CHAPTER THREE: MATERIALS AND METHODS

3.1 Study site

This study was carried out in the Mwea Rice Irrigation Scheme in Kirinyaga District of Kenya (Appendix 1). 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 1990; Mutero 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 1989b). 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 1990). The total area and the area devoted to rice cultivation at Mwea are 13,640 and 6,138 ha 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 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 1998).

3.1.1 Study villages

Three villages were selected from the Scheme for larval ecology work. The study was conducted in villages within and around the rice growing complex. 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 (Kamachiri) and 3) one village falling in 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.

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.

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.

3.1.2 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 include: distance to the nearest dwelling house, Surface debris, emergent plant cover, turbidity, presence of *Azolla*, habitat permanence (permanent or seasonal), rice growth cycle. Briefly distance to the nearest habitat was measured when less than 100 m but over 100 m it was estimated. Surface debris, emergent plant cover, and presence of *Azolla* were all estimated as percent of total surface covered. Habitat permanence was expressed in terms of the length of time the habitat contained water. Temporally habitats were therefore scored on basis of not holding water for a period no more that 2 months while permanent habitats had water for a period longer than this. Rice growing cycle was scored based on the stages of paddy preparation and subsequent rice development. This was categorized as land preparation, transplanting, tillering, flowering (consisting of panicle development, heading, flowering, meiosis), maturation and harvesting and ratoon crop. Turbidity was categorized in 4 classes namely clear, low, medium and high based on watercolor on a white background.

3.2 Collection of 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.

3.3 Mapping of Larval Habitats

In the study sites, the latitude and longitude coordinates of the productive larval habitats were mapped using a Geographic Positioning System (GPS). Geo-referenced layers of roads and major landmarks were overlaid onto the coverage to depict the distributions of larval habitats on a base map in ArcView 3.2a®.

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 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 larval habitat. Different rice 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 be 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 and aquatic nets for mosquitoes and invertebrates (Service 1993a; Danislozano 1997). In some cases (of smaller habitats), aquatic invertebrates were vacuum- and strainer- sampled (Settle 1996; Curtis 1998).

3.5 Characterization of larval Habitat

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 and k) Conductivity l) Temperature m) Relative humidity, n) Nitrate content, o) Phosphate content, p) Ammonia content, q) Sulphates and r) Chlorophyll *a* content.

3.6 Measurement of environmental variables

The environmental variables were measured using the technique described by (Minakawa 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 was 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 chlorophyll *a* content were measured using hand held field machines (HACH®).

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.

In experimental rice plots, comparisons were made between the numbers of mosquito larvae and aquatic invertebrates by standard dipping methods and aquatic nets for

different stages of *Anopheles* and other invertebrates from field preparation to rice harvesting.

A randomized complete block design was used to compare experimental control plots to those treated with the recommended rates of fertilizer and pesticide under standard rice cultivation practices. Treatments were assigned as follows: Ten plots (6.3m x 3.15m each) served as experimental controls where no fertilizer will be applied. Two replicates of ten plots each were treated to standard cultivation practices.

3.8 Influence of production of malaria vectors by Agricultural activities

In experimental rice plots, comparisons were made between the numbers of mosquito larvae and aquatic invertebrates by standard dipping methods and aquatic nets for different stages of *Anopheles* and other invertebrates. 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 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). The experiments used a complete randomized block design with two ponds per block and treatments allocated randomly among the ponds.

3.9 *Anopheles* mosquitoes Productivity from the habitats

A cross-sectional survey was done to assess the productivity of *Anopheles* mosquitoes from the rice fields. Eight cages measuring one metre by one metre by one metre (1 M^3) were placed on 8 sub-blocks habitats, randomly selected, at the Experimental plots to monitor the emerging adults for 14 days. One sub-block was selected from each of the 8 blocks. 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 at the MIAD field

station until they became gravid. Petri dishes containing a small amount of distilled water were placed inside the cages to attract oviposition. 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 habitats in the experimental plots (25 by 25 cm) at an initial density of 14 larvae/cm^2 of pan surface area (L/cm^2). The habitats were covered with a mosquito netting to exclude any ovipositing adults and converge the emergent mosquitoes. Duration of the preadult development period was determined by observing each cage at 0700 and 1600 h daily. The number of days taken 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

In the villages, larval sampling was done in two habitats per village based on their productivity. The larval instars were counted per dip in each sampling. Ten to twenty dips were taken depending on the habitat size. The habitats 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 and identified morphologically (Gillies 1987). The morphological features to be 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 mosquito was then be used for morphological identification.

3.12 Data Management

The data was analysed using SPSS for windows (Ver 11). Multiple logistic regression analysis was used to determine the association between the environmental and agricultural variables and the occurrence of anopheline and culicine larvae. The occurrence of a species (or a subfamily) was defined as the presence of a particular species (or a subfamily) 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)}. Principal factor component analysis was used to determine the factor/s, which are responsible for the abundance of *Anopheles* larvae in the aquatic habitat.

For the survival of the anopheline larvae vertical and horizontal tables (Reisen 1982;Service 1993a) were constructed.

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, will be:

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

where t_{i-1} will be the previous mean age at molting.

The percentage of total immature life spent at each instar will be:

$L_i = 100 \times D$ i $/ t_5$,

where t_5 will be the median time of adult emergence.

Relativized probability of capture in a vertical sample will be $P_i = D_i / D_p$,

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

Survivorship from L1 to adult emergence will be estimated by A/I,

where A = total number of adults

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

SECTION 2: RESULTS

CHAPTER 4: SPATIAL AND TEMPORAL DISTRIBUTION OF *ANOPHELES* LARVAE IN 3 ECOLOGICALLY DIFFERENT VILLAGES IN MWEA IRRIGATION SCHEME, KENYA.

4.1 Abstract

The use of irrigation to flood agricultural land during rice cultivation has over years been associated with an increase in number of disease vectors and corresponding increased health burden due to malaria and other vector and water-borne diseases. Flood irrigation provides water for breeding of *Anopheles* mosquitoes through out the rice growing cycle. Field studies were conducted to examine the primary factors responsible for regulating abundance and diversity of the aquatic stages of malaria vectors. The study was conducted in 3 villages in Mwea Rice Irrigation Scheme from April 2004 to March 2005. The villages were selected such that one village is within the scheme with organized rice cropping and irrigation (Mbui Njeru village), and one village falling on the periphery of the scheme with unorganized and limited rice growing (Kamachiri) while the third was a non-irrigated village outside the scheme (Murinduko). At each study site, all aquatic habitats were sampled every other week to generate stage-specific estimates of mosquito larval densities abundance and diversity. Sampling for mosquito larvae was conducted using standard dipping technique. In villages where rice growing was conducted, the 1st row of paddies from the edge of the village and associated habitats was sampled. A sampling unit of paddy field measured 60m by 60m and all habitats within this area were sampled. Canals, ditches and seep associated with each selected paddy were also

sampled. Records of distance to the nearest dwelling house, canopy coverage, surface debris, turbidity, flow rate, habitat permanence, habitat types, rice growth stage, number of tillers and presence of *Azolla* spp in the water were taken for each habitat. 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 stage. A total of 3,175 pupae were collected from the three villages. One-way ANOVA showed that there was a significant site-to-site variation in larval abundance and the Tukeys HSD (α = 0.05) further indicates that the 3 villages are different from each other in larval abundance. Paddy and associated canal were the most productive habitat type. Pools and puddles formed within the village were important during the rainy season. Mbui Njeru with organized rice growing had relatively lower mosquito larvae compared to Kiamachiri and Murinduko with unplanned rice growing. Multiple regressions showed that presence of other invertebrates, percentage *Azolla* cover, Distance to nearest homestead, water turbidity and rice height were the best predictors for *Anopheles* mosquito larval abundance in the habitats. This study shows that unplanned rice growing supports more mosquito larvae than planned rice growing system. From the results larval control activities should be initiated at early rice stages and during the rainy season and the unplanned rice system should be minimized.

Key words: Habitat characterization, larval diversity, *An. gambiae*, Paddy, Rice stage

4.2 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 1975; Oomen 1994; Ijumba 2001), with increased transmission in some situations (Coosemans 1985;Goonasekere 1988; Robert 1992) but not others (Robert 1988; Boudin 1992).

In Kenya, only a small fraction of potential area has been developed for irrigation. Rice is usually grown under irrigation throughout the cropping cycle in Kenya. Rice fields generally constitute to 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 nearby non-irrigated areas. Recently in Mwea irrigation Scheme of central Kenya, Mutero and colleagues (2004) 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 distribution and abundance of mosquito larvae reflect the oviposition preferences of adult females and the ability of immature stages to tolerate the conditions that prevail in aquatic habitats (Reisen 1981). Mosquito larval habitats are the location where many important life cycle processes take place: oviposition, larval development, adult emergence, resting, swarming and mating (Overgaard et al., 2002). Knowing the

ecological characteristics of the larval habitat and what environmental factors affect mosquito abundance is therefore essential for developing new mosquito control methods. Physical factors such as habitat permanence or degree of spatial heterogeneity and biotic factors such as predation are known to influence mosquito species assemblages (Bazter et al., 1996).

Effective control of malaria through vector management requires information on the distribution and abundance of vectors in the targeted areas. Anopheline larval control is a potentially important target in malaria vector control. 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 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). 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 is to describe key anopheline larval habitats, and to determine the spatial and temporal pattern in larval densities in 3 ecologically different villages in Mwea Irrigation Scheme, central Kenya.

4.3 Materials and methods

4.3.1 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 include: 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. Briefly distance to the nearest habitat was measured when less than 100 m but over 100 m it was estimated. Surface debris, emergent plant cover, and presence of *Azolla* were all estimated as percent of total surface covered. Habitat permanence was expressed in terms of the length of time the habitat contained water. Temporally habitats were therefore scored on basis of not holding water for a period no more that 2 months while permanent habitats had water for a period longer than this. Rice growing cycle was scored based on the stages of paddy preparation and subsequent rice development. This was categorized as land preparation, transplanting, tillering, flowering (consisting of panicle development, heading, flowering, meiosis), maturation and harvesting and ratoon crop. Turbidity was categorized in 4 classes namely clear, low, medium and high based on watercolor on a white background.

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. 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.

4.3.2 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 stages and scenarios 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 Coetzcee 1987). The pupae were kept in the insectary in cages to develop into adults and the emergent adults were then identified morphologically.

4.3 Statistical 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. 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. 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).

4.5 Results

4.5.1 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 stage (Table 4.1). Murinduko had significantly higher larvae than Kiamachiri and Mbui Njeru (ANOVA, $p< 0.01$). There is a significant site-to-site variation in larval abundance and the Tukeys Honest Significant Difference ($\alpha = 0.05$) further indicates that the 3 villages are different from each other in larval abundance. A total of 3,175 pupae were collected from the three villages. The mosquito larval densities confirm that there is a significant site-to-site variation in the 3 villages. Murinduko had significantly higher densities compared to Kiamachiri and Mbui Njeru.

4.5.2 Larval Identification and diversity in the 3 villages

Morphological identification of late stage instars of *Anopheles* larvae was done on 2,306 larvae (Table 4.2). This resulted in 1,893 (82.09%) *An. gambiae*, 58 (2.52%) *An. funestus,* 180 (7.81%) *An. pharoensis,* 47 (2.04%) *An. rivorulum,* 46 (1.99%) *An. maculipalpis,* 59 (2.56%) *An. rufipes,* and 23 (1.00%) *An. coustani.*

All the 7 different anopheline species were present in Murinduko and Kiamachiri whereas only 3 different species were represented in Mbui Njeru. *Anopheles gambiae* was the most predominant species followed by *An. pharoensis*.

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

Table 4.2 shows the anopheline late stage larval species composition identification from each village

Species	Kiamachiri	Mbui Njeru	Murinduko	Total
An. gambiae s.l.	414	174	1,333	1,921
An. funestus	10	0	48	58
An. pharoensis	23	14	143	180
An. rivulorum	7	1	39	47
An. maculipalpis	2	0	44	46
An. rufipes	4	0	55	59
An. coustani	2	0	21	23

4.5.3 Pupal densities and adult emergence

Of the 3,175 pupae collected, 1,104 pupated into adults that were then identified morphologically. The species composition of the emergent mosquitoes was made up of 16 species. *Anopheles gambiae* s.l. was the most predominant anopheline species while *C. quinquefasciatus* was the most common Culex species. Most of the emergent mosquitoes were from Murinduko which had the most pupae.

4.5.4 Mosquito larval densities from each habitat type

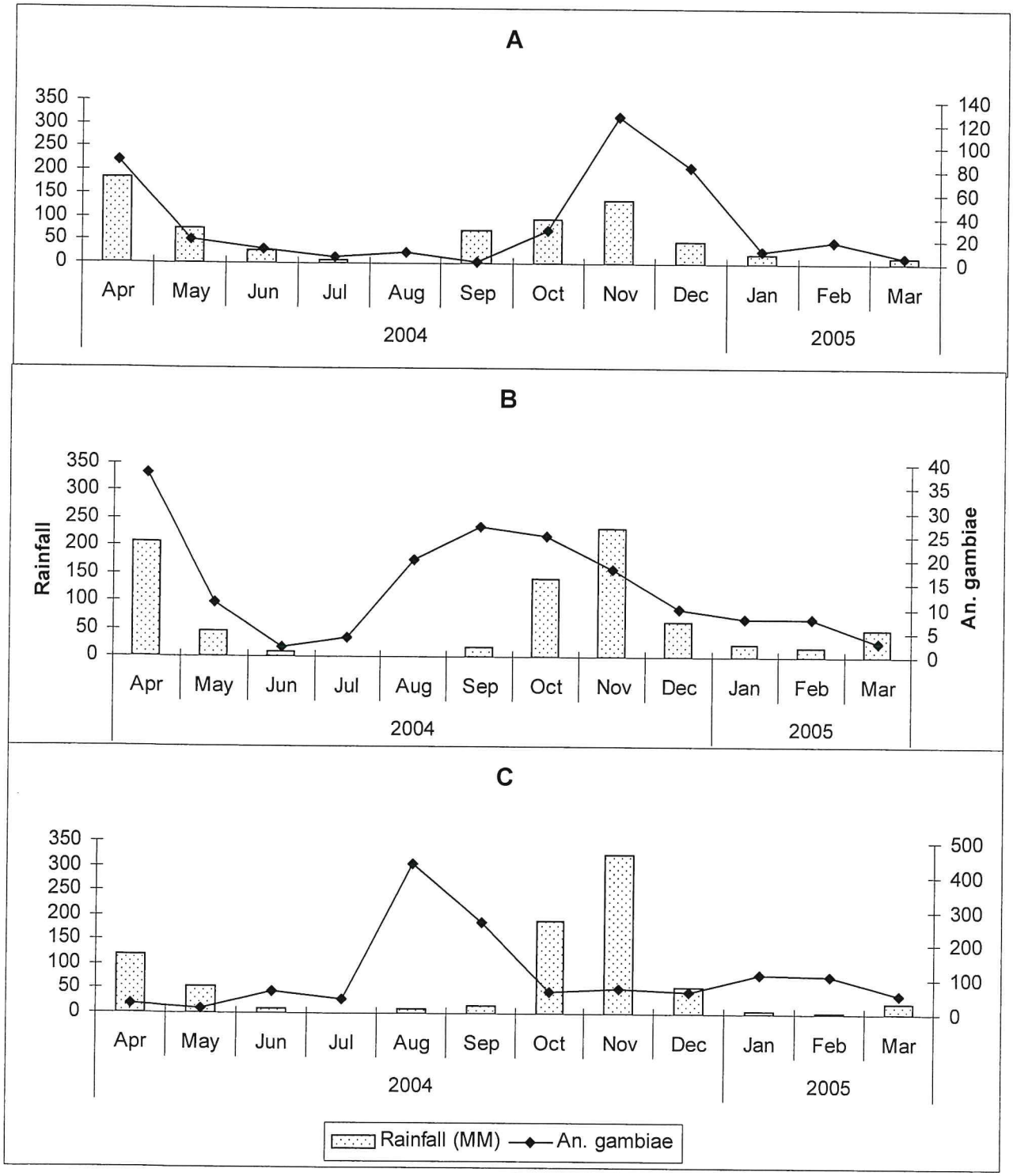
Habitat type is a significant factor in larval densities ($F = 16.113$, $p = 0.000$) in the 3 villages. In the 3 villages, paddy and canals had high densities of both anopheline and culicine larvae than the other habitat types (Table 4.1). In Kamachiri, holes and pit, seep and pools also had higher densities. Hoof prints and tyre tacks were important habitat types in Mbui Njeru. In Murinduko, stream pools, pools and seep are important habitat types in larval densities.

In Mbui Njeru and Kiamachiri, paddy and canals had higher densities of mosquito larvae. Murinduko there were various habitat types which had higher densities. This includes: paddy, canal, stream pools, seeps and pools. Hoof prints were important for larval development in Murinduko and Mbui Njeru. In Kiamachiri, no hoof prints were found. Trye tracks were predominant habitat type in Mbui Njeru and produced higher densities of larvae especially during the rain season.

4.5.5 Temporal variation of *An. gambiae s.l.* and rainfall pattern

Figure 3 shows the effect of rainfall on the abundance of *An. gambiae* mosquito larvae from each of the village. From this figure, the *An. gambiae* larval abundance correlated with the both short and long rains (r = 0.7585) with the peak of larvae and rainfall been April and November in Kiamachiri. In Mbui Njeru, the short rains corresponded with *An. gambiae* larval abundance with 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 increase in larval abundance, but with 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 larval abundance. Rainfall in this village had a low and negative correlation with *An. gambiae* abundance (r = -0.2671).

Figure 3 shows the relationship between Rainfall, *An. gambiae S.L.* larvae in A) Kiamachiri, B) Mbui Njeru, C) Murinduko from April 2004 to March 2005



4.5.6 Multiple Regression analysis

Stepwise logistic regression showed that presence of other invertebrates, percentage *Azolla* cover, Distance to nearest homestead, water turbidity and rice height were the best predictors for *Anopheles* mosquito larval abundance in the habitats (Table 4.3). An increase in percent *Azolla* cover, turbidity and rice height had a negative effect in *Anopheles* larval abundance. Whereas presence of other invertebrates and distance to the nearest homestead had a positive correlation with the larval abundance.

Table 4.3 Logistic regression for *Anopheles* larval abundance in the larval habitats

Variables	B	S.E.	Wald	df	Sig.	Odd Ratio	95.0% C.I.for OR	
							Lower	Upper
Distance (M)	0.011	0.004	10.558	1	0.001	1.012	1.005	1.019
Turbidity			8.937	2	0.011			
o Clear	0.827	0.277	8.896	1	0.003	2.287	1.328	3.938
o Low	0.624	0.253	6.092	1	0.014	1.867	1.137	3.065
Emergent vegetation	0.159	0.251	0.403	1	0.526	1.172	.717	1.916
Floating vegetation	0.335	0.211	2.524	1	0.112	1.398	0.925	2.114
Submerged vegetation	0.305	0.911	0.112	1	0.738	1.356	0.227	8.088
Water Depth	0.052	0.020	6.522	1	0.011	1.053	1.012	1.096
Other invertebrates	-0.769	0.163	22.255	1	0.000	0.463	0.337	0.638
Paddy category			9.425	4	0.093			
o Land preparation	-0.113	0.336	0.114	1	0.736	0.893	0.462	1.725
o Transplanting	-0.336	0.304	1.218	1	0.270	0.715	0.394	1.298
o Tillering	0.318	0.285	1.248	1	0.264	1.375	0.787	2.403
o Flowering	-0.014	0.346	0.002	1	0.968	0.986	0.501	1.942
Rice height	-0.001	0.004	0.098	1	0.754	0.999	0.990	1.007
#Tillers	-0.004	0.010	0.188	1	0.664	0.996	0.976	1.016
%Water cover	-0.003	0.003	1.238	1	0.266	0.997	0.991	1.002
%Azolla cover	-0.010	0.002	18.533	1	0.000	0.990	0.985	0.994
Distance category			3.948	2	0.139			
0 – 100 M	1.311	0.681	3.708	1	0.054	3.709	0.977	14.084
101 – 200 M	0.648	0.423	2.346	1	0.126	1.912	0.834	4.380
Season (Wet or Dry)	-0.363	0.178	4.179	1	0.041	0.696	0.491	0.985
Constant	-23.631	22003.100	0.000	1	0.999	0.000		

4.6 Discussion

Mosquito Larval habitat ecology is thought to be important in determining larval densities and species assemblage. These in turn influence malaria transmission in an area. Understanding larval habitat ecology is therefore important in designing malaria control programs. A renewed interest in mosquito larval control has been the central them in recent studies exploring the feasibility of reducing malaria vector populations through environmental and agro-ecosystem management approaches (Keiser et al., 2002; Killeen et al., 2002). We have studied the ecologies of several larval habitats in Mwea Irrigation Scheme in 3 ecologically diverse villages. This study has followed the larval habitat every other week for a year.

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 is 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 are very small in size. This could lead to high densities of *An. gambiae* larvae. At the main irrigation scheme, the paddies are 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.

Although even before introduction of rice cultivation, the three villages might differ in terms of hydrology and natural breeding sites (Briet et al., 2003), villages with unplanned and/or limited rice cultivation would be expected to have diverse breeding sites than those within the scheme. More mosquito species were observed outside the irrigation scheme than within the scheme, suggesting that in addition to paddies, villages' with unplanned rice cultivation may have diverse larval habitats supporting more species compared to those within the scheme. This is more so because within the scheme, most of the land is under rice cultivation and thus natural breeding sites may be rare unlike in areas with unplanned and/or limited rice cultivation where natural breeding sites are common and contribute significantly to mosquito production. It is well documented that villages with diverse breeding habitats have more mosquito species diversity compared to those with limited larval habitat diversity. In western Kenya, more species were reported in Kisian, a village with permanent and diverse larval habitats compared to Saradidi with little larval habitat diversity (Beier et al., 1990). Further studies are underway to identify the larval habitats for these mosquito species, since most of them might be breeding in

sites other than rice fields. This possibility is supported by significantly higher number of *An. funestus* and more species diversity in Murinduko where there was limited rice cultivation compared to the other villages.

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.

Several factors were to be found to be good indicators in the abundance and density of *Anopheles* larvae in the habitats. The distance to the nearest homestead is important for the larval abundance. *Anopheles gambiae* is known to be anthrophagic and endophilic (Mbogo et al., 1993; Mwangangi et al., 2003) and its oviposition is basically in open, sunlit habitats (Gillies and De Meillon, 1968, Gillies and Coetzcee 1987) basically found to be associated with human activities such as pools and ditches. Due to agricultural activities in Mwea Irrigation Scheme, the distance between larval habitat and nearest

human habitation is positively correlated. With increase of the distance there is also increase of larvae. This is because most productive habitats are associated with paddy and the canals. These habitats predominate throughout the year especially when the rice is growing. Consequently these habitats though have some distance to the homesteads have larvae. The gravid mosquitoes will fly in search of water and oviposit in the paddies. *Anopheles arabiensis* is the only sibling species of *An. gambiae* complex found in Mwea Irrigation scheme (Mutero et al., 2004a, b). Although *An. arabiensis* is anthrophagic, (Beier et al., 1990; Ijumba et al., 1990; Petrarca et al., 1991; Petrarca and Beier, 1992; Githeko et al., 1994) the availability of cows is a determining factor for blood feeding. The alternative sources of blood meal for *An. arabiensis* coupled with its exophilic behavior make them to be able to oviposit in habitats, which are far away from human dwelling.

Presence of other invertebrates is 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 (Beehler et al., 1995) while presence of tadpoles also attracted mosquito oviposition. 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.

Percent *Azolla* cover, turbidity, and rice height were negatively associated with anopheline larval abundance. *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 et al., 1969; Mogi et al., 1986; Baolin et al., 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 don't get attracted, as these mosquitoes prefer to use clear and "clean" water. Rice height is negatively associated with anopheline larval abundance. Various studies in schemes (Mukiama and Mwangi 1989a; 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. Our finding is similar with these studies. 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. Increase of tillers was also found to be negatively associated with culicine mosquito larval abundance.

Conclusively, rice growing is an important source of mosquito production. Rice paddy and canal are most productive habitats. This study also shows that unplanned rice growing supports more *Anopheles* larvae than planned rice growing system. From the results larval control activities should be initiated at early rice stages and during the rainy season. The unplanned rice system should be better managed and if possible control by the management system of the Mwea Irrigation Scheme where water for irrigation is put together with those of the scheme.

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

5.1 Abstract

The use of irrigation to flood agricultural land during rice cultivation has over years been associated with an increase in number of disease vectors and corresponding increased health burden due to malaria and other vector and water-borne diseases. Flood irrigation provides water for breeding of *Anopheles* mosquitoes through out the rice growing cycle. Field and experimental plots were used to examine the physicochemical factors responsible for regulating abundance and diversity of the aquatic stages of malaria vectors. The field study was conducted in 2 villages in Mwea Rice Irrigation Scheme from July 2004 to March 2005. The villages were selected such that one village is within the scheme with organized rice cropping and irrigation (Mbui Njeru village), and one village falling on the periphery of the scheme with unorganized and limited rice growing (Kamachiri). The experimental plot was developed at the Mwea Irrigation and Agricultural Development Center (MIAD), in Mwea Irrigation Scheme. One rice test plot (1 Acre; 63m x 63m) was established in the Mwea rice irrigation scheme. Within the 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. Thirty sub-blocks were randomly selected for larval survey and water analysis studies. At each stage of rice growth (transplanting, tillering, flowering and maturation, 3 water analysis were done. At each study site, all aquatic habitats were sampled every other week to generate stage-specific estimates of mosquito larval densities and abundance. Sampling for mosquito larvae was conducted using

standard dipping technique. During each larval collection, physicochemical variables such as pH, temperature, conductivity, salinity, and dissolved oxygen were collected. 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. For culicine mosquito larvae, 1,942 were collected of which, 79.66% (n = 1,547) were early stage instars and 20.34%(n = 20.34) were late stage instars. A total of 146 pupae were collected from the two villages. At the experimental plots, most of the larvae were collected at the transplanting and the tillering stage of rice development. Pearson correlation showed that water temperature was significantly and positive correlated to both anopheline (r = 0.122) and culicine (r = 0.125) larvae. Logistic regression model gave pH as the best indicator for anopheline larvae at the two villages while salinity was the best predictor the anopheline larvae at the experimental plots. None of the physicochemical variables were found to be significant for presence of culicine larvae in the habitats in both the villages and at the experimental plots. This study shows that pH, salinity, and temperature are key determinants in the abundance of anopheline larvae.

Key words: Experimental plots, physicochemical variables, standard dipping technique, *Anopheles,* Larval densities, Rice growth

5.2 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, Ijumba et al., 2002), its peak population coinciding with the transplanting of rice seedlings (Mutero et al., 2000).

Studies by Rejmankova and colleagues (1991, 1993), 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 (1999) in western Kenya did not detect any significant association between the occurrence of *An. gambiae* larvae and habitat variables. Recent studies by Gimnig and colleagues (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. Shililu and colleagues (2003) found that pH was associated with *Anopheles* larval diversity in Eritrea. This study examined the influence of physicochemical factors in the abundance and diversity of anopheline and culicine mosquitoes in Mwea Irrigation Scheme, Central province of Kenya.

5.3 Method and materials

5.3.1 Larval Sampling

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

At the study sites, 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.

5.3.2 Measurement of physicochemical variables

In the habitats, the physicochemical variable measured included: pH, conductivity, dissolved oxygen, temperature, salinity and Total Dissolved solids (TDS). The pH, conductivity, dissolved oxygen and temperature was 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).

5.3.3 Statistical 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. Pearson correlation was used to determine the association between occurrence of anopheline and culicine mosquito larvae with the physicochemical factors measured in the habitats. Logistic regression analyses were used to determine the correlation between physicochemical variables and the presence or absence of anopheline and culicine larvae in a habitat. The occurrence of a subfamily is defined as the presence of a particular subfamily in a sample regardless of its density. Anopheline and culicine larvae was 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). For culicine mosquito larvae, 1,942 were collected of which, 79.66% (n = 1,547) were early stage instars and 20.34%(n = 20.34) were late stage instars. 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). For culicine, 1,315 larvae were collected in which 95.74% (n = 1,259) were early instars and 4.26% (n = 56) were late instars. Only 31 pupae were collected during the sampling period. At the experimental plots, most of the larvae were collected at the transplanting and the tillering stage of rice development.

Table 5.2 shows the mean, standard error, minimum and maximum 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. Water temperatures in Mbui Njeru were higher than in Kamachiri.

Table 7 shows the coefficients of Pearson correlation at the experimental plots (5.3a) and at the 2 villages (5.3b). Water temperature is positively correlated to both early instars *Anopheles* and culicine mosquito in the larval habitats at the plots and at the two villages.

Logistic regression model gave pH as the best indicator for anopheline larvae at the two villages while salinity was the best predictor the anopheline larvae at the experimental plots. None of the physicochemical variables were found to be significant for presence of culicine larvae in the habitats in both the villages and at the experimental plots using regression model.

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

Rice stage/Village	Early instars *Anopheles*	Late instars *Anopheles*	Culicine early instars	Culicine late instars	Number of pupae
Land preparation	72 (0.98)	4 (0.08)	1 (0.03)	0 (0.00)	1 (0.03)
Transplanting	6,612 (5.74)	119 (0.13)	953 (0.93)	12 (0.03)	8 (0.02)
Tillering	841 (1.16)	144 (0.25)	272 (0.48)	39 (0.09)	19 (0.07)
Flowering	28 (0.35)	3 (0.08)	9 (0.20)	0 (0.00)	1 (0.05)
Maturity	37 (0.28)	0 (0.00)	24 (0.27)	5 (0.11)	2 (0.04)
Sub total	**7,590 (2.78)**	**270 (0.10)**	**1,259 (0.46)**	**56 (0.03)**	**31 (0.02)**
Kiamachiri	666 (0.421)	76 (0.084)	1,124 (1.228)	351 (0.455)	91 (0.082)
Mbui Njeru	400 (0.880)	14 (0.051)	423 (1.437)	44 (0.190)	55 (0.334)
Sub total	**1,066 (0.389)**	**90 (0.063)**	**1,547 (0.976)**	**395 (0.337)**	**146 (0.108)**

Table 5.2 The Descriptive statistics of the water analysis at different stages of rice development for the experimental plots and the villages

Rice stage/Village		Temp (°C)	Cond mS/cm	Salin(ppt)	Dissolved oxygen (%)	Do Concenmg/L	DO Charge	pH	PH MV
Land preparation	Mean	23.855	133.396	127.94	156.200	7.668	40.06	7.428	-31.052
	SE	0.608	21.615	25.543	9.358	0.783	0.683	0.072	4.110
	Minimum	18.98	0.002	0.00	43.3	0.53	31	6.47	-89.2
	Maximum	29.95	425.400	569	255.7	15.01	45	8.43	21.9
Transplanting	Mean	28.642	101.697	80.97	92.333	5.082	24.93	7.518	-36.184
	SE	0.337	5.126	6.149	5.281	0.365	1.084	0.022	1.855
	Minimum	18.77	0.002	0.00	3.6	0.14	4	6.52	-221.0
	Maximum	35.97	517.600	818	332.3	30.85	52	8.51	57.5
Tillering	Mean	26.851	145.227	123.76	168.658	7.331	40.95	7.389	-27.657
	SE	0.297	6.667	8.108	4.539	0.290	0.340	0.0369	2.054
	Minimum	19.48	0.001	0.00	20.6	0.33	31	6.26	-74.0
	Maximum	33.20	453.400	638	322.8	15.52	52	8.17	33.6
Flowering	Mean	24.629	98.569	72.53	122.590	6.805	35.05	7.025	-9.110
	SE	0.318	6.399	5.630	14.295	0.814	1.247	0.070	3.942
	Minimum	22.23	48.430	32	2.4	0.12	26	6.65	-50.3
	Maximum	27.91	159.600	130	245.3	15.06	46	7.75	11.9
Maturity	Mean	23.911	84.860	62.34	107.753	6.623	32.72	7.041	-9.975
	SE	0.242	6.894	6.510	7.040	0.483	0.492	0.076	4.271
	Minimum	21.31	30.750	19	45.3	1.61	29	6.46	-106.6
	Maximum	27.85	233.400	217	223.1	14.57	41	8.76	22.6
Kiamachiri	Mean	23.584	71.99	68.67	55.253	3.660	29.28	6.970	-7.930
	SE	0.223	6.922	8.154	4.196	0.255	0.716	0.026	1.509
	Minimum	17.54	0	0	-0.1	.00	0	5.94	-164.4
	Maximum	35.49	915	1211	395.9	27.78	56	9.74	50.7
Mbui Njeru	Mean	25.085	188.60	182.10	131.068	5.004	23.40	7.392	-26.347
	SE	0.247	13.628	18.461	15.331	0.776	2.098	0.027	1.979
	Minimum	21.21	0	0	-2.8	-0.15	0	6.72	-135.6
	Maximum	33.05	761	1000	595.6	28.85	63	7.99	25.6

Table 5.3a: Pearson correlation for the variables taken at the experimental plots

	Rice height	Rice stage	# tillers	Depth (cm)	Turbidity	Other inverts	*Anopheles*	Culicines	# pupae	Temp(C)	SPcond mS/cm	Salinity (ppt)	D. Oxygen (%)	Do Conc mg/L	DO charge	pH
Rice stage	0.918**															
# tillers	0.243	0.281														
Depth (cm)	0.348**	0.171**	-0.468*													
Turbidity	-0.459**	-0.418**	0.106	-0.183**												
Other Inverts	0.000	0.060	a	-0.040	-0.092											
Anopheles	-0.172**	-0.189**	-0.111	-0.131**	0.188**	-0.263**										
Culicines	-0.141*	-0.129*	0.016	-0.157**	0.155**	-0.052	0.422**									
# pupae	-0.049	0.039	a	0.061	0.055	0.036	0.038	0.044								
Temp (^{o}C)	-0.486**	-0.226**	-0.189	-0.391**	0.241**	-0.033	0.215**	0.195**	-0.002							
Cond mS/cm	-0.124*	-0.046	0.076	-0.181**	-0.104*	0.078	0.009	0.006	0.125**	0.152**						
Salinity (ppt)	-0.118	-0.072	0.094	-0.158**	-0.104*	0.068	-0.012	-0.014	0.115*	0.062	0.960**					
D. oxygen (%)	0.058	0.081	-0.224	0.065	-0.332**	0.097*	-0.053	-0.140**	0.051	-0.118*	0.148**	0.149**				
DO Conc (mg/L)	0.137*	0.090	-0.208	0.197**	-0.237**	0.046	-0.099*	-0.147**	-0.025	-0.321**	-0.454**	-0.403**	0.762**			
DO Charge	0.225**	0.182**	-0.108	0.213**	-0.443**	0.135**	-0.155**	-0.250**	0.043	-0.362**	0.160**	0.179**	0.866**	0.675**		
pH	-0.506**	-0.374**	-0.204	-0.121*	0.235**	-0.037	0.062	0.055	0.069	0.317**	-0.021	-0.025	0.267**	0.225**	0.016	
PHMV_MV	0.462**	0.326**	0.206	0.139**	-0.222**	0.032	-0.039	-0.069	-0.067	-0.311**	0.017	0.021	-0.210**	-0.173**	0.028	-0.891**

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

[a] Cannot be computed because at least one of the variables is constant.

Table 5.3b: Pearson Correlations of chemical parameters of the water in the two villages

	Temp (°C)	Cond (ms/cm)	Salinity (ppt)	D. Oxygen (%)	D. oxygen (mg/l)	D. oxygen charge	pH	pH charge	L1L2 *Anoph*	L3L4 *Anoph*	L1L2 culic	L3L4 culic
Conductivity (ms/cm)	0.278**											
Salinity	0.247**	0.820**										
D. oxygen (%)	0.181**	0.153**	0.093									
D. oxygen (mg/l)	0.032	-0.209**	-0.230**	0.790**								
D. oxygen charge	-0.112*	-0.150**	-0.159**	0.655**	0.596**							
pH	0.263**	0.060	0.057	0.374**	0.338**	0.132*						
pH charge	-0.203**	0.028	0.020	-0.305**	-0.319**	-0.152**	-0.923**					
L1L2 *Anoph*	0.122*	0.097	0.102	0.174**	0.125*	0.081	0.099	-0.057				
L3L4 *Anoph*	-0.058	0.027	0.011	0.012	-0.009	0.039	0.018	-0.013	0.321**			
L1L2 culic	0.049	0.007	0.002	0.016	0.021	0.044	0.050	-0.037	0.046	0.003		
L3L4 culic	-0.012	-0.052	-0.044	-0.006	0.042	0.035	0.001	-0.026	0.041	0.030	0.143**	
Pupae	0.004	0.085	0.065	-0.068	-0.079	-0.112*	0.014	-0.016	0.056	0.086	0.134*	0.125*

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

5.5 Discussion

This study examined the influence of physicochemical factors in the abundance and diversity of anopheline and culicine mosquitoes in Mwea Irrigation Scheme, Central province of Kenya. We found that larval abundance was highest in between transplanting and tillering stage. At this stages of rice growth cycle temperature and salinity is highest at the experimental plots. When the rice is 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). Our results further indicated that water temperatures were positively correlated with abundance of *Anopheles* larval abundance. This study shows that when the rice has not achieved fully vegetative cover, the larval abundance is highest. 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 achieves maximum vegetation, the temperatures decline 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 1972). Pearson correlation further showed that temperature has a negative association with depth. 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; Rao 1984; Palchick and 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 as an effect of sunlight is at

times depth of water decline. This provides conducive water temperatures, which have a direct influence on the anopheline larval densities.

Logistic regression model showed that pH and salinity are the key predictors for the presence of *Anopheles* larvae at the two villages and the experimental plots respectively. At the experimental plots, the salinity was found to be high between and transplanting which coincided with high densities of anopheline larvae. Grillet (2000) (Grillet 2000) found that the occurrence and abundance of anopheline larvae is closely associated with physicochemical variables such as salinity and dissolved oxygen. Our study using the experimental plots showed salinity is important for the occurrence, which is similar to Grillet (2000) findings. In the villages, pH was found to be an important factor for the larval abundance. In rice agro ecosystem, pH and conductivity have been shown to important for larval abundance (Cates 1968;Case 1975;Robert 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. pH increases with the application of nitrogenous fertilizers during top dressing. Fertilizer application results in increase in oviposition by gravid *Anopheles* mosquitoes which consequently increases the number of larvae in the paddies. None of the physicochemical factors tested in the multiple regression model was found to be associated with culicine larval abundance. This may be due to the wide range of habitats, which culicines occupy. Culicine mosquito larvae can occur in fresh habitats to very old and fowl habitats unlike A*nopheles*, which prefer fresh and open habitats.

In conclusion, *Anopheles* larval abundance is found to be associated with pH and salinity. These parameters in a rice agro ecosystem are associated with early stages of rice growth cycle, which is associated with larval abundance. For effective control of developmental stages of mosquito larvae, the application of the larvicide should be done after transplantation and the larvicide should persist until the reproductive stage of the rice.

CHAPTER 6: EMERGENCE OF MOSQUITO FROM DIFFERENT HABITATS: EFFECT OF PRESENCE OF OTHER INVERTEBRATES.

6.1 Abstract

Mosquito populations are regulated by a variety of factors including adverse climatic conditions, limited food supply, competition, parasites or pathogens and predators. 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. This study was carried out in Mwea Irrigation Scheme, Central Kenya from May to August 2005. Ten cages measuring 0.50 m by 0.5 m by 0.5 m were placed in different habitat types to investigate the effect of other invertebrate on the larval survival and consequently the emergent mosquitoes. Similarly another set of ten cages measuring 1 m by 1 m by 1 m were placed in different habitat types in Kiamachiri village to study the mosquito productivity of different habitat types. All these cages were covered with fine netting material to deter other mosquitoes and invertebrates from oviposition. Pools that were freshly formed immediately after the rains, the *An. gambiae* females oviposit immediately and the survival rate of these mosquitoes was high due to less pressure from predation. The only survival limitation was the tendency to dry quickly before all larvae

pupate. Overall the emergence rate was 35.39% (Range 21.05 – 97.87) from these temporary pools. With the progression of rains, the pools stabilize and other invertebrates start colonizing these habitats. Culicine mosquitoes also oviposit in these habitats. Due to presence of few other invertebrates, the survival of mosquitoes is decreased with the percent emergence being 14.29% for *Anopheles* mosquitoes and culicine 14.46%. When the densities of other invertebrates is very high, the emergence of mosquito is highly suppressed, with very few emerging as adults. Overall with many other invertebrates, the emergence rate for *Anopheles* was 2.41% and that of culicine was 5.65%. All the emerged *Anopheles* mosquitoes were *An. gambiae* s.l. while among the culicines, they were all *C. quinquefasciatus*. Productivity from different type shows that paddy had most emergent mosquitoes followed by marshes. The results further showed that when the structural complexity (in terms of debris and vegetation) of a habitat is increased, there is an increase in the number of other invertebrates which results in fewer emergent mosquitoes. In conclusion, this study shows that the presence of other invertebrates is playing a role in natural regulation of mosquito in the habitats.

Key words: Predation, *An. gambiae*, Emergence rate, Productivity, natural regulation

6.2 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 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 level 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 this 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. Much small mortality 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 (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 were limited to large deep containers.

6.3 Materials and Methods

6.3.1 Effect of other 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.

6.3.2 Productivity from different habitats

We carried out a study to determine the emergent mosquito productivity from different habitat types in Kiamachiri village, Mwea Division, Kirinyaga district, Central Kenya. The cage size was one metre by one metre by one metre and had an iron sheet at the lower side of the cage covering 30 centimeters of the height. These cages were covered with a fine netting material not to allow 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 emergent mosquitoes. The emergent mosquitoes were collected using aspiration method (WHO 1975). The emergent mosquitoes were identified morphologically using keys of Gillies and Coetzee, 1987) and Gillett (1972) and preserved in driarites. The invertebrates in the habitats were identified to family level using keys of Merritt and Cummins, (1996).

6.4 Results

Table 6.1 shows the emergent mosquito productivity for the pools, which were formed immediately after the rains. Pools that were freshly formed immediately after the rains, the *An. gambiae* females oviposit immediately and the survival rate of these mosquitoes was high due to less pressure from predation. The only survival limitation was the tendency to dry quickly before all larvae pupate. Overall the emergence rate was 35.39% (Range 21.05 – 97.87).

With the progression of rains, the pools stabilize and other invertebrates start colonizing these habitats. Culicine mosquitoes also oviposit in these habitats. Due to presence of few

other invertebrates, the survival of mosquitoes is decreased with the percent emergence being 14.29% for *Anopheles* mosquitoes and culicine 14.46% (Table 6.2).

When the densities of other invertebrates is very high, the emergence of mosquito is highly suppressed, with very few emerging as adults. Overall with many other invertebrates, the emergence rate for *Anopheles* was 2.41% and that of culicine was 5.65% (Table 6.3). All the emerged *Anopheles* mosquitoes were *An. gambiae* s.l. while among the culicines, they were all *C. quinquefasciatus*. Table 6.4 shows the other invertebrates composition in the cages. Dytiscidae was the most common species in the cages.

6.4.1 Larval productivity from different habitat types

Table 6.5 shows the emergent mosquitoes from each habitat type and the larvae at day 0. From this table, Paddies were the most productive habitat type for both *Anopheles* and culicine larvae. *An. gambiae* was the most predominant anopheline species although a few *An. coustani* emerged from the habitats. Over 90% of the culicines were *C. quinquefasciatus*.

6.4.2 Short-term survival of mosquito in dry soil

During one of the trials, two cages in a paddy habitat type, were enclosed with about 50% covered with water. Due to climate climatic condition the habitat dried up, but two days later before it was transferred, it rained and the entire cage was covered with water (100% at the base). Immediately, (after third day), mosquito larvae emerged most of them culicine (unable to identify at adults), and a few *An. gambiae*. This showed that mosquito

eggs could survive short- term dryness in soil especially during the rainy period. This could be used as an adaptive strategy to maintain the high population of mosquitoes during the rain season.

Table 6.1 the number of mosquito larvae emerging from cages on freshly formed habitats

Habitat type	Early instars *Anopheles*	Late instars *Anopheles*	*Anopheles* females	*Anopheles* males	% *Anopheles* emergence
Tyre track	32	15	35	11	97.87
Tyre track	82	45	20	7	21.26
Pool	64	50	12	12	21.05
Pool	95	41	23	15	27.94
Pool	116	42	41	15	35.44
Pool	103	49	34	16	32.89
Pool	90	60	39	13	34.67
Pool	70	37	27	15	39.25
Seep	6	1	2	4	85.71
Pool	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 invertebrates (Less than 10 per cage)

Habitat type	Early instars *Anopheles*	Late instars *Anopheles*	Early instars culicines	Late instars culicines	Pupae	*Anopheles* females	*Anopheles* males	Culicine females	Culicine males	% *Anopheles* emergence	% Culicine emergence
Tyre track	57	44	53	11	0	15	5	7	24	19.80	48.44
Tyre track	34	15	187	111	6	7	0	11	19	14.29	10.07
Pool	60	18	20	4	1	2	5	0	1	8.97	4.12
Pool	80	8	13	13	0	0	2	0	3	2.27	11.54
Pool	72	17	41	4	0	4	2	0	0	6.74	0.00
Pool	47	1	42	1	0	17	5	4	3	45.83	16.28
Pool	36	12	4	0	0	2	1	0	0	6.25	0.00
Pool	20	6	0	0	0	0	1	0	0	3.85	0.00
Seep	11	8	0	0	0	0	2	0	0	10.53	0.00
Pool	9	5	1	0	0	5	5	1	0	71.43	100
Total	**426**	**134**	**361**	**144**	**7**	**52**	**28**	**23**	**50**	**14.29**	**14.46**

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

Cage#	Early instars *Anopheles*	Late instars *Anopheles*	Early instars culicine	Late instars culicine	*Anopheles* females	*Anopheles* males	Culicine females	Culicine males	% *Anopheles* emergence	% Culicine emergence
1	3	0	13	1	0	0	0	1	0	7.14
2	2	1	7	3	0	0	2	3	0	50
3	14	1	45	10	0	1	0	0	6.67	0
4	20	1	7	0	1	0	0	0	4.76	0
5	5	0	5	7	0	0	0	1	0	8.33
6	6	1	3	1	0	0	0	0	0	0
7	6	1	2	0	0	0	0	0	0	0
8	3	1	5	1	0	0	0	0	0	0
9	12	1	8	2	0	0	0	0	0	0
10	3	1	8	1	0	0	0	0	0	0
Total	**74**	**9**	**103**	**21**	**1**	**1**	**2**	**5**	**2.41**	**5.65**

Table 6.4 The other invertebrates present in cages placed in the paddy.

	Hemiptera		Coleoptera		Ephemeroptera	Odonata		Mollusca	Amphibian
CAGE#	Notonectidae	Gerridae	Dytiscidae	Hydrophilidae	Ephemerellidae	Coenagrionidae	Lebullidae	Snails	Tadpoles
1		++	++						
2		+	+						+++
3		+	+		+	+	+	+	+++
4	+++		++		+		+		
5	+		+	+	++	+	++	+	+
6	+	+++	++	+	++		++	+	
7	++	+	++		++		++		
8			++			++			
9	+	+++	++					+	
10		++			+	++	+		+

[+] Less than 10

[++] Between 11-20

[+++]Over 21

Table 6.5 shows the number of anopheline and culicine larvae at day zero and the emergent mosquitoes from each habitat type.

		Larvae at day 0					Emergent mosquitoes			
Habitat type	#Cages	Early instars *Anopheles*	Late instars *Anopheles*	Early instars culicine	Late instars culicine	Pupae	*Anopheles* females	*Anopheles* males	Culicine females	Culicine males
Paddy	30	492	91	827	57	13	100	43	690	553
Swamp	11	14	0	160	978	0	0	0	21	5
Marsh	12	231	32	440	82	27	46	19	89	44
Ditch	3	57	16	175	22	19	5	3	76	31
Pool	3	35	8	57	8	1	7	11	1	2
Seep	5	10	5	21	30	3	2	1	10	11
Total	**60**	**829**	**148**	**1,680**	**1,177**	**63**	**160**	**77**	**887**	**646**

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. We covered pools immediately a few 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), did show that predation accounted for more than 95% of mortalities in the habitat. Our study showed that there is 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 damsel flies have been shown to be important in natural regulation. Further work is required to incriminate the other invertebrates as predators.

In Kiamachiri, it was found that paddies were the most productive. We placed the cages when the rice was at the tillering stage. Tillering stage has been shown to be the harbouring most larvae in the rice cycle. At this stage the water is most exposed due to the low vegetation. Marshes followed paddy in the habitat productivity. We found out that when, the structural complexity of the habitat was decreased, the number of emergent mosquitoes increased. By structural complexity we mean 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. We 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 1999;Yanoviak 2001a, 2001b; Finke 2002; Carlson et al., 2004).

In conclusion, this study shows that there is natural regulation for mosquito production in the aquatic habitats. Although the number of emergent mosquitoes decreases due to predation pressure, there is a need to effective control mosquito developmental stages using larvicides such as Bti and Bs.

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

7.1 Abstract

Experimental plots measuring (6.3m x 3.15m) within a 1 acre plot at the Mwea Irrigation and Agricultural Development Center (MIAD) experimental station, in Mwea Irrigation Scheme were used to examine the primary factors responsible for regulating the aquatic stages of malaria vectors, abundance and diversity and its implication in vector abatement programmes. Each plot was hydrologically isolated using unidirectional inflow and outflow canals to avoid mixing between plots. The plots were exposed to natural colonization of mosquitoes and larval sampling was done once every week using standard dipping technique from plot preparation, transplanting up to harvesting, covering the full cycle of rice cropping. Sampling was conducted over three rice-growing cycles. The plots were characterized visually and physicochemical determinants were measured using field equipment. A total of 21,325 *Anopheles* larvae were collected of which 91.93% (n = 19,604) were early instars (L1, L2) and 8.07% (n = 1,721) were late instars (L3, L4). For culicine larvae, a total of 19,926 were collected of which 87.33% (n = 17,401) were early instars and 12.67% (n = 2,525) were late stage instars. A total of 513 pupae were collected. 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% *An. funestus,* 0.26% *An. coustani* and 0.07% (n = 1) *An. maculipalpis.* A total of 891 late stage larvae were identified of which 65.66% (n = 585) were *C. quinquefasciatus,* 9.88% (n = 88) *C. annulioris,* 7.30% *C. poicilipes,* 7.18% (n = 64) *C. tigripes,* 0.56% (n = 5) *C. duttoni,* 5.27% (n = 47) *Ae. aegypti,* 3.48% (n = 31), *Ae. cumminsii,* and 0.67% (n = 6) *Ae. Vittatus.* Of the 171 correlation coefficients, 71 (41.52%) were statistically significant indicating that there was non-random association between some of the variables tested. In conclusion, these studies show that mosquito control programmes should be emphasized at the beginning of rice growing cycle targeting the developmental stages. Throughout the rice growing cycle, larvicidal control programmes should go hand in hand with other vector control tools such as insecticide treated bednets.

Key words: Rice cycle, *An. gambiae*, physicochemical variables, standard dipping technique

7.2 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 et al., 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 et al., 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 1991, Victor and Reuben, 2000). Source and water depth (Collins 1980), temperature (Mogi 1978), pH, ionic composition and conductivity (Kramer 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 2002).

In this study we investigated 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.

7.3 Materials and Methods

7.3.1 Larval habitat description

The plots were characterized visually and by use of hand held field equipment. Briefly, the depth of water was measured form the same point every visit using a ruler. Rice height was measured from the same rice plant every visit. Here we assumed 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. Water chemical parameters, which were determined included: pH, Dissolved oxygen, salinity, conductivity, and temperature. The pH, conductivity, dissolved oxygen and temperature was measured using hand held machine YSI 650