UNIVERSITY OF NAIROBI

ANOPHELINE MOSQUITO VECTORS AND MALARIA TRANSMISSION DYNAMICS ALONG AN ALTITUDINAL GRADIENT ON THE HIGHLANDS OF MAMBILLA PLATEAU, NIGERIA.

BY

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I80/50795/2016

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (MEDICAL ENTOMOLOGY) IN THE SCHOOL OF BIOLOGICAL SCIENCES OF THE UNIVERSITY OF NAIROBI.

2019

DECLARATION

This thesis is my originalwork and has not been presented for a degree in any other university.

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This thesis is dedicated to God Almighty for His sufficient grace and provision. Lord I lay all my trophies down at your feet in humble adoration.

ACKNOWLEDGEMENTS

My special appreciation goes to my supervisors Professor Florence Oyieke and Dr. Eunice Owino for their encouragement, useful suggestions and guidance throughout the course of writing this thesis. I also appreciate Professor Georgina Mwansat for providing the CDC-light traps and other equipment for the field work and for supervising the field work. I am grateful to Professor Richard Mukabana and Dr. George On'gamo for positive criticisms and corrections that have added value to the thesis. My gratitude goes to Prof. P. N. Ndegwa, Director School of Biological Sciences University of Nairobi for encouragement and kind advice.

My profound gratitude goes to the Vice Chancellor of Taraba State University Jalingo Taraba State Nigeria and the entire management staff for approving and providing the funds for the research. I would like to sincerely appreciate Dr. Nannim Nanvyat and Dr. Dinfa both of University of Jos Plateau State Nigeria for professional advice. My profound gratitude goes to Dr. T.S Awolola Head of Department of Parasitology and Entomology, Nigeria Institute for Medical Research Yaba, Lagos for granting me access to the use of the laboratory for the molecular analyses. My deep appreciation goes to Mr. Tola, Mr. Tayo and Miss Kemi all of NIMR for helping with the analyses.

Finally, I would like to sincerely appreciate my wonderful family for the prayers and best wishes. My gratitude goes especially to my husband Mr. Umar Columbus Garba for understanding, encouragements and support both financially and morally. Thank you for believing in me and allowing me pursue my dreams, may God enrich you greatly. To my sweet wonderful children; Tsyntop, Yonny, Winner and little Benjamin what can I say, thank you for your love and patience for the many times I had to leave you, your love was my motivation to success. To my nieces and nephew Nde, Fxafa, and Garkwe. I say thank you very much for taking care of my children in my absence, may God provide you with families of your own.

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ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of Variance
BB	Blocking Buffer
CDC	Center for Disease Control
CSP	Circumsporozoite
EIR	Entomological Inoculation Rate
ELISA	Enzyme Linked Immunosorbent Assay
FMoH	Federal Ministry of Health
GPS	Global Positioning System
HIV	Human Immunodeficiency Virus
IRS	Indoor Residual Spraying
ITNs	Insecticide Treated Nets
LGA	Local Government Area
LLINs	Long Lasting Insecticidal Treated Nets
mAb	monoclonal Antibody
MIS	Malaria Indicator Survey
NIMR	Nigeria Institute for Medical Research
NMCP	National Malaria Control Programme
NMFS	Nigeria Malaria Fact Sheet
PCR	Polymerase Chain Reaction
PSC	Pyrethrum Spray Collection
rDNA	ribosomal Deoxyribonucleic Acid
SR	Source Reduction
TDS	Total Dissolve Solutes
US	United States

ABSTRACT

The presence of a suitable and efficient vector is a major determinant of local malaria transmission intensity.In the last few years, the Mambilla Plateau has witnessed an influx of people from different parts of the country who engage in various anthropogenic activities that could have profound consequence on vectors and malaria transmission in the region.Until now, no data exist for vector anopheline species and malaria transmission intensity for the Mambilla Plateau, a highland region prone to malaria epidemics. The study aimed to provide the baseline data on the anopheline vectors of malaria and malaria transmission on the highlands of Mambilla Plateau, Nigeria.The study was conducted in five locations along an altitudinal gradient namely; Nguroje (1,885m), Yelwa (1,674m), Gembu (1,584m), Kakara (1,496m) and Mayo Selbe (484m), above sea level. Collections were conducted once every month from November 2015 to October 2017. Adult mosquitoes were captured by the use of Centre for Disease Control (CDC) modified light traps and Pyrethrum Spray Catches (PSC). Larvae were sampled using the standard dipping methodand reared to adulthood. Larval habitats were identified and characterized according to the features observed in the field. The physicochemical parameters were also measured. The mosquitoes were identified morphologically by microscopy using taxonomic keys.*Anopheles gambiae sensu lato* (*sl*)where further identified genetically to sub species and molecular levels (M and S) forms by Polymerase Chain Reaction (PCR).Enzyme Linked Immunosorbent Assay (ELISA) was used to test for *Plasmodium falciparum* infectivity as well as source of bloodmeal.A total of 878 anophelines comprising of five species namely; *Anopheles gambiae sl*,*An. coustani*, *An. funestus*, *An. pharoensis*, and *An. rufipes*,were collected. *An. gambiaesl*was the highest number collected in all the locations and seasons (757). *An. gambiaesensu stricto* was the only member of the*An. gambiae* complex identified by PCR, the S molecular form of the vector dominated over the M form (520 and 192) respectively.There was a very strong positive correlation between the S and M forms, $r^2 = 0.94204$.Mayo-selbe had the highest species abundance of 572 but lowest species diversity($H^{'}= 0.24$). Yelwa had the least species abundance (24) but very high diversity ($H^{'}=0.81$). December recorded highest abundance of anophelines (235) but very low diversity ($H^{'} = 0.28$). A total of 60 larval habitats were sampled and characterized. Swamps were the highest number of habitats encountered (15) and tree trunk was the least (1). There was relatively low occurrence of positive breeding habitats along the altitudinal locations. Only six were positive. This could be due to the temporary nature of the habitats. Larvae were associated with still, clear and sunlit temporary habitats with a wide range of physicochemical parameters.There were two peak malaria transmission seasons in the Mambilla Plateau, in December and June. All malaria transmission indices namely; sporozoites rates (SR), man biting rates (MBR) and entomological inoculation rates (EIR) decreased with increasing altitude. These were in the range of SR (0.17-3.55%), MBR (0.002-2.56) and EIR (0.003-9.09 ib/p/n). Only 22.67% of the mosquitoes had fed on human blood, other sources of blood meal were unknown.The human blood index ranged from (0.00 to 0.22). Nguroje and Kakararecorded the lowest while Mayo-selbe recorded the highest.In the current study malaria transmission is occurring in the Mambilla Plateau and *An. gambiae s. s* is the major species of *An. gambiae*complex involved. However, other species that were collected have been reported to play secondary roles in transmission in other parts of Africa. Malaria control in the study area should therefore focus on *An. gambiae s. s*. There should also be close monitoring and

surveillance of the other anophelines species that were encountered in the study area as these may become potential vectors in the future.This study has provided the baseline information about the anophelines malaria vectors and malaria transmission in the highlands of Mambilla Plateau, which would be helpful for the sustainable management of vector mosquitoes and also inform policy measures to prevent or counter malaria epidemics in the future.

CHAPTER ONE
1.0: GENERAL INTRODUCTION

1.1: Background

Malaria is a disease that is caused by aprotozoan parasite of the genus *Plasmodium*. It is transmitted through the infective bite of the female *Anopheles* mosquitoes and affects both humans and animals. Recent World Health Organization (WHO) malaria report of 2017, showed that almost half of the world's population was at risk of the disease. Malaria is however most prevalent in wet and humid tropical countries of the world. It is endemic in most of sub-Saharan African countries, where it causes substantial morbidity and mortality among populations. Almost all cases of malaria occur in tropical African countries, which are the worst affected by the burden of the disease. In 2016, 90% of cases and 91% deaths occurred in African countries and cost for control was put at an estimated US $ 2.7 billion. Governments of endemic countries contributed 31% of funding. In 2016 an estimated 216 million cases which translates to 5million cases increase over 2015 were reported in 91 countries. There was only a slight reduction in deaths, reported to have been 445,000 against 446,000 in 2015. Anyone can contact malaria, but certain populations of individuals are at greater risk of the disease. These are infants and children under five years old, HIV/AIDS patients, pregnant women, non-immune migrants, mobile populations and travelers.

Vector control is of significant importance in malaria control. The signing and declaration of action plan to reduce malaria burden by the year 2010 by African countries was in place by the year 2000. They were to constitute epidemic readiness and warning signal responses. These responses were to be efficient, and able to detect and manage promptly any disease outbreak (WHO, 2000).

Increase in malaria intervention through long-lasting insecticide nets (LLINs), indoor residual spraying (IRS), prevention with drugs, prompt diagnosis and treatment, has resulted in significant reduction in global malaria incidences (Wilson *et al*., 2015, Aribodor *et al*., 2016). Eradication of malaria is however faced with many challenges. Millions of people in resource poor countries do not have access to the needed

services and most malaria cases and deaths go unreported and unregistered (U.S global health policy, 2013, Aribodor *et al*., 2016). There are several challenges to achieving malaria eradication in sub-Saharan Africa in general. These include; Poverty, migration (this could arise from conflicts, terrorism, insurgency, and natural disasters), weak health systems, poor sanitation. Drug resistance also results in treatment failure. Insecticides resistance, global warming and climate change also compound the problem of malaria eradication. Other challenges are insufficient surveillance capacity, attitude and behavior of the people, lack of political will, poor leadership and inadequate funding (U.S global health policy, 2013, Aribodor *et al*., 2016).

Highland regions of Africa often experience malaria epidemics due to the unstable transmission pattern. Such unstable and low transmissions aretermed hypoendemictransmissions and is estimated at less than 10% (Hay *et al*., 2008). Unstable malaria transmission resultsfrom variations in highland temperatures which hitherto are low. This has led to an increase in the number of malaria epidemics in the past decades (Abeku, 2007; Munhenga *et al*., 2014). Malaria occurrence and transmission are mostly driven by climatic factors such as temperature, rainfall and humidity. Rainfall creates suitable habitats for the adult female mosquito vectors to lay their eggs. Sufficient humidity allows for high activity and survival of mosquitoes while temperature affects parasite development time within the mosquito vector and disease outbreaks (Beck-Johnson *et al*., 2013, Vajda *et al*., 2017). Enhancement in the vectorial capacity of the mosquito vectors is another factor that accounts for malaria epidemics. All of these factors lead to longer transmission (Pan *et al*., 2012).

There is no globally acceptable starting point for malaria epidemics, as these depend on the situation in a particular area. Epidemic cases are seasonal and exhibit variations yearly (Lynch, 2005). The highest disease risk is observed among persons in low-to-moderate transmission settings, with non-immunity and the mean age of patients increase with decreasing transmission intensity (Omumbo *et al*, 1997). Beside the volatile epidemics, there is an increase of endemic malaria transmission in the African highland regions (Woyessa *et al*., 2014). Most reports of malaria in the African highaltitudinal regions are ascribed to *Plasmodium falciparum.*Prevention of malaria epidemics through early detection in susceptible areas such as the highlands should

form the basisfor control hence, the need for immediate devices to predict malaria epidemics.Detection of epidemics that is based only on rise in the number of cases by a group of health workers in selected locations may be cost-effective.

This would not provide sufficient time for an adequate response. This is because malaria epidemics appear suddenly and terminate within a few months (Mueller *et al,* 2009). Epidemics usually require prompt emergency responses in order to be effective (Tchuinkam *et al*, 2010).The challenge however, is insufficient surveillance and response systems to monitor malaria transmission dynamics to guide the elimination process.Data on various entomological parameters of anopheline vectors in high risk regions would provide important information on malaria transmission intensity. These data can aid in scaling up control and assess effectiveness of control measures as well as predict malaria epidemics in the highland regions.

1.2: Problem statement

Malaria is a public health problem in Nigeria. The geographic location of the country makes the climate suitable for malaria transmission throughout the year (MIS 2010). The country alone accounts for up to 25% of the global cases and deaths (WHO 2015). Malaria also has economic implications on households and individuals due to the cost of treatment and protection from the disease. In highland regions, the temperature is low with a corresponding low *Anopheles* vector population. This results in low and intermittent malaria transmission causing instability or epidemics. High mortality results due to low immunity in the human populations. Changes in land use such as mining, grazing, logging, can impact on malaria transmission. Microclimate of the immature stages could be altered, resulting in increase in the population of adult mosquito vectors.

Adult vector population dynamics is important because it acts as an indicator of transmission risk of the disease. In the last few years, the Mambilla Plateau has witnessed an influx of people from different parts of the country who engage in illegal mining for blue sapphires, extensive grazing and logging for high quality timber. These activities could have profound consequence on malaria transmission in the region. Larval habitats could be proliferated by these activities. *Plasmodium falciparum*can be introduced into the highland areas from the lowlands. In highland

areas like the Mambilla Plateau, epidemics do build up therefore monitoring vector population in epidemic prone regions is useful in models that detect likelihood of epidemic. The present study was carried out in a highland region prone to malaria epidemics to ascertain differences in malaria vector population dynamics and transmission intensities along an altitude gradient. Results obtained would provide useful information to elucidate epidemicsand inform appropriate and effective control measures to counter epidemics in the region.

1.3: Aim of the study

To provide baseline data on the anopheline mosquito vectors and malaria transmission dynamics on the Mambilla Plateau, Nigeria.

1.3.1: Specific objectives

1. To determine the species diversity and relative abundance of the anopheline vectors of malaria along an altitudinal gradient on the Mambilla Plateau, Nigeria.
2. To identify and characterize the larval habitats of the anopheline vectors of malaria along an altitudinal gradient on the Mambilla Plateau, Nigeria.
3. To estimate the Entomological Inoculation Rates (EIRs) of the anopheline vectors of malaria along an altitudinal gradient on the Mambilla Plateau, Nigeria.
4. To determine the source of blood meal of anopheline vectors of malaria along an altitudinal gradient on the Mambilla Plateau, Nigeria.

1.3.2: Null hypotheses

1. The species diversity and relative abundance of anopheline vectors of malaria on the Mambilla Plateau Nigeria does not differ along altitudinal locations.
2. There are no larval habitats of anopheline vectors of malaria on the Mambilla Plateau, Nigeria.
3. Entomological inoculation rates (EIR) of anopheline vectors of malaria on the Mambilla Plateau, Nigeria are not affected by altitude.
4. Human blood does not constitute the source of blood meal of vectors of malaria on the Mambilla Plateau, Nigeria.

1.3.3: Alternative hypotheses

1. The species diversity and relative abundance of anopheline vectors of malaria onthe Mambilla Plateau Nigeria differ along altitudes.
2. There are larval habitats of anopheline vectors of malaria on the Mambilla Plateau, Nigeria.
3. Entomological inoculation rates (EIR) of anopheline vectors of malaria on the Mambilla Plateau are affected by altitude.
4. Human blood constitutes the source of blood meal of vectors of malaria on the Mambilla Plateau, Nigeria.

1.4: Justification and significance of the study

There is a general decline in malaria burden but new challenges are coming into existence. There is currently a growing problem, of particular concern in the transmission of malaria in the sub-Saharan highlands. Until now, these highlands were viewed as areas of little or no malaria transmission. The low environmental temperatures in these highlands negatively impacted on *Plasmodium* sporogony and development (Bodker *et al*., 2003). However, the situation appears to be changing as highland regions previously reckoned free of malaria, are recording increase in the number of malaria epidemics. The change in malaria epidemiology in the highlands has been attributed to several factors including, socio-economic status, climatic and ecological changes. To exacerbate the problem, high levels of mortality in both children and adults are usually witnessed in the highlands during malaria epidemics (Cox *et al*., 1999) due to low levels of functional immunity as a result of low previous exposure to malaria infections. This is further compounded by poor and inadequately funded health care systems in most of these areas.

The WHO global technical strategy for malaria control 2030 is aimed at achieving a reduction in global burden of malaria incidence and mortality, and prevention of a resurgence of the disease in all countries that are free of the disease (WHO 2015). This target is challenging yet achievable. One of the ways to achieve this is to identify the specific vectors of the disease in a particular disease setting and to understand the roles of the vectors in transmission of malaria during periods of epidemics.

Epidemic malaria in the highland region of Mambilla Plateau is a significant public health problem. There is need to develop a framework that comprises estimates of vector abundance and diversity, and malaria transmission intensity through sporozoite and entomological inoculation rates in this region. There is also need to identify and characterize larval habitats. It is hoped that this information will help to predict malaria transmission intensity, epidemics and the potential impacts of environmental changes. That the data will provide both the local community and the government a basis for risk assessment, and also as a prerequisite for formulation of more targeted control approach.

CHAPTER TWO

2.0: GENERAL LITERATURE REVIEW

2.1: Causative agent of malaria

Malaria is caused by a protozoan parasite *Plasmodium* which belongs to phylum Apicomplexa. Over 200 species of the genus have been described to be parasitic to mammals, birds and reptiles (Rich and Ayala 2006). Of the over 200 species of plasmodia about four affect man. These are; *P. malariae, P. falciparum, P. vivax* and *P. ovale.* However, there has been a recent documented report of an occasional infection of humans with a fifth species of the parasite, *P. knowlesi* in many countries in South-east Asia. However, the WHO is yet to establish the zoonotic transmission of such malaria. Reports of malaria infection with other species such as *P. cynomolgi, P. semiovale, P. brasilianum, P. cynomolgibastianellii, P. inui, P. eylesi, P. rhodiani, P. simium* and *P. schwetzi*are very uncommon (Daneshvar *et al*., 2009).

*Plasmodiumfalciparum*has the widest geographical distribution in tropical and subtropical countries. It is the predominant species in Africa. Almost 85% of cases are ascribed to it while the remaining 15% of cases are due to *P. malariae, P. vivax* and *P. ovale*. In Africa *P. falciparum* is responsible for severe and often fatal periodic fevers due to the nature of its rapid replication in the blood leading to anaemia. A fatal condition due to this species is cerebral malariawhich results from clogging of small blood vessels in the brain. *P. vivax* is the most famous in terms of geographic spread it is found in most parts of Asia, South and Central Americaand in some parts of Africa (Rich and Ayala, 2006). About 70-90% of malaria cases in the Middle East are ascribed to this species. The species has quiescence liver stages that can lead to relapse after many months or years after being bitten by an infective mosquito.

P. ovale is more restricted in terms of geographic distribution. It occurs in the Philippines, tropical Africa (especially West Africa) and New Guinea. This species shares morphologic and biologic resemblance with *P. vivax.* Both carry very low risk of fatal outcomes that are associated with *P. falciparum.* Unlike *P. vivax, P. ovale* has affinity for people with negative blood group and these are found mostly in sub-Saharan Africa. Malaria infections due to *P. malariae*are rare and scattered in nature.

These are in western Pacific and South America, parts of India and Africa. Fevers from *P. malariae* if untreated can lead to a lifetime condition and in some cases resulting in serious complications with the kidneys. *P. knowlesi* is predominant in all of Southeast Asia naturally infecting long-tailed and pig-tailed macaque monkeys. There have been recent reports of this species infecting people in the area especially in Malaysia. There arereports of fatal conditions of infection with this species. The pathogen has been linked to its short replication cycle of 24 hours, thereby resulting in quick progression from mild to intense infection (Carter and Mendis, 2002).

2.2: Malaria vectors

Malaria is transmitted from an infected person to a healthy individual by anopheline mosquitoes. About 3,500mosquito species have been described under four genera. Human malaria is transmitted only by the infective bite of females of the genus*Anopheles***.** Over 400 different species of *Anopheles* mosquitoes exist of which, about 30-40 species are of significant importance as vectors in nature and they bite between dusk and dawn (WHO 2017). *Anopheles* mosquitoes have evolved some characteristic behaviours that have enhanced their vector status and efficiency as transmitters of diseases particularly malaria (Killeen *et al*., 2013). The adult female mosquito requires the protein in blood to develop her eggs before they are laid in water and so she must feed on blood. However, the eggs are laid in batches so she goes in search of another blood meal to develop and lay the next batch of eggs (Sinka, 2013). Male mosquitoes do not lay eggs and therefore do not require a blood meal and naturally, do not have the mouthparts for piercing and sucking blood like the female mosquitoes. The energy needed for both male and female mosquitoes to fly and perform other physiological processes come from sugar. They get this sugar from nectar of flowersand plants, fruit juices, honeydew and those from other naturally occurring sugar-filled juices. Recent studies have reported that larvae of *Anopheles* mosquitoes utilize maize pollen as an important food source (Wondwosen *et al*., 2017). Feeding behaviours differ among different *Anopheles* species. Some females prefer to feed on humans. Thosespecies such as *An. gambiae* are said to be ’anthropophagic’. Others such as *An. arabiensis,* would rather feed on animals, these species are said to be ‘’zoophagic’’. Some *Anopheles* species bite indoors, these are said to be ‘’endophagic’’, while thosethat bite outdoors are termed ‘’exophagic’’.

Some females like to rest outdoors after taking a blood meal, these are ‘’exophilic’’. Some rest indoors and are known as ‘’endophilic’’ (Sinka, 2013).

An.arabiensis is exophilic and zoophilic hence has tendency to transmit malaria to animals rather than humans.here has been suggestion of rearing cattle close to human dwellings to divert biting activities of this species (which is also one of the established malaria vectors in Africa) from humans to animals thereby reducing its efficiency as human malaria vector (Mahande *etal*., 2007; Killeen, *etal*., 2013; Sinka, 2013).

2.3: Species diversity and relative abundance of the anopheline vectors of malaria in the highland areas

The presence of a suitable vector is a major determinant of local malaria transmission intensity. Different *Anopheles* mosquito species are distributed in various geographical locations around the world, including tropical and sub-tropical regions of Africa (Okonkwo *et al*., 2014; WHO 2017). The intricate topography and landscape in the highland regions are believed to have contributed significantly to the diversity and abundance of malaria vectors (Githeko *et al*., 2006; Zhou *et al*., 2007). Theincrease in human population in most African highlands in the recent past, has resulted in deforestation and cultivation of natural swamps (Afrane *et al*., 2006). Consequently, the ecology of African highlands has been changing, favouring mosquito survivorship and parasite development. Abundance of mosquitoes in the highlands is seen to have resulted from this change in land use (Li *et al*., 2008; Githeko *et al*., 2013).

Malaria transmission in the highlands is characterized by instability and high variability. Larval habitats of malaria vectors are not uniform and this has influence on the distribution of the *Anopheles* vector species. It has been suggested that as subsistence agriculture claims most of the forest in the highlands, there couldbe a variation in microclimate of larval and adult habitats alike. This could lead to increase in survival of the larvae and parasite development in the adults. Increased enhancement in vectorial capacity by nearly 100% has been reported in *An. gambiae* where deforestation has taken place (Wanjala and Kweka, 2016).

Several anopheline species have been sampled across various highlands. *An. superpictus* (*form A*) is the principal *Anopheles* species in the highlands of Iran. It is widely distributed in all the rural districts in the highlands. Other species include, *An. superpictus* (*form B*), *An. maculipennis*complex, *An. turkhodi, An. d′thali, An. clavigerAn. marterii An. stephensi, An. apoci, An. culicifacies,* and *An. moghulensis,* (Amani *et al*., 2014, Soleimani-Ahmadi *et al*., 2014). In Colombia, *An. nuneztovari s.s. An. nuneztovari* and *An. darlingi*are the most abundant species of anophelines, other species such as *An. pseudopunctipennis An. albitarsis s.l. An. triannulatus* lineage Northwest (NW) *An. punctimacula* and *An. argyritarsis,* occurring in lesser number (Naranjo-Diaz *et al*., 2013). Species such as *An. stephensiAn. Barbirostris, An. culicifacies, An. subpictus, An. vagus, An. nigerrimu* are predominant in India (Asha *et al*., 2014).

Studies across highland regions of Africa have established *An. gambiae s.s.*and *An. funestus* as the main malaria vector species in the African highlands (Mwangangi *et al*., 2013). In the highlands of western Kenya, these two species are the known malaria vectors (Afrane *et al*., 2006; Zhou *et al*., 2007; Imbahale *et al*., 2011; Githeko *et al*., 2013). *An. arabiensis* and *An. quadriannulatus* are reported from high elevation areas of Zambia (Kent *et al*., 2007) while *An. gambiae s.l., An. funestus* and *An. marshallii s.l.* are the main anopheline species in the highlands of Tanzania, *An. gambiae s.l* being the most abundant species (Maxwell *et al*., 2003).

2.4: Species diversity and the relative abundance of anopheline vectors of malaria inNigeria

Generally, *An. gambiae, s.s, An. funestus, An. arabiensis* are the predominant malaria vectors recorded across Nigeria. Other species such as *An. coustani, An. pseudopunctipennis, An. pharoensis, An. rhodesiensis, An. hancocki, An. ardensis, An. distinctus, An. wilsoni, An. moucheti nigeriensis, An. melas and* An. *rivulorum*are less frequently reported (Okorie *et al*., 2014). They play minor roles in malaria transmission. This is consistent with findings in the Garki project which was carried out in 1980. The anopheline species identified included, *An. gambiae s. s, An. arabiensis, An. funestus, An. rufipes. An. pharoensis,* and*An. Welcome.* Others are *An. squamosus, An. coustani, An. nili, An. maculipalpis,* and *An. pretoriensis.* Only

An. gambiae s.l, and *An. funestus* were implicated with malaria transmission (Molineaux and Gramiccia, 1980).Okwa *et al,* (2009), found *An. gambiae* and *An. funestus*occurring in sympatry with *An.moucheti nigeriensis* in all the zones of the country.This suggests the ability of the anopheline species to adapt to different environmental conditions. Consequently, this would ensure continuous production and abundance of adult vectors throughout the year.

2.5: Factors influencing anopheline species diversity and relative abundance

Mosquitoes are very sensitive to changes in their environment. Slight changes in environmental conditions and availability of suitable habitats, significantly influence their abundance, densities and survival (Afrane *et al,* 2012). Adult features of mosquitoes can be determined to some extent by conditions experienced during larval development (Moller-Jacobs *et al*., 2014). Studies have shown that changes in environmental characteristics such as humidity, rainfall and temperature influence larval densities (Zhou *et al*., 2007; Soleimani-Ahmadi *et al,* 2014). Christiansen-Jucht *et al*., (2014), demonstrated significant effect of environmental temperature on the survival of *An. gambiae s.s*., both during their immature stage development and during their lifetime as adults. Higher temperature and food availability were shown to reduce larval to adult development time. They were also associated with production of larger females (Munga *et al*., 2006).

Abundance and diversity of mosquitoes tend to increase during the rainy season (Zhou *et al*., 2007). For example, rainwater often forms pools that are ideal larval breeding sites, thereby resulting in the increase of adult malaria vectors (Hriber, 2005; Zhou *et al*., 2007). Both rainfall and humidity have significant effects on the longevity of adult mosquitoes (Li *et al*., 2008). Populations of mosquito larvae seem to increase with increase in humidity. Larvae of*An. gambiae*in particular predominate in environments with high humidity andrainfall (Mwangangi *et al*., 2007; Attaulah, *et al*, 2015). Seasonal changes bring about unstable population density of *Anopheles* larvae (Munhenga *et al.*, 2014). In the Garki project, *Anopheles* mosquitoes increased appreciably during the wet season. Large seasonal variations were observed in densities of the principal malaria vectors, *An. funestus* and *An. gambiae s.l.* (Molineaux and Gramiccia, 1980). In Karachi, seasonal variation in abundance of mosquitoes was the driving force of malaria incidence (Shaikh *et al*., 2014).

Relative abundance and diversity of both larval and adult mosquito population vary by type and size of aquatic habitats. Habitat type influence selection of oviposition sites by the adult mosquitoes (Asha *et al*., 2014). Development and survival rate of the adult malaria vectors has also been linked to the type of larval habitat (Bashar and Tuno, 2014). It has been suggested that significant relationship exist between locations where larval habitats are repeatedly observed, and distribution of adult mosquitoes (Zhou *et al*., 2007; Li *et al*., 2008). Population of adult vectors was shown to cluster around most of the larval habitats (Zhou *et al*., 2007). Some species are found in both temporary and permanent habitats (Vanlalruia *et al*., 2014), certain species show lowest diversity and highest abundance in the urban environments (Thongsripong *et al*, 2013).

Zhou *et al*., (2007) found that habitats which are in close proximity to human habitation harbor higher populations of anopheline larvae. For example, species like *An. gambiae* have orientation towards humans. The presence of such species close to human dwellings is viewed as a strategy by the ovipositing females for energy conservation (Mwangangi *et al*., 2010). Agricultural practices play significant roles on the diversity and abundance of mosquito species. Positive correlation exists between irrigation development and increased malaria transmission (Rejmánková *et al*., 2013). *An. gambiae* and *An. funestus* have been shown to thrive well in habitats that are linked with agricultural crops (especially rice) or swamp margins. It is observed that each species has preference for different phase of the rice development. Where rice production is done all year round, number of larval habitats increase resulting in increased malaria vectors production (Imbahale *et al*., 2011).

Anthropogenic activities such as open pit mining and irrigation, create larval habitats which sustain production of the adult mosquito vectors throughout the year (Surendran and Ramasamy, 2005). Risk of malaria transmission has been associated with artificial or manmade lakesthat are formed by dams, either for agricultural purposes or hydroelectric projects. These become ideal breeding sites for anopheline mosquitoes as they are colonized and overgrown with vegetation (Rejmánková *et al*., 2013). Predation is a natural regulatory force on anophelines larval production (Kweka *et al*., 2011). Predatory impact of other invertebrate species composition in a

habitat leads to significant reduction in the populations of anopheline larvae (Kweka *et al*., 2012). Densities of larvae in the end affect the abundance of the adult population (Knight *et al.,* 2004). The role of predators in the natural regulation of mosquito larvae has been reported. For instance, number of *An. gambiae s.l, An. funestus* and other anopheline species were significantly reduced by predators in Ighuhu, Kenya (Kweka *et al*., 2012).

2.6: Larval habitats of the anopheline mosquitoes

One of the most common strategies to eradicate malaria has focused on mosquito larval control (Dida *et al*., 2015). Mosquito larval habitats are places where mosquitoes breed. From the point of egg laying to completion of the life cycle, to emergence as adults (Eckhoff, 2011). The larval habitat is therefore very vital for mosquito population. This is because, the development and fitness of the adult mosquitoes are impacted by the status of their aquatic larval habitats (Onchuru *et al*., 2016). Larval habitats are made up of diverse water bodies. Theserange from temporary to permanent, natural to artificial, small to large fresh water to salt water, shaded to sunny (Amani *et al*., 2014; Asha *et al*., 2014). A greater number of anopheline species breed in a broad range of habitats (Reimer *et al*., 2016), buteach species shows preference for various breeding sitesfor oviposition (Afolabi *et al*., 2010).

In the Rift valley of Central Ethiopia for example, the major anopheline vectors of malaria *An. arabiensis* and *An. pharoensis* show preference for sand poolsand natural swamps respectively (Kenea *et al*., 2011). In western Kenya aquatic habitats such as stream pools, puddles, tire tracks, ponds and swamps are the major larval habitats identified along the coast. Puddles stream pools, and tire tracks are the most preferred and productive habitats for *An. gambiae* larvae (Mwangangi *et al*., 2007). Natural and permanent habitats like riverbeds, are the main habitats responsible for continuous production of the adult vectors throughout the year in Iran (Amani *et al*., 2014; Soleimani-Ahmadi *et al*, 2014).

Sibling species of the *An. gambiae s.l* differ in their preference for breeding habitats. *An. gambiae s.l.* can be found in both temporary and permanent habitats (Imbahale *et al*., 2011), polluted and unpolluted man-made habitats such as irrigation wells, gutters and polluted ponds (Kudom *et al.*, 2012). Larvae have also been collected from polluted water rich in organic matter (Awolola, *et al.*, 2007; Castro *et al.*, 2010). In large bodies of water such as flood plains (Majambere *et al.*, 2008), and in pools along lake shores, especially when there are changes in water level (Minawaka *et al.*, 2008). Larvae of *An. arabiensis* are most abundant in sand pools along the edges of rivers (Ye-Ebiyo *et al.*, 2003). *An. melas* and *An. merus* are most productive around the coastal areas where they exhibit great tolerance for salinity. They survive in relatively high degrees of salinity sometimes, reaching or even exceeding that of seawater (Hanafi-Bojd *et al*., 2012).

Several factors are known to impact on larval development and growth. Some of which are quality of habitat, and physicochemical parameters of the breeding habitat. Physicochemical parameters such as dissolved oxygen, temperature, conductivity, PH, alkalinity, hardness, total dissolved solids, turbidity. Presence of ions like sulphates, nitrates, lead, iron and arsenic also influence development and survival of larvae. These subsequently affect the abundance and species diversity of anopheline vectors of malaria (Afolabi *et al*., 2013; Dida *et al*., 2015). It is suggested that selection of larval habitats is likely determined among other factors, by the physicochemical parameters of the water bodies (Rejmánková *et al*., 2013). Anopheline vectors show varying tolerance for each physicochemical parameter. For example, physicochemical parameters such as pH, dissolved oxygen, electrical conductivity and temperature impact larval abundance (Afolabi *et al*., 2013). High levels of Nitrates and pH significantly influence the abundance of *An. gambiaes.l.* larvae. This is in contrast to *An. funestus* and other anophelines species (Kweka *et al*., 2011). Chemical characteristics including conductivity, total alkalinity, sulphate and chloride, have significant effects on distribution and abundance of anopheline species in Iran (Soleimani-Ahmadi *et al*., 2014).Water temperature, turbidity, and dissolved oxygen were the major determinants of the abundance and distribution of mosquito larva in the aquatic habitats in Mara River in Kenya (Dida *et al*., 2015).

Anopheline species are found in habitats associated with different vegetation types. Some occur in temporary shallow water bodies, with algae or short grasses or completely devoid of vegetation (Mwangangi *et al*., 2007). *An. pharoensis* breeds in large vegetated swamps and along lakeshores among floating plants (Amani *et al*., 2014). The presence of algae mats is a major environmental factor positively associated with the abundance of *An. pharoensis* and *An. squamosus* larvae (Azari-Hamidian *et al*, 2011). Freshwater marshes rich in filamentous algae and tall dense macrophyte are usually habited by *An. vestitipennis* and *An. Pseudopunctipennis*. *An. farauti* larvae are found in habitats with filamentous algae, while *An. darlingi* potential habitats include, streams and river edges, with predominantly submerged macrophytes (Achee *et al*., 2006).

The physicochemical parameters of larvae breeding habitats on the Mambilla plateau are unknown. The knowledge of the factors that favour the adaptation of larvae to their habitats in a targeted habitat, could be an effective measure to control malaria through vector management. One of the potentially significant points in malaria vector control in the highlands, is to understand each species threshold to environmental factors, and their adaptation across altitudinal gradients. This may provide useful insight into mosquitoes' congregation (Dida *et al*., 2015). The identification of larval habitats and mosquito tolerance range to environmental variables could inform measures to control the insurgent of the vector mosquitoes, and guide mechanisms to prevent epidemics.

2.7: Sporozoite rates in anopheline mosquitoes

The capacity of *Anopheles* mosquitoes to transmit malaria to humans, is determined by the presence of infective *Plasmodium* sporozoites in their salivary glands (Foley *et al*., 2012). The sporozoite rate is defined as the number of *Plasmodium*-infected mosquitoes, divided by the total number of mosquitoes sampled, expressed as a percentage (Kilama *et al*., 2014). The sporozoite infection rate (SR) of the mosquito population, is a responsive means to explain the pattern of malaria transmission in a given location. It is also used to determine the Entomological Inoculation Rate (EIR), which is an estimate of human vulnerability to *Plasmodium*-infected mosquitoes. It is the most common entomological measure for assessing malaria endemicity and transmission intensity. It is particularly useful when estimating the effect of human-

vector contact (Kelly-Hope and McKenzie, 2009). It is also a measure for monitoring malaria control programmes (Churcher *et al*., 2015).

Large variations have been recorded in malaria transmission intensities, between localities in different ecological zones. Even when these zones are separated by only short distances (Robert *et al.*, 2003). There has been high positive sporozoite mosquito estimates, from one house while none at all in other houses (Drakeley *et al*., 2003). Studies in other African countries also indicate significant variations in sporozoite rates (Pates *et al*., 2006). The opportunistic behavior of some anophelines such as *An. arabiensis*, could explain concentration of transmission within a particular location (Elmahdi *et al*., 2012). Transmission efficiency, length of the extrinsic incubation period, and the mosquito mortality rate are also some factors that determine sporozoites prevalence in mosquitoes. However, these factors are likely to vary from one location to another (Smith*et al*., 2004; Paaijmans *et al*., 2010). Another factor which influence sporozoite rates are, increased distance of houses from breeding sites which result in low sporozoite levels (Mendis *et al*., 2000). Urbanization, which comes with high population density, improved housing, expanded personal protection and effective diagnosis and treatment (Wilson *et al*., 2015) also influences sporozoite rates.

2.8: Entomological inoculation rate (EIR)

The entomological inoculation rate is the average number of infectious bites received by a person per year. It is a means of estimating human exposure to infectious mosquitoes (Kilama *et al*., 2014). Low entomological inoculation rate will mean low malaria transmission. It is an estimate of human vulnerability to *Plasmodium*-infected mosquitoes, and is seen as the best means to express malaria transmission intensity (Hay *et al*., 2008). The EIR is deduced from the density of man biting and sporozoite rates and the human blood index within the anopheline mosquito population (Drakeley *et al*., 2003). Entomological inoculation rate is very important in places with more than one malaria vector, to determine the role of each of the species in transmission (Galardo *et al*. 2007).

Several components affect entomological inoculation rate; among are altitude, temperature, rainfall and urbanization (Worral*et al*., 2002). Shililu *et al*., (1998) observed recurrent but low malaria transmission intensity in high altitude, with seeming seasonal variation in entomological inoculation rate. Temperature decrease with increasing altitude, and parasite development time is influenced by temperature. Entomological inoculation rates therefore decrease with increasing altitude (Lindsay and Martens, 1998). Entomological inoculation rates increase with increases in the amount of rainfall. Increase in rainfall provide the female mosquitoes with ideal habitats to oviposit (Robert *et al*., 2003). At the end of the transmission period, entomological inoculation rate values have been shown to have doubled compared to the figures at the beginning of the transmission period. Furthermore, higher densities of mosquito vectors and entomological inoculation rates are associated with nearness to farmlands or households and breeding sites, as well as irrigated areas ((Elmahdi *et al*., 2012).

Several studies across Africa have reported high EIR values usually during the wet seasons. This translates to high risk of exposure to infective bites (Ukpong *et al*., 2015). Wet season tend to witness highest number of mosquitoes with high entomological inoculation rates. This is most probable with areas within close distance but with differing land use patterns. A 50% reduction in entomological inoculation rates has been observed every 90m away from breeding sites in Mozambique (Mendis *et al*., 2000). Rural environments have been reported to experienc higher intensities of transmission than the urban environments (Drakeley *et al*., 2003).

There is pronounced heterogeneity in EIR estimates across Africa (De Silva *et al*., 2012). Generally, EIRs estimates in urban areas are lower than in rural areas. However, some urban areas have recorded very high EIRs (Wilson *et al*., 2015). For example, a study in Libreville Gabon, recorded the highest EIR of 87.9 infective bites per person per year in the urban area while the peri-urban area had EIR of 13.3 infective bites per person per year (Mourou *et al*., 2012). Annual EIRs in Columbia ranged from 3.5 to 4.8 infective bites per person per year (Naranjo-Diaz *et al*., 2013). Different highland ecosystems have recorded variations in EIR (Wanjala *et al*., 2011). For example, a study has revealed ecosystems characterized by narrow valleys with

fast flowing rivers, with EIRs in the range of 0.4-11 infective bites per person, while those characterized by flat-bottomed valleys with slow moving rivers, having annual EIRs of 16.6 infective bites per person (Himeidan *et al*., 2009). EIRs in Nigeria are in the range of 18 to 145 and 12 to 54 infective bites per person per year for *An. gambiae* and *An. funestus* respectively (NMCP-FMoH, 2009).

The lifespan of the female adult mosquito vector is significant in malaria transmission. For transmission to take place, the mosquito must live long enough for *Plasmodium* parasites to complete sporogony (Breman, 2001). The sporogonic cycle is the time from when the female *Anopheles* mosquito takes a *Plasmodium*-infected blood meal, to the time when the blood meal is digested, and the infective stages of parasite appear in the salivary glands. For *P. falciparum* this takes about 10 days (Killeen *et al*., 2002). EIR decrease if mosquito lifespan is reduced hence, variability exists in the EIR, with a resultant variation in malaria transmission intensity across different locations (Shaukat *et al*., 2010).

2.9: Stability of malaria transmission

Stability of malaria is defined by transmission of the disease throughout the year by man-biting vector anopheline mosquitoes (Kiszewski *et al*., 2004). Africa is situated in the tropics and is characterized by warm temperature, heavy rainfall, high relative humidity, as well as very efficient *Anopheles* mosquito vectors. These present conducive environment for malaria to thrive. The region more than other parts of the world, experiences the most intense malaria transmission (Breman, 2001; Kiszewski *et al*., 2004).

There is a general decline in malaria transmission. Globally among populations at risk, there is a 37% reduction in new cases and 65% decrease in malaria death rate among children less than five years. There is a 60% decrease in malaria death rate among all age groups between 2000 and 2015. However, the region continues to carry a high proportion of the global malaria burden with 88% of malaria cases and 90% deaths (WHO, 2015). Generally, sub-Saharan Africa seems to face greater challenges with malaria intervention. This is believed to arise from the interaction between climate and physiological characteristics of various mosquito vectors (Lindsay and Martens, 1998; Shaukat *et al*., 2010).

Malaria transmissions across different geographical zones show varying trends. Transmission is less stable in some parts of the tropics, and particularly least stable in the more temperate parts of the world (Blandford *et al*., 2013). Savannah regions of West and Central Africa experience stable malaria transmission. This is more intense due to the uninterrupted heat that characterizes the region, exacerbated by the anthropohilic nature of the malaria vector mosquitoes in the region (Kiszewski *et al*, 2004).

Malaria in the highlands is characterized by unstable and high transmission variability, that results in epidemics during periods of suitable climate conditions (Wanjala *et al*., 2011). The intensity of transmission in the highlands may be altered through anthropogenic factors such as occupational activities which increase man-vector contact and seasonal migration and decrease in malaria control operations. These may lead to the emergence of efficient vectors (Cox *et al*, 1999; Zhou *et al*., 2007). Average population densities in highland areas are usually relatively high. This renders large populations at risk from infection and epidemics, as malaria transmission cycle involves available human hosts (Cox *et al*., 1999). In holoendemic areas, people may harbor malaria parasites in their blood without showing any clinical symptoms of the disease. Consequently, this results in intense transmission with more pronounced levels of illness and death (Cox *et al*., 1999). Transmission is normally moderate and seasonal around the coastal regions (Reimer *et al*., 2016).

Malaria indicator models have demonstrated reduced malaria transmission, in countries with high human population in non-endemic regions. Malaria transmission depends on the mosquito vector and the human-vector interaction. Therefore, human population density plays a significant role in stability of malaria transmission. Economic development and urbanization have brought about great changes in malaria transmission (Smith *et al.*, 2013). Urban areas have higher human population density but limited mosquito density. This is due to the limited number of vector habitats hence, lower levels of malaria endemicity (Tattem, 2008), and therefore sustaining malaria transmission is difficult because, transmission is determined by the number of mosquito bites per person at a given time (Smith *et al.*, 2012). In the rural areas however, transmission is linked to nearness to breeding sites, and is most common in

irrigated areas such as rice paddies, rivers, ponds and unstable water bodies (Smith *et al*., 2012).

In Nigeria malaria is endemic in all parts of the country however; intensity of transmission varies according to geographic location. In most of the south, the duration of malaria is perennial and lasts between 7-12 months, but is epidemic or strongly seasonal lasting three months or less in the northeastern region bordering Chad (MIS, 2010). Results from the Garki project which was conducted in a savannah region of Nigeria, demonstrated very high intensity of malaria transmission. However, there was variation from one locality to the other, and year to year. One of the factors that contributed to transmission was the exophily of some anopheline species particularly *An. gambiae s.l.* (Molineaux and Gramiccia, 1980).

Studies on malaria transmission dynamics have not been conducted in The Mambilla Plateau, which has varying altitudinal gradients, hence the aim of this study.

CHAPTER THREE

3.0: GENERAL MATERIALS AND METHODS

3.1: Study Area

Mambilla Plateau is located at longitude 6.8212° N, 11.5345° E and latitude 7.3523° N, 10.7723° E in Taraba State North-Eastern Nigeria. Mambilla Plateau has an area of about 3765sq km while the adjoining lowland covers about 1,250sqkm. It has boundaries with Gashaka Local Government Area in the north-east, Kurmi Local Government Area in the north-west and Republic of Cameroonin the south. It is located in a savannah landscape with a peculiar topography and climate. The topography comprises undulating lowland, low hills and irregular plains, ridges, hills, and escarpment. Climate is semi-temperate with mean annual temperature of 16°C and average altitude is 1600m above sea level. Mean annual rainfall is 1800mm. The rainy season extends from early April to October while the dry season occurs from November to March.

Mambilla Plateau has a population of 224,357 people (National Bureau of Statistics, 2012). The people engage in agriculture and stock herding. Crops grown include; maize, millet, sorghum, and rice. Tea, coffee, cocoa and ginger are also grown as well as yams, sweet potatoes, Irish potatoes, cassavas, apples, avocadoes and pears among others. Figure 1 is a map of Nigeria showing Mambilla Plateau and the study locations.

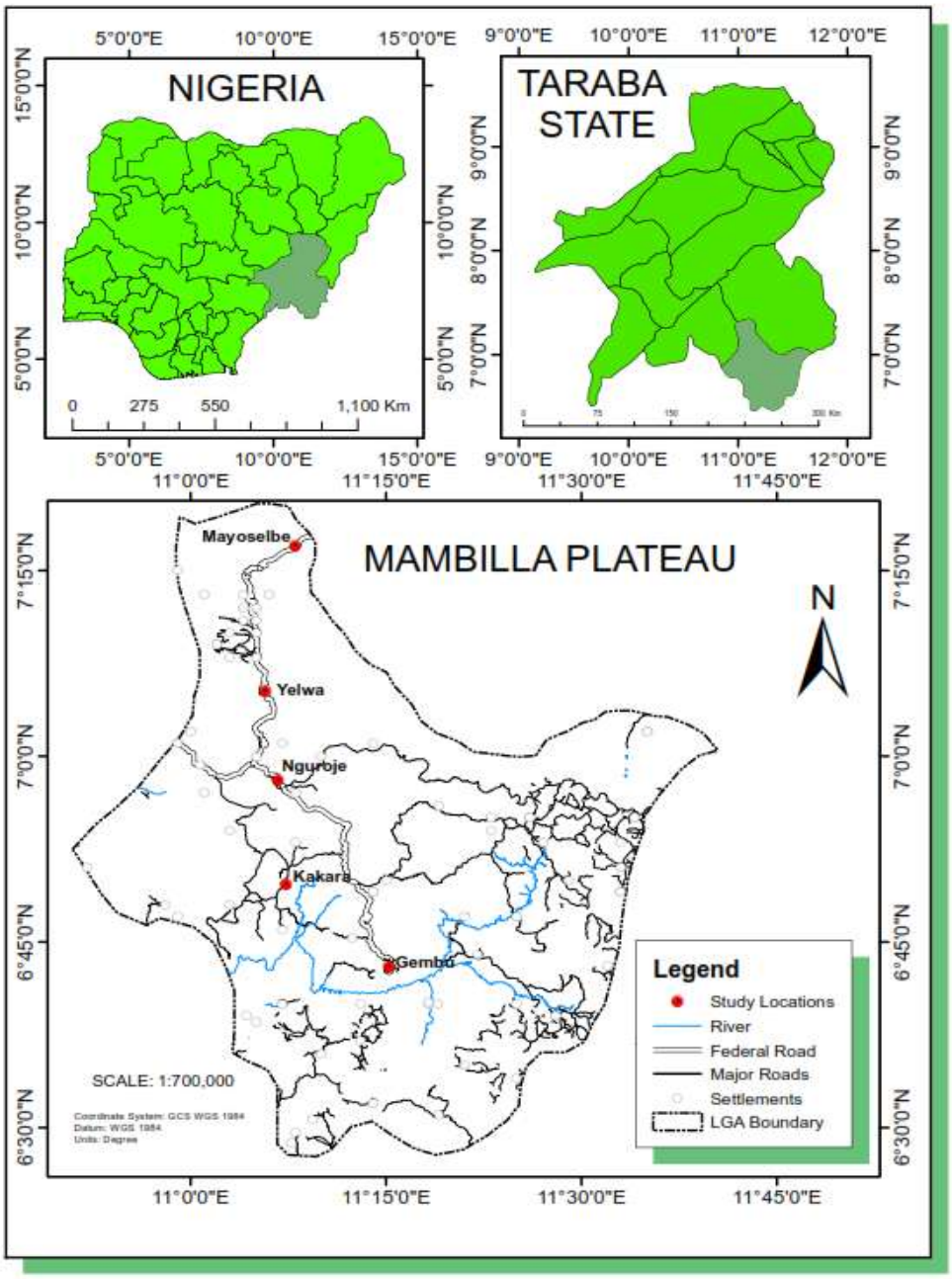

Figure 1: A Map of Nigeria showingMambillaPlateau and the study locations.

3.2: Study Design

The study was conducted from November 2015to October 2017 in five locations, selected along an altitudinal gradient to collect entomological data. The rationale for selection of the locations was based on entomological grounds, availability of larval habitats, altitudinal difference and access road throughout the year. The locations were Nguroje (1,885m), Yelwa (1,674m), Gembu (1,584m), Kakara (1,496m) and Mayo Selbe (484m), above sea level. Entomological surveys were carried out in all

the five altitudinal locations. Mosquito collections were conducted once every month. Larvae of anopheline vectors were sampled from various breeding sites in all the locations, using the standard dipping method, pipettes, and white plastic bowls (Service, 1993).

Larval habitats were characterized and described according to features observed in the field. Adult mosquitoes were captured by two methods, the use of Centre for Disease Control (CDC) modified light traps and Pyrethrum Spray Catches (PSC).

3.3: Approval to conduct research

There was no ethical board in place to give ethical clearance for the research so in place of clearance, a written approval letter was obtained from The Taraba State Ministry of Health (Ref. No: S/MOH 463 T.5/569) as shown in Appendix I.

3.4: Mosquito collections and preservation

3.4.1: Light trap collection (CDC)

A traditionally defined cluster of 10 houses in each of the altitudinal locations were chosen and three (3) houses equitably dispersed were randomly selected for inclusion in sampling. This gives a total of fifteen (15) houses. Mosquitoes were collected once every month. In every mosquito trapping night, light traps were positioned both inside and around the houses between 6pm in the evening and 6am in the morning. The traps were inspected every one hour. Members of *Anopheles* mosquitoes captured were aspirated into clearly labeled paper cups. Indoor traps were suspended from the ceiling at the foot end of the bed at approximately 1.5meters above the ground level in an occupied room (these were fixed stations for collection). Figure 2 shows indoor mosquito collection using the light trap. The outdoor traps were hung on a post around the same houses. Both indoor and outdoor temperatures and relative humidity were recorded as depicted in the raw data contained in Appendix II.

Figure 2: Indoor mosquito collection using CDC-light trap

3.4.2: Pyrethrum spray collection

Pyrethrum Spray Collections were carried out from an average of eighteen rooms where at least one inhabitant slept the previous night, but in which indoor residual spraying was not done as this may interfere with the result of the pyrethrum spray catch. PSCs were carried out in the morning between 6am and 10am by spreading white sheets on the entire floor and over the pieces of furniture that could not be moved, after which the rooms were sprayed with pyrethrum. The rooms were closed for about 10 to 15 minutes for mosquitoes to be knocked down. The knocked down mosquitoes were collected from the white sheets with forceps and put in petri dishes containing moist filter paper. The petri dishes were well labeled according to collection site, elevation and date of collection. The number of people sleeping in the room the night before collection period was also recorded. Female mosquitoes were classified according to their repletion status into unfed, fed, half-gravid and gravid specimens (WHO2009). Figure 3 shows indoor mosquito collection using PSC.

Figure 3: Pyrethrum spray collection of anopheline mosquito vectors.

3.4.3: Larval collection and rearing

Larvae were collected using the standard dipping method (Service, 1993) between 8am to 10.00am. In all the locations, larvae of all available instar were collected. Anopheline larvae were distinguished from culicines based on their resting habits in water and the respiratory siphons. The edge of the dipper was submerged, dipped at about 45 degrees about an inch below the surface of the water. This was done quickly but gently the dipper was moved along a straight line in the water. The stroke was ended just before the dipper was filled to avoid overflowing. The dipper was then gently raised out of the water without spilling the water and the larvae. Depending on the size of each larval habitat, 10-30 dips were taken at intervals along the edge for about 30minutes at each larval habitat (Kenea *et al*., 2011). The mosquito larvae were then transferred into plastic containers along with the breeding sites water. The plastic containers were labelled according to type of habitat, location of collection and coordinates of habitat. Time and date of collection were also recorded.

To rear the mosquitoes, larvae from the field were transferred into small white transparent plastic buckets. These were filled to two-thirds of their volume with the breeding site water. The mouths of the plastic buckets were covered with mosquito net. A small hole was made at the centre of the net and plugged with a dry cotton wool until adults emerged (Service, 1993). Larvae were on with baker's yeast.

All the collected mosquitoes were taken to the laboratory for morphological identification by microscopy**.** Mosquito samples were preserved individually in an Eppendorf tube each on silica gel. They were later transferred to the Molecular Entomology and Parasitology Laboratory at the Nigerian Institute for Medical Research (NIMR), Lagos for further analysis by Polymerase Chain Reaction (PCR).

3.5: Preparation of mosquito samples for analyses

Female anophelines were dissected transversely at the thorax between the 1st and 3rd pairs of legs to severe the mosquitoes into two parts. The wings and legs were used for DNA extraction, the head and thorax were used for sporozoite detection while the abdomens of the anophelines were used for blood meal analysis.

3.6: Data Analyses

Relative densities of species were calculated to compare the number of female anophelines captured in different altitude locations according to Bashar and Tuno, (2014). Shannon Wiener diversity index (H') Shannon, (1948) was used to estimate species diversity of the mosquitoes caught at different locations and each month of study. Larval density was determined from the breeding index according to Belkin (1954).

CHAPTER FOUR

4.0: SPECIES DIVERSITY AND RELATIVE ABUNDANCE OF ANOPHELINE VECTORS OF MALARIA

A component of this chapter was published as:

Species Diversity and Relative Abundance of Anopheline Vectors of Malaria on the Highlands of Mambilla Plateau Northeast, Nigeria, *Journal of biotechnology and bioengineering* Volume 1, Issue 1, pp 37-42 by Garba *et al*., (2017). (Appendix III).

4.1: Background

About 30-40 species of *Anopheles* species are vectors in nature. Most members of the *An. gambiaes.l* are responsible for transmitting*P.falciparum,* the parasite that causes the most severe form of malaria in sub-Saharan Africa (WHO, 2017). The *An. gambiae s.l* is a species complex that contains seven sibling species that are morphologically identical and indistinguishable. The sibling species include; *An. arabiensis, An. bwambae, An. gambiaesensostricto, An. melas, An. merus, An. quadrinnulatus A,* and*An. quadrinnulatus B.* The *An. gambiae s.s* species is now undergoing speciation. Five chromosomal forms are now known to include the Bamako, Bissau, Forest, Mopti and the Savannah, as well as two molecular forms, referred to as the M and S forms (Sinka, 2013).

These sibling species exhibit several behavioural differences which enhance their vector efficiency, these include their geographical distribution, biting and resting preferences. Other differences exhibited by the sibling species are, resistance to insecticides and host preference (Ebenezer *et al*., 2014;Wiebe *et al*., 2017).For example,*An. gambiae s.s, An. arabiensis, An. quadriannulatus A* and *B* are known to occur in freshwater, while *An. bwambae, An. merus* and *An. melas* are found in brackish water (Lehmann and Diabate, 2008). The molecular forms are found in sympatry however, the M form predominates in arid, regions and mostly in irrigated areas or rice fields in West Africa. The S form is dominant in humid, forest habitats across West and East Africa (Lehmann and Diabate, 2008; Sinka, 2013). Species diversity arises majorly from speciation, and this has serious epidemiological implications to disease transmission. New and more efficient vectors might arise, and this could further complicate malaria transmission intensity.

4.2: Materials and Methods

4.2.1: Morphological identification

All mosquitoes were identified by microscopy to species level using the morphological keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987). A stereomicroscope (Leica model NSW series IMNS 210) and Olympus Tokyo VT-II 225329 entomological were used. The identified mosquito samples were stored dry individually on silica gel. The key features that were used in identification include the following; the palps, which are are usually smooth and have three pale rings occasionally including a wide ring at the tip. In adult females the palps are as long as the proboscis and antennae lack plumes. The legs are irregularly speckled being very conspicuous in some and only just visible in others, the tibiae are narrow with pale ring at the tips. Tips of segments 1-4 of tarsi are pale while segments 2-4 are pale at the base. The pale areas of the wings are very variable in extent. Sometimes they are greatly reduced and the scutella are single lobed. Specimens that were positively identified as belonging to *An. gambiae s.l* were further tested by Polymerase Chain Reaction (PCR). Other species were not identified by PCR. Figure 4 is mosquito identification by microscopy.

Figure 4: Morphological identification of anopheline vectors by microscopy.

4.2.2: PCR identification of members of *Anopheles gambiae* complex and the molecular forms of *Anopheles gambiaesensu stricto*

All of the adult mosquitoes morphologically identified as *An. gambiae s.l.*, were differentiated to species level using PCR. This was performed with universal and species-specific primers for the *An. gambiaes.l.* Molecular identification of *An. gambiae* species complex is based on the species-specific nucleotide sequences in the ribosomal DNA (rDNA) intergenic spacers (IGS) following the procedure of Scott *et al*., (1993). Five sets of primers designed from the DNA sequences of the IGS region of *An. gambiae s.l* rDNA were used in PCR for the member species identification. The sequence details of the primers are abbreviated UN, GA, ME, AR and QD. The UN primer anneals to the same position on the rDNA sequences of all five species, GA anneals specifically to *An. gambiae sensu stricto*, ME anneals to both *An. merus* and *melas*, AR to *An. arabiensis* and QD to *An. quadriannulatus*.

The PCR reaction mix of 12.5µl contained 1X PCR buffer, 1.25µl of each of the 4 oligonucleotide triphosphates (dNTPs), 1.0µl of each ligonucleotide primers and 0.5 µl of Taq. DNA polymerase enzyme and 1.0µl of the genomic DNA was used as template for the amplification reaction. To improve specificity, primers that were not found in the sampling location were excluded in the master mix. Sterile double distilled water was used to make up the volume to 12.5µl for a single sample as contained in Table 4.1, and using pre-mix as in Table 4.2 using individual constituents. Each of these constituents of the master mix was multiplied by the number of samples identified (Scott *et al*., 1993).

Table 4.1: The reagents used for preparation of pre-mix for identification of *Anopheles gambiae s.l*

Reagent	**X1(µl)**
Pre-mix	4.0
ddH_2O	5.25
ME	0.5
AR	0.5
GA	0.5
UN	0.5
QD	0.25
DNA	1.0
Total	**12.5**

Table 4.2: The reagents used for preparation of master mix for identification of *Anopheles gambiae s.l* using individual constituents.

Reagent	X1 (µl)
PCR Buffer (X10)	1.25
dNTPs	1.25
GA	1.0
ME	1.0
AR	1.0
UN	1.0
QD	0.5
$MgCl_2$	0.5
ddH_2O	3.9
Taq	0.1
DNA	1.0
Total	12.5

4.2.3: PCR protocol for *Anopheles gambiae* complex

One leg or wing of each mosquito was placed in 1.0 µl centrifuge tubes and appropriately labelled. A mixture of the primers and 12.5 µl master mix was pipetted and added to the micro centrifuge tubes, containing the one leg or wing from each of the mosquitoes. A positive control with PCR products of *An. gambiae* of the same primer set, and a negative control without DNA template were included for each reaction. The PCR machine was programmed for *An. gambiae* complex. The reaction mixture was centrifuged for 3minutes at 14000 rpm in order to separate the template DNA from the tissues, and the reaction was placed in the PCR machine. The mixture was spun with the aid of a PTC 100 thermal cycler (MJ Research Inc., USA) according to the PCR conditions as follows; Initial Denaturation at95^{o}C briefly for 2 minutes, 30 cycles each of denaturation at 95^{o}C for 30seconds, annealing at 55^{o}C for 30seconds, extension at 72^{o}C for 40seconds and final extension at 72^{o}C for 7minutes. The amplified DNA was separated on a 1.0% agarose gel stained with ethidium bromide and viewed on a UV transilluminator (Scott *et al*., 1993).

4.2.4: PCR protocol for *Anopheles gambiaesensu stricto*

For the identification of molecular forms of *Anopheles gambiae s.s* the constituents of the digest is given in Table 4.3. 0.2 µl enzyme and 0.6 µl buffer. 1.0µlsterile double distilled water was used to make up the volume to1.8µl for individual constituents. Protocol involved adding 1.8 µl of digest mix and 10 µl of PCR product to each

tube.PCR was performed as in the case of *An. gambiae* complexand the product was run on 1.0% agarose gel as well.

Table 4.3: Substances used for preparation of Digest for identifying the M and S molecular forms of *Anopheles gambiae s.s*

Constituents	**X1(µl)**
Enzyme	0.2
Buffer	0.6
ddH_2O	1.0
Total	**1.8**

4.2.5: Preparation of 1.0% agarose gel

The PCR reaction product (DNA amplified) that were obtained, were examined in a 1.0% agarose gel electrophoresis. A gel mould was prepared by taping tightly to seal the two open ends. The comb was placed about 1.5cm from the left to the end, making sure the wells were not touching the side of the mould frame. The mould was kept in the refrigerator until required, during which 0.50g of agarose was weighed using an electric balance and poured into a 250ml beaker and 50ml of 1.0% Tris Boric EDTA (1XTBE) was added.

The top of the beaker was plugged loosely with paper towel and microwaved for 30seconds and mixed by swirling. It was microwaved again for an additional 25 seconds and swirled until the agarose was completely dissolved in the 1 XTBE. Cold water was poured over the flask for 3 minutes, this reduced the temperature to about 50˚C such that it could be held by hand. 10µl of Ethidium bromide (EtBr, Aldrich EISI0) was added to the agarose solution and mixed carefully by swirling. The solution was poured into the gel mould in the refrigerator and allowed to polymerize within 25minutes (Scott *et al*., 1993).

4.2.6: Gel electrophoresis of PCR product

The comb was carefully removed from the agarose gel by lifting up and pulling slightly on one side to ensure that the entire well remained intact. The tape was then removed from the frame and the gel in the mould frame was placed in the

electrophoresis tank. The wells were placed closest to the negative electrode and positioned to the left. IXTBE buffer was poured into the electrophoresis tank, such that it just covered the surface of the gel stage and to fill all wells (Scott *et al*., 1993).

4.2.7: Loading of PCR product

On waxy paper or paraffin, 2µl of loading dye was placed on the twenty spots from which each of the twenty wells (one for the reference ladder 100bp and for each of the test samples) was loaded. The reference lane (1st well in this case) was floaded first. This was done by mixing 5ul of 100bp DNA ladder with the loading dye on the wax paper. The seventeen test samples were similarly loaded, by mixing 5µl of each test samples with the 2µl of the loading dye on the wax paper and placed in the remaining lanes. Notice was made of each lane each sample was loaded onto. The positive and negative electrodes of the electrophoresis apparatus were connected to the power source and the power switched on. The gel was ran for 35min at 120v, after which the power was switched off. The gel was carefully removed with a spatula into a plastic bag and taken to the dark room for photography (Scott *et al*., 1993).

4.2.8: Photomicrography of agarose gel

The gel was placed on the transilluminator and covered with an ultra violet (uv) blocking shield for initial examination. The DNA amplification successes were indicated by the presence of white bands. The amplified DNA bands were photographed using a black and white Helcam Polaroid Camera. The transilluminator was switched off and the film tab was pulled straight and forcefully cut. The film tab was allowed to develop for 30 seconds. The photograph was pulled off from other film components. The DNA bands produced by PCR were promptly labelled, matching each lane with the test samples loaded. The gel was disposed in the laboratory waste bin in a sealed polythene bag (Scott *et al*., 1993).

4.2.9.: Data Analysis

Relative densities of species were calculated to compare the number of female anophelines captured in different altitude locations according to Bashar and Tuno, (2014). Shannon wiener diversity index (H') (Shannon1948), was used to estimate species diversity of the mosquitoes caught at various study altitudinal locations for the respective months of collection.

4.3: Results

4.3.1: Morphological identification of anophelines

A total of 878 anopheline mosquitoes comprising of five species were collected across the five altitudinal locations. *An. gambiae s.l*, was the most abundant of all the species collected that is; 757(86.22%), followed by *An. coustani* 73(8.31%), *An. funestus* 29(3.30%), 18(2.05%) and the least was *An. rufipes* 1(0.11%). Mayo-selbe recorded the highest number of mosquitoes collected 572(65.15%), Gembu was next with 163(18.56%), Nguroje 69(7.86%), Yelwa 24(2.73%) while Kakara had 50(5.69%) mosquitoes. Four out of the five anopheline mosquito species were caught inYelwa, Gembu andMayo-selbe while three species each were caught in Kakara and Nguroje each. The number and species composition of the *Anopheles* mosquitoes are represented in Table 4.4.

Table 4.4: Number and species composition of *Anopheles* mosquitoes along altitudinal locations

		Species					
Locations	**Altitude (m)**	***An. gambiae***	***An. coustani***	***An. funestus***	***An. Pharoensis***	***An. rufipes***	**Total**
Nguroje	1,885	66	2	0	0	1	69
Yelwa	1,674	17	4	2	1	-	24
Gembu	1,584	115	20	17	11	-	163
Kakara	1,496	34	12	4	0	-	50
Mayo-selbe	484	525	35	6	6	-	572
Total		**757**	**73**	**29**	**18**	**1**	**878**

Adult *Anopheles* mosquitoes made up 79.61% of the total collection along the altitudinal locations. Table 4.5 shows the number of adult mosquitoes sampled by both PSC and light trap. A total of 699 adult *Anopheles* mosquitoes were collected, 569(81.40%) by PSC and 130(18.60%) by light trap.

Table 4.5: Number of adult anopheline mosquitoes sampled by light trap and PSC along altitudinal locations.

Locations	Light-trap		PSC	Total
	Indoor	Outdoor		
Nguroje	2	5	4	11
Yelwa	7	3	15	25
Gembu	11	5	54	70
Kakara	5	2	18	25
Mayo-selbe	75	15	478	568
Total	**100**	**30**	**569**	**699**

More mosquitoes were collected indoors than outdoors these were, 100(76.92%) and 30(23.08%) respectively. Anophelines Peak biting period across the altitudinal locations was between 2-4am based on the highest collection of 57(43.86%). The least mosquito collections of 6(4.62%) was done between 6-8pm as presented in Table 4.6.

Table 4.6: Hourly anophelines mosquito collections by Modified CDC- light trap

	Time of collection						
Anophelines species	**6-8pm**	**8-10pm**	**10-12pm**	**12-2am**	**2-4am**	**4-6am**	**Total**
An. gambiae	6	9	12	29	42	7	105
An. coustani	-	1	3	4	8	-	16
An. funestus	-	1	-	-	4	-	5
An. Pharoensis	-	-	-	-	3	-	3
An. rufipes	-	1	-	-	-	-	1
Total	**6**	**12**	**15**	**33**	**57**	**7**	**130**

Table 4.7 shows the relative densities of the five anopheline species.*An. gambiae* had the highest relative density of 93.42, *An. coustani* 5.01, *An. funestus* 0.86, *An. pharoensis* 0.57 while *An. rufipes* had the least 0.14.

Table 4.7: Relative densities of female anophelines along altitudinal locations

Species	Locations															
	Nguroje			Yelwa			Gembu			Kakara			Mayo-selbe			Grand total	
	Light trap		PSC	Light trap		PSC	Light trap		PSC	Light trap		PSC	Light trap		PSC	N	RD
	In	Out		In	out		In	Out		In	Out		In	Out			
An. gambiae	3	3	3	7	2	8	11	4	48	1	0	12	68	11	472	653	93.42
An. coustani	0	0	0	0	0	2	0	0	0	3	2	1	5	1	21	35	5.01
An. funestus	0	0	0	0	0	0	0	0	0	0	0	0	2	1	3	6	0.86
An. pharoensis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3	4	0.57
An. rufipes	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.14
Total	**3**	**4**	**3**	**7**	**2**	**11**	**11**	**4**	**48**	**4**	**2**	**13**	**75**	**13**	**499**	**699**	**100**
Grand Total	**10**			**20**			**63**			**19**			**587**				

4.3.2: Species diversity and relative abundance of anophelines vectors in all the months and locations

Table 4.8 reveal that in the dry season which is from (Novemberto March), highest abundance of mosquitoes was recorded in December (235), when the species diversity was however low, 0.28. *An. gambiae* had high dominance, 0.75 over other anopheline species. The least species abundance of 6 mosquitoes was recorded in March, with a diversity index of 0, only *An. gambiae* were caught in March and these had a dominance of 1.00 over other species in that month. In the rainy seasonwhich started from (Aprilto October), June experienced more abundant mosquito collectionof 104. There was also a high diversity index of 0.99, but *An. gambiae* had low dominance, 0.29 over other species collected.

Table 4.8: Monthly species diversity of anopheline vectors of malaria

Months	Species richness (S)	Species abundance (N)	Diversity index (H')	Maximum diversity (H_{max})	Evenness (J)	Dominance (D)
November	4	95	0.59	1.39	0.42	0.58
December	3	235	0.28	1.10	0.25	0.75
January	2	58	0.10	0.69	0.14	0.86
February	2	55	0.17	0.69	0.25	0.75
March	1	6	0.00	0.00	0.00	1.00
April	3	49	0.64	1.10	0.58	0.42
May	3	65	0.46	1.10	0.42	0.58
June	4	104	0.99	1.39	0.71	0.29
July	1	7	0.00	0.00	0.00	1.00
August	2	16	0.23	1.10	0.21	0.79
September	4	98	0.58	1.39	0.42	0.58
October	4	91	0.60	1.39	0.43	0.57

Species abundance and diversity of anopheline vectors of malaria across the altitudinal locations as presented in Table 4.9. Mayo-selbe had the highest species abundance of 572 mosquitoes but lowest species diversity index of 0.24.*An. gambiae* dominated over other anophelines by 0.76. Gembu recorded the next higher abundance of 163 and the location also recorded the highest diversity index of 0.99 but it recorded the

lowest dominance of 0.14. Nguroje recorded 69 mosquito abundance and a species diversity index of 0.61 *An. gambiae* dominance over other species was 0.45. Kakara had abundance of 50 mosquitoes and a high diversity index of 0.81, but *An. gambiae* recorded low dominance of 0.26 over other anopheline species. Yelwa had the least abundance of 24, but very high diversity index of 0.81, while dominance was 0.41.

Table 4.9: Species abundance and diversity of anopheline vectors of malaria across the altitudinal locations

Location	Species richness (S)	Species abundance (N)	Diversity index (H')	Maximum diversity (H_{max})	Evenness (J')	Dominance (D)
Nguroje	3	69	0.61	1.10	0.55	0.45
Yelwa	4	24	0.81	1.39	0.58	0.41
Gembu	4	163	0.92	1.39	0.81	0.19
Kakara	3	50	0.81	1.10	0.74	0.26
Mayo-selbe	4	572	0.34	1.39	0.24	0.76

4.3.3: Identification of mosquitoes by PCR

4.3.3.1: Molecular (M and S) forms.

Molecular forms of *An. gambiae s.s.* identified along altitudinal locationsare shown on Table 4.10 below: Mosquitoes morphologically identified as *An. gambiae s.l,* were further identified to sub species and *An. gambiae s.s* molecular forms. Out of the 757*An. gambiae s.l* tested, 712(94.06%) of these were identified as *An. gambiae s.s* and they occurred in all the altitudinal locations and all months of collection. Figure 5 shows anamplified-fragments using the species-specific assay PCR assay for the identification of members of the *Anopheles gambiaes.l.* Lane 1kb is the molecular marker, lane 2 the negative control, lane 3 positive control and lanes 4-20 test lanes. DNA ladder size is 390bp for *An. gambiaes.s.*

Out of the 712 *An. gambiae s.s* which were further tested for molecular forms, 192(25.37%) were found to be the M form and 520(68.69%) were the S form.Figure 6shows an amplified fragment using the species- specific assay for the identification of member of the *An. gambiaes.s.* Lane 1 lader (molecular marker), lane 2 negative control:

lanes 3-20 test lanes of *An. gambiae s.s* (m-form=367bp and s-form=257bp.A total of 45(5.94%) of the mosquitoes collected from Gembu and Mayo-selbe could not be identified as depicted in Table 4.10.

Table 4.10: Molecular forms of *An. gambiae s.s.* along altitudinal locations

Location	Number sampled	*Anopheles gambiae* molecular forms		Not identified
		M form	S form	
Nguroje	66	21	45	-
Yelwa	17	5	12	-
Gembu	115	15	83	17
Kakara	34	11	23	-
Mayo-selbe	525	140	357	28
Total (%)	757	192(25.36)	520(68.69)	45(5.94)

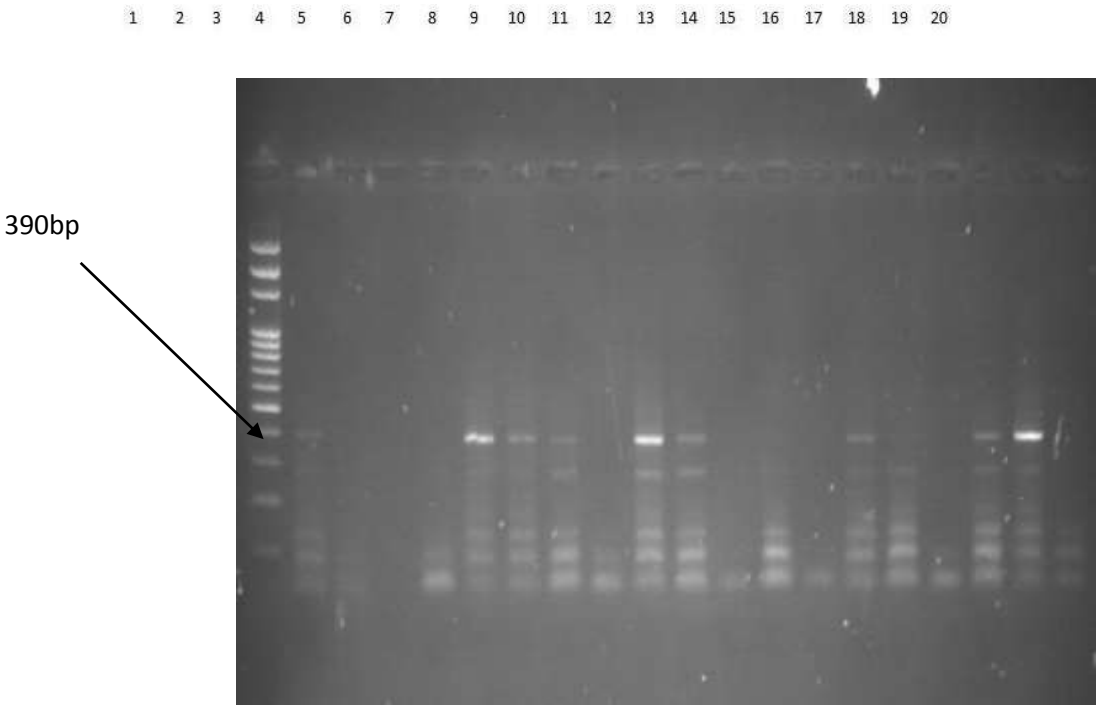

Figure 5: Amplified fragments using the species-specific assay PCR assay for the identification of member of the *Anopheles gambiae s.l* Lane 1kb molecular marker lane 2 negative control, lane 3 positive control lanes 4-20 test lanes. DNA ladder size is 390bp for *An. gambiae s.s*

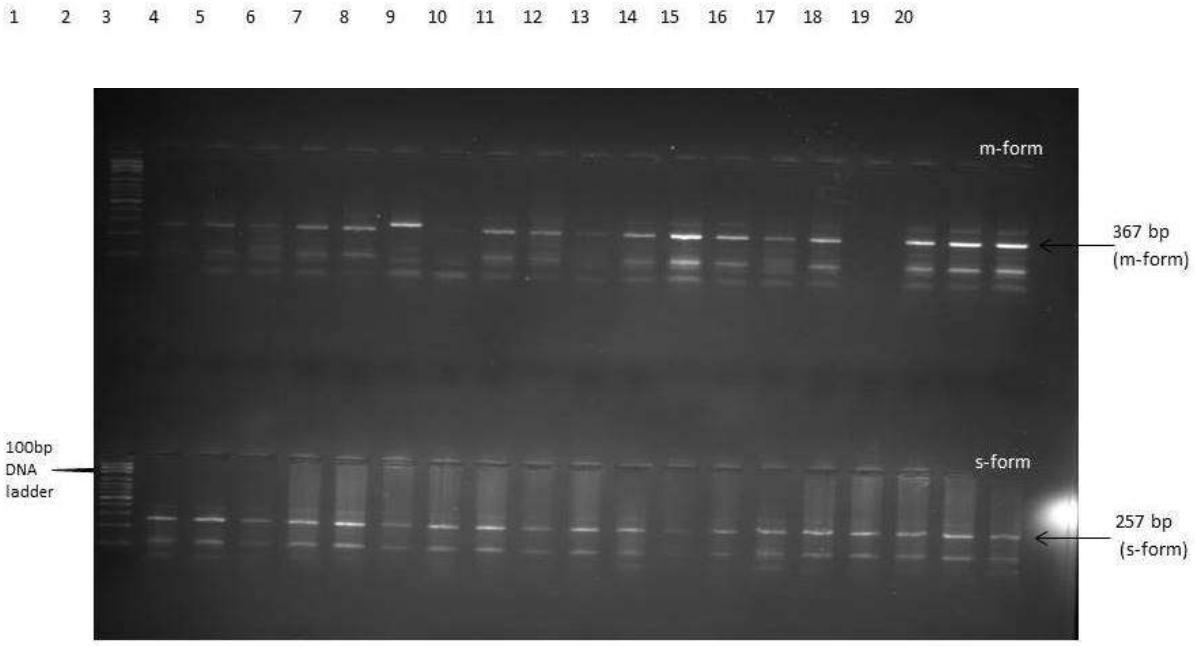

Figure 6: Amplified fragment using the species – specific assay for the identification of member of the An. *gambiae s.s Lane 1 lader* (molecular marker), lane 2 negative control: lanes 3-20 test lanes of An. *gambiae s.s* (m-form=367bp and s-form=257bp.

4.4: Discussion

From results of the current study, *An. gambiae sl* was the most abundant species in all the locations and months of study. Several studies in Nigeria and across Africa (Mwangangi *etal*., 2013, Okorie *et al*., 2014) have established the high abundance and wide geographic distribution of this species. This could be due to its high orientation towards humans and its habit of oviposition close to dwellings. Consequently, there is a high vecor-host contact that has resulted in it being a very efficient vector of malaria in humans (van Emden and Service, 2004).

More mosquitoes were collected by PSC than CDC light trap. Different *Anopheles* species exhibit varied feeding and resting behaviours. For example, species like *An. gambiae* which are endophagic and endophilic, rest indoors after a blood meal to digest the blood (Sinka, 2013). The mosquitoes that had fed during the night or early hours of the morning could still be digesting the bloodmeal thereby resulting in the high number collected by PSC than CDC light trap. Lower numbers of Anopheline mosquitoes were trapped indoors than outdoors. This could be due to the cold conditions in the Mambilla Plateau, where people hardly stayed up late outdoors. as opposed to warmer locations where people stay longer outdoors. Athropogenic

mosquitoes that could not locate hosts to feed on outdoors, would find their way indoors in search of blood meals (Falcuta *et al*., 2010, Sinka, 2013), thereby accounting for their high number indoors.

Temperature and relative humidity play significant roles in mosquito abundance. Peak mosquito abundance was between 2-4am in the morning. Temperature affect sporogony and the vector development time. Relative humidity ensures high activity of the mosquitoes. In the current study, both outdoor and indoor relative humidity were high (raw unanalysed data in appendix 2) between 2-4am thereby, resulting in high number being trapped within that period. Okorie *et al*., (2014) recorded increased in mosquito abundance as temperature and relative humidity increased. This concur with the present study.

Some mosquitoes from Mayo-selbe and Gembu could not be identified molecularly. Furthermore, higher mosquito abundance was also recorded for the two locations. The inability of some to be identified by PCR could have been due to initial incorrect morphological identification and or DNA degradation due to poor preservation or it could have been due to human error.

4.5: Conclusions

1. *Anopheles gambiae* mosquitoes have high abundance and wide geographic spread. Their habit of feeding and resting indoors further enhances their vector capacity and efficiency.

2. The Pyrethrum spray catches method is very effective for entomological mosquito sampling. The use of long lasting insecticidal treated nets and indoor residual spraying can go a long way to control adult anopheline vectors that bite at night and indoors.

3. Mosquitoes are very sensitive to changes in climatic factors (rainfall, temperature and relative humidity). These could influence abundance and malaria transmission seasons.

4. *An. gambiae* S and M forms are gaining wide spread in the Mambilla Plateau. New species which are better adapted and more efficient malaria vectors than the present vectors, could arise from these species in the future. This could have serious epidemiological implications to malaria eradication in the area.

CHAPTER FIVE

5.0: CHARACTERIZATION OF LARVAL HABITATS OF THE ANOPHELINE VECTORS OF MALARIA

Components of this section were published as:

Larval habitats of anopheline vectors of malaria on the highlands of Mambilla Plateau, Taraba state Northeast Nigeria. *International Journal of mosquito research,* 5(1):96-100. By Garba *et al.,* (2018). (Appendix IV).

5.1: Background

Members of the *An. gambiae* complex differ in their preference for breeding habitats and consequentl utilize various aquatic habitats for larval development. Larval habitats are made up of diverse water bodies, ranging from both temporary and permanent habitats (Imbahale *et al*., 2011), polluted and unpolluted, man-made habitats such as irrigation wells, gutters and polluted ponds (Kudom *et al.*, 2012). Larvae have also been collected from polluted water rich in organic matter (Awolola *et al.*, 2007, Castro *et al.*, 2010), in large bodies of water such as flood plains (Majambere *et al.*, 2008) and in pools along lake shores, especially when there are changes in water level (Minawaka *et al.*, 2006).Larvae of *An. arabiensis* are most abundant in sand pools along the edges of rivers (Ye-Ebiyo *et al.*, 2003). *An. melas* and *An. merus*, are most productive around the coastal areas where they exhibit great tolerance for salinity, surviving in relatively high degrees of salinity sometimes reaching or even exceeding that of seawater (Hanafi-Bojd *et al*., 2012).

These aquatic larval habitats are of distinct biotic and abiotic properties that substantially influence geographical distribution and abundance of these species (Okech *et al*., 2007). The physicochemical characteristics of the larval habitats are also very crucial to mosquito larval density (Amani *et al*., 2014). Physicochemical parameters include; dissolved oxygen, temperature, conductivity, PH, alkalinity, hardness, total dissolved solidsand turbidity. Others are presence of substances like sulphates, nitrates, lead, iron and arsenic, these all influence development and survival of larvae. The development and survival of larvae in turn, may affect the abundance and species diversity of anopheline vectors of malaria (Afolabi *et al*., 2013; Dida *et al*., 2015). It is suggested that selection of larval habitats is likely determined among other factors, by the physicochemical parameters of the water bodies (Rejmánková *et*

al., 2013). **L**arval habitats of the anopheline vectors of malaria were characterized in this study with a view to identify the characteristics that support larval abundance and development. This data would provide information to the relevant authorities in formulating effective larval control strategies in the study area.

5.2: Materials and Methods

5.2.1: Larval habitat characterization

Various anophelines larval habitats were sampled in all the five altitudinal locations. The habitats were visited once every month from November 2015 to October 2017 from 8am to 10am in the morning. Characterization of potential mosquito larval habitats was carried out. All the features of the habitats were observed and described as observed in the field. For example, habitat permanence, the presence or absence of vegetation, water condition and exposure to sunlight. Light intensity as either shady or light, area of the larval habitat covered by shade was visually estimated as percentage canopy cover. Water current was determined by visual scrutiny as still or slow-flowing. Other parameters were measured using hand-held digital meters.Temperature was measured with a thermometer, pH with Wagtech pH meter, electrical conductivity and total dissolved solids with Wagtech conductivity/TDS meter, turbidity with Wagtech turbidity meter, salinity and dissolved oxygen with Horiba Dip Electrode.

Water samples were collected from the various habitats in 1litre plastic bottles, they were tightly closed and labeled according to altitude, location, coordinates, and date of collection. The water samples were transported to Taraba State Ministry of water Resources and analyzed for other chemical parameters. The parameters were; alkalinity, hardness, dissolved oxygen, sulphate, iron, lead, arsenic and nitrate. These chemical parameters were analyzed using Wagtech photometer. The coordinates of each mosquito larval habitats were determined using hand-held global positioning system (GPS) receiver (Garmin e Trex® 10).

5.2.2: Data Analysis

Larval density for each study location was determined from the breeding index according to the formula of Belkin (1954): BI = TLP/ND×BP,

Where BI= breeding index, TLP = total number of larvae and pupae sampled

ND = number of dips

BP = number of breeding places /sampling sites

5.3: Results

5.3.1: Larval habitats of anopheline vectors of malaria

A total of 60 larval habitats along the altitudinal locations were sampled and characterized by analysing and recording physicochemical parameters. The larval habitats encountered were ponds, streams, wells, swamps, dug pits, rivers, tree trunk, discarded tyres, rock pools and building sites. The most common larval habitats encountered were swamps 15(25.00%), followed by discarded tyres at 9 (15.00%), streams and wells were 7(11.67%) each, dug pits were 6 (10.00%), rivers were 5(8.33%), building sites were 4(6.67%), ponds and rock pools were 3(5.00%) each, tree trunk had the least occurrence of 1(1.66%). The numbers of larval habitats examined were reflective of their relative availability in the various altitudinal locations. The highest number of habitats of 17 (28.33%) were recorded in Yelwa, followed by Gembu and Mayo-selbe with 12(20.00%) each, Nguroje had 11 (18.33%), Kakara had the least at 8(13.33%). These are indicated in Table 5.1.

Table 5.1: Larval habitats of anopheline vectors of malaria

Location	**Habitat Type**										
	Dug pit	**Pond**	**River**	**Stream**	**Swamp**	**Well**	**Tree trunk**	**Old tyres**	**Rock pools**	**Building sites**	**Total**
Nguroje	2	1	0	1	4	3	0	0	0	0	11
Yelwa	2	0	0	3	2	1	0	9	0	0	17
Gembu	0	1	0	2	6	3	0	0	0	0	12
Kakara	2	1	0	1	3	0	1	0	0	0	8
Mayo-selbe	0	0	5	0	0	0	0	0	3	4	12
Total	**6**	**3**	**5**	**7**	**15**	**7**	**1**	**9**	**3**	**4**	**60**

5.3.2: Larval abundancebreeding index ofanophelines along altitudinal locations

About 179 larvae were bred to adulthood. They comprised of four species namely *An. gambiae*, 133(74.30%), *An. coustani* 33(18.44%), *An. funestus* 20(11.17%) and *An. pharoensis* 13(7.26%). Larvae were sampled from only three altitudinal locations Kakara, Nguroje, and Gembu each having a breeding index of; 4.2, 11.4 and 20.2 respectively. Mayo-selbe and Yelwa were not productive for anopheline larvae. Gembu recorded the highest larval presence of 101(56.43%), Nguroje had 57(31.84%) larvae and Kakara had the least, at 21(11.73%). Anophelines larvae were found in only six of the 60 larval habitats sampled as depicted in Table 5.2.

Table 5.2: Larval abundance, species composition and breeding index of anophelines along altitudinal locations

	Species/Number of larvae					
Study locations	***An. gambiae***	***An. coustani***	***An. funestus***	***An. pharoensis***	**Total (%)**	**Breeding index**
Nguroje	47	7	1	2	57**(**(31.84)	11.4
Yelwa	-	-	-	-	0	0
Gembu	52	21	17	11	101(56.43)	20.2
Kakara	14	5	2	-	21(11.73)	4.2
Mayo-selbe	-	-	-	-	0	0
Total	**113**	**33**	**20**	**13**	**179(100)**	**35.8**

There was variability in the spatial distribution of anophelines larval populations. Larvae were associated with temporary habitats such as pits that were dug for blocks moulding and in clear, sunlit stream edges and swamps. Mosquito larvae also had preference for and they thrived in habitats with a wide range ofphysicochemical parameters as shown in Tables 5.3a and 5.3b.

Table 5.3a: Average Physicochemical Parameters of larval habitats

Location	Temp	pH	EC	TDS	Turbidity	Hardness
Nguroje	25.98	6.52	10.12	50.45	3.53	39.90
Yelwa	25.33	6.82	44.56	22.50	4.31	30.37
Gembu	21.39	7.11	83.89	42.16	22.88	41.00
Kakara	23.75	6.69	43.35	21.85	14.54	27.57
Mayo Salbe	31.92	5.88	87.02	43.80	5.36	51.80

Table 5.3b: Average physicochemical Parameters of larval habitats

Location	Salinity	Alkalinity	DO	NO3	SO4	Fe	Pb	As
Nguroje	14.36	17.63	5.63	15.37	5.54	.12	.01	.00
Yelwa	11.62	15.62	7.00	34.17	6.75	.03	.02	.00
Gembu	26.33	21.66	5.66	26.50	9.75	.12	.00	.00
Kakara	16.28	15.14	5.57	29.68	14.71	.06	.01	.00
Mayo Salbe	13.60	16.00	8.60	45.60	9.60	.02	.00	.00

5.4: Discussion

In the present study, very few larval habitats were productive. It was observed that most of these habitats were temporary in nature. For example, in two of the locations where larvae were initially collected (Nguroje and Kakara), were found to be non-existent with subsequent visits. Mosquitoes species can adapt to virtually every habitat, they are found to occur every where. The presence of stagnant water with enough nutrients to sustain the development of their larvae, could attract them to lay their eggs. Some species of mosquitoes have adapted to habitats where they only lay

their eggs in either naturally occuring or artificial containers (Amani *et al*., 2014, Reimer *et al*., 2016).

Characteristics of mosquito larval habitats are important determinants of their survival and subsequent development. Several factors influence the abundance and distribution of mosquito larvae in their habitats. Among these are climate, vegetation, sources of nutrients and anthropogenic activities (Mwangangi *et al*., 2007, Amani *et al*., 2014). Climate (rainfall) and anthropogenic activities also played major roles in larval abundance in the current study. Larvae were collected in the two locations during the dry season but in the rainy season, some were overgrown by vegetation as a result of the heavy rains while others were cultivated thereby rendering them non-productive. An earlier study in Eritrea recorded all year round collection of anopheline larvae, with more larval abundance during the wet season than in the dry season (Shililu *et al*., 2003). The study however acknowledged that the dry season larval habitats were temporary in nature, much like what was recorded in the current study. In the current study, four anopheline mosquito species were collected at larval stage and reared to adulthood. *An. gambiae* larvae dominated over other species. In contrast, a similar study in Kenya, *An. gambiae* recorded less abundance than other species particularly *An. arabiensis*. This could be due to the host preference of *An. arabiensis*, which is zoophilic in its feeding habit. In the present study, larvae were collected a few distances from human dwellings without major livestock activities. The study in Kenya collected larvae in habitats that were also associated with the rearing of animals close to human dwellings. The study reported significant association between the relative abundance of *An. gambiae* and the distance to the nearest house (Minakawa *et al*., 1999). Gimnig *et al*., (2001) suggested that the nearness of each species of the anophelines to their preferred hosts could play a major role in their abundance.

Among the larval habitats encountered in this study, swamps recorded the highest occurrence. This agrees with observations by Minakawa *et al*., (2004) where over 80% of *An. gambiae* larvae were sampled from isolated pools that were present in both swamp margins and ditches. This means that the cultivation of swamps due to the increasing human population could create more larval habitats for the malaria vectors. These results have serious epidemiological implications on malaria transmission in the highland regions

5.5: Conclusion

1. From results of the study, it was observed that the nature of larval habitats, seasonality as well as anthropogenic activities were key factors that influenced the productivity and abundance of larvae in the study locations.
2. Knowledge of the larval habitat preference of individual anopheline species is a usefull tool for targeted malaria transmission management through larval vector control. Larval habitats identification and surveillance should therefore be incorporated as major components of a successful integrated mosquito control programmes.

CHAPTER SIX

6.0: ENTOMOLOGICAL INOCULATION RATES (EIRs) OF THEANOPHELINE VECTORS OF MALARIA

6.1: Background

The entomological inoculation rate is often used as a measure to determine the number of infectious bites by mosquitoes per person per unit time mostly expressed per year.It is viewed as a more direct metric of malaria transmission intensity (Kelly-hope and Mckenzie, 2009). It is also a means of assessing the effect of malaria vector control tools such as insecticide-treated nets (ITNs), indoor residual spraying (IRS) and source reduction (SR) (Shaukat *et al*., 2010). The EIR is a product of the man biting rate and the sporozoites rateand is therefore dependent on these two indices (Shaukat *et al*., 2010).A decrease in the values of the MBR and SR will bring about a corresponding decrease in the EIR. Factors which affect the EIR include; population size of the malaria vectors, altitude, rainfall, temperature, socio-cultural attitudes and behaviour. Others are urbanization and existing malaria control actions and their effect on human infection and disease (Shaukat *et al*., 2010).

Man biting rate is the number of bites a person receives by vector mosquitoes over a fixed period of time. It can be estimated directly from the human landing catches (HLC), light trap catches or indirectly through the pyrethrum spray catches (PSC), by dividing the total number of blood-fed mosquitoes by the total number of persons in the rooms used for collection multiplied by the human blood index (Williams and Pinto, 2012, Bakhiet *et al*., 2017).

Sporozoites rate is a fraction of infectious vector mosquitoes. It is determined as the number of sporozoite-infective mosquitoes divided by the total number of mosquitoes and expressed as a percentage (Kelly-hope and Mckenzie, 2009, Kilama *et al*., 2014).

6.2: Materials and Methods

6.2.1: ELISA determination of circumsporozoite (CSP) proteins of *Plasmodium falciparum*

The assay involved two steps, first was the screening phase which involved identifying positive samples. The second phase was the quantification phase where, ELISA positive samples from the initial screening were retested to confirm positives.

The head and thorax of the mosquitoes were separated from the rest of the body. These were assayed by ELISA for the presence of circumsporozoite antigens (CSP) of *Plasmodium falciparum*, according to (Burkot *et al.*, 1984; Wirtz *et al*., 1987). The head and thorax of each mosquito was placed in a labeled 1.5ml micro centrifuge grinding tube. 50 µl of grinding buffer which consists of boiled casein containing (Igpal CA 630) was added then grounded. The pestle was rinsed with two 100 µl volumes of grinding solution, catching the rinses in the tube containing the mosquito triturate. The final volume was brought to 250 µl and tested. A working solution of monoclonal antibody (mAb) capture was prepared by adding phosphate buffer solution (PBS) to the reconstituted capture mAb based on the volumes indicated in Table 6.1 below. This was vortexed gently.

Table 6.1: Working solution of mAb capture by volumes by species

Species	mAb	µg/50µl/WELL	µg/5ml	µlSTOCK/5ml
Pf	Capture	0.200 µg/50µl	20.0 µg	40 µl stock+6ml PBS
Pv-210	Capture	0.025 µg/50µl	2.5 µg	5 µl stock+6ml PBS
Pv-247	Capture	0.025 µg/50µl	2.5 µg	5 µl stock+6ml PBS

50 µl of mAb solution (as in Table 6.1 above) was placed in each well of the ELISA micro titre plates. The plates were covered with foil paper and incubated for 0.5 hour at room temperature, after which the well contents were aspirated. The plates were banged upside down on paper towel 5 times into the sink and then again on paper towels. The wells were filled with 200µl Blocking buffer (BB) and the plates were covered, leaving spaces between the wells and the top of lid. These were incubated for 1hour at room temperature. After 1hour the well contents were aspirated, holding the sides of the plates, they were banged upside down on paper towel 5 times. The test samples and controls were loaded into the plate and tested. The test involved adding

50µl of positive controls to well A1 and 50µl of negative controls was added to wells B1-H1. 50µl of mosquito triturateper well, were added to the remaining wells. This was covered and incubated for 2hours.

For the confirmatory test, 50µl of negative control(s) were added to wells A1-H1 and 50µl of last vial dilution of positive control was added to wells A2, A3 and A4 and 50µl of each serial dilution to wells B2-H2, B3-H3 and B4-H4. 50µl of mosquito triturate per well was then added to the remaining wells, this was covered and incubated for two hours. Substrate was prepared by mixing substrate A and substrate B at a ratio of 1.1. A full 96-well was 5ml of substrate A+5ml of substrate B. A working solution of mAb conjugate was prepared by adding blocking buffer (BB) to the reconstituted conjugate mAb based on the volumes by species as listed inTable 6.2 below. This was vortexed gently (Burkot *et al.,* 1984; Wirtz *et al*., 1987).

Table 6.2: A working solution of mAb conjugate by volume by species

Species	**mAb**	**µg/50µl/WELL**	**µg/5ml**	**µl STOCK/5ml**
Pf	Peroxidase	0.050µg/50µl	5.0 µg	10µl stock +5ml BB
Pv-210	Peroxidase	0.050µg/50µl	5.0µg	10µl stock +5ml BB
Pv-247	Peroxidase	0.050 µg/50µl	5.0µg	10µl stock +5ml BB

Enzyme activity was checked by mixing 5µl of the mAb conjugate (as in table 6.2 above) with 100µl of the substrate in a separate tube and vortexed gently. A rapid colour change indicated that the peroxidase enzyme and the substrate are functional. The well contents were aspirated and the plate was banged upside down on paper towel 5 times holding the sides only. Wells were washed 2 times with 200µl of PBS-Tween, aspirating and banging plate 5 times with each wash. 50µl of peroxidase conjugate solution was added to each well, this was covered and incubated for 1hour. Well contents were aspirated and the plate banged upside down on paper towel 5times holding the sides only. The wells were then washed three times with 200µl of PBS-Tween, aspirating and banging plate 5 times with each wash. 50µl substrate solution was added to each well and the plate was covered and incubated for 30minutes. The plate was carefully handled to avoid splashing. Positive reactions were read visually (Burkot *et al.,* 1984; Wirtz *et al*., 1987).

6.2.2: Data Analysis

Entomological inoculation rates for the five locations were determined from the product of the sporozoite rates and the man biting rates.

6.3: Results

6.3.1: Determination of Sporozoite rates of anophelines

Only mosquitoes caught as adults were used for the CSP antigen ELISA as those reared from larvae were non-blood fed. Table 6.3 shows a total of 592 *Anopheles* mosquitoes that were tested and 33(5.57%) were positive for *P. falciparum* CSP. Figure 7 is amicro titre plate for Sporozoites identification by ELISA, the coloured portions indicate positive samples. Mayo-selbe recorded the highest number of infection and sporozoite rates at 21and 3.55% respectively. Gembu recorded 7 infections and 1.18% sporozoites rates, Yelwa and Kakara both recorded 2 infections with 0.33% sprozoites rates. The least number of *P. falciparum* infections and lowest sporozoites rate was recorded in Nguroje with 1 infection and 0.17% sporozoites rate.

Table 6.3: *P. falciparum* infectivity and Sporozoites rates of *An. gambiae s.l* along altitude locations

Location of Study	Number(%) tested	No. positive for *P. falciparum* CSP	Sporozoites Rates (%)
Nguroje	9(1.52)	1	0.17
Yelwa	17(2.87)	2	0.33
Gembu	63(10.64)	7	1.18
Kakara	13(2.20)	2	0.33
Mayo-selbe	490(82.77)	21	3.55
Total (%)	592(100)	33	5.57

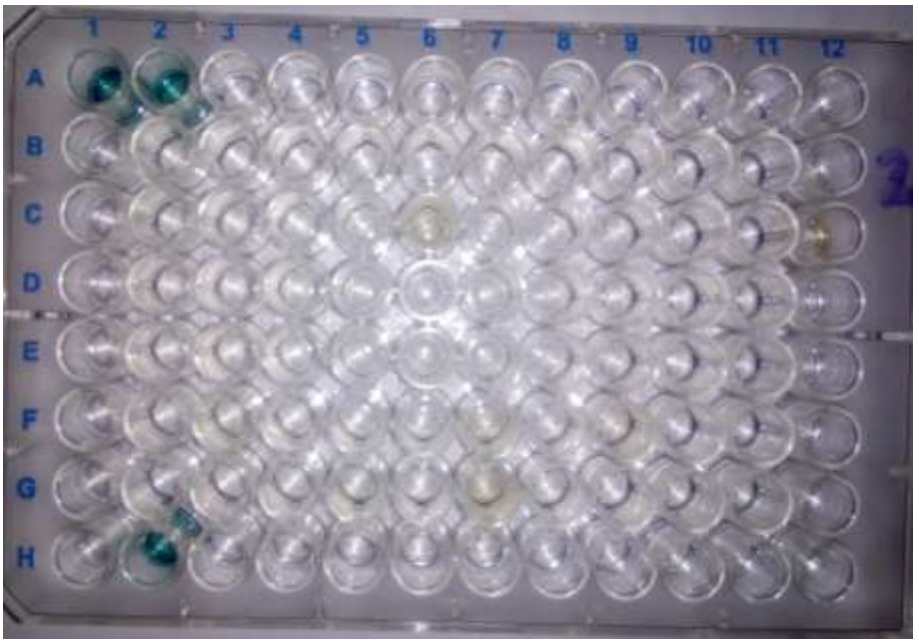

Figure 7: Micro titre plate for Sporozoiteidentification by ELISA

Likewise, for monthly *An. gambiae s.s.P. falciparum* infectivity, December had the highest number of infections as well as highest sporozoites rates. These were 8 and 1.35% respectively followed by June with 7 infective mosquitoes and 1.18% sporozoites rates. The lowest number of infections of 1 each were recorded in March and August and these months also had the least sporozoites rates of 0.17% each. In the months of February, April, May and July there were no recorded infections and therefore had 0 sporozoites rates.

Monthly *P. falciparum* infectivity and sporozoites rates of *An. gambiae s. l* along altitude locations are represented in Table 6.4.

Table 6.4: Monthly *P. falciparum* infectivity and sporozoites rates of *An. gambiae s. l* along altitude locations

	Month	Number tested	Number positive	Sporozoites rates (%)
1	November	57	5	0.84
2	December	144	8	1.35
3	January	49	2	0.51
4	February	44	0	0.00
5	March	6	1	0.17
6	April	38	0	0.00
7	May	51	0	0.00
8	June	55	7	1.18
9	July	7	0	0.00
10	August	16	1	0.17
11	September	67	6	1.01
12	October	58	3	0.51
	Total	592	33(5.57)	5.74(0.48*)

*Mean value

6.3.2: Determination of the man biting rates and entomological inoculation rates ofanophelines.

The man biting rate of *An. gambiae* along the altitudinal locations increased with decreasing altitude. Nguroje had a man biting rate of 0.02, Yelwa had 0.11, Gembu and Kakara had 0.12 each and Mayo-selbe had 2.56 as indicated in Table 6.5.

Table 6.5: Man biting rates of *An. gambiae* along altitudinal locations

Variables	Locations					
	Nguroje	**Yelwa**	**kakara**	**Gembu**	**Mayo-selbe**	**Total**
Mean No. of rooms	19	19	20	19	20	97
No. of rooms with *An.*	5	9	17	13	19	63
% of rooms with *An.*	26.32	47.37	85.00	68.42	95.00	64.42*
No. of *An.* caught	4	15	54	18	478	569
No. of persons in houses	97	101	105	68	116	487
No. of mosquitoes with blood in abdomen	2	11	13	8	297	331
Man biting rates	0.02	0.11	0.12	0.12	2.56	0.68

MBR = No. of mosquitoes with blood divide by No. of persons in room, *Mean value

The entomological inoculation rate was determined from the product of the sporozoites rates and the man biting rates. The mean entomological inoculation rates along the altitudinal locations over the twelve months were 0.003 for Nguroje, 0.04 for both Yelwa, and Kakara, 0.14 for Gembu, and 9.09 infective bites per person per night (ib/p/n) for Mayo-selbe as shown in Table 6.6.

Table 6.6: Entomological inoculation rates of An. gambiae along altitudinal locations

Variables	Locations					
	Nguroje	**Yelwa**	**Gembu**	**Kakara**	**Mayo-selbe**	**Total**
No.of mosquitoes tested	9	17	63	13	490	592
Sporozoites rates	0.17	0.34	1.18	0.34	3.55	5.58
Man biting rates	0.02	0.11	0.12	0.12	2.56	0.68
EIR	0.003	0.04	0.14	0.04	9.09	3.79

6.4: Discussion

Generally, the dry season months of December, November, January and March had more *P. falciparum* infectivity and highest SRs than the rainy season months of June, August, September and October. This is because higher number of mosquitoes were sampled in the dry season months than the rainy season. However, there was a difference of only one infective mosquito between the dry and rainy seasons. April, May and july which are all within the rainy season recorded no positive infectivity nor sporozoite rates. This implies that abundance of vectors did not play any role in malaria transmission between the two seasons. It could be infered that the mosquitoes only serve as nuissance biting pests and are not involved in malaria transmission within the period in question.Okorie *et al*., (2014) recorded similar higher vector abundance in the dry season months of December, January and february compared to the rainy season months.

There was also a reduction in mosquito abundance with higher rainfall, this is in agreement with the finding in the current study.This is evident in July and August (peak rainy months) which had lower number of vectors. Due to the heavy rain which comes with wind, larval habitats were washed away and consequently, a low number of adults were sampled .

Mayo-selbe recorded the highest mosquito abundance, high *P. falciparum*infectivity and highest SRs. Except for Gembu, all other locations recorded decreased infection and sporozoite rates as the altitude increased. This clearly shows that infection was influenced by altitude.

Both the man biting rates and entomological inoculation rates increased with increased altitude. Nguroje which is located at the highest altitude recorded least values of malaria transmission indices (MBRs, SRs and EIR). Mayo-selbe which is at the lowest altitudinal location, recorded the highest of these indices. This results is in tarndem with other similar studies. Shililu *et al*., (1998) recodred recurrent but low malaria transmission intensity in a high altitude location. Such variations in EIRs have also been reported from studies across Africa. This has brought about a resultant variation in malaria transmission intensity across different locations (Shaukat *et al*., 2010).

6.5: Conclusion

1. Highest number of infectivity and sporozoite rates were recorded in December which is in the dry season.Mosquito abundance was only slightly higher in the dry season than in the rainy season. There was also only a difference of one positive *P. falciparum* infection between the rainy and dry season months. This means that malaria transmission between the two seasons (rainy and dry), was not dependent on the number or abundance of mosquito vectors. It could also be infered that rainfall was also not a factor in malaria transmission intensity.
2. It is concluded that malaria transmission in the current study was driven by; location represented by altitude, temperature, and anophelines vectors abundance.

CHAPTER SEVEN

7.0: BLOODMEAL PREFERENCES OF THE ANOPHELINE VECTORS OF MALARIA

7.1: Background

Anopheles mosquitoes have evolved some characteristic behaviours that have enhanced their vector status and efficiency as transmitters of diseases particularly malaria (Killeen *et al*., 2013). The energy needed for both male and female mosquitoes to fly and perform other physiological processes come from sugar that they get from nectar of flowers and plants, fruit juices, honeydew and those from other naturally occurring sugar-filled juices. Male mosquitoes do not lay eggs and therefore do not require a blood meal. They naturally do not have the mouthparts for piercing and sucking blood like the female mosquitoes. The adult female mosquito requires the protein in blood to develop her eggs before they are laid in water, so she must feed on blood. However, the eggs are laid in batches so she goes in search of another blood meal to develop and lay the next batch of eggs (Sinka, 2013).

Feeding behaviours differ among different *Anopheles* species. Some females prefer to feed on humans, those species such as *An. gambiae* are said to be ‘’anthropophagic’’ while others such as *An. arabiensis* would rather feed on animals and are said to be ‘’zoophagic’’. Such species which are zoophagic have the tendency to transmit malaria to animals rather than humans (Sinka, 2013). .*An. arabiensis* are also opportunistic in their feeding pattern, they may have preference for animals but in the absence of animals they feed on humans. There have been suggestions that cattle that are kept close to human dwellings divert biting activities of this species (which is also one of the established malaria vectors in Africa), from humans to animals. This would in turn reduce its efficiency as a human malaria vector (Mahande *etal*., 2007; Killeen, *etal*., 2013; Sinka, 2013).

Among those species of *Anopheles* that feed on animals, some species prefer to feed on just one host while others may feed on two or multiple hosts (Falcuta *et al*., 2010). Some of the animals which some zoophagic *Anopheles* vector species feed on include bovines, pigs, horses, chicken and other birds. The choice of bloodmeal host could be

influenced by the availability of the host (Zimmaman *et al*., 2006). Knowledge of malaria vectors bloodmeal host selection is important as it would inform efficient malaria vector control programmes.

7.2: Materials and Methods

7.2.1: Blood meal identification

The engorged abdomens of the female mosquitoes were detached from the rest of the body and subjected to blood meal ELISA analysis, to determine the source of the blood meal according to (Beier *et al*., 1988). The engorged abdomens of the mosquitoes were grounded and homogenized in 50µl of phosphate-buffered saline. 950µl of phosphate buffered saline was subsequently added and stored at -20°C. 50µl of sample of human serum and the respective controls (positive control: 10 µl of human serum + 500 µl of PBS) were added and incubated for 1hour. After 1hour well contents were aspirated and the micro titre plate washed 2 times with 200µl PBS-Tween 20. (500 µl of Tween 20 was added to1l of PBS). 50µl of prepared enzyme conjugate solution was added to each well and incubated for 1hour. Affinity purified Antibody human IgG (H+L); 1˚ antibody 1: 500, Peroxidase-labeled affinity purified antibody to human IgG(H + L); 2˚ antibody 1: 500) that is , both 1˚ and 2˚ antibody were mixed to get the enzyme conjugate solution. Well contents were aspirated and the **micro titre plate** was washed 3 times with 200 µl PBS-Tween 20 (solution A and B were mixed together that is 5ml + 5ml per plate). The plate was covered with foil paper and incubated for 30minutes and the picture was snapped.

7.2.1: Data Analysis

The human blood index (HBI) was calculated from the formular; HBI= Number of mosquitoes with human blood divide by total number of mosquitoes with blood.

7.3: Results

A total of 592 female adult blood-fed mosquitoes that were tested for source of bloodmeal. A total of 85(14.36%) were positive for human blood meal. The source of blood meal for the rest of the mosquitoes were not verified. Figure 8 is amicro titre plate for blood meal source determination by ELISA. The coloured parts indicate positive human blood meals.

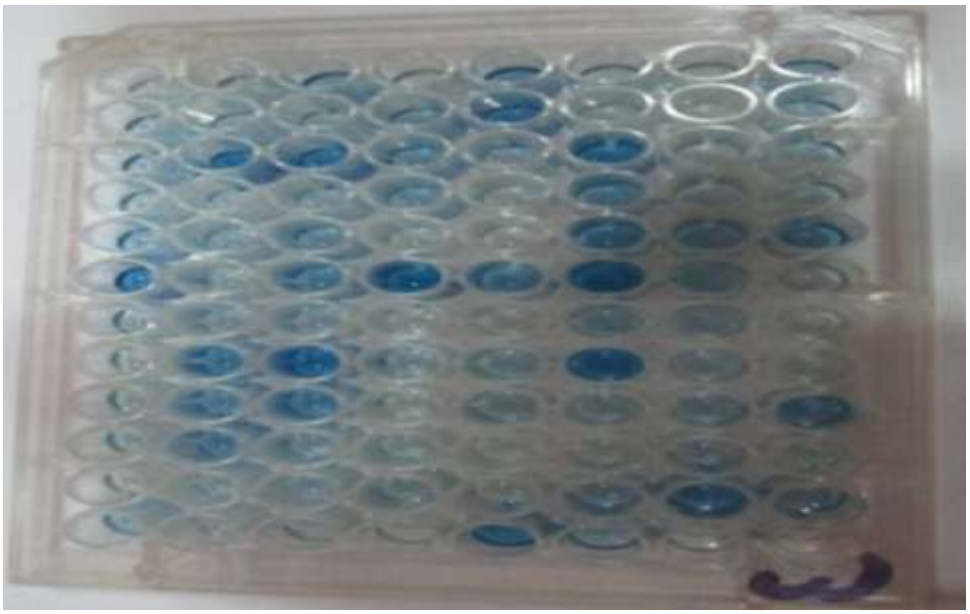

Figure 8: Micro titre plate for blood meal determination by ELISA.

Mayo-selbe recorded 71 positive human blood meal, this was the highest number among the samples tested. Next was 9 positive samples recorded in Gembu, Yelwa had 3 positive samples while Nguroje and Kakara both recorded 1 positive sample each as shown in Table 7.1.

Table 7.1: Blood meal preferences of *An. gambiae s.s.* along altitudinal locations

Location of Study	Number tested	No. positive for Human bloodmeal
Nguroje	9	1
Yelwa	17	3
Gembu	63	9
Kakara	13	1
Mayo-selbe	490	71
Total (%)	592	85(14.36)

Monthly blood-meal preferences, results in Table 7.2 showed that December yielded more positive human bloodmeal preference amounting to 36 positive mosquitoes. January had 12 mosquitoes that were positive for human blood meal. February and September had 9 each, November had 8, June had 5, March and August had 3 each. The remaining months; April, May, July and October did not record any positive results for human blood meal source.

Table 7.2: Monthly human blood meal preferences of *An. gambiae s. l.*

Month	Number tested	Human blood meal
November	57	8
December	144	36
January	49	12
February	44	9
March	6	3
April	38	0
May	51	0
June	55	5
July	7	0
August	16	3
September	67	9
October	58	0
Total	592	85(14.36)

The human blood index for the locations were; 0.22 for Mayo-selbe, 0.03 for Gembu and 0.01 for Yelwa. Nguroje and Kakara both recorded 0.

Human blood index of *An. gambiae* mosquitoes along altitudinal locations are summarized on Table 7.3

Table 7.3: Human blood index of *An. gambiae* mosquitoes along altitudinal locations

Location	Number with blood	Number with human blood	HBI
Nguroje	2	1	0.00
Yelwa	11	3	0.01
Gembu	13	9	0.03
Kakara	8	1	0.00
Mayo-selbe	297	71	0.22
Total	321	85	0.26

HBI= No. of mosquitoes with human blood divide by total no. of mosquitoes with blood

7.4: Discussion

There was generally low number of human blood fed mosquitoes, this may be due to the low temperature which necessitated individuals to retire early to bed and this might have led to less human blood meal recorded. Only few species of mosquito bite humans; most species tend to be opportunistic feeders. *Anopheles* species particularly *An. ganbiae* have however long been established as vectors that transmit malaria to humans due to their preference to feed on humans. Mosquito species are attracted to their hosts by CO_2. It has been reported that human odour harbour more lactic acid than other animals and this has been shown to play a major role along with CO_2 to attract blood seeking females to humans. Mosquitoes choice of blood meal from different people is perhaps better explained by the odour combinations that microbiota (non-pathogenic bacteria and fungi) on the skin and in pores and hair follicles emit. These emissions are in the form of volatile organic compounds and are extremely important mosquitoes host seeking signals.

Mayo-selbe recorded highest number of positive human blood meal mosquitoes along the altitudinal location while Nguroje recorded the least. The two locations represent the lowest altitude and the highest altitude respectively. As it is already known, temperature decrease with increasing altitude which is the case with the two locations. Temperature plays a significant role in mosquito host seeking behaviour and parasite

development time within the mosquito vector (Lindsay and Martens, 1998, Worral *et al*., 2002 and Christiansen-Jucht *et al*., 2014).

High relative humidity aid mosquitoes' survival. High humidity increases their agility and because the relative humidity is higher at night, hence their preference to feed at night. This could explain the higher number of mosquitoes captured between 2-4am along the altitudinal locations. It is believed that very little or no malaria transmission can take place with average monthly relative humidity of below sixty percent (Mwangangi *et al*., 2007, Li *et al*., 2008, Attaulah *et al*., 2015). This may explain the low values of malaria transmission indices at the high altitudinal locations. It should also be noted that between 2-4am most people are also in deep sleep and may not be in a position to repel the mosquitoes from taking a blood meal.

As mentioned earlier, a large percentage of the bloodmeal source of the mosquitoes tested were unknown. Mosquitoes are mobile by nature they can traverse ecological niches within a particular larval habitat in search of blood meal. Due to their varied pattern of feeding, mosquitoes can show preference for different vertebrate host even when exposed to the same individuals (vertebrates) in the same location (Stephenson *et al*., 2019).

Different mosquito species feed on specific hosts. However, there are basic factors that inform mosquitoes feeding choices these are genetics, nutritional state of the mosquitoes, host seeking behaviour, abundance of host, biomass. Others include relative humidity, characteristics of the habitat this determine availability and diversity of hosts (Stephenson *et al*., 2019). Identification of the source of the unknown blood meals wouild provide indication of the varied mosquito host preference among vertebrates.

7.5: Conclusion

1. Mosquitoes in the study region have a diverse host range. Only a little percentage of the mosquitoes tested had fed on human blood, the rest of the mosquito blood meal sources were unknown. This could be due to the feeding behaviour of the anopheline vectors, while some of them are host-specific, some are opportunistic feeders. This behaviour affords them access to diverse hosts.
2. The HBI is the proportion of mosquito blood meal that is derived from humans. In the present study, it is observed that the HBI varied with altitudinal location/gradient and the number of mosquitoes captured and tested for human blood meal.
3. An understanding of the feeding behaviour of Anophelines mosquito vectors would be very useful to better predict and direct malaria control towards a more targeted species for effectiveness.

CHAPTER EIGHT
8.0: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1: General Discussion

All the anopheline species reported in the current study have been reported in other parts of Nigeria and Africa (Okorie *et al*., 2011; Mala *et al*., 2011; Fornadel *et al*., 2011; Munhenga *et al*., 2014; Dida *et al*., 2015). This confirms the wide range of geographic distribution of these anophelines. *An. gambiae s.l* was the most abundant of all the anophelines species encountered. It was recorded monthly in all the five altitudinal locations however, the populations were not abundant throughout the season. Several studies have reported similar high abundances of *An. gambiae s.l* from different parts of Nigeria (Ayanda, 2009; Okorie, *et al*., 2011; Afolabi *et al*., 2013; Aigbodion *et al*., 2013) and it is among the most often reported malaria vector species in the country (Okorie *et al*., 2011). *An. gambiae* and *An. funestus* were two principal anopheline species identified in the current study and this agrees with reports by Ayanda, (2009) who recorded these two species out of the three *Anopheles* species recovered in Nassarawa State. *An. gambiae* and *An. funestus* complexes comprised 14.4% of the total species recorded in Benin City Nigeria (Aigbodion *et al*., 2013). According to Okorie *et al*., (2011), the two species are the most commonly reported malaria vectors in Nigeria, each having 65.2% and 17.3% occurrence respectively. An earlier study in another African highland had reported these two species as the predominant anophelines (Boedker *et al*., 2003). This has serious health implications as the two species have been identified as important malaria and lymphatic filariasis vectors in Nigeria (Aigbodion, *et al*., 2013). This is particularly so since Nigeria is one of the countries with high percentage of malaria prevalence in sub-Saharan Africa (WHO, 2015; 2017). *An. funestus* adapts well and has a wide geographic spread in sub-Saharan Africa, it has a high affinity for human blood and feeds indoors. The species is suggested as more efficient in malaria transmission at some instances than *An. gambiae* (Sinka, 2013).

An. gambiae s.s. was the main species playing a significant role in malaria transmission in the current study. This negates another study implicating other *Anopheles* mosquito species with these roles in other parts of Africa (Mwangangi *et al*., 2013). Several factors have enhanced the vector efficiency of *An. gambiae* in sub-Saharan Africa among being abundance and close association with humans. The species' anthropophilic behaviour which increases its vector-human contact (van Emden and Service, 2004) and the chance to transmit disease. This calls for concerted effort for the control of this species in order to achieve maximum success in the fight against malaria in Nigeria and Africa. This would be a significant step to prevent future epidemics of malaria in the highland regions of the country, particularly in Mambilla Plateau.

Several studies are suggestive of the fact that some *Anopheles* species which were previously not transmitting the disease are assuming vector status (Mwangangi *et al*., 2013). *An. coustani* recorded the second highest abundance of (4.05%) of anophelines along the altitudinal locations studied. The species is fairly abundant with a wide geographical distribution in many parts of Africa including; Nigeria (Okorie *et al*., 2011), Zambia (Fornadel *et al*., 2011), South Africa (Munhenga *et al*., 2014) Kenya and Tanzania (Mwangangi *et al*., 2013; Dida *et al*., 2015) among others. In the current study, the species was not tested for *Plasmodium* sporozoites. It was also found negative for *Plasmodium* parasites in Southern Zambia (Fornadel *et al*., 2011) but contributed a substantially high EIR with 23.91 infectious bites per person per year to the overall EIR recorded in Kenya (Mwangangi *et al*., 2013). It was found to be playing a major role in outdoor malaria transmission. The high demonstration of anthropophilic tendencies is suggestive of its potential as a secondary malaria vector in Africa (Fornadel *et al*., (2011). *An. coustani* has not been implicated with malaria transmission in Nigeria but its presence in the study area is of epidemiological concern. This species may have or develop the potential to be a vector in Nigeria and may play a significant role in malaria transmission in the future.

An. rufipes and *An. pharoensis* were less abundant among all anopheline species encountered.*An. rufipes* had the least abundance (0.11%) and only in Nguroje, this is in conformity with Munhenga *et al*., (2014), who recorded least abundance of *An. rufipes* (0.1%) and confined to only one location of the five locations sampled. *An.*

rufipes and *An. pharoensis* have not been incriminated in malaria transmission in the study area, and although both species are zoophilic and exophilic they are potential secondary vectors (Norirs and Norris, 2015).

Of the dry season months (November, December, January, February and March), December experienced more anophelines species abundance while March had the least numbers. Highest species diversity was recorded in November. December marked the beginning of the dry season and more larval breeding habitats were newly formed by the receding rains. This subsequently resulted in high emergence of adult mosquitoes. March indicates the peak of the dry season and it was observed that most of the breeding sites were dried up there by reducing adult abundance. Most of the potential breeding sites were man-made and these dried up as the dry season intensified and this could explain the high anophelines species abundance in December and significant reduction during March. A Similar trend was observed in the Usambara Mountains in Tanzania where highest anopheline mosquitoes were collected at the end of the long rains (Boedker *et al*., 2003). Shortly after rainfall, *Anopheles* mosquitoes tend to quickly recolonize habitats (Mala *et al*., 2011). In the rainy months (April, May, June, July, August, September and October), June and September recorded more anophelines abundances. June recorded the highest abundance and species diversity while least abundances were observed in July and August. July recorded the least abundance and least diversity of species. These differences could be as a result of the change in precipitation. In the study area, the rains were steady and moderate in June and September, larval habitats were well established and productive thereby resulting in more immature and larval presence. The temperatures and relative humidity were also favourable to both the larvae and adult developments during the period.

On the contrary, the months of July and August indicated the peak of the rainy season and it was observed that most of the breeding sites were flooded and some overgrown with tall grasses. Larvae were washed away by the heavy rains thereby reducing adult abundance. This result is in accord with an earlier study in Bali LGA of Taraba State in which *An. gambiae s. s.* were collected mostly in June and none during the peak rainy months of July and August (Lamidi, *et al*., 2017). It is a general consensus that precipitation is associated with extended duration of water bodies, enabling for the

female mosquitoes to successfully oviposit and for larvae to develop to adults. It is also a known fact that larval habitats can become flooded with excessive precipitation. This could result in reduced adult population density due to washing away of the larvae (Vajda *et al*., 2017).

The high species abundance recorded in Mayo-selbe has serious epidemiological consequences. Mayo-selbeis situated at the foot of the mountain has more favourable environmental conditions for both adult development and parasites development time within the vector. The location could serve as a reservoir of malaria transmission to the highlands. Highest species diversity index and lowest *An. gambiae* dominance was recorded in Gembu. This location is strategic in the Mambilla Plateau. It is the head quarter of the Mambilla Plateau (Sardauna LGA) of Taraba State. People from the lowlands and other smaller localities within the Mambilla Plateau converge there for various purposes. These could be for administrative, health care, research, commercial, educational and many more reasons. The high mosquito abundance recorded in Gembu means that high population of individuals are at risk of malaria in this location of the Mambilla Plateau. Some of the mosquito species sampled from this location have been reported to play significant and some secondary roles in malaria transmission elsewhere (Mwangangi *et al*., 2013; Sinka, 2013), these species could assume vector status in the future in the absence of *An. gambiae*which is the primary vector.

In the current study, *An. gambiae* S form dominated over the M form. A recent study in Bali LGA which is another part of Taraba State, the M form was found to be dominant over the S form (Lamidi *et al*., 2017). This is to be expected as the S form is known to show preference to breed in temporary pools which are formed shortly after the rains. The M forms on the however, prefer habitats such as rice fields or flooded areas which are more permanent in nature (Sogoba, *et al*., 2008; Sinka, 2013; Coetzee *et al*., 2013). These two habitat types typify the nature of larval habitats found in the Mambilla Plateau and Bali LGA respectively. Earlier studies by Onyabe *et al*., (2003) on the contrary found the two molecular forms to occur all over the country (Nigeria) irrespective of the ecological location. It is apparent that these two molecular forms of *An. gambiae* are gaining wide spread distribution in the country.

This is worrisome as it could have serious epidemiological implications to malaria transmission in Nigeria if the trend continues.

There was a relatively low occurrence of positive breeding sites along the altitudinal gradient. Only six out of the 60 potential breeding sites were positive for anopheline larvae. This could be due to their temporary nature and variability. Larvae were found breeding in only one site in the rainy season as compared with five during the dry season. The larvae might have been washed away by the heavy rainfall as mosquito larvae were observed to prefer still, clear and sunlit temporary habitats. Some of the habitats that were encountered during the dry season had been replaced by swamps that were formed from the rain pools. Some were overgrown with tall grasses and the temporary pits were filled up with refuse. This finding concurred with Aditya, *et al*., (2006) who observed that the mosquito habitats in the hill town of Darjeeling were temporary in nature and existed only for a brief period of time.

In the dry season, larvae were collected only in Nguroje and Kakara but in the rainy season they were collected only in Gembu, and none were collected in other altitudinal locations. Mayo-selbe is a lowland and recorded the highest number of adult mosquitoes but no larvae. This finding contrasts with the impression that flat areas provide more breeding sites than steep slopes (Bodker, *et al*., 2003). The non-availability of larvae in Mayo-selbe both in the dry and rainy seasons could be attributed to elevated temperatures which resulted in shorter development time of larvae and therefore more adult presence. Both larvae and adult mosquitoes are sensitive to temperature changes in their aquatic environment. Water temperatures of above 34°C generally have negative impact on the survival of vectors (Attaullah *et al*., 2015; Aribadoor *et al*., 2016). Some of the adult mosquitoes could also have been imported in vehicles due to migration.

In Kakara most of the breeding sites were observed close to the forest with banana and tea plantations about 50m away from houses and therefore beyond flight distance of the adult mosquitoes (Mala, *et al*., 2011). These temporary breeding sites were cultivated during the rainy season. In Nguroje the larval habitats were located just within 7m of residential houses and were also cultivated into vegetable gardens, this could account for their absence during the rainy season. In rural Gambia, a significant

decline in the number of mosquitoes with distance to nearest breeding habitats was observed. This is in agreement with the present study (Thomas, *et al*., 2013). It is suggested that for female mosquitoes seeking blood meals orientation towards a host accounts only for short range movement of over 30m or less (Thomas, *et al*., 2013).

In the current study temperature of water in the breeding habitats at the time of sampling ranged from 21.39°C to 31.90°C and pH of the breeding habitats varied from 5.88to 7.11. Earlier studies from other parts of Nigeria and Africa had reported temperatures and pH within the same range as the present study. Adebote, *et al*., (2008) reported temperature range of 14 to 40°C and pH range of 5.86 to 9.85, Afolabi, *et al*., (2013) recorded temperatures of 26.5 to 29.3°C and pH 7.1 to 7.3. Dida, *et al*., (2015) recorded a pH range of 6.7 to 8.4 along the Mara River in Kenya.

The anopheline larvae were associated with temperature range of 20.7 to 28.0°C and pH 5.8(slightly acidic) to 7.8(slightly alkaline). This is similar to a study by Hanafi-Bojd, *et al*., (2012) who found malaria vectors breeding in habitats of between 20°C - 30°C. Larvae thrived in habitats with 6 mg/L dissolved oxygen (DO), this was the amount of DO in all the habitats in the three altitudinal locations from where larvae were sampled,and that is Nguroje, Gembu and Kakara. This was identical to 6.4mg/L recorded by Dida *et al*. (2015)in Kenya. Other parameters which favoured larval productivity in the present study included, electrical conductivity in the range of 14.5mg to 159mg/L, total dissolved solids 8 to 87mg/L and turbidity which ranged from 1.4 to 138mg/L.

The entomological inoculation rate is an index for measuring malaria transmission intensity, and it is determined from the product of the sporozoites rates and the man biting rates. It was observed that malaria transmission indices SR, MBR and EIR values increased with decrease in altitude. These were highest during the peak malaria transmission season in June and lowest during the peak of the rainy season in July. Kilama *et al*., (2012) recorded similar seasonal differences in MBR and EIR pattern in Uganda. Malaria transmission intensity indices exhibited a similar trend along the altitudinal locations, except for Gembu where the values decreased with increasing altitude. Mayo-selbe recorded higher valueof the malaria transmission indices, that is (SR, MBR and EIR) than locations situated at higher altitudes. This could be because

of the location of Mayo-selbe, it is at the foot of the mountain and environmental conditions are more favourable for mosquitoes to survive and develop. It could also be because greater percentage of mosquitoes tested were from Mayo-selbe. Current results are in agreement with studies from other highland regions in Africa. For example, in a study in the Usambara Mountains in Tanzania Bodker *et al*., (2003) made similar observations. Maxwell *et al*., (2003) also recorded sporozoites rates which were slightly but not significantly greater in the lowlands than in the highlands. In the current study however, there were significant differences in the sporozoites rates between the high and low altitudinal locations. Studies from other parts of Africa have also revealed variation in sporozoite rates across locations (Mwangangi *et al*., 2013). The 2.74% mean sporozoites rates along the altitudinal locations is higher than the lower limit of SRs recorded in *An. gambiae s.s* species across Nigeria (0-91%) over a period of 100 years research (Okorie *et al*., 2011).

Gembu is located at a higher altitude than Kakara and yet it recorded higher values of sporozoites, man biting and entomological inoculation rates than the later. This could be that Gembu being the headquarters of the LGA experiences a rise in human population density and social activities are more pronounced. This could be as a result of the constant migration of people from morerural areas including the lowlands. These rural areas and lowlands, are believed to have higher malaria transmission. This has a significant impact on the health of the local populations as risk of malaria outbreaks tends to increase with each imported case of the disease (Smith *et al*., 2013).

Sporozoites, rates also differed with change in rainfall thereby creating two peak malaria transmission seasons along the altitudinal locations. One was in December after the long rainy season and the other in June shortly after the onset of the rainy season. This finding agrees with findings by Molineaux and Gramiccia, (1980) who reported an increase in sporozoites rates during the wet season. December marked the beginning of the dry season with more larval habitats formed by the receding rains and high emergence of adult mosquitoes. In June the rainfall was moderate and stable hence, breeding habitats were well established.

In the current **s**tudy, most of the mosquitoes that were assayed were unfed. Okorie *et al*., (2014) recorded a similar trend where an astonishing number of mosquitoes were unfed. An earlier study also reported blood meals that were negative for all tested hosts (Okwa*et al*., 2009). This could have been influenced by the use of blankets to protect individuals from cold during the night. It may also be due to some host-seeking factors; the mosquitoes may have been trapped indoors while searching for a blood-meal after emergence from the breeding sites.

About 22.67% of the mosquitoes had fed on human blood, other sources of blood meal were unknown. The current result concurr with reports by Boedker *et al*., (2003) who found that nearly 100% of all mosquitoes tested had fed on human blood. Molineaux and Gramiccia, (1980) also found that the main mosquito vectors (*An. gambiae* and *An. funestus*) had fed mainly on human blood. The research also reported that 79% of *An. gambiae* had fed on man even when the proportion of cattle to man were 10:1

An. gambiae s.s and *An. arabiensis* are the members of the *An. gambiae s.l* often reported to be involved in malaria transmission in Africa (Mwangangi *et al*., 2013; Molineaux and Gramiccia, 1980). Comparing the degree of anthropophily of these two species with respect to malaria transmission, *An. gambiae s. s.* tends to be stronger and more consistent than *An. arabiensis*. In a situation where these two species were in sympatry it was observed that *An. arabiensis* was less anthropophilic than *An. gambiae* though no clear-cut difference regarding indoor resting behaviour was observed (Molineaux and Gramiccia, 1980). In the current study the human blood index across the altitudinal locations, was highest in Mayo-selbe (0.22). This indicates the high vector efficiency of the anophelines vectors in Mayo-selbe.

Mayo-selbe recorded the highest number of positive human blood meal. This could be as a result of the high number of mosquitoes tested for blood meal from Mayo-selbe which is a lowland. Of the seasonal blood meal preference, December recorded highest mosquito abundance as well as human blood meal. This result is in accord with Zimamman *et al*., (2006) who opined that the choice of blood-meal could be influenced by the availability of the host. However, April, May, and October also recorded substantially high abundances of anophelines presence but all recorded zero

(0) human blood meal. This could also mean that season was a key factor in the choice of blood meal. April, May and October were all within the rainy season. This result strongly agrees with the findings of the Garki Project where malaria transmission was observed for to vary with both locations and seasons. (Molineaux and Gramiccia, 1980). From the results of the current study, it was further observed that the blood meal preference was influenced by some factors such as locations, abundance of the anopheline vectors and season.

8.2: Conclusions

1. Results of the current study revealed the abundance of anopheline mosquito species in the study area. *An. gambiae s.l* was the most abundant of the five species of anopheline mosquito collected. *An. gambiae s.s* was the main anopheline vector of malaria on the highlands of Mambilla Plateau Nigeria. This poses a serious health implication as *An. gambiae* is the major vector of malaria in sub-Saharan Africa and in Nigeria in particular where the burden of the disease is still high. Anopheline mosquito collections were made in all the altitude locations and all months although with varying abundance along an altitunal gradient. The S form was the dominant *An. gambiae ss* molecular form in Mambilla Plateau.
2. There were relatively low productive breeding larval habitats along the altitudinal locations. Mosquito larvae bred in still, clear sunlit temporary habitats that were formed from the rainfall as well as ditches that were created from moulding blocks. The larvae also thrived in habitats with a wide range of physicochemical parameters.
3. Malaria transmission indices namely; sporozoites rates, man biting rates and entomological inoculation rates all declined with increasing altitude. They also exhibited variation with season.
4. Human blood meal constituted the major blood meal source of anophelines vectors of malaria in the Mambilla Plateau. The choice of blood meal was influenced by location, altitude, abundance and availability of vectors as well as season.

5. Mayo-selbe recorded the highest number of mosquitoes collected as well as all the malaria transmission indices. Gembu recorded the next high abundance. These results have serious implications to malaria transmission in the Mambilla Plateau. Mayo-selbe is located at the foot of the mountain and could serve as a reservoir for both mosquitoes and *plasmodium* which can be transported into the highlands. Gembu is the central point of commerce and convergence of people in the Mambilla Plateau. This means that a high population of people with little or no functional immunity are at risk of malaria within the Mambilla Plateau.

8.3: Recommendations and Future work

From results of the study, malaria transmission is occurring in the highlands of Mambilla Plateau and *Anopheles gambiae s.s* mosquito is the major vector of the disease. It is therefore recommended that;

1. Control programmes should be targeted at the *An. gambiae s.s* species, and there should be close monitoring and surveillance of the other anophelines species that were encountered in the study.
2. Locations, altitude, vector abundance and season should be taken into account when evaluating effects of control programmes.
3. Other anopheline mosquito species that were identified in the study area should be screened through molecular means so as to establish the roles such species could be playing in malaria transmission in the study area.
4. A parasitological aspect should be included in such entomological studies so as to quantify the actual parasitaemia, and the level of malaria transmission in the study area.
5. Finally, the factors that predispose people in the Mambilla Plateau to malaria transmission should be investigated. This is with a view to adequately counsel and educate these vulnerable human populations concerning those behavioural activities that place them in danger of malaria.
6. The identification of mosquito blood meal sources other than from humans which was beyond the scope of this study should be considered as a major component for future research in Mambilla Plateau.

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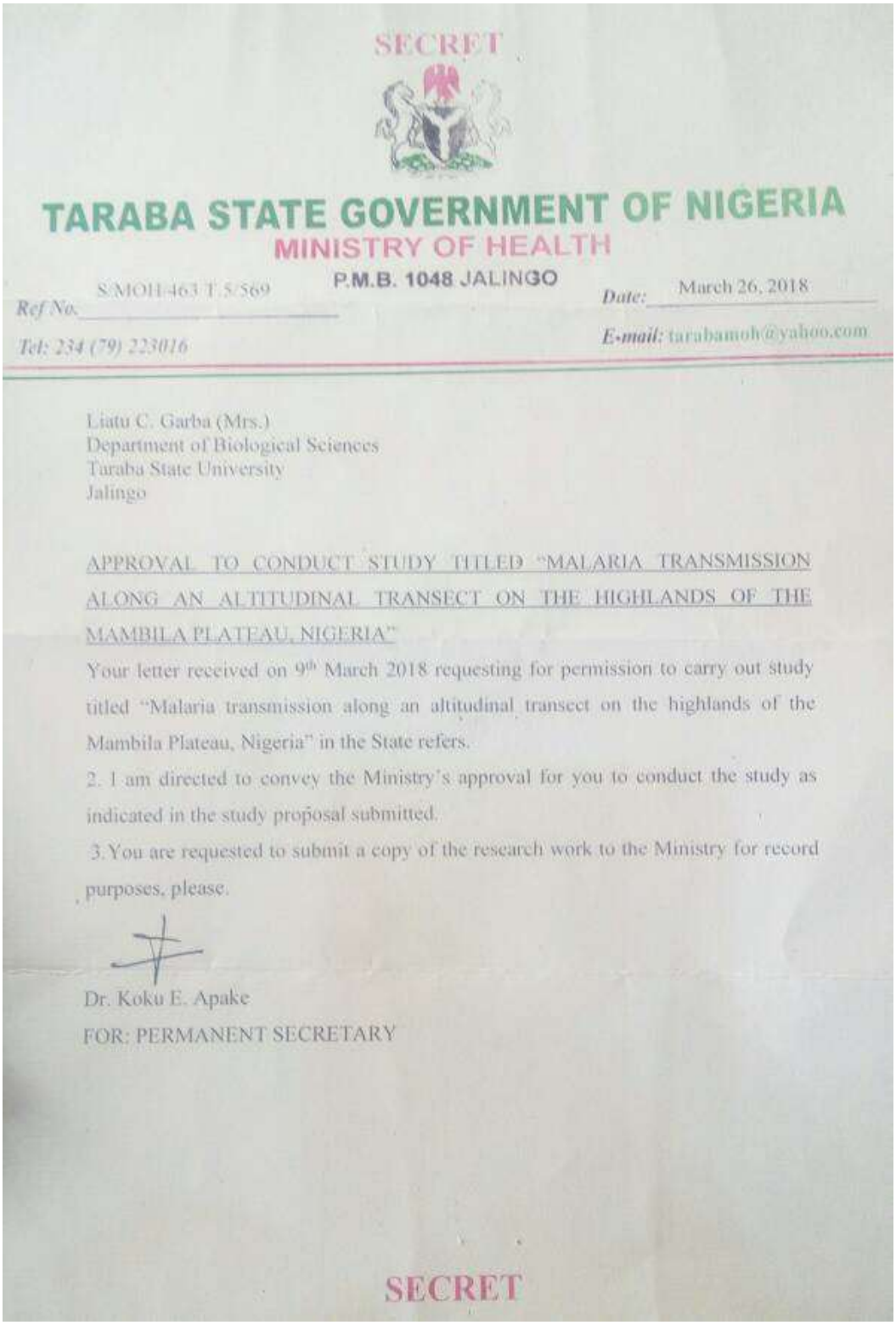
APPENDICES

APPENDIX I: APPROVAL LETTER TO CONDUCT RESEARCH



SECRET

TARABA STATE GOVERNMENT OF NIGERIA

MINISTRY OF HEALTH

P.M.B. 1048 JALINGO

Ref No. S/MOH/463/T.5/569

Date: March 26, 2018

Tel: 234 (79) 223016

E-mail: tarabamoh@yahoo.com

Liatu C. Garba (Mrs.)
Department of Biological Sciences
Taraba State University
Jalingo

APPROVAL TO CONDUCT STUDY TITLED "MALARIA TRANSMISSION ALONG AN ALTITUDINAL TRANSECT ON THE HIGHLANDS OF THE MAMBILA PLATEAU, NIGERIA"

Your letter received on 9th March 2018 requesting for permission to carry out study titled "Malaria transmission along an altitudinal transect on the highlands of the Mambila Plateau, Nigeria" in the State refers.

2. I am directed to convey the Ministry's approval for you to conduct the study as indicated in the study proposal submitted.

3. You are requested to submit a copy of the research work to the Ministry for record purposes, please.

Dr. Koku E. Apake
FOR: PERMANENT SECRETARY

SECRET

APPENDIX II: AVERAGE INDOOR AND OUTDOOR TEMPERATURES AND RELATIVE HUMIDITY FOR CDC LIGHT TRAP COLLECTIONS FROM NOVEMBER 2015 TO OCTOBER 2017.

Time ofcollection	Indoor		Outdoor	
	Temperature (°C)	Relative humidity	Temperature (°C)	Relative humidity
6-7 pm	23.74	36.32	23.98	34.17
7-8 pm	23.13	38.57	23.21	35.41
8-9pm	21.83	40.71	22.37	38.46
9-10 pm	20.87	41.33	22.16	39.12
10-11pm	20.69	42.26	21.38	40.30
11-12 pm	19.76	42.64	21.22	40.87
12-1am	19.54	43.75	20.33	41.93
1-2 am	19.32	43.89	20.14	41.98
2-3am	18.60	44.17	19.76	43.34
3-4am	18.25	44.53	19.42	43.15
4-5am	17.79	45.88	18.78	44.60
5-6 am	17.72	45.92	18.67	44.67

APPENDIX III: PUBLISHED PAPER 1

Journal of Biotechnology and Bioengineering, 20171(**1**): 37-42.

Species Diversity and Relative Abundance of Anopheline Vectors of Malaria on the Highlands of Mambilla Plateau Taraba State North East, Nigeria.

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Abstract

The increase in human population in the recent past has resulted in deforestation and cultivation of natural swamps consequently, the ecology of African highlands has been changing favouring mosquito survivorship and parasite development. The aim of the studywas to determine the effect of altitude on species abundance anddiversity of anopheline vectors of malaria along an altitudinal transect on the highlands of Mambilla Plateau Nigeria. Adults anopheline vectors were captured by the use Centre for Disease Control (CDC) modified light traps and Pyrethrum Spray Catches (PSC) while larvae were reared to adulthood. A total of 420 anopheline mosquitoes comprising of five species: *Anopheles ga*mbiae complex 394 (93.81%), *An. coustani* 17(4.05%), *An. funestus* 5(1.19%), *An. pharoensis* 3(0.71%) and *An.rufipes* 1(0.24%) were sampled along the altitude locations. A total of 342 (81.42%) adult *Anopheles* mosquitoes made up the total collection for the study period. *Anopheles gambiae sl* 333(97.36%), was the most abundant of all the adult species collected while *An.rufipes* 1(0.30%) was the least abundant species. Molecular analysis with PCR showed that *An. gambiae ss* was the main *An. gambiae* species in the study area. Peak biting period was between 12am-2am, temperature and relative humidity had no significant effect on mosquito abundance. This study has provided baseline data on the species and diversity of anopheline mosquitoes on the Mambilla Plateau.

Key words: *Anopheles*, Mambilla Plateau, Abundance, Diversity, PCR.

Journal of Biotechnology and Bioengineering
Volume 1, Issue 1, 2017, PP 37-42

Species Diversity and Relative Abundance of Anopheline Vectors of Malaria on the Highlands of Mambilla Plateau Northeast, Nigeria

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ABSTRACT

The increase in human population in most African highlands in the recent past has resulted in deforestation and cultivation of natural swamps consequently the ecology of African highlands has been changing favoring mosquito survivorship and parasite development. The aim of the study was to determine the effect of altitude on species abundance and diversity of anopheline vectors of malaria along an altitudinal transect on the highlands of Mambilla plateau Nigeria. Adult anopheline vectors were captured by the use of Centre for Disease Control (CDC) modified light traps and Pyrethrum Spray Catches (PSC) while larvae were reared to adulthood. A total of 420 anopheline mosquitoes comprising five species; An. gambiae sl 394(93.81%), An. coustani 17 (4.05%), An. funestus 5(1.19%), An. pharoensis 3(0.71%), and An. rufipes 1(0.24%) were sampled along the altitude locations. A total of 342 (81.42%) adult Anopheles mosquitoes make up the total collection for the study period An. gambiae sl 333(97.36%), was the most abundant of all the adult species collected while An. rufipes 1(0.30%) was the least abundant species. Molecular analysis with PCR showed that Anopheles gambiae ss was the main Anopheles gambiae species in the study area. Peak biting period was between 12am to 2am, temperature and relative humidity had no significant effect on mosquito abundance. There was no significant relationship between altitude and mosquito abundance and species diversity ($P > 0.05$). This study has provided baseline data on the species and diversity of anopheline species on the Mambilla plateau.

Keywords: *Anophelines, Mambilla Plateau, Abundance, Diversity, PCR*

INTRODUCTION

Malaria is caused by four species of protozoan parasites *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax* and *Plasmodium ovale*. The disease is transmitted by the bite of about 30-40 species of female *Anopheles* mosquitoes of about 515 known species [1]. The World Health Organization policy on Roll Back Malaria program (RBM), placed significant importance on vector control thereby the signing of the Declaration and Plan of Action to reduce malaria burden by the year 2010 by African countries [2]. Reduction was to be achieved through, access to the most desirable measures of protection both at personal and community levels and, population most at risk, children under five years and pregnant women. Countries were to constitute epidemic readiness and warning signals response that are efficient and able to detect and manage promptly any outbreak [2]. Highland regions of Africa are malaria hypoendemic due to climatic factors [3]. Hypoendemicity refers to unstable endemic malaria transmission which is estimated at less than 10% [4]. Topography of highland areas comprise of mountains, valleys and high plateaus with altitudes above 1500m above sea level the climate is cool and temperature inversely related to altitude [5]. Due to variations in highland temperatures which hitherto are low, malaria transmission is unstable leading to an

increase in the number of malaria epidemics in the past decades [6], [7].The increase in human population in most African highlands in the recent past has resulted in deforestation and cultivation of natural swamps [8]. Consequently, the ecology of African highlands has been changing favoring mosquito survivorship and parasite development. Abundance of mosquitoes in the highlands is seen to have resulted from this change in land use [9], [5].

Beside the volatile epidemics, there is an increase of endemic malaria transmission in the African highland regions [10]. Control strategies in highland areas should be much more based on prevention of epidemics through early detection. The challenge however, is insufficient surveillance and response systems to monitor malaria transmission dynamics to guide the elimination process. Data on various entomological parameters of anopheline vectors in high risk regions would provide important information on malaria transmission intensity. These data can aid in scaling up control and assess effectiveness of control measures as well as predict malaria epidemics in the highland regions.

MATERIALS AND METHODS

Study Area

Mambilla plateau is located at longitude 6.8212' N, 11.5345' E and latitude 7.3523' N, 10.7723' E in Taraba state North-Eastern Nigeria. Mambilla plateau has an area of about 3765sq km while the adjoining lowland covers about 1,250sqkm. The topography of Mambilla plateau comprises undulating lowland, low hills and irregular plains, ridges, hills, and escarpment. Climate is semi-temperate with mean annual temperature of 16^{0}C. Mambilla plateau has an average altitude of 1600m above sea level. Mean annual rainfall is 1800mm; the rainy season extends from late March to October while a short dry season occurs between November and early march. Mambilla plateau has a population of 224,357 people [11].

Study Design

The study was conducted from December 2016 to March 2017 in five communities selected along an altitudinal transect to collect entomological data. The rationale for selection of the communities was based on entomological grounds namely, areas prone to malaria epidemics access road throughout the year and altitude. The communities are Mayo-Selbe (484m), Kakara (1496m), Gembu (1584m), Yelwa (1674) and Nguroje (1885m) above sea level.

Ethical Clearance

Ethical clearance was obtained from Taraba State Ministry of Health and consent of Communities leaders was sought to carry out the research in their various communities.

Field Entomological Survey

Field entomological surveys were carried out in all the five communities, mosquito collections were conducted once every month in each of the study sites from December 2016 to March 2017. Anopheline vectors were captured by two methods the use of Centre for Disease Control (CDC) modified light traps and Pyrethrum Spray Catches (PSC). For the CDC collection, indoor traps were suspended from the ceiling at the foot end of the bed at approximately 1.5meters above the ground level in an occupied room from 6pm to 6am. The outdoor traps were hung on a post around the same houses. After every one hour traps were inspected and mosquitoes were aspirated into clearly labeled paper cups and kept in the cool box for transportation to the laboratory for further processing. PSC were carried out from 6am to 9am in at least 20 rooms in each of the communities by spreading white sheets on the entire floor and over the furniture that could not be moved after which the rooms were sprayed with pyrethrum. The rooms were closed for about 10 to 15 minutes for mosquitoes to be knocked down. The knocked down mosquitoes were collected from the white sheets with forceps into petri dishes. All *Anopheles* mosquitoes collected were then taken to the laboratory and identified morphologically using keys [12], these were sorted out according to species, site, methods, and time of collection, and stored individually in ependorf tubes and silica gel. Anopheles mosquitoes were further identified by molecular means using PCR. Female mosquitoes were classified according to their repletion status into unfed, fed, half-gravid and gravid specimens [13]. Elevations of houses from which both CDC-light trap and PSC samplings were made were recorded.

Data Analysis

$$RD = \frac{NA}{N} \times 100$$

RD= ..... (1)

Where RD = relative density of species
NA = number of all specimens of each species collected at each altitude
N = the number of specimens of all species collected at each altitude.

$$H' = -\sum \frac{ni}{n} \ln \frac{ni}{n}, \quad E = \frac{H}{H_{max}} \quad \text{.....} \quad (2)$$

Where
H' = Shannon diversity index
ni= number of species i
n = total number of samples
E = evenness
H_{max} = Maximum Diversity possible

Statistical Analysis

Pearson correlation coefficients were computed on the *Anopheles* mosquito species captured using SPSS 20v to study the correlations between mosquito abundance and altitude.

RESULTS

A total of 420 anopheline mosquitoes comprising five species; *An. gambiae sl, An. coustani, An. pharoensis, An. funestus* and *An. rufipes* were sampled along the altitude locations. *An. gambiae sl* 394(93.81%), was the most abundant of all the species collected followed by *An. coustani* 17 (4.05%), *An. funestus* 5(1.19%), *An. pharoensis* 3(0.71%), and *An. rufipes* 1(0.24%) was the least abundant species. The relative abundance and species of female *Anopheles* mosquitoes along altitude locations are represented in Table 1. Adult *Anopheles* mosquitoes make up 81.42% of the total collection for the study period along the altitude locations. A total of 342adult of which *An. gambiae sl* 333(97.36%), *An. coustani*, 5(1.46%), *An. pharoensis* 2(0.58%), *An. funestus* 1(0.30%) and *An.rufipes*1(0.30%).*An. gambiae sl* was the most abundant of all the adult species collected followed by *An. coustani, An. funestus, An. pharoensis* and *An. rufipes* was the least abundant species. There was significant difference between number and species of anophelines mosquitoes collected ($P < 0.05$).

Overall greatest species diversity three out of five species were recorded in Yelwa and Mayo-selbe next diversity two species out of five were recorded in Nguroje. Gembu and Kakara recorded the least species diversity (only one species (*An. gambiae*) out of the five anopheline species were collected.

Table1. *Species composition and relative abundance of anopheline mosquitoes on the highlands of Mambilla plateau*

Species Composition	Number sampled	Percentage (%)
Anopheles gambiae sl	394	93.81
Anopheles coustani	17	4.05
Anopheles funestus	5	1.19
Anopheles pharoensis	3	0.71
Anopheles rufipes	1	0.24
Total	420	100

Shannon wiener diversity index of 16.38 and evenness of 10.17 were recorded across the altitudinal locations. Highest relative density was observed in *An. gambiae* (97.37%) followed by *An. coustani* (1.46%) then *An. funestus* (0.59%). *An. pharoensis* and *An. rufipes* had the least relative densities of 0.29% each. There was significant difference between number and species of anophelines mosquitoes collected and altitude ($p<0.05$). Table 2 represents the diversity and relative density of *Anopheles* vectors of malaria along altitudinal locations. Table 3 shows hourly anophelines collection. Peak night biting period along the altitude was between 12-2am. Highest collection 0.18±0.38 and 0.28±0.51 occurred between this period and the least mosquito collections 0.08±0.27 was between 4-6am. There was no significant difference between time of collection and number of mosquitoes collected. There was significant difference between number of hourly night mosquitoes collections by CDC (6pm-6am) and the number collected by PSC (6am-9am) 13.85±36.60. There was a slight increase in indoor temperature and relative humidity over outdoor however there was no significant difference between them.

Polymerase Chain Reaction

Application of species-specific PCR analysis confirmed that *An. gambiae ss* is the only member of the *An. gambiae* complex in all the altitudinal locations. Out of the 300 *Anopheles* species selected [14], 197 (65.7%) were identified as *An. gambiae ss*. The remaining 103(34.3%) could not be identified. This could have resulted from incorrect morphological identification, DNA degradation due to bad preservation or human error. (Figure1).

Table2. *Relative density, species diversity and relative abundance of adult female anophelines across altitudinal locations*

	SPECIES Frequency (%)					
Location	***An. gambiae***	***An. coustani***	***An. funestus***	***An. pharoensis***	***An. rufipes***	**Mean± SD**
Nguroje	2	0	0	0	1	3
Yelwa	13	2	0	1	0	16
Gembu	58	0	0	0	0	58
Kakara	1	0	0	0	0	1
Mayo-selbe	259	3	2	0	0	264
N	333	5	2	1	1	342
RD	97.37	1.46	0.59	0.29	0.29	100.00
H'	-0.029	-0.063	10.27	3.12	3.12	16.38
E	10.17					

RD = Relative density, N = Number of samples, H' = Shannon weiner diversity index, E = evenness

Table3. *Number of adult mosquitoes caught at different time interval*

Time of collection	**Adult mosquito caught**	**Temperature outdoor**	**Temperature indoor**	**Relative humidity indoor**	**Relative humidity outdoor**
6-8pm	0.08 ± 0.27^{e}	23.74 ± 2.98^{f}	23.98 ± 3.09^{f}	36.32 ± 10.30^{b}	34.17 ± 10.16^{b}
8-10pm	0.10 ± 0.38^{e}	21.83 ± 2.55^{g}	22.37 ± 2.53^{g}	40.71 ± 11.69^{bc}	38.46 ± 12.33^{bc}
10-12pm	0.18 ± 0.38^{e}	20.69 ± 2.62^{gh}	21.38 ± 2.56^{gh}	42.26 ± 12.62^{bc}	40.30 ± 13.23^{bc}
12-2am	0.28 ± 0.51^{a}	19.54 ± 2.62^{ghi}	20.33 ± 2.45^{ghi}	43.75 ± 12.76^{c}	41.93 ± 13.84^{c}
2-4am	0.13 ± 0.33^{a}	18.60 ± 2.54^{hi}	19.42 ± 2.37^{hi}	44.17 ± 13.50^{c}	43.15 ± 15.18^{c}
4-6am	0.08 ± 0.27^{e}	17.79 ± 2.19^{i}	18.78 ± 2.06^{i}	45.88 ± 14.53^{c}	44.60 ± 15.66^{c}
6-9am	13.85 ± 36.60^{b}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Total	1.19±10.57	18.80±6.27	19.42±6.34	38.94±16.73	37.32±17.11

Values represent Mean ± SD of N=40 and r=5. Values in the same column with different superscript across the row differs significantly p<0.05).

DISCUSSION

All the anopheline species reported in the current study have been reported in other parts of Nigeria [15] and Africa in general. This confirms the wide range of geographic distribution of the anophelines. *An. gambiae sl* was the most abundant of all the anopheline species encountered it was recorded in all the five altitude locations. Several studies have reported similar high abundance of *An. gambiae sl* from different parts of Nigeria [16]; [15]; [17]; [18], it is among the most often reported malaria vector species in the country [15]. *An gambiae* and *An. funestus* were two principal anopheline species identified in the current study this agrees with [16] who recorded these two species out of the three *Anopheles* species recovered in Nassarawa state. *An. gambiae* and *An. funestus* complexes comprised 14.4% of the total species recorded in Benin City Nigeria [18], 65.2% and 17.3% respectively of most commonly reported malaria vectors in Nigeria [15]. Earlier study in other African highlands had reported these two species as the predominant anophelines [19]. This has serious health implications as the two species have been identified as important malaria and lymphatic filariasis vectors in Nigeria [18] particularly so that Nigeria is one of the countries with high malaria prevalence in sub-Saharan Africa [20]; [1].

Several factors have enhanced the vector efficiency of *An. gambiae* in sub-Saharan Africa among which are abundance and close association with humans, its anthropophilic behaviour which increases its vector-human contact [21] and the chance to transmit disease. This calls for concerted effort for the control of these species in order to achieve maximum success in the fight against malaria in Africa and Nigeria as a mechanism to prevent future epidemics of malaria in the highland regions of the country, Mambilla Plateau in particular.

An. coustani recorded the second highest abundance (4.05%) of anophelines along the altitudinal locations. The species is fairly abundant with a wide geographical distribution in many parts of Africa including; Nigeria [15]; Zambia [22], South Africa [7] Kenya and Tanzania [23]; [24] among others. The high demonstration of anthropophilic tendencies is suggestive of its potential as a secondary malaria vector in Africa [22] and although it has

not been implicated with malaria transmission in Nigeria, its presence in the study area is of epidemiological concern. This species may have or develop the potential to be a vector in the future and may play a significant role in malaria transmission in the country.

An. rufipes and *An. pharoensis* had the least abundances of all the anopheline species in the study area this is in conformity with [7] who recorded least abundance of *An. rufipes* 0.1% and confined to only one location of the five locations sampled. Contrary to the present result, *An. pharoensis* and *An. gambiae* jointly accounted for 96.7% of mosquitoes sampled in Baringo in Kenya [25]. *An. rufipes* and *An. pharoensis* have not been incriminated in malaria transmission in the study area and although both species are zoophilic and exophilic they are potential secondary vectors [26].

From our result, a slight change in temperature and relative humidity was observed between hourly anophelines collection however, these did not have any significant effect on the number of mosquito collection.

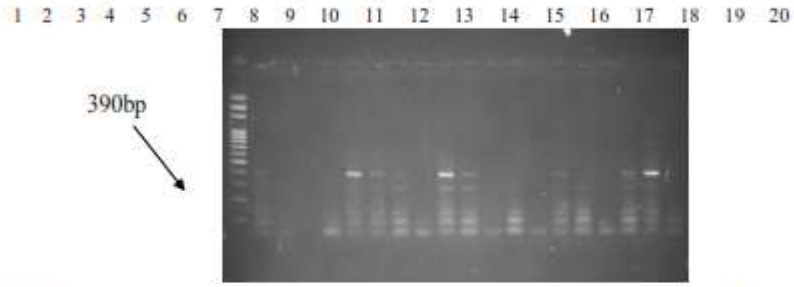

Figure1. *Amplified fragment using the species-specific assay for the identification of member of the Anopheles gambiae complex Lane 1 lader (molecular marker), lane 2 negative control: lane 3 positive control Anopheles gambiaes.s (390bp).*

CONCLUSION

Result of study revealed the abundance of anopheline mosquito species in the study area. Population of *An. gambiae sl* was the most abundant of the five species of anopheline mosquitoes and *An.gambiae ss* as the main anopheline vector of malaria on the highlands of Mambilla Plateau Nigeria. Anophelines mosquito collections were made in all the altitude locations although with varying abundance. With the exception of Kakara (1,496m), mosquito abundance declined either slightly or significantly with increasing altitude.

This study has provided information about diversity of *Anopheles* species on the highlands of Mambilla plateau. This would be helpful for the sustainable management of vector mosquitoes and to take precautionary measures against malaria epidemic. There was no significant relationship between altitude and mosquito abundance and species diversity. This study has provided useful data to elucidate malaria transmission intensity and epidemic on the Mambilla plateau.

ACKNOWLEDGEMENT

The authors wish to thank the management of Taraba State University Jalingo for Financial assistance to carry out this research. We also appreciate the community heads for granting us the permission to work in their various communities.

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APPENDIX IV: PUBLISHED PAPER 2

International Journal of mosquito research, 5(**1**):96-100.

Larval habitats of anopheline vectors of malaria on the highlands of Mambilla Plateau, Taraba State Northeast Nigeria.

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Abstract

Identification of larval habitats on the highlands ofMambilla Plateau and mosquito tolerance range to physico-chemical parameters may be helpful to control insurgent of vector mosquitoes and to put in place mechanisms to prevent epidemics. This study was conducted to determine and characterized the larval habitats of anophelinevectors of malaria in five altitudinal locations on the highlands of Mambilla Plateau. Larval density for each location was determined from the breeding index using the formula:BI= breeding index, TLP = total number of larvae and pupae sampled, ND = number of dips, BP = number of breeding places /sampling sites. Logistic regression analysis was used to determine relationship between larval abundance and altitude as well as mean physicochemical parameters. Larval mosquito abundance per study location was recorded and compared. In all, 60 larval habitats were sampled, only six were productive for anopheline larvae. These were from three altitudinal locations. About larvae 179 were collected from the breeding habitats and reared to adulthood. They comprised of four species; *An. gambiae*, *An. coustani*, *An. funestus*, and *An. pharoensis*. Mosquitoes thrived in still, clear sunlit temporary pools andswamps with wide range of physicochemical parameters. Altitude and physicochemical parameters of the larval habitats should be considered in planning and implementing targeted mosquito control programmes in the highlands

Keywords: Larval habitats, Mambilla Plateau, Nigeria, *Anopheles*

ISSN: 2348-5906
CODEN: IJMRK2
IJMR 2018; 5(1): 96-100

Received: 05-11-2017
Accepted: 07-12-2017

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Larval habitats of anopheline vectors of malaria on the highlands of Mambilla Plateau Taraba State North East Nigeria

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Abstract
Identification of larval habitats on the highlands of Mambilla Plateau and mosquito tolerance range to physico-chemical parameters may be helpful to control insurgent of the vector mosquitoes and to put in place mechanisms to prevent epidemics. This study was conducted to determine and characterize the larval habitats of anopheline vectors of malaria in five altitudinal locations on the highlands of Mambilla Plateau. Larval density for each study location was determined from the breeding index using the formula: BI= TLP/ND×BP, where BI= breeding index, TLP= total number of larvae and pupae sampled, ND= number of dips and BP= number of breeding places/sampling sites. Logistic regression analysis was used to determine relationship between larval abundance and altitude as well as mean physicochemical parameters. Larval mosquito abundance per study location was recorded and compared. In all, 60 larval habitats were sampled only six were productive for anopheline larvae these were from three altitudinal locations. About 179 larvae were collected from breeding habitats and reared to adulthood they comprised of four species; *Anopheles gambiae*, *An. coustani*, *An. funestus* and *An. pharoensis*. Mosquitoes thrived in still, clear sunlit temporary pools and swamps with wide range of Physico-chemical parameters. There was no significant relationship between larval abundance and physicochemical parameters as well as altitude $p<0.05$. Altitude and physicochemical parameters of the larval habitats should be considered in planning and implementing targeted larval mosquito control programmes in the highlands.

Keywords: larval habitat, mambilla plateau, nigeria, *Anopheles*

1. Introduction
One of the efforts to control malaria is reduction in the mosquito vector-humans contacts [1].Mosquito larval habitats are places where mosquitoes breed, from the point of egg laying to completion of the life cycle, to emergence as adults [2]. The larval habitat is therefore very vital for mosquito population dynamics because the development and fitness of the adult mosquitoes are impacted by the status of their aquatic larval habitats [3]. Larval habitats are made up of diverse water bodies ranging from temporary to permanent, natural to artificial, small to large fresh water to salt water, shaded or sunny [4-5]. A greater number of anopheline species have been observed to breed in a broad range of habitats [6] but each species shows preference for various breeding site for oviposition [7].

Larvae of *An. gambiae s.l.* can be found in both temporary and permanent habitats [9] in large bodies of water such as flood plains [10] and in pools along lake shores [11]. Sibling species of the *An. gambiae* complex differ in their preference for breeding habitats, *An. melas* and *An. merus*, are most productive around the coastal areas where they exhibit great tolerance for salinity, surviving in relatively high degrees of salinity sometimes reaching or even exceeding that of sea water [8].Quality of the breeding habitat and physicochemical parameters among others are factors that impact on larval development and growth [12]. The understanding of each species threshold to environmental variables and habitat adaptation across altitudinal gradients is one potentially significant point in malaria vector control in the highlands this may provide useful insight into mosquito congregation[25].

Until now, the larval habitats and the physicochemical parameters that influence anophelines mosquitoes in their breeding habitats on the Mambilla Plateau are unknown therefore knowledge acquired from the current study could help to develop an effective measure to

control malaria through vector management and to put in place mechanism to prevent epidemics.

2. Materials and Methods

2.1 Study area

Mambilla Plateau is located at longitude 6.8212˚ N, 11.5345˚ E and latitude 7.3523˚ N, 10.7723˚ E in Taraba state North-Eastern Nigeria with an area of about 3765sq km while the adjoining lowland covers about 1,250sqkm. It has boundaries with Gashaka Local Government Area (LGA) in the north-east, Kurmi Local Government Area in the north-west and Republic of Cameroun by an international boundary in the south. It is located in a savannah landscape with a peculiar topography and climate. The topography of Mambilla Plateau comprises undulating lowland, low hills and irregular plains, ridges, hills, and escarpment. Climate is semi-temperate with mean annual temperature of 16˚C. Mambilla Plateau has an average altitude of 1600m above sea level. Mean annual rainfall is 1800mm; the rainy season extends from early March to October while a short dry season occurs between November and February.

Mambilla Plateau has a population of 224,357 people [13]. The people engage in agriculture and stock herding. Crops grown on the Mambilla Plateau include; maize, millet, sorghum, sweet potatoes and Irish potatoes. Tea, coffee, cocoa Avocadoes and pears are also grown on the Mambilla Plateau. The study was conducted in five communities namely; Nguroje (N06˚57'50.1''E0 11˚07'00.7''; elevation 1885m above sea level), Yelwa ((N07˚05'10.5'' E011˚05'27.9''; elevation 1674m above sea level), Gembu ((N06˚42.640 E011˚15.486''; elevation; 1584m above sea level), Kakara ((N06˚49'23.8''E011˚07'26.7''; elevation 1496m above sea level) which are located on the highlands of Mambilla Plateau while Mayo-selbe ((N07˚16'54.8''E011˚81'14.5'' elevation; 484m above sea level) located at the foot of the mountain.

2.2 Sampling Design

Various anophelines larval habitats were sampled in all the five altitudinal locations.The habitats include ponds, wells, swamps, dug pits, rivers and streams among others. These were visited once every month from November 2016 to October 2017 geographical positions and elevations of each habitat were recorded using Global Positioning System (GPS Garmin Oregon 550).

2.3 Characterization of larval habitats

Characterization of larval habitats was carried out by observing and recording the features of the different breeding sites present within the sampling locations, for example habitat permanence, the presence or absence of vegetation, water condition and exposure to sunlight. Light intensity as either shady or light, area of the larval habitat covered by shade was visually estimated as percentage canopy cover. Water current was determined by visual scrutiny as still or slow-flowing. Other parameters were measured using hand-held digital meters, temperature with thermometer, pH with Wagtech pH meter, electrical conductivity and total dissolved solids with Wagtech conductivity/TDS meter, turbidity with Wagtech turbidity meter, salinity and dissolved oxygen with Horiba Dip Electrode.

Water samples were collected from the various habitats in 1litre plastic bottles tightly closed and labeled according to altitude, location, coordinates and date of collection. The water samples were transported to Taraba state ministry of water resources and analyzed for other parameters such as; alkalinity, hardness, dissolved oxygen, sulphate, iron, lead, arsenic and nitrate with Wagtech photometer.

2.4 Larval collection and rearing

Larval collections were done from various aquatic habitats inall the communitiesusing the standard dipping method [14] from between 0.8am to 10.00am. In all the locations where larvae were sighted larvae of all available instar were collected anopheline larvae were distinguished from culicines based on their resting habits in water and the siphon. The edge of the dipper was submerged, dipped at about 45 degrees about an inch below the surface of the water quickly but gently, the dipper was moved along a straight line in the water. The stroke was ended just before the dipper was filled to avoid overflowing. The dipper was then gently raised out of the water without spilling the water and the larvae.Depending on the size of each larval habitat, about 10-30 dips were taken at intervals along the edge for about 30minutes at each larval habitat [15] and transferred into plastic containers along with the breeding sites water and taken to the laboratory to be reared.

To rear the mosquitoes, larvae from the field were transferred into small white transparent plastic buckets filled to two-thirds of their volume with the breeding site water. The mouths of the plastic buckets were covered with mosquito net a small hole was made at the centre of the net and plugged with a dry cotton wool until adults emerged [16]. Larvae were fed with baker's yeast.

2.5 Data analysis

Larval density for each study location was determined from the breeding index according to the formula of Belkin: BI= TLP/ND×BP, where BI= breeding index, TLP= total number of larvae and pupae sampled, ND= number of dips and BP= number of breeding places/sampling sites. Logistic regression analysis was used to determine relationship between larval abundance and altitude as well as mean physicochemical parameters. The significance level was considered at $p<0.05$.

3. Results and Discussion

A total of 60 larval habitats were sampled these include swamps15 (25.00%), old tyres 9(15.00%), streams and wells7 (11.67%) each, dug pits6 (10.00%), rivers 5(8.33%), building sites 4(6.67%), ponds and rock pools 3(5.00%) and tree trunk 1(1.66 %). The highest number of habitats 17(28.33%) were observed in Yelwa, Gembu and Mayo-selbe had 12(20.00%) each, Nguroje 11(18.33%) while Kakara had the least 8(13.33%).This is indicated in Table 1.

About 179 larvae were bred to adulthood they comprised of four species *An. gambiae*113 (63.13%), *An. coustani* 33(18.44%), *An. funestus* 20(11.17%) and *An. pharoensis* 13(7.26%). Gembu recorded the highest number of larvae 101(56.43%), Nguroje 57(31.84%) and Kakara 21(11.73%).

Larvae were sampled from only three altitudinal locations, Gembu, Nguroje and Kakara.Gembu recorded the highest larval presence 101(56.43%), Nguroje 57(31.84%) and Kakara 21(11.73%). Only six of the larval habitats were positive for anophelines larvae breeding index was in the range of 0.0 to 20.2 (Table 2). There was variability in the spatial distribution of anopheline larval populations. Larvae were associated with temporary habitats such as pits that were dug for blocks moulding and in clear, sunlit stream edges and swamps. They also had preference for habitats with wide

range of Physico-chemical parameters and thrived in habitats with temperature range of 21.39±2.93-25.98±3.04, pH 6.52±.87-7.11±.56, Electrical conductivity (EC) 43.35±34.31-102±53.44, Total Dissolve Solutes (TDS)21.85±17.11-50.45±26.30, Turbidity 3.53±1.19-22.88±41.41, Hardness 27.57±13.01-41.00±8.20, Salinity 14.36±7.71-26.33±19.44, Alkalinity 15.14±7.51-21.66±10.22, Dissolve oxygen(DO) 5.57±1.13-5.66±1.66, Nitrates(NO_3^-)15.37±14.35-29.68±25.75, Sulphates (SO_4^{2-})5.54±3.18-14.71±9.03, Iron (Fe).06±.06-.12±.10, Lead (Pb)00±.00-.01±.00, Arsenic (As).00±.00. This is represented in Table 3.There was no significant relationship between larval abundance and physicochemical parameters and altitude at $p<0.05$.

Table 1: Larval habitats of anopheline vectors of malaria along altitudinal locations on the highlands of Mambilla Plateau

	Larval habitat										
Location	**Dug pit**	**Pond**	**River**	**Stream**	**Swamp**	**Well**	**Tree trunk**	**Old tyres**	**Rock pools**	**building sites**	**Total**
Nguroje	2	1	0	1	4	3	0	0	0	0	11
Yelwa	2	0	0	3	2	1	0	9	0	0	17
Gembu	0	1	0	2	6	3	0	0	0	0	12
Kakara	2	1	0	1	3	0	1	0	0	0	8
Mayo-selbe	0	0	5	0	0	0	0	0	3	4	12
Total	6	3	5	7	15	7	1	9	3	4	60

Table 2: Larval abundance, species composition and breeding index of anophelines along altitudinal locations on the highlands of Mambilla Plateau

	Species/Number of larvae					
Study locations	***An. gambiae***	***An. coustani***	***An. funestus***	***An. pharoensis***	**Total (%)**	**Breeding index**
Nguroje	47	7	1	2	57((31.84)	11.4
Yelwa	-	-	-	-	0(0.00)	0.0
Gembu	52	21	17	11	101(56.43)	20.2
Kakara	14	5	2	-	21(11.73)	4.2
Mayo-selbe	-	-	-	-	0(0.00)	0.0
Total	113	33	20	13	179(100)	35.8

There was a relatively low occurrence of positive breeding sites along the altitudinal locations only six out of the 60 breeding sites were positive for anopheline larvae. This could be due to their temporary nature and variability. In the dry season the anophelines larvae survived and proliferated by breeding in temporary stream edges and man-made habitats such as pits that were dug for moulding blocks in the rainy season however these were replaced by swamps that had formed from the rain pools. Most of the streams were overgrown with tall grasses and the temporary pits, filled up with refuse. This finding concurred with Aditya, *et al*., [25] who observed that the mosquito habitats in the hill town of Darjeeling were temporary in nature and existed only for a brief period of time. In the dry season, larvae were collected only in Nguroje and Kakara but in the rainy season they were collected only in Gembu and none in other altitudinal locations especially Mayo-selbe which is a lowland and recorded highest number of adult mosquitoes. This finding contrasts with the impression that flat areas provide more breeding sites than steep slopes [21].

The non-availability of larvae in Mayo-selbe both in the dry and rainy seasons could be attributed to elevated temperatures which resulted in shorter development time of larvae therefore more adult presence. Immature stages and adults of mosquitoes are sensitive to temperature changes in their aquatic environment. Water temperature of above 34ºC generally has a negative impact on the survival of vectors [22, 23]. It could also be due to the fast flowing nature of the rivers in Mayo-selbe which may not be conducive for the adult female *Anopheles* mosquitoes to breed.

Larvae were found breeding in only one site in the rainy season as compared with five during the dry season the larvae might have been washed away by the heavy rainfall as mosquito larvae were observed to prefer still, clear and sunlit temporary habitats. In Kakara most of the breeding sites were observed close to the forest with banana and tea plantations about 50m away from houses and therefore beyond flight distance of the adult mosquitoes [18] these temporary breeding sites were cultivated during the rainy season in Nguroje they were located just within 7m of residential houses and were also cultivated into vegetable gardens this could account for their absence during the rainy season. In rural Gambia, a significant decline in the number of mosquitoes with distance to nearest breeding habitats was observed in agreement with the present study [18]. It is suggested that for female mosquitoes seeking blood meals orientation towards a host accounts only for short range movement of over 30m or less[19] and mean flight range of female *An. gambiae* was estimated at 0.64miles [20].

Table 3: Average Physicochemical Parameters of larval habitats on the highlands of mambilla plateau

	Elevation	**Temp**	**pH**	**EC**	**TDS**	**Turbidity**	**Hardness**	**Salinity**	**Alkalinity**	**DO**	**NO_3^-**	**SO_4^{2-}**	**Fe**	**Pb**	**As**
Nguroje	1748 ±12.18	25.98 ±3.04	6.52 ±.87	102 ±53.44	50.45 ±26.30	3.53 ±1.19	39.90 ±13.23	14.36 ±7.71	17.63 ±8.84	5.63 ±1.28	15 ±14.35	5.54 ±3.18	.12 ±.10	.01 ±.00	.00 ±.00
Yelwa	1641 ±25.23	25.33 ±3.40	6.82 ±.62	44.56 ±31.28	22.50 ±15.47	4.31 ±2.49	30.37 ±11.74	11.62 ±4.80	15.62 ±6.30	7.00 ±2.97	34.17 ±22.75	6.75 ±4.55	.03 ±.02	.02 ±.00	.00 ±.00
Gembu	1548 ±6.02	21.39 ±2.93	7.11 ±.56	83.89 ±67.46	42.16 ±33.52	22.88 ±41.41	41.00 ±8.20	26.33 ±19.44	21.66 ±10.22	5.66 ±1.66	26.50 ±28.42	9.75 ±5.47	.12 ±.10	.00 ±.00	.00 ±.00

Kakara	1468 ±6.21	23.75 ±2.48	6.69 ±.61	43.35 ±34	21.85 ±17.11	14.54 ±29	27.57 ±13.01	16.28 ±11.94	15.14 ±7.51	5.57 ±1.13	29.68 ±25	14.71 ±9.03	.06 ±.06	.01 ±.00	.00 ±.00
Mayo Selbe	465 ±5.27	31.92 ±1.70	5.88 ±.22	87.02 ±46.	43.80 ±22.86	5.36 ±1.32	51.80 ±28.02	13.60 ±2.70	16.00 ±3.16	8.60 ±1.81	45.60 ±8.2	9.60 ±2.30	.02 ±.01	.00 ±.00	.00 ±.00

In the current study temperature of water in the breeding habitats at the time of sampling ranged from 21.39°C ±2.93to 31.90°C±1.70 and pH of the breeding habitats varied from 5.88± .22to 7.11±.46. Earlier studies from other parts of Nigeria and Africa had reported temperatures and pH within the same range as the present study. Adebote, *et al*., [24] reported temperature range of 14 to 40°C and pH range of 5.86 to 9.85, Afolabi, *et al*., [12] recorded temperatures of 26.5 to 29.3°C and pH 7.1 to 7.3, Dida, *et al*., [25] recorded a pH range of 6.7 to 8.4 along the Mara River in Kenya.

The anopheline larvae were associated with temperature range of 20.7 to 28.0°C and pH 5.8(slightly acidic) to 7.8(slightly alkaline) this is similar to study by Hanafi-Bojd, *et al*.,[8] who found malaria vectors breeding in habitats of between 20°C - 30°C. Larvae thrived in habitats with 6 mg/L dissolved oxygen this was the amount of DO in all the habitats in the three altitudinal locations from where larvae were sampled that is Nguroje, Gembu and Kakara this concurred with 6.4mg/L recorded by [25]in Kenya. Electrical conductivity in the range of 14.5mg to 159mg/L, total dissolved solids 8 to 87mg/L and turbidity was 1.4 to 138mg/L.

4. Conclusion

There was a relatively low occurrence of positive breeding sites along the altitudinal locations and with wide range of physico-chemical parameters. This study has provided baseline information on larval habitats and physico-chemical parameters of the larval habitats on the Mambilla Plateau that would be helpful for the sustainable management of vector mosquitoes and to take precautionary measures against malaria epidemics. Altitude and physicochemical parameters of the larval habitats should be considered in planning and implementing targeted larval mosquito control programmes in the highlands.

5. Acknowledgement

We wish to appreciate the authorities of Sardauna and Gashaka LGAs of Taraba State and leaders of all the communities for the permission and support granted us to carry out the research. We also want to thank the management of Taraba State University Jalingo for financing the research.

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