

***ANOPHELES* PRODUCTIVITY OF AQUATIC HABITATS CREATED
THROUGH ARTISANAL CAPTURE FISHING ON MAGETA ISLAND
IN WESTERN KENYA**

BY

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DECLARATION

I declare that this thesis is my own original work and has not been presented in part or whole for any award in any other Institution (See originality report in Appendix 1).

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DEDICATION

This thesis is dedicated to my loving parents George Mukachi Sifuna and Scholastic Sifuna for every effort made to ensure that I am successful.

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TABLE OF CONTENTS

DECLARATION	ii
ACKNOWLEDGEMENTS	iv
LIST OF FIGURES	ix
LIST OF APPENDICES	xi
LIST OF ABBREVIATIONS AND ACRONYMS	xii
ABSTRACT.....	xiii
CHAPTER ONE: INTRODUCTION.....	1
1.1 Problem statement.....	3
1.2 Justification and Significance of the Research	4
1.3 Research Objectives.....	6
1.3.1 General Objective	6
1.3.2 Specific Objectives	6
1.4 Research hypotheses	7
1.4.1 Null hypothesis	7
1.5.2 Alternative hypothesis	7
1.5.3 Assumptions.....	7
CHAPTER TWO: LITERATURE REVIEW.....	8
2.1 Malaria transmission.....	8
2.2 The malaria burden	10
2.3 Mosquito life cycle	11
2.4 The malaria vectors.....	13
2.5 Approaches for malarial control	14
2.5.1 Environmental management for mosquito control.....	14
2.5.2 Biological control of mosquito larvae.....	16
2.5.3 Chemical control of mosquitoes	16
2.6 The intricate lifecycle of malaria vectors.....	18

2.7 Mosquito breeding habitats.....	19
2.7.1 Choice of breeding sites by gravid <i>Anopheles</i> mosquitoes.....	21
2.7.2 Effect of physicochemical parameters on breeding of <i>Anopheles</i> mosquitoes	21
2.8 Ecology, a prerequisite for malaria control.....	32
CHAPTER THREE: MATERIALS AND METHODS	34
3.1. Study site.....	34
3.2 Study design.....	36
3.3 Mosquito larval and pupal sampling.....	37
3.4 Relationship between physical habitat characteristics and <i>Anopheles</i> productivity	38
3.5 Linking physical aspects of habitat water to <i>Anopheles</i> productivity.....	38
3.5.1 Measuring water temperature in <i>Anopheles</i> larval habitats	38
3.5.2 Measuring water turbidity/cloudiness in <i>Anopheles</i> larval habitats.....	39
3.5.3 Measuring total suspended solids (TSS) in water from <i>Anopheles</i> larval habitats	40
3.6 Relating between chemical characteristics of habitat water and <i>Anopheles</i>41 productivity	41
3.6.1 Measuring Dissolved Oxygen in <i>Anopheles</i> larval habitats.....	42
3.6.2 Measuring the Potential of Hydrogen (pH) in <i>Anopheles</i> larval habitats	43
3.6.3 Measuring Total Dissolved Solutes (TDS) in <i>Anopheles</i> larval habitats	44
3.6.4 Measuring conductivity of water in <i>Anopheles</i> larval habitats	45
3.7 Statistical analyses	46
CHAPTER FOUR: RESULTS	47
4.1 <i>Anopheles</i> productivity in different habitat types	47
4.2 Relationship between physical habitat characteristics and <i>Anopheles</i> productivity	49
4.3 Linking physical aspects of habitat water to <i>Anopheles</i> productivity.....	52
4.3.1 Temperature	52
4.3.2 Total suspended solids (TSS).....	53
4.3.3 Turbidity	55

4.4 Relationship between chemical variables of habitat water and <i>Anopheles</i>	55
productivity	55
CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS.....	58
5.1 Discussion.....	58
5.2 Conclusions.....	65
5.3. Recommendations.....	65
REFERENCES.....	66
APPENDIX 1: Typical Mageta Island fish landing beach	84
APPENDIX 2: Originality report.....	85
APPENDIX 3: Data sheet.....	86

LIST OF FIGURES

Figure 1: Conceptual framework	4
Figure 2: Malaria endemicity zones in Kenya	11
Figure 3: Lifecycle of <i>Anopheles</i> mosquitoes	13
Figure 4: Association between environmental factors with larval development of mosquitoes.....	19
Figure 5: An illustration of effects of sediments on macro-invertebrates	27
Figure 6: A topographic map showing location of Mageta Island in Siaya County, Western Kenya	36
Figure 7: Dried filter papers with sample	41
Figure 8: Measurement of Total Dissolved Solutes (TDS) in the laboratory	45
Figure 9: Mean \pm SE number of <i>Anopheles</i> larvae and pupae collected from ACF versus Non-ACF habitats	48
Figure 10: Relationship between larval <i>Anopheles</i> numbers and habitat perimeter in ACF versus non-ACF habitats.....	49
Figure 11: Mean number of <i>Anopheles</i> larvae collected from ACF versus non-ACF habitats with different habitat bottom surface types.....	51
Figure 12: Relationship between <i>Anopheles</i> larval numbers with temperature in ACF versus non-ACF habitats	52
Figure 13: Relationship between <i>Anopheles</i> larval numbers with TSS in ACF versus non-ACF habitats	53
Figure 14: Interaction between <i>Anopheles</i> larval numbers with total suspended solids and temperature in ACF versus non-AF habitats	54

Figure 15: Interaction between *Anopheles* larval numbers with dissolved oxygen and TSS in ACF verses non-ACF habitats56

Figure 16: Interaction between *Anopheles* larval numbers with total dissolved solutes and conductivity in ACF versus non-ACF habitats57

LIST OF APPENDICES

APPENDIX 1: Typical Mageta Island fish landing beach.....84

APPENDIX 2: Originality report85

APPENDIX 3: Mosquito larval collection data Sheet86

LIST OF ABBREVIATIONS AND ACRONYMS

ACF: Artisanal capture fishing

IRS: Indoor residual spraying

LLINs: Long Lasting Insecticidal Nets

TSS: Total suspended solids

TDS: Total dissolved solids

DO: Dissolved Oxygen

WHO: World Health Organization

s.s: sensu stricto

s. l: sensu lato

EC: Electric conductivity

NTU: Nephelometric Turbidity Unit

ABSTRACT

The effect of physicochemical characteristics of mosquito larval habitats on *Anopheles* productivity is not well known. A cross-sectional survey was carried out on Mageta Island, western Kenya, to fill this knowledge gap. All stagnant water bodies were sampled once from 0900hrs to 1100hrs. Habitats were classified either as those created by or associated with artisanal capture fishing (ACF) activities on the Island (i.e. ACF-related) or as non-ACF. Mosquito larvae and pupae were sampled using sweep nets and physicochemical parameters of habitat water determined simultaneously. The parameters studied included physical habitat characteristics (perimeter, depth and habitat bottom surface), physical characteristics of habitat water (Temperature, turbidity and total suspended solids (TSS) and chemical aspects of water (Dissolved Oxygen or DO, pH, total dissolved solutes or TDS and conductivity). The effect of physicochemical factors on *Anopheles* larvae/pupae abundance was determined using Generalized Linear Models. A total of 40 habitats were sampled. Only 50% of these had *Anopheles* larvae while 25% had *Anopheles* pupae. From these habitats, 862 *Anopheles* larvae and 230 *Anopheles* pupae were collected. High numbers of *Anopheles* pupae were found in ACF (10.90 ± 7.30) than in non-ACF habitats (1.11 ± 0.72). There were significantly more *Anopheles* larvae in ACF (mean = 36.95 ± 16.93) than non-ACF habitats (7.62 ± 3.04), ($P = 0.02$). Habitats with wooden bottom surfaces (34.85 ± 21.2) had more larvae than those with mud (21.35 ± 9.09) and rock bottom surfaces (1.375 ± 1.1). Of all factors studied, perimeter ($p=0.023$), TSS ($p=0.001$), temperature ($p=0.08$) and conductivity ($p=0.052$) influenced larval abundance significantly. Other factors interacted resulting in significant associations with *Anopheles* larval abundance. These were TDS with conductivity ($P = 0.035$), TSS with DO ($P = 0.003$) and TSS with temperature ($P = 0.001$). Even though individual physicochemical characteristics could be linked to density of *Anopheles* larvae and productivity of mosquito habitats, the results indicate that certain variables interact to regulate mosquito abundance. Malaria control measures tailored towards manipulating

physicochemical characteristics in mosquito breeding sites should be adopted in integrated mosquito programmes.

CHAPTER ONE: INTRODUCTION

Malaria remains a major public health problem despite the concerted control efforts (WHO, 2018). In 2018, approximately 228 million estimated cases and 405,000 malaria-related deaths were reported world-wide. Ninety four percent of these deaths occurred in sub-Saharan Africa (WHO, 2019). Currently, malaria is the leading cause of child mortality in Sub-Saharan Africa (WHO, 2019). The disease is transmitted by *Anopheles* mosquito vectors with *Anopheles gambiae* being the major species (WHO, 2018). Other species include *Anopheles funestus*, *Anopheles coustani* and *Anopheles arabiensis* (WHO, 2017). In Africa, the main parasite species that causes malaria is *Plasmodium falciparum* (WHO, 2018). *Plasmodium malariae*, *P. knowlesi*, *P. vivax* and *P. ovale* play lesser roles (WHO, 2017).

In Kenya, the western and coastal areas are most affected by malaria outbreaks (Njuguna *et al.*, 2019). A recent study in Western Kenya demonstrated that malaria is still a major cause of mortality in children aged below five years (Kapesa *et al.*, 2018). The study reported that 49% of all registered hospital admissions were due to malaria. A majority of these admissions were associated with severe forms of illness.

The Lake Victoria basin residents have constantly faced malaria incidents despite intervention efforts put in place over years. Artemisinin-based Combination Therapies (ACT), Indoor residual spraying and use of long-lasting insecticidal nets have been used and proved successful (Zhou *et al.*, 2014). However, despite considerable reduction in malaria cases through these methods, new challenges have come up. For example, there is an increased rise of mosquito resistance to insecticides (Wanjala *et al.*, 2015; Wanjala and Kweka, 2018). Furthermore, malaria parasites have also become resistant to anti-malaria drugs (Bloland, 2001) and mosquitoes are changing their behavior to avoid contact with insecticide treated surfaces (Govella *et al.*, 2013). In a recent study, for example, older children became susceptible to clinical malaria because of reduced exposure earlier in life to malaria parasites. This was due to prolonged use of

long-lasting insecticidal nets (Seidlein and Knudsen, 2016). This shows that malaria dynamics keep changing despite the implementation of various control methods (Kapesa *et al.*, 2018). Better planning and implementation of novel practices are needed to realize effective malaria control.

Furthermore, numerous mosquito breeding habitats associated with the fringes of Lake Victoria (Minakawa *et al.*, 2012) act as potential sources of malaria vectors. These habitats are diverse and different in terms of physical, chemical and biological characteristics (Gimnig *et al.*, 2001; Carlson *et al.*, 2004; Fillinger *et al.*, 2004). This diversity has greatly influenced the distribution and abundance of larval mosquitoes in nature (Ahmadi *et al.*, 2013). For instance, a study confirmed that artisanal capture fishing habitats exhibit high *Anopheles* abundance (Mukabana *et al.*, 2019). Despite the high numbers in these habitats, it is possible that not every ACF habitat is often colonized by the immature stages of malaria vectors. A number of mosquito habitats could contain no mosquito larvae while few other neighboring habitats contain high numbers of larvae. Such variability in terms of larval and pupal colonization need to be fully studied to understand the physicochemical basis of these habitats and applicability in mosquito control on Mageta Island.

Factors determining oviposition, survival and distribution of vectors in nature hence variability in breeding habitats (Mwangangi *et al.*, 2007; Ndenga *et al.*, 2012), need to be clearly defined and understood. Breeding water quality is important in explaining variability and productivity of habitat types (Kudom, 2015). Various studies suggest that intervention efforts for malaria should consider variability in productivity of different habitats (Gu and Novak, 2005). However, suppressing *Anopheles gambiae s.s.*, the major malaria vector in Western Kenya has been difficult because of the complexity of breeding habitats (Rejmánková, 2018). This study was designed to unravel key physicochemical factors that modulate breeding of malaria vectors in ACF habitats on Mageta Island. The knowledge of inherent characteristics of these habitats will enhance effective larval control programs through directing control efforts towards only productive habitats.

1.1 Problem statement

Malaria continues to be a huge burden and one of the most important public health problems worldwide (WHO, 2019). Africa is the most affected and records the highest number of deaths per year (WHO, 2018; WHO 2019). Malaria control in Africa is achieved mostly by use of long-lasting insecticidal nets and indoor residual spraying (WHO, 2018). These methods have made big gains by significantly reducing malaria cases. However, various challenges have also come up (Kokwaro, 2009). There is increased resistance of mosquito vectors to insecticide, increased outdoor biting by malaria mosquitoes (Benelli and Beier, 2017) and evolution of *Plasmodium* resistance to hitherto effective drugs (Bloland, 2001).

Increase in human activities that continually modify the environment creating potential breeding sites for mosquitoes (Ahmadi *et al.*, 2013), further escalates the problem of malaria. For instance, residents of the Lake Victoria basin depend on fishing as their main source of livelihoods. The fishing activities, otherwise known as artisanal capture fishing (ACF), have led to creation of numerous mosquito habitats (Mukabana *et al.*, 2019). The habitats are variable in nature, small, temporary sunlit pools and favor breeding of *Anopheles gambiae* mosquitoes (Minakawa and Sonye, 2005), the major malaria vector. Artisanal capture fishing habitats comprise a distinct group that is distinguishable by the type of habitat bottom surface. A complex set of physical and chemical factors interact forming a patchy habitat. Despite the importance of artisanal capture fishing in proliferation of a diverse group of mosquito breeding habitats, little is known about the factors that enhance mosquito preference for these habitats. Understanding the factors that contribute to relative abundance and distribution of malaria vectors on an artisanal capture fishing Island could be an important prerequisite of vector control operations. This study therefore sought to determine key factors that enhance high *Anopheles* productivity in artisanal capture fishing habitats. A conceptual framework illustrating how different

physicochemical factors affect mosquito productivity is provided below (Figure 1).

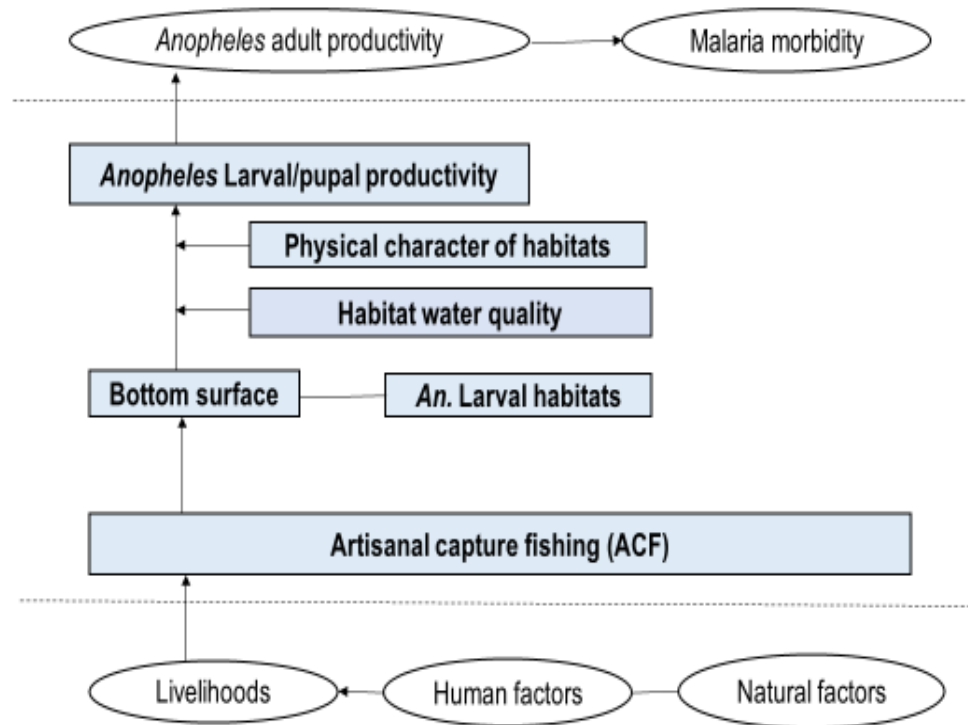


Figure 1: Conceptual framework showing the link between different physicochemical factors and *Anopheles* productivity on Mageta Island in Western Kenya. Arrows are used to show cause-effect relationships. Arrows start from cause variables and point towards outcome variables. Boxes indicate variables of interest. The lines indicate correlations between variables without cause-effect relationship.

1.2 Justification and Significance of the Research

Malaria is a major health threat in Sub-Saharan Africa (WHO, 2019). Effective control of this disease is still facing challenges (Benelli and Beier, 2017). Therefore, there is need to invent new strategies that can help curb this problem. Vector control has been recognized as one of the most effective control methods (Kokwaro, 2009). Most studies done focus on the adult vector of malaria, only

few studies try to understand the biology and ecology of aquatic stages of juvenile stages. It is well known that juvenile stages determine the dynamics, abundance and fitness of malaria mosquitoes (Rejmánková, 2018). Furthermore, there is a fundamental difference between mosquito adults and juvenile stages. Adults are usually mobile, they fly and can readily detect and circumvent intervention measures; mosquito eggs, larvae and pupae are confined within relatively small breeding sites and thus easy to control using appropriate methods because they cannot easily escape (Rejmánková, 2018). For effective intervention programs for mosquito control, biology and ecology of mosquito breeding, types of waters and range of habitats should be clearly known (Gu and Novak, 2008). Knowledge on larval ecology is limited and insufficient to achieve larval control which could be more promising (Gu and Novak, 2005). This study thus aims to fill this knowledge gap by exploring the different physical and chemical factors that affect aquatic stages and thus productivity of malaria vectors.

In order to understand the association between physicochemical parameters and *Anopheles* productivity in habitats associated with ACF activities, a cross-sectional survey was conducted on Mageta Island, Western Kenya. Instead of just focusing on identifying *Anopheles* mosquito habitats and designing effective control methods for malaria control, this study sought to provide precise valuable information on the ecology of *Anopheles* mosquito in relation to physicochemical characteristics of breeding habitats that have implication for vector distribution in a given area. This will enhance acquisition of accurate information on productivity of malaria vectors, which is essential for proper control interventions. Understanding of factors affecting *Anopheles* productivity in different habitats will enhance sustainability, cost-effectiveness and feasibility of mosquito control efforts.

1.3 Research Objectives

1.3.1 General Objective

To evaluate the physicochemical basis of higher *Anopheles* productivity in larval habitats created through artisanal capture fishing.

1.3.2 Specific Objectives

1. To determine the relationship between physical characteristics of mosquito larval habitats and *Anopheles* productivity on an artisanal capture fishing Island in western Kenya.
2. To associate physical aspects of mosquito habitat water to *Anopheles* productivity on an artisanal capture fishing Island in western Kenya.
3. To determine the relationship between chemical characteristics of mosquito habitat water and *Anopheles* productivity on an artisanal capture fishing Island in western Kenya.

1.4 Research hypotheses

1.4.1 Null hypothesis

Anopheles productivity in different larval habitat types is not affected by different physicochemical characteristics.

1.5.2 Alternative hypothesis

Anopheles productivity in different habitat types is affected by different physicochemical characteristics.

1.5.3 Assumptions

All mosquito larvae that developed into pupae emerged as adults.

CHAPTER TWO: LITERATURE REVIEW

It is over 100 years since *Plasmodium* species were described and confirmed to be transmitted by female anopheline mosquitoes. However, despite this, malaria remains a leading cause of morbidity and mortality worldwide (WHO, 2016; WHO, 2017; WHO, 2018). Artemisinin-based Combination Therapies (ACTs), Indoor residual spraying (IRS) and use of Long-lasting insecticidal nets (LLINs) have been used successfully as main malaria control methods (WHO, 2018). However, LLINs and IRS only target indoor resting and biting mosquitoes. On Mageta Island and most other fishing communities, fishing is the main economic activity. Most people carry out their activities outside in the evening. This therefore, renders LLINs and IRS less effective because they are mainly used indoors (Ogola *et al.*, 2017). This calls for alternative methods to curb malaria. Interest in malaria control methods that target aquatic immature stages has gained support (Gu and Novak, 2008; WHO, 2013; Tusting, 2014). The immature stages determine the dynamics, survival and number of adult mosquitoes that emerge from a habitat. Understanding the ecology of aquatic mosquito stages can greatly contribute to the implementation of current control methods and development of novel strategies.

2.1 Malaria transmission

Malaria in man is a deadly disease caused by several *Plasmodium* parasites namely *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. *Plasmodium falciparum* is responsible for most malaria cases (WHO, 2018). Human malaria parasites undergo development in two hosts, a human being and a female *Anopheles* mosquito (WHO, 2018). When a female mosquito bites an infected person, it carries the parasites then transfers them to another human being (Mogi, 1987). This disease presents a wide variety of symptoms from mild to severe. After being bitten by an infected *Anopheles* mosquito, incubation period takes between 7-30 days (Service, 1993). *Plasmodium falciparum* usually presents shorter incubation periods compared to other parasites such as human

malaria *Plasmodium malariae* (Strickland, 1991; Service 1993). Malaria occurrence depends on certain climatic factors such as temperature, humidity and rainfall (Strickland, 1991). Temperature is a key element in the transmission cycle of mosquitoes (Strickland, 1991).

Tropical and sub-tropical climates enhance survival and multiplication of *Anopheles* mosquitoes (Strickland, 1991). Warmer regions closer to the equator normally exhibit intense transmission of the parasite. Thus, malaria in these regions is transmitted year-round (WHO, 2018). Highest transmission has been experienced in Africa, South of the Sahara and parts of Oceania such as Papua New Guinea (WHO, 2017). In cooler regions, malaria transmission is less intense and therefore seasonal (WHO, 2016). The most affected people therefore are usually those from poor tropical and subtropical areas of the World, young children and pregnant mothers being the most vulnerable (WHO, 2018). This is a possibility because of the scarce resources and socio-economic instability that hinders effective control. The direct effects of treating and controlling malaria fall on the government and individuals affected. For instance, individuals incur costs when they have to purchase drugs, travel and treat the disease in various clinics and hospitals. Also, incur expenses in carrying out preventive measures such as insecticide spraying and burial costs. Also, the government uses a lot of funds for purchasing malaria drugs, supplying and purchasing health equipment, carrying out public health interventions and losing opportunities for joint economic ventures due to the disease (Guyant *et al.*, 2015).

2.2 The malaria burden

Malaria is a killer disease. It is caused by parasites that are transmitted from person to person through bites of female *Anopheles* mosquitoes (WHO, 2016). In 2018, 228 million cases of malaria were estimated worldwide (WHO, 2019). The estimated number of deaths that year was 405,000. Children under the age of 5 accounted for 67% of all malaria deaths worldwide in 2018. Poor tropical and subtropical areas of the World are the most affected (WHO, 2017; WHO 2019). Malaria in Kenya is endemic in Coastal areas near the Indian Ocean and the Lake Victoria basin (Fig 2). In these regions, early diagnosis and prompt treatment using antibiotics, use of long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the main intervention methods used (Zhou *et al.*, 2014; Njuguna *et al.*, 2019). Indoor residual spraying is mostly used in selected areas with high transmission rates around Lake Victoria.

Malaria is low in arid areas and the prevalence rate can only rise to 3% when the rains are heavy (WHO, 2016). In such regions, surveillance, effective diagnosis and treatment are the main tools used to control the disease (WHO, 2016). Long lasting insecticidal nets are presently distributed countrywide and also other preventive measures such as giving antimalarial drugs to pregnant mothers have been effected (WHO, 2016). This has influenced malaria drop from 11% to 8%, 2010-2015 (WHO, 2016). However, a recent study revealed that malaria is still the major cause of child mortality in Western Kenya (Kapesa *et al.*, 2018). This has been linked to mosquito resistance to various classes of insecticides (Ranson *et al.*, 2011), *Plasmodium* resistance to antimalarial drugs, and outdoor transmission (Cooke *et al.*, 2015). These consequently have greatly compromised intervention efforts (Guyant *et al.*, 2015)

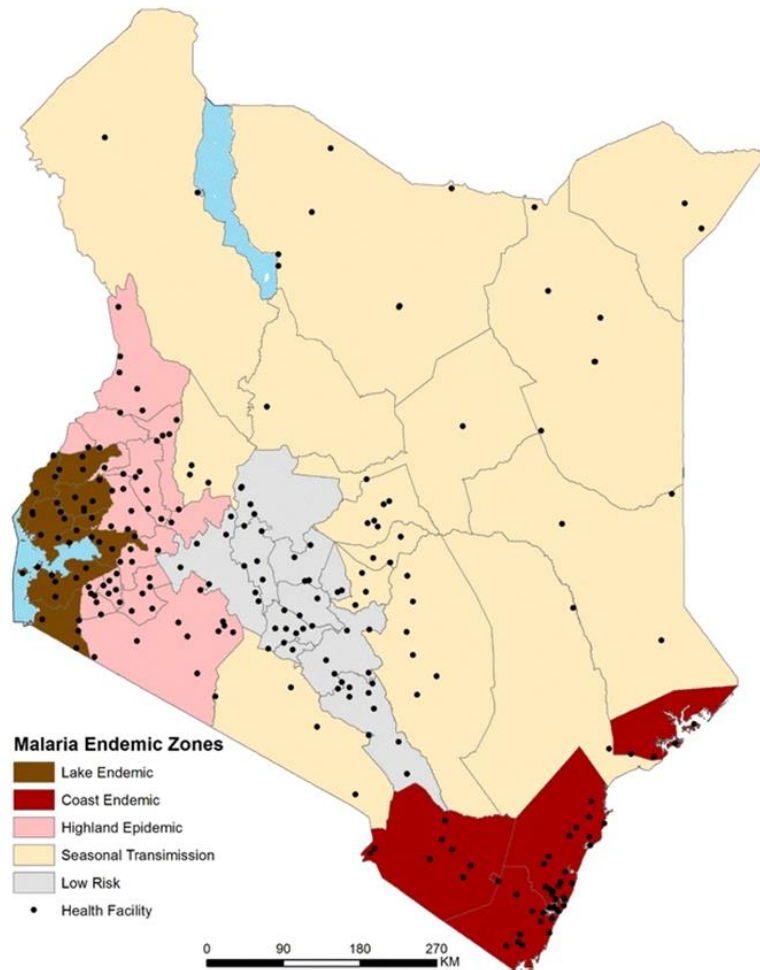


Figure 2: Malaria endemicity zones in Kenya (Githinji *et al.*, 2016)

2.3 Mosquito life cycle

Anopheles mosquitoes go through four developmental stages in their lifecycle (Figure 3). The first three stages (egg, larvae and pupae) are aquatic (WHO, 1982). The last stage (adults) is terrestrial (Mogi, 1987). In nature, females live up to approximately one month. A female mosquito can lay up to 50-200 eggs for every oviposition (Mogi, 1987). Eggs are laid singly on water surfaces. Larvae have four instars (first, second, third and fourth) (Figure 3) and have well-

developed head and mouth brushes for feeding. They lack a respiratory siphon but position themselves parallel to the water surface in order to take in atmospheric oxygen (Clements, 2011). They breathe through spiracles (Mogi, 1987). When disturbed, they quickly dive below the water surface (WHO, 1982). Larvae occupy different types of habitat ranges but in most cases, *Anopheles* mosquitoes prefer clean water that is open to direct sunlight (Minakawa *et al.*, 1999). There are also few species that occupy tree holes and leaf axils (Asir-uddin, 1952). However, preference for a certain habitat of each species varies. Pupae are comma-shaped. The head is merged with the thorax forming the cephalothorax and they frequently come to the surface to breathe through a pair of respiratory siphons (Clements, 2011). The pupal stage lasts for about 2-3 days prior to adult emergence (Paskewitz, 1995).

Development of different stages from egg to adult can take as little as five days but most take 10-14 days (Mogi, 1987) depending on temperature. The body size of an adult mosquito varies depending on the density of the larval population and availability of food in breeding habitats. Male mosquitoes live for about a week feeding on plant sugars (Mogi, 1987). Females can live for longer but their lifespan also depends on various factors like temperature, humidity and ability to obtain a blood meal (Paskewitz, 1995). Female *Anopheles* mosquitoes prefer feeding on humans but some also feed on animals as alternative hosts (Okara *et al.*, 2010). *Anopheles* mosquitoes mostly feed at dusk either indoors or outdoors. Their anthropophilic behavior makes them prone to transmitting parasites (Paskewitz, 1995).

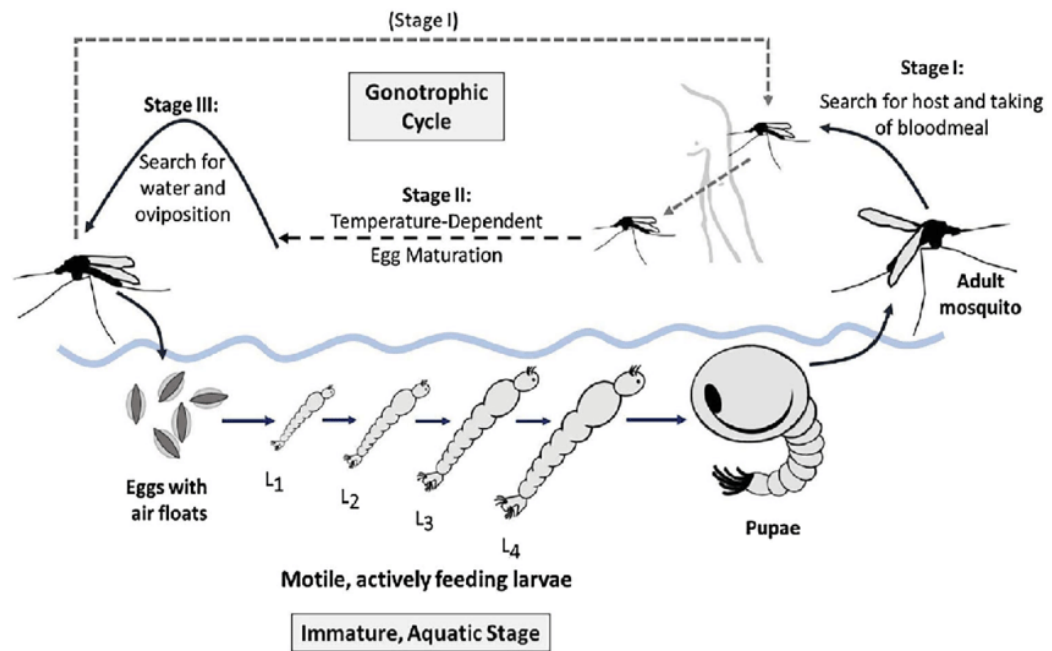


Figure 3: The lifecycle of *Anopheles* mosquitoes. The first three stages spend their life in water. Females require a blood meal for egg development. The female rests for a few days while the blood meal is digested and eggs develop after obtaining a full blood meal. This process is usually temperature dependent (Okuney *et al.*, 2019).

2.4 The malaria vectors

The local dominant *Anopheles* species in Kenya belong to two complexes: *Anopheles gambiae* and *Anopheles funestus* (Minakawa *et al.*, 1999; Mwangangi *et al.*, 2012). *Anopheles gambiae* complex are mostly found in areas with high malaria transmission (Okara *et al.*, 2010). In Western Kenya, malaria is transmitted primarily by *Anopheles gambiae sensu stricto*, *Anopheles arabiensis*, and *Anopheles funestus* (Imbahale *et al.*, 2012; Githeko *et al.*, 2006). *Anopheles gambiae s.s* is the most efficient vector within the *Anopheles gambiae* complex (Minakawa *et al.*, 2002). There is high variability in vector abundance and malaria transmission intensity in Western Kenya (Githeko *et al.*, 2006). This variability is as a result of factors that have been mentioned in literature (Githeko *et al.*, 2006). It has been difficult to identify a factor that contributes most to

vector abundance variations. This has led to difficulties in controlling *Anopheles gambiae s.s* and calls for new vector control strategies (Githeko *et al.*, 2012).

2.5 Approaches for malarial control

Mosquito control has been well explored to curb the malaria burden (Raghavendra *et al.*, 2011). However, the different control approaches used still present challenges (Guyant *et al.*, 2015). Resistance by *Plasmodium* parasites and *Anopheles* vectors to drugs and insecticides, respectively, (Guyant *et al.*, 2015), inadequate health care infrastructure and declining community acceptance considerably reduces effectiveness of control interventions (WHO, 2001). Because malaria vectors and disease pathogens are variable in nature (Hanafi-Bojd *et al.*, 2012) and that certain human populations are more vulnerable, intervention efforts need to be changed or a range of interventions need to be integrated in order to achieve success. One approach is to consider the variability and productivity of breeding habitats (Ndenga *et al.*, 2012; Abai *et al.*, 2013; Mereta *et al.*, 2013;). Identifying key factors driving selective mosquito oviposition in certain habitats will help to locate the most prolific habitats which will then inform on the best approaches for mosquito control (Rejmánková, 2018). This is especially useful for larval control measures (Gu and Novak, 2008) targeting outdoor transmission of malaria (Cooke *et al.*, 2015) e.g. in fishing communities. If larvae are targeted, the adult vector population can reduce drastically because larvae are easy target given that they cannot escape from breeding sites (Rejmánková, 2018). All this requires identification of mosquito breeding sites that are attractive and productive (Gu and Novak, 2005). The different approaches that have been used to control malaria mosquitoes are reviewed subsequently.

2.5.1 Environmental management for mosquito control

Environmental management is one of the oldest methods of control and has been widely adopted in various scenarios (Raghavendra *et al.*, 2011). Environmental

management encompasses plans directed towards carrying out monitoring activities that enhance modification or manipulation of environmental factors to minimize vector propagation and reduce man-vector-pathogen contact (WHO, 1982). It involves environmental modification, environmental manipulation and changes in human habitation (Randell *et al.*, 2010). Environmental modification involves long lasting physical transformation of the potential habitats to reduce, prevent or eliminate vector habitats through drainage or leveling up land without causing any adverse effects to the environment (WHO, 1982). Environmental manipulation consists of any recurrent activity that aims to produce unfavorable temporary conditions to breeding of vectors in habitats (WHO, 1982). Temporary changes involve changing water levels in reservoirs, flushing streams or canals, flooding or temporarily draining man-made or natural wetlands and changing water salinity (Ault, 1994). This method achieved success in early 20th century when it was integrated with other control approaches (Lacey and Lacey, 1990; Walker and Lynch, 2007). Earlier strategies of environmental management mostly involved draining wetlands, removal of breeding habitats and installation of house screens (Walker and Lynch, 2007). Environmental management however, requires high work force (Takken *et al.*, 1990), careful design and investment, regular maintenance and a site-specific approach (Konradsen *et al.*, 2004). Furthermore, its efficacy is dependent on specific ecological requirements of the vector species, precise information on habitat distribution and the local environmental conditions (Walker and Lynch, 2007). It is further documented that, this control approach works best when integrated with other interventions (Randell *et al.*, 2010).

2.5.2 Biological control of mosquito larvae

Biological control of mosquito has been in use and proven to be successful (Benelli *et al.*, 2016; Lacey, 2007; Moazami, 2011). There are natural enemies that feed on mosquito juvenile stages in aquatic environments and these considerably play a role in reducing mosquito larvae (Benelli and Walker, 2016). Larvivorous fish, for example, has been successfully used for mosquito control (Chandra *et al.*, 2008). Initially, the fish *Gambusia affinis* had been implemented as the most effective control agent throughout the World, but its negative impact on the environment was realized (Walker and Lynch, 2007). It is now recommended that larval control be done using indigenous fish species, and this has also shown success (Fletcher *et al.*, 1992). Use of fish for larval control is still facing challenges in terms of implementation due to the high initial capital required. Also, there is need to carefully consider the ecological cost of introducing predatory species (Chandra *et al.*, 2008). Apart from fish, parasitism and predation are also listed in the literature (Kamareddine, 2012), whereby individuals (natural enemies) are introduced and manipulated to suppress mosquito populations (Benelli *et al.*, 2016). However, these have their own challenges (Kamareddine, 2012).

The microbial agents *Bacillus thuringiensis* (*Bt*) and *Bacillus sphaericus* (*Bs*) (Kamareddine, 2012) have been adopted. These agents are non-toxic to non-target species and do not persist in the environment (Lacey, 2007). Furthermore, production cost is low. Reports about resistance are few (Hongyu *et al.*, 2004). Also, fungal pathogens (Blanford *et al.*, 2005), (*Metarhizium* and *Beuveria*), have shown promise although they have not been produced widely in commercial volumes for mosquito control (Scholte *et al.*, 2006).

2.5.3 Chemical control of mosquitoes

Chemical control of mosquitoes has evolved in years (Christophers, 1951). The earliest chemicals used were Paris green (Copper acetoarsenite) and petroleum

by products which majorly focused on larval control. These were later abandoned due to high toxicity and pollution to the environment (Walker and Lynch, 2007). Dichlorodiphenyltrichloroethane (DDT) was discovered and control shifted to adult mosquito populations (United States Environmental Protection Agency, 2016). However, malaria vectors became resistant to this chemical (Liu *et al.*, 2006) and also, its adverse effects on the environment makes it unsuitable (Guyant *et al.*, 2015). Currently, prompt diagnosis and treatment, use of long lasting insecticidal nets (LLINs) and indoor residual spraying are the most commonly used methods of control (WHO, 2018). Long lasting insecticidal nets have proved to be excellent tools in reducing child mortality and morbidity; for instance, nets treated with pyrethroids showed success in Asia (Hung *et al.*, 2002). It has been documented that for these methods to achieve success in Africa, wide distribution of nets must be maintained (Curtis and Mnzava, 2000) and for IRS, frequent supervision and inspection must be maintained (Shiff, 2002). However, the issues of resistance to insecticides, mosquito changing behavior to evade treated surfaces (Liu *et al.*, 2006; Nkya *et al.*, 2013; Benelli *et al.*, 2016) and increased outdoor activities (Cooke *et al.*, 2015) have presented difficulties in these control approaches.

2.6 The intricate lifecycle of malaria vectors

A mosquito is an integrant of a much larger metacommunity of interacting communities (Blaustein *et al.*, 2010). This means that biotic (predation and competition) and abiotic factors (environmental factors) significantly influence the growth, development and survival of the juvenile stages of malaria mosquitoes in a complex way (Mala and Irungu, 2011; Ndeng *et al.*, 2012; Garba and Olayemi, 2015; Emidi *et al.*, 2017). This plays a key role in spatial and temporal distribution of mosquito species, and thus on *Anopheles* productivity (Minakawa *et al.*, 1999; Sattler *et al.*, 2005; Grillet, 2009). Understanding the ecology of malaria vectors therefore can be critical in planning and implementing malaria control programs (Rejmánková *et al.*, 2018). However, this requires a deep understanding of factors affecting larval abundance as well as adult emergence from habitats (Gimnig *et al.*, 2001). The information regarding the precise identity of larval breeding sites and factors associated with them is contradictory. Most studies have focused more on designing mosquito control methods (Gu and Novak, 2005). To enhance efficiency of the different control methods, microhabitat factors that affect the occurrence and abundance of *Anopheles* larvae need to be clearly characterized (Minakawa *et al.*, 1999). This will enhance acquisition of accurate information on productivity of malaria vectors which is critical for proper control interventions. Figure 4 below explains the interactions between juvenile stages and their habitat characteristics in the context of the ecosystem. Humans can have an influence on the quality and availability of mosquito habitats mostly through ecosystem and landscape changes when they carry out their daily activities. Artisanal capture fishing (Mukabana *et al.*, 2019) is a good example of the effect of anthropogenic activities on mosquito breeding.

The main determinants of larval development have been given great consideration in the illustrating below.

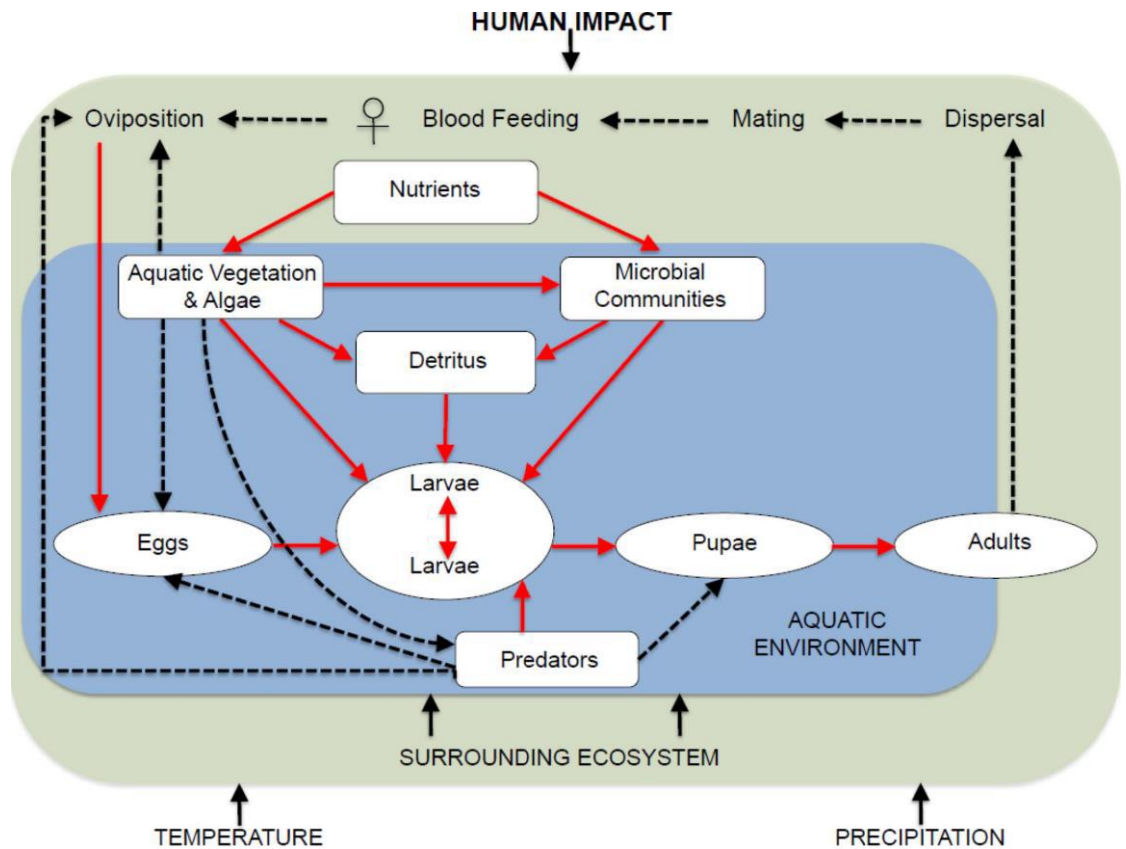


Figure 4: Association between environmental factors with larval development of mosquitoes (Rejmánková, 2013). The red arrows indicate physical cause-effect relationships. The arrow starts from the variable causing effect (dependent variable) pointing to the variable that is affected (independent variable). The dotted arrows indicate effects of human activities on the mosquito lifecycle.

2.7 Mosquito breeding habitats

Malaria transmission is highly dependent on availability of vectors (Christophers, 1951) and presence of a suitable host. Malaria vectors choose and occupy a nonrandom set of available habitats (Minakawa *et al.*, 2006; Okara *et al.*, 2010; Minakawa *et al.*, 2012). Productive larval habitats are linked to

producing competent malaria vectors. This is because larval habitats play a critical role in adult distribution, abundance and fitness (Ndenga *et al.*, 2012). *Anopheles* mosquitoes breed in a diversity of habitats (Fillinger *et al.*, 2004). They breed in pools, puddles, ditches, trenches, fishing boats (Mbida *et al.*, 2016; Mukabana *et al.*, 2019), river fringes, open drains, burrow pits (Mutuku *et al.*, 2006), rain water pools (Mala and Irungu, 2011), hoof prints, goldmines, drainage ditches (Kweka *et al.*, 2011), active and abandoned fish ponds among others (Fillinger *et al.*, 2009; Njunwa, 1993; Ndenga *et al.*, 2011). Both semi-permanent and permanent habitats contribute greatly to *Anopheles* larval populations (Mattah *et al.*, 2017; Ahmadi *et al.*, 2013). Mattah *et al.*, (2017) defined a semi-permanent habitat as a water body that contains *Anopheles* larvae at least once and which dries up at least once during the sampling period. A permanent habitat is one which contains *Anopheles* larvae at least once and has water throughout the sampling period (Mattah *et al.*, 2017). Man-made habitats occur in large numbers in the environment and contribute significantly to malaria transmission throughout the year (Fillinger *et al.*, 2004).

Habitats created through artisanal capture fishing were recently identified to be highly productive for mosquito larvae on Mageta Island in western Kenya (Mukabana *et al.*, 2019). Other habitats that are not associated with fishing activities on the Island included ditches, abandoned fish ponds and rock pools. Boats were the most abundant habitat type and favored high breeding of *Anopheles* larvae. The specific physicochemical characteristics that enhance high productivity in habitats associated with artisanal capture fishing have not been fully explored on this Island. In order to enact effective control interventions, the ecology of disease vectors should be clearly understood (Rejmánková *et al.*, 2018); and for this to succeed, the quality of larval habitats has to be realized (Minakawa *et al.*, 2006). This will help understand the factors that lead to spatial and temporal changes in mosquito abundance. However, the great diversity of mosquito larval habitats has presented difficulties in collecting data on ecology of *Anopheles* mosquito larval habitats.

2.7.1 Choice of breeding sites by gravid *Anopheles* mosquitoes

The selection of an oviposition site which guarantees egg and larval survival is a critical fitness aspect among mosquitoes questing for habitats. Larval habitats are important for distribution and abundance of adult mosquitoes (Gimnig *et al.*, 2001). Studies show that *Anopheles gambiae* lay eggs in both natural and man-made environments (Ahmadi *et al.*, 2013). However, most studies indicate that *Anopheles gambiae* has a great preference for small temporary sunlit pools with algae and little or no aquatic vegetation (Gimnig *et al.*, 2001; Minakawa *et al.*, 1999). A study conducted on oviposition preference by *Anopheles gambiae* mosquitoes revealed that mosquito habitat types significantly affect oviposition (Munga *et al.*, 2005). It is not clear however how a mosquito selects a site for oviposition. Some studies observed that *Anopheles* mosquitoes prefer turbid water (Ye-Ebiyo *et al.*, 2003; McCrae, 2017). Moreover, there are various factors which contribute to turbidity that are yet to be determined. There is also a possibility that the microbial fauna of larval habitats release volatiles that act as important cues for mosquito oviposition (Rejmánková *et al.*, 2005). It is not precisely described whether mosquitoes select a breeding site because of the inherent characteristics of the site. Understanding this aspect can help manipulate environmental factors with an aim of effective control.

2.7.2 Effect of physicochemical parameters on breeding of *Anopheles* mosquitoes

The density of larvae in various habitats depends on a number of physicochemical and biological characteristics (Ndenga *et al.*, 2012). Spatial heterogeneity of malaria vectors can be affected by factors such as water temperature (Beck-Johnson *et al.*, 2013), dissolved oxygen, chlorophyll a (Muturi *et al.*, 2007), pH (Mala and Irungu, 2011), turbidity (Mwangangi *et al.*, 2010), conductivity (Nikookar *et al.*, 2017), emergent vegetation, surface biofilm (Ndenga *et al.*, 2012), algal material and predators (Ndenga *et al.*, 2012). Increase in water temperature, coverage with vegetation (Mutuku *et al.*, 2006;

Ndenga *et al.*, 2012), water nutrient and algal content play a critical role in larval productivity (Mala and Irungu, 2011). Habitat conditions also have an effect on egg hatchability (Munga *et al.*, 2005). As illustrated from various studies, the available data is contradictory (Abai *et al.*, 2016) and further investigations are required to determine the characteristics that favor oviposition, larval and adult densities. Physicochemical parameters hypothesized to affect *Anopheles* productivity are described below.

2.7.2.1 Temperature

All insects are ectotherms. Increments in environmental temperature are associated with an increase in body temperature of insects, which consequently increases metabolism. Respiration and metabolism increase up to a critical thermal limit and death can occur soon after the respiration rate begins to drop, even if the optimal temperature is restored (Neven, 2000). This indicates a possibility of systematic cell death at high temperature in insects. Neven and Rehfield, (1995) noted that elevated temperatures have an effect on the nervous and endocrine systems. For instance, high temperatures can alter respiratory physiology and induce irregularities in nervous and endocrine systems (Neven, 2000).

It is clear that mosquitoes grow in stages (Paskewitz, 1995). Various studies have been done on the effect of temperature on mosquito life history and parasite development (Lyimo *et al.*, 1992; Beck-Johnson *et al.*, 2013). Lyimo *et al.*, (1992) reared *Anopheles* larvae at different temperatures, (24°C, 27°C and 35°C). Effects of density and temperature interacted strongly to determine the life history parameters of the mosquito. More so, survival was highest at intermediate temperature of 27 °C. Beck-Johnson *et al.*, (2013) observed that the rate of development of juvenile stages increased to a peak of around 28 °C, then declined.

Extremely low or high temperatures however can slow development or accelerate development leading to death. The number of anopheline larvae were considerably fewer (75% less) in shaded than unshaded habitats (Wamae *et al.*, 2010). High temperatures above 40°C hindered survival of mosquito larvae (Thomson, 1940). Low temperatures are rarely experienced in larval habitats of *Anopheles gambiae* compared to high temperatures which are frequently encountered in most tropical regions. However, these temperatures occur only for a few hours and larvae survive this short periods (Paaijmans *et al.*, 2010). Paaijmans *et al.*, (2010) noted the importance of these temperature fluctuations for larval development.

Generally, sensitivities to temperature vary between different developmental stages of mosquitoes (Bayoh, 2001). Pupation rates, larval-to-adult survivorship, larval to adult development time are all affected by temperature (Beck-Johnson *et al.*, 2013). Bayoh and Lindsay (2004) observed that the optimal survival temperature of mosquitoes was lower than the temperature at which development was quickest and this clearly suggests a critical relationship between temperature and the life cycle of an insect. A temperature-dependent, stage-structured delayed differential equation model with full mosquito life cycle included revealed that mosquito population abundance was highly sensitive to temperature (Beck-Johnson *et al.*, 2013). Moreover, the mosquito adult population abundance and ability to vector malaria was to a greater extent influenced by the dynamics of the juvenile mosquito stages which were also highly depended on temperature. Furthermore, Beck-Johnson *et al.*, (2013) noted that adult populations persisted at temperatures suitable for juvenile mosquitoes despite the fact that they had a high predicted survival across a broader range. There are few elaborative studies on temperature of larval habitats; more so, the available data are hard to compare because different methods of measurement have been used.

Small, shallow and open pools show fluctuations in temperature over a large range throughout the day (Paaijmans, 2008). Most habitats associated with artisanal capture fishing are mostly small with little water and exposed to direct

sunlight (Mukabana *et al.*, 2019). This therefore implies that the temperature in these heats up faster during the day (Paaijmans, 2008). The small size of these ACF habitats means that the water is well mixed and no stratification occurs during the day. It is clear from literature that temperatures above certain limits hinder proper development of mosquito larvae to an adult. If a mosquito larva develops fast, full development of a critical body mass needed for pupation will be hindered. Therefore, daily temperature fluctuations to which larvae are exposed needs to be clearly understood. Because most ACF habitats are wooden (Mukabana *et al.*, 2019), it is possible that they do not exhibit very high temperatures beyond normal range for mosquito development. This is because, wood is a bad conductor of heat. From literature, it is clear that daily variations in water temperature especially in natural breeding habitats like ACF habits are not clear. Temperature being a core factor in *Anopheles* productivity, elaborate studies need to be put forth to understand it. This could improve and verify model estimates of water temperature in different types of habitats in the field which can further enable estimation of average water temperature and temperature extremes that could have an effect on mosquito productivity.

2.7.2.2 Turbidity

Turbidity refers to water clarity (Wetzel, 2001). Turbidity can be caused by particulate matter such as clay, silt, fine organic matter, soluble organic compounds, algae and other microscopic organisms that accumulate in water (Wetzel, 2001). These particles modify light penetration (Gray *et al.*, 2000) impacting both organisms and their egg development. Turbid water for example, inhibits light penetration interfering with photosynthesis thus food availability for many invertebrates is restricted. Furthermore, there is a possibility of insects ingesting large volumes of soil particles from turbid waters (Gammon, 1970). The particles being inert and non-nutritional overwhelm the mosquito larvae hindering proper uptake of nutritional material. The effect of turbidity on mosquito abundance is not well elaborated. However, McCrae, (1984) document turbidity to alter the distribution of juvenile mosquitoes.

Some studies show that *Anopheles gambiae* prefer habitats with turbid water (Mala and Irungu, 2011; Mwangangi *et al.*, 2010). However, Dida *et al.*, (2015) found no association between turbidity and mosquito presence and abundance. *Anopheles Arabiensis* and *Anopheles gambiae* larvae considerably took more time to develop into pupae in water that was turbid compared to clear water. Furthermore, smaller mosquitoes emerged from turbid waters. This preference for turbid water could be a mechanism to evade predators. Turbidity can change throughout the day when fine particles sink over time or when pools are disturbed by human activities, drinking cattle, rain drops and water currents. Human activities in ACF habitat could cause turbidity in these habitats to fluctuate throughout the day. It is not clear however how this affects mosquito breeding in these habits. These fluctuations need to be put into consideration when measuring turbidity of water in the field. Overall, more research needs to be done about the effect of turbidity on *Anopheles* productivity.

2.7.2.3 Total suspended solids (TSS)

Total suspended solids measure the dry weight of suspended particles that are not dissolved in a water sample (Breu *et al.*, 2008). If particulate matter is high, light penetration is affected which could interfere with habitat productivity because light is necessary for most life processes (Gammon, 1970). Also, suspended solids absorb heat from sunlight and this increases water temperature (Paaijmans, 2008). Increase in water temperature decreases levels of dissolved oxygen (Fondriest Environmental, 2015). The effect of low levels of dissolved oxygen on aquatic species is clear (Sprague, 1963; Duodoroff and Shumway, 1970). However, it is not yet clear how low levels of DO affect mosquito productivity. Increased water temperature due to high TSS fastens growth rate of mosquitoes (Paaijmans, 2008). In a study, the near-surface water temperature increased due to suspended particles thus affecting the diurnal temperature behavior of small water pools during the day (Paaijmans, 2008). It is clear that too high temperatures lead to development of smaller mosquitoes (Christiansen-

Jucht *et al.*, 2015). Furthermore, changes to the aquatic environment due to increased suspended solids result to increased difficulties in finding food (Breu *et al.*, 2008).

Heavy rains, fast-moving water and seasonal changes in algal growth increases TSS in water (Breu *et al.*, 2008). Mosquito habitats associated with ACF activities are mostly made of wood bottom surface (Mukabana *et al.*, 2019). This could point to less TSS in these habitats as compared to other habitat types, like mud habitat bottom surface types. *Anopheles* mosquitoes could prefer these habitat types because they mostly breed in clear water that is open to direct sunlight. Arguable, constant human activities could increase the concentration of TSS in ACF habitats. However, this needs to be elaborately studied to come up with clear conclusions. Effects of total suspended solids on mosquito productivity are however limited, hence the need for further studies. Also factors such as sediment size, shape, composition, cumulative and stressor effects, overall habitat complexity and availability need to be clearly defined.

Direct and indirect effects of TSS are difficult to quantify (Jones *et al.*, 2012). For example, measuring the extent of stress and an appropriate scale of response of *Anopheles* mosquitoes on high TSS in habitats is difficult. Emphasis should be put on tolerance levels and the extent and duration of stress or exposure as a result of different particle sizes, shapes, concentrations and how often a mosquito habitat is disturbed (turbulence). However, it remains difficult to link available TSS data to mosquito productivity due to diverse habitats that TSS values relate to, a range of conditions experienced in these habitats and a large variability and uncertainty in the data available. Is it possible that a mosquito larva could have developed behavioral adaptations that enable them survive in water with high amounts of particles?

Figure 5 illustrates some of the effects of TSS on different macro-invertebrates in general.

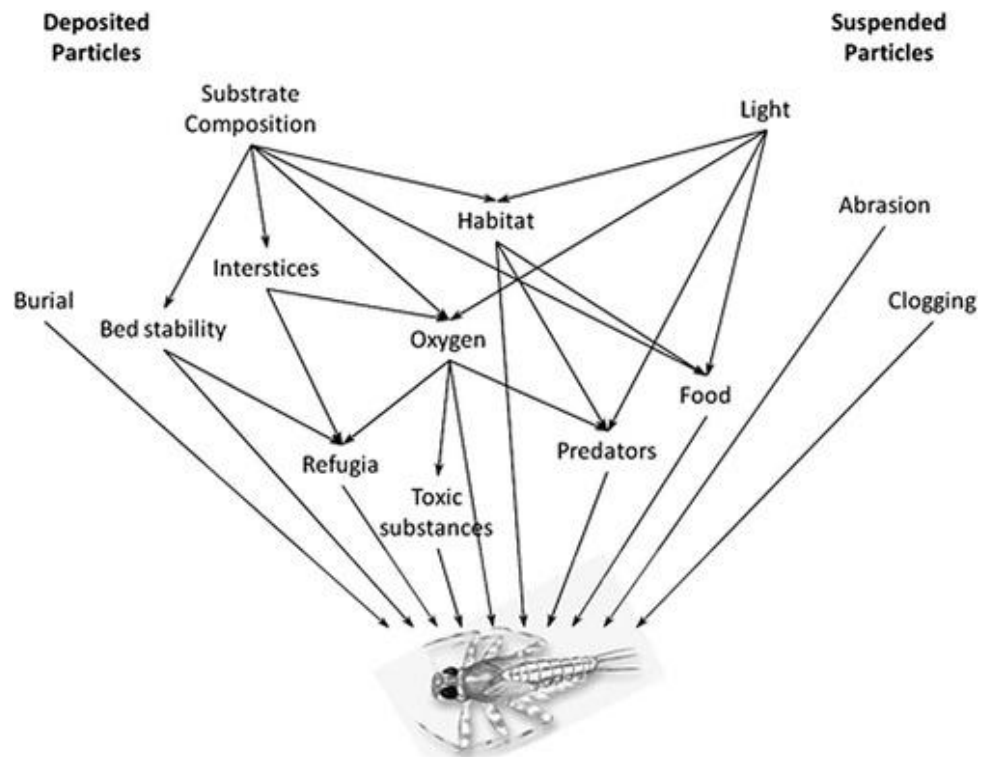


Figure 5: An illustration of the direct and indirect effects of sediments on macro-invertebrates. Both suspended and deposited particles impact the organism wellbeing. Arrows delineate interacting effects on the organism at an individual, species and community level. Response to changes in levels of sediments can be either positive or negative depending on the organism (Jones *et al.*, 2012).

2.7.2.4 Dissolved Oxygen

Movement of water over breathing structures of aquatic organisms cause microscopic bubbles of oxygen to diffuse from water to their blood/haemolymph. However, this is more efficient only above certain

concentrations. This means that oxygen can be present in water but can be too low to sustain aquatic life. Oxygen is an important indicator of water quality (Young, 1994) and directly influences organisms that are supported by a water body. A high amount of dissolved oxygen is expected to influence the diversity of organisms. Mosquito larvae are metapneustic thus they take in atmospheric oxygen (Clements, 2011). Despite using atmospheric Oxygen, larvae of several species of mosquitoes also use dissolved oxygen (Clements, 2011). Several studies have documented abundance of mosquitoes to vary with dissolved oxygen levels (Dida *et al.*, 2015). However, the nature of the variation has shown specificity in terms of context and mosquito species (Sunish and Reuben, 2002; Muturi *et al.*, 2008; Dejenie *et al.*, 2011; Kenawy *et al.*, 2013).

Elaborate work has been done on the effect of DO levels on *Culex* species, however little work has been done on *Anopheles* mosquito species. *Culex* can survive in conditions with extremely low levels of dissolved oxygen concentrations and this is not affected by the presence of atmospheric oxygen (Dale *et al.*, 2007). This mechanism is aimed at avoiding predation and competition by other organisms (Cech *et al.*, 1985). It is hypothesized that the survival of *Culex* mosquitoes at low DO levels could be due to less permeability of their cuticle to Oxygen (Reiter, 1978). Dissolved oxygen could not be a limiting factor for *Culex* larvae but it can lead to reductions in vector populations. In a number of studies, DO significantly influences *Anopheles* abundance (Kudom, 2015; Pinault and Hunter, 2012); while some studies have found *Anopheles* larvae breeding in polluted water (Awolola *et al.*, 2007). In another study, no significant association between *Anopheles* abundance and dissolved oxygen levels, was reported (Piyaratne *et al.*, 2005).

Habitats associated with ACF activities mostly contain little water, meaning little or no stratification occurs. From literature, in such water bodies DO remain at 100% saturation thus many dissolved gas molecules are held at equilibrium (Fondriest Environmental, 2013). Furthermore, ACF habitats are shallow and are always exposed to direct sunlight (Mukabana *et al.*, 2019), thus they require less

DO to reach 100% saturation. This is because increase in temperature increases the solubility of Oxygen in such habitats. The importance of DO in microbial decomposition thus food availability to mosquitoes cannot be neglected (Fondriest Environmental, 2013). Dissolved oxygen concentrations could be a pointer to high *Anopheles* abundance in habitats created through artisanal capture fishing. There is need to clearly describe how dissolved oxygen levels affect *Anopheles* productivity in different habitats to draw such relationships.

2.7.2.5 Potential of Hydrogen (pH)

Aquatic organisms have a well-defined pH range tolerance. If the pH goes below tolerance levels (too high or too low) then death occurs due to osmoregulatory failure (Rose *et al.*, 2013). The solubility and toxicity of chemicals including heavy metals in water is affected by pH (Rose *et al.*, 2013). Majority of organisms can withstand a pH range of 6.3-9.0 but they get stressed when pH ranges go below or beyond the normal range (European Inland Fisheries Advisory commission, 1969). Hatching and survival rates in animal natural systems are reduced by shifting of pH values away from the normal range. Further, more sensitive species of organisms are the ones affected by pH changes (Rose *et al.*, 2013). When pH levels are extreme, elements and compounds in water become more soluble and thus toxic compounds become more mobile putting aquatic life at risk because they can easily absorb them (Rose *et al.*, 2013). Also, when the pH of water slightly changes, phosphorus and other nutrients become more soluble thus readily available for plant growth. This makes more aquatic plants and algae to thrive, increasing the demand for dissolved Oxygen.

It is clear that low dissolved oxygen can be detrimental to aquatic organisms. The effect of pH on aquatic invertebrates was illustrated clearly when larval insects of emperopteral (flightless flies that were found in Hawaiian Islands but are now extinct) and trichopteran (caddisflies) declined at lower pH in a study by Sutcliffe & Hildrew (1989). Ranges of pH for mosquito larval habitats range from 3.3-8.1 but differ across different species of mosquitoes (Clements, 2011).

Both *Anopheline* and *Culicine* mosquitoes were positively associated with pH in a study by Dejenie *et al.*, (2011). In another study, *Anopheles* larvae preferred water with low PH values, i.e. acidic in nature (Adebote *et al.*, 2008). Natural changes in PH occur mostly due to surrounding rock interactions with other materials (Rose *et al.*, 2013). The bottom surface of most ACF habitats is wood. It is possible that precipitation and carbon dioxide concentrations (due to photosynthesis, respiration and decomposition) influence pH levels in such habitats. Nevertheless, the nature of most ACF habitats does not support vegetation growth thus lower rates of respiration and decomposition. It is not clear how PH levels in these habitats affect *Anopheles* productivity. As noted here, very few studies have attempted to link pH with *Anopheles* productivity. Elaborate studies therefore need to be carried out.

2.7.2.6 Conductivity

The capability of water to allow electrical flow is referred to as conductivity (Kevin *et al.*, 2014). It is directly related to ionic concentration (Wetzel, 2001). Ionic concentration is determined by dissolved salts and inorganic materials (Langland and Cronin, 2003). More ions therefore mean higher conductivity of water. Water conducts electricity due to the presence of positive and negative charges (Wetzel, 2001). However, the ions usually remain electrically neutral. This is because as they split in water into positively and negatively charged particles, the concentrations of each positive and negative charge remain equal (Gray *et al.*, 2000). Different aquatic organisms are adapted to certain ionic concentration. They absorb or excrete salts whenever there is a need (Kevin *et al.*, 2014). Any change in the conductivity of the ions by altering salt levels can negatively affect the metabolic abilities of the organisms. Sudden increase or decrease in conductivity levels is usually an indicator of pollution (Kevin *et al.*, 2014) resulting from human activities which can include agricultural practices.

In freshwater sources conductivity levels are also affected by the type of soil, underlying bedrock and ground water inflows (Wetzel, 2001). Clay soils for

example greatly contribute to conductivity because minerals in clay soil ionize as they dissolve. However, granite minerals remain inert and thus do not affect conductivity. In a community full of artisanal capture fishing activities, fluctuation in conductivity levels can occur as a result of pollution from agricultural runoff and human activities in habitats. Plausibility of this in habitats associated with ACF activities which are mostly made of wood is however debatable. A link between conductivity in ACF habitats could inform how this affects *Anopheles* productivity in mosquito habitats. Some studies associate conductivity and mosquito abundance but a few have been contradictory (Piyaratne *et al.*, 2005; Chirebvu and Chimbari, 2015; Dida *et al.*, 2015; Emidi *et al.*, 2017; Gopalakrishnan *et al.*, 2013; Musonda and Sichilima, 2019). A study by Awolola *et al.*, (2007) revealed that *Anopheles gambiae s.s* mosquitoes were readily found in habitats with high conductivity levels. This informs how well *Anopheles gambiae* can adapt to a wide range of habitats and how it is really important to understand its ecology before carrying out control methods.

2.7.2.7 Total dissolved solids (TDS)

Total dissolved solids include inorganic salts, organic matter and other dissolved materials in water (Fondriest Environmental *et al.*, 2014). The concentration of dissolved solids affects the spatial distribution of many fresh water invertebrates by balancing cell density of aquatic organisms. Very high concentration of TDS causes cells to shrink and this can affect the ability of an organism to move in the water column making it to either sink or float beyond normal range (Fondriest Environmental *et al.*, 2014). Increased levels of TDS can cause shifts in biotic communities, eliminate less tolerant species and can also cause serious effects on life stages of organisms (Kemker, 2014). Furthermore, if there are adverse changes in the ionic composition of water, some species of organisms can be eliminated while thriving of others is promoted (Derry *et al.*, 2003). It is hypothesized that excess TDS concentration can be toxic to aquatic organisms and their eggs, but all this depends on the ionic properties of the dissolved substances (Kemker, 2014). For fresh water sources, TDS should not exceed

2000mg/l. Very few studies have been done that associate total dissolved solids concentration with mosquito abundance. A recent study found no association between TDS and *Anopheles* abundance (Musonda and Sichilima, 2019). However, the adverse effects of TDS on *Anopheles* productivity should not be ignored.

2.8 Ecology, a prerequisite for malaria control.

The ecology of mosquitoes is the greatest impediment to malaria eradication and elimination (Rejmánková *et al.*, 2018). Many countries are struggling to enter the elimination phase (WHO, 2017). However, a lot of challenges resulting from poor understanding of mosquito ecology have made it hard to realize this goal. Despite the fact that major milestones have been made in understanding the ecology of malaria vectors, fully unraveling and understanding the interactions within the ecosystem is still presenting challenges (Rejmánková *et al.*, 2018). It is clear that larval ecology dictates vector-parasite interactions and successful transmission to a mammalian host (Ferguson *et al.*, 2010). It is also well known that mosquitoes get their vital resources from the immediate environment to complete their key life stages and successful transmission of malaria parasites to humans (Rejmánková *et al.*, 2018). The ecology of malaria vectors could thus help understand the ecological drivers that modulate the distribution and habitat segregation of *Anopheles* mosquitoes (Gu and Novak, 2005). This could be important for malaria control.

The high ownership of LLINs and intensive use of IRS in malaria hotspots has considerably reduced malaria cases (Raghavendra *et al.*, 2011); however, the entomological inoculation rate (EIR) is still above the level required for local elimination (WHO, 2017). Between 2015 to date, no significant reduction in global malaria has been observed (WHO, 2016; WHO, 2017; WHO, 2018). The number of cases reported in 2017 was 219 million (WHO, 2017) whereas 213 million cases were reported in 2015. More so, more deaths (435 0000) were recorded in 2017. A number of theories have been brought forth as to why

progress has stalled and there are increased malaria cases. Increasing resistance to insecticides and behavioral resistance of mosquitoes have been outlined as major challenges (Liu *et al.*, 2006; Kokwaro, 2009; Guyant *et al.*, 2015). Various studies propose that targeting the larval stage of the mosquito lifecycle could help curb the malaria problem (Gu and Novak, 2005; Gu and Novak, 2008). Strategies that are environmentally conscious are increasingly becoming important. Thus, there is need to embrace them especially under the potential threat of climate change. In this way, there will be no conflict between public health and environmental health. Furthermore, the breadth of factors involved in global mosquito control efforts will be considered. The ecological principles that have an influence on mosquitoes will therefore help in understanding the control of malaria transmission.

CHAPTER THREE: MATERIALS AND METHODS

Aquatic physical and chemical habitat characteristics influence mosquito oviposition, egg hatchability, pupation and adult emergence (Ndenga *et al.*, 2012; Selvan *et al.*, 2015; Abai *et al.*, 2016). These characteristics have a significant implication for larval source management measures. Some of the available data on the effect of aquatic physical and chemical characteristics on *Anopheles* productivity are limited and contradictory. Furthermore, deficiencies in field methodology for measuring mosquito productivity based on these characteristics have presented difficulties in interpreting data. While different habitat types resulting from ACF activities have been documented (Mukabana *et al.*, 2019), evidence on how different physicochemical factors affect variability of these habitats is limited.

3.1. Study site

This study was conducted on Mageta Island (33° 59'15"–34° 2'30" E and 0° 7'15"–0°8'15" N), located inside Lake Victoria in Siaya County, western Kenya (Figure 6). Mageta stands at an elevation of 1140 m above sea level with an estimated surface area of 7.02km² (Ogola *et al.*, 2017). Mageta is adjacent to Magare, Wayasi, Siamulala, Hama, Siro and Lolwe islands in Uganda. The Island is characterized by short (November and December) and long rains (March-May). Sometimes the area receives no rainfall the whole year. Fishing is the main economic activity. Thus, the Island has five fishing beaches namely Mitundu, Kuoyo, Mahanga, Sika and Wakawaka (Mukabana *et al.*, 2019). The main fish caught are tilapia (*Oreochromis niloticus*), the silver cyprinid (*Rastrioneobola argentea*), and Nile perch (*Lates niloticus*). Apart from fishing, small scale rearing of cattle, sheep, pigs, goats and chicken is practiced. Also, people practice crop farming of maize, sorghum, beans, vegetables, tangerines, tomatoes. The northern shore of the Island is muddy and supports most of the farming.

The major diseases and ailments in the Kenyan fishing communities include HIV/AIDS (Kwena *et al.*, 2019), waterborne diseases such as cholera (Nkoko, 2011) and malaria (Ogola *et al.*, 2017). A recent study found high *Plasmodium* parasite infection rates among major malaria vectors on Mageta Island (Ogola *et al.*, 2017). *Anopheles gambiae*, *Anopheles arabiensis*, *Anopheles funestus* and *Anopheles coustani* were the main malaria mosquito vectors on the Island (Ogola *et al.*, 2017). However, Mukabana *et al.*, (2019) found only *Anopheles gambiae* s.s on Mageta island in April 2018. This means vector dynamics keep changing. Mageta residents mainly use long lasting insecticidal nets (Ogola *et al.*, 2017) to protect themselves against indoor biting malaria mosquitoes. This clearly means that fishermen on Mageta Island are not protected because they engage in outdoor fishing activities as happens on adjacent islands (Olanga *et al.*, 2015). This renders them vulnerable to outdoor malaria. Furthermore, a study noted that there is low LLIN coverage on this Island (Ogola *et al.*, 2017). This suggests that more sustainable approaches should be deployed to realize considerable reduction of malaria cases on this Island. To achieve this, a better understanding of how different physicochemical factors affect *Anopheles* productivity in ACF habitats is needed which this study focuses on.

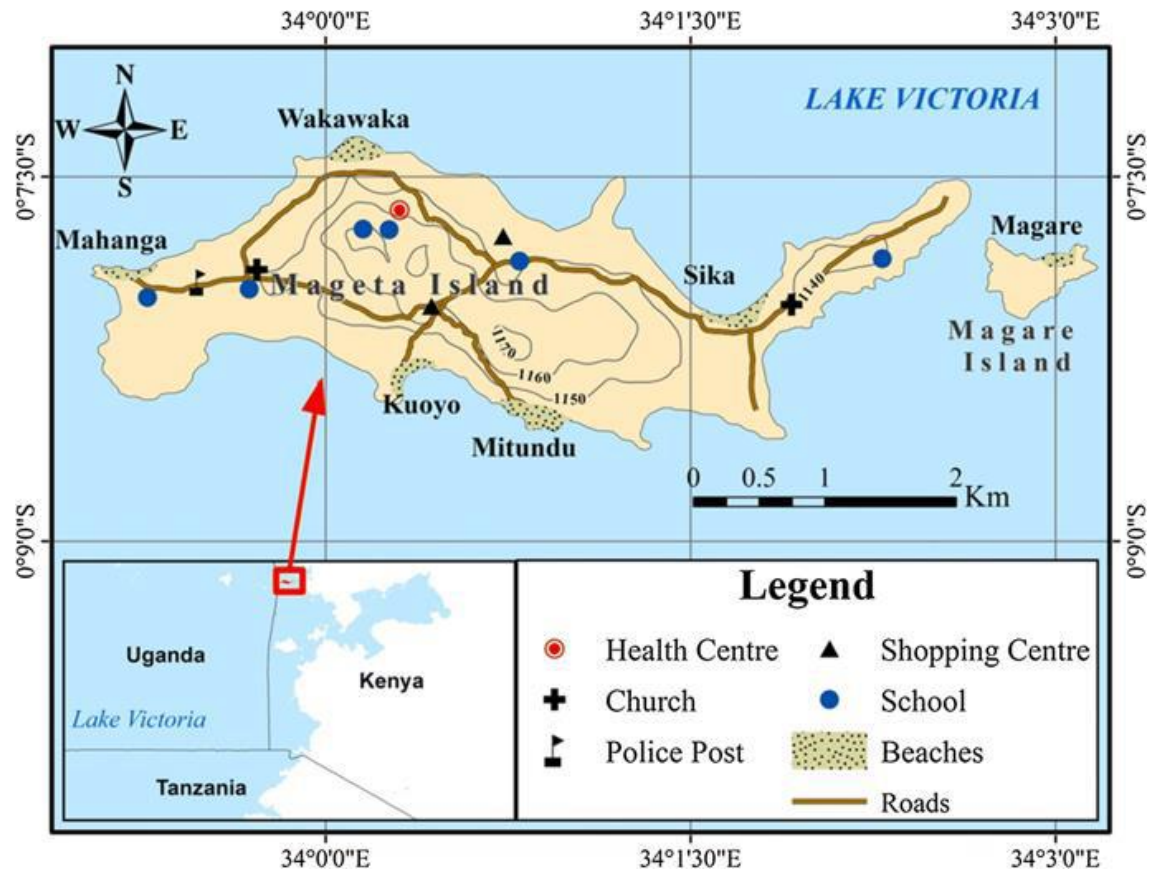


Figure 6: A topographic map showing location of Mageta Island in Siaya County, Western Kenya, (courtesy of Mukabana *et al.*, 2019).

3.2 Study design

A cross-sectional survey of all mosquito larval habitats was carried out on Mageta Island in April 2019 during the extended dry season to assess the abundance of different habitat types. The identified habitats were grouped into artisanal capture fishing (ACF) and non-ACF types. Artisanal capture fishing habitats were defined as those associated with fishing activities (Mukabana *et al.*, 2019) otherwise the habitats were classified as non-ACF. *Anopheles* larvae and pupae (outcome variable) were sampled daily from 0800hrs to 1100hrs and the number of larvae and pupae recorded. Each habitat was visited once during the sampling period. Simultaneously with larval and pupal sampling, water quality characteristics (predictors) of different mosquito habitat types were

collected and recorded. The location of each habitat was recorded using a hand-held global positioning system (GPS) unit.

3.3 Mosquito larval and pupal sampling

This objective sought to assess the effect of ACF activities on *Anopheles* productivity on Mageta Island. This determined the statistical relationship between habitat type (ACF versus non-ACF) and numbers of *Anopheles* larvae/pupae (productivity) inside individual habitats. Sampling was done using a sweep net for two to ten minutes depending on habitat size. Three different sweep net sizes including 6cm, 12cm and 24cm diameter were used depending on habitat size. The 6cm diameter sweep net was used in habitats ranging between 0.5-2m, 12cm in habitats between 3-15m and 24cm in habitats between 16-35m perimeter. The sweep net was made of a very fine netting material to enable collection of larvae of all sizes including newly hatched mosquito first instar larvae. The sweep net was inserted in water at an angle of 45 degrees, and gently dragged along the entire water surface of each habitat. In large habitats, larvae and pupae aggregated along the edges, so sweeping was carried out along the edges. Contents collected were emptied in a white tray to enhance visibility and counting of sampled of mosquitoes. Mosquito larvae and pupae were identified, counted and recorded. Water was allowed to settle so that larvae can re-surface before the next sweep was made. Up to three sweeps were made in each habitat. Care was taken not to cast a shadow over the water to prevent larvae and pupae from diving away and hiding. Larvae were collected in 20ml vials and preserved with absolute ethanol. The vials were labeled using a pencil and were taken to the laboratory for identification. Collected pupae were placed in plastic paper cups with water from habitats where they were collected awaiting adult emergence and identification.

3.4 Relationship between physical habitat characteristics and *Anopheles* productivity

The aim of this piece of work was to determine if the density of *Anopheles* larvae and pupae in habitats associated with ACF activities was affected by physical characteristics of these habitats. Thus, information on habitat bottom surface type, perimeter, and depth (predictors) was obtained and recorded for individual habitats (ACF versus non-ACF) to determine if they have an effect on *Anopheles* larval/pupal numbers (outcome). Habitat bottom surface was assessed by directly observing the habitat and categorizing it either as mud, wood, sand or rock. To measure depth of different habitats, a small weight was tied to a string and lowered carefully into the deepest section of habitats. The point where the water level reached on the string was marked. The submerged section of the string was measured using a meter rule and readings recorded to the nearest meter. The perimeter was measured using a measuring tape. Only the area covered by water was measured and recorded.

3.5 Linking physical aspects of habitat water to *Anopheles* productivity

Physical aspects of habitat water that enhance number of larvae and pupae productivity in habitats associated with ACF activities on Mageta Island were measured. These aspects; temperature, total suspended solids (TSS) and turbidity (predictors) depict the relationship between *Anopheles* productivity (outcome) in ACF versus non-ACF habitats.

3.5.1 Measuring water temperature in *Anopheles* larval habitats

Temperature was measured using a mercury thermometer. The thermometer was lowered gently into the water such that the bulb was a few centimeters below the water surface for 2 minutes. Thermometer readings were then read as rapidly as possible. Care was taken to avoid parallax errors by ensuring that there was a

straight line from the eye to the meniscus. The thermometer was allowed to equilibrate before the readings were taken and recorded. The temperature readings were taken at the same time interval (0900hrs-1000hrs) for all habitat types on Mageta Island. The weather was sunny throughout the study period.

3.5.2 Measuring water turbidity/cloudiness in *Anopheles* larval habitats

Turbidity readings were taken in the field in different *Anopheles* larval habitats categorized as ACF and non-ACF. A turbidity tube (Myre and Shaw, 2006) was used. Measurements were taken in daylight but not in direct sunlight. This was done by casting a shadow on the tube whereby the person stood between the sun and the tube. A clean container was used to collect water samples. The container was dipped in water and care was taken not to include sediment from the bottom of the habitats. Sample water from habitats was used to rinse the turbidity tube. The water sample in the container was stirred until it was homogenous. The viewer's head was held 15 cm directly above the tube to enhance clear visibility while pouring the sample into the tube. The sample water was slowly poured into the tube taking care not to introduce bubbles until the pattern on the disc became almost invisible. At this point, water was added even more slowly while viewing the disk closely. Pouring was stopped as soon as the pattern on the disk could no longer be seen. The turbidity value was then read from the scale on the side of the tube to the nearest centimeters. The value was then converted to Nephelometric Turbidity unit (NTU) using the Length-to-Turbidity Conversion Chart (Myre and Shaw, 2006) and recorded

3.5.3 Measuring total suspended solids (TSS) in water from *Anopheles* larval habitats

To measure TSS, about 100ml of water was collected in plastic bottles from ACF and non-ACF mosquito habitat types. The water was transported to the University of Nairobi Hydrobiology laboratory for analysis. Filter papers were placed onto the filtration apparatus, vacuum applied and were rinsed with three successive 20 mL volumes of deionized water. Air was drawn through the filters until all the water had been drawn off. The filters were placed in an oven set at 60 °C to dry. They were then left to cool to room temperature and weighed. The filter paper after cooling was inserted onto the filtration apparatus and vacuum applied. A small volume of deionized water was sprinkled on the filter to seat it. The sample water was shaken vigorously and 50 mL measured using a graduated cylinder. The sample was filtered and suction continued for around three minutes after filtration is complete to allow complete drainage. The filter paper with the sample (Figure 7) was carefully transferred to the oven where it was dried at 60 °C, left to cool to room temperature and then weighed.

The TSS was calculated using the following formula:

$$\text{TSS (mg/l)} = ((A-B) * 1000) / V$$

Where:

TSS=Total suspended solids

A = Weight in mg of the filter paper plus the dried residue

B = Weight in mg of the clean, unused filter

V= Volume of the sample filtered in mL

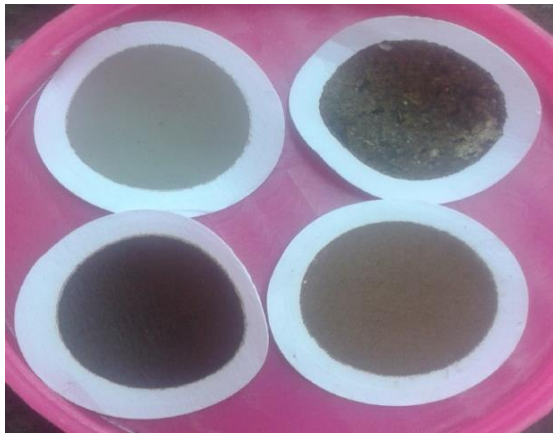


Figure 7: Dried filter papers with the sample. The different filter papers show the different sample concentration from different habitat types.

3.6 Relating between chemical characteristics of habitat water and *Anopheles* productivity

The chemical characteristics of water (predictors) in habitats on Mageta Island were assessed to determine whether they were associated with numbers of *Anopheles* larvae and pupae (outcome) in habitats associated with ACF activities. The chemical characteristics that were measured included dissolved oxygen (DO), pH, total dissolved solutes (TDS) and conductivity.

3.6.1 Measuring Dissolved Oxygen in *Anopheles* larval habitats

Winkler titration (Hassaan, 2016) was used to assess dissolved oxygen (DO) in mosquito larval habitats on Mageta Island. Water samples were collected in a special bottle, the biological oxygen demand (BOD) bottle, in the field. The samples were collected a few inches below the water surface. The BOD bottle was lowered about halfway into the water and let to fill slowly; for habitats that were large enough. For smaller habitats, the bottle was slowly lowered into the water until the lower lip of the opening had just submerged. The water was then allowed to fill slowly avoiding turbulence. When the water level in the bottle had stabilized, the bottle was slowly turned upright and filled slowly. For very small habitats, water for DO was not collected. Precaution was taken to ensure that the sample was not aerated and no air bubbles were trapped in the container as this could add oxygen to the sample thus skew the results. This was done by allowing the bottle to overflow 2-3 times and capping the bottle immediately it was full. Immediately, 3ml of manganese sulphate solution was added to the collection bottle by inserting the calibrated pipette just below the surface of the liquid. The pipette was squeezed slowly to avoid introducing bubbles. Also, care was taken not to add the reagent above the sample surface to avoid introducing oxygen in the sample. In the same manner, 3ml of alkali-iodide-azide reagent was added. The bottle was carefully stoppered to avoid any air pockets from forming below the cap. The sample was mixed by inverting several times. The sample was constantly checked for air bubbles and was discarded if they were present. A brownish-orange cloud of precipitate or floc appeared if oxygen was present. The sample was mixed by turning upside down when the floc had settled at the bottom after which it was left to settle again. Three milliliters of concentrated sulfuric acid was added using a pipette held just above the surface of the sample. The sample was then carefully stoppered and inverted several times to dissolve the floc. At this point, the sample was now “fixed.” The samples were then kept in a cool box and stored in a cool, dark place awaiting transportation to the hydrobiology laboratory at the University of Nairobi for DO analysis. As an added precaution, distilled water was squirted along the stopper, and the bottle

was capped with aluminum foil and a rubber band during the storage period. To assess the dissolved oxygen amount in the laboratory, 201 mL of the sample water was titrated with sodium thiosulfate to a pale straw color. Two to three drops of starch were added such that the mixture turned blue. The neutralizing solution was added carefully drop by drop from a calibrated pipette into the flask and the sample mixed completely by swirling the sample water. At the end point of the titration, one drop of starch was added to turn the mixture to a clear colour. The flask was held up to a white sheet of paper to check for absence of the blue colour. Clarity of the mixture meant that the acid had been neutralized. The amount of neutralizing sodium thiosulfate is always proportional to the amount of oxygen in the sample (Hassaan, 2016). For example, if the initial amount of sodium thiosulfate was 10 ml, then after titration 6 ml of sodium thiosulfate remains: Oxygen content will be $10\text{ml}-6\text{ml}=4\text{ml}$. Therefore, 4ml of sodium thiosulfate is equal to 4ml/l Oxygen content.

3.6.2 Measuring the Potential of Hydrogen (pH) in *Anopheles* larval habitats

The Potential of Hydrogen was measured using a pH meter. The meter was calibrated using buffers of PH 4, 7 and 10 two days before use. The probe was immersed in the first buffer (pH 2) solution and the meter calibrated to read the correct pH. After the initial buffer calibration, the meter was calibrated using other buffer solutions as appropriate. The probe was rinsed with de-ionized water and excess water removed between the different buffer solutions. The buffer solutions and temperature values used to calibrate the meter were recorded. The probe was then immersed into the buffer of pH 7 and the sample value noted. The value indicated by the meter was recorded. If the meter was outside of the acceptable accuracy range, it was recalibrated. After the meter had been properly calibrated, it was rinsed with de-ionized water and stored. When collecting water samples from the field, the pH electrode was dipped in the sample keeping it away from the sides and bottom of the sample container. The sample was stirred gently till the reading was stable. While suspending the probe away from the sides and bottom of the sample container, the pH value on the meter was noted

down. The pH meter was thoroughly rinsed with de-ionized water before taking the readings from the next habitat. While in use, the PH meter was periodically checked by rinsing the probe with de-ionized water, removing excess rinse water and immersing in the appropriate buffer solution.

3.6.3 Measuring Total Dissolved Solutes (TDS) in *Anopheles* larval habitats

For TDS, about 250 mL of water was collected in plastic bottles in the field. The water was stored in a cool box in a dark room awaiting transportation to the laboratory for assessment. In the laboratory, an empty petri dish was cleaned. A rinse of the inside surface of the petri dish with 4 mL of concentrated hydrochloric acid was done. The dish was tilted and rotated carefully and slowly so that the acid contacted any part of the inside of the hard-to-remove residue. All surfaces of the petri dish were rinsed first using tap water and then distilled water was used for final cleansing rinse. The clean petri dish was dried in an oven at a temperature of 60 °C for 60 minutes. The dish was cooled and dried at room temperature and weighed. A filter paper was inserted into the filtration apparatus (Figure 8) and vacuum applied. The filter was washed with three successive 20 mL of reagent water. Vacuum was applied continuously to remove all traces of wash water from the filter paper. The water in the filtration apparatus was discarded. Sample water was mixed well through repeated inversion and 50 mL of the sample measured. The sample was added to the filter holder. The filter was washed with three successive 10 mL portions of reagent water to ensure complete transfer of dissolved constituents into the filtrate. Suction was continuous until all visible water had been removed from the filter. The filtrate was transferred to an empty petri dish and evaporated in the oven to dryness at 60 °C. The petri dish plus the sample was left to cool and dry before re-weighing. TDS was calculated using the following formula:

$$\text{TDS (mg/l)} = ((A-B) * 1000) / \text{ml of sample}$$

Where:

TDS= Total dissolved solids

A = Weight of dish + dried residue, mg

B = Weight of dish, mg



Figure 8: Measurement of Total Dissolved Solutes (TDS) in the laboratory. The filtration apparatus contains water sample after filtration while labeled petri-dishes indicate presence of the sample left after drying in the oven respectively.

3.6.4 Measuring conductivity of water in *Anopheles* larval habitats

To assess conductivity, water samples were collected from the field in plastic bottles, stored in a cool box in a dark room awaiting transportation to the laboratory. Conductivity tests were completed using a conductivity meter in the laboratory. The conductivity meter was first calibrated using potassium chloride. The probe was then rinsed with de-ionized water. About 50 mL of sample water in 100 mL beaker was used. The electrode was completely submerged in the liquid and given time for electric conductivity (EC) readings to stabilize before taking the readings. The probe was rinsed with deionized water before taking conductivity measurements between different water sub-samples. Care was taken not to introduce air bubbles into the sample water as this could affect conductivity readings.

3.7 Statistical analyses

Each habitat was sampled once using a sweep net in three sweeps. *Anopheles* mosquito larval numbers collected in the three sweeps were summed up for each habitat type. Mosquito abundance was calculated as the total number of *Anopheles* larvae per habitat. The obtained data was entered in Microsoft Excel and analyzed using Statistical package for Social Sciences (SPSS) version 23. Generalized linear model (GLM) with count data fitted to a negative binomial distribution with a log link function was used to test whether physicochemical factors had an influence on *Anopheles* abundance in habitat types. Before subjecting variables to robust analysis, they were all first explored to assess their distribution using histograms and scatter graphs after which the appropriate link was selected. The initial model had presumed the distribution of the independent variable to be Poisson. Over dispersion was evaluated and the conditional variance exceeded the conditional mean, so, GLM with negative binomial was used. For binary data, presence or absence of *Anopheles* larvae, a negative binomial distribution with a log link function was fitted. Effects were considered significant at $P < 0.05$. An alpha level of 0.05 was used for all statistical tests of significance

CHAPTER FOUR: RESULTS

4.1 *Anopheles* productivity in different habitat types

During this study, a total of forty mosquito larval habitats, which is the total number that was present on Mageta Island, were surveyed. These included trenches (n=1), swamps (n=1), brick pits (n=1), sand mines (n=1), fish-bait mines (n=2), burrow pits (n=2), abandoned fish-ponds (n=4), ditches (n=5), rock pools (n=8) and boats (n=16). The total number of ACF habitats (boats, fish-bait mines and trenches) was 19 (47.5%) while non-ACF habitats (Abandoned fishponds, rock-pools, burrow pits, ditches, sand mines and swamps) were 21 (52.1%). A total of 862 *Anopheles* larvae and 230 pupae were collected from all habitats. Out of the 862 *Anopheles* larvae collected from habitats 81.4% (n=702) were from habitats associated with ACF activities while only 18.6% (n=160) were from non-ACF habitats. The highest number of larvae collected from a single habitat was 34.3% (n=296) and 58.7% (n = 135) for pupae. In all the 40 habitats (ACF and non-ACF) sampled, (50%) had *Anopheles* mosquito larvae while only 25% had *Anopheles* pupae. The mean number of *Anopheles* larvae in ACF (36.95 ± 16.93) and non-ACF (7.62 ± 3.04) habitats differed significantly ($P = 0.02$; Figure 9).

High numbers of *Anopheles* pupae were found in ACF (10.90 ± 7.30) than in non-ACF habitats (1.11 ± 0.72), Figure 9. Only few habitats contained *Anopheles* pupae despite intensive whole habitat census. Out of the 230 pupae collected from habitats, 90% (n=230) were found in ACF habitats while only 10% (n=23) were found in non-ACF habitats. The highest number of pupae found in non-ACF habitats was only 11. Of all the habitats that contained *Anopheles* pupae on Mageta Island, 70% were associated with ACF activities as compared to only 30% associated with non-ACF. The number of *Anopheles* larvae and pupae was higher in ACF habitats than non-ACF habits. Numbers of *Anopheles* larvae were generally higher than pupal numbers in habitats. The numbers of pupae were too low for any robust statistical analysis therefore they are not discussed henceforth.

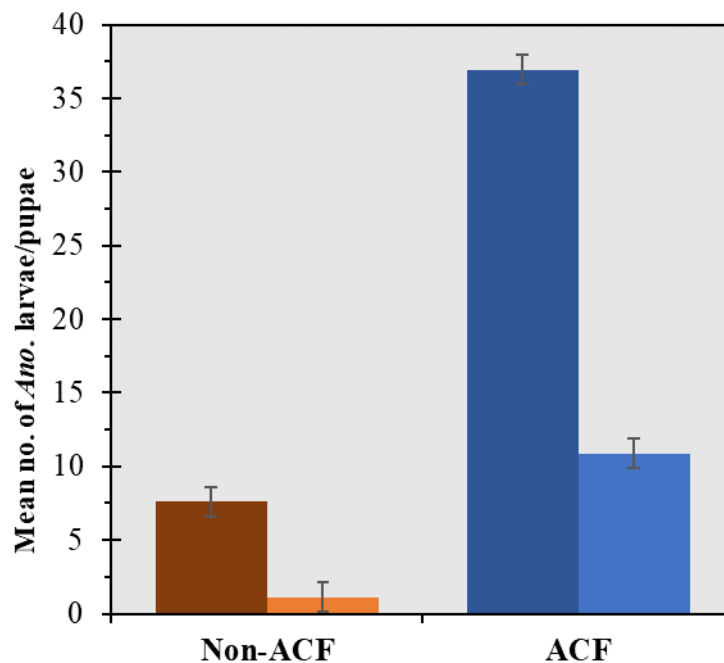


Figure 9: Mean \pm SE number of *Anopheles* larvae (indicated by dark brown for non-ACF and dark blue for ACF) and pupae (indicated by light brown for non-ACF and light blue for ACF) collected from artisanal capture fishing (ACF) versus non-artisanal capture fishing (non-ACF) habitats. Standard error of mean values for habitat types are indicated by bars.

4.2 Relationship between physical habitat characteristics and *Anopheles*

productivity

Three physical mosquito larval habitat characteristics namely perimeter, depth and habitat bottom surface type were assessed on Mageta Island in western Kenya. The mean perimeter of ACF ($4.62 \pm 0.53\text{m}$) and non-ACF habitats ($10.90 \pm 2.33\text{m}$) differed significantly ($P = 0.017$). The largest ACF habitat on Mageta Island was 9.50m while non-ACF was 35.60m . Non-ACF habitats were generally bigger and had significantly fewer *Anopheles* larvae. Perimeter significantly influenced *Anopheles* larval density inside habitats ($P = 0.023$; Figure 10).

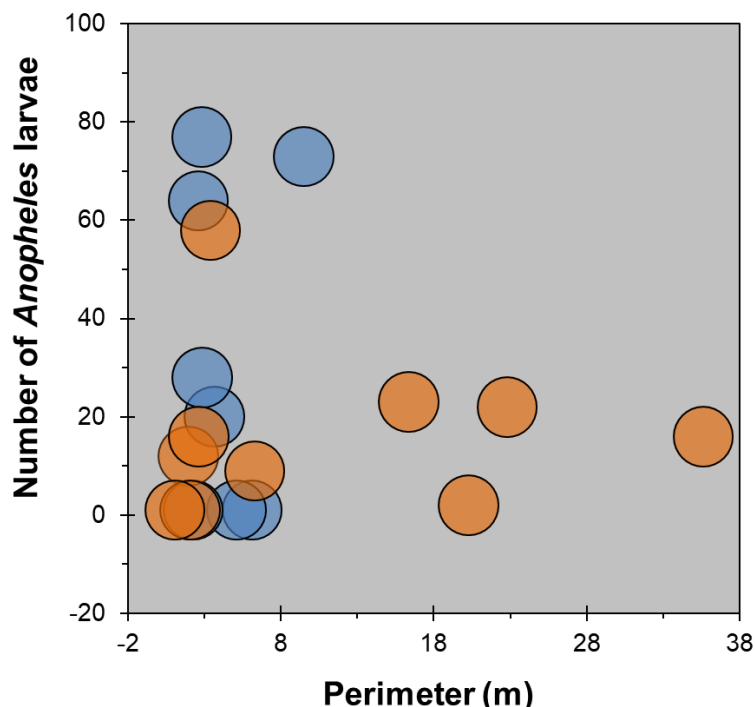


Figure 10: Relationship between larval *Anopheles* numbers and habitat perimeter in ACF (blue bubbles) versus non-ACF habitat (orange bubbles). Most ACF habitats were small and contained high number of mosquito larvae compared to non-ACF.

Depth in ACF versus non-ACF habitats was not significantly different ($P = 0.129$). The mean depth of ACF and non-ACF habitats was 0.10 ± 0.01 m and 0.17 ± 0.04 m respectively. The deepest ACF habitat on Mageta Island was 0.24m while non-ACF was 0.92m. Depth did not have a significant influence on *Anopheles* larval density ($P = 0.157$).

Mean numbers of *Anopheles* mosquito larvae collected were significantly influenced by habitat bottom surface type (Fig. 11). Mud (42.5%; $n = 17$) was the most abundant bottom surface type followed by wood (35%; $n = 14$) then rock (20%; $n = 8$). The least abundant bottom surface was sand (2.5%; $n = 1$). Wood (34.85 ± 21.2) contained most *Anopheles* larvae followed by mud (21.35 ± 9.09) then rock (1.375 ± 1.1). No larvae were found in the habitat that had a sand bottom surface.

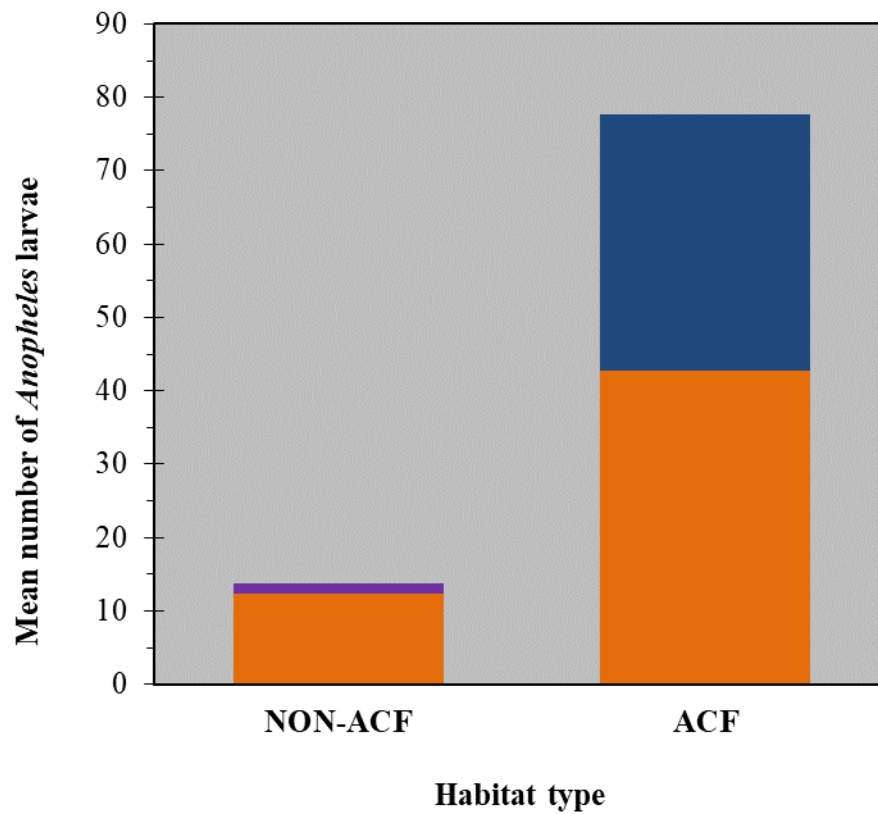


Figure 11: Mean number of *Anopheles* larvae collected from ACF versus non-ACF habitats with different habitat bottom surface types. The orange color represents mud bottom surface, the blue color represents wood bottom surface and the purple color represents the rock bottom surface type.

4.3 Linking physical aspects of habitat water to *Anopheles* productivity

4.3.1 Temperature

The mean temperature did not differ significantly between ACF (28.8 ± 0.89) and non-ACF habitats (28.1 ± 0.49 ; $P = 0.49$). However, temperature significantly influenced larval abundance ($P = 0.008$), with *Anopheles* larvae being constantly found in habitats with higher water temperatures (Figure. 12). The median temperature for ACF habitats was 30 while non-ACF was 28. Habitats associated with ACF activities constantly had higher temperature with higher *Anopheles* larvae populations compared to non-ACF habitats

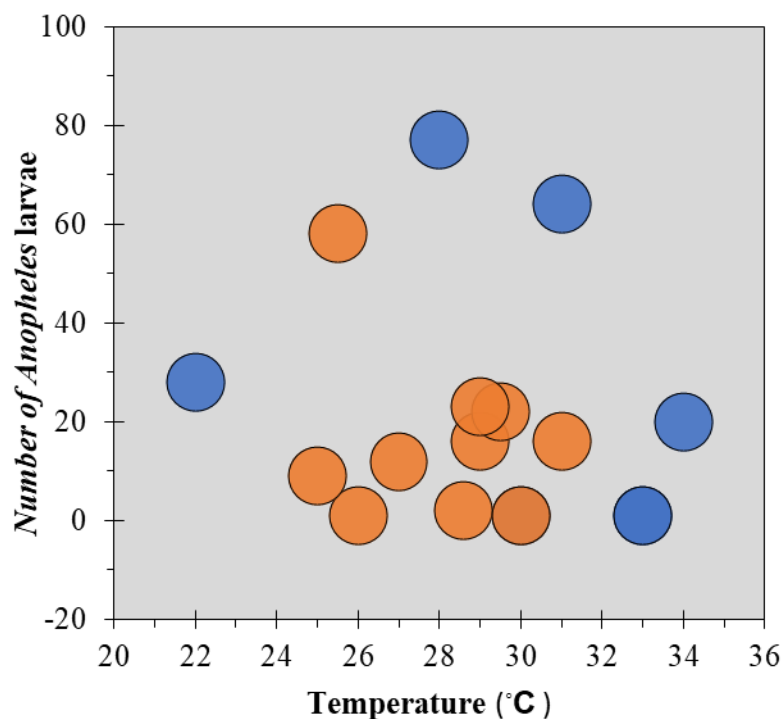


Figure 12: Relationship between *Anopheles* larval numbers and temperature in ACF (blue) versus non-ACF habitats (orange).

4.3.2 Total suspended solids (TSS)

Total suspended solids (TSS) were not significantly different between ACF (2.64 ± 0.11) and non-ACF (2.29 ± 0.09) habitats ($P = 0.86$). The highest concentration of TSS recorded in ACF habitats on Mageta Island was 23.30mg/L while non-ACF recorded 24.82mg/L. Total suspended solids significantly influenced larval abundance in habitats ($P = 0.012$, Figure 13). Most ACF habitats had lower TSS concentrations and are the ones that contained most larvae.

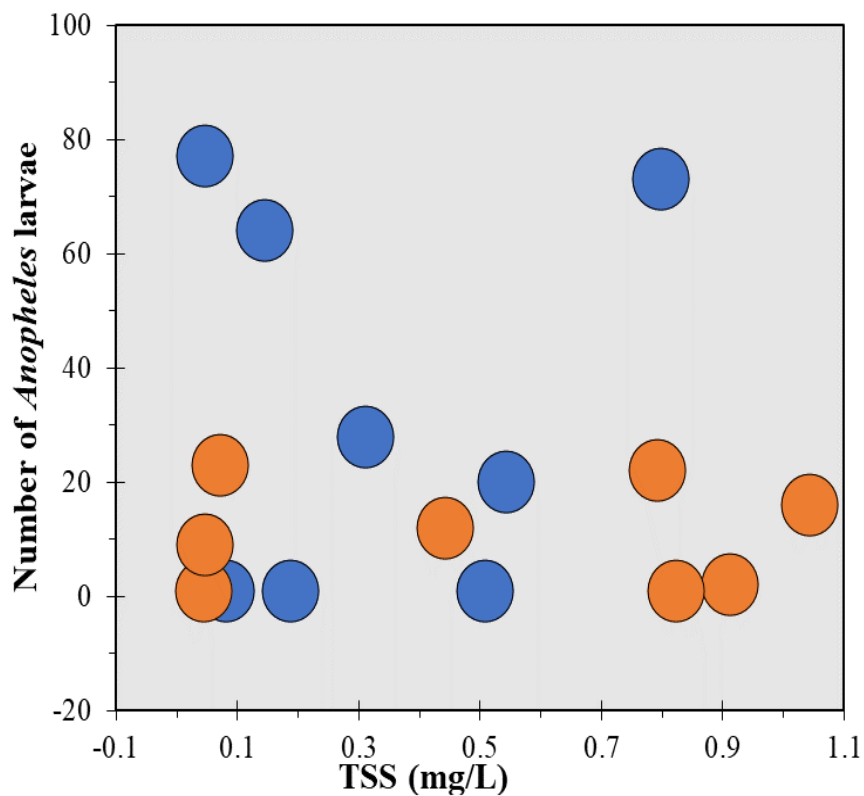


Figure 13: Relationship between *Anopheles* larval numbers and TSS in ACF (blue) versus non-ACF habitats (orange).

Further analysis revealed that TSS interacted significantly with temperature to influence *Anopheles* abundance ($P = 0.001$; Figure 14). The interaction between TSS and temperature was lower in habitats associated with ACF activities.

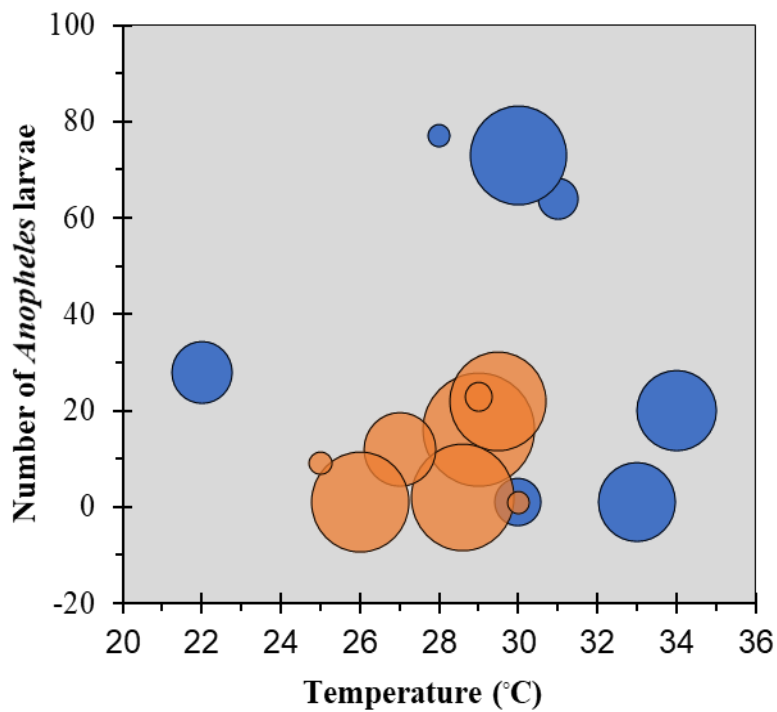


Figure 14: Interaction between *Anopheles* larval numbers with temperature and total suspended solids in ACF (blue) versus non-ACF (orange) habitats. The third variable (TSS) is indicated by the size of the bubble.

4.3.3 Turbidity

Turbidity significantly differed significantly between ACF (3.4 ± 0.52) and non-ACF habitats (2.0 ± 0.19 ; $P=0.012$) but was not significantly associated with *Anopheles* abundance ($P = 0.14$).

4.4 Relationship between chemical variables of habitat water and *Anopheles* productivity

Four chemical variables of mosquito habitat water, namely dissolved oxygen (DO), potential of hydrogen ions (pH), conductivity and total dissolved substances were assessed. Dissolved oxygen was not significantly different between ACF (4.2 ± 0.7132) and non-ACF habitats (3.9 ± 0.5759 ; $P=0.72$). Furthermore, there was no significant difference between *Anopheles* density and DO in ACF and non-ACF habitats ($P=0.25$). However, DO interacted significantly with TSS to influence *Anopheles* larval abundance in habitats ($P = 0.003$; Figure 15). Greater interaction between TSS and DO in ACF habitats resulted in low numbers of *Anopheles* larvae in ACF habitats.

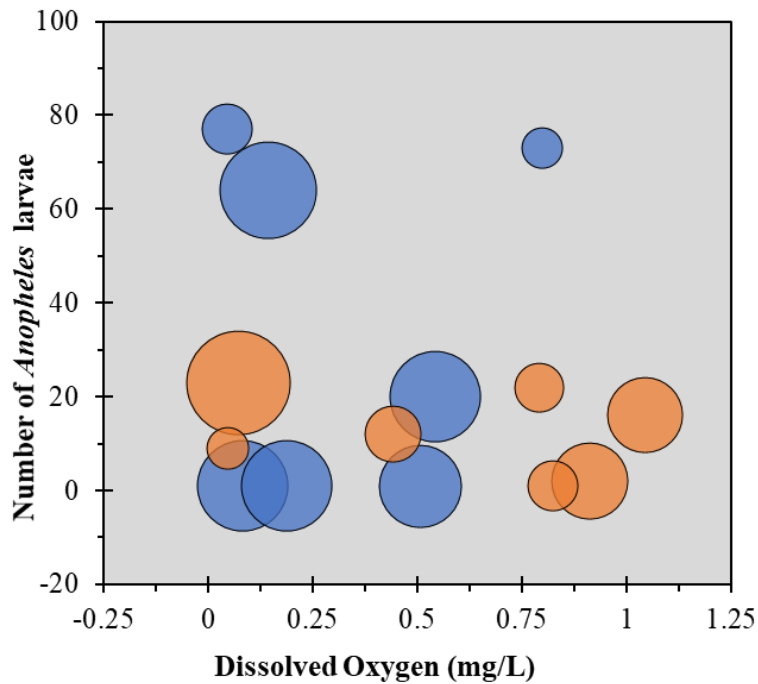


Figure 15: Interaction between *Anopheles* larval numbers with dissolved oxygen and TSS in ACF (blue colour) versus non-ACF habitats (orange colour). The third variable (TSS) is indicated by the size of the bubble.

Potential of hydrogen (pH) was not significantly different between ACF (7.8 ± 0.2956) and non-ACF habitats (8.1 ± 0.2926 ; $P=0.089$). Furthermore, pH had no influence on larval numbers in ACF and non-ACF habitats ($P=0.089$).

Comparing means of *Anopheles* larvae showed that conductivity was not different between ACF (15.4 ± 2.454) and non-ACF habitats (13.47 ± 1.724); $P=0.52$. Conductivity marginally influenced *Anopheles* larval abundance ($P=0.052$).

Total dissolved solutes (TDS) was not significantly differently between ACF (1.9 ± 0.2239) and non-ACF habitats (2.5 ± 0.5424); ($P=0.27$). Thus, TDS did not influence larval *Anopheles* abundance. Interestingly, TDS interacted significantly with conductivity to influence larval numbers ($P = 0.035$; Figure 16).

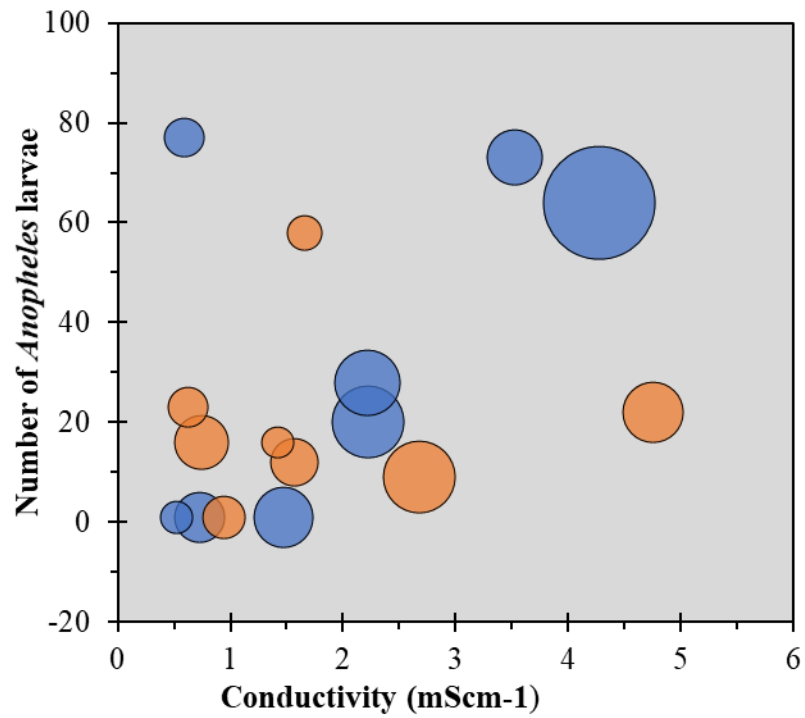


Figure 16: Interaction between *Anopheles* larval numbers with total dissolved solutes and conductivity in ACF (blue colour) versus non-ACF (orange colour) habitats. The third variable (TDS) is indicated by the size of the bubble. Greater interaction between TDS and conductivity (indicated by bubble sizes) consequently yielded high larval *Anopheles* numbers i.e. *Anopheles* numbers increased across habitats as the interaction increased.

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

A cross-sectional survey was carried out to determine the basis of preferential breeding of *Anopheles* mosquitoes in artisanal capture fishing habitats on Mageta Island in Western Kenya. Of all the mosquito habitats sampled on Mageta Island, about half (47.5%) were associated with artisanal capture fishing. High numbers of *Anopheles* mosquito larvae and pupae were found in fishing habitats compared to non-fishing habitats. Larval habitat bottom surface type, perimeter, total suspended solids and temperature were significantly associated with *Anopheles* mosquito abundance. Furthermore, the pairs of TSS and dissolved oxygen, TSS and temperature, TDS and conductivity interacted significantly to affect *Anopheles* mosquito productivity. This study emphasizes the role of physical and chemical factors on *Anopheles* mosquito productivity in artisanal capture fishing habitats.

The decrease in numbers of *Anopheles* mosquito larvae compared to a previous study on Mageta Island (Mukabana *et al.*, 2019) is attributed to several factors. Sampling was done during the extreme dry season when most habitats had dried up. Fishing was at its peak and most boats which act as active breeding sites were not available (Mukabana *et al.*, 2019). The number of pupae collected in this study was too low. This is not surprising as Mutuku *et al.*, (2006) also recorded very low numbers of *Anopheles* pupae from habitats; where he noted discontinuity in pupal production in habitats despite larvae occupying all habitats. The study noted that pupal densities were variable and were commonly absent in most habitats. It is interesting that ACF habitats recorded considerably high numbers of both *Anopheles* larvae and pupae compared to non-ACF habitats. Pupal density estimates being the best proxy measure of mosquito adult productivity (Mutuku *et al.*, 2006), clearly shows that these habitats are more productive despite the low numbers of *Anopheles* pupae. The very low numbers of *Anopheles* pupae compared to larvae could be pointing to several possibilities.

First, there is a possibility that predators and parasites if present in these habitats could have considerably reduced immature populations of mosquitoes. The effect of predators on mosquitoes has been elaborately reported elsewhere (Dida *et al.*, 2015). Secondly, the timing of sampling could have played a huge role here. Sampling of mosquito larvae and pupae is mostly done from mid-morning to noon. Is it plausible to say that most pupae emerge in the late afternoon? No single study has tried to determine how pupal emergence is affected by the time of the day. This could help device best sampling times for immature stages of mosquito and bridge the gap in different sampling methods that have presented contradicting results. Thirdly, it could be possible that mosquitoes could have laid their eggs in a wide range of habitats which promoted development of larvae to a certain extent but pupal production largely failed (Mutuku *et al.*, 2006). This third possibility could unravel the variability of habitats in terms of physical and chemical characteristics in promoting *Anopheles* productivity in different habitat types.

High numbers/densities of *Anopheles* larvae were constantly found in ACF habitats with smaller perimeter and wooden bottom surfaces. This is consistent with previous studies where *Anopheles* larval breeding occurred in small temporary sunlit pools (Minakawa *et al.*, 1999; Mereta *et al.*, 2013). Smaller habitats favor high breeding and development rates of mosquito larvae because they harbor fewer predators and have slightly higher temperature as the little water contained in such habitats heat up faster (Paaijmans, 2008). The effect of temperature on *Anopheles* productivity is clearly illustrated in various studies (Bayoh, 2001; Beck-Johnson *et al.*, 2013). Small habitats are unstable thus the probability of supporting a mosquito through its life cycle is unpredictable. However, large stable habitats usually favor breeding of predators (Washburn, 1995) and this is why small habitats are more important especially when they occur in large numbers in an area with stable rainfall over time. Some studies observed pupal occurrence and habitat stability decreasing constantly in habitats less than 1m³ (Minakawa and Sonye, 2005). Thus, a habitat should be stable enough to allow complete development of mosquitoes and unstable enough to

limit recolonization of predators such as fish and other predators (Mutuku *et al.*, 2006).

Little is known about habitat bottom surface and *Anopheles* mosquito productivity. Habitat bottom surface was positively associated with *Anopheles* larvae and ACF habitats with wood bottom surface favored high *Anopheles* abundance as observed by Mukabana *et al.*, (2019). Further, Dejenie *et al.*, (2011) also demonstrated a significant relationship between habitat bottom surface and mosquito numbers. Most ACF habitats with wood bottom surface on Mageta Island are boats prone to anthropogenic activities to enhance accessibility of emergent progenies to human blood source. The effect of anthropogenic activities on mosquito abundance is clear (Sinka *et al.*, 2010). *Anopheles* mosquito could also have preferred such habitats because the boats are parked ashore with little water exposed to full sunlight, which makes water suitable for *Anopheles* breeding. Furthermore, the possibility of boats holding water long enough to support breeding of predators is low because boats are emptied only after a few days thereby limiting chances of predator breeding (Dida *et al.*, 2015; Mukabana *et al.*, 2019).

The population dynamics of *Anopheles* mosquitoes were highly dependent on temperature (Bayoh and Lindsay, 2004). Temperature determines larval survival, growth rate, pupation age, and adult size (Paaijmans, 2008). In this study higher temperature in ACF habitats were associated with high *Anopheles* abundance. This agrees with previous studies, which found certain ranges of temperature to favor mosquito development (Bayoh and Lindsay, 2004; Beck-Johnson *et al.*, 2013; Lyimo *et al.*, 1992). Bayoh and Lindsay (2004) observed aquatic stages under laboratory conditions and noted that development rate of juvenile stages from one stage to the next increased at higher temperature to a peak of around 28 °C and then declined. It does not differ much from Lyimo *et al.*, (1992) who observed survival rate of mosquitoes being highest at 27°C which is similar to what Dale *et al.*, (2007) also observed. At 30 °C, survival decreased as density of mosquitoes increased. The present study is therefore in line with these findings because the mean temperature in ACF habitats was 28 °C with the highest

recorded temperature in these habitats being 34°C. Although the mean temperature between fishing and non-fishing habitats was almost similar, fishing habitats recorded slightly higher temperature. Fillinger *et al.*, (2009) noted that higher temperature pools were the most productive. However, future studies need to put into consideration the long-term effect of daily fluctuations on *Anopheles* mosquitoes in the field.

Total suspended solids (TSS) significantly influenced *Anopheles* numbers. *Anopheles* mosquito larvae were constantly found in habitats with less TSS. This could be linked to various factors. First, the high number of inert particles in these habitats could have made it difficult for larvae to feed by reducing accessibility of food available for mosquito larvae (Gammon, 1970). Various studies hypothesize that *Anopheles* larvae appear to take in large volumes of non-nutritional material non-selectively (Walker *et al.*, 1988). This thus makes it hard for the larvae to take in nutritious materials only, consequently causing adverse effects on mosquito larvae. Furthermore, the fact that mosquito larvae are filter feeders, high concentration of TSS could obturate feeding structures which reduces feeding efficiency thus reduced growth rates (Bilotta and Brazier, 2008). This stresses larvae and could eventually lead to their death (Caspers, 1979). Proper light penetration is necessary for optimal photosynthesis in water systems. However, too much suspended particles in mosquito habitats could hinder this. This in turn could lead to fewer photosynthetic plants such as algae thus restricting food availability to *Anopheles* larvae (Bond *et al.*, 2005; Fondriest Environmental, 2015). Furthermore, if suspended particles are large, they could have filled up habitats thus destroying eggs and larvae (Gammon, 1970). All these factors interfere with habitat productivity of *Anopheles* mosquitoes, the reason for low numbers. However, TSS needs to be monitored frequently and accurately because it can change throughout the day due to disturbance by for example human activities, cattle or rainfall. Thus, a better understanding of sediment shape, size, composition, cumulative and synergistic stressor effects and availability and complexity of habitats will play an important role in arriving at valid conclusions on the impact of TSS on *Anopheles* numbers.

A positive interaction between temperature and TSS significantly affected the number of *Anopheles* mosquito larvae. To some extent, TSS influences temperature which consequently affects *Anopheles* numbers. Total suspended solids in water absorb heat thus increasing the water temperature (Ling *et al.*, 2005). Increased water temperature in mosquito habitats can either be beneficial or detrimental to *Anopheles* larvae; which affects *Anopheles* productivity in the long run. Survival of mosquito larvae was shortest, less than seven days, when temperatures ranged between 38-40°C (Bayoh and Lindsay, 2004). In another study, suspended soil particles increased the near-surface temperature thus affecting the diurnal temperature pattern of small water pools (Paaijmans, 2008). It is possible that *Anopheles* larvae in habitats with high concentration of TSS could have been exposed to higher temperature during the day on both temporal and spatial scales consequently affecting *Anopheles* productivity (Paaijmans, 2008). However, extra attention is required to gain a better understanding of the effect of TSS on habitat water temperature and how the two interact to affect *Anopheles* numbers.

The effect of conductivity on presence and abundance of mosquitoes has been explored in a number of studies (Piyaratne *et al.*, 2005; Chirebvu and Chimbari, 2015; Olson and Hawkins, 2017). More *Anopheles* larvae were constantly found where conductivity was higher. However, conductivity levels were generally low across different habitat types indicating low levels of pollution on this Island and this is consistent with observations reported by Emidi *et al.*, (2017). A significant positive correlation between conductivity versus *Culex pipiens* and *Anopheles* abundance have also been demonstrated (Dejenie *et al.*, 2011; Nikookar *et al.*, 2017; Musonda and Sichilima, 2019). Elevated levels of salinity estimated by conductivity affect aquatic life negatively.

Total dissolved solutes (TDS) was not associated with *Anopheles* abundance and this is consistent with a recent study conducted in Zambia (Musonda and Sichilima, 2019). However, when TDS interacted with conductivity, a strong relationship with *Anopheles* abundance was observed. The fact that conductivity of water is directly related to dissolved ionized solids in water, makes the

interaction reasonable. Conductivity is highly depended on TDS (Kevin *et al.*, 2014). The source of the dissolved ions is dissolved salts which dissociate into ions forming electrolytes, thus, more ions contribute to higher conductivity. Most aquatic species can only tolerate a specific range of conductivity/TDS. When this range is exceeded, it can stress organisms. However, this study did not consider ionic composition of different habitat types and this could be an important aspect in future studies because different ions affect aquatic organisms differently (Kevin *et al.*, 2014).

Dissolved Oxygen (DO) had no significant effect on *Anopheles* abundance. However, despite this, *Anopheles* larvae were constantly found in habitats with higher DO levels and this is in line with a study by Piyaratne *et al.*, (2005). Interestingly, interaction between DO and TSS was significantly associated with low *Anopheles* numbers. Looking at the bigger picture, total suspended solids have an effect on DO. For instance, total suspended solids when in high concentration increase the temperature of larval habitats, then this leads to reduction in dissolved Oxygen levels. This is because suspended particles absorb more heat compared to water molecules themselves. The heat when transferred to the surrounding water by conduction makes water warmer, and it is well known that warm water holds little DO compared to cold water. Furthermore, when surface temperature of water in habitats increases, (in large habitats) stratification occurs. This prevents mixing of upper and lower layers of water. This in turn leads to low levels of dissolved oxygen that can hinder survival of aquatic organisms because most oxygen is used up in decomposition and respiration which mostly takes place in lower layers. This clearly explains how the effect of interaction between TSS and DO influences *Anopheles* productivity. However, the interaction effect needs to be studied further.

This study had various limitations. First, the sample size referring to the number of mosquito habitats in this study was limited. It is clear that more confident estimates of population parameters are obtained from much larger sample sizes. This however, is often difficult in certain field set ups. The sampling method used in this study was comprehensive. Using a sweep net gave better estimates

of mosquito productivity per habitat. The fewer habitats available for mosquito oviposition could be explained by the fact that sampling was done during the extremely dry season. Small sample size findings therefore should not be discarded as they still point to the existence of an effect depending on the context. Secondly, sampling of habitats for physicochemical parameters was done only once per habitat in the study area. Certain parameters change as a result of human activities or season. Future studies need to put into consideration the possibility of fluctuations of the different physical and chemical parameters so as to gain a better insight into their diurnal and seasonal changes for proper management efforts. More knowledge on physicochemical factors as key determinants of mosquito breeding will strengthen this case. If data is collected for sufficiently long periods of time in different parts of the World, this could be a great milestone in unraveling how various physicochemical characteristics affect *Anopheles* abundance. An accumulation of such facts will bring forth definite conclusions that could be crucial in planning malaria control interventions.

5.2 Conclusions

From this study it can be concluded that;

- a) Human activities, in this case artisanal capture fishing, play a key role in distribution and abundance of malaria vectors on Mageta Island.
- b) Physical character of mosquito larval habitats influence *Anopheles* productivity of aquatic habitats created through artisanal capture fishing on Mageta Island.
- c) Habitat water quality seem to play an important role in *Anopheles* productivity of aquatic habitats created through artisanal capture fishing on Mageta Island.
- d) It is likely that *Anopheles* productivity of aquatic habitats on Mageta Island is controlled by many interacting factors.

5.3. Recommendations

- a) More effort should be put in understanding the physicochemical characteristics that govern the presence and abundance of malaria vectors which can play a role in understanding and implementing targeted cost-effective malaria control interventions.
- b) Detailed, year-round investigation of larval habitats emphasizing on effect of physicochemical factors on *Anopheles* productivity in malaria endemic zones is fundamental in understanding seasonal malaria mosquito breeding dynamics with an aim of applying appropriate larval control measures.

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APPENDIX 1: Typical Mageta Island fish landing beach



A typical Mageta Island fish landing beach. Fishing is the main economic activities on this Island. Boats form the main breeding grounds for *Anopheles* mosquito. The boats with water are parked a shore open to direct sunlight thus enhancing mosquito breeding.

Prof Mukabana (Supervisor)
11 Sept 2020

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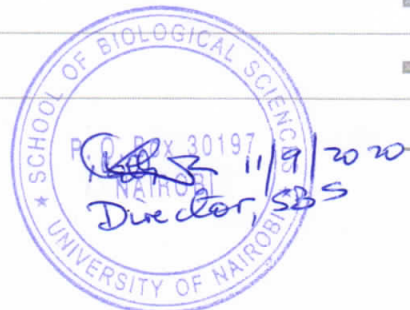
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APPENDIX 3: DATA SHEET

MAGETA MOSQUITO LARVAL / PUPAL / ADULT SAMPLING DATA 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, boat, swamp etc.): _____

Habitat location (e.g. ditch behind Smiths house): _____

Is habitat Manmade (Y/N): Is the habitat ACF based (Y/N): (ACF = Artisanal Capture Fishing) Is habitat open to direct sunlight? (Y/N):
 Stamp of human activity? (Y/N): Is habitat associated with supporting livelihoods? (Y/N): Associated livelihood type [examples ACF, sand harvesting, transport, aquaculture, ~~etc~~ farming, rental houses, livestock herding etc.]
Identity of stamp of human activity (Examples of stamps: bathing, digging, maize/fish drying, human stool, any human behaviours, cues in habitat, etc.)

Physicochemical characteristics of mosquito larval habitat:

Perimeter (m): Depth (m): Temperature (°C): PH:
 Dissolved Oxygen (mg/L): Nutrients: Total phosphorus (TP): Nutrients: Total Nitrogen (TN):
 Turbidity (NTU): Total dissolved solutes (mg/L): Habitat bottom surface type: sand (S), mud (M), wood (W) or rock (R)?

Biological characteristics of mosquito larval habitat:

Chlorophyll a (µg/L): Are emergent plants present?? (Y/N): Are algae present (Y/N):
 Are predators present (Y/N): Fish predators present (Y/N) / #: Amphibian predators present (Y/N) / #:

Mosquitoes in larval habitat:

Are *Anopheles* eggs present? (Y/N): Are *Anopheles* larvae present? (Y/N): Are *Anopheles* pupae present? (Y/N):
 # of *Anopheles* larvae: Sweep # 1: Sweep # 1:
 Sweep # 2: Sweep # 2:
 Sweep # 3: Sweep # 3:
 TOTAL: TOTAL:
 Total # of *Anopheles* adults collected: # of adult *Anopheles* mosquitoes collected per species: An. gambiae s.l.:
 An. fuscus group:
 An. coustali:

Comments:

Enumerator (names) _____ Signature _____