# MALARIA VECTOR BIONOMICS AND THE ROLE OF MICROBIAL LARVICIDES IN ANOPHELINE MOSQUITO LARVAL MANAGEMENT IN A RICELAND AGRO-ECOSYSTEM

By

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**DECLARATION**

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

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## DEDICATION

This work is dedicated to the memory of my late father Mr. Thomas M. Ngari.

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**ACKNOWLEDGEMENT**

I wish to express my deep and sincere gratitude to all those individuals who assisted me to realize my research goals. I'm particularly grateful to my Supervisors Prof. Lucy Irungu and Dr. Wolfgang Mukabana from the University of Nairobi and Dr. John Githure and Josephat Shililu from International Centre of Insect Physiology and Ecology (ICIPE), for their guidance, encouragement and commitment to supervise this work throughout the study period. I also appreciate the criticism, comments and advice from other scientists and colleagues at different stages of preparation of this thesis particularly from Dr. Ephantus Muturi.

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**ABSTRACT**

The current study was conducted with the aim of understanding the population dynamics of malaria vectors, their contribution to malaria transmission and potential role of *Bacillus thuringiensis* var *israelensis* and *B. sphaericus* (VBC- 60120) combined microbial formulations in malaria vector management in an irrigated rice agro-ecosystem. Field entomological studies were conducted between March 2005 and June 2007 in four villages in Mwea Rice Irrigation Scheme, Central Kenya. The population dynamics of *Anopheles* mosquitoes were examined by conducting weekly larval and adult mosquito sampling. Adult female anopheline mosquitoes were screened for blood meal sources and *Plasmodium falciparum* circumsporozoite proteins. The efficacy of the combined microbial formulation (VBC-60120) was evaluated and eventually applied in anopheline breeding habitas in Karima village. Eleven anopheline breeding habitats with differential spatial and temporal larval density were identified. Temporary pools and burrow pits were the most productive habitats but rice paddies and associated canals maintained mosquito production throughout the year. Nine *Anopheles* species were identified from larval and adult population in the area. *Anopheles arabiensis* Patton dominated both pre-adult and adult population. Time lag cross-correlation analysis revealed no association between adult malaria vector density and rainfall but the densities increased during rice planting period. All the mosquito species had a high preference for bovine (range 56.3-71.4%) over human (range 1.1-23.9%) or goat (0.1-2.2%) blood meals. Anthropophily was inversely proportional to land under rice cultivation. A resident of Mwea irrigated rice agro-ecosystem receive an average of 0.68±0.01 bites per person per night from *Anopheles* species with peak biting rates occurring during rice planting season. EIR values ranged between 0.02 infective bites per person per night for *An. funestus* Giles and 0.53 (ib/p/n) for *An. arabiensis* Patton. Microbial larviciding resulted

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# CHAPTER ONE

## 1.0 GENERAL INTRODUCTION AND LITERATURE REVIEW

### 1.1. INTRODUCTION

Malaria is a major public health concern in Africa where it is the greatest cause of morbidity and a large contributor to mortality (Breman, 2001) especially among pregnant women and children of tropical Africa (WHO, 2005). This parasitic infection caused by *Plasmodium* species continues to exert a heavy toll among a wide variety of individuals particularly in the tropics, yet it is a curable disease with effective drugs and preventive measures. According to WHO (2005) estimates, 2.6 billion people are at risk of malaria infection with 300-500 million infected annually. The disease is mainly confined to the tropical areas of Africa, Asia and Central America but hitting Africa hardest. Sub-Sahara Africa is heavily affected by the disease accounting for over 90% of malaria infections worldwide. Current estimates indicate that every year 1.5-2.7 million deaths occur due to malaria mainly among children under five years and pregnant women in the underprivileged societies (WHO, 1997; 2000; 2005). All of the four *Plasmodium* species (*Plasmodium falciparum* Welch , *Plasmodium vivax* Grassi and Feletti, *Plasmodium ovale* Stephens, and *Plasmodium malariae* Grassi and Feletti) are responsible for this debilitating disease in Africa but the bulk of deaths and associated malaria morbidity such as febrile illness, severe cerebral complications and anaemia are mainly due to *P. falciparum*. The problem of malaria in Africa is further aggravated by the presence of the highly efficient malaria vectors within the *Anopheles gambiae* Giles and *Anopheles funestus* Giles complexes that are widespread and difficult to control. The malaria crisis continues to worsen in the Afican continent despite ongoing efforts to battle the disease through integrated malaria management targeting both the vectors and the human reservoirs.

In Kenya, more than half of the population (25 millions out of 34 millions) is considered to live in areas vulnerable to malaria epidemics (MoH, 2007; Snow et al., 1999). Malaria endemicity in Kenya is largely driven by climate and temperature being holoendemic in Lakeside (Lake Victoria) and Coastal regions of the country where infections and transmissions are usually high and child mortality due to the disease alarming throughout the year. Other area include the highland districts e.g Kisii, Nyamira, Nandi and Uasin Gishu which are located in ecological regions traditionally regarded as highlands districts and intermediate altitudes where transmission is limited by rainfall and ambient temperature although malaria infection. In these areas, there is always a potential for limited transmission lending itself to an overall low disease risk on an average year and are prone to epidemics affecting all members of the community. In arid districts in north eastern and north western parts of the country where malaria is only experienced among communities that live near water. Malaria transmission is considered as low in other Central province and Nairobi area, including Kirinyaga district where maalria cases are few. In Kenya, malaria generally accounts for up to 30-50% of hospital outpatiuent attendances, 20 % of all admissions and more than 20% of all deaths in children under five years (MoH, 2006; Snow et al., 1999) mainly due to P. falciparum parasites (Snow et al., 1999). The malaria prevalence in Mwea irrigation scheme in the Kenyan Highlands is 26% higher than in the surrounding areas (WHO/FAO/UNEP, 1987, 1990; Hunter et al., 1993 Mutero et al., 2004).

Epidemiologically, malaria transmission revolves around factors affecting the human host, the arthropod vector, the parasite and the general environment. However, the primary variables affecting the risk of infection are the rate at which humans are bitten by vector mosquito species

and the proportion of mosquitoes that are infectious. When examined across different landscapes and seasons, these two factors have been shown to peak at different times and places. The distribution of humans and suitable habitats for mosquito larval development varies across the landscape and the density of the disease vectors fluctuates seasonally. These fluctuations are driven by environmental factors mainly rainfall, temperature and topography that eventually dictate the level of human-vector contact and consequently disease transmission. Ecological changes due to human activities like irrigated rice cultivation have also been blamed for some of the largest increases in the incidence of malaria and other vector borne diseases. This scenario is largely caused by the amplification of breeding habitats, increased vector competence and settlement of immunologically naïve population in endemic areas through employment (Lacey and Lacey, 1990). The human-vector-parasite interactions benefit greatly when the vectors involved are long-lived, highly competent and have strong avidity to bite humans as is common in Africa (Takken and Lindsay, 2003). The spatial and temporal variations in mosquito populations affects the rate at which humans get bitten as well as the proportion of infectious mosquitoes and the risk of infection in a locality.

The dynamism of the mosquito population, species diversity and the risk of malaria infections vary with locality and the ecological parameters that are associated with an area as well as the socio-economic activities of the local human population. In Africa there exists a large human population with a high growth rate of about 20 million people per year (WHO, 1997; WRI/UNEP/UNDP, 1995). Consequently food crisis is rampant in most African countries especially in the 41 Sub-Saharan countries where human population outstrips food production (WRI/UNEP/UNDP, 1995). Hence, irrigation is adopted as an effective way of increasing crop

production to feed the ever-growing population (WHO, 1997). However, introducing irrigation, especially irrigated rice cultivation into non-malarious or unstable malaria transmission regions causes a dramatic increase in the abundance of *Anopheles* vector species and brings vectors closer to human population centres (Gratz 1988, Lacey and Lacey 1990) thus enhancing conditions favourable for the transmission of communicable diseases (Hunter *et al.,* 1993). Irrigated rice cultivation elevates malaria endemicity status by providing ideal breeding sites and humidity necessary for the survival and transmission of malaria parasites by the highly efficient vectors, *Anopheles gambiae* complex, as well as increase in vector mosquito species. Adult mosquito numbers increase soon after the paddies are flooded attaining peak density when rice plants are small before declining as rice plant covers the surface water although they may persist in shallow puddles left after harvesting (Snow, 1983)

The current trends in disease vector control is to adopt Integrated Vector Management (IVM) strategy which is an ecologically based approach involving several complimentary interventions. Among such interventions is environmental management, chemical, biological and mechanical control measures which are especially recommended for use in or near rice fields (Lacey and Lacey, 1990). *Bacillus thuringiensis* var *israelensis* and *B. sphaericus* species have been used in control of anopheline in riceland ecosystem with considerable success (Romi *et al.,* 1993; Wirth *et al.,* 2004) and its integration with cattle rearing serve a great deal in reducing the number of vectors biting man especially from the highly zoophilic *Anopheles arabiensis* which is a common vector in East African riceland ecosystems (Ijumba *et al* 1990; Mutero *et al.,* 2004b; 2004c; Muriu *et al.*, 2008). It was the aim of this study to evaluate malaria transmission potential by anopheline species in Mwea irrigated rice agro-ecosystem and the role of microbial larvicides

in the Integrated Vector Management (IVM) in the area. This was tailored to focus on the immature stages of vector *Anopheles* species to reduce the transmission of malaria in rice-village complexes in Mwea, central Kenya. The study addressed issues of time, location and methodology i.e. "when", "where", and "how" to control *Anopheles* larvae in irrigated rice-village complexes based on the ecological principles of IVM (Metcalf & Novak 1994). Recently, Gu and Novak (2005) developed a new framework to evaluate impacts of larval interventions on adult populations and exposure intensity measured by entomological inoculation rates (EIR)) and unlike traditional models, which unrealistically assumed habitats were identical in adult productivity, their new models explicitly considered variability in productivity among habitats. The models identified critical knowledge gaps in relation to larval control interventions and provided qualitative and quantitative understanding of impacts of control interventions under various field conditions (Gu and Novak 2005). Their study demonstrated theoretically that significant reduction in populations of *Anopheles* mosquitoes, and consequently malaria parasite transmission, can be obtained through habitat-based targeted larval control interventions. Moreover, they highlighted the importance of ecological surveys of habitats and larval ecology in relation to estimation of adult productivity. In malaria control interventions the central goal is to reduce disease incidence (the number of potential new infections that people receive from mosquito bites) in order to reduce morbidity and mortality. This study set to carry out an assessment of malaria transmission risk and the role of microbial larvicides in mosquito larval control measures. As observed by Zhou *et al.,* (2004a) there is need for accurate information on the abundance of malaria vectors in order to determine malaria transmission intensity and the efficacy of vector control measures. This was achieved through monitoring various

epidemiological indicators especially malaria episodes reported among children under the age of five years, entomological inoculation rate as well as population dynamics of malaria vectors.

## 1.2. LITERATURE REVIEW

### 1.2.1. Rice Cultivation and Malaria

Rice is an annual crop belonging to the genus *Oryza* that is usually cultivated using either upland (dry rice) or lowland (wet rice) system. The upland rice does not require flooding as opposed to lowland rice, which needs constant irrigation and maintenance of water at a depth of 10-15cm (Chandler 1969; Grist 1986). Irrigation serves as the single most effective way of increasing crop production by increasing yield, acreage, number of cropping cycles per year and reducing the risk of crop failure (Oomen *et al.*, 1988; Weil *et al.*, 1990). The high population growth rate in sub-Saharan Africa outstrips crop production, coupled with urbanization and low and sparse rainfall unable to support rainfed agriculture (WRI/UNEP/UNDP, 1995) make it necessary for most countries to expand agriculture through irrigation of potentially arable land (WRI/IIED, 1986).

Rice is one of the most irrigated crops and is considered to pose the greatest risk of vector-borne diseases by providing suitable habitats for disease vectors like mosquitoes and snails (Service, 1989; Hunter *et al.*, 1993). Introduction of irrigated rice cultivation in areas characterized by unstable malaria transmission and low levels of immunity against malaria parasite is potentially dangerous as the disease is a threat to both the adults and the children. Malaria vectors are among the pioneer species colonizing recently flooded rice fields whereby *An. arabiensis* and occasionally *An. funestus* thrive (Ijumba and Lindsay 2001). Irrigated rice fields can produce large numbers of mosquitoes that may subsequently result in increased malaria transmission in the surrounding local communities. The farmers' practice of applying urea and organic fertilizers in rice fields to increase yield increases the population of mosquito aquatic stage and

consequently the resulting adult densities (Bradley, 1988). However, human exposure to malaria infectious *Anopheles* mosquitoes is more variable in irrigated areas than in neighbouring areas with various agro-economic systems (Ijumba and Lindsay 2001; Mutero *et al.*, 2004b). It has been observed that malaria transmission and incidence rates increase in the local communities in irrigated areas and surrounding villages after introduction of irrigation (Boudin *et al.*, 1992; Lindsay *et al.*, 1991; Ijumba, 1997) although in other cases the introduction of irrigated rice does not change the malaria risk (Faye *et al.*,, 1993; 1995) but results in a reduction in transmission (Manoukis *et al.*, 2006). Because of the complex association between rice cultivation and malaria, a thorough understanding of the local characteristics of the disease and their vectors is an essential pre-requisite to the development, implementation and assessment of vector control programs.

### 1.2.2. Common malaria vectors and their breeding sites

Female *Anopheles* mosquitoes are responsible for malaria transmission and about 40 species of approximately 500 anophelines species are capable of transmitting malaria. In Sub-Saharan Africa the most important vectors include *Anopheles gambiae* Giles complex, *An. funestus* Giles and *An. pharoensis* Theobald (Ijumba *et al.*, 1990; Service, 1993). In the gambiae complex, *An. gambiae* senso stricto Giles and *An. arabiensis* usually occur in sympatry exhibiting strong associations with the traditional rural life of many African communities. Both are excellent malaria vectors and often co-exist with *An. funestus*. The gambiae complex species share larval habitats although in environments with large water surface like irrigated rice fields *An. arabiensis* predominates (Service, 1993; Ijumba *et al.*, 1997).

The various *Anopheles* mosquito species breed in diverse forms of aquatic habitats although some species are known to prefer and predominate in certain habitat types. *Anopheles gambiae* s.l breed more prolifically in temporary and turbid water bodies such as ones formed by rain (Gillies & De Meillon, 1968) while *An. funestus* in contrast prefer more permanent water bodies (Gillies & de Meillon, 1968). The anopheline breeding habitats are usually shallow unprotected pools that have some degree of oxygen and protected from extreme heat (Sueur and Sharp, 1988: Gimnig *et al.*, 2001). Anopheline mosquito breeding habitats have been shown to include hoof and foot prints, burrow pits, water pools, fallen leaves, empty snail shells and tyretracks (Service, 1971; Lane and Crosskey, 1993; Gwadz and Collins, 1996; Minakawa *et al.*, 1999). In irrigated ricelands, *Anopheles* species have been shown to colonize recently flooded rice fields that serve as ideal breeding sites although they decline in abundance as the rice grows and begins to cover the water surface (Ijumba *et al.*, 2001). Other physicochemical factors that have been shown to influence the utilization and productivity of rice fields as anopheline breeding habitats include water, temperature, depth, turbidity and amount of dissolved oxygen (Muturi *et al.*, 2007). Intermittent irrigation practices and application of ammonium fertilizers in rice fields affects the density of anopheline mosquitoes (Mutero *et al.*, 2000; 2004a). Several other breeding habitats including tyretracks and temporary pools with differential productivity exist in irrigated rice lands (Ijumba *et al.*, 2001: Shililu *et al.*, 2003a). However, diverse larval habitats exist in different localities and understanding their spatial heterogeneity and productivity is essential in planning and implementing larval based control programs.

### 1.2.3. Malaria problem in Mwea rice irrigation scheme

Malaria parasitological survey in Mwea indicated the average *P. falciparum* parasite rate to be 23.5% among children up to nine years of age (Mutero *et al.*, 2004b). Results of the entomological evaluation showed 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 (Muturi *et al.*, 2006; 2008). However, malaria prevalence was significantly higher in the villages without irrigation (17-54%), compared to those with rice irrigation (0-9%), despite the former villages having significantly much lower numbers of *An. arabiensis*. This finding was consistent with recent findings in West Africa where it has been reported that although rice irrigation is usually associated with more anopheline mosquitoes, people living in irrigated areas had less incidence of malaria than those in adjacent non-irrigated areas. The failure of house spraying campaigns to break malaria transmission completely is well established in many parts of Africa where it is exacerbated by the presence of *An. arabiensis* that has a significant proportion of its population resting outside houses (Gillies and Coetzee, 1987).

### 1.2.4. Socio-economic burden of malaria

The economic burden of malaria to a country, a family and the individual is immense being estimated to reduce the annual per capita economic growth of malaria endemic countries by 1.3% (Sachs and Maloney, 2002). The long-term impact of this is a reduction of the Gross Net Product (GNP) by more than half.. In rural areas the economic burden of malaria is particularly apparent since the disease strikes the resourceful members of the community at the time of the year when there's greatest need for agricultural labour. Families that are affected by the disease turn away from cultivation of high yield crops like rice that are labour intensive to other less

demanding but low yield crops. The disease is also responsible for high rates of school absenteeism and the individuals become poorer and unable to afford protection measures and to seek health care when sick. According to WHO and UNICEF (2003) estimates, 34% of the total income in poor families is consumed by both the direct and indirect costs of health care. Irrigated rice cultivation is geared towards improving human nutrition and socio-economic status of individuals (van der Hoek, 2004) but the same irrigated systems are associated with water related diseases and human ill health (Service, 1989; Lacey and Lacey, 1990), diminishing their economic benefits. Hence, development of integral control mechanisms that lower disease burden have multiple effect of improving the welfare of households and ensure sustainability of control interventions due to associated benefits.

### 1.2.5. Epidemiological indicators of malaria transmission

The malaria parasite is typically transmitted to humans by mosquitoes belonging to the genus *Anopheles* and in rare cases, a person may contract malaria through contaminated blood, or a foetus may become infected by its mother during pregnancy. Natural transmission of malaria occurs through exposure to the bites of infective female *Anopheles* mosquitoes and the source of human infection is nearly always from human subjects either a sick person or an asymptomatic carrier of the parasite. Natural malaria transmission depends on presence of a relationship between the human host, the environment and agents of transmission and infection.

The overall vectorial situation in tropical Africa is dominated by *An. gambiae, An. arabiensis* and *An. funestus* (Gillies and Coetzee, 1987). The infection rates (sporozoite rate) tend to be higher in *An. gambiae* than in *An. arabiensis* due to the zoophilic nature of the latter although in absence of cattle in the village this difference disappears. In Africa, malaria transmission is without doubt governed by the presence of the highly efficient anopheline vectors that are capable of maintaining transmission even at extremely low levels of vector abundance and causing severe malaria cases even during periods when little or no transmission is detectable, highlighting the probability of severe malaria due to a single bite from infected mosquitoes (Mbogo *et al.,* 1995). Malaria transmission in Africa is not homogenous (Fontenille *et al.,* 1997) since human–parasite-vector relationship (EIR, parasite species, vector species and density) is dynamic. Intense malaria transmission occurs during and after rainy season although in some cases transmission may occur round the year especially in irrigated ricelands.

### 1.2.6. Entomological inoculation rate

Entomological inoculation rate (EIR) is the number of infective mosquito bites each human host receives in a unit time interval (per night, per month or per year). The EIR values are widely used to estimate the malaria risk (Rogers *et al.,* 2002) in an area. The relationship between EIR and malaria morbidity and mortality is determined by factors like human host immune status, vector competence and parasite virulence. Entomological inoculation rates (EIR) can be derived from both in-door human-bait collections (IR-HBC) and the proportion of human blood-fed females caught resting indoors (IR-HBF) with the latter being a more realistic index of EIR (Githeko *et al.*, 1993). Annual EIR values within Africa range between 0.1 to over 1000 depending on eco-epidemiological conditions of the locality (Hay *et al.,* 2000; Smith *et al.*,

2001). Accordingly, malaria status in the African region is determined by the EIR values with values <10 and >100 signifying areas of unstable and stable malaria. Areas with EIR values between 10 and 100 vary in malaria endemicity depending on underlying environment and demographic conditions such as rainfall, vegetation cover, human population density and land use patterns. For example, an unprotected person in rice irrigation scheme is estimated to receive 124 infective bites annually from *An. arabiensis* (Ijumba, 1997).

The biting rate of the disease vectors influences the transmission rate of vector-borne parasites especially malaria parasites where the infection status of the mosquito vector affects its biting rate. *P. falciparum* infected *Anopheles* mosquito requires a larger blood meal than an uninfected one which may consequently result in the vector biting several people per night in order to acquire enough blood meal (Koella *et al.,* 1998).

In Africa, substantial cross-continent variability in annual EIR value exists and land-use pattern having a major effect on annual EIR. In non-irrigated rural areas, EIR values range between zero and 884 while those within irrigated rice areas are less exposed, EIR range between 0-601 (Hay *et al.*, 2000). A complex relationship exists between malaria vectors, EIR values and irrigated rice cultivation depending on endemicity and seasonality. Irrigation farming has variously resulted in an increase (Coosemans, 1985), little effect (Robert *et al.*, 1985; Dossou-Yovo *et al.*, 1994) or decrease (Githeko *et al.*, 1993) in EIR values depending on locality. For example results of a study conducted in northern Tanzania showed more than 2.4-fold increase in malaria transmission in non-irrigated areas compared to rice irrigated areas (Ijumba *et al.*, 2002a). The apparent variation can be attributed to various factors including relative effect of irrigation on

species abundance and sporozoite rates that are specific to different localities. Hence, irrigated areas have different malaria transmission potentials even at low vector population density. This emphasizes the need of regular evaluation of disease transmission status especially prior to and after implementation of vector control measures that may affect the local malaria vector population.

### 1.2.7. Host-seeking and feeding behaviour

Different mosquito species exhibit different feeding and host-seeking characteristics. In the *Anopheles gambiae* s.l parous females tend to feed later in the night than nulliparous ones (Bockarie *et al.,* 1996). The late feeding habit of the parous females, which could have a high propability of being infected with sporozoites, reduces the exposure to infective bites in early hours of the night when people are engaged in outdoor activities (Bockarie *et al.,* 1996). Whereas *An. gambiae s.s* is anthropophilic, endophagic and endophilic, *An. arabiensis* is highly zoophilic, exophagic and exophilic in nature, spending more time outdoors than indoors. Under normal circumstances, the female mosquito requires a single blood meal to complete egg development but nutritional stress during larval development may cause the female to require multiple meals (Takken *et al.*, 1998). The need for multiple blood-meals increases the risk of malaria transmission since the infected mosquito may end up feeding on several individuals and infecting them with malaria parasites. Houses close to breeding habitats experience significantly higher biting rates than those further away due to their accessibility to newly emerged mosquitoes (Smith *et al.,* 1995; Carter *et al.*, 2000). Understanding the local vector population and their preferred blood meal host is an integral part in malaria control interventions since integration of several vector control methods is crucial in reducing disease transmission.

### 1.2.8. Malaria Control

The current trend in malaria control is to incorporate use of chemophylactic drugs, case management and malaria vector control. However, the use of chemophylaxis and chemoprophylaxis in malaria control have been reduced considerably and is only recommended as short term measure for international travellers and exceptional cases like combat troops in endemic areas (WHO, 2005). In case mangement, drugs with good patient compliance, safe and high efficacy are used to treat malaria. In this strategy of malaria control, complete treatment is given with artesunate-based combined therapy (ACT) currently being considered the most appropriate drugs (WHO, 2005). Mass treatments of fever cases with ACT help reduce morbidity and mortality in epidemic malaria situation. However, vector control remains the main and most effective intervention strategy in malaria control programs.

#### 1.2.8.1. Vector control

The current trends in disease vector control is to adopt Integrated Vector Management (IVM) strategy which is an ecologically based approach involving several complimentary interventions either used singly or in combination. Integrated vector management is a systemic approach to planning and implementation of vector control measure and is advocated as a global malaria control strategy to reduce or interrupt malaria transmission. The current basis of malaria control in Africa is that, malaria specific mortality can be reduced by vector control measures like use of ITNs (Snow *et al.,* 1988: Alonso *et al.,* 1991), and more recently adoption of the use of Long Lasting Impregnated Nets (LLINs), that last the life span of the net (Guillet et al., 2001), larvicides and source reduction. The suitability and success of each of these vector control measures in a given ecological set-up is a key element in the overall success of integrated anti-

vector strategy, hence the need to evaluate efficacy of each method separately or in combination. Among such interventions include environmental management, chemical, biological and mechanical control measures all of which are especially recommended for use in or near rice fields (Lacey and Lacey, 1990). A clear understanding of the local vector population bionomics is essential in planning and implementation of targeted and timely control measures that significantly reduce risk of malaria infection.

#### 1.2.8.2. Biological control

Biological control agents have been incorporated in malaria vector control programs in rice fields and other ecosystems (WHO, 1995). The approach involves utilization of biological toxins and natural enemies to achieve effective vector management and requires one to first ascertain the ecological changes that may follow the use of larvicides or biological agents to control larvae. Among the biological agents commonly used in malaria vector control are the larvivorous fish (*Gambusia affinis* Baird and Girard), mermithid nematodes (*Romanomermis culicivorax* Ross and Smith), fungus, (*Lagenidium giganteum* Couch) and bacteria (*Bacillus* spp) species. Use of aquatic plants such as *Azzola* species (Urticularia) around *An. gambiae* breeding habitats help in their control by making the habitats unattractive for habitation (Service, 1977). Mosquito larval predators such as notonectid and corixid eggs are also collected on artificial oviposition sites, and relocated to known mosquito sources, augmenting other control measures (Holling 1961). However, some of these intervention measures involve a great deal of work and might not show any immediate recognizable benefit resulting in lessening of interests in them from communities and thus difficult to sustain.

#### 1.2.8.2.1. Use of *Bacillus* species

The commercial formulations of *B. thuringiensis* var. *israelensis* and *B. sphaericus* are the most commonly used bacterial agents in the control of mosquito larval stages. The two bacteria produce insecticidal protein endotoxins used for mosquito control though they differ in their toxin composition, mode of action and risk for selecting insecticidal resistance. The two species have been shown to be highly effective against the target species (Mulla *et al.*, 1984; Fillinger *et al.*, 2003; Shililu *et al.*, 2003b) with few if any effects on non-target macro-invertebrates (Charles and Nielsen-LeRoux, 2000) hence becoming popular microbial insecticides for mosquito control. Although some resistance against *B. sphaericus* has been reported, this is overcome by combining with *B. thuringiensis var. israelensis* (B.t.i) whose endotoxins interact synergistically with *B. sphaericus* to enhance its high toxicity to mosquito larvae in absence of insecticide resistance in populations treated with the bacterium (Wirth *et al.*, 2004). However, the residual activity of these formulations is very short and re-colonization of the treated sites by anopheline larvae is witnessed between 5-7 days after treatment (Romi *et al.*, 1993; Skovmand and Sanogo, 1999). The granular formulations of the *B. thuringiensis* var. *israelensis* (Vectobac GR®) and *B. sphaericus* (ABG 6185®) have been found to be highly efficacious against *An. arabiensis* larvae in natural and artificial ditches and achieve complete control of the mosquito larvae in rice fields (WHO, 1995). Successful reduction of anopheline mosquito larval densities was realized recently in non-irrigated habitats in rural western Kenya recording up to 95 % reduction in late instars after *B. thuringiensis* var. *israelensis* and *B. sphaericus* application (Fillinger and Lindsay, 2006). However, most studies on use of microbial larvicides have mainly focused on malaria vector control and its success in relatively small breeding sites in non-

irrigated areas (Skovmand and Sanogo, 1999; Fillinger and Lindsay, 2006) with little information on large scale use of these in irrigated rice agro-ecosystems.

#### 1.2.8.2.2. Insecticide-treated Bed-nets and materials

Insecticide-impregnated bed nets (ITNs) and materials are effective in preventing malaria, decreasing the incidences of malaria by approximately 50% in field trials performed to date (Choi *et al.*, 1995; Howard *et al.*, 2000: Lengeler, 2001). The ITNs primarily affects malaria transmission by reducing mosquito-biting rate killing those that come into contact with the treated nets and through provision of a physical barrier that diminishes the chances of the female to reproduce due to lack of bloodmeal unless it gets it from an alternative source (Robert and Carnavale, 1991; Choi *et al.*, 1995; Lengeler, 2004). The use of insecticide-treated nets (ITN) and house spraying represent a quantum leap on the use of physical barriers and chemicals in malaria control.

Mosquito nets treated with pyrethroid insecticides provide a remarkable degree of protection against malaria in Africa. Curtis and others (1991) showed that use of ITNs and house spraying reduces the prevalence of anaemia and the number of malaria-infective mosquitoes biting each night by 90%. In addition, ITNs are cost-effective and preferred by most households. ITN coverage was not widespread previously (Alaii *et al.*, 2003b) but the coverage for children under five years of age has increased rapidly from 7% in 2004 to 67% in 2006 resulting in 44% reduction in malaria deaths (Fegan *et al.*,2007). Emphasis should be placed on the effective development and marketing of simple and affordable treatment kits for self-use in homes. This would be user-friendly, avoiding the cumbersome community-based treatment, and encourage

more people to use ITNs. Availability of ITNs to the most impoverished members in our rural communities, where malaria is prevalent is essential.

The effectiveness of ITNs faces several challenges since it is dependent on behavioural change which may limit its sustainability. Furthermore, the use ITNs is open to abuse as reported in western Kenya by Minakawa and others (2008) where families use the bednets to dry fish.

However, the development of mosquito nets pre-treated with insecticide, Long Lasting Impregnated Nets (LLINs) that last the life span of the net, is a solution to the difficulty of the re-impregnation of conventional nets (ITNs). One of the key challenges for use of ITNs on a large scale is the impregnation and the re-impregnation that needs technical skills and materials, which may not always be available (Lines, 1996). However, the current use of mosquito nets pre-treated with insecticide, Long Lasting Impregnated Nets (LLINs), that last the life span of the net, is a solution to this problem (Guillet et al 2001) and LLINs are now available and preliminarly recommended by WHO for malaria prevention (WHO, 2001)

The use of bednets help in preventing malaria transmission by mosquitoes only when an individual is sleeping under them and do not get exposed to infective mosquito bites before going to bed. This contrasts the approach of anti-larval measures where the vectors are controlled before dispersing and transmitting malaria (Killeen *et al.*, 2002a).

### 1.2.9. Vector control and community participation

Community and/or individual participation in vector control measures greatly affects disease transmission. Low levels of community involvement in antimalarial intervention activities results

in negative impact on disease incidence (Winch *et al.*, 1994). Community participation in malaria control measures is an integral aspect of the national fight against poverty since malaria and poverty are intimately related. The Gross Domestic Product (GDP) in malarious countries is much less compared to that in non-malarious regions with a more than fivefold difference (Gallup and Sachs, 2001) and being particularly burdensome to the poorest of the poor (Malaney *et al.*, 2004). In order to ensure success of antimalarial interventions, community participation should form part of the inception and planning of new interventions in their locality. This helps in promoting self-awareness and sense of control leading to a feeling of ownership that ensures sustainability. This was apparent in the successful implementation of microbial larviciding in rural western Kenya (Fillinger and Lindsay, 2006) where active collaboration with the local community members ensures access to all breeding habitats for larviciding is achieved. Such community-based operations can also be replicated in irrigated rice agro-ecosystems with few adjustments to cater for the agro-economic nature of these areas. Communiti participation is necessary since the presence of malaria infections may affect diverse features of human existence including mobility, investment choices, and even fertility decisions. In order to realize maximum success and benefits from malaria control intervention strategies it is important for the community to fully understand the intervention method and participate in its implementation. Alaii *et al.*, (2003a) observed that community participation in control measures is important as it helps ensure the success of the control measure and the eventual evaluation of the impact on the disease. Furthermore, building on existing infrastructure or experiences in the local community enhances the ability to succeed by ensuring community participation. In irrigated areas, infrastucture that are established to tackle various health and socio-economic problems facing the

community such as safe motherhood and integrated management of childhood illnesses can be exploited during anti-larval interventions (WHO, 2005).

### 1.2.10. Malaria treatment

Early diagnosis and provision of prompt and effective treatment is a fundamental component of the global strategy for malaria control. This helps in shortening the duration of malaria infection and prevents development of complications and mortality due to malaria. Nwaka and others (2004) observed that less than half of the children under five years infected with malaria are treated with anti-malarial drugs, a fundamental right for malaria risk populations (WHO, 2005). Until recently the usual first and second line drugs for malaria treatment have been Chloroquine and Sulfadoxine-pyrimethamine in most African countries including Kenya (WHO, 1993. However, drug resistance cases have been widely reported in Africa against chloroquine and more recently Sulfadoxine-pyrimethamine derivatives and hence their use in treatment and chemoprophylaxis in sub-Saharan Africa has been reduced (WHO, 1993; Ronn *et al.,* 1996). Comsequently, *P. falciparum* malaria is becoming more difficult to treat and control due to the emergence of parasite resistance to the major anti-malarials, notably chloroquine. This has necessitated the adoption of Arteseminin-based combination therapy (ACT) as first line of treatment for resistant malaria in Kenya and other African countries. For febrile malaria illness, Coartem (Artmether and Lumefantrine) is used while severe cases are usually treated with intravenous artesunate especially in children (Nwaka *et al.,* 2004).

Heterogeneity in therapeutic response to first-line anti-malarial drugs exists in different geographic localities and the ability of the health system to deliver the required drugs

successfully to the end user in malarious areas is essential (WHO, 2005). Thus, it is crucial to understand the extent of disease transmission risk and all possible control measures applicable in different ecological zones to limit its impact on vulnerable groups and plan implementation of different control strategies in tandem with existing ones.

## 1.3. Hypothesis

Microbial larvicide control interventions have significant effect on malaria vector dynamics and transmission risk in irrigated rice agro-ecosystem.

## 1.4. Objectives

### 1.4.1. Main Objective

To evaluate malaria vector bionomics and role of microbial larvicides in malaria vector control in an irrigated rice agro-ecosystem.

### 1.4.2. Specific objectives

1) To determine the spatio-temporal distribution of aquatic stages of malaria vectors in an irrigated rice agro-ecosystem
2) To assess the population dynamics and species diversity of *Anopheles* mosquitoes in an irrigated agro-ecosystem.
3) To determine the impact of irrigated rice cultivation on human biting rates of the malaria vectors.

4) To determine the relationship between irrigated rice cultivation and malaria transmission in a typical rice- village complex.

5) To assess the efficacy of microbial larvicides in malaria vector management in an irrigated rice-village complex.

# CHAPTER TWO

# 2.0 MATERIALS AND METHODS

## 2.1. Study area

The study was conducted in the Mwea rice irrigation scheme (MRIS), which consists of more than fourty villages in the Mwea Division of Kirinyaga district, central Kenya. The area is located 100 km North East of Nairobi and to the East of Mount Kenya with an altitude range of 1100-1200 metres (Fig. 2.1). Mwea region is mainly characterized by black cotton soil and the main agricultural activity is irrigated rice cultivation and subsistence farming in other areas. According to the 1999 national population census, Mwea division has approximately 150,000 persons in 25,000 households. The Mwea Rice Irrigation Scheme is located in the west central region of Mwea Division covering an area of about 13,640 ha with more than 50% of the scheme area being used for rice paddy cultivation. The remaining area is used for subsistence farming, grazing and other community activities.

The study was mainly focused in four irrigated villages namely; Kangiciri, Kiuria, Karima and Rurumi and conducted for three years. Baseline data to define the human population structure and other demographic attributes were collected at the beginning of the studies. Data relating to population size, house type, abundance of animals, distance of houses to mosquito breeding habitats and animal sheds, ITN coverage was collected for each of the four villages. These factors affect the presentation of malaria in the population.

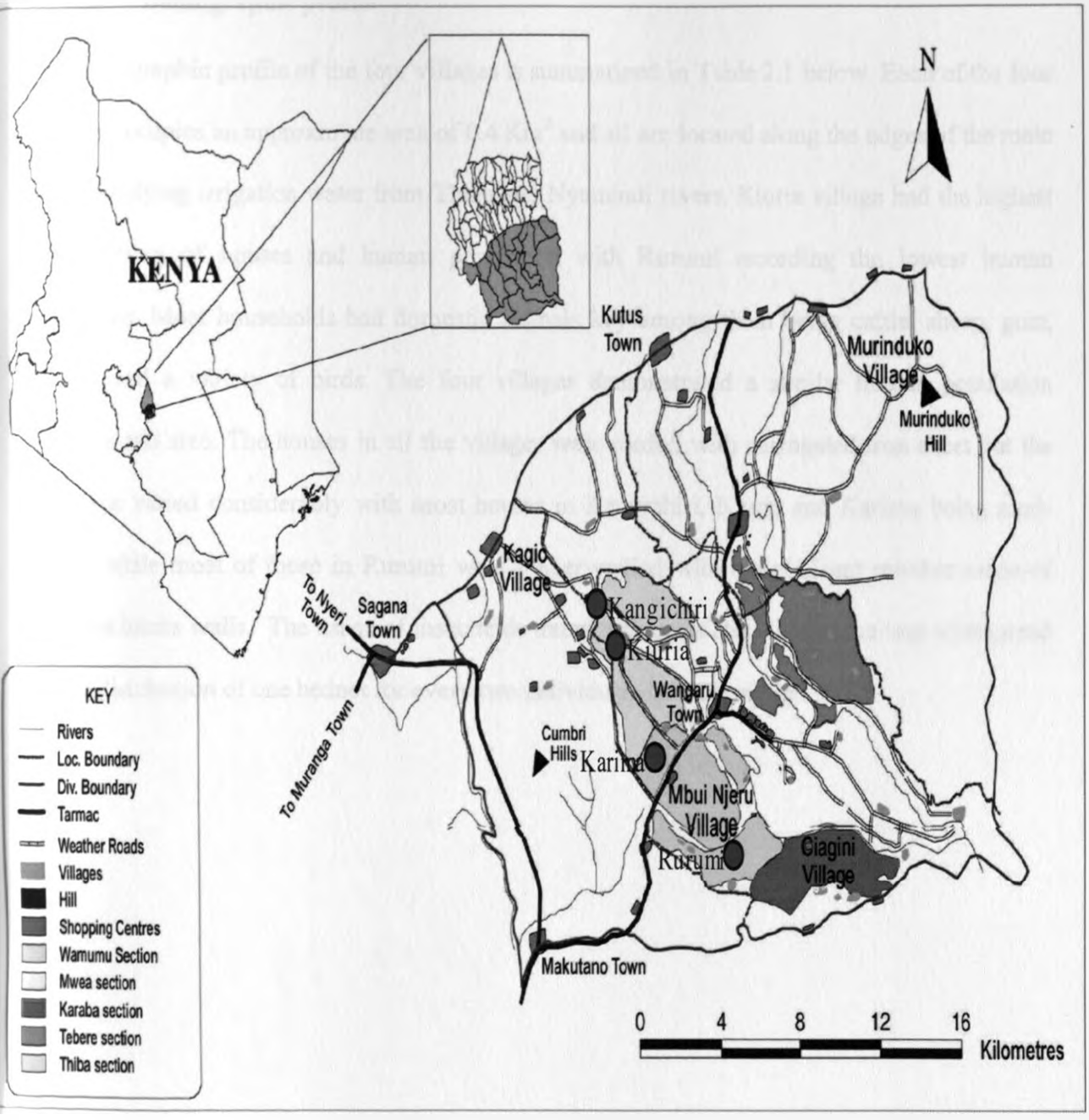



Figure 2.1: The map of study villages ( ● ) in the Mwea irrigation scheme of Kirinyaga district, Kenya

## 2.2. Demographic profile

The demographic profile of the four villages is summarized in Table 2.1 below. Each of the four villages occupies an approximate area of 0.4 $Km^2$ and all are located along the edges of the main canal supplying irrigation water from Thiba and Nyamindi rivers. Kiuria village had the highest concentration of houses and human population with Rurumi recording the lowest human population. Most households had domestic animals key among them being cattle, sheep, goat, donkey and a variety of birds. The four villages demonstrated a similar human population structure and size. The houses in all the villages were roofed with corrugated iron sheet but the wall type varied considerably with most houses in Kangichiri, Kiuria and Karima being mud-walled while most of those in Rurumi were timber-walled with a significant number made of concrete bricks walls. The usage of insecticide trated bednets (ITNs) in the area was widespread with a distribution of one bednet for every two individuals in each village.

Table 2.1: Demographic profile of the four study villages in the Mwea irrigated rice agro-ecosystem.

| Village | No. of homesteads | No. of households | Proportion of Population Structure (%) | | | Bed Net usage (%) | Goat | Sheep | Cattle | Chicken | Dog | Pig | Cat | Rabbits | Total animal population |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Children <5 Years | Children 5-10 yrs | Adult | | | | | | | | | | |
| Kangichiri | 155 | 276 | 14.40 | 13.40 | 72.20 | 51.94 | 70 | 34 | 322 | 891 | 73 | 0 | 77 | 8 | 1475 |
| Karima | 156 | 229 | 14.80 | 10.16 | 75.04 | 44.56 | 50 | 67 | 267 | 488 | 33 | 21 | 77 | 47 | 1050 |
| Kiuria | 240 | 422 | 15.98 | 10.32 | 73.70 | 45.96 | 98 | 27 | 325 | 1363 | 56 | 3 | 80 | 10 | 1962 |
| Rurumi | 220 | 400 | 16.18 | 13.24 | 70.59 | 37.09 | 122 | 57 | 233 | 613 | 29 | 0 | 44 | 6 | 1104 |
| Grand Total | | | 15.34 | 11.66 | 73.00 | 45.66 | 340 | 185 | 1147 | 3355 | 191 | 24 | 278 | 71 | 5591 |

## 2.3. Weather data

The weather data was collected through the use of a HOBO® Micro Station (Onset Computer Corporation, Bourne, Massachusetts, USA) that was set-up at each village to record the daily relative humidity, temperature, wind direction and speed, and solar radiation. The weather information was downloaded at the end of every month using BoxCar Pro® (Onset Computer Corporation, Bourne, Massachusetts, USA). A rain gauge, (Tru-Chek®, Rain Gauge Division, Edwards Manufacturing Co. Albert Lea, Minnesota USA) was installed in each of the four villages and locally recruited field assistants' recorded daily rainfall received at 0900 hrs.

### 2.3.1. Rainfall pattern

Malaria transmission is closely linked to rainfall patterns in an area since most of the disease vector species are dependent on surface water availability for breeding, thus the understanding of the rainfall regime of the study area was important. The rainfall pattern in the area is bimodal with two rainy seasons, a long one between March-July and a short one between October-December resulting in a mean annual rainfall of 950 ml. Monthly precipitation varied substantially from year to year as can be deduced from figure 2.2 with the highest rainfall being recorded in the months of May 2005 (377.3 mm) and November 2006 (413.1 mm) while the lowest (1.0 mm) was in February 2007 (Fig. 2.2). During the sampling period a total of 640.6 mm, 1269.0 mm and 501.6 mm of rainfall were recorded in 2005, 2006 and 2007 respectively.

### 2.3.2. Temperature

Over the sampling period, the mean monthly temperature varied between 20.5°C and 24.8°C. The minimum and maximum temperatures ever recorded in the area were 16.8°C and 30.3°C Mean monthly temperatures indicated that the hottest months were around February/March and October. Figure 2.3 shows the mean monthly temperature recorded in Mwea rice irrigation scheme during the study period.

### 2.3.3. Relative Humidity

Relative humidity (RH) is a crucial environmental variable in malaria transmission due to its influence on the growth of the malaria vectors. The mean monthly RH varied considerably with the most humid months during the entire study period being November and December 2006 recording relative humidity of over 82% while the least humid month was February 2006 with RH ≈ 58% (Fig. 2.3.). The mean RH recorded in the study area over the sampling period was 69.8%. The optimal RH for malaria vectors ranges from 70-80% and the RH in the study area favours the breeding of the vector mosquito species and the development of the malaria parasites (Wernsdorf and McGregor, 1986).

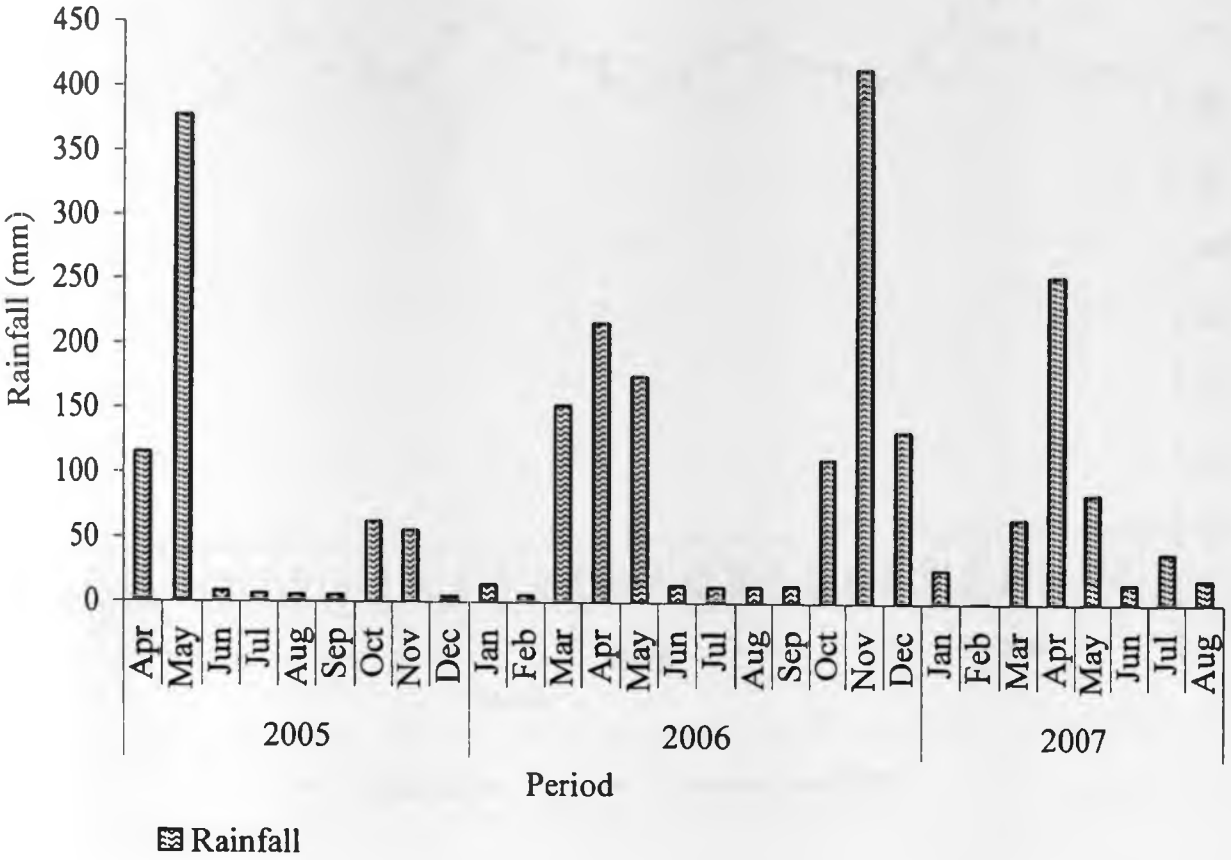



Figure 2.2: Monthly rainfall (mm) recorded in thestudy area from Arpil 2005 to August 2007

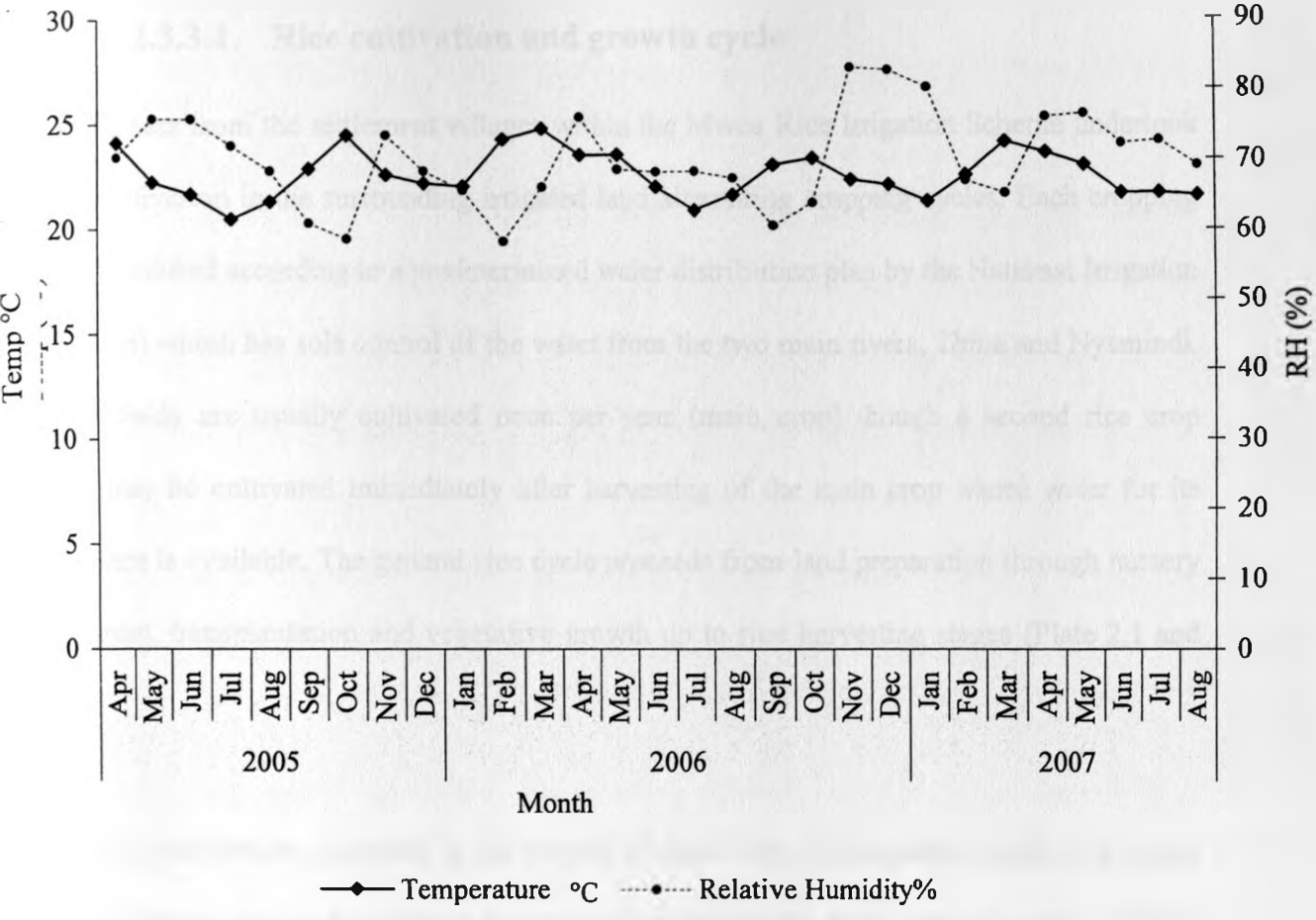



Figure 2.3: Mean monthly temperature (°C) and the mean relative humidity for the Mwea rice irrigation scheme.

### 2.3.3.1. Rice cultivation and growth cycle

Tenant farmers from the settlement villages within the Mwea Rice Irrigation Scheme undertook the rice cultivation in the surrounding irrigated land alternating cropping cycles. Each cropping cycle is scheduled according to a predetermined water distribution plan by the National Irrigation Board (NIB) which has sole control of the water from the two main rivers, Thiba and Nyamindi. The rice fields are usually cultivated once per year (main crop) though a second rice crop (ratoon) may be cultivated immediately after harvesting of the main crop where water for its maintenance is available. The general rice cycle proceeds from land preparation through nursery development, transplantation and vegetative growth up to rice harvesting stages (Plate 2.1 and 2.2).

During land preparation, occurring in the months of April-July, the vegetable waste /rice straws from the previous crop and weeds are burnt or decomposed. The water canals, roads and drains are repaired during this period to help ease flow of water and access to the rice farms. The land is flooded through the gravity irrigation system and then ploughed using rotavator machines, leveled using the oxen and post-rotavation weeding done by hand. Rice seeds are usually sown from mid July to August on nursery seedbeds set at the corners of selected paddies and the young seedlings then transplanted within 4-5 weeks (in September) onto the main paddies. In the paddies the vegetative growth phase /tillering commences and extends from the appearance of the first tiller until maximum tiller number is reached (September-early November). Application of fertilizers (Sulphate of Ammonia) is done two weeks after transplanting and a top dressing application done after four weeks. Chemical insecticides mostly Sumithion and Furadan are applied 5-6 weeks after transplanting to control rice pests especially stem borers and leaf miners.

During the vegetative growth, the rice plant stem elongates and the tillers continue to increase in number and height leading to an increase in ground cover and canopy formation (Plate 2.1).

The rice plant proceeds into the reproductive phase that lasts 20–30 days and includes the initiation of panicle, booting, heading and flowering stages. Plants are considered to be in the reproductive phase when more than 50% of plants have panicles usually in October to early November. Bird scaring is done during this period to reduce destruction and consumption of the newly formed embryo in the rice heads. The rice crop eventually matures after three to four months after transplanting and the paddies are drained towards the end of November to facilitate rice drying and eventual rice harvesting in December and early January (Plate 2.2.).

The above description was witnessed in all the villages simultaneously except in one half of Karima village where overlapping of the cropping cycle was observed. The overlapping cycle commenced in October with land preparation and nursery establishment in November. Transplanting was done in months of December/January with maturity and eventual rice harvesting in March/April period. This cycle ensured rice cultivation occurred around the village all year round. Rice was only planted when the artificial irrigation water was assured even though there was some natural flooding observed in the rice paddies during the rainy seasons.

Immediately after rice harvesting, the land is flooded with water when available in order to facilitate the cultivation of the second crop (ratoon). During this period the stumps of rice crop vegetates (tillering stage) and its development thereafter takes the course of the primary rice crop. The growth progresses till maximum tillers are attained and enter into the booting stage

hen the flowering, maturity and the eventual harvesting. Fertilizers and chemical insecticides are

ıpplied under the same regimen as in the primary crop.


**Plate 2.1:** Early stages in rice field development and rice growth in an irrigated agro-ecosystem


**Plate 2.2**: Later stages of rice development and eventual ratoon crop development

## CHAPTER THREE

## 3.0 Spatial heterogeneity and temporal distribution of *Anopheles* (Diptera: Culicidae) aquatic habitats in rice agro-village complexes in Mwea, Kenya


### ABSTRACT

A study was conducted to examine the spatial heterogeneity of *Anopheles* aquatic habitats in four rice agro-village complexes in Mwea, Kenya. Weekly larval sampling was conducted between April 2005 and November 2006 in all peridomestic (transient) larval habitats and randomly selected paddies and canals within 1.3 km radius from the village perimeter. In total, 54,648 anopheline larvae and 3,216 pupae were collected from eleven different aquatic larval habitats identified. Early instar larvae comprised 90.4% of the total number of larvae collected. The highest mean larval productivity was recorded in temporary water pools (2.26 ± 0.24) and burrow pits (1.90 ± 0.03) and the lowest densities occurred in hoof prints (0.14 ± 0.14). Seasonal variation in the overall mosquito larval density was evident but not strongly associated with rainfall (r = 0.177). Pools and burrow pits were the most important productive forms of habitats. A general model fitted on the anopheline larval data showed that habitat type and other physical characteristics within the habitats were important in predicting larval productivity. Larval and pupal productivity in the rice paddies and drainage canals were influenced by rice development cycle with the most productive phase occurring during early vegetative growth stages of rice. The density of all the anopheline pre-adult stages was inversely correlated (r = 0.7) with the distance from the habitat location relative to the nearest human dwelling.. Eight *Anopheles* species were identified from the pre-adult stages collected and were predominated by *Anopheles arabiensis* (68.73%). Other species identified included *An. pharoensis* Theobald (31.68%), *An. funestus* Giles (1.96%), *An. coustani* Laveran (0.94%), *An. rufipes* Gough (0.60%), *An. nili*

Theobald (0.04%), *An. rivolurum* Leeson (0.02%), and *An. pretoriensis* Theobald (0.02). In conclusion, Mwea irrigated rice agro-village complexes support a wide range of anopheline species and a fundamental difference in productivity of breeding sites occurs during the year with a small but significant important proportion of anopheline population breeding in small and transient habitats formed during the rainy season within the human settlement. However, rice paddies are the principal larval habitats throughout the year and managing these habitats would have an impact on mosquito abundance but larval control actions should be initiated during the early stages of rice growth.

## 3.1. Introduction

Ill human health is a major hindrance to sustainable intensification of lowland/irrigated rice cultivation and alleviating this problem may lead to a reduction in land pressure in the upland areas (Garrity 1988; Service 1989; Windmeijer and Andriesse 1993). In sub-Sahara Africa, irrigated rice cultivation has been known to enhance the development of several mosquito species most of which are vectors of human diseases (Lacey and Lacey 1990) in spite of helping in improving socio-economic status and ensuring food security in the region. In an effort to overcome the problem of malaria in Africa, integrated vector mangement measures targeting all stages of mosquito life cycle have been adopted with larval control being re-emphasized. For the development of sound control strategies for the malaria vectors, a good understanding of the vector dynamics and factors influencing their distribution is required as it would help in planning control programs based on accurate predictions of the likely effects. In Africa, irrigated rice cultivation with full or partial water control has been associated with high densities of the main malaria vectors than neighbouring areas without irrigated rice cultivation (Chandler *et al.* 1975; Faye *et al.* 1993a, 1995a; Dossou-Yovo *et al* 1995; Muturi *et al.* 2006). In central Kenya, the seasonal vector population increase has been associated with periodic flooding of the irrigated rice fields (Mukiama and Mwangi 1990; Mwangi and Mukiama 1992) with intermittent flooding in the area favouring oviposition by *An. arabiensis* though reducing larval survival rate (Mutero *et al.* 2000).

Most research on irrigation and malaria is focused on the prevalence of the disease within the human population close to the irrigation scheme. Few studies address the effects of the irrigation system, in particular, mosquito larval ecology and control. Vector control in Africa can target all

tages of the mosquito life cycle but has historically been focused almost exclusively on adult tages based on indoor residual spraying (Mnzava et *al.* 1993; Curtis 1994; Roberts *et al.* 2000) ›r, more recently, the use of insecticide treated bed-nets (ITNs) or curtains (Lengeler 2001). This ıpproach however, has also been complicated by the increasing insecticide resistance (Chandre *:t al.* 1999; Hargreaves *et al.* 2000) and high levels of drug resistance (Trape 2001) calling for :xceptionally high efforts from governments and research/control communities in order to ;ucceed in achieving the WHO roll back malaria campaign goals. In some areas, significant ;uccess has been recorded with bed nets and curative drugs but truly integrated and well managed efforts as staged in the early decades of the last century in Zambia (Utzinger *et al.* 2001) are needed in other parts of Africa especially those under irrigated rice cultivation in order to yield better results. Incorporation of larval control in these efforts would make malaria management more successful since, as shown by Killeen *et al.* (2002b), larviciding and source reduction control mosquitoes before they disperse and transmit disease thereby impacting positively in minimizing the disease effect.

Malaria is non-randomly distributed across a landscape in patches of higher or lower transmission intensity and malaria risk, which are separated by greater or lesser distances from each other (Carter *et al.* 2000). The spatial aspect of malaria risk with respect to human habitation in relation to specific types of environments and its relevance to protection against malaria has long been recognized (Celli, 1933). The association of malaria transmission with specific locations is attributable to the presence of suitable breeding habitats of the anopheline vectors with each habitat being the focus of malaria transmission. The *Anopheles* mosquito breeding habitats are highly diverse and species specific. For example, *Anopheles gambiae*

breeds in characteristically small, transient collections of water close to human habitation (Service 1993) although breeding also occurs in more extensive water surfaces like rice fields, seepage plains and river margins (Mwangi and Mukiama 1992; Shililu *et al.* 2003a). It has also been noted that, the dispersal and dimensions of malaria transmission depends on the productivity of the breeding habitats and the dispersal range of the vectors emanating from the breeding habitats (Carter *et al.* 2000; Thomson *et al.* 1995). The flight range of the malaria vectors has been shown to extend from 1-2 Km (Birley 1989; Dolo 1996) although the vectors tend to exhibit aggregated form of distribution with high densities within 100m range from human habitation (Zhou *et al.* 2004a).

Prior information on mosquito breeding habitats diversity and distribution pattern and the variation of larval density on temporal and spatial patterns is important in planning successful mosquito larval control programmes. The current studies were conducted with an objective of monitoring larval abundance and the productivity of the different habitat categories and to determine spatial distribution of *Anopheles* mosquito pre-adult stages with a view of providing vital information to guide in the implementation of microbial larvicides in irrigated rice cultivated areas in tropical Africa. Accurate knowledge of the distribution and density variation of malaria vectors is an important tool for planning and evaluating malaria control measures. The potential breeding habitats in the four study villages were identified and mapped out for sampling of mosquito pre-adult stages to give the baseline data on anopheline mosquito larval density variation and habitat diversity that enhance malaria transmission prior to implementation of large-scale microbial larvicide application.

## 3.2. Materials and Methods

### 3.2.1. Larval sampling

In this study, all the larval habitats within and around the four study villages of Kangichiri, Kiuria Karima and Rurumi as described in Chapter two were identified as well as paddies within a radius of 1.3 km from the outer boundaries of the villages. Mosquito larval sampling was conducted weekly over a 20-month study period (April 2005 – November 2006) using the standard dipping technique (Service, 1993). All small and transient aquatic habitats present in each village, hereafter referred to as non-irrigated habitat types, were identified, characterized and sampled for mosquito immature stages. In addition, thirty randomly selected paddies and associated canals, located within 1Km radius from the village perimeter were, also sampled. Each habitat was allocated a unique identifier throughout the sampling period and any newly formed habitat was recorded and included in the weekly sampling plan. The small forms of transient habitats such as hoof-prints occurring within a 10m radius from each other were pooled together and sampled as a unit. On each sampling occasion, 20 dips were made from each of the pooled units of the small and transient habitats as well as in all canals while 40 dips were randomly made along the edges of each rice paddy. The larvae and pupae collected in each habitat per sampling occasion were pooled together to represent the sample for the habitat and placed in labeled plastic bags and transported to the laboratory for further processing.

### 3.2.2. Laboratory processing

In the laboratory, mosquito samples were sorted by subfamily-Anophelinae or Culicinae- and then categorized as early instars ($1^{st}$ & $2^{nd}$), late instars ($3^{rd}$ & $4^{th}$) or pupae, counted and

recorded. The 3rd and 4th instars were preserved in 70% ethanol and the pupae were placed in emergent cages. The late instars and resultant emergent adults from pupae were identified morphologically to species using the identification key of Gillies and de Meillon (1968).

### 3.2.3. Larval habitat characterization

The characteristics of the habitats such as water depth, turbidity, presence/absence of other invertebrates, vegetation type, distance to the nearest house as well as rice height and growth stage were recorded. The distance from every larval habitat to the nearest household was measured using a 100m tape measure making the appropriate markings for every 100m length for longer distances. The distance to the village was categorized into twelve classes (i.e. 1=1-100m, 2-101-200m, 3=201-300m with 13 indicating distances beyond 1200m). The estimation of Azolla in each habitat, water movement, canopy cover or rice height and number of tillers were other factors assessed. Water bodies were inspected for mosquito larvae using standard dipping techniques with a 350-ml dipper to collect the mosquito larvae (Service, 1993). All data from the habitat characterization of each aquatic larval habitat was recorded on a field sampling form.

## 3.3. Data analysis

Data were analyzed using SPSS version 11.5 for windows (SPSS Inc., Chicago, IL). Data were transformed using the log transformation (log1+n) before statistical analysis to normalize distribution. Larval counts were calculated as the number of larvae per ten dips per sampling occasion because the number of larvae sampled was low. ANOVA tests were used to test variation in larval density among villages and habitat types. Tukey's HSD tests were used to

separate the means where significant differences were observed in ANOVA tests. Multiple regression analysis was used to test the significance of the various habitat characteristics on larval and pupal densities. Chi-square and correlation tests were also used to compare habitat diversity and larval productivity with rainfall. The wet and dry periods were derived by pooling all the months when rainfall was received (wet) and when no raining occurred (dry) in the area. Regression tests were conducted to determine the relationship between habitat productivity and its location in relation to human habitation.

## 3.4. Results

### 3.4.1. Larval habitat diversity and productivity

During the twenty months sampling period, a total of 54,648 anopheline larvae and 3,216 pupae were collected in eleven different types of aquatic habitats in the four irrigated rice agro-villages showing differential larval densities (Table 3.1). Early instars larvae comprised 90.4% of the total number of anopheline mosquito larvae collected from the four study villages. The diverse aquatic larval habitats types for anopheline mosquitoes identified during the study included rice paddies, canals, burrow pits, temporary pools, fishpond, tyretracks, hoof prints, quarry, ditches and marshy areas. In Kangichiri and Rurumi villages, eight habitat types were identified while seven habitat types were found in Kiuria and Karima. Most of the breeding habitats were common to all the villages except fishpond and quarry that were only found in Kiuria and Rurumi respectively, and marshes which occurred only in Kangichiri and Rurumi. The larval habitat types identified were not significantly different among the study villages ($\chi^2 = 6.975$, df 1, $p = 0.990$).

Among the villages, the anopheline mosquito larval density varied significantly ($F = 13.86$, df = 3, $p < 0.05$) with mean larval productivity in Rurumi being lower than that of other three villages. A one-way ANOVA and Tukey's honest test of significance indicated a significant difference ($F = 14.27$, df = 10, $p < 0.05$) in the larval densities in the eleven habitat types in all the villages. The highest mean larval productivity was recorded in temporary water pools ($2.26 \pm 0.24$) and the burrow pits ($1.90 \pm 0.03$) and the lowest density was recorded in hoof prints ($0.14 \pm 0.14$).

The proportion of times the habitats were positive for anopheline larvae during the 20-month sampling period differed, being highest in Kiuria 47.31% (n = 3752) and Karima 46.34% (n = 4573) compared to that of Rurumi 43.36% (n = 4368) and Kangichiri 43.05% (n = 4026). The rice paddies and the associated canals constituted most of the samples since they had water during much of the sampling occasions. Amongst the non-irrigated habitat types (other than paddies and canals) that were mainly found within the villages (peridomestic), tyretracks and temporary pools lead in sample collections in Kangichiri and Karima. In Kiuria and Rurumi villages, the marsh, temporary pools, quarry, fishpond and burrow pits registered most sample collections. The importance of each habitat type in terms of presence and productivity of *Anopheles* larvae was variable between the villages as shown in Table 3.2. Overall, the anopheline larval productivity among the various habitat types was significantly different ($F = 8.88$, $df = 9, 29$, $p < 0.05$) in the study area.

The utilization of the different habitat types by the anopheline mosquitoes for oviposition, and consequently its productivity, varied with habitat type amongst the four villages as shown by density variation of the early instar larvae (Table 3.2). Results of ANOVA and Tukey's honestly significantly difference tests indicated that the mean larval density of early instar larvae in Kangichiri were significantly higher in pits, paddies, pools and marshes compared to other habitat types ($F = 4.99$, $df = 7,416$, $p < 0.01$). In Karima, the pools and paddies were significantly more productive ($F= 3.77$, $df = 6, 364$, $p < 0.01$) and in Rurumi the ditches, paddies and quarries had significantly higher density of early instar larvae than other habitat types ($F = 6.58$, $df = 7, 344$ $p < 0.01$). In Kiuria, the density of the early instar larvae was not significantly different

amongst the different habitat types ($F = 1.78$, $df = 6,324$, $p > 0.05$). The ability of the larval habitat types to support anopheline larval development did not differ significantly as indicated by a two-way ANOVA for later instar larvae ($F= 1.6$, $df = 9,27$, $p < 0.05$) among the four villages.

Pupal productivity did not vary significantly variable between the different habitat types ($F = 1.06$ $df = 9, 20$ $p > 0.05$). A one-way ANOVA indicated significant difference in pupal density among the four villages ($F= 4.29$, $df = 3, 26$, $p < 0.05$) with highest mean pupal production noted in Kangichiri village ($0.38 \pm 0.09$).

Table 3.1: Anopheline larval density (#larvae/10 dips) in different aquatic habitats in four irrigated rice agro-village complexes in Mwea, Kenya

| | Larval Density | | | | |
|---|---|---|---|---|---|
| Breeding habitat | Kangichiri | Kiuria | Karima | Rurumi | Overall density |
| Canal | 0.86 | 1.51 | 1.05 | 0.66 | 1.06 |
| Ditch | 1.16 | 0.00 | 1.43 | 2.49 | 1.40 |
| Fishpond | - | 1.76 | - | - | 1.76 |
| Hoof print | 0 | - | 1.25 | - | 0.14 |
| Marsh | 1.70 | - | - | 1.06 | 1.38 |
| Paddy | 1.83 | 1.39 | 1.67 | 1.24 | 1.56 |
| Burrow Pit | 2.61 | 2.04 | 2.03 | 1.22 | 1.90 |
| Temporary Pool | 1.86 | 1.57 | 5.64 | 0.00 | 2.26 |
| Quarry | - | - | - | 1.77 | 1.77 |
| Seep | 2.00 | 2.23 | - | 0.48 | 1.62 |
| Tyretrack | 1.10 | 2.30 | 1.95 | 0.39 | 1.41 |
| **Mean Total** | **1.41** | **1.48** | **1.36** | **1.01** | **1.34** |

*Dash (-)=Habitat absent, 0.00=Habitat not productive*

Table 3.2: Anopheline larval habitat dynamics, number of habitats sampled and density of *Anopheles* larvae over the 20 months study period in Mwea, Central Kenya

| Village | Breeding habitat type | # Habitats sampled | % Habitats with water | # times Sampled | % habitat positive for anophelines | Early instars density | Late instars density | Pupae density |
|---|---|---|---|---|---|---|---|---|
| Kangichiri | Canal | 45 | 79.63 | 1994 | 31.06 | 0.75 | 0.07 | 0.08 |
| | Ditch | 4 | 69.35 | 62 | 27.91 | 0.99 | 0.13 | 0.14 |
| | Hoof print | 1 | 10 | 40 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Marsh | 4 | 61.9 | 168 | 50.00 | 1.54 | 0.18 | 0.49 |
| | Paddy | 30 | 76.38 | 2553 | 54.51 | 1.63 | 0.17 | 0.10 |
| | Pit | 3 | 27.36 | 106 | 34.48 | 1.99 | 0.48 | 0.45 |
| | Pool | 14 | 28.09 | 502 | 47.52 | 1.54 | 0.22 | 0.71 |
| | Tyretrack | 15 | 19.88 | 845 | 21.43 | 1.02 | 0.06 | 0.63 |
| | Total/means | 116 | 64.60 | 6234 | 43.05 | 1.25 | 0.13 | 0.15 |
| Karima | Canal | 55 | 67.03 | 3846 | 35.98 | 0.99 | 0.05 | 0.03 |
| | Ditch | 4 | 8 | 225 | 38.89 | 1.40 | 0.00 | 0.00 |
| | Hoof print | 2 | 25 | 86 | 25.00 | 1.25 | 0.00 | 0.00 |
| | Paddy | 30 | 60.03 | 2797 | 63.13 | 1.47 | 0.19 | 0.09 |
| | Pit | 7 | 12.03 | 326 | 40.00 | 2.39 | 0.31 | 0.11 |
| | Pool | 6 | 15.67 | 308 | 61.70 | 5.02 | 1.43 | 0.20 |
| | Tyretrack | 17 | 17.56 | 864 | 38.00 | 2.04 | 0.17 | 0.10 |
| | Total/means | 121 | 54.01 | 8470 | 46.34 | 1.25 | 0.12 | 0.05 |
| Kiuria | Canal | 58 | 80.26 | 2062 | 44.68 | 1.28 | 0.12 | 0.10 |
| | Ditch | 1 | 2.6 | 77 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Fishpond | 1 | 87.01 | 77 | 31.82 | 1.54 | 0.09 | 0.11 |
| | Paddy | 30 | 67.73 | 2628 | 51.80 | 1.23 | 0.11 | 0.07 |
| | Pit | 5 | 18.98 | 332 | 46.03 | 1.77 | 0.10 | 0.16 |
| | Pool | 21 | 13.73 | 1296 | 34.61 | 1.37 | 0.22 | 0.19 |
| | Tyretrack | 2 | 15.79 | 57 | 33.33 | 1.61 | 0.11 | 0.06 |
| | Total/means | 118 | 57.50 | 6529 | 47.31 | 1.28 | 0.12 | 0.09 |
| Rurumi | Canal | 40 | 80.40 | 2107 | 34.30 | 0.60 | 0.04 | 0.02 |
| | Ditch | 2 | 14.10 | 156 | 31.82 | 2.27 | 0.22 | 0.20 |
| | Marsh | 5 | 68.35 | 297 | 41.38 | 0.97 | 0.10 | 0.07 |
| | Paddy | 30 | 66.61 | 3103 | 51.45 | 1.11 | 0.13 | 0.05 |
| | Pit | 16 | 7.20 | 1250 | 21.11 | 1.19 | 0.03 | 0.26 |
| | Pool | 5 | 20.99 | 243 | 19.61 | 0.45 | 0.00 | 0.15 |
| | Quarry | 4 | 60.90 | 312 | 65.79 | 1.64 | 0.12 | 0.04 |
| | Tyretrack | 9 | 8.27 | 653 | 11.11 | 0.31 | 0.07 | 0.00 |
| | Total/means | 111 | 53.82 | 8121 | 43.36 | 0.92 | 0.09 | 0.04 |

**Density expressed as number of larvae/10dips/sampling*

A summary of a general model fitted on anopheline larval habitats is shown in Table 3.3 below. The model indicated that various characteristics in Mwea rice irrigation scheme were important in predicting mosquito larval productivity in the area. A stepwise elimination binomial dispersion analysis involving habitat type, distance to nearest house, percentage of water cover estimate in a habitat, turbidity and depth, presence or absence of floating , emergent or submerged vegetation types, amount of azolla and presence or absence of other invertebrates was conducted on the data collected taking into account data overliers. The model predicted that habitats characterized by low amounts of floating vegetation and Azolla, high turbidity and shortest distance from human habitations had relatively high anopheline larval densities. This helped in explaining the overall high productivity of temporary pools and burrow pits that exhibited these characteristics in Mwea. Thus, in the planning and implementation of larval control measures, specific habitats with unique characteristic that favour high larval productivity should be targeted during larval control programs.

Table 3.3: General model showing factors that predict anopheline larval productivity in Mwea irrigated rice scheme

The GENMOD Procedure

Analysis of Parameter Estimates

| meter | DF | Estimate | Standard Error | Wald 95% Confidence Limits | | Chi-Square | Pr > ChiSq |
|---|---|---|---|---|---|---|---|
| at type | 1 | 0.3321 | 0.1586 | 0.0211 | 0.6430 | 4.38 | 0.0363 |
| ing vegetation | 1 | -0.1607 | 0.0401 | -0.2394 | -0.0820 | 16.03 | <0.0001 |
| r Depth | 1 | -0.0056 | 0.0023 | -0.0101 | -0.0011 | 5.84 | 0.0156 |
| idity | 1 | -0.1591 | 0.0380 | -0.2336 | -0.0845 | 17.49 | <0.0001 |
| rtebrates | 1 | -1.1768 | 0.0829 | -1.3394 | -1.0142 | 201.31 | <0.0001 |
| nce | 1 | -0.0002 | 0.0001 | -0.0003 | -0.0001 | 19.55 | <0.0001 |
| la_cover | 1 | -0.0036 | 0.0008 | -0.0051 | -0.0021 | 22.06 | <0.0001 |
| ersion | 1 | 4.1821 | 0.0677 | 4.0494 | 4.3148 | | |

### 3.4.2. Seasonal variation in anopheline larval densities

A significantly high number of larvae were collected during the wet period (March-July; October-November) compared to the dry period ($F = 4.952$, $df = 1, 80$, $p < 0.05$) of December-February and August-September) especially within the small and transient larval habitats types ($F = 5.216$, $df = 1, 56$, $p < 0.05$). There were 2 peak larval density periods among the non-irrigated aquatic habitats, a major peak in March-May ($3.7 \pm 0.8$) and minor one in November, periods during which substantial amount of rainfall was recorded (Table 3.4 and 3.5). During the wet months, the burrow pits and water pools as well as the fishpond, marshes, among the non-irrigated habitats were important for mosquito breeding as demonstrated by their high larval densities (density range = 6.45- 2.22) in the different villages. In all the villages, the period between mid-December and February was characterized with low rainfall amounts (dry season) and overall low mean anopheline larval densit. During these months, most of these breeding habitats dried up and larval collection only occurred in the relatively stable habitats such as the marshy areas, paddies and canals (Table 3.5).

In the periods during and immediately after setting of rainfall, there was an increase in larval densities within the non-irrigated habitats in Mwea rice irrigation scheme (Table 3.5). Although rainfall was not significantly correlated with larval density variation in Mwea rice irrigation scheme ($r = 0.177$, $p > 0.05$) it was important in explaining the productivity of non-irrigated breeding habitats in the area.

Table 3.4: Figure: Seasonal variation of anopheline larval density in different breeding habitats of Mwea irrigation scheme

| | Larval density (larvae/10dips) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Kangichiri | | Karima | | Kiuria | | Rurumi | |
| Habitat type | Dry | Wet | Dry | Wet | Dry | Wet | Dry | Wet |
| Canal | 0.57 | 0.93 | 1.17 | 0.99 | 0.82 | 1.61 | 0.62 | 0.65 |
| Ditch | 2.36 | 0.59 | - | 1.40 | | 0.00 | 5.25 | 2.22 |
| Fishpond | - | 0.00 | - | - | 0.11 | 2.45 | - | - |
| Hoof print | - | - | - | 1.25 | - | - | - | - |
| Marsh | 0.83 | 2.08 | - | - | - | - | 0.62 | 1.27 |
| Paddy | 1.82 | 1.79 | 2.75 | 1.26 | 1.38 | 1.33 | 0.94 | 1.36 |
| Pit | 1.71 | 2.71 | - | 2.70 | 1.83 | 1.87 | 1.00 | 1.22 |
| Pool | 1.03 | 1.89 | - | 6.45 | 2.69 | 1.47 | 0.00 | 0.54 |
| Quarry | - | - | - | - | - | - | 1.60 | 1.82 |
| Tire track | 0.00 | 1.10 | 0.80 | 2.35 | | 1.72 | 0.00 | 0.40 |
| Total | 1.25 | 1.43 | 1.80 | 1.24 | 1.13 | 1.49 | 0.82 | 1.08 |

*Dash (-) = habitat absent, 0.00= not productive for larvae. Wet months (Mid Mar-July, Oct-Mid Dec), dry months (Mid Dec-Jan-Mid Mar July,- Sept, Dec)*

able 3.5: Temporal distribution of *Anopheles* mosquito aquatic stages in different habitat types Mwea rice irrigation scheme.

| Month | Rainfall | Canal | Ditch | Fishpond | Hoof print | Marsh | Paddy | Pit | Pool | Quarry | Tyre track |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Apr | 115.7 | 1.3 | 2.3 | 3.3 | 1.3 | 0.0 | 2.8 | 1.3 | 1.3 | 2.9 | 0.0 |
| May | 377.3 | 0.5 | 2.1 | 1.0 | 0.0 | 1.7 | 0.9 | 2.2 | 1.5 | 0.4 | 1.2 |
| June | 8.3 | 1.0 | 0.3 | 1.0 | - | 1.6 | 2.0 | 3.6 | 1.0 | 2.8 | 2.1 |
| July | 6.6 | 1.3 | 0.1 | 0.0 | - | 0.7 | 1.7 | 1.2 | 1.0 | 3.4 | 1.0 |
| Aug | 5.7 | 1.0 | 3.3 | 0.0 | - | 1.2 | 1.4 | 3.0 | 1.7 | 2.1 | 0.0 |
| Sep | 5.4 | 1.0 | 0.3 | 0.0 | - | 0.6 | 1.7 | 0.0 | 1.9 | 2.6 | 0.0 |
| Oct | 61.9 | 1.0 | 0.0 | 0.2 | - | 1.4 | 1.1 | 0.0 | 2.2 | 1.4 | 0.0 |
| Nov | 55.3 | 1.5 | 0.0 | 0.4 | 0.0 | 1.4 | 0.8 | 0.0 | 1.4 | 2.0 | 0.4 |
| Dec | 4.5 | 0.7 | 4.5 | 0.1 | - | 0.6 | 0.8 | 1.0 | 0.0 | 1.2 | 0.8 |
| Jan | 14.0 | 0.9 | 0.0 | 0.0 | - | 0.3 | 1.4 | 0.0 | 0.0 | 0.4 | 0.7 |
| Feb | 5.3 | 0.8 | 0.0 | 0.0 | - | 2.3 | 2.8 | 0.0 | 0.0 | 0.0 | 0.0 |
| Mar | 152.2 | 1.6 | 1.0 | 7.5 | 0.0 | 8.3 | 2.9 | 0.2 | 1.8 | 1.3 | 0.6 |
| Apr | 215.7 | 0.9 | 1.5 | 5.5 | 0.0 | 2.5 | 2.2 | 1.4 | 1.5 | 1.3 | 0.2 |
| May | 174.8 | 1.6 | 2.5 | 1.8 | - | 1.2 | 1.6 | 2.0 | 4.3 | 2.9 | 2.3 |
| June | 13.4 | 1.1 | 0.0 | 1.3 | - | 0.3 | 1.6 | 0.0 | 2.0 | 1.3 | 0.0 |
| July | 12.3 | 0.9 | 0.0 | 4.3 | - | 0.8 | 1.2 | 1.8 | 1.8 | 1.1 | 6.0 |
| Aug | 12.6 | 0.6 | 0.0 | 0.0 | - | 0.2 | 1.1 | 2.5 | 0.6 | 1.4 | 0.0 |
| Sep | 13.3 | 0.9 | 0.0 | 0.5 | - | 0.4 | 3.0 | 0.5 | 3.3 | 2.1 | 0.0 |
| Oct | 110.6 | 0.6 | 0.0 | 0.0 | - | 1.3 | 1.2 | 0.0 | 0.5 | 2.3 | 1.3 |
| Nov | 413.1 | 0.7 | 0.7 | 0.0 | - | 0.7 | 0.7 | 2.2 | 2.7 | 0.9 | 1.9 |

*Dash (-) = habitat absent, 0.0 = habitat not productiv, density= larve/10 dips*

### 3.4.3. Association between larval abundance and rice growth cycle

Figure 3.1 shows the anopheline larval and pupal density variation in relation to different developmental stages of the rice plant. Significantly higher densities of anopheline larvae were recorded during the tillering (2.42 ± 0.13), transplanting (2.25 ± 0.29) and nurseries (2.16 ± 0.40) stages of rice development than in the other rice stages ($F = 2.452$, $df = 11,132$, $p < 0.05$). Larval density was lowest during the rice maturation stages. The highest and lowest larval density during the development of the secondary (ratoon) crop was recorded in the harvesting (1.33 ± 0.14) and tillering (1.03 ± 0.41) stages respectively.

The pupae productivity varied significantly ($F= 2.067$, $df = 11,132$, $p < 0.05$) during the different rice development stages. An ANOVA and Tukey's HSD test of significance for the pupal stages indicated that fallow land (0.16 ± 0.06), nurseries (0.18 ± 0.10) and rice transplanting ( 0.16 ± 0.05) stage had the highest densities of pupae and lowest pupal production during harvesting (0.01 ± 0.0020 and flowering (0.02 ± 0.003) stages (Fig. 3.1). During ratoon crop growth, the anopheline pupal productivity was generally low with the peak density occurring during the tillering stages (0.1 ± 0.04) and few or no pupal during the ratoon harvesting stages. The apparent heterogeneity in mosquito productivity exhibited within the paddies during rice development emphasizes the need of habitat-based interventions measures targeting the early growth stages of rice when the habitat is highly productive.

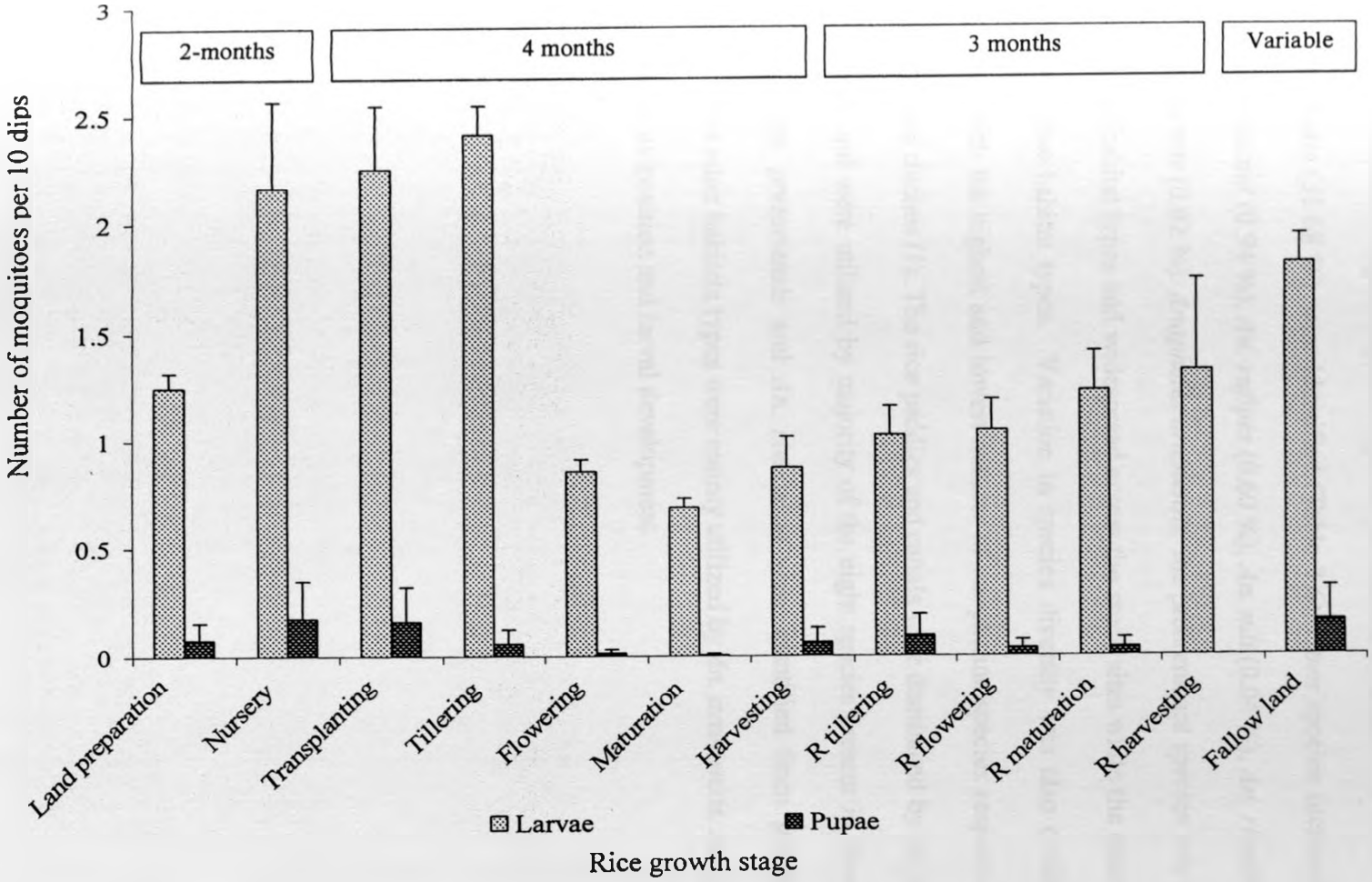



Figure 3.1: Temporal distribution *Anopheles* mosquito aquatic stages in relation to rice growth stages in the irrigated rice paddies of Mwea, Central Kenya.

### 3.4.4. Species diversity and abundance of anopheline larvae

Some 34.4% (n = 4,993) of the total anopheline larvae collected were identified to species by morphological characters. Eight *Anopheles* species dominated by *Anopheles arabiensis* (68.73%) and *An. pharoensis* (31.68 %) were identified (Table 3.6). Other species included *An. funestus* (1.96 %), *An. coustani* (0.94 %), *An. rufipes* (0.60 %), *An. nili* (0.04 %), *An. rivolurum* (0.02 %), and *An. pretoriensis* (0.02 %). *Anopheles arabiensis,* the predominant species was represented in a wide range of habitat types and widespread across the study sites while the other species were restricted to a few habitat types. Variation in species diversity was also evident among the habitats types with the highest and lowest number of anopheline species respectively occurring in paddies (7) and ditches (1). The rice paddies and canals were dominated by *An. arabiensis* and *An. pharoensis* and were utilized by majority of the eight species present in Mwea. The single specimens of *An. pretoriensis* and *An. rivolurum* were identified from paddies and canals respectively. The other habitats types were mainly utilized by *An. arabiensis, An. pharoensis* and *An. funestus* for oviposition and larval development.

*Table 3.6: Species distribution of anopheline larvae in diverse breeding habitats types in the Mwea rice irrigated agro-ecosystem*

| Habitat Type | *An. arabiensis* | *An. funestus* | *An. pharoensis* | *An. coustani* | *An. rufipes* | *An. nili* | *An. rivolurum* | *An. pretoriensis* | Total |
|---|---|---|---|---|---|---|---|---|---|
| Paddy | 2242 | 42 | 1300 | 32 | 24 | 1 | 0 | 1 | 3642 |
| Canal | 567 | 24 | 244 | 13 | 4 | 1 | 1 | 0 | 854 |
| Pool | 146 | 20 | 4 | 2 | 1 | 0 | 0 | 0 | 173 |
| Pit | 100 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 107 |
| Tire track | 81 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 87 |
| Marsh | 52 | 6 | 11 | 0 | 1 | 0 | 0 | 0 | 70 |
| Quarry | 16 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 29 |
| Seep | 12 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 14 |
| Fishpond | 12 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 13 |
| Ditch | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Hoof print | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 3232 | 98 | 1582 | 47 | 30 | 2 | 1 | 1 | 4993 |

### 3.4.5. Association between larval densities and habitat distance from human habitation (village)

The density of anopheline pre-adult stages was inversely correlated to the distance between breeding habitats and the nearest human habitation. The r values for early and late instars and pupal stages ranged between 0.57, 0.66 and 0.59 respectively ($p = 0.05$). A significantly higher density of *Anopheles* pre-adult stages were recorded from the larval habitats that were close to human habitation (Fig.3.2). Majority of the species had a higher proportion of the larvae and pupae identified from the habitats within the first 200 m from human habitation. The numbers of all immature stages identified decreased gradually as the distance from human dwellings increased but the counts for *An. pharoensis* showed little change with distance after the first 200 m (Table 3.7).

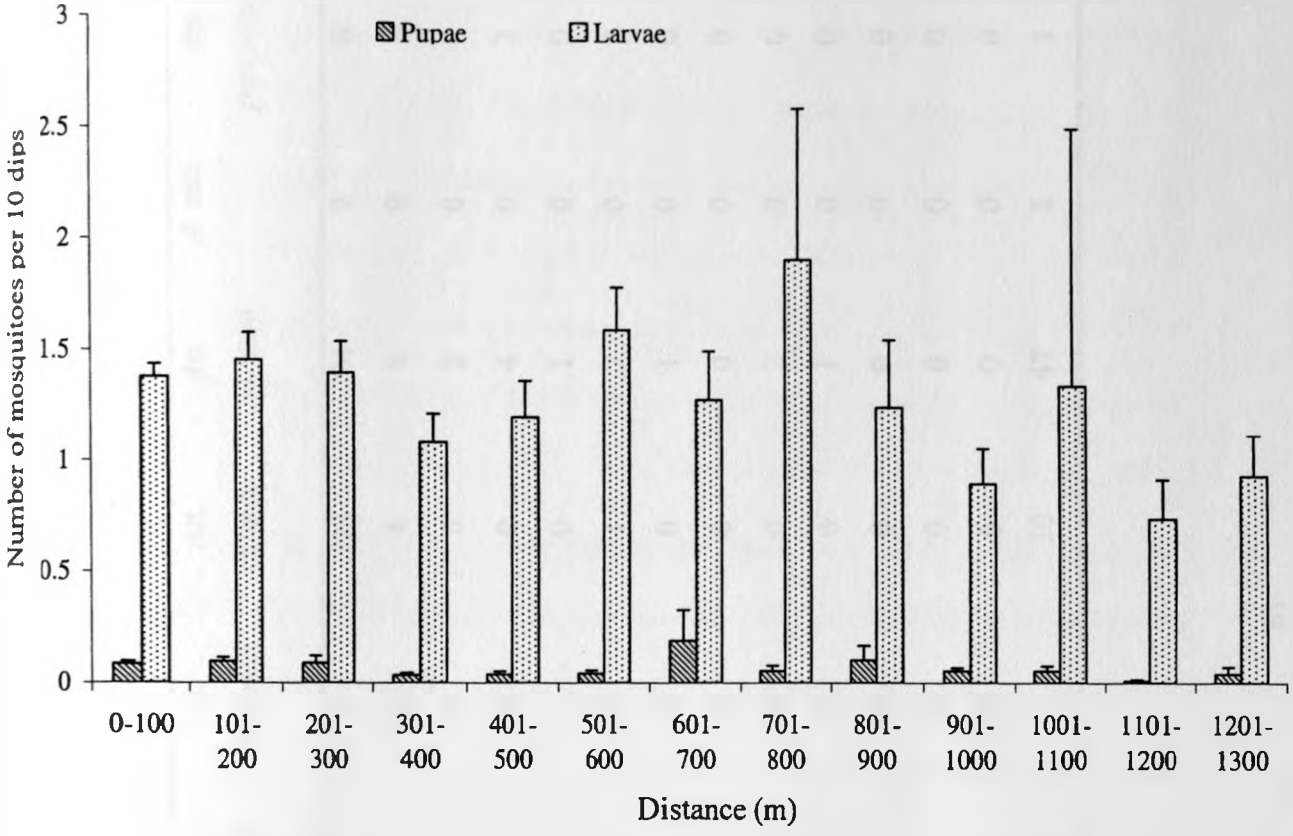



Figure 3.2: Anopheline mosquito pre-adult stages density variation in relation to habitat distance from the nearest human habitation.

Table 3.7: Anopheline species distribution relative to the distance from the nearest human habitation in Mwea.

| Distance Range (M) | *An. arabiensis* | *An. funestus* | *An. pharoensis* | *An. rivolurum* | *An. rufipes* | *An. coustani* | *A. nili* | *An. pretoriensis* | Total Number identified |
|---|---|---|---|---|---|---|---|---|---|
| 0-100 | 1701 | 56 | 556 | 1 | 25 | 24 | 2 | 0 | 2365 |
| 101-200 | 864 | 16 | 260 | 0 | 4 | 8 | 0 | 0 | 1152 |
| 201-300 | 218 | 7 | 63 | 0 | 0 | 3 | 0 | 0 | 291 |
| 301-400 | 48 | 7 | 92 | 0 | 0 | 4 | 0 | 1 | 152 |
| 401-500 | 74 | 2 | 98 | 0 | 0 | 1 | 0 | 0 | 175 |
| 501-600 | 51 | 6 | 59 | 0 | 1 | 3 | 0 | 0 | 120 |
| 601-700 | 49 | 3 | 53 | 0 | 0 | 1 | 0 | 0 | 106 |
| 701-800 | 17 | 0 | 92 | 0 | 0 | 0 | 0 | 0 | 109 |
| 801-900 | 34 | 0 | 62 | 0 | 0 | 2 | 0 | 0 | 98 |
| 901-1000 | 63 | 0 | 79 | 0 | 0 | 1 | 0 | 0 | 143 |
| 1001-1100 | 31 | 0 | 74 | 0 | 0 | 0 | 0 | 0 | 105 |
| 1101-1200 | 8 | 1 | 49 | 0 | 0 | 0 | 0 | 0 | 58 |
| 1201-1300 | 74 | 0 | 45 | 0 | 0 | 0 | 0 | 0 | 119 |
| **Total** | 3232 | 98 | 1582 | 1 | 30 | 47 | 2 | 1 | 4993 |

## 3.5. Discussion

The study has demonstrated the importance of diverse larval habitats types that are formed within the irrigated agro-ecosystems during the year. Prior information and correct understanding of vector mosquito abundance and distribution patterns are key elements for successful larval control interventions. Hence, in cases where habitat based (targeted) interventions are to be implemented the importance of the variation in mosquito productivity among different breeding habitats can only be re-emphasized. Since the distribution and abundance of the mosquito larvae reflects the oviposition preference of adult females and the ability of pre-adult stages to tolerate conditions that prevail in the aquatic habitats (Reisen *et al.*, 1981), understanding the role played by various habitat forms in supporting larval development is essential in the implementation of larval control measures in a given region. In the present study the mosquito breeding habitats within the irrigated systems were shown to play an important role of providing suitable breeding grounds for the anopheline species as espoused by the high larval densities in them in the absence of the transient peridomestic habitats that record peak larval densities in the wet period of the year. Due to the ease of identification and the expansive water surfaces provided by the irrigated systems related breeding habitats that are ideal for anopheline mosquito breeding, there is a risk of ignoring the potentially highly productive transient habitats found within human habitation during larval control intervention periods in the irrigated agro-ecosystems.

Species distribution within the irrigated agro-village complexes was diverse with the eight identified anopheline species presence in the villages and habitat forms varying. Most of the eight identified species in the study villages (Table 3.6) have been documented in the area as adults (Mukiama and Mwangi 1989; Ijumba *et al.*, 1990; Muturi *et al.*, 2006) except *An.*

*rivolurum* Leeson. This species was identified among the larvae that colonized water canals and is a sub-species in the *An. funestus* complex. Its' population could be high especially among the adult population where it could have been identified as *An. funestus,* mosquito species known to breed in irrigated ecosystems and represented in large numbers both in the irrigated and the non-irrigated habitat types. The dominant species however, were *An. arabiensis* and *An. pharoensis* being represented in large numbers in both peridomestic and the irrigated systems habitat types in all the villages. *Anopheles arabiensis* outnumbered all the other species both in the non-irrigated habitats and rice fields although within the quarries its number almost matched those identified as *An. pharoensis*. The dominance of the two species within the habitats forms is indicative of the malaria risk the communities in this region face. Although *An. arabiensis* has zoophilic tendencies, it poses the single most danger together with *An. funestus* in terms of malaria transmission in irrigated regions (Ijumba *et al.*, 1990; Klinkenberg *et al.*, 2003; Mutero *et al.*, 2004b; 2004c; Muriu *et al* 2008). On the other hand, *An. pharoensis*, an important malaria vector in some African countries like Egypt (Gillies and Coetzee 1987; Gillies and de Meillon, 1968) was found in relatively high numbers in the rice paddies and canals compared with other non-irrigated habitats. In previous studies of adult population in the area (Ijumba *et al.*, 1990; Mwangi and Mukiama 1992) the species had been shown to surpass that of *An. arabiensis* although it has not been implicated in malaria transmission in the region. This observation and also its uniform distribution in all distances shows that the species is important in colonizing the various habitats in the area and would form an important target for control as it has been noted to have high human-biting rates in the area particularly during the rainy season (Ijumba *et al.*, 1990). Two species, *An. funestus* and *An. coustani* formed an intermediate population group whose utilization of both the non-irrigated habitats and rice field breeding habitats was moderate

and well distributed. However, *An. nili* and *An. pretoriensis* were found colonizing canals and paddies in the peridomestic habitats and irrigated rice fields' habitats respectively but have been documented as important in malaria transmission in other parts of Africa (Gillies and de Meillon 1968; Shililu *et al.*, 2003a) hence important in targeted interventions.

Seasonally, the marshy areas, paddies and canals were the most important mosquito breeding habitats while other habitats provided focal breeding habitats in relation to blood meal sources during the wet periods of the year. The observed spatial heterogeneity in the larval densities can be attributed to the amount of rainfall and the irrigated rice growth pattern in the different villages. Within the peridomestic habitats, seasonal changes in the larval population followed the rainfall pattern. Non-irrigated habitats increased in number, stability and productivity for anopheline larvae during the rainy periods registering peak population during or shortly after prolonged precipitation. However, some non-irrigated larval habitats within the villages such as ditches, fishpond and marshes persisted throughout the dry season providing ideal vector breeding habitats. These habitats mostly harboured *An. arabiensis*, the major malaria vectors in the area (Mukiama and Mwangi 1989; Ijumba *et al.*, 1990) emphasizing their peculiar importance and contribution to malaria risk in the area. It is noted from the study that although these non-irrigated habitats were present and productive only for a few months during the year their overall productivity was higher than that of the expansive habitats provided by the rice paddies and associated drainage channels. The observation corroborates other results showing that the malaria vectors tend to utilize the small and transient water pools (Service, 1977) and the species only serves as a pioneer species in the irrigated rice fields (Ijumba , 1997). Thus, in irrigated agro-village complexes a fundamental difference in productivity of breeding habitats is

realized during the year with a small but significant important part of anopheline population breeding in non-irrigated habitats formed during the rainy seasonin close proximity to human settlements. In most of sub-Saharan Africa stable malaria transmission is due to the climatic conditions that are ideal for transmission and coincide with the ranges of *An. gambiae, An. arabiensis* and *An. funestus* the most efficient vectors of malaria in the world (Craig *et al.*, 1999; Beier *et al.*, 1999). From the current study the diverse habitats and the rice fields serve well to ensure availability of breeding habitats for the malaria vectors throughout the year near and farther a field from the village perimeter. Hence, mosquito breeding habitats are heterogeneous and larvicides application and environmental management would substantially reduce larval habitats within the vicinity of human dwellings and/or increases the time spent by the mosquito vectors searching for suitable aquatic habitat.

Our study has demonstrated the importance of the cultivated surfaces of the rice fields in relation to the anopheline mosquito pre-adult density variation with the irrigated rice plant development cycle. Within the cultivated irrigated systems, the pre-adult density tracked the rice plant growth patterns as this determined the presence or absence of open water surface suitable for *Anopheles* mosquitoes breeding. The population density of the aquatic stages was greater during the transplanting and tillering growth stages of the rice plant in both the primary and secondary (Ratoon) crop cultivation but generally low during the flowering and maturity stages of the crop. The increase in density during the transplanting and tillering stages as has been shown in other studies (Mutero *et al.*, 2000; Mwangangi *et al.*, 2006b) can be attributed to the application of sulfate of ammonia fertilizers in the cultivated rice fields. The high densities observed in the nursery seedbeds and fallow land is attributed to habitat stability in the small nurseries compared

to the expansive unstable flooded paddies where water is drained more often. In fallow land, small pockets of water ideal for mosquito breeding are formed by either grazing animals and other human activities as water seepage may occur from the canals and rainfall received during such periods. Hence it is essential during larval control interventions in the irrigated fields to target nursery seedbeds and other stages associated with high anopheline larval density. This would result in great reduction of resultant adult population that would be found in rice land agro-villages since intervention efforts are more efficient when more productive breeding habitats and periods are targeted (Gu and Novak, 2005). The ratoon tillering stage is characterized by high decomposition rate of the rice straws of the primary crop and high organic pollution unsuitable for anopheline mosquito breeding but as shown by Lawler and Dritz (2006) favour production of culicine mosquitoes. In some cases, after the harvesting of either primary or ratoon crop, rice straws were removed and the land was left fallow for a variable period of time depending on irrigation water availability. During such occasions grazing of animals would occur creating conducive small pockets of water within paddies and thus the fallow land (1.81±0.13) was noted of having a large population of anopheline larvae.

The habitats in the irrigated villages demonstrated density variation within them over distance where the malaria vector larval population was highly aggregated in habitats that were close to human habitation. This aggregation is observed despite the availability of habitats with similar condition over the extensive irrigated areas corroborating similar trends that were witnessed in other studies (Charlwood *et al.*, 1987; Takken *et al.*, 1998). Such studies has, shown the dispersal range of the malaria vectors to be less than 1km explaining why in our studies the densities declined as one progressed towards the one kilometer range. This gives an indication of how

malaria transmission trends may follow since the importance of host and larval habitat availability as determinants of malaria transmission intensity and distribution has long been recognized and discussed lucidly in qualitative terms (Kitron and Spielman 1989; Service 1991; Service 1997). Effective malaria transmission heavily depends on vector-human contact rate which in turn is highly influenced by the distance between the mosquito breeding habitats and the location of human habitation (Carter *et al*, 2000) and this distance may be large in irrigated rice-cultivated agro-villages. From the current study, the highest number of pre-adult stages was recorded within the 200m-distance range from villages where ready source of blood meal was available and hence the gravid females utilized habitats that were within their proximal range to oviposit eggs. However, though the densities of anopheline mosquitoes declined as one progressed away from the villages, a substantial number of the vector population was recorded indicating that females were able to fly across the distance to those far range habitats to breed and hence the residents of these areas remained vulnerable to mosquito related diseases. This is essential since as shown by Carter, *et al.*, (2000) the productivity of the breeding habitats and the effective dispersal range of the malaria vectors determines the dimensions of malaria transmission in a region. In the current study, the number of *An. arabiensis* and *An. pharoensis* identified from the habitats located up to 1km range was high remaining well within the maximum effective dispersal range (2km) of malaria vectors breeding habitats as was shown by Carter *et al.*, (2000). The understanding of the anopheline pre-adult stages ecology in different environments is important due to their potential as a target for malaria vector control. In previous studies (Minakawa *et al.*, 1999; Shililu *et al.*, 2003a, 2003b; Zhou *et al.*, 2004b; Fillinger and Lindsay 2006) elimination and treatment of larval habitats have registered dramatic effects on the vector population and level of malaria transmission. The current study has shown that in

irrigated ecosystems the vectors breed over a wide area though showing density clustering in areas close to the aggregated human habitation. Hence, in the event of implementing habitat based larval control measures, focusing on habitats within and close to human habitation is critical but those far beyond this aggregated areas are important in vector productivity and may act to reseed the environment with the vectors if they are not included in the control programs. However, it would be more crucial to study the contributions of each of these habitats to the adult mosquito population that actually seek blood meals from the human habitation.

The guiding principle of integrated malaria control in any area is to tailor interventions to the local entomological and epidemiological characteristics (Najera, 1999). One of the key local determinants of malaria transmission is abundance, distribution and adult productivity of larval habitats (Carter *et al.*, 2000) which re-emphasizes the importance of a prior knowledge and understanding of information regarding aquatic larval habitats productivity that would help in characterizing species specific oviposition site selection and planning of integrated mosquito management (Gu and Novak, 2005; Jacob *et al.*, 2007). In the current study the agronomic activities has also been show to have an impact on habiats productivity and thus synchronizing larviciding and other larval control activities would enhance the success of the intervention measures. As acknowledged by Fillinger and Lindsay (2006), successful larviciding would need vigorous mapping and complete coverage of aquatic habitats and subsequent monitoring. This would greatly help in the designing of control programs that are broadly acceptable in suppressing malaria vectors especially in tropical Africa where such interventions are complicated by the complexities of breeding habitats and the uncertainties of larval ecology (Killeen *et al.*, 2006). Hence our current results provide crucial baseline information necessary

for planning and implementation of malaria vectors larviciding activities in rural Africa especially in irrigated agro-ecosystems. For such measures to succeed in alleviating malaria incidence and intensity, the prolific habitats within and outside human settlement should be targeted. To be potentially effective, the timing of the intervention should be specific and habitat based especially in the transient habitats and the fallow land, saturated with rainwater during the rainy periods as well as the early stages of rice development and more importantly the rice nursery seedbeds.

## CHAPTER FOUR:

## 4.0 Population Dynamics and Distribution of Adult Anopheline Mosquitoes in relation to rice cultivation in the Mwea irrigated rice Agro-ecosystem


### ABSTRACT

Studies were conducted in four irrigated rice agro-village complexes in Mwea rice irrigation Scheme to determine the spatial-temporal dynamics of the malaria vectors between March 2005 and November 2006. A total of 344,891 adult anopheline mosquitoes belonging to nine species were collected indoor (5 species) and outdoor (9 species) resting sites. In both resting places, *Anopheles arabiensis* Patton was the predominant species constituting 85.4% (294,521) of the total anopheline collected. Other species collected included *An. pharoensis* Theobald (8.4%), *An. coustani* Laveran (3.1%), *An. funestus* Giles (3.0%), *An. nili* Theobald (0.03%), *An. pretoriensis* Theobald (0.01%), *An. maculipalpis* Giles (0.01), *An. rufipes* Gough (0.001%) and *An. moucheti* Evans (0.001%). A high proportion of *An. arabiensis* (79.9 %), *An. funestus* (52.2 %) and *An. nili* (53.2 %) were collected resting indoors as opposed to 99.4 - 100 % outdoor collections observed in other species. Seasonally, *An. arabiensis* had a large peak in September and a small one in May of every year with the lowest density occurring in the month of December. *Anopheles funestus* attained peak and lowest adult densities in the months December and September respectively. Time lag cross-correlation analysis did not reveal a strong association between adult malaria vector density and rainfall in Mwea, but closely followed the rice cultivation regime with high densities during early growth stages and maturity period of rice for *An. arabiensis* and *An. funestus* respectively.

## 4.1. Introduction

The devastating situation of malaria in sub-Saharan Africa has been blamed to a large extent on the increasing drug resistance (Breman *et al.*, 2004) and lack of a vaccine, calling for an integrated malaria control approach based on sound understanding of the mosquito ecology and transmission dynamics (Utzinger *et al.*, 2002). The tropical African climate is further favourable to a wide range of vector borne diseases including malaria with the continent also hosting a high diversity of vector species complexes that can potentially redistribute themselves in new locations driven by climate change and environmental manipulation by man. The suppression of malaria transmission over large areas depends upon population exposure of the vector species to the intervention measures and this in turn depends upon the level of coverage within the human community. Several species of malaria vectors thrive in irrigated agro-ecosystems (Carnevale *et al.*, 1999; Muturi *et al.*, 2006) where the flooded paddy fields provide ideal breeding habitats for the principal malaria vectors in Africa especially members of the *Anopheles gambiae* complexes.

Vector abundance is an important indicator of malaria transmission force (Garrett-Jones 1964) and hence, factors that increase or decrease vector abundance will have a profound impact on the prevalence of the disease. It is well known that malaria infections are not distributed homogenously, with some areas or some households within the same area showing higher malaria incidence than others (Carter *et al.*, 2000; Brooker *et al.*, 2004), which has been associated with the population ecology of the vector species. Several factors are associated with increased vector population density and species diversity with consequent malaria risk in a region. The distance from larval habitats to human dwellings has been associated with vector abundance in a house and therefore, with malaria risks (Var der Hoek *et al.*, 1998; Minakawa *et*

*al.*, 2002; Cano *et al.*, 2006; Zhou *et al.*, 2007). In irrigated ricelands agro-ecosystems, water management and application of ammonium sulphate fertilizers in rice fields has been shown to have a profound effect on the population dynamics of *Anopheles* mosquito larval stages (Mutero *et al.*, 2000; Mutero *et al.*, 2004a) which would be consequently expected to result in higher density of the adult population of the diverse vector species in such areas. All these factors have an influence on the implementation and success of vector control interventions in any region.

In integrated vector management, the success of control measures targeting the adult populations such as indoor residual spraying and insecticide treated bednets or curtains (Mnzava *et al.*, 1993; Curtis *et al.*, 1994; Roberts *et al.*, 2000; Lengeler, 2001) has been documented with extremely important results on the reduction of mortality and morbidity on human populations. However, truly integrated interventions need to involve measures against the larval populations which have also been shown to yield exceptionally high successes particularly during the eradication of *An. gambiae* from Brazil (Soper and Wilson, 1943) and Egypt (Shousha, 1948) as well as in USA and Europe (Kitron and Spielman, 1989; Hays *et al*2000). Mosquito larviciding and source reduction has proven crucial in malaria control efforts by eliminating the vectors before they disperse and transmit disease (Killeen *et al.*, 2002a). However, not all malaria vectors can necessarily be considered feasible targets for larvicidal control (Gratz and Pal, 1988). Thus, identifying the vector species in locality and their distribution and densities by methods of collection in place and time is vital in planning for successful control programs.

The current study sought to understand the population dynamics of the various malaria vectors present in Mwea rice irrigation scheme in relation to irrigated rice cultivation activities. The

information gathered has relevance in the development and implementation of integrated vector management strategies based on the productivity and variability of *Anopheles* mosquito adult populations. The principle objective of the study was to provide current information on anopheline mosquito diversity within the irrigated rice land agro-ecosystem prior to implementation of large-scale microbial larviciding in the area.

## 4.2. Materials and Methods

### 4.2.1. Adult mosquito sampling

Adult anopheline mosquitoes were sampled from the four study villages namely Kangichiri, Karima, Kiuria and Rurumi between March 2005 and November 2006 using two sampling methods to cater for indoor and outdoor resting vector populations. Indoor resting mosquito populations were sampled on weekly basis between 0700hrs-1130hrs using the Pyrethrum spray catch (PSC) method (WHO, 1975) in 20 randomly selected houses in each of the four study villages. The selected houses were evenly distributed in the central and peripheral localities of the villages and were assigned unique codes for identification and follow up during the entire sampling period. Houses were only substituted with a nearby one with similar characteristics when circumstances dictated. The household characterization was conducted in the field recording the number of people who slept in the house the night before sampling, presence of animals, distance of house to nearest larval habitat and animal shed, house roofing and wall type, eaves sizes and the usage of treated/non-treated bednets. During mosquito sampling, all rooms of each house were covered with white sheets spread on the floor for mosquito collection after ensuring all food material and utensils were well covered or removed from the house. The house was then sprayed using 0.3 % pyrethrin in water and left for 10-20 minutes to allow the knockdown effect to take place. All knocked-down female mosquitoes from each house were collected in individually labeled petri dishes fitted with an absorbent paper towel and transferred to the laboratory for further processing while the males were counted and identified to genus before discarding them.

Concurrent with indoor collections, six rechargeable-battery operated miniature CDC-light traps (J.W. Hock Ltd, Gainesville FL, USA) were operated nightly for 12h (1800hrs-0600hr) within each village twice every week to collect outdoor resting mosquitoes. The light traps were fitted with 6V 10A fluorescent bulbs and distributed evenly throughout the village to represent the central and peripheral sites of the village.The light traps were hang about 1.5m above the ground within the homestead, 10m from the nearest house and/or cowshed. The light traps were accorded identification codes and set at the same location for the entire sampling period only being substituted for another site when occasions dictated. The samples collected were then transferred to the laboratory for sorting and identification.

### 4.2.2. Laboratory processing of mosquito samples

In the laboratory, the contents of each light trap were killed either by freezing or suffocating them with chloroform and then all mosquitoes separated from other insects. The mosquito specimens collected by the two methods (PSC/light traps) were further categorized into either anopheline or culicine species. Anopheline mosquito specimens were identified morphologically to species using key by Gillies and de Meillon (1968) and sorted according to gonotrophic stages. These females' anopheline specimens were then counted, recorded and preserved in a silica-gel desiccator for use in blood meal source and sporozoite rate analysis (Chapter 6 and 7).

## 4.3. Data analysis

Data collected were categorized and recorded in terms of seasons and villages in Microsoft excel spreadsheets and analyzed using the SPSS software. ANOVA test were conducted on the data to

st spatial and temporal variation in anopheline mosquito population in the study villages. Cross orrelations with different time lags were used to compare the relationship between rainfall and nosquito density. The anopheline mosquito densities (indoor and outdoors) were calculated as ne number of female mosquitoes per house or light trap per week. Mean monthly densities for oth light trap and PSC collections were calculated by dividing the total number per month by he number of weekly collections made. The mean monthly mosquito catches per village were dded to give the total mean monthly catch for the whole study area.

## 4.4. Results

### 4.4.1. Vector species composition and abundance

During the study period, a total of 344,891 adult malaria vectors belonging to nine *Anopheles* species were collected. Of these, 240,986 mosquitoes comprising of five *Anopheles* species were collected resting indoors while a further 103, 905 mosquitoes composed of nine *Anopheles* species were collected from outdoor resting locations. The relative abundance of the *Anopheles* mosquitoes collected by the two methods in the four study villages are shown on Table 4.1. *Anopheles arabiensis*, the only member of the *An. gambiae* complex in Mwea according to previous studies in the area (Mukiama and Mwangi 1990; Ijumba *et al.*, 1990) was the most abundant species in all the villages constituting 85.4% (294,521) of the total anopheline collected. Other species collected during the study in decreasing order included *An. pharoensis* (8.4%), *An. coustani* (3.1%), *An. funestus* (3.0%), *An. nili* (0.03%), *An. pretoriensis* (0.01%), *An maculipalpis* (0.01), *An. rufipes* (0.001%) *and An. moucheti* (0.001%). Kangichiri village had the highest diversity of *Anopheline* species. Of the 9 *Anopheles* species sampled, 9 were found in Kangichiri, 8 each in Karima and Kiuria, and 7 species in Rurumi. In all the villages, only five species (all similar) were collected resting indoors while the number of species collected outdoors varied with village (Table 4.1).

A binomial dispersion analysis model was fitted onto the adult anopheline mosquito data to determine the variables that would help in predicting the density of adults collected resting inside houses. In this general model, the distance to nearest breeding habitat, presence or absence of

treated bed-net, presence or absence of domestic animals and month of the year were shown to be useful predictors of anopheline mosquito adult densities.

Table 4.1: Relative proportions of *Anopheles* mosquito species collected in four agro-village complexes in the Mwea Rice Irrigation Scheme, April 2005- November 2006.

| | Karima | | Kiuria | | Rurumi | | Kangichiri | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Outdoor | Indoor | Outdoor | Indoor | Outdoor | Indoor | Outdoor | indoor | |
| *An. arabiensis* | 42.5 (14,012) | 97.0 (90,038) | 64.8 (12,280) | 98.5 (42,056) | 30.3 (5,268) | 97.7 (50,514) | 80.2 (27,696) | 98.0 (52,657) | 85.4(294,521) |
| *An. funestus* | 6.6 (2,172) | 2.9 (2,717) | 4.4 (827) | 1.5 (625) | 3.4 (589 ) | 2.3 (1,187) | 4.1 (1,429) | 1.8 (959) | 3.0 (10,505) |
| *An. pharoensis* | 37.3 (12,314) | 0.1 (75) | 24.3 (4,608) | 0.03 (12 ) | 46.9 (8,164) | 0.002 (1) | 10.5 (3,638) | 0.2 (83) | 8.4 (28,895) |
| *An. coustani* | 13.6 (4,473) | 0.003 (3) | 6.5 (1,229) | 0.01 (2 ) | 19.4 (3,382) | 0.004 (2) | 4.9 (1,692) | 0.01 (5 ) | 3.1 (10,788) |
| *An. nili* | 0.03 (9 ) | 0.01 (11) | 0.04 (7 ) | 0.01 (5) | 0 | 0.02 (12) | 0.08 (28) | 0.04 (22) | 0.03 (94) |
| *An. pretoriensis* | 0.01 (4) | 0 | 0.03 (5) | 0 | 0.02 (3) | 0 | 0.1 (39) | 0 | 0.01 (51) |
| *An. maculipalpis* | 0.02 (7) | 0 | 0.01 (1) | 0 | 0.02 (4) | 0 | 0.06 (19) | 0 | 0.01 (31) |
| *An. rufipes* | 0.003 (1) | 0 | 0.01 (1) | 0 | 0 | 0 | 0.01 (2) | 0 | 0.001 (4) |
| *An. moucheti* | 0 | 0 | 0 | 0 | 0.01 (1) | 0 | 0.003 (1) | 0 | 0.001 (2) |
| **Total** | **32,992** | **92,844** | **18,958** | **42,700** | **17,411** | **51,716** | **34,544** | **53,726** | **344,891** |

***the number in parentheses indicates the sample size***

### 4.4.2. Spatial density variation of *Anopheles* species

A two way ANOVA test indicated that the density of the malaria vectors differed significantly ($F= 8.18$, $df_{7, 160}$, $p<0.05$) among the four villages with a 2.4-fold higher mean mosquito numbers recorded from indoor resting sites (39.22 ± 2.7) compared to outdoor resting locations (16.22 ± 2.5). Significantly higher densities of *Anopheles arabiensis* were collected from houses with a geometrical mean of 41.45 ± 3.36 *An. arabiensis* per house per night, than the 27. 15 ± 3.08 *An. arabiensis* per outdoor site per night in all the villages ($t = 2.97$ $df_{1, 166}$, $p = 0.003$). Comparatively higher densities of *An. arabiensis* were captured both outdoors and indoors in Kangichiri and Karima villages than those of Kiuira and Rurumi.

In contrast to *An. arabiensis* density, a significant majority of *An. pharoensis*, ($F = 17.48$, $df_{7, 138}$ $p < 0.05$), the second most abundant species in the area were principally collected resting outdoors as opposed to resting inside houses in all the villages. Site to site density variation indicated a 2-fold lower density in Kiuria and Kangichiri than Karima and Rurumi outdoor collections.

A significant majority of *An. funestus*, an important malaria vector in the area were captured resting outdoors ($F = 9.18$, $df_{7, 151}$, $p < .0.05$) than indoors in all the villages. A one-way ANOVA and Tukey's honest test of significance indicated a significantly higher density ($F = 3.93$, $df_{3, 80}$ $p = 0.01$) of outdoor collected *An. funestus* samples in Kangichiri and Karima than other villages. A similar test for those captured resting inside houses did not show any significant difference among the villages ($F = 1.68$, $df_{3, 71}$, $p >0.05$).

Other anopheline species with site to site variation included *An. coustani* mainly occurring outdoors in Karima and Rurumi, *An. nili*, mainly captured in Kangichiri, while others with few samples such as *An. pretoriensis*, *An. maculipalpis*, *An. rufipes* and *An. moucheti* were mainly collected outside houses in selected villages only.

Table 4.2: Density mean numbers ± SE) variation of three important *Anopheles* mosquito species in the four study villages within Mwea rice irrigation scheme.

| | Location | Kangichiri | Karima | Kiuria | Rurumi |
|---|---|---|---|---|---|
| *biensis* | Outdoors | 46.98±6.17 | 26.88±6.17 | 22.87±6.17 | 11.87±6.17 |
| | Indoors | 53.61±8.94 | 49.13±5.00 | 28.89±6.17 | 34.17±6.17 |
| | Overall | 50.30±5.43 | 38.01±3.97 | 25.88±4.36 | 23.02±4.36 |
| *estus* | Outdoors | 4.91±0.57 | 6.25±0.57 | 3.35±0.57 | 3.41±0.57 |
| | Indoors | 2.45±0.92 | 1.95±0.49 | 1.07±0.59 | 1.79±0.58 |
| | Overall | 3.68±0.54 | 4.10±0.37 | 2.21±0.41 | 2.60±0.41 |
| *aroensis* | Outdoors | 8.01±1.96 | 23.26±1.96 | 10.22±1.96 | 17.53±1.96 |
| | Indoors | 6.33±6.35 | 0.43±1.67 | 0.27±2.32 | 0.32±2.25 |
| | Overall | 7.17±3.33 | 11.84±1.29 | 5.25±1.52 | 8.93±1.49 |
| ecies | Outdoors | 20.88±4.98 | 18.48±4.98 | 13.04±4.98 | 12.50±4.98 |
| | Indoors | 48.65±7.21 | 47.58±4.03 | 27.53±4.98 | 33.13±4.98 |
| | Overall | 34.77±4.38 | 33.03±3.20 | 20.28±3.52 | 22.81±3.52 |

### 4.4.3. Seasonal patterns of anopheline species

There was a significant seasonal variation ($F = 6.21$, $df_{11, 156}$, $p < 0.05$) in the density of the three major *Anopheles* species namely; *Anopheles arabiensis*, *An. pharoensis* and *An. funestus* (Table 4.2). All the villages were located within the planned irrigated rice agro-ecosystem and received similar amounts of rainfall, thus the *Anopheles* species collected in the different villages were pooled together and compared with the rainfall amount received over time.

*Anopheles arabiensis* recorded two peak adult densities, a large one in September and a small one in May of every year. The highest densities of *An. arabiensis* captured from indoor and outdoor resting places were recorded in the month of September in both years coinciding with early vegetative growth stages of rice as described in chapter 2.2.3. A second peak and smaller peak occurred in months of April-May of every year. In May 2006, the geometrical mean of indoor resting *An. arabiensis* (74.17) was significantly lower than that recorded in May 2005 (31.86) coincident with comparative reduction of rainfall during the long rains in the same period (Fig. 4.1). The months of August and October also recorded relatively high *An. arabiensis* densities, periods occasioned by nursery rice development and onset of short rains in the region respectively. The month of December which was characterized by low rainfall and low rice cultivation activities recorded the lowest *An. arabiensis* density.

*Anopheles funestus* with high mean numbers collected outdoors had significantly higher density among the outdoor resting population in December when paddies are drained but associated canals having low water volumes ideal for species breeding. In contrast to *An. arabiensis* density, *An. funestus* density was lowest in September of each year both outdoors and indoors

Fig. 4.2) with a small peak in July 2005 and May-June 2006. The density of *An. funestus* resting inside houses attained maximum value in July and progressively increased from October to attain a minor peak in December.

*Anopheles pharoensis*, mainly collected outdoors, recorded two peak periods in adult density. A minor peak occurred in the month of June during land preparation after gradual increase since April and a major peak between September and November period (Fig. 4.3) which is characterized by vegetative (early and late) rice growth stages and onset of short rains in the Mwea irrigated rice agro-ecosystem.

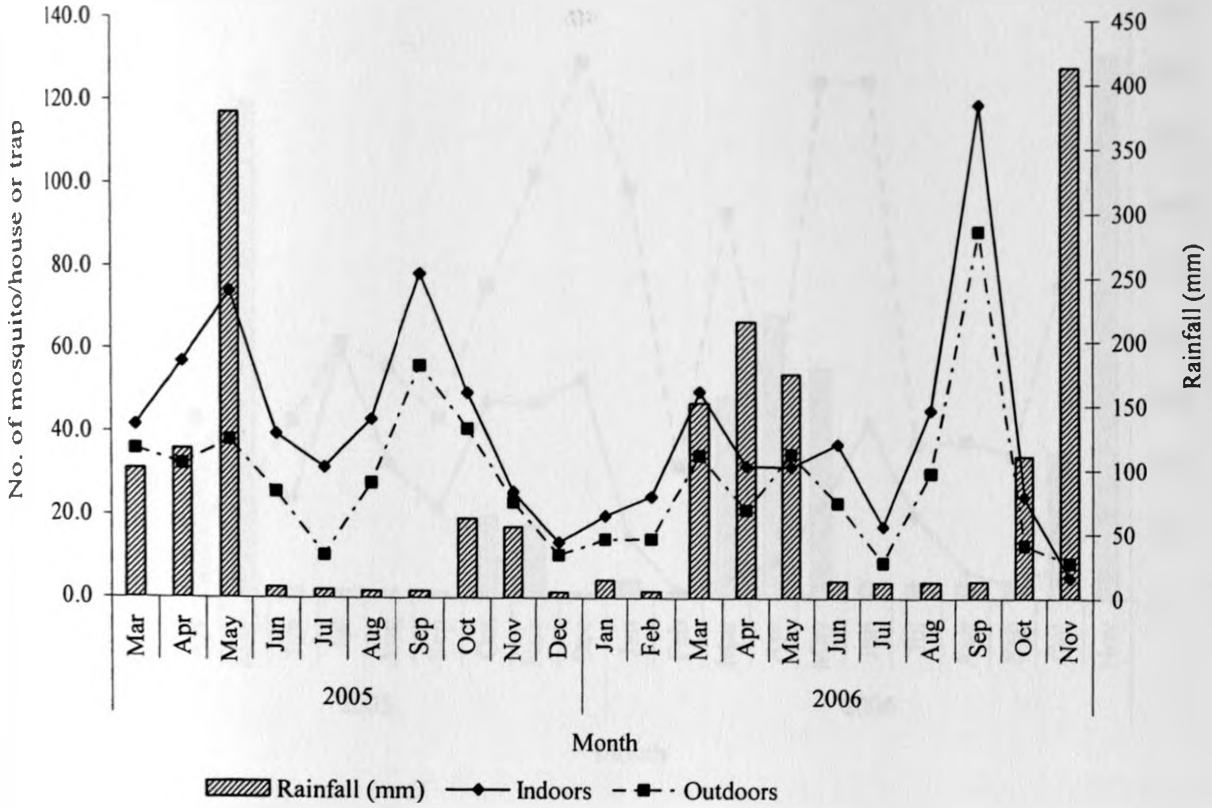



Figure 4.1: Density variations of adult *An. arabiensis* mosquitoes relative to monthly rainfall received in Mwea rice irrigation scheme.

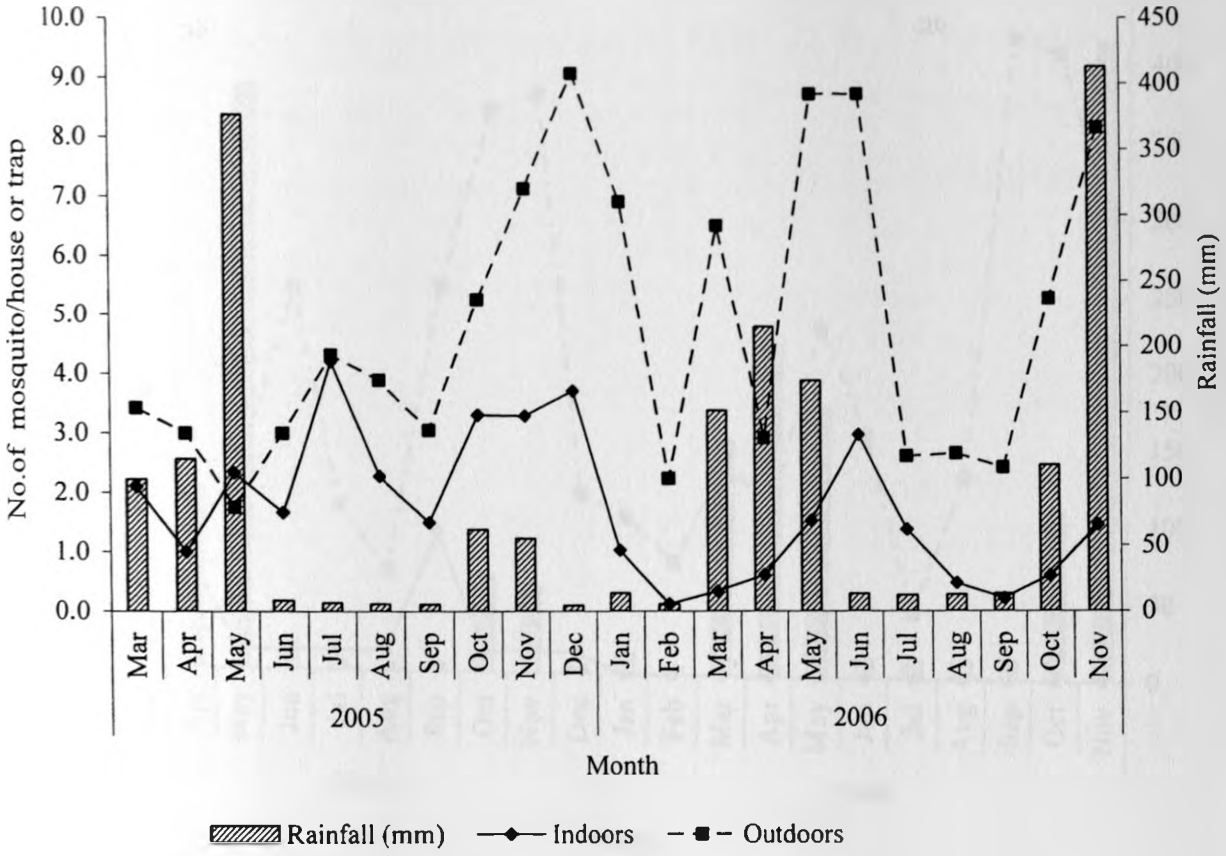



Figure 4.2: Population dynamics of *An. funestus* in relation to monthly rainfall received in the Mwea rice irrigation scheme between March 2005 and November 2006

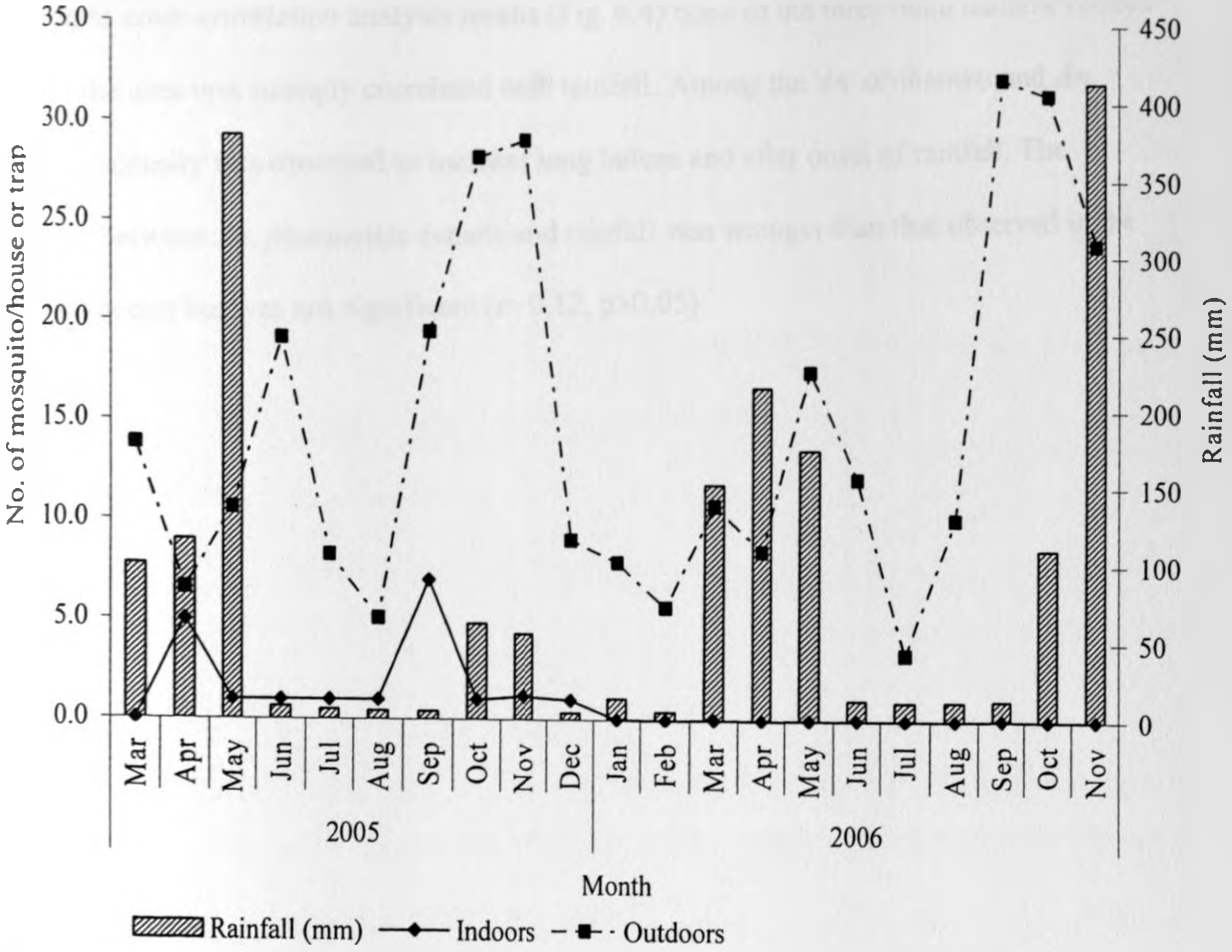



Figure 4.3: Temporal density variation of *An. pharoensis* adult population in the four study villages in Mwea irrigated rice agro-ecosystem

According to cross-correlation analysis results (Fig. 4.4) none of the three main malaria vectors density in the area was strongly correlated with rainfall. Among the *An. arabiensis* and *An. funestus* the density was observed to increase long before and after onset of rainfall. The correlation between *An. pharoensis* density and rainfall was stronger than that observed in the other two species but was not significant ($r= 0.12$, $p>0.05$).

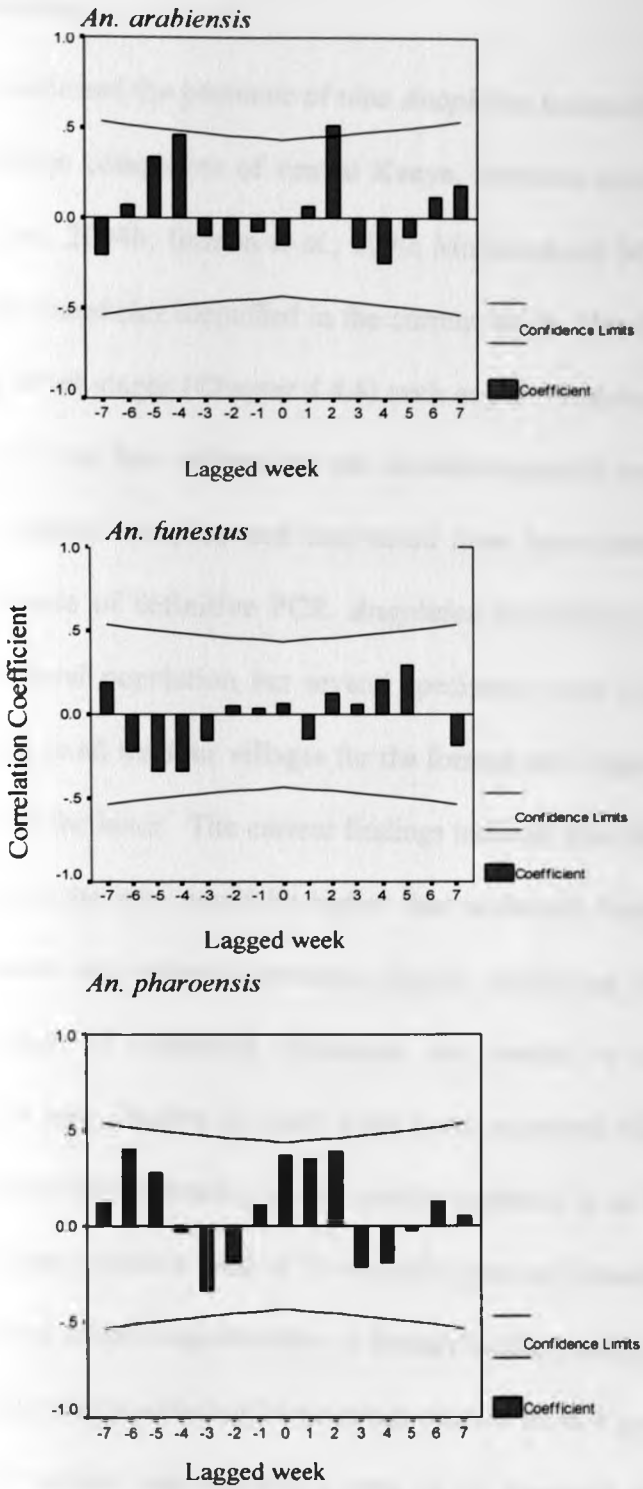



Figure 4.4: Cross-correlation between mean density of three *Anopheles* mosquitoes and Rainfall in the study area

## 4.5. Discussion

This study has confirmed the presence of nine *Anopheles* mosquito species within the Mwea rice irrigated agro-village complexes of central Kenya. Previous studies in the area (Muturi *et al.*, 2006; Mutero *et al.*, 2004b; Ijumba *et al.*, 1990; Mukiama and Mwangi, 1989) had reported the presence of some *Anopheles* identified in the current study. However, some of the species were identified in the larval stages (Chapter 4.4.4) such as *An. rivolurum* were not amongst the adult population in any of the four villages but this is understandable since this species is a subspecies within the *An. funestus* complex and thus could have been identified among the latter adult population in absence of definitive PCR. *Anopheles maculipalpis* and *An. moucheti* were not identified in the larval population but several specimens were identified amongst the outdoor resting populations in all the four villages for the former and single specimens in Kangichiri and Rurumi villages for the latter. The current findings indicate that the actual number of *Anopheles* mosquito species in the area could be higher than collected through adult mosquito surveys. Thus more intensive and extensive research studies employing both adult and larval surveys using a wide range of collection techniques are needed to establish the actual species composition in the area. Studies in other areas have supported this observation that mosquito collection techniques have a bearing on the species captured in an area. For example, modified CDC light traps alone yielded a total of 35 mosquito species (Chandler and Highton 1975) in the Kano Plain of Kenya while a combination of human-landing catches, modified CDC light traps and Monks wood type trap collected 54 mosquito species from 8 genera (Chandler *et al.*, 1975). Similarly a single suction trap captured a total of 32 mosquito species (Snow, 1983) in the Bansang rice agroecosystem in the Gambia compared to 74 mosquito species collected in Orissa, India through a combination of larval surveys and adult collections using light traps and oral and

nanical aspirators in indoor and outdoor shelters (Rajavel *et al.*, 2005). This indicates that or two sampling techniques may underestimate anopheline mosquito species in a given graphical area (Hii *et al.*, 2000)

e results of the current study further demonstrate the importance of *An. arabiensis* as the most portant and dominant malaria vector and the only sibling species of the *An. gambiae* complex Mwea riceland agro-ecosystem as was shown by Mukiama and Mwangi (1990). The species usually abundant and the most important malaria vector in irrigated rice areas (White, 1974; umba *et al.*, 1990; Ijumba *et al.*, 2002a; Klinkenberg *et al.*, 2002; 2003; Mutero *et al.*, 2004b). he importance of this species in human health especially within irrigated rice agroecosystem is urther emphasized by its involvement in the transmission of Bancroftian filariasis in Ghana Mawuli *et al.*, 1999) and o'nyong-nyong virus (Williams *et al.*, 1965). Udonsi (1988) further reported bancroftian filarial infection rates of about 21.7% resulting from transmission by *An. gambiae s.l.* in the Igwun agro-Basin in Nigeria. *Anopheles pharoensis* the second most abundant species during the current study especially in outdoor collections has not been incriminated in malaria transmission in the area (Ijumba *et al.*, 1990) although it has been shown to be an important malaria vector in Egypt (Gillies and Coetzee 1987; Gillies and de Meillon, 1968) and in Senegal (Carrara *et al.*, 1990). *Anopheles funestus,* an important malaria vector in Mwea irrigated rice scheme was represented in significantly high numbers although a high density was identified from outdoor population in contrast to its normally anthropophilic nature (Beier *et al.*, 1987) which would lead to majority of them occurring inside houses. However, *An. funestus* group consist of several sibling species, which are strongly zoophilic, that cannot be distinguished from *An. funestus s.s* morphologically (Gillies and Coetzee, 1987) such as *An.*

*rensis* (Kamau *et al.*, 2003a). The species is also implicated in filarial worm transmission and overall low density compared to *An. arabiensis* does not diminish its importance in disease ansmission in irrigated agro-ecosystems as emphasized by Charlwood and others (1997) and ould result in devastating consequences if ignored during control programs in irrigated areas. ther species identified were mostly caught outside human dwellings and were usually few in umbers apart from *An. coustani* but has not been incriminated in malaria transmission in the rea. Although *An. nili* is not considered as a major vector in Mwea, its presence in substantially arge numbers inside houses needs to be evaluated further since it has been reported as a major ector of malaria in forested and lowland areas of Africa (Service, 1964; Krafsur, 1970; Carnevale *et al.*, 1992). The species was shown to occur mainly in human dwellings with high malaria inoculation rates (Carnavale *et al.*, 1992) emphasizing its importance in malaria ransmission.

The rice growth cycle appeared to be a crucial determinant of the seasonal distribution of the malaria vectors in the area namely *An. arabiensis, An. funestus* and *An. pharoensis* though the prevailing rainfall pattern has minor impact on their density. This finding corroborates previously studies in the area (Mukiama and Mwangi, 1989; Muturi *et al.*, 2006) where rainfall was reported to have little effect on mosquito density within Mwea rice irrigation scheme. Two peak populations of *An. arabiensis* were observed, the first one in April-May a period coinciding with the long rains and onset of rice fields preparation while the second and the largest occurred in September, a period associated with vegetative rice growth after rice transplanting the previous month. Similar observations were made in Ahero Irrigation Scheme, Kenya and in Bansang area in the Gambia (Chandler and Highton 1975; Chandler and Highton 1976; Snow,

83) in which, *An. gambiae s.l* occurred throughout the rice growing period but in significantly gher densities during the early rice growth. The largest peak of *An. pharoensis* populations incided with the end of long rains and the late stage of rice cycle, a trend similarly observed by ıow, (1983). *Anopheles funestus* peak was associated with the end of the rainy period and the te stages of rice development or fallow land. The numerous open sun-lit pools created by foot -ints of rice workers during transplanting and application of fertilizers have been proposed to ıvor the breeding of *An. gambiae s.l* (Chandler and Highton 1976, Mutero *et al.*, 2004a). These bservations demonstrate the need for time specificity in control intervention programs targeting ıosquito species based on irrigated rice development cycle and the prevailing rainfall pattern for ıaximum impact of malaria vector control measures.

The objective of disease vector control interventions such as larviciding in malaria control programs is to reduce vector density to a point where transmission of malaria is minimal or totally interrupted. The impact of control strategies like use of insecticide treated bed nets or curtains (Lengeler, 2001) and indoor residual spraying (Mnzava *et al.*, 1993; Roberts *et al.*, 2000) on childhood mortality and morbidity are encouraging and motivating to vector control programs. However, as odserved in the current study a considerable proportion of the malaria vctors are captured from outdoors localities and present a major challenge in vector control especially where usage of IRS and LLINs is.advocated. Thus, the use of LLINs and IRS may not effectively target all sections of the vector populations and consequently emphasizing the need to employ other control measures that would target vector population not by indoor-based vector control measures. Hence, as pointed out by Beales and others (1988), the malaria control approaches may be based on imperfect knowledge especially in irrigated areas which the current

y sort to fill. But lack of information should not be a deterrent to the implementation of aria control interventions in any locality since in the course of carrying out malaria control grams, information can be gathered which would permit reprogramming. This study provides cial information on vector species, densities and their distribution in space and time at four i within the irrigated rice agro-ecosystem that targeted malaria vectors for microbial larval ntrol interventions. Furthermore, the range and pattern of geographical variation, seasonal riation and variation from year to year and the relation of these patterns to environmental riables and to the application of control measures has been elucidated. In this study area, *An. abiensis An. pharoensis* and *An. funestus* were the predominant and medically important osquito species with their density greatly influenced by the rice cropping cycle while rainfall layed a supplementary role in population variation.

## HAPTER FIVE

### ) Host choice and multiple blood feeding behaviour of malaria vectors and other Anophelines in Mwea rice scheme, Kenya


**)stract**

udies were conducted between April 2004 and February 2006 to determine the blood-feeding ttern of *Anopheles* mosquitoes in Mwea Kenya. Samples were collected indoors by pyrethrum ray catch and outdoors by Centers for Disease Control light traps and processed for blood meal alysis by an Enzyme-linked Immunosorbent Assay. A total of 3,333 blood-fed *Anopheles* osquitoes representing four *Anopheles* species were collected and 2,796 of the samples were ssayed, with *Anopheles arabiensis* comprising 76.2% (n=2,542) followed in decreasing order y *Anopheles coustani* 8.9% (n=297), *Anopheles pharoensis* 8.2% (n=272) and *Anopheles unestus* 6.7% (n=222). All mosquito species had a high preference for bovine (range 56.3-71.4%) over human (range 1.1-23.9%) or goat (0.1-2.2%) blood meals. Some individuals from all the four species were found to contain mixed blood meals. The bovine blood index (BBI) for *An. arabiensis* was significantly higher for populations collected indoors (71.8%), than populations collected outdoors (41.3%), but the human blood index (HBI) did not differ significantly between the two populations. In contrast, BBI for indoor collected *An. funestus* (51.4%) was significantly lower than for outdoor collected populations (78.0%) and the HBI was significantly higher indoors (28.7%) than outdoors (2.4%). Anthropophily of *An. funestus* was lowest within the rice scheme, moderate in unplanned rice agro-ecosystem, and highest within the non-irrigated agro-ecosystem. Anthropophily of *An. arabiensis* was significantly higher in the non-irrigated agro-ecosystem than in the other agro-ecosystems. These findings suggest that

: cultivation has an effect on host choice by *Anopheles* mosquitoes. The study further icates that zooprophylaxis may be a potential strategy for malaria control, but there is need to ess how domestic animals may influence arboviral epidemiology before adapting the strategy.

## 5.1. Introduction

*opheles* mosquitoes are important vectors of malaria and several arboviral infections. though more than 500 species of *Anopheles* have been described, only less than one third are nsidered vectors, and one or two species are known to be major drivers of disease transmission namics in a given area (White, 1974). The rate of disease transmission is dependent on vector stribution, abundance and lifespan, degree of host-vector-pathogen contact, susceptibility of e vector to the pathogen, and the effects of the pathogen on survivorship of both the vector and e host. These factors are further dependent on local ecologic factors such as local climatic nditions, topography, water table, occurrence and diversity of larval habitats and human festyles (Hadis *et al.*, 1997; Rwegoshora *et al.*, 2007).

or a mosquito to transmit an infection to humans, it must take at least two blood meals to acilitate uptake of the pathogens, and eventual transmission to a susceptible human. The degree f human-vector contact is, therefore, considered to be one of the most important components of isease transmission and is used in planning and evaluating the risk of vector-borne disease and he impact of vector control measures (Garrett-Jones *et al.*, 1980). *Anopheles* mosquitoes with preference for human blood are considered important vectors of malaria and Bancroftian ilariasis, and those with multiple blood meal hosts may increase the rate of arboviral ransmission (Anderson *et al.*, 1995; Snow, 1983). The choice of blood meal is influenced by several factors including host availability, nutritional requirements, intrinsic host preferences of the species, and vector density (Burkot, 1988; Loyala *et al.*, 1993; Githeko *et al.*, 1994; Zimmerman *et al.*, 2006)

igated rice agro-ecosystems are considered important "hotspots" for mosquito-borne diseases cause of the numerous mosquito species present. Worldwide, more than 89 species of *iopheles* are associated with rice cultivation (Lacey and Lacey, 1990), 23 of which occur in a riety of aquatic habitats present in African rice agro-ecosystems (Snow, 1983; Chandler *et al.*, 975; Chandler and Highton 1975; Muturi *et al* 2006). In Africa, the risk of human exposure to sease transmission by majority of these species is not fully understood because most studies are stricted to the main vectors of malaria and Bancroftian filariasis (Mutero *et al.*, 2004b; linkenberg *et al.*, 2003; Appawu *et al.*, 2001).

Review of the scanty literature indicates that rice land *Anopheles* mosquitoes tend to be highly zoophilic. Such findings have been documented for *Anopheles gambiae s.s.*, *Anopheles arabiensis*, *Anopheles funestus*, *Anopheles pharoensis* and *Anopheles rufipes* (Githeko *et al.*, 1994; Ijumba *et al.*, 1990; Ijumba *et al.*, 2002a ,2002b; Dolo *et al.*, 2004). Researchers have, therefore, suggested that zooprophylaxis could be an effective strategy for controlling malaria in these areas (Mutero *et al.*, 2004b, 20004c; Mahande *et al.*, 2007a, 2007b. This method has been used successfully in some parts of Africa (Burkot, 1988) and is also considered to have played a significant role in reduction of malaria in Europe and USA earlier in the last century (Bruce-Chwatt, 1985). However, studies have shown that the pattern of host choice and preference by *Anopheles* mosquitoes is site-specific and dependent on local ecologic factors (Garrett-Junes *et al.*, 1980). It is, therefore, conceived that decisions regarding zooprophylaxis can only be made based on local context of available hosts and blood feeding preferences (Kent *et al.*, 2007). The aim of the current study was to determine the blood-feeding patterns of *Anopheles* mosquitoes in Mwea Rice Irrigation Scheme in central Kenya.

## 5.2. Material s and Methods

### 5.2.1. Study area

he studies were conducted in Mwea Rice Irrigation Scheme in central Kenya, 100 km North-ast of Nairobi. The study area has been described (Chapter Two). Eight study sites were elected for the study based on rice growing practices and water management. These included six illages within the rice scheme (Ciagi-ini, Mbuinjeru, Rurumi, Karima, Kiuria and Kangichiri) here 75% of each village land is under rice cultivation, and two villages outside the scheme Kiamachiri and Murinduko). The two villages outside the irrigation scheme were included in the tudy to help in comparing the feeding behaviour in non-irrigated areas and implication on nalaria transmission. Kiamachiri is located immediately outside the scheme and 20% of the illage land is under rice cultivation. Murinduko is approximately 15 km away from the scheme nd only less than 5% of the village land is under rice cultivation because most of the land is hilly and unsuitable for rice cultivation. Rice cultivation in the villages within the scheme follows a definite cropping cycle as determined by the National Irrigation Board (planned rice cultivation) as described in Chapter two (2.1.3). In Kiamachiri and Murinduko villages, rice cultivation continues throughout the year as long as water is available (unplanned rice cultivation). Since only a small portion of land in Murinduko was under rice cultivation, the village was considered to be non-irrigated. Cattle, goats and chicken are the main domestic animals kept in the study area. Majority of the houses are mud-walled with iron roofing and unscreened eaves and windows.

s shown in Chapter four in this study and previous studies (Muturi *et al.*, 2006), nine species of *nopheles* are known to occur in the area with *An. arabiensis* as the dominant species and the nly member of the *An. gambiae* complex present (Mukiama and Mwangi, 1990). Previous udies have shown that both agricultural and environmental factors play a significant role in etermining the occurrence and abundance of these species (Mwangangi *et al.*, 2006a; Muturi *et l.*, 2007). *Anopheles arabiensis* and *An. funestus* are the known vectors of malaria in the area ljumba *et al.*, 1990; Mutero *et al.*, 2004b).

### 5.2.2. Mosquito collection

The sampling frame for adult mosquito collection extended between April 2005 and June 2007. For logistic purposes, samples for Mbuinjeru, Kiamachiri and Murinduko were collected between April 2004 and March 2005 and those for Ciagi-ini, Rurumi, Karima, Kiuria and Kangichiri were collected between March 2005 and June 2006. Indoor-resting mosquitoes were collected by pyrethrum spray catch (PSC) method (WHO, 1975) and outdoor populations were collected by Centers for Disease Control (CDC) miniature light traps (J.W. Hock Ltd, Gainesville, FL., USA). A detailed explanation of the sampling strategy has been described in chapter four.

### 5.2.3. Laboratory processing

All *Anopheles* mosquitoes collected by PSC and CDC light traps were transported to the laboratory and sorted by sex and species using morphological characteristics (Gillies and De Meillon, 1968). The females were further classified into their respective blood feeding stages

ıfed, blood-fed, semi-gravid and gravid) by examining their abdomen under a dissecting croscope (WHO, 1975). All blood-fed mosquitoes from each collection were preserved in ɔeled vials containing anhydrous calcium sulphate.

### 5.2.4. Blood meal identification

ımples of blood fed mosquitoes were cut transversely between the thorax and the abdomen, and e posterior portions containing the blood meal were placed individually in labeled vials. The ɔdomen of each mosquito was ground in 50 µl of phosphate-buffered saline (PBS) with ıbsequent addition of 950 µl of PBS and then stored at –20°C. Blood meals were identified by a irect enzyme-linked immunosorbent assay (ELISA) using anti-host (IgG) conjugates Kirkegaard and Perry, Gaithersburg, MD) against human, bovine and goat (Beier *et al.*, 1988). All blood meal samples were screened simultaneously for human, bovine and goat antibodies. A letailed protocol of the blood meal analysis is provided in Appendix I.

The ground mosquito triturates (50 µl) were added to wells of polyvinyl-chloride, U- shaped, 96-well micro-titre plates, which were covered and incubated overnight at room temperature. Each well was then washed twice with PBS containing 0.5% Tween 20 (PBS-TW 20). This was followed by the addition of 50 µl host specific conjugate (antihuman IgG, H&L) diluted 1:2,000 for 1:2,000 (or 1:250 for bovine) in 0.5% boiled casein containing 0.025% Tween 20. The boiled casein was prepared by dissolving 5g casein in 100 ml 0.1 N sodium hydroxide by boiling, adding 900ml PBS, adjusting pH to 7.4, adding 0.1g Thimerosal (sodium ethylmercurithiosalicylate) and 0.02 g phenol red, and storing at $4^0$C. After 1 hour, wells were washed three times with PBS-Tween 20, and 100 µl of ABTS (2,2'-azino-di-[3-

ylbenzthiazoline sulfonate]) peroxidase substrate was added to each well. The dark green sitives were identified visually after 30 minutes. A second host source was determined in the me microtitre plate where mosquitoes were screened for human blood. The second conjugate, osphatase-labelled anti-bovine IgG (1:250 dilution of 0.5 mg/ml stock solution) was added to e peroxidase-labelled antihuman IgG solution.

Bloodmeals were screened for human IgG by the addition of peroxidase substrate according to ie peroxidase-labelled antihuman IgG solution conjugate and then the phosphatase-labelled nti-bovine IgG (1:250 dilution of 0.5mg/ml stock solution) added to the peroxidase-labelled ntihuman IgG solution. After reading the plates at 30 minutes, the wells were washed 3 times with PBS-Tween 20, and 100 µl phosphatase substrate added to each well. Plates were read after hour to determine positive cow reactions.

In testing for goat IgG each test, 1:500 dilutions of human, cow, goat, dog, donkey and chicken serum were added to the conjugate solution to reduce background absorbance. Each plate contained control serum samples (1:500) dilution in PBS of human, cow, goat, dog, donkey and chicken and four field-collected male *Anopheles* mosquitoes ground in PBS at the same dilution as test samples.

### 5.3. Data analysis

Data were entered in Microsoft excel and analyzed using Epi-Info® software version 3.4.1 (CDC Atlanta, Georgia USA). Chi-square and Fishers exact tests were used (as appropriate) to compare the differences in the human blood index (HBI) and bovine blood index (BBI) between indoor

outdoor collected populations of *An. arabiensis* and *An. funestus*. The HBI and BBI were culated as the ratio of blood-fed mosquito samples that had fed on human and cattle pectively to the total tested. Chi-square test was also used to compare the differences in HBI *An. arabiensis* and *An. funestus* among planned, unplanned, and non-irrigated agro-systems. Data for the six villages within the rice scheme (planned rice) were pooled before alysis was done.

## 5.4. Results

### 5.4.1. Bloodmeal sources

total, 3,333 blood-fed *Anopheles* mosquitoes collected indoors and outdoors from eight sites Mwea, Kenya were tested by ELISA for host blood meal and 2,796 (83.9%) of the samples ›uld be identified. These comprised *An. arabiensis* (n = 2,542), *An. funestus* (n = 222), *nopheles coustani* (297), and *An. pharoensis* (n = 272). Overall, majority of the blood meals ;ere of bovine origin (69.9%) followed by human (8.1%). The remaining blood meals were rom goat (0.4%) and mixed blood meals from bovine and goat (3.0%), human and bovine 2.0%), human and goat (0.1%) and human, bovine and goat (0.3%) (Table 5.1).

Eighty two percent of *An. arabiensis* samples (n = 2,542) were positive for at least one of the three host blood meals tested. The majority of them had predominantly fed on cattle (70.9%), to a lesser extent on humans (7.8%) and rarely on goats (0.1%). Seventy-four samples (2.84%) proved to be of mixed origin mainly from human and bovine (1.6%) and bovine and goat (1.0%) (Table 5.1). Most of the blood fed *An. arabiensis* females were collected indoors as opposed to outdoors (Table 5.1). Although the human blood index for this species did not differ significantly between indoor and outdoor collected populations (Fisher exact test = 0.57 df = 1 P = 0.425), the bovine blood index was significantly higher among indoor (71.8%) than outdoor collected (41.3%) samples ($\chi 2$= 6.3 df = 1 P < 0.05). In contrast, the proportion of samples containing mixed blood meals was higher among outdoor than indoor collected mosquitoes (Table 5.1).

· *An. funestus*, 89.6% (n = 222) of the blood meal samples were successfully identified and
own to consist of at least one of the three hosts tested. The majority of blood meals were from
tle (56.3%) and humans (23.9%), and only one blood meal was of goat origin. Mixed blood
als mainly of bovine and goat (7.25%), and human and bovine (1.4%) were also obtained
able 5.1). The number of blood fed *An. funestus* was four-fold higher indoors than outdoors.
e human blood index for indoor collected *An. funestus* was 28.7% and significantly higher
an 2.4% for outdoor populations (Fisher exact test = 9.73 df =1 $P < 0.05$). In contrast, bovine
od index of 51.4% for indoor collected populations was significantly lower than 78.0% for
tdoor collected populations ($\chi 2$= 13.78, df = 1 $P < 0.05$). Mixed blood meals were common in
th indoor and outdoor samples.

ith a single exception, all blood-fed *An. coustani* were collected outdoors. This species had
redominantly fed on cattle (71.4%), over humans (5.4%) or goats (1.0%). Mixed blood meals
ainly of bovine and goat (9.5%), human and bovine (3.0%), and human and goat (1.0%) were
lso obtained. The single specimen from indoor collections had feed on cattle and 25 blood meal
amples were of unknown sources.

*Anopheles pharoensis* also fed predominantly on cattle (70.3%) over human (1.1%) or goat
2.2%). Mixed blood meals were mainly of bovine and goat (11.4%), and human and bovine
4.8%) origin. All but three samples of *An. pharoensis* were collected outdoors. The three
amples collected indoors had fed on cattle.

*Table 5.1: Blood-meal sources of Anopheles mosquitoes in four irrigated rice agro-*

| Anopheline Species | Location | # tested | Human (%) | Bovine | Goat | Human/bovine | Human/ Goat | Bovine/ goat | Human/ bovine/goat | Unknown |
|---|---|---|---|---|---|---|---|---|---|---|
| | Indoors | 2467 | 194 (7.9) | 1771 (71.8) | 3 (0.1) | 37 (1.5) | 0 (0) | 21 (0.9) | 6 (0.2) | 435 (17.6) |
| | Outdoors | 75 | 5 (6.7) | 31 (41.3) | 0 (0) | 4 (5.3) | 1 (1.3) | 5 (6.7) | (0) 0 | 29 (38.7) |
| *An. arabiensis* | Overall | 2542 | 199 (7.8) | 1802 (70.9) | 3 (0.1) | 41 (1.6) | 1 (0.04) | 26 (1.0) | 6 (0.2) | 464 (18.3) |
| | Indoors | 181 | 52 (28.7) | 93 (51.4) | 1(0.6) | 1 (0.6) | 0 (0) | 13 (7.2) | 0 (0) | 21 (11.6) |
| | Outdoors | 41 | 1 (2.4) | 32 (78) | 0 (0) | 2 (4.9) | (0) 0 | 3 (7.3) | 1 (2.4) | 2 (4.9) |
| *An. funestus* | Overall | 222 | 53 (23.9) | 125 (56.3) | 1 (0.5) | 3 (1.4) | 0 (0.0) | 16 (7.2) | 1 (0.5) | 23 (10.4) |
| | Indoors | 3 | 0 (0) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | Outdoors | 269 | 3 (1.1) | 189 (70.3) | 6 (2.2) | 13 (4.8) | 0 (0) | 31 (11.5) | 2 (0.7) | 25 (9.3) |
| *An. pharoensis* | Overall | 272 | 3 (1.1) | 192 (70.6) | 6 (2.2) | 13 (4.8) | 0 (0) | 31 (11.4) | 2 (0.7) | 25 (9.2) |
| | Indoors | 1 | 0 (0) | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | Outdoors | 296 | 16 (5.4) | 211 (71.3) | 3 (1.0) | 9 (3.0) | 3 (1) | 28 (9.5) | 1 (0.3) | 25 (8.4) |
| *An. coustani* | Overall | 297 | 16 (5.4) | 212 (71.4) | 3 (1.0) | 9 (3.0) | 3 (1.0) | 28 (9.4) | 1 (0.3) | 25 (8.4) |
| | Indoors | 2652 | 246 (9.3) | 1868 (70.4) | 4 (0.2) | 38 (1.4) | 0 (0) | 34 (1.3) | 6 (0.2) | 456 (17.2) |
| All species | Outdoors | 681 | 25 (3.7) | 463 (68) | 9 (1.3) | 28 (4.1) | 4 (0.6) | 67 (9.8) | 4 (0.6) | 81 (11.9) |
| combined | Overall | 3333 | 271 (8.1) | 2331 (69.9) | 13 (0.4) | 66 (2.0) | 4 (0.1) | 101 (3.0) | 10 (0.3) | 537 (16.1) |

### 5.4.2. Blood meal variation among study sites

en the blood feeding patterns of *An. arabiensis* and *An. funestus* were separated by village, human blood index (HBI) for *An. funestus* was significantly higher in the villages outside the e scheme (Kiamachiri and Murinduko) than those within the scheme ($\chi 2 = 35.02$ df = 1 P < 5). HBI for this species (*An. funestus*) was also significantly higher in Murinduko than in amachiri ($\chi 2 = 11.27$ df = 1 P < 0.05). The HBI for *An. arabiensis* was significantly higher in rinduko compared with the other villages ($\chi 2 = 25.86$, df = 1, P < 0.001) as shown inTable 2.

e 5.2: Proportion of human and bovine blood meals for *Anopheles arabiensis* and *An.* *stus* collected at eight sites representing three agro-ecosystems in Mwea, Kenya.

| | | *An. arabiensis* | | | *An. funestus* | | |
|---|---|---|---|---|---|---|---|
| ro-osystem | Village | No. tested* | % Human | % Bovine | No. tested | % Human | % Bovine |
| anned rice | Ciagi-ini | 227 | 2.2 | 84.1 | 30 | 0.0 | 96.7 |
| | Kangichiri | 240 | 2.1 | 51.7 | 7 | 14.3 | 71.4 |
| | Karima | 265 | 7.5 | 76.6 | 26 | 11.5 | 88.5 |
| | Kiuria | 309 | 6.1 | 78.0 | 48 | 0.0 | 93.8 |
| | Rurumi | 214 | 7.9 | 80.4 | 16 | 6.3 | 93.8 |
| | Mbui-njeru | 815 | 9.3 | 78.2 | 19 | 10.5 | 73.7 |
| | Overall | 2070 | 6.9 | 75.7 | 146 | 4.8 | 89.7 |
| Unplanned rice | Kiamaciri | 338 | 11.5 | 73.7 | 11 | 27.3 | 63.6 |
| Non-irrigated | Murinduko | 134 | 49.3 | 43.3 | 65 | 72.3 | 9.2 |

** Results for indoor and outdoor collected mosquitoes were pooled together*

## 5.5. Discussion

Out of the 9 species of *Anopheles* identified during this study (Chapter four) and in previous studies in Mwea Rice Irrigation Scheme (Ijumba *et al.*, 1990; Mutero *et al.*, 2004b), four of them were examined for blood meal sources and found to have higher preference for bovine over human hosts. Similar results have been reported in different rice growing areas in the African continent (Ijumba *et al.*, 1990; Ijumba *et al.*, 2002a; Dolo *et al.*, 2004; Mutero *et al.*, 2004b). Rice cultivating areas are often associated with higher mosquito densities and human communities in these areas enforce the use of bed nets and other protective measures against biting mosquitoes (Ijumba and Lindsay, 2001). Consequently, mosquitoes revert to feeding on domestic animals because humans are not easily accessible. These results demonstrated that majority of *An. arabiensis* gained entry into the house after feeding outdoors on bovine and that indoor-collected mosquitoes had no advantage over outdoor collected populations in terms of access to human blood meals. These findings confirmed that protection against mosquito bites is indeed one of the factors accounting for zoophilic tendency of *Anopheles* mosquitoes in rice irrigated areas. Interestingly, 16 % of the blood meal samples were not from any of the four hosts tested, an indication that anophelines in this area have a wide host range. These findings highlight the need to include a variety of possible hosts when conducting mosquito host choice studies.

Reduced anthropophily of *Anopheles* mosquitoes in rice cultivating areas has been suggested as one of the factors responsible for the low levels of malaria transmission in these areas despite the presence of higher vector densities (Ijumba *et al.*, 1990; Ijumba and Lindsay, 2001; Ijumba *et al.*, 2002a; Dolo *et al.*, 2004; Mutero *et al.*, 2004b). Zooprophylaxis is, therefore, considered a

ential malaria control strategy in rice growing areas of Africa (Mutero *et al.*, 2004b). wever, it should be noted that *An. funestus* differed from *An. arabiensis* in a way that is likely affect malaria epidemiology and the impact of zooprophylaxis on malaria control. *Anopheles nestus* was substantially more anthropophilic and endophagic than *An. arabiensis*. These servations indicate that inhabitants in the study area are at a greater risk of exposure to malaria nsmission by *An. funestus* than by *An. arabiensis* and that *An. funestus* may not be a good ndidate of zooprophylaxis. These findings therefore, emphasize the idea of Hadis and others 997) that conclusions regarding zooprophylaxis cannot be generalized for all mosquito species. *nopheles funestus s.l.* consists of nine members that are difficult to distinguish by orphological characteristics (Gillies and Coetzee, 1987). All except *An. funestus s.s* and to me extent *An. rivolurum* are believed to be zoophilic and non-vectors (Wilkes *et al.*, 1996). ue to limitation in resources, the species composition within the *An. funestus* group was not etermined in the current study. Previous studies (Kamau *et al.*, 2003a; Kamau *et al.*, 2003b) in he Mwea irrigation scheme using indoor collected samples found the species composition of the *An. funestus* complex within the Mwea Rice Scheme to be comprised mainly of *An. parensis* 99%) and *An. leesoni* (<1%). Based on these studies and the current HBI results, it is likely that *An. parensis* was the dominant species in villages within the irrigation scheme as opposed to *An. funestus s.s.* in the non- irrigated areas. However, further studies are necessary to define the species composition of the *An. funestus* complex in the area and their role in malaria transmission. Such studies should take into account the indoor and outdoor resting localities of members of this species complex.

onsiderable proportion of all *Anopheles* species examined contacted more than one host ng a single gonotrophic cycle. This is common among mosquitoes and its epidemiological ificance is controversial (Hadis *et al.*, 1997; Bruce-Chwatt *et al.*, 1966; Burkot *et al.*, 1988). nalaria transmission, the loss of certain number of sporozoites to non-human hosts during ed feeding could be of importance in malaria control. In fact, the presence domestic animals e been associated with a decrease in malaria transmission because of zoophilic deviation uce-chwatt *et al.*, 1966). For this reason, some African communities intentionally keep cattle r or inside houses to divert mosquitoes from humans to cattle (Burkot *et al.*, 1988). On the er hand, the presence of domestic animals may enhance or suppress transmission of oviruses depending on whether the vector feeds on potential or unimportant hosts. In stralia, close proximity of humans to domestic pigs and high mosquito densities was sociated with an outbreak of Japanese encephalitis (JE) (Ritchie *et al.*, 1997). In contrast, ultiple feeding from dead end hosts (cattle) was associated with a decrease in prevalence of JE India (Reuben *et al.*, 1992). Based on these findings, it is possible that multiple feeding bserved in this study could reduce malaria transmission but potentially enhance transmission of boviruses such as Rift Valley Fever Virus which is emerging as an important public health roblem in Kenya and other countries in Africa (Lincithicum *et al.*, 1999; Diallo *et al.*, 2005; DC, 2007). In the past, several *Anopheles* species including *An. pharoensis* and *An. coustani* ave been associated with transmission of the two arboviruses (Logan *et al.*, 1991; Gordon *et al.*, 992). Members of the *An. gambiae s.l.* and *An. funestus s.l* have also been implicated in the ransmission of O'nyong-nyong virus in east Africa (Williams *et al.*, 1965). This observation coupled with the high density of some of the vectors in the irrigated areas could easily initiate arboviral epidemics by the immigration of even a single viremic individual (Reiter *et al.*, 1998).

,ever, further studies are recommended to evaluate the potential role of domestic animals in ›viruses transmission in similar areas.

results of this study also demonstrated that land use had significant impact on blood feeding em of *An. arabiensis* and *An. funestus*. Anthropophily for *An. funestus* was lowest within the : scheme, moderate in the unplanned rice agro-ecosystem, and highest in the non-irrigated o-ecosystem and that of *An. arabiensis* was significantly higher in the non-irrigated agro-›system than in the other agro-ecosystems. The numbers of *Anopheles funestus* s.l. were low all agro-ecosystems especially when compared to those of *An. arabiensis*. For unknown sons, probably related to larval habitat characteristics, *An. funestus* is a rare species in African e agro-ecosystems except in Ahero Rice Scheme, Kenya and plateaus of Madagascar (Githeko *al.*, 1993; Marrama *et al.*, 1995; Klinkenberg *et al.*, 2003). Studies in the study area revealed at mosquito densities increased proportionally with increasing area under rice cultivation. The ea under rice cultivation in the villages within the rice scheme was approximately four and 15 mes greater than in Kiamachiri and Murinduko, respectively. It is, therefore, logical to expect ıcreasing anthropophilic tendency of these mosquito species as you move away from the rice :heme because protection against mosquito bites is directly proportional to mosquito densities Ijumba and Lindsay, 2001; Ijumba *et al.*, 2002; Dolo *et al.*, 2004; Mutero *et al.*, 2004b). It is, owever, worth to note that the limited resources neither allowed for replication of villages ›utside the rice scheme nor collection of mosquito samples at the same time frame in all villages. The villages within the irrigation scheme did not show great disparity in mosquito feeding ›ehavior and was consequently assumed that those outside the irrigation scheme would have similar characteristics and effect on mosquito host choice. Hence, the single villages outside the

ıe would present the unique features associated with land use patterns in relation to malaria rs and their choice of blood meal source. Nonetheless, further studies conducted ltaneously in equal number of villages for each agro-ecosystem and at the same sampling ıd would be more informative

current study adopted pyrethrum spray catch (PSC) and CDC light trap methods for ection of indoor and outdoor resting mosquito populations, respectively. This was essitated by the need to capture large representative samples of all *Anopheles* species present the study area for evaluation of the feeding behaviour and host choices in different ironments. The strongly exophagic and exophilic mosquito species such as *An. pharoensis* niedu, 1993) would be captured by light traps at night when most such species are active. On e other hand, some members of similar or different species spend most or part of their time sting indoors like *An. funestus s.l.* and thus majority would be captured entirely or partly rough PSC method. The combination of the two mosquito collection methods with individual ias towards different mosquito species helped increase the diversity and density of the species collected and tested for blood feeding behaviour.

This study has demonstrated that *Anopheles* mosquitoes in the Mwea Rice Scheme are highly zoophilic and that multiple feeding within the same gonotrophic cycle is common among these species. The study has further revealed that the degree of anthropophily among malaria vectors is directly proportional to the area of land under rice cultivation. By pooling together available literature and the findings of this study, it can be inferred that zooprophylaxis is a potential

aria control strategy in Mwea irrigated agro-ecosystem and similar areas especially through grated vector control programs.

## \PTER SIX:

## Effect of irrigated rice farming on *Anopheles arabiensis* and *Anopheles funestus* biting rates in Mwea irrigated rice agro ecosystem


### ;tract

dies were conducted in four irrigated rice agro-village complexes in Mwea from April 2005 to November 2006 to determine human biting rates of the major malaria vectors in the area. ;o methods were used to estimate and compare the biting rates in the region. Overall, a ;ident of Mwea irrigated rice agro-ecosystem was found to experience an average of 0.68±0.01 tes per person per night from both *Anopheles arabiensis* and *An. funestus*. The biting rates ffered significantly among the irrigated rice agro-village complexes and between months in the ear. *Anopheles arabiensis* showed two peaks in biting rates in each year. A high peak occurred luring early vegetative stages of the rice growth between August and September while a second ;maller peak occured in the month of May with lowest biting rates in December. The biting rates for *An. funestus* were highest in June and December while lowest biting rates were recorded in August / September period. Pearson correlation analysis indicated a close correlation between human biting rate estimates by the use of light traps and those from indoor resting mosquito collections hence both methods are good measure of biting rates. It is concluded that the biting rates of mosquitoes in irrigated rice agro-villages is highly influenced by the rice cultivation cycle and to a small extent by rainfall pattern and thus control efforts should be timely and site specific to have an impact on biting rates and malaria transmission in irrigated rice schemes.

## 6.1. Introduction

laria infections have immense economic burden to a country, family and an individual where as resulted in an estimated 1.3 % reduction in the annual per capita income growth rate in laria endemic countries and a long-term impact on the reduction of the GNP by more than half achs and Maloney 2002). Since the discovery of the relationship between *Anopheles* squitoes and malaria transmission, vector control programs have been intensified targeting th the immature and adult stages. One of the primary variables affecting the risk of infection is e rate at which humans are bitten by the mosquitoes.

the irrigated rice ecosystems, *Anopheles arabiensis* and *An. funestus* are the most abundant d common species that are largely blamed for malaria transmission. The population dynamics these species especially *An. arabiensis* is greatly influenced by the rice growth patterns which ovide ideal breeding habitats for the species and is known as the pioneer species in ricefields olonization (Ijumba, 1997, Mwangangi *et al.*, 2006a, 2006b; Muturi *et al.*, 2007). Though nophelism without malaria is a common phenomenon in irrigated agro-ecosystem (Diuk-wasser *al.*, 2005) malaria remains a challenge in rice irrigated regions due to the high numbers of dult vectors that come into contact with the human population. The propensity of malaria ectors to transmit malaria is greatly influenced by the rate at which the vectors' bite humans and ne proportion of the vector population that is infectious. However, the distribution of human opulation and suitable breeding habitats for mosquitoes varies across landscapes and influence he human-vector contact rates (Carter *et al* 2000; Minakawa *et al.,* 1999; Mutero *et al* 2004b; Zhou *et al* 2007; Muturi *et al* 2008). The density of the adult mosquito population also fluctuates

ɔnally with changes in weather parameters such as rainfall, humidity and temperatures ɛby affecting the human biting rates.

ɾious studies (Burkot and Graves, 1995) have shown that the human biting rates of malaria or species, form an essential component of the vectorial capacity and the entomological ɔulation rates which in turn are crucial parameters in describing and comparing malaria .smission intensities in entomological terms. However, as reported by Klinkenberg and ɛrs, (2003) most research on irrigation and malaria is usually focused on the prevalence of the ɛase within the human population close to the irrigation scheme with few studies addressing direct effect of the irrigation system, in particular rice cultivation cycle, on the vectors mselves and their human biting rates. Therefore, measuring the *Anopheles* mosquito species ing rates constitutes an important aspect of entomological monitoring (Githeko *et al.*, 1993) of ctor control programs targeting either the adult or larval stages of the vectors. Several methods e used to estimate human biting rates with human biting catches being the most direct method nce mosquitoes are caught while engaged precisely in the biting act (Service, 1993). However, is method faces ethical and logistical problems relating to standardization (Costantini *et al* 998; Magbity *et al.*, 2002). Sampling indoor resting mosquitoes is also useful though its success ɛpends upon the exophilic and endophilic nature of the vector mosquito species (Lines *et al.*, 991) and may fail to include those that leave houses immediately after feeding and may also ɪclude those entering houses after feeding outdoors on other hosts apart from human (Garrett-ones, 1970). Use of light traps has been adapted especially when they are hung beside occupied ɯtreated bed-net and has been shown to be more efficient and unbiased way of estimating biting ɾates (Lines *et al.*, 1991; Davis *et al.*, 1995; Costantini *et al.*, 1998). However, this method has

n reported in some studies (Mbogo *et al.*, 1993a) with failure to provide adequate estimates of ıan biting rates of *An. gambiae* mosquitoes. Thus, to get more representative estimates of ıan biting rates in an area, a combination of two or more biting rate measurement methods is ɔortant.

part of a study on irrigation and malaria infection risks in Mwea irrigated rice agro-ɔsystem, studies were undertaken to estimate the human biting rates in the region and the role ıyed by the rice growing season on their variation. The study evaluates the variation of human ing rates from indoor resting mosquito population and those caught by light traps with time, ace and rice growth cycle in Mwea with a view of providing necessary information for the anning and implementation of large scale microbial larviciding and other vector control tervention strategies in irrigated rice agro-ecosystem.

## 6.2. Materials and methods

### 6.2.1. Human biting rates estimation

e studies were conducted in the four irrigated rice agro-village complexes comprising of ngichiri, Karima, Kiuria and Rurumi as described in Chapter 2.1.1. The human-biting rates ere estimated by use of miniature CDC light traps and Pyrethrum Spray Catch collection (PSC) ethods in each of the four study villages. The CDC light traps (hereafter refered to as 'light ap') were operated with a rechargeable battery and fitted with a 4 W fluorescent UV lacklight-blue' in colour bulb. Sampling was done twice every week and the batteries were charged before every sampling occasion. The light trap catches were carried out in three ndomly selected houses in each of the four study villages in Mwea. In each house, a new ntreated bednet was provided to the occupants of each bed within the sampling house. During very mosquito sampling occasion, a single light trap was suspended about 1.5 m from the round and about 20 – 50 cm from the bed net. All the occupants in these houses were instructed o sleep under these nets especially the night when the traps were running. The rechargeable attery powering the light trap were left with one terminal connected and the householder was equested to set the trap on by connecting the other terminal when going to sleep and at dawn fter waking up to tie the neck of the trap bag to prevent mosquitoes from escaping before disconnecting the battery. The information on the number of occupants who slept in the house as well as the time the trap was set on and switched off (waking up time) was recorded by field assistants on field forms. The light trap catches were collected and transferred to the laboratory for sample processing after making enquires regarding whether the trap fan and light worked efficiently the whole night.

ncurrently with light trap collections, indoor resting mosquito collections were carried out rough PSC method as describe in section 4.2.1 of Chapter four. The mosquitoes were collected m twenty randomly selected houses once every week. The number of people who slept in each use in the night before the sampling day was recorded and the houses characterized. Female osquito samples collected were transferred to the laboratory for further processing and lentification.

### 6.2.2. Mosquito processing in the laboratory

All *Anopheles* mosquito specimens collected by PSC and light traps methods were identified to species level by morphological characteristics using the key by Gillies and De Meillon (1968). They were then categorized by abdominal conditions as fed, unfed, half-gravid or gravid, counted and recorded. All mosquitoes from each collection were dried in anhydrous calcium sulphate over cotton wool in individually labeled vials.

## 6.3. Data analysis

Data were entered in Microsoft excel spreadsheets and analysis was performed using SPSS version 11.0 (SPSS Institute, Chicago, IL). The total number of *Anopheles* mosquito species caught by the light traps was compared with the PSC collections by regression analysis using SPSS statistical software. The data for the night when either one or all light traps malfunctioned were excluded from the analysis. The biting rates for *Anopheles* were transformed to $\log_{10}(x + \frac{1}{2})$ to normalise the distribution. The human biting rate (HBR) for the light traps were derived by

ling the number of blood seeking (unfed) and fed mosquitoes collected by light traps with number of occupants of each house in each sampling occasion. This resulted in s/person/night for each mosquito species in each village. Similarly, the human biting rate R) for *Anopheles* species collected by the pyrethrum spray catch method were calculated by ding the total number of blood-fed and half-gravid female *Anopheles* mosquitoes caught by number of human occupants of each house, multiplied by the human blood index of 0.1 and 4 for *An. arabiensis* and *An. funestus* respectively. This resulted in bites/person/night for each squito species in each village. Analysis of variance was used to analyze difference in biting es between the two collection methods as well as between seasons, species and among lages.

## 6.4. Results

### 6.4.1. Biting density of *An. arabiensis* and *An. funestus*

*pheles arabiensis* accounted for 97.63 % and *An. funestus* 2.28% of the 240, 986 anophelines ected by PSC over the 20-month period of the study. Over the same period a total of 52,996, quitoes specimens were collected using light traps consisting of 47, 417 (89.47 %) *An. biensis* and 1, 070 (2.02%) *An. funestus*. The overall mean number of mosquitoes per person night in Mwea irrigated rice agro-ecosystem was 0.68 ± 0.01 b/p/n for both *An. arabiensis* l *An. funestus* combined (Table 6.1).

e biting rates of light trap collected *An. arabiensis* varied significantly among the study lages ($F = 9.45$, $df_{3, 76}$ $p < 0.05$) giving a mean HBR of 2.09, 1.89, 1.03 and 0.86 b/p/n in ngichiri, Karima, Kiuria and Rurumi, respectively. A similar test for PSC collected samples ereafter referred to as indoor resting human biting rates 'IR-HBR') indicate that residents of arima (1.56) received significantly higher bites per night from *An. arabiensis* ($F = 6.99$, $df_{3, 83}$ $< 0.05$) than those residing in either Kangichiri (0.90) Kiuria (0.70) or Rurumi (0.74).

esults of human biting rates for *An. funestus* indicated significantly higher bites per person per ight in Karima for both IR-HBR ($F = 7.01$ $df_{3, 80}$ $p < 0.05$) and light trap ($F = 7.12$, $df_{3, 72}$, $p <$ .05) collected samples compared with the other villages (Table 6.1.).

6.1: Mean biting densities (bites/person/night ± SE) of *An. arabiensis* and *An. funestus* in ur study villages.

| | *An. arabiensis* | | | *An. Funestus* | | |
|---|---|---|---|---|---|---|
| | Light Traps | IR-HBR | Total | Light Traps | IR-HBR | Total |
| gichiri | 2.09 ± 0.11 | 0.90 ± 2.03 | 1.04 ± 0.03 | 0.40 ± 0.04 | 0.10 ± 0.008 | 0.12 ± 0.008 |
| ma | 1.89 ± 0.10 | 1.56 ± 0.03 | 1.60 ± 0.03 | 0.67 ± 0.08 | 0.27 ± 0.014 | 0.30 ± 0.015 |
| ia | 1.03 ± 0.07 | 0.70 ± 0.02 | 0.74 ± 0.02 | 0.28 ± 0.03 | 0.05 ± 0.004 | 0.07 ± 0.005 |
| ımi | 0.86 ± 0.06 | 0.79 ± 0.02 | 0.79 ± 0.02 | 0.27 ± 0.04 | 0.09 ± 0.006 | 0.10 ± 0.006 |
| **ın Total** | **1.48 ± 0.05** | **0.99 ± 0.01** | **1.05 ± 0.01** | **0.43 ± 0.03** | **0.13 ± 0.005** | **0.15 ± 0.005** |

### 6.4.2. Seasonal variation in human biting rates

HBR (b/p/n) for *An. arabiensis* varied significantly between months ($F = 2.64$, $df_{19, 138}$ $p =$ ) over the twenty-month sampling period. The *An. arabiensis* biting activity was highly ntrated between August and September during rice transplanting and early vegetative s with a major peak in September. A progressive increase in biting rates was also observed March to achieve a small peak in May each year in the study villages during the long rain d. The highest HBRs of 4.31b/p/n in light traps and 3.11b/p/n from indoor resting mosquito ctions were recorded in month of September 2006 in Kangichiri and Karima respectively. monthly variations of the biting rates for all the villages and amount of rainfall received are wn in Table 6.2. Biting rates were generally low in all villages between the months of ember and February

ne-way ANOVA and Tukey's honest test of significance indicated a significant difference ($F$ .46, $df_{19, 136}$, $p = 0.002$) in HBR for *An. funestus* between the months in all the villages. Two ks in *An. funestus* biting rates in both light trap and PSC collections were observed in all the lages (Table 6.3). The first occurred in June and July with an observed reduction in rainfall ring the long rains and a second peak in biting rates occurred between November and January, end of short rains and rice growth cycle. The lowest human biting rates for *An. funestus* were corded during the early growth stages of rice (September) and at the onset of the long rains,.

ble 6.2: Seasonal variation in *An. arabiensis* human biting rates (bites/person/night) in four gated rice agro-village complexes, Mwea.

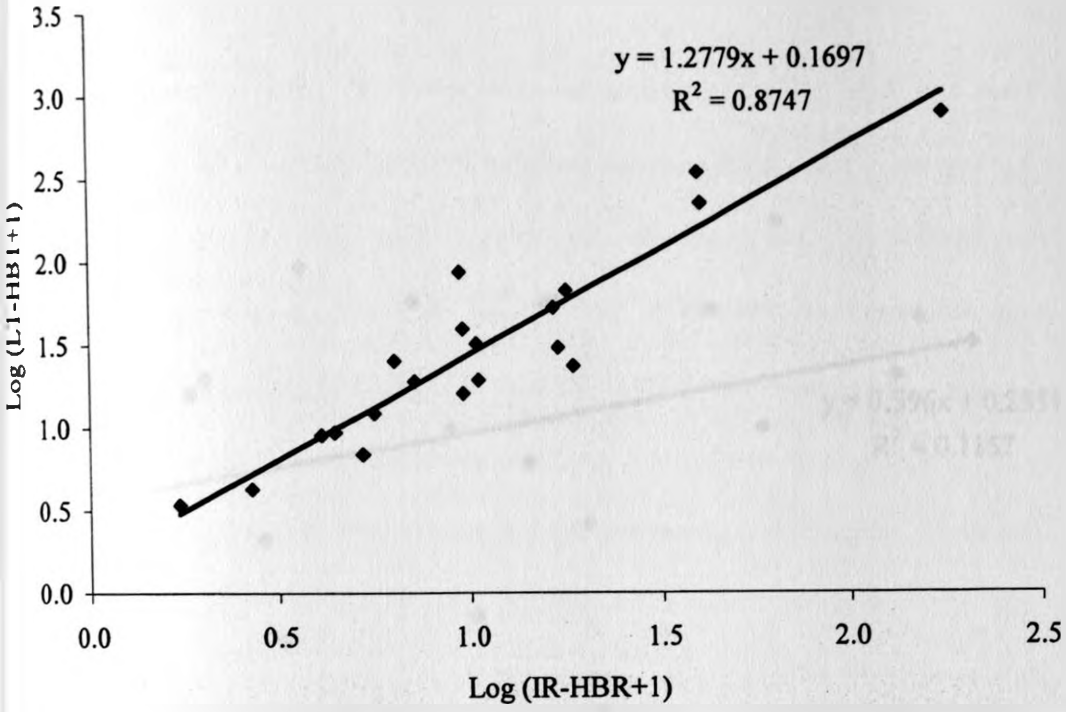
| ear | Month | Rainfall (mm) | Kangichiri | | Karima | | Kiuria | | Rurumi | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | CDC | PSC | CDC | PSC | CDC | PSC | CDC | PSC |
| 05 | Apr | 115.70 | 1.09 | 1.34 | 1.69 | 2.03 | 1.11 | 0.61 | 1.46 | 0.96 |
| | May | 377.25 | 2.88 | 0.85 | 2.53 | 2.90 | 1.80 | 1.03 | 2.20 | 1.52 |
| | June | 8.30 | 2.07 | 0.93 | 1.44 | 1.75 | 0.92 | 0.73 | 0.66 | 0.61 |
| | July | 6.55 | 2.16 | 1.03 | 1.00 | 1.01 | 0.60 | 0.61 | 1.39 | 0.56 |
| | Aug | 5.70 | 2.14 | 1.36 | 0.97 | 1.10 | 0.96 | 0.87 | 0.53 | 0.62 |
| | Sep | 5.35 | 4.02 | 1.89 | 2.07 | 2.00 | 2.68 | 1.57 | 0.84 | 0.92 |
| | Oct | 61.90 | 2.71 | 1.19 | 1.22 | 2.03 | 1.38 | 0.88 | 0.48 | 0.80 |
| | Nov | 55.30 | 1.88 | 0.61 | 1.44 | 1.19 | 0.75 | 0.44 | 0.30 | 0.74 |
| | Dec | 4.50 | 0.55 | 0.19 | 1.07 | 0.74 | 0.35 | 0.23 | 0.53 | 0.47 |
| 06 | Jan | 14.00 | 0.77 | 0.22 | 1.45 | 1.26 | 0.55 | 0.20 | 0.99 | 0.78 |
| | Feb | 5.25 | 0.74 | 0.46 | 1.95 | 1.72 | 0.45 | 0.23 | 0.25 | 0.45 |
| | Mar | 152.20 | 2.59 | 1.17 | 2.72 | 1.81 | 0.61 | 0.75 | 0.89 | 1.14 |
| | Apr | 215.70 | 1.99 | 0.75 | 2.72 | 1.68 | 0.76 | 0.61 | 1.03 | 0.89 |
| | May | 174.75 | 2.35 | 0.65 | 3.49 | 1.85 | 1.10 | 0.60 | 1.19 | 0.80 |
| | June | 13.35 | 1.66 | 0.85 | 2.59 | 1.91 | 0.53 | 0.47 | 1.13 | 0.84 |
| | July | 12.30 | 1.78 | 0.69 | 0.93 | 1.02 | 0.68 | 0.43 | 0.38 | 0.45 |
| | Aug | 12.58 | 2.67 | 1.24 | 1.80 | 1.56 | 1.65 | 1.18 | 1.14 | 1.04 |
| | Sep | 13.30 | 4.31 | 2.05 | 3.49 | 3.11 | 2.48 | 1.97 | 1.42 | 1.83 |
| | Oct | 110.55 | 2.10 | 0.72 | 2.11 | 1.27 | 0.73 | 0.66 | 0.67 | 0.48 |
| | Nov | 413.10 | 0.79 | 0.07 | 0.65 | 0.55 | 0.18 | 0.07 | 0.43 | 0.32 |

ble 6.3: Monthly variation in human biting rates (bites/person/night for *An. funestus* caught ough light traps and PSC in Mwea irrigated rice agro-ecosystem.

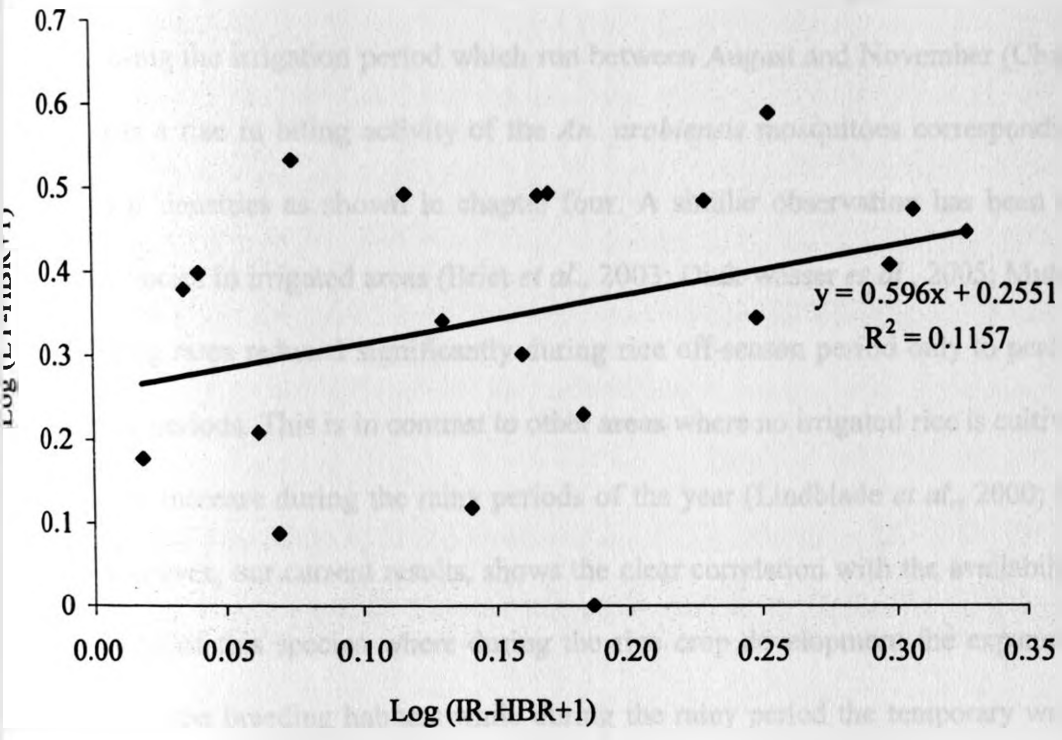
| ar | Month | Rainfall (mm) | Kangichiri | | Karima | | Kiuria | | Rurumi | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | PSC | CDC | PSC | CDC | PSC | CDC | PSC | CDC |
| 05 | Apr | 115.70 | 0 | 0 | 0 | 0 | 0.18 | 0 | 0 | 0 |
| | May | 377.25 | 0.07 | 0 | 0 | 0.37 | 0 | 0.05 | 0.29 | 0 |
| | June | 8.30 | 0.13 | 0.16 | 0 | 0.55 | 0 | 0.16 | 0.13 | 0.26 |
| | July | 6.55 | 0.21 | 0.39 | 0.46 | 0.67 | 0.19 | 0.17 | 0.18 | 0.86 |
| | Aug | 5.70 | 0.06 | 0.08 | 0.21 | 0.45 | 0.11 | 0.18 | 0.21 | 0.15 |
| | Sep | 5.35 | 0 | 0 | 0.40 | 0.20 | 0.18 | 0.40 | 0.13 | 0 |
| | Oct | 61.90 | 0.05 | 0.37 | 0.50 | 0.66 | 0.13 | 0.09 | 0.26 | 0.88 |
| | Nov | 55.30 | 0.31 | 0.53 | 0.45 | 0.64 | 0.26 | 0.36 | 0.22 | 0.19 |
| | Dec | 4.50 | 0.32 | 0.64 | 0.60 | 0.43 | 0.20 | 0.47 | 0.18 | 0.30 |
| 06 | Jan | 14.00 | 0.23 | 0.43 | 0.12 | 0.74 | 0.06 | 0.43 | 0.06 | 0.13 |
| | Feb | 5.25 | 0.01 | 0.20 | 0.04 | 0.25 | 0.001 | 0.12 | 0.02 | 0.07 |
| | Mar | 152.20 | 0.002 | 0 | 0.09 | 0.67 | 0.001 | 0 | 0.07 | 0.20 |
| | Apr | 215.70 | 0.03 | 0.05 | 0.15 | 0.34 | 0.01 | 0 | 0.06 | 0.10 |
| | May | 174.75 | 0.07 | 0.47 | 0.46 | 0.91 | 0.04 | 0.31 | 0.11 | 0.31 |
| | June | 13.35 | 0.18 | 0.42 | 0.63 | 1.25 | 0.06 | 0.11 | 0.14 | 0.54 |
| | July | 12.30 | 0.14 | 0.42 | 0.27 | 0.44 | 0.05 | 0.12 | 0.18 | 0.22 |
| | Aug | 12.58 | 0.06 | 0 | 0.15 | 0.00 | 0.01 | 0.20 | 0.07 | 0.17 |
| | Sep | 13.30 | 0.01 | 0.24 | 0.09 | 0.79 | 0.002 | 0.07 | 0.04 | 0 |
| | Oct | 110.55 | 0.06 | 0.60 | 0.19 | 0.76 | 0.02 | 0.22 | 0.02 | 0 |
| | Nov | 413.10 | 0.09 | 0.35 | 0.43 | 1.09 | 0.10 | 0.44 | 0.08 | 0.12 |

### 6.4.3. Relationship between light trap and indoor resting mosquito biting rates

relationship between the biting rates (including zero) estimated from light traps and indoor ing *An. arabiensis* and *An. funestus* is shown in figure 6.1. The Pearson correlation fficient (r) for *An. arabiensis* biting rates was 0.935 ($p < 0.05$) in the villages. A similar test the mean biting rates for light trap against indoor resting collected *An. funestus* (Fig. 6.2) ıples resulted in a Pearson correlation coefficient (r) of 0.340 ($p > 0.05$). These results clearly icate a correlation between the light traps and indoor resting populations for both *An. ıbiensis* and *An. funestus* in the irrigated rice agro-village complexes.




gure 6.1: The relationship between indoor resting human biting rate (IR-HBR) and light trap BR (Lt-HBR) of *An. arabiensis* in Mwea rice agro-village complexes.




gure 6.2: The relationship between Indoor resting human biting rate (IR-HBR) and light trap BR (Lt-HBR) of *An. funestus* in irrigated rice study villages.

## 6.5. Discussion

ed on the current results of biting rate, *An. arabiensis* is strongly associated with rice ivation. During the irrigation period which run between August and November (Chapter two 3), there was a rise in biting activity of the *An. arabiensis* mosquitoes corresponding to an ease in their densities as shown in chapter four. A similar observation has been made on lies of the species in irrigated areas (Briet *et al.*, 2003; Diuk-wasser *et al.*, 2005; Muturi *et al.*, 8). The biting rates reduced significantly during rice off-season period only to peak slightly ing the rainy periods. This is in contrast to other areas where no irrigated rice is cultivated and ng rates only increase during the rainy periods of the year (Lindblade *et al.*, 2000; Shililu *et* 2003c). However, our current results, shows the clear correlation with the availability of the eding habitats of this species where during the rice crop development the expansive water faces serve as the breeding habitats while during the rainy period the temporary water pools d other habitat types are the main source of *An. arabiensis* (Lacey and Lacey 1990; Mwangi d Mukiama, 1992). In contrast to *An. arabiensis,* the biting densities of *An. funestus* reduced gnificantly during the rice cropping period only to achieve maximum rates during the off- ason period. The biting rates increase between November and January, during harvesting and ost-harvesting periods. However, during the land preparion period occurring between April and ly there was progressive rise in biting rates which also coincided with the end of long rains in ie region.

Although the indoor biting rates for the two species were shown to differ among the villages, the overall pattern of increase and decrease was generally similar. However, it should be noted that, Karima village which was shown to have significantly higher biting densities than other villages

oth *An. arabiensis* and *An. funestus* from the two estimation methods is surrounded by rice s in a radius of more than 1 km. Hence the blood seeking anopheline mosquitoes with a t rage of approximately a kilometer (Thomson *et al.*, 1995; Dolo *et al* 1996) opted to seek d meals in nearest human habitation from their breeding locality (Faye *et al.*, 1992, 1995; ı *et al.*, 2004a; 2007). This contrasted with the other villages with distances between them other aggregated human habitation being less than a kilometer resulting in a reduced sure of anopheline mosquito biting densities. Most of the previous studies in rice irrigated s have shown significant variations of the malaria vector densities and consequently malaria smission potential between irrigated areas and neighboring non-irrigated villages (Dossou-ʻo *et al.*, 1994; Marrama *et al* 1995; Mutero *et al.*, 2004b; Muturi *et al.*, 2008) but few have wn these variations amongst the villages/sites within the irrigation agro-ecosystems. ɪefore, the current results re-emphasize the need for time and site specific targeting of vector trol interventions (Gu and Novak, 2005) even in loci perceived to share similar ʻironmental parameters.

e study demonstrated a clear correlation between the human biting rate estimates from light ps and indoor resting mosquito biting rates thereby showing the reliability of the light traps in imating the human biting rates. Although Mbogo and others (1993a) indicated that light traps uld not provide an adequate estimate of *An. gambiae* mosquitoes, other studies (Lines *et al.*, ⁹91; Davis *et al.* 1995; Costantini *et al.*, 1998; Magbity *et al.*, 2002) have shown light traps to : an efficient and unbiased estimate of human biting rates. In most studies, the efficiency of ght traps in human biting rates estimation has mostly been compared with human-bait ollections (Costantini *et al.*, 1998; Magbity *et al.*, 2002). However, the biting rates estimates

ed from the proportion of human blood-fed females caught resting indoors (IR-HBF) has n shown to be more realistic (Githeko *et al.*, 1993) and hence its comparison with light traps ne current study.

en mosquito density is high, more people receive mosquito bites, a common phenomenon in gated areas characterized with high anophelism (Diuk-wasser *et al.*, 2005) especially during rice cultivation period. The human biting rates of these disease vectors influence the smission rate of vector-borne parasites especially malaria parasites where the infection status the mosquito vector affect its biting rate. *Plasmodium falciparum* infected *Anopheles* squito requires a larger blood meal than uninfected one which may consequently result in the tor biting several people per night in order to acquire enough blood meal (Koella *et al.*, 98). Hence, it is possible to sustain malaria transmission even at low vector population density cessitating the evaluation of disease transmission status regularly as this is important in sessing the epidemiology of malaria in an area. Thus, the observed increase in biting rates thin the irrigated agro-village complexes may have a profound bearing on malaria nsmission risk and hence the planning and implementation of vector control interventions ould aim at reducing human biting rates. This can be achieved through integrated vector anagement aimed at the adult stages like use of bednets, indoor residual spraying, zoophylaxis nd those against larvae such as microbial larviciding.

APTER SEVEN

## *Plasmodium falciparum* Sporozoite and Entomological Inoculation Rates of Malaria Vectors in Mwea Irrigated Rice Agro-Ecosystem


STRACT

entomological study was conducted on the major malaria vectors in Mwea irrigated rice agro-system and their relative contribution to *Plasmodium falciparum* transmission in the irrigated agro-village complexes. A total of 9,251 samples of *Anopheles arabiensis* (81.37 %) and *funestus* (16.63 %) were tested by ELISA for *P. falciparum* circumsporozoite proteins and lded an overall positivity rate of 0. 43 % (40). The *An. arabiensis* sporozoite rate of 0.51 % (n 7,527) was higher than that of *An. funestus* at 0.02 % (n = 1,724) within the irrigated rice agro-lages. The malaria transmission intensity was not particularly high as was revealed by the latively low entomological inoculation rates (EIR) obtained from the two species. The EIR lues for *An. arabiensis* were 0.53 infective bites per person per night (ib/p/n) and 0.02 ib/p/n r *An. funestus* in Mwea irrigated agro-ecosystem. The highest inoculation rate for *An. rabiensis* (1.91 ib/p/n) was obtained in September, 2006 while inoculation rates for *An. funestus* ere only obtained in March, and December, 2006 at 0.42 and 0.15 ib/p/n respectively. The esults of this study shows that malaria transmission intensity in Mwea irrigated rice agro-village omplexes is low but perennial and largely maintained by *An. arabiensis* and *An. funestus*.

## 7.1. Introduction

laria vector control programmes aim at reducing the risk of malaria infections and it is ential to know and understand the level of transmission at the onset of vector control grammes and at the end (Githeko *et al.*, 1993). Many studies in Africa have demonstrated that ndard vector control measures are important in controlling and even eliminating malaria in tain areas where transmission levels are marginal (Mouchet *et al.*, 1998). As a foundation of laria vector control, actions that decrease vector-host contact through methods such as vicides application in larval habitats, larval habitat modification, indoor residual insecticide aying and use of insecticide treated bednets (ITNs) have shown correspondingly beneficial comes in terms of reduction of malaria morbidity. However, it is essential and necessary to fine quantitatively how much control is needed to achieve the desired impacts (Beier *et al.*, 99). Hence, measuring important indices related to malaria transmission would greatly help in e epidemiological assessment and control of malaria. Malaria transmission intensity in a gion can be evaluated by measuring important indices that quantify and numerically describe e dynamics of malaria infection. The main index used to estimate the intensity of malaria rasite transmission under field conditions is the entomological inoculation rate - EIR lacDonald, 1957; Rogers *et al.*, 2002; Vythilingam *et al.*, 2005).

IR contains two parameters that are directly measurable in a vector population, the human ting rates and proportion of mosquitoes carrying sporozoites in their salivary glands. The EIR alues are essential in assessing malaria situation and predicting epidemics (Onori and Grab, 980). It has also been shown (Takken and Lindsay, 2003) that, EIR has the best strategy for the levelopment of malaria control interventions aimed at lowering the force of transmission by

ducing in infectious mosquito bites. Beier and others (1994) showed a strong relationship :tween entomological inoculation rates and malaria incidence in Kenya where the EIR values :lped to explain 74% of child attack rates. Hence, effective vector control measures could :crease the incidence and prevalence of *P. falciparum* infections in Africa if the control easures reduce EIRs to levels that can be considered acceptable from a public health :rspective. Annual EIR values within Africa have been observed to range between 0.1 to over )00 depending on eco-epidemiological conditions of the locality (Hay *et al.,* 2000; Smith *et al.*, )01). Accordingly, the malaria status in African is determined by the EIRs with values <10 gnifying areas of unstable and values >100 indicating stable malaria while areas with values in :tween these values (10-100), vary in malaria endemicity depending on environment and emographic conditions such as rainfall, vegetation cover, human population density and land se patterns. For example, EIR estimates by Ijumba (1997) showed that an unprotected person in ice irrigation scheme receive 124 infective bites annually from *An. arabiensis*.

he importance of irrigated rice farming to the socio-economic development of a community and ounties cannot be underestimated. Currently, irrigated rice agro-ecosystems are estimated to epresent 55 % of the worlds harvested rice area contributing approximately three-quarters of the vorld's rice production (Keiser *et al.*, 2002). However, this apparent benefit comes along with dditional and more permanent mosquito breeding sites resulting in high malaria vectors density Chandler and Highton, 1975; Robert *et al.*, 1988; Lindsay *et al.*, 1991; Dolo *et al.*, 2004). The consequence of irrigation and increased vector density is rise in malaria prevalence and morbidity (Sissoko *et al.*, 2004) after pushing the transmission levels over a threshold (Bradley 1988), especially where conditions were previously unfavourable for malaria parasite and vector

velopment (Shililu *et al.*, 1998). For example, introduction of irrigation farming resulted in a ve-fold increase in malaria incidences in Mahaweli region of Sri Lanka (IIMI, 1986) and by ven fold in Tigray region of Ethiopia (Yohannes et al., 2005). However, irrigated areas are also sociated with reduced malaria incidences (Sissoko *et al.,* 2004) and sporozoite rates (Robert *et* ., 1985, 1992) especially compared to other non-irrigated areas (Githeko *et al.*, 1993; Ijumba *et* ., 2002a, 2002b). Thus, the malaria transmission intensity is complex and dependent on local arameters that affect the development of vectors capable of adapting to local ecological factors ecific to the area.

he understanding of the vectorial efficiency of *Anopheles* species in a region is important in the pidemiology of malaria since man-vector contact and sporozoite rates are good indicators of the vel of malaria transmission in a given area (MacDonald, 1957). This parameter of EIR, when roperly estimated, is important in evaluation of control measures. In the current study, porozoite rates and EIRs for two of the most important malaria vectors in Mwea irrigation cheme, *An. arabiensis* and *An. funestus* were determined in order to estimate the frequency of porozoite challenge to the human population living in villages within Mwea irrigated rice agro-cosystem.

## 7.2. Materials and methods

### 7.2.1. Mosquito collection for sporozoite analysis

The study was conducted within four villages out of the more than forty settlement villages in the Mwea-tebere rice irrigation scheme. The four irrigated villages involved in this study and (Kangiciri, Kiuria, Karima and Rurumi) are described in Chapter two. Indoor-resting mosquitoes were collected by pyrethrum spray catch (PSC) method (WHO, 1975) and outdoor populations were collected by Centers for Disease Control (CDC) miniature light traps (J.W. Hock Ltd, Gainesville, FL., USA). A detailed explanation of the sampling strategy has been described in Chapter four.

### 7.2.2. Laboratory processing of mosquito specimens

All specimens collected by PSC and Light traps methods were identified to species level by morphological characteristics using Gillies and De Meillon (1968). They were then categorized by abdominal conditions as fed, unfed, half-gravid or gravid. All mosquitoes from each collection were dried in anhydrous calcium sulphate for a minimum of five days and later placed individually in labeled vials. A subset of female mosquitoes representing all anopheline species collected both indoors and outdoors each month were selected for sporozoite and blood meal analysis.

### 7.2.3. Sporozoite ELISA testing

e method by Beier *et al.*, (1988) for processing the malaria vector mosquitoes for sporozoite ting was employed. Individual mosquitoes were cut transversely at the intersection between thorax and the abdomen. The abdomen section was placed in an individually labelled 1.5 ml crofuge tube for further processing of blood meals (Chapter Five) The anterior portion (head d thorax) was placed in a 1.5 ml microfuge tube containing 50µl boiled casein blocking buffer ich contained: 5.0g, casein in 100 ml 0.1 N sodium hydroxide, 0.1 thimerosal, 0.01 g phenol l, 900 ml phosphate buffered saline powder (PBS), Ph 7.4 with Nonidet P-40 (NP-40)(5 µl '40/1ml BB). The samples were triturated manually with plastic tissue grinders. After urating, 200µl of blocking buffer was added to each sample to bring the final volume to 250µl r mosquito sample. Samples of the mosquito triturates were stored at –20°C until they were ted.

96-well polyvinyl microtitre plate was used and all of its wells coated with 0.1 µg *P. lciparum* capture monoclonal antibody diluted in 50 µl PBS/well and incubated for 30 minutes room temperature in subdued light. Before the testing process, triturates were removed from e freezer and left to thaw at room temperature. After 30 minutes, the well contents were pirated and the wells filled with 200 µl of blocking buffer to block the remaining active sites. fter one hour, the blocking buffer was aspirated and 50 µl aliquots of each homogenized osquito triturate added to each well, leaving four wells for negative and two for positive ntrols. In the first well of the microtitre plate an aliquot containing 100 picograms (pg) of combinant *P. falciparum* positive control CS protein ($R32tet_{32}$) in 50 µl blocking buffer was dded as positive controls (Wirtz *et al.*, 1987). In the next four wells 50 µl triturates of wild-

aught males of *An. arabiensis* or *An. funestus* in blocking buffer was added as negative controls. After 2 hours of incubation, the mosquito triturate was aspirated and the wells washed two times with PBS-Tween 20 solution. The plates were then shaken dry and 50 µl of peroxides conjugated monoclonal antibody added to each well. After one hour, the solution was aspirated and the plates washed three times with PBS-Tween 20 solution and 100 µl of peroxides substrate added to each well. The plates were then placed in the dark for 30 minutes after which samples were assessed visually for positivity (Beier and Koros, 1991). A detailed explanation of the protocol followed is provided in appendix II.

### 7.3. Data analysis

Data were entered in Microsoft excel software and analyzed using SPSS version 11.0 software (SPSS Institute, Chicago, IL). The human biting rates (HBR) for calculation of EIR were adopted from chapter six but pooled from all the villages for the monthly calculations. The sporozoite rates were calculated as the proportion of female *Anopheles* mosquitoes from the two species carrying infective sporozoites in the head-thorax of the total number tested. Entomological inoculation rates expressed as the number of infective bites per unit time were derived directly from a product of the sporozoite rates and product of the human biting rates and human blood index. Analysis of variance and chi-square were used to analyze difference in sporozoite rates and EIRs between seasons, species and villages.

## 7.4. Results

### 7.4.1. Sporozoite rates

A total of 9,251 *Anopheles* mosquito samples from indoor and outdoor resting sites were tested for *Plasmodium falciparum* circumsporozoite (CS) antigen yielding an overall positivity rate of 0.43 % for the four irrigated rice agro-village complexes. Of these, 7, 527 (81.37 %) were *An. arabiensis* and 1, 724 (18.63 %) were *An. funestus* (Table 7.1). The difference between the mean *P. falciparum* sporozoite infection rates for *An. arabiensis* (0.51 %) and *An. funestus* (0.12 %) was significant among the study villages ($\chi^2 = 4.90$, df = 1, $p < 0.05$). None of the other species tested were positive for *P. falciparum* sporozoites.

Out of the 7,527 *An. arabiensis* samples tested for *P. falciparum* CS protein, only 38 were positive resulting in a sporozoite rate of 0.51 % (Table 7.1). A higher proportion (86.84 %) of those positive for *P. falciparum* CS proteins were collected resting indoors compared to those collected outdoors (13.16 %). Although the malaria sporozoite rates for this species were not significantly different (F= 1.02, df= 3, 100 p = 0.481) among the four villages over the entire sampling period, relatively high sporozoite rates were observed in Kangichiri (0.69 %) and Karima (0.53 % compared to Kiuria (0.49 %) and Rurumi (0.38) (Table 7.1). The *P. falciparum* sporozoite rates for *An. arabiensis* were generally low during the study period ranging from zero in some months to a maximum of 1.51 % in July 2006. The sporozoite rates were usually low during the first half of each year, only to peak during initial stages of the third and four quarter of

the year (Table 7.2). These are periods characterized by rice cultivation and it is immediately after peak densities of *An. arabiensis* adults.

Of the 1,724 *Anopheles funestus* specimens tested for *P. falciparum* CS antigens, only two individuals tested positive yielding an overall sporozoite rates of 0.12 % for Mwea irrigated rice agro-village complexes. Two villages only, Karima and Rurumi, recorded positive *P. falciparum* CS antigens for this species at 0.15 and 0.23 % respectively (Table 7.1). Except for the months of March and December 2006, when 9.09 % and 1.85 % sporozoite positive *An. funestus* were recorded, the parasites were absent in this species during the entire study period (Table 7.2).

Table 7.1: Sporozoite and entomological inoculation rates (EIR) of *An. arabiensis* and *An. Funestus* species, in the four study villages.

| | Kangichiri | Karima | Kiuria | Rurumi | Total |
|---|---|---|---|---|---|
| *An. arabiensis* | | | | | |
| No. ELISA tested | 1431 | 2082 | 1633 | 2327 | 7511 |
| Positive for sporozoite | 10 | 11 | 8 | 9 | 38 |
| Sporozoite rate | 0.69 | 0.53 | 0.49 | 0.38 | 0.51 |
| EIR | 0.72 | 0.85 | 0.36 | 0.30 | 0.53 |
| *An. Funestus* | | | | | |
| No. ELISA tested | 367 | 654 | 275 | 426 | 1722 |
| Positive for sporozoite | 0 | 1 | 0 | 1 | 2 |
| Sporozoite rate | 0.00 | 0.15 | 0.00 | 0.23 | 0.12 |
| EIR | 0.00 | 0.04 | 0.00 | 0.02 | 0.02 |

### 7.4.2. Entomological inoculation rates

The entomological inoculation rates (EIR) calculated as a product of human biting rates, human blood index and sporozoite rates for *An. arabiensis* and *An. funestus* during the study period is shown in Table 7.2. The mean sporozoite inoculation rates in Mwea irrigated rice agro-village complexes for the two-year study period was 0.53 infective bites per person per night for *An. arabiensis* and 0.02 ib/p/n for *An. funestus*. The combined sporozoite inoculation rate for both species was 0.30 ib/p/n giving an average of 109.62 ib/p/year in Mwea irrigated rice agro-ecosystem. A summary of the entomological inoculation rates for *An. arabiensis* and *An. funestus* in the study villages is shown in Table 7.1.

The EIRs for *An. arabiensis* were generally low throughout the study period, ranging from zero during dry months of the year and off season of rice cultivation to 0.35- 1.17 ib/p/n during long rain months and 0.53 – 1.91 ib/p/n during the rice cultivation season (Table 7.2.). The mean monthly EIRs differed significantly between the months during the study period ($\chi^2$= 49.85, df= 16, $p< 0.05$). Despite the monthly variation in *An. arabiensis* EIR, correlation analysis revealed no significant linear relation between EIR and weather variables including rainfall (r = 0.25), temperature (r = 0.22) and relative humidity (r = 0.34) all at p = 0.05.

Overall, *Anopheles funestus* accounted for relatively low levels and few cases of infective bites (0.02ib/p/n) from *Anopheles* mosquitoes compared to *An. arabiensis* (Table 7.1). However, in the months of March and December the species presents a real risk to malaria infections

recording high infective biting rates at 0.42 and 0.15 ib/p/n respectively (Table 7.2). However, during most of the year, the species apparently present minor risk in malaria transmission.

Table 7.2: Monthly entomological parameters of *Anopheles arabiensis* and *An. funestus* in the Mwea irrigated rice agro-ecosystem.

| Year | Month | Rainfall (mm) | *An. arabiensis* | | | | *An. funestus* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | #tested | HBR | SR | EIR | #tested | HBR | SR | EIR |
| 2005 | Mar | | 108 | 0.93 | 0.00 | 0.00 | 2 | 0.10 | 0 | 0.00 |
| | Apr | 115.7 | 315 | 1.29 | 0.63 | 0.82 | 10 | 0.19 | 0 | 0.00 |
| | May | 377.25 | 248 | 1.84 | 0.40 | 0.74 | 5 | 0.13 | 0 | 0.00 |
| | Jun | 8.3 | 279 | 1.09 | 1.08 | 1.17 | 9 | 0.29 | 0 | 0.00 |
| | Jul | 6.55 | 234 | 0.95 | 0.00 | 0.00 | 25 | 0.33 | 0 | 0.00 |
| | Aug | 5.7 | 190 | 1.01 | 0.53 | 0.53 | 40 | 0.19 | 0 | 0.00 |
| | Sep | 5.35 | 221 | 1.69 | 0.45 | 0.76 | 7 | 0.26 | 0 | 0.00 |
| | Oct | 61.9 | 178 | 1.26 | 0.00 | 0.00 | 21 | 0.31 | 0 | 0.00 |
| | Nov | 55.3 | 173 | 0.79 | 0.00 | 0.00 | 98 | 0.34 | 0 | 0.00 |
| | Dec | 4.5 | 106 | 0.45 | 0.94 | 0.43 | 117 | 0.34 | 0 | 0.00 |
| 2006 | Jan | 14 | 201 | 0.66 | 0.00 | 0.00 | 119 | 0.14 | 0 | 0.00 |
| | Feb | 5.25 | 184 | 0.73 | 0.00 | 0.00 | 15 | 0.02 | 0 | 0.00 |
| | Mar | 152.2 | 245 | 1.27 | 0.00 | 0.00 | 11 | 0.05 | 9.09 | 0.42 |
| | Apr | 215.7 | 305 | 1.06 | 0.33 | 0.35 | 26 | 0.07 | 0 | 0.00 |
| | May | 174.75 | 225 | 1.11 | 0.44 | 0.49 | 81 | 0.20 | 0 | 0.00 |
| | Jun | 13.35 | 314 | 1.07 | 0.00 | 0.00 | 134 | 0.27 | 0 | 0.00 |
| | Jul | 12.3 | 531 | 0.69 | 1.51 | 1.04 | 231 | 0.17 | 0 | 0.00 |
| | Aug | 12.575 | 664 | 1.31 | 0.45 | 0.59 | 60 | 0.07 | 0 | 0.00 |
| | Sep | 13.3 | 607 | 2.32 | 0.82 | 1.91 | 48 | 0.04 | 0 | 0.00 |
| | Oct | 110.55 | 519 | 0.88 | 1.16 | 1.01 | 112 | 0.10 | 0 | 0.00 |
| | Nov | 413.1 | 387 | 0.27 | 0.26 | 0.07 | 229 | 0.19 | 0 | 0.00 |
| | Dec | 131.9 | 265 | 0.57 | 0.38 | 0.22 | 54 | 0.08 | 1.85 | 0.15 |
| 2007 | Jan | 25.95 | 279 | 1.19 | 0.72 | 0.86 | 37 | 0.07 | 0 | 0.00 |
| | Feb | 1 | 236 | 1.09 | 0.00 | 0.00 | 50 | 0.11 | 0 | 0.00 |
| | Mar | 64.85 | 262 | 0.39 | 0.38 | 0.15 | 125 | 0.20 | 0 | 0.00 |
| | Apr | 252.1 | 251 | 0.59 | 0.00 | 0.00 | 57 | 0.10 | 0 | 0.00 |

HBR= human biting rates; SR= sporozoite rates; EIR= entomological inoculation rates. Results of indoor and indoor resting collections anophelines were pooled together.

## 7.5. Discussion

The current study has demonstrated that malaria transmission in Mwea irrigated rice agro-village complexes was primarily driven by *An. arabiensis* and, to a lesser extent, by *An. funestus*. The sporozoite rates for both species were generally low in the study area in most part of the study period. However, these results are in tandem with previous results in the area (Ijumba *et al.*, 1990; Mukiama and Mwangi, 1990; Muturi *et al.*, 2008) and other irrigated rice areas (Sissoko *et al.*, 2004), which showed low sporozoite rates in irrigated rice agro-ecosystems especially for *An. arabiensis*. The mean sporozoite rates for *An. arabiensis* of 0.51 % obtained in Mwea irrigated rice agro-ecosystem during the current study compares closely with those obtained by Githeko and others (1993) in Ahero but were lower compared to those obtained in neighbouring non-irrigated areas (Marrama *et al.*, 2004; Dolo *et al.*, 2004). All these data for *An. arabiensis* indicates that, its true sporozoite rates generally range between 0.50 and 1.0 % in irrigated rice agro-ecosystems. Although the sporozoite rates for this species were higher in some months than others there were no obvious peak periods. *Anopheles funestus* females in the Mwea rice irrigation scheme (MRIS) had sporozoites rates ranging between 0 to 9.09 % during the study period. However, sporozoite positive *An. funestus* were rarely encountered only appearing in March and December, 2006. However, the range in sporozoite rates for this species was similar to that found in Ahero (Githeko *et al.*, 1993) but the mean sporozoites rates were significantly lower especially compared to those recorded for the surrounding non-irrigated areas (Muturi *et al.*, 2008). Furthermore, the low sporozoite rates for *An. funestus* are expected since for unknown reasons, the species has been shown to be rare in irrigated rice agro-ecosystems of Africa with exceptions to Ahero rice scheme in Kenya (Githeko *et al.*, 1993), and rice growing areas of Madagascar (Marrama *et al.*, 1995).

Our results of EIRs indicated that a resident of Mwea irrigated rice agro-village complexes was at risk of receiving 0.30 % ib/p/n from both *An. arabiensis* and *An. funestus* females throughout the year. Probably owing to its high densities in the irrigated agro-villages much of the risk in malaria transmission was presented from *An. arabiensis* with inoculation rates ranging between 0 and 1.91 ib/p/n compared to the risk presented by *An. funestus* at 0-0.4 ib/p/n. The malaria transmission dynamics of the two species as indicated in the current study corroborates previous studies in the area and other irrigated areas (Ijumba *et al.*, 1990; Mukiama and Mwangi, 1990; Githeko *et al.*, 1993; Ijumba and Lindsay, 2001; Muturi *et al.*, 2008). However, the observed EIRs in the irrigated agro-villages do not present a high malaria transmission risk as that has been found in other parts of Kenya such as the Coastal and Western region (Beier *et al.*, 1990; Petrarca *et al.*, 1991; Mbogo *et al.*, 1993b). Several factors are likely to explain the malaria transmission risk by mosquitoes observed in the study area, especially the disparity in potential of the two species. Key among possible factors is the low human blood index (HBI). Blood feeding behaviour studies for the two species in irrigated areas have shown low preference for human blood and a high affinity for bovid blood even among the usually anthropophilic species like *An. funestus* (Ijumba *et al.*, 1990; Githeko *et al.*, 1994 Mutero *et al.*, 2004b; Muriu *et al.*, 2008; Muturi *et al.*, 2008) which eventually influences EIRs and hence malaria transmission risk.

In further relation to the infective bites of the malaria vectors, our results for *An. arabiensis* indicate that high rates were obtained when the biting rates were relatively high. Similar results have been reported in previous studies in irrigated areas (Koudou *et al.*, 2005) where high infective rates were shown to coincide with high human biting rates. The dynamics of the biting

ates is usually a reflection of the vector density variation thus correlations with factors nfluencing malaria vector density. Importantly, our results showed that high *An. arabiensis* nfectious biting rates in Mwea irrigation scheme coincided with rice cultivation season and long ain seasons or slightly thereafter when the human biting density by the vector peaks (Chapter our and Six). This helps in maintaining transmission during rice cultivation periods and links up with the transmission incidences by *An. funestus* an observation similarly made by Githeko and thers (1993) in Ahero. However, previous studies in irrigated areas (Briet *et al.*, 2003: Dolo *et l.*, 2004; Mutero *et al.*, 2004b; Muturi *et al.*, 2006) have reported higher densities of mosquitoes n irrigated rice regions that do not necessarily translate to production of more *P. falciparum* arasite infections (Ijumba and Lindsay 2001). Other factors that could have accounted for the lisparities in sporozoite and infective rates but were not evaluated in this study include lifferences in daily survivorship and vectorial capacity (Ijumba and Lindsay 2001; Diuk-Wasser *t al.*, 2005) and the high percentage of people currently sleeping under ITNs. These factors oupled with simple changes in the house design seen in Mwea to reduce mosquito's nuisance in eneral such as mosquito screening of windows and eaves has been suggested to contribute ignificantly in reduction of exposure to infectious mosquito bites (Lindsay *et al.*, 2003).

## CHAPTER EIGHT:

## 8.0 The Potential role of *Bacillus thuringiensis* var *israelensis* and *Bacillus sphaericus* combined formulation (VBC-60120) for the control of *Anopheles* (Diptera: Culicidae) mosquitoes in Mwea irrigated rice agro-ecosystem.


### ABSTRACT

Microbial larviciding is becoming a popular tool in the management of malaria vectors in sub-Saharan Africa where malaria has become a major hindrance to economic development of various communities. The current study evaluated the potential of a combine formulation of *Bacillus thuringiensis* var *israelensis* and *Bacillus sphaericus* (VBC- 60120) in managing malaria vectors in irrigated rice agro-ecosystems. The microbial larvicides were tested for efficacy in open fields and subsequently applied onto different mosquito breeding habitats in an irrigated rice agro-village. In open field trials, a reduction of 87.48 - 100 % was realized for the late instar larvae. In the irrigated agro-village complex, a total of 650, rice paddies, 150 drainage canals and 31 temporary larval habitats were treated once with the microbial larvicide and monitored for anopheline mosquito larval development. A 100 % reduction in total larvae was realized in the first three days while the late instar larvae recolonized the habitats seven days post-larviciding. An ANOVA test did not reveal any significant reduction of the adult mosquito population after the application of the microbial larvicides. The effects of the microbial larvicides on immature stages of anopheline mosquito showed the relevance and role that can be exploited for microbial larvicides in expansive water surfaces presented in irrigated rice agro-ecosystems.

## 8.1. Introduction

The communities within and around Mwea rice irrigation scheme in Central Kenya, depend primarily on irrigated rice cultivation for food and income. Unfortunately, although irrigated agriculture is known to improve human nutrition, survival and social economic status of the surrounding communities (van der Hoek, 2004), it is also associated with ill human health resulting from water-related diseases (Lacey and Lacey, 1990; Service, 1989). Of particular importance, irrigated areas support higher densities of malaria vectors than neighbouring non-irrigated areas (Chandler *et al.*, 1975a; Mutero *et al.*, 2004b). The numerous water bodies resulting from irrigation facilities leads to production of large numbers of malaria vectors and extends their breeding season (Chandler *et al.*, 1975b; Mwangi and Mukiama, 1992). It is therefore considered mandatory to control disease vectors in these areas if the benefits associated with irrigated agriculture are to be realized.

Integrated vector control efforts in Africa are possible and can target all stages of mosquito life cycle although most energy has been directed on adult stages (Curtis *et al.*, 1994; Roberts *et al.*, 2000). Targeting of mosquito larval stages through larviciding and source reduction is important in mosquito control interventions as mosquitoes are killed before they disperse to human habitations and its success is usually high since the larvae, unlike adults cannot change their behaviour to avoid control measures targeted at the breeding habitats (Charlwood and Graves, 1987; Fillinger *et al.*, 2003; Yohannes *et al.*, 2005). Historically, these strategies have been used as the first line tools for mosquito control in regions where malaria transmission has been interrupted (Kitron and Spielman, 1989). Mosquito larval control through biological agents is becoming increasingly popular (Asimeng and Mutinga, 1993; Fillinger and Lindsay, 2006) and

an integral part in vector control interventions. The realization by most tropical countries of the need to integrate microbial larvicides with other mosquito control tools calls for more studies on its role in malaria vector management on a large scale. In irrigated villages, there is fundamental difference in productivity of breeding habitats during the year with a small but significant part of anopheline population breeding in small and transient habitats formed during the rainy season within the human settlements. These forms of habitats are mostly found proximal to human settlement contributing highly to the adult mosquito population within the surrounding villages. This is of great importance in malaria epidemiology since effective malaria transmission heavily depends on human-vector contact rate, which in turn is highly influenced by the distance from the mosquito breeding habitats to the location of human habitation (Carter *et al.*, 2000; Minakawa *et al.*, 2002).

Because of the strong association between larval densities and adult mosquito populations (Grillet, 2000), malaria transmission can be limited by vector control efforts aimed at suppressing larval production. The larvicidal activity of microbial insecticides varies among larval habitats (Shililu *et al.*, 2003b) and has been shown to result in reduction of malaria vectors and a potential impact on the overall disease transmission in endemic areas. Furthermore, mosquito larviciding activities do not require substantial change in the human behaviour or the management of key resources like water for irrigation and land management and the equipment for larviciding activities are simple, inexpensive with the acquisition of larviciding skills being regarded as easy (Becker and Rettich, 1994; Becker, 1998; Mukabana *et al.*, 2006). The current study investigated the potential role of microbial larvicides in the control of malaria vectors and transmission intensity in irrigated rice agro-ecosystem with expansive mosquito breeding sites.

The main goal of this study was to provide vital information on the new microbial formulation and its implementation in malaria vector control program in the Mwea irrigated rice scheme and its potential as a tool in integrated malaria vector control strategies.

## 8.2. Materials and Methods

### 8.2.1. Study area

The study was conducted in two villages within Mwea irrigation scheme in Kirinyaga district an area located 100km North East of Nairobi and to the East of Mount Kenya with an altitude range of 1100 - 1200 metres. A detailed description of the study area has been given in chapter two in this work (2.1.1.). Two villages, Karima and Rurumi, lying within the larger Mwea rice irrigation scheme were used in this study as treatment and control village respectively. Using a satellite derived image, all the larval habitats types (Chapter four) within a radius of one kilometre from the village perimeter were selected for the study (Fig 8.1).

### 8.2.2. Open field efficacy trials for microbial formulations

The biological microbial larvicide used in the treatment of the larval habitats was a combined formulation of VectoBac® and VectoLex® corn granules (VBC-60120 CG) that were provided by Valent BioSciences Corporation IL, USA) with the active ingredients being *Bacillus thuringiensis var. israelensis* (B.t.i) and *B. sphaericus* (Bs). VectoBac® (*B. t. i*) has five active toxic crystal proteins while VectoLex® (*B.sph*) has two endotoxins and all are activated by the alkaline environment and enzymes in the mosquito larval gut.

Open field trials for the corn granules (CG) formulations were conducted between August–October 2006 at Mwea Irrigation Agricultural Development Centre (MIAD centre) experimental rice fields. Fifty plastic basins (diameter 1 m) were buried into an open sunlit field without vegetation one acre paddy subdivided into 100 plots of 5 x 3 m arranged in ten rows. Five rows

of 10 plots each were used where two basins were placed per plot 1.5 m apart buried in soil up to 5cm above the ground level. Dry soil from a known "virgin" land that has not been subjected to rice cultivation and thus various insecticides used for pest control in rice farms, was added to each basin (one-third of its volume) to provide suitable biotic and abiotic conditions for mosquitoes. The basins were subsequently filled with underground water from the well at MIAD centre and maintained up to a depth of 8-10 cm throughout the trials. After soil in the basin was saturated with water, 150 late instar larvae (L3 and L4) collected from rice nursery seedbeds that had not been subjected to any insecticide treatment were added onto each basin. Further, the early instar larvae (L1, L2) colonizing the basins were counted and recorded. Care was taken not to allow adult mosquitoes to emerge, by removing mature pupae from all basins on daily basis.

Treatment concentrations were calculated on the basis of a standard water depth of 0.1 m and fixed surface area (Schnetter *et al.*, 1981; Ragoonanansingh *et al.*, 1992) for an application rate of 20 kg/ha for the B.t.i/Bs for formulation (1.50 gm/basin). During each replicate, ten basins served as controls, ten for Vectolex only, whereas the other 3 sets of ten basins of the remaining 30 basins received the test formulation. The basins for different treatments were randomly assigned so that control and test treatment basins had equal chances of colonization by other mosquitoes and non-target invertebrates during the experiment.

The corn granule formulations weighed for each test basin were applied with hand (broadcasting) and sprayed evenly over the entire water surface. Afterwards, all basins were examined daily for mosquito larvae and the average number of larvae calculated per standard 350 ml capacity mosquito dipper by taking five dips per basin, four from the periphery and one from the centre.

To avoid contamination different set of dippers were used in sampling pre-adult mosquitoes from each set of treatment, a set for contols, vectolex and combined formulation respectively. Immature mosquitoes were classified in three categories: early instars (L1, L2), late instars (third and fourth) and pupae. All larvae were counted, classified to genus and development stage and then returned to their respective basins.

### 8.2.3. Community participation in larvicides application

Prior to the large-scale microbicides application, a community awareness meeting in the concerned villages was convened involving local leaders, rice farmers and village residents. During this meeting, the community was educated about microbial larvicides especially application in the mosquito breeding habitats. The bio-safety of the microbial larvicides used and its activity was explained to the community who then welcomed the initiative in their villages and rice fields. The application procedures, equipments and modalities were also demonstrated to the community and objectives of the work explained. They were requested to volunteer some members of the community to be trained and conduct actual larvicides application. The entire village community actively participated in identifying habitats for treatment and ensuring every potential larval habitat within their homesteads/village was treated with the microbial larvicide formulation.

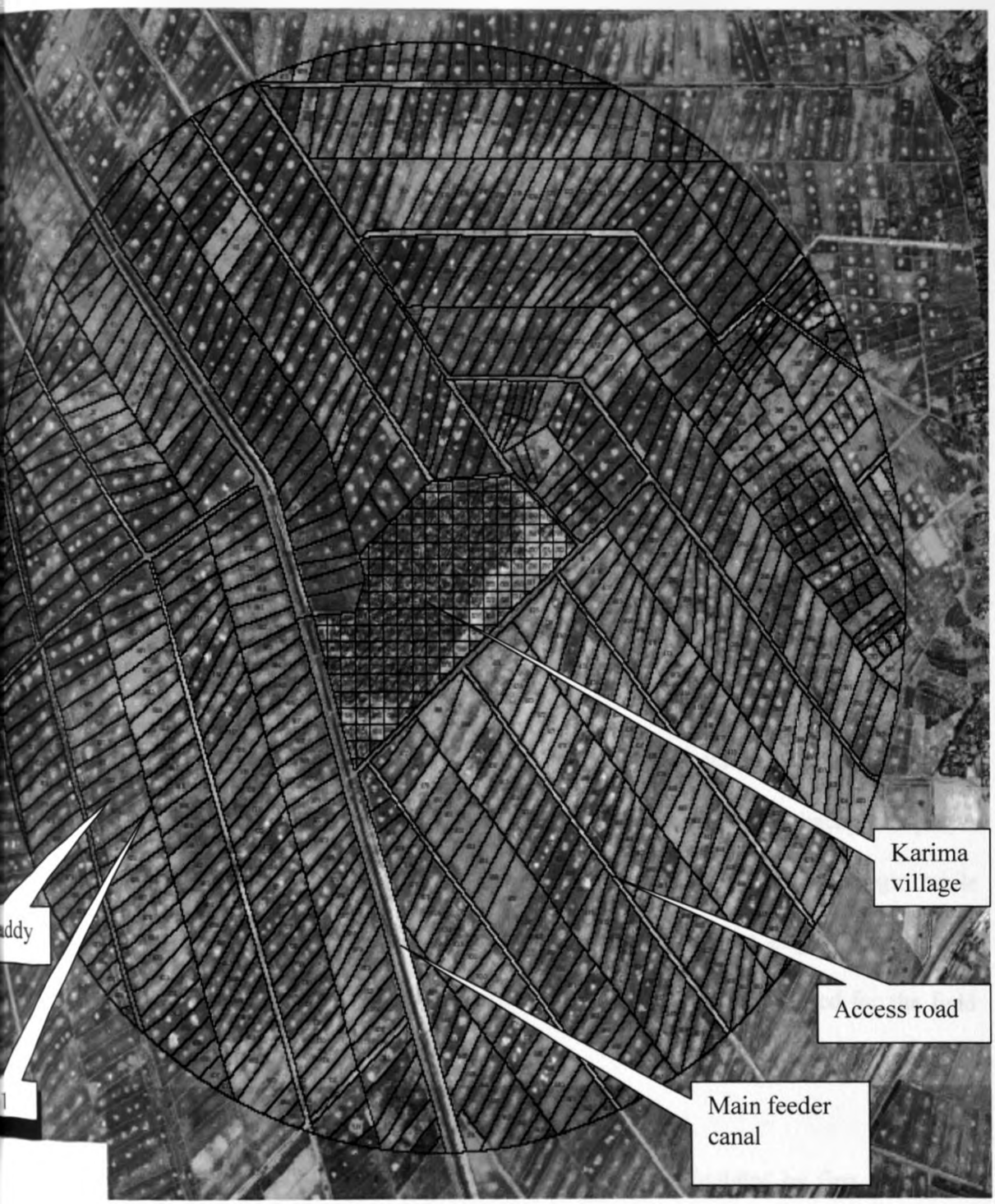



Figure 8.1: A grid based satellite image of Karima (intervention) village showing the village and rice paddies within the 1km radius.

### 8.2.4. Calibration of equipment

The application rate for *Bacillus thuringiensis* var *israelensis* and *Bacillus sphaericus* combined granular formulation (VBC-60120 CG) was 20kg/ha, which is dictated by the flow rate of the larvicide granules, speed of travel over the habitats while spraying and the width of the treated swath needed calibration. To achieve accurate dosage and complete coverage of the breeding habitats for a successful application rate, calibration of the application parameters was conducted in the field. A team of two specialists from Valent bioscience corporation laboratory trained project personnel (field assistants) and community members involved in the larvicide application on the calibrations and application of the larvicide in the field. The pump operated Maruyama power backpack sprayers (Maruyama U.S., Inc. MD157D/MD159: Duster, WA, USA) were used and calibrated to release 1kg of corn granules per minute to cover the required area.

Swath width was calculated by moving to an open flat field with the backpack sprayer fully filled with the larvicide granules. The application of the product was then done with backpack sprayer running at full throttle. The spray pipe was then swung (oscillated) left and to right while stationary and width covered with granules measured and then 10% of the length subtracted for overlap. After calibration, the swath width was set at 15m as standard to be used for the field larvicide application.

The applicators speed of travel in the flooded rice paddies was calculated by first measuring length and width in meters of typical rectangular rice paddy at the MIAD centre. The trained individual applicators (grouped in pairs) were guided through the flooded paddies while doing mock larvicide application and their comfortable working pace while carrying the power

ackpack sprayer for 100m length of the paddy timed. The exercise was repeated three times for ach group and the time then averaged to get the working speed for each team. For the whole roup the working speed of travel through the flooded rice paddies was set at 36m/min and twice hat speed while walking on dry surfaces.

he flow rate from the backpack machines was calibrated by first filling the backpack with ranules and the fuel tank with petrol mixed with engine oil in the ratio of 40:1. The entire ackpack sprayer with its contents was then weighed on a scale and the machine ran for two inutes at full throttle and expected flow setting. The backpack sprayer tank was then e-filled and weighed again. The second weight was subtracted from the first then divided by two o give the flow rate per minute and where necessary adjustments were made to the backpack prayer until the desired flow rate was achieved. Average readings with the granule tank at ¾ ull, ½ full and ¼ full were taken in order to get the most accurate flow rate reading. After alibration of every backpack sprayer, its flow rate and code number were marked on the granule nk. The verification of the best application rate was done by ensuring there are at least eight ranules/pellets of the larvicide in a 10 cm x 10cm quadrant on the water surface.

### 8.2.5. Microbial larvicides application

Karima (treatment) village, microbial larvicides were applied in all the rice paddies and canals ithin the one kilometre radius from the village perimeter and all other transient larval habitats ithin the peridomestic localities and along access roads and footpaths. In the control village Rurumi), a similar demarcation of habitats lying within the 1km radius was done but no rvicide application was conducted. Microbial larvicide application was done when the irrigated

elds were having the newly planted crop in the vegetative growth (transplanting/tillering stages) nd most of the paddies flooded with water and the temporary larval habitats were replenished y rainfall received during this period. Most of paddies in the region are rectangular in shape ith an average dimension of 100m x 60m. During application, a team of two applicators and ne field guide covered each paddy after calculating the number of times needed to move across ne paddy to effectively cover the entire habitat. Application was first done on the edges by aving one applicator moving on the dry edges of the paddy and spraying onto the paddy water urface to ensure complete and effective coverage. A guide helped the applicators in covering ach part of the paddy by ensuring they maintain the desired swath-length while in the flooded addies guiding on how to treat the various habitats with different dimensions. Canal treatment ith the larvicides was done whilst on the dry edges but varying the speed of movement to chieve the effective coverage. Treatment of the small water pools with the larvicides was done y broadcasting with hands or hand held sprayer over the water surface after calculating the mount needed for each habitat depending on its size. After treatment, each habitat was :ored/marked on the grid map in the hand held TDS recon computer units (Tripod Data ystems, Inc., OR, USA) detailing the date of treatment, grid code, and distance from the illage/house.

### 8.2.6. Mosquito larval sampling

total of 30 rice paddies and associated canals were randomly selected along four transects om the outer margins of each village to the maximum distance of 1km in addition to all eridomestic (temporary) larval habitats in each village. To increase the sensitivity of larval ollections, habitats were sampled by purposive sampling method whereby the sampler actively

earched for mosquito larvae and pupae making maximum number of dips where there was a igh concentration of mosquito immature stages. In the period immediately after application, the abitats were sampled on days 1, 3, 5, 7, 10, 15, 20 and 25. Larval sampling was done using a tandard dipper (350ml) whereby 40 dips and 20 dips were made from the rice paddies and eridomestic water pools/canals respectively. The larvae collected in each of the 40/20 dips were ooled together to constitute one larval/pupal collection for the particular habitat. The samples or each habitat were then placed in an individually labeled whirl pak and transferred to the aboratory for further processing

### 8.2.7. Adult mosquito sampling

dult mosquito collections were made through the Pyrethrum spray Catch (PSC) method and DC light traps (as described in chapter four). The PSC mosquito collections were made in venty houses every week in both the control and treatment villages. Six miniature CDC light aps were set at 1800hrs twice every week in each of the two villages for night collections until 500hrs the following day.

### 8.2.8. Processing of mosquito samples

ll the pre-adult samples collected in the field were placed into white enamel trays and sorted it into mosquito and other fauna per habitat. Mosquitoes sample were identified as either ulicine or anophelines and further categorized as early ($1^{st}$ and $2^{nd}$) or late $3^{rd}$ and $4^{th}$) instar ages. The fourth instar stages were preserved in 70% ethanol and used for identification to pecies level (Gillies and de Meillon, 1968). Pupal collections were placed in individually beled emergent cages for each habitat and the resultant adults identified to species. The adult

mosquito samples collected in the field through PSC and light trap methods were transferred to the laboratory for processing. The anopheline female samples were morphologically identified to species (Gillies and de Meilon, 1968).

### 8.3. Data analysis

Data on the larvae and adult mosquitoes were recorded in standard proforma prepared for both field and laboratory data entry. These data were then entered and cleaned in Microsoft excel computer program and analyzed by SPSS software. Descriptive statistics were used to show the variation in mosquito distribution at different locations and in the pre and post-treatment or intervention periods. ANOVA was used to test the difference between treated and untreated villages and changes in the populations of mosquitoes prior to, during and after microbial larviciding. The percentage reduction in larval mosquito densities was calculated using the formula of Mulla and others (1971) that takes into account the natural changes in the mosquito larval populations that are taking place at the same level and rate in both treated and untreated habitats such as predation:

Percentage reduction =100 - (T1/C1) x 100

Where, C1 is the average number of larvae in the control basins pre- and post-treatment, while T1 is the average number of larvae in the basins treated with experimental formulations.

## 8.4. Results

### 8.4.1. Field efficacy trials

The effect of the Bti/Bsp combined granular formulation and of *B. sphaericus* (vectolex) alone on *Anopheles* mosquitoes were determined and there was a significant reduction in larval population. Prior to treatment with the larvicides, both the control and treatment basins had statistically similar densities of anopheline larvae averaging between 16.88 and 17.96 larvae per dip. The percentage reductions and the mean numbers of total anopheline larval population, early instars, late instars and pupae during and after microbial larvicides application is shown on Table 8.1. The total larval density in the untreated controls increased during the first three days but then declined thereafter up to thirteenth day when a rise in population density was recorded. However, the total larvae in the vectolex and the combined formulation treatments decreased after the first day although that for vectolex increased from the fifth day, peaked on seventh and then declined progressive from tenth day. The test treatment (Bti/Bsp) basins showed a sharp decrease in total population by day three only to slightly peak on day seven. The trend observed in the total anopheline population is mirrored in the early instar larval populations in all the three experiments, control vectolex and combined formulation. The reductions rates of the late instar larvae, the targeted larval stages, in the combined formulation treatments ranged at 87.48 – 100% up to the seventh day after treatment. In comparison, the reduction in late instars caused by vectolex has significantly diminished by the seventh day at 35.58 % and none by the tenth day after treatment (Table 8.1). Pupation in the combined formulation treated basins remained low during most of the sampling period especially the first ten days where it remained in the range of

0.0 – 17.33 %. Similar treads were observed in the basins treated with vectolex but at a higher lever (range 0.0 - 43.56 %) than in the Bti/Bsp combined formulation.

Table 8.1: Effects of microbial larvicides corn granule formulations on anopheline mosquito density in open field trials.

| | Mean mosquito number per dip | | | | | | | | | | | | Percentage reductions | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total larvae | | | Early instars | | | Late instars | | | Pupae | | | Total larvae | | Early instars | | Late instars | |
| Day | UTC | VLX | TRT | UTC | VLX | TRT | UTC | VLX | TRT | UTC | VLX | TRT | VLX | TRT | VLX | TRT | VLX | TRT |
| 0* | 16.88 | 17.96 | 17.08 | 12.54 | 15.40 | 12.94 | 4.34 | 4.56 | 4.14 | 0.6 | 0.96 | 0.64 | - | - | - | - | - | - |
| 3 | 17.08 | 13.61 | 3.73 | 14.83 | 13.61 | 3.73 | 2.25 | 0 | 0 | 0.97 | 0 | 0 | 20.3 | 78.14 | 8.18 | 74.82 | 100 | 100 |
| 5 | 12.62 | 17.76 | 4.50 | 7.82 | 17.76 | 4.50 | 4.80 | 0 | 0 | 1.48 | 0 | 0 | 0 | 64.34 | 0 | 42.46 | 100 | 100 |
| 7 | 16.46 | 36.20 | 11.13 | 9.32 | 31.60 | 10.24 | 7.14 | 4.6 | 0.89 | 1.89 | 0 | 0 | 0 | 32.38 | 0 | 0 | 35.58 | 87.48 |
| 10 | 19.38 | 29.08 | 8.15 | 16.83 | 20.38 | 5.43 | 2.55 | 8.7 | 1.73 | 5.05 | 2.20 | 0.88 | 0 | 57.94 | 0 | 67.76 | 0 | 32.35 |
| 13 | 22.50 | 18.50 | 12.90 | 15.60 | 12.60 | 5.20 | 6.90 | 5.9 | 3.70 | 0.80 | 2.50 | 1.10 | 17.78 | 42.67 | 19.23 | 66.67 | 14.49 | 46.38 |
| 14 | 14.90 | 13.93 | 7.45 | 9.60 | 6.35 | 5.60 | 5.30 | 7.58 | 1.85 | 1.25 | 2.65 | 0.35 | 6.54 | 50.00 | 33.85 | 41.67 | 0 | 65.09 |
| 17 | 15.93 | 20.20 | 6.58 | 11.38 | 16.43 | 4.63 | 4.55 | 3.78 | 1.95 | 3.45 | 2.43 | 0.83 | 0 | 58.71 | 0 | 59.34 | 17.03 | 57.14 |
| 18 | 23.50 | 16.70 | 10.10 | 17.10 | 15.30 | 8.70 | 6.40 | 1.4 | 1.40 | 1.20 | 1.80 | 2.30 | 28.94 | 57.02 | 10.53 | 49.12 | 78.13 | 78.13 |
| 20 | 25.40 | 11.30 | 3.80 | 9.70 | 4.60 | 1.20 | 15.70 | 6.7 | 2.60 | 0.90 | 0.50 | 0.30 | 55.51 | 85.04 | 52.58 | 87.63 | 57.32 | 83.44 |
| 21 | 8.40 | 9.40 | 5.20 | 3.90 | 3.68 | 3.55 | 4.50 | 5.73 | 1.65 | 1.40 | 0.15 | 0.55 | 0 | 38.10 | 5.77 | 8.97 | 0 | 63.33 |
| 28 | 6.32 | 3.52 | 7.96 | 3.96 | 2.90 | 6.28 | 2.36 | 0.62 | 1.68 | 0.40 | 0.32 | 0.24 | 44.30 | 0 | 26.77 | 0 | 73.73 | 28.81 |

**Day of Treatment, UTC = Untreated Control, VLX = Vectolex, TRT = Bti/Bsp combined formulation*

### 8.4.2. Effect of microbial larvicides on mosquito larval population in irrigated ricefields

A total of 650 rice paddies, 150 canals and 31 temporary breeding habitats were identified and successfully treated with microbial larvicide consuming 6.3 tones of the Bti/Bsp combined formulation corn granules. Thirty paddies and 52 associated canals as well as all the 31-temoporary breeding habitats consisting of tyretracks and water pools, all with water were sampled during the study. Anopheline mosquito larval density variation during and after the two microbial larvicides application instances in Karima village is illustrated in Figure 8.2 and Figure 8.3.

The larval densities in all habitat categories evaluated, declined immediately after the application of the larvicides. In tyretracks and temporary pools, (transient habitats), the larval population was reduced to none following the application the microbial larvicides. There was significant decline in the density of late instar stages among the drainage canals ($F = 1.749$, $df = 27, 426$, $p = 0.02$) and rice paddies ($F = 4.5.45$ $df = 27, 205$, $p < 0.05$) the application of the larvicides. The late instar stages density showed increment in the seventh day through tenth to fourteenth and stabilized thereafter (Figure 8.2).

When all larvae, late instars and pupal stages collected from all habitats were plotted against application timeline, a 100 % larval mortality was demonstrated in the first three and seven days for total larvae and late instar larvae respectively (Fig. 8.3). However, the total larval density did not demonstrate considerable reduction owing to large proportion of early instars that were not greatly affected resulting from possible oviposition. In contrast to total larvae, complete

reduction of late instar larvae was realized within the first 24 hrs until the seventh day post treatment. Similarly, significant inhibition of anopheline adult emergence (pupae) was witnessed after larvicide application only the first few days' post treatment the density was relatively high (Fig. 8.3).

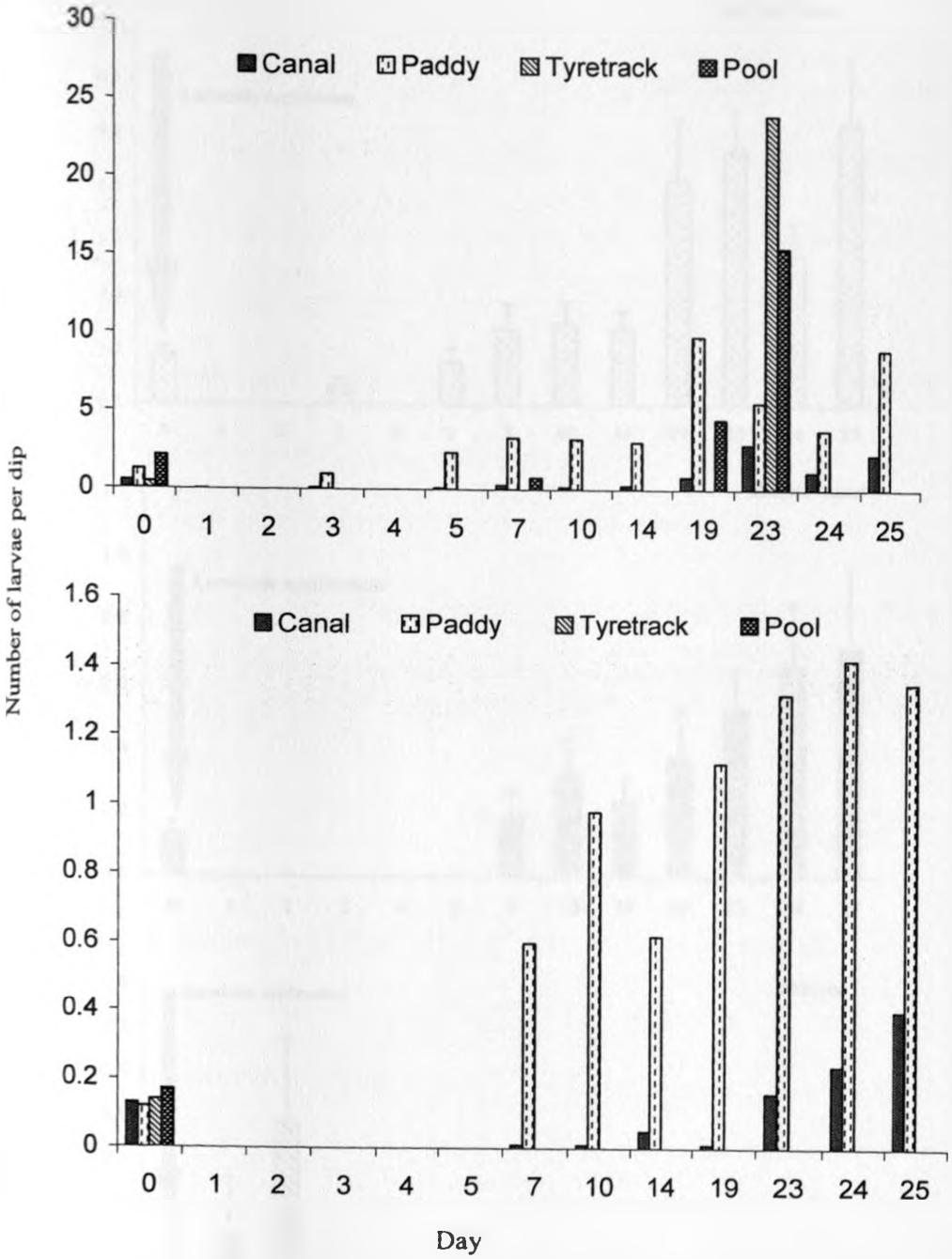



Figure 8.2: Effects of *Bacillus thuringiensis* var. *israelensis* (B.t.i) and *B. sphaericus* combined formulation application (day zero) on anopheline larval densities in different larval habitat types in Karima village.

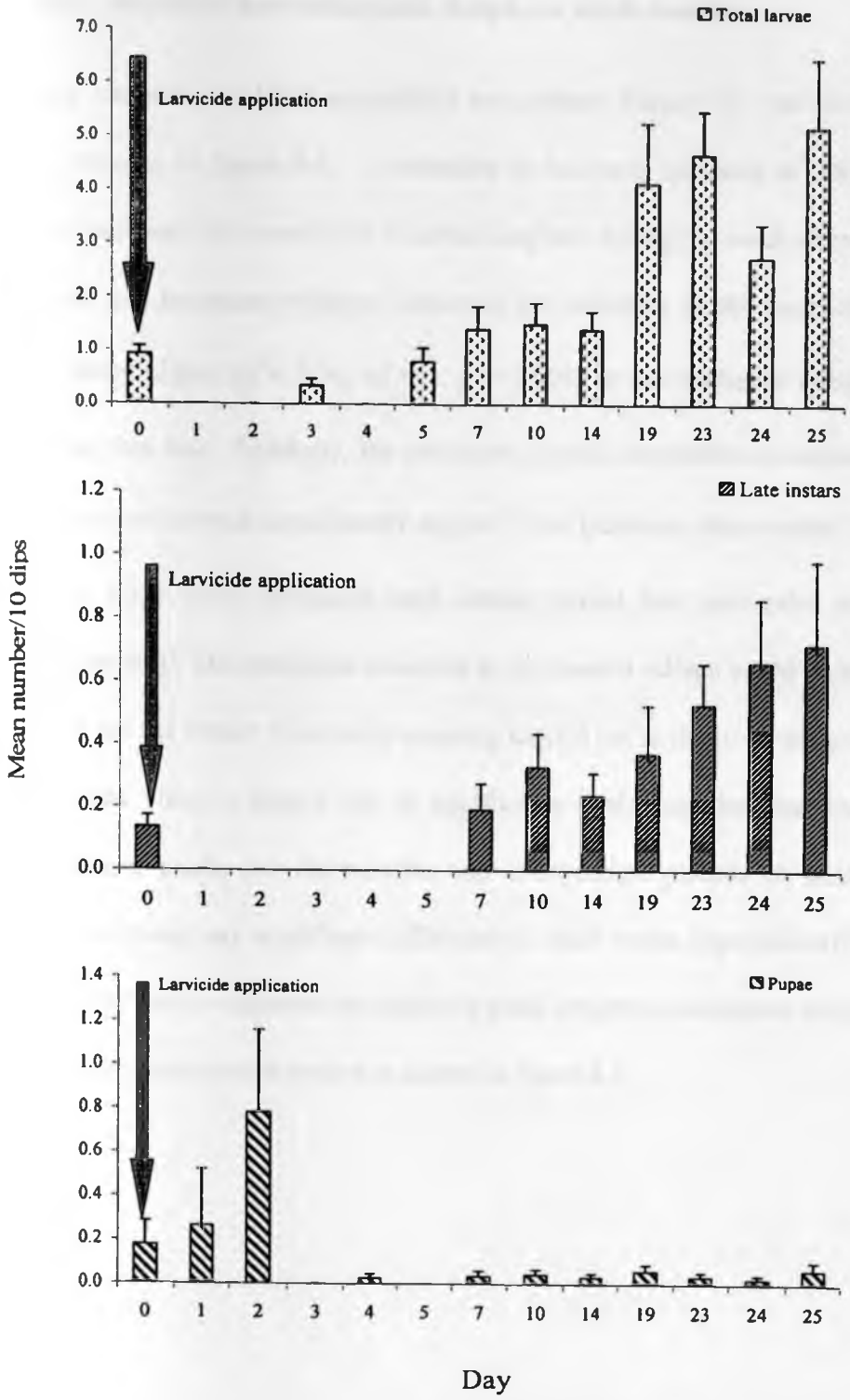



Figure 8.3: Population dynamics of all larvae, late instars and pupae of *Anopheles* mosquitoes in Karima village after application of Bti/Bsp combined formulation corn granules application.

### 8.4.3. Microbial larviciding and *Anopheles* adult densities

The density variation of adult anopheline mosquitoes during the two microbial larviciding occasions is shown in figure 8.4. A reduction in the mean numbers of *Anopheles* mosquitoes was realized between one week prior to larviciding and during the week of microbial larviciding in the control and treatment villages. However, the reduction in the anopheline adult densities was significantly higher ($\chi^2$= 5.36, df = 1, p = 0.004) in the treatment village than the control village during this time. Similarly, the reduction in adult anopheline mosquito densities during larvicide's application was significantly higher in the treatment than control villages ($\chi^2$= 6.92, df = 1, p < 0.05) when compared with similar period one year prior to larviciding (pre-intervention period). The reduction observed in the control village could be attributed to natural phenomenon and the indoor insecticide spraying carried out in the two villages. However, results of ANOVA and Tukey's honest test of significance evaluating the changes of vector density during the seven weeks pre-intervention and intervention periods in control and treatment villages did not reveal any significant difference in adult vector populations (F = 2.12 df= 3, 46, P > 0.05). There was no apparent reduction in adult *Anopheles* mosquito density one week after larviciding in the intervention period as shown in figure 8.4.

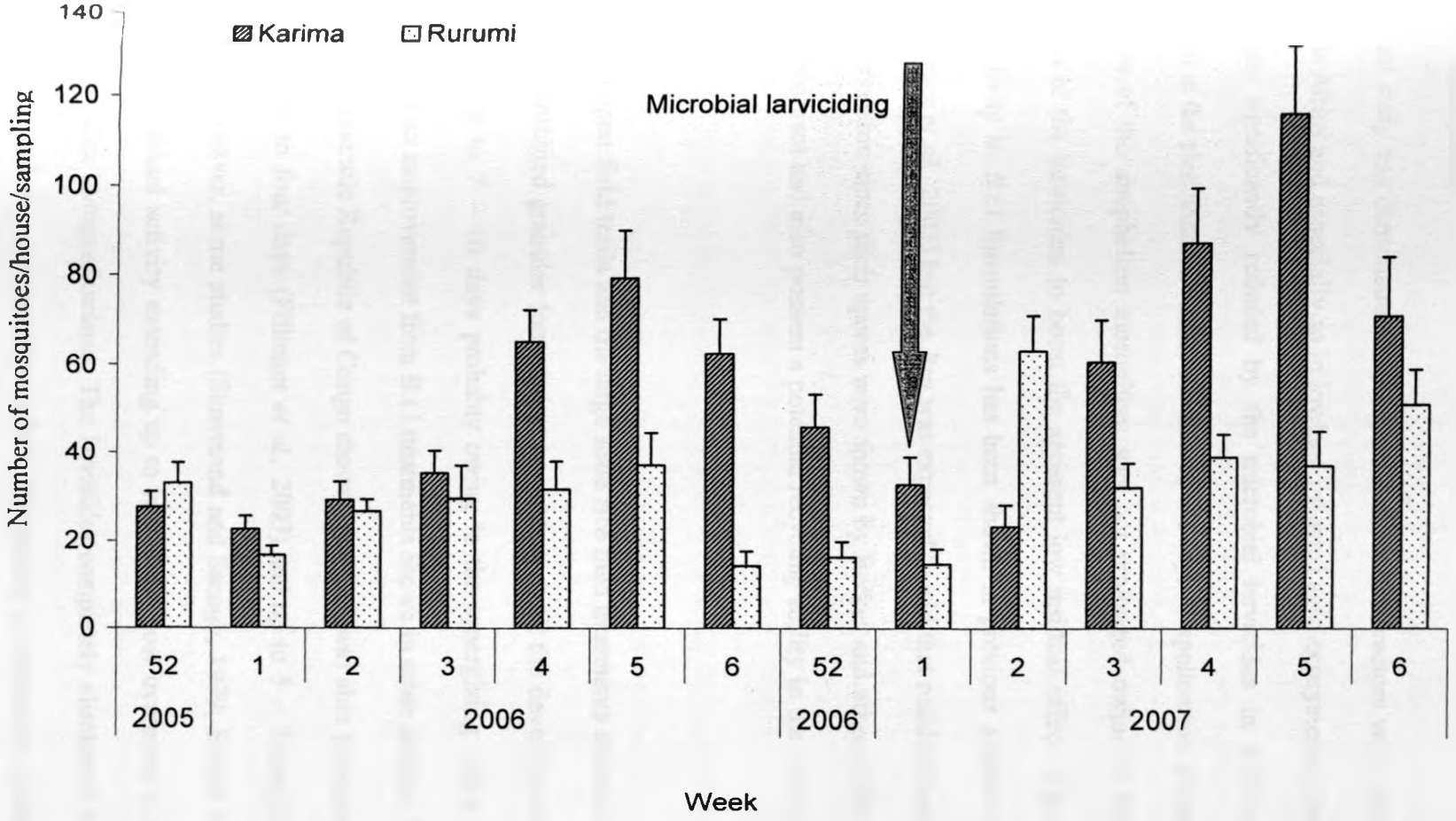



Figure 8.4: Summary of *Anopheles* mosquito density variation prior and during microbial larval control interventions in Karima (treated) and Rurumi villages in Mwea irrigated rice scheme.

## 8.5. Discussion

The current study has demonstrated that control of malaria vectors with microbial larvicides is possible in Africa and especially so in lowland irrigated agro-ecosystems. The immature aquatic stages were significantly reduced by the microbial larvicides in trial studies and during application in the rice paddies within the first seven days of application. However, the reduction in densities of the anopheline mosquitoes was not prolonged owing to lack of repeated re-application of the larvicides to boost the apparent low residual effect. The absence of a long residual activity in *B.t.i* formulations has been shown in previous studies (Das and Amalraj, 1997; Fillinger *et al.*, 2003) but the *Bsp* was expected to add that residual larvicidal effect in our current formulation since their spores were shown by Becker and others (1995) to persist longer in the environment and also possess a potential recycling ability in the mosquito larval gut after death.

Results from open field trails and the large scale rice field treatments showed that at the optimal dosage, the combined granular formulation was able to limit the development of the late larval instar stages up to 7 – 10 days probably owing to the synergizing effect of the two active ingredients and an improvement from B.t.i treatments shown in other studies. Previous studies in Kenya and Democratic Republic of Congo showed that B.t.i was able to suppress the late instars development up to four days (Fillinger *et al.*, 2003) and up to 5 – 7days (Karch *et al.*, 1991) respectively. However, some studies (Skovmand and Sanogo, 1999; Shililu *et al.*, 2003b) have shown longer residual activity extending up to two weeks post treatment in the control of *An. gambiae* and *Culex quinquefasciatus*. The larvicides completely eliminated mosquito larvae in temporary larval habitats demonstrating their importance in temporary habitats where habitat

instability affects productivity (Mutuku *et al.,* 2006) combined with microbial larvicides may help in annihilating such habitats. For example, in their study in Ouagadougou Burkina Faso, Skovmand and Sanogo, (1999) showed that the duration of B.t.i and Bsp control in cesspits and puddles was highly dependent on their transient nature. Thus, the current results show that microbial larvicides have a potential role in malaria control programs within irrigated rice agro-ecosystems but repeated applications as advocated in previous studies (Karch *et al.*, 1990, 1991; Shililu *et al.*, 2003b) are necessary when larval population's show recovery sign in order to have greater impacts on reduction of malaria transmission.

The densities of the adult malaria vectors were little affected by the microbial larvicide application in the rice fields according to the current results. However, this does not necessarily translate to lack of impact of the microbicides on adult vector population but rather an indication for the need of repeated applications and evaluation of other underlying factors with an influence on adult density such as adult mosquitoes flying from untreated zones outside the study areas into the village of focus (Service, 1997; Killeen *et al.*, 2003). In their study in Mbita, Suba district of Kenya, Fillinger and Lindsay (2006) were able to demonstrate a 92 % reduction in human exposure to mosquito bites after repeated and prolonged period of *B. thuringiensis* var *israelensis* and *B. sphaericus* application in larval habitats. Furthermore, several studies (Kumar *et al.*, 1995, 1998; Sharma *et al.*, 1998) involving malaria larval control measures have resulted in reduction of late instar stages with a consequent decline in the abundance of the adult vector population. Hence, the significant reduction of late instar larvae after short-term larviciding in the current clearly study shows the potential of microbiciding in reducing the high vector densities in the irrigated rice agro-ecosystems and thus malaria transmission.

In this study the cost implication of the microbial larviciding was not evaluated but the approach employed in its planning and implementation by involving the resident community would make it technically and financially feasible to its implementation and maintenance. The direct cost of microbial larvicide application calculated in previous studies (Fillinger and Lindsay, 2006; Samuelsen *et al.*, 2004) lie in the range of 0.85 – 0.89 USD per person per year, a reasonably lower amount compared to the direct cost of treating malaria estimated at 11.79 USD per household in addition to indirect costs and time lost due to illness (Morel *et al.*, 2008). Furthermore, active collaboration and participation of the community has been shown to be a key ingredient in the success of antilarval measure (Kitron and Spileman, 1989; Townson *et al.*, 2005; Fillinger and Lindsay, 2006; van der Berg and Knols, 2006) and an important strategy in community-based operations (Mukabana *et al.*, 2006). In the current study, the community participated in the scouting for larval habitats, larvicide application and training and showing great understanding by allowing access to their premises and breeding habitats in rice fields with young crops, their main source of food and income.

The apparent 100 % reduction of late instar stages within twenty four hours in larval habitats and potential of the combined formulations in reducing overall anopheline mosquito density in the rice fields and temporary habitats are encouraging for mosquito antilarval interventions. This emphasizes the importance of the strategy advocated by Gu and others (2008) for the need of habitat specific based interventions that appreciates the heterogeneity in mosquito production due to factors within and between habitats and across landscapes. In the current study, the behaviour of the total larvae was probably reflected more by the early instar population and could have been caused by the oviposition attractancy of the habitats. In such cases, targeting

individual habitats would reduce the availability of breeding habitats thereby diminishing adult mosquito emergence and compromise oviposition (Gu *et al.*, 2008). Furthermore, the success of microbial larvicides underlies the great advantage of larval control interventions especially use of *Bacillus* products over other control intervention strategies in malaria control (Killeen *et al.*, 2002a). For example, the larvicides have been shown to be selective in action and low probability of developing resistance (Charles and Nielsen-Leroux, 2000).

In addition to their acceptability, ease of handling and being regarded as intoxic, there are no reported cases of abuse of microbial larvicides unlike the misuse of bednets as fish drying material reported recently in western Kenya (Minakawa *et al.*, 2008). Similarly, their success as an antilarval measure serves to boost the overall success of integrated vector management in irrigated agro-ecosystems. For example, integration of microbial larviciding with zooprophylaxis which has been shown to be widespread in irrigated rice agro-ecosystems (Mutero *et al.*, 2004a; Muriu *et al.*, 2008) and insecticide treated bednet usage known to reduce malaria transmission intensity (Carnevale *et al.*, 1988; Magesa *et al.*, 1991) would result in a powerful tool for malaria vector management in sub-Saharan Africa.

## REFERENCES


Alaii, J.A., Van Den Borne H.W., Kachur S.P, Shelley, K, Mwenesi, H., Vulule, J.M., Hawley W A., Nahlen B L., and Phillips-Howard, P. A. **2003a.** Community reactions to the introduction of permethrin-treated bed nets for malaria control during a randomized controlled trial in western Kenya. *American Journal of Tropical Medicine and Hygiene,* **68:** 128-136

Alaii, J.A., Van Den Borne H.W., Kachur S.P., Mwenesi, H., Vulule, J.M., Hawley, W A., Meltzer, M.I., Nahlen B L., and Phillips-Howard, P. A. **2003b.** Perceptions of bed nets and malaria prevention before and after a randomized controlled trial of permethrin-treated bed nets in western Kenya. *American Journal of Tropical Medicine and Hygiene,* **68:** 142-148.

Alonso, P.L., Lindsay, S.W., Armstrong, J.R.M., Conteh, M., Hill, A.G., David, P.H., Fegan, G., DeFrancisco, A., Hall, A.J., Shenton, F.C., Cham, K., Greenwood, B.M.,**1991.** The effect of insecticide treated bed nets on mortality of Gambian children. *Lancet.* **337:** 1499–1502

Anderson, R.A., and R.A. Brust. **1995**. Field evidence of multiple host contacts during blood feeding by Culex tarsalis, Cx. restuans and Cx. Nigripalpus Diptera : Culicidae). *Journal of Medical Entomology*, **32:** 95–101.

Aniedu, I. **1993**. Biting activity and resting habits of Malaria vectors in Baringo district, Kenya. *Journal of Pest Science* , **66:** 72—76.

Appawu, A.M., Dadzie, S.K., A. Baffoe-Wilmot and D.M. Wilson. **2001**. DM: Lymphatic

filariasis in Ghana: entomological investigation of transmission dynamics and intensity in communities served by irrigation systems in the Upper East Region of Ghana. *Journal of Tropical Medicine and International Health*, **6:** 511–516.

Asimeng, E.J. and M.J. Mutinga. **1993**. Effect of rice husbandry on mosquito breeding at Mwea rice irrigation scheme with reference to biocontrol strategies. *Journal of the American Mosquito Control Association,* **9:** 17–22.

Beales, P.F., Goriup, S., Litsios, S., Molineaux, L., E. Onori and J.H. Pull. **1988**. *The planning of malaria control.* In ***Malaria:*** *Principles and Practice of Malariology* edited by; W.H. Wernsdorfer and Sir I. McGregor vol 2 by: Churchill Livingstone.

Becker, N. **1998**. The use of *Bacillus thuringiensis* subspecies *israelensis* (B.t.i) against mosquitoes, with special emphasis on the ecological impact. *Israel Journal of Entomology*, **32:** 63–69.

Becker, N., Zgomba, M., Petric, D., M. Beck and M. Ludwig. **1995**. Role of larval cadavers in recycling processes of *Bacillus sphaericus*. *Journal of the American Mosquito Control Association,* **11:** 329–334.

Becker, N. and F. Rettich. **1994**. Protocol for the introduction of new *Bacillus thuringiensis israelensis* products into the routine mosquito control program in Germany. *Journal of the American Mosquito Control Association*, **10:** 527–533.

Beier, J.C., G.E. Killeen, and J.I. Githure. **1999**. Short report: entomologic inoculation rate and Plasmodium falciparum malaria prevalence in Africa. *American Journal of Tropical Medicine and Hygiene,* **61:** 109–113.

Beier, J.C., Oster, C.N., Onyango, F.K., Bales, J.D., Sherwood, J.A., Perkins, P.V., Chumo, D.K., Koech, D.V., Whitmire, R.E., Roberts, C.R., C.L. Diggs, and S.L. Hoffman. **1994**.

Plasmodium falciparum incidence relative to entomological inoculation rates at a site proposed for testing malaria vaccines in Western Kenya. American Journal of tropical Medicine and hygiene, **50:** 529 - 536.

Beier, J. C. and J. K. Koros. **1991**. Visual assessment of sporozoite and bloomeal ELISA samples in malaria field studies. *Journal of Medical Entomology*, **28:** 805 - 808.

Beier, J.C., Perkins. P.V., Onyango, F.K., Gargan, T.P., Oster, M.C., Whitmire, R.E., Koech, D.K., and R.R. Clifford. **1990**. Characterization of malaria transmission by *Anopheles* (Diptera: Culicidae) in western Kenya in preparation for malaria vaccine trials. *Journal of Medical Entomology*, **27:** 570–577.

Beier, J. C., Perkins, P. V., Koros, J. D. Diggs, D. G. T. P., and D. K. Koech. **1988**. Blood meal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *Journal of Medical Entomology*, **25:** 9-16.

Beier, J.C., Perkins, P.V., Wirtz, R.A., Whitmire, R.E., M. Mugambi, and W.T. Hockmeyer. **1987**. Field evaluation of Enzimy Linked immunosobent assay (ILISA) for *Plasmodium falciparum* sporozoite detection in anopheline mosquitoes from Kenya. *American Journal of Tropical Medicine and Hygiene*, **36:** 459-468.

**van den Berg,** H. and B.G. Knols. **2006**. The Farmer Field School: a method for enhancing the role of rural communities in malaria control? *Malaria Journal* **5**:3.

**Birley**, M.H. **1989**. *Guidelines for forecasting of the Vector-borne disease implications of water resources development*. PEEM Guidelines, Series 2. WHO Geneva

**Bockarie**, M.J., Hexander, N., Bockarie, F., Ibam, E., Barnish, G. and Alpers, M. **1996.**

The late biting of parous *Anopheles* mosquitoes and pre-bedtime exposure of humans to infective female mosquitoes *Transactions of Royal Society of Tropical Medicine and Hygiene* **90:**23-25

Boudin, C., Robert, V., Carnevale, P. and Thomas, P.A. **1992.** Epidemiology of *Plasmodium falciparum* in a rice field and a savannah area in Burkina Faso. Comparative study on the acquired immunoprotein native populations. *Acta Tropica*, **51:** 103-111.

Bradley, D.J. **1988**. The epidemiology of ricefield-associated diseases. In: International Rice Research Institute in collaboration with WHO/FAO/UNEP, Panel of Experts on Environmental Management for Vector Control ed. Vector-borne disease control in humans through rice agroecosystems management. Philippines: *International Rice Research Institute, 29-39.*

Breman, J.G., M.S. Alilio, and A.Mills. **2004**. Conquering the intolerable burden of malaria: what's new, what's needed: a summary. *American Journal of Tropical Medicine Hygiene*, **71:**1–15.

Breman, J.G. **2001**. The ears of Hippopotamus: Manifestations, determinants and estimates of the malaria burden. *American Journal of Tropical Medicine and Hygiene,* **64**:1-11.

Briet, J., J. Dossou-Yovo, E. Akodo, N. van de Giesen, and M. Teucher. **2003**. The relationship between *Anopheles gambiae* density and rice cultivation in the savannah and forest zone of Cote d'Ivoire. *Journal of Tropical Medicine and International Health*, 8: 439-448.

Brooker, S. Clarke, S. Njagi, J.K., Polack, S., Mugo, S., Estambale, B., Muchiri, E., E.Magnussen, and J. Cox. **2004**. Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya. *Journal of Tropical Medicine and International Health,* **9:** 757–766.

Bruce-Chwatt, L,J, **1985**. *Essential malariology*. William Heinemann medical books 22 Bedford square, London Second Edition.

Bruce-Chwatt, L.J., C. Garreett-Jones and B. Weitz. **1966**. Ten year study (1955-1964) of host selection by anopheline mosquitoes. *Bulletin of the World Health Organanization,* 35: 405-439.

Burkot, T.R. and P.M. Graves. **1995**. The value of vector based estimates of malaria transmission. *Annals of Tropical Medicine and Parasitology* 89, 125–134.

Burkot, T.R. **1988**. Non-random host selection by Anopheline mosquitoes. *Parasitology Today*, 4:156-162.

Burkot, T.R., Graves, P.M., R. Paru and M. Lagog. 1988. Mixed blood feeding by the malaria vectors in the *Anopheles punctulatus* complex (Diptera: Culicidae). *Journa of Medical Entomology*, 1988, 25:205-213.

Carter, R., K.N. Mendis, and D. Roberts. **2000.** Spatial targeting of interventions against malaria. *Bulletin of the World Health Organanization,* **78:** 1401–1411.

Cano, J., Descalzo, M.A., Moreno, M., Chen, Z., Nzambo, S., Bobuakasi, L., Buatiche, J.N., Ondo, M., F. Micha, and A., Benito A. **2006**. Spatial variability in the density, distribution and vectorial capacity of anopheline species in a high transmission village (Equatorial Guinea). *Malaria Journal*, 5: 21.

Carnevale, P., Guillet P., Robert, V., Fontinelle, D., Doannio, J., M. Coosemans, and J. Mouchet. **1999.** Diversity of malaria in rice growing areas of Afrotropical region. *Parasitologia*, **41:** 273-276.

Carnevale, P., Le Goff, G., J.C. Toto, and V. Robert. **1992.** *Anopheles nili* as the main

vector of human malaria in villages of southern Cameroon. *Medical and Veterinary Entomology*, **6:**135-8.

**Carnevale**, P., Robert, V., Boudin, C., Halna, J.M., Pazart, L., Gazin, P., A. Richard and J. Mouchet. 1988. La lutte contre le paludisme par des moustiquaires impregnées de pyréthroides au Burkina Faso. *Bull Soc Path Exot* 81:832-846

**Carrara**, G.C., Petrarca, V., M. Niang, and M. Coluzzi. **1990.** *Anopheles pharoensis* and transmission of *Plasmodium falciparum* in Senegal River Delta, West Africa. *Medical and Veterinary Entomology*, **4:** 421- 424

**Centers for Disease Control (CDC). 2007.** Rift Valley Fever Outbreak - Kenya, November 2006--January 2007. MMWR, 56: 73-76. [www.cdc.gov/mmwr/preview/mmwrhtml/mm5604a3.htm] .

**Celli** A. **1933.***The history of malaria on the Roman Campagna from ancient times*. London, John Bale, Sons Danielsson.

**Chandler**, J., and R. Highton. **1976.** Breeding of *Anopheles gambiae* Giles (Diptera: Culicidae) in rice fields in the Kisumu area of Kenya. *Journal of Medical Entomology,* **13:** 211-115.

**Chandler**, J. A., and R. B. Highton. **1975**. The succession of mosquito species (Diptera, Culicidae) in rice fields in the Kisumu area of Kenya, and their possible control. *Bulletin of Entomological Research,* **65:** 295-302.

**Chandler,** J.A., Boreham, P.F.L., R.B. Highton and .M.N. Hill. **1975**. A study of the host selection patterns of the mosquitoes of the Kisumu area of Kenya. Transactions of the *Royal Society of Tropical Medicine and Hygiene* **69:** 415–425.

**Chandler**, J., R. Highton, and M. Hill. **1975.** Mosquitoes of the Kano plain, Kenya 1.

Results of indoor collections in irrigated and non-irrigated areas using human bait and light traps. *Journal of Medical Entomology,* **12:** 504-510.

**Chandler**, R..F.Jr **.1969.** *Plant morphology and stand geometry in relation to nitrogen.* In: Eastin J.D., Haskins F.A., Sullivan C.Y., van Bavel C.H.M., eds. *Physiological aspects of crop yield.* Madison, WI: ASA pp265-285.

**Chandre**, F., Darriet, F., Manga, L., Akogbeto, M., Faye, O., Mouchet, J. and P. Guillet **.1999.** Status of pyrethroid resistance in *Anopheles* gambiae sensu lato. *Bulletin of the World Health Organization* **77:** 230–234

**Charles**, J.F. and C. Nielsen-LeRoux. **2000**. Mosquitocidal bacterial toxins: diversity, mode of action and resistance phenomena. *Memorias Do Instituto Oswaldo Cruz* **95:** 201-206.

**Charlwood**, J. D., T. Smith, P. F. Billingsley, W. Takken, E. O. K. Lyimo, and J. H. Meuwissen. **1997.** Survival and infection probabilities of anthropophagic Anophelines from an area of high prevalence of *Plasmodium falciparum* in humans. *Bulletin of Entomological Research,* **87:** 445 - 453.

**Charlwood**, J.D and P.M. Graves. **1987**. The effect of permethrin impregnated bednets on a population of *Anopheles farauti* in coastal Papua New Guinea. *Medical and Veterinary Entomology* **1:** 319–327.

**Choi** HW, Breman JG, Teutsch S.M, Liu S, Hightower AW, Sexton JD**. 1995.** The effectiveness of insecticide-impregnated bed nets in reducing cases of malaria infection: a meta-analysis of published results. *American Journal of Tropical Medicine and Hygiene*; **52:** 377-382.

**Coosemans**, M. H. **1985**. Comparaison de l'endemie malarienne dans une zone de riziculture

et dans une zone de culture de coton dans la Plaine de la Rusizi, Burundi. *Annals de la Societe Belge de Medicine Tropicale*, **65:** 187-200.

Costantini, C., Sagnon, N.F., Sanogo, E., L. Merzagora and M. Colluzi. **1998**. Relationship to human biting collections and influence of light and bednets in CDC light-trap catches of West African malaria vectors. *Bulletin of Entomological Research*, **88:** 503–511.

Craig, M.H., R.W. Snow and L. le Sueur. **1999**. A climate-based distribution model of malaria transmission in Sub-Saharan Africa. *Parasitology Today* 15:105-111.

Curtis, C.F. **1994**. Should DDT continue to be recommended for malaria vector control? *Journal of Medical and Veterinary Entomology*. **8:** 107-112

Curtis, C.F., Lines, J.D., L. Boili and A. Renz. **1991**. Natural and synthetic repellents. In: *Control of Disease vectors in the community*. Curtis, C.F (editor) London:Wolfe Publishing, pp 75-92.

Das, P.K. and D.D. Amalraj. **1997**. Biological control of malaria vectors. *Indian Journal of Medical Research,* **106:** 174 – 197.

Davis, J.R., Hall, T., Chec, E.M., Majala, A., Minjas, J. and C.J. Shiff. **1995**. Comparison of sampling anopheline mosquitoes by light-trap and human-bait catches indoors at Bagamoyo. Tanzania. *Medical and Veterinary Entomolog,y* **9:** 249–255.

Diallo, M., Nabeth, P., Ba, K., Sall, A.A., Ba, Y., Mondo, M., Girault, L., M.O. Abdalahi and C. Mathiot. **2005**. C: Mosquito vectors of the 1998-1999 outbreak of Rift Valley Fever and other arboviruses (Bagaza, Sanar, Wesselsbron and West Nile) in Mauritania and Senegal. *Medical and Veterinary Entomology*, **19:** 119-126.

Diuk-Wasser, M. A., Toure, M. B., Dolo, G., Bagayoko, M., Sogoba, N., Traore, S. F., N.

Manoukis and C. Taylor. **2005**. Vector abundance and malaria transmission in rice-growing villages in Mali. American *Journal of Tropical Medicine Hygiene,* **72:** 725-731.

Dolo, G., Briet, O.J.T., Dao, A., Traore, S.F., Bouare, M., Sogoba, N., Niare, O., Bagayogo M,, Sangare, D., T. Teuscher and Y.T. Toure. **2004**. Malaria transmission in relation to rice cultivation in the irrigated Sahel of Mali. *Acta Tropica*, **89:** 147–159.

Dolo, G. **1996**. *Etude des populations d'An.gambiae s.l par marquage, lacher et recapture a Banambani en 1993 et 1994 (arrondissement central de Kati, Mali)*. Memoire de DEA en entomologie et Parasitologie medicales, ISFRA, Bamako.

Dossou-Yovo, J., Doannio, J.M.C., Rivie˙ re, F. and Chauvancy, G. **1995**. Malaria in Co^te d'Ivoire wet savannah region: the entomological input. *Tropical Medicine and Parasitology*, **46:** 263–269.

Dossou-Yovo J, Doannio, J, Riviere, F. and J. Duval .**1994**. Rice cultivation and malaria transmission in Bouake city (Cote d'Ivoire). *Acta Tropica* **57:** 91–94.

Faye, O., Fontenille D, Gaye O. **1995a**. Malaria and rice growing in the Senegal river delta (Senegal). *Annales de la Socie´te´ Belge de Me´decine Tropicale,* **75:** 179-189.

Faye, O., Fontenille, D., Gaye, O., Sy, N., Molez, J.F., Konate, L., Hebrard, G., Herve, J.P., Trouillet, J., S. Diallo and J. Mouchet. **1995b**. Malaria and rice growing in the Senegal river delta (Senagal). *Annales Societe Belge de Medicine Tropicale* **75:** 179–189.

Faye, O., Fontenille, D., Gaye, O., sy, N., Molez J..F., Konate, L.,Hebrand, G., Herve, J.P.,Trouillet, J. and S. Diallo .**1995c**. Paludise at riziculturedans le delta du Fleuve Seal (Senegal). *Anals de la Societe belge de Medicine tropicale*, **75:** 179-189.

Faye, O., Fontenille, D., Herve,, J.P., **1993a**. Malaria in the sahelian zone of Senegal. 1.

Entomological data concerning transmission. *Annales Societe Belge de Medicine Tropicale* **73:** 21–30.

**Faye**, O., Gaye, O., Herve, J.P., P.A. Diack and S. Diallo, **1993b**. Le paludisme en zone sahelienne du Senegal .2. indices parasitaires . *Annals de la Societe Belge de Medicine Tropicale*, **73:** 31-36.

**Faye**, O., Par, O., Diallo, S., O. Gaye and O. Ngir, **1992**. Comparative efficacy of the use of CDC light traps and humans to sample *Anopheles* populations. Results obtained in the Bignona zone of Senegal. *Bulletin de la Société de Pathologie Exotique et de sa Filiale de l'ouest Africa* **85 :** 185–189.

**Fegan**, G.W., Noor, A.M., Akhwale, W.S., S. Cousens and R.W. Snow. **2007**. Effect of expanded insecticide-treated bednet coverage on child survival in rural Kenya: a longitudinal study. *Lancet*, **370:**1035-1039.

**Fillinger**, U. and S.W. Lindsay. **2006**. Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. *Tropical Medicine and International Health*, **11:**1629-1642.

**Fillinger**, U., B.G. Knols and N. Becker. **2003**. Efficacy and efficiency of new *Bacillus thuringiensis* var *israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in western Kenya. *Tropical Medicine and International Health,* **8:** 37–47.

**Fontenille**, D., Lochouarn, L., Diagne,N., Sokhna , C., Lemasson J, Diatta, M., Konate, L., Faye,F., Rogier, C. and Trape, J. **1997.** High annual and seasonal variations in malaria transmission by Anophelines and vector species composition in Dielmo, a holoendemic area in Sengal. . *American Journal of tropical Medicine and Hygiene* **56:**247-253

**Gallup**, J. and J. Sachs. **2001.** The economic Burden of Malaria. *American Journal of*

*Tropical Medicine and hygiene,* **64:** 85-96.

Garrett-**Jones,** C., P.F.L.Boreham and C.P. Pant. **1980**. Feeding habits of anophelines (Diptera: Culicidae) in 1971–1978, with reference to the human blood index: a review. *Bulletin of Entomological Research*, **70:**165-185.

Garrett-**Jones,** C. 1970. Problems of epidemiological entomology as applied to malariology. *Miscellineous Publication of the entomological society of America*, 7: 168-177

Garrett-**Jones**, C. **1964.** Prognosis for interruption of malaria transmission through the assessment of mosquito vectorial capacity. *Nature,* **204:** 1173

Garrity, D.P. **1988**. Tropical rice agroecosystems: characteristics, distribution, and future trends. In: *Vector Borne Diseases Control in Humans Through Rice Agroecosystem Management.* Proceedings of a workshop jointly sponsored by International Rice Research Institute and the WHO/FAO/UNEP Panel of experts on Environmental Management for Vector Control. International Rice Research Institute, Manila, pp 13-28.

Gillies, M.T. and M. Coetzee. **1987**. A supplement to the Anophelinae of Africa south of the Sahara (Afro-tropical region). Johannesburg. Publication of the South Africa Institute of Medical Research, **55:** 1-143.

Gillies, M.T. and B. De Meillon. **1968**. The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Zone). Publication of the South African Institute for Medical Research, Johannesburg, **54:** 1–343.

Gimnig, J.E., Ombok, M., L. Kamau, and W.A. Hawley. **2001.** Characteristics of larval Anopheline (Diptera: Culicidae) habitats in Western Kenya. *Journal of medical Entomology* **38**: 282-288

Githeko, A.K., Service, M.W., Mbogo, C.M., F.K. Atieli and F.O. Juma. **1994**. Origin of

bloodmeal in indoor and outdoor resting malaria vectors in western Kenya. *Acta Tropica*, **58:** 307 – 316.

Githeko, A.K., Service, M.W., Mbogo, C.M., F.K. Atieli and F.O. Juma. **1993.** *Plasmodium falciparum* sporozoite and entomological inoculation rates at the Ahero rice irrigation scheme and the Miwani sugar-belt in western Kenya. *Annals of Tropical Medicine and Parasitology*. **87:** 379-91.

Gordon, S., Tammariello, R., Lincithicum, K., Dohm, D., Digoutte, J., M. Calvo-Wilson **1992**. Arbovirus isolations from mosquitoes collected during 1988 in the Senegal River basin. *American Journal of Tropical Medical Hygiene,* **47:**742-748.

Gratz, N.G.**1988.** The impact of rice production on vector-borne disease problems in developing countries. In: International Rice Research Institute in collaboration with WHO/FAO/UNEP, Panel of Experts on Environmental Management for Vector Control ed. Vector-borne disease control in humans through rice agroecosystems management. Philippines: *International Rice Research Institute,* 7-12

Gratz, N.G. and Pal. **1988.** *Malaria vector control larviciding* In *Malaria, Principles and Practice of Malariology* edited by; W.H. WERNSDORFER. and Sir I. McGregor Vol 2 By Churchill Livingstone

Grillet, M.T. **2000**. Factors associated with the distribution of *anopheles aquasalisI* and *an.oswaldi* (Diptera: Culicidae) in a malariaous area, Northeastern Venezuela. *Journal of Medical Entomology*, **37:** 231-238.

Grist, D.H. **1986**. *Rice*. Longman, London.

Gu, W., J. Utzingerand R.J. Novak. **2008**. Habitat-based larval interventions: A new perspective for malaria control. *American Journal of tropical Medicine and Hygiene*, **78:** 2 - 6.

Gu, W. and R.J. Novak. **2005.** Habitat-based modelling of impacts of mosquito larval interventions on entomological inoculation rates, incidence, and prevalence of malaria. *American Journal of tropical Medicine and Hygiene* **73:** 546-552.

Guillet, P., Alnwick, D., Cham, M.K., Neira, M., Zaim, M., Heymann, D., and K. Mukelabai. **2001,**: Long-lasting treated mosquito nets: a breakthrough in malaria prevention. *Bulletin of the World Health Organization* **79**:998.

Gwadz. R. and F.H. Collins. **1986**. *Anopheles Mosquitoes and the agents they transmit*. In: Beaty, B.J and Marquardt, W.C; *The biology of Disease vectors*; Published by the University Press Colorado.

Hadis, M., Lulu, M., Y. Makonnen and T. Asfaw. **1997**. Host choice by indoor resting *Anopheles arabiensis* in Ethiopia. *Transactions of Royal Society of Tropical Medicine and Hygiene*, **91:**376-378.

Hargreaves, K., Koekemoer, L.L., Brooke, B.D., Hunt, R.H., Mthembu, J. and Coetzee, M. **2000**. *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Medical and Veterinary Entomology,* **14:** 181–189.

Hay, S.I., Rogers, D.J., Toomer, J.F. and R.W. Snow **2000**. Annual *Plasmodium falciparum* inoculation rates (EIR) across Africa: Literature survey, Internet access and review. *Transactions of Royal Society of Tropical Medicine and Hygiene*. **94**:113-127

Hays, C.W. **2000.** The United States Army and malaria control in World War II. *Parasitologia* **42:** 47–52.

Hii, J.L., Smith, T., Mai, A., E. Ibam, and M.P. Alpers. **2000.** Comparison between anopheline mosquitoes (Diptera: Culicidae) caught using different methods in a malaria endemic area of Papua New Guinea. *Bulletin of Entomological Research* **90:** 211-219.

van der **Hoek,** W. **2004.** How can better farming methods reduce malaria?. *Acta Tropica*, **89:** 95 - 97

van der **Hoek,** W., Konradsen, F., Dijkstra, D.S., P.H. Amerasinghe, and F.P. Amerasinghe. **1998**. Risk factors for malaria: a micro epidemiological study in a village in Sri Lanka. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **92:** 265–269

**Howard,** S.C., Omumbo, J., Nevill, C., Some, E.S., Donnelly, C.A., R.W. Snow, **2000.** Evidence for a mass community effect of insecticide-treated bednets on the incidence of malaria on the Kenyan coast *Transactions of Royal Society of Tropical Medicine and Hygiene* **94**:357-60

**Hunter,** J.M., Rey, L., Chu, K.Y., E.O. Adekolu-John and K.E. Mott **.1993.** *Parasitic diseases in water resources development. The need for intersectoral negotiation. World Health Organisation: Geneva.*

**Ijumba,** J., F. Mosha, and S. Lindsay. **2002a**. Malaria transmission risk variations derived from different agricultural practices in an irrigated area of Northern Tanzania. *Medical and Veterinary Entomology,* **16:** 28-38.

**Ijumba,** J.N., Shenton, F.C., Clarke, S.E., Mosha, F.W. and S.W. Lindsay. **2002b**. Irrigated crop production is associated with less malaria than traditional agricultural practices in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **96:** 476 – 480.

**Ijumba,** J.N. and S.W. Lindsay. **2001**. Impact of irrigation on malaria in Africa: paddies paradox. *Medical and Veterinary Entomology.* **15**:1-11.

**Ijumba,** J.N. **1997**.*The impact of rice and sugarcane irrigation on malaria transmission in*

*the lower Moshi area of northern Tanzania.* PhD Thesis, Tropical Pesticides Research Institute and Danish Bilharziasis Laboratory, University of Copenhagen Charlottenlund, Denmark, 134 pp.

Ijumba, J.N., R.W. Mwangi, and J.C. Beier. **1990.** Malaria transmission potential of *Anopheles* mosquitoes in the Mwea-Tebere irrigation scheme, Kenya. *Medical and Veterinary Entomology* **4:** 425-432.

International **Irrigation Management Institute** (IIMI). **1986**. Proceedings of the workshop on irrigation and vector-borne disease transmission. International Irrigation Management Institute, Colombo, Sri Lanka.

Jacob, B.G., E. Muturi, P. Halbig, J. Mwangangi, R. K. Wanjogu, E. Mpanga, J. Funes, J. Shililu, J. Githure, J. L. Regens, and R.J. Novak. **2007**. Environmental abundance of *Anopheles* (Diptera: Culicidae) larval habitats on land cover change sites in Karima village, Mwea rice scheme, Kenya. *American Journal of tropical Medicine and Hygiene* **76:**73-80.

Kamau, L., Koekemoer, L.L., R.H. Hunt and M. Coetzee. **2003a**. *Anopheles parensis*: the main member of the *Anopheles funestus* (Diptera: Culicidae) species group found resting inside human dwellings in Mwea area of central Kenya. *Journal of American Mosquito Control Association*, **19:** 130–133.

Kamau, L., Munyekenye, G.O., Koekemoer, L.L., R.H. Hunt and M. Coetzee. **2003b.** Survey of the *Anopheles funestus* (Diptera:Culicidae) group of mosquitoes from 10 sites in Kenya with special emphasis on population genetic structure based on chromosomal inversion karyotypes. *Journal of Medical Entomology*,, **40:** 664-671.

Karch, S.,Z.A. Manzambi and J.J. Salaun. **1991**. Field trials with Vectolex (*Bacillus*

*sphaericus*) and Vectobac (*Bacillus thuringiensis* (H-14) against *Anopheles gambiae* and *Culex quinquefasciatus* breeding in Zaire. *Journal of the American Mosquito Control Association,* **7:** 176–179.

Karch, S., Monteny, N., Jullien, J.L., G. Sinegre and J. Coz. **1990**. Control of *Culex pipiens* by *Bacillus sphaericus* and role of none target arthropods in its recycling. *Journal of the American Mosquito Control Association,* **6:** 47–54

Keiser, J., J. Utzinger and B.H. Singer. **2002**. The potential of intermittent irrigation for increasing rice yields, lowering water consumption, reducing methane emissions, and controlling malaria in African rice fields. *Journal of the American Mosquito Control Association,* **18:** 329–340.

Kent, R.J., Thuma, P.E., S. Mhanrakurwa and D.E. Norris. **2007**. Seasonality, blood feeding behavior, and transmission of *Plasmodium falciparum* by *Anopheles arabiensis* after an extended drought in southern Zambia. *American Journal of tropical Medicine and Hygiene*, **76:** 267–274.

Killeen, G.F., Tanner, M., Mukabana, WR., Kalongolela, MS., Knandy, K., Lindsay, SW., Fillinger, U., and MC. De Castro. **2006**. Habitat targeting for controlling aquatic stages of malaria vectors in Africa. *American Journal of tropical Medicine and Hygiene*, **76:** 267–274.

Killeen, G.F., B.G. Knols and W. Gu. **2003**. Taking malaria transmission out of the bottle: implications of mosquito dispersal for vector-control interventions. *Lancet Infectious Diseases*, **3:** 297–303.

Killeen, G.F., U. Fillinger, and B.G.J. Knols. **2002a.** Advantages of larval control for

African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. *Malaria Journal* **1:** 8.

Killeen, G.F., Fillinger, U., Kiche, I., L.C. Gouagna and B.G. Knols. **2002b**. Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa? *Lancet Infectious Diseases,* **2:** 618-27

Kitron, U., and A. Spielman. **1989**. Suppression of transmission of malaria through source reduction: antianopheline measures applied in Israel, the United States, and Italy. *Reviews of Infectious Diseases,* **11:** 391–406.

Klinkenberg, E. W. Takken, F.Huibers, and Y.T.Toure. **2003**. The phenology of malaria mosquitoes in irrigated rice fields in Mali. *Acta Tropica* **85:**71-82.

Klinkenberg, E., F. Huibers, W. Takken, and Y. Touré. **2002.** Water management as a tool for malaria mosquito control? – The Case of the Office du Niger, Mali. *Irrigation and Drainage Systems,* **16:** 201- 212.

Koella, J.C., Sørensen, F.L., and Anderson, R.A. **1998**. The malaria parasite, *Plasmodium falciparum,* increases the frequency of multiple feeding of its mosquito vector *Anopheles gambiae. Proceedings of the Royal Society of London B.,* **256**:763-768

Koudou, B.G., Tano, Y., duombia, M., Nsanzabana, C., Cisse, G., Girardin, O., Dao, D., N'goran, E.K.N., Vounatsou, P., Bordmann, G., Keiser, J., Tanner, J., M. Tanner, and J. Utzinger. **2005**. Malaria transmission dynamics in central cote d'Ivore: the influence of changing patterns of irrigated rice agriculture. *Medical and Veterinary Entomology,* **19:** 27- 37.

Krafsur, E.S. **1970.** *Anopheles nili* as a vector of malaria in a lowland region of Ethiopia. *Parasitologia*, **12:** 47-61.

Kumar, A., Sharma, V.P., P.K. Sumodan and D. Thavaselvam. **1998**. Field trials of biolarvicide *Bacillus thuringiensis* var. *israelensis* strain 164 and the larvivorous fish *Aplocheilus blocki* against *Anopheles stephensi* for malaria control in Goa, India. Journal of the American Mosquito Control Association **14:** 457–462.

Kumar, A., Sharma, V.P., D. Thavaselvam and P.K. Sumodan. **1995**. Control of *Anophelesstephensi* breeding in construction sites and abandoned overhead tanks with *Bacillus thuringiensis* var. *israelensis*. Journal of the American Mosquito Control Association, **11:** 86–89.

Lacey, L. A. and C. M. Lacey. **1990**. The medical importance of rice land mosquitoes and their control using alternatives to chemical insecticides. *Journal of the American Mosquito Control Association*, **6:** 1-93.

Lane, R.P. and R.W. Crosskey. **1993**. *Medical insects and Arachnids*. Published by Chapman and Hall London.

Lawler, S.P. and Dritz, D.A. **2006**. Effects of rice straw and water management on the riceland mosquitoes. *Journal of Medical Entomology*, **43:** 828-832.

Lengeler, C. **2001.** *Insecticide-treated bednets and curtains for preventing malaria* (Cochrane Review). The Cochrane Library 4, Oxford pp. 1–70.

Lincithicum, K, Anyamba A, Tucket C, Kelly P, Myers M, C. Peters .**1999**. Climate and satellite indicators to forecast Rift Valley Fever epidemics in Kenya. *Science*, **285:** 397-400.

Lindblade KA, Walker ED, Onapa WA, Katungu J, Wilson ML, **2000**. Land use change alters malaria transmission parameters by modifying temperature in a highland area of Uganda. *Tropical Medicine and International Health*, **5:** 263–274.

Lindsay, S.W., Jawara, M., Paine, K., Pinder, M., G.E. Walraven and P.M. Emerson. **2003**. Changes in house design reduce exposure to malaria mosquitoes. *Tropical Medicine and International Health,* **8:** 512–517

Lindsay, S.W., Wilkins, H.A., Zieler, H.A., Daly, R.J., V. Petrarca and P. Byass, **1991**. Ability of *Anopheles gambiae* mosquitoes to transmit malaria during the dry and wet seasons in an area of irrigated rice cultivation in The Gambia. *Journal of Tropical Medicine and Hygiene,* **94:** 313 - 324.

Lines, J. **1996:** Mosquito nets and insecticides for nets treatment: a discussion of existing and potential distribution systems in Africa. *Tropical Medicine and International Health*, 1:616-632.

Lines, J.D., Curtis, C.F., T.J. Wilkes and K.J. Njunwa. **1991**. Monitoring human-biting mosquitoes (Diptera: Culicidae) in Tanzania with light-traps hung beside mosquito nets. *Bulletin of Entomological Research,* **81:** 77–84.

Logan, T., Lincithicum, K., Thande, P., Wagateh, J., C.Roberts. **1991**. Mosquito species collected from a marsh in Western Kenya during the long rains. *Journal of American Mosquito Control Association.* **7**:395-399.

Loyala, E.G., Gonzalez, L.C., Rodriguez, M.H., Arredondo, J.I., S. Bennett and D.N. Bown. **1993**. *Anopheles albimanus* (Diptera: Culicidae) host selection in three ecological areas of the coastal plains of Chiapas, southern Mexico. *Journal of Medical Entomology*, **30:**518-523.

MacDonald, G. **1957**. The epidemiology and control of Malaria. Oxford University Press, London.

Magbity, E.B., Lines J.D., Marbiah, M.T.,, K. David and E. Peterson. **2002**. How

reliable are light traps in estimating biting rates of adult *Anopheles gambiae s.l.* (Diptera: Culicidae) in the presence of treated bed nets? *Bulletin of Entomological Research*, **92:** 71–76.

**Magesa**, S.M., Wilkes, T.J., Mnzava, A.E.P., Njunwa, K.J., Myamba, J., Kivuyo, M.D.P., Hill, N., J.D. Lines and C.F. Curtis. **1991**. Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2 Effects on the malaria vector population. *Acta Tropica*, **49:** 97-108

**Mahande**, A., Mosha, F., J. Mahande and E. Kweka. **2007a**. E: Feeding and resting behaviour of malaria vector, *Anopheles arabiensis* with reference to zooprophylaxis. *Malaria Journal*, **6:** 100

**Mahande** AM, Mosha FW, J.M. Mahande and E.J. Kweka.**2007b**: Role of cattle treated with deltamethrine in areas with a high population of *Anopheles arabiensis* in Moshi, Northern Tanzania. *Malaria Journal*, **6:**109

**Malaney**, P., Spielman, A. and J. Sachs. **2004**. The malaria gap *American Journal of Tropical Medicine and Hygiene* **71**: 141–146.

**Manoukis**, N. C., Toure ´ M.B., Sissoko, I. Doumbia S. Traore´ S.F., M.A. Diuk-Wasser and C. E. Taylor. **2006**. Is vector body size the key to reduced malaria transmission in the irrigated region of Niono, Mali?. *Journal of Medical Entomology*, **43:** 820-827.

**Marrama**, L., R. Jambou, and I. Rakotoarivony. **2004**. Malaria transmission in southern Madagascar: influence of the environment and hydro-agricultural works in sub-arid and humid regions. Part 1. *Acta tropica* **89:** 193-203.

**Marrama**, L., Rajaonarivelo E, Laventure S, and Rabarison P. **1995**. *Anopheles funestus*

and rice culture on the Plateau of Madagascar. *Cahiers d'Etudes et de Recherches Francophones Sante'* **5:** 415-419.

Mawuli, D., S. K. Dunyo, C. K. Ahorlu, W. Z. Coker, M. A. Appawu, E. M. Pedersen, and P. E. Simonsen. **1999.** Bancroftian filariasis in an irrigated project community in southern Ghana. *Tropical Medicine and Intenational Health,* **4:** 13-18.

Mbogo, CM., Snow, RW., Khamala, CP., Kabiru, EW., Ouma, JH., Githure, JI., Marsh, K., JC Beier.**1995**. Relationships between *Plasmodium falciparum* transmission by vector populations and the incidence of severe disease at nine sites on the Kenyan coast. *American Journal of Tropical Medicine and Hygiene,* **49:** 245–253.

Mbogo, CN., Glass, GE., Forster, D., Kabiru, EW., Githure, JI., Ouma, JH. and JC. Beier, **1993a**. Evaluation of light traps for sampling anopheline mosquitoes in Kilifi, Kenya. *Journal of the American Mosquito Control Association.* **9:** 260–263.

Mbogo, CM, Snow RW, Kabiru EW, Ouma JH, Githure JI, Marsh K, JC, Beier. **1993b**. Low-level Plasmodium falciparum transmission and the incidence of sever malaria on the Kenyan coast. Am J Trop Med Hyg **49:** 245–253.

Metcalf, R.L. and Novak, R.J. **1994.** *Pest management strategies for insects affecting humans and domestic animals*. In Introduction to Insect Pest Management, Third Edition ed. Robert L. Metcalf & William H. Luckman John Wliey & Sons Inc.

Minakawa, N., Dida, G.O., Sonye, G.O. K. Futami and S. Kaneko. **2008**. Unforeseen misuses of bed nets in fishing villages along Lake Victoria. *Malaria Journal*, **7:** 165.

Minakawa, N., S. Pamela, and G. Yan. **2002**. Influence of host and larval Habitat distribution on the abundance of African malaria vectors in western Kenya. *American Journal of Tropical Medicine Hygiene*, **67:** 32–38.

Minakawa, N., Mutero, C.M., Githure, J.I., Beier, J.C. and Yan, G. **1999**. Spatial distribution and habitat characterization of anopheline mosquito larvae in Western Kenya. *American Journal of Tropical Medicine and Hygiene,* **62:**1010-1016.

Mnzava, A.E., Rwegoshora, R.T., Tanner, M., Msuya, F.H., C.F. Curtis, and S.G. Irare. **1993.** The effects of house spraying with DDT or lambda-cyhalothrin against *Anopheles arabiensis* on measures of malarial morbidity in children in Tanzania. *Acta Tropica* **54:** 141–151.

Morel, C.M., Thang, N.D., Xa, N.X., Hung, L.X., Thuan, L.K., Ky, P.V., Erhart, A., A.J. Mills and U. D'Alessandro. **2008**. The economic burden of malaria on the household in south-central Vietnam. *Malaria Journal*, **7:** 166.

Mouchet, J., Manguin, S., Sircoulon, J., Laventure, S., Faye, O., Onapa, A.W., Carnevale, P., and P. Julvez and D. Fontenille. **1998.** Evolution of malaria in Africa for the pas 40 years: impact of climate and human factors. *Journal of American Mosquito control Association*, **14:** 121 - 130.

Mukabana, W.R., Kannady, K., Ijumba, J., Mathenge, E.M., Kiche, I., Nkwengulila, G., Mboera, L., Mtasiwa, D., Yamagata, Y.,, van Schayk, I., Knols, B.G.J., Lindsay, S.W., de Castro, M.C., Mshinda H., Tanner M., U. Fillinger and G.F. Killeen. **2006.** Ecologists can enable communities to implement malaria vector control in Africa. *Malaria Journal* **5:** 9.

Mukiama, T.K. and R.W. Mwangi. **1990**. Population and cytogenetic observation of *Anopheles arabiensis* Patton of Mwea irrigation scheme, Kenya. *Insect Science applications*, **11:**119-131.

Mukiama T.K. and R.W. Mwangi. **1989**. Seasonal population changes and malaria

transmission potential of *Anopheles pharoensis* and the minor anophelines in Mwea Irrigation Scheme, Kenya. *Acta Tropical* **46:**181-9.

Mulla, M.S., Darwazeh, H.A., Davidson, E.W., H.T. Dulmage and S. Singer. **1984**. Larvicidal activity and field efficacy of *Bacillus sphaericus* strains against mosquito larvae and their safety to non-target organisms. *Mosquito news* **44**: 336-342

Mulla, M.S., Norland, R.L., Fanara, D.M., A. Darwazeh and D. McKean. **1971**. Control of chironomid midges in recreational lakes. *Journal of Economic Entomology*, **64:** 300–307.

Muriu, S.M., Muturi E.J., Shililu, J.I., Mbogo, C.M., Mwangangi, J.M., Jacob, B.G., Irungu, L.W., Mukabana, R.W., Githure, J.I. and Novak, R.J. **2008**: Host choice and multiple blood feeding behaviour of malaria vectors and other anophelines in Mwea rice scheme, Kenya. *Malaria Journal*, **7:** 43

Mutero, C., P. Ng'ang'a, P. Wekoyela, J. Githure, and F. Konradsen. **2004a**. Ammonium sulphate fertiliser increases larval populations of *Anopheles arabiensis* and culicine mosquitoes in rice fields. *Acta Tropica*, **89:** 187-192.

Mutero, C., C. Kabutha, V. Kimani, L. Kabuage, G. Gitau, J. Ssennyonga, J. Githure, L. Muthami, A. Kaida, L. Musyoka, E. Kiarie, and M. Oganda. **2004b**. A transdisciplinary perspective on the links between malaria and agroecosystems in Kenya. *Acta Tropica*, **89:** 171-186.

Mutero C., C. Kabutha L., Kabuage V., Kimani G., Gitau J., Ssennyonga L. Muthami and J. Githure. **2004c**. Livestock and Agroecosystem Management for Community-based Integrated Malaria Control. FINAL IDRC REPORT 2003.

Mutero, C.M. H. Blank F. Konradsen, W. van der Hoek. **2000**. Water management for

controlling the breeding of *Anopheles* Mosquitoes in rice irrigation schemes in Kenya. *Acta Tropica* **76:** 253-263

**Mutuku,** F.M., Bayoh, M.N., Gimnig, J.E.,Vulule, J.M., Kamau, L., Walker, E.D., E. Kabiru, and W.A. Hawley. **2006.** Pupal habitat productivity of *Anopheles gambiae* complex mosquitoes in a rural village in western Kenya. American *Journal of Tropical Medicine and Hygiene*, **74:** 54–61.

**Muturi,** E.J., Muriu, S., Shililu, J., Mwangangi, J., Jacob, B.G. Mbogo, C., J. Githure, and R. J. Novak. **2008**. Effect of Rice Cultivation on Malaria Transmission in Central Kenya. *American Journal Tropical Med.icine and Hygiene,* **78:** 270-275

**Muturi,** E. J., J. M. Mwangangi, J. Shililu, S. Muriu, B. Jacob, E. W. Kabiru, W. Gu, C. Mbogo, Githure J., and R. Novak. **2007**. Mosquito species succession and the physicochemical factors affecting their abundance in rice fields in Mwea, Kenya. *Journal. Medical Entomology*, **44:** 336-344.

**Muturi** E.J., J. Shililu, B. Jacob, W. Gu, J. Githure, R.Novak. **2006**. Mosquito species diversity and abundance in relation to land use in a riceland agroecosystem in Mwea, Kenya. *Journal of Vector Ecology* **31:**129-37.

**Mwangangi,** J.M., E.J. Muturi, J. Shililu, S.M. Muriu, B.Jacob, E.W. Kabiru, C.M Mbogo, J. Githure and R. Novak. **2006a**. Survival of immature *Anopheles arabiensis* (Diptera: Culicidae) in aquatic habitats in Mwea rice irrigation scheme, central Kenya. *Malaria Journal* **5:**114.

**Mwangangi**, J.M., J. Shililu, E.J. Muturi, G. Weidong, C. Mbogo, E. Kabiru, B. Jacob,

J. Githure and R. Novak. **2006b**. Dynamics of immature stages of *Anopheles arabiensis* and other mosquito species (Diptera: Culicidae) in relation to rice cropping in a rice agro-ecosystem in Kenya. *Journal of Vector Ecology* **31:**245-251.

**Mwangi,** R.W. and T.K. Mukiama. **1992.** Irrigation scheme or mosquito hazard: a case study in Mwea irrigation scheme. *Hydrobiologia,* **232:** 19-22

**Najera** JA, **1999.** Malaria control: Achievements, problems, strategies WHO/CDS/RBM/9910

**Nwaka,** S., Riopel, L., Ubben, D. and Croft, J.C. **2004.** Medicines for Malaria Venture new developments in antimalarials. *Travel Medicine and Infectious Disease* **2:**161–170 www.elsevierhealth.com/journals/tmid

**Oomen,** J.M.V., J. de Wolf and W.R. Jobin**. 1988**. *Health and irrigation. Incorporation of Disease-Control Measures in Irrigation, a multi-faceted Task in Design, Construction, Operation.* International institute of Land reclamation and Improvement/ILRI, Wageningen, The Netherlands.

**Onori,** E. and B. Grab. **1980**. Indicators for the forecasting of Malaria epidemics. *Bulletin of the World Health Organization*, **58:** 91 - 98.

**Petrarca,** V., Beier, J.C., Onyango, F., Koros, J., Asiago, C., D.K. Koech and C.R. Roberts. **1991**. Species composition of *Anopheles gambiae* complex (Diptera: Culicidae) at two sites in western Kenya. *Journal of Medical Entomology*, **28:** 307 - 313.

**Ragoonanansingh,** R.N., Njunwa, K.J., C.F. Curtis and N. Becker. **1992.** A field study of *Bacillus sphaericus* for the control of culicine and anopheline mosquito larvae in Tanzania. *Bulletin of the Society of Vector Ecology*, **17:** 45–50.

**Rajavel,** A., R. Natarajan, and K. Vaidyanathan. **2005**. Mosquito collections in the Jeypore

hill tracts of Orissa, India, with notes on three new country records, Culex (Lophoceraomyia) pilifemoralis, Culex (Lophoceraomyia) wilfredi and Heizmannia (Heizmannia) chengi. *Journal of American Mosquito Control Association,* **21:** 121-127.

**Reisen,** W. K., F. Mahmood, and K. Azra. **1981**. *Anopheles culicifacies* Giles : adult ecological parameters measured in rural Punjab province, Pakistan using capture-mark -release and dissection methods, with comparative observations on *An. stephensi* Liston and *An. subpictus* Grassi. *Research in Population Ecology.* **23:** 39-60.

**Reiter,** P., Cordellier, R., Ouma, J.O., B.C. Cropp, and H. Savage. **1998**. First recorded outbreak of yellow fever in Kenya, 1992-1993. II. Entomologic investigations. *American Journal of Tropical Medical Hygiene*, **59:** 650-656.

**Reuben,** R., Thenmozhi, V., Samuel, P.P., A. Gajanana and T.R. Mani. **1992**. Mosquito blood feeding patterns as a factor in the epidemiology of Japanese encephalitis in southern India. Am J Trop Med Hyg, 46: 654–663.

**Ritchie,** S. A. van den Hurk and J. Shield. **1997**. The 1995 Japanese encephalitis outbreak: why Badu? *Arbovirus Research Australia*, **7:** 224–227.

**Robert,** V., A. Van den Broek, P. Stevens, R. Slootweg, V. Petrarca, M. Coluzzi, G. Le Goff, M. A. Di Deco, and P. Carnevale. **1992**. Mosquitoes and malaria transmission in irrigated rice-fields in the Benoue valley of northern Cameroon. *Acta Tropica,* **52:** 201-204.

**Robert,** V. and P. Carnevale. **1991**. Influence of deltamethrin treatment of bednets on malaria transmission in the Kou valley, Burkina Faso. *Bulletin of World Health Organization*, **69:**735-740

Robert, V., Ouari, B., V. Ouedraogo and P. Carnevale. **1988.** Etude e´cologique des Culicidae adultes et larvaires dans une rizie`re en Vale´e du Kou, Burkina Faso. *Acta Tropica*, **45:** 351-359.

Robert, V., Gazin P, and Boudin C. **1985.** The transmission of malaria in a wooded savanna zone and a rice-growing zone in the vicinity of Bobo Dioulasso (Burkina Faso). *Annales de la Socie´te´ Belge de Me´decine Tropicale*, **65:** 201-214.

Roberts, D.R., S. Manguin, and J. Mouchet. **2000**. DDT house spraying and re-emerging malaria. *Lancet* **356:**330-332.

Rogers, D., Randolph, S.E., R.W Snow and S.I Hay. **2002**. Satellite imagery in the study and forecast of malaria. *Nature,* **415:** 710–715.

Romi R., Ravonihaimlina, B.Ramiakajoto, M.Mjiri, G. **1993.** Field trials of *Bacillus thuringiensis* H-14 and *Bacillus sphaericus* (strain 2362) formulations against *Anopheles arabiensis* in the central highlands of Madagascar. *Journal of American Mosquito Association,* **19**: 325-329.

Ronn, M.A., Msangeni, H.A., W.H. Wernsdorfer and I.C Bygbjerg.**1996**. High levels of resistance of *Plasmodium falciparum* to sulfadoxine-pyremethamine in children in Tanzania. *Transactions of Royal Society of Tropical Medicine and Hygiene,* **90:** 179-181

Rwegoshora, R.T., Simonsen, P.E., Meyrowitsch, D.W., Malecela-lazaro1, M.N., E. Michael and E.M. Pedersen. **2007**. Bancroftian filariasis: house-to-house variation in the vectors and transmission and the relationship to human infection in an endemic community of coastal Tanzania. *Annals of Tropical Medicine and Parasitology*, **101:** 51–60.

Sachs, J. and P. Malaney .**2002**. The Economic and Social Burden of Malaria. *Nature* **415:**

680-85

Schnetter, W., Engler, S., J. Morawcik and N. Becker. **1981**. Wirksamkeit von *Bacillus thuringiensis var. israelensis* gegen Stechmuecken und Non-Target Organismen. Mitteilungen der Deutschen Gesellschaft fuer Allgemeine und Angewandte Entomologie **2:** 195–202.

Service, M.W. **1997**. Mosquito (Diptera: Culicidae) dispersal – the long and short of it. *Journal of Medical Entomology*, **34:** 579–588.

Service, M.W.**1993**. *Mosquito Ecology. Field Sampling Methods,* second ed. Elsevier/Chapman and Hall, London

Service, M.W. 1991. Agricultural development and arthropod borne diseases: a review. Review Saude Publica 25: 165–178.

Service, M.1**989**. Rice, a challenge to health. *Parasitology Today*, **5:** 11-12.

Service MW. **1977**. Mortalities of the immature stages of species B of the *Anopheles gambiae* complex in Kenya: comparison between rice fields and temporary pools, identification of predators, and effects of insecticidal spraying. *Joural of Medical Entomology.* **13:** 535-45

Service, M.W. **1971**. Studies on sampling of larval populations of the *Anopheles gambiae* complex. *Bulletin of World Health organisation,* **45:169**-180

Service, M.W. **1964.** The behaviour of *Anopheles nili* Theobald in sprayed huts in Northern Nigeria. *Journal of Tropical Medicine and Hygiene*, **67:** 11-12.

Sharma, S.N., T. Sharma and H. Prasad. **1998**. Impact of Spherix (*Bacillus sphaericus* B-101, serotype H5a, 5b) spraying on the control of mosquito breeding in rural areas of Farrukhabad District, Uttar Pradesh. *Indian Journal of Malariology,* **35:** 185–196.

Shililu J, Ghebremeskel T, Seulu F, Mengistu S, Fekadu H, Zerom M, Ghebregziabiher

A, Sintasath D, Bretas G, Mbogo C, Githure J, Brantly E, Novak R, JC Beier. **2003a.** Larval habitat diversity and ecology of anopheline larvae in Eritrea. *Journal of Medical Entomology,* **40:** 921-9.

Shililu, J. I., Tewolde G.M., Brantly, E., Githure, J. I.,. Mbogo, C. M, Beier, J. C., R. Fusco and R. J. Novak. 2003b. Efficacy of *Bacillus thuringiensis israelensis*, *Bacillus sphaericus* and temephos for managing *Anopheles* larvae in Eritrea. *Journal of the American Mosquito Control Association*, 19: 251 – 258.

Shililu, J. Ghebremeskel, T., Mengistu, S., Fekadu, H., Zerom, M., Mbogo, C., Githure, J. Novak, R., E. Brantly, and J.C. Beier. **2003c**. High seasonal variation in entomologic inoculation rates in Eritrea, a semi-arid region of unstable malaria in Africa. *American Journal Tropical Medicine and Hygiene*, **69:** 607 – 613

Shililu, J.I., Maier, W.A., H.M. Seitz and A.S. Orago, A.S., **1998**. Seasonal density, sporozoite rates and entomological inoculation rates of *Anopheles gambiae* and *Anopheles funestus* in a high-altitude sugarcane growing zone in Western Kenya. Tropical Medicine and International Health **3:** 706–710.

Shope, R. E., **1980**, Arbovirus-related encephalitis. *Yale Journal of Biological Medicine*, **53:** 93-99.

Shousha, A.T. **1948.** Species-eradication. The eradication of *Anopheles gambiae* from Upper Egypt, 1942–1945. *Bulletin of the World Health Organisation* **1:** 309–353

Sissoko, M. S., A. Dicko, O. J. T. Brie¨ t, M. S. Sissoko, I. Sagara, H. Keita, M. Sogoba, C. Rogier, Y. Toure, and O. Doumbo. **2004**. Malaria incidence in relation to rice cultivation in the irrigated Sahel of Mali. *Acta Tropica* **89:** 161–170.

Skovmand, O. and E. Sanogo. **1999**. Experimental formulations of *Bacillus sphaericus*

and *B. thuringiensis israelensis* against *Culex quinquefasciatus* and *Anopheles gambiae* (Diptera: Culicidae) in Burkina Faso. *Journal of Medical Entomology,* **36:** 62–67.

**Smith,** T.M. Leuenberger, A.R. and C. Lengeler. **2001**. Child mortality and malaria transmission intensity in Africa. *Trends in Parasitology*, **17:**145-149

**Smith,** T., J.D. Charlwood and W. Takken. **1995**. Mapping the densities of malaria vectors within a single village. *Acta Tropica,* **59:** 1-18.

**Snow,** R.W.,Ikoku, I., J. Omumbo, and J. Ouma. **1999.** The epidemiology, politics and control of malaria epidemics in Kenya: 1900-1998. Report prepared for Roll Back Malaria, resource network on epidemics, World Health Organisation

**Snow,** R.W., Lindsay, S.W., Hayes, R.J., and B.M. Greenwood. **1988**. Permethrin-treated bednets (Mosquito nets) prevent malaria in Gambian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **82:** 838-842

**Snow,** W. F. **1983**. Mosquito Production and Species Succession from an Area of Irrigated Rice Fields in the Gambia, West Africa. *Journal of Tropical Medicine and Hygiene*, **86:** 237-245

**Soper,** F.L., and D.B. Wilson**. 1943**. *Anopheles gambiae in Brazil*. The Rockefeller Foundation, New York.

**Sueur,** D and Sharp, B. **1988.** The breeding requirements of three members of the *Anopheles gambiae* Giles complex (Diptera: Culicidae) in the endemic malaria area of Natal, South Africa. *Bulletin of Entomological Research*, **78:** 549-560.

**Takken**, W and S.W. Lindsay. **2003.** Factors affecting the vectorial competence of *Anopheles gambiae:* a question of scale *In: Takken W, Scott TW, eds. Ecological aspects for application of genetically modified mosquitoes. Ecological aspects for application of*

*genetically modified mosquitoes. Kluwer Academic Publishers, Dordrecht. Frontis series no.2*

**Takken**, W., M.J. Klowden, and G.M. Chambers, **1998**. Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae* sensu stricto (Diptera: Culicidae): the disadvantage of being small. *Journal of Medical Entomology* **35:** 639–645.

**Thomson**, M., Connor, S., Quinones, M., Jawara, M., J. Todd and B. Greenwood. **1995**. Movement of *Anopheles gambiae s.l.* malaria vectors between villages in the Gambia. *Journal of Medical and Veterinary Entomology*, **9:** 413–419.

**Townson**, H., Nathan, M.B., Zaim, M., Guillet, P., Manga, L., R. Bos and M. Kindhauser. **2005**. Exploiting the potential of vector control for disease prevention. *Bulletin of the World Health Organization*, **83:** 942–947.

**Trape**, J.F. **2001**. The public health impact of chloroquine resistance in Africa. *American Journal of Tropical Medicine and Hygiene* **64:** 12–17.

**Udonsi**, J. **1988**. Bancroftian filariasis in the Igwun Basin, Nigeria. An epidemiological, parasitological, and clinical study in relation to the transmission dynamics. *Acta Tropica,* **45:** 31-38.

**Utzinger**, J., Tanner, M., Kammen, D.M., G.F.Killeen, B.H Singer. **2002**. Integrated programme is key to malaria control. *Nature*, **419:** 431.

**Utzinger**, J., Tozan, Y. and B.H. Singer.**2001**. Efficacy and cost-effectiveness of environmental management for malaria control. *Tropical Medicine and International Health,* **6:** 677–687.

**Vythilingam**, I., Sidavong, B., Chan, S.T., Phonemixay, T., Vanisaveth, V., Sisouland P.,

Phetsoovanh, R., Hakim S.L. and S. Phompida. **2005**. Epidemiology of malaria in Attapeu Province, Lao PDR in relation to entomological parameters. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **99:** 833- 839.

Weil, D.E.C., Alicbusan, A.P., Wilson, J.F., Reich, M.R. and Bradley, D.J. **(1990).** *The Impact of Development. Policies on Health. A review of Literature.* World Health Organization Geneva.

Wilkes, T.J., Y.G. Matola and J.D. Charlwood. **1996.** *Anopheles rivulorum*, a vector of human malaria in Africa. *Medical Veterinary Entomology*, **10:** 108-110.

Williams, M., J. Woodall, P. Corbet, and J. Gillet. **1965**. O'nyong-nyong fever:an epidemic virus diseases in East Africa. VIII. Virus isolation from *Anopheles* mosquitoes. *Transactions of the Royal Society of Tropical Medicine Hygiene*, **59:** 300-306

Winch PJ, Lloyd LS, Hoemeke L, Leontsini E. **1994**. Vector control at the household level: an analysis of its impact on women. *Acta Tropica.* **56:**327-39

Windmeijer, P.N. and Andriesse, W. **1993**. *Inland Valleys in West Africa: an agro-ecological Characterization of Rice-Growing Environments.* 52, ILRI, Wageningen

Wirth, M.C., Jiannino, J.A.,Federici, B.A. and Wilton,W.E. **2004** :Synergy between toxins of *Bacillus thuringiensis* susp.*israelensis* and *Bacillus sphaericus. Journal of Medical Entomology,* **41:** 935-941.

Wirtz, R., Zavala, F., Charoenvit, Y., Campbell, G.H., Burkot, T.R., Schneider, I., Esser, K.M., Beaudoin, R.L., Andre, R.G., **1987**. Comparative testing of monoclonal antibodies against Plasmodium falciparum sporozoites for ELISA development. *Bulletin of World Health Organissation*, **65:** 39–45.

White, G.B. **1974**. *Anopheles gambiae* and disease transmission in Africa. *Transactions*

*of the royal society of Tropical Medicine and Hygiene,* **68:** 278-298.

WHO. **2005.** *Malaria control today.* Current WHO recommendations. Working document. Roll Back Malaria department, World Health Organization Geneva Switzerland. 1-75

WHO. **2000**. African Summit on Roll Back Malaria, Abuja, Nigeria, April 25, 2000. Geneva: World Health Organization. (WHO/CDS/RBM/2000.17).

WHO. **1997**. World malaria situation in 1994. *Weekly epidemiological record* **36:** 269-276. http://www.who.int/docstore/wer/pdf/1997/wer7236.pdf

WHO. **1995**. *Vector control for malaria and other mosquito-borne diseases*. Report of a WHO Study Group. WHO Technical Report Series No. 857. WHO, Geneva

WHO. **1993**. Global strategy for malaria. World Health Organisation. Geneva

WHO, World Health Organization. **1975**. *Manual on the practical entomology in malaria.* Part II. *Methods and techniques* Geneva, Switzerland: *World Health Organization Offset Publication*, 13.

WHO and UNICEF. **2003.** The Malaria Report, WHO/CDS/MAL/2003.1093.

WHO. **2001**. Report of the fifth whopes working group meeting (Review of VectoBaWG, Permanet® and Gokilaht-S5EC), Geneva,43.

WHO/FAO/UNEP. **1990**. *Waters Resources Development and Vector Borne Diseases Kenya.* World Health Organisation, Geneva.

WHO/FAO/UNEP. **1987**. *Effects of agricultural development on vector-borne diseases.* Working papers of 7th annual meeting, Joint WHO/FAO/UNEP Panel of Experts on Environmental management for Vector control, World Health Organisation, Geneva.

WRI/UNEP/UNDP. **1995**. *World Resources. A Guide to the Global Environment. People and the Environment.* Oxford University Press, Oxford.

**Yohannes**, M., Haile, M., Ghebreyesus, T.A., Witten, K.H., Getachew, A., P. Byass and S. W. Lindsay. **2005**. Can source reduction of mosquito larval habitat reduce malaria transmission in Tigray, Ethiopia? *Tropical Medicine and International Health* **10:** 1274–1285.

**Zimmerman**, R.H., Galardo, A.K.R., Lounibos, L.P., M. Arruda and R. Wirtz. **2006**. Bloodmeal hosts of *Anopheles* species (Diptera: Culicidae) in a malaria-endemic area of the Brazilian Amazon. *Journa lof Medical Entomology,* **43:** 947-956.

**Zhou**, G., Munga, S., Minakawa, N., A. K. Githeko, and G. Yan. **2007**. Spatial relationship between adult malaria vector abundance and environmental factors in Western Kenya Highlands. American *Journal of Tropical Medicine and Hygiene*, **71:** 29-35.

**Zhou**, G., Minakawa, N., Githeko, A. and G. Yan. **2004a**. Spatial distribution pattern of malaria vectors and sample size determination in spatially heterogeneous environments: A case study in West Kenyan highlands. *Journal of Medical Entomology* **41:** 1001-1009

**Zhou** G, Minakawa N, Githeko AK, Yan G, **2004b**. Association between climate variability and malaria epidemics in the East African highlands. *Proceedings of National Academies of Science, US,A* **101:** 2375–2380.

## APPENDIX I: BLOODMEAL ELISA

### BLOODMEAL ELISA SOLUTIONS

Phosphate buffered saline (PBS), Ph 7.4:

Use stock laboratory PBS or add 1 bottle of Dulbecco's BS to 1 litre of distilled water, mix and adjust pH if necessary. Store all of the following solutions at 4°c.

| 2. Boiled Casein, 0.5% (BC): | 500ml | 1 litre |
|---|---|---|
| Casein | 2.50 g | 5.00 g |
| 0.1 N NaOH | 50.0ml | 100 ml |
| PBS, Ph 7.4 | 450 ml | 900ml |
| Thimerosal | 0.05 g | 0.10 g |
| Phenol red | 0.01 g | 0.02 g |

a) Suspend casein in 0.1 N NaOH and bring boil.
b) After casein is dissolved, slowly add PBS, allow cooling and adjusting the pH to 7.4 with HCL.
c) Add the thimerosal and phenol red.
d) Shelf life – one week.

3. Wash solution (PBS – Tw);

PBS plus 0.05% Tween 20. Add 0.5 ml of Tween 20 to 1 L of PBS. MIX WELL. Do not store. Make each day.

4. Enzyme Diluent (BC-Tw):

100ml + 25µl Tween 20. Do not store. Make each day.

## HUMAN AND BOVINE HOST BLOODMEAL PROTOCOL FOR ELISA

A. Sample Preparation:

1. Negative controls – Grind male mosquitoes in 500 µl of PBS per mosquito.
2. Positive controls – For each host serum: To 500 µl PBS add 5 µl of host serum control.
3. Blood-meal Samples:- Dilute each mosquito abdomen sample in 1000 µl of PBS.
   - to ensure proper grinding, first put 100 µl PBS and grind with a grinder, then add 900 µl PBS to raise to the required volume.

**B Technique:**

To a PVC flex plate add:

- column 1 add 50 µl/well of eight negative controls
- column 2 add 50 µl/well of eight positive controls (chicken human, pig, cat, horse, cow, goat & dog).
- One well should be designated as a blank control and receive 50 µl of PBS alone.
- The remaining wells should receive 50 µl/well of mosquito blood meal sample.
- Incubate overnight at room temperature.
- Wash plate with PBS-Tween 2 times.

Enzyme – Conjugate preparation:

To 5 ml of BC-Tw (Enzyme diluent) add:

HRP phosphate anti-human ---------- 2.5 µl (1:2000 dilution)

Bovine phosphatase Conjugate ---------- 20.0 µl (1:250 dilution), and Sera of each host except the one being tested for ------ 10.0 µl (1: 500 dilution).

NOTE: Add serum from all hosts except those for which enzyme –conjugate was added. All HRP conjugates should be diluted 1: 2000. Cow which is a phosphatase conjugate should be diluted 1:250.

4. Add 50 µl/well of the prepared enzyme conjugate solution.
5. Incubate for 1 h at room temperature.
6. Wash plate with PBS-TW 3 times

Peroxidase substrate preparation: Mix solutions A and B together, 1:1 i.e.,
5 ml + 5 ml per plate.

7. Add 100 5 µl of substrate to each well
8. Incubate for 30 minutes
9. Read absorbance visually or at 414 nm with ELISA reader.
10. Wash plate with PBS-TW 3 times.

Phosphatase substrate preparation:

Add 2 tablets to 8 ml of distilled water and 2 ml of diethanolamine buffer.

11. Add 100 µl of the phosphatase substrate to each well.
12. Incubate for 5 hrs and read absorbance at 414 nm (you can
13. Read from 2 hrs onwards).

**Notes on bloodmeal ELISA**

Preparation for PBS (7.4), BC 0.5% and PBS-Tween are as for sporozoite ELISA.

For goat blood meal ELISA follow same directions except for use of conjugate use HRP goat dilution as in human i.e. 1:2000 (2.5 µl).

## APPENDIX II: SPOROZOITE ELISA TEST

SPOROZOITE ELISA SOLUTIONS

Reagents preparation:

1. Phosphate Buffered Saline (PBS-plain):

This reagent is used for the dilution of capture monoclonal antibody (Mab) before the plates are coated and when preparing the blocking buffer and PBS-Tween.

(i) Rinse the preparatory plastic bottle with distilled water.

(ii) Add 1 litre of distilled water into the bottle.

(iii) Pour the whole content of 1 Dulbecco's PBS BOTTLE (9.7GM) INTO THE BOTTLE AND PLACE A MAGNETIC STIRRER. Leave for 10 minutes to dissolve.

(iv) The solution is now ready for use. Store in fridge when not in use.

PBS-Tween 20

This is a wash solution. It is used to wash plates.

i. Put 1 litre of PBS plain in the bottle,

ii. Add 500 µl of Tween-20,

iii. Mix well using magnetic stirrer.

iv. Keep in the fridge when not in use.

NOTE: Make each day do not store.

3. Blocking Buffer or Boiled casein

To 1 litre of PBS plain add.

i. 10G bovine Serum Albumin (BSA)

ii. 5g casein

iii. 0.1 g thimerosal

iv. 0.02 g phenol red.

Or

Suspend casein on 0.1 N Sodium hydroxide and bring to boil. After casein has dissolved, slowly add PBS, allow to cool and adjust the pH to 7.4 with Hydrochloric acid (HCI). Add thimerosal and phenol red.

| | | |
|---|---|---|
| Casein (0.5%) | 2.5 g | 5.0g |
| 0.1 N NaOH | 50 ml | 100 ml |
| PBS (pH 7.4) | 450 ml | 900 ml |
| Thimerosal | 0.05 g | 0.1 g |
| Phenol red | 0.01 g | 0.02 g |

Mix well using a magnetic stirrer, leave it to mix for 2 hours or more. Keep in the fridge when not in use.

NOTE: This reagent is used to block the plates and prepare the Nonidet P-40.
Shelf life is 1 week at $4^0$c.

4. Nonidet P-40(NP-40)

To 40 ml of Blocking Buffer add 200 µl or NP-40, mix well and store in fridge when not in use.

5. 2A10 Monoclonal antibody (capture Mab)
This is usually in clear white bottles.

i. Put 5ml of PBS plain into a tube (for only one plate)
ii. Add 20 µl of the capture Mab.
iii. Mix well and dispense 50 µl into well of the PVC plate.

The amount is adjusted according to the number of plates.

6. Peroxidase Labelled Enzyme (2A10).
This is usually in **dark brown bottle.**

i. Put 5ml blocking buffer into a clear tube

ii. Add 10 µl of the 2A10 Peroxidase labeled enzyme (conjugate) into the tube and mix.

This is enough for only one plate.

7. Peroxidase substrate solution.

Mix equal parts 1:1 of solution A and solution B; i.e. for 1 plate mix 5ml of solution A and 5ml solution B. It should be noted that this mixing is only required when using the 2 component substrate. In a one component substrate no mixing is required. Store in the fridge.

NOTE

Solution A (ABTS) is 2.2' azino-di(30ethyl-benzethiazoline sulphonate.

Solution B is Hydrogen peroxide.

8. Enzyme check.

Put 100 µl of substrate into a vial and add 3 µl of fresh prepared enzyme. Mix and observe colour change for colourless to blue.

9. Positive control: $R32tet_{32}$ Reconstitution

Plasmodium falciparum

i. Dissolve lyophilized : $R32tet_{32}$ (10 µg) with 1000 µl distilled water to yield 100ng/10µl. This is normally refereed to as Vial I.

iii. Vial II stock solution

a. Put 1000 µl Blocking Buffer into a tube.

b. Add 10 µl (100ng) of the $R32tet_{32}$ from vial I, and mix well. This gives 1000 pg/10 µl BB

Store in freezer. It is also used to prepare the working control vial III when loading the triturates.

iv. Vial III working solution.

a. Put 1000 µl BB into a vial

b. Add 20 µl 200 pg) $R32tet_{32}$ from vial II, and mix well. This yield 100 pg/50 µl BB. (control I).

a. Put 500 µl BB into a vial

b. Add to µl (1000 pg) $R32tet_{32}$ from vial II, and mix well. This gives 100 pg/50 µl) of BB (control I)

Make 1:10 dilution to get 10 pg/50 µl blocking buffer.

e.g. Put 450 ml BB into a tube and transfer 50 ml from vial III (100 pg/50 ml) and mix well. This is control II. Keep in fridge.

10. Negative controls:

The negative controls are prepared from the head/thorax of the wild males of the same species or from the laboratory reared females.

(i) Put 50 µl of NP-40 into a vial

(ii) Cut the head/thorax and put into the vial

(iii) Leave for sometimes to soften

(iv) Grind with a pestle and adjust the volume by adding 200 µl of blocking buffer.

(v) Keep in the freezer when not in use.

11. Capture Mab (lyophilized Mab) and Peroxidase conjugate Mab Reconstitution.

Dissolve the lyophilized Mab (0.1 mg/vial) and peroxidase conjugated Mab (0.1 mg) in 0.2ml (200 µl) of diluent (1:1 distilled water and glycerine). Store at $4^0$c or $-20^0$c.

**TECHNIQUE**

Triture preparation

a. Label the vial with corresponding numbers as marked in the ELISA working sheet.
b. Add 50µl of BB-Np-40 into each vial
c. Using a sharp clean surgical blade cut the mosquito between the thorax and the abdomen.
d. Pick the head thorax with forceps and transfer to the sporozoite marked vial and the abdomen to the corresponding vial marked blood meal if the mosquito is blood fed. If not blood fed or no blood meal analysis required, discard.

e. Leave the head/thorax to soak in the NP-40 for 20 minutes.

f. Use a non absorbent glass or plastic rod (pestle) to grind the mosquito in the vial.

g. Adjust the volume by adding 200 µl of the blocking buffer.

h. Clean the pestle and wipe it dry with gauze before grinding the next sample to avoid contamination. This is repeated until all samples are prepared. Keep in the freezer until use.

II Plate coating

a. Label the PVC plate with appropriate number

b. Into each well of clean PVC add 50 µl of the diluted capture Mab.

c. Cover the plates and incubate for 30 min at room temperature in subdued light.

II Blocking the plate

a. Using an eight –channeled manifold to a vacuum pump, aspirate the capture Mab from the microtitre plate.

b. Bang the plate hard on an absorbent tissue paper or gauze to ensure complete dryness of the plate.

c. Fill each well with blocking buffer (pH 7.4) using a manifold attached to a 60 µl syringe. Incubate for 1 hour at room temperature in subdued light.

IV Loading the plate with triturates

a. Aspirate the blocking buffer from the wells using the manifold attached to a vacuum pump and bang to complete dryness.

b. Using labeled ELIXA processing sheets, as a guide, put 50 µl of 100, 50, 25, 12, 6, 3, 1.5, 0 pgs positive control in the first column wells. Into the next column wells add 50 µl of the negative controls.

c. Load the first mosquito sample (50 µl triturate) into the third column well (A3) and continue in the horizontal order up to the last well in the plate.

d. Cover the plate and incubate for 2 hours at room temperature in subdued light.

V. Addition of conjugate (Peroxidase enzyme)

a. After 2 hrs aspirate the triturate form the wells

b. Wash the plate 2 times with PBS-TWEEN 20

c. Add 50 µl of the peroxidase labeled enzyme and incubate for 1 h at room temperature

VI Adding of substrate

a. Aspirate the enzyme from the wells and wash 3 times with PBS-Tween 20 and banging it to dryness.

b. Using an octapete multichannel pipette add 100 µl of the substrate mixture and incubate for 30 minutes. Results are read visually or at 414 nm using an ELISA plate reader.