SPECIES COMPOSITION AND ABUNDANCE OF MOSQUITOES AND THEIR DISEASE TRANSMISSION POTENTIAL IN RURAL AND URBAN AREAS OF KILIFI COUNTY, KENYA

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DECLARATION

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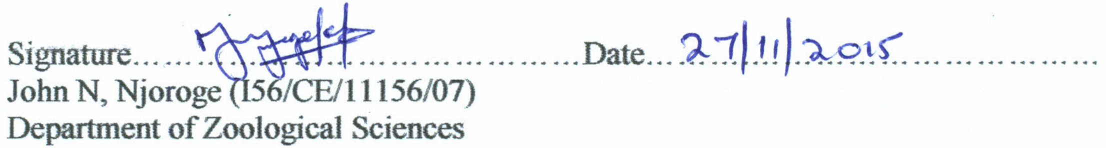

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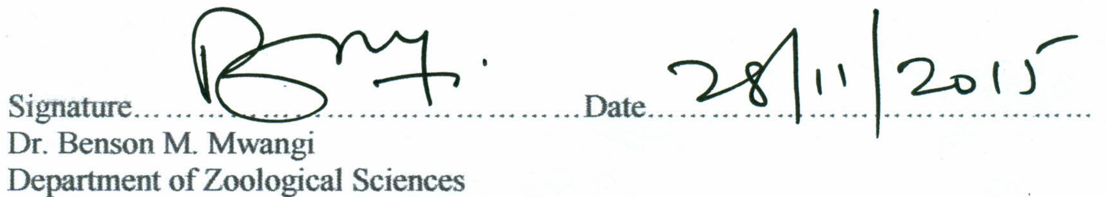

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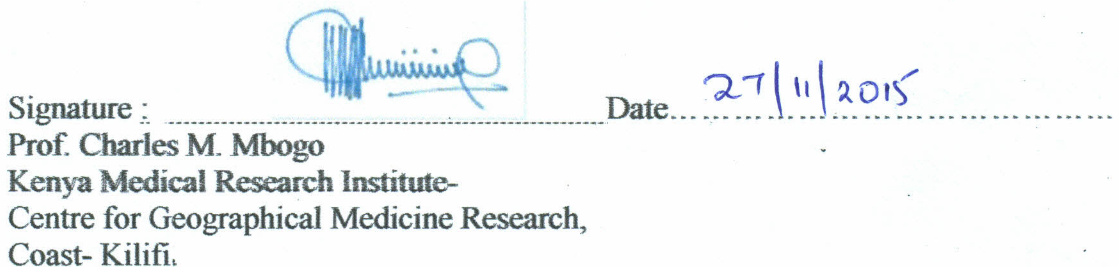

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DEDICATION

To my wife, Margaret Wairimu, for her love and dedication and our sons Anthony Njoroge and Charles Kabera and daughter Mary Nduta for their endurance and bravery during the time of this study.

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TABLE OF CONTENTS

DECLARATION........ii
DEDICATION........iii
ACKNOWLEDGEMENTS........iv
TABLE OF CONTENTS........v
LIST OF TABLES........viii
LIST OF FIGURES........ix
LIST OF PLATES........x
DEFINATION OF TERMINOLOGIES........xi
ACRONYMS AND ABBREVIATIONS........xii
ABSTRACT........xiii
CHAPTER 1........1
INTRODUCTION........1
1.1. Background information........1
1.2 Problem statement........5
1.3 Justification........6
1.4 Research questions........7
1.5 Hypotheses........7
1.6 Objectives........7
1.6.1 General objective........7
1.6.2 Specific objectives........8
1.7 Significance of the study........8
CHAPTER 2........9
LITERATURE REVIEW........9
2.1 Spatial and temporal dynamics of mosquitoes........9
2.2 Factors influencing spatial, temporal distribution and abundance of mosquitoes........11
2.3 Composition of mosquito species in urban and rural areas........16
2.4 Transmission diseases by mosquitoes........17
2.4.1 Vectors of lymphatic filariasis........17
2.4.2 Vectors of yellow fever........19

2.4.2 Vectors of yellow fever....19
2.4.3 Vectors of malaria....22
2.4.4 The *Anopheles gambiae* complex....23
2.4.5 The *Anopheles funestus* complex....24
2.5 Evaluation of malaria parasite transmission potential....26
2.5.1 Detection of mosquito blood meal sources....26
2.5.2 Determination of sporozoite infection rates in wild mosquitoes....28
2.5.3 Entomological inoculation rates (EIR)....29
CHAPTER 3....30
MATERIALS AND METHODS....30
3.1 Location of the study area....30
3.2 Climate and geomorphology....31
3.3 Land use practices....32
3.4 Research and sampling design....32
3.5 Selection of study sites....33
3.6 Species composition of mosquitoes found in Kilifi County....34
3.6.1 Sampling for indoor species of mosquitoes....35
3.6.2 Sampling for outdoor species of mosquitoes....36
3.7. Investigating the abundance of mosquitoes species in Kilifi County....36
3.8 Investigating disease transmission of mosquitoes in Kilifi County....37
3.8.1 Analysis of *Wuchereria bancrofti* in *Culequinquefasciatus*....37
3.8.2 *Plasmodium falciparum* circumsporozoite proteins and blood meal analysis in *Anopheles* mosquitoes....37
3.8.3 Preparation of cells and detection of arbovirus in Aedes mosquitoes....39
3.8.4 Indices of malaria parasite transmission....40
3.9 Data analysis....40
CHAPTER 4....41
RESULTS....41

4.1 Larval species of mosquitoes infesting urban and rural areas of Kilifi County....41
4.2 Adult species of mosquitoes composition in Kilifi County....42
4.3 Abundance of larval mosquito in urban and rural areas of Kilifi County....42
4.3.1 Abundance of larval and adult mosquito species in urban and rural areas of Kilifi County....43
4.4 Assessment of disease transmission potential of different species....47
4.4.1 Biting and entomological inoculation rates of *Anopheles gambiae* and *Anopheles funestus*....48
4.4.2 Filarial worms' infections in *Culex quinquefasciatus*....49
4.4.3 Arbovirus infections in *Aedes aegypti* and *Aedes* simpsoni....50
CHAPTER 5....51
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS....51
5.1 Discussion....51
5.2 Conclusion....60
5.3 Recommentations....60
5.4 Suggestions for further studies....61
REFERENCES....62
APPENDICES....73
Appendix i : CSAB elisa solutions....73
Appendix ii: Giemsa stain....74
Appendix iii: Lab processing form....75
Appendix iv: Laboratory processing form for mosquitoes....76
Appendix v: t- Test for indoor species sex differences in Kilifi and Jaribuni....77
Appendix vi : t- Test for outdoor species sex differences in Kilifi and Jaribuni....78
Appendix vii: Chi-square test for association between presence of sporozoites and physiological status of mosquitoes....79
Appendix viii: t-Test for human biting rates of *Anopheles gambiae* and *Anopheles funestus* from Jaribuni and Kilifi....80

LIST OF TABLES

Table 4.1: Larval species composition in rural and urban areas of Kilifi County......41

Table 4.2: Adult species composition in rural and urban areas of Kilifi County........42

Table 4.3: Mean numbers (± SE) of larval mosquito species in rural and urban areas of Kilifi County......43

Table 4.4: Mean numbers (± SE) of larval mosquito species collected from temporary rain pools in urban areas and River ponds in rural areas of Kilifi County......44

Table 4.5: Mean numbers (± SE) of adult mosquitos' species in rural and urban areas of Kilifi County......44

Table 4.6: Mean numbers (± SE) female and male larval mosquito species recorded in rural and urban areas of Kilifi County......45

Table 4.7: Mean numbers (± SE) of female and male adult mosquitoes recorded indoors in rural and urban areas of Kilifi County......46

Table 4.8 Mean numbers (± SE) of adult female and male mosquitoes species recorded outdoors in rural and urban areas of Kilifi County......46

Table 4.9: Sporozoites in Anophelines between August 2010 and June2011............47

Table 4.10: Physiological status and sporozoites in Anopheline Mosquitoes in Jaribuni and Kilifi......48

Table 4.11: The daily human biting rate and the entomological inoculation rates of *Anopheles gambiae* and *Anopheles funestus* in the rural areas of Kilifi County......49

Table 4.12: Filaria worms in *Culex quinquefasciatus* collected in Kilifi and Jaribuni......49

Table 4.13: Viral infection status in *Aedes spp* in the urban areas Kilifi County.......50

LIST OF FIGURES

Figure 2.1: Life cycle of mosquito....11

Figure 2.2: Life cycle of malaria parasites (Adapted from Strickland, 1991)....14

Figure 2.3: Transmission cycles of yellow fever virus (Adapted from Centers for Disease Control and Prevention, 2011)....21

Figure 3.1: Location of Kilifi County, Kenyan coast.... 30

Figure 3.2: Locations of Kilifi and Jaribuni in Kilifi County....31

LIST OF PLATES

Plate 1.1: Elephantiasis of the limbs (Adapted from White, 1989)........................4

Plate 1.2: Hydrocele, one of the clinical conditions of Lymphatic filariasis (Adapted from White, 1989)..4

Plate 3.1: Pools along the banks of Jaribuni River..33

Plate 3.2: Adult mosquitoes emerging from pupae in an emergence cage..........34

Plate 3.3: Center for disease control and prevention light trap (Adapted from Sudia and Roy 1981)..35

Plate 3.4: BG-Sentinel: Biogents' mosquito trap for researchers..........................36

DEFINATION OF TERMS

Term	Definition
Morbidity	Unhealthy state of individuals
Mortality	The state of being mortal
Endemic	Infection maintained in the population without external input
Spatial	Relationship between organisms and space
Temporal	Relationship between organisms and time
Optimal	Ideal conditions
Climatological	Conditions to do with climate
Dynamics	Changes
Abundance	The quantity of an animal or plant species, present in a particular area
Composition	Different species in a population
Geomorphology	Origin and evolution of topographic and bathymetric features
Arbovirus	Arthropod borne virus
Transmission	Transfer of a disease from one individual to another
Gravid	State of being pregnant

ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of variance
BB	Blocking buffer
CDC	Centre for disease control
CS	Circumsporozoite
DALY	Disability-adjusted life years
EIR	Entomologic inoculation rate
ELISA	Enzyme linked immunosorbent assay
GIS	Geographic information systems
GPS	Geographical positioning systems
HBR	Human biting rate
IBR	Infective biting rate
LF	Lymphatic filariasis
MAbs	Monoclonal antibodies
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PELF	Programme to eliminate lymphatic filariasis
SPSS	Statistical package for social scientists
UN	United Nations
WHO	World health organization
IVM	Integrated Vector Management

ABSTRACT

Mosquitoes (Diptera: Culicidae) are found in both urban and rural areas in Kenya. Many mosquito species are important vectors of human disease causing pathogens notably malaria, filariasis and yellow fever. The total days of labor lost coupled with costs of treatment and the high mortality associated with these diseases make them a serious obstacle for development. There is need therefore for measures to continuously control these insects. A study was conducted in Kilifi region of coastal Kenya to assess the mosquito's species composition, abundance and disease transmission potential in Kilifi town and Jaribuni village 20 kilometers from Kilifi town. Data collection started in August 2010 toward the end of long rains. Assessing the composition and abundance of the mosquito species was carried out by dipping and incubation to adult emergence for larvae followed by identification, counting and recording. Adult mosquitoes were trapped, identified, counted and recorded by species. Individual insects were also sorted by sex and enumerated. The total number of individuals per species was obtained. The mosquitoes species recorded were *Culex quinquefasciatus, Culex annulororis, Culex tigripes, Anopheles gambiae, Anopheles funestus, Anopheles pretoriensis, Anopheles squamosus, Aedes aegypti, Aedes simpsoni* and *Erepodites inornatus*.The data on species abundance was subjected to Analysis of Variance (ANONA), data on larval, indoor , outdoor sex occurrence, human biting rates and entomological inoculation rates was subjected to a paired t-test of SPSS (17.0) and data on physiological and sporozoites status was subjected to Chi-Square Test. The significance level was set at p=0.05. The larval and adult species mean numbers were not significantly different between Jaribuni and Kilifi. There were significant differences between the mean numbers of larval species recorded in River ponds and temporary rain pools. Adult females and males also differed significantly indoors as well as oudoors. The effect of mosquito blood meal on sporozoites presence was evident. There were significant differences between the human biting rates of *Anopheles gambiae* and *Anopheles funestus* as well as entomological inoculation rates. *Anopheles funestus* recorded higher mean. However no infections were recorded in *Culex quinquefasciatus* and *Aedes* species. The were more larval mosquitoes species recorded in Kilifi town area which could probably explain the role of temporaly ponds in species composition and abundance. The were almost equal numbers of adult species in the two sites probably indicating similar ideal ecological conditions for all the species.There is need therefore to encourage the members of the public to continue using treated bed nets to avoid infective mosquito bites.

CHAPTER 1

INTRODUCTION

1.1 Background

Mosquitoes belong to the family Culicidae which is further divided into three subfamilies: Anophelinae, Toxorhynchitinae and Culicinae, all of which are represented in the African continent. The subfamily Anophelinae comprises the genus *Anopheles, Bironella* and *Chagasia.* Subfamily Toxorhynchitinae includes the genus *Toxorhynchites* or *Megarhinus* while subfamily Culicinae comprises several genera including *Malaya, Ficalbia, Coquillettidia, Mansonia, Uranotaenia, Hodgesia, Orthopodomyia, Aedeomyia, Eretmapodites, Aedes, Culiseta, and Culex*. The genera *Anopheles, Culex* and *Aedes* are known vectors of vector borne diseases (Gillet, 1972).

Worldwide, malaria is estimated to cause up to 500 million infections annually, with 1-2 million deaths mostly in young children whose immune system is not fully developed (Greenwood *et al.* 1987). Malaria is a public health concern in over 90 countries of the world where 40% of the world population live (WHO, 1998a, 1998b). Majority of malaria related morbidity and mortality levels are due to *Plasmodium falciparum.* In Africa alone, malaria is responsible for an estimated 200-450 million cases of fever in children and up to 175 deaths per 1000 live births occur before the age of five years annually (Penelope and Kuile, 1994). This accounts for 90% of the global malaria cases (Breman *et al.,* 2001). Globally, an estimated 45,000,000 disability-adjusted life years (DALYs) are lost due to malaria. The total days of labor lost coupled with costs of treatment and the high mortality associated with the disease makes it a serious obstacle

for development (WHO, 1996). The persistence of endemic malaria in the tropics, and particularly in Africa, is contributory to the perpetual state of depressed economic growth within these regions (Breman *et al.*, 2001). EIR is the most accurate measure of transmission intensity particularly when efforts are made towards reducing human-vector contact (Nyaguara *et al.*, 2012). A change in either mosquito density or sporozoite rate or both affects the EIR. Similarly, mosquito population distribution is heterogeneous and even within a defined geographical area mosquito densities vary widely in space and time (Nyaguara *et al.*, 2012).

In Kenya, over 90% of all malaria cases are due to *Plasmodium falciparum* (Snow *et al.*, 1994a, 1994b). The disease accounts for 30% of all outpatients and 19% of all admissions to health facilities (Snow *et al.*, 1998). Perennial transmission of malaria occurs along Kenya's coast and around the Lake region (Roberts, 1974). Marked variation in the prevalence of malaria occurs at times in these areas due to the effect of topographical variation on local differences in entomological and parasitological parameters determining transmission (Copeland, 1994). Estimates of child morbidity and mortality levels on the Kenyan coast, show that one in every 15 children experience an episode of severe malaria before the age of five years (Marsh *et al.*, 1995) and that 58 infants per 1,000 live births and 12 per 1,000 children die of malaria annually (Snow *et al.*, 1994a and 1994b). This notwithstanding, severe anemia as a result of malaria was revealed to be the cause of 10 of the 43 maternal deaths reported in Kilifi District hospital between November 1994 and September 1997 (Shulman *et al.*, 1998). The long lifespan and strong human-biting habit of the *Anopheles* species is

the main reason why about 90% of the world's malaria deaths are in Africa (WHO, 2015).

Lymphatic filariasis is a major public health problem in over 80 countries throughout the tropical areas of Asia, Africa, the West Pacific and some parts of America (WHO, 1987,: 2001), where over 20% of the world population lives (Michael *et al*., 1996). More than 120 million people are affected, 44 million of them seriously incapacitated and disfigured by the disease and suffer from one or more clinical manifestations such as elephantiasis of the limbs, genitals hydrocele, chyluria and pneumonitis or recurrent infections associated with damaged lymphatics (Wamae *et al*., 2001). The condition causes serious psychosocial consequences such as sexual and social dysfunction of men with hydroceles or other genital abnormalities and of women with lymphoedema of breasts or genitals (Amuyunzu, 1997). Seventy six million of those that are infected have sub clinical hidden damage of their lymphatic and renal systems (Ottesen, 1994).

In Kenya, lymphatic filariasis is endemic in coastal counties of Kwale, Kilifi, Malindi, Tana River, and Lamu where over 2.5 million people live (Wamae *et al*., 2001). The inhabitants are at risk of infection since they are continuously exposed to infective mosquitoes due to favorable ecological conditions for vector species (Mwandawiro *et al*., 1997). About three decades ago, the prevalence of microfilaraemia, elephantiasis of the limb (Plate.1.1) and genitals hydrocele (Plate 1.2) was reported to be 22.0%, 17.0%, and 4.6% respectively in a rural village in Kilifi (Wijers and Kinyanjui, 1977).

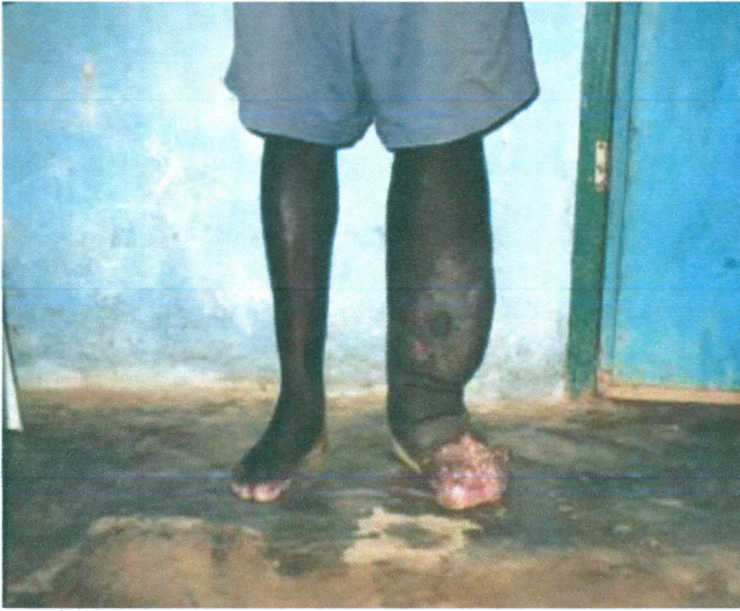

Plate 1.1: Elephantiasis of the limbs (Adapted from White, 1989)

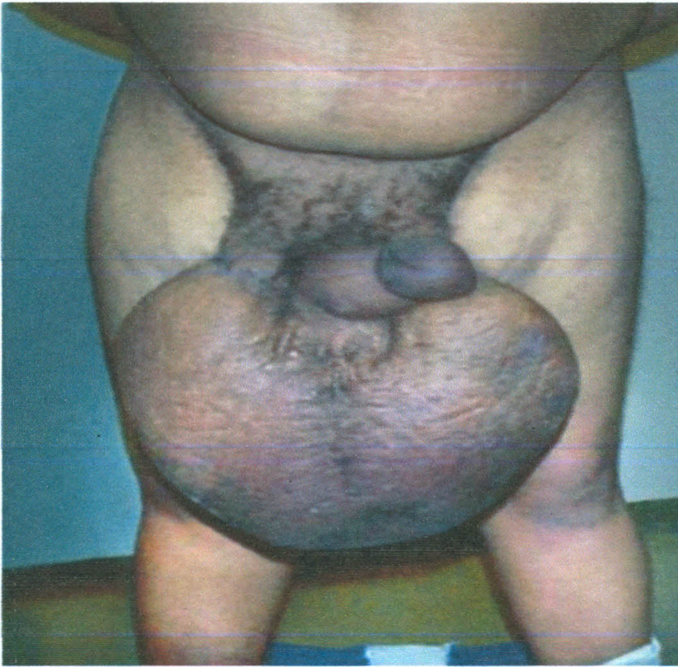

Plate 1.2: Hydrocele, one of the clinical conditions of Lymphatic filariasis (Adapted from White, 1989)

Yellow fever is endemic in tropical and sub-tropical areas of South America and Africa. Even though the main vector, *Aedes aegypti* also occurs in Asia, in the Pacific and the Middle East, yellow fever does not occur in these areas possibly because it has never been introduced (Barnett, 2007). Worldwide, there are about 600 million people living in endemic areas and the official estimates of the World Health Organization amounts to 200,000 cases of disease and 30,000 deaths a year (WHO, 2006). However the number of officially reported cases is far lower. An estimated 90% of the infections occur in the African continent (WHO, 2006). In 2008, the largest number of cases was recorded in Togo (WHO, 2008).

1.2 Problem statement

Malaria is a major cause of morbidity and mortality in Kilifi County. Its control in Kenya is mostly done through the use of Insectide Treated Bednets and treatment of the infected persons using antimalarials. Kilifi is one of the largest counties in coastal Kenya. Sand harvesting along Jaribuni river and blocked drainage channels in Kilifi urban area usually creates ideal sites for mosquito larval development. Different agricultural and environmental factors affect both culicine and anopheline larval instars development. In Kilifi there is a need to know the larval species composition and abundance, which in turn define the adult species composition and abundance. This knowledge would be important in implementation of Integrated Vector Management (IVM) policy in mosquito borne diseases control programmes in Kilifi County.

1.3 Justification of the study

Mosquitoes are among the most varied and abundant insect's species. Different mosquitoes species are competent vectors of various vector borne diseases notably malaria, bancroftian filariasis, and arboviruses (dengue fever, Yellow fever, Rift Valley fever, Chikungunya fever, and West Nile Virus fever) (Mwangangi *et al.*, 2012). Malaria is a public health problem in Kenya despite intense deployment of vector control tools.

Estimates of child morbidity and mortality levels on the Kenyan coast, show that one in every 15 children experience an episode of severe malaria before the age of five years (Marsh *et al.*, 1995) and that 58 infants per 1,000 live births and 12 per 1,000 children die of malaria annually (Snow *et al.*, 1994a, 1994b). This notwithstanding, severe anemia as a result of malaria was revealed to be the cause of 10 of the 43 maternal deaths reported in Kilifi District hospital between November 1994 and September 1997 (Shulman *et al.*, 1998). Lymphatic filariasis is also endemic in coastal counties of Kwale, Kilifi, Malindi, Tana River, and Lamu where over 2.5 million people live (Wamae *et al.*, 2001). Among the reported tropical diseases, LF is estimated to be responsible for the loss of 4.9 x 106 DALYs, ranking third (behind tuberculosis and malaria) in terms of the global disease burden (WHO, 2001). Mosquitos' diverse composition exposes the inhabitants of coastal counties to an array of risks of infections since they are continuously exposed to infective mosquitoes. The abundance of these insects is enhanced by favorable ecological conditions (Mwandawiro *et al.*, 1997). There is limited information on mosquitoes species composition and abundance

between urban and rural areas of the coastal counties in the country. The purpose of conducting this study was to investigate the effects of urbanization on the species composition and abundance in comparison with rural areas.

1.4 Research questions

i. What is the species composition of mosquitoes found in the urban and rural areas of Kilifi County?

ii. How does the species abundance vary between urban and rural areas in Kilifi County?

iii. Which mosquitos' species transmit diseases in Kilifi County?

1.5 Hypotheses

i. There is no difference in the species composition of mosquitoes found in the urban and rural areas of Kilifi County.

ii. There is no difference in the species abundance of mosquitoes found in the urban and rural areas of Kilifi County.

iii. There is no difference in the disease transmission potential of the mosquitoes found in the urban and rural areas of County.

1.6 Objectives

1.6.1 General objective

To assess the composition, abundance, vectorial competence of mosquitoes infesting Kilifi County.

1.6.2 Specific objectives

i. To determine the species composition of mosquitos found in the rural and urban areas of Kilifi County.

ii. To assess the abundance of different mosquito species in the urban and rural settings of Kilifi County.

iii. To assess the disease transmission potential of mosquitoes in Kilifi County.

1.7 Significance of the study

Mosquitoes are among the most successful insects and the main focal point of entomological studies in connection with their human-medical importance. Transmission of diseases by mosquitoes is more intense in places where the mosquito lifespan is longer (so that the parasite has time to complete its development inside the mosquito) and where it prefers to bite human rather than other animals. For example, the long lifespan and strong human-biting habit of the *Anopheles* species is the main reason why about 90% of the world's malaria deaths are in Africa (WHO, 2015). Knowledge of species composition, abundance and vectorial competence is therefore an important pre-requisite for the successful institution of integrated interventions and monitoring of mosquito borne diseases epidemiology.

CHAPTER 2

LITERATURE REVIEW

2.1 Spatial and temporal dynamics of mosquitoes

Spatial and temporal dynamics are seasonal changes that are cyclic, largely predictable, and arguably represent the strongest and most ubiquitous source of external variation influencing natural systems (Altizer *et al.*, 2006). There have been numerous studies demonstrating the spatial distribution of malaria vectors as a function of distance from known or suspected breeding sources. Larval mosquitoes are usually highly aggregated in pools of water with specific characteristics. The distribution of adults is largely dependent upon the distribution of these larval habitats and the flight range of the adults (Gimnig *et al.*, 1995). Many mosquito species feed on a restricted range of available hosts. Members of the *Anopheles gambiae* complex in East Africa exhibit distinct host-choice behavior with *Anopheles gambiae* being strongly anthropophilic and *Anopheles arabiensis* usually being strongly zoophilic. Charlwood and Edoh (1996) and Minakawa *et al.* (1999) reported that *Anopheles gambiae* larvae were more prevalent near human habitation while *Anopheles arabiensis* larvae were more common near animal habitations.

Keating *et al.* (2009) noted clustering in the distribution of *Anopheles gambiae* and *Anopheles funestus* adult mosquito populations along the Kenyan coast. These conform to findings of a previous study in an Ethiopian village by Ribeiro *et al.* (1996), who found out that clustering of *Anopheles gambiae* was common and changed as a function of time and mean mosquito density. The presence of clustering in all sampling periods,

irrespective of season or weather patterns, suggests extensive heterogeneity in the distribution of both *Anopheles gambiae* and *Anopheles funestus*. Throughout the study, 100% of *Anopheles funestus* mosquitoes and 90% of *Anopheles gambiae* mosquitoes were collected from less than one-half of the total number of houses (Keating *et al*., 2009). Furthermore, it was not necessarily the same houses that consistently harbored *Anopheles* species. In three villages, no *Anopheles funestus* were collected from any of the households, and very few *Anopheles gambiae* were collected within the same households. In other instances, some houses remained free of both species throughout the study, whereas nearby households had high densities of both *Anopheles gambiae* and *Anopheles funestus* (Keating *et al*., 2009). The results from the study gave clear implications for sampling design and also predicted mosquito distribution detection patterns to be harder in sites having high densities throughout the year (Ribeiro *et al*., 1996). As a result, non-random selection and sampling techniques, as well as information about house characteristics, may be needed to further quantify the distribution of *Anopheles sp* mosquitoes at high densities. This information also may be useful for standardizing measurements of Entomological Inoculation Rates (EIR) or for developing stratified sampling strategies for the surveillance and control of mosquito populations (Keating *et al*., 2009).

2.2 Factors influencing spatial and temporal distribution, composition and abundance of mosquitoes

In general, factors that affect the distributions of biological systems include physical e.g climate, biotic interactions, food resource and shelter. Many studies have shown that climate is a major factor governing the distribution of insects (Andrewartha and Birch, 1954; Southerst *et al*., 1995) either by acting directly on insect populations themselves or indirectly by affecting the structure of the ecosystem they inhabit. For mosquitoes, each species has unique environmental tolerance limits (Martens *et al*., 1995). Vector distribution is highly dependent upon the availability of suitable aquatic larval habitats and the proximity of the vertebrate host as a potential source of blood meals (Martens *et al*., 1995). The dependence of vector species on aquatic habitats is an invariant aspect of the mosquito life cycle (Figure 2.1), and the availability of an appropriate aquatic realm, necessary for egg, larval, and pupae development, critically determines mosquito species abundance (Service, 1993; Kettle, 1995).

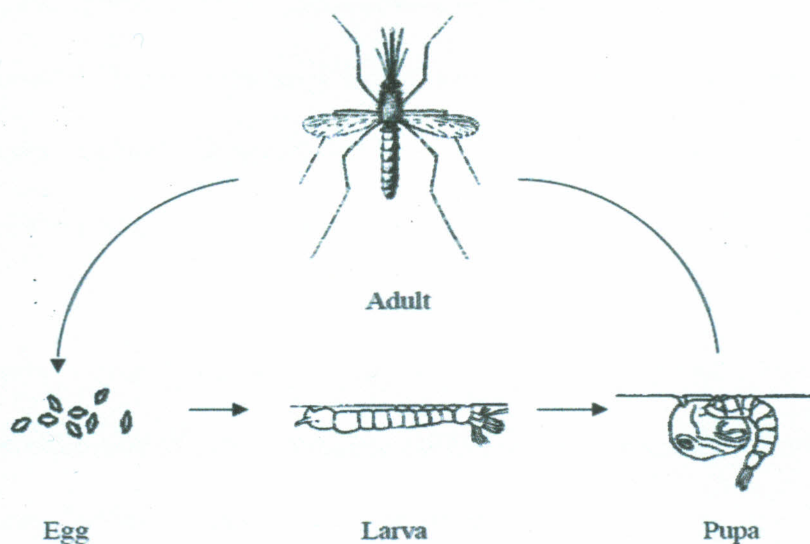

Figure 2.1: Life cycle of mosquito

Localized variations in the effect of climatic changes on the presence of suitable mosquito breeding conditions have been implicated in the variability observed in the ecology of the vectors and the distribution of the disease across different spatial scales (McMichael, 2003). Changes in temperature and humidity, could impact on the presence, development, activity, and survival of pathogens, vectors, zoonotic reservoirs of infection, and their interactions with humans which consequently lead to changes in their geographic distribution (Meade *et al*., 1988; McMichael, 2003).

In essence, as precipitation levels increase and/or favorable ecological conditions are created, mosquito densities increase and exhibit patterns of aggregation (Martens *et al*., 1995). Conversely, as conditions become less favorable, mosquito densities decline and the population surviving has an older age structure and tends toward randomness (Martens *et al*., 1995). This is potentially of epidemiological importance because it is the older mosquitoes that are the most dangerous in terms of sporozoite rates (Martens *et al*., 1995). Furthermore, when older mosquitoes are present in low density, the risk of encountering highly infectious mosquitoes within villages becomes essentially random. However, it should be noted that low mosquito densities and small sample sizes might reduce the power to detect over-dispersion (Meade *et al*., 1988; McMichael, 2003).

Mosquito's abundance is particularly influenced by climatic factors, mainly rainfall, temperature and relative humidity (WHO, 1990). Rainfall affects the availability of breeding habitats. The oviposition of mosquito eggs by gravid females and their maturation to larvae and subsequent adults requires the availability of aquatic breeding

habitats and is therefore dependent on rainfall (Molineux, 1988; Le Sueur and Sharp, 1991). Several studies have demonstrated the association between *Anopheles gambiae* abundance and rainfall but no direct predictable relationship has been established. *Anopheles gambiae* prefers to breed in temporary turbid waters, whereas *Anopheles funestus* prefers more permanent water bodies (Le Sueur and Sharp, 1991). However, the availability of both types of habitats depends on adequate rainfall, which is also related to the saturation deficit and affects mosquito survival (Molineux, 1988), providing a good basis for using rainfall as a predictor for the presence of vectors, their survival and possible malaria transmission.

Temperature, on the other hand plays an important role by driving malaria parasite development within the mosquitoes (Rueda *et al*., 1990). After oviposition, the rate of egg hatching, development through the larval and pupal stages to the adult mosquito largely depends on temperature. As temperature increases, the time required for mosquito development shortens (Rueda *et al*., 1990). For example at 16^0C, larval development may last more than 45 days, compared to only 10 days at 30^0C. At lower temperatures, the larval and pupal stages of mosquitoes take a longer time to complete (47 days) at 16°C in Ethiopia and a small increase in temperature substantially shortens the duration of these phases to 37 days at 17°C (Rueda *et al*., 1990). The effect of a long development cycle due to low temperatures is usually a reduction in the number of mosquito generations, in addition to putting larvae at the risk of predators, eventually reducing the adult population size. Thus, by affecting the duration of the aquatic stage

Low relative humidity levels cause the vectors to feed more frequently to compensate for dehydration (Lindsay and Mackenzie, 1997). Under conditions of optimal humidity, mosquitoes tend to survive for a longer period, which allows them to disperse farther and to have a greater opportunity to participate in malaria transmission cycles (Lindsay and Mackenzie, 1997; Liehne, 1998). *Anopheles* mosquitoes transmit malaria parasites when the environmental parameters such as water availability, temperature and humidity permit (Liehne, 1998). In many parts of the world where temperature is not a limiting factor, malaria transmission is highly seasonal, with its peak following the period of peak rainfall. Understanding how malaria varies in the community as a result of seasonal or year-to-year changes in environmental factors is important for the planning of national malaria control programs since it allows interventions to be adapted to specific sites or times of the year (Liehne, 1998).

The agro-ecosystem in which standing water is present throughout much of the crop growing season, is a complicated system involving higher aquatic plants, algae, mosquitoes and their natural predators. Biotic components such as the insect predators and phytoplankton have a profound impact on the abundance of mosquitoes breeding in rice fields (Mogi *et al.*, 1980). The impact of aquatic predators on rice field mosquito larvae has been shown earlier. In Japan a complex of predators was found to be an important mortality factor for the larval population of *Culex tritaeniorhynchus* in the fallow rice fields (Mogi *et al.*, 1980). In a field experiment, adult emergence rates were lower in experimental quadrats with a natural complex of predators. In Japan about 30 species of predators were confirmed to feed on mosquito larvae in the laboratory

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(Sugiyama *et al.*, 1985). Important groups of insect predators in rice fields include Coleoptera, especially Dytiscidae and Hydrophilidae, and Hemiptera, particularly Notonectidae, Odonata (Mogi, 1988). Predators usually have a limited breeding season and longer generation time than that of mosquitoes (Mogi, 1992).

Phytoplanktons which form a portion of the larval mosquito diet also influence the survival of mosquito larvae. Schaefer *et al.* (1983) and Kramer and Garcia (1989) reported a positive correlation between the presence of green algae and high numbers of mosquito larvae. By contrast, certain blue green algae were associated with an absence of mosquito larvae in rice fields.There are large numbers of contributory variables in the ecosystems, which influence the abundance of mosquitoes (Sunish, 2002).

2.3 Composition of mosquito species in urban and rural areas

Mosquitoes are found both in rural and urban areas. *Anopheles gambiae*, *Anopheles funestus* and *Anopheles coustani* are predominant in the rural stratum where there are fresh water aquatic habitats for breeding throughout the year while *Culex quinquefasciatus* is mostly found in urban and peri-urban strata while *Aedes aegypti* is mainly found in urban strata where limited aquatic habitats are polluted (Mwangangi *et al.*, 2012). Further, *Anopheles gambiae*, *Culex quinquefasciatus*, and *Aedes aegypti* mosquitoes are found occurring together inside the houses where they search for blood meal (Mwangangi *et al.*, 2012). This in turn exposes the inhabitants to an array of mosquito-borne diseases including malaria, bancroftian filariasis, and arboviruses (dengue fever, Yellow fever, Rift Valley fever, Chikungunya fever, and West Nile Virus fever). These findings provide useful information for the design of integrated

mosquito and disease control programs in East African environments (Mwangangi *et al.*, 2012).

The feeding and resting behavior of mosquito species may determine their importance in disease transmission (White, 1982). An efficient vector rests indoors and feeds primarily on humans rather than animals blood (Garret-Jones *et al.*, 1980). *Anopheles gambiae, Anopheles funestus,* and *Culex quinquefasciatus* are highly anthropophagic and endophagic and this guarantees them strong vector-host contact relationship (Garret-Jones, 1980; Gillies, 1988; Mbogo *et al.*, 1993a; Bogh *et al.*, 1998). In some areas, *Anopheles funestus* occurs in higher densities than *Anopheles gambiae* and contributes significantly to transmission of malaria (Charlwood *et al.*, 1997; Mbogo *et al.*, 2003). *Anopheles arabiensis* exhibit ecophenotypic plasticity varying from being anthropophilic to zoophilic depending on geographical location (Githeko *et al.*, 1996). Anthropophily, endophily and a relatively high survival rate of *Anopheles gambiae, Anopheles funestus* and *Culex quinquefasciatus* are considered to be the primary factors contributing to their efficiency in malaria and lymphatic filariasis transmission along the Kenyan Coast (Mbogo *et al.*, 1993a; Bogh *et al.*, 1998; Mwangangi *et al.*, 2003).

2.4 Transmission of diseases by mosquitoes

2.4.1 Vectors of lymphatic filariasis

Although many mosquito species may occur sympatrically in a given area, most of them are not important natural vectors. Usually one or two species in any given locality are responsible for disease transmission (White, 1982). Lymphatic filariasis is caused

by *Wuchereria bancrofti* which is transmitted by various genera of mosquitoes namely *Mansonia*, *Aedes*, *Culex*, and *Anopheles* species (Service, 1986).

The transmission dynamics of lymphatic filariasis are complex, involving two genera of parasite (*Wuchereria* and *Brugia*) and a number of genera of mosquito carriers. The four main genera are *Anopheles*, *Culex*, *Aedes* and *Mansonia* (WHO, 2013). The biological features of the vector–parasite relationship should be understood in order to define the entomological variables critical to lymphatic filariasis transmission and the threshold for interrupting transmission. Unlike the transmission of malaria and arboviruses, that of lymphatic filariasis is inefficient, and a large number of bites from infectious mosquitoes is required to initiate a new infection with microfilaraemia (WHO, 2013). Many factors contribute to the inefficient transmission of lymphatic filariasis. Firstly, microfilariae do not multiply in the mosquito body; hence, the number of L3 is limited by the number of microfilariae ingested. Second, only those mosquitoes that survive more than 10 days will contribute to transmission of the parasites. Those mosquitoes that die before the L3 develops cannot play a role in the transmission cycle. Third, the L3 are deposited on the skin and have to find their way into the bite wound (rather than being injected with the mosquito saliva like malaria sporozoites). In view of all these factors, the transmission of lymphatic filariasis parasites is considered to be less efficient than that of other vector-borne parasites (WHO, 2013).

Along the East African Coast, the known vectors are *Culex quinquefasciatus, Anopheles funestus,* and three members of *Anopheles gambiae* complex namely *Anopheles gambiae Anopheles arabiensis* and *Anopheles merus* (Mosha and Petrarca, 1983; Mwandawiro *et al*., 1997). The importance of these vectors varies from place to place depending on local ecological conditions. For instance Wijers and Kiilu (1977) reported *Culex quinquefasciatus* to be the main vector in Mambrui, a small Coastal town in Kilifi County, with infection rate of 5.7% while *Anopheles funestus* was the main vector in Jaribuni, a village more inland, with infection rate of 3.5%. However, a study by Mwandawiro *et al*. (1997) in rural inland villages of Kwale, reported the infection rates for *Anophheles gambiae, Anopheles funestus, and Culex quinquefasciatus* to be 1.0%, 1.1% and 0.8% respectively, confirming that *Culex quinquefasciatus* is also an important vector in rural villages. *Aedes aegypti, Aedes togoi, Mansonia uniformis,* and *Culex quinquefasciatus* are also important vectors of lymphatic filariasis in South East Asia and the Pacific (Service, 1993).

2.4.2 Vectors of yellow fever

Yellow fever virus is mainly transmitted through the bites of mosquitoes called *Aedes aegypti*. However other mosquitoes such as the "tiger mosquito" (*Aedes albopictus*), *Aedes africanus, Aedes opok, Aedes simpsoni, Aedes luteocephalus, Aedes taylori* and *Aedes vittatus*, which breed in natural sites (e.g. bamboo stumps, bromeliads and tree holes) also serve as vectors for the virus (Ngoagouni *et al*., 2012). Like other arboviruses which are transmitted by arthropods, the yellow fever virus is taken up by a female mosquito when it sucks the blood from an infected person. Viruses reach the

stomach of the mosquito, and if the virus concentration is high enough, the virions can infect epithelial cells and replicate there. From there, they reach the haemolymph (the blood system of mosquitoes) and from there they get into the salivary glands. When the mosquito sucks blood the next time, it injects its saliva into the wound, and thus the virus reaches the blood of the bitten person. There are also indications for transovarial and trans-stadial transmission of the yellow fever virus within *Aedes aegypti*, i.e., the transmission from a female mosquito to her eggs and then larvae. This trans-stadial infection seems to play a role in single, sudden outbreaks of the disease (Fontenille *et al*., 1997).

There are three epidemiologically different infectious cycles (Figure 2.3) in which the virus is transmitted from mosquitoes to humans or other primates (Barrett and Higgs, 2007). In the urban cycle, only the yellow fever mosquito *Aedes aegypti* is involved, which is well adapted to urban centers and can also transmit other diseases including Dengue and Chikungunya (Barrett and Higgs, 2007). The urban cycle is responsible for the major outbreaks of yellow fever that occur in Africa. Except for an outbreak in 1999 in Bolivia, this urban cycle no longer exists in South America and is only present in Africa (Barrett and Higgs, 2007).

Besides the urban cycle, there is both in Africa and South America, a sylvatic cycle (Forest cycle or Jungle cycle), where *Aedes africanus* (in Africa) or mosquitoes of the genus *Haemagogus* and *Sabethes* (in South America) serve as vectors. In the jungle, mainly non-human primates get infected with the virus. The disease is mostly

asymptomatic in African primates. In South America, the sylvatic cycle is currently the only way humans can get infected which explains the low incidence of yellow fever cases on this continent (Barrett and Higgs, 2007). People who become infected in the jungle can carry the virus to urban centers, where *Aedes aegypti* acts as a vector. It is because of this sylvatic cycle that yellow fever cannot be eradicated (Barrett and Higgs, 2007).

In Africa there is a third infectious cycle, also known as savannah cycle or intermediate cycle, which occurs between the jungle and urban cycle. Different mosquitoes of the genus *Aedes* are involved in this cycle. In recent years this is the most common form of yellow fever seen in Africa (WHO, 2006). Transmission in Africa is maintained by a high density of vector mosquito populations that are in close proximity to largely unvaccinated human populations. Although some countries have incorporated yellow fever vaccine into childhood immunization programs, vaccine coverage is not optimal (Hughes and Wilson, 2015).

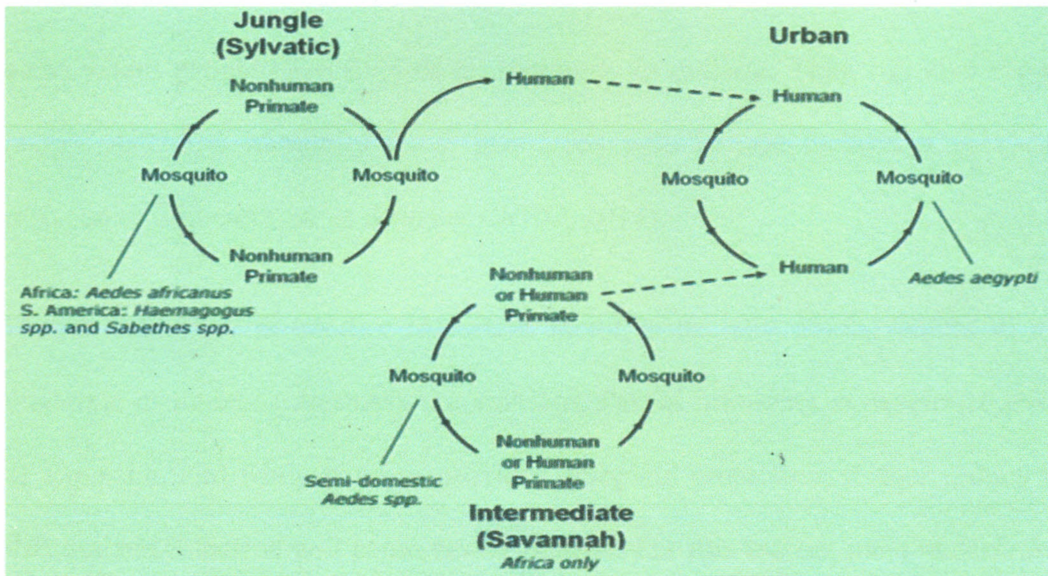

Figure 2.3: Transmission cycles of yellow fever virus (Adapted from Centers for Disease Control and Prevention, 2011)

2.4.3 Vectors of malaria

Over 80 species of *Anopheles* mosquitoes have been implicated in malaria transmission. The importance of each species in malaria transmission however varies by regions, as does their geographical distribution. For example, the *Anopheles minimus* complex, *Anopheles aquasalis, Anopheles albimanus* complex are very important vectors of *Plasmodium vivax* in South America (Marquetti *et al.*, 1991; Laubach *et al.*, 2001; Barrett and Higgs, 2007). In Sub-Saharan Africa, the *Anopheles gambiae* complex and the *Anopheles funestus* complex are the most important vectors of malaria, transmitting *Plasmodium falciparum*, which is the most pathogenic malaria parasite (WHO, 2000).

Transmission depends on climatic conditions that may affect the number and survival of mosquitoes, such as rainfall patterns, temperature and humidity. In many places, transmission is seasonal, with the peak during and just after the rainy season. Malaria epidemics can occur when climate and other conditions suddenly favour transmission in areas where people have little or no immunity to malaria. They can also occur when people with low immunity move into areas with intense malaria transmission, for instance to find work, or as refugees (WHO, 2015).

Human immunity is another important factor, especially among adults in areas of moderate or intense transmission conditions. Partial immunity is developed over years of exposure, and while it never provides complete protection, it does reduce the risk that malaria infection will cause severe disease. For this reason, most malaria deaths in

Africa occur in young children, whereas in areas with less transmission and low immunity, all age groups are at risk (WHO, 2015).

2.4.4 The *Anopheles gambiae* complex

The *Anopheles gambiae* complex comprises six sibling species, namely *Anopheles gambiae*, *Anopheles arabiensis*, *Anopheles melas*, *Anopheles merus*, *Anopheles bwambae* and *Anopheles quadriannulatus*, one unnamed species and several incipient species (new species from the process of speciation) all differing in various ways (Coluzzi *et al*., 1985; Gillies and Coetzee, 1987; Favia *et al*., 1997; Hunt *et al*., 1998). At least three species within *Anopheles gambiae* have been reported in West Africa (Fanello *et al*., 2003). Members of the *Anopheles gambiae* complex are morphologically similar, but exhibit distinct genetic and eco-ethological differences, as reflected in their ability to transmit malaria (Trape and Rogier, 1996).

Anopheles gambiae is the most important vector of *Plasmodium falciparum* malaria in sub-Saharan Africa. This species is reported to be the most specifically adapted to humans and has the highest malaria parasite inoculation rates with entomological inoculation rates ranging from between <1 to >1000 infective bites per person per year (Trape and Rogier, 1996).

Anopheles gambiae is also remarkably stable in a wide range of bio-ecological and seasonal conditions hence appears to be very flexible, both in exploiting new man-made environments and in their response to malaria control activities (Coluzzi, 1984). Members of the *Anopheles gambiae* complex have a wide geographical distribution and

have been reported from most African countries (Coluzzi *et al*., 1979; Chinery, 1984; Coetzee *et al*. 2000). *Anopheles melas* is confined to the West Coast of Africa (Coluzzi and Sabatini, 1968) whereas *Anopheles merus* is confined to the coast of East Africa and islands off its coast, and has also been recorded in Somalia in the North to Natal in the South (Paterson *et al*., 1964).

Two species of the *Anopheles gambiae* complex namely *Anopheles gambiae* and *Anopheles arabiensis* are the most broadly distributed and the most efficient vectors of malaria (Coetzee *et al*., 2000). The distribution range and relative abundance of *Anopheles gambiae* and *Anopheles arabiensis* appear to be strongly influenced by climatological factors, especially total annual precipitation (Lindsay *et al*., 1998). *Anopheles gambiae* is dominant in humid zones (Lindsay *et al*., 1998, Coetzee *et al*., 2000), whereas *Anopheles arabiensis* tends to predominate in arid savannas. Changes in species composition usually occur where these two species occur in sympatry with *Anopheles arabiensis* being predominant during the dry season and *Anopheles gambiae* more abundant during the rainy season. However, this pattern may vary depending on local ecological conditions and species adaptation (Lindsay *et al*., 1998).

2.4.5 The *Anopheles funestus* complex

Anopheles funestus is one of the three major vectors of malaria in Africa, together with *Anopheles gambiae* and *Anopheles arabiensis* of the *Anopheles gambiae* complex (Fontenille, 1997). *Anopheles funestus* is emerging as an important vector for malaria, with its major role sometimes being noticed during the dry season when *Anopheles*

gambiae are less active (Fontenille, 1997). Taxonomically, *Anopheles funestus* belongs to a group of at least nine morphologically similar species that were classified by Gillies and De Meillon in 1968. The first four species namely *Anopheles funestus, Anopheles aruni, Anopheles parensis,* and *Anopheles vaneedeni* which belong to the *funestus* sub-group have been described on the basis of characteristics in the adult (Gillies and Coetzee, 1987). *Anopheles confusus, Anopheles fuscivenosus, Anopheles leesoni* and *Anopheles rivulorum* can be distinguished from each other and from the *funestus* subgroup based on larval characters (Leeson, 1937).

Anopheles funestus is an adaptable species as shown by its wide distribution and ability to occupy regions ranging from lowland to high altitudes (Faye *et al.,* 1995). This species is abundant, widespread and is highly endophilic and anthropophilic and is found in almost all bioclimatic areas near swamps or rivers. The other species are more limited in density and distribution (Faye *et al.*, 1995). They are mainly zoophilic, but can avidly bite humans outdoors in the absence of other hosts (Gillies and De Meillon, 1968). *Anopheles rivulorum* is the second most abundant and widespread species in the group occasionally collected indoors along with *Anopheles funestus* and has been shown to be a vector of malaria in Africa (Wilkes *et al.*, 1986). Other studies have shown that *Anopheles vaneedeni* can be experimentally infected with *Plasmodium falciparum* in the laboratory (De Meillon *et al.*, 1977), but no evidence of its role in malaria transmission is available.

Studies conducted in Kenya have indicated the presence of *Anopheles funestus*, *Anopheles parensis, Anopheles leesoni,* and *Anopheles rivulorum* (Kamau *et al*., 2002; 2003). *Anopheles funestus* has been found almost exclusively inside human dwellings while *Anopheles rivulorum* was found exclusively outdoors, *Anopheles parensis* both indoors and outdoors and *Anopheles leesoni* was found indoors (Kamau *et al*., 2003). Only *Anopheles funestus* has been implicated as an important vector of *Plasmodium falciparum* in Kenya, even though its importance in malaria transmission varies depending on locality. The paucity of studies on this species group indicates the need for proper identification of members of the *Anopheles funestus* species complex to make available data on biological and behavioral characteristics that will elucidate their role in malaria transmission (Kamau *et al*., 2003).

2.5. Evaluation of malaria parasite transmission potential

The evaluation of malaria parasite transmission rates in nature is performed by the identification of the source of blood meals using direct Enzyme-Linked Immunosorbent Assay (ELISA) and determination of the sporozoite loads in the salivary glands of Anopheles mosquitoes (Beier *et al*., 1988).

2.5.1 Detection of mosquito blood meal sources

The identification of blood meals taken by malaria vectors is an important procedure in malaria epidemiology because the degree of human feeding influences the probability that mosquitoes will come into contact with gametocyte carriers and thus acquire and

transmit *Plasmodium* infections (Beier *et al.*, 1988). Garret-Jones *et al.* (1980) demonstrated that the most successful vectors of malaria fed most commonly on human and secondarily on cattle and other domestic animals' blood depending on host availability. In tropical Africa, it has been estimated that 80% of *Anopheles* species feed on any large mammal that is available (Garret-Jones *et al.*, 1980). In areas where malaria transmission is low after a period of eradication, the identification of blood meals from mosquitoes can provide important epidemiological information on host preference by the different species, and act as a useful guide on which species to target for control (Garret-Jones *et al.*, 1980).

Along the Kenyan coast, a high degree of preference to human feeding by Anopheline vectors has been reported as a major factor contributing to their efficiency in *Plasmodium falciparum* transmission. Mbogo *et al.* (1993b) reported a human blood feeding rate of 94.4% in *Anopheles gambiae* and 90.8% in *Anopheles funestus* along the Kenyan coast. The high preference for human feeding which facilitates frequent vector contact with gametocyte carriers provides evidence for the high malaria transmission observed in different malaria endemic areas (Mbogo *et al.*, 1993b)). Several methods have been developed for the detection of the different sources of blood meals in disease vectors. These include the use of enzyme linked immunosorbent assay (ELISA), precipitin tests, haemagglutination assays, counter current immuno-electrophoresis and immunoflourescence. The ELISA method developed by Beier *et al.* (1988) is the most commonly used test. The ELISA assay meets the criteria for a field operational kit for

the detection of blood meals in mosquitoes and has been the most commonly used technique (Beier *et al*., 1988).

2.5.2 Determination of sporozoite infection rates in wild mosquitoes

The rate of mosquito infectivity in the field has always been measured by determining the proportion found to be carrying *Plasmodium* sporozoites (Wirtz *et al*., 1985). Previously, the determination of sporozoites rates was done by the dissection of the salivary glands, and this was obviously a tedious and laborious process, especially in areas where mosquito density was high. In addition, this process requires fresh female mosquito samples. This method was not easily applicable in the field. Wirtz *et al*. (1985) developed an ELISA method for the detection of the *Plasmodium falciparum* circumsporozoite protein in field-collected mosquitoes. ELISA combines the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme.

There are two main variations of this method: the ELISA can be used to detect the presence of antigens that are recognized by an antibody or it can be used to test for antibodies that recognize an antigen. In the sporozoite ELISA technique, monoclonal antibodies are used to detect circumsporozoite proteins of *Plasmodium falciparum* (Wirtz *et al*., 1985) and the results are read visually or by using an ELISA reader (Wirtz *et al*., 1985; Beier *et al*., 1988). The sporozoite ELISA technique is more rapid, and is very useful in detecting infectious mosquito species from either fresh or dry stored mosquito specimens. Sporozoite rates are then inferred from the proportion of human

biting Anophelines that test positive for *Plasmodium falciparum* circumsporozoite protein by ELISA (Wirtz *et al.*, 1985; Beier *et al.*, 1988).

2.5.3 Entomological inoculation rates (EIR)

The intensity of malaria parasite transmission in the field is estimated by determination of the entomological inoculation rate (EIR). Entomological inoculation rate is the product of the mosquito biting rate and the proportion of mosquitoes carrying sporozoites in their salivary glands (Beier *et al.*, 1999). Entomological inoculation rate can be used to estimate the level of transmission to individuals living in a particular location at a time and as a measure to differentiate transmission intensity between geographic areas (different villages or parts of villages) over a period of time (Beier *et al.*, 1999). In Africa, EIRs in malaria endemic areas have been reported to range from between <1 to >1000 infective bites per person per year (Trape and Rogier, 1996). This reflects the extent to which the malaria transmission situation is variable in different parts of Africa, hence the need for more area specific control measures. Any control measures aimed at reducing malaria prevalence may need to substantially reduce EIR's in order to reduce the prevalence of malaria infection (Trape and Rogier, 1996).

MATERIALS AND METHODS

3.1 Location of the study area

This study was carried out in Kilifi town and Jaribuni Village of Kilifi County, coastal Kenya. Kilifi County borders Tana River County to the North, Taita Taveta to the West, Mombasa to the South and Indian Ocean to the East (Figure 3.1) and (Figure 3.2). Kilifi County is located on the Kenyan coast at a latitude $2^{0}20''$ and 4^{0} south and between longitude $39^{0}$and $40^{0}14''$ East. Kilifi County covers a total area of 12,384.2 km^{2} and according to the 2009 population census had a population of approximately 1,088.608 inhabitants (Kilifi and Malindi districts strategic plans, (2005 - 2010).

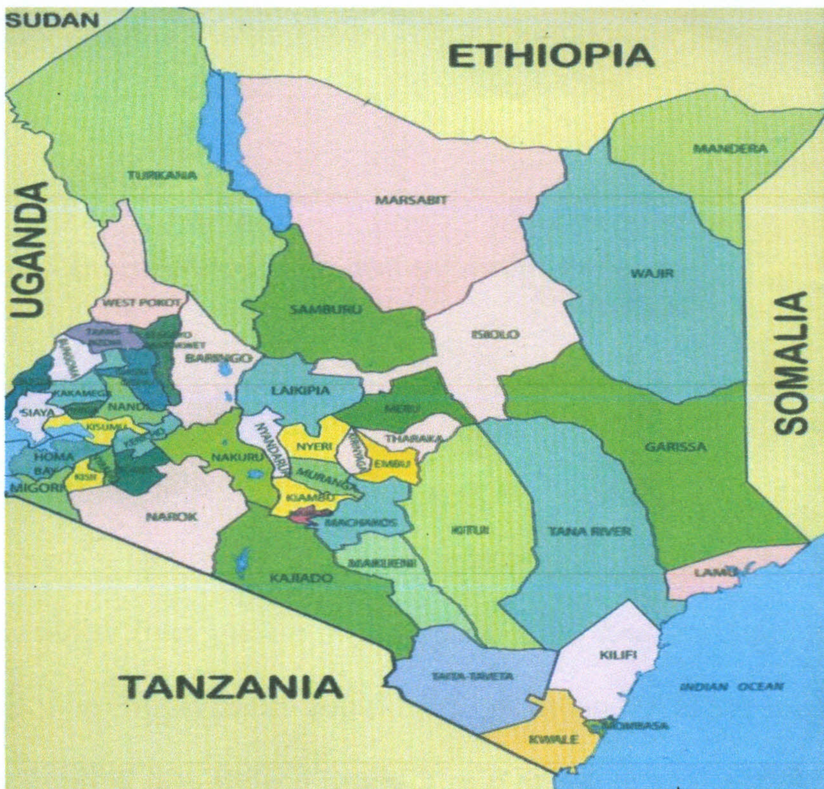

Figure: 3.1: Location of Kilifi County, Kenyan coast

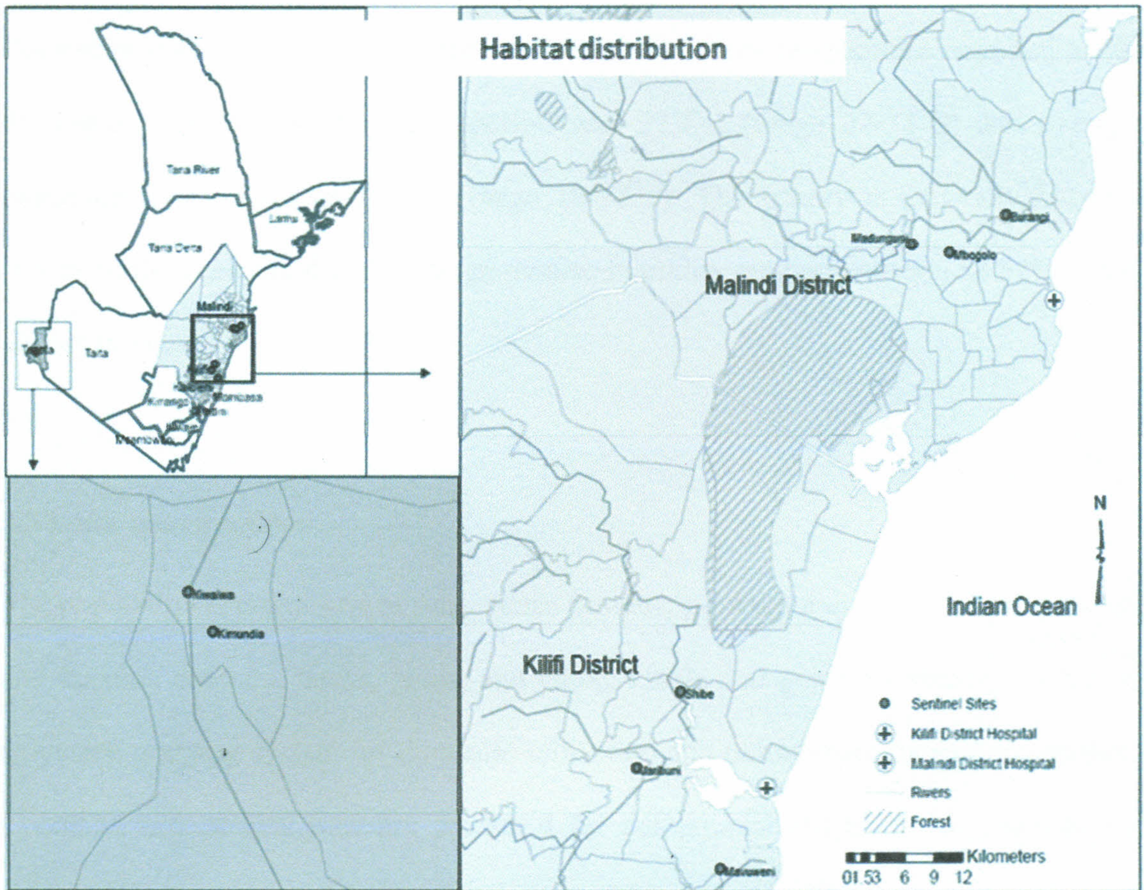

Figure 3.2: Location of Kilifi town and Jaribuni village within Kilifi County

3.2 Climate and geomorphology

The county is covered by forests and savanna type of vegetation. The natural vegetation varies widely from patches of rain forest in the Arabuko Sokoke forest to dry thorn bushes. Most of the human populations live within the 10 km wide coastal strip and in the hilly areas. The altitude ranges from 0 to 400 m above sea level. Average annual rainfall ranges from over 1,200 mm at the coastal belt to 400 mm in the hinterland (Drought Monitoring and Early Warning Bulletin, 2013). Rainfall pattern is bimodal

with long rains in April-June (with a peak in May) and short rains in October-December with a peak in November. Average maximum temperature recorded along the coastal belt during the study period was 27.5^{0}C (range 22-33^{0}C) and average minimum temperature of 22.8^{0}C (range 19-27^{0}C). The county is generally hot and humid all the year round with average relative humidity ranging between 58-92% along the coastal belt.

3.3 Land use practices

The population is composed of subsistence farmers, growing mainly maize and cassava and keeping chicken, ducks, goats, and cattle. Cattle and goats are mainly reared on extensive grassing system for domestic utilization and partially for income generation. Coconuts and cashew nuts are produced for commercial purposes. Homesteads are scattered and separated from one another either by agricultural land or small patches of natural vegetation. Each homestead has a number of houses, which are as a result of extended families sharing one compound (Department of Agriculture and Livestock Development, 2013).

3.4 Research and sampling design

Ten sampling sites for larval and adults were selected in Kilifi and Jaribuni. Season sampling was conducted between October- December 2010 and April-June 2011. Kilifi town represented the urban set up while Jaribuni was the rural set up. Selected mosquito larval habitats were sampled biweekly for a period of eleven (11) months.

Ten (10) houses and ten (10) outdoor vegetation sites were selected in each site for adult sampling and also sampled biweekly for both seasons. Species counts were made and recorded to obtain means.

3.5 Selection of study sites

The sites were selected based on ecological variations. Kilifi urban becomes heavily infested with mosquitoes during the rainy season due to numerous temporary water pools followed by a progressive decline as the dry season sets in. Jaribuni is a mosquito field (rural) study station for Kemri-Wellcome trust endowed with larval habitats year round along Jaribuni River (Plate 3.1). All aquatic habitats with larvae were sampled but 10 houses were randomly selected for adult mosquitoes sampling.The sites were selected for comparative analysis between rural and urban setups.

Plate 3.1: Pools along the banks of Jaribuni River

3.6 Determination of species composition of mosquitoes found in Kilifi County

Standard dipping techniques using a 350 ml dipper was used to sample mosquito larvae and pupae. On each sampling occasion a total of 10 dips were targetted for each sampling pond along Jaribuni River. However number of dips taken from temporary pools ranged between 1-10 depending on the habitat size. Pipetting and counting were done in very small habitats where dipping was not possible due to habitat size limitations. The samples were placed in plastic bags (whirl pak) with sufficient water and transferred to the laboratory for further processing (Plate 3.2). In the laboratory, the larvae were separated from predators and other non-mosquito organisms. The larvae were further sorted into culicines and anophelines. Larvae from each genus were counted and classified as either; early instars (L1, L2) or late instars (L3, L4). Larvae were then incubated to pupation and subsequent adult emergence.

Plate 3.2: Adult mosquitoes emerging from pupae in an emergence cage in the lab

3.6.1 Sampling for indoor mosquitoes

Adult mosquitoes were collected biweekly from ten (10) selected houses in Kilifi town and its environs and ten houses in Jaribuni using CDC battery powered light traps (Plate 3.3), a manual aspirator and a battery powered giant aspirator to enhance collection efficiency. The diverse collection methods were necessitated by different bahavioural orientations shown by different species.

Plate 3.3: Center for disease control and prevention light trap (Adapted from Sudia and Roy 1981)

Light traps were set in five houses in the evening between 1800hrs and 0600hrs. The remaining five houses were sampled the following week. Aspiration was always done during the day

3.6.2 Sampling for outdoor mosquitoes

Alternative resting habitats utilized by adult mosquitoes in both Kilifi and Jaribuni were identified including vegetation around human habitations and animal shelters and sampled using battery powered CDC light traps and *Aedes* trap (BG sentinel trap) (Plate 3.4). *Aedes* trap was set at 0700hours and collected at 1700hours. *Aedes* species specifically feed during the day while *Anopheles* species feed during the night and *Culex* species are all time feeders.

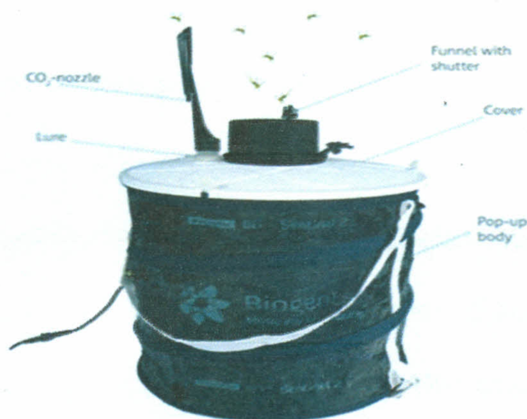

Plate 3.4: BG-Sentinel: Biogents' mosquito trap for researchers

3.7 Investigating the abundance of mosquito species

Samples from both and Jaribuni were sorted out into males and females separately and grouped into their respective species. Counts were made for each species and totals recorded. Male mosquito samples were then disposed off but females retained for further analysis.

3.8 Investigating the disease transmission potential of mosquitoes in Kilifi County

Adult mosquitoes obtained from indoor and outdoor sites were sorted to species level using keys by Gillies and Coetzee (1987) and Gillet (1972) and enumerated. The Culex females were tested for microfilaria worms while still fresh. The Aedes species females were stored under ultra cold conditions of -80^{0}C for a later screening for arbovirus. The Anopheles species females were further classified according to physiological status as unfed, blood-fed, half-gravid or gravid by examining the abdominal appearance and later subjected to Eliza test for *Plasmodium* circumsporozoite.

3.8.1 Analysis of presence of *Wuchereria bancrofti* in *Culex quinquefasciatus*

The head, thorax and abdomen of each *Culex pipiens* mosquitoes were dissected separately from each other in a drop of phosphate buffered saline (PBS) on a slide and searched for filarial worms. The worms are classified as L1 (sausage stage), L2 (motile short) and L3 (motile, infective and with caudal papillae) larvae.

3.8.2 Plasmodium falciparum circumsporozoite proteins and blood meal analysis in *Anopheles* mosquitoes

The *Anopheles* mosquitoes were placed singly in plastic vials paying much attention to avoid contamination. Eventually, 50 µl of grinding buffer (50 µl boiled casein containing Nonidet 40) was added into each vial, and the samples ground using sterile pestles. Subsequently, 200 µl of blocking buffer was added bringing the final volume to 250 µl. The samples were stored at -20°C until time of testing. The two hundred and sixty seven microlite aliquots were tested by an enzyme-linked immunosorbent assay

(ELISA) using monoclonal antibodies to detect circumsporozoite (CS) proteins of *Plasmodium falciparum*. The 96 well microtiter plates were labeled appropriately. Twenty (20) ml *of Plasmodium falciparum* monoclonal antibody (2A10-Kirkgaard and Perry®, Gaither, MD USA) were diluted in 5 ml PBS. In each micro titer well, 50 ml of the monoclonal antibody were placed and incubated for 30 minutes, and then the contents were aspirated.

The plate was banged to dry on soft tissue paper. Without washing, the unbound reactive sites were blocked with 200 ml of blocking buffer. After one hour of blocking, the contents were aspirated and the plate dried again. The plate was then charged with 50 ml of the mosquito triturate. Both negative and positive controls were included. The negative control consisted of four male *Anopheles* mosquitoes, which were homogenized in 1 ml of PBS. The positive controls consisted of commercially prepared recombinant *Plasmodium falciparum* CS protein. The plates were incubated for 2 hours. The contents were aspirated and washed twice with PBS-Tween 20. Fifty microlitres horseradish peroxidase conjugated Mab (Kirkgaard and Perry®) was added to each well. The plate was then incubated for one hour and then washed 3 times with PBS-Tween and after which 100 ml peroxidase substrate (2.2 Azino [3-ethyl-bennzethiozoline]sulphate)-ABTS was added. The plates were read visually after 30 minutes. For blood meal analysis, the same procedure was followed but involved additional step of enzyme-conjugate preparation. Serum from all hosts except those being tested, enzyme conjugate was added.

3.8.3 Preparation of cells and detection of arbovirus in *Aedes* mosquitoes

A T-75 flask with confluent vero cells was decontaminated using 70% ethanol and the media emptied into a waste container. The cells were washed twice with 1% PBS and 3 ml trypsin added to digest the intercellular matrix. The cells were incubated at 37^0C for 5 minutes and 20 ml of growth media (GM) containing 86% MEM, 10% FBS 2% L-glutamine, and 2% antimycotic/antibiotic solution. The cells were punched to separate those still attached followed by adding growth media as observation was made under a microscope until there was a moderate concentration of cells in the media. The cell suspension was then pippeted and added into the wells of a 24-well plate (1 ml/well). Plates were incubated at 37^0C overnight to become confluent.

The *Aedes* mosquito samples stored from the ultra low freezer (-65^0C to -86^0C) were obtained and grouped into fifty two (52) pools of three (3) mosquitoes each and homogenized in minimum essential media (MEM) containing 15% FBS, 2% antimycotic/ antibiotic solution, 2% L-glutamine, and 80% MEM. The homogenates were then spinned/centrifuged at 1200 rpm for 10 min to get rid of the mosquito debris. Fifty micro liters of each of the 52 homogenates were inoculated into individual well of the 24-Well plate containing confluent Vero cells and incubated at 37^0C for 45 minutes for virus adsorption to occur. One milliliter of maintenance media containing 94% MEM, 2% FBS, 2% antimycotic/antibiotic solution and 2% L-glutamine were added to each well and the cells incubated at 37^0C. The cells were observed daily for 14 days for development of cytopathic effect (CPE). All Samples showing CPE are further screened by RT-PCR to identify the infecting virus.

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3.8.4 Indices of malaria parasite transmission

Malaria transmission potential was determined according to Bruce-chwatt (1985). The sporozoite rate was taken as the proportion of mosquitoes positive for *Plasmodium falciparum* sporozoites out of the total number of mosquitoes tested. The daily human biting rate (HBR) was obtained by dividing the total number of blood fed and half gravid mosquitoes caught in a house by the total number of people who slept in that house the night preceding collection. The daily EIR was derived as the product of daily HBR and sporozoite rate.

3.9 Data analysis

The species of different mosquitoes identified in the two sampling sites were documented and presented in a tabular form. The data on the abundance of species was subjected to Analysis of Variance (ANONA). Data on larval, indoor , outdoor sex occurrence, human biting rates, entomological inoculation, and sporozoites rates from both Kilifi and Jaribuni was subjected to a paired t-test of SPSS (17.0). The significance level was set at p=0.05. The data on physiological and sporozoites status was subjected to Chi-Square Test at p=0.05.

CHAPTER 4

RESULTS

4.1 Identification of larval species of mosquitoes infesting urban and rural areas of Kilifi county

Nine species of larval mosquitoes namely *Culex quinquefasciatus*, *Culex annulororis*, *Culex tigripes*, *Anopheles gambiae*, *Anopheles funestus*, *Anopheles pretoriensis*, *Anopheles squamosus*, *Aedes aegypti* and *Erepodites inornatus* were recorded infesting Kilifi town and Jaribuni village. *Culex annulioris* and *Anopheles pretoriensis* were only recorded in Jaribuni where as *Anopheles squamosus*, *Culex tigripes* and *Erepodites inornatus* were only recorded in Kilifi. The rest of the species were common in both sites (Table 4.1).

Table 4.1 Larval species composition in rural and urban areas of Kilifi County

	Larvae	
Species	**Rural**	**Urban**
Aedes aegypti	0	+
Anopheles funestus	+	+
Anopheles gambiae	+	+
Anopheles pretoriensis	+	0
Anopheles squamosus	0	+
Culex annulioris	+	0
Culex quiquefasciatus	+	+
Culex tigripes	0	+
Eretmapodites inornatus	0	+

Key
+ = Present 0 = Absent

4.2 Identification of adult species of mosquitoes infesting urban and rural areas of Kilifi County

Nine species of adult mosquitoes namely *Culex quinquefasciatus, Culex annulororis, Culex tigripes, Anopheles gambiae, Anopheles funestus, Anopheles pretoriensis, Anopheles squamosus, Aedes aegypti* and *Aedes simpsoni* were recorded infesting Kilifi town and Jaribuni village. *Anopheles funestus, Anopheles pretoriensis* and *Culex tigripes* were only recorded in Jaribuni and *Anopheles squamosus, Aedes simpsoni* and *Aedes aegypti* were only reorded in Kilifi. The rest of the species were common in both sites (Table 4.2).

Table 4.2 Adult species composition in rural and urban areas of Kilifi County

	Adults	
Species	**Rural**	**Urban**
Aedes aegypti	0	+
Aedes simpsoni	0	+
Anopheles funestus	+	0
Anopheles gambiae	+	+
Anopheles pretoriensis	+	0
Anopheles squamosus	0	+
Culex annulioris	+	+
Culex quiquefasciatus	+	+
Culex tigripes	+	0

Key
+ = Present 0 = Absent

4.3 Abundance of mosquito species in urban and rural areas of Kilifi County

The abundance of mosquito species in Kilifi and Jaribuni was determined.

4.3.1 Abundance of larval mosquitoes based on emergent adults and adult species in urban and rural areas of Kilifi County

There were no significant differences in the mean numbers of larvae recorded between the rural and urban areas ($F = 0.44$, $p=0.509$). Rural area recorded a mean of (15.30 ± 28.74) and urban area (11.73 ± 11.00).

Table 4.3: Mean numbers (± SE) of larval mosquito species in urban and rural areas of Kilifi County.

Larval species	Urban	Rural
Aedes aegypti	18.67±12.67a	-
Anopheles funestus	1.00 ± 0.00a	8.40± 6.85a
Anopheles gambiae	9.33±4.93a	14.45±23.13a
Anopheles pretoriensis	-	7.11±8.10a
Anopheles squamosus	2.00±0.00a	-
Culex annulioris	-	12.43±12.41a
Culex quiquefasciatus	10.58± 11.12a	28.06±53.37a
Culex tigripes	7.67±2.89a	-
Eretmapodites inornatus	3.00±0.00a	-
F-value	1.13	1.29
P-value	0.37	0.28

Means denoted by same lower case letters in the same column are not significantly different at $p = 0.05$.

The mean numbers of larval mosquitoes collected from river ponds in Rural areas were not significantly different from those collected from temporary rain pools in urban areas ($t = 2.121$, $P = 0.078$) (Table 4.4).

Table 4.4: Mean numbers (± SE) of larval mosquito species collected from temporary rain pools in urban and rural areas of Kilifi County

Habitat	Mean
Temporary rain pools	92.86 ± 37.58b
River ponds	255.43 ± 150.23a

Means denoted by same lower case letters within the column are not significantly different at p =0.05

There were no significant differences in the mean numbers of adult mosquitoes recorded in urban areas (F = 0.28, p = 0.760) and rural areas (F = 1.29, p = 0.281). Rural recorded a mean of (15.30 ± 28.74) and urban (11.73±11.00) (Table 4.5).

Table 4.5: Mean numbers (± SE) of adult mosquitos' species in urban and rural areas of Kilifi County

Larval species	Kilifi	Jaribuni
Anopheles funestus	-	5.55±8.78a
Anopheles gambiae	1.00±0.00a	1.12±0.354a
Anopheles pretoriensis	-	1.00±0.00a
Anopheles squamosus	12.00±0.00a	-
Culex annulioris	-	2.16±1.17a
Culex quiquefasciatus	11.78±14.22a	2.18±1.18a
F	0.28	1.29
p	0.760	0.281

Means denoted by same lower case letters within the column are not significantly different at p =0.05

There were no significant differences between the mean numbers of female and male larval mosquito species recorded infesting the rural and urban areas of Kilifi County (Table 4.7). Males were not recorded in *Anopheles squamosus* and *Eretmapodites inornatus*.

Table 4.6: Mean numbers (± SE) female and male larval mosquito species recorded in rural and urban areas of Kilifi County

Species	Female	Male	t	p
Aedes aegypti	11.44 ± 3.48a	7.22 ± 1.89a	1.15	0.28
Anopheles funestus	3.91 ± 1.12a	3.82 ± 1.08a	0.12	0.91
Anopheles gambiae	7.18 ± 1.89a	6.82 ± 1.94a	0.70	0.49
Anopheles pretoriensis	3.33 ± 1.10a	3.78 ± 1.92a	0.28	0.79
Anopheles squamosus	2.00 ± 0.00	-	-	-
Culex annulioris	5.86 ± 1.61a	6.57 ± 1.35a	0.58	0.57
Culex quinquefasciatus	10.03 ± 3.92a	10.03 ±3.85a	1.24	0.22
Culex tigripes	4.00 ± 1.00a	3.67 ± 0.67a	1.00	0.42
Eretmapodites inornatus	2.00 ± 0.00	1.00 ± 0.00	-	-

Means denoted by same lower case letters in the same row are not significantly different at p =0.05

There were significant differences between the mean numbers of female and male *Culex annulioris* adult mosquitoes recorded infesting indoors (t =4.38, p = 0.005). The mean numbers of female and male *Anopheles funestus* (t = 1.35, p = 0.190) and *Anopheles gambiae* (t = 1.18, p = 0.272) and *Culex quinquefasciatus* (t = 1.15, p = 0.256) however never differed significantly. Males were not recorded in *Anopheles pretoriensis* and *Anopheles squamosus*. (Table 4.7)

Table 4.7 Mean numbers (± SE) of female and male adult mosquitoes recorded indoors in urban and rural areas of Kilifi County

Species	Females	Males	t	p
Anopheles funestus	3.32 ± 1.00a	2.24 ± 0.57a	1.35	0.190
Anopheles gambiae	0.78 ± 0.15a	0.38 ± 0.24a	1.18	0.272
Anopheles pretoriensis	1.00 ± 0.00	-	-	-
Anopheles squamosus	12.0 0± 0.00	-	-	-
Culex annulioris	1.57 ± 2.00A	0.43 ± 0.29A	4.38	0.005*
Culex quinquefasciatus	3.18 ± 0.59a	4.29 ± 1.21a	1.15	0.256

Means denoted by same lower case letters in the same row are not significantly different at p =0.05

Means denoted by same upper case letters in the same row are significantly different at p =0.05

There were significant differences between the mean numbers of female and male adult *Aedes aegypti* (t = 2.40, p =0.027), *Anopheles funestus* (t = 7.07, p = 0.001), and *Anopheles gambiae* (t =4.80, p = 0.003). The mean numbers of female and male adult *Anopheles pretoriensis* (t = 3.00, t = 0.205), *Culex annulioris* (t = 3.04, t = 0.140), and *Culex quinquefasciatus* (t = 0.83, t = 0.413) recorded infesting outdoors never differed significanly. Males were not recorded in *Aedes simpsoni* and *Culex tigripes* (Table 4.8).

Table 4.8 Mean numbers (± SE) of adult female and male mosquitoes species recorded outdoors in the urban and rural areas of Kilifi County

Species	Females	Males	t	p
Aedes aegypti	8.60 ± 3.82A	6.15 ± 3.82A	2.40	0.027*
Anopheles funestus	1.43 ± 0.20A	0.00 ± 0.00A	7.07	0.001*
Anopheles gambiae	1.43 ± 0.30A	0.00 ± 0.00A	4.80	0.003*
Anopheles pretoriensis	1.50 ± 0.00a	0.00 ± 0.00a	3.00	0.205
Aedes simpsoni	1 ± 0.00a	-	-	-
Culex annulioris	3.40 ± 0.98a	0.20 ± 0.13a	3.04	0.140
Culex quinquefasciatus	1.45±0.45a	2.5 ± 1.20a	0.83	0.413
Culex tigripes	1.0±0.00a	-	-	-

Means denoted by same lower case letters in the same row are not significantly different at p =0.05

Means denoted by same upper case letters in the same row are significantly different at p =0.05

4.4 Assessment of disease transmission potential of different species

Plasmodium falciparum circumsporozoite were only detected in *Anopheles funestus*. Approximately 5% of *Anopheles funestus* tested positive for *Plasmodium falciparum* circumsporozoite. The sporozoic *Anopheles funestus* samples were all captured in Jaribuni (Table 4.9). There was more of the blood fed mosquitoes (39.2%) which had sporozoites. However, there was not significant association in the presence of sporozoites to the physiological status of the mosquitoes ($\chi^2 = 1.188$, P = 0.552) Table 4.10).

Table 4.9: Sporozoites in Anophelines between August 2010 and June2011

Village	Mosquioto species	Number examined	%Total	Number +ve for sporozoites	% +ve for sporozoites
Kilifi	*An.Gambiae*	2	0.007	0	0
Kilifi	*An.squamosus*	6	2.26	0	0
Jaribuni	*An.gambiae*	12	4.53	0	0
Jaribuni	*An.pretoriensis*	4	1.51	0	0
Jaribuni	*An. Funestus*	241	90.9	12	4.98
Total		265	100	12	4.53

Table 4.10 Physiological status and sporozoites in Anopheline Mosquitoes in rural and urban areas of Kilifi County

	Physiological status				
Species	**Unfed**	**Blood fed**	**Half gravid**	**Gravid**	**Total**
An. gambiae	9	5	0	0	14
Number with sporozoites	0	0	0	0	0
Anopheles funetus	36	97	3	105	241
Number with sporozoites	7	3	0	2	12
An. Pretoriensis	3	1	0	0	4
Number with sporozoites	0	0	0	0	0
An. Squamosus	6	0	0	0	6
Number with sporozoites	0	0	0	0	0

4.4.1 Biting and entomological inoculation rates of *Anopheles gambiae* and *Anopheles funestus*

There were significant differences between the human biting rates of *Anopheles gambiae* and *Anopheles funestus*. *Anopheles funestus* recorded the highest mean of (1.146 ± 0.003) (t = 3.289, df = 6, $p<0.05$) and *Anopheles gambiae* (0.15 ± 0.067) (Table 4.11). There were significant differences between the entomological inoculation rates of *Anopheles gambiae* and *Anopheles funestus*. *Anopheles funestus* recorded the highest mean of (0.084 ± 0.028) (t= 3.005, df = 6, $p < 0.05$) and *Anopheles gambiae* (0.000 ± 0.000) (Table 4.11)

Table 4.11: The daily human biting rate and the entomological inoculation rates of *Anopheles gambiae* and *Anopheles funestus* in Kilifi County.

	Anopheles gambiae			*Anopheles funestus*		
Month	HBR	SPOR	EIR	HBR	SPOR	EIR
October	0.33	0.00	0.00	0.25	0.00	0.00
January	0.00	0.00	0.00	0.33	0.50	0.165
February	0.00	0.00	0.00	1.58	0.00	0.00
March	0.33	0.00	0.00	1.87	0.03	0.056
April	0.33	0.00	0.00	0.77	0.14	0.108
May	0.00	0.00	0.00	0.89	0.08	0.071
June	0.33	0.00	0.00	2.33	0.08	0.186
Mean	0.19± 0.003A	0.00	0.00B	1.15±0.067A	0.119	0.084±0.028B

Means denoted by same upper case letters in the same row are significantly different at p =0.05

4.4.2 Filarial infections in *Culex quinquefasciatus*

No filarial infections were observed among the 187 mosquitoes dissected and examined in both Kilifi town and Jaribuni village (Table 4.9).

Table 4.12: Filaria worms in *Culex quinquefasciatus* collected in Kilifi and Jaribuni.

Village	**Number examined**	**% +ve filarial worms L_1-L_3 (all stages)**	**(%) +ve for L_3 only**
Kilifi	158	0.0	0.0
Jaribuni	29	0.0	0.0
Total	187	0.0	0.0

4.4.3 Arbovirus infections in *Aedes aegypti* and *Aedes simpsoni*

No arbovirus infections were detected in all the 153 *Aedes sp* samples collected in Kilifi (Table 4.10).

Table 4.13: Viral infection status in *Aedes spp* in Kilifi Urban

Virus	*Aedes simpsoni* (n=1)	Aedes aegypti (n= 153)
Flavivirus	0	0
Alphavirus	0	0
Phlebovirus	0	0

CHAPTER 5

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Results of species composition of larval mosquitoes from urban and rural areas of Kilifi County revealed nine species.These are: *Culex quinquefasciatus, Culex annulororis, Culex tigripes, Anopheles gambiae, Anopheles funestus, Anopheles pretoriensis, Anopheles squamosus, Aedes aegypti* and *Erepodites inornatus*. *Anopheles squamosus, Erepodites inornatus*, and *Aedes aegypti* were only recorded in Kilifi while *Anopheles pretoriensis* was only recorded in Jaribuni. *Erepodites inornatus* was only recorded in larval form which was an indicator of its rare occurrence in the County.

The occurrence of the species in urban areas could be attributed to urbanization which creates numerous temporary aquatic habitats which are ideal for breeding of some mosquito species as reported by Gillet (1972). It has also been reported that *Aedes aegypti* acts as a vector for yellow fever in urban areas (Barrett and Higgs, 2007) and is mainly found in urban strata where limited aquatic habitats are temporary (Mwangangi *et al*., 2012). The presence of *Anopheles pretoriensis* in rural areas only could be attributed to larval habitats suitability. It has been reported that *Anopheles pretoriensis* prefer breeding in permanent aquatic habitats like *Anopheles funestus* which may explain their absence in urban areas. *Culex quinquefasciatus, Culex annulororis* and *Anopheles gambiae,* were recorded in both rural and urban areas coexisting with all other species in both temporary and permanent habitats which was an indicator of their wide distribution and adaptability (Mwangangi *et al*., 2012). It has also been reported

that *Culex quinquefasciatus* is mostly found in urban and peri-urban strata while *Aedes aegypti* is mainly found in urban strata where limited aquatic habitats are polluted (Mwangangi *et al*., 2012). This is in agreement with Service (1993) and Kettle (1995) who reported that aquatic habitat suitability is an invariant aspect of the mosquito life cycle. The almost unity ratio of females to males in larval habitats indicated that there was no sexual selection in mosquito's populations and the samples came from the same populations of species. Overproduction of males observed in the first hatches may also be counterbalanced by increased fitness of females which predominated in the delayed hatches as observed by Lounibos and Escher (2008).

Results of species composition of adult mosquitoes from urban and rural areas of Kilifi also revealed nine species. These are: *Culex quinquefasciatus, Culex annulororis, Culex tigripes, Anopheles gambiae, Anopheles funestus, Anopheles pretoriensis, Anopheles squamosus, Aedes aegypti* and *Aedes simpsoni. Aedes simpsoni, Aedes simpsoni* and *Anopheles squamosus* were only recorded in the urban areas while *Anopheles funestus, Anopheles pretoriensis*, and *Culex tigripes* were only recorded in rural areas. *Aedes simpsoni, Aedes aegypti* and *Anopheles squamosus* prefer breeding in temporaray aquatic habitats which were numerous in the urban areas during the rainy season hence their presence in urban areas only. The presence of *Anopheles* funestus, *Anopheles pretoriensis*, and *Culex tigripes* in rural areas is also attributable to permanent aquatic habitats. The species are reported to predominantly breed in habitats that are permanent (Mwangangi *et al*., 2012)

The survey of mosquito species abundance in rural and urban areas revealed equal numbers of larval species. The mean numbers of adult species also never differed significanly. This implied that urban and rural areas share similar ecological conditions and hence both larval and adult species numbers insignificant variations were possibly due to similar larval habitats with similar physical chemical characteristics within similar ecological setting as observed by Minakawa *et al*. (1999). This also concurs with the findings by Bashar and Tuno (2014), who reported that the abundance of larval and adult mosquito species and composition may be influenced by important, factors e.g. variation in adult species composition among the sites and climate. It has also been repoted that each mosquito species has unique environmental tolerance limits (Martens *et al*., 1995) and larval species abundance is also highly dependent upon the availability of suitable aquatic habitats.

The dependence of mosquito species on aquatic habitats and the availability of an appropriate aquatic realm, necessary for egg, larval, and pupae development, critically determines mosquito species abundance (Service, 1993; Kettle, 1995). The oviposition of mosquito eggs by gravid females and their maturation to larvae and subsequent adults also requires the availability of suitable aquatic breeding habitats (Molineux, 1988; Le Sueur and Sharp, 1991). The biotic and abiotic factors that affect life history traits such as growth, development and survival of the immature stages of the various species affect productivity in the breeding sites and determine the composition, abundance, distribution and the fitness of the resultant adult mosquito populations as observed by Paaijmans *et al*.(2007).

The mean larval numbers differed significantly between temporary rain pools in urban areas and River ponds in rural areas. Stream edge and streambed pools notably maintained larvae permanently in rural areas through out the sampling period although in lower numbers as water levels subsided. In urban areas, larval development was temporarily maintained in at least four different larval habitats namely rain pools, drainage channels, old tyres and water barrels. The diffefrences could be attributed to the nature of the habitats and biotic interactions within the habitats. It has been reported that the presence or absence of predators, parasites, pathogens (Aniedu *et al*., 1993) or cannibalism contributes to high larval mortality during development. Lower water levels bring prey, predator and cannibals closer as well as increasing levels of pollution (Okogun, 2005). Other biotic factors that may affect survival are predation by sibling species, and other interactions between sibling species as observed by Schneider *et al*. (2000) while abiotic factors such as temperature may also affect larval mortality as observed by Bayou and Lindsay (2003).

Comparatively, more adult species were collected indoors than outdoors suggesting a predominant endophilic behavior except *Aedes aegypti* which exhibited a predominant exophilic life style. The mean numbers of indoor females and males differed significantly. The predominant endophily among the adult species could be attributed to daily temperature inversions which make most species to seek refuge in human or animal shelters which are warmer at night. This endophilic behavior increases the degree of human-vector contact as vectors seek for blood meal which possibly explains the occurrence of more female mosquitoes indoors than outdoors as observed by

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Mbogo *et al.* (1993a). It has been reported that males follow the females only for mating purpose and are not known to take blood meal which may also explain the occurrence of more males indoors than outdoors (Mbogo *et al.*,1993a). The mean number of outdoor resting females and males also differed significantly. The differences could be attributed to behavioural patterns such as swaming, feeding, mating and flight range of different species as observed by Mwangangi *et al.* (2012).

Results on species composition revealed all nine species being of medical importance in Africa. *Anopheles gambiae* and *Anopheles funestus* are known vectors of malaria (Mbogo *et al.*, 1993a) and are also known vectors of O'nyong-nyong virus and bancroftian filariasis in East Africa (Williams *et al.*, 1965, Pedersen and Mukoko, 2002). *Anopheles squamosus* and *Anopheles pretoriensis* are regarded as occasional malaria vectors (Gillet, 1972). *Culex quinquefasciatus* transmits bancroftian filariasis and together with *Aedes aegypti, Erepodites inornatus* and *Culex annuloris* are vectors of West Nile Virus (Gillet, 1972). *Aedes aegypti* and *Aedes simpsoni* transmits yellow fever (Midega *et al.*, 2006).

The mean number of human biting rates between *Anopheles gambiae* and *Anopheles funestus* differed significantly. This study revealed *Anopheles funestus* to be the most competent malaria vector during the period. There were more of the blood fed *Anopheles funestus* mosquitoes (39.2%) which had sporozoites. However, there was not significant association in the presence of sporozoites to the physiological status of the mosquitoes.This could be attributed to the fact that mosquitoes bite any vertebrate for

blood meal and not necessarily humans. However, the sporozoic *Anopheles funestus* must have taken human blood meal as malaria is a human infection which is not known to infect other vertebrates as observed by Mbogo *et al.* (1993a). This concured with Garret-Jones *et al.* (1980) who reported that an efficient vector rests indoors and feeds primarily on humans. *Anopheles funestus* is a known adaptable species as shown by its wide distribution and ability to occupy regions ranging from lowland to high altitudes. This species is also abundant, widespread, highly endophilic and anthropophilic and is found in almost all bioclimatic areas near swamps or rivers (Faye *et al.*, 1995) which probably explains the transmission competence observed. Previous studies along the Kenyan coast have also reported *Anopheles gambiae* and *Culex quinquefasciatus* to have high human blood index (Mbogo *et al.*, 1993a; Bogh *et al.*, 1998; Mwangangi *et al.*, 2003). In both sites, it was not possible to compare the sporozoite rates of *Anopheles gambiae* in different physiological states due to the low numbers collected. However authors such as Mwangangi *et al.* (2003) and Mbogo *et al.* (2003) had found *Anopheles gambiae* to be the main malaria vector. The presence of *Plasmodium falciparum* circumsporozoite in unfed and gravid mosquitoes implied that the mosquito specimens might have completed oviposition cyle and must have had a human blood meal which concurs with Martens *et al.* (1995) who reported that it is the older mosquitoes that are the most dangerous in terms of sporozoite rates.

The mean number of entomological innoulation rates between *Anopheles gambiae* and *Anopheles funestus* also differed significantly. This agreed with findings from a report by Mbogo *et al.* (1995; 2003) who reported that *Anopheles gambiae* and *Anopheles*

funestus vary in sporozoite rates. The observation suggests that the entomological indices, which determine malaria transmission, are regional and species-specific. A similar variation in malaria transmission between species has been reported previously by Mbogo (1993b; 1995; 2003) along the Kenyan Coast suggesting the need to consider the local transmission characteristics prior to designation and implementation of mosquito borne diseases control program. For instance, any successful malaria control strategy should target all anophelines since anopheline species considered occasional vectors could easily become major vectors if *Anopheles funestus* and *Anopheles gambiae* were completely eliminated by differential control measures. This is because *Anopheles funestus* has also been observed to be the minor vector by Charlwood *et al.* (1997) in some areas where it occurred sympatrically with *Anopheles gambiae* in equal numbers.

This study exonerated *Culex quinquefasciatus* from being a principal vector of bancroftian filariasis in the study area. In both Jaribuni and Kilifi villages, no *Culex quinquefasciatus* was found infected with filarial worms despite several patients showing overt symptoms of the disease. This could be attributed to the fact that many factors contribute to the inefficient transmission of lymphatic filariasis as observed by WHO (2013). Firstly, microfilariae do not multiply in the mosquito body; hence the number of L3 is limited by the number of microfilariae ingested. Second, only those mosquitoes that survive more than 10 days will contribute to transmission of the parasites. Those mosquitoes that die before the L3 develops cannot play a role in the transmission cycle. Third, the L3 are deposited on the skin and have to find their way

into the bite wound (rather than being injected with the mosquito saliva like malaria sporozoites). In view of all these factors, the transmission of lymphatic filariasis parasites is considered to be less efficient than that of other vector-borne parasites (WHO, 2013). The findings contradicted Mwandawiro *et al.* (1997) who reported all the three species namely *Anopheles gambiae*, *Anopheles funestus* and *Culex quinquefasciatus* as vectors of lymphatic filariasis. This suggests that the vectorial system of a given area may change with time and hence the need for regular ecological and entomological surveys.

No *Aedes aegypti* was found infected with arbovirus. This was probably due to lack of infection of vectors with or without a previous blood meal. Yellow fever disease is characterized by single and sudden outbreaks when biotic and abiotic conditions are conducive as observed by Fontenille *et al.* (1997).

The effects urbanization as observed by Wang *et al.* (2005) have a significant impact on the economy, lifestyles, ecosystems and disease patterns, including malaria and other mosquito borne diseases. An estimated 39% of the population in sub-Saharan Africa (SSA) lived in urban areas in 2003, one hundred and ninety eight million Africans lived in urban malaria endemic areas and 24-103 million clinical attacks occur annually in those areas. Malaria and other mosquito borne diseases control strategies used in rural areas cannot be directly transferred to the urban context. The epidemiology of urban malaria therefore poses a number of specific challenges: The first malaria infection occurs often late in childhood and the acquisition of semi-immunity is delayed. The

intensity of the malaria risk is often heterogeneous over small distances being subjected to the degree of urbanization of particular sub-divisions and their proximity to possible breeding sites. Day to day rural-urban movement is likely to increase the endemicity of malaria; Agricultural and animal husbandry practices are important economic activities which create a favorable micro- environment for *Anopheles* mosquitoes breeding; while rural populations usually lack access to health care, which hampers the effectiveness of case confirmation and management and the promotion of intermittent antimalarials during pregnancy (Wang *et al*., 2005).

Previous studies show that the rate of clinical malaria attacks detected in urban health facilities is high and season-dependent (Wang *et al*., 2005). Transmission and severity of malaria are influenced by the geographic characteristics of a town and by the socio-economic environment (Baragatti, *et al*., 2009). The heterogeneity and seasonal variation of the entomological inoculation rate, depending on both vector densities and sporozoites rate, have been documented. Lindsay *et al*. (1990) showed a difference in the composition of vector species and the vector's adaptation in different sub-divisions in Banjul.This implies that to improve interventions, the determinants of the diversity of transmission levels within sub-divisions of a town should be understood. There are concerns about the association between urban agricultural activities and the creation of breeding sites for *Anopheles* species. Rural areas often lack infrastructure, including poor water supply, drainage and sanitation which provides an ideal environment for vector breeding (Prathiba *et al*., 2012).

5.2 Conclusions

From the results it is concluded that:

i. Kilifi urban set up recorded a higher number of larval mosquito species compared to Jaribuni. *Culex tigripes, Erepodites inornatus* and *Anopheles squamosus* were only recorded in Kilifi where as *Anopheles pretoriensis* and *Culex annulioris* were only recorded in Jaribuni.

ii. There were equal numbers of adult species in the two sites. *Culex tigripes Anopheles pretoriensis* and *Anopheles funestus* were only recorded in Jaribuni where as *Aedes simpsoni* and *Aedes aegypti* were only recorded in Kilifi.

iii. Jaribuni recorded a significantly higher mean number of larval mosquito species than Kilifi but no significant differences in adult mosquito species.

iv. No infections were detected in *Anopheles gambiae, Culex quinquefasciatus* and *Aedes aegypti*

v. *Anopheles funestus* was the most competent malaria vector, recorded significantly higher entomological inoculation and human biting rates.

5.3 Recommentations

i. There is need for research institutes such as KEMRI to work with the community in the identification and destroying potential larval habitats in both urban and rural settings as a primary intervention measure to control mosquito species populations.

ii. There is need for the local communities to embrace the use of treated

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bednets as a way of avoiding infective mosquito bites. Cultural beliefs should never be allowed to frustrate war on mosquito bites.

iii. More research needs to be done in all mosquitoes endemic areas to compare both the abiotic and biotic factors and mosquitoes species composition and abundance. This would help ascertain the factors with significant effects on specific species composition and abundance.

5.4 Suggestions for further studies

i. Mosquito species collected from both larval and terrestrial habitats need to be identified further by PCR to determine the sibling species and also detect any newly evolving species.

ii. More work is required to indentify and quantify the factors that contribute to the presence of some species in certain areas but absent in others within the same ecological setup.

iii. More work is required to investigate and assess the role of microbial communities (bacteria) in the mosquito larval habitats in relation to species abundance and composition.

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APPENDICES

Appendix i : CSAB elisa solutions

1. Phosphate buffered saline (PBS), pH 7.4.

Add one bottle Dulbeco's PBS to one liter of distilled water. Store in fridge (Shelf life is one week).

2. Boiled casein 0.5 % (BB)

Casein	gm	5.00
0.1 N NaOH	ml	100.00
PBS, 7.4	ml	900.00
Thimerosal	gm	0.01
Phenol red	gm	0.02

Suspend casein in 0.1 N NaOH and boil the contents. After the casein is dissolved, slowly add PBS; allow cooling and adjusting the pH to 7.4 with hydrochloric acid. Add phenol red and thimerosal (shelf life is one week).

3. Wash solution (PBS-Tween 20)

Add 0.5 ml of Poly-oxyethelene-sorbitan monolaurate (Tween 20) to one liter of PBS. Mix well and store in fridge. (Shelf life is two weeks).

Appendix ii: Giemsa stain

Giemsa powder	3.8 g
Glycerol (Glycerine)	250 ml
Methanol (Methyl alcohol)	250 ml

Weigh 3.8 g of Giemsa and transfer it to 500 ml bottle. Add 250 ml of methanol and 250

ml of glycerol and mix well. Place the bottle of stain in a water bath at 50-60°C for up to 2 hours to help the stain dissolve, mixing well at intervals. Label and store at room temperature in the dark. Stable for several months

Appendix iii: Lab processing form

DATE	SITE	HSE No	MOSQ No	SPP	SPORO	H	B	G	HGB	REMARKS

Appendix iv: Laboratory processing form for mosquitoes

Site of collection…………………… Code………………………………..

Time of collection…………………...Collection technique…………………

DATE	HSE #	MOSQ#	SPP	Total # of filarial worms					
				Head/Thorax			Abdomen		
				L1	L2	L3	L1	L2	L3

Appendix v: t-Test for indoor species sex differences in Kilifi County

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Jaribuni	1.5714	7	.53452	.20203
	Kilifi	.429	7	.7868	.2974

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1 Jaribuni - Kilifi	1.14286	.69007	.26082	.50465	1.78106	4.382	6	.005

Appendix vi: t-Test for outdoor adult species sex differences in Kilifi County

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Female	8.6000	20	17.06767	3.81645
	Male	6.1500	20	17.08577	3.82050

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1 Female Male	2.45000	4.57079	1.02206	.31081	4.58919	2.397	19	.027

Appendix vii. Chi-Square Test for association between presence of sporozoites and physiological status of mosquitoes

sporozoite * food statas codes Crosstabulation

	food status codes			Total
	empty	fed	gravid	
Count	51	98	104	253
% within sporozoite	20.2%	38.7%	41.1%	100.0%
Count	1	6	5	12
% within sporozoite	8.3%	50.0%	41.7%	100.0%
Count	52	104	109	265
% within sporozoite	19.6%	39.2%	41.1%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.188[a]	2	.552
Likelihood Ratio	1.376	2	.503
Linear-by-Linear Association	.312	1	.577
N of Valid Cases	265		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 2.35.

Appendix viii: t- Test for human biting rates of Anopheles gambiae and Anopheles funestus

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 AG - AF	-.95714	.7698	.29098	-1.66915	-.24514	-3.289	6	.017

Appendix ix: Entomological inoculation rates and sporozoite rates in *Anopheles gambiae* and *Anopheles funestus*

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 AG - AF	-.08371	.07372	.02786	-.15189	-.01554	-3.005	6	.024