PRESENCE, ABUNDANCE AND DISTRIBUTION OF MOSQUITO LARVAE AND THEIR PREDATORS ON THE MARA RIVER, KENYA AND TANZANIA

BY

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DECLARATION

I, Dida Gabriel Owino, declare that this thesis is my original work. It is being submitted for the degree of Doctor of Philosophy in Public Health of Maseno University, Kenya. This thesis has not been submitted before for any degree or examination at this or any other University.

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DEDICATION

To four most important people: God, Dida, Pricila and Ashley. Dida, thank you for passing your talent to me.

ABSTRACT

Vecor-borne diseases are becoming major health problem among communities living within major rivers of Africa. The major objective of this study was to determine the presence, abundance and distribution of mosquito larvae on the Mara River Basin, Kenya and Tanzania. The specific objectives were, 1) to determine the presence, abundance and distribution of malaria and non-malaria transmiting mosquito larvae on the Mara River, 2) to determine the presence, abundance and distribution of mosquito larvae predators and their relationship with mosquito larvae abundance and distribution on the Mara River, 3) to characterize different mosquito larvae habitats and determine how mosquito larvae and their predators prefer these habitats on the Mara River, 4) to determine the relationship between water physico-chemical parameters and the abundance of mosquito larvae and predators on the Mara River. In this cross-sectional survey, each identified habitats was dipped 20 times using standard dipper. Water physico-chemical parameters were determined using a multi-parameter-YSI meter, while a D-frame sampler was used to sample predators. The collected mosquito larvae and their predators were identified using standard keys. Mean mosquito larvae and predators per habitat types were compared using ANOVA, while relationship between mosquito larvae, predators, and physico-chemical parameters was evaluated using Generalized Linear Model (GLM). In total, 4,001 mosquito larvae were captured. *An. arabiensis* (25.9%) and *An. gambiae* s.s (24.3%) were the most dominant. Of the 297 predators captured, 54.2% of them were Hemiptera, 22.9% Odonata and 22.9% Coleoptera. Drying stream contained majority of mosquito larvae and their predators. A relationship between Dissolved Oxygen (DO) [Z=3.34, $p \leq 0.001$], temperature (Z=2.75, $p \leq 0.001$), turbidity, Z =-3.65, $p \leq 0.001$) and mosquito larvae (Z=6.49, $p \leq 0.001$) and predators were observed. Presence, abundance and distribution of mosquito larvae along the Mara River were confirmed. The three predator Orders; Hemiptera, Odonata and Coleoptera were captured in different habitats. Drying stream accounted for majority of mosquito larvae and their predators. A relationship between DO, temperature, turbidity, mosquito larvae and their predators was observed. Presence of vectors and non-vectors on the Mara River calls for their immediate control and education to help curtail the insurgent of vector-borne diseases in the area. Identification of indigenous predators is important in local vector control. Vector control program should be emphasized during dry period. Abiotic factors play significant roles in abundance and distribution of larval mosquitoes and their predators and should be manipulated to enable effective design for integrated vector control program within the Mara River basin.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
DO	Dissolved Oxygen
DNA	Deoxyribonucleic Acid
GOK	Government of Kenya
GPS	Global Positioning System
KEMRI	Kenya Medical Research Institute
LVB	Lake Victoria Basin
MOH	Ministry of Health
NTU	Nephelometric Turbidity Unit
PCR	Polymerase Chain Reaction
pH	Potential Hydrogen Concentration
s.l	*Sensu lato*
s.s	*Sensu stricto*
TDS	Total Dissolved Solids
Temp	Temperature
TSS	Total Suspended Solids
UN	United Nations
UNDP	United Nations Development Programme
UNEP	United Nations Environmental Programme
WHO	World Health Organization
WMO	World Meteorological Organization
YSI	Yellow Spring Instruments

DEFINITION OF TERMS

Abundance	Relative representation of species in a particular ecosystem.
Abiotic	Physical and chemical factors that may influence habitat such as temperature, humidity, wind, conductivity, among others.
Acidic	A substance with a pH less than 7 due to prevalent hydrogen ions. Acids tend to be sour and corrosive.
Alkaline	pH over 7; emits hydroxyl ions. Also called “basic.” It neutralizes the acids it combines with chemically.
Bio-control	Using natural means like predators to control pests, like Goldfish placed in water storage containers to eat incoming mosquitos.
Biodiversity	Biological variety of the kind that preserves species.
Biotic Factor	The environmental influence exerted naturally by living organisms
Brackish Water	Water contaminated by salt, but with salinity lower than 35 parts per thousand.
Distribution	This is the manner in which a biological taxon is spatially arranged.
Drying stream	A stream whose water id severely reduced to change in weather
Ecology	Branch of biology dealing with the relations and interactions between organisms and their environment, including other organisms.
GPS	GPS, which stands for Global Positioning System, is a radio navigation system that allows land, sea, and airborne users to determine their exact location, velocity, and time 24 hours a day, in all weather conditions, anywhere in the world.

Habitat	The home of a species (*Microhabitat*: of an individual organism).
Interaction	The primary ones are competition, mutualism, predation, parasitism, amensalism, and commensialism.
Lentic	Still water (pond, lake, etc.); also, organisms living in it.
Lotic	Flowing water, as in a river or stream.
Niche	An organism’s role, function, or position in an ecosystem.
Ontogeny	The development of an organism from the egg to maturity.
Presence	Availability of mosquito at a location we need to state a fixed time period.
Stream Order	A measure of stream size and branching, from first order streams (the largest) to twelfth.
Taxon	A classification category for a group of organisms (Cetaceans, Mammalia, Protista, etc.).
Tributary	Atreams channel that branches back toward its source.

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CHAPTER ONE: INTRODUCTION

1.1. Background

Malaria and other mosquito-borne diseases are now a major health problem among communities living along major rivers of Africa, among them the Mara River (Bussmann *et al.*, 2006). About 219 million malarial cases were reported in 2010 alone (with an uncertainty range of 154 million to 289 million) and an estimated 660,000 deaths (with an uncertainty range of 490,000 to 836,000), mostly among African children (World Malaria Report, 2012). Children are the most affected, with up to 90% of all deaths occurring in the sub-Saharan Africa.

Malaria is a vector-borne disease caused by protozoa of the genus *Plasmodium.* The parasite is transmitted by mosquito species of the genus *Anopheles*. Acoording to WHO, there are five species of *Plasmodium* that causes malaria: *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale, Plasmodium vivax* and most recently *Plasmodium knowlensi*. Among the five species, *P. falciparum* is the species that accounts for the most severe malaria infections in the world (World Malaria Report, 2012). Clinically, malaria is characterized by fever, which is often periodic with varying degrees of anaemia, splenic enlargement and various syndromes resulting from the physiological and pathological involvement of certain organs including the brain, the liver, and the kidneys (Brabin, 1983; Grau *et al.*, 1986; Duarte *et al.*, 2006).

The main malaria vectors in sub-Saharan Africa are larval mosquitoes of the *Anopheles gambiae* and *Anopheles funestus* complex (Gimnig *et al.*, 1999). *Anopheles gambiae* sensu stricto, (s.s) and *An. arabiensis* are the primary malaria vectors, while *Anopheles funestus* s.s and *Anopheles rivulorum* are the most important secondary vectors, especially around the Lake Victoria basin region (Taylor *et al.,* 1990; Kawada *et al.,* 2012). However, *An. gambiae* s.s. and *An. arabiensis* are the most efficient malaria vectors in the world (Levine *et al.,* 2004), because of their marked preference for human environments and for humans as hosts and also due to their rapid adaptation to changes in their environment induced by human habitation and agriculture *(*Patz *et al.,* 2000).

In the Lake Victoria basin, most cases of malaria transmission have been reported around the shores of the lake (Noor *et al.,* 2009). Malaria is reported as the leading cause of morbidity and mortality among children in many districts within the Lake Victoria basin, including parts of the Mara River basin of Kenya and Tanzania. Besides, the Maasai Mara game reserve is classified as low to moderate malaria epidemic area in East Africa (Schlagenhauf-lawlor & Scott, 2001). According to the Serengeti Mara Camp Fact sheet of 2013, the famous Serengeti National Park in Tanzania also falls within a malaria endemic zone.

Studies have also shown that malaria cases increases with decreasing distance to the shores of large water bodies such as lakes, rivers and dams (Lautze *et al.,* 2007; Yewhalaw *et al.,* 2009). Similarly, mosquito density inside houses decreases with increasing distance to the nearby breeding site (Minakawa *et al.,* 1999; Minakawa *et al.,* 2002). A previous study indicated that communities living in East and Central Africa are mostly concentrated around the shoreline of large water bodies (Moonen *et al.,* 2010; Warburg *et al.,* 2011). The primary malaria vectors in these areas are the *An. gambiae* s.s., *An. arabiensis,* and *An. funestus* s.s. Both *An. gambiae* s.s. and *An. arabiensis* belong to the *An. gambiae* complex (*An. gambiae* s.l.), a group considered as among the most important malaria vectors in Africa (Bass *et al.,* 2010). *Anopheles gambiae* s.s feeds preferentially indoors on humans and is one of the most competent malaria vectors known (Gillies & Coetzee, 1987). *Anopheles arabiensis* on the other hand, is regarded as zoophagic (and exophagic), whose major blood source is mainly cattle, but also feeds on humans indoors (Mahande *et al.,* 2007; Iwashita *et al.,* 2014). The *An. gambiae* complex larvae are known to be sympatric, with immature stages inhabiting sunlit, shallow and temporary bodies of fresh water such as ground depressions, puddles, artificial containers, swamps, pools and hoof-prints (Minakawa *et al.,* 2005; Mutuku *et al.,* 2006; Imbahale *et al.,* 2011).

It is logical to assume that the environment associated with any ecosystem can maintain a high number of malaria vectors. A recent study reported cases of malaria vectors breeding in elongated stagnant water pools (lagoons) separated from the lake by sand bars (Minakawa *et al.,* 2008). This implies that mosquitoes can also breed in isolated pools in river tributaries and streams, especially during dry seasons; which need investigations. Also, due to climate variability, drying of streams may result in creation of pockets of water, which are ideal breeding sites for the vectors. If large numbers of malaria vectors breed in such habitats, their contribution to local transmission would be substantial. Sometimes, these changes occur in areas where malaria disease was previously absent.

Malaria cases in the Mara River basin have been noticed for a decade now (Noor *et al.,* 2009). It is not clear whether these cases were introduced from the nearby lowland or resulted from local transmission because no record of larval mosquitoes along the Mara River has been reported. According to Bomet and Kericho counties report, malaria cases have increased from 5-10% between 2008 and 2011 and in Transmara, the Malaria test positivity rate was reported to be higher, to ranging between 10-30.1% in 2012 alone. Baringo County is classified as endemic for malaria, while cases of Rift Valley Fever outbreaks was reported in the past (2006-2007) causing high mortalities among small ruminant which account for about 3.41 million of total livestock population, with 117 humans having been affected leading to 3 deaths (WHO, 2007). It was therefore imperative to determine which species of mosquitoes are responsible for disease transmission in the area, especially along the Mara River and and its tributaries in order to establish their contribution to local disease transmissions in the area and epidemic risk assessment of malaria among both local residents and tourists visiting the Mara River basin.

Presence of disease transmiting mosquitoes along the Mara River and its tributaries, smaller streams and the adjacent terrestrial habitats may not only pose a health challenge to the local residents who may have lower resistance to malaria parasite infection, but also present serious risk to tourists visiting the region. According to the Mara travel information Fact Sheet (2010), each year about 1-2 million tourists from various parts of the world visit the Maasai Mara National Reserve in Kenya and Serengeti National Park in Tanzania and stay in the area for several days and malaria is thus ranked as the first concern for traveler's health in these tourist areas. The Mara River being transboundary is of particular importance to the inhabitants of the basin. However, since Mara region is largely known to be a moderate transmission zone, the immunity of inhabitants could be low, thus increasing their vulnerablility to malaria infection. This coupled with the currect degradation being witnessed along the Mara River, creates numerous microhibats that are potential vector breeding habitats, thus making them an area of particular imp[ortance to study.

Mosquito-borne diseases, such as malaria, arbovirus and Zika viruses, among others, have initiated an interest in understanding the factors that drive or constrict mosquito production (Ohba *et al.*, 2011; Warburg *et al.*, 2011). One major part of understanding influences on mosquito abundance is to look at the influence of mosquito predators. Predators can strongly influence populations of mosquitoes both by disturbing their development rate and also by consuming mosquito larvae and adults (Tuno *et al.*, 2007). Aquatic insects in the Orders Coleoptera, Hemiptera, Odonata, Diptera and fishes are also known to play a crucial role in mosquito control and have proven to be highly competent and widely used in putting into check the mosquito populations (Shaalan & Canyon, 2009). Studies in Kenya have reported higher predation capabilities of these orders mainly in rice irrigation schemes (Mwangangi *et al.*, 2008a), in wetlands around Lake Victoria where members of the *An. gambiae* s.l. dominate (Ohba *et al.*, 2010) and in Mwea in the then Central Province of Kenya (Muturi *et al.*, 2008). The relationship between prey and predator is a classic example of how nature works to regulate the increase and decrease of species. These relationships are what keep many ecosystems in balance. Understanding which predators play a key role in the mosquito lifecycle along the Mara River is therefore important.

Tuno *et al.* (2007) noted that biological control will give a long lasting effect if the biological agents can survive and recycle. This can only be successful if the agent is locally identified and used because of easier adaptability. Federici (1995) noted that although many biological agents including predators, parasites and microbial agents have been assessed in the laboratory as bio-control, few have been used because these agents are introduced into unfamiliar areas. Thus, there was need to establish the most appropriate predator that can be used to control mosquito larvae at the local setting.

Mosquitoes breed in varied habitats and different genera have shown specific habitat and breeding preferences, for example, *Anopheles* spp. are associated with fresh water habitats, whereas *Culex* spp. may also be found in polluted conditions including septic tanks and *Aedes* species breeds in peri-domestic and other small water collections including desert coolers (Parthiban and David, 2007). To comprehend this, there was need to establish where and which mosquito species breed within the Mara River basin. This study was particularly informed by the previous studies in Western Kenya which showed that malaria vectors larvae inhabit lagoons along Lake Victoria (Minakawa *et al.,* 2008; Minakawa *et al.*, 2012) when the lake water recedes during dry spells, a situation which can also occur when water volumes reduce due to climatic conditions and/or as a result of destruction of catchments along the river such us the Mara.

Evaluation of larval habitat for mosquitoes in terms of species composition and resources can help in understanding the bio-ecology and related control measures of larval mosquitoes more appropriately (Aditya *et al.,* 2006). Knowledge of larval vector ecology is a key factor in risk assessment and establishment of effective control measures, because the most effective method for controlling vector populations is to control the larvae in their aquatic habitats before they emerge as adults. A knowledge on where mosquitoes breed and why they prefer certain water bodies over others is very important for sound mosquito control strategies (Ijumba & Lindsay, 2001). However, the understanding of mosquito larval ecology is limited, and the knowledge is insufficient to achieve effective vector control through the means of larval control (Fillinger & Lindsay, 2011). For example, it is unknown what causes vector abundance and distribution, and how the mosquito larval abundance is regulated in the diverse aquatic habitats. Focusing efforts to larvae, however, requires sound knowledge of the local situation and the behavior of the available mosquito population. A basic understanding of the aquatic stages of mosquitoes would be extremely relevant for disease vector control on the Mara River basin.

Even though describing larval habitats in more general terms only tells a part of the tale, it may still give valuable insight on the suitability of different habitats for mosquito larvae. According to Kenea *et al.* (2011), the densities of total Anopheline larvae and *An. squamosus* in natural habitats were higher but lower for *An. pharoensis*. Habitat permanence is another factor that has been studied, classifying habitats as temporary, permanent or on a scale in between. Gimnig *et al.* (1999) showed that *An. gambiae* and *An. arabiensis* were both associated with temporary habitats while *An. funestus* s.s was associated with semi-permanent bodies of water. However, Kenea *et al.* (2011) showed a negative correlation between habitat permanence and total Anophelines, and a positive correlation with *An. arabiensis*, as opposed to Gimnig *et al.* (1999). Habitat classification has been shown to give good predictive power for some species of Anopheline larvae, especially during dry season (Rejmánková *et al.*, 2013). Thus, it was important to characterize habitat for larval mosquitoes during the dry period along the Mara River in order to inform intervention policies.

Currently, the Mara River basin has been impacted greatly by the wanton destruction of the Mau forest at the upper catchment region. This has led to the fluctuation of Mara River water volume, and subsequent changes in the physico-chemical parameters and hydrological characteristics (Defersha *et al.,* 2012; Matano *et al.,* 2015). However, it is not clear how and to what extent these factors influence the presence and distribution of larval mosquitoes and their predators within the Mara River basin. Changes in the physico-chemical and biotic characteristics of surface water habitats may create conditions either favourable or unfavourable to the breeding success of mosquitoes depending on the ranges of tolerance or adaptability of different species to these habitats (Herrel et *al.,* 2001; Mwangangi *et al.,* 2008). This can have implications for vector-borne diseases, because habitat changes that favour breeding of potential vector species can ultimately lead to increased rates of parasite or pathogen transmission. In areas around Lake Victoria basin, Anopheline control strategy is based mainly on Indoor Residual Spraying (IRS) and the distribution of Long Lasting Insecticidal Treated Nets (LLITNs), targeting the main malaria vector *Anopheles gambiae* complex (Noor *et al.,* 2009; Stell *et al.,* 2013).

Previous studies reported conflicting information on the physico-chemical conditions, as either favourable or unfavourable to the breeding success of larval mosquitoes. For intance, a study on malaria vector control in Ethiopia (Dejenie *et al.,* 2011) showed that almost all their study habitats were alkaline (pH>7.0) and both Anopheles and Culex larvae were positively associated with this high pH. Paaijmans *et al.,* (2008), established temperature and dissolved oxygen as important for larval mosquito development. However, Minakawa *et al.,* (1999) argue that a combined effect of physico-chemical can influence mosquito abundance. Since previous studies found conflicting information on influence of physico-chemical parameters on mosquito distribution and abundance, the current study established the physico-chemical characteristics of the different habitats and its relationship to the presence and abundance of larval mosquitoes and their predators in the Mara River basin.

1.2. Statement of the Problem

Although mosquito-borne diseases are major health concern worldwide, and larval control can be an important component of disease control program, little is known about the ecology of mosquito larvae. Usually, the description of mosquito larvae habitats has been given in more general terms such as permanent/temporary or natural/man-made habitats. More studies, such as those of (Munga *et al.*, 2005; Fillinger & Lindsay, 2011; Kweka *et al.*, 2012), are however, starting to focus on finding what factors are influencing the occurrence and abundance of Anopheline larvae. Destruction of forests and increased agricultural activities along the river may create suitable mosquito breeding micro-habitats, thus increasing the risk of disease transmission. This may in turn influence community health patterns and affect household incomes (Kioko, 2013).

Even though no such studies have been carried out on rivers, water level fluctuation in lakes and large dams has been associated with increase of malaria vectors, their survival rates and longevity, which could result in increased mosquito densities in Africa (Minakawa *et al.,* 2008). It is therefore important to establish the availability and suitability of mosquito and predator breeding habitats along the Mara River in order to predict their role in the plasmodia transmission in the area.

Furthermore, most previous studies on abundance and status of disease vectors in the Lake Victoria were limited to the detection of specific disease transmitting vectors and at times considered the role of only few or none of the factors that may influence mosquito abundance (Minakawa *et al.,* 1999; Muturi *et al.,* 2008). In addition, most of these studies did not establish the status of predators even as they shared the same habitats. In situations where predators were considered, their relationship with mosquito and how they are influenced by other ecosystem factors such as the physico-chemical parameters were not considered (Minakawa *et al.,* 1999; Muturi *et al.,* 2008). Little research has focused on the assessment of the available predators' local ecology to establish their impact on mosquito population. Particularly, the abundance and distribution of these mosquito predators and their relationship with mosquito larvae abundance and distribution within the Mara River basin remain unestablished, possibly because of the difficulties in identifying and quantifying the impact of the most common predators in the natural environment.

Predatory insects and their larvae (e.g. Dystiscidae, Notonectidae and Odonata) do not only prey on mosquito larvae, but also prevent adult mosquitoes from oviposition (Fincke, Yanoviak, & Hanschu, 1997; Stav and Blaustein, 2000; Lundkvist *et al.,* 2003; Fischer *et al.,* 2012). However, difficulties in colonization and management of insect predators, as well as, a lack of synchrony between predator and prey life cycle, impeded their deployment (Atwood & Richardson, 2012). Both mosquito-fish and insect predators occur mainly in large, permanent ponds, while most mosquito species prefer temporary ponds as breeding sites (Chumchal *et al.,* 2016). Furthermore, little is known on the predation of larval mosquitoes from rivers and streams in Kenya.

According to the East Africa trans-boundary report (2008), application of fertilizer especially to tea, coffee, and sugarcane plantations, as well as increased pollutants like sewage and waste water discharge into the Mara River, also significantly contributes a considerabe proportion of nutrient load into adjacent rivers that ultimately discharge into Lake Victoria (Matano *et al.*, 2013; Anyona *et al.*, 2014). These may impact negatively on water quality in both medium and long-term and affect its biota and the subsequent changes in mosquito larvae density and the subsequent disease transmission. This study, therefore also sought to establish the link between water physico-chemical parameters in the Mara River, its tributaries and adjacent terrestrial water bodies with the presence and distribution of larval mosquitoes and their predators.

1.3. Significance of the Study

This study inform formulation of sound and effective vector control strategies, aimed at reducing human mosquito contact and thus decrease in disease transmission within the Mara River basin. For current vector control efforts to achieve meaningful reduction in malaria transmission, it is important for control programme officers to have access to adequate information on the local malaria and non-malaria mosquito's ecology, distribution and transmission patterns as well as factors affecting their abundance such as the available predators. Understanding species interactions such as competition and predation across environmental gradients may also provide useful insight into how assemblages of mosquitoes are structured. Such information is critical for proper application of biological control measures.

1.4. Study Objectives

1.4.1. Broad Objective

To determine presence, abundance and distribution of malaria and non-malaria transmiting mosquito larvae and their predators on the Mara River, Kenya and Tanzania.

1.4.2. Specific Objectives

1. To determine the presence, abundance and distribution of malaria and non-malaria transmitting mosquito larvae on the Mara River basin, Kenya and Tanzania.
2. To determine the presence, abundance and distribution of mosquito larvae predators and their relationship with mosquito larvae abundance and distribution on the Mara River basin, Kenya and Tanzania.
3. To characterize different mosquito breeding habitats (in the main river, its tributaries, streams, rock pools, puddles, swamps, and river beds during low flows) and determine how the mosquito larvae and their predators prefer these habitats on the Mara River Basin, Kenya and Tanzania.
4. To determine the relationship between water physico-chemical parameters and the abundance of mosquito larvae and their predators on the Mara River basin, Kenya and Tanzania.

1.5. Research Questions

1. What are presence, abundance and distribution of malaria and non-malaria transmitting mosquito larvae on the Mara River basin, Kenya and Tanzania?

2. What are the presence, abundance and distribution of mosquito larvae predators and their relationship with mosquito larvae abundance and distribution on the Mara River basin, Kenya and Tanzania?
3. What are the different mosquito breeding habitats (in the main river, its tributaries, streams, rock pools, puddles, swamps, and river beds during low flows) and how do the mosquito larvae and their predators prefer these habitats on the Mara River basin, Kenya and Tanzania?
4. What is the relationship between water physico-chemical parameters and presence, distribution and abundance of mosquito larvae and their predators on the Mara River basin, Kenya and Tanzania?

1.6. Scope of the Study

This study covered a few purposively selected points along the Mara River, its tributaries and associated aquatic microhabitats within its basin in Kenya and Tanzania. The study focused on potential mosquito breeding habitats.

CHAPTER TWO: LITERATURE REVIEW

2.1. The Lake Victoria Basin

The Lake Victoria Basin (LVB) is one of Africa’s largest trans-boundary water resources covering 180,950 km^2 in surface area and surrounding the second largest fresh water lake in the world (68,800 km²), with the largest fresh water fishery resources (Odada *et al.*, 2003). A map of the Lake Victoria Basin showing the catcment area and towns within the basin is shown in Figure 2.1.

Figure 2.1: Map of Lake Victoria Basin (LVBC & WWF-ESARPO, 2010)

The Lake Victoria watershed is shared among five states in the following proportions; Tanzania 44% (85,448 km^2), Kenya 22% (42,724 km^2), Uganda 16% (31,072 km^2), Rwanda 11% (21,362 km^2) and Burundi 7% (13,594 km^2). The Lake is shared among three of the five partner states of the East African Community (EAC), i.e. Kenya, Uganda and Tanzania, with a shoreline of approximately 3,450 km long, demarcated among the riparian countries (Odada *et al.,* 2003).

2.1.1. Lake Victoria Basin Climate

The Lake Victoria Basin falls under the equatorial hot and humid climate with a bi-annual rainfall pattern, where the long rains are experienced from March to May and short rains from October to December (Kizza *et al.,* 2009). July is the coolest month of the year while the warmest fluctuates around October to February. According to the Regional Trans-boundary Diagnostic Analysis for East Africa (Bootsma *et al.,* 2003; Tungaraza *et al.,* 2012), rainfall varies considerably from one part of the Basin to another. The highest rainfall is normally reported in Uganda with Ssese Island recording about 2,400 mm annually, while Tanzania and Kenya receive between 1,350 and 2,447 mm annually. Burundi and Rwanda get an average rainfall of about 1800 mm annually. On the Northern and Western shores, the effects of rainfall do not extend more than 40 km inland. Rainfall amount increases from east to west, ranging between 600 to 2,800 mm annually. The temperature in the Lake Victoria Basin reaches maximum in February, just before the March equinox while the minimum is recorded in July after the June equinox. The maximum temperature ranges between 28.6˚C and 28.7˚C, while the minimum ranges between 14.7˚C and 18.2˚C. Comparison of temperature records for the period 1950-2000 and 2001-2005 shows that maximum temperatures have increased by an average of 4˚C (Miller, 2009).

2.1.2. Streams, Rivers and River Mouths

Streams and rivers affluent to Lake Victoria contribute about 20% of the water into the lake (Oteyo *et al.*, 2014). Since these streams and rivers flow through farmlands, towns and human settlements, they transport much of the common pollutants produced in these areas by human activities, loading the lake with heavy metals, agricultural chemicals, silt among other pollutants (Matano *et al.*, 2015). These lotic ecosystems therefore influence physico-chemical characteristics of the lake waters. At the points of entry into the lake (river mouths) they present a water environment different from the rest of the lake.

The principal tributary of Lake Victoria is the Kagera River, which enters the lake along its western shore, draining the highlands of Burundi and Rwanda. The Mara River traverses different land use types including forests, farmlands, open lands, urban centers, game reserves and conservancy before flowing into Lake Victoria through the Mara Swamp at Musoma Bay in the lower Mara basin (Hughes & Hughes, 1992).

2.2. The Mara River and its Watershed

The Mara River and its basin is an important freshwater ecosystem for Kenya and Tanzania. The river has a catchment area of 13,504 km^2, with 65% of the basin being in Kenya and 35% in Tanzania (Figure 2.2).

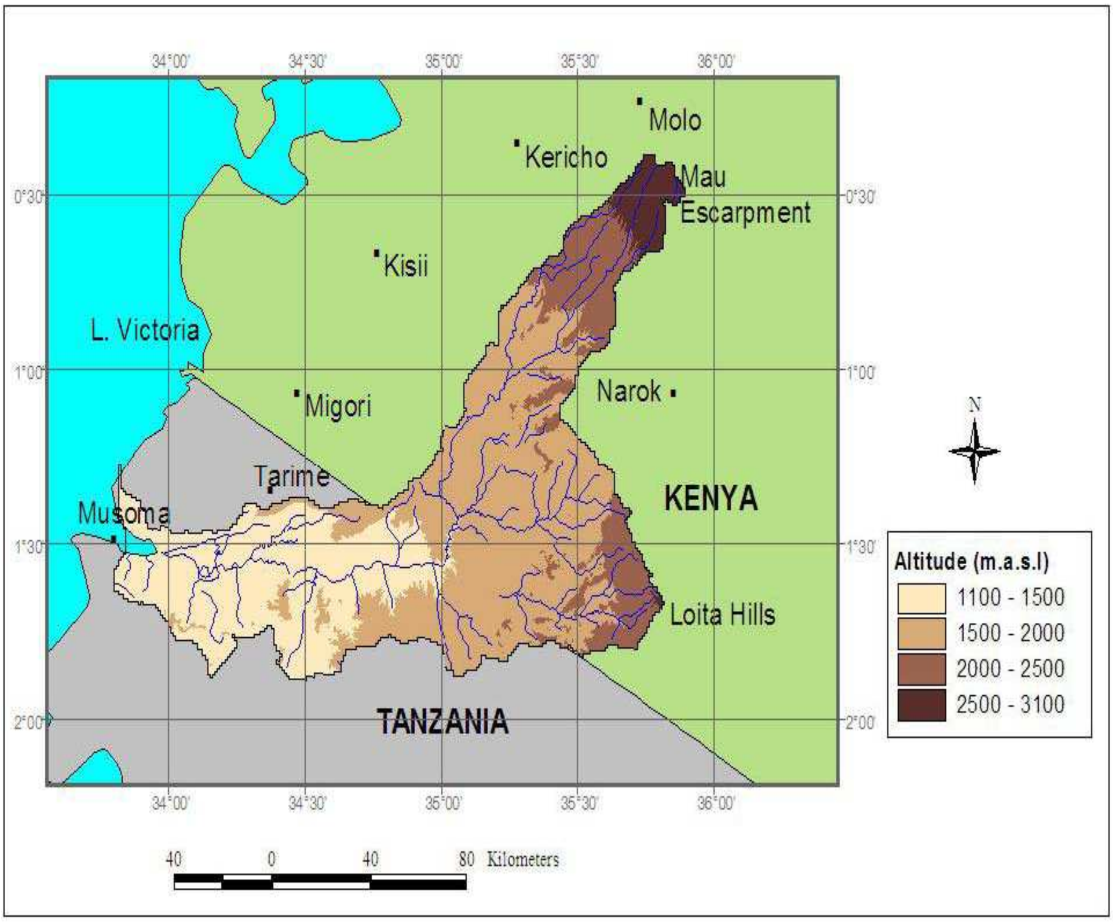


Figure 2.2: Location and Relief of Mara River Basin (LVBC & WWF-ESARPO, 2010)

Mara River originates from the Mau Escarpment in the Kenyan highlands, and flows for about 395 km draining into Lake Victoria at Kirumi swamp in Tanzania. Where forests still remain, rainwater percolates through the dense canopy into the soil and ultimately seeps into the Mara River tributaries with some forming springs that drain into Nyangores and Amala tributaries.

These rivers exit the forest and descend over 1000 m on the southern slope of the escarpment, supporting farmers, pastoralists, and the growing urban centers in the region. As the Mara River continues into the protected areas of Maasai Mara National Reserve and across the Tanzanian border into the Serengeti National Park, it is joined by the Talek and Sand Rivers (Mango *et al.*, 2011).

The Mara River provides food, important plants, fertile soils, and critical habitat to people and wildlife. However, in such a system, the many demands for these resources are sometimes incompatible. Clearing of forests and increased cultivation in the upper catchment is believed to have increased sediment loads and altered the hydrograph of the river (Odada *et al.*, 2003). Without the dense forest to moderate the flow of water into the system, both seasonal floods and droughts are becoming more extreme. Further downstream, increase in the area under irrigated agriculture and industrial activities such as mining have led to higher rates of water abstraction. In addition, the river provides the primary domestic water source for nearby towns and settlements, many of which lack sewage or waste water treatment facilities (Nyairo *et al.*, 2015). By the time the Mara River reaches the protected reserves, it has passed through hundreds of kilometers inhabited by thousands of Kenyans, and hundreds of thousands of Tanzanians await the river's waters downstream of Serengeti National Park (Mango *et al.*, 2011).

In order to cope with this high pressure, there have been, and continue to be, ongoing changes and regulations in land and water-use patterns in the Mara River basin (Onyando, 2013). The degradation of natural vegetation cover and soil conditions has led to changes in rainfall-runoff characteristics of the basin, which consequently changes the river flow regimes. Major environmental changes resulting from the basin surface modifications observed in Mara River basin include high-peak stream flows, reduced base flows, enlarged river channels, and silt build-up along the river bed (Mango *et al.,* 2011). This creates microhabitats that are suitable breeding habitats for mosquito vectors increasing the risk of malaria among the inhabitants of the basin.

2.3. Diseases in the Lake Victoria Basin

A number of water-associated pathogens of viral, bacterial and parasitic origin are endemic to the Lake Victoria basin including the Mara River basin (Mutie *et al.,* 2006). Viral infections include: rotavirus, vector-borne encephalitis and onyong-nyong fever; bacterial infections include: *Escherichia coli, Salmonella* and *Vibrio cholera*; while parasitic infections include unicellular protozoans, which cause diseases such as malaria, amoebiasis, and giardiasis, and also the multi-cellular metazoan helminthes: the cestodes, trematodes and nematodes which cause taeniasis, fascioliasis, schistosomiasis, trichinosis and filariasis in humans and animals (Harley *et al.,* 2001). Arthropod vectors like mosquitoes (*Anopheles, Aedes, Culex),* and black flies (*Simulium*), which are known disease transmitters may breed in such water pools, which need investigation. It is therefore important to establish the specific mosquito species that breed along the Mara River in order to quantify their potential in disease transmission.

Malaria is transmitted by a range of *Anopheles* mosquitoes and the risk of disease varies greatly across the continent (Kelly-Hope *et al.,* 2009). The vector groups for both human malaria (*Anopheles* mosquitoes) and other diseases such Yellow Fever, Zika virus (*Culex* mosquitoes, *Culex quinquefasciatus* and *Aedes* species) demonstrate complexity of diseseas vector species abundance. The Mara River transverses urban area where commercial activities are predominant, anthropological activities such as open drainage system and littering of environments with various peridomestic containers encourage the breeding of mosquitoes and consequently increase mosquito-borne diseases in the area. Therefore, a study of the biology of mosquitoes and physico-chemical parameters of the breeding sites was essential to determine their influence on mosquito distribution, abundance and diversity.

It has been emphasized that vector control could be the only means of eradication of the disease from the endemic regions, and has evidently cut the vector-human contact and reduced malaria incidences in some countries (Eziefula *et al.,* 2012; Kaneko, 2010). Nevertheless, in many areas of the Lake Victoria basin, including Mara River, the vector still remains the key link in the transmission of the disease, and this oviates the necessity of carrying out research to establish which species breed in the river catchment in order to identify the most appropriate control method for the area. Infact, a biological control such as use of indegenious predators could be the only available tool as attempts to employ other strategies have failed due to numerous reasons such as the development of resistance to the available drugs and insecticides by the vectors, and also due to lack of knowledge on the behavior of the vectors.

2.3.1. Epidemiology of Malaria – Global and Geographic Distribution of Malaria

The World Health Organization (WHO) estimated that about 219 million malaria cases and about 660,000 people died, mostly children, especially in sub-Saharan Africa and Asia (Duchet *et al.,* 2012). In Kenya, malaria accounts for 30% of outpatient attendance and 19% of hospital admissions. A six-year surveillance across Kenya-carried out from 2003 to 2009, indicated that out of the 166,632 paediatric admissions, [which included 78,530 (47%) admission due to malaria cases], western Kenya reported the highest cases of malaria (70%), followed by highlands areas of Rift Valley (45%) and along the Kenyan coast (22%) (Okiro *et al.*, 2010).

2.3.2. Parameters used to Measure Malaria Transmission

Malaria endemicity historically has been defined in terms of rates of parasitemia or palpable-spleen rates in children 2-15 years of age as hypoendemic (<10%), mesoendemic (11-50%), hyperendemic (51-75%), and holoendemic (>75%) (Shaukat *et al.,* 2010). While there are seasonal and geographic differences between areas, an EIR of <10 per year is a low transmission area, 10 – 49 per year is intermediate transmission, and >50 per year is high transmission (Kelly-Hope and McKenzie, 2009).

Constant, frequent, year-round infection is termed as stable transmission; generally in areas with EIRs of >100 per year. In stable transmission areas, most adults experience malarial infections that are asymptomatic, while in low or sporadic transmission areas, complete protective immunity is not acquired and symptomatic disease may occur at all ages that may result in epidemics in such areas (Shaukat *et al.,* 2010). An epidemic can develop when there are changes in environmental, economic, or social conditions, such as heavy rains following drought or migrations (usually among refugees or internally-displaced people [IDPs] (Opondo, 2013; Spencer *et al.,* 2004), from a non-malarious region to an area of high transmission; a breakdown in malaria control and prevention services can intensify epidemic conditions which usually results in considerable morbidity and mortality among all age groups (Kiszewski and Teklehaimanot, 2004).

2.3.3. Malaria in Africa

Present epidemiologic findings show that Africa is undergoing a reduction in malaria transmission which has been attributed to effective large scale malaria control programmes (Kariuki *et al.,* 2013). Interventions such as the use of effective anti-malarial therapeutics and insecticide treated bed nets (ITNs) have resulted in reduction of both the burden of malaria and its associated mortality in Africa. For instance, widespread use of ITNs in Kenya resulted in a 44% reduction in mortality in children below 5 years over a two year period (Eisele *et al.,* 2010). The combined use of ITNs and artemisinin-based combination therapy (ACT) in Zanzibar reduced mortality by 52% in under-fives over a two year period (Bhattarai *et al.,* 2007). Regardles, in Africa, the problem of malaria continues to present a big challenge, as not all households are able to afford ITNs or other alternative malaria control options. This affects the socio-economic development of the continent.

While previous studies indicated *An. gambiae* s.l as the primary malaria vector (Omumbo *et al.,* 1998; Koekemoer *et al.,* 2002; Ernst *et al.,* 2009), their main role in malaria transmission sustainability throughout the year is questionable in some places and during some periods of the year. For instance, Minakawa *et al.* (2008), highlighted the central role of vector-selection in localized malaria-risk area when they examined how the 1.5m drop in the water level of Lake Victoria affected the vector populations. The study found that on newly emerged land, *An.funestus* group benefited from new breeding sites during the high water period, being better able to reproduce during flooded conditions. *An.gambiae* s.l. on the other hand was disadvantaged during the low-water level period because it is far more sensitive to the drying out of habitats during the dry season. The drop in water level so far shifted the composition of mosquito populations, and moreover, malaria transmission towards *An. funestus* group. Therefore, the previous study demonstrated the complexity of relationships between water, vector populations, and malaria transmission in western Kenya (Minakawa *et al.*, 2008). It was therefore imperative to investigate the presence of mosquitoe larvae during the dry period along the Mara River in order to inform policy for localized vector-borne disease control in the area.

2. 4. Malarious Regions in Kenya and Tanzania

Figure 2.3 depicts the historical distribution of malaria in Kenya and Tanzania. In both countries, malaria endemicity is largely dependent on three factors; (1) the type of mosquito vector in an area, (2) the parasite species and (3) the climate (Omumbo *et al.*, 1998). These factors generally determine the intensity and length of transmission of malaria and the high malaria incidences in sub-Saharan Africa are attributed to these factors. The malarious regions in Kenya have been classified into various zones based on the transmission intensity. The various zones (Western and Nyanza regions), are endemic zones, with high intensities of malaria transmission. The transmission is continuous over many successive years. Endemic areas primarily exist in tropical Africa, except in highland areas and at the coast. In malaria endemic zones, children are the most vulnerable to the attack, as adults acquire a degree of immunity through continued exposure. In zones of less intense transmission, particularly in epidemic areas, a larger proportion of the population is likely to be non-immune and all are at risk of infection (DFID, 2010).

In Tanzania, malaria is the single most significant disease causing economic burden to health and economy of its 40 million inhabitants (projections from the population census of 2002). Similar to Kenya, the population groups most vulnerable to malaria are children under five years and pregnant women. It is estimated that 90% of about 40 million people in Tanzania are at risk of malaria infection resulting into 11 million clinical malaria cases per annum (Mboera *et al.,* 2013).

The disease is responsible for more than one-third of deaths among children under the age of 5 years and for up to one-fifth of deaths among pregnant women (Selemani *et al.,* 2015). Malaria contributes 39.4% and 48% of all outpatients less than 5 years of age and aged 5 years and above, respectively (Mwanziva *et al.,* 2011; Selemani *et al.,* 2015). In terms of hospital admissions, malaria accounts for 33.4% of children under the age of 5 years and 42.1% in children aged 5 years and above (MOH Tanzania, 2010). In Tanzania, most of the malaria attributable cases and deaths occur in rural villages away from effective diagnostic or treatment facilities.

Malaria poses many societal and economic burdens in Tanzania, ranging from school absenteeism to low productivity in the workplace. In the short term, widespread malaria reduces agricultural production and other economic outputs. Additionally, the cumulative effect in the long term may leade to a decrease in national economic capacity and development (Lowassa *et al.,* 2012; Mboera *et al.,* 2007).

The main focus of malaria control measures in Tanzania includes case management (early diagnosis and prompt treatment with effective drugs), vector control using insecticides treated mosquito nets (ITNs), malaria intermittent treatment in pregnant women, malaria epidemics prevention and control, information, education and communication, and operational research (Mboera *et al.,* 2013). Despite these strategies, malaria cases and deaths have been increasing in the country, mainly due to injudicious use of antmalarial drugs, delayed health-seeking behaviour, and reliance on the clinical judgement without laboratory confirmation in most of the peripheral health facilities (Mlozi *et al.*, 2015). Furthermore, most of the information on malaria cases are health facility-based; which is incomplete, while ecological data are untimely and unreliable (Monitoring & Change, 2015). The lack of reliable data on the magnitude of malaria and the vectors responsible for its transmission calls for investigation. On the Kenyan side, Western and Nyanza regions are endemic zones, while in Tanzania, high intensities of malaria transmission risk zones are near the coast, but the endemicity covers almost every zone in the country (Omumbo *et al.*, 1998) (Figure 2.3).

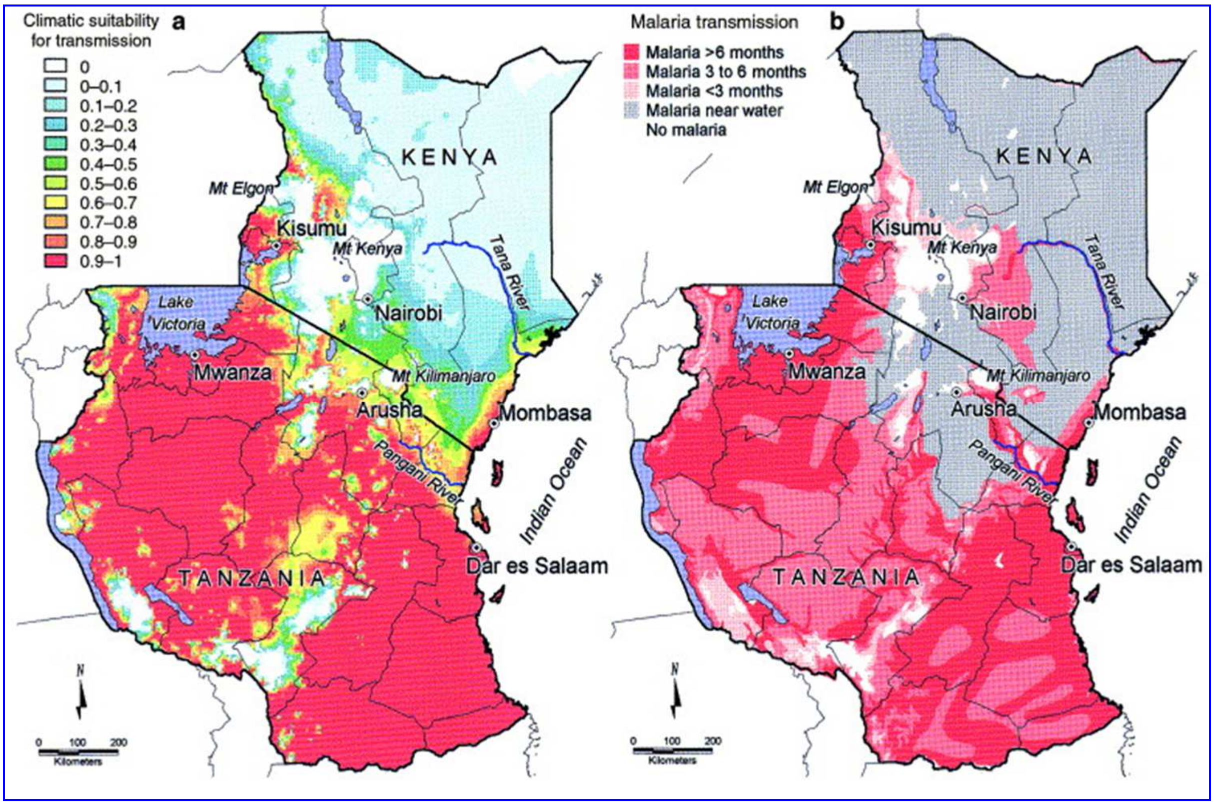


Figure 2.3: Historical Malaria Transmission in Kenya and Tanzania

2. 5. Malaria Control Strategies

2.5.1. Early Diagnosis and Treatment

Malaria is the leading cause of morbidity and mortality in Kenya. It accounts for 16% of all outpatient attendance (Kenya Malaria Indicator Survey, 2015) and 15% of all admissions to health facilities are based on passive case reports. This survey is designed to obtain national and epidemiological zone representative population-based estimates of malaria programme indicators to inform strategic planning and evaluation of relevant malaria control interventions. The greatest challenge to malaria control in the sub- Saharan region is proper diagnosis and treatment. Ensuring proper treatment adherence is challenged by self-medication and poor quality of treatment, particularly in the unregulated private sector (Karunamoorthi, 2014). In Kenya, for instance, there is a high prevalence of counterfeit and sub-standard antimalarial medicines, which can cause death, reduce confidence in malaria treatment, and increase drug resistance. Early diagnosis and treatment of patients as well as control of malaria vectors constitute key measures in mitigation of the disease and reduction in illnesses and deaths related to malaria as well as easing the socio-economic burden caused by the diseases. The main objective of the Kenya Malaria Indicator Survey (KMIS) 2015 was to measure progress achieved in key malaria indicators. Rapid diagnostic test (RDT) on site and malaria blood slide examination at a reference laboratory is conducted on children 6 months to 14 years proved to be effective.

2.5.2. Chemotherapy of Malaria

Antimalarial drugs are designed to prevent or cure malaria. Two types of antimalarial drugs are availabe, one taken as a preventive measure; called prophylactic drugs, and the other taken after the infection has already occured; called therapeutic drugs (Andrews *et al.*, 2014). Current recommended treatment regimens in Kenya are ACTs (Watsierah *et al.*, 2012; Watsierah &

Ouma, 2014). Antimalarial combination chemotherapy is widely advocated for delaying the development of resistance to the remaining armoury of effective drugs. The concept of combination therapy is based on the synergistic or additive potential of two or more drugs, with independent modes of action and different biochemical targets in the parasite (Enato & Okhamafe, 2005).

2.5.3. Vector Control

One of the most common strategies used to eradicate malaria is the use of various chemicals including insecticides. Currently, vector control is focused on the use of insecticide treated bed-nets (ITNs). Although ITNs have proved efficacious in reducing severe malaria morbidity and mortality among children, concerns have emerged over their sustainability and long-term effects on the development of malaria immunity, coupled with increased insecticide resistant mosquitoes (Okiro *et al.,* 2010; Snow and Marsh, 2005). Insecticide use has had a negative impact on non-target organisms and the environment. Studies have also shown that some of the chemicals used kill natural mosquito predators more effectively than the target mosquitoes and over time, predators such as fish and insects die out while mosquitoes develop resistance, multiplying in ever larger numbers in a losing battle often referred to as "the pesticide treadmill" (Wilson & Tisdell, 2001). Moreover, the application of insecticide strategies has also failed due to the development of insecticide resistance and lack of knowledge about the behavior of the vectors. However, there are hopes as other alternatve chemicals to be incorporated in bed nets are being evaluated (Kawada *et al.*, 2014). The non-selective nature and use of pesticides therefore leaves biological control of mosquito larvae as among the best and most environmentally friendly option for the control of mosquitoes.

Previous studies on the abundance and distribution of larval mosquitoes and their predators focused mostly on wetlands, shallow lakes, and almost all ornamental pools in different parts of the world. However, information on the presence of mosquito larvae and their predators in most rivers are lacking. Information on larval mosquitoes and their predators in the Mara River and its tributaries is important and necessary since some of these predatory species have been evaluated as bio-control agents in the worldwide campaign to control malaria vectors. The identification of indigenous predator populations is recommended worldwide due to their adaptability and may therefore help curtail the insurgent of the disease and non-disease vectors in the Mara River basin if a predator propagation program can be initiated. Currently, little is known about the ecology of larval mosquitoes and their predators in most rivers of Kenya. Furthermore, researches on indigenous mosquito predators are particularly scarce, making it crucial to determine the impacts of their abundance and interaction in ecosystems such as the Mara, which formed the basis for the current study.

Interest in formulating non-chemical approaches has been growing over the past four decades because of the limitations of chemical use, including mosquitoes' insecticide resistance, disturbances to the ecosystem, and the health risks for human and domestic animals (Yasuoka & Levins, 2007). Current biological control tools that are considered most promising for malaria prevention include fungi, bacteria, larvivorous fish, parasites, viruses, and nematodes (Kamareddine, 2012). Among these, the most commonly used biological control agents is larvivorous fish, which are introduced to aquatic habitats for larval mosquitoes. The challenge, however, has been the adaptability of such foreign bi-control agents to the local settings. It is thus important to evaluate the locally available predator candidates along the Mara River that can effectively suppress Anopheline larval population.

2.5.4. Malaria Vector Control using Insecticides

Malaria vector control remains key and most important part of the global malaria eradication strategy and is still the most effective approach for the prevention and control of malaria. While mosquitoes can be controlled by use of insecticides, the dangers presented by use of insecticides of chlorinated or organophosphate origin in the control of mosquitoes are numerous. Organophosphate larvicides are used infrequently because of their negative impacts on non-target organisms and the environment. Currently, most malaria endemic countries, in addition to bed nets, have opted for indoor residual spraying [IRS] (Hightower *et al.,* 2010). Indoor Residual Spraying (IRS) application reduced malaria cases successfully in Bioko Island, Equatorial Guinea (Kleinschmidt *et al.,* 2013), as its importance was previously evident in the decades long campaign in South Africa (Mabaso *et al.,* 2004). Insecticide treated nets (ITNs) act by repelling or killing the mosquitoes (Wilson *et al.,* 2014). Currently, Long Lasting Insecticide Nets (LLINs) have proved successful in reducing malaria morbidity and mortality in most countries across sub-Saharan Africa (Tambo *et al.,* 2012). As per the WHO (2010) guidelines, only pyrethroids can be used in treated nets and applied as IRS (Kawada *et al.,* 2014). Nevertheless, widespread resistance to these tools have been reported (Chanda *et al.,* 2011; Kawada *et al.,* 2011; Mulamba *et al.,* 2014) supporting the argument that ecological control of malaria vector could be the most ideal control strategy.

2.5.5. Biological Control of Malaria Vectors

The biological control effort of malaria vectors has mainly used mosquito larval predators such as fish and tadpoles (Peckarsky, 2006), while others have tried entomopathogenic bacteria such as *Bacillus thuringiensis* (Bti) and some species of fungus, *Metarhizium* (Scholte and Takken, 2008; Scholte *et al.*, 2004).

Biological control will give a long-lasting effect if the biological agents can survive and recycle. This can only be successful if the agent is locally identified and used because of easier adaptability. Many biological agents including predators, parasites and microbial agents have been assessed in the laboratory as bio-control agents for mosquitoes and other urban pests (Federici, 1995). However, the only bio-agents that are in operational use are bacteria *Bacillus thuringiensis* H-14 and *Bacillus sphaericus* 2362 (Romero *et al.*, 2001). In addition, specific microbial agents are targeted for certain pest species only (e.g. *Bacillus thuringiensis*). The obvious reason is that these agents are introduced into unfamiliar areas leading to enormous challenges in vectors control worldwide. It is thus important to identify indigenous predators within the Mara River basin that can be used as bio-control agents locally as opposed to use of insecticides.

2. 5.5.1. Predators

The role of predatory aquatic insects in the natural regulation of mosquito larvae has been reported. The mosquito-fish, *Gambusia affinis,* has been used for mosquito since the early decades (Kroeger *et al.*, 2013). However, mosquito-fish was found to affect not only mosquito larval populations, but also reduce or even displace other native species (Miura *et al.*, 1984;

Pyke, 2008; Leopard *et al.,* 2013). In addition, mosquito-fish sometimes failed to control mosquito larval populations, most likely due to reduction of other natural antagonists (Blaustein *et al.,* 2004). Predatory insects and their larvae (e.g. Dystiscidae, Notonectidae, and Odonata) do not only prey on mosquito larvae, but also prevent adult mosquitoes from oviposition (Fincke, Yanoviak, & Hanschu, 1997; Stav and Blaustein, 2000; Lundkvist *et al.,* 2003; Fischer *et al.,* 2012). However, difficulties in colonization and management of insect predators, as well as a lack of synchrony between predator and prey life cycle, impeded their deployment (Atwood & Richardson, 2012). As stated previously, both mosquito-fish and insect predators occur mainly in large, permanent ponds, while most mosquito species prefer temporary ponds as breeding sites (Chumchal *et al.,* 2016). Therefore, their impact on natural mosquito larval populations could be over-estimated. Particularly, the abundance and distribution of these mosquito predators and their relationship with mosquito larvae abundance and distribution within the Mara River basin remained undetermined.

2.5.6. Other Mosquito Vector Control Methods

Curently, genetically modified mosquitoes are being exploited as mosquito control agents. This has been tested through genetic engineering that transforms *Plasmodium* susceptible strain of mosquito to a more refractory form that cannot tranmsit *Plasmodium* (Beerntsen *et al.,* 2000; Ito *et al.,* 2002; James, 2003). Other methods include use of refractory genes that can be driven into the wild populations by using symbionts (Dotson and Beard, 2009). More recently, malaria vector control using the sterile insect technique (SIT) (Pates and Curtis, 2005), is in the pipeline. This employs laboratory male mosquitoes that are conspecific and compatible with the target population (Alphey and Alphey, 2014), which are then released to mate with the wild population. Interestingly, this method has not succeeded in producing any meaningful result because the laboratory reared mosquitoes have not shown any responsive trait and also the released males become weaker in the field (Reeves *et al.*, 2012). Larval mosquitoes can also be targeted using source reduction, such as elimination of mosquito vector breeding sites, usually reffered to as source reduction of the breeding sites. However, one demerit of this is that most of the breeding sites are small, dispersed and transient, making it even more complicated for effective vector control (Pates and Curtis, 2005).

2.5.7. Malaria: The Disease, Symptomatology and Life Cycle

Species belonging to the genus *Plasmodium* namely *Plasmodium falciparum, P. vivax, P. malariae*, *P. ovale,* and the recently reported *P. knowlesi* cause malaria in humans (Ndouo, 2009). Among these, the species that causes the greatest illness and death in Africa is *P. falciparum* (Ndouo, 2009). *Plasmodium falciparum* occurs in most malaria-affected areas of the world, *i.e.* tropical Africa and Asia. Up to 85-90% of malaria cases are due to *P. falciparum*. *Plasmodium vivax* is uncommon in sub Saharan Africa (Md Idris et al., 2014), but common in South Asia and Central America, and is predominant in South America. Similarly, *P. ovale* is found mainly in tropical Africa, in West and South Africa, with sporadic reports from other continents, e.g. the South Pacific islands. *P. malariae* is the least common species of malaria to infect humans, and is infrequent all over the world (Moresby, 1998). Malaria symptoms may appear and disappear in phases and may come and go at various time frames. These cyclic symptoms of malaria are caused by the life cycle of the parasites - as they develop, mature, reproduce and are once again released into the blood stream to infect more blood and liver cells. Fever is the main symptom of malaria (Odaga *et al.*, 2014). A high swinging fever can develop when this happens, with marked shivering and intense perspiration.

Furthermore, serious complication involving the brain and kidneys can then develop leading to delirium and coma. The most severe manifestations are cerebral malaria (mainly in children and persons without immunity), anaemia (mainly in children and pregnant women), kidney and other organ dysfunction (e.g. respiratory distress) (Bartoloni & Zammarchi, 2012; Newton *et al.,* 2000). Persons repeatedly infected with malaria will usually acquire a considerable degree of clinical immunity, which provides them with protection against future infections (Baird *et al.,* 1995).

During the 1960s, there were occasional reports of accidental infections with *P. cynomolgi, P. inui* and *P. knowlesi* in humans, a known primate malaria species; suggesting that some primates might act as reservoirs for human malaria, though it appeared that the chances of such naturally acquired infections were very remote (Cogswell, 1992). However, it is now apparent that humans are at risk from infection with *P. knowlesi*, a malaria parasite with a 24 hour erythrocytic cycle, found especially in Southeast Asia where its natural hosts are macaque and leaf monkeys (Jongwutiwes *et al.,* 2004). Until 1971, there had only been two authenticated cases of naturally acquired human infections with *P. knowlesi* both in peninsular Malaysia. No other cases were recorded until 2004 when a focus of human infections was identified in Sarawak, Malaysian Borneo (Singh & Daneshvar, 2013). Since then there have been several hundred reports of human infections in the region and there is now overwhelming evidence that *P. knowlesi* is a zoonosis involving macaque (*Macaca* spp.) and leaf monkeys (*Presbytis* spp.) as reservoir hosts with mosquitoes belonging to the Leucosphyrus group of *Anopheles* as the vectors, mainly distributed in Malaysia and other countries in Southeast Asia (Vythilingam, 2012). Retrospective examination of blood films and the application of the polymerase chain reaction (PCR) and other molecular techniques revealed that a number of malaria cases previously attributed to *P.*

malariae in Malaysia were misidentified and that they were in all probability due to *P. knowlesi* (Cox, 2010).

The life cycle of *Plasmodium* species is shown in Figure 2.4. *Plasmodium* have both human and mosquito cycles. Human infection with malaria is initiated when the female *Anopheles* injects into the human host saliva containing plasmodial sporozoites during feeding. Sporozoites enter the human blood circulation system and rapidly either enter hepatocytes or are cleared (Yamauchi, Coppi, Snounou, & Sinnis, 2007). Within the hepatocytes, sporozoites reproduce asexually (known as schizogony, forming hepatic schizonts). This stage is asymptomatic and reflects the primary incubation period. The period lasts on average from 5 to 6 days for *P. falciparum,* and 10 to 14 days for *P. vivax.* Occasionally it may take much longer (approximately 1 month), on average, for *P. malariae*. At the completion of this stage, hepatic schizonts rupture and release plasmodial merozoites into the circulation. *P. vivax* and *P. ovale* hypnozoites may remain dormant for prolonged periods of time. When these leave dormancy and enter schizogony, they may cause the characteristic relapses associated with these plasmodial forms. A proportion of merozoites released into the circulation develop into male and female gametocytes. When taken up by female *Anopheles* in a blood meal, gametocytes develop into microgametes in the mosquito stomach, fuse to form a zygote, ultimately penetrating the mosquito stomach to form an oocyst. Within the oocyst, motile sporozoites develop ultimately bursting the oocyst and migrating to the salivary glands, from which they will be injected into the next host at the mosquito's next blood meal (Deng *et al.,* 2014).

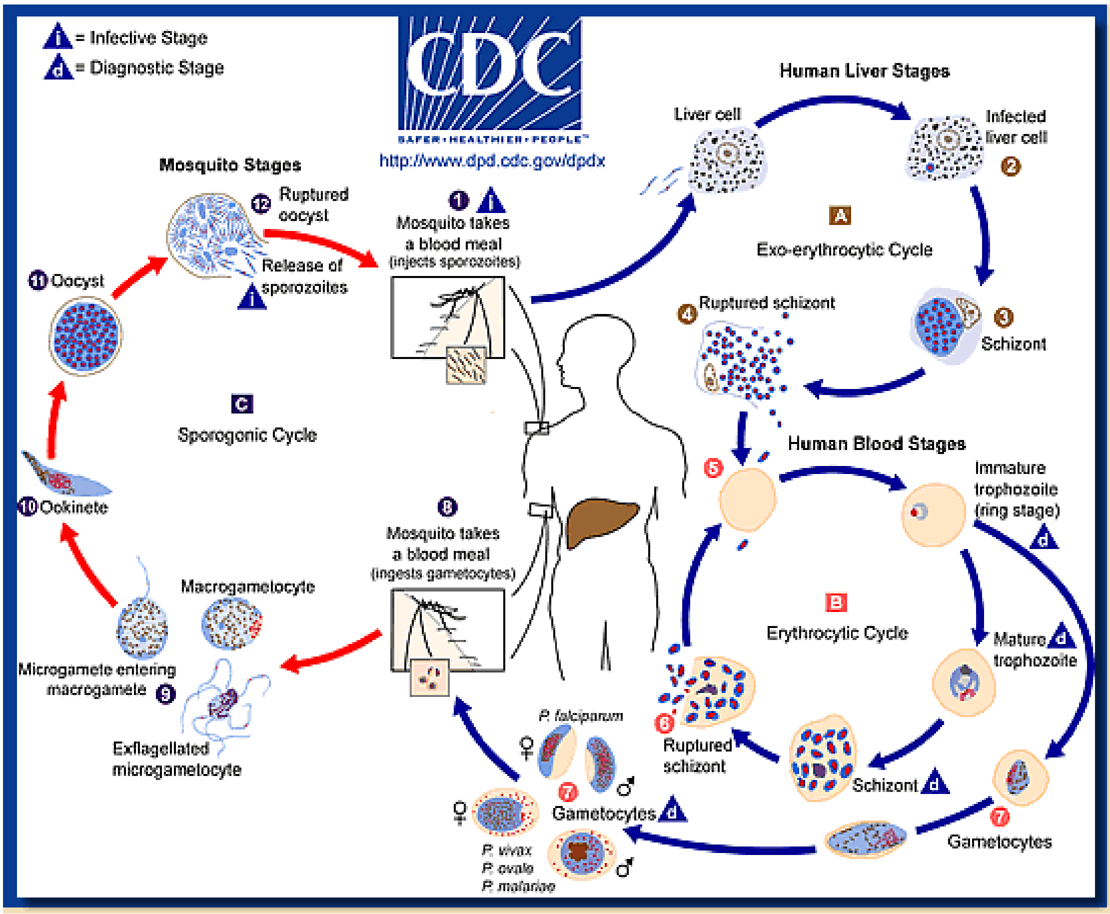


Figure 2.4: Life Cycle of Malaria Parasite

(Source, CDC: ***http://http://www.cdc.gov/malaria/about/biology/)***

The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host❶. Sporozoites infect liver cells ❷and mature into schizonts❸, which rupture and release merozoites❹. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogonyA), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogonyB). Merozoites infect red blood cells❺. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites❻. Some parasites differentiate into sexual erythrocytic stages (gametocytes)❼. Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes) are ingested by an *Anopheles* mosquito during a blood meal❽. The parasites' multiplication in the mosquito is known as the sporogonic cycle🅲. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes❾. The zygotes in turn become motile and elongated (ookinetes) ❿which invade the midgut wall of the mosquito where they develop into oocysts⓫. The oocysts grow, rupture, and release sporozoites⓬, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites ❶into a new human host perpetuates the malaria life cycle.

2.5.8. Malaria Situation in the Lake Victoria Basin

In the Lake Victoria basin, malaria transmission is intense and is also affected by climate and geography, and often coincides with the rainy seasons (Hashizume *et al.*, 2012). Studies showed increased incidence of malaria in the Lake Victoria region as early as 1980s (Imbahale *et al.*, 2011). This prompted a wake-up call to researchers and communities within the Lake Victoria region to consider the potential impacts of climate and health issues on their vulnerability and coping strategies. Although rural communities are particularly affected, urban areas are not spared either, because of the close link between urban malaria and migration, as well as drainage set-ups found within cities due to unplanned development (Siri *et al.*, 2010). Poorly planned and poorly drained informal settlements in cities and towns create the potential for an increase in malaria linked to rapid urbanization. According to Noor *et al.* (2009), malaria constituted approximately 32% of the total outpatient cases in Nyanza and Western provinces in Kenya, followed by upper respiratory tract infections, skin diseases and diarrhea.

Epidemics of the disease frequently occur in highlands, Lake Victoria basin and coastal regions with the following districts cited as being most at risk: West Pokot, Trans Nzoia, Uasin Gishu, Kericho, Nandi, Bureti, Kisii, Nyamira, Gucha, Transmara and Nyando-almost three quarters of which falls within the Mara River basin catchment of Kenya (Kenya Malaria Fact Sheet, 2014). According to Bomet and Kericho counties report, malaria cases have increased from 5-10% between 2008 and 2011 and in Transmara, the malaria test positivity rate was reported to be higher, and ranging between 10-30.1% in 2012 alone. Baringo County is classified as endemic for malaria, while cases of Rift Valley Fever outbreaks was reported in the past (2006-2007) causing high mortalities among small ruminant which account for about 3.41 million of total livestock population, with 117 humans having been affected leading to 3 deaths (WHO, 2007). It was therefore imperative to determine which species of mosquitoes are responsible for disease transmission along the Mara River and and its tributaries.

The most vulnerable groups to malaria in the population are children and pregnant women. For instance, a longitudinal cohort project undertaken between 1992 and 1994 in Asembo Bay of Western Kenya, reported malaria parasite prevalence to be 83% in 1-4 year olds and 60% in 10-14 year olds (Bloland *et al.,* 1999). Anaemia was reported in the same study to be consistently associated with high-density infection of malaria in children under the age of 10 years of age. More than half of all pregnant women had hemoglobin levels of <11.0 g/dl, with up to 40% having a Hb of <8.0 g/dl in the peak of malaria season.

Malaria control involves a number of different approaches. These include protection against infection through prophylaxis, control of development of the disease in infected individuals, personal protection through protective clothing, repellents, bed-nets, community/population protection through insecticide spraying, and environmental management (Ginn *et al.,* 2008). Meanwhile, previous studies reported the effectiveness of insecticide-treated bed-nets in reducing morbidity and mortality from malaria has been documented in many studies (Choi *et al.,* 1995; Abdulla *et al.,* 2001; Binka *et al.,* 2007;; Eisele *et al.,* 2010). This has been exploited comprehensively in the fight against the disease. However vector resistance often impedes progress in the fight against the diseases within the Lake Victoria basin region (Kawada *et al.,* 2011a; Kawada, *et al.,* 2011b).

2.6. Malaria Vectors and their Predators in the Lake Victoria Basin

Breeding of mosquitoes in aquatic habitats can be influenced by both abiotic and biotic factors some of which are dependent on certain locations (Wambold *et al.,* 2011; Gouagna *et al.,* 2012). The main abiotic factors that influence breeding habitats of mosquito larvae include water temperature, its chemical composition, water pH, depth and turbidity, while the biotic factors are mainly the predators, bacteria, fungi, and aquatic plants (Minakawa *et al.,* 1999; Ohba *et al.,* 2012). More importantly, habitat location is crucial because it can be influenced by local factors such as weather conditions (rainfall patterns, temperature), and physico-chemical parameters such as pH, alkalinity and turbidity.

Other important habitat factors include land use and degradation patterns (e.g. soil erosion, chemical pollutants) as well as land use and geological conditions (Matano *et al.,* 2014). Species assemblages and abundance in specific locations can also be influenced by historical factors and population dynamics, mainly previous colonization or non colonization of the area by the particular species, and how population increase or decrease is dependent on local environmental pressures (Kenawy *et al.*, 2013). Therefore, mosquito larval habitat location and ecology becomes important in determining larval densities and species assemblage which in turn influences malaria transmission in an area.

The mosquitoes that transmit malaria belong to the *Anopheles* group. However, not all *Anopheles* mosquitoes are vectors of malaria. For example, there are more than 200 species of *Anopheles* mosquitoes worldwide, but only four of these mosquitoes are known to carry malaria parasite (Carter & Mendis, 2002). In the sub-Saharan Africa, the *Anopheles gambiae* complex consists of six confirmed species, one unnamed species and several incipient ones (Maureen Coetzee *et al.*, 2013). The six species are *An. gambiae sensu stricto, An. arabiensi, An. merus, An. melas, An. quadrianmnulatus* and *An. bwambae*. More recently, the previously known forms of *An. gambie* s.s have evolved into to *An. coluzzi* and *An. gambiae* s.s species based on molecular forms (M and S) have been identified that appear to be reproductively isolated (Fossog *et al.*, 2015). The 'S' form is distributed widely throughout the *An. gambiae* species range, whereas the 'M' form is commonly restricted to western parts of Africa, and hybridization between them is rare in most areas of sympatry. The complex varies in their ability to transmit malaria and other diseases. Interestingly, due to climate change, other *Anopheles* species which are found outside Africa, and are known trammitters of various diseases have been reported to spatially exist in the area (Dida *et al.*, 2015), albeit the unknown species of disease vectors that may exist within the Mara River basin which needs investigation.

Overal, there are four larval stages, namely, 1st, 2nd, 3rd and 4th instars, respectively, of larval mosquitoes (Schaper & Hernández-chavarría, 2006). The morphological distinctive feature of the *Anopheles* larvae is its ability to lie parallel to the surface of the water, unlike *Culex* larvae which lies perpendicular to the water surface (Schaper & Hernández-chavarría, 2006). They breathe air via small caudal openings (spiracles). This surface position makes them susceptible to chemicals, which float on water. Yet larvae can also take up oxygen dissolved in the water but to a limited extent. This means therefore that an oil film with mechanical protection is only of limited benefit. The larvae are filter-feeders and have oral tufts of hair. They feed on all kinds of microscopic organisms (Ohba *et al.*, 2012).

Culex spp, together with *Anopheles gambiae* complex and *Anopheles funestus s.s.*, are the most important vector in sub-Saharan Africa. *Culex* mosquitoes can breed easily in polluted water (drainage canals, septic pits, etc). The vectors thrives better under urban conditions of poverty, poor water drainage and pollution (De Silva and Marshall, 2012). In the mosquito genus *Mansonia*, there are two subgenera: *Mansonia* and *Mansonoides.* Their larvae breathe air through a siphon via the roots and stems of water plants. *Pistia* spp., water hyacinth (*Eichhornia*) and marsh grass (*Isachne*) are the principal host plants (Ghosh *et al.*, 2006).

Mosquito breeding in aquatic habitats is also largely influenced by the presence of predators (Gouagna *et al.*, 2012). Mosquito predators and most of the other aquatic insects, which are associated with wetlands are, however, not well studied *(Gouagna et al.,* 2012). Yano *et al.* (1983) listed 117 species of aquatic coleopterans, in 14 families from rice fields worldwide. Majority of these have since been implicated as mosquito predators (Bambaradeniya &

Amarasinghe, 2004). A study in the rice-cultivating area of Malaysia showed that the orders Diptera (Families: Chironomidae and Culicidae), Coleoptera (Family Hydrophilidae), Hemiptera (Families: Dytiscidae, Corixidae, Pleidae, Nepidae, Belostomatidae), Odonata (Families: Libellulidae, Coenagrionidae), and Ephemeroptera (Family Baetidae) comprised the major aquatic insect fauna. The dominant aquatic insects were from the families Chironomidae, Dytiscidae, Corixidae and Belostomatidae (Bambaradeniya & Amarasinghe, 2004), of which the aquatic representatives of the Coleoptera, Hemiptera and Odonata were the most predatory insects in the aquatic ecosystem.

Nevertheless, predation of larvae by larvivorous fish (Chandra *et al.*, 2008) and cannibalism among larvae (Soleimani-Ahmadi *et al.,* 2014) also influence the population dynamics of mosquito larvae and are factors that play a major role in mosquito population size. Some of the larvivorous fish have also shown potential as bio-control agents in rice fields (Chandra *et al.,* 2008).

The larvivorous nature of *Oreochromis niloticus* was also reported, whereby zooplankton and insects form, including larval mosquitoes were shown to be their main food component in all the seasons; long dry, short dry, short rainy and long rainy seasons (Wijesinghe, Wickramasinghe, Kusumawathie, Jayasooriya, & De Silva, 2009). *Clarias gariepinus* fingerlings have also been reported to feed on insects including mosquito larvae/pupae and act as biological control agents (Ofulla *et al.,* 2010). A similar study also reported that Haplochromines (astatotilapia) feeds primarily on larval and adult insects, further reinforcing the role that fish species can play in controlling mosquito populations in aquatic habitats (Day *et al.,* 2015).

Although no single approach to mosquito control is appropriate for all locations, emphasis on natural control of mosquito larvae by their natural predators should be an important element worth considering in the long-term planning of mosquito control. Since the predators have a significant effect on overall mosquito populations, their role should be considered when implementing habitat management, mosquito control and when modeling mosquito population dynamics. However, it should also be noted that while there are various organisms known to prey on mosquito larvae, such as copepods, insects and fish, information on their presence, abundance and distribution are limited (Kumar & Hwang, 2006). This is because many predators that have been shown to be highly successful in eliminating target prey have been experimented in the laboratory. However, their relationship in natural habitats remains unexploited.

Various organisms, known as natural biological control agents, can be utilized to control mosquito populations along the Mara River, avoiding the use of chemicals that can cause harm to human and environment. The efficient selection of effective natural enemies has become increasingly important for the success of biological control programs. Control of mosquito larvae with biological agents like competitors and predators is more convenient and alleviates the need for frequent chemical applications. The selection of biological control agents should be based on their potential for unintended impacts, self-replicating capacity, climatic compatibility, and their capability to maintain very close interactions with target prey populations (Kluge, 2000). They eliminate certain prey and sustain such environments (i.e., as when prey is introduced, they eat the prey) for long periods thereafter (Kumar & Hwang, 2006). However, this will only be possible if the predator possesses extraordinary search efficiency irrespective of the illumination situation in response to the emergence of prey. Thus, the current study was designed to establish the predator-prey relationships, and particularly with reference to existence of other environmental parameter such as habitat types and water physico-chemical parameters.

2.7. Influence of Physico-Chemical Parameters on the Aquatic Habitats of Larval mosquitoes and their Predators

Invasion of the aquatic habitats by organisms including disease vectors can be influenced by abiotic and biotic factors (Gouagna *et al.,* 2012), some of which are dependent on certain locations. Habitat location is important because it can be influenced by local factors such as weather conditions (rainfall patterns, temperature), and even physio-chemical parameters such as pH, alkalinity and turbidity; most of which depend on adjacent land use practices and are influenced by the adjacent land degradation status and soil or geological conditions. Species assemblages and abundance in specific locations can also be influenced by historical factors and population dynamics such as previous colonization or non-colonization of the location or area by the particular species and how population increase or decrease depending on local environmental pressures. This can also be true for differential abundance of mosquito species in different locations (Mutuku *et al.,* 2006).

The main abiotic factors that can influence breeding habitats of mosquito larvae include water temperature, conductivity, salinity, water pH, depth and turbidity (Norkute, 2014). Studies in Ethiopia, showed that conditions which favoured *An. arabiensis* larvae in their breeding habitats were temperature greater than 27°C, water depth of less than 40cm, high carbonate concentration, high water pH, and presence of water lettuce (Abdelbasit & Fadlelmola, 2009). *An. merus* and *An. melas,* both members of *An. gambiae* complex, breed in salt water with a pH greater than 7.0

(Gillies & Coetzee, 1987). However, in Mbita point, western Kenya, water pH was shown not to determine the occurrence of Anopheline larval mosquitoes (Minakawa *et al.,* 1999). Turbidity of water has been reported to have an effect on larval populations by influencing adult oviposition behaviour. Adult females of *An. gambiae* s.s. were shown to prefer ovipositing on clear water rather than on turbid water (Parham *et al.,* 2012).

The physico-chemical microclimate is an important aspect trying to characterize larval habitats. Water temperature, is widely regarded to have a positive correlation with the densities of Anopheline larvae (Munga *et al.,* 2005; Muturi *et al.,* 2007; Kenea *et al.,* 2011). This is likely connected with Anopheline larvae being more frequent in less shaded waters, which naturally should be warmer than those in the shade. Dissolved oxygen and pH have been shown to have a positive correlation with distribution and abundance of both Anopheline and Culicine larvae (Adebote *et al.,* 2008). Other physico-chemical variables positively correlated with Anopheline larvae are concentration of nutrients phosphate (Rejmánková *et al.,* 2013) and nitrate (Norkute, 2014). Dejenie *et al.* (2011) in a study on malaria vector control in Ethiopia showed that almost all their study habitats were alkaline (pH>7.0) and both Anopheles and Culex larvae were positively associated with this high pH. Paaijmans *et al.* (2008) and Couret *et al.* (2014), established temperature and dissolved oxygen as important for larval mosquitoes development. However, Minakawa *et al.* (1999) argue that a combined physico-chemical effect can influence mosquito abundance.

Chemical composition of water influences mosquito larval species and their population and can also influence the abundance of predators. Since the previous studies found that various aquatic microhabitats tend to have different sets of physico-chemical parameters that influence the occurrence of the malaria vectors and their predators, it was thus necessary to establish the physico-chemical characteristics of the different habitats and relate it to the presence and abundance of larval mosquitoes on the Mara River.

2.8. Conceptual Framework

The invasion of the aquatic habitat by both larval mosquitoes and predators along the Mara River can be presented as an interlay of four important factors: larval mosquitoes, predator, habitat types and the physico-chemical parameters. However, other confounding factors such as destruction of forest, human settlement; irrigation and urbanization, are also major driving forces that can influence ecological change favourable for mosquaito breeding. Moreover, use of mechanized farming and application of ferterlizers in the surrounding catchment may alter water chemistry, which can eventually either influence or hinder the invasion of the river channel or its tributatries and the adjacent habitats by both larval mosquitoes and predators. The sustainability of this interplay can lead to the existence of malaria vectors capable of transmitting the disease. The main goal of establishing whether larval mosquitoes have invaded aquatic habitats is to safeguard human health. However, this goal can only be achieved through factual and reliable data on the existence of these larval mosquitoes and their influencers. It was, therefore, important to determine the presence of larval mosquitoes and their predators and the influence of the physico-chemical parameters on their breeding habitats along the Mara River and its tributaries that may contribute to their abundance. Figure 2.5 gives a flow chart of the conceptual framework.

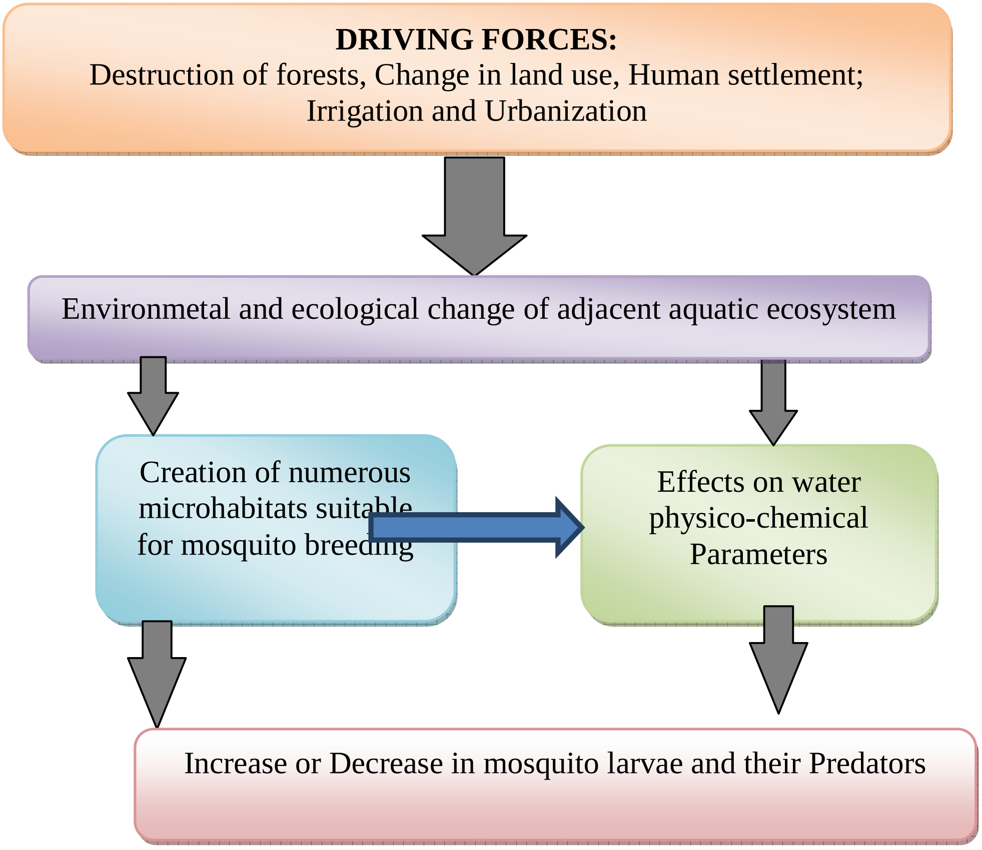


Figure 2.5: Conceptual Framework. ***Source: Researcher (2014).***

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study Area

This cross-sectional study was conducted along the trans-boundary Mara River basin which lies between Longitudes 33^{0}47’E and 35^{0}47’E and Latitudes 0^{0}28’S and 1^{0}52’S, traversing Kenya and Tanzania, in East Africa (Mutie *et al.*, 2006). This study was conducted at the upper-, mid- and and lower-Mara River Basin (South W. of Kenya and North Eastern part of Tanzania) and it’s two perennial tributaries of Amala and Nyangores, in the upper Mara River catchment area. Other smaller streams draining into the Mara River on the Kenyan and Tanzanian side of the basin were also included. Several streams from first to fourth order (Strahler system) feed the Amala and Nyangores tributaries in the upper catchment. The Mara River is a sixth order stream at the junction of these two tributaries (Figure 3.1).

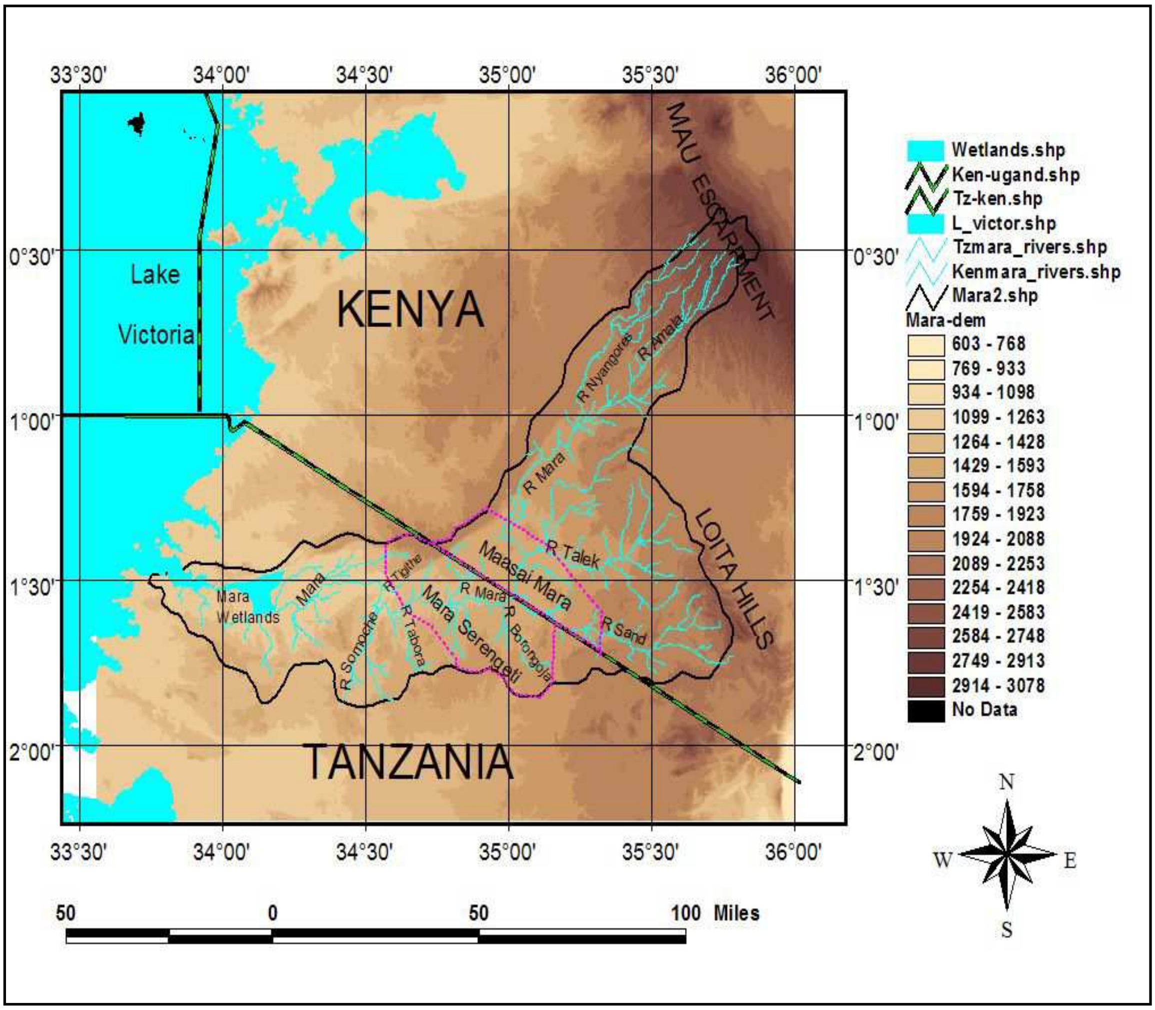


Figure 3.1: Map of Mara River basin showing Nyangores and Amala tributaries (Source: Hoffman, 2007).

The upper catchment of the Mara River basin has an average precipitation of about 1400 mm annually but varies among years. The evapotranspiration is around 1,090 mm per year (Mango *et al.*, 2011), temperature varies between 10.5°C and 15°C. The study area has two large towns on the Kenya side, Mulot and Bomet, and smaller town centres, which include Silibwet, Sierra

Leone, Tenwek, Tegat, Kembu and Mugango. On the Tanzania side, the main town centres are Kwebuse, Morito and Musoma. The main land uses within the basin are agropastoralism, livestock keeping, large scale and small scale irrigation agriculture, wildlife conservation, urban centres and human settlement (Mutie *et al.*, 2006).

3.2 Sampling Sites Selection and Description

Before the start of the sampling process, a GIS (Arc GIS) tool was used to demarcate boundaries and sampling sites within the Mara River basin. The selection of the sites was purposive aimed at covering both sub-catchments of Amala and Nyangores tributaries and lower catchments with their smaller feeder rivers, and the other sites downstream. The location of each of the sampling points was marked using GPS equipment (Appendix I). On these points, mosquito-breeding habitats were identified within a 100m stretch. On the main Mara River channel on the Kenyan side, the sites sampled included: Kapkimolwa, Ngerende 1, Lower Ngerende, Kabosom Bridge, Twenwek falls, Chemosit Bridge, Mara Bridge (site 3). Along the Mara River tributaries, the sites sampled included: Silibwet, Kapkimolwa, Kabosom, Isei Bridge, Chepterer and Chemosit Bridge. On the Tanzanian side of the Mara River, samples were collected from 5 sampling sites, namely: the new Mara Bridge at the Kenya –Tanzania border, Nyahenda Bridge, Tarime Serengeti Bridge, Morito Bridge at Kwebuse, Morito village and Kirumi wetland at the point of entry into Lake Victoria. Specific mosquito breeding sites such as river beds, swamps, drainages, open sunlit puddles and rock pools were all mapped and included in the study. Figure 3.1 shows geo-referenced location and distribution of the 39 sampling sites identified and sampled for the presence of mosquito larvae and their predators within the Mara River Basin.

3.3. Sample Size Determination

The technique used in determining sample sizes for biotic factors was non-statistical due to the infinite nature of target populations (water physico-chemical parameters, larval mosquitoes, macro-ivertebrates and fish). According to Kirkwood and Sterne (2006), purposive sampling is the method of choice for studies in which certain participant characteristics are desirable because of how well they match the goals of the research. As for this study, the selection of the sites were purposive because whenever a suitable and safe area along the Mara River (free of hippos, crocodiles and other hazards) was reached, the area was searched for potential mosquito breeding sites and their predators sampled. Therefore, the sampling sites were decided upon after assessing the prevailing field conditions without applying any approved mathematical formula. The sample size for biotic and abiotic factors in this study was however guided by the previous work of Fillinger & Lindsay (2011).

3.4. Study Design and Data Collection Methods

The study employed a purposive research design in which sampling of selected sites along the Mara River basin and its tributaries were done. The codes of the sampling sites were given based on the point of sampling. The points were strategically chosen and described based on available structure such as a bridge. For example, the sampling site on each side of the bridge was labeled based on the point of sampling relative to the bridge and direction of flow of the river by use of the first letters of upper and downstream sections (i.e. URS1-10 and DRS1-10) respectively, for the main river. For instance, URS 1-10 meant that the sampling site was located at the upper part of the main river or either of its tributaries before a bridge, while DRS 1-10 were located on lower side of the river after the bridge. Sites 1, 2, 3, etc, were given to any identified breeding terrestrial habitat adjacent to the main river or its surrounding with the nature and/or name of the habitat also described in detail. This kind of labeling was used for ease of recording the findings and to facilitate analysis of the specimen. At the sampling site, local assistance was sought to help navigate the area and explain the nature of the study area and the dangers that might be present.

3.4.1. Desription of the Sampling Procedures

In each of the selected sampling point, potential breeding habitats for larval mosquitoes identified in a 10m stretch along the Mara River basin were sampled and the larval mosquitoes and predators captured recorded as per the checklist attached (Appendix II).

3.4.2. Larval and Predators Sampling

Sampling of mosquito larvae were conducted using a standard mosquito dipper (350 ml; BioQuip Products, Rancho Dominguez, California, USA) to determine presence or absence of larval mosquitoes and to estimate their abundance (Minakawa *et al.*, 2012). Each sampling site was geo-recorded using handheld Global Positioning System (GPS) and their coordinates taken. A maximum of 20 dips were done on each site, distributed across the defined area at suitable places for Anopheline and Culicine larvae.

The habitats were categorized as main river water habitats, drying streams, swamps, rock pools, hippo hoof-prints and puddles. The habitats were categorized by the vegetation cover and characteristics as short grass (short vegetations), tall grass (tall vegetations) [see Appendix III] and open sunlit puddles depending on the presence or absence of different vegetation types. The open habitat was a distinct area larger than 1m^2. The captured mosquito larvae were immediately preserved in 90% ethanol for later identification and analysis.

A D-frame dip net sampler of 0.3m width attached to a long pole and with a cone shaped bag for capturing the mosquito larvae predators was used. Sampling was done from downstream end of the river to upstream. A total of three jabs were made at each sampling point, with a single jab consisting of a forceful thrust of the sampler into the sediment for a linear distance of 0.5m.

3.4.3. Determination of Water Physico-Chemical Parameters in Breeding Sites

The physico-chemical parameters were measured *in situ* with a handheld multi-parameter meter, YSI Professional Plus (YSI Integrated Systems and Services, St. Petersburg, FL 33716, USA). Prior to the readings at the sampling site, the YSI was dipped in water for 3 minutes to stabilize, and then the parameters recorded after the stabilization of values was noted.

3.5. Laboratory Identification and Analysis of Larval mosquitoes and their Predators

In the laboratory, all the collected larval mosquitoes were identified microscopically using standard taxonomic keys. The *An. gambiae* s.l. and *An. funestus* group larvae were further identified to species level by use of PCR as follows: Each individual larva was put into 1.5ml vial then dried in anhydrous Calcium Sulphate before being kept for analysis. The desiccation process was aimed at preventing the rotting of specimens, since rotting of specimens could cause degradation of DNA. During desiccation, uncorked vials containing the specimens were put in a container with anhydrous Calcium Sulphate, which were sealed and kept for three days for water to be absorbed from the specimens.

During the sampling process, the relatively large macro-invertebrate such as *Anisops wakefieldi* (back-swimmers), Rhantus larvae (diving beetles) and *Onychohydrus hookeri* (water beetles) were visually observed, classified and counted. Those that could not be identified to species level in the field were preserved for further identification using appropriate keys. The total number of mosquito larvae predators were counted and totaled up for each habitat sampled.

3.5.1. *Anopheles gambiae* Complex DNA Extraction

An extraction method as described by Kamau *et al.,* (1998) was used to evaluate mosquito larvae species (Appendix I). Individual mosquito larvae were put into a 1.5ml vial and 100µl of ground buffer (0.08M NaCl, 0.16M sucrose, 0.06M EDTA, 0.1M Tris-HCl and 0.05% SDS) added. A pestle was used to grind the larvae until homogenous lysate was formed. The lysate was then incubated for 30 minutes in a water bath at 65^0 C then 14µl of 8M Potassium Acetate added and the mixture vortexed. The mixture was then incubated in ice for 30 minutes and then centrifuged for 10 minutes at 10,000 rpm. Supernatant was picked, put into fresh 1.5ml vial and 200µl of 90% ethanol added. This mixture was then kept at $–20^{\circ}$ C for at least 20 minutes after which it was centrifuged at 10,000rpm for 20 minutes. The 90% ethanol was then discarded and replaced with 70% ethanol, which was then poured off and replaced with 90% ethanol again. The 90% ethanol was then poured off and the tubes dried inverted on blotting paper overnight at room temperature. The following day the pellet DNA was suspended in 100µl sterile distilled water and kept at $–20^0$C until it was needed for analysis.

3.5.2. *Anopheles* Sibling Species Identification

The *Anopheles gambiae* and *Anopheles arabiensis* sibling species were identified by Polymerase Chain Reaction (PCR) and electrophoresis technique. The PCR was done using PERKIN ELMER^{TM} GeneAmp PCR System 9600 machine (LabX Company, Midland, ON, Canada), using the ribosomal DNA based technique of Cornel and Collins (1996). During the rDNA-PCR, each reaction mixture of 15µl contained, 10X PCR buffer (10mM Tris (pH 8.3), 50mM KCl, 1.5mM (1.8$MgCl_2$), 25mM $MgCl_2$, 10mM dNTPs, (N=adenine, guanine, cytosine, and thymine), 0.5U *Taq* polymerase, 10pmol of each primerand 1µl DNA template: universal 20-mer primer (UN) = GTG TGC CCC TTC CTC GAT GT; *An. gambiae* primer (GA) = CTG GTT TGG TCG GCA CGT TT; *An. arabiensis* primer *(*AR) = AAG TGT CCT TCT CCA TCC TA. The cycling conditions were as follows: pre-cycle denaturation at 20 s at 95^0 C, 30 s at 55^0 C, and 30 s at 72^0C, the cycle was repeated 30 times and a final extension at 72^0C for 5 minutes. After the polymerase chain reaction (PCR), the amplified products were analysed by electrophoresis. The master mix protocol for *An. gambiae* s.s*, An. arabiensis* and the resulting bands is as shown shown in Appendix IV (Figure 1 and 2).

The amplified DNA was loaded onto a 15% agarose gel in the electrophoresis tank (E-C Apparatus Corporation, St. Petersburg, Florida) and an electric field applied. The 15% agarose gel contained 3µl of ethidium bromide, which enabled the separated bands to be visualized under UV transillumination. The bands were photographed for future reference. Sample within the *Anopheles gambiae* complex that were not identified using PCR methods were marked as unknown *Anopheles gambiae* species. All unused specimens were preserved in 99% ethanol for future reference.

The species within the *An. funestus* group were identified by DNA-based techniques. The DNA extraction was done as described by Collins *et al.* (1987) [Appendix II]. Preparation of DNA extraction solutions and protocol is as described in Appendix II. Species-specific identification of *An. funestus* siblings was performed according to the cocktail PCR assay of Koekemoer, Kamau, Hunt, & Coetzee, (2002). The primers specific to *An. funestus* s.s, *An. rivulurum*, *An. veneedini*, and *An. parensis* were available for the PCR.

3.6. Statistical Analysis

3.6.1. Data Exploration

The first step in data analysis involved evaluating the quality of data collected. Each individual set of variables was first checked for their distribution and homogeneity of variance using histograms and dot charts. Multiple logistic regression assumes that the variables have normal distributions as non-normally distributed variable (highly skewed or kurtotic or variables with substantial outlier) can distort relationships and significant tests. In this analysis, the data sets were found to be non-normally distributed, thus an option of analysis was chosen. Since transformation did not improve data quality, the dataset was analysed as per Soediono, (1989), using both Generalized Linear Model (GLM) and Canonical Correlation Analysis-CCA (Appendix V).

The GLMs extend the linear modeling capability of R to scenarios that involve non-normal error distributions or heteroscedasticity (Zuur *et al.,* 2009). In this aspect, all other classic assumptions (particularly independent observations) still apply. Under this concept, the linear functions of the predictor variables are obtained by transforming the right side of the equation (f(x)) by a link function. The data is then fitted in this transformed scale (using an iterative routine based on least squares), but the expected variance is calculated on the original scale of the predictor variables.

3.6.2. Deternination of differences in mosquito Larvae Species and their Predators

Mean differences in mosquito and predator’s abundance per habitat types and among species were compared using one-way analysis of variance (ANOVA). Chi-square ($\chi 2$ test) and student’s t-test were used to determine differnces in propotions of mosquito spp. and their predators, and differences in mosquito larvae abundance between habitat types. Shannon-Wiener diversity index (H’) was calculated to determine the variation in the larval mosquito diversity between the terrestrial and river environment. The river environment was within 30m, while distance between terrestrial was estimated as additional 70m (Minakawa *et al.*, 2008). This was important in determining species abundance, distribution and richness (Koller *et al.*, 1996; Magurran, 2004) since it is the most preferred index among the other diversity indices in ecology, providing values between 0.0 – 5.0. Results are generally between 1.5 –3.5, and can exceed 4.5 very rarely. The values above 3.0 indicate that the habitat structure is stable and balanced; the values under 1.0 indicate that there is pollution and degradation of habitat structure.

Differences in changes of the physico-chemical parameters were identified and analysis of variance (ANOVA) used to test if the magnitude of the changes were different between habitat types.

3.6.3. Determination of the relationship between water Physico-Chemical Parameters on Mosquito Larvae and Predators Abundance

All variables were first explored for their distribution and the homogeneity of variance checked using histograms and dot charts after which the most appropriate link function was chosen. Multicolinearity was assessed by means of Variance Inflation Factor (VIF), with a VIF above 2.5 considered to have a problem (Running, Ligon, & Miskioglu, 1999). A Chi-square test was used to establish the differences in the proportion of each species of larval mosquitoes between habitat types. A negative binomial Generalized Linear Model (nb-GLM) was used to assess the relationship between abundance of the mosquito larvae perdators (see the full model in output in Appendix VI) with mosquito larvae and the physico-chemical parameters. The response variable was the total number of larval mosquitoes (which included both total *Culex* spp. and total *Anopheles* spp.).

The predictor variables were mosquito predators and the physico-chemical parameters, which included: pH, conductivity, dissolved oxygen, temperature, turbidity, alkalinity, hardness and salinity. In a separate model, the explanatory variables were used to assess the suitability of the breeding habitats to both *Culex* spp. and the *Anopheles* spp. larval mosquitoes. In similarity with mosquito model, the influence of physico-chemical parameters and total larval mosquitoes on predators' abundance was built as follows: (predators' abundance) ~ pH + Cond. + DO + Temp + Turb + Alk + Salinity + Total larval mosquitoes (Culex and Anopheles species), (family = nb-GLM, data = Ecol.data). However, the two unidentified *Aedes* spp. sampled were not included in the above model because their identities were uncertian.

Mean range of water physico-chemical parameters requirements by both mosquito larvae and their predators in the same habitats was evaluated using Canonical Correlation Analysis (CCA) [see explanation on application in Appendix VII], while the overall relationship between mosquito larvae, their predators and the physico-chemical parameters in the shared habitats was determined using GLM and Ordination Analysis (OA).

3.6.4. Mosquito Diversity between Terresterial and Aquatic Environments

To compare the diversity and abundance of larval mosquitoes between terrestrial and river edge habitats, Shannon-Weiner diversity index was used to test the degree of dispersion of the micro-invertebrates between the habitats. Since the population of mosquito predators were few, the diversity index was only opted for the mosquito larvae samples.

Biodiversity Indices: Larval mosquito diversity was evaluated using Shannon-Weiner diversity index (H’) to assess the degree of biodiversity between the river edge and the adjacent terrestrial habitats. The Shannon-Weiner (H’) Diversity index and Shannon evenness index (Koller *et al.*, 1996) was worked out as follows:

Shannon - Weinner Diversity Index: $\mathbf{H^l = -\Sigma\ [(n_i / N) \times (\ln n_i / N)]}$

Where: $\mathbf{H^l}$: Shannon Diversity Index

$\mathbf{n_i}$: Number of individuals belonging to i species

N: Total number of individuals

Shannon Evenness Index: $\mathbf{E = H/log(S)}$

Where: **E**: Evenness index

H: Shannon Diversity Index

S: Species number

All statistical analyses were performed using R version 3.0 (The R Foundation for Statistical Computing, 2013). An alpha value ($p<0.05$) was consisdered statistically significant.

3.7. Ethical Consideration

Academic authority to conduct the research was sought from the School of Graduate Studies (SGS) of Maseno University (Appendix VIII). Prior authority to conduct the field study was obtained from the District Commissioner's offices in all the areas surveyed. District officers, area chiefs, and other stakeholders were also consulted. Also consents were obtained from landowners when larval mosquitoes were collected on their lands. This field study did not involve endangered or protected species.

CHAPTER FOUR: RESULTS

4.1. Description of some study sites in relation to Mosquito Larvae Breeding

Sampling sites for mosquito larvae and their predators along the Mara River and its tributaries are as shown in Figure 4.1 below.

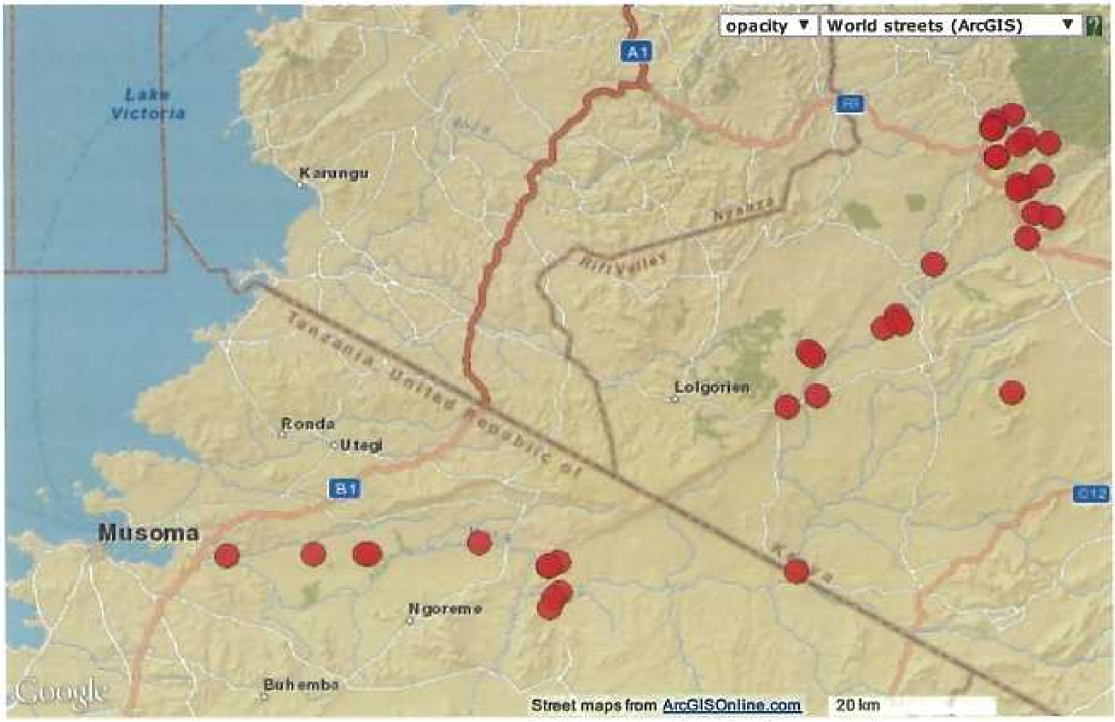


Figure 4.1: Sampling Sites along the Mara River and Its Tributaries in Kenya and Tanzania (n = 39)

Kapkimolwa Bridge: URS 1-10 and DRS 1-10

This sampling site was located on a small stream with a narrow width ranging between 0.30 to 0.60m and meanders before draining into Amala tributary. The stream was occasionally disturbed by livestock grazing along its banks. Around the bridge human activities such as collection of drinking water and washing of utensils were also on-going. The stream bed and banks were covered with sharp rocks some of which protrude above the water surface; this hindered the free flow of water, in the process creating natural breeding micro-habitats for larval mosquitoes and their predators.

Ngerende: URS 1-10 and DRS 1-10

These sampling sites were situated near Ngerende island camp and were located on the main Mara River after the confluence with a continuous steady stream flow. Site (i) comprised of rocky outcrops protruding above the river water. Several mosquito-breeding habitats were identified on the Rocky River banks (Plate 4.1). In this area, increased human activities were witnessed and the site also served as a watering point for cattle and other livestock. Site (ii) & (iii) were located approximately 100m from the main river. The site was swampy, characterized by *Typha domingensis* vegetation and surrounded by bushes. Sections of the swamp were exposed to sunlight thus creating suitable breeding habitats for larval mosquitoes.

Plate 4.1: Habitat types along Mara River at Ngerende area showing Rock Pools of Mosquito Breeding Habitats (see arrow) (July to August, 2011).

Plate 4.2: Habitat types along Mara River at Ngerende area showing Vegetated Pools of Mosquito Breeding Habitats (see arrow) (July to August, 2011).

Ngerende site was located by the roadside, about 110m from the main river and was characterized by several hippo hoof-prints, pudlles, drainages and open sunlit pools suitable for mosquito larvae development (Plates 4.2 and 4.3). This site was located opposite a large swamp by the Nile cabbage and with a width that ranged between 0.65m to 0.75m. The puddles and swamps served as wild animal watering points and were highly potential for mosquito breeding.

Plate 4.3A: Ngerende sampling point site comprising of vegetated pools (shown by arrow) (July to August, 2011).

Plate 4.3B: Ngerende sampling point site showing open sunlit puddles (July to August, 2011).

Kabosom Bridge: URS 1-10 and DRS 1-10

Kabosom sampling site was located in a wide stream that formed natural meanders of constant width ranging between 0.70m and 0.95m. The site also served as a water collection point for the locals living in the area. The site had several rock pools, vegetated pools and puddles suitable for mosquito breeding. This sampling site was located on a slow moving stream with a width ranging between 0.5m and 0.6m and discharges its waters into Nyangores tributary. It had little disturbances from animals and humans. The site had a strip of vegetated swamps at its banks, which made it an ideal breeding site for larval mosquitoes.

Olchoro Hot & Cold Spring: URS 1-10 and DRS 1-10

These sampling sites were located close to each other on the upper ridges of Mara River. The lower site (DRS 1-10) was located at a hot spring (which was designated as a men's bathing point) and also used as a domestic animal watering point while the cold spring was located a few meters from the hot spring and was mainly used as a domestic water collection point (Plates 4.4 and 4.5). Adjacent the men bathing place was a circular hot spring used as a bathing point for women. These sampling sites were highly disturbed by human activities. The surrounding area had several man made puddles and drainages, which were ideal habitats for *Anopheles* species breeding. Several sampling points were recorded in this area.

Plate 4.4: Drainage with Slow Moving Water (shown by arrow) at Olchoro Sampling Site (July to August, 2011)

Plate 4.5: Hot and Cold Spring at Olchoro Sampling Site (July to August, 2011)

Upper Nyangores Tributaries: URS 1-10 and DRS 1-10.

This sampling site was located along the Nyangores tributary and had a width of approximately 13m. It was characterized by a high diversity of microhabitats along the banks among them, muddy drainages with cattle and hippo hoof-prints arising from occasional disturbance by livestock and humans. A high presence of filamentous green algae were observed; a probable indicator of mosquito presence.

Tenwek Falls: URS 1-10 and DRS 1-10

This sampling site was located in a river of variable width of approximately 14m (Plates 4.6 and 4.7). The site had a wide variety of microhabitats particularly at the lower edge characterized by a tunnel for generating electricity. Beside it were several rock pools.

Plate 4.6: Open Puddle (shown by arrow) at Tenwek Sampling Point along the Mara River (July to August, 2011).

Plate 4.7: Swamp Pool at Tenwek Sampling Point along the Mara River (July to August, 2011).

Silibwet Bridge: URS 1-10 and DRS 1-10

This sampling site was located in a small stream of variable width ranging from 0.55m to 0.67m (Plates 4.8 and 4.9). It was disturbed by livestock and human activities, though some parts were vegetated. At this site a high presence of filamentous green algae was found; which indicates signs of mosquito presence. The habitat had scattered short grass with open puddles, suitable for anophelene breeding.

Aionet Spring (URS 1-10 and DRS 1-10)

This is one of the springs draining into Nyangores tributary and surrounded by tea and maize plantation. It appeared to be the main human water source, but with signs of livestock watering on its lower sections. On the drainage were several puddles formed as a result of several meanders and slow moving water pools. This sampling site was located on a slow moving stream with width that ranged from 0.65m to 0.71m. A lot of human activities took place here, including bathing and animal watering among other domestic activities. The area was surrounded by vegetation including grassland, maize and tea plantations. There was high presence of filamentous green algae indicative of possible presence of larval mosquitoes.

Ise Bridge: URS 1-10 and DRS 1-10

This sampling site was located in a small stream of variable width ranging between 0.45m and 0.60m that drains into Amala tributary. It is also disturbed by livestock grazing and human activities, such as washing of vehicles. There were open sunlit pools by the stream side about 2m from the river. Some parts were vegetated.

Chepterer Bridge (Simwaga): URS 1-10 and DRS 1-10

This sampling site was located in a wide stream that forms natural meanders. The site acts as a water collection point for communities living close by for domestic purposes. Beside it were several rock pools suitable for mosquito breeding.

Ngito River (A Tributary of Amala River): URS 1-10 and DRS 1-10

This sampling site was located in a small stream of variable width that ranged from 0.50m to 0.65m. The site was also disturbed by livestock and human activities. Some parts were also vegetated.

Mulot Water Pan

This sampling site was located at a large open dam, surrounded by vegetation including water Lillies, few Nile cabbages and few water hyacinths. The dam was used as a watering point for livestock. This site was potential for both *Anopeles and Culex* spp. breeding.

Trans Mara - Narok Brdge: Site 1-4 (URS 1-10 and DRS 1-10)

This sampling site was located in a large stream of variable width that ranged from 10 to 12m and formed natural meanders. The habitats were suitable for for *An. funestus* group breeding.

Plate 4.8: Trans Mara Bridge Sampling Site showing a Swamp along the Mara River (July to August, 2011).

Plate 4.9: Trans Mara Bridge Sampling Site showing open paddle along the Mara River (July to August, 2011).

New Mara Bridge: Site 1-3

This sampling site was located at a large stream of variable width ranging from 10 to 12m that formed natural meanders at the Kenya-Tanzania border after the expansive game reserve. It was located at the end of the Mara game reserve and characterized with increased tourist activities. Wild animals including hippos and crocodiles were present at some points along the Mara River. The site provided good breeding habitats for all types of mosquito species, especially on the several rocks pools, the puddles and swampy areas.

Kichwa Tembo Bridge: (URS 1-10 and DRS 1-10)

This sampling site was located in a small stream of variable width that ranged between 0.90 and 1.10m (Plates 4.10 and 4.11). It had some occasional disturbance especially from livestock. The targeted breeding habitats were open sunlit isolated pools, arising from a riverbed. The water flow was relatively slow creating suitable conditions for the anopheles mosquito breeding.

Plate 4.10: Kichwa Tembo Bridge Sampling Site Characterized by a large drying stream (July to August, 2011).

Plate 4.11: Kichwa Tembo Bridge Sampling Site Characterized by a large Open Puddle (July to August, 2011).

4.2. Presence, Abundance and Distribution of Malaria and Non-Malaria Transmitting Larval mosquitoes

The number of mosquito larvae collected per site during this survey is shown in Table 4.1. A total of 4,001 mosquito larvae were captured and identified from 1,600 individual dips *Anopheles gambiae* s.s., *An. Arabiensis* and *An. funestus* gruop.; the three most potent vectors of malaria in sub-Saharan Africa, together with other *Anopheles* spp. were the most dominant mosquito species (57.7%), followed by *Cx. quinquefasciatus* and *Cx. pipiens* complex (42.3%). Small proportions (0.3%) of *Aedes* spp. were also recorded though they were not identified to species level. *Anopheles pharoensis* constituted 12.0% of the 1,336 *An. gambiae* complex subjected to PCR analysis, 60.7% were *Anopheles gambiae* s.s. while 39.3% were *An. arabiensis*. Sibling species of the *An. funestus* comprised 1.5% (Table 4.1). The *An. funestus* species that failed to aplify were generally claffied and *An. funestus* group. Sites with altitudes below 1,700, especially those that were located on the Tanzanian side of the study were favourable to *An. arabiensis*, while those above 1,900 were particularly favourable to *An. gambiae*. Species of the *An. funestus* group and other Anophelines were few, thus their distribution could not be clearly depicted (Figure 4.2). The mosquito larvae were mainly collected in drying streams, swamps, vegetated puddles and open water pools. The majority was collected in drying streams where predators were also dominant.

An. gambie s.s dominated the upper part of Mara River in Kenya, while *An. arabiensis* showed dominance from the upper side of Tanzania preceded by lower elevation. *Culex* complex spp. were evenly distributed across the study sites. The three species were sparsely distributed, with *An. gambiae s.s* species dominating upper part of the Mara River in Kenya, while *An. arabiensis* showed a similar trend towards Tanzania. *Anopheles funestus* (complex) were few but evenly distributed along the Mara River in Kenya and Tanzania.

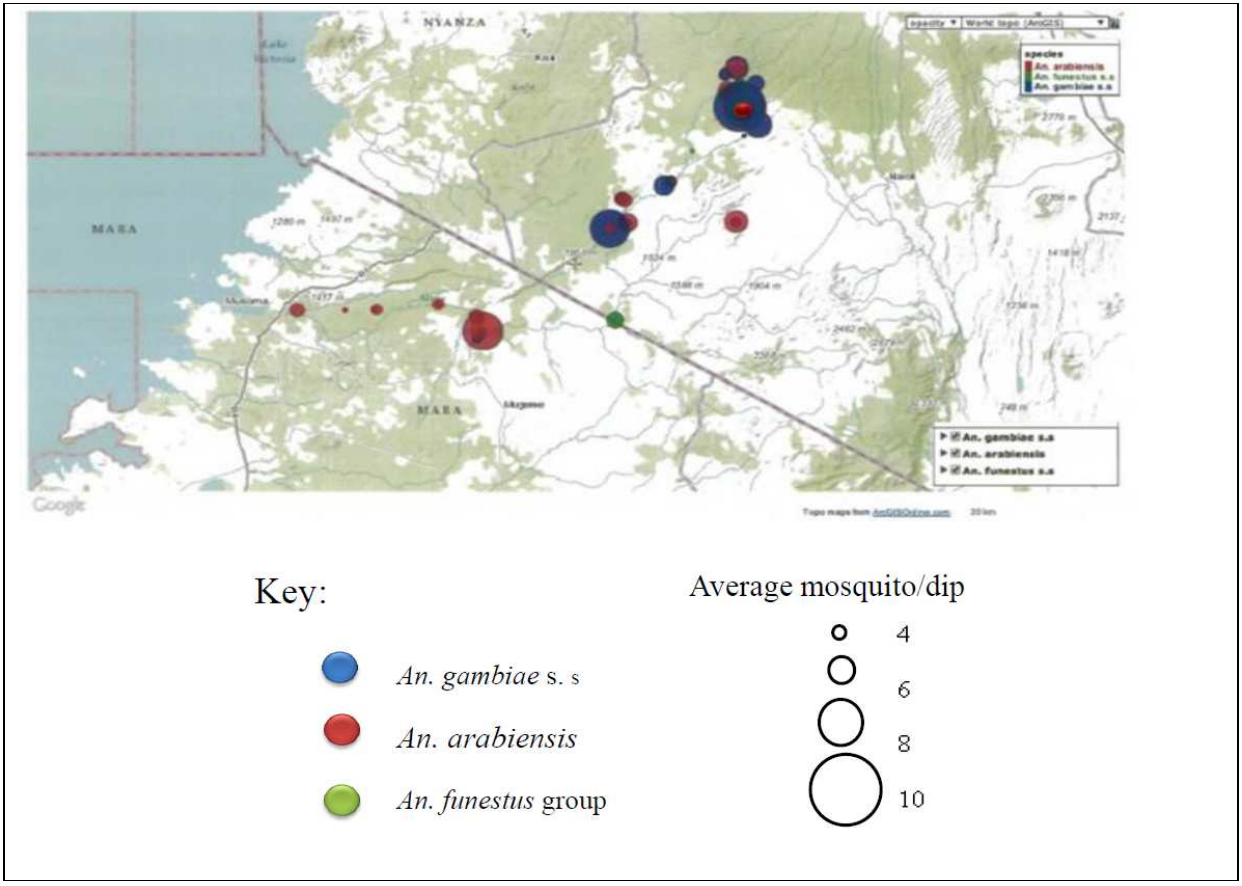


Figure 4.2: Distribution of Intensity of Three Main Malaria Vectors alogn the Mara River: The red dot refers to *An. arabiensis*, blue dot to *An. gambiae s.s*. and green dots to *An. funestus* group larvae.

Overall, the specific *Anopheles* and *Culex* spp. included *An. arabiensis*. (25.9%) followed by *An. gambiae s.s* (24.3%), *Culex quinquefasciatus* (19.0%), *Cx. pipiens complex* (10.5%), *An.*

coustani complex (8.0%) and *An. maculpalpis* (3.6%). Others that were identified though in relatively smaller numbers included: *An. rivulorum, An. azamiae, An. pharoensis, An. ardensis*, *An. faini*, *An. hamoni* and *An. sergeti*; all of which combined accounted for about 8.6% (Table 4.1).

Table 4.1: Mosquito Species, their Numbers and Percentage Composition along the Mara River

Mosquito species	No. of larval mosquitoes	% Composition
An. arabiensis	1038	25.9
An. gambiae s.s	973	24.3
Cx. quinquefasciatus	761	19.0
Cx. pipiens complex	420	10.5
An. coustani complex	321	8.0
An. maculpalpis	145	3.6
Unidentified *An. funestus group*	140	3.5
An. frivulorum	50	1.3
An. azamiae	45	1.1
An. pharoensis	44	1.1
An. hamoni	28	0.7
An. funestus s.s	15	0.4
An. christyi	12	0.3
Aedes spp.	5	0.1
An. ardensis	2	0.05
An. faini	1	0.02
An.sergeti	1	0.02
Total	**4001**	**100.0**

4.3. Presence, Abundance and Distribution of Mosquito Larvae predators and Their Relationship with Mosquito Larvae Abundance and Distribution along the Mara River.

4.3.1. Mosquito Larvae Predator Distribution and Abundance

Table 4.2 shows the distribution of mosquito larvae predators in the sampling sites. A total of 297 predators belonging to 3 orders were identified in 39 sampling sites. The predators were sparsely distributed in the habitats that were found to be colonized. Both larval mosquitoes and predators dominated drying stream. The three orders collected were: Hemiptera (54.2%), Odonata (22.9%) and Coleoptera (22.9%) across the sampling sites, (Figure 4.3). The differences were however not signicant ($\chi^2 = 1.0835$, d.f = 2, $p = 0.2731$). Order Hemiptera recorded a total of 7 Families, with members of family Velidae and genus *Rhagovelia* being the most dominant. Odonata recorded 3 Families dominated by Family Coenagrionidae, while order Coleoptera had 2 families dominated by Family Dytiscidae.

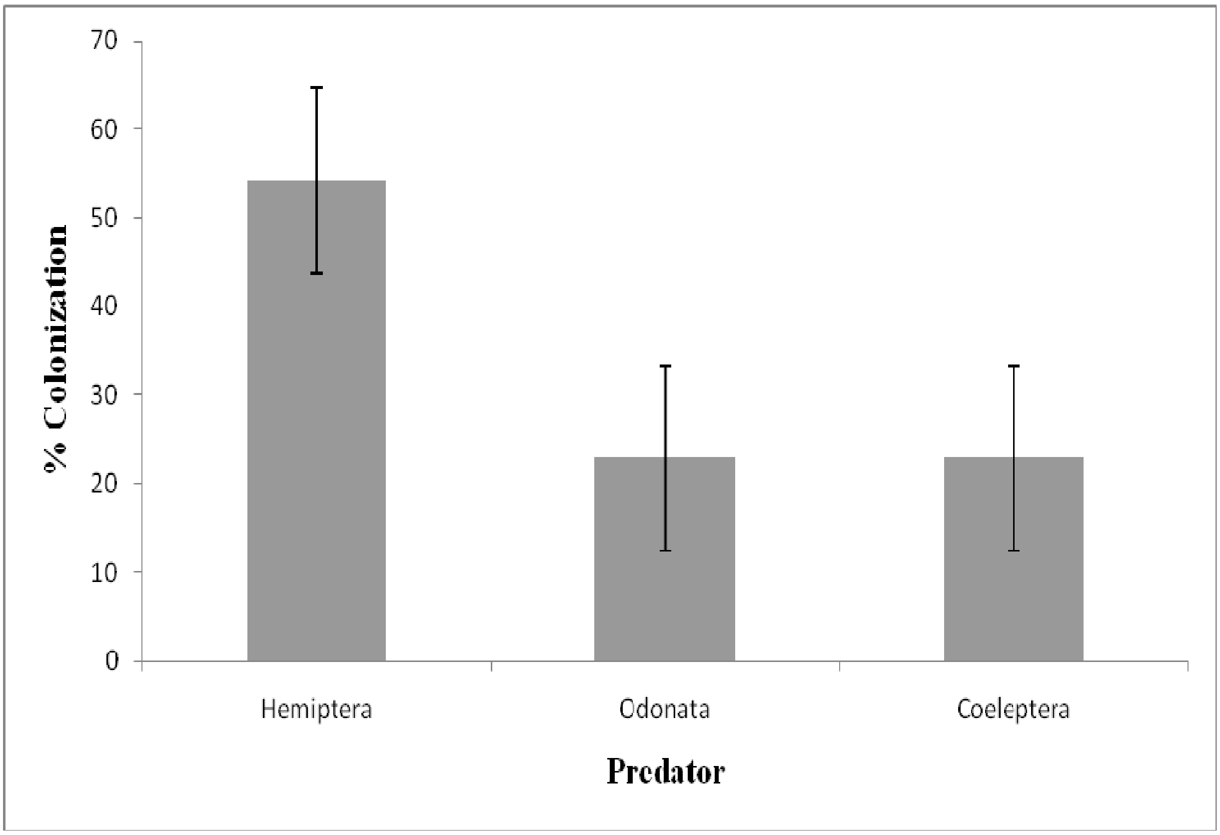


Figure 4.3: Distribution of Mosquito Larvae Predators by Order along the Mara River

Table 4.2: Distribution of Mosquito Larvae Predators along the Mara River

Order (N)	**Family**	**Genus**	**% Habitats colonized**
Hemiptera (157)	Gerridae	*Hynesionella* (7)	2.4
		Limnogonus (13)	4.4
	Hydrometridae	*Hydrometra* (15)	5.1
	Velidae	*Rhagovelia* (38)	12.8
	Notonectidae	*Anisops* (30)	10.1
		Enithares (9)	3.0
	Pleidae	*Plea* (8)	2.7
	Naucoridae	*Laccocoris* (7)	2.4
	Nepidae	*Ranatra* (5)	1.7
		Laccotrephes sp 1 (17)	5.7
		Laccotrephes sp 2 (7)	2.4
		Nepa (5)	1.7
Odonata (82)	Lestidae	*Lestes* (20)	6.7
	Coenagrionidae	*Enallagma* (21)	7.0
	Libellulidae	*Palpopleura* (14)	4.7
		Orthetrum (13)	4.4
Coleoptera (58)	Hydrophilidae	*Hydrochara* (8)	2.7
	Dystiscidae	*Laccophilini* sp. 1 (14)	4.7
		Laccophilini sp. 2 (3)	1.0
		Laccophilini sp. 3 (15)	5.1
		Laccophilini sp. 4 (6)	2.0
		Laccophilini sp. 5 (6)	2.0
		Laccophilini sp. 6 (5)	1.7
		Copelatus (4)	1.3
		Cybister (6)	2.0
		Hydaticus (1)	0.3
Total (297)			**100.0**

4.3.2. Canonical Correlation Analysis (CCA) between Mosquito Larvae and Predators in Shared Habitats along the Mara River

To establish the relationship between larval mosquitoes and predators in the habitats, a regression matrix between the three variables was developed. Data from some sites showed inverse correlation between predators and prey (mosquito larvae), suggesting effective predation. However, there was no particular pattern of relationship observed between the two variables, simply because higher numbers of predators were captured in habitats with a few mosquitoe numbers as shown in Figure 4.4. The strength of the relationship in the regression analysis was linear at $y = -0.0026x2 + 1.6252x - 31.084$, $R^2=0.6$, $p<0.001$. Data from some sites showed inverse correlation between predators and prey (mosquito larvae), suggesting effective predation.

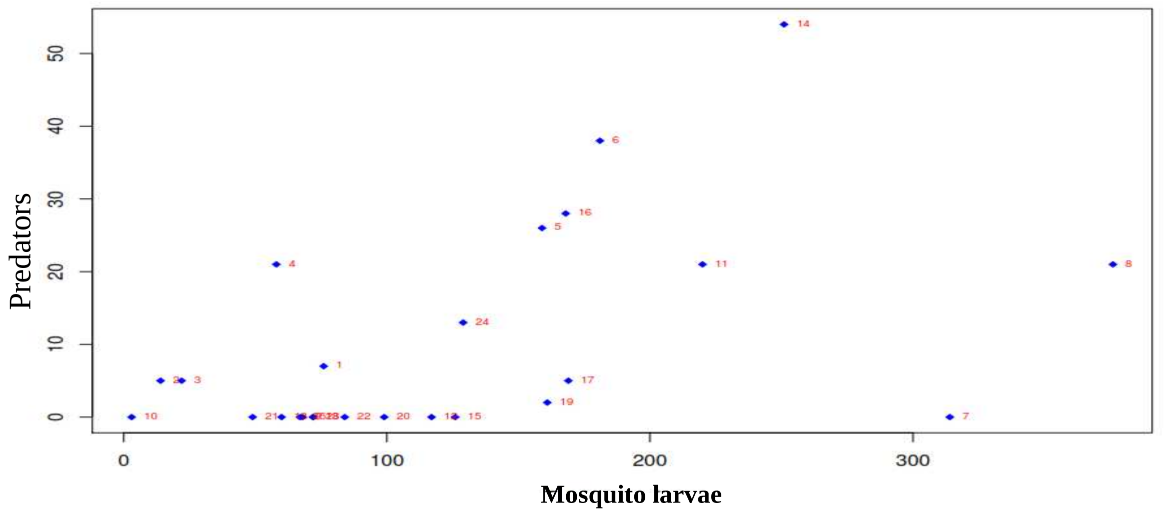


Figure 4.4: Relationship between Larval Mosquitoes and Predators in Shared Habitats along the Mara River (blue stars) and the mosquitoes (absolute number) in shared habitat along the Mara River Kenya and Tanzania. (n=39).

4.4. Characterization of the Different Mosquito Breeding Habitats and their Preference to these Habitats

4.4.1. The Anopheline and Culicine LarvaL Abundance in Habitats

The sampling points (n=39) were surveyed along the Mara River and its tributaries. The sampling points comprised of macro-habitats including: river (n=25), drying stream (n=48), swamps (n=18), open puddles (n=22), rock pools (n=17), dam sites (n=10), hoof-prints (n=20), vegetated pools (n=26) and drainages (n=56). Mean *Anopheles gambiae* s.l larvae were higher in the drying streams (μ=53.3, SE=33.1) and swamps (μ=23.1, SE=13.6), followed by drainages (μ=15.0, SE=9.6).

The drainages were classified separately from open puddles because they had unique vegetation that partially covered them. Dam and vegetated pools by the river and drainages recorded the same mean of *An. gambiae s.l* larvae (Table 4.3). Springs were however not classified as habitats, but their characteristics were noted and reported. One - way ANOVA indicated that the mean *An. gambiae* s.l varied significantly among habitat types (n=10, F= 8.2374, d.f. =9, 26, $p \leq 0.01$).

Similarly, *An. coustani* complex was highest in drying stream (μ=27.8, SE=10.2) and swamps (μ=26.5, SE=11.2). Apart from these, only vegetated pools by the river, drainages and dam contained the mosquito larvae. Open puddles, dams and livestock hoof-prints were not suitable for *An. coustani*. However, the main Mara River produced only one mosquito. One-way ANOVA indicated that the mean *Anopheles custani* complex varied significantly among vegetation types (n=10, F=4.513, d.f.=9, 26, p=0.03). *Anopheles funestus* group were found to mainly occupy swamps (μ = 15.3, SE = 8.7), vegetated pools (μ=12.5, SE=3.7) and drying stream (μ=7.6, SE=2.1). *An. maculpalpis* were found in two habitats namely; open puddles in a brick-making site (μ=24.2, SE=16.4), where their numbers were highest and in vegetated pools (μ=14.2, SE=5.0) of the terrestrial habitats. *An. pharoensis* were found mainly in drying streams (μ=14.0±4.0), swamps (μ=4.9, SE=2.8) and dams (μ=2.1, SE=0.9). Their differences were found to be statistically significant across the ten habitat types, using ANOVA (n=10, F=4.222, d.f.=9, 26, *p*=0.04). Other Anophelines such *An. ardensis*, *An. faini*, *An. hamoni*, *An. sergentii*, and *Aedes* spp. populations were lower compared to populations of other Anophelines sampled, and were mainly found in drainages and open puddles in both terrestrial water bodies and along the Mara River basin. *Culicine* spp. mainly dominated drying strans, open pudlles and artificial containers among other habitats (Table 4.3).

Table 4.3: Mean densities (± SE) of Mosquito Larvae per Habitat Type along the Mara River

Taxa	Drying streams	*Other habitat types	Open puddles	Vegetated pools	Rock pools	Animal hoofprints	Rivers
An. arabiensis	34±23.8	12.31±6.4	29.1±2.1	22.8±1.3	4.3±0.2	4.1±5.1	0.0
An. gambiae s.s	11.4± (0)	10.3± 5.0	14.2±4.1	9.1± 3.6	0.0	2.1±1.3	0.0
Cx. pipiens	12.2±7.4	3.8±0.8	16.2±1.4	5.1±0.9	0.0	4.6±1.5	0.0
An. coustani	20.3±9.7	7.3±6.6	1.0±0.4	11.0±4.4	0.0	0.0	0.0
Cx.quinquefasciatus	20.2(0)	7.2±0.2	18.1±8.0	2.1±0.1	0.3±0.2	0.1±0.1	0.0
An. funestus group	0.9±1.2	0.0	0.0	5.6±1.5	0.0	0. 0	0.0
An. pharoensis	2.1±0.1	5.2±1.2	0.0	0.0	0.0	0.0	0.0
An. azamiae	0.0	0.1±0.1	0.2±0.1	<0.1	0.0	0.0	0.0
An. christyi	0.3±0.1	0.3±0.1	0.0	<0.1	0.0	0.0	0.0
An. maculipalpis	0.0	<1.0	26.3±11.4	0.1±0.1	0.0	0.0	0.0
An. ardenis	0.0	0.0	2.2±1.3	0.0	0.0	0.0	0.0
An. sergentii	1.4±0.4	2.3±0.1	0.1±0.1	0.0	0.0	0.0	0.0
An. faini	0.0	0.0	3.4±1.4	1.6±0.5	0.0	0.0	0.0
An. lessoni	0.0	0.0	0.0	<0.1	0.0	0.0	0.0
Aedes spp.	0.0	0.0	< 0.1	0.0	0.0	0.0	0.0

The table shows six representatives of types of habitas as classified along the Mara River, in this table the rest were grouped as other habitat types.

The numbers of mosquito larvae were higher in drying streams followed by isolated swamps by the main river. Except for two specine of *An. coustani* larvae collected in the river environment, no other mosquito larvae species were sampled from the Mara River.

4.4.2. Mosquito Larvae Species Population in Open Sunlit Pools and in different Vegetation Types

The vegetation surveyed were mostly associated with the Mara River and its tributaries (Amala and Nyangores). Few open sunlit pools existed as it was a dry period. Puddles created as a result of brick making accumulated water from the previous rainy season creating suitable habitats for larval mosquitoes. Overall, a total of 220 macro-habitats were surveyed for presence of mosquito larvae based on vegetation type with respect to vegetation height (Table 4.4), as previously done by Minakawa *et al.* (2012) [see picture in Appendix I]. On overall, there were more larval mosquitoes in habitats with short grass, followed by open sunlit pools, and lowest in habitats with tall grasses. Most mosquito species including *Anopheles gambiae s.l.*, *An. funestus group*, *An. pharoensis, An. ardensis, An. azamiae, An. christyi, An. maculpalpis, An. hamoni* and *An. sergentii* were more in habitats with short grass compared to habitats with tall grass and those that were open and sunlit. Only *Culex* spp. and *An. faini* were more in open sunlit pools than in habitats with short grass and those with tall grass (Table 4.4).

Table 4.4: Percent Composition of Mosquito Larvae Species collected at various Habitat types based on Vegetation Characteristics

Mosquito species	***Short grass (%)**	**Tall grass (%)**	**Open sunlit (%)**	**Total (%)**
An. gambiaes.l	959 (60.7)	2 (0.13)	618 (37.6)	1579 (100)
An. funestus group	65 (81.3)	13 (16.3)	2(2.5)	80 (100)
An. coustani	299 (53.6)	255 (45.7)	4 (0.7)	558 (100)
An. pharoensis	40(62.5)	22 (34.4)	2 (3.13)	64 (100)
Culex spp.	620 (41.5)	37(2.5)	837 (56.0)	1494 (100)
An. ardensis	2 (66.7)	1(33.3)	0 (0.0)	3 (100)
An. azamiae	40 (76.9)	0(0.0)	12 (23.1)	52 (100)
An. christyi	8 (66.7)	3(25.0)	1 (8.3)	12 (100)
An. maculipalpis	75 (57.5)	0(0.0)	55 (42.3)	130 (100)
An. hamoni	15 (53.6)	0(0.0)	13 (46.4)	28 (100)
An. Sergentii	1(100)	0(0.0)	0 (0.0)	1 (100)
An. faini	0 (0.0)	0(0.0)	1 (100)	1 (100)
Total	**2124**	**333**	**1544**	**4001**

**Habitats with short grass produced the highest number of larval mosquitoes followed by open sunlit pools.*

4.4.3. Terrestrial versus River Edge Habitats

Mosquito larvae were found inhabiting both the terrestrial and river edge habitats. A total of 1,289 larval mosquitoes were collected from terrestrial aquatic habitats while 2,712 larval mosquitoes were collected at the river edge habitats. River edge habitats had a total of 70 mosquito-breeding habitats. About 36 (51.4%) were pools of slow moving water, while 20 (28.6%) were large swamps, the remaining 14 (20.0%) were mainly rock pools and small patches of pudlles. The result revealed that out of a total of 2,124 larval mosquitoes sampled in the habitats with short vegatations, *Anopheles* spp. were dominat (60.8%), over *Culex* spp. (39.2%), and the differences in the two species composition was statistically significant (t=12.667, df = 1, *p*=0.03).

The terrestrial habitats mainly comprised of open sun lit pools. A total of 80 microhabitats were sampled and found to habour different mosquito species. Nevertheless, *Culex* spp. were the majority comprising of about 58.9% (n=759) of the larval mosquitoes sampled. The remaining proportion (41.1%) comprised of the *Anopheles* spp., majority of which occupied habitats with littered dry leaves or scattered short grass. The population of both species were however similar (t = 5.618, df = 1, *p* = 0.11).

Further the mosquito and predators habitats were classified based on their location within the river or stream continuum or in the terrestrial habitats. The terrestrial aquatic habitatas were located approximately 50m away from the main river/streams. The Shannon-Weiner (H') Diversity Index was slightly higher for river edge habitats (2.17) compared to terrestrial habitats (1.43). However, the diversity index did not vary between the aquatic terrestrial habitat and river edge habitats (t=0.3120, df=1, *p*=0.342). The Shannon evenness index was higher in river edge habitats (2.13) than in terrestrial aquatic habitats (1.30) (Table 4.5), and significant differences was observed between the two broad habitat types (t=7.123, df=1, *p*=0.002). The findings showed that as the number of mosquitoe increased, the diversity of larval mosquitoes became linear, futher showing the diversity of larval mosquitoes and species richness along the Mara River.

Table 4.5: Summary of the Diversity Indices of Mosquito Species Richness along the Mara River, Kenya and Tanzania

Indices	**Terrestrial Habitats**	**Riveredge Habitats**	**Pr(>|z|)**
Species number	1289	2712	-
Species Richness	9	12	-
Shannon-Weinner Diversity Index	1.438	2.1747	0.342
Shannon Evenness Index	1.2640	2.1332	0.002

Predator numbers seemed to increase in vegetated pools, streams and swamps following increase in mosquito population. For instance, *Culex* spp. and the *Anopheles* mosquito larvae were collected in temporary water pools and puddles, even as more of the *Anopheles* spp. were observed to prefer aquatic environments such as swamps, slow flowing streams and vegetated pools compared to *Culex* spp. However, the highest numbers of predators were captured in drying streams (40.1%), followed by swamps (20.2%) and other vegetated pools adjacent to the river (17.8%). The least number of mosquito larvae predators were recorded in rock pools (0.7%) (Table 4.6). Figure 4.5 and 4.6, summarizes density of larval mosquitoes and their predators population in different habitats within the Mara River basin. The findings further showed that habitats favorable to *Culicine* spp. were also preferred by *Anopheles* spp. but the occurrence of the predators where *Culicine* spp. dominated remained low.

Table 4.6: Habitat Preference by both Mosquito Larvae and Predators along the Mara River

Habitat	Mosquito (n)	Propotion (%)	Predators (n)	Proportion (%)
Drying stream	1009	25.2	120	40.4
Swamps	830	20.7	92	31.0
Open puddle	524	13.1	4	1.4
Dams	510	12.8	13	4.4
Vegetated pools	455	11.4	45	15.2
Hoof-prints	250	6.3	4	1.4
Drainages	234	5.8	13	4.4
Rock pool	188	4.7	3	1.0
River	1	0.0	3	1.0
TOTAL(N)	**4001**	**100**	**297**	**100**

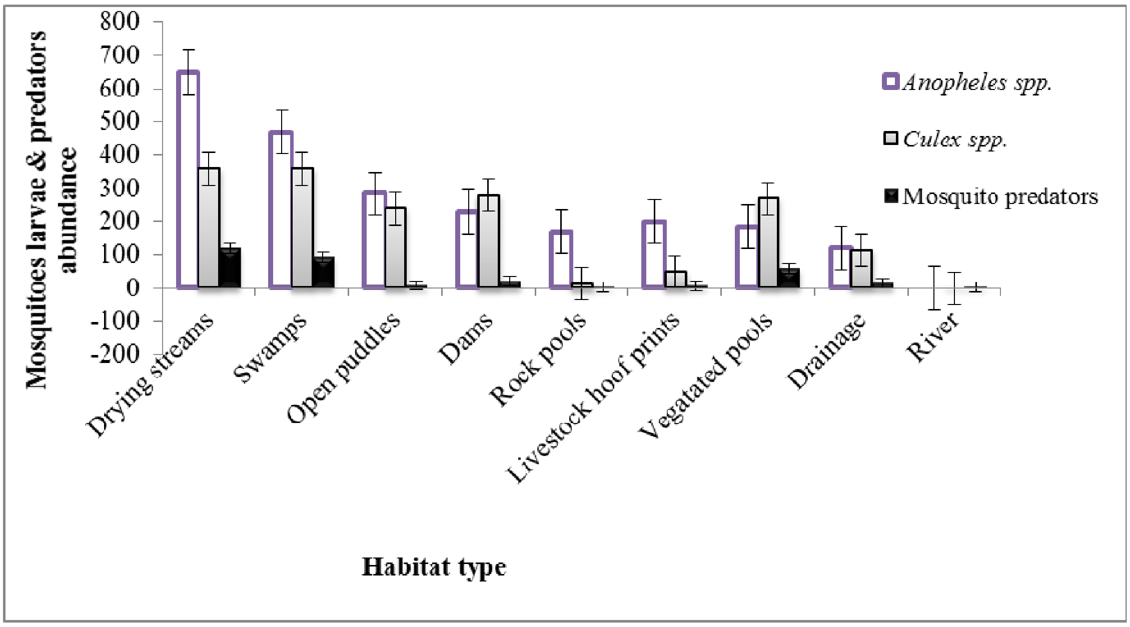


Figure 4.5: Distribution of Mosquito Species and their Predators in different Habitats along the Mara River.

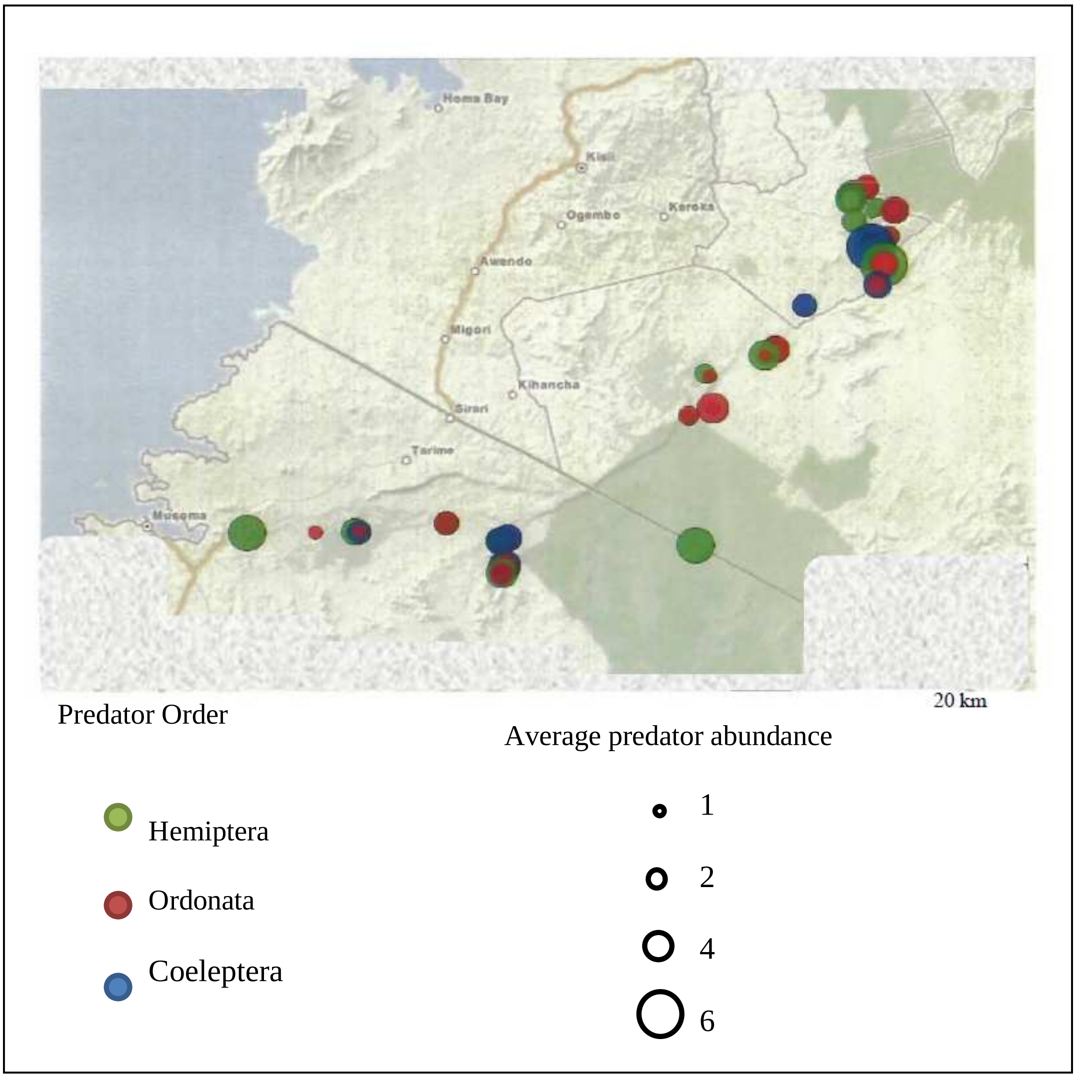


Figure 4.6: Predator Order Distribution and Intensity along Mara River as shown by dots of different sizes and colors (n = 39).

4.5. Relating Physico-chemical Parameters to Mosquito Larvae Abundance using GLM

In the final GLM model, three physico-chemical parameters (conductivity, dissolved oxygen, temperature and alkalinity) were found to be the most favorable factors for the immature mosquito survival (Table 4.7). In a separate Poisson-GLM model, water temperature, conductivity, dissolved oxygen, and alkalinity was predictive for both *Culex* spp. and *Anopheles* spp. abundance (Tables 4.8 and 4.9).

Table 4.7: Generalized Linear Model relating Total Larval mosquitoes to Physico-Chemical Parameters along the Mara River

Variable	**Estimate**	**SE**	**Z**	**Pr(>|z|)**
Dissolved oxygen	0.30	0.02	15.81	<0.001
Temperature	0.30	0.01	10.23	<0.001
Turbidity	-0.04	0.01	-13.16	<0.001

Table 4.8: Generalized Linear Model Relating Explanatory Variables (pH, Conductivity, Dissolved Oxygen, Temperature, Alkalinity and Turbidity) to *Culex* spp. along the Mara River

Variable	**Estimate**	**SE**	**Z**	**Pr(>|z|)**
pH	0.01	0.01	4.98	<0.001
Conductivity	0.02	0.01	5.22	<0.001
DO	0.25	0.03	9.68	<0.001
Temperature	0.25	0.02	7.65	<0.001

Table 4.9: Generalized Linear Model Relating Explanatory Variables (pH, Conductivity, Dissolved Oxygen, Temperature, Alkalinity and Turbidity) on *Anopheles* spp. Larval Mosquitoes along the Mara River

Variables	Estimate	SE	Z	P-value
pH	- 0.02	0.01	-6.03	<0.001
Conductivity	0.01	0.01	16.77	<0.001
DO	0.33	0.03	11.56	<0.001
Temperature	0.15	0.02	7.48	<0.001
Alkalinity	0.04	0.01	8.00	<0.001

4.5.1. Physico-Chemical Parameters across Habitats

Table 4.10 shows the physico-chemical parameter for each of the eight different habitats. The findings showed that DO was highest (6.4±0.7 mg/L) in rivers and lowest (2.4±2.7 mg/L) in swamps. Most habitats however had dissolved oxygen values ranging between 4.0 and 5.6 mg/L. Conductivity levels across different habitats showed wide variations ranging between a mean of 144.5±97.6 µS/cm for the rivers and 368.0±125.9 µS/cm for the rock pools. Dams and stream habitats also recorded relatively high mean conductivity levels of 290±186.5 µS/cm and 269.8±213.8 µS/cm, respectively. The pH levels varied only slightly between different habitats ranging between 7.0 and 8.2. Only the swamp habitats recorded a neutral (7.0±1.3) pH, while other habitats recorded alkaline pH, slightly above 7.0. Turbidity levels like conductivity varied highly between different habitats within the Mara River basin, with the highest mean turbidity of 542.6±2.3 NTU recorded within rock pools and the lowest mean turbidity of 95.2±131.9 NTU recorded in dams. Mean alkalinity and hardness were both highest 400±282.8 mg/L and

372±393.2 mg/L, respectively, in drainages. However, lowest mean alkalinity (100±62.4 mg/L) was recorded in dams while lowest mean hardness (58.5±46.7 mg/L) was recorded in swamps. There were slight variations in temperature between different habitats, ranging from 19.7±2.3^{0}C in the main river to 26.2±3.4 ^{0}C in rock pools. Only swamps recorded slight salinity of 0.4mg/L while all the other sites were zero (0). The physico-chemical parameters as measured on site are summarized on Table 4.10.

Table 4.10: Average Physico-Chemical Parameters at different Habitats along Mara River Basin

Habitats	DO (mg/l)	pH	Alkalinity (mg/l)	Hardness (mg/l)	Turbidity (NTU)	Conductivity (µS/cm)	Temperature (^{0}C)	Salinity (mg/l)
Dams	4.7±1.8	8.1±0.4	100±62.4	87.7±56.2	96.9±142.0	269.8±213.8	19.4±1.9	0.0 ±0.0
Streams	5.3±1.6	8.1±0.6	126.2±26.5	102.4±68.9	124.3±152.6	290±186.5	18.5±2.1	0.0 ±0.0
Swamps	2.4±2.7	7.0±1.3	244.5±274.6	58.5±46.7	142.2±108.5	174.3±59.2	20.2±4.9	0.4±0.0
Drainages	4.3±3.8	7.3±0.5	400±282.8	372±393.2	144.8±84.3	168.5±13.4	20.2±0.7	0.0±0.0
Rock pools	6.0±0.7	7.1±0.8	153±60.8	127±69.3	542.6±2.3	368.0±125.9	21.2±3.4	0.0±0.0
Puddles	5.6±0.8	8.2±0.5	104±103.0	188±247.7	95.2±131.9	168.8±87.3	18.2±2.3	0.0±0.0
Springs	4.0±0.3	8.3±0.6	124±113.2	183±148.4	134.5±121.7	155.7±88.4	24.3±2.2	0.0±0.0
Rivers	6.4±0.7	7.3±0.4	100±199.2	178±228.8	135.2±142.4	144.5±97.6	19.7±2.3	0.0±0.0

4.5.2. Influence of Physico-Chemical Parameters on Mosquito larvae and Predator in Shared Habitats along the Mara River

4.5.2.1. Acidity Level (pH)

The pH values were distributed differentially in the different habitats, with the rivers showing a more remarkable variation (Figure 4.7). The pH had three outlying values on dam, stream and spring. The highest mean pH value (8.3±0.6) was recorded in spring habitats, while the lowest (7.0±1.3) was recorded at the swamps. Stream and dam habitats had almost the same variation (8.1±0.6) and (8.1±0.4), respectively. Similarly, drainages and rivers showed similar trend of pH values. However, springs, puddles, rivers and rock pools had 8.3±0.6, 8.2±0.5, 7.1±0.8 and 7.3±0.4 respectively. One-way Anova indicated a significant difference in pH among the sites (ANOVA, n=10, F=11.2418, d.f.=9, 26, *p*<0.01).

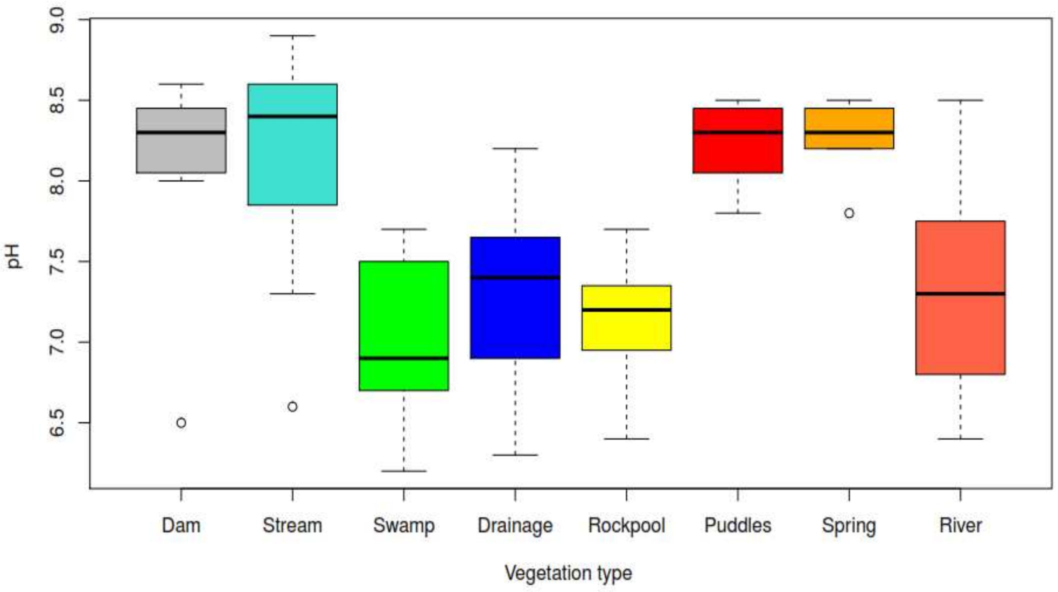


Figure 4.7: Boxplot and Whisker plot of pH levels across Different Habitat Types along the Mara River (July-August 2011, n=39).

In a correlation matrix to determine the direction of pH in the shared habitats (Figure 4.8), there was a positive correlation between larval mosquitoes and the predators (r=0.696, *p* <0.05) as well as pH and larval mosquitoes (r=0.192, *p*>0.05) and a negative association between pH and predators (r = -0.104, *p*>0.05) in the shared habitats.

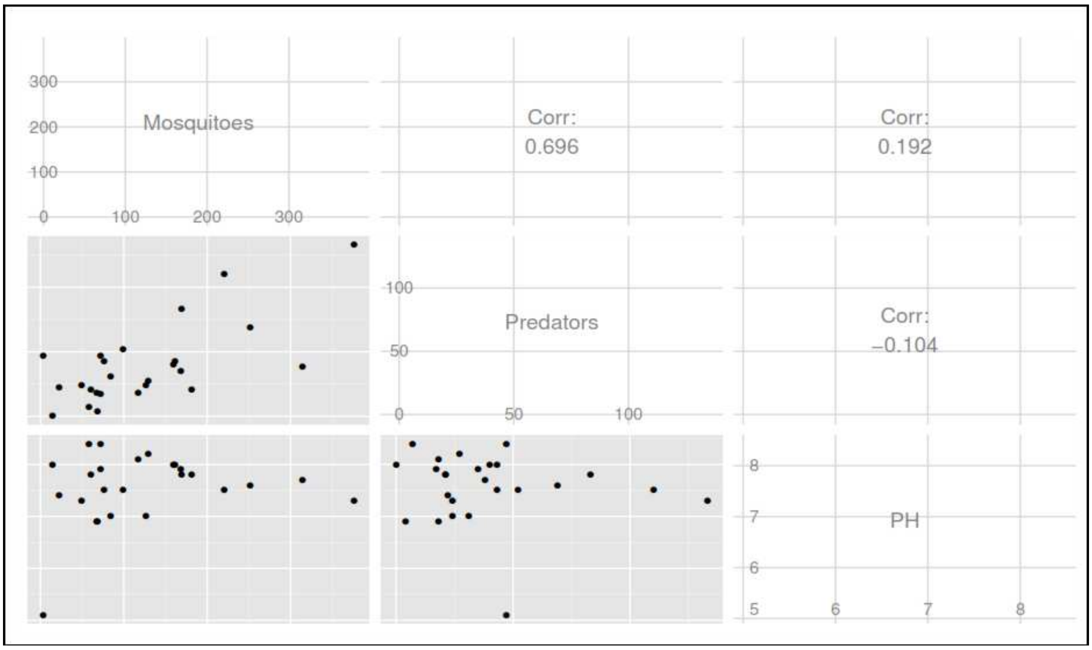


Figure 4.8: Correlation Matrix between Larval mosquitoes, Predators and pH Values in Habitats along the Mara River (July-August 2011).

Further analysis to determine preferable level of conductivity range requirement by both larval mosquitoes and predators and predators in the shared habitats indicated that, with values ranging between 5.2 to 8.4, the most prefered range was between 8.1 and 8.4 for both larval mosquitoes and predators in the shared habitats along the Mara River (Figure 4.8). However, some mosquitoes had specific pH requirements. For instance, while some larval mosquitoes preferred pH range of 6.7 to 7.0, others preferred a range of 7.4 to 8.2 and 7.9 to 8.2. Nevertheless, all the larval mosquitoes and predators had specific range of preferred values. There was a negative correlation between pH and predators abundance.

4.5.2.2. Conductivity Level

Conductivity is a measure of the ability of water to conduct electrical current. Drainages and open sunlit puddles recorded almost same variation, with a mean range of 168.5±13.4 µS/cm and 168.8±87.3 µS/cm, respectively. Swamps (174.3±59.2 µS/cm), dams (290±186.5 µS/cm) and streams (269.8±213.8 µS/cm) were also clustered with an almost similar conductivity range. Spring and river habitats recorded the lowest mean conductivity range of 155.7±88.4 µS/cm and 144.5±97.6 µS/cm, respectively (Figure 4.9). One-wayANOVA test indicated a significant difference in electrical conductivity among the habitat types (ANOVA, n=10, F=7.1433, d.f.= 9, 26, p<0.01).

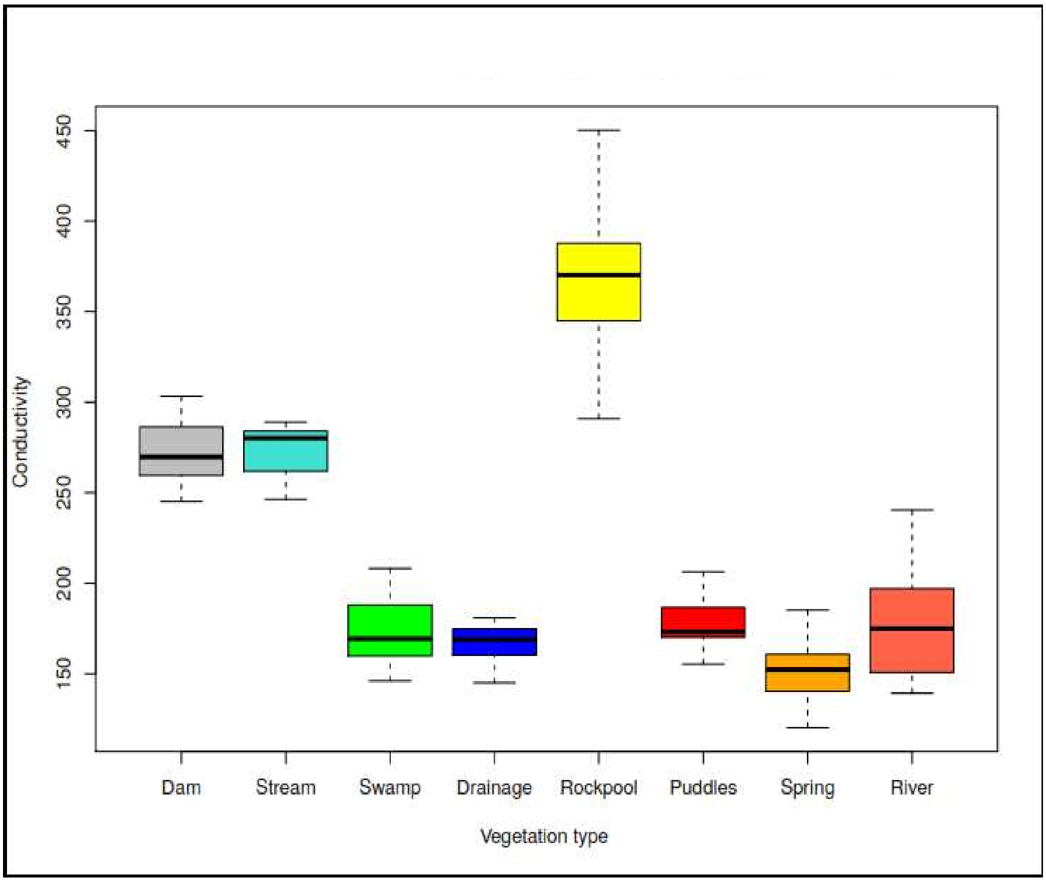


Figure 4.9: Boxplot and Whisker plot of Concentration of Conductivity per Habitat type along Mara River (July–August, 2011, n=39).

Further analysis to determine preferable conductivity range requirement for both larval mosquitoes and predators in the shared habitats indicated that, with values between 109.9 µS/cm and 382.6 µS/cm, a range between 162.9 µS/cm to 166 µS/cm was most preferrable by both larval mosquitoes and predators in the shared habitats along the Mara River (Figure 4.10). However, some larval mosquitoes had specific conductivity requirements. For intance, while some prefered conductivity range of between 109.9 to 165.0 µS/cm, others prefered a range of 166.6 to 276.3 µS/cm.

In a correlation matrix to determine the direction of conductivity in the shared habitats (Figure 4.10), there was a positive, but non-significant correlation between larval mosquitoes and predators (r=0.592, *p*<0.05), conductivity and larval mosquitoes (r=0.174, *p*>0.05) and conductivity and predators (r =0.148, *p*=>0.05), Figure (4.10).

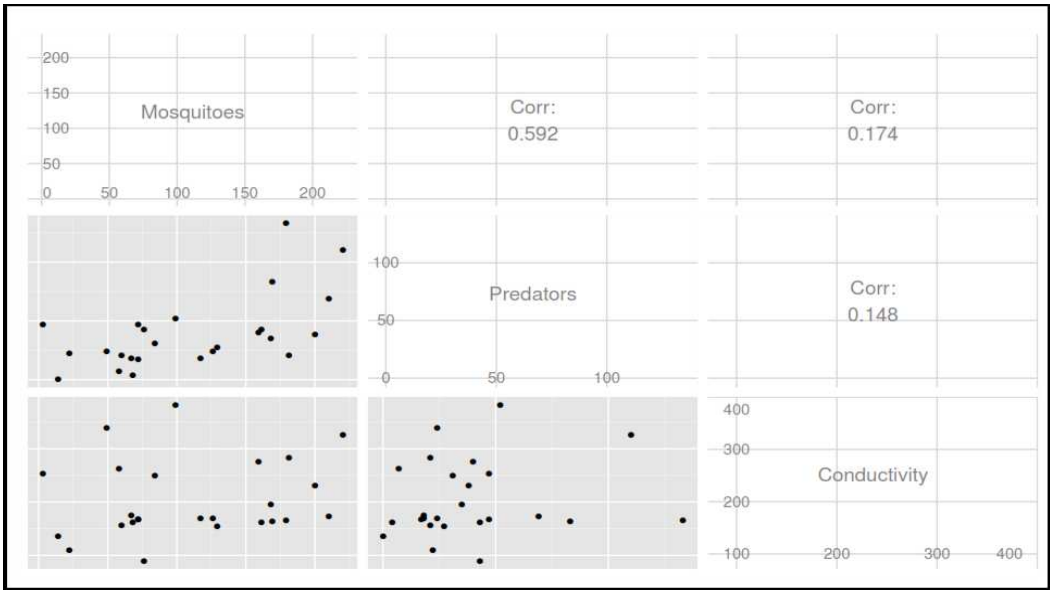


Figure 4.10: Correlation Matrix between Larval mosquitoes, Predators and Conductivity Values in Shared Habitats along the Mara River Basin (July-August 2011).

4.5.2.3. Turbidity Level

The turbidity levels in each habitat type indicated the level to which substances were suspended in the habitats along the Mara River. Mean turbidity was lowest in dams (96.9±142.0 NTU) and puddles (95.2±131.9 NTU) and the highest in rock pools (542.6±2.3 NTU). Springs and rivers had an almost closer range of 135.2±142.4 NTU and 134.5±121.7 NTU, respectively. Drainages, swamps and streams had 144.8±84.3 NTU, 142.2±108.5 NTU and 124.3±152.6 NTU, respectively. Rivers (135.2±142.4 NTU) and springs (134.5±121.7 NTU) almost had a common range. A significant difference in turbidity was observed among the habitat types (one–way ANOVA, n=10, F=4.6597, d.f.=9, 26, *p*<0.05). Rock pools had the highest turbidity levels. Boxplot presentation for turbidity in the different mosquito and predator breeding sites are as shown in Figure 4.11.

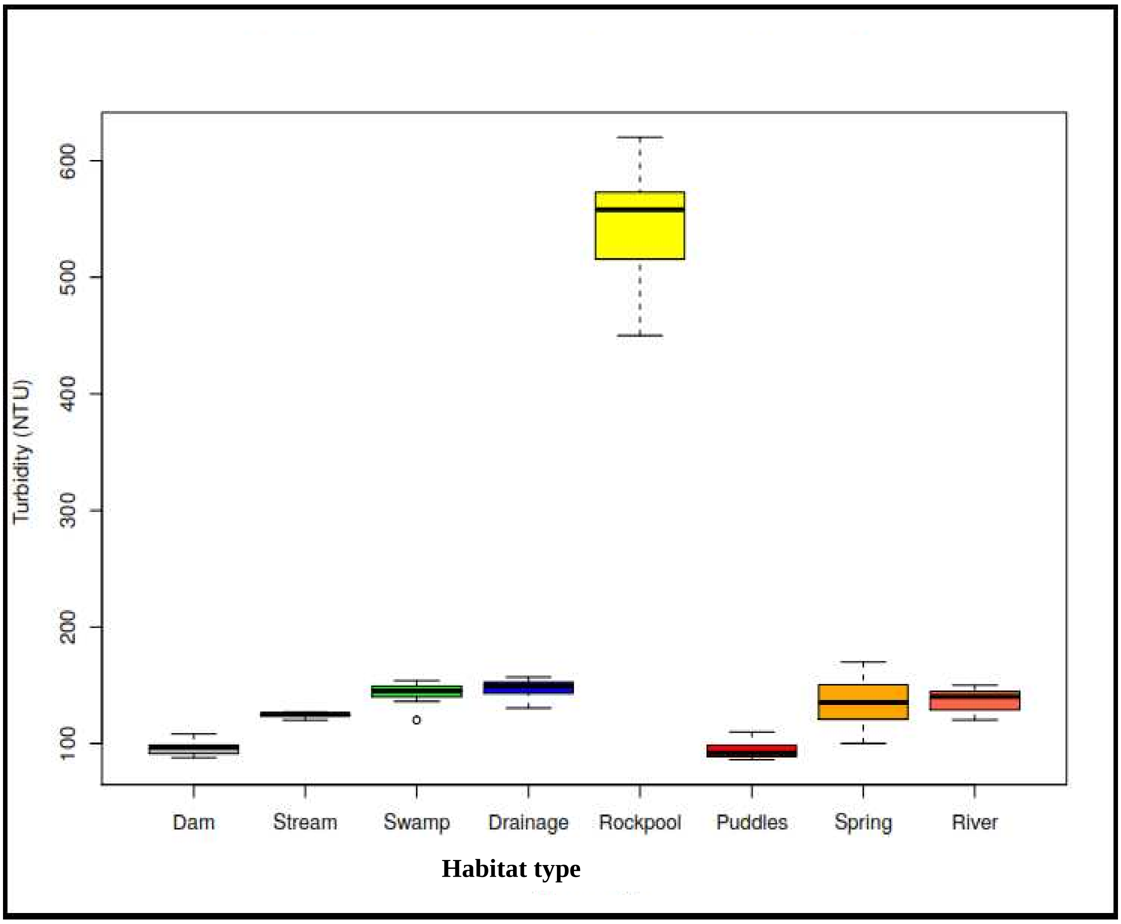


Figure 4.11: Boxplot and Whisker Plot of Total Turbidity along Mara River (July-August 2011, n=39).

Correlation matrix to determine the direction of relationship bwetween turbidity, larval mosquitoes and predators in the shared habitats is as shown in Figure 4.12. There was a weak positive, but non-significant correlation between larval mosquitoes and predators in the presence of turbidity (r=0.00396, $p>0.05$), a positive correlation between turbidity and larval mosquitoes (r=0.192) and a negative correlation between turbidity and predators (r=-0.315, $p>0.05$) in the shared habitat.

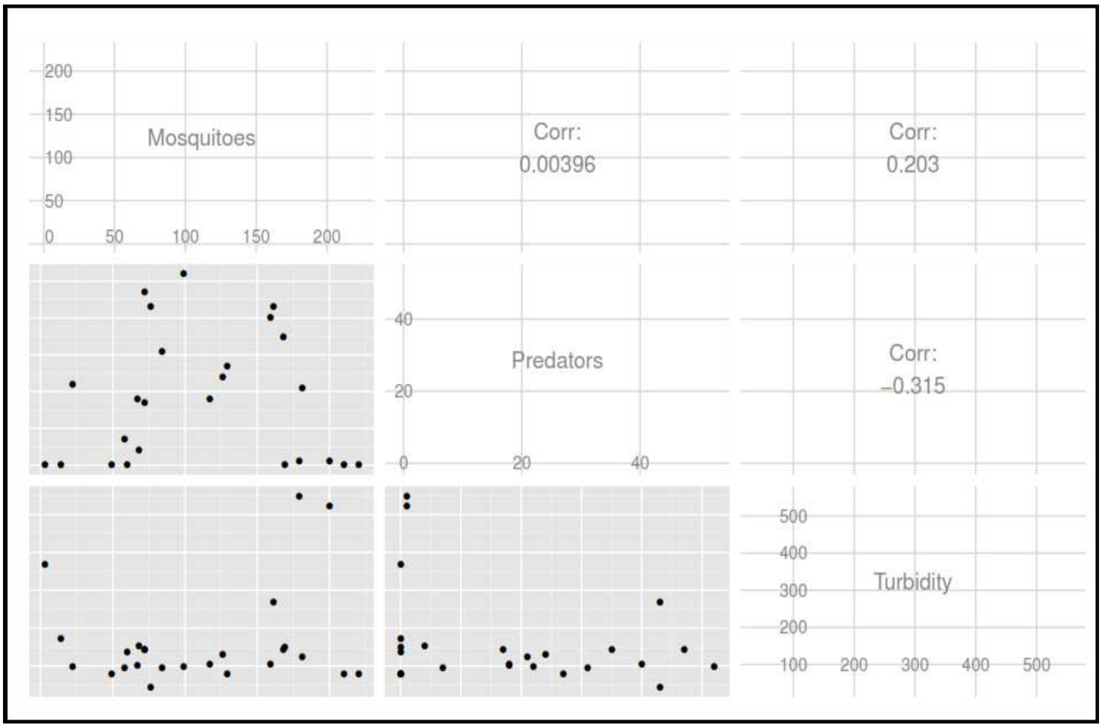


Figure 4.12: Correlation Matrix between Larval mosquitoes, Predators and Turbidity in shared Habitats along the Mara River Basin (July-August, 2011). Turbidity had a Negative Corelation with Predators.

A positive correlation (r=0.203, $p<0.05$) was observed between larval mosquitoes and turbidity, but non-significant correation between predators and larval mosquitoes (r=0.004, $p>0.05$) in the same habitat, while a negative correlation was observed between turbidity and predators in the same habitat. This required further analysis using in a more robust model, to establish the relationship between individual parameters and the bundance of larval mosquitoes and their predators.

Analysis to determine preferable level of turbidity requirement by both larval mosquitoes and predators in the shared habitat indicated that, with values ranging between 103.8 to 590.4 NTU, a range between 143.2 NTU and 144.0 NTU was most preferred by both larval mosquitoes and predators in the shared habitats along the Mara River. However, some larval mosquitoes had no specific turbidity requirement range.

4.5.2.4. Alkalinity

Alkalinity indicates the habitats' ability to absorb acidic substance. The water condition inhabited by larval mosquitoes and their predators along the Mara River is shown in Figure 4.13. Mean alkalinity was highest in drainages (400±282.8 mg/l), followed by swamps (244±274.6 mg/L), while the lowest alkalinity was recorded in dams (100±199.2 mg/L) and rivers (100±62.4 mg/L). Swamps produced a mean of 244±274.6 mg/L, while rock pools had 153±60.8 mg/L, followed by streams (126.2±26.5 mg/L) and springs (124.5±113.2 mg/L). A significant difference in alkalinity was observed among the different land uses (One–way ANOVA, n=10, F=3.7219, d.f.=9, 26, p=0.02). Boxplot presentation for alkalinity in the different mosquito and predators breeding sites are as shown in Figure 4.13.

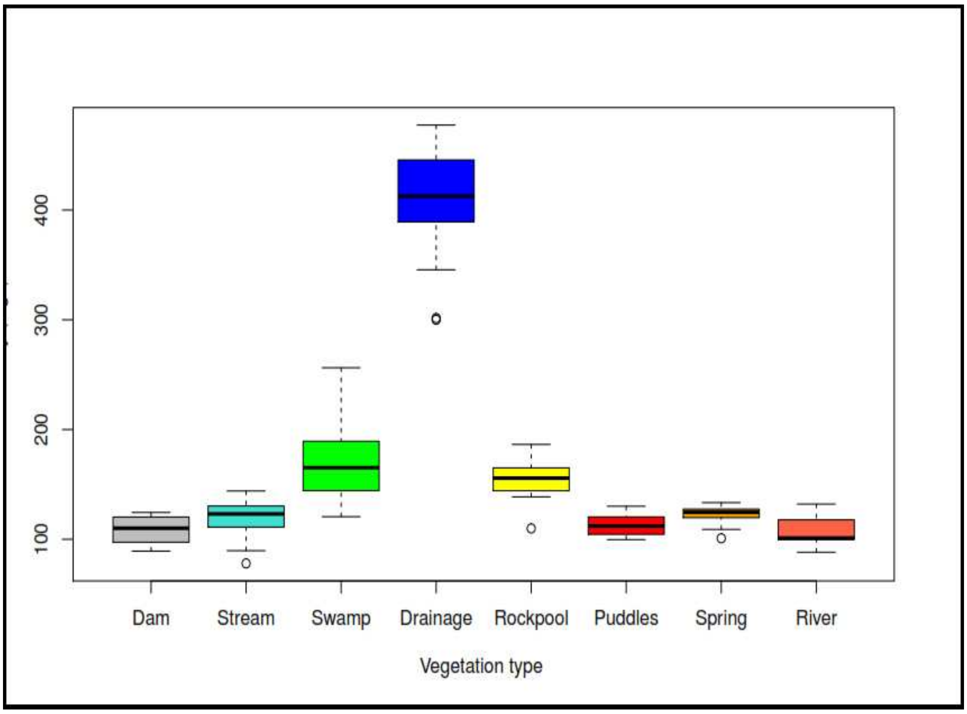


Figure 4.13: Boxplot and Whiskers of Average Alkalinity across different Habitat types along Mara River (July-August 2011, n=39).

Alkalinity level was highest in drainages followed by swamps. Dams and puddles had the lowest alkalinity level. Analysis by correlation matrix to determine the direction of alkalinity in the shared habitats is as shown in (Figure 4.14). There was a positive, but non-significant correlation between larval mosquitoes and predators (r=0.00171, $p>0.05$), a positive correlation between alkalinity and larval mosquitoes (r=0.128) as well as predators (r=-0.059, $p>0.05$) in the shared habitat was also observed.

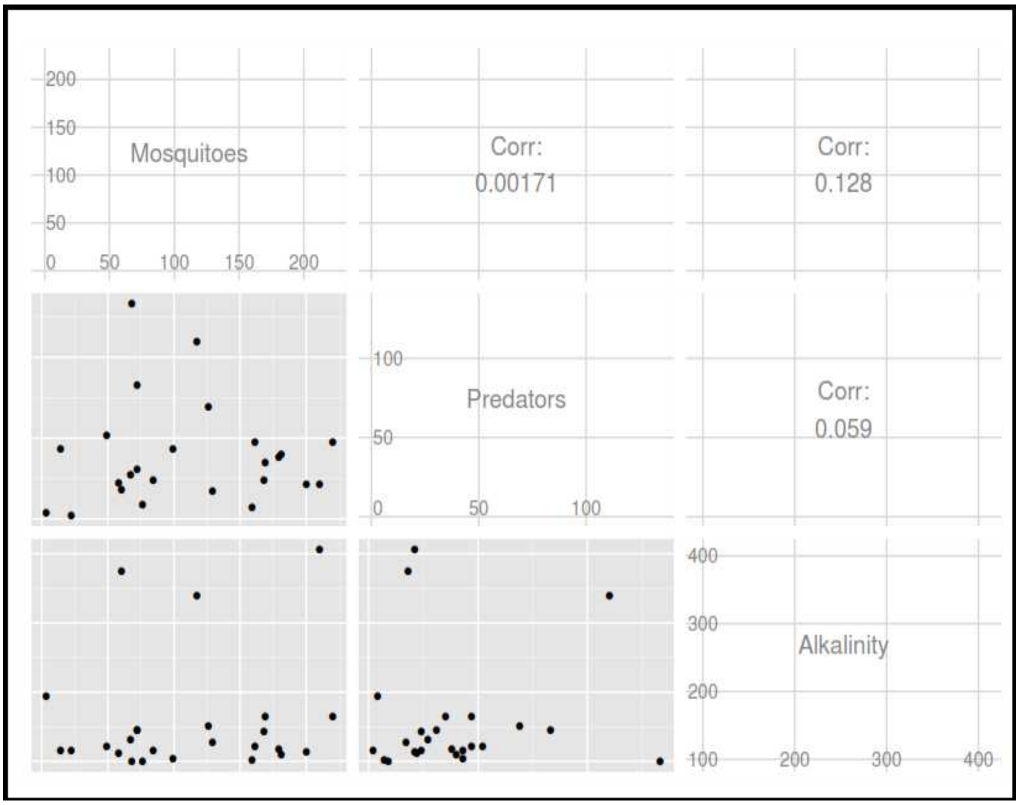


Figure 4.14: Correlation Matrix between Larval Mosquitoes, Predators and Alkalinity in Shared Habitats along the Mara River Basin (July-August 2011). Both Larval mosquitoes and the Predators Showed a Positive Correlation with Alkalinity.

Analysis to determine preferable level of alkalinity range requirement by both larval mosquitoes and predators in the shared habitat indicated that, with values ranging between 6.4mg/L, and 406.1mg/L, only few larval mosquitoes and predators prefered a range between 131.2mg/L, and 144.4mg/L, others had a more wider requirement range However, some mosquito had no specific alkalinity requirement range.

4.5.2.5. Salinity

The distribution of organisms within habitats is to some extend driven by salinity. Along the Mara River most aquatic habitats had zero levels of salinity. Only swamps recorded salinity level of 0.4 mg/L. Thus, the influence of salinity along the Mara River could not be statistically evaluated as a result of insufficient sample numbers. However, in the Ordination Analysis, both predators and mosquito larvae showed a positive relationship with salinity (CCA, $p<0.05$).

4.5.2.6. Dissolved Oxygen (DO)

As with other chemical parameters, dissolved oxygen levels were measured during sampling along the Mara River in every habitat. A habitable aquatic ecosystem requires a good supply of oxygen in the water system. The boxplots below (Figure 4.15) indicate results for the water condition inhabited by larval mosquitoes and their predators along the Mara River. Mean dissolved oxygen was highest in rivers (6.4±0.7 mg/L), followed by rock pools (6.0±0.7mg/L). The lowest was recorded in swamps (2.4±2.7mg/L). Dissolved oxygen in dams (4.7±1.8 mg/L), drainages (4.3±3.8 mg/L), and springs (4.0±0.3 mg/L) varied slightly but were almost of the same range. The mean dissolved oxygen in puddles was 5.6±0.8mg/L, while that of stream was 5.3±1.6mg/L. A significant difference in mean dissolved oxygen was observed among the different habitat types (One–way ANOVA, n=10, F=4.3261, d.f.=9, 26, $p=0.01$). Oxygen level was highest in rivers followed by rock pools. Swamps had the lowest oxygen levels.

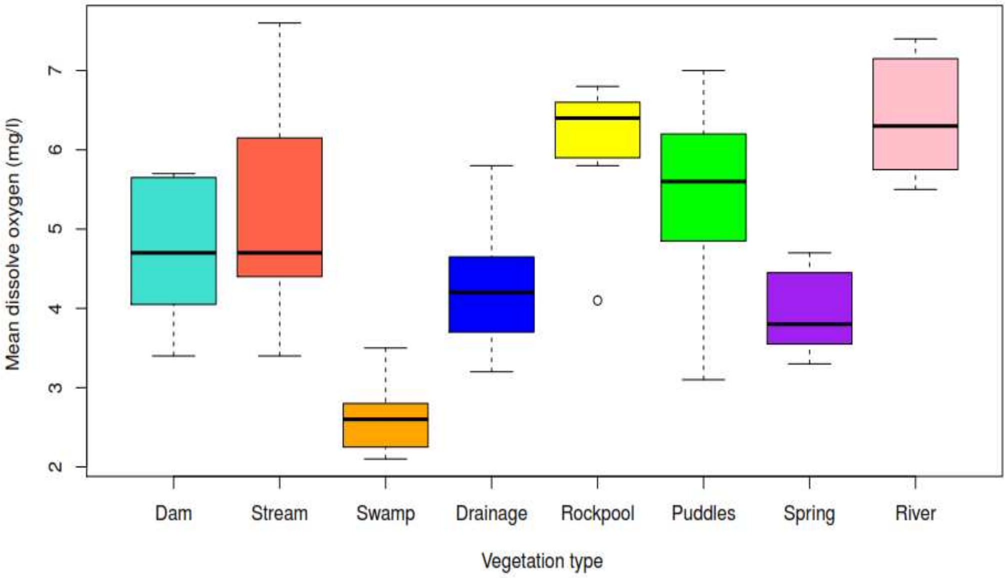


Figure 4.15: Boxplot and Whisker Plot of the Concentration of Dissolved Oxygen across Habitat Types along Mara River (July-August 2011, n=39).

Analysis by correlation matrix to determine the direction of dissolved oxygen in the shared habitats is as shown in (Figure 4.16). There was a positive, but non-significant correlation between larval mosquitoes and predators in the presence of oxygen (r=0.219, *p*>0.05), between dissolved oxygen and larval mosquitoes (r=0.0379, *p*>0.05) and dissolved oxygen and predators (r=0.0436, *p*>0.05) in the shared habitat (Figure 4.16).

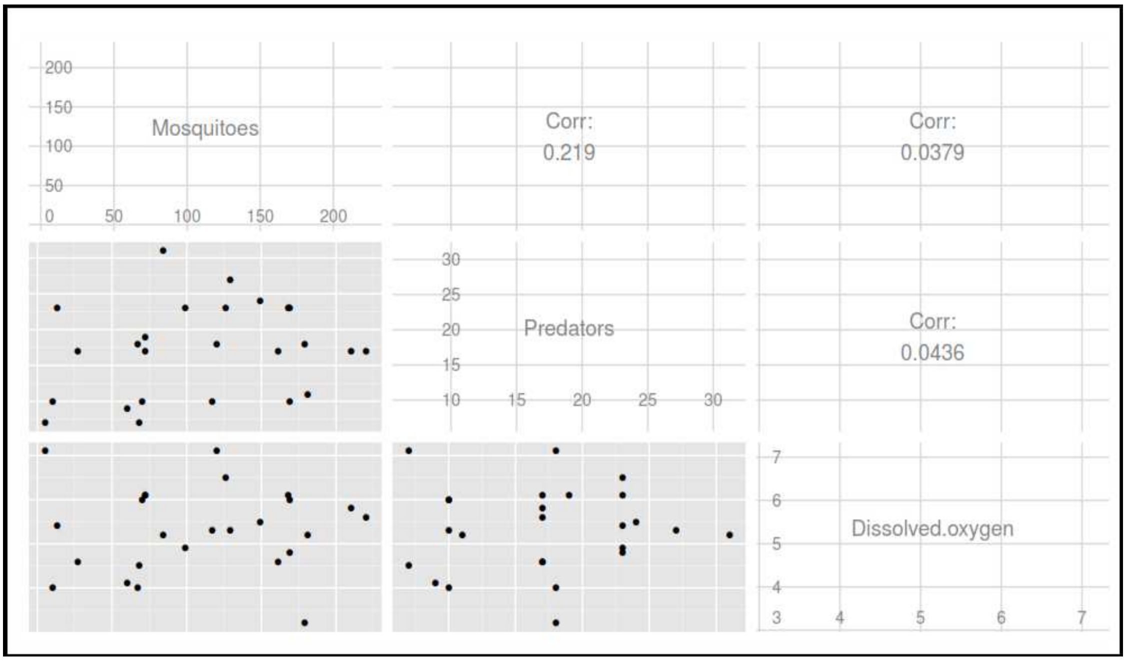


Figure 4.16: Correlation Matrix between Larval Mosquitoes, Predators and Dissolved Oxygen Values in Shared Habitats along the Mara River Basin (July-August, 2011). A Posive Correlation was observed between larval mosquitoes and Predators with Dissolved Oxygen.

Analysis to determine preferable level of dissolved oxygen range requirement by both larval mosquitoes and predators in the shared habitat indicated that values ranging between 6.0mg/L and 6.5mg/L were most preferable, however, some larval mosquitoes and predators also preferred a range of between 5.2mg/L and 5.3mg/L. Few preferred dissolved oxygen levels of between 5.3mg/L and 6.5mg/L, while others preferred DO levels of between 2.3mg/L and 6.1mg/L.

4.5.2.7. Temperature

Temperature influences the rates of metabolism and growth of aquatic organisms. It is also responsible for solubility of oxygen in river water, and neutralizes toxic materials. At a higher temperature, organisms perspire and die faster, leaving behind matter that requires oxygen for decomposition. Along the Mara River, the highest mean temperature was recorded in the springs (26.3±2.2°C), followed by rock pools (26.2±0.7°C) and puddles (25.2±2.3°C). The lowest temperature was recorded in rivers (19.7±2.3°C). Dams (24.4±1.9°C) and drainages (24.2±0.7°C) scored almost the same value, which differed only slightly. The mean temperature recorded at the swamps and streams were 23.2±4.9°C and 22.5±2.1°C, respectively (Figure 4.17). The temperature range of the Mara River as tested between July and August was 19.7 to 26.3°C. A significant difference in mean temperature was observed among the different habitat types (One–way ANOVA, n=10, F=5.3107, d.f.=9, 26, *p*=0.04).

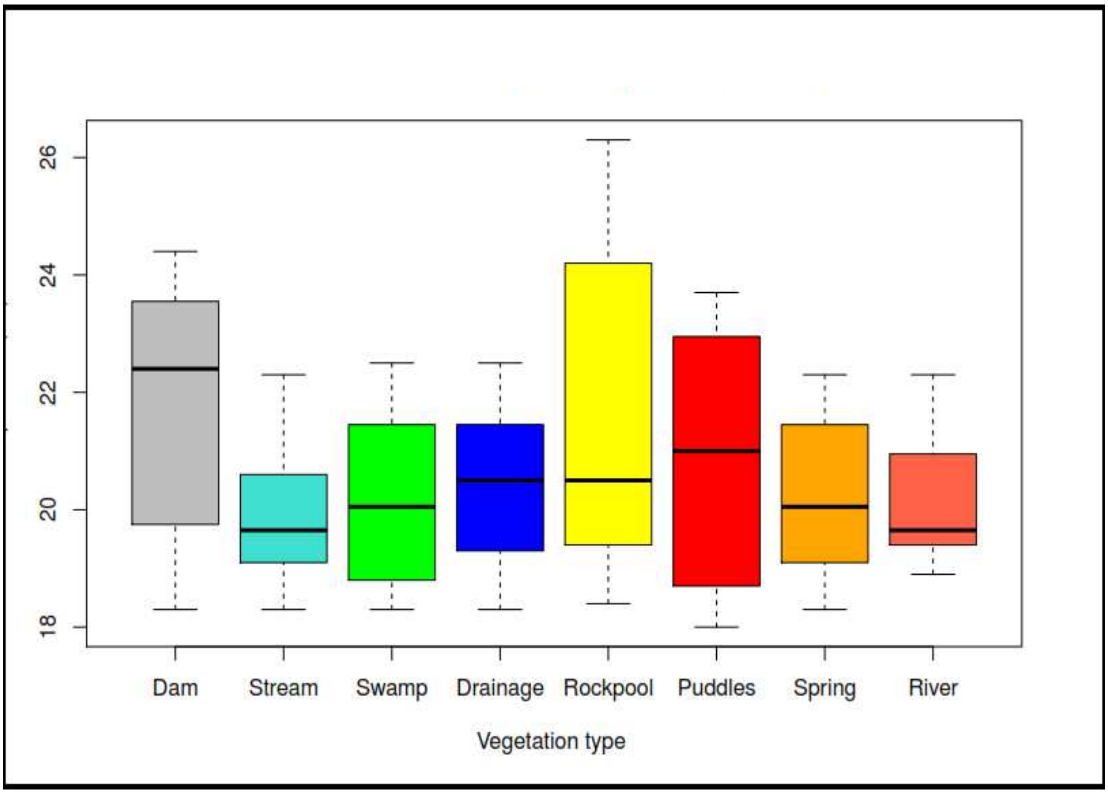


Figure 4.17: Boxplot and Whisker Plots of the Average Temperature Values across Habitat Types along the Mara River (July-August, 2011, n= 39).

Temperature level was highest in rock pools followed by dams. Streams and rivers had the lowest temperature levels. A correlation matrix to determine the direction of temperature in the shared habitats is as shown in Figure 4.18. There was a positive, but non-significant correlation between larval mosquitoes and predators (r=0.195, $p>0.05$); *i.e.* between temperature and larval mosquitoes (r=0.249, $p>0.05$) and between temperature and predators (r=-0.161, $p>0.05$) in the shared habitats.

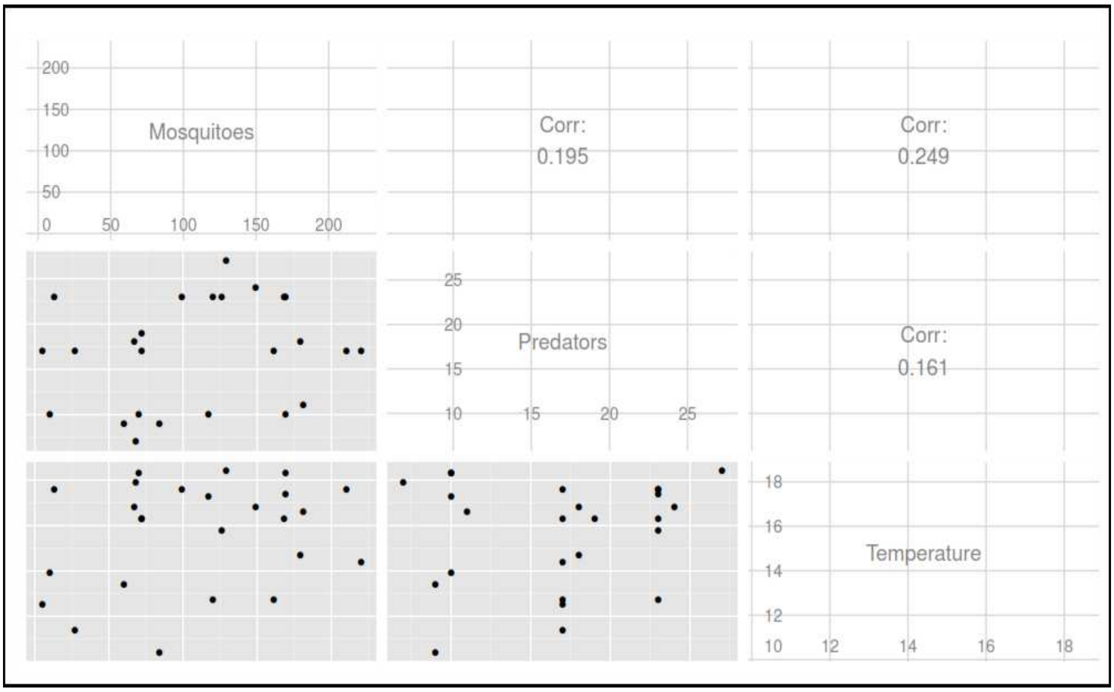


Figure 4.18: Correlation Matrix between Larval Mosquitoes, Predators and Temperature in Habitats along the Mara River Basin (July-August, 2011). A Positive Correlation between Larval mosquitoes and Predators with Temperature was observed.

Analysis to determine the preferable level of temperature range requirement by both larval mosquitoes and predators in the shared habitat indicated that values ranging between 19.3°C and 20.6°C were most preferred. However, some larval mosquitoes preferred a temperature of 22.3°C, though some larval mosquitoes preferred even lower temperatures at 18°C.

4.5.2.8. Water Hardness

Hardness of surface water is usually as a result of the presence of multivalent metal from minerals dissolved in the water. In the aquatic environment, ions result from abundance of calcium and magnesium in water. Figure 4.19 shows graphical distribution of hardness along the Mara River. The highest mean hardness (372±393.2mg/L) was recorded in the drainages while the lowest were recorded in dams and swamps (87.7±56.2mg/L and 58.5±46.7 mg/L, respectively). Streams recorded a mean average of 102.4±68.9 mg/L, while puddles, springs and rivers recorded almost the same mean values of hardness (188±247.7 mg/L, 183±148.4 mg/L and 178±228.8 mg/L, respectively). Rock pool had the intermediate value (178±228.8 mg/L). Mean hardness differed significantly among different habitat types along the Mara River (One–way ANOVA, n =10, F=5.1004, d.f.= 9, 26, $p \leq 0.001$).

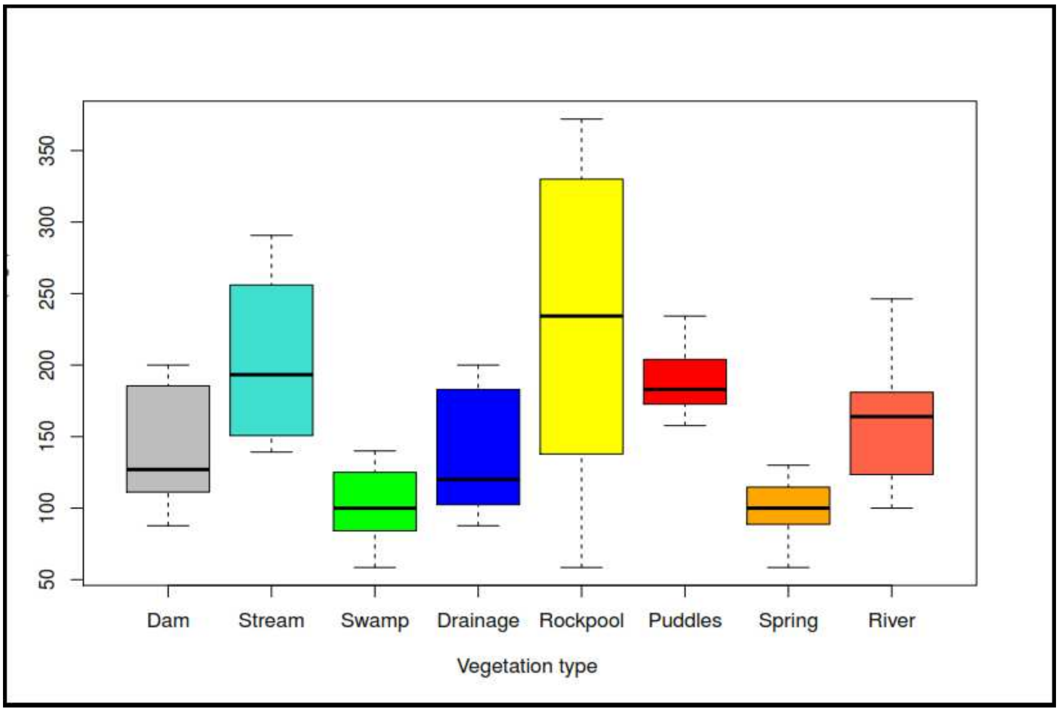


Figure 4.19: Boxplot and Whisker Plot of Mean Hardness across Habitat Types along Mara River (July-August, 2011, n = 39).

Hardness level was highest in rock pools followed by streams. Swamps and rivers had the lowest hardness levels. A correlation matrix to determine the direction of hardness in the shared habitats is as shown in Figure 4.20. There was a positive correlation between larval mosquitoes and predators in the presence of hardness (r=0.56, *p*<0.05), and between hardness and larval mosquitoes (r=0.0592, *p*>0.05). However a negative correlation was observed between hardness and predators (r=-0.2491, *p*>0.05) in the shared habitats.

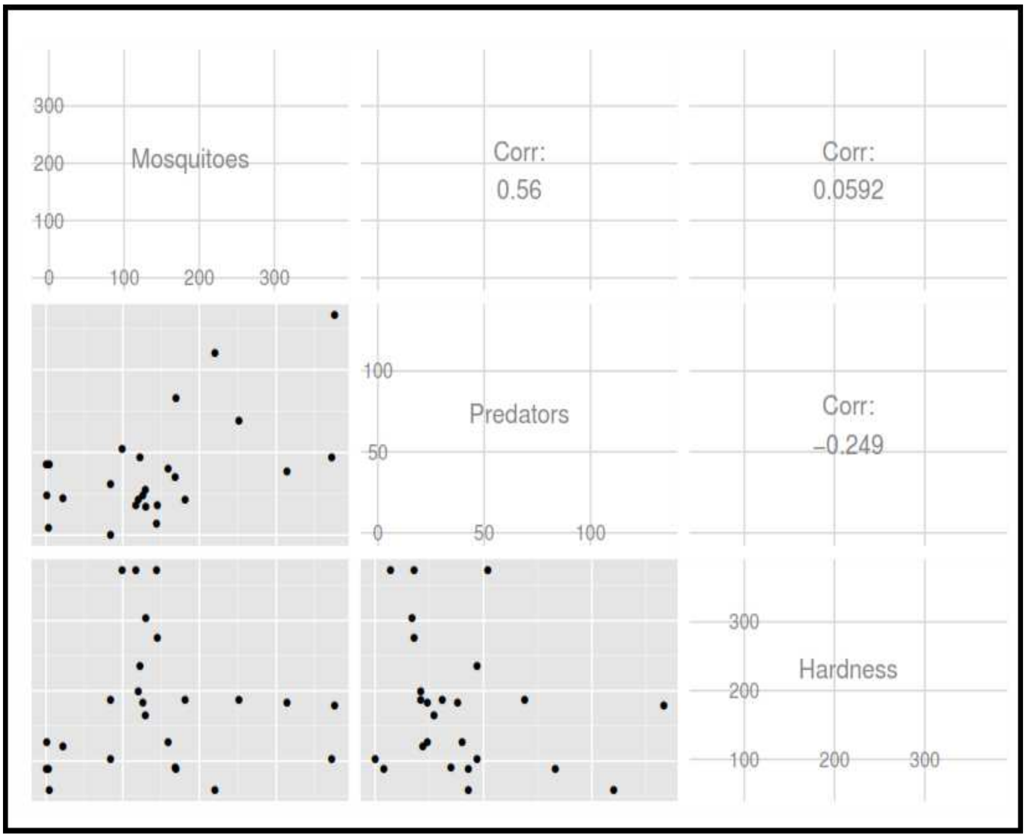


Figure 4.20: Correlation Matrix between Larval mosquitoes, Predators and Hardness Values in Shared Habitats along the Mara River basin (July-August 2011).

Analysis to determine the preferable level of hardness range requirement by both larval mosquitoes and predators in the shared habitats indicated that, given values ranging between 58.5mg/L, to 372mg/L, both larval mosquitoes and predators would have varied range of hardness requirements. This study found no specific preferences for hardness by mosquito larvae and their predators.

4.6. Relating Physico-Chemical Parameters to Mosquito Larvae Abundance using GLM

In the GLM model, the results established that the abundance of predators in habitats were partially driven by the presence of mosquito larvae (Z=6.49, $p \leq 0.001$), and the prevailing water physico-chemical parameters (dissolved oxygen, Z=3.34, $p \leq 0.001$; temperature, Z=2.75, $p \leq 0.001$; and turbidity, Z=-3.65, $p \leq 0.001$), based on the best model with the smallest AIC (Table 4.11).

Table 4.11: Negative Binomial-GLM Model for the Biotic and Abiotic Predictor Variables that influence Mosquito Predators' Abundance in Habitats along the Mara River

Physico-chemical parameters	Estimate	Std. Error	Z-value	Pr(>|z|)
Intercept	-3.42	1.22	-2.83	<0.001
Dissolved Oxygen (DO)	0.38	0.11	3.34	<0.01
Temperature	0.07	0.03	2.74	<0.001
Turbidity	-0.01	0.01	-3.65	<0.001
Total larval mosquitoes	0.01	0.01	6.49	<0.001

4.7. Canonical Correspondence Analysis (CCA) for the Individual Relationship between the Physico-Chemical Parameters, Larval mosquitoes and Predators

The Canonical Correspondence Analysis (CCA) to determine the role of each independent variables on the dependent variables (larval mosquitoes and predators), hardness (negatively) and salinity, pH and conductivity (CCA, $p \leq 0.05$) positively influenced the abundance of both mosquito and predators, while temperature, dissolved oxygen and turbidity significantly influenced mosquito larvae and predators abundance (CCA, $p \leq 0.01$). Biplot from a CCA on how variables correlated is presented. Quadrats represent uqniueness of the variables such that the more important variables are in the first quadrant and directly opposite their most important correlates (Figure 4.21). The raw numbers indicated are random numbers generated by the analysis on how the variables were added onto the model.

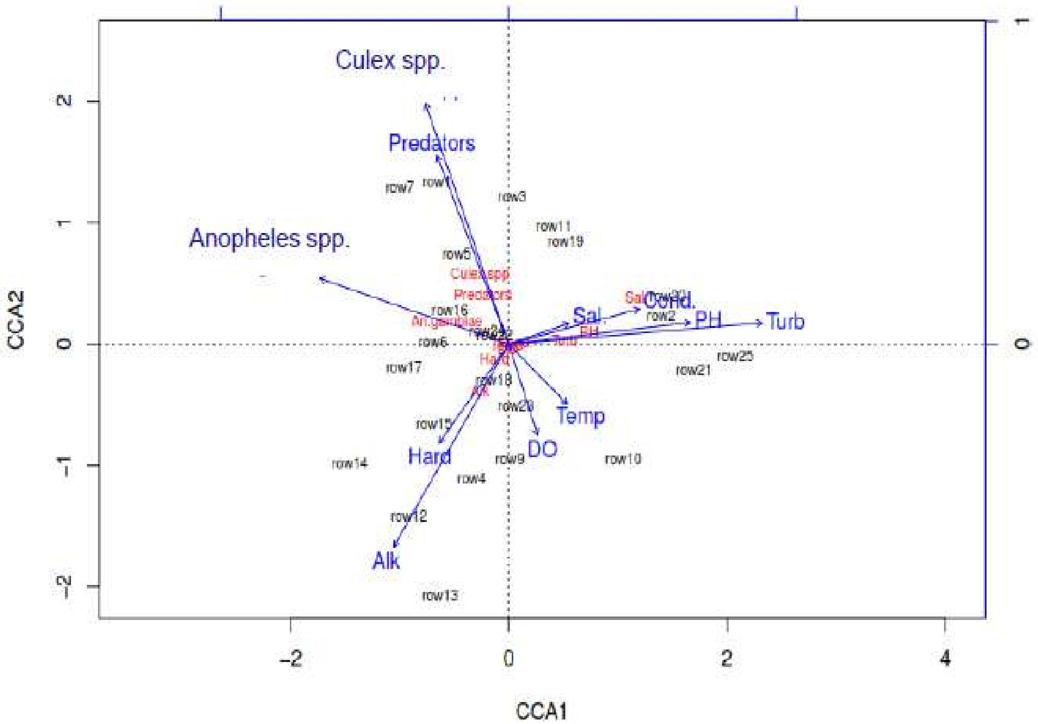


Figure 4.21: RDA Ordination Biplot of the Overall Effect of Various Enviromental Parameters Recorded Along the Mara River, (July to August, 2011, n=39). Dissolved Oxygen and Temperature Correlated with Mosquito Lravae and their Predators.

Key: Sal = Salinity, Turb = Turbidity, PH = pH, Temp = Temperature, DO = Dissolved Oxygen, Hard = Hardness, Alk = Alkalinity.

The sum of all canonical Eigenvalues was 0.440. Dissolved oxygen and temperature were the most important factors that positively and directly correlated with both larval mosquitoes and predators abundance based on quantrant reflection in the ordination analysis. Overall, the model explained 99.8% of all the nine variables.

To evaluate the strength and pattern of relationship between mosquito larvae and predators, a canonical correlation analysis was done. There was a strong correlation between the predators and mosquito larvae ($r=0.72$, $p\leq0.001$).

CHAPTER FIVE: DISCUSSION

5.1. Presence, Abundance and Distribution of Malaria and Non-Malaria Transmitting Vector Larval mosquitoes along the Mara River Basin

Several species, which included *Anopheles gambiae* complex, *Anopheles funestus* group *An. pharoensis, An. maculipalpis, An. coustani complex* larvae and few uncommon species such as *An. azamiae, An. christyi, An. hamoni* and *An. sergeti* were present in few of the Mara River habitats. Among these, *An. pharoensis, An. coustani* and *An. azamiae* have been reported as malaria vectors elsewhere. More specifically, *An. coustani* have been implicated as a transmitter of malaria parasite in Kenya, Ethiopia, Cameroon and Upper delta (Cohuet *et al.*, 2003; Massebo *et al.,* 2013; Mwangani *et al.*, 2008).

Most mosquito larvae were found in isolated pools of the receding waters or in temporary habitats near the main Mara River and along the perennial Mara River tributaries of Amala and Nyangores. Most habitats found along the Mara River were characterized by various types of vegetation, which in turn offered a variety of microhabitats for the larval mosquitoes. *Anopheles custani* complex and *An. funestus* group were found mainly in swamps, river edge and drying stream in higher densities. Previously, they have been reported to mainly occupy vegetated areas near the shore with large volume of unpolluted water (Minakawa *et al.,* 2012). However, several patches of open sunlit pools adjacent to the main Mara River were dominated mainly by *An. gambiae* s.l., *Culex* spp. and *An. maculipalpis*. For malaria transmitting vectors, *An. gambiae* s.l were the most dominant, especially in samples collected from open sunlit pools. Studies by Minakawa *et al.* (2002) suggested that *An. gambiae* s.l. tend to prefer open sunlit pools as was also evident in the current study.

Out of the 4001 larval mosquitoes collected, *An. gambiae* s.s. comprised 840 (25.9%), followed by *An. arabiensis* (24.3%) based on the results from the PCR technique. Other *Anopheles* species that did not belong to *An. gambiae* s.l also existed in the study sites as shown in Table 2. These species failed to amplify with primers specific for *An. gambiae* s.s and *An. arabiensis*. The specimens that failed to amplify after repeated trials and adjustments were initially microscopically identified into other *Anopheles* species and grouped. There was no further identification done for *Culex* spp., except for the use of standard morphological identification key. Future studies should therefore consider identifying all species that belong to the same genus in the study area using oligo primers specific for all the different sibling species of Anophiline and Culicine. The two species knwon to tramsmit malaria (*An. gambiae* s.s and *An. arabiensis)* and *Culex* spp. were sparsely distributed, with *An. gambiae* s.s species dominating upper part of the Mara River in Kenya, while *An. Arabiensis* showed a similar trend towards Tanzania. *Culex* spp. were evenly distributed among the sites.

In the Mara River basin, *An. funestus* group larvae were mostly found in swamps and few in rock pools, while, *An. pharoensis, An. azamiae, An. christyi, An. maculipalpis, An. hamoni* and *An. sergeti* dominated open sunlit puddles, hippo hoof-prints and drainages. As with similarity to other areas, *An. gambiae* s. l. were found in high abundance either on temporary sunlit pools or open habitats with scattered short grass (Minakawa *et al.,* 2002; Serneels & Lambin, 2001). Insignificantly few *An. funestus* group larvae were found in rock pools located at the Trans-Mara border site 4, Trans Mara bridge sites 2, 5 and 6, and a site located at Kichwa Tembo Bridge close to the border of the Mara Game Reserve, near Mara Safari Club in Kenya in the current study. Hardly have they been found inhabiting rock pool or open sunlit pools in other studies.

Culex spp. and *An. gambiae* s.l. dominated most habitats. The Mara River is perennial, flowing all year round, with levels of water fluctuating during the dry and rainy. As a result, small pools of water are present by the riverside during the rainy season, which dry as the amount of rainfall decrease. On the contrary, stream water becomes stable with reduced flow rate. The observation that *An. gambiae* s.l. and *An. funestus* group were abundant in drying stream tributaries of the Amala and Nyangores rivers was a clear confirmation that most malaria vector species prefer breeding on stable waters with less disturbances. Stable flows of the steam during dry period appear to support more larval mosquitoes along the river basin than rainy period. This contradicts earlier report that malaria transmitting vector population may only increase during rainy seasons (Odongo-Aginya *et al.*, 2005); (Manikandan & Sevarkodiyone, 2014). This study also showed that *Culex* spp. larvae were the most abundant and widespread mosquito larvae along the Mara river basin. They were collected from different habitats. This clearly indicates that *Culex* spp. larvae have great degree of adaptability to different habitats than other mosquitoes. The presence and wide distribution of Anophelines within the Mara River, the vector of human malaria constitutes a major potential health problem. Further studies on the vectorial capacity of these disease pathogens vectors are required and every effort should be made to prevent their spread along the Mara River.

At Transmara border site, the habitats were mainly rock pools with stagnant water created by the hydrologic effect of stream water, which hits the river banks and settles on pocket-like rock habitats. The water in these habitats was clear and shielded from direct sunlight. Consistent with the current findings, previous study reported that the presence of *An. gambiae* s.l. and *An. funestus* group in diverse natural aquatic habitats in the Western Kenya highlands were inversely correlated to canopy cover (Minakawa *et al.*, 2002). Similarly, Minakawa et al (2008) and Fillinger *et al.* (2004) studies indicated preference for open sunlit pools by the *An. gambiae* s.l and recommended that these habitats be closely monitored if the risk of malaria transmissions is to be reduced among the riparian communities living along the lake. In both the current and previuos studies, larval sampling has indicated that swamps and other potential habitas adjacent to either a river or lake becomes more potential for both *An. gambiae* s.l and *An. funestus* group. The Mara River is perennial, flowing all year round, with levels of water fluctuating during the dry and rainy seasons (Serneels & Lambin, 2001). As a result, small pools of water are created by the riverside and stream tributaries during the rainy season, which dry as the amount of rainfall decreases (Serneels & Lambin, 2001), making them stable with reduced flow rate suitable for *Anopheles* mosquito breeding.

On terrestrial habitats, open sunlit puddles were found to harbour more larval mosquitoes compared to roadside ponds with vegetated habitats. Consistent with the current findings, the behavioural avoidance patterns of standing waters characterized with vegetation by the *An. gambiae* s.l was also reported by Mutuku *et al.* (2006). In the river habitats, more larval mosquitoes were found in drying streams and riverbeds with little vegetation as compared to open water, thus an indication that aquatic vegetation plays an important role in harbouring these malaria-transmitting vectors. The stablility of a stream during dry periods appear to support more larval mosquitoes along the river tributary streams than in rainy periods. However, seasonality studies may be required to determine trend and density that may provide proof for comparison purposes. This study has also shown that *Culex* spp. were the most widespread mosquito larvae along the Mara River basin as they were collected from a variety of habitats. This is an indication that *Culex* spp. larvae have a great degree of adaptability to different habitats than any other mosquito species.

The main Mara River, with riffles and pools and characterized with fast flowing waters had no mosquitoes. However, large swamps with tall emergent vegetation adjacent to the Mara River were found to be dominated by *An. coustani*. The many habitats adjacent to the main river either created through human activities such as brick making or animal activities especially at watering points appeared to harbour most malaria transmitting vector, i.e. the *An. gambiae* s.l. The receding river and stream tributaries' water levels caused by the destruction of forests, rock pools which initially were below the water surface, especially during dry spells, were the main potential breeding micro- habitats for *An. gambiae* s.l and *An. funestus* group. Therefore, these conditions are potentially improving the habitat diversity for these vectors, which are good indicators of the health risk posed by the communities of the riverine ecosystem.

Open sunlit puddles, rock pools and drains, which produced the highest numbers of larval mosquitoes, were shallow, isolated and tended to limit predator access. Such habitats presented perfect-breeding sites for potentially harmful mosquito species some of which are known carriers of malaria parasites and the viruses.

This study compared terrestrial water pools with those adjacent to the river, because past studies suggest that the pools along the lake shore are more potential than their terrestrial counterparts (Minakawa *et al.,* 2002). Similarly, in this study the Shannon diversity index was slightly higher for river edge habitats (1.43) compared to terrestrial habitats (1.17), though both were still low considering that the typical value of the index ranges from 1.5 (low species richness and evenness) to 3.5 (high species evenness and richness), (Magurran, 2005), although values beyond these limits, up to a maximum of 5 may also be encountered. Consequently, the evenness index was also higher in river edge habitats (2.13) as compared to the terrestrial aquatic habitats (1.23), reflecting a variation in abundance of mosquito species between the two sites, along the Mara River. This study has also revealed that *Culex* spp. were the most widespread mosquito larvae along the Mara River basin as they were collected from a variety of habitats. The presence and wide distribution of *Anopheles* spp., the vector of human malaria, constitutes a major potential health problem.

Considering that similar proportions of all sub-species give an evenness index of one, with higher values reflecting very dissimilar proportions (some rare and some common species), it is apparent that mosquito sub-species were clearly dismal as reflected by the dominance of *Anopheles gambie* s.l and *Culex* spp. over other mosquito species in both habitats. This is an indication that some species are better adapted to the habitats sampled than others as was also observed in Western Kenya (Imbahale *et al.*, 2011).

Few uncommon species such as *An. pharoensis*, *An. faini*, *An. hamoni* and *An. azamiae*, have been implicated in malaria transmission elsewhere in Africa (Cohuet *et al.*, 2003; Massebo *et al.*, 2013). Although studies have been done on abundance and distribution of larval mosquitoes in Kenya previously (Kweka *et al.*, 2013; Minakawa *et al.*, 1999; Muturi *et al.*, 2008), majority were limited to the detection of known specific disease and non-disease transmiting mosquito larvae as in the current study. Although their densities were low, they have to be regularly monitored to avoid any future outbreak of diseases when their density explodes. However, the current study is consistent with that of Minakawa *et al.* (2002), Fillinger *et al.* (2004) and Minakawa *et al.* (2012) who also reported diverse natural aquatic habitats for both *An. gambiae* s.l. and *An. funestus* group in western Kenya.

5.2. Presence, Abundance and Distribution of Mosquito Larvae Predators and their Relationship with Mosquito Larvae Abundance and Distribution along the Mara River Basin

In the current study, the distribution of mosquito larvae predators along the Mara River was sparse with a total of 297 predators belonging to 3 orders identified in the 39 sites sampled. The sparse distribution and particular dominance of members of order Hemiptera over the other orders could have been due to their broad tolerance to a wide range of environmental conditions which probably enabled them to inhabit micro-habitats that other predator species may not prefer. Studies have also shown that throughout ontogeny, species will likely experience different effects of abiotic factors, depending on their developmental stage, thus creating conducive habitats for some species and not others (Eitam *et al.,* 2002).

Overall, drying streams supported the greatest numbers of both mosquito larvae and their predators during this sampling period and may be responsible for increasing natural predation in certain temporary habitats such as dams, open puddles and vegetated pools. This possibility is supported by the observation that certain terrestrial aquatic habitats had lower number of larval mosquitoes and higher predator abundance.

The relatively low mosquito and predator numbers observed in the ephemeral habitats as compared to drying streams and swamps might have been due to several reasons. As reported in earlier studies, adult mosquito may have the ability to detect the presence of predators and consequently avoid ovipositing in such habitats, preferring instead to inhabit areas with swamps and grassy patches that can protect the immatures (Blaustein *et al.,* 2004). Previously, larval mosquitoes of the species *Culiseta longiareolata* were reported to detect chemicals from notonecta predators, and the instinct/cue can exist in the habitat for up to a week or more after their disappearance from the pool (Blaustein *et al.,* 2004) and for *Culex* species, a period as low as two days have also been reported (Blaustein, Blaustein, & Chase, 2005).

Majority of insect predators recorded in the sampled habitats as already been mentioned were mainly the order Hemiptera, as compared to the other two aquatic insect orders; Odonata and Coleoptera. The Order Hemiptera were widespread representing 7 families. The 7 families were over-represented by Family Velidae and Genus Rhagovelia. Other predators of mosquito larvae belonged to the Order Odonata (which recorded 3 families dominated by family Coenagrionidae) and Order Coleoptera (which recorded 2 families dominated by Dytiscidae). Order Hemiptera were the majority. A previous study also reported high number of this Order in aquatic habitats in Japan. This was related to the presence of other preferred food items such snails, in addition to larval mosquitoes (Ohba & Nakasuji, 2006). In the current study, area snails were encountered in the habitats for mosquito larvae and their predators. Gilbert & Burns (1999) concluded that notonectid predators have the potential to alter mosquito communities via direct or indirect effects. Direct evidence of notonectid predation on mosquito larvae was obtained and this further confirmed their predominant role in mosquito larvae control (Kumar & Hwang, 2006). Most of the insects in the Order Hemiptera have also been acclaimed as pollution tolerant (Joshi *et al.*, 2014).

Evaluation of the effectiveness of the biological control of mosquito in nature is complicated by the fact that some larval mosquitoes that are vulnerable to predators such as *Notonecta* spp. and *Anisops* spp. avoid laying eggs in waters infested with these particular, thus making their control through biological means almost impossible (Blaustein *et al.,* 2004; Eitam *et al.,* 2002). Simulation models suggest that mosquito species of *Culiseta longiareolata*, though susceptible to *Notonecta maculata* predation, can be abundant owing to their strong avoidance of waters containing the predator (Warburg *et al.,* 2011). However, a similar trend has not been reported with regards to malaria vector species. Studies have also shown that some predators especially those of the genus *Notonecta* often show vertical migration, i.e. up and down the water column implying that *Notonecta* could be one of the most appropriate bio-control tool for *Anopheles* and *Culex* larvae, whereas *Buenoa* (Backswimmer) may be more effective for *Aedes* spp. larvae since they prefer visiting artificial containers (Suárez-Rubio & Suárez, 2004). Notonectids generally prefer mosquitoes to chironomids, ceratopogonids, cladocerans, among other aquatic insects (Blaustein *et al.*, 2004; Eitam *et al.*, 2002), but alternative prey may also be sought (Kumar & Hwang, 2006). Moreover, studies show that many predators prefer a particular developmental stage of larval mosquitoes. For example, it has been reported that while aquatic mites and copepods attack early instar larvae of larval mosquitoes, backswimmers have been reported to attack later instars (Fischer *et al.,* 2012).

In the current study, it was established that the only predator that could colonize hoof-prints and manmade pools belong to the Order Hemiptera. This successful colonization of isolated pools by this group could most likely be associated with the predator mobility by flight and tolerance to a wide range of environmental pressures (Sites, Nichols, & Permkam, 1997). It is well known that notonectids are largely mobile, voracious of mosquito larvae and have the potential to alter mosquito communities via direct or indirect effects (Blaustein *et al.,* 2004), through a reduction in oviposition by adult mosquitoes (Eitam *et al.,* 2002). The direct effects occur primarily through predation. Laboratory and field experiments demonstrated that notonectids may disrupt mosquito egg rafts, though evidence of a reduction in subsequent hatching success was not observed (Shaalan & Canyon, 2009). Notonectids dominance on predation of mosquito larvae has been largely appreciated. Unlike in other studies, the current study found Hemiptera invading cattle hoof-prints. Studies by Service (1985), Wondji *et al.,* (2005) and Coetzee *et al.,* (2005) reported that predators mainly visit swamps habitats with grassy patches which mosquito prefer to oviposit to protect their immatures. It was shown in these studies that they avoid open sunlit habitats.

Other studies have also shown that species of *Vellidae and Gerridae* (Miura & Takahashi, 1988), both of which are semi-aquatic, prey on mosquito larvae on the water surface while some like Belostomatidae, Naucoridae and Nepidae are capable of preying on mosquito larvae in the laboratory (Shahayaraj & Sathiamoorthi, 2002), a strong indicator that some of the predators along the Mara River could actually be controlling the mosquito population.

However, some ecological factors may also influence the abundance of predator and mosquito populations. For instance, a simulation study of wind speed was found to be particularly important in the movement of mosquitoes in Papua New Guinea. Wind speed of between 36 and 72 km/h were sufficient to carry mosquitoes from New Guinea to the Northern Peninsula of Australia (Ritchie & Rochester, 2001). However, reduced wind velocity was also shown to be important as it allowed female mosquitoes to deposit their eggs in artificial containers accurately without disturbance (Service, 1971).

In the ordination analysis results, factoring in all the variables showed that in shared habitats, turbidity, conductivity and salinity had indirect influence on mosquito larvae and predator abundance, while dissolved oxygen and temperature had direct influence on mosquito larvae and predator abundance, supporting the concept that within aquatic habitats macro-invertebrates can be sensitive to factors affecting water quality. Previous studies reported that thermal pollution, pesticides and organic compounds may alter the water physico-chemical parameters and thus interfere with aquatic invertebrate diversity and composition (Zimmerman, 1993). This may also partially explain the abundance of Hemiptera, as compared to the two other aquatic insect orders.

5.3. Characterization of the Different Mosquito Breeding Habitats and Determination of their Preference by Larval mosquitoes

Mosquito larval habitats are the locations where many important mosquito life-cycle processes like oviposition, larval development, and adult emergence, resting, swarming and mating take place (Karthikairaj, Ravichandran, & Sevarkodiyone, 2013). Mosquito larvae were found in isolated pools of the receding waters or in temporary habitats near the Mara River or along the perennial Mara River tributaries of Amala and Nyangores. Most habitats found along the Mara River were characterized by various types of vegetation, which in turn offered a variety of microhabitats for the larval mosquitoes.

Vegetation type was an important factor for both *Anopheles* and *Culex* larvae presence and abundance in the respective habitats. Similar observation was reported for *An. gambiae* larvae (Mwangangi *et al.*, 2008). The presence of vegetation could help the larvae to hide themselves away from their predators. Abundance of *Anopheles* and *Culex* larvae were higher with presence of aquatic fauna. However, existence of favorable environment for various aquatic fauna was also observed. For instance, drying stream, open puddles and drainages showed the highest abundance of *Culex* and *Anopheles* spp. and the least with rock pools. Although soil type may also have effects on mosquito distribution and abundance, it was not analyzed in this study, however, both current and previous reports indicated there could be variations in development of *Anopheles* larvae based on soil types (Lindh *et al.*, 2015; Pfaehler *et al.*, 2006).

Most mosquito larvae in the drying stream were found in isolated pools of the receding water body or in temporary habitats near the Mara River or on the drying sections of Amala and Nyangores tributaries, which are the two main tributaries of Mara River on the Kenyan side of the basin. There were several patches of open sunlit habitats adjacent the main Mara River in which the *An. gambiae* s.l., *Culex* spp. and *An. maculipalpis* dominated. However, in all the cases of abundance, the malaria transmitting vectors, *A. gambiae* s.l was the most dominant. *An. funestus* larvae dominated swamps and were few in rock pools, while, *An. pharoensis, An. azamiae, An. christyi, An. maculipalpis, An. hamoni* and *An. sergeti* were also recorded in open sunlit puddles and drainages. In other studies, *An. gambiae* s.l. have also been reported in high numbers either on temporary sunlit pools or open habitats with scattered short grass (Fillinger & Lindsay, 2011; Minakawa *et al.*, 1999; Ofulla *et al.*, 2013).

This variability in species abundance could be attributed to local ecological differences. For instance, at site 4, the habitats were mainly rock pools created as a result of water currents, which hits the riverbanks and splashes out to fill the pocket-like rocks. The water in these habitats are often clear and shielded from direct sunlight by vegetations, and this could be the reason as to why they were colonized by the *An. funestus* group larvae. Such shaded micro-habitats with stilth water are preferred by the group (Minakawa *et al.*, 2008). It is thus recommended that these habitats be closely monitored if the risk of malaria transmissions is to be reduced among the riparian communities within the Mara River basin.

In these areas, larval sampling indicated that swamps were more potential habitats for both *An. gambiae* s.l. and *An. funestus* group. The Mara River is perennial and flows all year round, with levels of the water fluctuating during dry and rainy seasons (Serneels & Lambin, 2001). As a result, small pools of water are present by the riverside during the rainy season, which dry as the amount of rainfall decreases. On the contrary, stream water of its tributaries becomes stable with reduced flow rate, actually drying up. The stable flow of the streams during dry period appears to support more larval mosquitoes along the river basin than rainy period. However, this has never been proved.

Studies have established that mosquito colonization of a habit depends on a number of factors and these factors may vary depending on mosquito species. In West Africa, for example, a closely related species of *An. gambiae* s.s (M and S form), initially orientating as same species, were reported to inhabit completely different habitats. For instance, in Mali M form was found to dominate open sunlit puddles while S form were mainly found in swamps and long vegetated pools (Edillo *et al.,* 2006). Previous studies reported the presence of *Anopheles* species concomitantly in open sunlit puddles, drainages, artificial containers and many other open habitats known to be free of predators (Coetzee *et al.,* 2000; Service, 1985), and where the water temperatures are ambient (Minakawa *et al.,* 1999). *An. funestus* group on the other hand have been reported to inhabit vegetated pools, mainly permanent or semi-permanent habitats such as rice irrigation schemes, wetlands, and river edges with short vegetation that can provide shade (Fillinger *et al.,* 2004; Minakawa *et al.,* 2012).

Habitats that had clear water and were shielded from direct sunlight presented perfect breeding grounds for larval mosquitoes as evidenced by the abundance of larval mosquitoes in these habitats. On terrestrial habitats, open sunlit puddles were found to harbor more mosquitoes as compared to roadside ponds with vegetated habitats. Consistent with the current findings, behavioral avoidance patterns of standing waters characterized with vegetation by *An. gambie* were also reported by Mutuku *et al.* (2006). In the river habitats, more larval mosquitoes were found in slow flowing streams and river-beds with little vegetation as compared to open water, thus an indication that aquatic vegetation plays an important role in harboring these malaria transmitting vectors. The stable flows of the stream during dry period appear to support more mosquitoes along the river tributary streams than during the rainy seasons. However, more seasonality studies may be required to determine trend and density and to provide proof for this speculation. This study has also shown that *Culex* spp. were the most widespread mosquito larvae along the Mara River basin as they were collected from a variety of habitats. This is a clear indication that *Culicine* spp. larvae have a great degree of adaptability to different habitats compared to other mosquitoes. The presence and wide distribution of *Anopheles* spp.; the vector of human malaria constitutes a major potential health problem. Further studies on the vectorial capacity of these disease pathogen vectors are required and every effort should be made to prevent their spread within the Mara River basin. The results of this study also showed that most mosquito larvae could survive well in neutral or slightly alkaline aquatic habitats. Similar results were also reported by Afrane *et al.* (2006).

In the current study, the main Mara River, with riffles and pools and characterized by fast flowing waters had no mosquitoes. However, large swamps with tall emergent vegetation adjacent the Mara River were found to habor only *An. coustani*, while short emergent vegetations such as short grass and sedge harbored *An. gabbiae* s.l., *Culex* and *An. funestus* group. The many habitats adjacent to the main river either created through human activities such as brick making or animal trampling especially at watering points appeared to harbor most malaria transmitting vector of the *Anopheles gambiae* s.l. and *Culex* species. The receding river and stream tributaries water levels caused by the destruction of forests, rock pools which initially were below the water surface especially during dry spells, are becoming potential breeding micro habitats for *An. gambiae* s.l. and *An. funestus* group. Therefore, these conditions are potentially improving the habitat diversity for these vectors, which are good indicators of the health of riverine ecosystem. Open sunlit puddles, rock pools and drains, which produced high numbers of mosquitoes, were shallow, isolated and tended to limit predator access. Such habitats then presented perfect-breeding sites for potentially harmful mosquito species some of which are known carriers of malaria parasites.

The Shannon diversity index was slightly higher for terrestrial habitats compared to river edge habitats, though both were still low considering that the typical value of the index ranges from 1.5 (low species eveness and richness) to 3.5 (high species evenness and richness) (Magurran, 2005) however, values beyond these limits upto a maximum of 5 may also be encountered. The evenness index was higher in terrestrial habitats than river edge habitats, reflecting a variation in abundance of mosquito species between the two sites, along the Mara River. Considering that similar proportions of all sub-species give an evenness index of one, with higher values reflecting very dissimilar proportions (some rare and some common species), it is apparent that mosquito sub species were clearly dismal as reflected by the dominance of *Anopheles gambie* s.l and *Culex* spp. over other mosquito species in both habitats. This could be an indication that some species are better adapted to the sampled habitats than others.

Overall, the indices of the mosquitoe species along the Mara River indicate diversity index value (H) of 1.43 for terrestrial habitats while river edge habitats had an index of 2.17 and the differences were not statistically significant. This was not satisfactory since ‘H’ value above 3 indicates a better aquatic balance and stable ecosystem (Koller *et al.,* 1996). However, evenness aspect varied significantly suggesting that with increasing sample size a population outburst is imminent in the Mara River ecosystem. In addition, a steady increase in the evenness as mosquito density increase as shown in the cumulative indices suggests that the ecosystem can be a function of many hiden factors. Hagy *et al.* (2011) reported that high variability of population during sampling period can be a subject of many factors such as normal die off, hatching of eggs, cannibalism, re-emergence, patterns of predation, local movements and uneven distribution, this tentatively oviates the necessity of carrying out a longitudinal study to in order determine the seasonal accumulative indices of these parameters over time.

Some organisms are more chemical-tolerant than others, and aquatic insects are sensitive to change of the environment. For instance, spraying of pesticides in the agricultural fields along the river channel has been reported by Gereta *et al.* (2003). Another factor could be competitive advantage, as some predators are more adaptive than others. Analysis of the data established significant differences between predators' density and habitat types. For instance, they were more likely to be captured in drying streams, swamps, vegetated pools and puddles. Such relationships could be attributed to the fact that these habitats harbored a high number of different species as compered to other habitats, which were located in the terrestrial sites. The terrestrial habitats mainly comprised open sunlit puddles that contained mostly the order Hemiptera and few Coleopterans, while habitats adjacent to the river contained many other species. Also, habitats adjacent to the river mainly comprised of vegetated habitats of which some species colonized to avoid risk of competition since they are unable to withstand environmental instability (Sites *et al.,* 1997). Furthermore, open habitats with water on land appeared to contain dirty water. Future studies need to be carried out to establish the tolerability of the aquatic predators to turbidity or water transparency.

Only members of Hemiptera were found in a few terrestrial habitats. Habitats on land are often shallow and could only retain water for short periods of time and as such they exhibited different patterns of population growth because of the effects of different environmental variables such as temperature, water levels fluctuations and life cycle strategies (Williams & Hynes, 1974). Previous studies reported that thermal pollution, pesticides and organic compounds may alter the water physico-chemical parameters and thus interfere with aquatic invertebrate diversity and composition (Hilsenhoff, 1988). This may also partially explain the abundance of Hemiptera, as compared to other two aquatic insect orders (Odonata and Coleoptera). Most of the insects in the Order Hemiptera have been acclaimed as pollution-tolerant (Joshi, 2012), and their population was found to be higher than any other order along the Mara River. Other known sensitive taxa such as Plecoptera were completely absent from all the sites along Mara River, suggesting that the waters were polluted.

In the present study, it was observed that despite the abundance of sunlit open water bodies, predators' density were low, especially in terrestrial habitats, whereas in the river fed pools with vegetations, the insect density was much higher. This suggest that the temporary water pools without vegetation might be the primary factor influencing the population dynamics of aquatic insects in all situations, especially for adventurous groups like the Hemipterans.

Attempts were made to establish correlations between larval mosquitoes and predators in the shared habitats using regression matrix, so that scatter plot could deduce valuable information on how the relationship between the two variables would complement. There was no particular pattern of relationship observed between the two variables, however higher numbers of predators were captured in habitats with lower densities of mosquitoes. This suggests that predators might have found these habitats suitable for them to inhabit and feed on larval mosquitoes. Similarly, Service, (1971), in their earlir studies also attempted to understand the differences in density of larval mosquitoes against their predators, they compared the voracity of various types of predators by counting the number of larval mosquitoes consumed against the number of adults that emerged, their overall aim was to try to quantify the potential of aquatic predators of mosquito larvae. They established that some mosquito predators feed more than others and even prefer larger instars.

Furthermore, it was observed in the current study that habitats with higher numbers of predators had larval mosquitoes of between 3rd and 4th instars, while habitats with higher number of larval mosquitoes but low number of predators had 2nd and 1st instar larval mosquitoes. Previous studies experimental studies showed that many predators prefer a particular developmental stage of larval mosquitoes. Our findings are thus consistent with that of Service (1971) who also observed that 3rd and 4th instar larvae of *Culex pipiens* s.l were preferred by predator of the order Odonata. Similarlay, in the Mara River it was observed that backswimmers dominated habitats with higher numbers of late instar larval mosquitoes, suggesting that could have been feeding on the older instars of larval mosquitoes.

5.4. Relationship between Water Physico-Chemical Parameters and the Presence, Distribution and Abundance of Mosquito Larvae and their Predators along the Mara River Basin

Mosquito larvae and predators are aquatic, and often share habitat with other species including predators. However, it has never been clear on how water physico-chemical parameters regulate mosquito and predator's populations. Mosquito populations are declining globally due to many factors including habitat destruction, interventions and climate change. It influences mosquito survivorship, for example, by reducing the rate at which larval mosquitoes lay their eggs or through direct predation of mosquito larvae, then the the role of water physico-chemical parameters may decline or increase mosquito populations.

In the current study, most mosquito larvae were collected from water accumulations with different degrees of turbidity. Post & Kwon (2000) attributed the favorable effect of sunlight on mosquito larval population to the requirement of algae to sunlight. These algae are frequently favorable as larval food and also aided in maintaining the balance of dissolved gases and in utilizing organic materials unfavorable to the larvae. Gouagna *et al.* (2012) however reported that turbidity had no significant effect on *Culex* spp. larvae; though habitats that were shaded, vegetated and had stagnant water were generally preferred for mosquito breeding.

Culex spp. have been reported as having a wide range of habitat preference and can breed in stagnated waters polluted or unpolluted. For instance, they have been found to breed in toilets, sewerages, containers, pits, ponds and many other habitats known to be unsuitable for breeding by the members of *Anopheles* (Vinogradova *et al.,* 2007). The *An. gambiae* complex and *An.*

funestus s.s prefer clean unpolluted waters and are never found in habitats contaminated with faeces or containing dead plants and foul smell (Gillies & Coetzee, 1987). However, both *Anopheles* and *Culex* spp. are influenced by physical and chemical parameters such as turbidity, temperature, alkalinity, pH, dissolved oxygen, nitrate, hardness, water current and vegetation types among others. All these actors have an overall effect on the quality of breeding habitats; though few are found to be important for specific species (Muirhead-Thomson, 1951). In a review by White *et al.* (2011) for instance, *An. arabiensis* was reported to breed in habitats with alluvial deposits while *An. gambiae* s.s. was found in brackish waters with modest salinity. In Mali, Diuk-Wasser *et al.* (2007) found higher densities of *An. gambiae* s.s in rice irrigation scheme with moderate equivalent of light penetration and shade during the initial period of rice germination, whilst *An. funestus* s.s. dominated area when the rice fully germinated and provided shade and thus overtaking the *An. gambiae* group. This suggests that larval mosquitoes generally prefer differing range of chemicals (Ye-Ebiyo *et al.,* 2000) as well as site characteristics (Fillinger *et al.,* 2004; Minakawa *et al.,* 2005).

The influence of vegetation on certain mosquito larvae species is debatable since the mosquito larvae may also be influenced by other factors such as light penetration and water temperature (Knight *et al.,* 2003). A previous study (Ye-Ebiyo *et al.,* 2000) reported that invasion of vegetation could also be due to availability of aquatic food sources. Proximity to maize was found to enhance development of *An. arabiensis* in Ethiopia, while in Kenya, Minakawa *et al.* (1999) and Fillinger *et al.* (2004) established that both artificial and natural habitats were preferred by the members of both *An. gambiae* s.s and *An. arabiensis* at equal measure. Minakawa *et al.* (2005) on the other hand reported that members of *An. gambiae* mainly preferred breeding in burrows of farms and animal gazing lands while, *An. funestus* preferred short-grass vegetated pools, swamps and grazing lands (Minakawa *et al.*, 2005; 2012). These results are also in line with the findings of (Kasangaki *et al.*, 2008), which reported that clearance of forests was endangering freshwater eco-systems in East Africa.

Removal of riparian vegetation has also been reported to modify stream hydraulics, substrate features, light and thermal system, water chemistry composition and organic matter contribution, all of which affect the riverine communities (Pusey & Arthington, 2003). Based on the findings of this study, the two most important factors found to influence the abundance and distribution of different mosquito species within the Mara River basin were habitat type and water chemistry. Ecological disturbance resulting from altered land use at the highland regions was initially reported as a possible cause for the puzzling increase in highland malaria (Imbahale *et al.*, 2011; Mutie *et al.*, 2006). Although larval abundance is only one factor influencing subsequent vector-biting rate and malaria transmission, reductions in malaria cases have been observed after large-scale implementation of larval control initiatives (Fillinger *et al.*, 2004).

Both anopheline and culicine larvae were positively associated with dissolved oxygen. Previous reports also indicated similar association between *Culex quinquefasciatus* and *Anopheles arabiensis* larvae with dissolved oxygen (Minakawa *et al.*, 2005). Oyewole *et al.* (2010), concurred that optimum dissolved oxygen might have contributed to the survival and breeding of *Anopheles* larvae in the Mara River. It has also been observed that dissolved oxygen saturation decreases when the bed sediment changes from stony substratum to soft sediments. Human settlements, urbanization and other pressures have been reported to influence changes in water chemistry as well as the reduction in dissolved oxygen levels (Ndaruga *et al.,* 2004).

The mosquitoe larvae sampled did show a significant association with water turbidity. Consistent with the current study findings, Kenawy *et al.* (2013) also showed that some larval mosquitoes prefer turbid water than clear water for oviposition. Critical to this study, the coeffient of turbidity was negative indicating the larval mosquitoes and predators preferred clean water. For the case of the previous study, this could be due to the fact that during the rainy season, *Anopheles* spp. seem to inhabit turbid waters, but during the dry season, when the water is relatively clear they still exist in the clear water, thus an indication that they can survive in both clear and turbid waters. However, in a separate GLM model in the current study, *Culex* spp. was shown to be influenced by turbidity. Previous studies have reported *Culex* spp. to survive better in turbid waters than the *Anopheles* spp. (Wang-Sattler *et al.,* 2007).

The finding of this study also suggests that both biotic (flora and fauna) and abiotic (chemical and physical) factors play a significant role in larval habitat preference by both *Culex* spp. and *Anopheles* spp. Thus, such factors should be taken into consideration when designing an integrated vector control program. Further longitudinal study of the aquatic mosquito larvae breeding habitats and non-breeding habitats are recommended; including all biotic and abiotic variables using accurate quantitative measurements. Abundance of *Culex* spp. and *Anopheles* spp. larval mosquitoes showed positive association with conductivity. As conductivity is the measure of the dissolved ions in water, there was no justification as to why conductivity was positively related with abundance of larval mosquitoes. However, unlike in the current study, Dejenie *et al.*

(2011) reported a negative association between conductivity and *Culex quinquefasciatus* larvae presence in tigray microdams in Ethiopia.

In this study, temperatures, dissolved oxygen and turbidity were found to be important determinants of predator abundance. The temperature recorded in the current study ranged between 18.0°C and 26.3°C, thus can be described as warm and more likely to support most of the predators especially the notonectids. Earlier studies showed that thermal conditions are especially important in predator–prey survival among aquatic organisms (Bertram, 1996), especially those that are involved in size-dependent predation. However, while much research has quantified the physiological effects of temperature on specific organisms, few studies have been conducted to evaluate the effect of temperature on species interactions in field conditions. In support of the current study, Paaijima *et al.* (2008) and Couret *et al.*, (2014) agree that indeed temperature and dissolved oxygen are important for larval mosquito development. However, Minakawa *et al.* (1999) argue that only combined effects of the physico-chemical parameters can influence mosquito abundance.

The pH was largely basic in all habitat types except for the swamps, which had near neutral pH. Alkalinity levels were equally high ranging between 100 and 400 mg/L. This pH range has been reported as optimal for most aquatic biota including mosquito larvae predators. Nevertheless, other findings agree with the positive association of mosquito larvae and other aquatic insects under a wide range of pH values (pH 5.86 – 9.85) (Adebote *et al.*, 2008). Earlier studies have also established correlations between temperatures and pH (Opoku & Amoako, 2002).

Mosquito larvae and predators share the same habitats and establishing the role the pH plays in the regulation of colonization is critical. Even though there was a range in pH requirement by mosquito larvae and predators in shared habitats, both mosquito larvae and predators were not affected by pH in a GLM model. This suggests that under the prevailing environmental conditions, both insects could tolerate a wide range of pH. Further analyses to determine preferable pH range requirement by both mosquito larvae and predators established that values between 5.2 and 8.4 were tolerable while values between 8.1 and 8.4 were most preferred, as evidenced by the highest number of both mosquito larvae and predators.

Similarly, a study by Dejenie *et al.* (2011) on malaria vector control in Ethiopia showed that almost all their study habitats were alkaline (pH >7) and both Anopheline and Culicine larvae were positively associated with this high (>7.0) pH. The current study thus is in agreement with the study of Dejenie *et al.* (2011) but do not support the findings of Adebote *et al.* (2008), which reported the preference of Anopheline species in low pH values.

Along the Mara River, the mean turbidity was highest in rock pools, while the lowest level was recorded in swamps and drainages. The findings showed that turbidity levels across all sampled sites were exceedingly high. This scenario could be as a result of increased particulate matter such as clay, silt, organic matter, plankton and other microscopic organisms, which have been reported to interfere with the passage of light through water (Sadar, 2004). The increased particulate matter could have been contributed by anthropogenic activities such as deforestation, riverbank cultivation, soil erosion (due to overgrazing among others), all occurring in the watershed. In addition, urbanization facilitates transportation of waste into the river channel through increased run-offs, while livestock trampling effect at watering points and along the riverbanks also contributes significantly to high turbidity levels of surface waters. All these activities can create suitable habitats for larval mosquitoes as was previously reported by Klinkenberg *et al.,* (2008).

A habitable aquatic ecosystem requires a good supply of dissolved oxygen in the water system (Hsieh *et al.,* 2015). Along the Mara River basin, the mean dissolved oxygen was highest in the river followed by rock pools, while the lowest was recorded in swamps. A significant difference in mean dissolved oxygen was observed among the different habitat types. Faster flowing sections of rivers and drying stream and sections that flow through riffles or small waterfalls have better oxygenated waters than slow flowing sections of rivers or rivers that have been modified as straight channels. Dissolved oxygen concentrations in water are dependent on physical, chemical, biological and microbial processes. Low dissolved oxygen concentrations (<3mg/L) in fresh water ecosystems are indicative of high pollution levels (Okbah *et al.,* 2013). However, in the current study, some aquatic habitats recorded dissolved oxygen levels insufficient to support aquatic life. Analysis to determine preferable level of dissolved oxygen range required by both mosquito larvae and predators in the shared habitat indicated that, values ranging between 6.0 mg/L, and 6.5 mg/L were most preferred. However, some mosquito larvae were found in water samples with dissolved oxygen concentration a low as 2.3 mg/L. The most common cause of low oxygen levels was the off-load of organic material into the water system (such as agricultural run-offs). Nevertheless, more mosquito larvae were collected in slow-flowing drying stream and swamps where the mean oxygen was relatively low. This may suggest that some predators could be less likely to survive in polluted waters without sufficient oxygen.

The majority of *Anopheles* and *Culex* spp. larvae were found inhabiting pools adjacent to the Mara River created by receding river waters, some of which had relatively high dissolved oxygen levels. These findings were consistent with those of Dejenie *et al.* (2011) which also reported that both Anopheline and Culicine larvae were positively associated with dissolved oxygen. Studies by Muturi et al. (2008) also indicated similar association of *Anopheles* spp. larvae and other mosquito larvae with dissolved oxygen. Likewise, Oyewole *et al.,* (2010) emphasized that optimum dissolved oxygen is superlative to the survival of the *Anopheles* larvae. Water hardness is usually a result of the presence of multivalent metal from minerals dissolved in the water. In the aquatic environment, ions result from abundance of Calcium and Magnesium in water. The highest mean hardness was recorded in the drainages, while the lowest were recorded in dams and swamps. A correlation matrix established that there was a positive correlation between mosquito larvae and predators in the presence of hardness. However, a negative correlation was observed between hardness and predators in the shared habitats suggesting that most predators require lower water hardness levels to survive in the habitat.

Analysis to determine the preferable level of hardness range requirement by both mosquito larvae and predators in the shared habitats indicated that values ranging between 58.5mg/L and 372.0mg/L, were favorable. The wide range of water hardness observed could be due to differences in buffering capacity of the waters across habitat types, as hardness values are not consistent across the basin. Elevated values in some areas could be due to sewer supply from the nearby towns or spills of fertilizer from the nearby farms. Other established sources could be the local geology (Lawrence, 2007). However, CCA results revealed that insects would prefer a varied range in hardness. Few insects showed preference for specific hardness values. It was also of interest to note that along the Mara River, most aquatic habitats had meagre detectable level of salinity. Only swamps recorded salinity level of 0.4mg/L. However, the influence of salinity along the Mara River could not be statistically evaluated as a result of insufficient sample numbers.

In the current study, rock pools, dams and drying stream recorded the highest mean conductivity, while swamps and drainages had the lowest conductivity values. For both mosquito larvae and predators, a perfect linear requirement with conductivity in the same habitat was demonstrated within the ranges of between 162.9µS/cm to166µS/cm by both mosquito larvae and predator residing in the same habitats. The high levels were due to elevated dissolved solids and contaminants especially electrolytes. Potential sources of these contaminants are destruction of the forest cover (which in the process, increase the litters) and human activities experienced along the river channel (that creates drainages and pools suitable for mosquito breeding). Mutie *et al.*, (2006) reported an increased destruction of the upper catchment of the Mau forest and elevated level of pollution, attributable to high levels of wastewater discharged into the river from different origins.

Previously, dissolved oxygen, temperature and conductivity were reported to positively correlate with community structure as a whole (Spieles & Mitsch, 1999). In the current study, no direct relationship was detected between conductivity and predator abundance in the GLM model, however, there was a positive insignificant relationship between conductivity, predators and mosquito population in the ordination analysis, pointing to the direction of the establishe result that limited range of conductivity levels is prefferable by the mosquito and predators' population.

The conductivity of a river or stream should remain within a specified range to allow for a succesful biologically functional system. Changes in conductivity are often used as water pollution indicator. Urban run-offs and industrial pollution are often characterized by high conductivity.

The rate at which a mosquito larva develops also dependents on the prevailing temperature, development of *An. gambiae* s.l. mosquito larvae ceases at temperatures below 16°C and below 14°C they die. Paaijmans *et al.,* (2008) and Couret *et al.,* (2014) also reported that temperature affects the rate of larval development, while Tuno (2005) reported that high temperatures influence pupation rates as well as larval survivorship. Larval-to-adult survivorship and larval-to-adult development time were also reported to be influenced by temperature by Afrane et al (2006).

In a canonical correspondence analysis, which assessed the contribution, it was noted that each of the response variables with the physico-chemical parameters, (conductivity, pH, hardness, salinity and turbidity) were less likely to influence predators' abundance while temperature, dissolved oxygen and presence of mosquito larvae were shown to be the predictors of predators' abundance. The ordination results from canonical correspondence analysis revealed the strongest variables that influenced the existence of predators and mosquito larvae in shared habitat that may aid in the effective biological control of larval mosquitoes. Anderson (2001), concur that ordination primarily endeavours to represent sample and species relationships as faithfully as possible in order to choose precisely which tool is necessary for immediate use. Predator abundance was strongly positively correlated with the increasing number of larval mosquitoes, suggesting that carefully selected predators may play a noble role in controlling larval mosquitoes as compared to the water chemical parameters. Dissolved oxygen and conductivity were also reported to correlate positively with community structure as a whole (Spieles & Mitsch, 1999). In the current study, no relationship was detected between conductivity and predator abundance in the GLM model, but a positive correlation analysis suggested that conductivity may or may not be an important factor for mosquito predator population depending on range requirement by specific group or orders of the predators. Previous studies have also established correlations with temperature and pH (Adebote *et al.,* 2008).

Nevertheless, more larval mosquitoes were collected in slow flowing streams and swamps where the mean oxygen was relatively low (2.4±2.7 mg/L), suggesting that some predators could be less likely to survive in polluted waters without sufficient oxygen. The majority of insects recorded in these habitats were mainly of order Hemiptera. It was established that turbidity had an effect on predator abundance in the current study. However, in the ordination analysis results factoring in all the variables showed that in shared habitats with both larval mosquitoes and predators' turbidity, conductivity and salinity had an indirect influence over the larval mosquitoes and predators' abundance, while dissolved oxygen and temperature had a direct influence. This further proves that in any aquatic habitats, invertebrates can be sensitive to factors affecting water quality. Previous studies reported that thermal pollution, pesticides and organic compounds may alter the water physio-chemical parameters and thus interfere with aquatic invertebrate diversity and composition (Szczytko & Dimick, 2005). This may also partially explain the abundance of Hemipterans, as compared to the other two aquatic insect orders; Odonata and Coleoptera.

Most of the insects in the order Hemiptera have been acclaimed as pollution tolerant (Mahavidyalaya, 2012), and their population found to be higher than any other order in the Mara River basin. Besides, other known sensitive taxa such as Plecoptera were completely absent from all the sites along the Mara River, which may suggest that the waters were polluted.

In the current study, a significant proportion of *Anopheles* spp. and other mosquito larvae were found inhabiting pools adjacent the Mara River created by receding Mara River waters some of which had relatively high dissolved oxygen levels. These findings were consistent with those of Dejenie *et al.* (2011) which also reported that both anopheline and culicine larvae were positively associated with dissolved oxygen. Studies by (Muturi et al., 2008) also indicated similar association of *Anopheles* spp. larvae and other larval mosquitoes with dissolved oxygen. Likewise Oyewole *et al.,* (2010) supported the idea that optimum dissolved oxygen might have contributed to the survival and breeding of *Anopheles* larvae.

The relatively low predator numbers in mosquito habitats observed in the current study indicated that, as earlier reported, adult larval mosquitoes may have the ability to detect the presence of predators and consequently avoid ovipositing in such habitats preferring instead to inhabit areas free of predators (Ohba, 2011). Previously, mosquitoes of the genus *Culiseta longireolata* were reported to detect chemicals from Notonecta predators, and the instinct/cue can exist in the habitat for up to a week or more after their disappearance from the pool (Blaustein *et al.,* 2004) and for *Culex* spp., this period was as low as two days (Blaustein *et al.,* 2005). Furthermore, it was expected that with increasing predator densities, the concentration of kairomones would increase and this may result in reduced oviposition by larval mosquitoes (Blaustein *et al.,* 2004). However, in the current study, we reliably noted in our multi-correlation matrix that majority of predators were bonded to where there were lower densities of larval mosquitoes, suggesting in addition to the already known theory of predator avoidance and the presence of kairomones, that higher number of predators and less larval mosquitoes could also be as a result of direct predation. It was therefore reasonable to expect less larval mosquitoes in habitats with higher number of predators and vice versa. Other factors that have previously been reported to play an important role in habitat selection by various species of larval mosquitoes are volatile compounds produced by microbial population in the breeding sites (Sumba *et al.,* 2014), chlorophyll-a content in the breeding sites (Mwangangi *et al.,* 2008) or the presence of conspecific larvae or aquatic predators (Minakawa *et al.,* 2005).

5.4.1. Biplot on the Overall Effects of Physico-Chemical Parameters and Larval mosquitoes on Predators' Abundance

Multivariate ordinations generally described connectivity among parameters in the Mara River. The results of ordination analyses of all the 9 variables from canonical correspondence analysis (CCA) indicated the strong variables that influenced the existence of predators as a factor that may aid in the effective biological control of larval mosquitoes. As explained by Clarke (1992), ordination primarily endeavors to represent sample and species relationships as faithfully as possible in order to choose pricely which tool is necessary for immediate use. Predators' abundance was strongly positively correlated with the increasing numbers of larval mosquitoes; suggesting that predators may play a noble role in controlling larval mosquitoes, therefore future studies should consider them.

CHAPTER SIX: SUMMARY OF FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

6.1. Summary of Findings

The findings reported herein provide new information on the presence of mosquito larvae and their predators within the Mara River and its tributaries. Some of these predatory species have been evaluated as bio-control agents in the worldwide campaign to control mosquito larvae. The results of this study have shown that mosquito larvae of different species are widely distributed in the Mara River, and more interestingly, they can survive in either neutral or slightly alkaline water habitats.

The main river, with open water bodies, steep edges, fast flowing water and little emergent vegetation had no larval mosquitoes. The many habitats adjacent to the main river either created through human activities such as brick making or animal watering points appeared to harbor most mosquito larvael. The receding river water body caused by the destruction of forests leaves bare rocks which initially were below the water surface, becoming potential breeding habitats for both and Anophiline and Culicine spp. Therefore, these conditions are potentially improving the habitat diversity for these larvae which are good indicators of the health of riverine ecosystem. The current study confirmed that several breeding sites occur along the Mara River basin. Among these sites, drying streams harbour a variety of microhabitats and large number mosquito larvae.

6.2. Conclusions

1. Presence, abundance and distribution of malaria and non-malaria transmitting mosquito larvae, were confirmed in the study area.
2. The three Orders; Hemiptera, Odonata and Coleoptera were present and uniformly distributed, with the Order Hemiptera were dominating the Mara River basin.
3. Drying stream accounted for the majority of mosquito larvae and their predators.
4. Relationship between Dissolved Oxygen (DO), temperature, turbidity and mosquito larvae and their predators was observed in Mara River basin.

6.3. Recommendations from Current Study

1. Presence of malaria and non-malaria mosquito larvae on the Mara River calls for their immediate control and education among the locals that can help curtail the insurgent of vector-borne diseases within the Mara River Basin.
2. Identification of mosquito larvae predators within the Mara River is an important finding since some of these predatory species have been evaluated as bio-control agents worldwide in campaign to control malaria vectors and may be usefull locally for control the larval mosquitoes.
3. Vector control program should be emphasized during dry period, targeting drying streams, shown to produce high number of larval mosquitoes.
4. Findings suggest that specific abiotic factors plays a significant role in the abundance and distribution of larval mosquitoes and their predators, these factors could be manipulated to enable effective design of a biolocally integrated vector control program with the Mara River basin.

6.4. Recomendations for Future Research

1. There is need to map out mosquito larvae hotspots within the Mara River basin so as to inform policy on vecorborne diseases eradication programme on the areas that need to be targeted most for effective mosquito control.
2. There is need to carry out a longitudinal study on mosquito larvae and their predators within the Mara River basin so as to elucidate the variations with respect to seasonality.
3. There is need for longitudinal study that can further elucidate the relationship between mosquito larvae, their predators and phyco-chemical paramers. This would reveal the temperal abundance and distribution of mosquito larvae and their predators, crucial for disease vector control on the Mara River.

REFERENCES

Abdelbasit, R., & Fadlelmola, K. (2009). Factors Affecting Oviposition and Larval Development of *Anopheles arabiensis* in East Nile Locality - Khartoum State. Masters thesis. The Graduate College Medical and Health Studies.University of Khartoum, Ethiopia.

A Abdulla, S., Schellenberg, J. A., Nathan, R., Mukasa, O., Marchant, T., Smith, T., Tanner, M., Lengeler, C. (2001). Impact on malaria morbidity of a programme supplying insecticide treated nets in children aged under 2 years in Tanzania : community cross sectional study. *British Medical Journal, 322*(2), 270–273.

Adebote, D. A., Oniye, S. J., & Muhammed, Y. A. (2008). Studies on mosquitoes breeding in rock pools on inselbergs around Zaria, northern Nigeria. *Journal of Vector Borne Diseases*, *45*(1), 21–28.

Aditya, G., Pramanik, M. K., & Saha, G. K. (2006). Larval habitats and species composition of mosquitoes in Darjeeling Himalayas, India. *Journal of Vector Borne Diseases, 43*(1), 7–15.

Afrane, Y. A., Zhou, G., Lawson, B. W., Githeko, A. K., & Yan, G. (2006). Effects of microclimatic changes caused by deforestation on the survivorship and reproductive fitness of Anopheles gambiae in western Kenya highlands. *American Journal of Tropical Medicine and Hygiene*, *74*(5), 772–778. http://doi.org/74/5/772.

Alphey, L., & Alphey, N. (2014). Five things to know about genetically modified (GM) insects for vector control. *PLoS Pathogens*, *10*(3), e1003909.

Anderson, M. T., Kiesecker, J. M., Chivers, D. P., & Blaustein, A. R. (2001). The direct and indirect effects of temperature on a predator–prey relationship. *Canadian Journal of Zoology*, *79*, 1834–1841. doi:10.1139/z01-158.

Anyona, D. N., Abuom, P. O., Dida, G. O., Gelder, F. B., Jackson, O., Matano, A., Kanangire, C. K., Bosire, E. K., . Oindo, B. O., Owuor, P. O., Ofulla, A. V. O. (2014). Effect of Anthropogenic Activities on Physico- chemical Parameters and Benthic s of Mara River Tributaries, Kenya. *Merit Research Journal, of Environmental Science and Toxicology 2*(5), 98–109.

Atwood, T., & Richardson, J. S. (2012). Trophic interactions between insects and stream-associated amphibians in steep, cobble-bottom streams of the Pacific Coast of North America. *Insects*, *3*(2), 432–441. http://doi.org/10.3390/insects3020432.

Baird, M. A., Nj, D., Abernethy, N., & James, D. (1995). Dendritic cell presentation of PPD and 19 kDa protein of *Mycobacterium tuberculosis* and emergent T helper cell phenotype, *Immunology & Cell Biology - Nature*, *73*(6): 537–543.

Bambaradeniya, C. N. B., & Amarasinghe, F. P. (2004). Biodiversity Associated with the Rice Field Agro-ecosystem in Asian Countries : A Brief Review. *Biodiversity Associated with the Rice Field Agroecosystem in Asian Countries: A Brief Review*, 1–29.

Bartoloni, A., & Zammarchi, L. (2012). Clinical aspects of uncomplicated and severe malaria. *Mediterranean Journal of Hematology and Infectious Diseases*, *4*(1), e2012026. http://doi.org/10.4084/MJHID.2012.026.

Bass, C., Nikou, D., Vontas, J., Donnelly, M. J., Williamson, M. S., & Field, L. M. (2010). The Vector Population Monitoring Tool (VPMT): High-Throughput DNA-Based Diagnostics for the Monitoring of Mosquito Vector Populations. *Malaria Research and Treatment*, *2010*, 190434. http://doi.org/10.4061/2010/190434.

Beerntsen, B. T., James, a. a., & Christensen, B. M. (2000). Genetics of Mosquito Vector Competence. *Microbiology and Molecular Biology Reviews*, *64*(1), 115–137. http://doi.org/10.1128/MMBR.64.1.115-137.2000.

Bertram, D. F. (1996). Size-dependent predation risk in larval fishes: Mechanistic inferences and levels of analysis. *Fishery Bulletin*, *94*(2), 371–373.

Bhattarai, A., Ali, A. S., Kachur, S. P., Mårtensson, A., Abbas, A. K., Khatib, R., Al-Mafazy, A. W., Ramsan, M., Rotllant, G., Gerstenmaier, J. F., Molteni, F., Abdulla, S., Montgomery, S. M., Kaneko, A., Björkman, A. (2007). Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS Medicine*, *4*(11), e309.

Binka, F. N., Kubaje, A., Adjuik, M., Williams, L. a., Lengeler, C., Maude, G. H., Armah, G., Kajihara, B., Adiamah, J.,Smith, P. G. (2007). Impact of permethrin impregnated bednets on child mortality in Kassena-Nankana district, Ghana: a randomized controlled trial. *Tropical Medicine & International Health*, *1*(2), 147–154.

Blaustein, L., Kiflawi, M., Eitam, A., Mangel, M., & Cohen, J. E. (2004). Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. *Oecologia*, *138*(2), 300–5. http://doi.org/10.1007/s00442-003-1398-x.

Blaustein, L., Blaustein, J., & Chase, J. (2005). Chemical detection of the predator Notonecta irrorata by ovipositing Culex mosquitoes, *30*(2), 299–301.

Bloland, P. B. D., Boriga, A., Ruebush ,T. K., McCormick, J. B., Roberts, J. M., Oloo, A. J., Hawley, W., Lal, A., Nahlen, B., & Campbell, C. C. (1999). Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission II. Descriptive epidemiology of malaria infection and disease among children, *American Journal of Tropical Medicine and Hygiene*, 60 (4) 641-648.

Bootsma, H. A., Hecky, R. E., & Ave, E. G. (2003). A Comparative Introduction to the Biology and Limnology of the African Great Lakes, *29*(Supplement 2), 3–18.

Brabin, B. J. (1983). An analysis of malaria in pregnancy in Africa, *Buletin of world Health Organization, 61*(6), 1005–1016.

Bussmann, R. W., Gilbreath, G. G., Solio, J., Lutura, M., Lutuluo, R., Kunguru, K., Wood, N., Mathenge, S. G. (2006). Plant use of the Maasai of Sekenani Valley, Maasai Mara, Kenya. *Journal of Ethnobiology and Ethnomedicine*, *2*, 22. doi:10.1186/1746-4269-2-22.

Carter, R., & Mendis, K. N. (2002). Evolutionary and Historical Aspects of the Burden of Malaria. *Clinical Microbiology Reviews*, *15*(4), 564–594. http://doi.org/10.1128/CMR.15.4.564-594.2002.

Chanda, E., Hemingway, J., Kleinschmidt, I., Rehman, A. M., Ramdeen, V., Phiri, F. N., Coetzer, S., Mthembu, D., Shinondo, C.J., Chizema-Kawesha, E., Kamuliwo, M., Mukonka, V., Baboo, K. S., Coleman, M. (2011). Insecticide resistance and the future of malaria control in Zambia. *PloS One*, *6*(9), e24336.

Chandra, G., Bhattacharjee, I., Chatterjee, S. N., & Ghosh, A. (2008). Mosquito control by larvivorous fish. *Indian Journal of Medical Research*, *127*(1), 13–27.

Choi, H. A. N. W., Breman, J. G., Teutsch, S. M., Liu, S., Hightower, W., & Sexton, A. D. (1995). The effectiveness of insecticide-impregnated bed nets in reducing cases of malaria infection: a meta-analysis of published results. *American Journal of Tropical Medicine & Hygiene*, *52*(5), 377–382.

Chumchal, M. M., Drenner, R. W., & Adams, K. J. (2016). Abundance and size distribution of permanent and temporary farm ponds in the southeastern Great Plains. *Inland Waters*, *6*, 258–264. http://doi.org/10.5268/IW-6.2.954.

Clarke, K. R. (1992). Nonparametric multivariate analyses of changes in community structure. *International Workshop on Solutions to Environmental Problems*, (MARCH 1993), 117–143. http://doi.org/10.1111/j.1442-9993.1993.tb00438.x.

Coetzee, M., Craig, M., & le Sueur, D. (2000). Distribution of African Malaria Mosquitoes Belonging to the Anopheles gambiae Complex. *Parasitology Today, 16*(2), 74–77. http://doi.org/10.1016/S0169-4758(99)01563-x.

Coetzee, M., Hunt, R. H., Wilkerson, R., Torre, A. Della, Coulibaly, M. B., & Besansky, N. J. (2013). *Anopheles coluzzii* and *Anopheles amharicus*, new members of the Anopheles gambiae complex. *Zootaxa*, *3619*(3), 246–274.

Cogswell, F. B. (1992). The Hypnozoite and Relapse in Primate Malaria, *5*(1), 26–35.

Cohuet, A., Simard, F., Toto, J. C., Kengne, P., Coetzee, M., & Fontenille, D. (2003). Species identification within the Anopheles funestus group of malaria vectors in Cameroon and evidence for a new species. *The American Journal of Tropical Medicine and Hygiene*, *69*(2), 200–205.

Couret, J., Dotson, E., & Benedict, M. Q. (2014). Temperature, larval diet, and density effects on development rate and survival of Aedes aegypti (Diptera: Culicidae). *PloS One*, *9*(2), e87468. http://doi.org/10.1371/journal.pone.0087468.

Cox, F. E. G. (2010). History of the discovery of the malaria parasites and their vectors. *Parasites & Vectors,* 3:5.

Dattalo, P. V. (2014). A Demonstration of Canonical Correlation Analysis with Orthogonal Rotation to Facilitate Interpretation. Unpublished manuscript, School of Social Work, Virginia Commonwealth University, Richmond, Virginia.

Day, S. W., Higham, T. E., Holzman, R., & Van Wassenbergh, S. (2015). Morphology, Kinematics, and Dynamics: The Mechanics of Suction Feeding in Fishes. *Integrative and Comparative Biology*, *55*(1), 21–35.

De Silva, P. M., & Marshall, J. M. (2012). Factors contributing to urban malaria transmission in sub-saharan Africa: a systematic review. *Journal of Tropical Medicine*, *2012*, 819563. http://doi.org/10.1155/2012/819563.

Defersha, M. B., Melesse, A. M., & McClain, M. E. (2012). Watershed scale application of WEPP and EROSION 3D models for assessment of potential sediment source areas and runoff flux in the Mara River Basin, Kenya. *Catena*, *95*, 63–72. http://doi.org/10.1016/j.catena.2012.03.004.

Dejenie, T., Yohannes, M., & Assmelash, T. (2011). Characterization of Mosquito Breeding Sites in the Vicinity of Tigray Microdams. *Ethiopian Journal of Health sciences*, 57–66.

Deng, C., Wang, Q., Zheng, S., Zhou, C., Gao, Y., Guo, J., Ahamada, M. S. A., Mliva, Oithik, F., Bacar, A., Attoumane, R., Song, J. (2014). Mass Drug Administration of Artemisinin-piperaquine on High Malaria Epidemic Area. *Tropical Medicine and Health*, *42*(2), 33–41.

DFID (2010). Malaria: Burden and Interventions, (December), 1–222.

Dida, G. O., Gelder, F. B., Anyona, D. N., Abuom, P. O., Onyuka, J. O., Matano, A.-S., Ofulla, A. V. (2015). Presence and distribution of mosquito larvae predators and factors influencing their abundance along the Mara River, Kenya and Tanzania. *SpringerPlus*, *4*, 136. http://doi.org/10.1186/s40064-015-0905-y.

Diuk-Wasser, M. a., Touré, M. B., Dolo, G., Bagayoko, M., Sogoba, N., Sissoko, I., … Taylor, C. E. (2007). Effect of rice cultivation patterns on malaria vector abundance in rice-growing villages in Mali. *American Journal of Tropical Medicine and Hygiene*, *76*(5), 869–874. http://doi.org/76/5/869 [pii].

Dotson & Beard CB (2009) Sequence and organization of the mitochondrial genome of the Chagas disease vector, Triatoma dimidiata. *Insect Molecular Biology*, 10: 205–215.

Duarte, A. M. R. D. C., Porto, M. A. L., Curado, I., Malafronte, R. S., Hoffmann, E. H. E., de Oliveira, S. G., da Silva, A. M., Kloetzel, J. K., Gomes, A. D. C. (2006). Widespread occurrence of antibodies against circumsporozoite protein and against blood forms of *Plasmodium vivax, P. falciparum and P. malariae* in Brazilian wild monkeys. *Journal of Medical Primatology, 35*(2), 87–96.

Duchet, C., Allan, R., & Carnevale, P. (2012). Vector Control : Some New Paradigms and Approaches. ISBN 978-953-51-1188-7, Published: July 24, 2013 under CC BY 3.0 license.

Edillo, F. E., Tripét, F., Touré, Y. T., Lanzaro, G. C., Dolo, G., & Taylor, C. E. (2006). Water quality and immatures of the M and S forms of *Anopheles gambiae s.s*. and *An. arabiensis* in a Malian village. *Malaria Journal, 5*, 35.

Eisele, T. P., Larsen, D., & Steketee, R. W. (2010). Protective efficacy of interventions for preventing malaria mortality in children in *Plasmodium falciparum* endemic areas. *International Journal of Epidemiology, 39 Suppl 1*, i88–101.

Eitam, A., Blaustein, L., & Mangel, M. (2002). Effects of Anisops sardea (Hemiptera: Notonectidae) on oviposition habitat selection by mosquitoes and other dipterans and on community structure in artificial pools. *Hydrobiologia*, *485*, 183–189. http://doi.org/10.1023/A:1021315309758.

Enato, E. F. O., & Okhamafe, A. O. (2005). *Plasmodium falciparum* malaria and antimalarial interventions in sub-Saharan Africa : Challenges and Opportunities, *African Journal of Biotechnology*, 4 (13), 1598–1605.

Equatorial, N., Subsidiary, L., & Program, A. (2008). Mara River Basin Transboundary Integrated Water Resources Management and Development Project Mara River Basin Monograph Final Report Water Resources and Energy Management (WREM), (December).

Ernst, K. C., Lindblade, K. a, Koech, D., Sumba, P. O., Kuwuor, D. O., John, C. C., & Wilson, M. L. (2009). Environmental, socio-demographic and behavioural determinants of malaria risk in the western Kenyan highlands: a case-control study. *Tropical Medicine & International Health, 4*(10), 1258–65. http://doi.org/10.1111/j.1365-3156.2009.02370.x.

Eziefula, A. C., Gosling, R., Hwang, J., Hsiang, M. S., Bousema, T., von Seidlein, L., & Drakeley, C. (2012). Rationale for short course primaquine in Africa to interrupt malaria transmission. *Malaria Journal*, *11*, 360. http://doi.org/10.1186/1475-2875-11-360.

Federici, B. A. (1995). The future of microbial insecticides as vector control agents. *Journal of the American Mosquito Control Association*, *11*(2 Pt 2), 260–268.

Fillinger, U., & Lindsay, S. W. (2011). Larval source management for malaria control in Africa: myths and reality. *Malaria Journal*, *10*(1), 353. http://doi.org/10.1186/1475-2875-10-353.

Fillinger, U., Sonye, G., Killeen, G. F., Knols, B. G. J., & Becker, N. (2004). The practical importance of permanent and semipermanent habitats for controlling aquatic stages of *Anopheles gambiae sensu lato* larval mosquitoes: operational observations from a rural town in western Kenya. *Tropical Medicine & International Health : TM & IH*, *9*(12), 1274–89.

Fincke, O. M., Yanoviak, S. P., & Hanschu, R. D. (1997). Predation by odonates depresses mosquito abundance in water-filled tree holes in Panama. *Oecologia*, *112*(2), 244–253. http://doi.org/10.1007/s004420050307.

Fischer, S., Pereyra, D., & Fernández, L. (2012). Predation ability and non-consumptive effects of Notonecta sellata (Heteroptera: Notonectidae) on immature stages of Culex pipiens (Diptera: Culicidae). *Journal of Vector Ecology*, *37*(1), 245–251. http://doi.org/10.1111/j.1948-7134.2012.00223.x.

Gereta, E. J., Wolanski, E., & Chiombola, E. A. T. (2003). Assessment of the environmental, social and economic impacts on the serengeti ecosystem of the developments in the Mara River catchment in Kenya. The Amala Project, Kenya.

Ghosh, A., Biswas, D., Chatterjee, S. N., & Bengal, W. (2006). Host Plant Preference of Mansonia Mosquitoes, *Journal of the American Mosquito Control Association*,142–144.

Gilbert, J. J., & Burns, C. W. (1999). Some observations on the diet of the backswimmer, Anisops wakefieldi (Hemiptera- Notonectidae, *Hydrobiologia*, (March 1997), 111–118.

Gillies, M., & Coetzee, M. (1987). A Supplement to the Anophelinae of Africa South of the Sahara. *Publications of the South African Institute for Medical Research, 55*(55), 63. Retrieved from http://ww.w.sandflycatalog.org/files/pdfs/190531.

Gimnig, J. E., Ombok, M., Kamau, L., & Hawley, W. A. (2001). Characteristics of Larval Anopheline (Diptera :Culicidae) Habitats in Western Kenya. *Journal of Medical Entomology*, 38(2):282-8.

Ginn, D. M. C., Frances, S. P., Sweeney, A. W., & Brown, M. D. (2008). Evaluation of Bistar 80SC (Bifenthrin) as a Tent Treatment for Protection Against Larval mosquitoes in Northern Territory , Australia, (306). *Journal of Medical Entomology*, 1087–1091.

Gouagna, L. C., Rakotondranary, M., Boyer, S., Lempérière, G., Dehecq, J.-S., & Fontenille, D. (2012). Abiotic and biotic factors associated with the presence of Anopheles arabiensis immatures and their abundance in naturally occurring and man-made aquatic habitats. *Parasites & Vectors*, *5*, 96. http://doi.org/10.1186/1756-3305-5-96

Grau, G. E., Piguet, P., Engers, H. D., Louis, J. A., Vassalli, P., & Lambert, P. (1986). L3T4+ T lymphocytes play a major role in the pathogenesis of murine cerebral malaria. *Journal of Immunology*, 2348–2354.

Hagy, H. M., Foth, J. R., & Kaminski, R. M. (2011). Invertebrate biomass in flooded corn and other wetlands managed for wintering waterfowl in the Mississippi Alluvial Valley. *Proceedings of the Annual Conference of the Southeast Association of Fish and Wildlife Agencies*, *65*, 56–61.

Harley, D., Sleigh, A., Ritchie, S., & Ev, C. L. I. N. M. I. R. (2001). Ross River Virus Transmission , Infection , and Disease : a Cross-Disciplinary Review. *Clinical Microbiology Review*, *14*(4), 909–932. doi:10.1128/CMR.14.4.909.

Hashizume, M., Chaves, L. F., & Minakawa, N. (2012). Indian Ocean Dipole drives malaria resurgence in East African highlands. *Scientific Reports*, *2*, 269. http://doi.org/10.1038/srep00269.

Herrel, N., Amerasinghe, F. P., Ensink, J., Mukhtar, M., van der Hoek, W., & Konradsen, F. (2001). Breeding of Anopheles mosquitoes in irrigated areas of South Punjab, Pakistan. *Medical and Veterinary Entomology*, *15*(3), 236–248. http://doi.org/10.1046/j.0269-283x.2001.00312.x.

Hightower, A., Kiptui, R., Manya, A., Wolkon, A., Leigh, J., Eng, V.,Hemel, M., Noor, A., Sharif, S., Buluma, R., Vulule, J., Laserson, K., Slutsker, L., Akhwale, W. (2010). Bed net ownership in Kenya : the impact of 3 . 4 million free bed nets. *Malaria Journal*, 9: 183.

Hilsenhoff, W. L. (1988). Rapid Field Assessment of Organic Pollution with a Family-Level Biotic Index, *Journal of the North American Benthological Society*, *7*(71), 65–6865. http://doi.org/10.2307/1467832.

Hsieh, H.-L., Lin, H.-J., Shih, S.-S., & Chen, C.-P. (2015). Ecosystem Functions Connecting Contributions from Ecosystem Services to Human Wellbeing in a Mangrove System in Northern Taiwan. *International Journal of Environmental Research and Public Health*, *12*(6), 6542–6560. http://doi.org/10.3390/ijerph120606542.

Hughes, R. H., & Hughes, J. S. (1992). A directory of african wetlands. Union, the World Wide Fund for Nature (WWF), and the United Nations Environment Programme (UNEP).

Ijumba, J. N., & Lindsay, S. W. (2001). Impact of irrigation on malaria in Africa: Paddies paradox. *Medical and Veterinary Entomology*, *15*(1), 1–11. http://doi.org/10.1046/j.1365-2915.2001.00279.x.

Imbahale, S. S., Paaijmans, K. P., Mukabana, W. R., van Lammeren, R., 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*(1), 81. http://doi.org/10.1186/1475-2875-10-81.

Ito, J., Ghosh, A., Moreira, L. A, Wimmer, E. a, & Jacobs-Lorena, M. (2002). Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature*, *417*(6887), 452–5. http://doi.org/10.1038/417452a.

Iwashita, H., Dida, G. O., Sonye, G. O., Sunahara, T., Futami, K., Njenga, S. M., Chaves, L., F., Minakawa, N. (2014). Push by a net, pull by a cow: can zooprophylaxis enhance the impact of insecticide treated bed nets on malaria control? *Parasites & Vectors*, *7*(1), 52.

http://doi.org/10.1186/1756-3305-7-52.

James, A. A. (2003). Blocking malaria parasite invasion of mosquito salivary glands. *Journal of Experimental Biology*, *206*(21), 3817–3821. doi:10.1242/jeb.00616.

Jongwutiwes, S., Putaporntip, C., Iwasaki, T., Sata, T., & Study, T. (2004). Naturally Acquired knowlesi Malaria, *Tropical Medicine and Health*, *10*(12), 2211–2213.

Joshi, P. P. (2012). Aquatic Hemipteran Diversity As Indicators of More Environmental Extremes : Relation To Tolerant of Some Physico-Chemical Characteristics. *Bioscience Discovery, 3*(1), 120–124.

Kamareddine, L. (2012). The biological control of the malaria vector. *Toxins, 4*(9), 748–767. http://doi.org/10.3390/toxins4090748.

Kamau, L., Hawley, W. A., Lehmann, T., Alloys, S. S., Cornel, A., Ke, Z., & Collins, F. H. (1998). Use of short tandem repeats for the analysis of genetic variability in sympatric populations of *Anopheles gambiae* and *Anopheles arabiensis*. *Heredity, 80* (April 1997), 675–682.

Kaneko, A. (2010). A community-directed strategy for sustainable malaria elimination on islands: short-term MDA integrated with ITNs and robust surveillance. *Acta Tropica*, *114*(3), 177–83. http://doi.org/10.1016/j.actatropica.2010.01.012.

Kariuki, S. K., Njunge, J., Muia, A., Muluvi, G., Gatei, W., Ter Kuile, F., Terlouw, D. J., Hawley, W. A., Phillips-Howard, P. A., Nahlen, B. L., Lindblade, K. A., Hamel, M. J., Slutsker, L., & Shi, Y. P. (2013). Effect of malaria transmission reduction by insecticide-treated bed nets (ITNs) on the genetic diversity of Plasmodium falciparum merozoite surface protein (MSP-1) and circumsporozoite (CSP) in western Kenya. Malaria Journal, 12(1), 295..

Karthikairaj, K., Ravichandran, N., & Sevarkodiyone, S. P. (2013). Bio-diversity of Larval mosquitoes in Sub-Urban Area , Thiruthangal , Virudhunagar District , Tamil Nadu , India. *World Journal of Zoology*, *8*(3), 319–323.

Karunamoorthi, K. (2014). The counterfeit anti-malarial is a crime against humanity: a systematic review of the scientific evidence. *Malaria Journal, 13*(1), 209. http://doi.org/10.1186/1475-2875-13-209.

Kasangaki, A., Chapman, L. J., & Balirwa, J. (2008). Land use and the ecology of benthic assemblages of high-altitude rainforest streams in Uganda. *Freshwater Biology, 53*(4), 681–697.

Kawada, H., Dida, G. O., Ohashi, K., Komagata, O., Kasai, S., Tomita, T., Sonye, G., Maekawa Y., Mwatele, C., Njenga, S. M., Mwandawiro, C., Minakawa, N., Takagi, M. (2011a). Multimodal pyrethroid resistance in malaria vectors, *Anopheles gambiae* s.s., *Anopheles arabiensis*, and *Anopheles funestus* s.s. in western Kenya. *PloS One*, *6*(8), e22574.

Kawada, H., Futami, K., Komagata, O., Kasai, S., Tomita, T., Sonye, G., Mwatele, C., Njenga, S., M., Mwandawiro, C., Minakawa, N., Takagi, M. (2011b). Distribution of a knockdown resistance mutation (L1014S) in Anopheles gambiae s.s. and Anopheles arabiensis in western and southern Kenya. *PloS One*, *6*(9), e24323. http://doi.org/10.1371/journal.pone.0024323.

Kawada, H., Dida, G. O., Ohashi, K., Kawashima, E., Sonye, G., Njenga, S. M., Mwandawiro, C., & Minakawa, N. (2014). A small-scale field trial of pyriproxyfen-impregnated bed nets against pyrethroid-resistant Anopheles gambiae s.s. in western Kenya. *PloS One*, *9*(10), e111195. http://doi.org/10.1371/journal.pone.0111195.

Kawada, H., Dida, G. O., Sonye, G., Njenga, S. M., Mwandawiro, C., & Minakawa, N. (2012). Reconsideration of Anopheles rivulorum as a vector of Plasmodium falciparum in western Kenya: some evidence from biting time, blood preference, sporozoite positive rate, and pyrethroid resistance. *Parasites & Vectors*, *5*(1), 230. http://doi.org/10.1186/1756-3305-5-230.

Kawada, H., Ohashi, K., Dida, G. O., Sonye, G., Njenga, S. M., Mwandawiro, C., & Minakawa, N. (2014). Preventive effect of permethrin-impregnated long-lasting insecticidal nets on the blood feeding of three major pyrethroid-resistant malaria vectors in western Kenya. *Parasites & Vectors*, *7*(1), 383. http://doi.org/10.1186/1756-3305-7-383.

Kelly-Hope, L. A., Hemingway, J., & McKenzie, F. E. (2009). Environmental factors associated with the malaria vectors Anopheles gambiae and Anopheles funestus in Kenya. *Malaria Journal*, *8*(1), 268. http://doi.org/10.1186/1475-2875-8-268.

Kelly-Hope, L. a, & McKenzie, F. E. (2009). The multiplicity of malaria transmission: a review of entomological inoculation rate measurements and methods across sub-Saharan Africa. *Malaria Journal*, *8*, 19. http://doi.org/10.1186/1475-2875-8-19.

Kenawy, M. A., Ammar, S. E., & Abdel-Rahman, H. a. (2013). Physico-chemical characteristics of the mosquito breeding water in two urban areas of Cairo Governorate, Egypt. *Journal of Entomological and Acarological Research*, *45*(3), 17. http://doi.org/10.4081/jear.2013.e17.

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*(2), 85–92.

Kioko, U. M. (2013). Economic Burden of Malaria on Subsistence Crop Production in Kenya. *International Journal of Education and Research (IJER)*, *1*(2), 1–20.

Kirkwood, B., & Sterne, J. (2006). Essential Medical Statistics. 2nd ed. Hoboken, NJ: Wiley-Blackwell; 2003.

Kiszewski, A. E., & Teklehaimanot, A. (2004). A review of the clinical and epidemiologic burdens of epidemic malaria. *American Journal of Tropical Medicine and Hygiene* *71*(Suppl 2), 128–135.

Kizza, M., Rodhe, A., Xu, C.-Y., Ntale, H. K., & Halldin, S. (2009). Temporal rainfall variability in the Lake Victoria Basin in East Africa during the twentieth century. *Theoretical and Applied Climatology*, *98*(1-2), 119–135.

Klinkenberg, E., McCall, P., Wilson, M. D., Amerasinghe, F. P., & Donnelly, M. J. (2008). Impact of urban agriculture on malaria vectors in Accra, Ghana. *Malaria Journal*, *7*, 151. doi:10.1186/1475-2875-7-151.

Kluge, R. L. (2000). The Future of Biological Control of Weeds with Insects : No More “ Paranoia ”, No More “ Honeymoon .” *Proceedings of the X International Symposium on Biological Control of Weeds*, *467*(July 1999), 459–467.

Knight, R. L., Walton, W. E., O’Meara, G. F., Reisen, W. K., & Wass, R. (2003). Strategies for effective mosquito control in constructed treatment wetlands. *Ecological Engineering*, *21*(4–5), 211–232. http://doi.org/10.1016/j.ecoleng.2003.11.001

Koekemoer, L. L., Kamau, L., Hunt, R. H., & Coetzee, M. (2002). A cocktail polymerase chain reaction assay to identify members of the Anopheles funestus (Diptera: Culicidae) group. *American Journal of Tropical Medicine and Hygiene*, *66*(6), 804–811.

Koller, D., Building, G., Wang, D. C. C. W. D. C. C., Wong, A. K. C. W. A. K. C., Strait, B. J., Dewey, T. G., Mathematical, A. (1996). NIH Public Access. *Biophysical Journal*, *334*(July), 148–155.

Kroeger, I., Liess, M., Dziock, F., & Duquesne, S. (2013). Sustainable control of mosquito larvae in the field by the combined actions of the biological insecticide Bti and natural competitors, *Malaria Journal*, 82–89.

Kumar, R., & Hwang, J. S. (2006). Larvicidal efficiency of aquatic predators: A perspective for mosquito bio-control. *Zoological Studies*, *45*(4), 447–466. http://doi.org/10.1007/s12630-011-9635-y.

Kweka, E. J., Zhou, G., Munga, S., Lee, M.-C., Atieli, H. E., Nyindo, M., Githeko, A. K., Yan, G. (2012). Anopheline larval habitats seasonality and species distribution: a prerequisite for effective targeted larval habitats control programmes. *PloS One*, *7*(12), e52084.

Kweka, E. J., Kamau, L., Munga, S., Lee, M.-C., Githeko, A. K., & Yan, G. (2013). A first report of Anopheles funestus sibling species in western Kenya highlands. *Acta Tropica*, *128*(1), 158–61. http://doi.org/10.1016/j.actatropica.2013.06.006.

Lautze, J., McCartney, M., Kirshen, P., Olana, D., Jayasinghe, G., & Spielman, A. (2007). Effect of a large dam on malaria risk: the Koka reservoir in Ethiopia. *Tropical Medicine & International Health*. http://doi.org/10.1111/j.1365-3156.2007.01878.x.

Lawrence, C. (2007). The husbandry of zebrafish (Danio rerio): A review. *Aquaculture*, *269*(1–4), 1–20. http://doi.org/10.1016/j.aquaculture.2007.04.077.

Laporta, G. Z., Ribeiro, M. C., Ramos, D. G., & Sallum, M. A. M. (2012). Spatial distribution of arboviral mosquito vectors (Diptera: Culicidae) in Vale do Ribeira in the South-eastern Brazilian Atlantic Forest. *Cadernos de Saúde Pública*, *28*(2), 229–238. doi:10.1590/S0102-311X2012000200003.

Levine, R. S., Peterson, A. T., & Benedict, M. Q. (2004). Geographic and ecologic distributions of the *Anopheles gambiae* Complex predicted using a genetic algorithm. *Journal of the American Soiety of Tropical Medicine& Hygiene*, *70*(2), 105–109.

Lindh, J. M., Okal, M. N., Herrera-Varela, M., Borg-Karlson, A.-K., Torto, B., Lindsay, S. W., & Fillinger, U. (2015). Discovery of an oviposition attractant for gravid malaria vectors of the Anopheles gambiae species complex. *Malaria Journal*, *14*(1), 119. http://doi.org/10.1186/s12936-015-0636-0.

Lowassa, A., Mazigo, H. D., Mahande, A. M., Mwang, B. J., Msangi, S., Mahande, M. J., , Kimaro, E. E., Elisante, E., Kweka, E. J. (2012). Social economic factors and malaria transmission in Lower Moshi , Northern Tanzania. *Parasites & Vectors*, 5:129 , *5*(1), 1.

Lundkvist, E., Landin, J., Jackson, M., & Svensson, C. (2003). Diving beetles (Dytiscidae) as predators of mosquito larvae (Culicidae) in field experiments and in laboratory tests of prey preference. *Bulletin of Entomological Research*, *93*, 219–226. http://doi.org/10.1079/BER2003237.

LVBC & WWF-ESARPO. (2010). Biodiversity Strategy and Action Plan for Sustainable Management of the Mara River Basin, 48.

Mabaso, M. L. H., Sharp, B., & Lengeler, C. (2004). Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying. *Tropical Medicine & International Health : TM & IH*, *9*(8), 846–56. http://doi.org/10.1111/j.1365-3156.2004.01263.x.

Magurran, A. E. (2004). Measuring Biological Diversity. 1ST edition Blackwell Science Ltd; 2005.

Magurran, a. E. (2005). Species abundance distributions: pattern or process? *Functional Ecology*, *19*(1), 177–181. http://doi.org/10.1111/j.0269-8463.2005.00930.x.

Mahande, A. M., Mosha, F. W., Mahande, J. M., & Kweka, E. J. (2007). Role of cattle treated with deltamethrine in areas with a high population of Anopheles arabiensis in Moshi, Northern Tanzania. *Malaria Journal*, *6*, 109. http://doi.org/10.1186/1475-2875-6-109.

Mahavidyalaya, A. (2012): In Joshi P (2012). Aquatic hemipteran diversity as indicators of more environmental extremes : Relation to tolerant of some physico-chemical characteristics of water. *Bioscience Discovery*, *3*(1), 120–124.

Malaria, N., & Programme, C. (2015). Malaria Indicator Survey 2015.

Mango, L. M., Melesse, a. M., McClain, M. E., Gann, D., & Setegn, S. G. (2011). Land use and climate change impacts on the hydrology of the upper Mara River Basin, Kenya: results of a modeling study to support better resource management. *Hydrology and Earth System Sciences*, *15*(7), 2245–2258. http://doi.org/10.5194/hess-15-2245-2011.

Manikandan, K., & Sevarkodiyone, S. P. (2014). Altitudinal Variation in Density of Mosquito , Thiruvannamalai Temple , A Hilly Area Srivilliputturtaluk, *Malaria Journal*, *9*(3), 149–154. http://doi.org/10.5829/idosi.wjz.2014.9.3.91101.

Massebo, F., Balkew, M., Gebre-Michael, T., & Lindtjørn, B. (2013). Blood meal origins and insecticide susceptibility of Anopheles arabiensis from Chano in South-West Ethiopia. *Parasites & Vectors, 6*, 44. http://doi.org/10.1186/1756-3305-6-44.

Matano, A., Kanangire, C. K., Anyona, D. N., Abuom, P. O., Gelder, F. B., Dida, G. O., Owuor, P.O., & Ofulla, A. V. O. (2015). Effects of Land Use Change on Land Degradation Reflected by Soil Properties along Mara River , Kenya and Tanzania. *Open Journal of Soil Science*, **5**, 20-38.

Mboera, L. E. G., Makundi, E. A., & Kitua, A. Y. (2007). Uncertainty in Malaria Control in Tanzania : Crossroads and Challenges for Future Interventions. *American Journal of Tropical Medicine & Hygiene*, *77*(Suppl 6), 112–118.

Mboera, L. E. G., Mazigo, H. D., Rumisha, S. F., & Kramer, R. A. (2013). Towards malaria elimination and its implication for vector control , disease management and livelihoods in Tanzania. *MalariaWorld Journal*, *4*(19), 18–20.

Md Idris, Z., Chim, W., Chang, S., Masatsugu, K., Isao, T., Ahmediin, O., Akhwale, W., Kongere, J., Akira, K. (2014). Geographic and seasonal variation in malaria prevalence on islands in Lake Victoria (western Kenya): results from three cross sectional studies. *Malaria Journal*, *13*(Suppl 1), P61.

Miller, N. L. (2009). Climate Vulnerability Assessments of Water Utilities in the Lake Victoria Basin United Nations – Habitat Project, (August).

Minakawa, N., Mutero, C. M., Githure, J. I., Beier, J. C., & Yan, G. (1999). Spatial distribution and habitat characterization of Anopheline mosquito larvae in western Kenya. *American Journal of Tropical Medicine & Hygiene*, *61*(6), 1010–1016.

Minakawa, N., Seda, P., & Yan, G. (2002). Influence of host and larval habitat distribution on the abundance of african malaria vectors in western Kenya. *American Journal of Tropical Medicine & Hygiene*, *67*(1), 32–38.

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. *American Journal of Tropical Medicine & Hygiene*, *73*(1), 157–165.

MinMinakawa, N., Dida, G. O., Sonye, G. O., Futami, K., & Njenga, S. M. (2012). Malaria vectors in Lake Victoria and adjacent habitats in western Kenya. *PloS One*, *7*(3), e32725.

http://doi.org/10.1371/journal.pone.0032725.

Minakawa, N., Sonye, G., Dida, G. O., Futami, K., & Kaneko, S. (2008). Recent reduction in the water level of Lake Victoria has created more habitats for Anopheles funestus. *Malaria Journal*, *7*, 119. http://doi.org/10.1186/1475-2875-7-119.

Miura, T., & Takahashi, R. M. (1988). A laboratory study of predation by damselfly nymphs, Enallagma civile, upon mosquito larvae, Culex tarsalis. *Journal of the American Mosquito Control Association*, *4*(2), 129–131. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-0024028859&partnerID=40&md5=ed35820c0a4bcf28b45ace00acea1445

Miura, T., & Takahashi, R. M. (1988). A laboratory study of predation by damselfly nymphs, Enallagma civile, upon mosquito larvae, Culex tarsalis. *Journal of the American Mosquito Control Association*, *4*(2), 129–131.

Mlozi, M. R. S., Rumisha, S. F., Mlacha, T., & Bwana, V. M. (2015). Challenges and opportunities for implementing an intersectoral approach in malaria control in Tanzania. *Tanzania Journal of Health*, *17*(1), 1–16.

Monitoring, E., & Change, B. (2015). President’s Malaria Initiative Technical Guidance Revised For Fiscal Year 2015 Planning, (April 2014).

Moonen, B., Cohen, J. M., Tatem, A. J., Cohen, J., Hay, S. I., Sabot, O., & Smith, D. L. (2010). A framework for assessing the feasibility of malaria elimination. *Malaria Journal*, *9*(1), 322. http://doi.org/10.1186/1475-2875-9-322

Moresby, P. (1998). Plasmodium ovale species in Papua New Guinea - lest we forget. *PNG Medical Journal*, *41*, 116–118.

Muirhead-Thomson, R. C. (1951). The distribution of anopheline mosquito bites among different age groups; a new factor in malaria epidemiology. *British Medical Journal*, *1*(4715), 1114–7. http://doi.org/10.1136/bmj.1.4715.1114.

Mulamba, C., Riveron, J. M., Ibrahim, S. S., Irving, H., Barnes, K. G., Mukwaya, L. G., Birungi, J., Wondji, C. S. (2014). Widespread pyrethroid and DDT resistance in the major malaria vector *Anopheles funestus* in East Africa is driven by metabolic resistance mechanisms. *PloS One*, *9*(10), e110058.

Munga, S., Minakawa, N., Zhou, G., Barrack, O. O., Githeko, A. K., & Yan, G. (2005). Oviposition site preference and egg hatchability of Anopheles gambiae: effects of land cover types. *Journal of Medical Entomology*, *42*(6), 993–997. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16465740.

Mutie, S. M., Mati, B., Home, P., Gadain, H., & Gathenya, J. (2006). Evaluating land use change effects on river flow using usgs geospatial stream flow model in mara river basin , Kenya. Proceedings of the 2nd Workshop of the EARSeL SIG on Land Use and Land Cover, (September), 28–30. Bonn, Germany.

Mutuku, F. M., Bayoh, M. N., Gimnig, J. E., Vulule, J. M., Kamau, L., Walker, E. D., Kabiru, E. & Hawley, W. A. (2006). Pupal habitat productivity of *Anopheles gambiae* complex larval mosquitoes in a rural village in western Kenya. *American Journal of Tropical Medicine & Hygiene*, *74*(1), 54–61.

Muturi, E. J., Mwangangi, J., Shililu, J., Jacob, B. G., Mbogo, C., Githure, J., & Novak, R. J. (2008). Environmental factors associated with the distribution of *Anopheles arabiensis* and C*ulex quinquefasciatus* in a rice agro-ecosystem in Mwea, Kenya. *Journal of Vector Ecology,* 33(1):56-63.

Muturi, E. J., Mwangangi, J., Shililu, J., Muriu, S., Jacob, B., Kabiru, E., Githure, J. I., Novak, R. (2007). Mosquito Species Succession and Physicochemical Factors Affecting Their Abundance in Rice Fields in Mwea, Kenya. *Journal of Medical Entomology*, *44*(2), 336–344.

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*(1), 129–138.

Mwanziva, C., Manjurano, A., Mbugi, E., & Mweya, C. (2011). Defining malaria burden from morbidity and mortality records , self treatment practices and serological data in Magugu , Babati District, northern Tanzania. *Tanzania Journal of Health Research*, *13*(2), 1–12.

Ndaruga, A., Ndiritu, G., Gichuki, N., & Wamicha, W. (2004). Impact of water quality on assemblages along a tropical stream in Kenya. *African Journal of Ecology*, *42*(3), 208–216.

Ndouo, F. S. T. (2009). Submicroscopic Human Parasitic Infections. Book edited by Patricia Hernandez-Rodriguez and Arlen Patricia Ramirez Gomez, ISBN 978-953-51-0612-8.

Newton, C. R. J. C., Hien, T. T., & White, N. (2000). Cerebral malaria. *Journal of Neurology, Neurosurgery, and Psychiatry*, 433–441.

Noor, A. M., Gething, P. W., Alegana, V. a, Patil, A. P., Hay, S. I., Muchiri, E., Juma, E., Snow, R. W. (2009). The risks of malaria infection in Kenya in 2009. *BMC Infectious Diseases*, *9*, 180. doi:10.1186/1471-2334-9-180.

Norkute, M. (2014). The effect of abiotic and landscape features on abundance of Anopheles larvae. Master´s Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Nyairo, W. N., Owuor, P. O., & Kengara, F. O. (2015). Effect of anthropogenic activities on the water quality of Amala and Nyangores tributaries of River Mara in Kenya. *Environmental Monitoring and Assessment*, *187*(11), 691.

Odada, E. O., Olago, D. O., Bugenyi, F., Kulindwa, K., Karimumuryango, J., West, K., Ntiba, M., Wandiga, S., Aloo-Obudho, P. & Achola, P. (2003). Environmental assessment of the East African Rift Valley lakes. *Aquatic Sciences - Research Across Boundaries*, *65*(3), 254–271.

Odada, E. O., Ochola, W. O., & Olago, D. O. (2009). Drivers of ecosystem change and their impacts on human well-being in Lake Victoria basin. *African Journal of Ecology*, *47*, 46–54. doi:10.1111/j.1365-2028.2008.01049.x.

Odaga, J., Sinclair, D., Ja, L., Donegan, S., Hopkins, H., Garner, P., Hopkins, H., Garner, P. (2014). Rapid diagnostic tests versus clinical diagnosis for managing people with fever in malaria endemic settings. *Cochrane Database Syst Review*, (4): 1-49.

Odongo-Aginya, E., Ssegwanyi, G., Kategere, P., & Vuzi, P. C. (2005). Relationship between malaria infection intensity and rainfall pattern in Entebbe peninsula, Uganda. *African Health Sciences*, *5*(3), 238–245.

Ofulla, A. V. O., Karanja, D., Omondi, R., Okurut, T., Matano, a., Jembe, T., Abila, R., Boera P. & Gichuki, J. (2010). Relative abundance of larval mosquitoes and snails associated with water hyacinth and hippo grass in the Nyanza gulf of Lake Victoria. *Lakes & Reservoirs: Research & Management*, *15*(3), 255–271.

Ofulla, A. V., Adoka, S. O., Anyona, D. N., Abuom, P. O., Karanja, D., Vulule, J. M., Okrut, T., Matano, A-S., Dida, G. O., Jembe, T. & Gichuki, J. (2013). Spatial distribution and habitat characterization of schistosomiasis host snails in lake and land habitats of western Kenya. *Lakes & Reservoirs: Research & Management*, *18*(2), 197–215.

Ohba, S., & Nakasuji, F. (2006). Dietary items of predacious aquatic bugs (Nepoidea:

Heteroptera) in Japanese wetlands. *Limnology, 7*(1), 41–43.

Ohba, S., Huynh, T. T. T., Kawada, H., Le, L. L., Ngoc, H. T., Hoang, S. Le, Higa, Y., Takagi, M. (2011). Heteropteran insects as mosquito predators in water jars in southern Vietnam, (June), 170–174.

Ohba, S.-Y., Ohtsuka, M., Sunahara, T., Sonoda, Y., Kawashima, E., & Takagi, M. (2012). Differential responses to predator cues between two mosquito species breeding in different habitats. *Ecological Entomology, 37*(5), 410–418.

Okbah, M. A, Ibrahim, A. M. A, & Gamal, M. N. M. (2013). Environmental monitoring of linear alkylbenzene sulfonates and physicochemical characteristics of seawater in El-Mex Bay (Alexandria, Egypt). *Environmental Monitoring and Assessment*, *185*(4), 3103–15. doi:10.1007/s10661-012-2776-9.

Okiro, E. A, Alegana, V. A, Noor, A. M., & Snow, R. W. (2010). Changing malaria intervention coverage, transmission and hospitalization in Kenya. *Malaria Journal*, *9*(1), 285. doi:10.1186/1475-2875-9-285.

Omumbo, J., Ouma, J., Rapuoda, B., Craig, M. H., Le Seur, D.,Snow, R. W. (1998). Mapping malaria transmission intensity using geographical information systems (GIS): an example from Kenya. *Annals of Tropical Medicine & Parasitology*, *92*(1).

Onyando, J. (2013). WWF Mara River Basin Management Initiative, Kenya and Tanzania, Phase III – Final evaluation report, (January 2013).

Opoku, A. A., & Amoako, J. (2002). The Occurrences and Habitat Characteristics of Mosquitoes in Accra , Ghana, *Journal of Vector Ecology*, *37*(1), 120–129.

Opondo, D. O. (2013). Erosive coping after the 2011 floods in Kenya. *International Journal of Global Warming*, *5*(4), 452. doi:10.1504/IJGW.2013.057285.

Oteyo, D. E., Obiero, F. O., Bay, H., Francis, K. J., Osogo, A. M., Kimeto, D. C., Kamande, T. (2014). Republic of Kenya, The parliamentary service commission interview notice, (March 2013), 15–17.

Oyewole, I. O., Ibidapo, C. A., Okwa, O. O., Oduola, A. O., Adeoye, G. O., Okoh, H. I., & Awolola, T. S. (2010). Species Composition and Role of Anopheles Larval mosquitoes in Malaria Transmission Along Badagry Axis of Lagos Lagoon, Lagos, Nigeria. *International Journal of Insect Science*, 51.

Paaijmans, K. P., Takken, W., Githeko, A. K., & Jacobs, A. F. G. (2008). The effect of water turbidity on the near-surface water temperature of larval habitats of the malaria mosquito Anopheles gambiae. *International Journal of Biometeorology*, *52*(8), 747–753. http://doi.org/10.1007/s00484-008-0167-2.

Parham, P. E., Pople, D., Christiansen-Jucht, C., Lindsay, S., Hinsley, W., & Michael, E. (2012). Modeling the role of environmental variables on the population dynamics of the malaria vector *Anopheles gambiae sensu stricto*. *Malaria Journal*, *11*(1), 271.

Parthiban M. and Dr. David B. V. (2007). Manual of Household and Public Health Pests and their Control, Namrutha Publications.

Pates, H., & Curtis, C. (2005). Mosquito behavior and vector control. *Annual Review of Entomology*, *50*, 53–70. http://doi.org/10.1146/annurev.ento.50.071803.130439.

Patz, J. a., Graczyk, T. K., Geller, N., & Vittor, A. Y. (2000). Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology*, *30*(12–13), 1395–1405. http://doi.org/10.1016/S0020-7519(00)00141-7.

Peckarsky, B. L., Kerans, B. L., McIntosh, A. R. & Taylor, B. W. (2006). Predator effects on prey population dynamics in open systems. *Oecologia* 156:431-440.

Pfaehler, O., Oulo, D. O., Gouagna, L. C., Githure, J., & Guerin, P. M. (2006). Influence of soil quality in the larval habitat on development of *Anopheles gambiae* Giles. *Journal of Vector Ecology : Journal of the Society for Vector Ecology*, *31*(2), 400–5.

Post, W. M., & Kwon, K. C. (2000). Soil carbon sequestration and land-use change: processes and potential. *Global Change Biology*, *6*(3), 317–327.

Pusey, B. J., & Arthington, A. H. (2003). Importance of the riparian zone to the conservation and management of freshwater fish: A review. *Marine and Freshwater Research*, *54*(1), 1–16.

Pyke, G. H. (2008). Plague Minnow or Mosquito Fish? A Review of the Biology and Impacts of Introduced Gambusia Species. *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 171–191.

Reeves, R. G., Denton, J. A, Santucci, F., Bryk, J., & Reed, F. A. (2012). Scientific standards and the regulation of genetically modified insects. *PLoS Neglected Tropical Diseases*, *6*(1), e1502.

Rejmánková, E., Grieco, J., Achee, N., & Roberts, D. R. (2013). Ecology of Larval Habitats. *Anopheles Mosquitoes - New Insights into Malaria Vectors*, *1*(2), 813. http://doi.org/10.5772/55229.

Report, W. M. (2012). World Malaria Report 2012, (December).

Ritchie, S. A., & Rochester, W. (2001). Wind-blown larval mosquitoes and introduction of Japanese encephalitis into Australia. *Emerging Infectious Diseases*, *7*, 900–903.

Romero, M., Gil, F. M., & Orduz, S. (2001). Expression of Mosquito Active Toxin Genes by a Colombian Native Strain of the Gram-negative Bacterium Asticcacaulis excentricus. *Memorias Do Instituto Oswaldo Cruz*, *96*(2), 257–263. doi:10.1590/S0074-02762001000200021.

Running, D. M., Ligon, J. B., & Miskioglu, I. (1999). from the SAGE Social Science Collections. All Rights Reserved. *Journal of Composite Materials*, *33*(10), 928–940.

Sadar, M. (2004). Making Sense of Turbidity Measurements–Advantages In Establishing Traceability Between Measurements and Technology, 10.

Schaper, S., & Hernández-chavarría, F. (2006). Scanning electron microscopy of the four larval instars of the Dengue fever vector Aedes aegypti (Diptera : Culicidae). *Revista de Biología Tropical*, 54(September), 847–852.

Schlagenhauf-lawlor, P., & Scott, J. (2001). Traveller's malaria, PMPH Publsher's, ISBN 155009157. USA.

Scholte, E.-J., Knols, B. G. J., Samson, R. A., & Takken, W. (2004). Entomopathogenic fungi for mosquito control: A review. *Journal of Insect Science*, *4*(19), 1–24.

Scholte, E., Bart, G. J. K. & Takken, W. (2008). An entomopathogenic fungus (Metarhizium anisopliae) for control of the adult African malaria vector *Anopheles gambiae*. *Entomologische Berichten*, *68*(1), 21–26.

Selemani, M., Mrema, S., Shamte, A., Shabani, J., Mahande, M. J., Yeates, K., Msengwa, A. S., Mbago, M. C. & Lutambi, A. M. (2015). Spatial and space-time clustering of mortality due to malaria in rural Tanzania: evidence from Ifakara and Rufiji Health and Demographic Surveillance System sites. *Malaria Journal*, *14*, 369.

Serneels, S., & Lambin, E. F. (2001). Proximate causes of land-use change in Narok district, Kenya: A spatial statistical model. *Agriculture, Ecosystems and Environment*, *85*(1-3), 65–81.

Service, M. W. (1971). Studies on Sampling Larval Populations of the *Anopheles gambiae* Complex. *Bulletin of the World Health Organization*, 169–180.

Service, M. W. (1985). Anopheles gambiae: Africa’s Principal Malaria Vector, 1902-1984. *Bulletin of the Entomological Society of America*, *31*(3), 8–14.

Shaalan, E. A., & Canyon, D. V. (2009). Aquatic insect predators and mosquito control. *Trop Biomed* (*Tropical Biomedicine*). Review Paper , *26*(3), 223–261.

Shahayaraj, K., & Sathiamoorthi, P. (2002). Influence of Different Diets of Corcyra Cephalonica on Life History of a Reduviid Predator Rhynocoris Marginatus (Fab .). *Journal of Central European Agriculture*, *3*, (1).

Shaukat, A. M., Breman, J. G., & McKenzie, F. E. (2010). Using the entomological inoculation rate to assess the impact of vector control on malaria parasite transmission and elimination. *Malaria Journal*, *9*, (122).

Singh, B., & Daneshvar, C. (2013). Human infections and detection of Plasmodium knowlesi. *Clinical Microbiology Reviews*, *26*(2), 165–84.

Siri, J. G., Wilson, M. L., Murray, S., Rosen, D. H., Vulule, J. M., Slutsker, L., & Lindblade, K. a. (2010). Significance of travel to rural areas as a risk factor for malarial anemia in an urban setting. *The American Journal of Tropical Medicine and Hygiene*, *82*(3), 391–7. http://doi.org/10.4269/ajtmh.2010.09-0047.

Sites, R. W., Nichols, B. J., & Permkam, S. (1997). The Naucoriae (Heteroptera) of Southern Thailand. *Pan-Pacific Entomologist*, *73*(2), 127–134.

Snapshot, C. G. (2014). The Global Malaria Epidemic, (April), 1–4.

Snow, B., & Marsh, K. (2005). Foresight Infectious Diseases : Preparing for the future. The Detection and Identification of Infectious Diseases www.bis.gov.uk/foresight/our-work/projects/published-projects/infectious-diseases.

Soediono, B. (1989). *Miscellaneous Papers*. *Journal of Chemical Information and Modeling* (Vol. 53). doi:10.1017/CBO9781107415324.004

Soleimani-Ahmadi, M., Vatandoost, H., & Zare, M. (2014). Characterization of larval habitats for anopheline larval mosquitoes in a malarious area under elimination program in the southeast of Iran. *Asian Pacific Journal of Tropical Biomedicine*, *4*(Suppl 1), S73–S80.

Spencer, S., Grant, A. D., Piola, P., Tukpo, K., Okia, M., Garcia, M., Salignon, P., Genevier, C., Kiguli, J. & Guthmann, J.-P. (2004). Malaria in camps for internally-displaced persons in Uganda: evaluation of an insecticide-treated bednet distribution programme. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *98*(12), 719–27.

Spieles, D. J., & Mitsch, W. J. (1999). The effects of season and hydrologic and chemical loading on nitrate retention in constructed wetlands: A comparison of low- and high-nutrient riverine systems. *Ecological Engineering*, *14*(1-2), 77–91.

Stav G, Blaustein L, M. Y. (2000). Influence of nymphal Anax imperator (Odonata: Aeshnidae) on oviposition by the mosquito Culiseta longiareolata (Diptera: Culicidae) and community structure in temporary pools. *Journal of Vector Ecology*, *25*(2), 190–202.

Stell, A. F. M., Roe, R. M., Arellano, C., & Apperson, C. S. (2013). Innovative Sugar — Insecticide Feeding Bioassay for Adult Female *Anopheles gambiae* (Diptera : Culicidae) Innovative Sugar – Insecticide Feeding Bioassay for Adult Female Anopheles gambiae (Diptera : Culicidae). *Journal of Medical Entomology*, *50*(4), 804–815.

Suárez-Rubio, M., & Suárez, M. E. (2004). The use of the copepod Mesocyclops longisetus as a biological control agent for Aedes aegypti in Cali, Colombia. *Journal of the American Mosquito Control Association*, *20* (4), 401–4.

Sumba, L. A., Okoth, K., Deng, A. L., Githure, J., Knols, B. G. J., Beier, J. C., & Hassanali, A. (2014). Daily oviposition patterns of the African malaria mosquito *Anopheles gambiae* Giles (Diptera : Culicidae) on different types of aqueous substrates Oviposition substrate preference, *2*(2004), 1–7.

Szczytko, S. W., & Dimick, J. J. (2005). 2005 Biological Water Quality Assessment of Logan Creek , Door Co ., WI. College of Natural Resources University of Wisconsin/Stevens Point Stevens Point, WI 54481: http://www.uwsp.edu/cnr-ap/watershed/. Accessed 6th April 2013.

Tambo, E., Adedeji, A. A., Huang, F., Chen, J.-H., Zhou, S.-S., & Tang, L.-H. (2012). Scaling up impact of malaria control programmes: a tale of events in Sub-Saharan Africa and People’s Republic of China. *Infectious Diseases of Poverty*, *1*(1), 7. doi:10.1186/2049-9957-1-7.

Taylor, K. A., Koros, J. K., Nduati, J., Copeland, R. S., Collins, F. H., & Brandling-bennett, A. D. (1990). Plasmodium falciparum infection rates in *Anopheles gambiae*, *An. arabiensis*, and *An. funestus* in western Kenya. *The American Journal of Tropical Medicine and Hygiene*, *43*(2), 124–129.

Tene Fossog, B., Ayala, D., Acevedo, P., Kengne, P., Ngomo Abeso Mebuy, I., Makanga, B., Magnus, J., Awono-Ambene, P., Njiokou, F., Pombi, M. & Costantini, C. (2015). Habitat segregation and ecological character displacement in cryptic African malaria larval mosquitoes. *Evolutionary Applications*, *8*(4), 326–45.

Tungaraza, C., Eliapenda, E., Osewe, K. O., & Palapala, P. M. (2012). Long-term climate impact on the Lake Victoria region influences water level fluctuation and resource availability. *International Journal of Environmental Sciences*, *2*(3), 1717–1732.

Tuno, N., Githeko, A., Yan, G., & Takagi, M. (2007). Interspecific variation in diving activity among *Anopheles gambiae* Giles (Diptera: Culicidae), *Anopheles arabiensis* Patton , and *Anopheles* funestus Giles (Diptera : Culicidae) larvae, *Journal of Vector Ecology June* (June), 112–117.

Venables, W. N., & Smith, D. M. (2012). *An Introduction to R. The R Manuals* (Vol. 2).

Vinogradova, E., Shaikevich, E., & Ivanitsky, A. (2007). A study of the distribution of the *Culex pipiens* complex (Insecta: Diptera: Culicidae) larval mosquitoes in the European part of Russia by molecular methods of identification. *Comparative Cytogenetics*, *1*(2), 129–138.

Vinogradova, E., Shaikevich, E., & Ivanitsky, A. (2007). A study of the distribution of the Culex pipiens complex (Insecta: Diptera: Culicidae) larval mosquitoes in the European part of Russia by molecular methods of identification. *Comparative Cytogenetics*, *1*(2), 129–138.

Wambold, L. J., Chan, D. O., Therriault, T. W., & Kolasa, J. (2011). Abiotic factors controlling species richness, diversity, and abundance in the Bromeliad *Hohenbergia polycephala* (Bromeliaceae), (1996), 91–101.

Wang-Sattler, R., Blandin, S., Ning, Y., Blass, C., Dolo, G., Touré, Y. T., delle Torre, A., Lanzaro, G. C., Steinmetz, L. M., Kafatos, F. C. & Zheng, L. (2007). Mosaic genome architecture of the Anopheles gambiae species complex. *PloS One*, *2*(11), e1249.

Warburg, A., Faiman, R., Shtern, A., Silberbush, A., Markman, S., Cohen, J. E., & Blaustein, L. (2011). Oviposition habitat selection by Anopheles gambiae in response to chemical cues by Notonecta maculata. *Journal of Vector Ecology : Journal of the Society for Vector Ecology*, *36*(2), 421–5.

Watsierah, C. A, Onyango, R. O., Ombaka, J. H., Abong'o, B. O., & Ouma, C. (2012). Provider knowledge of treatment policy and dosing regimen with artemether-lumefantrine and quinine in malaria-endemic areas of western Kenya. *Malaria Journal*, *11*(1), 436.

Watsierah, C. A, & Ouma, C. (2014). Access to artemisinin-based combination therapy (ACT) and quinine in malaria holoendemic regions of western Kenya. *Malaria Journal*, *13*(1), 290.

Welfare, S. (2010). Tanzania Malaria Programme Review 2010 Programme Review Proposal, 1–22. National Malaria Control Prigramme. Ministry of Health and Social Welfare Dar es Salaam, Tanzania.

White, B. J., Collins, F. H., & Besansky, N. J. (2011). Evolution of *Anopheles gambiae* in Relation to Humans and Malaria. *Annual Review of Ecology, Evolution, and Systematics*, *42*(1), 111–132.

Wijesinghe, W. M. G. S., Wickramasinghe, M. B., Kusumawathie, P. H. D., Jayasooriya, G. A. J. S. K., & De Silva, B. G. D. N. K. (2009). Studies on the efficacy of Toxorhynchites larvae and three larvivorous fish species for the control of Aedes larval populations in water-storage tanks in the Matale district of Sri Lanka. *Dengue Bulletin*, *33*, 140–147.

Williams, D. D., & Hynes, H. B. N. (1974). The occurrence of benthos deep in the substratum of a stream. *Freshwater Biology*. doi:doi:10.1111/j.1365-2427.1974.tb00094.x.

Wilson, A. L., Dhiman, R. C., Kitron, U., Scott, T. W., van den Berg, H., & Lindsay, S. W. (2014). Benefit of insecticide-treated nets, curtains and screening on vector borne diseases, excluding malaria: a systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, *8*(10), e3228. doi:10.1371/journal.pntd.0003228.

Wilson, C., & Tisdell, C. (2001). Why farmers continue to use pesticides despite environmental, health and sustainability costs. *Ecological Economics*, *39*(3), 449–462. doi:10.1016/S0921-8009(01)00238-5.

Wondji, C. S., Hunt, R. H., Pignatelli, P., Steen, K., Coetzee, M., Besansky, N., Lobo, N., Collins, F. H., Hemingway, J. & Ranson, H. (2005). An Integrated Genetic and Physical Map for the Malaria Vector Anopheles funestus, *1787*(December), 1779–1787. doi:10.1534/genetics.105.044800.

World Health Organization (2007). The *World Malaria Reports Fact Sheet* 2007: World Health Organization; http://www.who.int/features/factfiles/malaria/en/.

Yamauchi, L. M., Coppi, A., Snounou, G., & Sinnis, P. (2007). Plasmodium sporozoites trickle out of the injection site. *Cellular Microbiology*, *9*(5), 1215–22. doi:10.1111/j.1462-5822.2006.00861.x.

Yasuoka, J., & Levins, R. (2007). Impact of Deforestation and Agricultural Development on Anopheline Ecology and Malaria Epidemiology. *The American Journal of Tropical Medicine and Hygiene*, *76*(3), 450–460.

Ye-Ebiyo, Y., Pollack, R. J., & Spielman, A. (2000). Enhanced development in nature of larval Anopheles arabiensis larval mosquitoes feeding on maize pollen. *The American Journal of Tropical Medicine and Hygiene*, *63*(1-2), 90–3.

Yewhalaw, D., Legesse, W., Van Bortel, W., Gebre-Selassie, S., Kloos, H., Duchateau, L., & Speybroeck, N. (2009). Malaria and water resource development: the case of Gilgel-Gibe hydroelectric dam in Ethiopia. *Malaria Journal*, *8*, 21.

Zimmerman, M. C. (1993). The Use of the Biotic Index as an Indication of Water Quality. *Education*, 85–98.

Zuur, A. F., Ieno, E. N., & Meesters, E. H. W. G. (2009). *A Beginner's Guide to R*. *Applied Spatial Data Analysis with R*. doi:10.1007/978-0-387-78171-6.

APPENDICES

Appendix I: Location and Site Characteristics Questionnaire for the Mara River basin

River/stream/sampling point Name: ___________________ GPS point: _________
Date___________
Time: ____________ Area ID #:____
GPS location: Lat.______________ long.______________ UTM: ___________E
___________N ___________Elevation:

Conductivity: ________ DO: _______ Salinity: __________Hardness ___________________
Temp: ______ Turbidity: ________ pH: _________

Appendix II: Mosquito Larvae and predators Checklist

Mosquito larvae and predators habitats, characteristics and estimation of mosquito larvae, and predators densities, data collection form

SECTION A (Site/Area identification information)

1. Habitat area ID/Name---Date----------------
2. Habitat No.--------
3. Time of collection---

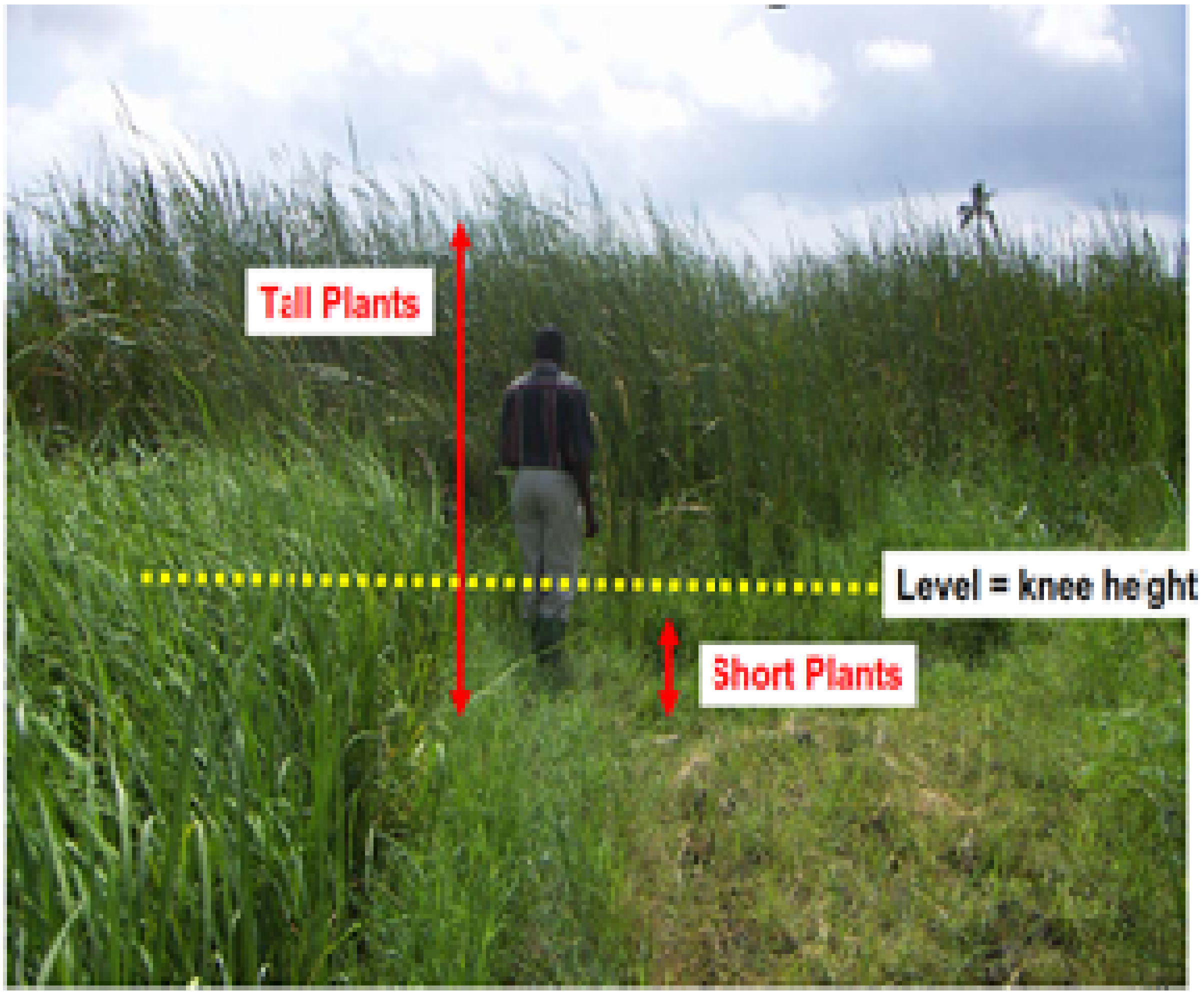
SECTION B (Habitat characteristics information)

1. Habitat type/manmade or natural: (a) Puddles (b) Pond (c) Stream/spring (d) Swamp (e) Rock pool
2. Presence of vegetation, in/around: (a) Grass (b) pappy reeds (c) Shrubs (d) water lilies
3. Presence of predators: (a)Dragonfly (b) water beetle (c) water scorpion (d)others
4. Light conditions: (a) Open and sunlight (b) Shaded (short grass, tall grass)
5. Water quality: (a) Foul smell (b) Clear (c) Turbid

SECTION C (Larvae species and abundance information)

1. Anopheline larvae species :(a) Present (b) Absent
2. Anopheline larvae stage found: (a) 1st instars (b) 2nd instars (c) 3rd instars (d) 4th instars (e) total # ----------------------------
3. Number of dips done : -------------
4. Culicine larvae species :(a) Present (b) Absent (c) total count ----------------------------
5. Other species---------------------------

Appendix III: Description of habitats based on plant height



Appendix IV: The Reaction Mixture for Species Identification. The Amount and Concentration is for Amplification of one Specimen.

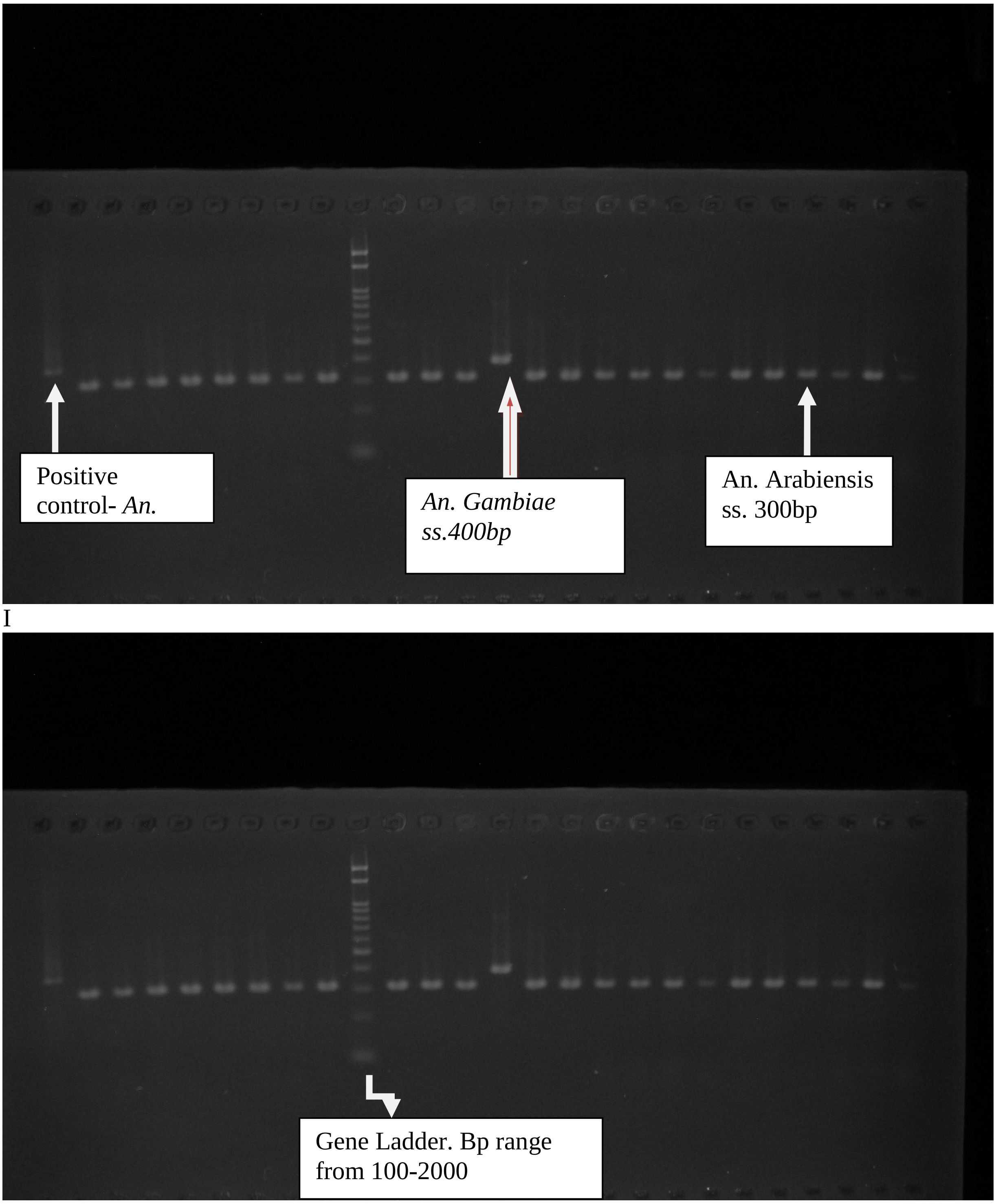
PCR 1: Species identification of *An. gambiae* complex (Larvae)

	Component	Volume for one sample
1	Distilled sterile water	8.8µl
2	10X PCR buffer	1.5µl
3	dNTP mix	1.14µl
4	Primers (GA, AR, UN) @	0.7µl
5	MgCl2	1.8µl
6	Taq polymerase	0.06µl
7	DNA template	1µl
	Total	15µl

The primer sequences to be used are indicated below:

Universal 20-mer primer (UN)	GTG TGC CCC TTC CTC GAT GT
An. gambiae primer (GA)	CTG GTT TGG TCG GCA CGT TT
An. arabiensis primer *(*AR)	AAG TGT CCT TCT CCA TCC TA

Figure 1: A plate for agarose gel. Shows how *An. gambiae* and *An. arabiensis* band fragments appear in the agarose gel after electrophoresis.



I. DNA Extraction

(1) Prepare ice and MilliQ water

(2) Switch ON the Thermo Block at 95°C

(3) Put a sample (legs of adult) into 1.5mL reaction tube

(4) DNA Extraction (REDExtract-N-Amp™ Tissue PCR Kit)

1) Mix Extraction Solution (20µL) + Tissue Preparation Solution (5 µ L)

2) Add the above mix solution into 1.5mL reaction tube (3)

3) Homogenize the sample

4) Wait for 10 min. at room conditions

5) Heat the tube at 95°C for 3 min.

6) Add the Neutralization Solution (20 µ L)

Preparation of Reaction mix Reaction volume : 5 µl/tube

Number of samples		**1**	**8**	**12**	**16**	**24**	**32**	**40**	**48**	**56**	**64**
Master mix (µl)	**ddw**	3.2	32	45	58	83	112	138	163	189	214
	REDExtract-N-Amp ReadyMix	1.0	10	14	18	26	35	43	51	59	67
	Primer* UNF	0.1	1	1	2	3	4	4	5	6	7
	FUN	0.1	1	1	2	3	4	4	5	6	7
	RIV	0.1	1	1	2	3	4	4	5	6	7
Total master mix (µl)		4.5	45	63	81	117	158	194	230	266	302
DNA template (µl/tube)		**0.5**									

* Two or 3-sample volume is added in excess (except 1)

II. PCR

	94°C	2 min	
Denature	94°C	30 sec	35 cycles
Anealing	40°C	30 sec	
Extension	72 °C	40 sec	
Last Extension	72 °C	5 min	

4 °C ∞

An. vanedeeni	*587 bp*
An. funestus	*505 bp*
An. rivulorum	*411 bp*
An. parensis	*252 bp*
An. leesoni	*146 bp*

iii. Electrophoresis

Marker	4µL	
Blue Juice	5µL	→ Use 5µL
TAE	32µL	

Appendix V: Full GLM model of the abiotic and biotic factors influence on mosquito predators abundance

Variable	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	-4.800802	3.349237	-1.433	0.151743
Total larval mosquitoes	0.014561	0.002213	6.579	4.75e-11 ***
Ph	0.166317	0.340036	0.489	0.624760
Conductivity	-0.004049	0.002680	-1.511	0.130842
Dissolved Oxygen (DO)	0.369071	0.112360	3.285	0.001021 **
Temperature	0.071721	0.026257	2.732	0.006304 **
Turbidity	-0.006546	0.001969	-3.325	0.000885 ***
Alkalinity	-0.003624	0.002075	-1.747	0.080698 .
Hardness	0.003582	0.003037	1.179	0.238203
Salinity	-0.434812	0.314544	-1.382	0.166862

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Negative Binomial (0.998) family taken to be 1)

Null deviance: 102.674 on 37 degrees of freedom

Residual deviance: 43.745 on 28 degrees of freedom

AIC: 311.58

Number of Fisher Scoring iterations: 1

Appendix VI: Method description of Generalized Linear Models and output for logistic regression model

Method description of Generalized Linear Models

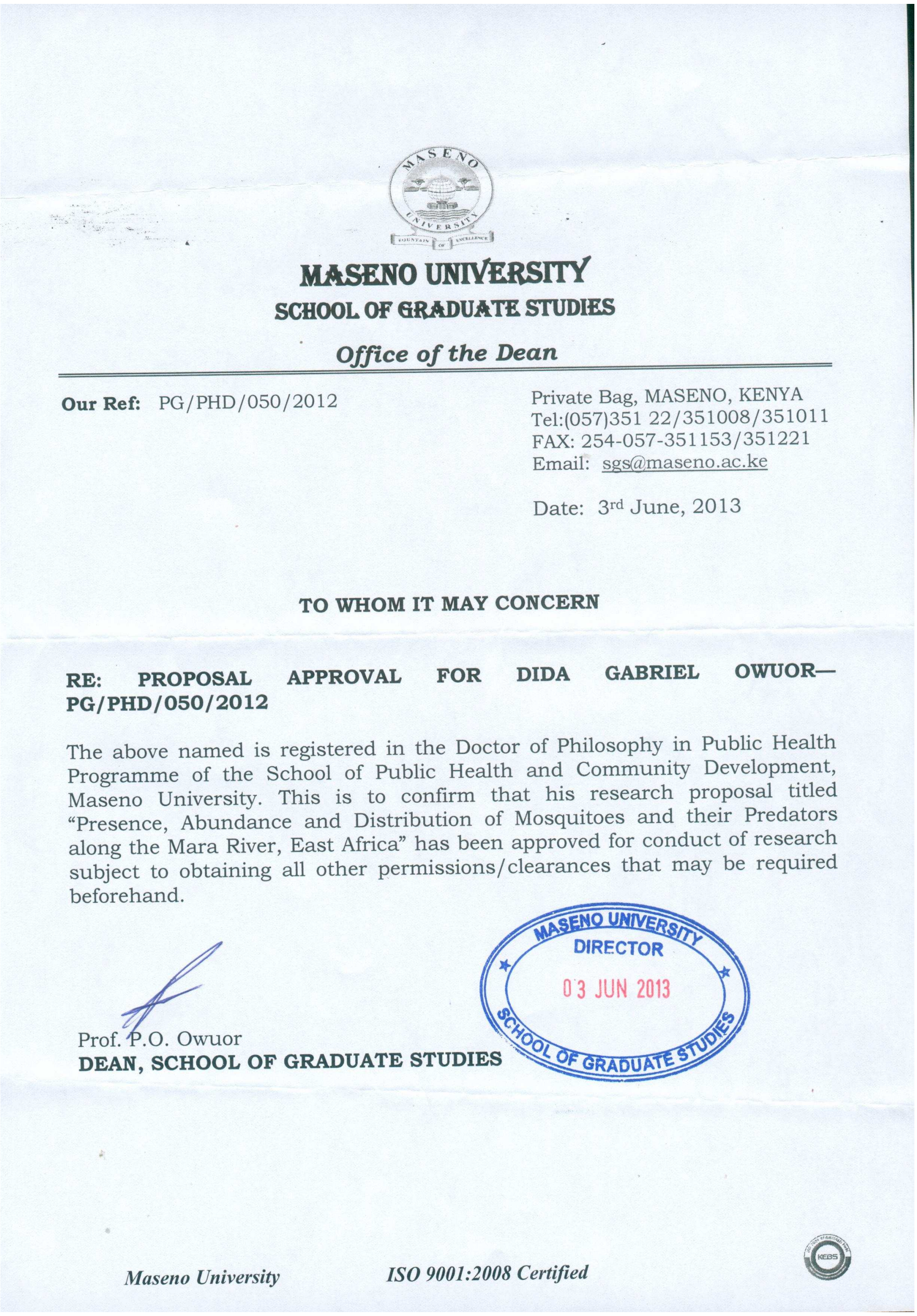
Generalized linear models (GLMs) is auseful mathematical extensions of linear models that provide a less restrictive form than classic multiple regressions by providing error distribution for the dependent variable other than normal and non-constant variance functions. They are also based on an assumed relationship called a link function between the mean of the response variable and the linear combination of the predictor variables (Zuur *et al.,* 2009).

Generalized linear models was developed in R (version 3.15.1, The R Foundation for Statistical Computing, 2012) to determine which environmental (biotic and abiotic) variables significantly explained the occurrence and abundance of anopheline larvae. For my case, prior to the modeling, I tested for collinearity among all predictor variables using Pearson correlation coefficient. If variables were highly correlated, one of both was removed ($r > 0.7$). Outliers were removed as well based on visual dot plots according to Zuur et al (2009). We used negative binomialregression (log link function) to model the abundance of of larval mosquitoes and predators. We started with a full model including all variables in the model. The forward–backward stepwise model selection method using Akaike's information criteria (AIC) was used to select the most appropriate (significant) model, (Zuur *et al.*, 2009). Homogeneity was checked by plotting residuals of every model against its respective predictors (also, see results of appendix IV).

Appendix VII: Canonical Correlation Analysis (CCA)

Canonical-Correlation Analysis (CCA) is a way of making sense of cross-covariance matrices. If we have two vectors $X = (X1, ..., Xn)$ and $Y = (Y1, ..., Ym)$ of random variables, and there are correlations among the variables, then canonical-correlation analysis will find linear combinations of the Xi and Yj which have maximum correlation with each other. All of the commonly encountered parametric tests of significance can be treated as special cases of canonical-correlation analysis, which is the general procedure for investigating the relationships between two sets of variables (Dattalo, 2014). The method was first introduced by Harold Hotelling in 1936.

Appendix VIII: Copy of Research Authorization Letter



MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES
Office of the Dean

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Email: sgs@maseno.ac.ke

Date: 3rd June, 2013

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR DIDA GABRIEL OWUOR—PG/PHD/050/2012

The above named is registered in the Doctor of Philosophy in Public Health Programme of the School of Public Health and Community Development, Maseno University. This is to confirm that his research proposal titled "Presence, Abundance and Distribution of Mosquitoes and their Predators along the Mara River, East Africa" has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

MASENO UNIVERSITY
DIRECTOR
03 JUN 2013
SCHOOL OF GRADUATE STUDIES

Prof. P.O. Owuor
DEAN, SCHOOL OF GRADUATE STUDIES

Maseno University *ISO 9001:2008 Certified*