KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

MOSQUITO LARVAE OCCURRENCE AND HABITAT CHARACTERIZATION ON URBAN WASTEWATER IRRIGATED VEGETABLE FARMS IN THE KUMASI METROPOLIS, GHANA

BY

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BSc. (Hons.) BIOLOGICAL SCIENCES, KNUST, KUMASI

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A THESIS SUBMITTED TO THE DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY, COLLEGE OF SCIENCE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY (PARASITOLOGY)

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JULY, 2016

DECLARATION

I hereby declare that this thesis submitted to the Department of Theoretical and Applied Biology in partial fulfillment of the award of MPhil Degree is a true account of my own research. To the best of my knowledge, except for the references that have been duly cited, this thesis contains no material previously published by another person or material which has been accepted for the award of any other degree by the University.

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DEDICATION

This work is dedicated to my beloved Father, Brother, Friend and Guardian; and to my family for their love and care.

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My profound gratitude goes to the Almighty God for His infallible sustenance and protection throughout this work; To Him be the Glory, for the great things He has done. I am highly grateful to my indispensable academic supervisors, Dr. John A. Larbi of the Department of Theoretical and Applied Biology and Prof. Razak Seidu of the Department of Mathematical Sciences, Norwegian University of Life Sciences for sacrificing their time, energy and resources to ensure the materialization of this work. Your impressive and challenging personal commitments, constructive criticism, patience, suggestions and extensive support has led to the successful completion of this great work.

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ABSTRACT

The risk of mosquito borne disease transmission is dependent on water-driven ecological regimes, such as the presence and persistence of favourable breeding habitats for the development of immature mosquitoes, which definitely influences vector competence. Wastewater irrigated farming systems provide food for more than 10% of the world's population, but also tend to create hotspots for the breeding of mosquito vectors, which could lead to malaria transmission. This study investigated the occurrences and habitat characterization of mosquito larvae on urban wastewater irrigated vegetable farms in the Kumasi metropolis.

Mosquito larvae were collected weekly from different sources of irrigation water and other pools from vegetable farms in the city of Kumasi in both the wet and dry seasons. At the same time, triplicate water samples were collected for microbiological and physicochemical analysis using standard methods. The collected larvae were microscopically identified using morphological keys.

Overall, 9,823 mosquito larvae were collected during the eight months study period and species identified were *Anopheles*, *Culex* and *Aedes*. Of these, *Culex* species were most abundant (57.3%), followed by *Anopheles* species (30.5%) and the *Aedes* species were the least abundant (12%). A total of 139 breeding habitats, composed of ponds, streams and furrows (including foot prints and storm drains), were observed throughout the study. Furrows were the most frequent (55.4%), followed by ponds (41%) and the streams were the least frequent (3.6%). Furrows had the highest larval densities (56.41%), followed by ponds (39.5%) and the streams had the least (4%). Furrows and streams had high species evenness, 0.614 and 0.639 respectively, whereas ponds had low species evenness (0.395). Total coliforms, phosphorus, temperature, electrical conductivity (EC) and pH were the environmental determinants that were positively associated with increasing abundance of the identified larval mosquitoes. Nitrite (p=0.291) and dissolved oxygen (p=0.001) had negative association with larval abundance; and their concentrations, nitrite (0-1.4 mg/L) and DO (0.01-12.25 mg/L), favoured larval persistence and survival. The *Culex* species were dominant in ponds which was rich in nitrite, and had low mean total coliform concentrations (30.33×10^7 MPN) and high mean *E. coli* concentrations (3.74×10^7 MPN). The *Anopheles* and *Aedes* species, on the other hand, occurred largely in furrows that had moderate mean concentrations of total coliforms (48.27×10^7 MPN) and *E. coli* (3.22×10^7 MPN). The mosquito larvae were abundant in the wet season than the dry season, however, the difference was significant for *Culex* (p=0.000), but not for *Anopheles* (p=0.874) and *Aedes* (p=0.093). There were no significant differences in the occurrence and abundance of the breeding habitats in the rainy and dry seasons (p=0.089).

The high abundance of larval mosquitoes and the favourable levels of microbial and physicochemical parameters suggest that the epidemiological importance of urban irrigated vegetable farms may be the provision of increased numbers of favourable larval habitats, as well as oviposition sites for the adult female mosquitoes. There is therefore a probable high risk of malaria transmission in the city of Kumasi due to the significant contribution of larval mosquitoes to the production of competent adult mosquito populations.

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CHAPTER ONE

INTRODUCTION

1.1 Background

The world's population is rapidly growing in size and most of these populations are concentrating in urban areas. This population growth predominant in the developing countries, where an extra 2.1 billion people are anticipated to be living in urban centres by 2030 (Ackerson and Awuah, 2010). This rapid growth in population is far outpacing sanitation provision, and as a result the greater proportion of domestic and industrial wastewater enters aquatic ecosystems untreated (Ackerson and Awuah, 2010). In Ghana, the Urban and peri-urban municipal or district assemblies are struggling with the increasing sanitation crisis in their localities (Abaitey, 2011). Although the state and civil society organizations have adopted various sanitation delivery approaches and intervening strategies, the problem still persist. For example, drains are currently being used as waste receptacles in most places of the country's urban areas. Residents often carry out such bad acts at night and during rainfalls. Industrial and domestic wastewaters are also very often untreated and discharged into drainage systems which mostly empty into water bodies like streams and rivers (Abaitey, 2011).

Approximately, 90% of wastewater produced around the world remains untreated, causing widespread water pollution, particularly in the low-income countries (Hussain *et al*., 2002). For example, the bulk of the generated wastewater in most urban areas of Ghana that enters the environment remains untreated. This is because there are no means of treating the wastewater and the available sewage network also serves only 4.5% of the entire population (Ackerson and Awuah, 2010). A well-controlled and appropriate reuse of wastewater can help in the effective use of water resources and the protection of the environment. Actions encouraging the reuse of wastewater are ongoing worldwide, but the underlying structures

in most developing countries are lacking measures for protecting the environment and human health (Hanjra *et al*., 2012).

The use of wastewater for irrigation in agriculture has become a widespread practice in most urban areas such as Accra and Kumasi. It has been practiced for many years in the arid zones of developing and industrialized countries. Many farmers, especially vegetables farmers, in the urban and peri-urban areas of most developing countries use this raw or diluted wastewater to irrigate their crops. Even though there is an extensive use of shallow dug wells by some farmers, about 70% of the farmers in Kumasi use polluted streams and rivers as their main sources of water for irrigation purposes (Habbari *et al*., 1999). This practice has been mainly controlled by several factors such as; social acceptance of the practice, the reliable supply of wastewater for a year round crop production, and the decreasing availability of water resources for irrigation in agriculture due to high demand for potable water in urban areas. To some extent, knowledge of the fact that wastewater contains nutrients that can increase crop yield is also a contributing factor (Mara and Cairncross, 1991).

Urban and peri-urban agriculture are seen as sustainable agriculture, this is because urban agriculture promotes the production of energy-saving local food, and contributes to food security and food safety. It increases the amount of food available to people living in the urban areas, and also make available to the urban consumers fresh vegetables, fruits, and meat products. Peri-urban agriculture provides employment, income, and access to food for people in the urban areas, which together helps to alleviate emergency and chronic food insecurity (Afrane *et al.,* 2004). For example, Accra has about 1000 active marketoriented urban vegetable farmers whose farm products are eaten by 200, 000 Accra residents daily (Abaidoo *et al.,* 2009). However, the effective use of untreated wastewater as a source of irrigation for urban agricultural practices can facilitate the spread of waterborne diseases

such as malaria, filariasis and dengue among the ambient human population. For example, urban agriculture in Kumasi is mostly practiced in low-lying areas where there is easy access to water, and in general, such places are known for breeding more mosquitoes. In addition, water in these places is increased by irrigated urban agricultural practices such as the construction of ponds and conduits which can serve as breeding sites for Anophelines (Afrane *et al.*, 2004).

With very favorable environmental conditions in anywhere of the world, vast populations of mosquitoes will practically breed in any collection of water that stands for not less than five days or a week long. The choice of breeding places varies in accordance with the type of mosquito, as some prefer shady places whereas others like sunlit places. However, some of the common breeding sites are ponds, swamps, slow-moving water bodies, and manmade containers of water such as drains and discarded cans. Medically, mosquitoes are the most important group of insects worldwide. This is because of the number of disease causing agents they transmit and the extent of the worldwide health problems such diseases cause (Merritt *et al.*, 1992).

There has always been a close link between the transmission of water related vector-borne diseases and the presence of excess water which is due to lack of adequate drainage. This is a major case in the tropical and subtropical regions where important water related vector-borne diseases such as malaria, lymphatic filariasis and schistosomiasis are common (Martin, 2013). Series of studies conducted in Kumasi and Accra on urban agriculture and the prevalence of malaria showed that, the distance of human residence from irrigated farming sites is inversely proportional to the prevalence of malaria. Therefore, the high incidence of malaria may be due to the closeness of residents to irrigated farming sites, as well as bad drainage practices and poor sanitation at various homes (Ackerson and Awuah, 2010). In addition, urban agriculture may promote the rapid development of insecticide

resistant mosquito species in urban areas. This is because urban agriculture, apart from being dependent on a continuous supply of water and nutrients, also uses high inputs of pesticides in intensive crop cultivation (Tallaki, 2005). Urban malaria is likely to increase in importance as rapid urbanization will result in the most of Africa's population living in cities in the near future (Klinkenberg *et al*., 2008). In the year 2000 for instance, Ashanti Region which falls within the forest zones of Ghana recorded the highest number of malaria reported cases and the highest prevalence of malaria parasitaemia (Coleman, 2009).

According to the latest estimates of the 2014 world malaria report of the WHO, about198 million episodes of malaria occurred with an estimated deaths of 584,000 as against about 219 million cases of malaria with an estimated 660,000 deaths in 2010. Most of these malaria cases and deaths occurred among children children under five years of age in subSaharan Africa. These estimates show that through the increased malaria prevention and control measures, the global malaria burden is drastically reducing. This is shown as the fall in malaria mortality rates increased from 25% globally since 2000 in 2010 to 47% in 2013 and from 33% in the World Health Organization (WHO) African Region in 2010 to 54% in 2013 (WHO, 2014). Even though malaria mortality rates have been reduced by an estimated 58% since 2000 among children living in Africa, a child dies every minute from malaria, and this result in the death of approximately a million of these children yearly (WHO, 2014). In terms of vulnerability to malaria, pregnant women and their unborn children are at risk the most, as malaria cases in pregnant women may result in maternal anaemia and low birth weight which may result in death during the first months of life. Furthermore, children who survive severe malaria attacks could develop some consequences such as brain dysfunction and convulsions (UNICEF, 2007).

1.2 Problem Statement and Justification

Currently, Africa has an urban population growth rate of 3.5%, which is more than three times the rate of rural population growth. Even though this growth rate of most of the African countries are outpacing the urban infrastructure such as sanitation, good roads, waste disposal systems, etc., it is still estimated that, more Sub-Saharan Africa countries will have higher urban population growth rate than the rural. However, these developing countries are already suffering from food shortages and health problems. Therefore, urban agriculture has been internationally recognized as a means to increase the growing urban food supply and also contribute to employment and poverty alleviation. For example, with the rest coming from peri-urban (PU) and rural areas, an estimated 90% of vegetables such as cabbage, spring onions and lettuce that are consumed in the city of Kumasi are produced in the city itself (Afrane *et al*., 2004).

The use of wastewater for irrigation in agriculture is on the increase in the urban and periurban areas, as well as in distant rural areas which are downstream the urban centres. Having no other choice due to the high demands of food and portable water by a fastgrowing urban population and the lack of proper functioning wastewater management system, many farmers rely on the use of wastewater as their only source of irrigation. For instance, in and around the city of Kumasi alone, the International Water Management Institute (IWMI) estimated that, untreated wastewater sources are used by farmers for irrigation on not less than 12000 hectares of land, which is more than twice the land covered by formal irrigation schemes in the country (Lightcap, 2010). Farmers therefore make use of makeshift irrigation schemes to sustain a year round vegetable production. This leads to the construction of shallow dug-out wells or conduits and these are linked by furrows to make irrigation easier for farmers who make use of watering cans. However, such irrigated farming areas have high number of adult Anophelines and therefore high levels of malaria transmission because

such water bodies together with human footprints in these irrigation schemes are associated with Anopheline breeding habitats (Afrane *et al*., 2012).

Even though there are some inconsistencies, some negative impacts on human health, mainly vector-borne diseases such as malaria, are highly associated with world-wide irrigation development projects (Herrel *et al*., 2011). Hence, it is anticipated that there may be worldwide health deterioration due to these irrigation development projects, if some good preventive measures are not properly put in place (Sanchez-Ribas *et al*., 2012). Moreover, the transmission of malaria is determined by many water-driven ecological factors, such as the provision of suitable habitats for the Anopheline and Culicine larvae to develop, that greatly affect the competence of the malaria vectors as they establish themselves in a new environment (Kengluecha, *et al*., 2005).

Even though the malaria burden in many places is dramatically reducing due to the increased malaria prevention and control measures, the disease is still the leading cause of illness and death in many countries (WHO, 2014; CDC, 2013a). Statistics of the Ghana Health Service (GHS) for half of the year of 2010 chronicles that, malaria was the leading cause of hospital admissions in the Ashanti region (12,143 admissions), as it recorded the highest number of 301,019 reported cases and taken the lives of 66 persons. This is of very much importance as the Kumasi Metropolis alone pulled 2,035,064 of the total population of 4,780,380 in the Ashanti region and has the highest number of facilities [29%] (GHS, 2010; GSS, 2012).

Vector control measures such as the use of pyrethroid insecticide-treated nets (ITNs) has led to much of the global success in malaria control and this is followed by the use of treatment drugs such as WHO recommended artemisinin-based combination therapy (ACT). However, the high levels of malaria transmission in sub-Saharan African countries have also

been characterized by widespread reports of the emergence of drug-resistant parasites and insecticide resistance of the adult Anophelines (WHO, 2014). Hence, malaria vector control measures that target the aquatic immature stages are gaining grounds. Moreover, the dynamics, abundance and fitness of the adult Anophelines are very much dependent on the aquatic stages of the vectors. Therefore, a better understanding of the biology, behavior and ecology of these important stages of the *Anopheles* mosquitoes could contribute to understanding how malaria is transmitted, the productivity of breeding habitats and aid in the implementation of well-designed appropriate control measures
(Paaijmans, 2008; WHO, 2014).

However, not much have been documented about the behaviour and ecology of these aquatic stages and therefore most vector control methods do not target these stages of the mosquito's life cycle. The aim of this present study, therefore, is to investigate the abundance of immature mosquitoes (larvae), their persistency and development in wastewater irrigation farms in the Kumasi Metropolis. More importantly, the study also focuses on the effects of some physicochemical and microbiological properties on the types, ecology and abundance of mosquito larvae, particularly the Anophelines which are the most broadly distributed and most efficient vectors of the malaria disease.

1.3 General Objective

To assess the occurrences of mosquito larvae and characterize their habitats on urban wastewater irrigated vegetable farms in the Kumasi metropolis.

1.4 Specific Objectives

(i) To determine the occurrence of mosquito larvae and species emanating from breeding habitats in the wastewater irrigated vegetable farm sites.

(ii) To determine the various mosquito breeding habitats (receptacles) on the wastewater irrigated vegetable farm sites.

(iii) To determine the breeding habitat characteristics (such as temperature, pH, dissolved oxygen, ammonia and nitrate, total coliforms and *Escherichia coli*).

(iv) To assess the relationship between the abundance of mosquito larvae and physicochemical and microbiological properties of breeding habitats on the wastewater irrigation farm sites.

CHAPTER TWO

LITERATURE REVIEW

2.1 Forms and Quality of Wastewater Reuse

Wastewater may be described as any water that has adversely been affected in quality as a result of the combination of surface, storm or ground water with liquid wastes emitted from different sources such as farms, institutions and domestic households, and commercial and industrial establishments. Wastewater varies widely in composition which may include bacteria, organic materials such as faecal matter, and many others (FAO, 2012).

According to the WHO's guidelines for the safe use of wastewater for irrigation purposes, water with 10^6 - 10^8 total coliforms per 100 ml and that with 10^4 -10^5 thermotolerant coliforms per 100 ml or less is considered of poor water quality and partially treated water respectively. On the basis of health risks associated with the use of wastewater for irrigation purposes, wastewater with the quality of 10^3-10^4 *E. coli* per 100 ml is unrestricted for irrigation purposes. On the other hand, waste water with the quality of 10^5 *E. coli* per 100ml is restricted for irrigation purposes (WHO, 2006a). Westcot (1997) in his studies about the quality control of wastewater for irrigated crop production made the important distinction between direct and indirect wastewater reuse. According to Westcot,

(i) Direct Reuse: This is where a decision had been made to make use of raw or treated wastewater for irrigation purposes. Therefore quality control measures are initiated to check on the wastewater conveyed from the point of collection or when it is discharged from a treatment plant before it is used for irrigation. To be able to monitor and control the quality of the water and the place where the irrigation occurs, most developed nations have well established physical and institutional infrastructure for the direct reuse procedure.

(ii) Indirect Reuse: This is the situation pertaining in many developing countries where no specific decision have been made to make use of raw or treated wastewater for irrigation purposes. Consequently, waste or marginal quality water of unknown composition is indirectly reused for irrigation or domestic purposes by many residents of urban downstream water bodies. This is as a result of the absence of monitoring or control measures over municipal and industrial wastewater, and therefore much of such wastewater is discharged into watercourses draining the urban areas without treatment. Consequently, the quality of such water bodies in most urban and peri-urban areas is dependent on the velocity of the water body, the volume and composition of the discharged effluent. Most of the health risks associated with this indirect reuse of wastewater is unknown since the quality of most of such watercourses is also unknown. However, this indirect reuse is estimated to expand rapidly in the future due to the high rate of urban population growth that outpaces the financial resources to establish adequate control measures.

2.1.1 Routes by Which Wastewater Reaches Farms

The routes by which wastewater arrives in farms depends on the location of the farm, season and the availability of other water sources. For instance in wet seasons, wastewater is commonly diluted by other water sources before reaching farms, whereas in dry seasons or during water scarcity, wastewater may directly reach farms with little or no dilution at all.

According to the FAO's on-farm practices for the safe use of wastewater in urban and periurban horticulture guide (FAO, 2012), the common routes by which wastewater reaches various farms include:

- Wastewater → Wastewater treatment plant → Vegetable farm;
- Wastewater → Shallow well →Vegetable farm;
- Wastewater → Stream → Vegetable farm;

- Wastewater →Stream → Farm pond →Vegetable farm;
- Wastewater → Drain/gutter →Vegetable farm;
- Wastewater →Drain/gutter →Farm pond → Vegetable farm;

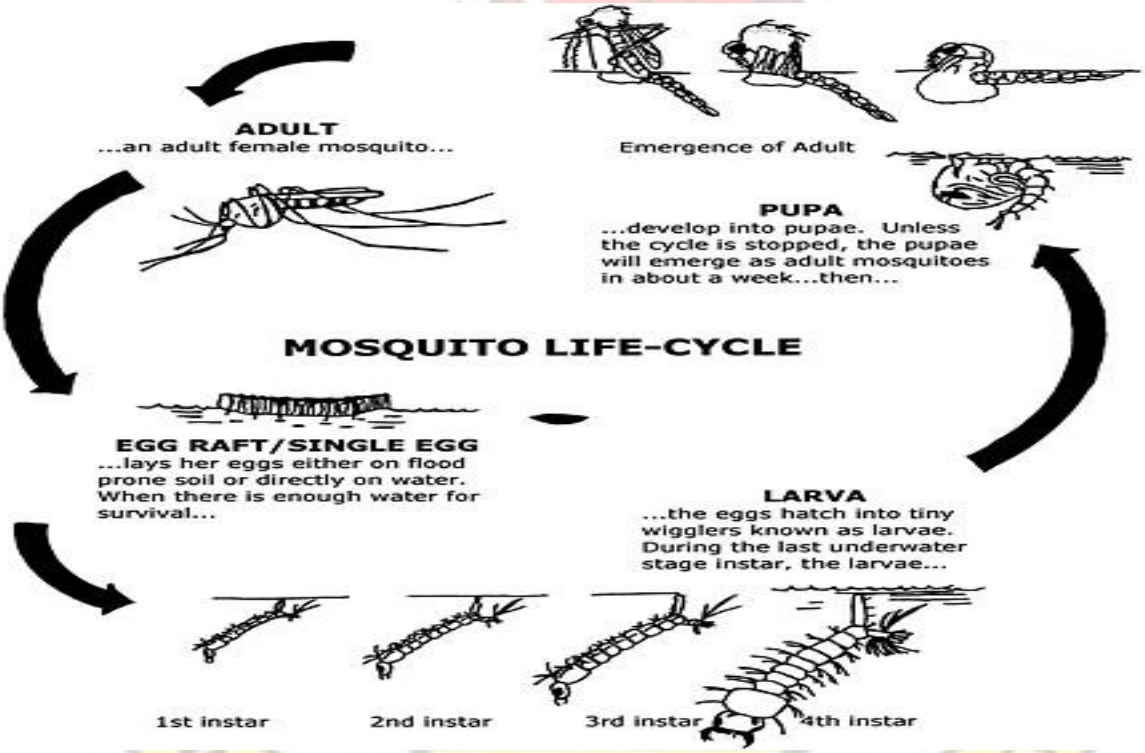
2.2 Mosquitoes

Globally, mosquitoes are by far one of the most important vectors that have major health impacts on the wellbeing of humans and even on domestic animals. In favorable environmental conditions, vast populations of these vectors can occur anywhere in the world (Gouge *et al*., 2001). With the exception of the permanently frozen areas, mosquitoes are found everywhere, including the warm moist climates, humid tropics and subtropics as well as the temperate and cool zones. However, the adults are very active all year round in the tropical areas whereas in the temperate climates, they become inactive and enter hibernation with the onset of winter to be able to survive. There are 41 genera of mosquitoes worldwide and about 3500 species have already been described from various parts of the world. The mosquitoes that are of medical importance are grouped into the Anopheline mosquitoes and the Culicine mosquitoes. Together, they belong to the order Diptera (flies) and the family Culicidae (small, midge-like flies). The Anophelines are the important genus *Anopheles* which is the vector for malaria, whereas the Culicine mosquitoes also contain three other important genera, *Culex*, *Aedes*, and *Mansonia* which are vector for Bancroftian filariasis (CDC, 2013a).

The female mosquitoes usually have a longer flight range than that of the males. Even though, some flight ranges as far as 75 miles from the breeding sites have been recorded, most species stay within a mile or two around their breeding habitat. The wind, at many times, has been a major factor in the migration of mosquitoes (Bhanot, 2008).

2.2.1 Mosquito Life Cycle

The life cycle of a mosquito is an example of complete metamorphosis (Figure 2.1), which has four distinct (special appearance) stages; the egg, larva, pupa and adult (Gouge *et al*., 2001). The first three stages, egg, larva and pupa, are aquatic. These stages last 5–14 days, depending on the type of species, ambient temperature, and other circumstances: but there are important exceptions. At very low ambient temperatures, these aquatic stages take longer time to develop and the longer they take, the more vulnerable they are to people, and predators such as birds and fishes. Their development depends on ambient temperature because they are cold-blooded and therefore rely on external heat sources to warm their bodies (CDC, 2013a).



Source: WUMCD, 2013

Figure 2.1: Life cycle of the Culicine mosquito

2.2.1.1 Mosquito Egg Biology

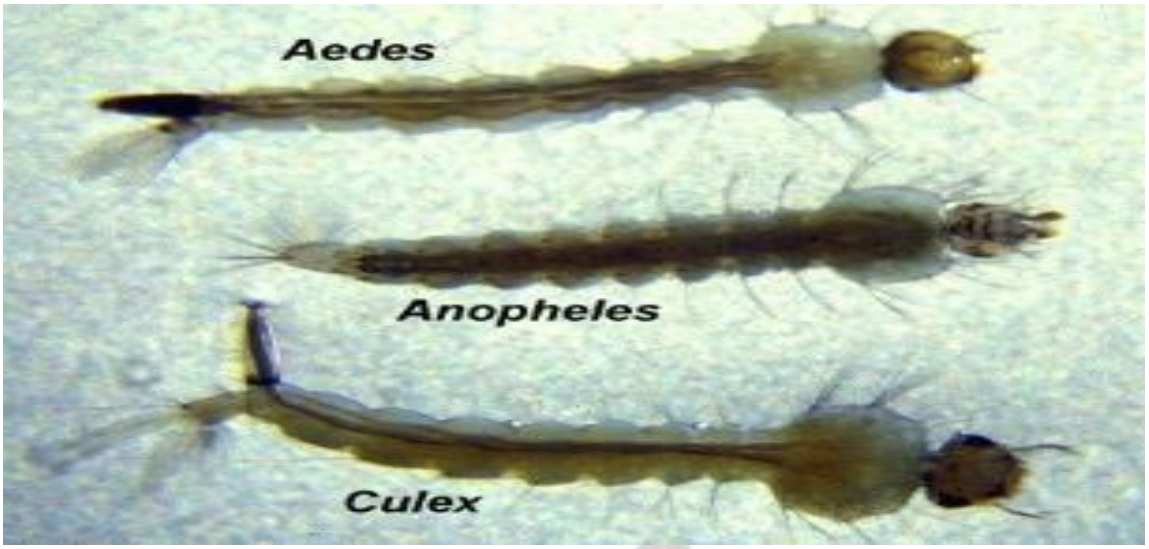
During the adult phase of the females of many common species of mosquitoes, they can lay 100–500 eggs before dying. Irrespective of the high egg and intergenerational mortality of mosquitoes for some period of weeks, thousands of mosquito populations can be created from

a single successful breeding pair. On the average, mosquito eggs take between 1-4 days to hatch (Spielman and D'Antonio, 2001; Jackman and Olson, 2001).

2.2.1.2 Larval Mosquito Biology

Unlike the egg stage, the larvae must always live in the water to survive and may take 7 days on the average. The rate of survival of the larvae in their habitats, especially the small sized habitats, is reduced by predation and cannibalism (Mwangangi *et al*., 2008). Reports from various studies indicated that, of a number of the larvae that hatched at a particular time period, only a small fraction (2-8%) survived to the adult stage (Paaijmans, 2008).The mosquito larva has a well-developed head with mouth brushes used for feeding (Mosquito Information Website, 2013a).

The larvae can stay submerged for some time and spend most of their time feeding on bacteria, algae, and other microbes in the surface micro-layer of the water. Feeding at this stage is accomplished by the ingestion of particles filtered from column or surface of the water, shredding of leaves, removal and ingestion of surface biofilms, and predation of insects or other larvae which are smaller or of their own size. The larvae stores up and provide nutrition for the non-feeding, yet active pupal stage. Breathing at this stage occurs through spiracles located on the eighth abdominal segment of the larvae, or through a snorkel-like device called siphon, and this demand that they frequently come to the water surface. The mosquito larvae develop through four major stages called instars, and at the end of each stage, the larvae molt (shedding their skins to allow for the next instar) and therefore further growth. Throughout the instar stages, the major features such as the presence or absence of a siphon, that differentiate the larvae of one species from the other becomes more visible and functional (Plate 2.1). The mosquito larva stops feeding during the 4th instar and metamorphose into pupae (CDC, 2013a)



Source: Russell, 2010

Plate 2:1: Distinguishing features of *Anopheles, Culex* and *Aedes* mosquito larvae

2.2.1.3 Pupal Mosquito Biology

The pupal stage is the transitional stage between the mosquito larvae, which live in water, and the adults, which live on land. The pupa, which is shaped like a comma, is also aquatic and this stage may last for a few days, from 1 to 10 days, or even more. During this stage, the development of the adult mosquito's mouthparts, legs and wings takes place in sheaths that are curled around the underside of the cephalothorax (Mosquito Information Website, 2013a). The pupae of most species, as with the larvae, must frequently come to the surface to breathe, and this occurs through a pair of respiratory trumpets on their cephalothorax. However, unlike the larvae, the pupae, as it has no functional mouthparts, do not feed; and therefore with their respiratory trumpets, they are always found hanging from the surface of the water. Consequently, the pupa has a lower activity as compared to the larva which feeds constantly (Spielman *et al*., 2001). At the end of the pupal stage, the pupa rises to the surface of the water, its cephalothorax splits at the dorsal surface using air pressure, and the adult mosquito emerges (Jackman and Olson, 2001).

2.2.1.4 The Adult Stage

Typically, due to the heavy predation that both the larvae and pupae are subjected to, only a few pupae are able to develop successfully to adults from the 100-500 eggs laid by a female mosquito (Bartlett, 1999). The time length of development of mosquitoes from egg to adult varies from one species to another and this period is influenced by the temperature of the immediate surroundings. While some species of mosquitoes take as little as 5 days to develop from egg to adult, a more typical period of development of species in the tropics takes 40 days or more (Mosquito Information Website, 2013a).

It usually takes a few days, after emerging from the pupal stage, for the adult mosquitoes to mate as the male mosquitoes are unfit to mate with the females who are always almost ready to mate immediately after eclosion. For instance, optimal mating for the males of *Anopheles arabiensis Patton* and *Anopheles gambiae Giles sensu stricto* (s. s.) occurs only within the 5–7 day old. Mating usually takes place quickly in the air closer to the site of the adult eclosion (Coleman, 2009). A newly emerged female, after mating and finding a blood source, can start a new cycle by laying her eggs on standing water (CDC, 2013a).

The life span of the adult mosquito is dependent on several factors such as temperature, humidity, sex of the mosquito, the time of year, and most importantly for the females, their ability to successfully obtain a blood meal while avoiding host defenses (CDC, 2013a). Nature also plays a major role in their survival; besides humans in various ways doing their best to get rid of them, they are also eaten by spiders, dragonflies and birds among many others. Due to the efficiency of all these life threatening barriers, only a small percentage of adult mosquitoes survive for their full potential lifespans. However, one female on the average, may lay hundreds of eggs, only if it can survive long enough to bite a blood source and lay a batch of eggs. Even though it is not possible to measure directly the length of life of mosquitoes in nature, but in general, the females live longer than the males. Depending on the above factors, most females probably may not live longer than 1-2 weeks in nature, but they can survive for up to a month

or two (or longer in captivity). Most males on the other hand live a very short life span, about a week; which is long enough to swarm and mate with the females (Miller, 2012; Bhanot, 2008).

2.2.2 Malaria Causing Mosquitoes

Human malaria is transmitted only by adult female mosquitoes belonging to the genus *Anopheles*. There are about 430 recognized *Anopheles* species, of which over 100 can transmit human malaria, but only 30–40 commonly transmit the *Plasmodium* parasites, which cause malaria in humans in the endemic areas. With the exception of Antarctica and at elevations above 2000-2500 meters (6500-8200 feet), Anophelines are found worldwide, even including areas where malaria has been eliminated. However, Anophelines are mostly found in malaria-endemic areas such as the tropical regions (CDC, 2013a; Coleman, 2009).

The predominant role of *Anopheles gambiae*, a complex of morphologically indistinguishable mosquitoes in the genus *Anopheles*, in the transmission of the most dangerous malaria parasite species (*Plasmodium falciparum*) has made it the best known *Anopheles* species. The *Anopheles gambiae* are the most efficient malaria vectors in the world and contains the most important malaria parasite vectors in Sub-Saharan Africa. This complex of species consists of the *Anopheles gambiae* s. s., *Anopheles merus*, *Anopheles arabiensis*, *Anopheles melas*, *Anopheles quadriannulatus*, and the *Anopheles bwambae* (Coleman, 2009).

A major important behavioral factor of the *Anopheles* species is the degree to which it prefers to feed on humans (anthropophily) or animals such as cattle (zoophily); even though most species are neither exclusively anthropophilic nor zoophilic. However, the main malaria vectors in Africa, *A. funestus* and *A. gambiae*, are mainly anthropophilic, and consequently, making them two of the most efficient malaria transmitting mosquitoes in the world. The anthropophilic species are

more likely to transmit the malaria parasites from person to persons since they prefer to feed on humans. Some *Anopheles* mosquitoes feed indoors

(endophagic), for example *A. funestus* and *A. gambiae s.s.* while others feed outdoors (exophagic) and seldom occur away from human habitations (CDC, 2013a).

The malaria parasites require from 10 to 21 days, depending on the ambient temperature and the parasite species, for their development within the mosquito. Before the next bite can be infectious to humans, this extrinsic incubation period of the malaria parasites is required within the mosquito. That is, if a female mosquito dies sooner than the extrinsic incubation period, then it will not be able to spread malaria since it cannot transmit any malaria parasites. Though scientists estimate that, fewer than 1 in 10 Anophelines survive long enough to transmit the malaria parasites, but that is still too many. Therefore scientists are hoping to shorten the lifespan of malaria mosquitoes even more (CDC, 2013a; Miller, 2012). By indirect estimates, the daily survivorship for several species of Anophelines such as the *A. gambiae* in Tanzania ranged from 0.77 to 0.84. This means that, between 77% and 84% will survive by the end of one day (Charlwood *et al*., 1997). Assuming these estimates remain constant through the adult life of a female *A. gambiae*, then less than 10% of them would survive longer than the 14-day extrinsic incubation period of the malaria parasite. However, if the daily survivorship estimate is increased to 0.9, then over 20% of the female *A. gambiae* would survive longer than the 14-day extrinsic incubation period (CDC, 2013a).

The adult Anophelines, on the average, has a flight range of between a few 100m and 2 Km. Therefore water collections very close to human settlements are more important sources of mosquitoes than those located far away from houses (HEAT Programme, 2013). The results of a study on open-space irrigated vegetable farms in the city of Kumasi by Afrane *et al*. (2004) showed that, suitable breeding habitats for Anophelines such as *A. gambiae* are being provided for by such farms. This accounts for the great numbers of adult *A. gambiae* in the communities close to urban irrigated agricultural fields as compared to the urban areas devoid of irrigated

agriculture. Furthermore, the study also reports of more reported cases of malaria within the urban areas of irrigated agricultural practices than the vicinities of no urban agricultural practices during both the rainy and dry seasons.

2.2.3 Breeding Habitats

Mosquitoes breed in a wide range of habitats. Each species, according to its own ecological adaptations, selects the best water condition as a habitat for its first three aquatic stages. Some mosquitoes overelaborate when selecting a breeding habitat, whereas others are generalists. The adult females of most species prefer to lay their eggs in stagnant water, some near the edge of the aquatic habitat and others have their eggs attach to aquatic floras (Gaines, 2011).

Species of the *Aedes* and *Anopheles* mosquitoes are found throughout the globe in tropical, subtropical, and temperate areas. With the exception of the extreme northern latitudes, species of the *Culex* mosquito can also be found throughout the world. *Culex* mosquitoes breed in various types of stagnant waters such as storm drains, rainwater barrels, septic tanks, and catch basins, which are rich in organic material. These stagnant waters are provided for by various types of sewerage systems, drainage systems and container sources. *Anopheles* mosquito larvae can be found in a wide variety of breeding habitats, but the key malaria causing vector, *Anopheles gambiae* complex, prefer open-water pools with minimal vegetation. The habitats of the *Aedes* mosquito larvae are dependent on the species, but generally, they are either container (artificial or natural containers such as waste tires and tree holes) mosquito species or floodwater mosquito species. The floodwater mosquitoes prefer habitats such as flood-irrigated habitats including flooded vegetable fields, and low-lying areas along lakes, streams and rivers (Valent BioSciences, 2014).

Much is not known about what makes vectors heterogeneous in their abundance and distribution, and as well as the regulation of the abundance of mosquito larvae in the various breeding habitats (Gaines, 2011).

The presence of any fleeting water body, such as inundated cattle hoof prints in muddy grounds, for more than a week makes it a potential mosquito breeding habitat. These aquatic habitats are only limited by their longevity, and the length of time a species needs to complete its life cycle from egg to adult stage. There is also a perception that selection of breeding habitats is to some extent affected by the topography of an area, since a breeding habitat, small or large, temporary or permanent, is most likely to occur at flat and relatively low-lying areas (Coleman, 2009; Gaines, 2011).

2.2.4 Mosquito Control

The establishment of an effective worldwide mosquito control system has proved to be intricate, costly and cooperative as there has been the need of the combined efforts of local governments, communities, industries, the agricultural sector and the state as a whole. In designing an effective control program, the foremost important factors that must be considered include the vulnerability of malaria vectors to insecticides, the vectors strict requirement of standing water or moist soil for the aquatic stages, and the specific feeding and resting preferences of the adult mosquitoes. The control of different species with different preferences necessitates different control methods. Therefore depending on the situation at hand, the most important approaches include those considered below (Mosquito Information Website, 2013b; CDC, 2013a).

2.2.4.1 Source Reduction

This involves the elimination or adroit control of aquatic habitats which are known to breed mosquitoes. Source reduction employs measures such as leveling, filling of holes, application of herbicides, and the manual removal of aquatic plants. This also includes the drainage of all potential breeding places such as sewage and other wastewater, discarded pots, tins, crockery, and coconut shells. It can also be accomplished through the management of water bodies such as intermittent irrigation, and by making standing waters unstable, such as changing the salinity

of water, for breeding by the mosquitoes. Mosquitoes which prefer breeding in containers are the most susceptible to source reduction, as containers are easily removed (Bartlett, 1999).

2.2.4.2 Mosquito Larviciding

Mosquito Larviciding involves the application of environmentally-benign larvicides to kill the larval forms of mosquitoes. This could be achieved through biocontrol measures which involve the process of importing natural predators to feed on the larvae. Examples of biological larvicides include the bacteria *Bacillus thuringiensis* var. *israelensis* (Bti), also called mosquito dunks, and the soil-inhabiting *Bacillus sphaericus* (Bs) which can be purchased in stores. *Bacillus sphaericus*, which is not toxic to non-target organisms, is frequently used in extremely polluted water, such as sewage treatment plants to control mosquito larvae (Bartlett, 1999; Gouge *et al*., 2001). Other organisms like fungi (e.g., *Laegenidium giganteum*) and mermithid nematodes (e.g., *Romanomermis culicivorax*) which parasitize on mosquito larvae are probable biological control agents but are not widely used for mosquito control because they don't give efficient results (CDC, 2012b). Other known mosquito predators include guppies and the mosquito fish, *Gambusia affinis*, which can be purchased for stocking pools and ponds (Bartlett, 1999).

Another form of larviciding is by the use of synthetic pesticides such as methoprene which mimics the juvenile hormone of the larvae. The presence of methoprene disrupts the development of the mosquito larvae to the adult stage. Methoprene is safe for use around humans but toxic to some other insects besides the immature forms of the mosquito. Other synthetic products such as oil, preferably rapidly biodegradable ones, can also be applied to the water surface and this suffocates the larvae and pupae (Bartlett, 1999; Gouge *et al*., 2001).

Ideally, the best approach to the control of mosquitoes seems to be by larval control as it eradicates mosquitoes before adulthood. However, the larvae escape their aquatic environment and move on to adulthood due to some limiting factors such as the short development time and

also because their habitats are small in nature, disseminated and fleeting. For instance, the *A. gambiae*, one of the principal malaria transmitting vectors in Africa, breeds in various small water bodies that are formed during precipitation. Therefore, the use of larval control measures on a large scale in mosquito control in Africa is yet to be attempted (WHO, 2012).

2.2.4.3 Adulticiding

Adulticiding involves the use of broad-spectrum adulticides, which can be harmful to other organisms such as birds and fish to kill adult mosquitoes. Natural predators such as the nymphs of damselfly and dragonfly can be imported to feed on mosquitoes at all of their developmental stages (Bartlett, 1999). In several small-scale vicinities, the introduction of sterile male mosquitoes into the environment has attained successful results. However, the difficulty of breeding more sterile males to be released remains an impediment for this approach (CDC, 2012b). Even though the use of these natural enemies does not provide adequate mosquito reduction, very high populations of such predators can be useful in reducing the problems associated with adult mosquitoes (Bartlett, 1999). Synthetic adulticides commonly used in mosquito control districts in the United States include malathion, permethrin and naled. Other adulticides such as methoxychlor are used to control the adult of certain mosquito species but have no effect on others (Bartlett, 1999; Gouge *et al*., 2001).

2.2.4.4 Exclusion (Use of Mosquito Nets and Window Screening)

This involves the use of mosquito nets and improved housing constructions, such as window screens, to prevent mosquito entry and bites. Others also employ practices such as the wearing of long sleeves and long pants, light-colored clothes, and limit evening outdoor activities. This is very effective when used in combination with a thorough attention to the control of breeding habitats. The use of insecticide-treated bed nets (ITNs) are more effective because besides

killing endophilic mosquitoes that try to feed on humans, they do not affect the general ecology in any way (CDC, 2013a).

2.2.4.5 Use of Repellents and Traps

This involves the application of insect repellents on skin to provide some form of personal protection from mosquito bites. The most effective of these repellents are those that contain DEET (N,N-diethyl-m-toluamide) which repels most mosquitoes as well as other harmful vectors. DEET containing repellents are not to be used around the mouth, nose and eyes, and on infants. Other repellents also contain certain insecticides that are suitable only for application to clothing and not to the skin. Therefore directions on the use of these products are supposed to be read very carefully (Bartlett, 1999; Gouge *et al*., 2001).

There is the availability of many kinds of traps that are asserted to provide mosquito control, but most of such traps have not been effective in controlling the vectors at the population level. For instance, many kinds of bug zappers and ultraviolet lights are extensively marketed worldwide but they trap insignificant numbers of mosquitoes (CDC, 2012b).

2.2.5 Insecticide Resistance

Insecticide-based control methods such as the use of ITNs and indoor spraying with recommended insecticides are the major ways of eliminating indoor biting mosquitoes. Conversely, mosquitoes may develop resistance to an insecticide after a lengthy exposure of several generations to a specific insecticide (CDC, 2013a). Currently, the effectiveness of insecticide-based vector control is very susceptible, since global malaria vector control measures are deeply dependent on a solitary insecticide class, pyrethroids, and also the great levels of resistance that could quickly arise due to the high number of generations per year mosquitoes can have. Resistance of Anopheline vectors to insecticides has the possibility of compromising the global achievements over the malaria disease through the control of malaria

vectors and also hindering future successes. Therefore, the only long-term way out from the extreme mosquito problem is by getting rid of their breeding habitats. However, the WHO recognizing the magnitude of the risk at hand with insecticide resistance, have introduced a Global plan for insecticide resistance management in malaria vectors (WHO, 2014).

2.3 Effect of Some Environmental Factors on Mosquito Larval Abundance

2.3.1 Physicochemical Parameters

Physicochemical parameters, composed of physical components such as conductivity, temperature, dissolved oxygen and pH, and nutrient components such as nitrates, sulphates, nitrites and phosphates, are water associated characteristics that have specific effects on the quality and biological components of the water.The development and survival of the mosquito larvae in their habitats are affected by several physicochemical factors such as optimum temperature, range of pH, and the concentrations of sulphate, ammonia and nitrate (Oyewole *et al*., 2009). Ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) are the most common ionic (reactive) forms of dissolved inorganic nitrogen (N) in aquatic environments. In general, because natural (unpolluted) aquatic environments have low levels of inorganic nitrogen as well as inorganic nitrogenous compounds, aquatic organisms are adapted to such low relatively levels of inorganic nitrogen concentrations. A rise in the concentration of the available inorganic nitrogen elevates life production by causing the numbers of primary producers to increase. However, organisms which are least tolerable to inorganic nitrogen are affected if there are high saturations of inorganic nitrogen that cannot be absorbed biologically. High saturations of nitrate, ammonia and nitrite resulting from anthropogenic activities can make these inorganic nitrogenous compounds toxic and therefore harmful to the survival and growth of aquatic organisms (Camargo and Alonso, 2006). Even though there are some other important unmeasured factors, the physicochemical factors of the aquatic habitats influence larval growth

rates. Larval survival, length of time for pupation and the size of the adult mosquitoes reflect the state of environmental conditions the aquatic stages were exposed to during their life stages (Paaijmans, 2008).

2.3.1.1 Temperature

Temperature has a major effect on several biological processes and physicochemical properties in the natural aquatic environments. It has a magnitude of influence on the rate of photosynthesis by aquatic photosynthetic organisms, amount of dissolved oxygen and the rates of metabolism by aquatic organisms. It also influences the activities of parasites, pathology of diseases, irritability of organism to toxic wastes and other behavioral activities such as aestivation, migration and periods of reproduction of aquatic organisms (Arroyo Seco Foundation, 2013).

The survival of the larval forms may be affected by temperatures that are near or above the upper lethal temperature. The immature forms of mosquitoes are poikilothermic and therefore the rate of their metabolism is dependent on the temperature of their aquatic habitat. Therefore, an increase in inhabited water temperature, within a certain temperature range, results in a faster development of the immature forms but a decrease in size of the emerging adults. The latter is as a result of the larvae not being able to accumulate the necessary nutrient reserves prior to pupation, due to accelerated rate of development and therefore the larvae developing too fast. On the other hand, an elongation in time of larval development due to a lower accelerating rate exposes the larvae to larval biopesticides, pathogens, rainfall and predators for longer periods. However, fewer adults are produced at higher temperatures due to the increased mortality of the immature forms and also the sizes of the adults affect their prey seeking behaviour and parasite infectivity rate. The intermediate-sized adult mosquitoes are more infectious to humans (Paaijmans, 2008).

Even though mosquito larvae cannot endure very high water temperatures, optimal warmer temperature, compared to cold water, makes available more larval food as it enhances the growth of aquatic plankton. The thermal death point (TDP), very high temperature that is lethal to all larvae, varies among species due to differences in the limit of biological tolerance to high temperatures, but all larvae are killed at 52 ^{0}C. For instance, the TDP for *Anopheles vagus* is 44 ^{0}C whereas that of *A. minimus* and the *A. gambiae* S.I is 41 ^{0}C. TDP (Vasudevan *et al*., 2001). However, no adults emerged when larvae were reared in the laboratory at 18 ^{0}C (Paaijmans, 2008).

2.3.1.2 Salinity

Salinity is the measure of the quantity of salts dissolved in water. The presence of salts in any aquatic environment critically affects the aquatic biota and the water quality for drinking or irrigation purposes. However, a characteristic tolerable salinity ranges for every type of organism (Arroyo Seco Foundation, 2013). For example, the larval forms of mosquitoes are found in various aquatic environments ranging from freshwater to hypersaline. Therefore, the larvae are frequently required to deal with rapid fluctuation in salinity in their aquatic habitats (Donini, 2007).

According to the results of a on salinity in aquatic habitats, about 96 hours of exposure of the *Culex* species larvae to habitats with sodium chloride (NaCl) concentrations of more than 6.2 ppt, the fatality rate of the larval population was more than 50%. For instance, in the first few hours of exposure larvae to 20 ppt concentrations of NaCl, all the larvae died and therefore, such levels of NaCl could be used in the control of mosquitoes. Furthermore, salinity has some effect on the growth rates of mosquitoes, as the growth rates of *Aedes aegypti* decreased with increasing salinity and also at some lower concentrations, the larval stage lasts longer (Thamer and Abdulsamad, 2005).

2.3.1.3 Electrical Conductivity (EC)

Electrical Conductivity is the property of water that measures the waters ability to conduct electrical currents. EC is dependent on the amount of dissolved ions in the water body and therefore varies with the source of the water such as municipal wastewater, and water drained from agricultural farms, rainfall and ground water. EC is related to, and correlated with the organic compounds and the concentration of total dissolved solids within an aquatic environment. However, EC is regulated by temperature which has large effect on conductivity. Therefore, EC is expressed at a standard temperature of 25 ^{0}C and reported as precise conductance (μS/cm at 25 ^{0}C). The specific conductance of natural surface waters occurs between the range of 50 to 1500 μS/cm .This is to allow samples collected at different temperatures to be compared directly (Navajo Reservoir Operations, 2013). Per any degree Celsius increase in temperature, the EC of any water body approximately increases by 2% (Pescod, 1992).

2.3.1.4 pH

pH is the hydrogen ion concentration of any water body. It is an expression of the acidity and alkalinity in an aquatic environment, and that makes available an indication of the balance between acids and bases dissolved in the water (Jayalakshmi *et al*., 2011). pH is an important indicator of the levels of contamination in a water body. It is therefore used as a quality control in the biological treatment of wastewater before usage and therefore important in determining the quality of wastewater effluent. The standard pH of any water for irrigation ranges from 6.5 to 8.4. Therefore, the pH of water bodies outside this range is considered abnormal in quality for irrigation (Pescod, 1992; González, 1996). At certain specific pH levels of a water body, the toxicity of some heavy metals is heightened as the solubility of many nutritive and toxic

chemicals is also affected. Therefore, the pH of an aquatic environment, to some extent, regulates the availability of soluble chemicals to aquatic organisms (Lokhande *et al*., 2011).

Even though pH is recognized as a simple water parameter, it is very important as a change in its value causes a change in most aquatic chemical reactions. Organisms in the aquatic environments are sensitive to changes in pH as higher levels of either acidity or alkalinity could exterminate marine life and also because pH monitoring is very necessary for most biological treatments. For instance, mosquito egg shells before hatching are weakened by aquatic habitats with pH near neutral levels of 6.8 - 7.2 (Oyewole *et al*., 2009). Very low pH levels such as 1.0 are lethal to mosquito larvae and pH range of 3.3 to 4.7 is very favourable for breeding (Water Watch, 2013). For example, according to the results of a study on the effect of pH levels on the survival of *Culex* larvae by Thamer and Abdulsamad (2005), up to 50% of the larvae were killed in the first few minutes of exposure to pH of 2. Mosquitoes are found to survive in both acid and alkaline aquatic environments as the pH of some major breeding sites range between 6.3 and 8.2 (Opoku *et al*., 2003).

2.3.1.5 Dissolved Oxygen (DO)

Dissolved Oxygen is the amount of oxygen dissolved in a water body through the process of diffusion of oxygen from the ambient air, high velocity of the water, and also as a by-product of photosynthesis. The total dissolved concentrations of gas in any aquatic environment should not be more than 110%, as exceeding this percentage can be detrimental to aquatic life (Tiimub *et al*., 2012). Characteristically, the solubility of DO in natural surface aquatic environments ranges from 1.5 mg/L at 0 ^{0}C to 8 mg/L at 25 ^{0}C. Oxygen tensions which can be lethal to mosquito larvae are often associated with breeding habitats with vegetation, and therefore most of mosquito species prefer open sunlight pools or habitats (Tiimub *et al*., 2012).

The survivability and growth of many aquatic organisms is dependent on the presence of oxygen. Therefore the absence of oxygen in any aquatic habitat may result in the destruction of mosquito eggs or larvae, death of adults, stunt growth and change in biota (Arroyo Seco Foundation, 2013). A widespread study on the egg-laying preference of mosquito species to aquatic habitats with different levels of DO and salts shows that, on the average, *Anopheles*, *Culex* and *Aedes* prefer a DO of 6.6, 2.1 and 6.2 ppm respectively. However, the general tolerable DO required by most mosquito species is 4 ppm or less (Vasudevan *et al*., 2001).

2.3.1.6 Total dissolved solids (TDS)

TDS is the sum total of all the substances such as mineral sources, dissolved in a water body. Due to the enormous amount of dissolved salts in natural water, salinity is measured for as TDS content in the water. TDS is primary composed of potassium, nitrates, phosphates, manganese, carbonates, chlorides, iron, sodium, calcium, sulphates and a few others, but specifically not of gases, sediments or colloids (Amankona, 2010).

High concentrations of the constituents of TDS in any water body has some regulatory effects such as reducing the utility and solubility of gases like oxygen, the water's density, and the osmoregulation of organisms in freshwater. Thus TDS affects the quality of water and therefore its uses for irrigational (up to 2000 mg/L), industrial and drinking (from desirable of 500 mg/L up to permissible of 1,000 mg/L) purposes. However, TDS concentration above 3,000 mg/L of any water body is classified not useful for irrigation and drinking purposes (Lokhande *et al*., 2011).

2.3.1.7 Ammonia

The relative concentrations of ionized ammonia NH_4^+ and unionized ammonia NH_3 are essentially dependent on the temperature and pH of a water body. Therefore, the concentration of NH_4^+ tend to increase or decreases as the levels of temperature and pH increases or decreases respectively. In

measuring the ammonia concentration of any water sample for any analysis, it is the total ammonia, which is the sum of the concentrations of NH_4^+ and NH_3 that is measured (Camargo and Alonso, 2006).

The readily breakdown of urea, which is commonly found in domestic wastewaters, into ammonia by resident microorganisms is what leads to the presence of ammonia in domestic effluents. The urea is an organic nitrogen compound that is excreted by humans and therefore their presence in domestic wastewaters. Aquatic invertebrates and fish directly excrete ammonium, which is a toxic waste product of the metabolism, into their aquatic ecosystems. Ammonium and ammonia salts are also present in rainwater but in small quantities. The density of saturated solution of ammonia in an aquatic environment is 0.880 g/cm^3 (Cooke, 2013a).

One major characteristic property of ammonia is its basicity, and is considered to be a weak base. The basis of the toxicity of ammonia is its characteristic basicity. Ammonia creates a solution which has a much higher pH than a neutral water solution. This property causes proteins (enzymes) to denaturate, leading to the damage of cells of an organism, death of the cells, and eventually death of the organism. On the other hand, ammonia contributes significantly to the nutritional needs of organisms living on land by serving as a precursor to fertilizers and food (Ceresana, 2012).

Ammonia, which is extremely poisonous to animal tissues, is a by-product of amino acid oxidation. Conversely, the female mosquitoes possess some physiological mechanisms which are effective in detoxifying ammonia. Therefore, they are able to survive the enormous deamination that occurs during the breakdown of the amino acids obtained from proteins in a blood meal. The lethality of ammonia on mosquitoes is still not well researched into.

However, reports from some researches carried out on aquatic organisms such as the juvenile shrimps showed that, high levels of ammonia concentrations can cause increase mortality, reduce growth, and increase vulnerability to pathogens in vulnerable populations (Fossog *et al*., 2012).

2.3.1.8 Nitrites

Nitrites in the aquatic ecosystem occur in the free-state of inorganic nitrogen, and are usually does not last for long since they are rapidly converted into nitrates by bacteria. The concentrations of nitrites (NO_2^-) in a water body are principally dependent and directly proportional to pH and therefore, the concentration of NO_2^- tends to increase when the value of pH of the water increases. As in the case of ammonia, much has not been documented about the direct toxicity of nitrite concentrations on aquatic animals (Camargo and Alonso, 2006).

2.3.1.9 Phosphorus

There has been an increasing attention on the occurrence of phosphorous in domestic wastewaters since the early 1970's. This has led to a thorough analysis of phosphorous, its presence in discharged wastewaters, and its effect on waters into which it is discharged (Cooke, 2013a). Phosphorus (P) is a nutrient that is of great environmental concern since together with nitrogen when in excess, may lead to the formation of algal bloom which depletes the aquatic ecosystem of dissolved oxygen and in turn affecting the aquatic wildlife. Total phosphorus, which is generally found in natural and waste waters in form of organic and inorganic phosphates (PO_4^3), is used as a sign of excessive nutrient increase which may lead to algae proliferation (Amankona, 2010).

The presence of Phosphates in water bodies may originate from sources such as sewage, liquid or solid wastes from industries, phosphates containing pesticides, agricultural run-off (fertilizers), and phosphate containing rocks (Jayalakshmi *et al*., 2011). In nature, the organic phosphates are of importance and are present as loose fragments or particles and also in the tissues of aquatic organisms. Phosphate induces the growth and development of aquatic plants and plankton which serves as food for other aquatic organisms such as the mosquito larvae (Amankona, 2010).

Ecologically, phosphate is a resource that is highly sought after, because of its important role in biological systems. Generally, phosphate is often a limiting nutrient in freshwater environments, and its availability may govern the rate of growth of organisms. However, some phosphorus is not in the molecular form which algae can break down for consumption. In the context of pollution, phosphates are one component of total dissolved solids, which is a major indicator of water quality (Hochanadel, 2010).

2.3.1.10 Potassium

In nature, potassium is found only in ionic salts in water bodies. The compounds of potassium are water soluble since they are ionic. The occurrence of potassium in wastewater may be from urine in sewage or from agricultural runoffs which carry along residues of potassium fertilizers and potassium from dead animal and plant material. The toxicity of potassium in water is often as result of other molecules with which it forms compounds, such as cyanide in potassium cyanide (Water Treatment Solutions, 2012).

The concentration of potassium in the mosquito larvae is 0.5 and 0.6% of dry mass. Irrespective of the changes in the external medium of potassium, the larvae undergo a series of regulations to maintain a constant potassium composition of the haemolymph (Ramsay, 1952). This is because the mosquito larvae use potassium in retaining fluid homeostasis and electrolyte balance through the formation of urine with their Malpighian tubules serving as kidneys. Any distortion in this intricate process of NaCl and KCl balance as well as other solutes during urine formation in the larvae may result in death. Currently, scientists are trying to use renal potassium transport as a new target in manufacturing mosquito insecticides (Rouzer, 2013). Moreover, the mechanism with which the larva regulates its volume is determined by the volume of the haemolymph, which is also dependent on the regulation of potassium and sodium present in the mosquito larva. An increase in the volume of haemolymph causes the body of the larva to stretch or be under tension and this acts as the stimulus that initiates volume

regulation which results in a decrease in larval volume through the excretion of fluid. Conversely, more fluid is maintained when the body wall is not under tension due to the less than normal volumes of haemolymph (Ramsay, 1952).

2.3.2 Microbial Load

Mosquito larvae depend on bacteria as an important source of food. A study conducted by Lindh (2007) showed that mosquito larvae become very small than normal or cannot grow in sterile media or water with added antibiotics. In contrast, a study by Mourya *et al*., (2002) showed a successful larval growth in water containing Tetracycline, even though there was no information given on time the larvae took to develop and on the adult body-size.

Besides the olfactory cues and odours that the female and male mosquitoes respectively depend on for feeding, resident bacteria of the various breeding sites are another probable source of Anopheles semiochemicals. A number of studies have shown that Bacteria themselves as well as volatiles from them have some effect on mosquito behavior. For instance, volatiles produced by some bacteria have shown to function as probable oviposition stimulants or attractants for several mosquito species. In line with this finding, *A. gambiae* gravid females had more eggs laid on water obtained from natural breeding sites containing bacteria and also on wet papers put on soil than on wet paper placed above heat sterilized substrates from the same sources (Lindh, 2007). Total coliform, fecal coliform and *E. coli* all serve as indicators of water contamination. Besides the presence of the indicator bacteria, there may also be other pathogens in the contaminated water. Therefore the level of contamination of any water body is determined by the indicator bacteria (Arroyo Seco Foundation, 2013).

2.3.2.1 Total Coliform

Total coliform is a group of rod shaped, aerobic and facultative anaerobic, nonspore-forming, gram-negative bacteria which ferment lactose with gas formation within 48 hours at an optimal

temperature of 35 ^{0}C (Cooke, 2013b). Coliforms are ubiquitous and can be found on vegetation, in soil, and in the aquatic environment. However, they are present in large numbers in the faeces of warm-blooded animals. They consist of several genera of bacteria of faecal and non-faecal origin such as *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Serratia*, faecal coliform and *Escherichia*. Coliform bacteria are commonly used as bacterial indicator of sanitary quality of foods and water. The use of water for a given purpose can be determined by measuring the number of total coliform present in a sample (Cooke, 2013b). In a study where the midgut flora and oviposition sites of field caught *A. gambiae* S.l. and *A. funestus* midguts were investigated using a culture dependent and a culture independent pathway, Gram negative bacterial species identified included members of the family Enterobacteriaceae (Lindh, 2007). The presence of faecal coliform bacteria in water bodies may indicate that such aquatic environments have been contaminated with the faecal material of humans or other animals. Faecal coliforms can enter water bodies through direct discharge of human sewage or waste from mammals and birds, and from storm and agricultural runoffs (Doyle and Erickson, 2006).

2.3.2.2 *Escherichia coli*

E. coli, comprising of up to 1% of bacterial biomass, is a rod-shaped member of the coliform group of bacterial species that are naturally found in the intestines of warm blooded animals such as humans and birds. *E. coli* are of fecal origin and therefore, unlike the general coliform bacteria group, their presence is thus an effective confirmation of fecal contamination. *E. coli* have an incubation period of 12-72 hours with between 30 ^{0}C – 37 ^{0}C optimal growth temperature (Todar, 2012; CDC, 2013b).

2.3.3 Effect of Seasonal Fluctuations on Mosquito Larval Abundance

Important factors in the transmission of vector-borne diseases include, survival and reproduction rate of the vector, time of year and level of vector activity, also the biting rate and

the rate of development and reproduction of the pathogen within the vector. Vectors, pathogens, and hosts survive and reproduce within certain optimal climatic conditions and changes in these conditions can modify these factors of disease transmission. The most influential climatic factors for vector borne diseases include temperature and precipitation but sea level elevation, wind, and daylight duration are additional important considerations (Patz *et al*., 2000).

Several approaches have been employed to better understand the mechanisms which lead to changes in the natural and controlled mosquito populations. Definitely, fluctuations in weather, overcrowding and competition for survival, the dynamics of natural preying and vulnerability to predators have been most stressed by most approaches to be the cause (Yang *et al*., 2009; Ackerson and Awuah, 2010).

2.3.3.1 Rainy (Wet) Season

In the tropics, rainfall influences malaria. Therefore, in places where the wet season overlaps with high temperatures, the incidence of malaria also tend to increase. Thorough studies have shown that rainfall is a determining factor in malaria transmission since it provides sufficient surface water at mosquito breeding sites which creates favourable conditions for the breeding of mosquito such as the *Anopheles* species (Basommi, 2011).

The intensity, duration and frequency of rainfall determine the presence, persistence and sizes of the habitats mosquito larvae inhabit. Therefore, an increase in precipitation increases the presence, persistence and sizes of larval breeding sites and also causes a fall in temperatures and the magnitude of the larvae's diurnal temperature behaviour in such habitats. However, an increase in precipitation may have negative effects on mosquito populations as it exposes the aquatic stage forms to excessive direct rain drop hits, water currents or flooding. This has definite consequences on the mosquito larvae as they are directly killed or flushed out of their aquatic habitat, or indirectly increasing their chances of survival by flushing out their previously colonized natural enemies. The results of experiments carried out in Kenya under

natural conditions showed that rainfall causes high mortality of mosquito larvae (Paaijmans, 2008).

2.3.3.2 Dry Season

The growth and development rate of the aquatic forms of Anopheline mosquitoes are very much dependent on the temperature of their aquatic habitat, which include shallow, small and tropical water bodies. The upper water layer of these breeding sites absorbs about 40-50% of the solar radiation. Therefore, in terms of growth and development of mosquito larvae, temperature is as important as nutrition and density. The water boundary layers as well as the daily natural variation in atmospheric energy fluxes, to which the larval forms of mosquitoes are exposed in the field, regulate the average and the daily variations in water temperature (Paaijmans, 2008). Though research reveals that mosquito breeding in the rainy season is at definitely higher levels than that of the dry season, the prevalence rates of malaria are relatively at the same levels and high. This indicates that the least activity required by the mosquitoes in the dry season to carry on malaria transmission exceeds the threshold required for definite malaria transmission. This is partly due to the fact that besides breeding sites that receive their water from rainfall, mosquitoes have adapted to breeding in other habitats that receive water from other sources (Olayemi *et al*., 2012). In addition, the survival of vectors are complemented by the easy access to permanent aquatic habitats which ensures that best adapted species like the Anophelines are being naturally selected by these kinds of habitats to sustain generations for the transmission of malaria (Mala *et al*., 2011).

2.4 Health Risks of Mosquitoes in Households in Communities Close to Wastewater Irrigation Sites

According to the WHO guidelines on the safe use of wastewater, excreta and greywater, health impact assessment should be carried out before the development of water resource management

projects such as wastewater irrigated vegetable farming. This is because; even though vector-borne diseases such as malaria, dengue and filariasis are not precisely related to the use of wastewater, should be considered in the endemic regions (WHO, 2006a). Examples of vector-borne diseases in relation to the use of wastewater that may lead to increased populations of disease vectors are indicated in Table 2.1. There have been reports of the potential production of disease transmitting mosquitoes in areas with ongoing wastewater use systems (Mukhtar *et al*, 2003). Studies by Klinkenberg *et al.* (2008) on the impact of agriculture on malaria vectors in Accra-Ghana showed that, malaria transmission was high in areas with ongoing irrigated farming. Similar studies in the city of Kumasi have shown that, over 80% of malaria vectors in the city are as a result of the ongoing irrigated vegetable farms within the city. Consequently, there exist same levels of malaria transmission between communities with irrigated farms and the surrounding areas without irrigated farms (Afrane *et al.*, 2012). Moreover, a considerable amount of money is demanded from citizens to be on mosquito control products and treatment drugs. In the year 2002, the cost of the disease in the country was estimated at US$ 13.51 per household or US$ 2.63 per capita (WHO, 2014; Coleman, 2009). A high transmission rate of greater than 1 case per 1000 and a per capita disease cost of US$ 2.63 would translate to a cost of US$ 4236.58 in a population of 1610867 of Kumasi as at the 2000 population census. In the year 2007, a total of 7451 hospital admissions made for uncomplicated malaria translated into a cost of US$ 19596.13 (supposing the exchange rate remains the same) (GSS, 2006; Coleman 2009). According to the WHO (2006a), protozoan pathogens including the *Plasmodium* species have a disease

burden of $\leq 10^{-6}$ DALY (Disability Adjusted Life Years) loss per person per year. However, this DALY is for treated irrigated water with the quality of $\leq 10^{3}$ *E. coli* per 100 ml, but the quality of the irrigated water was 10^{7} *E. coli* per 100 ml. This therefore predicts a more disease burden of DALY loss per person per year. Moreover, the estimated cost of work days lost to malaria in 2003 was at US$ 8.4 (Coleman, 2009).

Table 2.1: Possible relevant vector-borne diseases associated with wastewater irrigation

Disease	Vector	Relative risk of wastewater use in agriculture	Comments
Dengue	*Aedes aegypti*	Low	Vectors breed in standing water (e.g. tires, cans, bottles, etc.). Present in South-east Asia but not China.
Filariasis	*Culex quinquefasciatus*	Medium	Vectors breed in organically polluted water. Endemic in many countries where wastewater use in agriculture is practised.
Japanese encephalitis	*Culex* spp.	Medium	Vectors breed in flooded rice fields. Endemic in many countries where wastewater use in agriculture is practised.
Malaria	*Anopheles* spp.	Low	Vectors breed in uncontaminated water; 90% of malaria cases occur in Africa. *Anopheles* breeding has been reported from serial waste stabilization ponds.

Source: WHO, 2006b

2.5 Malaria

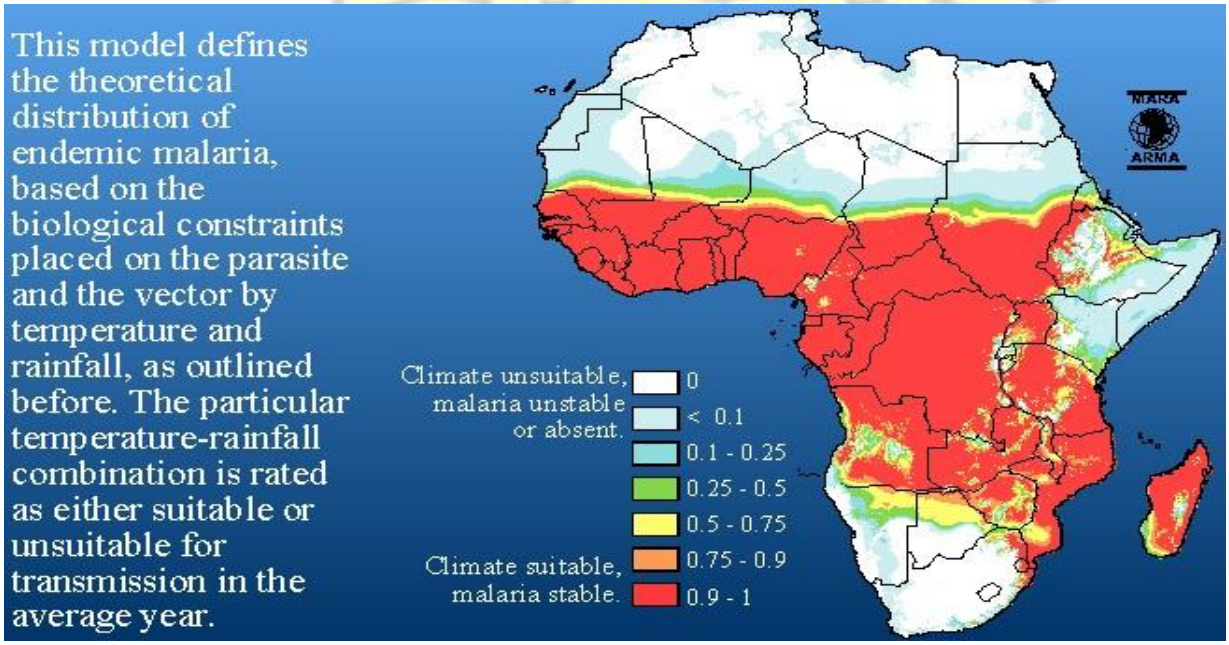
Malaria is a vector-borne infectious disease caused by protozoan parasites of the genus *Plasmodium* (Phylum Apicomplexa). Even though over 200 species of this genus have been identified, only four of these parasite species cause malaria in humans. These malaria causing species include the *Plasmodium vivax*, *P. ovale*, *P. malariae* and *P. falciparum*. Together, *P. falciparum* and *P. vivax*, accounts for up to 95% of malaria diseases diagnosed worldwide. The development and efficiency use of the *Plasmodium falciparum* is dependent on an optimal temperature which is predominant in the warmest areas of the world (CDC, 2012a). The malaria disease persistently affects large number of people worldwide than any other vector borne disease.

2.5.1 Distribution

The disease is far-flung in subtropical and tropical regions in a wideband around the equator, including much of Africa (Figure 2.2), many parts of Asia, and parts of the Americas.

According to the estimations of the WHO's 2014 world malaria report, 3.2 billion people are at risk of exposure to the parasite, with 1.2 billion at high risk, of being infected with the malaria parasites and developing the disease in 97 countries and territories with on-going malaria transmission. The report also showed that, in sub-Saharan Africa alone in 2013, 840 million people were at risk of the malaria transmission. The WHO Sub-Saharan African Region is the most burdened by the disease as the region accounts for about 90% of all deaths caused by malaria and an estimated 78% of all deaths of children below 5 years (WHO, 2014).

Malaria surveillance systems currently do not detect all malaria cases and deaths occurring in a country. These surveillance systems capture only 10% of the annual estimated malaria cases; these are lowest in countries with the highest risks of malaria transmission. The case detection rate in public sector health facilities in 39 of the 99 countries with on-going malaria transmission is less than 20%. As a result, these 30 countries according to the 2012 world malaria report recorded up to 185 million malaria cases, which were about 78% of the estimated worldwide malaria (WHO, 2012).



Source: (MARA/ARMA, 2009)

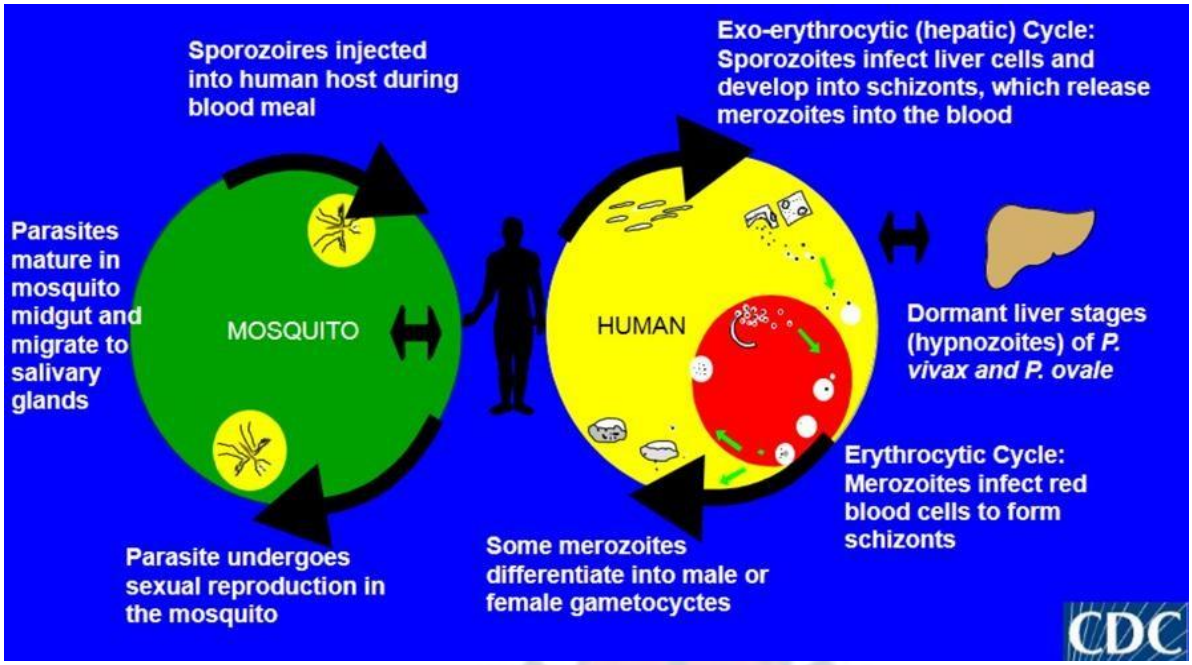
Figure 2.2: Distribution of endemic malaria in Africa

Environmental factors such as land use, temperature, topography, population movements, and rainfall are believed to have an intense impact on the spatial and temporal distribution of malaria vectors and the malaria disease. In spite of its importance, the study of environmental determinants of malaria has been hindered by the complications related to collecting and analyzing environmental data over enormous areas, and by the rate of variation in the malaria epidemiological condition (Coleman, 2009). The complex interactions between the environment, vector, parasite, and host are the key factors in the distribution of malaria. This results in different levels of malaria incidence rates in different areas (HEAT Programme, 2013).

Malaria is linked with poverty but is also a cause of poverty and a chief impediment to economic growth. According to the estimates of WHO (2012), the rates of malaria mortality are highest in countries with higher proportions of their population experiencing poverty (less than US$ 1.25 per person per day). Consequently, prevalence rates of the malaria parasite in children within such countries are highest in the rural areas and among poorer populations (WHO, 2012).

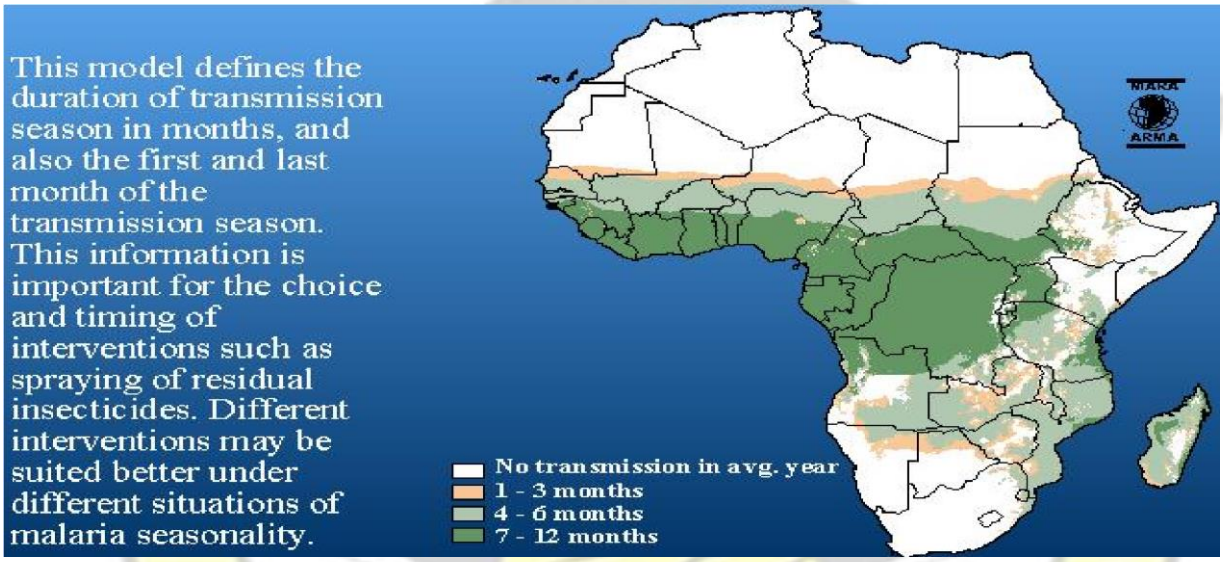
2.5.2 Transmission

Transmission of the disease begins with a bite from an infected female *Anopheles* mosquito vector, which through saliva, introduces the *Plasmodium* parasite into the hosts circulatory system during blood meals (Figure 2.3). The intensity of transmission is determined by factors related to the *Plasmodium* parasite, the malaria vector, the human host, and the environment. The transmission of the disease can be described as being stable or unstable depending on the seasonality (Figure 2.4) of the disease in an area (WHO, 2013).



Source: (Stennies, 2002)

Figure 2.3: Malaria transmission cycle



Source: (MARA/ARMA, 2009)

Figure 2.4: A malaria seasonality model describing the duration of malaria transmission season in Africa

At the community levels, the major way to reduce the transmission of malaria is by vector control. It has proved to be the only malaria control procedure that can reduce the disease transmission almost close to total elimination. Chemoprevention through intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine (SP) is also recommended by the

WHO for population groups, particularly pregnant women and infants, living in high risk malaria transmission areas (WHO, 2012).

Stable Malaria

Malaria transmission is said to be in a stable state when there is little seasonal or annual fluctuations in the occurrence of the disease. Stable malaria is found in warmer areas of the world, such as sub-Saharan Africa, where conditions encourage rapid sporogony associated with *P. falciparum* pathogen. The principal malaria vector species associated with such areas are the *A. gambiae* and *A. funestus.* Stable malaria transmission areas are characterized by a high natural immunity due to the immense levels of exposure to the disease, high infant mortality, and epidemics are unlikely events. Malaria transmission levels in these areas are also very high with some seasonal variations (Sanchez-Ribas *et al*., 2012; Keiser, 2005).

Unstable Malaria

Unstable malaria transmission areas are characterized by lower transmission rates, high potential epidemic incidence and are clearly noticeable with irregular transmission between months and years. The human populations of such areas have lower acquired immunity due to insufficient transmission in the dry season and also because the evenly infection among all age groups (Sanchez-Ribas *et al*., 2012). A study on the effect of irrigation and large dams on the burden of malaria on global and regional scale showed that the introduction of irrigation practices in unstable malaria transmission areas increases the risk of acquiring malaria by the non-immune populations. This may cause a change in the transmission of malaria from seasonal to perennial and therefore from epidemics to endemic (Keiser, 2005).

2.5.3 The Hidden Costs of Malaria

The cost of malaria is either exhibited directly or indirectly in countries where malaria occurs. The direct cost of the disease takes into account all individual and public expenses made on malaria prevention and treatment. For instance in Sub-Saharan Africa, where the disease is endemic, 30-50% of admitted patients and about 60% of outpatient cases are due to the malaria disease, and also takes about 40% of public health disbursement (Coleman, 2009). In 2013, the domestic and international funding for the global elimination and control of malaria was estimated to be US$ 2.7 billion, of which 72% was used in the countries of the WHO African Region alone. According to the 2014 world malaria report, even though the estimated US$ 2.7 billion was almost thrice the amount spent in 2005, it was definitely below the estimated US$ 5.1 billion that was needed to attain the global targets for the control and elimination of malaria. In West Africa, the cost for the control of malaria increased from US$ 89 million in 2005 to US$ 557 million in 2013, outstripping the US$ 4 per capita per year from 2011-2013 of Liberia, Cabo Verde and the Gambia (WHO, 2014). Besides all of the funds from internal bodies, a large portion of the national budget of most developing countries is being used for malaria control and eradication projects. In Ghana, 1-2% of the annual gross domestic product is estimated to be the country's economic burden of malaria (UNICEF, 2007).

Countries that are considered as low income countries are also malaria endemic, as the disease develops vigorously in areas that are impoverished and burdened with weak health systems, and environmental and social difficulties (WHO, 2013). Most families in such areas suffer loss of income as the expenditure for the treatment and prevention of malaria accounts for an average of over one quarter of their income. In Ghana for instance, because there is a high transmission risk of greater than 1 case per 1000 population, most of the citizens spend huge amounts of their income on control products such as malaria treatment drugs and mosquito repellents. The adult population is afflicted with the reduction in working hours and therefore

low productivity, whereas up to 60% of children's education and their general wellbeing are also hampered due to severe and repeated episodes of the malaria disease (UNICEF, 2007; WHO, 2014).

2.5.4 Malaria in Ghana

The global burden of malaria remains enormous, though access to malaria control interventions, especially bed nets in Africa, increased sharply in 2010. In Ghana for instance, health facilities of both the public and private sectors reach only about 60% of the country's population and cutting out a large proportion of children in rural areas from access to basic health care. Furthermore, inconsistency in access to recommended antimalarial drug combinations and rapid diagnostic tests (RDTs) for malaria treatment has also contributed to the endemic status of malaria in Ghana (WHO, 2014; MalariaCare, 2014).

In Ghana, malaria remains the leading cause of morbidity and mortality in children under five years of age. According to the 2014 health facility data of the Ghana Health Service, malaria caused 38% of all outpatient illnesses, 36% of all inpatient admissions and 33% of hospital deaths in children below five years of age. Malaria control activities in Ghana have recently been improved (MalariaCare, 2014). This has been by various Intervention policies and strategies such as the free distribution of ITNs/LLINs to all age groups, therapeutic efficacy tests (clinical and parasitological failure), and financing by interventions from sources like Government, Global Fund, USAID/PMI (President's Malaria Initiative), and WHO. Despite all relentless accelerating efforts towards malaria elimination, Ghana had 1 639 451 reported confirmed cases and a total of 2506 reported deaths in 2013 (WHO, 2014). This is because, in malaria control efforts, the key component is early, accurate diagnosis and rapid treatment with recommended antimalarial drug combinations. However, there has been a very slow progress

by Ghana toward the increase of access to universal malaria diagnostic testing materials and appropriate treatment measures (MalariaCare, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted within the city and peri-urban areas of Kumasi (Figure 3.1), the capital of the largest populated region (4,780,380; 19.4%) in Ghana; the Ashanti Region. Kumasi is the second largest city and one of the fastest growing cities in Ghana with an annual growth rate of 2.7% and an estimated population of 2,035,064 (GSS, 2012). The city is located in the rainforest zone of West Africa and lies between Latitude 6.35^0 – 6.40^0 and Longitude 1.30^0 – 1.35^0 with an average elevation of 270 meters (KMA, 2006). Kumasi lies within the middle belt of Ghana and covers a total area of 225 km², of which about 40 % is open land.

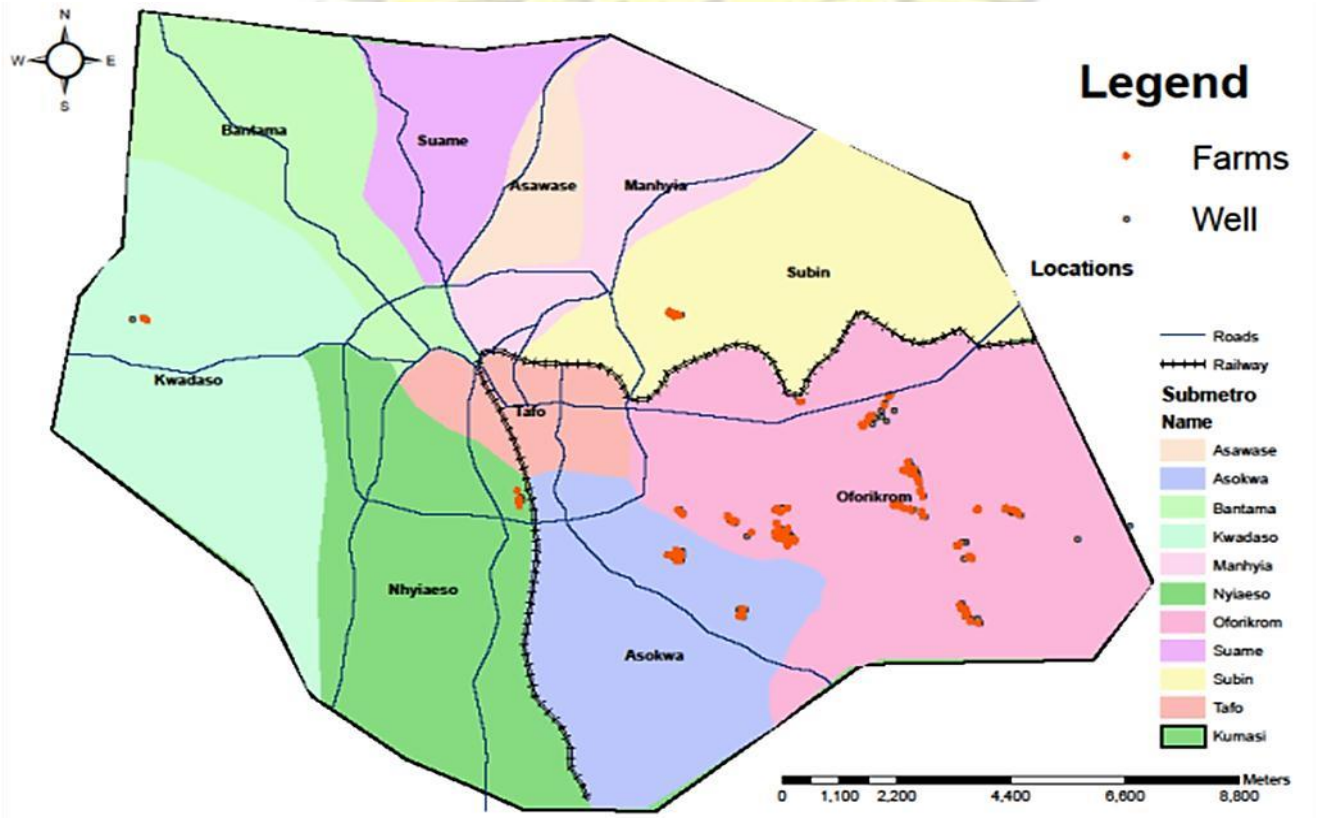


Figure 3.1: A map of the Kumasi metropolitan area showing the locations of wastewater irrigated vegetable farms

There are two major seasons in the metropolis, the dry and rainy seasons. The metropolis experiences two rainy seasons; a long major season from late March or early April to July and a short minor season from late August or early September to November each year with an average temperature range of 24 ^{0}C to 27 ^{0}C over the period (Ackerson and Awuah, 2010). The metropolis also experiences a dry season of about two to four months of no rainfall with temperatures of between 23 ^{0}C and 31 ^{0}C annually. Kumasi has a semi-humid tropical climate and an annual average rainfall of 1420 mm within the average 7 months of the rainy season (Ackerson and Awuah, 2010).

3.1.1 Wastewater Irrigation in the Urban Zone of Kumasi

The main drainage system in the city is an open sewer, which runs from north to south of the city and discharges into the Daban, Subin, Aboabo, Wiwi and Sisa rivers which flows into the Oda river about 9 km south of Kumasi. In effect, people who make use of these rivers from a number of miles downstream are the ones adversely affected (KMA, 2006). Many of the periurban areas within a 40 km radius of the city are engaged in widespread small scale vegetable (e.g., spring onions, lettuce, cabbage, and green pepper) farming in both the rainy and dry seasons. The areas which receive continuous water supply from close perennial rivers always have higher scale of farming operations. However, farmers at areas without such water supply obtain water either from shallow hand-dug wells or from fleeting streams which form several pools in the dry season (Cornish *et al*., 1999).

3.2 Study Site

Through a survey, all 16 market oriented wastewater irrigated vegetable farms located near human settlements identified, were considered for this study so as to provide a representation of the larval populations in the irrigation waters (mosquito breeding) within the Kumasi metropolis. Due to the close proximity of some of the farm sites, the farms were categorized into groups of eight vegetable farming sites as represented in Table 3.1. All these farm sites, apart from the farms at Georgia which were located on a slope of land, were located on low land areas. Gyinyase, Kentinkrono and some parts of Pokusika also have some farms located on slopes.

Table 3.1: Wastewater irrigated vegetable farms, their location and source of irrigation water

Site Name	Sub-Metro	Description
Ayeduase	Oforikrom	Farms are located at Ayeduase, Kotei, D and D, Deduako and Boadi. Source of irrigation water is stream
Appiadu/ Apemso	Oforikrom	Farms are located at Apemso and Appiadu. source of irrigation water is pond and stream
Georgia	Asokwa	Farms located close to Georgia hotel, Ahodwo. Source of irrigation water is a stream. It drains most liquid wastes of surrounding industries.
Gyinyase	Asokwa	Farmers on the slopes use pipe born water and ponds whereas those on low level lands uses ponds and a stream
Kentinkrono	Subin	Farms located on slopes and rely on ponds and a stream in the valley
Nima	Asawase	Farms located at Aboabo. Farms are close to refuse dump. Source of water is ground water.
Poku Sika	Oforikrom	Farms are located within KNUST campus. Source of irrigation water is a stream and ponds.
Ramseyer	Asokwa	Farms located within the Presbyterian Vocational Institute, Chirapatre. Source of irrigation water is ponds, pumped well, and a stream.

3.3 Sampling Sites

All the streams, rivers, ponds and dugout-wells, as well as all other various water bodies (e.g. furrows and storm drains) on the farm sites used by the farmers for irrigation purposes were sampled. All forms of shallow waters bodies created as a result of footprints, overflowed water during irrigation and the overflow of water from streams, rivers, ponds or dugout wells after rainfalls were recorded as furrows. All the shallow hand dug wells were also considered as ponds.

3.4 Sampling Methods

All samples were collected between the months of December 2013 and December 2014 on weekly basis. Samples were collected early in the mornings between the hours of 06:30 and 10:00 GMT each day of sampling before the water bodies become disturbed by the farmers during farm activities. Samples were collected from any water body, clear or dirty, with or without vegetation, with or without aquatic animals and with or without odour. Sterile containers were used for the collection and storage of samples. Hand gloves were worn during sample collection. Dry season samples were collected within the months of December 2013 and March 2014, whereas the wet season samples within the months of April 2014 and July 2014. During the rainy season, samples were not collected on the days with early morning rainfalls and on the days after a day's rainfall.

3.4.1 Sampling Mosquito Larvae

Standard white 100 ml, 150 ml and 350 ml dippers, depending on the size and depth on the breeding site, were used in collecting larvae from water body identified as a positive breeding site. The number of dips was dependent on the size of the water body and consequently the kind of dipper used. Much care was taken during each dip in order not to disturb the breeding

habitat. All collected larvae were immediately preserved in 100% ethanol and transported to the laboratory for larval identification and enumeration. The species were determined morphologically, using the identification features described by CDC [2004] (Table 3.2), first with a hand lens on field before preservation and then further examined microscopically in the laboratory. The microscopic examination was used in the identification of the *Culex* and *Aedes* species.

Table 3.2: Morphological identification features of the larval forms of *Anopheles, Culex* and *Aedes* mosquitoes

Larvae	Features
Anopheles	Rest parallel to water surface. Lack siphon on the eighth abdominal segment. Palmate or float hairs are present on at least some abdominal segments.
Culex	Rest at an angle to the water surface. Presence of a short, stout siphon on the eighth abdominal segment. Pectin present. Several pairs of tufts (basal tufts absent) or scattered single hairs occur along each side of the siphon.
Aedes	Rest at an angle to the water surface. Long, slender siphon on the eighth abdominal segment. Pectin present. Only one pair of siphonal tufts or hairs is present. Ventral brush is attached posteriorly to the saddle plate. Saddle does not encircle the anal segment completely (in most species).

Source: CDC, 2004

3.4.2 Irrigation Water Sampling

Representative samples of irrigation water were collected from all the breeding habitats from which mosquito larvae were collected. All irrigation water samples were collected using sterile 1.5 L bottles/ containers, and well labelled and preserved on iced packs for physicochemical and microbial analysis.

3.5 Laboratory Analysis

3.5.1 Physico-Chemical Parameters

All the physical parameters, pH, Temperature, Salinity, Total dissolved solids and Electrical Conductivity (EC), were analyzed in-situ using a DO meter (HANNA® instruments/H9828). Dissolved oxygen (DO), a chemical water quality parameter, was also measured in-situ in the field using the same DO meter. For the breeding habitats that were too small in size or shallow, the parameters were analyzed from collected water containing the larvae. This was done before the larvae were preserved in 100% ethanol. All the chemical parameters, Nitrite (NO_2^-), Ammonia (NH_4^+), Potassium ion (K^+) and Phosphorus (P) were also measured in the laboratory from the irrigation water collected from all the breeding sites.

3.5.1.1 Potassium

Potassium ion in the water samples was determined by the triplicate analysis method using a flame photometer [Jenway PFP7] (Jenway, 2013). A stock solution of 100 mg/L Potassium ion was prepared from a pure compound of Potassium (KCl). Three serial standards of 1, 2 and 3 mg/L concentrations were prepared from the stock solution. Distilled water was aspirated to blank the flame photometer. Each of the serial standard concentrations was aspirated starting from the least (1 mg/L) and readings were noted from the photometer. The water samples, marked as unknown were then aspirated and their readings recorded. Calibration curves were plotted using concentrations as abscissa and flame photometer readouts as ordinate values (Appendix 1.1). Concentrations of potassium ions in the water samples were calculated from the calibration curves.

3.5.1.2 Phosphorus

Phosphorus in the water samples was determined by the triplicate analysis method using a spectrophotometer [Optima SP-300] (Tsang, 2006). Reagents that were used included 0.1M

Ascobic Acid (AA), 4% Ammonium Molybdate (AM) solution, 2.5 M Sulphuric Acid (H_2SO_4) and 0.28% Potassium Antimonyl Tatrate (PAT). From the above reagents, a working colour developing reagent (CDR) was prepared (50 ml H_2SO_4 + 5 ml PAT + 30 ml of AM and of 15 ml AA). Three serial standard concentrations (1, 2 and 3 mg/L) of phosphate compounds were prepared from a stock solution of 100 mg/L, prepared from a pure compound of Sodium Hydrogen Phosphate (Na_2HPO_4).

The same quantity (0.25 ml) of distilled water, irrigation water samples and each of the serial standard solutions were pipetted into well labelled sterile cuvettes, and with the exception of the distilled water, 2.5 ml of CDR was added to each. The solutions were incubated at room temperature for 20 minutes. Absorbance of the solutions was then read at 770 nm on the spectrophotometer and values recorded. A standard curve was plotted from the standard values and an equation, as represented in Appendices 1.2 and 1.3, was generated from the curves. From the generated equations (Appendices 1.2 and 1.3), the concentrations of the water samples were calculated.

3.5.1.3 Nitrite

Nitrite in the water samples was determined by the Wagtech WTD Nitriphot method which is based on a calorimetric procedure using an iodide containing reagent system (Wagtech, 2013). The water samples were first filtered to obtain a clear solution whenever deemed necessary. Using a sterile measuring syringe, 1 ml of each sample was taken and transferred to a sterile test tube. The samples in the test tubes were then topped up to the 10 ml mark of the tubes with deionised water. One Nitriphot No 1 tablet was then added to the samples, crushed and mixed to dissolve. One Nitriphot No 2 tablet was then added to the samples, crushed and mixed to dissolve and capped immediately. Using a timer, the samples were left to stand for exactly two minutes to allow full colour development. Any further colour development after the thus time was ignored. This is because the intensity of the colour produced in the test is proportional to

the nitrite concentration in the water samples. Phot 43 on the Wagtech WTD Automatic Wavelength Selection Photometer was selected and the photometer readings were then taken and recorded as mg/$NaNO_2$.

3.5.1.4 Ammonia

Ammonia in the water samples was determined by the Wagtech WTD Ammonia test which is based on an indophenol method (Wagtech, 2013). Ten milliliter (10 ml) round glass (PT 595) test tubes was filed with the water samples to the 10 ml mark. One Ammonia No 1 tablet and one Ammonia No 2 tablet were added to each of the samples, crushed and mixed to dissolve. The samples were then left to stand for 10 minutes to allow colour development. The intensity of the colour produced in the test is proportional to the ammonia concentration in the water samples. The required wavelength (Phot 4) on the Wagtech WTD Automatic Wavelength Selection Photometer was selected to measure the ammonia (ammonical nitrogen) concentration in mg/L N and the results were then recorded.

3.5.2 Microbial Analysis

The presence of *E. coli* and total coliforms was determined using the Colisure-Quantitray technique according to the manufacturer's instructions (IDEXX, 2013). Ten milliliter (10mL) of the water samples was added to 90 ml of sterile non-buffered oxidant-free water in sterile conical flasks. A pack (2.43 g) of IDEXX dehydrated media (Colisure reagent powder) was then added to the sample and gently mixed thoroughly. The dehydrated media were allowed to dissolve completely and the resulting mixture was then poured carefully into the quantitrays (48 large x 48 small wells). The quantitrays were then heat sealed using the quantitray sealer and incubated at 36 ± 0.5 ^{0}C for 24 hrs.

Source: IDEXX, 2013

Plate 3.1: A Picture of a 48 x 24 well IDEXX Quanti-Tray

After the incubation period, the positive and negative wells were read according to the result interpretation table (Table 3.3) below.

Table 3.3: Result Interpretation for Incubated Irrigated Water Samples

Appearance	**Result**
Yellow/gold	Negative for total coliforms and *E. coli*
Red or magenta	Positive for total coliforms
Red/magenta and fluorescence	Positive for *E. coli*

The presence of *E. coli* in the samples was determined by passing a 365nm wavelength UV light within 12.7 cm of the sample in a dark environment. This was done to look out for red/magenta wells with fluorescence under the UV light. The Most Probable Number (MPN) for both the total coliforms and *E. coli* was quantified by counting all the positive wells and reference made to an IDEXX Quanti-Tray MPN table (Appendix 4) to obtain the Most Probable Numbers. This was done by matching the number of large positive wells against the number of small positive wells on the MPN table and the value recorded.

3.6 Diversity of Larval Mosquito Species at the Irrigated Fields

The Simpson's Index of Diversity (1-D) approach (Offwell, 2009) was used to quantify the variety (biodiversity) of larval mosquito species at the breeding habitats on the farm sites. The value of this index ranges between 0 and 1; the greater the value, the greater the sample diversity. The Simpson's Index of Diversity takes into account the richness and evenness of the larval species. The species richness measures the number of different kinds of larval mosquito species present at a specific breeding habitat on the farm sites. The species evenness measures the relative abundance of the different larval mosquito species that make up the richness of the farm and breeding sites. The Simpson's Index of Diversity has been used in determining the probability that any two individuals randomly selected from a sample will belong to the different species. The Simpson's Index of Diversity was therefore calculated using the following formula;

$$1 - D = 1 - \sum \frac{n(n-1)}{N(N-1)}$$

With D being the Simpson's Index, n =total number of larvae of a particular species, and

N = the total number of larvae of all species.

CHAPTER FOUR

RESULTS

A total of 9823 mosquito larvae, representing three genera, were collected from the study sites over a period of eight months. These three genera, the *Anopheles*, *Culex* and *Aedes* (Plate 4.1), were enumerated from furrow, pond and stream breeding habitats identified on the irrigation fields. Out of the total number of the collected larvae, 5632 (57.3%) were *Culex*, 3000 (30.5%)

were *Anopheles* and 1191 (12.1%) were *Aedes* (Figure 4.1). Mean values of 704 ± 181.01, 375 ± 88.78, and 148.88 ± 53.52 for *Culex*, *Anopheles* and *Aedes* respectively were also recorded.

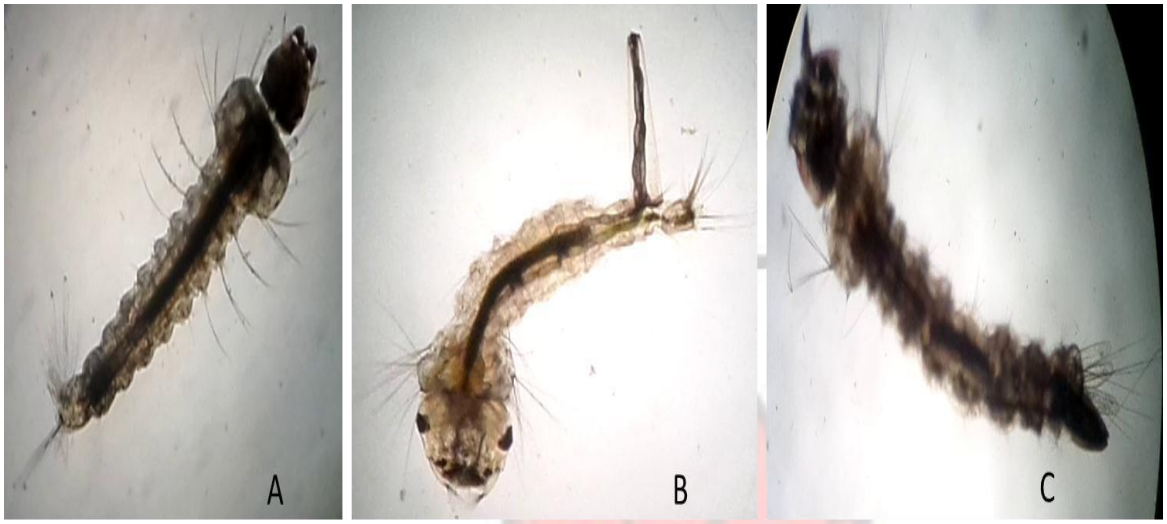


Plate 4:1: Pictures of the larval forms of *Anopheles* (A), *Culex* (B), and *Aedes* (C) as seen under scan (X10) lens of the light microscope

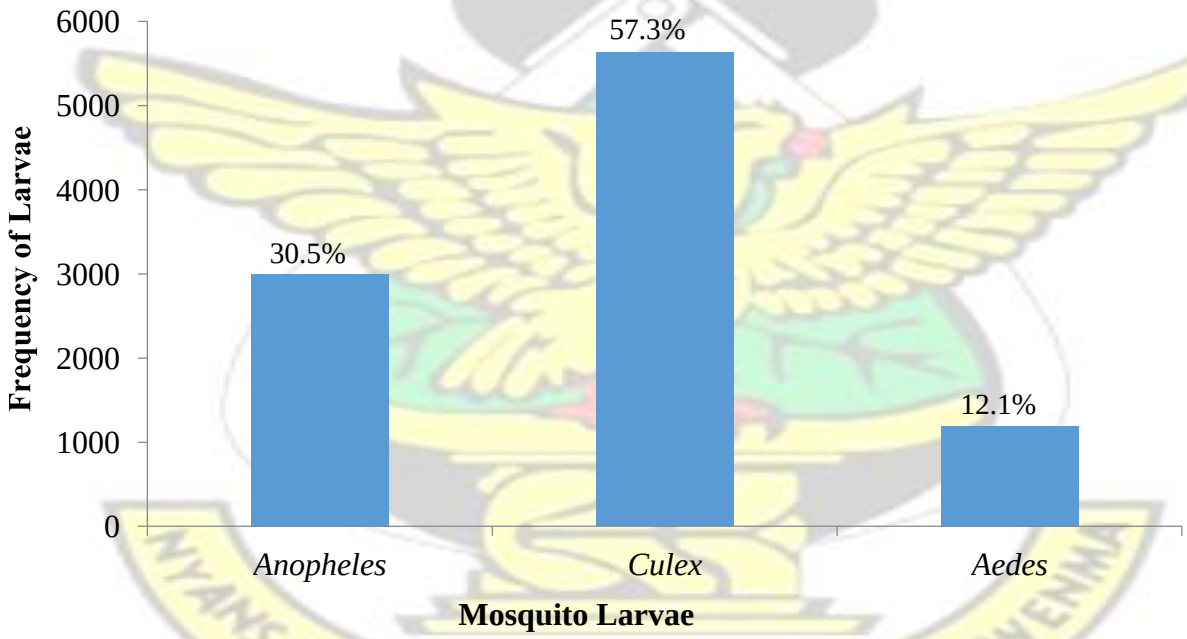


Figure 4.1: Distribution of *Anopheles, Culex* and *Aedes* mosquito larvae from the study area

4.1 Occurrence of Larval Mosquitoes in Irrigated Farm Sites

Culex larvae were collected from all the eight study sites whereas *Anopheles* and *Aedes* were collected from all the study sites with the exception of Georgia and Nima respectively. As represented in Appendix 2.1, Ayeduase recorded the highest number of mosquito larvae (2657;

27%), whereas Georgia recorded the lowest (81; 0.8%). As shown in Figure 4.2, Ayeduase recorded the highest numbers of *Culex* (1630) and *Aedes* (432), but not for *Anopheles*. Apemso/Appiadu recorded the highest number of *Anopheles* (669), followed by Kentinkrono (635) and then Ayeduase (595). Georgia again recorded the lowest number of *Culex* (76).

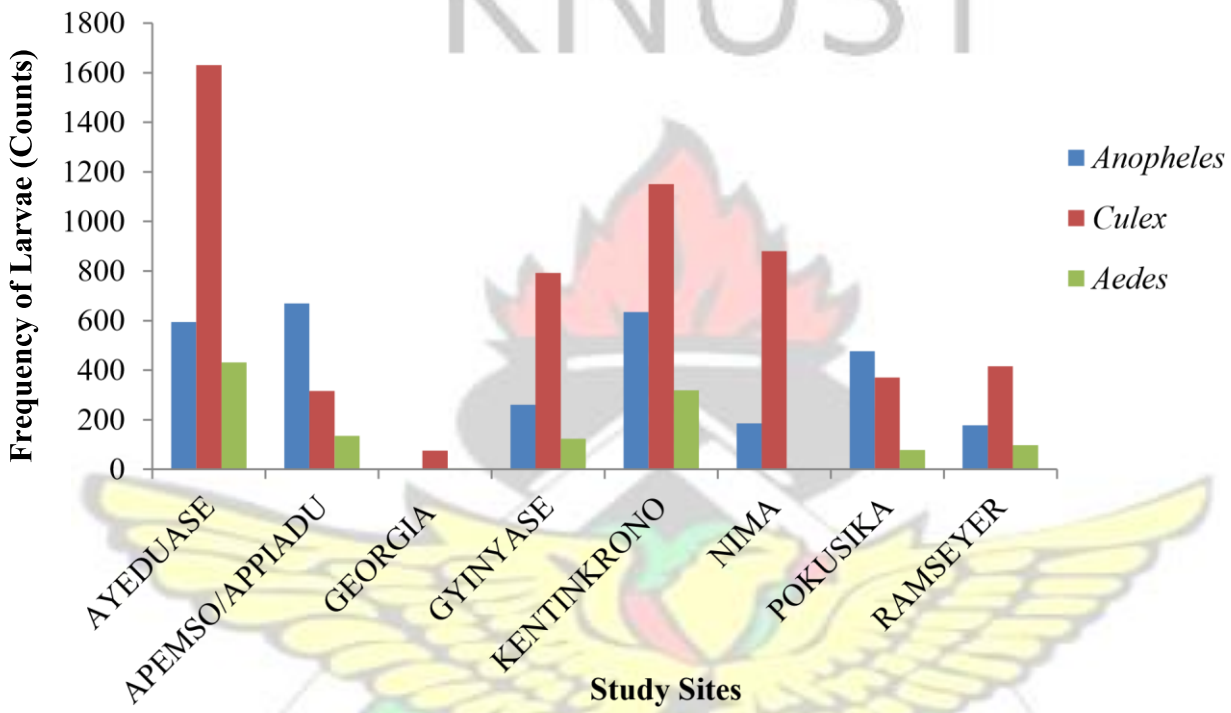


Figure 4.2: Distribution of *Anopheles, Culex* and *Aedes* mosquito larvae at the study sites

4.1.1 Seasonal Variation of Larval Mosquitoes in Irrigated Farm Sites

All the three larval mosquito species were present both in the wet and dry seasons, and their relative abundance is represented in Figure 4.3; *Culex* was abundant in both the wet (36.25%) and dry (21.08%) seasons than the other species. This was followed by the *Anopheles* (21.89% and 8.65%) and then the *Aedes* (4.85% and 7.28%). *Culex* and *Anopheles* were abundant in the wet season than in the dry season, whereas *Aedes* were abundant in the dry season than in the wet season.

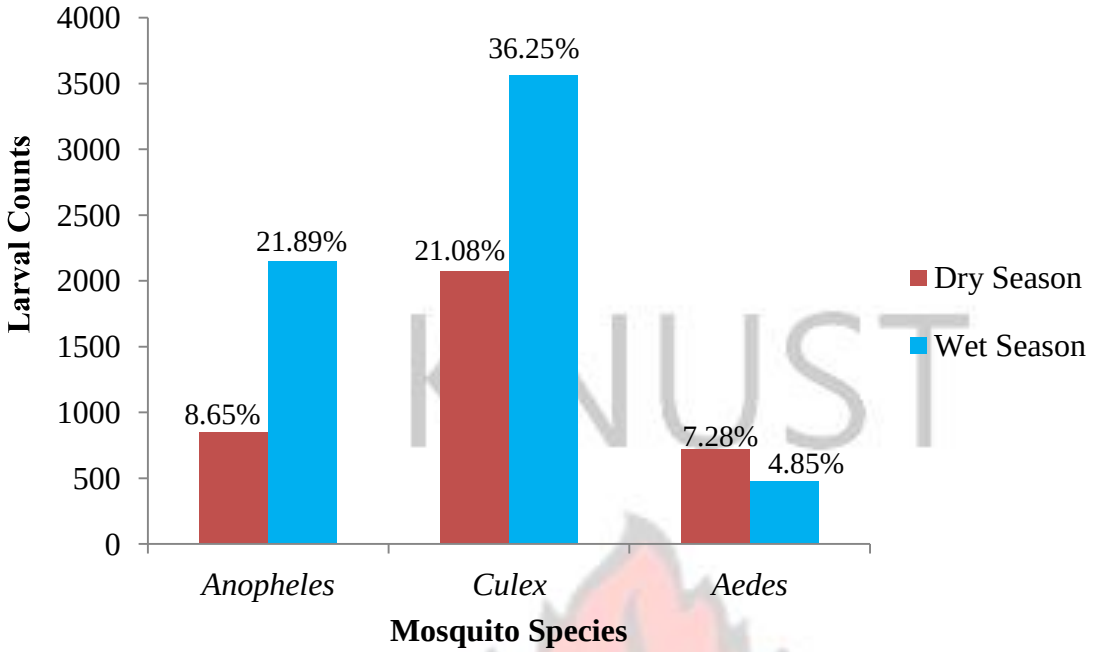


Figure 4.3: Occurrence and seasonal variation of larval mosquito species at the farm sites

The Simpson's Index of Diversity for the identified species at the farm sites is represented in Table 4.1. The index at the study sites ranged between 0.23 and 0.59 for the wet season, whiles it ranged between 0.00 and 0.62 in the dry season (Appendix 2.3). As shown in Appendix 2.4, Pokusika and Ayeduase relatively had the highest indexes for the dry season, whereas Kentinkrono and Ramseyer had the highest indexes for the wet season. Nima, followed by Georgia, all in the dry season, recorded the lowest indexes of 0.00 and 0.12 respectively.

Table [1].1: Simpson's Index of Diversity of the larval species at the farm sites

Farm Site	Simpson's Index of Diversity	
	Wet Season	**Dry Season**
Ayeduase	0.458	0.613
Apemso/Appiadu	0.393	0.535
Georgia		0.117
Gyinyase	0.419	0.496
Kentinkrono	0.588	0.434
Nima	0.279	0.000
Pokusika	0.500	0.618
Ramseyer	0.562	0.540

[1].2 The Association between Mosquito Larvae and Breeding Habitats or Sites

It was observed that, the main source of irrigation water differed from one farm to another. However, all the farmers employed practices such as the use of pipe borne water and deep wells with pumps, together with the use of water from ponds, hand dug wells and streams or rivers. Some streams are also blocked with sand bags at vantage points to impound enough water for irrigation purpose.

A total of 139 breeding habitats (sampling points) were recorded during the study period. These habitats were composed of furrows (Figures 4.4 and 4.5), ponds (Figure 4.6) and streams (Figure 4.7). The most frequent breeding site was furrow, followed by pond and stream, with frequencies of 77 (55.4%), 57 (41%) and 5 (3.6%) respectively.

Figure 4.4: Conduits of furrows between raised vegetables beds which serve as breeding habitats for mosquitoes

Figure 4.5: Examples of other water bodies on a vegetable farm considered as furrows which serve as breeding habitats for mosquitoes

Figure 4.6: Examples of ponds on vegetable farms which serve as breeding habitats for mosquitoes

Figure 4.7: A stream on a vegetable farm which serve as a breeding habitat for mosquitoes

As represented in Figure 4.8, Ayeduase recorded the highest number of furrows (17), ponds (19) and streams (3). With the exception of Ayeduase, Apemso/Appiadu and Gyinyase, there were no larval counts in streams at the remaining five farm sites. Georgia recorded the least number of furrows (1) and pond (1). Furrows recorded the highest occurrence of larvae, (5543;

56.41%), followed by pond with 3884 (39.5%) and then stream with 396 (4%). As represented in Figure 4.9, furrows recorded the highest numbers of *Anopheles* (2232) and *Aedes* (2495) larvae, but pond recorded the highest numbers of *Culex* larvae (2931). Stream recorded the lowest numbers of all the three kinds of mosquito larvae. The occurrence of *Anopheles* larvae were very abundant in furrows (2232) than that of both pond and stream combined (787).

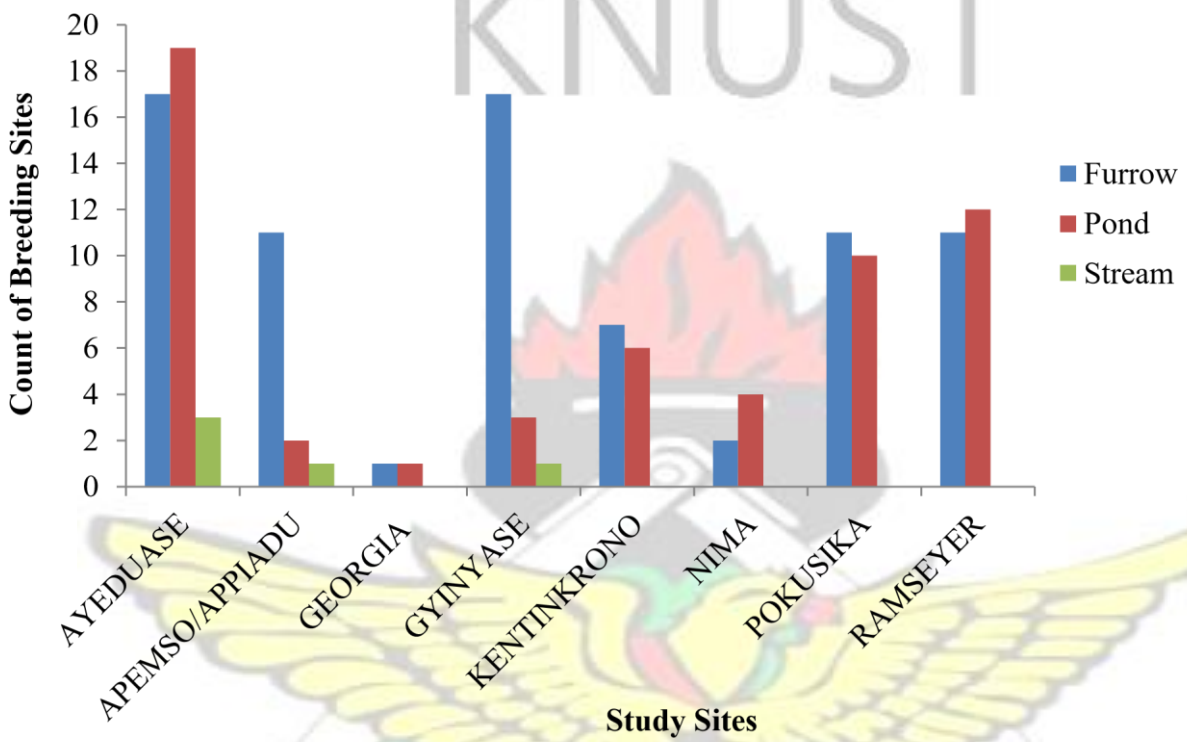


Figure 4.8: Occurrence and distribution of breeding sites at the study sites

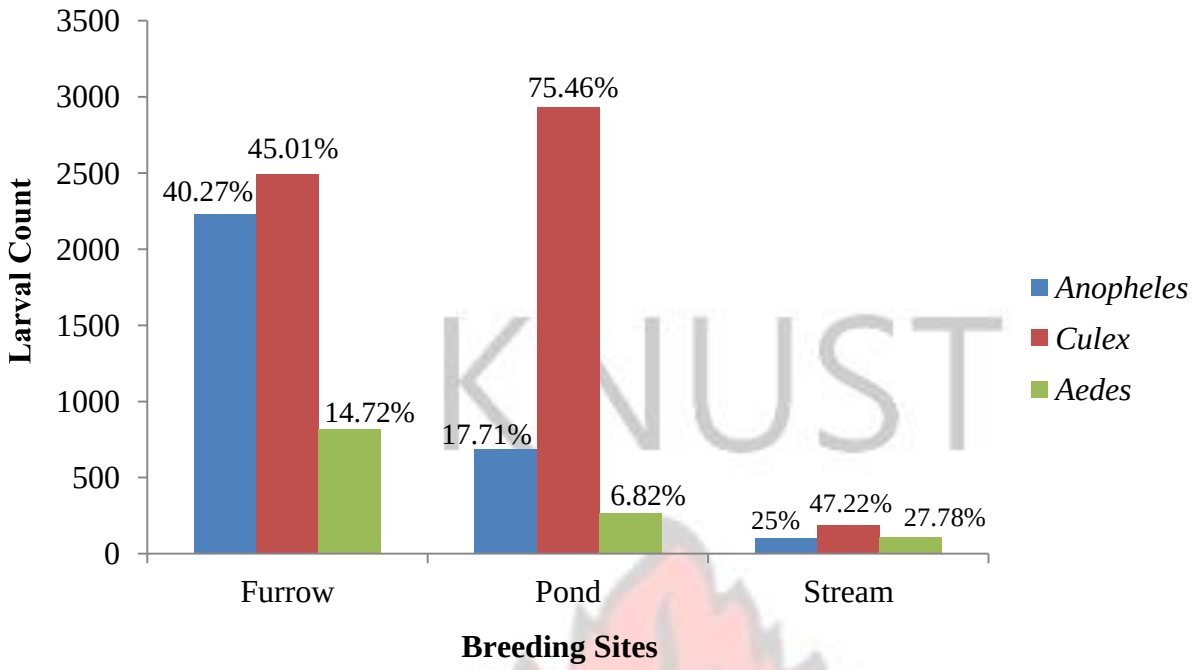


Figure 4.9: Frequency of *Anopheles, Culex* and *Aedes* larvae at the main breeding sites of mosquitoes

4.2.1 The Occurrence and Seasonal Variation of Larval Breeding Sites

The occurrence of the larval breeding sites for both the wet and dry seasons are represented in Figure 4.10. Furrow was prevalent in both the wet (30.94%) and dry (24.46%) seasons than the other breeding sites (Figure 4.10). This was followed by pond (20.86%; 20.14%) and then stream (3.60% for dry seasons). There was no larva found in streams at the sites during the wet season. Moreover, stream recorded the lowest number of habitats in the dry season. The pond breeding habitats was only one count higher in the wet season than the dry season.

As shown in Table 4.2, the Simpson's Index of Diversity for the identified species at the breeding sites ranged between a minimum of 0.29 in the wet season and a maximum of 0.64 in the dry season. Stream had the highest index, whereas pond had the lowest index.

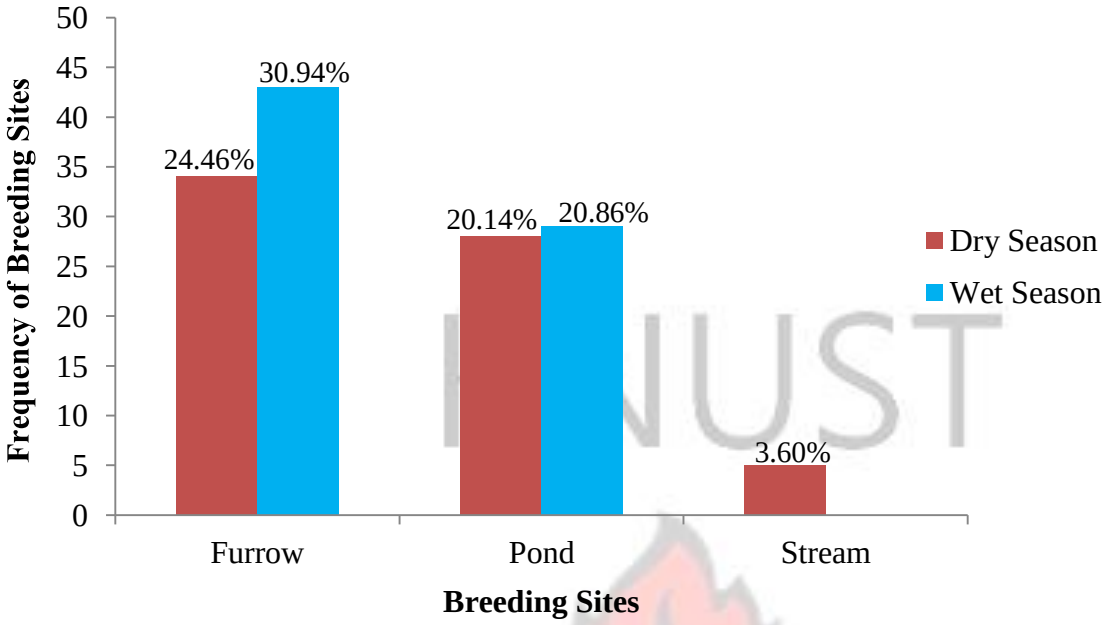


Figure [1].10: Occurrence and seasonal variation of larval breeding sites

Table [2].2: Simpson's Index of Diversity of the larval species at the breeding sites

Habitat	Simpson's Index of Diversity Breeding	
	Wet Season	Dry Season
Furrow	0.583	0.552
Pond	0.294	0.621
Stream		0.639

[1] .3 Quality of Water in which Mosquito Larvae Bred

[2] .3.1 Seasonal Variations of Physicochemical Characteristics

A summary of the descriptive statistics of the microbial and physicochemical characteristics at all the breeding habitats within the farm sites for both wet and dry seasons is shown in Table 4.3. The three sampling sites varied in the levels of physicochemical characteristics. As shown Table 4.3, furrow recorded relatively higher levels of DO (6.54 mg/L), EC (402 µS/cm) and potassium (29 mg/L), whereas pond recorded higher levels of salinity (0.32

mg/L) and nitrite (0.05 mg/L). Stream also recorded relatively higher levels of phosphorus than all the other sampling sites. However, pH, TDS and temperature varied slightly among the three sampling sites. Total dissolved solids recorded a minimum concentration of 60 ppm in furrow and a maximum of 705 ppm in pond, whereas pH ranged from a slightly acidic level of 5.39 to a maximum basic level of 10.56, all recorded in pond. Temperature also recorded a minimum level of 21 ^{0}C in pond and a maximum level of 38.12 ^{0}C in furrow. As represented in Table 4.3, wet season recorded high concentrations of EC, potassium, pH and DO than that of the dry season. On the other hand, dry season relatively recorded higher concentrations of TDS, ammonia, phosphorus, nitrite and salinity than the wet season. However, the mean concentration of temperature for both the wet and dry seasons was the same, 30 ^{0}C with respective standard errors of ±0.30 and ±0.48.

Furthermore, the mean levels and range of physicochemical characteristics of water among the breeding sites for both wet and dry seasons are shown in Table 4.3. No larvae were found in the streams at the various farm sites during the sampling period for wet season. With the exception of TDS, nitrite and ammonia, furrows in the wet season recorded high levels of physicochemical characteristics than that of the dry season (Table 4.3). Conversely, with the exception of DO, pH and potassium, ponds in the dry season recorded high levels of physicochemical characteristics than that of the wet season.

Table 4.3: Mean (± S.E.) and range (in parentheses) of physicochemical and microbial characteristics of water samples in breeding habitats within the farm sites for both the wet and dry seasons

Physicochemical and Microbial Characteristics	Season – Habitat					WHO Recommended Range of limits for irrigation wastewater (per 100ml)
	Wet		Dry			
	Furrow	Pond	Furrow	Pond	Stream	
DO (mg/L)	**6.54±0.36** (1.62-10.67)	**5.79±0.63** (0.1-12.25)	**1.11±0.10** (0.01-2.36)	**1.13±0.15** (0.06-3.41)	0.62±0.21 (0.09-1.15)	
pH (pH unit)	**9.22±0.10** (7.89-10.45)	**9.33±0.13** (8.41-10.56)	**7.43±0.09** (5.97-8.22)	**6.97±0.11** (5.39-7.76)	**7.04±0.20** (6.45-7.71)	6.5-8.4
EC (µS/cm)	**402±28.63** (119-898)	**334.45±22.39** (142-649)	**389.79±29.83** (100-916)	**365.79±43.63** (102-1073)	**183.2±20.18** (135-248)	700-3000
TDS (ppm)	**200.37±14.30** (60-450)	**185.24±14.69** (71-345)	**201.65±14.58** (35-458)	**202.21±27.41** (78-705)	**107±6.41** (89-125)	450-2000
Salinity (mg/L)	**0.21±0.01** (0.05-0.44)	**0.18±0.02** (0.06-0.45)	**0.19±0.02** (0.03-0.45)	**0.32±0.07** (0.07-1.24)	**0.08±0.01** (0.06-0.12)	
Temperature (^{0}C)	**30.56±0.35** (26.9-38.12)	**29.89±0.55** (21.65-35.12)	**29.79±0.66** (23-37)	**30.05±0.79** (23-38)	**27.74±1.04** (24.9-30.1)	≤ 40
Nitrite (mg/L)	**0.01±0.01** (0-0.34)	**0.05±0.05** (0-1.4)	**0.03±0.01** (0-0.17)	**0.06±0.04** (0-1)	**0.004±0** (0-0.01)	1.0
Ammonia (mg/L)	**2.55±0.36** (0.05-8.4)	**1.12±0.30** (0.04-6.3)	**3.02±0.51** (0.01-8.9)	**3.21±0.52** (0.05-9)	**2.82±1.03** (1.1-6.8)	
Potassium (mg/L)	**29.28±2.68** (7.08-73.26)	**21.85±2.73** (8.86-65.02)	**14.85±1.64** (3.06-41.56)	**19.55±2.65** (5.24-64.38)	**11.73±2.16** (7.35-19.82)	
Phosphorus (mg/L)	**1.33±0.08** (0.65-2.63)	**1.21±0.09** (0.71-2.69)	**1.11±0.21** (0.11-5.1)	**1.55±0.3** (0.15-6.13)	**2.09±0.91** (1.05-5.73)	0.1-3.0

Total Coliforms ($\times 10^7$) MPN	**9.83±0.88** (2.3-32.8)	**13.57±3.10** (1-87.8)	**96.89±70.56** (1-2419.6)	**47.7±9.02** (2-181.1)	**54.94±11.54** (30.9-93.4)	$<10^6$
E. coli ($\times 10^7$) MPN	**3.3±0.27** (1-8.5)	**4.07±0.57** (1-12.3)	**3.12±0.75** (1-23.1)	**3.39±0.67** (1-14.5)	**3.06±0.74** (1-5.2)	$\leq 10^5$

4.3.2 Microbial Characteristics

A total of 138 water samples from all the study farm sites were examined for the presence of total coliforms and *E. coli* (Figure 4.11). As shown in Appendices 2.5 and 2.6, respectively, total coliforms varied from a minimum of $1x10^7$ MPN /100ml to a maximum of 181.1×10^7 MPN/100ml, whereas *E. coli* also varied from a minimum of 1×10^7 MPN/100ml to a maximum of 23.1×10^7 MPN/100ml among the study farm sites. Georgia recorded the highest mean ($74.15\times10^7 \pm 37.75\times10^7$ MPN/100ml) for total coliforms, whereas Nima recorded the lowest mean ($8.917\times10^7 \pm 1.52\times10^7$ MPN/100ml).

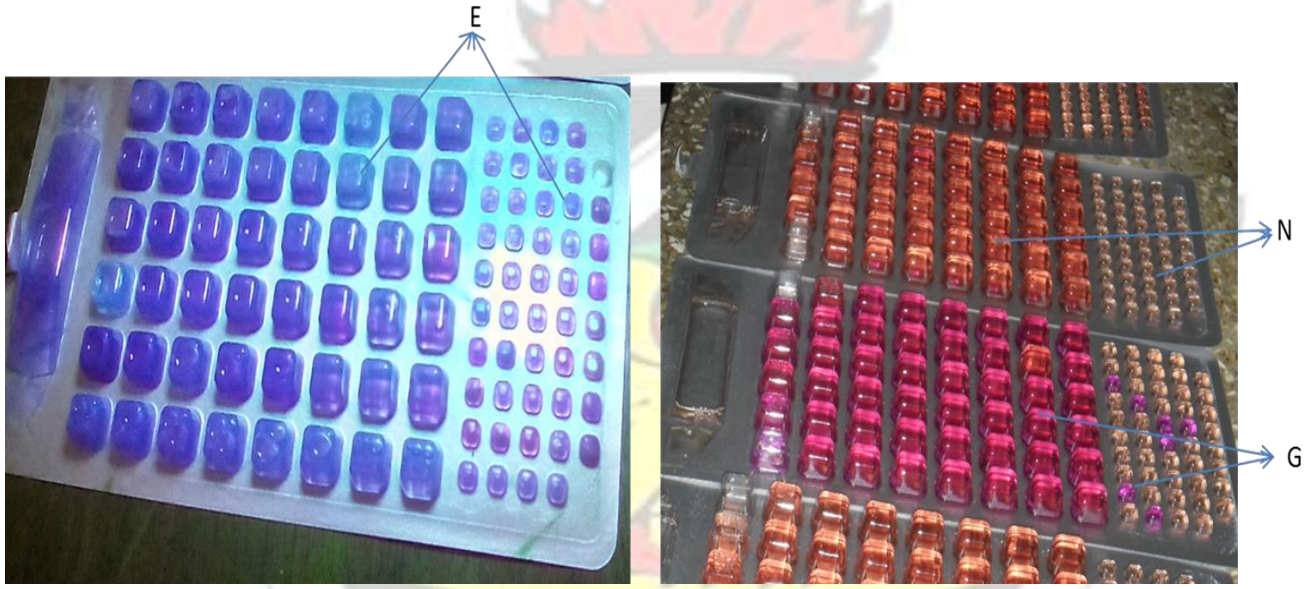


Figure 4.11: Wells of Colisure-Quantitrays showing the presence of *E. coli* (E) as wells fluoresce under a 365nm wavelength UV light, the presence of total coliforms (G), and no microbial growth (N) after incubation

The mean numbers of total coliforms and *E. coli* at the various farm sites and sampling sites are represented in Figures 4.12 and 4.13 respectively. Stream recorded the highest numbers of total coliform ($54.94\times10^7 \pm 11.54\times10^7$ MPN/100ml), whereas pond recorded the lowest numbers ($30.33\times10^7 \pm 5.19\times10^7$ MPN/100ml). Conversely, pond recorded the highest numbers of *E. coli* ($3.74\times10^7 \pm 0.43\times10^7$ MPN/100ml), whereas stream recorded the lowest numbers ($3.06\times10^7 \pm 0.74\times10^7$ MPN/100ml).

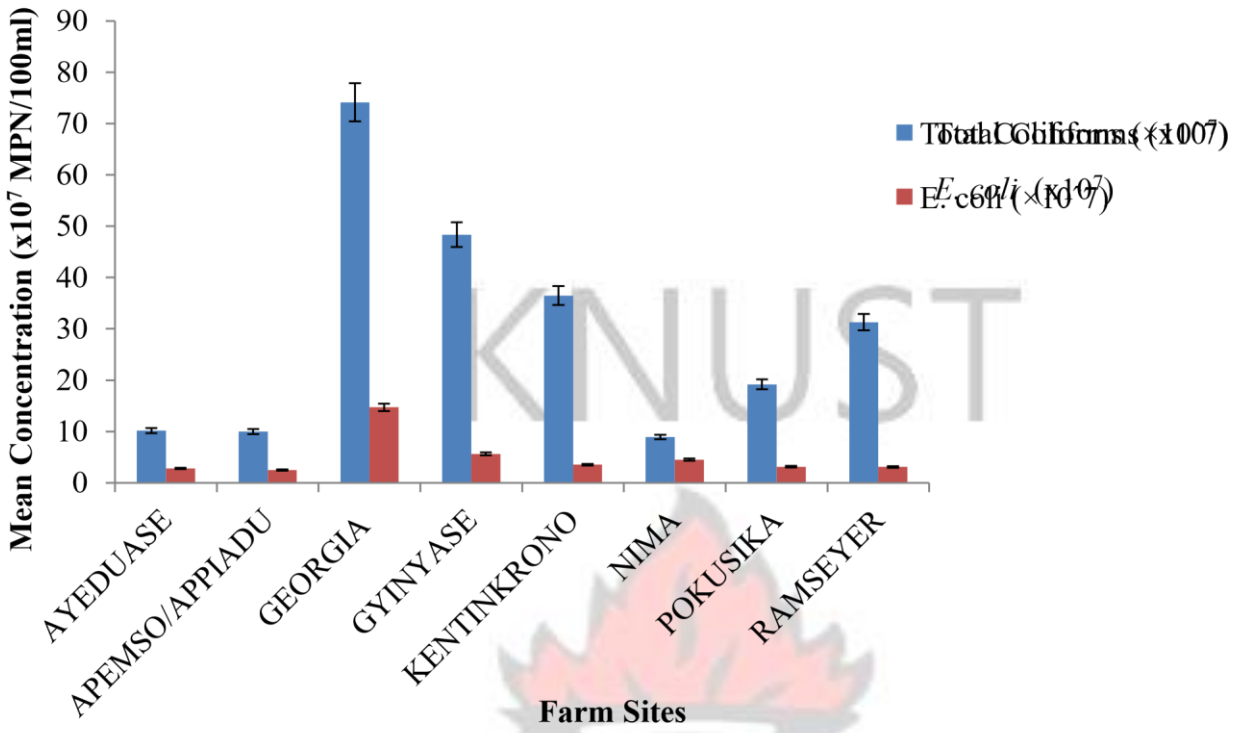
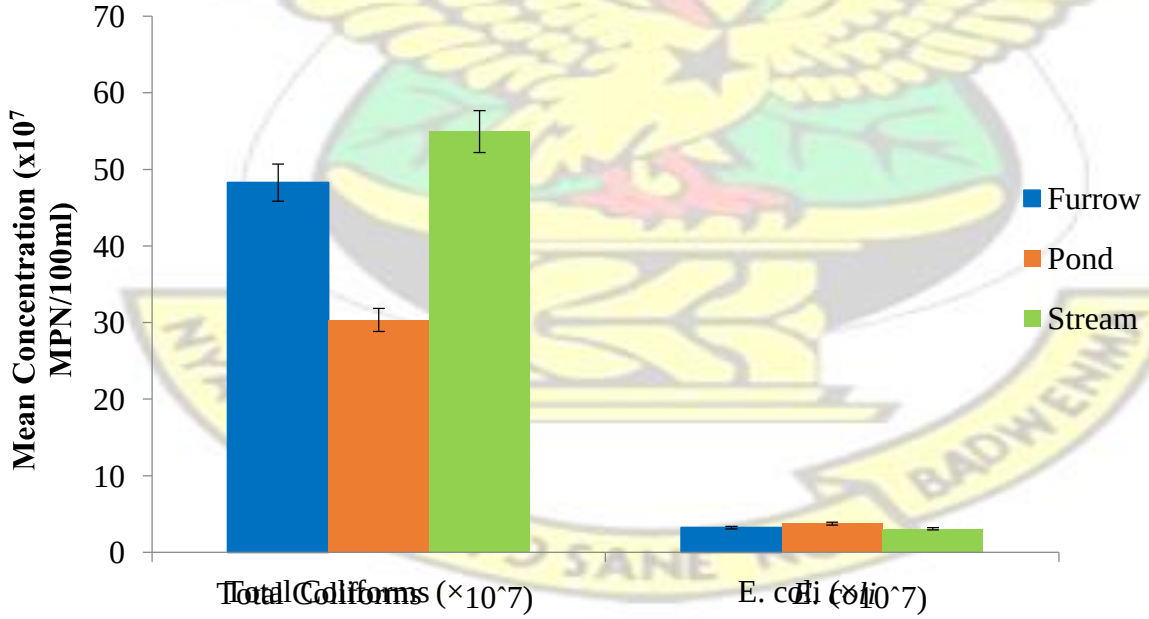


Figure 4.12: Comparison of the mean numbers of total coliforms and *E. coli* at the various farm sites



Microbial Characteristics Figure 4.13: Comparison of mean numbers of total coliforms and *E. coli* in furrow, pond and stream at all the farm sites

4.3.2.1 Seasonal Variation of Total Coliforms and *E. coli* in Water Sources in the Sampling Sites

Variations in the mean numbers and range of total coliforms and *E. coli* of water from the sampling sites for both the wet and dry seasons are shown in Table 4.3 above and also represented in Figure 4.14. Furrow and stream recorded higher concentrations of total coliforms during the dry season, $96.89\times10^7 \pm 70.56\times10^7$ MPN/100ml and $54.94\times10^7 \pm 11.54\times10^7$ MPN/100ml respectively, than that of the wet season. Moreover, the concentrations of total coliforms were higher than those of the wet season, as the minimum concentration was recorded by furrow ($9.83\times10^7 \pm 0.88\times10^7$ MPN/100ml) in the wet season.

However, pond recorded the highest concentrations of *E. coli* for both the wet season ($4.07\times10^7 \pm 0.57\times10^7$ MPN/100ml) and the dry season ($3.39\times10^7 \pm 0.67\times10^7$ MPN/100ml), and the minimum concentration was recorded by stream ($3.06\times10^7 \pm 0.74\times10^7$ MPN/100ml) in the dry season.

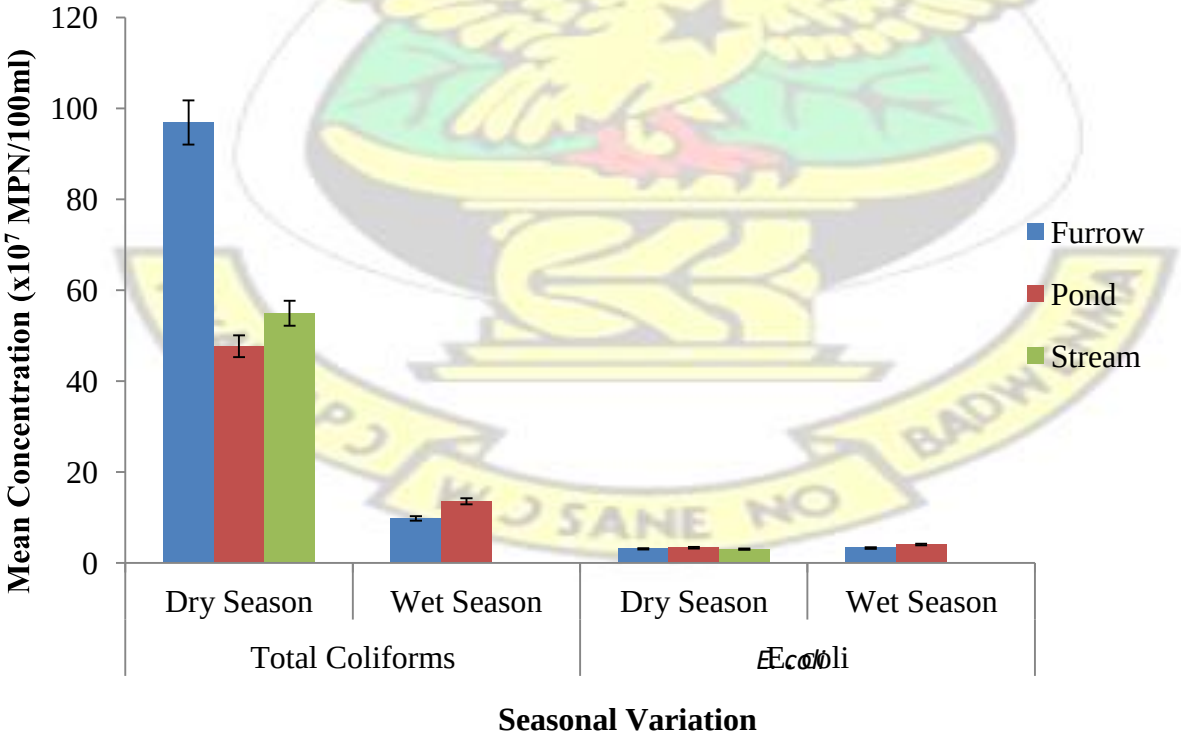


Figure 4.14: Seasonal variation of total coliforms and *E. coli* in furrow, pond and stream at all the farm sites

4.4

Strength of Association between Mosquito Larval Densities and Physicochemical Characteristics, and Seasonality

4.4.1 Strength of Mean Dispersion Relationship between Mosquito Larval Density and each of the Physicochemical and Microbial Properties, and Seasonality

The strength of the mean dispersion relationship between mosquito larval density and each of the physicochemical and microbial properties, using univariate analysis, has been presented in Tables 4.4 and 4.5. In determining this association, the three identified mosquito larvae species in the irrigated fields, irrespective of the type of species, were considered as the response or dependent variable. The physicochemical or microbial parameters, as well as larval counts in the wet and dry seasons were also considered as the predictor variable.

Using these indicators, DO, nitrite and ammonia (Tables 4.4) where observed to have negative relationship with larval densities, whereas the remaining predictor variables had positive relationship with larval densities. All the predictor variables had some effect on larval densities, but only pH and seasonality were statistically significant with *p* values of 0.006 and 0.014 respectively.

Table 4.4: Summary of univariate analysis of physicochemical parameters

Predictor Variables	Negative Binomial Regression Coefficient	P value at 95% Confidence Interval
DO	-0.0067	0.837
pH	0.2161	0.006
EC	0.0008	0.095
TDS	0.0005	0.636
Salinity	0.0172	0.976
Temperature	0.0135	0.686
Nitrite	-0.7188	0.291

Ammonia	-0.0203	0.560
Potassium	0.0013	0.846
Phosphorus	0.0026	0.978

As shown in Table 4.5, the coefficients of total coliforms (TC) and *E. coli*, as well as that of the wet and dry seasons were estimated as binary variables in this model. It was observed that, larval densities significantly (p=0.00) increased with increasing concentration of *E. coli*. The coefficient of TC (0.0001) as shown in Table 4.5 indicates that, with the same level of concentration increase in TC and *E. coli*, larval densities were observed to increase by 0.0001 times higher with TC than with *E. coli*. There was a significant (p=0.00) association between the densities of mosquito larvae and seasonal fluctuations. It was 0.4596 times more likely to find mosquito larvae in the wet season than in the dry season (p=0.014).

As represented in Table 4.5, the density of *Aedes* (Coefficient=4.2216) increased as the number of larvae at the sampling sites increased (p=0.00). The coefficient for *Anopheles* as shown in Table 4.5 is the expected difference in log count between *Anopheles* and the reference species, *Aedes*, whereas that for *culex* is also the expected difference in log count between *culex* and the reference species, *Aedes*. It was observed from the study that, *Anopheles* and *culex* larvae, with respective expected log counts of 0.0549 and 0.1504 occurred in higher abundance than the *Aedes*. Thus as the larval density increases at the sampling sites, *Anopheles* occurred more than *Aedes*, whereas *culex* occurred more than the *Anopheles*.

Table 4.5: Summary of univariate analysis of microbial indicators, and the wet and dry seasons, considered as binary variables

Predictor Variables	**Negative Binomial Regression Coefficient**	**P value at 95% Confidence Interval**
E. coli	4.2542	0.000
Total coliforms	0.0001	0.843
Aedes	4.2216	0.000
Anopheles	0.0549	0.779
Culex	0.1504	0.773

4.4

Dry season	3.5344	0.000
Wet season	0.4596	0.014

.2 Strength of Mean Dispersion Relationship between Mosquito Larval Density and all the Physicochemical and Microbial Properties together

The strength of the mean dispersion relationship between larval densities and all the predictor variables, considered in the univariate analysis model, were also examined by a multivariate analysis using the negative binomial regression model (Tables 4.6 and 4.7). This analysis measured the effect of all the predictor variables, when put together, on the dependent variable through the Incidence Rate Ratio (IRR). The IRR indicates the change in the larval densities in terms of a percentage increase or decrease, with the precise percentage determined by the amount the IRR is either above or below one (1).

As shown in Table 4.6, the incident rate ratios for temperature, ammonia, phosphorus, pH and EC indicate that for every unit increase in their values, larval densities at the sampling sites also had some respective percentage increase. Thus, the rate of larval densities increased by a factor equal to the IRR values of temperature (1.0199), ammonia (1.0302), phosphorus (1.1001), pH (1.1741) and EC (1.0026) shown in Table 4.6. Conversely, the incident rate ratios for DO, TDS, salinity, nitrite and potassium indicate that for every unit increase in their values, larval densities at the sampling sites also had some respective percentage decrease. Thus, the rate of larval densities decreased by a factor equal to the IRR values of DO (0.8668), TDS (0.9975), salinity (0.7563), nitrite (0.2994) and potassium (0.9886) shown in Table 4.6 below.

Total dissolved solids and potassium were observed to have the highest decreasing effect on larval counts, causing an about 100% and 99% decrease in larval densities respectively. On the other hand, phosphorus and pH were observed to have the highest increasing effect on larval

counts, causing an about 17% and 10% increase in larval densities respectively. However, only nitrite (p=0.048), EC (p=0.000) and DO (p=0.001) had statistically significant effects on larval densities.

Table 4.6: Summary of multivariate analysis of physicochemical parameters

Predictor Variables	Incidence Rate Ratio Interpretation (IRR)	P value at 95% Confidence Interval
DO	0.8668	0.001
pH	1.1741	0.334
EC	1.0026	0.000
TDS	0.9975	0.079
Salinity	0.7563	0.680
Temperature	1.0199	0.519
Nitrite	0.2994	0.048
Ammonia	1.0302	0.472
Potassium	0.9886	0.163
Phosphorus	1.1001	0.396

As represented in Table 4.7 below, total coliforms, *Anopheles*, *Culex* and wet season were considered as binary variables in the model. Total coliform had *E. coli* as its reference variable, that of wet season was dry season, and that of *Anopheles* and *Culex* was *Aedes*. The rate of occurrence of mosquito larvae in the wet season was observed to be 2.857 times higher than the dry season (p=0.037). As shown in Table 4.7, the IRR for *Anopheles* (1.1905) and *Culex* (1.8797) indicates that, larval densities for *Anopheles* increased by about 19% (p=0.39) with every one unit increase in *Aedes* larvae, whereas that of *Culex* increased by about 88% (p=0.30). The rate of occurrence of mosquito larvae was also higher for TC compared to *E. coli* whenever there was an equal increase in their concentration levels (p=0.390).

Table 4.7: Summary of multivariate analysis of microbial indicators, and the wet and dry seasons, considered as binary variables

Predictor Variables	Incidence Rate Ratio Interpretation (IRR)	P value at 95% Confidence Interval
Total coliforms	1.0004	0.390
Anopheles	1.1905	0.394

4.4

Culex	1.8797	0.301
Wet season	2.8566	0.037

.3 Strength of the Effects of Season, Breeding Habitats, and Physicochemical and Microbiological Properties on Larval Abundance

The strength of any possible differential effects of season, breeding habitats, and physicochemical and microbiological properties on the abundance of *Anopheles*, *Culex* and *Aedes* was determined by using Analysis of variance (ANOVA).

The strength of any possible differential effects of season on the abundance of the three identified larval mosquitoes and sampling points, and the concentration of microbiological and physicochemical properties on the irrigated fields are represented in Appendix 3.1. The study showed that there were significant ($p<0.05$) differences in the effect of season on the abundance of *Culex* ($p=0.000$) but not on *Anopheles* ($p=0.874$) and *Aedes* ($p=0.093$). Furthermore, significant ($p<0.05$) differences in the concentrations of all the physicochemical properties, as well as in the numbers of total coliforms but not in that of *E. coli* were observed. However, there were no significant ($p>0.05$) differences in the occurrence and abundance of the breeding habitats.

The strength of any possible differential influence of the breeding habitats on the abundance of the three identified larval species and the concentrations of the microbiological and physicochemical properties on the irrigated fields are represented in Appendix 3.2. As indicated in Appendix 3.2, the study showed significant differences in the abundance of *Anopheles* ($p=0.001$) and *Culex* (0.039) but that of *Aedes* was insignificant ($p=0.442$). With the exception of phosphorus ($p=0.004$) and EC ($p=0.016$) which recorded significant differences in their concentrations, that of the rest of the physicochemical and microbiological properties were insignificant.

The strength of any possible differential effects of physicochemical and microbiological properties on the abundance of *Anopheles*, *Culex* and *Aedes* are represented in Appendix 3.3.

Total coliforms and *E. coli* were observed to have significant (p<0.05) influence on the abundance of larval mosquitoes. Temperature, TDS and DO were revealed to have significant (p<0.05) influence on the abundance of *Anopheles*, whereas temperature, salinity, TDS, EC and DO were observed to have significant influence on the abundance of *Culex*. With the exception of ammonia and nitrite, the rest of the physicochemical properties were revealed to have significant (p<0.05) influence on the abundance of *Aedes*. However, temperature, DO and TDS were observed to have significant (p<0.05) influence on the abundance of all the three identified larval species.

CHAPTER FIVE

DISCUSSION

The use of wastewater for agricultural practices in both developing and industrialized countries is increasing. The major driving forces in this increasing usage include the increasing scarcity and stress on urban water due to increasing urban population, increasing environmental pollution coupled with inappropriate wastewater disposal and the recognition of the nutrient rich benefits of wastewater usage in irrigation.

5.1 Occurrence and Diversity of Mosquito Larvae in Wastewater used for Irrigation and in Pools Created on the Wastewater Irrigated Fields

Culex species was the most abundant of the three larval mosquito species identified in this study followed by the *Anopheles* species and then the *Aedes* species (Figure 4.1). This result is consistent with that of a recent study in Accra by Klinkenberg *et al*. (2008) which showed that *Culex* species are more abundant on urban agricultural fields, compared to other mosquito species. In the study by Opoku *et al*. (2003) on the occurrences and habitat characteristics of mosquitoes in Accra, similar results were also obtained, even though the study was not restricted to only vegetable irrigated farm sites. Also, this result is also consistent with that obtained in Pakistan, which showed the same order of dominance of larval mosquitoes in wastewater irrigated fields (Mukhtar *et al*., 2003). The high abundance of *Culex* and *Anopheles* may be due to their characteristic wide distribution and the availability of habitats conducive for breeding among the study sites. According to the findings of Valent BioSciences (2014), *Anopheles gambiae* complex, mostly prefer openwater pools with minimal vegetation whereas *Culex* species prefer various types of stagnant waters such as storm drains rich in organic materials. Similar to the findings of Opoku *et al*.

(2003), the dominance of *Culex* in all the breeding habitats indicates how versatile and adaptable they are at the different environmental conditions in the breeding sites. This feature of the *Culex* species is due to their high affinity for various types of stagnant waters rich in organic material as their preferred breeding habitats (Valent BioSciences, 2014).

Variation in the occurrence and abundance of the three larval species probably reflects alteration in the oviposition behaviour of these species. Spielman *et al.* (2001) have emphasized on the differential oviposition patterns in the mosquito species. However, an important alternative to the larval variations may be due to the differential survivorship among the three larval species found in the sampling sites (Mwangangi *et al.*, 2008). According to Mwangangi *et al.* (2008), predation and cannibalism are also influential factors in decreasing the survivorship of larvae in their specific habitats, particularly the small sized habitats. These factors may have contributed to the definite differences in the abundance of the identified larval species in this study.

The diversity of mosquitoes in the study area, in relation to the three identified larval species, were quite high; with a Simpson's Index Diversity of 0.46. Thus, the total abundance of the identified larval species, *Anopheles, Culex* and *Aedes*, are evenly distributed among the study sites. Furthermore, the relative abundance of the larvae making up the richness of the study area was also more distributed among the breeding habitats. These could be due to the availability of favourable environmental conditions at the study sites. An interesting finding from this study is that, the furrows and streams which the *Anopheles* species were more attracted to had very high species evenness, whereas the ponds which the *Culex* were attracted to had low species evenness. The study therefore showed that, all the three identified species were predominant at the study area.

5.2 Association between Mosquito Larval Abundance and the Identified Breeding

Habitats

In this study, furrows which were prevalent on the irrigated vegetable fields all year round had the highest larval abundance. This was followed by ponds which served as reservoirs for irrigation water. Streams on the farm sites recorded the least number of larval mosquitoes. These differences in larval abundance could probably be due to water quality and size of the breeding habitats, as well as the washing away of immature mosquitoes at the stream edges as a result of the continuous flow of these watercourses. These findings confirm the results obtained by Afrane *et al*. (2012), Klinkenberg *et al*. (2008) and Mukhtar *et al*. (2003) on the association between larval abundance and habitat type in Kumasi, Accra and Pakistan respectively. This study also showed that the type of the aquatic habitat influenced the persistency of mosquito larvae, and consequently adult mosquito productivity. This could be due to the high numbers of ponds (as reservoirs) on the irrigated fields, which ensured the persistence of some furrows such as foot prints and pools resulting from overflows during irrigation processes. Streams were observed to be present on all farm sites and their margins and pools were able to effectively contribute to larval breeding. The breeding habitat survey showed the predominance of furrows (55.4%) and ponds (41%) on all the farm sites, and consequently the high abundance of enumerated mosquito larvae. Similar to the study by Afrane *et al*. (2012), it was observed that these habitats were of adequate sizes, sunlit, always had water available and normally do not dry up. This may be due to the presence of watercourses and the low land nature of the fields. Therefore, the aquatic mosquito larvae are able to develop into adult mosquitoes. Definite association between the three identified larval species and their preferred breeding habitats within each of the farm sites confirms the assumptions that microhabitat colonization is influenced by oviposition patterns and behaviour by the gravid mosquitoes in selecting oviposition sites (Edillo *et al.,* 2006).

5.3 Association between Quality of Water at the Irrigated Fields and the Abundance

of Mosquito Larvae

Abundance of the three identified mosquito species larvae was found to vary (Table 4.4) according to differential microbial and physico-chemical factors which determined the quality of water of the breeding habitats. Thus combinations of these factors definitely contributed to the differential abundance of the larval mosquitoes observed at the sampling sites. Results obtained by Amerasinghe *et al.* (1995), Edillo *et al.* (2006) and Kengluecha *et al.* (2005) confirmed the definite correlation between physicochemical properties of aquatic habitats and larval abundance. According to Sanchez-Ribas *et al.* (2012), climatic and other abiotic and biotic factors have probable influential effects on the ability of Anophelines to adapt and flourish in water developmental project areas.

5.3.1 Microbiological Quality of the Water Samples

It was observed that concentrations of total coliforms and *E. coli* of sampled wastewater at the irrigated fields were of very poor quality (Appendices 2.5 and 2.6), exceeding the WHO (2006b) permissible limits of $<10^6$ total coliforms per 100ml and $\leq 10^5$ *E. coli* per 100ml for unrestricted irrigation and the recommended standard of $\leq 10^6$ *E. coli* per 100ml for restricted irrigation. According to the WHO guidelines for the health risks associated with the use of wastewater for irrigation, wastewater with total coliform concentrations of 10^6-10^8 is considered of poor water quality for irrigation purposes (WHO, 2006b). With respect to the mean concentration of total coliforms, the irrigation water at Nima was of less polluted compared with the other farm sites, whereas Georgia had the worst water quality. However, with respect to the mean concentration of *E. coli* in the irrigation water, Ramseyer and Pokusika had less polluted water quality compared with wastewater from the other sites, whereas Georgia had the poorest water quality. The variation in the quality of water at the irrigated fields may be accounted for by the sources of water, topography of the fields, and as well as some other

practices such as dumping of refuse on the fields (Table 3.1). Even though there were no significant differences in the concentrations of total coliforms (p=0.354) and *E. coli* (p=0.582) at the breeding habitats, the concentrations of total coliforms indicates that stream was less polluted compared to the other breeding habitats, whereas furrow had the poorest water quality. On the hand, stream was less polluted compared to the other breeding habitats, whereas pond recorded the poorest water quality. However, there is a perception that wastewater is of much benefits for crops than higher quality water, since they are rich in nutrients. Therefore in many cases, the poorer the quality of the wastewater, the more beneficial it is to the crops (WHO, 2006b).

There was a significant (p=0.00) influence of the microbial properties on larval abundance, which had more influence with an increase in total coliforms compared to an increase in *E. coli* concentration. An interesting finding in this study was that, furrows which recorded the highest densities of *Anopheles* species were also the most polluted with total coliforms, whereas stream which was less polluted recorded the lowest number of *Anopheles* species. Furthermore, pond which was polluted with *E. coli* recorded the highest abundance of *Culex.* These findings confirm the results obtained by Opoku *et al.* (2003) in a similar study carried out in Accra.

5.3.2 Physicochemical Quality of the Water Samples

Although the mean values of temperature (30.03 ^{0}C), EC (369.76 µS/cm) and TDS (194.54 ppm) were within the FEPA (1991) and WHO/UNEP (1997) recommended range of limits for irrigation wastewater, their levels varied among the breeding and study sites (Appendix 2.4). Conversely, even though the mean values of pH (8.27), nitrite (0.03 mg/L) and phosphorus (1.32 mg/L) were within the FEPA (1991) and WHO/UNEP (1997) recommended range of limits for irrigation wastewater, they also recorded very high values exceeding the FEPA (1991) and WHO/UNEP (1997) recommended range of limits

(Appendix 2.4). These differences in concentration levels could be due to ongoing different farming practices such as the application of different types of manure and pesticides, as well as the source of irrigation water. According to Ackerson and Awuah (2010) on the studies "Urban Agriculture Practices and Health Problems among Farmers Operating on a University Campus in Kumasi, Ghana", 97 % of the localized farmers used different types of pesticides and irrigation practices, and all the farmers either used organic (predominantly poultry manure) or inorganic (predominantly N-P-K, 15-15-15) fertilizers.

The levels of phosphorus, nitrite and ammonia (Appendix 2.4), indexed the extent of organic pollution, attributed mainly to contamination from human and domestic animal faecal matter (Amerasinghe *et al*., 1995). These nutrient levels at the study sites might have provided favourable conditions for the breeding of bacteria, yeast fungal spores, alga, and protozoa, which are the type of food that most of mosquito larvae ingest, hence, the prolific breeding in the study sites (Opoku *et al*., 2003). Moreover, the study showed moderate to favourable mean levels of DO (3.75 mg/L), salinity (0.22 mg/L) and pH (8.27) falling within the larval mosquito tolerable levels of 2–6.3 mg/L, 0.1–6.2 mg/L and 3.3–8.4, respectively (Vasudevan *et al*., 2001; Thamer and Abdulsamad, 2005; Opoku *et al*., 2003).

The concentrations of salinity, potassium, ammonia and TDS were within favourable ranges (Appendix 2.4) that supported the persistence and survival of mosquito larvae on the field and therefore the observed larval abundance. For instance, potassium is used by mosquito larvae in retaining fluid homeostasis and electrolyte balance through the formation of urine with their Malpighian tubules (Rouzer, 2013). The high prevalence of larvae in all breeding sites could therefore be attributed to the relatively high levels of nutrient potassium (3.06– 73.26 mg/L). The larvae may have used the tolerable potassium levels to retained fluid homeostasis in such polluted water bodies (WHO/UNEP, 1997; Rouzer, 2013). According to Lokhande *et al*. (2011), pH of an aquatic environment regulates the availability of soluble chemicals to aquatic

organisms, since certain specific pH levels of a water body affect the solubility of many nutritive and toxic chemicals. Moreover, the relative concentrations of ammonia are dependent on the temperature and pH of a water body, and consequently when resulting change in effect on larval abundance when put together with the other physicochemical parameters (Camargo and Alonso, 2005). The high larval abundance at the study area could therefore be due to the tolerable levels of ammonia which associated with increase in larval mortality, and TDS which determines the concentrations of salinity as well as an indicator of organic and inorganic pollution (Camargo and Alonso, 2005; Amankona, 2010).

The study showed that the levels of phosphorus, temperature, EC and pH contributed positively by increasing larval abundance, since mosquito larvae were abundant at the study area. This association might have relatively attracted gravid adults of the observed larval species to suitable breeding habitats within the study sites. Opoku *et al.* (2003) in Ghana and Amerasinghe *et al.* (1995) in Sri Lanka in similar studies found that temperatures between the ranges of 27 ^{0}C - 39 ^{0}C, as observed in this current study provide the most suitable conditions for the development of the identified larval species. Furthermore, the magnitude of microbial activities, which serve as food for the larvae, in aquatic environments is to a greater extent determined by the prevailing temperature (Arroyo Seco Foundation, 2013). In addition, the observed favourable temperatures might have suitably contributed to the poikilothermic activities of the larvae and therefore maintaining favourable metabolic rates (Paaijmans, 2008). Similar studies by Edillo *et al.* (2006) in a Malian village reported on the significantly influence of TDS and EC on the proportional abundance of mosquito larvae among the breeding sites. Previous studies have also revealed that aquatic habitats with pH units of neutral or slightly alkaline aid in the persistence and survival of mosquito larvae (Oyewole *et al.*, 2009).

More importantly, nitrite and DO which had negative association with larval abundance might have also favoured larval persistence and survival. Oxygen tensions (low or high) which can

be lethal to mosquito larvae are often associated with breeding habitats with vegetation, but contrary to this, the farm sites were inundated with favourable open sunlight pools or habitats with less or no vegetation at all, and therefore a probable provision of tension free breeding habitats (Tiimub *et al*., 2012). Moreover, the mean DO (3.75 mg/L) at the breeding habitats indicates a suitable breeding environment since the general tolerable DO required by most mosquito species is 4 mg/L or less (Vasudevan *et al*., 2001). The concentrations of nitrites in the breeding habitats are dependent and directly proportional to pH and therefore, the concentration of nitrites tends to increase when the value of pH of the breeding habitats increases (Camargo and Alonso, 2005). Hence the tolerable levels of pH at the breeding sites might have also provided concentrations of nitrites which favoured mosquito larval survival and abundance.

The relative levels of the microbial and physicochemical parameters significantly contributed to the favourable ecological regimes, such as nutrient availability and suitable temperatures, required for the survival and breeding activities of the identified larval species. It was observed that the identified larval mosquitoes preferentially exploited the quality of water at the aquatic habitats on the fields. *Culex* species were more attracted to ponds which were rich in nitrite and had the highest concentration of *E. coli*, whereas the *Anopheles* and *Aedes* species were attracted to furrows which were rich in salinity, potassium, DO, pH, EC, TDS, ammonia and temperature, and moderate concentrations of *E. coli* and total coliforms.

5.4 Seasonal Variation in Breeding Habitats, Occurrence and Diversity of Mosquito Larval Species, and the Quality of Water at the Irrigated Fields

The study showed that seasonal variation affected the occurrence and persistence of breeding habitats. However, due to the climatic conditions in Kumasi, breeding habitats were persistent during both the dry and wet seasons. Similar to the findings of Oyewole *et al.* (2009) and Mukhtar *et al.* (2003), no larva was found in the streams during the wet season and this might

be due to washing away by high currents resulting from rainfalls. Furrows were dominant in both seasons but occurred more in the wet season than in the dry season. This may be due to rainfall and the low land nature of the farm sites, and might have contributed to furrows recording the highest density of mosquito larvae. There were almost as many ponds in the wet season as in dry season, and this was due to the fact that the farmers create ponds all year round for water storage (Afrane *et al.*, 2012). The Simpson's Index of diversity showed that, the identified larval species were evenly distributed in the dry season than in the wet season. Thus, it was more likely to find the same number of the identified larval species at a particular breeding site in the dry season than in the wet season. This might have contributed to the larval dominance in the wet season, since some breeding sites had more abundance of one larval species as compared to another. Comparing the indexes of both the dry and wet seasons (Table 4.2), there was more evenness in the furrows than the ponds. This might have contributed to the high dominance of *Culex* in the study area since pond produced more *Culex* than the other larval species.

There was a significant association between seasonality and larval abundance. Similar to the findings of Opoku *et al.* (2003), this study revealed that mosquito larvae occur more in the wet season than the dry season (p=0.01). It was observed that *Culex* was dominant in both seasons but occurred more in the wet season than the dry season. Moreover, there were significant (p=0.00) differences in the abundance of *Culex* among the seasons. The *Anopheles*, which was next in abundance to *Culex*, was also abundant in the wet season (p=0.87). These may be due to the persistence of the breeding habitats caused by rainfall and the provision of suitable environmental conditions for effective breeding and survival of larvae (Afrane *et al.*, 2012; Opoku *et al.*, 2003; Coleman, 2009). Conversely, previous studies by Afrane *et al.* (2012) reports of rainfall, particularly heavy rains, washing away the immature forms of mosquitoes and therefore leading to low larval productivity and abundance.

It was observed that the wastewater at the irrigated fields was significantly (p=0.00) polluted with total coliforms in the dry season than in the wet season. Conversely, wastewater at the irrigated fields was less polluted with *E. coli* in the wet season than the dry season. With respect to the concentrations of *E. coli* and total coliforms, the irrigation water was generally polluted in the dry season than in the wet season. This may be accounted for by the rainfalls in the wet season which might have diluted the microbial and physicochemical properties of the breeding habitats. The differences in the microbial quality of the breeding habitats probably explain the larval abundance in the dry season, even though it recorded fewer breeding sites compared to that of the wet season (Basommi, 2011; Opoku *et al.*, 2003). However, for the exception of *Aedes*, *Anopheles* and *Culex* were abundant in the wet season than the dry season. Moreover, there were no significant (p=0.09) differences in the abundance *Aedes* with the seasons.

The study showed significant differences in the levels of physicochemical parameters among the seasons. Compare with the dry season, wet season had relatively higher levels of EC, pH and DO. This significantly (p<0.05) might have contributed to the dominance of *Anopheles* and *Culex* in the wet season. On the other hand, dry season had higher levels of TDS and nitrite than that of the wet season. This also might have significantly (p<0.05) contributed to the relatively high abundance of *Aedes* in the dry season. These variations might have contributed to the high larval abundance and persistence of the identified larval species in both the dry and wet seasons (Opoku *et al.*, 2003).

5.5 Health Risk Inference for the Communities with Irrigated Vegetable Farming Systems

This study showed that, there were significant differences in the abundance of *Anopheles* (p=0.001) and *Culex* (p=0.039) at the various breeding habitats. *Culex* significantly occurred in high numbers and is known to cause lymphatic filariasis in rural areas of Ghana, and also

have a potential for an established urban transmission of the disease (Gbakima *et al.*, 2005). However, according to reports by the Kumasi Metropolitan Assembly (2006), the disease is endemic in the three Northern Regions, Central, Brong Ahafo, Western and the Greater Accra Regions. On the other hand, though *Aedes* also definitely occurred in high numbers, and are known to cause dengue, they do not transmit any known disease in Ghana (Opoku *et al.*, 2003). Therefore, the identified *Culex* and *Aedes* in the study sites constitute more of public nuisance hazard to humans rather than a public health risk. Conversely, though the *Anopheles* did not dominate in abundance, they require a small number of species to sustain high levels of malaria transmission (Opoku *et al.*, 2003), as such, the health risk inference will cover health effects of only the *Anopheles* species.

The *Anopheles* species have been identified to be prevalent at the study and sampling sites. Studies by Mwangangi *et al.* (2008) have shown that, besides all anthropogenic and natural environmental hardships, the immature forms of the *Anopheles* species have an estimated average survivorship rate of 93.11%. Previous entomological studies conducted in Kumasi have shown a high prevalence of the three main malaria transmitting *Anopheles*, *A. gambiae* complex, *A. arabiensis* and *A. funestus*, in open space vegetable farms and their surrounding communities (Afrane *et al.*, 2012; Coleman, 2009). These *Anopheles* species have been documented to transmit the malaria causing parasite, *plasmodium*, worldwide, especially in sub-Saharan Africa. In Ghana, they are highly distributed in the forest zone where the city of Kumasi happens to be located (Coleman, 2009).

The different groups of people that are at risk from the irrigated vegetable farming include the farmers and other people who participate or live at the site of the activities, the immediate communities that are in close proximity to such activities, and other communities that surround such local communities. The exposure route for the *plasmodium* species is by contact (through

a bite) with the adult female mosquito vector, and the health threat for this exposure is the malaria disease (WHO, 2006a). Studies carried out by Mwangangi *et al.* (2008) on urban wastewater irrigation fields reported on the feasibility of finding mosquito larvae in abundance due to the presence of numerous potential breeding habitats on such fields. The mosquito larvae are strictly aquatic and require an average of up to seven days in water to survive. Most importantly, this is being provided for by the sources of water for irrigation and other pools prevalent on the fields (Mwangangi *et al*., 2008). The results of this study correlated the results of such previous studies (Mwangangi *et al*., 2008). The high occurrence of mosquito breeding habitats and the consequent abundance of mosquito larvae seemed reliable to predict the eclosion of more adult mosquitoes if all other requirements remain conducive for the immature mosquitoes. Studies conducted by Afrane *et al.* (2012) on the ecology of mosquitoes in an irrigated vegetable farm in Kumasi pointed out that, the abundance and survival of larval and adult mosquitoes were high in the irrigated fields, definitely contributing to the populations of adult mosquitoes and subsequently the transmission of malaria in the city. Moreover, the disease transmission is higher in communities closer to irrigated vegetable farms than in those further away (Klinkengerg *et al.*, 2008). More importantly, the vulnerability of malaria in Ghana is over 50% with a high transmission of greater than one case per 1000 population.

Therefore, the high abundance of the identified mosquito larvae on the irrigated vegetable farms in the city of Kumasi should be considered; since the larval stage of mosquitoes is important for the survival, persistence and vector competence of the adult mosquito (Klinkengerg *et al*., 2008).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study revealed a high larval occurrence and abundance on wastewater irrigated vegetable farms in the city of Kumasi, hence, possibly contributes significantly to the populations of adult female mosquitoes which consequently lead to high malaria transmission. Among the identified mosquito larvae, *Culex* species were the most abundant, followed by *Anopheles* species and then *Aedes* species. The study showed that the predominance of larval mosquitoes on the irrigated fields was as a result of the persistence of favourable breeding habitats, dominated by furrows, followed by ponds and streams. The microbiological and physicochemical quality of water on the wastewater irrigated fields was observed to have contributed significantly to the abundance and persistence of identified mosquito larvae. More importantly, total coliforms, EC, nitrite, DO, potassium, pH and temperature contributed significantly to the abundance and consequently survival of larval mosquitoes at the wastewater irrigated fields. Moreover, the even distribution of identified mosquito larvae reflected the adaptive differences and oviposition patterns in the adult female mosquitoes. In addition, the study revealed a significant impact of seasonality on the abundance and persistence of larval mosquitoes, and also on the quality of water on the irrigated fields. However, irrespective of the season, concentrations of total coliforms and *E*.

coli exceeded the recommended standard limits of WHO/UNEP and the Federal Environmental Protection Agency (FEPA) for both restricted and unrestricted irrigation practices.

RECOMMENDATIONS

To reduce the associated risks of high larval mosquito populations and the availability of favourable breeding habitats, as revealed in this study, and the consequent production of competent adult female mosquitoes, the following actions are recommended;

a. A study should be carried out to investigate how to employ the use of important physicochemical properties such as potassium, phosphorus, nitrite and ammonia in larval control measures and surveillance programmes.
b. Irrigated vegetable field farmers should be educated, encouraged, aided and be required by law to employ the use of mosquito predators such as the *Gambusia* species in their dug-out wells or ponds. This is because besides rainfall, these wells serve as water sources for the furrows, pools and human foot prints.
c. A study should be conducted with more emphasis on vector adaptability to ascertain the cause of the immerging trend of the *Anopheles* mosquito breeding suitably in highly polluted water bodies.
d. A study should be conducted to investigate the impact of irrigated wastewater quality on the vector competence of the adult female mosquito.

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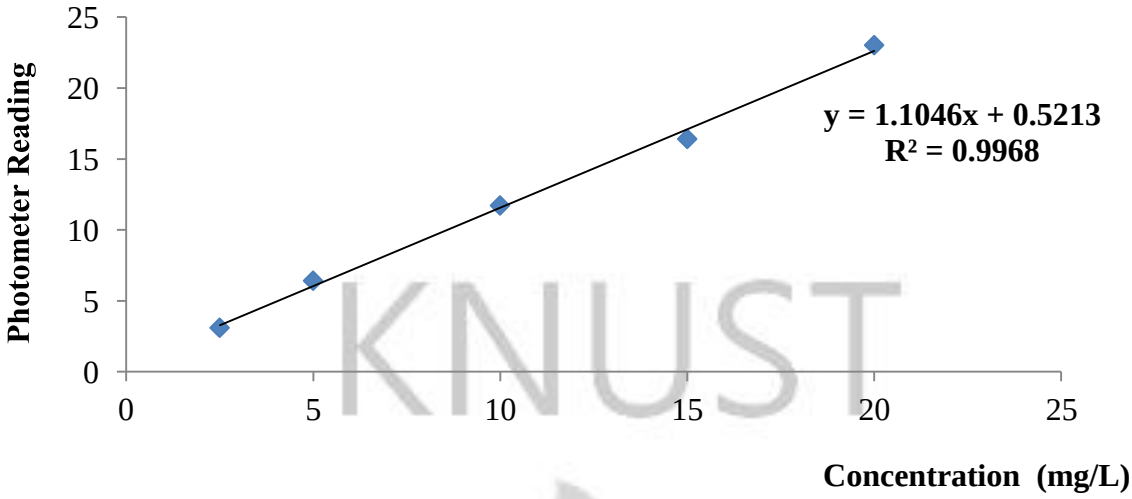
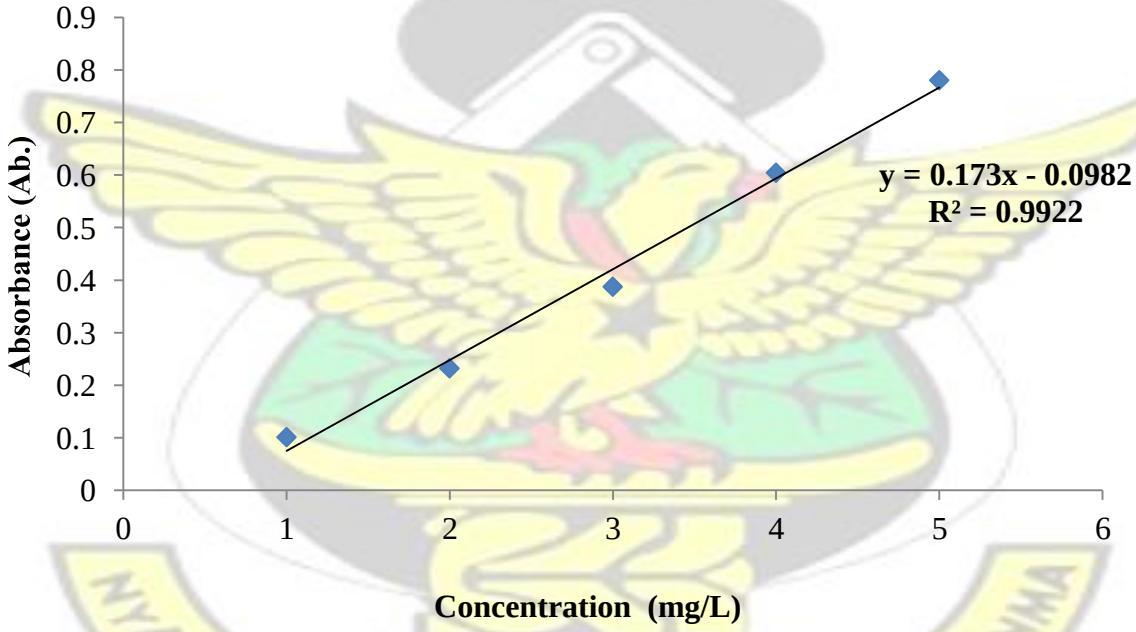
APPENDICES

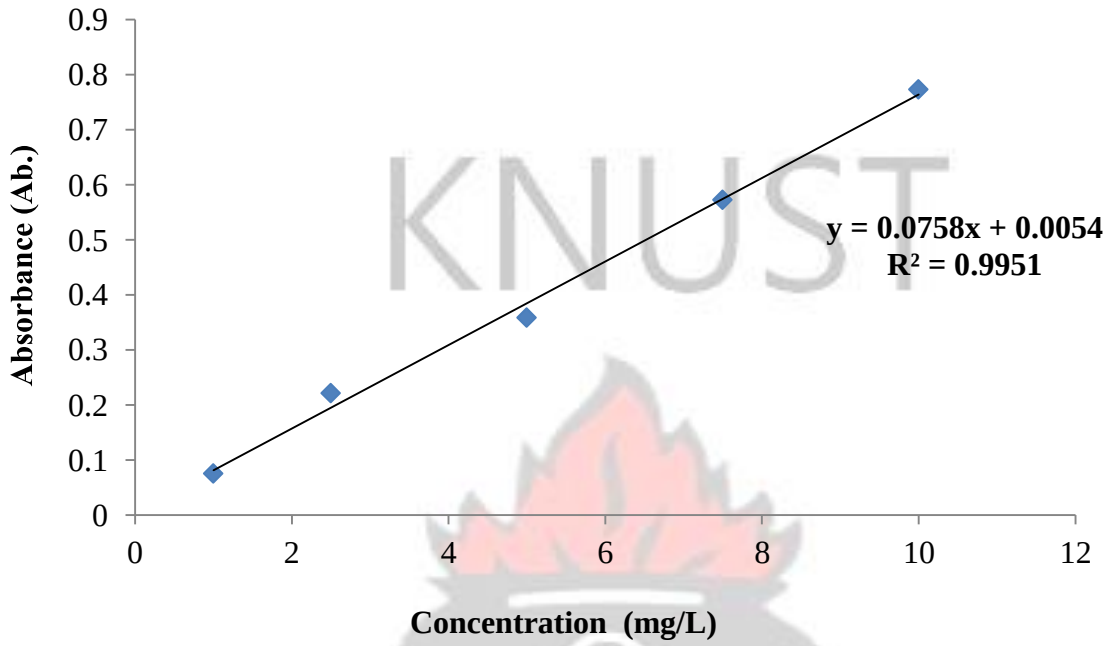
APPENDIX 1: Graph Representation of Calibration Curves



Appendix 1.1: A plotted calibration curve from standard potassium values



Appendix 1.2: A plotted calibration curve from standard phosphorus values for dry season



Appendix 1.3for wet season: A plotted calibration curve from standard phosphorus values

APPENDIX 2: Descriptive Statistics

Appendix 2.1: Occurrence of mosquito larvae in irrigated sites

FARM SITE	**FREQUENCY OF MOSQUITOE LARVAE**					
	Anopheles		***Culex***		***Aedes***	
	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season
AY	173	422	513	1117	346	86
APP	129	540	246	70	35	100
GEO	0	0	76	0	5	0
GY	231	30	688	104	116	8
KNT	0	635	115	1036	53	266
NM	8	177	0	880	0	0
PS	203	274	121	250	78	0
RAM	106	72	312	104	82	16
Total	850	2150	2071	3561	715	476
Percentage (%)	8.65	21.89	21.08	36.25	7.28	4.85
	30.54		57.33		12.13	

Appendix 2.2: Occurrence of mosquito larvae in breeding habitats

Breeding Habitats	Frequency of Habitats		Frequency of Larvae		Percentage of Mosquito Larvae (%)					
	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season			Wet Season		
					Anopheles	*Culex*	*Aedes*	*Anopheles*	*Culex*	*Aedes*
Pond	27	29	873	3011	26.12	50.97	22.91	14.65	83.19	2.16
Furrow	33	43	2365	3178	22.03	60.85	17.12	53.84	33.23	12.93
Stream	5	0	396	0	25	47.22	27.78	0	0	0

Appendix 2.3: Simpson's Index of Diversity of *Anopheles, Culex* and *Aedes* species at the farm and breeding sites

Farm Site	**Simpson's Index of Diversity (1 – D)**		
	Wet Season	**Dry Season**	**Combined**
Ayeduase	0.458	0.613	0.547
Apemso/Appiadu	0.393	0.535	0.550
Georgia		0.117	0.117
Gyinyase	0.419	0.496	0.487
Kentinkrono	0.588	0.434	0.587
Nima	0.279	0.000	0.287
Pokusika	0.500	0.618	0.568
Ramseyer	0.562	0.540	0.553
Sampling Site			

Furrow	0.583	0.552	0.614
Pond	0.294	0.621	0.395
Stream		0.639	0.639

Appendix 2.4: Summary statistics of the overall physicochemical parameters

Physicochemical Parameters	**Mean**	**Standard Error**	**Median**	**Minimum**	**Maximum**	**WHO Recommended Range of limits for irrigation wastewater**
Dissolved Oxygen (mg/L)	3.75	0.28	2.22	0.01	12.25	
Ph (pH unit)	8.27	0.10	8.22	5.39	10.56	6.5-8.4
Electrical Conductivity (µS/cm)	369.76	15.49	348	100	1073	700-3000
Total dissolved solids (ppm)	194.54	8.54	170	35	705	450-2000
Salinity (mg/L)	0.22	0.02	0.17	0.03	1.24	
Temperature (^{0}C)	30.03	0.28	30	21.65	38.12	≤ 40
Nitrite (mg/L)	0.03	0.01	0	0	1.4	1.0
Ammonia (mg/L)	2.51	0.22	1.4	0.01	9	
Potassium ion (mg/L)	21.61	1.29	16.47	3.06	73.26	
Phosphorus (mg/L)	1.32	0.09	1.05	0.11	6.13	0.1-3.0

Appendix 2.5: Summary statistics of the total coliforms ($\times 10^7$) in the irrigation water samples

Farm Sites	Mean	Standard Error	Range	Maximum	Minimum	Median	25%	75%
Ayeduase	10.138	1.064	31.8	32.8	1	10.138	5.6	15.575
Apemso/Appiadu	9.993	2.507	36.6	40.4	3.8	6.7	5.2	12.1
Georgia	74.15	37.75	75.5	111.9	36.4	74.15	36.4	111.9
Gyinyase	48.35	7.476	85.5	92.9	7.4	37.95	20.65	89.3
Kentinkrono	36.485	16.466	176.6	181.1	4.5	12.3	7.525	20.925
Nima	8.917	1.518	7.9	13.8	5.9	7.2	5.9	13.5
Pokusika	19.171	4.2	85.5	87.8	2.3	14	6.65	20.175
Ramseyer	31.309	6.833	129.4	131.4	2	15.6	8.675	52.4

Appendix 2.6: Summary statistics of the *E. coli* ($\times 10^7$) in the irrigation water samples

Farm Sites	Mean	Standard Error	Range	Maximum	Minimum	Median	25%	75%
Ayeduase	2.794	0.342	9.2	10.2	1	2.794	1.3	3.1

Apemso/Appiadu	2.475	0.487	5.8	6.8	1	2	1.25	3.1
Georgia	14.7	8.4	16.8	23.1	6.3	14.7	6.3	23.1
Gyinyase	5.61	0.915	13.5	14.5	1	5.3	2	8.05
Kentinkrono	3.518	0.729	9.7	10.7	1	2.7	1.95	4.533
Nima	4.5	0.604	3.5	6.7	3.2	4.2	3.575	5.2
Pokusika	3.133	0.538	11.2	12.2	1	2.4	1.675	3.775
Ramseyer	3.1	0.397	7.5	8.5	1	3	2	4.05

Appendix 2.7: Summary statistics for total coliforms ($\times 10^7$) and *E. coli* ($\times 10^7$) in irrigation water samples among the sampling sites

Summary Statistics	Total Coliforms ($\times 10^7$) MPN			*E. coli* ($\times 10^7$) MPN		
	Furrow	Pond	Stream	Furrow	Pond	Stream
Mean	48.27	30.33	54.94	3.22	3.74	3.06
Standard Error	31.29	5.19	11.54	0.36	0.43	0.74
Median	9.8	13.8	55.6	2.4	2.4	3
Range	2418.6	180.1	62.5	22.1	13.5	4.2
Minimum	1	1	30.9	1	1	1
Maximum	2419.6	181.1	93.4	23.1	14.5	5.2

APPENDIX 3: Analysis of Variance (ANOVA)

Appendix 3.1: The differential effects of season variability on sample points, mosquito larvae, and microbial and physicochemical properties

Dependent Variables	F Value	Significance ($p<0.05$)
Sample Point	2.936	0.089
Anopheles	0.025	0.874
Culex	13.556	0.000
Aedes	2.868	0.093
Dissolved Oxygen	7.555	0.007
pH	2227.785	0.000
Electrical Conductivity	54.687	0.000
Total dissolved Solids	326.394	0.000
Salinity	4130.979	0.000
Temperature	9260.630	0.000
Nitrite	85.911	0.000
Ammonia	109.883	0.000
Potassium ion	146.853	0.000
Phosphorus	60.547	0.000
Total coliforms	30.675	0.000
E. coli	0.650	0.422

Appendix 3.2: The differential effects of sample points on mosquito larvae, and microbial and physicochemical properties

Dependent Variables	F Value	Significance ($p<0.05$)
Anopheles	7.812	0.001
Culex	3.317	0.039
Aedes	0.821	0.442
Dissolved Oxygen	1.220	0.298
pH	0.017	0.983
Electrical Conductivity	4.284	0.016
Total dissolved Solids	2.378	0.097
Salinity	2.242	0.110
Temperature	3.066	0.050
Nitrite	0.918	0.402
Ammonia	0.811	0.446
Potassium ion	2.676	0.072
Phosphorus	5.876	0.004
Total coliforms	1.047	0.354
E. coli	0.543	0.582

Appendix 3.3: The differential effects of microbial and physicochemical properties on

Anopheles, Culex* and *Aedes

Factors	Dependent Variables					
	Anopheles		*Culex*		*Aedes*	
	F Value	Sig.	F Value	Sig.	F Value	Sig.
Dissolved Oxygen	1.924	0.015	8.252	0.000	3.049	0.000
pH	2.091	0.033	0.566	0.965	26.319	0.000
Electrical Conductivity	1.327	0.188	1.756	0.039	173.075	0.000
Total dissolved Solids	1.735	0.023	2.882	0.000	299.270	0.000
Salinity	1.040	0.428	1.952	0.003	7.271	0.000
Temperature	1.529	0.044	8.197	0.000	44.012	0.000
Nitrite	1.395	0.085	0.293	1.000	0.042	1.000
Ammonia	0.650	0.948	0.358	1.000	0.447	0.999
Potassium ion	0.859	0.691	7.910	0.000	30.240	0.000
Phosphorus	1.381	0.156	0.455	0.998	70.178	0.000
Total coliforms	1.011	0.478	1.252	0.176	0.768	0.855
E. coli	0.585	0.982	0.917	0.629	0.625	0.967

Significance ($p<0.05$)

APPENDIX 4: IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6

09-63235-01

APPENDIX 5: Field Images

Some selected farm sites

Some forms of irrigation practices

Sample collection