CHARACTERISATION OF *ANOPHELES* MOSQUITOES, BREEDING SITES AND RETROSPECTIVE STUDY OF MALARIA IN KATSINA STATE, NIGERIA

BY

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DECLARATION

I hereby declare that the work in this dissertation titled ‘‘Characterisation of A*nopheles* mosquitoes, breeding sites and retrospective study of malaria in Katsina State, Nigeria ’’ has been carried out by me in the department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of reference provided. No part of this dissertation was previously presented for another degree of diploma at this or any other institution.

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Sign Date

CERTIFICATION

This dissertation titled ‘‘CHARACTERISATION OF *ANOPHELES* MOSQUITOES BREEDING SITES AND RETROSPECTIVE STUDY OF MALARIA IN KATSINA STATE, NIGERIA” by Aliyu Mohammed UMAR, meets the regulations governing the award of the degree of Doctor of Philosophy (Ph.D) of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation

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DEDICATION

This dissertation is dedicated to all victims of Malaria throughout the world and my children Ummul Munzir and Abudhar.

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ABREVIATIONS

A	Adenine
AIDS	Acquired Immune Deficiency Syndrome
Af	*Anopheles funestus*
Ag	*Anopheles gambiae*
AHI	Adult House Index
Alt.	Altitude
Aq	*Anopheles quadrimaculatus*
Am	*Anopheles maculipennis*
An.	*Anopheles*
bp	base pair
C	Cytosine
CA	California
CEL-III	hemolytic C-type lectin
CDC	Centre for Disease Control
CS	circumsporozoite
Cm	Centimeter
dNTP,	deoxyribonucleoside triphosphate
E	east
EDTA	Ethylenediamine tetraacetic acid
e.g.	example
EIRs	Entomologic Inoculation Rates
Elev.	Elevation
ELISA	Enzyme-linked immunosorbent assay

ABREVIATIONS

et al.,	and others
EtBr gel	Ethine Bromide gel
FM	Frequency modulation
FMCK	Federal Medical Centre Katsina
G	Guanine
GHD	General Hospital Dutsinma
GHF	General Hospital Funtua
GIS	Geographic information system
GPS	Global positioning system
HBI	human blood index
HBR	Human Biting Rate
HCH	organophosphates
IGS	Intergenic spacer region
IGNP	Indira Gandhi Nahar Pariyojana
IMPs	Intentional Mismatch Primers
IRS	Indoor Residual Spraying
ITNs	insecticide-treated bed nets
ITS1	internal transcribed spacer 1
ITS2	internal transcribed spacer 2
Kdr	resistance allele
Km	Kilometer
Km^2	Kilometer square
KTSEPA	Katsina State Environmental Protection Agency

ABREVIATIONS

KTTV	Katsina State Television Station
Lat.	Latitude
LF	lymphatic filariasis
LLNs	long-lasting nets' (LLNs)
Lon.	Longitude
MARA	Malaria risk in Africa project
mm	Milimeter
M form	Mopti form
$MgCl_2$	Magnesium (iv) Chloride
ml	mililitre
N	north
NDVI	Normalized Difference Vegetation Index
OR	Odds ratio
PCR	polymerase chain reaction
PCR–RFLP	Polymerase chain reaction-restriction fragment length polymorphism
pers. comm.	Personal communication
Pf	*Plasmodium falciparum*
Popu.	Population
Pv 210	*Plasmodium vivax* starin 210
Pv 247	*Plasmodium vivax* starin 247
QD	*An. quadriannulatus* primers
QDA	*An. quadriannulatus* primers
RA	Rapid Assessment

ABREVIATIONS

rDNA	ribosomal DNA
S	south
S form	Savannah form
SIT	Sterile insect technique
s.l.	sensu lato
SNP	Single Nucleotide Polymorphisms
s.s	sensu stricto
T	Thymine
Tris-HCl	Tris-Hydrochloric acid
USA	United States of America
VecTest	Vector dipstick strips test
W	west
WHO	World Health Organisation
%	percentage
^{o}C	Degree Celsius
µM	micromolar
µL	microlitre

CHARACTERISATION OF *ANOPHELES* MOSQUITOES BREEDING SITES AND RETROSPECTIVE STUDY OF MALARIA IN KATSINA STATE, NIGERIA

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ABSTRACT

An entomological survey was conducted between 2009 and 2011 in some parts of Katsina State, Nigeria to establish the species of *Anopheles* present, the characteristics of their breeding sites and the prevalence of malaria infection from hospital records in selected hospitals. The state was zoned into three: A, B and C to represent the Guinea, Sudan and Sahel savannah respectively. Anopheline mosquitoes were collected indoors and from larval surveys. The collection was first identified morphologically using mosquito morphological keys, then subjected to PCR analysis, and Gel electrophoresis for M and S rDNA assay. Incrimination of malaria vectors was by detection of circumsporozoite protein using Vectest kits (MAS TM; Camarillo, CA, USA). A retrospective study of malaria prevalence during both dry and wet seasons was done by collecting hospital records of malaria in Dutsinma, Katsina and Funtua government hospitals. Geo-referencing and characterization of mosquito breeding sites were also carried out. Pictures of all man-made mosquito breeding sites sampled were taken using an ES 15 Samsung digital camera. A total of 3027 anopheline mosquitoes were collected and morphologically identified as *Anopheles* mosquitoes. Of the 1649 adults reared from larvae 1027 (62.28%) were *An. gambiae* s.l. followed by *An. funestus* 580 (35.17%). Of the 1378 adults collected indoors, 926 (67.2%) were *An. gambiae* while the least was *An. quadrimaculatus* with 9 (0.65%). In addition, *An. maculipennis* and *An. quadrimaculatus*, which are recognized as secondary malaria vectors, were also encountered. Larvae were most abundant in Zone A 555 (33.7%) at Dandume followed by Funtua with 298 (18.1%) while Zone C had the least with 148 (9.0%) from Katsina. Similarly, a preponderance of adults was observed in Zone A with 301 (21.8%) at Dandume followed by Funtua with 299 (21.7%) and the least was Zone C at Daura, with 98 (7.1%). *An. gambiae* adults were most abundant in homes. They were more preponderant in Zone B at Kankara with 200 (14.51%) and the lowest collection was made in Zone C at Katsina with 70 (5.08%). There was no significant difference between indoor collections between Zone A at Dandume, Zone B at Dutsinma and Zone C at Daura ($p > 0.05$, $F = 10.13$). In addition, *An. funestus* had its highest collection in Zone A at Funtua with 88 (6.39%) and the lowest in

Zone C with 28 (2.03%) in Daura. *An. quadrimaculatus* was the least abundant in all the zones combined, having only 2 specimens in Zone A (at Funtua). *An. gambiae* was most abundant in July, 2009 with 47 at Dandume in Zone A while An. funestus was the most abundant in February, 2009 also in Zone A (at Dandume). There was no statistically significant difference between *An. gambiae* and *An. funestus* population in Zone A, Zone B and Zone C ($p > 0.05$, $F = 5.05$). *Anopheles* mosquitoes were collected from five different types of breeding sites. *An. gambiae* were the most preponderant in pools with 332 (20.13%)] and least abundance were 120 (7.28%) in overhead tanks. *An. funestus* was most proponderant in concrete reservoirs. *An. maculipalpis* and *An. quadrimaculatus* were collected from rice farms only. There was no significant difference ($p > 0.05$, $F = 9.28$) between pool and pond nor between concrete reservoir and overhead tank in terms of collected larvae. The S form predominated over the M form in all the sampled towns. For indoor collections, the S form was the most preponderant in Zone B with 187 (19.75%) at Kankara and the lowest of 56 (5.91%) was in Zone C (at Daura). Of the 1069 adults reared from larvae, the S forms were the most preponderant with 354 (33.12%) in Zone A (at Dandume) and its lowest abundance was 45 (4.21%) in Zone C (at Katsina). The M forms were the most proponderant in Zone A (with 5 and 3 specimens reared from larvae at Funtua and Dandume respectively). There was no significant difference between the zones in terms of larval collections of M and S forms; however there was a significant difference in terms of indoor collections between Zones A, B and C and also between the two forms ($p > 0.05$, $F = 9.28$). The cocktail PCR-assay on the *An. funestus* group showed 518 (51.24%), to be *An. funestus* s.s and 67 (6.624%) to be *An. brucei* and 35 (3.46%) as *An. rivolurum*. The three species were identified from all the sampled towns. *An. funestus* adults and larvae identified by PCR showed no significant difference between the zones ($p < 0.05$, $F = 6.39$). The Vectest evaluation indicated that the most prevalent infection of *P. falcifarum* was 88 (6.61%) in Zone B (Kankara) and the lowest was 36 (2.70%) in Zone C (Daura). *P. malariae* recorded its highest prevalence of 206 (15.47%) also in Zone A (Funtua) and its least was 55 (4.13%) in Zone C (Daura). There was a very high significant difference between *P. falciparum* and *P. malariae* isolated from *An. gambiae* and *An. arabiensis* within the zones. No *P. Vivax* was collected during the study. The highest number of identified *Plasmodium* species both in 2009 and 2010 was in the month of July. This study has shown that *An. gambiae* was the most abundant species in wet months, followed by *An. funestus* at the end of the rainy season, and *An. arabiensis* in drier months. *An. funestus* has been confirmed to be responsible for transmission of malaria during the dry season. In addition, *Anopheles* mosquitoes survived and proliferated during the dry season by breeding in temporary ponds, concrete and overhead water reservoirs. *Plasmodium vivax* was not encountered in Katsina State.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

Tropical diseases such as dengue fever, lymphatic filariasis, malaria, yellow fever and those caused by the West Nile Virus and other viruses are all transmitted by mosquitoes. Malaria is the most common and important parasitic disease transmitted by mosquitoes. It is transmitted through the bite of an infected female *Anopheles* mosquito. The most important species of malaria parasites involved are *Plasmodium falciparum, P. ovale, P. malariae* and *P. vivax* (Midega, 2006). Diseases transmitted by mosquitoes have been responsible for killing more people than all the wars in history (Beerntsen, 2000). Worldwide, mosquitoes transmit diseases to more than 700 million people annually (Fradin, 1998) out of which about 300-500 million clinical cases are transmitted by the *An. funestus* complex. Malaria is responsible for the deaths of 1 child every 30 seconds (Shell, 1997; WHO, 2008). It is responsible for about 1 million deaths (range 744,000 – 1,300,000) in Africa every year; with about 75% of cases occurring in children that are 5 years and below (Snow *et al.,* 1999). Malaria is responsible for about 30% of all outpatient cases and between 20 to 50% of malaria patients admitted to hospitals in all malaria endemic countries in Africa. Between 18 and 37% of fatalities occured in Eastern and

Southern Africa in the 1980s and 1990s respectively (Breman, 2001). Worldwide malaria cases have gone up to about 41% and is worsening every year because of poor health systems, increasing resistance to drugs, changes in weather and conflicts (Breman, 2001). Malaria leads to a lot of social and economic problems, such as school absentism, lower agricultural production among others; consequently, more control efforts are required in order to reduce the rates of disease incidences and mortality. Furthermore malaria claim more lives than AIDS (Afandi, 2007). In 2006, 35 to 80 million Nigerians were infected with malaria, out of which 1 million people died, most of them children under five years (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, 2008). 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 (Carter Foundation, 2005). Malaria killed 401 people in the last four weeks of September (2011) in Katsina state, according to local health officials. Up to 50,311 malaria cases were recorded in Katsina State in 2011, and was attributed to the unusually heavy rainfall recorded that year in Daura, Funtua, Ingawa, Kurfi and Bindawa towns of Katsina State (WHO, 2011).

Anopheles mosquitoes contain about 400 species which includes *An. gambiae, An. funestus* and *An. minimus* complexes. However the transmission of malaria occurs only within about 40 species eventhough there are about 60 to 80 species of *Anopheles gambiae* (Bruce- Chwatt, 1985). In sub-saharan Africa, *P. falciparum* is mainly transmitted by *An. gambiae* and *An. funestus* species complexes. *An. gambiae* is the most ubiquitous in Africa as well as having the highest rates of sporozoite development (Coetzee *et al.*, 2000). The *An. gambiae* complex consists of six species, one unnamed species (Coetzee, 2004), and about three subspecies exist

within *An. gambiae* s.s in West Africa e.g. *An. gambiae* s.s. and *An. arabiensis* among others (Fanello *et al.,* 2003). *An. gambiae* s.s. is recognized as the world's most important vector of *P. falciparum* followed by *An. arabiensis* (Fanello *et al.,* 2003).

All the existing 3,000 species of mosquitoes spend part of their life cycle in water (Metzger, 2004). They can breed in virtually any natural or man-made deposit of water, some live and breed deep below the earth in mines, some have been found on top of mountains, others in highly polluted water, in snow pools, and some even in crab holes, others in water reservoirs like ponds and dams. In fact in sub-Saharan Africa, the 9.4 million people that live near dams and irrigation schemes are afflicted with 87.9% of the current global malaria burden (Keiser *et al*., 2005). They occur throughout the globe and can survive all weather conditions (WHO, 2005). However, variations in ecology and bahaviour play a big role in the life of mosquitoes. *An. gambiae* is known to proliferate in small temporary rain-dependent pools and puddles. *An. funestus* prefer large, permanent or semi-permanent water which contain plants. It increases malaria transmission and is also preponderant during the dry season when the population of *An. gambiae* and *An. arabiensis* are low (Gillies and De Meillon, 1968).

The *An. gambiae* have many chromosomal forms e.g. Forrest, Bissau, Mopti (M) and Savanna (S) as a result of variations in ecological zones which led to variations in the observed frequencies of chromosomal inversions in these chromosomal forms (Coluzzi *et al*., 1979).

Knowledge of distribution and abundance of mosquitoes is essential for controlling malaria in a particular area. Members of the *An. gambiae* sensu lato (s.l.) are closely related and morphologically similar and are sympatric (Coetzee *et al*., 2000), but differ in host-biting preference, abundance and vector competence which makes species identification and

distribution imperative towards effective control. Breeding sites of *Anopheles* mosquitoes are on the increase because of increase in human population, human migration to urban areas, urbanization and environmental degradation, and *Anophels* have become better in disease transmission because of changes in the environment. There is a need therefore for more research focusing on improving our understanding of malaria vector population ecology on a local scale to enable the formulation of effective vector control strategies, aimed at reducing human vector contact and disease transmission. Reduction of mosquito population has been used world over to control malaria (Killeen *et al.,* 2003). The vector remains the key link in the transmission of malaria, and hence, warrants research and control efforts.

Anopheles species composition and identification can be better studied through integration of molecular techniques with other tools such as a Global Positioning System (GPS) and Geographic Information System (GIS). The range and relative abundance of *An. gambiae* and *An. arabiensis* depends on rain and temperature. *An. gambiae* dominates in wet areas while *An. arabiensis* is more abundant in dry areas.

Mosquito control efforts can be facilitated by correct identification of available species and a lot of methods for identifying *Anopheles* species have been developed e.g. Ggas chromatography of cuticular hydrocarbons (Carlson and Service, 1980), polytene chromosome arrangements (Coluzzi *et al*., 1979) and allozyme analyses (Mahon *et al*., 1976).

About 90% of global malaria mortalities occur in Africa (African Malaria Report, 2003). This high figure is as a result of the efficient transmission of *Plasmodium falciparum* by *An. gambiae*,

An. arabiensis, and *An. funestus* (Fanello *et al*., 2003). *An. gambiae* and *An. funestus* both feed on human blood and that facilitate their vectorial capacity (Garrett-Jones, 1964). Diurnal differences exist between them, therefore there should be more reliable identification (Gillies and Coetzee, 1987; Fanello *et al*., 2003) so as to facilitate control of mosquitoes and malaria. Although the *An. funestus* group conceals one of the major malaria vectors in Africa, little is known about the dynamics of members of this group across the continent (Mulamba *et al*., 2014). 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 Katsina State. In addition, the control of malaria through mosquitoes should also be aimed at the specific biology of *An. funestus* and other non popular malaria vectors (Coetzee and Fontenille, 2004).

Not much is known of the species composition, distribution, abundance of *Anopheles* and *Plasmodium* species in Katsina 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, breeding sites, abundance and the prevalence of malaria in both dry and wet seasons across the three ecological zones of Katsina State. The data will be used to compare *Anopheles* species occurrence and abundance in relation to malaria episodes across the three ecological zones; this is more so when it was reported that there are few mosquito breeding sites but high numbers of mosquitoes and malaria cases in Katsina State in the dry season (KTSEPA, 2010; KTTV, 2010), raising questions on how and where *Anopheles* survives during the dry season in the State.

1.2 STATEMENT OF RESEARCH PROBLEM

Currently, there is no empirical data on *Anopheles* mosquito breeding sites and dynamics and species composition, vectoral capacity, malaria prevalence and distribution in Katsina State to warrant assessment of the mosquito and malaria situation towards prompt and effective intervention strategy.

1.3 JUSTIFICATION

Identification of *Anopheles* malaria vectors is essential for the identification of areas of Katsina State that are at risk of malaria and the formulation of strategies for effective control. 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. The transmission of malaria and the development of the *Anopheles* vector are directly linked to the breeding sites. Consequently, determination of the breeding sites of *Anopheles* in Katsina State during dry and wet seasons as a result of the current study is justified, because it will lead to efficient and effective larval control which will serve as a cost effective control strategy against malaria and its vectors. It is important to study breeding sites because *Anopheles* species utilize various habitats that differ in physicochemical properties, size, vegetation and productivity. Information on the spatial distribution of *Anopheles* vectors will facilitate the targeting of the prolific larval habitats thereby improving the preciseness of the control strategy and also reduce cost.

Location of larval habitats will provide information on *Anopheles* mosquito feeding-strategies and population structure which are essential for control measures such as Insecticide Treated Nets (ITNs) distribution in Katsina State. To incriminate the particular vector of malaria, *Plasmodium* parasites must be isolated from locally available mosquitoes prevalent in Katsina State through Rapid Assessment (RA). Similarly, RA can provide maps of areas of relative malaria risk as a result of the spatial heterogeneity of mosquito populations in Katsina State (Ribeiro *et al*., 1996). The most important is the production of physical maps for the assessment of such an area. Maps can be produced, and houses numbered, using a variety of techniques. Simple sketch maps can be used to localize each mosquito collection site in the State and provide reference points. Well-produced maps are necessary for a successful mosquito and malaria control strategies in Katsina State.

Besides, some *Anopheles* species like *An. funestus*, keep on appearing and disappearing from their known habitats. For instance *An. funestus* were thought to have disappeared from Niger republic, but recent studies have established their presence (Konate *et al*., 2001; Labbo *et al*., 2004); and some species have developed resistance to insecticides being utilized. It is essential to have proper species identification so as to determine how insecticide resistance occur in each species as well as to study the migration of resistance genes between populations. Besides, observations have shown that *An.gambiae* species can survive in relatively large numbers during the dry season but the manner by which they do so remains unknown.

This study will provide detailed information of the mosquito species composition, their vectoral capacity and malaria prevalence in Katsina State so as to facilitate mosquito control through the use of ITNs, insecticides etc. GPS guided maps to be produced by this study will identify areas where greatest mosquito and malaria control efforts should be focused on.

1.4 AIM AND OBJECTIVES

The aim of this study is:

to characterize the *Anopheles* species present in Katsina State and determine the most prevalent *Plasmodium* species in the area.

The objectives of this study were to determine:

i) the breeding sites of *Anopheles* mosquitoes among the different ecological zones of Katsina State during dry and wet seasons.

ii) the species composition and abundance of *Anopheles* in dry and wet seasons in Katsina State.

iii) the *Plasmodium* species in infected *Anopheles* mosquitoes among the different ecological zones.

iv) the prevalence of malaria retrospectively, using hospital records.

1.5 HYPOTHESES

i) Ho: There is no significant difference in the *Anopheles* breeding sites among the different ecological zones of Katsina State during dry and wet seasons.

ii) Ho: There is no significant differences in the *Anopheles* species composition and abundance

among the dry and wet seasons in Katsina State.

iii) Ho: There is no significant difference in the *Plasmodium* species being transmitted among the ecological zones of Katsina State.

iv) Ho: The is no significant difference in prevalence of malaria episodes among the dry and wet seasons based on retrospective hospital records of malaria.

CHAPTER TWO

2.0 LITERATURE REVIEW

The name *Anopheles* comes from the Greek words αν, *an*, meaning *not*, and όφελος, *ópheles*, meaning *profit*, it literary mean *useless*. There are approximately 460 recognized species while over 100 species have the potencial to transmit human malaria, only 30–40 species are actively involved in the transmission of human *Plasmodium* parasites, which cause malaria in humans in endemic areas. *An. gambiae* is one of the best known, because of its predominant role in the transmission of the most dangerous malaria parasite species, *Plasmodium falciparum* (Calvo *et al.*, 2009).

2.1 EVOLUTION OF ANOPHELES

The ancestors of *Drosophila* and *Anopheles* diverged 260 million years ago (Calvo *et al.*, 2009). The culicine and *Anopheles* clades diverged between 120 million and 150 million years ago respectively (Calvo *et al*., 2009). The Old and New World *Anopheles* species subsequently diverged between 80 million and 95 million years ago respectively (Calvo *et al*., 2009). The *An. gambiae* and *An. funestus* clades diverged 36 to 80 million years ago. There was an expansion of this genus during the Cretaceous period as shown by a molecular study of several genes in seven species (Dixit *et al*., 2010). The *Anopheles* genome (230–284 Mb) is similar in size to that of *Drosophila* but alot smaller than those found in other culicine genomes (528 Mb–1.9 Gb). The

genome is diploid with six chromosomes as is found in most culicine species, The only known fossils of this genus are those of *Anopheles (Nyssorhynchus) dominicanus* Zavortink and Poinar contained in Dominican amber from the Late Eocene (33.9 to 40.4 million years ago) and *An. rottensis* Statz contained in German amber from the Late Oligocene (23 to 28.4 million years ago) (Dixit *et al*., 2010).

2.2 THE *ANOPHELES* 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 (Dixit *et al*., 2010) important vectors, e.g. *An. paludis*, *An. hancocki*, *An. melas* among others

2.2.1 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, Craig and Le Sueur 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 completly absent.

This is why five 'chromosomal forms' named under the non-Linnaean nomenclature exists 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. Eventhough 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, 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).

*2.2.2 Anopheles funestus G*roup

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 was only possible through karyotyping (Green and Hunt 1980). Recently, however, easier PCR based assays have been developed which differenciate 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), or by a recently developed PCR assay (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 and Cameroon, 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*. (2009) 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*. 2005). 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.

2.3 Mosquito Habitats

Malaria is mostly found in tropical countries, especially in sub-Saharan Africa, even though *Anopheles* species live in colder countries. The colder countries have experience malaria outbreaks in the past, for example during the construction of the Rideau Canal in Canada during the 1820s. Since then, the *Plasmodium* parasite (not the *Anopheles* mosquito) has been eliminated from first world countries.

Mosquitoes prefer to lay their eggs in shallow, stagnant water such as ponds, marshes, swamps, floodwater, ditches and woodland pools. However, they have been living successfully in many different environments; they live even in environments that are not normally theirs. Mosquitoes grow well in hot, humid environments especially in tropical areas. Some species live in very cold countries, like those in the Arctic Circle (www.rci.rutgers.edu, 2013).

Mosquito larvae are usually found in different habitats including water bodies that contain vegetation which larvae attach to so as to avoid being swept away by water currents. The *Anopheles, Culex, Culiseta, Coquillettidia* and *Uranotaenia* species breed in permanent bodies of water and can survive in polluted water as well as freshwater, acid water and brackish water swamps. Other mosquito larvae may be present in container water sources such as puddles, upon leaves and stagnant water within small pools (www.rci.rutgers.edu, 2013).

Various mosquito species breed following habitats:

2.3.1. 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.

2.3.2. Running Water - 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. Eventhough *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.

2.3.3. 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 fload. Transient water bodies undergo water quality changes which results in various mosquito species using the same pool over a period of time.

Transient waters include:

2.3.4. 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.

Permanent waters include:

2.3.5. 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 utilise 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.

2.5 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 system 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.

2.6 PREFERRED SOURCES OF BLOOD MEALS

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* are 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).

2.7 PATTERNS OF FEEDING AND RESTING

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).

2.8 MOSQUITO VECTOR CONTROL

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. For a variety of reasons, these goals have not been met upto this moment (2014).

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. However less than 2% of the surveyed children sleep with ITNs. 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).

2.9 INSECTICIDE RESISTANCE

Indoor spraying with insecticides and ITNs are the methods to eliminate mosquitoe bites indoors. However, prolonged exposure to an insecticide leeds 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 fruatrating the Global Malaria Eradication Campaigns. Appropriate utilisation of insecticides during mosquito control can significantly stabilise the development and spread of resistance. However, this is continuously fruatrated 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).

2.10 *PLASMODIUM*

It is a genus of parasites which cause malaria. It was described in 1885 by Ettore Marchiafava and Angelo Celli. There are over 200 species in existance and new species are continuously being described (Coatney *et al*., 1966; Contacos *et al*., 1970). At least 11 of *Plasmodium* species infect humans however other species are zoophilic, infecting monkeys, rodents, birds, and reptiles among others. The parasite always has two hosts in its life cycle: a mosquito vector and a vertebrate host.

2.10.1 Species of Plasmodium *infecting Humans*

- *Plasmodium falciparum* (the cause of malignant tertian malaria)
- *Plasmodium vivax* (the most frequent cause of benign tertian malaria)
- *Plasmodium ovale curtisi* (another, less frequent, cause of benign tertian malaria)
- *Plasmodium ovale wallikeri* (another, less frequent, cause of benign tertian malaria)
- *Plasmodium malariae* (the cause of benign quartan malaria)
- *Plasmodium knowlesi* (the cause of severe quotidian malaria in Southeast Asia)

- *Plasmodium brasilianum*
- *Plasmodium cynomolgi*
- *Plasmodium cynomolgi bastianellii*
- *Plasmodium inui*
- *Plasmodium rhodiani*
- *Plasmodium schwetzi*
- *Plasmodium semiovale*
- *Plasmodium simium*

The first six listed here are the most common species that infect humans (Prugnolle *et al*., 2010; Krief *et al*., 2010). PCR has enabled the identification of new species that infect humans. For example an experimental infection has been reported with *Plasmodium eylesi*. Fever and low grade parasitemia were apparent at 15 days (Tsukamoto, 1977; Prugnolle *et al*., 2010; Krief *et al*., 2010). In addition, a possible infection with *Plasmodium tenue* has been reported by Russel (1928) in a three year old girl of the negro race from Georgia, USA; who had never travelled to places outside the United States. Confusingly *Plasmodium tenue* was proposed in the same year (1914) as a species that is found in birds.

Until recently man has been the only recognised host of *P. falciparum* but this species has also been reported in gorillas (*Gorilla gorilla*) (Prugnolle *et al*., 2010), bonbos (Krief *et al*., 2010), brown and black howler monkeys, *Alouatta guariba* and *Alouatta caraya*, respectively (Duarte *et al*., 2008).

A species that clusters with *P. falciparum* and *P. reichenowi* has been identified in Gabon, in chimpanzees (*Pan troglodytes*) (Ollomo *et al*., 2009). This appears to have diverged from these two species about 21 million years ago. It has only been identified from the sequence of its

mitochondrion therefore more research is needed to characterise the species which exists in chimpanzees (Prugnolle *et al*., 2010; Kaiser *et al*., 2010).

DNA sequences have identified two additional species within the subgenus *Laverania* i.e. *P. billbrayi* and *P. billcollinsi* (Prugnolle *et al*., 2010) and also in bonbos (Krief *et al*., 2010). *P. billbrayi* was identified in two subspecies of chimpanzee (*Pan troglodytes troglodytes* and *Pan troglodytes schweinfurthii*). *P. billcollinsi* was isolated in only one subspecies of chimpanzee (*Pan troglodytes troglodytes*). However more research should be carried out in order to characterise these species adequately.

Humans are currently considered to be the only host for *P. malariae*. However research reports have indicated that *P. malariae* can survivie in chimpanzees in Japan (Hayakawa *et al*., 2009), in wild chimpanzees (Kaiser *et al*., 2010), in the brown howler monkey (*Alouatta guariba*), black howler monkeys (*Alouatta caraya*) (Duarte *et al*., 2008) and in splectomised three-striped night monkey (*Aotus trivirgatus*) (Collins and Contacos, 1969). Independent reports suggest that *P. malariae* is only occasionally being hosted by the chimpanzees.

Plasmodium vivax produces low grade infection in chimpanzees but may be persistent and serve as a source of infection to man. *P. vivax* is also known to infect orangutans (Reid *et al*., 2006), brown howler monkey (*Alouatta guariba clamitans*) and chimpanzees (Kaiser *et al*., 2010). *Plasmodium ovale* can also be transmittable to chimpanzees. It is found in Africa, Myanmar (former Burma), the Philippines and New Guinea. In spite of its poor transmission to chimpanzees given its discontigous spread, it is suspected to be a zoonosis with a likely primate host. It has been suggested that *P. ovale* may be a natural parasite of chimpanzees (Duval *et al*.,

2009) but this needs confirmation. It has been recently shown that *P. ovale* is actually two genetically distinct species that coexist. These species are *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* (Surtherland *et al*., 2010). These two species probably separated between 1.0 and 3.5 million years ago. The remaining species capable of infecting humans have other primate hosts. *Plasmodium shortii* and *Plasmodium osmaniae* are now considered to be junior synonyms of *Plasmodium inui*.

2.11 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.

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, surportive leadership, absolute government backing, availability of funds, community participation, skilled technicians from different fields as well as an adequate implementation are all necessary (www.malariasymptoms.org).

Although mosquito control is inenevitable 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 leed to the elimination of malaria even if the vectors remain.

Some important measures in mosquito control are:

- To discourage egg laying,
- To prevent development of eggs into larvae and adults,
- To kill the adult mosquitoes,
- Not to allow adult mosquitoes into places of human dwelling,
- To prevent mosquitoes from biting human beings and deny blood meal (Anonymous, 2009).
- Using the sterile insect technique (SIT), in which sexually sterile male insects are released to wipe out a mosquito population,
- To study the parasite *Wolbachia* for use as a control agent.
- To use larvivorous fish e.g. the Empire Gudgeon (*Hypseliotris compressa*), the Pacific Blue-Eye (*Pseudomugil signifier*), and the mosquito fish, (*Gambusia* species).
- Use Parasites e.g. mosquitoes infected with a protozoan called microsporidia, *Amblyospora indicola* which kills mosquitoes at the larval stage.

- Use repellents which repel mosquitoes, ticks, biting flies and even leeches. The products contain DEET at a concentration less than, but close to 20% (Anonymous, 2009).
- Through Habitat modification by draining water from trap pools and permit predatory fish to gain access to the mosquito larvae (Anonymous, 2009).
- 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. (Anonymous, 2009).

On December 21, 2007, a study published in PLoS Pathogens found that the hemolytic C-type lectin CEL-III from *Cucumaria echinata*, a sea cucumber found in the Bay of Bengal, slowed the development of the plasmodium parasite when after being produced by transgenic *An. Stephensi* (Yoshida *et al*., 2007). This is a promising technique for control of malaria by genetically modifying mosquitoes refractory to *plasmodium*.

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 (1926) 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. 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. 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 depends on three factors: receptivity, infectivity and vulnerability of both vector and hosts (Ejov, 2004; Garrett-Jones, 1964).

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* (34.7%), *An. stephensi* (33.3%), *An. culicifacies* (18.0%), *An. annularis (12.1%), An. pulcherrimus* (1.1%) 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.

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. 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 bwtween 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 in East Africa, 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. Mosquito population 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*. (2000) 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.

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 *Anopheles 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 *et al*. (2004), 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. 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 differences 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 (OR [odds ratio] = 1.6 [95% CI: 1.2, 2.2], $p < 0.008$).

Marrama *et al*. (2004, 2009), 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.* *Anopheles 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 (SI = 1%) than the other species (SI = 0.1%). *An. funestus* was responsible for 90% of the infectious bites.

West Africa also experiences a remarkable mosquito and malaria abundance and distribution as enumerated from literature reported by the following authors: Takken *et al*. (2007) 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 2-km 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.

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. The authors examined 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. However, the S form were 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, Katsina 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 repiblic, 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 (t^2=13.62, P = 0.0002), 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 arears in the Southern Guinea Savanna, even though it was not present there about 20 years ago. The data suggest that *An. arabiensis* has extend 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 bas 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 shows an average of four bouts per person every year (WHO, 1995 and 2002). The situation is worsened by *plasmodium* resistance to first line anti-malarial drugs (WHO, 2000). 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.

One of the reports on mosquito and malaria species abundance and distribution from Northern Nigeria, is that of Lamidi (2009), who 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.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

Katsina State is 1,696 feet above sea level, it is located between 12°15' N - 7°30' E and 12° 25'N - 7.5° 0'E (Fig 1). It has an area of 24,194 km^2 ,with a population of 3,878,344 million and a population density of 160.3/km^2 (KTHSB, 2004). The climate of Katsina State is characterized by two well marked seasons, the rainy season, extending from May to September and the dry season from Octorber to April. The dry season is characterized by harmattan dust between November and February. The northeast harmattan laden dust lowers the day time temperature to about 25^o C and about 21^o C in the night. During the rainy season, the prevailing wind blows in a south-western direction and the humidity rises considerably. During this period (August – October), the mean daily temperature is between 28^{o}C - 33^{o}C. The maximum amount of rainfall occurs between August and September.

The topography of Katsina State is a gently undulating landscape. The vegetation is of three types: (i) the northern part (e.g Daura, Zango, Katsina, Jibia and Mai-Adua among others) experiences the lowest amount of annual rainfall about 25" – 28". The vegetation is of the Sahel savannah type and comprises of: Katsina (12^o 59" N: 7^o 36" E and Daura (13^o 2’ 11" N: 8^o 9’ 4" E. This zone is so dry and the few river systems dry up even during the rainy season. There is a large dam near Katsina town at Ajiwa town (7 km away) and other dams at Jibia and Zabge near Daura. Both dams are used for irrigation and fishing. There are small artificial dams erected for

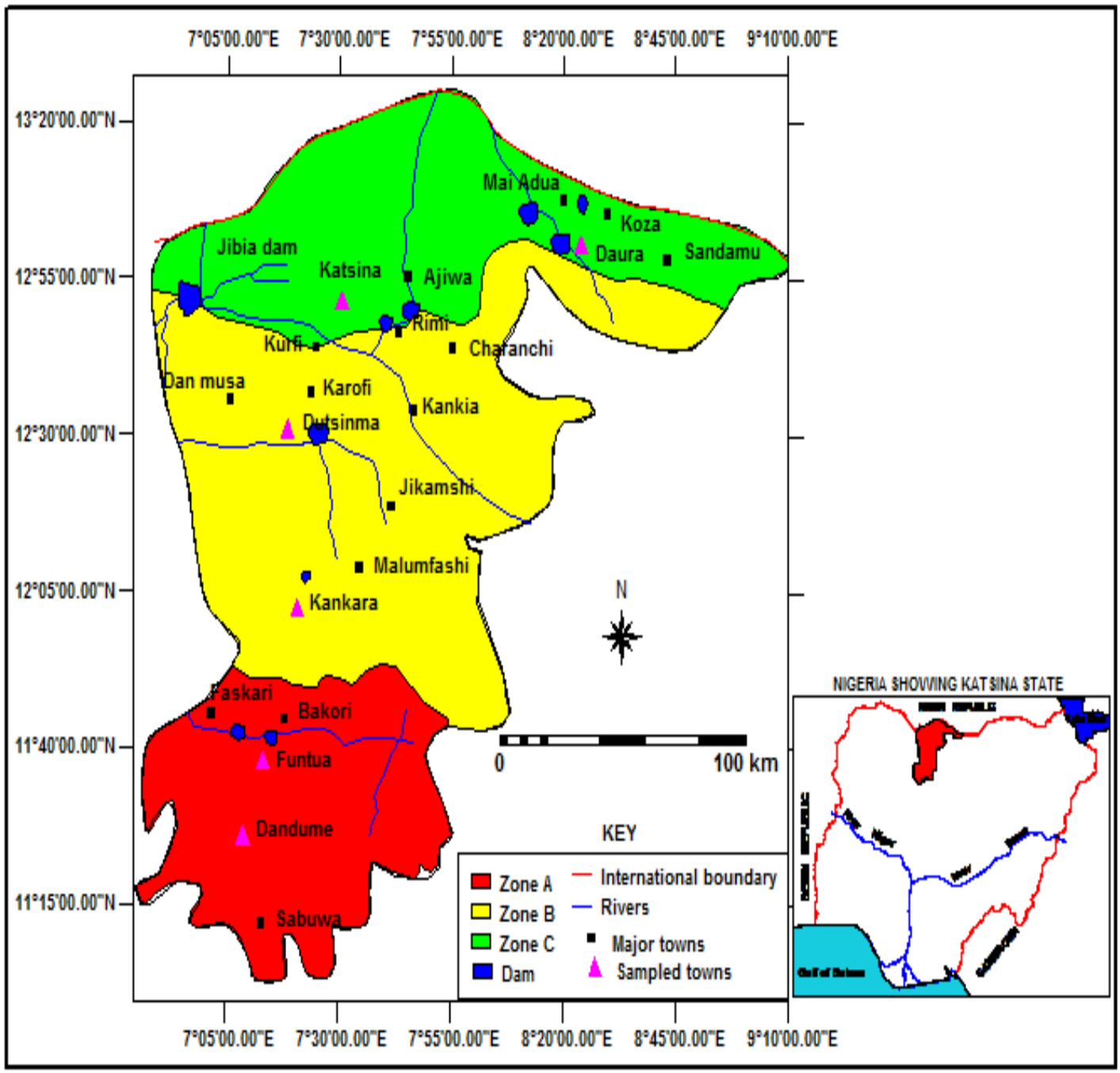


FIGURE 1 : KATSINA STATE SHOWING THE SAMPLED TOWNS AND VEGETATION ZONES

Source : Adapted and Modified from Katsina State Map, Ministry of Lands and Surveys

farming purposes in most of the towns in the zone. It is characrerised by very high temperature (40^oC - 47^oC) and low humidity. (ii) the middle area (e.g. Dutsinma, Matazu and Kafur etc.) experiences moderate amount of rainfall, 30" – 35". The vegetation is of the Sudan Savannah type and it experiences a moderate humidity. There are small artificial dams erected for farming purposes in most of the towns in the zone. There is also a large dam called Zobe dam, located 3 km outside Dutsinma for irrigation and fishing. The southern part (e.g. Funtua, Faskari etc.) has a vegetation similar to that of northern guinea savannah, and is characterized by low temperatures (18^oC - 26^oC), luxurious grass cover and high humidity. The highest amount of annual rainfall of about 35" – 55" is usally associated with the zone (KTHSB, 2004). The detailed account of the climate of Katsina State was obtained from the Katsina State Environmental Protection Agency KSEPA (2004) from which the description below were taken.

3.2 SAMPLING ZONES AND SITES

In order to adequately establish the *Anopheles* breeding sites and the *Anopheles* species transmitting malaria parasites in Katsina State, a total of 6 towns were selected using cluster sampling method. Two towns were selected randomly from each of the three ecological zones (Sahel, Sudan and Guinea savannah) of Katsina State.

3.2.1 Zone A (Guinea Savannah): Funtua (11^o 32" N; 7^o 30" E) (Fig 2) and Dandume (11^o 25" N ; 7^o 12" E) (Fig 3) were selected as representative towns of this zone.

3.2.2 Zone B (Sudan Savannah): The zone is represented by Dutsinma (12^o 27' 18" N: 7^o 29" E, Alt 604m above sea level (asl) (Fig 4) and Kankara (11^o 93" N : 7^o 41" E) (Fig 5) as sampled towns.

3.2.3 Zone C (Sahel Savannah): This zone is represented by Katsina (12^o 59" N: 7^o 36" E, (Fig 6) and Daura (13^o 2' 11" N: 8^o 9' 4" E, (Fig 7).

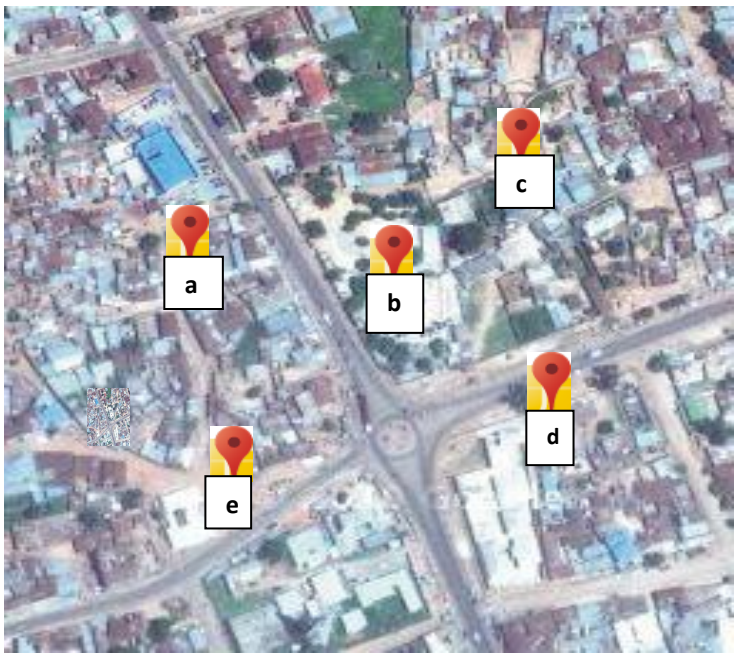


Fig 2: An aerial view of Funtua town (11^{o} 32" N; 7^{o} 30" E) showing sampling points (red pegs) within 1 km radius.

Legend

a	Pool
c	Pond
b	Concrete reservoir
d	Over head reservoir
e	Rice farm

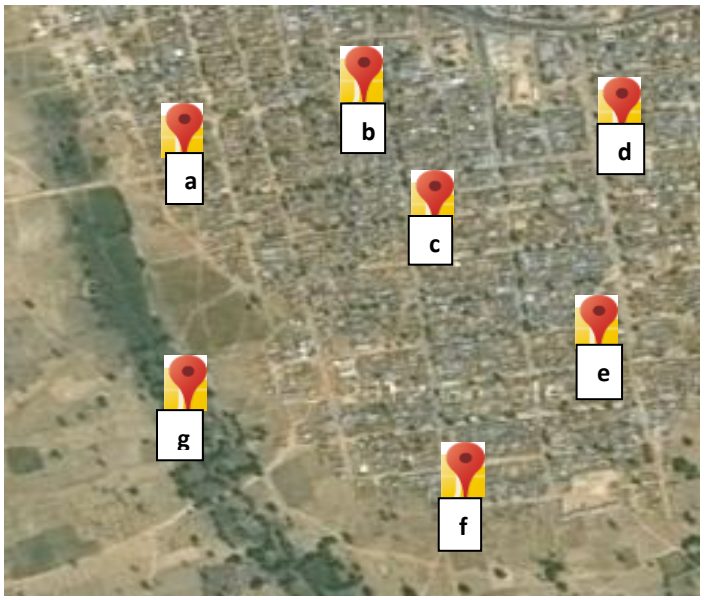


Fig 3: An aerial view of Dandume town (11^{o} 25" N ; 7^{o} 12" E) showing sampling points (red pegs) within 1 km radius. Sampling points f and g above all disappear during dry seasons.

Legend

a	Pool
b	Pond
c	Concrete reservoir
d	Over head reservoir
e	Rice farm

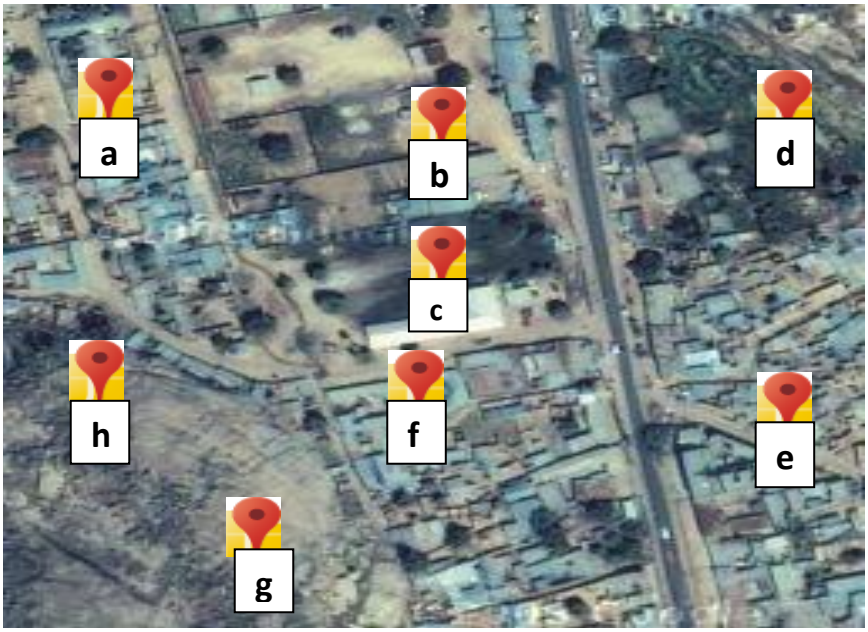


Fig 4: An aerial view of Dutsinma town (12^{o} 27" 18 N: 7^{o} 29" E) showing sampling points (red pegs) within 1 km radius. Sampling points f, g and h above all disappear during dry seasons.

Legend

a Pool

b Pond

c Concrete reservoir

d Over head reservoir

e Rice farm

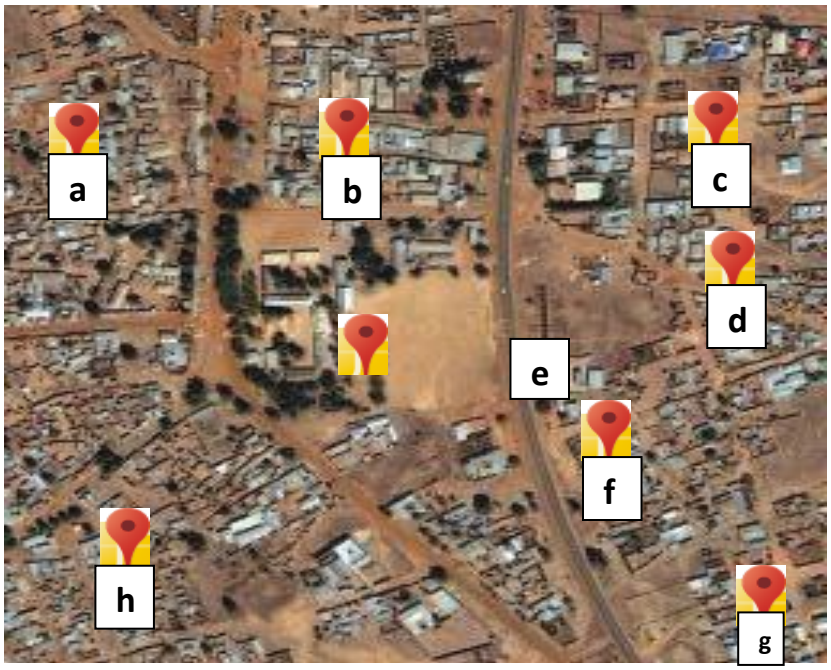


Fig 5: An aerial view of Kankara town (11^{o} 93" N : 7^{o} 41" E) showing sampling points (red pegs) within 1 km radius. Sampling points e, f, g and h above all disappear during dry seasons.

Legend

a	Pool
b	Pond
c	Concrete reservoir
d	Over head reservoir

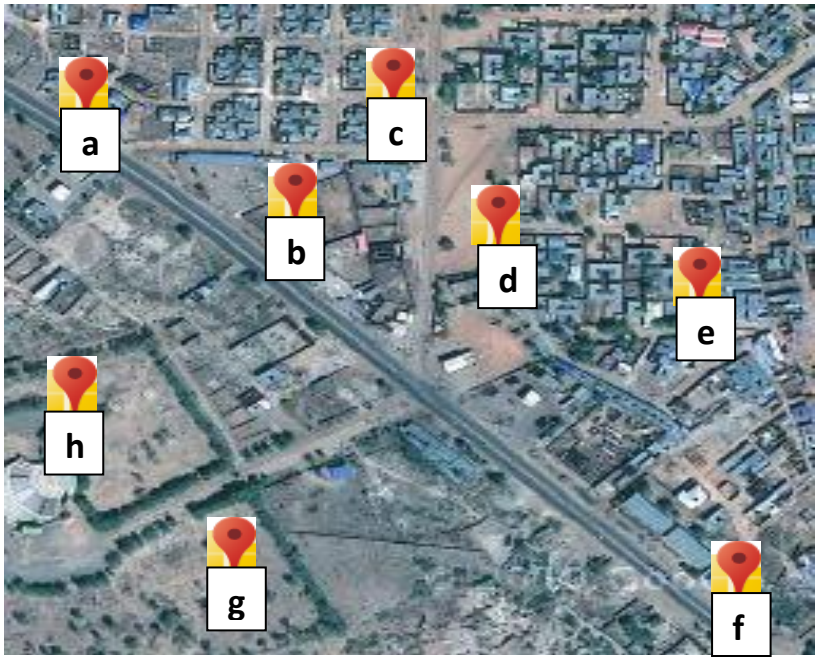


Fig 6: An aerial view of Katsina city (12^{o} 59" N : 7^{o} 36" E) showing sampling points (red pegs) within 1 km radius. Sampling points e, f, g and h above all disappear during dry seasons.

Legend

a	Pool
b	Pond
c	Concrete reservoir
d	Over head reservoir

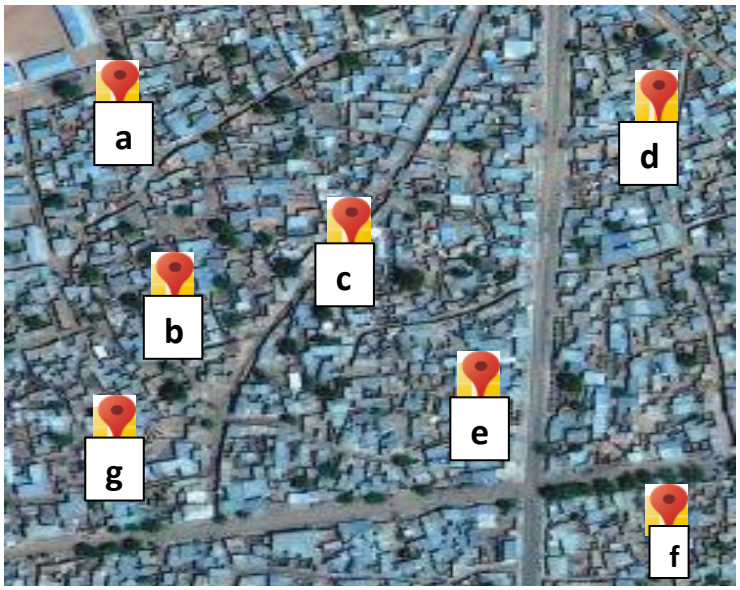


Fig 7: An aerial view of Daura town (13^{o} 2’ 11" N: 8^{o} 9’ 4" E) showing sampling points (red pegs) within 1 km radius. Sampling points e, f and g above all disappear during dry seasons.

Legend

a	Pool
b	Pond
c	Concrete reservoir
d	Over head reservoir

3.3 COORDINATES OF SAMPLED STUDY SITES

Table 3.1: Coordinates of indoor mosquito sampling points

Site	A		B		C	
	Lat.	Long.	Lat.	Long.	Lat.	Long.
Katsina	12° 44'56"	7° 25'55	12° 33'33"	7°27'71"	12°31'89"	7°29'54"
Daura	13°05'87"	8° 33'21"	13°00'34"	8° 34'42"	13°04'43"	8°31'11"
Dutsinma	12°15'77"	7° 12'27"	12°12'44"	7°13'36"	12°10'00"	7°16'66"
Kankara	11°78'88"	7° 25'88"	11°70'00"	7° 24'89"	11°72'93"	7°25'77"
Funtua	11°25'78"	7°20'85"	11°20'00"	7°20'54"	11°00'09"	7° 20'66"
Dandume	11°16'99"	7°05'67"	11°14'00"	7°04'88"	11°14'77"	7°05'33"

NB.
Only three mosquito resting sites were sampled in each of the sampled towns and their coordinates were as presented in Table 3.1.

Table 3.2: Coordinates of *Anopheles* breeding points

Locality	A (pool)		B (pond)		C (Overhead tank)		D (Cemented reservoir)		E (Rice farm)	
	Lat.	Long.	Lat.	Long.	Lat.	Long.	Lat.	Long.	Lat.	Long.
Katsina	12 °34’54"	7 °20’11"	12 °33’30"	7 °1999"	12 °31’80"	7 °18’22"	12 °33’77"	7 °17’55"	nil	nil
Daura	nil	nil	13 °00’31"	8 °30’00"	13 °04’41"	8 °33’33"	nil	nil	13 °01’11"	8 °30’02"
Dutsinma	12 °15’75"	7 °15’67"	12 °15’41"	7 °14’88"	12 °15’04"	7 °14’72"	12 °15’56"	7 °14’77"	nil	nil
Kankara	11 °78’86"	7 °25’66"	11 °70’02"	7 °25’00"	11 °72’99"	7 °24’77"	nil	nil	nil	nil
Funtua	11 °25’76"	7 °20’08"	11 °2007"	7 °20’00"	11 °20’09"	7 °20’11"	11 °20’33"	7 °.11’10"	11 °20’99"	7 °20’24"
Dandume	11 °16’97"	7 °05’77"	11 °14’09	7 °.0333"	11 °14’71"	7 °09’90"	11 °16’00"	7 °05’59"	11 °15’57"	7 °02’22"

NB.
Of the breeding points sampled, only the five shown in Table 3.2 were consistently present in all the three ecological zones and even then cemented reservoirs were not conspicuous in Daura and Kankara and rice farms were also not conspicuous in Katsina, Dutsinma and Kankara.

3.4 COLLECTION OF ADULT INDOOR RESTING MOSQUITOES

Indoor resting *Anopheles* were collected according to Faye *et al*. (1997), using Pyrethrum spray insecticide. In each town, 3 representative households were choosen and sprayed once bimonthly to determine the indoor resting density (as the number of female anophelines resting per room). Prior to spraying of the rooms, 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 pyrethrin insecticide was sprayed in a clockwise direction towards the ceiling until the whole room was filled with a fine mist of the insecticide. 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. All the mosquitoes on the sheet were transfered into a small bottle containing 70% alcohol and was labeled according to the name of collector, place of collection, mosquito species (based on pale spots on wings), date, among others (Service 1993).

3.5 COLLECTION OF *ANOPHELES* LARVAE

Anopheles breeding sites were identified for during the dry and wet seasons. In each locality, larvae of all available instars were collected from all water bodies within a radius of 1 km. Sample size per water body was not determined and samples were mixed (Onyabe and Conn, 2001). In water bodies (e.g. pools, reservoirs, puddles etc.) mosquito larvae were collected using standard dipping techniques with a 350ml dipper and 3 cm wide tea sieve (Service, 1993). The leading edge of the dipper was submerged, dipped at about 45 degrees, about an inch below the surface of the water quickly, but gently, the dipper was moved along a straight line in open water or in water with small floating debris. The stroke was ended just before the dipper was filled to prevent overflowing. The dipper was then raised out of the water gently without spilling the

water and or the mosquitoes. At each sampling location, anopheline and culicine mosquitoes that were collected were differentiated based on the floating habit of the mosquito larvae (Rozendaal, 1997). Because morphological differentiation of pupae into different genera is very difficult and impracticable under field conditions (Fillinger *et al*., 2004; Sattler *et al*., 2005), pupae were not differentiated. The immature stages were then sieved into a container which was labeled according to name of collector, place of collection, time and date.

Pictures of all *Anopheles* breeding sites within sampling areas across all the three ecological zones within Katsina State were taken using an ES 15 Samsung digital camera and was transferred to a computer laptop.

3.6 REARING OF LARVAE TO ADULTS IN THE LABORATORY

The larvae collected from the field were transferred into white plastic containers filled to two-thirds of their volume with non chlorinated distilled water. The mouth of the container was covered with a mosquito net. A small hole was made at the center of the net and plugged with a dry cotton wool until adults emerged and were to be fed (Service, 1993).

The larvae were fed with baker's yeast. Ten grams of yeast was dissolved in 50ml of distilled water. Ten percent sugar solution was prepared and a tea spoon was used to apply a 2ml of the sugar solution onto the cotton wool earlier inserted through the mosquito net on the mouth of the container, just enough to wet the cotton wool. That served as food source for the emerging adults. This was repeated after every two days using fresh cotton wool. Feeding continued for 5 days in order to allow the young mosquito to develop all adult features (Service, 1993).

Feeding was done once daily (Service, 1993) until pupae emerged. The adults were confined to the container by the mosquito netting applied.

3.7 CHARACTERIZATION OF POTENTIAL MOSQUITO BREEDING SITES

Each ward within the study areas was searched for open water bodies. To locate areas of potential breeding sites, the search was lead by the Ward head who has a good knowledge of the area. The search was done on foot and was limited to a 1 km radius, and was completed in 20 days. A sketch map was made of the area based on 50m transects made with a hand-held compass. Between January and April of every year of study. Potential surface pools were marked on the map. Similar comprehensive searches were done in April, May, and June 2009. All pools of water found, regardless of size, were noted on the map and sampled for *Anopheles* larvae. Those positive were assigned a number and marked with a stake. Similar searches were made once every two months from 2009 to 2011.

Characterization of potential mosquito breeding sites was carried out by recording the characteristics of the different categories of breeding sites present within the sampling areas. For example, size of water body, type of vegetation, habitat permanence, water condition and exposure to sunlight, daily temperature and rainfall were studied. All the features of pools, ponds, rice fields, wells, marshes, water reservoirs among others. were observed and described as observed in the field. Breeding sites were charecterised as stream, pond, swamp, pools, puddle, footprints, tyre track, cemented and overhead reservoirs, and others. Sites which were dry at the time of visit were excluded (Sattler *et al*., 2005).

The area of each body of water was evaluated by approximating the shape as a square, a rectangle, a circle or an ellipse. Turbidity was estimated by using a graduated transparent bottle with black letters written on the bottom. The bottle was gradually filled with water from the collection site and turbidity was evaluated by recording the mark reached by the water when the letters were no longer visible. Graduations ranged from 0 to 26 cm, starting from the top of the

bottle, so that a higher value indicated greater turbidity (Sattler *et al*., 2005). The proportion of the water surface covered by vegetation was estimated visually. The vegetation was not classified further, but included water lettuce, hypha grass species, water lentils and rotten vegetation.

The proportion of the water surface exposed to sunlight was estimated visually by assessing the proportion of the water surface shadowed at midday (Sattler *et al*., 2005).

Mosquito breeding sites were geo-referenced, using two hand-held global positioning system (GPS) receivers (eTrex and Garmin 12Xl, Garmin International Inc.; Olathe, U.S.A.). GPS was used to determine the exact coordinates of each beeding site. Maps of some of the study areas were obtained from google map and some were created in version 9.0 ESRI ArcMap (Redlands, CA, U.S.A.).

Daily temperature and amount of rainfall were recorded for each of the sampled towns.

3.8 MOSQUITO IDENTIFICATION

Mosquitoes analyzed were from two sources: 1) indoor resting adult catches and 2) adults reared from field collected larvae (series). The adult mosquitoes were first identified using morphological characteristics according to Gillies and Coetzee (1987), Hopkins (1952) and Proft *et al.* (1999) as outlined below. Members of the *Anopheles gambiae* s.l complex were identified into *An. gambiae* ss and *An. arabiensis* using species specific multiplex polymerase chain reaction (PCR). After morphological identification, specimens were stored in vials containing 70% ethanol for PCR.

3.8.1 PCR IDENTIFICATION OF A*nopheles gambiae*

3.8.1.1 *PCR Authentification of Anopheles gambiae* species complex according to Proft *et al*. (1999)

Samples of the adult mosquitoes morphologically identified as *Anopheles gambiae* s.l. were diferenciated to species level and also to M and S forms using PCR as outlined below.

Five sets of primers designed from the DNA sequences of the intergenic spacer (ITS) region of *An. gambiae* complex ribosomal DNA (rDNA) were used in PCR for the member species identification (Proft *et al*., 1999). The sequence details of these primers abbreviated UN, GA, ME, AR and QD and the expected sizes of the PCR products are given in Table 3.3. The UN primer anneals to the same position on the rDNA sequences of all five species, GA anneals specifically to *An. gambiae* s.s. ME anneals to both *An. merus* and *melas*, AR to *An. arabiensis* and QD to *An. quadriannulatus*.

The PCR reaction mix of 20 µl contained 1X PCR buffer supplied by the manufacturer (Sigma, USA), 200µM of each of the 4 oligonucleotide triphosphates (dNTPs), 10µM of each ligonucleotide primers and 0.125 U of *Taq* DNA polymerase enzyme (Sigma, USA), and 0.5 µl of the genomic DNA was used as template for the amplification reaction. Sterile double distilled water was used to make up the volume to 20µl. The reaction mix was spun down briefly at 14,000rpm. The amplification was carried out using a PTC 100 thermal cycler (MJ Research Inc., USA)

Table 3.3 PCR Master Mix for identifying *An. gambiae* species

1	96	Reagent
11.8 µl	**1180 µl**	**Sterile H_20**
2.5 µl	**250 µl**	**Taq 10X PCR Buffer with $MgCl_2$**
2.5 µl	**250 µl**	**dNTP (2 mM mix G, A, T, C)**
1.0 µl	**100 µl**	**$MgCl_2$ (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 µl**	**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)**
24 µl	**2.5 ml**	

NB. 24 µl was added to 1 µl 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 .

3.8.1.2 *PCR cycle conditions*

The cycling parameters for the reaction were as follows; initial denaturation was carried out at 94^{o} C for 3min, it was followed by annealing of 35 cycles of 94^{o}C for 30s, 50^{o}C for 30s, 72^{o}C for 60s and ended by a final of cycle of 94^{o} C for 30s, 50^{o} C for 30 s and 72^{o}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 electrophores 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.

3.8.2 *PCR discrimination of M and S forms of the An. gambiae s.s (Fanello et al., 2002)*

PCR Master Mix for 96 and 25µl PCR reactions were prepared and reagents were added in the order presented in Table 3.4 below.

Table 3.4 PCR Master Mix for *discrimination of Mopti and Savannah members of the Anopheles gambiae* s.s.

1	96	Reagent
14.8 µl	1480 µl	Sterile H_20
2.5 µl	250 µl	10X PCR Buffer
2.5 µl	250 µl	dNTP (2 mM mix G, A, T, C)
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 µl	GA (F, 25 pmol/µl) [CTGGTTTGGTCGGCACGTTT]
1.0 µl	100 µl	$MgCl_2$ (25mM)
0.2 µl	20 µl	Taq DNA polymerase (5U/µl)
24 µl	2.4ml	

NB. 24 µl was added to 1 µl template DNA

F and R indicate forward and reverse orientation and 1 µl DNA template was used.

3.8.3 PCR authentication for the members of the *Anopheles funestus* group (Cohuet *et al.*, 2003)

Members of the *An. funestus* complex were identified using the cocktail PCR method based on the ITS2 region developed by Koekemoer *et al.* (2002). The sequence details of the primers (abbreviated UV, FUN RIV, PAR, LEES and VAN), and expected diagnostic sizes of the amplified DNA products are shown in Table 3.5.

The PCR reaction mix of 25µl for each sample contained 10X reaction buffer, (500mM KCl, 100mM Tris- HCl pH 8.3), 1.5mM $MgCl_2$, 10pmol /primer of each primer, 10mM of each dNTP and 0.5 U of thermostable *Taq* DNA polymerase. DNA amplification was carried out using a PTC 100 thermal cycler, MJ Research Inc., USA.

PCR Master Mix for 96, 48 or 12.5µl PCR reactions was prepared as shown below and reagents were added in the order presented below.

Table 3.5 PCR Master Mix for authentication for the members of the *Anopheles funestus* group

1	96	Reagent
3.0 µl	300 µl	Sterile H_20
2.5 µl	250 µl	Taq 10X PCR Buffer with $MgCl_2$
2.5 µl	250 µl	dNTP (2 mM mix G, A, T, C)
1.5 µl	150 µl	$MgCl_2$ (25mM)
2.0 µl	100 µl	UV (F, 33pmol/ µl) [TGT GAA CTG CAG GAC ACA T]
2.0 µl	100 µl	FUN (R, 33 pmol/ul) [GCA TCG ATG GGT TAA TCA TG]
2.0 µl	100 µl	VAN (R, 33 pmol/µl) [TGT CGA CTT GGT AGC CGA AC]
2.0 µl	100 µl	RIV (R, 33 pmol/µl) [CAA GCC GTT CGA CCC TGA TT]
2.0 µl	100 µl	PAR (R, 33 pmol/µl) [TGA GGT CCC AAG CTA GGT TC]
2.0 µl	200 µl	RIVLIKA (R, 33 pmol/µl) [CCG CCT CCC GTG GAG TGG GGG]
2.0 µl	200 µl	LEES (R, 33 pmol/µl) [TAC ACG GGC GCC ATG TAG TT]
0.5 µl	20 µl	Taq DNA polymerase (5U/µl)
24 µl	2.5 ml	

NB. 24 µl was added to 1 µl template DNA

F and R indicate forward and reverse orientation. Volume can be reduced to 12.5 ul for economy. DNA extraction negative control was included in addition to PCR reaction mix negative control.

3.8.3.1 *Agarose gel electrophoresis*

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 0.5µg/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 Identification of Malaria Sporozoites

Anopheles mosquitoes caught indoors were subjected to VecTest, 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 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 247* CS 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 247* CS antigens and 1 vial of Grinding Solution (6 ml)

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.

3.9.1 Specimen Collection and Preparation

3.9.1.1. ***Mosquitoes***: Only female Anopheline mosquitoes were used. Homogenized samples of mosquitoes were stored at - 20°C until processed.

3.9.1.2. ***Homoginization***

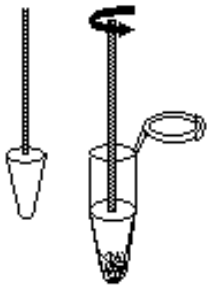
One to 10 female Anopheline mosquitoes were placed into the conical grinding tube provided in the kit, so as to provide the adequate DNA required, and then placed in the tube stand provided.



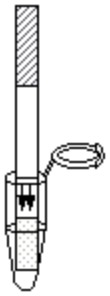
Thirteen drops (250µl) of Grinding Solution from the dropper bottle was dispensed onto the mosquitoes in the tube.



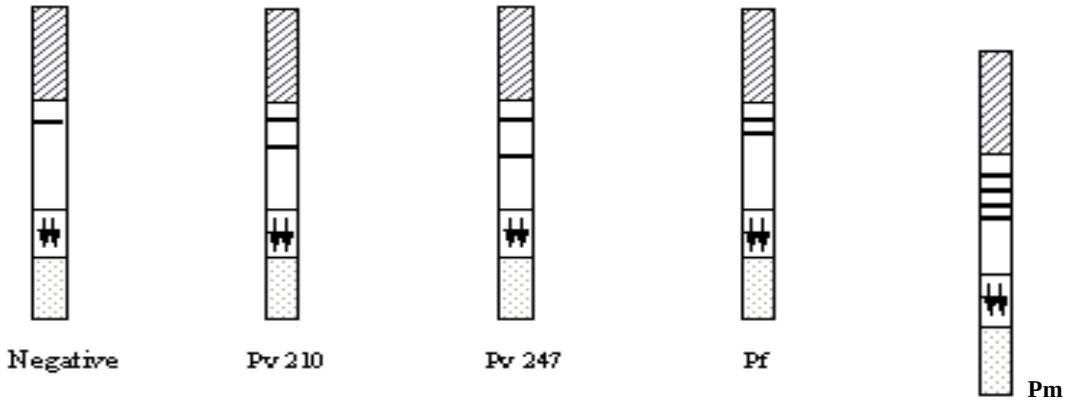
The mosquitoes were homogenized using a pestle provided in the kit.



A test strip from the canister was then placed into the mosquito suspension in the grinding tube with the arrows pointing down. The desiccant cap was replaced on the canister to protect the remaining strips from moisture. Fifteen minutes was allowed for the test to be completed.



The test results were determined by removing the test strip and comparing it to the pictorial sample provided on the container. The control line develops as a result of an antibody capture principle. The flow of the sample up the dipstick carries unreacted anti-*P. falciparum*, anti-*P. malariae*, anti-*P. vivax 210*, and anti-*P. vivax 247* conjugated with gold sol particles. When the sample reaches the Control Zone, these unreacted conjugates binds to the antibody immobilized on the membrane and a signal is produced.



3.9.1.3. *Handling the Solution*

Before use, the Grinding Solution was mixed by gently inverting the bottle five times. The bottle was held in an upside-down, vertical position and the white portion of the dropper cap just above the tip was squeezed. Care was taken when dispensing drops of grinding solution into tubes by holding the bottle firmly and not at an angle, because the size of the drops may vary, and can cause the total amount of dispensed solution to be incorrect.

The test results were rejected if the control line failed to develop and the tests were run again. The test was only interpreted as positive if two or more lines developed.

When only one control line developed on the dipstick, the result was treated as negative. However the presence of two close lines indicated *P. falciparum*, two widely separated lines indicate *P. vivax* 210 however two far apart lines indicated *P. vivax* 247. Two closely packed lines indicated the presence of *P. falciparum.*

Results were read within 30 minutes of performing the assay. Because thereafter, the dipsticks developed a gray colour that obscured accurate interpretation of results.

3.10 Retrospective Prevalence Study of Malaria

Information on the prevalence of malaria during dry and wet seasons were collected from hospital records at The Federal Medical Centre Katsina (ecological Zone A), General Hospital Dutsinma (ecological Zone B) and also General Hospital Funtua (ecological Zone C). Access to file records of laboratory test results on Malaria Parasite (MP) test were authorized by each hospital's management. Subsequently, the Malaria Parasite (MP) test results from 2009 to 2011 were analyzed and the monthly infection trends were noted.

3.11 Statistical Tools Used

ANOVA was used to determine the difference between *Anopheles* and *Plasmodium* species distribution and their abundance between towns, zones and seasons.

CHAPTER FOUR

4.0 RESULTS

4.1 MORPHOLOGICAL IDENTIFICATION

During the period of study (2009 – 2011) a total of 3,027 mosquitoes were collected across the three ecological zones and all were morphologically identified as anopheline mosquitoes. There was no significant difference between *An. gambiae* and *An. funestus* larvae collected from Zone A, Zone B and Zone C (x^2, p >0.05, F=5.05)(Appendix 34 & 35) during the period 2009 - 2011.

Of the 1649 adults reared from larval collections, *An. gambiae* s.l was the highest with 1027 (62.28%) followed by *An. funestus* with 580 (35.17%) and the least was *An. maculipennis* with 16 (0.97%) (Table 4.1). Of the 1378 adult mosquitoes from indoor adult collections, the preponderant species was *An. gambiae*, with 926 (67.2%), followed by *An. funestus,* 431 (31.3%) while the least aabundant was *An. maculipennis* with 9 (0.65%) (Table 4.2). *An. maculipennis* and *An. quadrimaculatus* were collected from Zone A only, (Funtua and Dandume towns) (Table 4.2). Of the mosquitoes collected indoors, *An. maculipennis* was the least abundant with 16 (0.9%).

Variability was observed in the spatial distribution of *An. gambiae* s.s and *An. funestus* larval populations. Zone A recorded the highest preponderance of larvae at Dandume, 555 (33.7%) and at Funtua with 298 (18.1%) while least abundance was 148 (9.0%) in Zone C at Katsina. Similarly, adults were more preponderant in Zone A at Dandume with 301 (21.8%) and Funtua 299 (21.7%) and were not abundant in Zone C at Daura with 98 (7.1%) (Table 4.2).

Table 4.1 Composition of *Anopheles* species larvae from different localities

Zone	Locality	*Anopheles* species				
		An. gambiae	*An. funestus*	*An. quadrimaculatus*	*An. maculipennis*	Total
Zone A	Funtua	169	109	11	9	298 (18.1%)
"	Dandume	356	178	15	6	555 (33.7%)
Zone B	Dutsinma	165	53	0	0	218 (13.2%)
"	Kankara	180	94	0	0	274 (16.6%)
Zone C	Katsina	108	40	0	0	148 (9.0%)
"	Daura	99	69	0	0	156 (9.5%)
	Total	1027(62.28)	580(35.17)	26 (1.58%)	16 (0.97%)	1649

Table 4.2 Species composition of *Anopheles* from indoor adult collections

Zone	Town/ Species	*An. gambiae*	*An. funestus*	*An. quadrimaculatus*	*An. maculipennis*	Total
Zone A	Funtua	211	88	7	2	299 (21.7%)
"	Dandume	214	75	5	7	301 (21.8%)
Zone B	Dutsinma	166	72	0	0	238 (17.3%)
"	Kankara	200	77	0	0	277 (20.1%)
Zone C	Katsina	111	54	0	0	165 (12.0%)
"	Daura	70	28	0	0	98 (7.1%)
	Total	926(67.2%)	431(31.3%)	12 (0.87%)	9 (0.65%)	1378

There was no significant difference in the numbers of larval collections between Zone A (Dandume), Zone B (Dutsinma) and Zone C (Daura) (p >0.05, F=10.13)(Appendix 35& 36). There was no significant difference in indoor collections between Zone A (Dandume), Zone B (Dutsinma) and Zone C (Daura) (p >0.05, F=10.13)(Appendix 38). There was no significant difference in indoor collections between *An. gambiae* and *An. funestus* within Zone A, Zone B and Zone C (p >0.05, F=5.05)(Appendix 65).

4.2 DISTRIBUTION OF *ANOPHELES* SPECIES ACROSS SAMPLED TOWNS

4.2.1 Adults

Anopheles gambiae adults caught were the most abundant in all the sampled resting places. Its adults were the most abundant in Zone A with 200 at Kankara and the lowest of 70 in Zone C at Katsina. On the other hand, the highest preponderance of *An. funestus* was 103 in Zone A at Funtua and the lowest of 28 in Zone C was found at Daura. Among all species sampled, *An. macilipalpis* was the least abundant with only 2 in Zone C (Funtua) (Fig 8 and Appendix 65). The highest number of *Anopheles* collected indoors was in Zone A, that is 301 at Dandume and their lowest abundance was in Zone C, that is 98 at Daura. The highest number of *An. gambiae* collected was 35 in Zone A at Dandume. The least collection was a single *An. funestus* at Daura in Zone C (Fig 9 and Appendix 66).

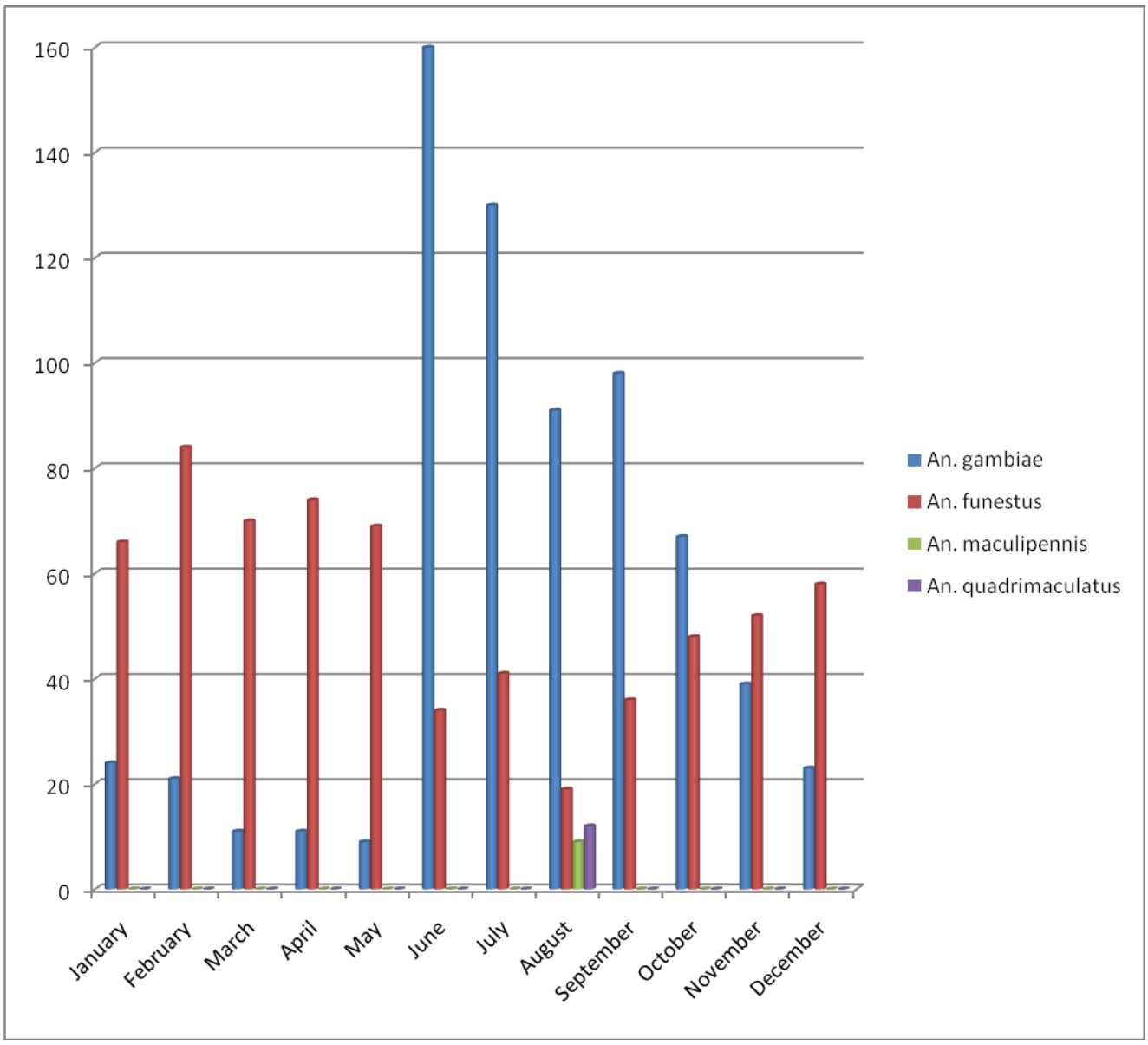


Fig 8: Poolled monthly distribution of *Anopheles* adults caught indoors

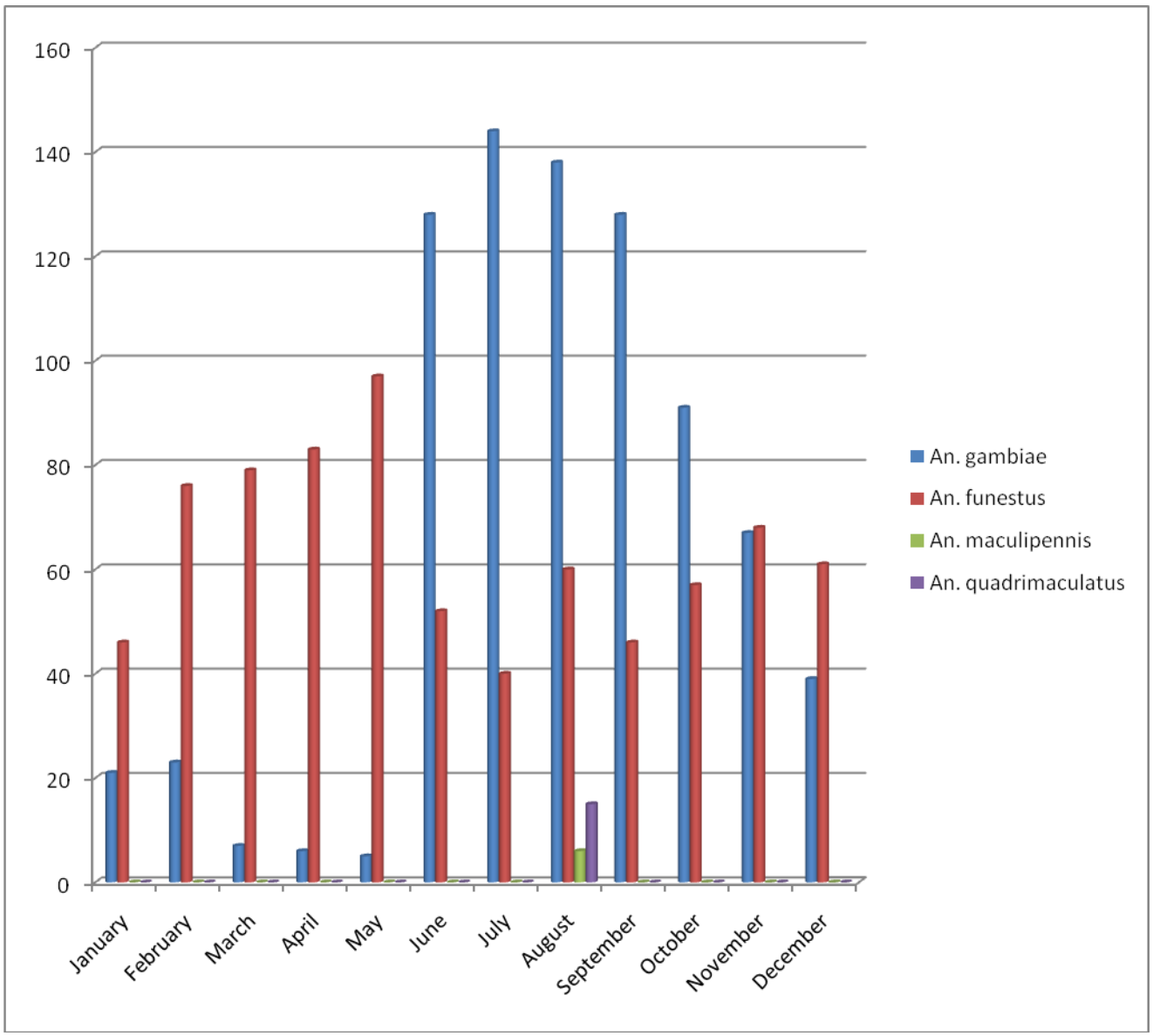


Fig 9: Poolled monthly distribution of *Anopheles* adults reared from larval collectoions

4.2.2. Larvae

The highest abundance of *An. gambiae* larvae collected in a pond was 17 in Zone B at Dutsinma in September, 2009. The highest number of 25 *An. funestus* larvae was recorded in Zone B in May in ponds at Dutsinma and Kankara and in a rice farm in June in Funtua (Zone A). Pools and rice farms breeding sites were only active during wet months while ponds and reservoirs were active during both wet and dry months. Fewer number of *An. gambiae* larvae were collected during the dry months of October to May, 2010. However, the *An. funestus* collections increased in number during the dry months but reduced during the wet months (Fig 10a, 10b, and 10c).

4.3 DISTRIBUTION OF *ANOPHELES* DURING THE MONTHS OF STUDY

The month of July recorded the highest abundance of *An. gambiae* within Zone A with 47 at Dandume while the highest abundance 37 *An. funestus*, was in February also at Dandume. On the other hand, *An. gambiae* have not been collected in the dry months between May and April in Zone C at Katsina and Daura and also in Zone B at Dutsinma and Kankara. *An. funestus* had only 5 collections in the months of January and February in Zone (Daura) and in July in Zone B (Kankara) and Zone A (Funtua) (Fig 11). *An. gambiae* larvae were not abundant (highest was 8) during the dry months while the reverse was the case for *An. funestus* (highest was 29) (Fig 10a, b, and Fig 11a, c). Upto 17% of reared *An. gambiae* were collected in June and July (Fig 10a, b and c) while 13 % of *An. funestus* were collected in December followed by 10% each in October and May (Fig 11a). Up to 24% of the *An. gambiae* collected indoors were collected in June and 18% were collected in August (Fig 11b). Twenty one percent of indoor *An. funestus* were collected in May and 18% each in March and April from 2009 – 2011 (Fig 10a, b, c and 11a,b,c).

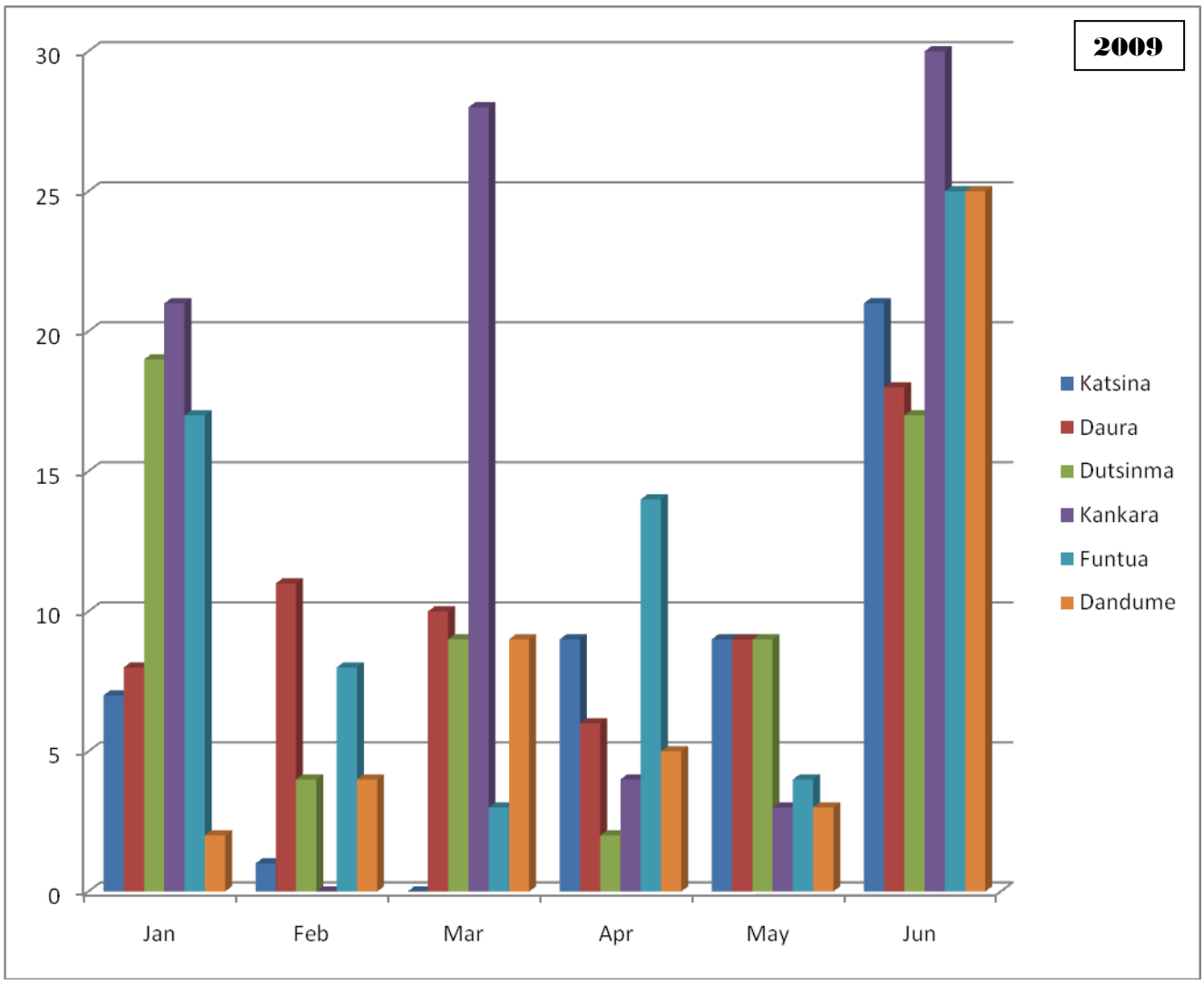


Fig 10a: Monthly distribution of *An. gambiae* adults reared from larvae

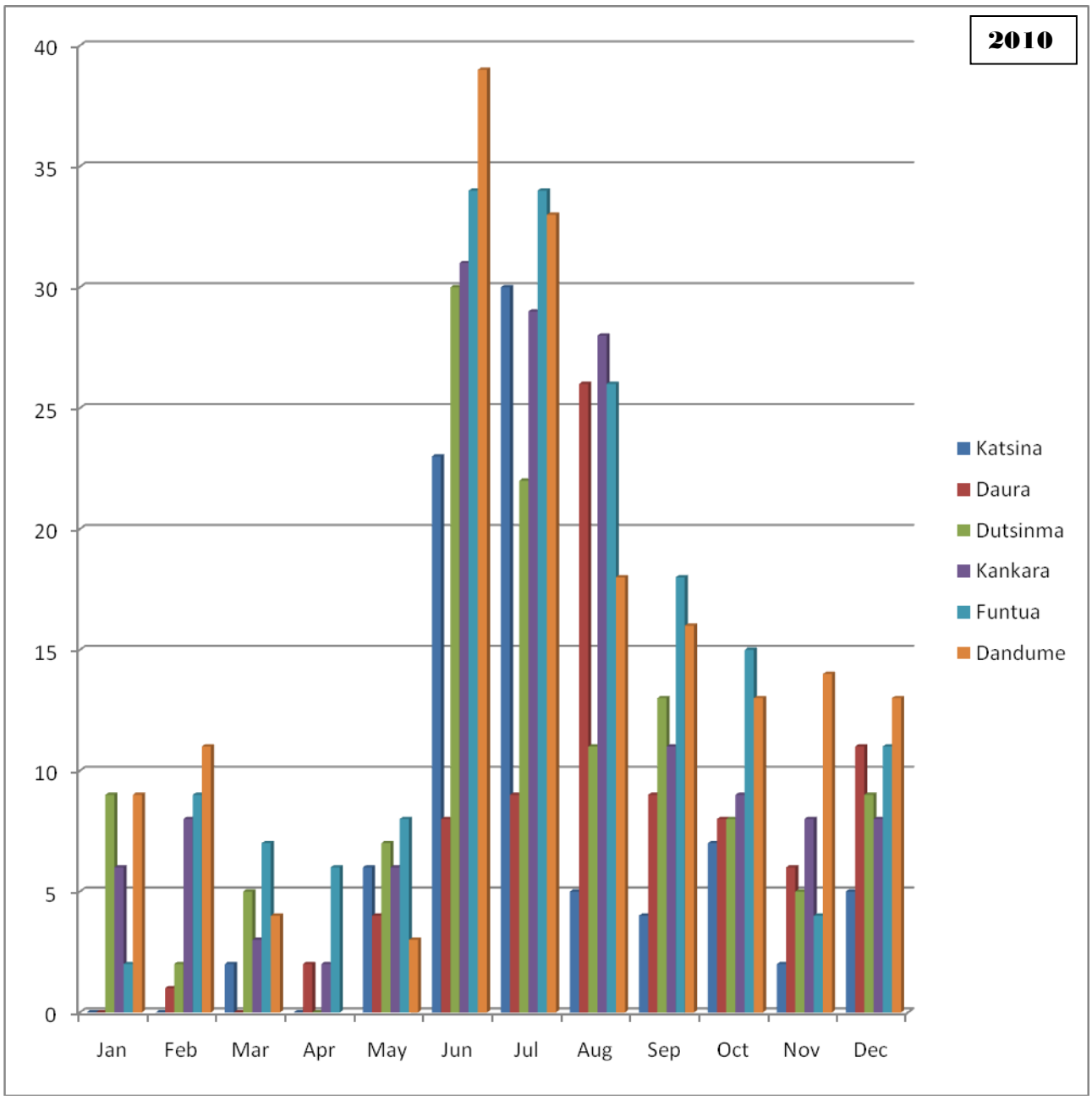


Fig 10b: Monthly distribution of *An. gambiae* adults reared from larvae

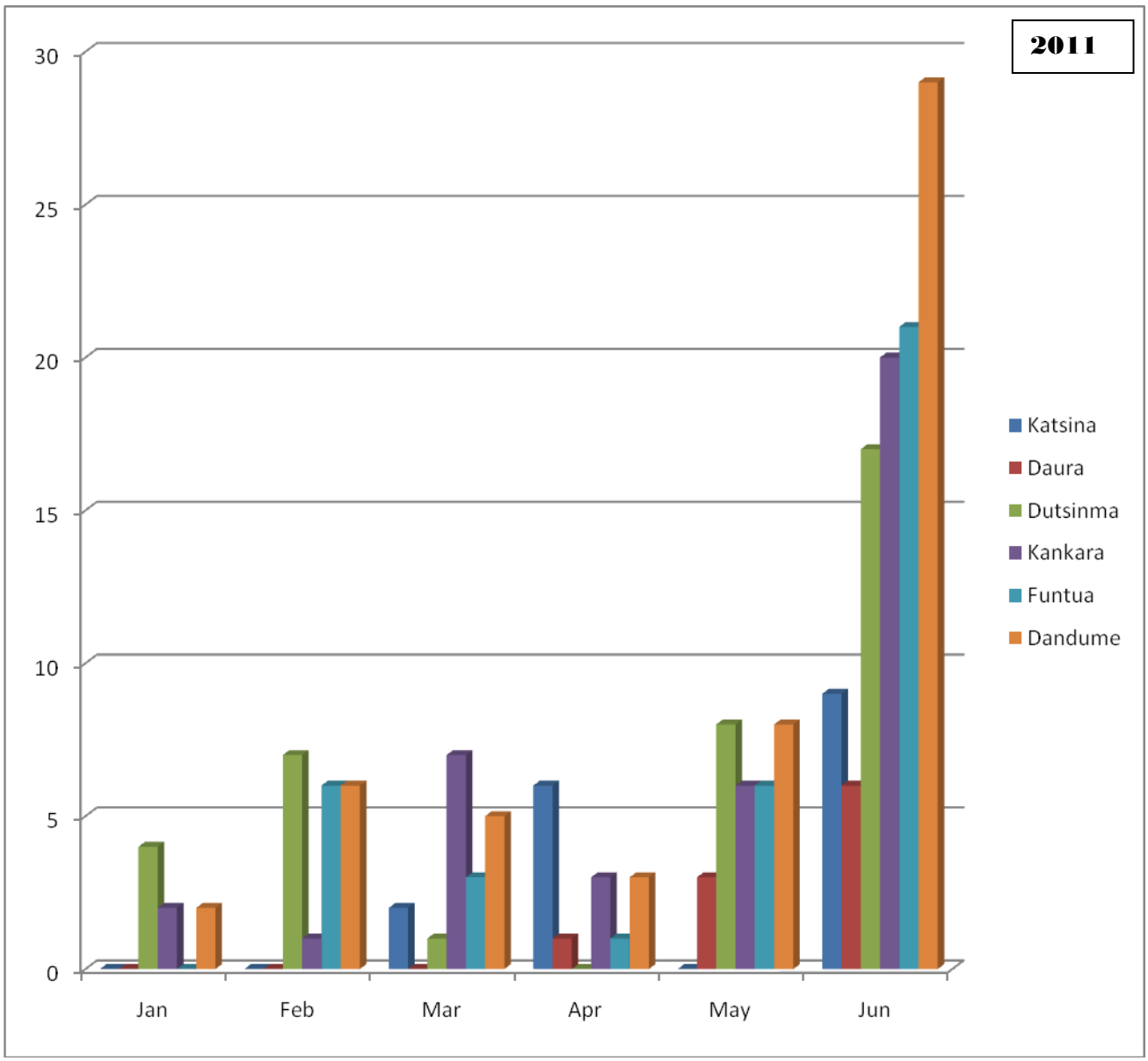


Fig 10c: Monthly distribution of *An. gambiae* adults reared from larvae

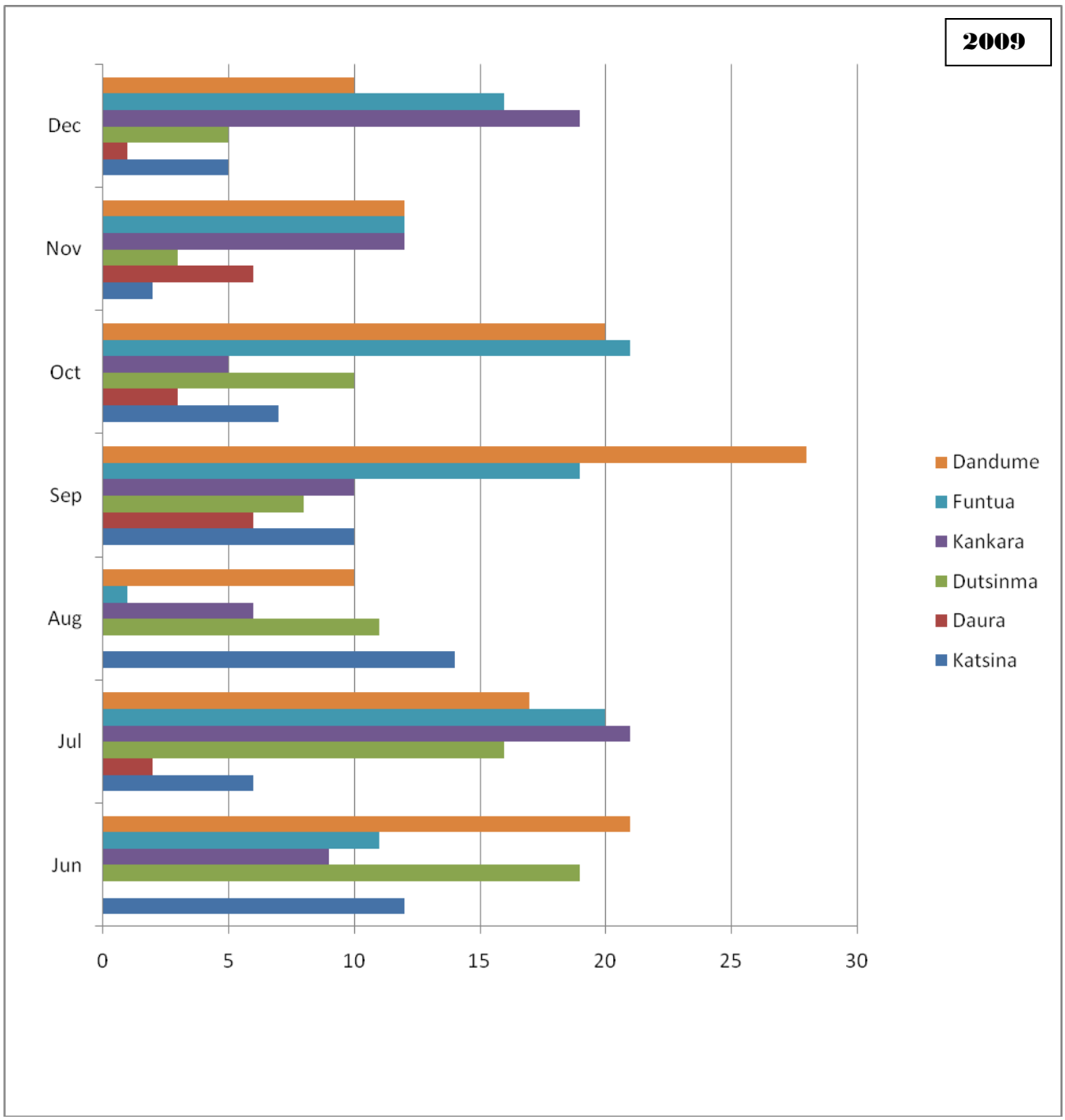


Fig 11a: Monthly distribution of indoor adult *An. gambiae* species

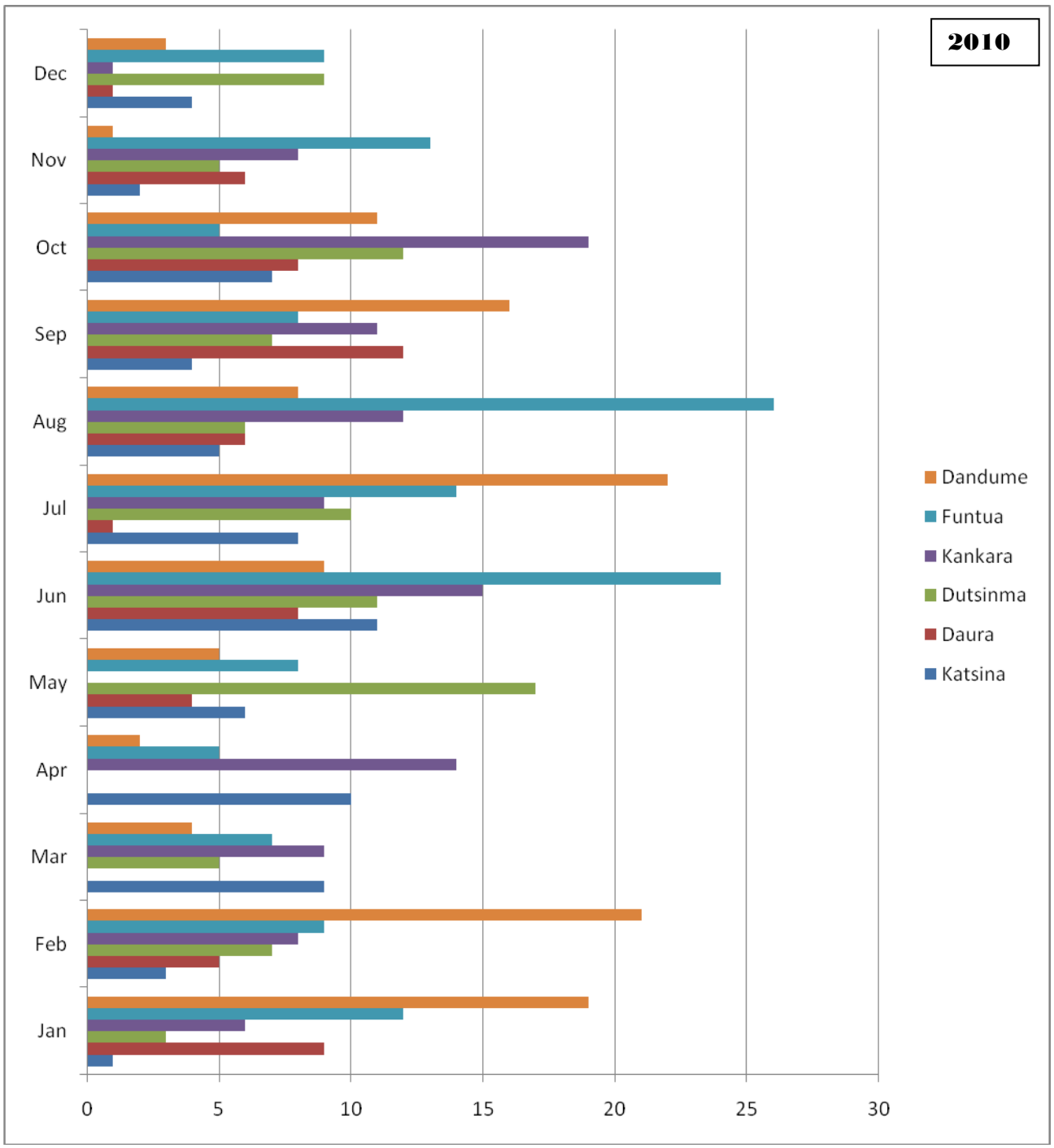


Fig 11b: Monthly distribution of indoor adult *An. gambiae* species

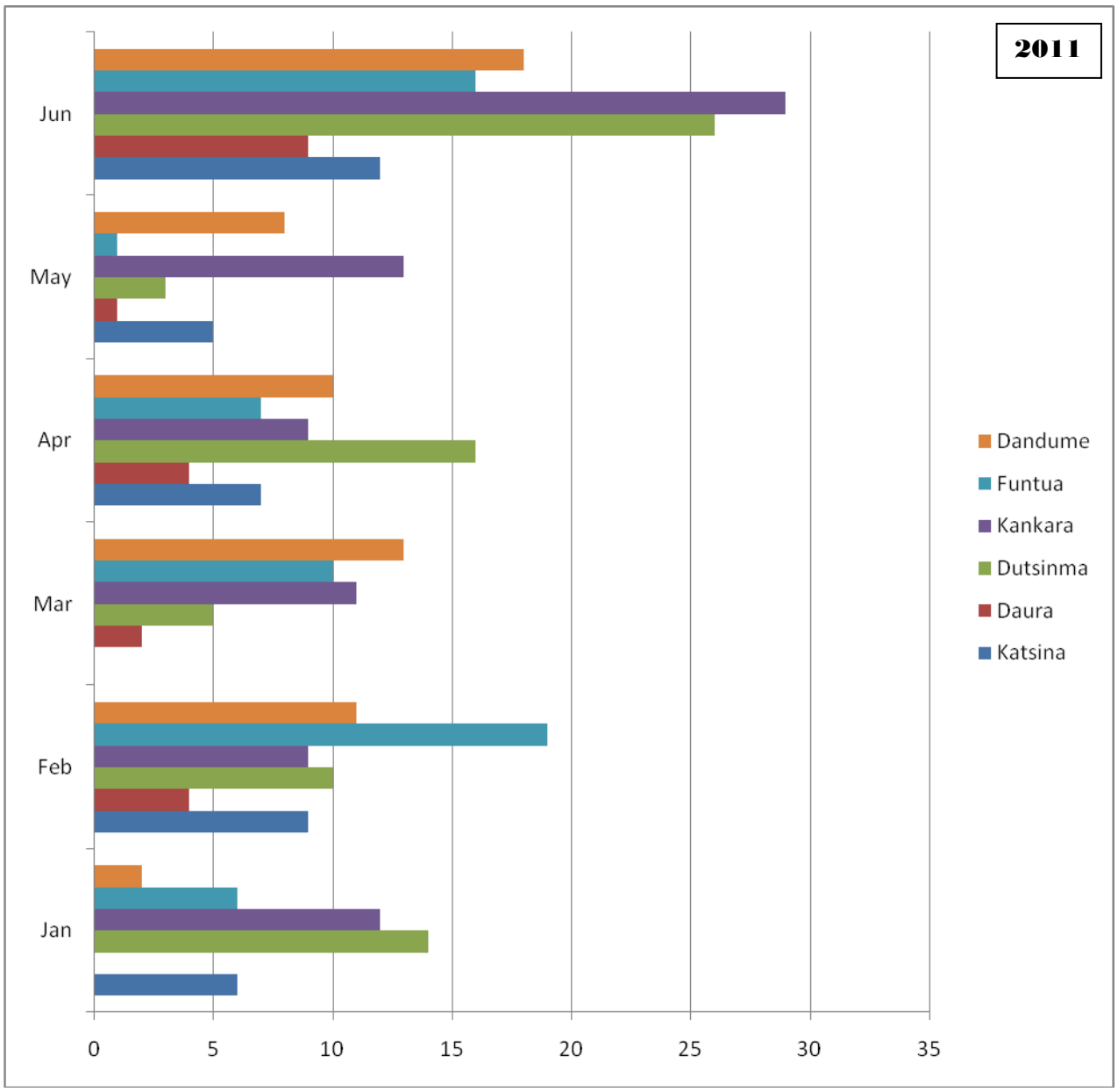


Fig 11c: Monthly distribution of indoor adult *An. gambiae* species from

4.4 DISTRIBUTION OF MOSQUITOES ACROSS BREEDING SITES

An. gambiae was the most proponderant species collected with 332 larvae from pools and the least abundant were 120 from overhead tanks. On the other hand, the highest number of *An. funestus* larvae were recovered from cemented reservoirs. There was no significant difference in number of *An. gambiae* and *An. funestus* collected from pools and ponds (p >0.05, F=9.28)(Appendix 34), from cemented reservoirs and overhead tanks (p >0.05, F=9.28)(Appendix 8) and those from rice fields and ponds (p >0.05, F=9.28)(Appendix 40 & 41). *An. maculipennis* and *An. quadrimaculatus* were found breeding in rice farms only (Table 4.5).

Table 4.5 Breeding sites and *Anopheles* species identified morphologically

Breeding site	*An. gambiae*	*An. funestus*	*An. macs*	*An. quad*	Total
Pool	332	31	0	0	363(22.0%)
Pond	165	60	0	0	225(13.7%)
Cemented reservoir	282	188	0	0	470(28.5%)
Overhead tank	120	94	0	0	214(13.0%)
Rice farm	235	100	16	26	377(22.9%)
Total	1027	541	16	26	1649

An. macs – An. maculipennis *An. quad – An. quadrimaculatus*

4.5 CHARACTERISTICS OF THE BREEDING SITES

Anopheles mosquitoes were collected from five different breeding sites within the three ecological zones. Some were collected from pools, ponds and cemented reservoirs while others were collected from overhead tanks and rice farms. Pools, ponds, cemented ground and overhead reservoirs were present in all the three ecological zones, however rice farms were fewer in Zones B and C but abundant in Zone A. The information on physical characteristics of the breeding sites observed during the study were: size of water body, type of vegetation, habitat permanence, water condition and exposure to sunlight are detailed below.

4.5.1 Size of water body

Anopheles larvae were found in water bodies ranged in size between 12 centimeters for e.g. rain pools to 50 m for earth dams.

4.5.2 Type of vegetation

Anopheles were found breeding in association with vegetation in the water bodies, e.g. *Typha* species (*Typha angustifolia)* and water lilies (Nyrnphaea spp.) and rotten vegetation.

4.5.3 Habitat permanence

Some of the observed ponds were permanent because they contain water throughout the year while others were temporary because they dried off during dry season between March and April of every study year from 2009 to 2011.

4.5.4 Water condition

The observed water bodies that were clean include rain pools while other pools were contaminated with oil, feaces, waste water, sewage etc. Others were polluted with organic matter, algal growth, deaying plant, animal waste etc. Some water bodies are turbid because they contain mud and other organic debris.

4.5.5 Exposure to sunlight

Habitats like earth dams and broken plastic reservoirs were completly exposed to sunshine however domestic water containers like buckets were only partially light and partial shaded.

The following pictures though not showing all the breeding points sampled, are however, a good representation of the different types of breeding sites peculiar to the ecological zones and sampled towns.

1. Pools: Some were brought about by water leaking from pipes (Plates I, II and III) mostly shallow and clear with little vegetation. However pools located in houses were polluted, deeper and contained more vollume of water (below) Pools located in gutters were more turbid, more polluted and smell (Appendices 21 & 22). They contain a lot of debri from vegetation, waste, sewage among others. Some pools were marshy (Appendix 20) while others were made by animal hoof prints and tyre marks (Appendix 19) and they contained plant debris. Pools were also observed at water fetching points. They were formed by seepage water from pipes and wells. There were also pools made on river beds during the dry season when there was little water. These pools ranged in size from 5 cm to 100 cm and contained rotten vegetation and other debris (Appendix 22)



Plate I: Pools within a house containing *Anopheles* larvae (arrow) at Funtua.



Plate II: Pools made by stagnant water at a washing point containing *Anopheles* larvae in a house at Daura



Plate III: Pools of water in a narrow alley containing *Anopheles* larvae at Katsina.

2. Rice farms: they contained numerous pools, some small (5 cm long) others were big (1 m long). They contained little vegetation at initial planting (Plate IV) but became filled with rice seedlings and rotten vegetation later in the farming season. Some became muddy especially during transplanting and other farm activities (Plate V).

3. Concrete reservoir

They were open reservoirs about 12 cm deep, with rotten vegetation and other debris. Some had clear water (Plates VII, VIII) while others have turbid water (Plate IX and Appendix 24). Some of them wee located in block making industries, some were abandoned fish ponds, others were reservoirs for boreholes etc.

4. Overhead tank

These were made of plastic. They contained from 1000 to 3000 litres water and abundant algal (spyrogyra) growth. Majority were clear and clean but some were turbid because of the presence of heavy algal growth (Plates X, XI & XII).

5. Ponds

These were big water bodies ranging from 2m to 20 m wide. Some contained aquatic plants all over the water surface e.g. water lilies (Plates XIII, XIV & XV) while others had vegetation only at their perimeter. Majority were turbid and contained rotten vegetation. Most were temporary because they dried up during the dry season (Appendix 26).

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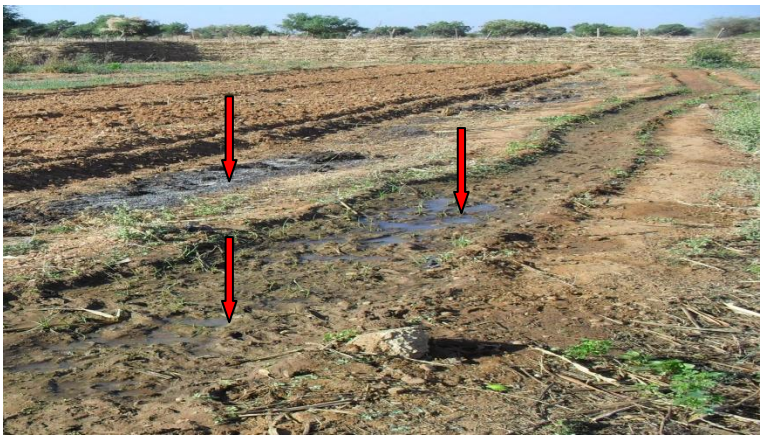


Plate IV: Pools at a farm containing *Anopheles* larvae at

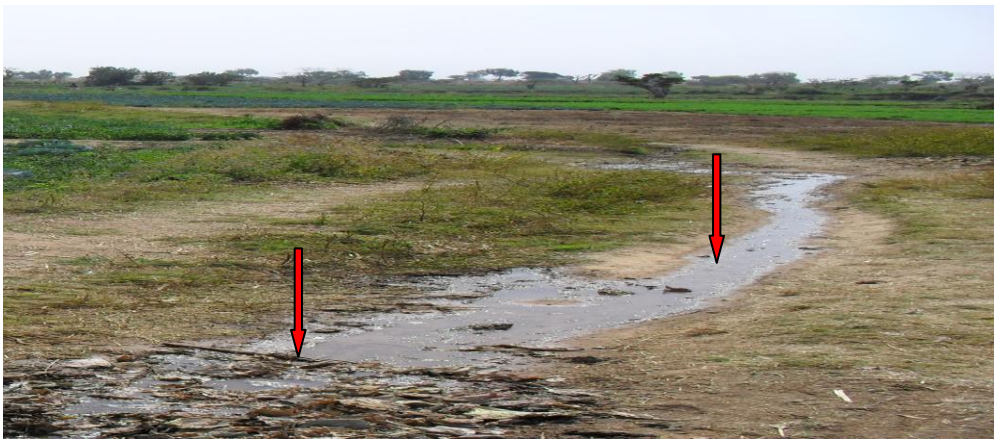


Plate V: Pools made from irrigation canals containing *Anopheles* larvae at Dutsinma



Plate VI: Puddles in a rice field where *Anopheles* larvae were collected at Dutsinma



Plate VII: Concrete water reservoirs containing *Anopheles* larvae at a block industry in Funtua



Plate VIII: Concrete water reservoirs containing *Anopheles* larvae in fish pond Dutsinma



Plate IX: Concrete water reservoirs containing *Anopheles* larvae in a water reservoir at Katsina.



Plate X: *Anopheles* larvae in plastic reservoirs at Katsina



Plate XI: *Anopheles* larvae in plastic reservoirs at Funtua



Plate XII: *Anopheles* larvae in plastic reservoirs at Dutsinma



Plate XIII: *Anopheles* larvae in temporary earth ponds during dry season at Daura



Plate XIV: *Anopheles* larvae in temporary earth ponds during dry season at Kofar Guga, Katsina

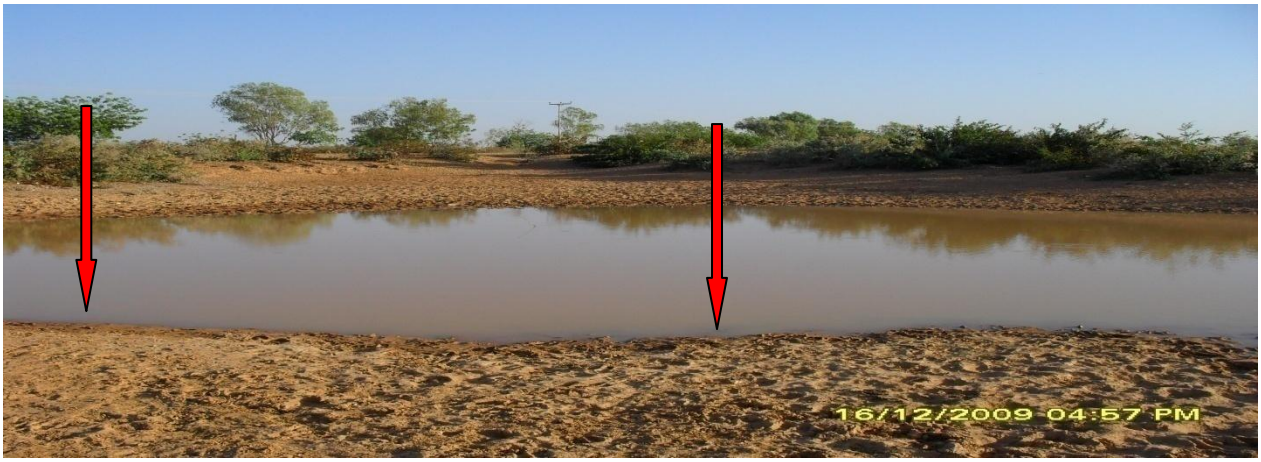


Plate XV: *Anopheles* larvae in temporary earth ponds during dry season at Daura

4.6 MOLECULAR CHARACTERISATION

4.6.1 PCR IDENTIFICATION OF THE *ANOPHELES GAMBIAE* COMPLEX

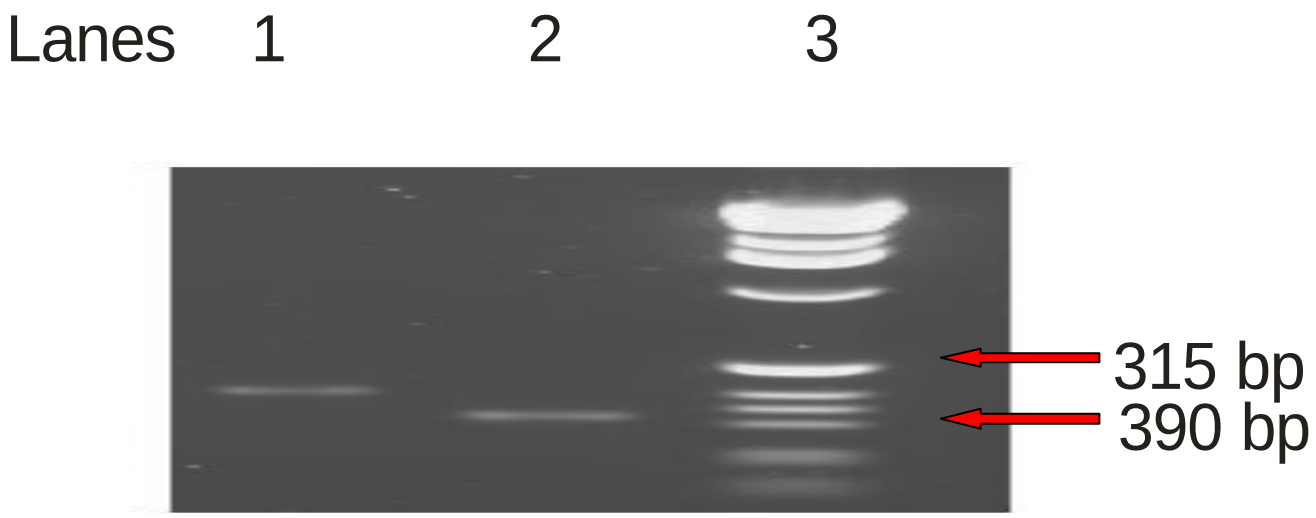
A PCR based test on the *Anopheles gambiae* reared from larvae showed that *An. gambiae* s.s. was the most preponderant, out of the 1069 adults reared from larvae, 955 (88.75%) were An. *gambiae* s.s. followed by *An. arabiensis* with 122 (11.3%). There was no significant difference between *An. gambiae s.s.* and *An. arabiensis* reared from larvae between Zones A, B and C. Also there was no significant difference between the two species (p >0.05, F=19)(Appendices 32 & 33). Nine hundred and one (97.3%) and 71 (2.7%) adult collections were found to be *An. gambiae s.s.* and *An. arabiensis* respectively. The cocktail PCR-assay on the total *An. funestus* group showed *An. funestus* s.s was the most abundant with 307 (76.6%), followed by 94 (23.4%) of *An. brucei* and 9% as *An. leesoni* (Table 4.6). There was no significant difference between *An. gambiae s.s* and *An. arabiensis* caught indoors in Zones A, B and C and also no significant difference between the two species (P >0.05, F=19)(Appendices 34 - 43). The highest number of *An. gambiae* s.s. were from Dandume with 333 and 205 for reared adults and indoor collections respectively. The lowest number of 9 *An. arabiensis* was also recorded at Dandume (Table 4. 6).

Table 4.6 Molecular charaterisation of *An. gambiae* s.l

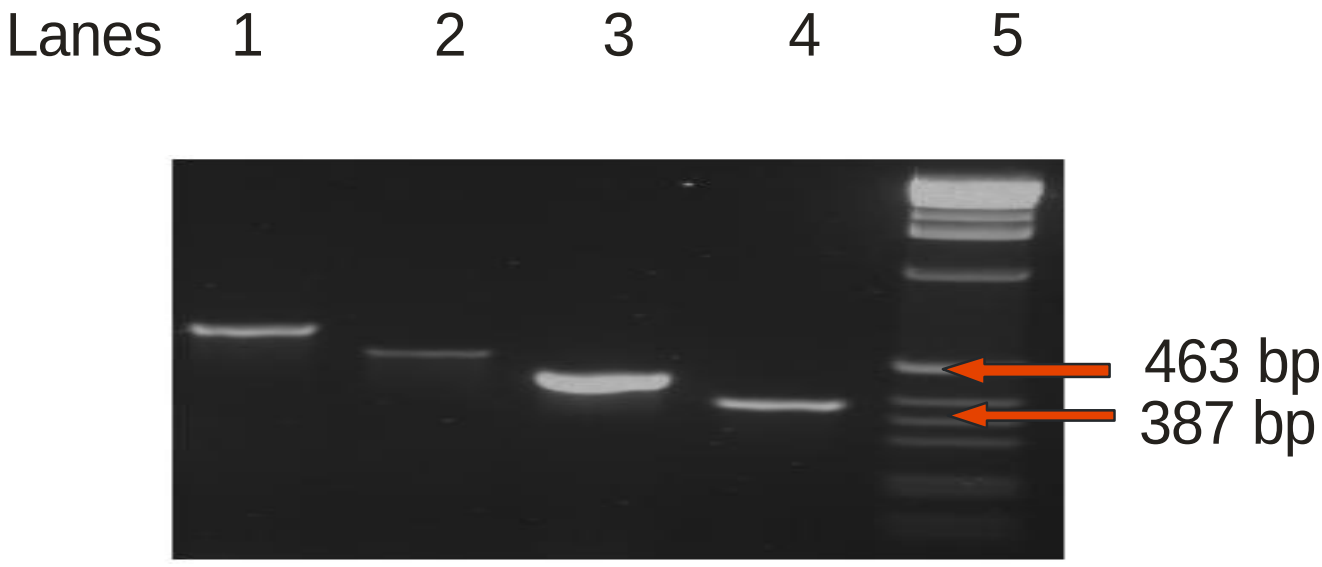
		Adults reared from larvae			Adults collected indoors		
		Anopheles			***Anopheles***		
Zone	Site	Ag. s.l	Ag. s.s	A. ar	Ag. s.l	Ag. s.s	A. ar
Zone C	Katsina	108	89	19	111	99	12
"	Daura	99	79	20	70	53	17
Zone B	Dutsinma	165	148	17	166	155	11
"	Kankara	180	161	19	200	188	12
Zone A	Funtua	169	145	24	211	201	10
"	Dandume	356	333	23	214	205	9
	Total	1069	955(88.7%)	122(11.3%	947	901(97.3%)	25 (2.7%)

Ag s.l = An. gambiae s.l Ag. s.s = An. gambiae s.s An. ar = An. arabiensis

4.7 PCR DEVELOPED PLATES FOR IDENTIFYING *An. gambiae s.s* and *An. arabiensis*



a)



b)

Plate XVI: *An. gambiae* PCR identification a) Lane 1, *An. gambiae (*top arrow*)*, Lane 2, *An. arabiensis (*down arrow*)*, Lane 3, 1 kb ladder. Primers create fragments of 415bp (QDA) *An. quadriannulatus*, 390bp *An. gambiae*, 315bp *An. arabiensis*. b**)** Lane 1 *An. quadriannulatus*, lane 3 *An. gambiae*, Lane 4 *An. arabiensis*, Lane 5 1kb ladder. Primers create fragments of 636 *An. quadriannulatus*, 463 *An. gambiae* and 387 *An. arabiensis*.

4.8 PCR IDENTIFICATION OF THE *Anopheles gambiae* COMPLEX INTO M AND S FORMS

Out of the total of 853 *An.gambiae* s.s adults collected indoors, the S forms were the most preponderant with its highest abundance of 186 (21.81%) in Zone B at Kankara and the lowest of 55 was recorded in Zone C at Daura. While of the 983 adults reared from larvae, the S form was also the most abundant collection with 341 (34.69%) in Zone A at Dandume and its lowest was 44 in Zone C at Katsina. There was no significant differenc between M and S forms of *An. gambiae s.s* larval collections however there was a significant difference in terms of indoor collections between Zones A, B and C and also between the two species (p >0.05, F=9.28) (Appendices 42 - 43). On the other hand, the highest collection of M forms were 5 and 3 reared from larvae in Zone A at Funtua and Dandume, respectively (Table 4.7)(Plate XVII).

4.9 MOLECULAR CHARACTERISATION OF *An. funestus* COMPLEX

Three *An. funestus* species were identified by PCR, that is *An. funestus* s.s., *An. rivulorum* and *An. brucei*. *An. funestus* s.s. was the most prolific with the highest collection of 149 in Zone A at Dandume followed by *An. brucei* with its highest number of 67 also in Dandume while *An. rivulorum* recorded its highest number of 35 also in Dandume. The three species were identified in all the sampled towns (Table 4.8)(Plate XVIII). There was no significant difference between *An. funestus* adults and larvae in the three zones (p <0.05, F=6.39)(Appendices 44 - 47).

Table 4.7 Locality, M and S forms identified by PCR

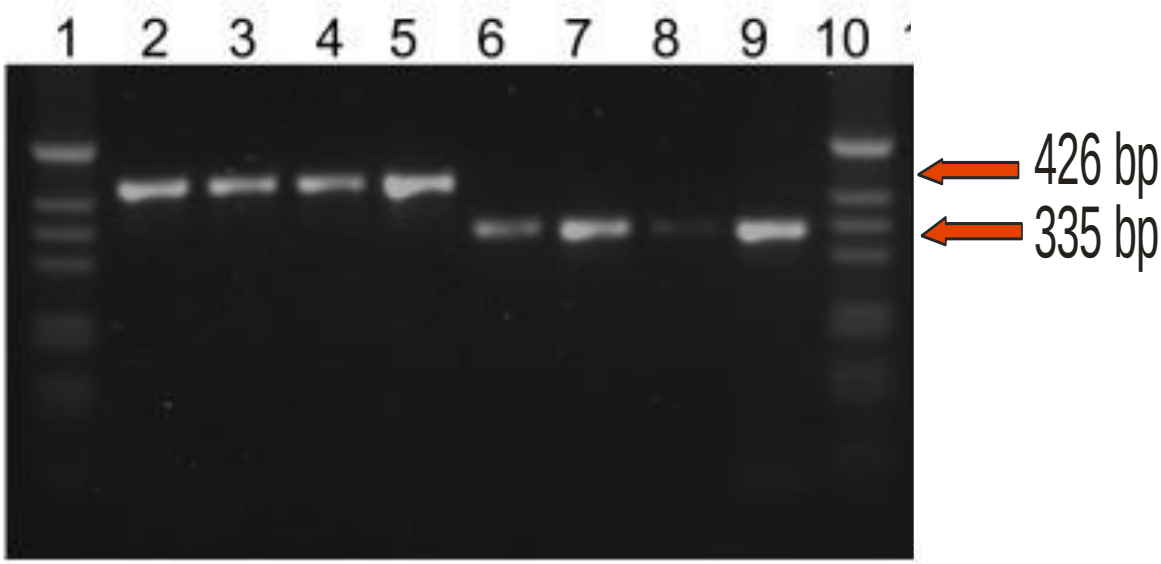
	An. gambiae adults caught indoors				*An. gambiae* larvae reared to adults			
Site	Total collected	Total examined	*An. gambiae* s.s		Total collecte	Total examined	*An. gambiae* s.s	
			M form	S form			M form	S form
Katsina	111	99	2(2%)	97(98%)	68	45	1(2.2%)	44 (97.8%)
Daura	70	56	1(1.8%)	55 (98.2%)	89	84	1(29.8%)	59(70.2%)
Dutsinma	199	180	4(2.2%)	176 (97.8%)	165	158	2(5.7%)	149(94.3%)
Kankara	200	187	1(0.5%)	186 (99.5%)	180	175	1(7.4%)	162(92.6%)
Funtua	180	152	5(3.3%)	147(96.7%)	169	167	2 (15%)	142 (85%)
Dandume	199	179	3(1.7%)	176(98.3%)	356	354	3 (3.7%)	341(96.3%)
Total	947	853	16	837	1069	983	11	897

Table 4.8 Distibution of the members of the *An. funestus* complex with respect to locality

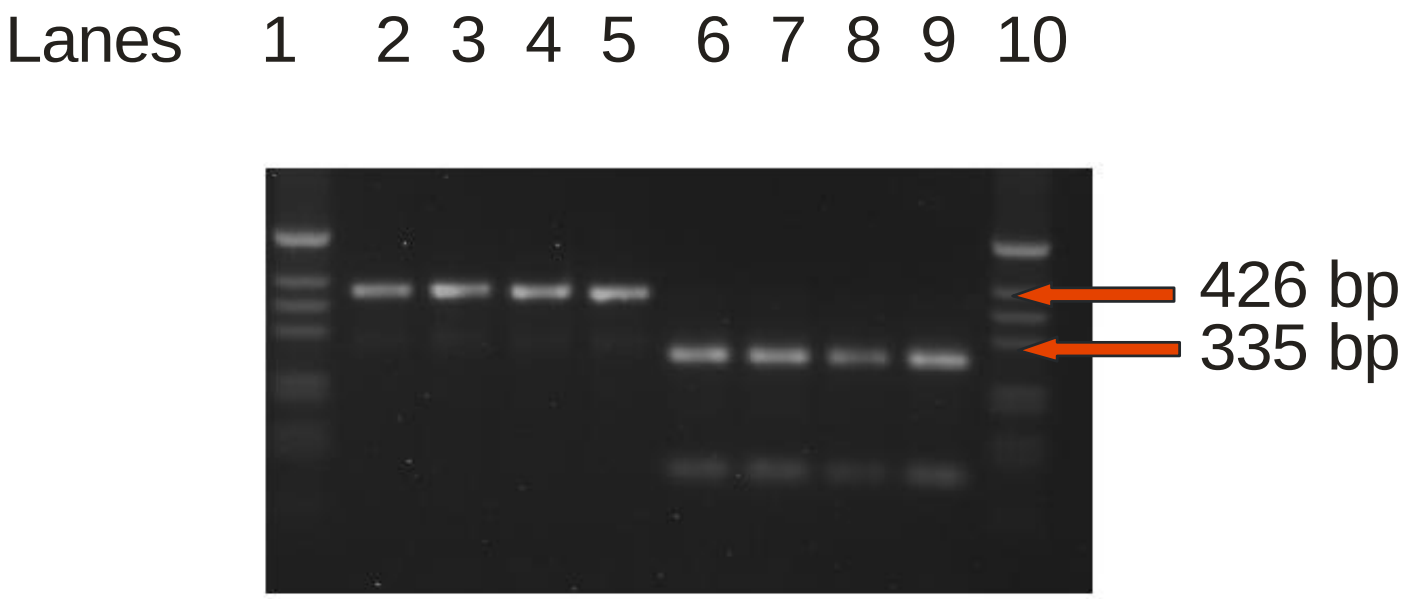
	Adults caught indoors					Larvae reared to adults				
Locality	Total collected	Total identified	Af	A r	Abr	Total collected	Total identified	Af	A r	Abr
Katsina	54	53 (98.2%)	29	9	15	40	36 (90%)	21	6	9
Daura	28	25 (89.3%)	16	2	10	69	69 (100%)	39	8	22
Dutsinma	72	68 (94.5%)	40	8	20	53	52 (98.1%)	25	11	16
Kankara	77	77 (100%)	42	8	27	94	94 (100%)	43	16	33
Funtua	88	88 (100%)	56	10	22	109	106 (97.3%)	58	19	29
Dandume	75	75 (100%)	50	7	19	178	175(98.3%)	99	28	48
Total	431	386	233	44	113	580	532	285	88	157

Anf = An. funestus Ar = An. rivulorum Abr = An. brucei

4.8.1 PCR DEVELOPED PLATES FOR IDENTIFYING *An. gambiae* M and S forms



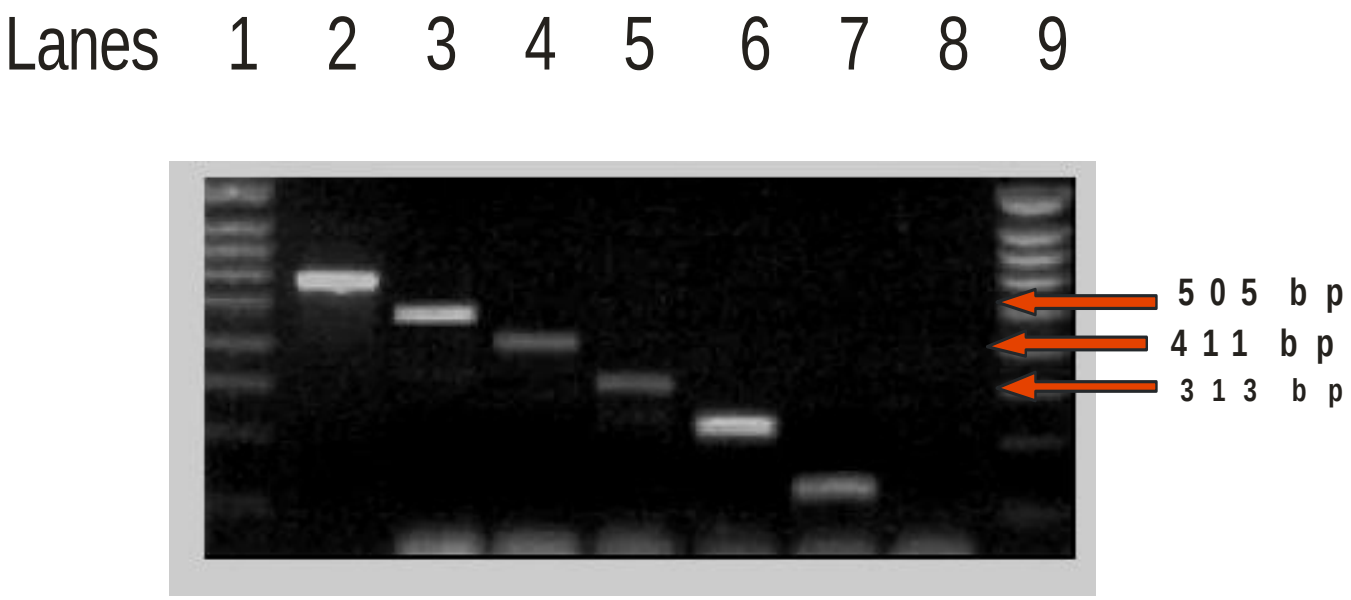
a)



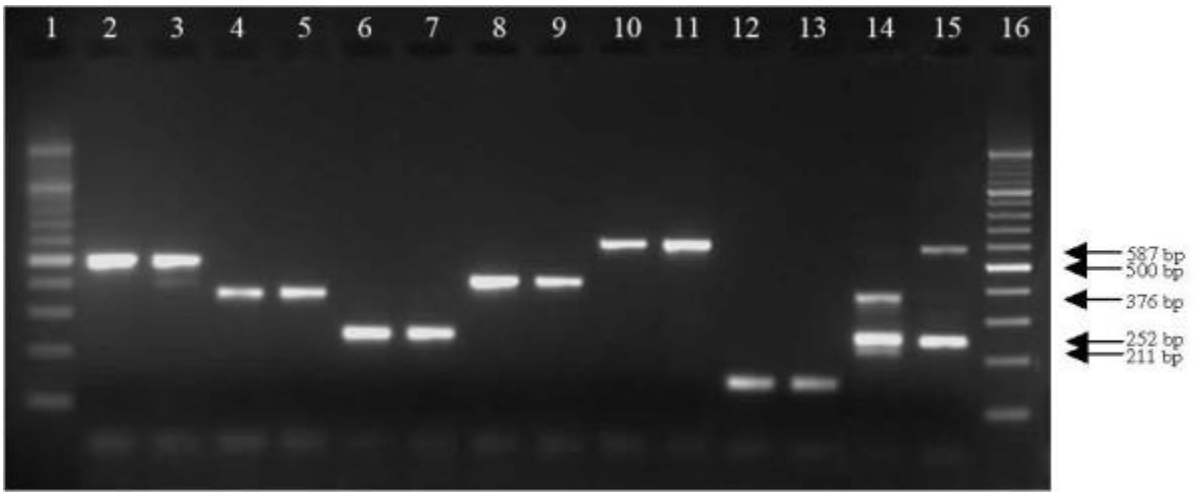
b)

Plate XVII: Gel electrophoresis of M – S rDNA assay a) Lanes 2–5 and 6–9 contain M and S PCR products respectively. Lanes 2–9 were performed using the IMP primers and reactions. Lanes 1, 10, and 19 contain 1 kb ladder marker. b) Gel electrophoresis of M/S rDNA assay. Lanes 2-5 and 6-9 contain M (426 bp)and S (335 bp) PCR products respectively. Lanes 1 and 10 contain 1kb ladder marker.

4.9.1 PCR PLATES IDENTIFYING *Anopheles funestus*



a)



b)

Plate XVIII: Amplified fragments using the species-specific polymerase chain reaction assay to identify members of the *An. funestus* group. a) Lanes 1 and 9, 100-basepair DNA size marker ladder; lane 3 (505bp), *An. funestus*; lane 4 (411bp), *An. rivulorum*; lane 5 (313bp), *An. brucei*; lane 8, negative control. The sizes of the fragments of the ladder are 1,000, 800, 700, 600, 500, 400, 300, 200, and 100 bp. b) An ethidium bromide stained 2.5% agarose gel showing the DNA bands after PCR-RFLP. Lanes 1 and 16 = 100 bp molecular marker; lanes 2 and 3 = *An. funestus* (500 bp) (first arrow); lanes 8 and 9 = *An. rivulorum* (376 bp) (third arrow); lanes 10 and 11 = *An. brucei* (587 bp) (first arrow)(Adapted from Koekermoer *et al*., 2002).

4.10 DETERMINATION OF *PLASMODIUM* SPOROZOITES USING VECTEST

Zone A recorded more *P. falciparum* and *P. malariae* than Zone B which in turn recorded more than Zone C. The Vectest evaluation dealt solely with indoor-collected specimens, that is the 926 of *An. gambiae* and 431 of *An. funestus* (Table 4.9). *P. falcifarum* was the most propoderant in Zone A, having the highest of 163 at Dandume and its lowest was 40 at Daura. While the highest abundance of *P. malariae* was in Zone C with 88 at Dandume and the least were in Zone C with 27 in Daura. There was no difference between *P. falciparum* and *P. malariae* within the zones (p<0.05, F=6.79). No *P. vivax* 210 nor *P. vivax* 247 were identified in any of the sampled towns during the current study. There was a very high significant difference between *P. falciparum* and *P. malariae* isolated from *An. gambiae* and *An. arabiensis* within the zones (p <0.05, F=161.44). There is a very high significant difference between malaria sporozoites isolated from *An. gambiae s.s*. which was higher than that of *An. arabiensis* which in was is higher than *An. funestus* which was also higher than those of *An. brucei* (p <0.05, F=161.44). *An. gambiae* s.s recorded the highest number of *P. falcifarum* at 190 and also the highest *for P. malariae* with 289. While *An. quadrimaculatus* had the lowest of 2 each for both *P. falcifarum* and *P. malariae* (Table 4.10).

4.11 DISTRIBUTION OF MALARIA ACROSS SAMPLED MONTHS

The highest infections with *Plasmodium* species in 2009 and 2010 was in the month of July with 82 and 78 respectively. There were more *P. malariae* than *P. falcifarum* species throughout 2009, 2010 and 2011 (Table 4.11). There was more *P. falciparum* and less *P. malariae* in all the three zones studied.

Table 4.9 Locality and *Plasmodium* sporozoites identified by Vectest

		Plasmodium sporozoites identified by Vectest				
Zone	Locality	*P. falcifarum*	*P. malariae*	*P. vivax (210)*	*P. vivax* (247)	Total
Zone C	Katsina	54 (33.3%)	108 (66.7%)	0	0	162
"	Daura	36 (39.6%)	55 (59.4%)	0	0	91
Zone B	Dutsinma	78 (33.9%)	168 (66.1%)	0	0	230
"	Kankara	88 (32.8%)	186 (27.2%)	0	0	268
Zone A	Funtua	80 (28.0%)	206 (72.0%)	0	0	286
"	Dandume	86 (29.2%)	109 (70.8%)	0	0	295
	Total	422 (29.4%)	832 (70.6%)	0	0	1332

Table 4.10 Prevalence of *Plasmodium* sporozoites isolated in the identified *Anopheles* spp.

Anopheles species	*Plasmodium* sporozoites identified by Vectest				Total
	P. fal.	*P. mal.*	*P. vivax (210)*	*P. vivax* (247)	
An. gambiae ss	277	381	0	0	479
An. arabiensis	95	133	0	0	168
An. funestus	120	193	0	0	153
An. brucei	30	51	0	0	81
An. rivulorum	11	24	0	0	431
An. maculipennis	3	7	0	0	12
An. quadrimaculatus	2	5	0	0	9
Total	501	744	0	0	1332

P. fal. = Plasmodium falciparum *P. mal. = P. malariae*

Table 4.11 Monthly prevalence of *Plasmodium* species

	Number of *Plasmodium* identified by Vectest								
		2009			**2010**			**2011**	
Month	Total	*P. fal.*	*P. mal*	*Total*	*P. fal.*	*P. mal*	*Total*	*P. fal.*	*P. mal*
June	42	25	17	45	16	29	40	14	26
July	48	29	19	40	15	25	110	22	40
August	25	10	15	34	14	20	41	15	26
September	22	7	18	31	13	18	53	18	35
October	21	8	13	40	15	25	31	12	19
November	19	6	13	62	25	37	62	35	75
December	15	5	10	98	28	70	0	0	0
January	0	0	0	63	19	44	0	0	0
February	0	0	0	58	17	41	0	0	0
March	0	0	0	62	22	40	0	0	0
April	0	0	0	35	12	23	0	0	0
May	0	0	0	27	6	21	0	0	0
Total	192	90	102	595	183	412	337	116	221

P. fal. = *P. falciparum* P. mal. = *P. malariae*

4.12 RETROSPECTIVE STUDY OF MALARIA IN HOSPITALS

In 2009, the highest prevalence of malaria was in the month of August where 187 confirmed cases were recorded while its least prevalence was 52 in December (Fig 12), in Zone C at the Federal Medical Centre, Katsina (FMCK) (Fig 12). In Zone B,the highest prevalence of malaria was 205 in July, recorded at the General Hospital Dutsinma (GHD) while November recorded the lowest (64) in 2009 (Fig 12). In 2010, July had the highest confirmed cases (205) of malaria in Zone C at FMCK while November had the lowest prevalence of malaria with 64 cases in Zone A at the General Hospital Funtua (GHF) (Figs 13 & 14). In 2010 there was a dramatic increase in confirmed malaria cases because the highest prevalence of 206 occurred in July in Zone C at FMCK while the lowest was 85 in Zone A in April at GHF. F-Test Two-Sample for Variances from hospital records of malaria in 2009 shows no significant difference between FMCK, GHD and GHF (p <0.05, F=2.82)(Appendices 49 - 50).

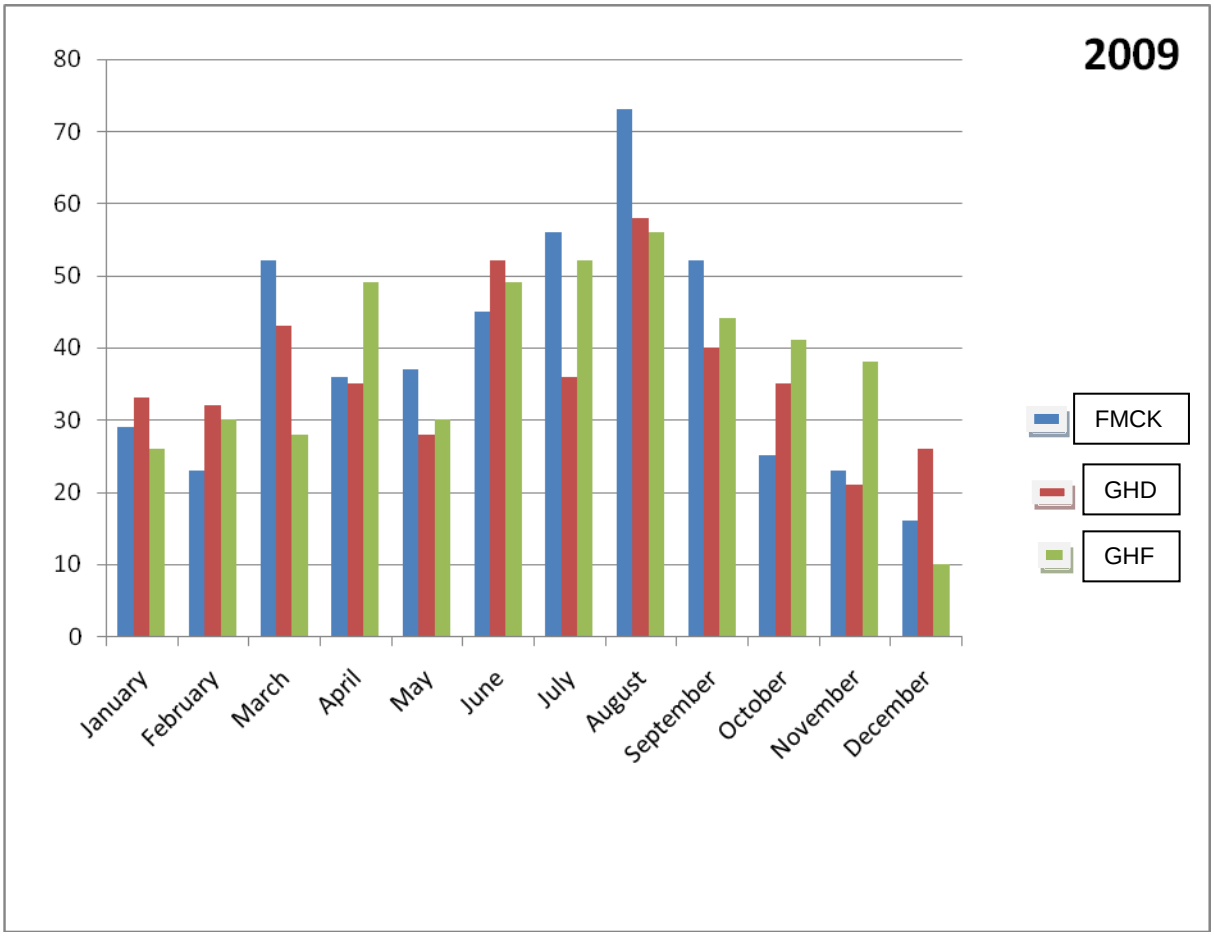


Fig 12 Monthly distribution of malaria cases in 3 hospitals in 2009

FMCK = Federal Medical Centre Katsina, GHD = General Hospital Dutsinma, GHF = General Hospital Funtua

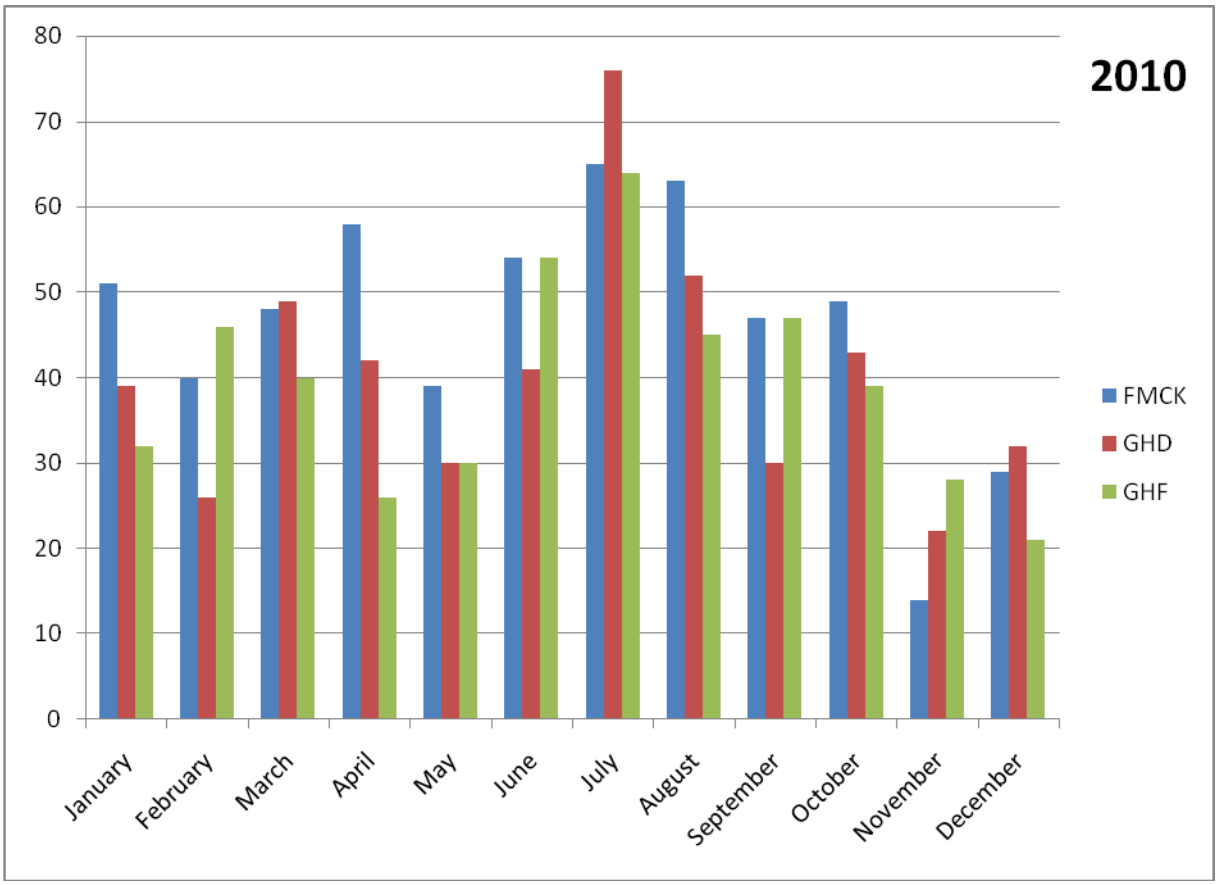


Fig 13 Monthly distribution of malaria cases in 3 hospitals in 2010

FMCK = Federal Medical Centre Katsina, GHD = General Hospital Dutsinma, GHF = General Hospital Funtua

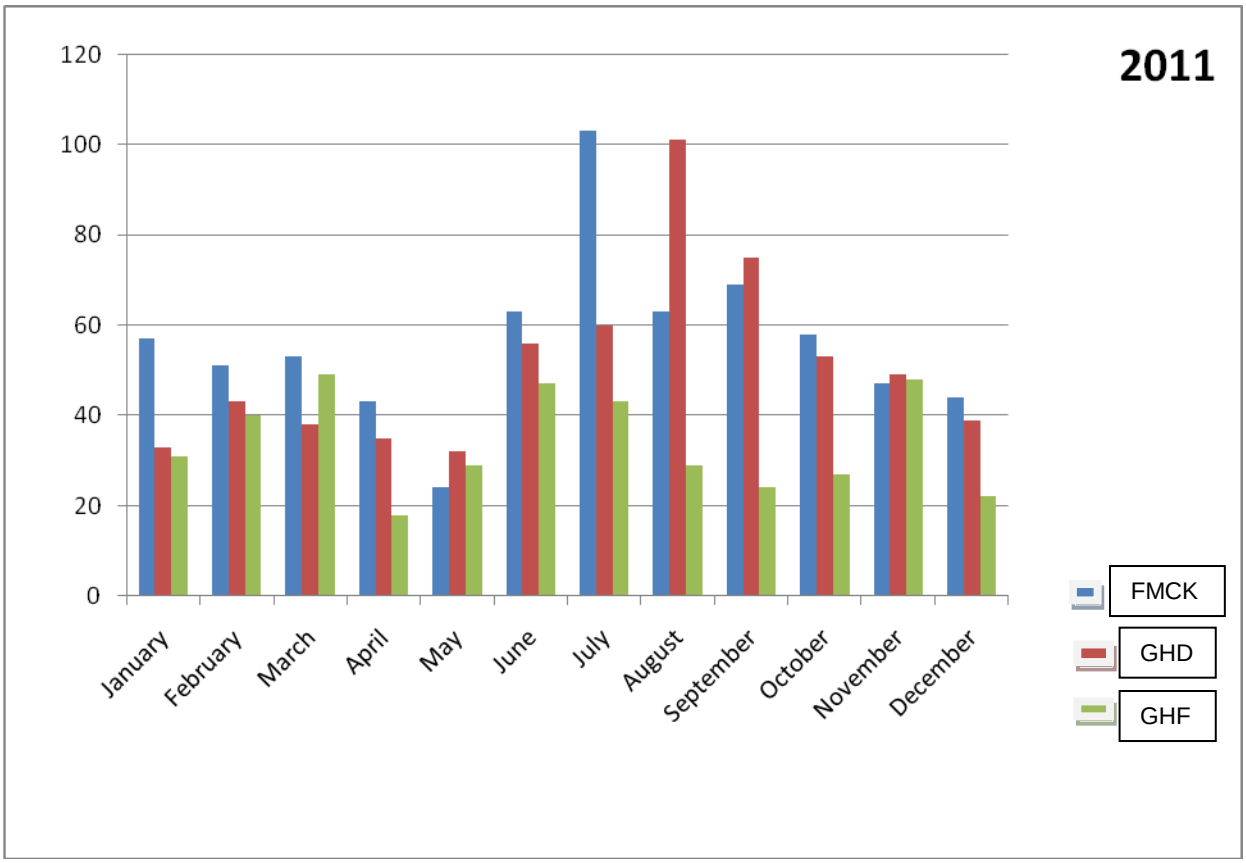


Fig 14 Monthly distribution of malaria cases in 3 hospitals in 2011

FMCK = Federal Medical Centre Katsina, GHD = General Hospital Dutsinma, GHF = General Hospital Funtua

CHAPTER FIVE

DISCUSSION

In this study, *Anopheles* mosquitoes were found breeding in all the ecological zones of Katsina State. Breeding was predominantly in pools (formed by rain), ponds, cemented reservoirs, overhead tanks and rice farms. They were also found in 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, the water supply system was erratic and this explains the use of numerous water storage containers to provide water for domestic chores, irrigation, car wash, block making and other construction purposes. These turned out to be conducive breeding sites for mosquitoes within and near human habitations. During all the three study years, it was 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 dry season. Similar findings were made by Sharma (2008) in the semi-arid districts of Rajasthan, India.

Zone A had a preponderance of mosquito breeding sites, which may be linked to the abundance of rainfall in that zone which is above that found in the other two zones. This may explain why *Anopheles* mosquitoes were highly prolific and thus highly abundant in the zone. This is similar to the findings made by Dunn (1926) in Northern Venezuela.

The presence of large water reservoirs (used for irrigation puposes) located close to some towns e.g. Sabke dam near Daura, Ajiwa dam near Kasina and Zobe dam at Dutsinma, were also responsible for the high proponderence of *Anopheles* species, which easily invaded nearby houses. According to Keiser *et al*. (2005), 87.9% of the world malaria cases occurred in 9.4 million people who live near dams and irrigation schemes in sub-Saharan Africa. Many

authors have reported that mosquitoes can travel up to 5 kilometers from their breeding points to invade human habitations (Takken *et al*., 2007). Besides that, extensive irrigation within the three zones has altered soil characteristics which has impacted negatively on the desert physiography, vector abundance, distribution and vectorial capacity, thus triggering off changes in *Plasmodium falciparum*-dominated malaria. The change in crop pattern, retention of high surface moisture, excessive use of irrigation canals and poor handling of irrigation water have attracted several anophelines to the three zones. The distribution of *Anopheles* and malaria observed in the three zones in Katsina State is more or less directly related to the distribution of irrigation water used for agriculture. Therefore water reservoirs for irrigation should be equipped with motorised aerators that will constantly stir the water so that it will be uninhabitable to the early stages of mosquitoes and thus contribute immensely to reduction of mosquitoes and by implication malaria.

In a similar vein, intense rains, farming activities undertaken through irrigation of enormous rice fields were also responsible for the very high population of *Anopheles* species recorded. In the irrigated areas and rice fields, mosquitoes and malaria transmission was very high. This is consistent with the reports of Marrama *et al*. (2004, 2009) who stated that malaria transmission was 150 times higher in irrigated areas than in the natural ecosystems and 90% of infections were caused by *An. funestus*. This study had observed that lands left fallow after harvesting rice, were highly suitable for mosquito breeding, causing large numbers of anophelines to emerge. By implication, the malaria transmission season underwent profound alterations when compared to areas where irrigation was not practiced (Takken *et al*., 2007).

Anopheles gambiae and *An. funestus* were the dominant species in rice farmlands, more than a third of *Anopheles* larval habitats were observed in farmlands, thus confirming farmlands as major contributors to mosquito and malaria prevalence in the three zones. This is consistent

with the report of Himeidan and Kweka (2012) that farmlands constituted 40% of anopheline larval habitats.

Numerous *Anopheles* breeding sites were found exposed to direct sunlight and in turbid water during the current study; this is consistent with the findings of Mutuku *et al*. (2006). According to Minakawa (1999), female *Anopheles* use open habitats, which are directly under the sun for oviposition because they are warmer and the warmth reduces the larva and pupal development time. In addition, there is less predation and more algae which served as food for larvae. Some of the breeding sites were very turbid, yet numerous *Anopheles* were observed from such sites. Consequently, the presence of *Anopheles* in turbid and polluted water is an indication that some physical qualities of a water body may not play a role in their proliferation. This is consistent with the report of Gimning *et al*. (2001).

Similarly, during the current study, contrary to conventional thinking that *A. gambiae s.l.* only breeds in clean and clear water, its larvae were found in habitats polluted by vegetation, human faeces, muddy water and even oil. This is contrary to the findings of Muirhead-Thompson (1951) but consistent with the recent findings of Sattler *et al*. (2005). These sites were the drain of mechanic workshops, polluted swamp used as garbage dump and one sewage pond with organic pollution from human faeces. It was also observed that breeding sites that were less than one meter in diameter e.g. buckets, small plastic tanks etc. were more likely to contain *Anopheles* larvae, as long as they contained some debris. Although *Anopheles* larvae were present in large drains, swamps and puddles, these sites contained much lesser densities of *Anopheles* larvae.

Aquatic plants like *Typha* species (*Typha angustifolia)* and water lilies (*Nyrnphaea* spp.) were present in some of the sampled breeding sites. These water plants facilitated *Anopheles*

breeding by slowing down water current, blocking water flow and providing shade and points for laying eggs. This is consistent with the report of Asaeda *et al*. (2005).

In this study, few *Anopheles* larvae were observed in swamps. This finding agrees with previous reports that *An. gambiae s.l.* prefers temporary breeding sites. Though *Anopheles* species are less likely to inhabit swamps if other habitats more preferable to *Anopheles* mosquitoes are present, the importance of swamp habitats to mosquito proliferation should not be under estimated, because of their great size and their role in supplying water for irrigation ditches, rice farms and various other agricultural activities. Consequently swamps should be targeted by appropriate control measures.

Anopheles funestus occurred at high densities in large non-moving water bodies like ponds, cemented reservoirs and overhead tanks especially to survive and proliferate during the dry season so as to remain closely associated with groups of houses. This is consistent with the reports of Charlwood *et al*. (2000) and Lamidi (2009).

Similarly, the high preponderance of *An. funestus* recorded during this study could be explained by the clearing of natural wooded savannah and increases in cultivated areas which modified soil surface characteristics. In addition, building of temporary ponds like dams and enhanced water run-off. This is also in agreement with the reports of Gillies and De Meillon (1968) and Gillies and Coetzee (1987).

In this study, seasonal variations in the population of *Anopheles* species collected across the three ecological zones of Katsina State and across the seasons were noted. *An. gambiae* was the most synnantrophic species and the most ubiquitous of all the anophelines collected within the three zones. The significantly higher *Anopheles* mosquitoes collected was as a result of a lot of breeding sites created by the abundant rainfall experienced, which is similar to the situation in the northern guinea savannah. In addition is high level of heterogeneity in

anopheline mosquito species composition at macro-geographic scale. Mbogo *et al.* (2003) reported differences in the relationship between mosquito population and rainfall in different districts of Kenya and narrowed that to environmental heterogeneity.

Similarly, the high preponderance of *Anopheles* in the wet seasons was because the range and relative abundance of *An. gambiae* and *An. arabiensis* are determined by the amount of annual rain, annual and wet season temperatures and high vegetation (Molineux, 1988; Le Sueur and Sharp, 1991), which are all adequate in Zone A. In addition, the oviposition of mosquito eggs by gravid females and their development to larvae and subsequently to adults depends on the presence of suitable breeding sites and is therefore dependent on rainfall (Le Sueur and Sharp, 1991; Molineux, 1988). This is also consistent with the findings of Lindsay *et al*. (1998), Shililu *et al*. (1998) and Gimnig *et al*. (2001). Numerous reasons were advanced by different authors to explain the afore-mentioned observations, for example; McMichael *et al.* (2003) reported that rainfall provides the medium for the acquatic stages of the mosquito life cycle and also increases the relative humidity and thus the life span of the adult mosquito. Hence, the association of mosquitoes and malaria with rainfall is due not only to greater breeding activity of mosquitoes, but also to the rise in relative humidity and higher probability of survival of female *Anopheles* mosquitoes. This varies with the circumstances of a particular geographic region and depends on local habits of mosquitoes. Malaria transmission can only be sustained by about 80mm of rain for 5 months however 60mm for as many months as well as 80mm for less than 5 months will be inadequate (Lindsay *et al.*, 1996).

The above information is important for mosquito control; firstly, determining the ecology and distribution of various *Anopheles* species is important in the determination of mosquito vector abundance and associated malaria prevalence. Secondly, *Anopheles gambiae* s.l. is a group of closely related and morphologically indistinguishable sympatric species. Thus the effective control of malaria through vector management requires information on distribution and abundance of vectors in a targeted area like Katsina State. Besides, individual species within the species complex differ in host-biting preference, abundance and vector competence, consequently, identification of the mosquito vectors to species level and mapping species distribution in heterogeneous environments are necessary for a successful control programme (Highton *et al*., 1979; Petrarca *et al*., 1991; Coetzee *et al*., 2000; Minakawa *et al*., 2002 and Godwin *et al*., 2005).

The data obtained shows that the population density of *Anopheles* larvae and adults increase as one moves from the arid towns of Zone C (Katsina and Daura) towards the less arid towns of Zone B (Dutsinma and Kankara) and culminates in a dramatic increase in population density of *Anopheles* in Zone A (Funtua and Dandume) because of abundant rain, which lead to the formation of water bodies like pools, ponds etc and the low temperatures experienced, which collectively provide conducive conditions suitable for *Anopheles* proliferation.

The recorded amount of rainfall in July and August resulted in temperatures that were favourable to mosquito proliferation. In fact, at 30^{o}C, larval and pupal development may last only 10 days (Rueda *et al.*, 1990; Tekleheimanot *et al.*, 2004). Martens (1995) reported that mosquito blood meals and length of the gonotrophic cycle increased during moderate temperatures. More cases of malaria were recorded in the study area during July and August throughout the period of study. In addition, low humidity levels caused the vectors to feed more frequently to compensate for dehydration. Under conditions of optimal humidity, mosquitoes tend to survive for a longer period, which allows them to disperse farther and to

have a greater opportunity to participate in malaria transmission cycles (Lindsay and Mackenzie, 1997; Liehne, 1998). Where temperature is not a limiting factor, malaria transmission is highly seasonal, with its peak following the period of peak rainfall. In addition, *Anopheles* species can aestivate as eggs for up to 20 months and majority of the eggs are able to hatch as soon as the rains resume thereby increasing the population density of *An. gambiae* s.l. (Jupp *et al*., 1980; Charlwood *et al*., 2000 and Kasili *et al*., 2009).

Anopheles gambiae s.s. was responsible for most of the malaria transmission during the wet season while *An. arabiensis* was responsible for most malaria transmission during the dry season as also reported by Moiroux *et al*. (2014), however malaria incidence is seasonal (Alam *et al.*, 2012) reported that.

Because *An. gambiae* were more abundant in the wet months, followed by *An. funestus* at the end of the rainy season, and then *An. arabiensis* in drier months. The three species seem to complement one another in order to sustain the endemicity of malaria in the sampled towns across the three zones. Faye *et al*. (1995) also observed a high variation of *An. gambiae* s.l. population density in the sahelian area of Republic of Niger. Similar findings were reported by Lamidi (2009). Consequently, *An.gambiae* s.s., *An. arabiensis,* and *An. funestus* s.s alternate in malaria transmission across the year (Ebenezer *et al*., 2014) especially during the rains as such all should be targeted for effective malaria control. Similarly, the high proliferation rate observed in the afore-mentioned species has been explained by other previous reports e.g. a study to examine the tolerance 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. Consequently a population explosion of *Anopheles* occurs immediately the rains set in. The present findings are also similar to those of Oyewole *et al*. (2005) and Kigadye *et al*. (2010).

The M and S forms differ by the ecotype of their larval sites, the S form prefers temporary breeding sites while the M form prefers permanent freshwater pools. This is similar to Caputo *et al*. (2008). Although for the current study, *An. gambiae* M and S forms were sympatric in most locations but the S form predominated because the Guinea savannah environment which is associated with more rain and vegetation provided abundant breeding sites which is much more suitable for the proliferation of the S forms. Temperature was a key factor distinguishing their distributions, because the S forms prefer areas of moderate to high temperature while the M forms prefers areas of low temperature. At the 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), Costantini *et al.* (2009), *Souza et al. (2010) and* Riehle *et al.* (2011). Behavioral and ecological differences between the M and S forms are likely to influence malaria epidemiology by spatially and temporally widening areas of transmission. All the above information on abundance and distribution are equally important for the control of *Anopheles* and malaria.

All three identified *An. funestus* subspecies i.e. *An. funestus, An. rivolurum and An. brucei* were all found positive for circumsporozoite protein, this is a confirmation that the afore-mentioned *Anopheles* species were all active in malaria transmission. This finding agrees with Mulamba *et al*. (2014).Therefore for any malaria control programme to be successful, *An. funestus, An. rivolurum and An. brucei* must all be targeted simultaneously and wholistically.

Heterogeneity in oviposition site preference between the *An. funestus, An. rivolurum and An. brucei* enabled vector densities to be high in both the wet and dry seasons, allowing for a year round transmission of malaria, especially during the dry season. Malaria transmission was maintained by these species which were anthropophilic as well as zoophilic, this behaviour allows for low level but persistent malaria transmission as reported by Manh *et al*. (2010).

Zoophilic behaviour was observed in some houses that kept animals such as cattle, camel, sheep, goats and donkeys. Therefore insecticides should be sprayed on and around these animals instead of only indoor spraying of insecticides, so as to reduce *Anopheles* populations.

Anopheles maculipennis and *An. quadrimaculatus* were identified morphologically during this study. They were collected only in zone A, from rice farms only and were absent from all other breeding points sampled, e.g. pools, ponds and reservoirs. Their presence was probably facilitated by the numerous breeding sites as a result of high rains and also the low temperatures prevailing in Zone A. A review of literature indicated that they are secondary vectors of malaria (Hopkins, 1987). The two species do not play a considerable role in malaria transmission in those towns where they are present. However control measures should also be directed towards them if the complete elimination of mosquitoes and malaria were to be accomplished.

Since malaria transmission is directly linked to mosquito proliferation, it will be expected that there will be more malaria cases in Zone A which has all the favourable weather conditions for mosquito proliferation. However hospital records of malaria transmission showed no significant difference in malaria cases in the three zones. This begs the question: is malaria prevalence related to other conditions apart from weather? The answer is yes, malaria transmission is promoted by the absence of proper drainage, inadequate sanitation, presence of adequate insecticide treated nets among others. Onyabe and Coon (2001) also reported similar observations.

There were more *Plasmodium* sporozoites in *Anopheles* during the rains. This finding actually coincides with the confirmed hospital cases of malaria observed based on

retrospective malaria records in the sampled hospitals. Therefore people should take stringent action against mosquitoes during the rains by using ITNs, seek medical help when they observe malaria symptoms, clearing drainage and vegetation within their surroundings etc. *Plasmodium malariae* was mostly responsible for sustaining malaria throughout the period of study. According to Kulwichit *et al.* (2000), *P. malariae* can persist in humans as an asymptomatic erythrocytic disease for many years if it is not treated conclusively, during which it causes problems in the liver. No *P. vivax* (210) and *P. vivax* (247) were identified during the study. A similar report was made by Gautret *et al*. (2001) and Culleton *et al*. (2008) who reported that *P. vivax* is absent from West and Central Africa due to the high prevalence of the Duffy negative phenotype in the indigenous populations. The Duffy binding proteins of *P. vivax* (PvDBP) are located on their merozoites (Fong *et al*., 2014).

In this study, the incidence of malaria varies with weather, which affects the ability of anopheline mosquitoes to survive or otherwise. Tropical areas including Nigeria have the best combination of adequate rainfall, temperature and humidity allowing for breeding and survival of anopheline mosquitoes. The burden of malaria varies across different regions of the world and even within a country. This is driven by the variation in parasite-vector–human transmission dynamics that increase or reduce the transmission of malaria infection and risk of disease and death associated with malaria. In support of the above observed pattern, Alaba and Alaba (2010) believe that of the four species of *Plasmodium* that infect humans (*P. falciparum, P. vivax, P. malariae and P. ovale*), *P. falciparum* is responsible for most of the pain and mortalities resulting from malaria in Africa and particularly in Nigeria. It accounts for about 50 percent of out-patient consultation, 15 percent of hospital admissions, and also prime among the top three causes of death in the country (National Malaria Control Plan of Action 1996 to 2001) (WHO, 1995; 2002 and 2005).

Hospital records of malaria in 2011 corroborated the high prevalence of malaria observed in the months of July and August during this study. This is consistent with IGNtake (2011) report that malaria killed 401 people in the last four weeks of September, in 2011 in Katsina State. Similarly, WHO (2011) recorded 50,311 malaria cases in Katsina State in September, 2011. Because of the heavy rainfall recorded in 2011 in Daura, Funtua, Ingawa, Kurfi, Dutsinma, Katsina and Bindawa towns (Legros *et al*., 2006; WHO-CISD, 2008).

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Anopheles mosquitoes were collected from five different types of breeding sites within the three ecological zones namely: pools, ponds and concrete reservours, overhead tanks and rice farms. Pools, ponds, concrete ground and overhead reservoirs were present in all the three ecological zones, however rice farms were fewer in Zones B and C but abundant in Zone A. The information on physical characteristics of the breeding sites observed during the study were: size of water body, type of vegetation, habitat permanence, water condition and exposure to sunlight.

An. gambiae was the most proponderant in pools (332) and least abundant 120 in overhead tanks. On the other hand *An. funestus* had its highest number in concrete reservoirs. *An. maculipennis* and *An. quadrimaculatus* were collected from only rice farms located in Zone A at Funtua and Dandume towns. It was also realized during the present study that there was a very high proliferation of *Anopheles* mosquitoes in water reservoirs such as overhead tanks and ground cemented reserviours in virtually all the sampled towns.

A total of 3,027 mosquitoes were collected and all were morphologically identified as *Anopheline* mosquitoes. Of the 1,649 adults reared from larvae, *An. gambiae* s.l was the highest with 1,027 (62.28%) followed by *An. funestus* with 580 (35.17%). However of the 1378 adults collected indoors, the highest was 926 (67.2%) of *An. gambiae* while the least was *An. maculipennis* with 9 (0.65%). In addition, *An. maculipennis* and *An. quadrimaculatus*, which are recognized as secondary malaria vectors, were collected in only Funtua and Dandume towns of Zone A. Of the mosquitoes collected indoors, *An. maculipennis* was the least abundant with 16 (0.9%). This species was also the least

abundant in larval collections. In terms of sites, the highest number of larvae collected came from Dandume in Zone A 555 (33.7%) followed by Funtua with 298 (18.1%) while Katsina in Zone C had the least with 148 (9.0%). Also the town with the highest collection of adults was Dandume with 301 (21.8%) catches followed by Funtua having 299 (21.7%) all in Zone A and the least was Daura in Zone C, with 98 (7.1%).

The highest number of adult collections were *Anopheles gambiae* in all the sampled houses. With the highest collection of 200 at Kankara and the lowest of 70 at Katsina. On the other hand *An. funestus* recorded its highest collection of 103 at Funtua and the lowest of 28 in Daura. In all, *An. macilipennis* had the lowest collection of only 2 at Funtua.

In September 2009, the highest collection of *An. gambiae* in a pond was 17 in Zone B at Dutsinma. The highest number of 25 *An. funestus* were recorded in May in ponds at Dutsinma and Kankara and in a rice farm in June at Funtua. Pools and rice farm breeding sites were only active during wet months while ponds and reservoirs were active during both wet and dry months. Low number of *An. gambiae* were collected during the dry months of October to May, 2009 however the *An. funestus* collections grew during the dry months but fell during the wet months. The total number of *An. gambiae* collected across the six sampled towns was significantly higher (p <0.05) than the number of *An. funestus* caught within the same period. However, there was a seasonal difference in the population of *Anopheline* species collected in which the wet season collections constitute 45.4% *An. gambiae* s.l and 17.7% *An. funestus* while the dry season population constitutes 32.3% *An. gambiae* s.l and 54.6% *An. funestus*. The overall number of *Anopheles* mosquitoes collected in the wet season was significantly higher than that of the dry season (p <0.01).

The month of July, 2009 recorded the highest number of *An. gambiae* with 47 in Zone A at Dandume while *An. funestus* was the most abundant in February, 2010 also at Dandume. On

the other hand *An. gambiae* was not collected in Zone C in the dry months between May and April in Katsina and Daura, and also in Zone B at Dutsinma and Kankara. *An. funestus* had only 5 specimens in the months of January and February, 2010 in Zone C at Daura and in July in Zone B at Kankara and in Zone A at Funtua. Fewer *An. gambiae* larvae were collected during the dry months while the reverse was the case for *An. funestus*. Obtained data shows that the population density of *Anopheles* species incresses as one moves from the arid towns of Katsina and Daura in Zone C towards the less arid towns of Dutsinma and Kankara in Zone B. There was a dramatic increase in population density of *Anopheles* in the northern savanna towns of Funtua and Dandume in Zone A. There is a significant difference in the population density of adults collected indoors between the dry and wet seasons (p <0.05). There were more adults in the wet season than in the dry season. More *Anopheles* species were collected during the months of July and August, 2009 which recorded the highest amount of rainfall. A high proponderence of *Anopheles* species were collected from Zone C at Daura and Katsina and in Zone B at Dutsinma towns of Katsina State. This is highly like as a result of the presence of large water bodies (used for irrigation puposes) located in these towns e.g. Sabke dam near Daura, Ajiwa dam near Kasina in Zone C and Zobe dam in Zone B at Dutsinma. There was a very high population of *Anopheles* species collected from Zone A at Funtua and Dandume. This is a consequent of intense farming activities undertaken through irrigation and of enormous rice fields present.

Out of the total of 853 *An.gambiae* s.s adults collected indoors, the highest collection of 186 of the S form was made in Zone B at Kankara and the lowest of 55 was recorded in in Zone C at Daura. While of the 983 adults reared from larvae, 341 S forms were the highest recorded in in Zone A at Dandume and the lowest was 44 from Zone C at Katsina.

Three *An. funestus* species were identified by PCR, that is *An. funestus, An. rivulorum* and *An. brucei*. *An. funestus* was the most abundant with highest collection of 149 in Dandume followed by *An. brucei* with its highest of 67 also in Dandume while *An. rivulorum* recorded its highest of 35 in Dandume all situated in Zone A.

The Vectest evaluation dealt solely with indoor-collected mosquitoes, that is 926 of *An. gambiae* and 431 of *An. funestus*. *P. falcifarum* was the most preponderant with 163 in Zone A at Dandume and its lowest was 40 in in Zone C at Daura. In addition *P. malaria* recorded its highest collection of 88 also in Zone A at Dandume and the least was 27 in Zone C at Daura. No *P. vivax* were recorded during this study. *An. gambiae* s.s recorded the highest number of *P. falcifarum* with 190 and also highest for *P. malariae* with 289. While *An. quadrimaculatus* had the lowest of 2 each for both *P. falcifarum* and *P. malariae*.

The highest number of identified *plasmodium* species both in 2009 and 2010 was in the month of July. This finding actually coincides with the confirmed hospital cases of malaria in the sampled hospitals where the highest number of confirmed cases were in July 2009 and 2010. There was more *P. malariae* than *P. falcifarum* through out 2009, 2010 and 2011.

The highest number of identified *plasmodium* infection in mosquitoes both in 2009 and 2010 was in the month of July. There was more *P. malariae* than *P. falcifarum* through out 2009, 2010 and 2011. In 2008, the month of August recorded the highest number (187) of confirmed malaria cases while December had the lowest with 52. The highest prevalence of malaria was in July at 205 while November recorded the lowest (64) in 2009. In 2010, July had the highest confirmed cases (205) of malaria while November had the lowest cases of 64. In 2010 there was a dramatic increase in confirmed malaria cases because the highest record of 206 occurred in July while the lowest of 85 was in May.

During the hot and dry season, *Plasmodium* species responsible for malaria (*P. falciparum and P. malariae*) were the dominant species in Katsina State.

In conclusion *An. gambiae* were the most abundant in wet months, followed by *An. funestus* at the end of the rainy season, and *An. arabiensis* in drier months. *An. gambiae* was responsible for malaria transmission during the wet season while *An. funestus* have been confirmed to be responsible for transmission of malaria during the dry season. *Anopheles* mosquitoes survived and proliferate during the dry season by breeding in temporary ponds, cemented and overhead water reservours.

RECOMMENDATIONS

a. To control *Anopheles,* all possible mosquito breeding sites such as open water reservoirs, pools and puddles should be covered or drained to prevent them from serving as breeding sites. In addition, all the three tiers of government should endevour to provide adequate pipe borne water such that there will be no need to reserve water and that will prevent the creation of preventable mosquito breeding points.

b. Focusing on larval control in Katsina State can lead to satisfactory results, as was the case in Palestine, Israel, Italy and the United States, where the modification or elimination of aquatic habitats was applied extensively and were responsible for eradicating malaria, particularly in urban areas for example, in Ethiopia, environmental management was responsible for a 49% decrease in *An. arabiensis* adult population (Yohannes *et al*., 2005). In Dar Es Salaam, the killing of larvae led to a drastic reduction of death caused by malaria (Fillinger *et al*., 2008).

c. Retrospective hospital records of malaria should be coupled with data on spatial distribution of Anopheles so as to determine areas or even households with malaria burden and then deploy appropriate control measures.

d. For every ecological factor identified as enhancing or reducing *Anopheles sp*. larvae productivity, at least one breeding site was found that contradicted these findings. Hence, all water bodies in an urban environments should be considered as potential breeding places and a target for larval control.

e. Rice fields have been implicated for malaria transmission by this study, therefore environmentally friendly larvicides should be applied to rice farms in order to capitulate mosquito proliferation.

f. *Anopheles gambiae*, *An. arabiensis*, *An. funestus*, *An. rivolurum* and *An. brucei* should be targeted simultaneously and wholistically for any mosquito control programme to succeed in Katsina State.

g. Knowledge of vector species and their correct identification should be saught continuously in Katsina State and beyond because it is a prerequisite for a proper understanding of the epidemiology and transmission dynamics of malaria in any given area.

h. *Anopheles funestus* is significant in its own right as a target of public health intervention, justifying further investment. Beyond that, comparative genomics involving *An. funestus* and additional anopheline genomes is further motivation, as it will provide both context for functional annotation of the reference *An. gambiae* genome, and a platform for the genetic analysis of traits associated with successful human malaria vectors.

i. The methods used during the current research can be used for discrimination of anopheline species of medical importance in this region, some of which have overlapping morphologic characters and for conducting complementary studies where rapid and accurate identification of large numbers of specimens is needed.

j. Although it seems likely that *An. funestus*, *An. rivulorum* and *An. brucei* are the predominant species of the complexes, there is a paucity of information on their species identification, distribution pattern and the presence of other members of these complexes cannot be ruled out. It is, therefore, important that exhaustive surveys covering diverse areas and using different collection.

k. Although 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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APPENDICES

Breeding sites of Mosquitoes

Appendix 1*: Running water habitat (Courtesy Florida Mosquito Control Association, 2013)*



Appendix 2: Woodland Pools - (Snowpool) (Courtesy Florida Mosquito Control Association, 2013)



Appendix 3: Woodland Pools - (Spring Rains) (Courtesy Florida Mosquito Control Association, 2013).



Appendix 4: Fresh Floodwater (Courtesy Florida Mosquito Control Association, 2013)



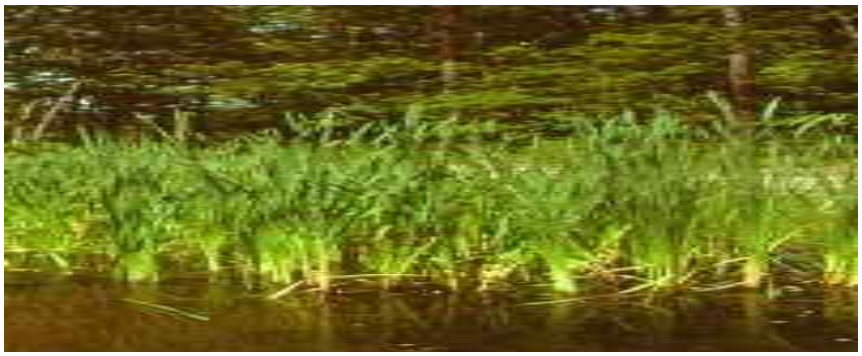
Appendix 5*: Tidal Floodwater (Courtesy Florida Mosquito Control Association, 2013)*



Appendix 6: Freshwater Swamp - (Tussocks - *Aedes abserratus*) an early season tussocks swamp.



Appendix 7: Another freshwater swamp are the cattail swamp



Appendix 8: Acid Water Swamp - (White Cedar Swamp).



Appendix 9: "crypts" formed by pockets of water surrounding tree roots.



Appendix 10: Brackish Water Swamps - (Salt Marsh



Appendix 11: Polluted Water



Appendix 12: Bromeliads (tropical)



Appendix 13: Pitcher plants



Appendix 14: Treeholes



Appendix 15: Bamboo Crab Holes Decaying Fruit



Appendix 16: Artificial - e.g., tyres



Appendix 17 container breeding sites



a)



b)



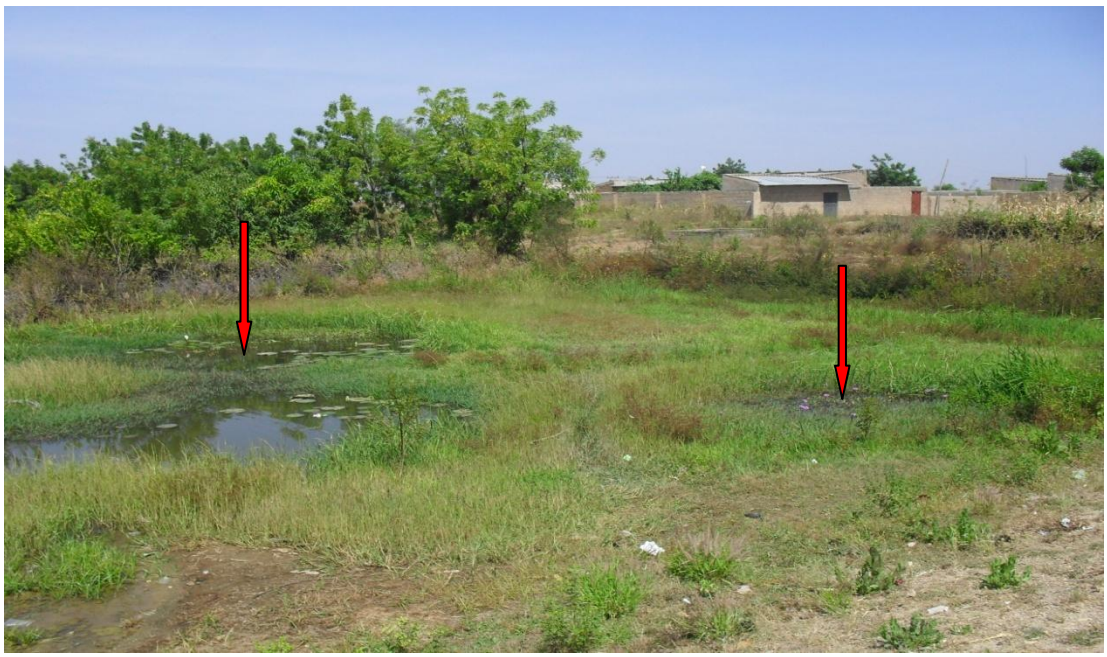
c)



d)

a) Police road-block drums containg *Anopheles* larvae at Dutsinma b) Drums used for house decoration containing anopheles larvae at Katsina c) Old tyres used as seat at a mosque containing *Anopheles* larvae at Dutsinma and d) abandoned coaltar drums containing anopheles larvae at Katsina

Appendix 18 Marshy pools containing *Anopheles* larvae



a)



b)

a) Kankara b) Marshy pools containing *Anopheles* larvae at Dandume.

Appendix 19 Pools made by marks serving as breeding sites.



a)



b)

a) Pools from animal hoofs prints where *Anopheles* larvae were collected at Funtua b) Pools from Tyre marks containing water from a burst water pipe containing *Anopheles* larvae at Katsina.

Appendix 20 Pools in drainages serving as breeding sites.



a)



b)



c)

a) Gutters containing *Anopheles* larvae at a) Kankara b) Dutsinma and c) Dandume

Appendix 21 Pools at water fetching points serving as breeding sites.



a)



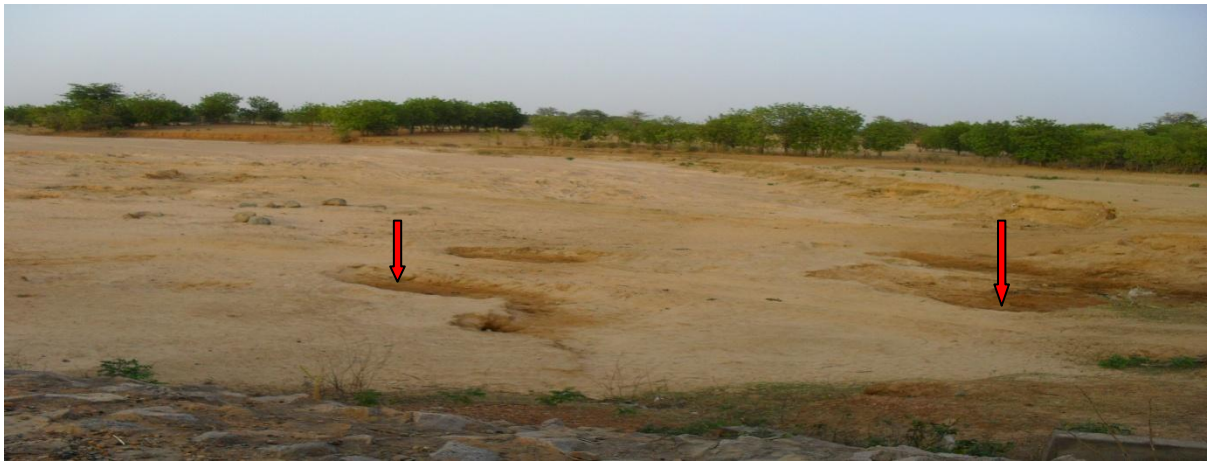
b)



c)

a) Kankara b) Daura and c) Katsina

Appendix 22 River bed pools serving as breeding sites.



a)



b)



c)

a) Katsina b) Dutsinma and c) Kankara

Appendix 23 Washing pits serving as breeding sites.



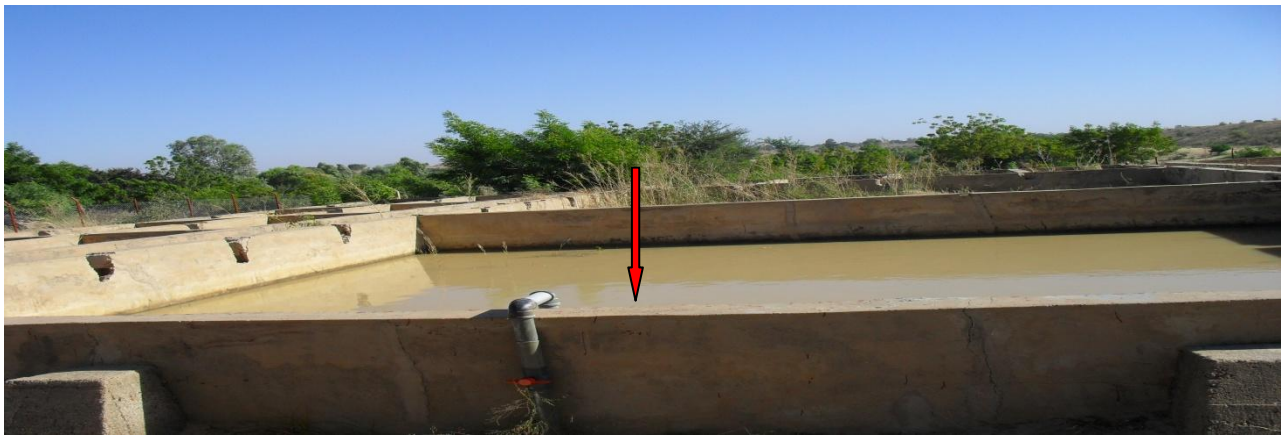
a)



b)

a) Daura and b) Katsina

Appendix 24 Ground Water reservours serving as breeding sites.



a)



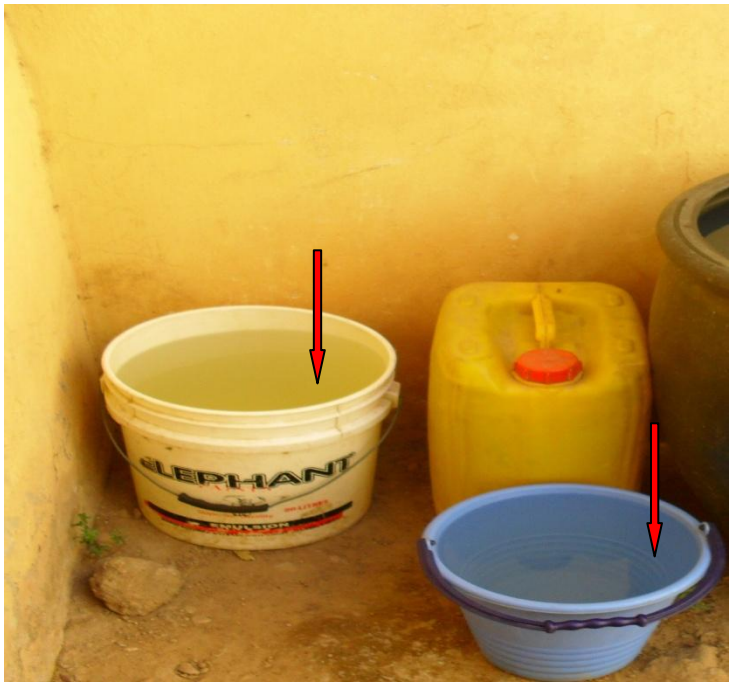
b)



c)

a) *Anopheles* larvae in an abandoned fish pond b) *Anopheles* larvae in a pool at a leaking borehole c) *Anopheles* larvae in a pipe borne water connection unit.

Appendix 25 a) Household reservoirs serving as breeding sites.



a)



b)

a) household water reservoirs b) *Anopheles* larvae breeding in a domestic sewage tank.

Appendix 26 Drying temporary ponds serving as breeding sites.



a)



b)



c)

a) Dutsinma b) Daura and c) Kankara

Appendix 27 Morphological identification of *Anopheles* adults



a)



b)



c)



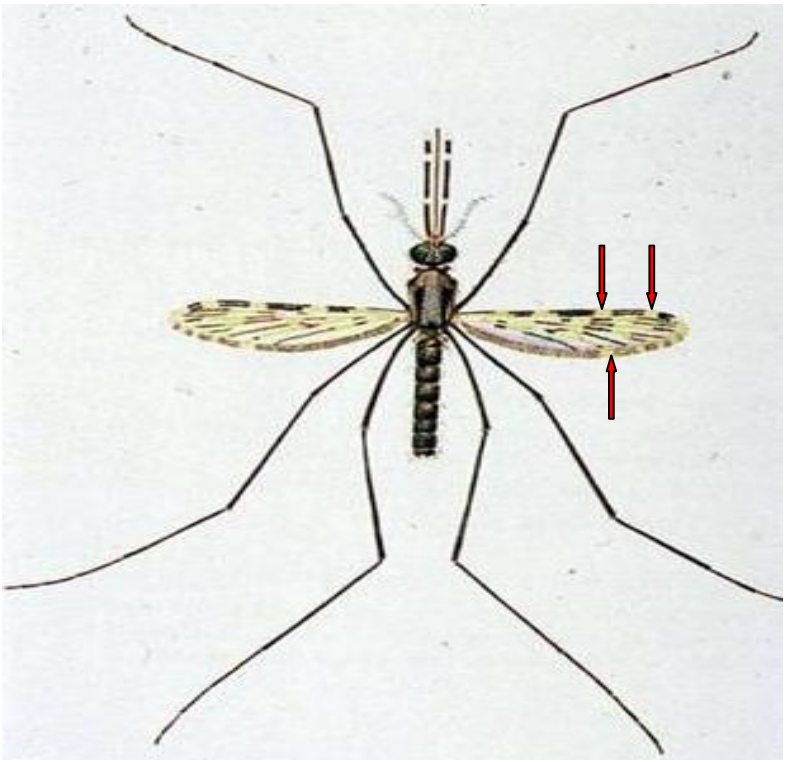
d)



e)

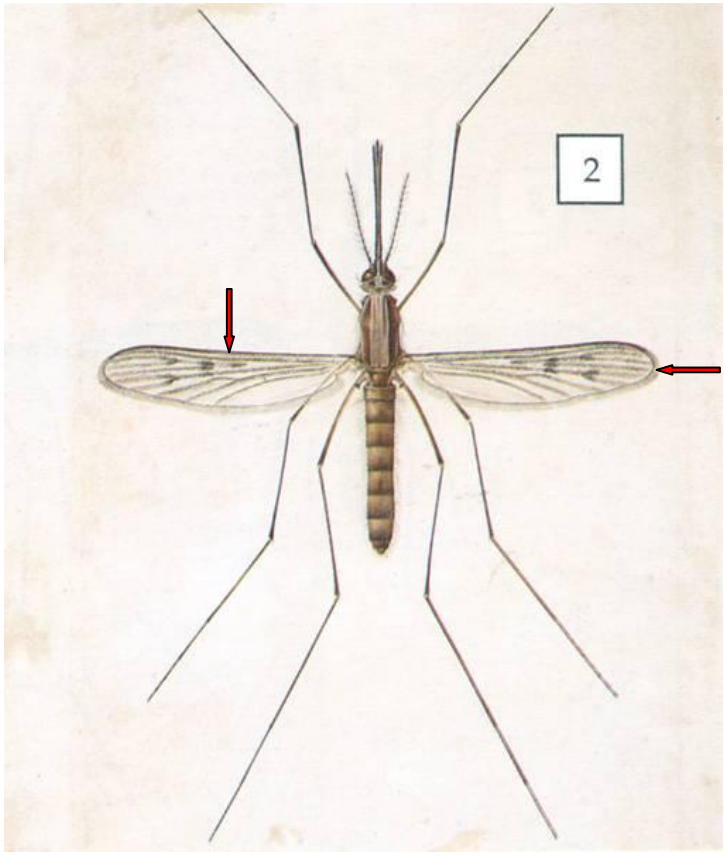
a) *An. gambiae* protarsi mostly pale, b) metatarsi with pale bands c) each abdominal segment have two white patches and a centrally located white line runing through the length of the abdomen d) wings with pale bands on forked vein and pale zone at the margin (right pointing arrow) e) palps with more than two pale rings.

Appendix 28 *Anopheles funestus s.l.*



Unspotted legs and dark body; apical and sub-apical white bands equal in length (downward arrows). Forked vein entirely dark (upward arrow)

Appendix 29 *An. maculipennis*

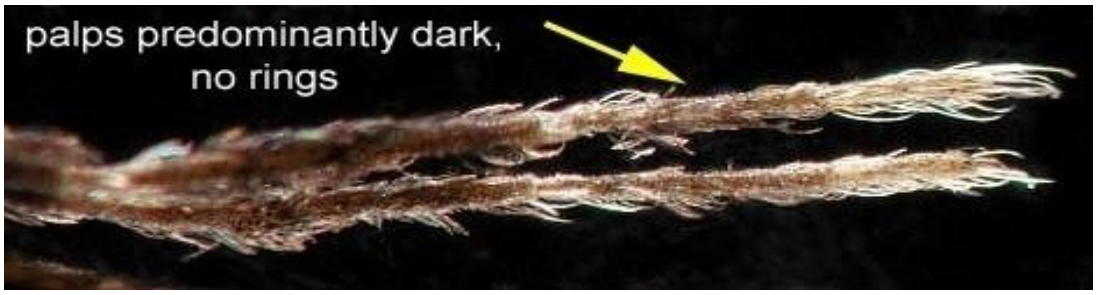


Complex with no bands on front edge of wing (left arrow); but has spots at junctions of wing veins (right arrow).

Appendix 30 *An. quadrimaculatus*



a)



b)



c)

a) Tuft on the head of adult of *An. quadrimaculatus* b) Haltares of *An. quadrimaculatus c)* Palps of *An. quadrimaculatus d)* Wing of *An. quadrimaculatus*

Appendix 31 STANDARD SOLUTIONS FOR PCR AND ELECTROPHORESIS
The following standard solutions were prepared using sterile double distilled water (sddw). Where appropriate, the solutions were autoclaved at 121lb/sq in. for 15 minutes in an Eyela Autoclave (Rikikakki Tokyo).

DNA Extraction
Bender buffer
0.1M NaCl, 0.2M sucrose. 0.1M Tris-HCl pH 7.5, 0.05M EDTA pH 9.1, 0.5% SDS. Stored at 40 C

0.5 M EDTA (pH8.0)
186.1 g/l in water, ph adjusted with NaOH pellets and stored at room temperature.

EtBr (10mg/ml)
1g of EtBr was completely dissolved in 100ml sddw and stored in the dark at room temperature**.**

KAc (5M K 8M Acetate)
60ml of 5 M KAc and 11.5 ml glacial acetic acid in 28.5 ml distilled water

TE (pH 8.0)
10Mm Tris-HCl (pH), 1mM EDTA (pH8.0). Stored at room temperature.

Solutions for Electrophoresis
Agarose Gels

10X TAE buffer
242 g Tris base, 57.1 ml glacial acetic acid, 100ml 0.5M EDTA, pH adjusted to 7.7 (with glacial acetic acid) and the volume made to 1000 ml with sddw.

0.5 M EDTA (pH 8.0)
186g of EDTA, dissolved in 800ml ddw, pH adjusted with NaOH pellets, the volume made to 1000 ml with sddw and stored at room temperature.

Li-Cor Ger Electrophoresis Protocol
1. 40% sequencing acrylamide stock was made as follows: (Wear a mask and double gloves while preparing this solution):

Chemical	Dry weight	Multiply by	Dry weight
Acrylamide	38.0g	6	228g
Bis-acrylamide	2.0g	6	12g
ddH_2O	Up to 100	6	Up to 600ml

The solution is then mixed thoroughly, and filtered through Whatmann paper then stored wrapped in aluminium foil at 40C.

2. Making a solution of 5.5% acrylamide for the 25cm gel:

Chemical	Amount	Multiply by	Amount
Urea	12.6g	5	63g
40% home-made acrylamide	4.125ml	5	20.625ml
5X TBE	3.0ml	5	30ml
ddH_2O	Up to 30ml	5	Up to 150ml

The solution is then mixed thoroughly.
This solution was made in large quantities (approx. 150ml volume) and stored as stock at 40c in a sealed bottle.

3. Preparation of the buffer solution.
The buffer solution in the gel is a 5X TBE buffer. The running buffer, however, is a 1X TBE buffer. The 5X TBE buffer was prepared by adding the following to a 2L beaker.

Chemical	Dry weight
Tris	108g
Boric Acid	55g
Na_2EDTA (with $2H_2O$)	9.3g

The solution was mixed by stirring until all salts have dissolved, hen brought to a final volume of 2L with ddH2O and divide into 2 IL bottles.

4. Preparation of the Ammonium Persulfate Solution (APS):
A 10% solution was made by adding 0.1g of APS to 1ml ddH2O.

5. The 25cm glass plates for the sequencer are then assembled and prepared. The inside of both glass plates were rinsed with 95% ethanol to clean. (The inside of a plate is determined by the beveled corners of the plate. The beveled corners help when prying the plates apart for cleaning; therefore they should go on the inside). The alcohol is allowed to dry before placing the 0.25mm spacers along the long edges of the rear plate.

The front plate plate was placed on top of the rear plate and the spacers aligned with the outside edges of the plates, making sure the plates are aligned evenly at the bottom. The left and right rail assemblies are placed over the long edges of the plates and the knobs tightened against the glass plate until finger tight.

6. The 5.5% acrylamide gel was prepareed and poured as follows:
Take 39ml of the 5.5% acrylamide gel.
Add 20ul of TEMED.
Add 20ul of the 10% APS solution.
The solution is swirled using a 60cc syringe with a 14-gauge needle. The glass plates are then angled slightly to deliver the acrylamide evenly across the top notch of the front plate, and the gel is let to flow at a steady, even rate until it reaches the bottom of the glass plates. At this point, the glass plates are laid flat and checked for any air bubbles, which are removed with wires if present. Preparation of the gel is followed by inserting the appropriate combs or piece of equipment for forming the wells (the trough to form the base of the wells). Any empty spaces are filled with Acrylamide.

The casting plate is then in the grooved area in the rails (which will eventually be occupied by the upper buffer chamber) and tightened until finger tight. The gel is then allowed to polymerize for about 1 hour.

Gel Loading Buffers
6x Bromophenol blue 0.25% bromophenol blue was added to 40% sucrose in water and stored at 40 C.
Bromophenol blue xylene cyanol : 1 volume of bromophenol blue xylene cyanol and 4 volumes of cyanide 5X orange G 20%w/v Ficoll, 25Mm EDTA, 2.5mM EDTA. 2.5 % (w/v) orange G. Stored at room temperature.

DNA Molecular weight & size markers
The 100 bp DNA molecular weight size marker obtained from Sigma were diluted according to the manufacturers recommendations and used. For the 100 bp ladder, the first band size is 100 bp, the subsequent ones are 200, 300…….1000bp.

APPENDIX 32 F-Test Two-Sample for Variances for *Anopheles* adults reared from larvae 2009-2011

	Dandume	*Katsina*
Mean	138.75	37
Variance	27224.91667	2596
Observations	4	4
Df	3	3
F	10.48725603	
P(F<=f) one-tail	0.042446724	
F Critical one-tail	9.276628154	

Appendix 33 t-Test: Paired Two Sample for Means for *Anopheles* larvae reared 2009-2011

	An. gambiae	*An. funestus*
Mean	179.5	90.5
Variance	8613.1	2485.9
Observations	6	6
Pearson Correlation	0.913569	
Hypothesized Mean Difference	0	
df	5	
t Stat	4.239377	
P(T<=t) one-tail	0.004087	
t Critical one-tail	2.015048	
P(T<=t) two-tail	0.008174	
t Critical two-tail	2.570582	

Appendix 34 F-Test Two-Sample for Variances for indoor collection 2009-2011

	Dandume	*Daura*
Mean	75.25	24.5
Variance	9614.916667	1094.333333
Observations	4	4
Df	3	3
F	8.786095035	
P(F<=f) one-tail	0.053721922	
F Critical one-tail	9.276628154	

Ftab=10.13

Appendix 35 F-Test Two-Sample for Variances for indoor collection 2009-2011

	An. gambiae	*An. funestus*
Mean	162	65.66666667
Variance	3526	461.8666667
Observations	6	6
Df	5	5
F	7.634237875	
P(F<=f) one-tail	0.021792347	
F Critical one-tail	5.050329058	

Ftab = 6.61

Appendix 36 F-Test Two-Sample for Variances for breeding sites

	Pool	*Pond*
Mean	90.75	56.25
Variance	26080.91667	6056.25
Observations	4	4
Df	3	3
F	4.306446508	
P(F<=f) one-tail	0.130743773	
F Critical one-tail	9.276628154	

Ftab=10.13

Appendix 37 F-Test Two-Sample for Variances for breeding sites

	Cemented reservoir	*Overhead tank*
Mean	117.5	53.5
Variance	19881	3929
Observations	4	4
Df	3	3
F	5.060066175	
P(F<=f) one-tail	0.107986392	
F Critical one-tail	9.276628154	

Appendix 38 F-Test Two-Sample for Variances for breeding sites

	Rice farm	*Pond*
Mean	94.25	56.25
Variance	10208.25	6056.25
Observations	4	4
Df	3	3
F	1.685572755	
P(F<=f) one-tail	0.339266573	
F Critical one-tail	9.276628154	

Appendix 39 F-Test Two-Sample for Variances for *An. gambiae* s.s and *An. arabiensis* reared from larvae

	Ag. s.s	*Ag. Arb*
Mean	159.1666667	20.33333333
Variance	8379.366667	7.066666667
Observations	6	6
Df	5	5
F	1185.759434	
P(F<=f) one-tail	1.11866E-07	
F Critical one-tail	5.050329058	

Ftab=6.61

Appendix 40 F-Test Two-Sample for Variances for for *An. gambiae* s.s and *An. arabiensis* reared from larvae

	Dandume	*Dutsinma*
Mean	237.3333333	110
Variance	34586.33333	6559
Observations	3	3
Df	2	2
F	5.273110738	
P(F<=f) one-tail	0.159410545	
F Critical one-tail	19	

Ftab=18.51

Appendix 41 F-Test Two-Sample for Variances for for *An. gambiae* s.s and *An. arabiensis* reared from larvae

	Dandume	*Kankara*
Mean	237.3333333	120
Variance	34586.33333	7741
Observations	3	3
Df	2	2
F	4.467941265	
P(F<=f) one-tail	0.182884188	
F Critical one-tail	19	

Appendix 42 F-Test Two-Sample for Variances for *Anopheles* M and S forms caught Indoors

	M form	*S form*
Mean	2.666666667	139.5
Variance	2.666666667	2765.9
Observations	6	6
Df	5	5
F	0.000964123	
P(F<=f) one-tail	1.56255E-07	
F Critical one-tail	0.1980069	

Ftab = 6.61

Appendix 43 F-Test Two-Sample for *Anopheles* M and S forms Reared and towns

	Dandume	*Daura*
Mean	139.25	45.5
Variance	8354.916667	927
Observations	4	4
Df	3	3
F	9.012855088	
P(F<=f) one-tail	0.051945968	
F Critical one-tail	9.276628154	

Ftab = 7.71

Appendix 44 F-Test Two-Sample for Variances *An. funestus* adults and larvae identified by PCR

	Dandume	*Katsina*
Mean	45.2	32
Variance	986.2	438
Observations	5	5
Df	4	4
F	2.251598174	
P(F<=f) one-tail	0.225569139	
F Critical one-tail	6.388232909	

Ftab=7.71

Appendix 45 F-Test Two-Sample for Variances for Variances for *An. funestus* adults and larvae identified by PCR (Indoors)

	Dandume	*Kankara*
Mean	45.2	46.2
Variance	986.2	935.7
Observations	5	5
Df	4	4
F	1.05397029	
P(F<=f) one-tail	0.480297475	
F Critical one-tail	6.388232909	

Ftab=7.71

Appendix 46 F-Test Two-Sample for Variances for *An. funestus* adults caught indoors

	Funtua	*Katsina*
Mean	52.8	32
Variance	1317.2	438
Observations	5	5
df	4	4
F	3.007305936	
P(F<=f) one-tail	0.15573755	
F Critical one-tail	6.388232909	

Appendix 47 F-Test Two-Sample for Variances for *An. funestus* adults Reared

	Dandume	*Daura*
Mean	105.6	41.4
Variance	4860.3	755.3
Observations	5	5
Df	4	4
F	6.434926519	
P(F<=f) one-tail	0.049404697	
F Critical one-tail	6.388232909	

Ftab=7.71

Appendix 48 F-Test Two-Sample for Variances from hospital records of malaria in 2008

	FMCK	*GHD*
Mean	38.91666667	36.58333333
Variance	288.0833333	110.6287879
Observations	12	12
Df	11	11
F	2.604053962	
P(F<=f) one-tail	0.063755017	
F Critical one-tail	2.81793047	

Ftab=4.84

Appendix 49 F-Test Two-Sample for Variances from hospital records of malaria in 2008/2009

	FMCK	*FMCK*
Mean	38.91666667	46.41666667
Variance	288.0833333	208.4469697
Observations	12	12
Df	11	11
F	1.382046157	
P(F<=f) one-tail	0.300340806	
F Critical one-tail	2.81793047	

Appendix 50 Monthly distribution of malaria cases in selected hospitals from 2008 - 2010

	2008				2009				2010			
	Total	FMCK	GHD	GHF	Total	FMCK	GHD	GHF	Total	FMCK	GHD	GHF
January	88	29	33	26	122	51	39	32	121	57	33	31
February	85	23	32	30	112	40	26	46	134	51	43	40
March	123	52	43	28	139	48	49	40	140	53	38	49
April	120	36	35	49	126	58	42	26	98	43	35	18
May	85	37	28	30	99	39	30	30	85	24	32	29
June	146	45	52	49	149	54	41	54	166	63	56	47
July	144	56	36	52	205	65	76	64	206	103	60	43
August	187	73	58	56	133	63	52	45	193	63	101	29
September	136	52	40	44	124	47	30	47	168	69	75	24
October	101	25	35	41	131	49	43	39	138	58	53	27
November	82	23	21	38	64	14	22	28	144	47	49	48
December	52	16	26	10	82	29	32	21	105	44	39	22
Total	1349	467	439	453	1486	557	482	472	1698	675	614	407

FMCK = Federal Medical Centre Katsina, GHD = General Hospital Dutsinma, GHF= General Hospital Funtua

lix 51 Locality, months and adults reared from larvae collected from a pool (breeding site A)

anuary		February		March		April		May		June		July		August		September		October		November		Dece
g	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag
	0	1	8	2	8	1	10	2	9	10	3	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	10	0	7	0	9	0	7	0	4	0	0	0	0
	0	0	0	0	0	0	0	0	0	9	3	10	4	8	2	3	3	0	2	1	0	
	10	1	11	14	0	1	11	0	12	13	2	5	0	4	0	5	0	11	8	12	10	8

lix 52 Locality, months and adults reared from larvae collected from a pond (breeding site B)

anuary		February		March		April		May		June		July		August		September		October		November		Dece
g	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	13	4	Ag	Af	Ag	Af	Ag	Af	Ag
	7	0	0	0	0	0	0	0	0	0	0	10	2	0	0	11	5	8 12	9	13	6	
	0	0	5	0	0	0	0	0	0	0	0	0	0	15	7	0	0	0	0	0	0	
2	9	1	11	2 17		1	2	0 25		11	5	10	3	14	4	17	6	5 10	6	15	5	
	11	4	15	3 17		1 23		0 25		15	4	15	5	5	3	9	3	10 8	14	13	5	
	0	0	0	0	0	0	0	0	0	4	0	7	0	6	0	7	0	0	0	0	0	
	5	0	8	0	7	0	7	0 16		4	3	9	0	4	0	7	0	13 4	10	3	0	

ocality, months and adults reared from larvae collected from overhead tank (breeding site C)

anuary		February		March		April		May		June		July		August		September		October		November		Dece
g	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag
	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4	0	0	0	0	0	0	
	5	1	0	0	7	1	11	0	9	10	5	12	5	14	7	15	6	12 2	9	11	6	
	0	0	0	0	0	0	0	0	0	12	2	10	2	11	2	4	0	3	0	0	0	
	0	0	0	0	0	0	0	0	0	8	3	15	0	10	3	7	3	0	3	0	0	
	8	3	10	0	3	0	0	0	0	8	0	10	1	11	4	3	1	0 5	8	4	6	
	0	0	0	0	0	0	0	0	0	11	0	11	0	10	2	9	0	6 0	0	0	0	

Locality, months and adults reared from larvae collected from a ground reservoir (breeding site D)

anuary	February	March	April	May	June	July	August	September	October	November	Dece

g	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	5	1	Ag	Af	Ag	Af	Ag	Af	Ag
																		0				
	0	0	0	0	0	0	0	0	0	6	4	8	3	0	0	5	1	0		0	0	0
																		0				
	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0		0	0	0
																		0				
	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	11	0	0		0	0	0
																		0				
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0
																		2				
	0	0	0	0	0	0	0	0	0	10	0	12	0	3	0	0	0	0		0	0	0
																		5				
	0	0	0	0	0	0	0	0	0	14	0	10	2	13	4	9	0	0		0	0	0

Locality, months and adults reared from larvae collected from Rice fields (breeding site E)

anuary		February		March		April		May		June		July		August				September		October		November		Dece
g	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Aq	Ac	Ag	Af	Ag	Af	Ag	Af	Ag
																				0				
	0	1	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0
																				0				
	0	0	0	0	0	0	0	0	0	5	2	5	0	5	2	0	0	5	0	0		0	0	0
																				0				
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0
																				0				
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0
				2		1		5												10				
	3	4	5	14		21		25		7	4	1	0	4	5	11	9	10	5	7		8	9	3
				0																4				
	0	0	0	0		0	0	0	0	14	0	12	3	11	17	15	6	9	6	0		0	0	0

Locality, months, indoor adult species and number collected from 2009 – 2011

anuary		February		March		April		May		June		July		August				September		October		November		Dece
g	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Aq	Ac	Ag	Af	Ag	Af	Ag	Af	Ag
				1																10				
	2	3	9	14		2	15	2	17	20	3	11	3	15	4	0	0	12	2	2		3	1	2
																				8				
	8	2	7	0	2	1	3	0	5	12	5	2	1	4	2	0	0	15	3	3		8	4	0
						1		3												19				
	3	2	5	2	8	15		17		31	7	20	23	15	2	0	0	11	4	3		5	3	5
				2		3		2												13				
4		2	15	18		20		11		32	4	32	6	14	4	0	0	12	9	11		9	20	10
				3		2														9				
2		7	21	14		10		1	7	32	8	40	4	24	3	7	2	18	8	9		11	14	4
				3		1		1												8				
4		5	27	14		11		12		33	7	35	4	19	4	5	7	30	8	20		3	10	2

Ag = An. gambiae *Af = An. funestus* *Ac = An. maculipennis* *Aq. = An. quadrimaculatus*

Locality, months, reared adult species and number collected from 2009 – 2011

anuary		February		March		April		May		June		July		August				September		October		November		Dece
g	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Aq	Ac	Ag	Af	Ag		Ag	Af	Ag

																				Af			
																				8			
	2	1	5	0	7	1	6	0	8	12	7	12	5	10	7	0	0	10	6	8	9	7	6
						1														18			
	5	1	5	0	7	11		0	9	10	7	12	5	14	9	0	0	15	6	2	6	11	6
				2		1														8			
	7	1	9	13		15		0	15	20	7	21	5	22	9	0	0	21	6	11	6	10	5
				3		1														12			
1		4	13	14		17		0	20	23	7	27	5	25	7	0	0	23	6	12	14	13	5
				2		1														10			
1		7	15	17		18		5	17	18	7	25	5	23	9	11	9	20	6	12	10	14	9
				0		1														35			
5		9	29	21		26		0	28	45	7	47	15	44	19	15	6	39	16	12	22	13	8

Ag = An. gambiae Af = An. funestus Ac = An. maculipennis Aq. = An. quadrimaculatus

Locality, months, and number of bitting *Anophele* collected from a room (resting site A)

anuary		February		March		April		May		June		July		August		September		October	November		Dece
																		Ag			
g	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Af	Ag	Af	Ag
																		5			
	0	0	0	1	6	0	3	0	4	3	0	7	0	6	3	2	2	2	3	1	2
																		2			
	4	2	2	0	1	0	3	0	3	6	0	0	0	3	2	3	0	0	3	4	0
																		9			
	7	2	4	0	3	1	7	0	5	10	4	11	7	6	0	4	1	0	0	1	2
																		3			
	4	2	8	0	9	2	7	0	2	12	4	11	2	0	2	2	1	1	3	4	3
																		3			
	6	2	7	1	4	0	1	0	7	12	1	10	2	9	0	2	0	1	3	6	0
				0														2			
	7	2	10	10		1	1	0	2	11	4	11	2	9	4	10	0	7	0	1	1

Locality, months, and number of bitting *Anopheles* collected from a hospital ward (resting site B)

anuary		February		March		April		May		June		July			August		September		October	November		Dece
																			Ag			
g	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag		Af	Ag	Af	Ag	Af	Af	Ag	Af	Ag
								2				2	1	0					0			
	2	3	7	0	0	0	7	10		5	3	0			8	0	7	0	0	0	0	0
												2	1	0					2			
	4	0	2	0	5	1	6	0	0	2	0	0			1	0	7	3	2	0	0	0
												8	7	0					5			
	4	0	3	2	3	0	5	3	9	11	2	0			7	2	4	2	3	5	1	2
												13	2	0					9			
	5	0	3	1	6	1	9	2	7	15	0	0			10	0	9	5	6	3	10	1
												10	0	0					3			
	6	2	9	1	7	2	5	0	0	8	3	0			9	0	9	5	4	7	5	4
												9	0	5					2			
	4	2	7	3	2	0	9	0	8	13	2	0			3	0	12	5	10	3	6	1

Locality, months, and number of bitting *Anopheles* collected from a hotel room (resting site C)

anuary		February		March		April		May		June		July				August		September		October	November		Dece
																				Ag			
g	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Ag	Af	Aq	Ac	Ag	Af	Ag	Af	Af	Ag	Af	Ag
	5	0	2	0	4	2	5	0	3	3	0	2	2	0	0	1	1	3	0	5	0	0	0

0	0	3	0	1	0	0	0	2	4	5	0	0	0	0	0	0	5	0	041	5	0	0
2	0	0	0	2	0	3	0	3	10	1	1	0	0	0	2	0	3	1	501	0	1	1
5	0	4	1	3	0	4	0	2	5	0	8	2	0	0	4	0	1	3	143	0	6	6
0	3	5	1	3	0	4	1	0	12	4	20	2	0	0	6	3	7	3	434	1	3	0
3	1	10	0	2	0	1	1	2	9	1	15	2	0	7	7	0	8	3	43	0	3	0

	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Katsina	37	37	35	36	35	32	31
Daura	39	39	36	35	35	34	34
Dutsinma	32	31	32	29	27	25	25
Kankara	33	32	30	30	31	29	25
Funtua	30	30	29	28	26	26	25
Dandume	26	27	25	26	26	23	23

a)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Katsina	31	34	38	38	38	36	36	35	35	34	33	32
Daura	34	35	40	40	41	38	37	37	36	36	33	32
Dutsinma	29	30	31	33	31	29	26	26	24	24	22	23
Kankara	29	29	30	32	35	31	31	28	26	27	26	25
Funtua	27	26	26	30	31	29	27	25	25	25	24	24
Dandume	28	28	28	30	30	29	27	26	24	24	26	22

b)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Katsina	37	36	37	37	40	38	37	36	35	36
Daura	40	40	42	41	43	40	34	34	33	33
Dutsinma	31	32	35	35	36	30	29	29	29	29
Kankara	32	31	34	31	35	33	33	33	33	32
Funtua	33	32	33	33	33	30	30	30	32	31
Dandume	31	32	33	34	33	28	29	28	28	26

Appendix 61 Average monthly temperature in ^{o}C in a) 2009 b) 2010 c) 2011

Appendix 62 Analysis of ANOVA for monthly temperature recorded in 2009

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*

Between Groups	614.7619	11	55.88745	8.419199	1.65E-06	2.125559
Within Groups	199.1429	30	6.638095			
Total	813.9048	41				

ANOVA Tem. 2010

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1171.903	11	106.5366	12.41002	9.06E-12	1.952212
Within Groups	515.0833	60	8.584722			
Total	1686.986	71				

a) 2009 b) 2010 c) 2011

Appendix 63 Average monthly Rainfall in millimeters in (mm) in a) 2009 b) 2010 and c) 2011

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Katsina	0	0	0	0	0	56.0	63.3	69.2	69.0	58.8	0	0
Daura	0	0	0	0	0	51.1	56.1	67.0	68.9	59.4	0	0
Dutsinma	0	0	0	0	0	62.1	71.1	68.9	69.9	61.8	0	0
Kankara	0	0	0	0	0	62.9	72.3	69.9	70.4	62.2	0	0
Funtua	0	0	0	0	0	67.7	73.0	70.2	71.5	71.1	0	0
Dandume	0	0	0	0	0	68.7	73.8	71.2	72.2	72.3	0	0

a)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Katsina	0	0	0	0	0	58.9	66.7	78.9	73.3	65.5	0	0
Daura	0	0	0	0	0	53.2	58.8	75.5	72.2	63.3	0	0
Dutsinma	0	0	0	0	0	64.4	74.4	78.7	76.6	66.5	0	0
Kankara	0	0	0	0	0	64.9	74.9	79.9	78.6	69.9	0	0
Funtua	0	0	0	0	50.0	73.3	79.3	85.5	79.9	76.6	70.0	0
Dandume	0	0	0	0	50.9	74.3	79.8	86.6	81.1	79.9	71.1	0

b)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Katsina	0	0	0	0	0	58.8	64.5	77.8	72.3	64.3	0	0
Daura	0	0	0	0	0	52.2	57.8	73.5	69.8	61.1	0	0
Dutsinma	0	0	0	0	568	63.3	73.3	78.8	75.5	65.4	0	0
Kankara	0	0	0	0	578	64.1	73.9	79.0	77.5	68.8	0	0
Funtua	0	0	0	0	652	70.1	78.8	84.5	79.0	77.5	0	0
Dandume	0	0	0	0	666	71.2	79.1	85.5	80.2	77.9	0	0

a) 2009 b) 2010 c) 2011

Appendix 64 ANOVA for average Rainfall in millimeters (mm)

ANOVA 2009

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	190.3744	11	17.30677	0.013185	1	1.952212
Within Groups	78758.14	60	1312.636			
Total	78948.52	71				

ANOVA 2010

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3329.701	11	302.7001	0.198498	0.99712	1.952212
Within Groups	91497	60	1524.95			
Total	94826.7	71				

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	50464.87	11	4587.715	0.20613	0.996594	1.952212
Within Groups	1335388	60	22256.47			
Total	1385853	71				

a) 2009 b) 2010 c) 2011

Appendix 65a Monthly distribution of adult *Anopheles* caught indoors

Month Locality	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Katsina	Ag 2 Af 5	Ag 3 Af 9	Ag 1 Af 14	Ag 2 Af15	Ag 2 Af 17	Ag 20 Af 3	Ag 11 Af 3	Ag 15 Af 4	Ag 12 Af 2	Ag 10 Af 2	Ag 3 Af 1	Ag 2 Af 7	165

Daura	Ag 1 Af 8	Ag 2 Af 7	Ag 0 Af 2	Ag 1 Af 3	Ag 0 Af 5	Ag 12 Af 5	Ag 2 Af 1	Ag 4 Af 2	Ag 15 Af 3	Ag 8 Af 3	Ag 8 Af 4	Ag 0 Af 2	98
Dutsinma	Ag 4 Af 13	Ag 2 Af 5	Ag 2 Af 8	Ag 1 Af 15	Ag 3 Af 17	Ag 31 Af 7	Ag 20 Af 23	Ag 15 Af 2	Ag 11 Af 4	Ag 19 Af 3	Ag 5 Af 3	Ag 5 Af 9	238
Kankara	Ag 4 Af 14	Ag 2 Af 15	Ag 2 Af 18	Ag 3 Af 20	Ag 2 Af 11	Ag 32 Af 4	Ag 32 Af 6	Ag 14 Af 4	Ag 12 Af 9	Ag 13 Af 11	Ag 9 Af 20	Ag 10 Af 8	277
Funtua	Ag 6 Af 12	Ag 7 Af 21	Ag 3 Af 14	Ag 2 Af 10	Ag 1 Af 7	Ag 32 Af 8	Ag 40 Af 4	Ag 24 Af 3 Aq 7 Ac 2	Ag 18 Af 8	Ag 9 Af 9	Ag 11 Af 14	Ag 4 Af 21	299
Dandume	Ag 7 Af 14	Ag 5 Af 27	Ag 3 Af 14	Ag 1 Af 11	Ag 1 Af 12	Ag 33 Af 7	Ag 35 Af 4	Ag 19 Af 4 Aq 5 Ac 7	Ag 30 Af 8	Ag 8 Af 20	Ag 3 Af 10	Ag 2 Af 11	301

Ag = An. gambiae Af= An. funestus Ac = An. maculipennis Aq = An. quadrimaculatus

Appendix 65b Poolled monthly distribution of adults caught indoors

	January	February	March	April	May	June	July	August
An. gambiae	24	21	11	11	9	160	130	91
An. funestus	66	84	70	74	69	34	41	19
An. maculipennis	0	0	0	0	0	0	0	9
An. quadrimaculatus	0	0	0	0	0	0	0	12
Total	90	105	81	85	78	194	171	131

Appendix 66a Monthly distribution of adults reared from larval collections

Month/ Locality	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Tota
Katsina	Ag 2 Af 4	Ag 1 Af 5	Ag 0 Af 7	Ag 1 Af 6	Ag 0 Af 8	Ag 12 Af 7	Ag 12 Af 5	Ag 10 Af 7	Ag 10 Af 6	Ag 8 Af 8	Ag 9 Af 7	Ag 6 Af 9	148
Daura	Ag 1 Af 5	Ag 1 Af 5	Ag 0 Af 7	Ag 1 Af11	Ag 0 Af 9	Ag 10 Af 7	Ag 12 Af 5	Ag 14 Af 9	Ag 15 Af 6	Ag 18 Af 2	Ag 6 Af 11	Ag 6 Af 7	156
Dutsinma	Ag 2 Af 7	Ag 1 Af 9	Ag 2 Af 13	Ag 1 Af 15	Ag 0 Af 15	Ag 20 Af 7	Ag 21 Af 5	Ag 22 Af 9	Ag 21 Af 6	Ag 8 Af 11	Ag 6 Af 10	Ag 5 Af 8	218
Kankara	Ag 3 Af 11	Ag 4 Af 13	Ag 3 Af 14	Ag 1 Af 17	Ag 0 Af 20	Ag 23 Af 7	Ag 27 Af 5	Ag 25 Af 7	Ag 23 Af 6	Ag 12 Af 12	Ag 14 Af 13	Ag 5 Af 9	274
Funtua	Ag 5 Af 11	Ag 7 Af 15	Ag 2 Af 17	Ag 1 Af 18	Ag 5 Af 17	Ag 18 Af 7	Ag 25 Af 5	Ag 23 Af 9 Aq11 Ac 9	Ag 20 Af 6	Ag 10 Af 12	Ag 10 Af 14	Ag 9 Af12	298
Dandume	Ag 8 Af 25	Ag 9 Af 29	Ag 0 Af 21	Ag 1 Af 26	Ag 0 Af 28	Ag 45 Af 17	Ag 47 Af 15	Ag 44 Af 19 Aq15 Ac 6	Ag 39 Af 16	Ag 35 Af 12	Ag 22 Af 13	Ag 8 Af16	555

Ag = An. gambiae Af= An. funestus Ac = An. maculipennis Aq = An. quadrimaculatus

Appendix 66b Poolled monthly distribution of adults reared from larval collections

	January	February	March	April	May	June	July	August
An. gambiae	21	23	7	6	5	128	144	138
An. funestus	46	76	79	83	97	52	40	60
An. maculipennis	0	0	0	0	0	0	0	6
An. quadrimaculatus	0	0	0	0	0	0	0	15
Total	67	99	86	89	102	180	184	219