DIVERSITY AND VECTORIAL SYSTEM OF ANOPHELINE MOSQUITOES (DIPTERA: CULICIDAE) IN SELECTED ECO-SETTINGS OF NASARAWA STATE, NIGERIA

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ABSTRACT

Mosquitoes are known to constitute nuisance as well as transmit disease-causing organisms (pathogens) to hosts. This study was aimed to determine species distribution, abundance, and diversity of mosquitoes in three selected eco – settings of Nasarawa State, Nigeria. *Anopheles* mosquitoes were sampled using Pyrethroid Spray Catch (PSC and Centre for Disease control light traps CDC) technique the mosquitoes were identified morphologically using keys. Knockdown resistance (Kdr) was determined following standard protocols. Meal preference was determined using Enzyme Linked Immuno Sorbent Assay (ELISA). Collected mosquitoes were classified based on physiological conditions. Molecular forms of the Anopheline species were determined following standard methods. Six (6) species of *Anopheles* mosquito vectors were encountered in all the selected eco – settings of Nasarawa State: *Anopheles gambiae s.l, An. funestus, An. nili, An. coustani, An. rufipes* and *An. pharoensis.* A total of Fifteen thousand, four hundred and seventeen (15,417) mosquitoes vector were encountered in the study areas between the months of January to December, 2017. Most of the mosquitoes encountered were Anopheline (64.09 %). Analysis revealed significant variations in the relative abundance of mosquito and distribution of the vector across the eco – settings studied. The highest number of mosquitoes was caught in the month of May 2017 (1,273; 12.88 %). *An. gambiae s.l* were the most dominant species (41.89%) encountered across the eco – settings during the two seasons followed by *An. coustani* (19.49 %). Indoors *An. gambiae s.l.* collection accounted for 68.21 %. The indoor resting density was 4.46, 3.99 and 3.65 man/hour/night, for sparse woodland, wooded grassland, and swampy grassland, respectively. Wet season accounted for 57.24 % of the vectors collected. Abdominal conditions analyses revealed 27.40 % were half gravid, 26.08 % were gravid and 25.97 % were freshly fed. The highest HBR was recorded in the month of May 2017 (18.30, 22.90, and 23.00 bites/man/hour, for sparse woodland, wooded grassland, and swampy grassland, respectively). Molecular analyses revealed S form *An. gambiae s.s* constituted 64.47 % of the collection, while 15.47 and 10.93 % were M form *An. colluzzi* and *An. arabiensis.* The sporozoite rate was 20.20 % in swampy grassland, 13.20 % in sparse woodland, and 12.80 % in wooded grassland. For the Kdr analyses, 19.02 % of the vectors were (RR) resistant, 31.66% were (RS) heterozygous susceptible and 50.51% were (SS) Susceptible in the study area. Blood meal source analyses revealed 47.47 % was from human, 30.54 % from bovine, and 21.72 % from goats for the study area. *Anopheles gambiae s.s* had 95.95 % blood meal from human, 45.07 % from bovine and 33.66 % from goats also for single blood meal source 98.56 % was from humans, 44.44% from bovine and 28.32% from goats. *Anopheles arabiensis* obtained 1.44 % of blood meal source from human, 55.56 % from bovine and 71.67 % from goats mixed blood meal source. *Anopheles arabiensis* obtained 24.30, 30.43, 33.85, 54.55 % from human/goat, human/bovine, human/bovine/goat and bovine/goats, respectively. This study revealed that *Anopheles* species were higher in terms of abundance which is very important vectors of malaria in Nigeria. These results indicated that vectors of mosquito-borne diseases are breeding in the study area, most of which are encouraged by human activities.

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LIST OF ABBREVIATIONS

AA	Amino Acid
ACT	Artemisinin-combination therapy
AMCA	American Mosquito Control Association
An.	*Anopheles*
CDC	Centre for Disease Control and Prevention
CSP	Circumsporozoite Surface Proteins
DDT	Dicholorodiphenyltriethane
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside Triphosphate
DR	Drug Resistance
DRC	Democratic Republic of Congo
ELISA	Enzyme Linked Immunosorbent Assay
FMOH	Federal Ministry of Health
IRS	Indoor Residual Spraying
ITNs	Insecticide Treated Bednets
LSM	Larval Source Management
MDR	Multidrug Resistance
P	*Plasmodiun*
PCR	Polymerase Chain Reaction
Pf	*Plasmodium falciparum*
PF	Production Function Approach
PLD	Population Level Diversity
PSC	Pyrethrum Spray Collections

Pv	*Plasmodium vivax*
RBM	Roll Back Malaria
RBP1, **2**	Reticulocyte Binding Proteins 1 and 2
RDTs	Rapid Diagnostic Tests
RELP	Restriction Fragment Length Polymorphism
SP	Sulfadoxine-pyrimethamine
USEPA	United State Environmental Protection Agency
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Malaria is among the world's most important parasitic diseases, causing approximately 438,000 deaths worldwide in 2015 (WHO, 2015). Controlling this disease is difficult due to many factors, including the emerging resistance to antimalarial drugs by the parasites, increasing resistance among some of the primary vectors, as well as a lack of knowledge about their biology and ecology. The human vector contact, particularly with *An. gambiae s.l.*, shows a remarkable stability and flexibility, producing extremely high inoculation rate in a range of geographic and seasonal ecological conditions (Adeleke *et al.*, 2010).

Malaria continues to be an ongoing problem in African countries south of the Sahara and although a lot has been achieved in the past 15 years, millions of people still remain at risk of contracting the parasite (Bhatt *et al.,* 2015) Africa provides a stable and ecologically diverse ecosystem and is home to the most efficient malaria vectors in the world (Sinka *et al.,* 2010; Wiebe *et al.,* 2017) and is likely to remain so in the face of global climate change. The major anopheline malaria vectors across sub-Saharan Africa are *Anopheles funestus s.s.* and three members of the *Anopheles gambiae* complex: *An. gambiae s.s.*, *Anopheles coluzzii* and *Anopheles arabiensis* (Gillies and Coetzee, 1987a; Sinka *et al.*, 2010). However, there are some additional species outside of these that play a role in malaria transmission within their geographic distribution, for example the *Anopheles moucheti* and *Anopheles nili* groups (Wiebe *et al.*, 2017). There are host of secondary or incidental vectors (Antonio-Nkondjio *et al.*, 2006; Tabue *et al.*, 2017). Considering that the genus *Anopheles* contains over 500 species globally, of which only a few are considered

important species for malaria transmission (Sinka *et al.*, 2010; Garros and Dujardin, 2013). The morphological identification of species is crucial in order to target scarce resources for controlling the malaria vectors only. Species groups and species complexes are common within the genus *Anopheles* (Harbach, 2004) and this complicates vector control since not all species within a complex have similar behaviours or similar roles in malaria transmission (Gillies and Coetzee, 1987b; Wiebe *et al.*, 2017). In the *An. gambiae* complex, for example, species range from the non-vectors *Anopheles quadriannulatus* and *Anopheles amharicus* to minor vectors *Anopheles melas, Anopheles merus* and *Anopheles bwambae,* to the major vectors *An.gambiae, An. coluzzii* and *An. arabiensis* (Gillies and Coetzee, 1987a; Coetzee *et al.*, 2013).

Mosquitoes of the family Culicidae are considered a nuisance and a major public health problem, because their females feeds on human blood and thus transmit extremely harmful diseases, such as malaria, yellow fever and filariasis. They are estimated to transmit diseases to more than 700 million people annually and responsible for the death of about 1 in 17 people (WHO, 2000a). Effective transmission of mosquito-borne disease requires successful contact between female mosquitoes and their hosts (Xu *et al*., 2014). Among Anophelinae, the members of the genus *Anopheles* are best known for their role in transmitting malaria and filariasis worldwide (Service, 1980; WHO, 2013). The malaria caused by *Plasmodium* parasite is one of the greatest killer diseases in the world (WHO, 2013). World Health Oorganisation (2013) reported an estimated 207 million cases of malaria in 2012 out of which 200 million cases (80.0 %) were in Africa continent. The distribution pattern, transmission and intensity of the disease are dependent on the degree of urbanization and the distance from vector breeding sites (Centre for Disease Control and

Prevention, CDC, 2015). The endemicity of malaria in any region is determined by indigenous *Anopheles* mosquitoes, abundance, feeding, restingbehavior and their *Plasmodium* infectivity, among other factors (Molta, 2000; WHO, 2003).

Federal Ministry of Health, Abuja reported that at least 50.0 % of Nigerians suffered from one form of malaria or the other making it the most significant health problem in Nigeria (Chukwuocha, 2012). The high transmission rate and prevalence of malaria is as a result of the diverse mosquitoes breeding sites, which include practically receptacle that holds water, such as tins, cans, old tyres, tree holes, cisterns, open pools, drainage, stream and pond (Ingstad *et al*., 2012). People living in poor rural areas are confronted with a multitude of barriers when assessing malaria prevention especially on the knowledge of the biology and ecology of the vectors, among others (Ingstad *et al*., 2012).

The mapping of malaria vectors is important in the control of malaria. This is because the species composition and distribution and other biological parameters of the mosquitoes are poorly known in different ecological zones of Nigeria and in most of the malaria endemic areas due to the difficulties in the morphological identification of certain complex species, theknowledge of which is required in the design of vector control programmes and in tackling the prevalence of the disease in endemic areas (Awolola *et al*., 2002).

Mosquitoes are responsible for the spread and transmission of several harmful diseases such as malaria and lymphatic filariasis. It is known to infect over 700 million people causing 1 million deaths each year especially in developing regions of the world including sub-Saharan Africa (WHO, 2016). Despite years of control efforts, malaria continues to be a major threat to public health in parts of sub-Saharan Africa, Nigeria inclusive. About 97%

of Nigeria's population is at risk of malaria where 60% of hospital outpatient visits and 30% of hospitalization among children under five years and pregnant women occur due to malaria (CDC, 2017). Entomological studies focused on the diversity, density, behavioral patterns and temporal variations of *Anopheles* species have long been found to be beneficial in the identification and monitoring of malarial vectors (Tadei *et al.*, 1998). A combination of factors that determine the capacity of a vector to transmit malaria include; abundance, anthropophily, zoophily, susceptibility to infection by the malaria parasite, infection rates and female longevity (Aniedu, 1992; Lounibos and Conne, 2000).

Vector-borne diseases remain a major public health issue in the tropical and subtropical regions of the world (WHO, 2016). Anopheline vector of malaria consists of various species with unique behaviour associated with their biting activities and transmission dynamics. Human malarial protozoa are transmitted by mosquitoes of the genus *Anopheles*. Mosquitoes of the family Culicidae are considered a nuisance and a major public health problem, because their females feeds on human blood and thus transmit extremely harmful diseases, such as malaria, yellow fever and filariasis (WHO, 2015). Malaria leads to a lot of social and economic problems, such as school absenteeism, lower agricultural production among others; consequently, more control efforts are required in order to reduce the rates of disease incidences and mortality.

The vectorial capacity of a mosquito population largely determines the intensity of vector-borne disease transmission. The vector competence is also a crucial parameter for the pathogen to be transmitted. In human malaria, vectorial systems are limited in number.
Only *Anopheles* females are able to transmit *Plasmodium* to humans, and, among the more than 450 *Anopheles* species known, 60 are considered to be actual vectors in the wild

(Manguin *et al*., 2008). Vectorial capacity and competence also present quantitative features in the sense that some species have a major role in malaria transmission, and others have a minor role. Even at the species level, some populations or individual mosquitoes can have different impacts on transmission (Manguin *et al*., 2008). Research to understand the genetic determinants of capacity and competence has greatly benefited from the availability of the whole genome sequence for *Anopheles gambiae* (Holt *et al*., 2002) with the identification of candidate genes in progress. However, the different aspects of vectorial capacity and competence have not been uniformly studied, and some have been largely overlooked.

There are 465 formally recognized species and more than 50 unnamed members of species complexes. Approximately 70 of these species have the capacity to transmit human malaria parasites and 41 species are considered to be dominant vector species complexes capable of transmitting malaria at a level of major concern to public health (Coetzee *et al.,* 2013; WHO, 2015).

The knowledge of major malaria vectors and their bionomics in Africa remains a problem. Hay *et al.* (2000) as a focal disease, malaria will therefore differ in its characteristics from place to place, since all malaria vectors do not exhibit identical behavior and ability to transmit parasites. Globally medical reports have shown that mosquito-borne diseases-are responsible for significant effect on human morbidity and mortality throughout the world (WHO, 2013). The global burden is 207 million malaria cases every year resulting into 627,000 deaths (WHO, 2013), sub-Saharan Africa being the most affected region. According to the latest WHO malaria report (WHO, 2014), there were in 2014 about 197

million malaria cases worldwide and an estimated 584 000 deaths, mostly among African children.

In Nigeria, malaria still remains a major health problem with about two-thirds of the population living in malarious areas (WHO, 2015). Among the malaria vectors in Nigeria *Anopheles gambiae* complex, *An. funestus* seems to be the major vectors of malaria transmission in the country though there are other non and minor vectors who are now incriminated to be responsible for the malaria transmission (Awolola *et al.* 2002).

1.2 Statement of the Research Problem

Vector borne diseases are among the major causes of illness and death, particularly in tropical and subtropical countries; vector control through the use of insecticide plays a key role in the prevention and control of infectious diseases. *Anopheles* mosquitoes are the major vectors responsible for malaria transmission in the tropical and subtropical regions of the world including Nigeria and Nasarawa state Mosquito control remains an important component of human and animal diseases. Currently, there is little or no empirical data on *Anopheles* mosquito vector population, dynamics in the eco-settings of Nasarawa State Malaria is characterized by it biological diversity and this diversity is conditioned mostly by the Anopheline mosquitoes that are involved in the transmission through their distribution, behaviour and vectorial capacity. The Anopheline mosquitoes responsible for malaria transmission differs in their distribution and major characteristics from different regions to another and may also differ across the selected eco-setting of Nasarawa state.

In Nigeria, mosquitoes are regarded as public health enemies because of their biting annoyance and noise nuisance, sleeplessness, allergic reaction and disease transmission due

to their bites. They transmit human diseases such as malaria, yellow fever, dengue, haemorrhagic fever, filariasis and encephalitis. Mosquito control remains an important component of human and animal diseases. However, this has been limited by the development and spread of resistance and limited knowledge of mosquito biology. Also significant changes in resistance patterns have been observed over the past 10 years in West Africa.Currently, there is little or no empirical data on *Anopheles* mosquito vector population, dynamics, species composition, vectorial capacity, malaria prevalence and distribution in Nasarawa State to warrant assessment of the mosquito and malaria situation towards prompt and effective intervention strategy. Malaria is characterized by its biological diversity. This diversity is conditioned mostly by the vector species that are involved in transmission (including their distribution, behavior and vectorial capacity). It is also conditioned, among others, by seasonality of transmission, pathogenicity of parasites species and by immune response of human hosts.

Malaria is a dreaded disease which differs in its distribution and major characteristics from different countries to another in the tropics and even within the same countries (Phillips, 2001). Nigeria has the largest burden of malaria and lymphatic filariasis in Africa yet very little is known about the distribution of *Anopheles* mosquitoes that act as vectors for both diseases, how the species interact, overlap or differ across the country Awolola *et al*., (2002)

1.3 Justification for the Study

Vector control is a major component of the national campaign against malaria in Nigeria. The global strategic plan for Roll Back Malaria recommends that by 2010, 80% of the population at risk need to be protected using effective vector control measures. *Anopheles gambiae* complex is a group of six morphologically indistinguishable yet genetically and behaviorally distinct mosquito species that vary dramatically in their importance as vectors of malaria in Africa as many as three and possibly four species may be sympatric in some regions, and at least two occur in most malaria-endemic areas. Identification of *Anopheles* malaria vectors is essential for the identification of areas in Nasarawa State that are at risk of malaria and to also help in the formulation of strategies for effective malaria vector control. Therefore, there is a need for using the Polymerase Chain Reaction assay (PCR) for clear distinct identification of the Anopheline mosquito species as this will help in adopting a specific control measures instead of targeting the assumed vectors.

Identification of *Anopheles* malaria vector species is essential for recognizing the eco-settings in Nasarawa State that are at risk of malaria as this will help in the formulation of strategies for effective malaria vector control. As not much is known of the species composition, distribution, and abundance of *Anopheles* and *Plasmodium* species in Nasarawa State. It is also not known how *Anopheles* species breed and sustain themselves within the selected eco-settings and also within the wet and dry seasons. The present study attempts to collate information on mosquitoes in terms of species distribution, abundance and the prevalence of malaria in both dry and wet seasons across the three ecological zones of Nasarawa State. It is important to establish the species composition, distribution and

abundance, vectorial capacity, ecological and behavioral differences across the selected eco-settings in Nasarawa State.

The understanding of the bionomics of *Anopheles* species responsible for malaria transmission, including correct and precise identification of the target species and its distribution will aid in deploying appropriate control measures. However, successful application of vector control measures in a given location requires the understanding of the bionomics of *Anopheles* species responsible for malaria transmission, including correct and precise identification of the target species and its distribution. When planning any vector control intervention, it is essential to assess the behavioural patterns of the local vector populations in order to select a suitable method for the vector control in that locality.The current study will identify *Anopheles* to species level so that control measures could target the specific malaria vectors instead of targeting the assumed vectors.

Therefore, monitoring of the vectors behaviour should be considered as integral component of any malaria control program. Therefore when planning any vector control intervention, it is essential to assess the behavioural patterns of the local vector populations in order to select a suitable method for the vector control in that locality.Because of the heterogeneity in behaviour, mosquitoes have different opportunities to escape from been killed or exit repellent actions of insecticides used in ITNs or IRS. For example many of the mosquitoe species shift to outdoor- or early biting, i.e. shift to zoophily or to exophily activities.Not much is known of the species composition, distribution, and abundance of *Anopheles* and *Plasmodium* species in Nasarawa State. It is also not known how *Anopheles* species breed and sustain themselves within the different ecological zones and also within the wet and dry seasons. Consequently, the present study attempts to collate information on mosquitoes in

terms of species distribution, abundance and the prevalence of malaria in both dry and wet seasons across the three ecological zones of Nasarawa State. Consequently, it is important to establish the species composition, distribution and abundance, vectorial capacity, ecological and behavioral differences and also the types of breeding sites utilized by *Anopheles* species in Nasarawa State.However, successful application of vector control measures in a given location requires the understanding of the bionomics of *Anopheles* species responsible for malaria transmission, including correct and precise identification of the target species and its distribution.Therefore, when planning any vector control intervention, it is essential to assess the behavioural patterns of the local vector populations in order to select a suitable method for the vector control in that locality.

In Nigeria, malaria still remains a major health problem with about two-thirds of the population living in malaria endemic areas (WHO, 2015). As this has continue to thrive due to the complex nature and the vectorial system of the Anopheline vectors which are the principal vectors that transmit the malaria parasites to various human host within the endemic areas in the world especially in the sub-Saharan African continent more so the complex behaviour of the vectors has contributed in sustaining malaria transmission alongside their resistance behaviour to various classes of insecticides as there is no recorded information on the seasonal abundance of anopheline mosquitoes in many regions within the, north central Nigeria. Therefore, this study was conducted to investigate the species composition and seasonal population dynamics and their possible association with rainfall and disease transmission prior to any implementation of a National malaria vector control.

1.4 Aim and Objectives of the Study

This study was aimed at assessing the diversity and vectorial system of anopheline mosquitoes (Diptera: Culicidae) in selected eco-settings of Nasarawa State, Nigeria.

The objectives of this study were to determine:

i. the diversity and abundance of *Anopheles* mosquitoes in the selected eco – settings of Nasarawa state, Nigeria
ii. the sibling composition of *Anopheles gambiae s.l* in the selected eco – settings of Nasarawa State, Nigeria
iii. the human biting and feeding habits of *An. gambiae s.l* in the selected eco-settings of Nasarawa State Nigeria
iv. the infection rate and Kdr resistance of the *Anopheles* mosquitoes in the selected eco-settings of Nasarawa State Nigeria.
v. To assess the Entomological Inoculation rates of the *Anopheles* mosquitoes in the selected eco-settings of Nasarawa State, Nigeria.

CHAPTER TWO

2.0 LITERATURE REVIEW

According to the World Health Organization, malaria is still the major cause of death in children in sub-Saharan Africa. The disease in this region takes the life of a child every 2 minutes (WHO, 2015). Recent studies also have shown that the abundance of Anopheline mosquito species is the most common entomological measurement used to determine the relationship between vectors and malaria incidence in any locality (Muturi *et al.,* 2006; Zimmerman *et al*., 2006). Changes that occur in the environment especially in climate have a great bearing on breeding habitats of different mosquito species that influences the population density of adult mosquitoes (Bashar and Tuno, 2014). Climatic factors such as rainfall affect adult mosquito abundance by drastically altering the quality and quantity of breeding habitats. To determine parasite activity levels and associated disease risk, the relationship between rainfall and mosquito abundance must be ascertained (Bashar and Tuno, 2014). A proper understanding of the relationship between despite years of control efforts, malaria continues to be a major threat to public health in parts of sub-Saharan Africa, Nigeria inclusive. About 97% of Nigeria's population is at risk of malaria where 60% of hospital outpatient visits and 30% of hospitalization among children under five years and pregnant women occur due to malaria (WHO, 2015). Entomological studies focused on the diversity, density, behavioral patterns and temporal variations of *Anopheles* species have long been found to be beneficial in the identification and monitoring of malarial vectors (Tadei *et al.,* 1998). A combination of factors that determine the capacity of a vector to transmit malaria include; abundance, anthropophily, zoophily, susceptibility to infection by the malaria parasite, infection rates and female longevity (Aniedu, 1992; Lounibos and Conne, 2000).

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2.1 Etiology of Malaria

The first evidence of malaria parasites was discovered in mosquitoes preserved in amber from the early Paleocene period approximately 30 million years ago (Poinar *et al.,* 2005). The name malaria derived from 'mal-aria' (bad air in ancient Italian) was probably first used by Leonardo Bruni in a publication in 1476 (James and Tate, 2004). The Malaria parasite discovery is credited to Alphonse Laveran who began his research at a military hospital in Algeria in the year 1879. He found black pigment in the blood as well as some entirely unknown bodies with certain characteristics which led him to believe that parasites were involved. He nonetheless was successful in carrying out investigations on fresh blood without chemical reactions or any staining process (Style *et al*., 2011). Laveran published his first great work on these parasites, *Traites des fievres palustres,* in 1884. He showed that the parasites destroy red blood corpuscles during development as well as changing the red pigment in malaria particles. He had established as far back as 1894 that the marsh

fever must undergo one phase of its development in mosquitoes. It was however, an Army Surgeon Ronald Ross, who while experimenting with less common species of mosquito larvae that were hatched in the laboratory and released to bite malarious patients that he found bodies that were in the evolutionary stage of human malaria parasite in the stomach wall of this uncommon mosquito species.

Laveran called the microscopic organism responsible for causing malaria, *Oscillaria malariae* (Cox and Sigh, 2010). Golgi observed in 1885 that all the parasites present in the blood divided almost simultaneously at regular intervals and that the division coincided with attacks of fever. He recognized that three types of malaria are caused by different protozoan organisms. Smith *et al.* (1995) stated that Marchiafara and Celli were however, the first to call this new organism *Plasmodium*.

2.2 Epidemiology of Malaria

Malaria still has a devastating impact on public health and welfare on the African continent. In Nigeria, over 30% of the population suffers from yearly malaria attacks in which 4,000 to 10,000 deaths occurs annually (WHO, 2015). In the year 2006, 35 to 80 million Nigerians were infected with malaria, out of which 1 million people died, most of them children under five years according to (WHO, 2008). The cost of malaria treatment and prevention in Nigeria has been estimated to be over $1 billion per annum (Odaibo, 2006; Olayemi and Ande, 2008a). Nigeria is the third most filarial endemic country in the world, with an estimated 22 million cases (WHO, 2004) and also the country with the highest number of yellow fever cases in Africa in 1994 (91% of cases in Africa, 85% of cases in the world), and in 1995 (CDC, 2017). The high densities of Mosquitoes in Nigeria are

estimated to transmit diseases to more than 700 million people annually and responsible for the death of about 1 in 17 people (WHO, 2000b).

The effective transmission of mosquito-borne disease requires successful contact between female mosquitoes and their hosts (Xu *et al.,* 2014). Among Anophelinae, the members of the genus *Anopheles* are best known for their role in transmitting malaria and filariasis worldwide (Service, 1963; WHO, 2013). Of these diseases, malaria caused by *Plasmodium* parasite is one of the greatest killer diseases in the world (WHO, 2013). WHO (2013) reported an estimated 207 million cases of malaria in 2012 out of which 200 million cases (80.0 %) were in Africa continent. The distribution pattern, transmission and intensity of the disease are dependent on the degree of urbanization and the distance from vector breeding sites (CDC, 2015). The endemicity of malaria in any region is determined by indigenous *Anopheles* mosquitoes, abundance, feeding, resting behavior and their *Plasmodium* infectivity among other factors (Molta, 2000; WHO, 2003). Federal Ministry of Health, Abuja reported that at least 50.0 % of Nigerians suffered from one form of malaria or the other making it the most significant health problem in Nigeria (Chukwuocha, 2012). The high transmission rate and prevalence of malaria is a result of the diverse mosquitoes breeding sites, which include practically receptacle that holds water, such as tins, cans, old tyres, tree holes, cisterns, open pools, drainage, stream and pond (Ingstad *et al.,* 2012). Part of the efforts being made is the official commemoration of April 25 every year, starting from 2008 as World Malaria Day (CDC, 2017) People living in poor rural areas are confronted with a multitude of barriers when assessing malaria prevention especially on the knowledge of the biology and ecology of the vectors, among others (Ingstad *et al.,* 2012). The mapping of malaria vectors is important in the control of

malaria. This is because the species composition and distribution and other biological parameters of the mosquitoes are poorly known in different ecological zones of Nigeria and in most of the malaria endemic areas due to the difficulties in the morphological identification of certain complex species, the knowledge of which is required in the design of vector control programmes and in tackling the prevalence of malaria infection diseases in endemic areas (Awolola *et al.*, 2002). According to the World Health Organization, malaria is still the major cause of death in children in sub-Saharan Africa. The disease in this region takes the life of a child in every 2 minutes (WHO, 2015).

Malaria has been around for thousands of years, and is still a major problem today. Despite efforts to eradicate malaria over the past 100 years, 149–274 million cases and 537,000–907,000 deaths from malaria occur in sub-Saharan Africa each year (Greenwood *et al.*, 2002). Malaria is caused by microorganisms belonging to the genus *Plasmodium*, and can infect reptiles, birds and mammals. Of more than 100 *Plasmodium* species, four of these infect humans. The transmission of the disease from one human to another involves mosquitoes of the genus *Anopheles* WHO (2011). *Anopheles gambiae* is the principal vector of malaria in sub-Saharan Africa (Gillies and Coetzee, 1987; Sinka *et al.*, 2010) where more than 90% of the World's clinical cases are recorded (Breman *et al.*, 2001). According to recent World Health Organisation's reports and statistics, malaria threatens the life and health status of about two-thirds of the world's human population; resulting in as much as 600 million clinical attacks and an estimated one million deaths annually. The disproportionately high intensity of malaria transmission in sub-Saharan Africa is due to the widespread distribution and high vectorial capacity of the primary vector, i.e., *An. gambiae* in the region (Hodges *et al.*, 2013). Studies have established this anopheline species as one of the most efficient transmitter of *Plasmodium* parasites in the world. The epidemiological

success of *An. gambiae* is largely dependent on its highly dynamic ecological behaviour White *et al.* (2011) that have evolved over a long time to take advantage of certain tropical clement weather conditions that promote mosquito proliferation and human/vector contact.

An. gambiae is widely distributed in sub-Saharan Africa, its behavior and ecological adaptability vary considerably from one locality to another, partly dictated by spatio-temporal differences in seasonal weather conditions (Olayemi *et al.*, 2011). Such temporal variations in anopheline vector behavior, in response to seasonal changes in weather conditions in an area, are responsible for enormous heterogeneity in the intensity of malaria transmission and efficacy of control measures. Studies have shown that in the rainy season, anopheline mosquitoes tend to be more endophagic, endophilic and anthropophagic, in order to avoid the hash environmental conditions outdoor Paisson *et al.,* 2004; Loaiza *et al.*, 2008; Olayemi and Ande, 2008b). Also, these mosquitoes breed more in natural larval habitats, such as temporary sunlit ground-pools, in the rainy season due to high proliferation of such sites during the period, as well as, guaranteeing faster developmental and higher survival rates (Olayemi and Ande, 2008c). The local interactions of combinations of these important entomological drivers of malaria transmission, occasioned by behavioural responses of anopheline mosquitoes to prevailing weather conditions, will go a long way in determining vectorial efficiency and hence, the patterns of malaria transmission, as well as, the efficacy of implemented vector-control measures. Anopheline mosquitoes pose less threat to human health during periods when they are compelled to be zoophilic or breed in less productive and hazardous sites ((Olayemi and Ande, 2008d). Also, residual indoor-spraying with insecticides such as pyrethroids and the use of

insecticide-treated bed nets are more effective at controlling malaria vectors, when such vectors prefer to feed and rest indoors (Roland *et al.*, 2000).

Nigeria, with its distinct tropical annual rainy and dry seasons, coupled with local heterogeneity in the intensity and distribution of climatic factors such as rainfall, relative humidity and temperature, in its different eco-geographical zones, is bound to elicit behavioural changes in anopheline mosquitoes at different times of the year, that may influence the epidemiology of malaria and efficacies of vector control measures. Though, the patterns of population dynamics of anopheline mosquitoes in almost all the geo-ecological zones of Nigeria have been elucidated (Awolola *et al.,* 2002; Oyewole *et al.,* 2005; Olayemi and Ande, 2008 d; e; White *et al.,* 2011). In Western Kenya and Southwest of Uganda, a low *P. falciparium* sporozoite infection rates of 6.30 % and 0.84 - 5.26 % for *An. gambiae* were recorded respectively (Echodu *et al.,* 2010). However, higher *P. falciparium* sporozoite infection rates for *An. gambiae* were also reported by other researchers. For instance, Okwa *et al.* (2006) reported *P. falciparium* sporozoite rates of 62.9 % in Badagry Area of Lagos. In another related study, Okwa *et al.* (2007) again also reported *P. falciparium* sporozoite rates of 50.00, 51.20, 53.30, 79.40 and 95.50 % for *An. gambiae* in Amuro odofin, Mushin, Ajeromi, Ojo and Alimosho areas of Lagos state, Nigeria respectively. In Benue state, Manyi *et al*., (2017) recorded an overall sporozoite infection rate of 54.90 %.

2.3 Global Indices of Malaria Cases

Malaria remains a major cause of morbidity and mortality in sub-Saharan Africa and represents one of the most critical public health challenges for Africa. More than two

billion people around the world, particularly people living in South America, south-eastern Asia and sub-Saharan Africa, are at risk of contracting malaria. Besides, one million deaths are recorded yearly of which, 91.00 % occur in sub-Saharan Africa (WHO, 2011; Omalu *et al.,* 2015).

Malaria is the 3rd leading cause of death for children under five years worldwide, after pneumonia and diarrhoeal disease (WHO, 2013; 2014). WHO estimates there were 655,000 malaria deaths in 2010, 91.00 % in the African Region, and 86.00 % were children under 5 years of age. WHO estimates 216 million cases of malaria occurred in 2010, 81.00 % in the African region as 3.3 billion people, or half of the world's population, in 106 countries and territories (WHO, 2014).

2.4 Malaria in Nigeria

Malaria is holoendemic in Nigeria, accounting for 25.00 % of infant mortality and 30.00 % of childhood mortality (WHO, 2016). Ninety five percent of malaria infections in Nigeria are caused by *Plasmodium falciparum* and five percent by *Plasmodium malariae.* According to Gallup and Sachs (2001) malaria transmission is however geographically specific. De Mellion (1951) also reported that malaria vectors exhibit behaviour variations in different localities. Malaria contributes to an estimated 11 % of maternal mortality (WHO, 2013; 2014). There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria. This compares with 215,000 deaths per year in Nigeria from HIV/AIDS (WHO, 2015). Malaria is a risk for 97.00 % of Nigeria's population. The remaining 3% of the population live in the malaria free highlands. Malaria is a major public

health problem in Nigeria where it accounts for more cases and deaths than any other country in the world.

2.5 Malaria Parasite in Human

Malaria is a vector born disease caused by protozoan parasites of the genus *Plasmodium*. There are four malaria parasite species in humans, namely *P. falciparum, P. vivax, P. malariae* and *P. ovale.* Parasites are transmitted from person to person by female mosquitoes of the genus *Anopheles*. Different species appear in different regions WHO (2015). The transmission can be seasonal, depending on the dynamics of the vector population. The life cycle of the parasite starts with the inoculation of the parasite into the human blood by the bite of a female *Anopheles* mosquito. Within half an hour, the sporozoites reach the liver and invade the liver cells. Within the liver cells, the trophozoites start their intracellular asexual division. At the completion of this phase, thousands of erythrocytic merozoites are released from each liver cell. The time taken for the completion of the tissue phase is variable, depending on the infecting species (5–6 days for *P. falciparum*). The merozoites invade the red blood cell (RBC), and then develop through the stages of rings, trophozoites, early- and mature schizonts; each mature schizont consists of thousands of erythrocytic merozoites (Wardrop *et al*., 2013). These merozoites are released by the lysis of the RBC and immediately invade uninfected red cells. This whole cycle of invasion - multiplication - release - invasion takes about 48 hours in *P. falciparum* infections. The contents of the infected cell that are released with the lysis of the RBC stimulate the Tumor Necrosis Factor and other cytokines, which results in the characteristic clinical manifestations of the disease. A small proportion of the merozoites undergo transformation into gametocytes. Mature gametocytes appear in the peripheral blood after a

period of 8–11 days of the primary attack in *P. falciparum* they rise in number until three weeks and decline thereafter, but circulate for several weeks. The gametocytes enter the mosquito when it bites an infected individual. The malaria parasite in the vector Human malaria is transmitted by mosquitoes of the genus *Anopheles*. Out of the 360 species there are about 45 with the ability to transmit malaria to humans. *Anopheles* are found worldwide, but the transmission of malaria occurs predominantly in tropical and subtropical zones of the world free of *Anopheles*, always means free of malaria, but not vice-versa. When, after the blood meal, the malaria parasite enters the mosquito, the gametocytes continue their development (Sporogony). The male and female gametes fuse and form into a zygote. This transforms into an ookinete which penetrates the gut wall and becomes an oocyst. The oocyst divides asexually into numerous sporozoites which reach the salivary gland of the mosquito, where they can be transmitted when the mosquito next takes a blood-meal. The sporogony in the mosquito takes about 10 – 20 days dependent on air temperature and thereafter the mosquito remains infective for 1–2 months, if it survives.

There is no sporogony at a temperature below 15 ^{o}C. Only the female mosquito takes a blood meal (male *Anopheles* feed on nectar) which is necessary for the development of eggs. Two to three days after the blood meal, which is taken during the night or at dawn, the female anopheline lays around hundred eggs (White *et al.*, 2011).

During her life of several weeks, she can therefore produce more than 1,000 eggs. The eggs are always laid on water surface, with preference for swamps or shallow water. They may also breed in water containers or tree holes. The oval eggs are one millimeter long and require about two weeks to develop into adult mosquitoes. They fly only short distances of a few kilometers. Their preferred location is close to human houses. There are behavioral

differences between mosquito species, which are important for the study of the geographical distribution of the vector (Wardrop *et al*., 2013).

The most important *Anopheles* species in Africa are members of the *An. gambiae* complex and *An. funestus*. Five species of the *An. gambiae* complex are vectors of malaria and two of them (*An. gambiae s.s* and *An.arabiensis*) are the most widely distributed throughout sub-Saharan Africa. *An. arabiensis* predominates in drier and *An. gambiae s.s* in more humid areas. Their preferred breeding sites are sunlit temporary pools or rice fields. *An. arabiensis* feeds on humans and animals while *An. gambiae* s.s feeds on humans predominantly, prefers indoor locations for biting and resting, and has a higher vectorial capacity than other species. Two salt water species of the *An. gambiae* complex (*An. melas* and *An. merus*) are found in West- and East Africa, respectively where *An. merus* feeds mainly on animals and *An. melas* bites humans or animals. Another major vector of malaria in many parts of tropical and sub-tropical Africa is *An. funestus* of the *An. funestus* group. It feeds mainly on humans and rests and bites indoors. It breeds in semi-permanent and permanent water with vegetation and swamps and is associated with all-year malaria transmission (White *et al.*, 2011).

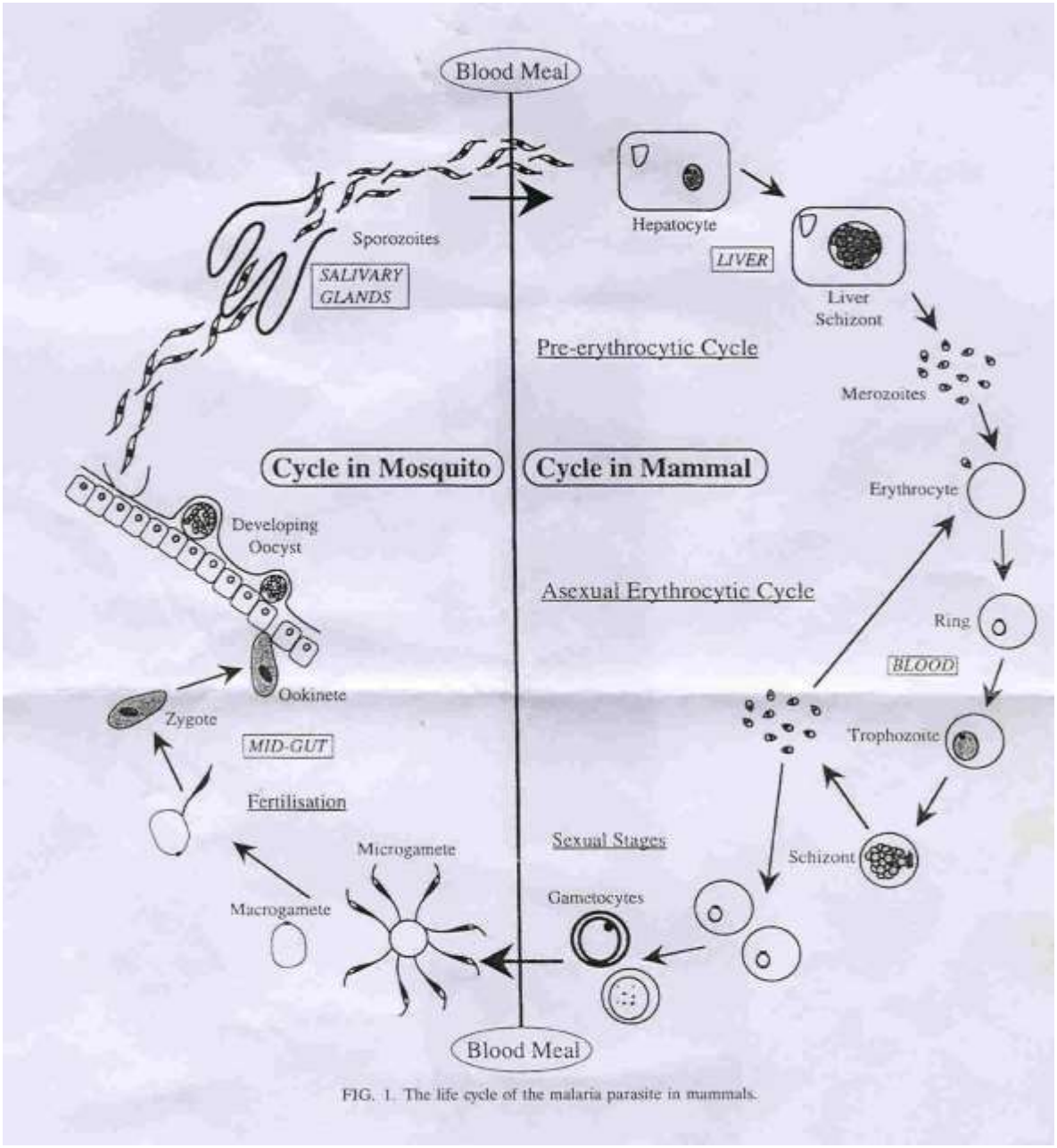

FIG. 1. The life cycle of the malaria parasite in mammals.

Figure 2.1: The life cycle of the Malaria Parasite. (Source: Phillips 2001).

2.6 Malaria Vector Ecology

Mosquitoes have through human history constituted a problem to man and animals. About 60 different genera of mosquitoes are found worldwide (Wardrop *et al*., 2013). Of these genera, members of the *Anopheles, Culex, Aedes, Hemagogus* and *Mansonia* complexes are important pests in Nigeria (Oyewole *et al.,* 2007). Mosquitoes not only inflict biting pains on man but also suck human blood and transmit disease pathogens and die soon after mating.

The female mosquito bites humans and animals because they need blood for the development of eggs. The males are short-lived, do not suck blood but nectars and plant juices, and die soon after mating. The haematophagous habit of the female mosquitoes is of public health importance. Various parasitic and viral diseases are transmitted through the biting mosquitoes. *Wuchereria bancrofti* and *Brugia malayi* which cause lymphatic Filariasis in humans (WHO, 2010) are transmitted by members of the *Aedes, Culex* and *Mansonia* complexes. Yellow fever and Dengue viruses are equally transmitted by these mosquitoes (Onyido *et al*., (2009)

The short fly range and the preferred locations for hosting and breeding are responsible for large local differences in the geographical distribution of the anopheline. The effect the environment on the malaria vector is further determined by rainfall and temperature which affect mosquito survival and the duration of the parasite life cycle in the vector (Onyido *et al*., (2009)).

2.6.1 Ambient temperature

Temperature influences the survival of the parasite during its life-cycle in the *Anopheles* vector. All species have the shortest development cycle around 27–31^{o}C which lasts from 8 to 15–21 days depending on species (Wardrop *et al*., 2013). The lower the temperature, the longer the cycle. Below 19^{o}C for P. falciparum, the parasites are unlikely to complete their cycle and hence to further propagate the disease. Temperature also modifies the vectorial capacity of the *Anopheles*. Optimal temperature values, ranging from 22^{o}C to 30^{o}C, lengthen the life-span of the mosquitoes and increase the frequency of blood meals taken by the females, to up to one meal every 48 hours. Higher temperatures also shorten the aquatic life cycle of the mosquitoes from 20 to 7 days and reduce the time between emergence and oviposition, as well as the time between successive ovipositions (Gething *et al*., 2011).

Temperature affects also the vector. In tropical climate the Anopheles eggs hatch within 2–3 days of laying, whereas for colder temperatures it can require 2–3 weeks. At minimum temperatures near the freezing point, African vector populations are effectively obliterated and at very high temperatures of above 40^{o}C, the *Anopheles* dies (Craig *et al*., 1999).

As a consequence of all the temperature requirements, malaria transmission becomes less frequent at high altitudes. Near the equator there are no *Anopheles* above 2,500 meters altitude and in the other regions there are none above 1,500 meters altitude.

2.6.2 Rainfall and humidity

Rainfall and humidity impact to a great extend the living conditions of the *Anopheles* (Thomson *et al.*, 1996). Temporal ponds, created by increasing rainfall, are responsible for ideal vector breeding conditions. However rainfall can also destroy existing breeding

places: Heavy rain can change breeding pools into streams, impede the development of mosquito eggs or larvae, or simply flush the eggs or larvae out of the pools (Ribeiro *et al.*, 1996). Conversely exceptional drought conditions can turn streams into pools. The appearance of such opportunistic mosquito breeding sites sometimes precedes epidemics. The interaction between rainfall, evaporation, runoff, and temperature modulates the ambient air humidity which in turn affects the survival and activity of *Anopheles* mosquitoes. Mosquitoes can survive if relative humidity is at least 50 or 60 percent. Higher values lengthen the life-span of the mosquitoes and enable them to infect more people. As a proxy for humidity and rainfall, the vegetation index is shown to be a successful indicator (Ribeiro *et al.*, 1996).

2.7 Distribution of *Anopheles* Mosquitoes

Mosquito distribution, abundance and the under lying causal factors vary from continent to continent. A review of available literature reveals that in South America, Dunn studied twenty-six cities, towns and villages located in various parts of the northern half of Venezuela and inspections were made to determine the extent to which the breeding of mosquito was occurring (Cuamba *et al*., 2006). Observations were also made at each place on the system of water supply and on other conditions having potential influence on the breeding and distribution of this species. The system of water supply was of such a nature as to necessitate the use of numerous containers for water storage in the houses, thus providing conditions favorable for breeding of domestic mosquitoes in the habitations. The water containers examined in the 23 towns numbered 9616 and consisted of the following vessels: 2725 tinajas, 2053 ollas, 1822 barrels, 1083 pilas, 824 filter stones, 288 tanks, 23 ornamental fountains, and 798 miscellaneous containers (Ejov, 2004). Of these vessels,

mosquito breeding was found in 2752, or 28.61 %. The positive containers included 1020 tinajas, 990 barrels, 278 ollas, 232 pilas, 95 filter stones, 70 tanks, 5 ornamental fountains, and 62 miscellaneous vessels. However in Europe, Poncon *et al*. (2008) stated that the chances of malaria re-emerging in an area depend on three factors: receptivity, infectivity and vulnerability of both vector and hosts (Ejov, 2004). Bansal and Singh (1993) determined the prevalence and distribution of anopheline mosquitoes in 12 villages located in all the 4 tehsils of arid Bikaner district, India. Six species, viz. *Anopheles subpictus* (34.70 %), *An. stephensi* (33.3 %), *An. culicifacies* (18.0 %), *An. annularis* (12.1 %), *An. pulcherrimus* (1.10 %) and *An. barbirostris* (0.8 %), were collected. *An. stephensi* was present throughout the year and the other species were present during the monsoon and post-monsoon periods. During the peak winter period (Dec-Jan) only *An. stephensi* was present and in low density. *An. culicifacies* made its appearance only during the spring season and continued up to the middle of November. *An. subpictus, An. pulcherrimus, An. barbirostris* and *An. annularis* were found only during the monsoon and post-monsoon periods. *An. subpictus* was the most abundant species during the monsoon, and so was *An. stephensi* during the spring season in indoor habitats (Cuamba *et al*., 2006).

In addition, Tyagi (2004) stated that, recently, there has been a resurgence of malaria in several parts of India, and the Thar Desert in north-western India, is currently suffering from the impact of repeated annual malaria epidemics which occur with the adoption of canal-irrigation work, especially the massive Indira Gandhi Nahar Pariyojana (IGNP). Before the advent of canalised irrigation, only *An. stephensi*, breeding mostly in household and community-based underground water reservoirs, and transmitting malaria at a low level, was prevalent in the interior of the Thar Desert (Ejov, 2004). Since the 1980s,

extensive irrigation from three different canal systems has changed the desert physiography, vector abundance, distribution and vectorial capacity, leading to the emergence of *P. falciparum*-lead malaria in the virgin levees of the Thar Desert. The change in crop pattern, retention of high surface moisture, and excessive canalisation added to mismanagement of irrigation water, have attracted several anophelines which were earlier not present e.g. *An. culicifacies*. According to Cuamba *et al*. (2006) malaria is responsible for 50 % of all outpatient attendance and around 22 % of all hospital deaths. A PCR presented a preponderance of *An. gambiae*, with indoor resting densities of between 0.9 to 23.5 per house. Of 403 *An. gambiae* identified to molecular form, 93.5 % were M-form and 6.5 % S-form. M and S were sympatric at 4 sites but no M/S hybrids were detected. *An. funestus* was found at one site near Luanda. They concluded that *An. gambiae* M-form was the most important and widespread malaria vector in the areas of study.

However, Minakawa *et al*. (2002) stated that in the basin region of Lake Victoria, there are three malaria vector species, *An. gambiae, An. arabiensis*, and *An. funestus,* but *An. arabiensis* does not inhabit highland areas in western Kenya (Gimnig *et al*., 2001; Shililu *et al*., 1998). The range and relative abundance of *An. gambiae* and *An. arabiensis were* defined by climatic factors such as annual precipitation and annual and wet season temperature. *An. gambiae* was more preponderant in moist environments, and *An. arabiensis* is more common in arid areas (Lindsay *et al*., 1998). Thus, other biotic or abiotic factors are responsible for species composition variation at micro-geographic scale. Kasili *et al*. (2009) collected day resting indoor mosquitoes and of those collected, 83 were *An. gambiae* s.l.

2.7.1 Monthly distribution of *Anopheles* mosquitoes

Mosquito populations were very high during the long rains in April to May and the short rains in November and December. Blood meal analysis of *An. gambiae* s.l. females showed 0.97 human blood index. *An. gambiae* s.l. breeds in polluted water in Nairobi and 95% of the larvae were *An. arabiensis. Anopheles arabiensis* was anthropophilic thus showing ecological flexibility within the species. Charlwood *et al*. (2000a) investigated the dry season survival of *An. funestus, An. gambiae* and *An. arabiensis* in the Kilombero valley a dry savannah zone of East Africa. *Anopheles gambiae* was found only in association with humans, in forested areas of high annual rainfall, while *An. funestus* occurred at high densities at the valley edge where large non-moving bodies of water remained. A large population of *An. arabiensis* was present along the river system throughout the middle of the valley, and mosquitoes probably derived from this population were occasionally caught in villages bordering the valley. *Anopheles funestus* was the most important dry season malaria vector in the valley, and remains in foci closely associated with groups of houses. All three species were highly abundant but as otherwise hidden refugia populations (Charlwood *et al*., 2000b).

Cano *et al*. (2006) reported from a small village in the mainland region of Equatorial Guinea, that malaria transmission varies from one country to another and that there are also local differences in time and space. A total of 1,173 anophelines were caught: 279 *An. gambiae* s.l. (217 *An. gambiae* s.s. and one *An. melas*), 777 *An. moucheti* and 117 *Anopheles carnevalei. An. moucheti* proved to be the main vector species. A significant association was found between the distance from the dwellings to the closest water point (River Ntem or secondary streams). Himeidan and Kweka (2012) reported that of the 4854

female anophelines they collected, 4847 (99.9%) were *An. arabiensis* and 7 (0.1%) *An. pharoensis.*Female *An. arabiensis* were breeding throughout the year, with 2 peak densities, during the rainy (158.4 females/room/day and 84.7 larvae/10 dips) and irrigated seasons (136.8 females/room/day and 44.8 larvae/10 dips). Shililu *et al*. (2003a), identified 13 anopheline species, with *An. gambiae* complex predominating during the first year (75.6%, n = 861) and the second year (91.9%, n = 1,262) of sampling. PCR indicated that 99% (n = 1,309) of the *An. gambiae s.l.* specimens were *An. arabiensis,* indicating that this was the only member of the *gambiae* complex present.

2.7.2 Seasonal patterns of *Anopheles* distribution

The global increase of seasonal potential malaria transmission zones causedby encroachment of seasonal zones on perennial ones and by the expansion ofseasonal malaria into areas formerly free of malaria is worrisome. Seasonal potentialmalaria transmission is most likely to foster epidemics, causing widespreaddebilitation, increased mortality, and high morbidity among unprepared or nonimmune populations (Awolola *et al.,* 2003). Malaria epidemics occurs mainly inhypo or mesoendemic areas. One of the characteristics of these of these epidemicstheir occurrence in cycles of 5-8 years; however, it is difficult to forecast a cyclicalepidemic as the cycles are far from regular. The most obvious pointers to a possibleepidemic are meteorological and environmental factors, but a reasonably goodcollection of vital statistical data may detect it at an early stage and facilitate theinitiation of appropriate measures (Awolola *et al.,* 2005).

West Africa also experiences a remarkable mosquito and malaria abundance and distribution as enumerated from literature reported by the following authors: Charlwood *et*

al. (2000b) reported that larval mosquito populations were found in rice fields, but *Anopheles gambiae s.s*. was significantly more abundant during the early stages of rice development than later in the growing season. It was also found that land left fallow after harvesting, served as highly suitable mosquito breeding sites, leading to a high population of anophelines. Although anopheline mosquitoes were also encountered in irrigation ditches, it is concluded that the rice fields were the main source of malaria vectors. Elsewhere in West Africa, Faye *et al*. (1995) carried out a longitudinal entomological study in two villages located in different ecological zones of Senegal (a sahelian area and a sudan-type savanna). In both villages, *An. gambiae* s.l. was the main vector with *An. gambiae* preponderant in the savanna area of Wassadou and *An. arabiensis* in the sahelian area of Thiaye. Malaria transmission is mainly seasonal with a man biting rate (ma) and an entomological inoculation rate (h) higher in Wassadou than in Thiaye. In the sahelian area, a high variation of *An. gambiae* s.l. density was observed because females disappear in the dry season. *An. gambiae* s.l. specific composition was observed with *An. gambiae* predominant in the rainy season and *An. arabiensis* generally more abundant in the dry season.

In addition, Pages *et al*. (2008) stated that the entomological studies conducted over the past 30 years, showed that, there was low malaria transmission in the suburb of Dakar, Senegal, but there was very few cases inside Dakar itself. However; there was some transmission based on reports of malaria among permanent residents. From May 2005 to October 2006, 4,117 and 797 *An. gambiae* s.l. were caught in Bel-air and Ouakam respectively. Three members of the complex were present: *An. arabiensis* (more than 98%), *An. melas* (less than 1%) and *An. gambiae* s.s. molecular form M (less than 1%). The

proportion of host-seeking *An. gambiae* s.l. captured indoors were 17% and 51% in Bel air and Ouakam, respectively. These data agreed with clinical data from a Senegalese military Hospital in Dakar (Hospital Principal) where most malaria cases occurred between October and December. It was the first detection of *An. melas* in Dakar. Briet (2009) reported that 13 villages in the savannah zone and 21 villages in the forest zone of Côte d'Ivoire experience a biting density of *An. gambiae* that was directly related to rice cultivation in the inland valleys in a 2km radius around each village. Biting population densities fall during wet season. In order words the onset of the rainy season was accompanied by a rise in mosquito biting density. In the forest zone, an annual population peak of *An. gambiae* was observed in the villages with one rice cropping cycle or without rice cultivation. In villages with two rice-cropping cycles, a second peak was observed during the dry (off) season cropping period. During the main season cropping period, both rice-cultivated and uncultivated inland valley surfaces have a positive correlation with the *An. gambiae* biting density, in the savannah villages of Côte d'Ivoire. In the forest zone, however, the *An. gambiae* biting population density was strongly correlated with the surface water availability in the rice-cultivated inland valleys, especially during transplanting, whereas the correlation with the surface water availability in other (uncultivated) inland valleys was weak or not significant. High density of anopheline required to sustain transmission (WHO, 2013). According to WHO (2013) areas of unstable (epidemic) malaria can beclassified into two distinct types of types of transmission, highly seasonal butintense transmission with more or less predictable pattern each year associated withexplosive epidemics at five to ten years intervals respectively, Highly seasonal with very littleor even no transmission for several years. These areas are also affected at times bydramatic and devastating

epidemic which often result from environmental ormeteorological changes at most times as that will enhance malaria transmission (WHO, 2013).

2.8 Spatio-temporal Malaria Transmission

Malaria transmission is through the bite of infected female *Anopheles* mosquito while the main vectors of malaria in Nigeria are: *An. gambiae s.s* (sensu stricto), which is predominantly in humid areas. It is therefore, present in highdensity, anthropophilic and is a very important vector of malaria. *An. arabiensis* is more dominant in the savannah ecotype. It prefers arid environment. It is also morezoophilic and exophilic, while An. melas is the salt-water species. It is generally more exophagic and zoophilic and thus a poorer vector than *An. gambiae* (Souza *et al.,* 2010).

There was a high degree of population of anophelines within zones and villages, with more than 80% of the total anophelines being collected from less than 20% of the villages and from only 10% of the houses sampled. Similarly, Shililu *et al*. (2003b) reported from Eritrea, where data revealed the presence of only one main malaria transmission period between July and October for the highlands and western lowlands. The highest inoculation rates were recorded in August and September (range = 0.29-43.6 infective bits/person/month) at all sites over the two-year period.

In addition, a group of authors represented by Sintasath *et al*. (2005), reported that a total of 12,937 individuals from 176 villages were screened for both *P. falciparum* and *P. vivax* parasite species using the OptiMal Rapid Diagnostic Test. Malaria prevalence was generally low but highly focal and variable with the proportion of parasitemia at 2.2% (range: 0.4% to 6.5%). Eventhough there was no significant difference in age or sex-

specific prevalence rates, 7% of households accounted for the positive cases and 90% of these were *P. falciparum*. Multivariate regression analyses showed that mud walls houses were positively associated with malaria infection. Marrama *et al*. (2004), conducted an entomological study, to identify malaria vectors and evaluate the transmission in the natural sub-arid ecosystem, in an irrigated area located in the sub-arid region and in rice fields located in the humid region in Madagascar. And of the 29,572 malaria vectors collected, 14,661 (49.5%) were *An. funestus,* 14,153 (47.9%) *An. gambiae* s.l. and 758 (2.6%) *An. mascarensis, An. arabiensis, An. merus* and *An. funestus* were present in all the villages while *An. gambiae* s.s and *An. mascarensis* (a mosquito native to Madagascar), were only found respectively in the two villages bordered with rice-fields and in the humid region. *Anopheles funestus, An. gambiae* s.s. and *An. mascarensis* were more frequently infected. *An. funestus* was responsible for 90% of the infectious bites (Charlwood *et al*., 2000b).

The spatial distribution of the *An. gambiae* (M and S) molecular forms and associated environmental factors, in relationship to disease prevalence, collected data showed that *An. gambiae* M and S forms were sympatric in most locations Ghana is one of the countries that share a similar weather and vegetation characteristics with Nigeria, therefore the mosquito and malaria situations of the two countries are similar. A report by Souza *et al.* (2010) showed that *An. gambiae s.s* mosquitoes are important vectors of lymphatic filariasis (LF) and malaria in Ghana. However, the S form was more preponderant in the central region, while the M form was more abundant in the northern and coastal savanna regions. Niger Republic, experiences a similar weather, climate and vegetation with the present study area, Nasarawa State. And one of the studies carried in the arid Niger Republic was that by Labbo *et al*. (2004), who reported members of *An. gambiae* complex in three zones of

Niger republic. *An. funestus* which was thought to have disappeared from Niger Republic reappeared in both the Sudan and Sahel zones. This was blamed on clearing of natural wooded savannah and enlargement of cultivated fields and that led to the modification of surface characteristics and the building of temporary ponds like dams, which enhance water run-off. The situation of mosquito and malaria species abundance, distribution and characterization in Nigeria differs as one moves across the various ecological zones, for instance, Onyabe and Conn (2001) investigated the distribution of *An. gambiae* and *An. arabiensis* across the ecological zones of Nigeria (arid savanna in the north gradually turns into humid forest in the south). They compared the study to the distribution determined from samples of indoor-resting females reported by an earlier study over 20 years ago. There was a change in the types of species present within the 10 localities in both years, but this observed change was very high in only four of the 10 localities (Onyabe and Conn, 2001). The identity of the more abundant species changed between 1997 and 1999 in only three of 10 localities. *An. arabiensis* was more preponderant in many areas in the Southern Guinea Savanna, even though it was not present there about 20 years ago. The data suggest that *An. arabiensis* has extended its range. Similarly, Godwin *et al.* (2005) stated that the ecology and distribution of various mosquito species is necessary when establishing mosquito vector abundance and related diseases prevalence. The distribution of various mosquito genera in natural and artificial habitats and their relative species abundance was studied between August 2002 and July 2003 in three foci i.e. Uromi, Ekpoma and Auchi, comprising the Esan and Etsako regions of Midwestern Nigeria. The study identified 17 vector species belonging to three genera (*Anopheles, Culex* and *Aedes*) as the vectors of four human diseases prevalent in the areas surveyed. A total of 736 mosquito larvae were encountered in artificial sources and 568 larvae were harvested from natural sources. Pools,

plastics and metal cans were the dominant artificial sources of mosquito larvae. Oyewole *et al*. (2005) collected a total of 790 *An. gambiae* (52.7%), 555 *An. arabiensis* (37%), and 155 *An. funestus* group (10.3%). The indoor catch of 807 (53.8%) predominated over the outdoors 693 (46.2%) which constituted mainly of *An. rivulorum* and *An. arabiensis*. The biting activity observed indoor was significantly higher than outdoor (p <0.05) with a ratio of 10.1: 9.60, indoor to outdoor. The implicated malaria vectors were *An. arabiensis, An. gambiae* s.s. and *An. funestus* s.s. with overall infection rates of 2.3%, 2.5% and 2.9% respectively. Elsewhere in Nigeria, Onyido *et al*., (2009) studied mosquitoes at the permanent site hostels of Nnamdi Azikiwe University, Awka. A total of 1265 mosquitoes made up of 5 mosquito species were collected as larvae. Seventy-two adult mosquitoes comprising 3 mosquito species were collected inside the university hostels. *Anopheles gambiae,* with 50 (69.45%), constituted the highest percentage of indoor biting and resting mosquitoes.

However, Okwa *et al.* (2009) stated that two of the problems of *Anopheles* control in Nigeria are the diversity of Anopheline vectors and large size of Nigeria. Consequently, anopheline distribution and transmission dynamics of malaria were compared between four ecotypes in Nigeria during the rainy season. Five species were identified out of 16,410 anophelines collected. *An. gambiae s.s* made up about 29.2% - 36.6% of the population in each zone. All five identified species vectored *P. falciparum. An. gambiae s.s* had the highest sporozoite rate. The most infected mosquitoes were found in the rain forest.

Alaba and Alaba (2010) believes that the incidence of malaria varies by weather, and that affects the ability of *Anopheles* to survive or otherwise. In addition, tropical areas including Nigeria have the best combination of adequate rainfall, temperature and humidity which

facilitate the breeding and survival of anopheline mosquitoes. The prevalence of malaria varies across different regions of the world and even within a country. This is facilitated by the variation in parasite–vector–human transmission dynamics that favour or curtail the transmission of *plasmodium* infection and the related risk of disease and death. Of the four species of *Plasmodium* that infect humans—*P. falciparum, P. vivax, P. malariae and P. ovale. Plasmodium falciparum* causes most of the severity and deaths associated with malaria, which is most prevalent in Africa south of the Sahara, where Nigeria has the largest population. And that malaria is responsible for about 50% of out-patient consultations, 15% of all hospital admissions, and the leading cause of death in the country (National Malaria Control Plan of Action 1996 to 2001). More importantly, it has become a social and economic problem, which consumed about US$3.5 million in government funding and US$2.3 million from other stakeholders spent on control efforts in 2003 (WHO, 2005). About 50% of Nigerians experience at least one episode per year. However, government figures show an average of four bouts per person every year (WHO, 2002). The situation is worsened by *plasmodium* resistance to first line anti-malarial drugs (WHO, 2000a). The 2005, malaria report on Nigeria indicated malaria has been on the rise over the recent years recording about 1.12 million at the beginning of 1990 and 2.25 million by the year 2000 and about 2.61 million cases were reported in 2003.

2.9 The Epidemiology of Malaria in Nasarawa North-Central Nigeria

Malaria in the guinea savannah fluctuates with a clear seasonal pattern (Samdi *et al.,* 2005; Federal Ministry of Health, FMOH, 2010). The relatively dry northern savanna of Nigeria demonstrates strong seasonality in Malaria transmission with a peak during the wet season. Therefore, the North has unstable hypoendemic or mesoendemic malaria (Molta, 2000).

Malaria is considered ‘seasonal’when potential transmission occurs between 1 to 7 months during the course of one year (Martin and Lefevre, 1995). Malaria prevalence in Nasarawa can be classified as mesoendemic. Because earlier works have showed that the prevalence of *Plasmodium* infection among the study communities ranged from 40.2% (80/200) in Nasarawa (Samdi *et al*., 2005) This classification is based on cumulative prevalence obtained for the population under study following the World Health Organization classification of malaria endemicity characterise the dry season while high values occur in the rainy season (Molta *et al*., 2000). Furthermore, Oguche *et al*. (2001) demonstrated this strong seasonality in astudy of the pattern of childhood cerebral malaria in the north eastern Nigeria, ninety-five (95) percent of patients presented with the infection between June andNovember with a peak in October. The seasonal pattern observed in the guinea savannah is incontrast with the wet forested areas of Nigeria where malaria transmission occurs at high levels all year round and is thus considered ‘perennial’. When malaria is potentially transmitted 8 to 12 months a year (FMOH, 2013), these areas particularly the rural settlement are usually in areas of stable malaria transmission is perennial and little affected by climatic changes and the malaria vectors in this part of Nigeria are highly infective, highly anthropophilic andhave high longevity, for instance a survey carried out in Ibeshe coastal region ofLagos state in September 1997, 1068 of the 1118 (96%) female *Anopheles* mosquitoes from the survey which were dissected were positive (Amajoh, 1997). A similar study was carried out the Sahel about the same period of the year (July-September) which corresponds with the peak malaria transmission period only 2.4% of female *Anopheles* were found to be ELISA positive (Samdi *et al*., 2006). However, vectors in the guinea and Sahel savannah incidentally have high number of infective bites per person about 60-100 infective bites per person per year in the savannah ecotype and about

30-60 infective bites per person per year in the forest ecotype. The cumulative entomological inoculation rate in the Sudan savannah reached a maximum of 145 sporozoite positive bites in 1year (out of which 132 were in the wet season) (Amajoh, 1997). Studies in the Garki district in Kano State had estimated that malaria transmission is sustainable when the human population receives about 0.33 infective bites per person i.e. transmission will be maintained as long as each person in the population is infected once every 3 years (Ekanem, 1997). In the Sudan Savanna, there are large seasonal, yearly and local variations in the level of malaria transmission. Molyneux and Gramiccia (1980) further observed during their six years malaria project in Garki, Kano State that, the cumulative prevalence of malaria was very high reaching 100% in the one to eight year age group. This could be ascribed to high levels of transmission resulting from the mosquito biting man at a very high rate, the capacity of the mosquitoes to pass malaria from one person to another was about 200- 2000 times greater than the critical value required to maintain malaria as an endemic disease (Molineaux and Gramiccia, 1980).

2.10 *Anopheles* Vectors Distribution in Different Eco-settings of Nigeria

Malaria vectors species abundance and distribution from Northern Nigeria which is characterized by harsh environmental conditions during the dry seasons with sparse grass land vegetation's in arid and savannah regions Lamidi (2009) identified *Anopheles* mosquito species in Nguru, Yobe State and determined their distribution and relative abundance during the months of the year. *Anopheles gambiae* (1145); *An. funestus* (1220) and *An. arabiensis* (827) were the major species prevalent in the town. *An. gambiae* were mostly abundant in wet months, followed by *An. funestus* at the end of the rainy season, and then *An. arabiensis* in drier months. Based on the observation of *Anopheles* monthly

distribution and supported data on malaria prevalence, the three species seem to complement one another and so sustain the endemicity of malaria in the town. The study indicated the existence of malaria vectors all year round due to the favourable environmental conditions associated with the Nigerian arid zone.

Table 1.1: Malaria Vectors of Bioclimatic Zones of Nigeria

Bioclimatic zones	Vector species	Site/localities	Authors
Northern Guinea Savanna	*An. nili, An. coustani, An. garnhami An. macullipennis, An. rufipes, An. gambiae*	An. Federe, Bassa, Foron, Jos, Vom, Bukuru	Anyawu and Iwuala, 1999
Sahel Savannah	*an. arabiensis, An. gambiae s.s, An. pharoensis, An. maculiplpis, An. rufipes, An. ziemanna, An. funestus, An. squamosus*	Koduga, Marte, Maiduguri, Damboa	Gadzama, 1983, Sara, 1990, Kalu, 1992, Molta *et al.* (2000), Samdi *et al.*(2006).
Forest	*An. gambiae s.s, An. arabiensis, An. funestus, An. rivulorum, An. hancocki*	Ibadan	Awolola *et al.* (2003)
Sudan Savannah	*An. gambiae, An. arabiensis, An. funestus, An. rivulorum*	Garki, Katsina	Molineaux & Gramiccia, 1980
Savanna Forest	*An. gambie s.s, An. arabiensis, An. hargreavesi, An. moucheti, An. nili, An. funestus*	Sapele, Benin, Auchi	Coluzzii *et al.* (1979)
Mangrove Forest/Swamp	*An. gambiae, An. arabiensis, An. funestus, An. rivulorum, An. melas, An. moucheti*	Akaka, Ilara, Ijesa-Isu	Awolola *et al.* (2002), Oyewole *et al.* (2007)

2.11 Malaria Mortality, Morbidity and Immunity

The incubation period for *P. falciparum* malaria (the time between the inoculation of the parasite and the first medical symptoms) is around 8–15 days. The main symptoms in all malaria forms are (periodic) fever outbreaks. The most severe form of malaria morbidity is cerebral malaria, which is characterized by coma with detectable parasitemia, and it is accompanied by the obstruction of capillaries in the central nervous system. Cerebral malaria is a severe complication of clinical malaria in areas with a malaria transmission of 10–20 infectious bites per year. Other major complications are severe anaemia, acute renal insufficiency or failure, hepatic or pulmonary problems, jaundice and gastrointestinal symptoms such as abdominal pain, nausea, vomiting, diarrhea or constipation (Gilles and Warrell, 1993).

Acquired immunity is developed after repeated infections. Adults can tolerate parasites without developing symptoms. Infants are protected due to maternal antibodies in the first 3–6 months of life. Until they have built their own immunity, they are vulnerable to clinical malaria episodes. Infant mortality in high endemic malaria regions is high (Kalipeni, 1993; Smith *et al.*, 2001). Pregnancy leads to suppression of immunity. High parasitemia is observed during the first pregnancy and is decreasing for further pregnancies (Brabin, 1983; McGregor, 1984; Steketee *et al.*, 2001). The malaria infection of the mother is a major reason for abortion and stillbirth and reduces the survival chances of a newborn (McCormick, 1985; Bouvier *et al.*, 1997).

2.12 Measures of Malaria Endemicity and Transmission

Malaria prevalence is the most widely available measure of endemicity. Prevalence data are obtained by community surveys of individuals who are tested for the presence of parasites

in their blood. The acquiring of partial immunity in older children and adults in endemic malaria areas leads to age-dependence of this measure. Prevalence is only an indirect measure of the amount of malaria transmission, because malaria infections may persist for varying length of time. A direct transmission measure is the incidence of the disease that is the number of new cases of malaria diagnosed per unit time and person. Incidence data can be biased when collected in health centers, because it may reflect patients' access to these centers. They also depend on accurate estimates of the population at risk (Samdi *et al.*, 2005).

The most common entomological measure of malaria transmission is the entomological inoculation rate (EIR), which is defined as the number of sporozoite positive mosquito bites per person and time unit (typically year) and is the product of the anopheline density, the human biting rate and the sporozoite index (the number of infective mosquitoes) (Macdonald, 1957; Hay *et al.*, 2000). The human biting rate can be measured by human bait catches or mosquito traps. One of the best documented studies on malaria transmission was conducted in 1971– 1973 in the Garki area of Northern Nigeria (Molineaux and Gramiccia, 1980). Using the Garki data, a mathematical model was formulated that makes 6 predictions of the age-specific prevalence of *P. falciparum* in humans as a function of the vectorial capacity (Dietz *et al.*, 1974). It can be used to link several measures of transmission (including the vectorial capacity and the entomological inoculation rate) and the malaria prevalence.

2.13 Measures of Malaria Mortality

There are basically four ways to measure mortality attributable to malaria: from clinical records, when the cause of death is identified; from observing the rise in mortality during

malaria epidemics; from observing the fall in mortality when malaria is brought under control; or by calculating the mortality necessary to maintain the observed level of the sickling gene in a balanced polymorphism (Molineaux, 1985).

Clinical records in Africa hardly ever include post-mortem series and, more seriously, introduce bias because they are only derived from tertiary-care facilities and very rarely include young children and infants. The fact that most people die outside the hospital and the limitation of paediatric beds in Africa make clear that information on death certificates are a poor measure of malaria mortality (Snow *et al.*, 2005).

Interactions between malaria and other diseases in areas of high malaria endemicity make it difficult to quantify the mortality attributable to malaria. Malaria may be a relevant risk factor for many deaths even when it is not the immediate cause (Molineaux, 1985). Moreover, low birth weight is an important risk factor for infant mortality and it is known to arise because of both prematurity and intrauterine growth retardation resulting from malaria infection of the mother during pregnancy (Steketee *et al*., 2001). Molineaux (1985) emphasized that is as important to look at the relationship of malaria endemicity with all-cause mortality as it is to look at its relationship with malaria specific deaths.

2.14 Spatial Epidemiology of Malaria

Spatial epidemiology is the study of the spatial/geographical distribution of the incidence of disease and its relationship to potential risk factors. The origins of spatial epidemiology go back to 1855 with the seminal work of Snow on cholera transmission. He mapped the cholera cases together with the locations of water source in London, and showed that contaminated water was the major cause of the disease. Spatial analysis in the nineteenth

and twentieth century was mostly employed by plotting the observed disease cases or rates (Howe, 2009). Recent methods make use of computer based cartographic methods, satellite derived data and modern statistical methods and allow an integrated approach to address both tasks; inference on the geographical distribution of a disease and its prediction at new locations.

Spatial epidemiological tools applied in malaria research can identify areas of high malaria transmission and assess potential environmental and other risk factors which can explain variation in space. Elucidating the relation between environment and malaria allows prediction of the impact environmental changes have on malaria risk, including the effect of global warming and of man-made interventions (dams, change in agriculture, urbanization, etc.). The understanding of environmental aspects of malaria is important for effective malaria interventions, which not only focus on the parasite directly, but also on the mosquito vector and its living conditions. Maps of malaria distribution provide estimates of the disease burden and assist in the evaluation of intervention programs (Samdi *et al*., 2005).

2.15 *Anopheles* Mosquito Vectors

The biology of the main African malaria vectors has been part of literature for over 50 years. The vectors have been variously described and identified as sub-species, forms, varieties, races, etc. These have been carried out in terms of morphological differences, distribution, biology, ecology, behavior among others. In West and Central Africa, five different species are considered major malaria vectors: *An. gambiae, An. arabiensis, An. funestus, An. nili* and *An. moucheti.* At least 4 or 5 other species are considered secondary

or locally important vectors, e.g. *An. paludis*, *An. hancocki*, *An. melas* among others (Dixit *et al*., 2010).

2.15.1 *Anopheles gambiae* complex

An. gambiae sensu stricto (s.s.), *An. arabiensis* and *An. melas* are *Anopheles* complexes found in West and Central Africa. *An. gambiae* is more preponderant in humid environments while *An. arabiensis* is more abundant in drier areas, but they are sympatric over a wide area. The salt-water species *An. melas* breeds in mangrove swamps along the west coast of Africa south till Namibia (Coetzee *et al*., 2000). Species are identified based on fixed paracentric inversions or on PCR based diagnostic tool, detecting species-specific sequence differences in the ribosomal-DNA intergenic spacer (rDNA-IGS) region (Scott *et al*., 1993). Furthermore, karyotype distributions in natural *An. gambiae* populations indicate strong and persistent deviations from Hardy-Weinberg equilibrium because certain heterokaryotypes are found to be deficient or even completely absent. This is why five chromosomal forms named under the non-Linnaean nomenclature exist in West Africa as Bamako, Bissau, Forest, Mopti and Savanna (Favia *et al*., 2001). Recently, analysis of the rDNA-IGS identified fixed sequence differences between sympatric and synchronous chromosomal forms of Savanna, Bamako and Mopti populations in Mali and Burkina Faso, leading to the designation of two nonpanmictic molecular forms named S and M forms. Both molecular forms are found throughout West and Central Africa (Favia *et al*., 2001).

All Mopti specimens identified so far belong to the M molecular form; however, outside Mali and Burkina Faso, the M form may exhibit chromosomal arrangements typical of the Bissau, Forest or Savanna forms. The S molecular form may also carry standard

chromosomes, indicative of the Forest form, or typical Savanna and Bamako karyotypes. Even though some very rare M/S hybrids have been found in Sierra Leone, Mali and Cameroon, evidence for reproductive isolation between molecular forms is so large that incipient speciation is being suggested (Favia *et al*., 2001) or example in South Cameroon, a population-genetic study based on microsatellite DNA markers reported high genetic difference between sympatric M and S populations, both within the standard Forest chromosomal form of *An. gambiae* (Wondji *et al*., 2002).

Insecticide resistance has been reported from almost all West African countries (Favia *et al*., 2001). Pyrethroid resistance due to *Kdr* mutation has been reported in S and M forms in every country in which this was investigated (i.e. Senegal, Sierra Leone, Burkina Faso, Mali, Côte d'Ivoire, Ghana, Benin, and Cameroon among others). One *An. arabiensis* specimen from Burkina Faso was also found to carry the resistance allele. Other resistance mechanisms (resistant AChE, esterases, oxydases, Rdl, GST) have also been reported in *An. gambiae* populations in West and Central African (Favia *et al*., 2001).

2.15.2 *Anopheles funestus* group

The *An. funestus* group consists of at least eleven species: *An. funestus* Giles, *An. vaneedeni* Gillies and Coetzee, *An. rivulorum* Leeson, *An. rivulorum*-like, *An. leesoni* Evans, *An. confusus* Evans and Leeson, *An. parensis* Gillies, *An. brucei* Service, *An. aruni* Sobti, *An. fuscivenosus* Leeson and an Asian member *An. fluviatilis* James (Favia *et al*., 2001). These species are not all sympatric. Originally, differenciating the members of the group were only possible through karyotyping (Green and Hunt 1980). Recently, however, easier PCR based assays have been developed which differentiate between members of the group. For

example the PCR assay based on species-specific single nucleotide polymorphisms (SNPs) in the internal transcribed spacer region 2 (ITS2) (Koekemoer *et al*., 2002; Cohuet *et al*., 2003).

Anopheles funestus is widespread throughout sub-Saharan West Africa. Since the 1930s this group is known as being composed of several species, which are very similar such that they can only be differentiated by very small morphological characters at larval or adult stages (Gillies and De Meillon, 1968; Koekemoer *et al*., 2002; Coetzee and Fontenille, 2004). *An. funestus*, *An. leesoni*, *An. rivulorum* and *An. brucei* are found in West and Central Africa. Their biology and vectorial capacity are very different. With the exception of *An. funestus*, these species feed on other animals rather than man therefore they are mostly not malaria vectors. In 2003, Cohuet *et al*. (2003) described a new taxon closely related to *An. rivulorum*, based on biological, morphological and genetic characteristics. This taxon, provisionally called *An. rivulorum*-like, has been reported from Burkina Faso andCameroon; it differs from the South African *An. rivulorum*, and does not seem to play any role in malaria transmission.

Anopheles funestus itself is highly polymorphic, both biologically and genetically, having at least 11 paracentric chromosomal inversions on chromosomes 2 and 3. In Burkina Faso, *An. funestus* exhibit a huge Hardy-Weinberg disequilibrium and linkage disequilibrium between inversions which led Costantini *et al*. (1996) to describe two chromosomal forms called Kiribina and Folonzo, based on the presence and association of paracentric inversions. In Senegal, 3 chromosomal populations exhibiting different anthropophilic activities were recognized, and are sometime found in sympatry. In Cameroon, a cline of inversion frequencies was reported from the humid forest in the South (with Folonzo-like

inverted populations) to the dry savannas in the North (with Kiribina standard populations), with both forms displaying strong heterozygote deficiency when sympatric. All these data suggest restricted gene flow between chromosomal forms of *An. funestus*. However, several observations from Cameroon (and East Africa) did not report any evidence of sympatry between Folonzo and Kiribina, and the reported heterokaryotypes were actually the expected frequencies within populations. Use of microsatellite markers in Senegal and Cameroon revealed that gene flow is permitted between chromosomal forms, and indicated isolation due to geographic distance between populations. These results strongly suggest that heterozygote deficits at chromosomal loci are mostly locus-specific and occur because of environmental selection on the inversions themselves (or the genes they contain) (Cohuet *et al*., 2003). No pyrethroid resistance has been reported in West-African populations of *An. funestus,* in contrast to findings in Mozambique and South Africa, and this seriously complicates vector control.

2.16 Molecular Identification of Sibling Species

Molecular identification technique for *Anopheles funestus* sibling species and *Anopheles gambiae* complex has been developed and has made it possible for molecular differentiation among the complex groups. This reliable species identification method of the complex groups has increased the precision which lead to effective control method selection and implementation for sibling species (Koekemoer *et al*., 2002). The molecular identification of *Anopheles gambiae* sibling species techniques utilizes oligonucleotides primers to differentiate five members of the sibling species of *An. gambiae*. This techniques uses species specific DNA sequences region, and the primers which consist of one universal primer that is complimentary to all five species and four species specific primers

for *An. arabiensis* Patton, 1905, *An. gambiae* Giles, 1902, *An. quadriannulatus* Theobald, 1911, *An. melas* Theobald, 1903 and *An. merus* Dönitz, 1902.The universal primer binds with one of the species specific primers to produce a DNA fragment of unique length for specific species (Scott *et al*., 1993). Molecular identification methods for *An. funestus* complexes have mainly used the rDNA locus because it is represented in multiple copies throughout the genome of mosquitoes, and it contains highly variable regions (Paskewitz *et al*., 1997). One transcription unit consists of three coding regions, 18S, 28S, and a small 5.8S gene, which are separated by noncoding regions called the Internal Transcribed Spacer regions 1 and 2 (ITS1 and ITS2) (Koekemoer *et al.,* 2002). The transcription units are separated by intergenic spacer regions (IGSs). The ITS regions shows relatively high levels of intraspecies variation but not as high as the IGS region. Variation found in these regions makes it possible to design species-specific diagnostic assays (Koekemoer *et al*., 2002).

2.17 Larval Habitats of Mosquitoes

Habitat and climate determine which mosquito species will be present in an area. Larval requirements can be quite specific and vary alot. Mosquito larvae can be found in numerous habitats. Each habitat produces and shows a seasonal progression of mosquito species. There are about four different types of mosquito habitats, e.g. Running Water, Transient Water, Permanent Water and Container habitats (Coetzee and Fontenille, 2004).

2.17.1 Running water

Mosquito larvae spend a lot of energy in order to avoid being flushed out of streams when the quantity of water increases tremendously. The tropical genus *Chagasia* and some *Anopheles* species breed in streams. Even though *An. quadrimaculatus, Culex territans*,

and Uranotaenia sapphirina breed in streams, they prefer other habitats. Larvae attached themselves to the vegetation along the banks of the streams so as to avaoid beng swept away by the water current (Coetzee and Fontenille, 2004).

2.17.2 Transient water

Aedes and *Psorophora* utilizes transient water sources, such as flooded areas, snowpools and ditches as breeding sites because their eggs cannot withstand desiccation. Their life cycles require alternating periods of wet and dry. Opportunistic species like *Culex* are capable of breeding even during an extended period of flood. Transient water bodies undergo water quality changes which results in various mosquito species using the same pool over a period of time (Charlwood *et al*., 1997).

2.17.3 Permanent water

These waters (also known as Semi-permanent) are present for long periods of time and support characteristic aquatic vegetation such as Cattail, rushes and sedges. *Anopheles, Culex, Culiseta, Coquillettidia,* and *Uranotaenia* all breed in permanent water to protect their eggs from dessication. *Aedes* adults lay eggs near the edge of the swamp or within tussocks of vegetation and requiring flooding to inundate the eggs for hatching. The species present, vegetation and water quality changes with the seasons (Charlwood *et al*., 1997).

2.17.4 Water containers

Container water habitats are located in natural settings, such as water held by plants (bromeliads) and artificial settings, such as water found in tyres. The container habitats are based on the containers themselves. Treehole sites generally have tannin-enriched water

which is characteristically clear, with rotting wood at the bottom. Many treehole species also utilize artificial containers, such as tyres, because they protect against harsh weather and are more common. Artificial containers are a convenient medium for transporting a species of mosquito to a place outside of its natural habitat (Manguin *et al*., 2008).

2.18 Susceptibility to become a Vector of Disease

Some species of *Anopheles* are poor vectors of malaria, as the parasites do not develop well (or at all) within them. Laboratory experiments have been able to select strains of *An. gambiae* that are refractory to infection by *Plasmodium* parasites. The immune systems of the refractory strains are capable of killing the malaria parasites after they have invaded the mosquito's stomach wall. The genetic mechanism for this response is currently being studied. It is hoped that one day, malaria may be contained or even be eliminated by genetically modified mosquitoes that are refractory to malaria by replacing those wild mosquitoes that are not resistant to the *Plasmodium* parasite (Holt *et al*., 2002).

2.19 Preferred Blood Meal Source

One important behavioral factor is the degree to which an *Anopheles* species prefers to feed on humans (anthropophily) or animals such as cattle (zoophily). Anthropophilic *Anopheles* is more likely to transmit the malaria parasites from one person to another. Most *Anopheles* mosquitoes are not exclusively anthropophilic nor zoophilic (Charlwood *et al*., 1997). 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. Once ingested by a mosquito, *Plasmodium* parasites must undergo development within the mosquito before they become infectious to man. The extrinsic incubation period ranges

from 10 – 21 days, depending on the parasite species and the temperature. If a female mosquito does not survive longer than the extrinsic incubation period, then she will not be able to transmit any *Plasmodium* parasites. It is difficult to directly determine the life span of mosquitoes in nature. The daily survivorship of *An. gambiae* in Tanzania ranged from 0.77 to 0.84 per day, meaning that at the end of one day between 77% and 84% will have survived (Charlwood *et al*., 1997). Assuming this survivorship is constant through the adult life of a mosquito, only about 10% of female *An. gambiae* would survive longer than a 14-day extrinsic incubation period. If daily survivorship increased to 0.9, over 20% of mosquitoes would survive longer than a 14-day extrinsic incubation period. Indoor residual spraying may affect malaria transmission more through their effect on adult longevity than through their effect on the population of adult mosquitoes (Charlwood *et al*., 1997).

2.20 Patterns of Feeding and Resting Behaviour

Some *Anopheles* mosquitoes are active at dusk or dawn while some are nocturnal (active only at night). Some *Anopheles* mosquitoes feed indoors (endophagic) while others are exophagic. After feeding, some mosquitoes prefer to rest indoors (endophilic) while others prefer to rest outdoors (exophilic), but this differs with region, local vector ecotype, vector chromosomal makeup, as well as housing type and local microclimatic conditions. Insecticides Treated Nets (ITNs) and improved housing construction which prevents against mosquito entry (e.g. window screens) can reduce biting by nocturnal, endophagic *Anopheles* mosquitoes. Endophilic mosquitoes can be easily controlled by indoor spraying with residual insecticides. In contrast, exophagic and exophilic vectors are best controlled through destruction of mosquito breeding sites (Charlwood *et al*., 1997).

2.21 Vectorial Capacity and Competence in Malaria Transmission

The vectorial capacity of a mosquito population largely determines the intensity of vector-borne disease transmission. The vector competence is also a crucial parameter for the pathogen to be transmitted. In human malaria, vectorial systems are limited in number.

Only *Anopheles* females are able to transmit *Plasmodium* to humans, and, among the more than 450 *Anopheles* species known, 60 are considered to be actual vectors in the wild (Manguin *et al*., 2008). Vectorial capacity and competence also present quantitative features in the sense that some species have a major role in malaria transmission, and others have a minor role. Even at the species level, some populations or individual mosquitoes can have different impacts on transmission (Manguin *et al*., 2008). Research to understand the genetic determinants of capacity and competence has greatly benefited from the availability of the whole genome sequence for *Anopheles gambiae* (Holt *et al*., 2002) with the identification of candidate genes in progress. However, the different aspects of vectorial capacity and competence have not been uniformly studied, and some have been largely overlooked.

For example, rapid progression has been recently made in mosquito immunity and olfaction genetics (Hallem *et al*., 2006; Lu *et al*., 2007). However, genetic determinants of parasite virulence and mosquito adaptation to human environments remain minor research areas. Moreover, evolutionary pressures on vectors, including the forces exerted by the parasites they transmit, can have major consequences on malaria transmission and are rarely considered for their impact on malaria control measures. Here, we address the major aspects of vectorial capacity and competence and the evolutionary forces that affect them in *Anopheles*: the vector longevity; the duration of sporogonic development; the contact

between the mosquito and vertebrate host suitable for the parasite; and the susceptibility/resistance of the vector to the parasite (Lu *et al*., 2007).

2.22 Vector Longevity

Malaria parasite development in vector mosquitoes requires passing through two epithelia and results in thousands of parasites (Vaughan, 2007). The sporogonic development might therefore impose some degree of virulence and affect the fitness of the vector host. The infection-induced fitness cost can be expressed as a reduction of survival or fecundity (Ferguson *et al.,* 2006) but an effect on survival would have a much higher impact on malaria transmission as the vector must live long enough to become infectious. Several mechanisms of *Plasmodium* virulence to mosquito vectors can be expected. Some were tested but mainly in experimental *Plasmodium–Anopheles* systems. The results on reduction of longevity are conflicting with many studies showing vector survival to be unaffected by infection, but some showing the opposite (Ferguson and Read, 2002a).

Potential mechanisms of virulence are:

i. The passage of parasites through the mosquito epithelia that can cause cell damage. It was observed that *Plasmodium* sp. causes host cell damage, followed by apoptosis (Hurd and Carter, 2004; Vlachou *et al.,* 2004); however, the effect of infection-induced apoptosis on mosquito survival remains unclear. Programmed cell death was observed in the midgut cells but also in the follicular epithelial cells in the ovaries, resulting in reduced egg production. The cell damage could therefore impact fecundity rather than survival (Hurd *et al.,* 2005b).

ii. The infection generates a mosquito immune response that can be costly. Parasite invasion of mosquito vectors in model and natural systems induces a massive immune response (Dong *et al.,* 2006; Mendes *et al.,* 2008) and several studies in insects demonstrate that such an induced response can affect fecundity (Schwartz and Koella, 2004) or longevity (Armitage *et al.,* 2003). In *Anopheles*, a reproductive cost was found (Ahmed and Hurd, 2006) and *An. gambiae* mosquito strains selected for refractoriness to rodent parasites show reduced fitness compared with susceptible strains (Hurd *et al.,* 2005; Voordouw *et al.,* 2009). This suggests that developing an immune response induces a fitness cost, but more research is needed to establish whether there is an effect on mosquito survival.

iii. Competition for energy resources between the host and the developing parasites can have a negative impact on the host. In agreement with this hypothesis, a reduced survival was observed in *Anopheles stephensi* infected with rodent parasites and was most striking under the condition of glucose deprivation (Lambrechts *et al.,* 2006). Moreover, infection increases mosquito sugar feeding (Rivero and Ferguson, 2003).

iv. Another potential cost of infection is that the parasite can affect mosquito behaviour and cause mortality. In the natural *An. gambiae–P. falciparum* system, a higher feeding associated mortality was observed, probably as a result of a decreased efficiency in bloodmeal intake, and increased feeding activity (Anderson *et al.,* 2000).

This is, in fact, the only effect of infection on mosquito survival to be observed in this natural system. It could be the result of compensatory behavior by the mosquito to obtain sufficient blood for its trophogonic cycle, or a manipulation of the behavior of the mosquito by the parasite to increase its transmission by multiplying mosquito blood feeds (Koella, 1999). Exploiting compensatory behavior can be considered a particularly well-developed strategy of manipulation by the parasite (Lefevre *et al.*, 2008).

Therefore, in spite of the potentially high virulence of *Plasmodium* to mosquito vectors, an effect on vector longevity is not always observed. The reduction of fecundity is a more common cost of infection and has only a minor effect on transmission. It is likely that evolutionary forces selected parasites affecting mosquito fecundity rather than that affecting mosquito survival, and this eventually benefited parasite transmission (Hurd *et al.*, 2005a). Moreover, meta-analysis of several published studies demonstrated that infection induced mortality is less likely to be found in natural systems than in experimental vector–parasite species combinations (Ferguson and Read, 2002b), which could be explained by evolutionary forces acting between coevolved parasites and hosts. As both vector and parasite share the interest of vector survival, parasites might evolve towards low virulence, whereas, in parallel, mosquitoes less affected by infection would be selectively advantaged. For this to be true, virulence in *Plasmodium–Anopheles* interactions must have a genetic basis; this has been shown in some experiments, with both parasite and mosquito genetics affecting infection success (Ferguson and Read, 2002a; Ferguson *et al.*, 2003b). However, the evolution of virulence is also highly dependent on environmental factors (Ferguson *et al.*, 2003a).

The fact that *P. falciparum and An. gambiae* share an evolutionary history, by contrast to laboratory model systems, might have led to the selection of mechanisms in both the parasite and the mosquito to reduce any negative impact on host survival and subsequently increase the chances of parasite transmission.

Recent studies emphasize the concept of tolerance that might play a role in host defense against the parasite in parallel to resistance. Tolerance is expected to be costly and could also impact survival or fecundity as the host spends resources to repair any damage the infection has caused. But, unlike resistance, tolerance to the effects of disease-induced mortality has a positive effect on parasite development (Best *et al.*, 2008; Read *et al.*, 2008) and could be an important mechanism in *Plasmodium–Anopheles* interactions. This opens a new avenue of research in the malaria vectorial system.

Today, there is a crucial need to decipher parasite effects on mosquito fitness, the mechanisms involved and their genetic and environmental determinants to predict how parasite virulence evolves. This is particularly relevant for the prospect of using genetically modified mosquitoes (GMMs) to control malaria transmission. A recent study showed that the cost of genetic transformation could be compensated for by the ability to resist infection in a laboratory model (Marrelli *et al.*, 2007). The potential success of the GMM strategy in the wild will be highly dependent on the balance between this cost and benefit to the mosquito, an area closely linked to the prevalence and virulence of infection. Moreover, vector longevity is likely to become a major target to control malaria transmission, for which we need a better understanding of the interactions between vectors, pathogens and insecticides in natural conditions.

2.23 Insecticide Resistance

Indoor spraying with insecticides and ITNs are the methods to eliminate mosquitoe bites indoors. However, prolonged exposure to an insecticide led to resistance. Resistance of mosquitoes to some insecticides has been discovered within just a few years after the insecticides were introduced. There are over 125 mosquito species that have demonstrated resistance to one or more insecticides, thus frustrating the Global Malaria Eradication Campaigns. Appropriate utilization of insecticides during mosquito control can significantly stabilize the development and spread of resistance. However, this is continuously frustrated by the inappropriate use of insecticides in agriculture which have for long been a contributor to resistance in mosquito populations. Consequently, all control measures should include an initial search for resistance against insecticides and drugs by mosquitoes and *Plasmodium* (Hougard *et al*., 2003).

2.23.1 Vector longevity, insecticide resistance and malaria transmission control

Insecticides reduce mosquito longevity, the most important parameter of vectorial capacity. However, insecticide resistance limits the efficacy of vector–control measures and can interact with the parasite (Ayala and Coluzzi, 2005).

2.23.2 New strategies could limit the emergence of resistance

The use of insecticides for agriculture or public health results in a strong selection pressure (Chouaibou *et al*., 2008). In malaria vectors, multiple resistance mechanisms appeared independently and/or were able to spread in spite of strong gene flow barriers. For instance, several mutation events were selected independently in *An. gambiae* (Weill *et al*., 2004),

and through introgression were passed to other members of the Gambiae complex (Diabate *et al*., 2004).

2.23.3 Effect of insecticide resistance on infection cost

Genetic insecticide resistance was shown to impact the infection level in the invertebrate host. For instance, several studies showed that insecticide resistant *Culex* were more heavily infected by Wolbachia and suffered higher infection costs (Duron *et al*., 2006). By contrast, in the case of filarial infection, insecticide resistant mosquitoes were less infected than susceptible ones. Insecticide resistance might affect parasite transmission in mosquitoes by changing potential redox reactions in several tissues and in doing so it was proposed that it could provide direct protection against infection (McCarroll and Hemingway, 2002). To our knowledge, no studies have been published on the relationship between insecticide resistance in *Anopheles* and infection level/cost of *Plasmodium*, although they would be highly relevant to malaria control (Lu *et al*., 2007).

2.24 Human–Mosquito contact and Human Biting Rate

The density of vectors in contact with humans and the vertebrate host preference for mosquito bloodmeals are closely related. The anthropophilic behaviour of *An. gambiae* is an important factor in its high vectorial capacity. The hypothesis developed by Coluzzi explains the enhanced contact between humans and *An. gambiae* some thousands of years ago and the subsequent drastic changes in vectorial capacity (Ayala and Coluzzi, 2005). An extensive penetration of forests began 3000 years ago by Bantu populations, which have established agriculture through deforestation (Willis *et al*., 2004). The *An. gambiae* ancestors, previously not able to survive in forests, could then find suitable sunny breeding sites and invade this new ecological niche. In parallel, a strong selection pressure against

cattle, owing to trypanosomiasis, had the consequence that humans were the most frequent large vertebrate hosts available in such areas (Ayala and Coluzzi, 2005). By providing the breeding sites and the bloodmeal to the newly arrived *Anopheles*, humans served as 'board and lodging', and selected the highly specialized species, *An. gambiae,* whose biology became very dependent on humans. This specialization to humans has been differentially selected in the members of the *An. gambiae* complex (Ayala and Coluzzi, 2005). The adaptation of these different species to diverse environments and their related trophic behaviour was accompanied by the fixation of different chromosomal arrangements, which are known to protect coadapted alleles from recombination (Coluzzi, 1982). The association between chromosomal inversions and host preference provides evidence of a genetic basis for trophic behaviour (Lacroix *et al*., 2005) and makes it susceptible to selective forces. The rapid adaptation of *An. gambiae s.s.* to humans and the specialization of the members of the complex to diverse environments is a clear illustration of their genetic diversity and plasticity.

The adaptation of *An. gambiae* ancestors to humans was accompanied by a dramatic increase in *P. falciparum* transmission. The traditional view of the story highlights the benefit of vector adaptation to its vertebrate host, but it could also be as a result of selection pressures exerted by the parasite to increase its transmission that might have strengthened the specialization. Several experiments revealed the ability of *Plasmodium* to modify the trophic behaviour of the mosquito host. Vectors show a preference for biting gametocyte-infected human hosts (Lacroix *et al*., 2005) and pregnant women (who are generally more heavily infected) (Ansell *et al*., 2005), and infected vectors are more aggressive (Wekesa *et al*., 1992; Koella *et al*., 1998). One might think that the proportion of infected mosquitoes

in nature would not allow strong selection pressure to be exerted by the parasite on mosquito behaviour. However, considering the daily mortality of *An. gambiae* (estimated at 10–18% (Costantini *et al*., 1996) and the long sporogonic development (10–14 days), the 5% sporozoite infection rate frequently observed (Bass *et al*., 2008) means that a large proportion of *An. gambiae* are in contact with the parasite during their lifespan, and suggests that trophic behavior might be under selective pressures to increase parasite transmission (Prugnolle *et al*., 2009). Genetic determinants of adaptation to human environments and trophic behaviour of malaria vectors remain almost unknown. Current investigation is based on the assumption that olfaction has a crucial role in behaviour, at least in mosquito host choice for a particular bloodmeal. Recent descriptions of cellular and molecular olfactory components (Hallem *et al*., 2006; Lu *et al*., 2007) open promising avenues for discovering how mosquitoes choose their vertebrate host for a bloodmeal and therefore the potential to modify its trophic behaviour to limit malaria transmission.

2.25 Mosquito Vector Control

With more than 120 years after the discovery of *Plasmodium* by Laveran, malaria remains one of the major public-health problems in Africa south of the Sahara. Between 1955 and 1968 the malaria control effort was to achieve global eradication of malaria through Indoor Residual Spraying (IRS) of every house with residual insecticides (DDT, DLN, HCH, various organophosphates). This programme did not involve Africa south of the Sahara, which remained in the pre-eradication stage, because of lack of funds, technical and operational issues among others. The afore-mentioned programme was in 1969 transformed to malaria control with 4 technical variants dealing solely with diagnosis and treatment. The 1992 WHO Global Strategy recommended not only case management but also selective and

sustainable vector control for malaria prevention. Insecticide-impregnated mosquito bednets (ITNs) and other materials, and IRS, which are still effective and widely used in several countries, mainly in Southern Africa, (Mabaso *et al*., 2004) and Burundi, were subsequently used for malaria control. This approach was able to stop the 1987 malaria outbreak in Madagascar and in KwaZulu Natal, South Africa. The 2000 African Summit on Roll Back Malaria held in Abuja, agreed to deploy appropriate and sustainable measures to strengthen the health systems. The summit agreed that 60% of malaria risk should be eliminated by the year 2005 especially in children less than five years of age and pregnant women through personal and community protective activities like the use of ITNs and other cheaply available measures to prevent infection and suffering.

In West-African countries *Anopheles* are controlled through the large scale use of ITNs and other impregnated materials, because of they are efficient in the reduction of incidence of malaria (Lengeler, 1998), and overall infant mortality in countries like Ghana (D'Alessandro *et al*., 1995), Kenya (Nevill *et al*., 1996), Burkina Faso (Habluetzel *et al*., 1997) and The Gambia (D'Alessandro *et al*., 1995). Moreover, trials have indicated a profound effect of permethrin-impregnated nets in Ghana (D'Alessandro *et al*., 1995), Kenya (Howard *et al*., 2000) and with impregnated curtains in Burkina Faso (Diallo *et al*., 2004), and there was no subsequent rebound mortality even after several years of ITN usage (Hawley *et al*., 2003; Maxwell *et al*., 2003). When 60 to 80% of the population is covered even people not covered by ITNs can be protected from malaria if they live inside treated compounds or around a 300m radius. These encouraging findings of efficacy and effectiveness facilitated the acceptance of ITNs for malaria control. Unfortunately, according to WHO (2005), only 15% of children under 5 years sleep under ordinary

mosquito nets in the 28 countries surveyed. In The Gambia and Sao Tomé and Principe, only a little over 10% of their population sleeps under mosquito nets, eventhough there was a large increase in the availability of mosquito nets in these countries within the last 10 years. However, more and more are using mosquito nets in African countries e.g. Burkina Faso, Ghana and Mali. In North Cameroon, mobile teams go directly to villages to treat the mosquito nets with insecticides and thus dramatically increasing the percentage of ITNs available and therefore their actual efficacy (Curtis *et al*., 2003). The prices of ITNs can be subsidized through: social marketing, low prices, tax-exemptions, offering ITNs gifts to pregnant women during antenatal clinic visit and all vaccination campaigns and to all staff of all companies and their families, and then establish centres for impregnating mosquito nets and deploy mobile teams to re-treat ITNs free of charge. These various methods need to be adapted and tailored towards the targeted population in terms of price, size, shape, colour of the nets and cultural behaviour of the people concerned (Curtis *et al*., 2003).

The main problems against large-scale use of ITNs before now are: human resistance to ITNs usage, high cost of nets, non-regular reimpregnation, non-availability and poor distribution of nets. However, recently, prices are falling and promotion, distribution and affordability of ITNs are on the increase as a result of social marketing programmes put in place. In addition, ITNs are now manufactured in African countries and are more acceptable in terms of quality, size, shape, colour, opacity, among others. It is hoped that in the future the ITNs will be distributed free (Curtis *et al*., 2003) for example during all vaccination programmes or as a kit for pregnant women (Guyatt *et al*., 2002) through local health systems and NGOs. A solution to the retreatment issue is the development of long-lasting nets‘ (LLNs) (Guillet *et al*., 2001), wash-resistant nets such as the Olyset Net (with

permethrin incorporated into the polyethylene fibre) or Permanet 2 (with deltamethrin imbibed onto the polyester fibre) which gives long lasting efficacy to the nets (N'Guessan *et al*., 2001). Another problem in the efficacy of ITNs is pyrethroid resistance by *An. gambiae* in West-African countries (Chandre *et al*., 1999), because of large-scale use of insecticides for agricultural purposes. Resistance to carbamates, organochlorines and organophosphates has been reported in *An. gambiae* populations in West and Central Africa. However, trials of ITNs in experimental huts against pyrethroid resistant *An. gambiae* indicated protection to users through a reduction of entry rates, increase of exit rates, decreased contact between *Anopheles* and humans and an increase of the mortality rate in resistant specimens (Darriet *et al*., 2000). Moreover the large-scale use of lambdacyhalothrin-treated nets in the Korhogo area (northern Ivory Coast) where *Kdr* allelic frequencies are less than 0.90 among *An. gambiae* populations facilitated a high decrease in inoculation rate, vectorial capacity and also about 50% reduction of incidence rate of malaria morbidity among children below 5 years. On the other hand, combination of different classes of insecticides tested in experimental huts in Ivory Coast indicated that that might be a promising technique for resistance management. The mixture is good because of its low cost and low toxicity (Hougard *et al*., 2003).

It is cheaper to prevent than treat malaria eventhough eliminating *Anopheles* mosquitoes is difficult. For effective prevention of malaria, collection of information about the disease, targeted technical approach to the disease, supportive leadership, absolute government backing, availability of funds, community participation, skilled technicians from different fields as well as an adequate implementation are all necessary (Lu *et al*., 2007).

Although mosquito control is inevitable in malaria control, it does not require the elimination of all *Anopheles* mosquitoes. For instance, in North America and Europe, eventhough *Anopheles* are still present, the *Plasmodium* has however been eliminated. There are also some socioeconomic improvements (e.g., houses with screened windows, air conditioning), which when combined with vector reduction efforts and effective treatment can led to the elimination of malaria even if the vectors remain. Some important measures in mosquito control are:

i. To discourage egg laying,
ii. To prevent development of eggs into larvae and adults,
iii. To kill the adult mosquitoes,
iv. Not to allow adult mosquitoes into places of human dwelling,
v. To prevent mosquitoes from biting human beings and deny blood meal (WHO, 2009).
vi. Using the sterile insect technique (SIT), in which sexually sterile male insects are released to wipe out a mosquito population,
vii. To use larvivorous fish e.g. the Empire Gudgeon (*Hypseliotris compressa*), the Pacific Blue-Eye (*Pseudomugil signifier*), and the mosquito fish, (*Gambusia* species).
viii. Use Parasites e.g. mosquitoes infected with a protozoan called microsporidia, *Amblyospora indicola* which kills mosquitoes at the larval stage.
ix. Use repellents which repel mosquitoes, ticks, biting flies and even leeches. The products contain DEET at a concentration less than, but close to 20% (Lu *et al*., 2007).

x. Through Habitat modification by draining water from trap pool and permit predatory fish to gain access to the mosquito larvae (Lu *et al*., 2007).

xi. Through chemical control e.g. Methoprene briquet, Temephos impregnated sand granules, Bti impregnated corn husks. Chemical spray outdoors and indoors can be achieved with a backpack spraying unit, via helicopter, via Argo, eight-wheeled all-terrain vehicle. These are useful for transporting insecticides, equipment and crew, over soft grounds such as saltmarshes, quicksands etc (Lu *et al*., 2007).

2.26 Mosquito and Malaria Control

Understanding the biology and behaviour of *Anopheles* mosquitoes can help understand how malaria is transmitted and can aid in designing appropriate control strategies. Factors that affect a mosquito's ability to transmit malaria include its innate susceptibility to *Plasmodium*, the choice of host and its life span. The susceptibility of *Anopheles* to insecticides and their preferred feeding and resting habitats should be highly considered when designing control programmes.

Eventhough malaria has existed since time immemorial, it was eliminated from Europe, North America, the Caribbean and parts of Asia and Southern Central America during the first regional elimination campaigns in the late 1940s. However, similar results are yet to be achieved in Sub-Saharan Africa.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out at Nasarawa State in three different Local Government Areas of Karu, Nasarawa Eggon and Doma. Nasarawa state is located in the North central part of Nigeria within the guinea savannah vegetation zone of the country Nigeria. Nasarawa state is located in the North central part of Nigeria within the guinea savannah vegetation zone of the country Nigeria. The state has a tropical climate conditions with a mean annual temperature and relative humidity ranging from 27 ^{0}C-33 ^{0}C and 65 %- 80 % respectively (Akwa *et al*., 2007).

3.2 Description of the Study Locations

The study was conducted in Nasarawa State. It is bounded in the North by Kaduna and Plateau, in the South by Benue, and the West to the Federal Capital Territory (FCT) to the East by Kogi State. The locations were spreads across the three Local Government Areas namely Karu, Nasarawa Eggon and Doma LGA's. The mean annual rainfall of Nasarawa is 1335 mm with August and September recording the highest monthly rainfall of about 300 mm. the highest monthly temperature is recorded in March with an average daily temperature of 29 ^{0}C -30 ^{0}C and the lowest in August at about 22 ^{0}C. Nasarawa has a tropical wet and dry climate with a pronounced dry season. Six (6) different locations were randomly selected, two from each site of the local Government. The map of the study area is found in Figure 3.1. Also, the GPS coordinates of the study sites were also taken and recorded as seen in Table 3.1

3.2.1 Description of wooded grassland eco-setting

The wooded grasslands eco-settings are lands covered with grasses and other herbs with woody plants [trees (≥7 m tall), bushes (3–7 m), dwarf trees, palm trees or shrubs (≤2 m) covering between 10 and 40 % of the ground. The eco-settings is characterized by the vegetation is dominated by grasses and other herbaceous (non-woody) plants. It is also called transitional landscape because grassland ecosystems which are dominated by the grass with few or no trees in the area where there is not enough for a forest and too much of a forest. (Akwa *et al*., 2007).

3.2.2 Description of sparse wood land eco-setting

This eco-setting made up of the crowns, or tops, of trees with an open canopy which allows full sunlight to enter the woodland, limiting shade and moisture. It is often regarded as a transition zones between different ecosystems, such as grasslands, true forests, and deserts. They are habitat where trees are the dominant plant form. The individual tree canopies generally overlap and interlink, often forming a more or less continuous canopy which shades the ground to varying degrees. (Akwa *et al*., 2007).

3.2.3 Description of swampy grassland eco-setting

The Swampy or wetland eco-settings are characterized by mineral soils with poor drainage and by plant life dominated by trees and grasses. There latter characteristic distinguishes a swamp from a marsh, in which plant life consists largely of grasses. They are very wet and soggy, like a swampy baseball field after four days of heavy rain. Some part of the swampy grassland resembles a wetland where trees, shrubs, and other plants grow and are habitats for different species of flora and fauna. (Akwa *et al*., 2007).

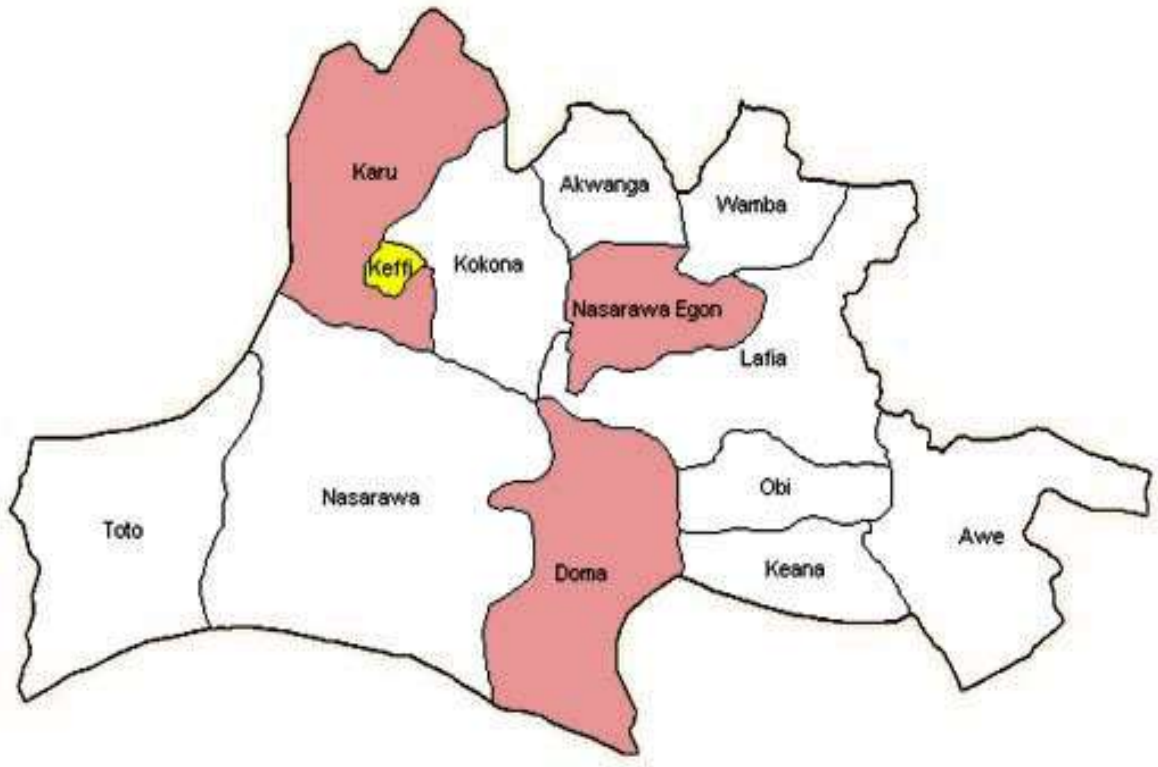

Figure 3.1: Map of Nasarawa State Showing the study sites. Nasarawa Geographic Information System (NAGIS).

Table 3.1: Global Positioning System Coordinates of the Study Sites

S/No	Study Sites	Latitude	Longitude
1	Gitata	9º36’50”N	6º32’35”E
2	Panda	9º38’42”N	6º32’31”E
3	Alogani	9º36’52”N	6º32’50”E
4	Aripka	9º36’50”N	6º32’01”E
5	Doma Town	9º36’48”N	6º32’49”E
6	Alagye	9º38’24”N	6º32’29”E

3.3 Study Design

Entomological surveillance wasconducted in the Local Government Areas of Doma Nasarawa Eggon and Karu Local Government Areas across the three senatorial zones of the state that represents the eco-settings within the state. The LGAs are located in the Guinea Savannah ecological zone; the landscape of the LGAs is mostly forested savannah. The climate also presents two distinct seasons i.e the rainy season which usually commences from the month of May to October and the dry seasons commences from the month of November to April with annual rainfall varying from 1,200 mm -1,500 mm. The prime period for malaria transmission is six months from the month of May to October (Ayanlade *et al.,* 2010).

3.4 Sample Collection

3.4.1 Centre for Disease Control light trap collection

Centre for Disease Control light trap methods (baited traps, was placed indoors and outdoors) in two different houses monthly for three (3) nights per site to measure mosquito biting time. The light trap bag was replaced every hour by two mosquito collectors from 18:00 hrs to 06:00 hrs per house per night in order to have proxy estimate on the peak biting time. One collector worked from 18:00 hrs to 24:00 hrs and was replaced by a second collector both indoor and outdoor from 24:00 hrs to 06:00 hrs following the methods of Yohannes and Boelee (2012). The trap was placed close to the legs of a person sleeping under an untreated bed net as bait both indoors and outdoors with the cups changed hourly. The mosquitoes collected were kept in separate labeled paper cups for identification and further analysis.

3.4.2 Pyrethrum spray collection

A total of 8 houses per LGA per month were sampled using the Pyrethrum Spray Collection (PSC) method as described by the WHO (2005) to sample indoor-resting mosquitoes. The houses sampled by two people, one inside and the other one outside, using an aerosol insecticide (Baygon) containing the active ingredients of 0.05 percent Imiprothrin, 0.05 percent Prallethrin, and 0.015 percent Cyfluthrin. Prior to spraying of the rooms from 5:30am to 8:30am all materials were removed from the rooms to be sampled. All openings like windows, doors, eaves among others were closed. A white sheet was spread to completely cover the whole floor. The pyrethrum insecticide (Raid) was sprayed in a clockwise direction towards the ceiling until the whole room was filled with a fine mist of the insecticide the two sprayers began spraying at the same time as they moved in opposite directions spraying inside the room as well as the eaves outside of the house The door was closed and left for 15 minutes. Thereafter, the door was opened and starting from the doorway, the sheets were folded and carried outside, the knocked down mosquitoes were collected using feather weight forceps and then placed in petri dishes or paper cups containing damp cotton wool and filter paper to maintain the physiological status of the mosquitoes. The Anopheline mosquitoes were preserved on damp absorbent paper in a cool box and later identified to the species level by morphological criteria (Gillies and De Meillon, 1968; Gillet, 1972; Gillies and Coetzee, 1987; Kent and Norris, 2005).

3.5 Morphological Identification of Mosquito Samples

All mosquitoes collected were identified and sorted out under a stereomicroscope (Leica model NSW series IMNS 210) and Olympus Tokyo VT-II 225329) Entomological microscope. All mosquitoes were identified using morphological keys of Gillies and De

Meillion (1968), Gillies and Coetzee (1987). After identification, the mosquitoes were preserved in dry labelled eppendorf tube over dry silica gel used for PCR identification. The mosquito identification was carried out at Abt Associates Entomological Laboratory and Insectary, Nasarawa State University, Keffi

3.6 PCR Identification of Members of the *Anopheles gambiae* Complex

Anopheles mosquitoes collected from the surveillance sites from the three LGA's were analyzed for species identification using the Polymerase Chain Reaction (PCR) at the Nigerian Institute of Medical Research (NIMR). All Mosquitoes presumed to be members of the *Anopheles gambiae* complex were analyzed using a standard method. The DNA was extracted and amplified using the *Anopheles gambiae* species-specific multiplex PCR (Scott *et al.,* 1993). PCR products were separated in Agarose gel, stained with ethidium bromide and visualized under UV trans illuminator. The PCR diagnosis bands for this assay include: a 464 base pair (bp) band for *Anopheles melas*, 390 bp for *An. gambiae*s.s. and 315bp for *An. arabiensis,*367 for the M form of *An. gambiae* and 267 for the S form of the *Anopheles gambiae s.s.* (Awolola *et al.,* 2005)

3.6.1 DNA extraction of mosquitoes

The whole body of the individual mosquitoes were placed separately in 1.5 ml Eppendorf tubes and grinded with pestles in 50 µL blocking buffer (1% bovine serum albumin, 0.5% casein and 0.00 2% phenol red made up in 0.01 mol/l Dulbecco's phosphate buffer saline (PBS) containing 0.5 %. The pestles were rinsed again with 200 µL blocking buffer. An aliquot of 50 µL was used for CS-ELISA. As heating was switch on and set to reach 65^{o} as the mosquitoes was placed in the 1.5 ml tube and was grind with 200 µL grinding buffer and was incubated in the heating block for 30 minutes 13µL 8M of potassium Acetate

(pH7.2) was added and mixed gently with a finger and then incubated for 30 minutes before centrifuging for 15 minutes at 13,000 rpm, then all the liquid (supernatant) was pipette out without disturbing the pellet and it was placed in a new tube and the old tube was discarded then 200 µL of ice cold ethanol (100 %) was added which was placed at at RT for 5mins and was then Centrifuge for 20 minutes at 13,000 rpm before washing with 200 µL 70 % ethanol (ice cold), without washing the pellet off It was then air dry on bench with tops open, overnight or until pellet is dry and re-suspend the pellet in 50 µL of double distilled water, making sure the pellet dissolves and then Store at -20 ^{o}C. Finally, the product was eluted in 500 µL of TE, incubated in a 100 °C water bath for 10 minutes and centrifuged at 15000 rpm for 10 second. An incubation of the extracted product at -20 °C for at least 1 hour before PCR amplification was found to be useful for increasing the yield (Collins, 1987).

3.6.2 PCR amplification and conditions

Initial Denaturation @ 95^{o}C – 2 mins
Denaturation @ 95^{o}C – 30sec
Annealing @ 55^{o}C – 30sec
Extension @ 72^{o}C – 40sec
Final extension @ 72^{o}C –7mins
} X 30 Cycles

12.5 µL of PCR was added to the master mix into each 200 µL tube and 1 µL of DNA into each tube and they were loaded in the PCR machine with a specified choosen program 1.5 % agarose gel was prepared with Tris-borate ethylene-di-amino tetraacetic acid (TBE) buffer; such as 1.5 g of agarose gel in 100 ml of TBE buffer it was Mixed and boil in microwave until the solution is clear it was allowed to cool down for 5 minutes then it was

remove and skin on top and 100 µL of ethidium bromide was added the gel was Pour into trough10 µL of PCR product was loaded with 1 µL of loading buffer into each well and 10 µL of standard marker per gel was loaded the PCR product was run at 100 volts and not more than 120-150 mA and the picture of the gel was taken under uv light using the gel documentation.

3.6.3 Interpretation of bands on gel

Anopheles gambiaes.s- 390 base pair; *Anopheles arabiensis* – 315 base pair ; *Anopheles merus* – 464 base pair ; *Anopheles quadriannulatus* –153 base pair

Table 3.2: PCR Master Mix for identifying *An. gambiae* species

1	96	Reagents
11.8 µL	1180 µL	Sterile H20
2.5 µL	250 µL	Taq 10X PCR Buffer with MgCl2
2.5 µL	250 µL	dNTP (2 mM mix G, A, T, C)
1.0 µL	100 µL	MgCl2 (25mM)
1.0 µL	100 µL	UN (F, 25 pmol/ul) [GTGTGCCCCTTCCTCGATGT]
1.0 µL	100 µL	AR (F, 25 pmol/µl) [AAGTGTCCTTCTCCATCCTA]
1.0 µL	100 µlL	GA (F, 25 pmol/µl) [CTGGTTTGGTCGGCACGTTT]
1.0 µL	100 µL	ME (F, 25 pmol/µl) [TGACCAACCCACTCCCTTGA]
2.0 µL	200 µL	QD (F, 25 pmol/µl) [CAGACCAAGATGGTTAGTAT]
2.0 µL	200 µL	QDA (F, 25 pmol/µl) [CATAATGAGTGCACAGCATA]
0.2 µL	20 µL	Taq DNA polymerase (5U/µl)
2.5 µL	250 µL	dNTP (2 mM mix G, A, T, C)
1.0 µL	100 µL	MgCl2 (25mM)

24μL was added to 1μl template DNA F and R indicate forward and reverse orientation. To improve specificity primers for species that do not occur in the area of sample collection were not included in the master mix. When removing a primer, its volume was replaced with an equal volume of sterile water. One microliter (μL) DNA template was utilized to make the 25 μL total reaction volume.

3.7 Polymerase Chain Reaction (PCR) Cycle Conditions

The cycling parameters for the reaction were as follows; initial denaturation was carried out at 94 ^{0}C for 3 minutes, it was followed by annealing of 35 cycles of 94 ^{0}C for 30 s, 50 ^{0}C for 30 s, 72 ^{0}C for 60 s and ended by a final of cycle of 94 ^{0}C for 30 s, 50 ^{0}C for 30 s and 72 ^{0}C for 10 min. For each reaction, a positive control with PCR products of *Anopheles* of the same primer set and a negative control that contained no DNA template were included. Samples amplicans were examined after being run in 2 % agarose gel electrophoresis and stained in Ethibium Bromide (EtBr) gel load 5 μL sample in a transilluminator and a picture was taken in a digital polaroid. Amounts for larger master mixes were adjusted upwards to be sufficient for 50 and 100 reactions to compensate for imprecise measurements. The remaining PCR product was loaded on NuSieve GTG agarose (Biowhittaker Molecular Applications, East Rutherford, NJ). PCR products were visualized under UV light on 1.5 % agarose, 0.5x TBE gels stained with ethidium bromide.

3.8 Agarose Gel Electrophoresis

Polymerase chain reaction (PCR) products were electrophoresed in 4% agarose gel stained with Ethidium bromide. DNA standards of the species were used as positive controls. A reaction mix with no template DNA was included as a negative control. Following the PCR for species identification, PCR products for both *An. gambiae* and *An. funestus* were

electrophoresed on separate 2 % agarose gels stained with 0.5 µg/ml EtBr to detect the presence of amplified DNA fragments. Eight microlitres of each sample was added to 1µL of (5X) bromophenol blue gel loading dye for the electrophoresis. The 2 % gel was prepared and electrophoresed in 1X TAE buffer using a mini gel system (BIORAD, USA) at 100 volts for one hour and then photographed over a UV transilluminator (UPC, USA) at short wavelength using a Polaroid camera and film type 667 (Polaroid, USA).

3.9 Blood-meal Molecular Assay

The abdomen of each mosquito was crushed in an Eppendorf tube containing 50 µL of water of high performance liquid chromatography (HPLC) quality. After a quick spin centrifugation to eliminate debris, 10µL of supernatant was used for DNA extraction analysis. Molecular analysis DNA extractions from individual mosquito abdomen samples were performed with the EZ1 DNA Tissue kit (Qiagen, Hilden, Germany) according to manufacturer recommendations. DNA extracted from unfed mosquitoes was used as a snegative blood meal control. To assess the kinetic degradation of vertebrate blood DNA, a set of the primers specifically amplifying the vertebrateNon-engorged mosquitoes were used as negative and positive controls for vCOI and mCOI PCR reactions, respectively. The PCR reaction contained 13 µL of sterile distilled water, 2.5µL of tampon 5X Phusion HF Buffer, 2.5µL of dNTPs, 0.5µL of each primer, 0.25 µL of Taq, 1 µL of MgCl2 and 5 µL of extracted DNA. Reactions were amplified through 35 cycles with the following parameters: 10 min at 95 ^{o}C, 1 min at 95 °C, 1 min at 40 °C for vCOI, and 1min at 52 °C for mCOI, 1.5 min at 72 °C, followed by a final extension step at 72 °C for 7min. Amplifications were assessed by gel electrophoresis, using 2 % agarose 0.5 % TBE, stained with ethidium bromide. Some vCOI and mCOI positive PCR products were purified using

the NucleoFast 96 PCR plate (Machery-Nagel EURL, France), as recommended by the manufacturer and sequenced using the same respective primers with the BigDye version 1-1 Cycle Ready Reaction Sequencing mix (Applied Biosystems, Foster City, CA, USA) and an ABI 3100 automated sequencer (Applied Biosystems) to control the amplified products.

3.10 Identification of Malaria Sporozoites

Head and thorax of each female *Anopheles* mosquito was crushed in Phosphate Buffered Saline (PBS) and tested for circumsporozoite (CSP) antigen using an ELISA assay (Charlwood *et al.,* 2015)**.** *Anopheles* mosquitoes caught indoors, outdoors and PSC were subjected to ELISA, using Dipstick assay, during which aliquouts (150 uL) of *Anopheles* mosquito triturates made from squashed mosquito thoraces using grinding tubes and were transferred into the wells of micro-titre plates (sero-wel; Bibby Sterilin Ltd, Stone Staffs, UK) for testing. Each Malaria Panel Assay kit which contains the following: i) Twenty Malaria Panel Assay dipsticks in a canister with a desiccant cap Test Zone. ii) Monoclonal antibodies to *P. falciparum, P.malariae*, *P. vivax 210*, and *P. vivax* 247CS antigens immobilized on a membrane Control Zone. iii) Immobilized polyclonal goat antibody to mouse immunoglobulins Conjugate Pad. iv) Gold complexed to monoclonal antibodies to *P. falciparum*, *P. malariae*, *P. vivax* 210, and *P. vivax* 247CS antigens and 1 vial of Grinding Solution (6 ml) 64 VicTest dipstick strips (MAS TM; Camarillo, CA, USA), which were placed separately in the test wells of the micro-titre plate and allowed to develop for 15 min at room temperature (22 ^{o}C – 25 ^{o}C). Positive dipstick result were indicated by a horizontal reddish purple line in addition to a control line on each test strip in a distinct detection zone.

3.11 Data Analysis

Data generated were analyzed using the SPSS software version 20.0 and Excel package. Chi – square ($\chi 2$) test was used to compare the mosquito species at various collection sites and seasons.The relationship between *Anopheles* species and months/season was carried using ANOVA, Chi – square and correlation (r) analysis. All significant difference was defined at 95 % confidence interval.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1a Spatial species composition and distribution across different eco – settings of Nasarawa State, Nigeria

Spatial species composition and distribution of *Anopheles* mosquitoes across the different eco – settings of Nasarawa State is presented in Table 4.1a. Six (6) different species of *Anopheles* mosquitoes were encountered in all the three eco – settings during the study period. These are: *Anopheles gambiae, An. funestus, An. nili, An. coustani, An. rufipes* and *An. pharoensis.* There was no variation in the composition and distribution of *Anopheles* mosquito species encountered in the three eco – settings of Nasarawa State.

The relative abundance of mosquito vector genera across the eco – settings is presented in Table 4.1a. A total of fifteen thousand, four hundred and seventeen (15,417) mosquitoes vector genera were encountered in the study areas between the period of January to December, 2017 and 2018. Among the collected mosquitoes (15,417 mosquitoes), 9,881 (64.09 %) were anopheline while 5,536 mosquitoes (35.91 %) were culicine. The highest number (5,644 mosquitoes; 36.61 %) of mosquitoes was collected in the Swampy grassland eco – setting (Doma Local Government Area, LGA) while Sparse woodland (Nasarawa Eggon LGA) had the least number (4,245 mosquitoes; 27.53 %) of mosquitoes. Among the collected *Anopheles* mosquitoes (9,881 mosquitoes), over 50 % (50.39 %; 3,006 mosquitoes) were recorded in Sparse woodland (Nasarawa Eggon LGA). This was followed by wooded grassland (Karu LGA) with 44.17 % (3463 mosquitoes) while the

Swampy grassland (Doma LGA) had the least (42.64 %; 3,412 mosquitoes). Analysis revealed significant difference ($p<0.05$) in the relative abundance of mosquito genera across the eco – settings.

4.1.1b Seasonal variation of *Anopheles* mosquitoes encountered in the study locations

Analyses revealed seasonal variations of *Anopheles* mosquitoes encountered in the study areas within the eco – settings (Table 4.1b). A total of 5,665 *Anopheles* mosquitoes (57.24 %) were encountered during the wet season as compared to 4,225 mosquitoes (42.76 %) encountered during the dry season. More *Anopheles* mosquitoes were encountered indoors (62.50 %) during the wet season than outdoors (37.50 %). In dry season, more *Anopheles* mosquitoes were collected indoors (2,571 mosquitoes; 60.85 %) than outdoors (1,654 mosquitoes, 39.15 %). Analysis revealed that there was a significant difference ($p<0.05$) in the population of *Anopheles* mosquitoes encountered in wet and dry seasons within the study areas.

4.1.2 Monthly variation of *Anopheles* mosquito in the selected eco – settings of Nasarawa State

The monthly variation of *Anopheles* mosquito vector in the selected eco – settings of Nasarawa State is presented in Table 4.2. Among the Anopheline mosquitoes caught during the study period, the highest number of mosquitoes (1,273 mosquitoes; 12.88 %) were caught in the month of May 2017 followed by June 2017 (1,222; 12.37 %). The least was recorded in the month of February 2017 (534 mosquitoes; 5.40 %). In relation to the eco – settings, the highest number of Anopheline mosquitoes were recorded in Swampy grassland

(3,835; 38.81 %) followed by wooded/grassland (3,175; 32.13 %), while the least anopheline mosquitoes were recorded in Sparse woodland (2,871; 29.06 %).

In sparse woodland eco – setting, anopheline mosquitoes were higher in the month of January and February 2017 (32.39 and 32.21 %, respectively) while the least was recorded in the month of May and June 2017 (27.42 and 27.33 %, respectively). Similarly, in wooded/grassland eco – setting, anopheline mosquitoes were peaked (34.27 %) in the month of February 2017 and least (30.05%) in the month of July 2017. The results of monthly dynamics of Anopheline mosquitoes varied significantly ($p< 0.05$) within the swampy grassland eco – setting. Anopheline mosquitoes were higher in the months of April, June and May 2017 (41.56, 41.52 and 41.33 %, respectively) while the least number of anopheline mosquitoes (33.52 %) was recorded in the month of February 2017. Analysis showed significant difference ($p<0.05$) in monthly variation of Anopheline mosquitoes among the selected eco – setting of Nasarawa State.

Table 4.1a Relative Abundance of Mosquito Genera across the Eco - Settings

Local Government Areas	Eco – settings	Anopheline (%)	Culicine (%)	Total (%)
Karu	Wooded/Grassland	3463 (44.17)	2065 (47.17)	5528 (35.86)
Nasarawa Eggon	Sparse woodland	3006 (50.39)	1239 (41.86)	4245 (27.36)
Doma	Swampy Grassland	3412 (42.64)	2232 (48.63)	5644 (36.61)
Total		9881 (64.09)	5536(46.41)	15417 (100)

χ^2 Cal =120.81; χ^2 tab = 5.99; df = 2

Table 4.1b: Seasonal Variation of *Anopheles* Mosquitoes encountered in the Study Area

Seasons	Wet Seasons		Dry Seasons		Total	
Locations	**Indoor (%)**	**Outdoor (%)**	**Indoor (%)**	**Outdoor (%)**	**Indoor (%)**	**Outdoor (%)**
Wooded/Grassland (Karu LGA)	1292(36.65)	648(30.55)	925(35.98)	511(30.89)	2217(36.31)	1159(30.70)
Sparse woodland (Nas. Eggon LGA)	3114(32.31)	603(28.43)	782(30.42)	487(29.44)	1924(31.51)	1090(28.87)
Swampy Grassland (Doma LGA)	1101(31.15)	870(41.01)	864(33.61)	656(39.66)	1965(32.18)	1526(40.42)
Total	**3535(62.50)**	**2121(37.50)**	**2571(60.85)**	**1654(39.15)**	**6106(61.70)**	**3775(38.20)**
Grand Total	5565 (57.24)		42215 (42.76)		9881(100)	

χ^2 Cal =71.63; χ^2 tab = 5.99; df = 2. LGA = Local Government Areas

Table 4.2: Monthly Variation of the Mosquito Genera in the Selected Eco-Settings of Nasarawa state

Months (2017)	Eco – settings			Total (%)
	Sparse Woodland (%)	Wooded/Grassland (%)	Swampy Grassland (%)	
January	184 (32.39)	189 (33.27)	195 (34.33)	568 (5.75)
February	172 (32.21)	183 (34.27)	179 (33.52)	534 (5.40)
March	171 (29.69)	185 (32.12)	220 (38.19)	576 (5.83)
April	266 (28.15)	295 (31.22)	384 (40.63)	945 (9.56)
May	349 (27.42)	395 (31.03)	529 (41.56)	1273 (12.88)
June	334 (27.33)	383 (31.34)	505 (41.33)	1222 (12.37)
July	280 (28.43)	296 (30.05)	409 (41.52)	985 (9.97)
August	206 (29.43)	232 (33.14)	262 (37.43)	700 (7.08)
September	209 (29.56)	236 (33.38)	262 (37.06)	707 (7.16)
October	239 (28.52)	270 (32.22)	329 (39.26)	838 (8.48)
November	236 (30.10)	260 (33.16)	288 (36.73)	784 (7.93)
December	225 (30.04)	251 (33.51)	273 (36.45)	749 (7.78)
Total	2871(29.06)	3175(32.13)	3835(38.81)	9881(100)

χ^2 Cal =29.02; χ^2 tab = 33.92; df = 22

4.1.3 Spatial composition of *Anopheles* mosquito species encountered across the selected eco – settings of Nasarawa State

Table 4.3 showed the spatial composition of *Anopheles* species encountered across the three selected eco – settings of Nasarawa State. Six (6) species of *Anopheles* vectors were encountered in all the selected eco – settings of Nasarawa State. The various species encountered are; *Anopheles gambiae* s.l, *An. funestus, An.nili, An.coustani, An.rufipes* and *An. pharoensis. An. gambiae* s.l were the most dominant species (41.89 %) encountered across the eco – settings during the two seasons followed by *An. coustani* (19.49 %) while *An. pharoensis* had the least number of species (5.83 %) across the eco – settings. There was a statistical difference ($p<0.05$) in the abundance of *Anopheles* species.

In respect to the eco – settings, *An. gambiae s.l* was higher (46.75 %) and *An. rufipes* had the least (3.39 %) number of *Anopheles* mosquito species in the woodland/grassland (Karu LGA) eco – setting; in the same way, *An. gambiae s.l* was higher (39.22 %) and *An. rufipes* had the least (4.70 %) number of *Anopheles* mosquito species in the sparse woodland (Nasarawa Eggon LGA) eco – setting while *An. gambiae s.l* was equally higher (39.37 %) and *An. pharoensis* had the least (4.77 %) number of *Anopheles* mosquito species encountered in the grassland (Doma LGA) eco – setting. Statistically, there is a significant difference ($p<0.05$) in the spatial composition of *Anopheles* mosquito species encountered across the selected eco – settings of Nasarawa State.

Table 4.3: Spatial Composition of *Anopheles* Mosquito Species encountered across the Selected Eco-Settings of Nasarawa State

Eco – settings	*An. gambiae*	*An. funestus*	*An. nili*	*An. coustani*	*An. rufipes*	*An. pharoensis*
Wooded/Grassland (Karu LGA)	517.5±68.50[a]	180.0±18.0[b]	120±14.0[a]	181.5±13.5[b]	37.5±2.5[b]	69.5±12.5[a]
Sparse woodland (Nas. Eggon LGA)	363±15.0[c]	210.5±13.5[a]	98.0±5.0[b]	148.5±13.5[c]	43.5±2.5[b]	62.0±10[ab]
Swampy Grassland (Doma LGA)	458.50±11.5[b]	91.0±9.0[c]	69.50±5.5[c]	293±28.5[a]	124.5±9.5[a]	55.5±35.67[b]
Average	223.17±15.83	80.25±6.75	47.92±4.08	103.83±9.25	34.25±2.42	31.1±9.70

Values with the same superscript within a column are not significantly different at P>0.05. LGA = Local Government Areas

4.1.4 Monthly spatial composition of *Anopheles* species encountered in the study locations

The monthly spatial composition of *Anopheles* species encountered in the three eco – settings is presented in Table 4.4. Six (6) species of *Anopheles* mosquito were encountered during the study period. The highest *Anopheles* mosquito population was recorded in the month of May (618±30.0 mosquitoes) followed by the month of June (465±26.0 mosquitoes) while the least *Anopheles* mosquito population was recorded in the month in the month of January (140±10.0 mosquitoes). The composition and distribution of *Anopheles* mosquito species varies significantly ($p<0.05$) on monthly basis.

Anopheles gambiae s.l was the most dominant (111.58±13.59 mosquitoes) species encountered throughout the months of the study period followed by *An. coustani* (51.96±4.61 mosquitoes) while *An. pharoensis* was the least encountered species (15.5±1.61 mosquitoes). Interestingly, all the six (6) species of *Anopheles* mosquito encountered during the study period were peaked in the month of May.

Table 4.4: Monthly Spatial Composition of *Anopheles* Species encountered in the Study Areas

Months (2017-2018)	*An. gambiae*	*An. funestus*	*An. nili*	*An. coustani*	*An. rufipes*	*An. pharoensis*	Total
January	75.00±2.00[a]	16.50±1.50 [a]	10.50±1.50 [a]	21.50±2.50 [a]	7.50±1.50 [a]	9.00±1.00 [a]	140±10.0
February	72.00±8.00 [a]	22.50±3.50 [a]	12.50±18.50[a]	18.50±0.50 [a]	6.50±1.50 [a]	10.00±1.00 [a]	142±33.0
March	80.50±5.50 [a]	24.50±3.50[a]	15.50±0.50 [a]	23.00±1.00 [a]	13.00±2.00 [a]	10.00±1.00 [a]	166.5±13.5
April	104.50±6.50[ab]	76.50±9.50 [c]	26.50±2.50[b]	66.50±6.50 [d]	10.00±1.00 [a]	9.00±1.00 [a]	293±27.0
May	287.00±11.00 [c]	85.50±5.50 [c]	86.50±0.50[c]	85.50±8.50 [f]	40.50±3.50 [d]	33.0±1.00 [c]	618±30.0
June	210.50±12.50 [b]	81.00±2.00 [c]	39.50±6.50[bc]	77.50±2.50 [e]	29.50±1.50 [c]	27.00±1.00 [b]	465±26.0
July	115.00±6.00[ab]	57.50±3.50[bc]	20.50±2.50[b]	55.50±9.50 [c]	20.50±1.50 [b]	21.50±.50[ab]	290.5±23.5
August	89.00±6.00 [a]	30.50±1.50[ab]	16.50±2.50[a]	43.50±8.50 [b]	18.00±1.00[ab]	17.50±1.50[ab]	215±21.0
September	82.00±7.00 [a]	34.00±5.00[ab]	12.50±0.50 [a]	50.00±2.00 [c]	16.00±1.00[ab]	16.00±1.00[ab]	210.5±16.5
October	71.00±16.00 [a]	51.00±10.00[bc]	9.50±1.50 [a]	58.50±9.50 [c]	16.50±1.50[ab]	14.50±3.50[ab]	221±42.0
November	82.50±4.50 [a]	34.00±2.00[ab]	11.5±2.50 [a]	63.00±13.00 [d]	15.00±2.00[ab]	10.00±1.00 [a]	216±25.0
December	70.00±6.00 [a]	40.00±5.00 [b]	11.00±3.00 [a]	60.50±10.50 [d]	12.50±1.50 [a]	9.50±1.50 [a]	203.5±27.5
Total	111.58±13.59	46.13±4.92	22.71±4.40	51.96±4.61	17.13±1.95	15.5±1.61	265.09±31.08

Values with the same superscript within a column are not significantly different at P>0.05

4.1.5a Species composition of *Anopheles* mosquitoes from indoor and outdoor collections

Of the 9881 (64.09 %) *Anopheles* mosquitoes caught across the selected eco – settings of Nasarawa State, 6392 (64.69 %) *Anopheles* mosquitoes were caught using CDC method; 6106 (61.80 %) indoors and 3775 (38.20 %) were caught outdoors. Indoors *Anopheles* mosquitoes were higher (65.66 %) in wooded/grassland (Karu LGA) eco – setting followed by sparse woodland (Nasarawa Eggon LGA) eco – setting (63.86 %) while the least (56.27 %) was recorded in the swampy grassland (Doma LGA) eco – setting. In the same vein, outdoor *Anopheles* mosquitoes were peaked in swampy grassland eco – setting (42.73 %) followed by sparse woodland (36.14 %) while woodland/grassland had the least number of outdoor *Anopheles* mosquitoes (34.32 %). The species composition of *Anopheles* mosquitoes from indoor and outdoor collections across the selected eco – settings of Nasarawa State varied significantly ($p<0.05$) (Table 4.5a).

4.1.5b Indoor resting density of *Anopheles* mosquitoes collected in the selected eco – settings of Nasarawa State

Table 4.5b showed the resting density of the *Anopheles* mosquitoes caught indoors across the three selected eco – settings of Nasarawa State. A total of ninety six (96) rooms each were sampled across the selected eco – settings. The highest indoor resting density was found in the sparse woodland (Nasarawa Eggon LGA) eco – setting (4.46 m/h/n), followed by wooded/grassland (Karu LGA) eco – setting (3.99 m/h/n) while the least was recorded in swampy grassland (Doma LGA) eco – setting (3.65 m/h/n).

4.1.6 Monthly Human Biting Rate (HBR) from CDC light trap collection across the selected eco – settings of Nasarawa State

The estimated human biting rates (HBR) were different in each eco – setting studied (Table 11a, b and c). The HBR being 16.08 bites per man per hour was recorded in the swampy grassland (Doma LGA) eco – setting, wooded grassland (Karu LGA) eco – setting being the immediate 15.4 bites per man per hour and sparse woodland (Nasarawa Eggon LGA) eco – setting being the lowest 12.7 bites per man per hour. The estimates also differed strongly by method of collection with CDC light trap (indoors) having the highest (30.0 bites per man hour) while the lowest was collected by CDC light trap (Outdoors) 14.2 per catching.

Human biting rate (HBR) also varies significantly on monthly basis across the selected eco – settings of Nasarawa State. The month of May recorded the highest HBR 18.3 bites per man per hour, 22.9 bites per man per hour and 23.0 bites per man man per hour in all the eco – settings respectively and lowest in March and December 2017 (10.4 bite per man per hour), December (11.8 bites per man per hour) and January and December (11.6 bites per man per hour) in sparse woodland, swampy grassland and wooded grassland eco – settings respectively (Table 6a, b and c).

Figure 4.1 presented below shows the biting activities of the Anopheline mosquitoes which commences from 6:00 pm to 6:00 am for both indoors and outdoors resting mosquitoes the figures clearly shows the biting rhythm was at it peak within the hours of 10:00 pm to 4:00 am and within this period the peak biting hour was recorded between the hours of 12:00 am

to 2:00am as the decline in the biting activities began from 3:00 am to 6:00 am, respectively.

Hourly biting activities of the mosquitoes at sparse woodland eco-setting (Nasarawa Eggon LGA) is presented in Figure 4.2. The biting activities of the Anopheline mosquitoes which commences from 6:00 pm to 6:00 am for both indoors and outdoors resting mosquitoes the figures clearly shows the biting rhythm was at its peak within the hours of 11:00 pm to 4:00 am and within this period the peak biting hour was recorded between the hours of 1:00am to 3:00 am as the decline in the biting activities began from 4:00 am to 6:00 am respectively.

Hourly biting activities of the mosquitoes at Swampy grassland eco-setting (Doma LGA) is presented in Figure 4.3 below. The results shows the biting activities of the Anopheline mosquitoes which commences from 6:00 pm to 6:00 am for both indoors and outdoors resting mosquitoes the figures clearly shows the biting rhythm was at its peak within the hours of 9:00 pm to 10:00 am and there was a decline within the hours of 11:00 pm and 12:00 am before the maximum peak was recorded within the period and biting hours of 2:00 am to 4:00 am as the decline in the biting activities began from 5:00 am to 6:00 am respectively.

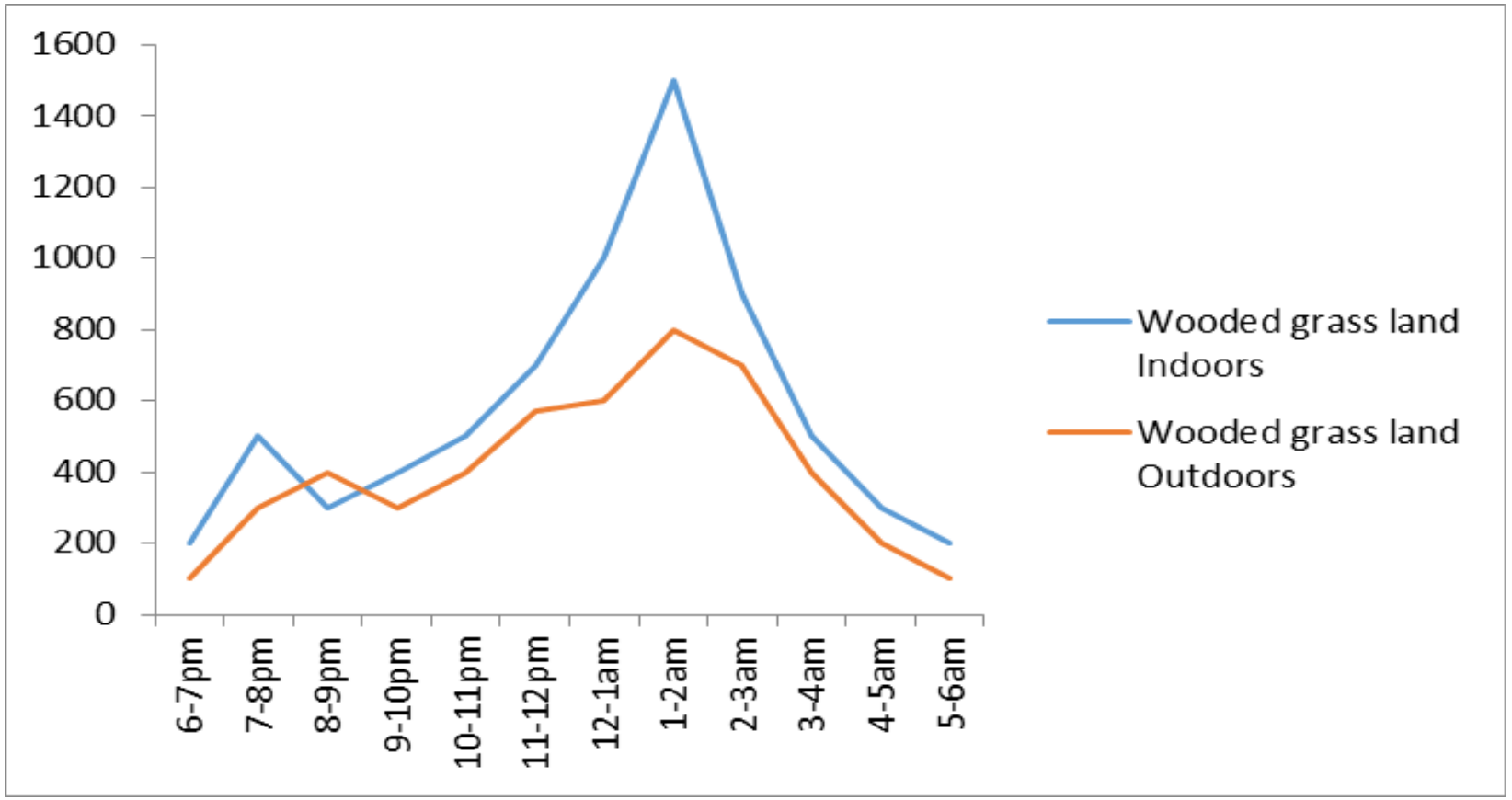

Figure 4.1. Hourly biting activities of the Mosquitoes at Wooded grassland eco-settings in Karu Local Government Area

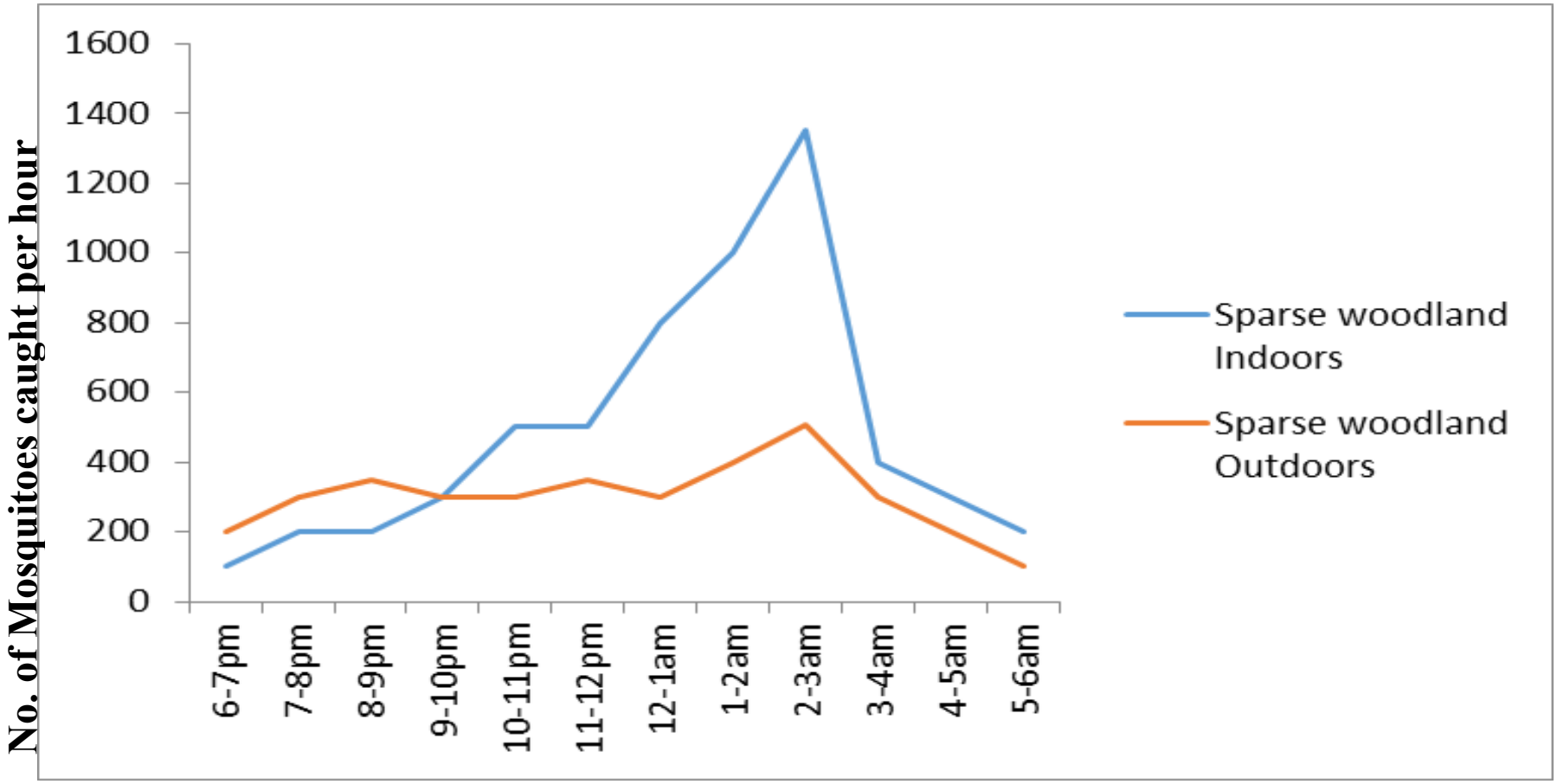

Figure 4.2 Hourly Biting Activities of the Mosquitoes at Sparse Woodland Eco-settings in Nasarawa Eggon Local Government Area

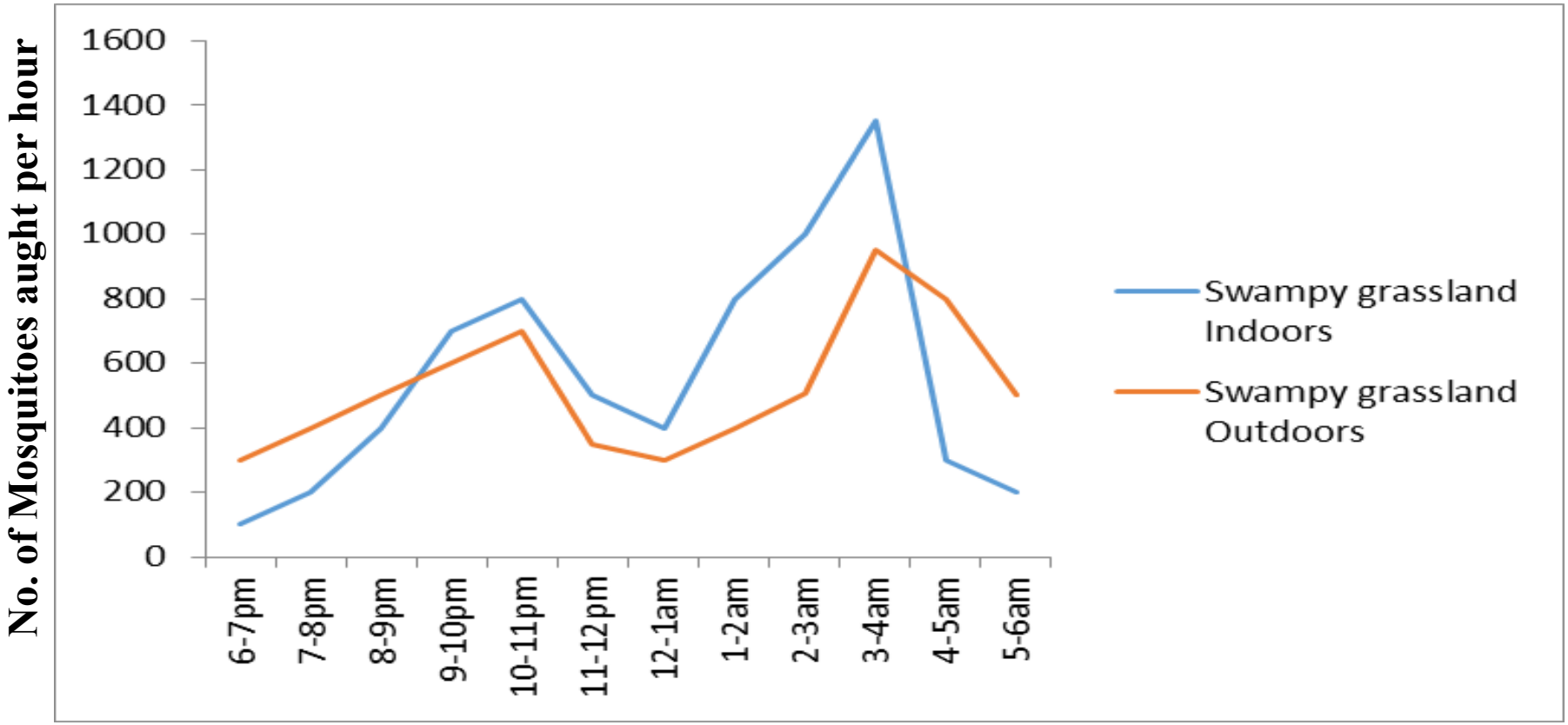

Figure 4.3. Hourly Biting Activities of the Mosquitoes at Swampy Grassland Eco-Settings in Doma Local Government Area

4.1.6d Spatial variations of abdominal conditions of anopheline mosquitoes collected across the selected eco – settings of Nasarawa State

Abdominal conditions of anopheline mosquitoes caught using PSC method were also analyzed. Three thousand, four hundred and eighty nine (3489) anopheline mosquitoes were collected using PSC method. The highest number of anopheline mosquitoes were caught in sparse woodland (Nasarawa Eggon LGA) eco – setting 1285 (36.83 %) followed by wooded/grassland (Karu LGA) eco – setting 1,150 mosquitoes (32.96 %) while swampy grassland had the least 1054 (30.21 % mosquitoes). Among the various abdominal conditions observed, half gravid (HG) were most abundant 947 mosquitoes (27.14 %) anopheline mosquitoes observed, followed by gravid (G) and fully fed (FF) 910 (26.08 %) and 906 (25.97 % mosquitoes) respectively, while unfed (UF) mosquitoes had the least 686 (19.66 %) anopheline mosquitoes observed.

However, the highest number 274 mosquitoes (42.39 %) of unfed (UF) anopheline mosquitoes was observed in sparse woodland (Nasarawa Eggon LGA) eco – setting while the least 239 (40.51 % mosquitoes) was recorded in wooded/grassland (Karu LAG) eco – setting. Fully fed (FF) mosquitoes were higher in wooded/grassland eco – setting 320 (56.02 % mosquitoes) and the least of 267 (50.63 % mosquitoes) was recorded in swampy grassland eco – setting; Gravid (G) and Half gravid (HG) abdominal conditions were higher in swampy grassland 287 mosquitoes (54.76 %) and 327 (62.13 % mosquitoes) respectively (Table 4.6d).

4.1.7 *Plasmodium* sporozoites identified by ELISA across some selected eco – settings of Nasarawa State

A total of one thousand, five hundred (1,500), five hundred (500) anopheline mosquitoes each from the three selected eco – settings were analyzed for *Plasmodium* sporozoite rate using ELISA. A total of 231 (15.40 %) *Plasmodium* sporozoite rate was recorded. The highest *Plasmodium* sporozoite rate 101 (20.20 %) was recorded in swampy grassland (Doma LGA) eco – setting, followed by wooded grassland (Karu LGA) eco – setting 66 (13.20 %) while sparse woodland (Nasarawa Eggon LGA) eco – setting had the least *Plasmodium* sporozoite rate 64 (12.80 %). With respect to methods of collection, indoors resting mosquitoes had the highest 88 (19.56 %) *Plasmodium* sporozoite rate followed by outdoors resting mosquitoes 81 (18.00 %) while PSC had the least 62 (10.33 %) *Plasmodium* sporozoite rate (Table 4.7).

4.1.8 *Anopheles gambiae s.s* M and S molecular forms identified by PCR

Ten percent (10%) of all the total mosquitoe collections from (CDC indoors, CDC outdoors and PSC methods) across the selected eco – settings of Nasarawa State were analyse using polymerase chain reaction (PCR) for the identification of species and molecular forms. The results showed that all *Anopheles gambiae s.l* mosquitoes were identified as *An. gambiae s.s* (Table 4.12a and b). Among the 1,500 (500, from each eco – setting) *An. gambiae s.s* samples analyzed for molecular forms, 1,012 mosquitoes (67.47 %) were identified as *An. gambiae s.s* Giles (Former Savannah (S) form), 232 mosquitoes (15.47 %) were *An. coluzzi* (M – form) and *An. arabiensis* 164 mosquitoes (10.93 %). The results therefore showed

that *An. gambiae s.s* exists in all the selected eco – settings of Nasarawa State (Table 4.8a and b).

4.1.9 Knock down resistant (Kdr) across the eco – settings of Nasarawa State

A total of six hundred (600) mosquitoes were analyzed for the presence of Kdr gene. This result shows high frequency of Kdr 201 mosquitoes (33.50 %) across the three (3) selected eco – settings of Nasarawa State. The gene type study showed that 39 mosquitoes (19.02 %) were homozygous resistant (RR), 63 (31.66 % mosquitoes) were heterozygous susceptible (RS) while 99 (50.51 %) were homozygous susceptible (SS). The highest being 69 (34.5 % mosquitoes) was recorded in the swampy grassland (Doma LGA) eco – setting while wooded grassland (Karu LGA) eco – setting and sparse woodland (Nasarawa Eggon LGA) eco – setting had Kdr of 66 (33.00 % mosquitoes) each (Table 4.9).

Table 4.5a: Species Composition of *Anopheles* Mosquitoes from Indoors and Outdoors Collections across the Selected Eco-settings

Eco – settings	Indoors (%)	Outdoors (%)	Total (%)	Predominant feeding Behaviour
Wooded/Grassland	2216 (65.66)	1158(34.32)	3374 (34.15)	Endophagic
Sparse woodland	1924 (63.86)	1089 (36.14)	3013 (30.49)	Endophagic
Swampy Grassland	1966 (56.27)	1528 (42.73)	3494 (35.36)	Endophagic
Total	6106 (61.80)	3775 (38.20)	9981 (100)	

χ^2 Cal =72.19; χ^2 tab = 5.99; df = 2.

Table 4.5b: Indoor Resting Density of *Anopheles* Mosquitoes Collected in the selected Eco-Settings in Nasarawa State

Eco – settings	Number of rooms sampled	*Anopheles* mosquitoes caught	Number of nights sampled	Indoor resting density (m/h/n)
Wooded/Grassland	96	1150	3	3.99
Sparse woodland	96	1285	3	4.46
Swampy Grassland	96	1054	3	3.65

Table 4.6a: Monthly Biting Rate from CDC light trap collection from Wooded Grassland (Karu LGA) Eco – setting of Nasarawa State

Months of collection (2017)	*Anopheles* per night per trap		Number of traps per night		Number of collection hours	Human Biting Rate (HBR) per night per trap (b/m/h)	
	Indoors	Outdoors	Indoors	Outdoors		Indoors	Outdoors
January	106	33	4	4	3	8.60	3.10
February	136	43	4	4	3	8.80	2.60
March	112	43	4	4	3	7.90	2.50
April	145	40	4	4	3	9.40	2.20
May	169	78	4	4	3	13.10	5.20
June	184	81	4	4	3	11.00	5.60
July	160	64	4	4	3	10.10	5.50
August	142	52	4	4	3	7.30	3.20
September	126	42	4	4	3	8.40	3.80
October	128	30	4	4	3	8.40	3.40
November	119	33	4	4	3	8.60	2.90
December	130	28	4	4	3	7.60	2.80
Average						9.10	3.60

b/m/h = bites per man per hour

Table 4.6b: Monthly Biting Rate from CDC light trap Collection from Sparse Woodland (Nasarawa Eggon) Eco – setting of Nasarawa State

Months of collection (2017)	*Anopheles* per night per trap		Number of traps per night		Number of collection nights	Human Biting Rate (HBR) per night per trap (b/m/h)	
	Indoors	Outdoors	Indoors	Outdoors		Indoors	Outdoors
January	102	37	4	4	3	8.6	3.1
February	106	31	4	4	3	8.8	2.6
March	94	29	4	4	3	7.9	2.5
April	113	26	4	4	3	9.4	2.2
May	158	62	4	4	3	13.1	5.2
June	131	66	4	4	3	11.0	5.6
July	121	66	4	4	3	10.1	5.5
August	87	39	4	4	3	7.3	3.2
September	100	45	4	4	3	8.4	3.8
October	100	40	4	4	3	8.4	3.4
November	102	34	4	4	3	8.6	2.9
December	92	33	4	4	3	7.6	2.8
Average						9.1	3.6

b/m/h = bites per man per hour

Table 4.6c: Monthly Biting Rate from CDC Light Trap Collection from Swampy grassland (Doma LGA) Eco – Setting of Nasarawa State

Months of collection (2017)	*Anopheles* per night per trap		Number of traps per night		Number of collection nights	Human Biting Rate (HBR) per night per trap (b/m/h)	
	Indoors	Outdoors	Indoors	Outdoors		Indoors	Outdoors
January	93	58	4	4	3	7.7	4.8
February	96	65	4	4	3	8.0	5.5
March	104	60	4	4	3	8.7	5.0
April	117	103	4	4	3	14.2	8.6
May	170	130	4	4	3	12.0	10.9
June	144	111	4	4	3	12.3	9.3
July	147	102	4	4	3	9.7	8.5
August	116	69	4	4	3	8.8	5.8
September	106	71	4	4	3	9.1	5.9
October	109	56	4	4	3	8.2	4.7
November	98	63	4	4	3	8.2	5.3
December	82	59	4	4	3	6.9	4.9
Average						9.5	6.6

b/m/h = bites per man per hour

Table 4.6d: Spatial Variations of Abdominal Conditions of Anopheline Mosquitoes Collected across the Selected Eco-Settings of Nasarawa State

Eco – settings	Unfed	Fed	Gravid	Half gravid	Total	No of occupants
Wooded/Grassland (Karu LGA)	239 (40.51)	320 (56.02)	277 (48.79)	314 (54.68)	1150 (32.96)	172 (36.13)
Sparse woodland (Nassarawa Eggon LGA)	274 (42.39)	319 (49.49)	346 (54.29)	346 (51.01)	1285 (36.83)	153 (32.14)
Swampy Grassland (Doma LGA)	173 (32.75)	267 (50.63)	287(54.76)	327 (62.13)	1054 (30.21)	151 (31.72)
Average	686 (19.66)	906 (25.97)	910 (26.08)	947 (27.14)	3489 (100)	476(100)

χ^2 Cal =17.19; χ^2 tab = 12.59; df =6; LGA = Local Government Area

Table 4.7: *Plasmodium* Sporozoites Identified by ELISA across the Selected Eco-settings in Nasarawa State

Eco – settings	CDC Indoors		CDC Outdoors		PSC		Total	
	No. examined (%)	No. +vepf (%)	No. examined (%)	No. +vepf (%)	No. examined (%)	No. +vepf (%)	No. examine (%)	No. +vepf (%)
Wooded grassland	150(30.0)	29 (32.9)	150 (30.0)	22 (27.)	200 (30.0)	15 (22.73)	500 (33.33)	66 (13.2)
Sparse Woodland	150 (30.0)	26 (29.54)	150 (30.0)	20 (31.25)	200 (30.0)	18 (28.13)	500 (33.33)	64 (12.8)
Swampy Grassland	150 (30.0)	33 (37.5)	150 (30.0)	39 (38.61)	200 (30.0)	29 (28.71)	500 (33.33)	101 (20.2)
Total	450 (30.0)	88 (19.56)	450 (30.0)	81 (18.0)	600 (40.0)	62 (10.33)	1500 (100)	231 (15.4)

χ^2 Cal =2.75; χ^2 tab = 9.48; df =4

Table 4.8a: *Anopheles gambiae s.l* M and S Molecular Forms Identified by PCR

Eco-Settings	CDC Indoors					CDC outdoors				
	No. PCR +ve	***An. coluzzi*(Mform)**	**Hybrid**	***An. gambiae* (S form)**	***An. arabiensis***	**No. PCR +ve**	***An. coluzzi*(Mform)**	**Hybrid**	***An. gambiae*(S form)**	***An. arabiensis***
Wooded grassland	150	15 (10.0%)	01(0.66%)	132(88.0%)	02 (1.33%)	150	13(8.67%)	0(0.0)	125(83.33)	12(8.0%)
Sparse Woodland	150	15 (10.0%)	02 (1.33%)	130(86.67%)	03 (2.0%)	150	18 (12.0%)	01 (0.66)	120 (80.0%)	11 (7.33%)
Swampy Grassland	150	20 (13.33%)	05(3.33%)	118(78.67%)	07 (4.67%)	150	21(14.0)%	02(1.33)	112(74.67)	15(10.0%)
Total	450	50 (11.11)	8 (1.7)	380 (84.44)	12 (2.67)	450	52 (11.56)	3 (0.67)	357 (79.33)	38 (8.44)

χ^2 Cal =27.01; χ^2 tab = 12.59; df =6

Table 4.8b: *Anopheles gambiae* s.l. M and S Molecular Forms Identified by PCR

Eco-Settings	CDC Indoors					CDC outdoors				
	No. PCR +ve	***An. coluzzi*(Mform)**	**Hybrid**	***An. gambiae* (S form)**	***An. arabiensis***	**No. PCR +ve**	***An. coluzzi*(Mform)**	**Hybrid**	***An. gambiae*(S form)**	***An. arabiensis***
Wooded grassland	200	35 (17.5)	1 (0.5)	64 (32.0)	28 (14.4)	500	63 (27.16)	2 (12.5)	321 (31.71)	42 (25.61)
Sparse Woodland	200	45 (22.5)	1 (0.5)	123 (61.5)	31 (15.5)	500	78 (33.62)	4 (25.0)	373 (36.86)	45 (27.44)
Swampy Grassland	200	50 (25.0)	3 (1.5)	88 (44.0)	55 (27.5)	500	91 (39.22)	10 (62.5)	318 (31.42)	77 (46.95)
Total	600	130(21.67)	5(2.5)	275(45.83)	114(19.0)	1500	232(100)	16(100)	1,012(100)	164 (100)

χ^2 Cal =27.01; χ^2 tab = 12.59; df =6

4.1.10 Blood meal sources across the eco – settings of Nasarawa State

Among the six hundred (600) blood – fed adult female *Anopheles gambiae s.l* mosquitoes collected, 465 (77.50 %) were analyzed by ELISA to determine the sources of their blood meals and also by PCR to determine their species. ELISA results for the sources of the blood meal indicated that most 222 (47.47 %) of the blood meals were from humans followed by bovine 142 (30.54 %) while the least were 101 (21.72 %) in goats (Table 4.10).

4.1.11 Blood meal source for *Anopheles gambiae s.s* and *An. arabiensis*

Blood meal sources for *Anopheles gambiae s.s* and *An. arabiensis* mosquitoes collected were analyzed by ELISA to determine the sources of their blood meals. Blood meal sources identified from *An. gambiae s.s* were (95.95 %) from humans, (45.07 %) from bovine and (33.66 %) from goats (Table 4.11). Moreso, some *An. gambiae s.s* mosquitoes obtained blood – meals from single sources, that is, 98.56 % from humans, 44.44 % from bovines, and 28.33 % from goats. *An. arabiensis* obtained blood meal from humans (1.44 %), bovines (55.56 %) and (71.67 %) from goats.

Mixed blood meal sources identified from *An. gambiae s.s* included 75.61% human/goat, 69.57 % human/bovine, 66.15 % human/bovine/goat and 45.45 % bovine/goat. Also, *An. arabiensis* are 24.39 % human/goat, 30.43 % human/bovine, 33.85% human/bovine/goat and 54.55 % bovine/Goat. The ELISA blood – meal results also showed that no blood – meal source from chickens was identified from both *Anopheles gambiae s.s* and *An. arabiensis* (Table 4.16).

Table 4.9: Knock Down Resistant (Kdr) across the Eco-Settings in Nasarawa State

Eco – settings	Knockdown Resistance (Kdr)							
	Kdr- West (RR)		Kdr-West (RS)		Kdr-West (SS)		Total	
	No. –ve	No. +ve	No. –ve	No. +ve	No. –ve	No. +ve	No. –ve	No. +ve
Wooded grass land	56 (35.82)	12(17.65)	49 (69.01)	22 (30.99)	29 (45.54)	32 (52.46)	134 (67.0)	66 (33.0)
Sparse Woodland	54(80.60)	13(19.40)	46 (68.66)	21 (31.34)	34 (51.52)	32 (48.48)	134 (67.0)	66 (33.0)
Swampy Grassland	56(80.0)	14(20.0)	41(67.21)	20(32.79)	34 (49.28)	35 (50.72)	131 (65.5)	69 (34.5)
Total	166(80.98)	39(19.02)	136(68.34)	63 (31.66)	97(49.49)	99 (50.51)	399 (66.5)	201 (33.5)

χ^2 Cal =0.34; χ^2 tab = 9.48; df =4

Table 4.10: Blood Meal Sources across the Eco- Settings in Nasarawa state

Eco – settings	Blood Meal sources			Total (%)
	Human (%)	Bovine (%)	Goat (%)	
Wooded grassland	69(49.29)	47(33.57)	24(17.14)	140(30.11)
Sparse Woodland	71(47.65)	42(28.19)	36(24.16)	149(32.04)
Swampy Grassland	82 (46.59)	53(30.11)	41(23.30)	176(37.85)
Total	222(47.74)	142(30.54)	101(21.72)	465(77.5)

χ^2 Cal =2.78; χ^2 tab = 9.48; df =4

Table 4.11: Blood Meal Source for *Anopheles gambiae* and *Anopheles Arabiensis*

Species	*Anopheles gambiae s.s* (%)	*Anopheles arabiensis*(%)	Total	χ^2 Calculated	χ^2 Tabulated
Blood Meal sources					
Human	213 (95.95)	9 (4.05)	222 (47.74)		
Bovine	64 (45.07)	78 (54.93)	142 (30.54)	45.07	5.99
Goat	34 (33.66)	67 (66.34)	101 (21.71)		
Single Blood meal sources					
Human	137 (98.56)	2 (1.44)	139 (49.64)		
Bovine	36 (44.44)	45 (55.56)	81 (28.93)	32.45	5.99
Goat	17 (28.33)	43 (71.67)	60 (21.43)		
Mixed Blood meal sources					
Human/Goat	31 (75.61)	10 (24.39)	41 (22.16)		
Human/Bovine	32 (69.57)	14 (30.43)	46 (24.86)	13.76	7.82
Human/Bovine/Goat	43 (66.15)	22 (33.85)	65 (35.14)		
Bovine/Goat	15 (45.45)	18 (54.55)	33 (17.84)		

4.1.12 Entomological Inoculation Rate (EIR)

Entomological Inoculation Rate (EIR) was determined across the various Eco – settings of Nasarawa State from the product of sporozoite rate (SR) and Human Biting (HBR). A total of 42.8 % Infective Bite per Person per night (IBP) was recorded as the highest in the month of October while the least 9.4 % IBP was recorded in the month of March for Indoors collected mosquitoes while the outdoors had the highest IBP of 15.9 % in the month of July and 3.6 % IBP lowest in the month of March at wooded grassland eco-settings of Karu LGA for the EIR (Table 4.12a).

In the Sparse woodland eco-setting, a total of 52.4 % IBP was recorded in the Month of May which is the highest and the lowest was 7.9 % IBP for indoor collected mosquitoes while in outdoors mosquitoes collection July recorded the highest 16.8 % IBP and the lowest was in the month of March 2.5 % IBP of the EIR (Table 4.12b)

The Swampy grassland eco-settings 49.2 % IBP which is the highest in the month of June for the mosquitoes collected indoors while 8.2 infective bite per person per night which is the lowest in the month of October. The outdoors collected mosquitoes, with 37.2 % infective bites per person per night was recorded which is the highest in the month of June while 14.1 % infective bite per person per night was recorded in the month of October which is the lowest EIR for outdoors at Swampy grassland (Table 4.12c)

Table 4.12a: Entomological Inoculation Rates for Outdoors and Indoors (EIR) for Wooded Grassland Eco – setting

Months	Indoor					Outdoor				
(2017)	No. Collected	No. examined	SR %	HBR (b/m/h)	EIR b/p/n)	No. Collected	No. examined	SR %	HBR (b/m/h)	EIR (b/p/n)
Jan	106	12	2.0	8.80	17.60	33	12	2.0	2.80	5.60
Feb	136	16	3.0	11.30	33.90	43	16	0.0	3.60	0.00
Mar	112	10	1.0	9.40	9.40	43	10	1.0	3.60	3.60
April	145	14	2.0	12.10	24.20	40	14	2.0	3.30	6.60
May	169	12	2.0	14.10	28.20	78	12	2.0	6.50	13.00
June	184	11	2.0	15.40	30.80	81	11	1.0	7.60	7.60
July	160	13	3.0	13.40	40.20	64	13	3.0	5.30	15.90
Aug	142	11	3.0	11.80	35.40	52	11	2.0	4.40	8.80
Sept	126	12	2.0	10.50	21.00	42	12	2.0	3.50	7.00
Oct	128	15	4.0	10.70	42.80	30	15	3.0	2.50	7.50
Nov	119	11	2.0	9.90	19.80	33	11	2.0	2.70	5.40
Dec	130	13	3.0	9.00	27.00	28	13	2.0	2.60	5.20
Total	1657	150	29	136.40	330.30	567	150	22	48.40	1.44

SR = Sporozoites Rates; HBR = Human Bitting Rate; EIR = Entomological Inoculation rate

B/M/H = Bites per Person per Hour; B/P/N = Bites per Person per Night

Table 4.12b: Entomological Inoculation Rates for Outdoors and Indoors (EIR) for Sparse Woodland Eco- setting

Months	Indoor					Outdoor				
	No. Collected	No. Examined	SR %	HBR	EIR	No. Collected	No. Examined	SR %	HBR	EIR
Jan	102	12	2.0	8.60	17.20	37	12	0.0	3.10	0.0 b/p/n
Feb	106	16	0.0	8.80	0.00	31	16	0.0	2.60	0.0 b/p/n
Mar	94	10	1.0	7.90	7.90	29	10	1.0	2.5 b/m/h	2.5 b/p/n
April	113	14	2.0	9.40	18.80	26	14	2.0	2.2 b/m/h	4.4 b/p/n
May	158	12	4.0	13.10	52.40	62	12	2.0	5.2 b/m/h	10.4 b/p/n
June	131	11	3.0	11.00	33.00	66	11	2.0	5.6 b/m/h	11.2 b/p/n
July	121	13	3.0	10.10	30.30	66	13	3.0	5.6 b/m/h	16.8 b/p/n
Aug	97	11	3.0	7.30	21.9 b/p/n	39	11	2.0	3.2 b/m/h	6.4 b/p/n
Sept	100	12	2.0	8.40	16.8 b/p/n	45	12	2.0	3.8 b/m/h	7.6 b/p/n
Oct	100	15	2.0	8.40	16.8 b/p/n	40	15	3.0	3.4 b/m/h	10.2 b/p/n
Nov	102	11	2.0	8.60	17.2 b/p/n	34	11	2.0	2.9 b/m/h	5.8 b/p/n
Dec	92	13	2.0	7.6 b/m/h	15.2 b/p/n	33	13	1.0	2.8 b/m/h	2.8 b/p/n
Total	1316	150	26	109.2	247.5	508	150	20	42.9 b/m/h	78.1 b/p/n

SR = Sporozoites Rates; HBR = Human Bitting Rate; EIR = Entomological Inoculation rate

B/M/H = Bites per Person per Hour; B/P/N = Bites per Person per Night

Table 4.12c: Entomological Inoculation Rates for Outdoors and Indoors (EIR) for Swampy Grassland Eco- setting

Months	Indoor					Outdoor				
	No. Collected	No. Examined	SR %	HBR B/M/H	EIR B/P/N	No. Collected	No. Examined	SR %	HBR B/M/H	EIR B/P/N
Jan	93	12	2.0	7.70	15.40	58	12	3	4.80	14.40
Feb	96	16	3.0	0.80	24.00	65	16	3	5.50	16.50
Mar	104	10	3.0	8.70	26.10	60	10	4	5.00	20.00
April	117	14	3.0	14.20	42.60	103	14	3	8.60	25.80
May	170	12	4.0	12.00	48.00	130	12	2	10.90	21.80
June	144	11	4.0	12.30	49.20	111	11	4	9.30	37.20
July	147	13	3.0	9.70	29.10	102	13	3	8.50	25.50
Aug	116	11	3.0	8.80	26.40	69	11	2	5.80	11.60
Sept	106	12	2.0	9.10	18.20	71	12	4	5.90	23.60
Oct	109	15	1.0	8.20	8.20	56	15	3	4.70	14.10
Nov	98	11	2.0	8.20	16.40	63	11	5	5.30	26.50
Dec	82	13	3.0	6.90	20.70	59	13	3	4.90	14.70
Total	1382	150	33	113.80	312.90	947	150	39	79.20	251.70

SR = Sporozoites Rates; HBR = Human Bitting Rate; EIR = Entomological Inoculation rate

B/M/H = Bites per Person per Hour; B/P/N = Bites per Person per Night

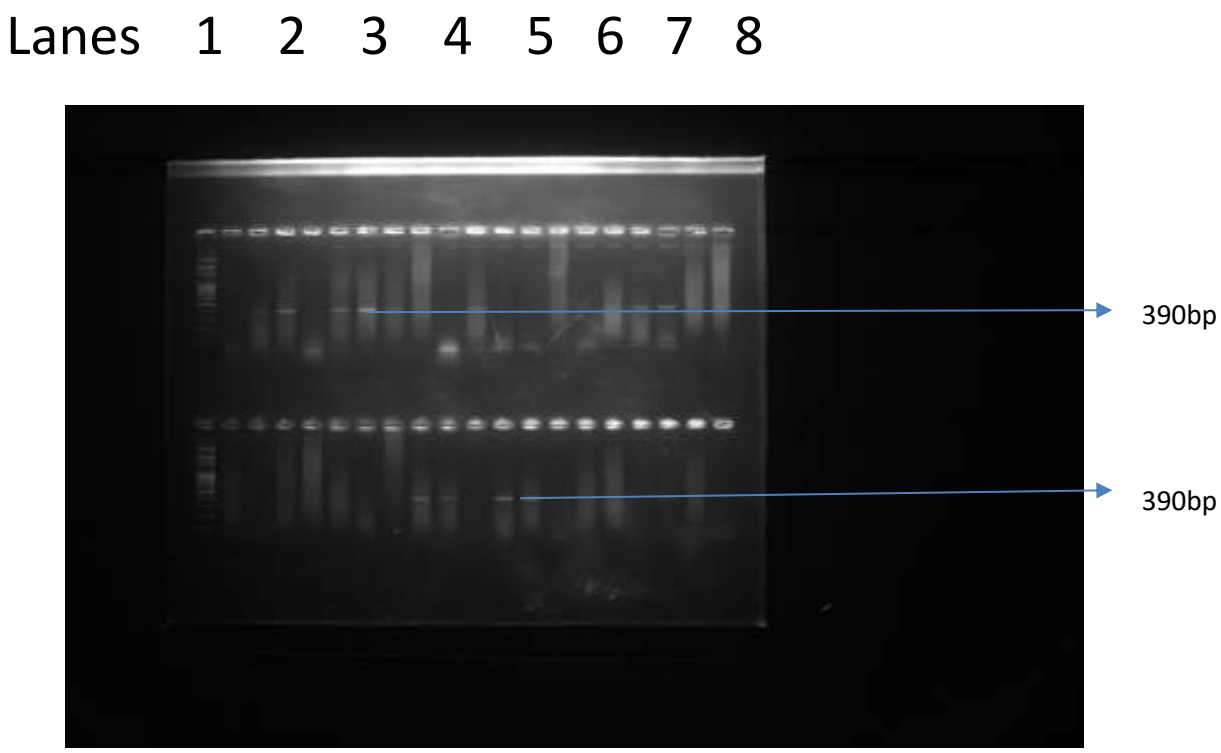

Plate I: Molecular form of *Anopheles gambiae s.s*

Plate I: *An. gambiae* PCR identification a) Lane 1, 1 kb ladder lane 3 Arrow at the top is *An. gambiae* 390bp (down arrow), Lane 4-5 still indicates *An.gambiae s.s* Primers create fragments of 390 bp *An. gambiae* s.s

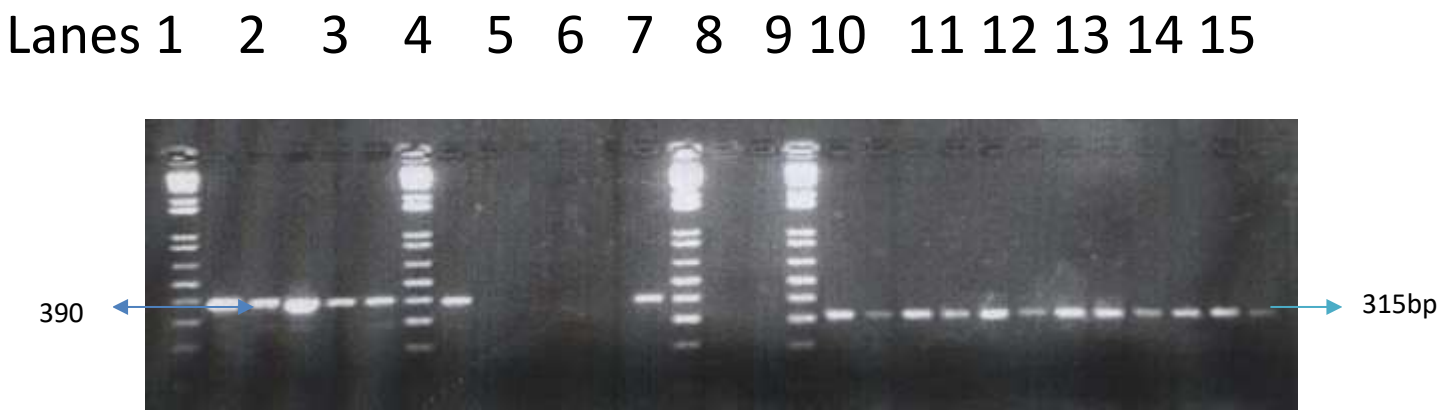

Plate II: Molecular forms for *Anopheles gambiae s.s*and *Anopheles arabiansis*

PCR identification of*Anopheles gambiae s.s*390 bp and *Anopheles arabiensis*

Plate II: *An. gambiae and An. arabiensis*PCR identification a) Lane 1, 1 kb ladder lane 2*An. gambiae (*left arrow*)*, lane 7-10 1kbladder, lane 11-15 *An. arabiensis (*down right,Primers create fragments of 390bp for *An. gambiae*, 315bp for *An. arabiensis*.

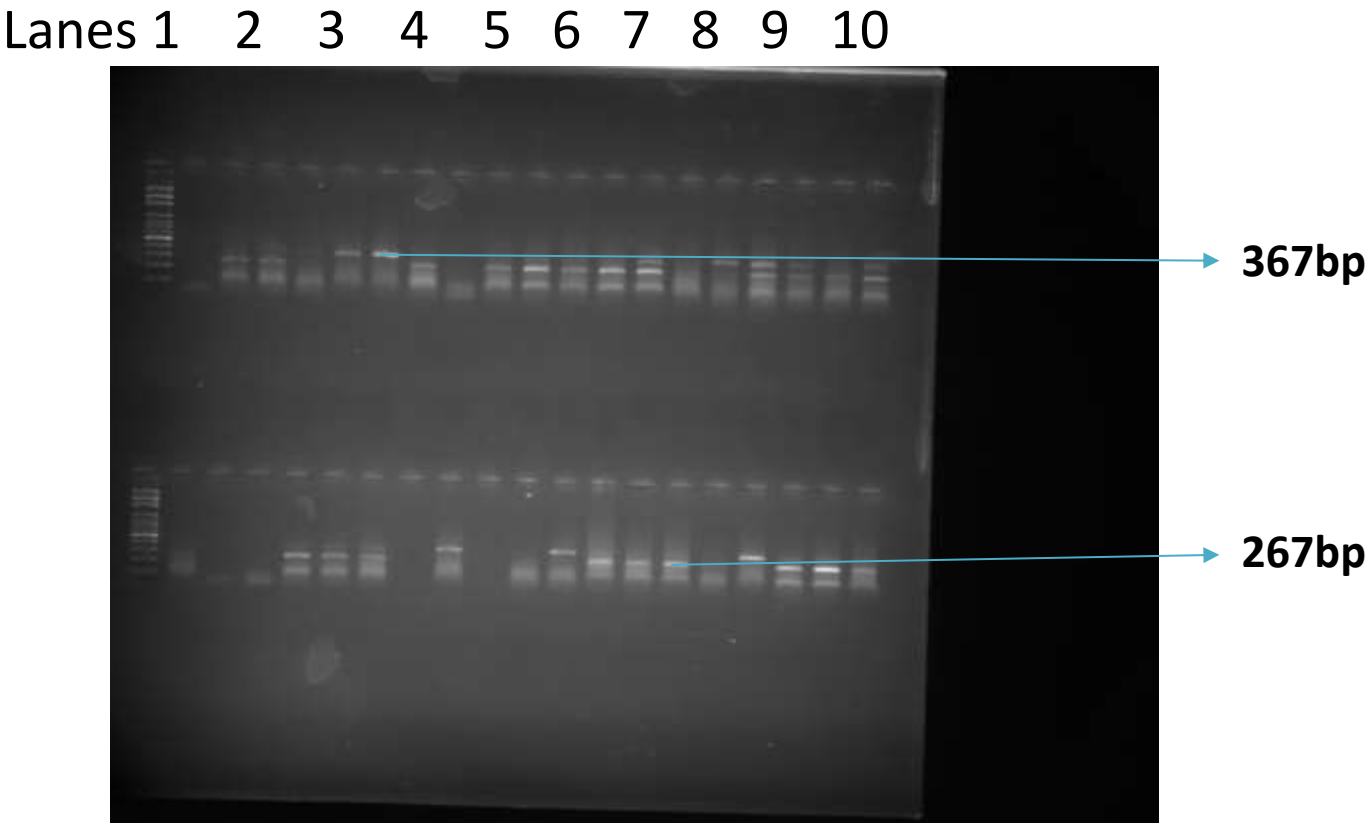

Plate III: Molecular forms of *Anopheles gambiae s.s* and *Anopheles colluzzi* (M & S forms)

The bands on the plate above clearly show that the mosquitoe vectors are *Anopheles gambiae s.s*due to the bands alignment at 257 and 367 base pairs (bp) respectively.

Plate III: *An. gambiae* PCR identification a) Lane 1, 1 kb ladder, lane 3-4 *An. gambiae M form An. colluzzii* (top arrow right), Lane 7-8, (down arrow right), *An. gambiae s.s* S form Primers create fragments of 367bp for *An. gambiae* M form (*An. colluzii*), and 267bp *An. gambiae s.s* S form *(An.gambiae s.s).*

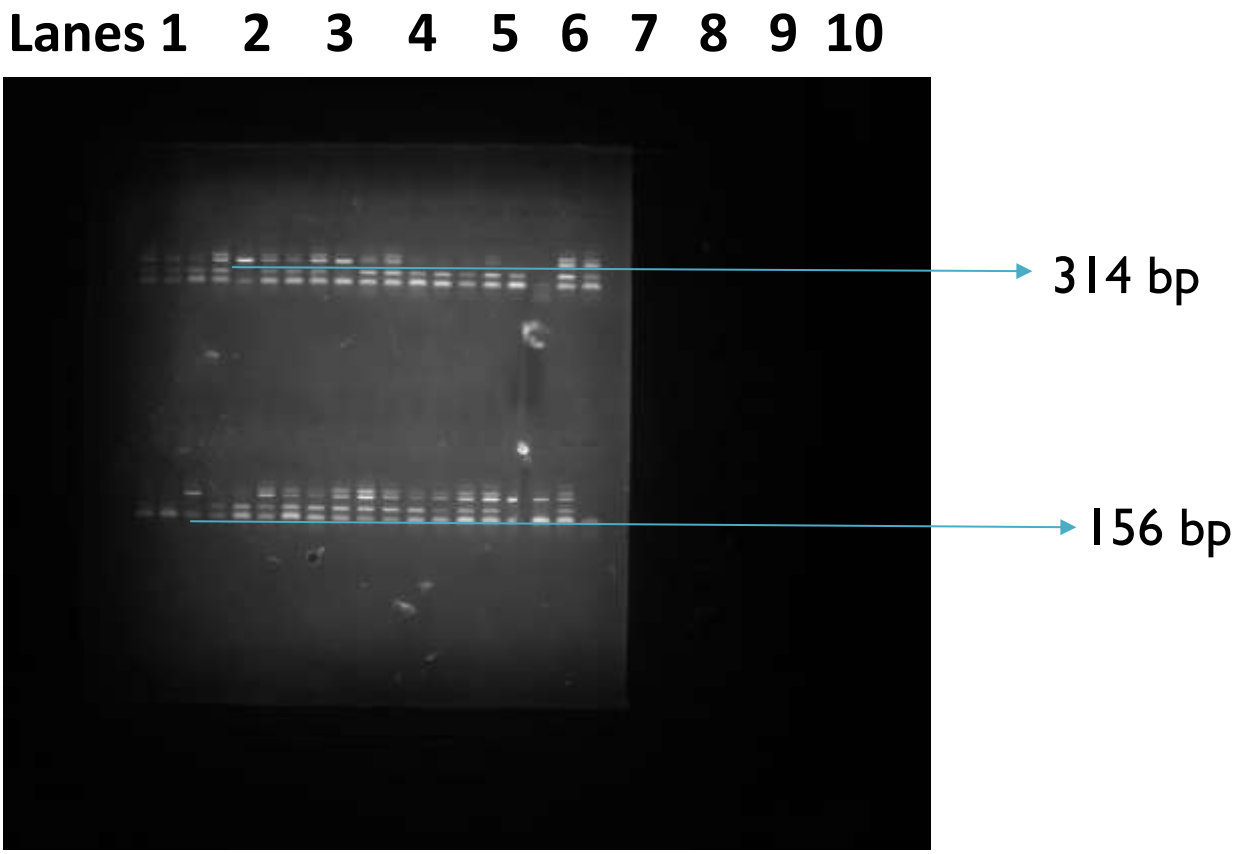

Plate IV: Kdr Homozygous resistant RR

Plate III: *An. gambiae* Kdr identification) Lane 1, 1 kb ladder, lane 3-4 *(*top arrow right indicates a suscesful reaction with a 314 base pair bp*)*, Lane 3-4, *(*down arrow right with 156 base pair bp*)*, indicates the the homozygous resistant allele of *An. gambiae s.s* Primers create fragments of 314bp for a successful reactions and and 156bp indicating a homozygous resistance allele.

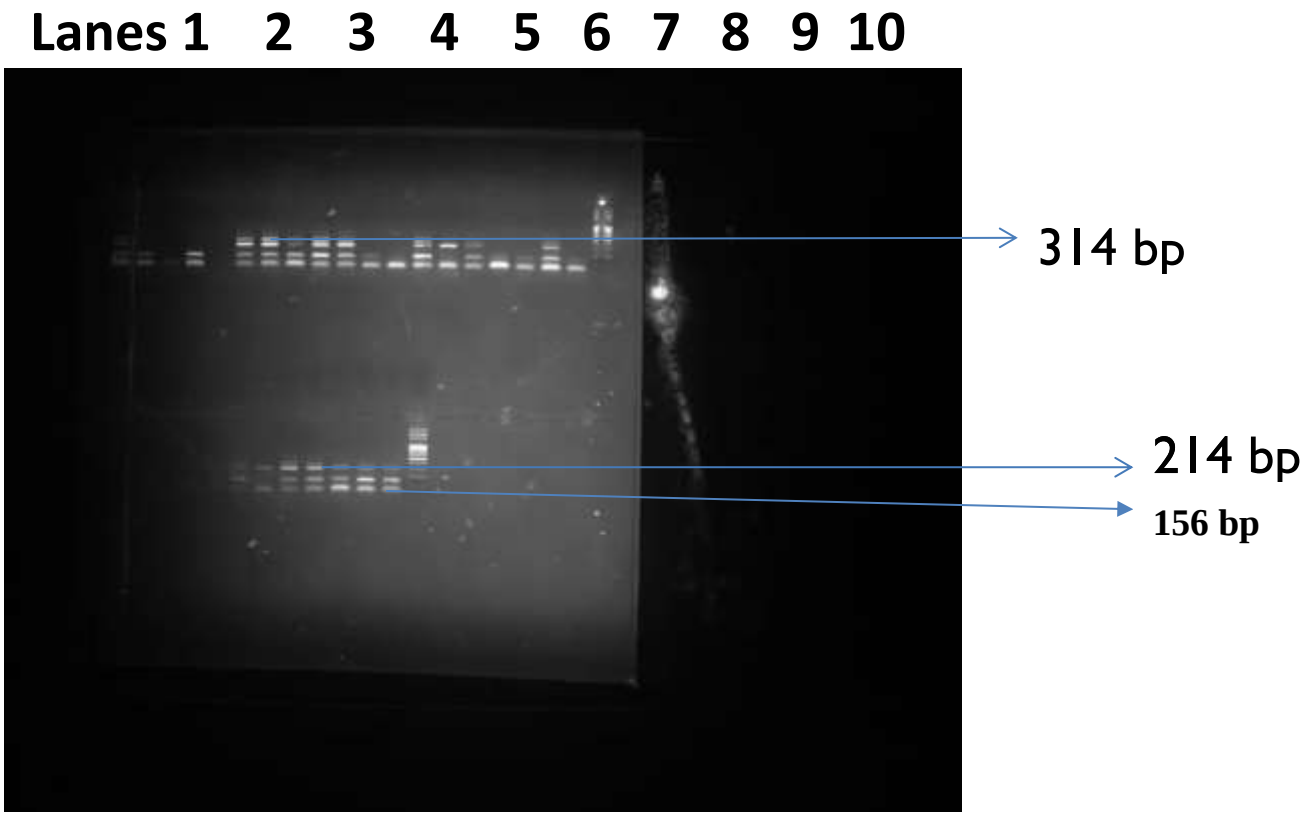

Plate V: Kdr Heterozygous resistance RS

Plate V: *An. gambiae* Kdr identification a) Lane 1, 1 kb ladder, lane indicates a successful reaction with 314 bp with arrow top right, lane 2-3 indicates susceptible allele with 214 bp arrow middle right and lane 3-4 indicates a resistant allele with 156 bp arrow dwn right of *An. gambiae s.s* the Primers create fragments of 314bp for a sucesful reaction, 214bp for a susceptible allele and 156bp of *An. gambiae s.s* indicating a heterozygous resistance allele.

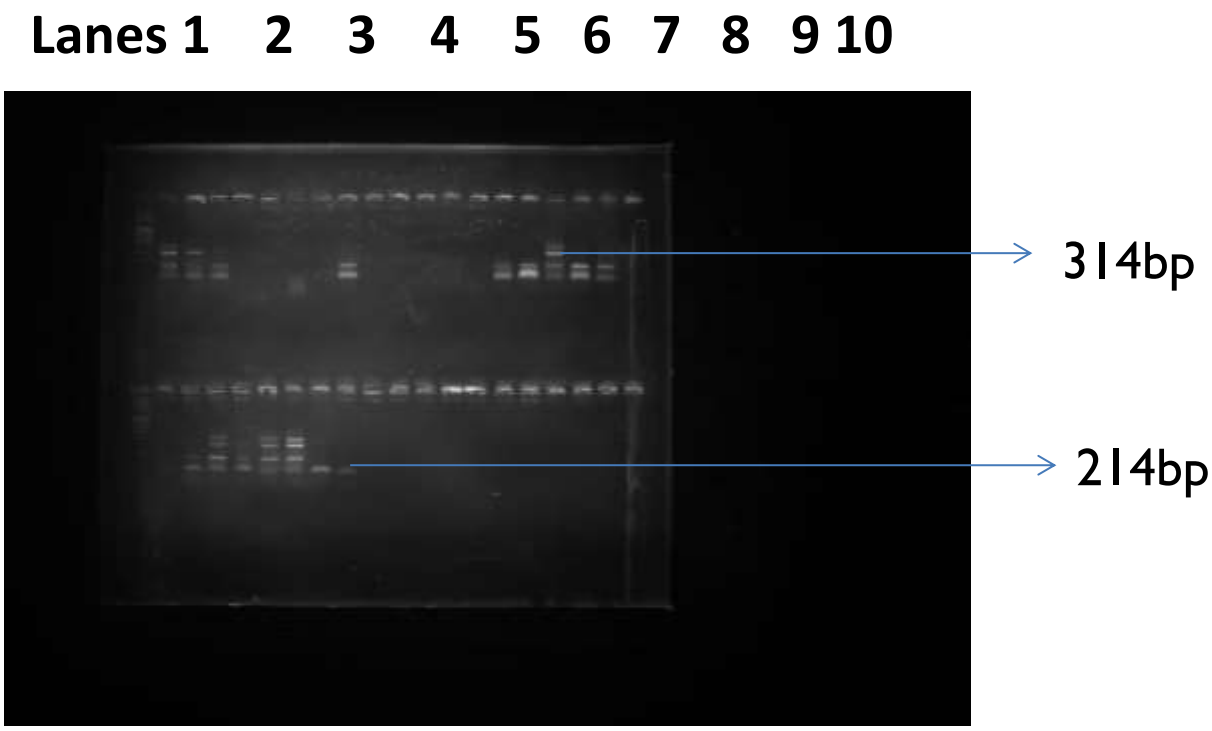

Plate VI: Kdr Homozygous susceptible SS

Plate V: *An. gambiae* Kdr identification a) Lane 1, 1 kb ladder, lane 5-6 indicates a successful reaction with 314 bp with arrow top right, and lane 3-4 indicates susceptible allele with 214 bp arrow down right of *An. gambiae s.s* the Primers create fragments of 314bp for a sucesful reaction, 214bp for a susceptible allele *gambiae s.s*is *indicating* a homozygous susceptible allele.

4.2 Discussion

The results of the present study revealed that the populations of *Anopheles* (64.09 %) mosquitoes were more abundant than the Culicines, (46.41 %). This observation may be attributed to rainfall pattern and water storage habits of the inhabitants across the study areas. The topography of the areas is undulating with some valleys which allows water to be retained that created a lot of water pools that serve as a potential breeding sites for the vectors as ground pools have been known to form the prolific breeding sites of the *Anopheles* mosquitoes as compared with culicines according to the findings of Olayemi and Ande (2008a) and Adeleke *et al.* (2018).

In all the three eco – settings studied, it was observed that there was presence of numerous water bodies created by rain in addition to breeding in small water storage containers utilized by people for household chores. In all the sampled towns and villages, the supply system was erratic and this explains the use of numerous water storage facilities to provide water for domestic chores, irrigation, car wash and other construction purposes. All these turned out to be condusive breeding sites for mosquitoes within and near human habitation. It was also observed that water storage in cemented and plastic tanks was largely responsible for the abundance of *Anopheles* mosquitoes in all the zones, especially in the rainy season. This finding is also similar report of Sharma (2008) in semi-arid district of Rajasthan, India and Oyewole *et al*. (2016) in Badagry axis of Lagos Lagoon, Lagos, Nigeria.The result of the morphological examination of the mosquitoes revealed the predominance of *Anopheles* mosquitoes over *Culex* mosquitoes. This observation is very important because it pomp out that *Anopheles* species of mosquitoes are breeding in the study areas most of which are encouraged by human activities. This finding is in conformity with work of A. A Madara and N.O. Abdulraheem (2013) in the University of Abuja main campus, Abuja

FCT, Nigeria, who reported that *Anopheles* mosquitoes are the most abundant as compared to *Culex* and *Aedes* mosquitoes.

The swampy grassland eco-settings had more and abundance of the vectors compare to other selected eco-settings of Sparse woodland and wooded grassland as this may be attributed and linked with the swampy areas that has created abundant and potential breeding sites than other eco-settings as this explain why the vectors were highly prolific interms of their feeding and distributions across the eco-setting this finding is in conformity with the findings of Lamidi *et al.* (2017) in Taraba state North east Nigeria. The presence of large water bodies across the eco-settings was also responsible for the distribution and abundance of the mosquitoes which easily invaded the nearby houses to feed and transmit Malaria. Kaiser *et al.* (2005) reported that 88 % of the World malaria cases occurred among 9.4 million individuals who live in a nearby dams, and irrigation schemes in sub-Saharan Africa which was the case across the selected eco-settings. As many authors also reported that mosquitoes can travel up to 5 kilometers from the breeding from their breeding sites to invade human habitations according to Charlwood *et al.* (2000b). Extensive farming activities within the selected eco-settings have negative implications on the mosquito's distribution, abundance, and vectorial capacity within the selected eco-settings, more so lack of proper and adequate canals and channels of sewage disposal, constuctions of road and other temporary/permanent breeding sites have attracted a lot of mosquitoes across the selected eco-settings all these human activities have contributed to the distribution and abundance of the mosquitoes due to the potential breeding sites created alongside Agricultural activities involved. In similar vein the intense Agricultural activities such as irrigation and enormous rice farms were also responsible for the high density and abundance of the Anopheline mosquitoes encountered this is in line with the findings of Marrama *et al.*

(2004) who stated that malaria transmission is 150 times higher in a Manmade breeding sites than the natural eco-settings and 90 % of the malaria infections is caused by *An. gambiae. s.s* and *An funestus* which is in line with the findings in this study.

This study has also observed that fallow lands left after rice harvesting were higly responsible and suitable potential breeding sites for the vectors that causes a large number of mosquitoes to emerge from such sites and invade the human habitations around as *An.gambiae* and *An. funestus* were the dominant species encountered which are the principal vectors of malaria transmission within the selected eco-settings this findings agrees with the findings of Himeidan and Kweka (2012) who reported that farmlands constitute about 40 % of the mosquitoes larval habitat.

In this study also interms of the seasonal variations in the population density of Anopheline mosquitoes collected across the eco-settings *An.gambiae* were the predominant species they were significantly high due to the availability of the potential breeding sites created by rainfall that is being experienced within the selected eco-settings which is a natural phenomenon and occurrence within the guinea savannah region in addition to the heterogeneity in Anopheline mosquitoes species composition at the macro-geographic scale as Mbogo *et al.* (2003) also reported the differences in the relationship between mosquitoes population density and rainfall in different district of Kenya and narrow that to environmental heterogeneity which is in agreement with the findings in this study. More so the preoponderence of *Anopheles* mosquitoes caught during bthe wet seasons compared to the dry seasons across the eco-settings were determined by the amount of annual and rainfall alongside temperatures, relative humidity and high vegetations which is inline with the In a similar report by Lamidi *et al*. (2017), they reported that *Anopheles* mosquitoes were most dominant in their study in three Riverine communities in Taraba state, North Eastern Nigeria. This finding is

also in conformity or agreement with the work of Bunza *et al*. (2010), in Katsina State and Oduola *et al*. (2016) in Kwara state respectively, who stated that *Anopheles* species were the most abundant mosquito species generally.

Although, the result of this study is not in conformity with the work of Afolabi *et al*. (2019) and Akunne *et al*. (2015) in Akure, Ondo state and Nnamdi Azikiwe University, Awka respectively. In their separate studies, they all reported that *Culex* mosquito species were the most abundant the study area, which comprised of three eco – settings and has a guinea savanna type of vegetation with high temperature all year round and rainfall lasting in six (6) months (Kowal and Knobe , 1972), therefore, might have occasioned the result obtained. The population of *Anopheles* mosquito is the highest in the adult mosquitoes collected from all the study sites because some *Anopheles* species do exhibit high sense of genetic heterogeneity that enables it to adapt to many ecological zones, as reported by Coluzzi *et al*. (1985). The adult stage can also withstand harsh environmental conditions when compared with other species.

On the basis of monthly variation of *Anopheles* mosquitoes collected in the study areas, the results revealed that the population density of the *Anopheles* species increased tremendously between the month of May and June and this corresponds to the onset of the rainy season. The monthly variation of *Anopheles* mosquitoes in the three eco – settings showed that *Anopheles* mosquitoes were most abundant in the month of May (12.88 %) followed by June (12.37 %) while the least was recorded in the month of February (5.40%). The variation in monthly abundance of *Anopheles* mosquitoes could be attributed to a number of factors, one of which is that the eco – settings are located around riverine areas and as such experienced seasonal flooding which usually provides favourable temporary and permanent breeding sites for *Anopheles* mosquitoes (Bunza *et al.*, 2010). This finding is similar to a recent study conducted in three selected areas of

Taraba by Lamidi *et al*. (2017). He reported that *Anopheles* mosquitoes were most abundant in the month of May and least in November. The result of this study is not in conformity with the work of Ebube *et al*., (2018), who in his study reported that *Anopheles* mosquitoes were most dominant in the month of July.

In this study, the seasonal variation in the population of *Anopheles* mosquitoes across the selected three eco – settings of Nasarawa state across the seasons were also studied. The result of this study showed high relative abundance of *Anopheles* mosquitoes in the rainy season (61.17 %) compared to (38.81 %) encountered in the dry season. The significantly higher *Anopheles* mosquitoes collected in the rainy was as a result of a lot of breeding sites created by the abundant rainfall experienced. The finding of this study is similar to that of Olayemi *et al*. (2011), and Ebenezer *et al* (2014), who reported a higher abundance of *Anopheles* mosquitoes in the rainy season and low in dry season in North Central Nigeria and Bayelsa state respectively.

Similarly, the high preponderance of *Anopheles* mosquitoes in the wet (rainy) season was because the range and relative abundance of *Anopheles* mosquitoes are determined by the amount of annual rain, annual wet season temperatures couple with high vegetation (Oyewole *et al.*,2007).

Six (6) species of Anopheline mosquitoes were encountered throughout the study period and in all the eco – settings. Depinay *et al*. (2004) put the usual number at less than five within a given area, and this has been confirmed in different localities in Africa (Manga *et al.*, 1995, Appawu *et al*., 2004; Muturi *et al*., 2006). The relative higher number of *Anopheles* species in the area may be as a result of the favourable tropical weather and breeding conditions of the six *Anopheles* species encountered in the study areas, *Anopheles gambiae s.l* and *An. coustani* were most dominant species encountered. The

high abundance of malaria vector (*Anopheles gambiae*) encountered in this study area means that there is a risk of malaria in the study areas and its environs. The unequal distribution of the *Anopheles* species within the area further suggests that the occurrence of the species truly varies according to the micro and macro environmental differences exhibited by different eco – settings as found in studies conducted by Keateng *et al.* (2003).

The environmental conditions of the area were favourable to support the continual breeding and survival of the mosquito vectors. The predominance of *An. gambiae* could be attributed to the adaptability of these species making it possible for them to survive in adverse environment as previously reported by Dondorp *et al*. (2009). The result of this finding is in conformity to the work of Okwa *et al*. (2006), Oguoma and Ikpeze, (2008) who in Lagos and Kano respectively, reported that *An.gambiae* was the most predominant species. However, this results contrasts with the findings of Simon – Oke and Ayeni (2015). The other species collected occurred in very low densities.

Monthly spatial composition of *Anopheles* species encountered in the study areas was also noted. The highest *Anopheles* species population was recorded in the month of May (618±30.0 mosquitoes) and least was recorded in the month of January (140±10.0 mosquitoes). The composition and distribution of *Anopheles* species varies significantly ($p<0.05$). *Anopheles gambiae s.l* was the most dominant (111.58±13.59 mosquitoes) species encountered throughout the months of the study. *An. gambiae s.l* abundance in this study is also in conformity with other studies in different geo – political zones within Nigeria (Awolola *et al*., 2005; Umaru *et al*., 2007; Okwa *et al.*, 2007; Oyewole *et al*., 2007; Oguoma and Ikpeze, 2008; Oguoma *et al*., 2010).

Anopheles gambiae s.l was more abundant in the wet months, followed by *An. coustani* and *An. funestus* at the end of the rainy season. These three species seems to complement each other in order to sustain the endemicity of malaria in the sampled towns/villages across the three eco – settings. Faye *et al.* (1995) also observed a high variation of *An. gambiaes.l* population in the Sahelian area of Niger Republic. Also, similar findings were reported by Lamidi (2009). Moreover, Ebenezer *et al.* (2014) reported that *An. gambiae s.s, An. arabiensis* and *An. funestus s.s* alternate in malaria transmission across the year especially during the rainy period and as such all should be targeted for effective malaria vector control. Consequently, the high proliferation rate of *An. gambiae s.l, An. funestus* and *An. coustani* observed have been explained by other previous reports, for example, a study was conducted to examine the tolerance level of *An. gambiae* eggs to desiccation during the dry season by Mbogo *et al.* (2004) indicated that *An. gambiae* eggs can survive up to 15 days under dry conditions, with their susceptibility to desiccation depending on type of soil. However, the population of *Anopheles gambiae* occurs immediately at the set of rainy season (Oyewole *et al*., 2005).

Species composition of *Anopheles* mosquitoes collected from indoor and outdoor was also analysed. The result of this study revealed that most *Anopheles* mosquitoes were caught indoors (68.21%) across the three eco – settings of Nasarawa state. This finding is in conformity with the reports of Getachuw *et al.* (2009), Ndiath *et al.* (2011) and Dehela *et al.* (2017), who reported that *Anopheles* mosquitoes were predominantly endophagic and as such tend to reside indoors than outdoors that was shown in the bitting rhythms which usually commences at 10 pm as the peak biting period was noticed at 2-4 am for both indoors and outdoors collections across the selected eco-settings in Nasarawa state as this has confirmed the endophagic and exophagic nature of

the vectors as the endophagic and endophilic nature of the vectors were eminent which is in conformity with the findings of Oduola *et al.* (2016) at Ilorin kwara state North Central Nigeria and (Sadanandane *et al.,* 2004; Mathenge *et al.,* 2004, 2005; Mbogo *et al.,* 2011; Govella *et al.,* 2011).

This work confirmed that the method used influences the quality and the variety of mosquitoes collected. In this study, the highest number of *Anopheles* mosquitoes were collected using CDC (64.69 %) method than PSC (35.31 %). This is probably due to the multiple attraction stimuli displayed by the method (light).

Adult mosquitoes were also collected by Pyrethrum Spray Catch (PSC) and identify and graded according to their abdominal conditions. Among the various abdominal condition observed, Half Gravid (HG) were most abundant. This high proportion of the half gravid females of *Anopheles* mosquitoes could be due to the non – use of protective clothing during the night and indigestion (Okorie *et al.,* 2011). The result of this study contrasts the findings of Okorie *et al*. (2014), who reported that a remarkable number of mosquitoes were unfed. This may be influenced by some host seeking factors; these mosquitoes may have been trapped indoors while searching for blood meal after their emergence from the breeding sites (Govella *et al.,* 2011).

This study also revealed the endophagic, exophagic behaviour of the malaria vectors across the selected eco-settings as this was observed through the hourly collections of the vectors of which at the wooded grassland eco-settings the bitting activities of the vectors commences from 8:00 pm through 5:00 am and the peaked biting activities were noticed within the hours of 12:00 to 2:00 am and the declined in bitting activities of the vectors was noticed within the hours of 3:00 am to 6:00 am respectively. At the Sparse woodland eco-settings the btting activities do commence within the hours of 9:00 pm

through 6:00 am and the peak period of the bitting was noticed within the hours of 2:00 am to 3:00 am and a declined was noticed within the hours of 3:00 am to 4:00 am at the sparse woodland eco-setting and at the Swampy grassland eco-setting the bitting activities do commence within the hours of 8:00 pm and 9:00 pm it declines within the hours of 11:00 pm and the peaked bitting activities was observed within the hours 2:00 am to 3:00 am and a decline in the bitting activities was observed within the hours of 4:00am to 6:00 am respectively this hourly bitting activities that was noticed at the early hours of the day across the selected eco-settings in Nasarawa is in conformity with the findings of Ngom *et al.,* (2013). who also report similar findings within the some eco-settings in Nasarawa Stateand the implications of the peak bitting activity of the vectors at those early hours is that there will be more malaria transmission at that period because many individuals are far asleep at those period without any active activities that will distract the vectors during that period.

Human Biting Rate (HBR) from CDC light trap collection across the selected eco – settings were also determined. The result revealed that the estimated Human Biting Rate (HBR) were different in all the eco – settings studied. The Human Biting Rate (HBR) recorded for *Anopheles gambiae* was very low. The HBR being 16.08 % was recorded in the Swampy grassland eco – setting while wooded grassland and sparse woodland had a HBR of 15.4 % and 12.7 % respectively. This result is not in conformity with the findings of Atting *et al*. (2016), who reported a higher percentage of 76.7 % Human Biting Rate (HBR) in Uyo, Akwa Ibom State. This finding also contrasts with previous findings by Awolola *et al.* (2003) who reported a higher (HBR) of *Anopheles gambiae*. A better understanding of each sibling species within the complex in quite important to help identify their respective roles in disease transmission and the Human Biting Rate

(HBR) can be an important factor in the epidemiology of malaria transmission and in estimating the vector – human contact.

Among the total of 1500 *An. gambiae* s.s adults collected indoors, the S forms were the most preponderant with its highest abundance of 1012 (67.47 %) and the M form (15.47 %) across the selected eco-settings, the wooded grass land had (88.0 %) of the S forms, 86.7 % for the sparse wood land and 78.67 % at the swampy grassland eco-settings for the S forms from the indoor collections and the hybrid forms are 10 and 13 % respectively while the outdoors collections had 83.35 % at the wooded grassland , 80.0 % at the sparse woodland and 74.67 % at the swampy grassland for the outdoor collections.The result of this study showed that of the *Anopheles gambiae s.l* mosquitoes analysed for molecular forms were identified as *Anopheles gambiae s.s* (S – form), *An. coluzzi* (M – form), the hybrid form and *An. arabiensis*. Two sibling species, *Anopheles gambiae s.s* and *An. arabiensis* are of the greatest medical importance in Nigeria. Onyabe and Conn (2001) disclosed that each of these two species were prevalent over the other in at least one locality in each five ecological zones of Nigeria. At adult stage, the M and S molecular forms are highly endophilic as earlier reported. This finding is also consistent with those of Cuamba *et al*. (2006), Souza *et al.* (2010), Richle *et. al.* (2011). The findings of this work conflict the work by Oyewole *et al.* (2010) who reported only M – molecular form in their work. However, this may be in conflict also with the previous reports in some parts of Nigeria where the molecular S – form was found to be the prominent species (Awolola *et al*., 2005). This study also established the predominant S – molecular form than the M – form. Meanwhile, the distribution of the molecular M and S forms of *An. gambiae s.s* is still being determined in most of the West African countries. Also the M & S forms of the An. gambiae encountered at the selected eco-settings were highly endophilic this finding is in

conformity with the findings of Cuamba *et al.* (2006), Riehle *et al.* (2011) and Souza *et al.* (2010). Again the behavioural and ecological differences between the M & S forms across the selected eco-settings are most likely to influence the malaria epidemiology by spatially and temporally widening the areas of the malaria transmission across the eco-settings as all the information obtained in this study on the relative abundance and distribution of the malaria vectors are equally very important for the control of the vectors and malaria infection transmission across the selected eco-settings in Nasarawa State.

In an earlier study of species and populations of the *An. gambiae* complex in Cameroon with special emphasis on chromosomal and molecular forms of *An. gambiae s.s*, Charles *et al.* (2005) reported that the molecular forms of M and S were widespread throughout Cameroon, and assort independently from the chromosomal forms. He further asserted that S – molecular form population were characterized by Karyotypes typical of forest and Savannah chromosomal forms, and M molecular form populations were characterized by karyotypes typical of forest, Savannah and Mopti. Behavioral and ecological differences between the molecular forms (M, S, H and *An. arabiensis*) are likely to influence malaria epidemiology by spatially and temporarily widening areas of transmission. Thus, it can be concluded that M, S, H forms along with *An. arabiensis* are all equally dangerous vectors of human malaria.

About 40 % of the *An. gambiae* mosquitoes caught across the selected eco-sittings were analysed for *Plasmodium* sporozoites identified by ELISA showed an overall (15.40 %) of *Plasmodium falciparum* sporozoite infection rate were positive and well active in malaria transmission this findings agrees with the findings of (Ayala and Coluzzi, 2005). This low sporozoite infection rates cut across the three eco – settings (Sparse woodland, swampy grassland and wooded grassland). There are a lots of reports on

sporozoites and infectivity rates in Lagos state, Nigeria by (Awolola *et al*., 2002; Okwa *et al.*, 2006, 2007), Igbo-Ora, Oya State (Okwa *et al.*, 2007, 2008; Okon *et al.*, 2010), are in conformity with the findings from this study but there is paucity of information on the *Plasmodium falciparium* sporozoites infectivity rates of *Anopheles* mosquitoes Nasarawa State as the information obtained from this study will aid in the control of An. gambiae s.s which was incriminated by the presence of the sporozoite alongside An. funestus and *An. arabiensis* who are also known to be very active in malaria transmission holistically because they maintained the transmission due to their Anthropophilic and zoophilic behaviour as mentioned above this may occur very low as encountered in this study but the malaria transmission will be very consistent which is in conformity with the findings of (Govella *et al.*, 2011). Zoophilic behaviour was observed in some houses that kept some animals such as cattle, sheep and goats at the swampy grassland eco-settings, therefore insecticides should be sprayed on and around the animal's dwellings instead of an indoor spraying of insecticides so as to reduce the population of the *Anopheles* mosquitoes.

The lack of significant differences among the sporozoite infectivity rates between the study areas might probably be due to the fact the parasite and the vector originated from the same geographical region (Awolola *et al.*, 2002). Sporozoite rate of 15.4 % recorded in this study is higher than the *P. falciparium* sporozoite infection rates for *An. gambiae* reported in other studies. For example, Oyewole *et al.* (2010) reported a *P. falciparium* sporozoite rate of 6.70 % and 6.30 % respectively in the year 2001 and 2002 in Igbo – Ora, a rural community of Oyo state, South – West, Nigeria. Also, Oduola *et al*. (2016) reported that *P. falciparium* sporozoite infection rates of *An. gambiae s.s* in six communities of Oyo state varied between 1.9 % and 3.1 %.

Blood fed adult female *An. gambiae s.l* mosquitoes collected were analysed by ELISA to determine the sources of their blood meals source and also by PCR to determine their species. ELISA result for the sources of the blood meals indicated that most (47.47 %) of the blood meal sources were from humans. Blood meals sources for *An. gambiae s.s* and *An. arabiensis* collected were also analysed by ELISA to determine the sources of their blood meals. The result showed that blood meal sources identified from *An. gambiae s.s* were 95.95 % from human, 45.07 % from Bovine and 33.66 % from Goats.

However, in this study, about 95 % of the *Anopheles gambiae s.s* analyzed for blood – meal source were Anthropophagic this revealed 47.47 % of the blood meals were taken from humans, 30.54 % from Bovine and the least was 21.72 % was from goats respectively as the shows that the vectors have high affinity for humans blood than the animals blood in the study areas which is in conformity with the findings of Oduola *et al.* (2014) and the implication is that there will be high rate of malaria transmission to the humans in those selected eco-settings. The mosquitoes in this study survey exhibited complex feeding behaviours. Individual mosquito species frequently fed on human and non – human hosts, even among species considered highly anthropophilic (Orsborne *et al.*, 2018). Also in terms of multiple blood meal sources obtained by the Anopheles gambiae .s.s mosquitoes shows that 98.56 % were from humans, follow by bovine with 44.44 %, 28.33 % from goats respectively while An. arabiensis blood meal obtained from humans were 1.44 %, bovine 55.56 %, and goats 71.67 %, and for the mixed blood meal sources 75.61% was obtained from humans/goats, 69.57 % from human/bovine, 66.15 % from humans/bovine/goats and 45.45 % bovine/goats all obtained by the *Anopheles gambiae .s.s* while *An. arabiensis* obtains blood meals as 24.39 % from humans/goat, 30.43 % from human/bovine, 33.85 % from humans/bovine/goats and 54.55 % from bovine/goats respectively this results has indicated that the malaria

vectors *An. gambiae* have high affinity for humans blood that makes them to be highly Anthropogenic, it also show clearly that *An. arabiensis* are mostly zoophagic base on the blood meal sources obtained and this implies that there will be high rate of malaria transmission in the study area due to the human preference for blood meal source by the vectors this findings agrees with the findings of Souza *et al.* (20110) and disagrees with the findings of Lamidi e*t al.* (2017) in Taraba state North east Nigeria. Also the blood – meal sources were tested only for origin from humans, bovine and goats and no blood – meal source from chickens was identified from both *An. gambiae s.s* and *An. arabiensis*, but there is a possibility that malaria vectors obtain blood meals from all available domesticated animals, as shown by a large number of previous and recent studies indicating that the major malaria vectors in sub-Saharan Africa really adapt to available blood-meal hosts even if they prefer human hosts (1994; Noor *et al.,* 2007; Animut *et al.,* 2013; Massebo *et al.,* 2013; Ngom *et al.,* 2013).

More importantly, the majority of those that had fed on humans had also fed on animals. This strongly suggests a shift in blood – meal sources as a result of the interaction between increased bed net coverage and close proximity of domesticated animals. Comparing the findings of this research with the data obtained from Shililu *et al.* (1998), blood meal source from humans have dropped and from bovines has increased. Mutuku *et al*. (2011) also reported a similar reduction in human blood – meal source and increased blood meal sources from cattle and goats following increased ITN use. The increased incidence of multiple blood feeding as compared to most surveys, could be a differences in blood meal assessment technique, with many studies choosing to pursue a narrower range of possible hosts (Onyango *et al.*, 2013; Keven *et al.*, 2017). High incidence of multiple blood feeding has important epidemiological implications (Tendrow *et al*., 2019). Numerous mosquitoes showed evidence of feeding on human

and one or two additional mammal species. This may increase the probability that a mosquito is also feeding on multiple individuals within a species, thereby amplifying the vectorial capacity of the potential malaria vectors in the selected eco – settings. The Zoophilic preferences of the mosquitoes in this study area, however, may temper their impact on malaria transmission by dedicating potentially infective bites to non – human hosts (Ndenga *et al.*, 2016).

Knock down resistant (KDR) was also determined for the presence of Kdr gene. The result of this finding revealed a high frequency of Kdr (33.50 %) across the three selected eco – setting of Nasarawa state. The gene types encountered were Homozygous Resistant (RR) with (19.02 %), heterozygous susceptible (RS) (31.66 %) and Homozygous Susceptible (SS) with (50.51 %). Across the selected eco-settings swampy grassland eco-settings have the highest resist alleles of 34.5 %, while the sparse woodland and the wooded grassland eco-settings had 33.00 % each. The highest resistant genes found in the swampy grassland eco-settings could be attributed to the high rate of Agricultural practices couple with the indiscriminant use ofpesticides and other herbicides on the farm lands that has brought the issue of resistance on the vectors due to the much and availability of potential breeding sites and the implications of this is that the resistance genes will further enhance and drive the rate of malaria transmission in the areas by the vectors as this will further complicates and lead to the failure of any intervention that is already put in place which is in line with the findings of Liu (2015). In a similar study on the detection of Kdr mutation in Pyrethroids susceptible *An. gambiae s.l* from Ladanai, Kano state, Northwest, Nigeria, Safiyanu *et al*. (2019) reported high frequency of Kdr mutation in Pyrethroids susceptible *An. gambiae s.s*. The presence of Kdr gene gives no indication of the actual strength of resistance level. Even if the Kdr gene is detected or not, the combination of other

resistance mechanism like metabolic based mechanism could also pay crucial role in the impact of resistance (Liu *et al.*, 2006; Liu, 2015; Safiyanu *et al.*, 2019).

Entomological Inoculation Rate (EIR) is an important index in measuring and determining malaria infection transmission in any given area. In this study, the EIR was determined using the product of Sporozoite Rate (SR) and Human Biting Rate (HBR) across selected eco-settings. In wooded grassland eco-settings, 13.20 % of the sporozoite rate was found from the vectors analyzed and 42.8 % infective bites per person per night was recorded in the month of May which is the highest and 9.4 % infective bites per person per night was recorded in the month of March which is the lowest respectively for the indoors mosquitoes collected for the EIR this implies that there will be high rate of malaria transmission. This result disagrees with the findings of Misracc *et al.* (2017) in Gamogofa zone of Southern Ethiopia who reported 17.1 and 3.66 infective bites per person per night.

In Sparse woodland eco-settings, 12.80 % of the sporozoites rates was found from the vectors analysed and the EIR of 52.4 infective bites per person per night was recorded in the months of May as the highest and 7.4 infective bites per person per night was recorded in the months of March which is the lowest in the mosquitoes collected indoors and for the outdoor collected mosquitoes while the month of July had 15.9 infective bites per person per night which is the highest and the lowest is 3.6 infective bites per person per night of the EIR this also implies that there will be high rate of malaria transmission in the study area. The result of this finding is also in disagreement with the findings of Tirados *et al.* (2016) in Konso district of Southern Ethiopia who recorded the EIR of 5.3b/p/n and 2.2 b/p/n infective bites per person per night respectively.

In Swampy grassland eco-settings the sporozoites rate of 20.20 % was found in the vectors analysed and the highest EIR was recorded in the month of June of 49.2 infective bites per person per night and 8.2 infective bite per person per night was the lowest recorded for the mosquitoes collected indoors in the months of March, for the outdoors collected mosquitoes the highest EIR was also recorded in the month of June with 37.2 infective bites per person per night with the lowest in the month of October with 14.1 infective bites per person per night as the EIR the findings of the sporozoites rates and the EIR across the selected eco-settings revealed the malaria transmission intensity within the eco-settings will be at the higher at the swampy grassland eco-settings follow by the wooded grassland eco-settings and the least transmission will occur at the sparse wood land eco-setings. This result is similar to the findings of Oyewole *et al.* (2010) in Lagos Southwest Nigeria who recorded 13.9 infective bites per person per night.

The EIR usually serves as an indicator for the level of parasite transmission in a given area. In this study, the EIR recorded across the various eco-settings seems to be a bit higher than the ones reported in some parts of the country and the implication is that there will be constant and steady transmission of malaria infections across the selected eco-settings. In addition the relative EIR maintained by *An. gambiae s.s.* in this present study indicates and showed the role played by these species in malaria transmission across the various selected eco-settings in Nasarawa state.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study established that six (6) species of *Anopheles* mosquitoes were prevalent during the study period; *Anopheles gambiae, An. funestus, An. rufipes, An. coustani, An. nili* and *An. pharoensis* across. The highest *Anopheles* mosquito species population was recorded in the month of May and June.

In respect to the eco – settings, *An. gambiae s.l* was the most encountered species, while *An. rufipes* had the least encountered in woodlandw/grassland and sparse woodland (Nasarawa Eggon LGA) eco – setting while *An. gambiae s.l* was equally higher (39.37%) and *An. pharoensis* had the least (4.77%) number of *Anopheles* mosquito species encountered in the grassland (Doma LGA) eco – setting. Statistically, there is a significant difference ($p<0.05$) in the spatial composition of *Anopheles* mosquito species encountered across the selected eco – settings of Nasarawa State.

Of the total number of *Anopheles* mosquitoes caught across the selected eco – settings of Nasarawa State, (64.69%) *Anopheles* mosquitoes were caught using CDC method; (68.21%) indoors and (31.79%) were caught outdoors. Indoors *Anopheles* mosquitoes were higher (73.92%) in wooded grassland (Karu LGA) eco – setting and the least (59.33%) was recorded in the swampy grassland (Doma LGA) eco – setting. In the same vein, outdoor *Anopheles* mosquitoes were peaked in swampy grassland (Doma LGA) eco – setting followed by sparse woodland (Nasarawa Eggon LGA) eco – setting while woodland/grassland (Karu LGA) eco – setting had the least number of outdoor *Anopheles* mosquitoes caught respectively. However, endophagy is the predominant

feeding behaviour of the *Anopheles* mosquitoes caught indoors and outdoors across the selected eco – settings.

The highest indoor resting density was found in the sparse woodland (Nasarawa Eggon LGA) eco – setting (26.8%), followed by wooded grassland (Karu LGA) eco – setting (23.9%) while the least was recorded in swampy grassland (Doma LGA) eco – setting (21.9%).

seasonal variations more mosquitoes were encountered during the wet season (61.17 % mosquitoes) compared to (38.81 % mosquitoes) during the dry season, with More of the *Anopheles* mosquitoes caught indoors (68.08 %) than outdoors (31.92%).

Acording to methods of mosquito collections CDC light trap method of collection had the highest number of mosquitoes caught (64.69 %) compared to PSC method (35.31 %). The Swampy grassland (Doma LGA) eco – setting had the highest number of anopheline mosquitoes collected by CDC (68.84%) and least in the number of anopheline mosquitoes collected by PSC (15.31%). Abdominal conditions of Anopheline mosquitoes caught using PSC method were also analyzed. Among the various abdominal conditions observed, half gravid (HG) were more abundant (27.14 %) anopheline mosquitoes, followed by gravid (G) and fully fed (FF) with (26.08 %) and (25.97 %) respectively, while unfed (UF) mosquitoes had the least (19.66 %) anopheline mosquitoes observed.

The Human biting rate (HBR) also varied significantly on monthly basis across the selected eco – settings of Nasarawa State. The month of May recorded the highest HBR 18.3 b/m/h, 22.9 b/m/h and 23.0 b/m/h in all the eco – settings respectively and lowest in the month of March, December and January with (10.4b/m/h), (11.6b/m/h), (11.8b/b/m/h) respectively. Polymerase Chain Reaction (PCR) showed that *Anopheles*

gambiae s.s is the predominant *Anopheles* in the study area, the vector is anthropophilic (preference for humans) and endophilic (bites indoors). *An. gambiae s.s* samples were further analyzed for molecular forms. They were identified as *An. gambiae s.s* Giles (Former Savannah (S) form), *An. coluzzi* (M – form), Hybrid forms and *An. arabiensis*. The results therefore, showed that *An. gambiae s.s* exists in all the selected eco – settings of Nasarawa State. *Plasmodium falciparum* sporozoite prevalence in the mosquito vector was also determined among the *Anopheles gambiae* s.l collected using Enzyme Linked Immunosorbent Assay (ELISA). A relatively lower *P. falciparum* sporozoite infection rate of (15.40%) was recorded. The highest sporozoite rate of *Plasmodium falciparum* was found in swampy grassland eco – setting, followed by wooded grassland while the least sporozoite infection rate was recorded in sparse woodland. *Anopheles* mosquitoes were analyzed for the presence of Kdr gene. This result shows high frequency of Kdr 201 (33.50 %) across the three (3) selected eco – settings of Nasarawa State. Blood – fed adult female *Anopheles gambiae s.l* mosquitoes were analyzed by ELISA to determine the sources of their blood meals and also by PCR to determine their species. ELISA results for the sources of the blood meal indicated that most (47.47 %) of the blood meals were from humans followed by bovine (30.54 %) while the least count of blood meal source (21.72 %) was recorded in goats.Blood meal sources for *Anopheles gambiae s.s* and *An. arabiensis* mosquitoes collected were analyzed by ELISA to determine the sources of their blood meals. Blood meal sources identified from *An. gambiae s.s* were (95.95%) from humans, (45.07 %) from bovine and (33.66 %) from goats Moreso, some *An. gambiae s.s* mosquitoes obtained blood – meals from single sources, that is, 98.56% from humans, 44.44% from bovines, and 28.33 % from goats. *An. arabiensis* obtained blood meal from humans (1.44 %), bovines (55.56 %) and (71.67 %) from goats.Mixed blood meal sources identified from

An. gambiae s.s included 75.61 % uman/goat, 69.57 % human/bovine, 66.15 % human/bovine/goat and 45.45% bovine/goat. Also, *An. arabiensis* are 24.39% human/goat, 30.43 % human/bovine, 33.85 % human/bovine/goat and 54.55% bovine/Goat. The ELISA blood – meal results also showed that no blood – meal source from chickens was identified from both *Anopheles gambiae s.s* and *An. arabiensis.*Entomological Inoculation Rate (EIR) was determined across the various Eco – settings of Nasarawa State from the product of sporozoite rate (SR) and Human Biting (HBR). Entomological inoculation rate was also determined and a total of 42.8 % Infective Bite per person per night (IBP) was recorded and the highest was in the month of October while the least 9.4 % IBP was recorded in the month of March for all Indoors collected mosquitoes while the outdoors had the highest IBP of 15.9 % in the month of July and 3.6 % IBP lowest in the month of March at wooded grassland eco-settings of Karu LGA for the EIR.In the Sparse woodland eco-setting of Nasarawa Eggon LGA, a total of 52.4 % IBP was recorded in the Month of May which is the highest and the lowest was 7.9 % IBP for indoor collected mosquitoes while in outdoors mosquitoes collection, the highest IBP was recorded in the month of July 16.8 % and the lowest was recorded in the month of March 2.5 %.Also, in the Swampy grassland eco-settings, the highest IBP/N 49.2 % was recorded in the month of June while the least IBP/N 8.2 % was recorded in the month of October for the for the mosquitoes collected indoors. The highest 37.2 s% infective bites per person per night was recorded in the month of June while the least 11.6 % infective bite per person per night was recorded in the month of August EIR for outdoors at Swampy grassland.

5.2 Recommendations

On the basis of the above conclusion, the following recommendations are therefore made;

i. Knowledge of vector species and their correct identification should be sought continuously in Nasarawa State and beyond because it is a prerequisite for a proper understanding of the epidemiology and transmission dynamics of malaria in any given area.
ii. *Anopheles gambiae, An. arabiensis, An. funestus, An. coustani* and *An. nili* should be targeted simultaneously and wholistically for any mosquito control due to their endophagic and endophilic behaviour that was encounterd at the course of this study to warrant the success of any intervention programme in Nasarawa State across the selected eco-settings.
iii. Integrated Vector management should be adopted as a control strategy to curtail the distribution and the proliferation of the vectors across the selected eco-settings which may include Indoor residual spray (IRS), the use of Long Lasting Insecticidal treated Nets (LLIN) and larvicing activities.
iv. PCR based identification methods have the advantage of being fast and reliable it is necessary to first validate a molecular identification method on local mosquitoes before it is used in a new geographical area due to the potential factor of inter and intraspecific genetic variability.

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