DIVERSITY AND DISTRIBUTION OF IMMATURE VECTORS OF MALARIA AND RIFT VALLEY FEVER IN HABITATS ALONG AN ALTITUDINAL GRADIENT IN BARINGO COUNTY, KENYA.

By

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DECLARATION

Candidate

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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DEDICATION

This thesis is dedicated to my loving wife Ann Njeri Chomba, my son Brayden Mugambi, dad Athinya Mathiu and mum Agnes Makandi

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TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

WHO	World health organization
TDR	Tropical diseases research
TDRC	Tropical diseases research Centre
DVBDU	Division of vector borne disease unit
MSc	Master of science
RVF	Rift valley fever
POPs	Persistent organic pollutants
DDT	Dichloro-diphenyl-trichloroethane
DNA	Deoxyribonucleic acid
GPS	Global positioning system
GLM	Generalized linear model

ABSTRACT

Malaria and RVF are two diseases whose onset of epidemics leads to massive losses in human lives. Both diseases are transmitted by infected mosquito vectors. Infected *Anopheles* mosquitoes transmit *plasmodium* parasites that cause malaria while infected flood water *Aedes* species is responsible for primary transmission of RVF viruses. Most scientists are biased on adult stage control of mosquito species. However, the high mobility of adults has enabled them to adopt, changing their biting and resting patterns such that interventions targeting their behavior are rendered ineffective. This makes interventions that target immature stages more advantageous. For effective implementation of immature stage based control strategies, information on their diversity and distribution in various habitats distributed along altitudinal gradients is important.This study investigated the diversity and distribution of malaria and RVF mosquito vectors at immature stages along an altitudinal gradient in Baringo County, Kenya. It was conducted between June and September 2014, which was during the short rains. The species identified in the entire study area (800m to 2300m altitude) were *Culex quinquefaciatus*, *Cx. annulioris*, *Cx. pipiens*, *Cx. poicilipes*, *Cx. tigripes*, *Anopheles pharoensis*, *An. gambiae s.l*, *An. coustani*, *An. funestus* and *Aedes taylori*. Altitude was divided into three classes; 800m to 1300m, 1301m to 1800m and 1801m to 2300m. *Aedes taylori* and *Culex tigripes* were only in the 1801m to 2300m altitudinal class while *An. funestus* was only in the 800m to 1300m altitudinal class. The altitudinal class between 1801m to 2300m, had the lowest Shannon-wiener diversity index (H'=0.9836) and the highest number of species (9species). Comparison of mosquitoes collected in habitats in different altitudinal classes revealed variations in the respective species counts (χ^2_9 =127.47; p-value < 0.001). The only species whose distribution showed correlation with altitude was *An. pharoensis* (r = -0.40; t_{32}=-2.50; p=0.02). The highest species diversity was recorded in river banks where the water was clear and vegetation present. Stepwise regression analysis revealed that suitability of a habitat for vector breeding was mainly dictated by water quality and the presence of vegetation. The results in this study reveal the need for continuous monitoring of vectors not only in the low land areas but also in the highland areas to avoid sudden epidemics of malaria and RVF.

CHAPTER ONE

1.0: INTRODUCTION

1.1 Background information

Malaria and Rift Valley Fever are some of the vector-transmitted diseases that have claimed many lives in tropical Africa (Woods *et al*., 2002; WHO, 2013b). Malaria is caused by protozoan parasites of the genus *Plasmodium* transmitted by infected female mosquitoes of the genus *Anopheles*. It is currently the leading cause of mortality and morbidity in many countries with 90% of the mortalities in Africa (WHO, 2013a). In Kenya, 20% of reported child mortalities under 5 years are as a result of malaria (KEMRI, 2014). Baringo County in Kenya is one of the malaria endemic zones and experiences seasonal epidemics.

Rift valley fever, the second vector transmitted disease is caused by a Phlebovirus of the family Bunyaviridae. Rift valley fever is maintained by trans-ovarian transmission in flood water *Aedes* mosquitoes. Outbreaks are associated with heavy, prolonged rainfall which are often associated with the El Niño phenomena. Secondary transmission in outbreaks is mainly by female *Culex* mosquitoes and biting flies (Swanepoel *et al*., 2011; El Vilaly *et al*., 2013). In the 2006 to 2007 Kenyan epidemic, a total of 684 cases were reported including 155 human deaths (23%). Amongst the 684 cases, about 183 were in the rift valley (WHO, 2007), which Baringo district, now Baringo County, was part of.

Like other insect species, the distribution range of many insect disease-vectors including the *Anopheles, Culex* and *Aedes* species, is defined by climatic factors that favor their respective physiological functions (Githeko *et al*., 2000). Factors such as temperature, humidity and precipitation tend to vary along the altitudinal gradient (Li *et al*., 2012). Altitude therefore indirectly defines the occurrence and distribution of insect vector species in many regions and

sometimes creates buffer zones for vector borne diseases (WHO, 1975; Cox, 1999). The altitudinal ranges of these climatic factors are changing with the general global climate change. These changes are likely to affect vector distribution ranges (Wettstein & Schmid, 1999; Kiratani, 2006). It is therefore important to continuously monitor changes in the diversity and distribution of these vectors with the aim of preventing outbreaks of vector-borne diseases (Wettstein & Schmid, 1999; Kiratani, 2006). Such information can be used to determine epidemic thresholds for purpose of vector management (Bacaer & Guernaoui, 2006).

1.2 Statement of the problem

Due to increasing malaria epidemics, proponents have advocated for integrated mosquito management as a strategy for combating and monitoring mosquito borne diseases (Schiff, 2002; Utzinger *et al*., 2002). This involves both adult and larva control methods using available resources with minimal disturbance/damage to the environment (Walton *et al*., 2013; Fonseca *et al*., 2013; Smith *et al*., 2013). However, most scientists are biased on the control of adult stages. This is currently proving difficult because of behavioral adaptations such as change in biting periods by mosquito adults (Russell *et al*., 2013; Aziz, 2014). This has led to shortcoming in interventions such as the use of bed nets.

With such behavioral trends in adult stages coupled with projected change in the distribution range, it is important to concentrate resources on control of aquatic stages. They are less mobile (Killeen *et al*., 2002) and targeting their habitats would be effective in controlling vector populations (Chaki *et al*., 2014). Due to the fact that distribution of vectors is affected by

various environmental factors, this approach can be effective with good knowledge on the diversity and distribution of different species in aquatic habitats along the altitudinal gradient.

1.3 Justification

Information on the diversity and distribution of immature stages of mosquitoes along the altitudinal gradient that would form the basis of management is scarce in Baringo County, Kenya. Most studies have been undertaken only in the low altitude areas which constitute the lake region of the county. This study will fill in the gaps by providing information on the diversity distribution of immature vectors of malaria and RVF in habitats within the high and mid altitude areas and add to the existing knowledge on the diversity and distribution of immature vectors of malaria and RVF in habitats within the low altitude areas of the county.

1.4 Hypothesis

Diversity and distribution of immature malaria and RVF mosquito vectors vary among habitats along the altitudinal gradient in Baringo County, Kenya.

1.5 Objectives

1.5.1: Broad objective

To determine the diversity and distribution of malaria and RVF mosquito vector larvae in different aquatic habitats along an altitudinal gradient in Baringo County, Kenya.

1.5.2 Specific objectives

a) To determine the diversity and distribution of malaria and RVF mosquito vector species larvae along the altitudinal gradient.

b) To evaluate habitat suitability for Malaria and Rift Valley fever vector breeding based on water quality, vegetation and presence of other organism in a habitat.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of Mosquitoes

Mosquitoes are insects classified in the order Diptera, suborder Nematocera and family Culicidae. They have two main subfamilies; Anophelinae and Culicinae with about 41 genera and over 3000 species (Harbach, 2007). The life span of adult mosquitoes vary among species and generally ranges between two weeks and one month (Levin, 2014). Life span is however determined by motility factors such as predators and parasites. Knowledge of motility factors is thus essential for Entomologists in the control of mosquito populations (Levin, 2014).

2.1.1 Life cycle of Mosquitoes

Mosquitoes are holometabolous insects with four distinct life stages; egg, larvae, pupae and adult. Eggs are oviposited by an adult female either in mud or in water with floats or as a raft depending on species. *Anopheles* species mostly oviposit their eggs in aquatic habitats, singly with floats while *Culex* species oviposit their eggs in aquatic habitats clustered together as a raft (Clements, 2011). Most *Aedes* species prefer to oviposit their eggs singly in muddy habitats. Hatching may take two days to months depending on the species and other parameters such as the photoperiod, humidity and temperature. *Aedes* eggs are very resistant and can diapause in mud for months. Eggs hatch into larvae (Clements, 2011).

There are four larval instars in mosquitoes. Depending on ambient temperature, larval development from the first instar to the pupae may take an average range of three days to seven days. Larvae are mostly filter feeders. They feed on algae, micro fungi, bacteria and micro plant debris in the habitats (Levin, 2014). However, larvae of some mosquito species such as *Culex tigripes* are predators and tend to feed on other larvae in the habitat (Appawa *et al*., 2000).

Unlike most Diptera where the pupal stage is a dormant stage, mosquito pupae are very active. Just like the larvae, they are aquatic. The Pupal stage lasts from one to three days, depending on environmental factors such as ambient temperature. Adults emerge from the pupae. The adult can either be Male or female, distinguishable by morphological features such as the antennae (Clements, 2011).

2.1.2 Morphological adaptations of Mosquitoes

The various mosquito stages have varied morphological adaptations that enable them survive in their varied habitats. As previously described, eggs have floats or are laid clustered as a raft to enable them float in water. The coloration of eggs varies in relation to species (Carpenter & La Casse, 1955).

Larvae are cylindrical in shape. The body is divided into head, thorax and abdomen, with nine abdominal segments. The eighth abdominal segment has a respiratory siphon in the family Culicinae while the family Anophelinae lacks a respiratory siphon and instead, has spiracles on the eighth abdominal segment. The siphon is directly connected to the tracheal system and is used for ventilation. The larvae swim to the surface of the water to breath. Due to lack of a respiratory siphon, larvae of the family Anophelinae orient parallel to the water surface in a habitat as they gain air while Species from the family Culicinae oriented at an angle to the water surface in a habitat to gain air. Adjacent to the siphon is the sandal. The arrangement of hairs and papillae on the abdominal segments are essential for taxonomic purposes. Other features used for taxonomic purposes include setae, siphon, sandal, combs and color of either the siphon or the head. Unlike larvae, the pupae are comma shaped, head and thorax fused into a cephalothorax, with a pair of breathing trumpets and the abdomen curving below the cephalothorax. Pupae also swim to the surface of the water to gain air (Levin, 2014).

Adult mosquitoes are terrestrial with the basic adult Diptera structure of head, thorax and abdomen, and a pair of wings on the thorax. The mouth parts of mosquitoes are elongated into a stylet, modified for piercing and sucking. Features on wings, legs and abdomen are important for taxonomic purposes (Levin, 2014).

2.2 Feeding behavior of Mosquitoes

Male mosquitoes mostly feed on nectar and plant sap. Apart from nectar and plant sap which provide energy for flight, female mosquitoes need vertebrate blood to obtain concentrated proteins for egg production. After a blood meal, Yolk protein precursors are produced in the fat body of the female mosquito. The yolk protein gene is only expressed after a blood meal related signal in anautogenous mosquitoes (Hansel *et al*., 2014). This blood sucking habit makes the female mosquito a potential vectors and cyclic hosts for some disease causing microorganisms.

Feeding preferences of mosquitoes are varied. Some species prefer to feed on certain vertebrates and not others. An example is the *Anopheles culicifacies* and *An. stephensi in india,* which predominantly prefers human blood (Swami & Srivastava, 2012). Endophagic species prefer to feed on vertebrates that are in door as compared to outdoor while exophagic species prefer to feed on vertebrates that are out door as opposed to indoor feeding. Exophagic species are mostly exophilic meaning they prefer to rest outdoor while endophagic species are mostly endophilic meaning they prefer to rest indoors. An example is; *Anopheles pharoensis* have been documented as exophilic and exophagic while *An. gambiae S.l* and *Anopheles funestus* have been documented as endophagic and endophilic (Aniedu, 1993).

2.3 Malaria and RVF vector species

Not all species are responsible for the spread of malaria. Only some subgenera in the genus *Anopheles* are known to be cyclic hosts of human infecting *Plasmodium* species, which

are; *Plasmodium falciparum*, *plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium vivax* (Sinka, 2013). However all biting mosquito species are potential secondary vectors of Rift Valley Fever (Linthicum *et al*., 1985; Sang *et al*., 2010).

Identification of these species is mostly based on distinct morphological characteristics on the head, thorax and abdominal segments (Harbach, 2004; Harbach, 2007). This requires skill and precise knowledge of the morphological features. With the advent of molecular techniques, DNA based identification procedures are becoming popular especially where morphologically indistinguishable sibling species exist such as in *Anopheles gambiae s.l.* mtDNA sequencing is the most widely used technique for species identification which is favored by an increasingly huge, easy to access data base of insect sequences worldwide (Wells & Škaro, 2014).

2.3.1 Malaria vector species

The genus *Anopheles* is divided into about six subgenera with over 400 species. The subgenera are *Celia, Anopheles, Kerteszia, Lophopodomyia, Nyssorhynchus* and *Stethomyia* (Harbach, 2004). Most of the species in the subgenus *Anopheles*, series *Myzorhynchus and Anopheles* spread malaria. In Kenya, the most efficient vectors are *Anopheles gambiae* complex and *Anopheles funestus* group (Minakawa *et al*., 2002 a, b). *Anopheles gambiae* is composed of six morphologically indistinguishable siblings. However, they portray a difference in behavior (Scot *et al*., 1993). These are *Anopheles gambiae s.s, An. arabiensis, An. melas, An. merus, An. bwambe, An. quadriannulatus* A and B. Among these, *An. quadriannulatus* do not spread malaria.

In Kenya, *Anopheles gambiae s.s, An. arabiensis* and *An. merus* are the most common. *Anopheles gambiae s.s* is adapted to cooler and more humid environment and is most common in rainy seasons. *Anopheles arabiensis* is adapted to drier regions. It is the most abundant during

the dry seasons because it prefers breeding in permanent water bodies. *Anopheles merus* breeds in salty waters and is found mainly on the coastal strip along the Indian Ocean. The first two are the most efficient transmitters of malaria in Kenya. *Anopheles merus* is regarded as a minor vector (Minakwa *et al.,* 2002a,b).

Anopheles funestus group is composed of nine morphologically similar species. According to classification by Gillies and De meillon (1968), they include; *An. funestus s.s* , *An. aruni* Sobti, *An. parensis* Gillies, *An. vaneedeni* Gillies and Coetzee, *An. confulis* Evans and Leeson, *An. fuscivenosus* Leeson, *An. leesoni* Evans and *An. rivulorum*. The first four are distinguishable by their adult characteristics. The rest are distinguishable by their larval characteristics. In Kenya, the most common are *An. funestus s.s, An. parensis, An. leesoni* and *An. rivulorum.*

2.3.2 Rift valley fever vector species

Rift valley fever is a zoonosis caused by rift valley fever virus. In animals, the main mode of transmission is by obtaining an inoculum of the virus from infected vectors as they feed on the animal's blood. In humans, the main mode of transmission is through direct or indirect contact with infected animals, such as drinking unpasteurized milk and feeding on infected meat (Linthicum *et al*., 1985; Gaff *et al*., 2007).

There are two types of vectors, primary vectors and secondary vectors. Primary vectors are flood water species of the genus *Aedes*. This is because they have been documented as reservoir hosts of RVF virus due to trans-ovarian transmission and ability of the eggs to diapause in soul for months or years until there is flooding (Sang *et al*., 2010). Flood water *Aedes* species identified in Kenya include *Aedes mintoshi, Ae. ocharacieus, Ae. sudanensis,* and *Ae. circumluteolus* (Lutomiah *et al*., 2013).

However, many species of mosquitoes and *Phlebotominae* are susceptible to RVF if they feed on an infected host and are able to cause secondary transition of the virus. The wide range of such secondary vectors is what causes sudden epidemics of the virus after primary infection by the flood water *Aedes* species (Linthicum *et al*., 1985). This is indicative of the fact that most female mosquitoes of the two sub families, *Culicinae* and *Anophelinae* are capable of secondary transmission of the virus.

2.4 Mosquito ecology

Larvae and pupae of mosquitoes are adapted to aquatic habitats while adult mosquitoes are adapted to terrestrial habitats. Mosquitoes, like most insects, are climate sensitive and altitude in many cases defines their niche breadth (WHO, 1975; Cox, 1999). A study in western Kenya and the Rift Valley found that in western Kenya, *Anopheles arabiensis* were not found in areas above 1400 m elevation while *Anopheles gambiae* and *Anopheles funestus* were found in areas above 1700 m. In the Rift Valley, *Anopheles funestus* was abundant while no *An. Gambiae* was recorded. (Minakawa *et al.,* 2002a).

Ecological preferences cause a differentiation in vector competence of various mosquito species. Temperature, humidity, breeding sites and host attractiveness are some of the factors that influence species abundance (Petrarca *et al*., 1999).

During the dry season, survival tactics of *Anopheles gambiae s.l* have been examined in western Kenya by Minakawa *et al.* (2001). It was found that mosquito eggs underwent an embryonic diapause for at least few days in moist soil. Adult mosquito's preferred to lay their eggs in flooded soil and if this was not available, they preferred to lay in moist soil. This was under laboratory conditions. Other factors observed were adults producing and laying more eggs continuously during the dry season and shorter larval development time during drier seasons.

In manmade habitats, mosquito larvae thrive in areas where human activity is more. This is because in these areas, biological succession is lower; implying a lesser number of natural enemies. Mosquitoes undertake short periods from egg to larvae in optimum conditions hence they are able to survive and undergo fast cycles despite the disturbance. This is as seen in a study done in western Kenya by Carlson *et al.* (2004). There were more larvae in active brick pits than in abandoned brick pits.

A study conducted by Mala *et al*. (2011) in two regions within Baringo; Kamirimar and Tirion, showed that *Anopheles arabiensis* was the only sibling species of *Anopheles gambiae s.l.* in both sites and it was also the dominant *Anopheles* species. Other species identified were, *An. Funestus, An. Pharoensis* and *An. Cousteni* in Kamarimar. These are regions where altitude is below 1200m.

2.4.1 Larva and pupae habitats

Mosquito larva and pupa can be found in a variety of aquatic habitats. They prefer habitats with shallow water with little to no flow. In permanent habitats such as lakes and swamps, mosquito larvae and pupa can be found mainly on temporary pools along the habitat such as animal hoof prints or on the shallow margins of the water body (Pemola & Jauhari, 2005).

Oviposition habitats vary with species in some cases. For instance, *Aedes aegypti* prefer to oviposit in stored water within human settlements such as open water tanks (Powell & Tabachnick, 2013). Other species prefer to oviposit along permanent water bodies such as *Anopheles arabiensis* (Norkute, 2014). Some species show no preference to special habitat conditions such as *Culex quinquefaciatus*. The species has been labelled as an invasive species and can be found all over the world in all forms of habitat. However, genetic sequences have

shown that the species has variant strains with some able to act as vectors of Bancroftian filariasis caused by *Wuchereria bancrofti* (Bockarie *et al*., 2009). Additionally, it has been found that mosquito species use chemical cues to determine habitats where conspecific larvae have been, which are preferred (Himeidan *et al*., 2013).

After eggs have been oviposited and larvae hatched, various biotic and abiotic habitat factors control larvae populations by either reducing them or maintaining their number. The biotic characteristics include vegetation, predators, crowding and abundance of food substances. Larvae thrive more in habitats with vegetation such as emergent and submerging plants. The vegetation has various advantages such as shielding the larvae from excessive sun light, predators such as fish and providing oxygen for larvae that gain oxygen by piercing into plant tissues, mainly larvae of *Mansonia* species (Merritt *et al*., 1992). Larvae in habitats with more micro fungi and other microbes that larvae feed on tend to have more larvae. In crowded habitats, larvae compete for food resource and space such that selection pressure causes death of weak larvae. However, before oviposition, female adult mosquitoes use chemical and visual cues to determine habitats with larvae food, vegetation and predators (Bentle & Day, 1989)

Abiotic factors that control larvae populations include chemical properties of water, temperature and duration of the habitat. Effects of abiotic factors such as salinity are dependent on the species. An example is the preference of salty water by *Anopheles merus* in Kenya (Minakwa *et al.,* 2002b).

2.5 Mosquito control

Mosquito breeding patterns require consistent monitoring and control to prevent malaria and RVF epidemics. There is a positive correlation between the rainy seasons and mosquito population increase. This is attributed to increase in larval habitats (WHO, 2013b). Mosquito

larvae control methods can be divided into three classes; biological control, chemical control and environmental control.

Biological control involves the use of natural enemies to control population levels of a vector. An example is the use of *Bacillus thuringiensis* var. israelensis in control of mosquito larvae (Mullar, 1990). The use of fish such as *Oreochromis niloticus* has been favored as a good biological control method in western Kenya. This is because *Oreochromis niloticus* is not only a good larval control measure, but also edible by human beings (Howard *et al*., 2007).

Chemical control involves the use of insecticides and larvicides in mosquito control. The mode of action is different in various categories of insecticides and larvicides. Resistance to various categories has been documented, which has led to augmentation between various categories for efficiency (Hemingway & Ranson, 2000). Some insecticides categorized in the persistent organic pollutants (POPs) are not used in control such as DDT. However, their use is allowed in vector control where the effects of not using them are greater than the effects of using them. Recently, the use of insecticide treated bed net has been advocated as good control measure for adult mosquitoes. The nets are treated with pyrethroids (WHO, 2006). The future of insecticides has an addition of insect growth regulators which unlike Pyrethroids, carbamates, organophosphates and organochlorines, are less harmful to non-target organisms such as fish and birds (Benelli, 2015). This is seen in studies on mosquito nets incorporating Pyriproxyfen, a known insect growth regulator (Ngufor *et al*., 2014).

Environmental control has been used even before the advent of insecticides. This involves manipulating the environment so that mosquito habitation is not favored. Some of the environmental practices involve draining of stagnant water in puddles and containers, ensuring proper management of household wastes especially empty cans and polythene bags ensuring

water does not accumulate in them creating temporary habitats, properly covering stored water in tanks and other forms of containers to avoid mosquito oviposition and clearing unnecessary bushes to reduce mosquitoes resting places (Utzinger *et al.,* 2001).

2.6 Species diversity and distribution

Diversity contains two distinct concepts: Species richness and species evenness. Species richness is a measure of the number of species in a community. Species evenness is concerned with the distribution of individual species within the ecological space. A combination of species richness and evenness is commonly referred to as heterogeneity. This term is the same as diversity. Species richness and species evenness can both be measured separately. However, there are various methods developed that combine both and these are the heterogeneity measures (South wood, 1978). There can be no ready-made formulae for the number of localities to be sampled and the amount of work to be carried out in a mosquito survey project. Obtaining a satisfactory and comparable sample is left to the judgment of the entomologist. It is however recommended to have at least four or three sample sites within an area for proper statistical analysis (WHO, 1975).

Factor that affect the manner in which taxons are arranged in space, species distribution, vary based on the type of species based on niche characteristics (South wood, 1978). A specialist species is only able to survive in a particular environment that it is adopted to, such as a particular temperature range, precipitation rage, vegetation type, and water body with specific chemical parameters (South wood, 1978). The distribution of specialist species tends to be narrow. On the other hand, generalist species tend to have very high limits in terms of environments that they can occupy, for example, such a species that can occupy areas with a wide variety of vegetative matter, very wide temperature tolerance range, has a wide range of

food matter. The distribution of generalist species tends to be very wide (South wood, 1978). These factor in turn affect the diversity of species in a particular genus within a habitat (South wood, 1978).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the study area

The study area is approximately 252Km, North West of Nairobi, and measuring approximately 3,500 km^2. It lies in an agro-pastoral zone within Baringo County. Temperature range is between 24° in the cold season and 30° degrees in the warm season. Average annual rainfall in the highland is between 1000mm and 1500m while the low lands experience an annual rainfall of about 600mm. It is located between 35.602 E, 0.541N and 36.277 E, 0.723 N with elevation ranging from 800m to 2300m (Fig. 1). This area is characterized by presence of lakes and rivers, some of which are seasonal.

3.2 Sampling points

Sixteen sampling points were established in the study area with the help of officers from Marigat DVBDU and google earth android application. The points were selected based on availability of potential larval habitats and accessibility. Coordinates and elevation of each point were recorded from a hand held GPS receiver (Garmin, model *e*trex 10).

Elevations were divided into three classes for the purpose of analysis, based on land cover as viewed on an Arc map 3.0 imagery base map (Fig. 1). They included 800m to1300m to represent low altitude gradient, 1301m to 1800m for mid-altitude and 1801m to 2300m for high altitude. The class range was obtained by subtracting the lowest (800m) from the highest point (2300m) in the study area. The difference was then divided by three. One was added to the lower limit of each class except for the first range to avoid points falling in two classes.

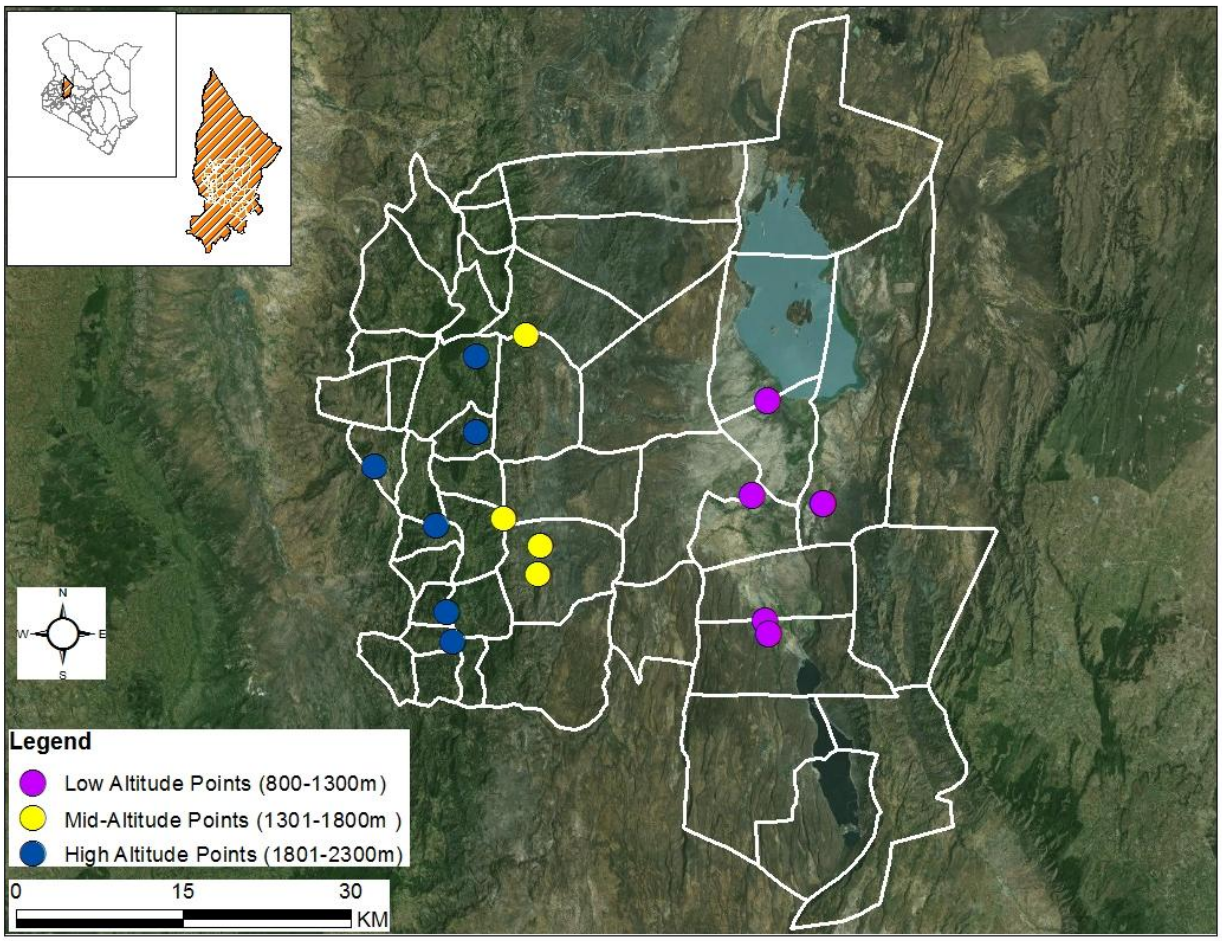

Figure 1*:* Map of Baringo County, Kenya, showing the study area and sampling points grouped into the three altitudinal classes.

The sampling points were grouped in the three altitudinal classes. The Low altitude points were, Kapkuikui, Loboi, Lake 94, Nteppes, Salabani and Kambi ya Samaki. The Middle altitude points included: Kipcherere, Kimau, Yomu, Sabor, Kabeswa and Sabor. While the high altitude points included: Kurget, Talai, Kaplewa, Kaptimbor, Borowonin, Tandui, Sacho, Kamonol and Sacho.

3.3 Habitat census

Potential habitats were identified within a 50m radius from the sampling point. The 50m radius was arrived at while considering individuals undertaking the sampling exercise on foot and the minimum distance recorded in adult mosquito flight experiments (Tsuda *et al*., 2008; Verdonschot & Besse-Lototskaya, 2014). An area was identified as a potential habitat if there was water with little to no flow (stagnant). This was because mosquitoes prefer shallow water with minimum flow/stagnant water (Norris, 2004).

The habitats were classified according to their nature, based on a combination of factors. There were habitat forms such as a hoof print, swamp, water pan, dam, stream margins, spring margins, pit, lake, flood zone and marsh (Fig. 2a-g), presence or absence of vegetation, presence or absence of any other aquatic organisms apart from immature mosquitoes and water quality which was qualitatively classified as clear or turbid. The various combinations of these factors were observed and recorded during collection of immature mosquitoes. Turbidity was estimated by dipping and collecting water with transparent 100ml container in the habitat from down-up. Collected water was allowed to settle for 2minutes in the container before checking the visibility of a three inch white tile placed under the container. If the tile was visible, the habitat was classified as clear and if it was not visible, the habitat was classified as turbid. In Habitats too shallow for using the 100ml container, Observation was done directly in the habitat.

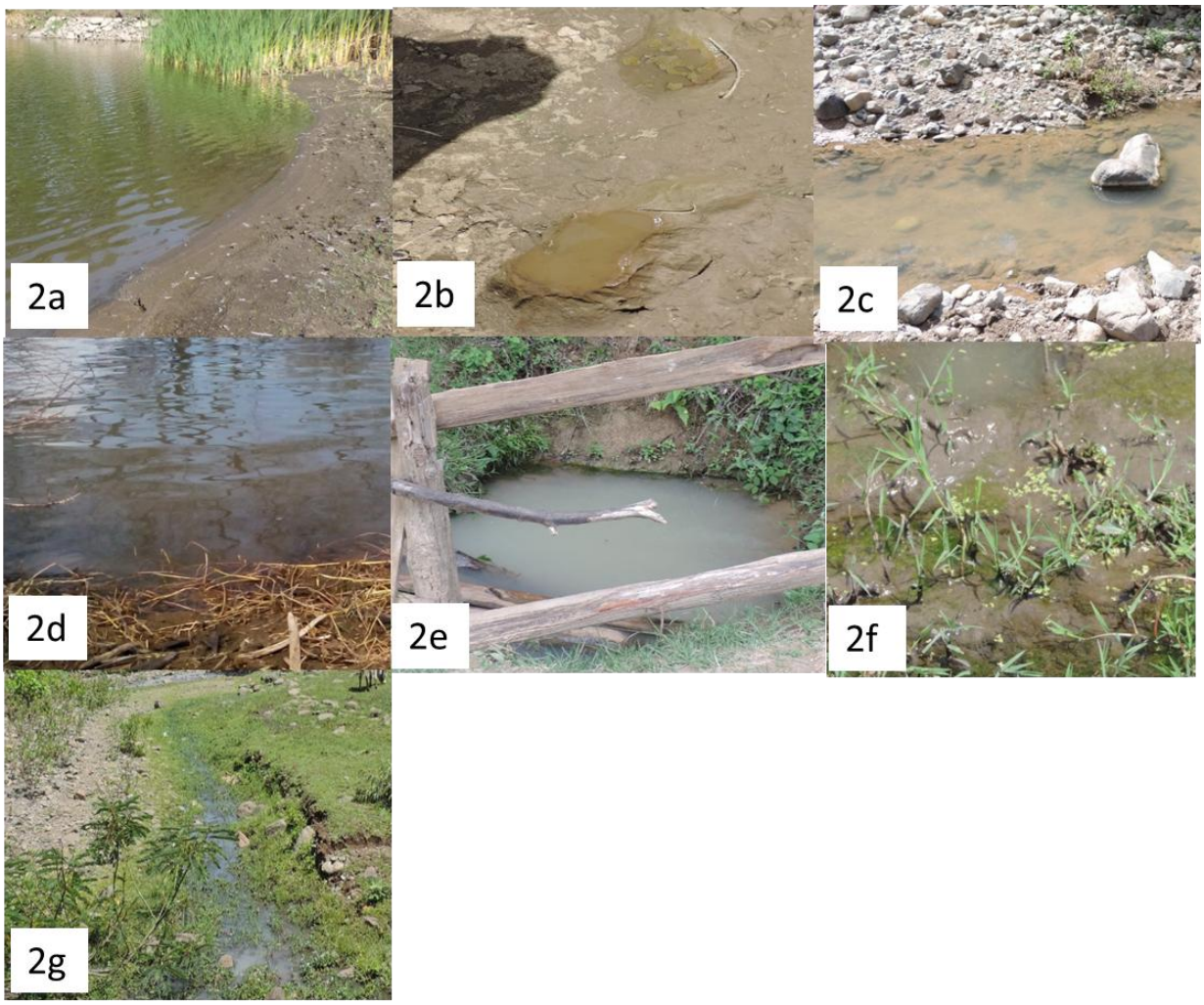

Figure 2: Images of different aquatic habitats: dam margin (2a); animal hoof print (2b); stream bank (2c); Lake Flood zone (2d); water pit (2e); mash (2f); spring bank (2g).

3.4 Sampling of mosquito larvae from aquatic habitats

Sampling for immature mosquitoes was carried out after every two weeks between 6th June 2014 and 28th August 2014. The sampling period coincided with the rainy season and a total of five sampling sessions were completed in all selected aquatic habitats.

During sampling, immature mosquitoes were collected using 350ml WHO standard dippers at a maximum of 30 dips per habitat (Fig. 3). Plastic pipette was used in extremely shallow habitats. The sampler ensured that his shadow was cast away from the habitat. This minimized chances of immature mosquitoes swimming to the bottom of the habitat. The dipper was lowered gently at an angle of 45^0 so that collection was by displacement suction. This way, there was minimal water disturbance, increasing the probability of capturing more immature mosquitoes. Where there was dense vegetation, water was disturbed, so that larvae and pupae moved downwards. Vegetation was then cleared using the dipper. A wait period of 3 to 4minutes would ensue before collecting the immature mosquitoes. In clumps of vegetation such as grass, the dipper was pressed gently into the vegetation so that water flowed in.

Figure 3: Researcher inspecting the dipper for immature mosquitoes.

After collection, the immature mosquitoes were transferred into a sealable collection cup using a plastic pipette, or directly from the habitat onto a pipette and finally into the sealable cup. The collection cups were filled with water sourced from respective sampled habitats to avoid desiccation of the specimen. A pencil written label, indicating the point and date of collection were immersed into the cup before sealing and subsequent transportation to the DVBDU laboratory in Marigat. In the laboratory, third and fourth instar larvae were identified (while still alive on a petri dish, using a dissecting microscope), and separated from second and first instar larvae. The third and fourth instar larvae were stored in labelled, sealable cups containing 80% ethanol, waiting identification to species level

The first and second instar larvae were put in labelled cups, three quarter full of water from the source habitat containing algae and loosely sealed to allow air in and out (Fig. 4a & b). They were left at room temperature (an average of 29^0C during the day) to allow development to fourth instar. Each cup contained not more than 12 larvae. An experiment conducted a week before the first collection showed that there is reduced development in instances where there were more than 12 larvae in a cup. It was also observed that development from second to fourth instar, at room temperature took a minimum of one and a maximum of two days in cup containing water and algae from the source habitat compared to four days when tap water was used.

Figure 4: Collection cup containing immature mosquitoes (4a), Algae in a habitat (4b).

3.5 Identification of larvae

Ethanol preserved larvae were identified to species level using third and fourth instar morphological keys under guidance from experts in the Marigat DVBDU (Mark Rotich and Richard Borr). This was by observing features such as color of the head, arrangement and shape of abdominal setae, number and type of combs, distinct hairs on the sandal, siphon index and other markings and features on the body surface as guided by the identification key (Gillies & de Meillon, 1968). This was done under a dissecting microscope (Fig. 5).

Figure 5: Identification of mosquito larvae under a dissecting Microscope in the laboratory

3.6 Data analysis

Species diversity analysis was performed on PAST version 2.17c. Other statistical analyses were conducted on R version 3.1.1. To standardize the abundance of a species collected in each habitat, the total number of individuals collected for that species in the habitat was divided by the average number of dips in the habitat and the quotient multiplied by 30. Thirty (30) was the maximum number of dips for all habitats. Standardization ensured that figures were comparable among all habitats. Comparison of mosquitoes collected in different altitudinal classes was done using the chi-square test. Generalized linear model (GLM) was used to estimate the effect of various habitat parameters on diversity and the abundance of species. Linear correlation analysis was applied to estimate the association between altitude and the diversity and distribution of species in the study area.

CHAPTER FOUR

4.0: RESULTS

4.1 Diversity along the altitudinal gradient

A total of 1,536 immature mosquitoes were collected from which 10 mosquito species were identified. With respect to distribution along altitudinal gradients, 8 species (*Cx pipiens, Cx. quinquefaciatus, Cx. annulioris, Cx. poicilipes, An. pharoensis, An. coustani. An. gambiae* and *An. funestus*) were found in altitudinal class range varying between 800m and 1300m (H'= 1.462), with 7 (*Cx pipiens, Cx. quinquefaciatus, Cx. annulioris, Cx. poicilipes, An. pharoensis, An. coustani.* and *An. gambiae*) found between 1301m and 1800m (H'= 1.686) and 9 species (*Cx pipiens, Cx. quinquefaciatus, Cx. annulioris, Cx. poicilipes, An. pharoensis, Cx. tigripes, An. coustani, An. gambiae* and *Ae. taylori*) between 1801m and 2300m (H'= 0.9836) (Table 1). Of all the species identified, only seven (7) species (*Cx pipiens, Cx. quinquefaciatus, Cx. annulioris, Cx. poicilipes, An. pharoensis, An. coustani* and *An. gambiae*) were common in the three altitudinal zones, with *An. funestus* limited to lower altitudinal zone while both *Cx. tigripes* and *Ae. taylori* were found in higher altitudinal zones (1801m-2300m) only.

The Buza and Gibson evenness (e^H'/S) showed that the 1301m to 1800m altitudinal class had higher evenness (0.77), followed by 800m to 1300m altitudinal class (0.53), and 1801m to 2300m altitudinal class (0.3) (Table 1).

Table 1: Different mosquito species collected in different altitudinal class ranges.

	Total abundance		
Species	800m-1300m	1301m-1800m	1801m-2300m
Culex pipiens	206.3	91.9	175.0
Cx. quinquefaciatus	886.6	409.0	1684.2
Cx. annulioris	76.0	126.5	130.0
Cx. poicilipes	18.8	126.3	105.9
Cx. tigripes	0.0	0.0	24.9
Anopheles pharoensis	385.2	205.0	44.3
An. coustani	108.7	56.3	9.5
An. gambiae	74.5	39.3	15.0
An. funestus	18.0	0.0	0.0
Aedes taylori	0.0	0.0	43.3
Taxa	8	7	9
Individuals	1774.1	1054.3	2232.1
D	0.318	0.2289	0.5821
H'	1.462	1.686	0.9836
e^H/S	0.5395	0.7712	0.2971

*In columns are standardized numbers of mosquito larvae (Relative abundance)

Comparison of mosquitoes collected in different altitudinal classes revealed variations in the respective species counts (χ_9^2 =127.47; *p*-value < 0.001). There was however no variation in the total number collected among the different altitudinal classes (χ_2^2 = 2.17; *p*-value = 0.34). Of the 1,536 immature mosquitoes collected, *Cx. quinquefaciatus* constituted 58.8%, dominating the species community, while *An. funestus* made only 0.04% of the total collection.

4.2 Distribution of mosquito species in the altitudinal ranges

The distribution of various species along the altitudinal ranges was varied (Table 1). However, most of the species showed no correlation with altitude. This is as described below.

4.2.1 *Culex* species

Four *Culex* species were identified in both 800m to 1300m and 1301m to 1800m altitudinal ranges with five *Culex* species identified in 1801m to 2300m altitudinal range (Table 1). *Culex* species identified in 800m to 1300m included *Cx. quinquefaciatus* (886.6), *Cx. pipiens* (206.3), *Cx. annulioris* (76.0) and *Cx. poicilipes* (18.8), while *Cx. quinquefaciatus* (409.0), *Cx. poicilipes* (126.3), *Cx. pipiens* (91.9) and *Cx. annulioris* (126.5) were identified in 1301m to 1800m altitudinal range. The five *Culex* species identified in 1801m to 2300m ranges included *Cx. quinquefaciatus* (1684.2), *Culex pipiens* (175.0), *Cx. annulioris* (130.0), *Cx. poicilipes* (105.9) and *Cx. tigripes* (24.9).

Culex quinquefaciatus was the most abundant mosquito species in the entire study area and the most abundant *Culex* species in the three altitudinal class ranges. *Culex poicilipes* was the least abundant in the class range between 800m to 1300m while *Cx. annulioris* and *Cx. pipiens* were the least abundant in the altitudinal class range between 1301m to 1800m. *Culex tigripes* was only in the altitudinal class range between 1801m to 2300m. It was also the least abundant in this altitudinal class range (Table 1). Further analysis showed that none of the *Culex* species had a significant correlation with altitude ($p>0.05$; Table 2).

Table 2: Correlation of the effect of altitude on the distribution of different mosquito species

	Correlation to Altitude			
	r	***T***	***Df***	***P***
Aedes taylori	0.32	1.91	32	0.07
Anopheles coustani	-0.24	-1.43	32	0.16
An. fenestus	-0.16	-0.92	32	0.37
An. gambiae s.l	-0.18	-1.01	32	0.32
An. pharoensis	-0.40	-2.50	32	0.02
Culex annulioris	0.05	0.31	32	0.76
Cx. pipiens	0.04	0.22	32	0.83
Cx. poicilipes	0.21	1.23	32	0.23
Cx. quinquefaciatus	0.26	1.49	32	0.14
Cx. tigripes	0.39	2.39	32	0.23
Diversity in habitats	-0.34	-2.07	32	0.05

4.2.2 *Anopheles* species

Distribution of *Anopheles* species in the altitudinal class ranges were, *Anopheles pharoensis* (385.2), *An. coustani* (108.7), *An. gambiae s.l* (74.5) and *An. funestus* (18.0) between 800m to 1300m; *Anopheles pharoensis* (205.0), *An. coustani* (56.3) and *An. gambiae s.l* (39.3) between 1301m to 1800m; *Anopheles pharoensis* (44.3), *An. gambiae s.l* (15.0) and *An. coustani* (9.5) between 1801m to 2300m (Table 2).

Anopheles pharoensis was the most abundant among *Anopheles* species in all altitudinal class ranges, with its population significantly correlated with altitude (r = -0.40; t_{32}=-2.50; p=0.02; Table 2). In comparison to *Culex* and *Anopheles* species, it was the second most abundant species after *Cx. quinquefaciatus*. *Anopheles funestus* was only found in the 800m to 1300m altitudinal class range with the least abundance.

4.2.3 *Aedes* species

Aedes taylori was the only *Aedes* species present. Its distribution was not correlated with altitude (r = 0.32; t_{32}=1.91; p=0.07; Table 2). It was found only in the 1801m to 2300m altitudinal class range, with a relative abundance of 43.3.

4.3 Effects of ecological factors on diversity and distribution of species

Statistical analysis showed that some ecological parameters significantly affected distribution of mosquito species. Turbidity significantly affected the number of *Culex tigripes* (Turbid; ß= 2.38; t= 2.256; p=0.0343; Appendix 1, Table IV). Habitat form significantly affected the number of *Culex tigripes* (spring bank; ß= 3.951; t=2.403; p=0.0251; Appendix 1, Table VII), *Culex annulioris* (Hoof print; ß= 27.2; t=-2.195; p=0.039; Appendix 1, Table VII) and *Anopheles pharoensis* (Marsh; ß = 33.235; t=2.319; p= 0.0301; Appendix 1, Table VIII).

4.4 Most Preferred habitat for larvae

Shannon-Weiner diversity index showed that a River bank, where turbidity was clear and both vegetation and other organisms were present, recorded the highest diversity of mosquito larvae species (H' = 1.721; Table 3). Diversity in habitats showed correlation with altitude (r= -0.34, t_{32} =-2.07, df =32, p=0.05: Table 2).

Statistical analysis showed that only hoof print (ß= -0.5168; t= -2.617; p = 0.0157; Appendix xi), Water pit (ß= -0.498; t= -2.345; p = 0.0284; Appendix 1, Table XI) and presence of vegetation (ß= 0.597; t= 2.558; p = 0.018; Appendix 1, Table XI) significantly influenced diversity. Further analysis showed that a combination of vegetation and water quality had the greatest effect on diversity (AIC= 29.9). The most preferred habitat for larval species was therefore dictated mainly by vegetation and the level of water quality.

Table 3: Habitat species diversity

Altitude	Habitat form	Water quality	Vegetation	Other organisms	Number of Species	H^1
1334	Riverbank	Clear	Present	Present	7	1.721
1457.79	Dammargin	Turbid	Present	Present	5	1.467
1450.17	Springbank	Clear	Present	Present	5	1.466
1019.81	Marsh	Clear	Present	Present	5	1.458
1457.79	Dammargin	Clear	Present	Present	5	1.271
1925	Dammargin	Clear	Present	Present	4	1.219
1450.17	Springbank	Clear	Present	Present	4	1.203
982.93	Lakemargin	Clear	Present	Present	4	1.193
1323	Riverbank	Clear	Present	Present	5	1.123
987.2	Floodzone	Turbid	Present	Present	3	1.048
982.93	Hoofprint	Clear	Present	Present	3	0.9802
1019.81	Marsh	Turbid	Present	Present	4	0.9764
987.2	Floodzone	Clear	Absent	Absent	3	0.9743
983.24	Lakemargin	Clear	Present	Present	3	0.9103
2212	Springbank	Clear	Present	Present	6	0.8957
1015.24	Marsh	Clear	Present	Present	5	0.8025
983.24	Lakemargin	Clear	Present	Present	4	0.7834
987.2	Floodzone	Clear	Present	Present	3	0.6883
1457.79	Dammargin	Clear	Absent	Absent	2	0.6735
987.2	Floodzone	Turbid	Present	Present	2	0.672
2140	Waterpit	Clear	Present	Present	2	0.6555
999.7	Hoofprint	Clear	Present	Present	2	0.6269
999.7	Hoofprint	Clear	Present	Absent	2	0.6211
2177	Waterpan	Turbid	Present	Absent	2	0.5196
1837	Dammargin	Clear	Present	Present	2	0.518
2177	Waterpan	Turbid	Present	Absent	3	0.4769
2140	Waterpit	Turbid	Present	Present	4	0.4699
999.7	Hoofprint	Turbid	Present	Present	2	0.3365
2212	Springbank	Turbid	Absent	Absent	1	0
2179	Waterpit	Clear	Absent	Absent	1	0
2179	Waterpit	Turbid	Absent	Absent	1	0
2179	Waterpit	Turbid	Absent	Absent	1	0
999.7	Hoofprint	Clear	Absent	Present	1	0
987.2	Floodzone	Turbid	Absent	Absent	1	0

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

The only *Aedes* species identified was *Ae. taylori* in the altitudinal range between 1801m to 2300m. This species has been implicated as a vector of yellow fever in selvatic transmission. Its ability to feed on monkeys and humans enables it to spread the yellow fever virus from monkeys to human beings (Digoutte, 1999). Primary infections of RVF are a result of flood water *Aedes* species which are considered as reservoir hosts of RVF virus due to trans-ovarian transmission and ability of the eggs to diapause in soil for months or years until there is flooding (Sang *et al*., 2010). Flood water *Aedes* species in Kenya include *Ae. mintoshi, Ae. ocharacieus, Ae. sudanensis,* and *Ae. circumluteolus* (Lutomiah *et al*., 2013). None of these species were identified in the entire study area within the study period. The results therefore indicate that there was no risk of RVF primary outbreak based on the identified vectors in the study area during the study period. This was consistent with Baringo county vector borne disease unit (VBDU) data and public health records. They indicated no cases of RVF were reported between January 2013 and September 2014 within the study region.

The RVF virus has previously been isolated in all the three genera identified in the study area (Sang *et al*., 2010). Many species of mosquitoes and sandflies are susceptible to RVF if they feed on an infected host and are able to cause secondary transmission of the virus. The wide range of such secondary vectors is what causes sudden epidemics of the virus after primary infection by the flood water *Aedes* species (Linthicum *et al*., 1985). This indicated that, although there were no primary vector larvae species identified in the study area, in case of entry of infected individuals such as cattle into the region, there would be a possible epidemic especially

if this was in the rainy season as mosquito species reach their peak abundances during such seasons (Uyi, 2013).

Among the identified 10 species, five were *Culex* mosquito species; *Cx quinquefaciatus, Cx. pipiens, Cx. annulioris, Cx. poicilipes* and *Cx. tigripes*. Apart from being secondary vectors of RVF, *Culex* species have been implicated as vectors of various other arbovirus diseases. An example of such a disease is the West Nile Virus. The west Nile Virus is transmitted by *Culex* species, from birds to humans and other mammals. This is a result of their ability to feed on both mammals and birds (Molei *et al*., 2006). Evidence of the west Nile virus transmission in Kenya was found in mosquitoes collected in various parts including the former Rift valley province which Baringo, currently Baringo County, was part of (LaBeaud *et al*., 2011). None of the *Culex* species showed a significant correlation to altitude. This implied that in case of an emergence of RVF, West Nile Virus or any other disease spread by the *Culex* species, whose distribution was not limited by altitude, the disease may spread rapidly in the entire county, if rapid interventions are not initiated.

Culex quinquefaciatus was the most abundant species in the study area and *Cx tigripes* was the least abundant. *Culex quinquefaciatus*, apart from being among secondary vectors of RVF in epidemics in Kenya (Sang *et al*., 2010), it is also the main vector of urban lymphatic filariasis, caused by the nematode *Wuchereria bancrofti* (Bockarie *et al*., 2009). However, there are no cases of vector transmitted filariasis in Baringo County. Any cases that come in are from the coastal regions of Kenya. Mosquito species in the region are not able to transmit the disease (unpublished data, Baringo County, VBDU). *Culex tigripes* is a predator of other mosquito larvae and can be used as larval biological control (Appawa *et al*., 2000). With the increase in

highland malaria all over Kenya and considering it was in the high altitude regions, it can be exploited as a measure of reducing highland malaria transmission.

Like *Culex quinquefaciatus* and *Cx. tigripes*, the other three *Culex* species, *Cx. pipiens, Cx. annulioris* and *Cx. poicilipes* did not show any significant correlation with altitude. This is an indication that any diseases they transmit can be spread both in the highlands and the lowlands leading to infections in the entire region. *Culex pipiens* was implicated as the main vector maintaining the RVF epidemic in Egypt 1971 to 1978 (Hoogstraal *et al*., 1979). Laboratory test of *Cx. pipiens* strains have also shown that apart from being susceptible to RVF virus, they are also susceptible to West Nile Virus (Amraoui *et al*., 2012). It is also a primary vector of the Ndumu Virus (NDUV) as reported in a study done in Garissa, Kenya, where evidence of trans-ovarian transmission of the virus was recorded (Lutomiah *et al*., 2014). Studies in Senegal indicated that *Cx. poicilipes* was the main RVF virus vector after the 1998 outbreak in Mauritania (Diallo *et al*., 2000). RVF viruses were isolated from *Cx. annulioris* species in the 2007/2008 epidemic in Kenya (Sang *et al*., 2010). These are further indications that all the *Culex* species identified in the study area are secondary vectors of RVF and therefore the fact that they are not limited by elevation indicates that all regions of Baringo County have a potential risk of RVF secondary outbreaks.

Among the four *Anopheles* species identified in the study area, only *An. pharoensis* showed a significant correlation with altitude. However, between the altitudinal classes, the least abundances of *Anopheles* species were in the high altitude class (1801-2300m). *Anopheles pharoensis* was the most abundant Anopheles species, and second most abundant after *Cx. quinquefaciatus* amongst all species identified in the study area. This is contrary to what a study in 2011 established, where a sibling species of *An. gambiae s.l*, and *An. arabiensis*, was the most

abundant (Mala *et al*., 2011). *Anopheles pharoensis* has been documented as an efficient malaria vector in Senegal (Carrara *et al*., 1990). It might also be an efficient vector in Baringo County considering the many cases of malaria, which were higher during the study period (Unpublished data, Baringo county public health records). Studies on its biting habits in Kapkuikui village, Baringo County indicated that it bites more often outdoor than indoor and is exophilic (Aniedu, 1993). This might be the reason for its success since interventions in Baringo County mostly involve use of insecticide treated bed nets and pyrethrum spraying inside houses. These affect indoor bitters. *Anopheles funestus* and *An. gambiae s.l* are documented as endophilic and prefer biting indoors than outdoors (Aniedu, 1993). This might explain their low larval abundances compared to *An. pharoensis*. *Anopheles funestus* larvae were the least abundant amongst *Anopheles* species. They were identified only in the low altitude region (800m to 1300m). This is consistent with findings in a study done in 2011 within the low altitude region where it was the least abundant species (Mala *et al*., 2011). *Anopheles coustani* had a higher abundance than *An. funestus* and *An. gambiae*, but lower than *An. pharoensis*.

Individual species responded to different ecological parameters in the same habitat differently while others were not affected by any of the recorded parameters. Results on *Culex* species are consistent with findings in a study carried out in villages within Mwea, Kenya, where *Culex* species responded differently to various ecological parameters in habitats (Muturi *et al*., 2007). In this study, only *Cx. annulioris* and *Cx. tigripes* responded to the recorded habitat parameters; hoof print habitat form for *Cx. annulioris*, spring bank habitats form and turbidity for *Cx. tigripes*. Vegetation and turbidity in habitats had the greatest influence on diversity. However, diversity in habitats had a negative correlation with altitude, indicating that habitat diversity reduced as altitude increased. On the interaction between species in a habitat, none of

the *Culex* species showed any significant interactions. However, there were significant interactions between *An. funestus* and *An. coustani*. *Anopheles pharoensis* showed significant interactions with *An. coustani* in the habitats.

5.2 Study Conclusion

The study hypothesis predicted that immature stages of malaria and RVF vector species vary amongst habitats along the attitudinal gradient. However, the results show only the distribution of *An. pharoensis* had a negative association with altitude. The implication of this is a need for continuous monitoring of vector species to avoid malaria and RVF outbreaks that would likely affect highlands and lowlands, assuming the vector competence of adult mosquitoes found in both regions is the similar. During monitoring, habitats that have clear water with vegetation would be the most probable culprit for larvae breeding.

5.3 Recommendations from the study

From the outcome of this study, I recommend that;

I. The diversity and distribution of adult vector species along the altitudinal gradient should also be investigated to compare the results with immature stages.

II. It is important to continuously monitor and control mosquito vectors in Baringo County since they are mainly not restricted by altitudinal barriers to minimize the probability of malaria and RVF outbreaks

III. The presence of *Ae. taylori* in the high altitude regions where monkey migrations are common, implicates that it is also important to monitor yellow fever in the region.

IV. This study did not monitor immature stages in tree holes, household water containers and leaf apexes where *Aedes* species that are not primary vectors of RVF are found. A survey of their diversity and diversity is recommended since they are vectors of other diseases such as chikungunya by *Ae. aegypti*.

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LIST OF APPENDICES

Appendix 1: GLM Tables

Table I: GLM of the of effect habitat parameters on *Aedes taylori*

GLM: *Aedes taylori*				
Coefficients:				
	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.602	3.935	0.407	0.688
org[T.Present]	-1.24	4.878	-0.254	0.802
qua[T.Turbid]	1.909	2.811	0.679	0.504
type[T.Floodzone]	-1.26	4.311	-0.292	0.773
type[T.Hoofprint]	2.745E-17	4.126	0	1
type[T.Lakemargin]	0.8779	4.834	0.182	0.858
type[T.Marsh]	0.2415	4.825	0.05	0.961
type[T.Riverbank]	0.8779	5.519	0.159	0.875
type[T.Springbank]	2.131	4.381	0.486	0.632
type[T.Waterpan]	-2.272	7.532	-0.302	0.766
type[T.Waterpit]	5.024	4.438	1.132	0.27
Veg[T.Present]	-1.24	4.878	-0.254	0.802

(Dispersion parameter for gaussian family taken to be 42.56499)

Null deviance: 1182.43 on 33 degrees of freedom

Residual deviance: 936.43 on 22 degrees of freedom

AIC: 235.22

Table II: GLM of the effect of habitat parameters on *Anopheles coustani*

GLM:*Anopheles coustani*				
Coefficients:				
	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	7.0454	7.0729	0.996	0.33
org[T.Present]	-1.1161	8.7696	-0.127	0.9
qua[T.Turbid]	-5.4384	5.0526	-1.076	0.293
type[T.Floodzone]	11.017	7.7503	1.421	0.169
type[T.Hoofprint]	-0.172	7.4174	-0.023	0.982
type[T.Lakemargin]	-4.8133	8.6897	-0.554	0.585
type[T.Marsh]	4.0995	8.6732	0.473	0.641
type[T.Riverbank]	1.6617	9.9214	0.167	0.869
type[T.Springbank]	1.6133	7.8759	0.205	0.84
type[T.Waterpan]	-0.4909	13.5392	-0.036	0.971
type[T.Waterpit]	-0.9895	7.9771	-0.124	0.902
Veg[T.Present]	-1.1161	8.7696	-0.127	0.9
(Dispersion parameter for gaussian family taken to be 137.5431)				
Null deviance: 3756.7 on 33 degrees of freedom				
Residual deviance: 3025.9 on 22 degrees of freedom				
AIC: 275.1				
Number of Fisher Scoring iterations: 2				

Table III: GLM on the effect of habitat parameters on *Culex poicilipes*

GLM: *Culex poicilipes*

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.7193	9.0419	0.19	0.8509
org[T.Present]	3.7802	11.2109	0.337	0.7392
qua[T.Turbid]	-3.9379	6.4591	-0.61	0.5483
type[T.Floodzone]	-3.8927	9.9078	-0.393	0.6982
type[T.Hoofprint]	-6.98	9.4822	-0.736	0.4694
type[T.Lakemargin]	-9.2797	11.1088	-0.835	0.4125
type[T.Marsh]	-1.7004	11.0876	-0.153	0.8795
type[T.Riverbank]	15.4703	12.6834	1.22	0.2355
type[T.Springbank]	10.6449	10.0684	1.057	0.3019
type[T.Waterpan]	30.3385	17.3082	1.753	0.0936
type[T.Waterpit]	0.7793	10.1978	0.076	0.9398
Veg[T.Present]	3.7802	11.2109	0.337	0.7392

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 224.7815)

Null deviance: 8274.5 on 33 degrees of freedom

Residual deviance: 4945.2 on 22 degrees of freedom

AIC: 291.8

Number of Fisher Scoring iterations: 2

Table IV: GLM on the effects of habitat parameters on *Culex tigripes*

GLM: *Culex tigripes*

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.998	1.477	1.353	0.1899	
org[T.Present]	-1.546	1.831	-0.844	0.4076	
qua[T.Turbid]	2.38	1.055	2.256	0.0343	*
type[T.Floodzone]	-1.571	1.618	-0.97	0.3424	
type[T.Hoofprint]	-4.666E-16	1.549	0	1	
type[T.Lakemargin]	1.094	1.814	0.603	0.5525	
type[T.Marsh]	0.3011	1.811	0.166	0.8695	
type[T.Riverbank]	1.094	2.072	0.528	0.6026	
type[T.Springbank]	3.951	1.645	2.403	0.0251	*
type[T.Waterpan]	-2.832	2.827	-1.002	0.3274	
type[T.Waterpit]	-0.589	1.666	-0.354	0.727	
Veg[T.Present]	-1.546	1.831	-0.844	0.4076	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 5.996841)

Null deviance: 274.37 on 33 degrees of freedom

Residual deviance: 131.93 on 22 degrees of freedom

AIC: 168.59

Number of Fisher Scoring iterations: 2

Table V: GLM on the effects of habitat parameters on *Culex quinquefaciatus*

GLM: *Culex quinquefaciatus*

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	56.53	86.5	0.653	0.5202
org[T.Present]	42.68	107.25	0.398	0.6945
qua[T.Turbid]	-31.67	61.79	-0.513	0.6134
type[T.Floodzone]	-71.7	94.79	-0.756	0.4574
type[T.Hoofprint]	-115.14	90.72	-1.269	0.2176
type[T.Lakemargin]	-95.02	106.28	-0.894	0.381
type[T.Marsh]	83.4	106.08	0.786	0.4401
type[T.Riverbank]	-71.44	121.34	-0.589	0.562
type[T.Springbank]	-36.4	96.32	-0.378	0.7091
type[T.Waterpan]	336.27	165.59	2.031	0.0545
type[T.Waterpit]	-22.19	97.56	-0.227	0.8222
Veg[T.Present]	42.68	107.25	0.398	0.6945

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 20573.77)

Null deviance: 817879 on 33 degrees of freedom

Residual deviance: 452623 on 22 degrees of freedom

AIC: 445.37

Number of Fisher Scoring iterations: 2

Table VI: GLM on the effect of habitat parameters on *Culex pipiens*

GLM: *Culex pipiens*

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	26.073	12.172	2.142	0.0435	*
org[T.Present]	27.918	15.092	1.85	0.0778	.
qua[T.Turbid]	-10.107	8.695	-1.162	0.2576	
type[T.Floodzone]	-21.33	13.338	-1.599	0.124	
type[T.Hoofprint]	-14.32	12.765	-1.122	0.274	
type[T.Lakemargin]	-30.409	14.955	-2.033	0.0542	.
type[T.Marsh]	3.727	14.926	0.25	0.8051	
type[T.Riverbank]	-27.459	17.074	-1.608	0.1221	
type[T.Springbank]	-25.323	13.554	-1.868	0.0751	.
type[T.Waterpan]	35.766	23.3	1.535	0.139	
type[T.Waterpit]	-17.823	13.728	-1.298	0.2076	
Veg[T.Present]	-20.382	15.092	-1.351	0.1906	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 407.3577)

Null deviance: 15936.0 on 33 degrees of freedom

Residual deviance: 8961.9 on 22 degrees of freedom

AIC: 312.02

Number of Fisher Scoring iterations: 2

Table VII: GLM on the effect of habitat parameters on *Culex annulioris*

GLM: *Culex annulioris*

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	18.49687	11.81854	1.565	0.132	
org[T.Present]	6.58898	14.65367	0.45	0.657	
qua[T.Turbid]	-9.19619	8.44266	-1.089	0.288	
type[T.Floodzone]	-17.88593	12.95045	-1.381	0.181	
type[T.Hoofprint]	-27.2	12.39415	-2.195	0.039	*
type[T.Lakemargin]	-24.50816	14.52017	-1.688	0.106	
type[T.Marsh]	-15.40943	14.49255	-1.063	0.299	
type[T.Riverbank]	-23.47483	16.57835	-1.416	0.171	
type[T.Springbank]	-0.05629	13.16028	-0.004	0.997	
type[T.Waterpan]	-15.88966	22.62348	-0.702	0.49	
type[T.Waterpit]	-18.25034	13.32953	-1.369	0.185	
Veg[T.Present]	6.58898	14.65367	0.45	0.657	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 384.0376)

Null deviance: 13740.9 on 33 degrees of freedom

Residual deviance: 8448.8 on 22 degrees of freedom

AIC: 310.01

Number of Fisher Scoring iterations: 2

Table VIII: GLM on the effects of habitat parameters on *Anopheles pharoensis*

GLM: *Anopheles pharoensis*

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	16.021	11.687	1.371	0.1843	
org[T.Present]	-4.268	14.49	-0.295	0.7711	
qua[T.Turbid]	-10.261	8.349	-1.229	0.232	
type[T.Floodzone]	20.137	12.806	1.572	0.1301	
type[T.Hoofprint]	-2.38	12.256	-0.194	0.8478	
type[T.Lakemargin]	-9.118	14.358	-0.635	0.5319	
type[T.Marsh]	33.235	14.331	2.319	0.0301	*
type[T.Riverbank]	28.415	16.394	1.733	0.097	
type[T.Springbank]	2.321	13.014	0.178	0.8601	
type[T.Waterpan]	-11.492	22.372	-0.514	0.6126	
type[T.Waterpit]	-7.59	13.181	-0.576	0.5706	
Veg[T.Present]	5.732	14.49	0.396	0.6962	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 375.5324)

Null deviance: 16104.3 on 33 degrees of freedom

Residual deviance: 8261.7 on 22 degrees of freedom

AIC: 309.25

Number of Fisher Scoring iterations: 2

Table IX: GLM on the effects of habitat parameters on *Anopheles gambiae s.l*

GLM: *Anopheles gambiae s.l*

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	5.044	4.733	1.066	0.2981
org[T.Present]	-11.68	5.868	-1.991	0.0591
qua[T.Turbid]	2.233	3.381	0.661	0.5157
type[T.Floodzone]	-5.566	5.186	-1.073	0.2948
type[T.Hoofprint]	-1.285E-14	4.963	0	1
type[T.Lakemargin]	6.319	5.814	1.087	0.2889
type[T.Marsh]	3.075	5.803	0.53	0.6015
type[T.Riverbank]	1.219	6.639	0.184	0.856
type[T.Springbank]	1.045	5.27	0.198	0.8446
type[T.Waterpan]	-17.6	9.059	-1.942	0.065
type[T.Waterpit]	-5.839	5.338	-1.094	0.2858
Veg[T.Present]	10.32	5.868	1.758	0.0926

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 61.57996)

Null deviance: 1982.8 on 33 degrees of freedom

Residual deviance: 1354.8 on 22 degrees of freedom

AIC: 247.78

Number of Fisher Scoring iterations: 2

Table X: GLM on the effects of habitat parameters on *Anopheles funestus*

GLM: *Anopheles fenestus*				
Coefficients:				
	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	2.61	1.847	1.413	0.1717
org[T.Present]	-1.36	2.29	-0.594	0.5587
qua[T.Turbid]	-2.17	1.32	-1.644	0.1144
type[T.Floodzone]	3.924	2.024	1.939	0.0655
type[T.Hoofprint]	2.889E-15	1.937	0	1
type[T.Lakemargin]	0.1101	2.269	0.048	0.9618
type[T.Marsh]	0.8332	2.265	0.368	0.7165
type[T.Riverbank]	0.1101	2.591	0.042	0.9665
type[T.Springbank]	-0.02751	2.057	-0.013	0.9894
type[T.Waterpan]	0.9197	3.536	0.26	0.7972
type[T.Waterpit]	-0.2201	2.083	-0.106	0.9168
Veg[T.Present]	-1.36	2.29	-0.594	0.5587

(Dispersion parameter for gaussian family taken to be 9.381548)				
Null deviance: 314.47 on 33 degrees of freedom				
Residual deviance: 206.39 on 22 degrees of freedom				
AIC: 183.8				
Number of Fisher Scoring iterations: 2				

Table XI: GLM of the effects of habitat parameters on species diversity (H′)

	Estimate	Std. Error	t value	Pr(>|t|)	
Intercept	0.60981	0.18826	3.239	0.00377	**
Other organisms	-0.02395	0.23342	-0.103	0.9192	
Turbidity	-0.19332	0.13449	-1.437	0.16465	
Floodzone	-0.16121	0.20629	-0.781	0.44285	
Hoofprint	-0.51676	0.19743	-2.617	0.01573	*
Lake margin	-0.22077	0.2313	-0.954	0.35021	
Marsh	-0.0396	0.23086	-0.172	0.86538	
Riverbank	0.239	0.26408	0.905	0.37527	
Springbank	-0.1002	0.20963	-0.478	0.63739	
T.Waterpan	-0.51538	0.36038	-1.43	0.16673	
T.Waterpit	-0.49801	0.21233	-2.345	0.02843	*
Other vegetations	0.59715	0.23342	2.558	0.01793	*

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1