USING AN ECOSYSTEM APPROACH TO UNDERSTAND THE LINK BETWEEN ARTISANAL CAPTURE FISHING AND MALARIA ON MAGETA ISLAND IN WESTERN KENYA

BY

ONYANGO, JANET ACHIENG (BSC. HONS)

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DECLARATION

I declare that this thesis is my original work and has not been presented for the award of a degree in any other University (see originality report in Appendix 1)

Signature……; Date……..

Ms Onyango, Janet Achieng

Supervisors:

This thesis has been submitted with our approval as university supervisors

Signature……; Date……

Prof. Wolfgang Richard Mukabana

School of Biological Sciences**,** University of Nairobi, Nairobi, Kenya; and

Science for Health, Nairobi, Kenya

Signature……; Date……

Dr. Collins Kalwale Mweresa

School of Biological and Physical Sciences, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya; and Science for Health, Nairobi, Kenya

DEDICATION

This thesis is dedicated to my beloved son Femi Ayodo Omondi

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LIST OF ABBREVATIONS AND ACRONYMS

ACF	:	Artisanal Capture Fishing
ACTs	:	Artemesinin-based Combination Therapies
Ecohealth	:	Ecosystem approach to human health
IRS	:	Indoor Residual Spraying
LLINs	:	Long Lasting Insecticidal Nets
LSM	:	Larval Source Management
PCR	:	Polymerase Chain Reaction
s.l	:	sensu lato
s.s	:	sensu stricto
WHO	:	World Health Organization

LIST OF DEFINITIONS

TERM		DEFINITION
Anthropophagy	:	Preference for feeding on human hosts
Artisanal capture fishing	:	Small scale activity in which fish are caught in the wild using rudimentary methods
Endophagy	:	Preference for feeding in-doors
Endophily	:	Preference for resting in-doors
Ecohealth	:	Ecohealth, short form for 'an ecosystem approach to human health', refers to systemic, participatory approaches to understanding and promoting health and wellbeing in the context of social and ecological interactions
Larval Source Management	:	Refers to the targeted 'care' of mosquito breeding sites, with the objective to reduce the number of mosquito larvae and pupae

ABSTRACT

In this study an ecosystem approach was employed to understand the association between artisanal capture fishing (ACF) and the problem of malaria on Mageta Island in western Kenya. In this work and in the local context, the term Mageta Island is generally used to include the adjacent Magare Island. The central goal was to establish whether actions of artisanal fishers, in their unrelenting quest for existence, surpass ecosystems' sustainability thresholds with potential negative repercussions on human health. This was achieved through a cross-sectional survey seeking to demonstrate the effect of ACF on creation of *Anopheles* larval habitats and its effect on *Anopheles* larval density. Mosquito larvae were sampled using a standard WHO dipper. The association between *Anopheles* presence/density and ACF activities was inferred.

A total of 87 mosquito larval habitats were identified. Stagnant water bodies created through ACF activities or otherwise are hereafter respectively referred to as 'fishing' and 'non-fishing' habitats. More fishing (77.8%) than non-fishing habitats (38.3%) contained *Anopheles* larvae. There was a significant negative association between ACF and the probability of finding *Anopheles* larvae in habitats. The mean number of *Anopheles* larvae in fishing boats, rock pools, ditches, lagoons and swamps was 40.08±10.16, 30.81±10.54, 5.71±3.11, 1.14± 0.9 and 1.09± 0.7, respectively. No *Anopheles* larvae were recovered from fishing ponds, fish bait mines and trenches. Although the total numbers of *Anopheles* larvae collected from both habitat types were about equal, the mean density in 'fishing habitats' (N=27; 35.7±1.15) was twice that in 'non-fishing habitats' (N=60; 17.4±0.539) (P = 0.001). The mean number of *Anopheles* mosquitoes in habitats with muddy, rocky and wooden bottom surfaces were 2.22 ± 0.29, 27.39 ± 0.87 and 40.08 ± 1.29, respectively. All pairwise comparisons of larval density revealed significant differences between habitats whose bottom surfaces were muddy, rocky or wooden (p = 0.001 in all cases). A map developed using computer algorithms showed that malaria hazard was strongly correlated to the spatial distribution of ACF activities on Mageta Island.

These results demonstrate that ACF is a key driver of malaria endemicity on Mageta Island. Larval source management strategies in the global south should be cognizant of the heterogeneity in *Anopheles* breeding habitats created by livelihood activities.

CHAPTER ONE: INTRODUCTION

1.1. Background

Malaria is a life threatening parasitic disease (WHO, 2018). The disease is transmitted between humans through bites of *Plasmodium*-infected female *Anopheles* mosquitoes (Gillies & Coetzee, 1987). The *Anopheles gambiae* complex and the *Anopheles funestus* group (Gillies and Coetzee, 1987) constitute the main mosquito vectors of malaria in sub-Saharan Africa. Malaria is a global burden. According to the World Health Organization, cases of malaria in 2017 were estimated to be 219 million. Malaria deaths recorded in the same year were 435,000 globally. Sub-Saharan Africa disproportionately bears the greatest burden of malaria. This region contained 92% of malaria cases and 93% of malaria deaths in 2017 (WHO, 2018). The presence of efficient *Anopheles* vectors that transmit the disease is among the reasons for persistence of malaria in Africa (WHO, 2018).

Malaria is caused by parasites belonging to the genus *Plasmodium* (WHO, 2018). Five main *Plasmodium* species namely *P. falciparum, P. knowlesi, P. malariae, P. ovale* and *P. vivax* are responsible for causing malaria in humans (WHO, 2018). Two species out of the five namely *P. falciparum* and *P. vivax* are reported to pose the greatest threat to man (WHO, 2018). *Plasmodium falciparum* is the most prevalent species in Africa and is also responsible for most malaria deaths globally. *Plasmodium vivax* is the most dominant malaria parasite species outside sub-Saharan Africa (WHO, 2018).

Malaria is preventable and curable. In 2017, an estimated US$ 3.1 billion was invested towards managing the disease globally by governments of malaria endemic countries and international partners (WHO, 2018). Malaria endemic countries contributed US$ 900 million, which was about 28% of the total funding (WHO, 2018). The recommended control strategies aimed at eradicating malaria include the use of effective anti-malaria drugs that target the parasite and use of insecticides that target the mosquito vector. Artemesinin-based Combination Therapies (ACT), long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the main strategies used for controlling malaria in Africa (Okumu & Moore, 2011; Fullman *et al*., 2013 ; WHO, 2018). LLINs and IRS target indoor, night-biting/resting mosquitoes only (Durnez & Coosemans *et al*., 2013). This implies that persons engaging in outdoor sociocultural/economic activities, at night, remain unprotected (Monroe *et al*., 2015). This protection gap also applies to artisanal capture fishers of Lake Victoria in western Kenya (Olanga *et al*., 2015), who are the focus of this study.

1.2. Problem Statement

The evolving and current threat of outdoor transmitted malaria (Durness & Coosemans, 2013; Killeen, 2014), especially in community groups engaging in compelling social, cultural and economic activities at night (Monroe *et al*., 2015), e.g. artisanal capture fishers exploiting the Lake Victoria fishery in Kenya (Olanga *et al*., 2015), can be viewed as an ecological disaster (Lebel, 2003).

Artisanal capture fishing forms the primary livelihood source for local inhabitants along the shores of Lake Victoria in Kenya (Njiru, 2008; Nathenson *et al*., 2017). The average daily income of about USD 2.00 (Omwega *et al*., 2006), resulting from selling the small fish stocks (Fiorella *et al*., 2015), is usually spent on food, medication and other basic needs (Omwega *et al*., 2006; Fiorella *et al*., 2014). Fishing crew are forced to relentlessly exploit fishery resources as a coping strategy (Fiorella *et al*., 2017; Larsen *et al*., 2018). This exerts big pressure on the environment (Groeneveld, 2016), which when done beyond a certain threshold poses negative repercussions on human health (Forget & Lebel, 2001; Lebel, 2003; Charron, 2012; Asakura *et al*., 2015). As noted elsewhere 'poor people are forced to overuse environmental resources to survive from day to day, and their impoverishment of the environment further impoverishes them, making their survival ever more difficult and uncertain' (WCED 1987). Thus, artisanal capture fishing, as practiced in the Lake Victoria fishery, is not a sustainable livelihood source (Geheb & Binns, 1997).

In this study an ecosystem approach (Forget & Lebel 2001; Lebel 2003; Charron 2012; Asakura *et al*., 2015) was employed to find out how, even if inadvertently, activities associated with artisanal capture fishing facilitate the breeding of malaria mosquito larvae on Mageta Island in western Kenya. In this work and in the local context, the term Mageta Island is generally used to include the adjacent Magare Island. A conceptual framework (figure 1), explaining how artisanal capture fishing leads to creation of suitable *Anopheles* larval habitats is shown on page 3. The framework illustrates the various conditions of the larval habitats that could mediate and moderate this relationship. The moderators considered included presence of fish predators in the habitats, exposure of the habitats to direct sunlight and presence of emergent plants in the habitats. The type of habitat bottom surface was considered as the main mediator of this relationship.

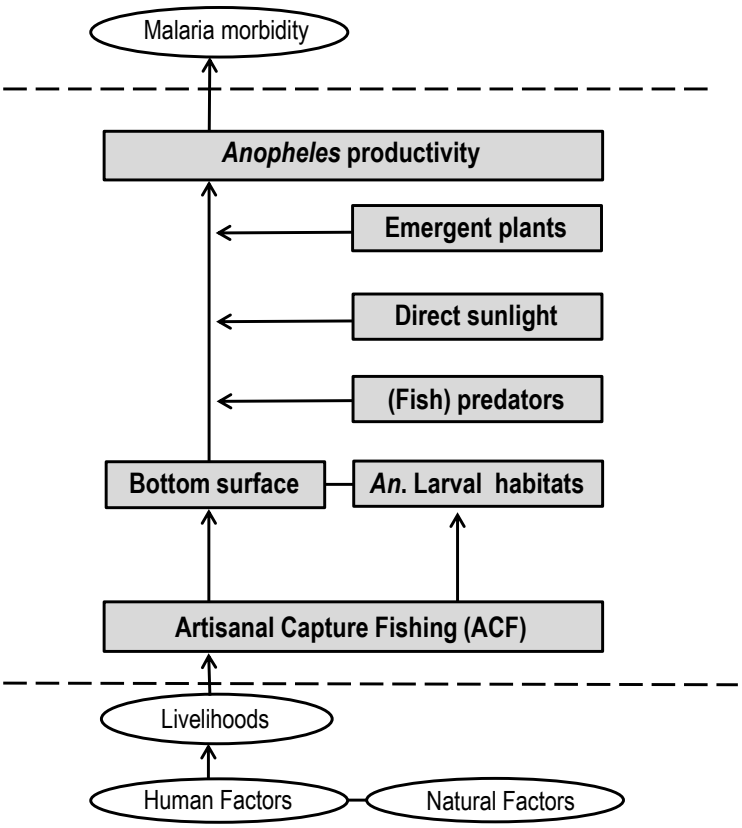

Figure 1: Conceptual framework illustrating the perceived relationship between artisanal capture fishing (ACF) and *Anopheles* productivity on Mageta Island in western Kenya. Boxes indicate variables of interest, arrows depict cause-effect relationships (starting from the variable that has cause influence and point to the variable that is being affected) and lines indicate correlation between two variables with no cause-effect relationships.

1.3. Justification and Significance of the Research

In Africa big gains have been made in eliminating malaria. Between 2000 and 2015, malaria incidence and mortality rates fell by 42% and 66%, respectively. Vector control strategies, including LLINs and IRS, contributed to these gains. LLINs and IRS only target anthropophagic mosquitoes feeding (endophagic) and resting (endophilic) indoors. This implies that people who engage in outdoor sociocultural and socioeconomic activities remain unprotected. Besides this protection gap, it is thought that human activities that support livelihoods enhance mosquito breeding. Suitable malaria mosquito breeding habitats are inadvertently created as man strives to improve his socioeconomic status. Socioeconomic issues are generally more important to the local inhabitants than the actual problem of malaria (Opiyo *et al.,* 2007). This implies that innovative, locally sustainable mitigating approaches that guarantee human health while safeguarding the environment and securing socioeconomic needs are urgently needed.

Artisanal capture fishing is the main livelihood activity on Mageta Island, where malaria is endemic (Ogola *et al*., 2017). Although some authors have pointed out the increased risk of malaria in the context of artisanal fishing (Barai *et al*., 1982; Akogbeto. 2000; Sogoba *et al*., 2007; Woodburn *et al*., 2009), no one has specifically assessed the link between artisanal fishing, environmental degradation and larval ecology of malaria vectors. This study sought to establish whether actions of artisanal fishers, in their unrelenting quest for existence, surpass ecosystems' sustainability thresholds with potentially negative repercussions on human health with respect to malaria.

An eco-health approach (Forget & Lebel 2001; Lebel, 2003; Charron, 2012; Asakura *et al*., 2015) was employed to understand the association between artisanal fishing and *Anopheles* larval productivity on Mageta Island in western Kenya. Rather than just mapping to know if breeding habitats of malaria mosquitoes are '*few, fixed* and *findable*', and therefore amenable to larval source management (WHO, 2013), this study sought to provide a deeper understanding that may be used in shaping and re-shaping decisions in malaria control policy with respect to larval source management strategies. Larval source management strategies in the global south should be cognizant of the heterogeneity in *Anopheles* breeding habitats created through livelihood activities. Embracing an ecosystem approach to human health (*ecohealth*) with respect to malaria can facilitate attainment of acceptable levels of health that will enable people to realize sustainable livelihoods (Dunn *et al*., 2011; Le Mare *et al*., 2014).

1.4. Research Objectives

1.4.1. General objective

To describe the association between artisanal capture fishing and *Anopheles* larval productivity on Mageta Island in western Kenya

1.4.2. Specific objectives

1. To assess the role of artisanal capture fishing in creation of *Anopheles* larval habitats
2. To determine the effect of artisanal capture fishing on *Anopheles* larval productivity
3. To develop a malaria hazard map of Mageta Island

1.5. Research Hypotheses

1.5.1. Null hypotheses

1. Artisanal capture fishing plays no role in creation of *Anopheles* larval habitats
2. Artisanal capture fishing has got no effect on *Anopheles* larval productivity

1.5.2. Alternative hypotheses

1. Artisanal capture fishing plays a role in creation of *Anopheles* larval habitats
2. Artisanal capture fishing affects *Anopheles* larval productivity

1.5.3. Assumptions made in this Study

This study assumed that the majority of *Anopheles* larvae found inside aquatic habitats eventually emerged into adult mosquitoes capable of transmitting malaria.

CHAPTER TWO: LITERATURE REVIEW

Human activities are closely linked to malaria endemicity. As community members strive to improve their socioeconomic status, they inadvertently create suitable breeding habitats for malaria vectors (Imbahale *et al.,* 2011b). Unfortunately, people's socioeconomic limitations are more important to them than the actual problem of malaria. This understanding was echoed by an exploratory study of community factors relevant for participatory malaria control in a fishing Island on Lake Victoria in Kenya (Opiyo *et al*., 2007). It was also recently demonstrated that small and innovative approaches for addressing root causes of social and economic inequalities can promote health as well as sustainable economic development (Nathenson *et al*., 2017). In this regard, applying the pillars of *Ecohealth* namely (a) community participation, (b) transdisciplinarity, and (c) gender equity (Lebel *et al*., 2003), can be a good approach in deciphering the link between artisanal capture fishing (ACF) and malaria. In the context of this study ACF is defined as a small scale activity in which fish are caught in the wild using crude/traditional methods.

2.1. Global burden of Malaria

Malaria mostly occurs in tropical and sub-tropical regions of the world (figure 2). In 1900 the burden was distributed worldwide except for some few countries (Hay *et al.*, 2004). A century later, 111 countries managed to eliminate the disease (Nkumama *et al.,* 2017). However, malaria prevalence was still reported in 106 developing countries. Between the years 2000 to 2015, incidence of malaria among the population at risk globally reduced by 37% whereas mortality rate reduced by 60%. In 2015, there were an estimated 438,000 malaria deaths worldwide. Most deaths occurred in Africa (90%), especially in children aged ≤5 years (WHO, 2016). Between 2000 and 2015, malaria incidence and mortality rates in Africa fell by 42% and 66%, respectively (WHO, 2017). These gains were attributable to (a) prompt diagnosis accompanied with appropriate treatment, and (b) protection of people from mosquito bites through indoor residual spraying (IRS) and long lasting insecticidal nets (LLINs). A total of 216 million malaria cases were reported in 91 countries in 2016. This was an increase over the previous year by 5 million. In 2017, there were an estimated 435, 000 deaths from malaria globally (WHO, 2018).

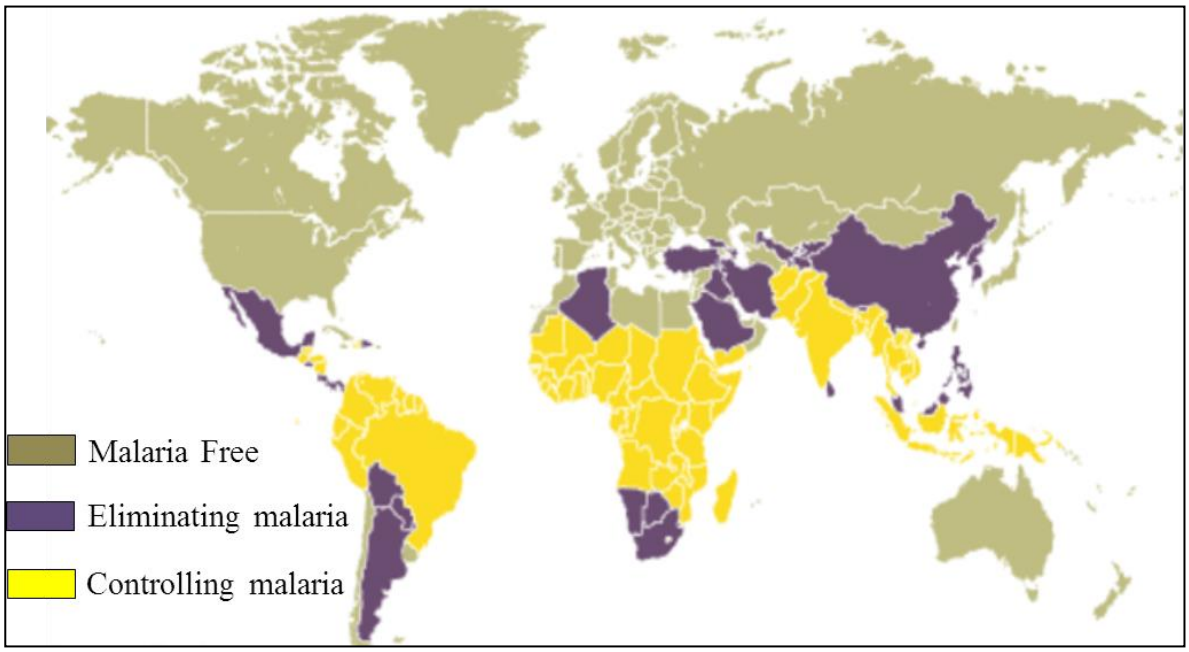

Figure 2.World malaria distribution map (WHO, 2017).

2.2. Epidemiology of malaria in Kenya

There are four main malaria epidemiological zones in Kenya. The four zones, grouped according to disease endemicity, include highland epidemic prone areas, endemic areas, semi-arid zones/seasonal malaria transmission areas and low risk malaria areas (figure 3). The risk diversity in the four zones is largely determined by altitude, malaria prevalence, rainfall patterns and temperature (DOMC, 2016). The western highlands of Kenya are the ones that are epidemic prone. Malaria transmission in the western highlands is usually seasonal, occurring during the long rains when the minimum temperatures are around 18˚C, favoring vector breeding. Lake Victoria and coastal regions are the malaria endemic areas. These areas have stable malaria transmission. Malaria vector survival rates are high in the malaria endemic areas and this could be due to suitable climatic conditions like altitudes ranging between 0 to 1,300m above sea level.

The Semi-arid zones also known as seasonal malaria transmission areas include areas in North eastern Kenya. These areas are characterized by extreme climatic conditions. Malaria transmission in such areas is usually intense occurring during the rainy seasons. The central highlands of Kenya are the low malaria risk areas. Such areas usually experience very low temperatures that cannot allow the completion of the sporogonic cycle of the malaria parasite (DOMC, 2016).

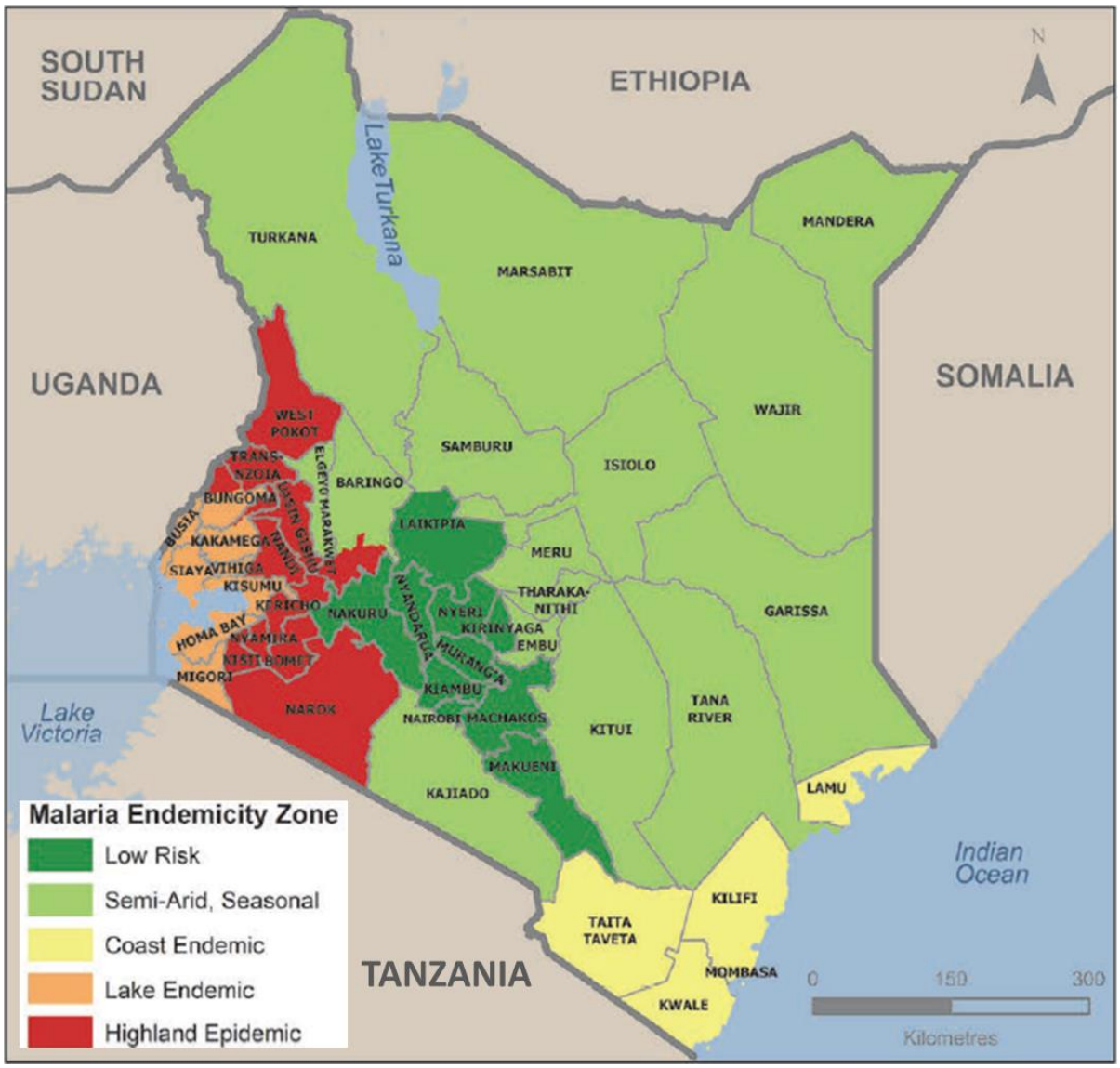

Figure 3: Malaria endemicity zones in Kenya (DOMC, 2016).

2.2.1. Transmission of Malaria

Malaria is a devastating vector borne disease (WHO, 2018) caused by a parasite belonging to the genus *Plasmodium.* Five main *Plasmodium* species namely *P. falciparum, P. knowlesi, P. vivax, P. malariae* and *P. ovale* are currently known to cause malaria in humans (WHO, 2018). Female *Anopheles* mosquitoes are responsible for transmitting malaria parasites. *Plasmodium falciparum* is the most dangerous out of the five malaria parasites (WHO, 2018). The *Plasmodium* parasite requires two hosts i.e. a human being (intermediate host) and a female *Anopheles* mosquito (definitive host) to complete its life cycle. Malaria transmission is influenced heavily by behavioral, environmental, economic and social factors (Qayum *et al.*, 2015; Nii-Trebi, 2017) and can be perennial or seasonal (Cairns *et al*., 2015).

2.2.1.1. Life cycle of malaria parasites

Plasmodium parasites undergo different developmental stages in their life cycle (figure 4) (Crompton *et al.*, 2010). Infected female *Anopheles* mosquitoes bite human hosts and inject sporozoites (Phattanawiboon *et al*., 2014) as they seek for blood meals to develop their eggs (Clement, 1999). Sporozoites invade hepatocytes and asexual reproduction then takes place to form schizonts, which contain merozoites. This stage is usually asymptomatic and lasts approximately 1-2 weeks. In case of *P. vivax* and *P. ovale*, a dormant phase known as hypnozoite, which is responsible for relapses in malarial disease when reactivated, is usually formed. After 1-2 weeks of the invasion, hepatocytes may rapture releasing schizonts, which burst releasing merozoites. Merozoites then invade erythrocytes and differentiate into the ring form (trophozoites). Trophozoites mature and reproduce asexually to form schizonts, which burst releasing merozoites that eventually invade erythrocytes to continue the cycle (Lefevre *et al.*, 2018). Erythrocytes may rapture releasing parasite debris and inducing the first symptoms of malarial disease in the human host signified by instant fever.

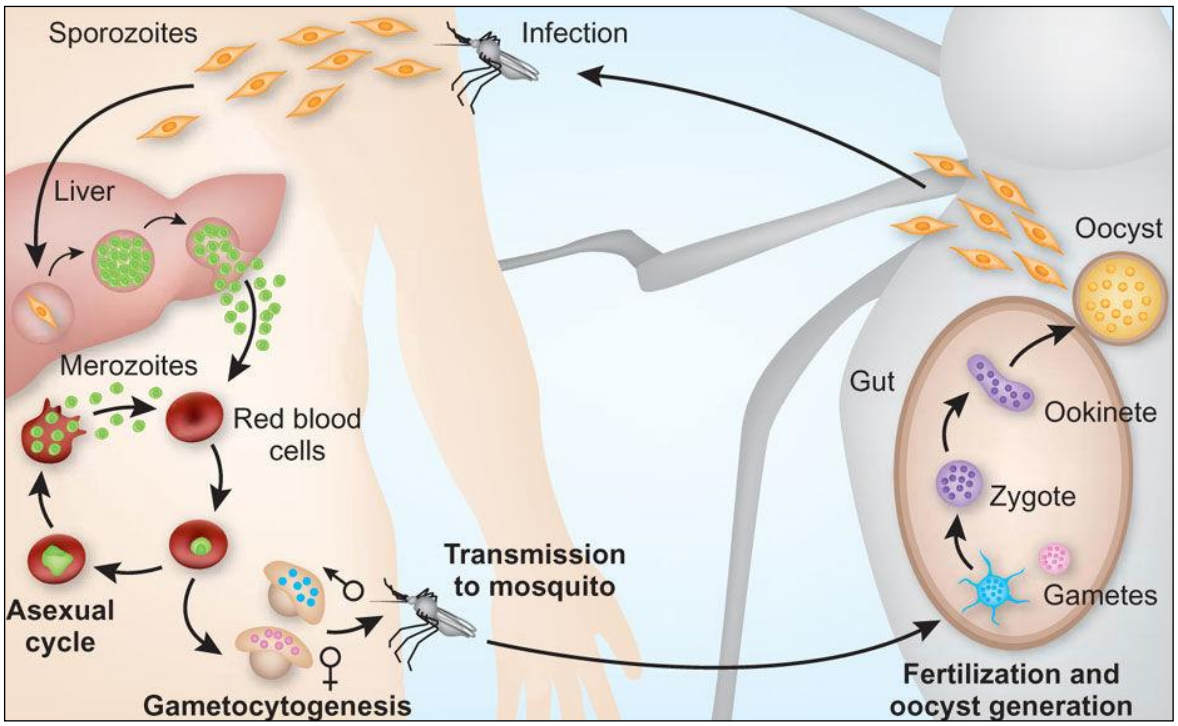

Figure 4: Generalized life cycle of the malaria parasite (Pasvol, 2010)

Some of the infected blood cells leave the cycle of asexual multiplication. Instead of replicating, the merozoites in these cells develop into sexual forms of the parasite, called gametocytes (Alano, 2007). In the blood stream gametocytes circulate awaiting ingestion by a female *Anopheles* mosquito seeking for a blood meal. The ingested gametocytes

develop further into mature male and female sex cells called gametes. The gametes fuse to form a zygote within the lumen of the mosquito's mid gut (Simonetti, 1996; Belachew, 2018). The resulting zygote develops into ookinetes which develop into oocysts. Oocysts undergo sporogony to form sporozoites. Sporozoites then migrate to the mosquito salivary glands through the haemocoel (Simonetti, 1996; Belachew, 2018) ready for transmission to the next human host (figure 4).

2.2.1.2. Malaria vectors in Africa

Malaria is transmitted by female *Anopheles* mosquitoes (Gillies and Coetzee, 1987). There are approximately 430 species of *Anopheles* and 70 of them are known to transmit malaria (Service, 2004). Though Africa has a vast number of *Anopheles* mosquitoes, the vectors have a worldwide distribution (Figure 5). In Africa the principal vectors of malaria are *An. gambiae*, *An. arabiensis* and *An. funestus* (Coetzee *et al*., 2013). *An. gambiae* and *An. arabiensis* belong to the *An. gambiae* complex, which consists of the most efficient vectors of malaria in Africa (Coetzee *et al*., 2013). This is mainly due to the mosquitoes' inherent behavioral traits namely anthropophagic, endophagic and endophilic. These traits enhance vector-host interactions that favor malaria transmission (Takken & Knols, 1999). The *An. funestus* group consists of *An. rivulorum*, which is a known vector of malaria. *An. funestus* is endophilic and anthropophilic whereas *An. rivulorum* is exophilic and zoophilic. All the other species within the *An. funestus* group are zoophilic. In Kenya *An. gambiae, An. arabiensis and An. funestus* are largely responsible for malaria transmission (Okara *et al*., 2010). These three species have been reported in the littoral zones of Lake Victoria (Minakawa *et al.,* 2012), including on Mageta Island where the work reported in this thesis was carried out (Ogola *et al*., 2017).

2.3. *Anopheles* larval habitats

The mosquito's life cycle encompasses four key life history stages namely eggs, larvae, pupae and adults (Clements, 1999). The first three stages are aquatic. In Africa the principle vectors of malaria namely *Anopheles arabiensis, Anopheles gambiae and Anopheles funestus* (Coetzee *et al*., 2013) often inhabit different habitat types. Poor drainage is a major culprit when it comes to occurrence of *Anopheles* larval habitats (Jacob *et al*., 2007). Temporal and spatial distribution patterns of *Anopheles* larval habitats vary seasonally and annually (Li *et al.,* 2009).

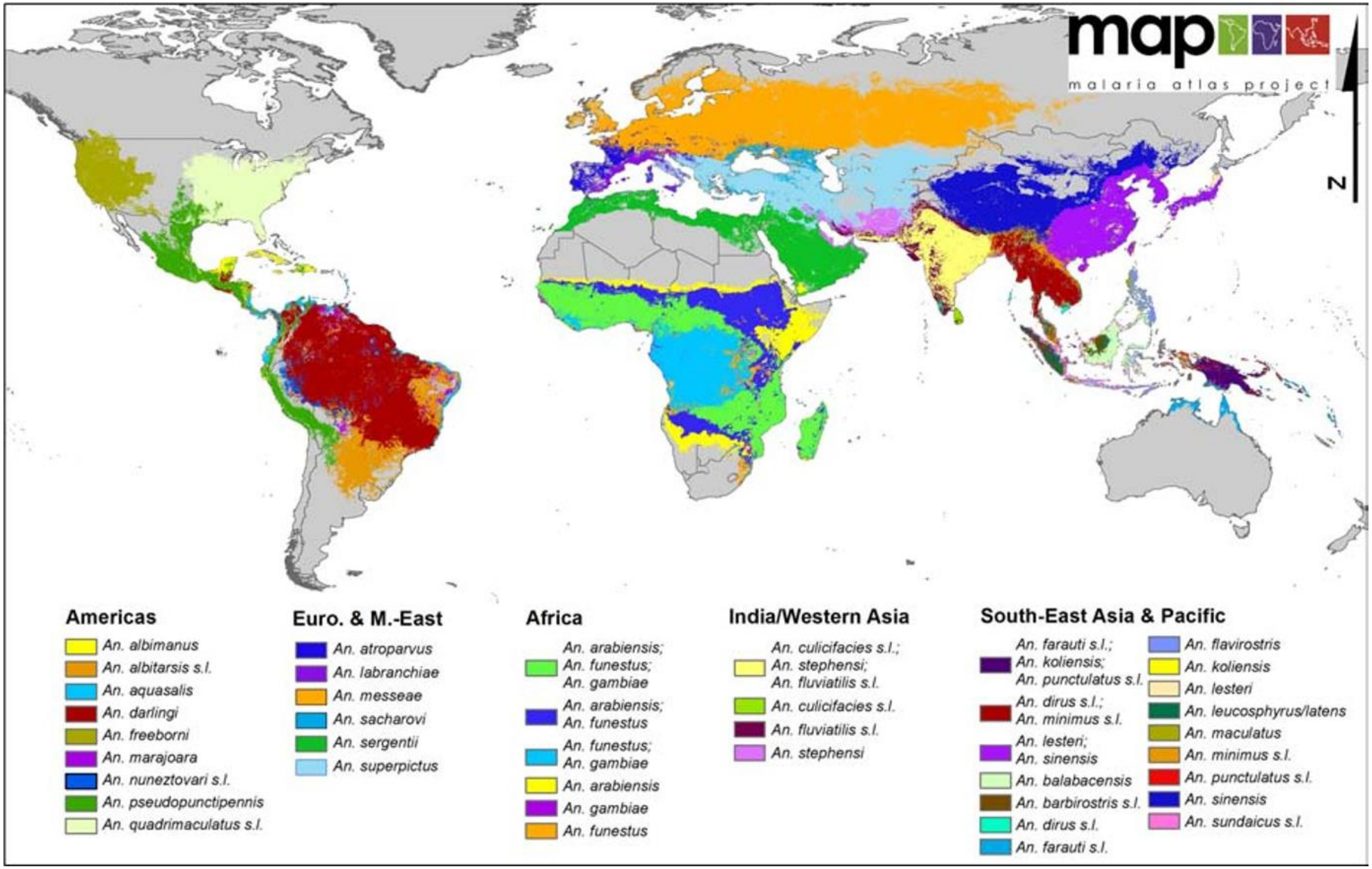

Figure 5: Global distribution map of *Anopheles* mosquito vectors of malaria (Sinka *et al*., 2012)**.**

2.3.1. *Anopheles* larval habitat diversity and characteristics

Anopheles larval habitats are diverse in nature. Some habitats are natural while others are man-made, being the result of human activities. Natural habitats include rock pools, lagoons swamps, tree-holes, stream-bed pools, river fringes etc. (Imbahale *et al.,* 2011b; Shililu *et al.,* 2007). Man-made habitats include dams, wells, domestic containers, drainages, burrow pits, goldmines, gutters, ponds, canoes, rice paddies etc. (Imbahale *et al.,* 2011b). Mosquito larval habitats have also been classified as temporary (Gimonneau *et al* 2012), semi-permanent and permanent (Fillinger *et al.,* 2004; Imbahale *et al.,* 2011b). The commonly observed mosquito breeding habitats in Africa include open sunlit puddles, vegetated pools, animal hoof prints, tire impressions, tire tracks etc. (Dida *et al.,* 2018; Mbida *et al.,* 2017).

2.3.1.1. *Anopheles* larval habitat diversity

Characterization of *Anopheles* larval habitats is based on factors like the habitat environment (Gimnig *et al.,* 2001), habitat size (Gimnig *et al*., 2001; Minakawa *et al.,* 2004) and habitat stability (Ndenga *et al*., 2011). Habitat environmental factors include turbidity, temperature, presence of algae, emergent plants and aquatic vegetation (Gimnig *et al.,* 2001; Fillinger *et al*., 2004; Minakawa *et al*., 2004; Dida *et al.,* 2018). Habitat characteristics generally inform the choice of *Anopheles* species residing in them. Habitat segregation has been observed with *Anopheles* larvae (Gimonneau *et al*., 2012). *Anopheles* species are reported in different geographical areas, during different seasons and in different habitats. As observed along the Kenyan coast *Anopheles gambiae* s.l prefers habitats with floating debris and emergent plants (Mwangangi *et al.,* 2007). As observed in cape coast Ghana *Anopheles gambiae* prefers to breed in temporary, sunny habitats which are small and relatively clean and without overhanging vegetation (Kudom, 2015).

Anopheles arabiensis and the S form of *An. gambiae* have been found to occur in temporary habitats e.g. rain pools, while the S form of *Anopheles gambiae* occurs in large breeding sites e.g. rice paddies (Gimonneau *et al*., 2012). In general, more *Anopheles* larvae have been reported to occur in man-made habitats compared to natural habitats (Imbahale *et al*., 2011a). *Anopheles gambiae* larvae are reported to occur in drinking water tanks and stand pipes in Mauritania (Salem *et al.,* 2013), but also in organically polluted habitats associated with human activities in the city of Dar es Salaam in Tanzania (Sattler *et al.,* 2005). Major larval habitats of *Anopheles gambiae s.l* are reported to be anthropogenic, being associated with human activities (Kudom *et al*., 2012). *Anopheles arabiensis* on the other hand has been

reported to be abundant in ephemeral natural aquatic habitats like streambed pools throughout the year (Shililu *et al*., 2007). As much as there is species segregation, niche overlap has also been reported in some cases. Several studies conducted in Kenya reported breeding of *Anopheles arabiensis* and *Anopheles gambiae* s.s. both in semi-permanent and permanent water pools (Gimnig *et al*., 2001, Fillinger *et al*., 2004; Imbahale *et al.*, 2011b). These two main vectors often inhabit small and sunlit temporary water pools (Gimnig *et al*., 2001; Minakawa *et al.,* 2004; Imbahale *et al.,* 2011b).

2.3.2. *Anopheles* habitat preferences and productivity

Habitat productivity in terms of larval density and abundance is a key factor to consider when looking at *Anopheles* larval habitats. Different factors are reported to influence larval productivity, some of the factors mostly reported are seasons (Mala *et al.,* 2011; Shililu *et al.,* 2003), human activities that alter land use/cover (Jacob *et al.,* 2007; Adekele*et al.,* 2013) and age of water in the larval habitats (Munga *et al.,* 2013). A study conducted in Eritrea reported more ephemeral larval habitats during the rainy seasons compared to dry seasons (Shililu *et al.,* 2003). However, year-round breeding has also been reported for *An. arabiensis* and *An. funestus* in villages in Baringo, Kenya (Mala *et al.,* 2011). Permanent water sources available during the dry seasons provide larval seed for the new rain fed habitats that freshly form after rains start (Mala *et al.,* 2011). *An. arabiensis, An. funestus* and *An. gambiae* have also been reported to occur in dry seasons supported by natural habitats like stream bed pools (Dida *et al*., 2018). Several studies have reported a positive association between human activities and larval productivity. Agricultural practices are the main human activity type reported to alter larval productivity.

A study conducted in Southwestern Nigeria (Adekela *et al.,* 2013) reported high *Anopheles gambiae* complex larval densities in highly flooded agricultural sites (Adekela *et al.,* 2013). Habitats located in agricultural land use land cover (LULC) change sites were reported to contain more *Anopheles* larvae compared to those located in LULC unchanged sites (Jacob *et al.,* 2007; Dongus *et al.,* 2009). Water age is another factor that has been reported to seriously influence larval productivity and *Anopheles* habitat preferences. Different water ages have been reported to contain different *Anopheles* species. As observed in western Kenya highlands, fresh water was more productive for *Anopheles gambiae s.l.* (Munga *et al.,* 2013) compared to old-age waters. Presence of predators like backswimmers and tadpoles (Mahgoub *et al.,* 2017) are some of the factors reported to contribute to low productivity of old-age water. Cannibalism and other complex interactions have also been reported to

influence stable age distribution of larval *Anopheles* stages (Edillo *et al*., 2004). Ecological succession of *Anopheles* larval habitats has also been associated with habitat age. It is reported that as habitats age they become less suitable for *Anopheles gambiae s.l.* but more suitable for *Anopheles coustani* (Kiszewki *et al*., 2014).

2.4. Malaria in the context of Ecosystem health

Malaria transmission patterns are largely dictated by the local *Anopheles-Homo-Plasmodium* relationships, which determine the level of contact between humans and mosquito vectors (Keiser *et al.*, 2005). The risk of acquiring malaria varies significantly between ecosystems and livelihoods (Mboera *et al*., 2015). There is a strong association between human activities and malaria and several studies attest to this (see review by Mboera *et al*., 2013a). The relationship between malaria and human activities can be explained from an ecological standpoint. In general, human activities linked to supporting livelihoods are responsible for ecosystem changes that enhance mosquito breeding. As humans strive to improve their socioeconomic status, they inadvertently create suitable breeding habitats for mosquito vectors. This is seen in crop farming (Diuk-Wasser *et al.*, 2007; Yasuoka and Levins, 2007; Jarju *et al.*, 2009), mineral extraction (Yapabandara and Curtis, 2004), fish pond construction (Maheu-Giroux *et al.*, 2010), brick making (Carlson *et al.*, 2004) and dam construction activities (Keiser *et al.*, 2005), among others.

Livelihood activities that are conducted outdoors at night are known to expose actors to a very high risk of acquiring malaria (Escovar *et al.*, 2013). The peak biting times of *Anopheles* mosquitoes mainly occur at night and during twilight (Durnez and Coosemans, 2013; Sougoufara *et al*., 2014). Occupational groups exposed to such risk include military personnel (Tuck *et al.*, 2003), rubber plantation workers (Pattanasin *et al.*, 2012) and fisherfolk (Escovar *et al.*, 2013). The relationship between ecosystem health, malaria and some of these livelihood activities are described below. This thesis mainly focuses on the relationship between malaria and fishing, hence the extensive review further below.

2.4.1 Malaria and agriculture

The link between crop agriculture and malaria can be explained from the context of production systems. Farm locations, different farming practices and farming technologies can create ecosystem changes leading to creation of suitable breeding habitats for malaria vectors. Human-vector-parasite contact is enhanced as a result of new breeding habitats created and this in turn facilitates the spread of malaria (Keiser *et al*., 2005). Ground digging to plant

crops is one of the ways through which mosquito breeding habitats are created (Jarju *et al*., 2009). Irrigated rice cultivation is probably the best known agricultural production system that enhances mosquito breeding. For example, one study in Mali reported an increase in *An. gambiae* density in a rice agroecosystem (Diuk-Wasser *et al.*, 2007). Another study conducted in western Kenya also reported increased breeding of *An. gambiae* and *An. arabiensis* in rice paddies (Imbahale, 2011a). Several other studies in central Kenya have also confirmed prolific breeding of *Anopheles* mosquitoes in rice paddies (Mwangangi *et al*., 2006a; Mwangangi *et al.*, 2006b; Muturi *et al*., 2007; Muturi *et al*., 2008; Mwangangi *et al*.,2008; Mwangangi *et al*.,2010). The relationship between malaria, sociocultural and socioeconomic factors linked to farming has also been reported in several studies (Somboon *et al*., 1998; Matthys *et al*., 2006; Pattanasin *et al*., 2012). These studies demonstrate how overnight stay in farming huts exposes farmers to malaria. The temporary farm structures used are located next to the rice fields and are porous to entry of malaria mosquitoes.

Furthermore, farming is usually done during the rainy seasons, which is the time when malaria prevalence is also high. At this time of the year farmers are usually under too much pressure to get a lot of work done around their farms. This means that a case of malaria can cause delays and loss of labor (man hours). Money may also be lacking for medication during such times because most people have usually exhausted their income reserves. This underscores the relationship between malaria and sociocultural/economic factors.

2.4.2 Malaria and soil excavations

Mining causes environmental degradation which leads to creation of breeding habitats of malaria mosquitoes. Excavation of soil from the ground to make bricks has been reported to form pits that accumulate water mainly during the rainy seasons. The brick pits are suitable breeding habitats for malaria mosquitoes. A study conducted in western Kenya confirmed high malaria transmission resulting from the brick pits (Carlos *et al*., 2004). In this study it was shown that brick pits had the highest number of *Anopheles* larvae and houses located next to the pits had higher numbers of adult mosquitoes compared to those that were further away. Mosquito larvae were found both in the abandoned and functional brick pits (Carlos *et al*., 2004). This was also the case in the Gambia (Majambere *et al*., 2008). In another study excavation of soil to re-plaster houses and/or make bricks created *Anopheles* breeding habitats that sustained malaria transmission during the dry season in the United Republic of Tanzania (Mboera *et al.,* 2013). Cement lined pits have also been reported as larval habitats

in Western Kenya (Fillinger *et al.*, 2004). According to this study *Anopheles* larvae were found to colonize the cement lined pits throughout the year.

2.4.3. Malaria and fishing

Fishing broadly entails aquaculture, which involves rearing fish under controlled conditions, and capture fishing, which involves hunting for fish in the wild. Although this thesis mainly focuses on the link between malaria and (artisanal) capture fishing a short treatise of the link between malaria and aquaculture is given immediately below.

2.4.3.1. Malaria and fish aquaculture

As early as the 1950s, the practice of fish farming had begun in the western parts of Kenya. These early fish farmers faced enormous, unanticipated challenges which forced many of them to abandon their fish ponds (Lockhart *et al*., 1969). Abandoned and active fish ponds can both serve as breeding habitats for *Anopheles* mosquitoes (Fletcher *et al*., 1992; Vittor *et al*., 2009; Imbahale *et al*., 2013) depending how they are maintained. A study done by Howard and Omlin (2008) in western Kenya demonstrated that aquatic stages of the Afrotropical malaria vector *An. gambiae* breeds both in abandoned and active fish ponds. In fact this was the most abundant mosquito species observed in fish ponds by Howard and Omlin (2008). Location of the fish ponds determines their contribution to malaria transmission. Ponds located near occupied houses are generally associated with a higher risk of malaria to house occupants (Simpson, 2006; Vittor, 2009). Finger ponds that are dug next to the existing water bodies by farmers to help them in irrigation also serve as suitable breeding habitats for malaria mosquitoes (Kipkemboi *et al*., 2007). These ponds are proven to harbor fish especially tilapia (Bailey *et al.*, 2005; Kipkemboi *et al.*,2006; Van Dam *et al*., 2006) but mosquitoes inadvertently breed in them. These studies underscore the relationship between aquaculture and malaria with respect to mosquito larval breeding.

2.4.3.2. Malaria and capture fishing

Fishing in most parts of the developing world is usually artisanal and various activities revolving around this industry place the fishermen at the risk of getting bites from *Anopheles* vectors of malaria (Escovar *et al.*, 2013). A study in South America demonstrated that activities involving catching fish and gathering fishing bait (crabs) from mangroves and marshlands coincided with biting times of a local malaria vector namely *An. neivai* (Escovar *et al.*, 2013). A study conducted by Omwega and others (2006) in western Kenya reported malaria as one of the leading diseases affecting fishermen along the shores of Lake Victoria.

In general fishing activities are carried out at night and during twilight when malaria mosquitoes are most active (Mathenge *et al.,* 2001; Durnez and Coosemans, 2013). Furthermore, actors spend a significant amount of time along the lake shores where malaria mosquitoes breed (Minakawa *et al*., 2012).

Associations between malaria and fishing activities have been documented in several other studies. In India a study reported nocturnal fishing as an enhancing factor for malaria transmission in rural areas (Barai *et al*., 1982). Diurnal activities have also been reported in Colombia to be exposing fishermen to a high risk of being bitten by *An. neivai,* i.e. the major malarial vector in the region (Carvajal *et al*., 1989; solarte *et al*., 1996). A study in the Brazilian Amazon reported that nocturnal fishing activities facilitate outdoor malaria transmission due to bites by malaria mosquitoes that prefer to rest and bite outdoors (Sa *et al*., 2005). Malaria episodes have also been reported among seamen engaging in the fishing industry in Lithuania (Scerbaviciene & Pilipavicius, 1999; 2009).

Migration by fishermen looking for higher fish catches has also been reported to increase the risk of exposure to malaria. For example, a study in Colombia reported that movement of fisherfolk around the river Naya basin increased their exposure to malaria (Sevilla-Casas, 1993). In this specific study, fishermen were reported to travel from South America, Africa and Asia into the Naya river basin which is a malaria endemic area. In Western Kenya, fishermen are reported to depart from their homes to various fishing beaches of Lake Victoria to participate in capture fishing (Omwega *et al*., 2006, Nunan, 2010; Nunan, 2018). A study conducted in Poland reported cases of malaria in fishermen and seafarers from endemic areas of West Africa (Jaremin *et al*., 1993; Jaremin *et al*., 1996).

A study done in West Africa by Akogbeto (2000) reported high malaria transmission in coastal areas and lagoons in Cotonou and this resulted from the high densities of *An. gambiae* and *An. melas*. A study in Mali reported high densities of adult and larval stages of malaria mosquitoes in fishing hamlets (Sogoba *et al*., 2007). In this study it was postulated that the fishing village was responsible for high malaria transmission in the neighboring villages experienced before the rainy season. Yet another study conducted in Uganda reported high malaria prevalence rates among pregnant women in a fishing community near Entebbe (Woodburn *et al*., 2009). These studies demonstrate the intricacies between malaria, outdoor biting of mosquitoes and capture fishing.

2.4.3.3. Complications of malaria control in fishing communities

Fishing communities comprise of fishermen, fish traders and fish processors, among other actors. Many people are involved in the fishing industry and malaria has been reported as one of the leading causes of death among fishing communities (Omwega *et al*., 2006). This underscores the importance of reviewing in detail the complications encountered in controlling this deadly vector borne disease in the fishing communities. The high vectorial capacity of *Anopheles* mosquitoes that transmit malaria is responsible for maintaining high malaria transmission in endemic areas in Africa. The three main vectors of malaria in Kenya namely *An. arabiensis, An. funestus* and *An. gambiae* were found to be breeding in the litoral zones of Lake Victoria (Minakawa *et al*., 2012). Environmental conditions associated with the lake were responsible for maintaining the vectors. The study reported *An. rivulorum,* which is a secondary malaria vector, to be breeding in the water hyacinth.

The fishing industry basically forms the main source of income for the fisherfolk in Kenya (Njiru, 2008; Nunan, 2010; Nunan, 2015; Nathenson *et al*., 2017). The fisherfolk continually exploit the diminishing fish stocks (Fiorella *et al*., 2015) and the resulting catches are hardly eaten by the members of the fishers' households (Fiorella *et al*., 2014). Poverty in the fishing communities has led to overexploitation of fisheries resulting in too much pressure being exerted on the environment (Larsen *et al*., 2018). The strategies employed by the fisherfolk to cope with the pressure have also lead to increased risk of malaria. Movement of fisherfolk in search of 'greener pastures' and bigger markets (Nunan, 2010; Nunan, 2018) increases their exposure to malaria (Sevilla-Casas, 1993; Jaremin *et al*., 1996). The use of modern fishing methods (Fiorella *et al*., 2017) is also expensive for most fisherfolk hence they prefer traditional methods which inadvertently create mosquito breeding habitats (Mukabana *et al*., submitted). The search of fish baits (Mkumbo & Mlaponi, 2007) during peak biting hours of *Anopheles* mosquitoes also exposes collectors to a high risk of being bitten (Escovar *et al*., 2013). A study conducted in Uganda by Kitakule and Reynolds (1991), cited malaria as a health hazard among poor people involved in fishing in Lake Victoria. In that study, most fisherfolk were reported to be living in temporary houses which were porous to mosquitoes.

The frontline malaria vector control strategies, which include LLINs and IRS (WHO, 2017), do not confer protection to people in the fishing industry. Actors in this industry spend a significant proportion of nights outdoors, during the peak biting times of *Anopheles* mosquitoes (Durnez& Cooseman, 2013). This is especially so along the lakeshores where most malaria mosquitoes reproduce (Minakawa *et al*., 2012). Unfortunately the LLINs are

abused by being put to unintended uses in fishing communities especially in Africa. A study conducted in western parts of Kenya reported use of LLINs for fish capture and drying (Minakawa *et al*., 2008). In Southeast Asia, Democratic Republic of Timor-Leste, bed nets have been used to capture small sized fish and shrimps (Lover *et al*., 2011). A recent study in Zambia has also reported widespread mosquito net fishing in the Barotse flood plains (Larsen *et al.,* 2018).

2.5. Malaria prevention and control strategies

Malaria control strategies mainly target *Plasmodium* parasites and *Anopheles* vectors (WHO, 2017). Parasite control involves the use of effective anti-malarial drugs (Wistanley & Ward, 2006), notably Artemisinin-based Combination Therapies (ACTs) (Banek *et al*., 2014; WHO, 2017). Vector control strategies are largely based on using LLINs and IRS, both of which remain the most effective malaria transmission preventive strategies (WHO 2017). The World Health Organization Global Malaria Program (WHO/GMP) advocates for the combined use of three malaria interventions namely ACTs, LLINs and IRS. The combined use of these three interventions is known to be effective in reducing malaria cases (Bhattarai *et al.*, 2007; Ceesay *et al.*, 2008; O'Meara *et al.*, 2008; Chizema-Kawesha *et al.*, 2010; Ngomane & de Jager, 2012).

The foraging and resting behavior of African malaria mosquitoes (i.e. anthropophagy, endophagy and endophily) is the functional basis of using LLINs and IRS. Several studies have documented the effectiveness of LLINs and IRS against indoor mosquitoes that are active at night (Mabaso *et al.*, 2004; John *et al.*, 2009; Kleinschmidt *et al.*, 2009; Pluess *et al.*, 2010; Hamel *et al.*, 2011; Okumu & Moore, 2011; Bekele *et al.*, 2012; Fullman *et al.*, 2013). However, vectors that mediate residual transmission by virtue of being zoophilic, exophagic and exophilic are missed by these front-line interventions (Pates & Curtis, 2005; Tirados *et al.*, 2006; Geissbuhler *et al.*, 2007; Griffin *et al.*, 2010; Van Bortel *et al.*, 2010; Yohannes & Boelee, 2012). LLINs act as a physical barrier between the person sleeping under them and host seeking mosquitoes. LLINs are impregnated with insecticides, which are proven to be very effective in providing protection against mosquitoes (Lengeler, 2004; Muller *et al.*, 2006). Other studies even show that LLINs have community level effects against unprotected individuals (Killeen & Smith, 2007; Le Menach *et al.*, 2007). The LLINs are impregnated with pyrethroid insecticides that repel mosquitoes, so reducing house entry (Lines *et al.*, 1987; Miller *et al.*, 1991). They also kill mosquitoes that get in contact with them. A study done by Charlwood and others (2001) documented the community wide effect of LLINs.

Reduced survival rates and mosquito densities are among the other reported effects (Magesa *et al.*, 1991; Robert & Carnevale, 1991).

Use of mosquito repellents has also been documented for malaria prevention and control (Rozendaal, 1997). A study done by Killeen and Moore (2012), discusses a vapor repellant which is sprayed in space and does not need to be applied onto the skin or on cloths (spatial repellent) hence effective for preventing outdoor malaria transmission. Insecticide-treated clothes have also been reported to reduce malaria transmission (Rowland *et al.*, 1999; Macintyre *et al.*, 2003; Kimani *et al.*, 2006)

Larval source management (LSM) is yet another vector control option. The use of microbial insecticides (Fillinger & Lindsay, 2006; Fillinger *et al.*, 2011; Tusting *et al.*, 2013) and environmental management tools (Killeen *et al.*, 2002; Keiser *et al.*, 2005) are some of the documented LSM strategies that have effectively reduced malaria transmission. However, LSM must be tailored to local environmental conditions (with respect to mosquito breeding ecology) and needs community support in order to be successful. Biological control for malaria mosquitoes has also been reported in the port city of Assab by Fletcher and others (1992). Use of fish as a mosquito predator reduces mosquito larval density in stagnant water, though it is unclear whether this approach reduces malaria prevalence in communities (Walshe *et al.*, 2017). Since each of the different malaria control strategies applied singularly may not be effective, it is advisable to combine the different strategies to maximize benefits.

2.6. Challenges in malaria elimination

Over the last few years malaria burden has been on the decline. This has been attributed to the fore-line interventions, which include ACTs, IRS and LLINs (WHO, 2013; WHO, 2017). However, challenges have been encountered in malaria prevention and control and for its elimination to be achieved these challenges need to be addressed (Cotter *et al.*, 2013, WHO 2016). Several studies have been done documenting some of these challenges (Sulistyaningsih *et al.*, 2010; Ranson *et al.*, 2011; WHO, 2012; Tulloch *et al.*, 2013, Liu *et al.*, 2013; WHO 2018). The WHO thinks the fact that malaria is widespread (WHO 2017) makes its worldwide elimination almost impossible due to many other logistical problems like different economic status and interests among endemic countries.

Extensive and long term use of insecticide-based interventions has significantly increased the fraction of malaria mosquitoes biting outdoors at night. These mosquitoes, which mediate

residual transmission of malaria by virtue of being zoophilic, exophagic and exophilic, are not targeted by IRS and LLINs (Bekele *et al*., 2012; Fullman *et al*., 2013). Furthermore, resistance by female *Anopheles* mosquitoes to insecticides has also been reported (Ranson *et al*., 2011). Parasite resistance to currently used antimalarial drugs (i.e. ACTs) has also been observed in Cambodia, Thailand and Burma (WHO, 2017).

Movement of people in search for greener economic pastures has been reported in several studies as a coping strategy among fishers seeking to increase their fish catches (Nunan, 2010; Nunan, 2018). However, this mobility increases exposure to malaria (Sevilla-Casas, 1993; Jaremin *et al*., 1993; Jaremin *et al*., 1996) and has made it almost impossible to contain malaria in areas were eradication could be possible. A study done by Liu and others also cited movement of people in malaria endemic areas as a cause of re-introduction of the disease in areas where it had been cleared (Liu *et al*., 2013).

Other challenges in malaria elimination include limited knowledge about malaria parasites. For example, not much is known about *Plasmodium vivax* i.e. the second most important malaria parasite after *Plasmodium falciparum.* This makes early diagnosis difficult until the parasite (*P. vivax*) has infected vital organs like the liver. These infections often remain dormant leading to relapses of malarial disease (Sulistyaningsih *et al*., 2010). Other malaria parasites, known to have a zoonotic reservoir like *Plasmodium knowlesi*, are difficult to eradicate (Liu *et al*., 2013). *Plasmodium knowlesi* was previously known to only infect old world macaque monkeys (Garnham, 1966; Lee *et al*., 2011) but has more recently been reported to have widespread distribution (Cox-Singh *et al.*, 2008; White, 2008; Figtree *et al.*, 2010; Khim *et al.*, 2011; William *et al.*, 2011; Goh *et al.*, 2013). Human conflicts as well as lack of adequate funds also hamper malaria control efforts (Liu *et al*., 2013).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study area

This study was carried out on Mageta Island located inside Lake Victoria (figure 6) in Siaya County in Western Kenya. Mageta lies very close to the Kenya-Uganda border. Administratively, Mageta Island (surface area, 7.24sq km) together with Magare Island (surface area = 0.27sq km) and the uninhabited Sirigombe Island constitute Mageta Location (coordinates: 33^{o} 59'2''E, 0^{o} 7'19''S and 34^{o} 3'10''E, 0^{o} 8'38''S). Mageta location has a population of approximately 7,000 persons and is adjacent to the islands of Wayasi, Siamulala, Hama, Siro and Lolwe with Uganda. Mageta Island lies at altitudes ranging between 1,125-1,174m above sea level.

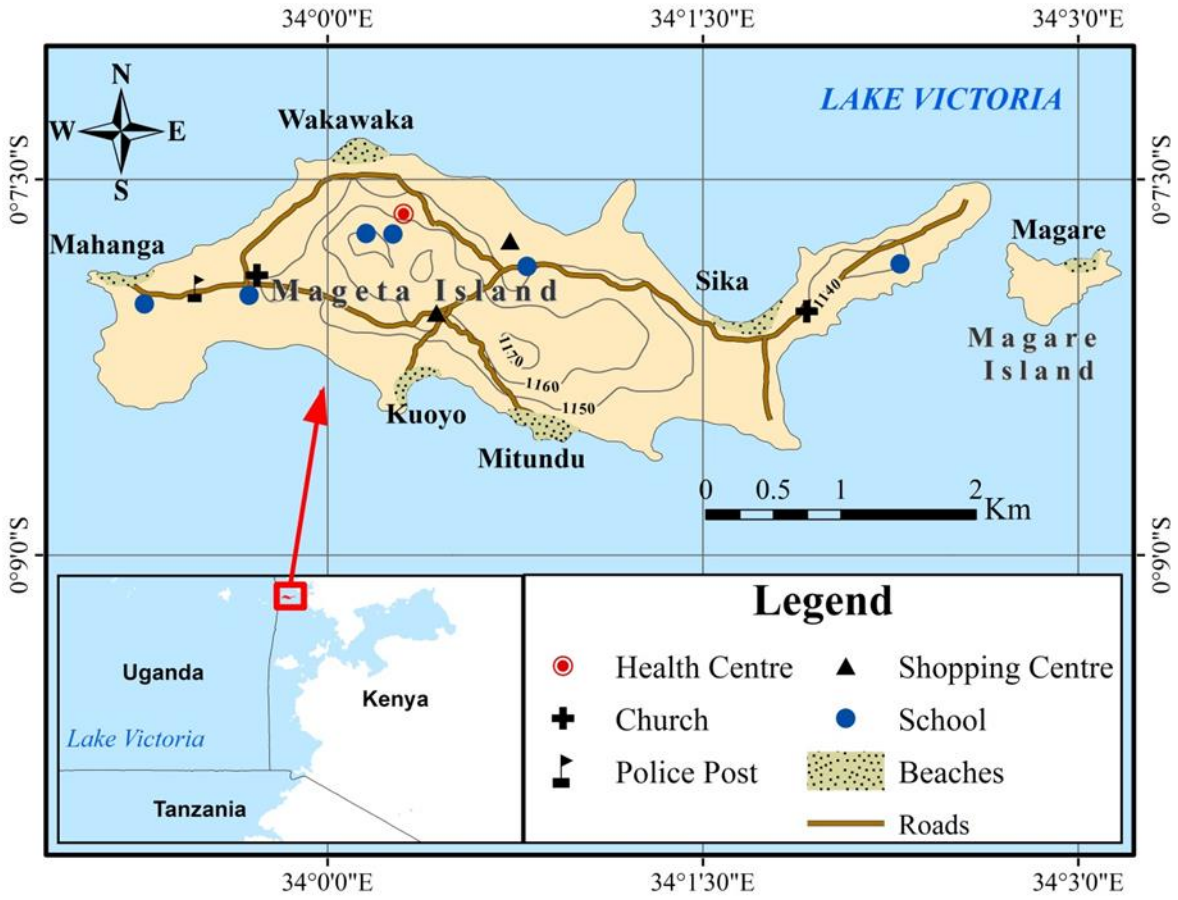

Figure 6: Study area map showing location of Mageta and Magare Islands in Western Kenya.

Mageta location has six fishing beaches namely Kuoyo, Magare, Mahanga, Mitundu, Sika and Wakawaka. The main fish species caught around Mageta include tilapia (*Oreochromis niloticus*), Nile perch (*Lates niloticus*) and the silver cyprinid (*Rastrioneobola argentae*). Other common fishes include species of *Haplochromis*, *Mormyrus* and *Protepterus*. Fresh

water shrimps (*Caridina nilotica*) and crabs are also common. These fish and crustaceans are caught using rudimentary methods in a locally active artisanal fishing industry, which constitutes the main livelihood activity for the local inhabitants. Animal husbandry and small scale crop farming are also practiced on Mageta Island. Crop agriculture is concentrated mainly to the island's muddy, northern shores. The dominant crop plants include beans, collard greens (*Brassica oleracea*) of the cultivar *Acephala,* maize and tomatoes. Tangerines are also present. Domesticated animals include cats, cattle, chicken, dogs, donkeys, geese, goats, pigs and sheep**.** Wild animals include lizards, snakes, monkeys, crocodiles, species of the otter and night-grazing hippopotami. Lake flies and many different avian species abound. The plants and animals most probably serve as sugar and blood meal sources for local mosquito species, respectively.

The main malaria mosquitoes on Mageta Island include *Anopheles gambiae, An. arabiensis, An. funestus* and *An. coustani* (Ogola *et al.,* 2017). *Plasmodium falciparum* is the main malaria parasite on the Island (pers. comm., Mageta Health Centre). *Anopheles gambiae* and *An. funestus* prefer to blood-feed on humans rather than animal hosts (Sinka *et al*., 2010). They also feed at night (Sinka *et al*., 2010) and rest indoors (Sinka *et al*., 2010). Unfortunately, the readily available malaria protection measures namely long lasting insecticidal nets target indoor, night-biting/resting mosquitoes only. Thus, persons engaging in outdoor socioeconomic activities at night, e.g. the artisanal capture fishers of Mageta Island remain unprotected (Olanga *et al*., 2015). This protection gap, together with increased resistance by malaria mosquitoes and parasites to hitherto effective insecticides and drugs (Bhattarai *et al*., 2007), respectively, escalates the vulnerability of local inhabitants of Mageta Island to malaria. Innovative, locally sustainable mitigating approaches are urgently needed.

3.2. Artisanal capture fishing and creation of *Anopheles* larval habitats

The central aim of this observational study was to find out if activities associated with artisanal capture fishing facilitate creation of stagnant water bodies suitable for breeding of malaria mosquitoes. Thus, the research team sought to find, ecologically characterize and map all potential mosquito larval breeding habitats present on Mageta Island, which includes Magare Island in the context of this work. This exercise was carried out through a cross-sectional survey, with community health volunteers, hereafter referred to as community actors, as liaison.

The community actors served to introduce members of the research team to community members whose farms had potential mosquito breeding habitats. The putative mosquito larval

habitats were accessed by walking from one to the next in each of the 22 villages on Mageta Island. The search focused on identifying all holes, depressions, grooves, furrows, gutters and all earthen, wooden or other containers that held stagnant water where *Anopheles* larvae may have lived. Characterization of habitats involved engaging community actors in an ecological dialogue (Smith & Morrow, 1991) that helped to succinctly describe individual habitats types. Discussions focused on how and why the habitats were created, even if inadvertently.

Habitat types created through artisanal capture fishing activities or otherwise were respectively classified as 'fishing habitats' or 'non-fishing habitats'. The outcome of finding ('*Anopheles* present') or not finding malaria mosquito larvae ('*Anopheles* absent') inside the habitats was determined and recorded. Analysis of the correlation between habitat types as a predictor of *Anopheles* presence in the habitats was used to fill up the research gap. Larval sampling was performed using a 350ml WHO dipper. Up to ten dips were taken from each larval habitat and inspected before a decision on presence (or absence) of *Anopheles* larvae in individual habitats was reached. Mapping of individual mosquito larval habitats was done with the aid of a GPS receiver (Garmin eTrex® 10). The GPS data points were transferred into ArcGIS software (10.2.2) and used to develop a spot map.

3.3. Artisanal capture fishing and *Anopheles* larval productivity

The aim of this part of the survey was to evaluate the effect of artisanal capture fishing on *Anopheles* larval density on Mageta Island. We sought to verify, and understand, if and how artisanal capture fishing contributes to *Anopheles* larval productivity. Causal effects were measured by determining the statistical relationship between habitat type (i.e. 'fishing' versus 'non-fishing') and the counts of *Anopheles* larvae found inside individual habitats. Habitat type was further classified using bottom surface type (i.e. wooden, rocky and muddy) as a possible mediator of this association (Minakawa *et al*., 1999). Three potential qualitative moderators of this relationship namely emergent plants (Gimnig *et al*., 2001), direct sunlight (Minakawa *et al*., 1999; Gimnig *et al*., 2001) and fish predators (Walshe *et al*., 2017; Gachelin *et al*., 2018) were also assessed. Each moderator was classified into two categorical predictors of *Anopheles* larval productivity i.e. exposure versus non-exposure of habitats to direct sunlight, presence versus absence of emergent plants in habitats and presence versus absence of fish predators in the habitats. These classifications of variables were superseded by diligent larval sampling using a WHO standard dipper (350 ml).

Mosquito sampling was done between 0900-1100 hours in all stagnant water bodies found on Mageta Island. Sampling was done when the water was calm and a moving shadow was not cast into the water to prevent the larvae from diving. The dipper was lowered gently at an angle of 45^{o} below the water surface to allow larvae to flow into it (figure 7). Sampling was done around floating debris and edges of habitats because larvae prefer such areas (WHO, 2013) and may not be evenly distributed on the water surface. The contents of the dipper were emptied into a white tray to help visualize the larvae.

Figure 7: Author of this thesis using a dipper to sample larvae from a mosquito breeding habitat on Mageta Island.

Anopheles mosquito larvae collected from each one of the 10 dips were counted and the number recorded into data entry sheets (Appendix 2) for individual habitats. The *Anopheles* larvae from each habitat were gently transferred into separate 1.5ml eppendorf tubes half filled with ethanol to preserve larval morphology. The tubes were then labeled by inserting a piece of paper written in pencil with the details corresponding to the larval habitat and stored. The stored samples were transported to the laboratory at the School of Biological Sciences, University of Nairobi, pending further processing.

Identification of *Anopheles* larvae was done morphologically using the keys of Gillies and Coetzee (1987) as shown in Appendix 3. A further identification of individual members of the *An. gambiae* complex (a group of morphologically indistinguishable sibling mosquito species) was done using a ribosomal DNA-polymerase chain reaction method (Scott *et al*., 1993), as described in Appendix 4. A total of 100 larvae of *An. gambiae* complex were subjected to confirmatory DNA tests to identify them to species level (Scott *et al.,* 1993).

3.4. Mapping malaria hazard on Mageta Island

This exercise sought to perform spatial analysis of *Anopheles* larval densities on Mageta Island. The aim was to provide a visual representation of areas with the highest *Anopheles* larval concentrations on the Island. To do this the researcher stood at the edge of a habitat holding a GPS receiver (Garmin eTrex® 10) above the water surface and captured the

geographic coordinates (latitude, longitude and elevation). The data were later transferred from the GPS receiver into ArcGIS software (10.2.2) and used to develop a hazard map.

Two important layers were considered in developing the hazard map. The base layer consisted of the geographic locational map of Mageta Island. This provided the template over which subsequent data were overlaid. The second layer consisted of the mapped *Anopheles* larval breeding habitats on the Island. Since the *Anopheles* larval density of each habitat had been predetermined (section 3.3), this raster layer of larval density was incorporated to complete the hazard map. This end product made it possible to quickly visualize *Anopheles* larval hotspots on Mageta Island.

3.5. Data analysis

A logistic regression model of the form "Logit (*Anopheles* present) = $\beta_o + \beta_1$ habitat type", where β_o is the intercept of the regression line on the y-axis and β_1 is the gradient, was fitted. This model was used to make sense from data collected in the cross-sectional survey and to describe the probability of finding *Anopheles* larvae in habitats created through artisanal capture fishing. Habitat type, classified as 'fishing' (coded '1') or non-fishing' (coded '0') depending on whether or not the habitats were created from activities associated with artisanal capture fishing, respectively, was used as the predictor variable. Habitat content i.e. presence (coded '1' or absence (coded '0') of *Anopheles* larvae in the stagnant water bodies constituted the response variable. A P-value of 0.05 or less denoted a significant effect of artisanal capture fishing on presence of malaria mosquitoes in water. The numbers of *Anopheles* larvae collected in 10 dips during the second cross-sectional survey were summed for individual larval habitats. The effect of human activities, livelihoods, habitat bottom surface type and artisanal capture fishing on the number of mosquito *Anopheles* larvae present in habitats was modeled using generalized linear models (GLM) with a Poisson distribution and a log link function (Field, 2011). Potential moderators of this relationship namely habitat bottom surface type, exposure to direct sunlight and the presence of emergent plants or fish predators in habitats were also considered in the models. All data were analysed using version 23 of the IBM Statistical Package for the Social Sciences (SPSS). A spot map showing spatial distribution of the different mosquito larval habitat types on Mageta Island was developed using ArcGIS software (10.2.2).

CHAPTER FOUR: RESULTS

This work was carried out in July 2017 during the dry season. Seventy seven percent of *Anopheles gambiae* s.l. larvae recovered from Mageta Island were identified as *An. gambiae* s.s. No other *Anopheles* mosquito species were found on the island.

4.1. Artisanal capture fishing and creation of *Anopheles* larval habitats

a) Habitat types

A total of 87 mosquito larval habitats were identified on Mageta Island. The habitats were classified into eight different types (figure 8). The habitats included rock pools (n = 32; 36.8%), wooden boats (n = 24; 27.6%), swamps (n = 11; 12.6%), ditches (n = 7; 8%), lagoons (n = 7; 8%), fish ponds (n = 3; 3.4%), fish bait mines (n = 2; 2.3%) and trenches (n = 1; 1.1%).

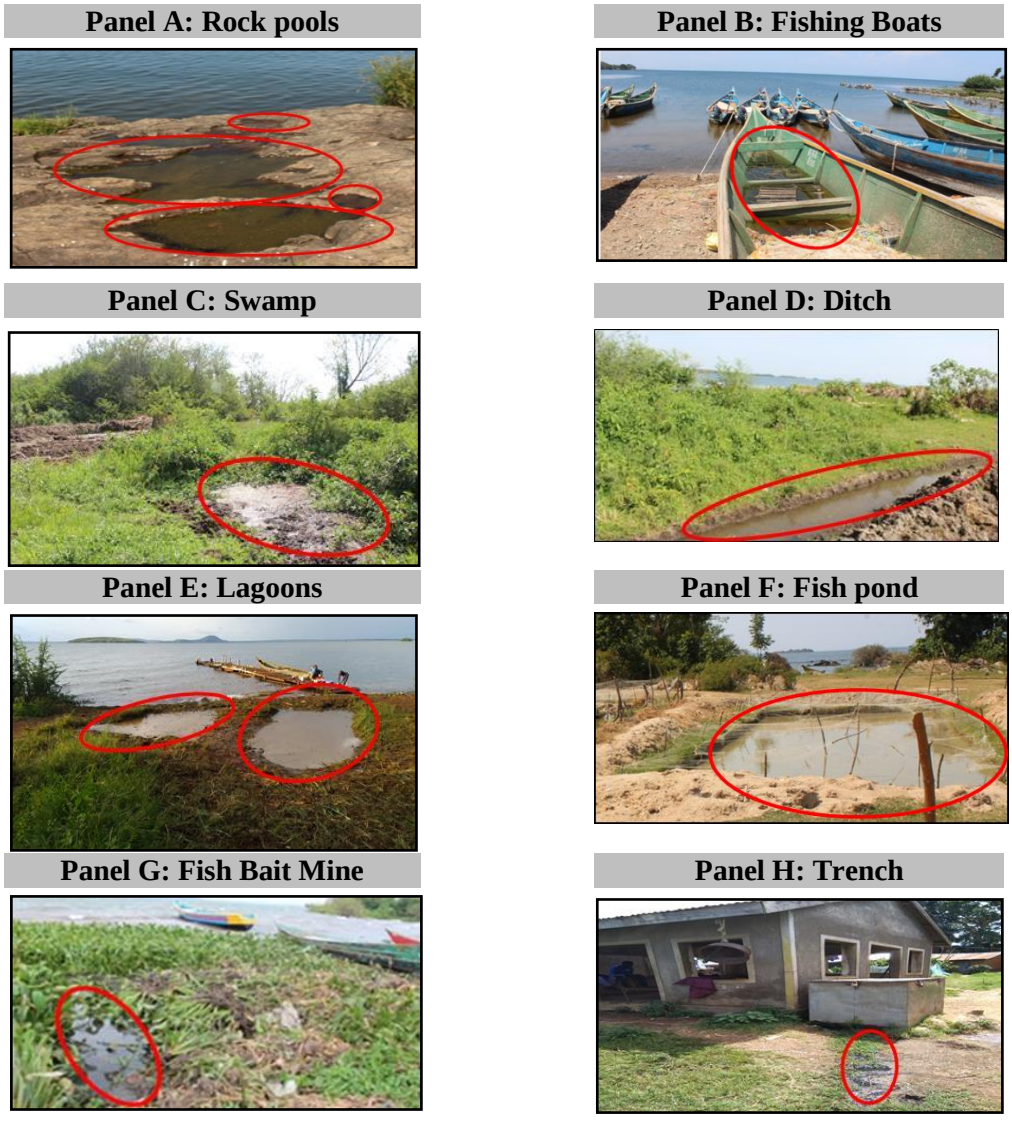

Figure 8: Mosquito larval habitat types found on Mageta Island in western Kenya. Areas with stagnant water are circled with red.

A spot map developed (figure 9) to show distribution of the various habitat types showed most of them to be on the eastern, western and southern shores of the Mageta Island. Fishing habitats (n=27; 31%) included all fishing boats (23), all fish bait mines, trenches and one ditch used as a docking place for a fishing boat. Non-fishing habitats (n=60; 69%) included all rock pools, swamps, lagoons, ditches (6), fish ponds and one boat used for public transport.

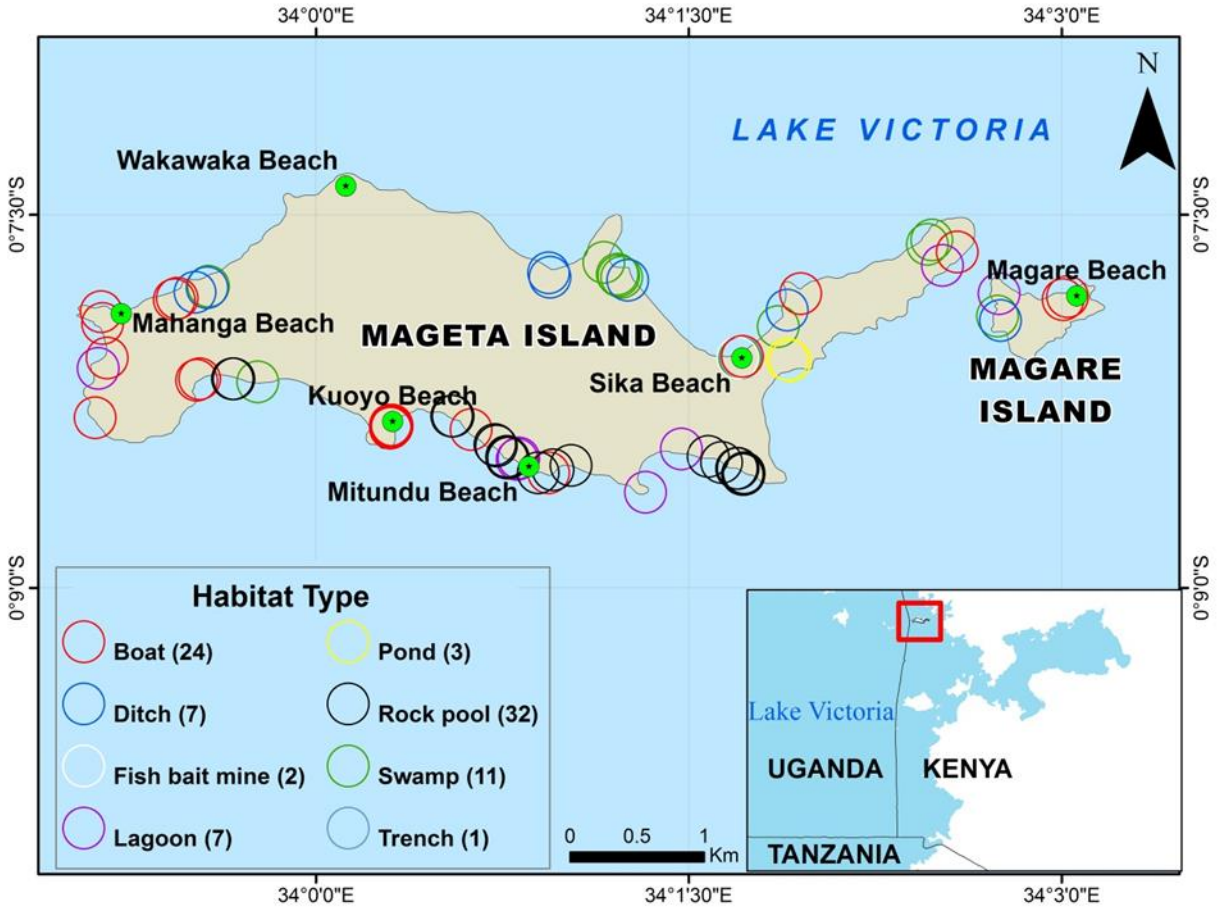

Figure 9: Geographical location of *Anopheles* larval habitats on Mageta and Magare Islands. The center of the rings is the exact location of the habitats. The figure legend details the different habitat types.

b) Origin of mosquito breeding habitats

Identified habitats were largely associated with man's efforts to support livelihoods. For example, at the end of each fishing round fishers customarily fetch and pour fresh lake water in fishing boats after parking them ashore. Ditches, which are normally sunk below the water table, were created to prevent access of night-grazing hippopotami to food crops. The ditch water was also used to irrigate crop plants and, in one special case, a ditch was used for docking a fishing boat. The three fish ponds found on Mageta Island were used to culture edible fish i.e. tilapia. Stagnant water pools associated with fish bait mines resulted from

excavation of earthworms from wet soil using bare hands or removing rocks/stones from crab traps. The earthworms and crabs were used locally as fish baits. Most temporary housing structures used for primary fish processing were connected to dug-out trenches. The trenches often contained water originating from cleaning activities or slow-melting ice blocks present in leaky, locally-made cooler boxes used for temporary storage of captured fish. The identified natural habitats were largely created through wave action on Lake Victoria. Waves deposited water near the shoreline in depressions on rocks to form rock pools, in the littoral zone to form swamps and behind sand bars to form lagoons (Minakawa *et al*., 2012). In all cases adult gravid female mosquitoes laid eggs in the stagnant water bodies, which acted as larval breeding resources.

c) Presence of Anopheles larvae in habitats

All the eight different habitat types identified on Mageta Island with the exception of fish bait mines, fish ponds and trenches contained *Anopheles* larvae. Stagnant water bodies containing *Anopheles* larvae formed about one half of all putative habitats (50.6%; 44/87). In terms of fishing verses non-fishing habitats, 77.8% (21/27) and 38.3% (23/60) of habitats contained *Anopheles* larvae, respectively. These data underscore the importance of artisanal fishing on the epidemiology of malaria on Mageta Island. Boats recorded the highest percentage of the fishing habitats that contained *Anopheles* larvae (90%; 20/23). The other fishing habitat that contained *Anopheles* larvae was the ditch used for docking a fishing boat.

The fitted logistic regression model ($\chi^2 = 12.11$, df = 1, N = 87, $p < 0.001$) found a significant negative association between artisanal capture fishing and presence of *Anopheles* mosquitoes in habitats (P = 0.001) (figure 10). The odds of finding *Anopheles* larvae in a habitat was 0.173 (95% CI = 0.062 – 0.505). This indicated a general decrease in the likelihood of finding *Anopheles* larvae in habitats for every unit increase in habitats associated with artisanal capture fishing. This showed the presence of other factors of malaria endemicity on Mageta Island beyond artisanal capture fishing.

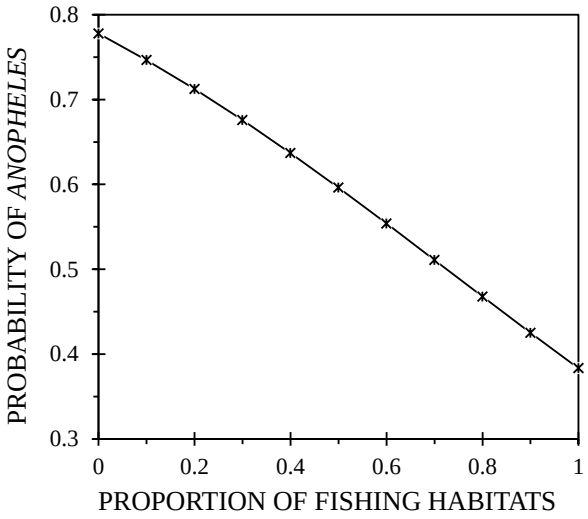

Figure 10: Modeled probabilities of finding *Anopheles* mosquitoes in larval habitats associated with (artisanal capture) fishing.

4.2. Artisanal capture fishing and *Anopheles* larval productivity

a) Anopheles density in different habitat types

The total number of *Anopheles* larvae collected in this study was 2008, with a mean density of 23.08±5.05 individuals per habitat. Forty eight percent of the larvae were recovered from fishing boats and 49% from rock pools. Despite being the most common habitat type, the mean number of malaria mosquito larvae present in rock pools (30.81±10.54) was significantly lower than those found inside fishing boats (40.08±10.16) (P = 0.001). The mean number of *Anopheles* larvae in ditches, lagoons and swamps was 5.71±3.11, 1.14± 0.9 and 1.09± 0.7, respectively (figure 11). These data underscore the importance of fishing boats (hence artisanal capture fishing), rock pools and, to a lesser extent, ditches on the overall epidemiology of malaria on Mageta Island.

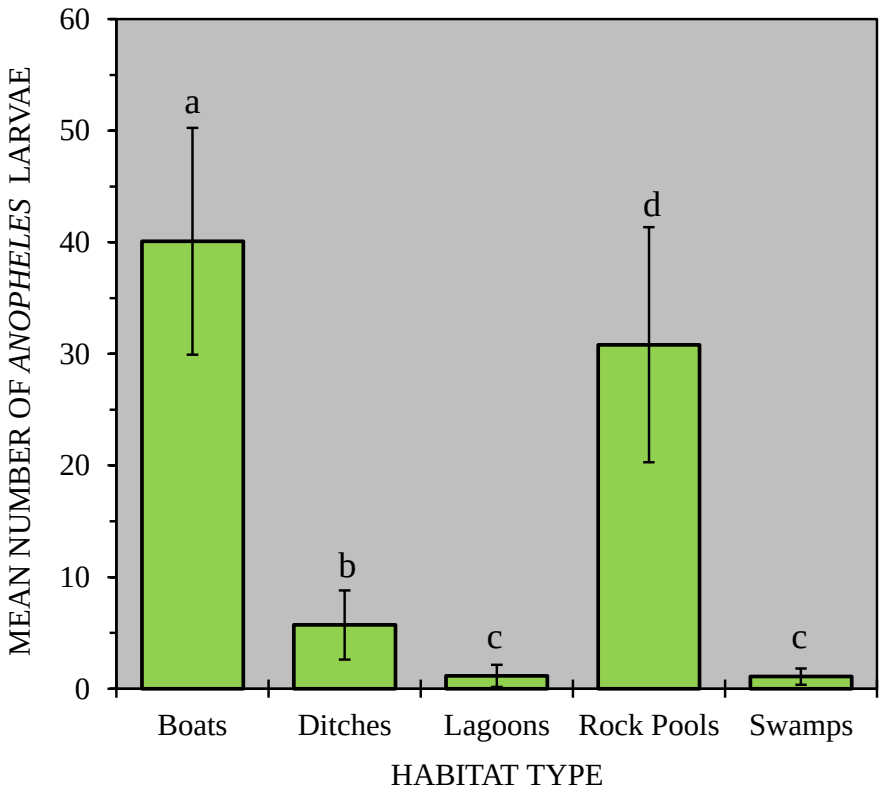

Figure 11: Mean number of *Anopheles* larvae collected from different mosquito habitat types on Mageta Island. The standard errors of the mean values are shown. Bars with different letters denote a significant difference in the mean number of mosquitoes collected. Similar letters indicate no difference in the mean number of mosquitoes collected. No *Anopheles* larvae were recovered from fishing ponds, fish bait mines and trenches.

(b) *Anopheles density in 'fishing' versus 'non-fishing' habitats*

Although about half (48%) of malaria mosquito larvae were recovered from 'fishing habitats', the mean *Anopheles* larval density in the fishing habitats (35.7±1.15) was significantly higher than in 'non-fishing habitats' (17.4±0.539) (P = 0.001) (Figure 12). This implies that there is a relationship between artisanal capture fishing and the density of *Anopheles* larvae found in habitats on Mageta Island. Fishing boats and the ditch used as a boat 'docking station' were the only 'fishing habitats' that contained *Anopheles* larvae. However, while no *Anopheles* larvae were found in fish bait mines and the trench, *Anopheles* eggs were observed in the fish bait mines and inside fishing boats.

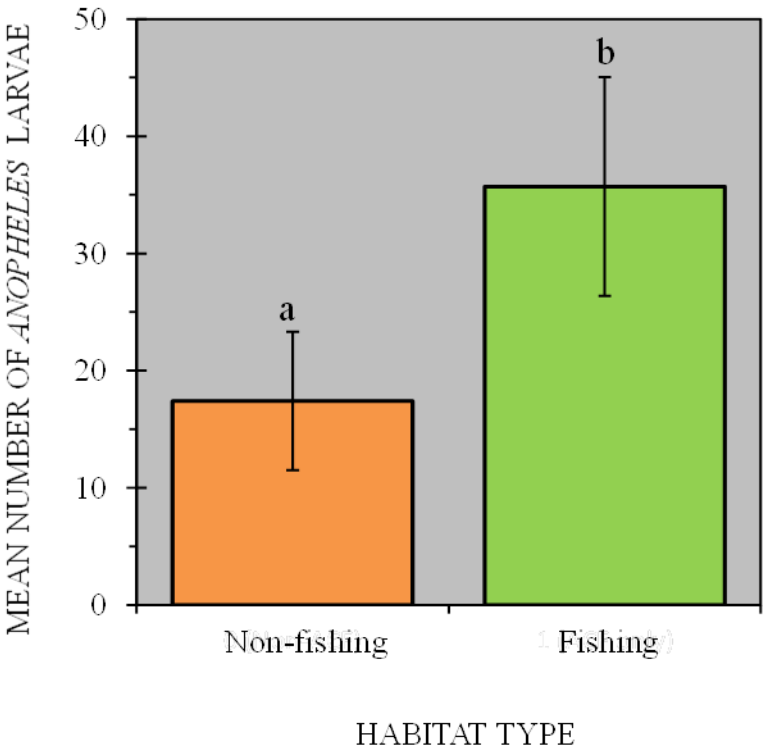

Figure 12: Mean numbers of *Anopheles* larvae collected from 'fishing' versus 'non-fishing' habitats on Mageta Island. The standard errors of the mean values are also shown. Bars with different letters denote a significant difference in the mean number of mosquitoes collected.

(c) *Anopheles density in habitats of different bottom surface types*

Inspection of bottom surfaces of identified habitats revealed that 27 had mud (3 lagoons, 11 swamps, 7 ditches, 2 fish bait mines, 1 trench and 3 fish ponds), 36 had rock (all rock pools and 4 lagoons) and 24 were wooden (all boats). The mean number of *Anopheles* mosquitoes in these habitats were 2.22 ± 0.29, 27.39 ± 0.87 and 40.08 ± 1.29, respectively (figure 13). Because all fishing boats were wooden the significant association between artisanal capture fishing and *Anopheles* larval productivity is likely to have been driven by some evolutionarily beneficial aspect(s) of the timber used to construct the boats. Similarly, the high number of *Anopheles* larvae encountered in rock pools is predictive of factors in this habitat type that promote colonization. Pairwise comparisons revealed significant statistical differences in *Anopheles* density between mud versus rock bottomed habitats (P = 0.001), mud versus wood bottomed habitats (P = 0.001) and wood versus rock bottomed habitats (P = 0.001).

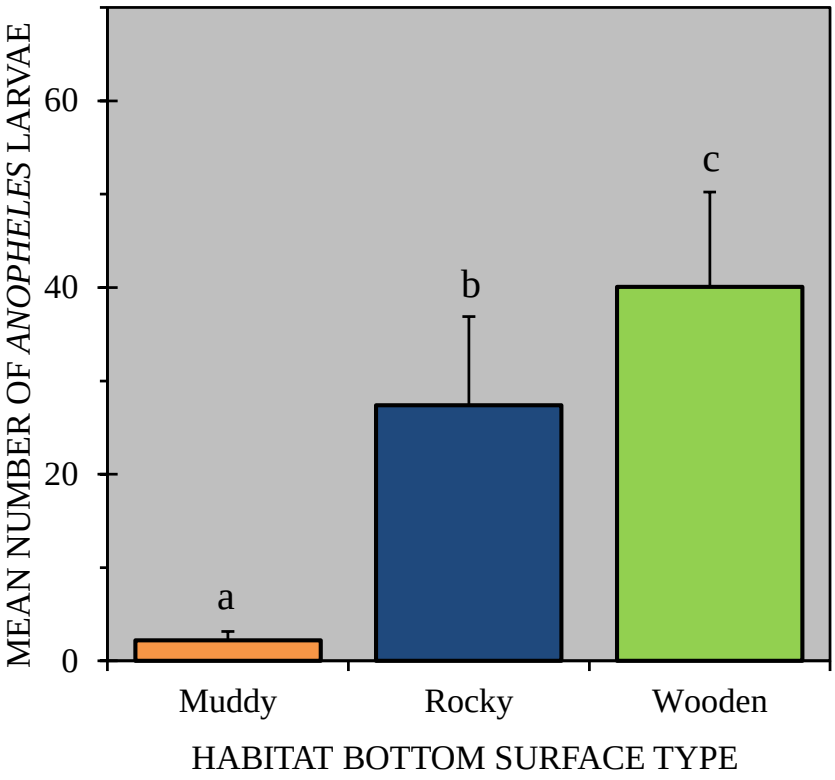

Figure 13: Mean numbers of *Anopheles* larvae collected from habitats with different bottom surface types. The standard errors of the mean values are shown. Bars with different letters denote a significant difference in the mean number of mosquitoes collected.

(d) *Effect of moderator variables on Anopheles larval productivity*

The correlation between *Anopheles* larval density and the interaction between artisanal fishing and exposure of habitats to direct sunlight could not be determined. This was also the case for the relationship between *Anopheles* larval density and the interaction between artisanal fishing and presence of emergent plants/fish predators in habitats. These potential moderator variables were all declared statistically redundant given that all fishing habitats were exposed to direct sunlight, no fishing habitats had emergent plants in them and only one fishing habitat contained fish predators. However, as expected from examining the main effects, exposure to direct sunlight had a significant effect on *Anopheles* larval density inside habitats (Minakawa *et al*., 1999; Gimnig *et al*., 2001) (P = 0.001). More *Anopheles* larvae were recovered from habitats exposed to direct sunlight. Similarly, presence of emergent plants and fish predators in habitats both had significant effects on *Anopheles* larval density (P = 0.001). Habitats that had emergent plants in them and those that harbored larvivorous fish had significantly fewer *Anopheles* larvae (P = 0.001 (Table 1).

Table 1: Mean number (±SE) of *Anopheles* larvae recovered from larval habitats exposed (predictor present) and not exposed (predictor absent) to different moderating variables on Mageta Island in western Kenya.

Predictor	N	Mean (±) number of *Anopheles* larvae		Exp (B)	P
		Predictor Present	Predictor Absent		
Emergent Plants	87	1.87±0.484	25.54±0.572	13.621	0.001
Fish Predators	87	0.10±0.067	30.85±0.689	323.885	0.001
Direct Sunlight	87	23.32±0.569	23.43±1.294	1.005	0.938

4.3. Mapping Malaria hazard on Mageta Island

Spatial analysis of *Anopheles* larval densities on Mageta Island revealed that larval density varied significantly (Figure 14). The busy fishing beaches recorded higher *Anopheles* larval densities compared to the less active fishing beaches. The deep red zone, as shown in Kuoyo and Mahanga beaches, signified very high malaria hazard levels. These two beaches are the main fish landing beaches on the Island. Mitundu beach had a moderate malaria hazard level. Wakawaka and Sika beaches recorded low malaria hazard levels. The last beach, which is Magare, had very low malaria hazard levels (see yellow zones in figure 14). The above analysis clearly shows that malaria hazard was strongly correlated to the spatial distribution of ACF activities on Mageta location.

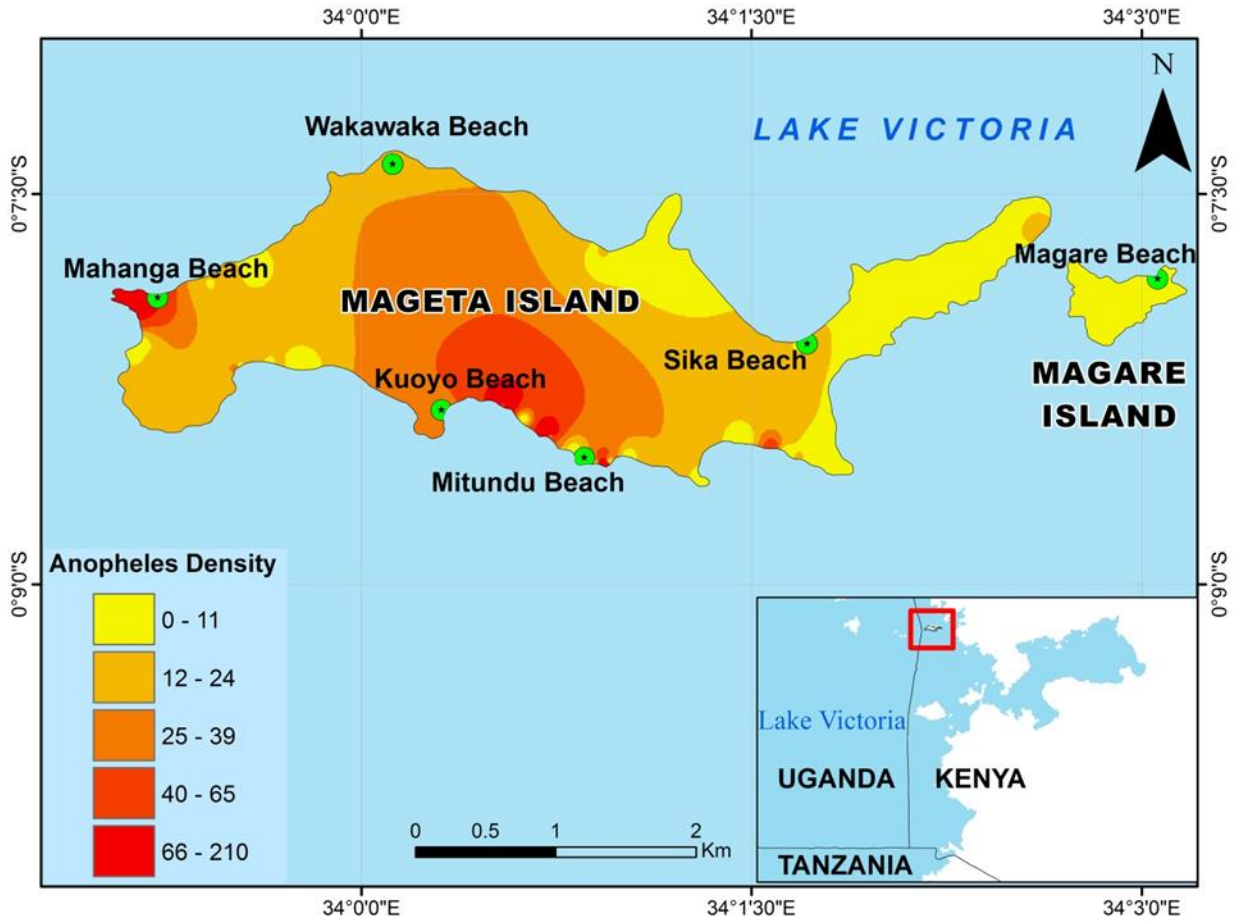

Figure 14: A visual representation of areas with the highest *Anopheles* larval concentrations on Mageta Island.

CHAPTER FIVE: DISCUSSION

5.1. Discussion

This study applied an ecosystem approach to find out if and how, even if inadvertently, artisanal capture fishing (ACF) processes facilitate breeding of malaria mosquitoes on Mageta Island in western Kenya. Of the 87 mosquito larval habitats identified 27 (fishing boats, trenches and fish bait mines) were created through ACF activities. Although the total numbers of *Anopheles* larvae collected were about equal, the mean density in 'fishing habitats' was twice that in 'non-fishing habitats'. Interestingly, 90% of the fishing boats, which formed the majority (85%) of 'fishing habitats' contained *Anopheles* larvae. Forty eight percent of the larvae were recovered from fishing boats and 49% from rock pools. Despite being the most common habitat type, the mean number of *Anopheles* larvae present in rock pools was significantly less than those found inside the wooden fishing boats. These data underscore the importance of artisanal capture fishing on the overall epidemiology of malaria on Mageta Island.

There was a significant negative association between artisanal capture fishing and the probability of finding *Anopheles* larvae in the habitat. Although this result is puzzling on initial thought, this relationship was not infinite. The fitted logistic regression equation predicted that 38% of stagnant water bodies would contain *Anopheles* larvae if 100% of breeding habitats on Mageta Island were created through artisanal capture fishing. On the other hand 78% of stagnant water bodies would contain *Anopheles* larvae if no single breeding habitat on Mageta Island was created through artisanal capture fishing. This analysis implies that although artisanal capture fishing is an important facet of malaria epidemiology on Mageta Island, other drivers of endemicity do exit. Thus, malaria control efforts need to be informed by holistic approaches that recognize the interdependent nature of health and other societal, developmental and ecosystem factors (Asakura *et al*., 2015).

Although little is known about breeding of *Anopheles* larvae in boats, traces of information relating to the role of boats and other transport vessels as agents for dispersal of arthropod vectors worldwide do exist (Mouchet *et al*., 1995; Guagliardo *et al*., 2015a). Studies outside Africa have looked at *Aedes* to a larger extent and *Culex* to a small extent (Pratt *et al*., 1946; Belton & Belton, 1990; Tsunoda *et al*., 2012; Guagliardo *et al*., 2015a; Guagliardo *et al*., 2015b). Furthermore, little discussion is made on why pools of water in boats are used for mosquito breeding. Two recent studies in Cameroon have documented utilization of boats for breeding by *Anopheles* larvae, specifically *Anopheles coluzzii* (initially the M form of

Anopheles gambiae ss) in two fishing communities near the port of Duala within the Wuori river estuary (Etang *et al*., 2016; Mbida *et al*., 2017). Our study corroborates these findings though with respect to *Anopheles gambiae* ss, which had an overwhelming presence in fishers' boats. Mbida *et al* (2017) explains the phenomenon of *An. coluzzii* breeding in boats, among other manmade habitats, as an adaptation to utilizing artificial habitats when natural ones become rare. We hypothesize that preferential colonization of pools of water in boats by *Anopheles* larvae as observed in the current study was partly due to lack of alternative oviposition sites in the highly populated fishing settlements.

It is documented that *Anopheles gambiae* utilize manmade habitats for larval breeding (Fillinger *et al*., 2011; WHO, 2013) but it is puzzling why fishers' boats were highly prolific. This study was carried out in the dry season, thus the finding that boats formed an important breeding habitat resource for malaria mosquitoes is confusing. By iteratively engaging local inhabitants in ecological dialogue it was explained that fishers engage in an active maintenance process that involves pouring fresh lake water in parked wooden boats to prevent them from cracking. Boats not-in-use are normally stationed ashore (Nathenson *et al*., 2017; Nunan, 2018) during months when fishing is illegal (Ntiba *et al*., 2001; Njiru *et al*., 2007; Njiru *et al*., 2008), when fish catches get seriously diminished, when actors are off duty and during tumultuous party times when fishermen revel after receiving cash bonuses from their cooperative societies. It is unlikely that the larvae found in boats were introduced therein with lake water through the maintenance process. Strong waves must have killed all mosquito larvae in the lake water around the open beaches where fishing boats were parked. Besides the open lake not being their typical breeding habitat (Minakawa *et al*., 2012), *Anopheles* eggs, possibly introduced through direct oviposition, were found in the boats.

Presence of high numbers of *Anopheles* larvae in fishers' boats can be probably explained borrowing from life history theory (Stearns, 1992; Reznick *et al*., 2002; Begon *et al*., 2006; Dobson, 2007). A mosquito's life cycle encompasses four key life history stages namely eggs, larvae, pupae and adults (Clements, 1999). The first three stages namely eggs, larvae and pupae are aquatic, and will most likely exist in pools of water in boats for the case of malaria vectors on Mageta Island. Utilization of boats as a breeding resource is a very risky phenomenon for *Anopheles* mosquitoes because this habitat type is highly ephemeral. Although water is placed in the boats in the morning hours and emptied in the late afternoon on the same day or after a few days, the most common malaria vectors in the area i.e. *Anopheles gambiae* complex mosquitoes (Ogola *et al*., 2017) need about seven days, often less, to complete the aquatic cycle (Mamai *et al*., 2016). From an evolutionary standpoint

selection pressure should favor traits that promote shorter aquatic developmental periods and production of large numbers of offspring by gravid female malaria mosquitoes. Alternatively, gravid malaria vectors may, through an ecological phenomenon referred to as 'bet-hedging' (Begon *et al*., 2006) cope by distributing single egg loads into several boats. Reproduction should also entail a relatively small energy investment in each offspring (Stearns, 1992). This should result in a sizeable number of young offspring able to evade extrinsic larval mortality (Reznick *et al*., 2002) and develop into terrestrial adult beings that live for long enough while accessing readily available blood meals from the vast human reservoir in the fishing hamlets.

In terms of habitat bottom surfaces and *Anopheles* larval productivity, the larger number of *Anopheles* larvae in rock pools relative to mud-bottomed habitats is not surprising. This is because larvae of *Anopheles gambiae* sl are often found in habitats containing algae (Gimnig *et al*., 2001) and rock offers a better substrate for algal growth than mud substrates (Minakawa *et al*., 1999). Besides, rock pools were all found near the shoreline and water in them was frequently refreshed by spilling waves. This served to oxygenate the water, which may have promoted *Anopheles* larval productivity (Kipyab *et al*., 2015; Dida *et al*., 2018). However, the fact that most rock pools were found under tree canopies could explain the relatively lower *Anopheles* productivity compared to boats. Generally, anopheline larvae prefer open sun-lit waters (Minakawa *et al*., 1999; Gimnig *et al*., 2001). *Anopheles gambiae* sl tolerates relatively high water temperatures (Dida *et al*., 2018), thus the warmer sun-lit water pools in boats may have been an important factor for larval development, because warm water accelerates larval development (Kipyab *et al*., 2015). In addition, the warm water in boats may have allowed more microorganisms to grow, which provided food sources for mosquito larvae (Ponnusamy *et al*., 2008; Kaufman *et al*., 1999; Kaufman *et al*., 2006; Kipyab *et al*., 2015). Fishing boats used on Mageta Island are constructed using timber from the Africa teak tree (*Milicia excelsa*), known as *Mvule* among locals. Unsubstantiated reports indicate that timber of this tree contains pores that harbor bacteria. We hypothesize that these bacteria may multiply rapidly and act as food for mosquito larvae (Kipyab *et al*., 2015; Kaufman *et al*., 1999), so increasing *Anopheles* productivity in boats. On the contrary, presence of aged water may have harbored larger numbers of predators that suppressed *Anopheles* larval density (Munga *et al*., 2013) in rock pools relative to boats.

In this study, the majority of *Anopheles* larval habitats were reported to result from human activities that supported livelihoods, notably artisanal capture fishing. This result goes in tandem with observations by other researchers in relation to crop cultivation (Dunn *et al*., 2011; Shayo *et al*., 2015), livestock herding (Shayo *et al*., 2015) and brick making (Carlson *et*

al., 2004). The findings underscore man's own contribution towards the viciousness of malaria and affirm the link between malaria and poverty, hence the poverty trap formed by the ecology of infectious diseases (Bonds *et al*., 2010; Berthélemy *et al*., 2013). This implies that the poor of the south (e.g. the artisanal fishers of Mageta Island) whose wealth, by definition, is primarily gained by extracting natural resources (Lebel, 2003) are unable to make enough to lift themselves out of poverty (Cinner *et al*., 2009). They are stuck in a cycle of poverty that is almost impossible to break (Bonds *et al*., 2010). As fishing activities intensify so does the chance of increasing *Anopheles* larval densities in breeding habitats. This fuels malarial disease, hence the need to extract more fish to generate income as a coping strategy towards treatment. Contrary to conventional wisdom there is limited evidence that illness, even in the context of our study areas, reduces fishing effort. Instead, illness maintains fishing effort but shifts it to practices that pose a negative impact on the environment (Fiorella *et al*., 2017).

Malaria hazard mapping clarified the link between ACF and *Anopheles* larval density. All the six beaches in Mageta location had *Anopheles* habitats whose numbers and distribution varied significantly. The busy fishing beaches, namely Kuoyo and Mahanga, recorded the highest malaria hazard levels. This could be explained by the intricate biological relationship between *Anopheles* mosquitoes and humans, coupled to the frequent migration of fisher folk between fishing beaches (Nunan, 2010; Nunan, 2018). Less active beaches like Magare recorded lower malaria hazard levels. These findings project to man's own contribution towards the viciousness of malaria and affirm the link between malaria and poverty.

The cross-sectional design used in this study, despite its inherent shortcomings (Aschengrau & Seage III, 2013) was ideal for this study. First, the design does not allow the inference of the temporal sequence between exposure and outcome, but it makes biological sense in this study to assume that the appearance of mosquito larvae in the boats (outcome) was due to presence of water in the boats (exposure). This is because stagnant water is a prerequisite for oviposition and larval development. Second, cross sectional study designs tend to identify high proportions of prevalent rather than incident outcomes. In the case of our study, it is unlikely that boats overstaying with water resulted in most of the larval breeding except for those boats that were spoilt and had been abandoned by the owners at the lake shores. From dialoguing with the Mageta fishing community we learnt that boats containing water were parked ashore just for a few hours or days (Camlin *et al*., 2013; Nathenson *et al*., 2017), hardly exceeding one week. Third, the fact that the abandoned boats exposed the fisher folks to a higher risk of contracting malaria compared to their counterparts who were rather

unemployed only means that this study although conducted in an informal occupational set up did not suffer a '*healthy worker survivor effect*' (Arrighi & Hertz-picciotto, 1994; ACSAL, 2017).

5.2. Conclusions

From this study it can be concluded that:

a) Socioeconomic and sociocultural factors are more important to the fishing community of Mageta Island than the actual problem of malaria. This was explained by the fact that *Anopheles* breeding habitats on Mageta Island were mainly created through man's efforts to support livelihoods i.e. through artisanal capture fishing activities.

b) Artisanal capture fishing is not the only driver of malaria epidemiology on Mageta Island. This was explained by the fitted logistic regression equation which predicted that 38% of stagnant water bodies would contain *Anopheles* larvae if all breeding habitats on Mageta Island were created through artisanal capture fishing

c) Although, the availability of stagnant water is an important consideration for breeding of mosquitoes, other factors (e.g. habitat bottom surface type) determine habitat colonization by gravid female *Anopheles* mosquitoes.

d) The stamp of human activity is an important factor for selection of stagnant water as a breeding resource for *Anopheles* larvae. This can be inferred by visualizing areas with the highest *Anopheles* larval concentrations as depicted on the malaria hazard map of the Island.

5.3. Recommendations

a) Rather than just mapping to know if breeding habitats of malaria mosquitoes are '*few*, *fixed* and *findable*', and therefore amenable to larval source management, a deeper understanding is critical in shaping and re-shaping malaria control policy.

b) Malaria control efforts need to be informed by holistic approaches that recognize the interdependent nature of health and other societal, developmental and ecosystem factors.

c) Disease control strategies should be combined with income generating activities to enhance community participation and sustainability.

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APPENDIX 1: ORIGINALITY REPORT- JANET ACHIENG ONYANGO (156/87643/16)

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APPENDIX 2: DATA ENTRY SHEET

General Information:

Date ..... Village ..... GPS site # .....

Name of Community Health Volunteer (CHV) .....

GPS co-ordinates of mosquito larval habitat:

Latitude ..... Longitude ..... Elevation (m) .....

Description of mosquito larval habitat:

Habitat type (e.g. lagoon, puddle etc.):					
Perimeter of habitat (m):		Depth of habitat (cm):		Distance to nearest occupied house (m):	
Are emergent plants present? (Y/N):		Are algae present? (Y/N):		Is the water hyacinth present? (Y/N):	
Is the water clear? (Y/N):		Is the water organically polluted? (Y/N):		Is habitat open to direct sunlight? (Y/N):	
Distance of habitat to the shoreline (m):		Is the habitat sandy (S) or muddy (M)?:		Is the habitat permanent? (Y/N):	
Is the habitat manmade? (Y/N):		Who created the habitat? (M/F):		Creator's education level (1º/2º/3º):	
Does habitat support livelihoods? (Y/N):		Types of livelihoods supported e.g. artisanal capture fishing (ACF) etc.:			
Are fish present in the habitat? (Y/N):		Edible fish species present in habitat:			
Culicine spp larvae (mean # in 10 dips):		*An. gambiae* larvae (mean # in 10 dips):		*An. funestus* larvae (mean # in 10 dips):	
Habitat location (eg ditch on Janet's farm):					

Comments:

.....

.....

.....

.....

Enumerator (names) _____ Signature _____

APPENDIX 3: MORPHOLOGICAL IDENTIFICATION OF ANOPHELES LARVAE

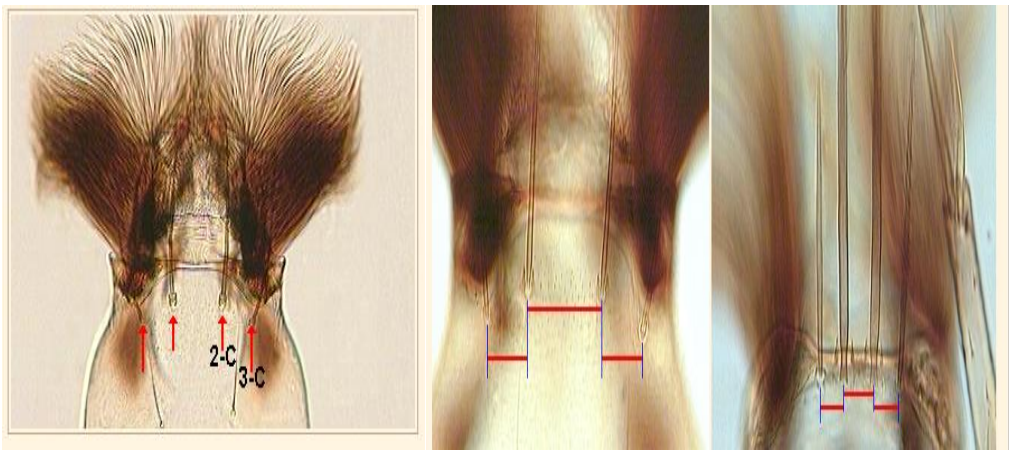
Different features including head, antennae, thorax and abdomen were used to distinguish *Anopheles gambiae* larvae from the other *Anopheles spp.* Identification key with the mentioned features was used to finally arrive at *Anopheles gambiae* (Hervey *et al.*, 1998).

Anopheles gambiae identification key

Head:	Distance between the two setae 2-c and 3-c = equal to or longer than between seta 2-c
Head:	Apex of seta 2-c is simple (not branched)
Head:	Ornamentation of seta 2-c is Smooth (not barbed)
Head:	Number of branches on seta 3-c is 3 or 4
Head:	Ornamentation of seta 3-c is Smooth (not barbed)
Head:	Ratio of a/b is greater than 1. Where a is the length of seta 5-c and b is the distance between 4-c and 5-c
Head:	Number of branches on seta 8-c (Sutural hair) is either 1 or 2
Antenae:	Number of branches on seta 1-A (Antennal hair) is1
Thorax:	Type of insertion of seta 1-P (Inner submedian prothoracic hair) is on a small not or slightly sclerotinized tubercle or directly on integument
Thorax:	Number of branches on setae 10-M and 9-M. where 9-M/10-M is 1/1 or 9-M/10-M is 2+3/1, 4+5/1, 6+7/1, >8/1
Abdomen:	Number of branches on seta 2-V (ante palmate hair) is either 1, 2 or 3

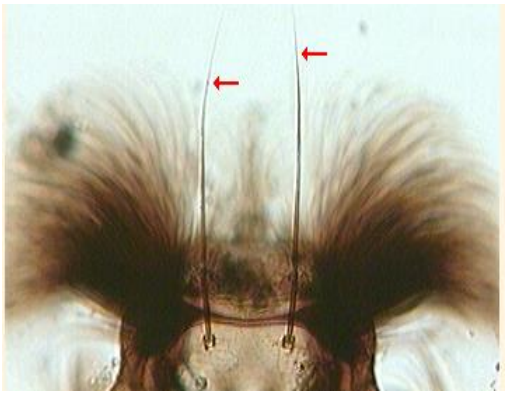
(a) ***Anopheles*** **larval head** ***containing*** **clypeal hairs (seta 2-c and 3-c)**

Distance between setae 2-c and 3-c is longer than or equal to between seta 2-c



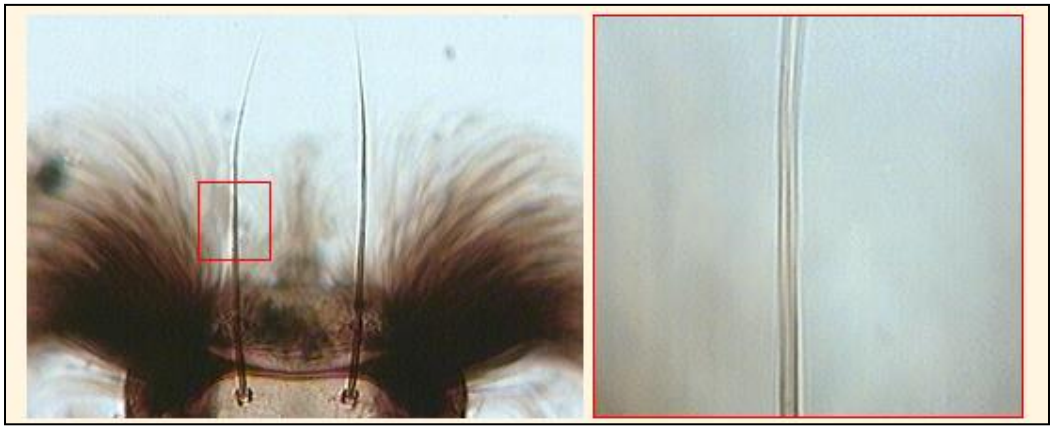
(b) ***Anopheles*** **larval head showing apex of inner clypeal hairs (seta 2-c)**

Apex of seta 2-c is simple (not branched).



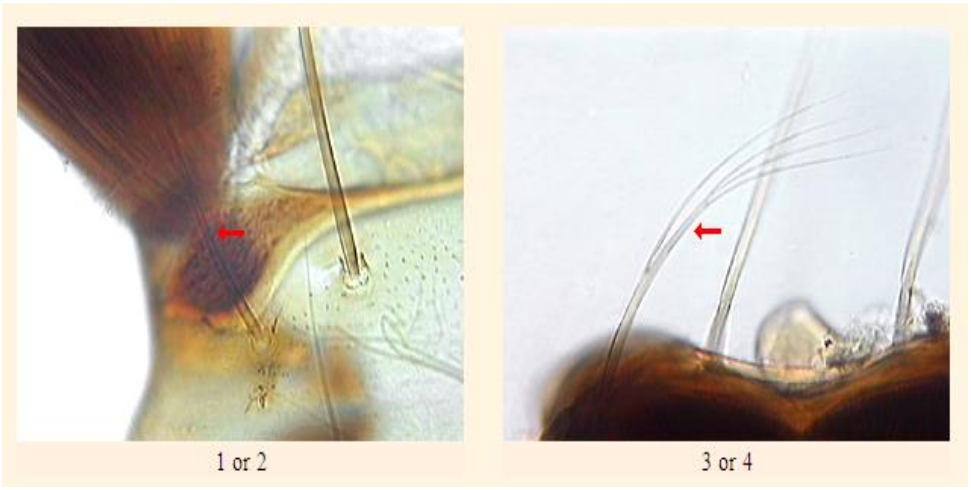
(c) *Anopheles* larval head showing Seta 2-c ornamentation

Seta 2-c ornamentation is smooth (not barbed) for *Anopheles gambiae.*



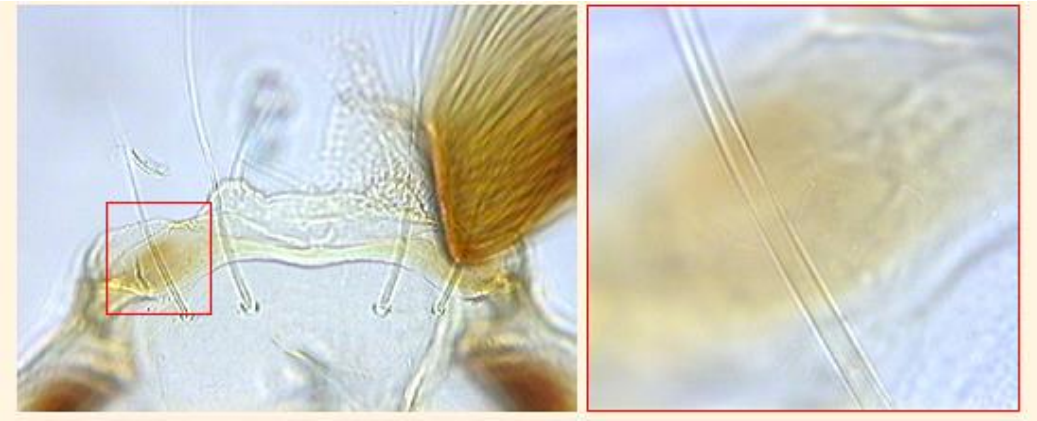
(d) *Anopheles* larval head showing number of branches on Seta 3-c

Number of branches on seta 3-c (outer clypeal hair) is 1 or 2 as shown on the left or 3 or 4 as shown on the right.



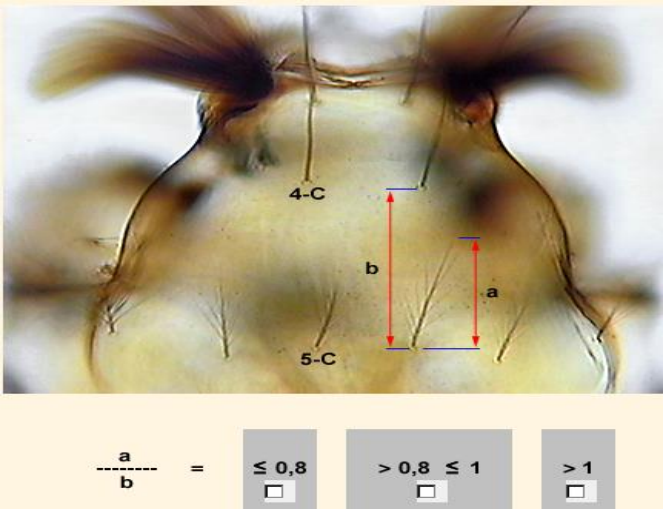
(e) *Anopheles* larval head showing seta 3-c ornamentation

Seta 3-c is not barbed (smooth) in the case of *Anopheles gambiae.*



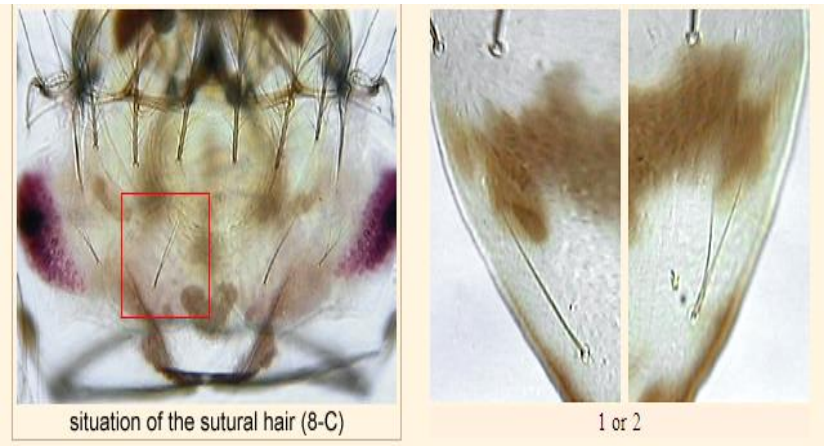
(f) *Anopheles* larval head showing distance between bases of seta 4-c and seta 5-c

Ratio of length of seta 5-c divide by the distance between bases of seta 4-c and seta 5-c is greater than one for *Anopheles gambiae.*



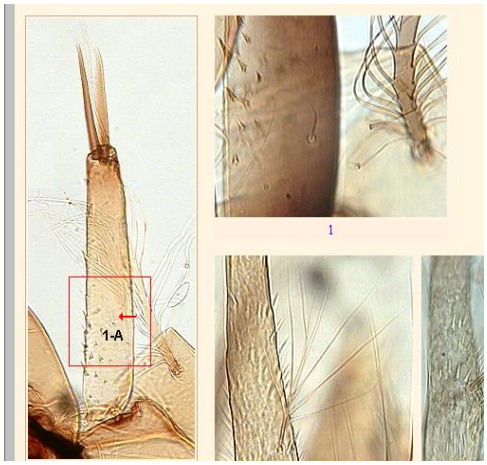
(g) *Anopheles* head indicating situation of the seta 8-c and the number of branches

The number of branches on seta 8-c (Sutural hair) is either 1 or 2 for *Anopheles gambiae.*



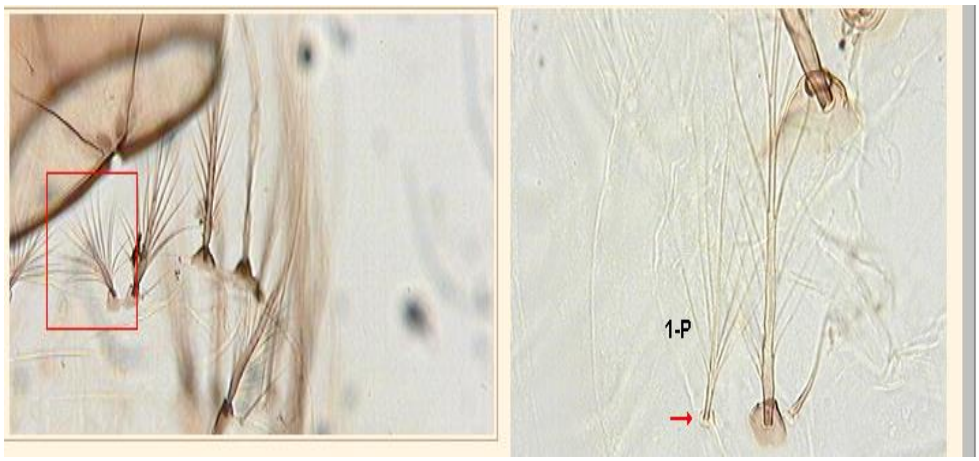
(h) *Anopheles* antennal hair (seta 1-A) situation and number of branches

Number of branches on seta 1-A (antennal hair) is 1 for *Anopheles gambiae.*



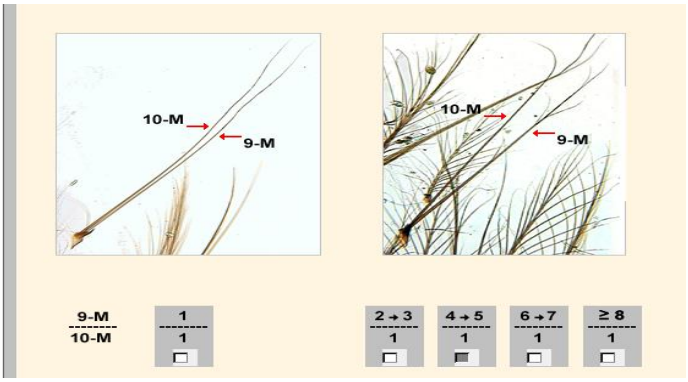
(i) ***Anopheles*** **larval thorax indicating insertion of Inner sub median prothoracic hair (seta 1-p)**

Insertion of seta 1-P (inner sub median prothoracic hair) is on a small note or slightly sclerotized tubercle or directly on integument for *Anopheles gambiae.*



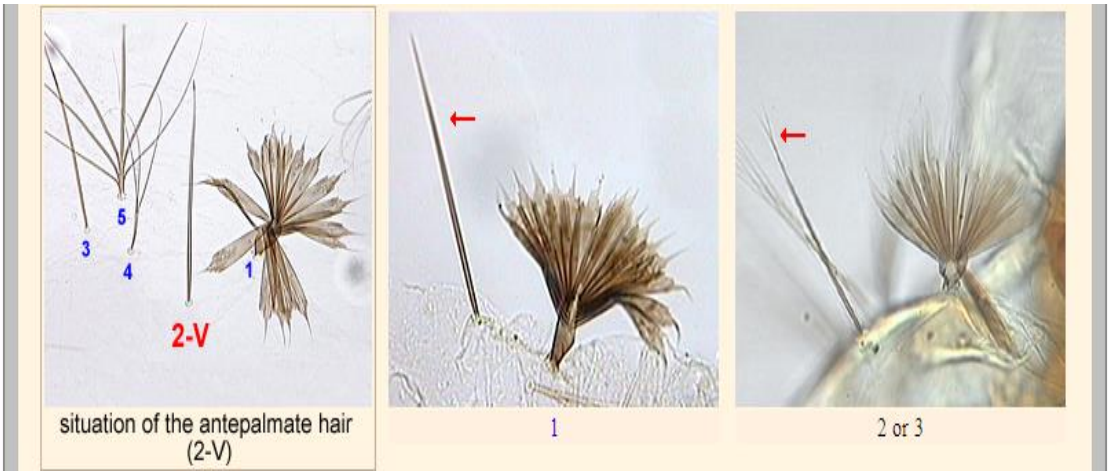
(j) ***Anopheles*** **thorax indicating the situation of setae 10-M and 9-M and number of branches**

Number of branches on setae 10-M and 9-, where 9-M/10-M=1/1 or 9-M/10-M=2+3/1, 4+5/1, 6+7/1, >8/1



(k) *Anopheles* abdomen indicating situation of ante palmate hair (seta 2-V) and number of branches

Number of branches on seta 2-V (ante palmate hair) is either 1, 2 or 3 for *Anopheles gambiae.*



APPENDIX 4: IDENTIFYING *ANOPHELES GAMBIAE* (S.S) COMPLEX MOSQUITOES USING THE POLYMEREASE CHAIN REACTION

A ribosomal DNA-polymerase chain reaction method was used to identify individual members of the *An. gambiae* complex (Scott *et al*., 1993), a group of morphologically indistinguishable sibling mosquito species that includes the major vectors of malaria in Africa.

Preparation of DNA samples

An. gambiae (s.l) DNA was extracted from abdomens using the method described by Collins *et al*. (1987). Abdomens were crushed individually in autoclaved 1.5ml micro centrifuge tubes containing 100ul grinding buffer. The lysates were incubated in a 65^{o}C water bath for 30 minutes. Fourteen microliters of 8M potassium acetate were added into each tube, the tubes vortexed and cooled on ice for 30 minutes. Precipitated proteins were voided by centrifuging the tubes for 10 minutes at 14,000 rotations per minute (rpm) and saving the supernatants into sterile similarly labeled 1.5ml micro centrifuge tubes. DNA was precipitated by adding 200µl 95% ethanol into each tube in the new set. The tubes were cooled for 20 minutes at -20^{o}C then spun at 14,000 rpm for 20 minutes. The 95% ethanol was then replaced with 200µl 70% ethanol. This was also poured off and replaced again with 200µl 95% ethanol. The final ethanol was discarded and the tubes dried in an incubator. The residual pellet (actual DNA) was resuspended in 100µl sterile distilled water and stored at -80^{o}C.

PCR amplification

Three different PCR primers sets were used. The pellets were resolubilised to 0.02µg/µl then stored at -20^{o}C. The primers (table2) are based on ribosomal DNA (rDNA) sequences (Scott *etal*., 1993) which in *An. gambiae* (s.l) show species-specificity in the intergenic spacers (Collins *etal*., 1989). All primers used were 20-mers.The universal plus-strand primer (UN) matches the rDNA of all species in the *An. gambiae* complex. AR was the *An. arabiensis*-specific primer and AG the *An. gambiae*-specific primer. PCR primers used in this assay were all derived from sequences in the intergenic spacer (IGS) regions.

Primer name	Primer DNA sequence (5'to 3')	Tm^{o}C.
UN	GTG TGC CCC TTC CTC GAT GT	58.3
GA	CTG GTT TGG TCG GCA CGT TT	59.3
AR	AAG TGT CCT TCT CCA TCC TA	47.4

Table 2. *Anopheles gambiae* complex ribosomal DNA (rDNA) intergenic spacer species-diagnostic primer sequences and their melting temperatures (Tm)*.

The UN primer anneals to the same position of the rDNA of all species in the *An. gambiae* complex, GA anneals specifically to *An. gambiae*, and AR anneals to *An. arabiensis*.

Reaction conditions

The PCR master mix comprised of the following ingredients

1. One microlitre sample DNA and
2. Fourteen microlitres PCR master mix containing;

1.5µl 10X PCR buffer (200mM Tris-HCl at pH 8.4 plus 500mM KCl).

0.15µl each 10mM deoxy nucleotide (dATP, dTTP, dCTP, and dGTP).

0.2µl 50mM Magnesium chloride.

0.25µl each of *arabiensis*, *gambiae* and *universal* primers (0.02µg/ml).

0.058µl Taq DNA polymerase (Amplitaq) (5µ/µl).

10.892µl sterile water.

The reactions were done using DNA Engine ® peltier Thermal cycler obtained from BIO-RAD. Initial and all subsequent melting of double-stranded DNA took place at 94^{o}C for 10 seconds. Annealing was for 10 seconds at 60^{o}C followed by polymerization at 72^{o}C for 20 seconds. No auto-extension time was used. After 30 cycles, the thermal cycler stabilized at 4^{o}C until samples could be removed. Each amplified sample was mixed with 3µl gel loading solution and electrophoresis done on agarose gels.

Gel electrophoresis and staining

Analysis of polymerase chain reaction (PCR) products was done by electrophoresis on 3.0% horizontal slab agarose-TBE gels stained with ethidium bromide (Sambrook *et al*., 1989). Three microlitres of gel loading solution (Sigma, St. Louis, MO) were added to each amplified sample and 17µl of each sample-dye mixture applied in separate slots on the gel. *An. gambiae* control DNA was loaded in the first slot in each of the two rows of wells while *An. arabiensis* control DNA was loaded in the last slot of the two rows. All other wells were loaded with amplified samples to be species identified. Continuous zone submarine electrophoresis using 1X TBE buffer was done until the blue zone had migrated about 1.5 inches away from its initial position in the sample wells. Visual inspection and photography of ethidium bromide-stained DNA was done with UV light on a UV transilluminator (Model UVT 400-M).