CHARACTERIZATION OF MICROHABITATS OF ARBOVIRAL MOSQUITO VECTOR LARVAE ON THREE ISLANDS OF LAKE VICTORIA, KENYA

Ogao Onchuru Thomas (B.Sc.)

I56/20571/2010

A research thesis submitted in partial fulfillment of the requirements for the award of Master of Science degree (Biotechnology) in the School of Pure and Applied Sciences of Kenyatta University

July 2014

DECLARATION

This thesis is my original work and has not been submitted to any other university for a degree award.

Signature: _____ Date_____

Ogao Onchuru Thomas

RECOMMENDATION

This thesis has been submitted with our approval for examination as per the University regulations.

Signature: _____ Date_____
Dr. Marion Burugu
Department of Biochemistry and Biotechnology
Kenyatta University, Nairobi, Kenya

Signature: _____ Date_____
Dr. Daniel Masiga
Molecular Biology and Bioinformatics Unit
International Center of Insect Physiology and Ecology, Nairobi, Kenya

Signature: _____ Date_____
Dr. Jandouwe Villinger
Molecular Biology and Bioinformatics Unit
International Center of Insect Physiology and Ecology, Nairobi, Kenya

DEDICATION

To my loving to my parents, Mr. and Mrs Onchuru, siblings and mentors for their prayers, encouragement, moral and financial support.

ACKNOWLEDGEMENTS

I appreciate a number of people for their intellectual, moral, spiritual and financial support while in the process of producing this thesis. To start with, I thank the almighty God for his strength that sustained me throughout the process. Much appreciation to my supervisors, Drs. Marion Burugu, Daniel Masiga and Jandouwe Villinger for their timely advice and wise counsel in every step of my research. Deep gratitude to Dr. Martin Kaltenpoth and his team at the Max Planck Institute of Chemical Ecology for hosting me in his lab and their timely contribution towards the success of my thesis. I am also grateful to the Molecular Biology and Bioinformatics Unit (MBBU) members at the International Centre of Insect Physiology and Ecology (ICIPE) for their support. Specifically I am grateful to my colleagues Yvonne Ukamaka, Purity Ngina and David Omondi whom I worked with, and whose ideas and corrections ensured the success of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

Arbovirus	Arthropod borne virus
BLAST	Basic Local Alignment Search Tool
CO	Cytochrome Oxidase
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
DGGE	Denaturing gradient gel electrophoresis
HRM	High Resolution Melting
ICIPE	International centre for insect physiology and ecology
ITN	Insecticide treated nets
ITS	Internal transcribed spacer
ND	Nitrogen Deoxylase
OR	Odds ratio
OTU	Operational taxonomic unit
PBS	Phosphate buffered saline
PCA	Principle component analysis
PCR	Polymerase Chain Reaction
PCoA	Principle coordinates analysis
RDA	Redundancy analysis
RFLP	Restriction Fragment Length Polymorphism
RVF	Rift Valley Fever
TGGE	Temperature gradient gel electrophoresis
TDS	Total dissolved salts
WHO	World Health Organization

ABSTRACT

Mosquitoes are the primary vectors of arboviral infections. As there are no vaccines or effective therapeutic treatments for these diseases, vector control is an important approach for curbing their circulation or transmission. These vectors spend the first three of their four life stages in aquatic habitats where fitness and efficiency of adult mosquitoes to transmit arboviral infections is greatly influenced. Understanding ecology of mosquitoes in their aquatic habitats is therefore a critical component of vector control. This study investigated competent mosquito vectors present, their distribution and characteristics of their breeding habitats in three Lake Victoria Islands where high seroprevalence rates for arboviruses have been reported. Mosquito larvae sampled from Mfangano, Rusinga and Ngodhe Islands, were reared and identified by morphological and molecular means to determine mosquito distribution. Environmental variables including pH, temperature, nitrates, dissolved oxygen, ammonium, phosphate, copper, fluoride, salinity, conductivity, resistivity and oxidation reduction potential in the each of the sampled microhabitats were measured with a YSI photometer and professional plus water meter. Redundancy analysis and spearman correlation were used to determine association of each of these factors with three genera of mosquitoes sampled (*Anopheles, Aedes and Culex*). In addition, using Polymerase chain reaction, temperature gradient gel electrophoresis and 454 sequencing, bacteria in the microhabitats were profiled to determine their influence on mosquito breeding patterns. Competent vectors were present in the three Islands but their distribution varied based on island topography and size as well as human influence. Physico-chemical factors influence presence of larvae of each genus differently. Whereas presence of *Aedes* mosquito larvae had a strong positive correlation with ammonium rich microhabitats (p-value = 0.0235), presence of *Anopheles* larvae had a significant positive correlation with microhabitat temperature (p-value =0.004). However, there was no significant correlation between any of the variables investigated with the presence of *Culex* mosquito larva. Bacteria from 29 phyla and 23 candidate phyla were present in larval microhabitats suggesting that mosquitoes breed in bacteria rich sites. Proteobacteria accounted for >40% of bacteria community composition in 86% of samples analyzed by 454 sequencing. Overall, there were differences in bacteria community composition even in microhabitats hosting similar mosquito species suggesting that bacteria alone do not influence breeding patterns. In conclusion, environmental factors, bacteria and human influence are important factors in determining presence and suitability of microhabitats to host mosquito larvae of a particular genus. Presence of *Aedes* in ammonium rich microhabitats suggests that use of ammonium rich fertilizers will indirectly encourage transmission of *Aedes* vectored arboviruses and hence should be monitored closely and farmers advised accordingly.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Mosquitoes, which fall in the *Culicidae* family within the order *Diptera,* consist of about 3,500 species distributed worldwide. Of these, only a few species within the *Anopheles, Aedes,* and *Culex* genera have been well described owing to their medical significance (Minard *et al.*, 2013a). Due to their hematophagous nature, species within these genera transmit disease-causing pathogens to humans and animals. For instance, mosquito species of the *Anopheles* genus transmit malaria causing *Plasmodium* parasites, while culicines are vectors of arboviruses. The latter also transmit *Wuchereria bancrofti,* a pathogen responsible for lymphatic filariasis (Diallo *et al.*, 2012). In Kenya, mosquito species of the genera *Aedes, Mansonia, Anopheles* and *Culex* have been implicated as vectors of various arboviruses, including West Nile, Rift Valley fever, Yellow fever, Dengue, O'nyong nyong, Chikungunya, Usutu and Sindbis viruses alongside malaria and lymphatic filariasis (Kasili *et al.*, 2009).

Although control measures are in place to minimize the spread of diseases as a result of these pathogens, they are increasingly emerging in new areas. This is attributed to enhanced international trade or travel and human activities that either distribute vectors to nontraditional regions or establish favorable ecosystems for virus evolution and transmission efficiency. The lack of effective treatment and vaccines for these diseases demands new strategies for their control. Vector control that has succeeded in curbing other diseases, such as trypanosomiasis and Malaria is a promising option to pursue. However, vector management requires a good understanding of vector ecology. For mosquitoes, knowledge about their microhabitats, especially aquatic ones that host three of the distinct four life stages of these holometabolous insects, is very important for designing control programmes. These habitats play a significant

role in the determination of adult population sizes, compositions, fitness as well as competency. Various studies support this. Mwangangi *et al.* (2010) found that the availability of larval microhabitats affects larval densities and the adult population sizes. Moreover, it has been shown that adult female mosquitoes aggregate around ovipositing microhabitats, which increases the risk of disease transmission to nearby susceptible hosts (Le Menach *et al.*, 2005).

Larvae survival and abundance within a habitat are under the influence of both biotic and abiotic factors. Mwangangi *et al.* (2007b) noted that *Anopheles* larval densities observed in rice fields were under the influence of temperature, dissolved oxygen (DO), pH, turbidity, and salinity. These results confirm findings of a study conducted by Salit *et al.* (1996) for species of a similar genus. Other factors identified to be responsible for variation in larvae abundance and diversity within habitats include, nitrates, ammonium, shade coverage, amount of debris, salinity, conductivity and TDS (Minakawa *et al.*, 1999; Rao *et al.*, 2011). Some of these studies recommend inclusion of a detailed water chemistry analysis to determine if other water factors such different types of salts and metals influence mosquito breeding behavior.

Recent studies have revealed that aquatic microbiota play an important role in development of larvae and fitness of newly emerged mosquitoes. Besides acting as a source of nutrition, bacteria in larval microhabitats influence oviposition via semiochemical compounds that they produce (Sumba *et al.*, 2004; Ponnusamy *et al.*, 2008). In addition, they act as a primary source of symbiotic bacteria necessary for vital processes in adults. Although bacteria acquired at larvae or pupae stages are lost during metamorphosis through the formation of the meconial peritrophic matrices, some persist and become transstadially transmitted to adults that emerge afterwards via

specialized structures (Briones *et al.*, 2008; Engel and Moran, 2013; Minard *et al.*, 2013b). Horizontal transmission is also possible when mosquitoes pick up bacteria from microhabitats when emerging and/or depositing eggs. In adults, symbiotic bacteria determine their fitness and efficiency at which they can transmit pathogens. Indeed, Hoffmann *et al.* (2011) and Walker *et al.* (2011) demonstrated that the ability of *Aedes aegypti* to transmit dengue fever virus greatly reduces when infected with certain strains of *Wolbachia* bacteria. This may explain why some species are more efficient vectors than others. Lastly, as is the case in most hematophagous insects, symbiotic bacteria in mosquito provide hemolytic enzymes required for blood digestion to aid in the development of eggs (Minard *et al.*, 2013a; Gaio *et al.*, 2011).

While these studies provide substantial evidence supporting the importance of larval habitats and environmental variables on species composition, fitness and competency, not much research has been done on microhabitats of arboviral vectors especially in different populations. In Lake Victoria, a region with long historical arboviral activity, most of the work has concentrated on species within the *Anopheles* genus, which are involved in malaria transmission. In addition, although microbial communities have been reported to influence oviposition preference, mosquito development, nutrition and pathogen transmission, a deeper understanding on variation in bacteria communities present in habitats hosting different species is necessary.

The aim of this study was to identify physico-chemical factors, human activities and bacteria communities making breeding sites conducive for mosquito development and subsequently pathogen transmission. If these factors influence presence and distribution of mosquito vectors, then it is possible to design and/or implement vector control mechanisms in order to reduce

transmission of pathogens with no effective vaccines and treatment such as arboviruses and malaria.

1.3 Statement of the problem

High seroprevalence rates for Dengue, West Nile, Chikungunya and Rift Valley fever viruses have been reported in various parts of Kenya, including Lake the Victoria basin (Sutherland *et al.*, 2011). On the other hand, entomological surveys have successfully identified the presence of mosquito species of *Mansonia*, *Aedes*, *Anopheles*, and *Culex* genera to be present in the region. These vectors are associated with the transmission of various arboviruses (Alatoom and Payne, 2009; Mwangangi *et al.*, 2012), and their presence is an indication of ongoing arboviral activities that are leading to the high seroprevalence observed in humans. The high abundance and diversity of the vectors points to existence of a wide variety of microhabitats with possible variable conditions in which the immature stages of the vectors thrive. Controlling the vector is essential in preventing arbovirus circulation. However, lack of information on the larval ecology and human contribution towards their presence can hinder successful implementation of vector control programmes. Therefore, there is a need to understand microhabitats where the vectors breed, identify factors that influence vector site preference and determine how human activities affect distribution, abundance and diversity of the vectors.

1.4 Justification

This is a novel study in a malaria and arbovirus endemic area, where there are known vectors but limited knowledge of their distribution and ecology. The presence of diverse and large numbers of adult mosquitoes is a key indicator of the existence of aquatic habitats in which the immature

stages of these mosquitoes thrive. Conditions within these sites significantly determine fitness, abundance and dynamics of the adult vectors as well as how efficiently they can transmit pathogens. Thus, understanding these microhabitats and subsequently targeting these sites in control programmes can largely reduce adult numbers and or hinder their fitness. In addition, even though it is clear that the region's warm and wet climate is favoring the establishment of the vectors, it is not well understood how human activities, microbial communities and other environmental factors are fuelling arbovirus circulation through the establishment and distribution of the vectors. Understanding how human activities as well as conditions within microhabitats are influencing vector presence and distribution will provide crucial information for vector control in the management of arbovirus vectors in Lake Victoria region and basic knowledge on larvae ecology.

1.5 Research questions

1. Is there a difference in distribution of mosquito larvae microhabitats in Rusinga, Mfangano and Ngodhe islands?
2. Are there competent vectors of arboviruses and malaria in the region?
3. Do physico-chemical factors and human activities affect suitability of breeding sites for each genus present differently?
4. Do bacteria communities in microhabitats influence mosquito breeding patterns?

1.6 Hypotheses

1. There is a difference in microhabitat distribution between Rusinga, Mfangano and Ngodhe islands.

2. There exist competent arboviral vectors in the three islands.
3. Physico-chemical and human factors influence suitability of breeding sites for competent arboviral vectors in the islands differently.
4. Bacteria communities in larvae microhabitats influence mosquito breeding patterns.

1.7 Objectives

1.7.1 General objective

To characterize mosquito larvae microhabitats and their distribution in three islands of Lake Victoria.

1.7.2 Specific objectives

i. To determine the distribution of mosquito larvae microhabitats in Rusinga, Mfangano and Ngodhe islands.
ii. To identify competent vectors of arboviruses present and mosquito species distribution in the areas sampled.
iii. To determine physico-chemical factors influencing breeding pattern of each mosquito genus sampled.
iv. To identify bacteria communities in mosquito larvae microhabitats and determine their influence on microhabitat choice.

CHAPTER TWO: LITERATURE REVIEW

2.1 Arboviruses vectors in Kenya

Arboviruses refer to viruses transmitted biologically between vertebrate hosts by hematophagous arthropods. Their presence in Kenya dates back many years (Henderson *et al.*, 1970; Johnson *et al.*, 1980). Currently, circulation is evident across the country as witnessed by high antiviral immunoglobulin G antibodies in humans for Dengue, Sindbis, Usutu, RVF, Chikungunya, West Nile, and Yellow fever viruses (Mease *et al.*, 2011). Additionally, presence and high abundance of arbovirus vectors is well documented (Yuill, 1986). A recent study (Lutomiah *et al.*, 2013) revealed an even distribution of arbovirus mosquito vectors in Kenya, including Lake Victoria basin. According to their study, *Aedes ochraceus*, *Aedes circumluteolus*, *Mansonia africana* and *uniformis* implicated in the transmission of RVF, were sampled in the lake basin although the area has few incidences of RVF outbreaks. The study also recorded a high number of *Culex quinquefasciatus*, a major vector of West Nile virus. Other competent vectors associated with the transmission of arboviruses in this area include; *Anopheles (funestus* and *gambiae*) for O'nyong nyong (Lutwama *et al.*, 1999), *Aedes aegypti* for Dengue and Yellow fever (Sang and Dunster, 2001), and *Aedes africanus*, a vector of slyvatic YFV (Lutomiah *et al.*, 2013).

Mosquitoes pick viruses when taking blood meals from natural viraemic vertebrate hosts (LaBeaud *et al.*, 2011b). The viruses initially grow in the arthropod gut cells followed by distribution to salivary glands for further development. Transmission occurs when this infected mosquito vector feeds on uninfected vertebrate hosts (Weaver and Reisen, 2010) in subsequent blood meals. Once infected, the mosquito remains infective throughout its life time and, depending on the vector species, the virus may be transmitted transovarially to the next

generation (Artsob *et al.*, 2009) or venereally by invading reproductive organs. This manipulation of the host ensures continued existence of the virus in following generations.

2.2 Economic and medical importance of arboviruses

To humans, arbovirus infections result in febrile illnesses, life threatening encephalitis and/or fatal hemorrhagic fevers that end up with high mortality rates (Tandale *et al.*, 2009). Survivors of arbovirus infections experience chronic pain (De Andrade *et al.*, 2010) that progress to long term neural or physical impairment and death more than thirty months after infection (Labeaud *et al.*, 2011a). In animals like goats and sheep, arbovirus infections result in abortions and high mortality rates in new born offspring (Bird *et al.*, 2009). The high mortality translates to huge economic losses to farmers.

2.3 Arbovirus control

The most effective way of combating any disease is by prevention. This includes the use of vaccines and improving diagnostic tests for accurate detection. However, currently there are no commercially available vaccines for most of the arboviruses (CDC, 2005). Insect repellants and insecticide treated mosquito nets (ITNs) are the current methods deployed in the control of mosquitoes transmitting pathogens (CDC, 2005). However, use of nets by the recipients is not guaranteed. For instance, in the western part of Kenya, a greater disparity has been shown to exist between owning nets and their correct use (Atieli *et al.*, 2011a) thereby hindering prevention efforts. In addition, since some arbovirus vectors bite outdoors during the day, use of ITNs may not be effective. This has necessitated search of new and effective control strategies. Focus on immature stages of the vectors is a good option since their habitats determine fitness,

abundance and dynamics of the adult populations as well as how efficiently they can transmit pathogens. Trials of vector control using insecticides like *Bacillus thuringiensis* in the laboratory and in the field have shown some degree of efficacy in the control of the vector at larvae stage (Jeffery *et al.*, 2007). These results concur with findings obtained from the use of microbial larvicides for malaria vector control which resulted in significant decrease of malaria incidences by Odds Ratio (OR) of 0.44 (Fillinger *et al.*, 2009). This was due to a 98% reduction of adult *An. gambiae* mosquitoes in larvicide treated sites. Similar success was achieved for larvae of *Ae. aegypti*, a major vector of dengue and yellow fever viruses from experiments done in a laboratory setting. Larvae of this species showed high mortality rates when plant extracts with larvicidal effects were applied on them (Langat *et al.*, 2012). Therefore, vector control on early stages of development can effectively manage arbovirus vectors. However, this requires a deeper understanding of the ecology of the vectors especially in their natural habitats.

2.4 Mosquito larvae microhabitats

2.4.1 Types of larval microhabitats and their importance

Mosquitoes are holometabolous insects with four distinct life stages i.e. egg, larva, pupa and adult. The first three (immature stages) develop in aquatic or moist microhabitats which range from small to large, clean to dirty, or fresh water to salty water depending on species. These habitats can be classified as temporary or permanent based on how long they are stable, man-made or natural based on the forces behind their formation. Animal hoof prints, rainwater pools, tire tracks, puddles, tree holes, water storage containers, rock pools, discarded containers and tires, pits, canals, ponds, river banks, ocean and lake shores are some of the commonly identified microhabitats (Imbahale *et al.*, 2011; Mwangangi *et al.*, 2008; Oyewole *et al.*, 2009). Manmade

habitats arise from human activities such as cultivation, construction and deforestation that create structures for holding water thus have the potential of becoming mosquito microhabitats. For example, rice cultivation in Mwea generates stagnant water bodies in which anopheles mosquito breed, this influences the transmission patterns of malaria in the region (Muriu *et al.*, 2008).

Abundance and distribution of mosquito larvae in a habitat indicates oviposition preferences of gravid adult female mosquitoes for that particular site. Mosquito larvae presence in a specific habitat provides evidence of their ability to adapt and survive in the conditions present in that particular habitat (Mwangangi *et al.*, 2007b), while their absence indicates inability to survive in a particular site due to unsuitable conditions. Suitability of a site therefore has a direct determination on larvae diversity and abundance hence population dynamics of adult mosquitoes in that particular and nearby places.

2.4.2 Factors influencing larvae habitat selection

Both abiotic and biotic factors influence the productivity of a microhabitat as well as the type of species a microhabitat can host as reported by findings of various studies.

a) Abiotic factors

Both physical and chemical factors have been associated with the choice of ovipositing site. According to, Gardner *et al.* (2013) aquatic ammonia and nitrate positively correlate with larvae abundance whereas pH has a negative correlation. Another study done at the Kenyan coast reported that chlorophyll, which indicates presence of phytoplankton in a habitat, a source of food for mosquito larvae, positively correlates with the wing length of *An. gambiae* adults that

emerge afterwards (Mwangangi *et al.*, 2007a). In a study done in western Kenya, Minakawa *et al.* (1999) identified canopy coverage and amount of debris in a microhabitat to positively correlate with culicine larvae. They also noted that topography and land cover types influence breeding behavior of anopheles mosquitoes. They found out that areas of higher elevation experienced lower numbers of larvae microhabitats compared to lowlands. This situation was more common during the dry seasons than the rainy seasons (Minakawa *et al.*, 2005). Whereas most species select permanent microhabitats to ensure that they fully complete their lifecycle, temporary microhabitats are more likely to be free of invertebrate and vertebrate predators which increase chances of survival (Mercer *et al.*, 2005). Furthermore, these sites are replenished periodically with dissolved nutrients from surface run-off, especially after the rains. Such nutrients are important as they support growth of algae and phytoplankton essential as food for the immature stages of mosquito.

Total salt concentration in water determines water salinity and conductivity which influence the type of mosquito species that can be hosted at a microhabitat (Roberts and Irving-Bell, 1997). Other environmental factors that affect larvae survival, growth rate, pupation age, and adult size are temperature, humidity, light and larval density (Bayoh and Lindsay, 2004). Warm temperatures of about 28-30°C have been shown to provide optimal growth conditions for most species (Robert *et al.*, 1998). However, it is reported that some species within the *Anopheles* genus can do well at temperatures as high as 35°C (Bayoh and Lindsay, 2003). Conditions in the same habitat may vary from time to time. These induce changes in water quality of the habitat from time to time. This therefore suggests that different species can utilize the same habitat for

breeding at different times. In conclusion, water quality influences egg hatching, pupation and adult emergence processes.

b) Biotic factors

Co-existence of organisms in the same habitat normally results in enhancement or destruction of the members through processes related to symbiosis, predation and competition. In larval microhabitats, predators such as tadpoles, fish and dragon fly larvae are responsible for the absence or low numbers of larvae (Willems *et al.*, 2005). In addition, larvae of *Toxorhynchite* mosquitoes have been reported to prey on larvae of other mosquitoes thereby influencing their abundance in a site (Jones and Schreiber, 1994). The carnivorous nature of these mosquitoes made them attractive for use as biological control agents of harmful mosquitoes. Besides, Intra- and interspecific competition among mosquito larvae significantly determines the fitness of adult mosquitoes that emerge from the site. Different vegetation types surrounding aquatic sites have also been reported to have varying effects on larval abundance depending on their height, density or flowering periods (Gardner *et al.*, 2013).

Bacteria that play important roles in the development of larvae naturally colonize mosquito aquatic habitats. These habitats also act as a source of symbiotic bacteria found in guts of adult mosquitoes. Several bacteria, mostly from proteobacteria, firmicutes and actinobacteria phyla have been isolated from mosquito larvae microhabitats. These include, *Pantoea stewartii*, *Acinetobacter* sp, *Pseudomonas* sp., *Bacillus* sp., *Exiguobacterium* sp., *Micrococcus* sp., *Proteus* sp., *Rhodococcus* sp., *Paenibacillus* sp. and *Comamonas* sp. (Sumba *et al.*, 2004; Lindh *et al.*, 2008b; Minard *et al.*, 2013a).

2.5 Microbes as a source of food and semiochemicals

The importance of bacteria in larval microhabitats cannot be under-estimated. Their presence significantly influences mosquito species abundance and diversity (Rejmankova *et al.*, 1996; Ponnusamy *et al.*, 2010). They accomplish this in several ways. To begin with, they act as a source of nutrition for the developing larvae (Merritt *et al.*, 1992; Wotton *et al.*, 1997). Therefore, a habitat that is rich in bacteria supports a higher number of larvae due to minimal competition. Secondly, bacteria breakdown complex organic matter into simpler molecules that can be easily assimilated by mosquito larvae (Reiter *et al.*, 1991). Lastly, during this breakdown and other metabolic processes, bacteria release volatile and non-volatile compounds that act as semiochemicals in attracting or repelling mosquitoes. To demonstrate importance of bacteria as semiochemicals, Sumba *et al.* (2004) set up experiments where they allowed gravid female *An. gambiae* mosquitoes to choose ovipositing sites between sterile and non-sterile microhabitat material. As hypothesized, unsterilized microhabitat soils were more attractive to ovipositing female *An. gambiae* mosquitoes than sterilized soils.

Chouaia *et al.* (2012) also stressed the critical role bacteria play in mosquito development. In their study, they demonstrated the beneficial role *Asaia* sp (acetic acid bacteria) play on the development of *An. stephensi* mosquito larvae. When larvae were treated with rifampicin antibiotic, they experienced delayed developmental rates compared to larvae that had high levels of *Asaia* bacteria symbionts. This symbiont dominates gut, salivary glands and reproductive system microflora of both larvae and adults of *An. stephensi* (Animut *et al.*, 2012), which explains their significance to the host.

Additionally, human skin microflora indirectly influences disease transmission by dictating attractiveness of individuals to vectors. By breaking down chemicals such as sweat on human skin, they produce various volatile compounds that either attract or repel vectors to that individual(Verhulst *et al.*, 2010; Verhulst *et al.*, 2011).

The positive association observed between bacteria and mosquitoes prompted researchers to investigate further the compounds produced by these bacteria. Compounds from Proteobacteria (Alpha, beta, delta and gamma), Firmicutes, Bacteriodetes and Cyanobacteria phyla were detected. In most cases, more than one compound was detected as being released at the same time by bacteria implying that these compounds do not function in isolation rather they act synergistically. Compounds identified include, carboxylic acids, alcohols (3-methyl-1-butanol, 2-phenylethanol), Indole, CO_2 and methyl esters (Rejmankova *et al.*, 2005; Lindh *et al.*, 2008b; Ponnusamy *et al.*, 2008; Ponnusamy *et al.*, 2010). Receptors for some of the compounds such as Indole have been found in *An. gambiae* showing that the insect perceives these compounds (Biessmann *et al.*, 2010).

2.6 Methods for characterization of microorganisms in microenvironments

The conventional way of classifying and identifying bacteria is by culturing. However, various studies have revealed that this is not an exhaustive technique of telling bacteria composition in the same sample. To begin with, bacteria of different species require different culture periods, media nutrients and incubation temperatures to grow. In their study, Kopke *et al.* (2005) showed that if the same sample is cultured under different nutrient concentrations, varying pH, temperature and oxygen levels, different bacteria types were isolated. In addition, it is difficult to

replicate in culture natural systems such as symbiotic relationships that exists in most bacteria colonies.

Due to these limitations, researchers are opting for molecular methods to complement existing culture methods in order to expand number of bacteria that can be identified in complex samples. These methods exploit the 16S rDNA gene as a marker (Rajendhran and Gunasekaran, 2011) which although well conserved has highly variable regions that can distinguish almost all bacteria species. These methods include cloning, probing, temperature gradient gel electrophoresis (TGGE), denaturing gradient gel electrophoresis (DGGE).

TGGE and DGGE use temperature and denaturing reagents respectively to separate amplified 16S rDNA fragments of the same length based on differences of their sequences rather than size. During a gradient electrophoresis, DNA is partially melted which affects its mobility and in the process becomes immobilized within the gel (Rosenbaum and Riesner, 1987). A mixture of DNA fragments with different sequences melt at different points of the gradient and this is the basis of separation. To ensure that dsDNA does not melt completely, a GC-clamp is added to one of the primers used to amplify the 16s rDNA fragment that is to be separated. Size of separated bands can be identified with the use of ladder containing a cocktail of DNA segments of known bacteria species with different and known migration rates. Alternatively, they can be isolated and sequenced to identify the bacteria. The labour intensive, time consuming and less sensitive nature of these methods renders them poorly suited for analyzing samples with complex microbial communities. As a result, their usage has been on a decrease in the past 2 decades (Muyzer and Smalla, 1998).

Because of these limitations, sequencing is increasingly becoming a method of choice for bacteria identification. Sequencing of 16S rDNA accurately identifies bacteria even at very low concentrations. The technique is also efficient in metagenomics for samples containing, rare bacteria, slow-growing bacteria, and uncultivable bacteria. General bacteria primers are used to amplify all bacteria present in a sample before sequencing machines can read the precise and accurate order of nucleotides in the DNA molecules amplified.

2.7 Mosquito identification

Different methods exist for identifying organisms. These include morphological as well as molecular identification techniques. Adult mosquitoes can be identified up to species level based on their morphological characteristics, resting position, while their larvae can be identified by swimming movements (Strickman, 1989), resting position below the water surface, breathing organs and other morphological characters (WHO, 2003). Whereas anopheline larvae do not have posterior breathing siphons and rest parallel to the water surface, culicine larvae rest at an angle from the water surface and possess breathing siphons. However, identification up to species level with morphological features requires experienced taxonomists who can correctly discriminate features that are almost similar in different species. Nevertheless, molecular techniques can correctly discriminate very similar species even when used with inexperienced taxonomists.

Polymerase Chain Reaction (PCR), sequencing and Restriction Fragment Length Polymorphism (RFLP) of Internal Transcribed Spacer (ITS) marker are some of the molecular methods that have been used to identify mosquito species (Beebe *et al.*, 2002). Other markers used in

molecular identification of mosquitoes include, Cytochrome Oxidase (*COI*) and Nitrogen Deoxylase (*ND1-4*). New analytical methods such as High Resolution Melting (HRM) analyses have been developed to identify species up to genotype level. The method identifies species or genotypes based on the strength of hydrogen bonds between DNA strands as determined by their DNA sequence. Using HRM, members of *An. funestus* species have been successfully identified (Vezenegho *et al.*, 2009). This technique is cheap, fast and less tedious.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study site

The study was conducted on three inhabited islands of Lake Victoria (Ngodhe, Rusinga and Mfangano) in Homa Bay County (Figure 1). Among the three, Mfangano island (~00°28′S, 34°1′E) is the largest (66 km^2) with a population of 26,120 followed by Rusinga (~00°24′S, 34°1′E, 43.8 km^2) with a population of 24,275 as per 2009 national census (KNBS, 2010). Ngodhe Island (~00°21′S, 34°11′E) although small, it is inhabited but no statistics exist as per 2009 census report. These Islands and areas along the Lakeshore are characterized by warm and wet weather conditions and experience both long (March to June) and short (October to December) rain periods.

Among the three, Ngodhe Island has a steep landscape compared to Rusinga that is relatively flat and Mfangano that combines landscape features of the two. Rusinga is easily accessible to the main land as it is connected to the mainland via a man-made, land-filled causeway while accessibility to the others is mainly via boats or ferry services. Ngodhe and Rusinga Islands are closer to each other (~5 km apart) while the distance between Mfangano and the two ranges from 11 to 16 km. Fishing is the main activity of the people in these Islands. However, agriculture and small-scale livestock farming are also important economic activities in the area. Since fishing occurs mainly at night, people in the region are exposed to bites from a variety of both nocturnal and diurnal mosquito species. The islands also act as a home to a variety of bird species that move freely between islands and the mainland as well as a nearby national park.

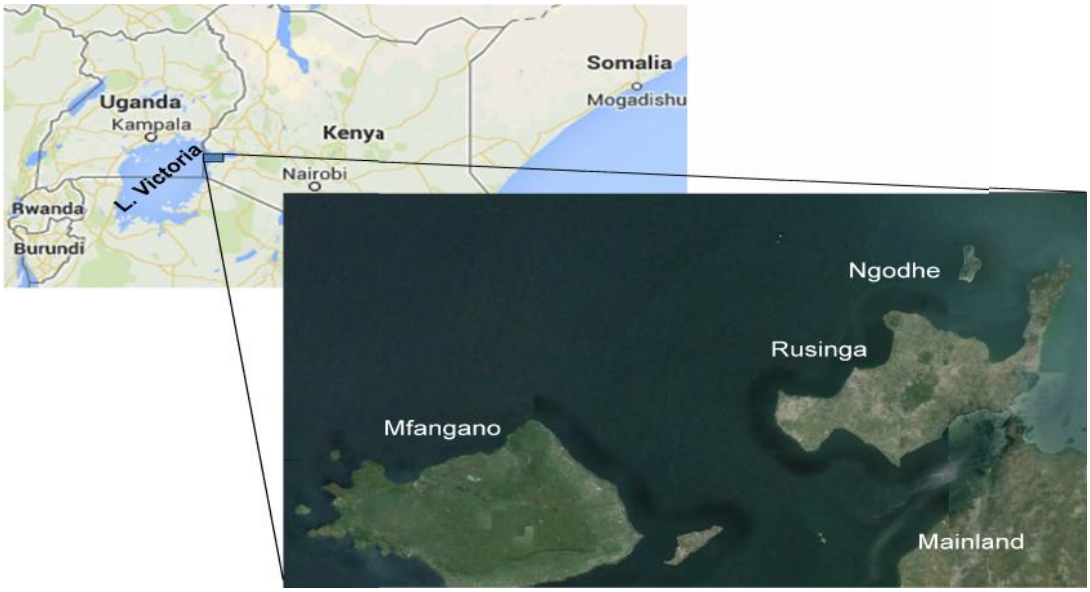

Figure 3.1: Map of the study area (Google maps 2013). Mfangano is the largest followed by Rusinga and Ngodhe, which are close to the mainland.

3.2 Vectors present, mosquito and microhabitat distribution

3.2.1 Sampling of immature mosquitoes

A sampling site/microhabitat was defined as an aquatic environment that hosts immature life stages of mosquitoes namely eggs, larvae instars and pupae. To ensure unbiased sampling, 32 sites selected from representative locations of the study sites were enrolled in the study. Selected sites were also reflective of defined categories (whether they were found in Lakeshore, drainage, pit, unused vehicle tires, ponds, puddles, swamps, boat, rock pool and tree hole). Distribution of habitats was analyzed based on its location, circumstances behind microhabitat existence, as well as category into which it belongs (Lakeshores, boats, tires etc). Sampling from the habitats was done using standard dippers (Bioquip, USA). Three to five dips were made per site. Larvae, pupae or eggs collected were transferred with some of the habitat water into perforated plastic

bottles and labeled with site name, date and time of collection. Using a Global Positioning System (GPS) device (Garmin, USA) and a camera, geo-reference data of the environment around each sampling site was recorded. Samples were then transported to an artificial insectary at International centre of insect physiology and ecology (*icipe*) Thomas Odhiambo field station in Mbita. The larvae were later transported to Duduville campus in Nairobi for final rearing, identification and processing. Sampling was carried out during the long and short rain seasons.

3.2.2 Larval rearing

In the insectary, larvae, pupae and eggs from the field were transferred into clean plastic troughs and reared in cages in their natural microhabitat water until they all emerged. Larvae were fed with ground Tetramin® fish food (Tetra, Germany) (Diallo *et al.*, 2012) until they pupated. For optimal development, temperature and humidity was maintained at 26-27°C and 80% respectively (American Mosquito Control Association, 1979). The imagines were aspirated from the cages and preserved in 2 ml micro centrifuge tubes at -80°C freezer awaiting morphological identification.

3.2.3 Identification of mosquitoes

Morphological identification keys (Edwards, 1941; Gillett and Coetzee 1987) were used to identify emerged mosquitoes to species level. Genomic DNA was extracted from a pair of mosquito legs using hotshot DNA extraction protocol (Montero-Pau *et al.*, 2008). Briefly, using 0.2 ml tubes, the tissues were incubated in a thermocycler for 40 minutes at 98°C with 30 µl of the alkaline lysis reagent (25 mM NaOH, 0.2 mM Na_2EDTA and distilled water, pH 12). The mixture was then transferred to 4°C for 5 minutes to cool down before centrifugation for 30

seconds and addition of 30 µl of neutralization buffer (40 mM Tris-HCl, pH 5). The mixture was vortexed slowly for one minute and stored at 4°C for short term (less than one week) or -20°C for long-term (more than a week). 1-3 µl of this solution were used as template for PCR.

LCO 1490 Forward and TLN2-3014 Reverse primers (Appendix I) were used to amplify long Cytochrome Oxidase I (*COI*) gene (~1700bp), a common and reliable marker used for insect identification and classification (Aly, 2014; Lunt *et al.*, 1996). A 20-µl PCR reaction was set up consisting of 2 µl of 50ng DNA template, 4 µl buffer, 0.4 µl of 10 mM dNTP mix, 0.5 µl of 10 mM of each of the primers, 0.2 µl of 0.5u Phusion polymerase and the rest PCR grade water. Forty cycles of PCR reaction were run using Bio-Rad thermo cycler (Biometra analytic, Jena, Germany) PCR machine. The following conditions were used; 98°C initial denaturation for 2 minutes, 98°C denaturation for 20 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 1 minute 30 seconds. PCR product was resolved on 1.2% agarose gel stained with ethidium bromide.

The PCR product (5 µl) was cleaned enzymatically with 2 µl of EXOSAP IT (Affymetrix Inc., USA) to eliminate unconsumed dNTPs and primers before Sanger sequencing. Sequences for the *COI* gene were visually inspected and curated using Geneious version 6.1.6 (Biomatters, http://www.geneious.com/). Similarity searching of the non-redundant nucleotide database was performed using BLAST (Altschul *et al.*, 1990) to confirm morphological identification done on mosquitoes.

3.2.4 Genetic variation between populations

Due to high costs of sequencing, representative sequences for *Culex pipiens, Aedes aegypti* and *Anopheles gambiae* were selected from different populations in the three Islands and their long *COI* gene (1700bp) sequenced. These sequences were used to construct a phylogenetic tree to determine how close genetically the different populations and compare evolutionary history of mosquitoes caught from different sites. Mega 5 program (Tamura *et al.,* 2011) was used to construct the maximum likelihood phylogenetic tree with 1000 bootstrap replications under different models.

3.3 Physico-chemical parameters associated with mosquito breeding sites

After sampling mosquito larvae from a site, its physico-chemical parameters were recorded *in situ*. Water conductivity, temperature, total dissolved solids (TDS), pH, nitrogen, ammonium (NH_4^+), nitrate (NO_3^-), and salinity were measured using the YSI professional plus multiparameter water meter (YSI, USA) while copper (Cu), phosphate (PO_4^{3-}), sulphate, and fluoride ions were measured using 9800 photometer. Measurements were done in triplicate and the mean was used for analysis. Principal coordinate analysis (PCoA) based on all measured variables was used to determine if sites hosting same species have similar physico-chemical parameters.

Multi-dimensional scaling method (Redundancy Analysis - RDA) (Kindt and Coe, 2005) was used to visualize correlations between mosquito genera and physico-chemical variables as well as associations between individual physico-chemical variables. In RDA, dependent variables (mosquito genera in this case) were modeled as functions of independent variables (physico-

chemical parameters) to produce correlation triplot (scaling two). In this triplot, correlation amongst two variables in consideration was determined by the respective angle between their vectors. Therefore, two variables with a small angle between them have a strong positive correlation, while those with an angle of 90° or 270° have no correlation among them and those with an angle of 180°C between them have a strong negative correlation between them. Type and significance of these correlations were determined spearman's correlation.

3.4 Bacterial community profiling

3.4.1 Water and sediment sampling

Water and sediments samples for bacteria community analysis were collected from 10 of the 32 sites where mosquito's immature stages (3.2.1 above) had been sampled and transferred into sterile falcon tubes. Five additional sites, 3 from the mainland in Mbita and 2 from adjacent Islands (Ringiti and Takawiri), were included in the study for comparison purposes. Selection of the 15 sites for bacteria profiling was based on the species of mosquito they hosted as well as category of the site i.e. if its location was on the Lakeshore, boat, rock pool and tree hole. The water and sediment samples were transported to the laboratory in a cool box and stored at 4°C awaiting further analyses.

3.4.2 Extraction of DNA from water and sediment samples

Samples from microhabitats (3.4.1 above) were centrifuged at maximum speed. The pellets were resuspended and washed three times in phosphate buffered saline (PBS). Using Fast DNA spin kit for soil (MP biomedical, USA), total genomic DNA was extracted following manufacturer's instructions. This DNA was used as template for PCRs for TGGE and 454 sequencing.

3.4.3 Bacterial profiling by PCR and TGGE

Extracted DNA was diluted in the ratio of 1:20 before it was used for PCR. Using universal bacteria primers i.e. EUB_933 Forward (attached to a 40-nucleotide GC sequence) and EUB_1387 Reverse primers (Appendix I), a fragment of approximately 450bp of the 16S rDNA gene was amplified. The 12.5 µl PCR reaction contained; 1 µl of VWR Taq polymerase, 1.25 µl taq polymerase buffer, 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.5 µM of each primer, 1µl of 50ng template and the rest PCR grade H_2O. Forty cycles of a touch down PCR reaction were run using Thermo cycler-T-Professional gradient 96 PCR machine (Biometra analytic, Jena, Germany). The following conditions were used; 94°C initial denaturation for 3 minutes, 20 cycles with each comprising of 94°C denaturation for 40 seconds, annealing of 68°C for 40 seconds and extension of 72°C for 40 seconds. This was followed by 20 cycles with similar conditions but with annealing temperature of 58°C. A final elongation of 10 minutes at 72°C was used. PCR products were resolved in a 1.2% agarose gel stained with gel green dye for 30 minutes at a voltage of 130V and then viewed under UV light.

Samples whose amplicons were about 450bp long were further analyzed by TGGE to determine variation in bacteria community within between samples. Briefly, using the TGGE MAXI system (Germany) with a temperature gradient of 40-55°C, the PCR products were resolved for 18 hours at 150V. Silver staining was used to visualize community profiles as resolved on the gel. Bands from the gels were cut out and dissolved in low TE buffer overnight. One micro liter of this solution was used for PCR with same conditions and reagents as described above. However, EUB_933 Forward primer without the GC clamp was used. The PCR products were purified using InnuPREP PCR pure kit (analytikjena, German). 25 ng/µl of the purified product was

Sanger sequenced. The sequences were curated using Geneious software. The closest database sequences were retrieved using NCBI blastn (www.ncbi.nlm.nih.gov/blast/) and RDPII (http://rdp.cme.msu.edu). Representative samples were selected for 454 sequencing. Selection criteria was based on species abundance obtained from the site and category of the sampling site.

3.4.4 Bacteria profiling by 454 sequencing

Bacterial 16S region was amplified by PCR using 16S universal eubacteria primers (Gray 28F and Gray 519R-Appendix I) with a sample specific 8-mer barcode DNA sequence, and HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA). The following conditions were used, 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds; 53°C for 40 seconds and 72°C for 1 minute; after which a final elongation step at 72°C for 5 minutes was performed. Following PCR, all the bar-coded amplicons from different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a Roche 454 FLX titanium instruments and reagents following manufacturer's guidelines. Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso *et al.*, 2010) was used to analyze sequences and determine alpha diversity (diversity within a sample) as well as beta diversity (diversity between the samples) respectively. To enable comparison of bacteria composition, OTU tables of all taxonomic levels tables were generated by QIIME software.

CHAPTER FOUR: RESULTS

4.1 Microhabitat distribution

Mfangano Island recorded the highest number of microhabitats of mosquito larvae among the three Islands (Figure 4.1a and 4.1b-i). Most of the microhabitats sampled were located along the lakeshore, followed by pools inside unused fishing/transport boats, drainage systems and rivers respectively (Figure 4.1b-iii). Other areas sampled include rock pools, swamps, tree holes, ponds, discarded containers and tires, water tanks, pit holes, pools, puddles and animal footprints (Figure 4.1c). Of these, sites on lakeshores, tree holes, riverbanks, discarded containers, water tanks, ponds and swamps were mostly stable while puddles, pools, unused tires, roadside drainages and animal footprints were temporary. Both human and natural factors were responsible for the existence of these microhabitats (Figure 4.1b-ii and 4.1c).

Figure 4.1a: Map of sampling sites in the three islands (Google map, 2014). Sampling points on map are based on their GPS coordinates. Mfangano Island has the highest number of microhabitats followed by Rusinga and Ngodhe respectively.

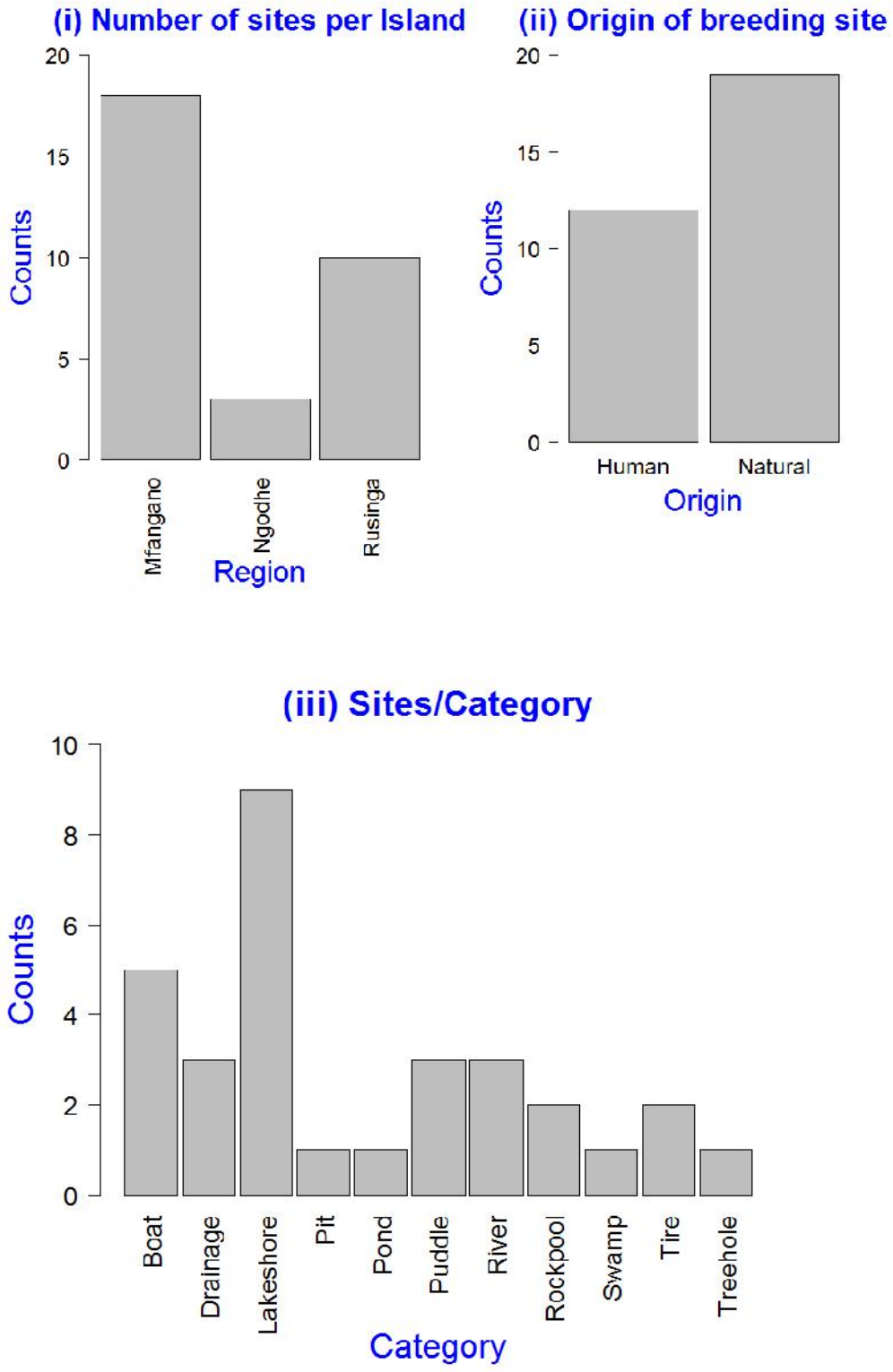

Figure 4.1b: Microhabitat distribution. (i) Number of microhabitats sampled from each island (ii) Frequency of microhabitats based on whether their existence was due to human or natural activities (iii) microhabitat distribution by category.

Figure 4.1c: Some of the microhabitats where mosquito larvae were sampled. Existence of these microhabitats was because of unmonitored human activities as well as natural factors.

4.2 Vector presence and mosquito species distribution

4.2.1 Mosquito distribution

A total of 1,178 mosquito larvae were successfully reared to adults for both May and November sampling periods. Table 4.1 shows a breakdown of this figure by genera for each category sampled.

Table 4.1. Number of mosquitoes sampled per category from each of the three islands in May and November 2012

		May-12			**Nov-12**		
		May 2012			November 2012		
Site	Category	*Anopheles*	*Aedes*	*Culex*	*Anopheles*	*Aedes*	*Culex*
Ngodhe	Lakeshore	9	0	48	2	0	32
Rusinga	Lakeshore	51	0	45	9	35	172
	Puddle	0	0	0	28	4	0
	Pond	0	0	0	3	0	15
	Boat	0	0	0	3	0	1
	Pit	0	25	55	1	0	119
	Drainage	4	0	0	0	0	9
Mfangano	Swamp	0	0	0	1	0	7
	Lakeshore	1	0	1	14	0	3
	Puddle	0	0	0	4	0	4
	River	2	0	13	0	0	15
	Rockpool	0	0	0	0	52	4
	Tree hole	0	0	0	0	54	0
	Drainage	25	0	7	4	0	4
	Tire	0	69	0	0	57	12
	Tank	1	8	18	0	0	3
	Boat	0	0	0	0	1	10
	Discarded containers	0	112	2	0	0	0

According to table 4.1, there was variation in the microhabitats occupied by different species. Whereas mosquito species of the *Aedes* genus dominated rock pools, tires, tree holes, discarded containers and tanks, those of *Culex* genus were found in pits, swamps and lakeshores. Members

of the *Anopheles* genus on the other hand were present in temporary drainages, puddles and lakeshores. In most cases, only one species dominated a microhabitat. However, some species of either the same or different genera were found to co-exist in the same microhabitat at the same time. For instance, *Culex pipiens* was mostly found sharing a microhabitat with species of the same genus e.g. *Culex watti* and *Culex univittatus*. On the other hand, *Aedes aegypti* was also found co-existing in same habitats with *Aedes metallicus, Aedes luteocephalus* and *Aedes vittattus*. In a few cases, two species from different genera e.g. *Anopheles* and *Culex* were found sharing the same microhabitat.

Details of species composition for every Island are shown in figure 4.2a. More mosquito species were present in Mfangano Island that also had a high number of *Aedes* species (*Ae. aegypti, Ae. metallicus, Ae. vittattus* and *Ae. Luteocephalus*). *Culex pipiens* mosquitoes dominated both Rusinga and Ngodhe Islands. Overall, more mosquitoes were sampled in November than in May (Figure 4.2a and Table 4.1). Both sampling periods experienced a higher number of *Culicine* mosquitoes as compared to *Anopheline* mosquitoes. However, there was an increase in the numbers of Culicines in November as compared to May. On the contrary, the number of *Anophelines* decreased for the same period.

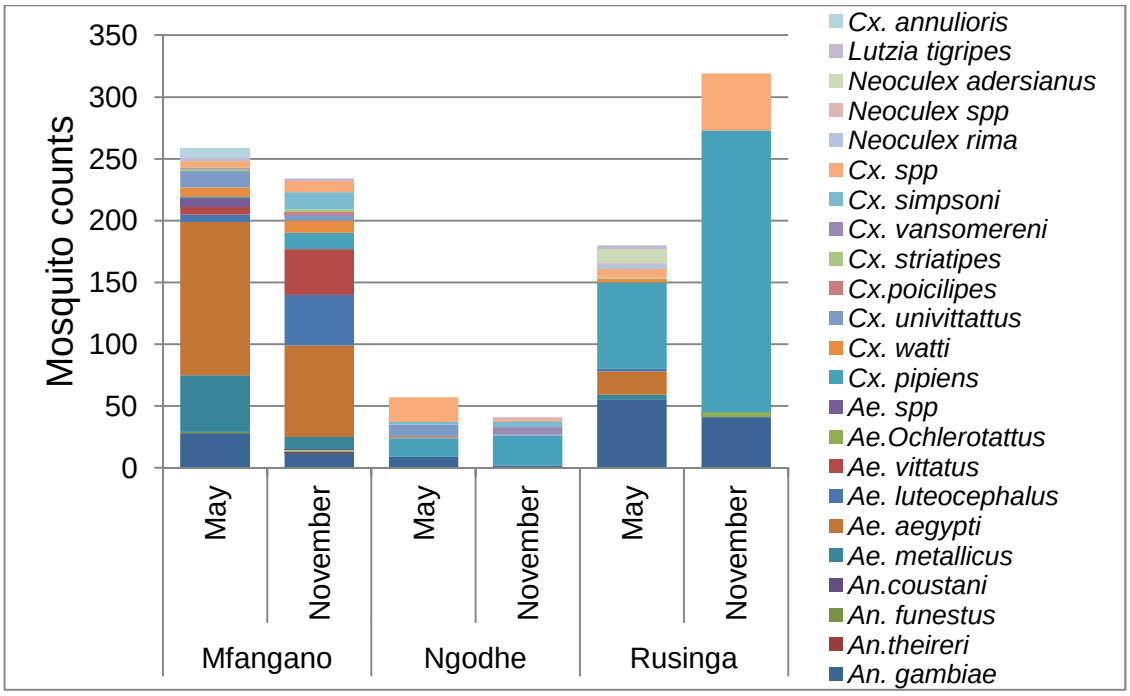

Figure 4.2a: Mosquito species composition for the three Islands. Mfangano had higher number of *Aedes* mosquito than Rusinga and Ngodhe that were majorly inhabited by *Culex* mosquitoes. Mfangano also recorded many mosquito species than the other two.

4.2.2 Vectors present

Out of the 21 species present (Figure 4.2a), about six of them are reported competent vectors of pathogens for arboviral infections and malaria (Table 4.2)

Table 4.2 Known competent mosquito vectors sampled from the three islands in this study with the pathogens they transmit as reported by various studies done in Kenya.

Mosquito vector	Virus	Reporting studies
Aedes aegypti	Flavivirus	Ochieng et al., 2013
Culex pipiens	Usutu, WNV, Sindbis	Jost et al., 2010, Lutomiah et al., 2011
Culex univittatus	West Nile virus (WNV)	Lutomiah et al. ,2011, Ochieng et al.,2013
Anopheles gambiae	O'nyong nyong	Kasili et al., 2009
Anopheles funestus	Ngari viruses	Ochieng et al., 2013
Culex species	Sindbis	Ochieng et al., 2013
Culex vansomereni	WNV	Lutomiah et al., 2011

4.2.3 Genetic drift between populations

There was no genetic variation between populations sampled. From the maximum likelihood tree (Figure 4.2b), individuals of the same species but from different islands show high genetic similarity due to high bootstrap values.

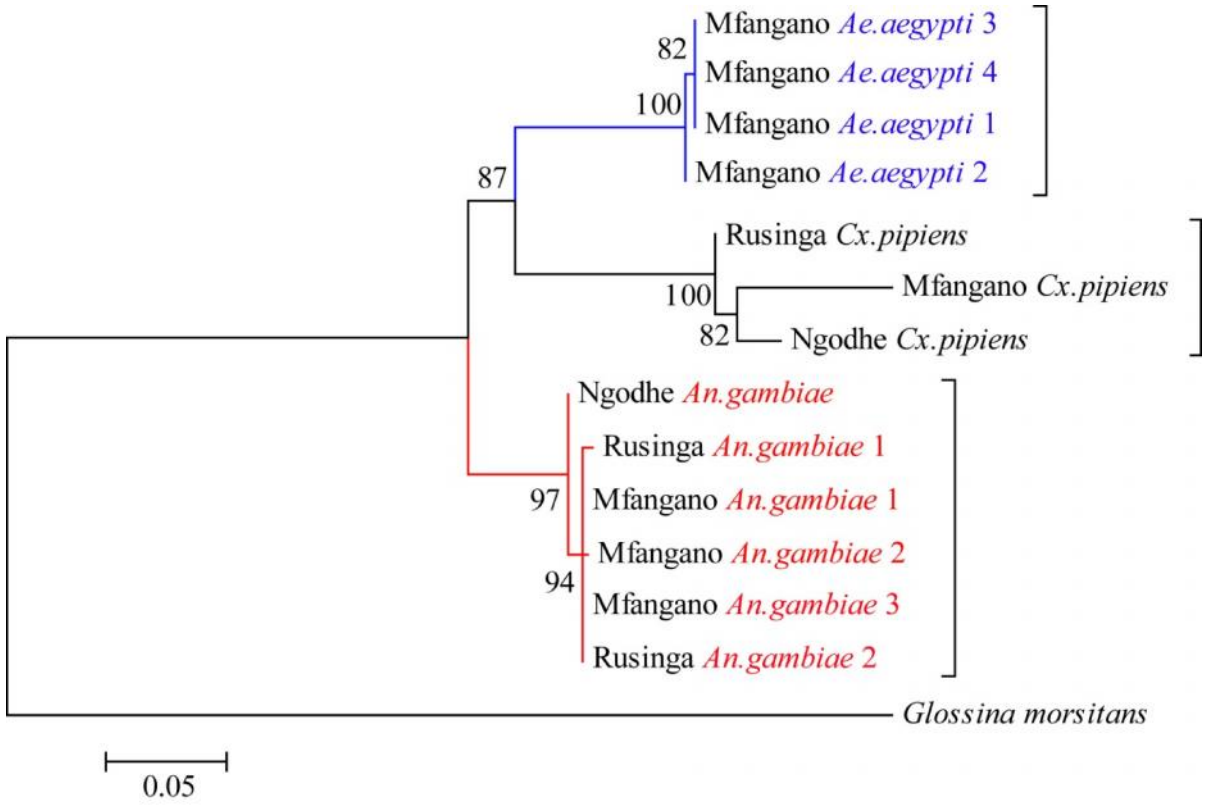

Figure 4.2b: Phylogenetic analysis of three vectors found in the three islands. Similar species from different regions cluster together due to their close genetic similarity (high bootstrap values). This maximum likelihood tree is based on *COI* gene

4.3 Physico-chemical characteristics of water in mosquito breeding sites

There was variation in microhabitat conditions between sites. For instance, phosphate concentrations were higher in rock pools, tires and tree holes while boats had alkaline pH values. Data for all measured variables is attached in Appendix IV. PCoA based on all measured variables showed that sites hosting similar genus do not necessarily cluster together (red colored

sites on row dendogram figure 4.3a). This suggests that not all variables measured were important in microhabitat selection. PCoA done on mosquitoes collected from all sites revealed that there is a high probability of the species in the *Culex* genus sharing habitats with those of the *Anopheles* genus than *Aedes* genus (column dendogram Figure 4.3a).

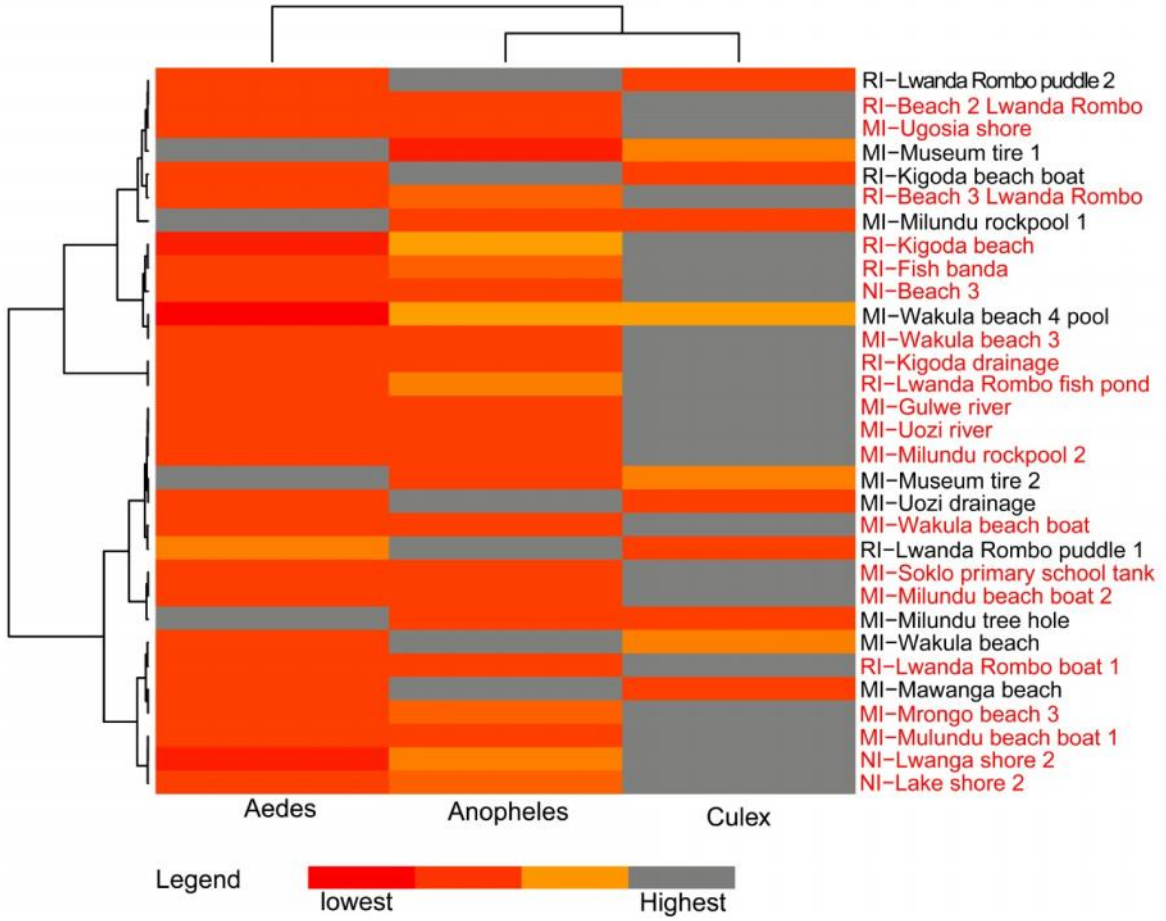

Figure 4.3a: Heat map of mosquito collection and PCoA of sampling sites based on all variables measured. Sites hosting similar mosquito genus do not necessarily cluster together e.g. sites where *Culex* was the major genus (colored red). There is a high probability of *Culex* sp sharing habitats with those of the *Anopheles* sp than *Aedes* sp (*Culex* and *Anopheles* cluster together column dendogram). Different row colors represent number of mosquitoes collected for each genus in the same site (red=lowest, grey=highest).

4.3.1 Correlation of mosquito genera with the physico-chemical parameters

From the triplot (Figure 4.3b), there was no correlation between any of the three genera (Angle between them is ~90°/270°) i.e. the genera are independent of each other, although there is a high probability of *Culex* and *Anopheles* occurring together than with *Aedes* genus. However, correlation was observed between some of the variables investigated in each of the three genera. Ammonium, phosphate and fluoride had a positive correlation among themselves and with *Aedes* spp (angle between them is <45°) while nitrates, dissolved oxygen, pH and temperature had a positive correlation amongst themselves and with *Anopheles* spp. On the other hand, ORP, free copper, TDS, salinity, resistivity and conductivity had a positive correlation with each other and with *Culex* species.

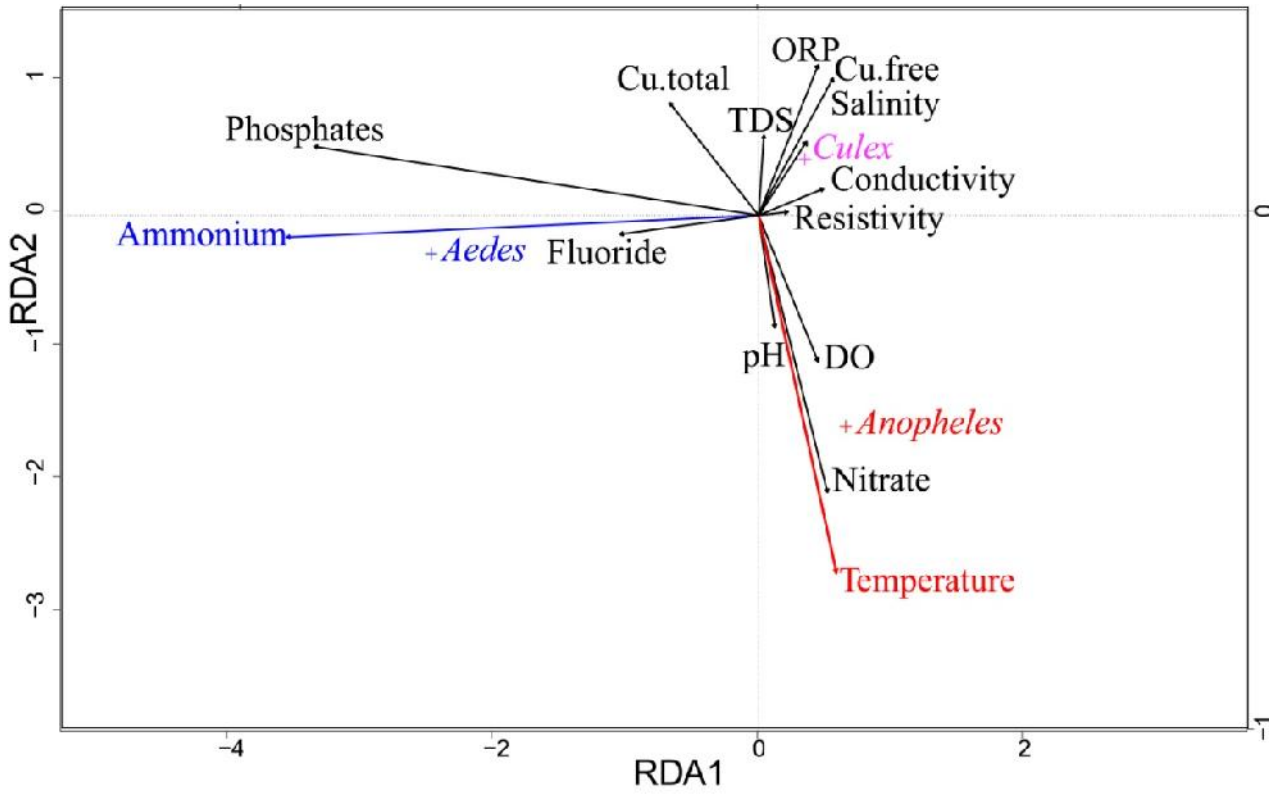

Figure 4.3b: RDA Correlation triplot of mosquito genera and environmental variables. Angle between two variables explains their correlation (small angle = positive correlation,

90° or 270° = no correlation and 180° = negative correlation). *Anopheles* larvae are positively correlated with temperature, DO, and NO_3^-, while *Aedes* larvae are positively correlated with Fluoride, Conductivity, TDS, Salinity, PO_4^- and NH_4^+. *Culex* larval presence was positively correlated with free copper and ORP. Matching colors indicate statistically significant correlations between specific mosquito genera and physico-chemical variables as determined by spearman's correlation test. (PO_4^- = Phosphate, NH_4^+ = Ammonium, DO = Dissolved oxygen, NO_3^- =Nitrate, Cu=Copper, ORP=Oxidation reduction potential).

4.3.2 Significance of correlations observed in RDA analysis

Spearman's correlation test was done to determine significance of correlations observed in RDA analysis. Of the three variables that showed positive correlations with *Aedes* and with each other in RDA (Figure 4.3b), only Ammonium was predictive of *Aedes* larval presence (r_S = 0.4961, df = 30, P = 0.0235), whereas Phosphate (r_S = 0.3094, df = 30, P = 0.0849) and Fluoride (r_S = 0.0920, df = 30, P = 0.6162) were not significant at a p-value of 0.005. Presence of *Anopheles* larvae, in turn, correlated significantly with increasing temperatures (r_S = 0.3708, df = 30, P = 0.0400) but not with DO (r_S = -0.1768, df = 30, P = 0.3334) or nitrates (r_S = 0.0038, df = 30, P = 0.9834). However, *Culex* had no significant correlation with any of the variables investigated.

4.4 Bacteria present in mosquito habitats and their influence on breeding patterns.

4.4.1 Bacteria community variation for sites hosting similar mosquito species.

With general bacteria primers a PCR segment of the 16S rDNA of about 450bp was amplified (Figure 4.4a).

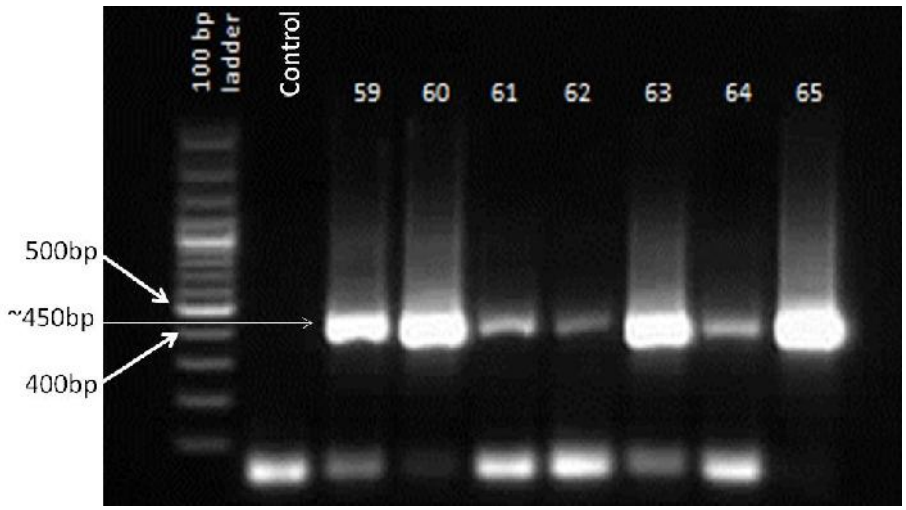

Figure 4.4a. PCR results for 450bp 16S rDNA fragment. Bacteria are present in all samples but with varying abundances as shown by the difference in band intensity.

This confirmed the presence of bacteria in the samples but did not reveal the communities present. Further analysis by TGGE these samples gave complete bacteria community profiles which varied even for samples hosting similar species (Figure 4.4b). Gel photos were used to study band profiles for each sample. Visible bands were cut out from the gel and amplified by PCR. The PCR product was purified and sequenced. However, most of the sequences obtained were of low quality and had double signals that limited their use in further identification of bacteria present in the samples.

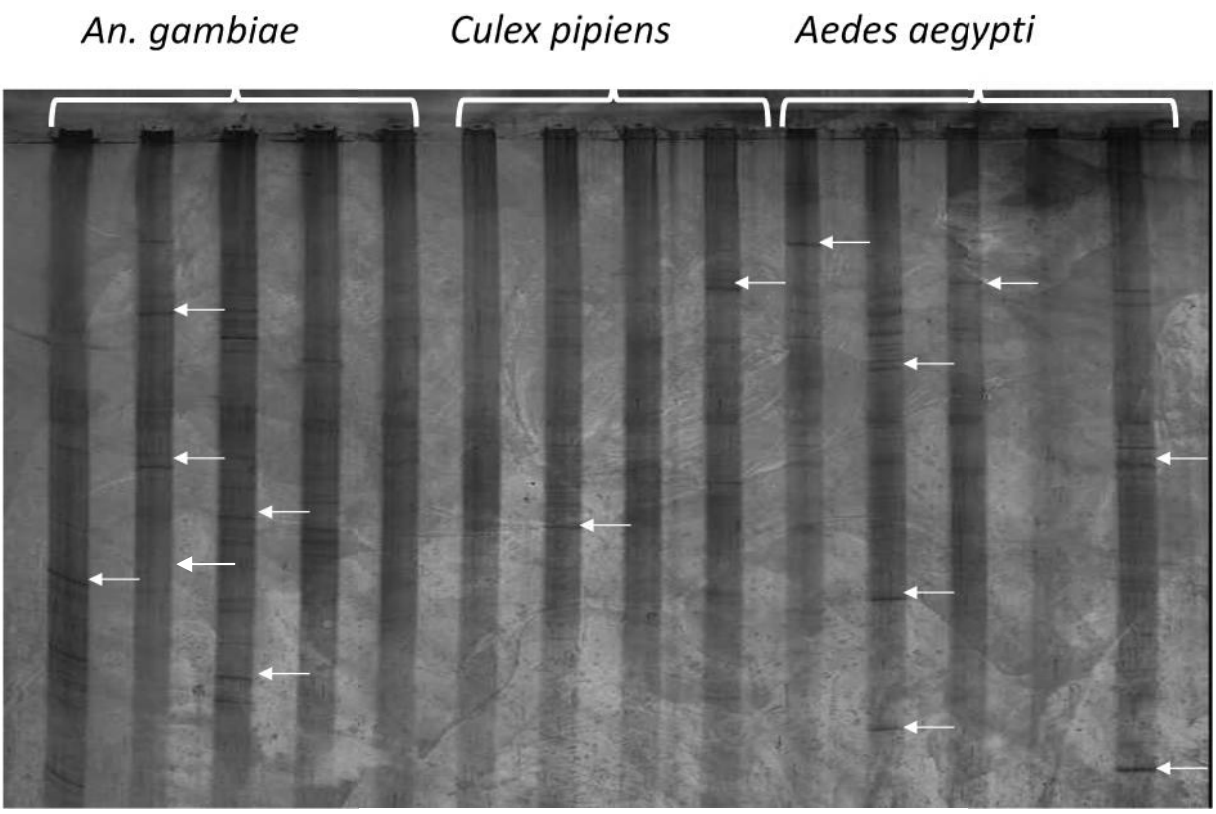

Figure 4.4b: TGGE gel photo showing variation in bacteria community profiles within and between samples. The dark lines (pointed with white arrows) along the migration path represents DNA bands of bacteria present. Sites hosting similar mosquito species do not necessarily have similar DNA band profiles.

4.4.2 Bacteria composition

Raw data from 454 pyrosequencing data analyzed by QIIME pipeline produced a summary of operational taxonomic unit tables for various levels of classification. Fifty-one phyla were present in all samples. These included phyla for bacteria that are culturable, as well as candidate phyla identified by metagenomics that have not previously been cultured. Out of the 51 Phyla, only 12 had a frequency of more than 1% in the number of operational taxonomic units (OTUs)

present (Figure 4.5a). The most abundant phylum across all 15 samples was Proteobacteria (with a frequency of >50% in two thirds of the samples analyzed) followed by Firmicutes and Bacteriodetes respectively (Figure 4.5a). Only one sample (M_Tank) had higher abundance (>90%) of a single phylum i.e. Cyanobacteria.

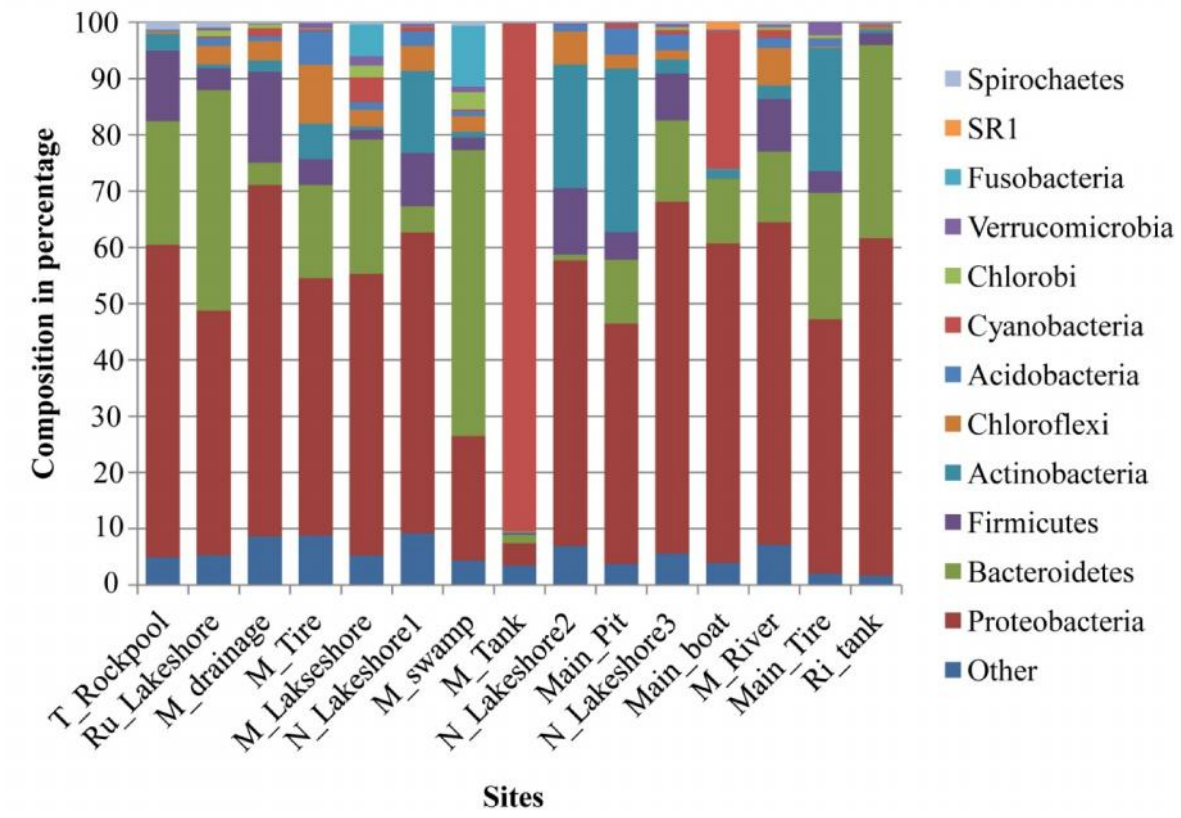

Figure 4.5a: Bacteria composition by phyla (>1%) found in mosquito breeding microhabitats. Mosquitoes select breeding sites rich in Proteobacteria, Bacterioidetes and Firmicutes (N-Ngodhe, M-Mfangano, Ru-Rusinga, T-Takawiri, Main-Mainland, Ri-Ringiti).

At class level, 29 classes had a frequency of greater than 1%. Just as in the phylum level, some bacteria OTUs could not be assigned any classes. Taxonomy was assigned to the OTUs using the Qiime pipeline (Caporaso *et al.*, 2010). At genus level, 921 OTUs were detected of which only 348 OTUs were classified to existing genera. Out of the 348 genera, only 31 had frequencies of >1%. At

genera level, bacteria distribution was not site or species specific. *Flavobacterium* which dominated M_Swamp (55.09%) and Ri_tank that hosted *Culex pipiens* and *Aedes vittattus* respectively, were also present in less quantity in other hosting similar and different mosquito species (Figure 4.5a and Appendix III). *Dechloromonas* that dominated a *Aedes aegypti* site (T_Rockpool) was less abundant in another site that hosted a similar species (M_Tire). Other bacteria genera that were present in unrelated sites but in varying quantities include, *Rubrivivax, Hydrogenophaga, Dechloromonas,* and *Acidovorax* (Betaproteobacteria), *Hyphomicrobium, Bradyrhizobium, Balneimonas, Paracoccus,* and *Porphyrobacter* (Alphaproteobacteria) *Clostridium* and *Bacillus* (Firmicutes) as well as *Mycobacterium* (Actinobacteria). Detailed composition of bacteria present in all samples identified to genus level is attached in appendix III.

4.4.3 Variation in bacteria diversity and influence on habitat choice

OTU tables from QIIME pipeline were used for alpha and beta diversity analysis. Using Chao1 method, alpha diversity analysis was performed to produce rarefaction curves showing bacteria species richness within each sample based on the number of different OTUs detected against the sequencing depth. According to figure 4.5b, sample 3, obtained from a Lakeshore site, had a higher bacterial diversity while sample 6 from a plastic tank, had the least bacteria diversity. Apart from a few, most of the samples are approaching a plateau phase suggesting that the sequencing depth was enough to detect most of the bacteria present.

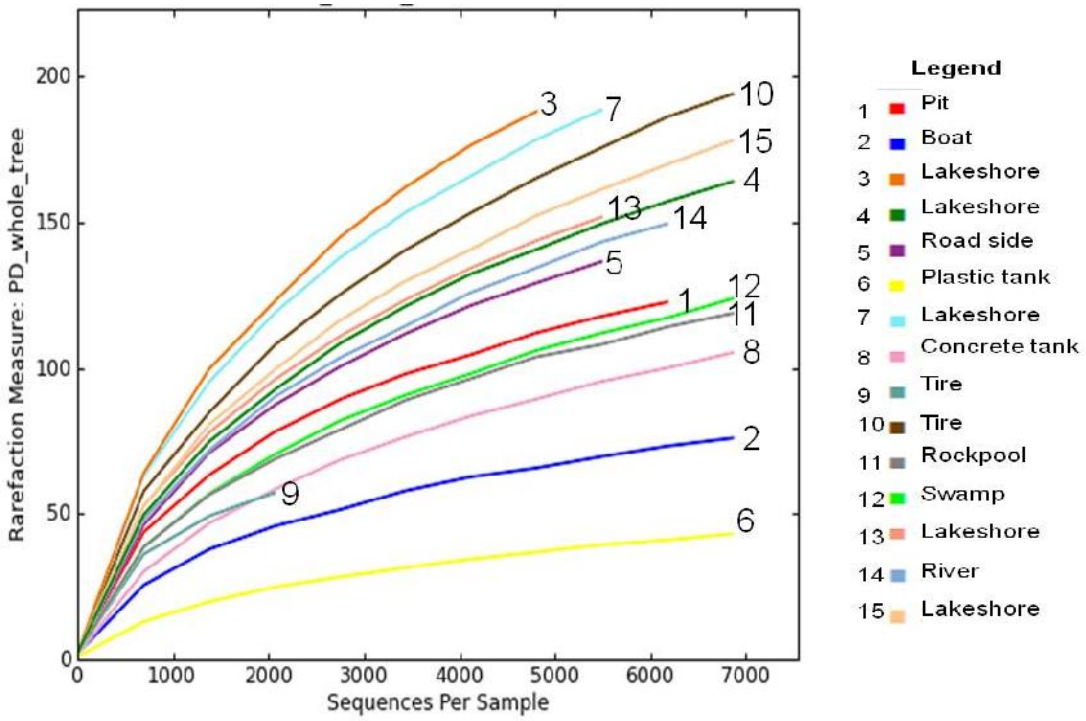

Figure 4.5b: Rarefaction curves of bacterial diversity in samples collected from different categories. Bacteria diversity exists in mosquito microhabitats. Sample 3 has a higher number of species while 6 has the lowest diversity. Sites of the same category e.g. samples 3 &4 from Lakeshore, display variation in bacteria diversity.

To compare species diversity between the samples and determine their influence on breeding patterns, a beta diversity analysis was done based on weighted and unweighted Unifrac distances. These distances were used to generate trees indicating similarity in bacteria diversity composition between samples (Figure 4.5c). More information about the sample was included in this analysis i.e. sample region and abundant mosquito species sampled from the site. Among the species sampled, some but not all of the *C. pipiens* mosquitoes from different regions formed five clusters. Two samples from lakeshore sites in Ngodhe Island that hosted a similar species (*C. pipiens*) showed similarity in their bacteria diversity composition. Bacteria diversity for tire samples was also similar. Other sites did not show any similarity.

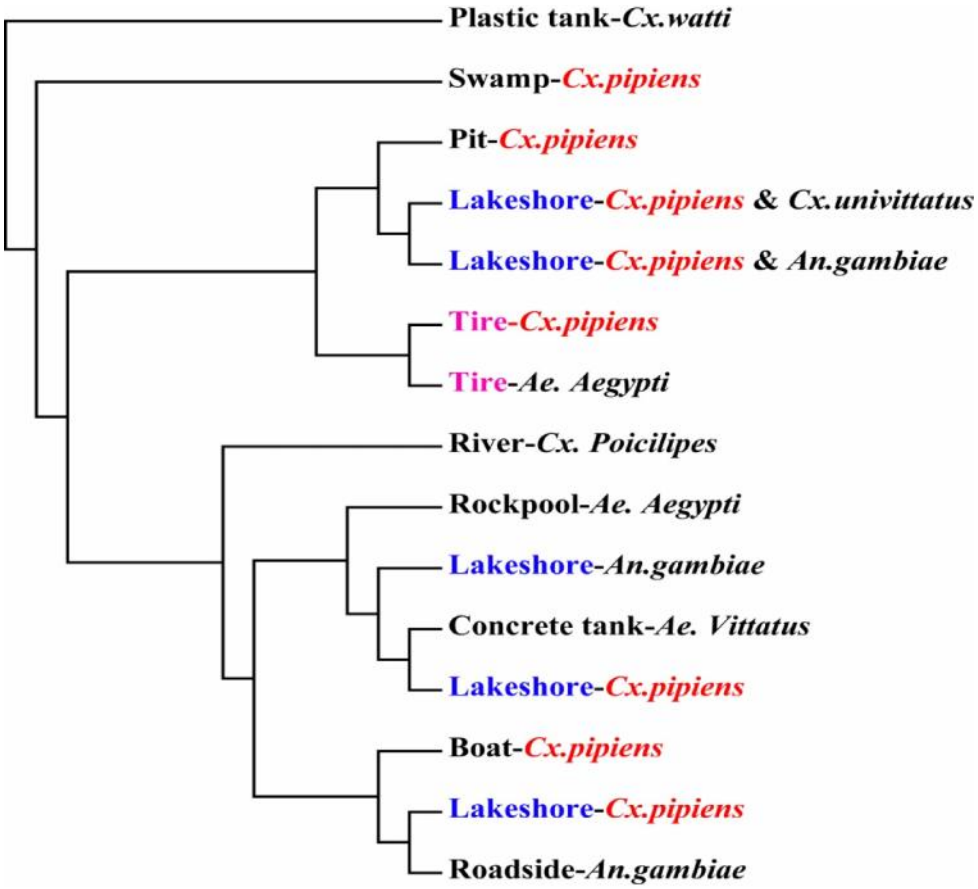

Figure 4.5c: Influence of bacteria community on mosquito species breeding patterns. Bacteria diversity is not the only significant factor in the choice of breeding site for mosquitoes. For instance, *Culex pipiens* has five separate clusters (blue boxes) suggesting that other factors play a role in selection of these sites. Bacteria diversity is also not influenced by category of a microhabitat (red boxes). (MA=Mainland, MF=Mfangano, NG=Ngodhe, TA=Takawiri, RU=Rusinga, RI=Ringiti).

CHAPTER FIVE: **DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS**

5.1 Discussion

Competent vectors of arboviruses and malaria as previously reported (Table 4.2) were amongst mosquito species sampled from the three study Islands. These include *Ae. aegypti, Cx. pipiens, Cx. univittatus,* and *An. gambiae* as reported by Weaver and Barrett (2004). Viruses have been successfully detected and isolated from these vectors. In their study, Ochieng *et al.* (2013)were able to isolate West Nile virus (WNV), Flavivirus, and Ngari viruses from *C. univittatus, Ae. aegypti* and *An. funestus* respectfully. In Kisumu, a city located along Lake Victoria shores experiencing similar climatic conditions as the study area, Ochieng *et al.* (2013) isolated from *C. pipiens* and *C. species* Usutu and Sindbis viruses respectfully. Elsewhere, Jost *et al.* (2010) identified Sindbis virus (SINV) in *C. pipiens* in Germany, which has also been reported as major vector of the same virus in neighboring countries like Sweden. Lutomiah *et al.* (2011) demonstrated vector competency for species within *Culex* genus when he showed the ability of *C. pipiens, C. univitattus* and *C. vansomereni* to be infected and subsequently transmit WNV under laboratory conditions. Therefore, higher abundance of these vectors whose competency is well known implies that should there be accessible reservoir hosts for viruses that have already been detected in the region (Geser *et al.*, 1970; Surtees *et al.*, 1970; Johnson *et al.*, 1977; Ochieng *et al.*, 2013), circulation and transmission of the viruses will occur easily.

Genetic variation findings based on *COI* gene revealed that mosquitoes of the same species from different populations were genetically similar suggesting that there is gene flow between populations in the three islands. This flow could be aided by human activities such as transport between Islands, which could be providing a means of even vector distribution in the islands. For instance, if old tires (which we found to be microhabitats) are transported to other regions for

recycling, then it is possible to transfer vectors from one island to another resulting to homogenous populations in all sites. The high genetic similarity is advantageous in control programmes because a similar control strategy can be adopted to target different populations thus reducing resources spent in control programmes.

Great variation in microhabitat and mosquito distribution exists in the three Islands. Microhabitat and mosquito abundance, diversity and population structures of adult mosquitoes are strongly under the indirect influence of Island size, its topography, human activities, physico-chemical factors as well as existing control programmes. These factors play a significant role in determining availability of microhabitats of immature stages of various mosquito species. For instance, Mfangano Island, which is the largest amongst the three, recorded a high number of mosquito larvae and diversity as compared to Rusinga and Ngodhe Islands in this study. Because of its large size, there were high chances of having microhabitats with varying conditions to support the observed species diversity and abundance.

The difference is further explained by the varying topographical and vegetation cover conditions existing between the islands. Whereas Mfangano has a mixture of flat and steep landscapes with good vegetation cover, Rusinga is majorly flat with good vegetation cover while Ngodhe Island is characterized by a steep gradient and shrubs as its main vegetation cover. Steep landscapes reduce significantly the number of larval habitats in that particular area (Minakawa *et al.*, 2005) while poor vegetation cover reduce chances of having tree hole microhabitats. This supports Atieli *et al.* (2011b) findings which showed that areas with steep gradients experience lower incidences of malaria because of low numbers of the vector as a result of few or absent

microhabitats for their larvae. In contrast, flat areas host a high number of microhabitats that increase vector numbers translating to high number of malaria cases. Lastly, differences in mosquito distribution are determined by existing vector control programmes. During the time of sampling, vector control was being implemented in Rusinga Island (International centre for insect physiology and ecology, 2012). As a result, mosquito collection from this island was low.

This study also revealed that humans influence vector distribution by establishing favorable microhabitats for mosquito breeding. These activities, when not well monitored, result in the creation of favorable sites for mosquito breeding. Unused boats, poorly discarded containers and tires, abandoned ponds, infrastructure development and poorly maintained water storage containers are some of the larval microhabitats established by various human activities. As previously observed by Yee (2008), water storage containers and improperly discarded tires and plastics, all of which are as a result of human activities, were good breeding sites for species in the *Aedes* and *Culex* genera, most of which are known vectors of arboviruses. This implies that vector distribution and possibly virus circulation to new ecological zones is possible, should these materials be transported for recycling in areas outside the point of origin. Furthermore, since most of the human established microhabitats are in close proximity to residential areas, arbovirus circulation or transmission may easily occur.

The stability of a microhabitat determines the type of genus or species it hosts. In this study, sampling was done from both temporary as well as permanent habitats. However, there was variation in the genus that was hosted in these habitats. Whereas *Anopheline* larvae mosquitoes were sampled from temporary habitats, species within *Aedes* and *Culex* genera were present in

permanent habitats. This is because apart from having a faster development process that can be accomplished in short lived habitats, anophelines prefer temporary or semi permanent habitats because they experience minimal competition and few or no predators (Seid *et al.*, 2013). In this study, stable microhabitats were found along the lakeshore where they could get a constant supply of water or sites that could hold water for a long period. Other permanent microhabitats include, rock pools, tree holes, discarded containers, water storage tanks and tires. These type of microhabitats mostly hosted species within the *Aedes* genus as reported elsewhere (Bartlett-Healy *et al.*, 2012).

In contrast, *Anopheles gambiae* larvae were sampled from open, sunlit temporary puddles and pools that hold water for a short time since they are subject to evaporation and depend on seasonal supply of rainwater. As reported before, these microhabitats experience long-term exposures to sun light (Mwangangi *et al.*, 2010) essential for generating high temperatures needed by *Anopheles* larvae for successful development. Wamae *et al.* (2010), proved this when he demonstrated that shading areas around a microhabitat results in a significant decrease in water temperatures, which subsequently lowers anopheline larval densities. However, direct exposure to sun light is responsible for the short lifespan of these microhabitats as it increases evaporation rates of habitat water. Therefore, it is important to note that both temporary and permanent or more stable microhabitats are responsible for species diversity since they each encourage breeding of different species. Successful vector control programmes should target both types.

Physical and chemical characteristics of water are strong determinants of species that can occupy a particular microhabitat. A strong positive correlation was observed between *Aedes* sp and ammonium rich microhabitats (r_S = 0.4961, P = 0.0235). Rao *et al.* (2011), reported a similar correlation for *Aedes albopictus* sampled from coconut shells in Calicut city. This suggests that continuous use of ammonium and phosphate rich fertilizers shall encourage transmission of *Aedes* vectored arboviruses such as Dengue and Chikungunya, whose outbreaks have been on the rise recently. For *Anopheles*, a positive correlation (r=0.46, p=0.008) was observed for temperature which agrees with a previous study done in Gambia where it was reported that *An. gambiae* mosquitoes select high temperature (up to 35^0 C) microhabitats for breeding (Bayoh and Lindsay, 2003). Similar positive correlations for *An. arabiensis* and *An. pharoensis* have also been reported (Mwangangi *et al.,* 2007b; Kenea *et al.*, 2011; Animut *et al.*, 2012; Seid *et al.*, 2013).

On the other hand, *Culex* genus had positive but insignificant correlations with conductivity, TDS, ORP, resistivity, free copper and salinity. Due to the promiscuous nature of *Culex* mosquitoes, they were present in most sites (>80%) sampled whereas *Aedes* and *Anopheles* mosquitoes that are more selective were found in 25% and 50% of sampled sites respectively. In addition, the study area hosts abundant and diverse species of birds, which are preferred blood meal sources by *Culex* mosquitoes (Garcia-Rejon *et al.*, 2008; Richards *et al.*, 2012). This ensures a stable supply of nutrients necessary for reproduction, which partially explains the higher numbers of *Culex* collected.

Mosquito larvae habitats are rich in bacteria diversity. Since bacteria act as a source of nutrition for developing larvae (Merritt *et al.*, 1992), adult female mosquitoes select sites rich in bacteria for laying eggs to ensure enough food for their offspring once they hatch. As previously described (Ponnusamy *et al.*, 2010; Dinparast Djadid *et al.*, 2011), Proteobacteria, Firmicutes and Bacteriodetes were identified as the major bacteria phyla found in larvae microhabitats. Interestingly, a relatively similar pattern of bacterial community composition exists in adult mosquito mid guts (Wang *et al.*, 2011).

Same bacterial genera that have been reported to be present in midguts of adults or larvae were present in breeding sites. These include *Acinetobacter, Bacillus, Hydrogenophaga, Brevundimonas, Lysinibacillus, Sphingomonas, Comamonas* and *Pseudomonas* identified in *Anopheles* adults and larvae (Rani *et al.*, 2009). Also present were *Rubrivivax, Hydrogenophaga, Rhodobacter, Pseudomonas* and *Flavobacterium* genera reported in *Culex* larvae (Duguma *et al.*, 2013), as well as *Acinetobacter, Pseudomonas* and *Bacillus* found in *Aedes* mosquitoes (Zouache *et al.*, 2011). The bacterial genera we identified to be present in breeding sites that have also been reported in larvae and adult digestive tracts have been shown to play important roles in the life cycles of various insect orders. For instance, several species within *Bacillus* and *Paenibacillus* genera in termites enable the insect to digest hemicelluloses or celluloses (Konig, 2005). If such and other bacteria play important roles in mosquitoes, their presence in breeding sites may be important in their acquisition, and possibly survival in later life stages. Such bacteria can be targeted in designing effective control strategies for the vectors.

Transstadial transmission mechanisms could be the reason behind the observed similarity in bacteria composition between habitats, larvae and adults. This is because ingested microhabitat bacteria may evade gut sterilization mechanisms during metamorphosis and in the process get transferred to later stages of development where they may be required as symbionts (Briones *et al.*, 2008; Lindh *et al.*, 2008a). These symbionts benefit the hosts by breaking down complex food substances for easy assimilation through provision of degradative enzymes or essential vitamins to the hosts (Minard *et al.*, 2013a). Apart from transstadial transmission, adults may horizontally acquire the bacteria from aquatic microhabitats when laying eggs or during adult emergence. Minard *et al.* (2013b) demonstrated that *Asaia* sp bacteria found in adult *Ae. albopictus* had a strong positive correlation with *Asaia* sp in the mosquitoes' sampling sites pointing towards either of these mechanisms as the primary form of acquisition.

As observed previously by Dinparast Djadid *et al.* (2011), there was no significant correlation between specific bacteria composition in larval microhabitats and mosquito species hosted in that particular site. It has also been noted that bacteria community in adult mosquito midguts varies extensively between individuals of the same species (Osei-Poku *et al.*, 2012). Whether this variation in adult gut is under the influence of composition within habitats or diet is not yet clear. Therefore, bacteria diversity may not be the only factor influencing mosquito-breeding patterns since the same species falls into different clusters e.g. *Culex pipiens* (Figure 4.5c). Other factors such as physico-chemical variables could be working synergistically with bacteria diversity to influence mosquito-breeding behavior. In addition, different populations within the same species may be responding to different bacteria communities. Lastly, only specific bacteria genera may

be involved in choice of microhabitats and therefore analysis on all bacteria diversity may not be giving a true picture of bacteria influence on mosquito breeding.

Successful identification of bacteria present in complex samples largely depends on the techniques used. In this study, the combined use of PCR, TGGE and 454 pyrosequencing provided a comprehensive, culture independent and unbiased way of revealing bacteria composition in larval microhabitats of medically important mosquitoes. However, of the three, sequencing turned out as a superior and more reliable technique in bacteria identification. From this technique, up to 9% of total OTUs in a sample were classified as other bacteria (Figures 4.5c) as they did not fall into any of the known bacteria taxonomic levels. In addition, sequencing was able to detect bacteria OTUs with a frequency of less than 1%, which in TGGE were too faint for detection. This clearly demonstrates how techniques such as TGGE or culture may lead to underestimation of bacteria community composition in complex samples. In addition, products obtained from these techniques may not be fit for further investigation. For instance, TGGE gel band extracts generated low quality sequences that had double signals, limiting further sequence analyses. This is caused by co-migration of fragments from different species which may result in clustering of bands in the same position in the gel thereby compromising the quality of the final band being sequenced (Muyzer and Smalla, 1998).

Even with the right technique, a high quality of DNA must be ensured in order to get the right results. DNA extracted from the mixture of water and soil or sediment samples in most cases presents challenges during PCR. DNA extracted from these samples normally has a brownish colour pointing to an underlying of problem of contamination with humic acids. Presence of

humic acids is a major hindrance for PCR as the acids inhibit polymerase enzyme during PCR (LaMontagne *et al.,* 2002). Therefore, before PCR is done, contaminated DNA should either be purified or diluted to reduce template contamination from humic acids (Tsai and Olson, 1992).

These results highlight the importance of microhabitat conditions in mosquito development and subsequent adult population dynamics for a particular region. It is clear that environmental factors are essential for breeding site selection. These factors are genus specific as different mosquito genera respond to different environmental conditions. In addition, some bacteria reported to be present in guts of adult mosquitoes are present in breeding aquatic microhabitats, which could be their primary source. Already, a number of studies have elucidated the important functions these bacteria have in mosquitoes as well as other insects suggesting their significance to the insects' adaptation to certain ecological zones, competency to transmit pathogens and digestion.

Because of their importance, these bacteria could have co-evolved with the mosquitoes and in the process devised mechanisms to evade metamorphosis mechanisms and gain transmission to adults from aquatic habitats. This detailed knowledge on bacteria associated with mosquitoes, the possibility of acquisition from aquatic habitats combined with information on how various environmental factors play a role in site selection, is vital for designing targeted mosquito control programmes. If well exploited, this knowledge is significant in reducing or eliminating the role of the vector in pathogen transmission and/or circulation in Lake Victoria basin, a highly arboviral activity area, as well as regions endemic for mosquito borne diseases.

5.2 Conclusion

- Mosquito distribution is under the influence of microhabitat distribution whose presence is directly determined by topography, human activities and size of an Island. This distribution does not vary with the distribution of rains since the mosquitoes utilize habitats that are able to hold water at any season.
- Humans influence presence and distribution of vectors by creating favorable conditions for vector establishment e.g. creation of breeding sites.
- Lake Victoria basin is a host to known mosquito vectors of arboviruses and malaria. This includes, *Ae. aegypti, C. pipiens, C. univittatus,* and *An. gambiae.* This poses danger of ease of pathogen circulation or transmission in case of an outbreak. Genetic variation of same species found in different islands is minimal.
- Specific environmental conditions within a microhabitat determine its suitability to host a particular genus of mosquitoes. Whereas *Aedes* sp mosquitoes prefer microhabitats rich in phosphate and ammonium salts, *Anopheles* sp mosquitoes prefer sites with high temperatures. However, *Culex* sp mosquitoes exhibit flexibility to most environmental parameters and as a result, they are present in high numbers.
- Mosquitoes breed in sites rich in bacteria communities. However, these bacteria do not influence mosquito-breeding patterns in isolation but in conjunction with other factors such as environmental parameters.
- Molecular techniques used for bacteria identification are more sensitive and detect bacteria under very low concentrations than traditional techniques.

5.3 Recommendations

- Mosquito vector control programmes should be guided by vector distribution of an area as determined by its topography and size. These control programmes should be implemented regardless of the season. Communal approach should be incorporated in curbing vector establishment through mobilization of individuals to reduce or minimize activities that result in creation of breeding sites.
- Diagnosis should target both malaria and arboviral infections since vectors of both are present.
- The usage of ammonium and phosphate rich fertilizers should be well monitored since they may encourage breeding of *Aedes* sp mosquitoes and subsequent transmission of *Aedes* vectored arboviruses such as Dengue and Chikungunya, whose outbreaks have been on the rise recently.
- Some of the bacteria species present in larvae and their microhabitats have been reported to be present in adult mosquito mid-guts where they play significant nutritional or defensive roles in both life stages. However, it is unclear if and which gut bacteria originate from microhabitats. To establish this, transmission mechanisms from one life stage to the other should be investigated further under controlled conditions.
- Similar study should be conducted in other areas with high incidences of malaria and arboviral infections such as western Kenya, Baringo and Kenyan coast. This will give a true picture of the characteristics of microhabitats of mosquito vectors, which will be significant in designing control programmes that target the country and East African region.

REFERENCES

Alatoom, A. & Payne, D. 2009. An Overview of Arboviruses and Bunyaviruses. *Laboratory Medicine,* 40: 237-240.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. 1990. Basic local alignment search tool. *Journal of molecular biology,* 215: 403-410.

Aly, S. M. 2014. Reliability of long vs short COI markers in identification of forensically important flies. *Croatian Medical Journal*, 55: 19-26.

American Mosquito Control Association 1979. Manual for Mosquito Rearing and Experimental Techniques. *American Mosquito Control Association Bulletin* No.3.

Animut, A., Gebre-Michael, T., Balkew, M. & Lindtjorn, B. 2012. Abundance and dynamics of anopheline larvae in a highland malarious area of south-central Ethiopia. *Parasites and Vectors,* 5: 117.

Artsob, H., Gubler, D. J., Enria, D. A., Morales, M. A., Pupo, M., Bunning, M. L. & Dudley, J. P. 2009. West Nile Virus in the New World: trends in the spread and proliferation of West Nile Virus in the Western Hemisphere. *Zoonoses and Public Health,* 56: 357-69.

Atieli, H. E., Zhou, G., Afrane, Y., Lee, M. C., Mwanzo, I., Githeko, A. K. & Yan, G. 2011a. Insecticide-treated net (ITN) ownership, usage, and malaria transmission in the highlands of western Kenya. *Parasites and Vectors,* 4: 113.

Atieli, H. E., Zhou, G., Lee, M. C., Kweka, E. J., Afrane, Y., Mwanzo, I., Githeko, A. K. & Yan, G. 2011b. Topography as a modifier of breeding habitats and concurrent vulnerability to malaria risk in the western Kenya highlands. *Parasites and Vectors*, 4: 241.

Bartlett-Healy, K., Unlu, I., Obenauer, P., Hughes, T., Healy, S., Crepeau, T., Farajollahi, A., Kesavaraju, B., Fonseca, D., Schoeler, G., Gaugler, R. & Strickman, D. 2012. Larval mosquito habitat utilization and community dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae). *Journal Medical Entomology,* 49: 813-24.

Bayoh, M. N. & Lindsay, S. W. 2003. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae* sensu stricto (Diptera: Culicidae). *Bulletin of Entomological Research,* 93: 375-81.

Bayoh, M. N. & Lindsay, S. W. 2004. Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Medical and Veterinary Entomology,* 18: 174-179.

Beebe, N. W., Hurk, A. F. V. D., Chapman, H. F., Frances, S. P., Cooper, R. D. & Williams, C. R. 2002. Development and Evaluation of a Species Diagnostic Polymerase Chain Reaction – Restriction Fragment – Length Polymorphism Procedure for Cryptic Members of the *Culex sitiens* (Diptera: Culicidae) Subgroup in Australia and the Southwest Pacific. *Journal of Medical Entomology,* 39: 362-369.

Biessmann, H., Andronopoulou, E., Biessmann, M. R., Douris, V., Dimitratos, S. D., Eliopoulos, E., Guerin, P. M., Iatrou, K., Justice, R. W., Krober, T., Marinotti, O., Tsitoura, P., Woods, D. F. & Walter, M. F. 2010. The *Anopheles gambiae* odorant binding protein 1 (AgamOBP1) mediates indole recognition in the antennae of female mosquitoes. *Public Library of Science One,* 5: e9471.

Bird, B. H., Ksiazek, T. G., Nichol, S. T. & Maclachlan, N. J. 2009. Rift Valley fever virus. *Journal of the American Veterinary Medical Association,* 234: 883-93.

Briones, A. M., Shililu, J., Githure, J., Novak, R. & Raskin, L. 2008. *Thorsellia anophelis* is the dominant bacterium in a Kenyan population of adult *Anopheles gambiae* mosquitoes. *International Society for Microbial Ecology,* 2: 74-82.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J. & Knight, R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods,* 7: 335-6.

CDC 2005. Information on Arboviral Encephalitides. Division of Vector-Borne Diseases (DVBD).

Chouaia, B., Rossi, P., Epis, S., Mosca, M., Ricci, I., Damiani, C., Ulissi, U., Crotti, E., Daffonchio, D., Bandi, C. & Favia, G. 2012. Delayed larval development in *Anopheles* mosquitoes deprived of Asaia bacterial symbionts. *BMC Microbiol,* 12 Suppl 1: S2.

De Andrade, D. C., Jean, S., Clavelou, P., Dallel, R. & Bouhassira, D. 2010. Chronic pain associated with the Chikungunya Fever: long lasting burden of an acute illness. *BioMedical Central Infectious Diseases,* 10: 31.

Diallo, D., Diagne, C. T., Hanley, K. A., Sall, A. A., Buenemann, M., Ba, Y., Dia, I., Weaver, S. C. & Diallo, M. 2012. Larval ecology of mosquitoes in sylvatic arbovirus foci in southeastern Senegal. *Parasites and Vectors,* 5: 286.

Dinparast Djadid, N., Jazayeri, H., Raz, A., Favia, G., Ricci, I. & Zakeri, S. 2011. Identification of the midgut microbiota of *An. stephensi* and *An. maculipennis* for their application as a paratransgenic tool against malaria. *Public Library of Science One,* 6: e28484.

Duguma, D., Rugman-Jones, P., Kaufman, M. G., Hall, M. W., Neufeld, J. D., Stouthamer, R. & Walton, W. E. 2013. Bacterial communities associated with *Culex* mosquito larvae and two emergent aquatic plants of bioremediation importance. *Public Library of Science One,* 8: e72522.

Engel, P. & Moran, N. A. 2013. The gut microbiota of insects - diversity in structure and function. *Federation of European Microbiological societies Microbiology Reviews,* 37: 699-735.

Fillinger, U., Ndenga, B., Githeko, A. & Lindsay, S. W. 2009. Integrated malaria vector control with microbial larvicides and insecticide-treated nets in western Kenya: a controlled trial. *Bulletin of the World Health Organization,* 87: 655-665.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* 3: 294-9.

Gaio, A. D., Gusmao, D. S., Santos, A. V., Berbert-Molina, M. A., Pimenta, P. F. P. & Lemos, F. J. A. 2011. Contribution of midgut bacteria to blood digestion and egg production in *Aedes aegypti* (diptera: culicidae) (L.). *Parasites & Vectors,* 4.

Garcia-Rejon, J. E., Blitvich, B. J., Farfan-Ale, J. A., Loroño-Pino, M. A., Chim, W. A. C., Flores-Flores, L. F., Rosado-Paredes, E., Baak-Baak, C., Perez-Mutul, J., Suarez-Solis, V., Fernandez-Salas, I. & Beaty, B. J. 2008. Host-feeding preference of the mosquito, *Culex quinquefasciatus,* in Yucatan State, Mexico. *Journal of Insect Science,* 10.

Gardner, A. M., Anderson, T. K., Hamer, G. L., Johnson, D. E., Varela, K. E., Walker, E. D. & Ruiz, M. O. 2013. Terrestrial vegetation and aquatic chemistry influence larval mosquito abundance in catch basins, Chicago, USA. *Parasites and Vectors,* 6: 9.

Geser, A., Henderson, B. E. & Christensen, S. 1970. A multipurpose serological survey in Kenya. 2. Results of arbovirus serological tests. *Bulletin of the World Health Organanization,* 43: 539-52.

Henderson, B. E., Kirya, G. B. & Hewitt, L. E. 1970. Serological Survey for Arboviruses in Uganda, 1967-69. *Bulletin of the World Health Organization,* 42: 797-805.

Hoffmann, A. A., Montgomery, B. L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P. H., Muzzi, F., Greenfield, M., Durkan, M., Leong, Y. S., Dong, Y., Cook, H., Axford, J., Callahan, A. G., Kenny, N., Omodei, C., McGraw, E. A., Ryan, P. A., Ritchie, S. A., Turelli, M. & O'Neill, S. L. 2011. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature,* 476: 454-U107.

Imbahale, S. S., Paaijmans, K. P., Mukabana, W. R., Lammeren, R. v., Githeko, A. K. & Takken, W. 2011. A longitudinal study on *Anopheles* mosquito larval abundance in distinct geographical and environmental settings in western Kenya. *Malaria Journal,* 10: 2-13.

International centre for insect physiology and ecology. 2012. *Mosquito Control by combining Chemical and biological measures in Africa (Mocc-A)* [Online]. Available: http://www.icipe.org/mbita/index.php/activities/mosquito-research/8-activities/10-mosquito-control-by-combining-chemical-and-biological-measures-in-africa-mocc-a 2014].

Jeffery, J. A. L., Kay, B. H. & Ryan, P. A. 2007. Control of Arbovirus Vector *Verrallina funerea* (Diptera: Culicidae) in Southeast Queensland , Australia. *Journal of Economic Entomology,* 100: 1512-1518.

Johnson, B. K., Chanas, A. C., Shockley, P., Squires, E. J., Gardner, P., Wallace, C., Simpson, D. I., Bowen, E. T., Platt, G. S., Way, H., Parsons, J. & Grainger, W. E. 1977. Arbovirus isolations from, and serological studies on, wild and domestic vertebrates from Kano Plain, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 71: 512-517.

Johnson, B. K., Chanas, a. C., Squires, E. J., Shockley, P., Simpson, D. I., Parsons, J., Smith, D. H. & Casals, J. 1980. Arbovirus isolations from ixodid ticks infesting livestock, Kano Plain, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 74: 732-7.

Jones, C. & Schreiber, E. 1994. The carnivores, Toxorhynchites. *Wing Beats,* 5: 4.

Jost, H., Bialonski, A., Storch, V., Gunther, S., Becker, N. & Schmidt-Chanasit, J. 2010. Isolation and phylogenetic analysis of Sindbis viruses from mosquitoes in Germany. *Journal of Clinical Microbiology,* 48: 1900-3.

Kasili, S., Oyieke, F., Wamae, A. K., Bisen, C. & Mbogo, C. 2009. Seasonal changes of infectivity rates of *Bancroftian filariasis* vectors in coast province, Kenya. *Journal of Vector Borne Diseases,* 46: 219-24.

Kawai, M., Yamaguchi, N. & Nasu, M. 1999. Rapid enumeration of physiologically active bacteria in purified water used in the pharmaceutical manufacturing process. *Journal of Applied Microbiology,* 86: 496-504.

Kenea, O., Balkew, M. & Gebre-Michael, T. 2011. Environmental factors associated with larval habitats of anopheline mosquitoes (Diptera: Culicidae) in irrigation and major drainage areas in the middle course of the Rift Valley, central Ethiopia. *Journal of Vector Borne Diseases,* 48: 85-92.

Kindt, R. & Coe, R. 2005. Analysis of ecological distance by ordination. *Tree diversity analysis: A manual and software for common statistical methods for ecological and biodiversity studies.* Nairobi, Kenya: World Agroforestry centre.

KNBS 2010. The 2009 Kenya Population and Housing Census. Nairobi, Kenya.

Konig, H. 2005. Bacillus species in the intestine of termites and other soil invertebrates. *Journal of Applied Microbiology,* 101: 620-627.

Kopke, B., Wilms, R., Engelen, B., Cypionka, H. & Sass, H. 2005. Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. *Applied Environmental Microbiology,* 71: 7819-30.

Labeaud, A. D., Bashir, F. & King, C. H. 2011a. Measuring the burden of arboviral diseases: the spectrum of morbidity and mortality from four prevalent infections. *Population Health Metrics,* 9: 1.

LaBeaud, A. D., Cross, P. C., Getz, W. M., Glinka, A. & King, C. H. 2011b. Rift Valley fever virus infection in African buffalo (*Syncerus caffer*) herds in rural South Africa: evidence of interepidemic transmission. *America Journal of Tropical Medicine and Hygiene,* 84: 641-646.

LaMontagne, M. G., Michel, F. C., Jr., Holden, P. A. & Reddy, C. A. 2002. Evaluation of extraction and purification methods for obtaining PCR-amplifiable DNA from compost for microbial community analysis. *Journal of Microbiological Methods,* 49: 255-64.

Lane, D. J. 1991. *16S/23S rRNA sequencing. In: Nucleic acid techniques in bacterial systematics.,* New York, NY, John Wiley and Sons.

Langat, K. B., Siele, K. D., Nanyingi, O. M., Mutai, K. C., Rukunga, M. G., Mwandawiro, C., Ireri, L. & Tonui, K. W. 2012. Larvicidal Action of Extracts from *Tithonia diversifolia* Against the Dengue Mosquito *Aedes aegypti* (Diptera: Culicidae). *Journal of Biologically Active Products from Nature,* 2: 46-49.

Le Menach, A., McKenzie, F. E., Flahault, A. & Smith, D. L. 2005. The unexpected importance of mosquito oviposition behaviour for malaria: non-productive larval habitats can be sources for malaria transmission. *Malaria Journal,* 4: 23.

Lindh, J. M., Borg-Karlson, A. K. & Faye, I. 2008a. Transstadial and horizontal transfer of bacteria within a colony of *Anopheles gambiae* (Diptera: Culicidae) and oviposition response to bacteria-containing water. *Acta Tropica,* 107: 242-50.

Lindh, J. M., Kannaste, A., Knols, B. G., Faye, I. & Borg-Karlson, A. K. 2008b. Oviposition responses of *Anopheles gambiae* s.s. (Diptera: Culicidae) and identification of volatiles from bacteria-containing solutions. *Journal of Medical Entomology,* 45: 1039-49.

Lunt, D. H., Zhang, D. X., Szymura, J. M. & Hewitt, G. M. 1996. The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology,* 5: 153-65.

Lutomiah, J., Bast, J., Clark, J., Richardson, J., Yalwala, S., Oullo, D., Mutisya, J., Mulwa, F., Musila, L., Khamadi, S., Schnabel, D., Wurapa, E. & Sang, R. 2013. Abundance, diversity, and distribution of mosquito vectors in selected ecological regions of Kenya: public health implications. *Journal of Vector Ecology,* 38: 134-42.

Lutomiah, J. L., Koka, H., Mutisya, J., Yalwala, S., Muthoni, M., Makio, A., Limbaso, S., Musila, L., Clark, J. W., Turell, M. J., Kioko, E., Schnabel, D. & Sang, R. C. 2011. Ability of selected Kenyan mosquito (Diptera: Culicidae) species to transmit West Nile virus under laboratory conditions. *Journal of Medical Entomology,* 48: 1197-201.

Lutwama, J. J., Kayondo, J., Savage, H. M., Burkot, T. R. & Miller, B. R. 1999. Epidemic O'Nyong-Nyong fever in southcentral Uganda, 1996-1997: entomologic studies in Bbaale village, Rakai District. *America Journal of Tropical Medicine and Hygiene,* 61: 158-62.

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J., Dymock, D. & Wade, W. G. 1998. Design and evaluation of useful bacterium-specific PCR primers that

amplify genes coding for bacterial 16S rRNA. *Applied and Environmental Microbiology,* 64: 795-9.

Mease, L. E., L., C. R., Musila, L. A., Trish, P., Ogolla, F., Randal, J. S., Cindy, A. R. & Adungo, N. 2011. Seroprevalence and distribution of arboviral infections among rural Kenyan adults: A cross-sectional study. *Virology Journal,* 8.

Mercer, D. R., Sheeley, S. L. & Brown, E. J. 2005. Mosquito (Diptera : Culicidae) Development within Microhabitats of an Iowa Wetland. *Journal of Medical Entomology,* 42: 685-693.

Merritt, R. W., Dadd, R. H. & Walker, E. D. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology,* 37: 349-76.

Minakawa, N., Clifford, M. M., John, I. G., John, C. B. & Guiyun, Y. 1999. Spatial distribution and habitat characterization of anopheline mosquito larvae in western Kenya. *America Journal of Tropical Medicine and Hygiene,* 61: 1010-1016.

Minakawa, N., Munga, S., Atieli, F., Mushinzimana, E., Zhou, G., Githeko, A. K. & Yan, G. 2005. Spatial distribution of anopheline larval habitats in Western Kenyan highlands: effects of land cover types and topography. *America Journal of Tropical Medicine and Hygiene,* 73: 157-65.

Minard, G., Mavingui, P. & Moro, C. V. 2013a. Diversity and function of bacterial microbiota in the mosquito holobiont. *Parasites and Vectors,* 6: 146.

Minard, G., Tran, F. H., Raharimalala, F. N., Hellard, E., Ravelonandro, P., Mavingui, P. & Valiente Moro, C. 2013b. Prevalence, genomic and metabolic profiles of *Acinetobacter* and *Asaia* associated with field-caught *Aedes albopictus* from Madagascar. *FEMS Microbiology Ecology,* 83: 63-73.

Montero-Pau, J., Gómez, A. & Muñoz, J. 2008. Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. *Limnology and Oceanography Methods,* 6: 218-222.

Muriu, S. M., Muturi, E. J., Shililu, J. I., Mbogo, C. M., Mwangangi, J. M., Jacob, B. G., Irungu, L. W., Mukabana, R. W., Githure, J. I. & Novak, R. J. 2008. Host choice and multiple blood feeding behaviour of malaria vectors and other anophelines in Mwea rice scheme, Kenya. *Malaria Journal,* 7: 43.

Muyzer, G. & Smalla, K. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Van Leeuwenhoek,* 73: 127-41.

Mwangangi, J. M., Mbogo, C. M., Muturi, E. J., Nzovua, J. G., Kabiru, E. W., Githure, J. I., Novak, R. J. & Beier, J. C. 2007a. Influence of biological and physicochemical characteristics of larval habitats on the body size of *Anopheles gambiae* mosquitoes (Diptera: Culicidae) along the Kenyan coast. *Journal of Vector Borne Diseases,* 44: 122-7.

Mwangangi, J. M., Midega, J., Kahindi, S., Njoroge, L., Nzovu, J., Githure, J., Mbogo, C. M. & Beier, J. C. 2012. Mosquito species abundance and diversity in Malindi, Kenya and their potential implication in pathogen transmission. *Parasitology Research,* 110: 61-71.

Mwangangi, J. M., Muturi, E. J., Shililu, I., Muriu, S., Jacob, B., Kabiru, E. W., Mbogo, C. M., Githure, J. I. & Novak, R. J. 2007b. Environmental covariates of *Anopheles arabiensis* in a rice agroecosystem in Mwea , Central Kenya. *The American Mosquito Control Association,* 23: 371-377.

Mwangangi, J. M., Muturi, E. J., Shililu, J., Muriu, S. M., Jacob, B., Kabiru, E. W., Mbogo, C. M., Githure, J. & Novak, R. 2008. Contribution of different aquatic habitats to adult

Anopheles arabiensis and *Culex quinquefasciatus* (Diptera: Culicidae) production in a rice agroecosystem in Mwea, Kenya. *Journal of Vector Ecology,* 33: 129-138.

Mwangangi, J. M., Shililu, J., Muturi, E. J., Muriu, S., Jacob, B., Kabiru, E. W., Mbogo, C. M., Githure, J. & Novak, R. J. 2010. *Anopheles* larval abundance and diversity in three rice agro-village complexes Mwea irrigation scheme, central Kenya. *Malaria Journal,* 9: 228.

Ochieng, C., Lutomiah, J., Makio, A., Koka, H., Chepkorir, E., Yalwala, S., Mutisya, J., Musila, L., Khamadi, S., Richardson, J., Bast, J., Schnabel, D., Wurapa, E. & Sang, R. 2013. Mosquito-borne arbovirus surveillance at selected sites in diverse ecological zones of Kenya; 2007 - 2012. *Virology Journal,* 10: 140.

Osei-Poku, J., Mbogo, C. M., Palmer, W. J. & Jiggins, F. M. 2012. Deep sequencing reveals extensive variation in the gut microbiota of wild mosquitoes from Kenya. *Molecular Ecology,* 21: 5138-50.

Oyewole, I. O., Momoh, O. O., Anyasor, G. N., Ogunnowo, A. A., Ibidapo, C. A., A.Oduola, O., Obansa, J. B. & Awolola, T. S. 2009. Physico-chemical characteristics of *Anopheles* breeding sites : Impact on fecundity and progeny development. *African Journal of Environmental Science and Technology,* 3: 447-452.

Ponnusamy, L., Ning, X., Satoshi, N., Dawn, M. W., Coby, S. & Apperson, C. S. 2008. Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. *Proceedings of the National Academy of Sciences,* 105: 9262-9267.

Ponnusamy, L., Wesson, D. M., Arellano, C., Schal, C. & Apperson, C. S. 2010. Species composition of bacterial communities influences attraction of mosquitoes to experimental plant infusions. *Microbial Ecology,* 59: 158-173.

Rajendhran, J. & Gunasekaran, P. 2011. Microbial phylogeny and diversity: small subunit ribosomal RNA sequence analysis and beyond. *Microbiological Research,* 166: 99-110.

Rani, A., Sharma, A., Rajagopal, R., Adak, T. & Bhatnagar, R. K. 2009. Bacterial diversity analysis of larvae and adult midgut microflora using culture-dependent and culture-independent methods in lab-reared and field-collected *Anopheles stephensi*-an Asian malarial vector. *BioMedical Central Microbiology,* 9: 96.

Rao, B. B., Harikumar, P. S., Jayakrishnan, T. & George, B. 2011. Characteristics of *Aedes (Stegomyia) albopictus Skuse* (Diptera:Culicidae) breeding sites. *The Southeast Asian Journal of Tropical Medicine and Public Health,* 42: 1077-82.

Reiter, P., Amador, M. & Colon, N. 1991. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. *Journal of the American Mosquito Control Association,* 7: 52-55.

Rejmankova, E., Higashi, R., Grieco, J., Achee, N. & Roberts, D. 2005. Volatile substances from larval habitats mediate species-specific oviposition in *Anopheles* mosquitoes. *Journal of Medical Entomology,* 42: 95-103.

Rejmankova, E., Roberts, D. R., Manguin, S., Pope, K. O., Komarek, J. & Post, R. A. 1996. *Anopheles albimanus* (Diptera: Culicidae) and cyanobacteria: an example of larval habitat selection. *Environmental Entomology,* 25: 1058-67.

Richards, S. L., Anderson, S. L. & Yost, S. A. 2012. Effects of blood meal source on the reproduction of *Culex pipiens quinquefasciatus* (Diptera: Culicidae). *Journal of Vector Ecology,* 37: 1-7.

Robert, V., Awono-Ambene, H. P. & Thioulouse, J. 1998. Ecology of larval mosquitoes, with special reference to *Anopheles arabiensis* (Diptera: Culcidae) in market-garden wells in urban Dakar, Senegal. *Journal of Medical Entomology,* 35: 948-55.

Roberts, D. M. & Irving-Bell, R. J. 1997. Salinity and microhabitat preferences in mosquito larvae from southern Oman. *Journal of Arid Environments,* 37: 497-504.

Rosenbaum, V. & Riesner, D. 1987. Temperature-gradient gel electrophoresis. Thermodynamic analysis of nucleic acids and proteins in purified form and in cellular extracts. *Biophysical Chemistry,* 26: 235-46.

Salit, A. M., Al-Tubiakh, S. S., El-Fik, S. A. & Enan, H. 1996. Physical and chemical properties of different types of mosquito aquatic breeding places in Kuwait state. *In:* Wildey, K. B. (ed.) *Proceedings of the Second International Conference on Urban Pests*

Sang, R. C. & Dunster, L. M. 2001. The growing threat of arbovirus transmission and outbreaks in Kenya:A review. *East African Medical Journal,* 78: 655-661.

Seid, T. M., Delenasaw, Y., Pieter, B., Abdulhakim, A., Luc, D., Niko, S., Sophie, O. V., Worku, L., Luc, D. M. & Peter, L. M. 2013. Physico-chemical and biological characterization of anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. *Parasites and Vectors,* 6: 320.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Annals of the Entomological Society of America,* 87: 651-701.

Strickman, D. 1989. Biosystematics of larval movement of Central American mosquitoes and its use for field identification. *Journal of the American Mosquito Control Association,* 5: 208-18.

Sumba, L. A., Guda, T. O., Deng, A. L., Hassanali, A., Beier, J. C. & Knols, B. G. J. 2004. Mediation of oviposition site selection in the African malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) by semiochemicals of microbial origin. *International Journal of Tropical Insect Science,* 24: 260-265.

Surtees, G., Simpson, D. I., Bowen, E. T. & Grainger, W. E. 1970. Ricefield development and arbovirus epidemiology, Kano Plain, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 64: 511-22.

Sutherland, L. J., Cash, A. A., Huang, Y. J., Sang, R. C., Malhotra, I., Moormann, A. M., King, C. L., Weaver, S. C., King, C. H. & LaBeaud, A. D. 2011. Serologic evidence of arboviral infections among humans in Kenya. *America Journal of Tropical Medicine and Hygiene,* 85: 158-161.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution,* 28: 2731-9.

Tandale, B. V., Sathe, P. S., Arankalle, V. a., Wadia, R. S., Kulkarni, R., Shah, S. V., Shah, S. K., Sheth, J. K., Sudeep, a. B., Tripathy, A. S. & Mishra, A. C. 2009. Systemic involvements and fatalities during Chikungunya epidemic in India, 2006. *Journal of Clinical Virology: the official publication of the Pan American Society for Clinical Virology,* 46: 145-9.

Tsai, Y. L. & Olson, B. H. 1992. Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. *Applied Environmental Microbiology,* 58: 2292-5.

Turner, S., Pryer, K. M., Miao, V. P. & Palmer, J. D. 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *Journal of Eukaryotic Microbiology,* 46: 327-38.

Verhulst, N. O., Andriessen, R., Groenhagen, U., Bukovinszkine Kiss, G., Schulz, S., Takken, W., van Loon, J. J., Schraa, G. & Smallegange, R. C. 2010. Differential attraction of malaria mosquitoes to volatile blends produced by human skin bacteria. *Public Library of Science ONE,* 5: e15829.

Verhulst, N. O., Qiu, Y. T., Beijleveld, H., Maliepaard, C., Knights, D., Schulz, S., Berg-Lyons, D., Lauber, C. L., Verduijn, W., Haasnoot, G. W., Mumm, R., Bouwmeester, H. J., Claas, F. H., Dicke, M., van Loon, J. J., Takken, W., Knight, R. & Smallegange, R. C. 2011. Composition of human skin microbiota affects attractiveness to malaria mosquitoes. *Public Library of Science One,* 6: e28991.

Vezenegho, S. B., Bass, C., Puinean, M., Williamson, M. S., Field, L. M., Coetzee, M. & Koekemoer, L. L. 2009. Development of multiplex real-time PCR assays for identification of members of the *Anopheles funestus* species group. *Malaria Journal,* 8: 282.

Walker, T., Johnson, P. H., Moreira, L. A., Iturbe-Ormaetxe, I., Frentiu, F. D., McMeniman, C. J., Leong, Y. S., Dong, Y., Axford, J., Kriesner, P., Lloyd, A. L., Ritchie, S. A., O'Neill, S. L. & Hoffmann, A. A. 2011. The *wMel Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature,* 476: 450-U101.

Wamae, P. M., Githeko, A. K., Menya, D. M. & Takken, W. 2010. Shading by napier grass reduces malaria vector larvae in natural habitats in Western Kenya highlands. *Ecohealth,* 7: 485-97.

Wang, Y., Gilbreath, T. M., 3rd, Kukutla, P., Yan, G. & Xu, J. 2011. Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya. *Public Library of Science One,* 6: e24767.

Weaver, S. C. & Barrett, A. D. 2004. Transmission cycles, host range, evolution and emergence of arboviral disease. *Nature Reviews Microbiology,* 2: 789-801.

Weaver, S. C. & Reisen, W. K. 2010. Present and future arboviral threats. *Antiviral Research,* 85: 328-345.

Willems, K. J., Webb, C. E. & Russell, R. C. 2005. A comparison of mosquito predation by the fish *Pseudomugil signifier Kner* and *Gambusia holbrooki* (Girard) in laboratory trials. *Journal of Vector Ecology,* 30: 87-90.

Wotton, R. S., Chaloner, D. T., Yardley, C. A. & Merritt, R. W. 1997. Growth of Anopheles mosquito larvae on dietary microbiota in aquatic surface microlayers. *Medical and Veterinary Entomology,* 11: 65-70.

Yee, D. A. 2008. Tires as habitats for mosquitoes: a review of studies within the eastern United States. *Journal of Medical Entomology,* 45: 581-93.

Yuill, T. M. 1986. Arthropod-Borne Viruses. *Annual review of ecology systems,* 17: 189-219.

Zouache, K., Raharimalala, F. N., Raquin, V., Tran-Van, V., Raveloson, L. H., Ravelonandro, P. & Mavingui, P. 2011. Bacterial diversity of field-caught mosquitoes, *Aedes albopictus* and *Aedes aegypti*, from different geographic regions of Madagascar. *Federation of European Microbiological societies Microbiology Ecology,* 75: 377-89.

APPENDICES

Appendix I: List of primers

Primer Name	Primer Sequence (5'-3')	target	Reference
Primers for molecular identification of mosquito			
TL2-N-3014 R	TCCAATGCACTAATCTGCCATATTA	1700	(Simon *et al.*, 1994)
LCO 1490 F	GGTCAACAAATCATAAAGATATTGG		(Folmer *et al.*, 1994)
Bacteria community profiling primers			
Eub_16s 933F	GC-clamp GCACAAGCGGTGGAGCATGTGG	454	(Kawai *et al.*, 1999)
Eub_16s 1387R	GCCCGGGAACGTATTCACCG		(Marchesi *et al.*, 1998)
Gray 28F	GAGTTTGATCNTGGCTCA	491	(Lane, 1991)
Gray 519R	GTNTTACNGCGGCKGCTG		(Turner *et al.*, 1999)
GC-clamp	CGCCCGCCGCGCGCGGCGGGCGGGGCG GGGGCACGGGGGG	40	(Kawai *et al.*, 1999)

Appendix II: Protocols

1. **Exosap it**
    a. Mix 5 µl of PCR product with 2 µl Exosap it reagent
    b. Mix and incubate at 37°C for 15 minutes
    c. Incubate at 65°C for 15 minutes to deactivate the enzyme
2. **InnuPREP PCR product purification protocol**
    a. Mix 10x binding buffer with DNA to be purified
    b. Transfer into spin filter with column and centrifuge at 10,000xg for 2 minutes
    c. Transfer spin filter to new 2.0 ml tube
    d. Add elution buffer (at least 10 µl) and incubate at room temperature for 1 minute
    e. Centrifuge at 6,000xg for one minute to recover pure DNA.

3. Silver staining protocol

a. Mix the developer (15g Na_2CO_3, 750 µl formaldehyde, 500 µl dH_2O and freshly prepared 50 µl of $Na_2S_2O_3$) and chill it to 4°C.

b. Prepare the fixer (75 ml of 100% HAc + 925 ml dH_2O). Fix the gel for 30 minutes with 500 ml of the fixer. Chill the remaining 500 ml to 4°C.

c. Add 750 µl of formaldehyde to stain (0.75g $AgNO_3$, 500 ml dH_2O).

d. After fixing for 30 minutes, wash the gel three times with distilled water

e. Stain the gel in $AgNO_3$ with formaldehyde for 50 minutes

f. Wash once with distilled water

g. Add 750 µl of formaldehyde to the developer. Submerge the gel in the developer, shake until the bands are visible.

h. The bands can be cut out for further processing, or the gel can be dried overnight and store in glycerol for a long time.

Appendix III: Bacteria genera present in aquatic sites with a frequency of greater than 1 %

Taxon / Site	T_Rock pool	Ru_Lakeshore	M_drainage	M_Tire	M_Lakeshore	N_Lakeshore1	M_Swamp	M_Tank	N_Lakeshore 2	Main_pit	N_Lakeshore3	Main_boat	M_River	Main_Tire	Ri_tank
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Hydrogenophaga	2.97	7.17	6.67	2.03	8.21	2.58	1.8	0	0.08	0.51	6.98	16.93	0.79	2.32	6.72
Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	7.67	1.22	1.13	4.98	0.16	1.02	1.01	0.17	11.54	3.45	2.92	0.28	3.01	4.55	0.8
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Rubrivivax	0	0.75	0.7	7.8	13.47	2.42	2.85	0.14	2.16	0.09	10.04	12.14	2.51	3.12	2.93
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium	2.45	0.04	0.04	20.83	10.62	0.59	55.09	0.14	0.16	1.24	6.13	4.78	0.04	12.13	55.56
Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Dechloromonas	38.75	18.52	17.25	2.41	1.2	4.62	0.66	0.04	0.44	0.05	5.56	0	4.56	0.27	0.18
Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__KD1-23	0.2	61.82	57.57	0.38	2.68	0.27	0.13	0.04	0.12	1.2	2.28	0	1.8	0.45	0.36
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae;g__Mycobacterium	0.51	0.04	0.04	1.81	0	3.49	0.28	0	11.94	9.48	0.57	0	0.25	5.53	0.14
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Rhodoferax	0	0	0	0.03	2.79	0	5.2	0.03	0	0	1.35	0.28	6.44	0.36	2.25
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Acidovorax	1.18	0.04	0.04	2.41	9.04	0.27	0.09	0	0.04	0.64	0.64	3.86	0	0.71	10.03
Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Azospira	0.15	0	0	1.84	1.48	0.48	0.02	0.02	4.39	0.51	1.57	0	0.38	1.87	0.16
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Chitinophagaceae;g__Flavisolibacter	0.02	0.59	0.55	1.52	0.05	2.52	0	0.05	0.32	1.01	1.35	1.38	0.08	0	0.02
Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	0.25	4.74	4.41	0.06	0	7.2	0.79	0	2.2	0.32	0.71	0	0.42	0	0.04
Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Rhodobacter	0	0.21	0.2	0.95	3.78	0.43	0.19	0	0.56	0.74	1.42	0.83	1.21	5	0.44
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Roseomonas	0.32	0.13	0.12	10.68	0	0.91	0.04	0.21	8.18	0.09	0.07	5.15	0	2.32	0.12
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__Rhodococcus	1.2	0.04	0.04	1.36	0	1.07	0.21	0	0.64	0.09	0	0	0	21.41	0.02
Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__PSB-M-3	0.76	1.68	1.56	0	0.66	0.7	0.04	0	0	0	3.28	0	6.19	0	0
Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Paracoccus	0.02	0.08	0.08	0.32	0.27	4.24	0	0	2.67	1.15	1.07	0.37	0	0.18	0.02
Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium	0	0	0	1.3	0	0.59	0.39	0.03	0.8	1.01	1.07	5.43	0.13	0.36	0.16
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Balneimonas	0	0.96	0.9	0.1	0.22	25.67	0.19	0	3.11	0.41	1.21	0	0	0	0.04
Acidobacteria;c__Holophagae;o__Holophagales;f__Holophagaceae;g__Geothrix	0	0	0	0	0.05	0	0.04	0	0	5.29	0.07	0	1.3	1.69	0
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Microbacterium	0	0	0	1.59	0	0.43	0.02	0	4.35	2.85	0.43	0.64	0.04	0.45	0
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Parabacteroides	5.02	0	0	0	0	0	0	0	0	0.09	2.21	0	0	0	1.06
Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Rubellimicrobium	0	0	0	0.38	0	11.49	0	0.05	1.64	0.05	0.07	4.14	0.13	0	0.02
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Limnohabitans	0.07	0	0	0.03	1.64	0	1.18	0.15	0.48	0.09	0.07	0.28	0.38	3.93	0.82

Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobulbaceae;g__Desulfobulbus	0.56	1.34	1.25	0.03	5.42	0.21	0	0	0	0.32	0	0	0.17	0.18	0.02
Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae;g__Candidatus Solibacter	0.1	0.08	0.08	2.25	1.1	0.32	0.36	0	0.72	0.09	0.64	1.29	0.17	0	0.02
Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Novosphingobium	0.05	0.54	0.51	1.08	0.71	0.38	0.09	0.01	0.48	0.37	1	0.37	1	2.23	0.16
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Gordoniaceae;g__Gordonia	0.02	0	0	0.73	0.11	0.21	0	0.05	5.55	0.6	0.07	0	0	9.99	0
Fusobacteria;c__Fusobacteria;o__Fusobacteriales;f__Fusobacteriaceae;g__u114	0.02	0	0	0	17.74	0	19.58	0	0	0	0	0	0.08	0	0
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Bosea	0.1	0	0	0.92	0.11	0	0	0.03	3.19	10.81	0.78	0.18	0	0	0.04
Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Geobacteraceae;g__Geobacter	0.91	0.21	0.2	0	0.82	0.21	0.19	0.15	0.48	11.14	0.43	0	13.67	0.54	0
Chloroflexi;c__Anaerolineae;o__Caldilineales;f__Caldilineaceae;g__Caldilinea	0	0	0	8.75	0.49	0.11	0.54	0	2.24	0	0.43	0	0.29	0.27	0.1
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__Bradyrhizobium	0.1	0	0	0.57	0.11	0.43	0.04	0.02	1.12	5.48	0.78	0	0.13	0.54	0
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes	0.54	0.17	0.16	0.86	0.05	0.97	0.04	0.04	5.43	1.89	0.85	0	0.54	0	0
Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Crenotrichaceae;g__Crenothrix	0	0	0	0	0.38	0	0.06	0	0	0	3.77	0	19.86	0	0
Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolinaceae;g__Anaerolinea	0.2	0.67	0.62	2.09	0.11	1.24	0.04	0	0.4	0.28	0.28	0	0.71	0	0
Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingopyxis	0	0	0	0	0	0	0	0	0	1.43	0.28	0	0	1.07	0.02
Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Zoogloea	0	0	0	0	0.44	0	0.02	0	0	1.84	1.21	0	0	0	0.02
Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	0.27	0	0	0.54	0.16	0	0.02	0	0.08	0.51	2.14	0.37	0.04	0.09	1.02
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Sinomonas	0	0	0	0	0	0	0	0	0	5.98	0	0	0	0	0
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Nocardioides	0	0	0	0	0	0.48	0	0	0	3.27	0	0	0.13	0	0
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Flexibacteraceae;g__Spirosoma	0	0	0	0	0	0	0	0	0	0	0	14.72	0	0	0
Cyanobacteria;c__Synechococcophycideae;o__Pseudanabaenales;f__Pseudanabaenaceae;g__Leptolyngbya	0	0.21	0.2	0	0	0	0.15	96.86	0	0	0	0	0	0	0.1
Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Trichococcus	0	0.04	0.04	0	0	0	0.02	0	0	0	5.7	0	0	0	0
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Hylemonella	0.05	0	0	0.16	0	0.05	0	0	0.24	3.22	0	0	0	0	0
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Janthinobacterium	0	0	0	0	0	0	0	0.04	0	0	0	0	0	3.21	0
Proteobacteria;c__Betaproteobacteria;o__Hydrogenophilales;f__Hydrogenophilaceae;g__Thiobacillus	3.95	0.04	0.04	0	0	0	0	0	0	0	0	0	0	0	0.06
Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae;g__Microvirgula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.51
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Myxococcaceae;g__Anaeromyxobacter	0.05	0.04	0.04	0.06	0.11	0.75	0.11	0.02	0.04	0	0.21	0	24.37	0	0.1
Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Arcobacter	20.59	0	0	0	0	0.05	0	0	0	0	0	0	0	0	0
Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Sulfurospirillum	0.25	0	0	4.76	0	0	0	0	0	0.14	0	0	0	0	0.3
Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Thermomonas	0	0	0	0.54	0	0.05	0.11	0	0	0.69	0.93	0	0	0	4.87
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae;g__Actinotalea	0	0	0	0	0	0.16	0	0	1.16	0	0	0	0	0	0
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae;g__Terracoccus	0	0.08	0.08	0	0	0	0	0	0	1.79	0	0.28	0.08	0	0

Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Leucobacter	0	0	0	0.1	0	0.05	0	0	1.08	0	0	0	0	0	0.3
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Yonghaparkia	0	0	0	0	0	0	0	0	0	0	0	2.12	0	0	0.04
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae;g__Nocardia	0	0	0	0	0	0.43	0	0	1.56	0.14	0	0	0	0	0
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Aeromicrobium	0	0	0	0.06	0	0.05	0	0	0.08	0.09	0.21	0	0	1.43	0
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae;g__Propionicimonas	0.29	0	0	0.22	0	0.11	0	0	0.4	1.06	0	0	0	0	0.04
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Promicromonosporaceae;g__Cellulosimicrobium	0	0	0	0.1	0	0	0	0	1.92	0.05	0	0	0	0	0.06
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae;g__Microlunatus	0	0	0	0	0	0.27	0	0	0.04	1.93	0.21	0.18	0	0	0
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Williamsiaceae;g__Williamsia	0	0	0	0.19	0	0.05	0	0	0	0	0	0	0	2.32	0
Actinobacteria;c__Rubrobacteria;o__Rubrobacterales;f__Rubrobacteraceae;g__Rubrobacter	0	0.92	0.86	0	0.22	2.42	0.02	0	0	0	0.36	0	0.29	0.09	0
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Flexibacteraceae;g__Emticicia	0	0	0	0.38	0.16	0	1.09	0.03	0	0	0	0	0	0	0
Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Acidaminobacter	0.05	0.67	0.62	0	0	0	0	0	0	0	1.92	0	0	0	0
Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Fusibacter	0	0.59	0.55	0	1.2	0.11	0.02	0	0	0.05	0.36	0	0.17	0	0.02
Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Sedimentibacter	1.69	0	0	0	0	0	0	0	0.2	0	0.71	0	0	0	0
Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	0	0	0	0.6	0	0.11	0	0	0	0.14	1.78	0	0.04	0	0.04
Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Gemmataceae;g__Gemmata	0	0	0	0	0.44	0	0	0	0.32	0	0	1.66	0.17	0	0
Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__Pirellula	0	0	0	0	0	0	0.02	0	0	0	0.28	2.76	0.17	0	0
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Devosia	0.07	0.04	0.04	0.54	0.11	0.38	0.04	0	0.36	0.32	0.14	3.31	0.04	0.89	0.08
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Pedomicrobium	0	0	0	0.1	0.11	0.11	0.11	0	0.04	0	0.36	0.09	0.13	1.16	0
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;g__Methylosinus	0	0	0	0.73	0	0.32	0.3	0	1.84	0.05	0	0	0.08	0	0
Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Amaricoccus	0	0	0	0.03	0	0.38	0	0	0	1.38	0	0	0	0	0.04
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Roseococcus	0	0	0	0.13	0.33	0.11	0	0.02	0.04	0.05	0	4.23	0	0.18	0
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__Skermanella	0	0.21	0.2	0	0.49	1.77	0.02	0	0.6	0.14	0.36	0.83	0	0	0.04
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Azohydromonas	0	0.04	0.04	0.22	0	0.27	0	0.03	0.68	0	0.14	1.47	0.04	0	0.16
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Mitsuaria	1.15	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0.02
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Pelomonas	0	0	0	0.03	0.22	0.16	0.06	0.49	0.12	0.6	0.14	3.4	0.42	0.45	0.38
Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Azoarcus	0	0.04	0.04	0	0.11	0.64	0	0	0	0	1.07	0	0.08	0	0
Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Sulfuritalea	0	0	0	0	0.44	0.21	0.17	0	0	0	1.07	0	0.13	0	0
Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae;g__Thauera	0	0	0	0	0	0.21	0	0	0	0	1.42	0	0	0	0.04
Proteobacteria;c__Gammaproteobacteria;o__Aeromonadales;f__Aeromonadaceae;g__Oceanimonas	0	0	0	0	0	0	0	0	0	0	2.56	0	0	0	0
Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__Methylococcaceae;g__Methylomonas	0	0	0	0	0.66	0.27	0.75	0	0	0	2.14	0	0.59	0	0
Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	0	0	0	0.03	0	0	0	0	0	1.52	0.28	0	0	0	0.04

Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Arenimonas	0	0.04	0.04	0	0.27	0.32	0	0	0	0	1.14	0	0	0	0
Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Rhodanobacter	0	0	0	0	0	0	0	0	0	2.07	0	0	0	0	0
Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae;g__Spirochaeta	0	0	0	0.22	0.71	0.11	1.09	0.12	0	0	0.14	0	0	0	0.18
Spirochaetes;c__WWE1;o__[Cloacamonales];f__[Cloacamonaceae];g__W22	1.35	0.13	0.12	0	0	0	0	0	0	0	0	0	0	0	0
Thermi;c__Deinococci;o__Deinococcales;f__Deinococcaceae;g__Deinococcus	0	0.13	0.12	0.1	0.27	0.05	0	0	0.04	0	0.21	1.84	0	0	0.08
Verrucomicrobia;c__Opitutae;o__Opitutales;f__Opitutaceae;g__Opitutus	0	0.13	0.12	0.38	0.33	0.43	0.41	0.03	0.08	0	0.07	0	0.5	2.05	0.1

Appendix IV: Physicochemical data

row.names	Cond (uS.cm)	Res (Ohms)	Sal ppt	Sp. Cond (uS.cm)	TDS (mg.L)	D O (mg.L)	NH_4^+ (mg.L)	NO_3^- (mg.L.)	ORP	pH	Temp	Cu.free (mg.l)	Cu total (mg.l)	Flu (mg.l)	PO_4^- (mg.l)	An	Ae	Cx
Rusinga fish banda a	879.8	1136.6	0.424	868.4	564.85	2.269	7.4	1.977778	0	7.921111	25.68	0	0	1.49	5.5	1	0	104
Ngodhe island beach	1009.333	990.7333	0.416667	859	559	7.76	3.1	157.5	36.3	7.195	34.16667	0	0	1.4	20.9	0	0	13
Ngodhe island lakeshore 2	274.9333	3638.067	0.12	252.3333	164	0.583333	0.8	161.8	35.7	7.135	29.66667	0	0	0.77	16.9	1	0	22
Ngodhe island lwanga shore 2	274.6333	3641.2	0.13	272.0667	176.8	0.09	1.3	80.2	-23.9	7.215	25.5	0	0	0.68	16.9	1	0	4
Rusinga lwanda rombo boat 1	440.45	2270.525	0.195	408.95	266.025	7.6125	1.4	196.9	38	7.72	29.05	0	0	0.73	0.1	0	0	1
Rusinga beach 3 lwanda rombo	731.3333	1367.433	0.35	723.3333	468	0.15	5.2	75.8	11.2	7.225	25.56667	0	0	0.925	17.7	1	0	17
Rusinga 2 lwanda rombo	664.75	1504.45	0.295	608.25	396.5	2.9825	1.65	65.4	23.5	7.1	29.925	0.1	0	1.04	11.7	0	0	121
Rusinga lwanda rombo puddle 1	635.5	1573.325	0.2625	553.5	359.125	10.74	1.4	229.6	12.9	8.16	32.85	0	0	0.925	1.4	20	4	0
Rusinga lwanda rombo fish pond	1986.75	503.35	0.91	1815.5	1181.375	0.0475	1.55	179.6	8.7	7.725	29.925	0	0	6.2	3	3	0	15
Rusinga lwanda rombo puddle 2	673	1486.833	0.3	618	403	0.543333	2.9	87.1	11.3	7.365	29.63333	0	0	0.925	16.9	8	0	0
Rusinga kigoda4 drainage	2093.4	477.7	1	1983.4	1287	0.312	0.9	101.9	-28.85	8.81	27.9	0.28	0.18	0.925	29.3	0	0	9
Rusinga kigoda beach	927.3333	1078.383	0.416667	854	555.75	0.498333	2.55	28.95	-65.55	7.8525	29.51667	0	0	0.925	16.9	5	0	13
Rusinga kigoda beach boat	825.2	1211.9	0.37	759.6	494	6.866	1.1	67.8	-12.55	8.66	29.48	0	0	1.03	16.9	3	0	0
M Mrongo beach 3	440.56	2269.98	0.19	408.04	265.2	0.302	0.9	23.35	-109.1	7.6875	29.2	0	0	0.32	5.9	1	0	10
M Wakula beach2	449.3667	2225.3	0.17	372.1833	241.8	7.411667	0.55	86.6	-6.4	7.925	35.86667	0.1	0.13	0.34	11.3	5	0	1
M Wakula beach boat	627	1595.083	0.26	552.6667	360.75	19.15	0.9	114.6	-7.7	8.6225	32.05	0.04	0	1.04	2	0	0	1
M Wakula beach 3	1107.333	902.95	0.506667	1033.667	672.75	1.056667	0.5	25.65	-61.85	7.46	28.73333	0	0.07	0.925	16.9	0	0	1
M Wakula beach 4 pool	1052.8	949.84	0.502	1023.6	665.6	2.2	0.5	27.3	-64.4	7.29	26.5	0.25	0.08	1.38	6.5	4	0	4
M Mawanga beach	472.74	2133.94	0.182	390.48	253.38	0.994	1.3	22.3	-111.7	7.073333	35.98	0	0	0.95	18.9	8	0	0
M Ugosia shore	695.7143	1437.343	0.314286	652.8571	423.4286	1.025714	0.6	0	53.82	7.058	28.44286	0.03	0.08	0.46	17.7	0	0	5
M Mulundu beach boat 1a	281.52	3552.26	0.13	274	178.1	5.842	3.6	0	51.375	7.46	26.42	0	0	0.925	16.9	0	0	2
M Uozi river	532	1486.833	0.3	618	403	1.7	0.28	28.12	-33.3	7.43	24.3	0.23	0.16	0.97	41.2	0	0	2
M Uozi Drainage	542	1486.833	0.3	618	403	3.54	0.23	103.77	-7.3	7.3	27.1	0	0.2	0.77	24.1	1	0	0
M Milundu Rock pool 1	1296	1486.833	0.3	618	403	2.03	32.33	18.98	-27.3	7.26	28.5881	0	0.2	7	61	0	44	0

M Milundu beach boat2	147.6	1486.833	0.3	618	403	5.44	1.31	87.86	41.1	7.59	26.2	0	0	0.76	4	1	1	1
M Museum Tire 2	603	1486.833	0.3	618	403	1.66	4.33	99.71	-9.9	7.83	26.6	0	0	0.02	39.9	0	48	10
M Milundu tree hole	11.8	1486.833	0.3	618	403	2.66	36.56	64.24	-31.2	7.61	28.588 1	0	0	0.925	68.6	0	63	0
M museum Tire 1	930	1486.833	0.3	618	403	3.99	7.91	97.76	-15.8	7.84	26.3	0.11	0.09	1.5	48	0	6	2
M Gulwe river	517	1486.833	0.3	618	403	2.41	0.54	89.73	-17.1	7.84	20.7	0.2	0.07	0.9	10.3	0	0	4
M Soklo/gulwe primary school tank 3	149.4	1486.833	0.3	618	403	5.27	0.24	90.09	14.6	8.06	23.6	0.1	0.12	0.24	6.7	0	0	3
M Milundu Rockpool 2	460.3	1486.833	0.3	618	403	1.41	11.88	18.62	24.1	7.26	28.588 1	0	0.09	0.925	53.5	0	0	10

(Cond=Conductivity, Sp. Cond=Specific Conductivity, Res=Resistivity, Sal=Salinity, TDS=Total Dissolved salts, DO=Dissolved oxygen, NH_4^+=Ammonium, NO_3^- =Nitrates, Temp=Temperature, PO_4^-=Phosphates, An=*Anopheles*, Ae=*Aedes*, Cx=*Culex*), M=Mfangano