CHAPTER ONE

INTRODUCTION

1.1 Background

Mosquitoes are important vectors of human diseases and the most common blood sucking arthropods. There are approximately 3,500 species of mosquitoes grouped into 42 genera (Leopoldo, 2008). They cause serious biting annoyance, noise nuisance, sleeplessness, allergic reactions and disease transmission through their bites on their victims (Onyido *et al.*, 2009). Worldwide, mosquito-borne diseases have emerged as a major public health concern. They are found mainly in Asian, African, South, and Central American regions. The diseases have also been reported in some developed and temperate regions including Europe and Asia countries (Fradin, 1998). Recently, the incidence of mosquito-borne diseases has peaked at an alarming rate, with approximately 700 million infected cases and more than one million deaths recorded annually (Caraballo and King, 2014). The main route of transmission of mosquito-borne diseases is through their bites, in which viruses, parasites, or bacterial agents are transmitted to humans by the infected mosquitoes (Caraballo and King, 2014). Dengue fever virus is transmitted by *Aedes* mosquitoes especially; *Aedes aegypti* and *Aedes albopcitus*. *Aedes aegypti* is also a vector for yellow fever (Black *et al*., 2002) and *Dirofilaria immitis* (Russell *et al*. 2005) while *Culex* mosquitoes transmit filiariasis (Onyido *et al*., 2016). The parasites of the malaria disease are transmitted by the *Anopheles* mosquitoes most common being *Anopheles gambiae* and *Anopheles funestus* (Onyido *et al.*, 2008).

Mosquitoes have worldwide distribution and are found in both tropics and temperate regions of the world. Some mosquito genera such as *Anopheles, Culex,*

Mansonia and Aedes are common in the tropics or warm climates while a few others are found in temperate regions of the world (Leopoldo, 2008). Mosquitoes generally lay their eggs on the open surface of water both in permanent and temporary water collections or attach them to some partially submerged objects depending on the species. Examples of such permanent water collections are fresh water swamps, edges of rivers, marshes, rice fields, slow flowing streams and irrigation dishes while the temporary water collection includes small ponds, tree holes, plant leaf axils, crab holes, puddles and rock pools. Man-made water breeding sites include water collections in vehicle tyres, tin cans, man and animal foot prints, coconuts shells, plastic containers, bamboo stumps, scoops in concrete slabs used in feeding animals, water holding cisterns and tanks, and cassava fermentation pots (Onyido *et al.*, 2006; Service). Mosquitoes are widespread and their distribution depends largely on ecological and environmental factors such as rainfall pattern, relative humidity, temperature, turbidity and vegetation amongst others. For example, rainfall generally brings new opportunistic breeding places. Nonetheless, rainfall can also destroy existing breeding places, heavy rains can change breeding pools into streams, impede the development of mosquito eggs or larvae or simply flush eggs or larvae out to the pools (Bruce-Chwatt, 1991). Also, Service (2008) observed that mosquito populations decrease in the dry season due to high temperatures and lack of breeding places.

The breeding habitats of mosquitoes vary by species. Oviposition takes place just above water level. *Aedes* species prefer to lay eggs in containers which include discarded old tyres of vehicles, flowerpots, gutters, trash cans or natural containers such as leaf axils and treeholes that can hold water (David *et al.,* 2004). However, *Aedes* species such as *Ae. aegypti* and *Ae. albopictus* which are the primary vectors of arboviruses worldwide, prefer to breed in containers. *Ae. albopictus* display

opportunistic behaviour, inhabiting both artificial and natural containers. *Ae. triseriatus*, *Ae. sierrensis* and *Aedes geniculatus* prefer to breed in tree holes while *Ae. japonicus*, and *Aedes atropalpus* breed in rock pools (David *et al.*, 2004; Talsania *et al*., 2013). *Ae. africanus* breeds in moist treeholes in tropical rain forests and also lays eggs bamboo stumps, and tree forks (Huang, 1990 and Schaeffer *et al*, 2008). *Ae. vittatus* breeds in in rock holes, hoofprints, boats, wells, tree trunks, treeholes, bamboo cups and pots, occasional utensils, rock pools, outcrops or river beds, and coral, and occasionally at the peak of the breeding season, in open concrete floodwater drains (Onyido *et al.*, 2009).

Culex mosquitoes breed in various types of stagnant water and lay their eggs in raft-shaped batches on a variety of standing water surfaces which may be found in sewage systems, drainage systems and container sources. These preferred oviposition habitats may include rainwater barrels, catch basins, storm drains, and septic tanks that are rich in decaying organic materials (Adebote *et al*., 2006; Ilahi and Suleman, 2013). *Culex pipiens* and *Culex quinquefasciatus* prefer to breed in sewage systems and drainages and containers. *C. molestus* and *C. restuans breeds in* dranaiges *while Culex tarsalis*, *Culex nigripalpus*, *C. salinarius*, *C. modestus*, *C. annulirostris*, *C. sitiens* breed in Open habitats (Foster and Walker, 2012; David, *et al.*, 2004).

Anopheles mosquitoes breed in ground pools. *Anopheles gambiae* the most efficient vector in sub Saharan Africa, breed in muddy sunlit ground pools of water of various sizes, animal foot prints and motor-vehicle tyre prints. It is occasionally found in man-made containers such as wheel barrows, motar pans, open tanks, canoes and abandoned concrete mixers, (Onyido *et al.*, 2009a). *An. melas*, a member of *An. gambiae* complex, is essentially a major vector of malaria around lagoons. *An. arabiensis* is a savannah vector with isolated populations in

deforested areas and are predominant in the dry season (Wagbatosoma and Ogbeide, 1995). *An. arabiensis* could be anthropophagous where there are fewer animal hosts. In the Savannah-forest, *An. arabiensis* appear to be a good vector of malaria and is responsible for 34.1% of human blood meals (Hougard *et al*., 2002). *Anopheles funestus* is a complex species with uneven distribution throughout Nigeria. It breeds in weedy parts of large collections of more or less permanent clear water such as swamps, edges of streams, rivers and other water pools often associated with water lettuce, *Pistia stratiotes* and grasses, even in ditches and ponds under shades. Also *Anopheles* species have been reported to adapt themselves to the various ecological circumstances provided by all stages of rice culture including nursery, watering, planting, growing, tillering, maturation, harvesting and land fallow (Service, 1980).

Some mosquitoes are species complexes that are difficult to separate morphologically. For example, the *An. gambiae sensu lato* is a species complex comprising seven sibling species that are morphologically indistinguishable. *An. gambiae sensu stricto* (s.s.) and *An. arabiensis* are the most common species. Of the remaining members *An. quadriannulatus* species A, which is widespread in southern Africa, and *An. quadriannulatus* species B, found in Ethiopia, are considered to be zoophilic (Coetzee, 2004; Coetzee *et al.*, 2000). *An. melas* and *An. merus* are both salt water breeding and consequently only important vectors in coastal regions (Moreno *et al.*, 2004; Tsy *et al.*, 2003). *An. bwambae* also a member of the complex is restricted to a region close to the Buranga hot springs in Uganda (White, 1985). The marked differences in the vectorial efficiency of the species within the complex mean that correct identification of species is vital for focussed effort in their disease control programmes.

The method to identify, label and classify organisms is largely built around morphological characteristics developed by Carl Linnaeus in the 18th century (Hebert *et al.,* 2003a). Morphological identification of species is obviously limited since it does not consider genetic variation of individuals or morphological complexity. Today taxonomists also consider physiology, behaviour and population biology in the classification of new species. Since the discovery of DNA and recognition of its role in inheritance, genetic variation plays a major role to distinguish the diversity of life. DNA based identification could fill these gaps and most importantly add new biological diversity to the already known. Furthermore, molecular based methods are generally used for species identification of viruses, bacteria and protozoa, in taxonomic studies (Adl *et al*., 2007; Pace, 1997). In a more general way, whole genome sequencing and other sequencing based methods are used to identify diversity. Another approach is screening for biological markers such as microsatellites, amplified fragment length polymorphism or single nucleotide polymorphism (Arif *et al*., 2010).

1.2 Statement of the Problem

In spite of the abundant literature on mosquito studies, none has given a detailed ecological work on the habitats, habits and influence of the ecological factors on the abundance of the mosquito species in Awka-South Local Government Area of Anambra State. Moreso, identification has relied mainly on morphological technique which has limited application on the identification of sibling species. It is therefore pertinent that detailed ecological work and molecular characterization of the mosquito vector species in Awka South Local Government Area should be done to provide baseline data for the control of the various mosquitoes species.

1.3 Justification of Study

The effective control of mosquito vector species demands that correct identification and ecological studies be carried out to elucidate the habitats and behaviours of the vectors before embarking on control programmes. This is necessary to avoid waste of resources in combating harmless species. WHO (2003) advised that planning, execution and evaluation of anti-vector measures has to be based on a perfect knowledge of the activities of the vector species. They further stressed that the knowledge of the breeding, resting and biting habits and longevity of the vector species is important for anti-vector measures and the evaluation of such measures.

1.4 Aim and Objectives

The study was aimed at investigating the ecology and molecular characterization of mosquito vectors of public health diseases in Awka-South Local Government Area, Anambra State, South Eastern Nigeria.

The specific objectives of the study were to determine:-

1. the abundance and breeding habitats of mosquito species.
2. the biting and resting behaviour of the adult mosquitoes.
3. the physiological states, Indoor resting density, biting rates and sporozoites rates of female mosquitoes collected indoors
4. physicochemical and climatic factors influencing the survival of larvae and adult mosquitoes in their environments
5. morphological and molecular identification of adult mosquitoes.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Mosquito Ecology

Mosquitoes occur in almost every continent in the world except Antarctica. The important man-biting mosquitoes belong to the genera *Aedes, Anopheles, Culex, Mansonia, Haemagogus, Sabethes,* and *Psorophora* (Foster and Walker, 2002). *Haemagogus*, *Psorophora* and *Sabethes* are found only in Central and South America. Mosquitoes are widespread and can breed in a wide range of aquatic habitats in both tropics and temperate regions of the world.

2.1.1 Some ecological studies on mosquitoes

Anosike *et al.* (2007) studied the tree-hole breeding mosquitoes in tropical rainforest of Imo State, south-eastern Nigeria. This was done in two forest reserves in Orlu Senatorial Zone, Imo State, Southern Nigeria between May and October 2002. Tree-holes, bamboo stumps, leaf axils of cocoyams, pineapples, plantain and banana were sampled. Mosquitoes were collected from all biotypes sampled. Sixteen species made up of 4 genera of mosquitoes were recovered. Kalu *et al.* (2012) published a study in Umuchieze and Uturu, Abia State, Nigeria, using all-night human-bait catches and indoor insecticide spray sheet method for two consecutive years. A total of 500 adult female anopholines were captured. *An. gambiae complex*, *An. funestus* and *An. moucheti* were found in the communities. *An. gambiae complex* was the dominant species in both study communities with the species relative abundance of 55.29% and 51.82% recorded in Umuchieze and Uturu communities, respectively. In a study of crepuscular man-biting mosquitoes of a tropical zoological garden in Enugu, Onyido *et al.* (2009a) recorded *Ae. aegypti, Ae. albopictus, Ae. luteocephalus, Ae. vittatus* and *Ae. africanus*.

Onyido *et al*. (2009b) studied man-biting mosquitoes at the permanent site hostels of Nnamdi Azikiwe University Awka, Southeastern Nigeria. Mosquito larvae were sampled from flood pools around the hostels using ladle. Indoor biting and resting adult mosquitoes were collected using pyrethrum knockdown collection method (PKC). Outdoor biting mosquitoes were collected using human volunteers as baits and collectors. A total of 1,265 mosquitoes consisting of five species collected by larval sampling, 72 indoor-biting adult mosquitoes by pyrethrum knockdown and 132 outdoor-biting mosquitoes by human bait catch. *C. quinquefasciatus* 466 (36.84%) and *Ae. aegypti* 400 (31.62%) formed the bulk of the larval collection. *Anopheles gambiae*, 50(69.45%), constituted the highest percentage of indoor biting and resting mosquitoes. An overall room density of 8.2 mosquitoes/room/night was recorded of which 6 mosquitoes/room/night were *An. gambiae complex*. Outdoor-biting mosquitoes were *Ae. aegypti* 67(57.5%) and *Ae. albopictus* 38(28.79%). The authors reported that students in the hostels were exposed to mosquito bites and mosquito-borne diseases. They recommended that the students should do some self-protection interventions while the institution should provide other mosquito control strategies including screening of the doors and windows of the student hostels.

Service (1974) carried out a survey of the relative prevalence of potential yellow fever vectors in North-West Nigeria. Examination of over 6,700 water pots in six areas in September, 1973 showed that 11-53% contained *Ae. aegypti* larvae. In some areas, larvae of *Ae. vittatus* were found in up to 18% of the pots. The authors reported that in the early evenings, *Ae. aegypti* was the most abundant man-biting mosquito in the villages; and *Ae. vittatus* was also caught at bait in some villages. Service concluded that the only yellow fever vectors in the areas surveyed were *Ae. aegypti* and *Ae. vittatus*.

Okogun *et al*. (2005) recorded in a study of ecology of mosquitoes of Midwestern Nigeria' 17 species of mosquitoes belonging to three genera; *Anopheles*, *Culex* and *Aedes*. A total of 736 mosquito larvae were encountered in artificial containers and 568 larvae were collected from natural sources. The authors concluded that human activities and increasing environmental modification contributed to breeding of the mosquitoes. The authors recommended selective vector control measures including larviciding, particularly before onset of rainy season.

Oyewole *et al.* (2010) studied species composition and roles of anopheles mosquitoes in malaria transmission along Badagry axis of Lagos, Nigeria. One thousand nine hundred and thirty-eight female anopheles mosquitoes were collected from the year 2005 to 2007. These were *An. gambiae* complex, *An. melas* and *An. nili*. Adeoye *et al.* (2012) studied the abundance and composition of endophilic mosquitoes in the University of Lagos and its environment. A total of 573 mosquitoes were collected. *C. quinquefasciatus* was the most abundant (90.6%). *An. gambiae* complex was 5.2% and *Ae. aegypti* was 4.2%.

Species composition of anopheles mosquito in three villages of Uratta Owerri North, Imo State, was studied by Oguoma *et al.* (2010). Nine hundred and fifty-three anopheles mosquitoes were collected. *An. gambiae complex* 536 (56.20%) was the most abundant species. This was followed by *An. funestus* complex 306 (32.10%), *An. coustani* 65 (6.80%) and *An. moucheti* 46 (4.80%).

Epidemiology of tree-hole breeding mosquitoes in the tropical rainforest of Imo State, South-east Nigeria was studied by Anosike *et al.* (2007). Species of mosquitoes encountered included: *Ae. aegypti*, *Ae. africanus*, *Ae. simpsoni*, *Ae. albopictus*, *Ae. stokesi*, *Ae. taylori*, *Ae. apicoargenteus*, *C. quinquefasciatus*, *C. nebulosus*, *C. tigripes*, *C. decens* and *Toxorhynchites viridibasis*.

Boorman (1960) reported two series of 12 monthly mosquito catches with human bait made by the 24-hour method at various stations at Ilobi, near Lagos to provide data on the habits of the commonest man-biting species of mosquitoes. *Ae. aegypti*, *Ae. grahami*, *Ae. simpsoni*, *Ae. africanus*, *Ae. luteocephalus*, *Ae. circumluteolus* *Mansonia africana* were identified.

In a study of Mosquito fauna of Museum and Zoological Garden Complex in Jos, Nigeria, a tourist attraction centre, Onyido, *et al.* (2009a) caught 627 mosquitoes consisting of 4 genera and 9 species. The species of mosquitoes were *Ae. aegypti*, *Ae. africanus*, *Ae. vittatus*, *C. quinquefasciatus* and *Eretmapodites chrysogaster*. *Ae. aegypti* was the most abundant mosquito, 455 (72.6%) collected. This was followed by *C. quinquefasciatus*, 100 (23.8%), in human bait collections. The authors reported that Jos Zoological Garden Complex as a holiday resort for visitors is a place of constant interaction between man and animal populations which provided a regular supply of blood-meals for mosquitoes and possibly the transmission of many diseases to man.

Oyewole *et al.* (2005) investigated the activities and habits related to malaria transmission, epidemiology and control of mosquitoes at Ilara and Ijesa-Isu communities in Western Nigeria. Adult mosquitoes were collected twice a month from December 2003 to January 2005. A total of 1,500 anopheline mosquitoes were collected. These mosquitoes were identified using morphological and molecular techniques. Malaria vectors identified included *An. gambiae* complex 1345 (89.7%) and *An. funestus* group 155 (10.3%). The authors recommended that there should be accurate identification of *Anopheles* mosquitoes attracted to man in order to determine vector-host contact necessary for malaria transmission. This would also support the information required to formulate vector control programme.

Adebote *et al.* (2006) studied the breeding of mosquitoes in peridomestic containers and its implication in yellow fever transmission in villages around Zaria, Northern Nigeria. Systematic sampling, using small soup ladle or dipper was adopted for larval sampling. Containers of water and tree-holes were sampled. The authors reported that five species of mosquito breed in various water storage containers in the peridomestic environments of the four villages around Zaria. *Ae. aegypti* and *C. nebulosus* were collected from discarded automobile tyres. *C. quinquefasciatus* larvae were only found in clay pots and wells in a village. The authors reported this to be a contrast to *C. quinquefasciatus* preference for foul and polluted water as breeding habitat. *C. tigripes* only occurred in a water tank. *C. horridus* species were collected in wells as peridomestic breeding sites.

Emidi (2012) in Tanzania reported that man-biting rates of mosquitoes correlated significantly with agricultural activities and other land use types. In the report, *An. gambiae complex* and *An. funestus* were found with high biting rates in the proximity to piggery and temporary wells. *Aedes* species was associated with proximity to bovid and poultry units. The diverse land types and the presence of multiple distinct habitat types promoted proliferation of mosquitoes from all genera.

Mboera, *et al*. (2006) reported the surveys carried out in six villages at different altitudes in two districts in central part of Tanzania. A total of 1,291 mosquitoes were collected, of which 887 mosquitoes were collected by light traps and 404 mosquitoes by indoor pyrethrum spray catch technique. One thousand and twenty-six (79%) of the mosquitoes were *An. gambiae complex*, 3 (0.3%) were *An. funestus* and 262 (20.3%) were *C. quinquefasciatus* while *C. cinereus*, *An. coustani* and *Aedes* species accounted for 0.5%. None of the *An. gambiae complex* dissected was infected with malaria sporozoites.

In Liberia, Fox (1958) was able to identify six species of *Anopheles,* three of *Mansonia,* seven of *Aedes* and eight of *Culex.* Twenty-four man-biting mosquito species with seasonal distribution were observed in Liberia.

Foote (1953) presented the pictorial keys of mosquitoes of medical importance around Anglo-Egyptian Sudan. These mosquitoes include *An. gambiae complex*, *An. funestus*, *An. nili*, *An. rufipes*, *An. cinereus*, *An. coustani coustani*, *An. coustani ziemanni*, *An. d'thali*, *An. implexus*, *An. leesoni*, *An. macmahoni*, *An. maculipalpis*, *An. marshalli marshalli*, *An. marshalli gibbinni*, *An. multicolor*, *An. obscurus*, *An. pharoensis*, *An. pretoriensis*, *An. rivulorum*, *An. rupicolus*, *An. squamosus squamosus*, *An. symesi*, *An. theileri septentrionalis*, *An. turkhudi* and *An. wellcomei*. The forest inhabiting monkeys had yellow fever. The monkey-to-monkey transmission was believed to be maintained by *Ae. vittatus* and *Ae. africanus.* At the ground level yellow fever was transmitted by *Ae. simpsoni (bromeliae).* Transmission from monkey to man under tree canopies was by *Ae. metallicus*, *Ae. luteocephalus*, *Ae. furcifer* and *Ae. taylori*. *Ae. aegypti* was prevalent only at certain times and localities.

Ilahi and Suleman (2013) carried out a comprehensive survey of mosquitoes in Swat Pakistan, from April to September during 2000. They sampled both, adult and immature stages of mosquitoes. A total of 21 species in five genera were observed. In the study, most of the species built up their populations in June, July and August. Few increased their populations in September. Rice field (harbouring 12 species) was the most favourable site for mosquito breeding, five species in river margins and four species each in temporary pools and springs. Composition and relative abundance of mosquitoes in Swat, Pakistan showed that *C. quinquefasciatus* occurred most in 48 samples (out of 138 samples). *An. maculates* (17 samples), *C. pseudovishnui* (14 samples), *An. annularis* and *An. stephensi* (13

samples each). Others were *C. bitaeniorhynchus* (11 samples), *An. splendidus* (5 samples) and *C. theileri* (4 samples). In the study *Ae. aegypti* was recovered from tyres. *Ae. aegypti* which has not been reported earlier from this area of Pakistan was also collected.

Amerasinghe (1982) recorded thirty-six species of mosquitoes in a Forest Reserve in Sri Lanka. Twenty-one species were taken at day-time in human bait catches. Immature stages of seventeen mosquitoes were collected from bamboo stumps, tree holes, kitul-palm stumps and temporary ground pools. Sixty-four types of single and multi-species occurrences were recorded from breeding sites. Utrio (1979) in Finland recorded thirty-eight stated species of mosquitoes in his study.

Foster and Walker, (2002) and Eldridge and Edman, (2004) reported that *C. quinquefasciatus*, a major vector of nocturnally periodic *Wuchereria*, breeds in man-made polluted water. *Mansonioides*, vectors of *Brugia*, breed in water bodies infested with certain preferred weeds (*Pistia*, *Eichornia*, *Lemna*) only. They cannot oviposit in water infested with ferns like *Azolla* and *Salvinia*. Vast water bodies infested with *Azolla* or *Salvinia* do not favour breeding of *Brugia* vectors.

Freeborn and Brookman (1943) working for United States Public Health service provided identification guide to the mosquitoes of the Pacific Coast States. The authors were able to prepare a list of Pacific Coast mosquitoes and compared them to similar species in various parts of the world as early as 1940s.

Lounibos (2010) in his work, 'Invasion Biology of *Ae. albopictus*' stated that *Ae. albopictus* had become one of the most common man-biting mosquitoes. This mosquito was introduced in other countries from South-East Asia in mid-1980s. The new ranges include South-Eastern USA, Brazil, Southern Europe and West Africa. The project was aimed at understanding the ecology of invasions by *Ae.*

albopictus and to use this understanding to predict the consequences for the transmission of arboviral diseases, especially for dengue.

Resting and biting habits of *An. sundaicus* were studied in Car Nicobar Island, India by Kumari and Sharma (1994). Substantial numbers of *An. sundaicus* were observed to rest outdoors, although the species prefer to rest indoors. Indoor man-biting of *An. sundaicus* was significantly higher than outdoors and the species showed bimodal biting activity with first peak between 2130 to 2230 hours and second between 0130 to 0230 hours. *An. sundaicus* prefers to feed on the people's legs and hands.

Marshall *et al.,* (1982) reported from Murray Valley of South-Eastern Australia of capturing 138,359 mosquitoes. Ninety-eight percent of these mosquitoes were *C. annulirostris*.

2.2 Mosquito Breeding Habitats

The immature stages (larvae and pupae) of mosquitoes are always found in stagnant water. Breeding sites may be anything from water in discarded automobile tyres and the leaf axils of plants, to pools, puddles, swamps, and lakes. It is very important to note that mosquito species differ in their habits especially breeding habits, biting behaviour and flight range (Onyido *et al.*, 2006; Service, 1980).

2.2.1 *Aedes* Mosquitoes Breeding Habitats

The breeding habitats of *Aedes* mosquitoes can be subdivided into two main categories namely container breeding mosquitoes and floodwater breeding mosquitoes. Container breeding *Aedes* species prefer to lay eggs in artificial containers (e.g., flowerpots, gutters, and trash cans) or natural containers (leaf axils

and tree holes) that can hold water. Oviposition takes place just above water level. *Ae. aegypti* prefer more artificial container types while *Ae. albopictus* is a more opportunistic species inhabiting both artificial and natural containers. *Aedes* mosquitoes breeding in tree holes include *Ae. triseriatus, Ae. sierrensis* and *Ae. geniculatus*. Rock pool breeding are *Ae.vittattus, A.africanus, A.luteocephalus, Ae. japonicus* and *Ae. atropalpus* (Adebote *et al.*, 2006; Ilahi and Suleman, 2013; Talsania *et al.*, 2013).

Floodwater mosquitoes lay eggs in wet or moist substrate or waterlogged soil in ground depressions subject to temporary floods. Females differentiate between certain soil types to find the most suitable place for egg laying. Eggs of floodwater mosquitoes remain dormant until they are flooded and conditions are favourable for hatching. Floodwater mosquito populations can even withstand extended dry or cold periods in the egg stage. Select species of floodwater mosquitoes are able to fly long distances to get a blood meal and are aggressive and painful biters. While floodwater *Aedes* will lay their eggs in a variety of habitats, these habitats can typically be categorized into four ecotypes namely floodplain, irrigation, woodland and coastal habitats (Valent, 2018).

Floodplains include low-lying areas along rivers, streams, and lakes that are temporarily inundated at various points in the year. Other low-lying depressions, such as potholes and floodplains that will hold water following rain events. Common floodplain mosquitoes are *Ae. aegypti*, *Ae. albopictus, Ae. vexans* and *Ae. sticticus* (Huang, 1977). Many species of floodwater mosquitoes have adapted to develop in flood-irrigated habitats including flooded pastures, rice fields, and duck refuges. Common flooded irrigation mosquitoes include *Ae. nigromaculis, Ae. melanimon, Ae. caspius* and *Ae. vexans* (Schaeffer *et al.*, 2008).

Woodland pool mosquitoes commonly develop during winter and early spring. Larvae can be found in pools in forested areas following spring snowmelt or rains. Common woodland mosquitoes *Ae. canadensis, Ae. stimulans, Ae. atlanticus, Ae. increpitus, Ae. communis*, *Ae. washenoi, Ae. rusticus, Ae. cantans* and *Ae. cataphylla* (Talsania, *et al*., 2013). Coastal, or saltwater, mosquitoes develop in low-lying plains in coastal areas which include salt marshes, brackish swamps, and dredge spoils. Common coastal mosquitoes: *Ae. sollicitans, Ae. taeniorhynchus, Ae. squamiger, Ae. caspius, Ae. detritus, Ae. vigilax* and *Ae. camptorynchus* (David *et al.,* 2004).

2.2.2 *Culex* Mosquitoes Breeding Habitats

Culex mosquitoes lay their eggs in a variety of standing waters, these habitats can typically be categorized into four ecotypes namely sewage, container sources, drainage and open habitat (Adebote *et al.*, 2006; Ilahi and Suleman, 2013; Foster and Walker, 2002; David *et al.*, 2004). The sewage habitats include sewage poluted environment with mixed sewage and rainwater. These habitats include cess pits, septic tanks, and blocked sewerage systems. Key sewage system breeders are *C. pipiens* and *C. quinquefasciatus* (Adebote *et al.*, 2006)

Container sources are generally man-made sources that will catch and hold water, such as discarded automobile tyres, buckets, and rain barrels. Key container *Culex* include: *C. pipiens C. quinquefasciatus* (Ilahi and Suleman, 2013). Drainage habitats include catch basins, channels, roadside ditches, and underground storm water/drainage systems. Key drainage system *Culex* mosquitoes include: *C. pipiens, C. quinquefasciatus, C. molestus* and *C. restuans* (David *et al.*, 2004). Open surface habitats include surface-water habitats that become stagnant and enriched to support *Culex* larval development, such as swamps, marshes, bogs, rice

fields, and pastures.Common open habitat *Culex* mosquitoes include, *C. pipiens C. quinquefascitus*, *C. tarsalis*, *C. nigripalpus*, *C. salinarius*, *C. modestus*, *C. annulirostris* and *C. sitiens* (Talsania, *et al*., 2013).

2.2.3 *Anopheles* Mosquito Breeding Habitats

Typical habitats of *An. arabiensis* and *An. gambiae* include puddles, shallow ponds, burrow-pits, brick-pits, tyre tracks, ditches, human foot and animal hoof prints which are often created by the activities of humans or domestic animals (Koenraadt *et al*., 2004). These habitats are open, containing no, little or low (grass) aquatic vegetation (Mwangangi *et al*., 2007a) and are often of a transient nature, as their availability corresponds to precipitation (Koenraadt *et al*., 2004). *An. gambiae* can colonize a breeding habitat within a few days after the site is created (Minakawa *et al*., 2005). Besides temporary habitats, *An. arabiensis* is also found in garden wells (Robert *et al*., 1998) and water storage tanks. Another typical characteristic of breeding sites of *An. gambiae* is their shallow nature. Gimnig *et al.* (2001) showed that water bodies inhabited by *An. arabiensis* were on average 18.0 (95% CI ± 3.5) cm deep, by *An. gambiae* 29.4 (± 10.7) cm and by both species 9.7 (± 4.1) cm on the average. In another field study, average depths of 6.2 (±5.3 SD) and 10.6 (± 7.2) cm were recorded in dirt tracks and in ditches, respectively (Koenraadt *et al*., 2004).

2.3 Mosquitoes Habits

2.3.1 Mosquito Feeding and Resting Habits

Mosquitoes in general feed and afterwards rest. Different species of mosquitoes belonging to different genera have feeding and resting behaviour peculiar to them. While some feed outdoors and rest indoors, others feed indoors and also rest

indoors. *Ae. aegypti* almost exclusively feed on humans 99%, in single host species, and 97% of multiple-host bloodmeals including at least one human host. A low frequency of other hosts, including bovine, swine, cat, rat, and chicken were detected, but they represented <1% of bloodmeals. An even higher percentage of human feeding was detected in *Ae. albopictus*. Hosts of *Ae. albopictus* collected in a study were entirely human (100%) from both single and mixed meals. In the small number of double-host meals from *Ae. albopictus*, the study reported 3.8% as swine–human and <1% from dog–human and cat–human. Forage ratios for *Ae. aegypti* indicated that human, dog, and swine were preferred hosts in order of preference. (Alongkot and Laura, 2005). *Aedes* mosquitoes are crepuscular (active at dusk or dawn) feeding mainly at sun dawn and stops biting shortly in the early hours of the night. *Ae. aegypti* and *Ae. albobictus* are mainly exophagic feeding mainly outdoors.

C. quinquefasciatus fed on man, *C. pipiens* and *C. restuans* fed primarily on birds. *C. salinarius* Coquillett and *Coquillettidia perturbans* fed mainly on mammals, with fewer blood meals taken from birds. Multiple blood feedings on avian hosts were detected in *C. pipiens* and *C. Restuans* (Charles *et al.*, 2002). *Culex* mosquitoes are crepuscular (active at dusk or dawn) and nocturnal feeding throughout the night. They feed mainly outdoors (exophagic) and sometimes indoors (endophagic) as with *C. quinquefasciatus* (Service, 1980).

Anopheles species may prefer to feed on humans (anthropophily) or animals (zoophily). Most *Anopheles* mosquitoes are not exclusively anthropophilic or zoophilic. However, the primary malaria vectors in Africa, *An. gambiae* and *An. funestus*, are strongly anthropophilic and, consequently, are two of the most efficient malaria vectors in the world (CDC, 2008). Most *Anopheles* mosquitoes

are crepuscular (active at dusk or dawn) or nocturnal (active at night). Some *Anopheles* mosquitoes feed indoors (endophagic) while others feed outdoors (exophagic) (Ebenezer *et al*., 2013 and Adeleke *et al*., 2010). After blood feeding, some *Anopheles* mosquitoes such as *An. gambaie* and *An. funestus* prefer to rest on the walls indoors (endophilic) while others may prefer to rest outdoors (exophilic) (Service, 1980).

2.3.2 Mosquito Dispersal and Flight Range

Female mosquitoes may disperse by flight to find mates, food or oviposition sites. Dispersal to seek a host is epidemiologically important as the mechanism whereby female mosquitoes acquire and disseminate pathogens. Additionally, dispersal for oviposition is relevant to disease propagation. Females of the classic dengue vector *Aedes aegypti* (Linnaeus) distribute their eggs among several oviposition sites (Reiter *et al*., 1995) and frequently take multiple blood meals within a single gonotrophic cycle (Scott *et al*., 1993), which increases dispersal of their progeny.

A Study of the flight range of *Ae. aegypti* reveal that females generally fly about 100-500m from their larval habitat, but study conducted in urban San Juan, Puerto Rico, specifically reported that female *Ae. aegypti* could, in a few days, travel at least 441 m from a releasing point (Reiter *et al.*, 1995). Recapture studies of marked female *Ae. albopictus* (Skuse) showed a maximum dispersal of 400-600 m (Niebylski and Craig, 1994).

Gabriel *et al.* (2014) studied the dispersal of adult *Culex* mosquitoes in an Urban West Nile Virus Hotspot and reported that about 90% of the female *Culex* mosquitoes stayed within 3 km of their larval habitat.

Kaufmann and Briegel (2004) who conducted a study on the flight performance of *An. gambiae* and *An. atroparvus* observed that the maximal flight distances of *An. gambiae* were 9 km when sugar-fed and 10 km when blood-fed, while in starved females it was below 3 km and the average speed was around 1 km/h. In *An. atroparvus,* the maximal flight distances were 10-12 km when sugar-fed, 4.5 km when blood-fed, and below 3.5 km when starved, with an average speed of 1.3 km/h. Flight performances consisted of 1-4 h intervals of continuous flights, but mainly of bouts shorter than one h, randomly distributed during the long flight trials in both species.

2.3.3 Mosquitoes Longevity

Adult mosquitoes' lifespan vary from a few days to several months, depending on the species. The males live only about a week, long enough to swarm and mate with the females. The female lifespan is longer, up to a month or two, though many die sooner as they are, eaten by predators, blown around by storms or killed as they were trying to suck blood to help them develop their eggs.

The lifespan of the adult female *Aedes* mosquito can range from about 14days to about 30days, depending on the surroundings and environmental conditions. The *Culex* mosquitoes that carry the West Nile virus in the United States live for 5 to 15 days after biting a virus-infected bird before they can pass the virus on to a human. Studies has reported that the longevity of the *Anopheline* mosquitoes ranged from 13 to 23 days, sufficient for completing the intrinsic incubation cycle and for malaria transmission (Ermi *et al.*, 2012; WHO, 2003).

2.4 Ecological factors affecting distribution and abundance of mosquitoes

Ecological factors affecting the distribution and abundance of mosquitoes includes climatic and physicochemical factors.

2.4.1 Climatic factors (temperature, humidity and rainfall)

Chandra (2008) in a review entitled 'Nature Limits Filarial transmission' noted that the effects of temperature and humidity are vital on the development of *Wuchereria* larvae in its vector. Under experimental conditions, development of lymphatic filarial parasites in the mosquito takes two weeks at 27^{o}C and 90% humidity. The period of mosquitoes species larval development varies with season. At high temperatures and low moisture the complete cycle occupies 10-14 days but it is retarded to 6 weeks by cold. Experiments on the filarial vector *C. quinquefasciatus* revealed that its density was found to be significantly lower in the rainy season in comparison to dry seasons in different endemic areas of the tropics as their breeding places become flooded during the time. The hot months of the rainy season and sometimes summer were found to be the high time for filarial transmission in most endemic areas. This is established by the highest infection and infectivity rates (with filarial parasites) of the vector. The season provides optimum conditions to raise the vector efficiency index to its peak. Vector efficiency index is based on rapid parasitic development, proper nursing and low parasitic damage or death (Sara, 1990).

It is also observed that rise or fall of temperature and fall of humidity caused deformity and degeneration of a large number of filarial parasites in the mosquito body. Meyrowitsch *et al*. (2011) noted a longitudinal decline in the density of malaria vectors during the period 1998-2009. Part of the decline was associated with changes in the pattern of monthly rainfall. A similar decline in malaria vector

densities contributed to the decrease in levels of malaria infection reported from many parts of Sub-Saharan Africa.

Umaru *et al*. (2007) observed that there were no significant changes in the relative abundance of different species of *Culex* mosquitoes due to changes in season, except *C. pipiens fatigans* (36.4%) and *C. tigripes* (12.4%).

2.4.1.1 Relative Humidity

The interaction between rainfall evaporation, runoff, and temperature modulates the ambient air humidity, which in turn affects the survival and activity of *Anopheles* mosquitoes. To survive, they need at least 60% humidity (Bruce-Chwatt, 1991; Dutta and Dutta, 1978). Higher levels lengthen the life span of the mosquitoes and enable them to infect more people (Dutta and Dutta, 1978). In the Sahel, Sara (1990) observed that a constant high temperature and low relative humidity led to drastic decline in the mosquitoes' populations between March and May a hot dry season which could affect their life-span, reproductive and feeding capacities. Most of the breeding grounds dry up during these months, and insect activities are correlated with daily rhythm of temperature and humidity. Such rhythm is most evident in areas with hot and relatively dry days (Gadzama, 1977; Sara, 1990). The best conditions for the development of *Plasmodia* in the *Anopheles* and the transmission of infection are when the mean temperature is within a range of 20-30^{o}C, while the mean relative humidity is at least 60%. A high relative humidity lengthens the life of the mosquito and enables it to live long enough to transmit the infection to several persons. In Northern Nigeria, Idris (2009) noted in his survey that high humidity favours the development of mosquitoes and it could be up to saturation of 80% relative humidity at 28^{o}C. They are active at high humidity provided the temperature is about 25^{o}C.

2.4.1.2 Rainfall

Rainfall generally provides opportunistic breeding places. The appearance of such opportunistic mosquito breeding sites sometimes preceeded epidemics (Bruce-Chwatt, 1991). Nonetheless, rainfall can also destroy existing breeding places, heavy rains can change breeding pools into streams and impede the development of mosquito eggs or larvae or simply flush eggs or larvae out to the pools. Conversely, exceptional drought conditions can turn streams to pools in which mosquitoes would breed in profusion. This was observed in Northwest zone of Sri Lanka, where during the years of relative drought, great breeding of *An. culifacies* took place in streams and was followed by severe epidemics of malaria (Bruce-Chwatt, 1986). Also, it was fortuitous that during the time period of the six years of the Garki Malaria project which spanned the 1973 drought period served as another example thus, it was possible to observe the effect of low rainfall on malaria in the Garki District of Kano State (Betterton and Gadzama, 1981).

Of the mosquitoes species found in Garki area, three were considered to be major malaria vectors. These were *An. funestus* and two sibling species of *An. gambiae*. The two siblings look alike, but one is more able to tolerate dry conditions of the Sudan savanna than the other and they also differ in aspect of their biology and behaviour. It was found that the numbers of *An. funestus* were severely depressed during the drought years, which this was not the case for *An. gambiae* (Molineux and Gramicca, 1980). This was probably due to the difference in the breeding habits of the two species; *An. funestus* prefers semi-permanent waters Shaded by vertical aquatic plants such as read, whereas. *An. gambiae* will breed in the small puddles left by animals hoof prints and the like. In time of drought there would be insufficient water to establish *An. funestus* breeding sites, but even a small amount of rain would be sufficient to create sites suitable for *An. gambiae* (Betterton and

Gadzama, 1981). Thus, it is erroneous to believe that malaria, as water-related disease is necessarily reduced by a period of drought (Betterton and Gadzama, 1981). Repeated rains cause severe flooding resulting in temporary flushing out of breeding places. Consequently the breeding of a vector population is greatly reduced, but becomes, re-established when favourable conditions are restored.

Uttah *et al*. (2013) did a longitudinal study to determine the abundance and biting patterns of *C. quinquefasciatus* in the coastal region of Nigeria from September 2005 to August 2006. The highest number of females was caught in the month of August (wet season) and it represented nearly a quarter (24.0%) of the total females collected. The abundance of *C. quinquefasciatus* followed the pattern of rainfall with the populations starting to expand at the onset of the rains. The highest increase was found when the temperature had peaked. The mean biting rate was 3.2 times more in the rainy season than in the dry season. The transmission potential was higher in the dry season.

Kalu *et al*. (2012) assessed the seasonal variations of nocturnal, endophagous and anthropophagus *Anopheles* species in rural communities in Uturu and Umuchieze communities in Abia State, Nigeria. The relative abundance of prevalent *Anopheles* species (*An. gambiae complex*, *An. funestus* and *An. moucheti*) were higher during rainy season than the dry season. The periodic occurrence of the important malaria vectors explains periodicity of malaria epidemiology in the two communities (Gadzama, 1983).

2.4.2 Physicochemical factors

Mosquitoes exploit almost all types of aquatic environments. *Anopheles* mosquito has been found to use fresh water habitats for breeding. Larvae of *Anopheles* mosquitoes were collected in clear water of pH near neutral point of 7.0 (Russel,

1999 and CDC., 2007). Water of a near neutral pH of 6.8 – 7.2 is preferable for the breeding of many species of mosquitoes (CDC, 2004; Okogun *et al*., 2003). Many mosquito species prefer habitats with low oxygen tension (Okogun, 2005). Various chemical properties of the larval habitat has been observed in gutters, peri-domestic and domestic breeding sites and are related to vegetation and a wide range of heavy metal, nutrients and physicochemical characteristics of the water, ranging from pH, optimum temperature, total suspended solids, total dissolved solids and electrical conductivity. They have all been found to affect larval development and survival (Mutero *et al*., 2004).

2.4.2.1 Turbidity

Despite the dogma that *An. gambiae* is most often found in turbid water collections, various studies that examined the characteristics of larval habitat or larval population dynamics, failed to give a clear relationship between the presence of immatures and the clarity of breeding sites. It is known that dark substrates receive more eggs than light ones and moist substrates more than dry ones. Minakawa *et al*. (1999) concluded that *An. gambiae* preferred turbid water over clear water. This was supported by Gimnig *et al*. (2001) who observed that *An. gambiae* and *An. arabiensis* were associated with habitats that were high in turbidity and that both species increased in larval densities with increasing water turbidity. In contrast, Munga *et al*. (2005) found that *An. gambiae* preferred clear rainwater over natural water from forests and natural wetlands, which contained more impurities and was supported by Sattler *et al.* (2005) who showed a preference of *An. gambiae* to breed in rather clear water bodies. Some studies reported no effect of turbidity on the occurrence of *An. gambiae* (Mwangangi *et al*., 2007b). However, *An. arabiensis* and *An. gambiae* are often found to share larval habitats (Edillo *et al*., 2002). A clear difference in requirements for the larval

environment of the two species has not been observed. Several studies suggest the requirements are similar (Gimnig *et al*., 2001), others think they differ, but were unable to show that explicitly (Minakawa *et al*., 1999).

2.5 Morphological and Molecular Identification of Mosquitos Sibling species

Correct identification of species is vital for focussed effort in their disease control programmes. Some *mosquito* vectors are species complexes that are difficult to separate morphologically. For example, the *An. gambiae sensu lato* species complex comprises seven sibling species of mosquitoes that are morphologically indistinguishable namely; *An. gambiae sensu stricto*, *An. arabiensis, An. quadriannulatus* species A, *An. quadriannulatus* species B, (Coetzee, 2004; Coetzee *et al*, 2000), *An. melas, An. merus* and *An. bwambae.*

The method to identify, label and classify organisms is largely built around morphological characteristics. It was developed by Carl Linnaeus in the 18th century and his taxonomic system is to a large extent still used. Today taxonomists also consider physiology, behaviour and population biology in the classification of new species. Morphological identification of species is obviously limited since it does not consider phenotypic plasticity, genetic variation of individuals or morphological complexity (e.g. cryptic taxa or keys only developed for certain gender or life stage) (Hebert *et al*., 2003a). Since the discovery of Deoxyribonucleic Acid (DNA) and recognition of its role in inheritance, genetic variation plays a major role to distinguish the diversity of life. DNA based identification could fill these gaps and most importantly add new biological diversity to the already known. Furthermore, molecular based methods are generally used for species identification of viruses, bacteria and protozoa, in taxonomic studies (Adl *et al*., 2007; Pace, 1997).

Fredrick *et al*. (2016) conducted a study on molecular identification of *An. gambiae* sensu stricto Giles in Kamuli District, Uganda. Sibling species under the *An. gambiae* complex were characterized by polymerase chain reaction using species specific single nucleotide polymorphism (SNPs) in the intergenic spacer region (IGS) with primers specific for *An. gambiae* s.s., *An. arabiensis*, *An. melas*, *An. merus* and *An. quadriannulatus*. Molecular forms of the *An. gambiae* s.s. were further discriminated using primers specific for Mopti and Savannah forms. Out of 300 *An. gambiae* s.l. amplified, 98% (n= 294) were *An. gambiae* s.s. Out of 142 *An. gambiae* s.s. samples analyzed for molecular forms, 78.9% (n=112) were identified as *An. gambiae* s.s. Giles (*An. gambiae* Savannah) form, while the other 21.1% were not identifiable. It was then concluded that *An. gambiae* s.s Giles is the principal vectors of malaria in the area.

Kampen *et al*. (2003) conducted a study on polymerase chain reaction–based differentiation of the mosquito sibling species *An. claviger* s.s. and *An.petragnani*. Of the 592 mosquitoes, 407 larval specimens had been identified morphologically prior to species-specific DNA amplification, and in all instances PCR identification corroborated with morphologic identification. Mosquitoes identified as *An. claviger* s.s. came from various localities all over Europe and from Israel. Those identified as *An. petragnani* were collected in southern France and Spain. The species-diagnostic PCR assay would facilitate data collection on the temporal and spatial distribution of the two *An. claviger* sibling species because they represent possible vectors of disease in Europe, Near and Middle East, and North Africa.

Kengne *et al.* (2003) conducted a study on molecular indentification of *An. nili* group of African malaria vectors. Specimens of the *An. nili* group from Cameroon, Burkina Faso, Ivory Coast and Senegal were successfully indentified to species.

The species An. nili (Theobald), *An. carnevalei* and *An. somalicus* were observed and named, giving more insight into the members of the group.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of Study Area

The study was done in Awka-South Local Government Area of Anambra State, South-eastern Nigeria (Figs. 1 and 2). Awka South L.G.A is situated between Longitude 7^0 04'E and Latitude $6^0$10'N (wikipedia.org). Awka is in the tropical rainforest zone of Nigeria and experiences two distinct seasons brought about by the two predominant winds that rule the area: the southwestern monsoon winds from the Atlantic Ocean and the northeastern dry winds from across the Sahara desert. The monsoon winds from the Atlantic Ocean creates eight months of heavy tropical rains between April and October and is followed by four months of dryness (November - March) due to North eastern dry wind from the Sahara desert. There is also a short spell of cold dry period (Harmattan) locally called *Ugulu* (Igbo language) at the beginning of the dry season. This is particularly windy and dusty and enters Nigeria in late December or in the early part of January and is characterized by a grey haze limiting visibility and blocking the sun's rays (wikipedia.org). The temperature range in Awka is generally 27-30˚C between June and December but rises to 32-34˚C between January and April, with the last few months of the dry season marked by intense heat. The relative humidity of the area is about 70% in the dry season reaching 80% during the wet season. The annual rainfall is between 2000-3000mm (myweather2.com). The community is about 150m above sea level. Awka south has a population of 189,049 inhabitants (NPC, 2006), living in nine communities, namely, Amawbia, Awka, Ezinato, Isiagu, Mbaukwu, Nibo, Nise, Okpuno and Umuawulu. The towns could be subdivided into urban, semi-urban and rural areas. The urban areas include Awka

and Amawbia, the semi-urban Areas include Nibo and Okpuno and the rural area include Mgbakwu, Nise, Ezinato, Isiagu and Umuawulu. The urban areas especially Awka has such social amenities such as hospitals, three universities inluding Nnamdi Azikiwe University, good road networks, regular electricity supply and many secondary and primary schools.

The inhabitants of the area are predominantly farmers, civil servants, traders, and blacksmiths. The agricultural produce of the area include cassava, yam, maize, palm produce, plantain and coconuts. Heaps and ridge making are the peoples most adopted method of cultivation and it makes room for collection of water in the ridge furrows and spaces between mounds thereby creating numerous favourable breeding sites for mosquitoes especially during the rainy season. Some of the communities have permanent natural streams. Each of these streams has extensive freshwater swamps that serve as mosquito breeding sites.

Fig 1: Map of Anambra State showing the location of Awka South Local Government Area. Insert is map of Nigeria showing the location of Anambra State (Map made by Cartographer at the Department of Geography, Nnamdi Azikiwe University, Awka)

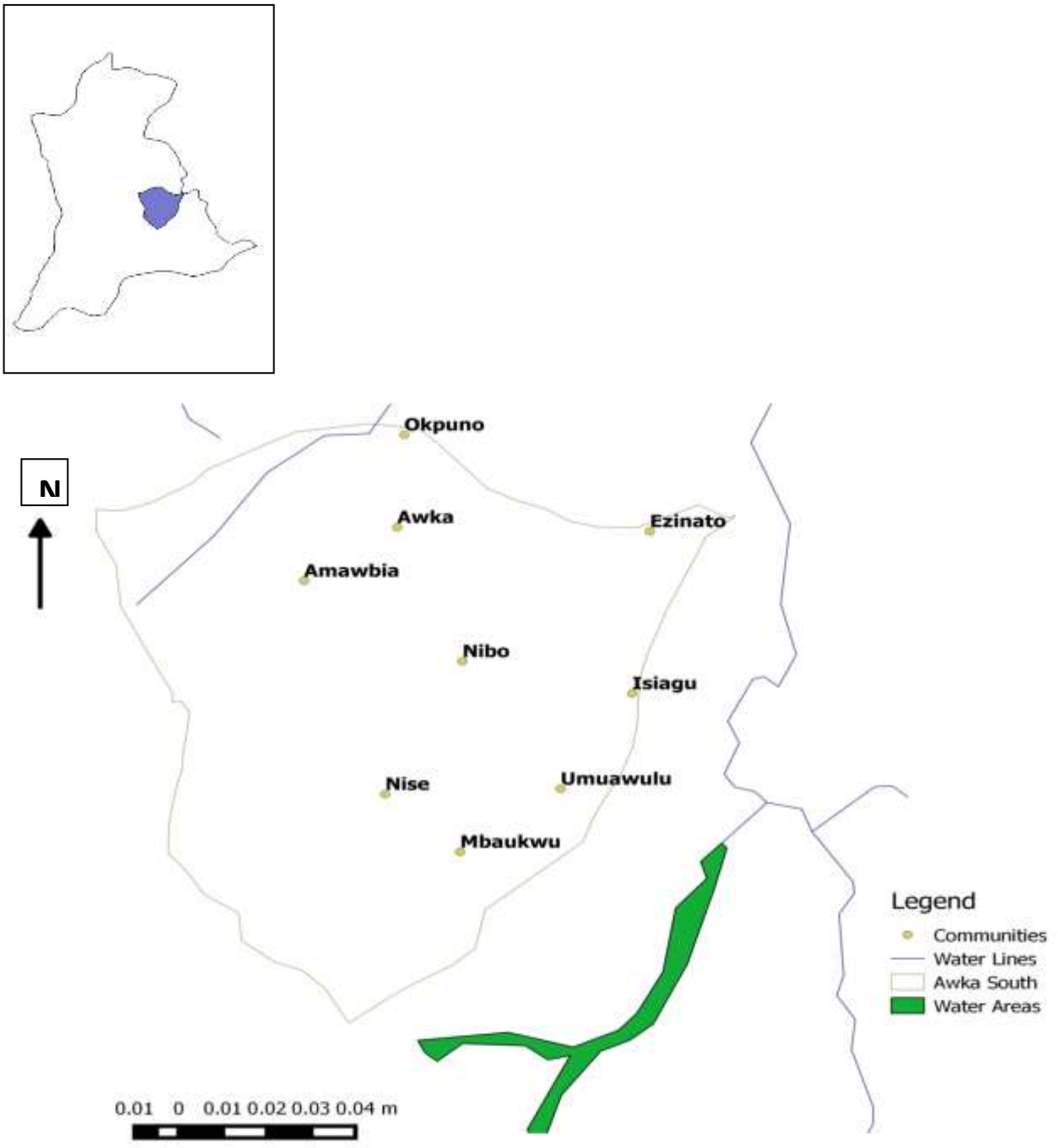

Fig 2: Map of Awka South Local Government Area, showing the study communities (using geographical coordinates of the communities with QGIS version 2.10.1. **(Appendix VII)** Insert is map of Anambra State showing the location of Awka South Local Government Area.

3.1.2 Study Design

The study was a longitudinal survey of mosquitoes breeding ecology, the ecological factors influencing both adult and immature stages survival in their environment. It also included a field survey of the biting and resting habits of the adult mosquitoes. Morphological and molecular identifications of the mosquitoes species were laboratory-based. The study was conducted over a period of twenty two (22) months starting from October, 2016 to July, 2018. Study communities were judgementally selected to represent urban, sub-urban and rural areas.

3.1.3 Advocacy visits and Community Sensitisation

Advocacy visits with an introductory letter from the Head of the Department of Parasitology and Entomology to the Chairman of Awka South Local Government Area, the traditional rulers and other opinion leaders of the communities were used to obtain permission to carry out the project (Appendix 1). The entire communities and the volunteer collectors were also sensitized and mobilised through meetings organized with the aid of their community leaders. The project intent and their significance were explained to them and their consent was obtained for the use of their environment for the study.

3.1.4 Selection of sampling Area

Six of the nine communities that made up Awka-South Local Government Area namely; Amawbia, Awka, Okpuno, Nibo, Nise, and Mbaukwu were judgementally selected (Onuoha *et al.*, 2011). This was done to properly represent the urban, sub-urban and rural communities in equal numbers. Thus; Amawbia and Awka communities are urban, Okpuno and Nibo are sub-urban while Mbaukwu and Nise are rural communities.

3.2 The abundance and breeding habitats of mosquito species through collection of their larval stages.

3.2.1 Collection of mosquito larvae

The breeding habitats of the mosquitoes were determined through collection of mosquito immature stages from standing water pools with the methods described by Onyido *et al*. (2016). Mosquito larvae were collected from the six selected communities in the study area. Six breeding sites were selected from each community. The larvae in ground pools and large domestic water containers were collected with the aid of ladles into a white plastic bowl. The larvae in discarded used automobile tyres were collected with the aid of large pipettes with rubber teat. The larvae in small containers were completely overturned into the sampling bowls. Coarse debris like sticks and plant leaves were handpicked and thrown away. A sieve of about 0.55mm mesh size (kitchen sieve), was used to separate the larvae from debris. Mosquito larvae in treeholes were collected with ladles into a white plastic bowl and larvae in plant leaf and axils were collected by overturning them into the bowl. At the end of each collection, larvae from different sampling sites were kept in seperate transparent and well-labeled bottles (Jam jars) and transported to the Entomology Laboratory of the Department of Parasitology and Entomology, Nnamdi Azikiwe University for rearing to adult stage and identification using an insect box (Appendix V).

3.2.2 Collection of mosquitoes breeding in septic tanks

Mosquitoes breeding in septic tanks were collected with locally made emergence traps. Each trap was made with a 20 litre bucket. A round hole of 4 inches circumference to serve as the adult mosquito entry point was made on the side of

the bucket. One end of a 4 inches rubber pipe was firmly fixed on the hole made on the bucket using glue. The other end of the pipe was fitted on a hole on septic tanks were adult mosquitoes leaving the breeding sites emerge. The bucket lid was removed and the openings fitted with nets of about 0.25mm to serve as barrier preventing trapped mosquitoes from escaping (Appendix VI). The trap was set up during evening hours and collected by the next morning. The Trap was taken with the mosquitoes inside to the Entomology Laboratory of The Department of Parasitology and Entomology, Nnamdi Azikiwe University for identification.

3.3 The biting and resting behaviour of the adult mosquitoes using Human Bait and Pyrethrum Knockdown methods.

The biting and resting habits of the adult mosquitoes were determined through collection of indoor and outdoor biting mosquitoes using Human Bait Collection (HBC) and Pyrethrum Knockdown (PKD) methods. The indoor and outdoor biting mosquitoes were collected using the methods also described by Onyido *et al*. (2016).

3.3.1 Collection of Indoor-biting and Resting Adult Mosquitoes

Indoor biting and resting adult mosquitoes were collected from the communities using pyrethroid-based insecticide knock down (PKD). The adult mosquitoes were collected from living rooms where people slept the previous night. Census of each selected household was taken and the number of persons that slept in each room was noted. Collections were made in 18 houses (3 houses / community) each month for 12 months of the year. Two rooms were used in each house. In each room, the doors and windows were shut and white spread sheets laid from wall to wall, covering furniture and other non-movable items in the rooms. Edible items, cooking utensils and movable household furniture were carried outside to avoid

contamination with insecticide. A pyrethroid-based insecticide aerosol (Baygon) were sprayed in the room and allowed to remain for 20 minutes before collection. In houses without ceiling, the eaves of the house were quickly sprayed from the outside before the inside. This was done to prevent the mosquitoes from escaping to the outside through the eaves. Cracks or any escape routes from the walls, doors and windows were closed with folded papers to prevent escape of mosquitoes through them.

At the end of the 20 minutes interval after spraying, the white spread sheets were folded starting from the edges to ensure that no knocked down mosquito escaped. As the white spread sheet were being folded, gentle shaking was applied to concentrate all the knocked down mosquitoes at the centre of the spread sheet. They were taken outside the room and spread out again to collect the knocked down mosquitoes using a pair of entomological forceps into a damped petri dish.

3.3.2 Collection of Outdoor-biting Mosquitoes

Outdoor-biting mosquitoes were collected using human-bait collection method (HBC). The collections were done all-night from 6.00pm-6.00am (local time). The volunteer collectors exposed their legs and hands for mosquito bites by rolling up their trousers and shirt sleeves to knee and elbow level respectively. At each occasion, four volunteers per house were employed. Mosquitoes alighting on the volunteers were collected with the aid of test tube vials and torchlight. The vials were quickly covered with a ball of cotton wool to avoid escape of the mosquito. The time of collection of each mosquito was properly recorded. Mosquito collections were collated at 15 minutes interval.

3.4 Determination of the physiological states, Indoor resting density, biting rates and sporozoites rates of female mosquitoes collected indoors

3.4.1 Physiological State of adult female mosquitoes collected indoors

The physiological states of female mosquitoes collected indoors was determined in order to observe mosquitoes that had blood meal and those that had not fed.

This was done following the model of Service (1985), who grouped them into four stages namely;

Unfed mosquitoes are mosquitoes that had flat abdomen indicating that they have not had any bloodmeal. Freshly fed mosquitoes are those that were freshly fed, their abdomens appeared reddish in colour. Half gravid mosquitoes are those that have fed and rested inside but have not completely digested their bloodmeal. The abdomen appeared whitish posteriorly and dark reddish anteriorly. Gravid mosquitoes are mosquitoes that have fed, rested and completely digested their bloodmeal and their abdomen becomes fully dilated and whitish due to the formation of fully developed eggs.

3.4.2 Indoor Resting Density of Mosquitoes

The indoor resting density of mosquitoes collected indoors in the study area was calculated from the result of PKD using the methods described by Ezihe *et al*, (2017). They were calculated by the number of mosquitoes collected divided by the total number of house sampled and the total number of night collections made. It is expressed as;

Indoor Resting Density (D) = (number of females ÷ number of houses) ÷ number of nights

3.4.3 Man biting rate of mosquitoes Collected

Man-biting rate is expressed as the number of bites a person receives from a specific vector species per night. This was calculated from PKC collections as the total number of freshly fed females of a species divided by the total number of occupants who spent the night in the rooms and then the total number of nights that were used for the collection (Ezihe *et al.* (2017). It is expressed as;

Man-biting rate (Mbr) = (number of freshly fed females ÷ total number of occupants) ÷ total number of nights

3.4.4 Determination of the sporozoite Rate of female *Anopheles gambiae* mosquitoes collected indoors

This was done at the Public health laboratory of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State. The Sporozoite index was determined with Enzyme-linked Immunosorbent Assays (ELISA). In the laboratory, the head and thorax of each of the mosquito samples was cut off using entomological dissecting blade. Each head and thorax of the mosquitoes was placed in labeled 1.5ml micro-centrifuge grinding tubes. Fifty microlitres (50µl) of grinding buffer was added before grinding very well. After grinding, the grinding pestles were rinsed with 100µl of the grinding solution into the tubes containing the mosquito triturates. The assay plates were coated with fifty microlitres (50µl) capture of monoclonal antibodies (mAb). The set-up was incubated for 30 minutes. Using an aspirator, the wells of the assay plates were filled with 200µl of blocking buffer (BB). The set-up was incubated for 1 hour. Fifty microlitres (50µl) of mosquito triturate and positive control were added added to the wells and incubated for 2 hours. The wells were washed twice using 200µl of phosphate buffered saline (PBS-0.05% Tween 20). fifty microlitres (50µl) peroxidase-monoclonal Antibodies (mixed 1:1) was added,

incubated in the dark for 1 hour and was washed three times with phosphate buffered saline (PBS-0.05% Tween 20). Then 100µl of substrate was added and absorbence was read using its characteristics colour change.

3.5 Physicochemical and climatic factors influencing the survival of larvae and adult mosquitoes in their environments

3.5.1 Collection of the physicochemical properties of the mosquito breeding sites

Water samples were collected from selected breeding sites in the study area and were analyzed at the Natural Product Research and Development Laboraty, Faculty of Physical Science building, Science Village, Nnamdi Azikiwe University, Awka. The water samples were collected once every month during mosquito collections for a period of 22 months that the study lasted. The water samples were kept in clean white jerrycans (Oyewole *et al*., 2009). The surface water temperature was measured in the field using a glass thermometer while the depth of the breeding sites were measured using a meter rule. Total dissolved oxygen, acidity and alkalinity were obtained by titration while pH, conductivity and salinity were analysed using the Myron L6psi Multiparameter water kit (Ramakanta and Tusharkanti, 2013). The Myron L multiparameter was calibrated using standard solutions of KCl, NaCl and Buffers of pH 4.0, 7.0 and 9.0. The sample cup and the pH/Oxidation Reduction Potential (ORP) sensor were rinsed three times with the sample to be analyzed. The sample was then placed in the sample cup and sensor of the kit. Each parameter was measured by pressing the respective parameter buttons on the kit and the reading on the screen was recorded after 30 secs.

3.5.2 Collection of the meteorological data

Monthly outdoor temperature, relative humidity and rainfall data of the collection period were obtained from the website of Weather Atlas. The monthly means of various weather parameters namely; maximum and minimum temperatures, rainfall, relative and humidity were obtained for the rainy seasons (March-October) and the dry season (November-February). The monthly meteorological data were related to the abundance of adult mosquitoes.

3.6 Morphological and molecular identification of adult mosquitoes after collection.

3.6.1 Morphological Characterisation

At the end of each collection period, all the mosquitoes collected were properly labeled and sent to the Entomology Laboratory of the Department of Parasitology and Entomology, Nnamdi Azikiwe University for identification. The morphological identifications were later confirmed at the Laboratory of National Arbovirus and Vectors Research Centre, Enugu. The mosquitoes were identified using the gross morphology of the species especially the body colour, patches of scales on the palps, antennae, proboscis, patches of pale and black scales on the wings and legs and the terminal abdominal segments using standard keys (Gillies and De Meillon, 1968; Gillet, 1972 and Gillies and Coetzee, 1987).

Anopheline mosquitoes were morphologically separated from culicine mosquitoes using the length of their palps, which were as long as their proboscis and spotted wings with alternate dark and pale bands arranged along the veins of the wings. Culicine mosquitoes were also separated from the Anophelines by their peg-like short palps and non-spotted wings.

An. funestus was identified by its small size, dark colour and almost entirely dark legs and three narrow white bands on the palps. *An. gambiae* sensu latum was identified by its relative medium size with irregulary spotted legs and palps with three pale rings including the wide ring at the tip.

Aedes group were separated from *Culex* group by the presence of dark and white silvery bands on their body and legs. *Culex* group had ash-grey to colourless appearance without any conspicuous body ornamentation. *Eretmapodites* was identified with a large patch of silver-white scales between the eyes, a mixture of yellow and black scales on the thorax and golden yellow patches underneath the abdomen.

3.6.2 Preservation of mosquitoes for molecular characterisation

All outdoor and indoor biting adult mosquitoes belonging to *An. gambiae* (Sensu lato-SL) and *C. pipiens* complexes were preserved in Ependorf tubes for molecular studies. An Ependorf tube was used to preserve a single adult mosquito. About 70% of the tube was filled with silica gel in its solid form. The silica gel served as a preservative to prevent the mosquito from decaying during transportation. A ball of cotton wool was placed in the tube to separate the preservative from the adult mosquito. Each adult mosquito was placed on the cotton ball and the Ependorf tube was covered. All the tubes containing the preserved mosquitoes were transported to the Laboratory of Nigerian Institute of Medical Research, Yaba Lagos State, for Polymerase Chain Reaction (PCR) studies (Appendix II, III and IV).

3.6.3 Molecular Identification of Sibling species of *Anopheles gambiae* (Sensu lato-SL) and *Culex pipiens* complex Using Polymerase Chain Reaction (PCR)

3.6.3.1 Step 1: DNA extraction

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

Four hundred microlitres (400µl) of the supernatant was transferred to Zymo-Spin IV Spin Filter (orange top) in a collection tube and centrifuged at 7,000rpm for 1 minute. One thousand two hundred microlitres (1200µl) of Genome Lysis Buffer was added to the filtrate in the collection tube and was mixed. Eight hundred microlitres (800µl) of the mixture was transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000rpm for 1 minute. The flow through the collection tube was discarded and the previous process was reapeted. Two hundred microlitres (200µl) 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 1 minute. Five hundred microlitres (500µl) g-DNA Wash Buffer was added to the Zymo-Spin IIC column and centrifuged at 10,000rpm for 1 minute. The Zymo-Spin IIC column was transferred to a clean 1.5ml microcentrifuge tube and 50µl DNA Elution Buffer was added directly to the column matrix. It was then centrifuged at 10,000rpm for 30 seconds and the DNA was eluted.

3.6.3.2 Step 2: Preparation of Master Mix

A master mix for *An. gambiae* and *C. pipiens* complex respectively were obtained by mixing the pre mix, primers and other reagents in the order listed in table 1. The total mixture obtained is called the master mix which was used for PCR in step 3. The sequence and barcodes of the primers used in identifying the sibling species of *An. gambiae* and *C. pipiens* complexes are shown in the table 2.

Table 1: Master Mix for *An. gambiae* complex and *C. pipiens* complex

Mosquitoes	Reagent	X1 (µl)
***An. gambaie* complex**	Pre-mix	3.0
	ddH_2O	8.0
	81F	0.5
	691R	0.5
	DNA	1.0
	TOTAL	12.0
***C. pipiens* complex**	Pre-mix	4.0
	ddH_2O	5.0
	BSA (X1)	1.0
	ACEpip 1	0.5
	ACEpip 2	0.5
	B1246s	0.5
	DNA	1.0
	TOTAL	12.5

Table 2: The names, sequence and barcodes of the primers

	Name	Barcode	Sequence
An. gambiae	**ME**	**S43FF**	**TGACCAACCCACTCCCTTGA**
complex	**AR**	**S4400**	**AAGTGTCCTTCTCCATCCTA**
	QD	**S4401**	**CAGACCAAGATGGTTAGTAT**
	UN	**S43FD**	**GTGTGCCCCTTCCTCGATGT**
	GA	**S43FE**	**CTGGTTTGGTCGGCACGTTT**
C. pipiens	**ACEpip**	**160524B002C12 1/9**	**5^1-GGAAACAACGACGTATGTACT-3^1**
complex	**B1246s**	**160524B002D12 2/9**	**5^1-TGGAGCCTCCTCTTCACGG-3^1**

3.6.3.3 STEP 3: PCR procedure for *An. gambiae* and *C. pipiens* complex after DNA extractions and obtaining the Master Mix

3.6.3.3.1 *Anopheles gambiae* Complex

Twelve and half microlitres (12.5 µl) of PCR master mix of each adult mosquito was added into each of the two hundred microlitres (200µl) tube. One microlitre (1µl) of DNA was added into each tube. Each of the tube was loaded in the PCR machine and an appropriate programme and PCR condition was chosen on the machine. The PCR conditions for *An. gambiae* complex chosen were; Initial Denaturation @ 95ºC – 2 mins, Denaturation @ 95ºC – 30sec, Annealing @ 55ºC – 30sec, Extension @ 72ºC – 40sec, Final extension @ 72ºC –7mins. All the conditions were set to run for 30 Cycles. 1.5% agarose gel was prepared with Tris-borate ethylene-di-amino tetraacetic acid (TBE) buffer; eg.1.5g of agarose gel in 100 ml of TBE buffer. It was mixed and boiled in microwave until solution was clear. It was cooled down for 5 minutes, the skin on top was removed and 100 µl of ethidium bromide was added. The gel was poured into trough. Ten microlitres (10 µl) of the PCR product was loaded with 1 µl of loading buffer into each well. 10 µl of standard marker per gel was loaded in the electrophoresis machine. PCR product was run at 100 volts and not more than 120-150 mA in the electrophoresis machine. The gel picture was taken under UV light using gel documentation machine.

3.6.3.3.2 *C. pipiens* Complex

Twelve and half microlitres (12.5 µl) of PCR master mix was added into each of the two hundred microlotres (200µl) tube. One microlitre (1µl) of DNA was added into each tube. Each of the tube was loaded in the PCR machine and appropriate programme and conditions was chosen. The PCR conditions for *C. pipiens*

complex chosen were, Initial Denaturation @ 94ºC – 5 mins, Denaturation @ 94ºC – 30sec, Annealing @ 58ºC – 30sec , Extension @ 72ºC – 1min, Final extension @ 72ºC –5mins. All the conditions were set to run for 35 cycles. 1.5qqq2w% agarose gel was prepared with Tris-borate ethylene-di-amino tetraacetic acid (TBE) buffer; eg.1.5g of agarose gel in 100 ml of TBE buffer. It was mixed and boiled in microwave until solution was clear. It was cooled down for 5 minutes and the skin on top was removed and 100 µl of ethidium bromide was added. The gel was poured into a trough. Ten microlitres (10 µl) of the PCR product was loaded with 1 µl of loading buffer into each well. 10 µl of standard marker per gel was loaded in the electrophoresis machine. PCR product was run at 100 volts and not more than 120-150 mA in the electrophoresis machine. The gel picture was taken under UV light using gel documentation machine.

3.6.3.3.3 Interpretation of bands on gel

The gel picture was taken under UV light using gel documentation machine and was read using the molecular weights of the *An. gambiae* and *C. pipiens* siblings species on the DNA ladder as shown in the table below.

Table 3: DNA ladder showing the molecular weights of sibling species of *An. gambiae* and *C. pipiens* complex

Mosquitoes	Molecular weights
Culex pipiens	610 base pair
Anopheles merus	464 base pair
Anopheles gambiae s.s	390 base pair
Anopheles arabiensis	315 base pair
Culex quinquefasciatus	274 base pair
Anopheles quadriannulatus	153 base pair

3.7 Statistical Analysis

Data collected from the study were analysed using the Statistical Package for Social Sciences (SPSS) version 2.10. Analysis of variance (ANOVA) at 5% significant level was used to compare the indoor and outdoor-biting adults and larval populations across breeding sites. Line graphs were used to show the response of mean monthly rainfall, temperature and relative humidity to the abundance of mosquito population and Pearson correlation were used to determine their relationships. Pearson correlation was also used to determine relationships between physicochemical properties of breeding sites and abundance of mosquito larvae. Shannon-Weiner Index was used to analyze the species diversity in the study area (Okonkwo *et al*., 2014). It is expressed as $H = (N \log N - \sum ni \log ni)/N$, where ni is the abundance and N the total number of individuals in the species. Also Simpson's dominance indices was used to evaluate the prevalence of each individual species and it measures the probability of picking two organisms at random that are of different species. It is expressed as $C = \Sigma(ni/N)2$ ni = number of individuals of nth species, N = total number of individuals for all species (Ogbeibu, 2005).

CHAPTER FOUR

RESULTS

4.1 Abundance of mosquitoes

A total of 3,478 mosquitoes were collected from the study using different methods (Table 4). Of this number, 815 (23.43%) were larvae, 1393(40.05%) were outdoor biting adults while 1270(36.51%) were indoor biting adults. The outdoor adult collections 1393(40.05%) were the highest and the larval collections were the least 815(23.43%). There was no significant difference in the numbers of different mosquito groups collected ($P > 0.05$, P value = 0.122) (Appendix IX).

The mosquitoes collected consisted of four genera and eight species. The mosquito genera were *Anopheles*, *Aedes*, *Culex* and *Eretmapodites*. The mosquito species were *Anopheles gambiae* 284(8.16%), *An. funestus* 143(4.11%), *Aedes aegypti* 333(9.57%), *Ae. albopictus* 227(6.52%), *Culex quinquefasciatus* 2448(70.38%), *C. tigripes* 41(1.17%) and *C. annulioris* and *E. chrysogaster* were 1(0.02%) each. The *Culex* species (*C.quinquefasciatus*, *C. tigripes* and *C. annulioris*) were the most abundant 2490(71.57%) while *E. chrysogaster* was least 1(0.02%). The *Anopheles* species (*An. gambiae* and *An.funestus*) 427(12.27%) and the *Aedes* group (*Ae. aegypti* and *Ae. albopictus*) was 560(16.09%). There was a significant difference in the numbers of different mosquito species collected in the study ($P < 0.05$, P value = 0.000) (Appendix X). Five of the eight species namely *An. gambiae*, *An. funestus*, *Ae. aegypti*, *Ae. albopictus* and *C.quinquefasciatus* were collected as larvae and biting adults both outdoors and indoors in the area. *Culex tigripes* was collected in larval stage only. *Culex annulioris* and *Eretmapodites chrysogaster* were collected in the outdoor adults biting catches.

Table 4: Mosquitoes species collected from Awka-South L.G.A, Anambra State.

Mosquitoes species	**Larval collection**	**Outdoor collection**	**Indoor collection**	**Total**	**Percentage (%)**
An.gambiae	73	80	131	284	8.16
An.funestus	34	62	47	143	4.11
Ae.aegypti	100	149	84	333	9.57
Ae.albopictus	93	77	57	227	6.52
C.quinquefasciatus	474	1023	951	2448	70.38
C.tigripes	41	0	0	41	1.17
C.annulioris	0	1	0	1	0.02
E.chrysogaster	0	1	0	1	0.02
Total	**815 (23.43%)**	**1393 (40.05%)**	**1270 (36.51%)**	**3478 (100%)**	**100**

P value for methods of collection = 0.122, P > 0.05
P value for abundance of different mosquitoes species collected = 0.000, P < 0.05

4.1.1 The monthly abundance of mosquito larvae in Awka South L.G.A

Most of the larvae, 529(64.79%), were collected from the month of March to November, corresponding to the wet season. Only 286(35.21%) larvae were collected from November to March corresponding to the dry season period. The highest number of larvae 91(11.16%) was collected in the month of September and the least 24(2.94%) was collected in the month of March. Larval collections in the other months of the year were 71(8.71%) in January, 74(9.07%) in February, 47(5.76%) in April, 53(6.50%) in May, 56(6.87%) in June, 71(8.71%) in July and August each, 75(9.20%) in October, 89(10.92%) in November and 52(6.38%) in December (Fig 3).

Figures 4 – 8 are barcharts of monthly abundance of the larvae of the most common five mosquito species. *Culex quinquefasciatus* larvae were collected from January to December. Most *C. quinquefasciatus* larvae (Fig. 4) were collected from November-February (dry Season) in septic tanks reaching the highest peak in February and less abundant from March-October (rainy Season) with the lowest population peak in the month of March (early rainy season). *Ae. albopictus* larvae were collected from the month of April to November corresponding to rainy season (Fig. 5). None was collected between December and March when the dry season was at its peak. *Ae. aegypti* (Fig. 6) was collected only from March-November during the wet season with the highest peak in August. None was collected between December and February during the dry season. *An. funestus* (Fig. 7) was collected from April to November with the highest peak occurring in October. No *An. funestus* larva was collected between December and March (dry season). *An. gambiae* was collected from April to November, with the highest population peak was in September (Fig. 8).

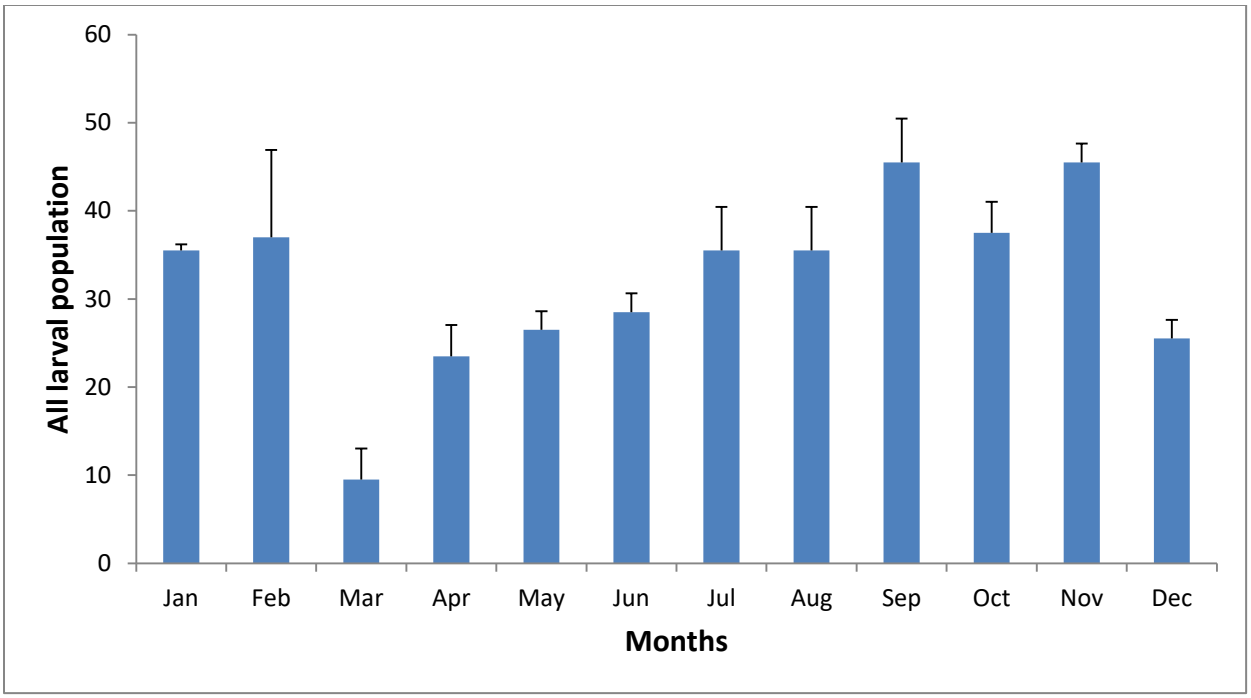

Fig 3: Monthly abundance of all Mosquitoes larvae populations

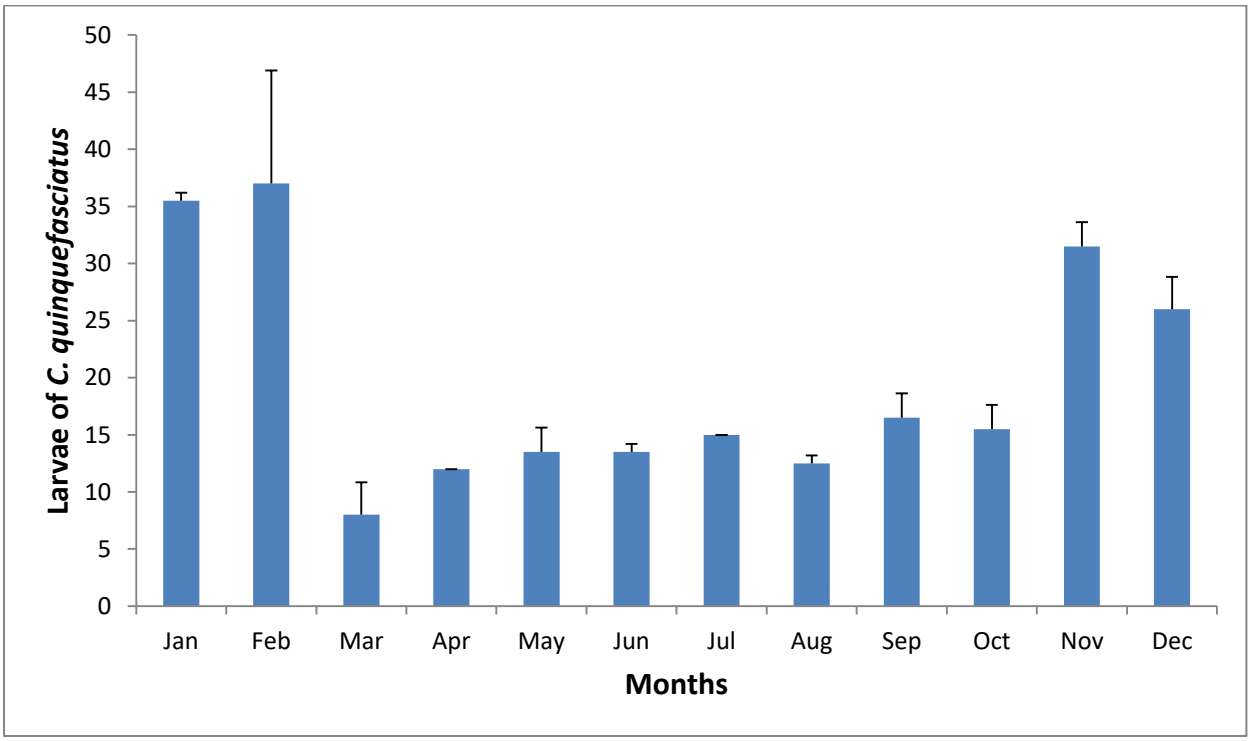

Fig 4: Monthly Abundance of *Culex quinquefasciatus* larvae

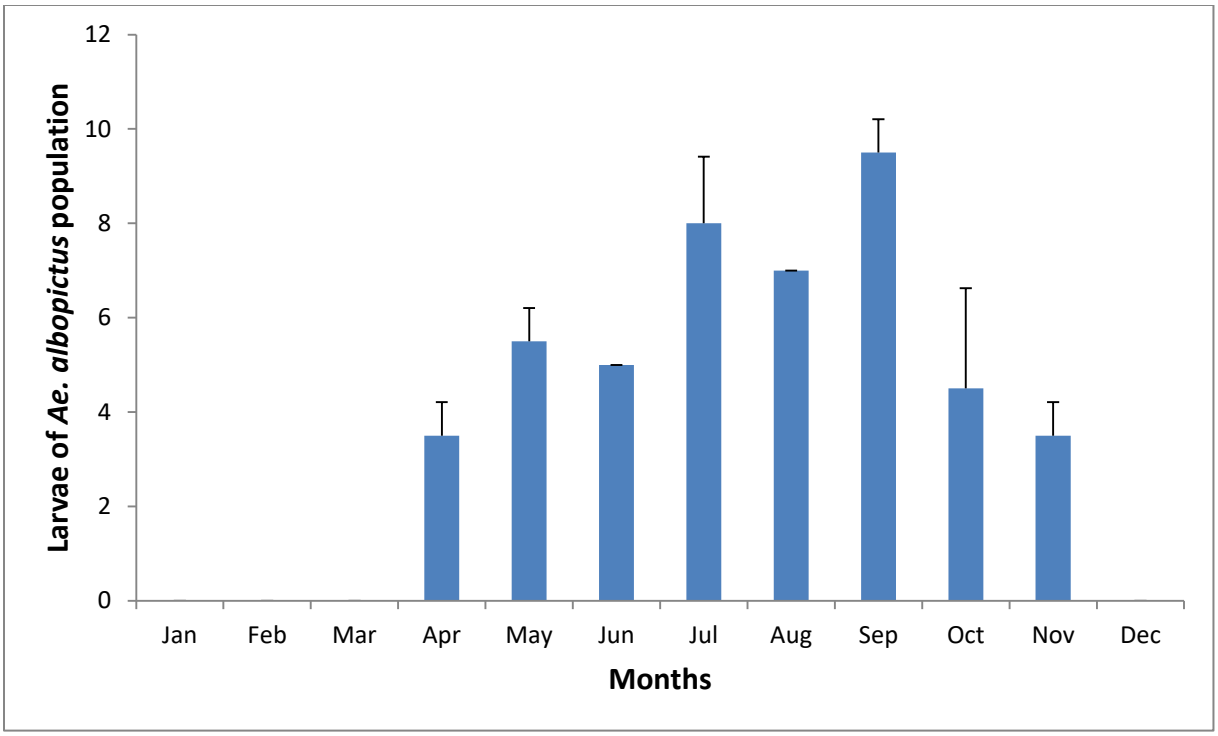

Fig 5: Monthly Abundance of *Aedes albopictus* larvae

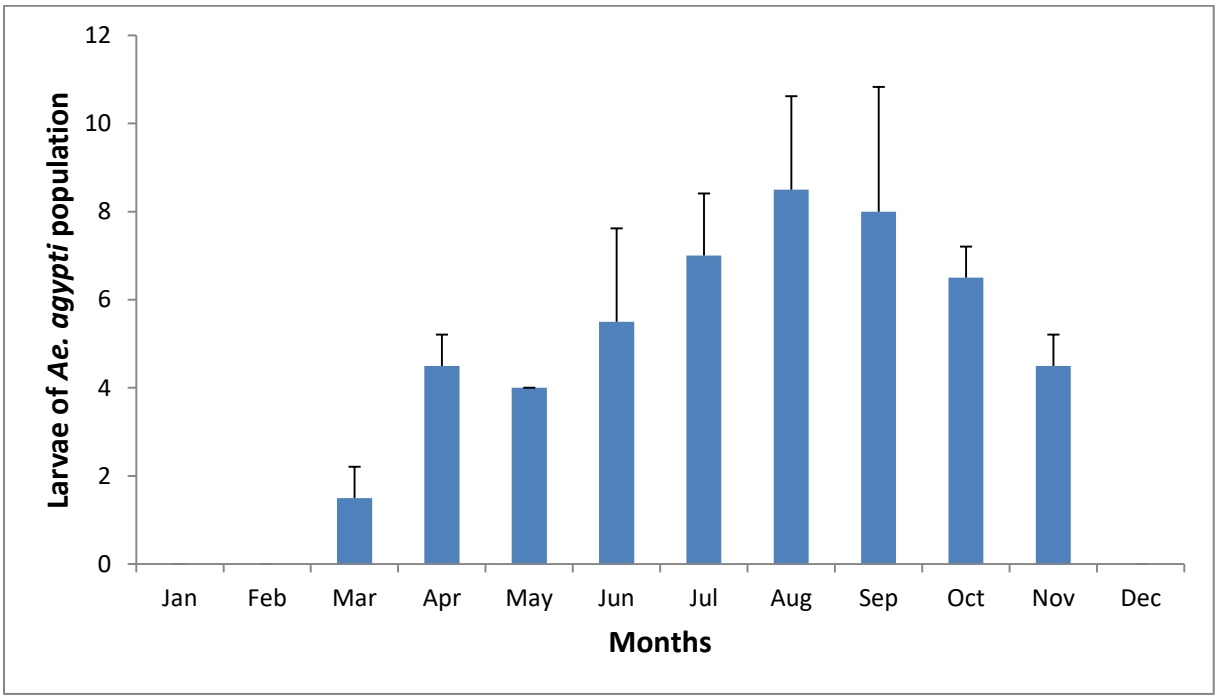

Fig 6: Monthly Abundance of *Aedes aegypti* larvae

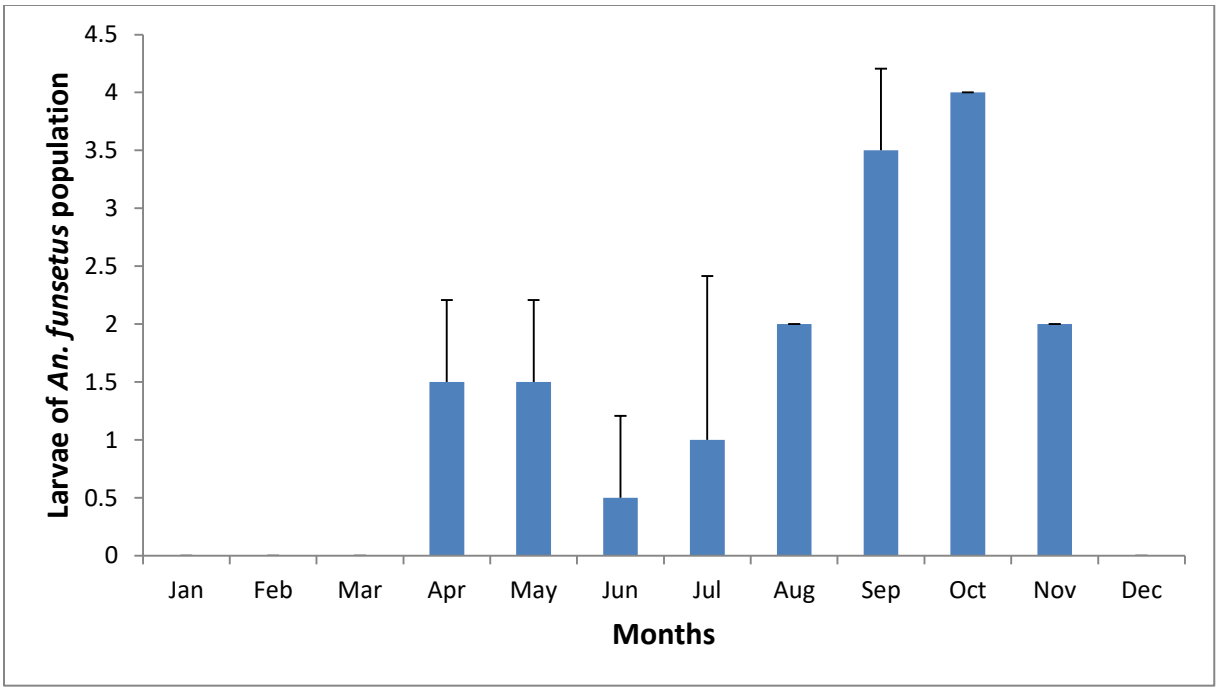

Fig 7: Monthly Abundance of *Anopheles funestus* larvae

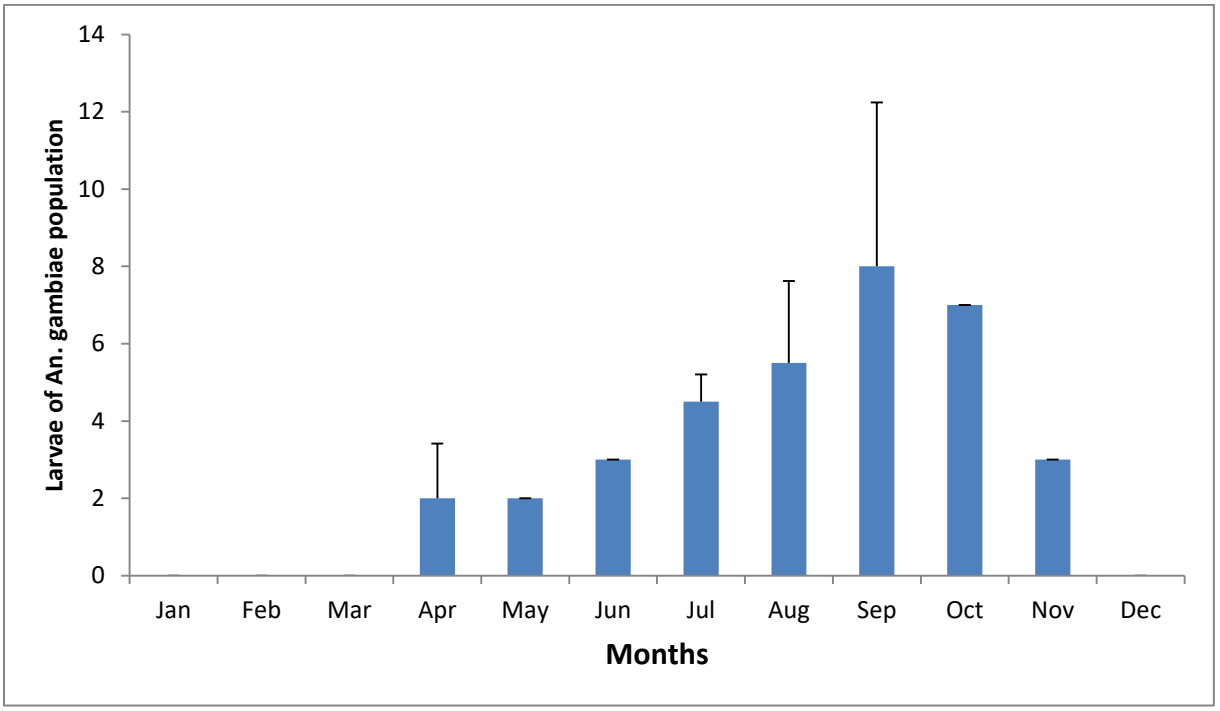

Fig 8: Monthly Abundance of *Anopheles gambiae* larvae

4.1.2 Monthly abundance of adult mosquitoes collected in the study area

All adult mosquitoes collected had the highest peak in September (rainy season) and the least in February at the height of the dry season (Fig. 9).

Figures 10 to13 are barcharts of monthly abundance of the adults of the five most common mosquito species. *Culex quinquefasciatus* was collected in all the months of the year. Most of the adult *C. quinquefasciatus* were collected from March to October (rainy Season) reaching the highest peak in September. Lower numbers of adult *C. quinquefasciatus* (Fig. 10) were collected from November to February (Dry Season) with the lowest population peak in the month of February (dry season).

Aedes albopictus adults (Fig. 11) were collected from the month of April to November corresponding to rainy season with the highest peak occurring in September. None was collected between December and March when the dry season was at its peak. Also, *Ae. aegypti* adults (Fig. 12) were collected from March to November during the wet season and shortly in early dry season with the highest in September. None was collected between December and February during the dry season.

Anopheles funestus adults (Fig. 13) were collected from March to December during rainy season and early part of the dry season with the highest population peak occurring in September. No *An. funestus* adult was collected between January to February during the dry season. Also, *An. gambiae* (Fig. 14) was collected from March to December during the rainy season and shortly into the dry season. With the highest population peak in September.

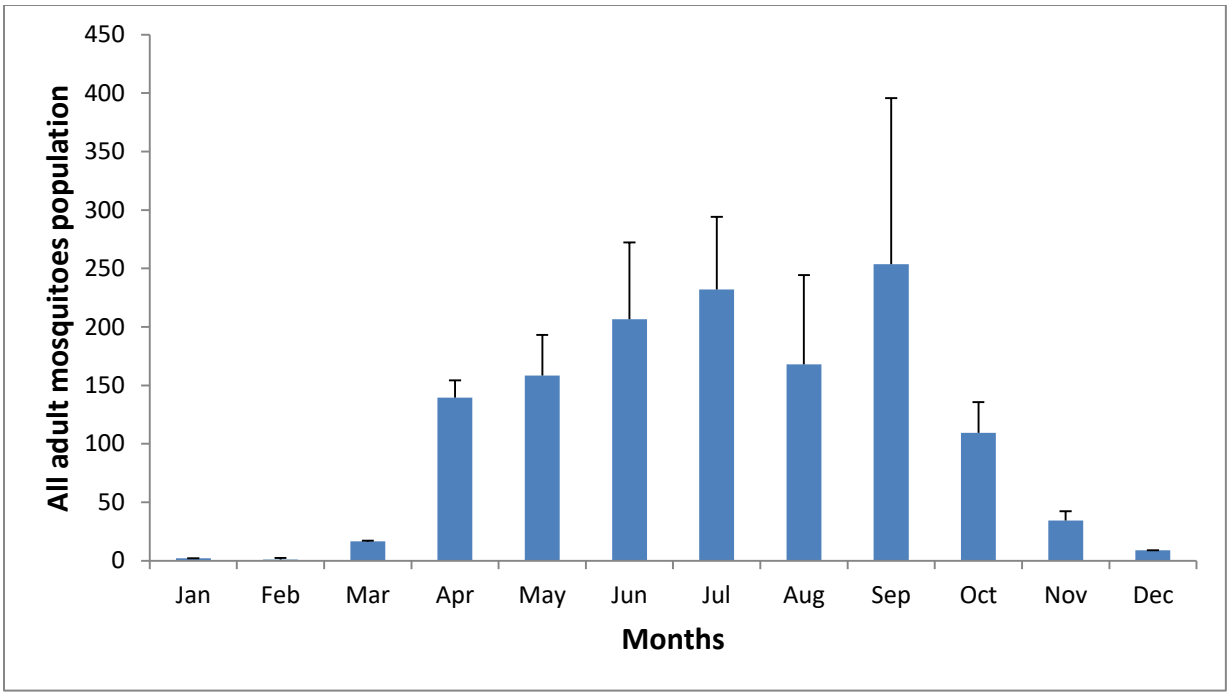

Fig 9: the Monthly abundance of all adult mosquitoes populations collected in the study area.

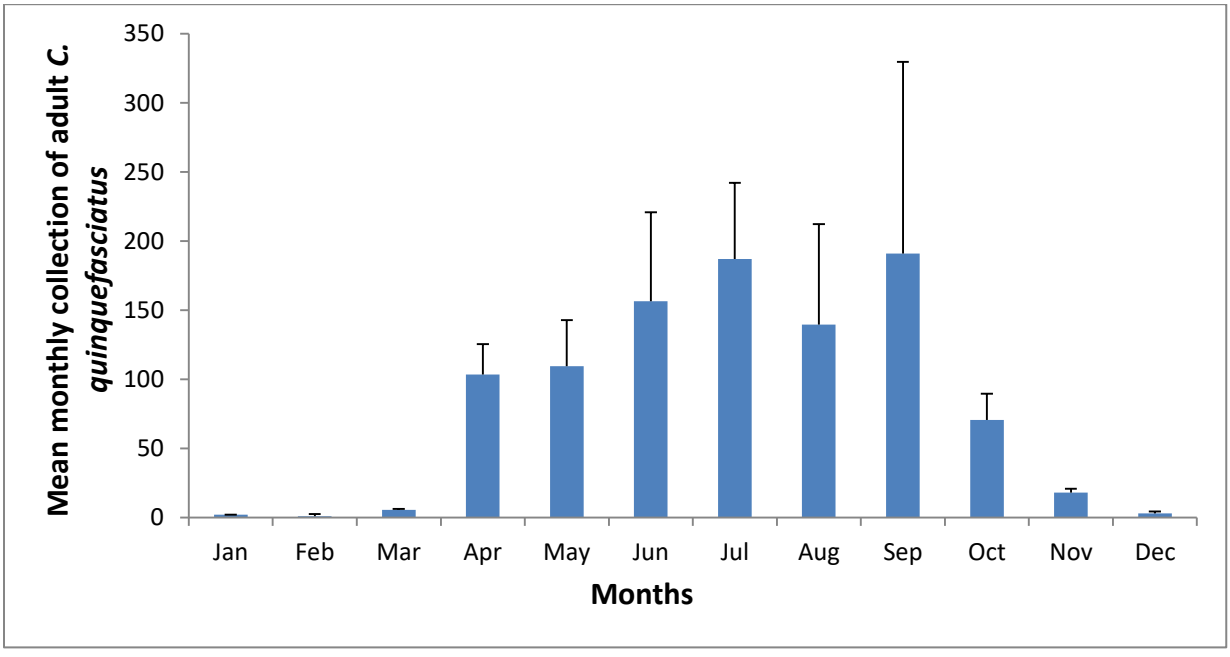

Fig 10: Monthly abundance of adult *Culex quinquefasciatus*

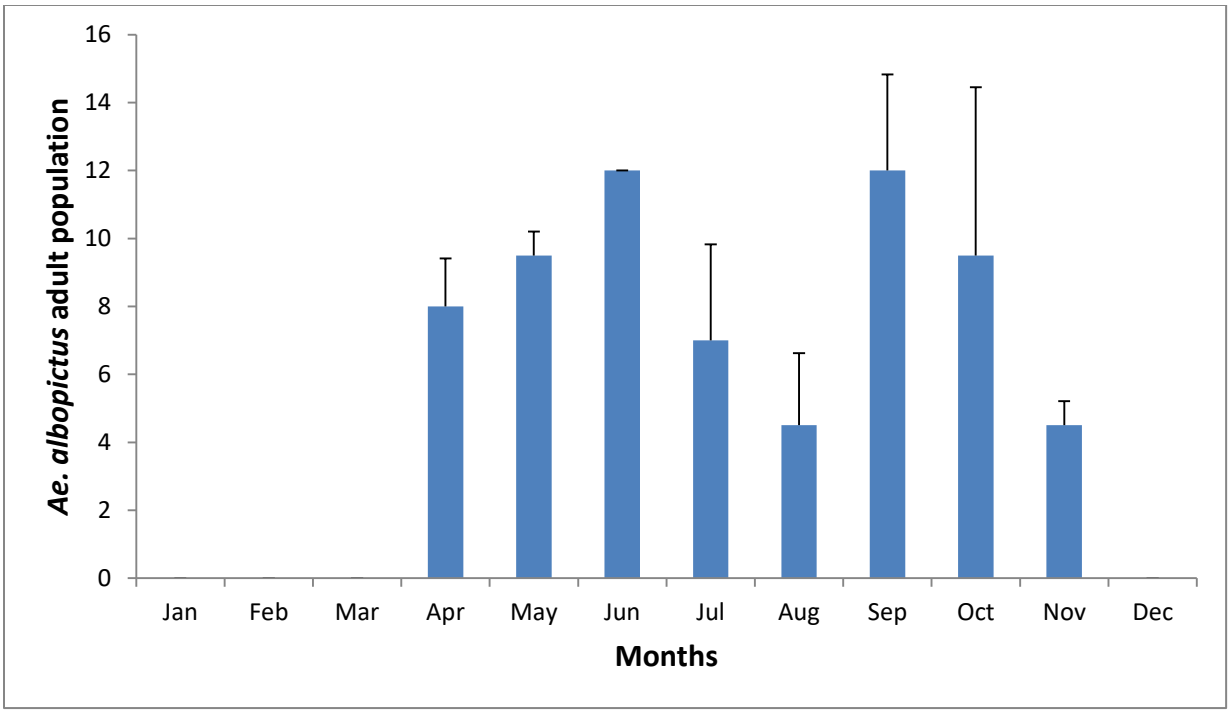

Fig 11: Monthly abundance of adult *Aedes albopictus*

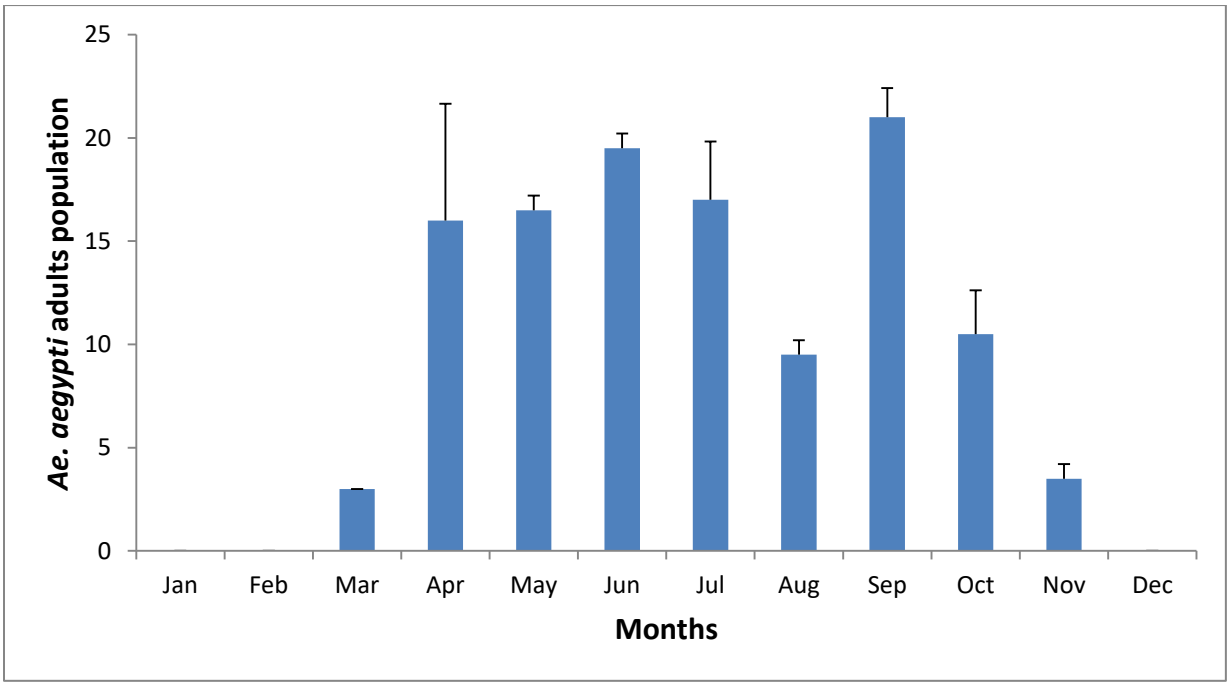

Fig 12: Monthly abundance of adult *Aedes aegypti*

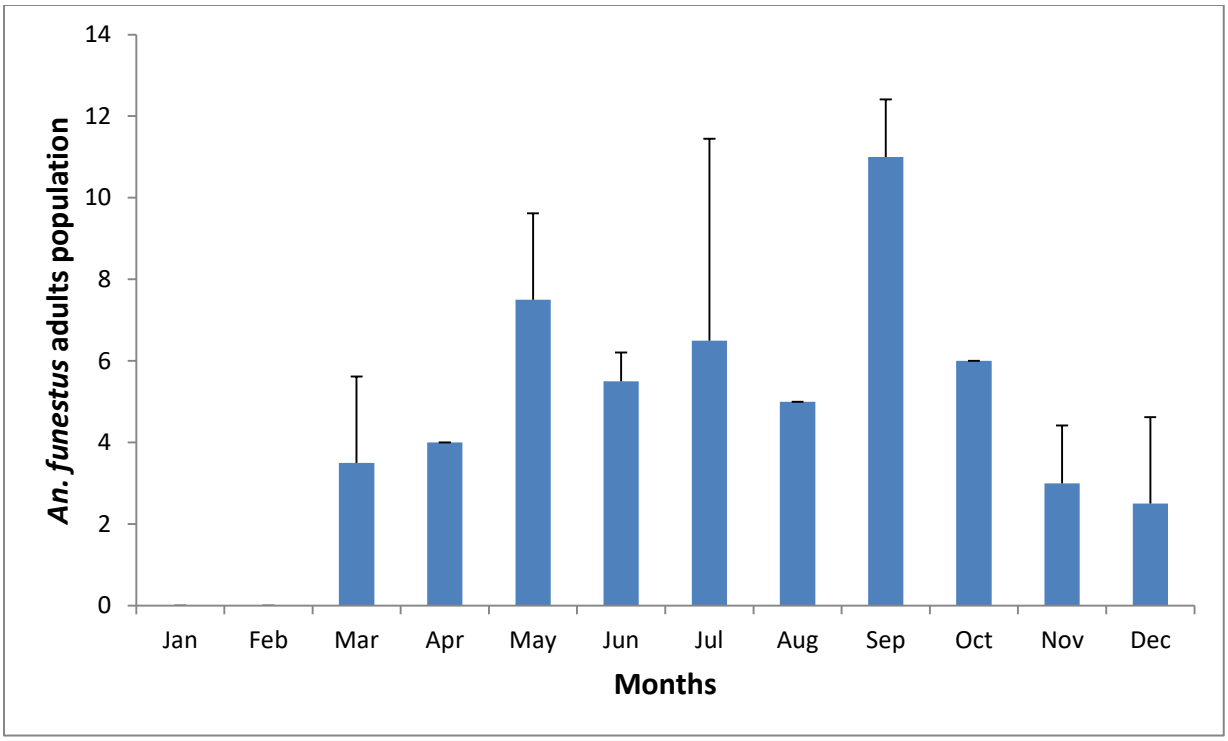

Fig 13: Monthly abundance of adult *Anopheles funestus*

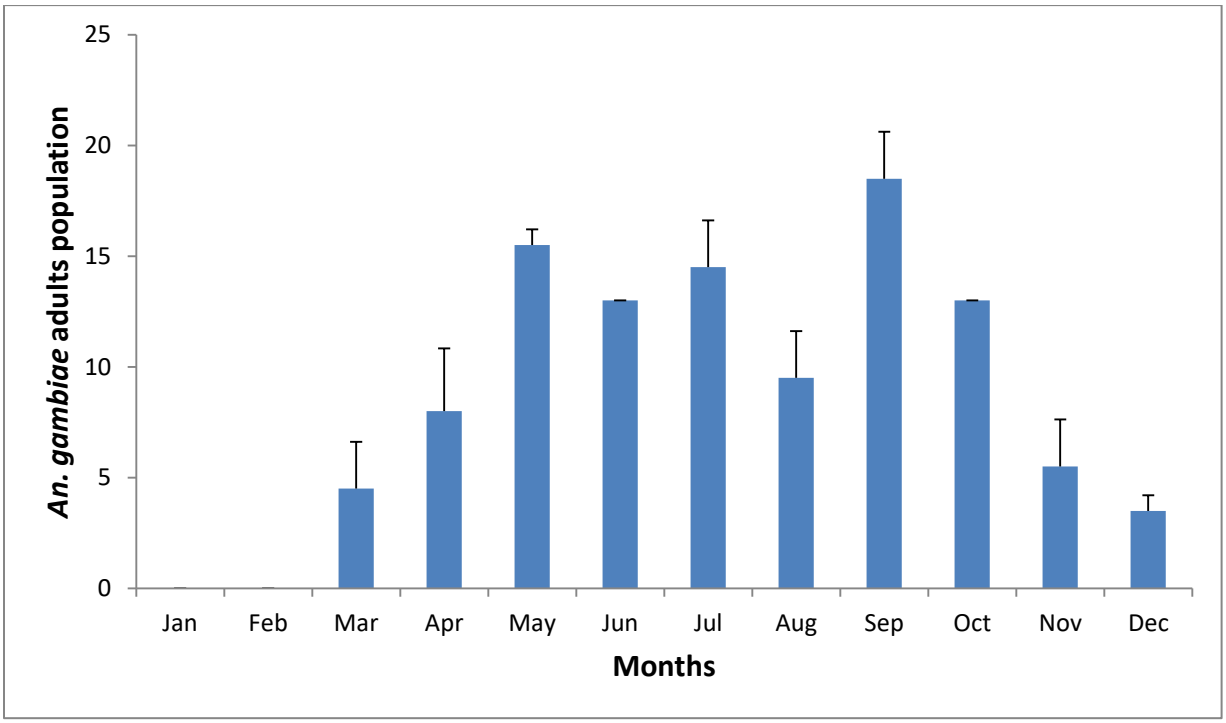

Fig 14: Monthly abundance of adult *Anopheles gambiae*

4.1.3 Mosquito Abundance in Rural in Rural, Surburban and Urban Ecotypes

Four species of mosquitoes namely; *Ae. aegypti, Ae. albopictus, C. quinquefasciatus and An. gambaie were found* in the three ecotypes (urban, sub-urban and the rural communities). *C annulioris, C. tigripes* were collected in sub-urban and rural communities. *E. chrysogaster* was collected only in the urban communities while *An. funestus* was not collected in sub-urban and rural communities (Table 5).

Table 5: Distribution of the mosquitoes in Urban, Surburban and Rural communities

Communities	Category	Mosquitoes species collected
Amawbia Awka	**Urban**	*Ae. aegypti* *Ae. albopictus* *C. quinquefasciatus* *C. annulioris* *E. chrysogaster* *An. gambiae*
Nibo Okpuno	**Sub-Urban**	*Ae. aegypti* *Ae. albopictus* *C. quinquefasciatus* *C. tigripes* *An. gambiae* *An. funestus*
Nise Mbaukwu	**Rural**	*Ae. aegypti* *Ae. albopictus* *C. quinquefasciatus* *C. tigripes* *An. gambiae* *An. funestus*

4.1.4 Diversity and Dominance indices of mosquitoes collected from Awka South L.G.A, Anambra State, Nigeria

Shannon-Wiener diversity index (H) and Simpson's dominance index (C) were calculated for all mosquito species collected in the study area (Appendix XVIII). With Shannon-Wiener diversity index, *C.quinquefasciatus* had the highest diversity index value of 0.107 while *C.annulioris* and *E.chrysogaster* had the least 0.0000003 each. Others in ascending order were *C.tigripes* 0.0004, *An.funestus* 0.057, *Ae.albopictus* 0.077, *An.gambiae* 0.089 and *Ae.aegypti* 0.098 (Table 6).

With Simpson's dominance, *C.quinquefasciatus* had the highest dominance index value of 0.495 while *C.annulioris* and *E.chrysogaster* had the least 0.00000004 each. Others were *C.tigripes* 0.0001, *An.funestus* 0.002, *Ae.albopictus* 0.004, *An.gambiae* 0.007 and *Ae.aegypti* 0.009 (Table 6).

Shannon-Wiener diversity index (H) was calculated for all mosquito species according to communities in the study area (Appendix XIX-XXIV). At Amawbia, *Ae.aegypti* had the highest diversity index value of 0.106, followed by *An.funestus, C.tigripes* and *C.annulioris* with 0.000 values each. Others were *E.chrysogaster* 0.005, *Ae.albopictus* 0.080, *An.gambiae,* 0.082 and *C.quinquefasciatus* 0.095 (Table 6).

Table 6: Species diversity and dominance indices for all mosquitoes collected from Awka South Local Government Area.

Mosquito species	Shannon-Wiener diversity index (H)	Simpson's dominance index (C)
An.gambiae	0.089	0.007
An.funestus	0.057	0.002
Ae.aegypti	0.098	0.009
Ae.albopictus	0.077	0.004
C.quinquefasciatus	0.107	0.495
C.tigripes	0.0004	0.0001
C.annulioris	0.0000003	0.00000004
E.chrysogaster	0.0000003	0.00000004
Total	**H=0.428**	**C=0.517**

In Awka, *Ae.aegypti* had the highest diversity index value 0.078 followed by *An.funestus, C.tigripes* and *E.chrysogaster* with 0.000 value each. Others were *C.annulioris* 0.005, *An.gambiae* 0.056, *Ae.albopictus* and *C.quinquefasciatus* 0.061 each (Table 7). In Nise community, *C.quinquefasciatus* had the highest diversity index value of 0.126 while *C.annulioris* and *E.chrysogaster were* 0.000 each. Others were *An.funestus* 0.031, *C.tigripes* 0.042, *An.gambiae* 0.090, *Ae.albopictus* 0.105, and *Ae.aegypti* 0.114 (Table 7).

In Nibo, *Ae.aegypti* had the highest diversity index value of 0.109 while *C.annulioris and E.chrysogaster* were 0.000 each. Others were *C.tigripes* 0.030, *C.quinquefasciatus* 0.073, *Ae.albopictus* 0.083, *An.funestus* 0.086 and *An.gambiae* 0.104 (Table 7). In Okpuno, *C.quinquefasciatus* had the highest diversity index value of 0.106 while *C.annulioris and E.chrysogaster* were 0.000 each. Others were *C.tigripes* 0.022, *Ae.albopictus* 0.073, *An.funestus* 0.076, *Ae.aegypti* 0.089 and *An.gambiae* 0.094 (Table 7). In Mbaukwu, *C.quinquefasciatus* had the highest diversity index value of 0.132 while *C.annulioris and E.chrysogaster* were 0.000 each. Others were *C.tigripes* 0.039, *Ae.albopictus* 0.066, *Ae.aegypti* 0.095, *An.funestus* 0.097 and *An.gambiae* 0.117 (Table 7).

Table 7: Shannon-Wiener index of diversity for mosquito species composition in the six study communities in Awka South Local Government Area

Mosquito species	**Shannon-Wiener index of diversity (H)**					
	Amawbia	**Awka**	**Nise**	**Nibo**	**Okpuno**	**Mbaukwu**
An.gambiae	0.082	0.056	0.090	0.104	0.094	0.117
An.funestus	0.000	0.000	0.031	0.086	0.076	0.097
Ae.aegypti	0.106	0.078	0.114	0.109	0.089	0.095
Ae.albopictus	0.080	0.061	0.105	0.083	0.073	0.066
C.quinquefasciatus	0.095	0.061	0.126	0.073	0.106	0.132
C.tigripes	0.000	0.000	0.042	0.030	0.022	0.039
C.annulioris	0.000	0.005	0.000	0.000	0.000	0.000
E.chrysogaster	0.005	0.000	0.000	0.000	0.000	0.000
Total	**H=0.368**	**H=0.261**	**H=0.508**	**H=0.485**	**H=0.460**	**H=0.546**

Simpson's index of dominance (C) was calculated for all mosquito species according to communities in the study area (Appendix XIX-XXIV). At Amawbia, *C.quinquefasciatus* had the highest dominance index value of 0.557 while *An.funestus, C.tigripes* and *C.annulioris* were 0.000 each. Others were *E.chrysogaster* 0.000004, *Ae.albopictus* and *An.gambiae*, 0.005 each and *Ae.aegypti* 0.012 (Table 8). In Awka, *C.quinquefasciatus* had the highest dominance index value 0.719 while *An.funestus*, *C.tigripes* and *E.chrysogaster* were 0.000 each. Others were *C.annuloris* 0.000003, *An.gambiae* and *Ae.albopictus* 0.002 and *Ae.aegypti* 0.004 (Table 8). In Nise community, *C.quinquefasciatus* had the highest dominance index value of 0.401 while *C.annuloris* and *E.chrysogaster were* 0.000 each. Others were *C.tigripes* 0.0007, *An.funestus* 0.0003, *An.gambiae* 0.007, *Ae.albopictus* 0.013, and *Ae.aegypti* 0.016 (Table 8). In Nibo, *C.quinquefasciatus* has the highest dominance index value of 0.367 while *C.annulioris and E.chrysogaster* were 0.000 each. Others were *C.tigripes* 0.0003, *Ae.albopictus* 0.005, *An.funestus* 0.006, *An.gambiae* 0.011 and *Ae.aegypti* 0.014 (Table 8).

In Okpuno, *C.quinquefasciatus* had the highest dominance index value of 0.502 while *C.annuloris and E.chrysogaster* were 0.000 each. Others were *C.tigripes* 0.0001, *Ae.albopictus* and *An.funestus* 0.004 each, *Ae.aegypti* 0.006 and *An.gambiae* 0.056 (Table 8). In Mbaukwu, *C.quinquefasciatus* had the highest dominance index value of 0.365 while *C.annuloris and E.chrysogaster* were 0.000 each. Others were *C.tigripes* 0.0006, *Ae.albopictus* 0.003, *Ae.aegypti* 0.008, *An.funestus* 0.009 and *An.gambiae* 0.018 (Table 8).

Table 8: Simpson's index of dominance for mosquito species composition in the six study communities in Awka South Local Government Area

Mosquito species	**Simpson's index of dominance (C)**					
	Amawbia	**Awka**	**Nise**	**Nibo**	**Okpuno**	**Mbaukwu**
An.gambiae	0.005	0.002	0.007	0.011	0.056	0.018
An.funestus	0.000	0.000	0.0003	0.006	0.004	0.009
Ae.aegypti	0.012	0.004	0.016	0.014	0.006	0.008
Ae.albopictus	0.005	0.002	0.013	0.005	0.004	0.003
C.quinquefasciatus	0.557	0.719	0.401	0.367	0.502	0.365
C.tigripes	0.000	0.000	0.0007	0.0003	0.0001	0.0006
C.annulioris	0.000	0.000003	0.000	0.000	0.000	0.000
E.chrysogaster	0.000004	0.000	0.000	0.000	0.000	0.000
Total	**C=0.579**	**C=0.727**	**C=0.434**	**C=0.403**	**C=0.572**	**C=0.404**

4.2 Larval abundance by breeding sites

A total of 815 mosquito larvae (Table 9) were collected from six types of breeding sites – blocked gutters, domestic water containers (drums, water storage cans, plastics, metal cans and cooking utensil), discarded automobile tyres, ground water pools, septic tanks and treeholes and plant axils identified in the study area. The highest number of larvae 202(24.78%) were collected from septic tanks and the least 41(5.03%) were from treeholes. The collections from blocked gutters were 135(16.56%), domestic containers 113(13.86%), discarded used tyres 126(15.45%) and ground water pools 198(24.29). There was no significant difference in the numbers of mosquito larvae collected from different breeding sites ($P > 0.05$). P value = 0.734 (Appendix XI).

Six mosquito species namely *An. gambiae*, *An. funestus*, *Ae. aegypti*, *Ae. albopictus*, *C. tigripes* and *C. quinquefasciatus* were collected in their larval stages. *C. quinquefasciatus* larvae 474(58.15%) was the most abundant while *An. funestus* larvae 34(4.17%) was the least. Other species in order of their abundance were *Ae. aegypti* 100(12.26%), *Ae. albopictus* 93(11.41%), *An. gambiae* 73(8.95%) and *C. tigripes* 41(5.03%). There was a significant difference in mosquito species collected from the breeding sites ($P < 0.05$). P value = 0.000 (Appendix XII).

Table 9: Mosquito larvae collected from different breeding sites in Awka-South L.G.A

Mosquioto species	**Breeding Sites**						
	Dirty gutters	**Domestic containers**	**Discarded tyres**	**Ground pools**	**Septic Tanks**	**Treeholes and plant axils**	**Total (%)**
An. gambiae	0	0	0	73	0	0	73(8.95)
An. funestus	0	0	0	34	0	0	34(4.17)
Ae. aegypti	0	67	33	0	0	0	100(12.26)
Ae. albopictus	0	0	93	0		0	93(11.41)
C. tigripes	0	0	0	0	0	41	41(5.03)
C. quinque-fasciatus	135	46	0	91	202	0	474(58.15)
Total	**135 (16.56%)**	**113 (13.86%)**	**126 (15.45%)**	**198 (24.29%)**	**202 (24.78%)**	**41 (5.03%)**	**815(100)**

P value for the abundance of larval species from breeding sites = 0.734, P > 0.05

P value for the abundance of mosquito larvae in different breeding sites = 0.000, P < 0.05

4.2.1 Larval abundance by communities in Awka South L.G.A, Anambra State

Mosquito larvae abundance by communities is shown in figure 15. Of the 815 larvae collected, the highest number of larvae 178(21.84%) were collected from Amawbia community and the least 106(13.00%) from Mbaukwu. Others, in order of abundance, were Awka 159(19.50%), Nibo 127(15.58%), Okpuno 125(15.33%) and Nise 120(14.72%). There was a significant difference in the abundance of mosquito larvae in different communities ($P < 0.005$). P value = 0.000 (see Appendix XII).

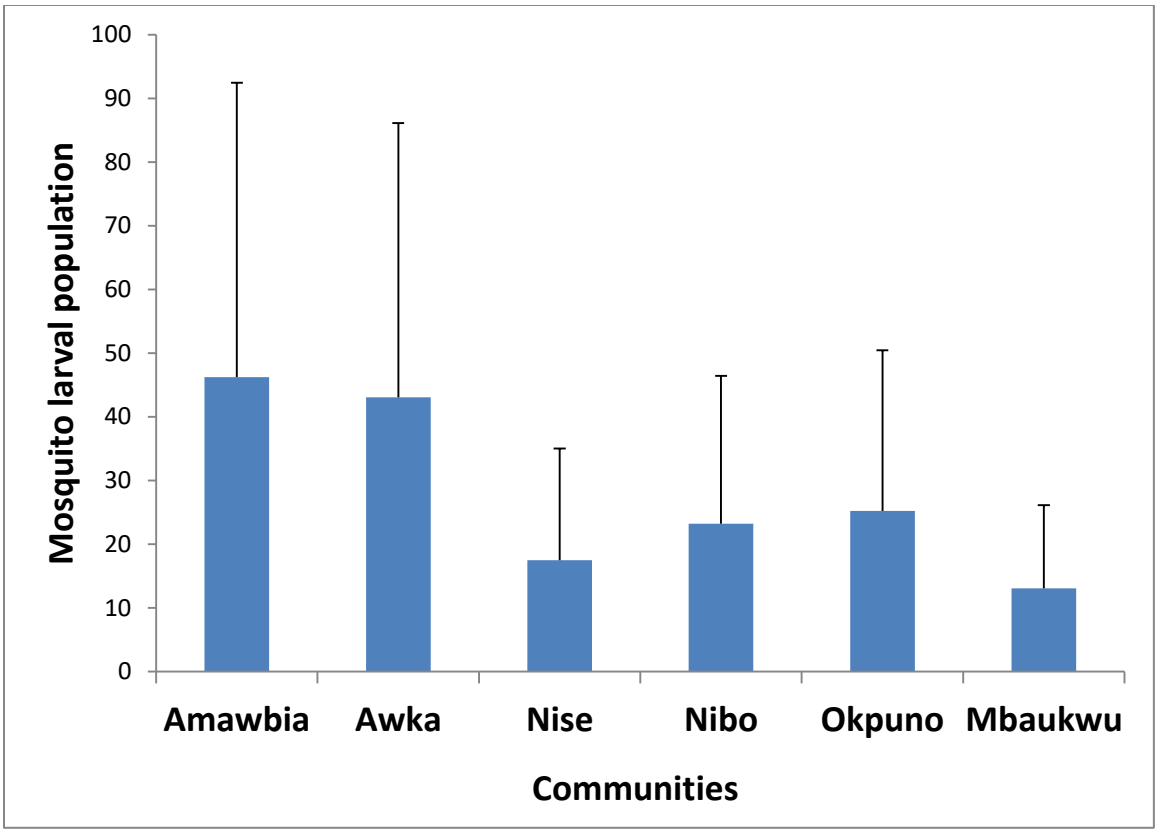

Fig 15. Mosquito larvae abundance from different communities of Awka-South L.G.A, Anambra State, Nigeria

(P < 0.005, P value = 0.000)

4.3 The biting and resting behaviour of the adult mosquitoes using Human Bait and Pyrethrum Knockdown methods.

A total of 2,663 adult mosquitoes comprised of 1,393(52.31%) outdoor biting adults and 1,270(47.69%) indoor biting and resting adults were collected from the study (Table 10). There was no significant difference between the numbers of mosquitoes collected outdoors and indoors ($P > 0.005$). P value = 0.122 (Appendix IX). Five species namely *An. gambiae*, *An. funestus*, *Ae. aegypti*, *Ae. albopictus* and *C. quinquefasciatus* were collected both indoors and outdoors and they constituted 2661(98.92%) of the total collection. In addition to these, two more species namely *C. annulioris* and *E. chrysogaster* were collected only outdoors in negligible proportions of 1(0.004%) each. Among the five common species, *C. quinquefasciatus* was the most abundant mosquito collected both outdoors 1,023(73.44%) and indoors 951(74.88%) while *An. funestus* was the least both outdoors 62(4.45%) and indoors 47(3.70%). Others in their order of abundance both outdoors and indoors were *Ae. aegypti* 149(10.69%) outdoors and 84(6.61%) indoors, *An. gambiae* 80(5.74%) outdoors and 131(10.31%) indoors, and *Ae. albopictus* 77(5.53%) outdoors and 57(4.49%).

Table 10: Outdoor and Indoor biting Mosquito population in Awka South L.G.A

Mosquito species	Outdoor biting population (%)	Indoor biting Population (%)	Total	Percentage (%)
An. gambiae	80(5.74)	131(10.31)	211	7.92
An. funestus	62(4.45)	47(3.70)	109	4.09
Ae. aegypti	149(10.69)	84(6.61)	233	8.75
Ae. albopictus	77(5.53)	57(4.49)	134	5.03
C. quinque fasciatus	1023(73.44)	951(74.88)	1974	74.13
C. annulioris	1(0.07)	0(0.00)	1	0.04
E. chrysogaster	1(0.07)	0(0.00)	1	0.04
Total	**1393 (52.31%)**	**1270 (47.69%)**	**2663 (100)**	**100**

P value of outdoors and indoor collection = 0.122, P > 0.005

4.3.1 Outdoor biting adult mosquitoes by community

Of the 1,393(100%) outdoor biting adult mosquitoes collected, the highest number 325(23.33%) were from Awka metropolis and the least 167(11.98%) were from Nise community (Fig 16). Outdoor mosquito collection from other communities were 205(14.72%) from Amawbia, 199(14.28%) from Nibo, 303(21.75%) from Okpuno and 194(13.92%) from Mbaukwu. There was no significant difference in the population of the outdoor biting mosquito population collected from different communities ($P > 0.05$). P value = 0.386 (Appendix XIII).

Seven mosquito species, including two *Anopheles* species and five Culicine mosquito species were collected outdoors (Fig 17). These were *An. gambiae* 80(5.79%), *An. funestus* 62(4.45%), *Ae. aegypti* 149(10.69%), *Ae. albopictus* 77(5.53%), *C. quinquefasciatus* 1023(73.44%), *C. annulioris* 1(0.07%) and *Eretmapodites chrysogaster* 1(0.07%). Among the Culicine mosquitoes, *C. quinquefasciatus* was the most abundant outdoor biting mosquitoes in the community followed by *Ae. aegypti* and *Ae. albopictus*. There was a significant difference in the numbers of the different outdoor mosquito species collected from different comunities ($P < 0.05$). P value = 0.000 (Appendix XIV).

4.3.2 All night hourly collections of outdoor biting adult mosquitoes in Awka South L.G.A, Anambra State, Nigeria

Five dominant mosquito species namely; *An. gambiae, An. funestus, Ae. aegypti, Ae. albopictus* and *C. quinquefasciatus* were collected (Fig 18). *Ae. aegypti* and *Ae. albopictus* had their peaks between 7.00-8.00pm and virtually stopped biting by 9.00pm. *C. quinquefasciatus* continued biting until dawn (6.00am) the next day, with a major peak between 9.00pm and 11.00pm. *An. gambiae* and *An. funestus* had their biting peaks by 1.00am followed by a gradual decline in population until dawn.

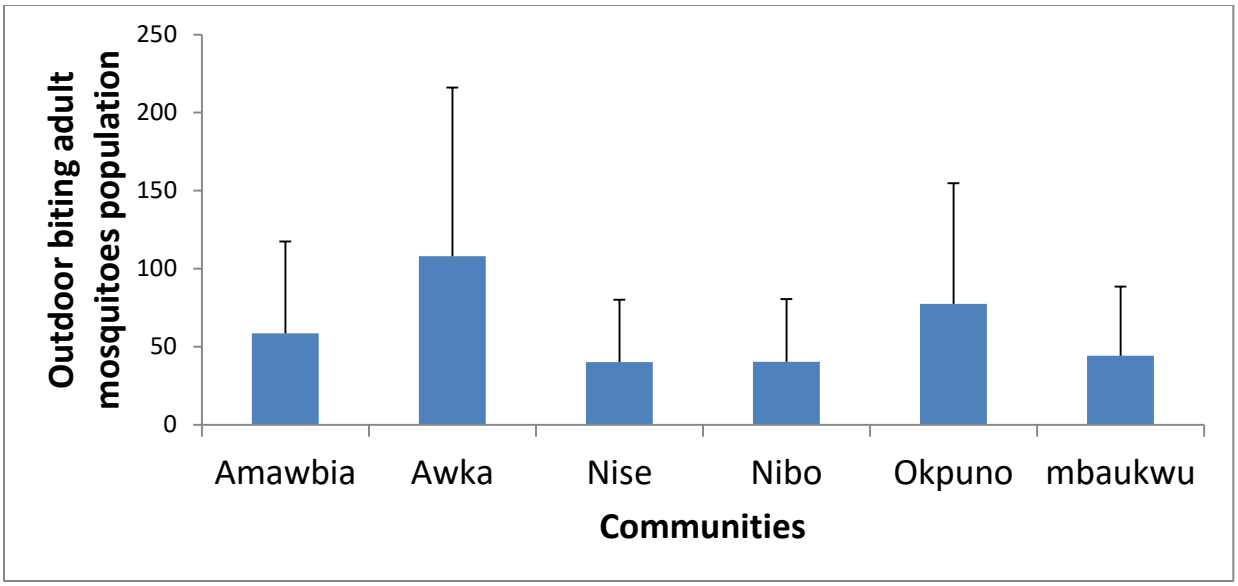

Fig 16. Outdoor biting adult mosquito populations from different communities

(P > 0.05, P value = 0.386)

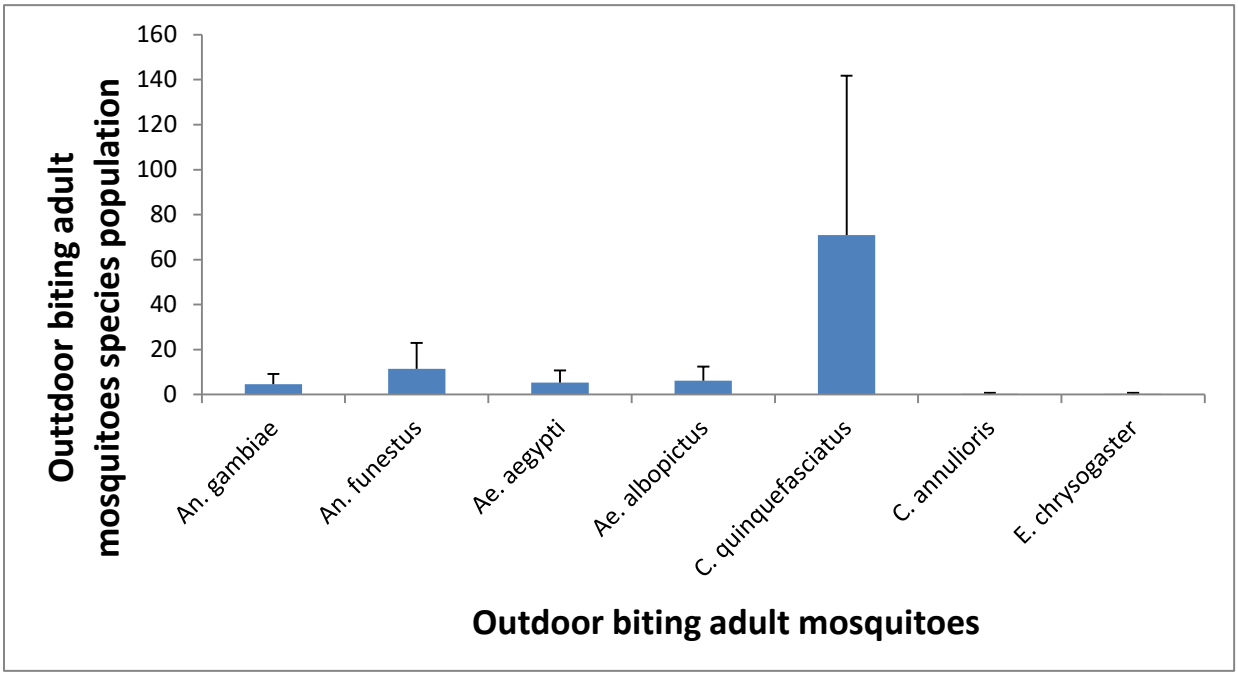

Fig 17. Outdoor biting adult mosquito species from different communities

(P < 0.05, P value = 0.000)

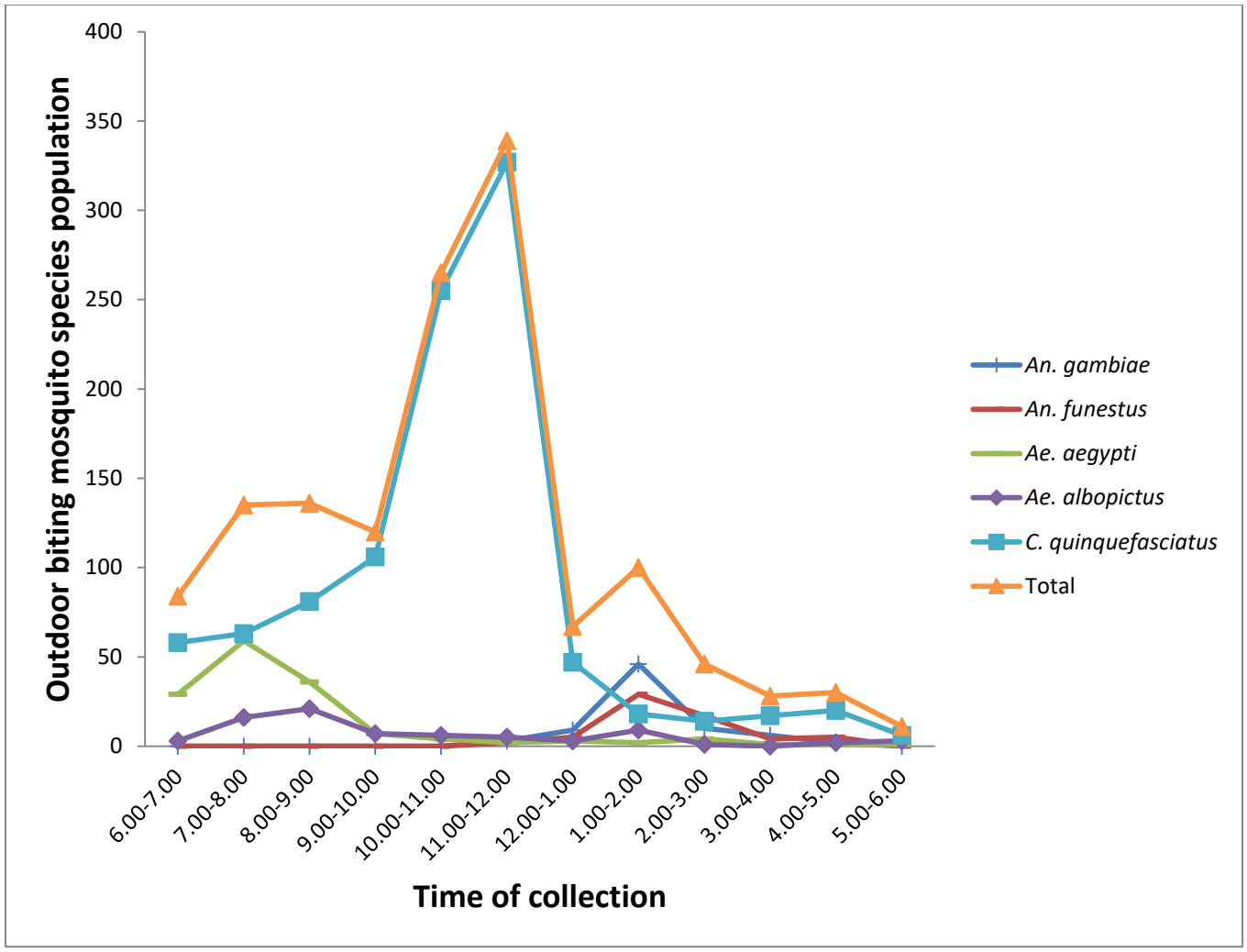

Fig 18: All night hourly collections of outdoor adult mosquitoes in Awka South Local Government Area, Anambra State using Human Bait Collection Method (HBC)

4.3.3 Indoor biting adult mosquitoes by community

Of the 1,270(100%) indoor biting adult mosquitoes collected (Fig. 19), the highest number 279(21.96%) were from Okpuno and the least 160 (12.59%), from Nise. The collection of indoor biting adult mosquitoes from other communities were 169(13.30%) from Amawbia, 257(20.23%) from Awka metropolis, 197(15.51%) from Nibo and 208(16.37%) from Mbaukwu. There was no significant difference in the distribution of the indoor biting mosquito population in the different communities ($P > 0.05$, P value = 0.796) (Appendix XV).

Five mosquitoes species namely *An. gambiae*, *An.funestus*, *Ae. aegypti*, *Ae.albopictus* and *C. quinquefasciatus* were collected indoors. *C. quinquefasciatus* 951(74.88%) was the most prevalent indoor-biting mosquitoes and *An. funestus* 47(3.70%) the least (Fig. 20). *An. gambiae* 131(10.31%) and *An. funestus* 47(3.70%) were important malaria vectors collected indoors. *Ae. aegypti* 84(6.61%) and *Ae. albopictus* 57(4.4%) were important vectors of arbovirus collected indoors. *C. quinquefasciatus* 951(74.88%) and *An. gambiae* were important vectors of lymphatic filariasis collected indoors. There was a significant difference in the distribution of the different indoor mosquito species collected in the different comunities ($P < 0.05$). P value = 0.000 (Appendix XVI).

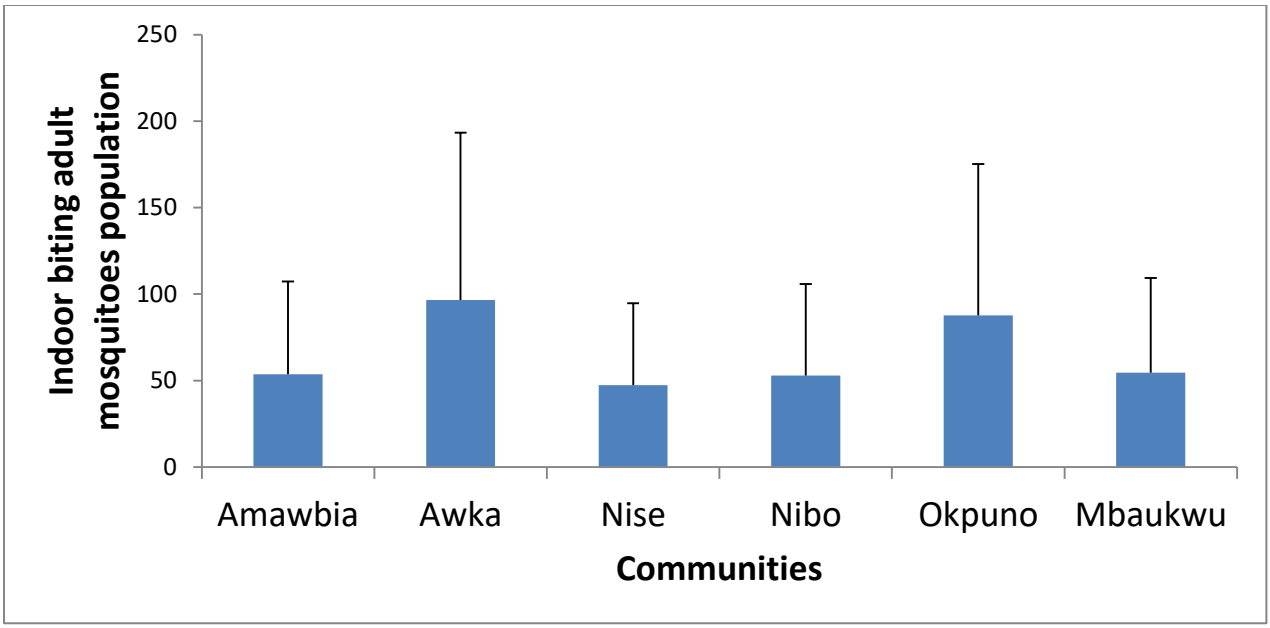

Fig 19. Indoor biting adult mosquito populations collected from different communities

(P > 0.05, P value = 0.796)

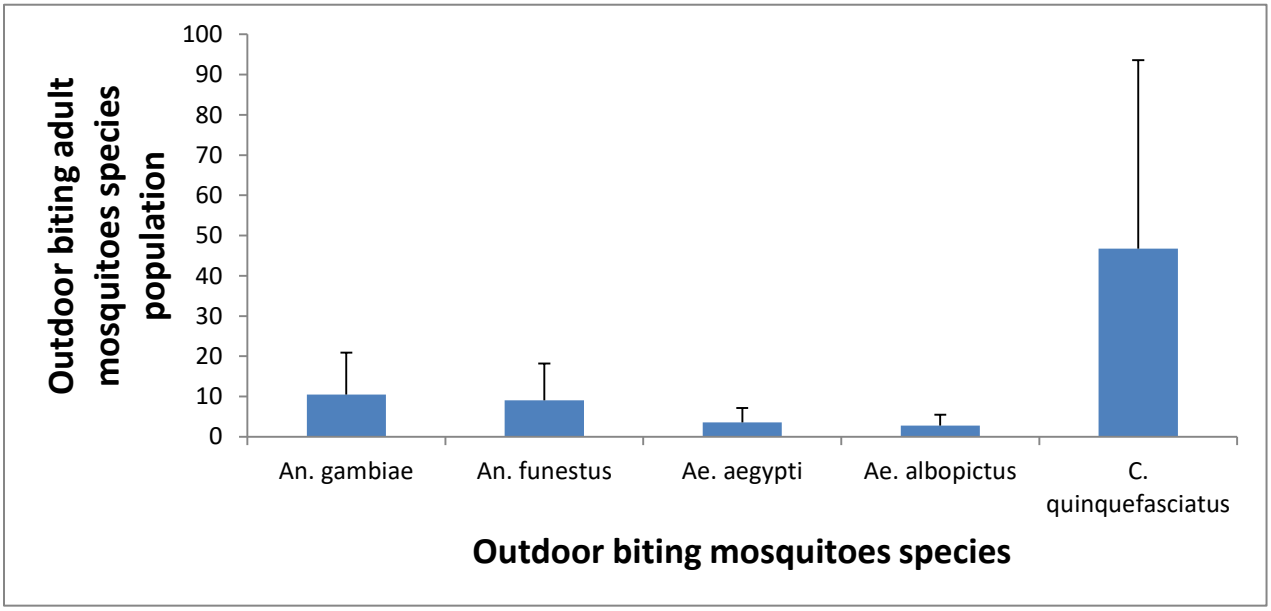

Fig 20. Indoor biting adult mosquito species collected from different communities

(P < 0.05, P value = 0.000)

4.4 Determination of the physiological states, Indoor resting density, biting rates and sporozoites rates of female mosquitoes collected indoors

4.4.1 Physiological state of indoor biting adult female mosquitoes.

Of the 1,270(100%) adult mosquitoes collected indoors (Fig 21), 125(9.84%) were unfed, 570(44.88%) were freshly fed, 409(32.20%) were half gravid and 166(13.07%) were gravid (Appendix XXXII).

4.4.2 Room densityof indoor biting adult mosquitoes collected.

Indoor Resting Density of different mosquito species were calculated (Appendix XVII). *Culex quinquefasciatus* had the highest room density of 2.20 mosquitoes/room/night followed by *An. gambiae* 0.30 mosquitoes/room/night, *An. funestus* 0.10 mosquitoes/room/night, *Ae. aegypti* 0.20 mosquitoes/room/night and *Ae. albopictus* 0.13 mosquitoes/room/night (Table 11).

4.4.3 Man-biting rates of indoor adult mosquitoes (MBR)

The man biting rate of different adult mosquitoes are shown in Table 11. *Culex quinquefasciatus* had the highest monthly indoor man biting rate of 1.0 bites/man/night followed by *An. gambiae* 0.017 bites/man/night, *An. funestus* 0.010 bites/man/nights, *Ae. aegypti* 0.020 bites/man/nights, and *Ae. albopictus* 0.014 bites/man/night.

4.4.4 Sporozoite index of adult *Anopheles* mosquitoes collected indoors

Of 50 bloodfed *An. gambiae* mosquitoes subjected to Enzyme-linked immunosorbent assays **(**ELISA) for analysis of sporozoite rate, only 5(10.0%) were positive for sporozoites and 45(90.0%) were negative (Table 12).

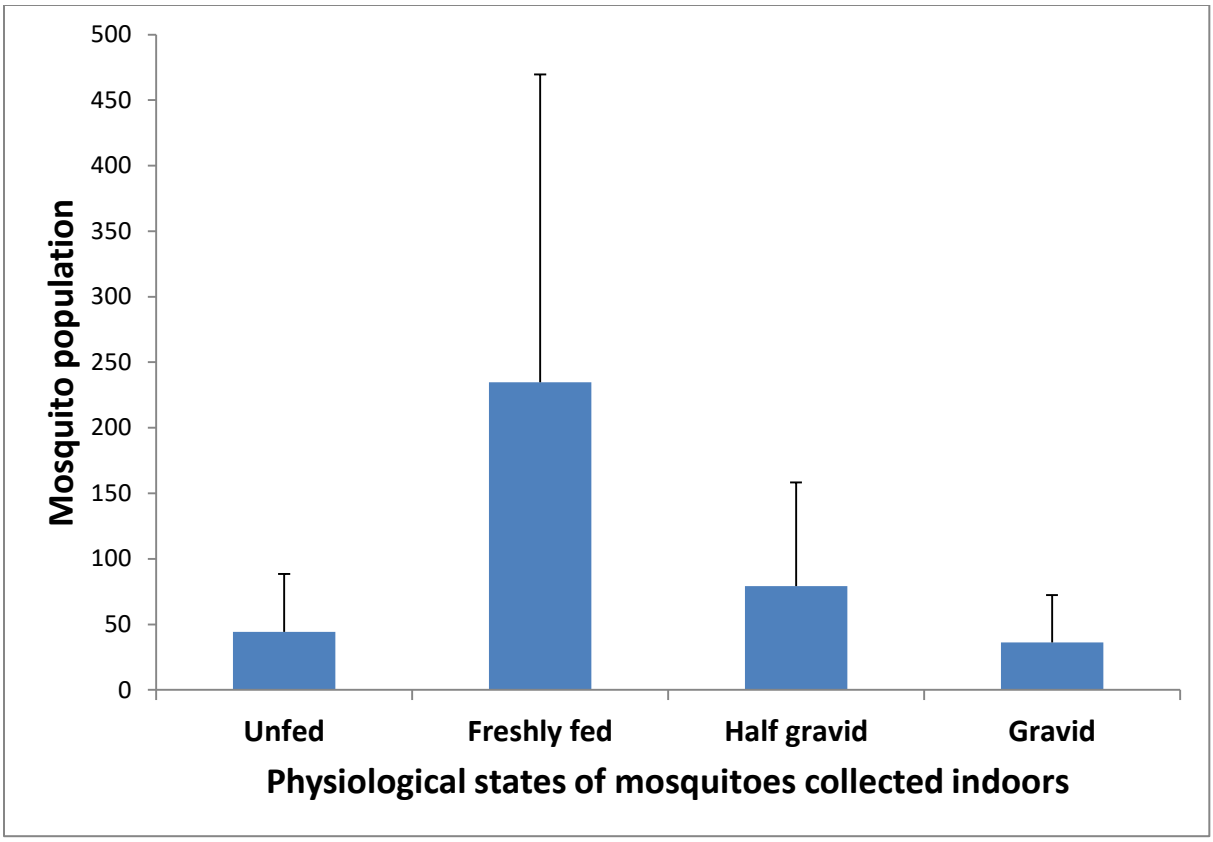

Fig 21. Abdominal Grading of Indoor-biting Adult Mosquitoes Collected using Pyrethroid Knock down (PKC) to determine their Physiological state

Table 11: Indoor Resting Density and Man-biting rate of different mosquito species in Awka South Local Government Area, Anambra State

Mosquitoes species collected indoors	No. of females collected	No. freshly fed females	Indoor Resting Density (IRD) No. of mosquitoes /room/night	Man Biting Rate (MBR) No. of bites/man/night
An. gambiae	131	11(1.9%)	0.30	0.017
An. funestus	47	6(1.0%)	0.10	0.010
Ae. aegypti	84	10(1.8%)	0.20	0.020
Ae. albopictus	57	9(1.6%)	0.13	0.014
C. quinque fasciatus	951	534(93.7%)	2.20	1.0
Total	1270	570(44.9%)	3.00	1.10

Table 12: Malaria sporozoites rate of the bloodfed *Anopheles gambiae* by Enzyme-linked Immunosorbent Assay (ELISA)

Mosquito complex	Positive (%)	Negative (%)	Total (%)
An. Gambiae	5(10.0)	45(90.0)	50(100.0)

4.5 Physicochemical and climatic factors influencing the survival of larvae and adult mosquitoes in their environments

4.5.1 Influence of rainfall on mosquito populations

Figure 22 is a plot of monthly mosquito collections (adults and larvae) against mean monthly rainfall. The mean monthly rainfall was very low between the months of December and February, but started increasing until it reached a peak in September, then it declined steadily to almost zero in December. The mosquito population followed the rainfall pattern and reached a high peak in the month of July, declined in the month of August and rose to the highest peak in October, from where it declined again to almost zero population in the month of December. Graph of mosquito larvae populations response to mean monthly rainfall were made for various mosquito larvae (Fig. 22 – 28). Correlation showed a significant positive relationship between rainfall and mosquito population ($r = 0.897$, see appendix XXV).

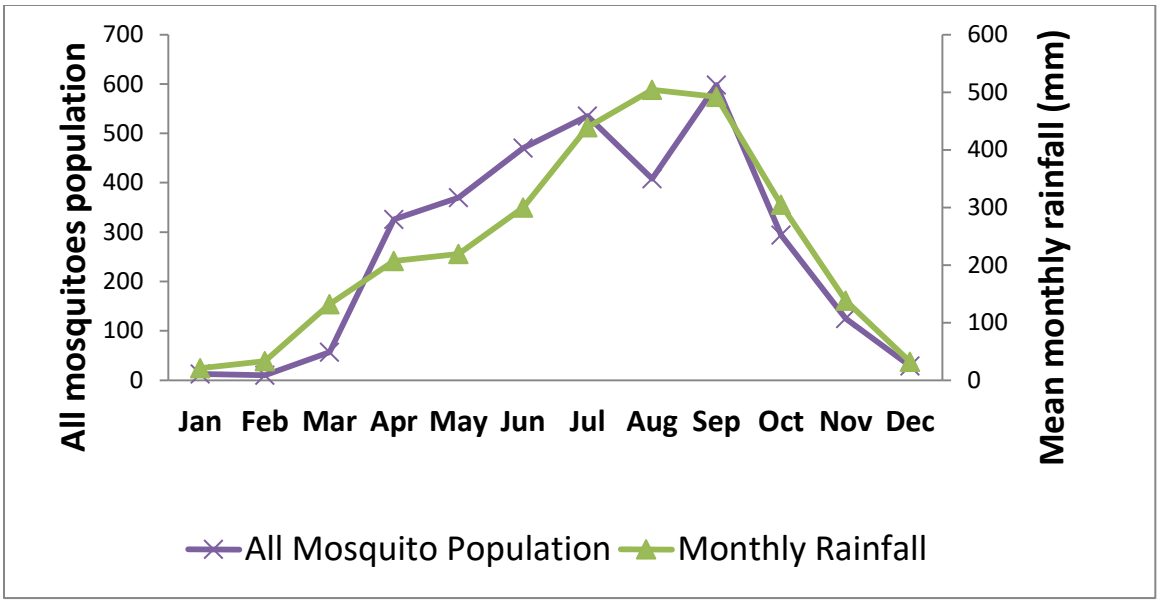

Fig 22. All Mosquito population (Larval and adults) Response to Monthly Rainfall patterns in Awka south Local Government Area, Anambra State

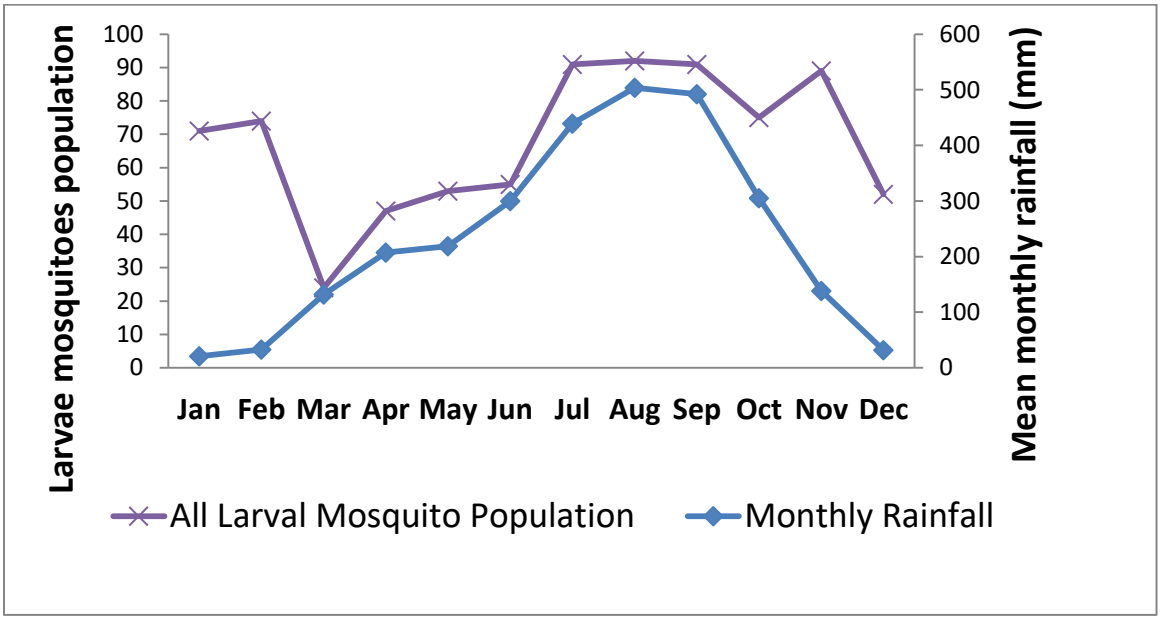

Fig 23. All Larval Mosquitoes Response to Monthly Rainfall patterns in Awka south Local Government Area, Anambra State

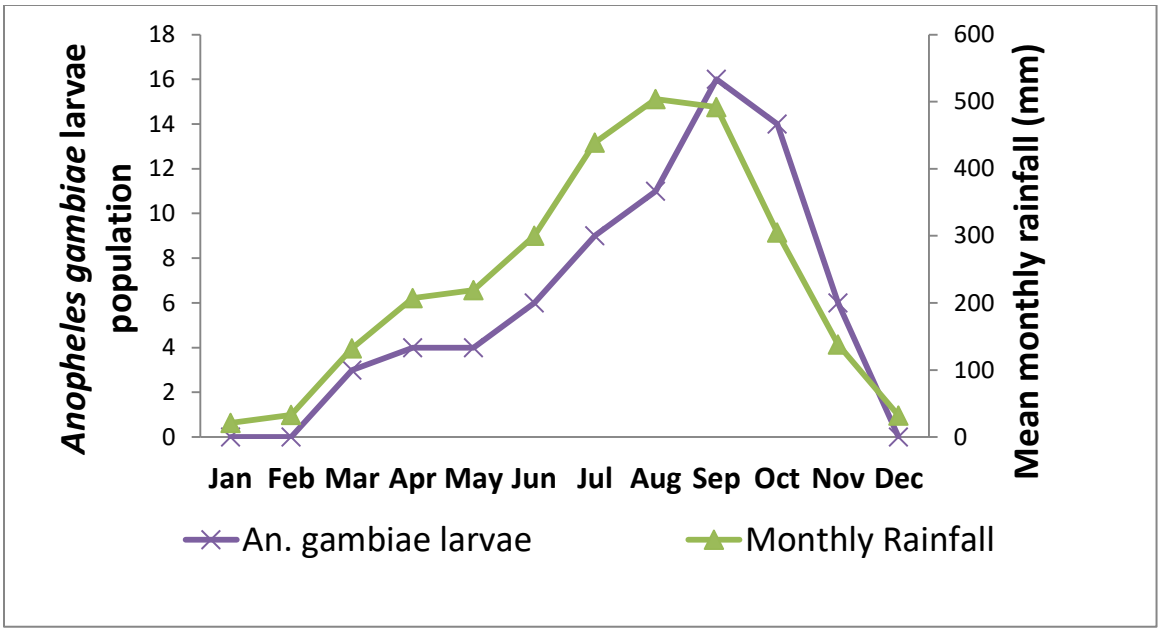

Fig 24. Response of *An. gambiae* Larvae to Monthly Rainfall patterns in Awka south Local Government Area, Anambra State

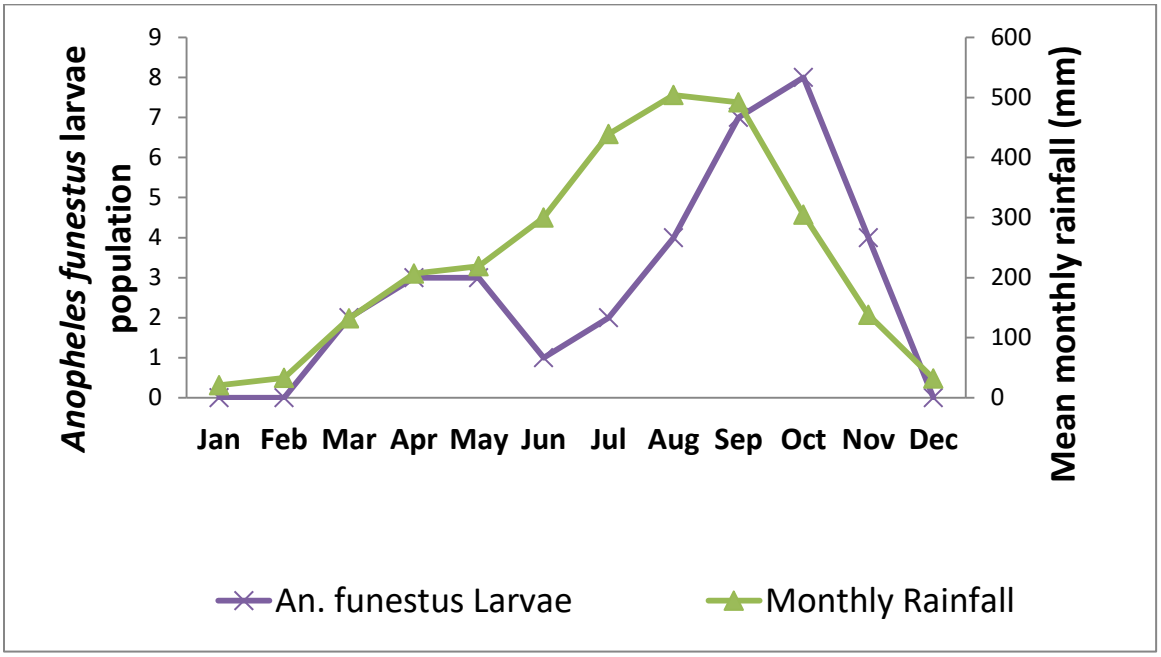

Fig 25. Response of *An. funestus* Larvae to Monthly Rainfall patterns in Awka south Local Government Area, Anambra State

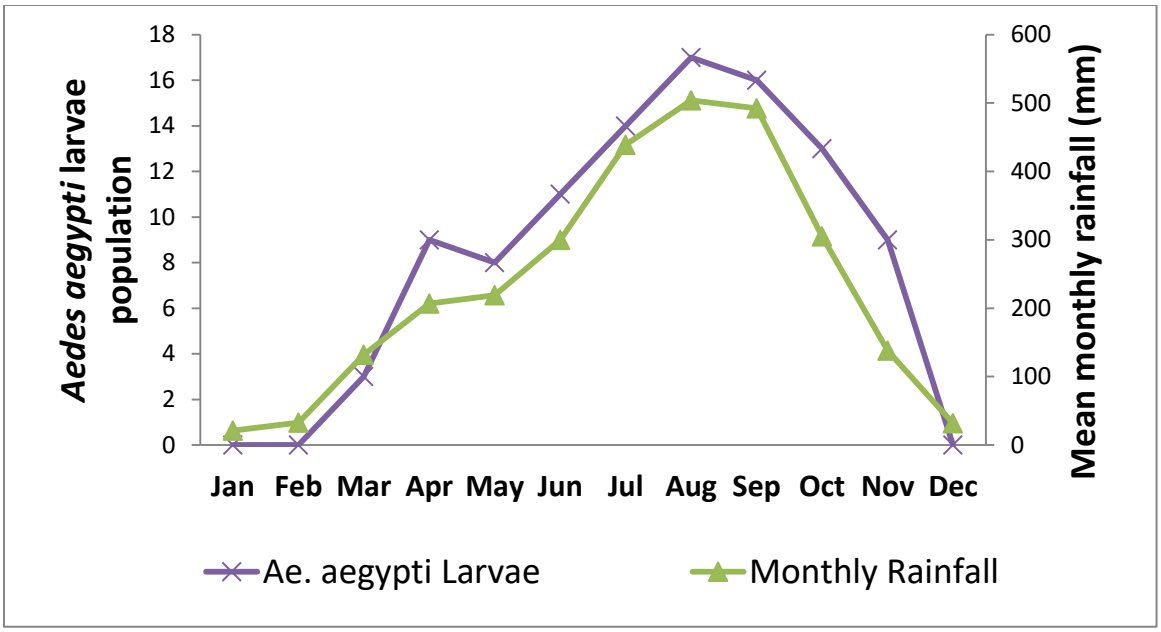

Fig 26. Response of *Ae. aegypti* Larvae to Monthly Rainfall patterns in Awka south Local Government Area, Anambra State

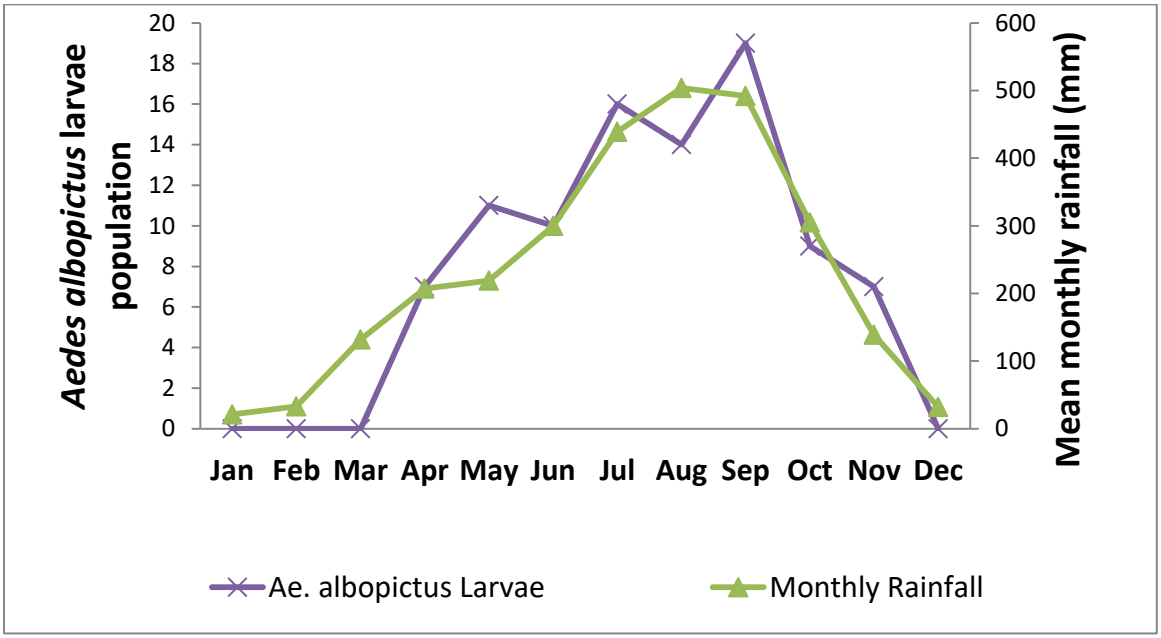

Fig 27: Response of *Ae. albopictus* Larvae to Monthly Rainfall patterns in Awka south Local Government Area, Anambra State

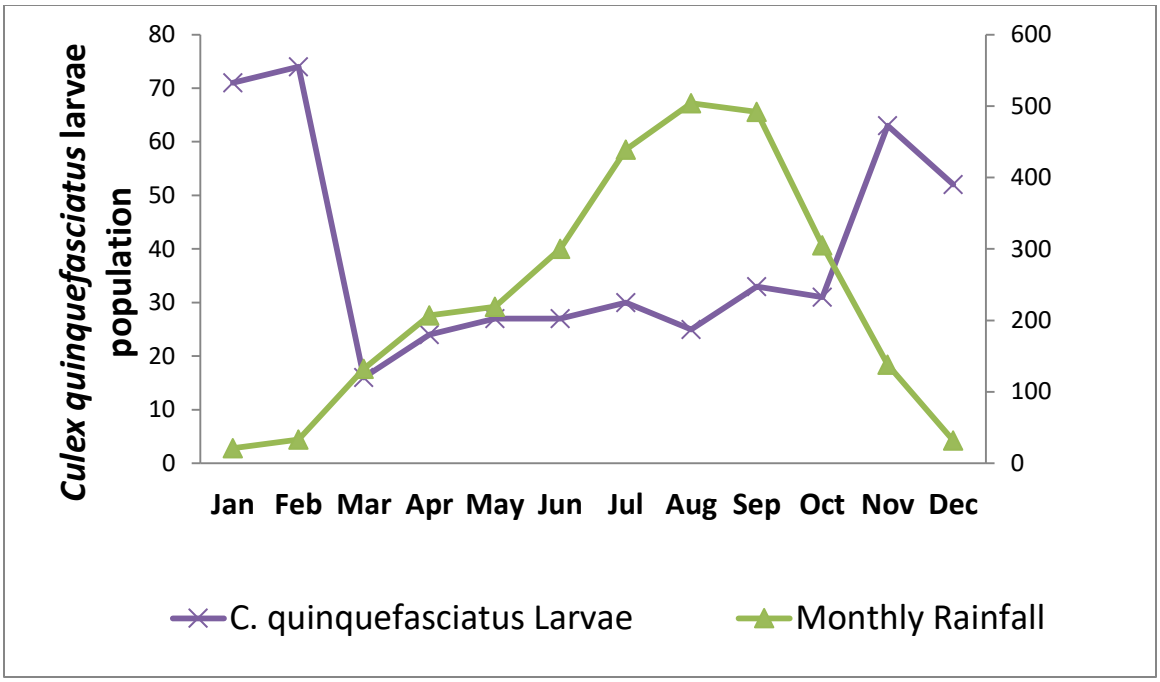

Fig 28: Response of *C. quinquefasciatus* Larvae to Monthly Rainfall patterns in Awka south Local Government Area, Anambra State

4.5.1.1 Adult mosquito population response to rainfall patterns

The response of adult mosquito populations to rainfall patterns were shown in figures 29 – 34. The adult populations followed rainfall pattern but contrary to the larval populations which started to increase in the month of March, adult mosquito populations increased rapidly from April and reached peak in July, declined slightly through August and rose to its highest peak in September. From October it declined rapidly to its lowest population in December. They all displayed strong positive relationship with rainfall, indicating that mosquito populations increased with increased rainfall until when heavy rainfall became detrimental to them.

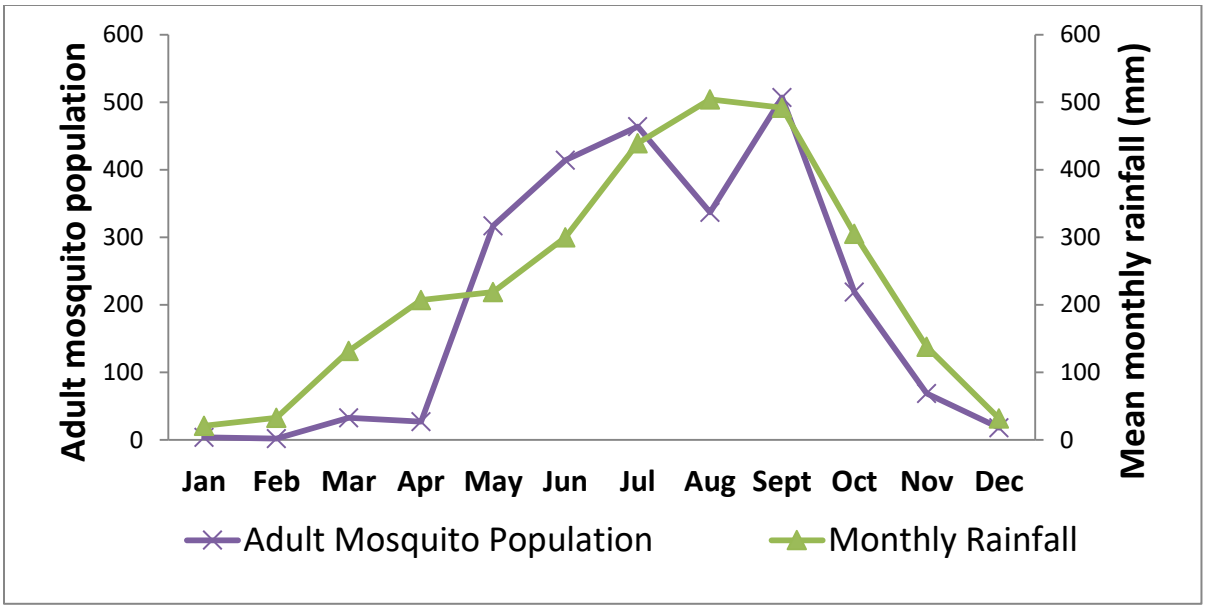

Fig 29: All Adult Mosquitoes Response to Monthly Rainfall patterns in Awka south Local Government Area, Anambra State

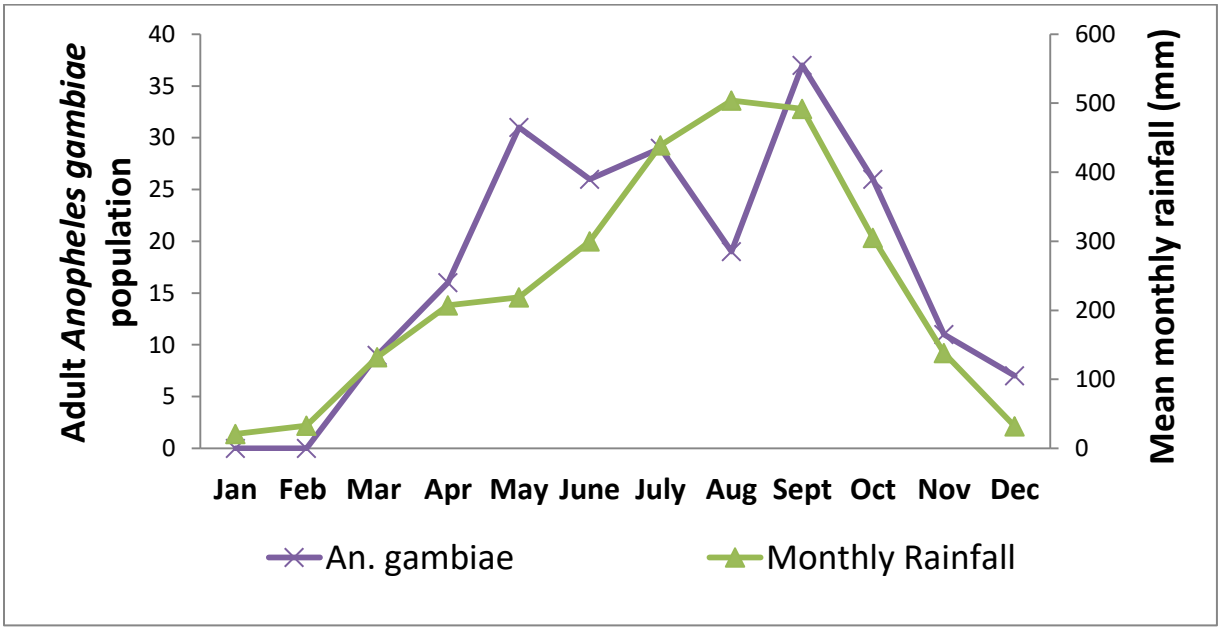

Fig 30: Response of Adult *Anopheles gambiae* to Monthly Rainfall Pattern in Awka South Local Government Area, Anambra State.

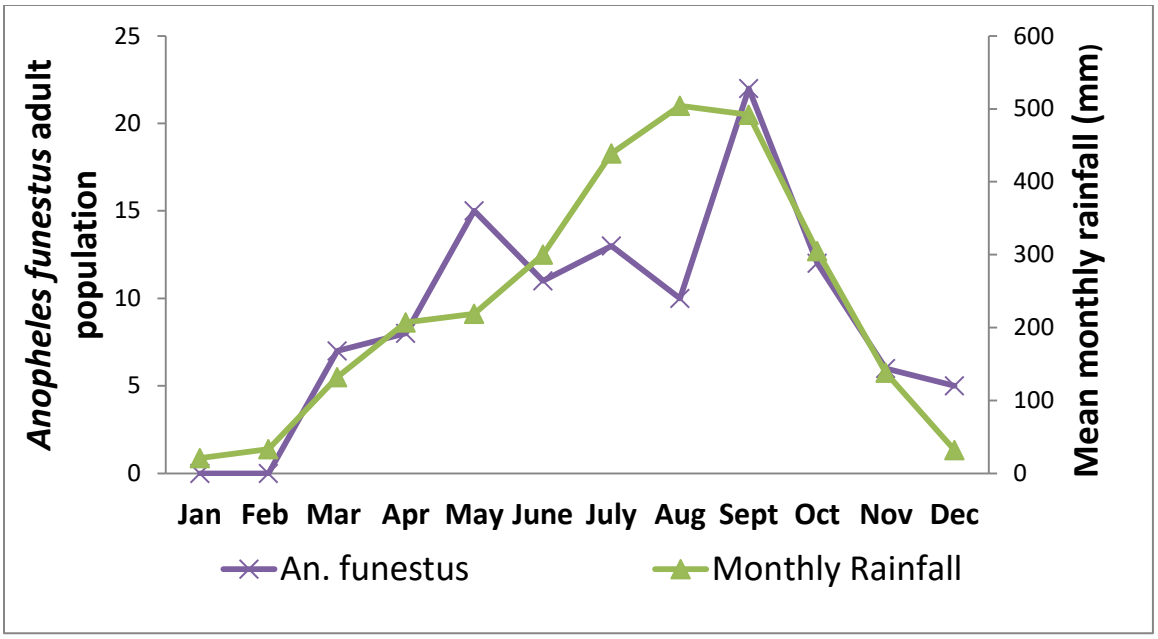

Fig 31: Response of Adult *Anopheles funestus* to Monthly Rainfall Pattern in Awka South Local Government Area, Anambra State.

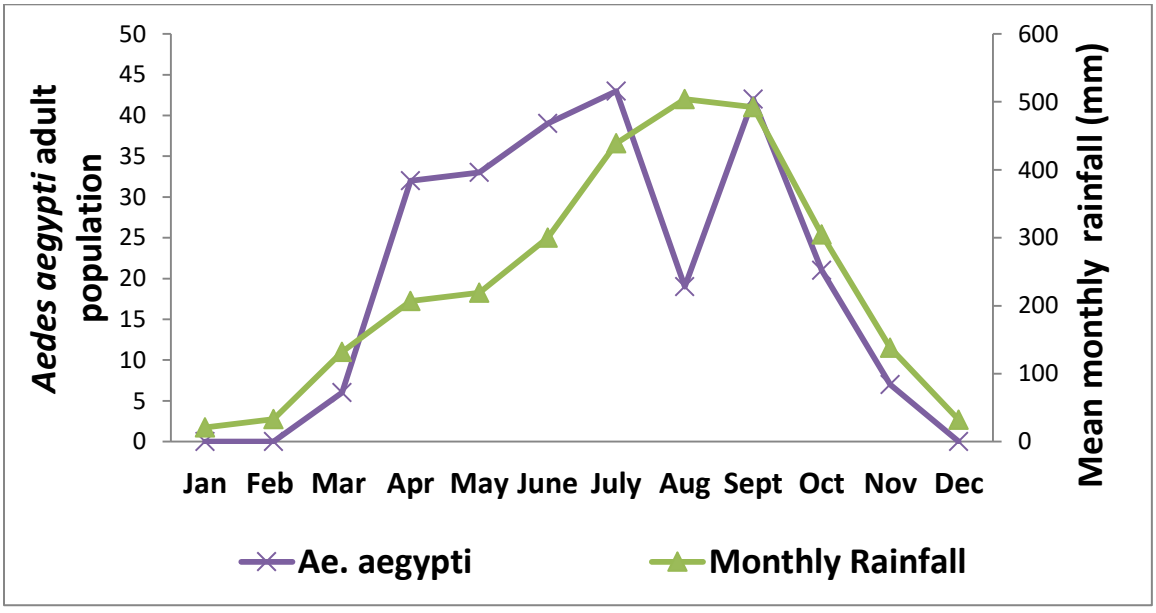

Fig 32: Response of Adult *Aedes aegypti* to Monthly Rainfall Pattern in Awka South Local Government Area, Anambra State

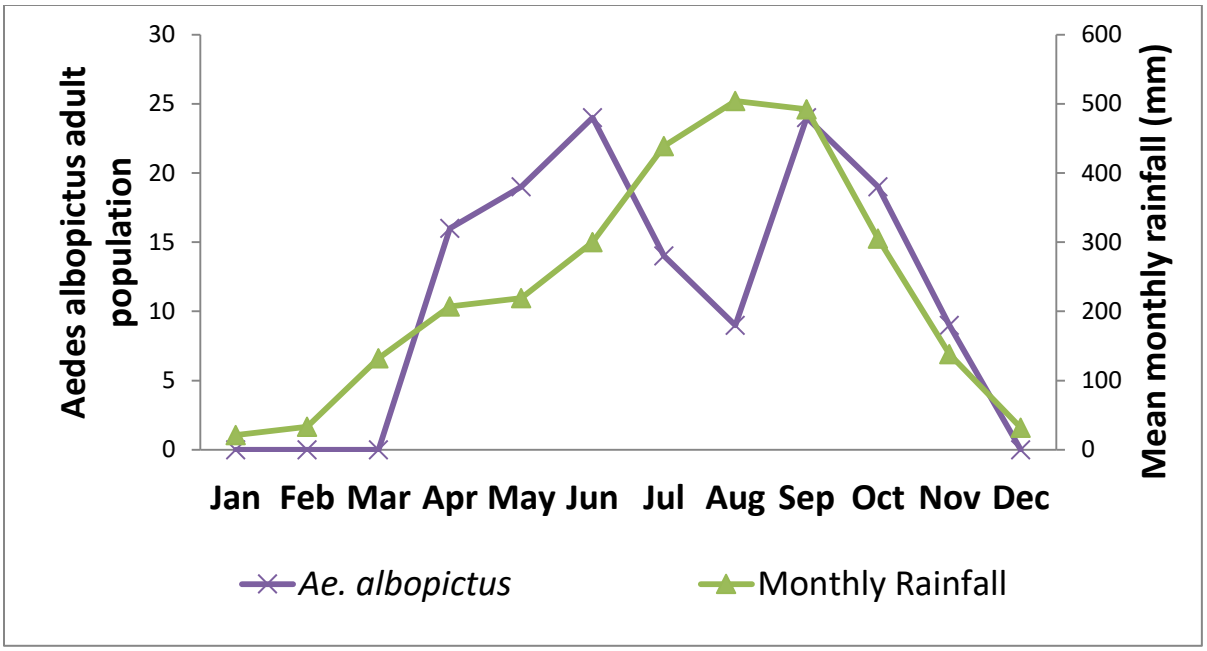

Fig 33: Response of Adult *Aedes albopictus* to Monthly Rainfall Pattern in Awka South Local Government Area, Anambra State.

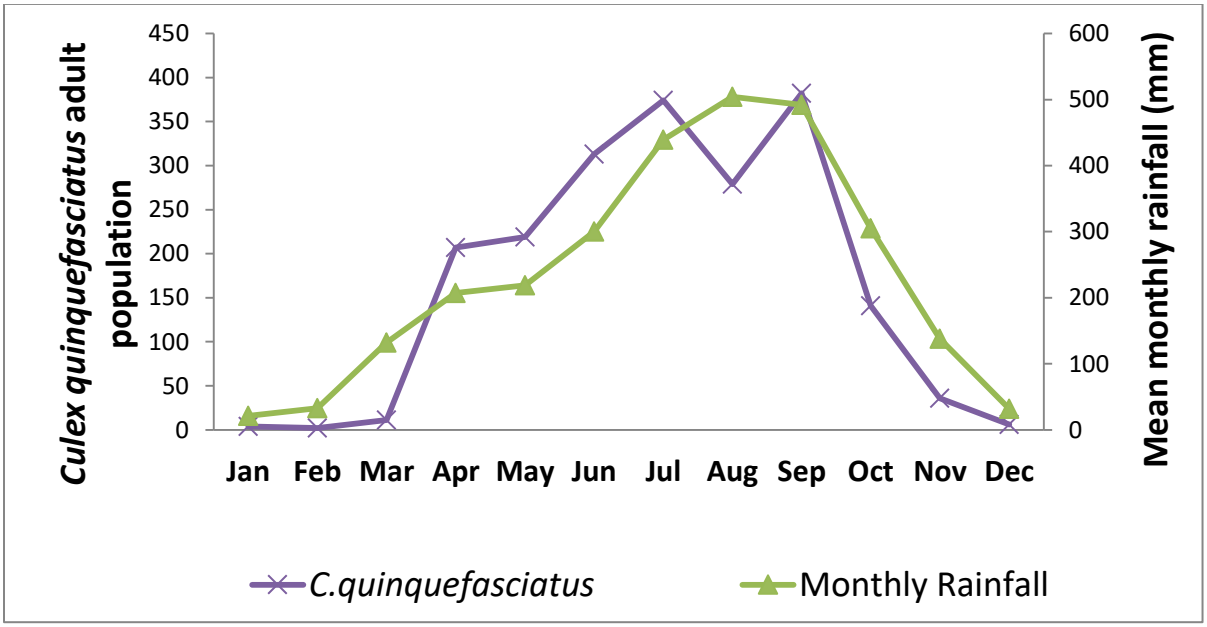

Fig 34: Response of Adult *Culex quinquefasciatus* to Monthly Rainfall Pattern in Awka South Local Government Area, Anambra State

4.5.2 Mosquito population response to environmental temperature

The adult mosquito population response to mean monthly temperature is shown in figure 35. The mean atmospheric temperature was more or less constant while the mosquito population increased between March and October months before it declined again to its lowest in December. Correlation of mosquito population against temperature indicated a negative relationship in which mosquito populations decreased with increasing temperatures. Correlation showed a significant negative relationship between temperature and mosquito population ($r = -0.775$, see appendix XXV).

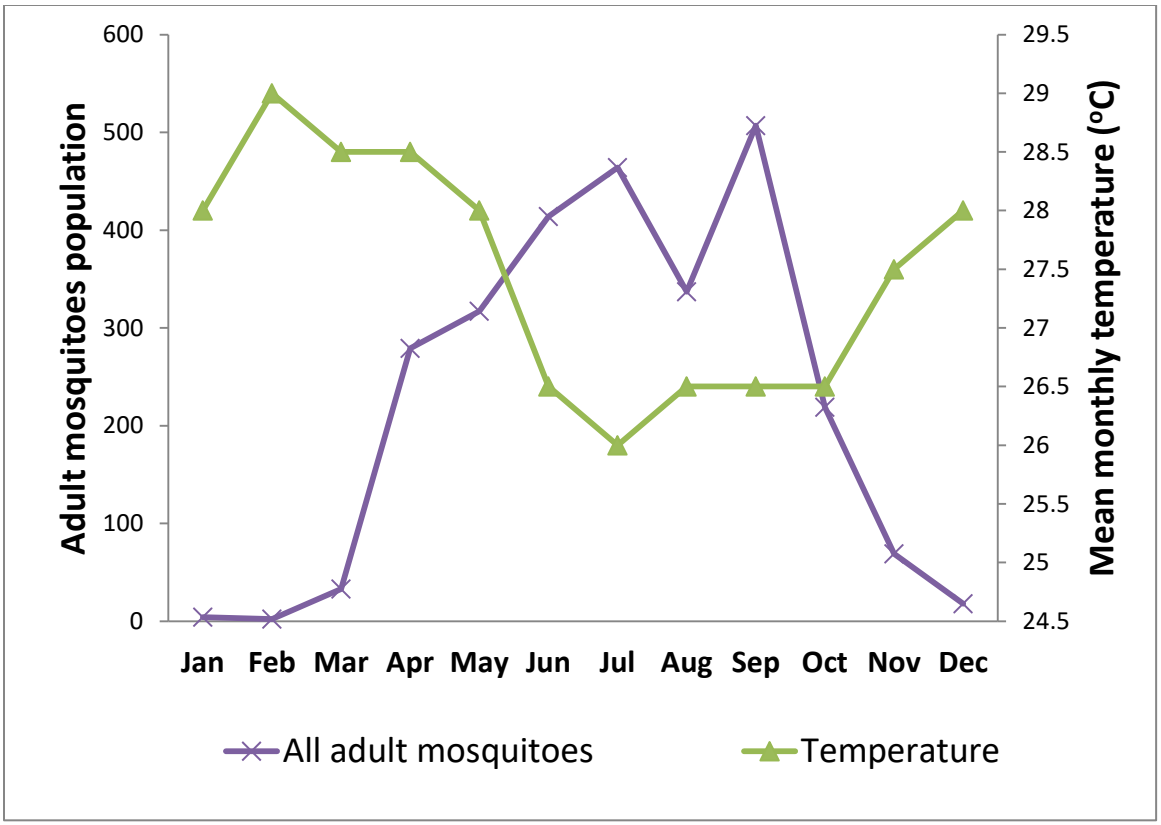

Fig 35. Response of All adult Mosquitoes to Temperature

4.5.3 Mosquito population response to mean environmental relative humidity

The response of adult mosquito populations to mean relative environmental humidity is shown in figure 36. The mean relative humidity fluctuated between 80% and 90% from December to January. The mosquito population started increasing from the month of March (beginning of wet season), until it reached its peak in the month of October (end of rainy season) to its lowest population in the month of December (beginning of dry season). Correlation showed a significant positive relationship between relative humidity and mosquito population ($r = 0.700$, see appendix XXV).

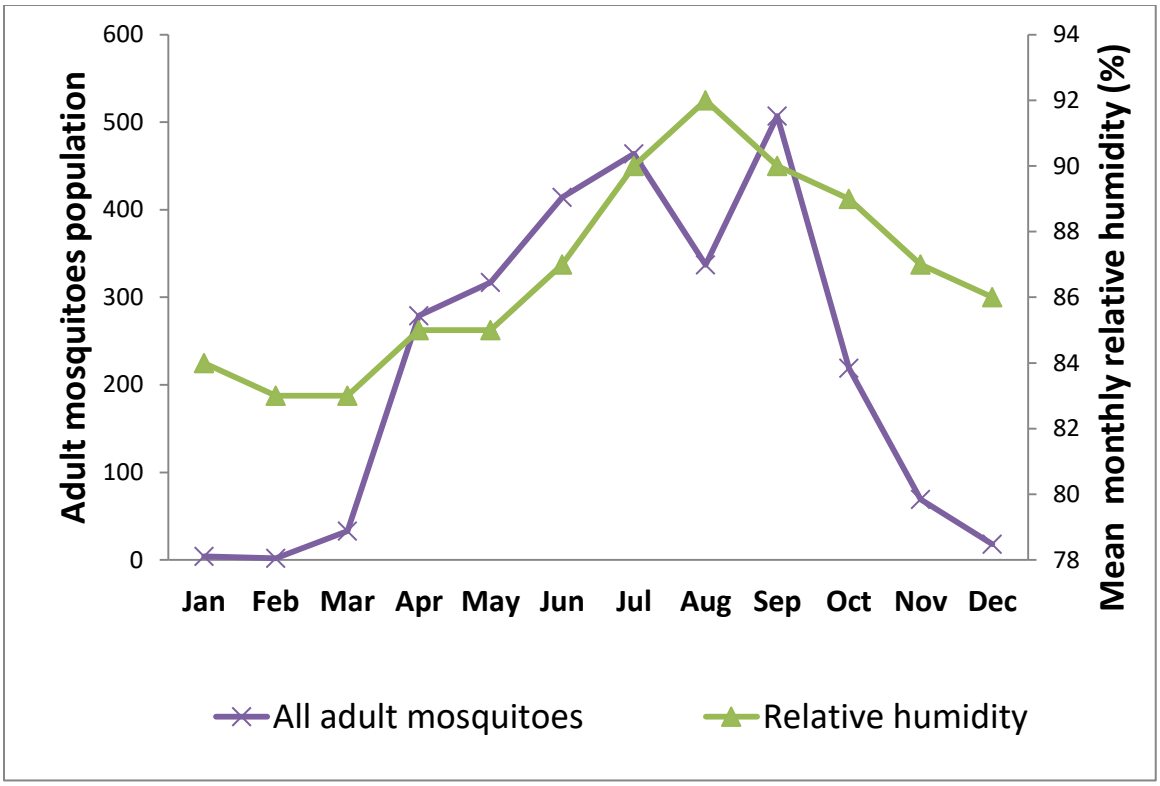

Fig 36. Response of All adult mosquitoes to Relative humidity

4.5.4 Response of mosquito larvae populations to physicochemical conditions of the breeding sites

Table 13 shows the range of mean values of physicochemical conditions of the mosquito breeding sites. Mosquito larvae were collected in the range values of pH 6.2 – 6.7, conductivity 69.2 – 150 **(**μS/cm), total dissolved oxygen 44.5 – 850 ppm, acidity 13.3 – 22.9 mg/l, alkalinity 181.3 – 227.2mg/l, salinity 0.038 – 0.065 PSU, surface water temperature 27.8 – 28^0C and water depth 6 – 13cm. The highest number of mosquitoes were collected in August when total dissolved oxygen was moderate while the least mosquitoes were collected in December when lowest oxygen content of the breeding sites were observed.

Table 13: Response of All mosquito larvae population to mean physicochemical conditions of all breeding sites in the study area

month	All mosquito larvae	mean pH	Mean Conductivity (µS/cm)	Mean Total Dissolved Oxygen (ppm)	Mean Acidity (mg/L)	Mean Alkalinity (mg/L)	Mean Salinity (PSU)	Mean Surface Water temp. (ºC)	Mean Depth of breeding site (cm)
Jan	71	6.2	69.2	45.2	22.9	181.3	0.038	28.3	6
Feb	74	6.2	76.3	49.5	22.6	185.4	0.040	28.6	6
Mar	24	6.3	90.7	44.5	20.8	198.2	0.045	28.8	8
Apr	47	6.5	127.2	67.8	15.2	203.9	0.061	28.5	8
May	53	6.5	130.3	71.1	15.1	205.8	0.061	28.2	9
June	55	6.6	133.6	74.7	15.0	210.9	0.061	27.8	13
July	91	6.6	138.1	77.0	14.6	214.7	0.063	27.8	13
Aug	92	6.6	141.8	79.9	14.4	218.1	0.064	27.8	12
Sept	91	6.7	150.0	85.0	13.3	227.2	0.065	28.0	9
Oct	75	6.6	134.6	79.5	14.6	212.4	0.061	28.2	8
Nov	89	6.3	130.0	72.1	17.6	193.7	0.056	28.6	7
Dec	52	6.2	78.6	49.6	22.9	184.7	0.038	28.0	7

4.5.5 Response of larvae of *Culex quinquefasciatus* to the physicochemical conditions of the breeding sites

Table 14 shows the range values of physicochemical conditions of the breeding sites of *Culex quinquefasciatus*. The larvae were collected in their breeding water in a pH range of 5.6 – 6.0, conductivity 83.1µS/cm – 141.3µS/cm, total dissolved oxygen of 58.6ppm – 96.8ppm, acidity 24.4mg/l – 29.2mg/l, alkalinlty, 138.3mg/l – 172.3mg/l, salinity 0.046PSU – 0.065PSU, surface water temperature 27.0ºC - 28.3ºC and depth of breeding site 5cm – 12cm. *Culex quinquefasciatus* was the only mosquito species that was collected from their breeding sites in each of the 12 months of the year. The mosquito has relationship to all physicochemical properties of the breeding sites except pH and the depth of breeding sites.

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Table 14: Response of larvae of *Culex quinquefasciatus* to physicochemical conditions of their breeding sites

Months	Larvae of *C. quinque-fasciatus*	pH	Conduct-ivity (µS/cm)	Total Dissolved Oxygen (ppm)	Acidity (mg/L)	Alkalinity (mg/L)	Salinity (PSU)	Surface Water temp. (°C)	Depth of breeding site (cm)
Jan	5	5.6	83.1	58.6	29.2	138.3	0.046	28.3	5
Feb	8	5.6	93.2	64.8	28.3	143.2	0.048	28.7	5
Mar	9	5.7	109.8	68.5	27.3	148.2	0.052	28.2	5
Apr	10	5.7	116.00	79.6	26.7	150.1	0.055	27.8	7
May	11	5.8	119.4	82.3	26.3	151.2	0.056	27.4	7
June	11	5.8	123.7	86.5	26.3	153.2	0.058	27.0	11
July	13	5.8	131.4	87.5	25.8	157.3	0.059	27.0	12
Aug	15	5.9	134.5	90.4	25.7	161.4	0.063	27.0	9
Sept	30	6.0	141.3	96.8	24.4	172.3	0.065	27.2	7
Oct	11	5.9	123.2	84.6	26.7	152.4	0.055	27.4	6
Nov	7	5.6	115.3	78.4	27.2	149.3	0.049	28.2	6
Dec	5	5.6	97.4	63.5	28.6	142.7	0.046	28.3	5

4.5.6 Response of larvae of *Anopheles gambiae* to physicochemical conditions of breeding sites

Table 15 shows the range values of physicochemical conditions of the breeding sites of *Anopheles gambiae*. The larvae were collected in their breeding water in a pH range of 6.1 – 6.4, conductivity 76.91µS/cm – 249.24µS/cm, total dissolved oxygen 48.25ppm – 166.3ppm, acidity 9.8mg/l – 31.2mg/l, alkalinlty 142.60mg/l – 177.43mg/l, salinity 0.040PSU – 0.098PSU, surface water temperature 31.0^{o}C – 31.8^{o}C and depth of breeding site range of 3cm – 6cm. The mosquito has positive relationship to all physicochemical properties of the breeding sites except acidity and the depth of breeding sites.

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Table 15: Response of larvae of *Anopheles gambiae* to physicochemical conditions of their breeding sites

Months	Larvae of *An.gambiae*	pH	Conduct-Ivity (µS/cm)	Total Dissolved Oxygen (ppm)	Acidity (mg/L)	Alkalinity (mg/L)	Salinity (PSU)	Surface Water temp. (°C)	Depth of breeding site (cm)
Jan	-	-	-	-	-	-	-	-	-
Feb	-	-	-	-	-	-	-	-	-
Mar	3	6.1	76.91	48.25	31.2	142.60	0.040	31.8	5
Apr	4	6.1	214.70	136.8	10.8	151.00	0.096	31.6	5
May	4	6.1	219.23	143.2	10.8	153.54	0.096	31.4	5
June	6	6.2	220.00	149.4	10.7	157.34	0.090	31.0	6
July	9	6.2	228.12	153.3	9.9	165.56	0.097	31.0	6
Aug	11	6.3	236.10	159.4	9.6	168.23	0.097	31.0	5
Sept	16	6.4	249.24	166.3	8.9	177.43	0.098	31.2	4
Oct	14	6.3	233.68	164.6	9.3	169.32	0.095	31.4	4
Nov	6	6.2	212.34	142.3	9.8	156.22	0.093	31.8	3
Dec	-	-	-	-	-	-	-	-	-

4.5.7 Response of larvae of *Anopheles funestus* to physicochemical conditions of breeding sites

Table 16 shows the range values of physicochemical conditions of the breeding sites of *An. funestus.* The larvae were collected in their breeding water in a pH range of 6.1 – 6.4, conductivity 76.91μS/cm – 249.24μS/cm, total dissolved oxygen 48.25ppm – 166.3ppm, acidity 9.6mg/l – 31.2mg/l, alkalinlty 142.60mg/l – 177.43mg/l, salinity 0.040PSU – 0.098PSU, surface water temperature 31.0^{o}C – 31.8^{o}C and depth of breeding site 3cm – 6cm. The mosquito has positive relationship to all physicochemical properties of the breeding sites except acidity and the depth of breeding sites.

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Table 16: Response of larvae of *Anopheles funestus* to physicochemical conditions of their breeding sites

Months	Larvae of *An. funestus*	pH	Conduct-Ivity (µS/cm)	Total Dissolved Oxygen (ppm)	Acidity (mg/L)	Alkalinity (mg/L)	Salinity (PSU)	Surface Water temp. (ºC)	Depth of breeding site (cm)
Jan	-	-	-	-	-	-	-	-	-
Feb	-	-	-	-	-	-	-	-	-
Mar	2	6.1	76.91	48.25	31.2	142.60	0.040	31.8	5
Apr	3	6.1	214.70	136.8	10.8	151.00	0.096	31.6	5
May	3	6.1	219.23	143.2	10.8	153.54	0.096	31.4	5
June	1	6.2	220.00	149.4	10.7	157.34	0.090	31.0	6
July	2	6.2	228.12	153.3	9.9	165.56	0.097	31.0	6
Aug	4	6.3	236.10	159.4	9.6	168.23	0.097	31.0	5
Sept	7	6.4	249.24	166.3	8.9	177.43	0.098	31.2	4
Oct	8	6.3	233.68	164.6	9.3	169.32	0.095	31.4	4
Nov	4	6.2	212.34	142.3	9.8	156.22	0.093	31.8	3
Dec	-	-	-	-	-	-	-	-	-

4.5.8 Response of larvae of *Aedes albopictus* to physicochemical conditions of breeding sites

Table 17 shows the range values of physicochemical conditions of the breeding sites of *Ae. albopictus*. The larvae were collected in their breeding water in a pH range of 6.2 – 6.8, conductivity 132.0µS/cm – 152.0µS/cm, total dissolved oxygen 33.80ppm – 41.7ppm, acidity 11.04mg/l – 14.31mg/l, alkalinlty 232.2mg/l – 267.2mg/l, salinity 0.061PSU – 0.067PSU, surface water temperature 26.0^{o}C - 26.8^{o}C and depth of breeding site range of 5cm – 9cm. The mosquito has positive relationship to all physicochemical properties of the breeding sites.

Table 17: Response of larvae of *Aedes albopictus* to physicochemical conditions of their breeding sites

Months	**Larve of *Ae.albopictus***	**pH**	**Con duct-ivity (**µS/cm)	**Total Dissolved Oxygen (**ppm)	**Acidity (**mg/L)	**Alkalinity (**mg/L)	**Salinity** (PSU)	**Surface Water temp. (ºC)**	**Depth of breeding site (cm)**
Jan	0	-	-	-	-	-	-	-	-
Feb	0	-	-	-	-	-	-	-	-
Mar	0	6.2	132.0	33.80	14.31	232.2	0.061	27.0	7
Apr	7	6.7	134.0	33.82	13.20	241.1	0.063	26.6	7
May	11	6.7	137.2	34.4	13.18	244.3	0.063	26.4	8
June	10	6.7	144.4	36.4	13.12	251.3	0.065	26.0	8
July	16	6.7	145.4	37.6	12.89	254.4	0.066	26.0	9
Aug	14	6.8	148.3	37.3	12.72	256.3	0.067	26.0	9
Sept	19	6.8	152.0	41.7	11.04	267.2	0.067	26.3	7
Oct	9	6.7	134.0	38.3	12.22	253.6	0.065	26.4	6
Nov	7	6.5	133.4	34.3	12.73	242.3	0.062	26.8	5
Dec	0	-	-	-	-	-	-	-	-

4.5.9 Response of larvae of *Aedes aegypti* to physicochemical conditions of breeding sites

Table 18 shows the range values of physicochemical conditions of the breeding sites of *Ae. aegypti*. The larvae were collected in their breeding water in a pH range of 7.3 – 7.6, conductivity 41.33μS/cm – 53.4μS/cm, total dissolved oxygen 18.45ppm – 35.2ppm, acidity 9.7mg/l – 12.4mg/l, alkalinlty 267.3mg/l – 291.7mg/l, salinity 0.023PSU – 0.029PSU, surface water temperature 27.0^{o}C - 28.3^{o}C and depth of breeding site 7cm – 24cm. The mosquito has positive relationship to all physicochemical properties of the breeding sites.

Table 18: Response of larvae of *Aedes aegypti* to physicochemical conditions of their breeding sites

Months	Larvae of *Ae.aegypti*	pH	Conduct - Ivity (µS/cm)	Total Dissolved Oxygen (ppm)	Acidity (mg/L)	Alkalinity (mg/L)	Salinity (PSU)	Surface Water temp. (°C)	Depth of breeding site (cm)
Jan	0	7.3	41.33	18.45	12.4	267.3	0.023	28.3	7
Feb	0	7.3	42.4	19.00	11.3	269.8	0.025	28.4	7
Mar	0	7.4	44.10	27.60	10.2	271.3	0.027	28.2	13
Apr	5	7.4	44.10	20.85	10.1	273.4	0.027	27.8	13
May	4	7.4	45.3	24.34	10.0	274.3	0.028	27.4	17
June	5	7.5	46.3	26.45	9.8	281.7	0.028	27.0	27
July	14	7.5	47.3	29.44	9.8	282.6	0.028	27.0	24
Aug	12	7.5	48.3	32.3	9.7	286.4	0.029	27.0	24
Sept	11	7.6	53.4	35.2	8.8	291.7	0.029	27.2	19
Oct	7	7.5	47.3	30.3	10.2	274.3	0.027	27.4	14
Nov	9	7.4	43.2	27.3	11.2	271.6	0.025	27.6	13
Dec	0	7.3	40.9	20.5	11.4	268.7	0.023	27.7	9

Table 19: Correlation coefficients (r) of response of larvae of mosquitoes to physicochemical conditions of their breeding sites

month	pH	Conductivity	Total Dissolved Oxygen	Acidity	Alkalinity	Salinity	Surface Water temp.	Depth of breeding site
All mosquitoes	0.340	0.430	*0.581	-0.359	0.352	0.374	-0.439	0.221
Culex quinquefasciatus	0.478	*0.773	*0.782	*-0.858	*0.937	*0.848	*-0.606	0.320
An. gambiae	*0.699	*0.797	*0.815	0.194	*0.761	*0754	*0.670	0.554
An. funestus	*0.669	*0.658	*0.737	0.242	*0.711	*0.699	*0.652	0.422
Ae. albopictus	*0.730	*0.758	*0.770	*0.621	*0.748	*0.738	*0684	*0.766
Ae. aegypti	*0.803	*0.745	*0.785	*-0.645	*0.791	*0.671	*-0.812	*-0.724

Table 19 above shows the correlation coeficients of each of the physicochemical properties of the mosquitoes breeding sites. Those marked (*) are significant (See appendix XXVI - XXXI). Those that has (*) with a negative sign (-) has negative relationships to the abundance of mosquitoes resulting in decrease in mosquitoes population when the parameter increase. Those with only (*) has positive relationships with abundance of mosquitoes and thus mosquitoes population increased with increase in the parameters.

4.6 Morphological and molecular identification of adult mosquitoes after collection.

4.6.1 Morphological Identification

Anopheline mosquitoes were morphologically separated from culicine mosquitoes using the length of their palps, which were as long as their proboscis and spotted wings with alternate dark and pale bands arranged along the veins of the wings. Culicine mosquitoes were also separated from the Anophelines by their peg-like short palps and non-spotted wings.

An. funestus was identified by its small size, dark colour and almost entirely dark legs and three narrow white bands on the palps. *An. gambiae* sensu latum was identified by its relative medium size with irregulary spotted legs and palps with three pale rings including the wide ring at the tip.

Aedes group were separated from *Culex* group by the presence of dark and white silvery bands on their body and legs. *Culex* group had ash-grey to colourless appearance without any conspicuous body ornamentation. *Eretmapodites* was identified with a large patch of silver-white scales between the eyes, a mixture of yellow and black scales on the thorax and golden yellow patches underneath the abdomen.

4.6.2 Molecular Identification of siblings species of *An. gambiae* and *Culex pipiens* complex by PCR

Of the 150 *An. gambiae* complex mosquitoes that were subjected to PCR, 124(82.67%) were amplified and identified while 26(17.33%) were unamplified and could not be identified. All the amplified 124(82.67%) were identified as *An. gambiae* s.s. (Fig. 37). Of a total of 100 *C. pipiens* complex subjected to PCR, none was amplified even after the process was repeated on another new 100

samples of *C. pipiens* complex. Thus they could not be identified molecularly although they were all previously identified under the objective lens of the microscope as *C. quinquefasciatus* using morphological features.

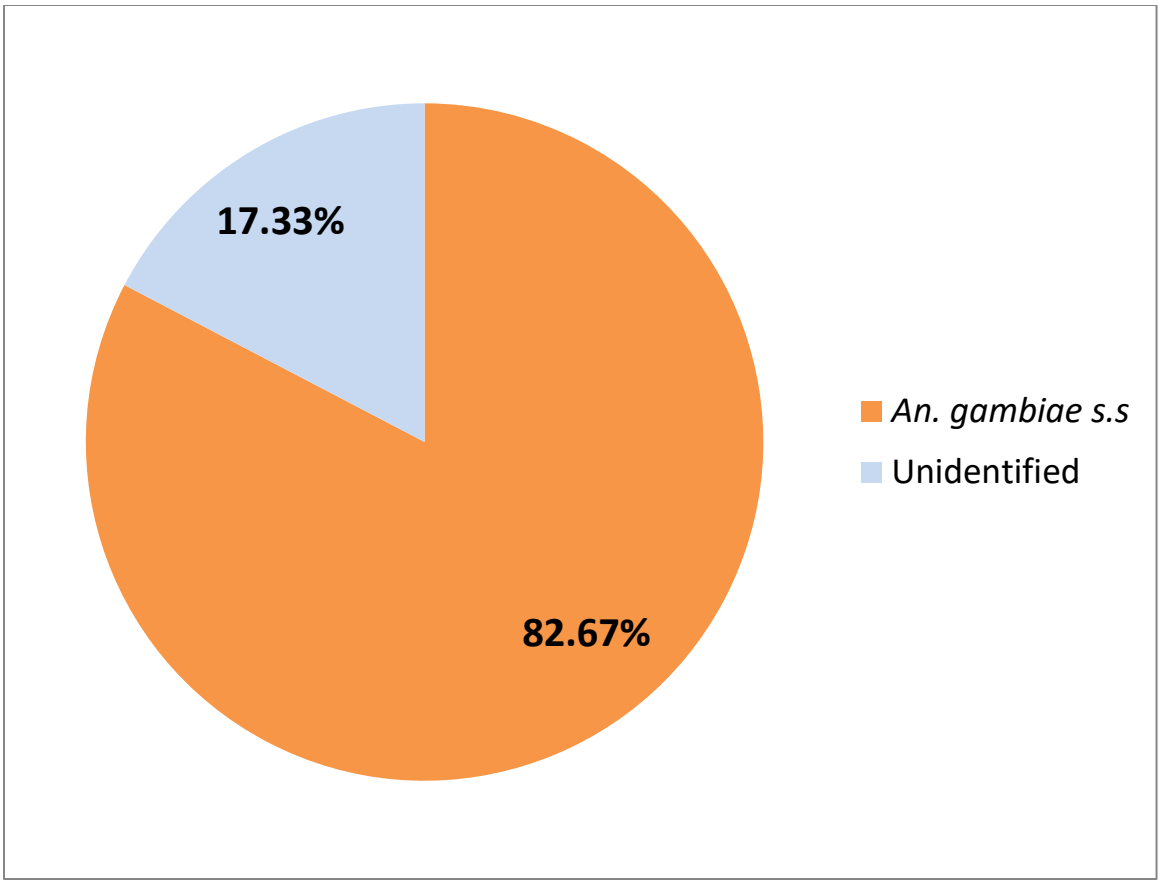

Fig 37. Percentage distribution of the identified and unidentified Anopheles gambaie complex mosquitoes

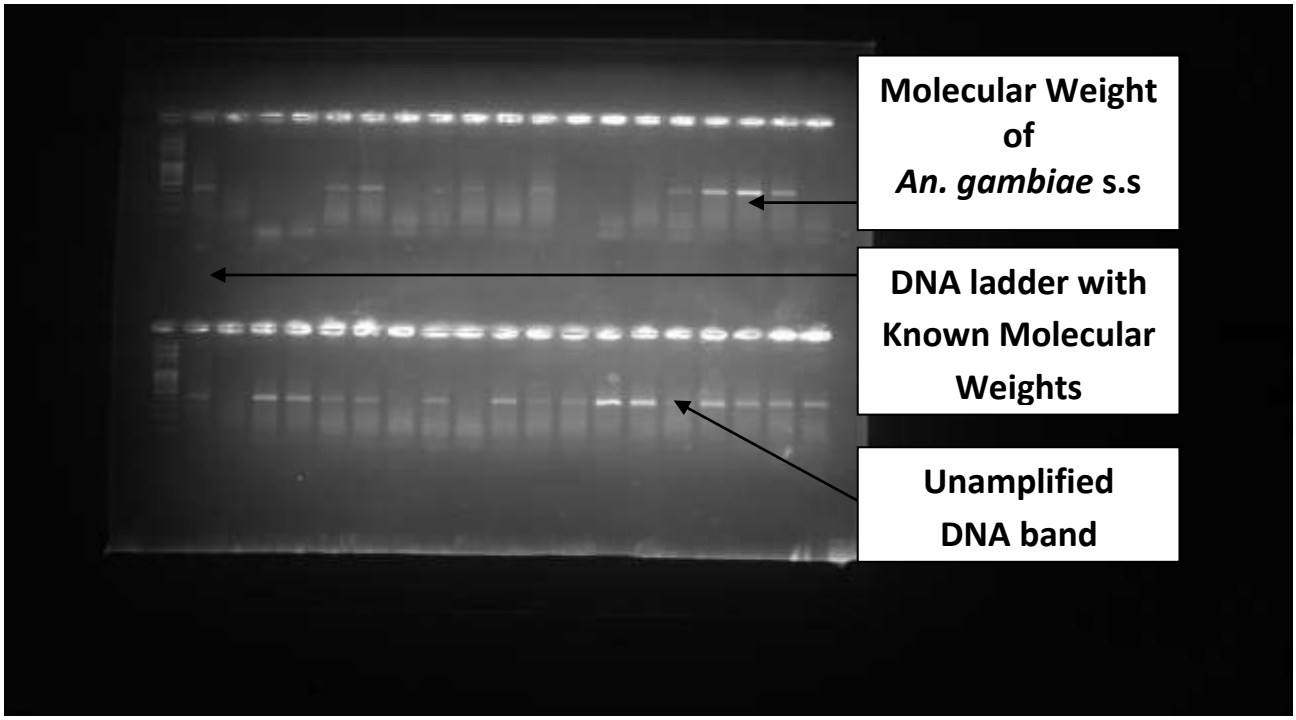

Fig 38. Gel image of *Anopheles gambiae* complex

CHAPTER FIVE

DISCUSSION

A substancial number of mosquitoes 3,478 were collected from the study. The number was greater than 1,150 mosquitoes collected by Onyido *et al*. (2016) in Nibo community. It was also greater than 516 mosquitoes collected by Umeanaeto *et al*. (2017) in the female hostel of Nnamdi Azikiwe University, Awka South Local Government Area. Okonkwo *et al*. (2014) also collected a total of 2,319 mosquitoes from Oba community in Anambra State. The high number of mosquitoes collected in this study may be as a result of the long period in which the study was conducted. While mosquito collections in each of the above mentioned studies lasted less than 6 months, collection in the present study lasted for 18 months. The high population of mosquitoes collected in this study may also be the result of combined methods of mosquitoes collection (Human bait, Pyrethrum knockdown and collection of larvae) employed.

A good number of mosquitoes were collected in larval stage in this study, although those collected in adult stage were greater, but there was no significant difference in the number of mosquito collected at different stages ($P > 0.05$). These collections in both larval and adult stages were indication that mosquitoes are breeding prolifically and biting in the community. The collection of mosquitoes both indoors and outdoor is an indication that the vectors live in close association with man and that most mosquitoes species distribution is mainly dependent on where man is found at every point in time.

Eight mosquito species made up of six *Culicines* and two *Anophelines* species were collected in the study. The species were *Ae. aegypti*, *Ae. albopictus*, *C. quinquefasciatus*, *C. annulioris*, *E. chrysogaster*, *C. tigripes*, *An. gambiae* and *An. funestus*. However, only five of the mosquito species (*An. gambaie*, *An. funestus*, *Ae. aegypti*, *Ae. albopictus* and *C. quinquefasciatus*) were abundant in the collections. Two species (*C. annulioris* and *E. chrysogaster*) were collected in adult stage and one species (*C. tigripes*) was collected in larval stage only. There was a significant difference mosquito species collected ($P < 0.05$). All the mosquitoes collected in this study have also been reported in different places in Nigeria. Okonkwo *et al.* (2014) collected *An. gambiae*, *An. funestus*, *Ae. Aegypti* and *Ae. albopictus* in Oba. Mbanugo and Okpalaononuju (2003) collected *Ae. aegypti* and *C. quinquefasciatus* in Awka metropolis. Umeanaeto *et al.* (2017) reported *An. gambiae, An. funestus, C. quinquefasciatus and C. annulioris* in Nnamdi Azikiwe University female hostels. Onyido *et al*. (2016) collected *An. gambiae, C. quinquefasciatus, Ae. aegypti* and *Ae. albopictus* in Nibo community. Also, Okogun *et al*. (2005), Umaru *et al.* (2006), Oguoma and Ikpeze (2008), Adeleke (2008), Onyido *et al*. (2009a&b) and Abdullahi *et al.* (2010) also made similar collections in different parts of Nigeria.

Of all the eight species of mosquitoes, *C. quinquefasciatus* had the highest indices of diversity and dominance. Okonkwo *et al.* (2014) also observed that *C. quinquefasciatus* had the highest indices of diversity and dominance in Oba, Anambra State. The implication is that *C. quinquefasciatus* is very abundant and breed in most breeding site where they would cause more biting nuisance than any other mosquito species in the study area.

The findings of this study showed that some mosquito species were collected from the three ecotypes of rural, suburban and urban environment while others were not.

Four species of mosquitoes namely: *Ae. aegypti, Ae. albopictus, C. quinquefasciatus and An. gambiae* were found in the urban, sub-urban and the rural communities. This indicates that these mosquito species can be found in all ecotypes. The main factor which may contribute to the availability of these mosquito species in all ecotypes is their peculiar breeding habitats which are all available in urban, suburban and rural communities (Gimnig *et al.*, 2001; Koenraadt *et al*., 2004). *C. annulioris* and *E. chrysogaster* were collected only in the urban communities. The breeding habitats of *C. annulioris* and *E. chrysogaster* were not determined in this study as these mosquitoes were not collected in larval stages. Also, the quantity collected as adult (one each) were too insignicant to conclude that they are urban mosquitoes. *An. funestus* was not found in the urban but were collected in sub-urban and rural communities. *An. funestus* mosquito was observed to breed in ground water pools under the shades of trees often created by the activities of humans or domestic animals. Koenraadt *et al*. (2004) also observed that *An. funestus* breed in sun lit pools but breed in pools under shaded trees. Such trees were not always available in the urban centres but are seen in sub-urban and rural communities. This may suggest why *An. funestus* was not collected in the urban communities.

Mosquito larvae were collected in six types of breeding sites namely – dirty gutters, domestic containers, discarded used tyres, ground pools, septic tanks and treeholes and plant axils. The highest numbers of larvae were from the septic tanks and the least were from tree holes and leaf axils. The proliferation of breeding sites agreed with the opinion of Mbanugo and Okpalaononuju (2003) who noted that the preponderance of mosquitoes in Awka metropolis was due to prevailing habitats in the area. These various sites identified collect and hold waters that form breeding sites for mosquitoes especially during the wet season. Onyido *et al.* (2009) and

Onyido *et al.* (2011) also observed that in Nigeria and most developing countries, the urban landscapes are littered with garbage, plastic and tin cans, bottles, disposable cups and discarded vehicle tyres and discarded earthen wares which form breeding grounds for mosquitoes especially during the wet season.

Six mosquito species were collected in larval stages. *An. gambiae* 73(12.76%) and *An. funestus* 34(5.94%) from ground water pools, *Ae. aegypti* 100(17.48%) from domestic containers and discarded used tyres, *Ae. albopictus* 93(16.26%) from discarded used tyres, *C. quinquefasciatus* 272(47.55%) from dirty blocked gutters, domestic containers and ground pools and *C. tigripes* 41(5.03%) from treeholes and leaf axils. There was a significant difference in mosquito species collected from the breeding sites ($P < 0.05$). The collection of different mosquito species from specific breeding sites showed that most mosquitoes have prefered breeding sites. The culicine mosquitoes (*Ae. aegypti, Ae. albopictus* and *C. quequefasciatus)* formed the bulk of the larvae collected 465(81.29%), with *C. quinquefasciatus* having the highest number 272(58.49%) and *Ae. albopictus* the least 93(20.0%). Most of the larvae 520(90.91%), were collected in the months of April to November, corresponding to the wet season. Only 52(9.09%) larvae were collected from December to March corresponding to the dry season period. Other works had also reported high prevalence of larvae in the rainy season than in the dry season (Okonkwo *et al.*, 2014; Mbanugo and Okpalaononuju, 2003). This is because breedings sites contain water for mosquitoes to breed in rainy season than in the dry seasons.

The highest number of larvae 91(15.90%) was collected in the month of September and the least 8(1.39%) was collected in the February. The volume of rainfall was highest in August but more larvae were collected in September when rain volume dropped and breeding sites became stagnant. It has been reported that periods of

heavy rain does not favour mosquito breeding as larvae are flushed out, but larval population would rise as soon as rain dropped. Also, least larval collectection has been reported to be least in the dry seasons as much breeding sites are not available (Onyido *et al*., 2009). The highest number of larvae was collected from Amawbia community and the least from Mgbakwu. There was asignificant difference in the abundance of mosquito larvae in different communities ($P < 0.05$). This indicates the larval mosquitoes were not evenly distributed across all the communities in the study area. The major factor that would influence the uneven distribution could be the availability of breeding sites in the different communities (Mbanugo and Okpalaononuju, 2003).

Five mosquitoes species which include *An. gambiae*, *An. funestus*, *Ae. aegypti*, *Ae. albopictus* and *C. quinquefasciatus* were collected biting both indoors and outdoors. There was no significant difference in the numbers of mosquitoes biting indoors and outdoors. *C. annulioris* and *E. chrysogaster* were collected outdoor only and in very negligible proportions. *An. gambiae* and *An. funestus* were collected only indoors. Outdoor biting and resting mosquitoes were highest in Awka and least in Nise community. *C. quinquefasciatus* were the most abundant culicine mosquitoes biting outdoors in the community. This agrees with observations made in Awka metropolis (Mbanugo and Okpalaononuju, 2003) and in Midwestern Nigeria (Okogun *et al.*, 2005). However it was in contrast with the findings from Katstina State Nigeria (Bunza *et al*, 2010) and North Central Nigeria (Oguoma and Ikpeze, 2008) where *Anopheles* species were the most abundant mosquito species. The variation in the abundance in the different regions may be because of the environmental factors such as availability of peculiar breeding sites of species which contributes to their abundance. Other culicines collected include the *Aedes* groups which are proven vectors of yellow fever and other arboviruses

and all have been variously involved in yellow fever epidemics in Nigeria (Lee and Moore 1972; Lee *et al*., 1974; Service 1974; Savage *et al.*, 1992). *An. gambiae* is an important vector of malaria and filariasis in Africa especially in rural communities.

Both outdoor and indoor biting adult mosquitoes were highest at Okpuno and Awka metropolis and least at Nise. There was no significant difference in the distribution of indoor biting and resting mosquitoes in the communities ($P > 0.05$). Indoor biting mosquitoes were not evenly distributed across all the communities in the study area. The mosquito species collected indoor were all known to be human biters, feeding on man and living in close affinity with man (Gordon and Lavoipierre, 1976). Therefore more mosquitoes were collected in highly populated communities like Awka and Okpuno where more human preys are found while least were collected from low population area such as Nise community. *C. quinquefasciatus* were the most prevalent indoor-biting mosquitoes. *C. quinquefasciatus* has been reported to be the most abundant in other studies in the different parts of the same study area (Mbanugo and Okpalaononuju 2003; Onyido *et al*., 2016; Umeanaeto *et al.*, 2017). This could be most likely explained by the presence of large blocked drainages with very dirty stagnant water, and septic tanks among others which serve as their breeding grounds.

From the all night hourly collections, most of the culicines (*Ae. aegypti* and *Ae. albopictus*) had their peaks between 7.00-8.00pm and virtually stopped biting by 9.00pm. This observation shows that these species are twilight biters. *C. quiquefasciatus* continued biting until dawn (6.00am) with a major peak between 9.00pm and 1.00am. This also shows that *C. quinquefasciatus* are nucturnal insects. The observations here are in agreement with Onyido *et al*. (2016) who made similar observation on Culicine species in Nibo community. *An. gambiae*

and *An. funestus* peaked between 1am - 2.00am with a gradual decline in population until dawn. This that they are norctunal insects The *Anopheles* species had their biting peak between 1am – 2am when human are fast asleep. The findings on *Anopheles* species which are important malaria vectors is in agreement with Aribodor *et al*., (2012) who reported that *Anopheles* species are midnight biters. Indoor resting density and man-biting rates showed that *C. quinquefasciatus* and *An. gambiae* has the highest indices. Aribodor (2012) stated that important vectors of mosquito-borne diseases are those which show close association with man and prefer man to other animals as source of food. *C. quinquefasciatus* and *An. gambiae* showed such a close association with man.

Of the 1,270 mosquitoes, 1, 145 (90.16%) were bloodfed while 125(9.84%) were not fed. This was an indication that greater number of the mosquitoes have had contact with human host during bloodfeeding and as such, there could be a greater tendency of the infected mosquitoes to have transmitted parasites like *Plasmodium* and filarial worms that cause malaria and filariasis respectively which are the two important human tropical infections that are endemic in Africa (Ejezie and Akpan, 1992). The proportion of *An. gambiae* and *C. quinquefasciatus* that were bloodfed were higher than the others. This was because both species are anthropophilic. Ebenezer *et al*. (2013) and Adeleke *et al*. (2010) made similar observations in Bayelsa and Abeokuta respectively. The high percentage of bloodfed mosquitoes clearly shows that the two genera; *Anopheles* and *Culex*, are both endophagic and endophilic as they were caught while resting on the walls.

Only 10% of *An. gambiae* subjected to Enzyme-linked immunosorbent assays **(**ELISA) for analysis of sporozoite rate were positive for malaria sporozoites. The prevalence here is higher than 4.7% reported by reported by Dery *et al*. (2010) in Ghana, but much lower than 35.5% of *P. falciparum* sporozoites rate in *An.*

gambiae reported by Okwa *et al*. (2007) in Lagos, Nigeria. The observations indicate that the *Anopheles* mosquito may have low natural infection rates to malaria. Also, the low prevalence of sporozoites in this study suggests that infection rate is low in the area.

There was a positive relationship between mosquito populations and rainfall. Mosquito population increased constantly as soon as the rainy season arrives. This was because rainfall provided breeding places for the mosquitoes (Mutero *et al*. 2004). *Culex quenquefasciatus* larvae had a negative relationship with rainfall. This means that the larvae do not depend solely on rainfall to breed. The mosquito was actively breeding during the dry season. In the dry season, their normal breeding sites are dry such that sewage systems and septic tanks become their breeding sites. Although Betterton and Gadzama (1981) reported that in dry periods mosquitoes would have insufficient water for breeding, *C. quiquefasciatus* is not affected as it has these altervative breeding places.

Adult mosquitoes declined in August before it peaked again in September. Rainfall was highest in August but reduced in September and the breeding water became more stagnant. These findings agrees with Uttah *et al*. (2013) who reported that repeated rains cause severe flooding resulting in temporary flushing out of mosquito breeding places. Consequently the breeding of a vector population is greatly reduced, but becomes, re-established when favourable conditions are restored. The least number of adult mosquitoes were collected in February when the monthly rainfall was low. In February, there were almost no visible mosquito breeding sites due to low rainfall thus; mosquito populations were least abundant. It was also observed that *Anopheles* mosquitoes were not breeding in the study area during the dry season because there was no water in the potholes was they breed. Betterton and Gadzama (1981) reported that in time of drought there would be

insufficient water to establish *Anopheles* breeding sites, but even a small amount of rain would be sufficient to create sites suitable for their breeding.

Adult mosquito populations had negative relationship to the mean environmental temperature. Mosquito populations decreased as temperature increased. This observation showed that, although adult mosquitoes survive more in the tropics than in temperate regions of the world, their population may decline sharply if the temperatures are high above 30˚C. This agrees with Sara (1990) who observed that a constant high temperature led to drastic decline in the mosquito populations.

Adult mosquito populations had positive relationship to the mean environmental relative humidity. Mosquito populations increased with increase in relative humididty. This demonstrated that a certain quantity of water is required in the atmosphere for adult mosquito populations to thrive. The observations here agree with some previous findings which reported that for adult mosquitoes to survive, they need at least 60% relative humidity (Bruce-Chwatt, 1991; Dutta and Dutta, 1978; Molineux, 1988). Higher levels of relative humidity lengthen the life span of the mosquitoes and enable them to infect more people (Dutta and Dutta, 1978). Also, Sara (1990) observed that a constant low relative humidity led to drastic decline in the mosquitoes' populations.

Mosquito larval population had positive correlation with pH, conductivity, total dissolved oxygen, alkalinity, salinity and water depth but had negative correlation with acidity and surface water temperature. In the study, mosquitoes were collected between the mean pH range of 6.2 – 6.7. This indicates that mosquitoes can breed in water with pH near neutral point of 7.0. The result of this study agrees with previous reports of CDC, 2004 and Okogun *et al*. (2003) who observed that water of a near neutral pH is preferable for breeding of many species of

mosquitoes. Also, all mosquito larvae were collected in water with mean conductivity range of 69.2µS/cm – 150.0µS/cm in all breeding sites. There was a positive relationship in response of all mosquito larvae population to the mean conductivity of all breeding sites. This may suggest that mosquitoes do not breed in waters with high electrical conductivity as was observed by Sumba *et al*., 2004.

Mosquitoes were also collected in waters with low oxygen contents ranging from 44.5ppm – 85.0ppm in all breeding sites. There was a moderate positive linear relationship in response of all larval mosquito populations to the mean total dissolved oxygen content of all breeding sites. This may suggest that mosquitoes breed in waters with moderate oxygen content but not in very low or very high oxygen content. The observation agreed with Mutero *et al*. (2004). The mean salinity of the breeding sites of all mosquitoes ranged from 0.038PSU - 0.065PSU. There was a weak positive linear relationship in response of all mosquito larvae population to the mean total salinity of all breeding sites. This suggests that increase in salt content of breeding water does not bring increase in mosquito populations. Also, mosquitoes may not survive in breeding waters with higher salt content.

Surface water temperature in all breeding sites ranged from 27.8˚C – 28.8˚C. There was a weak negative linear relationship in response of all larval mosquito population to the mean surface water temperature of the breeding sites. Increase in surface water temperature was observed to decrease the abundance of mosquito larvae populations. Mutero *et al*. (2004) observed that pH, optimum temperature, total suspended solids, total dissolved solids and electrical conductivity have been found to affect larval development and survival. The observations corroborates with the findings of the present study.

Virtually all the mosquitoes were found breeding in shallow waters between 6cm – 13cm, there was a weak positive linear relationship in response of all larval mosquito larvae population to the mean depth of the breeding sites. This is a pointer to the fact that very large bodies of stagnant water may not harbour mosquito larvae but only the shallow ones does.

Morphological identifications were applied in the identification of the mosquito species collected in this study. All mosquitoes collected were identified and distinguished from another using their unique features. Molecular identification was able to identify only 124(82.67%) of *An. gambiae* complex but was not able to identify *Culex* species. The 124 *An. gambiae* were identified as *An. gambaie* s.s. This may suggest that *An. gambiae* s.s is either the only sibling species of the complex available in the study area or it is the most prevalent species in the study area. Morphological identification techniques require simple skills but it has limitations in identifying species complexes. Molecular identification on the other hand is costly also requiring specialized techniques.

5.1 Conclusion

The findings of this study have shown that mosquitoes are breeding prolifically and biting in Awka South Local Government Area. Also, the results show that mosquito species already established as vectors of parasitic and viral diseases such as malaria and filariasis amongst others bite in the study area. Enzyme linked immunosorbent assay (ELISA) revealed that some female Anopheles mosquitoes were positive with sporozoites of malaria which they can transmit from person to person within the study population. It may suggest that other diseases which these mosquito species serve as their vectors may also exist in the population. Also,

Polymarase Chain Reaction (PCR) showed that *An. gambiae s.s* may be the only sibling species of the *Anopheles gambiae* complex in the study area.

5.2 Recommendations

The availability of mosquito breeding sites is an important ecological factor in mosquito abundance and diseases transmission in the community. The community should be educated on mosquito ecology and diseases. They should be enlightened on those factors that contribute to mosquito abundance in their environment and maintenance of a high level of environmental hygiene, including proper disposal of materials that can hold water for the breeding of mosquitoes. Integrated vector control which must include physical, chemical and biological methods, should be employed to effectively control the mosquito vectors. Further studies on the prevalence of the diseases such as malaria and filariasis amongst others which these mosquitoes can transmit should be undertaken regularly in the study community in order to determine their status and how to curb them.

5.3 Contribution to Knowledge

This work has established that the total dissolved oxygen content of breeding sites in the study area had positive relationship to mosquito abundance. This suggest that formulation of larvicides that can adversely affect the total dissolved oxygen content of breeding sites can negatively affect the developments of larval mosquitoes leading to their control. Also, through PCR, this work has implicated *An. gambiae* s.s likely to be the only member of the *An. gambiae* complex in the study area. Also, the specific breeding sites of *An. gambiae* s.s were identified in the area. This finding could help in the control the malaria vector without waste of resources by simply altering their specific breeding sites by both physical and chemical means.

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Appendix I

NNAMDI AZIKIWE UNIVERSITY

Faculty of Biosciences

Department of Parasitology and Entomology

Tel: _____

Our Ref: _____

Your Ref: _____

Head of Department

P.M.B. 5025
Awka
Anambra State Nigeria

Date: _____

18th July , 2016

The Chairman
Awka South Local Government Area
Anambra State.

Sir,

Letter of Introduction

The bearer, Irikannu Kindness Chidi, Reg. No NAU/PhD/2015587003F is a PhD student of the above named Department, in Nnamdi Azikiwe University, Awka. He is carrying out a project on "Ecology and Molecular characterization of Malaria Vectors in Awka South Local Government Area of Anambra State, Nigeria.

Kindly give him all necessary assistance to enable him successfully carry out the project.

SIGN
HEAD OF DEPT.

Prof. A.E. Onyido
HOD, Parasitology and Entomology

Appendix II

NNAMDI AZIKIWE UNIVERSITY

Faculty of Biosciences

Department of Parasitology and Entomology

Tel: _____

Our Ref: _____

Your Ref: _____

Head of Department

P.M.B. 5025
Awka
Anambra State Nigeria

Date: _____

19th March, 2018

The Director General,

Nigerian Institute of Medical Research,

6, Edmond Crescent (off Murtala Muhammed Way)

P.M.B, 2013 Yaba,

Lagos –Nigeria.

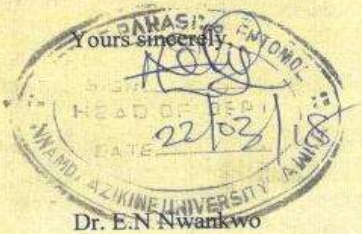
Sir,

A LETTER OF COLLABORATION

I write to introduce Mr. Irikannu Kindness Chidi to you. He is a Ph.D student of Pest Management in the Department of Parasitology and Entomolgy, Nnamdi Azikiwe University, Awka, Anambra State. He is carrying out his research Project on Ecology and Molecular Characterisation of Mosquito Vectors in Awka South Local Government Area, Anambra State, Nigeria. His Research work involves Molecular Entomology.

Therefore, I am seeking collaboration and assistance of the institute to allow him work in your Molecular Entomology Unit, Public Health Division. Thank you for your co-operation.

Yours sincerely,



Dr. E.N Nwankwo

HOD, Parasitology and Entomology

Appendix III

NIGERIAN INSTITUTE OF MEDICAL RESEARCH
FEDERAL MINISTRY OF HEALTH

Prof. Babatunde L. Salako
MBBS (Ib), FWACP, FRCP (Edin), FRCP (Lond), MNIM
Director-General/CEO

6, Edmund Crescent,
(off Murtala Mohammed Way)
P.M.B. 2013, Yaba, Lagos,
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Tel: +234-9092133886
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Website: www.nimr.gov.ng

Our Ref: MR/GEN.AD/1032/V/1341

Date: 17th April, 2018

Dr. E. N. Nwankwo
HOD, Parasitology and Entomology
Faculty of Biosciences
Department of Parasitology and Entomology,
Nnamdi Azikiwe University,
P.M.B. 5025, Awka,
Anambra State Nigeria.

RE: APPLICATION FOR BENCH SPACE IN THE DEPARTMENT OF PUBLIC HEALTH AND EPIDEMIOLOGY TO CARRY OUT Ph.D. RESEARCH PROJECT

I am directed to refer to your letter dated 19th March, 2018 on the above subject matter and to convey the Director-General's approval of your request to enable Mr. Irikannu Kindness Chidi carry out Ph.D. Research work in the Public Health and Epidemiology Department of the Institute.

However, your candidate is expected to pay the Bench space fee of **₦250,000.00 (Two Hundred and Fifty thousand naira only)** to the Finance and Accounts Department of the Institute before the commencement of the project.

Thank you.

B. N. Osuji
CEO (GAT&D)
for: Director-General

Appendix IV

NIGERIAN INSTITUTE OF MEDICAL RESEARCH
FEDERAL MINISTRY OF HEALTH

Prof. Babatunde L. Salako
MBBS (Ib), FWACP, FRCP (Edin), FRCP (Lond), MNIM
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6, Edmund Crescent,
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Tel: +234-9092133886
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Our Ref: MR/GEN.AD/1032/V/1403

Date: 5th June, 2018

Mr. Kindness Chidi Irikannu
Dept of Parasitology and Entomology,
Nnamdi Azikiwe University,
P.M.B. 5025, Awka,
Anambra State.

RE: PASIONATE APPEAL: REQUEST FOR REBATE IN BENCH SPACE FEE

I am directed to refer to your letter dated 24th May, 2018 on the above subject matter and to convey the Director-General's approval of 20% rebate on Bench Space Fee.

In the light of the above, you are to pay a Bench Fee of ₦200,000.00 to the Finance and Accounts Department of the Institute before the commencement of your Research Work.

Thank you.

B. N. Osuji
CEO (GAT&D)
for: Director-General

Appendix V

A cage used in rearing mosquito larvae to adult

Appendix VI

Locally made emergence trap used in collecting emerging adult mosquitoes mosquitoes from Septic tanks

Appendix VII

Global Positioning System (GPS) Data collected from the study Area

S/N	Location	Latitude	Longitude
1	Amawbia	6.20753	7.042822
2	Awka	6.222226	7.063910
3	Nise	6.149164	7.061196
4	Nibo	6.185513	7.078670
5	Okpuno	6.247550	7.065517
6	Mbaukwu	6.133355	7.078149
7	Ezinator	6.22111	7.12111
8	Isiagu	6.17673	7.11709
9	Umuawulu	6.1506	7.1009

Appendix VIII

Monthly average of metrological data in Awka South Local Government Area, Anambra State

Months	Temperature (^{0}C)	Relative humidity (%)	Rainfall (mm)
January	28.0	84	21
February	29.0	83	33
March	28.5	83	132
April	28.5	85	207
May	28.0	85	219
June	26.5	87	300
July	26.0	90	439
August	26.5	92	504
September	26.0	90	492
October	26.5	89	305
November	27.5	87	138
December	28.0	86	32

Appendix IX

Analysis of Data

(a) Comparison of mosquito populations collected in different forms using different methods

```
NPAR TESTS
  /FRIEDMAN=Larva Outdoor Indoor
  /MISSING LISTWISE.
```

Friedman Test

Ranks

	Mean Rank
Mosquito species collected as larvae	1.75
Mosquito species collected outdoors	2.56
Mosquito species collected indoors	1.69

Test Statistics[a]

N	8
Chi-Square	4.207
df	2
Asymp. Sig.	.122

a. Friedman Test

Appendix X

(b) Comparison of differences in mosquito species collected in different forms using different methods

```
ONEWAY Abundance BY Mosquitospp
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
```

ANOVA

Abundance of mosquitoes using 3 collection methods

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1581949.833	7	225992.833	19.598	.000
Within Groups	184502.000	16	11531.375		
Total	1766451.833	23			

Homogeneous Subsets

Abundance of mosquitoes using 3 collection methods

Duncan

Different species of mosquitoes collected	N	Subset for alpha = 0.05	
		1	2
Culex annuloris	3	.3333	
Eretmapodite chrysogaster	3	.3333	
Culex tigripis	3	13.6667	
Anopheles funestus	3	47.6667	
Aedes albopictus	3	75.6667	
Anopheles gambiae	3	94.6667	
Aedes aegypti	3	111.0000	
Culex quinquefasciatus	3		816.0000
Sig.		.279	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix XI

(c) Comparison of the distribution of each mosquito larvae in the breeding sites

```
NPAR TESTS
  /FRIEDMAN= dirty gutters domestic containers discarded tyres ground pools septic tanks tree holes and plant axils
  /MISSING LISTWISE.
```

Friedman Test

Ranks

	Mean Rank
Abundance of mosquito larvae in dirty gutters	4.33
Abundance of mosquito larvae in domestic containers	3.42
Abundance of mosquito larvae in discarded tyres	3.67
Abundance of mosquito larvae in ground pools	3.58
Abundance of mosquito larvae in septic tanks	2.58
Abundance of mosquito larvae in tree holes and plant axils	3.42

Test Statistics[a]

N	6
Chi-Square	2.780
df	5
Asymp. Sig.	.734

a. Friedman Test

Appendix XII

(d) Comparison of the distribution of mosquito larvae in the study communities

```
ONEWAY LAbundance BY Mosquitospp
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
```

ANOVA

Abundance of mosquitoes larvae in the study communities

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	23464.472	5	4692.894	26.277	.000
Within Groups	5357.833	30	178.594		
Total	28822.306	35			

Post Hoc Tests

Abundance of mosquitoes larvae in the study communities

Duncan

Different species of mosquitoes collected	N	Subset for alpha = 0.05	
		1	2
Anopheles funestus	6	5.6667	
Culex quinquefasciatus	6	6.8333	
Anopheles gambiae	6	12.1667	
Aedes albopictus	6	15.5000	
Aedes aegypti	6	16.6667	
Culex tigripis	6		79.0000
Sig.		.213	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Appendix XIII

(e) Comparison of the abundance of each outdoor biting adult mosquito species collected in the study communities

```
NPAR TESTS
  /FRIEDMAN=Amawbia Awka Nise Nibo Okpuno Mbaukwu
  /MISSING LISTWISE.
```

Friedman Test

Ranks

	Mean Rank
Abundance of mosquito species collected outdoors from Amawbia	3.14
Abundance of mosquito species collected outdoors from Awka	3.14
Abundance of mosquito species collected outdoors from Nise	2.71
Abundance of mosquito species collected outdoors from Nibo	4.14
Abundance of mosquito species collected outdoors from Okpuno	4.43
Abundance of mosquito species collected outdoors from Mbaukwu	3.43

Test Statistics[a]

N	7
Chi-Square	5.249
df	5
Asymp. Sig.	.386

a. Friedman Test

Appendix XIV

(f) Comparison of the abundance the different outdoor biting adult mosquito species collected in the study communities

```
ONEWAY Aabundance BY Mosquitospp
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
```

ANOVA

Abundance of Outdoor biting adult mosquito

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	134616.333	6	22436.056	29.918	.000
Within Groups	26247.500	35	749.929		
Total	160863.833	41			

Post Hoc Tests

Abundance of Outdoor biting adult mosquito

Duncan

Different species of mosquitoes collected	N	Subset for alpha = 0.05	
		1	2
Culex annuloris	6	.1667	
Eretmapodite chrysogaster	6	.1667	
Anopheles funestus	6	10.3333	
Aedes albopictus	6	12.8333	
Anopheles gambiae	6	13.3333	
Aedes aegypti	6	24.8333	
Culex quinquefasciatus	6		170.5000
Sig.		.180	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Appendix XV

(g) Comparison of the abundance of each indoor biting adult mosquito species collected in the study communities

```
NPAR TESTS
  /FRIEDMAN=Amawbia Awka Nise Nibo Okpuno Mbaukwu
  /MISSING LISTWISE.
```

Friedman Test

Ranks

	Mean Rank
Abundance of mosquito species collected indoors from Amawbia	3.10
Abundance of mosquito species collected indoors from Awka	2.80
Abundance of mosquito species collected indoors from Nise	3.30
Abundance of mosquito species collected indoors from Nibo	3.80
Abundance of mosquito species collected indoors from Okpuno	4.40
Abundance of mosquito species collected indoors from Mbaukwu	3.60

Test Statistics[a]

N	5
Chi-Square	2.367
df	5
Asymp. Sig.	.796

a. Friedman Test

Appendix XVI

(h) Comparison of the abundance the different indoor biting adult mosquito species collected in the study communities

```
ONEWAY Aabundance BY Mosquito spp
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA (0.05).
```

ANOVA

Abundance of Indoor biting adult mosquito

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	101916.000	4	25479.000	53.069	.000
Within Groups	12002.667	25	480.107		
Total	113918.667	29			

Post Hoc Tests

Abundance of Indoor biting adult mosquito

Duncan

Different species of mosquitoes collected	N	Subset for alpha = 0.05	
		1	2
Anopheles funestus	6	7.8333	
Aedes albopictus	6	9.5000	
Aedes aegypti	6	14.0000	
Anopheles gambiae	6	21.8333	
Culex quinquefasciatus	6		158.5000
Sig.		.322	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Appendix XVII

(i) Indoor Resting Density and Man-biting rate of different mosquitoes species in Awka South Local Government Area, Anambra State

Months	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Total**	**%**	**IRD**	**MBR**
Number collected	2	0	15	128	119	200	242	163	256	109	30	7	1270	100		
Fed	2	0	11	36	49	73	81	86	103	106	19	4	570	44.9		
An. gambiae (fed)	0	0	0	1	1	1	1	2	1	2	1	1	11	1.9	0.30	0.017
An. funestus (fed)	0	0	1	0	0	1	0	2	1	1	0	0	6	1.0	0.10	0.010
Ae. aegypti (fed)	0	0	1	1	0	1	1	1	2	2	1	0	10	1.8	0.19	0.020
Ae. Albopictus (fed)	0	0	2	1	1	1	1	2	0	1	0	0	9	1.6	0.13	0.014
C. quinque fasciatus (fed)	2	0	7	33	47	69	78	79	99	100	17	3	534	93.7	2.20	1.0

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

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

No. of houses = 18

No. of nights = 24

No. of room occupants = 26

NB: Total number of females of each species of mosquitos used in the calculation is shown in table of abdominal gradings of mosquitoes collected indoors in the main text.

Appendix XVIII

(j) Index of species diversity and dominance indices for all mosquitoes collected from Awka South Local Government Area.

Mosquito species	**ni**	**Pi= ni/N**	**P^2= $(ni/N)^2$**	**Pi Log Pi**	**Shannon-Wiener diversity index**	**Simpson's dominance index $C=\sum(ni/N)^2$**
An.gambiae	284	0.082	0.007	-0.089	0.089	0.007
An.funestus	143	0.041	0.002	-0.057	0.057	0.002
Ae.aegypti	333	0.096	0.009	-0.098	0.098	0.009
Ae.albopictus	227	0.065	0.004	-0.077	0.077	0.004
C.quinque fasciatus	2448	0.704	0.495	-0.107	0.107	0.495
C.tigripes	41	0.011	0.0001	-0.0004	0.0004	0.0001
C.annuloris	1	0.0002	0.00000004	-0.0000003	0.0000003	0.00000004
E.chrysogaster	1	0.0002	0.00000004	-0.0000003	0.0000003	0.00000004
Total	N=3478	∑ **1.000**	∑ **0.517**	∑ **-0.428**	H=0.428	**C=0.517**

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

Appendix XIX

(k) Index of species diversity and dominance indices for mosquitoes collected from Amawbia community, Awka South Local Government Area.

Mosquito species	**ni**	**Pi= ni/N**	**P^2= $(ni/N)^2$**	**Pi Log Pi**	**Shannon-Wiener diversity index**	**Simpson's dominance index $C=\sum(ni/N)^2$**
An.gambiae	40	0.072	0.005	-0.082	0.082	0.005
An.funestus	0	0.000	0.000	-0.000	0.000	0.000
Ae.aegypti	61	0.111	0.012	-0.106	0.106	0.012
Ae.albopictus	38	0.069	0.005	-0.080	0.080	0.005
C.quinque fasciatus	412	0.746	0.557	-0.095	0.095	0.557
C.tigripes	0	0.000	0.000	-0.000	0.000	0.000
C.annuloris	0	0.000	0.000	-0.000	0.000	0.000
E.chrysogaster	1	0.002	0.000004	-0.005	0.005	0.000004
Total	N=552	∑ **1.000**	∑ **0.579**	∑- **0.368**	H=0.368	**C=0.579**

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

Appendix XX

(l) Index of species diversity and dominance indices for mosquitoes collected from Awka community, Awka South Local Government Area

Mosquito species	ni	Pi= ni/N	P^2= $(ni/N)^2$	Pi Log Pi	Shannon-Wiener diversity index	Simpson's dominance index $C=\sum(ni/N)^2$
An.gambiae	30	0.040	0.002	-0.056	0.056	0.002
An.funestus	0	0.000	0.000	-0.000	0.000	0.000
Ae.aegypti	49	0.066	0.004	-0.078	0.078	0.004
Ae.albopictus	33	0.045	0.002	-0.061	0.061	0.002
C.quinque fasciatus	628	0.848	0.719	-0.061	0.061	0.719
C.tigripes	0	0.000	0.000	-0.000	0.000	0.000
C.annuloris	1	0.002	0.000003	-0.005	0.005	0.000003
E.chrysogaster	0	0.000	0.000	-0.000	0.000	0.000
Total	N=741	∑ 1.000	∑ 0.727	∑	H=0.261	C=0.727

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

Appendix XXI

(m) Index of species diversity and dominance indices for mosquitoes collected from Nise community, Awka South Local Government Area.

Mosquito species	**ni**	**Pi= ni/N**	**P^2= $(ni/N)^2$**	**Pi Log Pi**	**Shannon-Wiener diversity index**	**Simpson's dominance index C=$\sum(ni/N)^2$**
An.gambiae	37	0.083	0.007	-0.090	0.090	0.007
An.funestus	8	0.018	0.0003	-0.031	0.031	0.0003
Ae.aegypti	57	0.128	0.016	-0.114	0.114	0.016
Ae.albopictus	50	0.111	0.013	-0.105	0.105	0.013
C.quinque fasciatus	283	0.633	0.401	-0.126	0.126	0.401
C.tigripes	12	0.027	0.0007	-0.042	0.042	0.0007
C.annuloris	0	0.000	0.000	-0.000	0.000	0.000
E.chrysogaster	0	0.000	0.000	-0.000	0.000	0.000
Total	N=447	**$\sum$1.000**	**$\sum$0.434**	**$\sum$-0.508**	H=0.508	**C=0.434**

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 - $\sum$ni logni)/N

C = Simpson's index of dominance

Appendix XXII

(n) Index of species diversity and dominance indices for mosquitoes collected from Nibo communnity, Awka South Local Government Area

Mosquito species	**ni**	**Pi= ni/N**	**P^2= $(ni/N)^2$**	**Pi Log Pi**	**Shannon-Wiener diversity index**	**Simpson's dominance index $C=\sum(ni/N)^2$**
An.gambiae	56	0.107	0.011	-0.104	0.104	0.011
An.funestus	41	0.078	0.006	-0.086	0.086	0.006
Ae.aegypti	62	0.117	0.014	-0.109	0.109	0.014
Ae.albopictus	38	0.073	0.005	-0.083	0.083	0.005
C.quinque fasciatus	317	0.606	0.367	-0.073	0.073	0.367
C.tigripes	9	0.017	0.0003	-0.030	0.030	0.0003
C.annuloris	0	0.000	0.000	-0.000	0.000	0.000
E.chrysogaster	0	0.000	0.000	-0.000	0.000	0.000
Total	**N=523**	**∑1.000**	**∑0.403**	**∑-0.485**	**H=0.485**	**C=0.403**

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

Appendix XXIII

(o) Index of species diversity and dominance indices for mosquitoes collected from Okpuno community, Awka South Local Government Area.

Mosquito species	**ni**	**Pi= ni/N**	**P^2= $(ni/N)^2$**	**Pi Log Pi**	**Shannon-Wiener diversity index**	**Simpson's dominance index $C=\sum(ni/N)^2$**
An.gambiae	53	0.750	0.056	-0.094	0.094	0.056
An.funestus	45	0.064	0.004	-0.076	0.076	0.004
Ae.aegypti	58	0.082	0.006	-0.089	0.089	0.006
Ae.albopictus	42	0.059	0.004	-0.073	0.073	0.004
C.quinque fasciatus	501	0.709	0.502	-0.106	0.106	0.502
C.tigripes	8	0.011	0.0001	-0.022	0.022	0.0001
C.annuloris	0	0.000	0.000	-0.000	0.000	0.000
E.chrysogaster	0	0.000	0.000	-0.000	0.000	0.000
Total	**N=707**	**∑1.000**	**∑0.572**	**∑-0.460**	**H=0.460**	**C=0.572**

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

Appendix XXIV

(p) Index of species diversity and dominance indices for mosquitoes collected from Mbaukwu community, Awka South Local Government Area.

Mosquito species	**ni**	**Pi= ni/N**	**P^2= $(ni/N)^2$**	**Pi Log Pi**	**Shannon-Wiener diversity index**	**Simpson's dominance index $C=\sum(ni/N)^2$**
An.gambiae	68	0.134	0.018	-0.117	0.117	0.018
An.funestus	49	0.096	0.009	-0.097	0.097	0.009
Ae.aegypti	46	0.091	0.008	-0.095	0.095	0.008
Ae.albopictus	26	0.051	0.003	-0.066	0.066	0.003
C.quinque fasciatus	307	0.604	0.365	-0.132	0.132	0.365
C.tigripes	12	0.024	0.0006	-0.039	0.039	0.0006
C.annuloris	0	0.000	0.000	-0.000	0.000	0.000
E.chrysogaster	0	0.000	0.000	-0.000	0.000	0.000
Total	N=508	**∑1.000**	**∑0.404**	**∑-0.546**	**H=0.546**	**C=0.404**

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