ABUNDANCE OF INDOOR RESTING MOSQUITO POPULATIONS AND *PLASMODIUM FALCIPARUM* INFECTION OF *ANOPHELES* SPP IN SETTLEMENTS AROUND THE ZARIA DAM, ZARIA, KADUNA STATE

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DEDICATION

This research is dedicated to Almighty God for making everything possible in my life and fulfilling His covenant on me.

Declaration

I,declare that the work in this thesis titled "ABUNDANCE OF INDOOR RESTING MOSQUITO POPULATIONS AND *PLASMODIUM FALCIPARUM* INFECTION OF *ANOPHELES* SPP IN SETTLEMENTS AROUND THE ZARIA DAM, ZARIA, KADUNA STATE" has been carried out by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Comfort, MICHEAL _____ Signature _____ Date

Certification

This thesis entitled"ABUNDANCE OF INDOOR RESTING MOSQUITO POPULATIONS AND *PLASMODIUM FALCIPARUM* INFECTION OF *ANOPHELES* SPP IN SETTLEMENTS AROUND THE ZARIA DAM,ZARIA,KADUNA STATE" by Comfort,MICHEAL meets the regulation governing the award of the degree of masters of science (Zoology) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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Abstracts

The species composition of mosquitoes resting indoors in settlements surrounding the Zaria dam, Zaria, namely: Angwan Malami, Angwan Shika and Zaria water works quarters were investigated. Mosquitoes were caught twice a month for ten months January to October (2013),by application of pyrethrium spray in rooms where tenants had slept the previous night. The total mosquito caught were first identified microscopically,the *Anopheles gambiae*complex were morphologically identified. Using the Polymerase Chain Reaction (PCR) technique, the *Anopheles gambiae*complex were identified molecularly and further assyed by Enzyme Linked Immuno-sorbent Assay (ELISA) to determine the sporozoite infection rate. Microscopically, *Culex quinquefasciatus, Culex annulioris, Anopheles gambiae, Anopheles funestus, Anopheles rhodesiensis, Anopheles arabiensis* and *Aedes aegypti* were encountered. *Culex quinquefasciatus*wasthe most abundant with 254 (50.2%), followed by *Anopheles* species 134 (26.5%.) and only a single species of *Aedes aegypti* 1(0.2%) was collected in the study. *Anopheles gambiaes*.l samples identified by PCR (Polymerase Chain Reaction) were 104 out of which 66(63.5%) showed the diagnostic fragment (390bp) characteristic of *Anopheles gambiaes.s* while 27 (26%) showed the diagnostic fragment (315bp) of *Anopheles arabiensis*. The other 11(10.5%) of the sample were not amplified. The infectivity rates were 4.3% (2/46), 4.5%(1/22) and 5.1% (1/19) in Angwan Shika, AngwanMalami and Water Works Quarters respectively. The mean human biting rates were 0.84, 0.79 and 0.73 respectively while the entomological inoculation rate is 104.4.This study show *An.gambiae, An.arabiensis,Culex quinquefasciatus and Aedes aegyptia* abound in the study area. The infection rate, inoculation rate and human biting rate revealed during the study have incriminate *An.gambiae and An.arabiensis* as the main vectors of*falciparum*malaria in the settlements.

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CHAPTER ONE

1.0 INTRODUCTION

Mosquitoes are insects that comprised of over 3,500 known species, which are mostly associated with tropical,sub-tropical,temperate and snow region. They are ubiquitous dipterous insects of great medical and socio-economic importance as vectors of numerous disease causing parasites and pathogens (Service, 1993). It is known that a few species of mosquitoes are harmless and or useful to mankind, but most are nuisance because they suck blood from vertebrates, including man (www.bbc.co.uk/natural life/mosquitoes retrieved 16/08/2013). The female mosquitoes are blood sucking pests that transmit many harmful human and livestock diseases such as malaria, yellow fever and filariases. These diseases appeared to have defied several scientific advances and health services instituted to combat them (Service,1993).

Mosquito-borne diseases are major health problems in Nigeria in particular and in other part of Sub-Sahara Africa. Statistics show that malaria accounts for 660,000 deaths worldwide. Every year, more than 200 million cases occur(WHO, 2013). Recently it was discovered by the World Health Organisation that 90% of estimated 627,000 deaths caused by malaria in the world in the last two years were recorded in Africa.

Apart from malaria, other mosquito-borne disease have also accounted for large economic loss, social disgrace, low productivity,school and work related absenteeism, sleeplessness and so on in many parts of the Nigeria (Anosike*et al.,* 2003).

Several factors that have been reported to influence the role of mosquitoes in disease transmission are abundance, biting behaviour, host preference and longetivity

Noutcha and Anumdu (2009). The full understanding of these factors is a prerequisite to planning effective vector control measures.

1.1 Statement of the Problem

Human ecological transformation have occurred at an unprecedented rate over the past 50 years, prominent among them is water resource development project (WHO, 1996). The construction of dams and impoundment of reservoirs has been the principal means for the provision of irrigation water and supply of drinking water to many communities. However, approximately half of the large dams that serve as irrigation purposes,also serve as breeding grounds for vectors of diseases (Gujja and Perrin, 1999).

In 2001, the total area under irrigation worldwide was estimated to be 272 million hectares. Along potential advantage of increased water supply to the community and agricultural productivity, development of water resource may have both positive and negative impact on health (WHO, 1995). The development of water project and their operation has a history of facilitating increased transmission of vector-borne diseases (Service ,1991). The underlying reasons are the creation of new breeding sites and enhancement of human-vector contact due to ecological and demographic changes. For example, surface irrigation which is mainly used for flooding of rice fields creates temporary shallow water bodies which forms ideal breading sites for malaria vectors (Lacey and Lacey, 1990). There was also indication thatof the55 *Anopheles* species described worldwide that act as potential vectors, several are predominantly rice field breeders,particularly*Anopheles aconitus* and *Anopheles sinensis*.

1.2Justification

In the last three decades Nigeria had witnessed a number of human activities which include agricultural development, construction of dams for irrigation and domestic consumption, construction of human habitation and deforestation. All these anthropogenic activities have the potential to provide breeding grounds for mosquitoes and thus expand the range of mosquitoes in all geographical ranges of Nigeria (Ndams,2004).

Anopheles mosquitoes seldom occur in isolation from other Culicidae and malaria endemic areas have been found to have many species of mosquito fauna of which,the majority are completely harmless to humans (Davies and Crosskey, 1991). It is therefore essential to differentiate vector species from inocuous ones

Immunological test involving detection of circumsporoziote (CSP) antigens such as Enzyme Linked Immunosorbent Assay (ELISA) deosnot only distinguish human and non human malaria,but also individually recognize the sporozoite of all the four human infecting plasmodial parasites and can also quantify infections (Burkott and Wirtz , 1992).

ELISA techniques are currently in common use,providing results more quickly and equal or greater accuracy (Wirtz,*et al.*,1987,1991,1992) unlike the traditional microscopy techniques.

The importance of accurate identification of vectors of malaria cannot be over emphasized,because not all *Anopheles*are vectors of malaria. Therefore it is pertinent to discriminate between different species with precision on species to prove vectors of malaria and closely related harmless ones. This will reduce expenditure of time and money on superfluous malaria campaign (Smart, 1956).It is in the light of the above that this study was designed to shed light on species composition of mosquitoesbiting

indoors and determine the anopheline species for instance vectorial capacity status. A database for research and reference would be developed. It is hopedthat the information obtained from this research would provide invaluable information to the vector controller.

1.3 Aim

The aim of this study is to access the indoor resting populations of mosquitoesand*Plasmodium falciparum* infection of *Anopheles* species in three settlement around Zaria Dam,Zaria.

1.4 Objectives

I. To determine the occurrence and abundancespecies of indoor resting mosquitoes in the study area.

II. To determine the *Plasmodium falciparum*infection rate in theAnopheline population.

III. To determine the entomological innoculation and human biting index of the Anopheline population.

1.5 Hypotheses

I. The mosquitoesresting indoors arenot abundant in the study area.

II. The mosquitoes resting indoors are not related to transmissionof*Plasmodiumfaciparum.*

III. There are no infective bites by mosquitoes resting indoors in the study area.

CHAPTER TWO

2.0 LITERATURE REVIEW

The word mosquito is a Spanish meaning a little fly (Harold, 1904).This word as established in English and with variation in other languages refers to blood sucking flies of the family*Culicidae*.

2.1 Scientific Classification

Mosquitoes belong to Kingdom Animalia, Phylum Arthropoda, Class Insecta, Order Diptera, Suborder Nematocera, Infraorder Culicomorhpa, Super family Culicoidea, Family *Culicidae* and three sub families *Anophelinae, Culicinae, Toxorhynchitinae* (Meigen, 1818).

The mosquitoes are small, midge-like flies the *Culicidae*. Although a few species are harmless or even useful to humanity, most are a nuisance because they bite and suck blood from living vertebrates, including humans. In feeding on blood, various species of mosquitoes transmit some of the most harmful human and livestock diseases.(Service, 1993).

Mosquitoes have the following characteristics: A proboscis, long slender abdomen, scales on the wings bears venation, and palps are pendulous. They have a pair of compound eyes occupying a large part of the head surface, and antennae composing of segments (Goma, 1966). They originated in the Jurassic geological periods (Service, 1980).

2.2Evolution

The ancestors of *Drosophila* and mosquitoes diverged 260 million years ago (Moreno *et al.,* 2010). The Culicine and *Anopheles* Clades of mosquitoes diverged between 120 and 150 million years ago (Eric *et al*., 2009). The old world and new world *Anopheles* species subsequently diverged between 80 and 95 million years ago

(Eric *et al.*, 2009). Fossil records suggest that several existing genera were well differentiated in the mid-tertiary (35 million years ago). They might have evolved in the early tertiary (about 70 million years ago) or even earlier (Marshall, 1938; Edward, 1941).

2.3Distribution

Mosquitoes have a worldwide distribution, occurring throughout the tropical and temperate region and are only absent in Antartica (Smith,1980). However, some genera have restricted distribution and may be confined to certain areas; for instance, the *genus Haemagogus* is only found in central and South America (Service, 1980). Certain mosquito species are said to be ubiquitous but are more widespread in the tropical and sub tropical regions and are also found in temperate climates and even in the Arctic during summer (Bates, 1949). Species that are of medical importance (examples, *Anopheles gambiae* complex,*An. funestus* and *An. arabiensis*) co-exist widely over much of the African and Asian continents. Some species may appear to have disjunct distribution, although doubtless other localized population in the several zones remain to be discovered. *Anopheles gambiaes.l.* is evidently absent from all areas of the North and East Africa, whereas in West Africa its range extends well into the Sahel regions of Mauritanian zone (Gillies and Coetezee, 1987).

Culexpipiens complex includes, the anautogenous subspecies – *Culexpipienspipiens L,* the autogenous*Culexpipiensmolestusforskal* and *Culexpipiensquinquefasciatus.* They occur in Europe, extra tropical part of Asia, Africa, Middle part of North America, part of South America and Australia (Vinogradova, 1997). *Cx. p. pipiens*has also been recorded mainly in the Southern and Northern part of Africa. It is wide spread in Egypt, where both autogenous and anautogenous forms *(Cx. p. molestus*and Cx. *p. pipiens* respectively) coexist. Also,

these species coexist together with *Cx.p. quinquefasciatus* in South Africa. *Cx. p. pipiens* is the most common sub species even in some places of the Afro-tropical realm, totaling 92% of the collection from a Marsh in the Kenya highlands (Logan *et al*., 1991).

In Nigeria, mosquitoes are widely distributed throughout the country; the most common species belong to the genera *Anopheles*, *Culex, Aedes* (Surtees, 1959). The most notorious mosquito species which have been incriminated in the transmission of such diseases like malaria (*Anopheles gambiae*, *An.arabiensis*) yellow fever (*Aedesaegypti*) andfilariasis (*Anopheles gambiaes.l.* and *Culex p. quinquefasciatus*), have also been identified in Nigeria (Service, 1963). The major malaria vector in the rain forest part of Nigeria is *Anopheles gambiaes.l,* while *Anopheles funestus*is the major malaria vector in the Southern part, (Service, 1963). Recently an interesting research, rising *Anopheles gambiae s.l., An. funestus*and *Culex*species were collected in some households from a survey in four sentinel villages in Nasarawa and Plateau states. Only the Anopheline mosquitoes were found to harbour *Wuchereriabancrofti* larvae (Richards *et al*., 2005).

Gadzama (1976), carried out studies on the seasonal changes in mosquito population in Ahmadu Bello University,Zaria,Samaru campus and found correlation between the amount of rainfall and the number of Anophelines. The mosquitoes increased with higher rainfall. The most abundant in the dry season were the Culicines .Study carried out by Nendangtok (1991) on mosquitoes in Biological Sciences Department of Ahmadu Bello University,Zaria,831 mosquitoes were caught during the sampling period and these belong to three genera: *Culex, Aedes* and *Anopheles.* The species caught were *Culexquinquefasciatus, Culexannulioris, Culexpoicilipes, ,Aedesaegypti, Anopheles gambiae*and *Anopheles funestus* with *Culex* genus

predominating and *Culexquinquefasciatus* being the most abundant occurring throughout the dry season. Abdulkadir (2001), surveyed indoor resting populations of mosquitoes in some areas of Kadunametropolis,where he caught 380 mosquitoes belonging to genera *Culex* and*Aedes*with the *Culex* species predominating.

2.4 Ecology

The ecology of the adult mosquito of the Northern Guinea savannah in two villages of Kaduna (AngwanRimi, which is now urbanized and Kangimi) was studied (Service, 1963). The research showed that *Anopheles gambiaes.l, An. nili, An. funestus, Culexpipiensfatigan*. Now, *Cx. p. quinquefasciatus* and *Mansoniauniformis,*were the most common mosquito species during the period of study.

The occurrence and distribution of other mosquito species could be based on habitat availability, climate, environment and human activities. Larval surveys have shown that *Cx p. quinquefasciatus* is the commonest mosquito breeding in the numerous polluted streams, open drains etc of townships like Lagos (mangrove forest and coastal vegetation), Ibadan (low land rain forest),Bauchi-Jos-Plateau (Guinea savannah), Kano (Sudan savannah), and Sokoto to-BirninKebbi (Sahel savannah). (Service, 1963).Okogun*etal*., (2003) in Esan and Etsako region of Edo state, (Formerly Midwestern Nigeria) suggested that mosquito abundance is related to population and human activities: for example, the university town of Ekpoma is occupied predominantly by students and farmers had a higher relative abundance (35 – 70%) of mosquito larval species. Also, Amusan*et al*., (2004) reported that various habitat rice field within a populated village I near Abeokuta (rain forest zone) in Ogun state where *Aedesaegypti,* was most abundant species.

2.5 Biology

Anopheline eggs are laid singly on the water surface. In most species they are tropically boat shaped and laterally have a pair of floats. They are unable to withstand dessication. Culicine eggs never have floats. They are laid either singly (e.g. *Aedes)* or in the form of raft that float on the water surface (e.g. *Culex)* or are glued together as sticky masses that are stuck to the underside of floating vegetation (e.g. *Mansonia*) (Furman and Catts, 1970).

*Anopheles*larvae lack siphon, with the result that when they are at the water surface they (lie parallel to it and are not subtended at an angle as the*culciinae*). Microscopic examination show that their abdomen have small, brown, sclerotized plates, called targal plates, on the dorsal surface of abdominal tergites one to eight. In addition most or all of these segments have a pair of well developed palmate hairs sometimes called float hairs. On either side of abdominal segment eight there is a group of spines which are joined together at their base to form a structure called pectin. All these structure identify larvae as belonging to the genus *Anopheles* (Gillett, 1972; Service, 1976).

Culicine larvae all possess a siphon which may be long or short. They hang upside down at an angle from the water when they are getting air except*Mansonia* larvae which insert their specialized siphons into aquatic plants and remain submerged. There are no abdominal palmate hairs on tergal plates on *culicinae* larvae (Service, 1980).

In *Anopheline*pupae the respiratory trumpets are short and broad distally thus appearing conical whereas in most *culicinae* the trumpets are narrower and more cylindrical. The most reliable characteristic for identifying *Anopheline*pupae is the presence of short peg-like spines situated laterally near the distal margins of

abdominal segments two to seven. In Culicines there are no such spines (Gillett, 1972; Service, 1976, 1980).

The most reliable way to distinguish between adult Anophelines and Culicines is by examination of their heads. The first procedure is to determine the sex of adult: Female mosquitoes have non-plumose antennae, while males have plumose antennae. If the adult are females and also *Anopheles* thenthe palps will be about as long as the proboscis and annually lie closely alongside it. In male *Anopheles*, the palps are also almost the same length as the proboscis but are distinctly swollen at the ends and tend to look like clubs (Service, 1980).

Female *culicinae* also have non-plumose antennae and the palps are shorter than the proboscis, in males (which have plumose antennae) the palps are about as long as the proboscis but are not swollen distally and hence do not appear clubbed.

Within the sub-family *culicinae* there are three genera commonly found in Nigeria, and these are *Culex, Aedes,* and*Mansonia.* These three genera of mosquitoes can be differentiated by closely examining their adults and the larvae and eggs.

Culex eggs are usually brown, long and cylindrical, laid upright on the water surface and placed together to form an egg raft which can comprise up to about 300 eggs. Adhesion is due to surface forces holding the eggs together.

*Mansonia*egg raft are arranged in the form of rosettes and glued to the under surface of vegetation. Individual eggs are dark-brown, cylindrical but with tub-like extensions apically which are usually darker than the rest of the eggs. The eggs cannot withstand desiccation and hatch within a few days.

*Aedes*eggs are usually black, more or less out in shape and are always laid singly. Careful examination shows that the egg shell has a distinctive mosaic pattern. Eggs are laid on damp substrate just beyond the water line, such as on damp mud.

Aedes eggs can withstand dessication and can remain dry and viable for many months (Huang, 1981; Faran, 1981).

Culex adults are recognized more by their lack of ornamentation than any striking diagnostic characters. The tip of the abdomen of the females is blunt and the cerci retracted. Frequently, but not always, the thorax, legs, and wing veins are covered with dull, often brown scales although some whitish scales may occur on most segments.

Many adults *Aedes*have conspicuous patterns on the thorax formed by black, white or silvery scales, in some species yellow scales are present. The legs often have black and white rings. Scales on the wings of*Aedes* mosquitoes are narrow, and are usually more or less all black except at the siphons adapted for piercing aquatic plants to obtain air. The siphon is conical with the apical part dark and heavily sclerotized, it has prehensile hairs and serrated processes for inserting into plants (Service, 1980).

2.6 Morphological Identification

Morphological identifications are the baseline of any identificationtechnique. Basic descriptions of Anopheline mosquitoes and keys foridentification have been established by Gilles and De Mellon (1968) and later improved by Gilles and Coetzee(1987).Members of the *An. gambiae*complex are difficult to morphologically identify (Gilles and Coetzee, 1987). Hunt**,** *et. al*., (1998) stated that the only reliable morphological characters by which we can differentiate between the two saltwater and the fresh-water species is by using the number of antennal sensillaeand the palpal index pointed out by Coluzzi, (1964).

2.7 Molecular Techniques Used for Detection of *Anopheles* Species: Polymerase Chain Reaction:

The polymerase chain reaction (PCR) is an exquisitely, powerful *invitro*method for amplifying a defined target DNA sequence. It is newapproach for solving problems in molecular genetics, evolutionary anddevelopmental biology, medical diagnosis and forensic medicine. Thetechnique permits a sensitive, selective, accurate, and rapid detection of amount of an exact DNA region that lies between two regions of knownDNA sequences. It is based on the enzymatic amplification of a known specific DNA fragment using oligonucleotide primers, whichspecifically attach to the target DNA flanking region. The sequenceamplification uses a heat-stable DNA polymerase isolated from *Thermusaquaticus*(Saiki *et al*., 1988). Oligonucleotide primers are short (10- 20 nucleotides in length), singlestranded DNA molecules, which arecomplementary to the ends of a defined sequence of DNA template.Theprimers are extended on singlestranded denatured template DNA by aDNA polymerase in the presence of deoxynucleoside triphosphates(dNTPs) under suitable reaction conditions. This results in the synthesisof new DNA strands complementary to the template strands. Strand synthesiscan be repeated basically by sequential cycles composed of three steps: heatdenaturing of the double-stranded DNA at 93-95^{o}C to unwind the strandof starting DNA, followed by lowering the temperature of the mixture in therange of 50-60^{o}C to allow annealing of primers to the template of singlestranded DNA which target the complementary sequence and finallyextension of primers by DNA polymerase at 70-75^{o}C which suits theenzyme to complete the reaction (Saiki *et al*., 1988).

2.8 Enzyme-Linked Immunosorbent Assay (ELISA):

ELISA is a widely usedmethod for measuring the concentration of a particular molecule(e.g., a hormone or drug) in a fluidsuch as serum or urine. It is alsoknown as enzyme immunoassay or EIA. The molecule is detected byantibodies that have been made againstit; that is, for which it is the antigen.Monoclonal antibodies are often used.The test requires the antibodies fixed toa solid surface, such as the innersurface of a test tube a preparation ofthe same antibodies coupled to anenzyme. This is one (e.g., β-galactosidase) that produces a colouredproduct from a colourless substrate.The Test is carried as follows: The tubes are filled with the antigensolution (e.g., urine) to be assayed. Any antigen molecules present bind to the immobilized antibody molecules.The antibody enzyme conjugate is added to the reaction mixture. The antibody part of the conjugate binds toany antigen molecules that were bound previously, creating an antibodyantigen- antibody "sandwich". After washing away any unbound conjugate, the substrate solution is added. After a set interval, thereaction is stopped (e.g., by1%NaOH) and the concentration of colored productformed is measured in a spectrophotometer. The intensity ofcolor is proportional to the concentration of bound antigen.(www.user.rcn.com/jkimball.ma.ultane t/Biology Page/A/AIDS).

2.9 Medical Importance of Mosquitoes

Mosquitoes are the most widespread of the medically important insects. The tropical diseases they transmit are responsible for such ill-health and loss of human life which include, malaria, filariasis and the arbovirus diseases of yellow fever and dengue, being the best known. Mosquitoes are major health problem in Africa and other tropical areas of the world. They are estimated to transmit disease to more than

700 million people annually and are responsible for the death of about one in 17 people currently alive (WHO, 2000).

2.9.1 Malaria

Malaria is caused by *Plasmodium* parasites transmitted by *Anopheles* mosquitoes. The World Health Organization (2010) estimated that, there were 216 million documented cases of malaria. That year,between 655,000 and 1.2 million people died from the disease (roughly 2000-3000 per day), many of whom were children in Africa. Other high-risk groups include pregnant women and non-immune travelers, refugees, displaced persons and labourers entering endemic areas.

Hunt *et al* (1998) and Coetzee (2000)opined that although there are more than 400 known species of Anopheline mosquitoes, only about 30 species can be considered important malaria vectors. Only the female mosquito obtains blood meals. Malaria inhumans is caused by four species of protozoan parasites of the genus *Plasmodium:P. falciparum, P. vivax, P. ovale,* and *P. malariae.* Although *P. vivax*is responsible for most malaria infections in the world, the most severe form of malaria is caused by *P. falciparum.* The severity of malaria illness depends largely on the immunological status of the person who is infected. Partial immunity develops over time through repeated infection and without recurrent infection, but such immunityis relatively short-lived.

2.9.2 Yellow fever

The urban type of yellow fever is transmitted by *Aedesaegypti,*whereas other genera and species of mosquitoes may be involved with jungle yellow fever. Mosquitoes of the genera *Haemagogus* and Sabethes are vectors of jungle yellow fever in the Americas but species of*Aedes* are vectors in Africa. Yellow fever has not

been found in Asia. The development of effective vaccines and their mass application have reduced the occurrences of yellow fever, and it no longer has the global importance it had half a century ago (WHO, 2000).

The incidence of yellow fever in the world increased slightly in 1994 and 1995, compared with the previous 2 years, yet remained lower than the incidence reported before 1992 (Carter Foundation, 2005). In 1994, 1439 cases were notified to WHO, with 491 deaths (case fatality rate [CFR] 34%), and 974 cases were reported in 1995, with 247 deaths (CFR 25%). Yellow fever continues to be endemic exclusively in Africa and South America (Carter Foundation, 2005).

In 2005, the Carter Foundation noted that there were 1351 cases in total and 452 fatalities (CFR 33%) reported for Africa in 1994, and 459 cases reported with 34 deaths (CFR 7%) for 1995. Nigeria reported the highest number of cases in Africa in 1994 (91% of cases in Africa, 85% of cases in the world), and in 1995. Two yellow fever epidemics were reported in 1994 in Imo state, south-eastern Nigeria. The first outbreak began with cases reported in the Ihilala Local Government Area, Anambra state and Orsu LGA, Imo State in October 1993 and ended with 5 cases in January 1994. No further cases were reported from February to August 1994. In September, cases began to occur in Orsu LGA, Imo State. This second epidemic peaked in November and declined through December 1994 with a total of 1227 cases and 415 deaths (CFR 34%). The epidemic was brought under control by the combination of a vaccination campaign performed by the local primary health care department and ministry of Health teams and dry weather, which reduced the vector density.

In 1994, Liberia reported the highest percentage of cases in Africa (78% of cases in Africa, 37% of cases in the world). Outbreaks were reported in 1994 in Cameroon Gabonand Ghana 1995. In response to the large epidemic of 1992 – 1993,

Kenya established a sentinel surveillance system that detected continued low-level transmission of yellow fever virus. Such an initiative by the government of Kenya may serve as an example for epidemic prevention and control (WHO, 2000).

2.9.3 Filariasis

According to WHO (2000) lymphatic filariasis is a leading cause of permanent and long-term disability. In communities endemic with lymphatic filariasis, as many as 10 percent of women and 50 percent of men can be infected (WHO, 2000). In its severest form, lymphatic filariasis leads to elephantiasis a crippling condition in which limbs or other parts of the body are grotesquely swollen or enlarged. These conditions have a devastating effect on the quality of life of those affected impacting them not only physically, but also emotionally and economically. Mosquito-borne filariasis in human includes a group of disease caused by parasitic worms,the filarial nematodes. The species of filarial worms involved are *Wuchereriabancrofti, Brugiamalayi*and *B. timori. Wuchereriabancrofti*has an extensive geographical distribution and is found in many tropical countries. Several genera and species of misquotes are vectors of filariasis, especially*Culexquinquefasciatus*, which is found primarily in urban and suburban areas. *Anopheles*, *Mansonia* and *Aedes* species also can serve as vectors for filarial nematodes(WHO, 2000).

There are approximately 1.1 billion people at risk for contracting lymphatic filariasis and 120 million people infected worldwide. Nigeria is the third most endemic country in the world, with an estimated 22 million cases. According to WHO (2000), India, Nigeria, Bangladesh, and Indonesia account for 70 percent of infections. Estimates of annual economic loss in India due to lymphatic filariasis approach USD $ 1 billion. This economic loss is an even greater burden for the 32

least developed countries in the world endemic for lymphatic filariasis (Carter Foundation, 2005).

2.9.4 Dengue fever

It is transmitted primarily by *Aedesaegypti,* a container breeding mosquito that is closely associated with man and urban development. However, other *Aedes* species also may be involved. A more severe disease, dengue hemorrhagic fever, has produced high mortality among children in South East Asia and in 1981, reached the Caribbean region with outbreaks in Cuba (1981), Mexico (1985) and Puerto Rico (1986). No vaccine is available yet (WHO, 2000).

The World Health Organization estimates 50 million cases of dengue infections occur each year. This includes 100 to 200 cases reported annually to the U.S. centers for Disease Control and Prevention (CDC), mostly in people who have travelled abroad. From 1977 to 1994, U.S. health care workers reported to CDC 2,248 cases of dengue that have been imported in the country. Many more cases probably go unreported because some doctors do not recognize the disease (WHO,2000).

In 1997, dengue was the most important mosquito-borne viral disease affecting humans; its global distribution is comparable to that of malaria, and an estimated 2.5 billion people live in areas at risk for epidemic transmission. Each year, tens of millions of cases of dengue fever occur and, depending on the years, up to hundreds of thousands of cases of Dengue Haemorrhage fever (DHF). The case-fatality rate of DHF in most countries is about 5%. Most fatal cases are among children and young adults (Carter Foundation, 2005).

2.10 Control Measures Against Mosquitoes

Service (1995) noted that there are two kinds of mosquito control: large, organized programmes to reduce mosquito populations over a wide area, and actions

an individual can take to control mosquitoes with respect to themselves and their own property. Organized mosquito control programs today draw on the principles of integrated pest management. Many integrated mosquito control programmes are guided by surveillance of mosquito populations and knowledge of the mosquito life cycle: e.g. source reduction - the removal of mosquito breeding habitats.

While the US Environmental Protection Agency (USEPA) (2004) stated that since many mosquitoes breed in standing water, source reduction can be as simple as overturning an old tin can or can be as complex as permanently draining marshes. Much source reduction is a matter of education. For example, home owners can eliminate mosquito breeding grounds by removing unused plastic pools, old tires, or buckets by clearing clogged gutters and repairing leaks around faucets ; by regularly changing water in bird baths ; and by filling or draining puddles, swampy areas, and tree stumps. Eliminating such mosquito breeding areas can be an extremely effective and permanent way to reduce mosquito populations without resorting to insecticides (AMCA, 2004).

Service (1995) also noted that habitatmodification, such as ditching or diking marshes or manipulating daily Water flows can be effective at reducing mosquito populations by disrupting the mosquito life cycle. However, experience has shown that such large-scale programmes can be harmful to the ecosystem if not undertaken carefully. One example of a successful approach to habitat modification, open marsh water management, involves the use of shallow ditches to connect the shallow waters where mosquitoes breed to deeper waters where natural predators live. Simply giving the predators access to the mosquito larvae can result in long-term mosquito control (AMCA, 2004).

Service (1999) remarked that bio control is the direct introduction of predators to target mosquitoes. Effective biocontrol agents include predatory fish that feed on mosquito larvae such as *Gambesiaaffinis* and other minnows and killifish (*Fundulusmejalis*). Some other biocontrol agents that have had lesser degrees of success include the predator mosquito *Toxorhynchites* and predatory crustaceans, and parasitic nematodes, and fungi. Some public agencies also employ other predators such as birds, bats, dragonflies, and frogs, but evidence of effectiveness of these agents is only anecdotal. In particular, there is no documented study that establishes that bats or purple martins (a species of the Swallow bird of North America) consume enough mosquitoes to significantly control mosquito populations.

Service (1995) and USEPA (2004) stated that other materials used as biological control agents are the dead spores of varieties of the natural soil bacteria *Bacillus thuringiensis* (BT), especially *Bt.israelensis* (BTI). BTI is used to interfere in the digestion systems of larvae by deactivating digestive enzymes like amylase in the gut of the larvae (AMCA, 2004). It can be dispersed by hand or dropped by helicopter in large areas. BTI is no longer effective after the larvae turn into pupae, because they stop eating (AMCA, 2004).

At this point larviciding oils, such as Golden Bear, can be used, which increase the water tension until the pupae and larvae cannot break the surface to obtain air and therefore drown. A chemical commonly used in the United States is methoprene, considered slightly toxic to larger animals, which mimics and interferes with natural growth hormones in mosquito larvae, preventing development. Methoprene is frequently distributed in time-release briquette form in breeding areas (USEPA, 2004).

Adulticide, the ground or areal application of chemical pesticides, is less effective than the other methods of mosquito control and is generally considered the method of last resort. Nevertheless, careful application of adulticide is considered a critical part of integrated mosquito management. For example, ultra low volume (ULV) spraying of Malathion has been used in metropolitan areas like New York City to decrease the mosquito population and prevent the spread of West Nile Virus (USEPA, 2004).

Service (1995) noted that the most effective solutions for malaria control efforts in the third world are: mosquito nets (insecticide-laced mosquito nets, and Dichlorodiphenyltriethane (DDT). Plain mosquito nets are cheap, they are highly effective in protecting humans within the net, and do not adversely affect the health of natural predators such as dragonflies. Nor do they require sophisticated public health capacity on the part of the government.

Besides that Service (1995) also reported that the role of DDT in combating mosquitoes has been the subject of considerable controversy. While some argue that DDT greatly damages biodiversity, others argue that DDT is the most effective weapon in combating mosquitoes and hence malaria. While some of this disagreement is based on differences in the extent to which disease control is valued as opposed to the value of biodiversity, there is also genuine disagreement amongst experts about the costs and benefits of using DDT. Moreover, DDT-resistant mosquitoes have started to increase in numbers, especially in the tropics due to mutations, reducingthe effectiveness of this chemical (Service, 1995).

2.11 Mosquito Repellant and Personal Mosquito Control

Mosquito repellants generally contain one of the following active ingredients: DEET, Calnip, oil extract, nepetalactone, Citronella, or Eucalyptus oil extract. Often

the best “repellant” is a fan or gentle breeze as mosquitoes do not like moving air (Anonymous, 2001).

Other popular methods of household mosquito control include use of small electrical mats, mosquito repellent vapour, and mosquito coil, all containing a form of the chemical allethrin . Mosquito repellant candles containing Citronella oil another method to keep mosquitoes at bay. Some lesser known methods use the cultivation of plants like wormwood or sagewort, lemon balm, lemon grass, lemon thyme and the mosquito plant (Pelargoniuni) which act against mosquitoes. However scientists have determined that these plants are effective only when the leaves are crushed and used (Anonymous, 2001).

There are several widespread theories about mosquito control such as the assertion that Vitamin B, garlic, ultrasonic devices, incense, bats, purple martins and bug zappers can be used to repel or control mosquitoes .Whether these methods are effective at deterring mosquitoes or significantly reducing mosquito populations remains disputed. Moreover, some manufacturers of “mosquito repelling” ultrasonic devices have been found to be fraudulent because they sometimes exaggerate the capabilities of their products in order to sell (Fradin, 1998).

Although bats can be prodigious consumers of insects, many of which are pests, less than 1% of a bat’s diet will consist of mosquitoes; bats mostly feed on larger insects such as beetles. Bats are known carriers of rabies, so care must be taken if attempting to use them to control insects. Similarly, bug zappers kill a wide range of flying insects including many beneficial insects as well as mosquitoes, they are not effective at controlling mosquito populations (Fradin, 1998).

Some newer mosquito traps emit a plume of carbon dioxide together with other mosquito attractants such as chemical scents, warmth, water vapour and sounds. By mimicking a mammal, these factors draw female mosquitoes toward the trap, where they are typically sucked into a net or holder where they collect. These devices

will, indeed, trap and kill measurable numbers of mosquitoes, but their effectiveness in any particular case will depend on a number of factors such as the size and species of the mosquito population and the type and location of the breeding habitat (AMCA, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Area

The study was carried out in three settlements, Angwan Shika, AngwanMalami and the Water Works Quartersthat house the Zaria dam worker . The study area is characterised with a grassland ecosystem with trees being widely spaced so that the canopy does not close. The open canopy allows sufficient light to reach the ground to support an unbroken herbaceous layer consisting primarily of grasses. There are two seasons, a dry season that last between four to seven months and a rainy season that lasts between four to five months. The rainfall ranges between 1016mm and 1524mm with relative humidity between 60% and 80% . Mud houses are common with thatched roof and unkempt drainages and gutters that forms stagnant water are seen in the compounds.

3.2 The Zaria Dam

Zaria dam lies on longitude $11^0$07N and the latitude $7^0$46E. It is situated on the River Galma and was officially commissioned in 1975. The height of the spill way, above the river bed, is about 10.67km and extends up to 25km in eastern direction (Omor, 1987). The dam was constructed solely for the supply of drinking water to Zaria town and its environs. However, fishing and irrigation farming are also carried out. It also serves as recreational facility. During dry and rainy seasons it holds water that provide breedinggrounds for animals, including insects like mosquito.

Fig 1 shows map of Nigeria, indicating Kaduna state and Zaria respectively while Fig 2 is the map pf the study area showing the three settlements where the study was carried out.



Fig. I: Map of Nigeria showing Zaria.

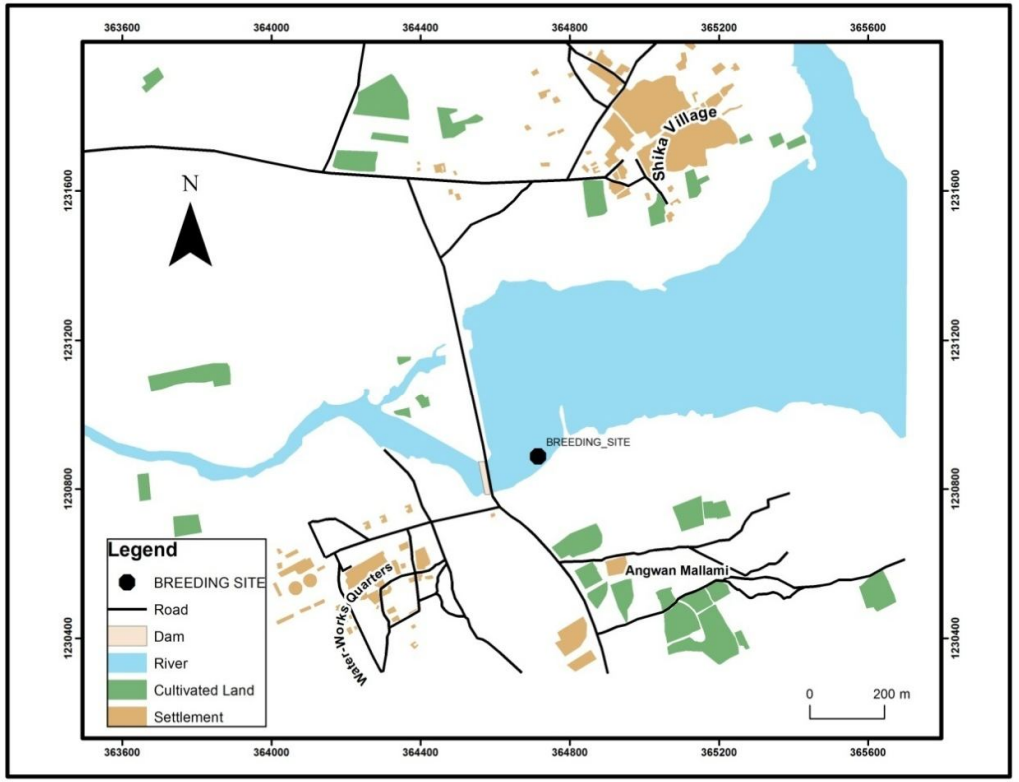


Fig II: Map of the study area showing the three settlements.

3.3Visitation and Recruitment of People for the Study

A letter of introduction was addressed to the village head of each of the three settlements, stating the name of the institution of learning, purpose and the importance of the research work. The letter was given to them by myself accompany by one of my supervisor s and someone that could convey the message to them appropriately in Hausa.After their acceptance,on the next visit, houses where the collection was done were marked and the tenants were informed and received their consent for the collection in their houses (Noutcha and Anumudu, 2009).

3.4Collection of Mosquitoes

Adult mosquitoes resting indoors in houses were collected by knock down spray according toWHO 1995, using indoor spray catches method (Pyrethrium Spray Collection or PSC). A total of 35 houses was selected from the three settlements. The houses where mosquitoes collection was done were randomly selected in the three settlements taking into consideration that each hut and house is occupied by number of persons with nearby domesticated animals and it is near to one or more breeding sites. In both cases only permanently inhabited houses were selected. The sampling was done twice a month from January to October 2013.

Before spraying, windows, doors and all opening through which mosquitoes could escape were closed and white sheets were spread out on the floor. The collection were done in the morning (6-9am). After spraying the huts were closed for 10 minutes and opened for collection. The mosquitoes that fell on the white sheets were picked up by forceps and placed in Petri dishes containing moist filter paper. All the collected mosquitoes were taken to parasitology and entomology laboratory for identification by microscopy. Specimens were then kept dry in Eppendort tubes

containing silica gel. The number of human being sleeping in the room the night before collection period was also recorded.

3.5Morphological Identification ofMosquitoes

The mosquitoes collected were identified using morphological key(Gillies and de Meillon,1968: Gillies and Coetzee, 1987). They were sorted by the appearance of abdominal blood repletion stage. The significantreduction or complete absence of scales on the abdominal sternites and the smoothly rounded posterior end of the scutellum were used to confirm members of the genus *Anopheles*. The Pattern of wings were used to separate *Anopheles gambiae*from *Anopheles funestus.* They were classified fed and unfed based on abdominal appearance. After identification mosquitoes were preserved in dry labelled Eppendorf tube over dry silica gel and the *Anopheles* later used for PCR identification.

3.6 PCR Amplification

Molecular characterization of *Anopheles* mosquitoes was done according to Scott *et al.*, (1993). One leg of each of the *Anopheles*species was placed in 1.0ml. micro-centrifuge tubes and labelled appropriately. A total of 104 *Anopheles* species were assayed using the PCR method.

3.6.1 Preparation of master mix

The components of the master mix was prepared in the following order

PCR buffer	1.25µl
Dntp	1.25µl
$Mgcl_2$	0.5µl
GA Primer	1.0µl

AR primer	1.0µl
UN primer	1.0µl
ddH_2O 6.0µl	
Taq polymerase	0.5µl
	12.5µl (for a single sample)

The 12.5ul was used for a single sample. To get the total master mix used for the 104 samples, each component of the master mix was multiplied by 104.

Using a pipette, 12.5µl master mix was added to the micro centrifuge tubes containing the one leg from each of the mosquitoes. The reaction mixture was centrifuge for 2minutes at 16000 revolution per minute in a micro centrifuge in other to release the template DNA from the tissues and the reaction was placed in the PCR machine. Kisumu strain of *Anopheles* species was used as negative control.

The machine was programmed as follows :-

An initial cycle of denaturizing at 94^0C for 2 minutes,30 cycles of denaturizing at 94^0C for 30 seconds each,Annealing at 50^0C for 30 seconds, Extension at 72^0C for 30 seconds, Final extra extension step at 72^0C for 8 minute.

3.6.2. PCR agarose gel electrophoresis

The product of the PCR reaction (DNA amplified) obtained, above were examined in a 1% agarose gel electrophoresis. The procedure is described below

3.6.2.1*Preparation Of 1% agarose gel*

A gel mould was prepared by taping tightly to seal the two open ends. The comb was placed about 1.5cm from the left to the end, making sure the wells were not touching the side of the mould frame. The mould was kept in the refrigerator until required during which 0.50g of agarose was weighed, using an electric balance and

poured into a 250ml beaker and 50ml of 1% Tris Boric EDTA (1XTBE) was added. The top of the beaker was plugged loosely with paper towel and micro waved for 30seconds and mixed by swirling. It was microwave again for an additional 25 seconds and swirled until the agarose was completely dissolved in the 1 XTBE. Running cold water over the flask for 3 minutes reduce the temperature to about 50 ^{0}C such that it could be held by hand. 10µl of Ethidium bromide (EtBr, Aldrich EISI0) was added to the agarose solution and mixed carefully by swirling. The solution was poured into the gel mould in the refrigerator and allowed to polymerise within 25minutes

3.6.2.2 *Gel electrophoresis of PCR product*

The comb was removed from the agarosegel carefully by lifting up and pulling slightly on one side and ensure that all the well remained intact. The tape was then removed from the frame and the gel in mould frame was placed in the electrophoresis tank with the wells closest to the negative electrode, which was positioned to the left. IXTBE buffer was poured into the electrophoresis tank such that it just covered the surface of the gel stage and to fill all wells.

3.6.2.3 *Loading of PCR product*

On waxy paper or paraffin, 2µl of loading dye was placed on the thirteen spot from which each of the thirteen wells (one for the reference ladder 100bp and twelve for each of the test samples) was loaded.

The reference lane (1st well in this case) was first loaded by mixing 5ul of 100bp DNA ladder with the loading dye on the wax paper and carefully taken in a x2 pipette. The twelve test samples were similarly loaded by mixing 5µl of each with the 2µl of the loading dye on the wax paper and placed in the remaining lanes noting to which lane each sample was loaded.

The positive and negative electrode of the electrophoresis apparatus were connected to the power source and the power switched on. The gel was ran for 35min at 120v, after which the power was switched off and the gel was carefully removed with a spatula into a plastic bag (Zip lock) and taken to the dark room for photography.

3.6.2.4 *Photomicrography of Agarose gel*

The gel was placed on the transilluminator and covered with a ultra violet (uv) blocking shield for initial examination. The DNA amplification successes were indicated by the presence of white bands. The DNA bands amplified were photographed using a black and white Helcam Polaroid Camera after which the transilluminator was switched off and the film tab was pulled straight and forcefully cut and allowed to develop for 30 seconds. The photograph was pooled off from other film components. The DNA bands produced by PCR were promptly labelled, matching each lane with the test samples loaded and notes kept. The gel was disposed in the laboratory waste bin in a sealed polythene bag.

3.7Determination of Plasmodium Infection in Adult*Anopheles* Resting Indoors.

ELISA was done according to the method described by Wirtz *et al*,(1987). The heads and thoraces of the mosquitoes collected were placed in individually labelled 1.5ml Eppendort tubes and each part of the mosquito was ground in 50µl grinding buffer and 150µl was used to rinse the pestle and to bring the total of the titrate to 200µl.

The ELISA test procedure employed involved the following:Each well of a 96 wells. Micro titre plate was coated with 50µl of IXPBS solution containing the *P.falciparium*antibody covered and incubated overnight at 11^0C. The following morning, the content of the plates were aspirated each well filled with blocking buffer

and incubated at room temperature for one hour. (20µl / well).After one hour, the blocking buffer was aspirated and 50µl of the mosquito Titurate was added to the apparatus well, positive and negative control were also added. It was covered and incubated for 2 hours .

After two hours, themosquito's samples were discarded. PBS. TWEEN 20 was used to wash the well twice. Then 50µl of the horseradish phosphatase labelled pf 2A10 antibody was diluted in blocking buffer, and was added to each well. The plate was covered and incubated in a dark place for 1 hour.The plate was washed with PBS-TW20 thrice and 100µl ofperoxidase substrate was added to each well. Colour change was noticed within two minutes.

Finally absorbance was read at 405nm using an ELISA plate reading after half an hour incubation. Each positive samples were re tested for confirmation, samples were considered positive if absorbance exceeds twice the mean of seven negative controls (Writz et al.,1987).

3.8 Estimation of Human Biting Rate and Entomological Inoculaton Rate

The human biting rate (HBR), was estimated as the ratio of blood fed *Anopheles* showing human blood in their abdomen, caught in a room and the number of humans that slept in the room for the study period. Sporozoite rates is a proportion of *Anopheles* mosquitoes with circumsporozoite protein (CSP) antigen in their salivary glands/ head thorax region to the number of mosquitoes under investigation while the entomologic inoculation rate (EIR) usually expresses the intensity of malaria transmission, which is the product of the vector biting rate and the proportion of mosquitoes infected with sporozoite Stage malaria parasitesand (Noutcha and Anumudu,2009).

HBR = No. of mosquito fed with blood / No. of persons in house

Sporozoite rate = No. of *Anopheles* with CSP / Total *Anopheles* mosquitoes under study

Entomological inoculation rate = vector biting rate x CSP infection rate

CHAPTER FOUR

4.0 RESULTS

A total of 506 adult mosquito were caught indoors during the study from January to October 2013.(Table 4.1). The collection comprised of 191(37.5%) males and female mosquitoes 315(62.3%).

Seven (7) species of adult mosquitoes were identified from the three villages namely *Culexquinquefasciatus* 254(50.2%), *Culex annulioris* 117(23.0%), *Anopheles gambiae*114(22.5%), *Anopheles arabiensis*5(1.0%), *Anopheles funestus* 4(0.8%), *Anopheles rhodesiensis*11(2.2%), *Aedesaegypti* 1(0.2%).(Table 4.2)

Six(6), mosquito species were collected and identified in AngwanMalami,where the dominant species was*Culexquinquefasciatus* with a total of 102(51.5%), 40(20,2%) were males 62(31.3%) females and 56 (28.2%) of which contained blood in the abdomen and the least was two species each of *Anopheles funestus*2(1.0%)

and*Anopheles arabiensis* 2(1.0%).Table 4.3.

Two species of adult indoor resting mosquitoes were collected from the water works quarters. *Culexquinquefasciatus* was the dominant species with a total of 36(64.3%). Whereas 14(25.0%) were males 22(39.3%) were females. Twelve females 12(21.4%) had blood in their abdomen.The second species was *An.gambiae* with 20 individuals representing 35.7% as shown in Table 4.4. Eleven of these were males while 9 individuals (16.1%) were females; of which only 6(10.7%) had blood in their abdomen.

Table 4.1 Monthly collection of mosquitoes during the sampling period

Month	Angwan Malami	Angwa Shika	Water works quarters	Total
January	6	8	1	15
February	5	11	0	16
March	8	7	0	15
April	7	15	5	27
May	17	16	6	39
June	28	33	9	70
July	41	49	8	98
August	18	19	11	48
September	32	43	9	84
October	36	51	7	94
Total	198	252	56	506

Table 4.2 Mosquito Species Distribution throughout the sampling period

Species	***cx.quinquefasciatus***	***cx.annulioris***	***An. gambiae s.s***	***An. rhodeseinsis***	***Anopheles funestus***	***An. arabiensis***	***Aedes aegypti***	***Total***
January	8	3	1	1	1	1	0	15
February	4	4	5	0	3	0	0	16
March	5	4	2	3	0	1	0	15
April	6	12	8	0	0	0	1	27
May	8	14	17	0	0	0	0	39
June	37	18	12	1	0	2	0	70
July	63	15	18	2	0	0	0	98
August	17	16	15	0	0	0	0	48
September	44	15	21	3	0	1	0	84
October	62	16	15	1	0	0	0	94
Total	254	117	114	11	4	5	1	506

Table 4.3: Adult Indoor resting Mosquitoes Caught During The Study

Species	Examined		Females collected		Total (%)
	Males (%)	Female (%)	With blood In abdomen	without blood in abdomen	
Culexquiquefasciatus	100(19.8)	154(30.4)	110(21.7)	44(8.7)	254(50.2)
Culexannulioris	43(8.50)	74(14.6)	8(1.60)	66(13.0)	117 (23.1)
Anophelesgambiae	47(9.3)	67(8.6)	51(10.0)	16(3.2)	114 (22.5)
Anophelesrhodesiensis		11(2.2)	7(1.4)	4(0.8)	11(2.2)
Anophelesfunestus		4(0.8)	2(0.21)	2(0.4)	4(0.8)
Anopheles arabiensis		5(1.0)	1(0.2)	4(0.8)	5 (1.0)
Aedesaegypti	1(0.2)		-		1(0.2)
Total	**191(37.7)**	**315(62.3)**	**179(35.4)**	**136(26.9)**	**506 (100)**

Table4.4: Mosquitoes collected from the three settlements in Zaria Dam

	Settlements								
	Angwan malam			**Angwan shika**			**Water works**		
Species	Males	Female	Total	Males	Females	Total	Males	Females	Total
Culex quinquefasciatus	40	62	102	46	70	116	14	22	36
Culex annulioris	16	31	47	27	43	70	0	0	0
Anopheles gambiae s.s	19	17	36	17	41	58	11	9	20
Anopheles rhodesiensis	0	9	9	0	3	3	0	0	
Anopheles funestus	0	2	2	0	2	2	0	0	
Anopheles arabiensis	0	2	2	0	2	2	0	0	
Aedes aegypti	0	0	0	1	0	1	0	0	
Total			198			252			56

Polymerase Chain Reaction

By using PCR technique the majority of the mosquitoes identified as *Anopheles gambiae s.l*showed the diagnostic fragment (315) of *Anopheles arabiensis* and (319) of *Anopheles gambiae s.s* species.

Out of 104 *Anopheles* species identified by PCR technique 66 (63.5%) showed the diagnostic fragment (319bp) of *Anopheles gambiae* species while 27(26%) showed the diagnostic fragment of (315bp) of *Anopheles arabiensis*. The other 11(10.5%) of the samples were not identified due to either misidentification during morphological identification process or DNA degradation due to bad preservation.(Plate 1).

Sporozoite Rate

Plasmodium falciparum sporozoite prevalence in the mosquitoes were determined in the 87*Anopheles s.l* collected using ELISA technique. Out of the 87 *Anopheles* only 4(4.6%) were infected(plate 2).The highest sporozoite rate of *Plasmodium falciparum* was found in Angwan Shika 4.3% (2/46), in AngwanMalami the prevalence was (1/22) 4.5% while in water works quarters the prevalence was(1/19) 5.1% (Table 4.6).

The result in Table 4.5 showed that 4 (4.60) of the 87 adults *Anopheles* collected indoor tested positive for *P.falciparum* CSP antigen. This was restricted to *Anopheles gambiae*s.s and *An. arabiensis* only. *Anopheles rhodesiensis*and *An.funestus* were not positive to CS antigen. 4.48% (3) of the 67 *Anopheles gambiae s.s* tested were positive to *P.falciparum* (4.48) while only (1) of the 5 *An.arabiensis* tested was positive to *P.falciparum* (20.00) respectively(Table 4.7).

Table 4.5: Distribution of Molecular Species of *Anopheles gambiae s.l*

	Anopheles species	
Village	*Anopheles gambiae*s.s	*Anopheles arabiensis*
Shika village	29	9
AngwanMalami	23	13
Water works	14	3
Grand total	66	27

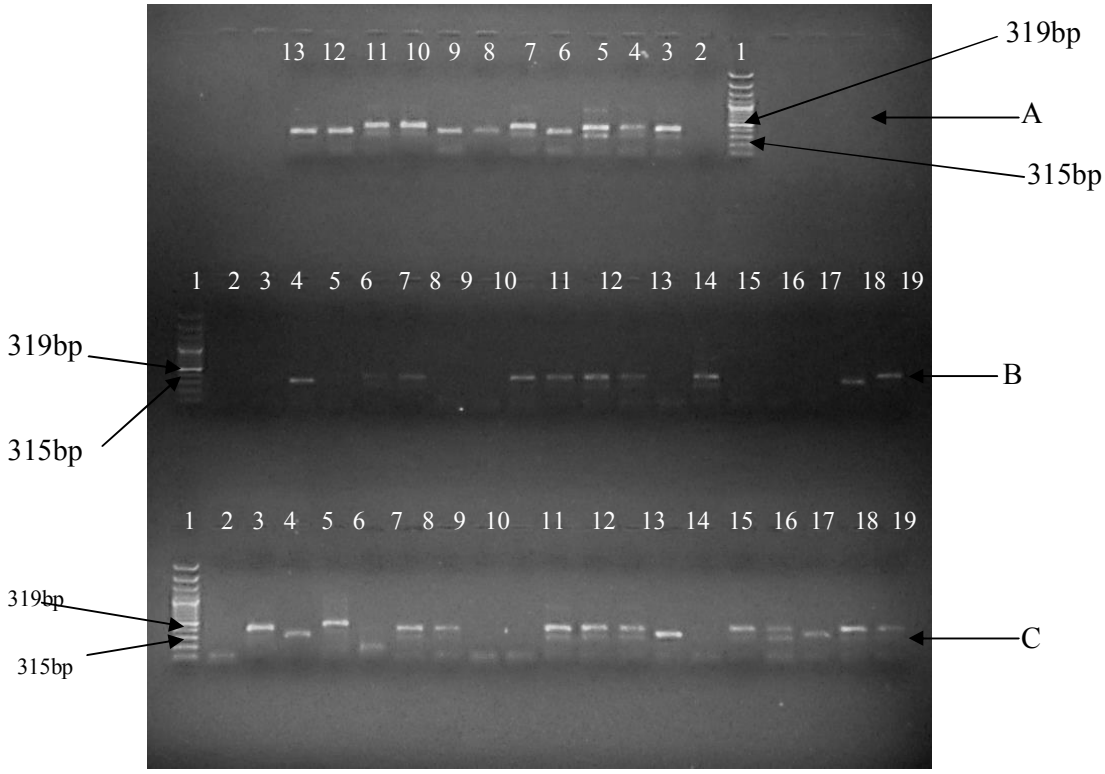


Plate I: Amplified fragment using the species- specific PCR assay for the identification of member of the *Anopheles gambiae* complex.

Lane 1 molecular marker, lane 2 negative control: lane 3 positive control *Anopheles gambiae* s.s (319bp), lane 4: positive control for *Anopheles arabiensis* (315bp),Lane 5 positive control for *Anopheles merus* and *Melas* (400bp), Lane: 6positive control for *Anopheles quadrianulatus* (415bp)

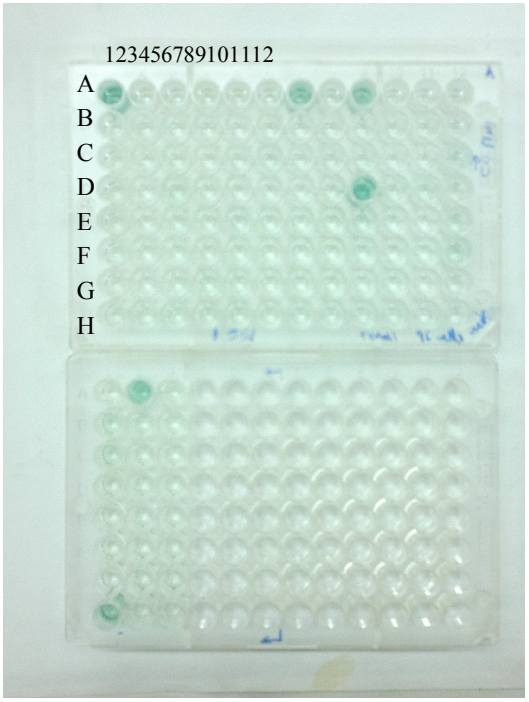


Plate II: Micro titre plate showing result of ELISA after PCR identification. The green colourindicates positive result for CS-ELISA well A1 is positive control and C1 to G1 are negative control. Sample showing dark green colours are positive test samples for CSP protein.

Table 4.6: Result of *Plasmodium falciparum* sporozoite infection rate in study area

Settlement	No. examined	No. positive	(% prevalence)
Angwan Shika	46	2	4.3%
Angwan Malami	22	1	4.5%
Water works quarters	19	1	5.1%
Total	87	4	4.6%

Table 4.7: Prevalence of *Plasmodium* CSP AntigenIn Indoor Resting Adult *Anopheles* species

Species	**No. examined**	**No. positive**	**Prevalence (%)**
An.gambiae s.s	67	3	(4.48)
An. arabiensis	5	1	(20.00)
An.funestus	5	0	(0.00)
An rhodesiensis	11	0	(0.00)
Total	**87**	**4**	**(4.50)**

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Indoor resting mosquitoes were collected in 35 houses in the 3 settlements. The result showed thatat least one *An. gambiaes s.s* was caught in each of the 24 houses during the sampling period, the percentage of houses with mosquito was in the ranges of 50-80% throughout the sample period. The mean vector population density was 5 *Anopheles* per house and estimated human biting rate of 0.79 (table 4.8).

From the 87 specimens tested for the presence of CSP antigen, 4(4.6%) were positive for*P.faciparum* sporozoite while 83(95.4%) were not infected (table4.9). Sporozoite rate was in the range of 2.0-4.0%. whereas the inoculation rate was 2.4

Table 4.8 Human Biting Rates Of *Anopheles gambiae s.l in* the Study Area

Variables	Villages			
	Shika village	**AngwanMalami**	**Water works Quarters**	**Total**
No. of houses	15	10	10	35
No. of houses harbouring *Anopheles*	12	7	5	24
% of houses with *Anopheles*	80	70	50	68.6*
No. of *Anopheles* caught	65	49	20	134
Mean No. of *An*. per house	5.4	7	4	5.5*
No. of persons in houses	55	28	26	109
No. of mosquito with blood in abdomen	46	22	19	87
Human biting rates	0.84	0.79	0.73	0.79*

Mean value *

Table 4.9: Sporozoite And Entomology Inoculation Rates Of*Anophelesgambiae*

Variables	Villages			Total
	Shika village	AngwanMalami	Water works Quarters	
Number of tenants at risk	39	28	14	81
Total number of *Anopheles*	65	49	20	134
No. fed	46	22	19	87
Female positive CSP	2	1	1	4
Sporozoite infection rate(%)	3.1	2.1	5.0	3.4
Inoculation rate	2.60	1.58	3.65	2.61*

Mean inoculation rate
104.4

CHAPTER FIVE

5.0DISCUSSION

Seven species of mosquitoes identified from the three settlements around Zaria Dam are,*Culexquinquefasciatus, Culexannulioris, Anopheles gambiae, Anopheles funestus, Anopheles rhodesiensis, Anopheles arabiensis* and *Aedes aegypti*.The dominant species were *Culexquinquefasciatus*, and *Anopheles gambiae*collected in all the three villages while the otherslike*Anopheles rhodesiensis* were infrequent. *Anopheles funestus*and*Anopheles arabiensis* are present only in AngwanMalami and Angwan Shika while*Aedesaegypti* was collected only in Angwan Shika.

In this study, female mosquitoes are higher than males and this may be associated with the fact that the females seek for blood meal for ovarian development. Unlike the results reported by Ideozu (1987) and Nendongtok (1991) where more males were collected than females because the collection was made outdoors.

Female mosquitoes with blood in the abdomen are mainly*Culexquinquefasciatus*,*Anopheles gambiae*, *Anopheles rhodesiensis,CulexannuliorisAnopheles funestus*,*Anopheles arabiensis* , but a single specie of*Aedesaegypti* had no blood meal.This maybe due to the established position that species with blood meals are strongly anthropophilic and those without blood in the abdomen had not fed by the time they were collected (Ndams, 2004).

Culex mosquitoes have the widest range of distribution and form the dominant genus in this study. Two species of *Culex*,*Culexquinquefasciatus*and *Culexannulioris* were caught. Recently, Noutcha (2014), recorded 46.5% of five Culicid species of the total mosquitoes collected and this form the dominant species obtained. Gillet (1972) described*Culexquinquefasciatus* as the commonest nuisance

mosquitoes in most parts of Africa and it is apotential vector of filariasis. Its presence in large numbers in these study areas should not be ignored because it could transmit *Wuchereriabancrofti* the causative agent of filariasis.

In this study,*Anopheles* species constitute 134(26.5%)of the total mosquitoes caught of which *Anopheles gambiae s.l.* constitute the highest out of the four *Anopheles* species collected. Out of the four *Anopheles* caught,*Anopheles funestus* and*An.gambiae* is the most important and efficient vectors of malaria (Colluzzi *et al.*,1978).

In Bayelsa state,more *An.gambiae s. l* were caught and implicated as the vector of malaria parasite (Noutcha,2014). Recent study by Umar (2014) on the characteristics and species composition of *Anopheles* in Katsina State,Nigeria, showed a preponderance of *Anopheles gambiae*over other species of mosquitoes.

The single *Aedes* specie caught may be due to the fact that it is a diurnal mosquito and has an ubiquitous mode of feeding. Also it is a container inhabiting mosquito, often breeding in unused flowerpots, spare tires, untreated swimming pool and drainage ditches, they thrive in urbanized areas. This finding is similar to those reported by others (Gadzama, 1977; Ideozu, 1987 and Nendongtok, 1991) . *Aedes aegypti* are known vector of yellow fever virus so the presence of this single specie may not pose any danger for now, but continious monitoring of this environment for *Aedes* species is advocated. However, the females of *Aedes* are known to readily fly from house to house over distance of 300m (Vincent,1984).

The results obtained from molecular characterization of *Anopheles gambiae*s.l by PCR amplification technique (Scott*et al.,*1993), yielded *Anopheles gambiae*s.s and *Anopheles arabiensis*, two siblings of the*gambiae* complex, based on their 319bp and 315bp DNA fragment lengths, respectively . The separation of the two taxa

provides the true identity of the complex and shows the distribution pattern of the *Anophelesgambiae* and *Anopheles arabiensis* collected in the study area. This information is necessary for mapping and planning control strategies for these species of mosquitoes.Unlike Nendongtok (1991) and Ideozu (1987) who reported on the distribution of *Anopheles gambiae*s.l (complex) in Zaria,this study has separated the complex into its sibling namely *Anopheles gambiae*s.s and *Anopheles arabiensis*, thus highlighting the advantage of PCR identification. These findings compare favourable with the report by Onyabe and Conn (2001), Ndams (2004) and Noutcha and Anumudu (2009).

The overall preponderance of *Anopheles gambiae*s.s was 63.5% showing that it was the most abundant in this study, which may be due toits ability to adapt to nearly all habitats. This is in line with the notion that this ability is conferred by genetic inversion polymorphism in this species of mosquito. Colluzzi *et al*.,(1987), which determines its heterogenous behaviours thatculminated in its distribution. In Sudan, out of 60 samples tested for PCR identification,52 (92.3%) were identified as *An.arabiensis,*which confirmed that *arabiensis* is the only member of the *gambiae* complex found in the study areas (Tasneem,2010).Similarly, Oduola *et al.*(2012), Ndams (2004) and Ebenezer *et al.*(2012) reported overall preponderance of *Anopheles gambiae*s.s over *Anopheles arabiensis* in their respective study areas.

With respect to site of collection, the result generally indicated that *Anopheles gambiae*s.s and*Anopheles arabiensis* were the predominant species in the population of *Anopheles* mosquitoes in Zaria dam area. Ndams (2004) however reportedthat*Anophelesarabiensis*were more than*Anopheles gambiae*s.s in Biye, HayinDogo, Sokadadi and Bomo in Zaria area, Kaduna state. This is in contrast to the present study in which*An. gambiae s.s*was more prevalent. Furthermore, the present

study was close to a dam,while that of Ndams(2004) was far away from aquatic environment. The few *An.arabiensis* found in Zaria dam area maybe associated with few animals encountered within inhabitant household. Service (1985) reported that.*Anopheles arabiensis* is both anthropophilic and zoophilic with greater tendency of zoophilism.

The ELISAassay show that *Anopheles gambiae* and *Anopheles arabiensis* were positive to *P.falciparum* CSP antigens, whereas *Anopheles funestus* and *Anopheles rhodesiensis* were negative to CSP antigen. Members of *Anopheles gambiae*s.*l* which usually have high sporozoite rates ranging from 1% to 5% but may approach 10% to 30% in extreme cases as explained by (Ndams 2004) and the two species exhibit high degree of anthropophillic and endophillic behaviours that bring them in close association with humans; thus the prevalence observed in this study corroborates with the result reported by others Noutcha and Anumudu (2009), Mbeora *et al,*(2010)and Umar(2014).*Anopheles gambiae*s.s and *Anopheles arabiensis*are the main vectors of *P.falciparum*having been found positive for *P.falciparum* CSP antigens. The present study did not incriminate *Anopheles funestus* with sporozoite CSPantigens,this may be attributed to the sample size (4/506) collected .But others Anon(2000) and Umar (2014) reported that *Anopheles funestus* was the sole transmitter of malaria in the dry season in Kenya and Nigeriarespectively.

Several factors contributes to the ability of a particular species to transmit malaria parasites, but the two most important are the mosquito's longevity and its propensity to feed on humans (Toure *et al.*,2004). Since individual mosquito must feed twice on human before transmission, the probability of the mosquitoes feeding on human twice was high, in the range 0.73-0.84 in this study.

The mean inoculation rate 104.4obtained in this study is lower than 128.7 and 131.3 reported by Noutcha and Anumudu (2009) in western Nigeria. Even at lowest EIRs, there were high rates of malaria prevalence and associated disease (Trape and Rogre, 1996).

The entomological inoculation rate (EIR) normally expresses the intensity of malaria parasite transmission and it is highly variable in Africa, with arrival EIRs ranging from >1 to <1000 infection bites person per year (Bruce, 1980). It is the most favoured measure for assessing malaria endemicity and transmission intensity. EIR assessment may be particularly useful when estimating the effect of the efforts to reduce human-vector contact (Burkottand Graves 1995).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, *Culex* species are more abundant than *Anopheles* species as indoor resting mosquitoes in Zaria dam area,while the least prevalent is *Aedes aegypti*.*Anophelesgambiaes.s*and *An. arabiensis*were positive to*P. falciparum* CSP antigen and therefore are considered as vectors of malaria in the study area .

6.2 RECOMMENDATIONS

I. Since *Culex* and*Anopheles* are abundant in the study area,mosquito bites can be prevented by the use of insecticide-treated bed nets,mosquito repellents and screening houses to prevent mosquitoes from entering.

II. *Anopheles gambiae s.s* and *Anopheles arabiensis* were positive *Plasmodium falciparum*antigen, further study to determine if they are transmitting other *plasmodium* species such as *P.ovale, P.vivax* and *P. malariae*is required

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APPENDIX

PCR - Polymerase Chain Reaction

WHO - World Health Organisation

ELISA - Enzyme Linked Immunosorbent Assay

DNA - Doxyribonucleic acid

dNTPs - Deoxynucleoside Triphosphate

NaOH - Sodium hydroxide

EIA - Enzyme Immunoassay

CFR - Case Fatality Rate

CDC - Centers for Disease Control and Prevention

DHF - Dengue Harmorrhage Fever

USEPA- United State Environmental Protection Agency

AMCA - American Mosquito Control Association

An - *Anopheles*

BT - *Bacillus thuringiensis*

ULV - Ultra low volume

DDT - Dicholorodiphenyl triethane

Km - Kilometer

PSC - Pyrethrium Spray Collections

P - Plasmodiun

µl - Micro litre

$MgCl_2$ - Magensium chloride

EDTA - Ethylenediaminetetraacetic acid

EtBr	-	Ethidium bromide
UV	-	Ultra violet
CSP	-	Circumsporozoite protein
EIR	-	Entomologic inoculation rate
HBR	-	Human biting rate
BP	-	Base pairs
No	-	Number
%	-	Percentage