquefasciatus and Cx. tigripes* have strong positive significant relationship (P<0.05) with dissolved oxygen, pH, sulphate and surface water temperature in the breeding sites. These findings contradict the

observations reported from Kenya by Emidi *et al.* (2017) indicating that none of the physicochemical parameters studied (pH, salinity, temperature, conductivity and total dissolved solids) was found to be significantly associated with the presence of *Culex* mosquitoes. However, increase in dissolved oxygen, pH, sulphate and surface water temperature increased the abundance of larvae species. This concurs with the observation of Mutero *et al.* (2004) that various physicochemical characteristics of the larval habitat observed in gutters, peri-domestic runoff and domestic containers including pH, optimum temperature, total suspended solids, total dissolved solids and electrical conductivity have been found to affect larval development and survival. It further agrees with the report of Oyewole *et al.* (2009) that physicochemical parameters such as temperature, salinity, conductivity, total dissolved solids and pH have significant influence on mosquito larval abundance.

The mean temperature of water from various mosquito breeding sites in Oraifite varied from 26.9±0.21^{0}C to 28.19±0.31^{0}C, respectively. Thus, the mean temperature of mosquito breeding sites observed in this study concurs with the range of 16 -32^{0}C specified by Bradley and Kutz (2006) at the US EPA as the best for the breeding of most mosquito species in the tropics. The result of this study corroborates with the findings of Oyewole *et al.* (2009) that factor such as optimum temperature (27.7^{0}C - 30.1^{0}C) might have provided conducive environment for survival and breeding activity of the *Anopheline* species. Unlike the present study, the mean temperature of water from various mosquito breeding sites sampled at the Obuasi Municipality in Ghana varied between 17.03 ± 0.18^{0}C and 24.06 ± 0.18^{0}C (Tiimub *et al.*, 2012). It could be as a result of differences in locations and environment. In line with the findings of Garba and Olayemi (2015), temperature was highest in ground pools that were usually fully exposed to the sun. The mean physicochemical parameters collected from man-made containers and ground pools breeding sites had no significant difference (P>0.05), P=0.952852.

All adult mosquitoes were collected within the temperature ranges of 26.0^{o}C – 29.1^{o}C. Mean atmospheric temperature values obtained in Oraifite during this study were suitable for the breeding, growth and survival of mosquitoes. This result agrees with the reports of Manyi *et al.* (2015), Githeko *et al.* (2000) and Service (2012) that optimum temperature of 29.1^{0}C helps in the development and hatching of mosquito eggs. This observation signifies increased transmission of the malaria, lymphatic filarial parasites and other mosquito-borne diseases in Oraifite. In line with the findings of Service (2012), the high temperature recorded in the present study would mean enhanced reproductive process in the mosquito vector populations in Oraifite. The study has shown that mosquito population increases as temperature decreases. In the present study, temperature had favourably enhanced the population of the vector species across the eight communities throughout the study period. Temperature had a strong negative correlation with indoor and outdoor mosquito population. Thus, the population of indoor and outdoor mosquitoes decreased as temperature increased.

The relative humidity values in Oraifite ranged from 83% to 92% with lowest values recorded from January, 2017 to March, 2017 (dry season) and higher values were recorded from July to October, 2017 (wet season). The records showed that relative humidity and rainfall have similar pattern of effects on the mosquito populations in the study area. Mosquito population increases with increase in relative humidity. This is similar with the reports of Githeko *et al.* (2000) and Service (2012). The seasonality trend observed in terms of the relative abundance of mosquitoes in this study conforms to the reports of Manyi *et al.* (2015) in Makurdi and two other studies in Southern Nigeria (Uttah and Uttah, 2009; Uttah *et al.*, 2013). This present result also agrees with the report of Mwangangi *et al.* (2009) in the Eastern part of Kenya. Meanwhile, annual rainfall and the corresponding relative humidity were observed to have proportional effects on the mosquito vector population in the area (i.e. the more the number of mosquitoes, the higher the amount of

rainfall and relative humidity). Relative humidity had a strong positive correlation with indoor and outdoor mosquito population. Thus, the population of indoor and outdoor mosquitoes increases as relative humidity increases. This in line with the observations of Olayemi (2011) that higher relative humidity of almost 60% indoors correlated strongly with indoor mosquito resting density (i.e., endophily), daily survival and adult longevity in North-Central Nigeria. This disagrees with the findings of Opayele *et al.* (2017) that fluctuations in relative humidity were not significantly correlated with mosquito abundance except for *Mansonia* species whose population increased with increased relative humidity in Ibadan, Nigeria.

In this study, the highest mosquito abundance did not occur in the month (August) with the highest amount of rainfall. This may be due to the effect of excess rainfalls resulting into flooding that could wash off mosquito larval habitats before their complete development. This is in agreement with the findings of Opayele *et al.* (2017) that local mosquito population decreases due to high rainfall that results to flood, thereby washing away mosquito larvae from their breeding sites in Ibadan, Nigeria. A sharp increase in mosquito population was recorded in the month following the month with high rainfall (September). This was because, mosquito larvae have the opportunity to complete their development since flooding stopped. Rainfall had a strong positive correlation with indoor and outdoor mosquito population. Thus, the population of indoor and outdoor mosquitoes increases as rainfall increases. This is in line with the observations of Manyi *et al.* (2015) that there was a strong positive correlation ($P<0.05$) between the mosquito populations and rainfall throughout the study period. The findings of this study conforms to Reudal *et al.* (2010) that reported the increase in mosquito population across all seasons of the year with peak in rainy season and least population in the dry season in Republic of Korea. The present observation is not in line with the report of Olayemi *et al.* (2014) that mosquito population development and intensity of disease transmission in North-Central Nigeria may not be so heavily dependent on rainfall, but

perhaps equally influenced by non-climatic factors, such as human behaviour.

Correlation on the effect of climatic factors on indoor and outdoor mosquito population shows that these temperature, relative humidity and rainfall have significant effects on indoor and outdoor mosquito population ($p<0.05$). The present study agrees with the report of Sutherst (2004) who observed that climate is an important determining factor in the distribution of vectors. This is contrary to the findings of Masaninga *et al.* (2012) who reported that temperature, rainfall and relative humidity played no significant roles in the mosquito distribution in their study area in an urban setting in Zambia. It is a general consensus among several researchers (Bradshaw *et al.*, 2000; Koenraadt *et al.*, 2004; Stoops *et al.*, 2007; Akram *et al.*, 2009) that abiotic factors of temperature, relative humidity, altitude and rainfall play a vital role in mosquito development which in turn influences their population density. Therefore, it is evident that malaria and other mosquito-borne disease cases are likely to increase during the wet season in Oraifite.

CONCLUSION

This study has revealed that mosquitoes in its large number are biting the inhabitants of Oraifite community. Also, that different species of mosquitoes belonging to different genera such as *Aedes*, *Culex*, *Anopheles*, *Mansonia, Eretmapodites* and *Coquillettidia* mosquitoes exist and bite man in Oraifite. The implication of the presence of these vectors is that Oraifite community is now at great potential risk of mosquito-borne disease out-breaks. Therefore, strict surveillance and control of mosquitoes are needed to prevent the outbreak of mosquito-borne diseases in Oraifite.

PCR on *An. gambiae* complex showed that *An. gambiae* s.s. is the main malaria vector in Oraifite communities.

The availability of many breeding sites of mosquitoes in the community which included man-made containers, ground pools and plant axils have resulted to the increase in larval population and indiscriminate biting of mosquitoes both indoors and outdoors in the area.

The physicochemical parameters (pH, salinity, total suspended solid, sulphate and surface water temperature) have relationships with larval abundance but dissolved oxygen had strong positive significant ($P<0.01$) relationship with all larval abundance while total dissolved solids had no significant ($p>0.05$) effect on mosquito larvae abundance.

There was highly significant ($P<0.05$) relationship between indoor and outdoor mosquito population and temperature, relative humidity and rainfall.

Recommendations

Further studies should be conducted on mosquito species distribution and the prevalence of the mosquito-borne diseases in Oraifite.

Government should continue to provide information to the community on eliminating artificially created mosquito breeding sites, the diseases mosquitoes transmit and personal protection measures against mosquito bite.

Further studies and systematic reviews with proper generalization on the distribution of mosquito larvae and physicochemical factors are required in the study area.

Strict surveillance and control of mosquitoes by the Government and inhabitants are required to improve environmental sanitation and prevent the outbreak of mosquito-borne diseases in Oraifite.

Contribution to knowledge

The study on mosquito bionomics and distribution of mosquitoes has not been reported in Oraifite, Anambra State. The result of the present studies would therefore, be useful in evidence-based policy making on surveillance and control of mosquito vectors in the study area. The result includes;

Aedes simpsoni which is a known vector of yellow fever virus was collected only at Umudisi. Umudisi has forest and stream where Monkeys, the intermediate hosts of yellow fever virus were said to be damaging crops. The inhabitants that visit the forest for any purpose might get bitten by infected mosquitoes. Therefore, there is need for vaccination of people in the area to prevent an outbreak of yellow fever virus.

PCR on *An. gambiae* complex showed that *An. gambiae* s.s. is the main malaria vector in Oraifite communities.

Dissolved oxygen significantly increased all larval abundance while total dissolved solids had no significant effect on mosquito larvae abundance in mosquito breeding sites in Oraifite.

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APPENDICES

Appendix 1: Global Positioning System (GPS) Data collected from the study Area (See fig. 2)

S/N	Location	Latitude	Longitude
1	Amakom	6.029008	6.859268
2	Umunakwa	6.030860	6.834042
3	Umudisi	5.997330	6.861527
4	Nkalafia	6.004097	6.861143
5	Umuafa	6.000612	6.844212
6	Umuezopi	6.011113	6.827545
7	Ibolo	6.022367	6.817198
8	Umuonyeagolu	5.995197	6.832913

Appendix 2a: Percentage distributions of indoor-biting and resting mosquitoes sampled in Oraifite communities

	Amakom	Umunakwa	Umudisi	Nkalafia	Umuafa	Umuezopi	Ibolo	Umuonyeagolu	Total (%)
Percentage of Indoor Mosquitoes sampled by PKC	7.68	11.28	12.9	14.17	16.85	12.48	13.33	11.28	99.97

Table 2b: Percentage of species composition of Indoor-biting and resting mosquito sampled in Oraifite communities

Species	(%)
Ae. aegypti	12.7
Ae. albopictus	14.25
An gambiae	20.73
An. funestus	16.08
Cx. decens	6.7
Cx. nebulosus	0.85
Cx. poicilipes	3.17
Cx. quinquefasciatus	19.1
Cq. maculipennis	0.07
An. moucheti	1.34
M. africana	3.46
M. uniformis	1.55
Total (%)	100

Appendix 4: Analysis of variance for mosquito species collected indoors in the eight villages in Oraifite from Jan-Dec, 2017(See table 2).

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Ae. aegypti	8	180	22.5	42.57143
Ae. albopictus	8	202	25.25	144.2143
An. gambiae	8	294	36.75	79.64286
An. funestus	8	228	28.5	141.7143
Cx. decens	8	95	11.875	212.6964
Cx. nebulosus	8	12	1.5	18
Cx. poicilipes	8	45	5.625	111.9821
Cx. quinquefasciatus	8	271	33.875	66.41071
Cq. maculipennis	8	1	0.125	0.125
An. moucheti	8	19	2.375	17.69643
M. africana	8	49	6.125	30.125
M. uniformis	8	22	2.75	27.07143

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	16493.21	11	1499.383	20.16541	3.38E-19	1.904539
Within Groups	6245.75	84	74.35417			
Total	22738.96	95				

POST HOC analysis of above table

t-Test: Two-Sample Assuming Equal Variances

	An. gambiae	*Cx. nebulosus*
Mean	36.75	1.5
Variance	79.64286	18
Observations	8	8
Pooled Variance	48.82143	
Hypothesized Mean Difference	0	
Df	14	
t Stat	10.08983	

P(T<=t) one-tail	4.18E-08
t Critical one-tail	1.76131
P(T<=t) two-tail	8.36E-08
t Critical two-tail	2.144787

t-Test: Two-Sample Assuming Equal Variances

	An. gambiae	*Cq. maculipennis*
Mean	36.75	0.125
Variance	79.64286	0.125
Observations	8	8
Pooled Variance	39.88393	
Hypothesized Mean Difference	0	
Df	14	
t Stat	11.59868	
P(T<=t) one-tail	7.23E-09	
t Critical one-tail	1.76131	
P(T<=t) two-tail	1.45E-08	
t Critical two-tail	2.144787	

t-Test: Two-Sample Assuming Equal Variances

	An. Gambiae	*An. moucheti*
Mean	36.75	2.375
Variance	79.64286	17.69643
Observations	8	8
Pooled Variance	48.66964	
Hypothesized Mean Difference	0	
Df	14	
t Stat	9.854705	
P(T<=t) one-tail	5.6E-08	
t Critical one-tail	1.76131	
P(T<=t) two-tail	1.12E-07	
t Critical two-tail	2.144787	

Appendix 3a: Gonotrophic stages of Adult mosquitoes collected indoors using Pyrethroid Knockdown Collection (PKC) Method.

Mosquito species	Unfed	Freshly fed	Half gravid	Gravid	Total (%)
Ae. aegypti	9	146	14	11	180(12.70)
Ae.albopictus	13	155	16	18	202(14.25)
An. gambiae	21	242	19	12	294(20.73)
An. funestus	16	176	17	19	228(16.08)
Cx. decens	7	76	9	3	95(6.70)
Cx. nebulosus	1	9	1	1	12(0.85)
Cx. poicilipes	3	35	4	3	45(3.17)
Cx. quinque fasciatus	14	229	16	12	271(19.11)
Cq. maculipennis	0	1	0	0	1(0.07)
An. moucheti	4	11	3	1	19(1.34)
M. africana	3	36	6	4	49(3.46)
M. uniformis	4	13	3	2	22(1.55)
Total(%)	**95(6.70)**	**1129(79.62)**	**108(7.62)**	**86(6.06)**	**1418(100)**

Appendix 3b: Percentage composition of gonotrophic stages of indoor-biting and resting species

Adult mosquito species	Unfed	Freshly fed	Half gravid	Gravid	Total (%)
Ae. aegypti	0.63	10.3	0.99	0.78	12.7
Ae. albopictus	0.92	10.93	1.13	1.27	14.25
An gambiae	1.48	17.07	1.34	0.85	20.74
An. funestus	1.13	12.41	1.20	1.34	16.08
Cx. decens	0.49	5.36	0.63	0.21	6.69
Cx. nebulosus	0.07	0.63	0.07	0.07	0.84
Cx. poicilipes	0.21	2.47	0.28	0.21	3.17
Cx. quinquefasciatus	0.99	16.15	1.13	0.84	19.11
Cq. maculipennis	0.0	0.07	0.0	0.0	0.07
An. moucheti	0.28	0.78	0.21	0.07	1.34
M. africana	0.21	2.54	0.42	0.28	3.45
M. uniformis	0.28	0.92	0.21	0.14	1.55
Total (%)	6.69	79.63	7.61	6.06	99.99

Appendix 3c: ANOVA comparing the population of mosquitoes in the various gonotrophic stages.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Unfed	12	95	7.916667	44.62879
Freshly fed	12	1129	94.08333	8160.992
Half gravid	12	108	9	48.90909
Gravid	12	86	7.166667	47.06061

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	66670.42	3	22223.47	10.70805	2.08E-05	2.816466
Within Groups	91317.5	44	2075.398			
Total	157987.9	47				

POSTHOC analysis

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Unfed	12	95	7.916667	44.62879
Half gravid	12	108	9	48.90909
Gravid	12	86	7.166667	47.06061

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	20.38889	2	10.19444	0.217522	0.805654	3.284918
Within Groups	1546.583	33	46.86616			
Total	1566.972	35				

Appendix 4a: Calculation of Indoor Resting Density (IRD) and Man biting Rate (MBR) of indoor biting adult mosquitoes collected in the study.

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Total	%	IRD	MBR
No. collected	14	9	22	110	107	112	262	197	233	163	112	77	1418			
Freshly Fed	12	0	15	89	84	78	206	160	195	140	83	58	1129			
Ae. Aegypti	0	0	0	13	9	9	22	23	26	20	14	10	146	13.0	0.31	0.12
Ae. Albopictus	0	0	0	14	17	16	19	16	22	21	16	14	155	13.7	0.35	0.13
An. gambiae	0	0	0	22	19	23	36	29	60	22	16	15	242	21.4	0.51	0.20
An. funestus	0	0	0	24	17	13	19	23	55	27	8	0	176	15.6	0.40	0.14
Cx. Decens	0	0	0	0	0	0	76	0	0	0	0	0	76	6.7	0.16	0.06
Cx. Nebulosis	0	0	0	0	0	0	0	1	0	4	2	2	9	0.8	0.02	0.007
Cx. Poicilipes	0	0	0	0	0	0	17	6	0	7	0	5	35	3.1	0.08	0.02
Cx. Quinque Fasciatus	12	9	14	16	22	16	17	18	42	30	21	12	229	20.3	0.47	0.19
Cq. maculipennis	0	0	0	0	0	1	0	0	0	0	0	0	1	0.1	0.002	0.0008
An. moucheti	0	0	0	0	0	0	0	11	0	0	0	0	11	1.0	0.03	0.009
M. Africana	0	0	1	0	0	0	0	20	0	9	6	0	36	3.2	0.09	0.03
M. uniformis	0	0	0	0	0	0	0	13	0	0	0	0	13	1.2	0.04	0.01

*Indoor Resting Density (IRD) = (number of mosquitoes ÷ number of houses) ÷ number of nights

*Man-biting rate (MBR) = (number of freshly fed mosquitoes ÷ total number of occupants) ÷ total number of nights

No. of houses =24

No. of nights = 24

No. of room occupants = 51

Appendix 4b: Relationship between Indoor resting density (IRD) and man-biting rate (MBR) (Figure 10)

Adult mosquito species	IRD mosquitoes/room/night	log_{10} (IRD+ 10)	MBR bites/man/night	Log_{10}(MBR+ 10)
Ae. Aegypti	0.31	9.491	0.12	9.079
Ae. albopictus	0.35	9.544	0.13	9.114
An gambiae	0.51	9.707	0.20	9.301
An. Funestus	0.40	9.602	0.14	9.146
Cx. Decens	0.16	9.204	0.06	10.06
Cx. nebulosus	0.02	8.301	0.007	7.845
Cx. Poicilipes	0.08	8.903	0.02	8.301
Cx. quinquefasciatus	0.47	9.672	0.19	9.279
Cq. maculipennis	0.002	7.301	0.0008	6.903
An. moucheti	0.03	8.477	0.009	7.954
M. africana	0.09	8.954	0.03	8.477
M. uniformis	0.04	8.602	0.01	8

Appendix 4c: Simple regression of Man-biting rate on Indoor Resting Density of different mosquito species in Oraifite (Fig. 10)

Simple regression of MBR on IRD

Summary output

Regression Statistics	
Multiple R	0.99972782
R Square	0.99944964
Adjusted R Square	0.99939607
Standard Error	0.00651944
Observations	13

ANOVA

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.849038318	0.849038318	19975.9138	2.79E-19
Residual	11	0.000467534	4.25E-05		
Total	12	0.849505852			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-0.007177131	0.002113369	-3.39606	0.005977054	-0.01183	-0.0025	-0.01183	-0.0025
IRD	0.408391448	0.002889504	141.3362	2.78643E-19	0.402032	0.414175	0.402032	0.414751

Appendix 5a: Outdoor biting adult mosquitoes collected using Human Bait Collection Method (HBC) (Figures 11 & 12)

Adult mosquito species	**Communities**								**Total (%)**
	Amakom	**Umunakwa**	**Umudisi**	**Nkalafia**	**Umuafa**	**Umuezopi**	**Ibolo**	**Umuonyeagolu**	
Ae. Aegypti	22	31	22	36	29	34	21	40	235 (24.68)
Ae. albopictus	24	19	34	31	24	39	28	39	238 (25.00)
Ae. africanus	15	4	9	12	7	0	0	0	47 (4.93)
Ae. circumluteolus	0	0	0	0	0	0	4	0	4 (0.42)
Ae. luteocephalus	16	13	0	12	12	14	0	0	67 (7.04)
Cx. decens	0	0	0	14	21	0	16	13	64 (6.72)
Cx. nebulosus	0	0	11	0	0	0	0	0	11 (1.16)
Cx. poicilipes	0	0	0	0	12	0	19	0	31 (3.26)
Cx. qiunquefasciatus	21	19	26	31	17	24	31	18	187 (19.64)
Cx. annulioris	0	0	0	0	6	0	0	0	6 (0.63)
Cx. nigripalpus	0	2	0	0	0	0	0	0	2 (0.21)
Er. Chrysogaster	0	0	6	9	0	3	0	0	18 (1.89)
M. africana	0	0	10	6	3	0	4	0	23 (2.42)
M. uniformis	0	0	0	12	0	7	0	0	19 (2.00)
Total (%)	**98 (10.29)**	**88 (9.24)**	**118 (12.39)**	**163 (17.12)**	**131 (13.76)**	**121 (12.71)**	**123 (12.92)**	**110 (11.55)**	**952 (100)**

Appendix 5b: Analysis of variance for all mosquito species collected outdoors in the eight villages in Oraifite from Jan-Dec, 2017 (Fig 12).

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Ae. aegypti	8	235	29.375	51.41071
Ae. albopictus	8	238	29.75	53.64286
Ae. africanus	8	47	5.875	34.125
Ae. circumluteolus	8	4	0.5	2
Ae. luteocephalus	8	67	8.375	49.69643
Cx. decens	8	64	8	78.57143
Cx. nebulosus	8	11	1.375	15.125
Cx. poicilipes	8	31	3.875	54.98214
Cx.qiunquefasciatus	8	187	23.375	31.125
Cx. annulioris	8	6	0.75	4.5
Cx. nigripalpus	8	2	0.25	0.5
Er. chrysogaster	8	18	2.25	12.21429
M. africana	8	23	2.875	13.55357
M. uniformis	8	19	2.375	21.125

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11906	13	915.8462	30.34244	1.3E-28	1.821327
Within Groups	2958	98	30.18367			
Total	14864	111				

There was a significant difference between mosquito species collected outdoors in Oraifite ($P<0.05$) P = 1.3E-28.

POST HOC ANALYSIS

t-Test: Two-Sample Assuming Equal Variances

	Ae. albopictus	*Ae. circumluteolus*
Mean	29.75	0.5
Variance	53.64286	2
Observations	8	8
Pooled Variance	27.82143	
Hypothesized Mean Difference	0	
Df	14	
t Stat	11.09088	
P(T<=t) one-tail	1.28E-08	
t Critical one-tail	1.76131	
P(T<=t) two-tail	2.55E-08	
t Critical two-tail	2.144787	

t-Test: Two-Sample Assuming Equal Variances

	Ae. albopictus	*Cx. annulioris*
Mean	29.75	0.75
Variance	53.64286	4.5
Observations	8	8
Pooled Variance	29.07143	
Hypothesized Mean Difference	0	
Df	14	
t Stat	10.75709	
P(T<=t) one-tail	1.88E-08	
t Critical one-tail	1.76131	
P(T<=t) two-tail	3.76E-08	
t Critical two-tail	2.144787	

Appendix 6: Out-door biting mosquito species collected by human bait catch (HBC) method

Mosquito species	Percentage distribution of out-door biting mosquito species by time of day												Total (%)
	4.30 - 4.45	4.45 - 4.50	5.00 - 5.15	5.15 - 5.30	5.30 - 5.45	5.45 - 6.00	6.00 - 6.15	6.15 - 6.30	6.30 - 6.45	6.45-7.00	7.00-7.15	7.15-7.30	
Ae. Aegypti	0.95	1.16	1.35	1.36	1.47	1.68	1.99	2.1	2.73	3.05	3.36	3.47	24.67
Ae. Albopictus	0.72	1.26	1.16	1.26	1.68	1.58	2.21	2.31	2.52	2.84	4.1	3.36	25.0
Ae. africanus	0.0	0.0	0.0	0.0	0.42	0.42	0.63	0.72	0.63	0.63	0.73	0.72	4.9
Ae. circumluteolus	0.0	0.0	0.0	0.11	0.0	0.11	0.0	0.21	0.0	0.0	0.0	0.0	0.43
Ae. luteocephalus	0.0	0.52	0.63	0.63	0.95	0.42	0.42	0.63	0.52	0.95	0.72	0.63	7.02
Cx. decens	0.0	0.42	0.63	0.52	0.52	0.84	0.52	0.42	0.84	0.63	0.63	0.72	6.69
Cx. nebulosus	0.0	0.0	0.11	0.21	0.0	0.11	0.0	0.11	0.21	0.32	0.11	0.0	1.18
Cx. poicilipes	0.0	0.32	0.52	0.52	0.42	0.32	0.52	0.63	0.0	0.0	0.0	0.0	3.25
Cx. quinquefasciatu	0.63	0.72	0.84	0.84	0.72	0.95	1.47	2.0	2.1	2.52	2.84	3.99	19.62
Cx. annulioris	0.0	0.0	0.0	0.0	0.0	0.11	0.21	0.11	0.0	0.11	0.11	0.0	0.65
Cx. nigripalpus	0.0	0.0	0.0	0.0	0.0	0.11	0.11	0.0	0.0	0.0	0.0	0.0	0.22
Er. chrysogaster	0.0	0.0	0.32	0.42	0.32	0.21	0.42	0.11	0.11	0.0	0.0	0.0	1.91
M. africanus	0.0	0.0	0.21	0.32	0.11	0.11	0.42	0.63	0.32	0.21	0.11	0.0	2.44
M. uniformis	0.0	0.11	0.11	0.42	0.32	0.21	0.21	0.32	0.0	0.21	0.11	0.0	2.02
Total (%)	2.3	4.51	5.88	6.61	6.93	7.18	9.13	10.3	9.98	11.47	12.82	12.89	100

Appendix 7: All adult Mosquitoes collected both Indoors and outdoors across all months of the year in the study area

Season	Months	*Ae. Aegypti*	*Ae. albopictus*	*Ae. africanus*	*Ae. circumluteolus*	*Ae. luteocephalus*	*An. gambiae*	*An. Funestus*	*Cx. decens*	*Cx. nebulosus*	*Cx. poicilipes*	*Cx. quinquefasciatus*	*Cx. annuliorris*	*Cx. nigripalpus*	*Cq. maculipennis*	*Er. chrysogaster*	*An. Mouchieti*
Dry	Dec	14	16	0	0	0	21	0	0	2	7	35	0	0	0	0	0
	Jan	0	0	0	0	0	0	0	0	0	0	26	0	0	0	0	0
	Feb	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0
	Mar	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0
	Sub Total	14	16	0	0	0	21	0	0	2	7	95	0	0	0	0	0
Wet	Apr	35	35	0	0	6	29	22	0	0	0	41	0	0	0	0	0
	May	43	50	4	0	6	24	21	0	0	0	44	0	0	0	0	0
	Jun	45	57	6	0	0	36	23	0	0	0	40	0	0	1	0	0
	Jul	65	45	0	0	7	43	29	159	0	28	40	0	0	0	0	0
	Aug	45	55	6	0	9	33	28	0	2	24	39	0	2	0	18	19
	Sept	83	81	24	0	28	63	59	0	0	0	69	0	0	0	0	0
	Oct	47	54	4	0	7	24	29	0	12	10	50	2	0	0	0	0
	Nov	38	47	3	4	4	21	17	0	7	7	40	4	0	0	0	0
	Total	415	440	47	4	67	294	228	159	23	76	458	6	2	1	18	19

Appendix 8: Monthly and Seasonal distribution (%) of all mosquito genera (Appendix 7)

Mosquito Genera	Dry season					Wet season									Annual
	Dec	Jan	Feb	Mar	**Total**	Apr	May	Jun	July	Aug	Sept	Oct	Nov	**Total**	Total (%)
Aedes	1.26	0	0	0	**1.26**	3.21	4.34	4.55	4.94	4.85	9.11	4.72	4.05	**39.77**	41.03
Anopheles	0.89	0	0	0	**0.89**	2.15	1.9	2.49	3.03	3.37	5.15	2.23	1.61	**21.93**	22.82
Culex	1.86	1.1	0.38	1.05	**4.39**	1.73	1.86	1.69	9.58	2.82	2.91	3.12	2.44	**26.15**	30.54
Coquillettida	0	0	0	0	**0**	0	0	0.04	0	0	0	0	0	**0.04**	0.04
Eretmapodites	0	0	0	0	**0**	0	0	0	0	0.76	0	0	0	**0.76**	0.76
Mansonia	0	0	0	0.38	**0.38**	0	0	0	0.17	2.49	0.29	0.84	0.59	**4.38**	4.76
Total (%)	4.01	1.1	0.38	1.43	**6.92**	7.09	8.1	8.77	17.72	14.29	17.46	10.91	8.69	**93.03**	99.95

Appendix 9: Monthly and Seasonal distributions (%) of all mosquito species (Appendix 7)

Species	Dry season					Wet season									Total
	Dec	Jan	Feb	Mar	**Total**	Apr	May	Jun	July	Aug	Sept	Oct	Nov	**Total**	
Ae. aegypti	0.59	0	0	0	**0.59**	1.48	1.81	1.9	2.74	1.9	3.5	1.98	1.6	**16.91**	17.50
Ae. albopictus	0.67	0	0	0	**0.67**	1.48	2.11	2.4	1.9	2.32	3.42	2.28	1.98	**17.89**	18.56
Ae. africanus	0	0	0	0	**0**	0	0.17	0.25	0	0.25	1.01	0.17	0.13	**1.98**	1.98
Ae. circumluteolus	0	0	0	0	**0**	0	0	0	0	0	0	0	0.17	**0.17**	0.17
Ae. luteocephalus	0	0	0	0	**0**	0.25	0.25	0	0.3	0.38	1.18	0.29	0.17	**2.82**	2.82
An gambiae	0.89	0	0	0	**0.89**	1.22	1.01	1.52	1.81	1.39	2.66	1.01	0.89	**11.51**	12.40
An. funestus	0	0	0	0	**0**	0.93	0.89	0.97	1.22	1.18	2.49	1.22	0.72	**9.62**	9.62
Cx. decens	0	0	0	0	**0**	0	0	0	6.71	0	0	0	0	**6.71**	6.71
Cx. nebulosus	0.08	0	0	0	**0.08**	0	0	0	0	0.08	0	0.51	0.29	**0.88**	0.96
Cx. poicilipes	0.3	0	0	0	**0.3**	0	0	0	1.18	1.01	0	0.42	0.29	**2.9**	3.20
Cx. quinquefasciatu	1.48	1.1	0.38	1.05	**4.01**	1.73	1.86	1.69	1.69	1.65	2.91	2.11	1.69	**15.33**	19.34
Cx. annulioris	0	0	0	0	**0**	0	0	0	0	0	0	0.08	0.17	**0.25**	0.25
Cx. nigripalpus	0	0	0	0	**0**	0	0	0	0	0.08	0	0	0	**0.08**	0.08
Cq. maculipenis	0	0	0	0	**0**	0	0	0.04	0	0	0	0	0	**0.04**	0.04
Er. chrysogaster	0	0	0	0	**0**	0	0	0	0	0.76	0	0	0	**0.76**	0.76
An. moucheti	0	0	0	0	**0**	0	0	0	0	0.8	0	0	0	**0.8**	0.8
M. africana	0	0	0	0.38	**0.38**	0	0	0	0.17	0.76	0.29	0.84	0.59	**2.65**	3.03
M. uniformis	0	0	0	0	**0**	0	0	0	0	1.73	0	0	0	**1.73**	1.73
Total (%)	4.01	1.1	0.38	1.43	6.92	7.09	8.1	8.77	17.72	14.29	17.46	10.91	8.69	93.03	99.95

Appendix 10: All adult mosquitoes collected both indoors and outdoors in Oraifite communities.

Season	Months	Outdoor biting adult (%)	Indoor biting adult (%)	Total (%)
Dry	Dec	18 (0.76)	77(3.25)	95(4.01)
	Jan	12(0.51)	14(0.59)	26(1.10)
	Feb	0(0.0)	9(0.38)	9(0.38)
	Mar	12(0.76)	22(0.93)	34(1.43)
	Sub total(%)	**42(1.8)**	**122(5.2)**	**164 (7)**
Wet	Apr	58(2.45)	110(4.64)	168(7.09)
	May	85(3.59)	107(4.51)	192(8.10)
	Jun	96(4.05)	112(4.73)	208(8.78)
	Jul	158(6.67)	262(11.05)	420(17.72)
	Aug	142(5.99)	197(8.31)	339(14.30)
	Sept	181(7.64)	233(9.83)	414(17.47)
	Oct	96(4.05)	163(6.88)	259(10.93)
	Nov	94(3.96)	112(4.73)	206(8.69)
	Sub total(%)	**910 (38.4)**	**1296 (54.6)**	**2206 (93)**
	Total (%)	**952 (40.2)**	**1418 (59.8)**	**2370 (100)**

Appendix 10a: Seasonal percentage distribution of indoor and outdoor adult mosquitoes (Appendix 10)

	Dry season	Wet season	Annual (%)
Indoor biting and resting mosquitoes collected by PKC method (%)	5.2	54.6	59.8
Outdoor biting mosquitoes collected by HBC method (%)	1.8	38.4	40.2
All Adults mosquitoes collected (%)	7	93	100

Appendix 10b: T-test comparing outdoor mosquito population in wet and dry season (Fig. 10)

t-Test: Two-Sample Assuming Equal Variances

	Outdoor biting adults (dry season)	Outdoor biting adults (wet season)
Mean	10.5	113.75
Variance	57	1747.643
Observations	4	8
Pooled Variance	1240.45	
Hypothesized Mean Difference	0	
Df	10	
t Stat	-4.78724	
P(T<=t) one-tail	0.000369	
t Critical one-tail	1.812461	
P(T<=t) two-tail	0.000738	
t Critical two-tail	2.228139	

There was a significant difference in outdoor mosquito population in the wet and dry season ($p<0.05$)

Appendix 10c: T-test comparing indoor mosquito population in wet and dry season (10)

t-Test: Two-Sample Assuming Equal Variances

	Indoor biting adults (dry season)	Indoor biting adults (wet season)
Mean	30.5	162
Variance	989.6667	3856.571
Observations	4	8
Pooled Variance	2996.5	
Hypothesized Mean Difference	0	
Df	10	
t Stat	-3.92286	
P(T<=t) one-tail	0.001427	
t Critical one-tail	1.812461	
P(T<=t) two-tail	0.002853	
t Critical two-tail	2.228139	

Appendix 11a: Monthly percentage distribution of indoor and outdoor adult mosquitoes (Appendix 10)

Mosquitoes	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov
Indoor biting and resting	3.25	0.59	0.38	0.93	4.64	4.51	4.73	11.05	8.31	9.83	6.88	4.73
Outdoor biting	0.76	0.51	0	0.51	2.45	3.59	4.05	6.67	5.99	7.64	4.05	3.96
All Adults	4.01	1.1	0.38	1.43	7.09	8.1	8.78	17.72	14.3	17.47	10.93	8.69

Appendix 11b: ANOVA checking for significant difference in adult mosquito collected in the various months of 2017 (Fig. 11).

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Dec	8	95	11.875	17.55357143
Jan	8	26	3.25	1.071428571
Feb	8	9	1.125	0.410714286
Mar	8	34	4.25	1.071428571
Apr	8	168	21	60.28571429
May	8	192	24.25	61.35714286
Jun	8	208	26.25	111.6428571
Jul	8	420	52.75	57.07142857
Aug	8	339	42.625	63.98214286
Sept	8	414	52	61.71428571
Oct	8	259	31.25	52.5
Nov	8	206	25.5	44.28571429

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	28094.36	11	2554.033114	57.50746432	2.34355E-34	1.904539
Within Groups	3730.625	84	44.41220244			
Total	31824.99	95				

Appendix 11c: Simple regression analysis to check for the effect of outdoor mosquito population on indoor mosquito population.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.955751081
R Square	0.913460129
Adjusted R Square	0.904806141
Standard Error	25.65882205
Observations	12

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	69493.92	6949 3.92	105.5537	1.24E-06
Residual	10	6583.751	658.3751		
Total	11	76077.67			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	14.67027217	12.50375	1.17327	0.267876	-13.1898	42.53037	-13.1898	42.53037
Outdoor adult mosquitoes collections	1.304576401	0.126979	10.27393	1.24E-06	1.021649	1.587504	1.021649	1.587504

Appendix 12: Larval species collected from different breeding sites in Oraifite

Larval species	Breeding sites			Total (%)
	Man-made containers(%)	Ground pools(%)	Plant axils(%)	
Ae. aegypti	126(10.9)	113(9.78)	36(3.11)	275(23.70)
Ae. albopictus	121(10.47)	98(8.48)	51(4.41)	270(23.36)
Ae. simpsoni	74(6.40)	0(0.0)	47(4.07)	121(10.47)
Ae. luteocephalus	66(5.71)	0(0.0)	0(0.0)	66(5.71)
An. gambiae	0(0.0)	156(13.49)	0(0.0)	156(13.50)
Cx. quinquefasciatus	102(8.82)	92(7.96)	0(0.0)	194(16.78)
Cx. tigripes	33(2.85)	41(3.55)	0(0.0)	74(6.40)
Total (%)	**522(45.16)**	**500(43.25)**	**134(11.60)**	**1156(100)**

Appendix 13: Percentage distribution of mosquito larvae collected from breeding habitats in communities sampled

Larval species	Communities								
	Amakom	Umuakwa	Umudisi	Nkalafi	Umuafa	Umuezeopi	Ibolo	Umuonyeagolu	
Ae. aegypti	2.77	4.15	2.94	3.98	2.51	1.47	3.29	2.68	23.79
Ae. albopictus	2.86	3.46	3.2	2.68	3.11	2.6	2.51	2.94	23.36
Ae. simpsoni	0.0	0.0	10.47	0.0	0.0	0.0	0.0	0.0	10.47
Ae. luteocephalus	0.0	0.0	0.0	0.0	5.71	0.0	0.0	0.0	5.71
An. gambiae	1.47	1.99	2.08	1.38	1.82	1.21	1.64	1.9	13.49
Cx. quinquefasciatus	1.82	2.16	2.85	2.51	1.04	1.64	1.47	3.29	16.78
Cx. tigripes	0.0	0.0	0.0	0.0	6.4	0.0	0.0	0.0	6.4
Total (%)	8.92	11.76	21.54	10.55	20.59	6.92	8.91	10.81	100

Appendix 14: T-test comparing population of amplified and unamplified *An. gambiae* complex (Figure 21)

t-Test: Two-Sample Assuming Equal Variances

	Amplified An. gambiae sl	*Unamplified An. gambiae* sl
Mean	15.5	4.5
Variance	11.14286	11.14286
Observations	8	8
Pooled Variance	11.14286	
Hypothesized Mean Difference	0	
Df	14	
t Stat	6.590592	
P(T<=t) one-tail	**6.04E-06**	
t Critical one-tail	1.76131	
P(T<=t) two-tail	1.21E-05	
t Critical two-tail	2.144787	

There was a significant difference between amplified and unamplified *An. gambiae* s.s.

Appendix 15a: Index of species diversity and dominance indices for all mosquitoes collected in Oraifite, Ekwusigo L.G.A, Anambra state

Mosquito species	**Ni**	**Pi = ni/N**	**$P^2 = (ni/N)^2$**	**Pi Log Pi**	**Shannon-Wiener diversity index**	**Simpson's dominance index.**
Ae. aegypti	690	0.196	0.038	-0.139	0.139	0.038
Ae. albopictus	710	0.201	0.041	-0.140	0.140	0.041
Ae. africanus	47	0.013	0.00017	-0.025	0.025	0.00017
Ae. circumluteolus	4	0.0011	0.0000012	-0.004	0.004	0.0000012
Ae. luteocephalus	133	0.038	0.0014	-0.053	0.053	0.0014
Ae. simpsoni	121	0.034	0.0012	-0.050	0.050	0.0012
An. gambiae	450	0.127	0.163	-0.114	0.114	0.163
An. funestus	228	0.065	0.0042	-0.077	0.077	0.0042
Cx. decens	159	0.045	0.0020	-0.061	0.061	0.0020
Cx. nebulosus	23	0.0065	0.000043	-0.015	0.015	0.000043
Cx. poicilipes	76	0.022	0.00046	-0.036	0.036	0.00046
Cx. quinquefasciatus	652	0.185	0.034	-0.136	0.136	0.034
Cx. annulioris	6	0.0017	0.0000029	-0.005	0.005	0.0000029
Cx. nigripalpus	2	0.00057	0.00000032	-0.002	0.002	0.00000032
Cx. tigripes	74	0.021	0.00044	-0.035	0.035	0.00044
Cq. maculipennis	1	0.00028	0.000000080	-0.0001	0.0001	0.000000080
Er. chrysogaster	18	0.0051	0.000026	-0.012	0.0012	0.000026
M. africana	72	0.020	0.00042	-0.034	0.034	0.00042
M. uniformis	41	0.012	0.00014	-0.023	0.023	0.00014
An. moucheti	19	0.0054	0.000029	-0.012	0.012	0.000029
Total	**N = 3526**	∑ **1.000**	∑ **0.287**	∑ **- 0.973**	H = 0.973	**C = 0.287**

ni = abundance of individual species in the ith , Pi = proportion of individuals in the ith species ie ni/N , N= total number of individuals of all species, H = Shannon-Wiener index of diversity H = (N log N - ∑ni logni)/N, C = Simpson's index of dominance =**$\sum(ni/N)^2$).**

Appendix 15b: Shannon-Wiener index of diversity for mosquito species composition in the eight communities of Oraifite, Ekwusigo Local Government Area (See Fig. 23)

Mosquito species	Shannon-Wiener index of diversity (H)							
	Amakom	**Umunakwa**	**Umudisi**	**Nkalafia**	**Umuafa**	**Umuezopi**	**Ibolo**	**Umuonyeagolu**
Ae. aegypti	0.143	0.153	0.118	0.140	0.119	0.140	0.143	0.151
Ae. albopictus	0.143	0.139	0.134	0.142	0.117	0.154	0.130	0.152
Ae. africanus	0.063	0.020	0.029	0.040	0.023	0.004	0.000	0.000
Ae. circumluteolus	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000
Ae. luteocephalus	0.066	0.050	0.000	0.040	0.114	0.004	0.000	0.000
Ae. simpsoni	0.000	0.000	0.144	0.000	0.000	0.000	0.000	0.000
An. gambiae	0.123	0.125	0.114	0.098	0.108	0.114	0.122	0.111
An. funestus	0.092	0.108	0.081	0.090	0.063	0.057	0.047	0.064
Cx. decens	0.000	0.000	0.000	0.064	0.094	0.000	0.092	0.110
Cx. nebulosus	0.000	0.000	0.058	0.000	0.000	0.000	0.000	0.000
Cx. poicilipes	0.000	0.000	0.000	0.000	0.076	0.000	0.095	0.000
Cx. quinquefasciatus	0.149	0.137	0.122	0.140	0.108	0.131	0.146	0.139
Cx. annulioris	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000
Cx. nigripalpus	0.000	0.052	0.000	0.000	0.000	0.000	0.000	0.000
Cx. tigripes	0.000	0.000	0.000	0.000	0.111	0.000	0.000	0.000
Cq. maculipennis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007
Er. chrysogaster	0.000	0.000	0.021	0.032	0.000	0.016	0.000	0.000
M. africana	0.000	0.000	0.054	0.047	0.029	0.004	0.045	0.000
M. uniformis	0.000	0.000	0.000	0.066	0.000	0.055	0.000	0.000
An. moucheti	0.000	0.047	0.000	0.017	0.011	0.004	0.000	0.000
Total	**H = 0.779**	**H = 0.831**	**H = 0.753**	**H = 0.876**	**H = 0.993**	**H = 0.683**	**H = 0.839**	**H = 0.734**
Log10(C + 10)	**1.0325**	**1.0346**	**1.0315**	**1.0364**	**1.041**	**1.0287**	**1.034**	**1.0307**

Appendix 15c: Simpson's index of dominance for mosquito species composition in the eight communities of Oraifite, Ekwusigo Local Government Area (See Fig. 23)

Mosquito species	Simpson's index of dominance (C)							
	Amakom	Umunakwa	Umudisi	Nkalafia	Umuafa	Umuezopi	Ibolo	Umuonyeagolu
Ae. aegypti	0.045	0.069	0.018	0.041	0.020	0.041	0.045	0.064
Ae. albopictus	0.045	0.039	0.038	0.043	0.018	0.073	0.028	0.066
Ae. africanus	0.0023	0.00011	0.00026	0.00061	0.00013	0.0012	0.000	0.000
Ae. circumluteolus	0.000	0.000	0.000	0.000	0.000	0.000	0.000093	0.000
Ae. luteocephalus	0.0027	0.0011	0.000	0.043	0.016	0.0012	0.000	0.000
Ae. simpsoni	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000
An. gambiae	0.022	0.00056	0.016	0.0093	0.013	0.016	0.021	0.015
An. funestus	0.0076	0.013	0.0050	0.0071	0.0023	0.0018	0.00098	0.0023
Cx. decens	0.000	0.000	0.000	0.0024	0.0081	0.000	0.0075	0.014
Cx. nebulosus	0.000	0.000	0.0017	0.000	0.000	0.000	0.000	0.000
Cx. poicilipes	0.000	0.000	0.000	0.000	0.0039	0.000	0.0084	0.000
Cx. quinquefasciatus	0.057	0.036	0.021	0.040	0.013	0.030	0.051	0.039
Cx. annulioris	0.000	0.000	0.000	0.000	0.000097	0.000	0.000	0.000
Cx. nigripalpus	0.000	0.0013	0.000	0.000	0.000	0.000	0.000	0.000
Cx. tigripes	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000
Cq. maculipennis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0000073
Er. chrysogaster	0.000	0.000	0.00012	0.00034	0.000	0.000055	0.000	0.000
M. africana	0.000	0.000	0.0014	0.00095	0.00027	0.0012	0.00084	0.000
M. uniformis	0.000	0.000	0.000	0.0026	0.000	0.0016	0.000	0.000
An. moucheti	0.000	0.00098	0.000	0.000068	0.000024	0.0012	0.000	0.000
Total	**C= 0.181**	**C = 0.161**	**C = 0.127**	**C = 0.190**	**C = 0.110**	**C = 0.138**	**C = 0.163**	**C=0.200**
Log10(C + 10)	**1.0077**	**1.0069**	**1.0054**	**1.0081**	**1.0047**	**1.0059**	**1.007**	**1.0086**

Appendix 15d : Comparison between Diversity and Dominance indices of mosquito species sampled in Oraifite using transformed data (Fig. 23)

Indices	Communities sampled for mosquitoes in Oraifite								
	Amakom	Umuakwa	Umudisi	Nkalafi	Umuafa	Umuezopi	Ibolo	Umuonyeagolu	Total
Shannon-Wiener's Diversity Index	1.0325	1.0346	1.0315	1.0364	1.0411	1.0287	1.0349	1.0307	1.0403
Simpson's Dominance Index	1.0077	1.0069	1.0054	1.0081	1.0047	1.0059	1.007	1.0086	1.0122

Appendix 15e: Analysis of variance for all mosquito species collected in the eight villages in Oraifite from Jan-Dec, 2017 (See table 9 & 10).

ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Ae. Aegypti	3	690	230	2275
Ae. albopictus	3	707	235.6667	1156.333
Ae. Africanus	3	47	15.66667	736.3333
Ae. circumluteolus	3	4	1.333333	5.333333
Ae. luteocephalus	3	133	44.33333	1474.333
Ae. Simpsoni	3	121	40.33333	4880.333
An. Gambiae	3	450	150	21636
An. Funestus	3	228	76	17328
Cx. Decens	3	159	53	2347
Cx. nebulosus	3	23	7.666667	44.33333
Cx. Poicilipes	3	76	25.33333	530.3333
Cx.quinquefasciatus	3	652	217.3333	2172.333
Cx. annulioris	3	6	2	12
Cx. nigripalpus	3	2	0.666667	1.333333
Cx. Tigripes	3	74	24.66667	1825.333
Cq. maculipennis	3	1	0.333333	0.333333
Er. chrysogaster	3	18	6	108
M. Africana	3	72	24	601
M. uniformis	3	41	13.66667	142.3333
An. Moucheti	3	19	6.333333	120.3333

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	371389.5	19	19546.82	6.811173	1.89E-07	1.852892
Within Groups	114792.7	40	2869.817			
Total	486182.2	59				

POST HOC analysis for the above table

t-Test: Two-Sample Assuming Equal Variances

	Ae. aegypti	*Ae. circumluteolus*
Mean	230	1.333333
Variance	2275	5.333333
Observations	3	3
Pooled Variance	1140.167	
Hypothesized Mean Difference	0	
Df	4	
t Stat	8.294006	
P(T<=t) one-tail	0.000577	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.001154	
t Critical two-tail	2.776445	

t-Test: Two-Sample Assuming Equal Variances

	Ae. Aegypti	*Ae. africanus*
Mean	230	15.66667
Variance	2275	736.3333
Observations	3	3
Pooled Variance	1505.667	
Hypothesized Mean Difference	0	
Df	4	
t Stat	6.765049	
P(T<=t) one-tail	0.001245	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.002491	
t Critical two-tail	2.776445	

t-Test: Two-Sample Assuming Equal Variances

	Ae. aegypti	*Ae. simpsoni*
Mean	230	40.33333
Variance	2275	4880.333
Observations	3	3
Pooled Variance	3577.667	
Hypothesized Mean Difference	0	
Df	4	
t Stat	3.88362	
P(T<=t) one-tail	0.008893	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.017787	
t Critical two-tail	2.776445	

Appendix 16: Physicochemical parameters of mosquito larvae in man- made container breeding sites in Oraifite

Month	Ph	Salinity Mg/L	TSS Mg/L	TDS Mg/l	DO Mg/L	Sulphate Mg/L	Temp °C
Mar	6.37	85.07	30	1870	3.55	805.37	27.8
Dec	5.19	77.04	30	670	3.58	826.21	27.4
Apr	5.8	76.59	26.67	800	4.15	710.12	27
May	5.63	65.23	30	400	5.97	811.33	26.8
June	5.37	49.16	20	330	5.65	775.61	26.4
July	5.04	31.19	10	200	7.83	808.35	25.5
Aug	5.41	58.33	23.33	400	4.51	772.63	26.6
Sep	4.83	48.21	16.69	200	6.4	766.68	27.5
Oct	5.3	62.39	20	530	4.38	638.69	26.8
Nov	5.76	73.74	26.67	600	3.98	799.42	27.2
Total	54.7	626.95	233.36	6000	50	7714.41	269
Average	5.47±0.139204	62.695±5.198965	23.336±2.107484	600±154.0418	5±0.445204	771.441±18.04673	26.9±0.206559

		pH	*Aedes aegypti*	*Aedes albopictus*	*Culex quinquefasciatus*	*Culex tigripes*
	Pearson Correlation	1	.533	.461	.763**	.403
Ph	Sig. (2-tailed)		.075	.131	.004	.194
	N	12	12	12	12	12
	Pearson Correlation	.533*	1	.945**	.634*	.927**
Aedes aegypti	Sig. (2-tailed)	.075		.000	.027	.000
	N	12	12	12	12	12
	Pearson Correlation	.461	.945**	1	.510	.902**
Aedes albopictus	Sig. (2-tailed)	.131	.000		.091	.000
	N	12	12	12	12	12
	Pearson Correlation	.763**	.634*	.510	1	.577*
Culex quinquefasciats	Sig. (2-tailed)	.004	.027	.091		.049
	N	12	12	12	12	12
	Pearson Correlation	.403	.927**	.902**	.577*	1
Culex tigripes	Sig. (2-tailed)	.194	.000	.000	.049	
	N	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 17: Correlation table of salinity and larvae species collected in man-made containers breeding sites in Oraifite.

Appendix 17: Correlation table of salinity and larvae species collected in man-made containers breeding sites in Oraifite.

		Salinity Mg/L	*Aedes aegypti*	*Aedes albopictus*	*Culex quinquefasciats*	*Culex tigripes*
Salinity Mg/L	Pearson Correlation	1	.268	.188	.600*	.096
	Sig. (2-tailed)		.400	.558	.039	.766
	N	12	12	12	12	12
Aedes aegypti	Pearson Correlation	.268	1	.945**	.634*	.927**
	Sig. (2-tailed)	.400		.000	.027	.000
	N	12	12	12	12	12
Aedes albopictus	Pearson Correlation	.188	.945**	1	.510	.902**
	Sig. (2-tailed)	.558	.000		.091	.000
	N	12	12	12	12	12
Culex quinquefasciats	Pearson Correlation	.600*	.634*	.510	1	.577*
	Sig. (2-tailed)	.039	.027	.091		.049
	N	12	12	12	12	12
Culex tigripes	Pearson Correlation	.096	.927**	.902**	.577*	1
	Sig. (2-tailed)	.766	.000	.000	.049	
	N	12	12	12	12	12

Appendix 18: Correlation table of total suspended solids and larvae species collected in man-made containers breeding sites in Oraifite.

		TSS Mg/L	Aedes aegypti	Aedes albopictus	Culex quinquefasciats	Culex tigripes
TSS Mg/L	Pearson Correlation	1	.253	.188	.596*	.098
	Sig. (2-tailed)		.428	.558	.041	.761
	N	12	12	12	12	12
Aedesaegypti	Pearson Correlation	.253	1	.945**	.634*	.927**
	Sig. (2-tailed)	.428		.000	.027	.000
	N	12	12	12	12	12
Aedesalbopictus	Pearson Correlation	.188	.945**	1	.510	.902**
	Sig. (2-tailed)	.558	.000		.091	.000
	N	12	12	12	12	12
Culexquinquefasciats	Pearson Correlation	.596*	.634*	.510	1	.577*
	Sig. (2-tailed)	.041	.027	.091		.049
	N	12	12	12	12	12
Culextigripes	Pearson Correlation	.098	.927**	.902**	.577*	1
	Sig. (2-tailed)	.761	.000	.000	.049	
	N	12	12	12	12	12

Appendix 19: Correlation table of total dissolved solids and larvae species collected in man-made containers breeding sites in Oraifite.

		TDS Mg/L	Aedes aegypti	Aedes albopictus	Culex Quinquefasciats	Culex tigripes
TDS Mg/L	Pearson Correlation	1	-.068	-.059	.177	-.298
	Sig. (2-tailed)		.833	.856	.582	.347
	N	12	12	12	12	12
Aedesaegypti	Pearson Correlation	-.068	1	.945**	.634*	.927**
	Sig. (2-tailed)	.833		.000	.027	.000
	N	12	12	12	12	12
Aedesalbopictus	Pearson Correlation	-.059	.945**	1	.510	.902**
	Sig. (2-tailed)	.856	.000		.091	.000
	N	12	12	12	12	12
Culexquinquefasciats	Pearson Correlation	.177	.634*	.510	1	.577*
	Sig. (2-tailed)	.582	.027	.091		.049
	N	12	12	12	12	12
Culextigripes	Pearson Correlation	-.298	.927**	.902**	.577*	1
	Sig. (2-tailed)	.347	.000	.000	.049	
	N	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 20: Correlation table of dissolved oxygen and larvae species collected in man-made containers breeding sites in Oraifite.

		DO Mg/L	*Aedes Aegypti*	*Aedes albopictus*	*Culex quinquefasciats*	*Culex tigripes*
DO Mg/L	Pearson Correlation	1	.806**	.781**	.763**	.765**
	Sig. (2-tailed)		.002	.003	.004	.004
	N	12	12	12	12	12
Aedes Aegypti	Pearson Correlation	.806**	1	.945**	.634*	.927**
	Sig. (2-tailed)	.002		.000	.027	.000
	N	12	12	12	12	12
Aedes albopictus	Pearson Correlation	.781**	.945**	1	.510	.902**
	Sig. (2-tailed)	.003	.000		.091	.000
	N	12	12	12	12	12
Culex quinquefasciats	Pearson Correlation	.763**	.634*	.510	1	.577*
	Sig. (2-tailed)	.004	.027	.091		.049
	N	12	12	12	12	12
Culex Tigripes	Pearson Correlation	.765**	.927**	.902**	.577*	1
	Sig. (2-tailed)	.004	.000	.000	.049	
	N	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 21: Correlation table of sulphate and larvae species collected in man-made containers breeding sites in Oraifite.

		Sulphate Mg/L	*Aedes aegypti*	*Aedes albopictus*	*Culex quinquefasciats*	*Culex Tigripes*
Sulphate Mg/L	Pearson Correlation	1	.628*	.555	.773**	.483
	Sig. (2-tailed)		.029	.061	.003	.111
	N	12	12	12	12	12
Aedes Aegypti	Pearson Correlation	.628*	1	.945**	.634*	.927**
	Sig. (2-tailed)	.029		.000	.027	.000
	N	12	12	12	12	12
Aedes albopictus	Pearson Correlation	.555	.945**	1	.510	.902**
	Sig. (2-tailed)	.061	.000		.091	.000
	N	12	12	12	12	12
Culex quinquefasciatus	Pearson Correlation	.773**	.634*	.510	1	.577*
	Sig. (2-tailed)	.003	.027	.091		.049
	N	12	12	12	12	12
Culex Tigripes	Pearson Correlation	.483	.927**	.902**	.577*	1
	Sig. (2-tailed)	.111	.000	.000	.049	
	N	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 22: Correlation table of temperature and larvae species collected in man-made containers breeding sites in Oraifite.

		Temperature ^{0}C	Aedes aegypti	Aedes albopictus	Culex quinquefasciats	Culex Tigripes
Temperature 0C	Pearson Correlation	1	.634*	.554	.795**	.501
	Sig. (2-tailed)		.027	.062	.002	.097
	N	12	12	12	12	12
Aedes Aegypti	Pearson Correlation	.634*	1	.945**	.634*	.927**
	Sig. (2-tailed)	.027		.000	.027	.000
	N	12	12	12	12	12
Aedes Albopictus	Pearson Correlation	.554	.945**	1	.510	.902**
	Sig. (2-tailed)	.062	.000		.091	.000
	N	12	12	12	12	12
Culex quinquefasciats	Pearson Correlation	.795**	.634*	.510	1	.577*
	Sig. (2-tailed)	.002	.027	.091		.049
	N	12	12	12	12	12
Culex Tigripes	Pearson Correlation	.501	.927**	.902**	.577*	1
	Sig. (2-tailed)	.097	.000	.000	.049	
	N	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 23: Physicochemical parameters of mosquito larvae in ground pools breeding sites in Oraifite

Month	pH	Salinity	TSS	TDS	DO	Sulphate	Temp
		Mg/L	Mg/L	Mg/l	Mg/L	Mg/L	°C
Mar	5.34	77.52	16.6	400	2.89	775.61	29.5
Apr	5.17	77.04	26.67	800	3.55	671.43	28.5
May	5.32	75.63	26.67	470	5.44	811.33	27.6
June	5.22	58.33	16.69	670	7.4	406.53	26.8
July	5.04	65.23	23.33	530	6.17	772.63	26.6
Aug	5.94	76.57	23.33	690	4.15	349.97	28.5
Sep	5.57	67.12	20	330	5.97	281.51	27.8
Oct	5.56	72.79	20	470	5.67	650.52	28.6
Nov	5.57	85.07	30	670	2.59	811.53	28.5
Dec	4.7	98.31	30	1800	2.26	507.73	29.5
Total	53.43	753.61	233.29	6830	46.09	6038.79	281.9
Average	5.343±0.108393	75.361±3.491501	23.329±1.573564	683±132.523	4.609±0.555541	603.879±63.89615	28.19±0.313564

Appendix 23: Correlation table of pH and larvae species collected in ground pools containers breeding sites in Oraifite.

		pH	*Aedes aegypti*	*Aedes albopictus*	*Anopheles gambiae*	*Culex quinquefasciatus*	*Culex tigripes*
pH	Pearson Correlation	1	.658*	.658*	.549	.812**	.489
	Sig. (2-tailed)		.020	.020	.064	.001	.107
	N	12	12	12	12	12	12
Aedes aegypti	Pearson Correlation	.658*	1	.969**	.953**	.943**	.951**
	Sig. (2-tailed)	.020		.000	.000	.000	.000
	N	12	12	12	12	12	12
Aedes albopictus	Pearson Correlation	.658*	.969**	1	.875**	.933**	.895**
	Sig. (2-tailed)	.020	.000		.000	.000	.000
	N	12	12	12	12	12	12
Anopheles gambiae	Pearson Correlation	.549	.953**	.875**	1	.877**	.983**
	Sig. (2-tailed)	.064	.000	.000		.000	.000
	N	12	12	12	12	12	12
Culex quinquefasciatus	Pearson Correlation	.812**	.943**	.933**	.877**	1	.868**
	Sig. (2-tailed)	.001	.000	.000	.000		.000
	N	12	12	12	12	12	12
Culex tigripes	Pearson Correlation	.489	.951**	.895**	.983**	.868**	1
	Sig. (2-tailed)	.107	.000	.000	.000	.000	
	N	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 24: Correlation table of salinity and larvae species collected in ground pools containers breeding sites in Oraifite.

		Salinity Mg/L	Aedes aegypti	Aedes albopictus	Anopheles gambiae	Culex quinquefasciatus	Culex tigripes
Salinity Mg/L	Pearson Correlation	1	.430	.445	.284	.624*	.234
	Sig. (2-tailed)		.163	.147	.372	.030	.464
	N	12	12	12	12	12	12
Aedes aegypti	Pearson Correlation	.430	1	.969**	.953**	.943**	.951**
	Sig. (2-tailed)	.163		.000	.000	.000	.000
	N	12	12	12	12	12	12
Aedes albopictus	Pearson Correlation	.445	.969**	1	.875**	.933**	.895**
	Sig. (2-tailed)	.147	.000		.000	.000	.000
	N	12	12	12	12	12	12
Anopheles gambiae	Pearson Correlation	.284	.953**	.875**	1	.877**	.983**
	Sig. (2-tailed)	.372	.000	.000		.000	.000
	N	12	12	12	12	12	12
Culex quinquefasciats	Pearson Correlation	.624*	.943**	.933**	.877**	1	.868**
	Sig. (2-tailed)	.030	.000	.000	.000		.000
	N	12	12	12	12	12	12
Culex tigripes	Pearson Correlation	.234	.951**	.895**	.983**	.868**	1
	Sig. (2-tailed)	.464	.000	.000	.000	.000	
	N	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

c. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

Appendix 25: Correlation table of total suspended solids and larvae species collected in ground pools containers breeding sites in Oraifite.

		TSS Mg/L	*Aedes aegypti*	*Aedes albopictus*	*Anopheles gambiae*	*Culex quinquefasciatus*	*Culex tigripes*
TSS Mg/L	Pearson Correlation	1	.419	.429	.331	.606*	.281
	Sig. (2-tailed)		.175	.164	.293	.037	.376
	N	12	12	12	12	12	12
Aedes aegypti	Pearson Correlation	.419	1	.969**	.953**	.943**	.951**
	Sig. (2-tailed)	.175		.000	.000	.000	.000
	N	12	12	12	12	12	12
Aedes albopictus	Pearson Correlation	.429	.969**	1	.875**	.933**	.895**
	Sig. (2-tailed)	.164	.000		.000	.000	.000
	N	12	12	12	12	12	12
Anopheles gambiae	Pearson Correlation	.331	.953**	.875**	1	.877**	.983**
	Sig. (2-tailed)	.293	.000	.000		.000	.000
	N	12	12	12	12	12	12
Culex quinquefasciatus	Pearson Correlation	.606*	.943**	.933**	.877**	1	.868**
	Sig. (2-tailed)	.037	.000	.000	.000		.000
	N	12	12	12	12	12	12
Culex tigripes	Pearson Correlation	.281	.951**	.895**	.983**	.868**	1
	Sig. (2-tailed)	.376	.000	.000	.000	.000	
	N	12	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

c. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

Appendix 26: Correlation table of total dissolved solids and larvae species collected in ground pools containers breeding sites in Oraifite.

		TDS Mg/L	*Aedes aegypti*	*Aedes albopictus*	*Anopheles gambiae*	*Culex quinquefasciatus*	*Culex tigripes*
TDS Mg/L	Pearson Correlation	1	.083	.109	-.075	.268	-.096
	Sig. (2-tailed)		.799	.737	.817	.400	.766
	N	12	12	12	12	12	12
Aedes aegypti	Pearson Correlation	.083	1	.969**	.953**	.943**	.951**
	Sig. (2-tailed)	.799		.000	.000	.000	.000
	N	12	12	12	12	12	12
Aedes albopictus	Pearson Correlation	.109	.969**	1	.875**	.933**	.895**
	Sig. (2-tailed)	.737	.000		.000	.000	.000
	N	12	12	12	12	12	12
Anopheles gambiae	Pearson Correlation	-.075	.953**	.875**	1	.877**	.983**
	Sig. (2-tailed)	.817	.000	.000		.000	.000
	N	12	12	12	12	12	12
Culex quinquefasciatus	Pearson Correlation	.268	.943**	.933**	.877**	1	.868**
	Sig. (2-tailed)	.400	.000	.000	.000		.000
	N	12	12	12	12	12	12
Culex tigripes	Pearson Correlation	-.096	.951**	.895**	.983**	.868**	1
	Sig. (2-tailed)	.766	.000	.000	.000	.000	
	N	12	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

c. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

Appendix 27: Correlation table of dissolved oxygen and larvae species collected in ground pools containers breeding sites in Oraifite.

		DO Mg/L	*Aedes aegypti*	*Aedes albopictus*	*Anopheles gambiae*	*Culex quinquefasciatus*	*Culex tigripes*
DO Mg/L	Pearson Correlation	1	.784**	.741**	.771**	.833**	.741**
	Sig. (2-tailed)		.003	.006	.003	.001	.006
	N	12	12	12	12	12	12
Aedes aegypti	Pearson Correlation	.784**	1	.969**	.953**	.943**	.951**
	Sig. (2-tailed)	.003		.000	.000	.000	.000
	N	12	12	12	12	12	12
Aedes albopictus	Pearson Correlation	.741**	.969**	1	.875**	.933**	.895**
	Sig. (2-tailed)	.006	.000		.000	.000	.000
	N	12	12	12	12	12	12
Anopheles gambiae	Pearson Correlation	.771**	.953**	.875**	1	.877**	.983**
	Sig. (2-tailed)	.003	.000	.000		.000	.000
	N	12	12	12	12	12	12
Culex quinquefasciatus	Pearson Correlation	.833**	.943**	.933**	.877**	1	.868**
	Sig. (2-tailed)	.001	.000	.000	.000		.000
	N	12	12	12	12	12	12
Culex tigripes	Pearson Correlation	.741**	.951**	.895**	.983**	.868**	1
	Sig. (2-tailed)	.006	.000	.000	.000	.000	
	N	12	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

c. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

Appendix 28: Correlation table of sulphate and larvae species collected in ground pools containers breeding sites in Oraifite.

		Sulphate Mg/L	*Aedes aegypti*	*Aedes albopictus*	*Anopheles gambiae*	*Culex quinquefasciatus*	*Culex tigripes*
Sulphate Mg/L	Pearson Correlation	1	.167	.221	.082	.348	.040
	Sig. (2-tailed)		.603	.491	.800	.268	.901
	N	12	12	12	12	12	12
Aedes aegypti	Pearson Correlation	.167	1	.969**	.953**	.943**	.951**
	Sig. (2-tailed)	.603		.000	.000	.000	.000
	N	12	12	12	12	12	12
Aedes albopictus	Pearson Correlation	.221	.969**	1	.875**	.933**	.895**
	Sig. (2-tailed)	.491	.000		.000	.000	.000
	N	12	12	12	12	12	12
Anopheles gambiae	Pearson Correlation	.082	.953**	.875**	1	.877**	.983**
	Sig. (2-tailed)	.800	.000	.000		.000	.000
	N	12	12	12	12	12	12
Culex quinquefasciatus	Pearson Correlation	.348	.943**	.933**	.877**	1	.868**
	Sig. (2-tailed)	.268	.000	.000	.000		.000
	N	12	12	12	12	12	12
Culex tigripes	Pearson Correlation	.040	.951**	.895**	.983**	.868**	1
	Sig. (2-tailed)	.901	.000	.000	.000	.000	
	N	12	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

c. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

Appendix 29: Correlation table of surface water temperature and larvae species collected in ground pools containers breeding sites in Oraifite.

		Temperature 0C	*Aedes aegypti*	*Aedes albopictus*	*Anopheles gambiae*	*Culex quinquefasciatus*	*Culex tigripes*
Temperature 0C	Pearson Correlation	1	.583*	.589*	.450	.754**	.395
	Sig. (2-tailed)		.047	.044	.142	.005	.204
	N	12	12	12	12	12	12
Aedes aegypti	Pearson Correlation	.583*	1	.969**	.953**	.943**	.951**
	Sig. (2-tailed)	.047		.000	.000	.000	.000
	N	12	12	12	12	12	12
Aedes albopictus	Pearson Correlation	.589*	.969**	1	.875**	.933**	.895**
	Sig. (2-tailed)	.044	.000		.000	.000	.000
	N	12	12	12	12	12	12
Anopheles gambiae	Pearson Correlation	.450	.953**	.875**	1	.877**	.983**
	Sig. (2-tailed)	.142	.000	.000		.000	.000
	N	12	12	12	12	12	12
Culex quinquefasciats	Pearson Correlation	.754**	.943**	.933**	.877**	1	.868**
	Sig. (2-tailed)	.005	.000	.000	.000		.000
	N	12	12	12	12	12	12
Culex tigripes	Pearson Correlation	.395	.951**	.895**	.983**	.868**	1
	Sig. (2-tailed)	.204	.000	.000	.000	.000	
	N	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

c. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

Appendix 30: Analysis of variance for Mean physicochemical parameters of mosquito larvae breeding sites in Oraifite (See table 7).

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MAN-MADE CONTAINERS MEAN±SE	7	1494.842	213.5489	106858.9
GROUND POOLS MEAN±SE	7	1423.711	203.3873	91443.69

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	361.4014	1	361.4014	0.003645	0.952852	4.747225
Within Groups	1189815	12	99151.27			
Total	1190177	13				

Appendix 31a: 2017 Weather data of Oraifite, Ekwusigo Local Government Area, Anambra State and adult mosquito population.

Months (2017)	Temperature (˚C)	Relative humidity (%)	Rainfall (mm)	Outdoor adult mosquitoes collections	Indoor adult mosquitoes collections
Jan	28.2	84	21	12	14
Feb	29.1	83	34	0	9
Mar	28.6	84	133	12	22
Apr	28.6	85	208	58	110
May	28.1	85	221	85	107
Jun	26.4	87	300	96	112
Jul	26.0	89	438	158	262
Aug	26.4	92	506	142	197
Sept	26.0	90	491	181	233
Oct	26.6	88	305	96	163
Nov	27.7	87	138	94	112
Dec	28.2	86	32	18	77

Appendix 31: Correlation analysis on the effect of climatic factors on indoor mosquito population (Appendix 31a)

Correlation between indoor biting mosquitoes and temperature

	Indoor adult mosquitoes collections	*Temperature*
Indoor adult mosquitoes collections	1	
Temperature (^{0}C)	-0.87643	1

Correlation between indoor biting mosquitoes and relative humidity

	Indoor adult mosquitoes collections	*Relative humidity (%)*
Indoor adult mosquitoes collections	1	
Relative humidity (%)	0.883495	1

Correlation between indoor biting mosquitoes and rainfall

	Indoor adult mosquitoes collections	*Rainfall (mm)*
Indoor adult mosquitoes collections	1	
Rainfall (mm)	0.911659	1

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	6592 1.81	21973 .94	17.3 0936	0.0007 39
Residual	8	1015 5.86	1269. 483		
Total	1 1	7607 7.67			

These climatic factors have a significant effect on indoor mosquito population ($p<0.05$).

Appendix 32: Correlation analysis on the effect of climatic factors on outdoor mosquito population (Appendix 31a).

Correlation between outdoor biting mosquitoes and temperature

	Outdoor adult mosquitoes collections	*Temperature*
Outdoor adult mosquitoes collections	1	
Temperature	-0.8978	1

Correlation between outdoor biting mosquitoes and relative humidity

	Outdoor adult mosquitoes collections	*Relative humidity (%)*
Outdoor adult mosquitoes collections	1	
Relative humidity (%)	0.885085	1

Correlation between outdoor biting mosquitoes and rainfall

	Outdoor adult mosquitoes collections	*Rainfall (mm)*
Outdoor adult mosquitoes collections	1	
Rainfall (mm)	0.936896	1

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	3699.2	1233.73	25.7081	0.000185
Residual	8	383.462	47.9828		
Total	11	4083.67			