

**THE ENVIRONMENTAL AND AGRICULTURAL
FACTORS THAT REGULATE MALARIA VECTOR
PRODUCTIVITY AND DIVERSITY IN MWEA
IRRIGATION SCHEME, KIRINYAGA DISTRICT, KENYA**

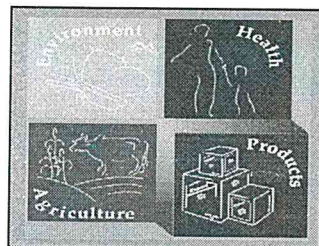
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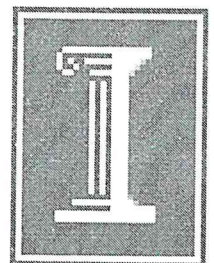
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DECLARATION

This Thesis is my original work and has not been presented for a degree in any other University or any other award.


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


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DEDICATION

To my family

My wife, Monica Kanini, for her love and dedication

And

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

And

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

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LIST OF ABBREVIATIONS AND ACRONYMS

Anopheles gambiae

s.s. *Anopheles gambiae sensu stricto*. One of the sibling species in the *An. gambiae* complex

s.l. *Anopheles gambiae sensu lato*; The *An. gambiae* complex

DDT: Dichlorodiphenyltrichloroethane

WHO: World Health Organization

pH: Hydrogen-ion exponent

ICIPE: International Centre for Insect Ecology and Physiology

DRIP: Dissertation Research Internship Programme

KEMRI: Kenya Medical Research Institute;

CGMR Coast: Centre for Geographic Medicine Research Coast

WHO: World Health Organization

ITBN: Insecticide Treated Bednet

ABSTRACT

Malaria continues to be a major cause of morbidity and mortality in Kenya, with the transmission caused by mainly *Anopheles gambiae* and *Anopheles funestus* complexes. It is a serious threat to the social and economic advancement of the country. In this study, Field and laboratory studies were used to examine the primary factors responsible for regulating the aquatic stages of malaria vectors and the abundance and diversity of non-mosquito invertebrates in rice fields. The temporal and spatial productivity of habitats for *Anopheles* species were analyzed for statistical correlations to various environmental variables. These include meteorological, hydrological, water chemistry, invertebrate abundance and diversity, and vegetation characteristics. The variables were measured using different field based hand held equipments or visually assessed. Experimental plots were used to closely monitor the events occurring in the rice fields associated with *Anopheles* larval densities. In this plots, agricultural-related events, such as flooding, seedling transplantation, tillering, booting, rice maturation and density, agrochemical use, and crop phenology, were recorded for correlation to changes in population dynamics of *Anopheles* and aquatic invertebrates.

A total of 26,077 *Anopheles* larvae were collected in the 3 villages in which, 87.76% (n = 22,885) were early instar stage and 12.24%(n = 3,192) were late stage. A total of 3,175 pupae were collected from the three villages. One-way ANOVA showed that there was a significant site-to-site variation in larval abundance and the Tukeys HSD ($\alpha = 0.05$) further indicates that the 3 villages are different from each other in larval abundance. Paddy and associated canal were the most productive habitat type. Pools and puddles

formed within the village were important during the rainy season. Multiple regressions showed that presence of other invertebrates, percentage *Azolla* cover, Distance to nearest homestead, water turbidity and rice height were the best predictors for *Anopheles* mosquito larval abundance in the habitats. At the experimental plots, most of the larvae were collected at the transplanting and the tillering stage of rice development. Pearson correlation showed that water temperature was significantly and positive correlated to both anopheline ($r = 0.122$) and culicine ($r = 0.125$) larvae. Logistic regression model gave pH as the best indicator for anopheline larvae at the two villages while salinity was the best predictor the anopheline larvae at the experimental plots. Pools that were freshly formed immediately after the rains with no other invertebrates the emergent mosquitoes were high compared to habitats with other invertebrates. When the densities of other invertebrates is very high, the emergence of mosquito is highly suppressed, with very few emerging as adults. Productivity from different type shows that paddy had most emergent mosquitoes followed by marshes. The results further showed that when the structural complexity (in terms of debris and vegetation) of a habitat is increased, there is an increase in the number of other invertebrates, which results in fewer emergent mosquitoes. In conclusion, larval control activities should be initiated at early rice stages (Between transplanting and tillering) and during the rainy season and the unplanned rice system should be better managed. Paddy and associated canals had more larvae and eventually had more emergent mosquitoes, which shows the importance of rice growing in mosquito abundance and diversity. This study shows that the presence of other invertebrates is playing a role in natural regulation of mosquito in the habitats.

SECTION 1

CHAPTER ONE: INTRODUCTION

1.1 Malaria epidemiology

Malaria continues to be a major cause of morbidity and mortality in tropical and subtropical countries of the world despite the enormous investment in control efforts. Malaria kills between 1.5 and 2.7 millions people each year in the world, and between 300 and 500 million other fall ill from it often severely (WHO 1998). Over a million of these deaths are in children aged under five but they also include women in their first or second pregnancy, older children, young adults and non-immune travelers. In Africa, it is responsible for an estimated 1 million deaths, mainly infants and children, annually. Approximately, 41% of the worlds population is at risk each year, there is an estimated 300 to 500 million cases of malaria (more than 95% of them in Africa), resulting in over 2 million deaths mainly in children under five years of age especially in remote areas with poor access to health facilities. With acute disease a child may die within 24 hours of infection. Pregnant women are 4 times more likely to suffer malaria attacks causing low weight babies and still births, endangering the health of the women and prospects for the new born (Lindsay 2000). Outside tropical Africa, malaria deaths occur mainly among non-immune new comers to endemic areas, for example among agricultural workers, miners and settlers in newly colonized areas (WHO 2000).

Malaria is most serious in the poorest countries and in populations living under the most difficult and impoverished conditions. The prevalence and severity as well as magnitude of associated social and economic effects vary widely in different geographical areas where the disease occurs. However, worst effects of the disease are

felt in Sub-Saharan Africa (WHO 2000). It undermines the health and welfare of families endangers the survival and education of children, disabilities, the active population and impoverishes individuals and countries

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

1.2 Malaria parasites

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

About 50 mosquito species of the genus *Anopheles* are responsible for the transmission of *Plasmodia*.

1.3 Malaria vectors

Out of more than 400 described species of *Anopheles* (White 1977) some 45 of them are implicated in the transmission of malaria. Different species of *Anopheles* are responsible for the transmission of malaria in specific geographic areas. The density of mosquito population is dependent on larval ecology. Irrigation schemes (particularly used for growing rice) are preferred breeding sites for *An. gambiae s.l.* and *An. funestus*. *Anopheles balabacensis* and *An. dirus* are abundant in the forested areas (Muirhead-Thomson 1951). *Anopheles merus* and *An. melas* have extensive breeding sites within the tidal limits of the Kenyan coastal line (Bryan 1983). The malaria vectors play an important role in the transmission of *P. falciparum* parasites in Kenya. These vectors generally cause high parasite inoculation rates and are also remarkably stable in a wide range of bio-ecological and seasonal conditions hence appears to be very flexible, both in exploiting new man-made environments and in their response to malaria control activities (Coluzzi 1984). There is a need to investigate in details to know the biology of the anopheline mosquitoes especially at the developmental stages.

1.4 Mosquito Habitats and the body size

Anopheles gambiae s.l. and *An. funestus* are the most important vector of human malaria in sub-Saharan Africa. Production of adults of *An. gambiae s.l.* occurs in small, temporary, sunlit, turbid pools of water (Gimnig 2002). Habitats are often created by human or animal activity wherein larvae are found in small depressions such as foot or hoof prints, the edges of bore holes and burrow pits, roadside puddles formed by tire tracks, irrigation ditches and other artificial bodies of water (Gillies 1968; White 1972; Minakawa 1999; Gimnig 2001).

The developmental stages of malaria vectors determine the body size, which in turn may influence the competence of the vector in *P. falciparum* transmission. The mosquito body size varies according to nutritional status of the breeding habitat and weather patterns. Conditions of larval development affect adult body size (Lyimo 1992; Koella 1996), which can influence adult survival (Hawley 1985) and vector competence (Paulson 1991; Nasci 1994). The degree of variation in wing length of *An. gambiae* complex and *An. funestus*, according to different habitats and ecological zones affect vector competence and malaria transmission along the Kenyan coast is unknown.

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

1.5 Statement of problem

Malaria is a major cause of morbidity and mortality in Mwea Irrigation Scheme. Mwea Irrigation scheme is one of the largest rice growing schemes in Kenya. Rice cultivation usually provides ideal sites for *Anopheles* larval development. In this agro-ecosystems different agricultural and environmental factors affect Anopheline larval instars development. In Mwea Rice Irrigation Scheme, there is a need to know the

factors, which affect the anopheline larval development, which will result in more adult production.

1.6 Justification of the study

In an irrigated agricultural ecosystem, *Anopheles* mosquito larvae in aquatic habitats are usually affected by different environmental and agricultural factors. These in turn affect the adult mosquito production in terms of vector densities, consequently influencing malaria parasite transmission. In the process of filter feeding, *Anopheles* larvae take in nutrients, which are essential for their growth and development. The number and productivity of larval habitats ultimately determines the density of adult mosquitoes. Different environmental and agricultural factors regulate the abundance of malaria vectors larvae in the habitat. This eventually affects the productivity and diversity of larval habitats. There is no much information available on factors regulating the productivity of the habitats. Consequently it is difficult to implement and eventually monitor and evaluate anopheline mosquito larval control programme in Mwea Irrigation Scheme. There is no information available for the survivorship of developmental stages of anopheline mosquitoes in Mwea irrigation scheme that could be used in models in productivity studies of anopheline from the aquatic habitats. The proposed study will provide information on malaria larval dynamics in the aquatic habitats, which will be useful in the implementation of larval control programme. Malaria vector variation as a result of different environmental and agricultural factors will be known to help implement the control measures at the appropriate time.

1.7 Research questions

- a) How does the productivity of *Anopheles gambiae s.l.* and *An. funestus* in the larval habitats fluctuate seasonally in the Mwea rice irrigation agro-ecosystem?
- b) Which environmental factors are associated with the productivity of *Anopheles* larvae in the aquatic habitats?
- c) How is the *Anopheles* larvae distribution in the aquatic habitats?
- d) What is the survivorship rate of the immature anopheline larvae in the aquatic habitats?
- e) How does agricultural activities influence the temporal colonization of aquatic habitats by mosquito larvae and other aquatic invertebrates?

1.8 General hypothesis

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

1.8.1 Specific hypotheses

- a) The population of *An. gambiae s.l.* and *An. funestus* fluctuates seasonally in the larval habitats and it is influenced by the weather pattern.
- b) Environmental factors determine the habitats capability to support malaria vectors developmental stages.
- c) There is *Anopheles* larval aggregation in the habitats during the development of larval instars.
- d) Agricultural activities influence the temporal colonization of habitats by mosquito larvae and other aquatic invertebrates.

- e) There is a significant reduction in numbers of *An. gambiae s.l.* larvae from one instar stage to the other and this influences the number of emergent adult mosquitoes

1.9 General Objective

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

1.9.1 Specific Objectives

- a) To determine the temporal productivity of *Anopheles gambiae s.l.* and *An. funestus* in the larval habitats in the Mwea rice irrigation agro-ecosystem.
- b) To determine the environmental factors associated with the productivity of *Anopheles* larvae in the aquatic habitats.
- c) To determine the spatial distribution of *Anopheles* larvae in the aquatic habitats.
- d) To determine the succession of mosquito larvae and other arthropod larvae in the aquatic habitats.
- e) To determine the survival of *An. gambiae s.l.* larvae in the aquatic habitats

CHAPTER TWO: LITERATURE REVIEW

2.1 General

Under natural conditions where man, vector and the infecting parasites co-exist, transmission is usually intense provided that the vector is abundant and feeds on human. The vectors must also live long enough to transmit infection. It has been shown that there is a high degree of specificity in the relationship between the mosquito and parasite (MacDonald 1967) and that susceptibility of an individual mosquito is genetically determined (Huff 1963).

2.2 Distribution of *Anopheles* mosquitoes in Kenya

The primary malaria vectors in Kenya are *Anopheles funestus* and three members of *Anopheles gambiae* complex: *An. gambiae s.s.*, *An. arabiensis* and *An. merus* (Coluzzi 1985; Collins 1988; Petrarca 1992). *Anopheles gambiae s.s.* and *An. arabiensis* are mostly closely associated with human and represent the major vectors of malaria (Muirhead-Thomson 1951; Highton 1979). The distribution of these two species overlaps and they occur sympatrically in large areas of tropical Africa. In Kenya *An. gambiae s.l.* and *An. funestus* are predominantly found along the Kenyan coast and western Kenya around Lake Victoria.

2.2.1 The *Anopheles gambiae* complex

The *An. gambiae* Giles complex comprises 6 sibling species, one unnamed species and several incipient species all differing in various ways (Mbogo 1993b; Service 1993b; Thomson and B.M. 1995; Ribeiro 1996). These vectors generally cause high parasite inoculation rates and are also remarkably stable in a wide range of bio-ecological and seasonal conditions hence appear to be very flexible, both in exploiting new man-made environments and in their response to malaria control activities (Toure

1997). Members of the *An. gambiae* complex have a wide geographical distribution and have been reported from most African countries (Gillies 1961;Blower 1981;Mbogo 1993a;Costantini and C. 1996;Ribeiro 1996;Charlwood and J.H. 1997;Takken 1998). While the existence of the six formally named species of the *An. gambiae* complex is well established, there is indication that further subdivision within the species of the complex may exist. This presents a need for the accurate characterization of these species and a definition of their local distributional limits, which is important in malaria epidemiology.

2.2.2 The *Anopheles funestus* Group

Anopheles funestus belongs to a group of at least eight morphologically similar species². Members of this group are difficult to identify because of the morphological overlap that exists within the group. This inability to distinguish species as well as the fact that they vary in their behaviour and biting preferences complicate the successful planning and maintaining of vector control activities in areas where *An. funestus* is the major vector (Gillies 1968;Rawlings 1981). *An. funestus* has been recognized as a vector of malaria parasites since the early part of the 20th century (Scott 1993;Service 1993b). Some of the species are sympatric in the savannas of West and East Africa (Clements 1981;White 1982). In some areas along the Kenya coast, *An. funestus* is more abundant, highly endophilic and anthropophilic. It is important to establish the role of the members of this group in malaria transmission along the coast.

2.3 The life cycle of *Anopheles* vectors of malaria

The complete cycle from egg to adult takes a minimum of about 2 weeks or slightly less. Figure 1 shows the life cycle of *Anopheles* mosquitoes.

The breeding habitats vary from large and usually permanent collections of water, such as fresh water swamps, marshes, rice fields and borrow pits to smaller collections of temporary water such as small pools, puddles, water filled car tracks,

Mosquito Lifecycle

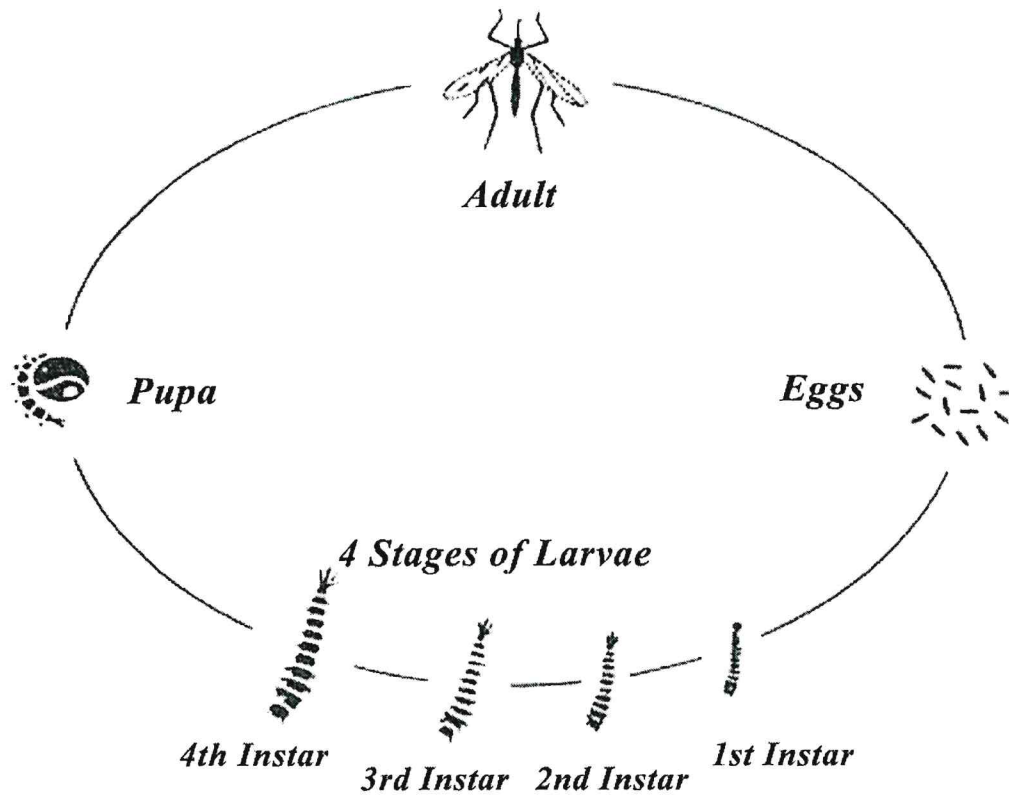


Figure 1: Schematic illustration of the life cycle of *Anopheles* vectors of malaria
(After Service, 1980).

ditches, drains, gulleys, and hoof prints. The most common breeding sites are the shallow open sun lit pools (Service 1993b). Larvae also occur in wells and manmade container habitats such as clay pots, motor vehicle tyres, water storage jars and tin cans (Chinery 1984). However *Anopheles gambiae s.s.* prefer small and undisturbed temporary pools of water exposed to sunshine (Muirhead-Thomson 1945)

The female *An. gambiae* after mating and blood feeding lays some 50-200 small (1mm long) brown or blackish boat-shaped eggs on the water surface. Viable eggs hatch into larvae within 2-3 days in the tropics, but in cooler temperate regions they may not hatch until after 4 -7 days or longer (Service 1980). The larvae, while on the water, lie parallel to the surface to allow water intake and surface feeding

At mean water temperatures of 25-28⁰ C the larvae undergo four moults within 6-9 days to reach the pupal stage, which lasts 2-3 days depending on temperature. Thus, the minimum duration for one generation may be as long as 10-11 days. The pupae bear respiratory trumpets that are short and broad distally thus appearing conical. The most distinctive characteristic of *Anopheles* pupae is the presence of short peg like spines situated laterally near the distal margins of abdominal segments. The pupal skin splits dorsally and the adult emerges. Careful movements are required to ensure that the adult mosquito does not fall sideways and be trapped in the surface film. This danger is particularly acute when the adult is largely out of the pupal exuviae but the terminal appendages are still not free. Finally the legs become free and spread on the water surface giving stability. The newly emerged adult inflates its wings, and separates and grooms its head appendages before flying away (Kettle 1992). When the progeny of any one egg batch emerge as adults the males emerge first. The males

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

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

2.4 Feeding and resting behaviour of *Anopheles* mosquitoes

Eighty percent of *Anopheles* feed on any large mammal that is available (Gillies 1972). The host preference by a particular species of mosquitoes is also likely to be influenced by environmental conditions.

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

Blood feeding by anopheline mosquitoes is essential for transmitting malaria parasites and characteristic of this behavior can have major implication for the epidemiology of disease. (Briegel 1993) showed that anopheline mosquitoes take multiple blood meals within the gonotrophic cycle. Because biting frequency is one of the main entomological factor determining the vectorial capacity of the vector species, (Garret-Jones 1964) multiple feeding has a profound effect on the rate of malaria transmission. The probability that an infected mosquito survives until it is able to transmit malaria parasites will increase when it takes a blood meal earlier in life thereby increasing the vectorial capacity (Garret-Jones 1964)

2.5 The Life cycle and Transmission of Human *Plasmodium* Parasite

Four protozoan parasites, namely *P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax* cause malaria in humans and they all have the similar life cycle (Figure 2).

During the infective bite of a female *Anopheles* mosquito numerous infective sporozoites are injected into the bloodstream, where they must remain for about 30 minutes and then disappear from the blood stream. Most of the sporozoites are destroyed by phagocytes whilst some enter the liver parenchymal cells, develop and undergo asexual multiplication (pre-erythrocytic schizogony). A large unpigmented schizont is formed containing several merozoites, which are released into the bloodstream to invade the erythrocytes. The merozoite attaches to an erythrocyte and is invaginated into the red cell through a parasitophorous vacuole, where it feeds and deposits a pigment called haemozoin as a by product (Aikawa 1980a). The ingested merozoite becomes a feeding trophozoite and in the early stages of an infection, the fully-grown trophozoite multiplies asexually to become a schizont (erythrocytic schizogony), producing a small number of merozoites (Aikawa 1980b).

Release of the merozoites from the erythrocytes brings on an attack of malaria, and the interval between attacks is the length of the schizogonic cycle. This may last several hours. The released merozoites repeat the cycle and invade other erythrocytes.

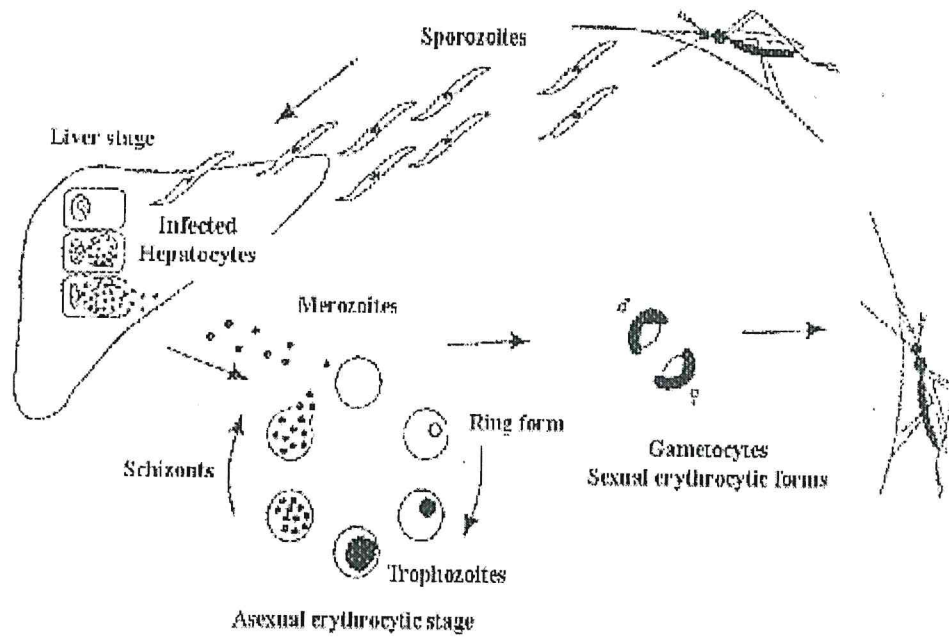


Figure 2: Schematic representation of the life cycle of *Plasmodium* species (After (Garnham 1966))

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

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

The effective transmission, high incidence and mortality of malaria is determined by a number of factors which affect the distribution, transmission intensity, disease outcome, small-scale variation and seasonality (MARA. 1998).

The number and type of anopheline mosquitoes determine to a large extent the level of transmission in a given area. Malaria transmission is influenced by climate and geography and often coincides with the rainy season (Craig 1999) when breeding sites are available, ensuring high numbers of *Anopheles*. The climatic conditions in tropical Africa favour intensive transmission of malaria parasites. Temperature affects the

sporogonic cycle of the parasite and vector survival. Very low temperatures limit the vector by lengthening the gonotrophic cycle and reducing the survival rate. *Anopheles gambiae* for example, only exists in frost-free (De Meillon 1934) or where absolute minimum temperatures in winter remain above 5°C (Leeson 1931). Rainfall provides mosquito-breeding sites and increases humidity, thus enhancing mosquito survival. However, the relationship between mosquito abundance and rainfall is not direct, as a specific amount of rain does not lead to a specific mosquito density. By examining rainfall patterns in known malaria and non-malarious areas, (Craig 1999) estimated that an average of 80mm per month for at least three to five months is a reasonable requirement for the availability of mosquito breeding sites to sustain malaria transmission.

Poor environmental sanitation, like poor drainage systems also increase mosquito-breeding sites. This, in addition to development projects in pursuit of economic development such as water schemes, construction of dams and bridges, oil drilling and mining activities, urban planning and development, logging activities inadvertently lead to an increase in mosquito breeding sites, thereby increasing their numbers, human-mosquito contact and therefore transmission (Okenu 1999).

Historically, population movement has also contributed to the spread of the disease (Prothero 1977). The movement of infected people from areas where malaria is still endemic to areas where the disease had been eradicated has led to the resurgence of the disease (Gubler 1998). For example, in 1985, 26 new active foci of malaria were

introduced in Brazilian states outside the Amazon region (Marques 1986). As people move, they increase their risk for acquiring the disease through the ways in which they change the environment and through the technology they introduce, for example through deforestation for resettlement and irrigation systems (Service 1991). Such environments create more favorable mosquito breeding habitats and at the same time, workers may have increased exposure to the vector. Furthermore, people can inadvertently transport infected mosquitoes to malaria free areas, reintroducing the disease (Martens 2000). Population movement is also intensely implicated in the spread of drug resistance in malaria (Rajagopalan 1986), with people carrying resistant parasites moving from endemic to non-endemic disease areas and accelerating transmission of resistant strains.

Weak health systems, poor strategic development and inadequate funding of control programmes all play a role in the transmission of malaria. Rural to urban migration in search of economic and educational opportunities has created densely populated communities, especially in peri-urban areas and weakened health systems with the attendant increase in human activity and adverse effects on the environment, thus creating breeding sites. This promotes vector abundance leading to increased human-vector contact.

2.6 Malaria transmission

Several studies have related the dynamics of vector populations to the incidences of *P. falciparum* infection and disease in human population (Mbogo 1993a;Beier 1994;Mbogo 1995;Kabiru 1997). To transmit malaria, an individual *Anopheles* has to feed on humans

at least twice within a week successively: the first time to acquire an infection and the second time to transmit the parasite. This means that the ability of a species to transmit malaria is related to the product of the two probabilities of an individual feeding on human twice and not directly to the proportion feeding on human.

2.7 Entomologic Inoculation Rate

Malaria transmission intensity is best expressed as the entomologic inoculation rate (EIR), which directly reflects the exposure of humans to pathogenic *Plasmodium* parasites (Burkot 1990;Beier 1999;Killeen 2000a). EIR Levels of one infective bite per year or less can readily sustain prevalence in excess of 40% for *P. faciparum*, the most pathogenic of species of human malaria parasites (Beier 1999).

Although the clinical outcome of malaria infection depends on the specific immunological and pathological interactions of parasites strains and individuals humans, (Snow 1993;Rihet 1998;Striradel 1999) most if not all, strains of *P. faciparum* are potentially life threatening (Alles 1998;Gupta 1999).

The EIR as a marker for malaria transmission intensity has been intensively studied. (Trape 1993) found that malaria transmission levels in tropical Africa varied considerably being influenced by ecologic conditions, from approximately 10^{-2} to 10^3 infective bites per person per year. (Snow 1994) reported that people living in the rice growing area of Kilombero District in Tanzania received at least 300 infective bites each year. (Beier 1990) recorded that residents of Sarididi and Kisian area of western Kenya received an average of 237 infective bites per year.

(Onori 1980) and (Oaks 1991) reported that the intensity of malaria parasites transmission by mosquito populations was a key component in the epidemiology of the disease. The two important aspects of malaria transmission they considered were entomological inoculation rates (EIR) and vectorial capacity (VC). The EIR is a measure of the number of infective bites each person receives per unit time (e.g. per night) and is a direct measure of the risk of human exposure to the bites of infective mosquitoes. In malaria endemic parts of Sub-Saharan Africa and the Southwest Pacific, transmission intensity can vary from undetectable levels to more than 1,000 infective bites per year (Beier 1999; Killeen 2000b). (Garret-Jones 1964) observed that EIR varied with time, vector species, and parasite species. (Dye 1986) pointed out that vectorial capacity that measures the potential for malaria transmission was based on several key parameters of vector populations.

2.8 Factors affecting distribution of malaria

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

Factors pertaining to the natural environment are for example, the availability of the breeding habitats for malaria vectors, which influences the distribution of malaria in an area. The local rainfall produces rain pools favoured by most malaria vector species for

in the rice growing areas during the dry season at Ahero (Githeko 1993) and at Mwea-Tebere irrigation scheme (Ijumba 1990) were maintained by irrigation water.

(Schofield 1984) demonstrated that the house design and situation was important in protecting its residents from mosquitoes.

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

In domestic situation zoophylaxis was important in reducing the frequency of mosquito feeding on humans and hence malaria transmission (Hess 1970). All these factors contributed to affecting the degree of man-vector contact.

2.9 Malaria control

The strategy for malaria control is based on breaking the chain of transmission of the parasites between humans and mosquitoes. There are presently two approaches: control of parasites by chemotherapy, chemoprophylaxis; or vaccination and breaking the contact between host and vectors by controlling the vector population by using insecticides, screens such as bed nets, window gauzes and curtains, or environmental management.

2.9.1 Chemoprophylaxis

Various synthetic anti-malarial drugs have been used for curative or prophylactic purposes. Use is being made of drugs such as quinine, chloroquine, mefloquine, proguanil, 4-aminoquinolines, halofantrin, artemisin and pyrimethamine sulfadoxine. However, increased resistance to some of these drugs by *P. falciparum* has been reported (Leonardo 1994). The efficacy of chloroquine in malaria chemotherapy has been compromised with the development of resistance to the drug by malaria parasites. In many parts of Africa, the drug is no longer used alone for therapy (Brasseur 1998). Malaria parasite resistance to alternative anti-malarial drugs such as 4-aminoquinolines, antifolics, quinine, fansidar and halofantrin has also been reported (Watkins 1993; Leonardo 1994) (Figgitt *et al.*, 1992). These synthetic anti-malarial drugs have therefore not achieved the main goal of malaria eradication due to increased cases of drug resistance, high costs, high failure rates and side effects being reported in many parts of the world (Leonardo 1994). For prophylaxis, other drugs including amodiaquine, pyrimethamine, proguanil, and sulfadoxine may be used in areas where chloroquine resistance is high. The antibiotic deoxycycline (a tetracycline derivative) is also an effective prophylactic. However, WHO discourages the use of these second line drugs for prophylaxis, because long-term usage may contribute to resistance development.

Chemoprophylaxis as a control strategy has been attempted and is still debated. Treatment is considered necessary for pregnant women, but prophylaxis for small children is debated because of the risk of long-term side effects and danger of increased selection for resistant parasite strains (Carnevalle and Mouchet, 1987). In addition, drug administration can prevent development of natural immunity or simply delay disease

development until children are older (Carnevalle & Mouchet, 1987). Furthermore, a countrywide prophylaxis exercise is expensive and requires strong organization. Therefore, there is need to change focus from treatment to prevention from infective bites. Hence the needs for vector control.

2.9.2 Vaccine development

An effective malaria vaccine has been a goal of researchers for almost 30 years. Repeated exposure does result in a degree of natural immunity, primarily to the erythrocytic or blood stages. Immunity does not prevent infection, but death and morbidity are reduced and infections in adults are mild or asymptomatic. The search is underway for pre-erythrocytic stages (Butcher, 1989; Carnevale and Mouchet, 1987; Carlson *et al.*, 1994). These vaccines need to be long lasting or boosted by natural reinfections as well as cheap to produce. Nevertheless, even if a highly effective vaccine is available, problems of poverty, lack of public infrastructure, management constraints and community mobilization may contribute to incomplete coverage of the populations most at risk (Hopkins, 1985).

2.9.3 Vector control

This includes activities that reduce the number of infective or infectious bites of the vector by reducing the vector density, longevity and or preventing human – vector contact. The principal aim of vector control is the reduction of disease morbidity and mortality by reducing the level of transmission. It involves the use of methods targeted at controlling the mosquito population at larval or adult stages of their life cycle. Vector control has proved to be the most effective method for malaria control since the lower the *Anopheles* densities, the lower the cases of malaria. It is also easier to control mosquito

populations within a given geographical area than giving vaccines for protection or administration of prophylactic drugs to individual persons.

2.9.3.1 Adult *Anopheles* control

Insecticides such as pyrethrum extracts have been used extensively in mosquito control, but the quick bio-degradability and high costs of isolation of natural pyrethrins reduced their use. This accelerated the development of affordable and persistent synthetic pyrethrins like permethrin and allethrin. Synthetic pyrethroids such as allethrin and permethrin have been effective insecticides. These compounds have however been found to be toxic to many non-target organisms and their persistence in the ecosystem. Moreover, increased vector resistance has been reported against synthetic pyrethroids (Shidrawi, 1990). The reported resistance to the synthetic pyrethrins calls for use of other more effective insecticides. Vector resistance to insecticides is a recurring theme and a major problem in malaria control programmes (Shidrawi, 1990). By 1985, at least 117 mosquito species had been reported as resistant to one or more of insecticides with 67 of these in the genus *Anopheles* (Pant, 1988; Shidrawi, 1990). The best evidence of resistance to pyrethroids in the *An. gambiae* complex is the 5.8 – fold tolerance to bioallethrin seen in a strain of *An. gambiae* from Burkina Faso (Malcolm, 1988; Pant, 1988). A population of *An. gambiae* in an area of western Kenya experienced a 2.5 - fold increase in the LT_{50} to permethrin exposure one year after a permethrin-impregnated bed net study was implemented (Vulule et al., 1994). Organophosphate pesticides are alternatives to pyrethrins since they have short persistence. However, resistance has been reported to these insecticides as well (Lines *et al.*, 1984). Malathion resistance has been

recorded from *An. arabiensis* in the Gezira District of Sudan (Lines *et al.*, 1984; Lines, 1988). The widespread use of persistent insecticides facilitates resistance development, especially when selection is applied against a large proportion of the population. DDT and other organochlorine insecticides exemplify this. Besides resistance development to these insecticides, they are toxicants to other non-target organisms including mammals, are a risk to the ozone layer and may therefore enhance global warming. The evolution of resistance to most insecticides by the vector has prompted the need to develop new tools for vector control.

Repellents have also been used in vector control. Synthetic repellents such as dimethyl phthalate and 2-ethyl-1, 3-hexanediol have not provided a great impact in controlling the rate of inoculation and transmission of malaria parasite since most of these repellents are highly volatile and thus provide only short lived protection time against the vector. The most common mosquito repellent formulations available on the market contain deet (N, N-diethyl-3-methylbenzamide), which has shown excellent repellency against mosquitoes and other biting insects (Yap 1986; Coleman 1993, Walker *et al.*, 1996). The use of plants as traditional natural repellents has been documented from many areas (Curtis *et al.*, 1990), but most of the products from plants have not been carefully analyzed.

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

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

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

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

2.9.3.2 *Anopheles* larval control

The various means that are used for the control of malaria epidemics have not achieved much in the control of malaria parasite transmission in Kenya. A potential target of malaria control is the anopheline larva. This is because the life cycle can be interrupted before the emergence of adults that bite and transmit malaria parasites. Parasitic source reduction through modification of larval habitats was the key to malaria parasite eradication efforts in the United States, Italy and Israel (Kitron 1989). It is therefore

rational that appropriate management of larval habitats in the sub Saharan Africa may help to suppress vector densities and malaria transmission rates. The control of mosquito larvae before emergence of adults thus remains the most efficient and economical means of controlling malaria epidemics.

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

2.9.3.2.1 Environmental management

This involves practices that create unfavorable habitats for larval breeding. It may also involve the elimination of aquatic habitats. A simple approach is to fill with rubble, sand, and earth larval habitats of different sizes (Service, 1996). Other environmental modifications include the removal of overhanging vegetation to reduce breeding by shade loving mosquitoes such as, *An. dirus* (Service, 1996). Deforestation can also eliminate the malaria vector by removing adult mosquito resting habitats. Planting vegetation along

streams and reservoirs make habitats inimical to sun loving *An. gambiae*. However, this approach has not achieved much because it is impossible, to fill in all the scattered, small and temporary collections of water (Service, 1996). Secondly, the environmental changes such as agricultural irrigation schemes, creation of dams for water reservoirs and road construction or mining sites may favour the breeding of other species that were previously present in only small numbers or absent altogether (Service, 1996). Besides, the approach is labor intensive and costly thus untenable. There is, therefore, need to focus on more practical larval control methods.

2.9.3.2.2 Biological control

Biological control implies the use of predators, parasites or entomo-pathogens. Because of insecticide resistance and the adverse environmental impact of insecticide use, considerable resources have been devoted to the search for biological control agents. Several attempts have been made to control mosquito larvae by biological means. To date, only larvivorous fish have been used successfully in malaria control projects, and these cases are few. The use of North American fish *Gambusia affinis* successfully reduced malaria incidences in Italy and Greece, where malaria transmission was unstable (Wickramasinghe and Costa, 1986). In other studies vector densities and biting rates decreased or introduction of larvivorous fish. Previously, other fishes such as Armagosa pupfish (*Cyprinoden nevadensis armagosae*) and Guppies (*Poecillia reticulata*) were used (Moyle 1976). In Kenya, *Tilapia* species are under trial in Kitale to control mosquito larvae. The disadvantage is in the mass rearing and the restocking programmes required in the approach. Besides the fishes may not survive in some temporary breeding sites.

Invertebrate predators such as Coleopterans, Dipterans and Hemipterans have also been examined but they are difficult to rear *en mass*; they feed non-specifically, and they do not persist once vector target densities are reached (Rishikesh *et al.*, 1988).

Rishikesh *et al.* (1988) have summarized efforts to identify useful pathogens and parasites including viruses, fungi, nematodes and sporozoa. The main pathogens include: the fungi *Coleomyces* spp, *Culicinomyces clavosporus*, *Metarhizium anisopliae*, and the *Lagenidium giganteum* which have demonstrated little or no adverse effects on populations of invertebrate and vertebrate non-target organisms (Lawrence 1990), the protozoan *Nosema algerae* and the mermithid nematode *Romanomermis culicivorax*. None of these agents have shown any promise for wide scale larval control, having proven difficult to rear and store, as well as unstable or inefficient under the field conditions.

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

application or are only suitable for environments where a one-time control measure produces a valuable outcome.

2.9.3.2.3 Synthetic larvicides

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

However, this compound is expensive due to its high copper content. However, there have been no instances of mosquito larvae developing resistance to most of these larvicides (Service, 1996). This may be explained by the limited use. Most of these inorganic larvicides are highly toxic to aquatic organisms and plants because of relatively large amounts of water-soluble arsenic in them, which pose environmental pollution problems. Synthetic organic chemicals have also been used in mosquito larval control. Use of emulsions or granular formulations of DDT, dieldrin, heptachlor, or lindane has been attempted (WHO, 1984). Where resistant strains are encountered, parathion or baytex has been used (Metcalf 1962). However, most of these larvicides are organochlorines, organophosphates, or carbamates that are toxic and have cumulative environmental effects and persistence in the ecosystem due to their resistance to enzymatic degradation by soil and other environmental micro-organisms and chemical

reactions (Charlese 1995). Besides, widespread use of the same pesticides for the control of agricultural pests has led to rapid resistance development in vector populations. Temephos (Abate), an organophosphate of very low mammalian toxicity, has also been used to treat portable waters to control *Aedes aegypti* breeding in water storage pots (Service, 1996).

For most malaria vectors, reducing mosquito population densities by means of larvicides application is generally an inefficient way of reducing malaria transmission, because larval mortality among anopheline populations may be density dependent. Besides, when a large proportion of the larval habitat can be easily identified and targeted, larval control can be effective. The behaviour and ecology of the target vector determines the efficacy of the larvicide. For example, *An. gambiae* often breeds in small temporary rain pools. The number and wide distribution of these small pools may present insurmountable difficulties in control efforts using larvicides except in circumstances such as the eradication campaigns where the introduced species occurred in a limited geographic region.

The use of insect growth regulators (IGR) to control mosquitoes has also been attempted. IGR are chemicals which inhibit/disrupt growth of the insects. Most of these compounds have been grouped as: juvenile hormone mimics or chitin synthetase inhibitors (Laird 1985). These compounds generally have no toxicity to other non-target organisms. They are relatively specific to the insect and primarily active against the immature stages of mosquitoes. However, they may kill beneficial insects. Currently, the most widely used

IGR is Altosid[®] (Laird 1985) that has no remarkable effects on non-target aquatic organisms. However, there is a great desire to obtain larvicides or IGR from inexhaustible natural sources such as plants that can be cultivated, extracted, and biodegradable compounds obtained to avoid environmental pollution (WHO 1996).

2.9.3.2.4 Natural organic larvicides

Various natural organic chemicals have been extracted from plants and bioassays carried out to determine their effectiveness as larvicides. One of the earliest reports of the use of plant extracts against mosquito larvae is credited to (Campbell 1933) who found out that plant alkaloids like nicotine, anabasine, methyl-anabasine, and lupinine extracted from the Russian weed *Anabasis aphylla* killed larvae of *Culex pipiens*, *Cx. quinquefasciatus*, and *Cx. territans*. Haller (1940) noted that extracts for Amur cork tree fruit *Phellodendron amurense*, yielded a quick acting mosquito larvicide. The chemicals can be extracted from either whole plants or specific parts of the plants such as leaves, fruits, roots, and bark depending on the activity of the derivatives.

It has been shown that some limonoids (azadirachtin), quinones (plumbagin), alkaloids, flavonoids, terpenoids, polyacetylenes, and butyl-amides extracted from plants show a high degree of larvicidal activity against mosquito larvae (Kubo *et al.*, 1984). For instance, piperine and wisanine are alkaloids that were isolated from *Piper guineense* and were found to be very active on *Aedes aegypti* larvae (Addae-Mensah 1986). The same extract has been shown to have larvicidal activity against *An. gambiae* (Okinyo 2002). Limonoids such as azadirachtin from *Azadirachta indica* and terpenoids such as 5-E-

ocimene from *Tagetes minuta* have been reported to possess larvicidal activity against mosquito larvae (Maradufu 1978). Larvicidal activity of long chain fatty amides such as N-isobutyl-2E, 4E, 8Z, 10Z-dodeca-2, 4, 8, 10-tetraenamide isolated from *Spilanthes mauritiana* have been reported (Jondiko 1989). The amides from *Zanthoxylum gilletii* (*Fagara macrophylla*) have also been reported as larvicides against *Culex* species (Kubo *et al.*, 1984). Their efficacy against *Anopheles gambiae* has since been demonstrated (Okinyo 2002). The same compound was reported to have tyrosinase inhibition activity (Kubo *et al.*, 1999) and flavouring activity (Mukonyi and Ndiege, 2001). Other plants that have been successfully tested for larvicidal activity include: *Vernonia ammobila*, *Swartzia madagarensis*, *Pogestemon cablin*, *Sium suave*, *Datura candida*, *Achryolcline satureoides*, *Petiveria alliacea*, and *Gardenia lutea* amongst others (Michael *et al.*, 1991). The efficacy of most of these plant extracts as potential larvicides have only been tested under laboratory conditions. However, their efficacy under natural field conditions against natural anopheline larval populations has not been investigated. As much as these investigations have not been done, their potential in mosquito control is thought to be high.

2.10 Environmental Heterogeneity

The extent to which environmental heterogeneity affects patterns of vector production that are important for malaria parasite transmission is unknown (Grillet 2000). The factors affecting larval survival and the mechanism controlling adult production are also largely unknown for even most important vector species. A potentially important target of malaria vector is the anopheline larva and source reduction through modification of

larval habitats was the key to malaria eradication efforts in the United States, Israel and Italy (Kitron 1989). It is conceivable that appropriate management of larval habitats in sub Saharan countries particularly during dry season may help suppress vector densities and malaria transmission (Minakawa 1999). The understanding of anopheline larval ecology is limited and insufficient to achieve effective vector control through means of larval control (Oaks 1991).

2.11 Nutritional relationship of habitat to larvae

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

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

Recently, slightly reduced rates of larval drinking were measured in several fresh water species for which the function of drinking would be primarily for nutrient ingestion (Aly 1989). They argued that mosquito larvae drink copiously, that drinking rate can be increased by the presence of dilute colloids, and that it can be regulated independently of other mouth part activities that occur during feeding. Given these findings, there are natural circumstances when dissolved nutrients, could be an important food resource for mosquito larvae as recently suggested by (Wotton 1990). For all suspension feeders useful nutrients could come through drinking if concentrations of dissolved materials were high enough. Higher concentration of dissolved organic material may occur adjacent to leaf and substrate surfaces or near decaying tissue their associated precipitin and bacteria. Such zones would be rich in gelled and colloidal solutions of macromolecular nutrients (Costern 1987). If such "einfluss" zones have a few percents of balanced nutrients in solution, they could support mosquito growth.

(Howland 1930) concluded that the abundance of algae in the larval food was correlated with algal abundance in the habitats and that culicines consumed more algae than anophelines.

2.12 The nutritional status of the habitat

The relationships between habitat selection and intrinsic chemical properties of food and microhabitats have received much attention especially in phytophagous insects (Sota 1993). For detritus feeders like larva of mosquitoes in aquatic habitats, the habitat water contains both nutrients and deterrents (Parker 1982;Fisher 1990;Sota 1993). However,

there is little experimental analysis of the significance of particular water conditions along the Kenyan coast and the rest of Africa. Various chemical properties of the breeding habitat related to leaf litter such as pH, and concentration of ammonia, nitrate and sulphate affect larval development and survival (Peterson 1969; Zain 1979; Carpenter 1982). In this study investigations on the effect of the nutritional status of habitats on the productivity of the habitat and the mosquito body size from the habitats were carried out. Inorganic ions and organic carbon sources such as nitrogen, phosphorus, sulphur, and carbon, which provide essential nutritional substances for microbial growth, were considered.

2.13 Survival of immature *Anopheles arabiensis*

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

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

the duration of the sampling period. Service (1971, 1973, 1977, 1993) Reisen and Siddiqui (1979) and Reisen et al. (1982) provide extensive discussions about how such information can be analyzed.

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

2.14 The spatial and temporal distribution patterns of disease and vectors

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

Geographic information systems (GIS) and spatial statistics provide tools for studies of population dynamics of disease vectors in association with environmental and habitat features on multiple spatial scales. GIS is a computer-based system for automating, storing, manipulating and displaying mapped information and data (Chrisman 1989). It includes spatial data (locations) in form of geographic coverages (maps) and descriptive data (attributes) in the form of a relational database associated with the mapped features.

GIS therefore allows for the overlaying of a variety of data coverages (e.g climate, vegetation type, habitat distribution, vector abundance and infection rates) to identify factors that may explain the spatial and temporal distribution patterns of vector and disease. Spatial statistics on the other hand are a set of tools developed largely by geographers and geologists to describe, explain, extrapolate and predict the distribution of objects and processes in space (Getis 1992;Kitron 1998).

Non-geographic methods used in ecology include measures of aggregation, which are based on frequency counts without consideration of the actual geographic location. However, spatial statistics methods such as spatial autocorrelation, local spatial statistics, kriging and spatial-temporal statistics⁴⁷ consider both location and attributes or values of variables at particular locations and can be used to explain the distribution patterns of vectors, hosts and diseases. Thus, with the aid of spatial analysis, GIS can offer a way to identify and map the larval habitats of vector species, and their relationships to human settlements, thus predicting the potential risk for disease transmission.

The application of GIS and spatial analysis to define the epidemiology of vector borne diseases has been documented for a number of diseases, such as Lyme disease (Kitron 1991), Trypanosomiasis (Rogers 1993) and malaria (Beck 1997;Omumbo 1998). The study (Omumbo 1998). presents the first attempts to develop an empirical map of malaria endemicity for Kenya.

2.15 Rice cropping cycle

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

2.15.1 Rice management in the nursery bed.

The nursery bed is first raised by wet bed method that involves puddling and leveling. Prior to introduction of rice seeds into the nursery, the seeds are first soaked in water for 24 hours followed by incubation under rice straw for a period of 48 hours to encourage germination. Thereafter, the seeds are uniformly broadcasted on wet nursery bed at a seedling rate of 100g/m^2 . 57.5 kg/ha of sulphate of ammonia are also applied as a source of nitrogen at the time of sowing while an additional 57.5 kg/ha of the same is applied 14 days later. Immediately after sowing, the water level in the nursery is adjusted to an average depth of 3 cm. Bird scaring should also be done until the rice germinates and becomes well established. Fentrothion is applied 7-10 days after sowing at a rate of

400ml/acre to control insect pests mainly the stem borers and leaf miners. Most of the rice varieties grown in the scheme are disease resistant and therefore not treated before sowing. However, in cases where a susceptible variety such as IR is sowed, the seeds are usually top dressed with the fungicide benlate, which is protective against fungal diseases including stem rot and brown-leaf-sheath-rot. Seedlings are ready for transplanting 28 days after the sowing date. The timing of the sowing of the rice seeds in the nursery should correspond with the cropping cycle. For the long rain crop, it should be done between mid December and mid January and that of short rain crop between July and end of August.

2.15.2 Management in the main field

2.15.2.1 Land preparation

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

2.15.2.2 Transplanting

During transplanting, the water level is lowered to an average depth of 3cm. 60 kg/ha of muriate of potash and 39kg/ha of sulphate of ammonia (or urea at 80kg/ha) are applied during transplanting to provide source for potassium and nitrogen, respectively. Seedlings are transplanted at a rate of two per hole and 20 x 20cm spacing and the water level raised to an average depth of 5cm immediately after. Ten days after transplanting, gapping is done to replace dry or weak seedlings.

2.15.2.3 Insect control

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

2.15.2.4 Weed control

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

2.15.2.5 Top dressing

During panicle initiation stage 39kg/ha of sulphate of ammonia are applied to the crop to top up the nitrogen content of the soil.

2.15.2.6 Other field operations

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

2.15.2.7 Harvesting

When the paddy is 85% mature, the level of the water is left to go down (for dry season) or drained (for wet season) in preparation for harvesting. Two weeks later, the rice is harvested and the paddy separated manually from the straw. The yield per hectare varies with the rice variety. For instance, BASMATI 217 yields between 4.6 and 7.4 tonnes per hectare in research fields and between 3.3 and 6.5 tonnes per hectare in farmers' fields. The total growth period is usually 4-5 months. After harvesting, the land is left dry until the commencement of the second season if the farmer is not interested in raising up the ratoons.

2.16 Rice cultivation and malaria

Although many species of mosquito thrive in ricefields (Carnevale et al., 1999), flooded paddy fields provide ideal breeding sites for the principal vectors of malaria in Africa:

members of the *Anopheles gambiae* complex, especially *An. arabiensis* (White, 1972), prefer to breed in open sunlit pools (Gillies & De Meillon, 1968; Surtees, 1970; White, 1974; Snow, 1983; Coluzzi, 1984). These vectors are pioneer species which rapidly colonize recently flooded fields, although they decline in abundance as the rice grows and begins to cover the water surface (Snow, 1983; Lindsay et al., 1991; Ijumba et al., 1997). Irrigated-rice cultivation, depending on the number of cropping cycles, may also extend their breeding season and hence increase the annual duration of transmission. Moreover, in dry regions, irrigation will elevate relative humidity that aids survival of these vectors. Although *An. gambiae* sensu lato is generally associated with irrigated rice, in certain situations *An. funestus* also thrives in paddy fields, especially in parts of Madagascar. *Anopheles funestus* typically favours breeding sites shaded by vegetation (Gillies & De Meillon, 1968) and its presence tends to be indicative of more persistent wetland habitats. In ricefields *An. funestus* occurs later in the growth of rice and when the land is fallow (Marrama et al., 1995). Under some circumstances, however, *An. funestus* becomes excluded by well managed irrigation schemes such as those maintained under guidance of the West African Rice Development Authority, WARDA (Henry et al., 1998; Dolo et al., 2001; Dossou-Yovo et al., 2001). In general, the predominant vector in irrigated rice systems is that found in surrounding areas, although there is at least one notable exception to this rule. The Mopti form of *An. gambiae* sensu stricto found in West Africa thrives in ricefields located in the northern fringes of the Sahel. Thus, in Burkina Faso this cytotype was common in the centre of the ricefields, but at the edge of the irrigated area the Savanna form was more abundant (Robert et al., 1989). Irrigated ricefields represent ideal breeding sites for mosquitoes and they can generate large numbers of

individuals, although a smaller proportion are infective in ricefield villages than control communities. However, there is no simple association between irrigated ricefields and the degree of exposure to malaria parasites as measured using classical entomological methods. Thus, transmission intensity in irrigated settlements can appear higher, similar or less than in neighbouring villages outside the irrigation scheme. For example, in the rice-growing area of the Rusizi valley, Burundi, the vectorial capacity of *An. gambiae* s. l. was 150 times higher in the rice irrigation scheme than in an adjacent area growing cotton (Coosemans, 1985b). Alternatively, in the rice-growing areas of Bobo Dioulasso, Burkina Faso, the number of infective bites received in the local community was similar to that in the control area (Robert et al., 1985). Whilst in the Lower-Moshi rice irrigation scheme, Tanzania, the number of infective bites was 2.6 times lower in the irrigation scheme than in the control village (Ijumba et al., 1997). However, when entomologists measure exposure they may not accurately reflect the levels experienced by individuals in the study community. Mosquitoes collected off human baits or from light traps will overestimate true biting rates, particularly when large numbers of mosquitoes are biting, since people will often avoid receiving large numbers of bites by sleeping under a bednet or use some other means of protection.

2.17 Reduced malaria in areas of stable transmission

An alternative method for assessing transmission intensity is to determine the level of infection experienced in the human population. The observation that ricefields frequently generate large numbers of mosquitoes suggests that malaria transmission will increase in local communities. However, there is an increasing body of evidence that indicates that

this is not the case, at least in areas of stable transmission, where malaria may be less of a problem than in surrounding communities outside the ricefields. A review of the literature shows that high vector density does not necessarily imply an increased risk of exposure to malaria parasites. In a study of two communities in the Vallée du Kou, Burkina Faso, malaria prevalence varied between 35±83% in the savannah and 16±36% in the rice-growing area (Boudin et al., 1992). A recent study in the Lower Moshi rice irrigation scheme, Tanzania, showed that malaria prevalence was four times lower in children living near irrigated rice cultivation compared with a nearby savannah village (Ijumba, 1997). When a large-scale rice irrigation scheme was introduced in The Gambia, there were anecdotal reports of increased malaria in local communities. However, on closer examination, it was apparent that there was less malaria near the ricefield than in other rural communities (Lindsay et al., 1991). Another important finding from this study was that during the dry season there was 'anophelism without malaria'. Whilst enormous numbers of vectors were produced by the rice fields during the dry season there was little, or no, malaria in children in this community at this time of year. The main reason for this finding was probably related to the extremely high temperatures experienced during the dry season, often rising above 40°C during the day. Such exceptionally hot weather reduced the survival of adult mosquitoes and perhaps, more importantly, killed the developing parasites within the vector. The critical finding was that there were few infective mosquitoes during the hot, dry season. Similar levels of malaria transmission have also been reported in rice field communities and control villages in the same district. For example, following rice irrigation development in the Senegal River valley, the prevalence of malaria transmission and incidence rates

remained unchanged (Faye et al., 1995). This general observation that rice fields do not increase the risk of malaria, is also characteristic of some areas with exceptionally high numbers of mosquitoes. In The Gambia most *An. gambiae* s.l. breed on the edges of large pools bordering the River (Bugh, 2000; Thomas & Lindsay, 2000), which generate large numbers of adult mosquitoes. Interestingly villages closest to the breeding sites had less malaria than those further away (Thomas & Lindsay, 2000). Such effects also operate at a coarser spatial scale, with areas with the largest numbers of mosquitoes having less malaria than those with fewer mosquitoes (Thomson et al., 1994).

2.18 Reasons for protection

Reasons for this protection are many and varied. One plausible explanation is that the introduction of irrigated-rice cultivation results in wealth creation in local communities (Robert et al., 1985; Audibert et al., 1990; Boudin et al., 1992). And with this additional wealth, farmers make improvements to their homes, their standard of living rises and they have more disposable income with which to use to protect themselves from nuisance mosquitoes and malaria. In Kenya, it was found that at a certain threshold of income the situation becomes favourable for adoption of malaria control measures at a family level, and this goes hand in hand with improvement of the living standard of the family or community (Mwabu, 1991). Therefore, a community with relatively higher economic development would be associated with greater use of anti-malarial measures, and also, within a community, wealthy individuals would be more likely to use such measures.

Support for this comes from studies that have shown that bednet ownership was related to affluence in The Gambia (D'Alessandro et al., 1994) and Tanzania (Ijumba, 1997).

Income and wealth clearly affect the severity of the malaria problem. If the population has the financial resources to build housing inhospitable to mosquitoes, is knowledgeable about the use of personal protection measures and can afford them, understands the importance of seeking effective treatment at the first sign of illness, and can pay for health services and drugs, the rates of severe morbidity may be substantially reduced, despite being in an area of high risk (Oaks et al., 1991). Implicitly, severe environmental health problems affect countries and people who lack access to economic and other resources, people who are denied opportunities to improve their lot. There is enough recent and historical evidence to support the view that economic development in general has an overall positive impact on health (Carrin, 1984; Stewart, 1985). The disappearance of malaria in some parts of Europe was associated with economic development as a result of agricultural expansion rather than vector control or chemotherapy (Cambournac, 1994; Najera, 1994; Zulueta, 1994). At a certain threshold level of income, it becomes possible for the family to invest in antimalaria measures in order to save on direct costs associated with malaria treatment (Wang'ombe & Mwabu, 1993). Increased incomes lead to better nutrition, habitation and protection factors that often influence the overall health status more than public health expenditure on health care. Therefore, if irrigation contributes to increased incomes, this will be reflected in better health \pm although this process may not be particularly rapid (Oomen et al., 1988).

Large-scale irrigated rice cultivation can result in several thousand mosquitoes entering a local house during the night. For most people such high biting rates are unacceptable and

they will protect themselves by sleeping under bednets. Studies in The Gambia have shown that more people start using bednets when mosquito numbers begin to increase (Aikins et al., 1993; Thomson et al., 1994; Bùgh, 2000). Bednets in good condition reduce biting rates (Lindsay et al., 1989) and can protect against malaria (Bùgh, 2000). Thus, it is likely that part of the association between rice and moderate malaria may be explained by the high bednet coverage in communities living near irrigated rice production. Thus, high net use has been reported from rice villages in Burkina Faso (Robert et al., 1985), Cameroon (Robert et al., 1992) and The Gambia (Lindsay et al., 1991). Moreover, at high mosquito densities, density-dependent effects become important. With high net use, individual mosquitoes find it difficult to locate and obtain a human bloodmeal. Reanalysis of data from The Gambia (Lindsay et al., 1993) shows that the proportion of human-bloodfed mosquitoes collected from individual villages declined with increasing numbers of mosquitoes collected from light traps ($z = 3.059$, $P = 0.002$). Even with badly torn nets, blood feeding is density dependent (Lindsay et al., 1992).

It has been postulated that mosquitoes that find it difficult to feed on humans may be displaced and feed on other animals, such as cattle. Such a marked shift in feeding behaviour was suggested as the reason for the low sporozoite rates found in three villages in the Senegal River delta where *An. gambiae* s. l., *An. funestus* and *An. pharoensis* were vectors (Faye et al., 1995). Whilst *An. gambiae* s.l. will take proportionately more bloodmeals on cattle when cattle numbers increase (Lindsay et al., 1991), there is no evidence that zooprophylaxis is protective when cattle are kept close to households either when the principal vector is *An. gambiae* s.s. (Bùgh, 2000) or *An. arabiensis* (Ghebreyesus et al., 2000). Widespread use of anti-malarials (Carnevale & Robert,

1987; Faye et al., 1993b) in ricefield communities and the existence of a well established health infrastructure (Faye et al., 1993a) may also contribute to the general lower level of malaria, although the growing problem of chloroquine resistance will make control more difficult in the future. It is of course also important that irrigation schemes are well designed and maintained to reduce standing water to a minimum and thus limit the opportunities for mosquito breeding.