### MOSQUITO SPECIES COMPOSITION, ABUNDANCE AND PHYSICOCHEMICAL FACTORS OF RICE FIELDS IN MINNA, NIGER STATE, NIGERIA

BY

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#### ABSTRACT

The mosquito species diversity and fitness of rice fields in Minna, Niger State Nigeria were studied. Four selected Rice fields located in Fadikpe, Bosso, Maitumbi and Chanchaga areas of Minna, were investigated for the Ecology of Rice field Mosquitoes, as a prerequisite for developing effective control measures. Mosquito Larvae and Water samples were concurrently collected in the sampled Habitats between August and October 2012, while Soil samples were collected in November same year. Immature mosquitoes recovered were reared to adults and identified in the Laboratory using Standard Morphological keys. Ten Mosquito species were encountered in the following order of decreasing abundance: Culex pipiens pipiens 651(21.9%)> Culex quinquefasciatus 421(14.2%)> Anopheles gambiae 400 (13.5%)> Anopheles funestus 334 (11.2%)> Culex restuans 297 (10.0%)> Anopheles maculipalpis 187(6.3%)> Anopheles quardrimaculatus 178(6.0%)> Aedes dorsalis 173 (5.8%)> Aedes aegypti 169 (5.7%)> Aedes vexans 162 (5.5%), with species density significantly higher in rainy season than dry season. The results of water quality analyses indicated different degrees of variations in physicochemical parameters of the Rice fields in different locations. Statistically, Temperature, pH, Turbidity, Alkalinity, Hardness, Phosphate, Ammonia, Carbon dioxide, Chloride, Dissolved Oxygen and Biochemical Oxygen Demand were not significantly different (P>0.05) among Rice fields. However, Conductivity, Sodium, Potassium, Nitrate varied significantly (P<0.05). The results of dry season Soil analyses revealed the following mean values of physicochemical parameters as follows; Nitrogen=0.14%, Phosphorus=5.6mg/kg, Sodium=600mg/kg, Potassium=442.5mg/kg, Conductivity=224µS/cm, pH=7.61, Organic matter=1.59%, Sand=62.1%, Clay=30.1% Silt=7.8%. Statistically, the soil physicochemical parameters varied significantly (P<0.05) among Rice fields. The fluctuating asymmetry of Rice field mosquitoes revealed a high vectorial fitness. Significant Correlations between Mosquito abundance and physicochemical parameters of breeding Habitats were recorded for all species in both rainy and dry seasons. The Mosquito species encountered in this study are of Public Health importance, and the findings of the study should guide implementation of adequate vector control in Rice fields.

# TABLE OF CONTENTS

Title	Page
Cover page	i
Title page	ii
Declaration	iii
Certification	iv
Dedication	v
Acknowledgement	vi
Abstract	viii
Table of Contents	ix
List of Table	xiii
List of figures	XV
List of Appendix	xvi
CHAPTER ONE	
1.0 INTRODUCTION	1
1.1 Background of the Study	1
1.2 Justification of the Study	3
1.3 Aim of the Study	4
1.4 Objectives of the Study	4
1.5 Significance of the Study	5
1.6 Scope and Limitations of the Study	5
CHAPTER TWO	

2.0 LITERATURE REVIEW	6
-----------------------	---

2.1 Mosquito Biology	6	
2.2 Mosquito Life Cycle	8	
2.3 Mosquito-Borne Diseases	10	
2.3.1 Filariasis	11	
2.3.2 Encephalitis	12	
2.3.3 Dengue Fever	13	
2.3.4 Yellow Fever	14	
2.3.5 Malaria	15	
2.4 Evolution, Taxonomy and Distribution of Mosquitoes	16	
2.5 Rice fields as Source of Mosquitoes and Mosquito-Borne Diseases.	20	
2.6 Effect of Rice Management Practices on Mosquito Vector Abundance.	22	
2.7 Influence of Physicochemical Characteristics of Breeding Habitat		
on the Distribution of Mosquitoes Species in Rice agro- Ecosystem	24	
2.8 Mosquito Diapause in Dry Season (Aestivation)	27	
2.9 Egg Diapause in Mosquitoes	28	
2.10 Vectorial Fitness of Mosquitoes	29	
CHAPTER THREE		
3.0 MATERIALS AND METHODS	32	
3.1 Study Area	32	
3.2 Selection of Sampling Sites and Periods of Sample Collection	32	
3.3 Mosquito Larval Collection and Rearing	33	
3.4 Identification of Mosquito Species	33	
3.5 Collection and Fixing of Water Samples for Physicochemical Analyses.	33	
3.6 Physicochemical Analyses of Water from Rice field Larval Breeding Habitats	34	

3.7 Determination of Wing Symmetry	40
3.8 Collection of Soil Samples to obtain Aestivating Mosquitoes.	41
3.9 Physicochemical Analyses of Soil Samples.	41
3.10 Statistical Analysis	43
CHAPTER FOUR	
4.0 RESULTS AND DISCUSSIONS	44
4.1 Species Composition, Relative Abundance and Spatial Distribution of Mosquitoes in Rice fields	44
4.2 Physicochemical characteristics of rice fields mosquito breeding habitats.	51
4.3 Mean wing length (MWL) and Fluctuating asymmetry (FA) of regular and aestivating Mosquitoes in rice fields in Minna	57
4.4 Relationship between abundance of Mosquito genera in rice fields.	63
4.5 Relationship between mosquito abundance and physicochemical variables of rice field breeding habitats.	65
4.6 Relationship between wing length of breeding and aestivating mosquitoes in rice fields.	69
4.7 Relationship between wing length and physicochemical variables of rice fields breeding habitats	71
4.8 Discussion	75
CHAPTER FIVE	
5.0 CONLUSION AND RECOMMENDATIOS.	86
5.1 Conclusion	86
5.2 Recommendations	87
REFERENCES	88

## LIST OF TABLES

Table	Page
4.1.1 Species Composition, Relative Abundance and Spatial Distribution of Mosquitoes breeding in Rice fields' Larval Habitats in Minna during the Rainy Season of 2012	46
4.1.2 Species Composition, Relative Abundance and Spatial Distribution (Mean ±S of Mosquitoes in Rice fields' Larval Habitats in Minna during the Rainy Season of 2012	SE) 47
4.1.3: Species Composition, Relative Abundance and Spartial Distribution of Mosquitoes Aestivating in Rice fields in Minna during the Dry Season of 20	012 49
<ul> <li>4.1.4: Species Composition, Relative Abundance and Spatial Distribution (Mean ±SE) of Mosquitoes in Rice fields Larval Habitats in Minna during the Dry Season of 2012</li> </ul>	50
4.2.1 Mean physicochemical Characteristics of Rice fields Mosquito Larval Habita Minna during the Rainy Season of 2012	ats in 52
4.2.2: Physicochemical Characteristics (Mean ±SE) of water in Rice fields Mosquito Larval Breeding Habitats in Minna during the Rainy Season of 20	)12 52
4.2.3 Physicochemical Characteristics of Soil Samples from Rice field Mosquito Breeding Habitats in Minna during the Dry Season of 2012	55
4.2.4 Physicochemical Characteristics (Mean ±SE) of Soil Samples in Rice field Mosquito Breeding Habitats in Minna during the Dry Season of 2012	56
4.3.1: Mean wing length (MWL) and Fluctuating Asymmetry (FA) of Mosquitoes breeding in Rice field in Minna during the Rainy Season of 2012	58
4.3.2: Fluctuating Asymmetry (FA) of wings (Means ± SE) of Mosquitoes Breeding in Rice fields in Minna during the Rainy Season of 2012	59
<ul><li>4.3.3: Mean wing length (MWL) and Fluctuating Asymmetry (FA) of Mosquitoes Breeding in Rice field in Minna during the Dry Season of 2012</li></ul>	61

4.3.4	Fluntuating Asymmetry (FA) of wings (Means $\pm$ SE) of Mosquitoes Breeding in Rice fields in Minna during the Dry Season of 2012.	62
4.4.1	Correlation between Mosquito Species Abundance in Rice field Larval Habitats in Minna during the Rainy and Dry Seasons of 2012.	64
4.5.1	Correlation between Mosquito Abundance and Physicochemical properties of water in Rice fields Larval Breeding Habitats during the Rainy Season of 2012.	66
4.5.2	Correlation between Mosquito Abundance and Physicochemical properties of Soil in Rice fields Habitats in Minna during the Dry Season of 2012.	68
	Correlation between Wing length of Mosquitoes breeding in rice fields during rainy season and that of aestivating Mosquitoes in dry season of 2012.	70
4.7.1	Correlation between Wing Length and Physicochemical properties of Water in Rice fields Mosquito Larval Breeding Habitats in Minna during the Rainy Season of 2012.	72
4.7.2	Correlation between Wing Length and Soil Physicochemical properties of Rice field Mosquito Habitats in Minna during the Dry Season of 2012.	74

## LIST OF FIGURES

# Figure

# Page

2.1.1 Principal characteristics of mosquito identification	8
2.2.1 Life Cycle of Mosquitoes	10

## LIST OF APPENDIX

Appendix	Page
A: Mosquito species composition in rice fields in Minna, during the rainy season (September, 2012)	101
B: Mosquito species composition in rice fields in Minna, during the rainy season (October, 2012)	102
C: Species composition of mosquitoes aestivating in rice fields during the dry season (November, 2012)	103
D: Species composition of mosquitoes aestivating in rice fields during the dry season (December, 2012)	104
E: Species composition of mosquitoes aestivating in rice fields during the dry season (January, 2013)	105
F: Species composition of mosquitoes aestivating in rice fields during dry season (February, 2013)	106
G: Species composition of mosquitoes aestivating in Rice fields (March, 2013)	107
H: Species composition of mosquitoes aestivating in rice fields during the dry season (April, 2013)	108
I: Physicochemical characteristics breeding water (August, 2012)	109
J: Physicochemical characteristics of breeding water (September, 2012)	110
K: Physicochemical characteristics of breeding water (October, 2012)	111
L: Physicochemical characteristics of soil in breeding site during dry Season (NOV, 2012)	112
M: Physicochemical characteristics of soil in breeding site during the dry season (DEC, 2012)	113
N: Mean Wing Length of mosquitoes breeding in rainy season. (September, 2012)	114
O: Mean Wing Length of mosquitoes breeding in rainy season. (October, 2012)	115
P: Mean Wing Length of mosquitoes aestivating in dry Season (NOV, 2012)	116
Q: Mean Wing Length of mosquitoes aestivating in dry season (Jan, 2013)	117

R: Mean Wing Length of mosquitoes aestivating in dry season (Feb, 2013)	118
S: Mean Wing Length of mosquitoes aestivating in dry season (April, 2013)	119

#### **CHAPTER ONE**

#### 1.0 INTRODUCTION

#### **1.1** Background of the study

According to fossil evidences, it was estimated that mosquitoes originated in the early tertiary period, some 70 million years ago or even earlier (Al-Sariy, 2007). They are distributed throughout the world. However, the majority are found in the tropics and subtropics. The warmer temperature in the tropics allows them to be more active and the rainfall provides them with aquatic sites for larval and pupal stages (Service, 2004)

Mosquitoes are significant pests not only to humans but also to domestic livestock with potential fatal out come. Although control measures are been taken but mosquito bornediseases still thrive in many countries and cause millions of deaths (Tren and Bate, 2001). Mosquitoes are insect arthropods belonging to the Order *Diptera* and Family *Culicidae* with 3 sub families, namely; *Anophelinae, Culicinae,* and *Toxorhynchitinae*. About 43 genera and over 3,500 species of mosquitoes have already been described (Harbach, 2011)

Mosquitoes exploit all Kinds of lentic aquatic habitats for breeding, but Rice fields are amongst the most productive sites for mosquitoes. Rice agro- ecosystem perfectly fits the ecological requirement of mosquito vectors and specifically suitable for the pioneer species, members of the *Anopheles gambiae* complex (Al-Sariy, 2007). Water quality of aquatic habitats is an important determinant of female mosquito oviposition and successful larval development (Joseph, 2007). Larval abundance reflects oviposition preference of female. Changes in physicochemical characteristics of Rice field water may create conditions that are either favourable or unfavourable to breeding success. This can have implication for the transmission of vector-borne diseases.

Mosquitoes sometimes go into their own form of off season aestivation as the rainy season winds down. Depending upon the species and sometimes climate, mosquitoes can successfully survive the unfavourable dry period in the egg, larval or adult stage. The resumption of direct development in the resting or diapausing eggs is commonly influenced by a variety of environmental factors such as the rise in temperature and oxygen levels or exposure to light (Ricci, 2001; Gyllostrom and Hansson, 2004; Vanderkhove, Decbrick,Bredock,Condeporcuna, Jeppesen and deMecster, 2005). In some cases, specific abiotic factors such as seasonal changes in physicochemical characteristics and biochemistry of the host soil can stimulate the resumption of direct development.

The Vectorial fitness of mosquitoes is greatly influenced by its breeding ecology (Olayemi, 2008). Rice Management practices certainly have an influence on larval development and adult body size of mosquitoes. Body size is a pivotal trait for mosquitoes, because it has been related to survival, blood feeding behavior, reproductive success and vectorial fitness (Agnew, Hide, Sidoboc, and Michalakis, 2002). Wing size reflects body size, which is related to survival and reproductive success (Carron, 2007). Adult body size is often correlated with higher vectorial fitness. Larger body size confers better fitness (Carron, 2007)

The diverse mosquito species occurring in African Rice agro- ecosystems have been scarcely studied, despite the strong link between irrigated rice cultivation and mosquito-borne diseases (Muturi, 2007). This no doubt left farmers with little or no knowledge of

the potential health hazards' associated with mosquitoes arising from flooded rice fields. However, the knowledge of mosquito species, occurrence and distribution is an essential component of vector ecology and a guiding principle to the formulation and implementation of integrated vector management program (Ephantus, 2008).

As in other parts of the country, Rice fields in Minna constitutes potential breeding site for various mosquitos' species (Olayemi, 2008). Rainfall and high temperature in the city area favours the bionomics of mosquitoes. Although, vector control is a major component of the Global Malaria Control Strategy (GMCS) and still remains the most effective measure to prevent Malaria transmission (Oruganje, Alaribe, Oduola, Adeogun, and Awoola, 2011). Successful application of vector control measures in a given location requires the understanding of the bionomics of the mosquito species responsible for the disease transmission, including the correct and precise identification of the target species and its distribution. Unfortunately the correct and precise identification and contribution of rice fields to the mosquito diversity and its physicochemical characteristics are yet to be determined from Minna in the North Central region of Nigeria.

#### **1.2 Justification of the Study.**

Irrigation is a key factor to enhance crop production, but it often results in negative health outcomes and consequential to the increased frequency and transmission dynamics of water-related vector-borne diseases. The widely practiced system of growing rice in mostly stagnant water, inevitably results in expansion of mosquito larval habitats, specifically suitable for *Anopheles* species. This is true in many parts of Nigeria, especially Minna in the North Central Zone as Malaria is strongly associated with water logging, poor maintenance of irrigation systems and rice cultivation. Similarly, the use of herbicides and insecticides to protect the crops from weeds and rice pests as well as the use of fertilizers to improve yield, inevitably results in changes in surface water quality and physicochemical parameters of breeding habitats, which affects the survival and development of Mosquitoes. However, studies on mosquito vectors in Nigeria, are not only few but limited to vector control measures which are further confounded by the presence of different ecological zones that support the breeding of diverse mosquito species. Unfortunately, there have been little or no entomologic studies to assess the impact of rice cultivation on mosquito production and disease transmission. This study seeks to fill the gap in the knowledge of mosquito bionomics, species composition and density of breeding and aestivating mosquitoes in the wetland rice agro ecosystem of Minna in north central Nigeria.

#### **1.3** Aim of the Study

The aim of this study is to elucidate the ecology of Rice fields Mosquitoes, as a prerequisite for developing effective control strategies.

#### **1.4** Specific Objectives of the Study

- a. To determine species composition, relative abundance and spartial distribution of mosquitoes breeding in rice fields in Minna during the rainy season.
- b. To determine species composition, relative abundance and spatial distribution of mosquitoes aestivating in rice fields in Minna during the dry season.
- c. To determine the body size and wing symmetry of both breeding and aestivating mosquitoes in rice fields in Minna.
- d. To determine the physicochemical characteristics of rice field mosquito larval habitats in the area.

e. To determine the physicochemical properties of soil samples from rice field mosquito habitats.

#### **1.5 Significance of the Study**

The diverse mosquito species breeding in Nigerian rice fields are scarcely studied despite the strong link between rice cultivation and mosquito-borne diseases. The outcome of this research will provide public health stakeholders with adequate knowledge of the potential health hazards associated with mosquitoes arising from flooded Rice fields and reduce the high health and socioeconomic burdens of mosquito borne-diseases. Understanding of mosquito species occurrence and fitness is an essential component of vector ecology and a guiding principle to the formulation and implementation of integrated vector control strategies.

#### **1.6 Scope and Limitations of the Study**

The study is limited to rice fields' mosquito larval breeding habitats during the rainy and dry seasons. Larval collection and water quality analyses were conducted only during the rainy season in the various sampling locations. Hatching and rearing of aestivating mosquitoes and the analyses of soil physicochemical properties were conducted during the dry season only. Species composition and relative abundance were determined at adult stage.

#### **CHAPTER TWO**

#### 2.0 LITERATURE REVIEW

#### 2.1 Mosquito Biology

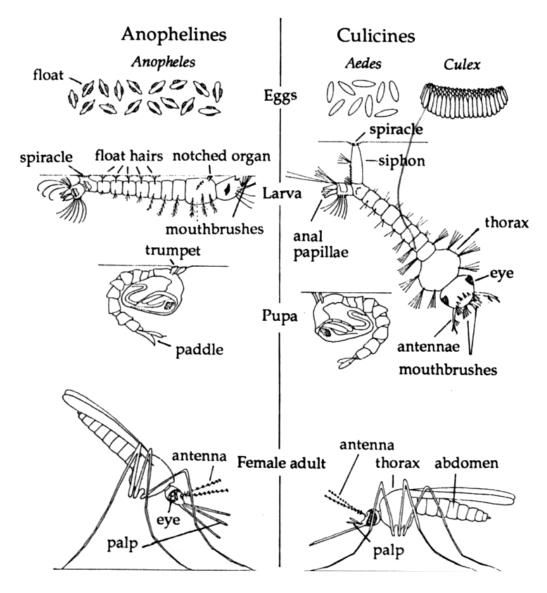
The word mosquito is from the Spanish and Portuguese for "little fly (mosca) and diminutive (ito)" (Lesley, 1993). Superficially, mosquitoes resemble crane flies (Family *Tipulidae*) and chironomid flies (Family *chiromidae*), as a result observers seldom realize the important differences between the members of the respective Families (Fang, 2010). Biologists however used certain distinguishing characters to identify mosquitoes (Fig.2.1.1).

All mosquitoes must have water to complete their life cycle. The water range in quality from melted snow to sewage effluent and it can be in any container imaginable (Williams, Leach, Wilson, and Swart, 2008). The feeding habit of mosquitoes is quite unique in that it is only the adult female that bites man and other animals. The male feeds only on plant juices. Some female mosquitoes prefer to feed on only one type of animal or a variety of animals. Females mosquitoes feed on man, domesticated animals, such as cattle, horses, goats etc; all types of birds including chickens; all types of wild animals including deer, rabbits, and they also feed on snakes, lizards, frogs and toads (Service, 2004).

The flight habit of mosquitoes depends on the species. Most domestic species remain close to their point of origin while some species known for their migration habits are often an annoyance far from their breeding place. The flight range of female is usually longer than that of males. Mosquitoes stay within a mile or two of their source. However, some have been recorded as far as 75 miles from their breeding source (Fang, 2010).

The length of life of the adult mosquito usually depends on several factors; temperature, humidity, sex of the mosquito and time of the year. Most males live a very short time, about a week, and females live about a month depending on the above factors (Mc Cafferty, 1983).

Mosquitoes are amongst the anthropophagic insects because they feed on human blood (Snow, 1990). Since the discovery of the link between mosquitoes and transmitting viruses, it has been a major priority to prevent the spread of diseases and to control mosquito populations (Medlock, Snow, and Leach, 2006). Mosquitoes are capable of breeding in a variety of environments. Many mosquitoes are generalists and choose a variety of oviposition sites, where as others are specialists and choose unique habitats for laying eggs (Rattanarithikul, Harbach, Harrison, Panthuzri, Jones and Colemann, 2005).



**Figure 2.1.1** Principal characters for mosquito identification. Source: (http://entomology.unl.edu/urbanent/mosquito.htm)

#### 2.2 Mosquito Life Cycle

Mosquitoes have four distinct stages in their life-cycle; eggs, larva, pupa, and adult (Fig 2.2.1). The female usually mate only once but produces eggs at intervals throughout their life. In order to be able to do so, most female mosquitoes require blood meal. The digestion of blood meal and the simultaneous development of eggs take about 2-3 days in the tropics but longer in the temperate zones (Crans, 2004). The gravid females search for

suitable places to deposit their eggs, after which another blood-meal is taken and another batch of eggs is laid. Depending on the species a female lays between 30 - 300 eggs at a time (Clement, 1992). Many species lay their eggs directly on the surface of water, either singly (i.e. *Anopheles*) or stuck together in floating rafts (i.e. *Culex*). In the tropics, the eggs usually hatch within 2-3 days.

Some species (i.e. *Aedes*) lay their eggs just above the water level or on wet mud; these eggs hatch only when flooded with water (Clement, 1992; WHO, 2006). Once hatched, the larvae do not grow continuously but metamorphose in four different instars. The fist instars measures 1.5mm in length, while the forth instance is about 8.10mm (Al-Sariy, 2007). Mosquito larvae feed on yeast, bacteria and small aquatic organisms (Noris, 2004). However, a few non-vector mosquitoes can be predaceous. Most mosquito larvae have a siphon located at the tip of the abdomen through which air is taken in and out to the water surface to breath. They dive to the bottom for short period in order to feed or escape danger (Rattanarithikul *et al.*, 2005). In Warm climate, the larval period last about 4-7 days or longer, if there is shortage of food (Becker *et al.*, 2010). The full-grown larva then metamorphoses into a comma-shaped pupa. When mature, the pupal skin splits at one end and a fully developed adult mosquito emerges. In the tropics, the pupal period last 1-3 days. The entire period from egg to adult takes about 7-13 days, under favourable condition (Clement, 1992, World Health Organization, 2006).

Studies have suggested that female may be able to detect the level of predation or potential competition from other mosquitoes in aquatic habitats, which affect where eggs are laid (Williams *et al.*, 2008). After the female mosquito has laid her eggs, the cycle is repeated.

19

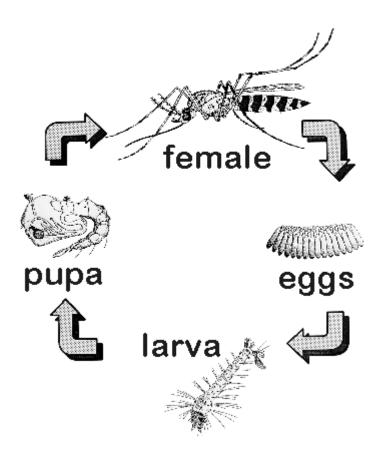


Figure 2. 2.1: Life cycle of a mosquito

Source: (http://www.mosquitoes.org/LifeCycle.html)

#### 2.3 Mosquito-Borne Diseases.

Mosquitoes are important vectors (agents) in the transmission of diseases. Mosquito-borne diseases involve the transmission of viruses and parasites from animal-to-animal, animal-to-person or person-to-person, without the insect catching the disease themselves (Crosby, 2005). Mosquitoes are estimated to transmit disease to more than 700 million people annually in Africa, South and Central America and much of Asia with millions of resulting deaths. But in temperate and developed countries, mosquito bites are now mostly an irritating nuisance, with some deaths each year (Fradin, 2006). Mosquitoes are known to carry many infectious diseases from several different classes of micro organisms, including

Viruses and Parasites. Mosquito-borne illnesses include; Filariasis, Encephalitis, Dengue fever, Yellow fever, and Malaria.

#### 2.3.1 Filariasis

Filariasis is a parasitic disease (usually an infectious tropical disease) that is caused by thread like nematodes (round worm) belonging to the super family *Filarioidea*, also known as "filariae" (Center for Disease prevention and Control, 2012). These are transmitted from host to host by blood feeding arthropods, mainly black flies and mosquitoes.

Eight known filarial nematodes use human as their definitive hosts. These are divided into three groups according to the niche they occupy within the body.

- Lymphatic Filariasis is caused by the worms' *Wuchereria bancrofti, Brugia Malayi* and *Brugia timori*. These worms occupy the lymphatic system including the lymph nodes. In chronic cases these worms leads to the disease *Elephantiasis*.
- Subcutaneous Filariasis is caused by Loa loa (the eye worm) Mansonella streptocerca, and Onchocerca volvulus. These worms occupy the subcutaneous layer of the skin in the fat layer. Loa loa causes loa loa Filariasis, while Onchocerca volvulus causes River blindness.
- Serous cavity Filariasis is caused by the worms *Mansonella persitans* and *mansonella orzardi* which occupy the serous cavity of the abdomen.

About 120 million people are affected by lymphatic Filariasis worldwide (Palumbo, 2008). The most spectacular symptoms of lymphatic Filariasis is Elephantiasis – edema with thickening of the skin and underlying tissues which was the first disease discovered to be transmitted by mosquito bites (Wrenger, Schettert, and Liebau, 2013). Filariasis is considered endemic in tropical and subtropical regions of Asia, Africa, Central and South America and Pacific Island nations, with more than 120 million people infected and one billion people at risk of infection (The Carter Center, 2002). Filariasis is considered endemic in 73 countries, 37 of these are in Africa (The Carter Center, 2002).

#### 2.3.2 Encephalitis

Encephalitis is an acute inflammation of the brain. Encephalitis with Meningitis is known as *Meningoencephalitis*. Symptoms include Headache, Fever, Confusion, Drowsiness, and Fatigue. More advanced and serious symptoms include Seizures, or Convulsion, Tremors, Hallucinations and Memory problem.

Viral Encephalitis can occur either as a direct effect of acute infection or as one of the sequelae of a latent infection. The most common causes of acute Viral Encephalitis are Rabies virus, Herpes simplex, Poliovirus and measles virus. Other causes include infection by Flavivirus such as *Japanese encephalitis* virus, *St louis encephalitis* virus, West Nile virus or by *Togaviridae* such as Eastern Equine *encephalitis* virus, Western Equine *encephalitis* virus or Venezuelan *encephalitis* virus (Solomon, Ooi, Beasley and Mallewa, 2003). The epidemiology of Flavivirus Encephalitis is governed by a complex interplay of climatic, entomologic, human behavioral, viral and host factors that are not completely understood (Solomon *et al.*, 2003)

Viruses are transmitted naturally among birds in enzootic cycles by bird-biting mosquitoes especially the *Culex* genus. Humans becomes infected inadvertently when they encroach on this cycle, but they are considered "dead-end" hosts because normally they do not have sufficiently higher or prolonged viraemia to transmit the virus further. However it becomes apparent that West Nile virus can be transmitted among humans through infected transplanted organs and blood products (Iwamoto *et al.*, 2003; Alpert, Fergerson, & Noel, 2003). The main vectors of Encephalitis includes; *Cx. tritaeniorhynchus, Cx. vishnui, Cx. pipiens pipiens, Cx. restuans, Cx. quinquafasciatus, Cx. tarsalis, Cx. annulirostris, Cx. gelidus,* and *Ae. normanensis.* The main vertebrate hosts includes; migratory birds e.g. Asiatic cattle egret (*Bubulcus ibis coramandus*), Domestic fowls, Pigs, Birds of the family *corvidae* (e.g. blue jays finches, blackbird), Pigeons, Sparrows, Herron and Feral pig (CDC,2004).

During the summer of 2002 and 2003, North America was affected by its largest ever outbreak of arboviral *Encephalitis*. West Nile virus caused 2,942 cases of Meningitis in 2002 with 276 deaths, and 2,866 cases in 2003 with 246 deaths (CDC, 2004)

#### 2.3.3 Dengue Fever

Dengue fever, also known as break bone fever is an infectious tropical disease caused by the dengue virus. Symptoms include Fever, Headache, Muscles and Joint pains and a characteristic skin rash that is similar to measles. In small proportion of cases, the disease develop, into the life threatening dengue hemorrhagic fever, resulting in bleeding, low level of blood platelets and blood plasma, where dangerously low blood pressure occur (Whitehorn, 2002).

Dengue is transmitted by several species of mosquito within the genus *Aedes*, principally *Aedes aegypti*. The virus is of four different types. Infection with one type usually gives lifelong immunity to that type, but only short time immunity to others (Reiter, 2010). Subsequent infection with a different type increases the risk of severe complication.

As there is no commercially available vaccine, prevention is sought by reducing the habitat and the number of mosquitoes and limiting exposure to bites. Dengue fever has become a global problem since the Second World War and is endemic in more than 110 countries. Apart from eliminating the mosquitoes, work is on going on a vaccine, as well as medication targeted at the virus (Scott, 2008).

#### 2.3.4 Yellow Fever

Yellow fever also known as yellow jack is an acute viral hemorrhagic disease (Schmal John and Mc Clain, 1990). The virus is a 40 to 50 nm enveloped positive sense RNA virus, the first human virus discovered (Brett, Thiel and Rice, 2007). The virus is transmitted by the bite of female mosquito *Aedes aegypti* and other species and is found in tropical and subtropical areas in South America and Africa but not in Asia (CDC, 2012). The only known host of the virus is primates and several species of mosquitoes.

Yellow fever presents in most cases in humans with Fever, Chills, Anorexia, Nausea, Muscles pain (with prominent backache) and Headache, which generally subsides after several days.

In some patients, toxic phase follows in which liver is damage with jaundice and lead to death (Old Stone, 2002). Because of increased bleeding tendency (bleeding diathesis), yellow fever belong to the group of hemorrhagic fevers. The World Health Organization(W.H.O) estimated that yellow fever cause 200,000 illnesses and 30,000 deaths every year in unvaccinated populations (WHO, 2006) and nearly 90 percent of the infections occur in Africa (Tolle, 2009).

24

#### 2.3.5 Malaria

Malaria is a mosquito-borne disease of humans and other animals caused by Protists (a type of microorganism) of the genus *plasmodium*. It begins with a bite from an infected female *Anopheles* mosquito, which introduces the protists through saliva into the circulatory system. In the blood, the protists travel to the liver to mature and reproduce. Malaria causes symptoms that typically include Fever and Headache, which in severe cases can progress to Coma or death. The disease is widespread in Tropical and Sub Tropical regions in broad band around the equator, including much of Sub-Saharan Africa, Asia, and the Americans.

Five species of *Plasmodium* can infect, and be transmitted by humans. The vast majority of deaths are caused by *Plasmodium falciparum* and *plasmodium vivax, while plasmodium ovale* and *plasmodium malariae* cause a generally milder form of malaria that is rarely fatal (Fairhurst and Wellems, 2010). Malaria is prevalent in Tropical and Sub Tropical regions because rain fall, warm temperatures, and stagnant waters provide habitats ideal for mosquito lavae.

Disease transmission can be reduced by preventing mosquito bite by distribution of mosquito nets and insect repellents, or with mosquito control measures such as spraying insecticides and draining standing water (Nadjm and Behrens, 2012).

The World Health Organization estimated that in 2010, there were 219 million documented cases of malaria. That year, between 660,000 and 1.2million people died from the disease (Nayyar, Preman, Newson, and Hermington, 2012) many of whom were children in Africa. The actual number of deaths is not known certainly, as accurate data is unavailable in many

rural areas, and many are undocumented. Malaria is commonly associated with poverty and may also be a major hindrance to economic development.

#### 2.4 Evolution, Taxonomy and Distribution of Mosquitoes

Based on fossil evidences, it was estimated that mosquitoes originated in the early tertiary period, some 70 million years ago or even earlier (Al-Sariy, 2007). The oldest known mosquito with an anatomy similar to modern species was found in 79-million-year-old Canadian amber from the crustaceous (Poinar, 2000). An older sister species with more primitive features was found in amber that was 90 – 100 million years old (Berkent and Grimaldi, 2004). Genetic analyses indicated that the *Culicinae* and *Anophelinae* clades may have diverged about 150 million years ago (Calvo, Pham, Marinostti, Anderson and Ribeiro, 2009) The Old and New World *Anopheles* species are believed to have subsequently diverged about 95 million years ago The mosquito *Anopheles gambiae*, is currently undergoing speciation into M & S molecular forms (Calvo *et al.*, 2009). This means that, some pesticides that work on the M form will not work on the S form (Favia, Lanfrancotti, Spanos, Siden- Kiamos and Louis, 2001).

Mosquitoes are insect arthropods belonging to the order *Diptera* and family *Culicidae* with 3 genera namely; *Anophelinae*, *Culicinae*, and *Toxorhynchitinae*. Harbach (2008) outlined the scientific classification of mosquitoes as thus;

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Diptera
Suborder	Nematocera

Infra orderCulicomorohaSuperfamilyCulicoideaFamilyCulicidaeSubfamiliesAnophilinaeCulicinaeToxorhynchitinae

Over 3,500 species of the *Culicidae* have already been described (Harbach, 2011). They are generally divided into two Subfamilies which in turn comprise some 43 genera. These figures are subject to continual change, as more species are discovered, and DNA studies compel the rearrangement of the taxonomy of the family.

The two main subfamilies are the *Anophilinae* and *Culicinae*, with their genera as outlined by (Harbach, 2008) thus;

Subfamilies and genera

- Anophelinae
  - Anopheles
  - Bironella
  - Chagasia.
- Culicinae
  - Aedeomyia.
  - Aedes
  - Armigeres

- Ayurakitia
- Barachinda
- Coquilletidia
- Culex
- Culiseta
- Deinocerites
- Eretmapodites
- Ficalbia
- Galindomyia
- Haemagogus
- *Heizmann*ia
- Hodgesia
- isostomyia
- Johnbelkinia
- Kimia
- Limatus
- Lutzia
- Malaya
- Mansonia
- Maorigoeldia
- Mimomyia
- Onirion
- Opifex

- Orthopodomyia
- Psorophora
- Runchomyia
- Sabethes
- Shannoniana
- Topomyia
- Toxorhynchites
- Trichoprosopon
- Tripteroides
- Udanya
- Uranotaenia
- Verrallina

Mosquitoes are distributed throughout the world. Some species exist at altitudes of >14,000 feet, while others can inhabit mines that are 3,760 feet below the sea level (Al-Sariy, 2007). Species range in latitudes northward from the Tropics to Arctic regions and southward to the end of the continents. A wingless species have been reported to exist in Antarctica, while many species do exist in the remote desert (Al Sariy, 2007). However, majority of mosquitoes are found in the tropics and subtropics. The warmer temperatures in the tropics allow them to be more active and the rainfall provides them with aquatic sites for larval and pupal stages.

#### 2.5 Rice fields as Source of Mosquitoes and Mosquito-Borne Diseases.

Increased rice production inevitably results in expansion of mosquito larval habitats. About a quarter of the 60 odd Anopheles species listed as important vectors of human Malaria breed in Rice fields (Al-Sariy, 2007). Furthermore, Rice fields also produce important mosquito vectors of human Filariasis and Viral diseases. The relative abundance of mosquitoes breeding in Rice field varies extensively by season and spatially. This variation has been attributed to the different rice cultural practices (Ahmed, Shaalam, Abdoul Soud, Tripet and Al- Khedhairy., 2011). Although, the principal vector of Japanese Encephalitis (JE), *Culex tritaeniorhynchus* and other *Culicine* mosquitoes that can transmit this disease (i.e. Culex bitaeniorynchus, Culex epidesmus, Culex fuscocephala, Culex gelidus, Culex pseudovishnui, Culex sitiens, Culex vishnui and Culex whitmore (Ahmed et al., 2011) are able to breed in ground water habitat such as sunlit pools, roadside ditches, tidal marshes and low salinity, or man-made containers, their preferred major larval habitats are Rice fields (Al-Sariy,2007). Rice agro-ecosystem perfectly fits the ecological requirement of vectors and in fact Malaria and Japanese Encephalitis (JE) are important vector-borne diseases associated with rice production in developing countries (Al-Sariy, 2007).

In Africa, Rice fields provide a wide range of mosquito breeding habitats, specifically suitable for pioneer species, such as members of the *Anopheles gambiae* comlex, the main vector of malaria (Ahmed *et al.*, 2011). In Madagascar, (Mutero, Wekoyela, Githure, and Kondradsen, 2004) observed that Rice fields are efficient breeding places for Malaria vector *Anopheles gambiae*. The larvae were found in Rice-fields that do not have emergent vegetation and thus exposed to the sun.

In Texas and Arkansas, USA (Mutero *et al.*, 2004) reported that, Rice field and associated pastures represent a significant source of mosquito breeding sites for dark rice mosquito, *Psorophora columbiae* and the Malaria mosquito *Anopheles quadrimaculatus* and several other species in Arkansas.

In Malaysia, Al-Sariy (2007) found that *Anopheles campestric, Culex tritaeniorhynchus* and several other species of *Anopheles* and *Culex* commonly occur in Rice field.

In Indonesia, dominant rice field *Anopheline* mosquitoes are *Anopheles aconifus*, *Anopheles barbirostris*, and an increased prevalence of Malaria even leading to epidemics has been attributed to double rice cropping in a year (Service,2004).

In India, Sunish and Reuben (2001) observed 14 *Anopheline* and 15 *Culicine* species in Rice fields of Gujrat with *Anopheles subpictus* and *Culex vislinui* as the two dominant species. Amerasnighe *et al.*, (1995) reported that 26 species of mosquitoes were found in Rice fields of the dry zone in Eastern Province of Sri Lanka while Bambarademiya (2002) recorded 14 species in Rice fields in central zone.

The widely practiced system of growing rice in mostly stagnant water provides the habitat for mosquitoes that serve as vectors of Malaria, Filariasis, Arborviruses as well as the Snail, which serve as the intermediate host of Schistosomiasis. Over 40 viruses have been identified in studies on rice field agro ecosystems, but by far the most important is Japanes Encephalitis – JE (keiser *et al.*, 2002). The development of rice field very often leads to an increase in Malaria, Filariasis, or Arthropod-borne virus infections in humans. Irrigation is a key factor to enhance crop production system, but it often results in negative health outcomes and consequential to the increased frequency and transmission dynamics of water associated infectious diseases (i.e. Schistosomiasis) or water-related vector-borne diseases (i.e. Malaria) (Herrel *et al.*, 2001). Among the common mosquito-borne diseases of humans, Malaria and Filariasis have been the most common for centuries. This is true in many parts of the tropics and subtropics. Malaria was strongly associated with water logging, with poor maintenance of irrigation systems and with rice cultivation (Tren and Bate, 2001). The immature stages of mosquitoes are aquatic, and need water for their development. The practice of flooding Rice fields to grow the crop encourages of population of immature mosquitoes.

#### 2.6 Effect of Rice Management Practices on Mosquito Vector Abundance.

The Agricultural Research Service-ARS (2003) has estimated that 400 million tons of rice is grown annually in more than 145 million ha worldwide. Over 90 percent of the production occurs in Asia, while the remainder is divided among Latin America, Africa, Australia, Europe and the USA (Agricultural Research Service, 2003). Rice is one of the most important crops in the world, and it provides the main source of energy for more than half of the world population (Business Group International, 2001). According to the National Agricultural Statistics Service NASS (2006) the average yield per acre for all U.S rice is an estimated 6,636 pounds per acre. This high yield is achieved in part by the use of efficient management practices. Among these practices are the use of herbicides and insecticides to protect the crop from weeds and rice pest respectively, as well as the use of fertilizers to improve yield.

Ecological studies carried out by Lee (1998) to compare organically and conventionally farmed rice fields in Korea during the rice growing period revealed abundance of two

mosquitoes, *Anopheles sinensis* and *Culex tritaeniolynchus*, which were lower in the organically farmed rice field compared to conventionally farmed Rice fields. The application of Urea, a nitrogenous fertilizer in Rice fields, significantly increases the grain yield and the population densities of mosquito larvae and pupae (Dasrie and Ward, 2000). In Indian rice fields, synthetic nitrogenous fertilizers were found to be responsible for a significant increase in *Anopheline* and *Culicine* larvae populations (Victor and Reuben 2000). Fields treated with inorganic fertilizer (N.P.K) had significantly increased population densities of immature mosquitoes than field treated with organic manure (i.e. farm yard manure and green manure).

In Taiwan, studies on Ten Rice fields indicated that, the size of the mosquito population was mainly related to flooding and drying practices and application of insecticides against insect pests of rice (Al Sariy, 2007). It was also observed that, the manipulation of arable land for rice production has created another environment for *Anopheline* mosquitoes.

Similarly, results of a study by Mutero *et al.*, (2004) on the effect of Ammonium Sulphate fertilizer on mosquito larvae population in Rice fields showed a significant overall increase in the larvae population of *Anopheles arabiensis* and *Culicine* mosquitoes after ponds were treated with fertilizer. Significantly, more fourth instars larvae of *Anopheles arabiensis* were collected in fertilized plots than in control plots. It was also found that the first application had the most impact compared with second and third application. The studies suggested that, Ammonium Sulphate fertilizers reduce Turbidity of water in Rice fields, thereby making them visual and attractive for egg laying by *Anopheles arabiansis* and *Culicine* mosquitoes.

# 2.7 Influence of Physicochemical Characteristics of Breeding Habitat on the Distribution of Mosquitoes Species in Rice agro- Ecosystem

Large-scale environmental modification concomitant with processes such as forest clearing, irrigation development, human settlement and rice cultivation inevitably result in changes in surface water quality, and affect the survival of mosquito's species breeding in surface water habitat.

Mosquitoes exploit all kinds of lentic aquatic habitats for breeding, prevailing physicochemical parameters in these habitats are important factors for survival and development of mosquitoes. Larvae of Anopheles mosquitoes have been found to thrive in aquatic bodies such as Fresh water marshes, Mangrove swamp, Rice fields, Grassy ditches, the edges of streams and rivers and small temporary rain pools (Oyewole *et al.*, 2009). Many species prefer habitats with vegetation while some breed in open, sunlit pools. A few species breed in tree holes or leaf axils of some plants (C.D.C, 2004). However, high water current and flooding have been reported to lead to Anopheles species larvae death due to reduction in oxygen tension causing physical harm to larvae (Okogun, 2005). Water of near neutral pH 6.8 - 7.2 was found most optimal for the weakening of the egg shells for first instars stage to emerge (Okogun *et al.*, 2008). Various chemical properties of the larval habitat related to vegetation, ranging from pH, Optimum Temperature, Concentration of Ammonia, Nitrate and Sulphate have been found to affect larval development and survival (Mutero et al., 2004). Rice agro- ecosystem perfectly fit the ecological requirement of mosquitoes vectors and specifically suitable for pioneer species, members of the Anopheles gambiae complex (Al-Sariy, 2007).

Physicochemical and biotic characteristics of surface water habitats may create conditions either favourable or unfavourable to breeding success of mosquitoes, depending on the range of tolerance of different species. This can have implication for the transmission of vector-borne diseases, because habitat changes that favour potential vector species can ultimately lead to increased rate of parasite or pathogen transmission.

The attractiveness of gravid females for oviposition largely depends on the interactions between the physicochemical parameters and also on the availability of suitable water bodies (Filinger, Sonye, Killeen and Berker, 2004). Mosquitos' species differs in the type of aquatic habitats they prefer for oviposition based on location, the physicochemical conditions of water body, and the presence of potential predators (Shililu *et al.*, 2003; (Piyarantnea, Amerisinghe, and Konradsen, 2005) Physicochemical factors that influence oviposition, survival and the spartio-temporal distribution of mosquito species includes Salts, Dissolved organic and inorganic matter, Degree of eutrophication, Turbidity, Presence of suspended mud, Presence or absence of plants, Temperature, Light, Shade and Hydrogen ion concentration (Ephantus, 2008).

Several studies have examined the relationship between habitat characteristics and larvae abundance. In Sri Lanka, *Anopheles culicifacies* Giles was positively associated with light and vegetation (Piyarantnea *et al.*, 2005). In Venezuela, Salinity and Dissolved Oxygen were associated with the spatial distribution of *Anopheles aquasalis* Curry and *Anopheles Oswaldoi* Peryussu (Gillet, 2000). *Culex qunquefasciatus* larvae, in Peninsular Malaysia, were most abundant in polluted drains containing 1.0 to 2.0 g/liter of Dissolved Oxygen and 0.1 - 2.4 g/litre of soluble reactive Phosphate and 0.1 - 0.9 g/liter of Ammonia and Nitrogen (Hassan, Narayana, and Salmah, 1993).

In Africa, similar studies with Malaria vectors *Anopheles gambiae* Giles S.S. and *Anopheles arabiansis* Patton have yielded variable results. While some studies failed to detect any significant relationship between *Anopheles gambiae* S.L and environmental variables (Minakawa, Mutero, Githure, Becker and Guiyu, 1999), others have reported significant relationship. For example in Eritrea, *Anopheles arabiensis* was associated with shallow, clean water and sunlit habitats (Shililiu *et al.*, 2003) as were those reported for *Anopheles gambiae* S.S. in western Kenya. Gimnig, Ombok, Kamau, and Hawley (2001) recorded larvae in ponds at water temperatures of 25.7°C to 27.1°C, while Tuno *et al* (2005) gave a range of 31.6°C to 34°C as optimal for larvae development. Most observations noticed that the thermal death point is in the range of 41°C to 42°C. Average in water pH value of 7.2 to 9.3 with mean value of 8.2 has been recorded in harboring immature *Anopheles gambiae* and *Anopheles arabiensis*, but larvae have been found in natural water with pH reading as low as 4.0 (Gillet, 2000).

Physicochemical factors of Rice field agro- ecosystem also impacted significantly on temporal distribution and abundance of mosquito species (Muturi *et al.*, 2007) Nitrogenous fertilizer could enhance the mosquito larvae population in Rice field (Simpson and Roger, 1991). Sunish and Reuben (2001) found that the height of the rice plant, water temperature, dissolved Oxygen, Ammonia, and Nitrate Nitrogen strongly influence the abundance of immature mosquitoes in India. Application of synthetic Nitrogen fertilizers to the Rice field was followed by rise in concentration of Ammonia Nitrogen and subsequent increase in Nitrate Nitrogen level in the Rice field water which can increase the density of Mosquito larvae (Sunish and Reuben, 2002).

The question of physicochemical content of potential water has been investigated by several authors, but no precise conclusions were drawn beyond the fact that it can be highly variable.

#### 2.8 Mosquito Diapause in Dry Season (Aestivation)

Diapause is the primary factor synchronizing insect life cycle with seasonal changes in the environment. Diapause is the major factor regulating the timing of growth, development and reproduction, both before and after the period of dormancy. Kostal *et al*, (2004) defined diapauses as "a neurohormonally mediated, dynamic state of low metabolic activity. Associated with this, is reduced morphogenesis, increased resistance to environmental extremes, and altered or reduced behavior activity. Diapause occurs during genetically determined stage(s) of metamorphosis, and its full expression develops in a species-specific manner, usually in response to a number of environmental stimuli that precedes unfavourable conditions (Horie, Kanda, and Mochida, 2000). Once diapause begins; metabolic activity is suppressed even if conditions favourable for development prevail (Kostal, 2006).

In mosquitoes the environmental control of diapause have been investigated from the early 1960s coinciding with the beginning of intensive study in the field of Seasonality, Diapauses and Photoperiodism in insects (Blitvich, Rayms-Keller, Blair and Bearty, 2007). The majority of mosquito species enters and overwintering diapause as eggs (55 species), as adults about (30 species) and as larvae (16 species) (Delinger, 2002).

Some trends in the relationship between the diapausing stage and systematic position of mosquitoes are evident. Egg stage diapause is typical for *Ochlerotatus, Aedes,* and *Psorophora*. Adults' diapause occurs mainly in *Anopheles, Culex and Culiseta*.

Larval diapauses is encountered in distinct representatives of many genera such as *Anopheles, Ochlerotatus, Culiseta, Mansonia, Orthopodomyia, Toponyia, Tripteroides, Armigeres, Wyeomyia* and *Toxorhyhchites* (Blitvich *et al.*, 2007).

#### 2.9 Egg Diapause in Mosquitoes

Egg diapause is manifested as a long stable arrest of hatching even when environmental conditions are favourable for this process. Diapause is terminated as a result of the reactivation of development. Egg diapause is an adaptation to the seasonality of climatic conditions, an adaptation that promote successful survival in winter (winter diapauses) or summer (aestivation) (Bale, 2002). The winter egg diapause is typical for Mosquito species occurring in the temperate zone.

In addition to egg diapauses in mosquitoes, there is aseasonal quiescence, a state of inactivity induced by infavourable environmental conditions and which ceases shortly after exposure to adequate hatching stimuli (Missrie, 2002). Aseasonal quiescence is an adaptation to peculiar conditions of special larval habitats, such as tree and rock holes, or transient ground pools, where water level may be subjected to large and abrupt fluctuations. Such aseasonal quiescence results in an asynchronous hatching of eggs (Danks, 2001). Usually the first flooding induces hatching of the majority of the egg; where as the remainder of the eggs may hatch much later after subsequent flooding episode (Delinger, 2002).

38

Though egg diapause is quite distinct from aseasonal quiescence, in some cases it is very difficult to distinguish these phenomena, and it takes special experiments to do so (Danks, 2002). Egg diapauses may be obligate or facultative. Obligate diapause is common in monovoltine mosquito species such as *Ochlerotatus canadensis, Ochlerotatus hexadontus, Ochlerotatus squamiger, Ochlerotatus excrucians,* and *Ochlerotatus Communis* (Daink, 2003). It occurs spontaneously in each generation irrespective of environmental condition and last usually for a longer period, up to one year, from the end of the spring – beginning of summer to the spring of the next year (Danks, 2000).

On the contrary, facultative diapause is recorded in the multivoltive species (*Aedes vexans*, *Ochlerotatus caspius*, *Aedes cinereous* etc) (Danks, 2003). This diapause is control by environmental conditions, mainly photoperiod and temperature. Law temperature is usually responsible for diapause termination in both monovoltine and multivoltine species (Gillot, 2005). The ability to pass through adverse periods in diapauses helps mosquitoes to exploits seasonally fluctuating resources, to diversify in tropical habitats, and to allow them to colonize temperate and Polar Regions.

#### 2.10 Vectorial Fitness of Mosquitoes

The terms vectorial capacity, competence, and fitness are often used interchangeably to describe the ability of a mosquito to serve as a disease vector. Vectorial capacity is defined quantitatively and is influence by such variables as vector density and longevity as well as vector competence (Black and More (1996). Estimates of vectorial capacity take into account all the environmental, behavioural, cellular, and biochemical factors that influence association between a vector, the pathogen transmitted by the vector, and the vertebrate host to which the pathogen is transmitted (Beerntsen, 2000).

39

Both behavioural and environmental factors can play a decisive role in determining Vectorial fitness. For example, a particular mosquito species might be genetically and biochemically compatible for the complete development of a particular pathogen, but if this species does not coexist temporally and spatially with vertebrate host that harbors the parasite, or if the preferred blood source for this species does not include that vertebrate, the mosquito is not a suitable vector for the pathogen (Beerntsen, 2000).

Body size is a pivotal trait for mosquitoes, because it has been related to survival, blood feeding behavior, reproductive success and Vectorial fitness. The best measure of body size is assumed to be dry weight; therefore weight is used in many studies involving association with body size (Carron, 2007). However, the weight of an adult mosquito varies considerably and depends on whether the mosquito has recently had blood or sugar meal. For example, mosquito weight can sometimes give unreliable results due to different factors such as gravidity or recent intake of blood meal (Carron, 2007). To circumvent this problem, many researchers have used wing length as an indicator of body size. This approach is justified with the observation that wing length generally is correlated closely to a power function of body weight. Body size therefore confers better fitness to mosquitoes in a natural population. Wing length which is correlated with dry weight can be used as a proxy for body mass (Takken, Klowden, Chambers, 1998). At times of stress smaller larvae will not survive and hence adult size at emergence will be skewed. This is seen to happen in tree-hole mosquitoes as was the case for the univoltine temperate mosquito, Aedes cantans (Renshaw, Service and Birley, 1994).

It might be expected that mosquitoes occupying temporary, resource-limited habitat as do *Anopheles gambiae* would suffer such stress. Nevertheless, despite high mortality among

larvae, wing length distributions of both males and females *Anopheles gambiae* from Tanzania (lyimo and Takken, 1993) and Sa'o Tome are close to normal.

The size of the emerging adult is of importance as larger females survive longer and greater fecundity (Takken *et al.*, 1998). More over smaller and virgin females requires a second or third blood meal in order to develop mature eggs, prolonging the time of their first oviposition (lyimo & Takken, 1993). Adult size furthermore, affects host seeking behavior and parasite infectivity (Ameneshawa and Service, 1996). Intermediate-size mosquitoes were found to be more infectious to humans (Lyimo and Koella, 1992). Various biotic and abiotic factors affects the growth, development and survival of the immature mosquitoes and consequently affects their Vectorial fitness.

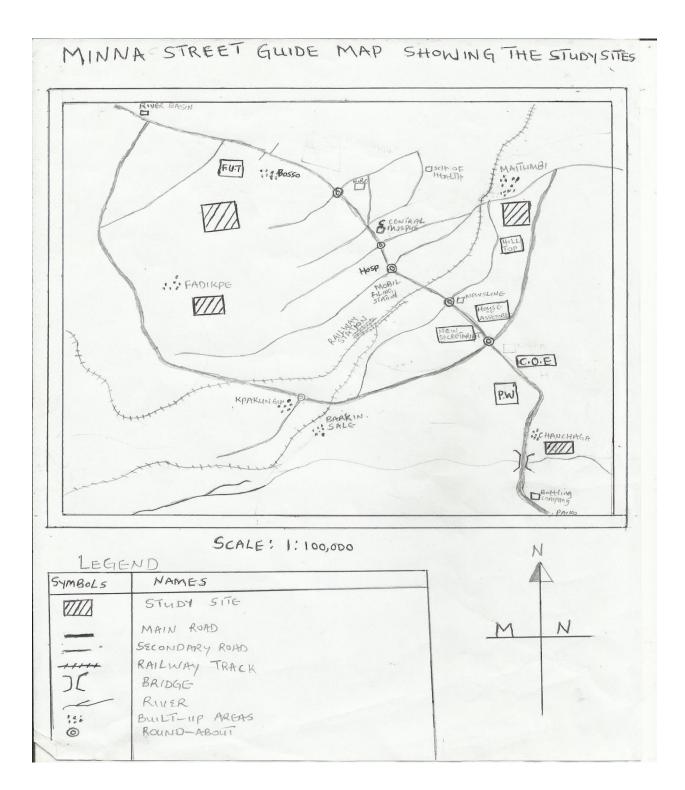
#### **CHAPTER THREE**

#### MATERIALS AND METHODS

#### 3.1 Study Area.

3.0

The study was carried out in Minna, the Capital of Niger State, North- central Nigeria. Minna, is located within longitude 6°33'E and latitude 9° 37'N, covering a land area of 88km<sup>2</sup> with an estimated human population of 1.2 million. The area has a tropical climate with mean annual temperature, relative humidity and rainfall of 30.20°C, 61.00% and 1334.00mm, respectively. The climate presents two distinct seasons; a rainy season between May and October, and a dry season between November and April. The vegetation in the area is typically grass dominated Savannah with scattered trees (Olayemi, 2008) The study covers four rice fields in four widely spaced sites located in Fadikpe, Bosso, Maitumbi and Chanchaga areas of the city. The ecotype of all these four sites is that of Fadama wetland rice agro-ecosystem with temporary stagnant fresh water pools of various -sizes constituted by rains. Green algae, short herbs, in addition to the growing rice crops are found in all the sites. Additionally, all the sites are rich in rotting organic materials.



#### **3.2 Selection of Sampling Sites and Periods of Sample Collection**

Sampling Sites (i.e. Rice fields) were randomly selected in May and June, 2012 prior to the inundation of Rice fields with water at the beginning of the rainy season. Four sampling stations were randomly selected in each rice field. Mosquito larvae were collected in each Sampling station in the four sites between August and October 2012, while soil samples were collected in Nov 2012 for hatching and rearing of aestivating of mosquitoes.

#### **3.3 Mosquito Larval Collection and Rearing**

Mosquito larvae were collected by dipping (using an improvised dipper 1.5 liters capacity) at depths of no more than 5cm at the varioys sampling stations between 08:00- 10:00 hours on each collection day. Larvae were transferred into large plastic buckets (5 liters size) to transport live larvae from the field to the laboratory. Total larvae number and larval instars were recorded within 24 hours of collection. Larvae were reared in white plastic bowls (2 liters size) in the laboratory of the Department of Biological Sciences, Federal University of Technology Minna. Each breeding site was represented with four replicate bowls covered with mosquito net to prevent emerging adult mosquitoes from escaping. Rearing was done according to the methods described by Pecor and Graffigan (1977) and Gerberg (1970). At the end of the day, emerging adults were counted and recorded. The adult were demobilised by spraying with insecticide (Raid) for identification.

#### **3.4 Identification of Mosquito Species**

The adult mosquitoes were carefully removed from the rearing bowls with a pair of forceps and identified under microscope, using standard morphological and Taxonomic keys, i.e (Gillet and Smith, 1972; Gordon and Lavoipierre, 1978 and Service, 2000).

#### **3.5** Collection and Fixing of Water Samples for Physicochemical Analyses.

Water samples were collected concurrently with larval sampling from the four Rice fields investigated, between 08:00 – 10:00hours at depths of about 5cm on each sampling day. From each sampling station, 125ml of water was collected to make 500ml per sampling site using 500ml capacity specimen bottles to ensure adequate representation. The water was fixed immediately using the procedures described by (APHA, 1999) in preparation for laboratory analysis. Water Temperature, pH and Conductivity were determined at the sites during larval collection using ordinary mercury thermometer and conductivity meter respectively.

#### 3.6 Physicochemical Analyses of Water from Rice field Larval Breeding Habitats

Water samples were analyzed for the following physicochemical parameters. Temperature, P<sup>H</sup>, Conductivity, Turbidity, Alkalinity, Hardness, Potassium (K), sodium (N), phosphate (PO<sub>4</sub>), Nitrate (NO<sub>3</sub>), Ammonia (NH<sub>4</sub>), Carbon dioxide (CO<sub>2</sub>) chloride (CL), Dissolved Oxygen (D.O) and Biochemical Oxygen Demand (B.O.D). Analyses of these parameters were carried out in Water Resources and Fisheries Technology (WAFT) Laboratory, Federal University of Technology, Minna, Nigeria.

TEMPERATURE: Temperature was determined using Mercury Thermometer (Model: Combo) manufactured by Hanna Instruments (P) Ltd in (<sup>0</sup>C). The instrument was immersed in thoroughly shaken water sample and the readings were (Ramteke and Moghe, 1988).

pH: pH was determined using pH meter (Model:Combo) manufactured by Hanna Instruments(P) Ltd. The pH meter was calibrated with buffer solutions and the instrument

was immersed in a well-mixed sample and readings were noted (Ramteke and Moghe, 1988).

CONDUCTIVITY: Conductivity was determined using conductivity meter (Model: Combo), manufactured by Hanna Instruments (P) Ltd. The Electrical Conductivity (EC in  $\mu$ S/cm) of the water sample was obtained by immersing the electrodes in a well-mixed sample (Ramteke and Moghe, 1988).

TURBIDITY: Turbidity was determined using Turbidity tube - manufactured by Jal-Tara in (JTU). A well-mixed sample is poured into the cleaned turbidity tube that was placed above the white sheet placed on the floor. The open end of the tube was observed to visualize the black markings from the distance of 7 to 10cm. The level of water at which the black mark was seen was noted.

ALKALINITY: Alkalinity was determined using titrimetric method. Standardization of sulphuric acid: About 50ml of 1 N sodium carbonate solution was taken in a conical flask and added few drops of methyl orange. Titrated against 0.1 N sulphuric acid until the colour changed from yellow to orange.

TOTAL ALKALINITY: Added 3-4 drops of methyl orange to the same sample. The solution turned yellow which was titrated against 0.02 N sulphuric acid until the colour changed to orange. The volume of sulphuric acid consumed was noted down (Sunil kumar and Shailaja, 1998). Total alkalinity calculated as,

TOTAL ALKALINITY = (ml\*N) of  $H_2SO_4*50*1000$ ml of sample taken HARDNESS: Hardness in( mg/l) was determined using titrimetric method. Total Hardness: To 25ml of the well-mixed sample taken in a conical flask, 2ml of buffer solution and 1ml of Sodium hydroxide was added. A pinch of eriochrome black T was added and titrated immediately against 0.01 M EDTA till the wine red colour changes to blue.

Total Hardness mg/l CaCO3 was then calculated as, (ml\*N) of EDTA \*1000 ml of sample taken

NITRATE: Nitrate was determined in(mg/l) using Spectrophotometer – PRIM Light and Advanced 70C10382 with LCD display manufactured by Secomam, France. About 50ml of standard, samples and blank (distilled water) were taken in separate crucibles and heated to dryness and cooled. The residue was dissolved in 2ml phenol disulphonic acid and the contents were diluted to 50ml in a nessler's tube. Added 6ml of liquid ammonia to develop a yellow colour and mixed the solution thoroughly. The colour developed was read at 40nm spectrophotometrically. The concentration of nitrate was noted (Trivedy and Goel, 1987).

PHOSPHATE: Phosphate was determined in( mg/l) using Spectrophotometer – PRIM Light and Advanced 70C10382 with LCD display manufactured by Secomam, France. About 50ml of samples, standard and blank (distilled water) were taken in a Nessler's tube. Added 2ml of ammonium molybdate solution and 5 drops of stannous chloride reagent. The tubes were mixed thoroughly and the intensity of blue colour obtained is proportional to the amount of phosphates and read spectrophotometrically at 690nm. The concentration

of phosphates was noted (APHA, 1985).

AMMONIA: Ammonia was determined in (mg/l) using Spectrophometer- PRIM Light and Advanced 70C10382 with LCD display, manufactured by Secomam, France. About 50ml of sampled water was filtered through whatman N0.42 and10.00ml of the filtered samples was pipette into a 50ml beaker and stirred. While stirring, 1 drop of MnSO4.H<sub>2</sub>O, 0.5ml oxidizing solution, and 0.6ml of Phenate solution were added. Allow for 15 minutes for maximum colour development. Carry 10.00ml of ammoniac free distilled water and 10.00ml of the 0.30mg/l solution of total ammonia nitrogen through the procedure. With the Spectrophotometer at630nm, set 0.0 absorbance (100% transmittance) with the reagent blank. The absorbance of the standard and samples were read.

CARBON DIOXIDE: Carbon dioxide was determined in (mg/l) using titrimetrc method. About 100ml of the sample was taken in a conical flask and added a few drops of phenolphthalein indicator. The solution was titrated against 0.05 N sodium hydroxide. The endpoint is the appearance of pink colour.

NOTE: The colour change to pink after the addition of phenolphthalein to the sample, but before titration indicates the absence of free carbondioxide (Trivedy and Goel, 1987).

Free CO<sub>2</sub>, mg/L = 
$$(ml*N)$$
 of NaOH \* 44 \* 1000  
ml of sample taken

CHLORIDES: Chloride was determined (mg/l) using titrimetric method. Standardization of silver nitrate: About 25ml of 0.0141 N sodium chloride was taken in a conical flask and 2ml of potassium chromate indicator was added. The solution was titrated against silver

nitrate until a brick red precipitate of silver chromate appeared. The volume of silver nitrate consumed was noted (Ramteke and Moghe, 1988).

Determination of chlorides in the sample: About 25ml of water sample was taken in a conical flask and 2ml of potassium chromate indicator was added.

The solution was titrated against standardized silver nitrate until a brick red colour precipitate of silver chromate started precipitating. The volume of silver nitrate consumed was noted down.

Chlorides, mg/L =  $(\underline{ml^*N})$  of AgNO<sub>3</sub> \* 35.5 \* 1000 ml of sample taken

DISSOLVED OXYGEN: Dissolved Oxygen was determined in (mg/l) using Winkler's Iodimetric method. Standardization of sodium thiosulphate: About 100ml of boiled, cooled distilled water was taken in 500ml standard flask. Added 3 g of potassium iodide and 2 g of sodium bicarbonate and mixed well. Added 6ml of concentrated hydrochloric acid and pipetted out 25ml of 0.1 potassium dichromate solution. Covered the flask with a watch glass and kept in the dark for 5 minutes. Made up the volume to 250ml with boiled, cooled distilled water. Titrated against sodium thiosulphate until the solution changed yellowish green in colour. 1ml of starch was added. The titration was continued until the colour changes from blue to light green.

Determination of Dissolved Oxygen: The sample was collected in 125ml BOD bottle carefully without allowing air bubbles. Added 1ml of manganous sulphate and 1ml of alkali iodide – azide reagent. A brown precipitate of basic manganic oxide formed was allowed to settle. Added 1ml of concentrated sulphuric acid and mixed well until the precipitate dissolved. About 25ml of the solution was taken and titrated against sodium thiosulphate

until a straw yellow colour appeared. Few drops of starch indicator was added and titrated again until the blue colour disappeared (Manivasakam, 1997).

Dissolved oxygen, mg/L = (ml\*N) of sodium thiosulphate \* 8 \* 1000 V<sub>2</sub>[(V<sub>1</sub>-V)/V<sub>1</sub>]

Where,

V1 = Volume of sample bottle

V2 = Volume of contents titrated

V = Volume of MnSO4 and KI added (2ml)

BIOCHEMICAL OXYGEN DEMAND: BOD is determined by determining the dissolved oxygen of the water sample on the first day and same water was incubated at room temperature for 5 days in the dark before the titration for Oxygen using Winkler-azides method. B.O.D (mg/l) = D.O on day 1 - D.O on day 5

SODIUM AND POTASSIUM: Sodium and Potassium were determined in (mg/l) using Flame Photometric method. The compressor was switched on and the pressure of air was adjusted to 0.45 kg/cm<sup>2</sup>. The gas supply was switched on to maintain the air gas mixture to get a blue flame. The blue flame was adjusted into cone shaped and aspirated with distilled water. The sodium and potassium standards were mixed in equal proportion. The instrument was calibrated by curve-fit method by aspirating with series of standards of known concentration. The samples were then introduced and the readings were noted. The gas supply was put off followed by the air supply (Ramteke and Moghe, 1988).

Sodium, mg/L = Observed values \* Calibration factor

Potassium, mg/L = Observed values \* Calibration factor

#### **3.7 Determination of Wing Symmetry**

Both left and right wings of individual adult mosquitoes were removed carefully with forceps and mounted on a Microscope glass slide. The wings were measured using calibrated Dissecting Microscope.

#### **3.8** Collection of Soil Samples to obtain Aestivating Mosquitoes.

At the onset of the dry season (Nov. 2012) when all stagnant rain pools in the sampling sites (rice fields) had dried off, soil samples were collected by excavation at a shallow depth of about 5cm, 10cm and 15cm using local farm tool at each sampling station in the four sites. About 0.75kg of soil samples were transferred into plastic bowls and flooded with about 2 liters of borehole water to form a water layer – soil depth of about 5cm in the bowls. Each breeding site was represented with four replicate bowls covered with mosquito net to prevent emerging adults from escaping. The experiment was repeated Six times between Nov. 2012 and April 2013 in the laboratory of the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria.

#### 3.9 Physicochemical Analyses of Soil Samples.

Soil samples were analyzed for the following variables: Nitrogen (N), phosphorus (P), Sodium (N), Potassium (K), Conductivity, pH, Organic matter as well as the textual classes of the soil (i.e. sand, clay, silt). Analyses of these variables were carried out in the laboratory of Soil Science Department, Federal University of Technology, Minna, Nigeria. The quantity of mineral elements such as Nitrogen (N), Phosphorus (P), Sodium (Na), Potassium (K) as well as Organic matter content were determined in as described by Black *et al.*, (1965) and Basu (2011).

Soil texture was determined using the following formulas;

% sand=51(H<sub>1</sub>-2)  $\times$ 3 (T<sub>1</sub>-20)  $\div$ 51 $\times$ 100

% clay= (H<sub>2</sub>-2) ×3 (T<sub>2</sub>-20)  $\div$ 51×100

% silt =100-(% sand+% clay)

Where, H<sub>1</sub> and H<sub>2</sub> were hydrometer readings 1 and 2 respectively.

T<sub>1</sub> and T<sub>2</sub> were temperature readings 1 and 2 respectively.

Nitrogen was determined by Kjedhal method using the following formula;

 $N = \underline{\text{ml of } H_2SO_4 \times \text{normality of acid} \times 0.014 \times 100}$ Oven dry weight of the soil

Sodium and Potassium was determined using flame photometer.

Phosphorus was determined by Trough method using the following formula;

 $P (mg/kg) = \frac{Absorbance(reading) \times 0.16 \times 25 \times 25}{Atomic number of Phosphorus}$ 

Organic matter content of the soil was determined using the following formula;

= Organic carbon $\times$  Van Bemmelen factor

=Organic carbon×1.74.

#### **3.10 Statistical Analysis**

Data obtained were analyzed using SPSS Software (Version 20). The results of Species composition, Relative abundance and Spartial distribution of Mosquitoes, Physicochemical Variables of Rice field breeding habitats, as well as Wing length (MWL) and Fluctuating Asymmetry (FA), were analyzed using one-way ANOVA, and Duncan multiple range test was employed to separate their means, and P<0.05 was considered significant. The relationships between Mosquito abundance, Mosquito Wing length and Physicochemical Variables of breeding habitats, were compared and correlated using Paired Sampled t-test to determine the degree of association between the variables.

#### **CHAPTER FOUR**

4.0 RESULTS AND DISCUSSIONS

# 4.1 Species Composition, Relative Abundance and Spatial Distribution of Mosquitoes in Rice fields.

Table 4.1.1 presents detailed results of species composition, relative abundance and spartial distribution of mosquitoes breeding in rice fields during the rainy season. Ten mosquito species were encountered each of which make up in more than 5% of the total samples collected in the rainy season. *Aedes* (3 species), *Anopheles* (4 species) and *Culex* (3 species) were the genera represented. Of the three genera represented, *Culex* were the dominant mosquitoes with 1,369 (46%) followed by *Anopheles* 1,099 (37%) and then *Aedes* 504 (17%) out of the total 2,972 mosquitoes collected. However, the frequency of occurrence of the ten species occurred in the following order of decreasing abundance: *Culex pipiens pipiens* 651(21.9%)> *Culex quinquefasciatus* 421(14.2%)> *Anopheles gambiae* 400 (13.5%)> *Anopheles funestus* 334 (11.2%)> *Culex restuans* 297 (10.0%)> *Anopheles maculipalpis* 187(6.3%)> *Anopheles quardrimaculatus* 178(6.0%)> *Aedes dorsalis* 173 (5.8%)> *Aedes aegypti* 169 (5.7%)> *Aedes vexans* 162 (5.5%).

The distribution of mosquito species in rice fields varied considerably in Minna. *Aedes aegypti* occurred more frequently in Chanchaga, followed by Fadikpe, with equal presence in Bosso and Maitumbi. *Aedes dorsalis and Aedes vexans* also occurred more frequently in chanchaga than the rest areas with low abundance in Fadikpe and Bosso.

Bosso recorded higher abundance of Anopheles mosquitoes (i.e An. Funestus, An. quadrimaculatus and An.maculipalpis), while Chanchaga recorded lower abundance of

*An.quardrimaculatus* and *An.maculipalpis*. However, *An. gambiae* was highly encountered in Fadikpe with low abundance in Bosso.

The frequency of occurrence of *Culex pipiens pipiens* was high in Bosso, Chanchaga and Maitumbi. However, the range of abundance and distribution of *Culex* among the sampled sites was negligible.

Table 4.1.2 provides details of statistical analysis of species composition, relative abundance and spartial distribution (Mean±SE) of mosquitoes breeding in rice fields during the rainy season. Statistically, the distribution and relative abundance of eight out of the ten mosquito species was not significantly different (P>0.05) among the four rice fields larval habitats. This is however not true with *Aedes dorsalis* and *Anopheles quadrimaculatus* as their distribution and relative abundance varied significantly (P<0.05) among rice fields with Chanchaga and Maitumbi having the highest frequency of occurrence of *Aedes dorsalis* and *Anopheles quadrimaculatus* respectively.

Mosquitoes Species	FAD	BSO	MTB	CHG	SUM	MEAN	SD	SE
Aedes Aedes aegypti	46(27.2)* (37.4)**	36(21.3) (31.6)	36(21.3) (29.8)	51(30.2) (34.9)	169 (5.7)	42	7.55	3.78
Aedes dorsalis	36(20.8) (29.3)	40(23.3) (35.1)	45(26) (37.2)	52(30.1) (35.6)	173 (5.5)	43	6.90	3.45
Aedes vexans	41(25.3) (33.3)	38(22.6) (33.3)	40(24.7) (33.1)	43(26.5) (29.5)	162 (5.5)	40	2.15	1.15
Sub Total	123	114	121	146	504 (17)			
Anopheles Anopheles gambiae	107(26.8) (39.6)	84(21) (28.9)	106(26.5) (39.3)	103(25.8) (39.3)	400 (13.5)	100	10.80	5.40
Anopheles funestus	80(24) (29.6)	90(27) (30.9)	89(26.6) (32.2)	75(22.5) (28.6)	334 (11.2)	84	7.23	3.62
Anopheles quardrimaculatu	39(21.9) s(14.4)	53(29.2) (18.2)	50(28.1) (18.5)	37(20.8) (14.1)	178 (6.0)	45	7.64	3.82
Anopheles maculipalpis	44(24.6) (16.3)	65(34.8) (22.3)	31(16.6) (11.9)	47(25.1) (17.9)	187 (6.3)	47	14.00	7.00
Sub Total	270	291	276	262	1099 (37)			
Culex								
Culex Pipiens pipriens	150(23) (45.1)	155(23.8) (47.9)	167(25.2) (47.2)	179(27.5) (49.7)	651 (21.9)	74	4.90	2.45
Culex quinquaefasciatu	104(24.7) as (31.3)	97(23) (30)	108(25.7) (30.5)	112(26.6) (31.1)	421 (14.2)	105	6.40	3.20
Culex restuans	78(26.3) (23.5)	71(23.9) (21.9)	79(26.6) (22.3	69(23.2) (19.2)	297 (10.0)	74	4.9	2.45
Sub Total	332	323	354	360 (46)	1369			
Aggregate	725	728	751	768	2,972			

Table 4.1.1SpeciesComposition, Relative Abundance and Spatial Distribution of Mosquitoesbreeding in Rice fields' Larval Habitats in Minna during the Rainy Season of 2012

\* indicates spartial species percentage among sampling sites.

\*\*indicates species percentage of mosquito genera within sampling sites.

FAD=Fadikpe, BSO=Bosso, MTB=Maitumbi, CHG=Chanchaga

Mosquitoes species	FADIKPE	BOSSO	MAITUMBI	CHANCHAGA	AGGREGATE
Aedes Aedes aegypti	23.00±3.00ª	18.00±0.30ª	18.00±2.00ª	26.00±5.00ª	21.25±1.85
Aedes dorsalis	18.00±2.00 <sup>ab</sup>	20.00±2.00 <sup>ab</sup>	22.50±0.50 <sup>ab</sup>	26.00±2.00 <sup>ab</sup>	21.62±1.31
Aedes vexans	20.50±3.50 <sup>a</sup>	19.00±2.00 <sup>a</sup>	20.50±2.00ª	21.50±0.50 ª	50.00+-2.14
Anopheles Anopheles gambiae	53.50±5.50 <sup>a</sup>	42.00±1.00 <sup>a</sup>	5.00±3.00 <sup>a</sup>	51.50±0.50ª	50.00±2.14
Anopheles funestus	40.00±1.00 <sup>a</sup>	45.00±1.00 <sup>a</sup>	44.50±3.50 <sup>a</sup>	37.50±2.50 <sup>a</sup>	41.75±1.46
Anopheles quardrimaculati	19.50±0.50 <sup>b</sup> us	26.00±0.00 <sup>a</sup>	25.00±1.00 <sup>a</sup>	18.50±0.50 <sup>b</sup>	22.25±1.35
Anopheles maculipalpis	22.00±2.00 <sup>a</sup>	32.50±1.05 <sup>a</sup>	15.50±0.50 <sup>a</sup>	23.50±0.50 <sup> a</sup>	23.38±3.06
Culex Culex pipiens pipiens	75.00±9.00 ª	77.50±2.50 ª	83.50±2.50 ª	94.50±4.50 ª	82.63±3.48
Culex quinquaefasciat	52.00±6.00 <sup>a</sup> us	48.50±3.50 ª	54.00±3.00 <sup>a</sup>	56.00±2.00 <sup>a</sup>	52.63±1.81
Culex restuans	39.00±4.00 <sup>a</sup>	35.50±2.50 ª	39.50±1.50 ª	34.50±0.50 ª	37.13±1.25

 Table 4.1.2 Mosquito Species Abundance and Spatial Distribution (Mean ±SE) in Rice fields' Larval Habitats in Minna during the Rainy Season of 2012

Values followed by same superscript alphabets in row are not significantly different at P> 0.05 level of significance.

Table 4.1.3 shows species composition, relative abundance and spatial distribution of mosquitoes aestivating in rice fields in Minna during the dry season. The results obtained in this study shows similar trends to the one obtained during the rainy season. A total of 261 mosquitoes aestivated from the soil samples collected. Ten species were encountered and they occurred in the following order of decreasing abundance: *Cx. pipiens pipiens* 78(30%)> *An. gambiae* 6(23%)> *Cx. quinquefasciatus* 34 (13%)> *An.funestus* 32 (12.3%)> *Ae. aegypti* 20 (7.7%)> *An. maculipalpis* 10 (3.8%)> *An. quadrimaculatus* 9(3.4%)> *Cx. restuans* 8(3.1%)> *Ae. dorsalis* 6(2.3%)> *Ae vexans* 4(1.5%).

The aestivation of mosquito species in the breeding habitats is variable. For instance, *Aedes vexans* did not occurred in Bosso and Maitumbi. Similarly, there was no encounter with *Anopheles maculipalpis* and *Culex restuans* in Chanchaga and Maitumbi respectively. However, *Culex pipiens pipiens, Anopheles gambiae*, and *Anopheles funestus* occurred more abundantly in Chanchaga. Generally, *Aedes dorsalis* and *Anopheles quadrimaculatus* occurred in very low proportions in all the breeding habitats.

Table 4.1.4 provides details of statistical analysis for species composition, relative abundance and spartial distribution (Mean±SE) of mosquitoes aestivating in rice fields during the dry Season. The abundance of *Aedes* species were not significantly different (P>0.05) in the four rice fields. Same can be said for *Anophels funestus, Anopheles maculipalpis, Culex pipiens pipiens* and *Culex quinquefasciatus*. The densities of *Anopheles gambiae* and *Anopheles quardrimaculatus* varied significantly (P<.0.05) between Fadikpe and Chanchaga. *Culex restuans* in Fadikpe and Chanchaga were not significantly different (P>.0.05) but varied significantly (P<0.05) in Bosso and Maitumbi.

Mosquitoes Species	FAD	BSO	MTB	CHG	SUM	MEAN	SD	SE
Aedes aegypti	7(35)* (70)**	4(20) (80)	3(15) (75)	6(30) (54.5)	20 (7.7)	5	1.80	0.90
Aedes dorsalis	1(16.7) (10)	1(16.7) (20)	1(16.7) (25)	3(50) (27.3)	6 (2.3)	2	1.00	0.50
Aedes vexans	2(50) (20)	0(0) (0)	0(0) (0)	2(50) (9.1)	4 (1.5)	1	1.20	0.60
Sub Total	10	5	4	11	30 (11.5)			
Anopheles gambiae	15(2.5) (60)	11(18.3) (40.7)	12(20) (54.5)	22(36.7) (595)	) 60 (23)	15	4.90	2.45
Anopheles funestus	6(18.8) (24)	9(28.1) (33.3)	6(18.8) (27.3)	11(34.4) (29.7)	) 32 (12.3)	8	2.20	1.10
Anopheles quardrimaculati	2(22.2) ıs(8)	2(22.2) (7.4)	1(11.1) (4.5)	4(44.4) (10.8)	9 (3.4)	2	1.30	0.65
Anopheles maculipalpis	2(20) (8)	5(50) (18.5)	3(30) (13.6)	0(0) (0)	10 (3.8)	3	2.10	1.10
Sub Total	25	27	22	37	111 (42.5)			
Culex Pipiens pipiens	23(29.5) (60.5)	17(21.8) (58.6)	14(17.9) (66.7)	24(30.8) (75)	) 78 (30)	20	4.80	2.40
Culex quinquaefasciat	11(32.4) us (28.9)	10(29.4) (34.5)	7(20.6) (33.3)	6(17.6) (18.8)	34 (13)	9	2.40	1.20
Culex restuans	4(50) (10.5)	2(25) (6.9)	0(0) (0)	2(25) (6.3)	8 (3.1)	2	1.60	0.80
Subtotal	38	29	21	32	120 (46)			
Aggregate	73	61	47	80	261			

 Table 4.1.3: Species Composition, Relative Abundance and Spartial Distribution of Mosquitoes

 Aestivating in Rice fields in Minna during the Dry Season of 2012

\*indicates spartial species percentage among sampling sites.

\*\*indicates species percentage of mosquito genera within sampling sites.

FAD=Fadikpe, BSO=Bosso, CHG=Chanchaga, MTB=Maitumbi

Mosquito species	FADIKPE	BOSSO	MAITUMBI	CHANCHAGA	AGREEGATE
Aedes Aedes aegypti	1.17±0.17 <sup>a</sup>	0.67±0.21 <sup>a</sup>	0.50±0.22ª	1.50±0.56 <sup>a</sup>	0.96±0.18
Aedes dorsalis	0.17±0.17 <sup>a</sup>	0.17±0.17 <sup>a</sup>	0.17±0.17 ª	0.50±0.22 ª	0.25±0.09
Aedes vexans	0.33±0.21 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.33±0.21 ª	0.17±0.08
Anopheles					
Anopheles gambiae	2.50±0.34 <sup>ab</sup>	1.83±0.40 <sup>b</sup>	2.00±0.63 <sup>b</sup>	3.66±0.67 <sup>a</sup>	2.50±0.29
Anopheles funestus	1.00±0.26 <sup>a</sup>	1.50±0.34 <sup>a</sup>	1.00±0.26 <sup>a</sup>	1.83±0.31 <sup>a</sup>	1.33±0.16
Anopheles quardrimaculati	0.33±0.21 <sup>ab</sup>	0.83±0.30 <sup>a</sup>	0.50±0.22 <sup>ab</sup>	0.00±0.00 <sup>b</sup>	0.42±0.11
Anopheles maculipalpis	0.33±0.21 <sup>a</sup>	0.33±0.21 <sup>a</sup>	0.17±0.17 <sup>a</sup>	0.67±0.21 <sup>a</sup>	0.37±0.10
Culex					
Culex Pipiens pipiens	3.83±1.01 <sup>a</sup>	2.83±0.75 <sup>a</sup>	2.33±0.71 ª	4.00±1.59 <sup>a</sup>	3.25±0.52
Culex quinquaefasciat	1.83±0.60 <sup>a</sup> us	1.67±0.33 <sup>a</sup>	1.67±0.31 ª	1.00±0.37 <sup>a</sup>	1.42±0.21
Culex restuans	0.66±0.21 ab	0.17±0.17 <sup>a</sup>	0.00±0.00 <sup>b</sup>	0.40±0.24 <sup>ab</sup>	0.30±0.09

Table 4.1.4:Mosquito Species Abundance and Spatial Distribution (Mean ±SE) in Rice fieldsLarval Habitats in Minna during the Dry Season of 2012

Values followed by same superscript alphabets in a row are not significantly different at P>0.05 level of significance.

#### 4.2 **Physicochemical characteristics of rice fields mosquito breeding habitats.**

Tables 4.2.1 provide details of physicochemical conditions of breeding water in the various rice fields from which mosquitoes were collected. The results show that water samples from Chanchaga had the highest level of Ammonia (0.106 mg/l), but with equal values of Alkalinity and Phosphate in Maitumbi. The highest levels of Sodium (12.7mg/l), Dissolved Oxygen (D.O) (8mg/l) and Biochemical Oxygen Demand (B.O.D) (3.7mg/l) were also recorded in Maitumbi, but Bosso recorded the highest levels of pH (7.4), Conductivity (189.7µS/cm), Hardness (105mg/l) and Potassium (11.4mg/l). Turbidity (0.44 JTU), Nitrate (2.9mg/l), Chloride (28mg/l), and Carbon dioxide (CO<sub>2</sub>) (2.62mg/l) were very high in Fadikpe.

Table 4.2.2 provides results for statistical analysis of the physicochemical characteristics (Mean $\pm$ SE) of water in rice field's mosquito larval breeding habitats. Statistically, Temperature, pH, Turbidity, Alkalinity, Hardness, Phosphate, Ammonia, Carbon dioxide (CO<sub>2</sub>), chloride, Dissolved oxygen (D.O), and Biochemical Oxygen Demand (B.O.D) were not significantly different (P>0.05) among the four rice fields breeding habitats. However, the same cannot be said for the remaining physicochemical parameters that varied significantly (P<0.05) among rice fields.

Conductivity in Bosso was significantly higher than recorded in Fadikpe, Chanchaga and Maitumbi. Potassium in Maitumbi and Chanchaga was not significantly different (P>0.05), but differ significantly (P<0.05) in Fadikpe and Bosso.

Physicochemical Parameters FAI	DIKPE	BOSSO	MTB	CHG	SUM	MEAN	SD	SE
Temperature ( <sup>0</sup> C)	29.6	29.3	29.8	30.1	118.3	29.7	0.30	0.15
P <sup>H</sup>	7.1	7.4	7.1	7.3	28.9	7.2	0.15	0.08
Turbidity (J.T.U)	0.44	0.23	0.24	0.25	1.16	0.29	0.10	0.05
Conductivity (µS/Cn	n) 178	189.7	184.′	7 180.7	733.1	183.3	5.10	2.55
Alkalinity (mg/l)	117.8	116.9	118	118	470.7	117.7	0.50	0.25
Hardness (mg/l)	94	105	100.7	103.7	403.4	100.8	4.90	2.45
Phosphate (mg/l)	0.1	0.06	0.35	0.35	0.86	0.22	1.20	0.60
Sodium (mg/l)	11.3	12.3	12.7	10.2	46.5	11.6	1.10	0.50
Potassium (mg/l)	5.3	11.4	4.6	4.7	26	6.5	3.30	1.65
Nitrate (mg/l)	2.9	1.29	1.87	1.58	7.64	1.91	0.70	0.35
Ammonia (mg/l)	0.13	0.104	0.07	0.106	0.41	0.103	0.20	0.10
Carbon dioxide (mg/	1) 2.62	1.35	2.2	1.24	7.41	1.85	0.70	0.36
Chloride (mg/l)	28	20.7	24	27.8	100.5	25.1	3.50	1.75
Dissolve Oxygen (m	g/l) 7.3	7	8	7	29.3	7.3	0.50	0.25
Biochemical Oxygen demand (mg/l)	3.	2.7	3.7	3	12.4	3.1	0.40	0.20

<b>Table 4.2.1</b>	Mean physicochemical	Characteristics	of Rice	fields	Mosquito	Larval	Habitats	in
Minna during th	ne Rainy Season of 2012							

Note: 0C= degree celcius, JTU= Jackson Turbidity Unit,  $\mu$ S/cm= micromhos per centimeter, mg/l = milligram per liter.

Physicochemical Parameters.	FADIKPE	BOSO	MTB	CHG A	GREEGATE
Temperature (°C)	29.66±0.13 <sup>a</sup>	29.37±0.34 ª	29.80±0.129 <sup>a</sup>	29.87±0.49 ª	29.68±0.14
PH	7.07±0.13 <sup>a</sup>	7.37±0.18 <sup>a</sup>	7.07±0.09 <sup>a</sup>	7.27±0.49 <sup>a</sup>	7.19±0.07
Turbidity (J.T.U)	0.44±2.00 <sup>a</sup>	0.33±0.15 ª	$0.24\pm0.00^{a}$	0.25±0.01 <sup>a</sup>	0.79±0.51
Conductivity (µS/cm)	78.00±4.00 ab	189.66±2.33 <sup>a</sup>	184.66±2.19 <sup>ab</sup>	180.66±0.67 <sup>b</sup>	184.00±1.52
Alkalinity (mg/l)	117.17±2.17 <sup>a</sup>	116.80±3.43 <sup>a</sup>	118.33±4.41 <sup>a</sup>	118.33±3.33 <sup>a</sup>	118.66±1.54
Hardness (mg/l)	94.00±3.46 <sup>a</sup>	105.00±0.58 <sup>a</sup>	100.67±2.19 <sup>a</sup>	103.67±2.19 <sup>a</sup>	103.33±1.18
Phosphate (mg/l)	$0.05 \pm 0.00^{a}$	0.66±0.02 <sup>a</sup>	0.20±0.15 <sup>a</sup>	0.20±0.08 <sup>a</sup>	0.13±0.43
Sodium (mg/l)	11.30±0.76 <sup>ab</sup>	12.27±0.22 ª	12.70±0.64 ª	$10.20 \pm 0.05$ <sup>b</sup>	11.62±0.36
Potassium (mg/l)	5.30±13.12 <sup>ab</sup>	11.32±1.75 ª	4.58±0.05 <sup>b</sup>	4.69±0.05 <sup>b</sup>	7.26±1.14
Nitrate (mg/l)	2.90±0.37 ª	1.96±0.45 <sup>b</sup>	1.88±0.19 <sup>b</sup>	1.58±0.30 <sup>b</sup>	2.14±0.22
Ammonia (mg/l)	0.13±0.01 <sup>a</sup>	0.10±0.00 <sup>a</sup>	0.07±0.00 <sup>a</sup>	0.11±0.00 <sup>a</sup>	0.10±0.01
Carbon dioxide (mg/l)	2.64±0.49 <sup>a</sup>	1.35±0.21 <sup>a</sup>	2.18±0.02 <sup>a</sup>	1.24±0.09 <sup>a</sup>	1.73±0.17
Chloride (mg/l)	28.43±3.64 ª	20.78±3.09 ª	24.03±0.49 ª	27.80±040 ª	24.51±1.28
Dissolve Oxygen) (mg/l)	7.33±0.67 <sup>a</sup>	7.10±0.59 ª	8.00±0.58 ª	7.00±1.00 <sup>a</sup>	7.36±0.33
Biochemical Oxyg demand (mg/l)	gen 3.00±0.58 ª	2.67±0.33 <sup>a</sup>	3.67±0.33 <sup>a</sup>	3.33±0.33 ª	3.17±0.21

Table 4.2.2:Physicochemical Characteristics (Mean ±SE) of water in Rice fields Mosquito LarvalBreeding Habitats in Minna during the Rainy Season of 2012

Values followed by same superscript alphabets in rows are not significantly different at P > 0.05 level of significance.

Table 4.2.3 presents results of physicochemical characteristics of soil samples fields mosquito breeding habitats during the dry season. Deta. The highest levels of Potassium (270mg/kg), Organic matter (1.68%), and Percentage clay (59.0%) were recorded in Fadikpe, while Bosso recorded the highest level of Sodium (695mg/kg), Conductivity (395µS/cm), pH (8.21) and Percentage silt (12.4). Nitrogen (0.224), Phosphorus (17.95) and Percentage sand (67.9%) were higher in Chanchaga.

Table 4.2.4 provides results for statistical analysis of physicochemical characteristics (Mean±SE) of soil samples in rice fields' mosquito breeding habitats during the dry season. All the physicochemical parameters of the soil samples varied significantly (P<0.05) among the rice fields. For instance, Nitrogen, Phosphorous, Sodium, P<sup>H</sup> and Percentage silt varied significantly (P<0.05) in the four rice fields. The level of Potassium in Chanchaga and Maitumbi was not significantly different (P.>0.05), but varied significantly (P<0.05) in Fadikpe and Bosso. Conductivity in Bosso and Maitumbi differ significantly (P<0.05), but was not significantly different (P.>0.05) in Fadikpe and Chanchaga. There was no significant difference (P>0.05) in the level of organic matter in Bosso, Maitumbi, and Chanchaga, but they were significantly lower than recorded in Fadikpe. Percentage sand was not significantly different (P>0.05) in Chanchaga and Maitumbi, but varied significantly (P<0.05) in Fadikpe and Bosso. The same can also be said for Chanchaga and Maitumbi

Physicochemica Parameters	l FADIKPE	BOSO	MTB	CHG	SUM	MEAN	SD	SE
Nitrogen (%)	0.14	0.091	0.119	0.224	0.57	0.14	0.06	0.03
Phosphorous (Mg/kg)	0.35	2.40	1.60	17.95	22.3	5.6	8.20	4.10
Sodium (mg/kg)	505	695	690	510	2400	600	106.80	53.40
Potassium (Mg/kg)	270	103.5	235	230	1770	442.5	395.40	197.70
Conductivity (µS/cm)	165	395	123	214	897	224	119.70	59.85
рН	7.4	8.21	7.74	7.03	30.42	7.61	0.50	0.25
Organic matter (	(%) 1.68	1.63	1.57	1.48	6.36	1.59	0.08	0.04
Sand (%)	59.0	56.7	64.8	67.9	248.4	62.1	5.10	2.55
Clay (%)	31.3	30.9	29.8	28.5	120.5	30.1	1.60	0.80
Silt (%)	9.65	12.4	5.36	3.6	31.01	7.8	4.10	2.05

Table 4.2.3 Physicochemical Characteristics of Soil Samples from Rice field Mosquito BreedingHabitats in Minna during the Dry Season of 2012

Note: mg/kg = milligram per kilogram,  $\mu S/cm = micromhos$  per centimeter, % = percentage.

Physicoche Parameters.		BOSO	MAITUMBI	CHANCHAGA	AGGREGATE
Nitrogen (%	6) 0.14±0.00 <sup>b</sup>	0.09±0.00 <sup>d</sup>	0.12±0.00 °	0.22±0.00 <sup>a</sup>	0.14±0.02
Phosphorou (Mg/kg)	us 0.35±0.00 <sup>d</sup>	2.41±0.00 <sup>b</sup>	1.61±0.01 °	17.90±0.052ª	5.58±2.16
Sodium (m	g/kg) 504.67±0.33	3° 69.20±0.35 <sup>d</sup>	691.00±0.58 <sup>a</sup>	510.67±0.67 <sup>b</sup>	443.88±69.02
Potassium (Mg/kg)	270.00±3.51ª	103.83±0.33 b	235.67±0.67 <sup>b</sup>	231.00±0.58b	209.38±8.86
Conductivit (Us/cm)	ty 165.00±1.15 <sup>b</sup>	385.67±8.35ª	82.38±41.13°	215.00±0.58b	212.01±34.61
P <sup>H</sup>	7.44±0.10°	8.19±0.04ª	7.76±0.01 <sup>b</sup>	7.06±0.02 <sup>d</sup>	7.59±0.02
Organic matter(%)	1.68±0.03ª	1.59±0.03 <sup>b</sup>	$1.57 \pm 0.01^{b}$	1.52±0.03 <sup>b</sup>	1.59±0.02
Sand (%)	58.70±0.35 <sup>ab</sup>	55.6±0.24 <sup>b</sup>	64.53±0.17 <sup>a</sup>	$67.9 \pm 1.88^{a}$	62.16±1.91
Clay (%)	30.97±0.28 <sup>a</sup>	30.87±0.03ª	29.60±0.11 <sup>b</sup>	29.03±0.58 <sup>b</sup>	30.12±0.29
Silt (%)	9.57±0.08 <sup>b</sup>	12.50±0.06ª	5.38±0.02°	$3.47 \pm 0.13^{d}$	7.73±1.06

Table 4.2.4Physicochemical Characteristics (Mean ±SE) of Soil Samples in Rice field MosquitoBreeding Habitats in Minna during the Dry Season of 2012

Values followed by same superscript alphabets in a row are not significantly different at P> 0.05 level of significance.

### **4.3** Mean wing length (MWL) and Fluctuating asymmetry (FA) of regular and aestivating Mosquitoes in rice fields in Minna.

Table 4.3.1 shows the mean length of wings measured from mosquito genera collected from the four rice fields during the rainy season. *Aedes* mosquitoes are larger in size than *Anopheles* and *Culex*, with Fadikpe producing larger *Aedes* mosquitoes (MWL 3.1). Although *Anopheles* and *Culex* had a better fitness than *Aedes* because their fluctuating asymmetry is perfect or near perfect (i,e 0.0 or 0.1). *Culex* was better fit in Chanchaga (FA 0.0) while *Anopheles* was more fit in Fadikpe (FA 0.0) and Maitumbi (FA 0.0). The Fluctuating asymmetry of *Aedes* in the four rice fields is not perfect except in Chanchaga (FA 0.0).

Table 4.3.2 provides statistical analysis for Fluctuating Asymmetry (FA) of wings (Mean $\pm$ SE) of mosquitoes breeding in rice fields during the rainy season. The results revealed that the fitness of the three mosquito genera were not significantly different (P>0.05) among the rice fields.

	Aede	5			Anoph	neles			Cul	'ex		
Location	LW	RW	MWL	FA	LW	RW	MWL	FA	LW	RW	MWI	L FA
Fadikpe	3.2	3.0	3.1	0.2	2.8	2.8	2.8	0.0	2.9	3.0	2.95	0.1
Bosso	3.1	2.9	3.0	0.2	2.7	2.8	2.75	0.1	3.0	2.9	2.95	0.1
Maitumbi	3.1	2.9	3.0	0.2	2.8	2.8	2.8	0.0	2.9	2.8	2.8	0.1
Chanchaga Aggregate	2.9	2.9	2.9	0.0	2.8	2.9	2.85	0.1	2.9	2.9	2.9	0.0

Table 4.3.1:Mean wing length (MWL) and Fluctuating Asymmetry (FA) in (mm) ofMosquitoes breeding in Rice field in Minna during the Rainy Season of 2012

Note: LW = left wing, RW = right wing, MWL = mean wing length, FA = fluctuating asymmetry.

Location	Aedes	Anopheles	Culex
Fadikpe	0.20±0.06ª	0.00±0.00ª	0.10±0.06 <sup>a</sup>
Bosso	0.20±0.06 <sup>a</sup>	0.10±0.06 <sup>a</sup>	0.10±0.06 <sup>a</sup>
Maitumbi	0.20±0.06 <sup>a</sup>	$0.00 \pm 0.00^{a}$	0.10±0.06 <sup>a</sup>
Chanchaga	0.00±0.00 <sup>a</sup>	0.10±0.06 <sup>a</sup>	$0.00{\pm}0.00^{a}$
Aggregate	0.12±0.05	0.05±0.03	0.08±0.05

### Table 4.3.2:Fluctuating Asymmetry (FA) in (mm) of wings (Means ± SE) ofMosquitoes Breeding in Rice fields in Minnaduring the Rainy Season of 2012

Values followed by same superscript alphabets in a row are not significantly different at P> 0.05 level of significance.

Table 4.3.3 presents the results of the Mean Wing Length (MWL) and Fluctuating Asymmetry (FA) of mosquitoes breeding in rice fields during the dry season. The results revealed that the sizes of the three mosquito types aestivating in rice fields during the dry season have little variations among the four sites. However, larger *Aedes* (i.e MWL 3.0) were encountered in Chanchaga and Fadikpe produced smaller *Aedes* mosquitoes (MWL 2.75). However, all mosquito types have good fitness (0.0 or 0.1) except for *Aedes* and *Anopheles* encountered in Bosso and Chanchaga respectively (MWL 0.2).

Table 4.3.4 provides results for statistical analysis of the Fluctuating Asymmetry (FA) of wings (Mean $\pm$ SE) of mosquitoes breeding in rice fields during the dry season. The results revealed that, there was no statistical difference (P>0.05) in the fitness of mosquito types among the four rice fields.

Aedes			A	Anopheles			Culex					
Location	LW	RW	MWL	FA	LW	RW	MWL	FA	LW	RW	MWI	L FA
Fadikpe	2.7	2.8	2.75	0.1	2.7	2.6	2.65	0.1	2.7	2.7	2.7	0.0
Bosso	2.9	2.7	2.8	0.2	2.6	2.8	2.7	0.1	2.8	2.7	2.75	0.1
Maitumbi	2.9	2.8	2.85	0.1	2.8	2.7	2.75	0.1	2.8	2.8	2.8	0.0
Chanchaga	3.0	3.0	3.0	0.1	2.8	2.6	2.7	0.2	2.8	2.9	2.85	0.1
Aggregate												

## Table 4.3.3:Mean wing length (MWL) and Fluctuating Asymmetry (FA) in (mm) ofMosquitoes Breeding in Rice field in Minna during the Dry Season of 2012

Note: LW = left wing, RW = right wing, = MWL = mean wing length, FA = fluctuating asymmetry.

Location	Aedes	Anopheles	Culex
Fadikpe	$0.10{\pm}0.06^{a}$	$0.10{\pm}0.06^{a}$	0.00±0.00ª
Bosso	$0.20{\pm}0.06^{a}$	$0.10{\pm}0.06^{a}$	0.10±0.06 <sup>a</sup>
Maitumbi	$0.10{\pm}0.06^{a}$	0.10±0.06 <sup>a</sup>	$0.00{\pm}0.00^{a}$
Chanchaga	0.10±0.06 <sup>a</sup>	0.20±0.06 <sup>a</sup>	0.10±0.06 <sup>a</sup>
Aggregate	0.15±0.06	0.13±0.06	0.05±0.03

Table 4.3.4:Fluntuating Asymmetry (FA) in (mm) of wings (Means  $\pm$  SE) ofMosquitoes Breeding in Rice fields in Minna during the Dry Season of 2012.

Values followed by same superscript alphabets in a row are not significantly different at P > 0.05 level of significance.

## 4.4 Relationship between abundance of Mosquito genera in rice fields.

Table 4.4.1 shows the correlation analysis of the relationship between abundance of mosquito genera in rice fields during the rainy and dry seasons. During the rainy season, *Anopheles* mosquitoes correlated negatively with *Aedes* and *Culex*, while *Aedes* correlated positively with *Culex*. All such correlations were however, strong. But for dry season aestivating mosquitoes, *Aedes* correlated positively strong with *Anopheles* and *Culex*, and a weak positive correlation also exist between *Anopheles* and *Culex*.

	Correlations	
Variables Combination	Rainy season	Dry season
Aedes Vs Anopheles	-0.866*	0.694
Aedes Vs Culex	0.738	0.819*
Anopheles Vs Culex	-0.612	0.355

# Table 4.4.1:Correlation between Mosquito Species Abundance in Rice field Larval<br/>Habitats in Minna during the Rainy and Dry Seasons of 2012.

\* indicates strong correlations.

## 4.5 Relationship between mosquito abundance and physicochemical variables of rice field breeding habitats.

Table 4.5.1 shows the relationships between mosquito abundance and physicochemical parameters of water in rice fields during the rainy season. The correlation between *Aedes* mosquitoes and physicochemical properties of water in the rice fields were variable. Though, while Hardness had a modest negative correlation and potassium also correlated negatively very weak, other parameters correlated positively with *Aedes* mosquitoes. There exist a strong positive correlation between *Aedes* mosquitoes and physicochemical parameters such as pH, Phosphate, Sodium, Ammonium and Chloride, and a modest positive correlation between *Aedes* and parameters such as Temperature, Conductivity, Alkalinity, Carbon dioxide, Dissolved Oxygen and Biochemical Oxygen Demand. But Turbidity Correlated positively weak with *Aedes* Mosquitoes.

*Anopheles* mosquitoes were negatively correlated with all the physicochemical properties except with Hardness and Sodium that correlated positively very strong. Apart from Potassium that correlated negatively very weak, the negative correlation that exist between *Anopheles* mosquitoes and the rest physicochemical parameters is however, very strong.

The abundance of *Culex* mosquitoes had a modest negative correlation with Hardness and a very weak negative correlation with Potassium, but correlated positively with the rest parameters. There exist a strong positive correlation between the abundance of *Culex* mosquitoes and parameters such as pH, phosphate, Sodium, Nitrate, Ammonia, and Chloride, and a modest correlation between Temperature, Conductivity, Alkalinity, Carbon dioxide, Dissolved Oxygen and Biochemical Oxygen Demand. But Turbidity correlated positively weak with *Culex* mosquitoes.

Correlations				
Physicochemical Variables	Aedes	Anopheles	Culex	
Temperature	0.584	-0.888*	0.585	
P <sup>H</sup>	0.607	-0.902*	0.607	
Turbidity	0.478	-0.830*	0.478	
Conductivity	0.577	-0.884*	0.577	
Alkalinity	0.580	-0.886*	0.580	
Hardiness	-0.553	0.870*	-0.553	
Phosphate	0.745	-0.523	0.745	
Sodium	0.612	0.903*	0.612	
Potassium	-0.138	-0.271	-0.138	
Nitrate	0.697	-0.940*	0.697	
Ammonium	0.714	-0.933*	0.714	
CO <sub>2</sub>	0.552	-0.878*	0.552	
Chloride	0.605	-0.906*	0.605	
D.O	0.505	-0.886*	0.565	
B.O.D	0.512	-0.839*	0.512	

Table 4.5.1CorrelationbetweenMosquitoAbundanceandPhysicochemicalproperties of water in Rice fieldsLarval Breeding Habitats during the Rainy Seasonof 2012.

\*indicates strong correlations.

Table 4.5.2 shows the correlation coefficient between mosquito abundance and Soil physicochemical properties in the sampled rice fields during the dry season. *Aedes* mosquitoes strongly correlated positively with Nitrogen and Phosphorus, and had very weak positive correlation with conductivity and a modest positive correlation with percentage sand. *Aedes* mosquitoes correlated negatively weak with Sodium, Potassium and Percentage silt, and negatively strong with pH, Organic matter and Percentage clay.

There is a strong positive correlation between *Anopheles* mosquitoes aestivating in rice field with soil Nitrogen, a modest positive correlation with Phosphorus and Potassium and a weak positive correlation with Sodium and percentage sand. But the correlation between *Anopheles* mosquitoes with Conductivity, Organic matter, Percentage clay and silt is negatively weak. However, there exist a strong negative correlation between *Anopheles* mosquitoes and Soil pH. The abundance of *Culex* mosquitoes was negatively correlated with Sodium, pH and Percentage sand, but positively correlated with the rest of the physicochemical properties of the soil. All such correlations are however, weak .

Correlations				
Physicochemical Variables	Aedes	Anopheles	Culex	
Nitrogen	0.836*	0.812 *	0.334	
Phosphorus	0.953*	0.596	0.124	
Sodium	-0.134	0.165	-0.218	
Potassium	-0.031	0.519	0.259	
Conductivity	0.224	-0.203	0.097	
P <sup>H</sup>	-0.628	-0.842*	-0.407	
Organic Matter	-0.703	-0.210	0.351	
Sand	0.504	0.306	-0.294	
Clay	-0.693	-0.268	0.312	
Silt	-0.427	0.309	0.280	

Table 4.5.2CorrelationbetweenMosquitoAbundanceandPhysicochemicalproperties of Soil in Rice fieldsHabitats in Minna during the Dry Season of 2012.

\*indicates strong correlations.

# 4.6 Relationship between wing length of breeding and aestivating mosquitoes in rice fields.

Table 4.6.1 shows the correlation between wing lengths of mosquitoes breeding in rice field during the rainy season, with mosquitoes aestivating in rice fields during the dry season. There was a negative but strong correlation between the wing length of *Aedes* and *Culex* mosquitoes breeding in the rice fields in both rainy and dry seasons. However, there was no correlation between *Anopheles* in rainy season and *Anopheles* in dry season.

# Table 4.6.1Correlation between Wing length of Mosquitoes breeding in rice fields<br/>during rainy season and that of aestivating Mosquitoes in dry season of<br/>2012.

Seasonal mosquito variations.			
Rainy Season / Dry Season		Dry Season	Correlation
Aedes	Vs	Aedes	-0.945*
Anopheles	Vs	Anopheles	0.00
Culex	Vs	Culex	-0.629

\*indicates strong correlations

## 4.7 Relationship between wing length and physicochemical variables of rice fields breeding habitats.

Table 4.7.1 shows the correlation between wing lengths and physicochemical characteristics of water in rice field during rainy season. All the physicochemical variables correlated positively with the wing length of *Aedes* except for Hardness that correlated negatively very strong (-1.000). The positive correlations are however, very strong, except for Phosphate and Potassium that correlated positively weak at (0.414) and (0.353) respectively. *Anopheles* wing length correlated negatively weak with Temperature,  $P^H$ , Turbidity, Conductivity, Hardness, Sodium, Nitrate, Ammonia and Chloride, but correlated negatively strong with Phosphate. There also exist a strong positive correlation between the wings of *Anopheles* mosquitoes with Potassium and a weak positive correlation with Carbon dioxide, Dissolved Oxygen and Biochemical Oxygen Demand. Apart from Potassium that correlated positively weak with *Culex*, all other parameters had a negative correction with *Culex*. However, all such negative correlations are weak, except Phosphate and Biochemical Oxygen Demand that had a strong and modest correlations of (-0.622) and (0.525) respectively.

Correlations			
Physicochemical Variables	Aedes	Anopheles	Culex
Temperature	1.000	-0.013	-0.474
рН	0.999*	-0.022	-0.449
Turbidity	0.993*	-0.108	-0.479
Conductivity	1.000	-0.001	-0.472
Alkalinity	1.000	-0.003	-0.469
Hardiness	-1.000	-0.020	0.484
Phosphate	0.414	-0.887*	-0.622
Sodium	0.999*	-0.035	-0.458
Potassium	0.353	0.816*	0.452
Nitrate	0.988*	-0.133	-0.435
Ammonium	0.980*	-0.195	-0.487
CO <sub>2</sub>	0.998*	0.050	-0.436
Chloride	0.998*	-0.001	-0.419
D.0	0.999*	0.040	-0.429
B.O.D	0.996*	0.350	-0.528

Table 4.7.1:Correlation between Wing Length and Physicochemical properties of<br/>Water in Rice fields Mosquito Larval Breeding Habitats in Minna during the Rainy<br/>Season of 2012.

\*indicates strong correlations.

Table 4.7.2 shows the correlation coefficient between mosquito wing lengths with physicochemical properties of soil in the rice fields breeding habitats. *Aedes* wings correlated positively strong with Nitrogen, phosphorus and Percentage sand, but correlated positively weak with Sodium and Potassium. *Aedes* wings also correlated negatively strong with pH, Percentage clay and Percentage Silt, but correlated negatively weak with Conductivity. The correlation between *Anopheles* wings with Nitrogen, Potassium, Conductivity, Organic matter, Percentage clay and sand was negatively weak, but had a modest negative correlation with Organic matter. Phosphorus, Sodium and Percentage sand correlated positively weak with *Anopheles* wing length. There exists a strong positive correlation between the wing length of *Culex* with Nitrogen, Phosphorus and Percentage sand as well as a modest and weak positive correlation with Sodium and Potassium respectively. However, pH, Organic matter, Percentage clay and Percentage silt correlated negative but weak correlation.

Correlations				
Physicochemical Variables	Aedes	Anopheles	Culex	
Nitrogen	0.811*	-0.150	0.747	
Phosphorus	0.944*	0.062	0.782	
Sodium	0.263	0.286	0.583	
Potassium	0.098	-0.196	0.341	
Conductivity	-0.107	-0.143	-0.433	
рН	-0.620	-0.246	-0.650	
Organic Matter	-0.987*	-0.522	-0.956*	
Sand	0.868*	0.459	0.983*	
Clay	-0.983*	-0.488	-0.978*	
Silt	-0.805*	-0.437	-0.956*	

Table 4.7.2Correlation between Wing Length and Soil Physicochemical propertiesof Rice field Mosquito Habitats in Minna during the Dry Season of 2012.

\*indicates strong correlations.

#### **4.8 Discussion**

Ten species of mosquitoes were encountered with considerable variation in their distribution among the sampled Rice fields in Minna. Result of this study demonstrated coexistence of *Aedes, Anopheles* and *Culex* mosquito's larvae in the same Rice field aquatic habitats (Table 4.4.1). This confirms an earlier work by Lacey and Lacey (1990) who found pests and vectors of *Anopheline* and *Culicine* mosquito species in association with rice field habitats.

The major concern about Rice field is that it may serve as breeding sites for potential mosquito vectors of diseases such as Malaria, Yellow fever, Dengue, Encephalitis as well as Filariasis. Unfortunately, the major species of mosquitoes (i.e., *Aedes, Anopheles* and *Culex*) that are capable of transmitting such diseases are found coexisting in the rice agro ecosystem of Minna. The Center for Disease Prevention and Control has reported a list of 60 species that have been found positive for west Nile virus since 1999( C.D.C, 2007) to include rice fields species such as *Ps. Columbiae, An. crucians, An quadrimacultus, Cx. erraticus, Cx salinarius.* The abundance of some of these species in Minna, calls for active larviciding interventions through integrated pest management program. Mosquito occurrence in various habitats has been investigated by different researchers (Lamidi, 2009).

Mosquito breeding activities in conventional larval habitats in Minna is heterogeneous, with the swamps been the most active source of mosquito production (Olayemi, Omalu, Famotele, Shega and Idris, 2010). The occurrence and abundance of mosquito larvae in different habitats reflects the egg- laying preference of females as well as the ability of

85

immature mosquitoes to survive under the prevailing conditions (Mukthurl, Amerisinghe, Ensink, and Vanderhook, 2002).

Proximity and number of host animals, water quality, presence and absence of water current, degree of shading and plant composition and the density and height of crops are also factors that encourage oviposition by female mosquitoes (Lacey, and Lacey, 1990). Rice fields could be good breeding sites for mosquitoes as they provide water with organic content, nearby pastures (with mammal host), shade and plant biomass for protection against predators, thereby increasing the survival rate of adult mosquitoes (Muturi *et al.*, 2007)

The occurrence of mosquito species in Rice fields in Minna revealed that, *Culex* species appeared as the most dominant among the three mosquito genera, with *Culex pipiens* pipiens taking the lead. This is in agreement with the results of some previous works (Olayemi *et al.*, 2010; Mgbemena and Ebe, 2012). The abundance of *Anopheles* species in Minna was second only to *Culex* with *Anophelles gambiae* taking the lead. This also agreed with Adeleke *et al.*, (2008) in Abeokuta, Nigeria and Vujic *et al.*, (2010) elsewhere in Serbia. However, studies on mosquito diversity in Imo state, South east Nigeria, provides contradictory information as Nwosu, Iwu and Nwosu (2011) incriminated mosquito species in the following order of decreasing abundance: *Anopheles gambiae* (39.6%)> Anopheles funestus (18.5%)> Culex quinquefasciatus (13%)

>Anopheles Pharoensis (19%)> Aedes aegypti (8.5%)> Culexx Pipien fatigans (6%)> Anopheles rhodesiensis (2.49%)>Culex trigripe (2%). Minna, a Cosmopolitan city in North Central Nigeria is characterized by several polluted gutters and drainages that drains and floods some the wetland Rice fields; thereby providing preferential breeding sites for *Culex* mosquitoes, hence their abundance. However, *Anopheles* mosquitoes thrive very well in some of these Rice fields, making their abundance second only to *Culex*. The relationship between the three mosquito genera (i.e. *Aedes Anopheles* and *Culex*) and the diseases they transmit is not established in this work, but Olayemi (2008) and Olayemi *et al.*, (2010) previously incriminated *Anopheles* mosquitoes and malaria transmission in Minna, North Central Nigeria.

The occurrence of *Culex* in this study was higher in Chanchaga. This could probably be explained by the release of pollutants by domestic and anthropogenic activities in Chanchaga area to the rice fields through flooding of the Chanchaga River. This is because *Culex* mosquitoes are known to prefer polluted water for breeding (Olayemi *et al.*, 2010). The larval habitats in Bosso and Maitumbi have clearer waters that were less polluted, and thus favour the breeding of *Anopheles* species. However, there were no previously published reports on the distribution of mosquito species in Minna rice fields to provide information for comparison. By implication, Bosso and Maitumbi are more prone to malaria transmission than Chanchaga because of *Anopheles* vector abundance in the areas. Meanwhile, the distribution and relative abundance of *Culex* and *Anopheles* mosquitoes were not significantly different (P>0.05) among the rice fields larval habitats. This confirms the findings of Olayemi (2008). But there is a significant different (P<0.05) in the distribution and abundance of *Aedes dorsalis* in Fadikpe and Chanchaga with later having the highest frequency of occurrence (Table 4.1.2)

The contribution of aestivating mosquitoes to the persistence of Anopheline malaria vector was evaluated by Lehmann, Dao, Yaro and Adamu (2011), and it was revealed that aestivating mosquitoes constituted the main source of the population after the 6-7 months of long dry season (Lehmann *et al.*, 2011).

The mechanism that allows *An.gambiae* to survive diverse habitats in sub-Saharan Africa during the dry season has been debated for over 70 years (Della Torre and Petracrca, 2002). Two explanations were proposed; first, by aestivation (Omar, Cloudslay and Thomson, 1966) and second by migration from permanent water surface in distant location soon after the rains (Chariwood and Billingslay, 2000). However, records of movement of mosquitoes in distance beyond 2km are rare (Blivitct *et al.*, 2001) and no reports exceed 10km.

The result of this study shows that both the three mosquito genera (i.e *Aedes, Anopheles* and *Culex*) aestivated in the Rice fields during the dry season, thereby constituting a good source of mosquito-vectors and mosquito-borne diseases (Table 4.1.3). This result can be juxtapose with the findings of Blitvich *et al*, (2001) who posited that egg aestivation is typical for *Ochlerotatus, Aedes* and *Psorophora,* while *Anopheles* and *Culex* undergoes larval and adult aestivation respectively. But the occurrence of diapause at more than one point in the life cycle of an insect has been documented for the Carabid beetles, *Nebria brevicollis* and *Patrobus atrorufus* (Thiele, 1969). Diapause is also known to occur in both egg and larva of *Ae. triseriatus* (Clay and Venard., 1972).

In some cases, seasonal changes and physicochemical characteristics of host soil can stimulate hatching of aestivating eggs (Gjullin -Yates and Stage, 1950) and research have shown that eggs of both *Aedes vexans* and *Aedes sticticus* will survive in large numbers, for two to three years.

Generally, information on surface water quality of Rice field larval habitats in Minna is scarce. Available data on physicochemical characteristic of water in mosquito larval breeding habitats in Minna shows that Temperature, Phosphate, Carbonate, and Transparency are not significantly different among habitats (P>0.05) (Olayemi, 2008; Olayemi et al., 2010). The present study revealed that Temperature, pH, Turbidity, Alkalinity, Hardness, Phosphate, Carbon dioxide Chloride, Dissolved Oxygen and Biochemical Oxygen Demand do not varied significantly (P>0.05) among the various rice fields habitats (Table 4.2.2). Rice fields in Chanchaga recorded high Ammonia level. This perhaps results from high amount of domestic and industrial effluents in the area, because waste water from residential homes as well as the Scientific Equipment Center, and the IBB specialist Hospital, all floods towards the sampling Rice fields. The high level of Dissolved Oxygen recorded in Maitumbi could result from photosynthetic activities of the rice crops and other aquatic vegetations .Pesticides and Herbicides applications played a role in the high levels of Turbidity, Alkalinity, Hardness, Conductivity, Potassium, Nitrate, Chloride and Carbon dioxide recorded in Fadikpe (Table 4.2.1).

All the physicochemical parameters of the Soil samples varied significantly (P<0.05) among the four rice fields (Table 4.2.4). However, Bosso recorded high levels of Sodium, Conductivity, pH, and Percentage silt. But Potassium, Organic matter, and Percentage clay are high in Fadikpe, while Chanchaga recorded high levels of soil Nitrogen Phosphorus and Percentage sand. This could be attributed to the different edaphic factors in the soil as well as agrochemicals usage and rice cultivation practices.

The body size of mosquitoes is influenced by water temperature (Day, Ramsey and Zhag, 1990), larval nutrition (Briegel, 1990) and in part photoperiod (Lanciani, 1992). Wing

length which is correlated with dry weight can be used as proxy for body mass (Takken *et al.*, 1996). In this study, wing length was used as proxy for body size and the results shows that *Aedes* mosquitoes are larger in size than *Anopheles* and *Culex* both in the rainy and dry seasons (Table4.3.1) and (Table 43.3) respectively.

The wing length of *Aedes* ranged from 2.9mm to 3.1mm, *Anopheles* ranged from 2.75mm to 2.85mm and *Culex* ranged from 2.8mm to 2.9mm during the rainy season, while for dry season aestivating mosquitoes, *Aedes* wing length ranged between 2.75mm to 3.0mm, *Anopheles* ranged between 2.65mm to 2.75mm and *Culex* wing length ranged between 2.7mm to 2.85mm. In comparison, Jocob and Lyimo (1996) measured the wing length of *Aedes* mosquitoes to range from 2.4 to 3.3mm with a mean of 2.83mm. Rice field in Fadikpe harbored larger *Aedes* and *Culex* while larger *Anopheles* was encountered in Chanchaga. Female with long wings (large body size) had higher fecundity than females with short wings (Small body size) (Xue and Ali, 1994) and mosquitoes that harbor malaria parasites appear to be less fit, because they lay fewer eggs and may have a shorter life (Hogg and Hurd, 1995; Anderson *et al.*, 2000; Luciano, Wang, Collins, and Jocobs-Lorena, 2003). This implies that, *Anopheles* mosquitoes in Chanchaga would have higher fecundity and better chance of progeny development and survival, hence the abundance of malaria vectors in the area.

Although, fitness estimation is however very difficult, largely because of its biological complexity. But the present study revealed that *Anopheles* and *Culex* mosquitoes breeding in Rice fields during the rainy season have better fitness than *Aedes*, because their fluctuating asymmetry (FA) is perfect or near perfect (i.e. 0.0 or 0.1). For dry season aestivating mosquitoes, all of them have good fitness (0.0 or 0.1). However, there was no

significant difference in fitness of both rainy and dry season mosquitoes (P>0.05) among the rice fields (Table 4.3.3) and (Table 4.3.4) respectively. This means that, *Anopheles* and *Culex* mosquitoes can serve as good disease vectors in both seasons, provided they are genetically and biochemically compatible for the complete development of a particular pathogen.

Although, variation in the wing lengths of *Culex quinquefasciatus* and *Mansonia africana* was reported to be higher in wet season than dry season, but was not statistically significant (P>0.05) (Adeleke *et al.*, 2008), and the average wing length of *Anopheles* was 3.2mm (Grieco *et al.*, 2007). Physicochemical parameters of larval habitats alone might account for much of the seasonal size variation in Adult mosquitoes (Corenz *et al.*, 1990). Research also revealed that cool temperature prolong larval development and result in the emergence of large adults (Mullens, 1987). Lanciani and Edwards (1993) established that short photoperiod mosquitoes have greater wings than long photoperiod mosquitoes.

In this study, photoperiod is not considered, but other variables including Temperature might play an important role in the body size of all the mosquito types.

However, the study established a relationship between rain fall and mosquito vector abundance because all the species were highly abundant during the rainy season than the dry season. The same relationship was established in Lafiagi and Calabar by Ajao (2005) and Origanje *et al.*, (2011) respectively. The implication is that mosquito-borne diseases would be more prevalent during the rainy season which is the wetland rice cultivation season. Vector control measures should therefore be taken more seriously at this period. However, dry seasons are also not disease-free period as mosquitoes thrive well in this period and can therefore, transmit diseases.

91

The correlation analysis of the relationship between the abundance of mosquitoes in Rice fields during the rainy season shows that *Anopheles* mosquitoes correlated negatively with *Aedes* and *Culex*, while *Aedes* correlated positively with *Culex*. But for dry season aestivating mosquitoes, *Aedes* correlated positively strong with *Anopheles* and *Culex* and a positive correlation also exist between *Anopheles* and *Culex* (Table 4.4.1).

This implies that, an increase in the population of *Anopheles* mosquitoes, decreases the abundance of *Aedes* and *Culex* during the rainy season, and this explains why malaria fever is more prevalent than any other mosquito-borne diseases during the rainy season. On the contrary, there is the possibility of same prevalence of mosquito-borne diseases in the dry season, because the abundance of all the three mosquito types increases simultaneously during the dry season.

The distribution of mosquito larvae in various breeding habitats has been attributed to the association of mosquito species to varying degree of physicochemical parameters in larval habitats. Although, several studies have examined the influence of physicochemical characteristics of breeding habitats on the abundance of mosquito species with contradicting results (Okogun, 2005). The abundance of *Aedes* mosquitoes in Minna Rice fields correlated positively with all the physicochemical variables of the breeding water except for Hardness and Potassium. But *Anopheles* mosquitoes had a negative correlation with all the physicochemical parameters. *Culex* mosquitoes also correlated negatively with Hardness and potassium but positively correlated with the rest variables (Table 4.5.1); in agreement with previous similar studies (Minakawa *et al.*, 1999; Filinger *et al.*, 2004; Olayemi *et al.*, 2010). The implication of these findings is that, increase in Hardness and Potassium levels can cause reduction in vector abundance of *Aedes* and *Culex* mosquitoes,

where as *Anopheles* vector abundance can be, reduce by increasing the levels of these physicochemical parameters in the breeding water habitats.

*Anopheles* mosquitoes exploits a wide range of breeding habitats with different limnological characteristics (Surendran and Ramasamy, 2005), however *Anopheles culicifacies* were positively correlated with Dissolved Oxygen (D.O) and the production of *Anopheles arabiensis* was favoured by moderately Turbid water (Giming *et al.*, 2001). Physicochemical factors such as pH, and Dissolved Oxygen might provide a perfect environment for survival and breeding activity of *Anopheles* species (Oyewole, *et al.*, 2009).

Geller et al., (2000) reported that under laboratory conditions, Anopheles gambiae carries out normal development when pH varies as much as from 4.0 to 7.8 as long as there are sufficient phytoplanktons and zooplanktons for it to feed on. Grillet (2000) reported a positive association between Dissolved Oxygen and the abundance of Anopheles oswadi and Amerasinghe et al., (1995) reported a negative association between Dissolved Oxygen and Anopheles culicifacies. Anopheles funestus breeds preferentially in large bodies of water including swamps and rice fields (Chan, Ho and Chan, 1971; Burkot et al., 2007; Minakawa et al., 2001). Sunish, Reuben and Rajandran, (2006) suggested that high algal productivity and associated photosynthesis is responsible for high Dissolved Oxygen concentration in aquatic habitats, thereby favoring higher survival of mosquito larvae. In the current study, algal growth was not quantified, but considering that the sampled Rice field's habitats had algal biomass and other floating and submerged vegetation, it is likely that algal productivity resulted in higher concentration of Dissolved Oxygen, thereby promoting larval productivity. This agreed with the work of Muturi et al., (2008). The results of study also, suggest that algal biomass and physicochemical factors may play significant role in determining larval abundance in breeding sites as more or less all habitats had similar values of the variables.

However, the strong correlations found between certain physicochemical parameters and larval abundance, perhaps, confirms the influence of these parameters on the breeding activities of mosquitoes, and indicates the possibility of mosquito larval control through the manipulation of such parameters.

The aestivation of the three mosquito genera occurred in rice fields larval habitats during the dry season. It is possible that the physicochemical characteristics and biochemistry of the host soil plays a role in the aestivation of Anopheles and Culex, because Anopheles mosquitoes correlated positively with Nitrogen, Phosphorus, Potassium, Sodium, and Percentage sand. Culex mosquitoes also correlated positively with Nitrogen, Phosphorus, Potassium, Conductivity, Organic matter, Percentage clay and silt (Table 4.5.2). Increase in these physicochemical parameters might influence the hatching of aestivating eggs. Experiments with the eggs of Aedes vexans shows that reduction in Dissolved Oxygen influence hatching in nature (Thiele, 1969). Short photoperiod and low temperature also influence diapauses termination (Gillot, 2005). In addition to egg diapauses in mosquitoes, there is aseasonal quiescence, a state of inactivity induced by unfavorable environmental conditions which ceases shortly after exposure to adequate hatching stimuli (Missrie, 2002). Though egg diapause is different from aseasonal quiescence, in some cases it is very difficult to distinguish these phenomena, and it takes special experiments to do so (Danks, 2002).

Correlation analysis between the wing lengths of breeding and aestivating mosquitoes shows a negative correlation between *Aedes* and *Culex* mosquitoes in both rainy and dry seasons, but no correlation between the wing lengths of *Anopheles* mosquitoes in rainy season, and *Anopheles* mosquitoes in dry season (Table 4.6.1). This implies that larger *Aedes* and smaller *Culex* coexist in the rice fields in both seasons. Competition for the same resources such as food and space can cause such relationship. However, the sizes of *Anopheles* mosquitoes in the rainy and dry seasons have no relationship what so ever. The availability of all the physicochemical parameters in the breeding water provides a good breeding ground that produces larger *Aedes*, except Hardness. Larger *Anopheles* are found in association with Potassium, Carbon dioxide, Dissolved Oxygen and Biochemical Oxygen Demand, whereas *Culex* mosquitoes respond negatively with such parameters in breeding water, as it make them smaller in size (Table 4.7.1). The relationship between the body sizes of aestivating mosquitoes and soil physicochemical characteristics is variable. While some of the physicochemical parameters make them grow large, others retard their growth and make them smaller (Table 4.7.2).

#### **CHAPTER FIVE**

## 5.0 CONLUSION AND RECOMMENDATIOS.

#### **5.1 Conclusion**

The presence and occurrence of ten species of three mosquito genera (Aedes, *Anopheles* and *Culex*) vectors in rice fields, and the physicochemical characteristics of breeding habitats have been established in both rainy and dry seasons. The body sizes of *Aedes* mosquitoes are larger than *Anopheles* and *Culex*. However, the vectorial fitness of rice fields' mosquitoes is perfect. The study has also established that, the distribution of mosquito larvae in various habitats has been attributed to the association of mosquito species to varying degree of physicochemical parameters in larval. However both species may exploits the same habitats for larval development, they respond differently to both water and soil qualities; thus influencing their distribution, body size and vectorial fitness.

### **5.2 Recommendations**

The strong correlations found between certain physicochemical parameters and larval abundance in rice fields can be use to alter the breeding activities of mosquitoes. Therefore, the possibility of mosquito larval control through the the manipulation of such parameters is strongly recommended.

Finally, the mosquito species encountered in this study are of public health importance, hence the need to incorporate integrated pest management and control strategies in rice cultivation practices. This will not only ride these species out of the breeding sites but also free the state of the disease associated with these organisms.

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## APPENDIX

Mosquitoes species	FAD	BSO	MTB	CHG	SUM
An. gambiae	59	43	56	51	209
An. funestus	41	44	48	40	173
An.quardrimaculatus	20	26	26	21	93
An.maculipalpis	24	43	16	24	107
Sub.Total	144	156	146	136	582
Ae. Aegypti	26	21	20	31	98
Ae. Dorsalis	20	22	23	24	89
Ae.vexans	24	21	22	23	90
Sub. Total	70	64	65	78	277
Cx.pipiens pipiens	84	80	86	90	340
Cx. quinquefaciatus	58	52	57	58	225
Cx. Restuans	43	33	41	35	152
Sub.Total	185	165	184	183	717

Appendix A: Mosquito species composition in rice fields in Minna, during the rainy season (September, 2012)

	FADK	BSO	MTB	CHG	SUM	
Mosquitoes species						
An. gambiae	48	41	50	52	191	
An. funestus	39	46	41	35	161	
An.quardrimaculatus	19	26	24	16	85	
An.maculipalpis	20	22	15	23	80	
Sub.Total	126	135	130	126	517	
Ae. Aegypti	20	15	16	21	72	
Ae. Dorsalis	16	18	22	28	84	
Ae.vexans	17	17	18	20	72	
Sub. Total	53	50	56	89	228	
Cx.pipiens pipiens	66	75	81	99	311	
Cx. quinquefaciatus	46	45	51	54	196	
Cx. Restuans	35	38	38	34	145	
Sub.Total	147	158	170	177	652	

Appendix B: Mosquito species composition in rice fields in Minna, during the rainy season (October, 2012)

Mosquitoes species	FADK	BSO	MTB	CHG	SUM
An. gambiae	04	03	04	05	16
An. funestus	01	02	00	03	06
	00	00	00	01	01
An.quardrimaculatus					
-	00	00	00	00	00
An.maculipalpis					
	01	00	00	01	02
	00	00	00	00	00
`Ae. Dorsalis					
	00	00	00	00	00
Ae.vexans					
	08	06	04	09	27
Cx. pipiens pipiens					
	01	03	01	02	07
Cx. Quinquefaciatus					
	00	00	00	00	00
Cx. Restuans					
Total	15	14	09	21	59

Appendix C: Species composition of mosquitoes	aestivating in rice fields during the dry
season (November, 2012)	

Mosquitoes species	FAD	BSO	MTB	CHG	SUM
An. gambiae	02	01	03	06	12
	00	00	01	02	03
An. funestus					
	00	00	01	01	02
An.quardrimaculatus					
	00	00	00	00	00
An.maculipalpis	01	00	01	02	0.4
A a A accurati	01	00	01	02	04
Ae. Aegypti	00	00	00	00	00
Ae. Dorsalis	00	00	00	00	00
	00	00	00	01	01
Ae.vexans	00	00	00	01	01
	05	04	05	09	23
Cx. pipiens pipiens					
	00	02	01	02	05
Cx. quinquefaciatus					
	01	00	00	00	01
Cx. Restuans					
Гotal	09	07	12	23	51

Appendix D: Species composition of mosquitoes aestivating in rice fields during the dry season (December, 2012)

Msosquitoes species	FAD	BSO	MTB	CHG	SUM
An. gambiae	02	03	00	04	09
An. funestus	02	02	01	01	06
An.quardrimaculatus	01	00	00	01	02
An.maculipalpis	00	01	01	00	02
Ae. Aegypti	01	01	00	00	02
Ae. Dorsalis	00	00	00	00	00
Ae.vexans	00	00	00	00	01
Cx.pipien	04	02	02	02	10
Cx. Quinquefaciatus	04	02	02	00	08
Cx. Restuans	01	01	00	00	02
Total	16	13	06	08	43

Appendix E: Species composition of mosquitoes aestivating in rice fields during the dry season (January, 2013)

Mosquitoes species	FAD	BSO	MTB	CHG	SUM
An. gambiae	02	02	01	03	08
An. funestus	01	02	01	02	06
An.quardrimaculatus	00	01	00	01	02
An.maculipalpis	00	01	01	00	02
Ae. Aegypti	02	01	01	01	05
Ae. Dorsalis	00	00	00	01	01
Ae.vexans	01	00	00	01	02
Cx.pipien	03	02	01	02	08
Cx. Quinquefaciatus	03	01	01	01	06
Cx. Restuans	01	00	00	01	02
Total	14	11	06	13	44

Appendix F: Species composition of mosquitoes aestivating in rice fields during dry season (February, 2013)

Mosquitoes species	FADK	BSO	MTB	CHG	SUM
An. gambiae	02	01	01	02	06
An. funestus	01	01	02	02	06
An.quardrimaculatus	00	01	00	00	01
An.maculipalpis	01	02	01	00	04
Ae. Aegypti	01	01	01	01	02
Ae. Dorsalis	01	00	00	01	02
Ae.vexans	00	00	00	00	00
Cx.pipien pipiens	01	02	01	02	05
Cx. quinquefaciatus	02	01	00	01	04
Cx. Restuans	01	00	00	01	02
Total	10	09	07	09	35

Appendix G: Species composition of mosquitoes aestivating in Rice fields (March, 2013)

Mosquitoes species	FADK	BSO	MTB	CHG	SUM
An. gambiae	03	01	03	02	09
An. funestus	01	02	01	01	05
An. quardrimaculatus	01	00	00	00	01
An.maculipalpis	01	01	00	00	02
Ae. Aegypti	01	01	00	01	03
Ae. Dorsalis	00	01	01	01	03
Ae. Vexans	00	00	00	00	00
Cx.pipien pipien	02	01	01	01	05
Cx. Quinquefaciatus	01	01	02	00	04
Cx. Restuans	00	00	00	00	00
Total	14	11	06	06	33

Appendix H: Species composition of mosquitoes aestivating in rice fields during the dry season (April, 2013)

Parameters.	CHG	MTB	FAD	BSO	SUM
Temperature ( <sup>0</sup> c)	29.8	30.0	29.8	28.7	117.3
pH	7.2	6.9	7.2	7.1	28.4
Turbidity (JTU)	0.24	0.24	0.44	0.22	1.14
Conductivity (µS/cm)	180	182	180	189	731
Alkalinity (mg/l)	115	125	111	121	472
Hardness (mg/l)	102	99	89	106	396
Potassium (mg/l)	4.74	4.44	5.2	14.7	29.1
Sodium (mg/l)	10.11	11.6	12.7	12.7	46.41
Phosphorus (mg/l)	0.051	0.052	0.211	0.043	0.36
Nitrate (mg/l)	1.634	1.504	1.098	2.611	7.68
Ammonia (mg/l)	0.11	0.07	0.13	0.1	1.04
Carbon dioxide (mg/l)	1.41	2.21	2.62	1.15	7.39
Chloride (mg/l)	28.1	24.2	270	18.2	97.5
Dissolved Oxygen (mg/l)	9	7	8	8	32
B.O.D (mg/l)	4	4	3	2	13

Appendix I: Physicochemical characteristics breeding water (August, 2012)

JTU=Jackson Turbidity Unit,  $\mu$ s/cm= micromhos per centimeter, mg/l= milligram per liter, BOD=Biochemical Oxygen Demand.

Parameters.	CHG	MTB	FAD	BSO	SUM
Temperature ( <sup>0</sup> C)	29.9	29.6	29.6	29.6	118.9
pH	7.1	67.2	6.8	7.3	28.4
Turbidity (JTU)	0.25	0.24	0.45	0.21	1.15
Conductivity (µs/cm)	180	183	177	194	734
Alkalinity (mg/l)	115	120	125	110	470
Hardness (mg/l)	101	98	94	104	397
Potassium (mg/l)	4.73	4.6	5.3	10.4	25.03
Sodium (mg/l)	10.3	12.7	11.1	12.1	46.2
Phosphorus (mg/l)	0264	0.51	0.052	0.041	0.81
Nitrate (mg/l)	1.53	1.99	2.99	1.1	7.6
Ammonia (mg/l)	0.105	0.07	0.141	0.105	0.42
Carbon dioxide (mg/l)	1.21	2.14	2.62	1.14	7.11
Chloride (mg/l)	27	23.1	28.3	17.2	95.6
Dissolved Oxygen (mg/l)	9	7	8	8	32
B.O.D (mg/l)	4	4	3	2	13

Appendix J: Physicochemical characteristics of breeding water (September, 2012)

Parameter.	CHG	MTB	FAD	BSO	SUM
Temperature ( $^{0}$ c)	30.7	29.8	29.4	29.8	11.7
pH	7.5	7.1	7.2	7.7	29.5
Turbidity (JTU)	0.26	0.24	0.44	0.26	1.05
Conductivity (µS/cm)	182	189	1.77	186	734
Alkalinity (mg/l)	125	110	117.5	119.4	471.9
Hardness (mg/l)	108	105	100	105	418
Potassium (mg/l)	4.6	4.7	5.4	8.85	35.4
Sodium (mg/l)	10.2	13.8	10.1	12	48
Phosphorus (mg/l)	0.288	0.046	0.052	0.106	0.48
Nitrate (mg/l)	1.583	1.145	3.86	2.161	8.64
Ammonia (mg/l)	0.105	0.07	0.14	0.105	0.44
Carbon dioxide (mg/l)	1.1	2.2	2.64	1.77	7.08
Chloride (mg/l)	28.3	24.8	29.8	26.95	100.60
Dissolved Oxygen (mg/l)	6	9	6	7.3	28.3
B.O.D (mg/l)	3	3	4	3	12

Appendix K: Physicochemical characteristics of breeding water (October, 2012)

JTU=Jackson Turbidity Unit,  $\mu$ s/cm= micromhos per centimeter, mg/l= milligram per liter, BOD=Biochemical Oxygen Demand

PARAMETERS.	FAD	BSO	MTB	CHG	SUM
Nitrogen ( <sup>0C</sup> )	0.114	0.090	0.117	0.224	0.57
Phosphorus (mg/kg	0.35	2.42	1.62	17.96	223
Sodium (mg/kg)	505	69.6	69.2	512	240
Potassium (mg/kg)	271	103.5	237	232	1.770
Conductivity (µs/cm)	165	396	126	216	897
P <sup>H</sup>	7.45	8.20	7.76	7.04	30.41
Organic matter (%)	1.68	1.63	1.56	1.58	6.36
Sand (%)	59.1	56.8	64.6	62.2	248.6
Clay (%)	31.2	30.9	29.4	30.2	120.6
Silt (%)	9.65	12.6	5.37	3.6	31.02

APPENDIX L: Physicochemical characteristics of soil in breeding site during dry Season (NOV, 2012)

Mg/kg=milligram per kilogram,  $\mu$ s/cm= micromhos per centimeter, <sup>0</sup>C=degree celcius, %=percentage.

Parameters.	FAD	BSO	MTB	CHG	SUM
Nitrogen ( <sup>0C</sup> )	0.15	0.081	0.119	0.225	0.57
Phosphorus (mg/kg)	0.34	2.41	1.61	17.8	22.4
Sodium (mg/kg)	504	68.5	69.1	510	24.02
Potassium (mg/kg)	260	104.5	235	231	17.72
Conductivity (µs/cm)	163	393	124	215	89.8
P <sup>H</sup>	7.41	8.11	7.77	7.10	30.46
Organic matter (%)	1.67	1.52	1.58	1.50	6.38
Sand (%)	58.0	540	64.2	67.8	248.4
Clay (%)	30.4	30.8	29.6	28.4	121.5
Silt (%)	9.40	12.5	5.41	3.7	32.01

Appendix M: Physicochemical characteristics of soil in breeding site during the dry season (DEC, 2012)

Mg/kg=milligram per kilogram,  $\mu$ s/cm= micromhos per centimeter, <sup>0</sup>C=degree celcius, %=percentage.

	Aede	es			And	opheles	5		Culey	K		
	LW	RW	MEAN	FA	LW	RW	MEAN	FA	LW	RW	MEAN	FA
Location Fadk	3.1	2.9	3.0	0.2	2.8	2.9	2.85	0.1	2.9	2.9	2.9	0.0
BSO	2.9	2.9	2.9	00	2.8	2.8	2.8	0.0	2.9	3.0	2.95	0.1
MTB	3.2	2.9	3.1	0.3	2.7	2.8	2.75	0.1	3.0	2.9	2.95	0.1
CHG	3.1	2.9	3.1	0.2	2.8	2.8	2.8	0.0	2.9	2.8	2.85	0.1

Appendix N: Mean Wing Length of mosquitoes breeding in rainy season. (September, 2012)

LW= left wing, RW=right wing, FA=fluctuating asymmetry

	Aede	s			Anop	oheles		Culex				
	LW	RW	MEAN	FA	LW	RW	MEAN	FA	LW	RW	MEAN	FA
Location Fadk	3.2	2.9	3.1	0.3	2.8	2.8	2.8	0.0	2.9	3.0	2.95	0.1
BSO	3.1	2.9	3.0	0.2	2.7	2.8	2.75	0.1	3.0	3.9	2.95	0.1
MTB	3.1	2.9	3.0	0.2	2.8	2.8	2.8	00	2.9	2.8	2.8	0.1
CHG	2.9	2.9	2.9	0.0	2.8	2.9	2.85	0.1	2.9	2.9	2.9	0.0

Appendix O: Mean Wing Length of mosquitoes breeding in rainy season. (October, 2012)

LW= left wing, RW=right wing, FA=fluctuating asymmetry

	Aedes					Anopheles				K		
	LW	RW	MEAN	FA	LW	RW	MEAN	FA	LW	RW	MEAN	FA
Location Fadk	3.0	3.0	3.0	0.0	2.8	2.6	2.7	0.2	2.8	2.9	2.9	0.1
BSO	2.9	2.8	2.85	0.1	2.8	2.7	2.75	0.1	2.8	2.8	2.8	0.0
MTB	2.9	2.7	2.8	0.2	2.6	2.8	2.7	-0.2	2.8	2.7	2.75	0.1
CHG	2.7	2.8	2.75	0.1	2.7	2.6	2.65	0.2	2.7	2.7	2.7	0.0

Appendix P: Mean Wing Length of mosquitoes aestivating in dry Season (NOV, 2012)

LW= left wing, RW=right wing, FA=fluctuating asymmetry

	Aede	S			And	pheles	5		Culex			
	LW	RW	MEAN	FA	LW	RW	MEAN	FA	LW	RW	MEAN	FA
Location Fadk	2.9	2.8	2.85	0.1	2.8	2.7	2.75	0.1	2.8	2.8	2.8	0.0
BSO	3.0	3.0	3.0	0.0	2.8	2.6	2.7	0.2	2.8	2.9	2.85	0.1
MTB	2.7	2.8	2.75	- 0.1	2.7	2.6	2.65	0.2	2.7	2.7	2.7	0.0
CHG	2.9	2.2	2.8		2.6	2.8	2.7	0.2	2.8	2.7	2.75	0.1

Appendix Q: Mean Wing Length of mosquitoes aestivating in dry season (Jan, 2013)

LW= left wing, RW=right wing, FA=fluctuating asymmetry

	Aed	es		Anopheles				Culex				
	LW	RW	MEAN	FA	LW	RW	MEAN	FA	LW	RW	MEAN	FA
Location Fadk	2.9	2.7	2.8	0.2	2.6	2.8	2.7	2.8	2.7	2.7	2.75	0.1
BSO	2.7	2.8	2.75	0.1	2.7	2.6	2.65	2.7	2.8	2.7	2.7	00
MTB	3.0	3.0	3.0	0.0	2.8	2.6	2.7	2.8	2.7	2.9	2.85	0.1
CHG	2.9	2.8	2.85	0.1	2.8	2.7	2.75	0.1	2.8	2.8	2.8	0.0

Appendix R: Mean Wing Length of mosquitoes aestivating in dry season (Feb, 2013)

LW= left wing, RW=right wing, FA=fluctuating asymmetry

	Ae	des		Anopheles				Culex				
_	LW	RW	MEAN	FA	LW	RW	MEAN	FA	LW	RW	MEAN	FA
Location Fadk	3.0	3.0	3.0	0.1	2.8	2.6	2.7	0.2	2.8	2.9	2.85	0.1
BSO	2.9	2.8	2.85	0.1	2.8	2.7	2.75	0.1	2.8	2.8	2.8	0.0
MTB	2.9	2.7	32.8	0.2	2.6	2.8	2.7	0.1	2.8	2.7	2.75	0.1
CHG	2.7	2.8	2.75	0.1	2.7	2.6	2.65	0.1	2.7	2.7	2.7	0.0

Appendix S: Mean Wing Length of mosquitoes aestivating in dry season (April, 2013)

LW= left wing, RW=right wing, FA=fluctuating asymmetry