ANOPHELES LARVAL PRODUCTIVITY AND DIVERSITY IN MWEA IRRIGATION SCHEME, KIRINYAGA DISTRICT, KENYA

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JUNE 2006

DECLARATION

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

Signature ..... Date 3-2-2007

We confirm that the work reported in this thesis was carried out by the candidate under our supervision as the university supervisors.

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DEDICATION

To my family

My wife, Monica Kanini, for her love and dedication

and

My sons, Alan Mwangangi and Timothy Mwendwa for their endurance and bravery during the time of this study.

To the Mwea Rice farmers who invest all their lives to toil in water and mud growing rice.

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LIST OF ABBREVIATIONS AND ACRONYMS

Bs:	*Bacillus sphaericus*
Bti:	*Bacillus thuringiensis var israelensis*
DDT:	Dichlorodiphenyltrichloroethane
DRIP:	Dissertation Research Internship Programme
EIR:	Entomological Inoculation Rates
ICIPE:	International Centre of Insect Ecology and Physiology
ITN:	Insecticide Treated Bednet
IVM:	Integrated Vector Management
KEMRI:	Kenya Medical Research Institute; CGMR Coast: Centre for Geographic Medicine Research Coast
MIAD:	Mwea Irrigation and Agricultural Development Centre
MIS:	Mwea Irrigation Scheme
pH:	Hydrogen-ion exponent
WARDA:	West African Rice Development Authority
WHO:	World Health Organization

ABSTRACT

The use of irrigation to flood agricultural land during rice cultivation has over the years been associated with an increase in the number of disease vectors and corresponding increase in health burden due to malaria and other vector and water-borne diseases. In this study, field and laboratory studies were used to examine the primary factors responsible for regulating the aquatic stages of malaria vectors in a rice agro-ecosystem prior to implementation of a larval control programme. The objective of this study was to determine the environmental and agricultural factors that regulate malaria vector productivity and diversity in Mwea Irrigation Scheme, Kirinyaga district, Kenya. The study was conducted in 3 villages representing planned (Mbui Njeru), and unplanned rice cultivation with varying amount of land under rice (Kiamachiri and Murinduko). The physico-chemical variables were measured using different field based hand held equipments or visual assessment. Experimental plots were used to closely monitor the factors associated with *Anopheles* larval densities in the rice fields. A total of 29,252 immature stages of anopheline mosquitoes were collected in the three villages comprising of 78.23% (n = 22,885) early instars, 10.91% (n = 3,192) late instars and 10.85 % (n = 3,173) pupae. *Anopheles gambiae s.l.* was the most abundant and was found in all habitats that were positive for late anopheline instars in the 3 villages. Larval abundance was significantly higher in Murinduko compared to the other villages ($F_{(2, 182)} = 38.685$, $p < 0.01$). Rainfall was positively associated with *Anopheles gambiae s.l.* larval abundance in Kiamachiri (r = 0.759) and Mbui Njeru (r = 0.602) but negatively associated in Murinduko (r = -0.267). Multiple logistic regressions showed *Anopheles* larval density to be significantly associated with many interrelated biotic and abiotic variables including presence of other invertebrates, percentage *Azolla* cover, distance to nearest homestead, water turbidity, water temperature, conductivity, pH, and water depth. *Anopheles* productivity from different habitat types showed that paddies had most emergent mosquitoes (n = 143) followed by marshes (n = 65). Succession of *Anopheles* species was evident with *An. gambiae* colonizing the paddies throughout the rice growth cycle with peaks during the early stage of rice growth while *An. rufipes* and *An. coustani* occurred during the late vegetative stages. Larval densities were significantly higher at the centre of the paddy compared to the periphery during the transplanting period ($F_{(1,166)} = 4.809$, $P = 0.030$) but the difference was not significant during the tillering period ($F_{(1,362)} = 0.037$, $P = 0.848$). The survivorship of immatures in the paddies showed that there was 98.26% mortality of larvae. In conclusion, rice paddies and associated canals are the most productive habitats types throughout the year while peridomestic habitats are important during the long and short rains. The results further indicate that several biotic and abiotic factors interact to regulate *Anopheles* larval densities in aquatic habitats. These findings demonstrate the need to target larvicidal application in the entire paddy between transplanting and tillering stages in order to achieve effective larval control.

CHAPTER 1: INTRODUCTION

1.1 Background information

Malaria continues to be a major cause of morbidity and mortality in tropical and subtropical countries of the world despite the enormous investment in the control efforts. Malaria kills between 1.5 and 2.7 millions people each year in the world, and between 300 and 500 million fall ill from it oftenly (WHO, 1998). Over a million of these deaths are in children aged less than five years but also include women in their first or second pregnancy, older children, young adults and non-immune travelers. In Africa, the disease is responsible for an estimated 1 million deaths, mainly infants and children, annually. With acute disease a child may die within 24 hours of infection. Pregnant women are four times more likely to suffer malaria attacks and may lead to giving birthe to babies with low birth weight and still births, endangering the health of the women and prospects of survival for the new born (Lindsay *et al.,* 2000). Outside tropical Africa, malaria deaths occur mainly among non-immune new comers to endemic areas, for example among agricultural workers, miners and settlers in newly colonized areas (WHO, 2000).

Malaria is most serious in the poorest countries and in populations living under the most difficult and impoverished conditions. The prevalence and severity as well as magnitude of associated social and economic effects vary widely in different geographical areas where the disease occurs. However, worst effects of the disease are felt in Sub-Saharan Africa (WHO, 2000). It undermines the health and welfare of families, endangers the survival and education of children, causes disabilities, to the active population and impoverishes individuals and countries

In Kenya, malaria is responsible for approximately 30% of the total out patient clinic visits (Snow *et al.*, 1998). Annually some 26,000 children aged five years and below die from malaria related cases translating to 72 deaths daily (Snow *et al.*, 1998). Estimates of infant and child mortality on the Kenyan coast show that at least 58 infants per 1000 life births and 12 children per 1000 children aged between one and four years die each year (Snow *et al.*, 1994). With regard to morbidity, people in areas of high transmission usually go through several attacks every year. Each such episode may last about 5 to 15 days often incapacitating the victim(WHO, 1995).

1.2 Statement of problem

Malaria is a major cause of morbidity and mortality in Mwea Irrigation Scheme. Its control in Kenya is mostly done through the use of Insectide Treated Bednets and treatment of the infected persons using antimalarials. Other methods of malaria control based on the mosquito larval methods are rearly advocated even in the National Malaria Control Strategy. Mwea Irrigation scheme is one of the largest rice growing schemes in Kenya. Rice cultivation usually provides ideal sites for *Anopheles* larval development. In these agro-ecosystems different agricultural and environmental factors affect anopheline larval instars development. In Mwea Rice Irrigation Scheme, there is a need to know the factors, which affect the anopheline larval development, which in turn result in more adult production. Knowledge of these factors would be important in implementation of Integrated Vector Management (IVM) policy in malaria control programmes in Mwea Irrigation Scheme.

1.3 Justification of the study

In an irrigated agricultural ecosystem, *Anopheles* mosquito larvae in aquatic habitats are usually affected by different environmental and agricultural factors. These in turn affect the adult mosquito production in terms of vector densities, consequently influencing malaria parasite transmission. The number and productivity of larval habitats ultimately determines the density of adult mosquitoes. In an agroecosystem, different environmental and agricultural factors regulate the abundance of malaria vectors larvae in the habitat. This eventually affects the malaria vectors productivity and diversity from the larval habitats. There is little information available on factors regulating the productivity of the habitats. Consequently it is difficult to implement and eventually monitor and evaluate anopheline mosquito larval control programme in Mwea Irrigation Scheme. There is no information available on the survivorship of developmental stages of anopheline mosquitoes in Mwea irrigation scheme that could be used in models in productivity studies of anopheline from the aquatic habitats. This study would provide information on malaria larval dynamics in the aquatic habitats, which is useful in the implementation and evaluation of a larval control programme. Variation of malaria vector populations as a result of different environmental and agricultural factors would be known to help implement the control measures at the appropriate time. This would ensure that the burden of malaria among inhabitants of Mwea Irrigation Scheme is reduced consequently leading to increased economic productivity.

1.4 Research questions

a) How does the productivity of *Anopheles gambiae s.l.* and *An. funestus* in larval habitats fluctuate seasonally in the Mwea rice irrigation agro-ecosystem?

b) Which environmental factors are associated with the productivity of *Anopheles* larvae in the aquatic habitats?

c) What is the distribution pattern of *Anopheles* larvae within the paddy habitats?

d) What is the survivorship rate of the immature anopheline larvae in the aquatic habitats?

e) How do agricultural activities influence the temporal colonization of aquatic habitats by mosquito larvae and other aquatic invertebrates?

1.5 General hypothesis

Environmental factors and agricultural practices directly affect *Anopheles* larval densities and aquatic invertebrate population dynamics in the larval habitats in rice growing ecosystem.

1.5.1 Specific hypotheses

a) The population of *An. gambiae s.l.* and *An. funestus* fluctuates seasonally in the larval habitats and it is influenced by the rice growth cycle.

b) Environmental factors determine the capability aquatic habitats to support malaria vectors developmental stages.

c) There is *Anopheles* larval aggregation within the paddies during the development of larval instars.

d) Agricultural activities influence the temporal colonization of habitats by mosquito larvae and other aquatic invertebrates.

e) The survival of immature stage of *An. gambiae s.l.* larvae vary significantly from one instar stage to the other and this influences the number of emergent adult mosquitoes

1.6 General Objective

To determine the environmental and agricultural factors that regulate malaria vector productivity and diversity in Mwea irrigation scheme, Kirinyaga district, Kenya

1.6.1 Specific Objectives

a) To determine the temporal variation in productivity of *Anopheles gambiae s.l.* and *An. funestus* in larval habitats in the Mwea rice irrigation agro-ecosystem.

b) To determine the spatial distribution of *Anopheles* larvae in the aquatic habitats.

c) To determine the environmental factors associated with the productivity of *Anopheles* larvae in the aquatic habitats.

d) To determine the succession of mosquito larvae in the aquatic habitats.

e) To determine the *Anopheles* larval distribution within the paddies.

f) To determine the survival of *An. gambiae s.l.* larvae in the aquatic habitats

CHAPTER 2: LITERATURE REVIEW

2.1 Malaria vectors

Out of more than 400 described species of *Anopheles* (White, 1977) some 45 of them are implicated in the transmission of malaria. Different species of *Anopheles* are responsible for the transmission of malaria in specific geographic areas. The density of mosquito population is dependent on larval ecology. Irrigation schemes, particularly those which used for growing rice, are preferred breeding sites for *An. gambiae s.l.* and *An. funestus*. *Anopheles merus* and *An. melas* have extensive breeding sites within the tidal limits of coastal line (White, 1972, Bryan, 1983; Mbogo *et al.,* 2003). The malaria vectors play an important role in the transmission of *P. falciparum* parasites. These vectors generally have high parasite inoculation rates and are also remarkably stable in a wide range of bio-ecological and seasonal conditions hence appears to be very flexible, both in exploiting new man-made environments and in their response to malaria control activities (Coluzzi *et al.,* 1984).

2.2 Mosquito larval habitats

Anopheles gambiae s.l. and *An. funestus* complex are the most important vectors of human malaria in sub-Saharan Africa. Production of adults of *An. gambiae s.l.* occurs in small, temporary, sunlit, turbid pools of water (Gimnig *et al.,* 2001). Habitats are often created by human or animal activity wherein larvae are found in small depressions such as foot or hoof prints, the edges of bore holes and burrow pits, roadside puddles formed by tyre tracks, irrigation ditches and other artificial bodies of water (Gillies and De Meillon, 1968; White, 1972; Minakawa *et al.,* 1999; Gimnig *et al.,* 2001). The

developmental stages of malaria vectors determine the body size of the adults, which in turn may influence the competence of the vector in *P. falciparum* transmission. The mosquito body size varies according to nutritional status of the breeding habitat. Conditions of larval development affect adult body size (Lyimo and Koella, 1992; Koella and Lyimo, 1996), which can influence adult survival (Hawley, 1985) and vector competence (Paulson and Hawley, 1991; Nasci and Mitchell, 1994).

The adaptability to environmental changes leading to marked contrasts in vector bionomics has led to the development of various levels of vectorial efficiency for populations of *Anopheles* species in heterogenous environments within the same locality and has thus become important factor in determination of epidemiology of malaria (Toure *et al.,* 1994). Environmental heterogeneities have arisen mainly as a result of human activities which act as a means of constant evolutionary challenge as they provide a source of environmental change to which anthropophilic *Anopheles* have to respond by developing a highly dynamic vector-host relationship (Mulla *et al.,* 1990; Mutero *et al.,* 2000).

2.3 *Anopheles* mosquito distribution in Kenya

The primary malaria vectors in Kenya are *Anopheles funestus* and three members of *Anopheles gambiae* complex: *An. gambiae s.s., An. arabiensis* and *An. merus* (Coluzzi *et al.,* 1985; Collins *et al.,* 1988; Petrarca and Beier, 1992). *Anopheles gambiae s.s* and *An. arabiensis* are mostly closely associated with human and represent the major vectors of malaria (Muirhead-Thomson, 1951; Highton *et al.,* 1979). The distribution of these two species overlaps and occurs sympatrically in large areas of tropical Africa. In Kenya *An.*

gambiae s.l. and *An. funestus* are predominantly found along the Kenyan coast, western Kenya around Lake Victoria and in central Kenya at Mwea Irrigation Scheme.

2.3.1 The *Anopheles gambiae* complex

The *An. gambiae* Giles complex comprises 6 sibling species, one unnamed species and several incipient species all differing in various ways (Githeko *et al.,* 1993a; Service, 1993b; Thomson *et al.,* 1995; Ribeiro *et al.,* 1996). These vectors generally cause high parasite inoculation rates and are also remarkably stable in a wide range of bio-ecological and seasonal conditions hence appear to be very flexible, both in exploiting new man-made environments and in their response to malaria control activities (Favia *et al.,* 1997). Members of the *An. gambiae* complex have a wide geographical distribution and have been reported from most African countries (Gillies, 1961; Blower *et al.,* 1981; Mbogo *et al.,* 1993; Costantini *et al.,* 1996; Ribeiro *et al.,* 1996; Charlwood *et al.,* 1997; Takken *et al.,* 1998). While the existence of the six formally named species of the *An. gambiae* complex is well established, there is indication that further subdivision within the species of the complex may exist (Toure *et al.,* 1994). This presents a need for the accurate characterization of these species and a definition of their local distributional limits, which is important in malaria epidemiology.

2.3.2 The *Anopheles funestus* Group

Anopheles funestus belongs to a group of nine morphologically similar species (Kamau *et al.,* 2003). Members of this group are difficult to identify because of the morphological overlap that exists within the group. This inability to distinguish species as well as the

fact that they vary in their behaviour and biting preferences complicate the successful planning and maintaining of vector control activities in areas where *An. funestus* is the major vector (Gillies and De Meillon, 1968; Rawlings *et al.,* 1981). *Anopheles funestus* has been recognized as a vector of malaria parasites since the early part of the 20th century (Scott *et al.,* 1993; Service, 1993b). Some of the species are sympatric in the savannas of West and East Africa (Clements and Paterson, 1981; White, 1982). It is important to establish the role of the members of this group in malaria transmission.

2.4 The life cycle of *Anopheles* vectors of malaria

Figure 1 shows the life cycle of *Anopheles* mosquitoes. The female *Anopheles* after mating and blood feeding lays some 50-200 small (1mm long) brown or blackish boat-shaped eggs on the water surface. *Anopheles* eggs are white when freshly laid, they turn to brown, then black respectively as they mature. Viable eggs hatch into larvae within 2-3 days in the tropics, but in cooler temperate regions they may not hatch until after 4 -7 days or longer (Service, 1980). The larvae, while on the water surface, lie parallel to the surface to allow air intake and surface feeding.

At mean water temperatures of 25-28^0 C the larvae undergo four moults within 6-9 days to reach the pupal stage, which lasts 2-3 days depending on temperature (Lindsay and Bayoh, 2004). Thus, the minimum duration for one generation may be as long as 10-11 days. The pupae bear respiratory trumpets that are short and broad distally thus appearing conical. The most distinctive characteristic of *Anopheles* pupae is the presence of short peg like spines situated laterally near the distal margins of abdominal segments.

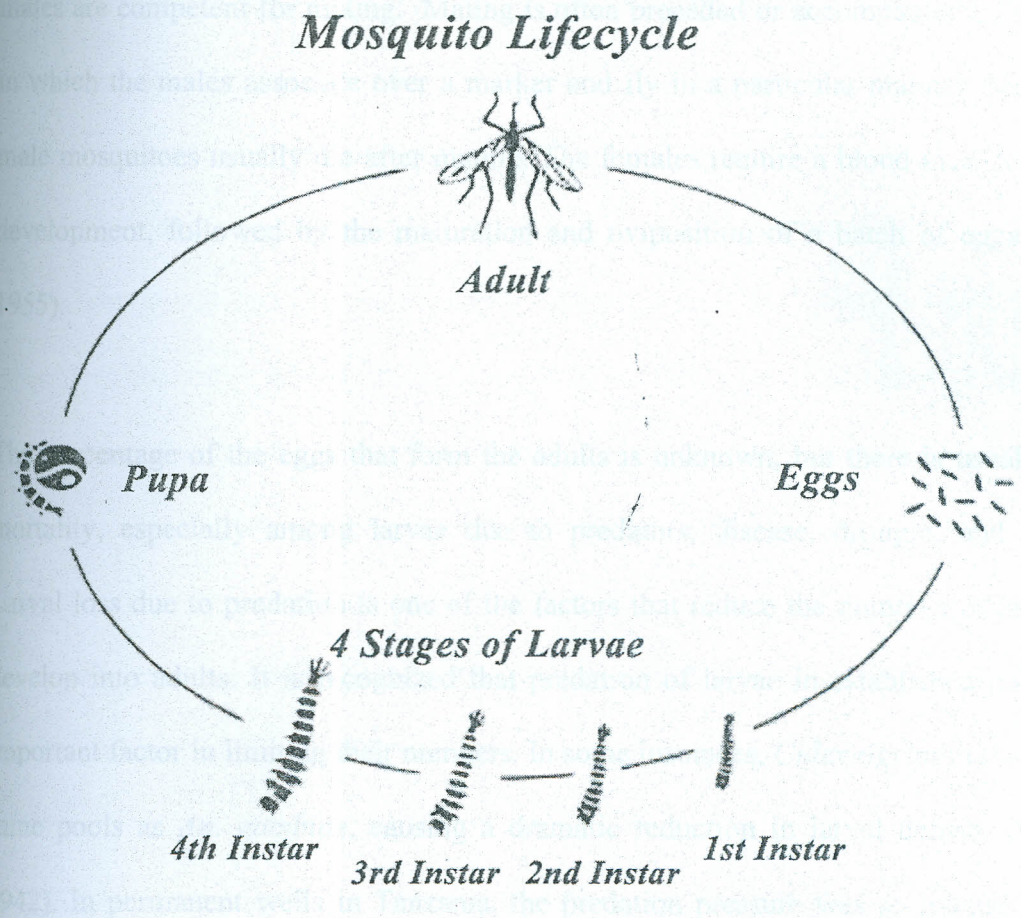

Figure 1 Schematic illustration of the life cycle of *Anopheles* vectors of malaria (After Service, 1980).

The pupal skin splits dorsally and the adult emerges. Careful movements are required to ensure that the adult mosquito does not fall sideways and be trapped in the surface film. This danger is particularly acute when the adult is largely out of the pupal exuviae but the terminal appendages are still not free. Finally the legs become free and spread on the water surface giving stability. The newly emerged adult inflates its wings, and separates and grooms its head appendages before flying away (Kettle, 1992). When the progeny of any one egg batch emerge as adults the males emerge first. The males become ready for

mating within 24 hours after emergence such that by the time the females emerge, the males are competent for mating. Mating is often preceded or accompanied by swarming in which the males associate over a marker and fly in a particular manner. Most of the male mosquitoes usually die after mating. The females require a blood meal for ovarian development, followed by the maturation and oviposition of a batch of eggs (Gillies, 1955).

The percentage of the eggs that form the adults is unknown, but there is usually heavy mortality, especially among larvae due to predators, disease, drought, and flushing. Larval loss due to predation is one of the factors that reduce the numbers of larvae that develop into adults. It is recognized that predation of larvae in established pools is an important factor in limiting their numbers. In some instances, *Culex tigripes* colonizes the same pools as *An. gambiae*, causing a dramatic reduction in larval density (Haddow, 1942). In permanent wells in Tanzania, the predation pressure was so intense that few larvae survived to pupae (Christie, 1958). It is possible that the same pressures exist in other types of permanent waters, thus limiting their productivity for *An. gambiae*. It may be noted that the agility often displayed by *An. gambiae* larvae, in contrast to species such as *An. funestus*, would tend to increase their vulnerability to attack by predators (Service, 1980).

2.5 Malaria parasites

There are four species of the genus *Plasmodium* that cause human malaria namely: *P. falciparum, P. malariae, P. ovale* and *P. vivax. Plasmodium falciparum* is probably the single most important parasite an African child encounters during his or her first few years of life. The transmission of *P. falciparum* is highly variable and is associated with severe disease and death for persons with little or no acquired immunity, such as infants, when the immunity gained through maternal antibodies during gestation has waned (McGregor, 1964; Greenwood, 1991). About 50 mosquito species of the genus *Anopheles* are responsible for the transmission of Plasmodia.

2.6 Feeding and resting behaviour of *Anopheles* mosquitoes

Eighty percent of *Anopheles* feed on any large mammal (Gillies, 1972). The host preference by a particular species of mosquitoes is also likely to be influenced by environmental conditions. Some of the mosquitoes are strictly zoophilic while others are anthropophilic. Of the 3 species of *An. gambiae* complex, *An. arabiensis* and *An. merus* are partially zoophilic and partially endophilic (White, 1974; Mosha and Petrarca, 1983; Mutero *et al.,* 1984). Studies in western Kenya show that *An. arabiensis* has a lower proportion (in terms of frequency) of human meals, which reflects a higher degree of exophily (Joshi *et al.,* 1975; Highton *et al.,* 1979; Githeko *et al.,* 1994). Petrarca *et al.,* (1991) found that a significant proportion of *An. arabiensis* fed on cattle but were collected indoors. *Anopheles arabiensis* is generally diverted to cattle feeding than *An. gambiae s.s.* (Githeko *et al.,* 1994). *Anopheles gambiae s.s.* is primarily endophilic and endophagic whereas *An. arabiensis* and *An. merus* show some degree of partial exophily and zoophagy (White, 1974; Coluzzi *et al.,* 1979; Gillies and Coetzee, 1987).

Blood feeding by anopheline mosquitoes is essential for transmitting malaria parasites and characteristic of this behavior can have major implication for the epidemiology of desease. Briegel and Horler, (1993) showed that anopheline mosquitoes take multiple blood meals within the gonotrophic cycle. Because biting frequency is one of the main entomological factor determining the vectorial capacity of the vector species, multiple feeding has a profound effect on the rate of malaria transmission (Garret-Jones, 1964).

2.7 The life cycle and transmission of human *Plasmodium* parasite

Four protozoan parasites, namely *P. falciparum, P. ovale, P. malariae* and *P. vivax* cause malaria in humans and they all have the similar life cycle (Figure 2). During the infective bite of a female *Anopheles* mosquito numerous infective sporozoites are injected into the bloodstream, where they must remain for about 30 minutes and then disappear from the blood stream. Most of the sporozoites are destroyed by phagocytes whilst some enter the liver parenchymal cells, develop and undergo asexual multiplication (pre-erythrocytic schizogony). A large unpigmented schizont is formed containing several merozoites, which are released into the bloodstream to invade the erythrocytes. The merozoite attaches to an erythrocyte and is invaginated into the red cell through a parasitophorous vacuole, where it feeds and deposits a pigment called haemozoin as a by product (Aikawa, 1980). The ingested merozoite becomes a feeding trophozoite and in the early stages of an infection, the fully-grown trophozoite multiplies asexually to become a schizont (erythrocytic schizogony), producing a small number of merozoites (Aikawa and Seed, 1980). Release of the merozoites from the erythrocytes brings on an attack of malaria, and the interval between attacks is the length of the schizogonic cycle. This may

last several hours. The released merozoites repeat the cycle and invade other erythrocytes.

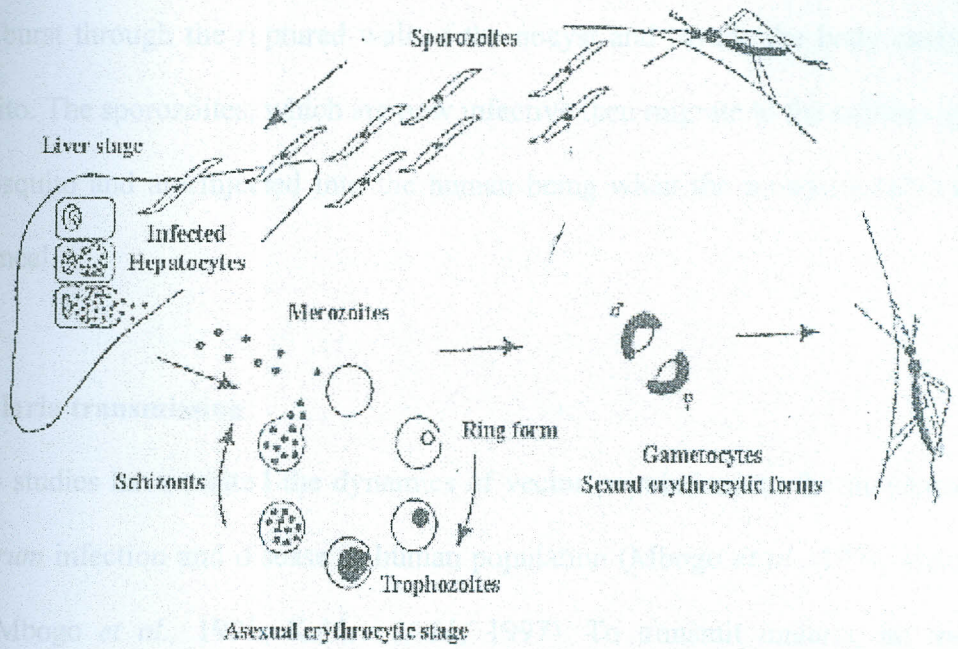

Figure 2: Schematic representation of the life cycle of *Plasmodium* species (After Garnham, 1966)

After a number of cycles of schizogony, the trophozoites do not divide but become gametocytes, which develop no further in man but circulate in the bloodstream until a mosquito takes them up during a blood meal (Kettle, 1992).

In the *Anopheles* mosquito, microgametocytes exflagellate to become microgametes. The microgametes move away to find and fuse with macrogametes to form zygotes, which remain motionless for 18-24 hours then elongate to form motile ookinetes (Aikawa and Seed, 1980; Kettle, 1992). The ookinete penetrates the wall of the midgut and forms an oocyst and through sporogony, the oocyst develops into enlarged motile sporozoites, which burst through the ruptured wall of the oocyst and invade the body cavity of the mosquito. The sporozoites, which are now infective then migrate to the salivary glands of the mosquito and are injected into the human being when the mosquito takes the next blood meal.

2.8 Malaria transmission

Several studies have related the dynamics of vector populations to the incidences of *P. falciparum* infection and disease in human population (Mbogo *et al.,* 1993; Beier *et al.,* 1994; Mbogo *et al.,* 1995; Kabiru *et al.,* 1997). To transmit malaria, an individual *Anopheles* has to feed on humans at least twice within a week successively: the first time to acquire an infection and the second time to transmit the parasite. This means that the ability of a species to transmit malaria is related to the product of the two probabilities of an individual feeding on human twice and not directly to the proportion feeding on human.

2.9 Entomologic Inoculation Rate

Malaria transmission intensity is best expressed as the entomologic inoculation rate (EIR), which directly reflects the exposure of humans to pathogenic *Plasmodium* parasites (Burkot *et al.,* 1990; Beier *et al.,* 1999; Killeen *et al.,* 2000b). EIR Levels of

one infective bite per year or less can readily sustain prevalence in excess of 40% for *P. faciparum*, the most pathogenic of species of human malaria parasites (Beier *et al.*, 1999).

The EIR as a marker for malaria transmission intensity has been intensively studied. (Trape *et al.*, 1993) found that malaria transmission levels in tropical Africa varied considerably being influenced by ecological conditions, from approximately 10^{-2} to 10^{3} infective bites per person per year. Snow *et al.*, (1994) reported that people living in the rice growing area of Kilombero District in Tanzania received at least 300 infective bites each year. Beier *et al.*, (1990) recorded that residents of Saridiidi and Kisian area of western Kenya received an average of 237 infective bites per year. Onori and Grab, (1980) and Oaks *et al.*, (1991) reported that the intensity of malaria parasites transmission by mosquito populations was a key component in the epidemiology of the disease. The two important aspects of malaria transmission they considered were entomological inoculation rates (EIR) and vectorial capacity (VC). The EIR is a measure of the number of infective bites each persons receives per unit time (e.g. per night) and is a direct measure of the risk of human exposure to the bites of infective mosquitoes. In malaria endemic parts of Sub-Saharan Africa and the Southwest Pacific, transmission intensity can vary form undetectable levels to more than 1,000 infective bites per year (Beier *et al.*, 1999; Killeen *et al.*, 2000a). Garret-Jones, (1964) observed that EIR varied with time, vector species, and parasite species. Dye and Hasibeder, (1986) pointed out that vectorial capacity that measures the potential for malaria transmission was based on several key parameters of vector populations.

2.10 Factors affecting distribution of malaria

Several factors significantly affect the distribution of malaria in space and time, between persons, and the resulting morbidity and mortality. Some of these factors include; the natural environment through its vector populations, interaction between vector and parasite, parasite determinants and some of its genetically controlled characteristics, host-biological factors, behavioral, social and economic elements.

Factors pertaining to the natural environment for example, the availability of the larval habitats for malaria vectors, influences the distribution of malaria in the area. The local rainfall produces rain pools favored by most malaria vector species for example *An. gambiae s.s.* and *An. arabiensis*. The slope of the land and the nature of the soil are some of the other environmentally related factors, which affect the type of surface water available and its persistence and subsequently the increase of local malaria vector populations. The optimal range of temperature and the relative humidity for most malaria vectors is 20-30°C and 70-80% respectively (Wernsdorfer and McGregor, 1986). Increasing the temperature increases the growth of vector population by shortening the interval from oviposition to adult emergence and vice versa. Biological factors such as immune response and genetics, as well as socio-economic status, living and working conditions, exposure to vectors and human behaviour, all play a critical role in determining a persons risk of malaria infection and hence illness.

Greenwood, (1989) found that climatic and topographic features determine the ecology of both human and arthropod hosts as well as their contacts. Ponds and reservoir in an area were important in malaria transmission, as they were the breeding sites for mosquitoes.

Many other environmental factors have been found to influence the level of exposure to the mosquitoes of an individual resident in a malaria endemic area. Greenwood, (1989) listed some of them as place and type of residence, the use of anti-mosquito measures and the position of the house.

Irrigation schemes and hydroelectric projects were likely to increase the intensity of malaria transmission and may change the seasonal transmission dependent on rainfall into perennial transmission by maintaining a population of the vector anophelines mosquitoes through out the year (Robert *et al.,* 1985). Large numbers of *An. gambiae s.l.* found in the rice growing areas during the dry season at Ahero (Githeko *et al.,* 1993b) and at Mwea-Tebere irrigation scheme (Ijumba *et al.,* 1990) were maintained by irrigation water.

Schofield and White, (1984) demonstrated that the house design and situation was important in protecting its residents from mosquitoes. Better-designed houses with mosquito prove screens at the windows and insectide treated curtains have less mosquitoes compared to poor designed houses with large eaves and many openings at the walls.

The role of human behavior in relation to vector and the transmission of malaria was summarized by Greenwood, (1989). He asserted that human behavior which operates at several different levels depending on the number of others involved and the social structure within a community could greatly influence malaria transmission. He quantified human behavior in terms of the methods of avoidance of mosquito bites which included

insecticide treated nets, house screening, mosquito coils, smoky fires, household insecticide and specialized house construction.

In domestic situation zooprophylaxis was important in reducing the frequency of mosquito feeding on humans and hence malaria transmission (Hess and Hayes, 1970). Mosquitoes especially *An. arabiensis* would be defracted to feed on cattle and other vertebrates hosts if an attempt to feed on humans is thwarted. All these factors contributed to affecting the degree of man-vector contact.

2.11 Vector control

This includes activities that reduce the number of infective or infectious bites of the vector by reducing the vector density, longevity and or preventing human – vector contact. The principal aim of vector control is the reduction of disease morbidity and mortality by reducing the level of transmission. It involves the use of methods targeted at controlling the mosquito population at larval or adult stages of their life cycle. Vector control has proved to be the most effective method for malaria control since lowering the *Anopheles* densities, the reduces the cases of malaria. It is also easier to control mosquito populations within a given geographical area than giving vaccines for protection or administration of prophylactic drugs to individual persons.

2.11.1 Insecticides

Insecticides such as pyrethrum extracts have been used extensively in mosquito control, but the quick bio-degradability and high costs of isolation of natural pyrethrins reduced their use. This accelerated the development of affordable and persistent synthetic

pyrethrins like permethrin and allethrin. Synthetic pyrethroids such as allethrin and permethrin have been effective insecticides. These compounds have however been found to be toxic to many non-target organisms and their persistence in the ecosystem. Moreover, increased vector resistance has been reported against synthetic pyrethroids (Shidrawi, 1990). The reported resistance to the synthetic pyrethrins calls for use of other more effective insecticides. Vector resistance to insecticides is a recurring theme and a major problem in malaria control programmes (Shidrawi, 1990). By 1985, at least 117 mosquito species had been reported as resistant to one or more of insecticides with 67 of these in the genus *Anopheles* (Pant, 1988; Shidrawi, 1990). The best evidence of resistance to pyrethroids in the *An. gambiae* complex is the 5.8 – fold tolerance to bioallethrin seen in a strain of *An. gambiae* from Burkina Faso (Malcolm, 1988; Pant, 1988). A population of *An. gambiae* in an area of western Kenya experienced a 2.5 - fold increase in the LT_{50} to permethrin exposure one year after a permethrin-impregnated bed net study was implemented (Vulule *et al.,* 1994). Organophosphate pesticides are alternatives to pyrethrins since they have short persistence. However, resistance has been reported to these insecticides as well (Lines *et al.,* 1984). Malathion resistance has been recorded from *An. arabiensis* in the Gezira District of Sudan (Lines *et al.,* 1984; Lines, 1988). The widespread use of persistent insecticides facilitates resistance development, especially when selection is applied against a large proportion of the population. DDT and other organochlorine insecticides exemplify this. Besides resistance development to these insecticides, they are toxicants to other non-target organisms including mammals, are a risk to the ozone layer and may therefore enhance global warming. The evolution of resistance to most insecticides by the vector has prompted the need to develop new tools for vector control.

2.11.2 Repellents

Repellents have also been used in vector control. Synthetic repellents such as dimethyl phthalate and 2-ethyl-1, 3-hexanediol have not provided a great impact in controlling the rate of inoculation and transmission of malaria parasite since most of these repellents are highly volatile and thus provide only short lived protection time against the vector. The most common mosquito repellent formulations available on the market contain Deet® (N, N-diethyl-3-methylbenzamide), which has shown excellent repellency against mosquitoes and other biting insects (Yap, 1986; Walker *et al.*, 1988; Coleman *et al.*, 1993)

2.11.3 Ethnobotanical plants

The use of plants as traditional natural repellents has been documented from many areas (Curtis, 1992), but most of the products from plants have not been carefully analyzed. In western Kenya, (Seyoum *et al.*, 2002a; Seyoum *et al.*, 2002b), showed that ethnobotanical studies of the traditionally used plants are indeed effective against malaria vectors when burned or thermally expelled with domestic charcoal stoves. These studies further showed that some plants were commonly applied by cutting the branches and placing them inside houses particularly around beds. Potted live plants have been shown to repel mosquitoes from human habitations in western Kenya (Seyoum *et al.*, 2002a).

Citronella products are used in India and are effective against anopheline mosquitoes, but their protective effects do not last long. In Tanzania, the smoke from burning plants provided some protection. However, the effectiveness of these methods is probably limited and will depend on both the biology of the local vectors and the intensity of the

transmission. In China, *Eucalyptus* spp and *Artemisia* have been used for years as traditional repellents for mosquitoes. The active principle, p – methane -3, 8 - diol has been isolated and is now used commercially as a repellent, Mosiguard® for personal protection (Kumar *et al.,* 1991).

2.11.4 Insecticide Treated Bed nets

The use of insecticide treated bed nets (ITNs) and curtains with pyrethroids seems to be the most promising available method of controlling malaria in endemic tropical countries. Several studies have shown that the uses of ITNs are effective in reducing morbidity and mortality due to malaria (Lengeler, 2000). A trial of permethrin-impregnated bed nets in Gambia resulted in a 70% reduction in clinical cases of malaria in children who slept under the nets (Alonso *et al.,* 1991). A series of impregnated bed net studies in Kenya documented a reduction in incidence of infections in children under 6 years during both the high and low transmission seasons. Entomological inoculation rates (EIR) declined by 50% during the high season. Nevertheless, acquisition of new infections still occurred at a very high rate during the high transmission season, and it was estimated that 100% of the children would have been infected with *P. falciparum* within 13.6 weeks in the bed net villages and within 10.6 weeks for the controls (Beach *et al.,* 1993). Mbogo *et al.,* (1996) observed that permethrin impregnated bed nets exert a major impact upon abundance of the indoor-resting principal vectors of *P. falciparum* in the coastal villages of Kenya. Indoor-resting densities of *An. gambiae s.l* and *An. funestus* were 9 times lower in the houses where ITNs were in use as compared to households where no nets were used (Mbogo *et al.,* 1996). The behaviour of the vectors largely affects the success of the control method. During the high transmission season, substantial numbers of

vectors may be feeding outdoors, often during the early evening before the usage of bed nets. Thus bed nets may be most useful in areas where transmission is less stable, seasonal or of low intensity. ITNs are now regarded as the panacaea to malaria control. This is due to its encouraging effects in reducing both morbidity and mortality among children. However, it has been observed that, there is a tendency of behaviour change by vector species in the areas where bed nets are in use (Mbogo *et al.,* 1996). Studies in Kilifi showed that, a significant proportion of malaria vectors appeared to bite earlier in the evening in the houses where ITN were used, with greater tendency towards exophagy rather than the typical endophagy of most anthropophilic *An. gambiae s.l* (Mbogo *et al.,* 1996). This change in behaviour renders the use of ITN less effective, as mosquitoes will often bite when bed nets are not in use. The use of ITNs in Kenya is not as widespread as might be desired. This is because; neither the nets nor the required insecticides are widely available or affordable to most communities. Other problems include non-compliance in the proper use of nets and failure to maintain the insecticide treatment rhythm.

2.12 *Anopheles* larval control

A potential target of malaria control is the anopheline larva. This is because the life cycle can be interrupted before the emergence of adults that bite and transmit malaria parasites. Source reduction through modification of larval habitats was the key to malaria eradication efforts in the United States, Italy and Israel (Kitron and Spielman, 1989). It is therefore rational that appropriate management of larval habitats in the sub Saharan Africa may help to suppress vector densities and malaria transmission rates. The control of mosquito larvae before emergence of adults thus remains the most efficient and economical means of controlling malaria epidemics.

The classical method that has been used to kill mosquito larvae involves the application of oil on water. The oil contains poisons that presumably affect the nervous system (Wigglesworth, 1976). *Anopheles* larvae below such film at 24°C should be dead in 2 to 3 hours. The mosquito larvae may also die from suffocation, the oil also reduces the surface tension hence the larvae cannot come out of the water for air. However, the oil film on the water surface is likely to prevent free exchange of oxygen between the water surface and the free air thus leading to suffocation of other non-target aquatic organisms. This factor has prompted the employment of other means of controlling mosquito larval populations. These methods include: environmental management, biological control, natural organic larvicides and botanicals or use of plant materials.

2.12.1 Environmental management

This involves practices that create unfavorable habitats for larval breeding. It may also involve the elimination of aquatic habitats. A simple approach is to fill with rubble, sand, and earth larval habitats of different sizes (Service, 1986). Other environmental modifications include the removal of overhanging vegetation to reduce breeding by shade loving mosquitoes such as, *An. dirus* (Service, 1986). Clearing of bushes can also eliminate the malaria vector by removing adult mosquito resting habitats. Planting vegetation along streams and reservoirs make habitats inimical to sun loving *An. gambiae*. However, this approach has not achieved much because it is impossible, to fill in all the scattered, small and temporary collections of water (Service, 1986). Secondly, the environmental changes such as agricultural irrigation schemes, creation of dams for water reservoirs and road construction or mining sites may favor the breeding of other species that were previously present in only small numbers or absent altogether (Service,

1986). Besides, the approach is labor intensive and costly thus untenable. There is, therefore, need to focus on more practical larval control methods.

2.12.2 Biological control

Biological control implies the use of predators, parasites or entomo-pathogens. Because of insecticide resistance and the adverse environmental impact of insecticide use, considerable resources have been devoted to the search for biological control agents. Several attempts have been made to control mosquito larvae by biological means. To date, only larvivorous fish have been used successfully in malaria control projects, and these cases are few. The use of North American fish *Gambusia affinis* successfully reduced malaria incidences in Italy and Greece, where malaria transmission was unstable (Wickramasinghe and Costa, 1986). In other studies vector densities and biting rates decreased or introduction of larvivorous fish. Previously, other fishes such as Armagosa pupfish (*Cyprinoden nevadensis armagosae*) and Guppies (*Poecillia reticulata*) were used (Moyle, 1976). Invertebrate predators such as Coleopterans, Dipterans and Hemipterans have also been examined but they are difficult to rear *en mass*; they feed non-specifically, and they do not persist once vector target densities are reached (Rishikesh *et al.,* 1988).

Rishikesh *et al.,* (1988) have summarized efforts to identify useful pathogens and parasites including viruses, fungi, nematodes and sporozoa. The main pathogens include: the fungi *Coleomomyces* spp, *Culicinomyces clavosporus*, *Metarhizium anisopliae*, and the *Lagenidium gigantium* which have demonstrated little or no adverse effects on populations of invertebrate and vertebrate non-target organisms (Lawrence and Cynthia,

1990), the protozoan *Nosema algerae* and the mermithid nematode *Romanomermis culicivorax*. None of these agents have shown any promise for wide scale larval control, having proven difficult to rear and store, as well as unstable or inefficient under the field conditions.

The bacterial endospore toxins produced by various strains of *Bacillus* species such as *B. thuringiensis israelensis* H-4 and *B. sphaericus* have also been used as larvicidal agents (Lacey and Undeen, 1986; De Berjac and Sutherland, 1989; David *et al.*, 1990). Their most attractive feature in vector control stems from the purported failure to induce mechanisms of resistance that confer cross-resistance to other classes of insecticides. They can also be produced on a local level with far less capital outlay than would be required for more traditional insecticides. Unfortunately, the *Bacillus* toxins are still relatively expensive. Since they have no residual activity, they either require frequent application or are only suitable for environments where a one-time control measure produces a valuable outcome.

2.12.3 Synthetic chemical larvicides

Vector control by synthetic chemical larvicides has been implemented in some circumstances, especially when the use of residual adulticides was not effective or too expensive. The choice of such larvicides for mosquito control has been based on the species and behaviour of the mosquitoes, hazards to domestic animals, wildlife, fish, other aquatic organisms, environmental pollution, presence of insecticide resistant mosquitoes and cost factors (Michael *et al.*, 1996). Paris green dust has been used to control larvae (Service, 1986). However, this compound is expensive due to its high

copper content. However, there have been no instances of mosquito larvae developing resistance to most of these larvicides (Service, 1986). This may be explained by the limited use. Most of these inorganic larvicides are highly toxic to aquatic organisms and plants because of relatively large amounts of water-soluble arsenic in them, which pose environmental pollution problems.

Synthetic organic chemicals have also been used in mosquito larval control. Use of emulsions or granular formulations of DDT, dieldrin, heptachlor, or lindane has been attempted (WHO, 1983). Where resistant strains are encountered, parathion or baytex has been used (Metcalf *et al.,* 1962). However, most of these larvicides are organochlorines, organophosphates, or carbamates that are toxic and have cumulative environmental effects and persistence in the ecosystem due to their resistance to enzymatic degradation by soil and other environmental micro-organisms and chemical reactions (Charlese *et al.,* 1995). Besides, widespread use of the same pesticides for the control of agricultural pests has led to rapid resistance development in vector populations. Temephos (Abate), an organophosphate of very low mammalian toxicity, has also been used to treat portable waters to control *Aedes aegypti* breeding in water storage pots (Service, 1986).

For most malaria vectors, reducing mosquito population densities by means of larvicides application is generally an inefficient way of reducing malaria transmission, because larval mortality among anopheline populations may be density dependent. Besides, when a large proportion of the larval habitat can be easily identified and targeted, larval control can be effective. The behaviour and ecology of the target vector determines the efficacy

of the larvicide. The number and wide distribution of these small pools may present insurmountable difficulties in control efforts using larvicides except in circumstances such as the eradication campaigns where the introduced species occurred in a limited geographic region.

2.12.4 Insect growth Regulators

The use of insect growth regulators (IGR) to control mosquitoes has also been attempted. IGR are chemicals which inhibit/disrupt growth of the insects. Most of these compounds have been grouped as: juvenile hormone mimics or chitin synthetase inhibitors (Laird and Miles, 1985). These compounds generally have no toxicity to other non-target organisms. They are relatively specific to the insect and primarily active against the immature stages of mosquitoes. However, they may kill beneficial insects. Currently, the most widely used IGR is Altosid® (Laird and Miles, 1985) that has no remarkable effects on non-target aquatic organisms. However, there is a great desire to obtain larvicides or IGR from inexhaustible natural sources such as plants that can be cultivated, extracted, and bio-degradable compounds obtained to avoid environmental pollution (WHO, 1996).

2.12.5 Natural organic larvicides

Various natural organic chemicals have been extracted from plants and bioassays carried out to determine their effectiveness as larvicides. One of the earliest reports of the use of plant extracts against mosquito larvae is credited to (Campbell *et al.,* 1933) who found out that plant alkaloids like nicotine, anabasine, methyl-anabasine, and lupinine extracted from the Russian weed *Anabasis aphylla* killed larvae of *Culex pipiens, Cx. quinquefasciatus*, and *Cx. territans.* Haller (1940) noted that extracts for Amur cork tree

fruit *Phellodendron amurense*, yielded a quick acting mosquito larvicide. The chemicals can be extracted from either whole plants or specific parts of the plants such as leaves, fruits, roots, and bark depending on the activity of the derivatives.

It has been shown that some limonoids (azadirachtin), quinones (plumbagin), alkaloids, flavonoids, terpenoids, polyacetylenes, and butyl-amides extracted from plants show a high degree of larvicidal activity against mosquito larvae (Kubo *et al.*, 1984). For instance, piperine and wisanine are alkaloids that were isolated from *Piper guineense* and were found to be very active on *Aedes aegypti* larvae (Addae-Mensah and Achieng, 1986). The same extract has been shown to have larvicidal activity against *An. gambiae* (Okinyo, 2002). Limonoids such as azadirachtin from *Azadirachta indica* and terpenoids such as 5-E-ocimenone from *Tagetes minuta* have been reported to possess larvicidal activity against mosquito larvae (Maradufu *et al.*, 1978). Larvicidal activity of long chain fatty amides such as N-isobuyl-2E, 4E, 8Z, 10Z-dodeca-2, 4, 8, 10-tetraenamide isolated from *Spilanthes mauritiana* have been reported (Jondiko, 1989). The amides from *Zanthoxylum gilleti* (*Fagara macrophylla*) have also been reported as larvicides against *Culex* species (Kubo *et al.*,, 1984). Their efficacy against *Anopheles gambiae* has since been demonstrated (Okinyo, 2002). Other plants that have been successfully tested for larvicidal activity include: *Vernonia ammophila, Swartzia madagarensis, Pogestemon cablin, Sium suave, Datira candida, Achryrolcline satureoides, Petiveria alliacea,* and *Gardenia lutea* amongst others (Michael *et al.*, 1991). The efficacy of most of these plant extracts as potential larvicides have only been tested under laboratory conditions. However, their efficacy under natural field conditions against natural anopheline larval

populations has not been investigated. As much as these investigations have not been done, their potential in mosquito control is thought to be high.

2.13 Environmental heterogeneity and larval productivity

The extent to which environmental heterogeneity affects patterns of vector production that are important for malaria parasite transmission is unknown (Grillet, 2000). The factors affecting larval survival and the mechanism controlling adult production are also largely unknown for even most important vector species. A potentially important target of malaria vector is the anopheline larva and source reduction through modification of larval habitats was the key to malaria eradication efforts in the United States, Israel and Italy (Kitron and Spielman, 1989). It is conceivable that appropriate management of larval habitats in sub Saharan countries particularly during dry season may help suppress vector densities and malaria transmission (Minakawa *et al.,* 1999). The understanding of anopheline larval ecology is limited and insufficient to achieve effective vector control through means of larval control (Oaks *et al.,* 1991).

2.14 Nutritional status of larval habitats

Since 1930 evidence has accrued that mosquito larvae could utilize dissolved nutrients (Beklemishev, 1930; Shipitzina, 1930). Filtered pond or infusion waters supported slow, larval development, the best growth occurring in waters thought to contain colloidal materials (Hinman, 1932; Trager, 1936). However, subsequent acceptance of the idea that mosquito larvae take very little dissipated interest in dissolved nutrients as a possible natural food resources, even as the burgeoning use of artificial diet showed that some drinking must be possible (Clements, 1963).

Experiments with holidic diets demonstrated that mosquito larvae could develop solely by taking dissolved nutrients (Dadd and Kleinjan, 1976). Actual uptake rates of two or three times the larval body weight per day were measured in osmotic-balance studies of salt water mosquito species in which oral intake of water countered osmotic loss through the cuticle (Clements, 1992).

Slightly reduced rates of larval imbibing were measured in several fresh water species for which the function of taking in water would be primarily for nutrient ingestion (Aly and Dadd, 1989). They argued that mosquito larvae drink copiously, that drinking rate can be increased by the presence of dilute colloids, and that it can be regulated independently of other mouth part activities that occur during feeding. Given these findings, there are natural circumstances when dissolved nutrients, could be an important food resource for mosquito larvae as recently suggested by (Wotton, 1990). For all suspension feeders useful nutrients could come through drinking if concentrations of dissolved materials were high enough. Higher concentration of dissolved organic material may occur adjacent to leaf and substrate surfaces or near decaying tissue their associated precipitin and bacteria. Such zones would be rich in gelled and colloidal solutions of macromolecular nutrients (Costern *et al.*, 1987). If such zones have a few percentages of balanced nutrients in solution, they could support mosquito growth. Howland, (1930) concluded that the abundance of algae in the larval food was correlated with algal abundance in the habitats and that culicines consumed more algae than anophelines. The relationships between habitat selection and intrinsic chemical properties of food and microhabitats have received much attention especially in phytophagus insects (Sota, 1993). For detritus feeders like larva of mosquitoes in aquatic habitats, the habitat water

contains both nutrients and deterrents (Parker, 1982; Fisher *et al.*, 1990; Sota, 1993). Various chemical properties of the larval habitat related to leaf litter such as pH, and concentration of ammonia, nitrate and sulphate affect larval development and survival (Peterson and Chapman, 1969; Zain and Newsen, 1979; Carpenter, 1982). In this study investigations on the effect of the physicochemical and agricultural factors on the productivity of the habitat were carried out. Inorganic ions and organic carbon sources such as nitrogen, phosphorus, sulphur, and carbon, which provide essential nutritional substances for microbial growth, were considered.

2.15 Survival of immature *Anopheles gambiae* s.l.

Life tables provide a structured framework for identifying developmental stages most susceptible to mortality and, under some conditions, for inferring sources of mortality (Service, 1993a). The life tables for the developing immatures can be constructed using either horizontal or vertical methods (Reisen and Siddiqui, 1979; Reisen *et al.*, 1982; Service, 1993a). Horizontal life table methods are appropriate for distinct cohorts that can be followed through time, whereas vertical life table methods are appropriate for populations with overlapping generations and age distributions that remain stationary for the duration of the sampling period. Service, (1973, 1977, 1993a), Reisen and Siddiqui, (1979) and Reisen *et al.*, (1982) provide extensive discussions about how such information can be analysed. In Kenya, Service, (1973; 1977) and Aniedu *et al.*, (1993) studied the survival of immature *An. gambiae* complex in the larval habitats. The survival of immature stages is important in determining the success of larval control programme. It is usually very important to determine the survivorship of larvae in aquatic habitats, which would be useful in evaluation of larval abatement programme in a locality.

2.16 The spatial and temporal distribution patterns of vectors

The natural distribution pattern of most disease vectors is largely determined by environmental conditions. For malaria vectors, each species has unique environmental tolerance limits. Vector distribution is highly dependent upon the availability of suitable breeding habitats and the proximity of the human host as a potential source of blood meals. The combined effects of the physical environment, the presence of compatible vectors and the degree of population mobility influence the malaria situation most (Bergquist, 2001).

Geographic information systems (GIS) and spatial statistics provide tools for studies of population dynamics of disease vectors in association with environmental and habitat features on multiple spatial scales. GIS is a computer-based system for automating, storing, manipulating and displaying mapped information and data (Chrisman *et al.*, 1989). It includes spatial data (locations) in form of geographic coverages (maps) and descriptive data (attributes) in the form of a relational database associated with the mapped features. GIS therefore allows for the overlaying of a variety of data coverages (e.g climate, vegetation type, habitat distribution, vector abundance and infection rates) to identify factors that may explain the spatial and temporal distribution patterns of vector and disease. Spatial statistics on the other hand are a set of tools developed largely by geographers and geologists to describe, explain, extrapolate and predict the distribution of objects and processes in space (Getis and Ord, 1992; Kitron *et al.*, 1998).

Non-geographic methods used in ecology include measures of aggregation, which are based on frequency counts without consideration of the actual geographic location. However, spatial statistics methods such as spatial autocorrelation, local spatial statistics,

kriging and spatial-temporal statistics consider both location and attributes or values of variables at particular locations and can be used to explain the distribution patterns of vectors, hosts and diseases. Thus, with the aid of spatial analysis, GIS can offer a way to identify and map the larval habitats of vector species, and their relationships to human settlements, thus predicting the potential risk for disease transmission.

The application of GIS and spatial analysis to define the epidemiology of vector borne diseases has been documented for a number of diseases, such as Lyme disease (Kitron *et al.,* 1991), Trypanosomiasis (Rogers and Williams, 1993) and malaria (Beck *et al.,* 1997; Omumbo *et al.,* 1998).

2.17 Rice cultivation and malaria

Although many species of mosquito thrive in rice fields (Carnevale *et al.,* 1992), flooded paddy fields provide ideal breeding sites for the principal vectors of malaria in Africa: Members of the *Anopheles gambiae* complex, especially *An. arabiensis* (White, 1972), prefer to breed in open sunlit pools (Gillies and De Meillon, 1968; Surtees, 1970; Coluzzi *et al.,* 1984). These vectors are pioneer species which rapidly colonize recently flooded fields, although they decline in abundance as the rice grows and begins to cover the water surface (Snow, 1983; Lindsay *et al.,* 1991; Ijumba, 1997). Irrigated-rice cultivation, depending on the number of cropping cycles, may also extend their breeding season and hence increase the annual duration of transmission. Moreover, in dry regions, irrigation will elevate relative humidity that aids survival of these vectors.

Although *An. gambiae sensu lato* is generally associated with irrigated rice, in certain situations *An. funestus* also thrives in paddy fields, especially in parts of Madagascar. *Anopheles funestus* typically favours breeding sites shaded by vegetation (Gillies and De Meillon, 1968) and its presence tends to be indicative of more persistent wetland habitats. In ricefields *An. funestus* occurs later in the growth of rice and when the land is fallow (Marrama *et al.,* 2004). In general, the predominant vector in irrigated rice systems is that found in surrounding areas, although there is at least one notable exception to this rule. The Mopti form of *An. gambiae* sensu stricto found in West Africa thrives in ricefields located in the northern fringes of the Sahel. Thus, in Burkina Faso this cytotype was common in the centre of the rice fields, but at the edge of the irrigated area the Savanna form was more abundant (Robert *et al.,* 1992). Irrigated rice fields represent ideal breeding sites for mosquitoes and they can generate large numbers of individuals, although a smaller proportion is infective in rice field villages than control communities. Thus, transmission intensity in irrigated settlements can appear higher, similar or less than in neighbouring villages outside the irrigation scheme. For example, in the rice-growing area of the Rusizi valley, Burundi, the vectorial capacity of *An. gambiae* s. l. was 150 times higher in the rice irrigation scheme than in an adjacent area growing cotton (Coosemans, 1985). Alternatively, in the rice-growing areas of Bobo Dioulasso, Burkina Faso, the number of infective bites received in the local community was similar to that in the control area (Robert *et al.,* 1985). Whilst in the Lower-Moshi rice irrigation scheme, Tanzania, the number of infective bites was 2.6 times lower in the irrigation scheme than in the control village (Ijumba, 1997). However, when entomologists measure exposure they may not accurately reflect the levels experienced by individuals in the study community. Mosquitoes collected off human baits or from light traps will overestimate

true biting rates, particularly when large numbers of mosquitoes are biting, since people will often avoid receiving large numbers of bites by sleeping under a bed net or use some other means of protection.

2.18 Malaria transmission intensity in rice agro-ecosystem

An alternative method for assessing transmission intensity is to determine the level of infection experienced in the human population. The observation that rice fields frequently generate large numbers of mosquitoes suggests that malaria transmission will increase in local communities. However, there is an increasing body of evidence that indicates that this is not the case, at least in areas of stable transmission, where malaria may be less of a problem than in surrounding communities outside the rice fields. A review of the literature shows that high vector density does not necessarily imply an increased risk of exposure to malaria parasites. In a study of two communities in the Valleae du Kou, Burkina Faso, malaria prevalence varied between 35±83% in the savannah and 16±36% in the rice-growing area (Boudin *et al.,* 1992). A study in the Lower Moshi rice irrigation scheme, Tanzania, showed that malaria prevalence was four times lower in children living near irrigated rice cultivation compared with a nearby savannah village (Ijumba, 1997). When a large-scale rice irrigation scheme was introduced in The Gambia, there were anecdotal reports of increased malaria in local communities.

However, on closer examination, it was apparent that there was less malaria near the rice field than in other rural communities (Lindsay *et al.,* 1991). Another important finding from this study was that during the dry season there was anophelism without malaria. Whilst enormous numbers of vectors were produced by the rice fields during the dry

season there was little, or no, malaria in children in this community at this time of year. The main reason for this finding was probably related to the extremely high temperatures experienced during the dry season, often rising above 40°C during the day. Such exceptionally hot weather reduced the survival of adult mosquitoes and perhaps, more importantly, killed the developing parasites within the vector. The critical finding was that there were few infective mosquitoes during the hot, dry season. Similar levels of malaria transmission have also been reported in rice field communities and control villages in the same district. For example, following rice irrigation development in the Senegal River valley, the prevalence of malaria transmission and incidence rates remained unchanged (Faye *et al.,* 1995). This general observation that rice fields do not increase the risk of malaria is also characteristic of some areas with exceptionally high numbers of mosquitoes. In The Gambia most *An. gambiae* s.l. breed on the edges of large pools bordering the River (Bogh, 2000; Thomas and Lindsay, 2000), which generate large numbers of adult mosquitoes. Interestingly villages closest to the breeding sites had less malaria than those further away (Thomas and Lindsay, 2000). Such effects also operate at a coarser spatial scale, with areas with the largest numbers of mosquitoes having less malaria than those with fewer mosquitoes (Thomson *et al.,* 1994).

2.19 Malaria protection in rice agro-ecosystems

Reasons for malaria protection are many and varied. One plausible explanation is that the introduction of irrigated-rice cultivation results in wealth creation in local communities (Robert *et al.,* 1985; Boudin *et al.,* 1992). And with this additional wealth, farmers make improvements to their homes, their standard of living rises and they have more disposable income with which to use to protect themselves from nuisance mosquitoes and malaria. In Kenya, it was found that at a certain threshold of income the situation becomes favourable for adoption of malaria control measures at a family level, and this goes hand in hand with improvement of the living standard of the family or community (Mwabu, 1991). Therefore, a community with relatively higher economic development would be associated with greater use of anti-malarial measures, and also, within a community, wealthy individuals would be more likely to use such measures.

Support for this comes from studies that have shown that bednet ownership was related to affluence in The Gambia (D'Alessandro *et al.,* 1995) and Tanzania (Ijumba, 1997). Income and wealth clearly affect the severity of the malaria problem. If the population has the financial resources to build housing inhospitable to mosquitoes, is knowledgeable about the use of personal protection measures and can afford them, understands the importance of seeking effective treatment at the first sign of illness, and can pay for health services and drugs, the rates of severe morbidity may be substantially reduced, despite being in an area of high risk (Oaks *et al.,* 1991). Implicitly, severe environmental health problems affect countries and people who lack access to economic and other resources, people who are denied opportunities to improve their lot. There is enough historical evidence to support the view that economic development in general has an

overall positive impact on health (Carrin, 1984; Stewart, 1985). The disappearance of malaria in some parts of Europe was associated with economic development as a result of agricultural expansion rather than vector control or chemotherapy (Cambournac, 1994; Najera, 1994; Zulueta, 1994). At a certain threshold level of income, it becomes possible for the family to invest in antimalaria measures in order to save on direct costs associated with malaria treatment (Wang'ombe and Mwabu, 1993). Increased incomes lead to better nutrition, habitation and protection factors that often influence the overall health status more than public health expenditure on health care. Therefore, if irrigation contributes to increased incomes, this will be reflected in better health although this process may not be particularly rapid (Oomen *et al.,* 1988).

Large-scale irrigated rice cultivation can result in several thousand mosquitoes entering a local house during the night. For most people such high biting rates are unacceptable and they will protect themselves by sleeping under bednets. Studies in The Gambia have shown that more people start using bednets when mosquito numbers begin to increase (Aikins *et al.,* 1993; Thomson *et al.,* 1994; Bogh, 2000). Bednets in good condition reduce biting rates (Lindsay *et al.,* 1989) and can protect against malaria (Bogh, 2000). Thus, it is likely that part of the association between rice and moderate malaria may be explained by the high bednet coverage in communities living near irrigated rice production. Thus, high net use has been reported from rice villages in Burkina Faso (Robert *et al.,* 1985), Cameroon (Robert *et al.,* 1992) and The Gambia (Lindsay *et al.,* 1991). Moreover, at high mosquito densities, density-dependent effects become important. With high net use, individual mosquitoes find it difficult to locate and obtain a human blood meal. It has been postulated that mosquitoes that find it difficult to feed on

humans may be displaced and feed on other animals, such as cattle. Such a marked shift in feeding behaviour was suggested as the reason for the low sporozoite rates found in three villages in the Senegal River delta where *An. gambiae* s.l., *An. funestus* and *An. pharoensis* were vectors (Faye *et al.,* 1995). Whilst *An. gambiae s.l.* will take proportionately more blood meals on cattle when cattle numbers increase (Lindsay *et al.,* 1991), there is no evidence that zooprophylaxis is protective when cattle are kept close to households either when the principal vector is *An. gambiae s.s.* (Bogh, 2000) or *An. arabiensis* (Ghebreyesus *et al.,* 2000). Widespread use of anti-malarials (Carnevale and Robert, 1987; Faye *et al.,* 1993b) in ricefield communities and the existence of a well established health infrastructure (Faye *et al.,* 1995) may also contribute to the general lower level of malaria, although the growing problem of antimalarial resistance will make control more difficult in the future. It is of course also important that irrigation schemes are well designed and maintained to reduce standing water to a minimum and thus limit the opportunities for mosquito breeding.

CHAPTER 3: GENERAL MATERIALS AND METHODS

3.1 Study area

This study was carried out in the Mwea Rice Irrigation Scheme in Kirinyaga District of Kenya (Figure 3). This site is near a riverine system, which provides the main source of water, used to periodically irrigate fields for rice and other crops. The average temperature is about 23^0C-25^0C, with about 10^0C difference between the minimum temperatures in June/July and the maximum temperatures in October/March (Ijumba *et al.*, 1990; Mutero *et al.*, 2000). The area has four seasons, which are typical of tropical areas; a hot, dry period followed by a short rain period, which, in turn, is followed by a cool, dry period and then a long rain period. The irrigation phase of the rice cultivation cycle in August, during a normal dry period, links the flooding effects of the two rainy seasons (Mukiama and Mwangi, 1989a). However, as reported widely, unexpected periods of drought may occur at both sites, which have an impact on vector abundance. Several agricultural practices regarding flooding and planting times are utilized at this rice-growing complexes (Ijumba *et al.*, 1990). The total area and the area devoted to rice cultivation at Mwea are 13,640 and 6,138 hactare, respectively. The human population density of Mwea is 1.8/ha. The annual average precipitation for Mwea is 950 mm. Furthermore, the long rains fall between March and May while short rainy period is between October and December.

As is characteristic of many East African rice irrigation systems, prevalence of malaria in humans in Mwea, was 26% higher in the rice villages than the surrounding areas (Ijumba

et al., 1990). Vectorial capacity in these rice irrigation-villages is high, primarily due to the high biting rates of *An. arabiensis*. Personal protection rates against mosquitoes in Mwea exhibit mixed results, but this method alone do not significantly reduce transmission of malaria in humans (Mwabu, 1991; Snow *et al.*, 1998).

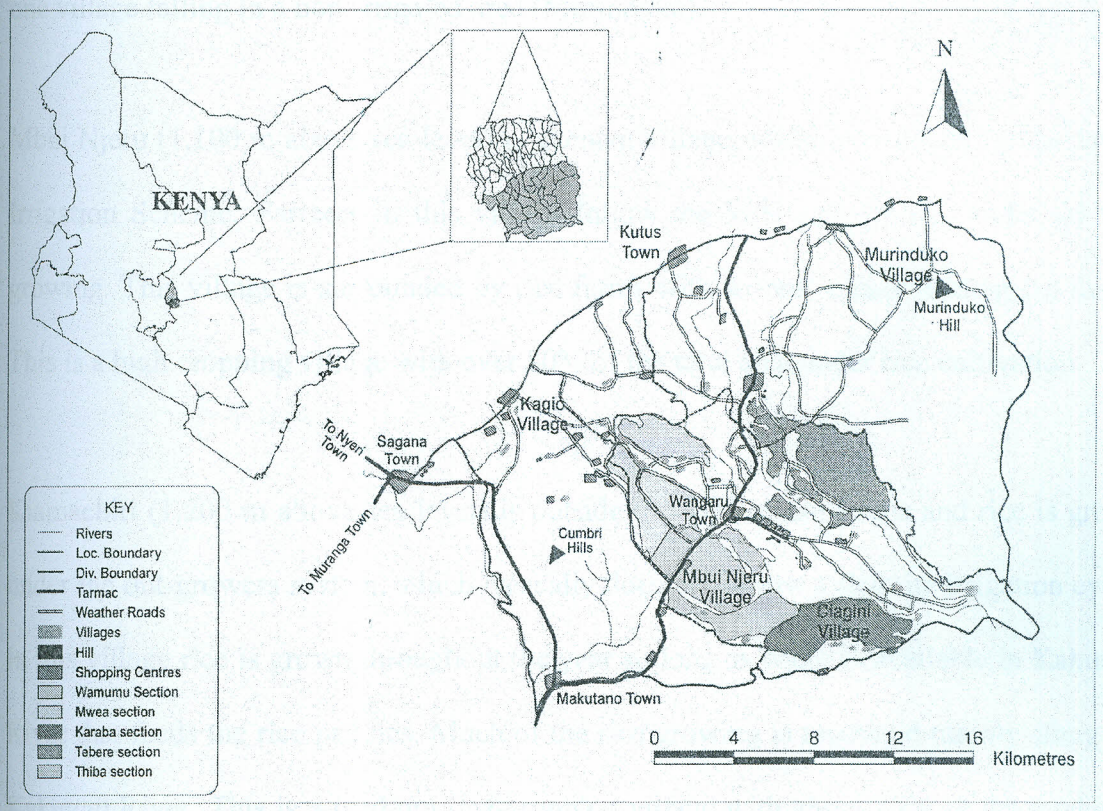

Figure 3: Mwea Rice Irrigation Scheme and study villages.

(Source: Mutero *et al.*, 2004)

3.1.1 Study villages

Three villages within and around the rice growing complex were selected from the Scheme for larval ecology work (Figure 3). The selection plan was to assess villages within 3 categories: 1) One village within the planned irrigation scheme (i.e. Mbui Njeru), 2) one village falling in the "unplanned" rice growing area (Kiamachiri) and 3) one village falling in a non-irrigated area (Murinduko).

Mbui Njeru (1,100 m above sea level) is a tenant village, which was set up by the Mwea Irrigation Scheme. Farmers in this village follow the MIS irrigation calendar for rice growing. This village is surrounded by rice farms with several canals feeding the farms. This is a high cropping village with over 80% of the total area under rice cultivation.

Kiamachiri (1,200 m above sea level) is outside the MIS tenant farms and rice is grown under the out-growers system, which basically does not follow a definite irrigation cycle. In this village rice is grown throughout the year so long as water is available in Kakungu River that feeds the rice paddies. Much of the rice growing is restricted mainly along the Kakungu River. This is a medium rice-cropping village with about 25% of the total area with rice.

Murinduko is a high attitude village (1,350 m above sea level) falling outside the MIS. This village has a topography tapering towards valleys at lower part of the village. Two springs of water originate from this village and flow as streams at the edges of the village. The soil in this area is highly porous and seepage of rain water in quite high.

Although it is a non-irrigated village, the inhabitants have started using the stream water for rice growing on a small scale. This is a village with minimum rice cropping (low) with less than 5% of the total area under rice cultivation.

3.2 Rice cropping cycle

In Mwea rice irrigation scheme, two rice crops are grown annually. The long rain crop is grown between January and June and the short rain crop between August and December. Between the two cycles, it is recommended that the land be left furrow for a minimum of 21 days. However, currently ratooning is being encouraged over the second season crop since it is more economically viable. The schedule of husbandry could be conveniently described under four operations namely land preparation, nursery development, transplantation and harvesting. The cycle begins with preparation of the land, which involves burning of vegetable wastes, and repair of canals, roads and drains. This is closely followed by leveling and banding after which the land is ploughed and flooded with water. Following the ploughing, nursery beds are prepared in the corners of the paddies.

3.2.1 Rice management in the nursery bed

The nursery bed is first raised by wet bed method that involves puddling and leveling. Prior to introduction of rice seeds into the nursery, the seeds are first soaked in water for 24 hours followed by incubation under rice straw for a period of 48 hours to encourage germination. Thereafter, the seeds are uniformly broadcasted on wet nursery bed at a seedling rate of $100g/m^2$. 57.5 kg/ha of sulphate of ammonia are also applied as a source of nitrogen at the time of sowing while an additional 57.5 kg/ha of the same is applied 14

days later. Immediately after sowing, the water level in the nursery is adjusted to an average depth of 3 cm. Bird scaring should also be done until the rice germinates and becomes well established. Fentrothion is applied 7-10 days after sowing at a rate of 400ml/acre to control insect pests mainly the stem borers and leaf miners. Most of the rice varieties grown in the scheme are disease resistant and therefore not treated before sowing. However, in cases where a susceptible variety such as IR is sowed, the seeds are usually top dressed with the fungicide benlate, which is protective against fungal diseases including stem rot and brown-leaf-sheath-rot. Seedlings are ready for transplanting 28 days after the sowing date. The timing of the sowing of the rice seeds in the nursery should correspond with the cropping cycle. For the long rain crop, it should be done between mid December and mid January and that of short rain crop between July and end of August.

3.2.2 Management in the main field

3.2.2.1 Land preparation

On the flooded paddy, animal paddling is done to soften and mix the mud. At final paddling time, triple super phosphate (TSP), di-ammonium phosphate (DAP) or single super phosphate (SSP) is applied at a rate of 50kg/acre for TSP and DAP or 100kg/ha for SSP after which the land becomes ready for transplanting.

3.2.2.2 Transplanting

During transplanting, the water level is lowered to a depth between 5 – 10 cm. 60 kg/ha of muriate of potash and 39kg/ha of sulphate of ammonia (or urea at 80kg/ha) are applied

during transplanting to provide source for potassium and nitrogen, respectively. Seedlings are transplanted at a rate of two per hole and 20 x 20cm spacing and the water level is raised to an average depth of 5cm immediately thereafter. Ten days after transplanting, gapping is done to replace dry or weak seedlings.

3.2.2.3 Insect control

The main pests of rice in the area are leaf miners (genus *Leucoptera*), stem borers and armyworms. Up to the early 1990s, agricultural spraying of fentrothion and occasionally DDT 25% wettable powder and furadan 5G were used to control these pest populations. However, owing to the residue effect of DDT and furadan as well as insecticide resistant development, the two chemicals have been phased out. Currently fentrothion is the main chemical used to control insect pests. It is applied at a rate of 400ml/acre 35 days after transplanting. This dose is quite effective against a wide rage of insect pests including leaf miners, stem borers, cutworm.

3.2.2.4 Weed control

The weeds of economic significant in the scheme are *Vandelia anagallis*, *Lythraceae*, *Juncus effuses* (commonly known as *kitunguu*) and sedges. They are controlled by application of satunil herbicide at a rate of 90ml/ha at 1-2 leaf-stage of weed followed by manual weeding when necessary. The first, second and third weeding is done 16 days, 35 days, and 50-60 days, respectively after transplanting (only if necessary). Most parts of the scheme are covered by an aquatic plant known as *Azolla*, which has been observed to suppress other weeds, by covering the entire surface of the paddy field with no effect on

rice production. Unconfirmed reports also suggest that this characteristic also inhibit the breeding of mosquitoes.

3.2.2.5 Top dressing

During panicle initiation stage 39kg/ha of sulphate of ammonia are applied to the crop to top up the nitrogen content of the soil. Addition of the nitrogenous fertilizer increases production of tillers, increases spikelet numbers and has an overall increase in grain yield. The fertilizers ensure the crop development is enhanced and its vegetation generally flourishing well.

3.2.2.6 Bird scaring and rogueing

Other important operations include bird scaring and rogueing. Rogueing involves removal of the unwanted and damaged parts of the rice crop and is carried out before the onset of panicle formation and near maturity. Bird scaring starts at the onset of flowering and goes on until harvesting to minimize crop loss from birds.

3.2.2.7 Harvesting

When the paddy is 85% mature, the level of the water is left to go down (for dry season) or drained (for wet season) in preparation for harvesting. Two weeks later, the rice is harvested and the paddy separated manually from the straw. The yield per hectare varies with the rice variety. For instance, BASMATI 217 yields between 4.6 and 7.4 tonnes per hectare in research fields and between 3.3 and 6.5 tonnes per hectare in farmers' fields. The total growth period is usually 4-5 months. After harvesting, the land is left dry until

the commencement of the second season if the farmer is not interested in raising up the ratoons.

3.3 Meteorological data

In each village, a rain gauge (Tru-Chek®, Rain Gauge Division, Edwards Manufacturing Co. Albert Lea, Minnesota, USA) was placed and read daily at 0900 Hrs. One HOBO® Micro Station (Onset Computer Corporation, Bourne, Massachusetts, USA) was set at Mwea Irrigation and Agricultural Development (MIAD) Centre. Rainfall, Relative humidity and Temperature were read daily every 3 hours. BoxCar Pro (Onset Computer Corporation, Bourne, Massachusetts, USA) was used to download the weather information every month end.

3.4 Anopheline larval sampling

At the study site, rice fields were sampled biweekly along cross-sectional transects to generate stage-specific estimates of *Anopheles* larval density. Rice paddy measuring 60 m by 60 m was treated as one larval habitat. Different rice growth stages and scenarios in the rice paddy were treated as sub-blocks and sampled differently. These sub-blocks made up the main rice paddy. Habitats that were within the village (non-rice fields) were also sampled during the rainy season. These non-rice field habitats were followed until when they dried up and monitored every time to check whether they had water. Samples were taken using standard dippers (Danislozano *et al.*, 1997; Service, 1993a).

3.5 Physicochemical characterization of larval habitats

The larval breeding habitats were characterized to determine the environmental and chemical factors, which may influence the productivity of anopheline mosquitoes. The factors which were assessed include: a) distance to the nearest dwelling house, b) surface debris, c) emergent plant cover d) algae cover e) turbidity f) presence of *Azolla* g) volume of the habitat (length, width and depth) h) habitat permanence (permanent or seasonal) i) rice growth j) pH k) conductivity l) temperature m) relative humidity, n) nitrate content, o) phosphate content, p) ammonia content, q) sulphates.

3.6 Measurement of environmental and physicochemical variables

The environmental variables were measured using the technique described by Minakawa *et al.,* (1999). In brief, emergent plants included both aquatic and immersed terrestrial vegetation. Plant coverage of a habitat was measured in percentage of water surface covered by placing a square frame (1 m^2) with grids above the habitat. Distance to the nearest house (human habitation) was measured when it was shorter than 100 metres. When the distance exceeds 100 metres it was estimated. Algae cover and debris were estimated as a percentage of the part of the larval habitat, which they cover using a square grid. The presence of *Azolla* was estimated as a percent of the area covered by the floating *Azolla* in the paddy. All the estimations done in this study were done by one person in each village throughout the sampling period to avoid discrepancies. The habitat types included pond, pool, puddle, canal, ditch, tyre truck, hoof prints, seep, marsh and swamp. Rice growth was determined by assessing the rice growth cycle. Rice growth cycle was categorized as: ploughed, transplanted, tillering, booting and maturation stage,

and harvesting. The pH, conductivity, dissolved oxygen and temperature were measured using hand held machine YSI 650 Multiparameter Display System (YSI Environmental, YSI Incorporated, Yellow Springs, USA). Salinity and Total Dissolved solids (TDS) were measured using field hand held equipment YSI EC 300 (YSI Environmental, YSI Incorporated, Yellow Springs, USA). Turbidity was measured by placing water samples in a glass test tube and holding against a white background and will be classified into four levels: clear low, medium and high. The micronutrients i.e. nitrates, phosphates, ammonia and sulphate content were measured using hand held field machines (HACH®, DR/2400 Spectrophotometer, Hach Company, Ames, Iowa).

3.7 Experimental plots

The experimental plot was developed at the Mwea Irrigation and Agricultural Development (MIAD), experimental station, in Mwea Irrigation Scheme. One rice test plot (1 Acre; 63m x 63m) was established in the Mwea rice irrigation scheme. Within each acre plot, 8 blocks (50.4m x 3.15m) each with 8 sub-blocks (6.3m x 3.15m) were established. Each block was hydrologically isolated using unidirectional inflow and outflow canals to avoid mixing between plots. The plots were exposed to natural colonization of *An. gambiae* complex.

3.8 Larval productivity and agricultural practices

The influence of agricultural practices used at the scheme and rice farmers such as fertilizers, and herbicides usage, on the anopheline larval abundance was studied. At the experimental plots, conditions similar to those used by the farmers in the field was used.

A control plot was used in which the rice was grown without using the agricultural chemicals. The plots were sampled at pre-treatment, 1 day post-treatment, and then every 3-5 days post-treatment for approximately 4 weeks. Anopheline larval densities were determined using standard dipping technique to assess the larval abundance in the plots. Weekly sampling of larvae and other aquatic invertebrate was done in order to monitor weekly trends of their densities over the rice cropping cycle. At each sampling, rice growth was monitored (plant height, rice stage, number of tillers and water depth).

3.9 *Anopheles* larval productivity from different habitats

A cross-sectional survey was done to assess the productivity of *Anopheles* mosquitoes from the rice fields. Ten cages measuring one metre by one metre by one metre (1 m^3) were placed on different habitats types, randomly selected, to monitor the emerging adults for 14 days. The cages were constructed from metal frames and a fine netting material was placed over it. The breeding habitats were selected based on productivity of *Anopheles* mosquito larvae. The breeding habitats were covered to exclude any adult mosquito from oviposition.

The cages were inspected daily for the presence of emergent mosquitoes and if present were collected by use of aspiration method (WHO, 1975). The mosquitoes in the cage were aspirated and placed in a paper cup. The mosquitoes were provided with 6% sucrose solution (w/v) placed in a cotton wool placed on the paper cups. These mosquitoes were kept in a cool box and transported to the Laboratory for identification.

3.10 Survival of *Anopheles gambiae* s.l. larvae

3.10.1 Horizontal life tables

Blood-fed *An. gambiae* s.l. females were collected with aspirators from houses throughout the villages and kept in cages containing 2% sugar solution in a semi field condition at the MIAD field station until they become gravid. Petri dishes containing a small amount of distilled water were placed inside the cages to attract oviposition in a screen house. Newly laid eggs in these containers were monitored every 0.5 h to determine their time of eclosion. Approximately 2h after eclosion, 100 first-instar larvae (L1) were collected for each replicate. These were transferred to larval trays in the screen house at an initial density of 14 larvae/cm^2 of pan surface area (L/cm^2). The screen house provided a semi field condition to the mosquito larvae during the entire development period. Briefly the screen house was constructed with wooden framework and a plastic mosquito netting pinned on the sides. The plastic netting material was reinforced with chicken mesh wire. At the roof, transluscent roofing material was used. This ensured that the there was penetration of sunlight to the trays naturally. The mosquito proof netting ensured that any ovipositing adults excluded from the structure. The mosquito larvae rearing was done using water collected from the inlet canal to the paddies in the field station. Duration of the preadult development period was determined by observing each cage at 0900 and 1600 h daily. The time taken in days for the larval developmental stages was recorded till emergent of adults. HOBO® was used to record daily hourly temperature profiles for the experimental plots during the time of study.

3.10.2 Vertical life tables

Five paddies were selected at the Mwea Irrigation and Agricultural Development Centre (MIAD) for the sampling in this study. The 5 paddies were measuring 40 m by 80 m and were followed from transplanting till tillering. The numbers of larvae were counted per dip and recorded in each sampling. Twenty dips were taken from each paddy. The sampling was done twice every week (Mondays and Thursdays). The paddies were characterized visually and using hand held field machines.

3.11 Identification of anopheline larvae

The mosquito larvae collected were sorted out according to the sub-families. The anopheline larvae were grouped according to the instar stage. The early instars consisted of L1 and L2 while late stage instars comprised of L3 and L4 stages. The late instars were identified morphologically (Gillies and Coetzee, 1987). The morphological features, which were examined, included the distance between inner clypeal hairs, long mesopleural hairs, which are simple, thoracic hairs, palmate hairs, saddle hair, main tergal plates and the accessory plates. The pupae were kept in the insectary in cages to develop into adults. The emergent mosquitoes were then used for morphological identification (Gillies and Coetzee, 1987).

3.12 Data Management

The data was analysed using SPSS for windows Ver 11 (SPSS Inc., Chicago, IL). Multiple logistic regression analysis was used to determine the association between the environmental and agricultural variables and the occurrence of anopheline larvae. The

occurrence of a species was defined as the presence of a particular species in a sample regardless of its density. Multiple regression analyses were used to determine the correlation between environmental and agricultural variables and the relative abundance of *Anopheles* larvae in a habitat. The relative abundance of *Anopheles gambiae* was calculated as the number of *An. gambiae* larvae divided by the number of dips taken from each larval habitat. The dependent variable (relative abundance of a species) was transformed using the log transformation method {log (x+1)}. One-way Analysis of Variance (ANOVA) was used to test for site-to-site variation in larval densities.

For the survival of the anopheline larvae vertical and horizontal tables were constructed (Reisen *et al.*, 1982; Service, 1993a) . When all adults had emerged, stage-specific survivorship for the pooled data were estimated as:

$$S\,\mathrm{i} = n_{\mathrm{i}} / (n\,\mathrm{i} - 1),$$

where

n i = total number of immatures entering life instar i,

and n i - 1 = the number alive in the previous instar.

Mean instar duration in hours at molting, T i, was:

$$D\,\mathrm{i} = T\,\mathrm{i} - (t_{\mathrm{i}-1}),$$

where $t_{\mathrm{i}-1}$ was the previous mean age at molting.

The percentage of total immature life spent at each instar was:

$L_i = 100 \times D_i / t_5$,

where t_5 was the median time of adult emergence.

Relativized probability of capture in a vertical sample was:

$P_i = D_i / D_p$,

where D_p was the duration of the shortest-lived life stage, which was taken as the standard.

Survivorship from L1 to adult emergence was estimated by:

A/I,

where A = total number of adults

and I = total number of L1 originally counted into the rearing trays.

CHAPTER 4: SPATIAL AND TEMPORAL DISTRIBUTION OF *ANOPHELES* LARVAE IN THREE AGRO-ECOLOGICAL VILLAGES IN MWEA IRRIGATION SCHEME, KENYA.

4.1 Introduction

In developing countries, irrigated farming has become increasing important as a means of boosting food production. Irrigation development projects worldwide have been associated with negative impacts on human health, particularly with respect to vector-borne diseases. Evidence for a direct relationship between irrigation development and increased malaria transmission is inconsistent (Harrison and Scanlon, 1975; Ijumba and Lindsay, 2001; Oomen *et al.,* 1994), with increased transmission in some situations (Coosemans, 1985; Goonasekere and Amerasinghe, 1988; Robert *et al.,* 1992) but not others (Boudin *et al.,* 1992; Robert *et al.,* 1988).

In Kenya, only a small fraction of the potential area has been developed for irrigation. Rice is usually grown under irrigation throughout the cropping cycle in Kenya. Rice fields generally constitute an important source of vector mosquitoes (Lacey and Lacey, 1990). The provision of mosquito breeding sites associated with irrigation for rice usually results in a corresponding increase in prevalence of malaria and other water borne diseases. Surtees (1970), working in Ahero irrigation scheme of western Kenya showed that there was a 70-fold increase in the number of malaria vectors, mainly *An. arabiensis* in the scheme compared to the nearby non-irrigated areas. Recently in Mwea irrigation Scheme of central Kenya, (Mutero *et al.,* 2004a) showed that there is a 30 – 300-fold increase in the number of the local malaria vector, *An. arabiensis* in villages with rice irrigation compared to those without irrigation.

The suppression and even eradication of malaria from vast areas has been attributed to effective large-scale programs to kill the immature *Anopheles* species vectors or reduce the amount of suitable habitat for them in proximity to vulnerable human populations (Killeen *et al.,* 2002; Gu and Novak, 2005). The appropriate management of larval habitats during the dry season may help suppress vector densities and consequently, malaria transmission. However, our understanding of anopheline larval ecology in Africa is insufficient and this affects the design and implementation of larval control. The objective of this study was to describe key anopheline larval habitats, and to determine the spatial and temporal pattern in larval densities in 3 agro-ecological villages in Mwea Irrigation Scheme, central Kenya.

4.2 Materials and methods

4.2.1 Larval habitat characterization

The larval breeding habitats were characterized to determine the environmental factors, which may influence the productivity of anopheline mosquitoes. The factors which were assessed included: distance to the nearest dwelling house, Surface debris, emergent plant cover, turbidity, presence of *Azolla*, habitat permanence (permanent or seasonal), rice growth cycle and presence or absence of other invertebrates. The habitat characterisation was done as described in section 3.6 of this thesis.

4.2.2 Rainfall and relative humidity

In each village, a rain gauge (Tru-Chek®, Rain Gauge Division, Edwards Manufacturing Co. Albert Lea, Minnesota, USA) was placed and read daily at 0900 Hrs. One HOBO®

Micro Station (Onset Computer Corporation, Bourne, Massachusetts, USA) was set at Mwea Irrigation and Agricultural Development (MIAD) Centre. BoxCar Pro (Onset Computer Corporation, Bourne, Massachusetts, USA) was used to download the weather information every month end.

4.2.3 Larval sampling and identification

At the study sites, rice fields were sampled biweekly along cross-sectional transects to generate stage-specific estimates of *Anopheles* larval density and invertebrate abundance and diversity (samples of invertebrates supported their potential as predators). Rice paddy measuring 60 m by 60 m was treated as one sampling block. Different rice growth stages in the rice paddy were treated as sub-blocks. Peri-domestic larval habitats within the village were sampled mainly during the rainy season. These non-rice field habitats were followed until they dried up and monitored biweekly to check for presence of water. Samples were taken using standard dipping technique and a plastic dipper (BioQuip Products, Inc. California, USA) with a wooden ladle was used. Depending on the habitat type, 5 to 25 dips were made.

The mosquito larvae collected were sorted out according to the sub-families as either anopheline or culicine. The anopheline larvae were grouped according to the instar stage and identified morphologically (Gillies and Coetzee, 1987). The pupae were kept in the insectary in cages to develop into adults and the emergent adults were then identified morphologically.

4.3 Data analysis

The statistical analyses were done using SPSS software (Version 11.5 for windows, SPSS Inc., Chicago, IL). Descriptive statistics was used to tabulate the larval densities and abundance from each village. The dependent variable (relative abundance of a species) was transformed using the log transformation method {log (x+1)}. One-way Analysis of Variance (ANOVA) was used to test for site-to-site variation in larval densities. Pearson correlation was used to determine the association between *An. gambiae* and rainfall. Multiple regression analyses were used to determine the correlation between environmental and agricultural variables and the relative abundance of *Anopheles* larvae in a habitat.

4.4 Results

4.4.1 Larval densities

A total of 26,077 *Anopheles* larvae were collected in the 3 villages in which, 87.76% (n = 22,885) were early instar stage and 12.24% (n = 3,192) were late instar stage (Table 4.1). Murinduko had significantly higher larvae than Kiamachiri and Mbui Njeru ($F_{(2, 182)}$ = 38.685, $p < 0.01$). There was a significant variation between sites in larval abundance and the Tukeys Honest Significant Difference ($\alpha = 0.05$) further indicated that the 3 villages are different from each other in larval abundance. A total of 3,173 pupae were collected from the three villages. The mosquito larval densities confirm that there was a significant site-to-site variation in the 3 villages.

In the 3 villages Murinduko had 7 habitat type categories while Mbui Njeru and Kiamachiri had each 5 habitat types. Paddies and canals had the most larvae followed by the marshes. Tree holes and rock pools were only found in Murinduko. In Murinduko water reservoirs had more larvae than in Mbui Njru and Kiamachiri. Pools formed during the rainy season were important larval habitats in Murinduko and Kiamachiri but harboured fewer larvae in Mbui Njeru.

4.4.2 Pupal densities and adult emergence

Of the 3,173 pupae collected, 1,104 pupated into adults and these were then identified morphologically. The species composition of the emergent mosquitoes was made up of 16 species. The 16 species included: *An. gambiae*, *An. funestus*, *An. pharoensis*, *An. rivorulum*, *An. maculipalpis*, *An. rufipes*, *An. coustani*, *Cx. quinquefasciatus*, *Cx.*

annulioris, Cx. tigripes, Cx. poicilipes, Ae. circumluteolus, Ae. taylori, Ae. aegypti, Mansonia spp., and Filcabia spp. Anopheles gambiae s.l. was the most predominant anopheline species while *C. quinquefasciatus* was the most common Culex species.

Table 4.1 Total number of *Anopheles* larvae collected from each habitat type and the larval density from each habitat (number of larvae per dip)

Village	Habitat type	Early stage *Anopheles*	Late stage *Anopheles*	Pupal stage	Early Instars *Anopheles* Densities	Late Instars *Anopheles* Densities	Pupal densities
Kiamaciri	Paddy	3,945	305	168	0.38	0.03	0.02
	Canal	856	59	83	0.18	0.01	0.02
	Pools	537	52	104	0.20	0.02	0.04
	Marsh	1,270	163	160	0.24	0.04	0.03
	Water reservoirs	17	2	21	0.06	0.01	0.07
	Total	6,625	581	536	0.28	0.03	0.02
Mbui Njeru	Paddy	1,626	168	228	0.20	0.02	0.04
	Canal	372	32	8	0.10	0.01	0.00
	Pools	45	2	6	0.16	0.01	0.02
	Marsh	807	141	93	0.18	0.06	0.02
	Water reservoirs	8	0	137	0.03	0.00	0.56
	Total	2,858	343	472	0.17	0.03	0.03
Murinduko	Paddy	8,523	1,508	844	0.75	0.13	0.07
	Canal	718	188	93	0.53	0.14	0.07
	Pools	1,197	124	104	0.73	0.07	0.06
	Marsh	2,765	436	564	0.92	0.13	0.27
	Water reservoirs	121	2	457	1.76	0.00	2.16
	Tree holes	14	0	15	0.85	0.00	0.38
	Rock pool	64	10	88	1.07	0.09	1.59
	Total	13,402	2,268	2,165	0.78	0.13	0.14

4.4.3 Temporal variation of *An. gambiae s.l.*

Figure 4 shows the effect of rainfall on the abundance of *An. gambiae* mosquito larvae from each of the villages. From this figure, the *An. gambiae* larval abundance positively correlated with the both short and long rains (r = 0.759) with the peak of larvae and rainfall being April and November in Kiamachiri. In Mbui Njeru, the short rains corresponded with *An. gambiae* larval abundance with the peak being in April when there were high rains. The rice cycle for this village begins in July with flooding of farms and consequent growing of rice until December. In this village, there was positive correlation between rainfall and larval abundance (r = 0.602). This resulted in high abundance of *An. gambiae* during the rice growing cycle. In Murinduko, the long rains with a peak in April did not result in the increase in larval abundance, but with the clearing of marshes changing land use to agricultural the larval abundance increased with a peak in August. The short rains also did not have effect on the larval abundance. Rainfall in this village had a low and negative correlation with *An. gambiae* abundance (r = -0.267).

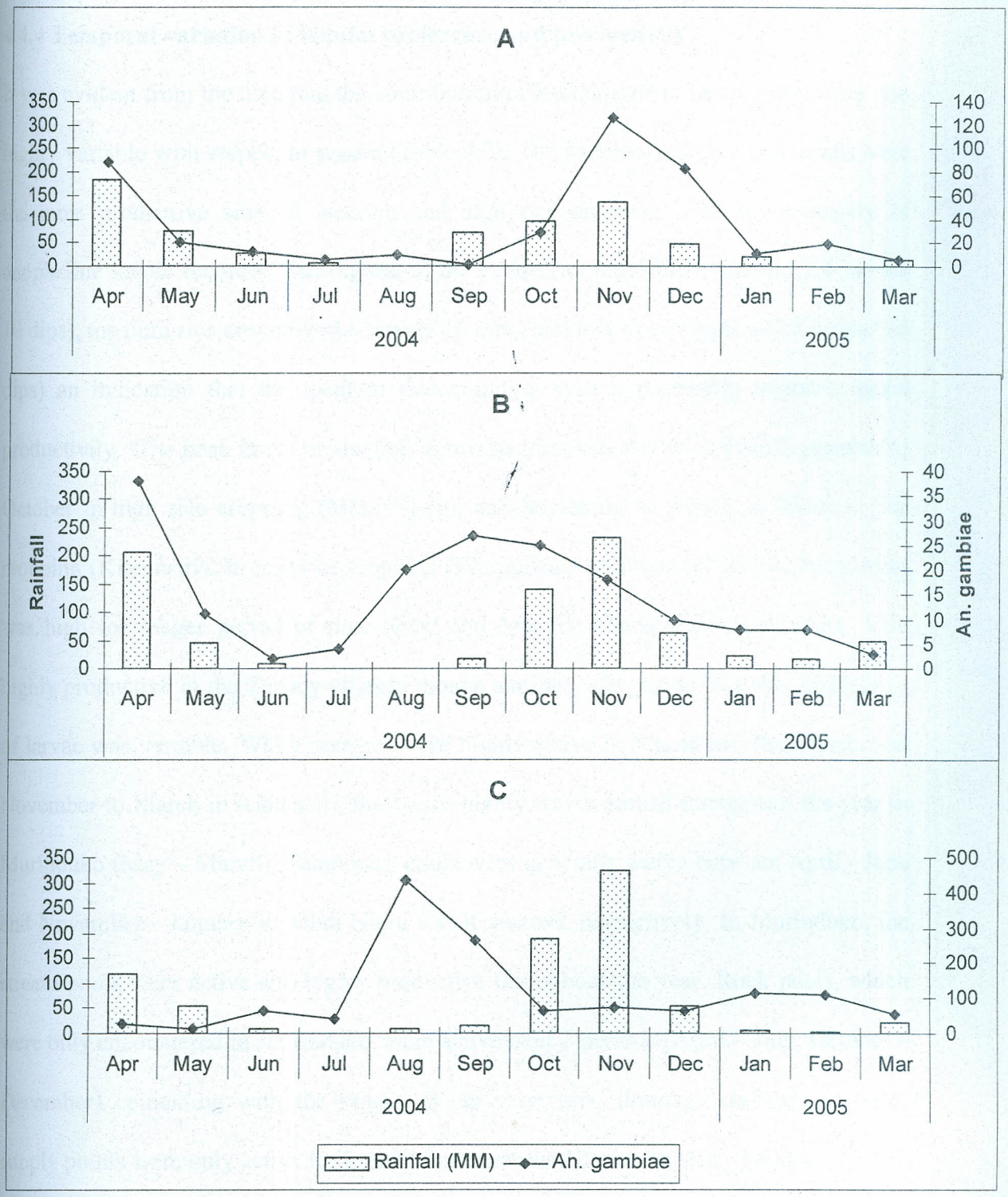

Figure 4 The relationship between rainfall and *An. gambiae s.l.* larvae in A) Kiamachiri, B) Mbui Njeru, C) Murinduko from April 2004 to March 2005

4.4.4 Temporal variation in habitat preference and productivity

It was evident from the data that the contribution of habitat type to larval production was highly variable with respect to season (Table 4.2). The paddies, marshes and canals were the most productive sites in medium and high rice cropping. The mean density of anopheline larvae recorded was highest in the paddies in high rice cropping (2.4 larvae/ 10 dips), medium rice cropping (4.6 larvae/ 10 dips) and low rice cropping (7.8 larvae/ 10 dips) an indication that the medium rice cropping system preferably supports larval productivity. The peak larval production in rice paddies was recorded from September to October in high rice cropping (Mbui Njeru), and September to March in Medium rice cropping (Kiamaciri). In low rice cropping (Murinduko) productivity within the paddies was high for longer period of time (June and March). The marshes and canals were highly productive in the 2 study villages though similarly the period of active production of larvae was variable. While marshes were highly active in March in Mbui Njeru, and November to March in Kiamaciri, they were highly active almost throughout the year in Murinduko (May – March). Temporary pools were generally active between April - June and November - January in Mbui Njeru and Kiamaciri, respectively. In Murinduko, the stream pools were active and highly productive throughout the year. Rock pools, which were only encountered in Murinduko, were active over 2 periods (April – July; October – December) coinciding with the rains. Water reservoirs/ drainage channels at water supply points were only active for limited time over the 12 months study period.

Table 4.2 Seasonal variations in *Anopheles* larval densities over a 12-month sampling period among different aquatic habitats in three study sites in Mwea, Kenya (*Density is expressed as number of larvae per 10 dips*)

		Month												
Village	Habitat type	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Mean
Kiamaciri	Canal	1.4	0.1	0.4	1.1	2.0	2.8	4.9	3.7	1.7	3.0	1.3	1.4	1.99
	Marsh	1.1	0.5	1.2	2.7	2.7	1.8	1.3	4.2	4.0	2.5	2.2	2.0	2.18
	Paddy	2.1	1.3	1.7	1.4	1.8	7.9	4.5	7.3	8.6	9.8	3.0	6.2	4.64
	Temporary pool	1.9	2.0	1.8	0.9	0.2	3.2	0.6	6.9	2.8	2.4	1.7	0.7	2.09
	Water reservoir	0.2	0.0	1.3	0.0	-	-	1.0	1.8	0.0	2.0	0.0	-	0.52
	Total	**6.7**	**3.9**	**6.3**	**6.2**	**6.7**	**15.6**	**12.4**	**23.8**	**17.1**	**19.7**	**8.2**	**10.3**	**11.40**
Mbui Njeru	Canal	0.7	0.7	0.1	1.1	0.3	2.5	1.3	1.6	1.4	1.1	1.0	1.0	1.05
	Marsh	0.0	0.0	0.2	-	0.7	-	2.1	1.0	0.0	-	0.7	16.7	1.78
	Paddy	2.2	0.7	1.0	0.7	1.4	6.0	3.0	0.4	4.2	2.0	3.0	4.4	2.41
	Temporary pool	7.9	2.9	0.5	0.0	0.9	0.7	0.5	1.1	1.8	0.3	0.1	0.5	1.42
	Water reservoir	0.0	-	-	-	-	-	0.0	0.0	2.7	-	-	0.0	0.22
	Total	**10.9**	**4.3**	**1.8**	**1.8**	**3.2**	**9.1**	**7.0**	**4.0**	**10.0**	**3.4**	**4.7**	**22.5**	**6.88**
Murinduko	Canal	2.5	2.8	4.9	4.9	17.9	8.0	3.4	1.8	2.9	3.9	4.9	8.9	5.58
	Marsh	2.3	5.7	6.8	3.9	8.2	27.1	9.4	7.9	3.9	4.2	6.6	8.5	7.86
	Paddy	2.1	3.8	11.3	6.1	12.0	16.3	6.9	5.1	4.1	6.9	7.4	11.2	7.77
	Rock pool	3.0	3.4	-	10.0	-	-	13.0	9.5	3.5	-	-	-	3.53
	Stream pool	7.6	3.5	10.5	3.9	11.8	5.0	3.0	3.5	5.7	8.6	14.2	14.3	7.64
	Temporary pool	8.5	1.4	12.7	7.1	6.8	11.0	9.3	21.9	8.6	9.2	8.8	5.0	9.18
	Tree hole	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	9.0	-	-	0.82
	Water reservoir	0.5	0.0	-	-	-	-	0.0	8.7	9.0	0.0	0.0	0.0	1.51
	Total	**26.5**	**20.4**	**46.2**	**35.8**	**56.7**	**67.4**	**45.1**	**58.4**	**38.6**	**41.8**	**42.0**	**47.9**	**43.90**

-The habitat was dry/not sampled

4.4.5 Habitat diversity and productivity

The different larval habitats encountered in the study sites included water paddies, canals, marshes, temporary pools, water reservoirs, rock pools, stream pools, tree holes. Eight habitat types supported larval development in non-irrigated agro ecosystem compared to 5 in each of the two rice agro-ecosystem. The number of habitats encountered in each site was variable, with the highest number of habitats sampled being recorded in Kiamaciri (n = 226). The number of habitats sampled in Mbui Njeru and Murinduko were 201 and 170, respectively. The period of active productivity of the larval habitats, assessed by the amount of sampling efforts when the habitats had water and proportion of times the habitat was positive for anopheline larvae was also variable between sites (Table 4.3).

Among the permanent or stable aquatic habitat categories (paddies, canals and marshes) paddies and associated canals had high densities of anopheline larvae in both High rice cropping and Medium rice cropping whereas the marshes had the highest *Anopheles* density in Low Rice cropping (Table 4.3). In Kiamaciri (unplanned rice cropping), paddies were the most preferred habitat (density = 4.1 larvae/ 10 dips) followed by temporary pools (2.8 larvae/ 10 dips) and marshes (2.1 larvae/ 10 dips). The paddy contributed 35.2 % of total *Anopheles* larvae collected in this site and this habitat type was positive 56.3% of the total times sampled (n= 749). In Mbui Njeru, other than the temporary pools (2.4 larvae/ 10 dips), the paddies (2.2 larvae/ 10 dips) were equally productive with the habitat having water 57.6% of the times monitored. The marshes and temporary pools which include hoof prints were only of low significance in the rice

growing sites both seen from the densities of larvae and pupae and the duration of active life of the habitat throughout the year.

The unstable or temporary aquatic habitats (temporary pools, water reservoirs/tanks, tree holes and rock pools) showed variable significance with drainage channels at water reservoirs showing the highest productivity assessed both by pupal and larval density. The significance of these temporary pools was limited since the habitats were dry 85.7% of the times monitored. Although canals in the two rice growing sites had generally low larval densities, they were active almost over the 12 months of the study as they held water 83.0% and 37.3% of the times assessed/ sampled in Mbui Njeru and Kiamaciri, respectively, and were positive for anopheline larvae ≈ 40% of the sampling effort for this habitat. Water reservoirs including drainage channels at water collection points were only of limited significance in the 2 sites (Table 4.3). Murinduko (<5% of area under rice) had the highest number of larval habitats with the highest larval density obtained from the temporary pools (9.9 larvae/ 10 dips). Larval production from canals, marshes, paddies and stream pools was not highly variable (range: 6.7 – 8.8 larvae/ 10 dips). Rock pools (n = 4) and tree holes (n = 3), a rare aquatic habitat for *Anopheles* breeding, were only encountered in Murinduko, a village with < 5% of the area under rice on the valley bottoms.

Table 4.3 Habitat dynamics, productivity and diversity of *Anopheles* larval habitats sampled over 12 months in three ecologically varied villages in the Mwea Rice Irrigation Scheme (April 2004 – March 2005)

	Habitat Category	# Habitats sampled	% Larval habitats dry	% Larval habitats with water	# Samples taken	% Larval habitats negative	% Larval habitats positive for *Anopheles* larvae	% Total *Anopheles* larvae	Density (no./10 dips)
Kiamaciri	Canal	30	37.26	62.74	330	60.30	39.70	16.6	1.9
	Marsh	27	51.75	48.25	193	51.30	48.70	18.3	2.1
	Paddy	108	47.29	52.71	749	43.66	56.34	35.2	4.1
	Temporary pool	59	50.00	50.00	423	55.08	44.92	23.8	2.8
	Water reservoir	2	53.85	46.15	24	75.00	25.00	5.8	0.7
Mbui Njeru	Canal	19	16.97	83.03	225	68.44	31.56	13.7	1.1
	Marsh	6	80.46	19.54	17	52.94	47.06	21.2	1.7
	Paddy	80	42.45	57.55	545	53.21	46.79	28.4	2.2
	Temporary pool	96	85.65	14.35	374	63.90	36.10	30.5	2.4
	Water reservoir	2	76.92	23.08	6	83.33	16.67	5.7	0.4
Murinduko	Canal	9	2.92	97.08	133	34.59	65.41	12.4	6.7
	Marsh	22	14.29	85.71	162	20.37	79.63	14.5	7.8
	Paddy	83	13.58	86.42	1126	30.73	69.27	16.3	8.8
	Rock pool	4	64.52	35.48	11	27.27	72.73	10.9	5.9
	Stream pool	10	6.09	93.91	108	23.15	76.85	15.8	8.5
	Pools	35	9.30	90.70	234	23.08	76.92	18.3	9.9
	Tree hole	3	55.56	44.44	4	25.00	75.00	5.3	2.9
	Water reservoir	4	38.89	61.11	21	71.43	28.57	6.3	3.4

4.4.6 *Anopheles* species composition and distribution among habitats.

Morphological identification of late stage instars of *Anopheles* larvae (n = 2,313) yielded 7 anopheline species with *An. gambiae* comprising 82.1%, *An. pharoensis* 7.8%, *An. rufipes* 2.6%, *An. funestus* 2.5%, *An. rivorulum* 2.1%, *An. maculipalpis* 2.0%, and *An. coustani* 1.0% (Table 4.4). All the 7 anopheline species were present in non irrigated and unplanned agroecosystems whereas only 3 species were represented in planned agroecosystems.

Anopheles gambiae s.l. was the most abundant and was found in all habitats that were positive for late anopheline instars in the 3 villages. The relative importance of aquatic habitats in supporting larval development was variable amongst the villages. The low rice-cropping village (Murinduko) had diverse habitat types and most of the *Anopheles* was found in almost all these habitats. In planned cropping village (Mbui Njeru) paddies, canals and temporary pools were the habitats producing *An. gambiae* and *An. pharoensis* whereas other habitat type did not produce any *Anopheles*. In the unplanned rice cropping (Kiamaciri), besides the rice paddies, canals and temporary pools, marshes were also important habitats producing anophelines. It was also noted that the *Anopheles* species diversity increased in the unplanned rice growing. In the village with limited rice growing activities i.e. Murinduko, the species diversity was high with 7 different *Anopheles* species being found in the habitats. In this village, besides the other habitat types, stream pools were also important source of *Anopheles* mosquito. The habitats in the planned rice growing system produced only 2 *Anopheles* species, the unplanned rice growing system 5

species were identified while in the village with limited rice growing 7 species were found (Table 4.4).

4.4.7 Factors associated with habitat preference

Multiple logistic regressions showed that turbidity, water depth, presence of other invertebrates, percentage *Azolla* cover, and distance to nearest homestead were the best predictors for *Anopheles* mosquito larval abundance in the habitats (Table 4.5). Turbidity and depth of the habitat had a positive association with the larval abundance while presence other invertebrates; percent *Azolla* cover and distance had a negative effect on the larvae. There was a negative association between presence of other invertebrates, distance to the nearest homestead or house, the percent *Azolla* cover and larval densities. The water turbidity was important indicator of larval abundance, whereby habitats with clear or low turbidity tended to harbor most of the anopheline larvae.

Table 4.4 Species distribution of 3rd and 4th instar stage *Anopheles* larvae in different larval habitats in 3 villages in Mwea

Village	Habitat type	*An. gambiae s.l.*	*An. funestus*	*An. pharoensis*	*An. rivulorum*	*An. maculipalpis*	*An. rufipes*	*An. coustani*	Total
Kiamaciri	Paddy	228	5	13	5	1	0	0	252
	Canal	25	0	1	1	0	0	1	28
	Marsh	26	1	1	0	0	0	0	28
	Temporary pool	107	4	8	1	1	4	1	126
Mbui Njeru	Paddy	84	0	4	1	0	0	0	89
	Canal	24	0	6	0	0	0	0	30
	Temporary pool	66	0	4	0	0	0	0	70
Murinduko	Paddy	871	33	92	18	38	19	9	1,080
	Canal	165	2	6	2	1	0	4	180
	Marsh	46	3	4	5	0	14	1	73
	Stream pool[a]	92	6	19	14	2	15	0	148
	Temporary pool	166	4	22	0	3	7	7	209
	Rock pool	0	0	0	0	0	0	0	0
Total		1,900	58	180	47	46	59	23	2,313

[a] Stream pool denotes stream edges and streambed pools of varying sizes

Table 4.5 Parameter estimates of a logistic regression model fitted to explain abundance of *Anopheles* larvae in habitats in Mwea

	B	S.E.	Wald	df	Sig.	OR[a]	95.0% C.I.for OR[b]	
							Lower	Upper
Turbidity			10.919	2	0.004			
i. Clear	0.890	0.274	10.532	1	0.001	2.436	1.423	4.169
ii. Low	0.731	0.247	8.795	1	0.003	2.078	1.281	3.369
Presence of emergent vegetation	0.161	0.232	0.481	1	0.488	1.174	0.746	1.848
Presence of Floating vegetation	0.296	0.205	2.075	1	0.150	1.344	0.899	2.011
Presence of submerged vegetation	0.086	0.878	0.010	1	0.922	1.090	0.195	6.095
Water Depth (cm)	0.048	0.020	5.900	1	0.015	1.049	1.009	1.091
Presence of other invertebrates	-0.862	0.156	30.689	1	0.000	0.422	0.311	0.573
Rice height (cm)	-0.002	0.003	0.577	1	0.448	0.998	0.991	1.004
Number of tillers	-0.001	0.009	0.007	1	0.934	0.999	0.981	1.018
% Water cover	-0.003	0.003	.998	1	0.318	0.997	0.992	1.003
% Azolla cover	-0.010	0.002	17.211	1	0.000	0.990	0.986	0.995
Distance category			21.762	2	0.000			
i. 0 – 100 M	-0.734	0.180	16.646	1	0.000	0.480	0.337	0.683
ii. 101 – 200 M	-0.600	0.176	11.664	1	0.001	0.549	0.389	0.774
Constant	0.466	0.964	0.234	1	0.629	1.594		

Table Legend: OR[a] Odds Ratio; 95.0% C.I. for OR[b] 95% Confidence Interval for Odds Ratio

4.5 Discussion

The understanding of larval habitat ecology is important in designing targeted malaria control programs. Currently there is renewed interest in mosquito larval control and recent studies explore the feasibility of reducing malaria vector populations through environmental and agro-ecosystem management approaches (Keiser *et al.*, 2002). Ecologies of larval habitats in Mwea Rice Irrigation Scheme in three agro ecologically different villages based on agricultural practices and water management in order to understand the variation in larval habitat dynamics and productivity were studied. Both rice growing and rainfall significantly contributed to high abundance of mosquito larvae but the importance was site-specific. In 'planned' rice growing system, the larval abundance and densities corresponded well with the rice-growing season. Larval habitats in villages with unplanned rice cropping tended to have higher larval densities than villages planned rice-cropping (organized) rice growing. The paddies and canals in the medium rice cropping are poorly drained which makes them more favourable for anopheline larval development, whereas the paddies and the irrigation canals in the organized/ planned rice growing are well drained. The effect of the unplanned rice cropping which is an unorganised rice growing and the subsequent unorganised water management meant that rice growing was undertaken throughout the year with rice paddies at different growth stages throughout the year. This phenomenon not only increased the number of habitats but prolonged the period of active life of the larval habitat for production of *Anopheles* larvae.

In the low rice cropping (Murinduko), where < 5% of the area is under rice, rice cultivation has only recently been introduced along the river valleys. This has resulted in an exponential increase in breeding sites for mosquitoes. Initially most of habitats were concentrated in stream edges and stream pools. The soils and topography of this village does not allow formation of rain-fed pools hence the negative association between rainfall and larval densities. Although even before introduction of rice cultivation, the three villages were different in terms of hydrology and natural breeding sites, villages with unplanned and/or limited rice cultivation would be expected to have diverse breeding sites than those within the scheme (Briet *et al.,* 2003). Species diversity was higher in villages outside the irrigation scheme suggesting the presence of diverse and productive larval habitats in these sites unlike in the rice growing villages where larval breeding is limited to rice paddies and associated canals. For example, studies in western Kenya have shown similar trends where greater assemblage of anopheline species were associated with villages with permanent and diverse larval habitats (Beier *et al.,* 1990).

Paddies and irrigation canals were the most productive habitat types throughout the sampling period in the rice growing villages. These habitat types had water most of the rice growing cycle. The peri-domestic habitats (including pools, tyre tracks and pits) were productive for only limited periods corresponding to the rainy season. However, these habitats were very productive for the short periods when they contained water making them important sources of *Anopheles* mosquitoes during the wet season. Pools associated with streams were equally important sources of *An. gambiae* for extended periods of time in the study sites.

Azolla cover was negatively associated with anopheline larval abundance. *Azolla* provides a mat-like structure on the surface of the habitat thus reducing penetration of sunlight which in turn affects photosynthetic activity of the algae and other aquatic forms that serve as a food source for mosquito larvae. The macrophyte mat may also inhibit oviposition in these habitats. In some instances there was complete coverage at the surface of the paddies and canals by the *Azolla,* which made the water underneath could not be available for mosquito oviposition. The negative effect of the *Azolla spp* on mosquito production has been documented by other investigators (Mogi *et al.,* 1986; Baolin, 1988).

Increase in turbidity resulted in a significant increase in anopheline larval densities in the habitats. It is likely that increase in turbidity tended to affect the attractiveness of these breeding sites to ovipositing female *Anopheles* mosquitoes. McCrae, (1984) found that *An. gambiae* preferred a dark to a light background as an oviposition substrate. In this study clear and low turbid water had the most abundant *Anopheles* larvae. Mosquitoes view water bodies by the reflecting dark background of the soil substrate. The dark background increased the attractiveness of the breeding sites to ovipositing female *Anopheles* mosquitoes.

The study observed a decrease in larval abundance with increasing distance from the homesteads. This is likely due to feeding preferences and availability of blood meal sources for the anophelines close to human habitation. Studies have shown that *An. arabiensis*, the predominant anopheline species in the Mwea Irrigation Scheme, feeds

predominantly on cattle and humans (Mutero *et al.,* 2004a). Gravid mosquitoes utilize the habitats within close proximity to the homesteads for oviposition as an evolutionary mechanism for energy conservation. This consequently meant that habitats far away from the homesteads had low larval densities due to few numbers of gravid females using them for oviposition.

The presence of other invertebrates was negatively associated with anopheline larval abundance. An increase in the densities of other invertebrates in the habitats resulted in the decrease in the larval densities. The other invertebrates' composition in the habitats included some of which may be important in predation. Predation usually has a negative effect on the populations, consequently an inverse correlation with mosquito larvae (Service 1977).

The data generated from this study suggest that implementation of larval control activities should be targeted based on habitat productivity, which is governed by rainfall, rice cropping season and water management. However, the application of interventions would have to consider habitat and site specific attributes of larval productivity. The fact that medium and low rice growing supports more *Anopheles* larvae than high rice cropping system calls for better management of the rice cultivation and subsequent water distribution so as to reduce the active period when the rice fields are flooded.

CHAPTER 5: PHYSICOCHEMICAL FACTORS ASSOCIATED WITH *ANOPHELES* LARVAL DENSITIES IN MWEA IRRIGATION SCHEME, CENTRAL KENYA

5.1 Introduction

For larval control to be an integral part of a vector management programme, a sound understanding of the factors responsible for larval activity of principal vectors of malaria is crucial (Molineaux, 1997). A strong association exists between the density and distribution of the preadult stages and that of adult vectors. Therefore vector control strategies aimed at suppressing larval production would subsequently affect adult population densities thereby limiting malaria transmission. Knowledge of the influence of habitat factors on larval production is critical for understanding the spatial and temporal distribution patterns of the anopheline species, and in planning and implementing appropriate larval control strategy.

In Kenya and Tanzania, *An. arabiensis* is the predominant malaria vector and the only member of *An. gambiae s.l* present in inland areas with rice cultivation (Ijumba *et al.*, 1990; Mutero *et al.*, 2000; Mutero *et al.*, 2004a), its peak population coinciding with the transplanting of rice seedlings (Mutero *et al.*, 2000).

Studies by Rejmankova and colleagues (Rejmankova *et al.*, 1993; Rejmankova *et al.*, 1991), demonstrated that there was a strong association between larval distribution and the distribution of some habitat factors such as cyanobacterial mats and filamentous algae. Minakawa and colleagues (Minakawa *et al.*, 1999) in western Kenya did not detect any significant association between the occurrence of *An. gambiae* larvae and habitat variables.

Studies by Gimnig *et al.*, (2001) showed that small size, the presence of turbid water and algae, and the absence of emergent vegetation was associated with the presence of *An. gambiae* larvae in western Kenya. Grillet, (2000) found that salinity and dissolved oxygen were the only physicochemical variables associated with anopheline larvae in larval habitats in Venezuela. Shililu *et al.*, (2003) found that pH was associated with *Anopheles* larval diversity in Eritrea. This study examined the influence of physicochemical factors in the abundance of anopheline mosquitoes in Mwea Irrigation Scheme, Central province of Kenya prior to implementation of a malaria vector control program.

5.2 Method and materials

5.2.1 Larval Sampling

At the experimental plots, a weekly larval sampling was done to generate stage-specific estimates of *Anopheles* larval abundance. Samples were taken using standard dipping technique and a plastic dipper (Mosquito Control Service and Supplies, USA) with a wooden ladle. Twenty dips were taken from each sub-plot.

At the study villages, larval habitats were sampled biweekly along cross-sectional transects to generate stage-specific estimates of *Anopheles* larval density. Five to twenty dips were taken from each habitat depending on habitat type. The mosquito larvae collected were sorted out according to the sub-families as either anopheline or culicine.

5.2.2 Measurement of physicochemical variables

In the habitats, the physicochemical variables which were measured included: pH, conductivity, dissolved oxygen, temperature, salinity and Total Dissolved solids (TDS). The pH, conductivity, dissolved oxygen and temperature were measured using hand held machine YSI 650 Multiparameter Display System (YSI Environmental, YSI Incorporated, Yellow Springs, USA). Salinity and Total Dissolved solids (TDS) were measured using field hand held equipment YSI EC 300 (YSI Environmental, YSI Incorporated, Yellow Springs, USA).

5.3 Statistical analysis

Descriptive statistics was used to tabulate the larval densities and abundance from each village. Logistic regression analyses were used to determine the correlation between physicochemical variables and the presence or absence of anopheline in a habitat. The occurrence of anopheline larvae was defined as the presence of anophelines in a sample regardless of its density. Anopheline larvae were categorized as 1 if present and 0 if absent in each habitat at each sampling time.

5.4 Results

A total of 1,156 *Anopheles* larvae were collected in the 2 villages in which, 92.21% (n = 1,066) were early instar stage and 7.79% (n = 90) were late stage (Table 5.1). All the 90 late stage instars were morphologically identified as *An. gambiae s.l.* A total of 146 pupae were collected from the two villages.

In the experimental plots, 7,860 anopheline larvae were collected in which 96.56% (n = 7,590) were early instar stages and 3.44% (n = 270) were late stage instars (Table 5.1). Only 31 pupae were collected during the sampling period. Of the 270 late stage anophelines collected, 95.62% (n = 258) were *An. gambiae s.l.* while 4.38% (n = 12) were *An. pharoensis.* At the experimental plots, 98.17% (n = 7,716) of the larvae were collected between transplanting and the tillering stage of rice development. Few larvae were collected between the flowering stage and the maturation stage.

Table 5.2 shows the mean and standard error of the water analysis parameters tested at the experimental plots and the two villages. At the experimental plots, temperatures raised from land preparation (23.86°C) to the maximum at the transplantation stage (28.64°C). It slightly went down during tillering stage and at the flowering and maturation stages the temperatures declined significantly. The pH at the experimental rice plots was high between land preparation and tillering (range 7.39 – 7.52), which was as a result of addition of basal fertilizers and top dressing. Salinity was found to be highest also between land preparations and tillering (Range: 80.97 – 127.94)

In the villages water temperatures in Mbui Njeru were higher than in Kamachiri although the difference was not significant (ANOVA, p = 0.416). Conductivity, salinity and pH were significantly higher in Mbui Njeru than in Kiamachiri (ANOVA, P < 0.001). Dissolved oxygen was found to be slightly higher in Mbui Njeru than in Kiamachiri but this difference was not statistically different (ANOVA, P = 0.333).

Table 5.3 shows the multiple logistic regressions of the physicochemical parameters in the villages and the experimental test plots. Logistic regression model gave pH, depth and conductivity as the best indicator for anopheline larvae at the two villages. pH (r = -0.625; p 0.05) and water depth (r = -0.031; p = 0.012) were negatively associated with the presence of *Anopheles* larvae in the habitats while conductivity (r = 0.007; p = 0.019) was weakly but positively associated with the presence of *Anopheles* larvae.

At the experimental plots, low turbidity, temperature and conductivity were the best predictors of the anopheline larvae at the experimental plots. Low turbid water (r = 1.00; p = 0.004), temperature (r = 0.187; p <0.001) and conductivity (r = 0.015; p = 0.009) were positively associated with the presence of *Anopheles* larvae in the paddies. At the experimental plots, *An. gambiae was* seen to be positively associated with low turbid water (OR = 2.72; 95% CI = 1.366 – 5.431) while in the villages it was negatively associated with pH (OR = 0.535; 95% CI = 0.282 – 1.015).

Table 5.1 Mosquito larvae collected at different stages of rice development at the experimental plots and the 2 villages

Sampling site	Habitat type/Rice stage	Early instars *Anopheles*	Late instars *Anopheles*	Pupae
Kiamachiri	Paddy	536	62	32
	Canal	72	5	12
	Pool	1	0	0
	Seep	26	6	31
	Marsh	19	3	10
	Ditch	0	0	0
	Tank	0	0	0
	Pit	12	0	6
Mbui Njeru	Paddy	336	9	55
	Canal	40	3	0
	Seep	24	2	0
	Hoof prints	0	0	0
Experimental plots	Land preparation	72	4	1
	Transplanting	6,612	119	8
	Tillering	841	144	19
	Flowering	28	3	1
	Maturity	37	0	2

Table 5.2 Descriptive statistics of the water analysis at different stages of rice development for the experimental plots and the villages (Mean±SE)

Site	Habitat type/Rice stage	Depth (cm)	Temperature (°C)	Conductivity (ms/cm)	Salinity (ppt)	Dissolved oxygen (mg/l)	pH
Kiamachiri	Paddy	10.24 ± 0.45	24.29 ± 0.32	74.40 ± 9.08	65.17 ± 10.04	3.60 ± 0.31	6.97 ± 0.03
	Canal	15.00 ± 1.19	22.95 ± 0.44	61.31 ± 11.82	53.14 ± 10.97	3.57 ± 0.60	6.96 ± 0.07
	Pool	9.00 ± 0.01	23.19 ± 1.40	19.50 ± 5.40	235.30 ± 35.25	1.51 ± 0.32	6.95 ± 0.01
	Seep	7.78 ± 0.68	23.22 ± 1.01	100.52 ± 43.15	97.16 ± 45.12	3.86 ± 1.86	7.03 ± 0.12
	Marsh	18.00 ± 5.00	22.61 ± 0.68	86.13 ± 32.13	101.93 ± 48.06	3.53 ± 0.68	6.97 ± 0.08
	Ditch	6.00 ± 0.01	19.96 ± 0.01	0.76 ± 0.01	0.37 ± 0.01	3.75 ± 0.51	7.38 ± 0.97
	Tank	11.00 ± 0.01	24.55 ± 0.01	0.22 ± 0.10	0.10 ± 0.01	24.09 ± 0.87	8.30 ± 1.26
	Pit	51.63 ± 12.47	21.88 ± 0.44	69.53 ± 28.88	78.73 ± 33.87	3.59 ± 0.75	6.89 ± 0.08
Mbui Njeru	Paddy	9.11 ± 0.58	25.25 ± 0.29	185.31 ± 14.07	173.54 ± 19.19	5.29 ± 0.91	7.39 ± 0.03
	Canal	32.58 ± 7.03	24.20 ± 0.44	203.67 ± 41.95	217.56 ± 56.55	4.65 ± 1.78	7.46 ± 0.07
	Seep	3.50 ± 0.50	25.44 ± 2.27	130.82 ± 65.43	113.54 ± 57.31	1.81 ± 0.80	7.13 ± 0.10
	Hoof prints	6.00 ± 0.01	28.71 ± 0.01	318.90 ± 10.15	346.99 ± 12.11	0.20 ± 0.01	7.35 ± 1.15
Experimental plots	Land preparation	8.58 ± 0.39	23.86 ± 0.61	133.40 ± 21.62	127.94 ± 25.54	7.67 ± 0.78	7.43 ± 0.07
	Transplanting	6.93 ± 0.23	28.64 ± 0.34	101.70 ± 5.13	80.97 ± 6.15	5.08 ± 0.37	7.52 ± 0.02
	Tillering	7.70 ± 0.26	26.85 ± 0.30	145.23 ± 6.67	123.76 ± 8.11	7.33 ± 0.29	7.39 ± 0.04
	Flowering	8.25 ± 0.69	24.63 ± 0.32	98.57 ± 6.40	72.53 ± 5.63	6.81 ± 0.81	7.03 ± 0.07
	Maturity	9.39 ± 0.52	23.91 ± 0.24	84.86 ± 6.89	62.34 ± 6.51	6.62 ± 0.48	7.04 ± 0.08

Table 5.3 Parameter estimates of a logistic regression model fitted to explain abundance of *Anopheles* larvae and the physicochemical analysis at the experimental plots and the villages

	Variable	B	Wald	df	Sig.	OR	95.0% C.I. for OR	
							Lower	Upper
Experimental plots	Water depth	0.026	0.502	1	0.479	1.026	0.955	1.103
	Turbidity		8.619	3	0.035			
	Clear	0.654	2.567	1	0.109	1.923	0.864	4.279
	Low	1.002	8.099	1	0.004	2.724	1.366	5.431
	High	0.733	2.995	1	0.084	2.081	0.907	4.774
	Temperature	0.187	25.257	1	0.000	1.206	1.121	1.298
	Conductivity	0.015	6.755	1	0.009	1.015	1.004	1.027
	Salinity	-0.009	3.358	1	0.067	0.991	0.982	1.001
	Dissolved oxygen	0.013	0.124	1	0.725	1.013	0.943	1.088
	pH	-0.323	1.085	1	0.298	0.724	0.394	1.330
	Constant	-4.146	3.818	1	0.051	0.016		
Villages	pH	-0.625	3.669	1	0.050	0.535	0.282	1.015
	Dissolved oxygen	0.034	1.940	1	0.164	1.034	0.986	1.084
	Turbidity		1.058	2	0.589			
	Clear	-0.350	1.044	1	0.307	0.705	0.360	1.379
	Low	-0.190	0.354	1	0.552	0.827	0.442	1.546
	Depth	-0.031	6.287	1	0.012	0.969	0.946	.993
	Conductivity	0.007	5.516	1	0.019	1.007	1.001	1.012
	Salinity	-0.004	2.213	1	0.137	0.996	0.992	1.001
	Constant	4.393	3.752	1	0.053	80.908		

5.5 Discussion

This study examined the influence of physicochemical factors in the abundance of anopheline mosquitoes in Mwea Irrigation Scheme, Central province of Kenya. Larval abundance was found to be highest in between transplanting and tillering stage. At these stages of rice growth cycle water temperatures was highest at the experimental plots. When the rice was at the reproductive stage, these two parameters decline subsequently. A number of workers have documented the effects of temperature on development of temperature on development and relative abundance of Riceland mosquito larvae (Lacey and Lacey, 1990). The results of this study further indicated that water temperature was important factor for the abundance of *Anopheles* larval in the larval habitats. At transplanting and tillering there are many open spaces in between the plants, which provide good conditions for the growth of mosquito larvae. When the rice plants achieved maximum vegetation, the temperatures declined due to the effect of the rice canopy that make the water to be under shade throughout the day. It is know that *An. arabiensis*, which is the most predominant species in Mwea Irrigation scheme (Mutero *et al.*, 2000), has been shown to have a preference for shallow and exposed ground pools (Gillett and Smith, 1972).

Depth was found to be important factor for anopheline larval abundance. Increase in depth of a habitat increases the water volume in a habitat consequently having a negative impact on water temperatures. Water depth has been found to have an influence on larval densities (Chandler and Highton, 1976; Collins and Washino, 1980; Palchick and Rao, 1984; Washino, 1985). Rice fields in Mwea Irrigation scheme are maintained at 10 cm

water depth through out the growth cycle but due to evapotranspiration and direct evaporation at times depth of water decline. This provided conducive water temperatures, which have a direct influence on the anopheline larval densities.

Other important factors for the abundance of anopheline mosquito larvae in the habitats were pH and conductivity. In rice agro ecosystem, pH and conductivity have been shown to be important for larval abundance (Cates, 1968; Case and Washino, 1975; Robert *et al.,* 1988). Although pH had a wide range in our study at the village level, the mean was 6.97 for Kiamachiri and 7.32 in Mbui Njeru. Fluctuations in pH are mostly associated with addition of nitrogenous fertilizers. Nitrogenous fertilizers have been documented to be increasing anopheline and culicine larvae densities in rice agro-ecosystem (Victor and Reuben, 2000; Mutero *et al.,* 2004b). Consequently in in this study pH was key factor associated with anopheline larval abundance.

In conclusion, *Anopheles* larval abundance was found to be associated with several factors at the rice agro-ecosystem. These parameters in a rice agro ecosystem were associated with early vegetative stages of rice growth cycle, which were associated with larval abundance. For effective control of developmental stages of mosquito larvae, the application of the larvicides should be done at the vegetative stage and the larvicides should persist until the reproductive stage of the rice.

CHAPTER 6: MOSQUITO PRODUCTIVITY FROM DIFFERENT HABITATS IN MWEA IRRIGATION SCHEME: EFFECT OF PRESENCE OF OTHER NON-MOSQUITO INVERTEBRATES.

6.1 Introduction

Mosquito populations are regulated by a variety of factors including adverse climatic conditions, limited food supply, competition, parasites or pathogens and predators (Service, 1973). The importance of any of these factors in different environments is poorly understood, thus affecting proper understanding of the factors that affect production of adult populations. Predation is recognized as an important factor in the organization of many ecological communities (Sih *et al.*, 1985) including aquatic communities (Zaret, 1980). Species sharing the same trophic level as mosquito larva often share predators, which in turn affects predation intensity on mosquitoes (Bence, 1988; Chesson, 1989).

Predation is reported as one of the most limiting factors causing a high rate of mortality to immature stages of mosquitoes (Service, 1973). Together with insect pathogens, predation can significantly limit numbers of mosquitoes depending on the species and type of habitat. From five separate estimates in 1969 and 1971, Service found high mortalities of the pre-adults of *An. gambiae* in small ponds near Kisumu (Service, 1973). He attributed this high mortality rates to predators, and high infection levels of larval nematodes and Coelomomyces contributed to these high mortality rates. Apart from the natural regulation of mosquito numbers, predators and pathogens have raised interest due

to their potential for manipulation for biological control as part of Integrated Pest Management.

Larvae and their predators occur in a variety of habitats ranging from large and more permanent sites to very small and temporary collections of water. In a study carried out by (Service, 1977) on natural mortalities and predation of immature stages of *An. gambiae*, mortality was observed to be higher in the rice pools than in the small pools and ponds. Small mortality rate was estimated in the first two larval instars. Precipitin tests ran on smears of gut contents of possible predators showed that Coleoptera larvae, Hemiptera and predacious adult Diptera were important predators. Although not all Hemipterans tested produced a positive reaction to *An. gambiae* antisera, species of *Laccotrephes, Enithares, and Anisops* and various *Corixidae* are known predators (Jenkins, 1964). In Tanzania, (Christie, 1958) observed intense predation pressure by *Notonectidae* on mosquito larvae and pupae. However, in Japan, (Toshihiko *et al.*, 2002) observed that, *Notonectidae* and *Chaoborus* species impacted upon mosquitoe larvae than other predators. The impact of other predators such as the dragonfly nymphs, *Dytiscid* and *Hydrophilid* beetles was limited to large deep containers.

6.2 Materials and Methods

6.2.1 Effect of other non-mosquito invertebrates

Ten cages measuring 50 cm by 50 cm by 50 cm were placed in different habitats to determine the effect of other invertebrates on the larval abundance. The cage at lower

side had 30 cm iron sheet high to prevent movement of enclosed immature mosquitoes in and out of the cage. These cages were covered with a netting material to deter gravid mosquitoes and other insects from oviposition. The cages were placed in temporary pool, trye track, seepage and the paddy. Temporary pools were formed immediately after rains. These pools were open devoid of vegetation and shallow.

6.2.2 Productivity from different habitats

A study was carried out to determine the emergent mosquito productivity from different habitat types in Kiamachiri village, Mwea Division, Kirinyaga district, Central Kenya. Ten cages measuring 1m X 1m X 1m with an iron sheet on the lower side of the cage covering 30 centimeters of the height were used for the study. These cages were covered with a fine netting material in order to prevent the oviposition of the gravid mosquitoes and other invertebrates. These cages were placed in different habitats, which included paddy, ditch, marsh pool and seep. The cages were monitored daily to assess the emerging mosquitoes. The emergent mosquitoes were collected using aspiration method (WHO, 1975). The emergent mosquitoes were identified morphologically (Gillies and Coetzee, 1987) and preserved in drierite: Anhydrous Calcium Sulfate® (W.A. Hammond Drierite Company Ltd, Xenia Ohio, USA). The non-mosquito invertebrates in the habitats were identified to family level (Merritt and Cummins, 1996).

6.3 Statistical analysis

The sum of the each larval instar was computed from each trial and the emergent mosquitoes were counted. The emergent mosquito per species was expressed as a percent

based on the number of mosquito larvae at day 0. One-way Analysis of Variance (ANOVA) was used to test for variation in emergent mosquitoes from different habitats. The emergent mosquitoes density defined as the number of emergent mosquitoes divided by the number of cages in a particular habitat type.

6.4 Results

Table 6.1 shows the emergent mosquito productivity for the pools, which were formed immediately after the rains. Overall the emergence rate was 35.39% (Range 21.05 – 97.87). Tyre tracks, pools and seep were the habitats, which were enclosed. Only a small fraction of the main habitats was covered by the cage. Three habitats lasted for longer period of time and the productivity was quite high (range 84.00 – 97.87). The mosquitoes in these habitats took about ten days for all of them to emerge as adults. In some instance the habitats dried up fast but as the water levels diminished some mosquitoes managed to emerge. Of the 362 emergent mosquitoes, 245 (68.0%) were females while 117 (32.0%) were males. It was observed that male mosquitoes emerged faster than the female mosquitoes. All the emergent mosquitoes were all identified morphologically as *An. gambiae s.l.*

Table 6.2 shows the production of *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes from temporary habitats, which have low densities of the invertebrates present. When the habitats stayed for two weeks after the onset of rains, besides *An. gambiae* other invertebrates and culicine mosquitoes started colonizing these habitats. The emergent cages were placed in habitats with other invertebrates and the overall

emergence was 14.29% for *An. gambaie* mosquitoes and *C. quinquefasciatus* 14.46% (Table 6.2). None of the other species of anophelines and culicine emerged. Compared to the cages, which was set immediately after the rains, these cages had lower number of larvae. In these cages more *An. gambiae* females emerged (65%; n = 52), compared to the males (35%; n = 28). For *C. quinquefasciatus*, more males emerged (68.49%; n = 50) compared to the females (31.51%; n = 23).

Table 6.3 shows the number of *An. gambiae* and *C. quinquefasciatus* emerging from paddy habitat type with many other invertebrates present. Overall with many other invertebrates, the emergence rate for *An. gambiae* was 2.41% and that of *C. quinquefasciatus* was 5.65% (Table 6.3). All the emerged *Anopheles* mosquitoes were *An. gambiae* while among the culicines they were all *C. quinquefasciatus*. The larval densities per cage were very low. In most cages none of the larvae made it to emerge as adults.

Table 6.4 shows the emergent mosquitoes from each habitat type and the larvae at day zero. From this table, paddies were the most productive habitat type for both *Anopheles* and culicine mosquitoes. *Anopheles gambiae* was the most predominant anopheline species (98.0% n = 232) although a few *An. coustani* (2.0% n = 5) emerged from the habitats. For the culicines, all the emergent mosquitoes were *C. quinquefasciatus*. Paddy and marsh habitat types were most productive for *An. arabiensis* while paddy, marsh and ditch were the most productive habitats for *C. quinquefasciatus*. Swamps, temporary pools and seep were least productive habitat types in this study area.

Table 6.5 shows the other invertebrates composition in the cages. Dytiscidae (Coleoptera) was the most common of the other invertebrates in all cages. The dystiscidae were collected as either in the larval forms or as adults. Belostomatidae and notonectidae were the commonest hemipterans. For the ephemeroptera, the only family present was ephemerellidae. In order odonata, coenagrionidae and lebullidae were the only families observed in the cages. Other invertebrates found were the snails (mollusca).

Table 6.1 The number of mosquito larvae emerging from cages in freshly formed habitats

Habitat	Early instars *Anopheles*	Late instars *Anopheles*	*Anopheles* females	*Anopheles* males	% *Anopheles* emergence
A1	32	15	35	11	97.87
A2	82	45	20	7	21.26
A3	64	50	12	12	21.05
A4	95	41	23	15	27.94
A5	116	42	41	15	35.44
A6	103	49	34	16	32.89
A7	90	60	39	13	34.67
A8	70	37	27	15	39.25
A9	6	1	2	4	85.71
A10	8	17	12	9	84.00
Total	**666**	**357**	245	117	35.39

Table 6.2 The number of mosquitoes emerging from cages with very low densities of other non-mosquito invertebrates (less than 10 per cage)

Habitat	Early instars *Anopheles*	Late instars *Anopheles*	Pupae	*Anopheles* females	*Anopheles* males	% *Anopheles* emergence
A1	57	44	0	15	5	19.80
A2	34	15	6	7	0	14.29
A3	60	18	1	2	5	8.97
A4	80	8	0	0	2	2.27
A5	72	17	0	4	2	6.74
A6	47	1	0	17	5	45.83
A7	36	12	0	2	1	6.25
A8	20	6	0	0	1	3.85
A9	11	8	0	0	2	10.53
A10	9	5	0	5	5	71.43
Total	**426**	**134**	**7**	**52**	**28**	**14.29**

Table 6.3 The number of mosquito emerging from the cages with high densities of other non-mosquito invertebrates (more than 10 per cage), placed in a paddy

Cage#	Early instars *Anopheles*	Late instars *Anopheles*	*Anopheles* females	*Anopheles* males	% *Anopheles* emergence
A1	3	0	0	0	0
A2	2	1	0	0	0
A3	14	1	0	1	6.67
A4	20	1	1	0	4.76
A5	5	0	0	0	0
A6	6	1	0	0	0
A7	6	1	0	0	0
A8	3	1	0	0	0
A9	12	1	0	0	0
A10	3	1	0	0	0
Total	**74**	**9**	**1**	**1**	**2.41**

Table 6.4 The number of anopheline larvae at day zero and the emergent mosquitoes from each habitat type.

		Larval instars			Emergent mosquitoes	
Habitat type	**#Cages**	**Early instars *Anopheles***	**Late instars *Anopheles***	**Pupae**	***Anopheles* females**	***Anopheles* males**
Paddy	30	492	91	13	100	43
Swamp	11	14	0	0	0	0
Marsh	12	231	32	27	46	19
Ditch	3	57	16	19	5	3
Pool	3	35	8	1	7	11
Seep	5	10	5	3	2	1
Total	**60**	**829**	**148**	**63**	**160**	**77**

Table 6.5 The other invertebrates present in cages placed in the paddy.

	Hemiptera		Coleoptera		Ephemeroptera	Odonata		Mollusca
Cage#	Notons[a]	Belost[b]	Dytiscid[c]	Hydro[d]	Ephem[e]	Coenagrion[f]	Lebullidae	Snails
A1	-	++	++	-	-	-	-	-
A2	-	+	+	-	-	-	-	-
A3	-	+	+	-	+	+	+	+
A4	+++	-	++	-	+	-	+	-
A5	+	-	+	+	++	+	++	+
A6	+	+++	++	+	++	-	++	+
A7	++	+	++	-	++	-	++	-
A8	-		++	-	-	++	-	-
A9	+	+++	++	-	-	-	-	+
A10	-	++		-	+	++	+	-

Table legend: Notons[a]: Notonectidae; Belost[b]: Belostomatidae; Dytiscid[c] : Dytiscidae; Hydro[d]: Hydrophillidae; Ephem[e]: Ephemerellidae; Coenagrion[f]: Coenagrionidae

-Invertebrate absent
+ Less than 10
++ Between 11-20
+++Over 21

6.5 Discussion

Mosquito populations are regulated by a variety of factors including adverse climatic conditions, limited food supply, competition, parasites or pathogens and predators (Service, 1973; Service, 1977). This study investigated the effect of the presence of other aquatic invertebrates on the emergent mosquitoes. Pools were covered immediately 2 -3 days after rains, these pools had already been colonized by *Anopheles* larvae, but no other invertebrates were in these pools. These pools produced the highest number of emergent mosquitoes, which were all *An. gambiae*. The only constrains was the quick drying up of these pools. But the ones, which persisted for a longer period of time, the emergent rate of the anopheline was quite high (97.87%) and the least productive were the ones, which had water for short duration of time (21.05%). Adverse weather is known to be a limiting factor to the populations of *Anopheles* mosquitoes. The drying habitats make the larvae which have not yet pupated die off and make it impossible for them to emerge as adults. Although as the habitats dry, the temperatures increase hastening the rate of development for *Anopheles* larvae which emerge as adults.

When the pools stayed with water for a week, culicines and other invertebrates colonized the habitat together with *An. gambiae*. In these pools at this condition were covered with cages and the emergent mosquitoes counted. The number of emergent mosquitoes decreased significantly compared to cages without other invertebrates. When the cages were placed in habitats, which had a very high density of other invertebrates, the emergent mosquitoes.were reduced to very low numbers. (Service, 1977), showed that predation accounted for more than 95% of mortalities in the habitat. The present study

showed that there was a significant role played by predation of mosquito immature stages at the habitats. Dytiscidae were the most common other invertebrates in these habitats. Dytiscidae, notonectidae, dragonfly and damselflies have been shown to be important in natural regulators.

In Kiamachiri, it was found that paddies were the most productive habitat. The cages were placed when the rice was at the tillering stage. Tillering stage has been shown to harbour most larvae in the rice cycle. At this stage the water is most exposed due to the low vegetation. Marshes followed the paddy in the habitat productivity. This study found out that when, the structural complexity of the habitat was decreased, the number of emergent mosquitoes increased. Structural complexity is defined as the increase in vegetation cover and floating debris. The high vegetation cover and debris were found to harbour more aquatic stages of other invertebrates consequently having a high predation pressure. It was observed that when the marshes were cleared, the number of emergent mosquitoes from these habitats was more and there was a decline in the number of other invertebrates inhabiting these habitats. Habitat structural complexity increases when the habitat has had water for a longer period of time. These habitats provide favourable condition for development of other invertebrates that coexist with mosquito larvae. Due to intra- and interspecific competition for resources, the number of emergent mosquitoes decline significantly. A reduction in the structural complexity of a habitat leads to increase in intraguild predation (Predator-predator antagonism) thus reducing the predation pressure on the prey species. The structural complexity of habitats and the age of temporal habitats have been shown to influence arthropod populations in both natural

and agricultural environments (Rypstra *et al.,* 1999; Yanoviak, 2001a, b; Finke and Denno, 2002; Carlson *et al.,* 2004).

In conclusion, this study shows that there was natural regulation for mosquito production in the aquatic habitats. Although the number of emergent mosquitoes decreases due to predation pressure, the control mosquito developmental stages using larvicides such as Bti and Bs is required. The mosquito control programme should be iniatiated at the beginning of the rain season both the short and long rains and at the transplanting and tillering stage of the rice growth cycle.

CHAPTER 7: THE EFFECT OF RICE GROWING CYCLE ON MOSQUITO LARVAL ABUNDANCE AND ITS IMPLICATION ON MOSQUITO CONTROL

7.1 Introduction

Irrigation development projects worldwide have been associated with negative impacts on human health, particularly in respect to vector-borne diseases, and rice fields in particular constitute an important source of vector mosquitoes (Lacey and Lacey, 1990). Malaria has been associated with irrigation development and hence the necessity to include health and environmental assessments in irrigation development planning to prevent adverse health effects (Klinkenberg *et al.*, 2004; Mutero *et al.*, 2004a). It is notable that presence and abundance of mosquito breeding sites associated with rice irrigation often results in a corresponding increase of malaria vectors and water borne diseases. In Ahero irrigation Scheme of western Kenya, Surtees (1970) showed a 70-fold increase in the number of malaria vectors mainly *Anopheles arabiensis* compared to nearby non-irrigated areas.

The rice land agro-ecosystem, presents a complex system in which water is present throughout much of the crop-growing season hence the sustained vector production throughout the year with the somewhat limited variability as a result of changes in habitat properties. The physical and chemical properties of rice field water exhibit marked variations during crop cycle (Roger and Kurihara, 1988). These changes have a tremendous impact on the relative abundance of mosquitoes breeding in rice fields and associated habitats. Variations may occur in response to dilution effect by rain, dispersion of the surface soil by cultivation practices, biological phenomena and fertilizer application (Sunish and Reuben, 2001). Agricultural operations like weeding and drying

up of the field have a transient effect on the larval population (Rajendran, 1987). Broadcasting nitrogenous fertilizers in rice fields has been found to enhance mosquito larval populations (Simpson and Roger, 1991; Victor and Reuben, 2000). Source and water depth (Collins and Washino, 1980), temperature (Mogi, 1978), pH, ionic composition and conductivity (Kramer and Garcia, 1989) have been reported to influence larval density and their rate of development.

Source reduction through modification of larval habitats was the key to malaria eradication efforts in the United States, Italy, and Israel (Kitron and Spielman, 1989). The suppression and even eradication of malaria has to date been attributed to effective large-scale programs to kill the immature *Anopheles* species vectors or reduce the amount of suitable habitat for them in proximity to vulnerable human populations (Killeen *et al.*, 2002). In this study, the effect of rice growth cycle on abundance and diversity of mosquito larvae and how this could be used in the effective larval abatement programme in Mwea Rice Agro-ecosystem, Kenya was investigated.

7.2 Materials and Methods

7.2.1 Larval habitat description

The experimental plots were characterized visually and by use of hand held field equipment. Briefly, the depth of water was measured from the same point every visit using a ruler. Rice height was measured from the same rice plant every visit. Here the assumption was that the rice development is homogenous and development was at the same rate. Rice growth cycle was characterized using the agronomic standard of rice

growing into 5 categories namely: land preparation, transplanting, tillering, flowering (consisting of booting, meiosis, heading, panicle development and flowering), maturation and harvesting and post harvesting (ratoon development). Water turbidity was examined against a white background and scored in 4 classes namely: Clear, Low, Medium and High. To reduce the magnitude of human sampling error, one trained field assistant made the classification.

7.2.2 Larval sampling and larval identification

Larval sampling was done once weekly to generate stage-specific estimates of *Anopheles* abundance and diversity. Samples were taken using standard dipping techniques and a plastic dipper (Mosquito Control Service and Supplies, USA) with a wooden ladle was used. Five to twenty dips were taken from each sub-plot depending on water volume.

The mosquito larvae collected were sorted out according to the sub-families as either anopheline or culicine. The anopheline larvae were grouped according to the instar stage and identified morphologically (Gillies and Coetzee, 1987). The pupae were maintained in the insectary in emergent cages (Bioquip Products, Inc., Rancho Dominguez, CA, USA) to develop into adults. The emergent adult mosquitoes were identified by morphological criteria.

7.3 Data analysis

Simple descriptive statistics was used to tabulate the larval densities and abundance from the experimental plots. Pearson correlation was used to determine the association between mosquito larvae and the different physicochemical variables measured in the

plots during the larval sampling. The relative abundance of *Anopheles gambiae* was calculated as the number of mosquito larvae divided by the number of dips taken from each larval habitat. The dependent variable (relative abundance of a species) was transformed using the log transformation method {Log (x+1)}.

7.4 Results

7.4.1 *Anopheles* species composition

A total of 21,325 *Anopheles* larvae were collected of which 91.93% (n = 19,604) were early instars and 8.07% (n = 1,721) were late instars. A total of 513 pupae were collected. Table 7.1 shows the anopheline larval composition based on each rice growth cycle. Most of the larvae were collected between land preparation and tillering stage. Between flowering and rice maturation, there was a decline of the number of mosquito larvae from the plots. When the rice was harvested, there was an increase in larval abundance in the rice plots.

Table 7.2a shows the morphological identification of the late stage instars of *Anopheles* mosquito larvae. Morphological identification of *Anopheles* larvae yielded 84.09% (n = 1,274) *An. gambiae s.l.,* 13.47% (n = 204) *An. pharoensis,* 1.32% (n = 20) *An. rivulorum,* 0.79% (n = 12) *An. funestus,* 0.26% (n = 4) *An. coustani* and 0.07% (n = 1) *An. maculipalpis.*

Table 7.2b shows the morphological identification of emergent mosquitoes from the pupae. Most of the emergent mosquitoes were identified as *An. gambiae s.l.* (57.97%, n = 80), *C. quinquefasciatus* accounted for 23.91% (n = 33). Other species identified from the emergent mosquitoes included *An. coustani* (n = 4), *An. rufipes* (n = 2), *C. poicilipes* (n = 1), *Ae. taylori* (n = 4), and *Ae. circumluteolus* (n = 14).

Table 7.1 The *Anopheles* larval abundance from the experimental plots for the entire the rice growing cycle

Rice Stage	Early Instars *Anopheles*	Late Instars *Anopheles*	Pupae
Land preparation	2,554	99	72
Transplanting	7,081	143	11
Tillering	3,750	765	214
Flowering	1,219	170	28
Maturation	208	41	23
Harvesting/Ratoon	1,051	35	31
Total	**15,863**	**1,253**	**379**

Table 7.2a Morphological identification of late stage instars of *Anopheles* mosquito larvae

Species	Sum	Percentage
An. gambiae s.l.	1,274	84.09
An. funestus	12	0.79
An. pharoensis	204	13.47
An.rivulorum	20	1.32
An.maculipalpis	1	0.07
An. coustani	4	0.26
Total	**1,515**	**100.00**

Table 7.2b Identification of emergent mosquitoes from pupae

Genera	Mosquito species	Female	Male	Total	Percentage
Anopheles	*An. gambiae*	76	4	80	57.97
	An. coustani	0	4	4	2.90
	An. rufipes	2	0	2	1.45
Culex	*C. quinquefasciatus*	28	5	33	23.91
	C. poicilipes	1	0	1	0.72
Aedes	*Ae. taylori*	4	0	4	2.90
	Ae. circumluteolus	4	10	14	10.14
Total	Total	115	23	138	100

7.4.2 Temporal patterns of *Anopheles* larval densities

A bimodal pattern in distribution of the predominant species *An. gambiae s.l.* was observed over the 120 days (17 weeks) of rice growth (Figure 5). Overall, larval densities of *Anopheles* species increased in early stages of rice vegetative growth (tillering stage) and during late ripening and harvesting stage. During paddy preparation (denoted as week 0) the densities were low but rose in week 1, but rose again to reach a peak in week 3 with a mean density of 11.2 anophelines per 10 dips. A second peak was recorded in week 17 with a mean density of 8.7 anophelines/ 10 dips. A slight increase in density was observed from week 4 to week 6. From week 7 to week 14 the density of anopheline larvae was generally low, averaging less than 1 larva per 10 dips. The data shows that the distribution of the major anopheline vector species, *An. gambiae s.l.*, followed a pattern closely linked to rice crop phenology and condition of the rice field. Application of sulphate of ammonia fertilizer (18.5 g/m^2) at day 10 post transplanting (week 2) tended to promote larval productivity thereby leading to the peak seen in week 3 (11.2 larvae per 10 dips). Low larval densities were found during the late vegetative, reproductive and maturation phases of rice between tillering and harvesting.

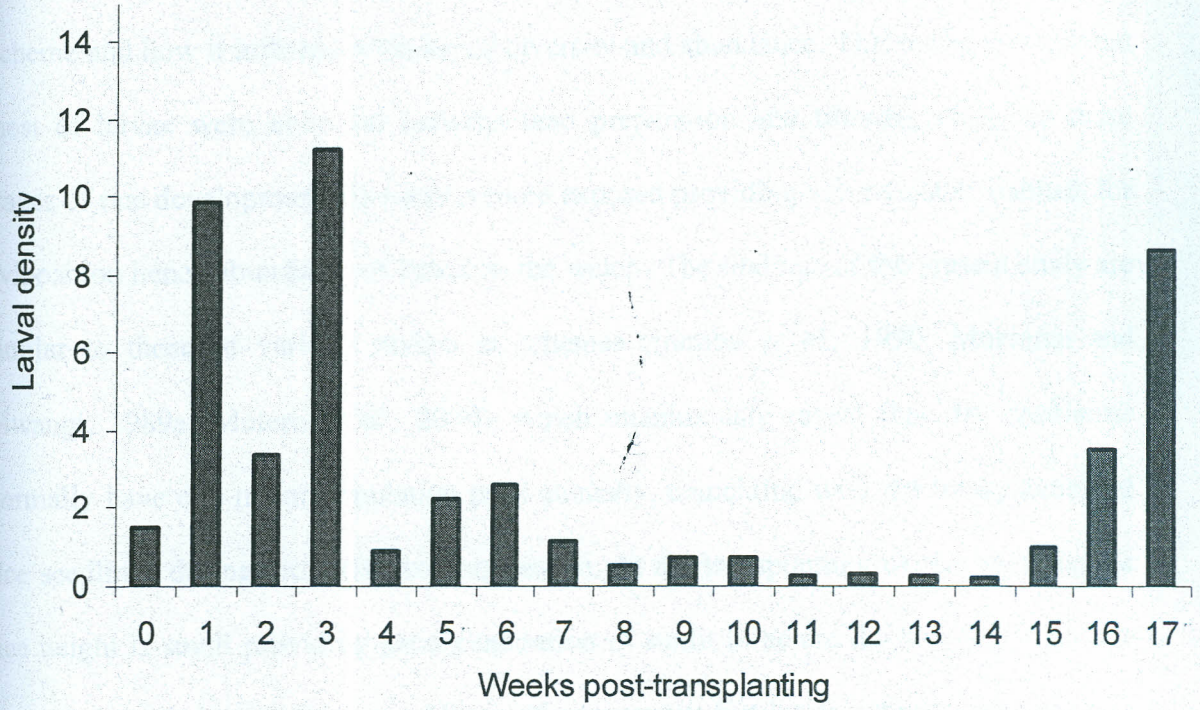

Figure 5 Weekly density fluctuations in mosquito larvae over the 120-day rice growing cycle

Foot note: Larval density is expressed as number of larvae per 10 dips; Weeks 0–1 = Field preparation; Week 1 = Transplanting; Weeks 2–3: Early vegetative phase; Weeks 4–8: Late vegetative phase; Week 9–13: Reproductive phase; Weeks 14–17: Ripening and Maturation phase.

7.5 Discussion

This study describes, the rice growing cycle as used by farmers in Mwea Irrigation Scheme and how it influences the larval diversity and abundance. This study showed that most of larvae were collected between land preparation and tillering stage. At these stages of rice development the water is more exposed providing more suitable habitats for oviposition hence abundance of larvae in the water. The findings of the present study are similar to those of various studies in schemes (Ijumba *et al.,* 1990; Mukiama and Mwangi, 1989a; Mutero *et al.,* 2000), which unanimously reveal that *An. arabiensis* normally have one major population peak annually, coinciding with the transplanting of rice seedlings during the main growing season. At the transplanting of rice seedling the rice height is small providing good penetration of sunlit to aerate the habitats. Also few days after transplanting there is addition of nitrogenous fertilizers, which were shown to attract *Anopheles* and culicine mosquito to oviposition (Victor and Reuben, 2000; Mutero *et al.,* 2004b). For effective control of the mosquito menace in Mwea Irrigation Scheme, consolidated efforts should be levelled against the pre-adult stage between the transplanting and the tillering stage. At these stages the abundance of mosquito larvae is highest, consequently larvicidal pesticides application would yield maximum reduction in adult population. At this stage, the vector control programmes should use integrated vector control mechanisms (Lacey and Lacey, 1990) targeting both the adult mosquito populations and larval control. Considering Mwea Irrigation Scheme, the rice growing is in phases; the control should be done all year round based on the rice-growing circle.

Morphological identification of the mosquito larvae yielded significantly more *An. gambiae s.l.* larvae than the other species. Further, the identification of emergent mosquito also gave more *An. gambiae s.l.* This indicates that the rice paddy produce more *An. gambiae* mosquitoes than the other species. This means that the rice farmers are exposed more to the malaria risk and there is a possibility of continuous parasite transmission. The implication of this is that, the people living within the scheme should use insecticide treated bednets (ITN) to minimize the human-vector contact. This would lower the chances of getting the malaria parasites hence remaining healthy.

The goal of this study was to evaluate the potential for targeted larval control in rice and the data generated provides sufficient evidence for targeted application of larvicides taking advantage of the 3-week window of productivity following transplanting of rice. The targeted plan would be more effective in situations where there is planned rice cultivation cycle and synchronous rice transplanting thereby minimizing the duration of time the rice fields are under rice cultivation. For effective control of the mosquito problem in Mwea Irrigation Scheme, consolidated efforts should therefore be targeted against the pre-adult stage between transplanting and early tillering stage. At the same time water management before rice transplanting should be considered, as there is strong evidence that continuous flooding reduces survival and productivity (Mutero *et al.*, 2000). Water management through intermittent flooding and draining of the rice plots applied during the first 3 weeks after transplanting would aid in flushing any mosquito larvae in the rice fields hence have an impact on mosquito densities. A more integrated approach to vector control using larval control strategy, approaches to reduce man-vector

contact would be a feasible plan of reducing adult mosquito and larval populations in similar rice agro-ecosystems in sub Saharan Africa.

In conclusion, these studies show that larval control programmes should be emphasized at the beginning of rice growing cycle when the larval productivity is highest. Throughout the rice growing cycle, larvicidal control programmes should go hand in hand with other vector control tools such as insecticide treated bednets. In Mwea Irrigation Rice Scheme Integrated Vector Management (IVM) programme would be ideal combining bednets and larvicidal application which targets larvae at the most productive times in the habitats.

CHAPTER 8: *ANOPHELES* SPECIES SUCCESSION IN RICELAND ECOSYSTEMS, MWEA, KENYA

8.1 Introduction

Development of water projects meant to increase agricultural production particularly in arid areas comes along with increased risk of mosquito-borne diseases. Numerous studies have demonstrated the complex malaria-agriculture linkages with more or less malaria depending upon local conditions and vectors (Ghebreyesus *et al.,* 2000; Ijumba and Lindsay, 2001). Increased prevalence of mosquito related diseases in these areas is attributed to increased vector densities and changes in vector phenology as a result of irrigation. However, in some areas increased vectors densities have been associated with low sporozoite rates and hence a reduction in malaria prevalence (Ijumba, 1997).

In Africa, few existing studies have demonstrated a strong relationship between rice cropping cycle and mosquito species succession. In Mali, *An. gambiae s.l* and *An. pharoensis* dominated the first 6-8 weeks of rice cycle followed by a sharp decline thereafter and dominance of *An. rufipes* and *An. funestus* (Klinkenberg *et al.,* 2004). In the Gambia, Snow, (1983) demonstrated succession of different mosquito genera in the rice fields. In this study *An. gambiae s.l.*, *An. rufipes*, and *C. neavei* were predominant during the early stages of rice development, *C. ethiopicus* and *C. poicilipes* around the middle of the rice cycle while *An. ziemanni* peaked as the rice matured. In addition, *C. antennatus*, *Mansonia uniformis* and *M. Africana* occurred throughout the growing season, but had little relation with the cycle of rice growth.

In Kenya, the only documented attempt to relate the rice cycle and mosquitoes species succession was conducted three decades ago in Kisumu (Chandler *et al.,* 1975). Like in the Gambia, this study documented *An. gambiae s.l* and *An. pharoensis* to occur during the early growing cycle while *M. uniformis, M. Africana* and *Mimomyia splendens* had no relation with rice cycle. In contrast however, *An. ziemmani* was abundant during the middle of rice cycle together with *C. poicilipes* while *C. antennatus* occurred towards the end of the cycle. Thus as demonstrated by these studies, the sequence of species succeeding each other during the rice development is location specific and should be addressed in this way. For instance, the replacement of *An. gambiae* by *An. funestus* as the rice develops may ensure continued transmission of malaria and filariasis throughout the cropping cycle. In this respect, having precise knowledge of the phenology of mosquito vectors in relation to rice growing cycle would facilitate their timely control. This study investigated the relationship between rice cropping cycle and mosquito species succession prior to implementation of an integrated vector control strategy based on microbial formulations.

8.2 Materials and Method

8.2.1 Experimental rice paddies

This study was undertaken in the experimental rice plots as described in section 3.7.

8.2.2 Larval sampling and identification

Larval sampling was done once weekly to generate stage-specific estimates of larval densities. Ten to twenty dipper samples depending on the amount water in each sub-plot

were taken at intervals throughout the sub-plot using a standard mosquito dipper (350 ml). If the sub-plot was covered with floating vegetation, the vegetation was carefully opened up to allow for water pooling before dipping was done. Samples from each sub-plot were pooled together in plastic bags (whirl paks) and transported to the laboratory where they were sorted into different instar stages of either anopheline or culicine counted and recorded. All third and fourth instar larvae were immediately preserved in 95% ethanol and later identified morphologically to species using taxonomic keys (Gillies and Coetzee, 1987). The early instars were kept in screen house and raised to 3rd and 4th instars and identified morphologically. The screen house was made of wooden frame work and the sides were covered using a plastic netting material (natural colour i.e. clear) which was fortified from inside by chicken mesh wire. The roof was made from translucent plastic fibre. This allowed the mosquito larvae to develop in the natural sunlight and relative humidity. The daily temperature and relative humidity fluctuation were monitored using a HOBO®.

8.3 Data analysis

The relative abundance of mosquitoes was expressed as the number of mosquito larvae per dip. The difference between the proportion of the physical and biological characteristic of the subplots in different stages of rice development was compared by chi-square test.

8.4 Results

8.4.1 Species composition and abundance

A total of 240 collections were made from 15 experimental plots over the 15-week rice growing cycle during the period 26th January 2006 and 11th May 2006. Anopheline larvae were found in 196 collections and 33 of these collections (13.8%) had only anophelines. A total of 1,683 late instar anopheline larvae were collected during the study period. Morphological identification of these larvae yielded 5 species of *Anopheles*. The *Anopheles* larval composition included *An. gambiae s.l* (n = 1,361; 80.87%), *An. pharoensis* (n = 272; 16.16%), *An. rufipes* (n = 37; 2.20%), *An. coustani* (n = 10; 0.59%) and *An. maculipalpis* (n = 3; 0.18%). Table 8.1 shows the *Anopheles* species composition at different stages of rice growth cycle. The duration of these stages are: Weeks 0–1 = Land preparation; Week 1 = Transplanting; Weeks 2–8 = Tillering (comprising of Early vegetative phase week 2-3 and Late vegetative phase Weeks 4–8), Week 9–13: Reproductive phase; Weeks 14–17: Ripening and Maturation phase.

8.4.2 Relationship between mosquito densities and rice growth cycle

The mean number of *Anopheles* larvae collected per dip during the rice growing cycle is shown in table 8.2. From this table, the *Anopheles* larvae increased immediately after transplanting and this was the highest numbers collected at the paddies. Thereafter the *Anopheles* larval densities were also high during booting stage (late vegetative stage) after addition of second top dressing with sulfate of ammonia fertilizer. From flowering, the *Anopheles* larval densities greatly declined upto the maturation stage.

Table 8.1 The morphological identification of *Anopheles* larvae at different stages of rice growth

Rice stage	*An. gambiae*	*An. Pharoensis*	*An. coustani*	*An. rufipes*	*An. maculipalpis*
Flooding	166	30	1	0	0
Transplanting	479	74	0	3	0
Tillering	335	132	0	14	0
Booting	183	3	0	9	0
Flowering	169	20	0	11	3
Maturity	29	13	9	0	0
Total	**1,361**	**272**	**10**	**37**	**3**

Table 8.2 Density of anopheline (± SE[a]) collected in different stages of rice growth.

Rice stage	Early *Anopheles* density	Late *Anopheles* density	Pupae density
Flooding	0.34 ± 0.06	0.11 ± 0.03	0.02 ± 0.01
Transplanting	1.04 ± 0.19	0.16 ± 0.05	0.14 ± 0.05
Tillering	0.32 ± 0.08	0.10 ± 0.03	0.02 ± 0.01
Booting	0.98 ± 0.16	0.17 ± 0.08	0.00 ± 0.003
Flowering	0.21 ± 0.03	0.04 ± 0.01	0.02 ± 0.01
Maturity	0.12 ± 0.03	0.02 ± 0.01	0.01 ± 0.01

Table legend: SE[a] Standard Error

Figure 6 shows the changes in mosquito species composition in relation to rice growing cycle. It is important to note the fertilizer treatment was: Diammonia phosphate (DAP) and murate of potash (MoP) was added at transplanting (Week 0), while top dressing with sulfate of ammonium (SA) was added at week 3 and week 7. *An. gambiae s.l.* was common throughout the rice growing cycle but their densities were 3.5-16 folds higher during the early stage of rice development than in the other periods. Three peaks were observed for *An. gambiae s.l.*, and these were preceded by fertilizer application the previous week. Di-ammonium sulphate and muriate of potash were applied during the rice seedlings transplanting phase while sulphate of ammonia was applied third and seventh week post transplanting. The other anophelines including *An. pharoensis*, *An. rivulorum*, *An. coustani* and *An. maculipalpis* were encountered. *Anopheles pharoensis* was commonly found from flooding stage upto week 7 post transplanting in higher numbers but after this period they population declined. After second top dressing with Sulfate of ammonia (week 3), *An. rufipes* started colonizing the paddies though in very small numbers. *An. maculipalpis* colonized the paddies at the flowering stage while *An. coustani* colonized the paddies when the rice was fully mature.

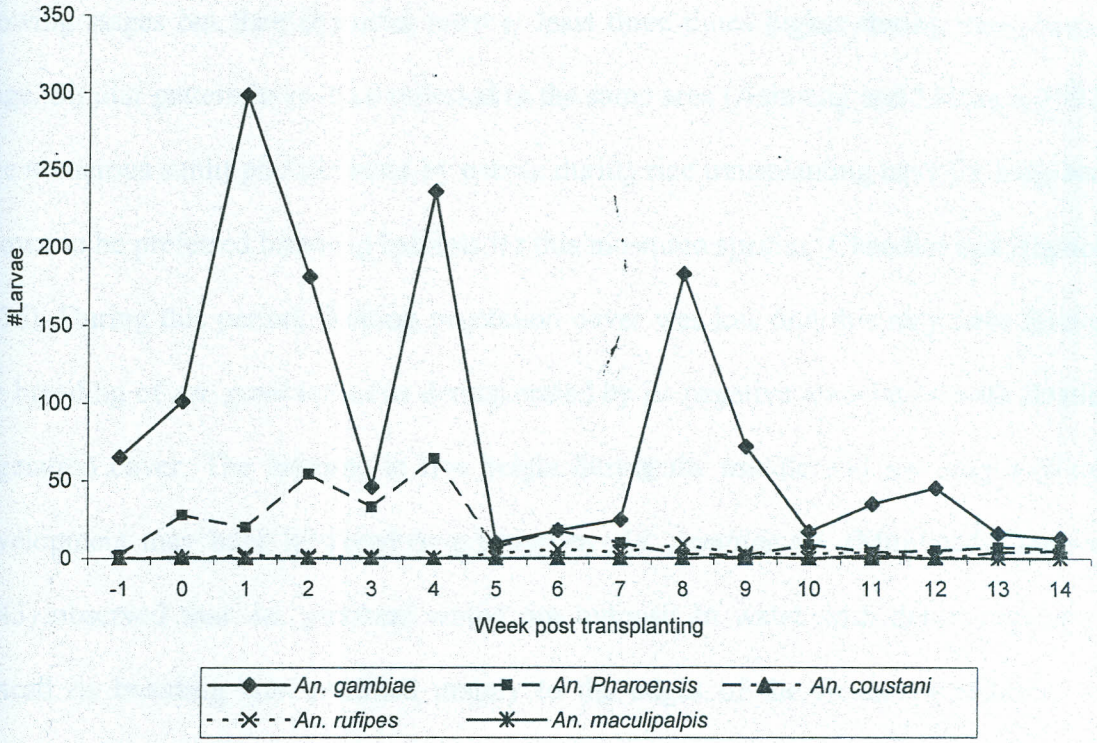

Figure 6: The *Anopheles* larval species succession from flooding to maturation of rice

8.5 Discussion

The rice fields in Mwea irrigation scheme in central Kenya seem to be excellent aquatic habitats for several genera of mosquitoes. *An. gambiae s.l* predominated most of the rice growing stages but their densities were at least three times higher during transplanting stage. Similar pattern have been reported in the same area (Asimeng and Mutinga, 1993). The numerous sunlit pools created by people during rice transplanting have for long been known to be preferred breeding habitats for this mosquito species (Chandler and Highton, 1976). During this period, floating vegetation cover was low and this may have favored the breeding of *An. gambiae s.l* as demonstrated by its negative association with floating vegetation cover. The increase in rice height during the middle and late stages of rice development may have hindered oviposition by *An. gambiae s.l.* (Muirhead-Thomson, 1945) observed that *An. gambiae* would not oviposit in water with dense vegetation; instead its breeding was confined mainly to the edges of the fields. In addition, the number of predators are known to increase as emergent vegetation becomes dense, considerably reducing survival of *An. gambiae s.l* larvae (Christie, 1958). In this study, a high proportion of rice plots harbored other aquatic invertebrates including potential predators of mosquitoes during the middle stage of rice development. It is therefore possible that increasing height favored predator population growth, which together with mechanical obstruction might explain the low density of mosquitoes during the middle and late stage of rice development. The support for this comes from the observed negative association between rice height and *An. gambiae s.l* density.

The results further showed that *An. rufipes* and *An. coustani* colonized the paddies after the booting stage. This signifies that these two *Anopheles* species preferred to oviposit in shaded habitats. Although they were found in low numbers, they seemed to be important in the paddies during the fully vegetative stage of rice. At this period, it was further observed the numbers of *An. pharoensis* declined in the paddies implying that too much canopy (shade) inhibits the oviposition of gravid *An. pharoensis* mosquitoes. Only *An. gambiae* was collected in the paddies throughout the rice cycle though there was a decline at the very late vegetative stages. In conclusion this study shows that there was succession found in the paddies. *Anopheles gambiae* colonized the paddies throughout the cycle, while *An. pharoensis* declined when the rice was fully at the vegetative stage. *Anopheles rufipes and An. coustani* colonized the paddies when they were rice was fully matured (from flowering stage). The larval control programme in the rice agro-ecosystem should target both the major malaria (*An. gambaie* and *An. pharoensis*) species colonizing the paddies at early stages of rice growth and also target the minor vectors (*An. coustani,* and *An. rufipes*), which come late in the rice cycle.

CHAPTER 9: THE SURVIVAL AND SPATIAL DISTRIBUTION OF IMMATURE *ANOPHELES* IN PADDIES

9.1 Introduction

Anopheles gambiae complex and *An. funestus* complex are the primary vectors of malaria in Mwea rice irrigation scheme. The distribution and abundance of mosquito larvae is as a result of availability of oviposition sites; the oviposition preferences of females; and the ability of the immatures to tolerate and develop after the eggs were laid.

Life tables provide a structured framework for identifying developmental stages most susceptible to mortality and, under some conditions, for inferring sources of mortality (Service, 1993a). The life tables for the developing immatures can be constructed using either horizontal or vertical methods (Reisen *et al.,* 1982; Reisen and Siddiqui, 1979; Service, 1993a). Horizontal life table methods are appropriate for distinct cohorts that can be followed through time, whereas vertical life table methods are appropriate for populations with overlapping generations and age distributions that remain stationary for the duration of the sampling period. Service, 1971, 1973, 1977), Reisen and Siddiqui, (1979) and Reisen *et al.,* (1982) provide extensive discussions about how such information can be analyzed.

In Kenya, Service, (1971, 1973, 1977) and Aniedu *et al.,* (1993) studied the survival of immature An. gambiae complex in the larval habitats. The objective of this study was to determine survival of immature *An. arabiensis* in different habitats in Mwea Irrigation Scheme and under the experimental rice growing conditions.

9.2 Materials and Methods

9.2.1 Horizontal and vertical Life Tables

This study was conducted as described in section 3.10. Briefly, for horizontal life table blood-fed *An. gambiae* s.l. females were collected with aspirators from houses throughout the villages and kept in cages containing 2% sugar solution in a semi field condition at the MIAD field station until they become gravid. Petri dishes containing a small amount of distilled water were placed inside the cages to attract oviposition in screen house. Newly laid eggs in these containers were monitored every 0.5 h to determine their time of eclosion. Approximately 2h after eclosion, 100 first-instar larvae (L1) were collected for each replicate. Duration of the preadult development period was determined by observing each cage at 0900 and 1600 h daily. The time taken in days for the larval developmental stages was recorded till emergence of adults. For vertical life tables, five paddies were selected at the Mwea Irrigation and Agricultural Development Centre (MIAD) for the sampling in this study. The 5 paddies were measuring 40 m by 80 m and were followed from transplanting till tillering. The numbers of larvae were counted per dip and recorded in each sampling. Twenty dips were taken from each paddy.

9.2.2 Measurement of the physicochemical factors

This study was conducted as described in section 3.6. Briefly, the pH, conductivity, dissolved oxygen and temperatures were measured using hand held machine YSI 650 Multiparameter Display System (YSI Environmental, YSI, Incorporated, Yellow Springs, USA). Salinity and Total Dissolved solids (TDS) were measured using field hand held

equipment YSI EC 300 (YSI Environmental, YSI Incorporated, Yellow Springs, USA). Turbidity was measured by placing water samples in a glass test tube and holding against a white background and was classified into four levels: clear low, medium and high. The micronutrients i.e. nitrates, phosphates, ammonia and sulphate content were measured using hand held field machines (HACH®, DR/2400 Spectrophotometer, Hach Company, Ames, Iowa).

9.3 Results

9.3.1 Age distribution and survivorship curves

Pre-adult developmental time in the experimental set up at the screen house was 11.85 days (d) (284.40 h) from eclosion to emergence (Table 9.1). The mean duration of each instar stage *i*, D_i, were estimated to be 1.40 days (d) (33.60 h) for first instars, 2.90 d (69.60 h) for second instars, 1.85 d (44.40 h) for third instars, 3.80 d (91.20 h) for fourth instars and 1.90 d (45.60 h) for pupae. The survivorship rate was estimated to be 92.94% during L1 stage, 98.1% for the L2 stage, 93.55% for the L3 stage, 83.44% for the L4 stage, and 97.52% for pupae. A total of 590 individuals emerged into adults, giving an overall survivorship from L1 to adult emergence of 69.41%. The emergent *An. gambiae* mosquitoes were composed of 354 females (60%) and 236 males (40%). It was observed that the males emerged first, then followed by the females and mostly the mosquitoes emerged in the evening.

The larvae per instars stage were plotted into a histogram and this resulted into the age distribution histograms, which was then fitted with a best-fit curve to give the age specific curve, which simulates the time specific survivorship curves (Figure 7). Overall survivorship curves for immature stages of *Anopheles gambiae* in cages in the paddy habitats were pooled due to the general similarity in the two-week period survivorship curves. Using the outline of Southwood, (1966) life tables for *Anopheles gambiae* immature stages were calculated from these survivorship curves as given in figure 7.

Table 9.1 Instar mortalities of *An. gambiae* in the screen house studies

Instars (i)	Age at the beginning of Instar (ti-1)	No. entering instar (Sti-1)	Deaths in instar (Di)	Relative proportion dying in Instar (Di/Sti-1)	Proportion dying daily in instars $1-(Sti/Sti-1)^{1/d}$
L1	0	850	60	0.071	0.051
L2	1.40	790	15	0.019	0.007
L3	4.30	775	50	0.065	0.035
L4	6.15	725	120	0.166	0.047
Pupa	9.95	605	15	0.025	0.013
Adult	11.85	590			

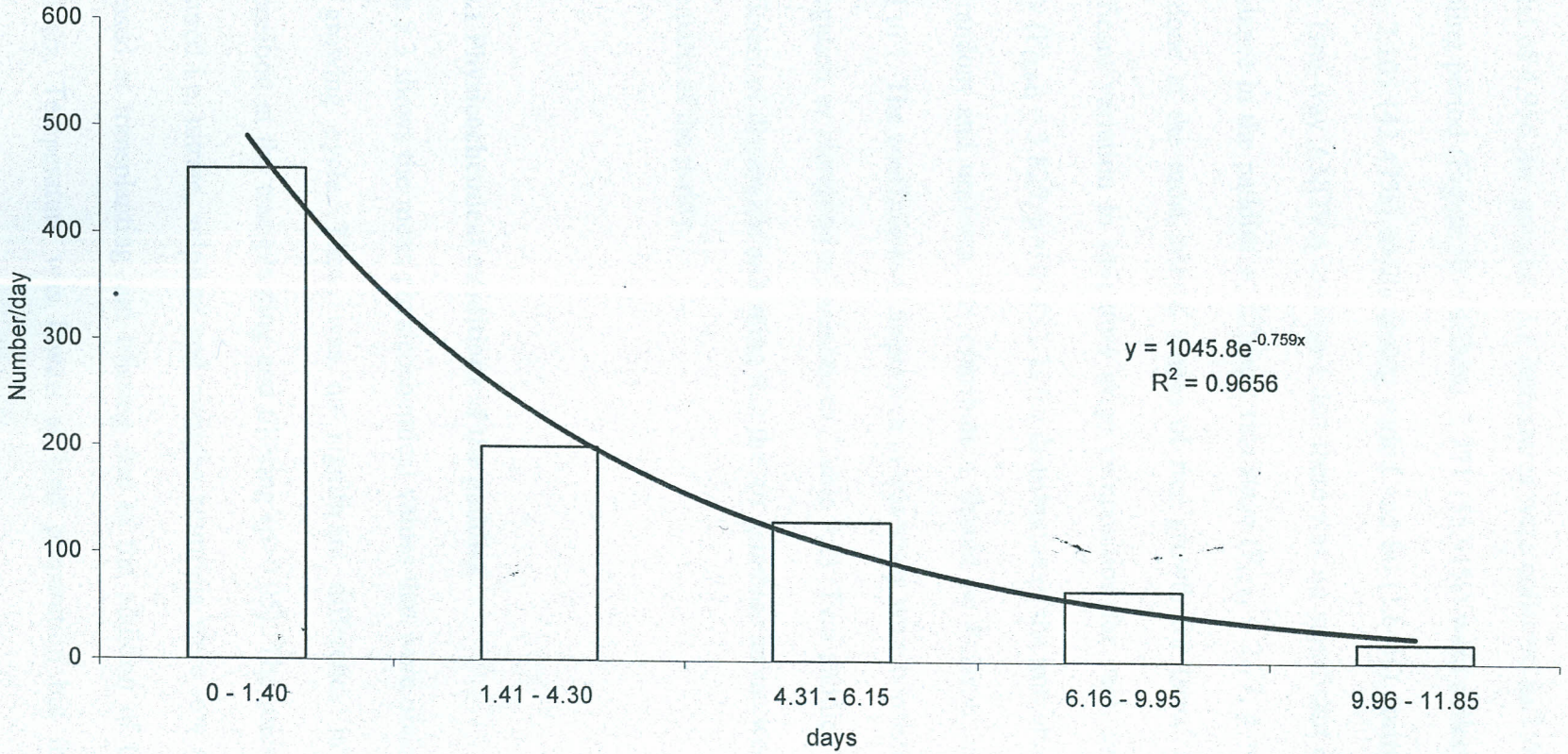

Figure 7 Age distribution and survivorship curve for the immature stages (1st instar –Pupae) of *Anopheles gambiae*

9.3.2 Vertical life tables

A total of 4,956 *An. gambiae s.l.* immatures were collected in 1,400 dips throughout the sampling period (Figure 8). Of these, 2,771 (55.91%) were collected during the tillering stage, 2,105 (42.47%) transplanting period and 80 (1.61%) during the land preparation stage. One-way ANOVA indicated that there was no significant difference in the larval abundance in the paddies at different rice stage ($F_{(2,6)} = 2.77$, $p = 0.14$) as the sampling was done at the most critical stages of rice growth for larval abundance. There was significant variation in immature stage composition for each day's collection in each paddy ($F_{(2,64)} = 3.829$, $p = 0.007$). If the distribution of the immature stages in the paddies was random and uniform, the distribution should be Poisson, with the variance/mean equal to 1. The coefficient of dispersion in this study were consistently was >1, implying aggregation or clumping of immatures (Table 9.2). Two paddies (C and D) had a large coefficient of dispersion indicating that the *An. gambiae* larvae were highly aggregated in some parts of the paddy.

9.3.2.1 Physicochemical conditions of the paddies

Table 9.3 shows the mean physicochemical values that were observed in paddies during rice growing cycle. There was no significant difference in the physicochemical composition at the transplanting and tillering ($p>0.05$). The micronutrients which were measured i.e. nitrate, sulphate and nitrogen ammonia were low at land preparation but increased at transplanting and tillering due to the addition of basal and top dressing fertilizers. Temperature was lowest at land preparation but increased slightly after

transplanting. pH remained was stable during the sampling period. Salinity and conductivity was highest at the land preparation but declined after transplanting. There was however no significant difference in the physicochemical composition between transplanting and tillering ($p>0.05$).

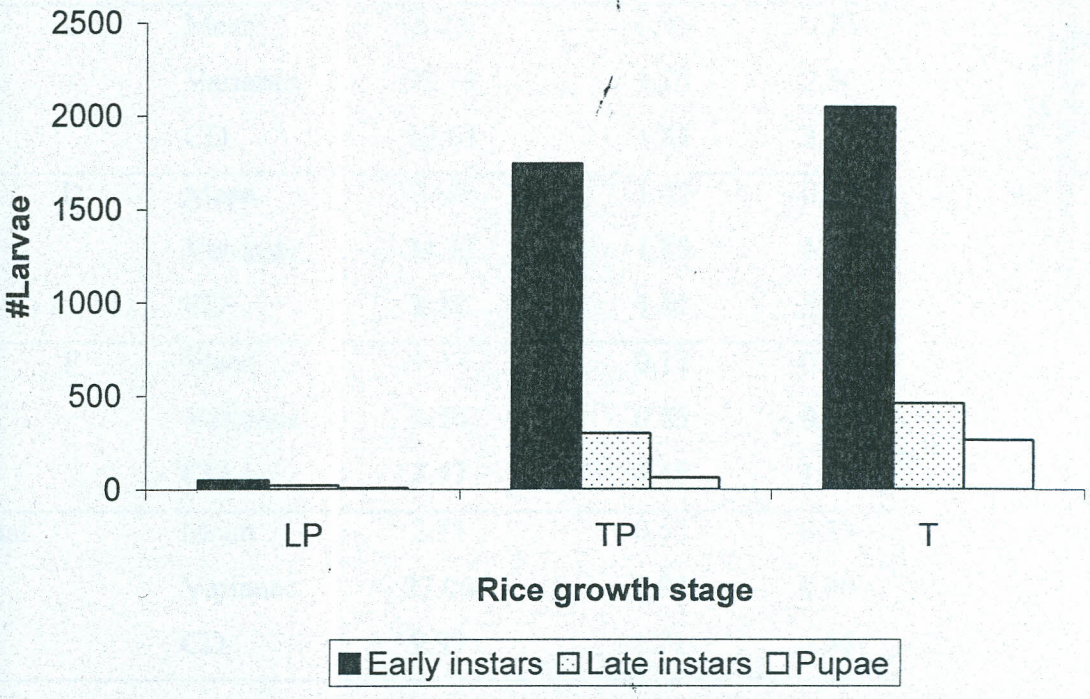

Figure 8 The total number of *Anopheles* immatures collected from paddies at each rice growth stage

Figure legend: LP, Land preparation; **TP,** Tranplanting; **T,** Tillering

Table 9.2. The Mean, variance (s^2) and coefficient of dispersion (CD) of the densities of *An. gambiae s.l.* larvae collected from each paddy.

Paddy number	Statistics	Early instars	Late instars	Pupae
A	Mean	1.18	0.34	0.05
	Variance	2.57	0.44	0.07
	CD	2.18	1.27	1.46
B	Mean	0.94	0.23	0.007
	Variance	1.00	0.96	0.033
	CD	1.066	4.12	4.62
C	Mean	6.20	0.96	0.75
	Variance	78.34	1.76	2.50
	CD	12.63	1.83	3.32
D	Mean	3.90	1.02	0.25
	Variance	34.32	1.85	1.31
	CD	8.81	1.81	5.30
F	Mean	1.33	0.17	0.11
	Variance	4.76	0.75	0.15
	CD	3.57	4.48	1.30
Total	Mean	2.71	0.55	0.23
	Variance	27.06	0.93	0.60
	CD	9.99	1.71	2.56

Table 9.3 Mean and SE of physicochemical conditions of water samples taken from paddies with different rice stage

Rice stage	Land preparation	Transplanting	Tillering
Temperature	22.8 ± 0.87	24.25 ± 1.07	24.22 ± 0.70
Conductivity (ms/cm)	301.5 ± 45.11	317.20 ± 60.30	178.72 ± 15.45
Salinity (ppt[a])	319.54 ± 23.01	240.44 ± 45.07	159.52 ± 18.32
DO[b] (%)	0.2 ± 0.01	64.92 ± 14.04	91.89 ± 13.71
pH	7.86 ± 0.15	7.85 ± 0.10	7.61 ± 0.06
Nitrate (mg/l)	13.6 ± 3.44	25.9 ± 4.99	12.64 ± 5.09
Sulphate (mg/l)	80 ± 5.25	54.4 ± 11.00	68.22 ±28.59
Nitrogen Ammonia (mg/l)	0.01 ± 0.001	4.1 ± 0.080	0.14 ± 0.02

Legend: ppt[a] Parts per thousand, DO[b] Dissolved Oxygen

9.3.2.2 Distribution of immatures *Anopheles* in the paddy

The distribution of immatures in the age classes for each age class and rice growth cycle is shown in figure 8. There was a significant difference in the *Anopheles* larval densities among the 5 paddies ($F_{(2,12)} = 4.94$, $p = 0.027$). These results further indicate that the survival of the immatures was better in some paddies than others. In the paddies, most early stages larvae were collected during transplanting and tilleing rice growth stages while most late stage instars and pupae were collected during tillering stage. The mortality rate during the transplanting was 99.95% and at tillering was 96.61% and the overall mortality was 98.26%.

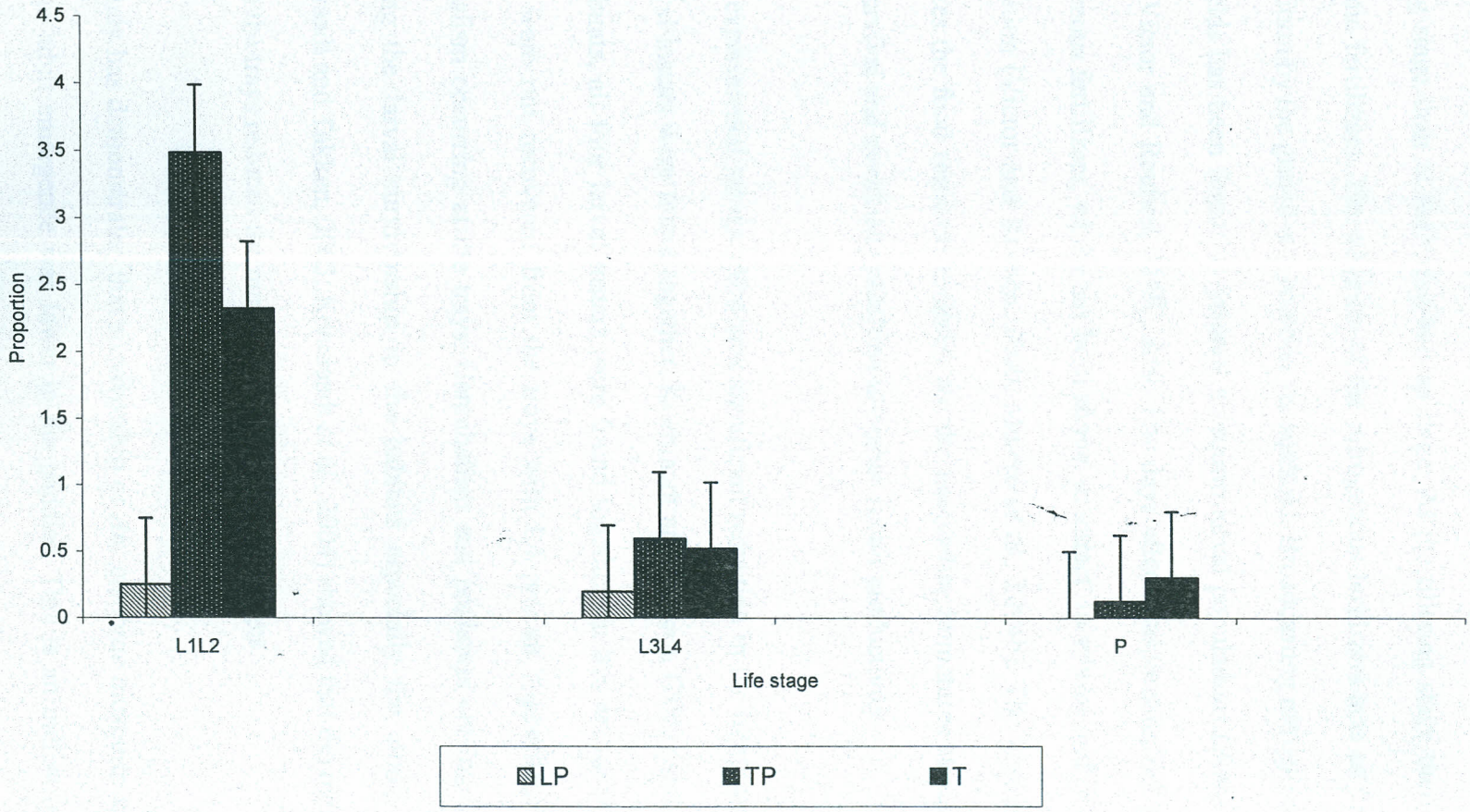

Figure 9 Proportion of *Anopheles* immatures in the paddy for each rice growth stage sampled at the MIAD center at the Mwea Irrigation Scheme

Legend: **LP** Land preparation, **TP** Transplanting, **T** Tillering

9.4 Discussion

This study also found a higher survivorship of *An. gambiae* larvae in paddies at the tillering stage than at the transplanting stage. At the tillering stage there is addition of inorganic fertilizers. The addition of the nitrogenous fertilizers acts as the attractant for oviposition by the gravid *An. gambiae* mosquitoes. Broadcasting nitrogenous fertilizers in rice fields has been found to enhance mosquito larval populations (Simpson and Roger, 1991; Victor and Reuben, 2000). Also few days after transplanting there is addition of nitrogenous fertilizers, which has been shown to attract *Anopheles* and culicine mosquito oviposition (Victor and Reuben, 2000; Mutero *et al.,* 2004b). The nitrogenous fertilizer enhances the food resource available for the anopheline immatures stages that increases their survival and eventually resulting to higher vector productivity.

In the experimental set up, there was significant reduction in the immatures. In the trays all larval instars were found together. Koenraadt and Takken, (2003) showed that within the habitats, all four larval instars were found together. In this set up majority of dead larvae were not recovered from the trays with L4 present suggesting that there was cannibalism occurring at the trays. Cannibalism and predation are important factors in reducing the larval survivorship in the habitats especially the small sized habitats. (Koenraadt and Takken, 2003; Koenraadt *et al.,* 2004) showed that the older larval instars (L4) in the trays reduced the numbers of earlier instars stages.

The study has demonstrated that survivorship of *An. gambiae* mosquito larvae from first instar to adult emergence was lower in the paddies. This is an indication that a higher

proportion of larvae and pupae died in the paddy habitats. This implied that the there was lower chances of the *An. gambiae* mosquito larvae attaining maturity due to high mortality rates. The findings in this study were similar to that of Sunahara *et al.,* (2002) who found out that the causes of mortality in the rice fields were caused by both abiotic and biotic factors. Service, (1977) attributed the efficacy of the natural enemies in such small habitats that may be inhabited by mosquito larvae to the small volume of water in them. When eggs are laid in a given habitat, mosquito competitors and predators can reduce mosquito densities by direct mortality and/or reduced growth rates due to reduced activity and due to reduced food availability.

This study also demonstrated that high proportion of larvae died in the 1st and 3rd instars as well as in pupal stage. The overall mortality was significantly high in all the habitats, being 98.26%. These high mortality rates are consistent with survivorship rates results of *Anopheles gambiae* and with larval mortality rates results by other workers under field conditions. Service, (1977) recorded 92.6-100% mortality for *Anopheles gambiae* rice fields and temporary pools, Kano plains Kisumu, while Aniedu *et al.,* (1993) recorded a 91.9% for *Anopheles gambiae* in Baringo, Kenya. The high mortality in the present study can be attributed partly to predation since presence of known and potential *Anopheles gambiae* larval and pupal predators were recorded although quantification of the relative contribution or proportion of mortality of the mosquito immature stages that was due to predation was not conducted in this study. However, previous estimates indicate that significant mosquito larval and pupal population reduction, 50-90% in *Culex*

tritaeniorhynchus (Reisen and Siddiqui, 1979) and 82% in *Anopheles gambiae* mosquitoes was due to predation effects (Mbogo *et al.*, 2003).

It was further observed that when the paddies were sprayed with fenitrithion (applied at a rate of 400ml/acre), 35 days after transplanting to control insect pests, all the *An. gambiae* larvae died and the survival rate was zero. The fenitrithion also killed all the other invertebrates within the paddy indiscriminately. The zero percent mortality continued for about ten days when the colonisation started again in the paddies.

More detailed ecological studies are required to elucidate the importance of predators and pathogens in regulating population size of the immature stages of *An. gambiae* in the rice fields of Mwea Irrigation Scheme during the rice growing cycle and to identify other limiting factors governing their numbers and local distribution. In conclusion, the survival of immatures at the rice fields is influenced by the availability of other invertebrates, which acts as predators and competitors in the habitats.

CHAPTER 10: THE DISTRIBUTION OF MOSQUITO LARVAE WITHIN THE PADDY AND ITS IMPLICATION FOR LARVAL CONTROL IN RICE AGROECOSYSTEM

10.1 Introduction

Although many species of mosquito thrive in rice fields, flooded paddy fields provide ideal breeding sites for the principal vectors of malaria in Africa: Members of the *Anopheles gambiae* complex, especially *An. arabiensis* (White, 1972), prefer to breed in open sunlit pools (Surtees, 1970; Gillies and Coetzee, 1987). These vectors are pioneer species, which rapidly colonize recently flooded fields, although they decline in abundance as the rice grows and begins to cover the water surface (Snow, 1983; Lindsay *et al.,* 1991; Ijumba, 1997). Irrigated-rice cultivation, depending on the number of cropping cycles, may also extend their breeding season and hence increase the annual duration of transmission.

Although, rice field is covered with water most of the time of the rice cycle, it is not clear which sites mosquitoes prefer to oviposit. The larval distribution within a paddy may vary depending on the oviposition behaviour of gravid mosquitoes. The objective of these sets of experiments was to access the larval distribution within a paddy prior to implementation of larval control programme in Mwea Irrigation Scheme.

10.2 Material and methods

The study was conducted in the Mwea Rice Irrigation Scheme (00°67'S, 37°35'E), at the MIAD centre rice farms. The paddy size was selected at Mwea Irrigation and Agricultural Development Centre (MIAD), which was the common size used by farmers

at the main rice scheme and the rice was grown following the normal agronomic cycle. The paddy size was measuring 40 M by 80 M.

Twenty-eight cages were distributed randomly within the paddy to assess the larval densities within different parts of the paddy. These cages measured 50 cm by 50 cm. A thirty centimeters high iron sheet was placed at the lower side to prevent movement of enclosed immature mosquitoes in and out of the cage. The cages were examined twice a week (Monday and Thursday) to examine the oviposition preference of mosquitoes within the paddy as measured by presence and density of mosquito larvae. The cages were placed between transplanting and flowering (Week 1 to week 14 post transplanting).

The edge was defined as 1 M from the periphery of the habitat, whereas the center was defined as a distance of 20 M from the edges of the paddy. In this paddy 15 cages were placed at the edge whereas 13 cages were placed at the center. Three rice cycles were followed in this study and the data collected was merged for analysis.

10.3 Data analysis

The larvae collected were expressed as densities (Larvae collceted/number of dips) for analysis. The larval densities where then log transformed to normalise the data. One-way Analysis of Variance (ANOVA) was used to test for variation in larval densities for cages at the centre and periphery. Two way ANOVA was used to determine the larval densities between cage position and rice growth cycle.

10.4 Results

The cages were sampled on 1,020 occasions in which they were found to have *Anopheles* larvae only, culicine larvae only and both *Anopheles* and culicine larvae co-existing. Table 10.1 shows the distribution of *Anopheles* and culicine larvae in the cages at the periphery and the center. The *Anopheles* larvae were found only at the cages at the periphery 30.7% (n = 84) and at the center 39.2% (n = 74). Both *Anopheles* and culicines larvae were found absent in the cages at periphery on 69.3% occasions (n = 190) and at the center 60.8% (n = 115). The cages at the center had *Anopheles* larvae co-existing together with culicines on 78.6% of the occasions (n = 235) while at the periphery they co-existed on 74.8% of the occasions (n = 193). Culicines were found alone at the cages at the periphery on 25.2% of the occasions (n = 65) while they were found at the center on 21.4% of the occasions (n = 64). The differences in the presence or absence of both the *Anopheles* and culicine larvae at the cages both at the center and periphery were highly significant ($\chi^2 = 262.24$, $p < 0.001$).

Table 10.1 The distribution of *Anopheles* and culicine larvae in the cages at the periphery and the center.

Cage position	Culicine category	*Anopheles* absent	*Anopheles* present	Total
Periphery	Culicine absent	190 (69.3)	84 (30.7)	274
Centre		115 (60.8)	74 (39.2)	189
	Sub total	**305 (65.9)**	**158 (34.1)**	**463**
Periphery	Culicine present	65 (25.2)	193 (74.8)	258
Centre		64 (21.4)	235 (78.6)	299
	Sub total	**129 (23.2)**	**428 (76.8)**	**557**

The periphery was visited 590 occasions (53.0%) and the center 524 (47.0%). In this study, 34,788 mosquito larvae were collected of which, 17,218 were collected from the periphery and 17,570 were from center. For *Anopheles* 2,731 larvae were collected at the periphery while 4,730 larvae were collected at the center. For culicine larvae a total of 14,487 larvae were collected at the periphery while 12,840 larvae collected at the center. A total of 650 pupae was collected at the periphery while 1,004 was collected at the center. The larvae collected were however expressed as densities (larvae/dip) for analysis. Figure 10 shows the mosquito larval densities collected during the sampling period. The densities of early and late stage instars *Anopheles* were higher at the centre than at the periphery and this difference was significant ($F_{(1,29)} = 10.472$, $p = 0.003$). However more culicine early instars were collected at the periphery than from the center while more late stage instars were collected at the center compared to the periphery but there was no significant difference for culicine densities which collected at the center and

the periphery ($F_{(1,29)} = 0.070$, $p = 0.792$). The densities of the pupae were higher at the center than at the periphery although this difference was not significant ($F_{(1,29)} = 3.774$, $p = 0.061$). The densities of culicine larvae were significantly higher than the densities of *Anopheles* larvae collected both at the center and the periphery ($F_{(142,971)} = 5.270$, $p < 0.001$).

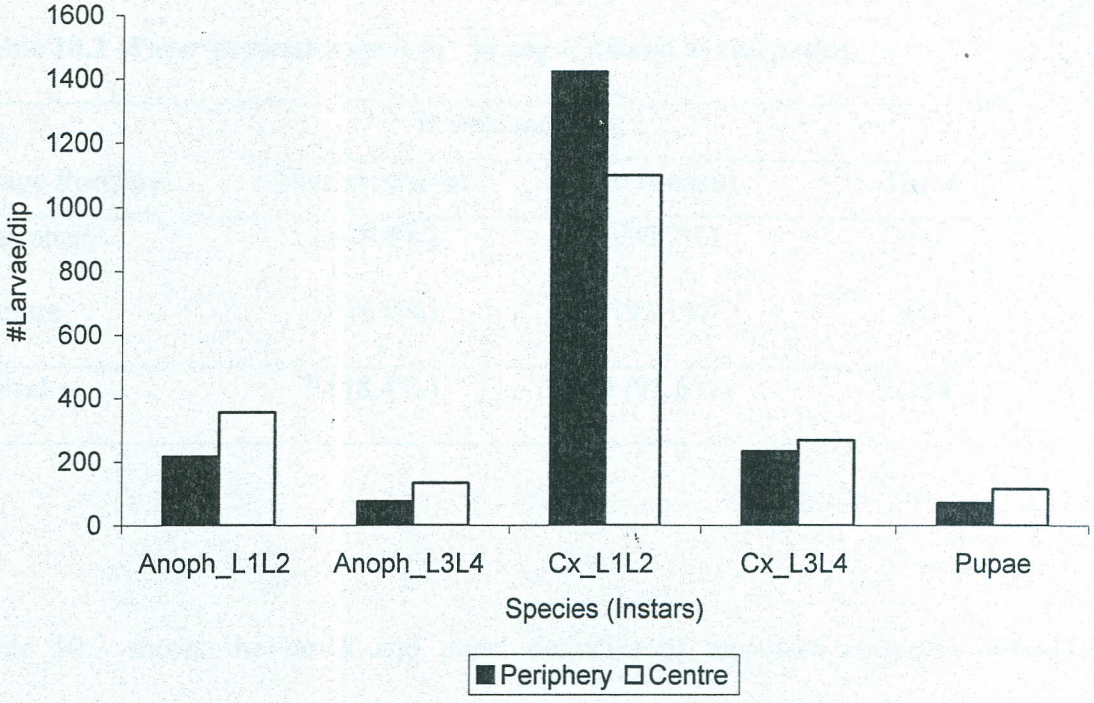

Figure 10 The densities of mosquito larvae collected from the cages at the periphery and the center

Figure legend:
Anoph_L1L2: Early instars *Anopheles;* **Anoph_L3L4:** Late instars *Anopheles;* **Cx-_L1L2:** Early instars Culicines; **Cx_L1L2:** Early instars Culicines

The cages were visited for sampling on 1,114 ocassions of which 94 times (8.4%) were found to be dry while 1,020 times (91.6%) had water. The cages at the periphery were dry in 58 occasions (9.8%) and were sampled 532 times (90.2%). Table 10.2 shows the times at which the habitat was sampled. The cages at the center were found dry in 36 occasions (6.9%) and were sampled in 488 occasions (93.1%). There was a significant difference ($F_{(1,782)} = 5.399$, $P = 0.020$) in the presence of water in the cages at the periphery and the center with the cages at the center having more times with water present.

Table 10.2 Water present/absent in the cages placed at the paddy

	Water category		
Cage Position	**Water Absent**	**Water Present**	**Total**
Periphery	58 (9.8%)	532 (90.2%)	590
Centre	36 (6.9%)	488 (93.1%)	524
Total	**94 (8.4%)**	**1,020 (91.6%)**	**1,114**

Table 10.3 shows the larval and pupal densities of mosquito collected during the sampling period. From this table, the densities of larvae and pupae were higher for both *Anopheles* and culicine at the center than at the periphery. There was significant difference for early instars *Anopheles* ($F_{(65,1048)} = 1.490$, $p = 0.008$) and early instars culicine ($F_{(137,976)} = 1.225$, $p = 0.05$) between the center and periphery while there was no significant difference for late stage *Anopheles* ($F_{(40,1073)} = 1.268$, $p = 0.125$) and late

stage culicines ($F_{(59,1054)} = 1.488$, $p = 0.11$) between the center and periphery. The densities of pupae were significantly different between the center and periphery ($F_{(37,1076)} = 1.601$, $p = 0.013$). The larval densities were higher at the transplanting and tillering stage both at the center and at the periphery but were lower at the flowering stage. Further analysis using two way Analysis of Variance (ANOVA) showed that the location of the cage was significant ($F_{(1,1108)} = 41.453$, $p < 0.001$) and the rice growth stage was also significant ($F_{(2,1108)} = 86.983$, $p < 0.001$) for *Anopheles* larval densities and the interactions between the cage location and rice stage was also found to be significant ($F_{(2,1108)} = 41.453$, $p < 0.001$).

Table 10.3 Densities of mosquito immature stages collected in the cages during the rice growth cycle

Cage Position	Rice stage	Early instars *Anopheles*	Late instars *Anopheles*	Early instars Culex	Late instars Culex	Pupae
Periphery	Transplanting	57.53	26.50	633.27	98.60	11.80
	Tillering	121.48	38.03	756.15	119.24	50.54
	Flowering	37.20	11.56	31.89	14.31	8.45
	Sub total	**216.21**	**76.08**	**1,421.30**	**232.15**	**70.79**
Centre	Transplanting	121.30	61.70	295.10	21.10	18.70
	Tillering	182.65	57.08	735.77	200.93	77.52
	Flowering	51.50	14.90	69.40	45.80	18.50
	Sub total	**355.45**	**133.68**	**1,100.27**	**267.83**	**114.72**
Total	Transplanting	178.83	88.20	928.37	119.70	30.50
	Tillering	304.13	95.11	1491.91	320.17	128.06
	Flowering	88.70	26.46	101.29	60.11	26.95
	Grand total	**571.66**	**209.77**	**2,521.57**	**499.99**	**185.51**

10.5 Discussion

This study sought to understand the distribution of mosquito larvae within the paddy, which would form the basis for larvicidal control products. This study found out that *Anopheles* and culicine larvae co-existed in the larval habitats. Most of the habitats sampled were inhabited by the two species occurring together. *Anopheles* and culicine larvae were found only in few occasions existing alone in the cages. The *Anopheles* were found existing mostly alone at the cages during the transplanting and the early tillering (vegetative stages) while the culicine were found existing alone at the late tillering and flowering stages. At the middle of the vegetative stages the both *Anopheles* and culicine larvae were found to exist together in most of the occasions.

The mosquito larvae were distributed in the paddy both at the periphery and at the center, although the center had more *Anopheles* larvae than the edge. The center had water most of the times during the sampling, which acted as oviposition cue for the gravid mosquitoes. Periphery had more culicine larvae than the center.

The study found out that there was no significant difference in mosquito larval abundance during the tillering stage. The tillering stage has been found to be highly associated with the highest larval densities due to the addition of nitrogenous fertilizers at this stage. The larval composition is same at the center and the edge. For effective control of mosquito larvae, the larvicidal agents should be applied at the entire plot at the tillering stage. This ensures a maximum reduction in the larval densities. The present study further showed that there was a significant increase of larval abundance from transplanting to tillering,

but after tillering stage there was a significant reduction in larval densities (Mulla *et al.*, 1990; Mutero *et al.*, 2000). In conclusion, the mosquito larvae were randomly distributed in the whole paddy. This implies that in mosquito abatement programme in Mwea Irrigation Scheme, the larvicidal agents should be applied in the entire paddy. The application should be made at the early vegetative stage to ensure maximum larval reduction.

CHAPTER 11: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

11.1 General discussion

11.1.1 Temporal and spatial distribution

Rice growing and rainfall significantly contribute to high abundance of mosquito larvae in the habitats. In Mbui Njeru, which is in the organized rice growing system, the larval abundance and densities correspond with the rice-growing season. Villages with unorganized rice growing (outgrowers) contribute more larvae than villages with organized (controlled) rice growing. The irrigation system, in the outgrowers was unorganized. The paddies and canals in the outgrower rice system tend to be poorly drained which makes them more favorable for anopheline larval development. The paddies were very small in size consequently leading to high densities of *An. gambiae* larvae. At the main irrigation scheme, the paddies were big (one single paddy is approximately one acre). This could lead to dilution effect of larvae in these paddy based on the size of the paddy. Moreover the paddy and the irrigation ditches (canals) in the organized rice growing are well drained. The outgrower rice growing occupies a small area compared to the organized rice growing system, which is under a big acreage.

Murinduko village initially was a non-irrigated village but rice was introduced later as an outgrower. This resulted in an exponential increase in larval densities. Introduction of irrigation created more breeding sites for mosquitoes. Initially most of habitats were concentrated in the stream at the edges of the village and stream pool at the Lower side

river. The soils and topography of this village does not allow formation of pools associated with rainfall, thus rainfall had no effect on increase of mosquito densities as the introduction of the creation of paddies had.

Paddy and irrigation canals were the most productive habitat types through out the sampling time frame. These habitat types have water during most of the rice growing cycle. The presence of water provides conducive habitats for larval development. During the rainy period, pools tyre tacks and pits (peri-domestic habitats) become productive, but after the rain period is over these habitats dry up. Even though the life period (the number of days with water) for these habitats is less, they are very productive. This makes these habitat types important sources of *Anopheles* mosquitoes during the wet season. In Murinduko, the stream pools are important habitat type. The stream has water through out the year. This stream is slow flowing and has several pools formed at the edges of the stream, which provides shallow and open habitats favorable for *An. gambiae* larval development.

Presence of other invertebrates was positively associated with *Anopheles* larval abundance. The mosquito oviposition is guided by biological and chemical cues to locate favorable habitats. Mokany and Shine, (2002, 2003), found that oviposition site selection by mosquitoes was affected by cues from conspecific larvae and anuran tadpoles. More larvae were found in habitats with presence of mosquito larvae while presence of tadpoles also attracted mosquito oviposition (Beehler and Mulla, 1995). The presence of other invertebrates indicates more permanent and stable habitats, hence the mosquitoes in

spite of the risk of competition, the anopheline will oviposit their eggs due to presence of water.

Azolla cover provides a mat-like structure on the surface of the habitat. This at times covers the entire habitat consequently reducing sunlit penetration. This inhibits mosquito oviposition in these habitats. The negative effects of the *Azolla spp.,* on mosquito production have also been documented by other investigators (Chow, 1969; Mogi *et al.,* 1986; Baolin, 1988). Increase in turbidity results in a significant reduction in anopheline larval densities in the habitat. Clear water provides a good attractant to *Anopheles* mosquito oviposition. McCrae, (1984) found that *An. gambiae* preferred a dark to a light background as an oviposition substrate. Clear water makes the substrate of the habitat visible, which the mosquitoes perceive as a dark background, consequently are attracted to oviposit. When the water is highly turbid, the *An. gambiae* mosquitoes do not get attracted, as these mosquitoes prefer to use clear and "clean" water.

Rice height was negatively associated with anopheline larval abundance. Various studies in schemes (Mukiama and Mwangi, 1989b; Ijumba *et al.,* 1990; Mutero *et al.,* 2000) uninamously reveal that *An. arabiensis* normally have one major population peak annually, coinciding with the transplanting of rice seedlings during the main growing season. At transplanting of rice seedling the rice height is small providing good penetration of sunlit to aerate the habitats. Also few days after transplanting there is addition of nitrogenous fertilizers, which were shown to attract *Anopheles* mosquito to oviposition (Victor and Reuben, 2000; Mutero *et al.,* 2004b). As the rice develops it

increases in height and the number of tillers increase, covering the habitat and this prohibits the breeding of *An. gambiae* mosquitoes.

11.1.2 Physicochemical factors regulating *Anopheles* abundance

This study examined the influence of physicochemical factors in the abundance of anopheline mosquitoes in Mwea Irrigation Scheme, Central province of Kenya. Larval abundance was found to be highest in between transplanting and tillering stage. At these stages of rice growth cycle water temperatures were highest at the experimental plots. When the rice was at the reproductive stage, these two parameters decline subsequently. A number of workers have documented the effects of temperature on development of temperature on development and relative abundance of Riceland mosquito larvae (Lacey and Lacey, 1990). The results of this study further indicated that water temperature was important factor for the abundance of *Anopheles* larval in the larval habitats. At transplanting and tillering there are many open spaces in between the plants, which provide good conditions for the growth of mosquito larvae. When the rice plants achieved maximum vegetation, the temperatures declined due to the effect of the rice canopy that make the water to be under shade throughout the day. It is know that *An. arabiensis*, which is the most predominant species in Mwea Irrigation scheme (Mutero *et al.*, 2000), has been shown to have a preference for shallow and exposed ground pools (Gillett and Smith, 1972).

Depth was found to be important factor for anopheline larval abundance. Increase in depth of a habitat increases the water volume in a habitat consequently having a negative

Anopheles mosquitoes. The drying habitats make the larvae which have not yet pupated die off and make it impossible for them to emerge as adults. Although as the habitats dried, the temperatures increase hastening the rate of development for *Anopheles* larvae, which eventually emerge as adults.

When the pools stayed with water for a week, culicines and other invertebrates colonized the habitat together with *An. gambiae*. In these pools at this condition were covered with cages and the emergent mosquitoes counted. The number of emergent mosquitoes decreased significantly compared to cages without other invertebrates. When the cages were placed in habitats, which had a very high density of other invertebrates, the emergent mosquitoes were reduced to very low numbers. (Service, 1977), did show that predation accounted for more than 95% of mortalities in the habitat. The present study showed that there is a significant role played by predation of mosquito immature stages at the habitats. Further work is required to incriminate the other invertebrates as predators.

In Kiamachiri, it was found that paddies were the most productive. Marshes followed paddy in the habitat productivity. It was found out that when, the structural complexity of the habitat was decreased, the number of emergent mosquitoes increased. The high vegetation cover and debris were found to harbour more aquatic stages of other invertebrates consequently having a high predation pressure. When the marshes were cleared, the number of emergent mosquitoes from these habitats was more and there was a decline in the number of other invertebrates inhabiting these habitats. Habitat structural complexity increases when the habitat has had water for a longer period of time. These

habitats provide favourable condition for development of other invertebrates that coexist with mosquito larvae. Due to intra and interspecific competition for resources, the number of emergent mosquitoes decline significantly. A reduction in the structural complexity of a habitat leads to increase in intraguild predation (Predator-predator antagonism) thus reducing the predation pressure on the prey species. The structural complexity of habitats and the age of temporal habitats have been shown to influence arthropod populations in both natural and agricultural environments (Rypstra *et al.,* 1999; Yanoviak, 2001a, b; Finke and Denno, 2002; Carlson *et al.,* 2004).

11.1.4 Rice cycle and larval control

This study shows that most of larvae were collected between land preparation and tillering stage. At these stages of rice development the water is more exposed providing more suitable habitats for oviposition consequently abundance of larvae in the water. This finding is similar to various studies in schemes (Ijumba *et al.,* 1990; Mukiama and Mwangi, 1989a; Mutero *et al.,* 2000), which unanimously reveal that *An. arabiensis* normally have one major population peak annually, coinciding with the transplanting of rice seedlings during the main growing season. At transplanting of rice seedling the rice height is small providing good penetration of sunlit to aerate the habitats. Also few days after transplanting there is addition of nitrogenous fertilizers, which were shown to attract *Anopheles* and culicine mosquito to oviposition (Victor and Reuben, 2000; Mutero *et al.,* 2004b).

11.1.5 Succession of *Anopheles* mosquitoes

The rice fields in Mwea irrigation scheme in central Kenya seem to be excellent aquatic habitats for several genera of mosquitoes. *An. gambiae s.l* predominated most of the rice growing stages but their densities were at least three times higher during transplanting stage. Similar pattern have been reported in the same area (Asimeng and Mutinga, 1993). The numerous sunlit pools created by people during rice transplanting have for long been known to be preferred breeding habitats for this mosquito species (Chandler and Highton, 1976). During this period, floating vegetation cover was low and this may have favored the breeding of *An. gambiae s.l* as demonstrated by its negative association with floating vegetation cover. The increase in rice height during the middle and late stages of rice development may have hindered oviposition by *An. gambiae s.l.* (Muirhead-Thomson, 1945) observed that *An. gambiae* would not oviposit in water with dense vegetation; instead its breeding was confined mainly to the edges of the fields. In addition, the number of predators are known to increase as emergent vegetation becomes dense, considerably reducing survival of *An. gambiae s.l* larvae (Christie, 1958).

The results further showed that *An. rufipes* and *An. coustani* colonized the paddies after the booting stage. This signifies that these two *Anopheles* species preferred to oviposit in shaded habitats. Although they were found in low numbers, they seemed to be important in the paddies during the fully vegetative stage of rice. At this period, it was further observed the numbers of *An. pharoensis* declined in the paddies implying that too much canopy (shade) inhibits the oviposition of gravid *An. pharoensis* mosquitoes. Only *An.*

gambiae was collected in the paddies throughout the rice cycle though there was a decline at the very late vegetative stages.

11.1.6 Distribution of mosquito larvae within the paddy

The mosquito larvae were distributed in the paddy both at the edges and at the center, although the center had more *Anopheles* larvae than the edge. The center had water most of the times during the sampling, which acted as oviposition cue for the gravid mosquitoes. Periphery had more culicine larvae than the center. This study showed that for effective larvicidal control programme the application of larvicidal agents should be applied more to center of the paddy.

The study found out that there was no significant difference in mosquito larval abundance during the tillering stage. The tillering stage has been found to be highly associated with the highest larval densities due to the addition of nitrogenous fertilizers at this stage. The larval composition was same at the center and the periphery. For effective control of mosquito larvae, the larvicidal agents should be applied at the entire plot at the tillering stage. This ensures a maximum reduction in the larval densities. The present study further showed that there was a significant increase of larval abundance from transplanting to tillering, but after tillering stage there was a significant reduction in larval densities (Mulla *et al.,* 1990; Mutero *et al.,* 2000).

11.1.7 Survivorship of *Anopheles* immatures

This study found a higher survivorship of *An. gambiae* larvae in paddies at the tillering stage than at the transplanting stage. At the tillering stage there was addition of inorganic

fertilizers. The addition of the nitrogenous fertilizers acts as the attractant for oviposition by the gravid *An. gambiae* mosquitoes. The nitrogenous fertilizer enhances the food resource available for the anopheline immatures stages that increases their survival and eventually resulting to higher vector productivity.

At the experimental set up, there was significant reduction in the immatures. In the trays all larval instars were found together. In this set up majority of dead larvae were not recovered from the trays with L4 present suggesting that there was cannibalism occurring at the trays. Cannibalism and predation are important factors in reducing the larval survivorship in the habitats especially the small sized habitats.

The study has demonstrated that survivorship of *An. gambiae* mosquito larvae from first instar to adult emergence was lower in the paddies. This is an indication that a higher proportion of larvae and pupae died in the paddy habitats. This implied that the there was lower chances of the *An. gambiae* mosquito larvae attaining maturity due to high mortality rates. When eggs are laid in a given habitat, mosquito competitors and predators can reduce mosquito densities by direct mortality and/or reduced growth rates due to reduced activity and due to reduced food availability.

It was further observed that when the paddies were sprayed with Fenitrithion was applied at a rate of 400ml/acre, 35 days after transplanting to control insect pests, all the *An. gambiae* larvae died and the survival was zero. The fenitrithion also killed all the other

invertebrates within the paddy indiscriminately. The 100% mortality continued for about ten days when the colonisation started again in the paddies.

11.2 Conclusions

1. Rice growing is an important source of malaria vectors in Mwea Irrigation scheme. Rice paddies and associated canals are most productive habitats throughout the year while peridomestic habitats are important only during the long and short rains.
2. Turbidity, temperature, conductivity, pH, and depth are strongly associated with *Anopheles* larval abundance. These parameters in a rice agro ecosystem are associated with the early stages of rice growth cycle, a period when high densities of larvae are present. For effective control of mosquito larvae, the application of larvicides should be done between transplantation and the reproductive stage of the rice.
3. *Anopheles* mosquitoes colonize the paddies at different times during the rice growth cycle. *Anopheles gambiae* colonizes the paddies throughout the rice growth cycle, while *An. pharoensis* is high from land preparation but declines when the rice is fully at the vegetative stage. *Anopheles rufipes* and *An. coustani* colonizes the paddies when the rice is fully mature (from flowering stage).
4. High larval mortality is observed in the rice paddies with most of the larval mortalities being experienced in L1 and L4 stages. Larval survivorship reduced significantly due to presence of other aquatic invertebrates.

5. The distribution of mosquito larvae within the paddy varies spatially. During the transplantation stage of the rice, the paddy has more mosquito larvae distributed at the centre compared to the periphery of the paddy. During the tillering stage, there is no significant difference in mosquito larval distribution at the paddy. Larval control in the rice agro ecosystem should therefore be done at the tillering stage.

11.3 Recommendations

1. The larval control of immature stages of anopheline mosquitoes should be implemented during the vegetative stage (early and late tillering). At the ratoon stage, the control should be applied after the paddies are flooded to encourage the flourishing of the tillers
2. Larval control programme should be implemented by individual farmers in Mwea Irrigation Scheme. This would ensure each farmer applies the larvicides at the appropriate stage to maximize on the larvicidal effect.
3. Addition of nitrogenous fertilizers results in increased oviposition, consequently the application of larvicides should coincide with fertilizer application (Week 3 and week 7 for Sulphate of Ammonia top dressing).
4. There is a significant role played by other invertebrates in the habitats, consequently further studies should be initiated on the predation ecology and how it regulates the anopheline larval abundance and diversity. The study should be designed to show the most effective predators in the habitats and how they can be used for larval control.

5. During rain season, the temporally pools should be targeted for control as they contribute more *An. gambiae* mosquitoes. They should be back filled and those that are big should be applied with larvicides.

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