MALARIA TRANSMISSION AND VECTOR ECOLOGY IN A REFORESTED SWAMPY RIVER VALLEY IN MBALE, VIHIGA DISTRCT OF WESTERN KENYA.

BY

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DECLARATION

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

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DEDICATION

To my parents Ngũgĩ Ndagũi and Wanjĩra Ngũgĩ, my love Wambũi and to God be the glory for great things He has done.

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ABSTRACT

The ecology of malaria vectors and malaria transmission in a reforested swampy river valley in a western Kenya highland site were investigated, and the findings compared to those of a deforested site within the region. Data was collected from January 2005 to June 2005. Larval sampling was done to determine the relative abundance of anopheline larvae in habitats in the reforested area. Sampling of indoor resting *Anopheles* mosquitoes was done by Pyrethrum Spray Catch method (PSC), and the relative abundance/densities determined. Parasitological surveys were done to determine malaria parasite prevalence in school children within the sites. Survivorship of anopheline larvae in artificial habitats was investigated together with characteristics of natural habitats to determine the factors for the low survivorship of *Anopheles* larvae observed in the reforested site. More anopheline larvae were collected in habitats in the open sunlit patches than in the habitats under forest cover (χ^2=24.3524, df=1, P<0.0001) and canopy cover was negatively associated with larval abundance (P=0.0152). The relative density of adult anopheline mosquitoes was significantly lower (F=39.16, df=1, 5 P<0.0001) in the reforested site compared to the density of the same in the deforested area. The prevalence of *P. falciparum* parasites in school children at the reforested site was found to be significantly lower (χ^2=5.6, df=1, P=0.01796) than that of the deforested site. The overall mean larval survival time was shorter in the open sunlit habitats compared to that of the habitats under forest cover. (χ^2=110.5, df=1, P<0.0001) and χ^2=10.02, df=1, P=0. 0015 February 2005 and May 2005, respectively). On average it took a shorter time period (17 days) for larvae to develop into female adult stage in the habitats exposed to sunlight and a much longer time (27 days) in habitats under forest cover. Mean pupation rate was higher in

open sunlit habitats than in forest habitats especially in the experiment done in May 2005. This study demonstrated a significant reduction in the survivorship of *An. gambiae* and *An. funestus* larvae in the reforested larval habitats and hence a low abundance of the same. The adult vector mosquito population in the reforested site was far much less than the vector population in the deforested site; the same was also true for *Plasmodium falciparum* prevalence. The results of the study therefore, suggest that reforestation of the swampy river valleys may reduce survivorship of *An gambiae* and *An funestus* larvae, which may lead to a low abundance of vector mosquitoes. Low abundance of vectors may result to a reduction in the entomological inoculation rate which may be one of the potential factors that may reduce malaria transmission in this area.

CHAPTER ONE

1. INTRODUCTION AND LITERATURE REVIEW.

1.1 General introduction.

1.1.1 Public health importance of malaria.

Malaria remains a major public health problem across the tropical and subtropical areas of the world. At present, malaria kills about twice as many people as does AIDS and as many as half a billion people worldwide are left with chronic anemia due to malaria infection (Tasawar *et al,* 2003). At present it is estimated that approximately 350 – 500 million clinical malaria episodes occur annually, with over a million deaths each year and some 3.2 billion people living in 107 countries or territories are at risk (WHO 2005).

Over 80% of malaria deaths occur in Africa south of the Sahara, where approximately 66% of the population is thought to be at risk (WHO/UNICEF 2005). More than 1 million Africans, who die from malaria each year, are children under 5 years of age (WHO/UNICEF 2005). In contrast, less than 15% of the global total of malaria deaths occurs in Asia (including Eastern Europe), despite the fact that an estimated 49% of the people in this region are living under threat from the disease. In the Americas 14% of the population are at risk, but the region sees only a tiny fraction of global malaria-related deaths. As these figures make clear, malaria exacts its heaviest toll on the African continent. Chiefly there are two explanations. First, the climate and ecology of tropical Africa provide ideal conditions for *Anopheles gambiae* the most efficient of the mosquitoes carrying the malaria parasite to thrive and it is here also that *Plasmodium*

falciparum the most deadly species of the malaria parasite is most common. This fatal combination greatly increases the transmission of malaria infection and the risk of disease and death. Second, poverty and lack of good-quality health care have hindered the control and treatment efforts that have had a significant impact elsewhere in the world (WHO/UNICEF, 2005). The disease is estimated to be responsible for an estimated average annual reduction of 1.3% in economic growth for those countries with the highest burden (WHO, 2005). Malaria is a problem to which answers are available. The know-how, the plans and the technologies are all in place. And they are beginning to work. Just two things stand in the way of taking treatment and prevention measures to scale: a shortage of funds and a shortage of in-country capacity to put plans into action on the ground (WHO/UNICEF, 2005).

Though the burden of malaria is still worst in Africa, more people are accessing prevention and treatment services for malaria, and that progress has been made in preventing and treating malaria since the year 2000, sparking hope that the number of people who become sick and die from malaria will begin to decline (WHO/ UNICEF, 2005). However, challenges remain to reduce the burden of the disease, given that the fight against the disease has not yet hit a breakthrough. Moreover, malaria has come back to areas where it had been previously eliminated (Mathenge, 1999), and recent epidemics in the highlands regions of Africa have raised concerns (Lindblade *et al.*, 2000). Therefore, research leading into new control methods and the improvement of the existing ones is needed, including effective environmental management policies

(Minakawa *et al.*, 2002a), which can offer cheaper alternatives to the otherwise expensive intervention methods (WHO UNICEF /, 2005).

1.1.2 Human malaria parasites.

Malaria of humans is caused by the protozoan parasites belonging to the genus *Plasmodium.* Of the four species of *Plasmodium* that infect humans —*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* — *P. falciparum* causes most of the severe disease and deaths attributable to malaria and is most prevalent in Africa south of the Sahara and in certain areas of South- East Asia and the Western Pacific (WHO, 2005). *P. vivax* is cosmopolitan in tropical areas except in parts of tropical Africa, *P. malariae is* distributed principally in Southeast Asia, Africa and Indian Subcontinent and *P. ovale* is distributed in tropical Africa on the West Coast and Ethiopia (Tasawar, et al 2003). In general, fever is the primary symptom of malaria in humans, in which the severity of attack and reaction to treatment differ according to the particular malaria parasite. There are four main categories of malaria that correspond to the type of parasite involved, malignant tertian malaria (*P. falciparum*), tertian malaria (*P. ovale*), benign malaria (*P. vivax*) and quartan malaria (*P. malariae*). Periodicity of fever is characteristic in malaria cases, in which the febrile paroxysms occur at every 24- hour period (quotidian) or recur every third day (tertian). In case of falciparum malaria, low-grade fever is observed between paroxysms, and quartan malaria, paroxysms recur every fourth day (CDC, http://www.cdc.gov/malaria/disease.htm). Malaria parasites are transmitted by haematophagous female mosquitoes of the genus *Anopheles*. The most common malaria

vectors in the Afro-tropical region are *Anopheles gambiae* complex and *Anopheles funestus* (White 1972, Coluzzi *et al.*, 1979).

1.1.3 Problem statement

Malaria was a rare occurrence in the African highlands, however in the recent past these areas have recorded increased epidemics raising the concern that malaria outbreaks are on the increase (Lindblade *et al.*, 2000). Land use change, such as swamp cultivation has been pointed out as one of the potential factors for the increased malaria epidemics in western Kenya highlands. Cultivation of swamps creates suitable breeding conditions for *Anopheles gambiae,* which is the main malaria vector in these areas. Reforestation of swampy river valleys in western Kenya highlands may interrupt *An.gambiae* breeding, leading to a significant reduction in malaria mosquitoes. Thus this study was aimed at establishing whether reforestation of swampy river valleys may reduce significantly the population of malaria vectors, and thus malaria transmission in western Kenya highlands.

1.2 LITERATURE REVIEW.

1.2.1 Malaria in the highlands.

It has been documented that malaria transmission in the highland areas is hindered by low temperatures, which limit the development of the parasites in the mosquito (Garnham, 1945; Hay *et al.*, 2002). Recent epidemics in these areas have raised concerns that high elevation malaria transmission may be increasing. Countries affected by highland malaria in Africa include Kenya, Ethiopia, Madagascar, Tanzania, Uganda, Rwanda, South Africa and Zimbabwe (Mouchet *et al.*, 1998; Githeko *et al.*, 2000). In Uganda, malaria incidence has increased more than 30 times in the highlands (1500-1800m) (Mouchet *et al.*, 1998). Cultivation at the bottom of valleys and extension of settlements are in large part responsible for this increase, along with abnormally heavy rainfall that favored a severe epidemic in 1994 (Mouchet et al., 1998). A similar increase in malaria was observed in the neighboring highlands of Rwanda and Burundi, and epidemics have been recorded in Ethiopia since 1958 (Fontaine 1961), highlands of Madagascar (1987) and Swaziland (1985-86) (Mouchet *et al.*, 1998).

Epidemics in western Kenya generally occur in areas at altitudes of between 1500-2200 meters above sea level, where the annual mean daily temperature varies between 18-22°C (Githeko and Ndegwa 2001). Malaria transmission in these areas has been found to be focal and mostly confined to valleys and along streams (Githeko and Shiff, 2005), it is also highly seasonal (Brooker *et al.*, 2004). Studies on land use change has identified that

major malaria vector breeding sites are cultivated swamps at the bottom of valleys (Zhou *et al.*, 2004). According to Brooker *et al.*, (2004), malaria prevalence can reach 60% in areas close to the major habitats and sporozoite rates can be as high as 9%. Epidemics are likely to occur where malaria transmission is low, unstable and low level of immunity in the human population, as observed in the highlands areas.

1.2.2 History of Malaria in the Highlands of Western Kenya.

Epidemic malaria in the Kenya highlands is caused by *Plasmodium falciparum* species and transmitted by *Anopheles gambiae* s.s. and *An.funestus* mosquitoes (Shililu *et al.*, 1998; Githeko and Ndegwa 2001). The highlands of western Kenya were regarded by colonial settlers as safe havens from the surrounding malarious areas of Uganda and Kenya. After World War I, malaria encroached into these highland communities (Hay *et al.*, 2002). The first recorded epidemic of malaria in the highlands of western Kenya occurred in 1918/19 (Matson, 1957) and malaria epidemics were frequently reported by the early 1930s (Garnham, 1945; Robert 1964; Hay *et al.*, 2002). These initial epidemics were associated with both population movements and progressive construction of roads and a railway through the highlands (Garnham, 1945). With these human activities, new aquatic habitats were created, facilitating a gradual spread of parasites and vector mosquitoes into the highlands from the low-lying hyper endemic-disease areas (Garnham, 1948). During the 1950s and 1960s, control efforts such as indoor residual house spraying, mass drug administration, or chemoprophylaxis effectively contained or prevented epidemics in some of these high-altitude areas (Robert, 1964).

Since the 1980s, however, the incidence of highland malaria and frequency of epidemics have been increasing, with severe outbreaks in 1995, 1998/99, (Hay *et al.*, 2002) and most recently in May through July 2002 (Carlson *et al.*, 2004; Brooker *et al.*, 2004). Currently, in western Kenya, 15 districts, compared to 3 in 1988, are under constant threat of epidemics (Githeko *et al.*, 2000; Minakawa *et a*l., 2002b). Hypotheses about the reasons for these epidemics include climate change, land use change, demographic pattern, cessation of control activities and antimalarial drug resistance (Lindsay and Martens, 1998; Mouchet *et al.*, 1998; Lindblade *et al.*, 2000). The underlying cause of malaria epidemics in the East African highlands remains a subject of debate (Githeko and Ndegwa, 2001; Hay *et al* 2002; Zhou *et al.,* 2004). The epidemics normally occur from May to August, following the long rains. However, during the 1997/98 El Nino, the epidemic occurred from January through March 1998, following unusually heavy rains caused by El Nino weather (Githeko and Ndegwa, 2001).

1.2.3 Ecology of the malaria vectors.

The success of malaria transmission depends greatly on abundance and composition of efficient vector species (Shililu *et al.*, 1998). Members of the *Anopheles gambiae* complex are major malaria vectors in sub-Saharan Africa, with *Anopheles gambiae* Giles being the most important vector of human malaria in this region (Gimnig *et al.*, 2002). This complex consists of a number of mosquito species that vary in their ability to transmit falciparum malaria in Africa (White 1972; Coluzzi *et al.*, 1979; Coluzzi 1984, 1992). The sibling species of the complex include *An. gambiae s.s, An. arabiensis, An. quadriannulatus, An. merus, An. melas and An. bwambae* (Coluzzi, 1984). In Central and

East Africa, the three major vectors involved are *Anopheles gambiae* Giles, *Anopheles arabiensis* Patton and *Anopheles funestus* Giles (Mouchet *et al.*, 1998). The 1st two species are mainly linked to rainfall, and the 3rd is dependent on accumulation of rainwater in pools (Mouchet et al., 1998). The three species are also widely distributed from south of the Sahara to northern South Africa (Gillies and De Meillon, 1968).

The two principal vectors of the complex are *An. gambiae s.s.* which is the most efficient vector, and *An. arabiensis.* According to Shililu *et al.,* (1998), *An. gambiae s.s* is the only member of the *An. gambiae* Giles complex in the high altitude zones of western Kenya. Based on it's highly anthropophagic and endophilic behaviour relative to *An. arabiensis*, the vector contributes significantly to malaria transmission in the high altitude areas. *An. gambiae* usually predominates on most environments, but is known to prefer humid environments (Lindsay *et a*l., 1998) while *An. arabiensis* is more common in hotter, drier parts of Africa. (Coetzee *et al.*, 2000; Minakawa *et al.*, 2002b; Lindsay *et al.*, 1998; Lindsay, 2004).

In western Kenya, *An. gambiae, An. arabiensis*, and *An. funestus,* have been shown to be the major vectors for *P. falciparum* (Mathenge, 1999). The three species are abundant in the lower parts of western Kenya, while *An. arabiensis* is absent in highland areas with elevations above 1,400m. (Minakawa *et al.*, 2002b), where *An. gambiae s.s* and *An. funestus* dominate (Shililu *et al.*, 1998), *An. gambiae* s.s has been found to prefer breeding in small transient pools of water that are clear and open to sunlight (Gimnig *et al.*, 2002). In such habitats larval predation is less prevalent than in large, permanent

habitats and also has increased resources (Minakawa *et al.*, 2004). *An. funestus* on the other hand, tend to occur more in large habitats that are semi permanent or permanent with emergent plants (Minakawa *et al.*, 2005a) preferably in habitats that have a moderate amount of shade (Hopkins, 1940), it may also breed in marshes choked with weeds and other densely growing aquatic vegetation (Kirk, 1936).

1.2.4. Climate change and variability.

Weather conditions, principally rainfall, temperature and humidity, are considered to be the most important causes of malaria epidemics in non-endemic areas (Gilles, 1993). Key malaria transmission parameters, such as mosquito population density and longevity, and the extrinsic incubation period of the parasite in the mosquito, are extremely sensitive to weather conditions (Lindblade *et al.*, 1999). In areas of unstable transmission such as highlands, small changes in transmission parameters can have substantial impacts on human morbidity and mortality (Onori and Grab, 1980).

Many studies have shown that climate is a major factor governing the distribution of insects, either by acting directly on the insect populations themselves or indirectly by affecting the structure of the ecosystem they inhabit (Lindsay *et al.*, 1998). Climatic conditions influence the development, reproduction, and survivorship of anopheline mosquitoes and malaria parasites inside the mosquitoes (Zhou *et al.*, 2004; Lindblade *et al.*, 1999); they also strongly affect the distribution and abundance of malaria vectors, particularly moisture index and temperature (Lindsay *et al.*, 1998). Temperature affects the development rates and survivorship of malaria parasites and mosquito vectors, while

rainfall influences the availability of mosquito larval habitats and thus mosquito demography (Zhou *et al.*, 2004). If water temperature rises, the larvae take a shorter time to mature (Bayoh and Lindsay, 2004) and consequently there is a greater capacity to produce more offspring during the transmission period (Githeko *et al.*, 2000). In warmer climates adult female mosquitoes digest blood faster and feed more frequently (Gillies, 1953), thus increasing transmission intensity. Similarly, malaria parasites complete extrinsic incubation within the female mosquito on a shorter time as temperature rises (Turell, 1989), thereby increasing the proportion of infective vectors (Githeko *et al.*, 2000).

Climate variability, defined as the short-term fluctuations around the mean climate state (Zhou *et al.*, 2004), has been associated with some of the recent epidemics in western Kenya (Minakawa *et al.*, 2002b; Githeko and Shiff, 2005). Alterations in global climate can disrupt the natural ecosystem and increase the risk of transmission of malaria (Patz, *et al.*, 2000). Higher temperatures, changes in precipitation, and changes in climate variability can alter the geographic ranges and seasonality of transmission of vector-borne infectious diseases - extending the range and season for some infectious diseases and contracting them for others (IPCC 1998; IPCC 2001; Kovats *et al.*, 2001). According to Githeko *et al.*, (2000), current evidence suggests that inter-annual and inter-decadal climate variability have a direct influence on epidemiology of vector-borne diseases, such as malaria, and that by 2100 it is estimated that average global temperatures will have risen by 1.0-3.5°C, increasing the likelihood of malaria in new areas.

The effects of climate change, defined as a change in the mean meteorological values and the departure from the mean (Githeko and Shiff, 2005) on vector-borne diseases, has been extensively reviewed by Githeko *et al.*, (2000) The greatest effect of climatic change on transmission is likely to be observed at the extremes of the temperatures at which transmission occurs (14-18°C and 35-40°C). Climatic anomalies associated with El Nino- Southern Oscillation phenomenon are expected to increase in frequency and intensity. These anomalies have been linked to outbreaks of malaria in Africa, Asia and South America (Githeko *et al.*, 2000; Moshe *et al.*, 2004). There is emerging evidence that, in addition to seasonal extreme climatic events there is a general elevation of mean temperatures and in some cases precipitation (Githeko *et al.*, 2000). For example, Hulme *et al.*, (2001) have shown that Africa is now warmer than it was 100 years ago and the mean rate of warming has been 0.5°C per century. Such changes are likely to support rapid development of malaria vectors and parasites in regions where there has previously been a low-temperature restriction on transmission. Current episodes of climate variability in Africa are likely to become more intense and frequent (McMichael and Githeko, 2001) and this will intensify the transmission of malaria in the eastern and southern highlands (Githeko *et al.,* 2000).

1.2.5 Land use change and malaria.

Land use change can have direct and indirect impacts on malaria (Patz *et al.*, 2000). A diverse range of land-use factors may be involved in malaria's re-emergence. These could include an increase in mosquito breeding habitats. Many species of malaria vectors and

their parasites react sharply to changes in the ecology of their habitat: climate, deforestation, vegetation, human population density, water bodies, and their location (Patz and Wolfe, 2002). Human alteration of the environment, regardless of the intent (i.e., clearing land for subsistence agriculture or dam construction for hydroelectric power and recreation use) often exacerbates existing mosquito-associated problems by expanding habitats, creating new habitats, or altering habitats in such a way that limited mosquito populations may explode with the availability of new habitats (Norris, 2004). This may be due to accelerated larval development and increased survivorship associated with the change in water chemistry and temperature of larva habitats, the alterations also affect the microclimate of the adult mosquitoes and accelerated malaria parasite development (Lindblade *et al.*, 2000). Some forms of habitat alteration have been well studied and documented while the effects of other human environmental changes are just now being recognized or rediscovered (Norris, 2004). These alterations can be placed into several broad and overlapping categories including water retention systems, deforestation, agricultural development, and urbanization. In addition to these alterations, human behavior associated with each of these landscape modifications may contribute significantly to disease transmission (Norris, 2004).

The effects of land use change by humans have long been recognized as a major factor exacerbating the transmission of mosquito-borne diseases. Land use changes that have been associated with an increase in malaria transmission include deforestation in Tanzania (Matola *et al.*, 1987; Mboera and Kitua, 2001) and cultivation of swamps in Uganda (Lindblade *et al.*, 2000; Mouchet *et al.*, 1998), brick making in western Kenya

(Carlson *et al.*, 2004). In Central African mountains, cultivation on the bottom of valleys was detrimental to the original papyrus marshes; the oil produced by this plant served to inhibit development of anophelines (Mouchete *et al.*, 1998). Now the new environment is suitable for development of *An. gambiae* s.s and *An. funestus* (Mouchet *et al.*, 1998). Cultivation of swamps can substantially increase the water temperature, by exposing pools of water to direct sunlight thus increasing the potential for prolific breeding of malaria vectors particularly *An. gambiae* (Yan G and Githeko, unpub data, Minakawa *et al.*, 2004). While deforestation on the slopes of the highlands primarily affects the rate of parasite development in adult mosquitoes, cultivation of swamps causes an increase in the rate of production of adult mosquito due to higher water temperature in the larval breeding habitats (Yan and Githeko, unpublished data; Minakawa *et al.*, 2004). Changes in land use often result in changes in human settlement pattern and habitat fragmentation may provide opportunities for exchange and transmission of malaria to uninfected population.

1.2.6 Antimalarial Drug resistance.

Drug resistance has been cited as one of the several factors responsible for the recent malaria epidemics in the western Kenya highlands (Lindsay and Martens, 1998; Githeko *et al.*, 2000; Shanks, 2000; IPCC, 2001; Hay *et al.*, 2002). With the possible exception of artemisinin derivatives, resistance to all antimalarials has been recorded (White, 1992) but has developed at different rates for different compounds (Phillips, 2001). The effectiveness of Chloroquine for the treatment of *falciparum* malaria has long been compromised in many parts of the world (Fogh *et al.*, 1979; Oloo *et al.*, 1996; Githeko *et*

al., 2000). Widespread resistance to sulphadoxine/pyrimethamine (SP) is emerging at a time when there seems to be an increasing number of epidemics in the African highlands (Githeko and Shiff, 2005). Though it cannot initiate an epidemic, drug resistance aggravates malaria case fatality after an epidemic (Zhou *et al.*, 2004).

The emergence of resistance to anti-malarial drugs has several implications in terms of treatment policy and efficacy (Githeko and Shiff, 2005). The case of chloroquine resistance in Kenya serves as an example. Following several studies in the 1980s that demonstrated that the drug had lost its efficacy, the ministry of health had difficulties changing the first line of treatment from chloroquine to sulphadoxine/pyrimethamine. The failure to change drug policy had a particularly severe implication during epidemics in western Kenya where up to 80% (Githeko and Shiff, 2005) of self-diagnosed malaria cases are treated at home. Many patients continued to use chloroquine despite its failure to clear parasites in the blood though clearing clinical symptoms quickly.

1.2.7 Vector control

Current vector control methods include indoor residual house spraying, insecticide impregnated materials and larval control. Potential methods include genetically modified mosquitoes and other applications based on new technology like the sterile insect technique (WHO/TDR, 2002). One potentially important target of malaria vector control is the immature stages of anopheline mosquitoes. To design efficient larval control methods, mechanisms regulating mosquito productivity in natural habitats must be understood (Minakawa *et al.*, 2005b)

There have been successful programs of residual spraying in houses, which resulted in eradication of *An. gambiae* from Brazil in 1934-1949 and from Egypt in 1948 (Soper, 1949). Control efforts in early 1930s on the Western Kenya highlands by residual spraying with dichlorodiphenyltrichloroethane (DDT) effectively controlled the epidemics, until the resurgence of epidemics in 1980s (White, 1972; Hay *et al.*, 2002).

The introduction of DDT revolutionized agricultural production and has been credited with the elimination of malaria from the United States and Europe (Beard, 2005). However, DDT is also known to have had major environmental consequences and has been associated with dramatic declines in many animal populations. Although DDT use has generally been restricted since the early 1970s, in developing countries, the pesticide continues to be used for vector control (Beard, 2005). Chlordane, DDT, toxaphene, mirex, aldrin, dieldrin and heptachlor are now banned under the Stockholm Convention on persistent organic pollutants. However, DDT has been retained for vector control in some countries until an alternative safe, effective and affordable insecticide is found (Githeko and Shiff, 2005).

Vector ecology studies in the highlands indicate that malaria transmission is focal and this raises the possibility of limited application of residual insecticides around the foci of transmission. Also given that vector breeding is restricted to the bottom of valleys (Zhou *et al.*, 2004, Minakawa *et al.*, 2005b), larval control can be done to achieve source reduction. Targeted control of malaria vectors, using larvicides at the larval stage and

using adulticides at the adult stage, in the valleys and basin-like depression in the plateau may be a cost–effective approach to reduce malaria transmission. Malaria transmission in the valleys can be further reduced if insecticide-based mosquito control is combined with elimination of larval habitats through appropriate land –use management (Zhou *et al.*, 2004).

Recent development in malaria control has found that permethrin impregnated bed nets and curtains can reduce malaria morbidity and mortality (Shiff *et al.*, 1996; Langeler *et al.*, 1997; Mathenge, 1999). A trial in the Gambia showed that insecticide impregnated bed nets with permethrin reduced overall deaths by over 50% (Alonso *et al.*, 1991). Further trials however, showed a reduction in overall child morality of 17-33% (Mutambu and Shiff, 1997) These encouraging results have generated interest in ITNs as a viable malaria control strategy in many malaria endemic countries (Adongo, 2005). Despite these encouraging results acceptability and affordability of impregnated bed nets has remained a barrier to their use. Currently only about 5% of the populations under threat from malaria are using insecticide impregnated bed nets (Githeko and Shiff, 2005).

1.2.7.1 Environmental management for mosquito control.

Environmental methods and biological control are alternatives to chemical control and are key components of the integrated strategy, in which the various methods used in malaria vector control are applied together. Environmental management for vector control is a term adopted in recent years to broadly cover activities previously classified

as source reduction, naturalistic and physical measures of vector control and species sanitation (Rafatjah, 1988). World Health Organisation expert committee on vector biology and control defined the term environmental management as the planning, organization, carrying out and monitoring of activities for modification and/or manipulation of environmental factors and their interactions with man with a view to preventing or minimizing vector propagation and reducing man-vector-pathogen contact. This approach, which should be carried out skillfully, is naturalistic and involves an attempt to extend and intensify natural factors, which limit vector breeding, survival and contact with man. Environmental management measures generally are not intended to replace other control measures but rather to complement them and contribute to the development of integrated control strategies (Mitchell, 1996).

In the early 1990s the control of both *P. falciparum* and *P. vivax* malaria in parts of East Asia and Pacific was highly successful largely because of environmental measures for the control of mosquito larvae. This concept was based on the pioneering work of Watson in Malaysia, who showed that by selective clearing of the forest around settlements the mosquito *Anopheles umbrosus*, a forest vector, resulted in the elimination of malaria (Bradley, 1994) Thereafter, environmental management was widely used in South East Asia, with the advantage that it could be applied with relatively few skills and local technology. Environmental management was relegated from the malaria control agenda when DDT became the main tool for the World Health Organization's (WHO) malaria eradication programme from 1956-67. Here was a relatively cheap and highly effective product for residual house spraying against malaria vectors (Lindsay *et al.*, 2004) that

promised to end malaria. This period also coincided with a shift from health protection and promotion in an integrated rural development context, based on sound ecological principles, to vertical control programmes, lodged solely in the health sector, where the research assessment focused largely on the resting habits of vectors and their susceptibility to insecticides. Since the failure of the above measures to sustainably eliminate malaria from the tropics, the effectiveness of environmental management has remained tragically under-exploited (Lindsay *et al.*, 2004).

1.2.7.2 Effects of deforestation and re forestation on malaria vectors.

Deforestation has been one of the major human activities associated with the resurgence of yellow fever and malaria in the Americas as well as the emergence of lesser-known mosquito viruses (Norris, 2004). Not only has removal of intact forest resulted in the emergence of newly recognized pathogens, but these activities also allow for shifts in relative vector species that may not have been previously incriminated (Tadei et al., 1998). A study by Manga *et al.*, (1995), demonstrated that deforestation favored the breeding of *Anopheles gambiae* in Cameroon. Similar observations have been reported by Matola *et al.*, (1987), Lindsay and Martens (1998), Tadei *et al.*, (1998), Mboera and Kitua (2001), Lindblade *et al.*, (2000), Tuno, *et al.*, (2005) and Minakawa *et al.*, (2005a; 2005b). Deforestation, may also lead to the establishment of a vector in an area where it had no role in malaria transmission, as was the case for *An. gambiae*, which became established in an area around a newly constructed airport (Manga *et al.*, 1995). The removal of forest canopy creates a suitable habitat for the expansion of vector mosquitoes

(Norris, 2004). Deforestation attracts and clusters humans for agriculture, road building, and logging. As *Plasmodium*-infected individuals enter the area, the vector and environment have already been modified in favor of transmission resulting in both small and large epidemics (Norris, 2004).

Conversely, growing of dense vegetation over *An. gambiae* and *An. funestus* breeding habitats has reverse effects as shown by Hopkins, (1940) and Blacklock, (1936), According to Blacklock (1936), gradual control of malaria was attained in Assam by encouragement and augmentation of naturally growing plants, called Basak, whose dense shade suppressed *An. minimus* breeding in small streams and marshy areas. It is suggested that the shade should be sufficiently dense to render the breeding habitats unsuitable for *An. gambiae* and *An. funestus*, for this reason *Eucalyptus sp.* may be unsuitable (Hopkins, 1940). Shade is thought to deter mosquito breeding, by its role in the inhibition of growth of organisms consumed as food by the larvae of certain species of *Anopheles* (Kirk, 1936). Warmer, open habitats tend to produce more algae (the main food source for *An. gambiae* s.s) than shaded habitats (Gimnig *et al.*, 2002). For *An. gambiae*, dense shade provided by forest trees may play a role in decreasing the optimum temperatures suitable for the development and survival of larvae. Survivorship of *An. gambiae* larvae reduced from 55-57% in habitats fully exposed to sunlight to 1-2%in habitats with full forest canopy coverage and partial canopy coverage (Tuno *et al.*, 2005).

Water from forests and natural wetlands may contain toxic substances from leaf litter, and some harmful microorganisms may be present in stagnant water (Munga *et al.*,

2005). Leaf litter may produce phenolic compounds (tannin-phenolics) that are harmful for some mosquito species (Rey *et al.*, 1998, 1999; David *et al.*, 2000). Lignin-like compounds from some tree species are also known to be toxic to *Culex* and *Aedes* (David *et al.*, 2000, 2001).

1.2.7.3 The *Eucalyptus* trees and malaria vector control.

Many swampy river valleys in western Kenya highlands are undergoing extensive reforestation mainly by the exotic *Eucalyptus* spp (Family Myrtacea), the popular species being *Eucalyptus globulus* (Blue gum). The trees are primarily planted for firewood, and also as a source of income from sales of timber and poles. Eucalyptus trees contribute to over 70 per cent of rural energy needs in Kenya. Tea and tobacco industries use eucalyptus as wood fuel in curing these products.

Reforestation of swamps with *Eucalyptus* spp. dries up swamps and also creates a canopy over water pools hence reducing breeding sites for mosquitoes (Ndenga, 2005). Lemon eucalyptus (*Eucalyptus maculata citriodon*) has been shown by Trigg (1996) to provide protection against *An. gambiae* and *An.funestus*. Repellency assays with natural products obtained from *Eucalyptus citriodora*, showed that they were active against *Anopheles gambiae s.s.* (Barasa *et al.*, 2000).

Due to its rapid growth, *Eucalyptus globulus* is used in Africa to drain swamps, and thus may play an important role in the elimination of breeding grounds for mosquitoes

(Santos, 1997). However, according to Hopkins (1940), Eucalyptus does not provide very dense shade over water surfaces to render them unsuitable for *An. gambiae* and *An. funestus*, and therefore may be unsuitable for this purpose (Hopkins, 1940).

1.3 Main objective.

To investigate the effect of reforestation of swampy river valley on the population of malaria vectors and the transmission of malaria parasites in western Kenya highlands.

1.3.1 Objectives of the Present Study.

The specific objectives in this study were to determine:

1. The distribution and relative abundance of the immature stages and adults of *Anopheles* mosquitoes in the study sites.

2. The prevalence of malaria parasites in school children in the ages 5 to 12 residing in the area adjacent to study sites.

3. The characteristics of the natural mosquito larval habitats and the survivorship of anopheline larvae in the reforested valley.

1.4 Hypothesis.

Reforestation of swampy river valleys may disrupt breeding of *Anopheles* mosquitoes thus reducing the population of malaria vectors and malaria parasite transmission in a western Kenya highland site.

CHAPTER TWO

2. MATERIALS AND METHODS.

2.1. Study area

The main study site was in Mbale, while another site selected at Iguhu served as a relative comparison area. The sites are located in Vihiga and Kakamega districts respectively, in western Kenya (Figure 1). Both are highland sites, within an altitude range of 1,400-1,600m above sea level, and about 8kms apart. The site in Mbale (about $1km^2$) is a reforested swampy river valley, which has numerous drainage ditches, some of which are cleaned regularly while papyrus reeds and/or grass cover some. The ditches drain into river Ehedue that flows through the study area. The dominant vegetation type is *Eucalyptus globulus* (Plate1a), which is planted primarily for commercial purposes but also used as a source of wood fuel. A comparison site was established at Iguhu. This site falls in a section of the Yala river valley, which is undergoing deforestation for food crop cultivation (Plate1b) and is characterized by numerous drainage ditches. The area was selected to serve as a relative reference site for parasitological surveys and for adult mosquito collections under conditions devoid of the forest cover, since it was not possible to have suitable control site under natural conditions. Houses at both sites are located between 100-200m from the rivers. Cultivated parts of the valleys have maize, beans, vegetables and napier grass. These two sites have the peak of the long rains in the month of April and the peak of the short rains in September. Data was collected for a period of six months (January-June 2005).

Fig 1. A map of Kenya showing relative positions of the study sites.

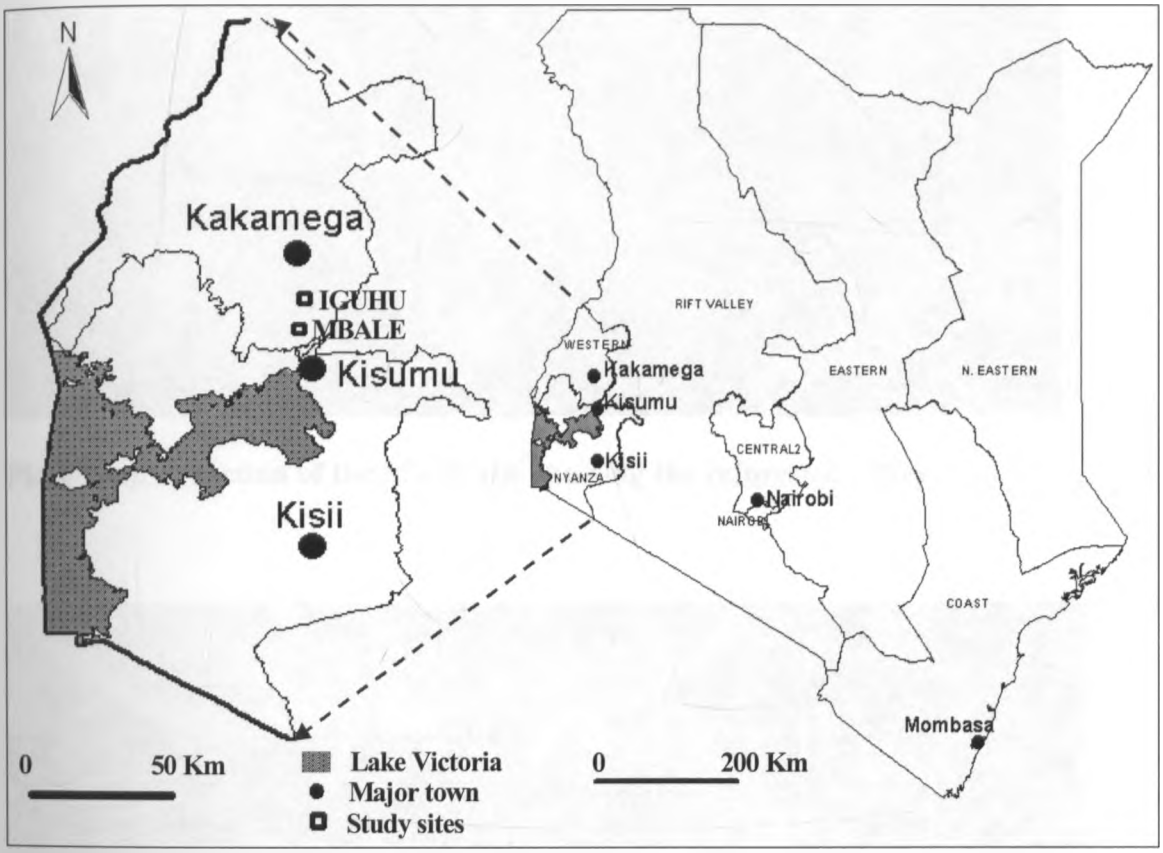

Plate 1. a). A section of the Mbale site showing the reforested valley.

b). A section of the Iguhu site showing the deforested area.

2.2 Ethical Clearance

Written informed consent to participate in the study from parents of school children (ages 5-12 years) was sought with the assistance of local administrators (area chiefs) and school Head teachers. Consent forms were duly signed before the parasitological surveys were done. Oral permission was obtained from members of individual households for spray catches. Ethical clearance was obtained from the Kenya Medical Research Institute.

2.3 Sampling of indoor resting adult *Anopheles* mosquitoes.

A total of 20 houses were randomly selected in each site. Adult mosquitoes were collected from selected houses at 09 00 hrs and 12 00 hrs by the Pyrethrum spray sheet collection method (PSC) (WHO, 1975) where white calico sheets were spread on the floor. A pyrethrum knockdown spray (containing 5ml pyrethrum and 6ml piperonyl butoxide in 5 litres of paraffin) was then sprayed into the houses to knock down mosquitoes that were resting indoors. After a knockdown time span of 10 minutes, mosquitoes were collected from the sheets and placed in petridishes lined with moist cotton wool and filter paper and labelled with the date, the house number, and the number and age of sleepers. The mosquitoes were transported in a cooling box to the laboratory for sorting and identification. Mosquito sampling was done once in a month for six consecutive months (January-June 2005).

2.3.1 Processing of the mosquitoes.

In the laboratory, identification of the mosquitoes was done by external morphology under a dissecting microscope, using the keys of Gilles and De Meillon (1968) and Gilles and Coetzee (1987). The mosquitoes were identified as *An. gambiae* complex *An. funestus* complex or Culicine. The abdomens of the female mosquitoes were examined directly and the mosquitoes were classified as unfed, fed, half-gravid, and gravid (WHO, 1975).

2.4 Sampling *Anopheles* larvae.

All aquatic habitats, in the reforested site were identified and inspected for the presence of anopheline larvae. A total of twenty-six (26) larval habitats were randomly selected in the study, this included those located in the forest and those located in small patches of land deforested for food crop cultivation. Sampling for mosquito larvae was done by dipping method (WHO, 1975) using a standard larval dipper (350ml) (Plate 2). In each habitat at least 3-40 dips were made depending on the size of the habitat. Sampling was done bi-monthly (at fourteen (14) days interval) for a period of six (6) months, from January 2005 to June 2005. The water sample obtained by dipping was transferred to a white tray measuring 40cm long by 25 cm wide by 5 cm deep. Then using plastic pipettes larvae and pupae were individually picked from the tray and put in white paper cups, where they were identified, sorted by instar stages counted, and recorded in the field note book. Identification was done by external morphology to the *Anopheles gambiae* complex, *Anopheles funestus* complex according to the keys of Gilles and DeMeillon

(1968); these were then counted and recorded. Larvae and pupae of species that could not be identified by external morphology in the field were held in paper cups and maintained at the insectary where after emerging into adults their identity was determined and recorded accordingly.

Plate 2. A photograph showing a man using a standard larval dipper at the site

2.5 Parasitological Surveys.

Two parasitological surveys were done. The first survey was done during the dry season (February 2005) and the second survey at the peak of the rainy season in May 2005. A sample of 100 school children in the ages between 5-12 years, from each site was recruited in the study. However, in the Mbale site, 100 blood smears were prepared in the

first survey, and 93 in the second survey, while at the Iguhu site, 95 blood smears were prepared in the first survey and 91 in the second (some children were absent from school during surveys). Thick and thin blood smears were prepared from drops of blood obtained by finger prick using sterile lancets. The slides were air-dried. Thin smears were fixed in methanol and stained with 4% Giemsa stain together with thick smears, Thick smears were examined under the ×1,000 magnification to identify and count the malaria parasite species. Thin smears were used for the detailed identification of the particular parasite species in case they were not clear in the thick smears. Parasite density was scored against 200 leukocytes when the slide was positive; otherwise, the whole slide was carefully scanned before being declared negative. Parasite densities were converted to number of parasites per microliter of blood, assuming a leukocyte count of 8,000 cells/μL (Prybylski, 1999, Munyekenye, *et al.*, 2005).

2.6 Survivorship of anopheline larvae in the reforested habitats.

The survivorship of anopheline larvae in reforested larval habitats was determined and compared with three controls. The first control was made up of a larval habitat containing swamp water and placed in the open sunlit area; the second control was a habitat containing rainwater and food (yeast powder) and placed in forested area and control three, was a habitat with rainwater and food (yeast powder) and placed in the open sunlit area. The experiment was replicated three times, that is, there were three larval habitats for each of the treatments above. Artificial larval habitats were created in small sized plastic washbasins; 14cm deep and 24cm in diameter. Half a kilogram (0.5kg) of soil from reforested site was added to each of the three washbasins. These washbasins were

then flooded with about 3 litres of swamp water from reforested area and placed in the forest; three other washbasins were filled with about 3 litres of rainwater and also placed in the forest (Plate 4). The same design was repeated but this time the washbasins were placed in an open sunlit area within a distance of approximately 20m from the edge of forest (Plate 3). Thirty (30) newly hatched cohort of first instar larvae of *An. gambiae s.s,* from eggs laid by mosquitoes from Iguhu highland site in western Kenya, and maintained in a colony at the insectary at the Kenya Medical Research Institute (KEMRI), Kisumu, were put in each washbasin. Each basin was then screened with a mosquito netting material to keep off wild mosquitoes and predators. The washbasins were covered by placing a polythene paper every evening and during the day whenever there was rain. Larvae in washbasins containing rainwater were fed on 0.05 g of brewers yeast (Pharmadass Ltd, Middlesex, UK) once everyday. Surviving larvae were counted on each day until they changed into pupa. The pupa were picked using plastic pipettes and put in screened paper cups where they were held until they emerged into adults. The adults were separated according to sexes. Water temperature in each basin was recorded on an hourly basis by the use of StowAway-TidbiT Temperature Loggers. Boxcar Pro 4.0 (Onset Computer Corporation) was used to launch, download and export the data to MS Excel through MS Notepad.

Plate 3. Experimental set up with washbasins placed in the open sunlight.

Plate 4. Experimental set up with washbasins placed in the forest

2.7 Habitat Characteristics.

The 26 randomly selected larval habitats were further categorized as drainage ditches (88%) and pools. The location and elevation of each habitat was recorded using the global positioning system (GPS) unit. This information was used to generate a map showing the spatial location and distribution of larval habitats (Figure 5). The habitats were characterized using the following variables: 1) canopy cover, 2) presence of filamentous algae, and 3) habitat size. Canopy cover was estimated with a spherical densitometer (Lemmon, 1956). Habitat size was estimated by measuring the length and width using a tape measure; most of the drainage ditches were regular in shape.

2.8 Data analysis.

Data was entered in MS Excel; analysis was performed using SAS 8.01 (SAS, Inc., Cary, NC) and JMP 5.0.1, statistical packages.

The means procedure in SAS was used to calculate the total sum, means and standard errors for larvae in natural habitats. The number of larvae per dip was used as a unit to estimate the relative abundance/density of larvae in each habitat. Kruskal Wallis test was used to compare larval abundance between habitats in the open and habitats under forest cover, while the Student t-test was performed to determine the differences in relative abundance between *An gambiae* complex and *An funestus* complex in the forest and open

habitats. Habitats were grouped into two main categories based on canopy cover, group 1, were habitats that had a canopy cover of >50% and group 2 had a canopy cover of <50%. The Correlation coefficient was used to establish the association between larval abundance and canopy cover.

Kaplan-Meier survival analysis was used to calculate mean larval survival times in the artificial habitats in the forest and in the open sunlit habitats. This is a time to event analysis in which mean development time and pupation success for *An. gambiae* larvae were determined and compared between the main habitat types (forest vs. open). In this study, mean survival time refers to the number of days the larvae survive to develop into adults or number of days to their death. Maximum, minimum and mean temperatures between the forest and open sunlit habitats were compared using ANOVA.

Data on adult mosquito collections was log transformed (log to base 10) and subjected to repeated measures of ANOVA, to determine the differences in vector abundance per house between the two sites and between months. This test could not be performed for *An. funestus* due to their small number in both sites. The relative density in this study was estimated as number of female anopheline mosquitoes per house.

Parasite prevalence for *P. falciparum* and *P. malariae* was calculated in excel and the Yates Chi-square was used to test for statistical significance of the differences in parasite prevalence between sites and among the sites. Parasite density was estimated based on

8000 leucocytes in a micro liter of blood. Since the parasites were counted against 200 white blood cells, density was obtained by multiplying the number of parasites in an individual by 40. Geometric mean of parasite density for *P. falciparum* was calculated in excel, the data was log transformed and subjected to a one-tailed t- test to determine whether there were significant differences in parasite densities between the two sites and between the two survey periods.

CHAPTER THREE

3. RESULTS.

3.1 Relative abundance of anopheline larvae.

Mosquito larvae were encountered in all the habitats except one during the study period. More anopheline larvae were observed in the months of January and February 2005, with the peak in the month of February, and their population decreased in the wet months of March, April and May 2005 (Figure, 2), with the lowest numbers in May, which was the peak of the rain season in the study site that year. More *An. gambiae* larvae were encountered than *An. funestus* larvae in all the months' except in the month of March (Figure, 2).

3.1.1. Larval abundance in relation to habitat type

Twenty-six (26) larval habitats were identified; this was more than 90% of all the habitats present in the study site. Fourteen (14) of these were under forest cover and 12 were located in the open sunlit patches under cultivation. Significantly more *An.gambiae* complex larvae ($\chi^2 = 24.3524$, df = 1, P<0.0001) and *An. funestus complex* larvae ($\chi^2 = 4.5896$ df = 1, P = 0.0322) were collected in the habitats in the open sunlight than the larvae of the same species collected in habitats under forest cover (Table. 1). *An.funestus* complex larvae were found to be more (0.14±0.03 larvae/dip) than *An. gambiae* larvae (0.08±0.02 larvae/dip) in the forest habitats, while *An. gambiae* complex larvae were comparatively more (0.58±0.34) in the habitats that were located in open sunlight (Table 1).

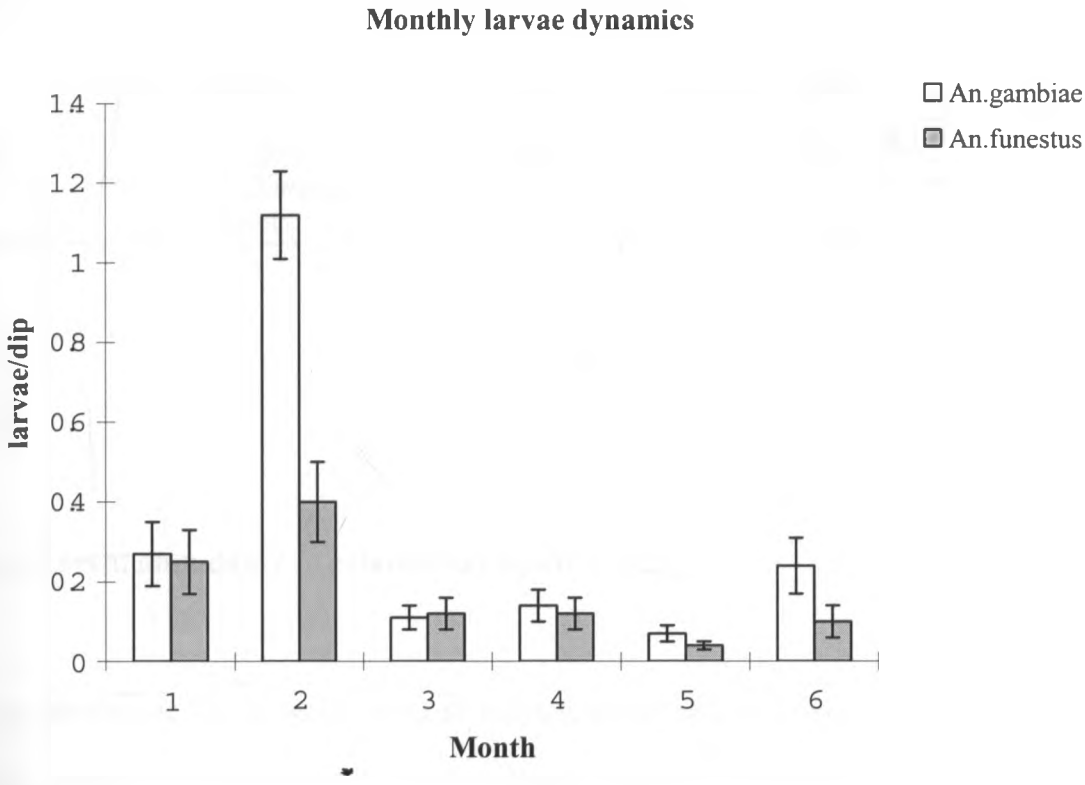

Fig. 2 Monthly dynamics of the mean number of anopheline larvae per dip for the period of January –June 2005

Table 1. The sum and the mean number (±SE) of anopheline immature stages per dip in the forest and open habitats.

Habitat type	Variable	Sum	Mean (larvae/dip)
Forest	*An.gambiae*	241	0.08±0.02
	An.funestus	420	0.14±0.03
	Total	661	0.22±0.04
Open	*An.gambiae*	489	0.58±0.34
	An.funestus	320	0.20±0.04
	Total	871	0.79±0.34

3.1.2. Larval abundance in relation to canopy cover.

More anopheline larvae were found in habitats under canopy cover category 2 (canopy cover below 50 %) than in the habitats under canopy cover category 1 (canopy cover above 50%).

There was a significant association between the presence and abundance of anopheline larvae and canopy cover (Correlation coefficient: P = 0.0152), where fewer anopheline larvae were encountered in habitats with more canopy cover (above 50%) and more larvae being found in habitats with a lower canopy cover (below 50%).

3.2 Relative abundance of indoor resting adult mosquitoes.

A total of 850 adult mosquitoes were collected from 20 houses in the reforested site in Mbale during the study period. Out of the total, 10.12% (86) were anopheline mosquitoes and 89.88% (764) were culicine. A total of 606 mosquitoes were collected from the same number of houses (20) at a deforested site in Iguhu, with anopheline comprising 78.22% (474) and culicine constituting the remaining 21.78% (132). Among the two anopheline vectors (*An.gambiae* and *An. funestus* complexes) identified at the two sites, *An. gambiae* complex was more abundant than *An. funestus* (Figure 3 & 4 and Table 2). The number of female vectors was also higher at the deforested site than at the reforested site, though the proportion of blood fed mosquitoes was higher at the reforested site (Table 2). The reforested site had a higher number of culicine mosquitoes than in the deforested site (figure 3).

The relative indoor resting density of *An. gambiae* mosquitoes remained low in the months of January to March in both sites (Figure 3 & 4) and peaked in the month of April in the reforested site (figure 3) and the of May in the deforested site (figure 4). The density of *An. funestus* remained low throughout the sampling period except for a slight increase in month of June 2005 (Figure 3&4). The density of culicine mosquitoes remained fairly low at the deforested site with a sudden peak in the month of June 2005 (Figure 4) while at the reforested site, the density short up as from the month of March 2005 (Figure 3).

Table 2. Number of female anopheline mosquitoes collected at the study sites and the proportion of the blood fed females.

Site	*An. gambiae*		*An. funestus*	
	Number of females	Blood fed	Number of females	Blood fed
Mbale (reforested)	71	0.72	9	0.56
Iguhu (deforested)	351	0.67	18	0.39

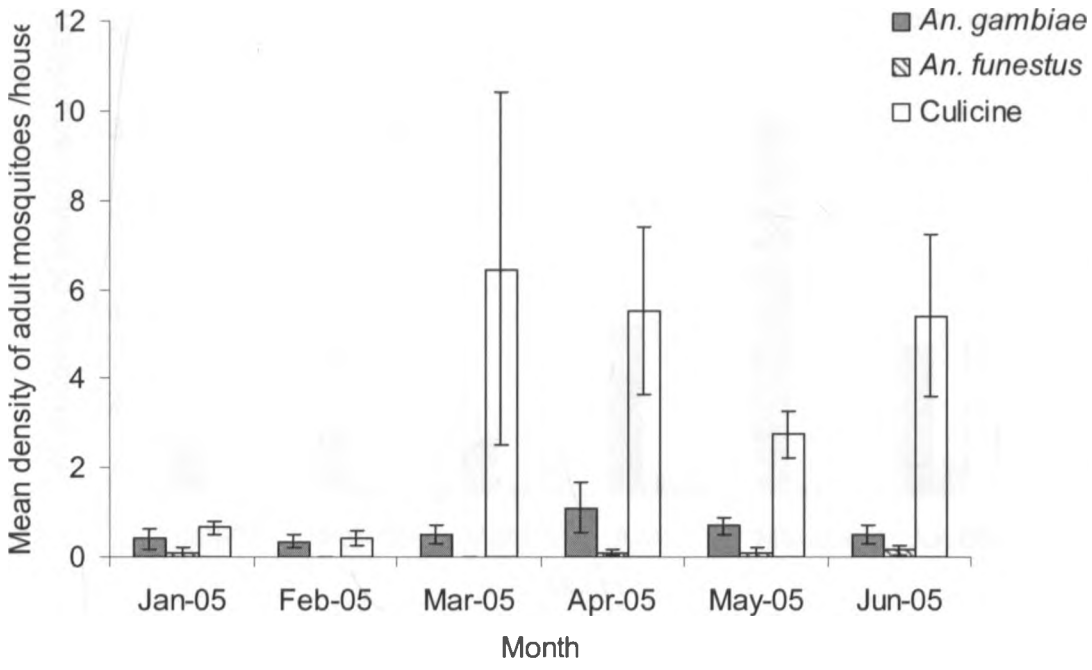

Fig. 3. Relative densities/abundance of indoor resting mosquitoes at the reforested site in Mbale during the sampling period

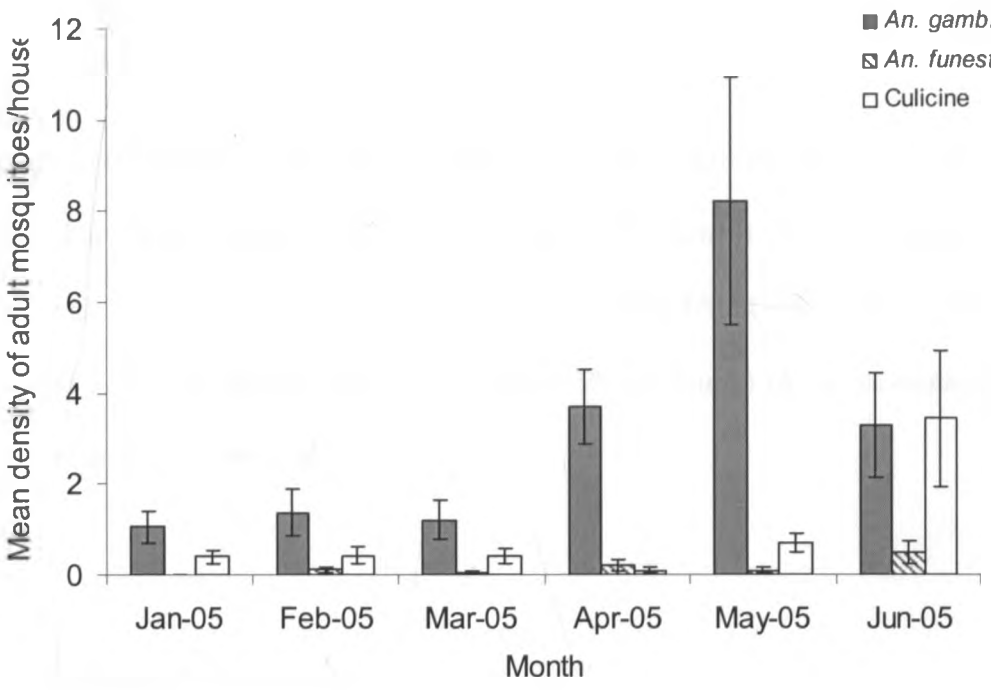

Fig. 4 Relative densities/abundance indoor resting mosquitoes at the deforested site in Iguhu during the sampling period.

NB. Relative mosquito density in this study refers to the mean number of female mosquitoes per house.

The density of mosquito vectors was significantly higher in the deforested site than in the reforested site (ANOVA; $F = 39.16$, $df = 1, 5$, $P<0.0001$). Monthly variations in the density of *An. gambiae* complex was also found to be significant (ANOVA; $F = 4.05$, $df = 1, 5$, $P = 0.0017$), with a minimum of 0.35 ± 0.13, (in February) and 1.05 ± 0.37, (in January) females mosquitoes per house in reforested and deforested sites, respectively,

and a maximum of 1.1 ± 0.59 and 3.7 ± 0.83 (both in April), females mosquitoes per house in reforested and deforested sites, respectively (Fig.3 & 4). The number of *An. funestus* complex mosquitoes was too small to be subjected to a similar statistical analysis.

Examination of mosquito abdominal appearance to determine the inoculation rate indicated that 74.64% and 8.50% (in Mbale), 70.75% and 4.44% (in Iguhu) of *An. gambiae* and *An. funestus* complexes respectively were blood fed or half gravid and 21.12% and 3.33% (in Mbale) and 20.22% and 0.5% (in Iguhu) of *An. gambiae* and *An. Funestus,* respectively, were gravid.

3.3 Parasitological surveys.

3.3.1 Prevalence of malaria parasites and parasite densities.

Plasmodium falciparum and *Plasmodium malariae* were the two types of malaria parasites identified at the study sites, during the study period.

The prevalence of *P. falciparum* parasites in School children from homes adjacent to the reforested site was comparatively lower than that of the children from homes adjacent to the deforested site, in both the February 2005 and May 2005 surveys (Table 3). *Plasmodium falciparum* prevalence in the reforested site was found to be significantly lower ($\chi^2 = 5.6$, df = 1, P = 0.018) than prevalence of the same parasite species in the deforested site, during the February survey (Table 4). Prevalence of *Plasmodium malariae* parasites at the two sites was very low (below 5%) in both surveys (Table 3).

When the parasite prevalence between the two survey periods was compared, prevalence of *P. falciparum* at the reforested site in May was found to be higher than that of February, while the prevalence of *P. falciparum* at the deforested site in May was lower than that of February. However these differences in the prevalence were not significant (Table 4).

Table 3. Malaria parasite prevalence's in children from the reforested and deforested sites during the February and May 2005 parasitological surveys.

Month/ Site	Sample size n	*P. falciparum* prevalence	*P. malariae* prevalence
February '05			
Reforested	100	37.00%	3.00%
Deforested	94	56.38%	3.20%
May '05			
Reforest	93	42.00%	1.00%
Deforested	91	47.25%	2.18%

Table. 4. Result of the Chi-square test for parasite prevalence between the two surveys and between the sites.

Month/Site	Study site/month	χ^2	P-value
Feb. vs. May	Deforested	1.04	0.244
	Reforested	0.2	0.554
Deforested vs. reforested	Feb.	5.6	0.018
	May	0.33	0.566

The density of *P. falciparum* parasites in school children at the reforested site was found to be slightly higher compared to that of school children at the deforested site in the two survey periods (Table 5). However, these differences were not significant (t-test.)

Table 5. The parasite densities per site in the February and May surveys

Site	Month	Geometric mean
deforested	Feb.	573.3
	May	744.38
reforested	Feb.	783.91
	May	750.02

3.4 Survivorship of *An.gambiae* larvae in artificial habitats.

3.4.1 Mean survival time.

Mean survival time analysis was done to compare larvae survival in the forest and in the open sunlit habitats. Rain and swamp water types were used in each habitat, and the results analyzed separately. Overall mean survival time (Table 6) was significantly shorter in the open sunlit habitat during February experiment (χ^2 = 109.19, df = 1,

P<0.0001 - in rain water, and $\chi^2 = 110.5$, df = 1, P<0.0001, - in swamp water) and May experiments ($\chi^2 = 12.89$, df = 1, P = 0.0003, - Rain water, $\chi^2 = 10.02$, df = 1, P = 0.0015 - in swamp water). Larvae took a longer period (before development to adult or death), in forest habitat.

Table 6. Mean survival time (days) of *Anopheles gambiae* larvae, in the forest and open habitats placed in rain and swamp water.

Month	Habitat	Rain water	Swamp water
February	Forest	24.09 ± 0.57	24.08 ± 0.57
	Open	12.39 ± 0.34	14.23 ± 0.22
May	Forest	15.92 ± 0.24	23.73 ± 1.20
	Open	14.91 ± 0.27	17.45 ± 0.64

3.4.2 Pupation success.

Pupation success in this study is defined as the proportion of first-instar larvae that developed to the pupa stage.

More pupae were observed in the experiment that was done in May 2005 both in habitats with rainwater and swamp water (except in the forest habitats with swamp water which had 9 pupae), than the number of pupae recorded in the experiment that was done in February 2005 (Table 7).

Overall mean pupation success was higher in the open sunlit habitats than in the habitats under forest cover (Table 8). Pupation was also observed to be higher in the habitats with rainwater (Table 8) except in the February experiment (open) in which one of the replicate had been interfered with.

Table 7. The number of pupae observed out of 90 larvae (3 replicates of 30 each) in the two habitat types, during the February and May experiments.

Habitat type	Water type	No. of pupae	% Pupation
February			
Forest	Swamp	59	66
	Rain	67	74
Open	Swamp	63	70
	Rain	29	32
May			
Forest	Swamp	9	10
	Rain	74	82
Open	Swamp	71	78
	Rain	67	74

Table 8. Mean pupation success in the forest and open sunlit habitats.

Month	Forest		Open	
	Rainwater	Swamp water	Rainwater	Swamp water
February	0.74 ± 0.05	0.66 ± 0.04	0.32 ± 0.03	0.7 ± 0.04
May	0.82 ± 0.05	0.07 ± 0.02	0.92 ± 0.23	0.68 ± 0.03

3.4.3 Comparison of water temperature in the forest and open artificial habitats.

The maximum water temperature was higher in month of February 2005 compared to that of the month of May 2005 in both habitat types (Table 9). The maximum, minimum and mean water temperatures were significantly higher in habitats in the open than in habitats in the forest ($P<0.0001$) both in February 2005 and May 2005 experiments except, the minimum water temperature of habitats in the open for the month of February 2005, which had a slight drop that was not significant (ANOVA $P=0.9436$) (Table 9).

Table 9. Comparison of maximum, minimum and mean water temperatures for artificial habitats under forest cover and in the open area at Mbale.

Month	Forest habitats	Open habitats	F	P-value
February				
Max. Water temp.	28.51 ± 0.13	39.20 ± 0.22	1828.5	<0.0001
Min. water temp	16.28 ± 0.17	16.26 ± 0.29	0.005	0.944
Mean water temp.	21.32 ± 0.11	24.50 ± 0.11	392.2	<0.0001
May				
Max. water temp.	25.08 ± 0.13	34.55 ± 0.23	1434.5	<0.0001
Min. water temp	17.23 ± 0.07	17.75 ± 0.08	25.08	<0.0001
Mean water temp.	20.27 ± 0.07	23.20 ± 0.10	716.05	<0.0001

3.4.4 Larval development time.

Development time is the length of time that a first-instar larva requires to emerge as an adult. On average it took 14.65 ± 0.20 days for larvae in swamp water to develop into female adult stage in the open and 24.19 ± 0.81 in the forest during the February experiment. In the May experiment, the average number of days was longer; 19.79 ±1.00 (open) and 29.67 ± 3.38 (forest) (Table 10). In general the development time for both males and females was shorter in habitats in the open sunlight and longer in habitats located in the forest. (Table10).

Table 10. Mean development time (days) for adult mosquitoes in the forest and open habitats during the two experimental periods

Dev. Time (days)	Forest		Open	
February	**Rainwater**	**Swamp water**	**Rainwater**	**Swamp water**
Female	15.94 ± 0.16	24.19 ± 0.81	14.69 ± 0.36	14.65 ± 0.20
Male	16.29 ± 0.21	23.12 ± 0.52	14.27 ± 0.32	14.85 ± 0.20
May				
Female	17.23 ± 0.29	29.67 ± 3.38	15.62 ± 0.46	19.79 ± 1.00
Male	17.05 ± 0.26	30.00 ± 2.00	14.97 ± 0.41	18.94 ± 0.41

3.5 Larval habitat characteristics.

Twenty six (26) larval habitats were identified and a map showing their spatial location and distribution in the site was produced (Figure 5). Twenty two (22) were drainage ditches (plates 6 and 7) whose lengths varied from 1 – 30 m, the remaining 4 larval habitats, being small pools with an average diameter range of 0.2 - 0.5 m. Filamentous algae were found in 3 habitats only. Twelve (46%) of the habitats had a canopy cover of over 70%, 6 (23%) between 20 -70%, and 9 (35%) below 20% canopy cover.

Among the three habitat characteristics that were considered (habitat size, presence of filamentous algae and canopy cover), only canopy cover was found to have significant influence on anopheline larval habitats. Most of the habitats were characterized by the presence of short grass and papyrus on the edges, with varying degrees of emergent vegetation (Plate 5, 6 &7). Vegetation in drainage ditches was cleared occasionally to improve on the drainage. Fewer larvae were encountered in ditches that were cleared and water was flowing fast. *An. funestus* larvae were common in habitats that were choked with grass and/or reeds (Plate 5), while *An. gambiae* larvae were abundant in habitats with little or no vegetation. More habitats were aggregated on the right hand side of the Kisumu – Kakamega road (Figure 5).

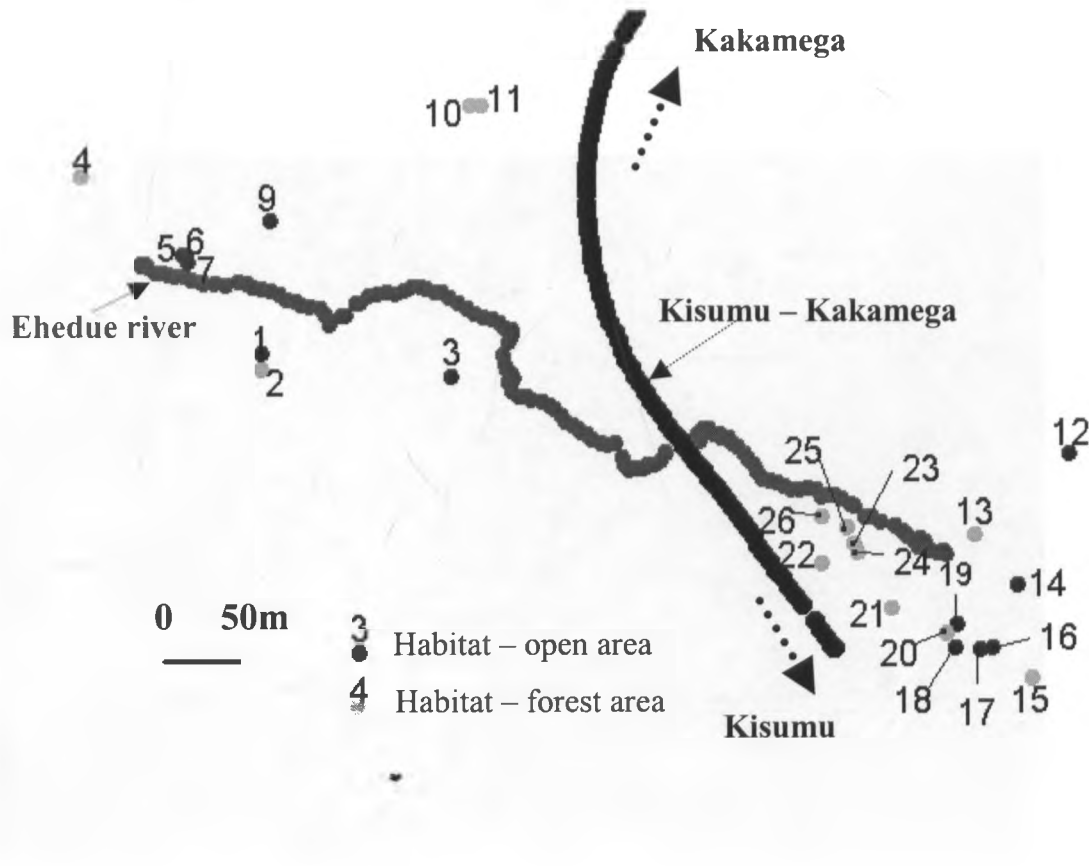

Fig. 5. A map showing spatial location and distribution of larval habitats in the reforested valley.

Plate 5. A habitat chocked with grass- productive for *An.funestus* larvae.

Plate 6. A drainage ditch in a cultivated patch in the valley

Plate 7. A Typical drainage ditch in the forest- with little emergent vegetation.

CHAPTER FOUR

DISCUSSION

Results of this study indicate that, habitats in the reforested area had a lower number of anopheline larvae than the habitats in the open sunlight. Anopheline larvae had higher chances of surviving and developing into pupae in the habitats exposed to sunlight than in the habitats located in the forest. The density of adult anopheline mosquitoes together with the malaria parasite prevalence were found to be lower in the reforested site than in the deforested site.

Forest cover has been found to influence growth conditions for *An.gambiae* larvae (Tuno *et al.*, 2005), by limiting the growth of algae, which is one of the main food source for anopheline larvae especially *An.gambiae* s.s. Warmer, open habitats tend to produce more algae than do shaded habitats and the number and productivity of larval habitats ultimately determines the density of adults (Gimnig *et al.*, 2002). When food was provided in form of yeast to larvae in artificial habitats in the forest, their growth in terms of pupation success was found to be fairly faster than in larvae that received no yeast.

The forest cover has the effect of lowering the water temperature to levels that can be inhibitive to larval survival, since warmer temperatures encountered in small and open habitats shorten larval-to-pupae development time of *An.gambiae* (Gillies and De Meillon, 1968; Bayoh and Lindsay, 2004). Survival of the immature stages determines how many adults are available at a specific location (Bayoh and Lindsay, 2004).

In the experiment done in the artificial habitats, the maximum, minimum and the mean temperatures were found to be significantly lower in the forest than in the open, and larvae-to-adult development time was significantly longer in forest than in the open. It is important to note that, during the February experiment the maximum water temperature in the forest habitats was relatively high (28°C) than the temperature for May (25°C). This was followed by significantly higher pupation success than in the cooler May experiment, suggesting that low water temperature caused by the forest cover may indeed retard larval growth. However, the length of time larvae are exposed to water temperatures below the minimum or above the maximum limits may influence larval survival rate.

Water temperature is a critical factor determining the survival of *An. gambiae*, prolonged exposure to temperatures below the minimum (16°C) retards growth and temperatures above 40°C are lethal (Bayoh and Lindsay, 2004). The optimum rearing temperature for *An. gambiae* mosquitoes is 27°C (Lymo *et al.*, 1992). This observation also suggest that, abnormally high air temperature in reforested valleys in the highlands as observed during the February experiment may result in warming up of water in the breeding habitats to levels that are optimum for larval growth. This may lead to a high density of adult mosquitoes, making such habitats that are otherwise inhibitive to larval growth under normal air temperature, very productive. The cessation of larval development at 14-16 °C, with continuation of the larval period for >30 days, may have important public health consequences in the African highlands if such individuals can develop into adults as temperatures warm (Bayoh and Lindsay, 2004).

Forest canopy cover seems to affect not only water temperature of the larval habitats but also the occurrence of other aquatic insects, which in turn influence the survivorship of *An. gambiae* larvae (Tuno *et al.*, 2005). This suggests that the forest may provide conditions suitable for anopheline mosquito predators (and probably competitors). In natural conditions the predation effect might be enhanced in the forest habitats with lower water temperature because the prolonged larval development period of *An. gambiae* in the forest habitats increases the chances of larvae encountering predators (Tuno *et al.*, 2005). Larval predation has been observed to be less prevalent in temporary habitats than in large, permanent habitats (Sunahara *et al.*, 2002). Since most larval habitats in the study area were of the latter type predation may have been high.

In this study *An. funestus* larvae were found to thrive in habitats with dense growth of grass and reeds. Minakawa *et al.*, (2005b), reported that *An. funestus* were mostly found in natural swamps and pastures characterized by the presence of emergent vegetation. This observation is consistent with earlier findings (Hopkins, 1940). *An. funestus* larvae show a marked preference for waters densely shaded from the sun, or marshes choked with reeds and other densely growing aquatic vegetation (Kirk, 1936). Since Eucalyptus trees do not have a dense shade *An. funestus* larvae can thrive. However, it would be important to determine the amount of shade that would be deterrent to anopheline mosquitoes. Most of the larval habitats were drainage ditches. Frequent clearing of emergent vegetation in the drainage ditches may create conditions unsuitable for *An. funestus* breeding in the reforested habitats. Once the ditches are cleared water may flow fast enough to deter mosquito breeding. It was observed that, some habitats that were

cleared had fewer larvae than adjacent habitats with vegetation growth. Emergent vegetation interrupts water flow providing ideal conditions for mosquito breeding and predation.

The habitats in the forest were observed to have more leaf litter mainly from *Eucalyptus* trees, than those in the open. Leaf litter may produce phenolic compounds (tannin-phenolic) that are harmful to some mosquito species (Rey *et al.*, 1999). *Eucalyptus* leaves are known to have a repulsive effect on adult *An. gambiae s.s* (Barasa *et al.*, 2000) and they could possibly have negative effect on larval growth in the forest habitats.

In addition to the possible effects of Eucalyptus leaf litter and shade on growth and survival of Anopheline larvae, their role in draining up of swamps and marshes is worth noting. Due to its rapid growth, *Eucalyptus globulus* is used in Africa to drain malarial swamps, and thus may play an important role in the elimination of breeding grounds for mosquitoes (Santos, 1997).

In this study, the month of February had the highest larval count (449) and the month of May had the lowest larval count (75). This observation is rather interesting, because the vector population is normally expected to be high as rains subside and the breeding sites increase in number and vice versa during the dry period. During the sampling period, the month of May had more heavy rain, which may have flushed out and killed most larvae. Heavy rains experienced in the months of November and December may have been responsible for a significant increase in larvae population in January and the peak in February (personal observation) as a result of an increase in breeding sites.

The relative indoor resting density/abundance of *An.gambiae* mosquitoes in the reforested site (Mbale) was significantly much lower ($F=39.16$, $df=1$, $P<0.0001$) than the relative density found in the deforested site (Iguhu). The low relative density in the reforested site could be attributed to the low survival rate of larvae in the habitats located in the forest as a result of the factors discussed above. However, in the reforested site culicine mosquitoes were predominant indicating that the forest provides suitable conditions for culicine mosquitoes to thrive. It is interesting to note that a very low relative density of *An.funestus* was found in both sites, and this may suggest that it may be a minor vector in the study sites.

Low relative densities of Anopheline mosquitoes may translate into low entomological inoculation rate (Bødker *et al.*, 2003) and vector abundance is an important determinant of malaria transmission force (Garrett et al., 1964) and thus factors that increase or decrease vector abundance will have an impact on malaria prevalence. In this study, that the reforested site had a significantly lower prevalence of malaria parasites than the deforested site during the two survey periods. Among other factors, this may have been due to the low relative vector density observed in the reforested site. This observation suggests that reforestation of swampy river valleys may have an important role in disrupting the breeding of *An. gambiae* and *An. funestus* mosquitoes thus reducing the malaria vector population to significant levels. This in turn may lower entomological inoculation rate and subsequently reduce malaria transmission in western Kenya highlands.

Conclusion

In summary this study demonstrated a significant reduction in the survivorship of *An. gambiae* larvae in the reforested larval habitats and hence a low relative abundance of the same. Some plausible reasons (which relate to the forest canopy cover), are; 1) reduced availability of food (mainly algae) for *Anopheles* larvae, 2) lower water temperature, 3) increased presence of predators and competitors, 4) harmful chemical substances released by decomposing eucalyptus leaf litter (or may be root exudates).

Since most anopheline larval habitats in the study site are drainage ditches, regular maintenance practice by clearing emergent vegetation would increase the speed of water flow in the drainage ditches. Further, breeding of mosquitoes may be deterred by the effect of shade by the *Eucalyptus* trees. It was observed that, some habitats that were cleared had fewer larvae than adjacent habitats with emergent vegetation.

The adult mosquito vector population in the reforested site was far much less than the vector population in the deforested site; the same was also true for *Plasmodium falciparum* prevalence.

Taken together these results suggest that reforestation of the swampy river valley may reduce survivorship of anopheline larvae, leading to a low abundance of adult vector mosquitoes. Low relative densities of anopheline mosquitoes may translate into low entomological inoculation rate (EIR), which may result to the low transmission of the malaria parasite.

Recommendations.

This study was faced with a major challenge of coming up with a suitable control site within the limited study period hence the selection of a relative comparison area which had many factors in common with the reforested except forest cover. In addition, more comprehensive results would have been obtained if a similar study was conducted in other several highland sites. However, the following are recommended from this study;

1. Studies on the effect of *Eucalyptus* leaf litter and root exudates on larvae of Anopheline vectors in this site are needed. In this study it was only hypothesized that they might influence larval development. Bioassays of the chemical extracts on larvae may prove useful in this case.
2. The physical and chemical characteristics of water in the reforested habitats in the study site need to be analyzed to determine the effect of *Eucalyptus* trees on the quality of water with respect to the chemical substances that may influence larval growth,
3. A study on anopheline larvae nutrition in reforested habitats in relation to larval growth is recommended for this site. Alongside this, the role of predators needs to be examined since it was observed that indeed there were numerous early instar larvae but very few late instar stage larvae.
4. A larger sampling area for larvae and adult mosquito vectors, a suitable comparison area, together with a larger sample size for parasitological surveys with increased frequency of surveys (maybe monthly) and for a longer period of time, will be ideal for similar studies in future for more comprehensive and reliable results.

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